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A Simple Total Synthesis of Both Enantiomers of γ-Amino-β-hydroxybutanoic Acid (GABOB) by Enzymatic Kinetic Resolution of Cyanohydrin Acetates.

Yang Lu[+], Christine Miet[+], Nicole Kunesch[+]* and J. Poisson
Laboratoire de Chimie des Substances Thérapeutiques Naturelles
Centre d'Études Pharmaceutiques, 92296 Châtenay-Malabry CEDEX, France
[+]CNRS-URA 1310

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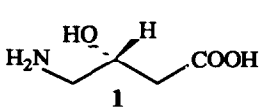
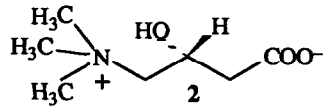
Abstract : Both (*R*)-and (*S*)-3-cyano-3-hydroxy-propionic acid ethyl ester have been obtained *via* enzymatic kinetic resolution of the racemic acetate using lipase from *Candida cylindracea* and lipase from porcine pancreas respectively. Their selective reduction affords the corresponding (*R*)- and (*S*)-GABOB with high optical purity.

(*R*)-4-Amino-3-hydroxybutanoic Acid (GABOB) **1** has been found to be a remarkable antiepileptic and hypotensive drug.[1] (*R*)-carnitine **2**, available by methylation of **1**[2], plays an important role in the transport of long-chain fatty acids through the membranes of mitochondria.[3] It is also an effective drug to improve myocardial function and to treat carnitine deficiency [4] and myopathic deficiencies.[5]

On the other hand, the enantiomeric (*S*)-carnitine **3** is a competitive inhibitor of (*R*)-carnitine acyl transferases.[6]



1



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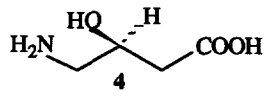

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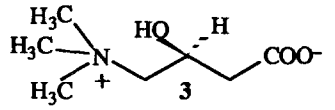


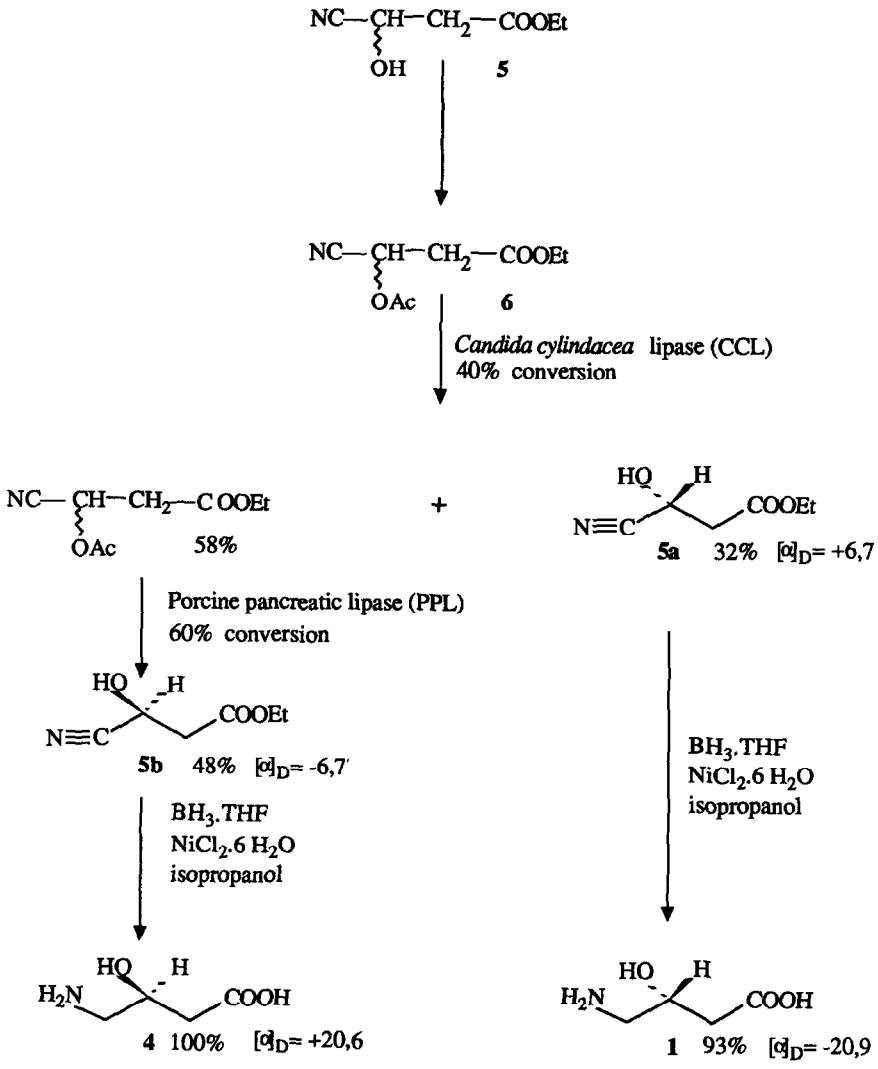
3

Several syntheses of enantiomerically pure (*R*)-GABOB **1** or (*S*)-GABOB **4** and either (*R*)- or (*S*)-carnitine **2** and **3** have been described. Most of these involve the formation of chiral salts and the separation of the resulting diastereoisomers.[7] The chiral template approach has provided several syntheses using chiral carbohydrates[8a] or other chiral precursors[8b-d] as starting point. Syntheses using either isolated enzymes or microorganisms have also been investigated.[9] More recently, the synthesis of both (*R*)- and (*S*)-GABOB using 2-hydroxy-1,2,2-triphenyl acetate as chiral auxiliary reagent has been reported.[10]

In this communication we report a simple, cheap and efficient three or four step synthesis of **1** and **4** starting from the cyanohydrin **5** as the key intermediate.

The addition of cyanides to a carbonyl group is a method of choice for the preparation of α-hydroxy carboxylic acids and 2-aminoalcools.[11] The enantioselective synthesis of cyanohydrins through chiral[12] or enzymatic[13] catalysis as well as through enantioselective transesterification of O-acetyl cyanohydrins[14] or lipase-catalyzed enantioselective esterification of racemic cyanohydrins[15] has been described.

SCHEME 1


NC—CH—CH2—COOEt
OH
5
NC—CH—CH2—COOEt
OAc
6
Candida cylindacea lipase (CCL)
40% conversion
NC—CH—CH2—COOEt
OAc
58%
+
HO H
N≡C COOEt
5a 32% [α]D= +6,7
Porcine pancreatic lipase (PPL)
60% conversion
HO H
N≡C COOEt
5b 48% [α]D= -6,7
BH3.THF
NiCl2.6 H2O
isopropanol
BH3.THF
NiCl2.6 H2O
isopropanol
HO H
H2N COOH
4 100% [α]D= +20,6
HO H
H2N COOH
1 93% [α]D= -20,9

Most asymmetric syntheses involving chiral and enzymatic catalysts provide good results only with simple aliphatic or aromatic aldehydes and lack general applicability. On the other hand, the direct resolution through the use of lipases and esterases[14] or the above mentioned oxynitrilases[13] involves buffer solutions which induce racemization *via* rapid cyanohydrine-aldehyde interconversions. More recently, the transesterification or esterification by lipases in organic solvents has been described.[16] Although these reactions proceed much slower than in aqueous medium[17], they show much greater enantiomeric selectivity.[16b,d]

In order to minimize the reversibility of the enzyme catalyzed transesterification or esterification, it is essential to design the substrates in such a way that the products formed will not take part in the reverse reaction.[16e,f] In the case of racemic cyanohydrins, the use of enol esters provides a good solution.[15,16f]

An alternate way is to choose an ester substrate furnishing a less reactive alcohol, such as sterically hindered secondary alcohols. Since the enol ester appoach[15] gave no satisfactory results when applied to the resolution of **5**, we investigated the transesterification of cyanohydrin acetate **6** in heptane in the presence of *n*-butanol.

Our synthetic strategy is based on selective enzymatic transesterification by the lipase from the yeast *Candida cylindracea* (CCL) of one of the enantiomers of an easily accessible starting material followed by a similar reaction of the remaining enantiomer by the lipase from porcine pancreas (PPL). (*R*)-GABOB and (*S*)-GABOB could be prepared in only three or four steps respectively after selective reduction by BH_3.THF under carefully chosen reaction conditions of the two enantiomeric cyanohydrins thus obtained.

Scheme 1 shows the synthetic pathway towards the enantiomeric cyanohydrins **5a** and **5b** starting from the racemic cyanohydrin **5**, which was synthesized in the following manner:ethyl-3,3-diethoxypropionate[18] provides quantitatively ethyl formylacetate after hydrolysis. Very mild reaction conditions like those used in enzymatic conversions of aldehydes into cyanohydrins had to be applied, particularly buffered solutions at pH = 5.4 in water/ethanol = 1/1.[13c] A 1N KCN/HOAc buffer[13d] gave ethyl 3-cyano-3-hydroxy-propionate[19] **5** within 15 minutes with a yield of 54%, while the reaction in a 1N NaOAc/HOAc buffer in water:ethanol = 1/1 with $(CH_3)_3SiCN$ was quantitative.

Acetylation of **5** (Ac_2O/pyridine) provided compound **6**, which developed rapidly into a substrate of choice, especially since the enantiomeric excess observed with lipase is generally higher with less polar, hydrophobic products.[20] Indeed, when the O-acetyl cyanohydrin **6** was treated with the lipase from *Candida cylindracea* (CCL) in heptane in the presence of *n*-butanol and the reaction stopped after about 40% overall conversion (monitored by GC analysis), optically active cyanohydrin **5a**[21] ($[\alpha]_D$ = +6.7 c=2, CH_2Cl_2) could be isolated in 32% yield along with (*S*)-enriched starting material ($[\alpha]_D$ = -32.0 c=1, CH_2Cl_2). Reduction of **5a**[22] in isopropanol with catalytic amounts of $NiCl_2.6H_2O$[23] led to the isolation of (*R*)-GABOB **1**, ($[\alpha]_D$ = -20.9 c=1.6, H_2O, literature[2]: $[\alpha]_D$ = -20.8) in 93% yield.

Finally the recovered (*S*)-enriched O-acetyl cyanohydrin was treated with the lipase from porcine pancreas (PPL), which hydrolyzes preferentially (*S*)-enantiomers while yeast lipase selectively catalyzes the cleavage of the (*R*)-enantiomer.[16e] When the reaction was interrupted after 60% conversion, the (*S*)-cyanohydrin **5b** ($[\alpha]_D$ = -6.7 c=1.9, CH_2Cl_2) was obtained in 48% yield. Its reduction under the above mentioned reaction conditions provided quantitatively (*S*)-GABOB **4** ($[\alpha]_D$ = + 20.6 c=1.9, H_2O, literature [8c]: $[\alpha]_D$ = +20.1).

Thus, two biologically important enantiomers can be prepared in a few steps from cheap, easily available starting material through sequential, judicious application of enzymes in organic solvents.

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19 **5**: calc. for $C_5H_9NO_3$: C % 50.15 H % 6.47 N % 9.68, found : C % 50.34 H % 6.34 N % 9.79; bp 102-106°C (1 mm); IR ($CHCl_3$) ν cm-1 : 3450 (OH, s), 2240 (CN, w), 1730 (ester, s); RMN^{1}H($CDCl_3$), 300MHz, δ ppm : 1.31 (t, 3p, J = 7 Hz, C<u>H</u>$_3$), 2.88 (d, 2p, J = 5.5 Hz, -C<u>H</u>$_2$-), 3.95 (bs, 1p, O<u>H</u>), 4.25 (q, 2p, J = 7 Hz, O-C<u>H</u>$_2$-CH$_3$), 4.83 (t, 1p, J = 5.5 Hz, -C<u>H</u>-OH); RMN^{13}C : ($CDCl_3$), 50.32 MHz, δ ppm : 169.3 (ester), 118.7 (CN), 61.5 (-CH-OH), 57.2 (O-CH_2), 39.4 (CH_2), 13.8 (CH_3).
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21 To a solution of 5g of **6** in 60 ml heptane/*n*-butyl alcohol = 5/1, 7,5 g of C.C.L. (Sigma) are added and the mixture is stirred at room temperature for 24 hours. After filtration, **5a** and **6** are separated by flash chromatography on silica.
22 To 858 mg (6 mmoles) of **5a** in 30 ml of isopropanol saturated with $NiCl_2.6H_2O$ are added 45 mmoles of BH_3.THF at 0°C during 15 minutes. After 2 hours at room temperature the reaction mixture is evaporated and the residue is dissolved in 30 ml of 6N HCl. After stirring for 1 hour the solution is concentrated to 10 ml and absorbed on Amberlite IR-120, H^+-form. Elution with 5% aqueous ammonia provides 655 mg (93%) of **1**, which was recrystallized from H_2O/MeOH, m.p. 213-215°C, literature[8d] : 213-214°C .
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