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ENZYMATIC RESOLUTION OF HINDERED SECONDARY ALCOHOLS: EFFICIENT ACCESS TO A NEW SIMPLIFIED CHIRAL AUXILIARY

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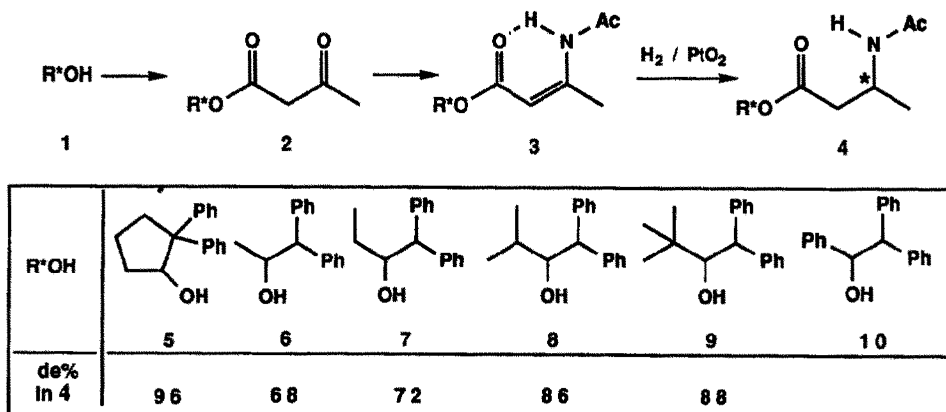
Abstract: 2,2-Diphenylcyclopentanol, an efficient chiral auxiliary which bears only one stereogenic center, as well as some related hindered secondary alcohols, were resolved by means of pig liver acetone powder (PLAP) and horse liver acetone powder (HLAP).

Introduction:

Recently, three of us have described the synthesis of chiral β-amidoesters **4**, with high diastereomeric excess, through the asymmetric hydrogenation of stereogenic β-acetamidocrotonates **3**[1]. For this purpose, new efficient chiral auxiliary alcohols **1** bearing only one stereogenic center, such as 2,2-diphenylcyclopentanol **5**, 1,1-diphenyl-3-methyl-2-butanol **8**, and 1,1-diphenyl-3,3-dimethyl-2-butanol **9** were designed (Scheme 1). Interestingly, the former alcohol **5** gave a similar stereochemical result compared to the one obtained with 8-phenylmenthol[2].



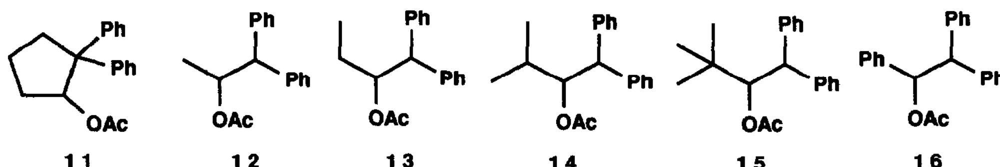
Scheme 1

Alcohols **5-10** are easily accessible in their racemic form[3]. The utilization of these materials as chiral auxiliaries in asymmetric synthesis requires either their own asymmetric synthesis[4], or a practical, large-scale method for their separation into individual pure enantiomers[5]. In this paper, we describe our studies on the resolution of alcohols **5, 8, 9** and related compounds **6, 7** and **10**, by enzymatic hydrolysis[6] of their corresponding acetates[7].

Analytical studies:

All attempts at enzymatic esterification (with acetic, butyric or higher anhydrides) or transesterification (with vinyl or isopropenyl acetate) of alcohols **5-10** have failed in the present case[8]. However, we have found that the simple enzymatic hydrolysis of racemic acetates **11-14**, derived from the alcohols **5-8** is effective, yielding the desired optically active alcohols with good enantiomeric excess.

We have first determined the enantioselectivity coefficient E [9] for several enzymatic preparations on the milligram scale. For this purpose, the racemic acetates **11-16** were treated with various enzymatic preparations in 0.1 M sodium phosphate buffer, pH 7.4, containing 10% of an organic cosolvent, at 30°C [10].



Evolution of the reaction was monitored by GC analysis. The ee of the alcohol formed and of the remaining acetate were determined by GC analysis after derivatization with (*S*)-O-acetyllactyl chloride[11]. Correlation of these measurements allowed the calculation of the E coefficients[9], except when the rate of hydrolysis was too slow (Table 1).

Table 1: Enantioselectivity Coefficients (E) and Rates (V) Obtained in the Hydrolysis of Racemic Acetates **11-14** Catalyzed by PLAP or HLAP in the Presence of Various Added Organic Solvents[a].

	E (V)[b] calculated (and measured) in the presence of the cosolvent :						
	Acetone	Methanol	Ethanol	DMSO	CH_2Cl_2	Hexane	Ether
Acetate (Enzyme)	E (V)	E (V)	E (V)	E (V)	E (V)	E (V)	E (V)
11 (HLAP)	64 (34)	nd	nd (0.7)	**>100 (15)**	nd	53 (0.7)	>100(4.8)
11 (PLAP)	33 (33)	nd	nd	**75 (13)**	nd	14 (0.9)	nd
12 (HLAP)	9 (43)	4 (27)	nd	7 (74)	nd (1.8)	7 (13)	21 (13)
12 (PLAP)	19 (53)	19 (22)	nd	**22 (79)**	nd (1.4)	26 (21)	38 (16)
13 (HLAP)	>100 (36)	>100 (22)	>100 (73)	**>100 (24)**	nd (1)	>100 (9)	>100 (6)
14 (HLAP)	13 (4)	nd	58 (3)	**>100 (11)**	nd (1.6)	23(0.2)	nd (0.6)
14 (PLAP)	nd (0.5)	nd	nd	nd (0.4)	nd (0.2)	nd (0.1)	nd

a: 10% organic solvent added to 0.1 M sodium phosphate buffer, pH 7.4, 30°C; b: rate of hydrolysis in μmoles of hydrolyzed acetate/h and /g of enzyme[10]. nd : not determined.

Among the studied enzymatic preparations [horse liver acetone powder (HLAP)[12a], pig liver acetone powder (PLAP)[12a], α-chymotrypsin[12a], porcine pancreatic lipase (PPL)[12a], lipase from *Candida cylindracea*[12b], lipase R10[12c], lipase from *Pseudomonas fluorescens*[12d], lipase from *Mucor miehei*[12e], lyophilized yeast[12f], dry acetone powders prepared from *Rhizopus arrhizus*, *Geotrichum candidum*, or *Mucor plumbeus* mycelium[8a]], only PLAP and HLAP were found to be efficient on acetates **11-14**. Acetates **15** and **16** suffered very slow hydrolysis, if any, and without any selectivity. As shown in table 1, variation of the organic cosolvent proved to have a weak influence on the value of the E coefficients, but a marked one upon the rate of the reaction, DMSO giving the best results. In view of these analytical results, we decided to upscale the resolution of acetates **11-14** using HLAP and PLAP with DMSO as the cosolvent.

HLAP or PLAP-catalyzed preparative resolution of alcohols 5-7:

Treatment of the racemic acetates **11-13** by PLAP or HLAP was done on the gram scale in a mixture of 0.1 M sodium phosphate buffer (pH 7.4) and DMSO (9 : 1 respectively) at 30°C[13]. Under these conditions the rate of enzymatic hydrolysis of the racemic acetate **14** was definitely too slow for the preparative point of view.

The racemic acetates were first hydrolyzed (about 40-60% hydrolysis) into a mixture of (*R*)-alcohols and (*S*)-acetates. These compounds were separated by flash chromatography. Assignment of the absolute configuration of the resulting alcohols was ascertained by comparison of their optical rotation with the literature data[1,3]. (*R*)-Alcohols **5** (96.5% ee) and **7** (94% ee) proved to be suitable for most synthetic purposes (Table 2).

Table 2: Gram Scale Enzymatic Hydrolysis of Racemic Acetates **11**, **12** and **13**.

			(*R*)-Alcohol[a]				(*S*)-Acetate[a]		
(±) Acetate	Enzyme	% Hydrolysis		Yield[b] %	ee % (observed)	ee % (simulated)[c]	Yield[b] %	ee % (observed)	ee % (simulated)[c]
11	HLAP	54	**5**	28	**96.5**	87	46	56[d]	98
12	PLAP	60	**6**	55	60	62	39	88[d]	99
13	HLAP	45	**7**	43	**94**	96	44	84	84

a: for $[\alpha]_D^{25}$, see note 14; b: isolated yields, calculated from total (±)-acetate; c: by calculation from the enantioselectivity coefficients previously determined (see table 1); d: the fact that some reprecipitation of racemic acetates **11** and **12** in the phosphate buffer-DMSO solution occurred concurrently with enzymatic hydrolysis could explain the difference between the simulated and observed ee%: the precipitated racemic acetates are not hydrolyzed and contaminate the resolved acetates during the work up.

As alcohol **6** was obtained in a lower ee (60%), this was reacetylated[13] and the resulting enantiomerically enriched acetate (*R*)-**12** was submitted again to an enzymatic hydrolysis (Table 3). This allowed the formation of (*R*)-alcohol **6** of high optical purity (96.5% ee). Conversely, (*S*)-acetates **11** (56% ee) and **12** (88% ee), unconsumed in the previous hydrolyses, were further submitted to enzymatic hydrolysis. This allowed the formation of materials of high optical purity : (*S*)-acetates **11** (92% ee) and **12** (98% ee); these esters afforded (*S*)-alcohols **5** and **6** upon alkaline hydrolysis, without any loss of optical purity. Using such a procedure, the overall yields of enantiomeric alkohols **5**, **6** and **7** (above 92% ee) were respectively 68, 62 and 65%.

Table 3: Enzymatic Hydrolysis of Enantiomerically Enriched Acetates **11** and **12**.

			Alcohol[a]			(*S*)-Acetate[a]		
Acetate (ee%)	Enzyme	Hydrolysis %	Yield[b] %	ee % (observed)	ee % (simulated)[c]	Yield[b] %	ee % (observed)	ee % (simulated)[c]
(*S*)-**11** (56)	HLAP	8	(*R*)-**5** 6	**93**	94	78	**92**	98
(*S*)-**12** (88)	PLAP	20	(*S*)-**6** 15	69.5	74	61	**98**	98
(*R*)-**12** (60)	PLAP	77.5	(*R*)-**6** 69	**96.5**	96	17	46	10

a: for $[\alpha]_D^{25}$, see note 14; b: isolated yields, calculated from the total enriched acetate used in these reactions; c: by calculation from the enantioselectivity coefficients previously determined (see table 1)

Conclusion:

Esterase activities cheaply available in commercial pig liver acetone powder (PLAP) and horse liver acetone powder (HLAP) were found to be effective for the preparation of both enantiomers of several hindered alcohols. Among them, 2,2-diphenylcyclopentanol **5**, which bears a quaternary carbon center in the α-position to the hydroxyl function, is an efficient chiral auxiliary for the asymmetric hydrogenation of stereogenic β-acetamidocrotonates. To our knowledge, it is one of the few reported examples[15] of an efficient enzymatic resolution of a secondary alcohol having a quaternary carbon center in the α-position. Moreover, although PLAP has been demonstrated to be efficient in two related cases[16], here we report on the use of HLAP in such a process for the first time. Compared to the conventional chemical ways for the resolution of racemic alcohols, the present method exhibits outstanding advantages: easier procedure, better chemical yield and lower cost.

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7. **11** : (92 %); **Mp**: 76-77°C; **IR** : 1728 cm^{-1}; **^{1}H-NMR** (250 MHz, $CDCl_3$), δ ppm: 1.75 (s, 3H) 1.75-2.3 (m, 4H), 2.55 (m, 2H), 6.06 (m, 1H), 7.1-7.35 (m, 10H); **^{13}C-NMR** (63 MHz, $CDCl_3$), δ ppm: 20.41, 21.03, 30.53, 35.02, 59.24, 79.56, 125.73, 126.08, 126.55, 127.79, 127.87, 128.34, 144.92, 145. 35, 170.49.

   12 : (91 %); **Mp**: 75-76°C; **IR** : 1728 cm^{-1}; **^{1}H-NMR** (250 MHz, $CDCl_3$), δ ppm: 1.18 (d, J= 6 Hz, 3H), 1.82 (s, 3H), 4.0 (d, J= 9.5 Hz, 1H), 5.72 (dq, J= 9.5 and 6 Hz, 1H), 7.15-7.6 (m, 10H); **^{13}C-NMR** (63 MHz, $CDCl_3$), δ ppm: 19.18, 20.95, 57.50, 71.81, 126.36, 126.66, 128.06, 128.21, 128.57, 141.49, 170.39.

   13 : (89 %); oil; **IR:** 1740 cm^{-1}; **^{1}H-NMR** (250 MHz, $CDCl_3$), δ ppm: 0.86 (t, J= 7.5 Hz, 3H), 1.50 (m, 2H), 1.80 (s, 3H), 4.06 (d, J= 9.5 Hz, 1H), 5.68 (ddd, J= 4, 7.5 and 9.5 Hz, 1H), 7.1-7.42 (m,

10H); **^{13}C-NMR** (63 MHz, $CDCl_3$), δ ppm: 20.56, 25.85, 55.76, 75.95, 126.27, 126.51, 128.03, 128.11, 128.48, 141.35, 170.33.

14 : (90 %); **Mp**: 58-59°C; **IR**: 1735 cm^{-1}; **^{1}H-NMR** (250 MHz, $CDCl_3$), δ ppm: 0.88 and 0.90 (2d, J= 6 Hz, 6H), 1.76 (s, 3H), 1.82 (m, 1H), 4.17 (d, J= 10 Hz, 1H), 5.68 (dd, J=10 and 3.5 Hz, 1H), 7.1-7.35 (m,, 10H); **^{13}C-NMR** (63 MHz, $CDCl_3$), δ ppm: 15.47, 20.02, 20.48, 29.30, 54.16, 78.72, 126.36, 126.56, 128.11, 128.39, 128.56, 141.35, 170.39.

15 : (87 %); **Mp**: 130°C; **IR**: 1739 cm^{-1}; **^{1}H-NMR** (250 MHz, $CDCl_3$), δ ppm: 0.84 (s, 9H), 1.57 (s, 3H), 4.25 (d, J= 8.5 Hz, 1H), 5.59 (d, J= 8.5 Hz, 1H), 7.1-7.45 (m, 10H); **^{13}C-NMR** (63 MHz, $CDCl_3$), δ ppm: 20.22, 26.71, 35.76, 41.73, 53.74, 80.90, 125.96, 126.39, 127.83, 128.07, 128.25, 128.42, 128.71, 142.13, 142.62, 170.28.

16 : (84 %); **Mp**: 155°C.; **IR**: 1722 cm^{-1}; **^{1}H–NMR** (250 MHz, $CDCl_3$), δ ppm : 1.83 (s, 3H), 4.41 (d, J= 10 Hz, 1H), 6.49 (d, J= 10 Hz, 1H), 7.0-7.35 (m, 15H); **^{13}C-NMR** (63 MHz, $CDCl_3$), δ ppm : 20.80, 57.38, 77.00, 126.42, 127.31, 127.72, 127.91, 128.15, 128.24, 128.48, 140.37, 141.13, 170.1.

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10. **Typical analytical procedures** : Kinetics were made with five points. Enzymatic hydrolyses: for each cosolvent (DMSO, hexane, dichloromethane, ethyl ether, methanol or acetone), five identical tubes were prepared with a solution of the racemic acetate in the cosolvent (2mg/100µL) which was poured into 0.1M phosphate buffer (900µL) containing the enzyme (10-20 mg). The tubes were vigourously shaken at 30°C. At intervals, the complete mixture from one tube was extracted with diethylether and analyzed Enzymatic acylations: 30-60 mg of enzyme were added to a solution of racemic alcohol in hexane (2mg/1ml), followed by 3 equivalents of butyric, valeric or hexanoic anhydride, isopropenyl acetate, or vinyl acetate. The suspension was stirred at 30°C. At intervals, a 100 µL-aliquot was taken and analyzed.

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Table 4: GC Retention Times of Diastereomeric Esters Derived from (S)-Acetyllactic Acid

Alcohol	Column / Temperature	**retention time** (min) of the diastereomeric acetyllactyl esters (*Abs. conf.of alcohols*)		R_S [a]	α [b]
5	DBwax 30 [c] / 230°C	17.21 (*R*)	18.44 (*S*)	3.9	1.08
6	DBwax 30 [c] / 220°C	11.05 (*R*)	11.81 (*S*)	5.2	1.08
7	DBwax 30 [c] / 220°C	11.87 (*R*)	12.58 (*S*)	5.67	1.06
8	DBwax 30 [c] / 140°C to 220°C (2°C/min)	41.23 (*R*)	41.49 (*S*)	1.68	1.01
9	BP 10 [d] / 220°C	19.37 [e]	19.67 [e]	2.0	1.02
10	BP 10 [d] / 240°C	24.91 [e]	25.35 [e]	1.95	1.02

a: resolution factor; b: separation factor; c: (30m x 0.25mm) capillary column on Shimadzu GC.8A, carrier gas: He (1 bar). d: (20m x 0.25mm) capillary column on Varian 3700, carrier gas: He (1 bar); e: absolute configuration not determined.

12. Purchased from **a:** Sigma; **b:** Meito-Sangyo (lipase My); **c:** Amano; **d:** Fluka; **e:** Novo (lipozyme); **f:** Alsa (Briochin).

13. **Typical procedure for preparative resolution:** racemic acetate **12** (1g) dissolved in DMSO (9mL) was poured under vigorous stirring into 0.1 M sodium phosphate buffer (81 mL, pH=7.4) containing PLAP (2g). The pH of the resulting suspension was maintained at pH 7 (Radiometer pHstat) by monitored addition of 0.1 M aqueous sodium hydroxide. The suspension was stirred at 30°C until 60% conversion to alcohol (5 days). After extraction with dichloromethane, centrifugation and concentration, the crude was purified by silica gel column chromatography (dichloromethane/ethyl acetate, 95 : 5). The ee of the alcohol **6** was determined by GC analysis after derivatization with (*S*)-O-acetyllactic acid chloride[9]. The residual acetate was treated with 1 equivalent of sodium methoxide in methanol and the ee of the obtained alcohol was analyzed by the same method. The enantiomerically enriched (*S*)-acetate **12** (88% ee) (354 mg), dissolved in 6mL of DMSO, was submitted again to PLAP (700 mg)-catalyzed hydrolysis (20%, 6 days) in 0.1M sodium phosphate buffer (54 mL) to give (*R*)-alcohol **6** (69.5% ee) and (*S*)-acetate **12** (98% ee). The previously obtained (*R*)-alcohol **6** (60% ee) was acetylated. The resulting acetate, dissolved in DMSO (6mL), was submitted to PLAP (700mg)-catalyzed hydrolysis (77.5%, 19 h.) in 0.1M sodium phosphate buffer (54 mL) yielding (*R*)-alcohol **6** (96.5% ee) and (*S*)-acetate **12** (46% ee).

14. $[\alpha]_D^{25}$ of the resolved acetates and alcohols after enzymatic hydrolysis :

Compound, ee (%)	$[\alpha]_D^{25}$ (c, solvent)	Compound, ee (%)	$[\alpha]_D^{25}$ (c, solvent)
(*R*)-**5** 96.5	– 104.9 (1.1, EtOH)	(*R*)-**5** 93	– 78.1 (1.15, EtOH)
(*R*)-**6** 60	+ 29.1 (3.0, MeOH)	(*R*)-**6** 96.5	+ 44.0 (0.55, MeOH)
(*R*)-**7** 94	+ 35.4 (3.2, MeOH)	(*S*)-**6** 69.5	– 27.9 (2.54, MeOH)
(*S*)-**11** 56	+ 97.9 (0.62, EtOH)	(*S*)-**11** 92	+ 121.8 (0.62, EtOH)
(*S*)-**12** 88	– 41.3 (3.27, MeOH)	(*S*)-**12** 46	– 20.5 (3.27, MeOH)
(*S*)-**13** 84	– 9.5 (3.25, MeOH)	(*S*)-**12** 98	– 50.7 (3.25, MeOH)

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