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A simple modular synthesis of pyridinoline a collagen cross-link of biochemical interest

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Abstract—A simple convergent synthesis of the collagen cross-link pyridinoline starting from glycine is reported.

1. Introduction

Pyridinoline (Pyd; **1**; Fig. 1) and deoxypyridinoline (dPyd; **2**) are two pyridinium cross-links formed in the mature form of collagen from lysine and hydroxylysine residues.[1] Currently the urinary levels of these two collagen cross-links are used in the clinic as reliable biomarkers for the non-invasive assessment of bone degradation in various pathologies, such as osteoporosis, bone cancer and alteration of bone metabolism.[2] Thus it is particularly important to have available suitable amounts of Pyd **1** and dPyd **2** in pure form for use as reference standards in diagnostics. As a consequence of both, the difficulties involved in the procurement of Pyd **1** and of dPyd **2** from natural sources[3] and of the chemical interest for the structural features of these compounds, various synthetic protocols for their synthesis have recently been reported.[4] Moreover, while the routes to obtain dPyd **2**, starting from natural amino acids, have been widely exploited and well set-up, those affording Pyd **1** are far less advanced, due to the difficulties connected with the presence of an additional stereogenic centre in the chain bonded to the heterocyclic nitrogen of the pyridine portion of the molecule. Thus, we started this work with the aim of solving the pending troubles connected with the modular preparation[4a,g,i] of Pyd **1**, from the protected pyridines **3a**[4b] or **3b**, and regarding the availability of the stereomerically pure amino acidic halohydrins **4a**–**e**.

In fact, the amino acidic iodohydrin **4a** was obtained[4g] from the corresponding bromohydrin **4b**. This derived

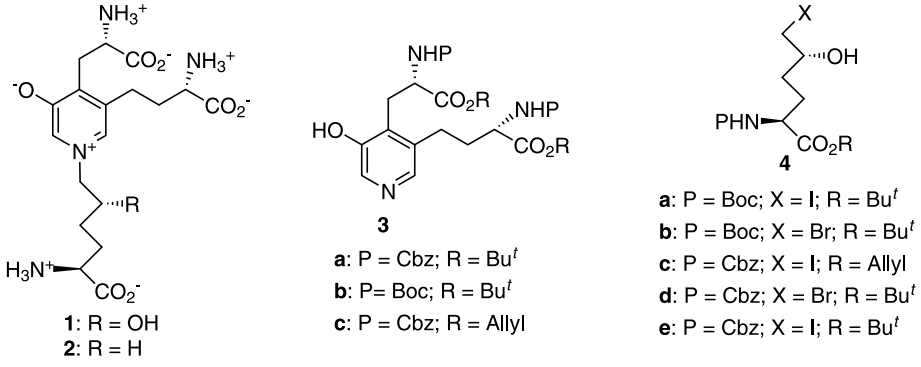

Figure 1.

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from glutamic acid as a 1:1 mixture with the 2*S*,5*S* stereoisomer from which it can not be directly resolved by common methods, not even qualitatively. Moreover, both free and the derivative esterified at the alcoholic group, even as Mosher esters, behave similarly on TLC and reverse phase HPLC.[4g] Similarly, the diastereomeric mixture of the parent epoxides is unseparable under several different conditions. As a consequence, the 2*S*,5*R* diastereomer **4b** useful for the preparation of the iodohydrin **4a** and of the Pyd **1**, needed be isolated by esterification of the alcoholic group with a thyroxin acid derivative, followed by separation of the esters through preparative HPLC and subsequent selective hydrolysis thereof.[4g] Similar difficulties were observed in the preparation of the iodohydrin **4c** which was only recently obtained in our laboratory from the corresponding bromohydrin **4d** which was separated by a relatively simple artifice[4a] from its (2*S*,5*S*)-diastereomer. Moreover, in no case was the possibility of constructing the stereogenic centres of the halohydrins **4** considered.

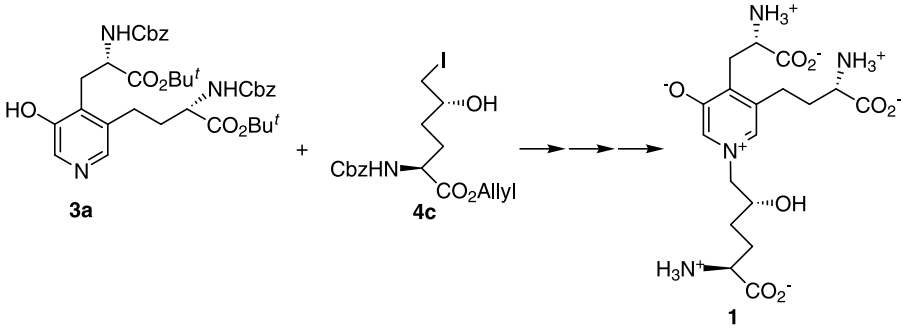
2. Results and discussion

Here we report the modular synthesis of Pyd **1** by alkylation of the substituted pyridine **3a** with the iodohydrin **4c** (Scheme 1). Both the pyridine nucleus and the aminoacidic iodohydrin are obtained in a short stereoselective way, starting from the achiral amino acid glycine, which is the starting material for the preparation of the key (*S*)-2-benzyloxycarbonylamino-5-hexenoic acid **5** (Scheme 2).

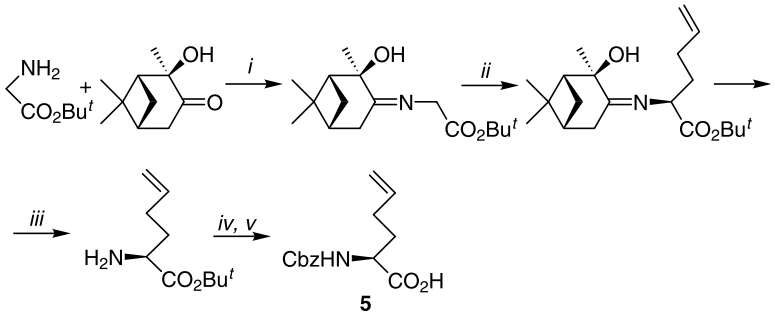
In fact, for the construction of the stereogenic centre at C-5 of the iodohydrin **4c**, one available option was to perform a stereoselective iodolactonization of the unsaturated acid **5** (Scheme 3) hoping to obtain the (2*S*,5*R*)-δ-valerolactone **6** and modest quantities of the undesired isomeric (2*S*,5*S*)-δ-valerolactone **7**. The lactone **6**, by successive alcoholysis, could afford the iodohydrin **4c**.

With this goal in mind, we first obtained the unreported L-amino acid **5** by asymmetric alkylation of a chiral derivative of glycine (Scheme 2), according to the method suggest by Hoarau et al.[5] for the synthesis of the (*R*)-isomer of **5**, used in the synthesis of pipecolic acid. The enantiomeric excess of the amino acid **5** was shown to be higher than 98% by chiral GLC of the L-glutammic acid diethyl ester, obtained via initial oxidation of the double bond of **5** (see Section 3).

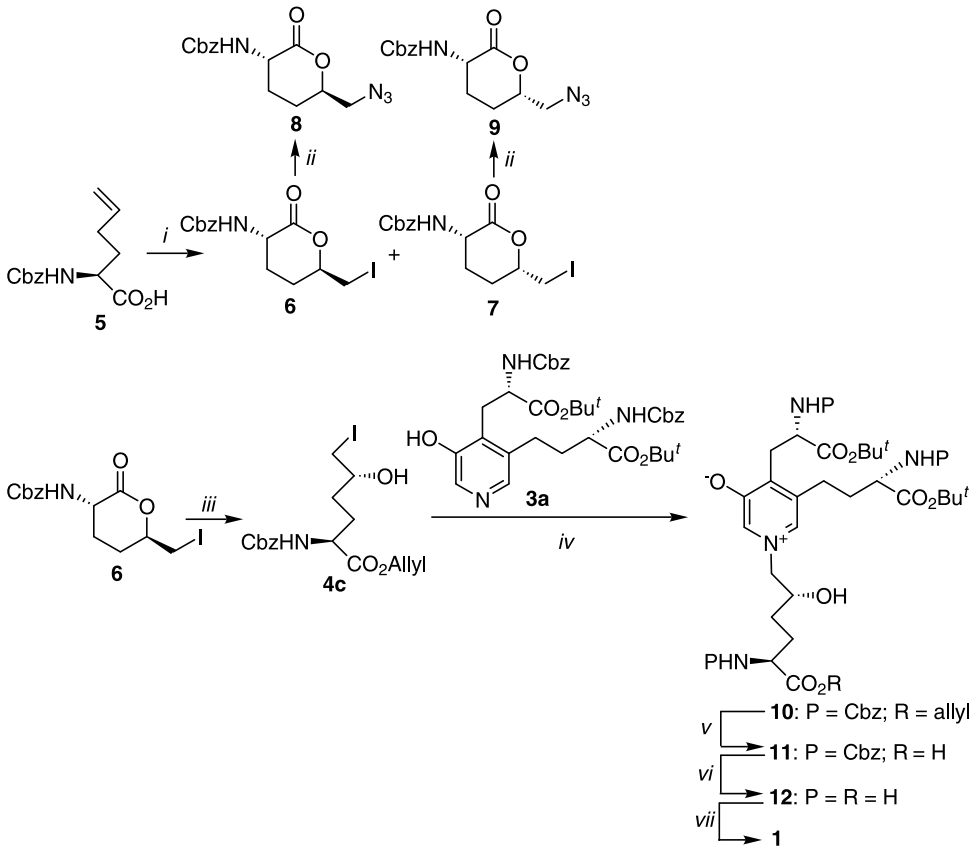
The iodolactonization of the amino acid **5** could be performed under two sets of conditions: under thermodynamic control, involving the equilibration of a cyclic intermediate by action of hydrogen iodide formed in the reaction, or under kinetic control, preventing the action of the acid with a base.[6] In a preliminary step we performed MM+ and AM1 calculation of the desired lactone **6** and of its diastereomer **7**, in order to have an estimate of the relative thermodynamic stability to help us choosing the most favourable conditions to run the iodolactonization reaction. Using both computational methods, we found very low energy differences between the *trans*- and *cis*-lactones **6** and **7** showing that the



Scheme 1.



Scheme 2. *Reagents and conditions:* (i) $BF_3{\cdot}Et_2O$, toluene, reflux, 2.5 h, 72%; (ii) LiHMDS, 4-bromo-1-butene, THF, −100→−30°C, 20 h, 85%; (iii) citric acid, H_2O/THF, room temp., 84 h, 85%; (iv) CbzCl, dioxane/H_2O, room temp., 2.5 h, 89%; (v) TFA, room temp., 1 h, 98%.



Scheme 3. *Reagents and conditions:* (i) I_2, KI, $NaHCO_3/H_2O$, 0°C, 24 h, **6** (39%) and **7** (18%); (ii) NaN_3, DMF, room temp., 12 h, 96%; (iii) Cs_2CO_3, CH_2=$CHCH_2OH$, reflux, 8 min, 82%; (iv) MeCN, reflux, 16 h, 65%; (v) $(Ph_3)_4Pd$, *N*-methylaniline, THF, rt, 76 h, 60%; (vi) H_2, Pd/C, EtOH–H_2O (80:20), room temp., 95%; (vii) CF_3CO_2H–H_2O (95:5), room temp., 40 min, 65%.

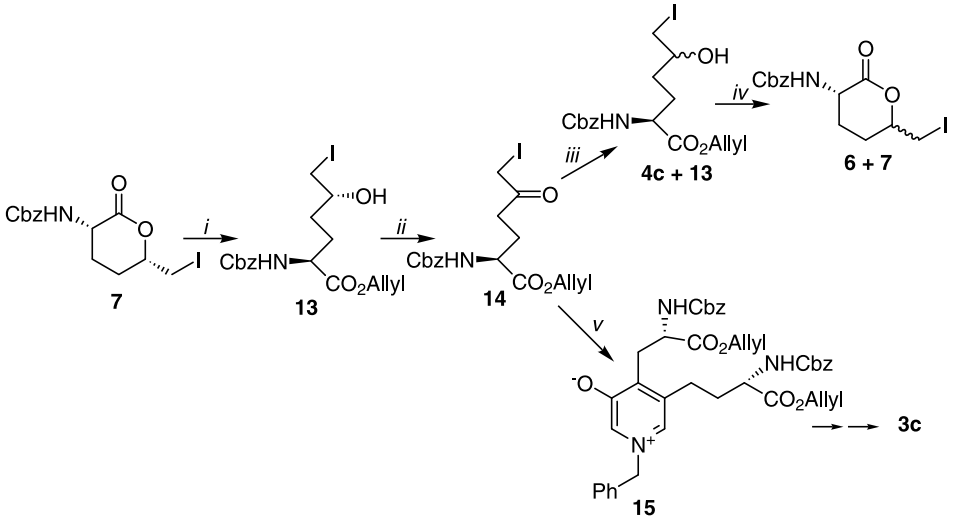
cis-lactone is a little more stable ($\Delta_{cis\rightarrow trans}$ $E=1.0$ kJ mol^{-1}, for MM+, and $\Delta_{cis\rightarrow trans}$ $E=1.1$ kJ mol^{-1}, for AM1). This corresponds to a very close equilibrium mixture of these lactones (*trans*/*cis* ratio = 0.6–0.7:1).[7] Moreover, this result prompted us to study first the iodolactonization reaction under kinetic control, treating the amino acid **5** with a mixture of iodine and potassium iodide in dichloromethane–water saturated with sodium hydrogen carbonate. A mixture of the iodolactones **6** and **7** was then obtained in satisfactory yields and in a 7:3 molar ratio.

The iodolactones **6** and **7** were easily separated by rapid chromatography and their stereochemistry was assigned by transformation into the corresponding azido lactones **8** and **9** to which we had recently assigned a complete structure.[8] To confirm that under the reaction conditions no equilibration of the isomeric iodolactones **6** and **7** occurs, each iodolactone was isolated and stirred in the presence of a new mixture of the solvent and reagents used in the iodolactonization. In each case, after 3 h, the same pure starting lactone was detected by HPLC.

In order to improve the diastereoselectivity observed in the lactonization, we tried to optimise the reaction conditions (varying solvent, temperature etc.) and also performed the reaction under thermodynamic control.[6a] However, under thermodynamic conditions, the reaction does not occur at all, and surprisingly our initial conditions were found to be the best ones so far to obtain the iodolactone **6**. Fortunately, the undesired (2*S*,5*S*)-lactone **7** could be recycled and reutilised for the synthesis of Pyd **1** and so the obtained diastereoselectivity was considered acceptable.

The iodolactone **6** was then treated with allyl alcohol (Scheme 3), in the presence of caesium carbonate, to afford by transesterification the iodohydrin **4c**. These controlled conditions allow the loss of iodine and the formation of the corresponding epoxide to be avoided which smoothly forms in the presence of anhydrous sodium carbonate.[6a,9] The iodocompound **4c** was then reacted with the trisubstituted pyridine **3a** in boiling acetonitrile to afford the fully protected Pyd **10** and finally Pyd **1**, after gradual regeneration of the aminoacidic functions via compounds **11** and **12**.

With the synthesis of Pyd **1** in hand, we improved it by recycling the (2*S*,5*S*)-lactone **7**. This was transesterified under the same conditions studied for its stereomer **6** to afford the iodohydrin **13** (Scheme 4). This was then oxidized to the iodoketone **14** which afforded additional pure iodohydrin **4c** in three steps: sodium borohydride reduction of the keto group to afford a diastereomeric mixture of the two iodohydrines **4c** and



Scheme 4. *Reagents and conditions:* (i) Cs_2CO_3, CH_2=$CHCH_2OH$, reflux, 8 min, 80%; (ii) pyridinium chlorochromate, CH_2Cl_2, room temp., 5 h, 81%; (iii) $NaBH_4$, MeOH, 0°C, 20 min, 90%; (iv) CF_3CO_2H, room temp., 40 min, **6** (32%) and **7** (36%); (v) benzylamine, K_2CO_3, MeCN, room temp., 8 h, then TMG, O_2, MeOH, room temp., 6 h, 46%.

13, lactonization of the obtained iodohydrin mixture to the separable iodolactones **6** and **7**, separation and transesterification of **6** with allylic alcohol. Moreover, the iodoketone **14** was also a good starting material for the preparation of the substituted pyridine **3c** since it reacts with benzylamine to afford the pyridinium salt **15**, the proximate precursor of **3c**.[10] The 'one pot' construction of the pyridinium nucleus, starting with the iodoketone **14**, proceeds even better than using the analogue bromoketone studied in our original protocol.[4b]

Thus, we have set-up a concise and efficient protocol for the synthesis of pyridinoline **1** starting from glycine. The results allow a more direct and less expensive synthesis of this precious collagen cross-link.

3. Experimental

3.1. General

Nuclear magnetic resonance spectra were recorded at 298 K on 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 solvent signal (residual proton signal for proton spectra or carbon signal for carbon spectra).[11] Proton and carbon assignments were established, if necessary, with homonuclear and heteronuclear 2D *J*-revolved experiments. ^{1}H 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). 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}. 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)[12] capillary column (25 m, 0.25 mm ID, purchased from Macherey-Nagel); carrier gas was He set at 88 kPa column head pressure and the column temperature was set at 220°C. HPLC analyses were carried out on a silica direct phase column (superspher Si-60, 12.5 cm, 4 mm ID, purchased from Merck); the mobile phase was hexane/2-propanol, 93:7, v:v; the flow rate was 1 mL/min and the detection was performed at 221 nm. UV spectra were obtained using a Perkin–Elmer Lambda11 UV/VIS spectrometer. 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% 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.[13] Usual work-up refers to washing the organic layer with water, drying over Na_2SO_4, and evaporating the solvent under reduced pressure.

3.2. (*S*)-2-Benzyloxycarbonylaminohex-5-enoic acid 5

The acid **5** was obtained using the same asymmetric alkylation reported by Hoarau et al.[5] for the preparation of the *tert*-butyl ester of the D-isomer of **5**. In our preparation we started with the *tert*-butyl glycinate Schiff base of (1*R*,2*R*,5*R*)-2-hydroxypinan-3-one and obtained the *tert*-butyl (2*S*)-2-aminohex-5-enoate (75% yield from Schiff base) as an oil: $[\alpha]_D$ = +26.6 (*c* 1, MeOH) (lit.[5] –25.2 for its enantiomer); ^{1}H NMR ($CDCl_3$): δ 7.34–7.28 (5H, aromatics-H), 5.78 (1H, m, 5-H), 5.26 (1H, d, *J* = 6.3, N*H*), 5.09 (2H, s, OCH_2Ph), 5.02 (1H, dd, *J* = 16.8 and <1, 6-Ha), 4.97 (1H, dd, *J* = 9.8 and <1, 6-Hb), 4.26 (1H, m, 2-H), 2.08 (2H, m, 4-H_2), 1.89 (1H, m, 3-Ha), 1.71 (1H, m, 3-Hb), 1.44 [9H, s, C(CH_3)$_3$]. Anal. calcd for $C_{10}H_{19}NO_2$: C, 64.83; H, 10.34; N, 7.56. Found: C, 64.67; H, 10.41; N, 7.48%.

^{1}H NMR is in agreement with that of the reported D-isomer by Hoarau et al.[5] After carbobenzylation of the amino group,[5] the *tert*-butyl (2*S*)-2-benzyloxycarbonylaminohex-5-enoate (85% yield) was obtained: an oil; $[\alpha]_D = +1.04$ (*c* 1, $CHCl_3$) [lit.[15] −15 (*c* 1, MeOH)]; ^{1}H NMR ($CDCl_3$): δ 7.35–7.30 (5H, aromatics-H), 5.77 (1H, m, 5-H), 5.28 (1H, d, $J = 7.7$, N*H*), 5.08 (2H, s, OCH_2Ph), 5.02 (1H, dd, $J = 17.0$ and <1, 6-Ha), 4.97 (1H, dd, $J = 10.1$ and <1, 6-Hb), 4.26 (1H, m, 2-H), 2.08 (2H, m, 4-H_2), 1.89 (1H, m, 3-Ha), 1.74 (1H, m, 3-Hb), 1.44 [9H, s, C(CH_3)$_3$]. Anal. calcd for $C_{18}H_{25}NO_4$: C, 67.69; H, 7.89; N, 4.39. Found: C, 67.80; H, 7.81; N, 4.43%. ^{1}H NMR is in agreement with that of the D-isomer reported by Hoarau et al.[5]

The *tert*-butyl (2*S*)-2-benzyloxycarbonylamino-5-hexenoate (1.0 g; 3.1 mmol) was dissolved in aqueous trifluoracetic acid (8 ml; 95%) and the resulting solution was stirred at room temperature for 1 h. The solvent was then removed under reduced pressure to afford the pure acid **5** (807 mg, 98%): mp 69–71 (from CH_2Cl_2–diisopropylether); $[\alpha]_D = +21.6$ (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.36–7.26 (5H, aromatics), 6.36 (1H, m, 4-H), 5.27 (1H, d, $J = 7.9$, N*H*), 5.16–4.99 (4H, overlapping, 6-Ha, 6-Hb and CH_2Ph), 4.40 (1H, m, 2-H), 2.16–2.12 (2H, overlapping, 4-Ha and 4-Hb), 1.98 (1H, m, 3-Ha), 1.78 (1H, m, 3-Hb). Anal. calcd for $C_{14}H_{17}NO_4$: C, 63.87; H, 6.51; N, 5.32. Found: C, 63.60; H, 6.40; N, 5.45%.

The enantiomeric excess (>98%) of the amino acid **5** was detected by chiral GLC (see Section 3.1 for conditions) after transformation of **5** into glutammic acid diethyl ester *N*-trifluoroacetate. The transformation requires: an initial esterification of **5** (20 mg) with EtOH (in 1 M HCl at 25°C for 24 h) followed by: an oxidation with $NaIO_4$–$KMnO_4$ (*t*BuOH–H_2O at 25°C for 12 h);[14] a second esterification with EtOH of the obtained acid; the decarbobenzylation of the amino group (by hydrogenolysis in the presence of Pd/C); and a trifluoracetilation (with trifluoroacetic anhydride).

The retention time of the obtained trifluoroacetate was identical to that of an authentic sample obtained from L-glutamic acid (31.87 min) and differed from that of the D-isomer derivative (29.26 min).

3.3. Iodolactonization of (*S*)-2-benzyloxycarbonylamino-hex-5-enoic acid 5

A saturated aqueous solution of $NaHCO_3$ (200 mL) containing iodine (14.48 g, 57.00 mmol) and KI (28.96 g; 174.44 mmol) was added to a solution of the acid **5** (5.00 g, 19.0 mmol) in CH_2Cl_2 (200 mL), at 0°C under vigorous stirring. After 48 h, a saturated aqueous solution of $Na_2S_2O_4$ was added to the reaction mixture which was extracted with dichloromethane and worked up. The obtained residue, after separation of the organic phase and usual work-up, was quickly chromatographed on column (eluting with CH_2Cl_2–AcOEt; 100:10 v/v) to afford first the (2*S*,5*S*)-2-benzyloxycarbonylamino-5-iodomethyl-δ-valerolactone **7** (1.33 g, 18%): mp 142–143°C (from CH_2Cl_2–diisopropyl ether); HPLC: $R_t = 7.7$ min; $[\alpha]_D = +41.5$ (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.36–7.28 (5H, aromatics-H), 5.59 (1H, d, $J = 4.5$, N*H*), 5.11 (2H, s, OCH_2Ph), 4.48–4.40 (2H, overlapping, 2-H and 5-H), 3.34 (1H, dd, $J = 10.5$ and 5.1, CH*H*I), 3.27 (1H, dd, $J = 10.5$ and 6.2, C*H*HI), 2.64 (1H, m, 3-Ha), 2.28 (1H, m, 4-Ha), 1.80 (1H, m, 4-Hb), 1.63 (1H, m, 3-Hb). Anal. calcd for $C_{14}H_{16}INO_4$: C, 43.21; H, 4.14; N, 3.60. Found: C, 43.13; H, 4.22; N, 3.69%.

Further elution gave the (2*S*,5*R*)-2-benzyloxycarbonylamino-5-iodomethyl-δ-valerolactone **6** (2.88 g, 39%): mp 112–114°C (from CH_2Cl_2–diisopropyl ether); HPLC: $R_t = 13.2$ min; $[\alpha]_D = 7.0$ (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.36–7.28 (5H, aromatics-H), 5.50 (1H, d, $J = 3.0$, N*H*), 5.10 (2H, s, OCH_2Ph), 4.30 (1H, m, 5-H), 4.15 (1H, m, 2-H), 3.34 (2H, br s, CH_2I), 2.47 (1H, m, 3-Ha), 2.24 (1H, m, 4-Ha), 1.94–1.85 (2H, overlapping, 3-Hb and 4-Hb). Anal. calcd for $C_{14}H_{16}INO_4$: C, 43.21; H, 4.14; N, 3.60. Found: C, 43.32; H, 4.10; N, 3.57%.

3.4. Separate transformation of the (2*S*,5*R*)- and (2*S*,5*S*)-2-benzyloxycarbonylamino-5-iodomethyl-δ-valerolactones 6 and 7 into the corresponding azido-lactones 8 and 9

Each lactone **6** and **7** (200 mg, 0.51 mmol), dissolved in DMF (2 mL), was treated with NaN_3 (66 mg, 1.02 mmol) at room temperature for 12 h. Then the reaction mixture was poured in ice cold water and extracted with AcOEt to afford, after usual work-up, the appropriate crystalline azidolactone. In particular, starting from the lactone **6**, the (2*S*,5*R*)-5-azidomethyl-2-benzyloxycarbonylamino-δ-valerolactone **8** (96% yield) was obtained: mp 86–87°C (from CH_2Cl_2–diisopropyl ether); identical in all respects with that previously described.[8] Starting from the (2*S*,5*S*)-lactone **7**, the pure (2*S*,5*S*)-5-azidomethyl-2-benzyloxycarbonylamino-δ-valerolactone **9** (97% yield) was obtained: mp 90–92°C (from CH_2Cl_2–diisopropyl ether); identical in all respects with that previously described.[8]

3.5. Attempts of separate equilibration of the (2*S*,5*R*)- and (2*S*,5*S*)-2-benzyloxycarbonylamino-5-iodomethyl-δ-valerolactones 6 and 7 under the conditions used for their preparation by iodolactonization

Each lactone **6** and **7** (74 mg, 0.19 mmol) was separately dissolved in CH_2Cl_2 (2 mL) and treated at 0°C for 48 h with a saturated solution of $NaHCO_3$ containing iodine (145 mg, 0.57 mmol) and KI (290 mg; 1.75 mmol). The mixture was then diluted with a saturated aqueous solution of $Na_2S_2O_4$ and worked up to afford a crude residue which was directly subjected to HPLC analysis. This showed that each starting lactone was uncontaminated by the respective diastereomer (the lactone **6** showed $R_t = 13.2$ min; the lactone **7** showed: $R_t = 7.7$ min).

3.6. Allyl (2*S*,5*R*)-2-benzyloxycarbonylamino-5-hydroxy-6-iodohexanoate 4c

The (2*S*,5*R*)-lactone **6** (2.00 g, 5.1 mmol), dissolved in allylic alcohol (60 mL) was treated with Cs_2CO_3 (1.20 g) at reflux for 7–8 min. The time control is very important since, at longer reflux time, the progressive formation of the corresponding epoxide is observed. The reaction mixture was then diluted with AcOEt (20 mL), washed with water and worked up. Column chromatography (eluting with hexane–AcOEt; 80:20 v/v) afforded the pure iodohydrin **4c**[4a] (1.87 g; 82%): mp 72–73°C (from CH_2Cl_2–diisopropyl ether); $[\alpha]_D = +7.0$ (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.36–7.30 (5H, aromatics-H), 5.88 (1H, m, CH_2=C*H*-CH_2O), 5.42 (1H, d, $J = 6.5$, N*H*), 5.32 (1H, dd, $J = 16.8$ and <1, C*H*H=CH-CH_2O), 5.25 (1H, dd, $J = 10.3$ and <1, CH*H*=CH-CH_2O), 5.12–5.07 (2H, AB system, OCH_2Ph), 4.62 (2H, d, $J = 4.0$, CH_2=CH-CH_2O), 4.45 (1H, m, 2-H), 3.54 (1H, m, 5-H), 3.30 (1H, dd, $J = 9.9$ and 3.5, 6-Ha), 3.18 (1H, dd, $J = 9.9$ and 6.6, 6-Hb), 2.04 (1H, m, 3-Ha), 1.75 (1H, m, 3-Hb), 1.64 (1H, m, 4-Ha), 1.57 (1H, m, 4-Hb). Anal. calcd for $C_{17}H_{22}INO_5$: C, 45.65; H, 4.96; N, 3.13. Found: C, 45.52; H, 4.88; N, 3.21%.

3.7. 4-[(*S*)-2-Benzyloxycarbonylamino-2-(*tert*-butyloxycarbonyl)ethyl]-5-[(*S*)-3-benzyloxy carbonylamino-3-(*tert*-butyloxycarbonyl)propyl]-1-[(2*R*,5*S*)-5-benzyloxycarbonylamino-5-(allyloxycarbonyl)-2-hydroxypentyl]-3-pyridiniumolate 10

The 4-[(*S*)-2-benzyloxycarbonylamino-2-(*tert*-butyloxycarbonyl)ethyl]-5-[(*S*)-3-benzyloxycarbonylamino-3-(*tert*-butyloxycarbonyl)propyl]-3-hydroxypyridine **3a**[4b] (795 mg, 1.2 mmol) and the allyl (2*S*,5*R*)-2-benzyloxycarbonylamino-5-hydroxy-6-iodohexanoate **4c** (2.142 g, 4.8 mmol) were dissolved in CH_3CN (15 mL) and refluxed under argon atmosphere for 16 h. The solvent was then evaporated under reduced pressure to give a crude product which was chromatographed (eluting with AcOEt–MeOH; 100:7 v/v) to afford the title compound **10** (764 mg, 65%): a resinous material; $[\alpha]_D = -2.6$ (*c* 1, $CHCl_3$). The compound was identical (^1H and ^{13}C NMR, mass spectra) to that previously obtained.[4a]

3.8. 4-[(*S*)-2-Amino-2-(*tert*-butyloxycarbonyl)ethyl]-5-[(*S*)-3-amino-3-(*tert*-butyloxycarbonyl)propyl]-1-[(2*R*,5*S*)-5-amino-5-carboxy-2-hydroxypentyl]-3-pyridiniumolate 12

A solution containing the allylic ester **10** (885 mg, 0.9 mmol), *N*-methylaniline (2.89 g, 27.00 mmol) and tetrakis(triphenylphosphine) palladium(0) (104 mg, 0.09 mmol) in anhydrous THF (15 mL) was stirred, under argon, at room temperature for 76 h. After evaporation of the solvent under reduced pressure, the residue was chromatographed (eluting with CH_2Cl_2–MeOH; 9:1 v/v) to afford the 4-[(*S*)-2-benzyloxycarbonylamino-2-(*tert*-butyloxycarbonyl)ethyl]-5-[(*S*)-3-benzyloxycarbonylamino-3-(*tert*-butyloxycarbonyl)propyl]-1-[(2*R*,5*S*)-5-benzyloxycarbonylamino-5-carboxy-2-hydroxypentyl]-3-pyridiniumolate **11** (508 mg, 60%) as a resinous material.

The crude acid (450 mg, 0.48 mmol), dissolved in aqueous EtOH (180 mL, 80%), was then hydrogenated in the presence of 10% Pd/C (80 mg) at room temperature and atmospheric pressure. Filtration of the catalyst and evaporation of the solvent give a residue, which was chromatographed (eluting with MeOH–NH_3; 100:5 v/v) to afford the title compound **12** (244 mg, 95%) as a glass. The compound was identical (^1H and ^{13}C NMR, mass spectra) to that previously obtained.[4a]

3.9. Pyridinoline 1

The *tert*-butyl derivative **12** (230 mg, 0.43 mmol) was dissolved in aqueous CF_3CO_2H (8 mL, 95%: v/v) and the resulting solution was stirred at room temperature for 40 min. The solvent was then removed under reduced pressure and the residue was purified by column chromatography (eluting with MeOH–NH_3; 80:20 v/v). The obtained residue was then dissolved in aqueous HCl (7.0 mL, 1.0 M) and the solution was lyophilised to afford the pyridinoline **1** as tetrachloride dihydrate (167 mg, 65%): λ_{max} (HCl 0.1 M)/nm 242 (ε/dm^3 mol^{-1} cm^{-1} 3850), 294 (6520); λ_{max} (50 mM phosphate buffer, pH 7.5)/nm 252 (ε/dm^3 mol^{-1} cm^{-1} 3710), 324 (6150); ESI/MS m/z 429 (M^+). Anal. calcd for $C_{18}H_{36}N_4Cl_4O_{10}$: C, 35.42; H, 5.95; N, 9.18; Cl, 23.24. Found: C, 35.48; H, 6.00; N, 9.22; Cl, 23.36%. Other physico-chemical properties (^1H and ^{13}C NMR) confirmed those reported.[4a]

3.10. Allyl (2*S*,5*S*)-2-benzyloxycarbonylamino-5-hydroxy-6-iodohexanoate 13

The (2*S*,5*S*)-lactone **7** (2.00 g, 5.1 mmol), dissolved in allylic alcohol (60 mL) was treated with $NaHCO_3$ (1.20 g) at reflux for 7–8 min. At longer reflux time (15–20 min) the formation of the corresponding epoxide starts. Dilution with AcOEt (20 mL) and usual workup afforded a residue which, after column chromatography (eluting with hexane–AcOEt; 80:20 v/v), afforded the pure iodohydrin **13** (1.83 g; 80%): an oil; $[\alpha]_D = +3.1$ (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.36–7.29 (5H, aromatics-H), 5.88 (1H, m, CH_2=C*H*-CH_2O), 5.36 (1H, d, $J = 6.3$, N*H*), 5.31 (1H, dd, $J = 17.0$ and <1, C*H*H=CH-CH_2O), 5.24 (1H, dd, $J = 10.2$ and <1, CH*H*=CH-CH_2O), 5.12–5.06 (2H, AB system, OCH_2Ph), 4.62 (2H, d, $J = 4.2$, CH_2=CH-CH_2O), 4.41 (1H, m, 2-H), 3.56 (1H, m, 5-H), 3.31 (1H, dd, $J = 9.8$ and 3.0, 6-Ha), 3.17 (1H, dd, $J = 9.8$ and 7.0, 6-Hb), 2.09 (1H, m, 3-Ha), 1.97 (1H, m, 3-Hb), 1.84 (1H, m, 4-Ha), 1.66 (1H, m, 4-Hb). Anal. calcd for $C_{17}H_{22}INO_5$: C, 45.65; H, 4.96; N, 3.13. Found: C, 45.78; H, 5.05; N, 3.08%.

3.11. Allyl (2*S*)-2-benzyloxycarbonylamino-6-iodo-5-oxohexanoate 14

The iodohydrin **13** (1.0 g, 2.24 mmol), dissolved in CH_2Cl_2 (90 mL), was treated with pyridinium

chlorochromate (966 mg, 4.48 mmol) at room temperature for 5 h. Then isopropanol was added, followed by ice cold water and the solution was worked up to afford a residue which, after column chromatography on silica (eluting with hexane–AcOEt; 80:20 v/v), afforded the pure iodo ketone **14** (806 mg, 81%): mp 79–81°C (from CH_2Cl_2–diisopropyl ether); $[\alpha]_D = -3.0$ (*c* 1, $CHCl_3$). ^1H NMR ($CDCl_3$): δ 7.36–7.29 (5H, aromatici), 5.88 (1H, m, CH_2=C*H*-CH_2O), 5.36 (1H, d, $J = 4.0$, N*H*), 5.32 (1H, dd, $J = 17.4$ e <1, C*H*H=C*H*-CH_2O),), 5.25 (1H, dd, $J = 10.7$ e<1, CH*H*=C*H*-CH_2O), 5.12–5.05 (2H, sistema AB, CH_2Ph), 4.62 (2H, d, $J = 5.5$, CH_2=CH-CH_2O), 4.36 (1H, m, 2-H), 3.75 (2H, s, 6-H_2), 2.80 (2H, m, 4-H_2), 2.22 (1H, m, 3-Ha), 1.94 (1H, m, 3-Hb). Anal. calcd for $C_{14}H_{16}INO_5$: C, 41.50; H, 3.98; N, 3.46. Found: C, 41.62; H, 4.08; N, 3.37%.

3.12. (*S*,*S*)-1-Benzyl-4-[2-benzyloxycarbonylamino-2-(allyloxycarbonyl)ethyl]-5-[3-benzyloxycarbonylamino-3-(allyloxycarbonyl)propyl]-3-pyridiniumolate 15 starting from benzylamine and iodo ketone 14

To a solution containing benzylamine (0.109 mL, 1 mmol) and the iodo ketone **14** (979 mg, 2.2 mmol) in CH_3CN (20 mL), anhydrous K_2CO_3 (460 mg, 3.3 mmol) was added and the mixture was stirred under nitrogen for 8 h. At this time the disappearance of the starting iodo ketone was monitored (TLC), the solid K_2CO_3 was filtered on a pad of Celite and the solvent was evaporated under reduced pressure. The residue was then dissolved in MeOH (20 mL) and tetramethylguanidine (0.375 mL, 3 mmol) was added to the mixture, which was vigorously stirred at 25°C under oxygen atmosphere for 6 h. The solvent was then removed under reduced pressure and the residue was purified by rapid chromatography (eluting with CH_2Cl_2–MeOH; 9:1 v/v) to afford the title compound **15** (332 mg, 46%) as a glass: ^1H NMR ($CDCl_3$): δ 7.50 (1H, br s, pyridinium-H), 7.39–7.22 (15H, aromatics-H), 7.04 (1H, br s, pyridinium-H), 5.83 (2H, m, 2×CH_2=C*H*-CH_2O), 5.59 (2H, m, 2×N*H*), 5.31–4.94 (10H, overlapping, 2×OCH_2Ph, $N^+C H_2$Ph, 2×CH_2=CH-CH_2O), 4.62 (2H, m, CH_2=CH-CH_2O), 4.56 (2H, m, CH_2=CH-CH_2O), 4.34 (2H, m, 2×C*H*(N-HCbz)CO_2Allyl), 3.29 (1H, m), 2.98 (1H, m), 2.64 (2H, m), 2.01 (1H, m), 1.75 (1H, m). Anal. calcd for $C_{41}H_{43}N_3O_9$: C, 68.22; H, 6.00; N, 5.82. Found: C, 68.06; H, 5.85; N, 5.73%.

3.13. Transformation of the iodo ketone 14 into a diastereoisomeric mixture of iodo lactones 6 and 7

$NaBH_4$ (65 mg; 1.7 mmol) was gradually added to a solution of the iodo ketone **14** (700 g; 1.57 mmol) in MeOH (100 mL), at −5°C. The mixture was stirred at −5°C for 20 min, then was poured into an ice cold aqueous solution of HCl (2 M) and extracted with AcOEt. Usual work-up afforded a chromatographically unseparable mixture of diastereoisomeric iodohydrines **4c** and **13** (633 mg; 90%, in a 1:1 ratio).

The obtained mixture of iodohydrines was dissolved in CF_3CO_2H (1.5 mL) and the solution was stirred at room temperature for 40 min. The solvent was then removed under reduced pressure (under 40°C) and the residue (492 mg), was quickly chromatographed on column (eluting with CH_2Cl_2/AcOEt; 100:10; v:v) to afford first the lactone **7** (154 mg; 36%) and then the lactone **6** (137 mg; 32%) described above.

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