*Tetrahedron* Vol. 45, No. 19, pp. 6127 to 6134, 1989
Printed in Great Britain.

0040-4020/89 $3.00+.00
Pergamon Press plc

# SYNTHESIS OF ω-HYDROXY ANALOGUES OF VALINE, LEUCINE AND ISOLEUCINE

Sabine Englisch-Peters

Max-Planck-Institut für Experimentelle Medizin, Abteilung Chemie, Hermann-Rein-Str. 3, D-3400 Göttingen, FRG

(*Received in Germany* 15 *May* 1989)

The isomers of γ-hydroxy-valine (**6**) were prepared by a modified Erlenmeyer synthesis and the δ-hydroxy analogues each of leucine (**16a**) and isoleucine (**16b**) were synthesized via 1,4-Michael addition. The diastereomers of **6** could be separated by column chromatography, those of **16b** by reverse phase HPLC. The enantiomers of allo- and iso-γ-hydroxy-valine (**6**) were separated enzymatically using D- or L-amino acid oxidase which selectively oxidized the α-amino function of the D- or L-enantiomer to the corresponding α-keto acid.

## INTRODUCTION

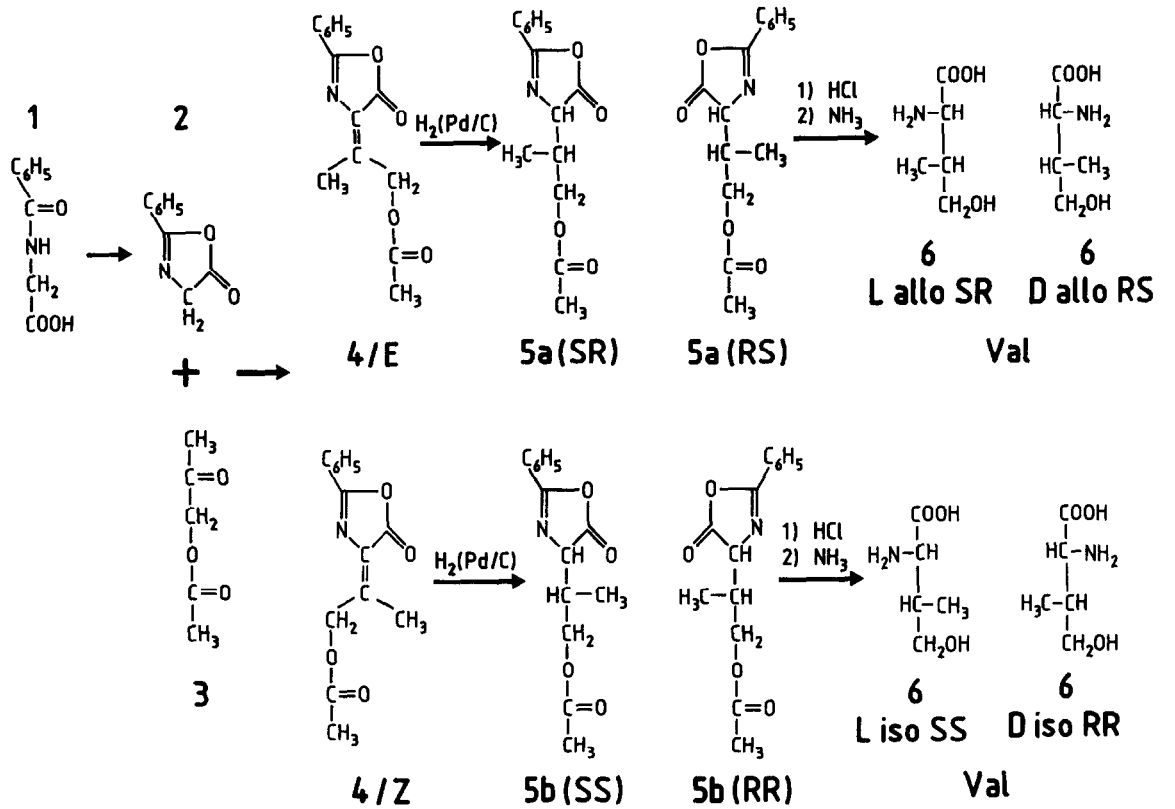
A key reaction in protein biosynthesis is the esterification of the natural amino acids to their corresponding transfer RNAs (tRNAs) catalyzed by the aminoacyl-tRNA synthetases.[1] The accuracy of these aminoacylations is a crucial problem because of the structural similarity among several amino acids. Isoleucyl-tRNA synthetase, for example, misactivates noncognate amino acids like valine and leucine. In a two-step "proofreading" mechanism the enzyme hydrolyzes these wrong aminoacyl-adenylates or misaminoacylated tRNAs prior to release of the product.[2] In order to investigate this "proofreading" activity in more detail we synthesized the substrate analogues γ-hydroxy-valine, δ-hydroxy-leucine and δ-hydroxy-isoleucine. The advantage of these analogues is that they can potentially form lactones during the hydrolysis and are thus good mechanistic probes. As well, these lactones are easily distinguishable from the free amino acids by thin layer chromatography.

## RESULTS AND DISCUSSION

### 1. Diastereoselective Synthesis of γ-Hydroxy-valine (6)

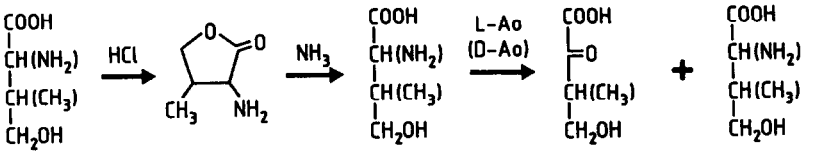
The amino acid γ-hydroxy-valine (α-amino-ß-methyl-γ-hydroxy-butyric acid) contains two asymmetric centres, and therefore exists as four stereoisomers: L allo SR, D allo RS, L iso SS and D iso RR. The synthesis was carried out using a modified protocol of the method described by E. Galantay et al..[3] Based on the classical Erlenmeyer method a diastereoselective preparation of

the diastereomers is achieved in four steps. Hippuric acid (**1**) is converted to the corresponding azlactone **2** by acetic acid anhydride. The azlactone serves as the methylene component in the aldol condensation with acetoxyacetone[4] (**3**) resulting in the formation of **4** in E- and Z-configuration. The E-diastereomer is formed with an excess of 99%, probably due to steric hindrance in the transition state leading to the Z product, thus the reaction is kinetically controlled. The diastereomers are separated by silica gel column chromatography and cis-addition of hydrogen led to the formation of the diastereomeric enantiomer pairs of the protected γ-hydroxy-valine (**5a**, **b**). The protecting azlactones were removed with hydrochloric acid and the resulting lactones were opened to the corresponding γ-hydroxy-valine isomers (**6**) by boiling with aqueous ammonia.



## 2. Purification of the γ-Hydroxy-valine Isomers and Preparation of Pure Enantiomers

To separate traces of valine γ-hydroxy-valine was converted to the corresponding lactone **7** and then extracted with chloroform, the contaminant valine remaining in the water phase. The lactone was then opened to the free amino acid. We separated the γ-hydroxy-valine enantiomers by oxidation of the D- or L-enantiomer with either D- or L-amino acid oxidase to give the corresponding α-keto acids (**8**).[5] The amino acids were separated by ion exchange column chromatography from the keto acids.

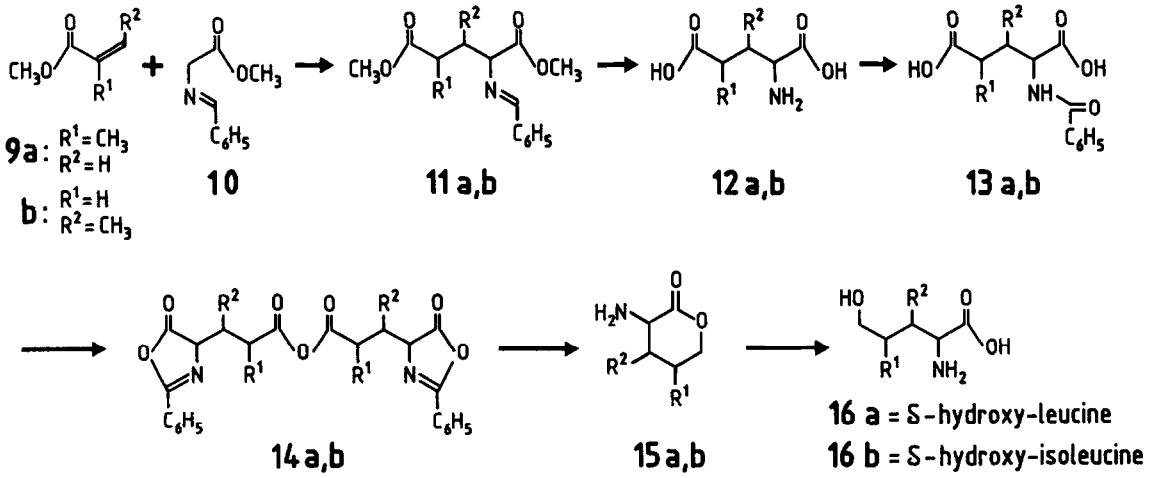



**6** **7** **6** **8** **6**

## 3. Synthesis of δ-Hydroxy-leucine (**16a**) and -isoleucine (**16b**)

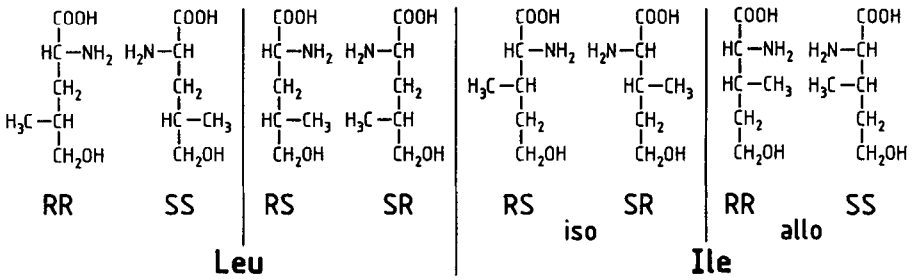
The two appropriate carbon skeletons for the δ-hydroxy analogues were formed by a 1,4 Michael addition of N-benzylidene-glycine methyl ester[6] (**10**) to either 2-methyl-propenoic acid methyl ester (**9a**) or methacrylic acid methyl ester (**9b**).[7] The resulting 1,4 adducts, N-benzylidene-γ-methyl-glutamic acid methyl ester (**11**) or N-benzylidene-β-methyl-glutamic acid methyl ester (**11b**), were hydrolyzed, through treatment with concentrated hydrochloric acid, yielding the corresponding γ- or β-methyl-glutamic acids (**12a** and **12b**). The overall yield of the synthesis was about 80% in both systems. In order to reduce the γ-carboxyl group of γ- or β-methyl-glutamic acid specifically both α-amino and α-carboxy functions had to be protected. The α-amino group was benzoylated and afterwards reacted with the α-carboxyl group to a five membered azlactone ring using dicyclohexyl-carbodiimide (DCC) as condensation reagent.[8] This intramolecular reaction is followed by an intermolecular anhydride formation upon activation of the γ-carboxy group by DCC. The resulting dimers (**14a** and **14b**) were obtained in a yield of about 65%. The NMR data and the elementary analysis confirmed the dimer formation. To get selective reduction of only the anhydride we used borane-tetrahydrofuran $BH_3$·THF.[9,10] The dimer was dissolved in tetrahydrofuran and $BH_3$·THF was added in a molar excess of 2:1. The reduced δ-hydroxy compounds were deprotected with concentrated hydrochloric acid and the lactone intermediates (**15a,b**)

formed were converted to the free δ-hydroxy-leucine (**16a**) and δ-hydroxy-isoleucine (**16b**) by alkaline hydrolysis. The yield of this reaction was about 50% for both amino acid analogues.




4. Separation of δ-Hydroxy-isoleucine Diastereomers (**16b**)

The resulting diastereomers of δ-hydroxy-isoleucine (allo RR;SS and iso RS; SR) were separated by HPLC on a reverse phase column. The retention times of the allo and iso form differed by about 30 seconds under the conditions used. The separated diastereomers were identified by $^1$H NMR spectroscopy.




5. Aminoacylation by Aminoacyl-tRNA Synthetases of these Analogues

Aminoacyl-tRNA synthetases are absolutely specific for the L-enantiomers of the corresponding amino acids. Therefore, in aminoacylation experiments pure L-enantiomers and DL-compounds as well can be applied. These synthesized compounds were used in aminoacylation assays of valyl-,[11] leucyl-[12] and isoleucyl-tRNA synthetases[13] from different organisms.

## EXPERIMENTAL

Column chromatography was performed using Merck (Darmstadt, FRG) silica gel, (0.063 - 0.2 mm) or Amberlite IR 120 (Serva, Heidelberg, FRG). The enzymes D- and L-amino acid oxidase and catalase were bought from Boehringer (Mannheim, FRG). Melting points were determined on a Reichert micromelting point apparatus and are uncorrected. The NMR spectrometers CU Bruker HX 60 and Bruker WH 270 were used for the collection of $^1H$ NMR data ($CDCl_3$, DCl or NaOD solution). Chemical shifts δ (ppm) are reported relative to tetramethylsilane as internal standard. Upon recording spectra in NaOD or DCl the chemical shifts are reported as well relative to the internal standard despite of using the $H_2O$ as indicator. Infrared spectra were recorded on a Perkin Elmer 325 IR spectrometer. Circular dichroism data were measured on a Jacso J-500 A spectropolarimeter. Optical rotation was performed using a Perkin-Elmer 141 polarimeter.

2-Phenyl-4(2-acetoxy-1-methylethylidene)-2-oxazoline-5-one (**4**): A mixture of 35 g (0.2 mol) hippuric acid (**1**), 61.2 g (0.6 mol) acetic anhydride, 27.8 g (0.24 mol) acetoxyacetone (**3**), 37.9 g (0.1 mol) anhydrous lead(II)acetate in 500 ml tetrahydrofuran was heated under reflux for 16 h in nitrogen atmosphere. After cooling to room temperature the mixture was filtrated and evaporated to dryness in vacuo. The resulting yellow oil was taken up in 700 ml of benzene and treated with hydrogen sulfide for 5 min at 10°C to precipitate the lead ions. The filtrated solution was again evaporated and dissolved in 10 ml chloroform. The two isomers were separated by column chromatography on silica gel (1.5 x 25 cm) using n-hexane/chloroform (92:8, v/v) as eluant. The E-isomer elutes from the column with 1400 ml of the eluant and the Z-isomer after 2500 ml. After evaporation colourless crystals of each isomer appeared. Recrystallization was performed using ethanol/ water.

E-2-Phenyl-4(2-acetoxy-1-methylethylidene)-2-oxazoline-5-one (**4**/E): Yield: 19.0 g (73.1 mmol, 63%). M.p.: 102°C, reported 100°C[3]. $^1H$ NMR ($CDCl_3$, tetramethylsilane): 2.40 (s, $CH_3$-C), 2.15 (s, $CH_3$-COO), 5.24 (s, Ar-O-$CH_2$) ppm. IR: 775, 695 (monosubst. ar.) 1640 (C=N), 1780 (C=O aryl ester), 1735 (C=O saturated ester) $cm^{-1}$. UV (max.): 314 nm. - Calc. C, 64.82; N, 5.40; H, 5.05; found C, 64.80; N, 5.40; H, 5.16%.

Z-2-Phenyl-4(2-acetoxy-1-methylethylidene)-2-oxazoline-5-one (**4**/Z): Yield: 0.1 g (0.4 mmol, 1.9%). M.p.: 86°C, reported 76°C[3]. $^1H$ NMR ($CDCl_3$, tetramethylsilane): 2.33 (s, $CH_3$-C), 2.10 (s, $CH_3$-COO), 5.37 (s, Ar-O-$CH_2$) ppm. IR: 775, 635 (monosubst. ar.), 1640 (C=N), 1780 (C=O), 1735 (C=O) $cm^{-1}$. UV (max.): 314 nm. - Calc. C, 64.82; N, 5.40; H, 5.05; found C, 65.02; N, 5.33; H, 5.20%.

D,L-Allo-γ-hydroxy-valine (**6**, allo): To a solution of 8.25 g (31.8 mmol) of **4**/E in 150 ml dioxane was added 1 g palladium-on-charcoal (10%) catalyst. The mixture was stirred in hydrogen atmosphere at room temperature for 4 - 6 h until 10 mol hydrogen was taken up (840 ml $H_2$ at 21°C/760 torr, saturated with dioxane). The filtrated solution was evaporated to dryness. The residue was boiled in 60 ml concentrated hydrochloric acid and 40 ml water for 4 h and further incubated at 4°C overnight. The filtrated solution was evaporated, the precipitate dissolved in 50 ml water and purified by ion exchange column chromatography (20 x 2.5 cm, Amberlite IR 120, $H^+$). After washing with 500 ml water, **6** was eluted with 300 ml $NH_3$ (aq). The eluate was boiled until no ammonia was detectable, evaporated and recrystallized from ethanol/ water. Yield: 3.41 g (26 mmol, 91%). M.p.: 225°C, reported 206°C.[3] $^1H$ NMR (1N NaOD): 0.80 (d, $CH_3$-C), 2.03-2.15 (m, $CH_3$-C), 3.27 (d, CH-$NH_2$), 3.40-3.62 (m, $CH_2$) ppm. IR: 1555 (C-$NH_3$+), 1635 (C=O), 2575 (OH), 3120, 3305 (NH) $cm^{-1}$. - Calc. C, 45.10; N, 10.52; H, 8.33; found C, 45.21; N, 10.44; H, 8.36%.

D,L-Iso-γ-hydroxy-valine (**6**, iso): Starting with a solution of 0.8 g (3.7 mmol) of 4/Z it was prepared as 6, allo. Yield: 329.6 mg (2.5 mmol, 78%). M.p.: 230°C. $^1H$ NMR (1N NaOD, $H_2O$): 0.93 (d, $CH_3$-C), 1.79-1.92 (m, $CH_3$-C-

$NH_2$), 3.18 (d, CH-$NH_2$), 3.55-3.69 (m, $CH_2$) ppm. IR: 1595 (C-$NH_3$+), 1635 (C=O), 3120, 3305 (NH), 2975 (OH) $cm^{-1}$. - Calc. C, 45.10; N, 10.52; H, 8.30; found C, 43.35; N, 10.50; H, 8.46%.

Lactones of Allo- and Iso-γ-hydroxy-valine (**7**): 300 mg (25 mmol) γ-hydroxy-valine was stirred with 20 ml 2.5% hydrochloric acid at room temperature for 15 min. After titration to pH 8 with 3% $NH_3$ (aq.) the solution was evaporated to dryness and extracted overnight in a Soxhlet extractor with chloroform. The solvent was removed and the lactone of γ-hydroxy-valine was remaining. Yield: 100%.

Allo-γ-hydroxy-valine-lactone (**7**): M.p.: 182°C. $^1H$ NMR (1N DCl): 1.05 (d, $CH_3$), 2.35 (m, CH-C-$CH_3$), 3.65 (d, CH-$NH_2$), 4.22 (d, $CH_2$) ppm. IR: 1810 (C=O), 1959 (NH), 3335 (NH) $cm^{-1}$.

Iso-γ-hydroxy-valine-lactone (**7**): M.p.: 181°C. $^1H$ NMR (1N DCl): 1.30 (d, $CH_3$), 2.35 (m, CH-$CH_3$), 3.98 (d, CH-$NH_2$), 4.22 (d, $CH_2$) ppm. The IR data are identical to the allo lactone.

L-Allo-(iso)-γ-hydroxy-valine (**6**) and α-Keto-β-methyl-γ-hydroxy-butyric Acid (**8**): 25 ml 0.2 M phosphate buffer pH 8.3 was saturated with oxygen by bubbling the gas through the solution for 20 min. Afterwards, 2 ml 1% $H_2O_2$, 10 µl catalase, (260000 U/ml; 30% glycerol; 10% ethanol), 140 mg (1 mmol) D,L-allo-(iso)-γ-hydroxy-valine and 300 µl D-amino acid oxidase (2.5 mg) were added. This mixture was incubated for one day at room temperature. After evaporation the residue was dissolved in 10 ml 2% acetic acid and applied to an ion exchange chromatography column (Amberlite IR 120, 2.5 x 20 ml). The column was washed with 1000 ml water and eluted with 1000 ml 1.5 N $NH_3$ (aq). The flow-through contains α-keto-β-methyl-γ-hydroxy-butyric acid which was isolated by filtration on a silica gel column (2 x 10 cm) with chloroform. The eluate contains L-allo-γ-hydroxy-valine (L-iso-γ-hydroxy-valine). After recrystallization from ethanol/water the yield was 70 mg (50%).

L-Allo-γ-hydroxy-valine: M.p.: 274°C. $[\alpha]_{20}^{D}$ = +8.6° (c = 1). CD: $\Delta\varepsilon$ = 0.117 (in water, $\lambda_{max}$ = 209 nm).

L-Iso-γ-hydroxy-valine. M.p.: 248°C. $[\alpha]_{20}^{D}$ = +10.6° (c = 1). CD: $\Delta\varepsilon$ = 0.21 (in water, $\lambda_{max}$ = 209 nm).

α-Keto-β-methyl-γ-hydroxy-butyric Acid: M.p.: 91°C. $^1H$-NMR ($CDCl_3$, tetramethylsilane) 2.0 (t, $CH_3$), 4.67 (q, $CH_2$) ppm. IR: 1700, 1725, 1740 (C=O), 2900 (OH) $cm^{-1}$.

D-Allo-(iso)-γ-hydroxy-valine (**6**) and α-Keto-β-methyl-γ-hydroxy-butyric Acid (**8**): 2 ml 0.2 M phosphate buffer pH 7.6 were saturated with oxygen and 1 ml 1% $H_2O_2$ solution, 2 ml catalase (260000 U/ml in 30% glycerol and 10 % ethanol), 1 ml 100 mM D,L-allo-(iso)-γ-hydroxy-valine in water and 200 µl L-amino acid oxidase (0.2 mg) solution were added. This mixture was incubated for two days at room temperature. The separation of the amino acid and the α-keto acid was performed as above.

D-Allo-γ-hydroxy-valine: M.p.: 248°C. $[\alpha]_{20}^{D}$ = -10.2° (c = 1). CD: $\Delta\varepsilon$ = -0.23 (in water, $\lambda_{max}$ = 209 nm).

D-Iso-γ-hydroxy-valine: M.p.: 274°C. $[\alpha]_{20}^{D}$ = -8.1° (c = 1). CD: $\Delta\varepsilon$ = -0.119 (in water, $\lambda_{max}$ = 209 nm).

γ-Methyl-glutamic Acid (**12a**): 5 g sodium (200 mmol) were dissolved in 250 ml dry ethanol. At 0°C 5 g (28 mmol) N-benzylidene-glycine methyl ester (**10**) and dropwise 2.8 g (28 mmol) 2-methyl-propenoic acid methyl ester (**9a**) were added. Stirring was continued for 2 h. After warming to room temperature the solution was diluted with 500 ml ether and washed with saturated aqueous $NaHCO_3$. The ether was removed in vacuo and the intermediate N-benzylidene-γ-methyl-glutamic acid dimethyl ester was hydrolyzed with 2 N HCl at room temperature by stirring overnight. The aqueous phase was extracted with ether several times. The product was purified by ion exchange chromatography (Amberlite IR 120, $H^+$). It was eluted with 1 N $NH_3$ (aq.), and then the solvent was removed in vacuo. Crystallization from aqueous ethanol gave a pure product in a yield of 3.6 g (22.4 mmol) = 78%. M.p. 200°C. - Calc. C, 44.41; N,

8.64; H, 7.42; found C, 44.60; N, 8.91; H, 7.06%. - $^1$H NMR ($CDCl_3$, tetramethylsilane) 0.9 (m, CH-CH), 2.1 (m, $CH_2$-COOH; CH-$CH_3$), 3.1 (3.2) (d, CH-$NH_2$) ppm.

β-Methyl-glutamic Acid (**12b**): In analogy to the preparation of γ-methyl-glutamic acid, N-benzylidene-glycine methyl ester (**10**) was treated with an equimolar amount of methacrylic acid methyl ester (**9b**). Yield: 3.2 g (22.0 mmol) = 79%. M.p. 205°C. - Calc. C, 44.42; N, 8.64; H, 7.4; found C, 44.81; N, 8.23; H, 7.90%. - $^1$H NMR ($CDCl_3$, tetramethylsilane) 1.1 (m, $CH_3$), 2.4 (m, $CH_2$), 3.4 (t, CH-$NH_2$), 3.6 (q, CH-COOH) ppm.

N-Benzoyl-γ-methyl-glutamic Acid (**13a**): 4 g (25 mmol) **12a** were dissolved in 20 ml 2 N NaOH at 0°C. 3.446 g (25 mmol) benzoyl chloride was added dropwise and the mixture was stirred at room temperature for 2 - 3 h. The solution was titrated to pH 3 using citric acid. The benzoylated amino acid was precipitated and filtered off. Yield: 6.3 g (24 mmol) = 96%. M.p. 110°C. - Calc. C, 58.87; N, 5.28; H, 5.66; found C, 59.01; N, 5.22; H, 5.92%. - $^1$H NMR ($CDCl_3$, tetramethylsilane) 0.9 (m, $CH_3$-CH), 2.1 (m, $CH_2$-COOH; CH-$CH_3$), 3.2 (3.3) (d, CH-NH-R), 2.8 (m, NH), 7.6 (m, Ar.), 10.7 (s, COOH) ppm.

N-Benzoyl-β-methyl-glutamic Acid (**13b**): **13b** was synthesized analogously to **13a**. Yield: 6.3 g (24 mmol) = 96%. M.p. 107°C. Calc. C, 58.87; N, 5.28; H 5.66; found C, 59.23; N, 5.32; H, 5.72%. $^1$H NMR ($CDCl_3$, tetramethylsilane) 1.2 ($CH_3$-C), 1.6 (m, $CH_2$), 2.8 (m, CH-$NH_2$), 3.7 (m, CH-$NH_2$), 7.6 (m, Ar.), 10.7 (s, -OH) ppm.

3-[4'(2'-Phenyloxazole-5'-one)]-2-methyl-propionic Acid Anhydride (**14a**): 10 g (38 mmol) **13a** were suspended in 500 ml dry tetrahydrofuran and at 0°C under argon flow 18 g (38 mmol) dicyclohexylcarbodiimide (solved in 50 ml tetrahydrofuran) was added. This mixture was warmed to room temperature and stirring was continued for 2 days. The dicyclohexylurea was filtered off and the tetrahydrofuran removed in vacuo. The crude product was solved in a small amount of $CHCl_3$/$CH_3OH$ and purified by silica gel column chromatography (2.5 x 40 cm). The column was washed with $CHCl_3$ and the pure product was eluted with 1 l $CHCl_3$/$CH_3OH$ (4:1, v/v). Yield: 6.8 g (13 mmol) = 66%. - Calc. C, 60.15; N, 5.01; H, 4.78; found C, 59.23; N, 4.92; H, 4.97%. - $^1$H NMR ($CDCl_3$, tetramethylsilane) 0.9 (m, $CH_3$-CH), 2.1 (m, $CH_2$-COOH; CH-$CH_3$), 3.2 (3.3) (d, CH-N=R), 7.6 (m, Ar.) ppm.

3-[4'(2'-Phenyloxazole-5'-one)]-3-methyl-propionic Acid Anhydride (**14b**): **14b** was synthesized analogously to **14a**. Yield: 6.8 g (13 mmol) = 66%. - Calc. C, 62.15; N, 5.01; H, 4.78; found C, 60.29; N, 5.27; H, 4.76%. - $^1$H NMR ($CDCl_3$, tetramethylsilane) 1.2 (m, $CH_3$-C), 1.6 (m, $CH_2$), 3.5 (m, $CH_3$-CH), 3.7 (m, CH-$NH_2$), 7.6 (m, Ar.) ppm.

δ-Hydroxy-leucine (**16a**): 5 g (10 mmol) of the azlactone was dissolved in 5 ml tetrahydrofuran, cooled to 0°C and treated dropwise with an equimolar amount of $BH_3$ over a period of one day. To hydrolyze the rest of THF·$BH_3$ a little bit water was added. After removing the solvent the product was incubated overnight with 7.2 N HCl. The solution was again evaporated to dryness, redissolved in a small amount of water and purified by ion exchange chromatography (Amberlite IR 120, $H^+$). The free amino acid was eluted from the column with 1 N $NH_3$ (aq.). After evaporating to dryness the crude product was recrystallized from ethanol/water. Yield: 0.68 g (5.6 mmol) = 50%. M.p. 190 - 210°C (decomposition). - Calc. C, 33.34; N, 9.60; H, 8.62; found C, 33.23; N, 9.12; H, 8.72%. - $^1$H NMR (NaOD) 0.9 (m, $CH_3$-C), 1.2 (sext., CH-$CH_3$), 2.1 (m, C-$CH_2$-CH), 3.1 (q, CH-$NH_2$), 3.7 (m, C-$CH_2$-OH) ppm.

δ-Hydroxy-isoleucine (**16b**): δ-Hydroxy-isoleucine was prepared analogously to δ-hydroxy-leucine. Yield: 0.68 g (5.6 mmol) = 50%. - Calc. C, 33.4; N, 9.60; H, 8.62; found C, 33.11; N, 9.12; H, 8.72%.

Separation of allo- and iso-δ-hydroxy-isoleucine was carried out by HPLC on a C18 reverse phase column. First, allo-δ-hydroxy-isoleucine (Rt 2.5 min) and then iso-δ-hydroxy-isoleucine (Rt 2.8 min) was obtained by isocratic elution with sodium acetate buffer pH 3.

Allo-δ-hydroxy-isoleucine: M.p. 190 - 210°C (decomp.). $^1$H NMR (NaOD) 0.9 (m, $CH_3$), 1.2 (sext., $CH-CH_3$), 2.3 (m, $CH_2$); 3.2 (d, $CH-NH_2$), 3.45 (m, $CH_2-OH$) ppm.
Iso-δ-hydroxy-isoleucine: M.p. 190 - 210°C (decomp.). $^1$H NMR (NaOD) 0.9 (m, $CH_3$), 1.2 (sext., $CH-CH_3$), 2.0 (m, $CH_2$), 3.1 (d, $CH-NH_2$), 3.62 (m, $CH_2-OH$) ppm.

REFERENCES

1. Schimmel, P. *Annu. Rev. Biochem.* **1987**, *56*, 125-158.
2. Englisch, U.; Gauss, D.; Freist, W.; Englisch, S.; Sternbach, H.; von der Haar, F. *Angew. Chem.* **1985**, *97*, 1033-1043; *Angew. Chem. Int. Ed. Engl. 24*, 1015-1025.
3. Galantay, E.; Szabo, A.; Fried, J. *J. Org. Chem.* **1963**, *28*, 98-102.
4. Levene, P. A.; Walti, A. *J. Biol. Chem.* **1928**, *79*, 363-368.
5. Neims, A. H.; Hellerman, L. *Annu. Rev. Biochem.* **1970**, *39*, 867-888.
6. Stork, G.; Leong, A. Y. W.; Touzin, A. M. *J. Org. Chem.* **1976**, *41*, 3491-3493.
7. Gerngross, O.; Olzay, A. *Chem. Ber.* **1963**, *96*, 2550-2555.
8. Touzin, A. M. *Tetrahedron Lett.* **1975**, 1477-1480.
9. Yoon, N. M.; Pak, C. S.; Brown, H. C.; Krishnamurthy, S.; Stocky, T. P. *J. Org. Chem.* **1973**, *38*, 2786-2792.
10. Brown, H. C.; Krishnamurthy, S. *Tetrahedron* **1979**, *35*, 567-607.
11. Englisch, S.; von der Haar, F.; Cramer, F. *Biochemistry* **1989**, in preparation.
12. Englisch, S.; Englisch, U.; von der Haar, F.; Cramer, F. *Nucleic Acids Res.* **1986**, *14*, 7529-7539.
13. Englisch, S.; von der Haar, F.; Cramer, F. **1989**, in preparation.