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Tetrahedron: Asymmetry
Tetrahedron: *Asymmetry* 16 (2005) 3059–3069

Reduced collagen cross links: the first synthesis of all the possible (2*S*,2′*S*)-stereoisomers of 5-hydroxylysinonorleucine and of 5,5′-dihydroxylysinonorleucine in enantiomerically pure form

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Received 8 July 2005; accepted 12 August 2005

Abstract—The paper reports the first enantioselective synthesis of all the possible collagen reduced cross links as described: (2*S*,2′*S*,5*R*)- and (2*S*,2′*S*,5*S*)-5-hydroxylysinonorleucine **3a** and **3b**, (2*S*,2′*S*,5*R*,5′*R*)-5,5′-dihydroxylysinonorleucine **4a**, (2*S*,2′*S*,5*R*,5′*S*)-5,5′-dihydroxylysinonorleucine **4b** and (2*S*,2′*S*,5*S*,5′*S*)-5,5′-dihydroxylysinonorleucine **4c**. The Williams' glycine template methodology was used both for the introduction of a stereogenic at the α-position and for an easy protection of the amino acidic functionalities during the synthesis of the dimeric amino acids.

1. Introduction

Collagen, the most abundant structural protein of the human body, is responsible for the mechanical properties of bones, cartilage, skin, tendon, ligament and other tissues.[1] Collagen is secreted as a procollagen molecule which, during the maturation, suffers some post-transcriptional modifications ending with the formation of intra and intermolecular cross links, which are responsible for the mechanical strength of collagen fibrils.[2] The extent of the cross links formation starts with the enzymatic oxidative deamination of the α-amino group of the telopeptide lysine and hydroxylysine residues to their respective aldehydes, allysine and hydroxyallysine, which spontaneously form some aldimines, known as 'immature collagen cross links', by reaction with intact lysine or hydroxylysine (Fig. 1).[3]

With the aging of the collagen fibrils, some immature cross links evolve, via an initial rearrangement to ketoamines, to more complex cross links, called 'mature cross links', which, during bone remodelling, are excised and excreted unchanged in human urine, as two pyridinium cross links,[2d,4] pyridinoline **1a** (Py) and deoxypyridinoline **1b** (Dpy) (Fig. 1).[5] Py **1a** and Dpy **1b** are now considered important biochemical markers both of total collagen turnover and of bone resorption, useful for the diagnosis of osteoporosis and other bone diseases.[2a,6] Thus, different protocols are available for their synthesis[7] and quantification in various tissues.[8] Surprisingly, less attention has been devoted to the immature collagen cross links. In fact, after the studies of Tanzer[9] and of Bailey,[10] which have permitted their localization in various tissues, and their isolation, after stabilization by reduction, only the stereoselective synthesis of lysinonorleucine **2**, which lacks hydroxyls, has been reported.[11] The native products lysinonorleucine **2** (Fig. 2; LNL), 5-hydroxylysinonorleucine **3** (HLNL) and 5,5′-dihydroxylysinonorleucine **4** (DHLNL) have still not had the stereochemistry of their hydroxylated centres determined. While the stereochemistry of the centres binding the 2-amino groups of the products **2**–**4** may be reasonably considered as (*S*)-configuration, that of the 5 and 5′ hydroxylated carbons could be different from that of hydroxylysine and should be demonstrated.

Considering that the availability of all the (2*S*,2′*S*)-isomers in enantiomeric pure form and suitable amounts could be useful not only for a definitive assessment of the stereochemistry and of the biological significance of the native compounds,[12] but also to exclude or to give evidence of their possible presence in biological fluids, where they could form in small amounts by the enzymatic reduction of the parent imines, we herein report the synthesis of compounds **3a**, **3b** and **4a**–**c**.

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0957-4166/$ - see front matter © 2005 Published by Elsevier Ltd.
doi:10.1016/j.tetasy.2005.08.023

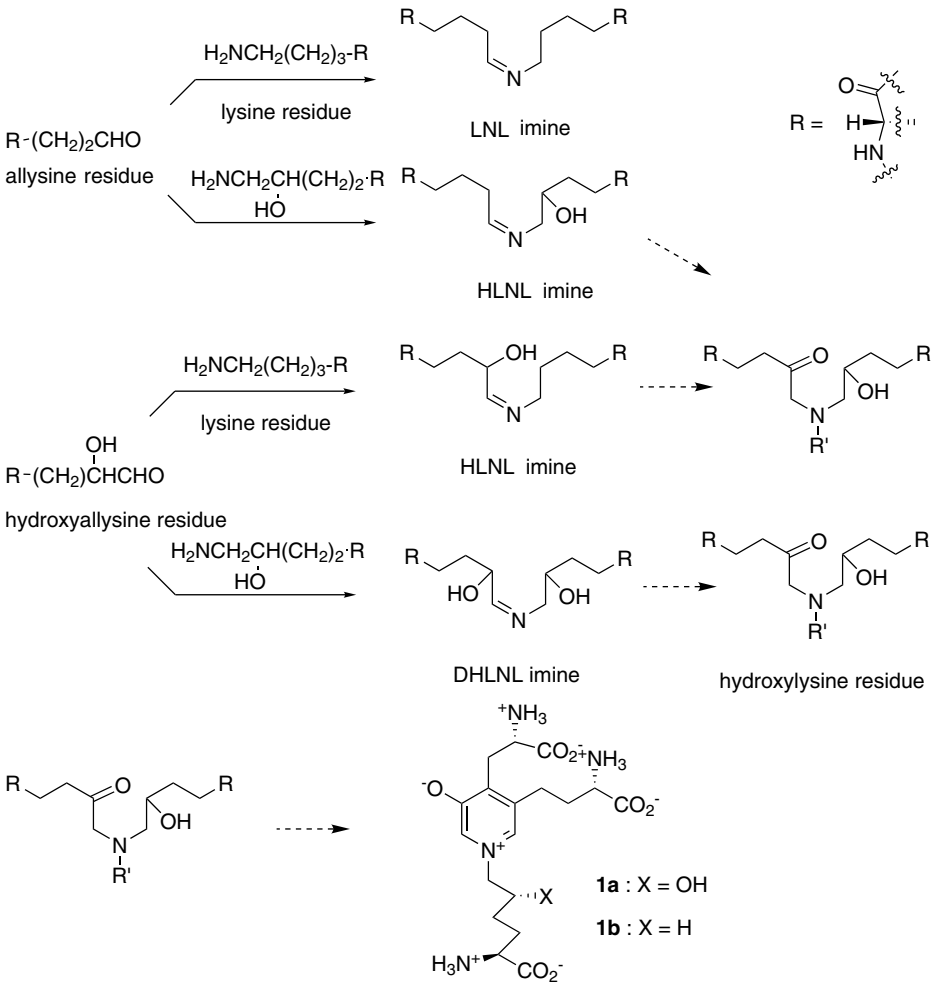


Figure 1. Formation of immature and mature collagen cross links.

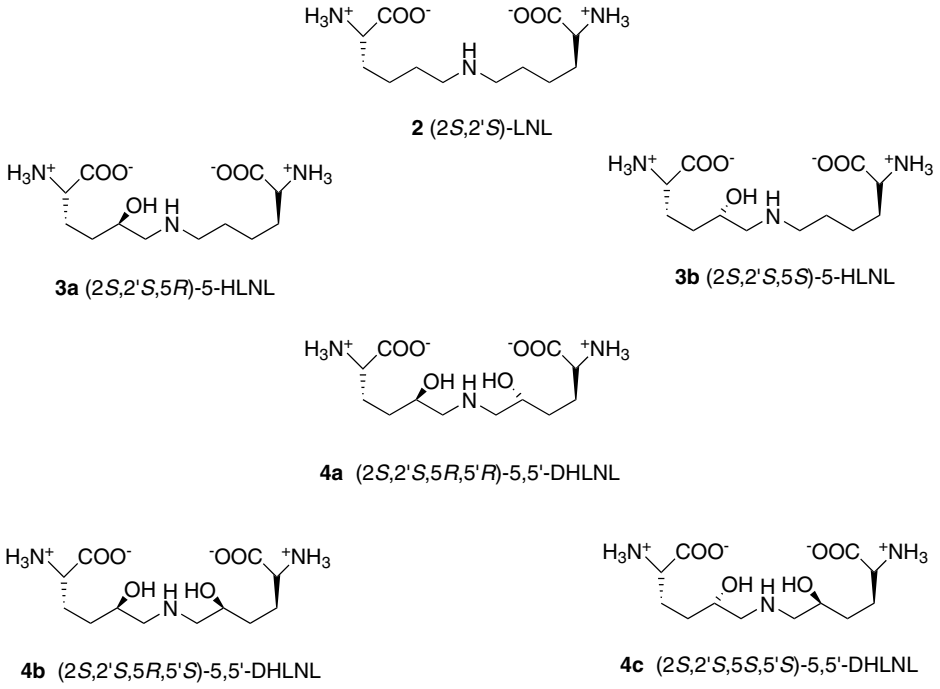


Figure 2. Possible reduced collagen cross links.

2. Results and discussion

Herein, we report the first stereoselective synthesis of the two possible (2*S*,2′*S*)-isomers of 5-hydroxylysinonorleucine **3a** and **3b** [(2*S*,2′*S*,5*R*)- and (2*S*,2′*S*,5*S*)-5-HLNL Fig. 2], and of the three possible (2*S*,2′*S*)-isomers of 5,5′-dihydroxylysinonorleucine **4a–c** [(2*S*,2′*S*,5*R*,5′*R*)-, (2*S*,2′*S*,5*R*,5′*S*)- and (2*S*,2′*S*,5*S*,5′*S*)-5-5′-DHLNL].

We decided to use the Williams' glycine template methodology[13] for the introduction of a stereogenic centre at the α-position of the desired dimeric amino acids and for the easy protection of the amino acidic functionalities during the successive synthetic elaborations. In fact, a simple retrosynthetic disconnection of the structure of the monohydroxylated cross links **3a** and **3b** suggested that they could be obtained (Fig. 3) by simple alkylation of the 6-amino group of a conveniently protected lysine **5**, with an appropriate amino acidic bromohydrin able to introduce either a natural or unnatural hydroxylysine side chain.

With this in mind, we treated the inseparable mixture of diastereomeric epoxides **6** and **7**, prepared in our laboratory[7b] using the Williams' glycine template methodology,[13] with lithium bromide (Scheme 1) and obtained the two bromohydrins **8** and **9**, which were separable by simple column chromatography. The structure of these bromohydrins was assigned on the basis of their ability to afford the corresponding (*R*)- and (*S*)-hydroxyazides under the conditions previously described for the analogue iodohydrins.[7b] Moreover, when we treated each bromohydrin with the protected hydroxylysine **5**, in the presence of various bases (K_2CO_3 or Cs_2CO_3),[14] necessary as scavengers of HBr, no alkylation occurred. Even at room temperature, bromohydrins **8** and **9** were dehydrohalogenated to the parent epoxide **6** or **7**, respectively (Scheme 1).

Considering that the regeneration of the epoxides could be due to the action of the base present for the alkylation, we decided to take advantage of this undesired reaction to prepare in diastereomeric pure form, epoxides **6** and **7**, by separate treatment with Cs_2CO_3, in CH_3CN at room temperature of each bromohydrin **8** or **9**. In our opinion, epoxides **6** and **7** could be useful to synthesize protected (2*S*,2′*S*,5*R*)- and (2*S*,2′*S*,5*S*)-5-HLNL **3a** and **3b** by a direct attack of the oxirane ring

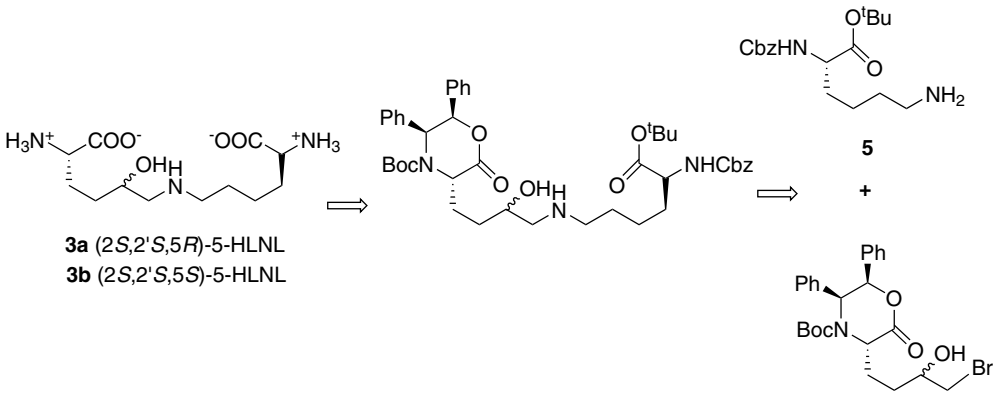
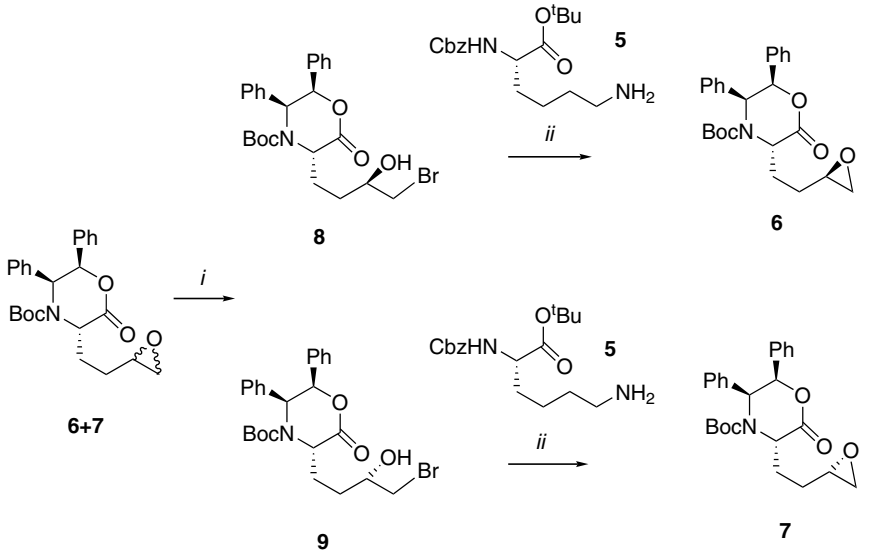
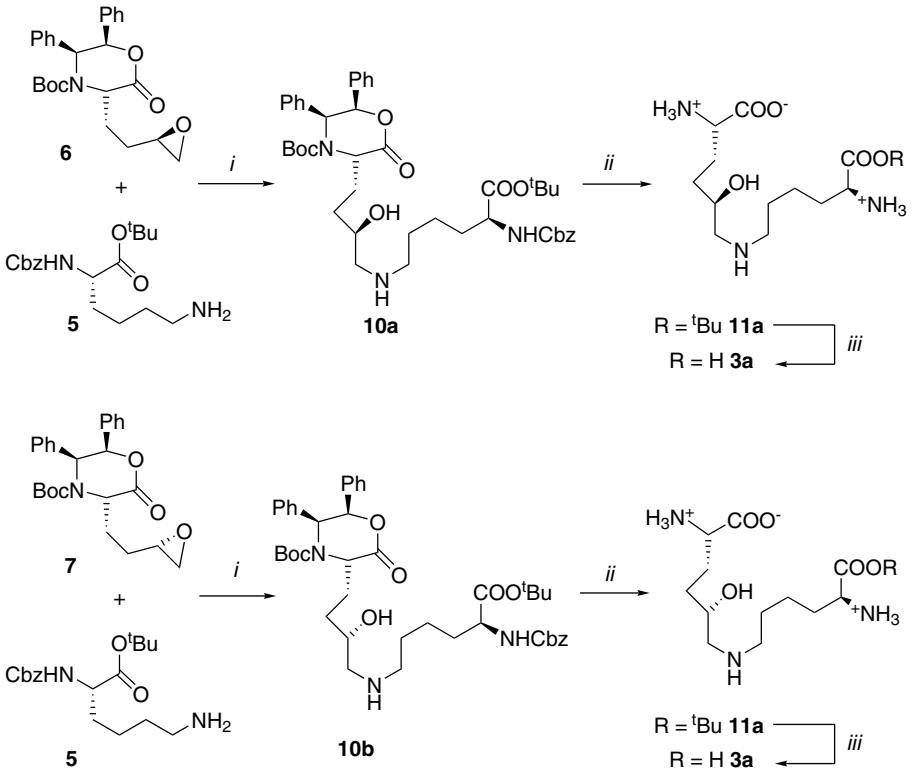


Figure 3. Retrosynthetic disconnection of the structure of the hydroxylated cross links **3a** and **3b**.



Scheme 1. Reagents and conditions: (i) LiBr, THF, CH_3COOH, 4h, rt, 82%; (ii) Cs_2CO_3, CH_3CN, 2 h, rt, 92%.



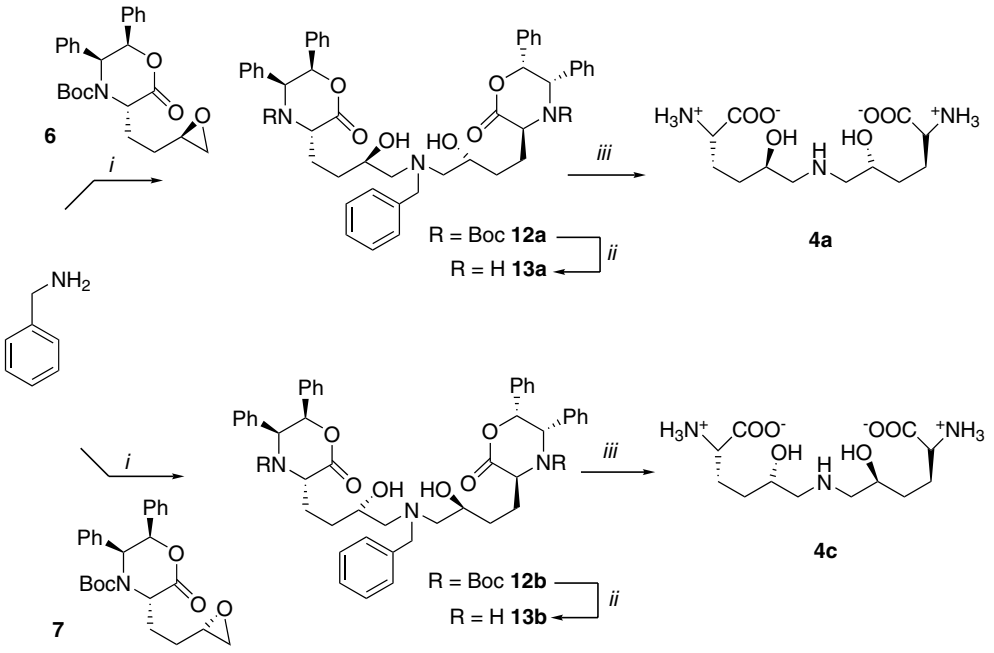
Scheme 2. Reagents and conditions: (i) $LiClO_4$, CH_3CN, 12 h, rt, 65%; (ii) Na, NH_3 liq, 15 min; (iii) HCl aq 1 M, 2 h, 52%, from **10a** or **10b**.

with the protected lysine **5** (Scheme 2). In fact, the reaction of the protected hydroxylysine **5** with the enantiomerically pure epoxide **6**, in the presence of lithium perchlorate,[7k] which activates the opening of the epoxide ring, affords the completely protected dimeric amino acid **10a** in satisfactory yield (70%), accompanied only by minor amounts (<5%; TLC, NMR evidences) of the less polar trimeric homologue. Crucial for a successful reaction were the molar ratio of epoxide **6** on amine **5** (1:2), and the reaction temperature (22–25 °C). A higher reaction temperature or larger excess of the amine caused the opening of the oxazinone ring lactone with the formation of the corresponding amide.

Next, we attempted the regeneration of the protected amino acidic functionalities of compound **10a** by a selective removing of its *N*-*t*-Boc protecting group, by treatment with aqueous trifluoracetic acid (TFA),[15] followed by catalytic hydrogenation under moderate pressure ($PdCl_2$, /EtOH/THF, 25 °C, 40 psi).[13] However, using this procedure, we observed that the expected compound **11a** was accompanied by the corresponding lactone which, probably formed by inner transesterification of the oxazinone lactone **10a** with the hydroxyl group of the hydroxylysine chain. To circumvent this problem, we decided to attempt deblocking the protected amino acidic functionalities of the compound **10a** using sodium in liquid NH_3 at −40 °C, the alternative procedure of Williams.[13] We were confident that these conditions would be suitable for cleaving both the oxazinone ring and the carbobenzyloxy group.[13,16] In fact, the crude product of the reaction, purified from the inorganic salts by short column chromatography on ion-exchange resin (Dowex 50W-X8, H^+ form), was the amino acid **11a**, which lacks the oxazinone ring, the carbobenzyloxy group and even the *N*-*t*-Boc, a protecting group which generally survives the opening of the oxazinone ring performed under chemical reduction conditions.[13] The hydrolysis of the *N*-*t*-Boc group probably occurs during the chromatographic purification under acidic conditions of the crude product, to afford the *tert*-butyl ester **11a**. The crude the *tert*-butyl ester **11a** was then transformed into the desired dimeric amino acid **3a** by treatment with aqueous HCl (1 M) at room temperature. The use of this aqueous acid avoids the concurrent lactonization of the hydroxylysine branch, which was observed when the hydrolysis of the *tert*-butyl ester **11a** is carried out with aqueous TFA (80–90%).[15]

Similar results were obtained performing a parallel sequence of reactions with the (5*S*)-epoxide **7** and amine **5**. In this case, (2*S*,2′S,5*S*)-5-HLNL **3b** was obtained through the diastereomeric intermediates **10b** and **11b**.

For the synthesis of dihydroxylated reduced cross links (2*S*,2′*S*,5*R*,5′*R*)- and (2*S*,2′*S*,5*S*,5′*S*)-5,5′-DHLNL **4a** and **4c** (Scheme 3), we decided to react each epoxide **6** and **7** with benzylamine, which in our mind, appeared a good auxiliary molecule not only for the introduction of the secondary amino group of the target amino acids **4a** and **4c**, but also for a series of other reasons, including its easily alkylation ability and cleavability.



Scheme 3. Reagents and conditions: (i) $LiClO_4$, CH_3CN, 12 h, 60 °C, 78%; (ii) $ZnBr_2$, CH_2Cl_2, 2 h, room temp.; (iii) H_2, $PdCl_2$, THF–C_2H_5OH–H_2O (4:4:2, v/v/v), 67% from **12a** or **12b**.

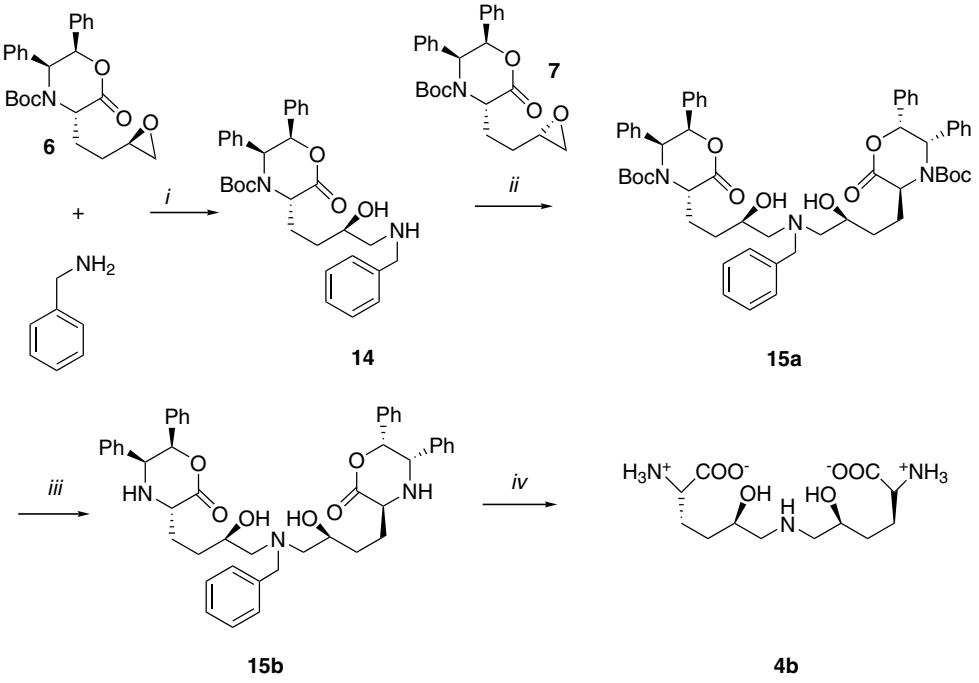
In fact, by reacting the benzylamine with two molecules of epoxide **6**, the tertiary amine **12a**, containing the masked functions of the dimeric dihydroxylated amino acid **4a**, was obtained in satisfactory yields (78%). The deblocking of compound **12a** was complicated by the presence of the two lysine hydroxyl groups and of the benzyl group and required the investigation of different procedures. The best results were obtained using a 'one-pot' cleavage of the oxazinone ring and of the benzyl group, by acidic elimination of the *N*-*t*-Boc group, followed by catalytic hydrogenation in the presence of $PdCl_2$. In fact, treatment of compound **12a** with $ZnBr_2$ in dichloromethane[17] caused the exclusive cleavage of the *N*-*t*-Boc groups and afforded the free dioxazinone **13a**, which by catalytic hydrogenation afforded the desired (2*S*,2′*S*,5*R*,5′*R*)-5,5′-DHLNL **4a**. On the contrary, when the cleavage of the *t*-Boc groups was performed treating compound **12a** with TFA, an inner transesterification occurred to afford a mixture of products mainly containing the *mono* and dilactones (MS and ^1H NMR evidences), deriving from the transesterification of the oxazinone lactone(s) with the hydroxyls of the chains. Similar, unsatisfactory results were obtained using the dissolving metal reduction (sodium in liquid NH_3/EtOH/THF) at −40 °C to cleave the oxazinone ring. This procedure, which was successful in the cleavage of the oxazinone ring of the protected monohydroxylated diamino acids **10a** and **10b**, shows in this case the survival of the benzyl group bonded to the secondary amino group, in spite of the reported general cleavability of the *N*-benzyl groups, under dissolving metal reduction conditions.[16] Thus, a successive catalytic reduction was necessary to cleave the benzyl group and to afford the free dimeric amino acid **4a**.

Parallel results, starting with diastereomeric epoxide **7**, allowed us to obtain the intermediate tertiary amines **12b** and **13b** and, successively, of the (2*S*,2′*S*,5*S*,5′*S*)-5,5′-DHLNL **4c**. The use of benzyl amine as a donor of the nitrogen atom was useful also for the concise preparation of the third isomeric (2*S*,2′*S*,5*R*,5′*S*)-5,5′-DHLNL **4b** which has two hydroxylysine branches with opposite geometries at the hydroxylated carbons (Scheme 4).

As reported in Scheme 4, an excess of benzyl amine was reacted with epoxide **6** (in a 2:1 ratio) to afford, in the first step, the secondary amine **14**, which after isolation and purification, was reacted with the diastereomeric epoxide **7** (in a 1:1 ratio) to form the completely protected dimeric amino acid **15a**, a key precursor of target compound **4b**. The regeneration of the amino acidic functionalities of **15a** was then performed by a short treatment of this dioxazinone with $ZnBr_2$ in dichloromethane, followed by catalytic hydrogenation. The Lewis acid cleaves the *N*-*t*-Boc groups to afford the free dioxazinone **15b** which was then reduced to the dimeric amino acid (2*S*,2′*S*,5*R*,5′*S*)-5,5′-DHLNL **4b**.

This procedure for the regeneration of the masked functionalities of **4b** affords more satisfactory results with respect to the cleavage of the dioxazinone of **15a** by means of sodium dissolved in liquid ammonia at −40 °C. In fact, in the last case, a partial survival of the benzyl group, which was bonded to the secondary amino group, was observed and the crude product of the reaction requires a catalytic hydrogenation to liberate the free amino acid **4b**.

The preparation of dimeric amino acid **4b** completed the synthesis of the possible reduced forms of collagen immature cross links, which are now available in enantiomeric pure form for biological experiments. Moreover, since the (2*S*)-stereochemistry of the starting



Scheme 4. Reagents and conditions: (i) $LiClO_4$, CH_3CN, 3 h, 60 °C, 55%; (ii) $LiClO_4$, CH_3CN, 72 h, 60 °C, 65%; (iii) $ZnBr_2$, CH_2Cl_2, 2 h, room temp.; (iv) H_2, $PdCl_2$, THF–C_2H_5OH–H_2O (4:4:2, v/v/v), 68% from **15a**.

amino acids used for the synthesis is obtained with one of the two possible Williams glycine templates, our procedure is also useful for the synthesis of all unnatural dimeric amino acids with a (2*R*)-configuration.

3. Experimental

3.1. General

Melting points were measured on a SMP3 mp apparatus (Stuart Scientific, USA) and are uncorrected. Nuclear magnetic resonance spectra were recorded at 298 K 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 for million (ppm, δ units) relative to the solvent signal.[18] Proton and carbon assignments were established, if necessary, with homonuclear and heteronuclear 2D *J*-revolved experiments. ^1H NMR data are tabulated in the following order: number of protons, multiplicity (s, singlet; d, doublet; bs, broad singlet; m, multiplet), coupling constant(s) in Hertz, assignment of proton(s). ^1H NMR and ^{13}C NMR spectra of compounds containing oxazinone ring(s) were complicated by the presence of distinguishable rotamers.[13] Optical rotations were taken at 24 °C on a Perkin–Elmer 241 polarimeter and $[\alpha]_D$ values are given in 10^{-1} deg cm^2 g^{-1}. The enantiomeric purity of the final amino acids was ascertained by gas chromatography of the corresponding trifluoracetylated methyl esters obtained as described in previous work on the synthesis of hydroxylysine.[19] 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-cyclodestrin (LipodexE) 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. Mass spectra were obtained using a Finnigan LCQdeca (ThermoQuest) ion trap mass spectrometer fitted with an electrospray source (ESI). 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. E. Merck 230–400 mesh silica gel was used for flash column chromatography. Usual work-up refers to washing the organic layer with water, drying over anhydrous Na_2SO_4 and evaporating the organic solvent under reduced pressure. Used Resin was a DOWEX 50X 8-200 was used as the cation exchange resin.

3.2. *tert*-Butyl(3*S*,5*S*,6*R*)-3-[(3*R*)-3-hydroxy-4-bromobutyl]-2-oxo-5,6-diphenyl-1,4-oxazinane-4-carboxylate 8 and *tert*-butyl(3*S*,5*S*,6*R*)-3-[(3*S*)-3-hydroxy-4-bromobutyl]-2-oxo-5,6-diphenyl-1,4-oxazinane-4-carboxylate 9

A mixture of the previously obtained[7b] diastereomeric epoxides **6** and **7** (846 mg, 2 mmol), dissolved in dry THF (20 mL) containing AcOH (0.34 mL, 6 mmol), was treated with anhydrous LiBr (280 mg, 3.2 mmol) and stirred at room temperature for 4 h. The mixture was then concentrated, diluted with water and extracted with ethyl acetate. The usual work-up afforded a crude residue (825 mg; $Y = 82\%$) formed by a stereomeric mixture of the bromohydrins **8** and **9**. Rapid chromatography (eluting with dichloromethane–acetone; 100:2, v/v) gave first the pure *tert*-butyl(3*S*,5*S*,6*R*)-3-[(3*R*)-3-hydroxy-4-bromobutyl]-2-oxo-5,6-diphenyl-1,4-oxazinane-4-carboxylate **8** (397 mg; $Y = 41\%$): mp 157–158 °C, (from

dichloromethane–benzene); $[\alpha]_D^{25} = -46.6$ (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.29–6.97 (8H, aromatics), 6.53 (2H, aromatics), 5.98 (0.2H, d, $J = 3$ Hz, 6-H; minor conformer), 5.95 (0.8H, d, $J = 3$ Hz, 6-H; major conformer), 5.25 (0.2H, d, $J = 3$ Hz, 5-H; minor conformer), 5.03 (1.6H, overlapping, 5-H; major conformer and 3-H; major conformer), 4.86 (0.2H, dd, $J = 10.5$, 4.5 Hz, 3-H; minor conformer), 4.14 (0.8H, m, 3′-H; major conformer), 3.96 (0.2H, m, 3′-H; minor conformer), 3.59 (1H, dd, $J = 10.5$, 3.8 Hz, 4′-Ha), 3.46 (1H, dd, $J = 10.5$, 6.6 Hz, 4′-Hb), 3.32 (1H, br s, O***H***), 2.34 (1H, m, 1′-Ha), 2.21 (1H, m, 1′-Hb), 1.93 (1H, m, 2′-Ha), 1.81 (1H, m, 2′-Hb), 1.41, 1.07 [9H, 2 × s, 2 × C(C***H***$_3$)$_3$; minor conformer and major conformer]; ^{13}C NMR ($CDCl_3$): 168.97, 153.89, 136.12, 134.08, 128.61–126.03, 81.75, 79.14, 71.13, 69.43, 61.43, 55.30, 39.37, 31.16, 30.85, 27.81; ESI-MS (positive) *m*/*z*: 526 ($^{79}Br-M+Na^+$), 528 ($^{81}Br-M+Na^+$), 1031 ($^{79}Br-2M+Na^+$), 1033 ($^{81}Br-2M+Na^+$). Anal. Calcd for $C_{25}H_{30}BrNO_5$: C, 59.53; H, 5.99; N, 2.78. Found: C, 59.38; H, 6.12; N, 2.86.

Additional elution afforded the diastereomeric *tert*-butyl (3*S*,5*S*,6*R*)-3-[(3*S*)-3-hydroxy-4-bromobutyl]-2-oxo-5,6-diphenyl-1,4-oxazinane-4-carboxylate **9** (389 mg; $Y = 41\%$): mp 156–158 °C, (from dichloromethane–benzene); $[\alpha]_D^{25} = -45.7$ (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.29–6.58–6.92 (8H, aromatics), 6.58 (2H, aromatics), 5.95 (1H, d, $J = 3$ Hz, 6-H), 5.26 (0.2H, d, $J = 3$ Hz, 5-H; minor conformer), 5.16 (0.8H, dd, $J = 9.3$, 5.4 Hz, 3-H; major conformer), 5.06 (0.8H, d, $J = 3$ Hz, 5-H; major conformer), 4.89 (0.2H, dd, $J = 10.5$, 4.5 Hz, 3-H; minor conformer), 3.97 (0.8H, m, 3′-H; major conformer), 3.88 (0.2H, m, 3′-H; minor conformer), 3.61 (0.8H, dd, $J = 10.5$, 3.5 Hz, 4′-Ha; major conformer), 3.58 (0.2H, dd, $J = 10.5$, 3.5 Hz, 4′-Hb; minor conformer), 3.50 (0.8H, dd, $J = 10.5$, 6.5 Hz, 4′-Hb; major conformer), 3.46 (0.2H, dd, $J = 10.2$, 6.5 Hz, 4′-Hb; minor conformer), 2.53 (0.2H, m, 1′-Ha; minor conformer), 2.39 (0.8H, m, 1′-Ha; major conformer), 2.11 (0.8H, m, 1′-Hb; major conformer), 2.05 (0.2H, m, 1′-Hb; minor conformer), 1.93 (1.8H, m, overlapping, 2′-Ha 2′-Hb; major conformer and 2′-Ha; minor conformer), 1.83 (0.2H, m, 2′-Hb; minor conformer), 1.41, 1.07 [9H, 2 × s, 2 × C(C***H***$_3$)$_3$; minor conformer and major conformer]; ^{13}C NMR ($CDCl_3$): 168.97, 153.80, 136.22, 134.23, 129.93–126.27, 81.42, 79.07, 70.61, 61.59, 56.12, 40.05, 31.74, 31.34, 27.82; ESI-MS (positive) *m*/*z*: 526 ($^{79}Br-M+Na^+$), 528 ($^{81}Br-M+Na^+$), 1031 ($^{79}Br-2M+Na^+$), 1033 ($^{81}Br-2M+Na^+$). Anal. Calcd for $C_{25}H_{30}BrNO_5$: C, 59.53; H, 5.99; N, 2.78. Found: C, 59.50; H, 6.02; N, 2.80.

3.3. *tert*-Butyl (3*S*,5*S*,6*R*)-3-[(3*R*)-3,4-epoxybutyl]-2-oxo-5,6-diphenyl-1,4-oxazinane-4-carboxylate 6 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 7

tert-Butyl (3*S*,5*S*,6*R*)-3-[(3*R*)-3-hydroxy-4-bromobutyl]-2-oxo-5,6-diphenyl-1,4-oxazinane-4-carboxylate **8** (1.12 g; 2 mmol) was dissolved in CH_3CN (15 mL) containing $CsCO_3$ (1.95 g; 6 mmol) and stirred for 2 h. At this point, the mixture was poured into water and extracted with ethyl acetate. Usual work-up afforded a solid residue, which is crystallized to afford the *tert*-butyl (3*S*,5*S*,6*R*)-3-[(3*R*)-3,4-epoxybutyl]-2-oxo-5,6-diphenyl-1,4-oxazinane-4-carboxylate **6** as a solid (0.867 mg, $Y = 92\%$): mp 129–130 °C (diisopropyl ether); $[\alpha]_D^{25} = -48$ (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.28–6.95 (8H, aromatics), 6.54 (2H, aromatics), 5.91 (1H, d, $J = 3.00$ Hz, 6-H), 5.21 (0.2H, d, $J = 3.00$ Hz 6-H; minor conformer), 5.02 (0.8H, dd, $J = 10.52$, 4.21 Hz, 3-H; major conformer), 4.99 (0.8H, d, 6-H, $J = 3.00$ Hz; major conformer), 4.84 (0.2H, dd, $J = 10.52$, 4.21 Hz, 3-H; minor conformer), 3.03 (1H, m, 3′-H), 2.79 (1H, m, 4′-Ha), 2.57 (1H, m, 4′-Hb), 2.32 (1H, m, 2′-Ha), 2.12 (1H, m, 2′-Hb), 1.96 (1H, m, 1-Ha), 1.70 (1H, m, 1-Hb), 1.45, 1.08 [9H, s, C(C***H***$_3$)$_3$; minor and major conformer]; ^{13}C NMR ($CDCl_3$): 168.91, 153.65, 136.32, 134.25, 128.90–126.19, 81.25, 78.94, 61.56, 56.14, 51.43, 47.35, 31.24, 28.94, 27.76; ESI-MS (positive) *m*/*z*: 446 ($M+Na^+$), 869 ($2M+Na^+$). Anal. Calcd for $C_{25}H_{29}NO_5$: C, 70.90; H, 6.90; N, 3.31. Found: C, 70.50; H, 6.70; N, 3.20.

Similar treatment of the *tert*-butyl (3*S*,5*S*,6*R*)-3-[(3*S*)-3-hydroxy-4-bromobutyl]-2-oxo-5,6-diphenyl-1,4-oxazinane-4-carboxylate **9** (1.12 g; 2 mmol) afforded the *tert*-butyl(3*S*,5*S*,6*R*)-3-[(3*S*)-3,4-epoxybutyl]-2-oxo-5,6-diphenyl-1,4-oxazinane-4-carboxylate **7** as a solid (0.862 mg; $Y = 92\%$): mp 120–122 °C (diisopropyl ether); $[\alpha]_D^{25} = -71$ (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.28–6.95 (8H, aromatics), 6.54 (2H, aromatics), 5.91 (1H, d, $J = 3.00$ Hz, 6-H), 5.19 (0.8H, d, $J = 3.00$ Hz, 5-H minor conformer), 5.02 (0.8H, dd, $J = 9.82$, 5.61 Hz, 3-H; major conformer), 4.98 (0.8H, d, $J = 3.00$ Hz, 5-H major conformer), 4.80 (0.2H, dd, $J = 9.82$, 4.82 Hz, 3-H; minor conformer), 3.03 (1H, m, 3′-H), 2.79 (1H, m, 4′-Ha), 2.57 (1H, m, 4′-Hb), 2.32 (1H, m, 2′-Ha), 2.06 (1H, m, 2′-Hb), 1.97 (1H, m, 1-Ha), 1.97 (1H, m, 1-Hb), 1.78 (1H, m, 1-Hb), 1.46, 1.08 [9H, s, C(C***H***$_3$)$_3$, minor and major conformer]; ^{13}C NMR ($CDCl_3$): 169.18, 153.73, 136.40, 134.09, 129.98–126.42, 81.29, 78.94, 61.53, 56.16, 51.46, 46.79, 31.47, 28.80, 27.78; ESI-MS (positive) *m*/*z*: 446 ($M+Na^+$), 869 ($2M+Na^+$). Anal. Calcd for $C_{25}H_{29}NO_5$: C, 70.90; H, 6.90; N, 3.31. Found: C, 70.60; H, 6.80; N, 3.40.

3.4. Alkylation of the 6-amino-(2*S*,5*R*)-2-benzyloxycarbonylaminohexanoate *tert*-butyl ester 5 with *tert*-butyl(3*S*,5*S*,6*R*)-3-[(3*R*)-3,4-epoxybutyl]-2-oxo-5,6-diphenyl-1,4-oxazinane-4-carboxylate 6 and with *tert*-butyl(3*S*,5*S*,6*R*)-3-[(3*S*)-3,4-epoxybutyl]-2-oxo-5,6-diphenyl-1,4-oxazinane-4-carboxylate 7

The protected lysine *tert*-butyl ester **5** (830 mg; 2.5 mmol) dissolved in acetonitrile (5 mL) was treated with the epoxide **6** (530 mg; 1.25 mmol) in the presence of $LiClO_4$ (130 mg; 1.25 mmol) at room temperature for 12 h and then diluted with water and extracted with ethyl acetate. After the usual work-up, the residue was purified by rapid chromatography, eluting with a solution formed by $CHCl_3$/MeOH/NH_3 (100:2:0.25; v/v/v), to afford the dialkylated compound **10a** as a glass (617 mg; $Y = 65\%$ from **6**): $[\alpha]_D^{25} = -23.5$ (*c* 1, $CHCl_3$);

^{1}H NMR ($CDCl_3$): δ 7.37–6.95 (8H, m, aromatics), 6.60 (2H, m, aromatics), 6.01 (0.7H, d, $J = 3$ Hz, PhC***H***OCHPh; major conformer), 5.98 (0.3H, d, $J = 3$ Hz; PhC***H***OCHPh; minor conformer), 5.45 (0.7H, d, $J = 8.4$ Hz, N***H***; major conformer), 5.42 (0.3H, d, $J = 8.4$ Hz, N***H***; minor conformer), 5.24 (0.3H, d, $J = 3.0$ Hz, PhC***H***NBoc; minor conformer), 5.12 (2H, s, PhC***H***$_2$O), 5.02 (0.7H, d, $J = 3.0$ Hz, PhC***H***NBoc; major conformer), 5.01 (0.7H, dd, $J = 9.8$, 5.9 Hz COC***H***N; major conformer), 4.94 (0.3H, dd, $J = 9.8$ Hz, 4.5 Hz COC***H***N; minor conformer), 4.26 (1H, m, COC***H***NHCbz), 3.90 (0.7H, m, CH$_2$C***H***OH; major conformer), 3.77 (0.3H, m, CH$_2$C***H***OH; minor conformer), 2.79–2.54 (4H, m, overlapping, NHC***H***$_2$CHOH and NHC***H***$_2$CH$_2$), 2.33–2.20 (2H, m, NCHC***H***$_2$CH$_2$), 1.87–1.35 (8H, m, overlapping, C***H***$_2$CH$_2$CH$_2$CH$_2$, CH$_2$C***H***$_2$CH$_2$CH$_2$, CH$_2$CH$_2$C***H***$_2$- CH$_2$, CH$_2$CH$_2$CH$_2$-C***H***$_2$), 1.47 [9H, s, COOC(C***H***$_3$)$_3$], 1.12 [9H, s, NCOOC(C***H***$_3$)$_3$]; ^{13}C NMR ($CDCl_3$): 171.56, 169.14, 155.86, 153,68, 136.41, 134.26, 131.40, 129.07–126.20, 82.03, 81.37, 78.91, 67.92, 66.85, 61.41, 55.93, 55.01, 54.29, 49.08, 32.67, 31.15, 31.21, 28.29, 28.01, 22.68; ESI-MS (positive) m/z: 760 (M+H$^+$), 782 (M+Na$^+$), 1519 (2M+H$^+$), 1542 (2M+Na$^+$). Anal. Calcd for $C_{43}H_{57}N_3O_9$: C, 67.96; H, 7.56; N, 5.53. Found: C, 67.70; H, 7.70; N, 5.40.

Similar treatment of the protected lysine *tert*-butyl ester **5**, (830 mg; 2.5 mmol) with the *tert*-butyl (3*S*,5*S*,6*R*)-3-[(3*S*)-3,4-epoxybutyl]-2-oxo-5,6-diphenyl-1,4-oxazinane-4-carboxylate **7** (530 mg; 1.25 mmol) afforded, after rapid chromatography, eluting with $CHCl_3$/MeOH/NH_3 (100:2.5:0.25; v/v/v), the dialkylated compound **10b** as a glass (605 mg $Y = 64\%$ from **7**); $[\alpha]_D^{25} = -21.1$ (c 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.37–6.95 (8H, aromatics), 6.95 (2H, aromatics), 6.01 (0.7H, d, $J = 3$ Hz, PhC***H***OCHPh; major conformer), 6.00 (0.3H, d, $J = 3$ Hz, PhC***H***OCHPh; minor conformer), 5.44 (0.7H, d, $J = 8.4$ Hz, N***H***; major conformer), 5.42 (0.3H, d, $J = 8.4$ Hz, N***H***; minor conformer), 5.23 (0.3H, d, $J = 3.0$ Hz, PhC***H***NBoc; minor conformer), 5.12 (2H, s, PhC***H***$_2$O), 5.04 (0.7H, dd, $J = 9.4$, 5.6 Hz, COC***H***N; major conformer), 5.02 (0.3H, d, $J = 3.0$ Hz, PhC***H***NBoc; minor conformer), 4.82 (0.3H, dd, $J = 9.4$, 4.2 Hz COC***H***N; minor conformer), 4.26 (1H, m, COC***H***NHCbz), 3.74 (0.7H, m, CH$_2$C***H***OH; major conformer), 3.64 (0.3H, m, CH$_2$C***H***OH; minor conformer), 2.76–2.60 (3H, m, overlapping, NHCH***H***$_a$-CHOH and NHC***H***$_2$CH$_2$), 2.55 (0.7H, dd, $J = 12.2$, 9.5 Hz, NHC***H***$_b$CHOH; major conformer), 2.50 (0.3H, dd, $J = 12.2$, 9.5 Hz, NHCH***H***$_b$CHOH; minor conformer), 2.39 (1H, m, NCHCH***H***$_a$CH$_2$), 2.05 (1H, m, NCHCH***H***$_b$CH$_2$), 1.85–1.35 (8H, m, overlapping, C***H***$_2$CH$_2$CH$_2$CH$_2$, CH$_2$C***H***$_2$CH$_2$CH$_2$, CH$_2$CH$_2$C***H***$_2$-CH$_2$, CH$_2$CH$_2$CH$_2$C***H***$_2$), 1.47 [9H, s, COOC- (C***H***$_3$)$_3$], 1.12 [9H, s, NCOOC(C***H***$_3$)$_3$]; ^{13}C NMR ($CDCl_3$) δ 171.62, 169.57, 155.93, 153.72, 136.54, 136.39, 134.36, 128.80–126.34, 82.07, 81.18, 78.82, 68.87, 66.83, 61.51, 56.39, 54.92, 54.21, 49.15, 32.63, 31.67, 31.24, 28.27, 27.99, 22.68; ESI-MS (positive) m/z: 760 (M+H$^+$), 782 (M+Na$^+$), 1519 (2M+H$^+$), 1542 (2M+Na$^+$). Anal. Calcd for $C_{43}H_{57}N_3O_9$: C, 67.96; H, 7.56; N, 5.53. Found: C, 67.85; H, 7.65; N, 5.60.

3.5. (2*S*,5*R*)-2-Amino-6-[(5*S*)-5-amino-5-carboxypentylamino]-5-hydroxyhexanoic acid 3a

A flame dried, two-necked, round-bottomed flask (100 mL), equipped with a magnetic stirring bar was charged with liquid ammonia (17 mL) and sodium metal (40 mg; 2 mmol). The resulting solution was stirred at −40 °C. Meanwhile, a flame-dried round-bottomed flask, fitted with a rubber stopper and an argon inlet, was charged with the completely protected dimeric amino acid **10a** (135 mg; 0.18 mmol), anhydrous THF (1.7 mL) and absolute ethanol (0.100 mL). The resulting mixture was then transferred to the sodium/liquid ammonia solution. After 20 min, the reaction was carefully quenched with solid ammonium chloride and allowed to warm to room temperature. After evaporation of the remaining ammonia, the residue was diluted with ice cold water and the mixture extracted with ethyl acetate. Then, the aqueous layer was acidified to pH 1 with HCl (1 M) and the obtained solution was applied on a DOWEX 50X 8-200 cation exchange resin column activated with HCl 2 M to afford the amino acid **11a**, eluting with an aqueous ammonia solution (0.5 M). The crude compound **11a** was then dissolved in aqueous HCl 1 M (5 mL) and the solution stirred for 2 h. Successive evaporation of the solvent afforded the amino acid **3a** as a chloridrate salt (36 mg; $Y = 52\%$); $[\alpha]_D^{25} = +4.4$ (c 0.33, H_2O); ^{1}H NMR (D_2O): δ 4.12 (2H, m, 2-H, 5′-H), 3.89 (1H, m, 5-H), 3.23 (1H, dd, 6-Ha, $J = 13.30$, 2.45 Hz, 6-Ha), 3.15 (2H, t, $J = 8.05$ Hz, 1-H), 3.05 (1H, dd, $J = 12.60$, 2.45 Hz 6-Hb), 2.19 (1H, m, 3-Ha), 2.02 (3H, m, overlapping, 3-Hb, 4′-H), 1.81 (2H, m, 2′-H), 1.68 (2H, m, 4-H), 1.56 (2H, m, 3′-H); ^{13}C NMR (D_2O): δ 173.19, 172.99, 67.21, 53.83, 53.13, 48.20, 30.55, 30.30, 26.96, 25.96 22.57; ESI-MS (positive) m/z: 292 (M+H$^+$). Anal. Calcd for $C_{12}H_{28}Cl_3N_3O_5$: C, 35.97; H, 7.04; N, 10.49. Found: C, 36.05; H, 7.15; N, 10.60.

3.6. (2*S*,5*S*)-2-Amino-6-[(5*S*)-5-amino-5-carboxypentylamino]-5-hydroxyhexanoic acid 3b

Starting from the compound **10b** (135 mg; 0.18 mmol), and using the same procedure described above for the dioxazinone **10a**, the amino acid **3b** was obtained (37 mg $Y = 52\%$); $[\alpha]_D^{25} = +8.7$ ($c = 0.33$, H_2O); ^{1}H NMR (D_2O): δ 3.98 (1H, m, 5-H), 3.79 (2H, dd, $J = 12.60$, 6.30 Hz, 2-H, 5′-H), 3.23 (1H, dd, $J = 12.95$, 3.15 Hz, 6-Ha), 3.14 (2H, t, $J = 8.05$, 1′-H), 3.07 (1H, dd, $J = 12.95$, 3.15 Hz, 6-Hb), 1.98 (1H, m, 3-Ha), 1.86 (3H, m, overlapping, 3-Hb, 4′-H), 1.71 (2H, m, 2′-H), 1.63 (1H, m, 4-Ha), 1.44 (3H, m, overlapping, 4-Hb, 3′-H); ^{13}C NMR (D_2O): δ 175.43, 175.25, 67.22, 63.56, 55.45, 53.05, 49.05, 30.08, 27.48, 25.97, 22.49; ESI-MS (positive) m/z: 292 (M+H$^+$). Anal. Calcd for $C_{12}H_{28}Cl_3N_3O_5$: C, 35.97; H, 7.04; N, 10.49. Found: C, 35.85; H, 9.95; N, 10.55.

3.7. Preparation of the tertiary benzylamines 12a and 12b

To a stirred solution of the epoxide **6** (250 mg, 0.59 mmol) in acetonitrile (0.8 mL), $LiClO_4$ (62.6 mg, 0.59 mmol) and benzylamine (32 μl, 0.29 mmol) were

added. The resulting solution was stirred for 12 h at 60 °C. After complete conversion of epoxide **6** (TLC), the reaction mixture was diluted with ethyl acetate and worked-up. Evaporation of the solvent under reduced pressure and purification of the crude product on silica gel, [eluting with a mixture of $CHCl_3$/solvent A 80:20 v/v, where solvent A is a solution of $CHCl_3$/MeOH/NH_3 (100:4:0.5 v/v/v)], gave the pure trialkylamine **12a** as a glassy material which was crystallized from CH_2Cl_2/benzine to afford the pure compound **12a** as a white solid (221 mg, $Y = 78\%$); $[\alpha]_D^{25} = -63.5$ (c 1, $CHCl_3$); mp 120–122 °C; ^{1}H NMR ($CDCl_3$): δ 7.42–6.87 (21H, aromatics), 6.55 (4H, aromatics), 5.94 (1H, d, $J = 2.80$ Hz, PhC***H***OCHPh), 4.96 (3.4H, m, overlapping, 2 × PhC***H***Nboc and COC***H***N major conformer), 4.79 (0.6H, dd, $J = 10.6$, 4.7 Hz COC***H***N; minor conformer), 3.94 (2.8H, m, overlapping, PhC***H***$_2$N, and CH_2C***H***OH, major conformer), 3.80 (0.6H, m, CH_2C***H***OH; minor conformer), 3.56 (0.6H, d, $J =$ 13.8 Hz, PhC***H***$_2$N; minor conformer), 2.53 (4H, m, PhNC***H***$_2$CHOH), 2.23 (4H, m, NCHC***H***$_2$$CH_2$CHOH), 1.65–1.47 (4H, m, NCHCH_2C***H***$_2$CHOH), 1.43, 1.09 [2 × 9H, 2 × s, 2 × NCOOC(C***H***$_3$)$_3$; major and minor conformer]; ^{13}C NMR ($CDCl_3$): δ 169.22, 153.80, 136.44, 134.29, 129.80–125.70, 81.41, 78.87, 67.04, 61.30, 60.41, 59.67, 56.02, 31.17, 30.58, 27.80; ESI-MS (positive) m/z: 954 ($M+H^+$), 1908 ($2M+H^+$). Anal. Calcd for $C_{57}H_{67}N_3O_{10}$: C, 71.75; H, 7.08; N, 4.40. Found: C, 71.65; H, 7.30; N, 4.30.

Using the same procedure for the synthesis of **12a**, starting from the epoxide **7** (250 mg; 0.59 mmol) the protected dimeric amino acid **12b** was obtained (212 mg Y = 75%): $[\alpha]_D^{25} = -21.4$ (c 1, $CHCl_3$); mp 125–126 °C, CH_2Cl_2/benzine; ^{1}H NMR ($CDCl_3$): δ 7.43–6.90 (21H, m, aromatics), 6.55 (4H, m, aromatics), 5.91 (1H, d, $J = 3$ Hz, PhC***H***OCHPh;), 5.18 (0.6H, d, $J = 3$ Hz, PhC***H***Nboc; minor conformer), 4.99 (1.4H, dd, $J = 9.3$, 5.6 Hz COC***H***N; major conformer), 4.95 (1.4H, d, $J = 3$ Hz, PhC***H***Nboc; major conformer), 4.76 (0.6H, dd, $J = 10.2$, 4.1 Hz COC***H***N; minor conformer), 3.88 (1H, dd, $J = 13.5$, 7.6 Hz PhC***H***$_a$N), 3.77 (1.4H, m, CH_2C***H***$_a$OH), 3.69 (0.6H, m, RCH_2C***H***$_b$OH), 3.53 (1H, dd, $J = 13.5$, 4.3 Hz PhC***H***$_b$N), 2.53 (4H, m, PhNC***H***$_2$CHOH), 2.39 (2H, m, NCHCH***H***$_a$CH2-CHOH), 2.10 (2H, m, NCHCH***H***$_b$$CH_2$CHOH), 1.66 (4H, m, NCHC$H_2$C***H***$_2$CHOH), 1.40, 1.09 [2 × 9H, 2 × s, 2 × NCOOC(C***H***$_3$)$_3$; major and minor conformer]; ^{13}C NMR ($CDCl_3$): δ 169.61, 153.80, 136.63, 134.09, 129.80–126.90, 81.14, 78.83, 67.74, 61.55, 60.47, 59.85, 56.33, 31.90, 31.00, 27.82; ESI-MS (positive) m/z: 954 ($M+H^+$), 1908 ($2M+H^+$), 1931 ($2M+Na^+$). Anal. Calcd for $C_{57}H_{67}N_3O_{10}$: C, 71.75; H, 7.08; N, 4.40. Found: C, 71.85; H, 7.20; N, 4.50%.

3.8. (2*S*,5*R*)-2-Amino-6-[(2*R*,5*S*)-5-amino-5-carboxy-2-hydroxypentylamino]-5-hydroxyhexanoic acid 4a and (2*S*,5*S*)-2-amino-6-[(2*S*,5*S*)-5-amino-5-carboxy-2-hydroxypentylamino]-5-hydroxyhexanoic acid 4c

Tertiary amine **12a** (120 mg; 0.125 mmol) was dissolved in (0.7 mL) of anhydrous dichloromethane and the resulting solution was treated with anhydrous $ZnBr_2$ (113 mg; 0.5 mmol). After 3 h, the reaction mixture was diluted with dichloromethane and washed with water. Usual work-up afforded the crude compound **13a**, which was dissolved in THF/ethanol/water 4:4:2 v/v/v (50 mL) and hydrogenated at 60 psi over $PdCl_2$.

After 48 h, filtration of the catalyst on Celite and removal of the solvent, gave the crude dimeric amino acid **4a**, which was then dissolved in HCl 0.1 M and purified through a cationic exchange resin DOWEX 50X 8-200. Successive elution with an aqueous ammonia solution (0.5 M) and lyophilization gave the pure compound **4a** as a solid (25 mg, $Y = 65\%$); $[\alpha]_D^{25} = -3.4$ (c 0.5, H_2O); ^{1}H NMR (D_2O): δ 3.91 (2H, m, 5-H), 3.66 (2H, t, $J = 6.30$ Hz, 2-H), 2.98 (1H, dd, $J = 12.95$, 3.15 Hz, 6-Ha), 2.87 (1H, dd, $J = 12.95$, 3.15 Hz, 6-Hb), 2.01 (2H, m, 3-Ha), 1.86 (2H, m, 3-Hb), 1.59 (4H, m, 4-H); ^{13}C NMR (D_2O): δ 177.71, 68.77, 55.81, 53.88, 31.00, 28.69; ESI-MS (positive) m/z: 308 ($M+H^+$)$^+$, 330 ($M+Na^+$). Anal. Calcd for $C_{12}H_{25}N_3O_6$: C, 46.89; H, 8.20; N, 13.67. Found: C, 47.00; H, 8.30; N, 13.50.

Using the same reaction sequence described above for the synthesis of **4a**, starting from the compound **12b** (120 mg; 0.125 mmol), the free amino acid **4c** was obtained (26 mg $Y = 67\%$); $[\alpha]_D^{23} = +9.1$ (c 0.5, H_2O); ^{1}H NMR (D_2O): δ 3.84 (2H, m, 5-H), 3.48 (2H, t, $J = 6.30$ Hz, 2-H), 2.83 (1H, dd, $J = 12.95$, 3.15 Hz, 6-Ha), 2.76 (1H, dd, $J = 12.95$, 3.15 Hz, 6-Hb), 1.82 (4H, m, 3-H), 1.61 (2H, m, 4-Ha), 1.50 (2H, m, 4-Hb); ^{13}C NMR (D_2O): δ 181.20, 69.78, 56.32, 54.56, 31.34, 30.37; ESI-MS (m/z): 308 ($M+H^+$). Anal. Calcd for $C_{12}H_{25}N_3O_6$: C, 46.89; H, 8.20; N, 13.67. Found: C, 46.75; H, 8.15; N, 13.70.

3.9. *tert*-Butyl(3*S*,5*S*,6*R*)-3-[(3*R*)-4-benzylamino-3-hydroxybutyl]-2-oxo-5,6-diphenyl-1,4-oxazinane-4-carboxylate 14

To a stirred solution of epoxide **6** (140 mg, 0.34 mmol) in 0.5 mL of acetonitrile (0.5 mL), $LiClO_4$ (36 mg 0.34 mmol) and benzylamine (74 μL, 0.68 mmol) were added. The resulting solution was stirred for 3 h at 60 °C. After complete conversion of the epoxide **6** (TLC), the reaction mixture was cooled to room temperature, diluted with ethyl acetate and washed with water/brine. Usual work-up afforded a crude product which, after purification on silica gel, eluting with $CHCl_3$/MeOH/NH_3; 100:4:0.5 v/v/v, gave the pure dialkylamine **14** as a glassy material, which was crystallized with CH_2Cl_2/benzine to afford a solid compound **14** (99 mg, $Y = 55\%$); $[\alpha]_D^{25} = -44.8$ ($c = 1$, $CHCl_3$); mp 103–104 °C; ^{1}H NMR ($CDCl_3$): δ 7.36–6.96 (13H, m, aromatics), 6.56 (2H, m, aromatics), 5.98 (1H, d, $J = 3.0$ Hz, PhC***H***OCHPh), 5.24 (0.2H, d, $J = 3.0$ Hz, COC***H***Ph; minor conformer), 5.05 (0.8H, dd, $J = 9.1$, 5.7 Hz, COC***H***N; major conformer), 5.01 (0.8H, d, $J = 3.0$ Hz, COC***H***Ph; major conformer), 4.82 (0.2H, dd, $J = 9.1$, 4.2 Hz, COC***H***N; minor conformer), 3.87–3.69 (3H, m, PhC***H***$_2$N and CH_2C***H***OH), 2.83 (1H, dd, $J = 12.1$, 3.0 Hz PhNC***H***$_a$CHOH), 2.60 (1H, dd, $J = 12.1$, 9.2 Hz, PhNC***H***$_b$CHOH), 2.32–2.13 (2H, m, NCHC***H***$_2$$CH_2$CHOH), 1.76–1.56 (2H, m, NCHCH_2-

CH_2CHOH), 1.41, 1.08 [9H, s, NCOOC- (CH_3)$_3$, major and minor conformer]; ^{13}C NMR ($CDCl_3$): δ 169.57, 154.01, 136.36, 134.28, 131.47, 128.7–125.37, 81.42, 78.98, 68.05, 61.42, 55.84, 54.51, 53.34, 31.12, 28.44, 27.81; ESI-MS (positive) m/z: 531 ($M+H^+$), 1061 ($2M+H^+$). Anal. Calcd for $C_{32}H_{38}$- N_2O_5: C, 72.43; H, 7.22; N, 5.28. Found: C, 72.60; H, 7.30; N, 5.50.

3.10. Preparation of the tertiary benzylamine 15a

To epoxide **7** (92 mg, 0.17 mmol) dissolved in acetonitrile (0.6 mL), $LiClO_4$ (0.17 mmol, 19 mg) and trialkylamine **14** were added. The resulting solution was then stirred for 72 h at 60 °C. After complete conversion of the epoxide **7** (TLC), the reaction mixture was cooled to room temperature, diluted with ethyl acetate and washed with water. Usual work-up afforded a crude product, which was purified on silica gel, [eluting with a mixture of $CHCl_3$/solvent A 80:20 v/v where solvent A is a solution of $CHCl_3$/MeOH/NH_3 (100:4:0.5 v/v/v)] and gave the pure dialkylamine as a glassy material, which was crystallized from CH_2Cl_2/benzine to afford compound **15a** as a white solid (105 mg, $Y = 65\%$); $[\alpha]_D^{25} = -44.0$ (c 1, $CHCl_3$); mp 123–124 °C; ^{1}H NMR ($CDCl_3$): δ 7.37–6.89 (21H, m, aromatics), 6.52 (4H, m, aromatics), 5.90 (1H, d, $J = 3$ Hz, PhC*H*OCHPh; in oxazinone of the *S* branch), 5.87 (1H, d, $J = 3$ Hz, PhC*H*OCHPh; in oxazinone of the *R* branch), 5.16 (0.3×2H, d, $J = 3$ Hz, COC*H*Ph; minor conformer), 4.99 (0.7H, dd, $J = 8.99$, 6.14 Hz, COC*H*N; in oxazinone of the *S* branch; major conformer), 4.94 (2.1H, m, overlapping, $2 \times$ PhC*H*NBoc and COC*H*N of the *R* branch; major conformer), 4.76 (2×0.3H, dd, $J = 8.99$, 6.14 Hz, 5-H; COC*H*N; minor conformer), 3.95 (1, 2H, m, overlapping; PhCH_2N and CH_2C*H*OH; minor conformer), 3.74 (2.8H, m, overlapping; PhCH_2N and CH_2C*H*OH; major conformer), 2.72–2.51 (4H, m, PhNCH_2CHOH), 2.31 (1H, m, NCHC$H_a$$CH_2$CHOH, in *S* branch), 2.24–2.09 (2H, m, NCHC$H_2$$CH_2$CHOH, in *R* branch), 1.98 (1H, m, NCHC$H_b$$CH_2$CHOH, in *S* branch), 1.70–1.47 (4H, m, NCHCH_2CH_2CHOH, in *S* and *R* branch), 1.40, 1.07 [2×9H, $2 \times$ s, $2 \times$ NCOOC(CH_3)$_3$; major and minor conformer]; ^{13}C NMR ($CDCl_3$): δ 169.57, 169.03, 153.80, 136.58, 136.29, 134.33, 134.26, 129.46, 126.20, 81.48, 81.14, 78.98, 78.80, 68.98, 67.97, 61.55, 61.30, 60.28, 60.13, 56.32, 55.52, 31.81, 31.02, 30.73; ESI-MS (positive) m/z: 954 ($M+H^+$), 1908 ($2M+H^+$). Anal. Calcd for $C_{57}H_{67}N_3O_{10}$: C, 71.75; H, 7.08; N, 4.40. Found: C, 71.55; H, 7.10; N, 4.20.

3.11. (2*S*,5*R*)-2-Amino-6-[(2*S*,5*S*)-5-amino-5-carboxy-2-hydroxypentylamino]-5 hydroxyhexanoic acid 4b

The protected functions of compound **15a** (95 mg; 0.10 mmol) were deblocked adopting the procedure described for compounds **12a** and **12b**, starting from **15a**. The compound **15b** was obtained and directly hydrogenated to afford the dimeric amino acid **4b** (*meso form*): (21 mg, $Y = 68\%$); ^{1}H NMR (D_2O): δ 3.85 (2H, m, 5-H, 5′-H), 3.66 (2H, m, 2-H, 2′H), 3.08 (2H, dd, 6-Ha, $J = 12.95$, 2.80 Hz), 2.92–2.86 (2H, dd, 6-Hb, $J = 12.95$, 2.80 Hz,), 2.02–1.89 (2H, m, 3-H, 3′-H), 1.88–1.76 (2H, m, 3-H, 3′-H), 1.65, 1.39 (2H, m, 4-H, 4′-H); ^{13}C NMR (D_2O) δ 183.66, 67,71, 67.63, 55.49, 55.43, 53.44, 30.88, 30.73, 27.72; ESI-MS (positive) m/z: 308 ($M+H^+$). Anal. Calcd for $C_{57}H_{67}N_3O_{10}$: C, 71.75; H, 7.08; N, 4.40. Found: C, 71.80; H, 7.20; N, 4.50.

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