A Practical Synthesis of (2*S*,3*R*,4*S*)-4-Hydroxyisoleucine, A Potent Insulinotropic α-Amino Acid from Fenugreek

Qian Wang,[a] Jamal Ouazzani,[a] N. André Sasaki,*[a] and Pierre Potier[a]

Keywords: Amino acids / Natural products / Biotransformations / Asymmetric synthesis / Total synthesis

An efficient eight-step synthesis of optically pure (2*S*,3*R*,4*S*)-4-hydroxyisoleucine (**1**), a potent insulinotropic α-amino acid found in the seeds of fenugreek (*Trigonella foenum-graecum* L.), is achieved in 39% overall yield. The method is suitable for large-scale production of the title compound. The key steps involve the biotransformation of ethyl 2-methylacetoacetate to ethyl (2*S*,3*S*)-2-methyl-3-hydroxybutanoate (**2**) with *Geotrichum candidum* and an asymmetric Strecker synthesis.

Introduction

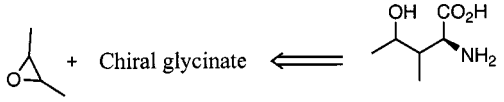
Diabetes mellitus is a metabolic disorder characterized by impaired glucose homeostasis. About 90% of patients have type 2 diabetes that leads to chronic complications such as retinopathy, neuropathy, nephropathy, and macrovascular diseases.[1−3] These complications can be further aggravated and lead to blindness, foot ulceration, end-stage renal disease, stroke and heart attack. Several types of pharmacological agents have been used extensively for the treatment of type 2 diabetes. Other than insulin, these drugs include sulfonylureas, biguanides, thiazolidinediones, α-glucosidase inhibitors and meglitinides. Although they have been proven effective in controlling hyperglycemia, there are troublesome side effects such as hypoglycemia, gastrointestinal discomfort, nausea, lactic acidosis, hepatotoxicity and gastrointestinal flatulence.[4] Taking into account an estimation that more than 200 million of the world's total population will have diabetes mellitus by 2010,[5] it is obvious that the search for new improved antidiabetic drugs is of great public interest.[6,7]

Recently Sauvaire has reported that (2*S*,3*R*,4*S*)-4-hydroxyisoleucine, extracted from the seeds of fenugreek (*Trigonella foenum-graecum* Leguminosae), possesses an interesting insulinotropic property.[8] Indeed, fenugreek has been traditionally used in northern African countries to lower blood sugar. The specific feature of this nonproteinogenic amino acid is that it increases glucose-induced insulin release, in the concentration range of 100 μmol/L to 1 μmol/L, through a direct effect on isolated islets of Langerhans for rats and humans. The insulin response is amplified proportionally by the increase of glucose concentration. Furthermore, the stimulating effect is strictly glucose dependent. Whereas the amino acid provides no effect at low (3 mmol/L) or basal (5 mmol/L) glucose concentration, it induces insulin secretion at the supranormal (6.6−16.7 mmol/L) level of glucose concentration in the absence of any change in pancreatic activity of α- and β-cells.

4-Hydroxyisoleucine was first isolated from fenugreek seeds and identified as the (2*S*,3*R*,4*R*)-isomer by Fowden in 1973.[9] Much later (1989), assignment of the correct absolute stereochemistry was established as 2*S*,3*R*,4*S*.[10] Wieland has also reported the occurrence of this amino acid in γ-amanitine, one of the cyclic octapeptides found in the highly poisonous mushroom, *Amanita phalloides*.[11] Recent studies on the structure-activity relationships have demonstrated that the (2*S*,3*R*,4*S*)-configuration is the absolute requirement for the insulinotropic effect of this amino acid.[12] Despite its attractive pharmacological properties as an antidiabetic agent, the total synthesis of this amino acid has not yet been reported to the best of our knowledge.[13] We describe here an economic, efficient and stereoselective synthesis of optically pure (2*S*,3*R*,4*S*)-4-hydroxyisoleucine that can be applied to large-scale production.

Results and Discussion

The most obvious conceivable approach to the construction of (2*S*,3*R*,4*S*)-4-hydroxyisoleucine (**1**) is the nucleophilic addition of chiral glycine enolate equivalents[14] to *cis*-2,3-dimethyloxirane, as depicted in Scheme 1.

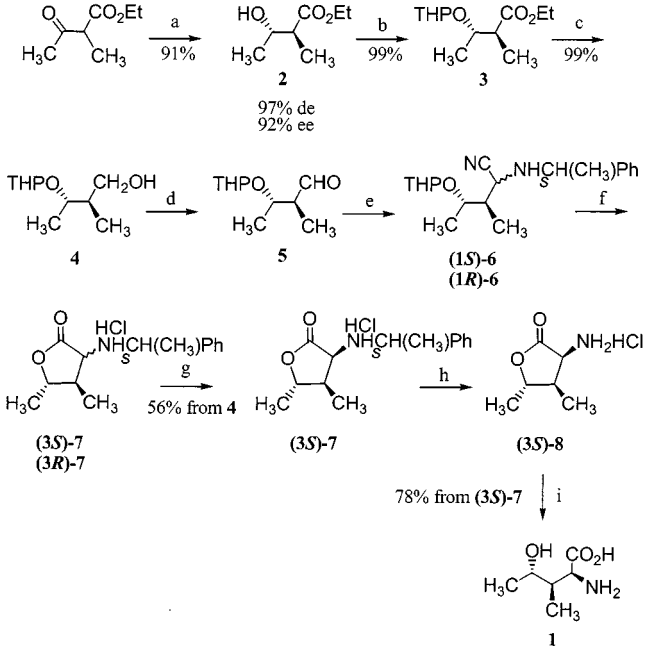


Scheme 1. Chiral glycine enolate approach

[a] Institut de Chimie des Substances Naturelles, CNRS, Avenue de la Terrasse, 91198 Gif-sur-Yvette Cedex, France
Fax: (internat.) + 33-1/6907-7247
E-mail: andre.sasaki@icsn.cnrs-gif.fr

However, the difficulties that can arise from the separation of the resulting diastereomers and the high costs of both starting materials are the unfavorable factors in investigating this route for large-scale synthesis of **1**. We therefore opted for the possibility of converting ethyl (2*S*,3*S*)-3-hydroxy-2-methylbutanoate (**2**) to **1** by an asymmetric Strecker synthesis. Although the enantioselective synthesis of diastereomers of alkyl 3-hydroxy-2-methylbutanoate has been widely investigated,[15] the only method that affords the alkyl 3-hydroxy-2-methylbutanoate with the desired (2*S*,3*S*) configuration is microbial reduction with *Geotrichum candidum* in 80−93% chemical yield with 92−99% *de* and 92−97% *ee*.[15f−15i] Like baker's yeast, the biomass prepared with *Geotrichum candidum* is environmentally completely harmless.

Thus, ethyl 2-methylacetoacetate was submitted to reduction by the biomass prepared with *Geotrichum candidum* to provide ethyl (2*S*,3*S*)-3-hydroxy-2-methylbutanoate (**2**) in 91% yield with 97% diastereomeric excess and 92% enantiomeric excess.[16] For large-scale synthesis of **1** (tonne scale), biomass production is certainly the most constraining step and requires at least 20 to 100 m^3 microbial culture facilities. In this work the bioconversion step did not require any specific microbiological equipment and could be conducted in any vessel or reactor equipped with a temperature regulator. In order to simplify the process, we tried to dissociate the biomass production step from the bioconversion step. This consists of the production of large quantities of *Geotrichum candidum* biomass that can be stored until the need for synthesis of compound **2**. Biomass aliquots were then conserved either by lyophilization or freezing. These aliquots were incubated every two weeks with ethyl 2-methylacetoacetate under standard conditions and compared with the initial fresh biomass. While lyophilization of the biomass affects both conversion and selectivity, these properties remain intact for at least 6 months after freezing at −20 °C. We investigated the role of substrate concentration from 10 to 50 g/L as well as the possibility of recovering the biomass and of using it for further biotransformations. All these optimizations were made in 15 liter fermenters. At 20 or 50 g/L level, the biomass was degraded and the conversion of the keto ester did not exceed more than 20% for 50 g/L and 60% for 20 g/L. For 15 g/L of the keto ester, the conversion was slower than for 10 g/L (one week instead of four days). We nevertheless could obtain the same yield, *ee* and *de*. The advantage of using 10 g/L of the keto ester is not only a high conversion rate but also that the biomass remains intact, as noticed by microscopic observations. The biomass was recovered and used for a second bioconversion leading to the same results as with the first incubation. We are currently investigating the biomass immobilization for continuous process evaluation. The resulting alcohol **2** was treated with DHP in the presence of TsOH (0.2%) to give **3** in almost quantitative yield (Scheme 2). Reduction of **3** was carried out with $LiAlH_4$ to provide **4** again in quantitative yield. Among the conventional methods for the oxidation of alcohols to aldehydes, the use of 2,2,6,6-tetramethyl-1-piperidinyloxy radical (TEMPO)[17] with sodium hypochlorite as the oxidant was found to be the most efficient one. This method provided the aldehyde **5** in quantitative yield without epimerization. The key intermediate **5** was then subjected to a Strecker synthesis. The aldehyde **5** was dissolved in a solvent mixture of methanol/water and treated with (*S*)-(−)-α-methylbenzylamine hydrochloride followed by KCN to give a mixture of the amino nitriles **(1*S*)-6** and **(1*R*)-6**. Reflux of the diastereomeric mixture of **(1*S*)-** and **(1*R*)-6** in 6 N HCl provided a mixture of the lactones **(3*S*)-** and **(3*R*)-7** in the ratio of 82:18 in 83% yield. The use of (*R*)-(+)-α-methylbenzylamine hydrochloride as chiral auxiliary under the same reaction conditions gave the (*R*)-(+)-α-methylbenzylamine analogs of **(3*S*)-** and **(3*R*)-7** in the ratio of 22:78. After washing with heptane/EtOAc, the aqueous phase was evaporated to dryness and the residue was dissolved in 2-propanol to crystallize **(3S)-7** in 56% yield (three steps from crude **4**). Although the *ee* and the *de* of ethyl (2*S*,3*S*)-3-hydroxy-2-methylbutanoate (**2**) attained by biotransformation of achiral ethyl 2-methylacetoacetate are 92% and 97%, respectively, recrystallized **(3S)-7** turned out to be a single diastereomer on the basis of the NMR analyses. Hydrogenolysis of **(3*S*)-7** in the presence of 10% Pd/C provided the hydrochloride of the amino lactone **(3*S*)-8**, which was directly subjected to hydrolysis by LiOH. The resulting lithium salt of the amino acid **1** was carefully neutralized with 1 equiv. of AcOH. After removal of water, the residue was recrystallized from 95% ethanol to provide



Scheme 2. Synthesis of (2*S*,3*R*,4*S*)-4-hydroxyisoleucine (**1**); a) *Geotrichum candidum*. b) 1.05 equiv. DHP, TsOH (0.2%), toluene, 0 °C to room temp., 2 h. c) $LiAlH_4$, toluene/THF, 0 °C to room temp., 1 h. d) TEMPO (2%), NaOCl, NaBr, $NaHCO_3$, 0 °C, 1 h. e) 1 equiv. (*S*)-$NH_2CH(Me)Ph{\cdot}HCl$, MeOH/H_2O, KCN, 0 °C to room temp., 48 h. f) 6 N HCl, reflux, 6 h. g) recrystallization (2-propanol). h) H_2/10% Pd/C, MeOH, room temp., 5 h. i) LiOH/H_2O, room temp., 24 h; acidification with AcOH; recrystallization (95% EtOH); purification of the mother liquor by Dowex 50WX8-200 ion-exchange resin (H^+ form)

optically pure (2*S*,3*R*,4*S*)-4-hydroxyisoleucine (**1**). The mother liquor was passed through a column of ion-exchange resin to recover the remaining amino acid **1**. It is worth noting that although the purification of amino acids by ion-exchange resin chromatography is practical, recrystallization is a much simpler process to provide the majority (84%) of optically pure **1** in our case. Thus, we obtained the desired optically pure (2*S*,3*R*,4*S*)-4-hydroxyisoleucine (**1**) in 78% yield from **(3*S*)-7** and in 39% overall yield from the easily accessible ethyl 2-methylacetoacetate. In order to confirm the optical purity of the final product and its precursors, separation of **1** from its (2*R*)-diastereomer by HPLC was attempted under different conditions with no significant results. Although the HPLC characterization of 2,4-dinitrobenzene-derivatized amino acids has been reported,[18] we have found for the first time that 2,4-dinitrobenzene derivatization by 1-fluoro-2,4-dinitrobenzene is also a fast, simple and reliable method to detect the presence of diastereomers of complex amino acids. This is demonstrated in Figure 1.

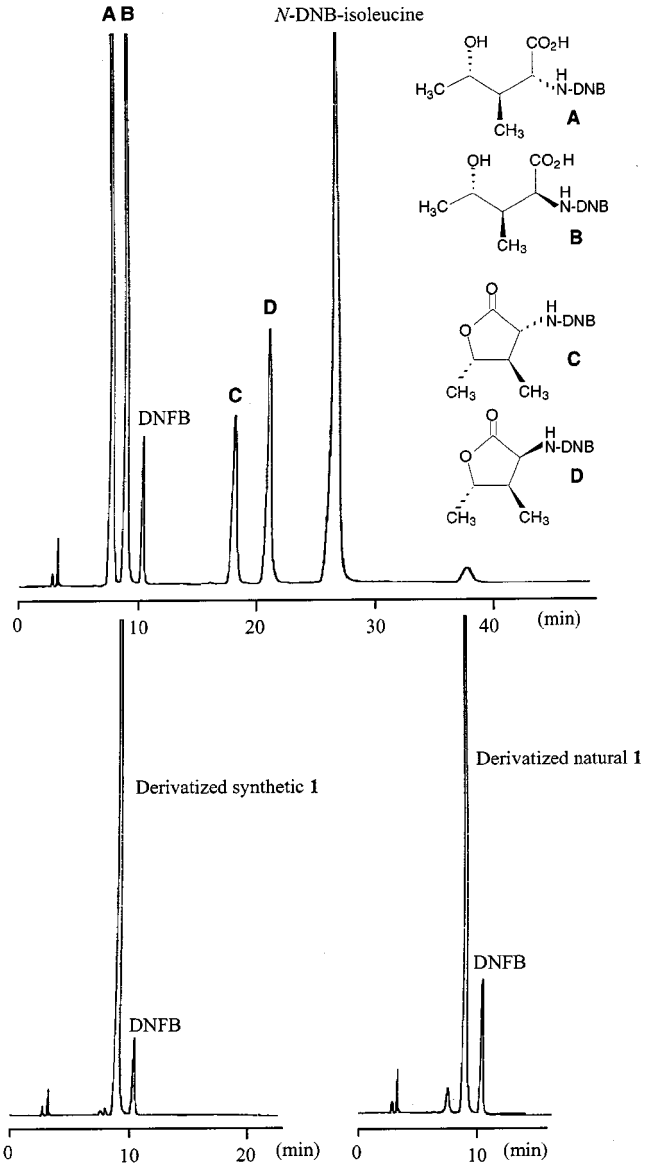


Figure 1. HPLC analysis after DNFB derivatization

The ^{1}H NMR spectrum, m.p. and $[\alpha]_D$ of the final product **1** are in complete agreement with the data reported in the literature.[9,10] In the present work, all reactions from ethyl 2-methylacetoacetate to the amino lactone **7** (six steps) were carried out without any kind of purification of the crude reaction products. We have routinely been able to prepare (2*S*,3*R*,4*S*)-4-hydroxyisoleucine (**1**) in about 40% overall yield.

Conclusion

An efficient method for the synthesis of optically pure (2*S*,3*R*,4*S*)-4-hydroxyisoleucine (**1**) from readily available ethyl 2-methylacetoacetate has been developed. The reaction sequences require no particular purification process for the intermediate compounds except for simple recrystallization for the compound **(3*S*)-7**. The final product is obtained in 39% overall yield. The insulinotropic activity of synthetic **1** has been proven to be slightly higher than that of natural **1** (vide infra). In view of the reaction conditions and reagents needed, the present method is suitable for large-scale production of (2*S*,3*R*,4*S*)-4-hydroxyisoleucine (**1**).

Experimental Section

General Remarks: Infrared spectra were recorded on a Perkin−Elmer Spectrum BX spectrometer. ^{1}H and ^{13}C NMR spectra were measured on Bruker AM-300 and AC-250 spectrometers (^{1}H at 300 and 250 MHz, respectively). Flash chromatography was performed using Kieselgel 60 (230−400 mesh, E. Merck). Solvents and reagents were purified according to standard laboratory techniques. Optical rotations were determined on a Perkin−Elmer 241 polarimeter at room temp. Mass spectra were run on an AEI MS-9 spectrometer (CI) and a Nawigator Thermoquest spectrometer (ESI). Elemental analyses were carried out at the ICSN.

Ethyl (2*S*,3*S*)-3-Hydroxy-2-methylbutanoate (2): The strain *Geotrichum candidum* LCM I-2366 (CNCM, Institut Pasteur, France) was cultivated in a sterile medium consisting of KH_2PO_4 (1 g), K_2HPO_4 (2 g), $MgSO_4$ (0.5 g), $FeSO_4$ (20 mg), KCl (0.5 g), $NaNO_3$ (3 g), glucose (30 g) and corn steep liquor (10 g) per liter of distilled water. Cells from agar slides were suspended in distilled water and used to inoculate the medium (2 L). This preculture was placed on a rotary shaker for two days (200 rpm, 27 °C), and then used to inoculate a 70 L fermenter containing 50 liters of growth medium (Chemap, France). The strain was grown in the fermenter for a further two days (27 °C, 300 rpm, pH 5, air 1VVM, 300 mL antifoam PEG), and then filtered on a paper sheet to give 3.2 kg of wet biomass. The recovered biomass was used in nonsterile conditions and media.

2.5 kg of this biomass were incubated in distilled water (30 L) containing 1.5% glucose, 1% NaCl and ethyl 2-methylacetoacetate (300 g, 2.08 mol). Samples of the incubation (1 mL) were extracted with EtOAc (0.5 m) and analyzed by GC to determine the percentage of conversion and the isomeric excess.[15g] When these values reached their maximum (94 to 98% conversion, with 92 to 98% *de* in 3 to 4 days), the medium was filtered on a paper sheet and

extracted twice with 10 L of EtOAc and twice with 10 L of CH_2Cl_2. The organic layers were pooled, dried over anhydrous Na_2SO_4, and the solvents evaporated under reduced pressure to give ethyl (2*S*,3*S*)-3-hydroxy-2-methylbutanoate (**2**) (276 g, 91%) with 92% *ee* and 97% *de* as an oil. $[\alpha]_D^{20} = +26$ (c = 3.0, $CHCl_3$). ^{1}H NMR (250 MHz, $CDCl_3$): δ = 4.18 (q, J = 7.1 Hz, 2 H), 3.89 (quint, J = 6.5 Hz, 1 H), 2.64 (br. s, 1 H), 2.44 (quint, J = 7.2 Hz, 1 H), 1.28 (t, J = 7.1 Hz, 3 H), 1.22 (d, J = 6.4 Hz, 3 H), 1.19 (d, J = 7.2 Hz, 3 H). ^{13}C NMR (62.5 MHz, $CDCl_3$): δ = 175.2, 68.6, 59.9, 46.7, 19.8, 13.7, 12.8. MS (CI): m/z = 147 [MH^+].

Ethyl (2*S*,3*S*)-2-Methyl-3-(tetrahydropyranyloxy)butanoate (3): Dihydropyran (13.5 mL, 148 mmol) was added dropwise at 0 °C to a stirred solution of ethyl (2*S*,3*S*)-3-hydroxy-2-methylbutanoate (**2**) (20 g, 140 mmol) in toluene (200 mL) in the presence of $TsOH{\cdot}H_2O$ (53 mg, 0.28 mmol, 0.2%) and stirred at room temperature for 2 h. The progress of the reaction was checked by TLC (heptane/EtOAc, 2:1). After removal of the solvent on a rotavapor to afford **3** (32.0 g, 99%) as a pale yellow oil, the crude product was used directly for the next reaction. The analytical sample was obtained by flash column chromatography (silica gel, heptane/EtOAc, 20:1). IR ($CHCl_3$): $\tilde{\nu}$ = 3009, 2982, 2945, 2872, 1727, 1455, 1381, 1324, 1262, 1235, 1190 cm^{-1}. ^{1}H NMR (250 MHz, $CDCl_3$): δ = 4.80−4.77 (m, 0.5 H), 4.65−4.62 (m, 0.5 H), 4.21−3.77 (m, 4 H), 3.56−3.45 (m, 1 H), 2.71−2.53 (m, 1 H), 1.81−1.47 (m, 6 H), 1.28 (t, J = 7.2 Hz, 1.5 H), 1.26 (t, J = 7.1 Hz, 1.5 H), 1.24 (d, J = 6.2 Hz, 1.5 H), 1.15 (d, J = 7.1 Hz, 1.5 H), 1.12 (d, J = 6.2 Hz, 1.5 H), 1.10 (d, J = 7.1 Hz, 1.5 H). ^{13}C NMR (62.5 MHz, $CDCl_3$): δ = 174.2, 99.4, 94.4, 75.8, 71.6, 62.1, 61.2, 59.6, 45.5, 30.7, 30.5, 25.1, 19.4, 18.7, 18.1, 14.9, 13.8, 12.0, 11.9. MS (CI): m/z = 231 [MH^+]. $C_{12}H_{22}O_4$ (230.3): calcd. C 62.58, H 9.63; found C 62.71, H 9.67.

(2*R*,3*S*)-2-Methyl-3-(tetrahydropyranyloxy)butanol (4): Crude ethyl (2*S*,3*S*)-2-methyl-3-(tetrahydropyranyloxy)butanoate (**3**) (30 g, 130 mmol) was added dropwise to a stirred suspension of $LiAlH_4$ (5.5 g, 143 mmol) in THF/toluene (3:1, 500 mL) at 0 °C. After stirring at room temperature for 1 h, the reaction mixture was treated by successive dropwise addition of ice-cold water (5 mL), an aqueous 15% NaOH solution (5 mL), and ice-cold water (3 × 5 mL) (“Fieser’s workup”). The mixture was then stirred for 30 min and filtered through Celite. The precipitated aluminum salts were washed with EtOAc. The solvent was evaporated to give crude **4** as a colorless oil (24.3 g, 99%) that was used directly for the next reaction. The analytical sample was obtained by flash column chromatography (silica gel, heptane/EtOAc, 5:1 then 2:1). ^{1}H NMR (250 MHz, $CDCl_3$): δ = 4.69−4.67 (m, 0.5 H), 4.58−4.56 (m, 0.5 H), 3.98−3.43 (m, 5 H), 1.80−1.48 (m, 7 H), 1.29 (d, J = 6.3 Hz, 1.5 H), 1.18 (d, J = 6.1 Hz, 1.5 H), 0.95 (d, J = 7.0 Hz, 1.5 H), 0.94 (d, J = 7.0 Hz, 1.5 H). ^{13}C NMR (50 MHz, $CDCl_3$): δ = 99.9, 97.4, 78.4, 74.9, 65.2, 64.2, 62.7, 41.2, 40.6, 31.3, 31.0, 25.3, 25.1, 20.7, 19.8, 18.9, 17.5, 14.0, 13.0. MS (CI): m/z = 189 [MH^+]. HRMS: $C_{10}H_{21}O_3$ [MH^+]: calcd. 189.14906; found 189.14755.

(2*S*,3*S*)-2-Methyl-3-(tetrahydropyranyloxy)butyraldehyde (5): NaOCl (122 mmol, 65 mL of 12.5% solution) and $NaHCO_3$ (30 g, 354 mmol) were added dropwise over a period of 1 h to a cold (0 °C), rapidly stirred (>1000 rpm) biphasic mixture consisting of (2*R*,3*S*)-2-methyl-3-(tetrahydropyranyloxy)butanol (**4**) (23 g, 122 mmol), TEMPO free radical (382 mg, 2%), sodium bromide (12.58 g, 122 mmol), toluene (240 mL), EtOAc (240 mL) and water (120 mL). The aqueous layer was separated and extracted with EtOAc (500 mL). The combined organic layers were washed sequentially with a solution of KI (980 mg) dissolved in 10% aqueous $KHSO_4$ (240 mL), 10% aqueous sodium thiosulfate (120 mL), and brine (120 mL), and dried over $MgSO_4$. Filtration and concentration in vacuo afforded the desired aldehyde **5** as a light brown oil (due to contamination by trace amounts of TEMPO) (22.5 g, 99%) that was used for the next step without purification. ^{1}H NMR (250 MHz, $CDCl_3$): δ = 9.78 (d, J = 2.7 Hz, 0.5 H), 9.74 (d, J = 2.0 Hz, 0.5 H), 4.76−4.62 (m, 1 H), 4.15−3.73 (m, 2 H), 3.53−3.43 (m, 1 H), 2.62−2.46 (m, 1 H), 1.82−1.51 (m, 6 H), 1.29 (d, J = 6.2 Hz, 1.5 H), 1.18 (d, J = 6.2 Hz, 1.5 H), 1.11 (d, J = 7.2 Hz, 1.5 H), 1.07 (d, J = 7.1 Hz, 1.5 H). ^{13}C NMR (62.5 MHz, $CDCl_3$): δ = 203.5, 203.1, 99.0, 95.1, 74.4, 71.0, 62.0, 51.6, 51.1, 30.5, 25.0, 19.2, 19.1, 18.6, 16.0, 9.7, 9.1. MS (CI): m/z = 187 [MH^+].

(1*R*,2*R*,3*S*)-1-Cyano-2-methyl-3-(tetrahydropyranyloxy)-*N*-[(*S*)-1′-phenylethyl]butylamine [(1*R*)-6], and (1*S*,2*R*,3*S*)-1-Cyano-2-methyl-3-(tetrahydropyranyloxy)-*N*-[(*S*)-1′-phenylethyl]butylamine [(1*S*)-6]: (*S*)-(−)-α-Methylbenzylamine hydrochloride (38.8 g, 247 mmol) and KCN (16.08 g, 247 mmol) were added successively to a suspension of crude (2*S*,3*S*)-2-methyl-3-(tetrahydropyranyloxy)butyraldehyde (**5**) (46.0 g, 247 mmol) in methanol (310 mL) and water (310 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 30 min then at room temperature for 48 h, diluted with water (620 mL), and extracted five times with EtOAc. The organic extracts were washed with brine, dried and filtered through Celite and Na_2SO_4. The solvent was evaporated to dryness to give the crude product as an oil (74.0 g, 95%), which was used directly for the next reaction without purification. The analytical sample was obtained by flash column chromatography on silica gel eluting with heptane/EtOAc (10:1 then 8:1).

(1*S*)-6: Viscous oil. IR ($CHCl_3$): $\tilde{\nu}$ = 3500, 3316, 3028, 3012, 2968, 2947, 2854, 2226, 1494, 1453, 1376, 1356, 1275, 1260, 1234, 1186, 1132 cm^{-1}. ^{1}H NMR (250 MHz, $CDCl_3$): δ = 7.39−7.23 (m, 5 H), 4.59 (m, 0.5 H), 4.50−4.47 (m, 0.5 H), 4.11−4.03 (m, 1 H), 3.88−3.74 (m, 1 H), 3.68−3.55 (m, 1 H), 3.46−3.40 (m, 2 H), 1.92−1.34 (m, 7 H), 1.40 (d, J = 6.5 Hz, 1.5 H), 1.38 (d, J = 6.4 Hz, 1.5 H), 1.15 (d, J = 6.2 Hz, 1.5 H), 1.06 (d, J = 6.1 Hz, 1.5 H), 1.02 (d, J = 6.9 Hz, 1.5 H), 1.00 (d, J = 6.9 Hz, 1.5 H). ^{13}C NMR (62.5 MHz, $CDCl_3$): δ = 143.6, 143.1, 128.6, 128.5, 127.5, 127.4, 127.0, 126.9, 120.1, 119.5, 100.2, 96.0, 76.4, 71.4, 63.2, 62.7, 56.6, 56.4, 50.5, 50.4, 42.7, 31.0, 30.7, 25.3, 24.7, 24.6, 19.9, 19.7, 18.5, 16.4, 13.1, 11.7. MS (CI): m/z = 317 [MH^+]. HRMS: $C_{19}H_{29}N_2O_2$ [MH^+]: calcd. 317.22289; found 317.22647.

(3*S*,4*R*,5*S*)-4,5-Dimethyl-3-*N*-[(*S*)-1′-phenylethylamino]-2-oxotetrahydrofuran [(3*S*)-7] and (3*R*,4*R*,5*S*)-4,5-Dimethyl-3-*N*-[(*S*)-1′-phenylethylamino]-2-oxotetrahydrofuran [(3*R*)-7]: A solution of the above mixture of (1*S*,2*R*,3*S*)- and (1*R*,2*R*,3*S*)-1-cyano-2-methyl-3-(tetrahydropyranyloxy)-*N*-[(*S*)-1′-phenylethyl]butylamine (**6**) (74.0 g) in 6 N HCl (820 mL) was refluxed for 6 h. The dark-brown reaction mixture was washed with heptane/EtOAc (1:1) (three times) and the aqueous phase was evaporated to dryness to give a mixture of **(3*S*)-7** and **(3*R*)-7** in quantitative yield [(2*S*)/(2*R*) = 4.5:1]. The residue was then dissolved in hot 2-propanol. After the removal of insoluble inorganic salts by filtration, the mixture was cooled to room temperature to crystallize out **(3S)-7** (37.3 g, 56% for three steps from the crude alcohol **4**) as fine white needle-shaped crystals. m.p. 228−229 °C. $[\alpha]_D^{20} = -7.8$ (c = 2.0, CH_3OH). ^{1}H NMR (250 MHz, D_2O): δ = 7.54 (s, 5 H), 4.88 (q, J = 6.9 Hz, 1 H), 4.53 (q, J = 6.6 Hz, 1 H), 4.27 (d, J = 7.7 Hz, 1 H), 2.60 (quint, J = 7.3 Hz, 1 H), 1.72 (d, J = 6.9 Hz, 3 H), 1.26 (d, J = 6.7 Hz, 3 H), 1.12 (d, J = 7.2 Hz, 3 H). ^{13}C NMR (62.5 MHz, CD_3OD): δ = 171.6, 136.6, 130.9, 130.5, 129.3, 83.9, 59.3, 56.2, 39.0, 20.0, 19.8, 14.5. For characterization of the products, a small portion of the aqueous solution was washed three times with heptane/EtOAc (1:1), neutralized with $NaHCO_3$ solution and extracted with EtOAc. The EtOAc extracts were washed

with brine, dried and the solvents evaporated. The residue was passed through a chromatography column eluting with heptane/EtOAc (6:1) to give the free amines of **(3*S*)-** and **(3*R*)-7**.

(3*S*)-7, Free Amine: Viscous oil. $[\alpha]_D^{20} = -94$ (c = 1.7, $CHCl_3$). IR ($CHCl_3$): $\tilde{\nu}$ = 3568, 3330, 3028, 2980, 2933, 2875, 1769, 1494, 1453, 1383, 1354, 1301, 1224, 1220, 1172, 1146 cm^{-1}. ^1H NMR (250 MHz, $CDCl_3$): δ = 7.39−7.22 (m, 5 H), 4.23 (qd, J = 6.5, 3.7 Hz, 1 H), 4.17 (q, J = 6.6 Hz, 1 H), 3.38 (d, J = 7.4 Hz, 1 H), 1.89 (quint. d, J = 7.2, 3.7 Hz, 1 H), 1.61 (br. s, 1 H, NH), 1.38 (d, J = 6.6 Hz, 3 H), 1.27 (d, J = 6.5 Hz, 3 H), 0.99 (d, J = 7.1 Hz, 3 H). ^{13}C NMR (62.5 MHz, $CDCl_3$): δ = 177.5, 144.8, 128.5, 127.3, 127.1, 81.3, 57.4, 57.0, 40.6, 24.6, 19.6, 12.7. MS (CI): m/z = 234 [MH^+]. HRMS $C_{14}H_{20}NO_2$ [MH^+]: calcd. 234.14939; found, 234.15075.

(3*R*)-7, Free Amine: Viscous oil. $[\alpha]_D^{20} = -39$ (c = 0.7, $CHCl_3$). IR ($CHCl_3$): $\tilde{\nu}$ = 3693, 3329, 3030, 2967, 2933, 2877, 1765, 1603, 1494, 1453, 1388, 1329,1247,1175 cm^{-1}. ^1H NMR (250 MHz, $CDCl_3$): δ = 7.38−7.24 (m, 5 H), 4.01 (q, J = 6.6 Hz, 1 H), 3.89 (qd, J = 6.1, 9.7 Hz, 1 H), 3.08 (d, J = 11.1 Hz, 1 H), 2.05 (s, 1 H, NH), 1.91 (m, 1 H), 1.38 (d, J = 6.6 Hz, 3 H), 1.35 (d, J = 6.1 Hz, 3 H), 1.12 (d, J = 6.5 Hz, 3 H). ^{13}C NMR (62.5 MHz, $CDCl_3$): δ = 177.2, 144.7, 128.7, 127.2, 126.4, 79.5, 61.9, 56.5, 46.9, 24.7, 18.4, 14.8. MS (CI): m/z = 234 [MH^+]. HRMS: $C_{14}H_{20}NO_2$ [MH^+] calcd. 234.14939; found 234.15006.

(3*S*,4*R*,5*S*)-3-Amino-4,5-dimethyl-2-oxotetrahydrofuran Hydrochloride [(3*S*)-8]: A solution of **(3*S*)-7** (37.0 g, 137 mmol) in methanol (1.3 L) was subjected to hydrogenolysis in the presence of 10% Pd-C (3.0 g, 2%) under atmospheric pressure at room temperature for 5 h. The catalyst was then removed by filtration. The solvent was evaporated to give (3*S*,4*R*,5*S*)-3-amino-4,5-dimethyl-2-oxotetrahydrofuran hydrochloride **(3*S*)-8** (22.6 g, 100%) as fine needle-shaped crystals. m.p. 232−233 °C (206 °C[10]). $[\alpha]_D^{20} = -18$ (c = 1.4, MeOH). IR (nujol): $\tilde{\nu}$ = 3526, 2924, 2854, 1771, 1581, 1504, 1462, 1385, 1355, 1315, 1260, 1209 cm^{-1}. ^1H NMR (250 MHz, D_2O): δ = 4.61−4.54 (m, 2 H), 2.72 (br. quint, J = 7.5 Hz, 1 H), 1.40 (d, J = 6.6 Hz, 3 H), 1.11 (d, J = 7.3 Hz, 3 H). ^{13}C NMR (50 MHz, D_2O): δ = 174.3, 85.2, 52.3, 38.1, 19.5, 13.0. MS (CI): m/z = 130 [MH^+]. HRMS: $C_6H_{12}NO_2$ [MH] calcd. 130.08680; found 130.08604.

(3*R*,4*R*,5*S*)-3-Amino-4,5-dimethyl-2-oxotetrahydrofuran Hydrochloride [(3*R*)-8]: A suspension of **(3*R*)-7** (120 mg, 0.47 mmol) and 10% Pd-C (24 mg) in methanol (10 mL) was hydrogenated under atmospheric pressure at room temperature for 5 h. The catalyst was removed by filtration. The solvent was evaporated to give **(3*R*)-8** (78 mg, 100%). $[\alpha]_D^{20} = -3.4$ (c = 1.6, MeOH). IR (nujol): $\tilde{\nu}$ = 3411, 2923, 2853, 1783, 1762, 1588, 1557, 1491, 1457, 1390, 1340, 1300, 1207 cm^{-1}. ^1H NMR (300 MHz, D_2O): δ = 4.47 (dq, J = 9.7, 6.2 Hz, 1 H), 4.19 (d, J = 11.7 Hz, 1 H), 2.46−2.32 (m, 1 H), 1.49 (d, J = 6.2 Hz, 3 H), 1.29 (d, J = 6.6 Hz, 3 H). ^{13}C NMR (62.5 MHz, D_2O): δ = 174.1, 83.1, 56.5, 43.5, 18.1, 13.2. MS (CI): m/z = 130 [MH^+]. HRMS: $C_6H_{12}NO_2$ [MH^+] calcd. 130.08680; found 130.08625.

(2*S*,3*R*,4*S*)-4-Hydroxyisoleucine (1): A solution of **(3*S*)-8** (22.6 g, 137 mmol) prepared as above in water (1300 mL) was treated with $LiOH{\cdot}H_2O$ (11.16 g, 266 mmol) at room temperature for 24 h. AcOH (8.2 g, 7.8 mL, 137 mmol) was then added to the reaction mixture, and the solvent was evaporated in vacuo. The residue was recrystallized from 95% EtOH to give (2*S*,3*R*,4*S*)-4-hydroxyisoleucine (**1**) (13.49 g) as fine white plate-like crystals. The mother liquor was passed through a column of Dowex 50WX8-200 ion-exchange resin (H^+ form), washed thoroughly with water and then with 2 M NH_4OH to give an additional 2.57 g of **1** (78% for two steps). m.p. 224 °C (224−225 °C[10]). $[\alpha]_D^{20} = +31.5$ (c = 1, H_2O) [ref.[9] +31 (c = 1, H_2O)]. IR (nujol): $\tilde{\nu}$ = 3301, 3132, 2923, 2853, 1631, 1463, 1377, 1310, 1270, 1176, 1103, 1052, 1020, 1033, 965, 934, 900, 857, 815 cm^{-1}. ^1H NMR (250 MHz, D_2O): δ = 3.88 (d, J = 4.4 Hz, 1 H), 3.78 (m, 1 H), 1.91 (m, 1 H), 1.23 (d, J = 6.4 Hz, 3 H), 0.95 (d, J = 7.0 Hz, 3 H). ^{13}C NMR (50 MHz, D_2O): δ = 174.2, 70.4, 57.5, 41.9, 21.3, 12.7. MS (ESI): m/z = 148 [MH^+]. $C_6H_{13}NO_3$ (147.17): calcd. C 48.97, H 8.90, N 9.52; found C 49.04, H 8.83, N 9.60.

Purification of 1 by Ion-Exchange Resin: The residue obtained above (the lithium salt of **1**) was dissolved in water, and passed through a column of Dowex 50WX8-200 ion-exchange resin (H^+ form). The column was washed thoroughly with water and the amino acid was eluted with 2 M NH_4OH to give (2*S*,3*R*,4*S*)-4-hydroxyisoleucine (**1**).

(2*R*,3*R*,4*S*)-4-Hydroxyisoleucine. (2*R*)-Diastereomer of 1: Obtained from **(3*R*)-8** in the same manner described for the preparation of **1**. M.p. 207−208 °C, $[\alpha]_D^{20} = +0.5$ (c = 1, H_2O) [ref.[9] +1 (c = 1, H_2O)]. IR (nujol): $\tilde{\nu}$ = 3301, 3132, 2923, 2853, 1633, 1590, 1524, 1463, 1398, 1351, 1326, 1285, 1145, 1128, 1089, 1061, 1033, 918, 826 cm^{-1}. ^1H NMR (250 MHz, D_2O): δ = 4.01 (d, J = 2.7 Hz, 1 H), 3.80 (quint, J = 6.3 Hz, 1 H), 2.11 (d quint, J = 2.7, 7.0 Hz, 1 H), 1.27 (d, J = 6.3 Hz, 3 H), 0.97 (d, J = 7.3 Hz, 3 H). ^{13}C NMR (62.5 MHz, D_2O): δ = 174.8, 69.9, 56.1, 40.3, 21.1, 11.9. MS (ES): m/z = 148 [MH^+]. $C_6H_{13}NO_3$ (147.17): calcd. C 48.97, H 8.90, N 9.52; found C 49.00, H 8.87, N 9.57.

DNB Derivatization: Amino acid **1**, its (2*R*)-diastereomer and the lactones **(3*S*)-8** and **(3*R*)-8** (each 2 mg) were mixed with 1-fluoro-2,4-dinitrobenzene (DNFB) (5 mg, about 1.5 equiv.) in phosphate buffer (1 mL, 20 mM, pH 7.4). The mixture was placed in an ultrasonic bath for 15 min at room temperature. A portion of the mixture (20 μL) was directly injected onto a reverse HPLC column (Hypersil, 5 μm, 250 × 4.6 mm i.d., Shandon HPLC, France). The eluent consisted of a mixture of acetonitrile (40%), water (60%) and trifluoroacetic acid (0.05%). Chromatograms were recorded at 347 nm.

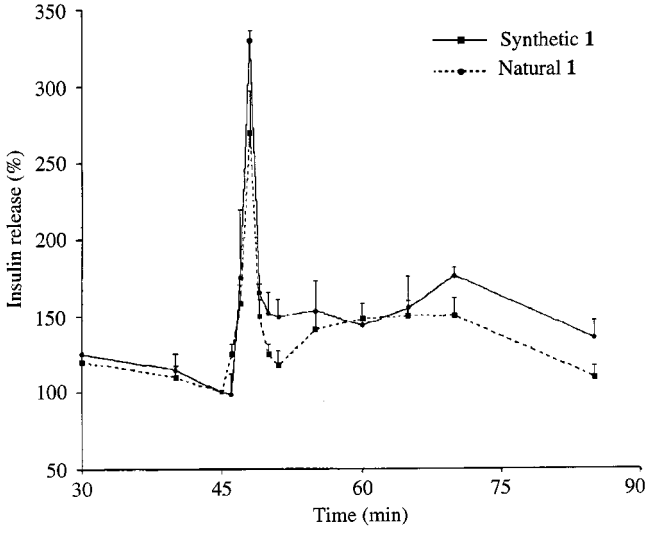


Figure 2. Effects of the synthetic and natural **1** on insulin released by the isolated, rat ex vivo pancreas, perfused in the presence of a slightly stimulating glucose concentration

Biological Activity of 1: In order to evaluate the insulinotropic potency of **1**, the effects of synthetic and natural 4-hydroxyisoleucine on insulin secretion were compared according to the literature method.[12] The results are shown in Figure 2. It should be noted that maximum insulin release is higher for synthetic **1**. This may be due to the fact that natural 4-hydroxyisoleucine contains approximately 4% of its (2*R*)-diastereomer as indicated in Figure 1.

Acknowledgments

We thank Sylvie Cortial and Philippe Lopes for their technical assistance and Jacques Rebello (Société Expansia, France) for helpful suggestions. We also thank Prof. Yves Sauvaire for providing us with authentic (2*S*,3*R*,4*S*)-4-hydroxyisoleucine and Prof. Gerard Ribes for performing biological tests.

[1] D. Porte, Jr., M. W. Schwartz, *Science* **1996**, *272*, 699−700.
[2] C. M. Clark, D. A. Lee, *N. Engl. J. Med.* **1995**, *332*, 1210−1217.
[3] G. Williams, *Lancet* **1994**, *343*, 95−100.
[4] L. Sorensen, D. K. Yue, page 41−49, in *Diabetes in Old Age*, 2nd ed. (Ed.: A. J. Sinclair, P. Finucane) **2001**, John Wiley & Sons Ltd. Chichester, UK.
[5] A. F. Amos, D. J. McCarthy, P. Zimmet, *Diabetic Medicine* **1997**, *14*, 1−85.
[6] J. Cobb, I. Dukes, *Annu. Rep. Med. Chem.* **1998**, *33*, 213−222.
[7] H. Shinkai, *Exp. Opin. Ther. Patents* **2000**, *10*, 59−66.
[8] Y. Sauvaire, P. Petit, C. Broca, M. Manteghetti, Y. Baissac, J. Fernandez-Alvarez, R. Gross, M. Roye, A. Leconte, R. Gomis, G. Ribes, *Diabetes* **1998**, *47*, 206−210.
[9] L. Fowden, H. M. Pratt, A. Smith, *Phytochemistry* **1973**, *12*, 1707−1711.
[10] N. W. Alcock, D. H. G. Crout, M. V. M. Gregorio, E. Lee, G. Pike, C. J. Samuel, *Phytochemistry* **1989**, *28*, 1835−1841.
[11] A. Gieren, P. Narayanan, W. Hoppe, M. Hasan, K. Michl, T. Wieland, H. O. Smith, G. Jung, E. Breitmaier, *Justus Liebigs Ann. Chem.* **1974**, 1561−1569.
[12] C. Broca, M. Manteghetti, R. Gross, Y. Baissac, M. Jacob, P. Petit, Y. Sauvaire, G. Ribes, *Eur. J. Pharmacology* **2000**, *390*, 339−345.
[13] For the synthesis of the (2*R*,3*R*,4*R*)- and (2*S*,3*R*,4*R*)-diastereomers see: T. Inghardt, T. Frejd, G. Svensson, *Tetrahedron* **1991**, *47*, 6469−6482.
[14] R. M. Williams, *Synthesis of Optically Active* α-*Amino Acids*, Pergamon: Oxford, **1989**.
[15] [15a] A. Tai, H. Watanabe, T. Harada, *Bull. Chem. Soc. Jpn.* **1979**, *52*, 1468−1472. [15b] R. W. Hoffman, W. Ladner, K. Steinbach, W. Massa, R. Schmidt, G. Snatzke, *Chem. Ber.* **1981**, *114*, 2786−2801. [15c] G. Fráter, U. Müller, W. Günther, *Tetrahedron* **1984**, *40*, 1269−1277. [15d] D. Seebach, I. Zimmermann, *Helv. Chim. Acta* **1986**, *69*, 1147−1152. [15e] M. Kitamura, T. Ohkuma, M. Tokunaga, R. Noyori, *Tetrahedron: Asymmetry* **1990**, *1*, 1−4. [15f] D. Buisson, C. Sanner, M. Larcheveque, R. Azerad, *Tetrahedron Lett.* **1987**, *28*, 3939−3940. [15g] D. Buisson, R. Azerad, C. Sanner, M. Larcheveque, *Biocatalysis* **1990**, *3*, 85−93. [15h] Y. Kawai, K. Takanobe, M. Tsujimoto, A. Ohno, *Tetrahedron Lett.* **1994**, *35*, 147−148. [15i] Y. Kawai, K. Takanobe, A. Ohno, *Bull. Chem. Soc. Jpn.* **1997**, *70*, 1683−1686. [15j] H. J. Bestman, B. Liepold, A. Kress, A. Hofmann, *Chem. Eur. J.* **1999**, *5*, 2984−2989.
[16] The *de* value was determined by GC analysis, *ee* was estimated by ^{1}H and ^{13}C NMR spectra of the (*S*)-*O*-acetyl lactate of **2**.
[17] P. L. Anelli, C. Biffi, F. Montanari, S. Quici, *J. Org. Chem.* **1987**, *52*, 2559−2562.
[18] F. Wang, X. Chen, Q. Chen, X. Qin, Z. Li, *J. Chromatogr.* **2000**, *883*, 113−118.

Received October 19, 2001
[O01505]