JOC*Article*

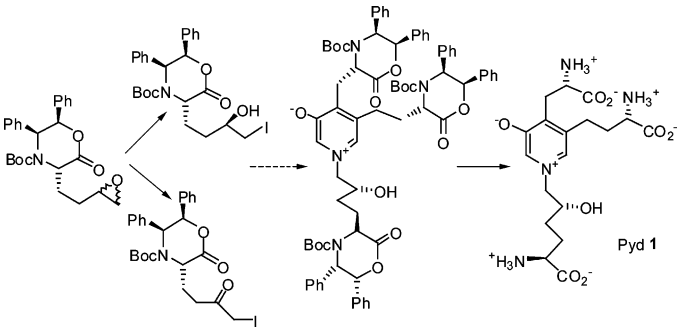
Practical Syntheses of Pyridinolines, Important Amino Acidic Biomarkers of Collagen Health

Pietro Allevi, Riccardo Cribiù, Elios Giannini, and Mario Anastasia*

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

mario.anastasia@unimi.it

Received January 23, 2007



The paper reports some successful results on the first fully stereoselective total synthesis of the collagen cross-link pyridinolines. All stereogenic centers are stereoselectively introduced using Williams glycine template methodology, and oxazinones are used as a source of chirality and as easily removable protecting groups of the amino acidic functionalities during the assembly of the pyridinoline nucleus.

Introduction

Pyridinoline (Pyd; **1**) and deoxypyridinoline (dPyd; **2**) are two collagen cross-links considered, to this day, as useful biochemical markers of bone resorption which can be correlated with diseases such as osteoporosis, bone cancer, and arthropathies and used to assess fracture risk prediction in older persons.[1−3] They are detected in human urine to permit early diagnosis of osteoporosis, for monitoring drug therapy of this bone disease, or for the follow-up of patients with malignant bone disease.[4a−f] As a consequence, it is important to have suitable amounts of pure Pyd (**1**) and dPyd (**2**) to be used as primary reference standards in analytical protocols. These cross-links, especially pyridinoline, are now obtained from natural sources,[5] after some tedious purifications, and by synthesis.[6] However, although the synthetic routes for preparing dPyd (**2**) have been exploited and well set up, those affording Pyd (**1**) are far less satisfactory due to some difficulties related to the stereoselective introduction of the hydroxy group of the hydroxylysine residue bonded to the heterocyclic nitrogen.[7] These difficulties were partially overcome by a more efficient protocol for the preparation of Pyd (**1**), developed in our laboratory.[7] Herein, we report two new efficient routes for the preparation of Pyd (**1**) and dPyd (**2**), based on the Williams morpholinone glycine synthons,[8] both to generate the stereogenic α-amino acidic centers and to suitably protect the amino acidic functions

(1) (a) Kleerekoper, M.; Camacho, P. *Clin. Chem.* **2005**, *51*, 2227. (b) James, I. T.; Walne, A. J.; Perrett, D. *Ann. Clin. Biochem.* **1996**, *33* 397. (c) For a comprehensive review on the biology of bones, see: *Priciples of Bone Biology*; Bilezikan, J. P., Raicz, L. G., Rogan, G., Eds.; Academic Press: New York, 2001.

(2) Stone, K. L.; Seeley, D. G.; Lui, L. Y.; Cauley, J. A.; Ensrud, K.; Browner, W. S. J.; Nevitt, M. C.; Cummings, S. R. *Bone Miner. Res.* **2003**, *18*, 1947.

(3) Schuit, S. C. E.; van der Klift, M.; Weel, A. E. A. M.; de Laet, C. E. D. H.; Burger, H.; Seemen, E.; Hofman, A.; Uitterlinden, A. G.; van Leeuwen, J. P. T. M.; Pols, H. A. P. *Bone* **2004**, *34*, 195.

(4) (a) Roth, M.; Uebelhart, D. *Clin. Chim. Acta* **2003**, *327*, 81. (b) Garnero, P.; Delmas, P. D. Biochemical markers of Bone turnover. In *Osteoporosis*; Marcus, R., Feldman, D., Kelsey, J., Eds.; Academic Press: San Diego, 1996; pp 1075−85. (c) Seibel, M. *Clin. Prat. Oncology* **2005**, *2*, 504. (d) Angeliewa, A.; Budde, M.; Schlachter, M.; Hoyle, N. R.; Bauss, F. *J. Bone Miner. Metab.* **2004**, *22*, 192. (e) Garnero, P.; Landewè, R.; Boers, M.; Verhoeven, A.; van der Linden, S.; Chiristgau, S.; van der Heijde, D.; Boonen, A.; Geusens, P. *Artritis Reum.* **2002**, *46*, 2847. (f) Vesper, H. W.; Demers, L. M.; Eastell, R.; Garnero, P.; Kleerekoper, M.; Robins, S. P.; Srivastava, A. K.; Warnick, G. R.; Watts, N. B.; Myers, G. *Clin. Chem.* **2002**, *48*, 220.

(5) (a) Robins, S. P.; Duncan, A.; Wilson, N.; Evans, B. *J. Clin. Chem.* **1996**, *42*, 1621. (b) Fujimoto, D.; Moriguchi, T.; Ishida, T.; Hayashi, H. *Biochem. Biophys. Res. Commun.* **1978**, *84*, 52. (c) Ogawa, T.; Ono, T.; Tsuda, M.; Kawanishi, Y. *Biochem. Biophys. Res. Commun.* **1982**, *107*, 1252.

10.1021/jo070136g CCC: $37.00
Published on Web 03/31/2007

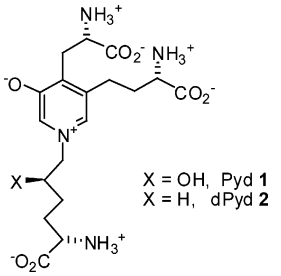


FIGURE 1. Chemical structures of pyridinoline **1** and deoxypyridinoline **2**.

during the assembly of the aromatic nucleus and to simplify the preparation of the 5*R*-hydroxylysine chain of Pyd (**1**) (Figure 1).

Results and Discussions

On the basis of our previous experience[6,7] with the synthesis of pyridinolines, we hypothesized a possible route for the preparation of Pyd (**1**), programming a chemoselective reaction of a halogen ketone **B** with a suitable amine **A**, followed by a cyclization−aromatization sequence affording a substituted hydroxypyridine nucleus with three amino acidic side chains completely protected with identical protective groups (Scheme 1).

With this in mind, we envisioned a synthesis which uses Williams' morpholinones[8,9] for a convenient preparation of the pyridinoline precursors **A** and **B** (Scheme 1). As we anticipated, our results show that Williams' morpholinone glycine synthon **3**, prepared by alkylation of the appropriate chiral oxazinone[9] and transformed in our laboratory into the epoxidic mixture **4**, could be a key substance for the preparation of both the α-hydroxyamine **7a** and the iodoketone **9** (Scheme 2) necessary for the synthetic project.[10]

Epoxidation of the olefin **3** affords an inseparable mixture of epoxides **4** which, however, by reaction with sodium iodide affords a diastereomeric mixture of iodohydrins **5** and **6**. These, on the contrary, are easily separated by rapid flash chromatography.[11a] The amino acidic iodohydrin **5**, having the required *R*-stereochemistry of the hydroxylated side chain of Pyd **1**, was then transformed into the azido alcohol **7a**, which, by catalytic reduction, affords the amino alcohol **8a**. On the other hand, the epimeric iodohydrin **6**, having the opposite *S*-stereochemistry at the hydroxylated center, was used to prepare the desired α-iodoketone **9** by oxidation with pyridinium chlorochromate.[12]

Moreover, considering that the hydroxy group of **7a** could interfere with the iodoketone **9** during the assembly of the pyridinium nucleus, thus depleting the reaction yields, we decided to use as starting material the silyloxyamine **8b** easily prepared via silylation of the hydroxyazide **7a** and successive catalytic hydrogenation, in the presence of Pd/C (Scheme 2). Then, we started the assembly of the pyridinoline nucleus (Scheme 3), setting up the bisalkylation of the hydroxyamine **8a** with the iodoketone **9** (reacted in a 1:2.5 molar ratio), in acetonitrile, in the presence of sodium carbonate, at room temperature.[6c] The reaction was followed on TLC to monitor the complete transformation of the starting amine first into the monoalkylated derivative and then into the diketoamine **10a**. At this point, the simple exchange of the reaction solvent (THF for CH_3CN) and of the base (DBU for Na_2CO_3) induced the diketoamine **10a** to undergo cyclization and aromatization to afford the protected pyridinoline **12a** via the intermediate formation of the α,β-unsaturated ketone **11a**.

These optimized reaction conditions are the result of several less satisfactory attempts in which the cyclization−aromatization reaction was performed in methanol containing Na_2CO_3. In fact, under these other conditions, a partial transesterification of the lactonic groups of the glycine synthons with formation of a mixture of methyl esters occurred. On the other hand, using tetrahydrofuran as solvent and Na_2CO_3 as base, the reaction was too slow and incomplete. The intermediate α,β-unsaturated ketone **11a** could not be isolated by standard procedures, being always accompanied (TLC evidence) by the starting diketoamine **10a** during its transformation into the protected pyridinoline **12a**. Compound **12a** was purified by rapid column chromatography to afford a glassy product (in about 21% yield) which showed the correct molecular formula and the expected physical chemical properties. At this point, with the aim to improve the yields obtained in the assembly of the aromatic nucleus of pyridinoline, starting from the hydroxyamine **8a**, we performed a parallel reaction sequence starting with the *O*-trimethylsilyloxy amine **8b** (Scheme 3). While monitoring this reaction by TLC, together with the expected silylated compound **12b**, we observed the formation of a more polar compound corresponding to the previously obtained protected pyridinoline **12a** lacking the silyl protection. Considering that the silyl group suffered a partial hydrolysis on TLC, we subjected the reaction mixture to a rapid chromatography on silica and obtained the desilylated compound **12a** in higher yield (34%) than starting with the free hydroxyamine **8a**. At this point, the accomplishment of the synthesis required only the deblocking of the protected amino acidic functions of compound **12a**. This could be performed following the reductive protocols reported by Williams et al.[8,9] (i.e., using alkali metals dissolved in liquid ammonia or a catalytic hydrogenation over a Pd° catalyst). Thus, we first performed the deblocking using sodium in liquid ammonia which, in our recent work on the synthesis of reducible collagen cross-links, caused a rapid and complete cleavage of the

(6) (a) Allevi, P.; Ciuffreda, P.; Longo, A.; Anastasia M. 4th Convegno Nazionale di chimica organica, Salerno, Italy, September 21−25, 1997; Italian Chemical Society; Commun. No. 03. (b) Waelchli, R.; Beerli, C. H.; Meigel, H.; Révész, L. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 2831. (c) Allevi, P.; Longo, A.; Anastasia, M. *Chem. Commun*. **1999**, 559. (d) Allevi, P.; Longo, A.; Anastasia, M. *J. Mol. Sci. (China)* **1999**, *15*, 154. (e) Hatch, R. P. U.S. Pat. 5723619, 1998; *Chem. Abstr*. **1998**, *128*, 205137H. (f) Adamczyk, M.; Johnson, D. D.; Reddy, R. E. *Tetrahedron* **1999**, *55*, 63. (g) Adamczyk, M.; Johnson, D. D.; Reddy, R. E. *Tetrahedron Lett.* **1999**, *40*, 8993. And corrigendum: *Tetrahedron Lett.* **2002**, *42*, 767. (h) Adamczyk, M.; Johnson, D. D.; Reddy, R. E. *Tetrahedron: Asymmetry* **2000**, *11*, 2289. And corrigendum: *Tetrahedron*: *Asymmetry* **2000**, *11*, 5017. (i) For a complete record of more recent work, see: Allevi, P.; Cribiù, R.; Giannini, E.; Anastasia, M. *Bioconjugate Chem.* **2005**, *16*, 1045 and references therein.

(7) Allevi, P.; Anastasia, M. *Tetrahedron: Asymmetry* **2003**, *14*, 2005 and references therein.

(8) Williams, M. R.; Sinclair, J. P.; DeMong, D. E.; Chein, D.; Zhai, D. *Org. Synth.* **2003**, *80*, 18 and references therein.

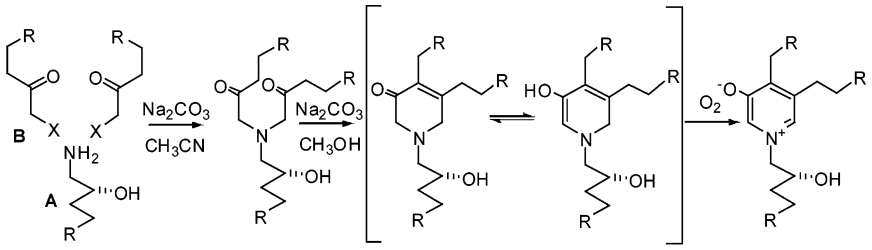
(9) Williams, M. R.; Im, M.-N.; Cao, J. *J. Am. Chem. Soc*. **1991**, *113*, 6976.

(10) For a recent successful use of William's morpholinones for the synthesis of hydroxylysine, see: (a) van den Nieuwendijk, A. M. C. H.; Kriek, N. M. A. J.; Brussee, J.; van Boom, J. H.; van der Gen, A. *Eur. J. Org. Chem*. **2000**, 3683. (b) Allevi, P.; Anastasia, M. *Tetrahedron: Asymmetry* **2004**, *15*, 2091.

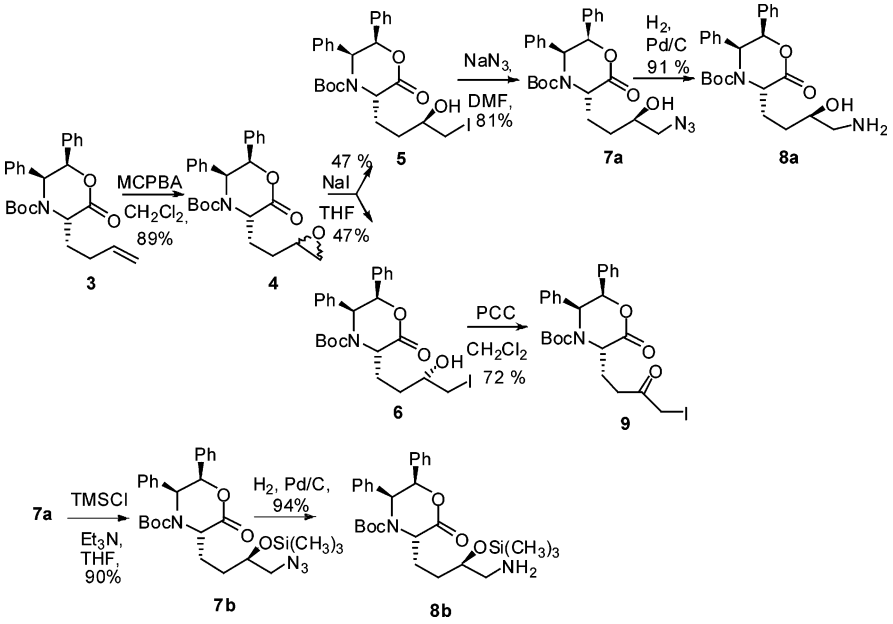
(11) (a) Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem*. **1978**, *43*, 2923. (b) The assignment of the stereochemistry of these compounds was previously reported by us in ref 10b.

(12) Corey, E. J.; Suggs, J. W. *Tetrahedron Lett.* **1975**, 2647.

SCHEME 1



SCHEME 2



oxazinones.[13] Moreover, after some unsuccessful results, probably due to the high functionalization of the molecule and to the difficulty of separating the water-soluble reaction product from the inorganic and organic byproducts, we turned our attention to the hydrogenolytic cleavage of the oxazinone rings present in compound **12a**. This deblocking procedure requires a preliminary acidic cleavage of the Boc groups, a generally simple reaction which, in our case, caused some initial difficulties due to the presence of the hydroxyl group in the hydroxylysine residue of **12a**. In fact, any attempt to cleave the Boc protecting groups in the usual conditions (using aqueous trifluoroacetic acid[14a] or formic acid)[14b] resulted in the contemporaneous partial trans-lactonization between the alcoholic hydroxy group of **12a** and the near lactonic group of the oxazinone ring. Finally, using $ZnBr_2$ in dichloromethane,[15] the cleavage of the Boc groups of compound **12a** occurred in good yields and without any undesired inner trans-lactonization. The deprotected oxazinone **13** formed in the reaction (Scheme 4) was then hydrogenated in the presence of palladium chloride at room temperature and pressure to regenerate the free pyridinoline (**1**) in good yields (80% from **12a**). Moreover, we were also able to extend the scope of our result, setting up a different procedure (Scheme 4) in which the iodoketone **9** and a self-immolative allylamine, for the introduction of the aromatic nitrogen atom in the pyridine ring, were used. Also in this case, reacting the iodoketone **9** with allylamine in the presence of Na_2CO_3 causes the bisalkylation to occur via formation of the intermediate diketoamine which, after exchange of the reaction solvent (THF for acetonitrile) and addition of DBU, suffers cyclization and oxidative aromatization to afford the fluorescent pyridinium salt **14** (in 27% yields).

This compound was completely characterized and subjected to the successive deallylation, performed using tetrakispalladium triphenyl phosphine and thiosalicylic acid as an allyl group scavenger,[16a,b] during the dealkylation. The hydroxypyridinium derivative **15** was obtained in good yields (78%) and in a satisfactory purity, as a white solid stable at 4 °C for almost 1 year. Successive alkylation of **15** with iodohydrine **5** in acetonitrile at 80 °C afforded the protected pyridinoline **12** which was then deprotected, following the procedure described for the synthesis of Pyd (**1**). In principle, alkylation of the compound **15** should afford also dPyr (**2**) or any other pyridinoline, simply changing the alkyl iodide to construct the chain bonded to the

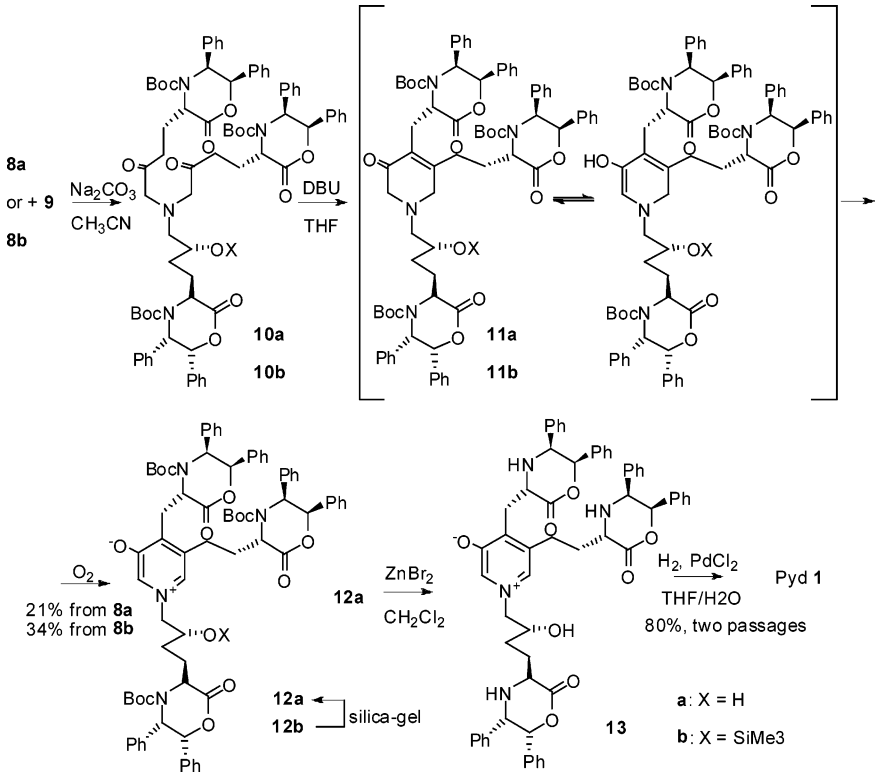
(13) Cribiù, R.; Allevi, P.; Anastasia, M. *Tetrahedron: Asymmetry* **2005**, *16*, 3059.

(14) (a) Schanabel, E.; Klostermeyer, H.; Berndt, H. *Liebigs Ann. Chem.* **1971**, *749*, 90. (b) Kinoshita, H.; Hammam, M. A. S.; Inomata, K. *Chem. Lett.* **2005**, *34*, 800.

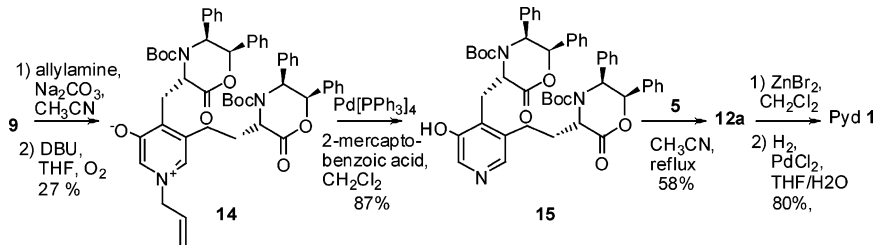
(15) Nigam, S. C.; Mann, A.; Taddei, M.; Wermuth, C. G. *Synth. Commun.* **1989**, *19*, 3139.

(16) (a) Hayakawa, Y.; Kato, H.; Kajino, M. U. H.; Noyori, R. *J. Org. Chem.* **1986**, *51*, 2402. (b) Bailey, W. F.; Jiang, X. L. *J. Org. Chem.* **1996**, *61*, 2956.

SCHEME 3



SCHEME 4



nitrogen atom of the pyridine ring. Thus, we may conclude that we have developed two routes for an efficient synthesis of pyridinoline overcoming the difficulties with the introduction of the correct stereochemistry of the 5-hydroxy group and also have set up a useful and simple method to obtain a proximate precursor of Pyd (**1**) and deoxypyridinoline dPyd (**2**), by a modular approach.

Experimental Section

General Methods. Melting points are uncorrected. Nuclear magnetic resonance spectra were recorded at 298 K on a spectrometer operating at 500.13 MHz for ^{1}H and at 125.76 MHz for ^{13}C. Chemical shifts are reported in parts per million (ppm, δ units) relative to the $CHCl_3$ signal fixed at 7.26 ppm for ^{1}H spectra and to the $CDCl_3$ signal fixed at 77.0 ppm for ^{13}C spectra. Proton and carbon assignments were established, if necessary, with ^{1}H−^{1}H and ^{1}H−^{13}C correlated NMR experiments. ^{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 Hz, assignment of proton(s). ^{1}H NMR and ^{13}C NMR spectra of compounds containing oxazinone ring(s) were complicated by the presence of distinguishable rotamers.[17] For some more complex compounds, the signals of major conformers are described, even if signals of minor rotamers were present in the spectra. Optical rotations were taken at 23 °C and $[\alpha]_D$ values are given in 10^{-1} deg cm^2 g^{-1}. HPLC analyses were carried out on a RP-18 column (125 mm, 4 mm ID, 5 μm), and 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% of 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. Mass spectra were obtained using a 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 silica gel plates (60 F_{254}) using UV light, 50% sulfuric acid or 0.2% ninhydrin in ethanol, and heat as the developing agent. Silica gel (230−400 mesh) was used for flash silica gel chromatography.[11a]

***tert*-Butyl (3*S*,5*S*,6*R*)-3-[(*R*)-4-Amino-3-hydroxybutyl)-2-oxo-5,6-diphenylmorpholine-4-carboxylate (8a).** A solution of *tert*-butyl (3*S*,5*S*,6*R*)-3-[(*R*)-4-azido-3-hydroxybutyl)-2-oxo-5,6-diphenylmorpholine-4-carboxylate (**7a**)[10b] (980 mg, 2.10 mmol) in AcOEt (170 mL) was hydrogenated in the presence of 10% Pd/C (40 mg) at room temperature and atmospheric pressure. The mixture was

(17) (a) William, M. R.; Yuan, C. *J. Org. Chem.* **1992**, *57*, 6519. (b) William, M. R.; Sinclair, J. P.; DeMong, D. E.; Chein, D.; Zhai, D. *Org. Synth.* **2003**, *80*, 18 and references therein.

filtered through a pad of Celite, and the solvent was evaporated under reduced pressure to afford the hydroxyamine **8a** (842 mg, 91%) as a white solid: mp 155−158 °C (from CH_2Cl_2/hexane); $[\alpha]_D^{25}$ −43.9 (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.28−7.00 (8H, aromatics), 6.59 (2H, aromatics), 6.01 (0.3H, d, J = 3.2 Hz, 6-H, minor conformer), 5.98 (0.7H, d, J = 3.2 Hz, 6-H, major conformer), 5.25 (0.3H, d, J = 3.2 Hz, 5-H, minor conformer), 5.02 (1.4H, m, overlapping, 5-H, 3-H, major conformer), 4.86 (0.3H, dd, J = 10.5, 4.6 Hz, 3-H, minor conformer), 3.83 (0.7H, m, 3′-H, major conformer), 3.65 (0.3H, m, 3′-H, minor conformer), 2.94 (0.3H, dd, J = 12.6, 3.5 Hz, 4′-H, minor conformer), 2.91 (0.7H, dd, J = 12.6, 3.5 Hz, 4′-H, major conformer), 2.64 (0.7H, dd, J = 12.6, 8.1 Hz, 4′-H, major conformer), 2.59 (0.3H, dd, J = 12.6, 8.1 Hz, 4′-H, minor conformer), 2.32 (1H, m, 1′-Ha, both conformers), 2.22 (1H, m, 1′-Hb, both conformers), 1.77 (1H, m, 2′-Ha, both conformers), 1.65 (1H, m, 2′-Hb, both conformers), 1.48, 1.14 [9H, 2 × s, (CH_3)$_3$C, minor and major conformers]; ^{13}C NMR ($CDCl_3$) (major conformer) δ 169.1 (2-C), 153.8 (^{t}BuO*C*═O), 136.2 (aromatic), 134.2 (aromatic), 131.7−124.2 (aromatics), 81.5 [(CH_3)$_3$*C*], 79.0 (6-C), 70.3 (3′-C), 61.3 (5-C), 55.4 (3-C), 47.4 (4′-C), 31.1 (1′-C), 30.3 (2′-C), 27.8 [(*C*H$_3$)$_3$C]; IR ($CHCl_3$) ν 3330, 1758, 1682 cm^{-1}; ESI-MS (positive) *m*/*z* 441 (M + H$^+$), 463 (M + Na$^+$), 903 (M + M + Na$^+$). Anal. Calcd for $C_{25}H_{32}N_2O_5$: C, 68.16; H, 7.32; N, 6.36. Found: C, 68.45; H, 7.50; N, 6.40.

***tert*-Butyl (3*S*,5*S*,6*R*)-3-[(*R*)-4-Azido-3-(trimethylsilyloxy)butyl]-2-oxo-5,6-diphenylmorpholine-4-carboxylate (7b).** To a stirred solution of the hydroxyazide **7a**[10b] (395 mg, 0.85 mmol), triethylamine (232 μL, 1.66 mmol), and *N*,*N*-dimethylaminopyridine (20 mg, 0.16 mmol) in anhydrous THF (6.0 mL) was added TMSCl (127 μL, 1.00 mmol). The resulting mixture was stirred at room temperature for 7 h and then diluted with AcOEt (50 mL). The solution was washed with brine, dried over anhydrous Na_2SO_4, and evaporated under reduced pressure. The crude residue was crystallized to afford the trimethylsilyl derivative **7b** (410 mg, 90%) as white solid: mp 149 °C (from CH_2Cl_2 /hexane); $[\alpha]_D^{25}$ −39.5 (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.29−7.00 (8H, aromatics), 6.59 (2H, aromatics), 5.93 (1H, br s, 6-H, both conformers), 5.26 (0.3H, d, J = 3.5 Hz, 5-H, minor conformer), 5.04 (0.7H, d, J = 3.5 Hz, 5-H, major conformer), 5.01 (0.7H, dd, J = 9.5, 5.3 Hz, 3-H, major conformer), 4.83 (0.3H, dd, J = 9.8, 5.0 Hz, 3-H, minor conformer), 3.94 (0.7H, m, 3′-H, major conformer), 3.89 (0.3H, m, 3′-H, minor conformer), 3.27 (2H, m, 4′-H$_2$, both conformers), 2.17 (1H, m, 1′-Ha, both conformers), 2.09 (1H, m, 1′-Hb, both conformers), 1.83 (2H, m, 2′-H$_2$, both conformers), 1.48, 1.12 [9H, 2 × s, 2 × (CH_3)$_3$C, minor and major conformers], 0.22, 0.21 [9H, 2 × s, (CH_3)$_3$Si, major and minor conformers]; ^{13}C NMR ($CDCl_3$) (major conformer) δ 169.1 (2-C), 153.6 (^{t}BuO*C*═O), 136.4 (aromatic), 134.3 (aromatic), 128.6−126.3 (aromatics), 81.2 [(CH_3)$_3$*C*], 79.0 (6-C), 71.2 (3′-C), 61.5 (5-C), 56.8 (4′-C), 56.4 (3-C), 31.2 (1′-C), 30.9 (2′-C), 27.9 [(*C*H$_3$)$_3$C], 0.3 [(*C*H$_3$)$_3$Si]; IR ($CHCl_3$) ν 3520, 2143, 1763, 1700 cm^{-1}; ESI-MS (positive) *m*/*z* 561 (M + Na$^+$), 1100 (M + M + Na$^+$). Anal. Calcd for $C_{28}H_{38}N_4O_5Si$: C, 62.43; H, 7.11; N, 10.40. Found: C, 62.63; H, 6.86; N, 10.51.

***tert*-Butyl (3*S*,5*S*,6*R*)-3-[(*R*)-4-Amino-3-(trimethylsilyloxy)butyl]-2-oxo-5,6-diphenylmorpholine-4-carboxylate (8b).** The trimethylsilyloxyazide **7b** (250 mg, 0.46 mmol), dissolved in AcOEt (40 mL), was hydrogenated in the presence of 10% Pd/C (15 mg) at room temperature and atmospheric pressure. After 20 h, the mixture was filtered through a pad of Celite and the solvent was evaporated under reduced pressure. The crude residue was then crystallized to afford the hydroxyamine **8b** (223 mg, 94%) as a white solid: mp 102 °C (from CH_2Cl_2/hexane; compound starts to reduce its volume at 95 °C); $[\alpha]_D^{25}$ −26.5 (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.29−7.00 (8H, aromatics), 6.58 (2H, aromatics), 5.95 (0.3H, d, J = 3.2 Hz, 6-H, minor conformer), 5.93 (0.7H, d, J = 3.2 Hz, 6-H, major conformer), 5.24 (0.3H, d, J = 3.2 Hz, 5-H, minor conformer), 5.03 (0.7H, d, J = 3.2 Hz, 5-H, major conformer), 5.00 (0.7H, dd, J = 9.5, 5.4 Hz, 3-H, major conformer), 4.81 (0.3 H, dd, J = 9.8, 4.6 Hz, 3-H, minor conformer), 3.74 (0.7H, m, 3′-H, major conformer), 3.70 (0.3H, m, 3′-H, minor conformer), 2.72 (2H, m, 4′-H$_2$, both conformers), 2.16 (1H, m, 1′-Ha, both conformers), 2.07 (1H, m, 1′-Hb, both conformers), 1.78 (2H, m, 2′-H$_2$, both conformers), 1.46, 1.17 [9H, 2 × s, 2 × (CH_3)$_3$C, both conformers], 0.18 [9H, s, (CH_3)$_3$Si, both conformers]; ^{13}C NMR ($CDCl_3$) (major conformer) δ 169.2 (2-C), 153.6 (^{t}BuO*C*═O), 136.4 (aromatic), 134.3 (aromatic), 128.5−126.4 (aromatics), 81.1 [(CH_3)$_3$*C*], 78.9 (6-C), 73.5 (3′-C), 61.4 (5-C), 56.5 (3-C), 47.7 (4′-C), 31.1 (1′-C), 30.8 (2′-C), 27.7 [(*C*H$_3$)$_3$C], 0.4 [(*C*H$_3$)$_3$Si]; IR ($CHCl_3$) ν 1760, 1705 cm^{-1}; ESI-MS (positive) *m*/*z* 513 (M + H$^+$). Anal. Calcd for $C_{28}H_{40}N_2O_5Si$: C, 65.59; H, 7.86; N, 5.46. Found: C, 65.45; H, 7.81; N, 5.40.

***tert*-Butyl (3*S*,5*S*,6*R*)-3-(4-Iodo-3-oxobutyl)-2-oxo-5,6-diphenylmorpholine-4-carboxylate (9).** To a solution of iodohydrin **6**[10b] (800 mg, 1.45 mmol) dissolved in CH_2Cl_2 (35 mL) was added pyridinium chlorochromate[12] (625 mg, 2.90 mmol). The initially orange solution was then stirred at room temperature for 2 h. At this time, the complete disappearance of the starting material was observed (TLC). The reaction was quenched with 2-propanol (1.0 mL), and the solvent was evaporated under reduced pressure. The residue was purified by flash silica gel chromatography (hexane/AcOEt 7:3 v:v) to afford, after crystallization, the iodoketone **9** (574 mg, 72% yield) as a white solid: mp 173−174 °C (from CH_2Cl_2 /diisopropyl ether); $[\alpha]_D^{25}$ −48.9 (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.29−7.00 (8H, aromatics), 6.58 (2H, aromatics), 6.06 (0.7H, d, J = 2.8 Hz, 6-H, major conformer), 6.01 (0.3H, br s, 6-H, minor conformer), 5.24 (0.3H, d, J = 2.8 Hz, 5-H, minor conformer), 5.00 (1.4H, m, overlapping, 5-H and 3-H, major conformer), 4.83 (0.3 H, dd, J = 9.8 and 5.6 Hz, 3-H, minor conformer), 3.92 (2H, s, 4′-H, both conformers), 3.15 (1H, t, J = 7.0 Hz, 2′-Ha, both conformers), 3.08 (1H, dd, J = 13.7, 7.0 Hz, 2′-Hb, both conformers), 2.55 (1H, m, 1′-Ha, both conformers), 2.33 (1H, m, 1′-Hb, both conformers), 1.49, 1.11 [9H, 2 × s, (CH_3)$_3$C, minor and major conformers]; ^{13}C NMR ($CDCl_3$) (major conformer) δ 201.9 (3′-C), 169.3 (2-C), 153.9 (^{t}BuO*C*═O), 135.2 (aromatic), 134.1 (aromatic), 128.6−125.9 (aromatics), 81.4 [(CH_3)$_3$*C*], 78.9 (6-C), 61.5 (5-C), 55.6 (3-C), 35.5 (2′-C), 27.8 [(CH_3)$_3$C], 28.9 (1′-C), 5.9 (4′-C); IR ($CHCl_3$) ν 1752, 1728, 1705 cm^{-1}; ESI-MS (positive) *m*/*z* 572 (M + Na$^+$). Anal. Calcd for $C_{25}H_{28}INO_5$: C, 54.65; H, 5.14; N, 2.55. Found: C, 54.49; H, 5.31; N, 2.61.

1-4-[(3*S*,5*S*,6*R*)-4-(*tert*-Butoxycarbonyl)-2-oxo-5,6-diphenylmorpholin-3-yl]-2-hydroxybutyl)-5−2-[(3*S*,5*S*,6*R*)-4-(*tert*-butoxycarbonyl)-2-oxo-5,6-diphenylmorpholin-3-yl]ethyl)-4-([(3*S*,5*S*,6*R*)-4-(*tert*-butoxycarbonyl)-2-oxo-5,6-diphenylmorpholin-3-yl]methyl)pyridinium-3-olate (12a). (i) Starting from Iodoketone 9 and Hydroxyamine 8a. To a solution of hydroxyamine **8a** (300 mg, 0.68 mmol) and iodoketone **9** (896 mg, 1.63 mmol) in CH_3CN (30 mL) was added Na_2CO_3 (350 mg). The resulting mixture was stirred at room temperature until the complete disappearance (2 h) of the starting amine **8a** (TLC, R_f = 0.18, CH_2Cl_2/MeOH 100:5 v:v), and the solvent was evaporated under reduced pressure at a temperature below 40 °C. The solid residue was suspended in CH_2Cl_2 (30 mL) and filtered to eliminate the inorganic salts. The glassy residue obtained by evaporation of the solvent under reduced pressure was dissolved in anhydrous THF (45 mL) containing DBU (270 μL, 1.80 mmol). The reaction mixture was then stirred under an oxygen atmosphere for 4 days at room temperature. The solvent was removed by evaporation, and the residue was purified by flash silica gel chromatography (CH_2Cl_2/MeOH 10:1 v:v), to afford the compound **12a (**181 mg, 21%) as a glass: $[\alpha]_D^{25}$ −23.8 (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) (major conformer) δ 7.59−6.94 (26H, aromatics, 2-H and 6-H), 6.73 (1H, br s, 6′$_{chain}$-H), 6.56 (6H, m, aromatics), 6.09 (1H, br s, 6′$_{chain}$-H), 5.95 (1H, br s, 6′$_{chain}$-H), 5.34 (1H, m, 3′$_{4chain}$-H), 5.29 (1H, br s, 5′$_{chain}$-H), 5.19 (1H, m, 3′$_{5chain}$-H), 5.15 (1H, br s, 5′$_{chain}$-H), 5.00 (1H, br s, 5′$_{chain}$-H), 4.93 (1H, br s, 3′$_{Nchain}$-H), 4.22 (1H, m, 1$_{Nchain}$-Ha), 4.12 (1H, m, 2$_{Nchain}$-H), 3.88 (1H, m, 1$_{Nchain}$-Hb), 3.58 (2H, m, 1$_{4chain}$-H$_2$), 3.17 (2H, m, 1$_{5chain}$-

H_2), 2.55 (1H, m, 2_{5chain}-Ha), 2.41 (1H, m, 2_{5chain}-Hb), 2.20 (2H, m, 4_{Nchain}-H_2), 1.77 (1H, m, 3_{Nchain}-Ha), 1.68 (1H, m, 3_{Nchain}-Hb), 1.12, 1.09, 1.01 [27H, 3 × s, 3 × C(CH_3)$_3$]; ^{13}C NMR ($CDCl_3$) (major conformer) δ 169.1, 169.0, 168.8 (3 × 2′-C), 153.9, 153.7, 153.6 (3 × ^{t}BuO*C*═O), 153.2 (3-C), 140.6−126.6 (aromatics), 81.6, 81.3, 80.6 [3 × (CH_3)$_3$*C*], 79.0, 78.9, 78.5 (3 × 6′-C), 69.1 (2_{Nchain}-C), 66.3 (1_{Nchain}-C), 61.4, 61.3, 60.9 (3 × 5′-C), 55.8 ($3'_{Nchain}$-C), 55.8 ($3'_{5chain}$-C), 54.6 ($3'_{4chain}$-C), 35.4 (2_{5chain}-C), 30.8 (4_{Nchain}-C), 30.5 (3_{Nchain}-C), 30.4 (1_{4chain}-C), 27.7 [3 × (*C*H_3)$_3$C], 26.6 (1_{5chain}-C); IR ($CHCl_3$) ν 3360, 1760, 1706 cm^{-1}; ESI-MS (positive) *m*/*z* 1264 (M + H$^+$). Anal. Calcd for $C_{75}H_{82}N_4O_{14}$: C, 71.30; H, 6.54; N, 4.43. Found: C, 71.59; H, 6.45; N, 4.49.

(ii) Starting from Iodoketone 9 and Trimethylsilyloxyamine 8b. To a solution of trimethylsilyloxyamine **8b** (300 mg, 0.58 mmol) and iodoketone **9** (771 mg, 1.40 mmol) in CH_3CN (30 mL) was added Na_2CO_3 (300 mg), and the resulting mixture was stirred at room temperature until the complete disappearance (2 h) of the starting amine **8b** (TLC, R_f = 0.18, CH_2Cl_2 /MeOH 100:5 v:v). The solvent was evaporated under reduced pressure at temperatures below 40 °C, and the solid residue was suspended in CH_2Cl_2 (30 mL) and filtered to eliminate the inorganic salts. The filtrate was evaporated under reduced pressure to afford a glassy residue which was dissolved in anhydrous THF (45 mL) containing DBU (230 μL, 1.54 mmol). The reaction mixture was then stirred under an oxygen atmosphere for 4 days at room temperature. The solvent was removed by evaporation, and the crude residue was purified by flash silica gel chromatography (CH_2Cl_2/MeOH 10:1 v:v), to afford the compound **12a** (251 mg, 34%) as a glass: $[\alpha]_D^{25}$ −24.0 (*c* 1, $CHCl_3$), identical in all respects with that described above.

Pyridinoline (1). To a solution of protected pyridinoline **12a** (160 mg, 0.127 mmol) in anhydrous CH_2Cl_2 (6.0 mL) was added anhydrous $ZnBr_2$[15] (172 mg, 0.76 mmol), and the resulting mixture was stirred under argon at room temperature for 3 h. The mixture was then diluted with AcOEt (50 mL), washed with a saturated aqueous solution of $NaHCO_3$ (30 mL), with water (305 mL), and dried over anhydrous Na_2SO_4. The evaporation of the solvent under reduced pressure afforded the crude pyridinoline precursor **13** (113 mg) which was dissolved in an aqueous 0.01 M HCl/THF 50:50 v:v mixture (60 mL) and hydrogenated in the presence of $PdCl_2$ (48 mg), at room temperature for 48 h. The reaction was monitored on HPLC (see General) until the complete conversion of starting material and of less polar intermediates into a single peak (*R*t = 10.56 min) corresponding to a pure sample of pyridinoline. The mixture was then filtered through Celite, to remove the catalyst; the solvent was evaporated; and the solid residue was dried in vacuo. The solid was then triturated with diethyl ether (which dissolves the formed bibenzyl) and filtered to afford the pyridinoline **1** (62 mg, 80%) as tetrachloride dihydrate: λ_{max} (HCl 0.1 M)/nm (ϵ/dm^3 mol^{-1} cm^{-1}), 242 (3850), 294 (6520); λ_{max} (50 mM phosphate buffer, pH 7.5)/nm (ϵ/dm^3 mol^{-1} cm^{-1}), 252 (3710), 324 (6150); ESI/MS *m*/*z* 429 (M + H$^+$). Other physicochemical properties (^{1}H and ^{13}C NMR) confirmed those reported.[7]

1-Allyl-5-2-[(3*S*,5*S*,6*R*)-4-(*tert*-butoxycarbonyl)-2-oxo-5,6-diphenylmorpholin-3-yl]ethyl-4-([(3*S*,5*S*,6*R*)-4-(*tert*-butoxycarbonyl)-2-oxo-5,6-diphenylmorpholin-3-yl]methyl)pyridinium-3-olate (14). To a stirred solution of the iodoketone **9** (900 mg, 1.64 mmol) in CH_3CN (30 mL) were added allylamine (51.0 μL, 0.68 mmol) and Na_2CO_3 (350 mg), and the resulting mixture was stirred at room temperature for 2 h. The solvent was evaporated under reduced pressure at temperatures below 40 °C, and the solid residue was suspended in CH_2Cl_2 (30 mL) and filtered to eliminate the inorganic salts. The filtrate was evaporated under reduced pressure to afford a glassy residue which was dissolved in anhydrous THF (45 mL) containing DBU (270 μL, 1.80 mmol). The reaction mixture was then stirred under an oxygen atmosphere at room temperature for 4 days. The solvent was removed under reduced pressure, and the crude residue was purified by flash silica gel chromatography (CH_2Cl_2/MeOH 10:1 v:v) to afford, after crystallization, the compound **14** (162 mg, 27%) as a white solid: mp 167 °C (from CH_2Cl_2/isopropyl ether); $[\alpha]_D^{25}$ −44.6 (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) (major conformer) δ 7.24−6.99 (18H, aromatics, 2-H and 6-H), 6.87 (1H, br s, $6'_{4chain}$-H or $6'_{5chain}$-H), 6.58 (4H, m, aromatics), 6.06 (1H, br s, $6'_{5chain}$-H or $6'_{4chain}$-H), 5.99 (1H, m, 2_{Nchain}-H), 5.43−5.40 (2H, m, 3_{Nchain}-H_2), 5.30 (1H, m, $3'_{4chain}$-H), 5.17 (1H, br s, $5'_{4chain}$-H or $5'_{5chain}$-H), 5.15 (1H, br s, $3'_{5chain}$-H), 5.07 (1H, br s, $5'_{5chain}$-H or $5'_{4chain}$-H), 4.65 (2H, br s, 1_{Nchain}-H_2), 3.71 (1H, m, 1_{4chain}-Ha), 3.56 (1H, m, 1_{4chain}-Hb), 3.16 (2H, m, 1_{5chain}-H_2), 2.56 (1H, m, 2_{5chain}-Ha), 2.40 (1H, m, 2_{5chain}-Hb), 1.11, 1.00 [18H, 2 × s, 2 × C(CH_3)$_3$]; ^{13}C NMR ($CDCl_3$) (major conformer) δ 169.2, 168.9 (2 × 2′-C), 154.0, 153.7 (2 × ^{t}BuO*C*═O), 152.7 (3-C), 141.2−126.5 (aromatics), 130.4 (2_{Nchain}-C), 122.2 (3_{Nchain}-C), 81.2, 80.4 [2 × (CH_3)$_3$*C*], 79.3, 79.2 (2 × 6′), 62.6 (1_{Nchain}-C), 61.9, 61.2 (2 × 5′-C), 56.2 ($3'_{5chain}$-C), 55.1 ($3'_{4chain}$-C), 35.0 (2_{5chain}-C), 30.4 (1_{4chain}-C), 27.8 [2 × (*C*H_3)$_3$C], 26.7 (1_{5chain}-C); IR ($CHCl_3$) ν 1765, 1703 cm^{-1}; ESI-MS (positive) *m*/*z* 880 (M + H$^+$), 902 (M + Na$^+$). Anal. Calcd for $C_{53}H_{57}N_3O_9$: C, 72.33; H, 6.53; N, 4.77. Found: C, 72.08; H, 6.57; N, 4.87.

5-2-[(3*S*,5*S*,6*R*)-4-(*tert*-Butoxycarbonyl)-2-oxo-5,6-diphenylmorpholin-3-yl]ethyl-4-([(3*S*,5*S*,6*R*)-4-(*tert*-butoxycarbonyl)-2-oxo-5,6-diphenylmorpholin-3-yl]methyl)-3-hydroxypyridine (15). To a stirred solution of allyl derivative **14** (150 mg, 0.17 mmol) and 2-mercaptobenzoic acid (128 mg, 0.83 mmol) in CH_2Cl_2 (15 mL) was added tetrakis(triphenylphosphine)palladium(0) (10 mg, 0.00865 mmol).[16] The resulting orange solution was stirred at room temperature under argon for 1.3 h. The solution was washed with a saturated $NaHCO_3$ aqueous solution (10 mL) and dried over anhydrous Na_2SO_4, and the solvent was evaporated under reduced pressure. The crude residue was purified by flash silica gel chromatography (CH_2Cl_2/MeOH 100:7 v:v) to afford the hydroxypyridine **15** (125 mg, 87%) as a white solid: mp 201 °C; $[\alpha]_D^{25}$ −48.2 (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) (major conformer) δ 8.27 (1H, br s, 2-H), 8.12 (1H, br s, 6-H), 7.37−6.52 (20H, aromatics), 6.25 (1H, d, *J* = 3.2 Hz, $6'_{4chain}$-H or $6'_{5chain}$-H), 5.90 (1H, d, *J* = 3.2 Hz, $6'_{5chain}$-H or $6'_{4chain}$-H), 5.07 (1H, dd, *J* = 11.2 and 3.0 Hz, $3'_{4chain}$-H), 5.01 (1H, d, *J* = 3.2 Hz, $5'_{4chain}$-H or $5'_{5chain}$-H), 5.00 (1H, dd, *J* = 11.2 and 3.0 Hz, $3'_{5chain}$-H), 4.96 (1H, d, *J* = 3.2 Hz, $5'_{5chain}$-H or $5'_{4chain}$-H), 3.61 (1H, dd, *J* = 14.7 and 11.2 Hz, 1_{4chain}-Ha), 3.44 (1H, dd, *J* = 14.7 and 3.2 Hz, 1_{4chain}-Hb), 3.03 (1H, ddd, *J* = 13.3, 13.3, 4.9 Hz, 1_{5chain}-Ha), 2.82 (1H, ddd, *J* = 13.3, 12.2, 5.3 Hz, 1_{5chain}-Hb), 2.33 (2H, m, 2_{5chain}-H_2), 1.20, 1.14 [18H, 2 × s, 2 × C(CH_3)$_3$]; ^{13}C NMR ($CDCl_3$) (major conformer) δ 169.7, 167.4 (2 × 2′-C), 154.9, 153.5 (2 × ^{t}BuO*C*═O), 152.6 (3-C), 142.0 (6-C), 137.6 (2-C), 136.6−126.4 (aromatics), 83.1, 81.2 [2 × (CH_3)$_3$*C*], 79.4, 79.2 (2 × 6′-C), 61.1, 60.9 (2 × 5′-C), 56.4 ($3'_{5chain}$-C), 55.4 ($3'_{4chain}$-C), 36.6 (2_{5chain}-C), 30.9 (1_{4chain}-C), 27.9 [2 × (*C*H_3)$_3$C], 26.9 (1_{5chain}-C); IR ($CHCl_3$) ν 1770, 1700 cm^{-1}; ESI-MS (positive) *m*/*z* 840 (M + H$^+$). Anal. Calcd for $C_{50}H_{53}N_3O_9$: C, 71.49; H, 6.36; N, 5.00. Found: C, 71.37; H, 6.52; N, 5.05.

Protected Pyridinoline 12a by Alkylation of Hydroxypyridine 15. The hydroxypyridine **15** (800 mg, 0.95 mmol) and the iodohydrin **5**[10b] (2.65 g, 4.8 mmol) were dissolved in CH_3CN (20 mL) and refluxed under an argon atmosphere for 20 h. The mixture was cooled at room temperature and treated with a strong basic resin (Dowex 1 100−200 mesh, X8; 860 mg) for 15 min. After filtration, the solvent was evaporated under reduced pressure to afford a crude product which was purified by flash silica gel chromatography (CH_2Cl_2/MeOH 10:1 v:v) to give the protected pyridinoline (**12a**, 698 mg, 58%) as a glass: $[\alpha]_D^{25}$ −25.2 (*c* 1, $CHCl_3$), identical in all respects with that described above.

Acknowledgment. This work was supported financially by Italian MiUR (Ministero dell'Università e della Ricerca).

Supporting Information Available: ^{1}H and ^{13}C NMR spectra for compounds **7b**, **8a**, **8b**, **9**, **12a**, **14**, and **15**. This material is available free of charge via the Internet at http://pubs.acs.org.

JO070136G