Pergamon

Tetrahedron: *Asymmetry* 11 (2000) 3151–3160

TETRAHEDRON: *ASYMMETRY*

Synthesis of all four possible stereoisomers of 5-hydroxylysine

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Received 21 June 2000; accepted 17 July 2000

Abstract

A simple protocol for the transformation of L- and D-glutamic acids into the enantiopure forms of all four isomers of 5-hydroxylysine is described.

1. Introduction

(2*S*,5*R*)-5-Hydroxylysine **1** (L-*normal*-5-hydroxylysine; Fig. 1) is an amino acid exclusive to collagen protein. It forms by post-translational hydroxylation of lysine and can be successively glycosylated with either a β-D-galactopyranosyl- or an α-D-glucopyranosyl-(1-2)-β-D-galactopyranosyl residue.[1,2] The extent of glycosylation differs for each collagen type but, in all cases, during collagen degradation, (2*S*,5*R*)-5-hydroxylysine is excreted in urine mainly as glycoside.[3] Thus, after an acidic hydrolysis, the (2*S*,5*R*)-5-hydroxylysine levels in human urine are commonly

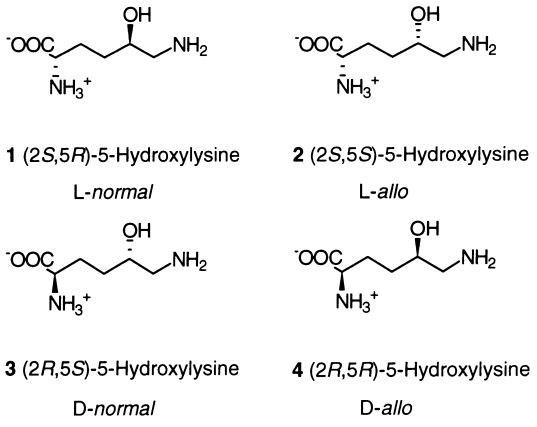

Figure 1.

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PII: S0957-4166(00)00281-0

measured to evaluate the collagen breakdown.[4] Moreover, during the hydrolysis, the (2*R*,5*R*)-5-hydroxylysine **4** (D-*allo*-5-hydroxylysine) is also formed and any analytical method must detect this 2-epimer also.[4]

The hydroxylysine glycosylates are also of relevance for immunological studies concerning the autoimmune response evidenced in rheumatoid arthritis.[5]

Surprisingly, while many authors have reported[6–8] convenient protections of (2*S*,5*R*)-5-hydroxylysine **1** to allow the study of the glycosylation of its hydroxy group, no stereoselective synthesis of the possible stereoisomers of this amino acid has been reported. All published syntheses[9–12] afford a mixture of the four possible 5-hydroxylysine stereoisomers and delicate chemical and enzymatic procedures are then necessary for their separation.[13,14] On the other hand, (2*R*,5*R*)-5-hydroxylysine **1**, the only pure isomer commercially available, is expensive since it is obtained by tedious procedures from gelatine acid hydrolysates which also contain the (2*R*,5*R*)-epimer **4** which forms by epimerization during the acidic hydrolysis.[15]

In a very recent paper,[16] a strategy for the stereoselective synthesis of 5-hydroxylysine was proposed which, however, is satisfactory only[16] for the preparation of (2*S*,5*S*)-hydroxylysine **2**. Thus, we decided to report on the synthesis of both (2*S*,5*R*)- and (2*S*,5*S*)-5-hydroxylysine **1** and **2** and of their diastereoisomer (2*R*,5*R*)- and (2*R*,5*S*)-5-hydroxylysine **3** and **4**, planned as part of a larger program concerning various markers of collagen turnover.[17,18]

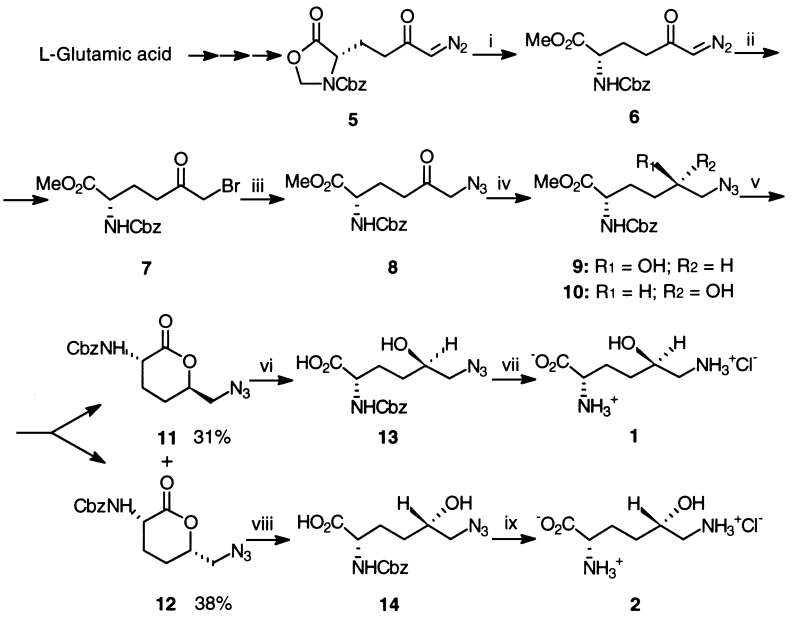
The results, here reported, concern the synthesis of (2*S*,5*R*)-5-hydroxylysine **1** and of (2*S*,5*S*)-5-hydroxylysine **2** starting with L-glutamic acid. However, they represent a verification of a general strategy which, starting from commercial D-glutamic acid, also allows the preparation of the corresponding D-enantiomers **3** and **4**.

2. Results and discussion

According to our protocol, the known diazo ketone **5**, prepared[19] in three steps from L-glutamic acid, is transformed into the azido ketone **8** which, by reduction with sodium borohydride, affords the diastereoisomeric hydroxy esters **9** and **10** (Scheme 1).

In this sequence of well-established reactions, the easy transformation of the oxazolidinone **5** into the corresponding methyl ester **6**, performed by mild treatment with $NaHCO_3$ in methanol, is new and noteworthy. In previous works[20,21] similar transformations were performed in the presence of sodium methoxide which could put in danger the enantiomeric purity of the amino acid.

Unfortunately, all attempts to separate the diastereoisomeric hydroxy esters **9** and **10** were unsuccessful even when the hydroxy group was esterified with achiral or chiral acids (acetates, trifluoroacetates, benzoates, Mosher esters).[22] However, the intramolecular transesterification of the 5-hydroxyl group with the methyl ester of the amino acid afforded a mixture of diastereoisomeric *cis* and *trans* lactones (2*S*,5*R*)-**11** and (2*S*,5*S*)-**12**, which showed a different polarity on normal phase HPLC and were easily separated by rapid chromatography on silica. The lactonization of the hydroxy esters **9** and **10** was performed with TFA, an acid commonly used in amino acid chemistry for the regeneration of the amino acid functionalities from *tert*-butyl derivatives without affecting their stereochemical integrity. In any case, the presence of a stereogenic center α to the lactonic carbonyls of **11** and **12**, suggests a possible epimerization of the lactones in the acid condition necessary for their formation. This unfavourable event could transform, at least in part, the initially formed lactones (2*S*,5*R*)-**11** and (2*S*,5*S*)-**12** into the



Scheme 1. Reagents and conditions: (i) $NaHCO_3$, MeOH, reflux, 10 min, 85%; (ii) HBr 33% in AcOH, THF, 0°C, 82%; (iii) NaN_3, DMF, rt, 1 h, 86%; (iv) $NaBH_4$, MeOH, 0°C, 20 min, 92%; (v) TFA, rt, 40 min; (vi) Cs_2CO_3, MeOH:H_2O 1:1 v/v, rt, 35 min, 85%; (vii) H_2 Pd/C, MeOH:H_2O 1:1 v/v, then pH 6.5–7.0, 75%; (viii) α-chymotrypsin, phosphate buffer (pH 7.4)/Me_2CO, 25°C, 12 h, 96%; (ix) H_2 Pd/C, MeOH:H_2O 1:1 v/v, then pH 6.5–7.0, 78%

reversed pair of epimers (2*R*,5*R*)-**12** and (2*R*,5*S*)-**11** (Table 1), with a consequent reduction of the enantiomeric purity of the diastereoisomers isolated at the end of the reaction.

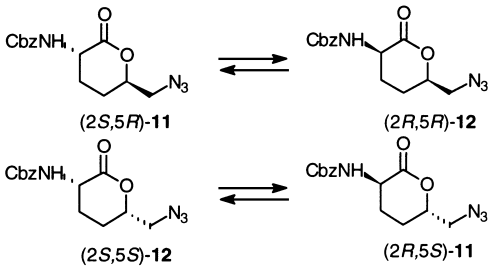
In order to check this possibility, we separately tested the stability of lactones **11** and **12** under various lactonization conditions. The results of this study led to the choice of the conditions (TFA, rt, 40 min) which caused the complete lactonization of hydroxy methyl esters **9** and **10** without modifying the geometry of the initially formed lactones.

The next step of the synthesis required the transformation of lactones **11** and **12** into the corresponding hydroxy acids **13** and **14** under basic conditions. Thus, a possible α epimerization of lactones **11** and **12** was taken in account. In order to exclude this event, the lactone **11** was hydrolyzed under various basic conditions and the crude product of the reaction (the hydroxy acid **13**) was directly treated with TFA (rt, 10 min) to regenerate the starting lactone group (Scheme 2). The stereochemical purity of the resulting lactone was then checked by HPLC and it was possible to select the milder conditions for the opening of the lactone **11** to the hydroxy acid **13** without α-epimerization: a simple treatment with Cs_2CO_3 of the lactone **11**, dissolved in a mixture of methanol and water (50%, v/v), followed by acidification. In this way, only the (2*S*,5*R*)-hydroxy acid **13** was obtained which, by cyclization, afforded the stereochemically pure starting lactone (2*S*,5*R*)-**11**.

In the case of the *cis* lactone **12** a slower hydrolysis reaction was observed (6 h with Cs_2CO_3 in MeOH/H_2O) with a longer exposure of *cis* lactone **12** to basic conditions. This resulted in a partial equilibration of this lactone with the epimer (2*R*,5*S*)-**11** and, consequently, the desired hydroxy acid **14** was accompanied by 12% of inseparable diastereoisomer (2*R*,5*S*)-**13**.

Under different hydrolysis conditions (Table 2) the hydroxy acid **14** was obtained in nearly satisfactory diastereoisomeric purity (see entry 3 in Table 2). However, in order to improve its

Table 1
Study on α-epimerization of lactones (2*S*,5*R*)-**11** and (2*S*,5*S*)-**12**

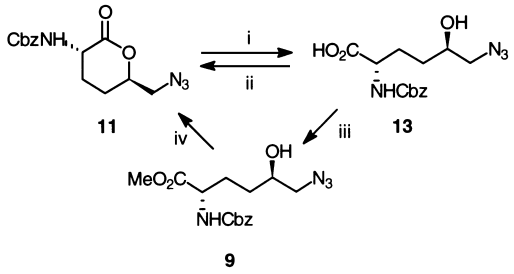


Starting lactone[a]	Conditions[b]		%
	Medium	Temperature; time	of α epimerization[c]
(2*S*,5*R*)-**11**	25% TFA in toluene	80°C; 20 min	5.3
(2*S*,5*S*)-**12**	25% TFA in toluene	80°C; 20 min	4.5
(2*S*,5*R*)-**11**	25% TFA in toluene	25°C; 2.0 h	2.4
(2*S*,5*S*)-**12**	25% TFA in toluene	25°C; 2.0 h	2.1
(2*S*,5*R*)-**11**	TFA	25°C; 40 min	0.0
(2*S*,5*S*)-**12**	TFA	25°C; 40 min	0.0

a For a 0.1 M solution.

b These conditions cause the complete lactonization of the methyl esters **9** and **10**.

c Evaluated by HPLC.



Scheme 2. Reagents and conditions: (i) Cs_2CO_3, MeOH:H_2O 1:1 v/v, rt, 35 min, 85%; (ii) TFA, rt, 10 min; (iii) CH_2N_2, Et_2O, rt, 10 min; (iv) TFA, rt, 40 min

preparation, the possibility of performing the hydrolysis under neutral conditions mediated by enzymes was considered.

Initial attempts to use various lipases,[23] operating in a phosphate buffer (pH 7.4), were unsuccessful and the unchanged starting *cis* lactone (2*S*,5*S*)-**12** was recovered. The goal was then achieved using commercial α-chymotrypsin[24] which permits the desired hydroxy acid (2*S*,5*S*)-**14** to be obtained quantitatively in pure form.

Table 2
Hydrolysis of the lactone **12** to the acid **14**

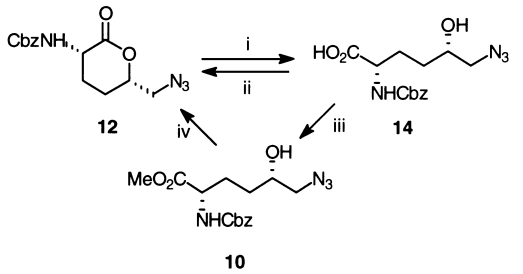
Conditions[a]	Time (h); yield (%)	α epimerization[b](%)
Cs_2CO_3 0.3 M(H_2O)/MeOH; 1:1	6.0; 87	12
Li_2CO_3 0.2 M(H_2O)/MeOH; 1:1	5.5; 83	8.9
Li_2CO_3 0.2 M(H_2O)/THF; 1:1	6.0; 85	4.3
KOH 1 M(H_2O)/THF; 1:20	2.5; 89	8.3
KOH 1 M(H_2O)/dioxane; 1:20	3.0; 80	9.0
KOH 1 M(H_2O)/THF; 1:1	1.0; 83	12
KOH 1 M(H_2O)/MeOH; 1:1	1.0; 85	11

a All reactions was performed at 25°C.

b Determined by HPLC after recyclization with TFA.

With hydroxy acids **13** and **14** in hand it was possible to prove experimentally that no α-epimerization had occurred during the initial transformation of the methyl esters **9** and **10** into the corresponding lactones (Schemes 2 and 3). In fact, separate treatment with TFA of the methyl esters (**9** and **10**, obtained from the pure acids **13** and **14**) give the diastereoisomeric pure lactones (2*S*,5*R*)-**11** and (2*S*,5*S*)-**12** (HPLC).

The synthesis was then accomplished in one step by simple hydrogenolysis of the hydroxy acids **13** and **14**. The formed hydroxylysines **1** and **2** were isolated as crystalline monohydrochlorides which showed physicochemical properties (specific rotation, ^{13}C NMR) in agreement with those reported.[13,25] Their stereoisomeric purity (> 98%) was more accurately established by means of GLC using a suitable chiral column.[26] This verification, performed on the corresponding *tris*-trifluoroacetates methyl esters, allowed retention times to be assigned to each isomer. These data should permit the detection of the presence of other isomers, now considered unnatural, in biological materials and solve such various analytical problems.



Scheme 3. Reagents and conditions: (i) α-chymotrypsin, phosphate buffer (pH 7.4)/Me_2CO, 25°C, 12 h; (ii) TFA, rt, 10 min; (iii) CH_2N_2, Et_2O, rt, 10 min; (iv) TFA, rt, 40 min

3. Experimental

3.1. General

Nuclear magnetic resonance spectra were recorded at 303 K on a Bruker AM-500 spectrometer operating at 500.13 MHz for ^{1}H and 125.76 MHz for ^{13}C. Chemical shifts are reported in parts per million (ppm, δ units) relative to $CHCl_3$ fixed at 7.24 ppm or to HDO fixed at 4.54 ppm for the ^{1}H spectra and relative to dioxane fixed at 67.60 ppm for the ^{13}C spectra. ^{1}H NMR data are tabulated in the following order: number of protons, multiplicity (s, singlet; d, doublet; bs, broad singlet; m, multiplet), coupling constants (J) in hertz, assignment of proton(s). IR spectra were determined with a Perkin–Elmer 1420 infrared recording spectrophotometer. Optical rotations were taken at 25°C on a Perkin–Elmer 241 polarimeter. Chiral GLC analyses were carried out on a Hewlett–Packard 5890 gas chromatography equipped with an octakis(3-*O*-butyryl-2,6-di-*O*-pentyl)-γ-cyclodextrin (Lipodex E)[26] capillary column (25 m, 0.25 mm ID, purchased from Macherey–Nagel); carrier gas was He set at 85 kPa column head pressure and the column temperature was set at 170°C. HPLC analyses were carried out on a silica direct phase column (superspher Si-60, 25 cm, 4 mm ID, purchased from Merck); the mobile phase was hexane:2-propanol, 75:25, v/v; the flow rate was 1 mL/min and the detection was performed at 221 nm. All reactions were monitored by thin-layer chromatography (TLC) carried out on 0.25 mm E. Merck silica gel plates (60 F_{254}) using UV light, 50% sulphuric acid or 0.2% ninhydrin in ethanol and heat as developing agent. E. Merck 230–400 mesh silica gel was used for flash column chromatography.[27] Usual work-up refers to washing the organic layer with water, drying over Na_2SO_4, and evaporating the solvent under reduced pressure. α-Chymotrypsin (EC 3.4.21.1), type II from Bovine Pancreas was obtained from Aldrich (Milwaukee, WI; cat. no. C 4129).

3.2. Methyl (S)-2-benzyloxycarbonylamino-6-diazo-5-oxohexanoate **6**

A mixture of (*S*)-3-(benzyloxycarbonyl)-4-(4-diazo-3-oxobutyl)-5-oxazolidinone **5** (5.0 g, 15.8 mmol, $[\alpha]_D$ +98.2 (*c* 1, $CHCl_3$), lit.[28] +99.8), $NaHCO_3$ (3.0 g) and MeOH (500 mL) was refluxed for 10 min. After cooling at room temperature and filtration on Celite, the solvent was evaporated, the residue oil was dissolved in AcOEt (80 mL) and worked up to afford a crude oil which, after column chromatography (eluting with hexane:AcOEt, 50:50, v:v), afforded the pure **6** (4.3 g, 85%): an oil; $[\alpha]_D$ +13.8 (*c* 1, $CHCl_3$) [lit.[29] –14.4 (*c* 1, $CHCl_3$)]; $[\alpha]_D$ –4.2 (*c* 1, AcOEt) [lit.[30] –7.5 (*c* 1, AcOEt)]; IR (film) 2100, 1730, 1710, 1630 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 7.36–7.29 (5H, aromatics-H), 5.45 (1H, d, J = 5.6, N*H*), 5.20 (1H, bs, 6-H), 5.12–5.06 (2H, AB system, OCH_2Ph), 4.35 (1H, m, 2-H), 3.73 (3H, s, OCH_3), 2.43–2.32 (2H, overlapping, 4-Ha and 4-Hb), 2.20 (1H, m, 3-Ha), 1.99 (1H, m, 3-Hb). Anal. calcd for $C_{15}H_{17}N_3O_5$: C, 56.42; H, 5.37; N, 13.16. Found: C, 56.87; H, 5.51; N, 13.03.

3.3. Methyl (S)-2-benzyloxycarbonylamino-6-bromo-5-oxohexanoate **7**

To a light yellow solution of methyl (*S*)-2-benzyloxycarbonylamino-6-diazo-5-oxohexanoate **6** (4.0 g, 12.5 mmol) in THF (40 mL) containing Thymol Blue (5 mg), a solution of HBr in AcOH (33%) was added dropwise under stirring at –5°C. When the solution colour turned violet, a saturated solution of $NaHCO_3$ was added to neutralize the acids. Then AcOEt (150 mL) was added and the mixture was worked up to afford a crude product which, after column chromatography

(eluting with hexane:AcOEt, 70:30, v:v), gave pure **7** (3.8 g, 82%): m.p. 86–87°C (from CH_2Cl_2/diisopropyl ether); $[\alpha]_D$ +2.1 (*c* 1, $CHCl_3$); IR (Nujol) 1735, 1690 cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.35–7.27 (5H, aromatics-H), 5.39 (1H, d, $J=6.7$, N*H*), 5.11–5.04 (2H, AB system, OCH_2Ph), 4.34 (1H, m, 2-H), 3.83 (2H, s, 6-H_2), 3.72 (3H, s, OCH_3), 2.78–2.65 (2H, overlapping, 4-Ha and 4-Hb), 2.20 (1H, m, 3-Ha), 1.91 (1H, m, 3-Hb). Anal. calcd for $C_{15}H_{18}BrNO_5$: C, 48.40; H, 4.87; N, 3.76. Found: C, 48.13; H, 4.96; N, 5.37.

3.4. *Methyl (*S*)-6-azido-2-benzyloxycarbonylamino-5-oxohexanoate* **8**

To a solution of methyl (*S*)-2-benzyloxycarbonylamino-6-bromo-5-oxohexanoate **7** (3.5 g, 9.4 mmol) in DMF (15 mL) cooled at 0°C, NaN_3 (0.85 g, 13 mmol) was added under stirring. After stirring at 0°C for 1 h, the mixture was diluted with AcOEt (100 mL) and worked up to afford, after column chromatography (eluting with hexane:AcOEt, 50:50, v:v), the pure azido ketone **8** (2.7 g, 86%): m.p. 70–72°C (from CH_2Cl_2/benzene); $[\alpha]_D$ +14.9 (*c* 1, $CHCl_3$); IR (film) 2100, 1780, 1520 cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.36–7.29 (5H, aromatics-H), 5.14 (1H, d, $J=7.2$, N*H*), 5.10–5.04 (2H, AB system, OCH_2Ph), 4.34 (1H, ddd, $J=8.2$, 7.2, 5.1, 2-H), 3.91–3.83 (2H, AB system, 6-H_2), 3.72 (3H, s, OCH_3), 2.55 (1H, m, 4-Ha), 2.45 (1H, m, 4-Hb), 2.23 (1H, m, 3-Ha), 1.91 (1H, m, 3-Hb). Anal. calcd for $C_{15}H_{18}N_4O_5$: C, 53.89; H, 5.43; N, 16.76. Found: C, 53.61; H, 5.27; N, 16.81.

3.5. *Methyl (2*S*,5*R*)- and (2*S*,5*S*)-6-azido-2-benzyloxycarbonylamino-5-hydroxyhexanoates* **9** *and* **10**

To a solution of methyl (*S*)-6-azido-2-benzyloxycarbonylamino-5-oxohexanoate **8** (4 g, 12 mmol) in MeOH (50 mL), $NaBH_4$ (590 mg, 15.5 mmol) was gradually added at –5°C. The mixture was stirred at –5°C for 20 min, treated with water (10 mL), acidified with aqueous HCl (2 M) and extracted with AcOEt (100 mL). Usual work-up afforded a chromatographically inseparable mixture of diastereoisomers **9** and **10** (3.7 g, 92%, in a 1:1 ratio) which showed in the ^1H NMR spectrum, diagnostic signal at δ 5.44 and 5.37 ppm corresponding to N*H* signal, respectively, for **9** and **10**.

3.6. *(2*S*,5*R*)-5-Azidomethyl-2-benzyloxycarbonylamino-δ-valerolactone* **11** *and (2*S*,5*S*)-5-azidomethyl-2-benzyloxycarbonylamino-δ-valerolactone* **12**

The mixture of diastereoisomeric azido esters **9** and **10** (4.0 g, 12 mmol) was dissolved in CF_3CO_2H (6.0 mL) and the solution was stirred at room temperature for 40 min. The solvent was then carefully removed under reduced pressure (under 40°C) and the residue (3.2 g) was quickly chromatographed on column (eluting with CH_2Cl_2:AcOEt, 100:10, v:v) to afford first the lactone **12** (1.37 g, 38%): m.p. 90–92°C (from CH_2Cl_2/diisopropyl ether); HPLC: $R_t=8.9$ min; $[\alpha]_D$ +56.2 (*c* 1, $CHCl_3$); IR (film) 2110, 1755, 1715 cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.36–7.29 (5H, aromatics-H), 5.62 (1H, d, $J=4.2$, N*H*), 5.10 (2H, s, OCH_2Ph), 4.51 (1H, m, 6-H), 4.46 (1H, m, 3-H), 3.46 (2H, d, $J=4.4$, CH_2-N_3), 2.63 (1H, m, 4-Ha), 2.00 (1H, m, 5-Ha), 1.87 (1H, m, 5-Hb), 1.62 (1H, m, 4-Hb). Anal. calcd for $C_{14}H_{16}N_4O_4$: C, 55.26; H, 5.30; N, 18.41. Found: C, 55.49; H, 5.41; N, 18.35.

Further elution gave the lactone **11** (1.12 g, 31%): m.p. 85–86°C (from CH_2Cl_2/diisopropyl ether); HPLC: $R_t=11.7$ min; $[\alpha]_D$ –19.9 (*c* 1, $CHCl_3$); IR (film) 2110, 1755, 1715 cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.36–7.29 (5H, aromatics-H), 5.52 (1H, bs, N*H*), 5.10 (2H, s, OCH_2Ph), 4.51 (1H, bs, 6-H), 4.14 (1H, m, 3-H), 3.52 (1H, dd, $J=13.0$, 3.5, C*H*H-N_3), 3.40 (1H, dd, $J=13.0$, 3.9, CH*H*-N_3),

2.47 (1H, m, 4-Ha), 1.98–1.82 (3H, overlapping, 4-Hb, 5-Ha and 5-Hb). Anal. calcd for $C_{14}H_{16}N_4O_4$: C, 55.26; H, 5.30; N, 18.41. Found: C, 55.60; H, 5.25; N, 18.70.

Finally, a washing of the column with CH_2Cl_2:MeOH (70:30, v/v) afforded an inseparable mixture of corresponding acids **13** and **14** (0.57 g, 15%), probably formed by a silica gel opening of the lactonic ring. These acids could be recovered and subjected again to the lactonization.

3.7. (2S,5R)-6-Azido-2-benzyloxycarbonylamino-5-hydroxyhexanoic acid **13**

To a solution of (3*S*,6*R*)-3-benzyloxycarbonylamino-6-azidomethyltetrahydro-2*H*-2-pyranone **11** (1.0 g, 3.29 mmol) in MeOH (25 mL) an aqueous solution of Cs_2CO_3 (0.27 M, 25 mL) was added. The resulting light opalescent mixture was stirred at room temperature for 35 min and then acidified with aqueous HCl (4 M). Addition of AcOEt (100 mL) and the usual work-up afforded pure acid **13** (995 mg, 94%): an oil; $[\alpha]_D$ +13.2 (*c* 1, $CHCl_3$); IR (film) 3350, 2100, 1730, 1710, 1690 cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.36–7.29 (5H, aromatics-H), 5.68 (1H, d, $J=7.0$, N*H*), 5.11–5.05 (2H, AB system, OCH_2Ph), 4.39 (1H, m, 2-H), 3.74 (1H, m, 5-H), 3.28 (1H, dd, $J=11.8$, 2.4, 6-Ha), 3.21 (1H, dd, $J=11.8$, 6.6, 6-Hb), 2.02 (1H, m, 3-Ha), 1.75 (1H, m, 3-Hb), 1.54 (2H, m, 4-H_2). Anal. calcd for $C_{14}H_{18}N_4O_5$: C, 52.17; H, 5.63; N, 17.38. Found: C, 52.04; H, 5.80; N, 17.27.

3.8. (2S,5S)-6-Azido-2-benzyloxycarbonylamino-5-hydroxyhexanoic acid **14**

A solution of (3*S*,6*S*)-3-benzyloxycarbonylamino-6-azidomethyltetrahydro-2*H*-2-pyranone **12** (400 mg, 1.32 mmol) in acetone (5.2 mL) was added to a solution of α-chymotrypsin (220 mg) in 0.1 M phosphate buffer (52 mL, pH 7.4). The suspension was stirred at 25°C for 12 h. When a solution formed this was acidified with aqueous HCl (4 M) and extracted with AcOEt (60 mL). The usual work-up afforded the pure acid **14** (406 mg, 96%): $[\alpha]_D$ +16.9 (*c* 1, $CHCl_3$); IR (film) 3350, 2100, 1730, 1710, 1690 cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.36–7.29 (5H, aromatics-H), 5.63 (1H, d, $J=7.2$, N*H*), 5.11–5.05 (2H, AB system, OCH_2Ph), 4.41 (1H, m, 2-H), 3.77 (1H, m, 5-H), 3.29 (1H, dd, $J=11.4$, 2.5, 6-Ha), 3.21 (1H, dd, $J=11.4$, 6.8, 6-Hb), 1.95 (1H, m, 3-Ha), 1.88 (1H, m, 3-Hb), 1.52 (2H, m, 4-H_2). Anal. calcd for $C_{14}H_{18}N_4O_5$: C, 52.17; H, 5.63; N, 17.38. Found: C, 52.35; H, 5.51; N, 17.49.

3.9. Regeneration of the lactonic ring

3.9.1. Directly from hydroxy acid **13** or **14**

Each acid **13** or **14** (10 mg) was dissolved in CF_3CO_2H (0.2 mL) and the solution was kept at room temperature for 10 min. The solvent was then removed under a nitrogen stream to afford the corresponding diastereoisomeric pure azido lactones **11** or **12** in quantitative yields (diastereoisomeric purity was checked by HPLC).

3.9.2. From methyl esters **9** or **10**

Each acid **13** or **14** (30 mg) dissolved in AcOEt (1 mL) was treated with excess CH_2N_2 in Et_2O at room temperature for 10 min. The solvent was then removed under a nitrogen stream to afford, in quantitative yields, the corresponding methyl ester **9** [^1H NMR ($CDCl_3$) δ 7.36–7.29 (5H, aromatics-H), 5.44 (1H, d, $J=6.9$, N*H*), 5.12–5.07 (2H, AB system, OCH_2Ph), 4.42 (1H, m, 2-H), 3.73 (4H, bs, overlapping, 5-H and OCH_3), 3.31 (1H, dd, $J=12.2$, 3.6, 6-Ha), 3.23 (1H, dd,

J = 12.2, 7.0, 6-Hb), 2.02 (1H, m, 3-Ha), 1.73 (1H, m, 3-Hb), 1.53 (2H, m, 4-H_2)] or **10** [^{1}H NMR ($CDCl_3$) δ 7.36–7.29 (5H, aromatics-H), 5.37 (1H, d, J = 7.2, N*H*), 5.11–5.07 (2H, AB system, OCH_2Ph), 4.40 (1H, m, 2-H), 3.73 (4H, bs, overlapping, 5-H and OCH_3), 3.33 (1H, dd, J = 12.4, 2.2, 6-Ha), 3.21 (1H, dd, J = 12.4, 7.7, 6-Hb), 1.94 (1H, m, 3-Ha), 1.82 (1H, m, 3-Hb), 1.49 (2H, m, 4-H_2)].

The methyl ester **9** or **10** (25 mg) was dissolved in CF_3CO_2H (0.2 mL) and the solution was kept at room temperature for 40 min. The solvent was then removed under a nitrogen stream to afford the corresponding diastereoisomeric pure azido lactones **11** or **12** in quantitative yields (diastereoisomeric purity was checked by HPLC).

3.10. (2S,5R)-5-Hydroxylysine monohydrochloride *1*

A solution of (2*S*,5*R*)-6-azido-2-benzyloxycarbonylamino-5-hydroxyhexanoic acid **13** (800 mg, 2.48 mmol) in MeOH:H_2O (80 mL, 1:1, v/v) was hydrogenated for 15 h, in the presence of palladium on charcoal at room temperature and atmospheric pressure. Filtration of the catalyst, evaporation of the MeOH and lyophilization afforded a residue which was dissolved in water (1.5 mL) and the pH adjusted to 6.5–7.0 with HCl (4 M). The addition of EtOH (2.5 mL) and the storage at 0°C effected the precipitation of (2*S*,5*R*)-5-hydroxylysine (*normal*-L-hydroxylysine) monohydrochloride **1** (368 mg, 75%): $[\alpha]_D$ +15.2 (HCl 6 M, *c* 1) [lit.[13] +14.5 (*c* 2)]; ^{1}H NMR (D_2O): δ 3.69 (1H, m, 5-H), 3.59 (1H, dd, J = 6.1, 6.1, 2-H), 2.97 (1H, dd, J = 13.3, 3.1, 6-Ha), 2.73 (1H, dd, J = 13.3, 9.6, 6-Hb), 1.87 (1H, m, 3-Ha), 1.72 (1H, m, 3-Hb), 1.45–1.38 (2H, overlapping, 4-H_2); ^{13}C NMR (D_2O): δ 175.37 (C-1), 68.27 (C-5), 55.43 (C-2), 45.35 (C-6), 30.56 (C-4), 27.59 (C-3).[25] Anal. calcd for $C_6H_{15}ClN_2O_3$: C, 36.28; H, 7.61; N, 14.10. Found: C, 36.35; H, 7.69; N, 14.08.

3.11. (2S,5S)-5-Hydroxylysine monohydrochloride *2*

The (2*S*,5*S*)-6-azido-2-benzyloxycarbonylamino-5-hydroxyhexanoic acid **14** (600 mg, 1.86 mmol) in MeOH:water (60 mL, 1:1, v/v) was hydrogenated for 15 h, in the presence of palladium on charcoal at room temperature and atmospheric pressure. Filtration of the catalyst, evaporation of the MeOH and lyophilization afforded a residue which was dissolved in water (1.0 mL) and the pH adjusted to 6.5–7.0 with HCl (4 M). The addition of EtOH (2.0 mL) and the storage at 0°C effected the precipitation of (2*S*,5*S*)-5-hydroxylysine (*allo*-L-hydroxylysine) monohydrochloride **2** (288 mg, 78%): $[\alpha]_D$ +25.5 (HCl 6 M, *c* 1) [lit.[13] +25.8 (*c* 2)]; ^{1}H NMR (D_2O): δ 3.68 (1H, m, 5-H), 3.56 (1H, dd, J = 5.7, 5.7, 2-H), 2.96 (1H, dd, J = 13.1, 1.7, 6-Ha), 2.73 (1H, dd, J = 13.1, 10.0, 6-Hb), 1.84 (1H, m, 3-Ha), 1.73 (1H, m, 3-Hb), 1.49 (1H, m, 4-Ha), 1.33 (1H, m, 4-Hb); ^{13}C NMR (D_2O): δ 175.38 (C-1), 68.16 (C-5), 55.47 (C-2), 45.35 (C-6), 30.64 (C-4), 27.59 (C-3).[25] Anal. calcd for $C_6H_{15}ClN_2O_3$: C, 36.28; H, 7.61; N, 14.10. Found: C, 36.18; H, 7.45; N, 14.23.

3.12. GLC derivation of 5-hydroxylysines

Hydroxylysines (1–2 mg) were esterified with methanolic HCl (1 M, 0.2 mL, 25°C, 12 h). The solvent was removed under a nitrogen stream and the residue was treated with a 50% mixture of $(CF_3CO)_2O$ in CF_3CO_2H (0.2 mL, 25°C, 2 h). After removal of the solvent, the residue (*tris*-trifluoroacetates of 5-hydroxylysine methyl ester) was dissolved in AcOEt and injected.

In the used analyses conditions (see General, Section 3.1), the derivatives showed the following retention times: 23.35 min for (2*R*,5*R*)-5-hydroxylysine **4**; 24.26 min for (2*S*,5*R*)-5-hydroxylysine **1**, 24.59 min for (2*S*,5*S*)-5-hydroxylysine **2** and 28.67 min for (2*R*,5*S*)-5-hydroxylysine **3**.

Acknowledgements

This work was supported financially by MURST COFIN progetto di ricerca 'Nuove metodologie e strategie di Sintesi di Composti di Interesse Biologico'. The authors are grateful to the student Vincenza Vau for technical assistance. This paper is dedicated to the memory of Professor Giorgio Traverso.

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