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Horse liver esterase catalyzed enantioselective hydrolysis of *N,O*-diacetyl-2-amino-1-arylethanol

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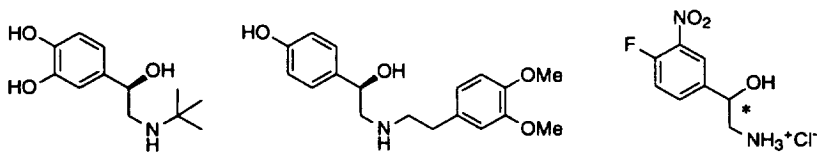
Abstract

N,O-Diacetyl-2-amino-1-arylethanol can be efficiently resolved by horse liver esterase (HLE). A remarkable organic co-solvent effect on the enantioselectivity of HLE was observed.

Many substituted 2-amino-1-arylethanols are of great pharmaceutical importance. For example, (*R*)-(−)-terbutaline **1**[1] is an important adrenergic bronchodilator while (*R*)-denopamine **2**, (Fig. 1)[2] was the first orally active and long acting positive inotropic agent. Amino alcohols[3] in general have also found widespread use in modern organic synthesis as chiral inducers. These two aspects have made them important synthetic targets and various asymmetric syntheses have been reported.[4]

In connection with our work on the total synthesis of cyclopeptide alkaloids,[5] both antipodes of optically active 2-amino-1-(4′-fluoro-3′-nitro)phenylethanol **3** were required. From the viewpoint of practical synthesis, enantiomerically pure cyanohydrin appeared to be an attractive precursor as it is readily available by both chemical[6] and enzymatic procedures.[7] However, careful analysis of literature data revealed that electron deficient aromatic aldehydes, such as nitrobenzaldehyde, were generally poor substrates in terms of enantioselectivity, most probably due to the background reaction and the configurational lability of the resulting cyanohydrin.[8,9] Furthermore, although (*R*)-oxynitrilase was easily available and showed low substrate specificity, the corresponding (*S*)-oxynitrilase was less accessible



1 **2** **3**

Fig. 1.

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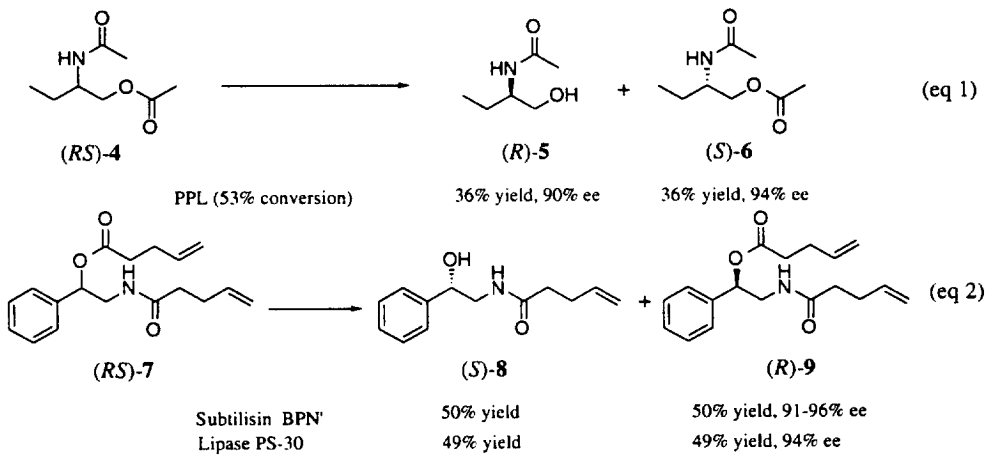
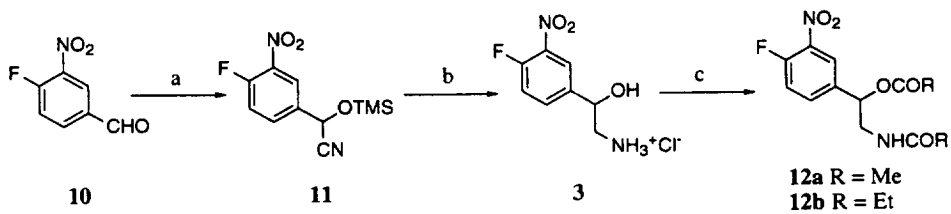
and exhibited a more narrow substrate tolerance.[9] These observations together with our interest in chemoenzymatic synthesis of chiral building blocks[10] prompted us to examine an alternative way, i.e., via enzymatic resolution of racemic amino alcohols. Interestingly, while much attention has been paid to the enzymatic resolution of 2-amino-2-alkylethanol[11] (Eq. 1), studies on that of 2-amino-1-arylethanol (Eq. 2) were relatively rare and to date, lipase has been elucidated as the enzyme of choice for performing the desired transformation.[12] We report herein that HLE (horse liver esterase) is an efficient alternative biocatalyst for resolving the *N,O*-diacetyl derivatives of 2-amino-1-arylethanol in the presence of suitable organic co-solvent.



Racemic amino alcohol **3** and its acyl derivatives **12a** and **12b** were prepared as shown in Scheme 1. Reaction of 4-fluoro-3-nitrobenzaldehyde **10** with TMSCN in the presence of a catalytic amount of anhydrous ZnI_2[13] gave cyanohydrin **11** which was, without purification, chemoselectively reduced to the corresponding amino alcohol **3** in excellent overall yield. Acylation with acetic anhydride or propionyl chloride gave **12a** and **12b**, respectively, ready for resolution studies.



Reagents and Conditions: a) TMSCN, ZnI_2; b) BH_3-THF, reflux, 90%; c) Ac_2O, DMAP, Et_3N, (**12a**, 80%); EtCOCl, DMAP, Et_3N (**12b**, 85%)

Scheme 1.

In a preliminary experiment, several commercially available lipases, acylases and esterases have been screened for resolution by stirring racemic **12a** or **12b** in a biphasic solution (phosphate buffer pH 7.5 and CH_2Cl_2) at 37°C. Disappointingly (Table 1), no hydrolysis of compound **12a** was observed with lipases such as PPL (porcine pancreas lipase), PCL (pseudomonas cepacia lipase), WGL (wheat germ lipase), *Aspergillus niger* and acylase PS Amano. It was interesting to note that PPL had been employed to resolve efficiently the structurally related amino alcohols (Eqs. 1 and 2) in organic solvent such as diisopropyl ether, thus efficiently indicating a remarkable solvent effect. Candida cyclindracea lipase (CCL, entry 7) did promote the hydrolysis, however, only racemic alcohol was obtained under these conditions. More promising results were obtained with protease from *Bacillus licheniformis* (entries 8

Table 1
Survey of enzyme catalyzed hydrolysis of **12a** and **12b**[a]

Entry	Substrate	Enzyme	Time, h	Solvent[b]	Convn[c], %	(*S*)-**13**, ee%[d]	(*R*)-**14**, ee%
1	**12a**	PPL	36	P.B./CH_2Cl_2 = 10/1	0		0
2	**12a**	PSL	36	P.B./CH_2Cl_2 = 10/1	0		0
3	**12a**	PLE	36	P.B./CH_2Cl_2 = 10/1	0		0
4	**12a**	WG	36	P.B./CH_2Cl_2 = 10/1	0		0
5	**12a**	PS Amano	36	P.B./CH_2Cl_2 = 10/1	0		0
6	**12a**	Asp	36	P.B./CH_2Cl_2 = 10/1	0		0
7	**12a**	CCL	24	P.B./CH_2Cl_2 = 10/1	40	0	
8	**12a**	*Bacil. Lichen.*	36	P.B./CH_2Cl_2 = 8/1	41	28	
9	**12a**	HLE	11.5	P.B./n-hex. = 10/1	45	79	62
10	**12b**	*Bacil. Lichen.*	36	P.B./CH_2Cl_2 = 8/1	41	28	
11	**12b**	HLE	2.2	P.B./n-hex. = 10/1	45	65	

a) All reactions were performed at 37°C; b) P.B. stands for phosphate buffer (40 mM, pH 7.5); c) The conversion was followed by HPLC; d) The ee was measured by HPLC analysis of the corresponding (*S*)-acetoxy propionyl ester. The ee of (*R*)-**14** was similarly determined by first removal of the ester function by chemical method.

and 10) and especially with HLE (entries 9, 11) although the *ee* of alcohol remained moderate under these conditions.

In searching for an efficient enzymatic process, the organic co-solvent was generally selected based on its ability to solubilize the substrates and to enhance the reaction rate, its influence on the enantioselectivity of a given enzyme was frequently overlooked. Following Kilbanov's observation[14] that the enantioselectivity of an enzymatic process may depend on the reaction medium, the organic co-solvent effect was next examined in detail on the HLE catalyzed hydrolysis of substrate **12a**. As seen in Table 2, both reaction rate and enzyme enantioselectivity were indeed influenced markedly by the organic co-solvent. The best result was obtained when HLE mediated hydrolysis was carried out in a phosphate buffer (pH 7.5) in the presence of acetone (10% volume, entry 6) as an organic co-solvent at 27°C. Under these conditions, a reasonable reaction rate was observed and (*S*)-**13** together with unreacted diacetyl derivative (*R*)-**14** were obtained with an *ee* greater than 90% at 50% conversion (E=55–60, Scheme 2). Treatment of (*S*)-**13** and (*R*)-**14** in refluxing HCl (6 N) gave the corresponding (*S*)-**3** and (*R*)-**3** in quantitative yield without loss of enantiomeric excess. The enantiomeric excess of (*S*)-**13**, (*S*)-**3** and (*R*)-**14**, (*R*)-**3** (90%) was measured by HPLC analysis of the corresponding (*S*)-lactate[15] **15** and **16**, respectively (Fig. 2).

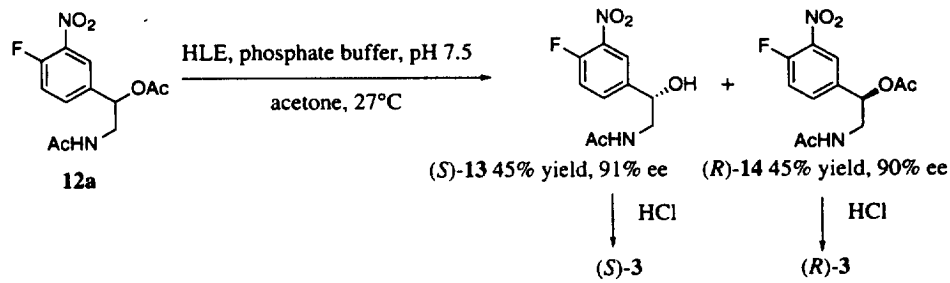
The absolute configuration of **13** and **14** was determined by a modified Mosher's method[16] (Fig. 3). Thus, coupling of (*S*)-**13** with (*S*)- and (*R*)-MTPA acids in the presence of DCC and DMAP afforded the corresponding (*S*)- and (*R*)-MTPA ester in excellent yield. Large negative $\Delta\delta$ ($\delta_S - \delta_R$) values were found for all protons on the left side of the MTPA plan, while all but one diastereotopic proton[17] on the right side of the MTPA plane displayed a large positive $\Delta\delta$. Therefore, the (*S*)-configuration was assigned for alcohol **13**. The (*R*)-configuration was determined for compound **14** following the same procedure.

The generality of the present enzymatic procedure was briefly examined. As shown in Scheme 3, treatment of *N,O*-diacetyl-2-amino-1-phenylethanol **17** with HLE under the above-developed conditions gave (*S*)-**18** and (*R*)-**19** (Scheme 3). Once again, the *ee*s of (*S*)-**18** and (*R*)-**19** (>90% ee) were determined

Table 2
Solvent effect of HLE catalyzed hydrolysis of **12a**[a]

Entry	Co-Solvent (v/v)[b]	Time, h	Convn[c], %	(*S*)-**13**, ee%[d]	(*R*)-**14**, ee%
1	hexane (10/1)	11.5	45	79	62
2	hexane (10/1)	17	61	74	65
3	hexane (1/10)	3	40	40	
4	Pr^i_2O (10/1)	11.5	48	78	68
5	EtOH (10/1)	16	55	61	0
6	acetone (10/1)	12	51	90	90

a): All reactions were performed at 27°C; b) ratio of phosphate buffer pH 7.5 *vs* organic co-solvent; c) The conversion was followed by HPLC; c) The ee was measured by HPLC analysis of the corresponding (*S*)-acetoxy propionyl ester. The ee of (*R*)-**14** was similarly determined by first removal of the ester function by chemical method.



Scheme 2.

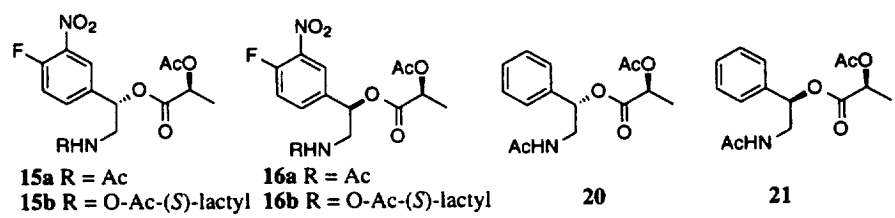
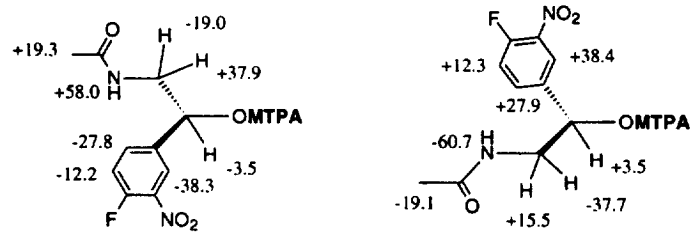


Fig. 2.

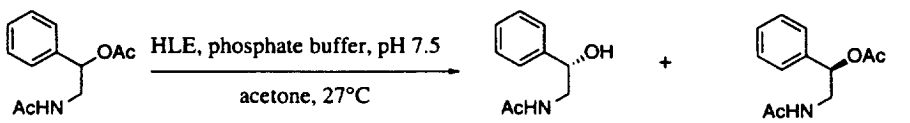
by HPLC analysis of their corresponding (*S*)-lactate **20** and **21**, respectively (Fig. 2), while their configuration was deduced by the modified Mosher's ester. Comparison of optical rotations of (*S*)-**18** and (*R*)-**19** with literature values[12b] (*cf.* experimental section) proved not only the enantiomerical purity but also the assignment of their absolute configuations.

In summary, HLE has been shown for the first time, to our knowledge, to be an efficient biocatalyst for the enantioselective hydrolysis of *N,O*-diacetyl-2-amino-1-arylethanol. The procedure should find application in the synthesis of other optically pure aminoalcohols.



$\Delta\delta$ (δ_S–δ_R) values for MTPA esters of **13** and **14**.

Fig. 3.



(*RS*)-**17** (*S*)-**18** 42% yield, 90% ee (*R*)-**19** 43% yield, 90% ee

Scheme 3.

1. Experimental section

Melting points were determined with a Kofler apparatus and were uncorrected. Infrared (IR) spectra were recorded on a Nicolet-205 spectrometer. ^{1}H NMR spectra were measured on Brucker AC-200 (200 MHz), Bruker AC-250 (250 MHz), Bruker (300 MHz) and Bruker WM-400 (400 MHz) spectrometers with tetramethylsilane as an internal standard (δ ppm). Flash chromatography was performed using Kieselgel 60 (230–400 mesh, E. Merck) and usually employed a stepwise solvent polarity gradient, correlated with TLC mobility. Solvents and reagents were purified according to standard laboratory techniques. Optical rotations were determined on a Perkin–Elmer automatic polarimeter at room temperature. Mass spectra were run on AEI MS-50 (EI), AEI MS-9 (CI) and Kratos MS-80 (FAB), respectively. All reactions requiring anhydrous conditions or an inert atmosphere were conducted under an atmosphere of Argon.

1.1. (RS)-2-Amino-1-(4′-fluoro-3′-nitro)phenylethanol 3

To a suspension of anhydrous ZnI_2 (2.27 g, 7.1 mmol) in CH_2Cl_2 were added, successively, TMSCN (12.3 mL, 92 mmol) and 4-fluoro-3-nitro benzaldehyde (12.0 g, 71.0 mmol) at room temperature. After being stirred for 1 h, the reaction mixture was diluted by addition of water and extracted with CH_2Cl_2. The combined organic extracts were washed with brine, dried (Na_2SO_4) and evaporated under reduced pressure to give a quantitative yield of cyanohydrin **11**: ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.18 (s, 9H), 5.50 (s, 1H), 7.40 (dd, J=8.6, 10.0 Hz, 1H), 7.78 (ddd, J=2.3, 4.0, 8.6 Hz, 1H), 8.19 (dd, J=2.3, 6.8 Hz). To a solution of the obtained crude cyanohydrin **11** in anhydrous THF, was introduced BH_3–THF (142 mL, 1 M in THF, 142 mmol) at 0°C. The resulting solution was heated to reflux for 3 h. After being cooled to 0°C, excess BH_3 was transformed into volatile trimethylborate by careful addition of anhydrous methanol. The volatiles were removed and the residue was redissolved in MeOH followed by slow addition of concentrated aqueous HCl solution. Evaporation of organic solvent gave a pale yellow solid which, after washing with ether afforded an analytically pure hydrochloride salt of the title compound (*RS*)-**3** (15.1 g, 90%): mp 205°C; IR (KBr), ν 3325, 3079, 1623, 1546, 1539, 1258 cm^{-1}; ^{1}H NMR (CD_3OD, 250 MHz): δ 3.01 (dd, J=9.2, 12.7 Hz, 1H), 3.22 (dd, J=3.5, 12.7 Hz, 1H), 5.0 (dd,

J=3.5, 9.2 Hz, 1H), 7.45 (dd, J=8.5, 9.1 Hz, 1H), 7.8 (ddd, J=2.4, 4.3, 8.5 Hz, 1H), 8.18 (dd, J=2.4, 7.1 Hz, 1H); ^{13}C NMR (CD_3OD, 62.5 Hz) δ 46.8, 69.2, 119.7 (d, J=21.4 Hz), 124.6, 134.4 (d, J=8.2 Hz), 138.0, 140.0, 156.1 (d, J=264.0 Hz); MS (CI) *m/z* 201 $(M-HCl)^+$; Anal. Calcd for $C_8H_{10}N_2O_3FCl$: C, 40.61; H, 4.26; N, 11.84; Found: C, 40.44; H, 4.48, N, 11.67.

1.2. (S)-2-Amino-1-(4′-fluoro-3′-nitro)phenylethanol 3

A solution of (*S*)-**13** (1.0 g, 4.13 mmol) was refluxed in HCl (6 N, 10 mL) for 3 h. Evaporation of the volatiles afforded an analytically pure hydrochloride salt of amino alcohol (*S*)-**3** (970 mg, 99%); $[\alpha]_D$=+39 (MeOH, *c* 0.3).

1.3. (R)-2-Amino-1-(4′-fluoro-3′-nitro)phenylethanol 3

Following the same procedure as described for (*S*)-**3**, (*R*)-**3** was prepared from (*R*)-**14** in 98.5% yield: $[\alpha]_D$=−39 (MeOH, *c* 0.5).

1.4. (RS)-N,O-Diacetyl-2-amino-1-(4′-fluoro-3′-nitro)phenylethanol 12a

To a solution of compound **3** (8.10 g, 34.32 mmol) in CH_2Cl_2 was added, successively, triethylamine (9.6 mL, 68.0 mmol), acetic anhydride (6.5 mL, 68 mmol) and DMAP (834.0 mg, 6.84 mmol) at 0°C. After being stirred for 4 h at room temperature, the reaction mixture was diluted by addition of saturated aqueous NH_4Cl solution and extracted with CH_2Cl_2. The combined organic extracts were washed with brine, dried (Na_2SO_4) and evaporated under reduced pressure to give the crude product **12a**. Recrystallization (EtOAc/heptane) gave pure **12a** (5.0 g). The filtrate was evaporated and purified by flash chromatography (EtOAc) to give another 2.8 g of **12a**. The total yield of **12a** was 80%: mp 85–90°C; IR ($CHCl_3$), ν 1750, 1679, 1546, 1349 cm^{-1}; ^{1}H NMR ($CDCl_3$, 250 MHz): δ 1.95 (s, 3H), 2.16 (s, 3H), 3.56 (ddd, J=5.9, 7.6, 14.2 Hz, 1H), 3.70 (ddd, J=4.5, 6.3, 14.2 Hz, 1H), 5.85 (dd, J=4.5, 7.6 Hz, 1H), 6.15 (br s, 1H), 7.32 (dd, J=8.6, 10.4 Hz, 1H), 7.66 (ddd, J=2.2, 4.1, 8.6 Hz, 1H), 8.05 (dd, J=2.2, 7.0 Hz, 1H); ^{13}C NMR ($CDCl_3$, 62.5 Hz) δ 21.0, 23.1, 43.9, 73.0, 118.8 (d, J=21.1 Hz), 124.1 (d, J=3.5 Hz), 133.9 (d, J=9.5 Hz), 135.2, 155.2 (d, J=271.0 Hz), 170.0, 170.4; MS (CI) *m/z* 285 $(M+H)^+$; Anal. Calcd for $C_{12}H_{13}N_2O_5F$: C, 50.71; H, 4.61, N; 9.86; Found: C, 50.77; H, 4.81; N, 9.56.

1.5. (RS)-N,O-Dipropionyl-2-amino-1-(4′-fluoro-3′-nitro)phenylethanol 12b

Using propionyl chloride instead of acetic anhydride, (*RS*)-**12b** was prepared in 85% yield following the procedure described above: mp: 95°C; IR ($CHCl_3$), ν 3456, 1744, 1675, 1625, 1543, 1506, 1350 cm^{-1}; ^{1}H NMR ($CDCl_3$, 250 MHz): δ 1.12 (t, J=7.5 Hz, 3H), 1.16 (t, J=7.5 Hz; 3H), 2.2 (q, J=7.5 Hz, 2H), 2.42 (q, J=7.7 Hz, 2H), 3.58 (ddd, J=5.9, 7.7, 14.1 Hz, 1H), 3.7 (ddd, J=4.4, 6.3, 14.1 Hz, 1H), 5.82 (br s, 1H, NH), 5.88 (dd, J=4.4, 7.7 Hz, 1H), 7.3 (dd, J=8.6, 10.4 Hz, 1H), 7.66 (ddd, J=2.3, 4.2, 8.6 Hz, 1H), 8.05 (dd, J=2.3, 7.7 Hz, 1H); ^{13}C NMR ($CDCl_3$, 50.2 MHz) δ 9.1, 9.8, 27.6, 29.7, 44.0, 72.9, 118.9 (d, J=20.3 Hz), 124.0, 138.8 (d, J=8.5 Hz), 133.8, 134.4, 155.3 (d, J=267 Hz), 173.6, 174.0; MS (EI) *m/z* 313 $(M+H)^+$.

1.6. (RS)-N,O-*Diacetyl-2-amino-1-phenylethanol* **17**

Following the same procedure as described for **12a**, compound (*RS*)-**17** was prepared in 75% yield: IR ($CHCl_3$), ν 1743, 1672, 1525, 1370, 1229 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz): δ 2.01 (s, 3H), 2.10 (s, 3H), 3.60 (ddd, *J*=5.7, 7.9, 14.2 Hz, 1H), 3.72 (ddd, *J*=4.7, 6.1, 14.2 Hz, 1H), 5.7 (br s, 1H), 5.84 (dd, *J*=4.7, 7.9 Hz, 1H), 7.3–7.4 (m, 5H); ^{13}C NMR ($CDCl_3$, 50.2 Hz) δ 21.2, 23.2, 44.4, 74.6, 126.4, 128.4, 128.7, 137.7, 170.3, 170.4; MS (CI) *m/z* 221 $(M+H)^+$.

1.7. (RS)-*2-Acetylamino-1-(4′-fluoro-3-nitro)phenylethanol* **13**

To a solution of compound **3** (210 mg, 0.84 mmol) in a mixed solvent CH_2Cl_2 and water (1/1) was added, successively, NaOH (68 mg, 1.7 mmol), and acetic anhydride (88 μL, 0.93 mmol). After being stirred for 3 h at room temperature, the reaction mixture was diluted by addition of saturated aqueous NH_4Cl solution and extracted with CH_2Cl_2. The combined organic extracts were washed with brine, dried (Na_2SO_4) and evaporated under reduced pressure. Recrystallization of the crude product (EtOAc/heptane) gave pure (*RS*)-**13** (152 mg, 75%): mp 100°C; IR ($CHCl_3$), ν 3355, 1660, 1628, 1598, 1538 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 2.05 (s, 3H), 3.37 (ddd, *J*=5.5, 7.3, 14.4 Hz, 1H), 3.65 (ddd, *J*=2.8, 6.4, 14.4 Hz, 1H), 4.30 (s, 1H, OH), 4.98 (dd, *J*=2.8, 7.3 Hz, 1H), 7.30 (dd, *J*=8.6, 10.5 Hz, 1H), 7.71 (ddd, *J*=2.2, 4.1, 8.6 Hz, 1H), 8.10 (dd, *J*=2.2, 7.1 Hz, 1H); ^{13}C NMR ($CDCl_3$, 62.5 Hz) δ 23.1, 47.8, 72.4, 118.5 (d, *J*=21.0 Hz), 123.5, 133.1 (d, *J*=9.1 Hz), 154.8 (d, *J*=271.0 Hz), 172.5; MS (EI) *m/z* 243 $(M+H)^+$; Anal. Calcd for $C_{10}H_{11}N_2O_4F$: C, 49.59; H, 4.57; N, 11.56; Found: C, 49.42; H, 4.48; N, 11.83.

1.8. (RS)-*2-Acetylamino-1-phenylethanol* **18**

Compound (*RS*)-**18** was prepared as described for (*RS*)-**13**. IR ($CHCl_3$), ν 3381, 1665, 1518, 1459, 1419 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 1.96 (s, 3H), 3.30 (ddd, *J*=4.8, 7.9, 13.9 Hz, 1H), 3.64 (ddd, *J*=3.4, 7.0, 13.9 Hz, 1H), 3.84 (s, 1H, OH), 4.80 (m, 1H), 6.30 (s, 1H, NH), 7.1–7.3 (m, 5H); ^{13}C NMR ($CDCl_3$, 62.5 Hz) δ 23.2, 47.6, 73.6, 126.0, 128.0, 141.8, 171.8; MS (EI) *m/z* 179 $(M)^+$; Anal. Calcd for $C_{10}H_{13}NO_2$: C, 67.02; H, 7.31; N, 7.81; Found: C, 66.97; H, 7.41; N, 7.53.

1.9. *Enzymatic resolution of* (RS)-**12a**

To a solution of racemic (*RS*)-**12a** (7.7 g, 27.1 mmol) in mixed solvent (phosphate buffer and acetone (10/1, total volume 1 L) was added HLE (4.0 g). The reaction mixture was vigorously stirred at 27°C and the reaction course was monitored by HPLC (hypersil, 5 μm, ODS, 0.4×250 mm; eluant: 35% CH_3CN in H_2O, containing 0.01% of TFA). The reaction was stopped at the 50% hydrolysis point (12 h) by addition of methanol and then filtered. After removal of volatiles from the filtrate, the residue was extracted with EtOAc. The combined organic extracts were washed with brine, dried (Na_2SO_4) and evaporated under reduced pressure. Recrystallization of the crude product (CH_2Cl_2/heptane) gave pure (*S*)-**13** (2.3 g). The filtrate was evaporated and purified by flash chromatography (EtOAc/heptane=9/1, then EtOAc, EtOAc/MeOH=9/1) gave another 0.7 g of (*S*)-**13** (total 3.0 g, 45.7%) and unhydrolyzed ester (*R*)-**14** (3.4 g, 44.2%). (*S*)-**13** $[\alpha]_D$=+21 (acetone, *c* 0.2); (*R*)-**14** $[\alpha]_D$=−32 ($CHCl_3$, *c* 0.9).

1.10. Enzymatic resolution of (RS)-17

The same procedure applied to (*RS*)-**17** gave (*S*)-**18** and (*R*)-**19**. (*S*)-**18**: $[\alpha_D]$=+73 {$CHCl_3$, *c* 0.2; lit.[12b]: the (*R*)-enantiomer: $[\alpha]_D$=−79, ($CHCl_3$, *c* 0.3)}; (*R*)-**19**: $[\alpha]_D$=−55 {($CHCl_3$, *c* 0.5; lit.[12b]: the (*S*)-enantiomer: $[\alpha]_D$=+60, ($CHCl_3$, *c* 0.27)}.

1.11. 2 (2S)-Acetoxypropionic acid 2-acetylamino-1 (1S)-(4-fluoro-3-nitrophenyl)-ethyl ester ***15a***

To a solution of (*S*)-**13** (30 mg, 0.124 mmol) in CH_2Cl_2 were added triethylamine (35 μl, 0.248 mmol), (*S*)-2-acetoxypropionyl chloride (31.4 μl, 0.248 mmol) and a catalytic amount of DMAP (3 mg). After being stirred at room temperature for 1 h, the reaction mixture was diluted by addition of saturated aqueous NH_4Cl solution and extracted with CH_2Cl_2. The combined organic extracts were washed with brine, dried (Na_2SO_4) and evaporated under reduced pressure. Purification by preparative TLC (EtOAc/acetone=9/1) afforded **15a** (34 mg, 80%): $[\alpha]_D$=+21 ($CHCl_3$, *c* 0.45); IR ($CHCl_3$), ν 1743, 1679, 1546, 1384, 1342, 1272, 1236 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 1.52 (d, *J*=7.2 Hz, 3H), 1.98 (s, 3H), 2.18 (s, 3H), 3.44 (ddd, *J*=5.5, 8.5, 14.2 Hz, 1H), 3.80 (ddd, *J*=3.5, 6.8, 14.2 Hz, 1H), 5.04 (q, *J*=7.1 Hz, 1H), 5.96 (dd, *J*=3.5, 8.5 Hz, 1H), 5.97 (br s, 1H), 7.32 (dd, *J*=8.7, 10.3 Hz, 1H), 7.64 (ddd, *J*=2.3, 4.0, 8.6 Hz, 1H), 8.05 (dd, *J*=2.3, 7.0 Hz, 1H); MS (EI) *m/z* 357 $(M+H)^+$.

1.12. 2 (2S)-Acetoxypropionic acid 2-acetylamino-1 (1R)-(4-fluoro-3-nitrophenyl)-ethyl ester ***16a***

$[\alpha]_D$=−86 ($CHCl_3$, *c* 0.9); IR ($CHCl_3$), ν 1757, 1736, 1680, 1623, 1546, 1370, 1349, 1251 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 1.50 (d, *J*=7.0 Hz, 3H), 1.98 (s, 3H), 2.16 (s, 3H), 3.35 (ddd, *J*=4.6, 9.1, 14.2 Hz, 1H), 3.94 (ddd, *J*=3.2, 7.6, 14.2 Hz, 1H), 4.95 (q, *J*=7.0 Hz, 1H), 6.04 (dd, *J*=3.2, 9.1 Hz, 1H), 6.18 (br s, 1H), 7.32 (dd, *J*=8.6, 10.3 Hz, 1H), 7.64 (ddd, *J*=2.2, 4.1, 8.6 Hz, 1H), 8.07 (dd, *J*=2.2, 6.8 Hz, 1H); MS (EI) *m/z* 357 $(M+H)^+$.

1.13. 2 (2S)-Acetoxypropionic acid 2-[2(2S)-acetoxypropionylamino)]-1 (1S)-(4-fluoro-3-nitrophenyl)-ethyl ester ***15b***

$[\alpha]_D$=+7.3 ($CHCl_3$, *c* 0.8); IR ($CHCl_3$), ν 1746, 1680, 1543, 1374, 1347, 1232, 1095 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.45 (d, *J*=6.8 Hz, 3H), 1.53 (d, *J*=7.1 Hz, 3H), 2.15 (s, 3H), 2.16 (s, 3H), 3.42 (ddd, *J*=5.4, 8.7, 14.3 Hz, 1H), 3.85 (ddd, *J*=3.3, 7.0, 14.3 Hz, 1H), 5.11 (q, *J*=7.1 Hz, 1H), 5.18 (q, *J*=6.8 Hz, 1H), 6.00 (dd, *J*=3.3, 8.7 Hz, 1H), 6.60 (m, 1H), 7.33 (dd, *J*=8.7, 10.4 Hz, 1H), 7.64 (ddd, *J*=2.3, 4.0, 8.7 Hz, 1H), 8.06 (dd, *J*=2.3, 6.8 Hz, 1H), MS (EI) *m/z* 429 $(M+H^+)$.

1.14. 2 (2S)-Acetoxypropionic acid 2-[2(2S)-acetoxypropionylamino)]-1 (1R)-(4-fluoro-3-nitrophenyl)-ethyl ester ***16b***

$[\alpha]_D$=−59 ($CHCl_3$, *c* 0.7); IR ($CHCl_3$), ν 1742, 1689, 1543, 1377, 1350, 1244, 1098 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.42 (d, *J*=6.8 Hz, 3H), 1.48 (d, *J*=7.1 Hz, 3H), 2.13 (s, 6H), 3.52 (ddd, *J*=5.5, 8.0, 14.0 Hz, 1H), 3.85 (ddd, *J*=3.3, 6.8, 14.4 Hz, 1H), 4.95 (q, *J*=7.1 Hz, 1H), 5.12 (q, *J*=6.8 Hz, 1H), 6.00 (dd, *J*=3.3, 8.0 Hz, 1H), 5.60 (m, 1H), 7.31 (dd, *J*=8.7, 10.3 Hz, 1H), 7.64 (ddd, *J*=2.3, 4.1, 8.7 Hz, 1H), 8.06 (dd, *J*=2.3, 6.9 Hz, 1H), MS (EI) *m/z* 429 $(M+H^+)$.

1.15. 2 (2S)-Acetoxypropionic acid 2-acetylamino-1 (1S)-phenyl ethyl ester **20**

$[\alpha]_D$=+28 ($CHCl_3$, *c* 0.25); IR ($CHCl_3$), ν 1743, 1675, 1525, 1375, 1268 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 1.52 (d, *J*=7.0 Hz, 3H), 2.00 (s, 3H), 2.16 (s, 3H), 3.40 (ddd, *J*=4.9, 9.3, 14.3 Hz, 1H), 3.82 (ddd, *J*=3.5, 7.2, 14.3 Hz, 1H), 5.05 (q, *J*=7.0 Hz, 1H), 5.90 (br s, 1H), 5.96 (dd, *J*=3.5, 9.3 Hz, 1H), 7.3 (m, 5H); MS (EI) *m/z* 293 $(M)^+$.

1.16. 2 (2S)-Acetoxypropionic acid 2-acetylamino-1 (1R)-phenyl ethyl ester **21**

$[\alpha]_D$=−97 ($CHCl_3$, *c* 0.3); IR ($CHCl_3$), ν 1743, 1675, 1525, 1375, 1256 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 1.50 (d, *J*=7.0 Hz, 3H), 2.02 (s, 3H), 2.14 (s, 3H), 3.38 (ddd, *J*=4.1, 9.8, 14.1 Hz, 1H), 3.9 (ddd, *J*=3.2, 7.8, 14.1 Hz, 1H), 4.95 (q, *J*=7.0 Hz, 1H), 6.02 (dd, *J*=3.2, 9.8 Hz, 1H), 6.12 (br s, 1H), 7.3 (m, 5H,); MS (EI) *m/z* 293 $(M)^+$.

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