Pergamon

Tetrahedron: *Asymmetry* 12 (2001) 869–876

TETRAHEDRON: *ASYMMETRY*

Chemoenzymatic synthesis of chiral substituted acrylate and acrylonitrile precursors for the synthesis of 3-deoxy-2-ulosonic acids and α-methylene-γ-lactones

D. Crestia, C. Guérard, H. Veschambre, L. Hecquet, C. Demuynck and J. Bolte*

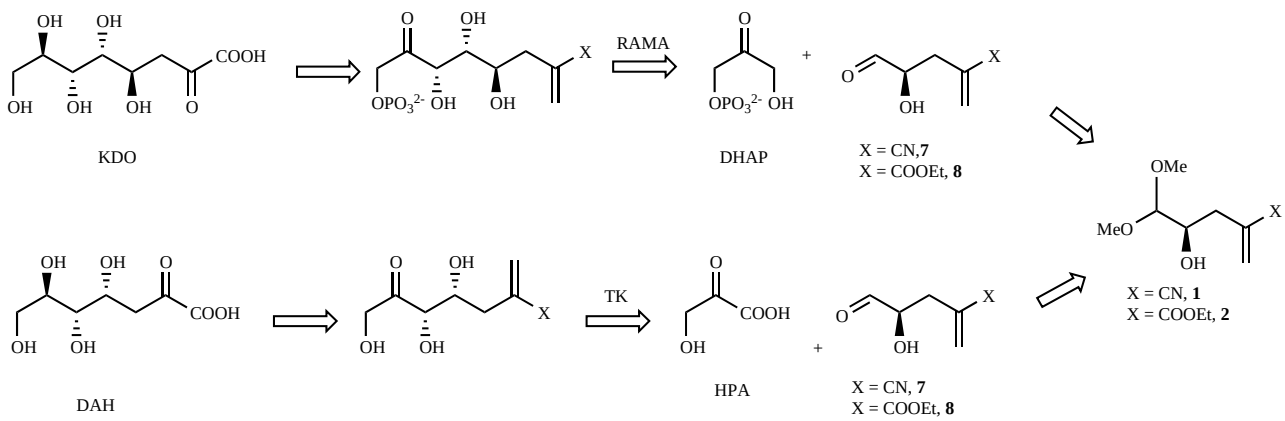
Laboratoire de Synthèse, Electrosynthèse et Etude de Systèmes à Intérêt Biologique, UMR 6504 du CNRS, Université Blaise Pascal, 63177 Aubière Cedex, France

Received 13 February 2001; accepted 2 April 2001

Abstract—Substituted acrylonitrile and ethyl acrylate bearing a masked α-hydroxyaldehyde were easily synthesised by a Barbier type reaction between the monoacetal of glyoxal and bromomethyl acrylonitrile or ethyl bromomethyl acrylate. We prepared these interesting synthons in an enantiomerically pure form by enzymatic resolution with *Candida rugosa* lipase, or by microbial reduction of the corresponding ketones using *Aspergillus niger* and *Lactobacillus kefir*. © 2001 Elsevier Science Ltd. All rights reserved.

1. Introduction

In the course of our studies on the enzymatic synthesis of ulosonic acids by a non-metabolic pathway, we became interested in the homochiral synthons **1** and **2**. Indeed, the acryloyl pattern in **1** is a good precursor for the pyruvyl moiety of 3-deoxy-2-ulosonic acids, and numerous syntheses of 3-deoxy-D-*manno*-2-octulosonic acid (KDO) and sialic acids are based on the reaction of ethyl bromomethacrylate with an aldehyde.[1] In fact, the aldehyde produced after acidic hydrolysis of **1** and **2** can lead to KDO, the key step being the condensation of dihydroxyacetone phosphate catalysed by fructose-1,6-bisphosphate aldolase from rabbit muscle (RAMA). In the same way, the transfer of a hydroxyacetyl group onto the same aldehyde, catalysed by transketolase (TK), would afford a precursor of 3-deoxy-D-*arabino*-2-heptulosonic acid (DAH) (Scheme 1). The full syntheses of KDO and DAH are in progress in our laboratory and have been the subject of preliminary papers.[2]



Scheme 1.

* Corresponding author. Tel.: 33 (0)4 73 40 71 28; fax: 33 (0)4 73 40 77 17; e-mail: jbolte@chisg1.univ-bpclermont.fr

PII: S0957-4166(01)00155-0

Moreover, **1** and **2** and its precursors **3** and **4** are of interest in the synthesis of naturally occurring α-methylene-γ-lactones and α-keto-γ-lactones which often present biological activities.[3] Herein, we describe the synthesis of both isomers of **1** and **2**, the chirality being obtained by lipase mediated resolution as well as by microbial reduction of the ketone precursors **3** and **4** (Scheme 2).

2. Results and discussion

2.1. Synthesis of racemic alcohols 1 and 2 and enantiomeric excess (e.e.) determination

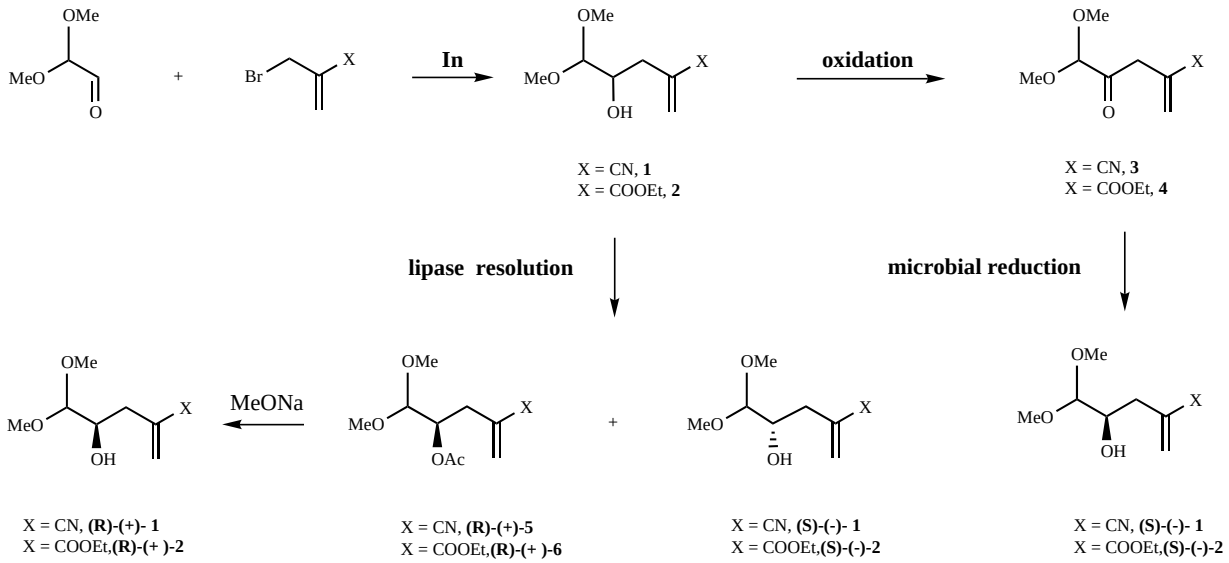
2-(2-Hydroxy-3,3-dimethoxy-propyl)acrylic acid ethyl ester **2** was prepared by a Barbier type reaction between the monoacetal of glyoxal and ethyl bromomethyl acrylate in the presence of indium as previously described.[2a] The corresponding nitrile **1** was obtained in the same way from bromomethyl acrylonitrile. Having in hand (±)-**1** and (±)-**2**, we looked for a method for the determination of e.e. The racemates were not resolved by GPC nor by HPLC using the chiral column available in the laboratory, so we turned our attention to spectroscopic methods. The ^{1}H NMR spectra of **1** and **2** resolved upon addition of Eu(hfc)$_3$. The olefinic protons of both enantiomers were the best separated, with a $\Delta\delta$ of 0.02 ppm which allowed calculation of the e.e. with satisfactory precision.

2.2. Enzymatic resolution

In order to perform the resolution, compounds **1** and **2** were subjected to transesterification by vinyl acetate in the presence of lipases. Of the enzymes tested,[4] the lipase from *Candida rugosa* was very effective for the resolution of the nitrile **1**. The results are reported in Table 1. The racemic alcohol was dissolved in vinyl acetate (75 mM) and *C. rugosa* lipase was added. The mixture was gently stirred at 25°C, the reaction was stopped after 25 or 30 h and the products were purified by chromatography. After 25 h the e.e. of the acetate produced and of the residual alcohol were more than 95 and 87%, respectively. The reaction occurred with a 48% conversion and an enantioselectivity coefficient (*E*) of more than 111.[5] This resolution was performed on a 10 g scale and led to the desired synthon with satisfactory e.e. and yield. In contrast, the ester **2** was poorly recognised by the enzyme, since after 30 h the conversion rate was only 20% and the e.e. of acetate produced was also only 20%.

2.3. Microbial reduction

Microbial reduction of ketones is a very useful method for the production of homochiral alcohols.[6] The appropriate choice of the microorganism often allows the preparation of both enantiomers, and moreover, when only one enantiomer is needed, the method is preferred



Scheme 2.

Table 1. *C. rugosa* lipase catalysed transesterification of **1** and **2** at 25°C

Substrate	Reaction time (h)	Conv. (%)	Produced ester			Residual alcohol			*E*
			Yield (%)[b]	E.e. (%)[a]	$[\alpha]_D^{25}$	Yield (%)[b]	E.e. (%)[a]	$[\alpha]_D^{25}$	
(±)-**1**	25	48	78	≥95	+12	77	87	−10	≥111
(±)-**2**	30	20	48	20	+2.6	58	5	−1	1.6

[a] Determined by ^{1}H NMR experiments in the presence of Eu(hfc)$_3$.
[b] Yields are given in comparison with 50% chemical yield for one enantiomer which can be isolated.

Table 2. Microbiological reductions of **3** and **4** at 27°C

	Alcohol **1**				Alcohol **2**			
	$[\alpha]_D^{25}$	E.e. (%)[a]	Yield (%)[b]	Incubation time (h)	$[\alpha]_D^{25}$	E.e. (%)[a]	Yield (%)[b]	Incubation time (h)
A. niger	−10.5	≥95	57	72	−9	90	47	48
L. kefir	No reduction				−11	≥95	30	72
Baker's yeast	Not carried out with **3**				−5.4	47	37	48
R. glutinis	Not carried out with **3**						No reduction	
Y. farinosa	Not carried out with **3**				−7.5	65	26	48
Z. rouxii (growth)	Not carried out with **3**				+6.6	57	62	48

[a] Determined by ^{1}H NMR experiments in the presence of Eu(hfc)$_3$.
[b] Yield after purification on silica gel flash chromatography. Presence in small quantities of unidentified products.

to enzymatic resolution, where the yields are limited to 50% if the wrong enantiomer cannot be recycled. In order to implement this strategy, **1** and **2** were oxidised to the corresponding ketones **3** and **4**. However, of the oxidants tested,[7] only pyridinium chlorochromate afforded **3** in a low 26% yield. In contrast, **2** was easily oxidised using pyridinium dichromate in the presence of pyridinium trifluoroacetate leading to ketone **4** in 87% yield (Scheme 2).

Ketones **3** and **4** were subjected to reaction with various microorganisms. The results are reported in Table 2. In the case of baker's yeast, we used commercial freeze-dried cells under non-fermenting conditions. For *Aspergillus niger*, *Lactobacillus kefir*, *Rhodoturula glutinis* and *Yamadazyma farinosa*, the bioconversions were carried out using washed resting cells. But, in the case of *Zygosaccharomyces rouxii*, no reduction was observed under these conditions, so the substrate was added in the fermentation medium at the end of the growth phase. In the reduction of **4**, only *Z. rouxii* gave a dextrogyre alcohol, with only modest e.e. The best results were obtained with *L. kefir* and *A. niger*, which afforded the levogyre alcohol with 90 and >95% e.e., respectively. Although no ketone remained in the reaction mixture after 48 h of reaction, the yield of the purified alcohol was only around 50%, probably due to its difficult extraction from water. In the case of the nitrile **3**, no reduction was observed with *L. kefir*, but *A. niger* gave the levogyre alcohol with a satisfactory e.e. of 95%.

2.4. Determination of the absolute configurations

None of the optically active alcohols isolated from microbial reduction or lipase catalysed resolution have been previously described, and it was necessary to establish their absolute configuration. Lipase from *C. rugosa* is one of the most often employed for laboratory and industrial applications.[8] Its chiral preference was established by Kazlauskas et al.[9] by analysis of the structure of covalent complexes formed with transition-state analogues. An empirical rule for enantiorecognition by *C. rugosa* lipase and eleven other hydrolases is represented schematically in Fig. 1. When the alcohol is drawn with the hydroxyl group pointing forward, out of the plane of the page, the favoured enantiomer bears a large substituent on the left and a medium substituent on the right. If we consider that, in **1** and **2**, the large substituent is the acetal group, where the atom directly linked to the asymmetric carbon is the most substituted, and the medium substituent is the acryloyl group, then the dextrogyre alcohol which is the substrate of the lipase should have (*R*)-configuration. Of course, this conclusion must be confirmed by other evidence since it is based on an empirical rule and a rather subjective classification of the substrate molecule. The enantiopreference of the lipase for ester **2** is also very low.

Compounds **1** and **2** are precursors of α-hydroxyaldehydes which are supposed to be substrates of fructose-1,6-bisphosphate aldolase and transketolase as shown in Scheme 1. In the case of transketolase, their configurations are essential since this enzyme only accepts α-hydroxyaldehydes of (*R*)-configuration at C-(2). This specificity was observed in all cases studied by us[10] and other groups.[11] Taking advantage of this property, Effenberger and co-workers[12] have prepared various (*S*)-α-hydroxyaldehydes. We thought that the measure of the activity of transketolase with both isomers of the aldehydes **7** and **8** provided by **1** and **2** could afford good evidence of their absolute configuration.

(+)-**1** and (−)-**1** were hydrolysed in acidic medium in the presence of Dowex H^+ resin. No racemisation occurred during the hydrolysis as indicated by the specific rotation of the resulting aldehydes. These compounds were then studied as aldehydic substrates of transketolase. In these reactions a hydroxyacetyl group is transferred from xylulose-5-phosphate to the aldehyde leading to

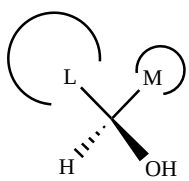
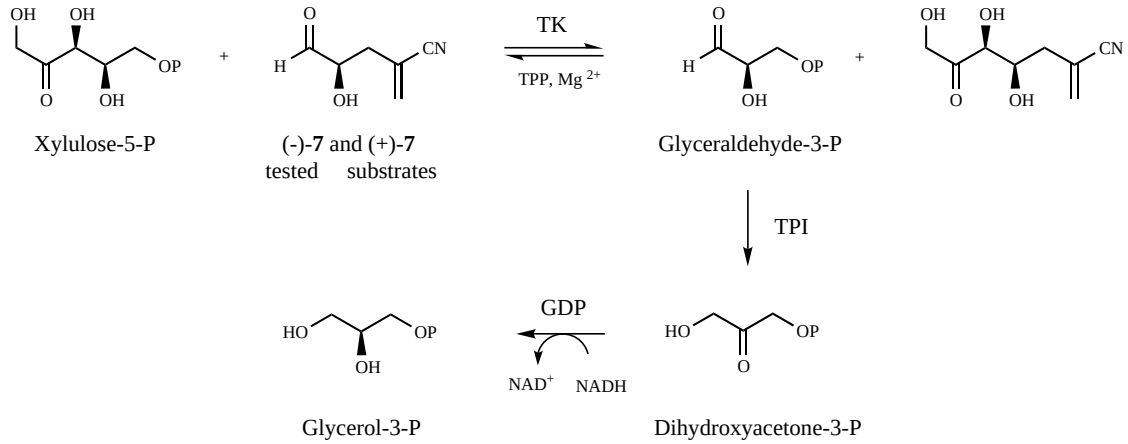


Figure 1. This figure represents the enantiomer of a secondary alcohol which reacts faster with *Candida rugosa* lipase in the transesterification reaction. The hydroxyl group points forward out the plane of the page; M represents the medium substituent (i.e. in our case the acryloyl group), L represents a large substituent (i.e. in our case the acetal group).



Scheme 3.

Table 3. Kinetic constants of TK towards aldehydic substrates

Substrate	E.e. (%)	$V_{rel.}$ at 50 mM	V_{max} (U/mg)	K_m (mM)	V_{max}/K_m (U/mg/mM)
(−)-**1**→(−)-**7**	95	0.01	0.3	273	1.1
(+)-**1**→(+)-**7**	95	0.309	0.4	9	44.4

the formation of a new ketose and the liberation of glyceraldehyde-3-phosphate, which is reduced by NADH in the presence of triose phosphate isomerase and glycerol phosphate dehydrogenase.[13] The oxidation of NADH can be monitored by UV spectroscopy at 340 nm (Scheme 3) and this method was applied in this study to follow the transketolase reactions. The rate of the TK reaction was measured at various concentrations of aldehyde which allowed calculation of the reaction rate at 50 mM concentration in aldehyde, the maximum reaction rate (V_{max}) and the Michaelis constant (K_m). The results are reported in Table 3.

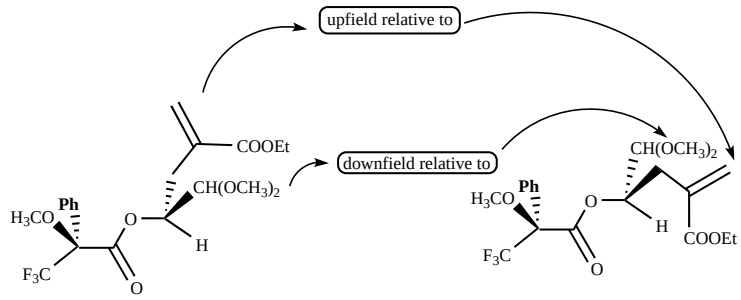
Of the substrates tested, (+)-**7** was best recognised by the enzyme with a K_m of about 9 mM versus 273 mM for the levogyre alcohol (−)-**7**. V_{max} was in the same range for the two enantiomers, but it is an extrapolated value without experimental meaning for (−)-**7** due to the high value of K_m. At a concentration of 50 mM in substrate, only (+)-**7** presents a significant activity. In view of this result, we attributed (*R*)-configuration to (+)-**1**, as is consistent with the presupposed lipase specificity. The same study could not be completed with ester **2**. Indeed, we did not have in hand the two enantiomers in a sufficient enantiomeric purity, and when the racemate was tested with transketolase, the activity was too low to determine the K_m values.

It could be supposed that, due to the small structural differences between **1** and **2**, the alcohols with the same optical rotation sign should be of the same configuration. However, we looked for additional evidence. Previously, the absolute configuration of alcohols was attributed by analysis of the ^{1}H NMR spectra of their Mosher's esters.[14] Indeed, it appears that, in solution, these esters adopt a preferential conformation where the methine proton, the carbonyl, and the methoxy groups are all *syn*- and coplanar. Consequently, certain groups of the alcohol moiety are, according to their position in the anisotropic magnetic field around the phenyl ring, either shielded or non-shielded. Mosher's esters were synthesised by reaction of (*S*)-(+)-α-methoxy-α-(trifluoromethyl)-phenyl-acetyl chloride (MTPA-Cl) with racemic alcohol **2** and enantiomerically pure (−)-**2** (Scheme 4).[15] The chemical shifts of the most differentiated protons are reported in Table 4.

In the (*S*,*S*)-ester, the acryloyl group is juxtaposed with the phenyl ring and the acetal with the methoxy while in the (*S*,*R*)-diastereomer, it is the opposite (Scheme 4). For the (*S*,*S*)-ester the resonance for the acryloyl group is shifted upfield from that of the alternate diastereomer as shown in Table 4. Additionally, the resonance for the acetal group of the (*S*,*R*)-ester is upfield from that of the (*S*,*S*)-ester. In our case, the differences between chemical shifts for the two diastereoisomers are sufficient (between 0.1 and 0.2 ppm) to apply this method for analytical purposes. The results obtained with the alcohol (−)-**2** show that it has (*S*)-configuration as with the parent nitrile (−)-**1**.

3. Conclusion

Both enantiomers of 2-(2-hydroxy-3,3-dimethoxy-propyl)-acrylonitrile have been prepared with satisfactory yields and e.e. by enzymatic resolution. The lipase from *C. rugosa* presents an enantioselectivity coefficient *E* of more than 100 in the esterification of these alcohols by vinyl acetate. The (*S*)-enantiomer and the (*S*)-enantiomer of the parent ethyl acrylate, were obtained by microbial reduction of the corresponding ketones using *A. niger* or *L. kefir*. Absolute configurations were attributed on the basis of the enantioselectivity of the



(*S*,*S*)- ester (*S*,*R*)- ester

Scheme 4. Configurational correlation model for (*S*)-MTPA derivatives of **2**.

Table 4. ^{1}H NMR chemical shift differences for MTPA derivates

	Substituent of alcohol moiety	Substituent chemical shift difference, [(*S*,*S*)-ester−(*S*,*R*)-ester] of MTPA derivatives of (−)-**2** δ [(*S*,*S*) ester−(*S*,*R*)-ester][a]
Acryloyl group	H olefin	[6.215−6.028] +0.187
	H olefin	[5.582−5.354] +0.228
	CH_2	[2.918–2.700] +0.218 and [2.529–2.400] +0.129
Acetal group	OCH_3	[3.362−3.462] −0.100 and [3.311–3.441] −0.130
	CH acetal	[4.314–4.416] −0.102

[a] Values are differences in δ ($\Delta\delta$), ppm, obtained by subtracting the chemical shifts of the designated group (either the acryloyl or acetal group) of the (*S*,*S*)-ester from that of the same group in the (*S*,*R*)-ester. A positive number means that the signal from the specified group in the (*S*,*R*)-ester is downfield from that of the corresponding group in the (*S*,*S*)-ester, a negative number means that the signal from the specified group in the (*S*,*R*)-ester is upfield from that of the corresponding group in the (*S*,*S*)-ester.

transketolase towards α-hydroxyaldehydes as substrates, and by analysis of the Mosher's ester derivatives by ^{1}H NMR spectroscopy in the presence of a chiral shift reagent.

4. Experimental

4.1. General methods

^{1}H (400.134 MHz) and ^{13}C (100.61 MHz) spectra were recorded on a Bruker AC 400 spectrometer. Mass spectra were obtained on a Hewlett–Packard 5989 A spectrometer. GC analysis was carried out on a Delsi Nermag chromatograph. Optical rotations were determined on a Jasco polarimeter. IR spectra were recorded on a Perkin–Elmer 881 spectrophotometer. UV analyses were performed on a Hitachi (U-2010) spectrophotometer. Solvents were distilled from an appropriate desiccant and stored under argon. Microanalyses were performed by the Service Central d'Analyses du CNRS, Vernaison, France. Transketolase was produced and purified in our laboratory as precedently described.[10] *Pseudomonas fluorescens* lipase (PFL) was purchased from Fluka. The other enzymes were purchased from Sigma and all reagents from Aldrich. TLC plates of silica gel 60F254 from Merck and Merck silica gel for column chromatography 60/230–400 and 60/40–63 mesh were used.

The microorganisms were all laboratory-grown except freeze-dried baker's yeast which was a commercial product (VAHINE, Monteux, France). Preculture and culture conditions for the fungus *A. niger* ATCC 9142, for the yeasts *R. glutinis* NRLLY 1091, *Z. rouxii* ATCC 13356, *Y. farinosa* IFO 10896 and for the bacterium *L. kefir* DSM 20587 have already been described elsewhere.[6]

4.2. Synthesis of the ketone derivatives

4.2.1. Synthesis of 2-(2-hydroxy-3,3-dimethoxy-propyl)-acrylonitrile 1. Indium powder (9.4 g, 81.86 mmol) was added to ethanol (25 mL) containing 2,2-dimethoxyacetaldehyde (108.8 mmol, 60 wt% solution in water) and α-(bromomethyl)-acrylonitrile[16] (12 g, 82.21 mmol). 0.1 N HCl (4 mL) was added and the mixture was stirred at 40°C for 7 h. The pH of the solution was readjusted to 7.5 by the addition of 1N NaOH and the white precipitate formed was removed by centrifugation. The supernatant was decanted and the pellet was washed with ethanol (20 mL). The combined organic phases were evaporated to dryness and the residue was purified by flash chromatography over silica gel (6:4 cyclohexane:ethyl acetate). Compound **1** was isolated in 96% yield (colourless oil). R_f=0.54 (6:4 cyclohexane:ethyl acetate). ^{1}H NMR (400.134 MHz) δ: 5.95 (s, 1H); 5.84 (s, 1H); 4.2 (d, 1H, J=4 Hz); 3.85 (m, 1H); 3.47 (s, 3H); 3.46 (s, 3H); 2.55 (dd, 1H, J=1.5 Hz); 2.51 (s, 1H,

OH); 2.34 (dd, 1H, $J=1.5$ Hz). ^{13}C NMR (100.61 MHz) δ: 133.0 (C olefin); 119.6 (CH_2 olefin); 115.7 (C≡N); 105.9 (CH acetal); 69.2 (CHOH); 55.4 (CH_3 acetal); 55.3 (CH_3 acetal); 36.8 (CH_2). MS (CI) m/z: (MH^+) 172; 154; 140. IR (neat) 2225; 2837; 2940; 3435 cm^{-1}. Anal. calcd for $C_8H_{13}NO_3$: C, 56.12; H, 7.65; N, 8.18. Found: C, 56.13; H, 7.72; N, 8.23%.

4.2.2. Synthesis of 2-(2-hydroxy-3,3-dimethoxy-propyl)-acrylic acid ethyl ester 2. Indium powder (4 g, 35.05 mmol) was added to ethanol (25 mL) containing 2,2-dimethoxyacetaldehyde (60 wt% solution in water, 46.58 mmol) and ethyl α-(bromomethyl)-acrylate[17] (10 g, 55.24 mmol). The mixture was stirred at 40°C for 2 h and treated as described for **1**. The crude residue was purified by flash chromatography over silica gel (5:5 cyclohexane:ethyl acetate). Compound **2** was isolated in 91% yield (colourless oil). $R_f=0.38$ (5:5 cyclohexane:ethyl acetate). ^{1}H NMR (400.134 MHz) δ: 6.26 (s, 1H); 5.71 (s, 1H); 4.22 (d, 1H, $J=2$ Hz); 4.20 (q, 2H, $J=7$ Hz); 3.83 (m, 1H); 3.44 (s, 3H); 3.43 (s, 3H); 2.69 (dd, 1H, $J=2$, 5 Hz); 2.49 (s, 1H, OH); 2.38 (dd, 1H, $J=1.5$ Hz); 1.31 (t, 3H, $J=7$ Hz). ^{13}C NMR (100.61 MHz) δ: 167.5 (C=O). 137.2 (C olefin); 127.3 (CH_2 olefin); 106.2 (CH); 70.0 (CHOH); 60.8 (CH_2 ester); 54.8 ($2CH_3$ acetal); 34.7 (CH_2); 14.1 (CH_3 ester). MS (CI) m/z: 246; 218 ($M^{+\bullet}$); 200; 141. IR (neat) 1076; 1439; 1738; 2952; 3465 cm^{-1}. Anal. calcd for $C_{10}H_{18}O_5$: C, 55.03; H, 8.31. Found: C, 55.03; H, 8.17%.

4.2.3. Synthesis of 2-(3,3-dimethoxy-2-oxo-propyl)-acrylonitrile 3. To a mixture of pyridinium chlorochromate (0.566 g, 2.63 mmol) and 4 Å molecular sieves (1.75 g) in anhydrous CH_2Cl_2 (15 mL) was added **1** (0.3 g, 1.75 mmol) in of anhydrous CH_2Cl_2 (5 mL). After 5 h, dry ether (45 mL) was added and the supernatant decanted from the black gum. The insoluble residue was washed 3 times with anhydrous ether (50 mL) whereupon it became a black granular solid. The combined organic solution was passed through a short pad of florisil (60–100 mesh, from Accros), and the solvent was evaporated. Column chromatography on silica gel gave a colourless oil, **3** (78.7 mg, 26%). $R_f=0.73$ (7:3 cyclohexane:ethyl acetate). ^{1}H NMR (400.134 MHz) δ: 6.09 (s, 1H); 5.84 (s, 1H); 4.49 (s, 1H); 3.54 (s, 2H); 3.45 (s, 6H). ^{13}C NMR (100.61 MHz) δ: 199.6 (C=O); 135.0 (C olefin); 118.0 (CH_2 olefin); 115.55 (C≡N); 104.3 (CH acetal); 55.4 ($2CH_3$ acetal); 41.2 (CH_2). MS (EI) m/z: 210; 198; 170 (MH^+); 130. IR (neat) 1625; 1740; 2227 cm^{-1}. Anal. calcd for $C_8H_{11}NO_3$: C, 56.79; H, 6.55; N, 8.28. Found: C, 56.82; H, 6.52; N, 8.28%.

4.2.4. Synthesis of 2-(3,3-dimethoxy-2-oxo-propyl)-acrylic acid ethyl ester 4. To a mixture of pyridinium dichromate (2.8 g, 7.44 mmol), 4 Å molecular sieves (0.5 g/mmol of alcohol) and pyridinium trifluoroacetate (1.4 g, 7.25 mmol) in anhydrous CH_2Cl_2 (25 mL) was added **2** (1.07 g, 4.908 mmol). After stirring for 3 h, the mixture was treated as described for **3**. Column chromatography on silica gel gave a colourless oil (0.866 g, 81%), **4**. $R_f=0.55$ (6:4 cyclohexane:ethyl acetate). ^{1}H NMR (400.134 MHz) δ: 6.32 (s, 1H); 5.61 (s, 1H); 4.51 (s, 1H); 4.15 (q, 2H, $J=7.5$ MHz); 3.6 (d, 2H); 3.40 (s, 6H); 1.25 (t, 3H, $J=7.0$ Hz). ^{13}C NMR (100.61 MHz) δ: 202.3 (C=O ketone); 166.2 (C=O ester); 133.9 (C olefin); 128.8 (CH_2 olefin); 103.7 (CH acetal); 61.0 (CH_2 ester); 54.7 ($2CH_3$ acetal); 41.0 (CH_2); 14.2 (CH_3 ester). MS (CI) m/z: 257; 245; 217 (MH^+). IR (neat) 1637; 1715; 1803; 2835; 2934 cm^{-1}. Anal calcd for $C_{10}H_{16}O_5$: C, 55.54; H, 7.45. Found: C, 55.21; H, 7.07%.

4.3. Microbiological reductions of 2-(3,3-dimethoxy-2-oxo-propyl)-acrylonitrile 3 and 2-(3,3-dimethoxy-2-oxo-propyl)-acrylic acid ethyl ester 4

After culture at 27°C for the times as indicated by Besse et al.,[6] the microorganisms were filtered through a glass sintered funnel or centrifuged, and then washed with aqueous NaCl (4 washes, 8 g/L). Mycelium (5 g) was placed in a 500 mL conical flask with distilled water (50 mL) and the substrate (50 μL). After incubation at 27°C on a rotating table set at 200 rpm, the mixture was filtered on sintered glass or centrifuged for 10 min at 8000 rpm. The liquor was then continuously extracted with ethyl acetate for 24 h. The ethyl acetate phase was dried on $MgSO_4$ and the solvent evaporated off under vacuum. The products extracted after bioconversion were separated on a silica gel column. The eluent was cyclohexane:ethyl acetate, 8:2 or 7:3. The results are reported in Table 2.

4.4. General procedure for the lipase-catalysed transesterification of 1 and 2

To a solution of the racemic alcohol (1.17 mmol of **1** or **2**) in vinyl acetate (15.6 mL) was added *Candida rugosa* lipase (200 mg). The mixture was stirred at 25°C for the time given in Table 1. The enzyme was removed by filtration through a pad of celite, and the solvent was evaporated. The unreacted alcohol and the acetate were separated by silica gel column (6:4, cyclohexane:ethyl acetate).

4.4.1. (*S*)-(−)-2-(2-Hydroxy-3,3-dimethoxy-propyl)acrylonitrile 1. Yield = 77%. $[\alpha]_D^{25}=-10$ (c 2.36, $CHCl_3$); e.e. = 87%. They were the same physical constants and NMR spectra as those described previously for racemic **1**.

4.4.2. (*R*)-(+)-2-(2-Acetoxy-3,3-dimethoxy-propyl)acrylonitrile 5. Colourless oil, yield = 78%; $R_f=0.46$ (6:4 cyclohexane: ethyl acetate); e.e. = ≥95%; $[\alpha]_D^{25}=+12$ (c 2.0, $CHCl_3$); ^{1}H NMR (400.134 MHz) δ: 5.90 (s, 1H); 5.77 (s, 1H); 5.04 (m, 1H); 4.30 (d, 1H, $J=5$ Hz); 3.38 (s, 3H); 3.36 (s, 3H); 2.60 (dd, 1H, $J=3.14$ Hz); 2.51 (dd, 1H, $J=9.15$ Hz); 2.06 (s, 3H). ^{13}C NMR (100.61) δ: 171.6 (C=O); 133.4 (C olefin); 118.8 (CH_2 olefin); 118.3 (C≡N); 103.4 (CH acetal); 70.4 (CHOAc); 55.7 (CH_3 acetal); 54.6 (CH_3 acetal); 33.9 (CH_2); 20.9 (CH_3 ester). MS (CI) m/z: 214 (MH^+); 182. IR (neat) 1077; 1234; 1623; 1747; 2224; 2941 cm^{-1}. Anal. calcd for $C_{10}H_{15}NO_4$: C, 56.32; H, 7.09; N, 6.57. Found: C, 56.33; H, 7.02; N, 6.52%.

4.4.3. (*R*)-(+)-2-(2-Hydroxy-3,3-dimethoxy-propyl)-acrylonitrile 1. To a solution of (*R*)-(+)-**5** (0.66 mmol) in anhydrous methanol was added sodium methylate (0.03 mmol). The mixture was stirred at 25°C for 4 h. The solvent was evaporated. The crude mixture was submitted to column chromatography on silica gel (6:4 cyclohexane:ethyl acetate). Compound (*R*)-(+)-**1** was isolated in 99% yield. Same physical constants and NMR spectra as those described previously for **1**. $[\alpha]_D^{25}=+10$ (*c* 2.0, $CHCl_3$); e.e. = ≥95%.

4.4.4. (*S*)-(−)-2-(2-Hydroxy-3,3-dimethoxy-propyl)-acrylic acid ethyl ester 2. Yield = 58%. $[\alpha]_D^{25}=-1$ (*c* 3.5), $CHCl_3$); e.e. = 5%. Same physical constants and NMR spectra as those described previously for racemic **2**.

4.4.5. (*R*)-(+)-2-(2-Acetoxy-3,3-dimethoxy-propyl)-acrylic acid ethyl ester 6. Colourless oil, yield = 48%. R_f = 0.61 (1:1 cyclohexane:ethyl acetate); $[\alpha]_D^{25}=+2.6$ (*c* 1.92, $CHCl_3$); e.e. = 20%; ^{1}H NMR (400.134 MHz) δ: 6.19 (s, 1H); 5.96 (s, 1H); 5.21 (m, 1H, J = 3 Hz); 4.32 (d, 1H, J = 5 Hz); 4.23 (q; 2H; J = 7 Hz); 3.42 (s, 3H); 3.40 (s, 3H); 2.82 (dd; 1H; J = 3.14 Hz); 2.44 (dd; 1H; J = 10.14 Hz); 2.03 (s, 3H); 1.31 (t, 3H, J = 7 Hz). ^{13}C NMR (100.61 MHz) δ: 170.3 (C=O); 166.7 (C=O); 136.6 (C olefin); 127.3 (CH_2 olefin); 104.2 (CH acetal); 70.55 (CHOAc); 60.9 (CH_2 ester); 54.9 (CH_3 acetal); 54.6 (CH_3 acetal); 32.4 (CH_2); 21.0 (CH_3 acetate); 14.25 (CH_3 ester). MS (CI) m/z: 261 (MH^+); 229; 215; 201. IR (neat): 1077; 1440; 1745; 2953 cm^{-1}. Anal. calcd for $C_{12}H_{20}O_6$: C, 46.14; H, 7.74. Found: C, 46.28; H, 7.75%.

4.4.6. (*R*)-(+)-2-(2-Hydroxy-3,3-dimethoxy-propyl)-acrylic acid ethyl ester 2. After alkaline methanolysis as described for (*R*)-(+)-**6**, (*R*)-(+)-**2** was isolated in 99% yield. Same physical constants and NMR spectra as those described previously for racemic **2**. $[\alpha]_D^{25}=+3.1$ (*c* 1.3, $CHCl_3$); e.e. = 20%.

4.5. Determination of enantiomeric excess

The e.e. of each alcohol and the ester produced either by microbial reduction or by lipase-catalysed transesterification were determined by NMR using Eu(hfc)$_3$ in $CDCl_3$ as chemical shift reagent on olefin protons.

4.6. Absolute configuration of (−)-1 and (+)-1

4.6.1. Procedure for acetal hydrolysis. A solution of acetal (−)-**1** or (+)-**1** (0.1–0.3 M) in water was treated with ion-exchange resin (AG-50 W-X8, H^+ form, 0.1–0.2 g). The suspension was stirred for 24 h after which time it was filtered to remove the resin beads. The pH of the solution was then adjusted to 7 by addition of 0.1N NaOH solution. The solution was freeze-dried and the specific rotation of the residue was determined ($[\alpha]_D^{25}=-12$ (*c* 2.07, H_2O) and +13 (*c* 1.42, H_2O)) to ensure that no racemisation occurred during acid hydrolysis. The product α-hydroxyaldehyde was used without further purification.

4.6.2. Measurement of kinetic constants, K_m and $V_{max\ rel.}$ ($V_{max\ rel.} = V_{max}$ tested substrate/V D-ribose-5-phosphate). D-Xylulose-5-phosphate (5 mg/mL, 21 μmol/mL, 25 μL), the substrate (at different concentrations (5–200 mM)), thiamine phosphate (10 mg/mL; 21 μmol, 5 μL), $MgCl_2$ (10 mg/mL; 10 mM, 5 μL) and NADH (10 mg/mL; 14 μmol/mL, 10 μL) were placed in a spectrophotometer cell at 25°C. The optical density A_1 was recorded. To this solution was added 1 U of transketolase, 2 U of triose phosphate isomerase and 21 U of glycerophosphate dehydrogenase. The optical density A_2 was recorded every minute at 340 nm; the $(A_1–A_2)/\Delta t$ (min) slope was determined and the activity of enzyme per mL of protein was determined using the following equations:

U/mL = μmoles of substrate formed/min/mL = $((A_1–A_2)/\Delta t \times$ cell volume$)/(6.22 \times$ light path (1 cm)$\times$ TK sample volume)

U/mg = mmoles of substrate formed/min/mg proteins = (U/mL)/mg protein/mL = rate of reaction for aldehyde at given concentration

A plot of (1/rate of reaction for aldehyde substrate) versus (1/aldehyde substrate concentration) was made. From the slope and the intercept of the Lineweaver–Burk plot, V_{max} substrate and K_m values were calculated. These values for **7** are given in Table 3.

4.7. Determination of absolute configuration of (−)-2 using NMR chemical shifts of α-methoxy-α-trifluoromethylphenylacetate (MTPA) esters

(*S*)-(−)-MTPA esters of (−)-**2** were synthesised according to the literature.[12] Chemical shifts of α-methoxy-α-trifluoromethylphenylacetate (MTPA) esters are given in Table 4.

References

1. (a) Enoch, K.; Gordon, D. M.; Whitesides, W. G. *J. Org. Chem.* **1993**, *58*, 5500–5507; (b) Chan, T.-H.; Lee, M.-C. *J. Org. Chem.* **1995**, *60*, 4232–4428; (c) Prenner, R. H.; Schmid, W. *Monatsch. Chem.* **1996**, *127*, 1045–1050; (d) Martin, L. D.; Stille, J. K. *J. Org. Chem.* **1982**, *47*, 3630–3633.
2. (a) Crestia, D.; Guérard, C.; Bolte, J.; Demuynck, C. *J. Mol. Cat. B.: Enzymatic* **2001**, *11*, 207–212; (b) Guérard, C.; Demuynck, C; Bolte, J. *Tetrahedron Lett.* **1999**, *40*, 4181–4182.
3. (a) Hoffmann, H. M. R.; Rabe, J. *Angew. Chem., Int. Ed. Engl.* **1985**, *24*, 94–116; (b) Das, B.; Venkataiah, B.; Kashinatham, A. *Tetrahedron* **1999**, *55*, 6585–6594; (c) Pitacco, G.; Sessanta o Santi, A.; Valentin, E. *Tetrahedron: Asymmetry* **2000**, *11*, 3263–3267 and references cited therein.
4. *Pancreas porcine* lipase, *Porcine fluorensens* lipase, *Candida antartica* lipase, *Aspergillus niger* lipase and *Candida rugosa* lipase (previously named *Candida cylindracea* lipase).
5. The enantiomeric ratio (*E*) was calculated from the measured e.e. according to: Rakels, J. L. L.; Straathof, A. J. J.; Heijnen, J. J. *Enzym. Microb. Technol.* **1993**, *15*, 1051.

6. Besse, P.; Baziard-Mouysset, G.; Boubekeur, K.; Palvadeau, P.; Veschambre, H.; Payard, M.; Mousset, G. *Tetrahedron: Asymmetry* **1999**, *10*, 4745–4754.
7. Pyridinium dichromate–pyridinium trifluoroacetate; pyridinium dichromate; 1,3-dicyclohexylcarbodiimide–DMSO.
8. (a) Sweers, H. M.; Wong, C. H. *J. Am. Chem. Soc.* **1986**, *108*, 6421; (b) Pedrocchi-Fantoni, G.; Servi, S. *J. Chem. Soc., Perkin Trans. 1* **1992**, 1029; (c) Wong, C. H.; Whitesides, W. G. *Enzymes in Synthetic Organic Chemistry*; Elsevier Science: Oxford, 1994; (d) Faber, K. *Biotransformations in Organic Chemistry*; Springer-Verlag: Berlin, Heidelberg, 1995; (e) Cornil, B.; Herrman, W. A. *Applied Homogeneous Catalysis with Organometallic Compounds*; VHC: New York, 1996; pp. 782–793.
9. Cygler, M.; Grochulski, P.; Kazlauskas, R. J.; Schrag, J. D.; Bouthillier, F.; Rubin, B.; Serreqi, A. N.; Gupta, A. K. *J. Am. Chem. Soc.* **1994**, *116*, 3180–3186.
10. Hecquet, L.; Demuynck, C.; Bolte, J. *J. Mol. Cat. B.: Enzym.* **2001**, *6*, 797–802.
11. (a) Kobori, Y.; Myles, D. C.; Whitesides, G. M. *J. Org. Chem.* **1992**, *57*, 5899–5907; (b) Sprenger, G. A.; Pohl, M. *J. Mol. Cat. B.: Enzym.* **1999**, *6*, 145–159; (c) Turner, N. *Curr. Opin. Biotechnol.* **2000**, *11*, 527–531.
12. Effenberger, F.; Null, V.; Ziegler, T. *Tetrahedron Lett.* **1992**, *33*, 5157–5160.
13. Bergmeyer, H. U. In *Methods of Enzymatic Analysis*, *Verlag Chemie*; Bergmeyer, H. U., Ed., 2nd ed.; Academic Press: New York, 1974; pp. 1304–1307.
14. Dale, J. A.; Mosher, H. S. *J. Am. Chem. Soc.* **1973**, *95*, 512–519.
15. ^{1}H NMR spectra confirm the values of the e.e. for (–)-**2**.
16. Csuk, R.; Hoering, U.; Schaade, M. *Tetrahedron* **1996**, *52*, 9759–9776.
17. Villieras, J.; Rambaud, M. *Synthesis* **1982**, *82*, 924–926.