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Synthesis of unnatural homologues of deoxypyridinoline as possible internal standards in analytical detection of pyridinolinic cross-links of collagen

Pietro Allevi, Eti Alessandra Femia, Paola Rota, Maria Letizia Costa, Mario Anastasia *

Dipartimento di Chimica, Biochimica e Biotecnologie per la Medicina, Università di Milano, Via Saldini 50, I-20133 Milano, Italy

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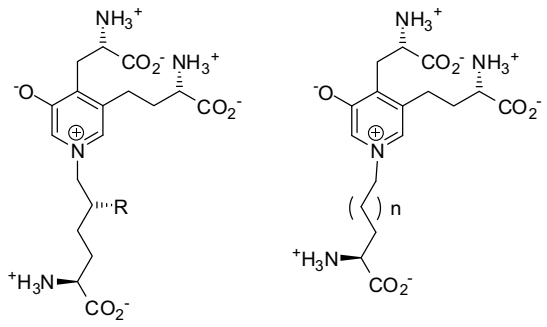
ABSTRACT

Three homologues of the collagen cross-links deoxypyridinoline, differing in the length of the side chain at the aromatic nitrogen, have been efficiently synthesized as possible internal standards in the quantitative analyses of pyridinolines. The first has a one-carbon shorter N-chain, while other two have a one-carbon and a two-carbon longer N-chain. The stereogenic centers are introduced stereoselectively using Williams' glycine template methodology and oxazinones to generate chirality and to suitably protect the amino acid functionality during assembly of the pyridinoline nucleus.

1. Introduction

Over the past decade, deoxypyridinoline 1 (dPyd) and to a minor extent pyridinoline **2** (Pyd; Fig. 1), two fluorescent cross-links derived from the degradation of bone collagen, have attracted increasing attention as biochemical markers of bone resorption, which are useful for assessing fracture risk prediction in persons suffering from osteoporosis, bone cancer, or arthropathies.[1–5]

Pyridinolines **1** and **2** are commonly detected in human urine to allow early diagnosis or for monitoring drug therapy of these and other metabolic bone diseases.[6,7] Currently, analytical methods for the accurate evaluation of these fluorescent cross-links in human urine and other various tissues, preferably use HPLC and a fluorescence detector, since fluorimetric methods of detection are more sensitive than those based on mass spectrometry or alternatives.[8–11] As a consequence, it is essential not only to have authentic samples of pure dPyd **1** and Pyd **2** to be used as primary reference standards, but also to dispose of some pyridinolines congeners (stereoisomers or near homologues), with very closed chemico-physical properties and polarity, to be used as suitable internal standards to minimize errors related to the pretreatment of the samples. However, until now, the reported quantitative determinations of pyridinolines, carried out by HPLC, did not use any internal standard, apart from one protocol, where a pyridinoline acetylated at the hydroxy group[11] of the hydroxylysine chain is used. Moreover, even this pyridinoline derivative is not completely satisfactory as an internal standard since it cannot be used to evaluate the efficiency of cross-link hydrolysis. In fact, its acetate group can be destroyed during hydrolysis, which is necessary, before analyses, in order to cleave pyridinolines bonded to carbohydrates or to peptide chains. Thus, the acetylated pyridinoline may only be added after the accomplishment of the hydrolytic procedures, and does not follow the same fate of pyridinolines during the preparation of the analytical samples. In previous work from our laboratory, we accomplished the synthesis of pyridinolines **1** and **2** and of all other known, free[12–20] or glycolsylated,[21] collagen cross-links which are now available in suitable amounts by synthesis. Herein, we report the first synthesis of three homologues **3a–c** of dPyd **1**, differing in the length of the chain bonded to the aromatic nitrogen. The homologue **3a** has a one-carbon shorter N-chain, while the homologues **3b** and **3c** have, respectively, a one-carbon and a two-carbon longer N-chain. In addition, we report a preliminary analysis of the chromatographic (HPLC) behavior of these homologues, which allows us to discriminate the homologous **3b** from dPyd **1** and Pyd **2** by this analytical technique.



1: R = H; deoxypyridinoline
2: R = OH; pyridinoline

3 **a**: n = 1
b: n = 3
c: n = 4

Figure 1. Pyridinolines structures.

* Corresponding author. Tel./fax: +39 0250316042.
E-mail address: mario.anastasia@unimi.it (M. Anastasia).

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This and the observation that other physico-chemical properties of compound **3b** are obviously very close to those of natural pyridinolines allow us to select the homologous **3b** as a convenient candidate to be used as the internal standard in the quantitative analysis of pyridinolines by HPLC.

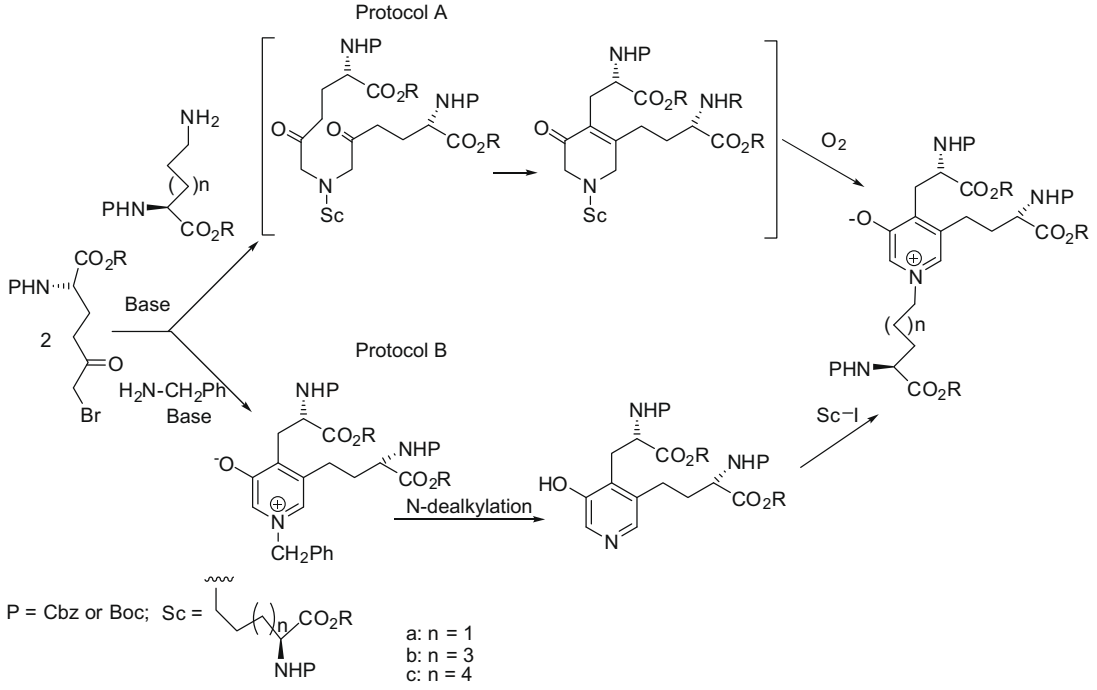
2. Results and discussion

On the basis of our previous work, we were able to perform the synthesis of the three homologous deoxypyridinolines **3a**, **3b**, and **3c**, adopting either our one-pot protocol[13,14,17] (Scheme 1, A) or our modular protocol[21–23] (Scheme 1, B) for the assembly of the pyridinium ring.

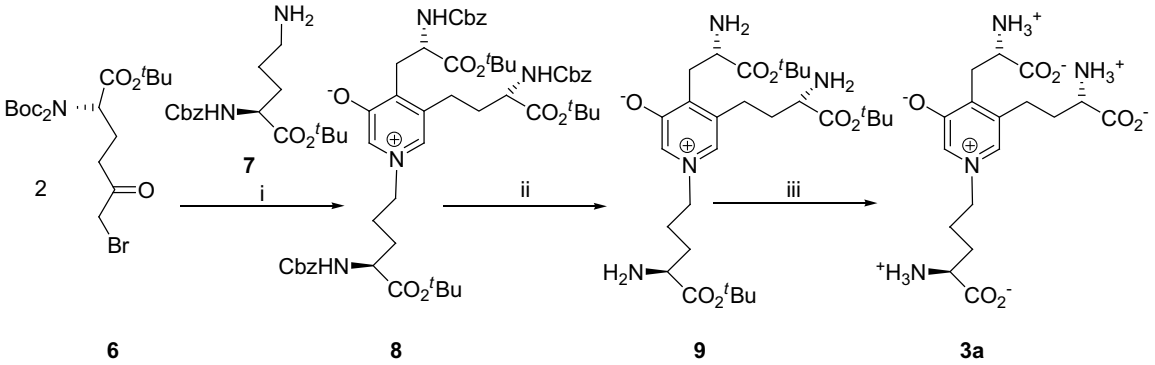
In the one-pot protocol, the pyridinium ring is assembled convergently from 2 M equiv of an aminoacidic bromoketone and a primary amine via a bis-alkylation and a successive cyclization–aromatization sequence of reactions. In the modular protocol, an auxiliary sacrificial amine (benzylamine or allylamine), easily cleavable, is used to introduce the pyridine nitrogen atom and for assembling the pyridinium nucleus, which after dealkylation of the aromatic nitrogen affords a substituted pyridine, which is able to be transformed into the desired pyridinium salt by alkylation of the aromatic nitrogen with a conveniently protected aminoacidic iodide generating the lysine chain.

Each of these protocols has some advantages. The first protocol is shorter and is very useful when the necessary protected amine is easily or commercially available, as in the case of lysine or hydroxylysine. The second protocol is longer but is of practical utility to prepare a series of congeners differing in the side chains at the aromatic nitrogen atom.

In programming our synthetic work, we started with the synthesis of the dPyd homologue **3a**, which appeared relatively more simple and had a fast preparation. In fact, we considered that by using our shorter one-pot protocol, bromoketone **6** and 2,5-diaminopentanoic acid *tert*-butyl ester **7**, both required for the assembling of **3a** (Scheme 2), could be conveniently obtained from L-glutamic acid.[18] We were also confident that homologue **3a**, with a one-carbon shorter lysine chain, could be differently retained in



Scheme 1. Protocols for pyridinolines synthesis.



Scheme 2. Synthesis of the pyridinoline analogue **3a**. Reagents and conditions: (i) K_2CO_3, MeCN, rt, 18 h, then O_2, K_2CO_3, MeOH, rt, 72 h, 55%; (ii) H_2, Pd/C, MeOH, 88%; (iii) TFA/H_2O; 95:5; v/v, rt, 95.5%.

HPLC with respect to natural pyridinolines, thus satisfying our needs and avoiding the synthesis of the other homologues **3b** and **3c**.

Next, we reacted 2 M equiv of bromoketone[13] **6** with the diamino acid *tert*-butyl ester[13] **7** in acetonitrile containing anhydrous sodium carbonate, and obtained, after exchanging the solvent and a one-pot oxidation by oxygen, the protected pyridiniumolate **8** in 55% total yields. Compound **8** showed the expected physico-chemical properties and ^{1}H and ^{13}C NMR spectra in agreement with the assigned structure and with those of the natural homologues obtained by synthesis in our laboratory.[13] The regeneration of the protected functions of **8** to afford compound **3a** required first a hydrogenolysis, assisted by Pd on carbon, to afford the corresponding triamino ester **9**, and then, a treatment with aqueous CF_3COOH. Homodeoxypyridinoline **3a** was isolated as a mono-trifluoroacetate salt, which was crystallized from ethanol and was completely characterized. This showed the correct molecular formula and mass spectrum in addition to other physico-chemical properties similar to those detected for its natural homologue **1**.[13] In particular, it showed superimposable UV molar extinction coefficients (see Section 3) and fluorescence characteristics in dilute solution, practically identical to those described for the natural[8,24–26] and the synthetic pyridinolines.[13] With the unnatural deoxypyridinoline **3a** in hand, we searched for chromatographic conditions (HPLC) suitable for separating it from the natural dPyd **1** and Pyd **2**. Unfortunately, after various experimentations, in which we tested different columns and eluents, we realized that the homodeoxypyridinoline **3a** was separable from pyridinoline **2**, but not from deoxypyridinoline **1** (Fig. 2).

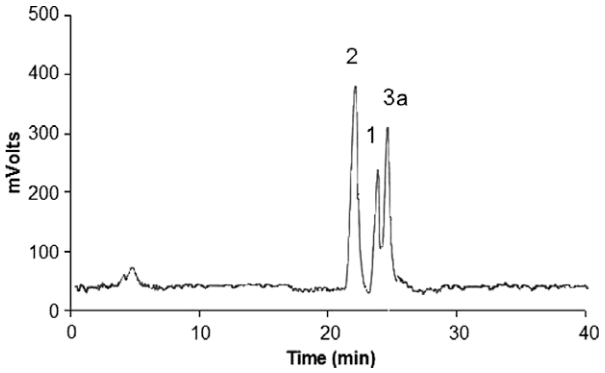
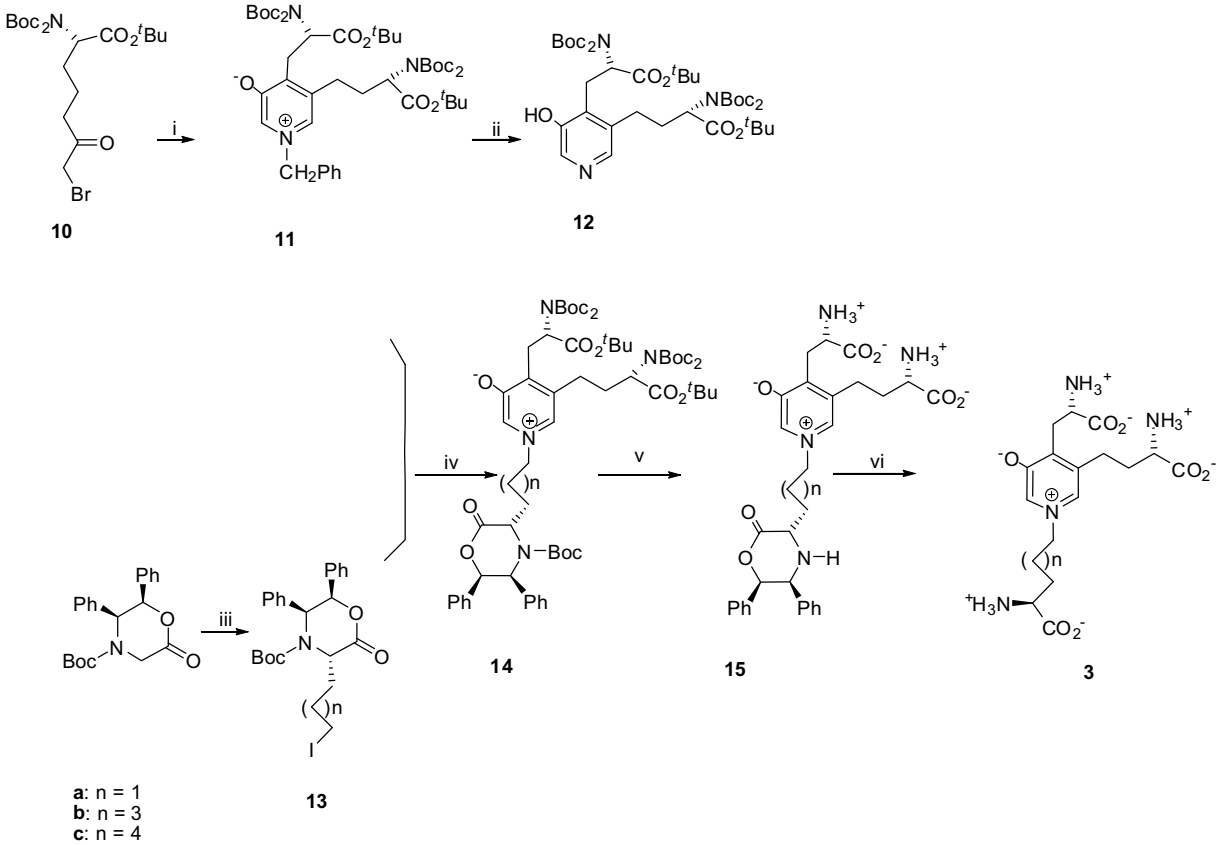


Figure 2. HPLC profile of natural and unnatural pyridinoline **3a**. The numerical numbers denote the number of the pyridinolines in the text. Chromatographic conditions are reported in Section 3.

Thus, we proceeded with the synthesis of homologues **3b** and **3c**, for which we decided to use our modular procedure[21] (Scheme 1, B), which appeared to be more convenient since no suitable commercial diamino acid was available for the construction of the unnatural side chain on the aromatic nitrogen. Furthermore, for the preparation of both deoxypyridinoline homologues **3b** and **3c**, (Scheme 3) we prepared a common 4,5-disubstituted pyridinium compound **12**, starting with bromoketone **10**, obtained from L-glutamic acid,[20] and the two aminoacidic iodo derivatives **13b** and **13c**, necessary for building two longer side chains on the aromatic nitrogen, using the Williams morpholinone chemistry.[27,28] We first prepared pyridinium salt **12** and iodo derivatives



Scheme 3. Modular synthesis of the pyridinoline analogues **3b** and **3c**. Reagents and conditions: (i) $PhCH_2NH_2$, Na_2CO_3, MeCN, rt, 12 h, then O_2, Na_2CO_3, MeOH, rt, 72 h, 71%; (ii) H_2, Pd/C, MeOH, 95%; (iii) 1,5-diiodopentane (or 1,6 diiodohexane), THF, HMPA, LiBTMSA, −78 °C, 45 min, 69%; (iv) CH_3CN, reflux, 6 h, 80–81%; (v) TFA/H_2O; 95:5; v/v, rt, 2 h, 84–85%; (vi) H_2, $PdCl_2$, MeOH/H_2O/AcOH; 10:2:1; v/v/v, 75%.

13b and **13c**, after which we alkylated pyridine **12** with iodo derivative **13b** in acetonitrile. The reaction was completed in 4 h and afforded the protected homopyridinoline **14b** in nearly quantitative yield (81%) (Scheme 3). This by a simple treatment with trifluoroacetic acid afforded amino acid **15b** in good yields (85%), by simultaneous regeneration of all aminoacidic functions, apart from those masked as an oxazinone ring. Moreover, compound **15b** suffers the loss of the Boc protection at the oxazinone ring and becomes ready for the final deblocking, which was performed by simple catalytic hydrogenolysis to afford the homodeoxypyridinoline **3b**.

The homopyridinoline **3b** shows physico-chemical properties and in particular UV and fluorescence properties, very close to those of the natural and synthetic homologues. Then, we explored the HPLC behavior of the deoxypyridinoline **3b** by subjecting it to the same analytical procedures used with the shorter homologue **3a** and with natural pyridinolines. Moreover, in this case, we observed that compound **3b** was retained differently from the natural pyridinolines **1** and **2** on a reversed-phase HPLC column (Fig. 3).

Thus deoxypyridinoline **3b** appears to be a suitable candidate to act as an internal standard in the analytical protocols used to quantify pyridinolines by HPLC. We also accomplished the synthesis of the homologous deoxypyridinoline **3c** by performing the alkylation of the substituted pyridine **12** with the iodine **13c** and by following a parallel sequence of reactions (Scheme 3) including the formation of the congeners **14c** and **15c**. The final compound **3c** was isolated by chromatography on resins (Dowex 50X 8-200 H^+) in a sufficiently pure form to show a ^1H NMR spectrum in complete agreement with the assigned structure. However, on repeating the spectrum after 20–30 min, the appearance of a second product, clearly formed from **3c**, could be monitored (^1H NMR). This unexpected liability of compound **3c** (confirmed in other successive preparations) prompted us to discontinue the preparation of **3c**

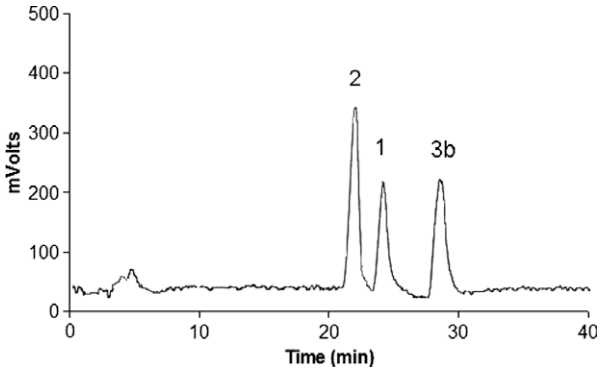
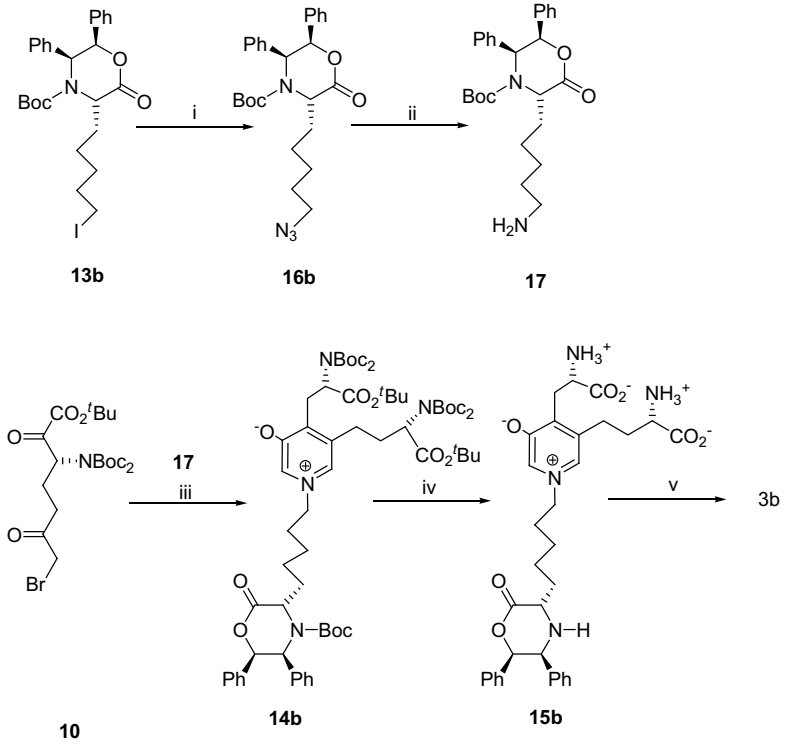


Figure 3. HPLC profile of natural and unnatural pyridinoline **3b**. The numerical numbers denote the number of pyridinolines in the text. Chromatographic conditions are reported in Section 3.

and to direct our efforts to verify a possible shortening of the synthesis of **3b**, considering that it was potentially useful as an internal standard for pyridinoline **1** and **2** analyses. We found that this was possible adopting our one-pot protocol (Scheme 4).[13] We reacted [13] amine **17** with bromoketone **10** and obtained homodeoxypyridinoline **14b** with physico-chemical properties identical to those of the above-described compound transformed by us into the free homopyridinoline **3b**.

Amine **17** was easily obtained by using Williams' oxazinones[27,28] from iodide **13b** (Scheme 4). In conclusion, the possibility to prepare homodeoxypiridinoline **3b** via this shorter and efficient way, together with the chromatographic profile herein evidenced for **3b**, allows us to consider this compound as a useful internal standard available for analytical procedures directed toward evaluating the levels of pyridinolines. Work is currently ongoing in our



Scheme 4. One-pot synthesis of protected pyridinoline analogue **3b**. Reagents and conditions: (i) NaN_3, DMF, rt, 1 h, 92%; (ii) H_2, Pd/C, AcOEt, 80%; (iii) Na_2CO_3, MeCN, rt, 10 h, then O_2, DBU, THF, rt, 120 h, 55%; (iv) TFA/H_2O; 95:5, v/v, rt. 2 h, 85%; (vi) H_2, $PdCl_2$, MeOH/H_2O/AcOH; 10:2:1, v/v/v, rt, 75%.

laboratory, in which this is demonstrated by the use of compound **3b** in a protocol for the evaluation of pyridinolines in human urine.

3. Experimental

3.1. General methods

Melting points are uncorrected. Nuclear magnetic resonance spectra were recorded at 303 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) and are referenced to residual $CHCl_3$ (δ_H = 7.28 ppm) and to $CDCl_3$ (δ_C = 77.0 ppm) for solutions in $CDCl_3$ or to internal CH_3OD (δ_H = 3.30 ppm and δ_C = 49.0 ppm) for solutions in D_2O. ^{1}H NMR data are tabulated in the following order: number of protons, multiplicity (s, singlet; d, doublet; br s, broad singlet; m, multiplet), coupling constant(s) in Hertz, assignment of signal(s). The ^{1}H and ^{13}C resonances were assigned by ^{1}H decoupling, ^{1}H–^{1}H COSY, and ^{1}H–^{13}C correlation experiments. ^{1}H NMR and ^{13}C NMR spectra of compounds containing the bis(*tert*-butoxycarbonyl)amino portion were complicated by the presence of distinguishable rotamers in a ratio close to 1:1, and the spectra of compounds containing an oxazinone ring showed the presence of a minor rotamer (30%). Where possible, the signals of both rotamers are described, otherwise, the signals of major rotamer are reported. The nomenclature of the single positions are given as in Figure 4.

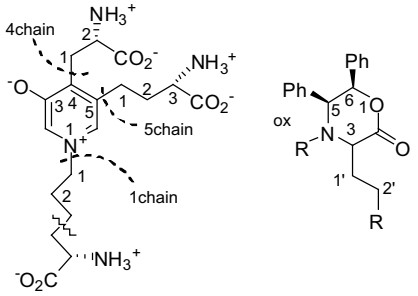


Figure 4. Carbon numeration used in this work.

Optical rotations were taken on a Perkin–Elmer 241 polarimeter, and $[\alpha]_D$ values are given in 10^{-1} deg cm^2 g^{-1}. Mass spectra were obtained using a Finnigan LCQdeca (ThermoQuest) ion trap mass spectrometer fitted with an electrospray source (ESI). The spectra were collected in continuous flow mode by connecting the infusion pump directly to the ESI source. Solutions of compounds were infused at a flow rate of 10 μL/min. The spray voltage was set at 5.0 kV in the positive ion mode and at 4.5 kV in the negative ion mode with a capillary temperature of 220 °C. Full-scan mass spectra were recorded by scanning a *m*/*z* range of 100–2000. HPLC analyses were carried out on a RP-18 column (LiChroCART, 125 mm, 4 mm ID, 5 μm purchased from Merck); elution was performed with a 20 min linear gradient from 100% of solvent A [0.01 M heptafluorobutanoic acid (HFBA) in CH_3CN/water 10:90 v/v] to 100% solvent B (0.01 M HFBA in CH_3CN/water 90:10 v/v); the flow rate was 1.0 mL/min and the detection was performed at 293 nm. All reactions were monitored by thin-layer chromatography (TLC) carried out on 0.25 mm E. Merck silica gel plates (60 F_{254}) using UV light, 50% sulfuric acid, anisaldehyde/H_2SO_4/EtOH solution or 0.2% ninhydrin in ethanol, and heat as developing agent. E. Merck 230–400 mesh silica gel was used for flash column chromatography. Work-up refers to washing with water, drying over Na_2SO_4, and evaporation of the solvent under reduced pressure.

3.2. Preparation of *tert*-butyl (*S*)-5-amino-2-(benzyloxycarbonylamino)pentanoate 7

3.2.1. Preparation of *tert*-butyl (*S*)-2-(benzyloxycarbonylamino)-5-iodopentanoate

tert-Butyl (*S*)-2-(benzyloxycarbonylamino)-5-hydroxypentanoate[29] (337 mg; 1.04 mmol) was dissolved in dry THF (3 mL) and treated in sequence with Ph_3P (382 mg; 1.46 mmol), imidazole[30] (113 mg; 1.66 mmol), and I_2 (266 mg; 1.05 mmol). The mixture was stirred for 2 h at room temperature, and then was diluted with AcOEt and worked up. The crude product was purified by rapid chromatography on silica (eluting with CH_2Cl_2) to afford the pure *tert*-butyl (*S*)-2-(benzyloxycarbonylamino)-5-iodopentanoate (391 mg; *Y* = 87%) as an oil: $[\alpha]_D^{20} = +10.2$ (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.39–7.33 (5H, aromatic protons), 5.36 (1H, d, *J* = 7.4 Hz, N*H*), 5.13 (2H, s, O*CH*$_2$Ph), 4.28 (1H, m, H-2), 3.21 (2H, m, H-5), 1.95 (2H, m), 1.81 (2H, m), 1.50 [9H, s, C(*CH*$_3$)$_3$]; ^{13}C NMR ($CDCl_3$): δ 171.0 (*COO*), 155.8 (*NCOO*), 136.2, 128.5, 128.1, and 128.0 (aromatic carbons), 82.4 [(CH$_3$)$_3$*C*], 66.9 (Ph*CH*$_2$O), 53.4 (C-2), 33.7, 29.0, 27.9 [C(*CH*$_3$)$_3$], 5.5 (C-5); ESI-MS (positive) *m*/*z*: 456 [M+Na]$^+$. Anal. Calcd for $C_{17}H_{24}INO_4$: C, 47.12; H, 5.58; N, 3.23. Found: C, 47.30; H, 5.25; N, 3.10.

3.2.2. Preparation of *tert*-butyl (*S*)-5-azido-2-(benzyloxycarbonylamino)pentanoate

To a solution of tert-butyl (*S*)-2-(benzyloxycarbonylamino)-5-iodopentanoate (100 mg; 0.23 mmol) in DMF (2 mL), NaN_3 (88 mg; 1.35 mmol) was added and the mixture was stirred at room temperature for 1 h. Then, the reaction mixture was diluted with AcOEt and worked up to afford the *tert*-butyl (*S*)-5-azido-2-(benzyloxycarbonylamino)pentanoate (77 mg; *Y* = 96%): $[\alpha]_D^{20} = +9.7$ (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.39–7.32 (5H, aromatic protons), 5.41 (1H, d, *J* = 7.6 Hz, N*H*), 5.13 (2H, s, O*CH*$_2$Ph), 4.30 (1H, dd, *J* = 13.4, 7.6 Hz, H-2), 3.33 (2H, m, H-5), 1.93 (1H, m) 1.72 (2H, m), 1.59 (1H, m), 1.49 [3H, s, C(*CH*$_3$)$_3$]; ^{13}C NMR ($CDCl_3$): δ 171.1 (*COO*), 155.8 (*NCOO*), 136.2, 128.5, 128.1, and 128.0 (aromatic carbons), 82.4 [(CH$_3$)$_3$*C*], 66.9 (Ph*CH*$_2$O), 53.8 (C-2), 50.9 (C-5), 30.1, 24.6, 27.9 [C(*CH*$_3$)$_3$]; ESI-MS (positive) *m*/*z*: 371.3 [M+Na]$^+$. Anal. Calcd for $C_{17}H_{24}N_4O_4$: C, 58.61; H, 6.94; N, 16.06. Found: C, 58.40; H, 7.10;N, 16.10.

3.2.3. Preparation of *tert*-butyl (*S*)-5-amino-2-(benzyloxycarbonylamino)pentanoate 7

To a solution of *tert*-butyl (*S*)-5-azido-2-(benzyloxycarbonylamino)pentanoate (228 mg; 0.65 mmol) in THF (10 mL), Ph_3P[31] (223 mg; 0.85 mmol) was added and the mixture was stirred overnight at room temperature. Then, the solvent was evaporated under reduced pressure to afford a crude product, which was purified by rapid chromatography on silica (eluting with CH_2Cl_2 to remove impurities and then with CH_2Cl_2/MeOH/concd NH_3; 100:3:0.3; v/v/v) to give amine **7** (153 mg; *Y* = 73%) as a glass: $[\alpha]_D^{20} = +8.2$ (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.38–7.20 (5H, aromatic protons), 5.43 (1H, d, *J* = 7.0 Hz, NH), 5.12 (2H, s, O*CH*$_2$Ph), 4.26 (1H, m, H-2), 2.75 (2H, m, H-5), 1.86 (1H, m), 1.73 (1H, m), 1.53 (2H, m), 1.48 [9H, s, C(*CH*$_3$)$_3$]; ^{13}C NMR ($CDCl_3$): δ 171.1 (*COO*), 155.8 (*NCOO*), 136.4, 128.5, 128.1, and 128.0 (aromatic carbons), 82.0 ((CH$_3$)$_3$*C*), 66.8 (Ph*CH*$_2$O), 54.0 (C-2), 41.2 (C-5), 30.2, 28.6, 28.0 [C(*CH*$_3$)$_3$]; ESI-MS (positive) *m*/*z*: 323.1 [M+H]$^+$, 345.1 [M+Na]$^+$. Anal. Calcd for $C_{17}H_{26}N_2O_4$: C, 63.33; H, 8.13; N, 8.69. Found: C, 63.40; H, 8.20; N, 8.50.

3.3. Synthesis of homodeoxypyridinoline 3a

3.3.1. One-pot synthesis of the protected homopyridinoline 8

To a solution containing amine **7** (387 mg; 1.20 mmol) and bromoketone[18] **6** (1.00 g; 2.41 mmol) in CH_3CN (35 mL), anhydrous

K_2CO_3 was added (500 mg; 3.62 mmol) and the mixture was stirred at room temperature under nitrogen for 18 h. After the disappearance of the starting amine, the solvent was evaporated under reduced pressure and the crude residue was recovered with MeOH (30 mL). Additional K_2CO_3 (250 mg; 1.80 mmol) was then added and the mixture was shaken under a slight pressure of oxygen (1.3 atm) at room temperature for 72 h. Then, the mixture was diluted with dichloromethane (50 mL) and filtered on a pad of Celite. After evaporation of the solvent, a crude residue was obtained, which was chromatographed on silica gel to afford, eluting with CH_2Cl_2/MeOH (100:7; v/v), the desired protected homodeoxypyridinoline **8** (640 mg; Y = 55%) as a glass $[\alpha]_D^{20} = +4.5$ (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 8.27 (1H, br s, pyridinium proton), 7.38–7.18 (15H, aromatic protons), 7.08 (1H, br s, pyridinium proton), 5.9–5.7 (3H, overlapping, N*H*), 5.06 (6H, m, O*CH*$_2$Ph), 4.37 (1H, m, H_{5Ch}-3), 4.33 (1H, m, H_{4Ch}-2), 4.25 (1H, m, H_{1Ch}-4), 4.05 and 3.92 (2 × 1H, 2 × m, H_{1Ch}-1), 3.34 (1H, dd, *J* = 11.2, 12.7 Hz, H_{4Ch}-1a), 2.87 (1H, dd, *J* = 12.7, 3.0 Hz, H_{4Ch}-1b), 2.66 (2H, m, H_{5Ch}-1), 2.07 (1H, m, H_{5Ch}-2a), 1.97 (3H, overlapping, H_{5Ch}-2b and H_{1Ch}-2), 1.85 and 1.66 (2 × 1H, 2 × m, H_{1Ch}-3), 1.47 [18H, s, 2 × C(*CH*$_3$)$_3$], 1.31 9H, s, [C(*CH*$_3$)$_3$]; ^{13}C NMR ($CDCl_3$): δ 172.2, 172.1, 170.5 (*COO*^{t}Bu), 156.6, 156.2 (N*COO*), 142.6 and 138.4 (pyridinium carbon), 130.6 (pyridinium carbon), 136.6, 136.2, 136.0, 128.6-127.5 (aromatic carbons), 82.7, 82.4 and 82.1 [3 × *C*(CH$_3$)$_3$], 67.2, 67.0, and 66.5 (O*CH*$_2$Ph), 59.8 (C_{1Ch}-1), 56.0 (C_{4Ch}-2), 53.4 (C_{5Ch}-3), 53.2 (C_{1Ch}-4), 33.1 (C_{5Ch}-2), 29.6 (C_{1Ch}-3), 27.9 [C(*CH*$_3$)$_3$], 27.9 (C_{4Ch}-1), 26.8 (C_{1Ch}-2), 26.1 (C_{5Ch}-1); ESI-MS (positive) *m*/*z*: 918.5 [M−Bu^{t}OH+Na]$^+$. Anal. Calcd for $C_{53}H_{68}N_4O_{13}$: C, 65.62; H, 7.17; N, 5.78. Found: C, 65.50; H, 7.35; N, 7.70.

3.3.2. Preparation of the homopyridinoline 3a by regeneration of the protected functionalities of 8

A solution of protected homodeoxypyridinoline 8 (300 mg; 0.31 mmol) in methanol (75 mL) was added with Pd/C (100 mg; 10%) and shaken under hydrogen for 4 h. At this time, the catalyst was filtered over a pad of Celite and the solvent was evaporated under reduced pressure to afford a crude product **9** (154 mg; 88%). The product showed the correct mass spectrum [*m*/*z* 516 (M−^{t}BuOH+Na)$^+$] and showed only the characteristic aromatic proton signals of the pyridinium ring (δ 8.09 and 6.90 ppm). Without any additional purification it was used in the following reaction. Crude compound **9** (100 mg; 0.176 mmol) was dissolved in aqueous CF_3COOH (1.8 mL; 95%; v/v) and left at 23 °C for 45 min. Then the acid was evaporated under reduced pressure to afford the pure homodeoxypyridinoline **3a** (89 mg; *Y* = 95.5%) as a monotrifluoroacetate salt, which was crystallized from ethanol as a solid monohydrate showing: $[\alpha]_D^{20} = +22.2$ (*c* 0.9, H_2O); λ_{max} (HCl 0.1 M)/nm (ε/M^{-1} cm^{-1}), 239 (3840), 293 (6490); ^{1}H NMR (D_2O): δ 8.18 and 8.11 (2 × H, 2 × s, H_{pyd}), 4.72 (2H, m, H_{1Ch}-1), 4.4.39 (1H, m, H_{4Ch}-2), 4.18 (1H, m, H_{5Ch}-3), 4.11 (1H, m, H_{1Ch}-4), 3.62 (2H, m, H_{4Ch}-1), 3.30 and 3.11 (2 × 1H, 2m, H_{5Ch}-1), 2.1–1.9 (4H, overlapping, H_{5Ch}-2 and H_{1Ch}-2), 2.23 (2H, m, H_{1Ch}-3); ^{13}C NMR (D_2O): δ 174.8, 174.4, 174.2 (3 × *COO*^{t}Bu), 165.1, 143.3, 141.0, 131.0, 130.7 (pyridinium carbons), 163.6 (q, CF_3COO), 117.5 (q, CF_3COO), 60.8 (C_{1Ch}-1), 55.3, 55.1, 55.0 (C_{4Ch}-2, C_{5Ch}-3 and C_{1Ch}-4), 31.9 (C_{1Ch}-2), 29.1, 27.9, 27.2, 26.8 (C_{5Ch}-1, C_{5Ch}-2, C_{4Ch}-1 and C_{1Ch}-3); ESI-MS (negative) *m*/*z*: 413 [M−H+MeOH]$^+$. Anal. Calcd for $C_{19}H_{29}F_3N_4O_{10}$: C, 42.94; H, 5.69; N, 10.54. Found: C, 43.20; H, 5.55; N, 10.40.

3.4. Preparation of 1-benzyl-4-[(2*S*)-2-bis(*tert*-butoxycarbonylamino)-2-*tert*-butoxycarbonyl ethyl]-5-[(3*S*)-3-bis(*tert*-butoxycarbonylamino)-3-*tert*-butoxycarbonylpropyl] pyridinium-3-olate 11

The protected bromoketone[20] **10** (275 mg; 0.55 mmol) was added to a solution of benzylamine (25 μL; 0.23 mmol) in CH_3CN (30 mL) containing Na_2CO_3 (243 mg; 2.29 mmol), and the mixture was stirred at room temperature under nitrogen for 12 h. The disappearance of the starting amine and the formation of the dialkylated benzylamine (TLC: CH_2Cl_2/MeOH; 100:7; v/v; R_f = 0.1 and 0.25, respectively) were then observed. The solvent was then evaporated under reduced pressure and the crude residue was recovered by MeOH (40 mL) and addition of Na_2CO_3 (243 mg; 2.29 mmol), and then shaken under a slight pressure of oxygen (1.3 atm) at room temperature for 72 h. At this time, the mixture was diluted with dichloromethane (50 mL) and filtered on a pad of Celite, and the solvent was evaporated under a reduced pressure to afford a crude residue, which was chromatographed on silica gel (eluting with CH_2Cl_2/MeOH; 100:7; v/v) to afford the desired compound **11** (145 mg; *Y* = 71%) as a glass: $[\alpha]_D^{20} = -34.0$ (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.57 (1H, pyridine proton), 7.39–7.19 (5H, aromatic protons), 7.00 (1H, pyridine proton), 5.59 (1H, m, 4.6, H_{4ch}-2), 5.14 (2H, s, PhC*H*$_2$), 4.64 (0.6H, dd, *J* = 9.1, 5.3 Hz, H_{5ch}-3 major), 4.60 (0.4H, dd, *J* = 9.1, 5.6 Hz, H_{5ch}-3 minor), 3.55 (0.4H, dd, *J* = 12.9, 3.8 Hz, H_{4ch}-1a minor), 3.52 (0.6H, dd, *J* = 13.3, 4.1 Hz, H_{4ch}-1a major), 3.27 (1H, m, H_{4ch}-1b), 2.77 (1H, m, H_{5ch}-1a), 2.57 (0.6H, m, H_{5ch}-1b major), 2.49 (0.4H, m, H_{5ch}-1b minor), 2.36 (0.6H, m, H_{5ch}-2a major), 2.19 (0.4H, m, H_{5ch}-2a minor), 2.05 (0.4H, m, H_{5ch}-2b minor), 1.97 (0.6H, m, H_{5ch}-2b major), 1.53, 1.50, 1.49, 1.46, 1.45, 1.44, 1.39, 1.36 [54H, 8 × s, C(CH$_3$)$_3$]; ^{13}C NMR ($CDCl_3$): δ 169.4, 169.3 (*COO*), 152.7, 152.5, 152.1, 152.0 (N*COO*), 144.6, 128.2 (aromatic carbons), 83.0–81.1 [*C*(CH$_3$)$_3$], 63.8 (PhCH$_2$), 57.9 and 57.7 (C_{5ch}-3), 56.6 and 56.5 (C_{4ch}-2), 29.8 and 29.7 (C_{5ch}-2), 28.0, 27.9, 27.8, 27.7 [C(*CH*$_3$)$_3$], 27.5 (C_{4ch}-1), 27.2 and 26.8 (C_{5ch}-1); IR ($CHCl_3$, ν_{max}, cm^{-1}): 2980, 2940, 1730, 1690, 1385, 1370, 1150; ESI-MS (positive) *m*/*z*: 886.4 [M+H]$^+$, 908.4 [M+Na]$^+$, 1171.6 [2M+H]$^+$, 1794.2 [2M+Na]$^+$. Anal. Calcd for $C_{47}H_{71}N_3O_{13}$: C, 63.71; H, 8.08; N, 4.74. Found: C, 63.60; H, 7.90; N, 4.70.

3.5. Preparation of 4-[(2*S*)-2-bis(*tert*-butoxycarbonylamino)-2-*tert*-butoxycarbonylethyl]-5-[(3*S*)-3-bis(*tert*-butoxycarbonylamino)-3-*tert*-butoxycarbonylpropyl]-3-hydroxypyridine 12

The pyridinium derivative **11** (152 mg; 0.17 mmol) was dissolved in methanol (25 mL) and hydrogenated in the presence of Pd/C (35 mg; 10%) for 1 h. After filtration on a pad of Celite, the solvent was evaporated under reduced pressure to afford a residue, which after column chromatography (eluting with CH_2Cl_2/MeOH; 100:5; v/v) afforded the 3-hydroxypyridine **12** in quantitative yield (129 mg; Y = 95%). Compound **12**, a glass, showed: $[\alpha]_D^{20} = -11.3$ (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 8.12 (1H, pyridine proton), 7.96 (1H, pyridine proton), 5.15 (0.3H, dd, *J* = 6.8, 6.8 Hz, H_{4ch}-2 minor), 5.11 (0.7H, dd, *J* = 6.8, 6.8 Hz, H_{4ch}-2 major), 4.74 (0.3H, dd, *J* = 9.4, 5.5 Hz, H_{5ch}-3 minor), 4.72 (0.7H, dd, *J* = 8.1, 6.2 Hz, H_{5ch}-3 major), 3.50 (1H, m, H_{4ch}-1a), 3.25 (1H, m, H_{4ch}-1b), 2.82 (0.7H, ddd, *J* = 13.6, 9.9, 6.7 Hz, H_{5ch}-1a major), 2.71 (0.6H, m, H_{5ch}-1 minor), 2.64 (0.7H, ddd, *J* = 13.9, 9.9, 5.7 Hz, H_{5ch}-1b major), 2.44 (0.7H, m, H_{5ch}-2a major), 2.36 (0.3H, m, H_{5ch}-2a minor), 2.11 (0.3H, m, H_{5ch}-2b minor), 2.01 (0.7H, m, H_{5ch}-2b major), 1.52, 1.50, 1.48, 1.45, 1.43, 1.42 [54H, 6 × s, 6 × C(CH$_3$)$_3$]; ^{13}C NMR ($CDCl_3$): δ 170.5 and 170.2 (*COO* minor), 169.5 and 169.4 (*COO* major), 152.9 and 152.8 (pyridine carbon), 152.4 and 152.3 (N*COO*), 141.5 and 141.4 (pyridine carbon), 137.2 and 137.0 (pyridine carbon), 132.6 and 132.2 (pyridine carbon), 83.1–81.2 [*C*(CH$_3$)$_3$], 58.5 and 58.3 (C_{4ch}-2 and C_{5ch}-3), 30.6 (C_{5ch}-2), 28.0-27.8 [C(*CH*$_3$)$_3$], 26.9 and 26.7 (C_{5ch}-1), 26.6 (C_{4ch}-1); IR ($CHCl_3$, ν_{max}, cm^{-1}): 2980, 2930, 1730, 1690, 1380, 1370, 1140; ESI-MS (positive) *m*/*z*: 796.3 [M+H]$^+$, 818.4 [M+Na]$^+$. Anal. Calcd for $C_{40}H_{65}N_3O_{13}$: C, 60.36; H, 8.23; N, 5.28. Found: C, 60.30; H, 8.10; N, 5.40.

3.6. Preparation of *tert*-butyl (3*S*,5*S*,6*R*)-3-(5-iodopentyl)- and of *tert*-butyl (3*S*,5*S*,6*R*)-3-(5-iodohexyl)-2-oxo-5,6-diphenylmorpholine-4-carboxylate 13b and 13c

To a stirred solution of (2*R*,3*S*)-*tert*-butyl 6-oxo-2,3-diphenylmorpholine-4-carboxylate (2.0 g; 5.66 mmol) and 1,5-diiodopentane (4.21 mL; 28.29 mmol) in THF (180 mL) containing HMPA (18 mL), lithium bis(trimethylsily1)amide (8.4 mL; 8.4 mmol; 1 M solution in THF) was added dropwise at −78 °C. After 45 min, the dry ice bath was removed and the reaction mixture was warmed up to room temperature under stirring for 30 min. Then, the reaction mixture was poured into ice cold water, extracted with ethyl acetate, and worked up. The crude residue obtained was purified by rapid chromatography on silica (eluting with hexanes/AcOEt; 9:1; v/v) to afford iodide **13b** (2.14 g; *Y* = 69%) as a white solid: mp 123–125 °C (from hexane); $[\alpha]_D^{20} = -45.4$ (*c* 1, $CHCl_3$). ^1H NMR (500 MHz): δ 7.27–7.01 (8H, aromatic protons), 6.59 (2H, m, aromatic protons), 5.96 (1H, br s, H_{ox}-6), 5.25 (0.3H, H_{ox}-5 minor), 5.03 (1.4H, overlapping, H_{ox}-5 major and H_{ox}-3 major), 4.84 (0.3H, dd, *J* = 6.9 Hz, H-3 minor), 3.23 (2H, t, *J* = 6.9 Hz, H-5′), 2.18 and 1.99 (2 × 1H, 2 × m, H-1′), 1.88 (2H, m, H-4′), 1.64 (2H, m, H-2′), 1.50 (2H, m, H-3′), 1.48 [3H, C(CH_3)$_3$ minor], 1.12 [6H, C(CH_3)$_3$ major]; ^{13}C NMR ($CDCl_3$): δ 169.2 (*C*OO), 153.6 and 152.9 (N*C*OO), 136–126 (aromatic carbons), 81.6 and 81.1 [*C*(CH_3)$_3$], 79.4 and 78.9 (C_{ox}-6), 61.6 and 60.4 (C_{ox}-5), 57.7 and 56.5 (C_{ox}-3), 35.6 and 35.0 (C-1′), 33.3 and 33.2 (C-4′), 28.4 and 28.1 (C-3′), 28.0 and 27.8 [C(CH_3)$_3$], 25.9 and 25.7 (C-2′), 7.0 and 6.7 (C-5′); IR ($CHCl_3$, ν_{max}, cm^{-1}): 2910, 1745, 1680, 1380, 1350, 1155, 1125, 900; ESI-MS (positive) *m*/*z*: 572.1 $[M+Na]^+$. Anal. Calcd for $C_{26}H_{32}INO_4$: C, 56.84; H, 5.87; N, 2.55. Found: C, 56.66; H, 5.60; N, 2.70.

By a similar procedure, starting with *tert*-butyl(2*R*,3*S*)-6-oxo-2,3-diphenylmorpholine-4-carboxylate (2.0 g; 5.66 mmol) and 1,5-diiodohexane (4.66 mL; 28.29 mmol) in THF (180 mL), iodide **13c** was obtained (2.2 g; *Y* = 69%): a solid: mp 140–141 °C (from hexane); $[\alpha]_D^{20} = -46.7$ (*c* 1, $CHCl_3$) ^1H NMR ($CDCl_3$): δ 7.29–7.05 (8H, aromatic protons), 6.58 (2H, aromatic protons), 5.96 (1H, br s, H_{ox}-6), 5.24 (0.3H, H_{ox}-5 minor), 5.02 (1.4H, overlapping, H_{ox}-5 major and H_{ox}-3 major), 4.83 (0.3H, dd, *J* = 10.2, 4.8 Hz, H_{ox}-3 minor), 3.20 (2H, m, H-6′), 2.20 and 1.99 (2 × 1H, 2 × m, H-1′), 1.91 (2H, m, H-5′), 1.66 (2H, m, H-2′), 1.56–1.48 [5H, m, overlapping, H-3′,H-4′ and C(CH_3)$_3$ minor], 1.12 [6H, C(CH_3)$_3$ major]; ESI-MS (positive) *m*/*z*: 586 $[M+Na]^+$, 1149 $[2M+Na]^+$. Anal. Calcd for $C_{27}H_{34}INO_4$: C, 57.55; H, 6.08; N, 2.49. Found: C, 576.60; H, 6.20; N, 2.60.

3.7. Preparation of the completely protected homodeoxypyridinoline 14b or 14c

Hydroxypyridine **12** (132 mg; 0.17 mmol) and iodide **13b** (274 mg; 0.50 mmol) were dissolved in CH_3CN (3 mL) and refluxed under an argon atmosphere for 6 h. The mixture was cooled at room temperature and the solvent was evaporated under reduced pressure. The crude product was purified by rapid chromatography on silica (AcOEt/MeOH; 100:8; v/v) to give the protected deoxypyridinoline **14b** (168 mg; *Y* = 81%) as a glass: $[\alpha]_D^{20} = -26.7$ (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 8.21 (1H, s, pyridinium proton), 7.77 (1H, s, pyridinium proton), 7.3–7.0 (8H, aromatic protons), 6.55 (2H, aromatic protons), 6.03 (0.8H, br s, H_{ox}-6 major), 5.99 (0.2H, br s, H_{ox}-6 minor), 5.34 and 5.28 (2 × 0.5, 2 × m, H_{4ch}-2), 5.19 (0.2H, d, *J* = 3.0 Hz, H_{ox}-5 minor), 5.03 (0.8H, d, *J* = 3.0 Hz, H_{ox}-5 major), 4.94 (0.8H, dd, *J* = 9.9, 5.5 Hz, H_{ox}-3 major), 4.77 (0.2H, dd, *J* = 10.6, 4.0 Hz, H_{ox}-3 minor), 4.62 (1H, m, H_{5ch}-3), 4.38 (2H, m, H_{1ch}-1), 3.48 (2H, m, H_{4ch}-1), 2.88–2.74 (2H, m, H_{5ch}-1), 2.39 and 2.32 (2 × 0.5H, 2 × m, H_{5ch}-2a), 2.16 (1H, m, H_{1ch}-5a), 2.10 (1H, m, H_{5ch}-2b), 2.02 (3H, overlapping, H_{1ch}-5b and H_{1ch}-2), 1.63 (3H, overlapping, H_{1ch}-3 and H_{1ch}-4a), 1.50–1.38 [64H, overlapping, C(CH_3)$_3$ and H_{1ch}-4b]; ^{13}C NMR ($CDCl_3$): δ 169.4, 169.2, 168.6, 168.5 (*C*OO), 153.7, 152.7, 152.3, 152.2 (N*C*OO), 144.3, 141.3 (pyridinium carbons), 136.7–127.6 (aromatic carbons), 83.4, 83.3, 83.2, 82.0, 81.9, 81.8, 81.1 [*C*(CH_3)$_3$], 79.4 and 78.9 (C_{ox}-6), 61.4 and 60.4 (C_{ox}-5), 61.1 and 60.9 (C_{1ch}-1), 57.8 (C_{5ch}-3), 57.6 and 56.2 (C_{ox}-3), 57.3 and 57.0 (C_{4ch}-2), 34.7 (C_{1ch}-5), 31.2 (C_{1ch}-2), 29.8 and 29.7 (C_{5ch}-2), 28.4–27.8 [C(CH_3)$_3$], 27.3–27.1 (C_{4ch}-1 and C_{5ch}-1), 27.1, 27.0, 25.8, 25.7 (C_{1ch}-3), 25.4 (C_{1ch}-4); IR ($CHCl_3$, ν_{max}, cm^{-1}): 2970, 2940, 1740, 1690, 1380, 1370, 1150; ESI-MS (positive) *m*/*z*: 1217.6 $[M+H]^+$, 1239.6 $[M+Na]^+$. Anal. Calcd for $C_{66}H_{96}N_4O_{17}$: C, 65.11; H, 7.95; N, 4.60. Found: C, 65.00; H, 7.80; N, 4.70.

By a similar procedure, starting with hydroxypyridine **12** (132 mg; 0.17 mmol) and iodide **13c** (185 mg; 0.33 mmol) dissolved in CH_3CN (3 mL) and refluxed under an argon atmosphere for 6 h, the protected deoxypyridinoline **14c** (164 mg; Y = 80%) was obtained as a glass: $[\alpha]_D^{22} = -30.6$ (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 8.23 (1H, s, pyridinium proton), 7.75 (1H, s, pyridinium proton), 7.3–7.0 (8H, aromatic protons), 6.55 (2H, aromatic protons), 6.00 (0.8H, br s, H_{ox}-6 major), 5.98 (0.2H, bs, H_{ox}-6 minor), 5.39 and 5.31 (2 x 0.5, 2 x m, H_{4ch}-2), 5.19 (0.2H, d, *J* = 3.0 Hz, H_{ox}-5 minor), 5.03 (0.8H, d, *J* = 3.0 Hz, H_{ox}-5 major), 4.94 (0.8H, dd, *J* = 9.9, 5.5 Hz, H_{ox}-3 major), 4.77 (0.2H, dd, *J* = 10.6, 4.0 Hz, H_{ox}-3 minor), 4.63 (1H, m, H_{5ch}-3), 4.38 (2H, m, H_{1ch}-1), 3.47 (2H, m, H_{4ch}-1), 2.88–2.74 (2H, m, H_{5ch}-1), 2.39 and 2.32 (2 × 0.5H, 2 × m, H_{5ch}-2a), 2.16–1.97 (5H, overlapping H_{5ch}-2b, H_{1ch}-6, H_{1ch}-2), 1.70–1.38 [69H, overlapping, H_{1ch}-3, H_{1ch}-5, H_{1ch}-4, C(CH_3)$_3$]; ^{13}C NMR ($CDCl_3$): δ 169.4, 169.2, 168.6, 168.5 (*C*OO^{t}Bu), 153.7, 152.7, 152.3, 152.2 (N*C*OO), 144.3, 141.3 (pyridinium carbons), 136.7–127.6 (aromatic carbons), 83.4, 83.3, 83.2, 82.0, 81.9, 81.8, 81.1 [*C*(CH_3)$_3$], 79.4 and 78.9 (C_{ox}-6), 61.4 and 60.4 (C_{ox}-5), 60.8 and 60.3 (C_{1ch}-1), 57.8 (C_{5ch}-3), 57.6 and 56.2 (C_{ox}-3), 57.3 and 57.0 (C_{4ch}-2), 34.7 (C_{1ch}-5), 31.4 (C_{1ch}-2), 29.8 and 29.6 (C_{5ch}-2), 28.4–27.8 [C(CH_3)$_3$], 27.3–27.1 (C_{4ch}-1 and C_{5ch}-1), 27.0, 25.7, 25.6, 25.4, 25.2; ESI-MS (positive) *m*/*z*: 1231.6 $[M+H]^+$, 1254.6 $[M+Na]^+$. Anal. Calcd for $C_{67}H_{98}N_4O_{17}$: C, 65.34; H, 8.02; N, 4.55. Found: C, 65.50; H, 8.20; N, 4.65.

3.8. Preparation of homodeoxypyridinolines 3b and 3c

3.8.1. Preparation of the partially protected pyridinolines 15b and 15c, by hydrolysis of BOC groups and of *tert*-butyl esters

The completely protected homodeoxypyridinoline **14b** (162 mg; 0.13 mmol) was dissolved in CF_3COOH/H_2O (2 mL; 95:5; v/v), and the resulting solution was stirred at room temperature for 2 h. Then, the solvent was evaporated under reduced pressure and the residue was triturated with diisopropyl ether to afford the partially protected homodeoxypyridinoline **15b** as its *tetra*-trifluoroacetate salt (120 mg; *Y* = 85%) as a powder: $[\alpha]_D^{20} = -10.6$ (*c* 1, CD_3OD); ^1H NMR (CD_3OD): δ 8.38 (1H, s, H_{Pyd}-6), 8.29 (1H, s, H_{Pyd}-2), 7.2–7.0 (10H, aromatic protons), 5.50 (1H, d, *J* = 3.7 Hz, H_{ox}-6), 4.25 (1H, d, *J* = 3.7 Hz, H_{ox}-5), 4.45 (2H, t, *J* = 7.5 Hz, H_{1ch}-1), 4.37 (1H, ddd, *J* = 7.7, 7.5, 3.4 Hz, H_{4ch}-2), 4.10 (1H, dd, *J* = 15.7, 6.3 Hz, H_{5ch}-3), 3.74 (1H, dd, *J* = 8.2, 5.2 Hz, H_{ox}-3), 3.49 (2H, m, H_{4ch}-1), 3.12 and 3.02 (2 × 1H, 2 × m, H_{5ch}-1), 2.29 and 2.23 (2 × 1H, 2 × m, H_{5ch}-2), 1.97 (4H, overlapping, H_{1ch}-5 and H_{1ch}-2), 1.53 (1H, m, H_{1ch}-4a), 1.37 (3H, overlapping, H_{1ch}-4b and H_{1ch}-3); ^{13}C NMR (CD_3OD): δ 172.0, 171.9, 171.4 (*C*OO), 172.0, 171.9, 171.4 (*C*OO), 163.5 (q, CF_3COO), 157.4 (C_{pyd}-3), 142.6 (C_{pyd}-5), 141.9 (C_{pyd}-4), 136.8 (C_{pyd}-6), 129.4 (C_{pyd}-2), 140.6, 131.8, 131.1, 130.5, 129.4, 129.1, 127.2 (aromatic carbons), 117.0 (q, *C*F_3COO), 72.7 (C_{ox}-6), 67.3 (C_{ox}-5), 62.7 (C_{1ch}-1), 59.9 (C_{ox}-3), 53.5 (C_{5ch}-3), 52.4 (C_{4ch}-2), 31.7 (C_{1ch}-2), 31.5 and 31.4 (C_{5ch}-2), 29.7 (C_{1ch}-5), 28.7 and 28.6 (C_{4ch}-1), 27.1 and 26.9 (C_{5ch}-1), 26.4 (C_{1ch}-3), 25.6 (C_{1ch}-4); ESI-MS (positive) *m*/*z*: 623.5

$[M+H_2O+H]^+$, 645.4 $[M+H_2O+Na]^+$. Anal. Calcd for $C_{41}H_{44}F_{12}N_4O_{15}$: C, 46.38; H, 4.27; N, 5.28. Found: C, 46.30; H, 4.40; N, 5.50.

By a similar procedure, starting from the completely protected homodeoxypyridinoline 14c (164 mg; 0.13 mmol) dissolved in CF_3COOH/H_2O (2 mL; 95:5; v/v), the partially protected homodeoxypyridinoline **15c** as its *tetra*-trifluoroacetate salt was obtained (120 mg; $Y = 84\%$) as a glass $[\alpha]_D^{20} = -10.5$ (c 1, CH_3OH); ^1H NMR (CD_3OD): δ 8.42 (1H, s, H_{Pyd}-6), 8.31 (1H, s, H_{Pyd}-2), 7.2–7.0 (10H, aromatic protons), 5.47 (1H, d, $J = 3.7$ Hz, H_{ox}-6), 4.77 (3H, overlapping, H_{ox}-5 and H_{1ch}-1), 4.36 (1H, ddd, $J = 7.7$, 7.5, 3.4 Hz, H_{4ch}-2), 4.10 (1H, dd, $J = 15.7$, 6.3 Hz, H_{5ch}-3), 3.77 (1H, dd, $J = 8.2$, 5.2 Hz, H_{ox}-3), 3.52–3.44 (2H, m, H_{4ch}-1), 3.12 and 3.05 (2 × 1H, 2m, H_{5ch}-1), 2.31 and 2.23 (2 × 1H, 2m, H_{5ch}-2), 1.96 (4H, overlapping, H_{1ch}-6 and H_{1ch}-2), 1.50 (1H, m, H_{1ch}-5a), 1.36–1.28 (5H, overlapping, H_{1ch}-5b, H_{1ch}-3 H_{1ch}-4); ^{13}C NMR (CD_3OD): δ 172.0, 171.9, 171.4 (*C*OO), 163.6 (q, CF_3COO), 157.7 (C_{pyd}-3), 142.5 (C_{pyd}-5), 142.1 (C_{pyd}-4), 136.8 (C_{pyd}-6), 129.4 (C_{pyd}-2), 140.6, 131.8, 131.1, 130.5, 129.4, 129.1, 127.2 (aromatic carbons), 117.0 (q, *C*F_3COO), 72.9 (C_{ox}-6), 67.3 (C_{ox}-5), 62.7 (C_{1ch}-1), 59.9 (C_{ox}-3), 53.4 (C_{5ch}-3), 52.5 (C_{4ch}-2), 32.1 (C_{1ch}-2), 31.5 and 31.4 (C_{5ch}-2), 29.8 (C_{1ch}-6), 29.2 (C_{1ch}-3) 28.7 and 28.6 (C_{4ch}-1), 27.1 and 26.9 (C_{5ch}-1), 26.7 (C_{1ch}-4), 26.0 (C_{1ch}-5); ESI-MS (positive) m/z: 637.5 $[M+H_2O+H]^+$, 659.4 $[M+H_2O+Na]^+$, 681.4 $[M+H_2O+2Na\text{-}H]^+$ 703.4 $[M+H_2O+3Na-2H]^+$, 725.4 $[M+H_2O+4Na-3H]^+$. Anal. Calcd for $C_{42}H_{47}F_{12}N_4O_{15}$: C, 46.89; H, 4.40; N, 5.21. Found: C, 46.80; H, 4.30; N, 5.40.

3.8.2. Hydrogenolysis of the oxazinone ring of 15b or 15c, preparation of 3b or 3c

The partially protected homodeoxypyridinoline **15b** (249 mg; 0.23 mmol) dissolved in a mixture of $MeOH/H_2O/AcOH$ (66 mL; 10: 2:1; v/v/v) was hydrogenated in the presence of $PdCl_2$ (70 mg) for 12 h at room temperature. The catalyst was then filtered off and the solution was concentrated under reduced pressure to a residue, which was diluted with water and loaded on a strong acidic resin column (2 mL; Dowex® 50WX8-200). Then the resin was washed with water, and finally the product was eluted with NH_3 in aqueous methanol (H_2O/MeOH; 2:1; v/v). After evaporation of MeOH, the solution was freeze-dried twice to afford the homodeoxypyridinoline **3b** (74 mg; 75% yield) as a fluffy material, which showed: $[\alpha]_D^{20} = +15.2$ (c 0.5, H_2O); its UV spectrum (in MeOH) shows λ_{max} at 231, 260, and 336 nm, and this indicates that it is in the 3-pyridiniumolate form (a zwitterion); ^1H NMR (D_2O): δ 8.38 (1H, s, H_{Pyd}-6), 8.29 (1H, s, H_{Pyd}-2), 7.2–7.0 (10H, aromatic protons), 5.50 (1H, d, $J = 3.7$ Hz, H_{ox}-6), 4.25 (1H, d, $J = 3.7$ Hz, H_{ox}-5), 4.45 (2H, t, $J = 7.5$ Hz, H_{1ch}-1), 4.37 (1H, ddd, $J = 7.7$, 7.5, 3.4 Hz, H_{4ch}-2), 4.10 (1H, dd, $J = 15.7$, 6.3 Hz, H_{5ch}-3), 3.74 (1H, dd, $J = 8.2$, 5.2 Hz, H_{ox}-3), 3.49 (2H, m, H_{4ch}-1), 3.12 and 3.02 (2 × 1H, 2 × m, H_{5ch}-1), 2.29 and 2.23 (2 × 1H, 2 × m, H_{5ch}-2), 1.97 (4H, overlapping, H_{1ch}-5 and H_{1ch}-2), 1.53 (1H, m, H_{1ch}-4a), 1.37 (3H, overlapping, H_{1ch}-4b and H_{1ch}-3); ^{13}C NMR (D_2O): δ 173.2, 172.6, 172.2 (*C*OO^{t}Bu), 155.9 (C_{pyd}-3), 141.6 (C_{pyd}-5), 141.2 (C_{pyd}-4), 136.5 (C_{pyd}-6), 129.5 (C_{pyd}-2), 62.1 (C_{1ch}-1), 53.7 (C_{ch1}-6), 53.4 (C_{5ch}-3), 52.4 (C_{4ch}-2), 30.8 and 30.7 (C_{1ch}-2 and C_{5ch}-2), 30.2 (C_{1ch}-5), 28.1 (C_{4ch}-1), 26.3 (C_{5ch}-1), 25.5 (C_{1ch}-3), 24.3 (C_{1ch}-4); ESI-MS (positive) m/z: 449.4 $[M+Na]^+$. Anal. Calcd for $C_{19}H_{30}N_4O_7$: C, 53.51; H, 7.09; N, 13.14. Found: C, 53.30; H, 7.20; N, 13.20.

By a similar procedure, starting with the partially protected homodeoxypyridinoline **15c** (145 mg; 0.14 mmol) dissolved in a mixture of $MeOH/H_2O/AcOH$ (66 mL; 10: 2: 1; v/v/v) and hydrogenated in the presence of $PdCl_2$ (70 mg) for 12 h at room temperature, the homodeoxypyridinoline **3c** was obtained (47 mg; $Y = 76\%$) as a fluffy material, which showed the appropriate physico-chemical properties: ^1H NMR (D_2O): δ 8.33 (1H, s, H_{Pyd}-6), 8.25 (1H, s, H_{Pyd}-2), 4.52 (2H, m, H_{1ch}-1), 4.32 (1H,m, H_{4ch}-2), 4.12 (1H, m, H_{5ch}-3), 4.04 (1H, m, H_{1ch}-7), 3.00 (2H, m, H_{4ch}-1), 3.12 and 3.02 (2 × 1H, 2m, H_{5ch}-1), 2.40 (2 × 1H, 2 × m, H_{5ch}-2), 1.92 (4H, overlapping, H_{1ch}-6 and H_{1ch}-2), 1.76 (8H, overlapping, H_{1ch}-3 H_{1ch}-4 and H_{1ch}-5); ESI-MS (positive) m/z: 463.4 $[M+Na]^+$. After a 30 min standing in D_2O at room temperature, compound 3c was transformed into a mixture containing at least a second compound (ca. 30%), which complicated the ^{13}C NMR spectrum interpretation. TLC and HPLC inspection confirmed that some decomposition of **3c** had occurred. Similar behavior was observed in two successive preparations.

3.9. Preparation of *tert*-butyl (3*S*,5*S*,6*R*)-3-(5-aminopentyl)-2-oxo-5,6-diphenylmorpholine-4-carboxylate 17

3.9.1. Preparation of the *tert*-butyl (3*S*,5*S*,6*R*)-3-(5-azidopentyl)-2-oxo-5,6-diphenylmorpholine-4-carboxylate 16b

To a solution of *tert*-butyl (3*S*,5*S*,6*R*)-3-(5-iodopentyl)-2-oxo-5,6-diphenylmorpholine-4-carboxylate **13b** (550 mg; 1.00 mmol) in DMF (4 mL), NaN_3 (130 mg; 2.00 mmol) was added and the mixture was stirred at room temperature for 1 h. Then, the reaction mixture was poured into ice cold water (25 mL) and stirred for 20 min to form the *tert*-butyl (3*S*,5*S*,6*R*)-3-(5-azidopentyl)-2-oxo-5,6-diphenylmorpholine-4-carboxylate **16b** (427 mg; $Y = 92\%$) as a white solid showing: mp 132–133 °C; $[\alpha]_D^{20} = -61.2$ (c 1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.29–7.00 (8H, aromatic protons), 6.59 (2H, m, aromatic protons), 5.96 (1H, br s, H_{ox}-6), 5.24 (0.3H, H_{ox}-5 minor), 5.03 (1.4H, overlapping, H_{ox}-5major and H_{ox}-3 major), 4.83 (0.3H, dd, J 6.9, H-3 minor), 3.32 (2H, m, H-5′), 2.20 and 1.99 (2 × 1H, 2 × m, H-1′), 1.70 (4H, overlapping, H-4′ and H-2′), 1.56 (2H, m, H-3′), 1.48 [3H, C(*CH*$_3$)$_3$ minor], 1.12 [6H, C(*CH*$_3$)$_3$ major]; ^{13}C NMR ($CDCl_3$): δ 169.2 (*C*OO), 153.6 and 152.9 (N*C*OO), 136–126 (aromatic carbons), 81.6 and 81.2 [*C*(CH$_3$)$_3$], 79.5 and 78.9 (C_{ox}-6), 61.5 and 60.4 (C_{ox}-5), 57.51 and 56.4 (C_{ox}-3), 51.3–51.2 (C-5′), 35.5 and 35.0 (C-1′), 28.7 and 28.6 (C-4′), 28.3–27.8 [C(*CH*$_3$)$_3$ and C-3′], 26.4 and 26.3 (C-3′), 25.6 and 25.5 (C-2′); ESI-MS (positive) m/z: 487.2 ($M+Na^+$), 951.1 ($2M+Na^+$). C, 67.22; H, 6.94; N, 12.06. Anal. Calcd for $C_{26}H_{32}N_4O_4$: C, 67.30; H, 6.75; N, 11.90. Found: C, 67.40; H, 6.80; N, 12.10.

3.9.2. Reduction of the azide 16b, preparation of the amine 17

A solution of the azide **16b** (700 mg; 1.51 mmol) in ethyl acetate (150 mL) was selectively hydrogenated in the presence of Pd/C (100 mg; 10%) to afford a crude product, which was purified by rapid chromatography on silica to afford the amine **17** (529 mg; $Y = 80\%$), a solid: 117–119 °C (from hexane); $[\alpha]_D^{20} = -54.9$ (c 1; $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.29–7.01 (8H, aromatic protons), 6.59 (2H, m, aromatic protons), 5.95 (1H, br s, H_{ox}-6), 5.24 (0.3H, H_{ox}-5 minor), 5.03 (1.4H, overlapping, H_{ox}-5 major and H_{ox}-3 major), 4.84 (0.3H, m, H-3 minor), 2.72 (2H, m, H-5′), 2.19 and 1.99 (2 × 1H, 2 × m, H-1′), 1.62 (2H, m, H-4′), 1.65 (2H, m, H-2′), 1.56–1.48 [11H, overlapping, H-3′, H-4′ and C(*CH*$_3$)$_3$]; ^{13}C NMR ($CDCl_3$): δ 169.2 (*C*OO), 153.5 and 152.8 (N*C*OO), 136–126 (aromatic carbons), 81.5 and 81.1 (*C*(CH$_3$)$_3$), 79.4 and 78.8 (C_{ox}-6), 61.6 and 60.3 (C_{ox}-5), 57.6 and 56.4 (C_{ox}-3), 41.5 (C-5′), 35.5 and 34.9 (C-1′), 33.2 and 32.9 (C-4′), 28.3 and 28.0 (C-3′), 28.1 and 27.8 [C(*CH*$_3$)$_3$], 25.8 and 25.7 (C-2′); ESI-MS (positive) m/z: 461.2 $[M+Na]^+$. Anal. Calcd for $C_{26}H_{34}N_2O_4$: C, 71.21; H, 7.81; N, 6.39. Found: C, 71.40; H, 7.90; N, 6.50.

3.10. Shortened *one-pot* preparation of homodeoxypyridinoline 3b

To a solution containing amine **17** (1.19 g; 2.71 mmol) and bromoketone **10** (2.00 g; 7.86 mmol) in CH_3CN (50 mL), anhydrous Na_2CO_3 was added (1.36 g; 12.83 mmol) and the mixture was stirred at room temperature under nitrogen for 10 h. At this time, after the disappearance of the starting amine, the solvent was evapo-

rated under reduced pressure and the crude residue was recovered with anhydrous THF (150 mL) and filtered. Then, 1,8-diazabicyclo[5,4,0]undec-7-ene (DBU) was added (1 mL; 989 mmol) and the mixture was shaken under a slight pressure of oxygen (1.3 atm) at room temperature for 120 h. The mixture was then diluted with dichloromethane (50 mL) and filtered on a pad of Celite. After evaporation of the solvent, a crude residue was obtained, which was chromatographed on silica gel to afford (eluting with CH_2Cl_2/MeOH, 100: 7; v/v) the desired protected homodeoxypyridinoline **14b** (1.81 g, *Y* = 55%) as a glass, $[\alpha]_D^{20} = -26.7$ (*c* 1; $CHCl_3$). The compound was identical in all respects to that obtained and described above. Since it was transformed into **3b**, its preparation represents a formal synthesis of compound **3b**.

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