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Chemoenzymatic preparation of all the stereoisomers of 2-(1-hydroxyethyl)- and 2,6-bis(1-hydroxyethyl)pyridines and their acetates

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Dedicated to Professor Károly Lempert on the occasion of his 80th birthday

Abstract—Several lipases were tested for the enantiomerically selective acetylation of racemic 1-[6-(1-hydroxyethyl)-pyridin-2-yl]ethanone *rac*-**2** to yield alcohol (*S*)-**2** and acetate (*R*)-**3**. Acetylation of a diastereomeric mixture of racemic and *meso*-2,6-bis(1-hydroxy-ethyl)pyridine, *rac*/*meso*-**4**, with the most selective Novozym 435 lipase in vinyl acetate resulted in a mixture of enantiopure diol (*S*,*S*)-**4**, monoacetate (*R*,*S*)-**5** and diacetate (*R*,*R*)-**6**. Hydrolysis of the mixture of racemic and *meso*-2,6-bis(1-acetoxy-ethyl)pyridine *rac*/*meso*-**6** by the same enzyme gave the pure enantiomers of diol (*R*,*R*)-**4**, monoacetate (*S*,*R*)-**5** and diacetate (*S*,*S*)-**6**. Using further chemical and enzymatic steps alcohol (*R*)-**2**, acetate (*S*)-**3**, (*S*,*S*)- and (*R*,*R*)-monoacetates (*S*,*S*)-**5** and (*R*,*R*)-**5**, *meso*-**4** and its acetate *meso*-**6** were also prepared and characterized.

1. Introduction

Optically active pyridyl alcohols are not only useful key compounds for pharmaceutical intermediates,[1,2] but also for chiral ligands and auxiliaries in asymmetric synthesis.[3,4] Enantiopure 2,6-bis(1-hydroxy-ethyl)pyridines (*S*,*S*)-**4** and (*R*,*R*)-**4** are useful chiral building blocks in the syntheses of optically active crown ethers.[5,6]

A number of methods have been published for the stereoselective production of 2,6-bis(1-hydroxyethyl)-pyridines (*S*,*S*)-**4** and (*R*,*R*)-**4**. Microbial reductions of the 2,6-diacetyl-pyridine **1** by baker's yeast[7,8] or *Pseudomonas putida* UV4 strain[9] have provided (*S*,*S*)-**4** in high enantiomeric purity. Asymmetric chemical reactions were also successfully applied for producing the enantiomers of diol **4**. The (*S*,*S*)-enantiomer (*S*,*S*)-**4** was obtained by reduction with a chiral borane reagent[10] or by transfer hydrogenation using a chiral Ru catalyst,[11] whereas (*R*,*R*)-**4** was produced by using a chiral borane reagent[10] or the (+)-DIP-Cl catalyst.[12] Although these methods resulted in one of the enantiomeric forms of diol **4** in various, usually high enantiomeric purity, none of them were flexible enough for the production of all stereoisomers of **4** in pure form (Figs. 1 and 2).

By exploiting the stereoselectivity of hydrolases, the acetylation of the isomeric mixture *rac*/*meso*-**4** with acetic anhydride catalyzed by lipoprotein lipase from *Pseudomonas* sp. (Amano P) was applied to produce a mixture of optically active diol (*S*,*S*)-**4**, monoacetate (*S*,*R*)-**5** and diacetate (*R*,*R*)-**6**.[13] From the diastereomeric mixture of *rac*/*meso*-**4**, highly enantiopure diacetate (*R*,*R*)-**6** was prepared by an elegant combination of a highly selective lipase catalyzed acetylation and ruthenium catalyzed in situ racemization.[14]

Although many of these methods are applicable for obtaining some of the stereoisomers of **4**, **5** and **6** in optically active forms, they were either not flexible enough or not selective enough for producing all stereoisomers of diol **4** and its acetylated derivatives **5** and **6** in pure form. Moreover, we have found inconsistencies between

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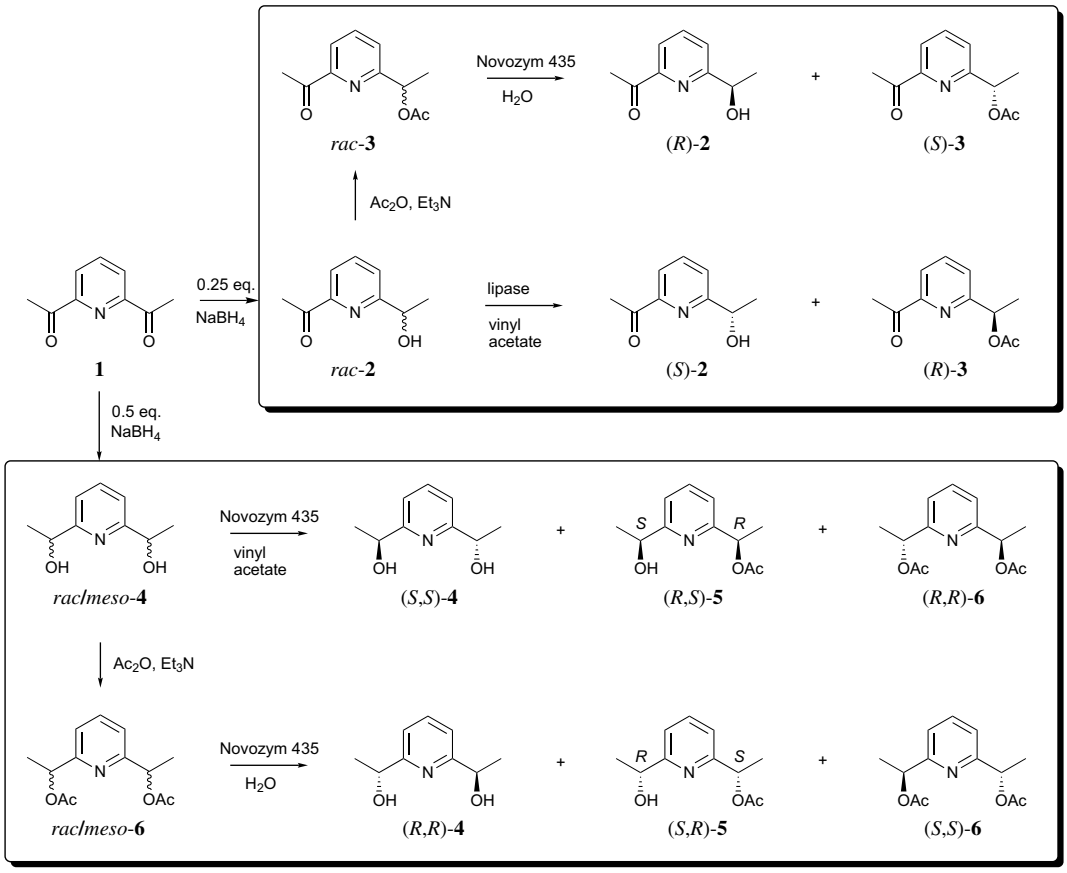

Figure 1. Preparation of enantiopure stereoisomers of 2-(1-hydroxyethyl)- and 2,6-bis(1-hydroxyethyl)pyridines **2** and **4** and their acetylic esters **3**, **5** and **6**.

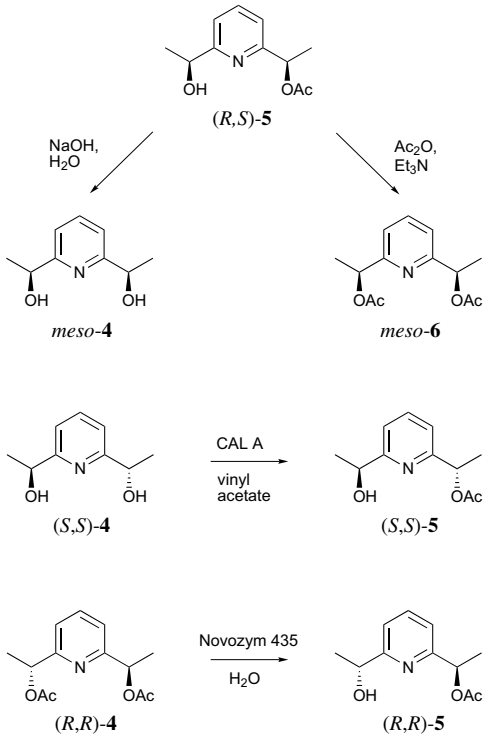

Figure 2. Preparation of pure *meso*-**4**, its diacetate *meso*-**6** and the two enantiomeric forms of monoacetates (*S*,*S*)-**5** and (*R*,*R*)-**5**.

the reported enantiomeric compositions and the corresponding specific rotation values.

We thought therefore that it was important to enhance the selectivity of the most flexible enzymatic acetylation method[13] by searching for a highly selective and efficient enzyme and extend the use of this method for the preparation of all pure stereoisomers of diol **4** and their acetylated derivatives **5** and **6**.

2. Results and discussion

Due to the stereoselective acetylation of the isomeric mixture of diol *rac*/*meso*-**4** being rather complex and that the intrinsic selectivity of the process cannot be detected directly, the enzyme catalyzed enantiomeric selective acetylation (i.e., simple kinetic resolution) of racemic 1-[6-(1-hydroxy-ethyl)pyridin-2-yl]ethanone *rac*-**2** was chosen for comparing the selectivities of several lipases (Fig. 1, Table 1). Enzymatic acetylations in vinyl acetate show applicable activity on *rac*-**2** revealing Novozym 435 as the most selective enzyme (E >250). The high enantiomeric selectivity of this enzyme was confirmed by preparative scale acetylation of *rac*-**2** yielding enantiopure alcohol (*S*)-**2** and acetate (*R*)-**3** (ee >99.5%, each). In the hydrolysis of *rac*-**3**, catalyzed by Novozym 435, the high degree of enantiomeric selectivity was preserved resulting in enantiopure (*R*)-**2** and (*S*)-**3** (ee >99.5%, each).

Table 1. Lipase-catalyzed acetylation of 2-(1-hydroxyethyl)pyridine *rac*-**2**[a]

Enzyme	Time (h)	*c* (%)	(*S*)-**2** Ee[b] (%)	(*R*)-**3** Ee[b] (%)	*E*[c]
Novozym 435	24	50.0	>99.5	>99.5	>250
Lipozyme TL IM	24	18.7	21.5	99.0	~250
Lipase PS	24	11.9	13.4	98.6	167
Lipozyme IM 20	24	8.1	9.4	98.0	109
PPL	24	5.0	5.0	96.7	62
Lipase AK	24	47.7	86.5	89.5	46
Candida cylindracae lipase	24	5.3	10.9	68.5	5.6
Lipase AY	24	5.7	3.2	63.9	4.7
Candida rugosa lipase	24	10.9	5.7	36.0	3.0
PLE	24	12.7	5.2	34.4	2.2
Candida antarctica lipase A	24	>99.0	<0.1	<0.1	~1.0

[a] Reactions with c >5% within 24 h. For details, see Experimental.
[b] Ee of (*S*)-**2** and (*R*)-**3** and c: GLC/Beta-DEX 120 column.
[c] Degree of enantiomeric selectivity (E) was calculated from c and ee_3,[15] and confirmed by independent calculation from ee_2 and ee_3[16] (due to sensitivity to experimental errors, E-value over 500 is given as >250).

In a report on acetylation using Ac_2O and Amano lipase P for the separation of the stereoisomers of **4**,[13] it was speculated that a highly stereoselective reaction would result in a mixture of the less reactive enantiomer of **4** along with the diacetate **6** from its reactive enantiomer and monoacetate **5** from *meso*-**4**. However, separation and proper characterization of the stereoisomers **4** and its acetate derivatives **5** and **6** is not a trivial task, as the experimental part of this work[13] indicated, stating that the 250 MHz ^{1}H NMR spectrum of *meso*-**4** 'was identical with that of the mixture of diol stereoisomers' *rac*/*meso*-**4**.

On the basis of our results on the enantiomeric selectivity of the enzymatic acetylation of racemic 1-[6-(1-hydroxy-ethyl)pyridin-2-yl]ethanone *rac*-**2** (Table 1), a high degree of stereoselectivity was expected in the acetylation of the isomeric mixture *rac*/*meso*-**4** with the most selective Novozym 435 in vinyl acetate. As expected, the reaction performed on a preparative scale stopped after reaching the expected conversions to yield a separable mixture of (*S*,*S*)-**4**, (*R*,*S*)-**5** and (*R*,*R*)-**6** in high purity (ee >99%, each, by GLC and 500 MHz ^{1}H NMR).

Similar to the simple kinetic resolution of *rac*-**2** catalyzed by Novozym 435, the high degree of stereoselectivity in the acetylation process was preserved in the hydrolysis as well. Thus, from the mixture of diacetates *rac*/*meso*-**6** the opposite enantiomeric forms of pure (*R*,*R*)-**4**, (*S*,*R*)-**5** and (*S*,*S*)-**6** (ee >99%, each, by GLC and 500 MHz ^{1}H NMR) were obtained without special care of controlling the degree of conversion.

With the highly enantiopure samples in our hands, some unexpected specific rotations were determined (Table 2). In many cases, our results (we always observed higher rotation values than reported previously) were in disagreement with the existing data, which prompted us to clarify this situation.

The effect of incomplete selectivity on the product composition of stereoselective baker's yeast reduction of 2,6-diacetyl-pyridine **1** was analyzed in detail:[7] a mixture of (*S*,*S*)-**4** (85.56%), *meso*-**4** (13.88%) and (*R*,*R*)-**4** (0.56%) is forming if one reduction step happens with a degree of selectivity of 85% ee, whereas practically pure (*S*,*S*)-**4** (99.8%) is forming [along with traces of *meso*-**4** (0.18%) and (*R*,*R*)-**4** (0.01%)] if one reduction step proceeded with a degree of selectivity of 99.8% ee. Accordingly, noncomplete stereoselectivity results in practically inseparable mixtures of diastereomers, for which accurate specific rotation determination is not really possible.

The chiral Ru(II)-catalyzed stereoselective reduction of 2,6-diacetylpyridine **1**[11] was not completely selective, resulting in a 91:9 mixture of (*S*,*S*)-**4** (99.6% ee by HPLC) and *meso*-**4**. The rotation data reported for (*S*,*S*)-**4** (Table 2), therefore, was apparently determined from this mixture.

It was supposed that the acetylation with Ac_2O catalyzed by lipoprotein lipase from *Pseudomonas* sp. (Amano P) produced a mixture of monoacetate (*R*,*S*)-**5** and 'optically pure' diol (*S*,*S*)-**4** and diacetate (*R*,*R*)-**6**.[13] However, NMR data of the bis-MTPA derivative from (*R*,*R*)-**4**, which was obtained by hydrolysis of the enzymatically produced (*R*,*R*)-**6** published in the same paper,[13] indicated the presence of 18% *meso*-**4** and 12% (*S*,*S*)-**4** in the (*R*,*R*)-**4** sample (Table 2).

When the enantiomeric composition was deduced from the diastereomeric ratio after diastereomeric derivatization of a compound, the different diastereomers may be formed at significantly different rates. Therefore, a strictly complete conversion of the original enantiomeric mixture into the desired derivative is an essential requirement in this case. No solid proof or definitive statement on the total conversion, however, was found for the MCF derivative of **2**[10] or the di-MTPA derivative of **4**.[10]

It is noteworthy that some other factors may also influence the specific rotation data. A strong concentration dependency of the specific rotation in acetone was found for diol (*S*,*S*)-**4**: $[\alpha]_D = -48.0$, -84.5 and -99.5 were determined for a freshly prepared sample at c 0.5,

Table 2. Specific rotations of pure stereoisomers of **2**–**6**[a]

Compound[a]	$[\alpha]_D^{25}$[a]		Lit. $[\alpha]_D$ (Ee %)
	c 2, EtOH	*c* 2, acetone	
(*S*)-**2**	−62.0	−91.3	−4.1 (99)[b] −7.5 (99.8)[c] −40.16 (>95)[d]
(*R*)-**2**	+61.8	+91.2	+40.53 (>95)[d]
(*S*)-**3**	−75.8		
(*R*)-**3**	+75.1		
(*S*,*S*)-**4**	−69.6	−99.5	−26.6 (99)[e] −26.84 (99.92)[f] −45.99 (>98)[g] −61.0 (99.6)[h] −63.6[i]
(*R*,*R*)-**4**	+70.0	+99.6	+44.01[j] +44.6 (>98)[k]
meso-**4**	−0.2	−0.3	+10.21[i]
(*S*,*S*)-**5**	−138.1	−131.3	
(*R*,*S*)-**5**	+49.5	+56.5	+29.43[i]
(*S*,*R*)-**5**	−49.8	−56.0	
(*R*,*R*)-**5**	+138.4	+131.2	
(*S*,*S*)-**6**	−181.9	−171.9	
(*R*,*R*)-**6**	+182.6	+172.6	+73.33[i]
meso-**6**	+0.2	+0.2	

[a] Ee of all chiral stereoisomers in this work were >99% (GLC/Beta-DEX 120 or HP Chiral columns). Chemical purity of all stereoisomers were checked by GLC and 500 MHz ^{1}H NMR.
[b] *c* 1.0, $CHCl_3$ (Ee of (*S*)-**2**: HPLC/Chiralcel OB column).[8]
[c] *c* 1.5, $CHCl_3$ (Ee: HPLC of di-*p*-bromobenzoate derivative of (*S*)-**2**/Chiracel OD column).[7]
[d] *c* ~4, acetone (Ee: GLC of MCF derivative of **2**/SBP-5 column)[10].
[e] *c* 0.51, $CHCl_3$ (Ee of (*S*,*S*)-**4**: HPLC/Chiralcel OB column).[8]
[f] *c* 2.98, $CHCl_3$ (Ee and diastereomeric composition: HPLC of bis-*p*-bromobenzoates of (*S*,*S*)-**4** and *meso*-**4**/Chiracel OD column).[7]
[g] *c* 1.9, acetone (Ee: di-MTPA derivative of **4**/^{19}F NMR).[10]
[h] *c* 1.9, acetone (data refers to a 91:9 mixture of (*S*,*S*)-**4** and *meso*-**4**).[11]
[i] *c* 2, acetone.[13]
[j] *c* 2, acetone {^{1}H NMR of the di-MTPA derivative from (*R*,*R*)-**4** of $[\alpha]_D$ +44.01 indicated the presence of 18% *meso*-**4** and 12% (*S*,*S*)-**4**}.[13]
[k] *c* 2.3, acetone (Ee: di-MTPA derivative of **4**/^{19}F NMR).[10]

1 and 2, respectively. Dependency of the rotation on the solvent is indicated by the $[\alpha]_D = -33.4$ value determined for the same sample in chloroform, *c* 1.

Initiated by the deviations of our specific rotation data from the reported ones (Table 2), we repeated rotation measurements for several samples, which were stored in stopped glass flasks at room temperature for four weeks. It was surprising to learn that the optical rotations for (*S*,*S*)-**4**, (*R*,*S*)-**5** and (*R*,*R*)-**6** differed significantly from the previously measured values. For example, $[\alpha]_D = +67.3$ (*c* 2, acetone) was observed for a (*R*,*S*)-**5** sample, which exhibited $[\alpha]_D = +56.5$ (*c* 2, acetone) previously. TLC check of this sample indicated substantial (ca. 70%) conversion into a novel less polar product. Separation and structure elucidation revealed that this 'novel' product was ketone (*R*)-**2**. This was further confirmed by the specific rotation determinations, because the separated (*R*,*S*)-**5** and (*R*)-**2** fractions exhibited the previously determined $[\alpha]_D = +56.5$ and +91.3 (*c* 2, acetone) values, respectively (Table 2).

As final proof of the reproducibility, the Novozym 435 mediated acetylation of *rac*/*meso*-**4** was repeated to give (*S*,*S*)-**4** (22%), (*R*,*S*)-**5** (41%) and (*R*,*R*)-**6** (20%), which exhibited essentially the same $[\alpha]_D = -98.9$, +56.3 and +173.0 (*c* 2, acetone), respectively, as determined in the first experiment (Table 2).

3. Conclusions

Among the several lipases tested for the enantiomeric selective acetylation of racemic 1-[6-(1-hydroxyethyl)-pyridin-2-yl]ethanone *rac*-**2**, Novozym 435 was found to exhibit excellent selectivity. With the aid of this highly selective enzyme, acetylation of a diastereomeric mixture of racemic and *meso*-2,6-bis(1-hydroxy-ethyl)pyridine *rac*/*meso*-**4** with vinyl acetate resulted in a mixture of enantiopure diol (*S*,*S*)-**4**, monoacetate (*R*,*S*)-**5** and diacetate (*R*,*R*)-**6**. Using further chemical and enzymatic steps all the possible enantiomers of alcohol **2**, acetate **3**, (*S*,*S*)-, (*R*,*R*)-, and *meso*-diols **4**, all stereoisomers of monoacetate **5** and diacetate **6** were also prepared and characterized. Accurately measured optical rotation data for the products and analysis of their deviation from the previously reported values have also been given.

4. Experimental

4.1. Materials and methods

4.1.1. Reagents and solvents. Vinyl acetate, sodium borohydride, acetic anhydride and triethyl amine were purchased from Aldrich. 2,6-Diacetyl-pyridine **1** was prepared according to the reported procedure.[17] All solvents were of analytical grade or freshly distilled while the sodium phosphate buffer (pH 7.0, 50 mM) was freshly made.

4.1.2. Biocatalysts. Lipase AK, lipase AY, lipase F, and lipase PS were obtained from Amano Europe. Lipozyme IM 20, Lipozyme TL IM, Novozym 435 and *Candida antarctica* lipase A (CAL A) were products of Novozymes, Denmark. Lipases from *Candida rugosa* and *Pseudomonas fluorescens* were purchased from

Fluka. PPL and lipase from *Candida cylindracae* were obtained from Sigma. Pig liver acetone powder (PLE) was prepared in our laboratory.

4.1.3. Analytical methods. NMR spectra were recorded on a Bruker DRX-500 spectrometer (500 MHz for ^{1}H and 125 MHz for ^{13}C; TMS; ppm on δ scale) in $CDCl_3$ if not stated otherwise. IR spectra were taken on a Specord 2000 spectrometer in film and the wave numbers reported in cm^{-1}. GC analyses were carried out on Agilent 4890D or HP 5890 instruments equipped with FID detector using H_2 as the carrier gas (injector: 250 °C, detector: 250 °C, head pressure: 12 psi). For the separations HP Chiral (30 m × 0.32 mm × 0.25 μm film of 20% permethylated β-cyclodextrin, HP Part No.: 190916-B213) with 1:50 split ratio or Beta-DEX 120 (30 m × 0.25 mm × 0.25 μm film, Supelco Column No.: 16161-0413) with 1:80 split ratio were used. Optical rotations were determined on a Perkin–Elmer 241 polarimeter. TLC was carried out on Kieselgel 60 F_{254} (Merck) sheets. Spots were visualized by treatment with 5% ethanolic phosphomolybdic acid solution and heating of the dried plates.

4.1.4. Racemic 1-[6-(1-hydroxyethyl)-pyridin-2-yl]-ethanone *rac*-2. To a solution of 2,6-diacetylpyridine **1** (4 g, 25 mmol) in anhydrous ethanol (120 mL) $NaBH_4$ (0.87 g, 3.7 mmol) was added portionwise at rt and the resulting mixture stirred overnight. After evaporating the solvent in vacuum, the residue was dissolved in ethyl acetate (50 mL) and washed by water (10 mL), 5% HCl solution (10 mL), saturated $NaHCO_3$ solution (10 mL) and brine (10 mL) and dried over sodium sulfate. From this solution, ethyl acetate was distilled off by rotary evaporator and the residue purified by column chromatography (silica gel/hexane:acetone 10:1) to give *rac*-**2** (1.67 g, 41%) as a colourless oil. IR: 3448, 2958, 1700, 1588, 1448, 1416, 1360, 1288, 1224, 1120, 1080, 1020, 912, 816; ^{1}H NMR: 1.53 (3H, d, J = 6.6 Hz, CH_3), 2.72 (2H, s, CO–CH_3), 3.9 (1H, br s, OH), 4.95 (1H, q, J = 6.6 Hz, O–CH), 7.48 (1H, d, J = 7.7 Hz, Py_5–H), 7.84 (1H, t, J = 7.7 Hz, Py_4–H), 7.93 (1H, d, J = 7.7 Hz, Py_3–H); ^{13}C NMR: 24.50, 26.10, 68.97, 120.27, 123.49, 137.81, 151.89, 162.64, 199.33; Anal. Calcd for $C_9H_{11}NO_2$: C, 65.44; H, 6.71; N, 8.48. Found: C, 65.37; H, 6.74; N, 8.44.

4.1.5. Analytical scale enzymatic acetylation of racemic 1-[6-(1-hydroxyethyl)pyridin-2-yl]ethanone *rac*-2. Enzyme (5 mg, see Table 1) was added to a solution of racemic 1-[6-(1-hydroxyethyl)-pyridin-2-yl]ethanone *rac*-**2** (5 mg) in vinyl acetate (1 mL) and the resulting suspension shaken at rt/1000 rpm in a sealed glass vial for the time indicated in Table 1, after which the reaction was analyzed by GC. The conversion and enantiomeric compositions of the resulting alcohol (*S*)-**2** and acetate (*R*)-**3** are listed in Table 1.

4.1.6. Chiral GC analysis of acylation reactions from racemic 1-[6-(1-hydroxyethyl)pyridin-2-yl]ethanone *rac*-2. R_t (Beta-DEX 120; 125–150 °C, 1 °C/min)/min: 20.59 (*S*)-**2** and 21.26 (*R*)-**2** (base line separation); 22.24 (*S*)-**3** and 22.56 (*R*)-**3** (base line separation).

4.1.7. Novozym 435 catalyzed acetylation of 1-[6-(1-hydroxyethyl)pyridin-2-yl]ethanone *rac*-2. Racemic 1-[6-(1-hydroxyethyl)pyridin-2-yl]ethanone *rac*-**2** (250 mg, 1.52 mmol) and Novozym 435 (150 mg) in vinyl acetate (4 mL) were shaken at rt for 24 h. After removing the enzyme by filtration, vinyl acetate was evaporated off under reduced pressure and the residue was purified by column chromatography (silica gel/hexane:acetone 10:1) to yield (*S*)-**2** (113 mg, 45%) and (*R*)-**3** (125 mg, 40%) as colourless oils.

4.1.8. (*S*)-[6-(1-Hydroxyethyl)pyridin-2-yl]ethanone (*S*)-2. $[\alpha]_D^{25} = -62.0$ (c 2.0 in EtOH) {lit.[8] (>99% ee): $[\alpha]_D = -4.1$ (c 1.0, $CHCl_3$); lit.[7] (>99.8% ee): $[\alpha]_D = -7.5$ (c 1.5, $CHCl_3$)}; IR, ^{1}H and ^{13}C NMR data were indistinguishable from the spectra of *rac*-**2**.

4.1.9. (*R*)-[6-(1-Acetoxyethyl)pyridin-2-yl]ethanone (*R*)-3. $[\alpha]_D^{25} = +75.1$ (c 2.0, EtOH); IR: 1744, 1700, 1592, 1456, 1424, 1360, 1288, 1228, 1112, 1080, 1032, 952, 816; ^{1}H NMR: 1.63 (2H, d, J = 6.7 Hz, CH_3), 2.14 (2H, s, CO–CH_3), 2.71 (3H, s, CO–CH_3), 5.98 (1H, q, J = 6.7 Hz, O–CH), 7.51 (1H, d, J = 7.7 Hz, Py_5–H), 7.81 (1H, t, J = 7.7 Hz, Py_4–H), 7.92 (1H, d, J = 7.7 Hz, Py_3–H); ^{13}C NMR: 20.65, 21.39, 25.80, 72.77, 120.21, 123.49, 137.39, 152.70, 159.61, 169.97, 199.83; Anal. Calcd for $C_{11}H_{13}NO_3$: C, 63.76; H, 6.32; N, 6.76. Found: C, 63.67; H, 6.38; N, 6.71.

4.1.10. Racemic 1-[6-(1-acetoxyethyl)pyridin-2-yl]ethanone *rac*-3. Racemic 1-[6-(1-hydroxyethyl)pyridin-2-yl]ethanone *rac*-**2** (2.0 g, 12.2 mmol), acetic anhydride (1.24 g, 12.2 mmol) and triethylamine (10 mL) were stirred at 50 °C for 8 h. The reaction mixture was diluted with chloroform (50 mL) and washed with water (3 × 15 mL), 5% HCl solution (15 mL), saturated $NaHCO_3$ solution (15 mL) and brine (15 mL). The organic solution was dried over sodium sulfate and concentrated under reduced pressure. The residue was purified by column chromatography (silica gel/hexane:acetone 10:1) to give *rac*-**3** (1.7 g, 66%) as a colourless oil. IR, ^{1}H and ^{13}C NMR data were indistinguishable from the spectra of (*R*)-**3**.

4.1.11. Novozym 435 catalyzed hydrolysis of 1-[6-(1-acetoxyethyl)pyridin-2-yl]ethanone *rac*-3. 1-[6-(1-Acetoxyethyl)pyridin-2-yl]ethanone *rac*-**3** (250 mg, (1.21 mmol) and Novozym 435 (150 mg) in 50 mM pH 7.0 sodium phosphate buffer were shaken at rt/1000 rpm for 48 h. The enzyme was filtered off and the filtrate extracted with chloroform (3 × 3 mL). The combined extracts were washed with brine (3 mL), dried over sodium sulfate and concentrated under reduced pressure. The residue was purified by column chromatography (silica gel/hexane:acetone 10:1) to yield (*R*)-**2** (90 mg, 45%) and (*S*)-**3** (115 mg, 46%) as colourless oils.

4.1.12. (*R*)-[6-(1-Hydroxyethyl)pyridin-2-yl]ethanone (*R*)-2. $[\alpha]_D^{25} = +61.8$ (c 2.0, EtOH); IR, ^{1}H and ^{13}C NMR data were indistinguishable from the spectra of (*S*)-**2**.

4.1.13. (*S*)-[6-(1-Acetoxyethyl)pyridin-2-yl]ethanone (*S*)-3. $[\alpha]_D^{25} = -75.8$ (*c* 2.0, EtOH); IR, ^{1}H and ^{13}C NMR data were indistinguishable from the spectra of (*R*)-**3**.

4.1.14. Bis(1-hydroxyethyl)-pyridine *rac/meso*-4. To a solution of 2,6-diacetylpyridine **1** (4 g, 25 mmol) in anhydrous ethanol (120 mL), $NaBH_4$ (1.75 g, 7.5 mmol) was added portionwise at rt and the resulting mixture stirred overnight. After evaporating the solvent in vacuum, the residue was dissolved in ethyl acetate (50 mL) and washed by water (10 mL), 5% HCl solution (10 mL), saturated $NaHCO_3$ solution (10 mL) and brine (10 mL), and dried over sodium sulfate. From this solution, ethyl acetate was distilled off by rotary evaporator and the residue purified by column chromatography (silica gel/hexane:acetone 10:1) to give *rac/meso*-**4** (2.55 g, 66%) as a white solid. Mp 49–51 °C; IR: 3360, 1596, 1576 1448, 1432, 1400, 1368, 1120, 1076, 1016, 928, 816; ^{1}H NMR: 1.51 (6H, d, $J = 6.5$ Hz, 2 CH_3), 4.11 (2H, br s, 2 OH), 4.90 (2H, m(q), 2 O-CH), 7.23 (2H, m, $Py_{3,5}$–H), 7.63 (1H, m(t), Py_4–H); ^{13}C NMR: 24.31/24.35, 69.30/69.32, 118.39, 137.86/137.90, 161.97; GC R_t (min) (HP Chiral; 125–155 °C, 1 °C/min): 14.36 (*S*,*S*)-**4** (28.2%), 15.07 *meso*-**4** (44.0%), 16.00 (*R*,*R*)-**4** (27.8%); Anal. Calcd for $C_9H_{13}NO_2$: C, 64.65; H, 7.84; N, 8.38. Found: C, 64.81; H, 7.72; N, 8.41.

4.1.15. Novozym 435 catalyzed acetylation of 2,6-bis(1-hydroxyethyl)-pyridine *rac/meso*-4. 2,6-Bis(1-hydroxyethyl)pyridine *rac/meso*-**4** (2.0 g, 10 mmol) and Novozym 435 (0.5 g) in vinyl acetate (20 mL) were shaken at rt/1000 rpm for 24 h. The enzyme was filtered off and the filtrate concentrated under reduced pressure. The residue was purified by column chromatography (silica gel/hexane:acetone 10:1) to give (*S*,*S*)-**4** (414 mg, 21%), (*R*,*S*)-**5** (999 mg, 40%) and (*R*,*R*)-**6** (581 mg, 20%) as colourless oils.

4.1.16. (*S*,*S*)-2,6-Bis(1-hydroxyethyl)pyridine (*S*,*S*)-4. $[\alpha]_D^{25} = -69.6$ (*c* 2.0, acetone), $[\alpha]_D^{25} = -99.5$ (*c* 2.0, EtOH) (lit.[8] (>99% ee): $[\alpha]_D = -26.6$ (*c* 0.51, $CHCl_3$), lit.[7] (99.92% ee): $[\alpha]_D = -26.84$ (*c* 2.98, $CHCl_3$), lit.[10] (>98% ee): $[\alpha]_D = -45.99$ (*c* 1.9, acetone), lit.[11] (99.6% ee): $[\alpha]_D = -61.0$ (*c* 1.9, acetone), lit.[13] (64% ee): $[\alpha]_D = -63.6$ (*c* 2, acetone); IR: 3376, 1596, 1576, 1448, 1432, 1400, 1368, 1120, 1076, 1016, 928, 816; ^{1}H NMR: 1.44 (6H, d, $J = 6.6$ Hz, 2 CH_3), 4.29 (2H, br s, 2 OH), 4.83 (2H, q, $J = 6.6$ Hz, 2 O–CH), 7.16 (2H, d, $J = 7.7$ Hz, $Py_{3,5}$–H), 7.63 (1H, t, $J = 7.7$ Hz, Py_4–H); ^{13}C NMR: 24.05; 69.14, 118.00, 137.38, 161.76; Anal. Calcd for $C_9H_{13}NO_2$: C, 64.65; H, 7.84; N, 8.38. Found: C, 64.31; H, 7.77; N, 8.36.

4.1.17. (*R*,*S*)-1-[6-(1-Hydroxyethyl)pyridin-2-yl]ethyl acetate (*R*,*S*)-5. $[\alpha]_D^{25} = +49.5$ (*c* 2.0, acetone), $[\alpha]_D^{25} = +58.5$ (*c* 2.0, EtOH) {lit.[13], $[\alpha]_D = +29.43$ (*c* 2, acetone)}; IR: 3400, 1740, 1596, 1464, 1372, 1244, 1080, 1044, 816; ^{1}H NMR (DMSO-d_6): 1.35 (3H, d, $J = 6.5$ Hz, CH_3), 1.48 (3H, d, $J = 6.7$ Hz, CH_3), 2.08 (3H, s, CO–CH_3), 4.72 (1H, m, O–CH), 5.35 (1H, d, OH), 5.74 (1H, q, $J = 6.7$ Hz, O–CH), 7.23 (1H, d, $J = 7.7$ Hz, Py_5–H), 7.43 (1H, d, $J = 7.7$ Hz, Py_3–H), 7.78 (1H, t, $J = 7.7$ Hz, Py_4–H); ^{13}C NMR (DMSO-d_6): 20.77, 21.13, 24.42, 69.45, 72.55, 117.64, 118.10, 137.34, 158.55, 165.00, 169.53; Anal. Calcd for $C_{11}H_{15}NO_3$: C, 63.14; H, 7.23; N, 6.69. Found: C, 63.25; H, 7.18; N, 6.73.

4.1.18. (*R*,*R*)-2,6-Bis(1-acetoxyethyl)pyridine (*R*,*R*)-6. $[\alpha]_D^{25} = +182.6$ (*c* 2.0, acetone), $[\alpha]_D^{25} = +172.6$ (*c* 2.0, EtOH) {lit.[13] (~50% ee): $[\alpha]_D = +73.33$ (*c* 0.58, $CHCl_3$)} IR: 1746, 1662, 1644, 1586, 1464, 1370, 1240, 1158, 1080, 1030, 950, 810; ^{1}H NMR (DMSO-d_6): 1.49 (6H, d, $J = 6.7$ Hz, 2 CH_3), 2.08 (6H, s, 2 CO–CH_3), 5.77 (2H, q, $J = 6.7$ Hz, 2 O–CH), 7.32 (2H, d, $J = 7.7$ Hz, $Py_{3,5}$–H), 7.81 (1H, t, $J = 7.7$ Hz, Py_4–H); ^{13}C NMR (DMSO-d_6): 20.30, 20.82, 72.05, 118.67, 137.48, 158.90, 169.21; Anal. Calcd for $C_{13}H_{17}NO_4$: C, 62.14; H, 6.82; N, 5.57. Found: C, 62.07; H, 6.96; N, 5.61.

4.1.19. Chiral GC analysis of 2,6-bis(1-hydroxyethyl)-pyridine *rac/meso*-4 and its acetylated derivatives 5, 6. R_t (HP Chiral; 125–155 °C, 1 °C/min)/min: 14.36 (*S*,*S*)-**4**, 15.07 *meso*-**4**, 16.00 (*R*,*R*)-**4**, 16.38 (*S*,*S*)-**5**, 16.56 (*R*,*S*)-**5**, 16.62 (*S*,*R*)-**5**, 16.76 (*R*,*R*)-**5**, 21.64 (*S*,*S*)-**6**, 21.83 (*R*,*R*)-**6**, 21.95 *meso*-**6**.

4.1.20. 2,6-Bis(1-acetoxyethyl)pyridine *rac/meso*-6. 2,6-Bis(1-acetoxyethyl)-pyridine *rac/meso*-**4** (2.0 g, 12.1 mmol), acetic anhydride (2.84 g, 27.8 mmol) and triethylamine (10 mL) were stirred at 50 °C for 8 h. The reaction mixture was diluted with chloroform (50 mL) and washed with water (3 × 15 mL), 5% HCl solution (15 mL), saturated $NaHCO_3$ solution (15 mL) and brine (15 mL). The organic solution was dried over sodium sulfate and concentrated under reduced pressure. The residue was purified by column chromatography (silica gel/hexane:acetone 10:1) to give *rac/meso*-**6** (2.49 g, 82%) as colourless oil. IR: 1748, 1664, 1648, 1588, 1464, 1372, 1240, 1160, 1080, 1032, 952, 812; ^{1}H NMR: 1.58 (~3.3H, d, $J = 6.7$ Hz, CH_3), 1.59 (~2.7H, d, $J = 6.7$ Hz, CH_3), 2.13 (6H, s, 2 CO–CH_3), 5.94 (2H, m, O–CH), 7.25 (2H, m, $Py_{3,5}$–H), 7.68 (1H, m, Py_4–H); ^{13}C NMR: 20.93, 21.44, 72.96, 118.82/118.87, 137.38/137.48, 159.61, 169.95; Anal. Calcd for $C_{13}H_{17}NO_4$: C, 62.14; H, 6.82; N, 5.57. Found: C, 62.25; H, 6.79; N, 5.52.

4.1.21. Novozym 435 catalyzed hydrolysis of 2,6-bis(1-acetoxyethyl)-pyridine *rac/meso*-6. 2,6-Bis-(1-acetoxyethyl)pyridine *rac/meso*-**6** (500 mg, 1.99 mmol) and Novozym 435 (250 mg) in 10 mL of 50 mM pH 7.0 sodium phosphate buffer were shaken at rt/1000 rpm for 48 h. The enzyme was filtered off and the reaction mixture was extracted with chloroform (3 × 5 mL). The combined extracts were washed with brine (5 mL), dried over sodium sulfate and concentrated under reduced pressure. The residue was purified column chromatography (silica gel/hexane:acetone 10:1) to give (*R*,*R*)-**4** (59 mg, 18%), (*S*,*R*)-**5** (150 mg, 36%) and (*S*,*S*)-**6** (85 mg, 17%) as colourless oils.

4.1.22. (*R*,*R*)-Bis-(1-hydroxyethyl)pyridine (*R*,*R*)-4. $[\alpha]_D^{25} = +70.0$ (*c* 2.0, acetone), $[\alpha]_D^{25} = +99.6$ (*c* 2.0, EtOH) {lit.[13] (64% ee): $[\alpha]_D = +44.01$ (*c* 2, acetone),

lit.[10] (>98% ee): $[\alpha]_D = +44.6$ (*c* 2.3, acetone)}; IR, ^1H and ^{13}C NMR data were indistinguishable from the spectra of (*R*,*R*)-**4**.

4.1.23. (*S*,*R*)-1-[6-(1-Hydroxyethyl)pyridin-2-yl]ethyl acetate (*S*,*R*)-5. $[\alpha]_D^{25} = -49.8$ (*c* 2.0, acetone), $[\alpha]_D^{25} = -56.0$ (*c* 2.0, EtOH); IR, ^1H and ^{13}C NMR data were indistinguishable from the spectra of (*R*,*S*)-**5**.

4.1.24. (*S*,*S*)-2,6-Bis(1-acetoxyethyl)pyridine (*S*,*S*)-6. $[\alpha]_D^{25} = -181.9$ (*c* 2.0, acetone), $[\alpha]_D^{25} = -171.9$ (*c* 2.0, EtOH); IR, ^1H and ^{13}C NMR data were indistinguishable from the spectra of (*S*,*R*)-**5**.

4.1.25. *meso*-2,6-Bis(1-hydroxyethyl)pyridine *meso*-4. A mixture of (*S*,*R*)-1-[6-(1-hydroxyethyl)pyridin-2-yl]ethyl acetate (*S*,*R*)-**5** (200 mg, 0.96 mmol) and aqueous sodium hydroxide (5%, 5 mL) was heated to 60 °C for 15 min. The reaction mixture was extracted with chloroform (4 × 5 mL). The combined extracts were washed with brine (3 mL), dried over sodium sulfate and concentrated under reduced pressure to give *meso*-**4** (158 mg, 98%) as white solid. mp 58–59 °C; IR: 3412, 1598, 1576, 1450, 1434, 1404, 1370, 1122, 1078, 1020, 930, 820; ^1H NMR (DMSO-d_6): 1.35 (6H, d, $J = 6.5$ Hz, 2 CH_3), 4.70 (2H, m, 2 O–CH), 5.30 (2H, d, 2 OH), 7.35 (2H, d, $J = 7.7$ Hz, $Py_{3,5}$–H), 7.75 (1H, t, $J = 7.7$ Hz, Py_4–H); ^{13}C NMR (DMSO-d_6): 24.32, 69.26, 116.91, 136.71, 163.88; Anal. Calcd for $C_9H_{13}NO_2$: C, 64.65; H, 7.84; N, 8.38. Found: C, 64.72; H, 7.77; N, 8.25.

4.1.26. *meso*-2,6-Bis(1-acetoxyethyl)-pyridine *meso*-6. A mixture of (*R*,*S*)-1-[6-(1-hydroxyethyl)pyridin-2-yl]ethyl acetate (*R*,*S*)-**5** (150 mg, 0.72 mmol), acetic anhydride (144 mg, 1.44 mmol) and triethylamine (5 mL) was stirred at rt for 6 h. The reaction mixture was diluted with 15 mL chloroform and washed with water (3 mL), 5% HCl solution (3 mL), saturated $NaHCO_3$ solution (3 mL) and brine (3 mL). The organic solution was dried over sodium sulfate and concentrated under reduced pressure. The residue was purified by column chromatography (silica gel/hexane:acetone 10:1) to give *meso*-**6** (138 mg, 75%) as a colourless oil. $[\alpha]_D^{25} = +0.2$ (*c* 2.0, acetone), $[\alpha]_D^{25} = +0.2$ (*c* 2.0, EtOH); IR: 1746, 1662, 1644, 1586, 1464, 1370, 1240, 1158, 1080, 1030, 950, 810; ^1H NMR: 1.55 (6H, d, $J = 6.7$ Hz, 2 CH_3), 2.10 (6H, s, 2 CO–CH_3), 5.89 (2H, q, $J = 6.7$ Hz, O–CH), 7.21 (2H, d, $J = 7.7$ Hz, $Py_{3,5}$–H), 7.64 (1H, t, $J = 7.7$ Hz, Py_4–H); ^{13}C NMR: 20.54, 21.06, 72.87, 118.66, 137.16, 159.71, 169.98; Anal. Calcd for $C_{13}H_{17}NO_3$: C, 62.14; H, 6.82; N, 5.57. Found: C, 62.09; H, 6.75; N, 5.64.

4.1.27. (*R*,*R*)-1-[6-(1-Hydroxyethyl)-pyridin-2-yl]ethyl acetate (*R*,*R*)-5. (*R*,*R*)-2,6-Bis(1-acetoxyethyl)-pyridine (*R*,*R*)-**6** (400 mg, 1.59 mmol) and Novozym 435 (200 mg) in 5 mL of 50 mM pH 7.0 sodium phosphate buffer were shaken at rt/1000 rpm for 48 h. The enzyme was filtered off, and the reaction mixture extracted with chloroform (3 × 5 mL). The combined extracts were washed with brine (5 mL), dried over sodium sulfate and concentrated under reduced pressure. The residue was purified by column chromatography (silica gel/hexane:acetone 10:1) to yield (*R*,*R*)-**5** (264 mg, 79%) as a colourless oil. $[\alpha]_D^{25} = +138.4$ (*c* 2.0, acetone), $[\alpha]_D^{25} = +131.2$ (*c* 2.0, EtOH); IR: 3432, 1740, 1596, 1464, 1372, 1240, 1080, 1048, 816; ^1H NMR: 1.43 (3H, d, $J = 6.5$ Hz, CH_3), 1.53 (3H, d, $J = 6.7$ Hz, CH_3), 2.06 (3H, s, CO–CH_3), 4.4 (1H, br s, OH), 4.81 (1H, m, O-CH), 5.86 (1H, q, $J = 6.7$ Hz, O–CH), 7.11 (1H, d, $J = 7.7$ Hz, Py_5–H), 7.17 (1H, d, $J = 7.7$ Hz, Py_3–H), 7.62 (1H, t, $J = 7.7$ Hz, Py_4–H); ^{13}C NMR: 20.71, 21.38, 24.23, 68.38, 72.73, 118.58, 118.59, 137.47, 158.51, 162.06, 169.95; Anal. Calcd for $C_{11}H_{15}NO_3$: C, 63.14; H, 7.23; N, 6.69. Found: C, 63.07; H, 7.26; N, 6.65.

4.1.28. (*S*,*S*)-1-[6-(1-Hydroxyethyl)-pyridin-2-yl]ethyl acetate with enzymatic reaction (*S*,*S*)-5. (*S*,*S*)-2,6-Bis(1-hydroxyethyl)-pyridine (*S*,*S*)-**4** (150 mg, 0.89 mmol) and CAL-A (15 mg) in vinyl acetate (2 mL) were shaken at rt/1000 rpm for 3 h. The enzyme was filtered off and the filtrate concentrated under reduced pressure. The residue was purified by column chromatography (silica gel/hexane:acetone 10:1) to give (*S*,*S*)-**5** (126 mg, 68%) as a colourless oil. $[\alpha]_D^{25} = -131.8$ (*c* 2.0, acetone), $[\alpha]_D^{25} = -131.3$ (*c* 2.0, EtOH); IR, ^1H and ^{13}C NMR data were indistinguishable from the spectra of (*R*,*R*)-**5**.

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