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A Simple Total Synthesis of Naturally Occurring Hydroxy-amino Acids by Enzymatic Kinetic Resolution

Yang Lu, Christine Miet, Nicole Kunesch*[1], and Jacques E. Poisson
Laboratoire de Chimie des Substances Thérapeutiques Naturelles
Centre d'Études Pharmaceutiques, 92296 Châtenay-Malabry CEDEX, FRANCE

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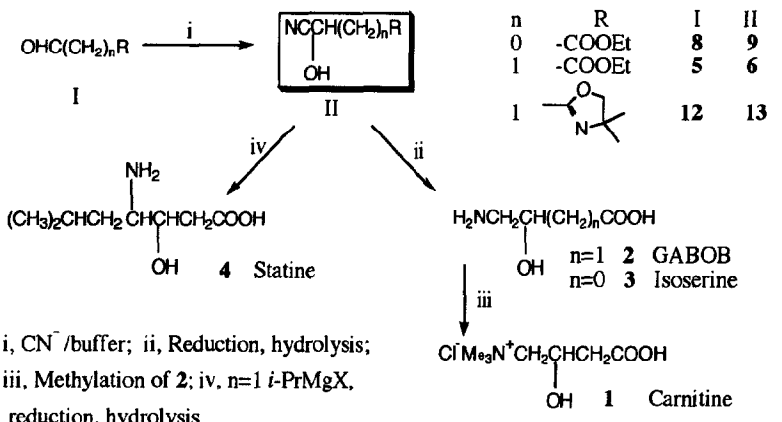
Abstract : Both optically pure enantiomers of GABOB and isoserine were obtained by enzymatic kinetic resolution of acetylated precursors in three or four steps. The key intermediates were cyanohydrins available from simple aldehydes. This procedure can be applied to other unusual hydroxy amino acids widely distributed in biologically important peptides.

A large number of biologically active natural compounds such as carnitine[2] **1**, GABOB[3] **2** - its chiral precursor -, isoserine[4] **3**, and statine[5] **4** possess a β-aminoalcohol functionality associated with a carboxylic group.

Among the many synthetic routes developed for their synthesis, none uses cyanohydrins, although they are interesting starting materials since they are easily transformed into β-hydroxyamines. More recently, a large number of methods was designed to afford optically active cyanohydrins. They constitute an attractive and general process to synthesize biologically active β-aminoalcohols starting from cyanohydrins as key intermediates and particularly to extend this method to compounds bearing an additional carboxylic group.

Thus, we conceived a simple and efficient chemical route to optically active carnitine **1**, GABOB **2** and isoserine **3** (in three or four steps) applicable to both enantiomeric forms starting from easily available aldehydes (scheme 1). This scheme could also be applied to the synthesis of statine **4**.



Scheme 1

The major practical difficulty was the selective reduction of the nitrile functionality in the presence of an ester group and the stereoselectivity of the reduction steps in the case of statine **4**. These reactions were performed in the racemic series before being applied to the optically active compounds.

(*R*)-4-Amino-3-hydroxybutanoic Acid (GABOB) **2a** has been found to be a remarkable antiepileptic and hypotensive drug.[6,7] (*R*)-carnitine **1a**, available by methylation of **2a** is a vitamin-like substance and it plays an important role in human metabolism and the transport of long-chain fatty acids into mitochondria.[8] Moreover, (*R*)-carnitine is an efficent drug to improve myocardial function[9,10] and to treat myopathic deficiencies.[11] It is

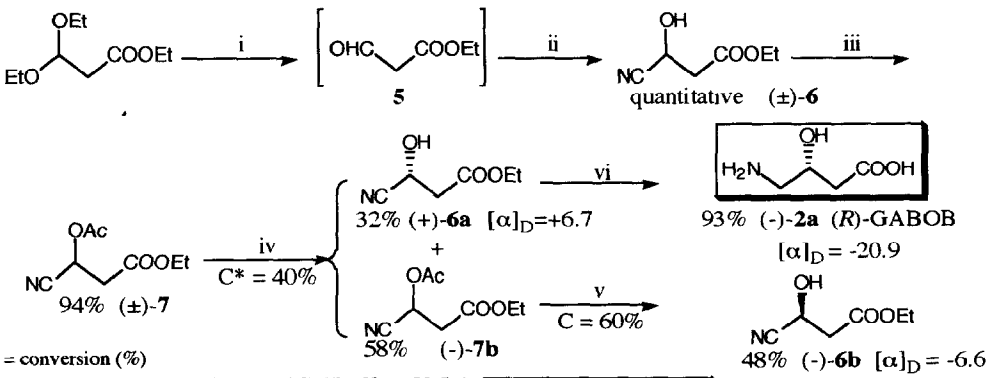
also used as a hypolipidemic agent in hemodialysis patients.[12] Furthermore, (*S*)-carnitine is a competitive inhibitor of (*R*)-carnitine acyl transferase.[13] Over the past decade, numerous preparations of both enantiomers of GABOB[14] **2** and carnitine **1** have been described.[15]

Our synthetic scheme starts from ethyl 3,3-diethoxypropionate[16], an easily available precursor of ethyl formylacetate **5**.

The preparation of chiral cyanohydrins starting from achiral aldehydes has been performed in 1890 by E. Fischer[17] with very low optical purity. The development of numerous methods was accomplished in the last years providing excellent enantiomeric excess. Optically active cyanohydrins could be obtained through chiral catalysts such as boryl compounds[18], titanium alkoxides[19], synthetic dipeptides[20] or oxynitrilases.[21] The latter provide good results, especially with aromatic aldehydes.

Alternatively, optically active cyanohydrins were conveniently prepared by stereoselective hydrolysis or transesterification of racemic cyanohydrins catalyzed by lipases.[22] Nevertheless, the employment of lipases in organic solvents[23] led to the recovery of optically active cyanohydrins susceptible to racemization in aqueous media. More recently, a kinetic resolution by lipases coupled with *in situ* formation and racemization of cyanohydrins allowed a quantitative synthesis of optically active cyanohydrins, starting mostly from aromatic aldehydes.[24]

Since our attempts to use oxynitrilases with ethyl formylacetate failed, we based our strategy on selective enzymatic transesterification of cyanohydrins by lipases.



Scheme 2

Scheme 2 shows the synthetic pathway towards the enantiomeric cyanohydrins **6a** and **6b** starting from the racemic cyanohydrin **6**, which was synthesized in the following manner : the hydrolysis of ethyl 3,3-diethoxypropionate provided quantitatively ethyl formylacetate **5**. 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.[21b] While a 1N KCN/HOAc buffer[21c] gave ethyl 3-cyano-3-hydroxy-propionate **6** within 15 minutes with a yield of 54%, the reaction in a 1N NaOAc/HOAc buffer in water:ethanol = 1/1 with $(CH_3)_3SiCN$ was quantitative.[25]

The transesterification or esterification by lipases in organic solvents has been amply described.[23,26] 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. In the case of racemic cyanohydrins, the use of enol esters provides a good solution.[26g,27] Since the enol ester approach gave no satisfactory results when applied to the resolution of **6**, we investigated the transesterification of cyanohydrin acetate **7** in heptane in the presence of *n*-butanol.

Acetylation of **6** (Ac_2O/pyridine) provided compound **7**, which developed rapidly into a substrate of choice. Indeed, when the racemic *O*-acetyl cyanohydrin **7** 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 NMR), the optically active cyanohydrin **6a** ($[\alpha]_D$ = +6.7, c=2, $CHCl_3$) could be isolated in 32% yield in addition to (*S*)-enriched starting material **7b** ($[\alpha]_D$ = -31.9, c=1, $CHCl_3$).

Finally, the recovered (*S*)-enriched *O*-acetyl cyanohydrin **7b** 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.[26f] When the reaction was interrupted after 60% conversion, the (*S*)-cyanohydrin **6b** ($[\alpha]_D$ = -6.6, c=2, $CHCl_3$) was obtained in 48% yield.

Selective reduction of **6a** to (*R*)-GABOB **2a** was then achieved after a careful study of the experimental conditions. The results after hydrolysis are summarized in Table 1. Reagents such as $LiAlH_4$, BH_3.THF reduced the ester function and should be avoided (Table 1, entry 1). The use of Raney nickel led to complex mixtures. The combination of $NaBH_4$ with $CoCl_2$[28], $NiCl_2$[29], $CuCl_2$[30] can reduce functional groups such as nitriles or amides which are inert to $NaBH_4$ alone. Indeed, the reduction of **6a** with $NaBH_4$ in the presence of $NiCl_2.H_2O$ in methanol followed by hydrolysis led to (*R*)-GABOB **2a** with 42% yield (Table 1, entries 2, 3) and 99% *ee* as calculated from the optical rotation ($[\alpha]_D$ = - 20.9, c = 1.7, H_2O; Lit.[2] $[\alpha]_D$ = - 21.1, c = 2.2, H_2O). While the combination of BH_3.THF with the same catalyst in THF afforded only a small amount of the desired compound (Table 1, entry 4), the same mixed system in more polar solvents such as methanol or isopropanol furnished good to quantitative yields (Table 1, entries 5,6,7). Thus the modification of the reactivity of diborane by adding an alcohol in addition to a specific catalyst accomplished a selective and mild reduction of the functional nitrile group of the cyanohydrin **6a**. Reduction of **6b** under the same conditions provided quantitatively (*S*)-GABOB **2b** ($[\alpha]_D$ = + 20.7, c = 1.9, H_2O; Lit.[14f] $[\alpha]_D$ = +20.1, c = 1.7, H_2O). It should be noted that this reagent lost its efficiency when applied to **7**, whose hydroxyl group is not free.

Table 1 : Reduction of cyanohydrin **6a** followed by hydrolysis into GABOB **2a**

Entry	Reagent	Catalyst	Solvent	Yield %
1	BH_3.THF (3 equivs)	none	THF	0
2	$NaBH_4$ (1.5 equivs)	$NiCl_2.6H_2O$ (0.5 equivs)	MeOH	42
3	$NaBH_4$ (2 equivs)	$NiCl_2.6H_2O$ (2 equivs)	MeOH	43
4	BH_3.THF (5 equivs)	$NiCl_2.6H_2O$ (1.5 equivs)	THF	15
5	BH_3.THF (4 equivs)	$NiCl_2.6H_2O$ (1.5 equivs)	MeOH	52
6	BH_3.THF (8 equivs)	$NiCl_2.6H_2O$ (1.5 equivs)	Isopropanol	74
7	BH_3.THF (8 equivs)	$NiCl_2.6H_2O$	Isopropanol*	quantitative

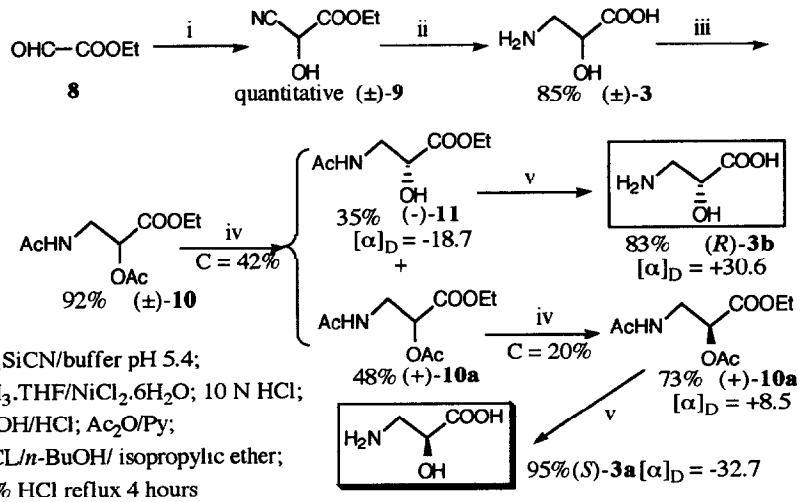
*saturated solution of $NiCl_2.6H_2O$

Having prepared chiral (*R*)- and (*S*)-GABOB, we turned our attention to the synthesis of (*R*)- and (*S*)-isoserine. Several syntheses of racemic[31] as well as (*S*)-[32] and (*R*)-isoserine[33] have been reported. In our procedure ethyl glyoxylate **8** was converted quantitatively into cyanohydrin **9** (ethyl 2-cyano-2-hydroxyacetate) by Me_3SiCN in a pH = 5.4 buffer similar to the preparation of ethyl 3-cyano-3-hydroxypropionate. The enzymatic resolution of cyanohydrin **9** or its *O*-acetyl derivative by several lipases was unsuccessful probably because of the similar size of the substituents attached to the stereocenter in agreement with the rules recently published by Kazlauskas.[34] Asymmetrically induced addition of HCN or KCN to ethyl glyoxylate by oxynitrilases was also uneffective.

Selective reduction of cyanohydrin **9** using BH_3.THF in the presence of $NiCl_2.6H_2O$ provided a three step synthesis of racemic isoserine **3** from ethyl glyoxylate (overall yield 85%, scheme 3). Since racemic isoserine possesses substantial differences in the size of the substituents attached to the stereocenter, the enantioselective transesterification of its diacetate **10** was undertaken.

In a preliminary communication[35] we reported the obtention of natural (*S*)-isoserine **3a**. This was achieved by enantioselective transesterification by *Candida cylindracea* lipase. As predicted by the above mentioned rules, this compound was obtained from the unreacted diacetate **10a** after 52% conversion.

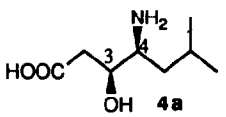
To obtain both enantiomers of isoserine **3**, the enzymatic transesterification with *Candida cylindracea* was stopped after 42% conversion (scheme 3). This led to the (*R*)-alcohol **11** which was hydrolyzed to provide pure (*R*)-isoserine **3b** (*ee* = 94% as calculated from the optical rotation, $[\alpha]_D$ = +30.6, c = 2, H_2O, Lit.[32b]: $[\alpha]_D$ = +32.5, c = 1, H_2O). The unreacted enriched diacetate **10a** was then submitted to another enzymatic transesterification which was stopped after 20% conversion. Hydrolysis of the unreacted diacetate **10a** furnished (*S*)-isoserine **3a** with an excellent optical purity (*ee* = 100%, $[\alpha]_D$ = - 32.7, c = 0.5, H_2O; Lit.[32c] $[\alpha]_D$ = - 32.2, c = 1, H_2O) and good chemical yield illustrating the versatility of tkinetic enzymatic resolution.



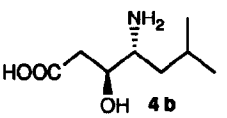
Scheme 3

Having achieved excellent results in the preparation of the above mentioned compounds, we turned our attention to the synthesis of more complex molecules.

Statine, (3*S*,4*S*)-4-amino-3-hydroxy-6-methylheptanoic acid **4a**, an unusual γ-aminoacid is a key component of pepstatin[5] and other synthetic inhibitors of aspartyl proteases such as renin[36], an important enzyme in the renin-angiotensin system. The isomer (3*S*,4*R*) **4b** was reported as a building block of didemnins[37], cyclodepsipeptides isolated from marine tunicates showing interesting antitumor and antiviral activity.



4a

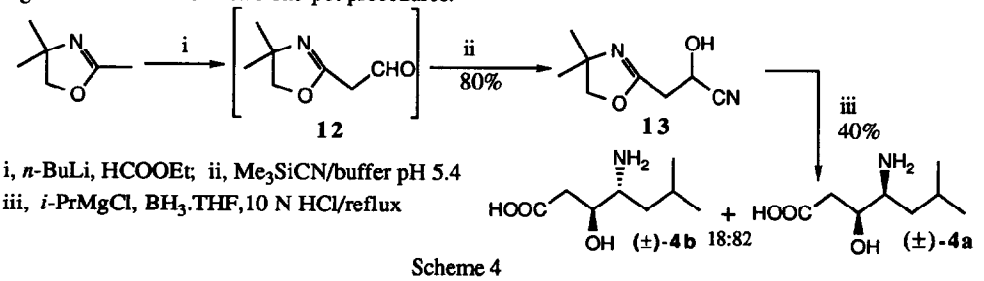


4b

Because of the increasing interest in a renin inhibitor for an antihypertensive agent, numerous syntheses of statine **4a** and its analogues have been published.[38] Most of them use a chiral precursor such as L-leucine or its derivatives having the desired chirality (*S*) at C-4. We chose a preparatively useful alternative to this approach, based on our previous work on optically active cyanohydrins. In our strategy, the first introduced chiral center would be fixed at C-3 (scheme 4), taking advantage of a cyanohydrin intermediate to provide the desired aminoalcohol.

Since Grignard reagents add smoothly to *O*-protected cyanohydrins and particularly to *O*-trimethylsilylcyanohydrins[21c,39], this reaction, followed by reduction of the intermediate imine[39a,40] constitutes an efficient synthesis of 2-aminoalcohols. In addition, a method of preparation of enantiomerically pure branched 2-aminoalcohols was described.[41] Racemic cyanohydrins were converted to diastereoisomeric chiral compounds by a chiral acetal protective group.[42] After separation they were subjected to addition of Grignard reagents followed by reduction *in situ* by lithium aluminium hydride.

In our case, preliminary attempts to add isopropylmagnesium halides to cyanohydrin **6** (scheme 1) presented no selectivity between the ester and nitrile functionalities toward Grignard reagents. It was therefore necessary to protect the ester function with the aid of the oxazoline protective group.[43] To avoid further reaction of the unstable aldehyde **12** (scheme 4), 2,4,4-trimethyl-2-oxazoline was treated in a one-pot synthesis with ethyl formate in the presence of *n*-BuLi and the reaction mixture poured into a NaOAc-AcOH water-EtOH 1:1 buffer pH 5-6. Reaction as before with Me_3SiCN afforded an excellent yield of racemic cyanohydrin **13**. Addition of an excess of isopropylmagnesium chloride to the unprotected cyanohydrin **13** followed by reduction of the intermediate imine by the above mentioned methods[39a,40] yielded a complex mixture, while, once more, reduction by diborane and hydrolysis *in situ* furnished a mixture of *threo* and *erythro* isomers of statine (±)-**4a** and (±)-**4b** respectively in a 82:18 ratio with 40% yield. We wish to underline the simplicity of our method leading to racemic statine in two one-pot procedures.



i, *n*-BuLi, HCOOEt; ii, Me_3SiCN/buffer pH 5.4
iii, *i*-PrMgCl, BH_3.THF, 10 N HCl/reflux

Scheme 4

The racemic cyanohydrin **13** thus obtained should be easily convertible into enantiomerically pure compound either through kinetic enzymatic resolution by the above mentioned techniques or by applying a more recently published procedure[41] to our substrate of choice.

Acknowledgment : The authors thank Hoechst-France for a generous gift of ethyl glyoxylate.

EXPERIMENTAL

Melting points were determined on a Kofler hot stage apparatus and are uncorrected. NMR (200 MHz) spectra were recorded using a Bruker AM 200 SY instrument. Electron impact (EI) and chemical ionisation (CI) mass spectra were recorded on Nermag/Sidar V2.3. IR spectra were determined on a Perkin-Elmer 682 spectrophotometer. Optical rotation data were taken with a Zeiss polarimeter for solutions in a 1 dm cell. Flash chromatography was carried out on Merck Kieselgel 60 (230-400 mesh). The lipase porcine pancreas (PPL, Type II) and *Candida cylindracea* (CCL, Type VII) were obtained from Sigma Chemical Co.

***(±)-Ethyl 3-cyano-3-hydroxypropionate* (6)** : To a solution of ethyl 3,3-diethoxypropionate[16] (13.3 g, 70 mmol) in CH_2Cl_2 (80 mL) trifluoroacetic acid (20 mL) and water (20 mL) were added. The solution was stirred for 2 hours at room temperature. The reaction mixture was poured into 1 L of a 1:1 ethanol/water solution buffered at pH 5.4 with sodium acetate/acetic acid. The pH was adjusted to 5.4 by sodium acetate. After addition of Me_3SiCN (12 mL, 90 mmol), the solution was stirred for 1 hour at room temperature, then neutralized by sodium hydrogen carbonate and extracted with dichloromethane. The organic phase was washed with brine, dried over sodium sulfate and the solvents were removed *in vacuo*. The residue was purified by flash chromatography on silica gel, using 7 : 3 cyclohexane / ethyl acetate as eluent, to yield the cyanohydrin **6** (8 g, 98%). I.R. (neat) 3400 (s), 2240 (w), 1708 (s), 1265 (m); ^1H NMR ($CDCl_3$): δ 1.30 (t, 3H, J = 7Hz), 2.88 (d, 2H, J = 5 Hz), 3.80 (s, 1H, broad), 4.28 (q, 2H, J = 7 Hz), 4.85 (s, 1H, J = 5 Hz); ^{13}C NMR ($CDCl_3$): δ 13.8, 39.3, 57.2, 61.5,118.7, 169.3; ms (I.E.) *m/z* (%) 116 (7, M^+-HCN), 98 (88), 88 (37), 71 (65), 55 (68), 43 (100); Anal. Calcd. for $C_6H_9O_3N$: $C_{50.34}$, $H_{6.34}$, $N_{9.79}$; Found: $C_{50.15}$, $H_{6.47}$, $N_{9.68}$.

***(±)-Ethyl O-Acetyl-3-cyano-3-hydroxypropionate* (7)** : To a solution of ethyl 3-cyano-3-hydroxypropionate **6** (1.3 g, 9.1 mmol) in pyridine (2 mL) acetic anhydride (2 mL) was added. The solution was stirred overnight at room temperature. The reaction mixture was quenched with water (20 mL), neutralized with a satured aqueous solution of sodium hydrogen carbonate, and then extracted with dichloromethane. The combined organic phases were washed with 1N HCl (50 mL) and brine (50 mL), dried over sodium sulfate and evapoated. The product was purified by flash chromatography on silica gel, using 8 : 2 cyclohexane / ethyl acetate as eluent, to yield the acetate **7** as colorless oil (1.57 g, 94%). I.R. (neat) 1730 (s), 1275 (m) cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.30 (t, 3H, J = 7.Hz), 2.15 (s, 3H), 2.97 (d, 2H, J = 5 Hz), 4.23 (q, 2H, J = 7 Hz), 5.57 (t, 1H, J = 7 Hz); ^{13}C NMR ($CDCl_3$): δ 13.6, 19.7, 36.4, 56.7, 61.2, 115.7, 167.0, 168.4; ms (IC, NH_3) *m/z*: 203 $(M+18)^+$, 186 $(M+1)^+$.

***(+)-Ethyl 3-cyano-3-hydroxypropionate* (6a); *(-)-Ethyl O-acetyl-3-cyano-3-hydroxypropionate* (7b)** : To a solution of (±)-ethyl *O*-acetyl-3-cyano-3-hydroxypropionate **7** (5 g, 27 mmol) in heptane (50 mL) were added *n*-butanol (10 mL) and CCL (7.5 g). The mixture was stirred at room temperature. After 24 hours, the reaction was stopped by filtering off the enzyme and washing the solid with heptane. The combined filtrates and washings were evaporated to dryness. By NMR spectroscopy the conversion of the transesterification was shown to be 39%. The residue was loaded into a 150 g silica gel column and the products were eluted from the column with a 10-30% gradient of ethyl acetate in cyclohexane. A clean separation of acetate and alcohol was thus obtained. The faster moving component was identified as (+)-ethyl *O*-acetyl-3-cyano-3-hydroxypropionate **7b** as a colorless oil (2.90 g, 58%) $[\alpha]_D$ -31.9 (c = 1, $CHCl_3$). The second component was a colorless oil identified as ethyl 3-cyano-3-hydroxypropionate **6a** (1.23 g,

32%) $[\alpha]_D$ +6.7 (c = 2, $CHCl_3$). **7b** was subjected to repeated transesterification as described above for **7** (1.57g, 8.5 mmol) $[\alpha]_D$ -31.9, *n*-butanol (4.8 mL), PPL (4.8 g), and heptane (14.5 mL). After 139 hours, 60% conversion was accomplished. Flash chromatography provided alcohol **6b** (591 mg, 48%) $[\alpha]_D$ -6.6 (c = 2, $CHCl_3$).

***(R)-GABOB* (2a)** : To a suspension of (+)-ethyl 3-cyano-3-hydroxypropionate **6a** (858 mg, 6 mmol $[\alpha]_D$ +6.7 in isopropanol saturated with $NiCl_2.6H_2O$ was added BH_3.THF (45 mL of a 1 M solution in THF) at 0°C during 15 minutes. After 2 hours at room temperature the reaction mixture was evaporated and the residue was dissolved in 6N HCl (30 mL). After stirring for 1 hour the solution was concentrated to 10 mL and adsorbed on Amberlite IR-120, H^+-form. Elution with water followed by 5% aqueous ammonia provided 655 mg (93%) of **2a**, which was recrystallized from H_2O/MeOH, m.p. 213-215°C, Lit.[14e] m.p. 213-214°C; $[\alpha]_D$ -20.9 (c = 1.7, H_2O), Lit.[2] $[\alpha]_D$ = - 21.1 (c = 2.2, H_2O); I.R. (KBr) 3130, 1575; ^1H NMR (D_2O): δ 2.23 (d, *J* = 7 Hz, 2H), 2.65-3.05 (m, 2H), 4.05 (m, 1H); ^{13}C NMR (D_2O): δ 42.8, 45.2, 66.4, 178.7; ms (I.E.) *m/z* (%) 101 (M^+-H_2O, 100), 83 (18), 73 (55), 44 (16); Anal. Calcd. for $C_4H_9O_3N$: $C_{44.33}$, $H_{7.62}$, $N_{11.76}$; Found $C_{40.25}$, $H_{7.68}$, $N_{11.69}$.

***(S)-GABOB* (2b)** : This compound was prepared as described for (*R*)-GABOB **2a** from **6b** $[\alpha]_D$ -6.6 (429 mg, 3 mmol). The product was quantitatively obtained as a cristal. mp 215-216°C, Lit.[14f] m.p. 212-214°C; $[\alpha]_D$ +20.7 (c = 1.9, H_2O), Lit.[14f] $[\alpha]_D$ = +20.1 (c = 1.7, H_2O).

***(±)-Ethyl 2-cyano-2-hydroxyacetate* (9)** : To a solution of acetate buffer (pH 5.4), prepared with 19.5 mL of a 0.2 M solution of sodium acetate, 30.5 mL of a 0.2 M solution of acetic acid and 50 mL of ethanol, were added freshly distilled ethyl glyoxylate[44] (1.02 g, 10 mmol) and trimethylsilyl cyanide (1.8 mL, 13.5 mmol). The reaction was performed over 10 minutes at room temperature. The solution was neutralized by the addition of a saturated aqueous solution of sodium hydrogen carbonate and extracted with dichloromethane. The organic layer was dried over sodium sulfate and the solvent evaporated under vacuum. The resulting residue was purified by flash chromatography with cyclohexane: ethyl acetate (8 : 2) as eluent to give quantitatively product **9** as a colorless oil (1.29 g). b.p. 125°C / 25 mmHg, 71-72°C / 0.5 mmHg; I.R. (neat) 3500, 2250, 1750, 1210 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.43 (t, 3H, *J* = 7.5Hz), 3.20 (s, 1H), 4.40 (q, 2H, *J* = 7.5 Hz), 4.75 (s, 1H); ^{13}C NMR ($CDCl_3$): δ 13.3, 59.8, 63.5, 115.1, 165.5; Anal. Calcd. for $C_5H_7O_3N$: $C_{46.51}$, $H_{5.47}$, $N_{10.85}$; Found: $C_{46.30}$, $H_{5.65}$, $N_{10.60}$.

***(±)-Isoserine* (3)** : To a suspension of $NiCl_2.6H_2O$ (5 g, 21 mmol) in THF (35 mL) and ethyl 2-cyano-2-hydroxyacetate **9** (4.3 g, 33.3 mmol) was slowly added BH_3.THF (135 mL of a 1 M solution in THF, 135 mmol) at 0°C. The mixture was stirred for 1.5 hours at room temperature. 10 mL of methanol was added. After removal of the solvents, the residue was dissolved in 10 N HCl (35 mL), stirred overnight at room temperature and then evaporated under vacuum. The residue was purified by ion-exchange chromatography on Amberlite IR-120H (H^+ form), eluting with water followed by 5% NH_4OH to yield (±)-isoserine **3** as white solid (3.0 g, 86%) which could be crystallized from water : methanol 1 : 1; m.p. 245-247°C; I.R. (KBr) 3070 (s), 1580 (s); ^1H NMR (D_2O): δ 2.60-3.15 (m, 2H), 4.08 (dd, 1H, *J* = 3.5 Hz, 6 Hz); ^{13}C NMR (D_2O): δ 43.8, 69.5, 177.7; ms (IC, NH_3) *m/z*: 106 $(M+1)^+$; Anal. Calcd. for $C_3H_7O_3N$: $C_{34.29}$, $H_{6.67}$, $N_{13.23}$; Found: $C_{34.35}$, $H_{6.77}$, $N_{13.22}$.

***(±)-Ethyl N-O-Diacetyl-3-amino-2-hydroxypropionate* (10)** : Dry HCl gas was introduced into a suspension of (±)-isoserine **3** (2.07 g, 19.7 mmol) in absolute ethanol (30 mL) without cooling until a clear solution formed. The reaction mixture was then boiled under reflux for 6 hours. The resulting solution was cooled to room temperature and evaporated to dryness *in vacuo*. The residue was dissolved in a solution of acetic

anhydride (10 mL) and pyridine (15 mL). The mixture was stirred overnight at room temperature, quenched with cold water, neutralized with a saturated aqueous solution of sodium hydrogen carbonate and then extracted with dichloromethane. The combined organic phases were washed with 10% HCl, and water, dried over sodium sulfate and evaporated, to yield the diacetate **10** as a colorless oil (3.94 g, 92%); I.R. (neat) 3280 (s), 1725 (s), 1640 (s), 1200 (s) cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.28 (t, 3H, J = 7 Hz), 1.97 (s, 3H), 2.16 (s, 3H), 3.75 (m, 2H), 4.21 (q, 2H, J = 7 Hz), 5.08 (t, 1H, J = 5 Hz), 5.90 (s, 1H, broad); ^{13}C NMR ($CDCl_3$): δ 13.8, 20.3, 22.8, 39.6, 61.7, 71.0 168.2, 169.9, 170.2; ms (IC, NH_3) m/z: 235 $(M+18)^+$, 218 $(M+1)^+$.

***(+)-Ethyl N-O-diacetyl-3-amino-2-hydroxypropionate* (10a); *(-)-Ethyl N-acetyl-3-amino-2-hydroxypropionate* (11)** : To a solution of (±)-ethyl *N-O*-diacetyl-3-amino-2-hydroxypropionate **10** (1500 mg, 6.9 mmol) in isopropylic ether (25 mL) were added *n*-butanol (540 mg, 7.3 mmol) and CCL (3 g). The mixture was stirred for 75 hr at room temperature. The reaction was stopped by filtering off the enzyme and washing the solid with isopropylic ether. The combined filtrates and washings were evaporated under reduced pressure to dryness. The conversion of the transesterification was shown by NMR spectroscopy to be 42%. The residue was loaded into a 30 g silica gel column and the products were eluted with methanol: dichloromethane 5 : 95 as eluent. A clean separation of diacetate and monoacetate was obtained. The faster moving component was identified as (+) ethyl *N-O*-diacetyl-3-amino-2-hydroxypropionate **10a** (715 mg, 48%); $[\alpha]_D$ +7.6 (c = 0.7, $CHCl_3$). The slower moving monoacetate **11** was a colorless oil (426 mg, 35%) $[\alpha]_D$ -18.7 (c = 3, $CHCl_3$). I.R. (neat) 1730 (s), 1650 (s), 1205 (m) cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.29 (t, 3H, J = 7 Hz), 2.02 (s, 3H), 3.40 (s, 1H, broad), 3.50-3.75 (m, 2H), 4.30 (m, 3H), 5.90 (s, 1H); ^{13}C NMR ($CDCl_3$): δ 13.6, 22.2, 42.6, 61.2, 69.5, 171.6, 172.1; ms (IC, NH_3) m/z: 193 $(M+18)^+$, 176 $(M+1)^+$. The enriched (+)-diacetate **10a** was subjected to repeated transesterification as described above for **10** (715 mg, 3,3 mmol) with *n*-butanol (220 mg), CCL (1.5 g), and isopropylic ether (15 mL). After 6 days, 20% conversion was accomplished. The flash chromatography gave diacetate (+)-**10a** (519 mg, 73%) $[\alpha]_D$ +8.5 (c = 3, $CHCl_3$) and monoacetate **11** (122 mg, 21%) $[\alpha]_D$ -4.4 (c = 3, $CHCl_3$).

***(S)-Isoserine* (3a)** : A solution of **10a** $[\alpha]_D$ +8.5 (300 mg; 1.38 mmol) in 1N HCl (15 mL) was refluxed for 4 h. After concentration under vacuum, the residue was purified by ion-exchange chromatography on Amberlite IR-120H (H^+ form), eluting first with water and then with 5% NH_4OH, to yield **3a** (140 mg; 96%) as a white solid that was repeatedly crystallized from H_2O: methanol 1:4; m.p. 191-193°C, Lit.[32c] m.p. 188-190°C; I.R. (KBr) 3025, 1540 cm^{-1}; $[\alpha]_D$ -32.7 (c = 0.5; H_2O); Lit.[32c] $[\alpha]_D$ = - 32.2 (c = 1, H_2O).

***(R)-Isoserine* (3b)** : This compound was prepared as described for (*S*)-isoserine **3a** from **11** $[\alpha]_D$ -18.7, (85 mg, 0.39 mmol). The product was obtained as a white solid (50 mg; 83%); m.p. 191-193°C, Lit.[33] m.p. 199-201°C; I.R. (KBr) 3025, 1540 cm^{-1}; $[\alpha]_D$ +30.6 (c = 2; H_2O); Lit.[32b] $[\alpha]_D$ = +32.5 (c = 1, H_2O).

***2(2'-Cyano-2'-hydroxyethyl)-4,4-Dimethyl-2-oxazoline* (13)** : To a stirred solution of 2,4,4-trimethyl-2-oxazoline (3.55 g, 31.4 mmol) in dry THF (10 mL) at -78°C was slowly added *n*-butyllithium (18 mL of a 2M solution in pentane, 36 mmol) under nitrogen. There was an immediate precipitation of the yellow anion. The reaction mixture was stirred for 20 min at the same temperature and ethyl formate (2.65 mL, 33 mmol) was then added dropwise. The reaction was allowed to warm up. After 1 hour, the reaction mixture was poured into 200 mL of a 1:1 ethanol/water solution buffered at pH 5.4 with sodium acetate/acetic acid. After addition of Me_3SiCN (5 mL, 37.7 mmol), the reaction mixture was stirred for 1 hour, then neutralized with a saturated aqueous solution of sodium hydrogen carbonate and extracted with dichloromethane. The combined organic phases were washed with brine, dried over sodium sulfate and evaporated to give the cyanohydrin **13** as a crystalline solid (4.22 g, 80%); mp 92-93°C; I.R. (KBr) 3200 (m), 1705 (S) cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.29

(s, 3H), 1.30 (s, 3H), 2.67 (dd, 1H, J = 18Hz, 6Hz), 2.80 (dd, 1H, J = 18Hz, 6Hz), 3.92, 3.96, 3.98, 4.02 (AB system, 2H), 4,75 (t, 1H, J = 6Hz), 6.20 (s, broad, 1H); ^{13}C NMR ($CDCl_3$): δ 27.8, 33.2, 57.3, 67.0, 79.1, 119.2, 162.4; ms (IC, H_3N) *m/z* 169 $(M+1)^+$, 142 $(M-H_2O)^+$; Anal. Calcd. for $C_8H_{12}O_3N_2$: $C_{57.14}$, $H_{7.14}$, $N_{16.67}$; Found $C_{57.04}$, $H_{7.27}$, $N_{16.45}$.

***(±)-Statine* (4)** : Isobutylmagnesium chloride (9 mL of a 2 M solution in ether, 18 mmol) was added dropwise to a solution of **13** (1.0 g, 6 mmol) in dry THF (30 mL). The solution was stirred under nitrogen at room temperature for 2 hours and BH_3.THF (40 mL of a 1 M solution in THF, 40 mmol) was added slowly. After 1.5 hours, 10 mL of methanol was added in order to destroy the excess of BH_3.THF. The solvents were removed under reduced pressure. The residue was hydrolyzed by refluxing it in 1N HCl (50 mL) for 1 hr. After removal of the solvents, the residue was purified by ion-exchange chromatography on Amberlite IR-120 H (H^+ form), eluting first with water and then with 5% NH_4OH to give the mixture of statine **4** and 2-amino-2-methylpropanol-1. The addition of ethanol (10 mL) yielded (±)-statine as white solid (415 mg, 40%) in a ratio of 82:18 (*threo:erythro*). ^1H NMR (D_2O): δ 0.75 (d, 6H, J = 6Hz), 1.20-1.60 (m, 3H), 2.15-2.45 (m, 2H), 3.10 and 3.20* (m, 1H), 3.85 and 4.05* (m, 1H); ^{13}C NMR ($CDCl_3$): δ 21.6*, 21.9, 23.1, 23.4*, 24.7*, 24.9, 36.7*, 39.4, 40.8*, 42.4, 54.4*, 54.8, 69.3, 69.6*, 179.9; Anal Calcd for $C_8H_{17}O_3N$: $C_{54.83}$, $H_{9.78}$, $N_{7.99}$; found: $C_{54.75}$, $H_{9.70}$, $N_{7.91}$.

* for the erythroisomer

References and Notes

1 Present address : Laboratoire de Pharmacognosie, CNRS-URA 1310, Faculté de Pharmacie, 4, Avenue de l'Observatoire, 75270 Paris CEDEX 06
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