Resolution of *N*-Protected Amino Alcohols by Porcine Pancreatic Lipase

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Abstract: The resolution of 2-amino alcohols protected by urethane-type groups either *via* porcine pancreatic lipase (PPL) hydrolysis of the corresponding racemic acetates or *via* PPL catalyzed transesterification of racemic alcohols was studied. In both cases, Boc protecting group led to better chemical yields and enantiopurities than Z and Fmoc protecting groups. Furthermore, a simple and efficient method for the synthesis of the medicinally interesting optically pure (*R*)-2-aminohexadecanol was developed.

Keywords: 2-Amino alcohols, enzymatic hydrolysis, enzymatic resolution, porcine pancreatic lipase, transesterification.

INTRODUCTION

Optically pure 2-amino alcohols are versatile intermediates for the synthesis of peptaibiotics and peptaibols [1, 2], as well as for the synthesis of a variety of optically active intermediates, for example diamines and triamines [3-5]. Their conversion to amino aldehydes and peptide aldehydes [6] is of particular interest, because it may lead to bioactive compounds [7, 8] and non-natural amino acids and their bioisosteric analogues [9-12]. In addition, saturated and unsaturated long chain 2-amino alcohols, which may be considered as sphingosine analogues, have been reported to display various biological activities [13], for example cytotoxic activity against various cancer cell lines [14], *in vivo* antiinflammatory activity [15, 16] and immunosuppresant activity [17]. (*R*)-2-Aminohexadecanol displays activity comparable to 2-amino-2-[2-(4-octylphenyl)ethyl]-1,3-propanediol hydrochloride (FTY720) [18], which is a novel synthetic immunosuppressant with remarkable activity both *in vitro* and *in vivo*. (*R*)-2-Aminohexadecanol has been reported to be more potent than the (*S*)-isomer [17].

N-Protected amino alcohols can be prepared from the corresponding chiral amino acids, by a variety of methods, for example by reduction of mixed anhydrides [19, 20], acyl fluorides [21] or pentafluorophenyl esters [22]. Enzymes nowadays are widely recognized among the most active and selective catalysts for the preparation of optically active compounds such as *N*-protected amino alcohols [23], even though there are reports for the kinetic resolution using non-enzymatic asymmetric acylations [24, 25]. In the present study our aim was to investigate the resolution of 2-amino alcohols protected by urethane-type groups using the inexpensive and commercially available porcine pancreatic lipase (PPL). We demonstrate that (*R*)-amino alcohols may be produced by the resolution of racemic amino alcohols.

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RESULTS AND DISCUSSION

Resolution of racemic alcohols may be accomplished either by enzymatic hydrolysis of racemic acylated alcohols or by enzymatic transesterification of racemic alcohols. In the present work we studied the efficiency of PPL (EC 3.1.1.3) Type II from Sigma, using racemic *N*-protected 2-amino alcohols and the corresponding acetates as substrates.

Racemic *N*-protected 2-amino alcohols **1a-f** were prepared by reduction of the corresponding amino acids as described in literature [19], and were acetylated by treatment with Ac_2O in the presence of DMAP (Table **1**). Three urethane-type protecting groups, namely *tert*-butoxycarbonyl (Boc), benzyloxycarbonyl (Z) and fluorenylmethoxy-carbonyl (Fmoc), widely used in peptide chemistry, were used in the present study. The PPL mediated hydrolysis of acetates **2a-f** was performed with a pH-stat apparatus. The reaction took place in a two-phase system of phosphate buffer pH 7.0 and a 1:1 mixture of toluene/hexane at 37 ºC for 3 h. The configuration of the products was determined comparing their specific rotation values with those existing in the literature. The enantiomeric purity of the amino alcohols produced was measured either by chiral HPLC analysis or by NMR analysis of the corresponding Mosher esters. We have previously shown [21] that an amino alcohol can be esterified with (*R*)-(+)-α-methoxy-α-trifluoromethyl phenyl acetic acid (Mosher acid) [26] using the DCC/DMAP method. The absence of diastereomeric signals in the ^1H and ^{19}F NMR spectra of the Mosher ester indicates >95% ee. Table **1** summarizes the data of products **3a-f**.

As shown in Table **1**, PPL hydrolyzed the acetate derivatives of *N*-protected phenylalaninol **2a-c** producing the (*R*)-enantiomer in good to high yield and in excellent

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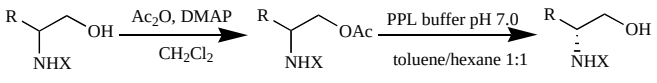
Table 1 Data for the PPL Catalyzed Enantiomer Selective Hydrolysis of Acetates



1a-f **2a-f** **3a-f**

1-3	R	X	Hydrolysis Yield[a] (%)	$[\alpha]_D$ observed	$[\alpha]_D$ lit[b]	% ee
a	$C_6H_5CH_2$	Boc	42	+22.0[c]	-23.5[c] [21]	>95%[d]
b	$C_6H_5CH_2$	Z	35	+38.0[e]	-42.5[e] [27]	95[f]
c	$C_6H_5CH_2$	Fmoc	32	+23.0[c]	-23.2[c] [28]	99[f]
d	$(CH_3)_2CHCH_2$	Boc	26	+23.0[e]	-28.0[e] [29]	75[f]
e	$(CH_3)_2CHCH_2$	Z	32	+12.8[c]	-28.0[g] [30]	50[f]
f	$nC_{14}H_{29}$	Boc	40	+10.7[c]	-8.5[h] [31]	>95%[d]

[a]After column chromatography. [b]Specific rotation of the enantiomer. [c](*c* 1.0, $CHCl_3$). [d]Enantiomeric excess was determined by NMR analysis of the corresponding Mosher esters.[e](*c* 1.0, CH_3OH). [f]Enantiomeric excess was determined by chiral HPLC analysis [Chiralpak® AS-H column, 2-propanol (5-20%), acetic acid (0.4%), in hexane mobile phase, flow rate 0.8 ml/min]. [g](*c* 1.0, EtOH). [h](*c* 2.0, $CHCl_3$).

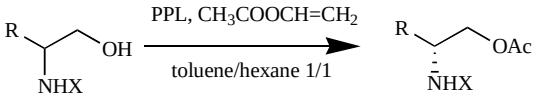
enantioselectivity. PPL catalyzed hydrolysis of Boc and Z acetate derivatives of leucinol **2d**,**e** occurred in considerably lower enantioselectivity compared to the corresponding phenylalaninol derivatives, clearly showing the importance of the side chain of the 2-amino alcohol used for the reaction. Furthermore, the acetate derivative of 2-amino hexadecanol **2f** was tested. We demonstrate that lipase catalysed resolution of **2f** is an interesting route for the synthesis of optically pure (*R*)-2-aminohexadecanol.

We also studied the lipase-catalyzed transesterification of racemic *N*-protected 2-amino alcohols **1a-f** using PPL and vinyl acetate as acylating agent in a 1:1 mixture of toluene/hexane at room temperature for 48 h (Table **2**). The absolute stereochemistry of acetates **4a**, **d**, **f**, was confirmed by comparison of the specific rotation with optically pure acetates that were synthesized by chemical methods. The PPL catalyzed transesterification reaction yielded the (*R*)-enantiomers. The detailed data for products **4a-f** are presented in Table **2**.

As shown in Table **2**, the *N*-protecting group was found to significantly influence the enzymatic transesterification. Higher enantioselectivities were observed when Boc group was used as protecting group in substrates **1a**, **d**, **f**. The lipase catalyzed transesterification of Z and Fmoc derivatives of phenylalaninol and leucinol **1b**,**c**,**e** proceeded in low yields and moderate enantioselectivities, indicating that the aromatic rings of Z and Fmoc groups obstruct the interaction with the active site of the enzyme.

In conclusion, we studied the resolution of *N*-protected 2-amino alcohols either *via* PPL hydrolysis of the corresponding racemic acetyl derivatives or *via* PPL catalyzed transesterification of racemic alcohols. In both cases the presence of Boc group led to better chemical yields and enantiopurities than Z and Fmoc groups. The Boc group gave the best chemical yields (**3a** and **3f**) in the case of the enzymatic hydrolysis with an ee >95%, as well as in the enzymetic transesterification reaction with chemical yield of 24% and an ee of 97% for acetate **4a**. Comparing PPL

Table 2. Data for the PPL Catalyzed Transesterification Products



1a-f **4a-f**

4	R	X	Yield[a] (%)	$[\alpha]_D$ observed	$[\alpha]_D$[b]	% ee[c]
a	$C_6H_5CH_2$	Boc	24	+14.9[d]	+14.5[d]	97
b	$C_6H_5CH_2$	Z	10	+12.0[e]		74
c	$C_6H_5CH_2$	Fmoc	11	+10.4[e]		56
d	$(CH_3)_2CHCH_2$	Boc	28	+31.2[e]	-37.5[e]	n.d.[f]
e	$(CH_3)_2CHCH_2$	Z	10	+21.3[e]		63
f	$nC_{14}H_{29}$	Boc	15	+14.9[e]	-12.3[e]	n.d.[f]

[a]After column chromatography. [b]Specific rotation of products of the same or opposite stereochemistry prepared by chemical methods. [c]Enantiomeric excess were determined by chiral HPLC analysis [Chiralpak® AS-H column, 2-propanol (5-20%), in hexane mobile phase, flow rate 0.5 ml/min]. [d](*c* 1.0, CH_3OH). [e](*c* 1.0, $CHCl_3$). [f]Not determined.

catalyzed hydrolysis and transesterification, significant improvements in reaction rates and enantioselectivities in the case of PPL hydrolysis of acetates were observed. Moreover, a simple and efficient method to prepare the medicinally interesting optically pure (*R*)-2-aminohexadecanol was developed.

MATERIALS AND METHODS

Melting points are uncorrected. Specific rotations were measured on a Perkin Elmer 841 polarimeter using a 10 cm cell. ^{1}H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$ using a Varian 200 MHz spectrometer. Chemical shifts are reported in δ units relative to tetramethylsilane (TMS) set at 0 δ, and coupling constants are given in Hz. Porcine pancreatic lipase (PPL) was purchased from Sigma (type II, crude) and was used without further purification. All other chemical reagents were purchased from Sigma-Aldrich Chemicals C. and were of analytical grade. TLC plates (silica gel 60 F_{254}) and silica gel 60 (230-400 mesh) for column chromatography were purchased from Merck. Visualization of spots was effected with UV light and/or phosphomolybdic acid and/or ninhydrin, both in EtOH stain.

General Procedure for the Enzymatic Hydrolysis of the Acetyl Derivatives

In a pH-stat apparatus, acetyl derivative **2a-f** (100 mg) was added to a 50:50 mixture of hexane and toluene (3 mL). Then, 7 mL of phosphate buffer 100 mM pH 7.0 were added. Finally, PPL (25 mg) was added and the hydrolysis was monitored at the pH-stat. After 3 hours the reaction was stopped and ethyl acetate (20 mL) and H_2O (20 mL) were added. The aqueous phase was washed twice with ethyl acetate (2 × 20 mL). The combined organic phases were washed with brine, dried, the solvent was evaporated and the product was purified by column chromatography using petroleum ether/EtOAc 8:2 as eluent. Physical constants and spectroscopic data of **3a-f** are in accordance with literature.

General Procedure for the Enzymatic Transesterification of Racemic Alcohols

Alcohol **1a-f** (100 mg) was dissolved in a 50:50 mixture of hexane and toluene (3-4 mL, 0.1 M). Then, vinyl acetate (184 μL, 2 mmol) and finally PPL (60 mg) were added. The mixture was left under stirring for 2 days at room temperature. Filtration of the enzyme and purification by column chromatography using petroleum ether/EtOAc 8:2 as eluent yielded the pure acetyl derivatives.

(R)-2-(tert-Butoxycarbonylamino)-3-phenylpropyl acetate (4a)

White solid; mp 68-69 ºC; ^{1}H NMR ($CDCl_3$) δ 7.32-7.15 (5H, m, arom. CH), 4.63 (1H, m, OCONH), 4.10-4.00 (3H, m, CH, CH_2OCO), 2.82 (2H, m, $CH_2C_6H_5$), 2.08 (3H, s, $OCOCH_3$), 1.40 [9H, s, $C(CH_3)_3$]; ^{13}C NMR ($CDCl_3$) δ 170.8, 155.1, 137.1, 129.2, 128.5, 126.5, 79.4, 65.0, 50.5, 37.8, 28.2, 20.8. Anal. calcd for $C_{16}H_{23}NO_4$: C, 65.51; H, 7.90; N, 4.77. Found: C, 65.27; H, 8.07; N, 4.86.

2-(Benzyloxycarbonylamino)-3-phenylpropyl acetate (4b)

White solid; mp 69-71 ºC; ^{1}H NMR ($CDCl_3$) δ 7.38-7.17 (10H, m, arom. CH), 5.09 (2H, s, $OCH_2C_6H_5$), 4.99 (1H, m, OCONH), 4.16-4.00 (3H, m, CH, CH_2OCO), 2.86 (2H, m, $CH_2C_6H_5$), 2.06 (3H, s, $OCOCH_3$); ^{13}C NMR ($CDCl_3$) δ 170.8, 155.7, 136.8, 136.1, 129.1, 128.6, 128.4, 128.1, 128.0, 126.7, 66.7, 64.8, 51.1, 37.7, 20.7. Anal. calcd for $C_{19}H_{21}NO_4$: C, 69.71; H, 6.47; N, 4.28. Found: C, 69.57; H, 6.58; N, 4.39.

2-(((9H-Fluoren-9yl)methoxy)carbonylamino)-3-phenyl-propyl acetate (4c)

White solid; mp 125-127 ºC; ^{1}H NMR ($CDCl_3$) δ 7.72 (2H, m, arom. CH), 7.51 (2H, m, arom. CH), 7.42-7.00 (9H, m, arom. CH), 4.92 (1H, d, J = 8.0 Hz, OCONH), 4.70-4.52 (3H, m, OCH_2CH), 4.20-4.00 (3H, m, CH, CH_2OCO), 2.82 (2H, d, J = 6.6 Hz, $CH_2C_6H_5$), 2.02 (3H, s, $OCOCH_3$); ^{13}C NMR ($CDCl_3$) δ 170.9, 156.4, 143.8, 141.3, 137.5, 129.2, 128.6, 127.7, 127.0, 126.6, 124.4, 119.9, 66.6, 63.8, 54.0, 47.2, 37.8, 20.8. Anal. calcd for $C_{26}H_{25}NO_4$: C, 75.16; H, 6.06; N, 3.37. Found: C, 74.88; H, 6.25; N, 3.44.

(R)-2-(tert-Butoxycarbonylamino)-4-methylpentyl acetate (4d)

White solid; mp 45-46.5 ºC; ^{1}H NMR ($CDCl_3$) δ 4.51 (1H, m, NH), 4.03 (2H, m, CH_2OCO), 3.92 (1H, m, CHNH), 2.12 (3H, s, $OCOCH_3$), 1.71 [1H, m, $CH(CH_3)_2$], 1.40 [9H, s, $C(CH_3)_3$], 1.34 (2H, m, CH_2CH), 0.91 [6H, d, J = 7.0 Hz, $CH(CH_3)_2$]; ^{13}C NMR ($CDCl_3$) δ 171.0, 155.3, 79.0, 66.7, 47.7, 40.9, 28.3, 24.7, 22.1. Anal. calcd for $C_{13}H_{25}NO_4$: C, 60.21; H, 9.72; N, 5.40. Found: C, 59.97; H, 9.88; N, 5.52.

2-(Benzyloxycarbonylamino)-4-methyl pentyl acetate (4e)

Oil; ^{1}H NMR ($CDCl_3$) δ 7.38-7.31 (5H, m, arom. CH), 5.11 (2H, m, $CH_2C_6H_5$), 4.71 (1H, d, J = 7.8 Hz, OCONH), 4.10-4.00 (3H, m, CH, CH_2OCO), 2.04 (3H, s, $OCOCH_3$), 1.66 [1H, m, $CH(CH_3)_2$], 1.34 (2H, m, CH_2CH), 0.94 [6H, d, J = 6.8 Hz, $CH(CH_3)_2$]; ^{13}C NMR ($CDCl_3$) δ 171.0, 155.1, 140.9, 128.5, 128.0, 127.4, 66.7, 66.5, 48.4, 40.8, 24.6, 23.0, 22.1, 20.8. Anal. calcd for $C_{16}H_{23}NO_4$: C, 65.51; H, 7.90; N, 4.77. Found: C, 65.32; H, 8.02; N, 4.81.

(R)-2-(tert-Butoxycarbonylamino)hexadecyl acetate (4f)

White solid; mp 47-48 ºC; ^{1}H NMR ($CDCl_3$) δ 4.50 (1H, m, OCONH), 4.06 (2H, m, CH_2OCO), 3.84 (1H, m, CH), 2.08 (3H, s, $OCOCH_3$), 1.45 [9H, s, $C(CH_3)_3$], 1.25 (26H, m, 13×CH_2), 0.88 (3H, t, J = 6.2 Hz, CH_3); ^{13}C NMR ($CDCl_3$) δ 171.0, 155.4, 79.6, 66.3, 49.5, 31.9, 29.6, 29.5, 29.4, 29.3, 28.3, 25.8, 22.7, 20.9, 14.1. Anal. calcd for $C_{23}H_{45}NO_4$: C, 69.13; H, 11.35; N, 3.51. Found: C, 68.87; H, 11.49; N, 3.86.

General Procedure for the Chemical Acetylation of 2-Amino Alcohols

To a solution of alcohol **1a-f** (1 mmol) in dichloromethane (3 mL), acetic anhydride (110 μL, 1.2 mmol) and then 4-dimethylaminopyridine (183 mg, 1.5 mmol) were added. The solution was stirred at room temperature. After 30 min, the starting material was consumed and the solvent was evaporated. The reaction mixture was dissolved in ethyl acetate (20 mL) and was quenched with H_2O (20 mL). The aqueous phase was

washed once more with ethyl acetate (20 mL). The combined organic phases were washed with 5% $NaHCO_3$, brine, 10% citric acid, brine, were dried and the solvent was evaporated to yield the crude acetyl derivative. The product was purified by column chromatography using petroleum ether/EtOAc 8:2 as eluent. All acetyl products were identified by ^1H NMR and ^{13}C NMR spectrometry and their spectra were identical to those of the corresponding acetyl derivatives prepared by the enzymatic transesterification procedure.

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