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A simple and convenient transformation of L-lysine into pyridinoline and deoxypyridinoline, two collagen cross-links of biochemical interest

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Abstract—Starting from L-lysine as the only chiral building block, pyridinoline and deoxypyridinoline are efficiently synthesised, thus mimicking the postranscriptional formation of these collagen cross-links.

1. Introduction

Pyridinoline **1** and deoxypyridinoline **2** (Fig. 1) are two cross-links found in the mature form of collagen.[1] At present, **1** and **2** are considered the most effective biochemical markers of various bone diseases, including osteoporosis,[2] arthropathies and bone cancer.[3] They were initially obtained and characterised from bones after several laborious purifications,[4] but protocols have recently been reported, by us[5,6] and others,[7] for the synthesis of these collagen cross-links from linear compounds. Our first protocol,[5] also adopted by Adamczyk at al.,[8] allows the direct 'one-pot' assembly of the 3-pyridiniumolate nucleus **3** while our second procedure,[6] as first proposed by Waelchli et al.,[7] allows the assembly of the 4,5-disubstituted-3-hydroxypyridine **4** followed by successive insertion of the L-5-hydroxylysine or L-lysine side chain. Both synthetic pathways involve the formation of diketoamine as **5** as a common key intermediate (Scheme 1). This diketoamine is obtained by a single selective reaction[5,6,8] between an amino acid α-bromoketone, obtained from glutamic acid and a suitable primary amine (allylamine, benzylamine or protected L-lysine) or via a multistep route, starting with a mixture of amino acid epoxides and benzylamine.[7]

The diketoamine **5** can undergo a base-catalysed intramolecular condensation to form a cyclic α,β-unsaturated ketone which, depending on the nature of the side chain at the heterocyclic nitrogen, affords the 3-pyridiniumolate **3** or the 4,5-disubstituted-3-hydroxypyridine **4**. Transformation of 3-hydroxypyridine **4** into the 3-pyridiniumolate **3** requires alkylation with a suitable iodoalkyl amino acid **6**.[6–8]

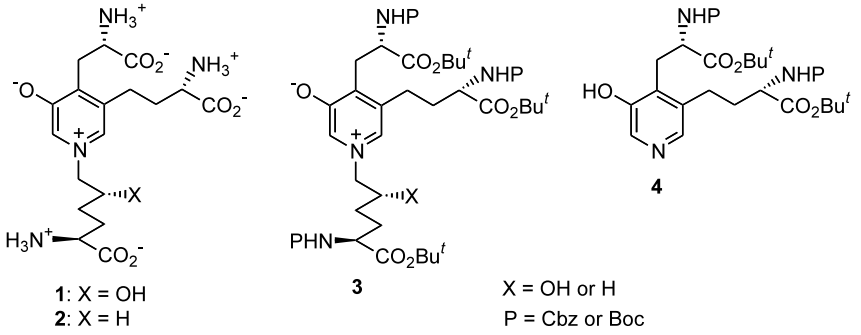

Figure 1.

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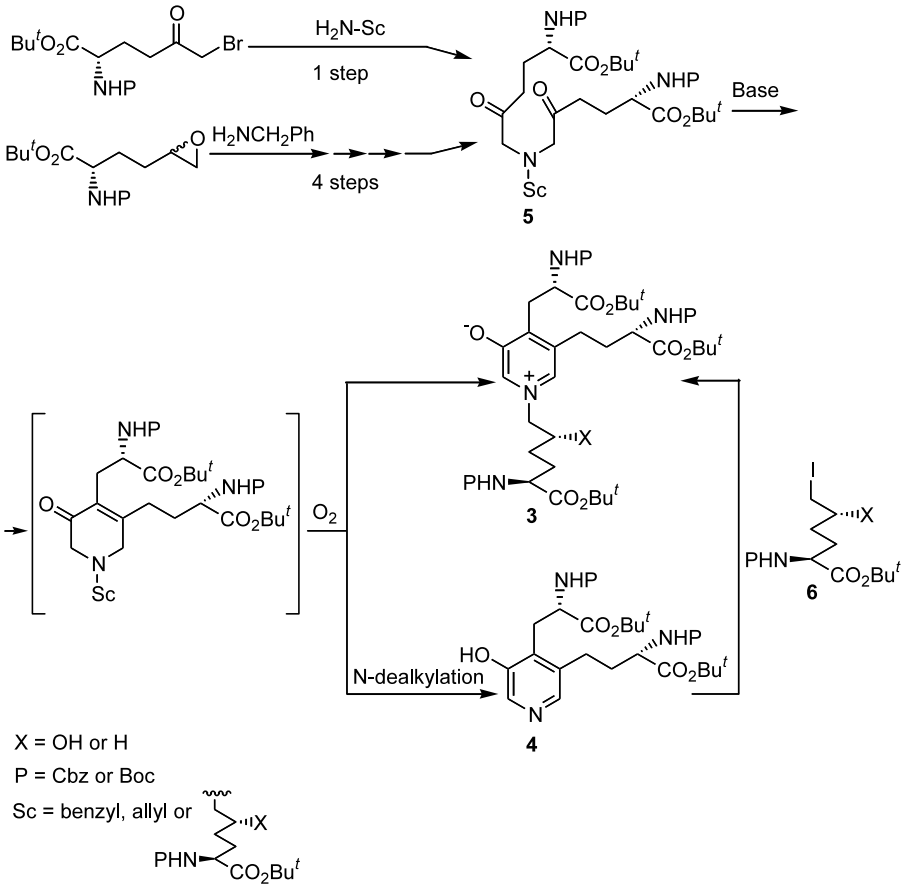


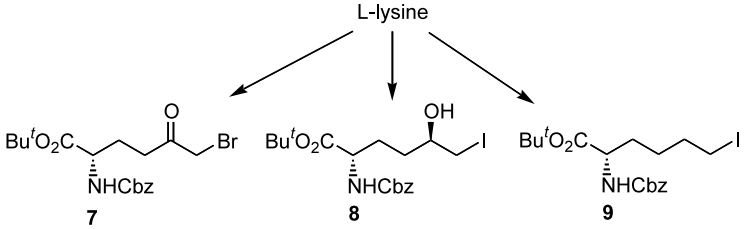
Scheme 1.

Our continuing interest in the chemistry of collagen cross-links has led us to explore the preparation of pyridinoline **1** and deoxypyridinoline **2** from L-lysine, thus mimicking the formation of these cross-links in collagen. The results of this work, reported herein, are of chemical interest since they allow convenient preparation of pyridinoline **1** using a new and simple preparation of its alkyl chain at the heterocyclic nitrogen, and a new and simple route to iodohydrin **6** (Scheme 1, X = OH) which has previously proven very troublesome to obtain.[7c]

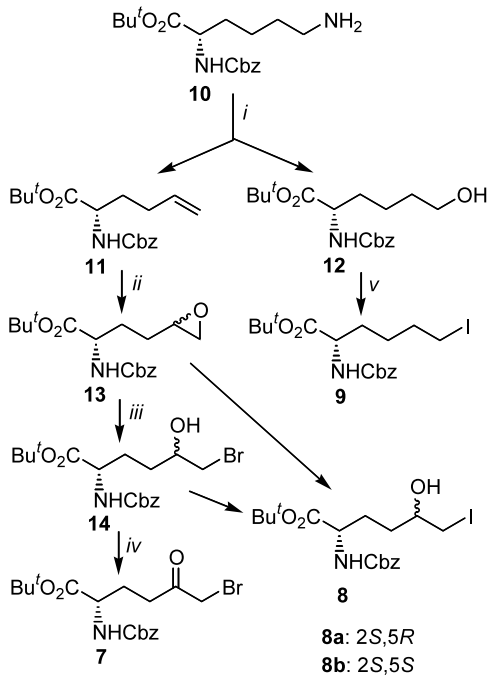
2. Results and discussion

In the light of previous work,[5–8] the preparation of **1** and **2** from L-lysine requires the transformation of this amino acid into the α-bromoketone **7** (Scheme 2), which is necessary for assembling the nucleus, and into the amino acid iodo derivatives **8** and **9**, which are useful for the construction of the side chains at the heterocyclic nitrogen of **1** and **2**, respectively. With this in mind, we performed the diazotisation of L-lysine protected as its N^2-benzyloxycarbonyl *tert*-butyl ester derivative **10** under controlled conditions (Scheme 3).[9] The reaction affords a mixture of the olefinic (2*S*)-amino acid **11** and the hydroxyalkyl (2*S*)-amino acid **12**, both of which are useful compounds in the synthesis of the first target, **2**.

The olefin **11** was then epoxidised to give an inseparable mixture of diastereomeric (2*S*,5*R*)- and (2*S*,5*S*)-epoxides **13a** and **13b**, according to the procedure of Hoarau et al.[10] for its 2*R*-enantiomer. Treatment of the epoxides **13** with LiBr following Hoarau's procedure afforded the inseparable bromohydrins **14a** and **14b**



Scheme 2.



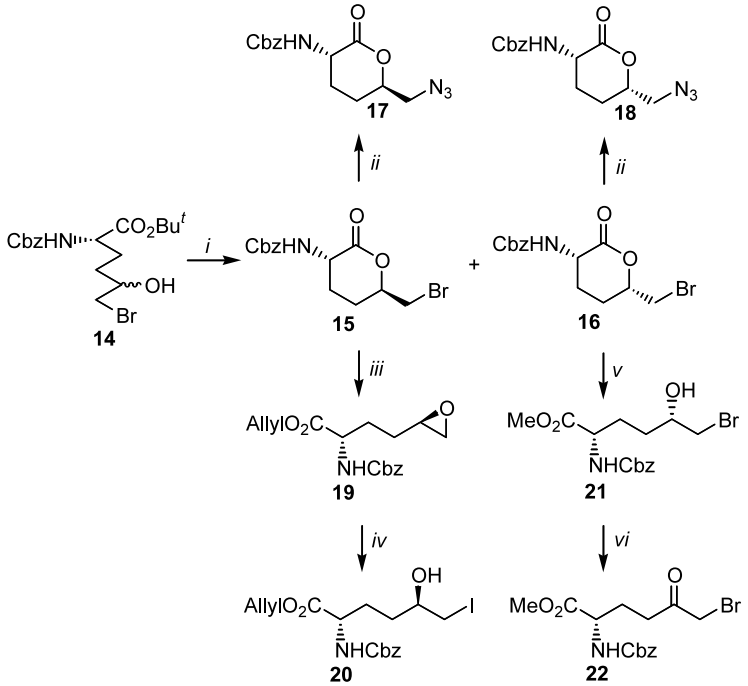
Scheme 3. *Reagents and conditions*: (i) $NaNO_2$, AcOH–H_2O, rt, 4 h, then KOH 0.1 M, rt, 2 h; (ii) *m*-CPBA, CH_2Cl_2–phosphate buffer (pH 8), rt, 12 h; (iii) LiBr, THF–AcOH, rt, 20 h; (iv) pyridinium chlorochromate, CH_2Cl_2, rt, 12 h; (v) Ph_3P, imidazole, I_2, THF, rt, 1 h.

which, upon oxidation with pyridinium chlorochromate, afforded the corresponding α-bromoketone **7**, as a single isomer and in satisfactory yields. This simple preparation of **7** is of interest since it avoids the use of large amounts of diazomethane required in the alternative method involving homologation of the glutamic acid.[5b]

The other diazotisation product, hydroxy amino acid **12**, was transformed into the iodide **9** in order to allow the synthesis of deoxypyridinoline **2** by alkylation of the appropriate pyridine nucleus. Our major objective was the synthesis of the (2*S*,5*R*)-iodohydrin **8a** which, in principle, appeared to be easily obtainable by opening of the epoxide **13a**, or from the bromohydrin **14a**, provided that they could be separated from the corresponding diastereomers **13b** and **14b**. Otherwise, separation of the final mixture of diastereomeric iodohydrins **8a** and **8b** could be attempted.

However, in agreement with the data reported by Hoarau et al.[10] and those observed for analogous iodohydrins,[†] all attempts to separate the diastereomeric epoxides or the halohydrins were unsuccessful even when the hydroxy group of **8** or **14** was esterified with either achiral or chiral acids (acetate, trifluoracetate, benzoate and Mosher's ester).

After these unsuccessful attempts, we turned to the possibility of a simple and elegant separation of the isomeric bromohydrins **14a** and **14b** via intramolecular esterification (Scheme 4), anticipating that the resulting diastereomeric lactones **15** and **16** would show an enhanced polarity difference as a result of the presence



Scheme 4. *Reagents and conditions*: (i) CF_3CO_2H, rt, 40 min; (ii) NaN_3, DMF, rt, 12 h; (iii) Cs_2CO_3, CH_2=$CHCH_2OH$, rt, 45 min; (iv) NaI, THF–AcOH, rt, 1 h; (v) MeOH, reflux, 9 h; (vi) pyridinium chlorochromate, CH_2Cl_2, rt, 12 h.

[†] The mixture of diastereomeric iodohydrins was separated[7c] only by preparative HPLC after esterification with a complex ester derived from tyrosine.

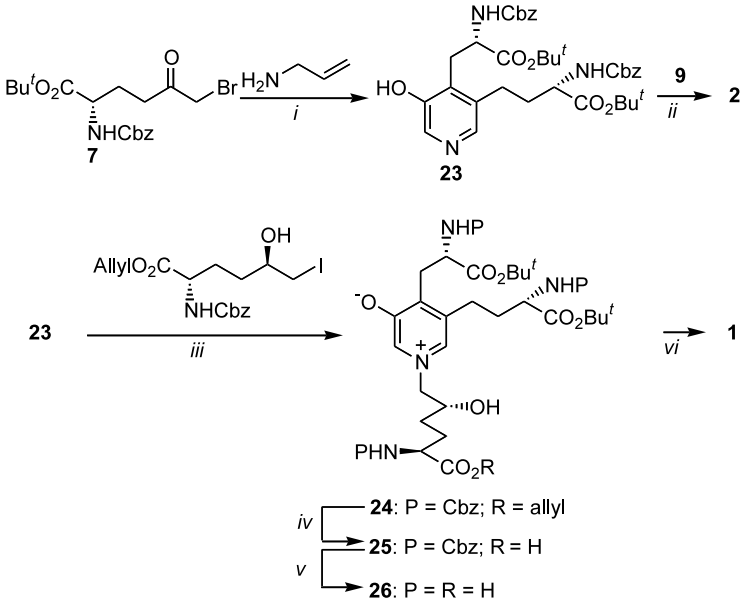
of a disubstituted ring in the molecule. In fact, treatment of the bromohydrins **14** with trifluoracetic acid afforded the lactones **15** and **16** (as a 1:1 mixture; ^{1}H NMR), which were easily separated by chromatography on silica. The stereochemistry of these lactones was then assigned by transforming each isomer into the corresponding azido lactone **17** or **18** to which we had recently assigned a complete structure.[11] The lactone **15**, possessing the correct stereochemistry for our objective, was then transesterified to the corresponding epoxidic allyl ester **19** by treatment with allyl alcohol in the presence of cesium carbonate. This ester was chosen since the allylic group could be selectively removed in the presence of various other esters or protective groups (e.g. the benzyloxycarbonyl and the *tert*-butoxycarbonyl) by palladium(0)-catalysed allyl transfer under mild and practically neutral conditions. By monitoring the reaction by TLC, it was possible to observe that the first compound formed is the bromohydrin allylic ester, which then undergoes ring closure to the epoxide **19**. Considering that for the alkylation of the 3-hydroxypyridine nucleus it was necessary to dispose of the iodohydrin, we decided to prolong the reaction until complete formation of the epoxide **19**. This was then easily transformed into the corresponding iodohydrin **20** by reaction with lithium iodide.

On the other hand, the lactone **16** was similarly transesterified with methanol to afford the diastereomeric (5*S*)-bromohydrin methyl ester **21**, which was then oxidised to the ketone **22**. This allows recycling to lactones **15** and **16** by reduction of the keto group with sodium borohydride and treatment of the obtained diastereomeric mixture of the bromohydrin **21** and its 5*R* diastereomer with trifluoroacetic acid.

With the acidic iodohydrin **20** in hand, we reached our target to transform L-lysine into the collagen cross-links, pyridinoline **1** and deoxypyridinoline **2** (Scheme 5). In fact, the reaction of the α-bromoketone **7** with allylamine afforded, after deallylation, the trisubstituted pyridine **23**,[6c] a common key intermediate for both of the desired compounds. Alkylation of this intermediate with iodide **9**, followed by regeneration of the protected functions (hydrogenolysis and TFA treatment),[6a] afforded the deoxypyridinoline **2**.

Similarly, alkylation of the intermediate **23** with the iodohydrin **20** in boiling acetonitrile afforded a fully protected pyridinoline **24**, thus showing that alkylation occurred only at the heterocyclic nitrogen atom (^{1}H NMR and mass and UV spectra). Selective deprotection of the allyl ester, by treatment with $Pd[Ph_3P]_4$ and *N*-methylaniline afforded the protected pyridinoline **25** with a single free carboxylic acid group.[12] Successive hydrogenolysis of the benzyloxycarbonyl groups afforded the diester **26**, which, by treatment with aqueous trifluoracetic acid and purification by chromatography, afforded the pyridinoline **1** which was characterised as the solid hydrochloride dihydrate salt.

In conclusion, we have demonstrated the possibility of transforming L-lysine into pyridinoline **1** and deoxypyridinoline **2** and more importantly we have set-up a new and elegant way to obtain the longest side chain of pyridinoline **1** in enantio- and diastereomerically pure form, starting from the olefinic amino acid **11**. This starting olefin, herein obtained by diazotisation of L-lysine, is obtainable by a variety of methods[13] using asymmetric synthesis of amino acids and thus its utility in the construction of a pyridinoline fragment



Scheme 5. *Reagents and conditions*: (i) Ref. 6c; (ii) MeCN, reflux, 8 h, then H_2, Pd/C, MeOH, rt, then CF_3CO_2H–H_2O (95:5), rt, 40 min; (iii) MeCN, reflux, 16 h, then column chromatography; (iv) $(Ph_3)_4Pd$, *N*-methylaniline, THF, rt, 76 h; (v) H_2, Pd/C, EtOH–H_2O (80:20), rt; (vi) CF_3CO_2H–H_2O (95:5), rt, 40 min.

appears to be general in scope. Efforts to extend this work to the syntheses of other collagen cross-links are being pursued in these laboratories and will be reported in due course.

3. Experimental

3.1. General

Melting points were measured on a SMP3 mp apparatus (Stuart Scientific, USA) and are not corrected. 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 for million (ppm, δ units) relative to solvent signal (residual proton signal for proton spectra or carbon signal for carbon spectra).[14] 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}. 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.[15] 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.

3.2. Diazotation of *tert*-butyl (*S*)-2-benzyloxycarbonylamino-6-aminohexanoate, 10

A solution of *tert*-butyl (*S*)-2-benzyloxycarbonylamino-6-aminohexanoate **10** (2.35 g, 7.0 mmol) in aqueous AcOH (50 mL; 50% v/v) was treated with $NaNO_2$ (5.5 g, 80 mmol) and the resulting mixture was stirred at room temperature for 4 h. The mixture was then basified with aqueous NaOH (40%) and extracted with diethyl ether. The organic layer was treated with a methanolic solution of KOH (50 mL, 0.1 M) for 2 h in order to complete hydrolysis of the formed acetyl derivate of the alcohol **12**. The reaction mixture was then concentrated under reduced pressure, diluted with water and extracted with AcOEt. Usual work-up gave a crude product which was chromatographed (eluting with hexane–AcOEt; 1:1 v/v) to afford first the crude olefinic amino acid **11** (670 mg) and then *tert*-butyl (*S*)-2-benzyloxycarbonylamino-6-hydroxyhexanoate **12** (990 mg, 42%) as an oil: $[\alpha]_D = +6.5$ (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.33–7.26 (5H, aromatics-H), 5.35 (1H, d, $J = 7.5$, N*H*), 5.07 (2H, s, OCH_2Ph), 4.22 (1H, m, 2-H), 3.58 (2H, t, $J = 6.1$, 6-H_2), 1.78 (1H, m), 1.63 (1H, m), 1.54 (2H, m), 1.43 [9H, s, C(CH_3)$_3$] 1.41–1.36 (2H, overlapping). Anal. calcd for $C_{18}H_{27}NO_5$: C, 64.07; H, 8.07; N, 4.15. Found: C, 64.13; H, 8.00; N, 4.13%.

Chromatographic purification of the crude olefinic product (eluting with hexane–AcOEt; 8:2 v/v) afforded pure *tert*-butyl (2*S*)-2-benzyloxycarbonylaminohex-5-enoate **11** (602 mg, 27%) as an oil: $[\alpha]_D$ +1.1 (*c* 1, $CHCl_3$) [lit.[13b] −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, $J = 7.7$, NH), 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%.

3.3. *tert*-Butyl (5*R*)- and (5*S*)-2-benzyloxycarbonylamino-4-(2-oxiranyl)butanoates, 13a and 13b

The amino acid olefin **11** (500 mg, 1.57 mmol) dissolved in 1,2-dichloroethane (10 mL) was added to an aqueous solution of a phosphate buffer (20 mL, 0.2 M; pH 8) followed by 3-chloroperoxybenzoic acid (550 mg of a 50% moist mixture; 1.6 mmol). The mixture was shaken at room temperature for 12 h, then the organic layers were separated to afford, after usual work-up, a crude product which was chromatographed (eluting with hexane–AcOEt; 70:30 v/v) to afford an inseparable mixture[10] of the title compounds **13a** and **13b** (420 mg, 80%): ^{1}H NMR ($CDCl_3$): δ 7.34–7.24 (5H, aromatics-H), 5.36 and 5.30 (0.5H, d, $J = 7.0$, N*H* of each isomer), 5.08 (2H, s, OCH_2Ph), 4.27 (1H, m, 2-H), 2.89 (1H, m, 5-H), 2.73 (1H, m, 6-Ha), 2.45 (1H, m, 6-Hb), 1.97 (1H, m, 3-Ha), 1.77 (1H, m, 3-Hb), 1.66 (1H, m, 4-Ha), 1.60 (1H, m, 4-Hb), 1.44 [9H, s, C(CH_3)$_3$]. Anal. calcd for $C_{18}H_{25}NO_5$: C, 64.46; H, 7.51; N, 4.18. Found: C, 64.60; H, 7.49; N, 4.23%.

3.4. *tert*-Butyl (2*S*,5*R*)- and (2*S*,5*S*)-2-benzyloxycarbonylamino-6-bromo-5-hydroxyhexanoates, 14a and 14b

The epoxide mixture **13** (670 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 20 h.[10,15] The mixture was then concentrated, diluted with water and extracted with diethyl ether. Usual work-up afforded a crude residue which, after chromatography (eluting with hexane–AcOEt; 80:20 v/v), gave an inseparable mixture of pure bromohydrins **14a** and **14b** (665 mg, 80%) as an oil: ^{1}H NMR ($CDCl_3$): δ 7.34–7.29 (5H, aromatics-H), 5.51 and 5.45 (0.5H, d, $J = 7.0$, N*H* of each isomer), 5.08 (2H, s, OCH_2Ph), 4.40 (1H, m, 2-H), 3.75 (1H, m, 5-H), 3.44 (1H, m, 6-Ha), 3.32 (1H, m, 6-Hb), 2.05–1.51 (4H, m, 3-H_2 and 4-H_2), 1.44 [9H, s, C(CH_3)$_3$]. Anal. calcd for $C_{18}H_{26}BrNO_5$: C, 51.93; H, 6.29; N, 3.36. Found: C, 52.08; H, 6.32; N, 3.30%.

3.5. *tert*-Butyl (2*S*)-2-benzyloxycarbonylamino-6-bromo-5-oxohexanoate, 7

A mixture of diastereomeric bromohydrins **14** (510 mg; 1.23 mmol) was dissolved in CH_2Cl_2 (50 mL) and treated with pyridinium chlorochromate (500 mg; 2.3 mmol) at room temperature for 12 h. The mixture was then diluted with water and the organic layer was separated. Usual work-up afforded a residue which, after chromatography (eluting with hexane–AcOEt; 80:20 v/v) gave the pure bromo ketone **7** (433 mg, 85%) as an oil; $[\alpha]_D = -0.6$ (*c* 1, $CHCl_3$) (lit.[5b] −0.4); ^{1}H NMR ($CDCl_3$): δ 7.31–7.38 (5H, aromatics-H), 5.37 (1H, d, *J* 8.0, N*H*), 5.06–5.14 (2H, AB system, OCH_2Ph), 4.24 (1H, ddd, *J* 4.5, 8.0, 8.5, 2-H), 3.87 (2H, s, 6-H_2), 2.75 (1H, ddd, *J* 7.0, 8.5, 18.0, 4-Ha), 2.67 (1H, ddd, *J* 5.5, 8.5, 18.0, 4-Hb), 2.20 (1H, m, 3-Ha), 1.89 (1H, m, 3-Hb), 1.46 [9H, s, C(CH_3)$_3$]. Anal. calcd for $C_{18}H_{24}BrNO_5$: C, 52.18; H, 5.84; N, 3.38. Found: C, 52.22; H, 5.79; N, 3.39%.

3.6. *tert*-Butyl (2*S*)-2-benzyloxycarbonylamino-6-iodohexanoate, 9

The alcohol **12** (394 mg, 1.17 mmol) was dissolved in dry THF (3 mL) and treated sequentially with Ph_3P (456 mg, 1.74 mmol), imidazole (126 mg, 1.86 mmol) and I_2 (300 mg, 1.17 mmol). After 1 h at room temperature the mixture was diluted with water and extracted with AcOEt. Usual work-up afforded a crude residue which was chromatographed (eluting with hexane–AcOEt; 90:10 v/v) to gave the pure iodide **9** (450 mg, 86%) as an oil; $[\alpha]_D = +11.8$ (*c* 1, $CHCl_3$) [lit.[6a] +11.0 (*c* 2.6, $CHCl_3$)]; ^{1}H NMR ($CDCl_3$): δ 7.40–7.28 (5H, aromatics-H), 5.33 (1H, d, *J* = 7.4, N*H*), 5.11–5.05 (2H, AB system, OCH_2Ph), 4.23 (1H, m, 2-H), 3.14 (2H, t, *J* = 6.7, 6-H_2), 1.87–1.75 (3H, overlapping), 1.64 (1H, m), 1.48–1.40 (2H, overlapping), 1.45 [9H, s, C(CH_3)$_3$]. Anal. calcd for $C_{18}H_{26}INO_4$: C, 48.33; H, 5.86; N, 3.13. Found: C, 48.25; H, 5.88; N, 3.23%.

3.7. (2*S*,5*R*)- and (2*S*,5*S*)-2-Benzyloxycarbonylamino-5-bromomethyl-δ-valerolactones, 15 and 16

A mixture of diastereoisomeric bromohydrins **14** (4.99 g, 12 mmol) was dissolved in CF_3CO_2H (6.0 mL) and the solution was stirred at room temperature for 40 min. The solvent was then carefully removed under reduced pressure (<40°C) and the residue (3.93 g) was quickly chromatographed on column (eluting with CH_2Cl_2–AcOEt; 100:10 v/v) to afford first the (2*S*,5*S*)-lactone **16** (1.48 g, 36%): mp 123–124°C (from CH_2Cl_2:diisopropyl ether); $[\alpha]_D = +58.2$ (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.38–7.31 (5H, aromatics-H), 5.83 (1H, d, *J* = 4.6, N*H*), 5.12 (2H, s, OCH_2Ph), 4.61 (1H, m, 5-H), 4.48 (1H, m, 2-H), 3.53 (1H, dd, *J* = 11.1 and 5.2 Hz CH*H*Br), 3.46 (1H, dd, *J* = 11.1 and 5.6 Hz, C*H*HBr), 2.65 (1H, m, 3-Ha), 2.23 (1H, m, 4-Ha), 1.90 (1H, m, 4-Hb), 1.66 (1H, m, 3-Hb). Anal. calcd for $C_{14}H_{16}BrNO_4$: C, 49.14; H, 4.71; N, 4.09. Found: C, 49.18; H, 4.65; N, 4.02%.

Further elution gave the (2*S*,5*R*)-lactone **15** (1.19 g, 29%): mp 127–128°C (from CH_2Cl_2–diisopropyl ether); $[\alpha]_D = +5.4$ (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.36–7.29 (5H, aromatics-H), 5.54 (1H, d, *J* = 3.2, N*H*), 5.10 (2H, s, OCH_2Ph), 4.57 (1H, m, 5-H), 4.14 (1H, m, 2-H), 3.49 (2H, br s, CH_2Br), 2.46 (1H, m, 3-Ha), 2.17 (1H, m, 4-Ha), 1.99–1.84 (2H, overlapping, 3-Hb and 4-Hb). Anal. calcd for $C_{14}H_{16}BrNO_4$: C, 49.14; H, 4.71; N, 4.09. Found: C, 49.07; H, 4.76; N, 4.15%.

3.8. Separate transformation of the (2*S*,5*R*)- and (2*S*,5*S*)-2-benzyloxycarbonylamino-5-bromomethyl-δ-valerolactones 15 and 16 into the corresponding azidolactones 17 and 18

Each lactone **15** and **16** (480 mg, 1.4 mmol) was dissolved in DMF (5 mL) and treated with NaN_3 (180 mg, 2.77 mmol) at room temperature for 12 h. The mixture was then poured in ice cold water and extracted with AcOEt. Usual work-up afforded quantitatively the appropriate crystalline azidolactone. In fact, starting from the lactone **15**, (2*S*,5*R*)-5-azidomethyl-2-benzyloxycarbonylamino-δ-valerolactone **17** was obtained: mp 86–87°C (from CH_2Cl_2–diisopropyl ether); identical in all respects with that described.[11] Starting from the (2*S*,5*S*)-lactone **16**, pure (2*S*,5*S*)-5-azidomethyl-2-benzyloxycarbonylamino-δ-valerolactone **18** was obtained: mp 90–92°C (from CH_2Cl_2–diisopropyl ether); identical in all respects with that described in the literature).[11]

3.9. Allyl (2*S*,5*R*)-2-benzyloxycarbonylamino-4-(2-oxiranyl)butanoate, 19

The (2*S*,5*R*)-lactone **15** (1.0 g, 2.9 mmol) was dissolved in allyl alcohol (70 mL) and stirred with cesium carbonate (0.840 g) at room temperature for 45 min. Then, the mixture was diluted with water and extracted with AcOEt. Usual work-up afforded a crude residue which, after column chromatography (eluting with CH_2Cl_2–AcOEt; 9:1 v/v), gave the epoxide **19** (778 mg, 84%) as an oil: $[\alpha]_D = +10.8$ (*c* 1, $CHCl_3$); ^{1}H NMR: δ 7.35–7.27 (5H, aromatics-H), 5.87 (1H, m, CH_2=C*H*-CH_2O), 5.39 (1H, d, *J* = 7.0, N*H*), 5.31 (1H, dd, *J* = 17.3 and <1, C*H*H=C*H*-CH_2O), 5.24 (1H, dd, *J* = 10.4 and <1, CH*H*=C*H*-CH_2O), 5.09 (2H, s, OCH_2Ph), 4.61 (2H, d, *J* = 5.1, CH_2=CH-CH_2O), 4.42 (1H, m, 2-H), 2.88 (1H, m, 5-H), 2.72 (1H, dd, *J* = 8.9 and 8.8, 6-Ha), 2.45 (1H, m, 6-Hb), 2.03 (1H, m, 3-Ha), 1.81 (1H, m, 3-Hb), 1.66 (1H, m, 4-Ha), 1.54 (1H, m, 4-Hb). Anal. calcd for $C_{17}H_{21}NO_5$: C, 63.94; H, 6.63; N, 4.39. Found: C, 63.89; H, 6.58; N, 4.43%.

3.10. Allyl (2*S*,5*R*)-2-benzyloxycarbonylamino-5-hydroxy-6-iodohexanoate, 20

Following Bajwa's procedure,[16] the epoxidic allyl ester **19** (638 mg, 2 mmol) was dissolved in THF (20 mL) and treated with NaI (600 mg, 4 mmol) and AcOH (0.343 mL, 6 mmol) at room temperature for 1 h. The mixture was poured in ice cold water and extracted with AcOEt. Usual work-up afforded a crude residue

which was chromatographed (eluting with hexane–AcOEt; 80:20 v/v) to afford the pure iodohydrin **20** (796 mg; 89%): mp 72–73°C (from CH_2Cl_2–diisopropyl ether); $[\alpha]_D = +6.9$ (*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, O*C*H_2Ph), 4.62 (2H, d, $J = 4.0$, CH_2=CH-C*H*$_2O$), 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, 45.63; H, 5.02; N, 3.09%.

3.11. Methyl (2*S*,5*S*)-2-benzyloxycarbonylamino-6-bromo-5-hydroxyhexanoate, 21

A solution of (2*S*,5*S*)-lactone **16** (1.0 g, 2.9 mmol) in MeOH (50 mL) was heated under reflux for 9 h. The solvent was evaporated under reduced pressure and the residue was purified by column chromatography (eluting with CH_2Cl_2–AcOEt; 9:1 v/v), to give the bromohydrin **21** (976 mg, 90%) as an oil: $[\alpha]_D = +7.6$ (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.36–7.28 (5H, aromatics-H), 5.39 (1H, d, $J = 7.7$, N*H*), 5.10–5.06 (2H, AB system, O*C*H_2Ph), 4.40 (1H, ddd, $J = 7.7$, 7.7 and 4.5, 2-H), 3.79 (1H, m, 5-H), 3.73 (3H, s, O*C*H_3), 3.46 (1H, dd, $J = 10.5$ and 3.5, 6-Ha), 3.32 (1H, dd, $J = 10.5$ and 7.0, 6-Hb), 2.33 (1H, br s, O*H*) 1.96 (1H, m, 3-Ha), 1.84 (1H, m, 3-Hb), 1.61 (1H, m, 4-Ha), 1.53 (1H, m, 4-Hb). Anal. calcd for $C_{15}H_{20}BrNO_5$: C, 48.14; H, 5.39; N, 3.74. Found: C, 48.42; H, 5.46; N, 3.56%.

3.12. Methyl (2*S*)-2-benzyloxycarbonylamino-6-bromo-5-oxohexanoate, 22

(2*S*,5*S*)-Bromohydrin **21** (460 mg, 1.23 mmol), dissolved in CH_2Cl_2 (50 mL), was treated with pyridinium chlorochromate (500 mg, 2.3 mmol) at room temperature for 12 h. The mixture was diluted with water and extracted. Usual work-up afforded a residue which, after chromatography (eluting with hexane–AcOEt; 80:20 v/v), afforded the pure bromo ketone **22** (398 mg, 87%): mp 86–87°C (from CH_2Cl_2–diisopropyl ether); $[\alpha]_D$ +2.2 (*c* 1, $CHCl_3$). Anal. calcd for $C_{15}H_{18}BrNO_5$: C, 48.40; H, 4.87; N, 3.76. Found: C, 48.49; H, 4.92; N, 3.68%. All other physico-chemical properties were identical to that previously described.[11]

3.13. Preparation of valerolactones 15 and 16 from bromoketone 22

To a solution of bromoketone **22** (4.77 g; 12.8 mmol) in MeOH (50 mL), $NaBH_4$ (590 mg; 15.6 mmol) was gradually added at −5°C. The mixture was stirred at −5°C for 20 min, treated with water (10 mL), acidified with aqueous HCl (2 M) and extracted with AcOEt. Usual work-up afforded a chromatographically inseparable mixture of bromohydrin **21** and its 5*R* epimer (4.32 g; 90%, in a 1:1 ratio) which showed in the ^1H NMR spectrum, diagnostic signals at δ 5.44 and 5.37 ppm corresponding to NH signal, respectively, for **21** and its 5*R* epimer. The crude mixture obtained (4.30 g) was dissolved in CF_3CO_2H (6.0 mL) and the solution was stirred at room temperature for 40 min. The solvent was then carefully removed under reduced pressure (<40°C) and the residue (3.47 g) was quickly chromatographed on column (eluting with CH_2Cl_2–AcOEt; 100:10 v/v) to afford first the lactone **16** (1.40 g, 32%) and then the lactone **15** (1.05 g, 24%): identical in all respects with those above described.

3.14. Deoxypyridinoline, 2

A solution of 4-[(*S*)-2-benzyloxycarbonylamino-2-(*tert*-butyloxycarbonyl)ethyl] - 5 - [(*S*) - 3 - benzyloxycarbonylamino - 3 - (*tert* - butyloxycarbonyl)propyl] - 3 - hydroxypyridine **23**[6c] (400 mg, 0.6 mmol) and *tert*-butyl (2*S*)-2-benzyloxycarbonylamino-6-iodohexanoate **9** (1.08 g, 2.4 mmol) in MeCN (10 mL) was heated under reflux for 8 h under an argon atmosphere. The solvent was then evaporated under reduced pressure to give a crude product whose UV spectrum (in MeOH) shows λ_{max} at 296 nm indicating (unpublished results from our laboratory) the presence of 3-hydroxypyridinium iodide form of the alkylated compound.

This crude product was chromatographed (eluting first with AcOEt and then with AcOEt–MeOH; 100:7 v/v) to afford, in the order, the excess of iodide **9** (626 mg), and then the 4-[(*S*)-2-benzyloxycarbonylamino-2-(*tert*-butyloxycarbonyl)ethyl] - 5 - [(*S*) - 3 - benzyloxycarbonylamino-3-(*tert*-butyloxycarbonyl)propyl]-1-[(*S*)-5-benzyloxycarbonylamino - 5 - (*tert* - butyloxycarbonyl)pentyl] - 3-pyridiniumolate (436 mg, 74%), a resinous material which resisted all efforts of crystallisation. Its UV spectrum (in MeOH) shows λ_{max} at 231, 260 and 336 nm and thus indicates that it is in the 3-pyridiniumolate form (a zwitterion) supported also by an appropriate elemental analysis; $[\alpha]_D^{20}$ +5.5 (*c* 1, $CHCl_3$) (lit.[6a] +5.1); ^1H NMR ($CDCl_3$): δ 8.09 (1H, br s, pyridinium-H), 7.40–7.20 (15H, aromatics-H), 6.90 (1H, br s, pyridinium-H), 5.61 (1H, d, *J* 7.0, N*H*), 5.49 (2H, overlapping, 2×N*H*), 5.16–5.05 (6H, overlapping, 3×O*C*H_2Ph), 4.24–4.14 [3H, overlapping, 3×C*H*(NHCbz)CO_2Bu^t], 3.87 (2H, m, $N^+CH_2CH_2$), 3.37 [1H, dd, $J = 12.5$ and 11.4, ring-4: C*H*HCH(NHCbz)CO_2Bu^t], 2.88 [1H, dd, $J = 12.5$ and 3.6, ring-4: CH*H*CH(NHCbz)CO_2Bu^t], 2.68 [2H, m, ring-5: C*H*$_2$CH$_2$CH(NHCbz)CO_2Bu^t], 2.08 [1H, m, ring-5: C*H*HCH(NHCbz)CO_2Bu^t], 1.92–1.80 [4H, overlapping, ring-5: CH*H*CH(NHCbz)CO_2Bu^t, ring-N: C*H*HCH(NHCbz)CO_2Bu^t and $N^+CH_2CH_2$], 1.65 [1H, m, ring-N: CH*H*CH(NHCbz)CO_2Bu^t], 1.45, 1.42 and 1.40 [3×9H, 3×s, 3×C(C*H*$_3$)$_3$], 1.40–1.28 (2H, $N^+CH_2CH_2CH_2$ masked by Bu^t signals); ^{13}C NMR ($CDCl_3$): δ 170.8 and 169.4 (3×*C*O$_2$Bu^t), 156.5, 156.1 and 156.0 (3×NH*C*O$_2$CH$_2$Ph), 142.9, 138.2, 137.0 and 136.2 (quaternary-C of pyridinium and phenyl rings), 130.3 and 122.0 (C-2 and C-6 pyridinium ring), 128.4, 128.1 and 127.5 (tertiary phenylic C), 82.7, 82.3 and 81.3 [3×*C*(CH$_3$)$_3$], 66.9 and 66.0 (3×O*C*H$_2$Ph), 60.2 ($N^+CH_2CH_2$), 57.0 [ring-4: *C*H(NHCbz)CO_2Bu^t], 53.7 [ring-5: *C*H(NHCbz)CO_2Bu^t and ring-N: *C*H(NHCbz)CO_2Bu^t], 33.2 [ring-5: *C*H$_2$CH(NHCbz)CO_2Bu^t],

32.2 [ring-N: $CH_2CH(NHCbz)CO_2Bu^t$], 30.2 ($N^+CH_2CH_2$), 27.8 [3×C(CH_3)$_3$ and ring-5: $CH_2CH(NHCbz)CO_2Bu^t$], 26.1 [ring-5: $CH_2CH_2CH(NHCbz)CO_2Bu^t$], 22.0 [ring-N: $CH_2CH_2CH(NHCbz)CO_2Bu^t$]. Anal. calcd for $C_{54}H_{70}N_4O_{13}$: C, 65.97; H, 7.18; N, 5.70. Found: C, 65.82; H, 7.23; N, 5.66%.

The obtained benzyloxycarbonyl derivative (400 mg, 0.41 mmol), dissolved in MeOH (90 mL), was hydrogenated in the presence of 10% Pd/C (70 mg) for 12 h at room temperature and atmospheric pressure. Filtration of the catalyst on a pad of Celite and evaporation of the solvent under reduced pressure afforded the 4-[(*S*)-2-amino-2-(*tert*-butyloxycarbonyl)ethyl]-5-[(*S*)-3-amino-3-(*tert*-butyloxycarbonyl)propyl]-1-[(*S*)-5-amino-5-(*tert*-butyloxycarbonyl)pentyl]-3-pyridiniumolate (215 mg, 90%) as an resinous material: $[\alpha]_D^{20}$ +32.8 (*c* 1, $CHCl_3$) (lit.[5b] +32.5); ^{1}H NMR (CD_3OD): δ 7.57 (1H, br s, pyridinium-H), 7.48 (1H, br s, pyridinium-H), 4.34 (2H, t, J=7.3, $N^+CH_2CH_2$), 4.20 [1H, dd, J=7.3 and 3.0, ring-4: $CH(NH_2)CO_2Bu^t$], 3.73 [1H, m, ring-5: $CH(NH_2)CO_2Bu^t$], 3.64 [1H, m, ring-N: $CH(NH_2)CO_2Bu^t$], 3.33 [1H, m, ring-4: $CHHCH(NH_2)CO_2Bu^t$], 3.20 [1H, m, ring-4: $CHHCH(NH_2)CO_2Bu^t$], 2.91 [1H, m, ring-5: $CHHCH_2CH(NH_2)CO_2Bu^t$], 2.81 [1H, m, ring-5: $CHHCH_2CH(NH_2)CO_2Bu^t$], 2.07–1.78 (6H, overlapping), 1.52, 1.48 and 1.38 [3×9H, 3×s, 3×C(CH_3)$_3$], 1.40–1.28 (2H, $N^+CH_2CH_2CH_2$ masked by Bu^t signals); ^{13}C NMR (CD_3OD): δ 172.7, 171.1 and 169.5 (3×CO_2Bu^t), 168.2, 142.5 and 141.4 (C-3, C-4 and C-5 pyridinium ring), 132.4 and 128.6 (C-2 and C-6 pyridinium ring), 85.1, 84.5 and 84.0 [3×$C(CH_3)_3$], 61.3 ($N^+CH_2CH_2$), 54.8, 54.5 and 53.7 [3×$CH(NH_2)CO_2Bu^t$], 34.6 [ring-5: $CH_2CH(NH_2)CO_2Bu^t$], 32.1, 31.9 and 29.9 [ring-4: $CH_2CH(NH_2)CO_2Bu^t$, ring-N: $CH_2CH(NH_2)CO_2Bu^t$, ring-5: $CH_2CH_2CH(NH_2)CO_2Bu^t$ and $N^+CH_2CH_2$] 28.2 [3×C(CH_3)$_3$], 23.1 [$N^+CH_2CH_2CH_2$].

The obtained *tert*-butyl derivative (204 mg, 0.35 mmol) was dissolved in CF_3CO_2H–H_2O (8 mL, 95:5, 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 product obtained was dissolved in aqueous HCl (4.0 mL, 1.0 M) and the solution was lyophilised to afford the deoxypyridinoline **2** as the hydrochloride monohydrate (141 mg, 70%): $[\alpha]_D^{20}$ +33.4 (*c* 1, H_2O)) [lit. +32.1[6a] (*c* 0.9, H_2O) +31.6[7c] (*c* 0.25, MeOH); +36.2[8b] (*c* 0.535, MeOH)]; ^{1}H NMR (D_2O): δ 8.19 (1H, br s, pyridinium-H), 8.13 (1H, br s, pyridinium-H), 4.43 (2H, t, J=7.2, $N^+CH_2CH_2$), 4.05 [1H, t, J=7.2, ring-4: $CH(NH_3^+)COO^-$], 3.82 [1H, t, J=5.5, ring-5: $CH(NH_3^+)COO^-$], 3.70 [1H, t, J=5.7, ring-N: $CH(NH_3^+)COO^-$], 3.34–3.26 [2H, m, ring-4: $CH_2CH(NH_3^+)COO^-$], 2.93 [1H, m, ring-5: $CHHCH_2CH(NH_3^+)COO^-$], 2.82 [1H, m, ring-5: $CHHCH_2CH(NH_3^+)COO^-$], 2.12 [2H, m, ring-5: $CH_2CH(NH_3^+)COO^-$], 1.98 (2H, ddt, J=7.2, 7.2 and 7.0, $N^+CH_2CH_2$), 1.84 [2H, m, ring-N: $CH_2CH(NH_3^+)COO^-$], 1.42 (1H, m, $N^+CH_2CH_2CHH$), 1.34 (1H, m, $N^+CH_2CH_2CHH$); ^{13}C NMR (D_2O): δ 174.4, 173.8, and 172.8 (3×CO_2^-), 156.2, 141.5 and 141.2 (C-3, C-4 and C-5 pyridinium ring), 135.3 and 129.0 (C-2 and C-6 pyridinium ring), 61.1 ($N^+CH_2CH_2$), 54.5, 54.2 and 53.0 [3×$CH(NH_3^+)CO_2^-$], 30.7, 30.0, 29.8, 27.9, 25.6 and 25.6. ^{1}H and ^{13}C NMR spectra are in agreement with the literature data;[4,5b,7c] ESI/MS m/z 413 (M^+). Anal. calcd for $C_{18}H_{34}Cl_4N_4O_8$: C, 37.51; H, 5.95; N, 9.72; Cl, 24.61. Found: C, 37.66; H, 6.02; N, 9.88; Cl, 24.25%.

3.15. 4-[(*S*)-2-Benzyloxycarbonylamino-2-(*tert*-butyloxycarbonyl)ethyl]-5-[(*S*)-3-benzyloxycarbonylamino-3-(*tert*-butyloxycarbonyl)propyl]-1-[(2*R*,5*S*)-5-benzyloxycarbonylamino-5-(allyloxycarbonyl)-2-hydroxypentyl]-3-pyridiniumolate, 24

A solution of 4-[(*S*)-2-benzyloxycarbonylamino-2-(*tert*-butyloxycarbonyl)ethyl]-5-[(*S*)-3-benzyloxycarbonylamino-3-(*tert*-butyloxycarbonyl)propyl]-3-hydroxypyridine **23**[6c] (265 mg, 0.4 mmol) and allyl (2*S*,5*R*)-2-benzyloxycarbonylamino-5-hydroxy-6-iodohexanoate **20** (714 mg, 1.6 mmol) in CH_3CN (5 mL) was heated under reflux for 16 h under an argon atmosphere. The solvent was then evaporated under reduced pressure to give a crude product whose UV spectrum (in MeOH) shows λ_{max} at 296 nm indicating (unpublished results from our laboratory) the presence of the 3-hydroxypyridinium iodide form of the alkylated compound.

This crude product was chromatographed (eluting first with AcOEt and then with AcOEt–MeOH; 100:7 v/v) to afford, in order, the excess iodohydrin **20** (445 mg), the starting pyridine **23** (16 mg) and then the title compound **24** (247 mg, 63%): a resinous material which resisted all efforts of crystallisation. Its UV spectrum (in MeOH) shows λ_{max} at 231, 260 and 336 nm and thus indicates that it is in the 3-pyridiniumolate form (a zwitterion) supported also by an appropriate elemental analysis; $[\alpha]_D$=−2.3 (*c* 0.6, $CHCl_3$); ^{1}H NMR ($CDCl_3$–D_2O): δ 7.39 (1H, br s, pyridinium-H), 7.35–7.20 (15H, aromatics-H), 6.95 (1H, br s, pyridinium-H), 5.85 (1H, dddd, J=16.8, 10.52, 5.0, 5.0, CH_2=CH-CH_2O), 5.29 (1H, dd, J=16.8, <1, CHH=CH-CH_2O),), 5.20 (1H, dd, J=10.52 and <1, CHH=CH-CH_2O), 5.10–5.05 (4H, overlapping, 2×OCH_2Ph), 5.00 (1H, d, J=12.6, A part of AB system, OCHHPh), 4.90 (1H, J=12.6, B part of AB system, OCHHPh), 4.60 (2H, m, CH_2=CH-CH_2O), 4.68 [1H, dd, J=7.0 and 5.6, ring-N: CH(NHCbz)CO_2Allyl], 4.24 [1H, dd, J=9.8 and 3.5, ring-4: CH(NHCbz)CO_2Bu^t], 4.19 [1H, dd, 7.0, 4.9, ring-5: CH(NHCbz)CO_2Bu^t], 3.87–3.81 [2H, overlapping, $N^+CHHCH(OH)$ and $N^+CH_2CH(OH)$], 3.69 [1H, dd, J=13.3 and 7.5, $N^+CHHCH(OH)$], 3.62 [1H, dd, J=13.3 and 9.8, ring-4: $CHHCH(NHCbz)CO_2Bu^t$], 3.26 [1H, dd, J=13.3 and 3.5, ring-4: $CHHCH(NHCbz)CO_2Bu^t$], 2.72–2.63 [2H, m, ring-5: $CH_2CH_2CH(NHCbz)CO_2Bu^t$], 2.08–1.95 [2H, overlapping, ring-5: CHHCH(NHCbz)CO_2Bu^t and ring-N: CHHCH(NHCbz)CO_2Allyl], 1.84 [1H, m, ring-5: $CHHCH(NHCbz)CO_2Bu^t$], 1.77 [1H, m, ring-N: $CHHCH(NHCbz)CO_2$Allyl], 1.51 [1H, m, $N^+CH_2CH(OH)CHH$], 1.44 and 1.36 [2×9H, 2×s, 2×C(CH_3)$_3$], 1.7–1.12 [$N^+CH_2CH(OH)CHH$ masked by Bu^t signals]. ^{13}C NMR ($CDCl_3$): δ 171.8,

170.7 and 170.5 (2×CO_2Bu^t and CO_2Allyl), 156.2 (3×$NHCO_2CH_2Ph$), 142.5, 139.5, 136.4, 136.2 and 136.0 (quaternary C of pyridinium and phenyl rings and CH_2=$CHCH_2O$), 131.5 (C-2 or C-6 pyridinium ring), 128.5, 128.4, 128.3 and 128.0 (tertiary phenylic C), 118.7 (C-2 or C-6 pyridinium ring and CH_2=$CHCH_2O$), 82.7 and 82.3 [2×$C(CH_3)_3$], 69.5 [$N^+CH_2CH(OH)$], 67.0, 66.8 and 65.9 (3×OCH_2Ph and CH_2=$CHCH_2O$), 66.6 [$N^+CH_2CH(OH)$], 53.9 [ring-4, 5 and N: CH(N-HCbz)CO_2], 27.9 and 27.8 [3×$C(CH_3)_3$], 33.0, 30.1, 29.6, 28.6 and 26.4. Anal. calcd for $C_{53}H_{66}N_4O_{14}$: C, 64.75; H, 6.77; N, 5.70. Found: C, 64.70; H, 6.81; N, 5.65%.

3.16. 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, 26

To a solution of allylic ester **24** (295 mg, 0.3 mmol) and *N*-methylaniline (963 mg, 9 mmol) in anhydrous THF (5 mL), tetrakis(triphenylphosphine) palladium(0) (34.7 mg, 0.03 mmol) was added and the resulting solution was stirred under an argon at room temperature for 76 h. After evaporation of the solvent under reduced pressure, the residue was purified by column chromatography (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 **25** (175 mg, 62%) as a resinous material.

The benzyloxycarbonyl derivative **25** (236 mg, 0.25 mmol), dissolved in aqueous EtOH (100 mL, 80%), was hydrogenated in the presence of 10% Pd/C (50 mg) for 15 h at room temperature and atmospheric pressure. After filtration of the catalyst on a pad of Celite and evaporation of the solvent under reduced pressure, the residue was chromatographed (eluting with MeOH–NH_3; 100:5 v/v) to afford the title compound **26** (124 mg, 92%) as a glass: ^{1}H NMR (CD_3OD): δ 7.81 (1H, br s, pyridinium-H), 7.73 (1H, br s, pyridinium-H), 4.48 [1H, dd, J=13.2 and 3.3, $N^+CHHCH(OH)$], 4.32 [1H, dd, J=7.2 and 2.8, ring-4: $CH(NH_2)CO_2Bu^t$], 4.25 [1H, m, $N^+CHHCH(OH)$], 4.05–3.98 [2H, overlapping, $N^+CH_2CH(OH)$ and ring-5: $CH(NH_2)CO_2Bu^t$], 3.71 [1H, m, ring-N: $CH(NH_2)CO_2H$], 3.37 [1H, dd, J=14.2 and 7.2, ring-4: $CHHCH(NH_2)CO_2Bu^t$], 3.26 [1H, dd, J=14.2 and 2.8, ring-4: $CHHCH(NH_2)CO_2Bu^t$], 3.08 [1H, m, ring-5: $CHHCH_2CH(NH_2)CO_2Bu^t$], 2.93 [1H, m, ring-5: $CHHCH_2CH(NH_2)CO_2Bu^t$], 2.21–2.02 [4H, overlapping, ring-5: $CH_2CH(NH_2)CO_2Bu^t$ and ring-N: $CH_2CH(NH_2)CO_2H$], 1.62 [2H, m, $N^+CH_2CH(OH)CH_2$], 1.57 and 1.39 [2×9H, 2×s, 2×$C(CH_3)_3$]; ^{13}C NMR (CD_3OD): δ 174.7 (CO_2H), 168.9 and 168.3 (2×CO_2Bu^t), 166.2, 141.7 and 139.7 (C-3, C-4 and C-5 pyridinium ring), 133.6 and 129.9 (C-2 and C-6 pyridinium ring), 85.4 [2×$C(CH_3)_3$], 70.5 [$N^+CH_2CH(OH)$], 65.6 ($N^+CH_2CH_2$), 55.0 [ring-N: $CH(NH_2)CO_2H$], 53.4 [ring-5: $CH(NH_2)CO_2Bu^t$], 53.7 [ring-4: $CH(NH_2)CO_2Bu^t$], 32.1 [ring-5: $CH_2CH(NH_2)CO_2Bu^t$], 30.3 [ring-4: $CH_2CH(NH_2)CO_2Bu^t$], 29.8 [ring-N: $CH_2CH(NH_2)CO_2H$], 29.1 [ring-5: $CH_2CH_2CH(NH_2)CO_2Bu^t$], 28.2 and 28.1 [2×$C(CH_3)_3$], 27.6 [$N^+CH_2CH(OH)CH_2$]. Anal. calcd for $C_{26}H_{44}N_4O_8$: C, 57.76; H, 8.20; N, 10.36. Found: C, 57.52; H, 10.41; N, 10.31%.

3.17. Pyridinoline, 1

The *tert*-butyl derivative **26** (108 mg, 0.2 mmol) was dissolved in aqueous CF_3CO_2H (5 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 (3.0 mL, 1.0 M) and the solution was lyophilised to afford the pyridinoline **1** as the hydrochloride dihydrate (81 mg, 67%): λ_{max} (0.1 M HCl)/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); ^{1}H NMR (D_2O): δ 8.26 (1H, br s, pyridinium-H), 8.23 (1H, br s, pyridinium-H), 4.67 [1H, dd, J=13.2 and 2.2, $N^+CHHCH(OH)$], 4.32 [1H, dd, J=13.2 and 8.6, $N^+CHHCH(OH)$], 4.18 [1H, t, J=8.0, ring-4: $CH(NH_3^+)COO^-$], 4.08 [1H, m, N^+CH_2CH-(OH)], 3.98 [1H, t, J=5.7, ring-5: $CH(NH_3^+)COO^-$], 3.95 [1H, t, J=5.7, ring-N: $CH(NH_3^+)COO^-$], 3.42–3.34 [2H, m, ring-4: $CH_2CH(NH_3^+)COO^-$], 3.02 [1H, m, ring-5: $CHHCH_2CH(NH_3^+)COO^-$], 2.91 [1H, m, ring-5: $CHHCH_2CH(NH_3^+)COO^-$], 2.25–2.10 [3H, overlapping, ring-5: $CH_2CH(NH_3^+)COO^-$ and ring-N: $CHHCH(NH_3^+)COO^-$], 2.01 [1H, m, ring-N: $CHHCH(NH_3^+)COO^-$], 1.75 (1H, m, $N^+CH_2CH_2CHH$), 1.64 (1H, m, $N^+CH_2CH_2CHH$); ^{13}C NMR (D_2O): δ 173.2, 172.9, and 172.3 (3×CO_2^-), 155.2, 141.0 and 140.9 (C-3, C-4 and C-5 pyridinium ring), 136.5 and 129.5 (C-2 and C-6 pyridinium ring), 69.8 [$N^+CH_2CH(OH)$], 66.1 ($N^+CH_2CH_2$), 53.5, 53.4 and 52.4 [3×$CH(NH_3^+)CO_2^-$], 30.4, 28.9, 27.8, 26.5 and 25.6; ^{1}H and ^{13}C NMR spectra are in agreement with the literature data;[5b,7c] 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, 35.56; H, 6.08; N, 9.22; Cl, 23.45%.

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