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A practical and simple synthesis of (2*S*,5*R*)- and (2*S*,5*S*)-5-hydroxylysine and of a related α-amino acid required for the synthesis of the collagen cross-link pyridinoline

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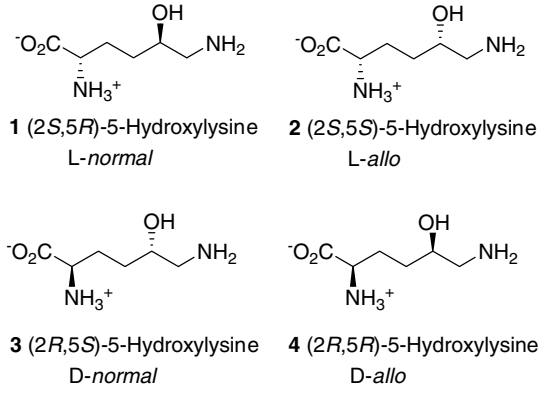
Abstract—A four step synthesis of (2*S*,5*R*)- and (2*S*,5*S*)-5-hydroxylysine using Williams' glycine template methodology for controlling the stereogenicity at the α-position is reported. This route offers the possibility for the synthesis of all possible isomers of 5-hydroxylysine and of an important α-amino acidic iodohydrin required for the construction of the hydroxylated side chain of the collagen cross-link pyridinoline.

1. Introduction

(2*S*,5*R*)-5-Hydroxylysine (L-*normal*-5-hydroxylysine) **1** is the natural isomer of four possible 2,6-diamino-5-hydroxyhexanoic acids **1**–**4** (Fig. 1) and it is exclusive to collagen protein, which is formed by posttranslational hydroxylation of some lysine residues.[1]

In collagen, (2*S*,5*R*)-5-hydroxylysine **1** can be glycosylated with either β-D-galactopyranosyl or an α-D-glucopyranosyl-(1 → 2)-β-D-galactopyranosyl residue[2] to afford galactosyl-*O*-hydroxylysine (5*R*-GaHyl) **5** or glucosylgalactosyl-*O*-hydroxylysine (5*R*-GGaHyl) **6**. Moreover, during maturation of collagen, its (2*S*,5*R*)-5-hydroxylysine residues interact with those of an adjacent lysine and/or hydroxylysine and form some important cross-links which, during collagen turnover, generate deoxypyridinoline **7** and pyridinolines **8a** and **8b**. In all cases during collagen degradation, (2*S*,5*R*)-5-hydroxylysine **1** and collagen cross-links **7** and **8** are not metabolised but are excreted in human urine mainly as glycosides **5**, **6** and **8b** (Fig. 2).[3,4] Thus, after an acidic hydrolysis, the total levels of the amino acid and of collagen cross-links are measured in this medium to evaluate the collagen breakdown[5] and to aid diagnosis of osteoporosis and of other metabolic bone diseases.[6] Under these conditions some amount of the isomerised (2*R*,5*R*)-5-hydroxylysine **4** also forms and must be evaluated.[5]



1 (2*S*,5*R*)-5-Hydroxylysine L-*normal*
2 (2*S*,5*S*)-5-Hydroxylysine L-*allo*
3 (2*R*,5*S*)-5-Hydroxylysine D-*normal*
4 (2*R*,5*R*)-5-Hydroxylysine D-*allo*

Figure 1.

(2*S*,5*R*)-5-Hydroxylysine **1** is commercially available both in enantiomerically pure form and as a mixture with its possible isomers **2**–**4** (Fig. 1). Moreover, the pure enantiomer **1** is quite expensive, since it is produced by tedious procedures from gelatine acid hydrolysates,[7] or it is isolated from a mixture of isomers by very laborious methods, involving the fractionated crystallization of appropriate derivatives.[8] In addition, the use of (2*S*,5*R*)-5-hydroxylysine **1** in the synthesis of collagen metabolites[9] or of peptides[10] requires lengthy procedures

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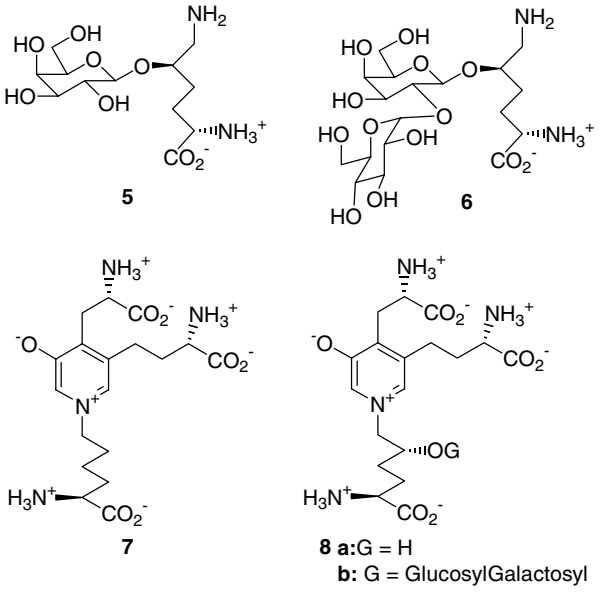
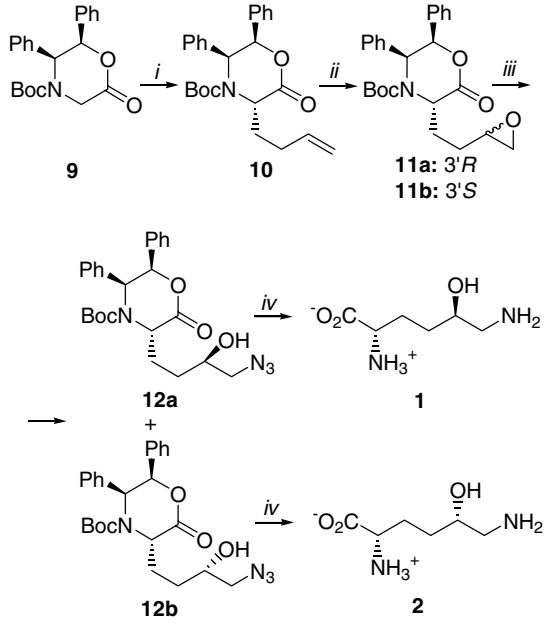


Figure 2.



Scheme 1. Reagents and conditions: (i) $CH_2{=}CH(CH_2)_2I$, $(Me_3Si)_2NLi$, THF-HMPA, −78 to −40 °C, 4 h; (ii) *m*-ClPBA, 1,2-dichloroethane-buffer pH = 7, room temp., 12 h; (iii) NaN_3–NH_4Cl, $MeOCH_2CH_2OH$, 50 °C, 4.5 h; (iv) TFA–H_2O (95:5), room temp., 1 h, H_2, Pd/C, H_2O–MeOH–HCl.

for protection prior to its use. Thus, in recent years, there has been a renewed interest for the preparation[11] not only for free and glycosylate hydroxylysines **1**, **5** and **6** but also for their epimers, as possible internal standards in clinical analyses of collagen metabolites. In addition protected hydroxylysines are important for the synthesis the collagen cross-links pyridinolines **8a** and **8b**, both needed for studies in clinical chemistry and biochemistry.[11b,12] Moreover, despite the apparent simplicity of the (2*S*,5*R*)-5-hydroxylysine **1** molecule, to date there is no short synthesis of this amino acid. In fact, the most recent synthetic strategies involve multistep, direct or convergent, procedures and often also the separations of diastereomers is laborious. This is due to the fact that in all these approaches the α-stereogenic center cannot efficiently direct the stereoselective introduction of the second stereogenic center at C-5 position.[11a,d,13]

As a result of our continuing interest in the synthesis of collagen cross links,[12] we report herein a simple four steps procedure for the synthesis of both (2*S*,5*R*)-5-hydroxylysine **1** and of (2*S*,5*S*)-5-hydroxylysine **2**, based on the use of diphenyloxyazinones, Williams' glycine templates.[14] Moreover the commercial availability[15] of both enantiomers of the starting oxazinone renders the procedure adaptable to preparing also the corresponding (2*R*)-hydroxylysines **3** and **4**. In addition we report the convenient preparation of a masked (2*S*,5*R*)-5-hydroxylysine, which helps to solve another synthetic problem associated to the construction of the hydroxylated side chain of pyridinoline **8a**, deriving from 5-hydroxylysine.[12]

2. Results and discussion

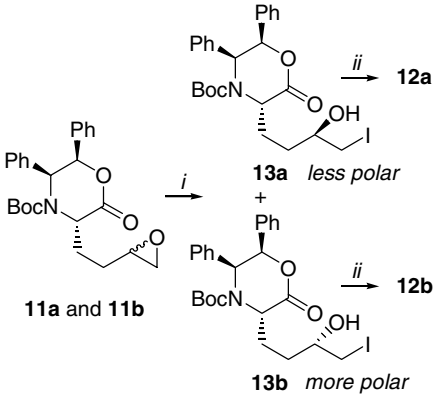
Our very simple and short protocol (Scheme 1) uses the glycine template **9** for the preparation, according to the Williams' procedure,[14] of the homoallyloxazinone **10**, the key compound of the synthesis (Scheme 1). Our idea was that this masked amino acid, by means of its carbonyl group, could form an intramolecular hydrogen bond in each of the hydroxylated epimers previewed as intermediates in our synthetic procedure (Scheme 1). This should increase the differences of polarity between the epimers and avoid the tedious separation of stereoisomers by subsidiary methods. In fact, the alkylation of the oxazinone **9**[14] with homoallyl iodide, at low temperature, in the presence of lithium bis(trimethylsilyl)amide, gave the homoallyloxazinone **10** in satisfactory yield, according to the Williams' results,[14] without any detectable amount of the epimeric alkylation product in the crude reaction mixture (^{1}H NMR analysis in DMSO-d_6 at 393 K, Scheme 1). The homoallyloxazinone **10** was then epoxidized by simple treatment with 3-chloroperbenzoic acid to afford a diastereomeric mixture (1:1; HPLC of the crude product) of epoxides **11a** and **11b**, in quantitative yield. These epoxides, even if resistant to all efforts of separation (TLC, column chromatography, crystallization), by reaction with sodium azide in the presence of ammonium chloride, afford a mixture of the hydroxyazides **12a** and **12b**, which could be easily separated by simple rapid chromatography on silica gel, due to their different polarities (TLC on silica, eluting with dichloromethane–acetone; 100:2; v/v). Initially, the structure **12a** was assigned tentatively to the less polar compound, however this assignment was subsequently substantiated by transforming the hydroxyazide **12a** into (2*S*,5*R*)-5-hydroxylysine **1**.

In the same way, the epimeric structure **12b** was assigned to the more polar hydroxyazide.

Each hydroxyazide was then transformed in one-pot into the corresponding 5-hydroxylysine **1** or **2** by simple treatment with trifluoroacetic acid followed by hydrogenation over Pd on carbon[14a] (Scheme 1). The obtained (2*S*,5*R*)-5-hydroxylysine **1** and of (2*S*,5*S*)-5-hydroxylysine **2** showed an high stereoisomeric purity (>98%; GLC on a suitable chiral column, see experimental) and all other physicochemical properties (specific rotation, ^{13}C NMR) were in agreement with those reported.[11c,d,e]

Interestingly, the mixture of epoxides **11** could be opened, by treatment with sodium iodide, to the mixture of easily separable iodohydrins of assigned structures **13a** and **13b** (Scheme 2).

These epimeric iodohydrins show a different polarity on silica gel and can be separated by rapid chromatography on silica. Their structure was easily demonstrated by simple separate transformation into the corresponding hydroxyazides **12a** and **12b**, described above (Scheme 2).



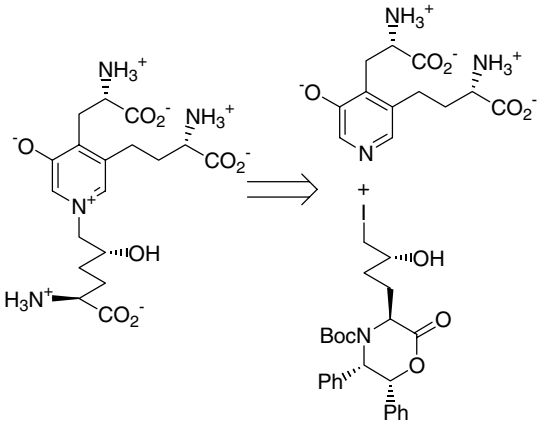
Scheme 2. Reagents and conditions: (i) NaI, THF–AcOH, room temp., 1 h; (ii) NaN_3, DMF, room temp., 12 h.

The iodohydrin **13a** is a very useful masked hydroxylysine since has a structure required in many synthesis of the pyridinoline **8**. In fact, it allows the construction of the hydroxylated side chain of the pyridinoline by alkylation of the nitrogen atom of an appropriate 4,5-disubstituted-3-hydroxypyridine (Scheme 3).[12]

3. Conclusion

Thus, the possibility herein reported for obtaining this amino acidic iodohydrin **13a** in a simple way is an important self-consistent result for the chemistry of collagen cross-links. This is evident from the documented difficulties in preparing similar iodohydrins,[12] which only recently have been obtained in a relatively satisfactory way according to some methods set-up in our laboratory.[12h]

In conclusion, herein we have reported a very short synthesis of enantiopure (2*R*,5*R*)- and (2*R*,5*S*)-5-hydroxylysines **1** and **2**, using a methodology which also allows the preparation of the corresponding (2*R*)-enantiomers **3** and **4**[15] and permits the preparation of an important iodohydrin **13a**, required by many protocols for the construction of pyridinoline hydroxylated side chain.[12]



Scheme 3.

4. Experimental

4.1. General

Nuclear magnetic resonance spectra were recorded on a Bruker AM-500 spectrometer operating at 500.13 MHz for ^1H 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 ^1H spectra and relative to dioxane fixed at 67.60 ppm for the ^{13}C spectra. ^1H NMR data are tabulated in the following order: number of protons, multiplicity (s, singlet; d, doublet; br s, broad singlet; m, multiplet), coupling constant(s) 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 a octakis(3-*O*-butyryl-2,6-di-*O*-pentyl)-γ-cyclodextrin (Lipodex E)[16] 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 chiral column (LiChroCART 250-4 (*R*,*R*)-Whelk-01, 5 μm, Merck, eluent hexane–2-propanol 60:40, 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% sulfuric acid or 0.2% ninhydrin in ethanol and heat as developing agent. Merck 230–400 mesh silica gel was used for flash column chromatography.[17] Usual work-up refers to washing the organic layer with water, drying over Na_2SO_4, and evaporating the solvent under reduced pressure. Mass spectra were obtained using a Finnigan LCQdeca (ThermoQuest) ion trap mass spectrometer fitted with an electrospray source (ESI).

4.2. GLC analysis of 5-hydroxylysines on a chiral column

5-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), 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**.

4.3. *tert*-Butyl (3*S*,5*S*,6*R*)-3-(3-butenyl)-2-oxo-5,6-diphenyl-1,4-oxazinane-4-carboxylate 10

The homologate oxazinone **10** was prepared according to the method of Williams and co-workers.[14] Starting with a stirred solution of the lactone **9**[15] (10.1 g, 30 mmol) and 4-iodobutene (27.3 g, 150 mmol), in anhydrous THF (225 mL) and HMPA (22.5 mL), cooled to −78 °C, lithium bis(trimethylsilyl)amide (45 mL of a 1 M solution in dichloromethane, 45 mmol) was added dropwise, under argon. After 10 min the reaction mixture was stirred at −40 °C for 4 h. At this time, the reaction was quenched with ethyl acetate and poured into a mixture of ethyl acetate (50 mL) and an aqueous solution of NH_4Cl (50 mL, 1 M). The organic layer were worked up to afford a crude residue which was chromatographed to afford the title compound **10** (7.3 g, 60%). Compound **10** showed: mp 148–152 °C (from methanol–diisopropyl ether); $[\alpha]_D^{23} = -57$ (*c* 0.5, $CHCl_3$) [lit.[14a] mp 157–152 °C; $[\alpha]_D^{23} = -56.7$ (*c* 0.54; CH_2Cl_2)]; ^{1}H NMR (393 K, DMSO-d_6): δ 7.25–7.05 (8H, aromatics), 6.56 (2H, d, $J = 7.7$, aromatics), 6.18 (1H, d, $J = 3.2$, 6-H), 5.92 (1H, ddt, $J = 16.6$, 10.2 and 6.5, 3′-H), 5.16 (1H, d, $J = 3.2$, 5-H), 5.15 (1H, dd, $J = 16.6$ and 1.7, 4′-Ha), 5.05 (1H, dd, $J = 10.2$ and 1.7, 4′-Hb), 4.82 (1H, dd, $J = 7.0$ and 7.0, 3-H), 2.34–2.23 (2H, m, 2′-H_2), 2.22–2.17 (2H, m, 1′-H_2), 1.19 [9H, s, $C(CH_3)_3$]; ESI-MS *m*/*z* (relative intensity): 837.0 (40, M+M+Na^+), 430.2 (100, M+Na^+). Anal. Calcd for $C_{25}H_{29}NO_4$: C, 73.68; H, 7.17; N, 3.44. Found: C, 73.92; H, 7.24; N, 3.31.

4.4. *tert*-Butyl (3*S*,5*S*,6*R*)-3-[(3*R*)-3,4-epoxybutyl]-2-oxo-5,6-diphenyl-1,4-oxazinane-4-carboxylate 11a and *tert*-butyl (3*S*,5*S*,6*R*)-3-[(3*S*)-3,4-epoxybutyl]-2-oxo-5,6-diphenyl-1,4-oxazinane-4-carboxylate 11b

To a solution of the homoallyloxazinone **10** (1 g, 2.45 mmol) and 3-chloroperbenzoic acid (1.02 g, 50%, 2.95 mmol) in 1,2-dichloroethane (60 mL), a buffered phosphate solution (120 mL, pH = 7) was added and the mixture was shaken for 12 h at room temperature. At this time the organic phase was separated and worked up to afford a crude residue, which was chromatographed (eluting with hexane–AcOEt 75:25) to afford an unseparable mixture of the epoxides **11a** and **11b** (920 mg, 88.7%) in a 1:1 ratio (HPLC: 7.7 and 9.5 min) as a white solid: mp 125–127 °C; $[\alpha]_D^{23} = -57$ (*c* 1, $CHCl_3$); ^{1}H NMR (303 K, $CDCl_3$): δ 7.25–6.95 (2×8H, aromatics), 6.54 (2×2H, d, $J = 7.2$, aromatics), 5.92 (2×1H, m, 6-H), 5.02 (2×1H, m, 3-H), 4.99 (2×1H, m, 5-H), 3.03 (2×1H, m, 3′-H), 2.80 (1H, dd, $J = 4.9$ and 4.0, 4′-Ha), 2.78 (1H, dd, $J = 4.9$ and 4.0, 4′-Ha), 2.59 (1H, dd, $J = 4.9$ and 2.6, 4′-Hb), 2.57 (1H, dd, $J = 4.9$ and 2.6, 4′-Hb), 2.33 (2×1H, m, 2′-Ha), 2.09 (2×1H, m, 2′-Hb), 1.98 (2×1H, m, 1′-Ha), 1.78 (1H, m, 1′-Hb), 1.69 (1H, m, 1′-Hb), 1.08 [9H, s, $C(CH_3)_3$], 1.06 [9H, s, $C(CH_3)_3$]; ESI-MS *m*/*z* (relative intensity): 446.2 (100, M+Na^+), 390.2 (60, M+Na^+−56). Anal. Calcd for $C_{25}H_{29}NO_5$: C, 70.90; H, 6.90; N, 3.31. Found: C, 70.73; H, 7.05; N, 3.25.

4.5. *tert*-Butyl (3*S*,5*S*,6*R*)-3-[(3*R*)-4-azido-3-hydroxybutyl]-2-oxo-5,6-diphenyl-1,4-oxazinane-4-carboxylate 12a and *tert*-butyl (3*S*,5*S*,6*R*)-3-[(3*S*)-4-azido-3-hydroxybutyl]-2-oxo-5,6-diphenyl-1,4-oxazinane-4-carboxylate 12b

To a solution of the epoxides **11** (600.0 mg, 1.42 mmol) in ethylene glycol mono methyl ether, NaN_3 (272.0 mg, 4 mmol) and NH_4Cl (60 mg, 1.07 mmol) were added and the mixture was heated at 50 °C for 4.5 h. At this time, the mixture was cooled at room temperature and poured into water to afford a solid, which was filtered and chromatographed (eluting with dichloromethane–acetone, 100:2; v/v) to afford first the (3′*R*)-hydroxyazide **12a** (268 mg, 40.4%): mp 110–112 °C (from methanol–water); $[\alpha]_D^{23} = -57.0$ (*c* 1, $CHCl_3$); ^{1}H NMR (295 K, DMSO-d_6): δ 7.26–6.69 (8H, aromatics), 6.54 (2H, d, $J = 7.9$, aromatics), 5.89 (1H, d, $J = 2.8$, 6-H), 4.98 (1H, d, $J = 2.8$, 5-H), 4.96 (1H, dd, $J = 7.0$ and 3.7, 3-H), 4.17 (1H, m, 3′-H), 3.55 (1H, br s, OH), 3.37 (1H, dd, $J = 12.4$ and 3.3, 4′-Ha), 3.29 (1H, dd, $J = 12.4$ and 7.2, 4′-Hb), 2.30 (1H, m, 2′-Ha), 2.14 (1H, m, 2′-Hb), 1.78 (1H, m, 1′-Ha), 1.68 (1H, m, 1′-Hb), 1.10 [9H, s, $C(CH_3)_3$]; ESI-MS *m*/*z* (relative intensity): 955.1 (54, M+M+Na^+), 489.2 (100, M+Na^+). Anal. Calcd for $C_{25}H_{30}N_4O_5$: C, 64.36; H, 6,48; N, 12,01. Found: C, 64.28; H, 6.44; N, 12.11.

Further elution afforded the (3′*S*)-hydroxyazide **12b** (263 mg, 39.8%): mp 106–108 °C (from methanol–water); $[\alpha]_D^{23} = -52.0$ (*c* 1, $CHCl_3$); ^{1}H NMR (295 K, DMSO-d_6): δ 7.25–6.74 (8H, aromatics), 6.54 (2H, d, $J = 7.4$, aromatics), 5.89 (1H, d, $J = 2.7$, 6-H), 5.05 (1H, dd, $J = 8.6$ and 5.4, 3-H), 4.99 (1H, d, $J = 2.6$, 5-H), 3.91 (1H, m, 3′-H), 3.37 (1H, dd, $J = 12.3$ and 3.8, 4′-Ha), 3.31 (1H, dd, $J = 12.3$ and 6.8, 4′-Hb), 2.31 (1H, m, 2′-Ha), 2.07 (1H, m, 2′-Hb), 1.80 (2H, m, 1′-H_2), 1.09 [9H, s, $C(CH_3)_3$]; ESI-MS *m*/*z* (relative intensity): 955.1 (51, M+M+Na^+), 489.2 (100, M+Na^+). Anal. Calcd for $C_{25}H_{30}N_4O_5$: C, 64.36; H, 6.48; N, 12,01. Found: C, 64.52; H, 6.34; N, 11.87.

4.6. (2*S*,5*R*)-5-Hydroxylysine monohydrochloride 1

The hydroxyazide **12a** (150 mg; 0.32 mmol) was dissolved in CF_3COOH–H_2O (5 mL; 95:5; v/v) and the

solution was stirred for 1 h at room temperature. At this time, a solution of CH_3OH–H_2O–HCl (8:3:1; v/v/v, 120 mL) was added and the resulting solution was hydrogenated at room temperature for 24 h at 25 °C, in the presence of Pd on carbon (80 mg, 10%). Filtration of the catalyst through Celite, evaporation of the solvent afforded a residue, which was dissolved in water (1.5 mL) and the pH adjusted to 6.5–7.0. The addition of EtOH and the storage at 0 °C effected the precipitation of (2*S*,5*R*)-5-hydroxylysine **1** as the monohydrochloride (56.0 mg, 88%): $[\alpha]_D = +15.0$ (*c* 1, HCl 6 M), [lit.[11c] $[\alpha]_D = +15.2$ (*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). 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.

4.7. (2*S*,5*S*)-5-Hydroxylysine monohydrochloride 2

The hydroxyazide **12b** (150 mg; 0.32 mmol) was dissolved in CF_3COOH–H_2O (5 mL; 95:5; v/v) and reacted as described for the isomer **12a** to afford (2*S*,5*S*)-5-hydroxylysine monohydrochloride **2** (49.2 mg, 78%): $[\alpha]_D = +25.5$ (*c* 1, HCl 6 M) [lit.[11c] $[\alpha]_D = +25.8$ (*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). 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.

4.8. *tert*-Butyl (3*S*,5*S*,6*R*)-3-[(3*R*)-3-hydroxy-4-iodobutyl]-2-oxo-5,6-diphenyl-1,4-oxazinane-4-carboxylate 13a and *tert*-butyl (3*S*,5*S*,6*R*)-3-[(3*S*)-3-hydroxy-4-iodobutyl]-2-oxo-5,6-diphenyl-1,4-oxazinane-4-carboxylate 13b

To a solution of the epoxide **11** (990 mg; 2.33 mmol) in THF (40 mL) containing acetic acid (0.4 mL), NaI was added (710 mg; 4.73 mmol) and the mixture was stirred at room temperature. After 1 h the solution was poured in a cold aqueous solution of $NaHCO_3$ and extracted with ethyl acetate. After usual work-up, the crude residue was chromatographed to afford the iodohydrin **13a** (605 mg, 47%); mp 167–168 °C; $[\alpha]_D^{23} = -43.2$ (*c* 1, $CHCl_3$); ^{1}H NMR (295 K, DMSO-d_6): δ 7.26–6.97 (8H, aromatics), 6.54 (2H, d, $J = 7.7$, aromatics), 5.90 (1H, d, $J = 2.8$, 6-H), 5.00–4.97 (2H, overlapping, 3-H and 5-H), 3.91 (1H, m, 3′-H), 3.41 (1H, dd, $J = 10.4$ and 3.5, 4′-Ha), 3.32 (1H, br s, OH), 3.27 (1H, dd, $J = 10.4$ and 7.0, 4′-Hb), 2.31 (1H, m, 2′-Ha), 2.15 (1H, m, 2′-Hb), 1.94 (1H, m, 1′-Ha), 1.75 (1H, m, 1′-Hb), 1.10 [9H, s, $C(CH_3)_3$]; ESI-MS *m*/*z* (relative intensity): 974.1 (100, M+M+Na^+), 518.1 (48, M+Na^+), 446.2 (25, M+Na^+ of epoxide formed in the MS source from iodohydrin). Anal. Calcd for $C_{25}H_{30}INO_5$: C, 54.45; H, 5.48; N, 2.54. Found: C, 54.59; H, 5.36; N, 2.48.

Further elution affords the iodohydrin **13b** (600 mg, 47%); mp 155–156 °C; $[\alpha]_D^{23} = -44.7$ (*c* 1, $CHCl_3$); ^{1}H NMR (295 K, DMSO-d_6): δ 7.27–6.97 (8H, aromatics), 6.55 (2H, d, $J = 7.5$, aromatics), 5.92 (1H, d, $J = 2.8$, 6-H), 5.06 (1H, dd, $J = 9.0$ and 5.6, 3-H), 5.00 (1H, d, $J = 2.8$, 5-H), 3.68 (1H, m, 3′-H), 3.42 (1H, dd, $J = 9.8$ and 3.5, 4′-Ha), 3.31 (1H, dd, $J = 9.8$ and 6.3, 4′-Hb), 2.59 (1H, br s, OH), 2.35 (1H, m, 2′-Ha), 2.07 (1H, m, 2′-Hb), 1.95–1.84 (2H, overlapping, 1′-Ha and H1′-Hb), 1.09 [9H, s, $C(CH_3)_3$]. Anal. Calcd for $C_{25}H_{30}INO_5$: C, 54.45; H, 5.48; N, 2.54. Found: C, 54.38; H, 5.32; N, 2.55.

4.9. Preparation of *tert*-butyl (3*S*,5*S*,6*R*)-3-[(3*R*)-4-azido-3-hydroxybutyl]-2-oxo-5,6-diphenyl-1,4-oxazinane-4-carboxylate 12a and *tert*-butyl (3*S*,5*S*,6*R*)-3-[(3*S*)-4-azido-3-hydroxybutyl]-2-oxo-5,6-diphenyl-1,4-oxazinane-4-carboxylate 12b from the analogues iodohydrins 13a and 13b

Each iodohydrin **13a** and **13b** (100 mg; 0.18 mmol) was dissolved in DMF (1 mL) and treated with NaN_3 (23.30 mg; 0.36 mmol) at room temperature for 12 h. Then the reaction mixture was poured into ice cold water and extracted with AcOEt to afford, after usual work-up, the appropriate crystalline hydroxyazide. In particular, starting from the iodohydrin **13a**, the azide **12a** was obtained (68 mg, 81%); mp 109–111 °C (from methanol–water); $[\alpha]_D^{23} = -56.2$ (*c* 1, $CHCl_3$); identical in all respect with that described above. Starting from the iodohydrin **13b**, the azide **12b** was obtained (70 mg, 83%); mp 106–108 °C (from methanol–water); $[\alpha]_D^{23} = -51.4$ (*c* 1, $CHCl_3$); identical in all respect with that described above.

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