A practical protocol for the synthesis of 3-hydroxy-4,5-disubstituted pyridine derivatives from acyclic compounds

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The paper reports a simple protocol for assembling a 3-hydroxy-4,5-disubstituted pyridine ring by reaction of allylamine or benzylamine and an α-bromo ketone and successive *N*-dealkylation.

Introduction

Pyridinoline **1** and deoxypyridinoline **2** are two collagen cross-links with a 3-hydroxypyridine diamino acidic nucleus **3** as a common structural feature (see Fig. 1).[1] They are considered effective biochemical markers of bone resorption[1,2] and are detected in the urine of patients for early diagnosis of osteoporosis or for monitoring drug therapy of this and other metabolic bone diseases. On the other hand, from a synthetic point of view, the construction of the nucleus of these compounds has created a considerable challenge, most notably because the established general methods for preparing the 3-hydroxy-4,5-substituted pyridine system involve the often tedious elaboration of the existing nucleus of various heterocyclic compounds.[3–5] In fact these procedures do not allow a ready and stereoselective incorporation of different side chains, especially if they contain an amino acidic portion. Therefore, a number of synthetic efforts are being directed to the development of general methodologies for the synthesis of the collagen cross-links **1** and **2**.

So far only two general routes for assembling the 3-hydroxy-4,5-disubstituted pyridiniumolate system of pyridinolines **1** and **2** have been published. The first one, reported by our group,[6] begins with the selective alkylation of a suitably protected L-lysine with an appropriate α-bromo ketone (Scheme 1, P = Cbz). The so-formed diketo amine is self condensed to afford a cyclic α,β-unsaturated ketone, followed by aromatisation of the system to a 3-pyridiniumolate ring without losing the substituent at the nitrogen atom.[6b] We conducted these three steps in a 'one-pot' reaction with a simple model study, and we then demonstrated the feasibility of the method in the synthesis of deoxypyridinoline **2**, where we isolated and characterised the crucial intermediate diketo amine and observed that the successive reactions of this compound proceed in a satisfactory way in methanol. Our new, efficient protocol was successively applied by M. Adamczyk *et al.*,[7] who adopted *vis-à-vis* the 'one-pot' protocol and confirmed its generality using amino acids protected as *tert*-butoxycarbonyl derivatives at the amino groups, in place of benzyloxycarbonyls (Scheme 1, P = Boc).

The second procedure (Scheme 2), published by R. Waelchli *et al.*,[8] noticeably differs from our protocol[6] since it involves the reaction of an epoxide with benzylamine and a cyclisation–aromatisation sequence of reactions, which ends with the loss of the substituent at the heterocyclic nitrogen atom. Thus a 3-hydroxypyridine nucleus is obtained but a subsequent alkylation at the nitrogen atom of the ring is required to afford protected pyridinolines **1** and **2**. This route is considerably longer and less straightforward compared with ours. Moreover it requires an undesirable replacement of the benzyl protective group of the pyridine nitrogen atom by Boc in one of the steps.

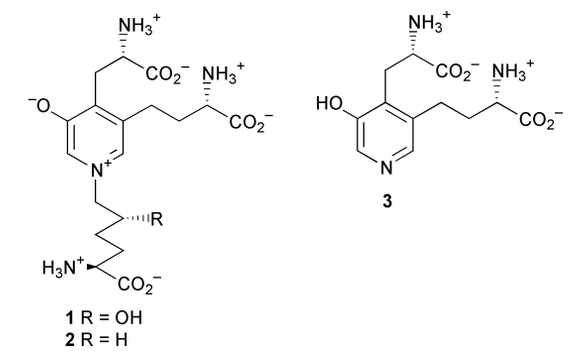
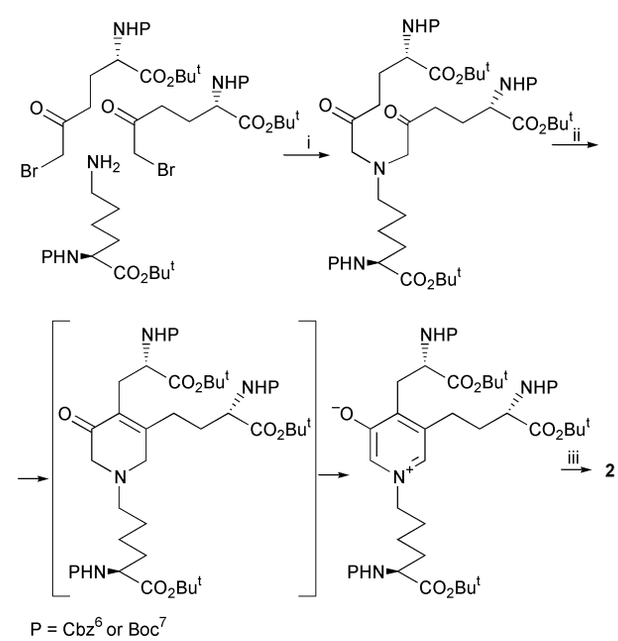


Fig. 1



Scheme 1 *Reagents and conditions:* i, K_2CO_3, MeCN, room temp.; ii, K_2CO_3, air, MeOH, room temp.; iii, H_2, Pd/C, MeOH, room temp.; TFA, room temp.

This procedure was also applied by M. Adamczyk *et al.*,[9] starting from natural amino acids. A protocol similar to that of R. Waelchli's was also reported in a patent by R. P. Hatch.[10]

With the aim of developing a more general approach to 3-hydroxy-4,5-disubstituted pyridines that allows a ready

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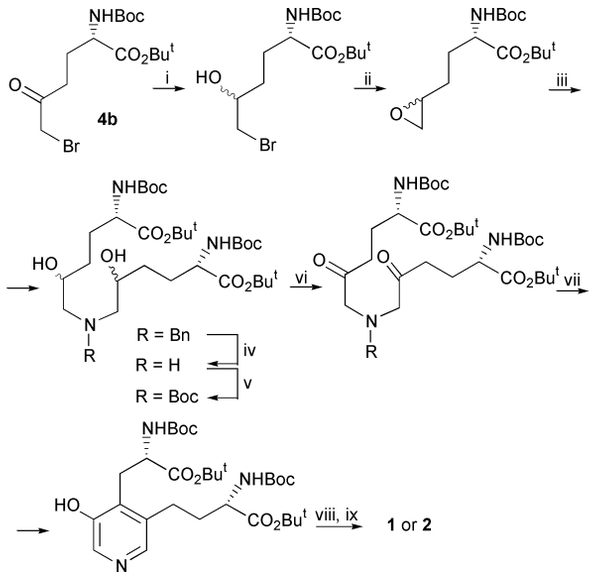
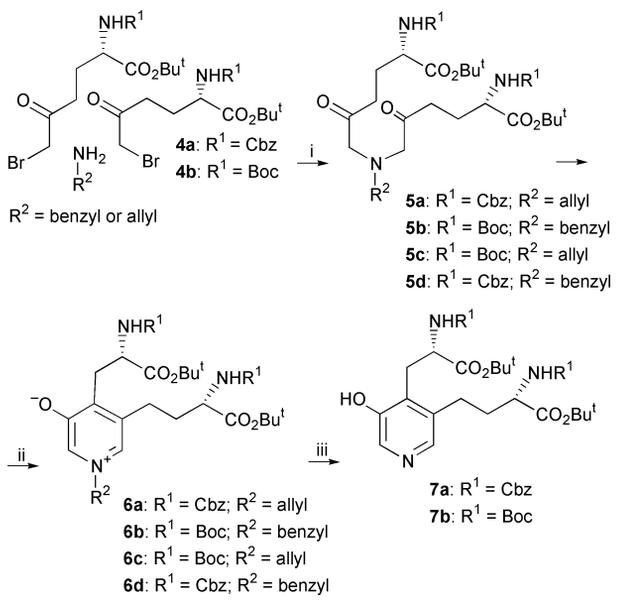


Scheme 2 *Reagents and conditions:* i, $NaBH_4$, MeOH, 0 °C to room temp.; ii, KOH, EtOH, room temp.; iii, $PhCH_2NH_2$, 75 °C; iv, H_2, Pd/C, EtOH; v, $(Boc)_2O$, THF, room temp.; vi, $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, −78 °C; vii, DBU, THF, room temp.; viii, alkylation; ix, TFA–water, room temp.

incorporation of a variety of different chains at the pyridine nitrogen, we were attracted by the possibility of constructing various congeners of pyridinolines, assembling different amino acidic chains to the nucleus of suitably protected **3**. At the end of our preliminary studies[11] we assembled the 4,5-disubstituted 3-hydroxypyridinoline nucleus by using our protocol and allylamine, as temporary chemical auxiliary for the introduction of the pyridine nitrogen atom (Scheme 3).



Scheme 3 *Reagents and conditions:* i, K_2CO_3, MeCN, room temp.; ii, base, O_2, MeOH, room temp.; iii, $Pd(PPh_3)_4$, 2-mercaptobenzoic acid, CH_2Cl_2, room temp., 1.5 h for **6a** and **6c** or H_2, Pd/C, MeOH, room temp, 1 h, for **6b**.

In fact, the reaction of the bromo ketone **4a** with this amine afforded the diketo amine **5a** and then the 3-hydroxypyridinium compound **6a** which, by deallylation [performed by means of $(\eta^2$-propene)$Ti(OPr^i)_4$], gave the desired key compound **7a**. Alkylation with appropriate amino acidic iodides allowed a ready preparation of various 3-hydroxypyridinolines and analogues, useful as internal standards in HPLC or mass spectrometric analysis or for immunoassay development.[11b]

Results and discussion

In the course of our efforts toward the synthesis of collagen cross-links **1** and **2**, in part described in preliminary accounts, we tried to improve the accessibility of these compounds by simplifying the preparation of the 4,5-disubstituted 3-hydroxypyridine nucleus, using benzylamine as an inexpensive carrier of the heterocyclic nitrogen atom as an alternative to allylamine. By using this amine and amino esters protected as Boc, it appeared possible to assemble in just two steps the heterocyclic nucleus **7b**, useful for the synthesis of compound **1**or **2** and of other congeners (Scheme 3).

With this goal in mind, we first examined the reaction of benzylamine with the bromo ketone **4b**, having the amino group protected as its *N-tert*-butoxycarbonyl derivative (Boc). We used the same conditions reported in our protocol for the assembly of the 3-hydroxypyridinoline nucleus, and we followed the reaction by HPLC, using naphthalene as internal standard. The reaction, which involves three steps (an alkylation, a condensation, and an oxidation), was conducted in MeCN containing solid potassium carbonate, since this system was shown to be the best one for the selective reaction of the amine with the carbon carrying the bromine atom. In fact we have experienced that the use of methanol in place of MeCN, or the use of tetramethylguanidine (TMG) or of DBU in place of K_2CO_3, did not allow the initial alkylation of the amine.

Our attention was then directed to a study of the successive steps of the synthesis: the inner condensation of the amino ketone **5**, and the final oxidative aromatisation of the resulting α,β-unsaturated ketone. After extensive experimentation using unpurified **5b**, we found that the reaction gives optimal results when conducted under oxygen atmosphere, using methanol as solvent for the inner condensation and aromatisation sequence (Table 1). In fact, the simple *one-pot* dilution with methanol of the solvent used in the initial alkylation (MeCN) affords poor or erratic results.‡ Analogous poor results are obtained by diluting the initial reaction mixture with THF. We also found that the reaction times are noticeably shortened and the yield slightly improved when organic bases such as DBU or TMG were used instead of K_2CO_3. Using the system THF–K_2CO_3, the reaction is slower and the yields are lower. The reaction was studied in 0.055 mmol scale but, for synthetic purposes, is usually performed in our laboratory in 4 mmol scale.

The cyclic compound **7b**, which is easily alkylated[8,9,11] to the pyridinoline **1** and deoxypyridinoline **2**, was obtained by simple hydrogenation of the salt **6b**.

The results of this study were also useful in allowing us to set up the reactions of the benzylamine with the bromo ketone **4a**, of the allylamine with the bromo ketone **4b**, and to improve the preliminary conditions reported for the reaction of allylamine with the bromo ketone **4a**. In all these cases, the best conditions require initial use of MeCN containing K_2CO_3, and successive evaporation of the solvent and replacement with MeOH containing TMG or DBU to afford **6a**–**d**. Moreover, in the present work the deallylation of compounds **6a** and **6c** was noticeably simplified and the yields improved (>88%) by the use of the previously unreported combination of $Pd[Ph_3P]_4$[12] and 2-mercaptobenzoic acid, as allyl group scavenger.[13] In this contest Pd^0 chemistry is shown to be clearly superior to the known[11a] employed titanium(II) compounds.

Thus as a result of our work, 4,5-disubstituted 3-hydroxypyridines **7a**, and **7b**, both useful for the preparation of the collagen cross-links **1** and **2** and of other congeners, are now easily accessible. Compound **7a** is obtained from the bromo ketone **4a** and allylamine, after deallylation of the intermediate

‡ Simple dilution of initial MeCN with MeOH, reported in our original model study, could be responsible for the low yields observed by M. Adamczyk *et al.*,[7] who used our *one-pot* protocol but neglecting the fact that we did a complete solvent substitution for the synthesis of the deoxypyridinoline.

Table 1 Cyclization and aromatisation of the amino diketone **5b**, at 25 °C under O_2 atmosphere

Entry	Solvent	Base	Time (*t*/h)	Yield of **6b** (%)[a]
1	MeOH	K_2CO_3	48	66
2	MeOH	TMG	6	70
3	MeOH	DBU	6	68
4	CH_3CN–CH_3OH (1 : 1, v/v)	K_2CO_3	48	11
5	CH_3CN–THF (1 : 1, v/v)	K_2CO_3	48	10
6	THF	K_2CO_3	48	29
7	THF	DBU	24	49
8	THF	TMG	24	22

[a] Yield by HPLC calculated on starting benzylamine.

salt **6a**. The analogous compound **7b**, differing only in the protection of the α-amino groups, is obtained by reaction of the bromo ketone **4b** with allylamine or benzylamine, after deallylation or hydrogenolysis of the intermediate salts **6c** and **6b**.

In conclusion, we herein show that the selective reaction of a bromo ketone with allylamine or benzylamine represents a general useful protocol for assembling 4,5-disubstituted 3-hydroxypyridines **7a** and **7b**, compounds of wide synthetic and biological utility. This route complements our previous protocol useful for the assembly, in a simple way, of the 4,5-substituted 3-hydroxypyridine nucleus[11a] having an alkylated heterocyclic nitrogen atom. Moreover our work allows us to prepare the parent amino acidic 3-hydroxypyridine **3**, potentially useful for studies of biological activity or for the evaluation of the specificity of antibodies against pyridinoline **1** and deoxypyridinoline **2** present in diagnostic kits. In fact compound **3** is obtained from **7a**, or more directly from **6d**, by hydrogenolysis followed by trifluoroacetic acidic hydrolysis, or from **7b** by simple acidic hydrolysis.

In light of the results herein reported, the use of a starting bromo ketone protected as its bis(*tert*-butoxycarbonyl) derivative at the amino group, to improve the yields[7] of the 4,5-disubstituted pyridine-assembling nucleus, appears not to be useful, considering the time-consuming and expensive preparation of the starting material.

Experimental

General

Mps were measured on a SMP3 mp apparatus (Stuart Scientific, USA) and are not corrected. Proton nuclear magnetic resonance spectra were recorded in $CDCl_3$ or D_2O at 303 K on a Bruker AM-500 spectrometer operating at 500.13 MHz. Chemical shifts are reported as δ-values in ppm, relative to residual $CHCl_3$ (δ 7.24) or HDO (δ 4.54) as internal standard. Multiplicities are described using the following abbreviations: s = singlet, d = doublet, t = triplet, q = quartet. Coupling constants (J) are reported in Hz. 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}.

HPLC analyses were carried out on an RP-18 column (LiChroCART, 125 mm, 4 mm ID, 5 μm purchased from Merck); the mobile phase was MeCN–0.2% TFA in water, 70 : 30, v/v; the flow rate was 1 cm^3 min^{-1} and 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 or 0.2% ninhydrin in ethanol and heat as detecting agent. E. Merck 230–400 mesh silica gel was used for flash column chromatography.[14]

(*S*)-2-Benzyloxycarbonylamino-6-bromo-5-oxohexanoic acid *tert*-butyl ester 4a

(i) Preparation of (*S*)-2-benzyloxycarbonylamino-6-diazo-5-oxohexanoic acid *tert*-butyl ester. To a solution of 2-(benzyloxycarbonylamino)pentanedioic acid *tert*-butyl ester[15] (10 g, 29.7 mmol) and 4-methylmorpholine (3.58 cm^3, 32.6 mmol) in THF (200 cm^3) cooled at −20 °C was added dropwise a solution of isobutyl chloroformate (4.21 cm^3, 32.4 mmol) [or benzyl chloroformate (4.61 cm^3, 32.4 mmol)] in THF (20 cm^3). After stirring of the mixture for 30 min at −20 °C, the solution was filtered on a pad of Celite and added dropwise a solution of diazomethane (237 mmol) in diethyl ether cooled to −20 °C. The reaction mixture was kept at 0 °C for 12 h, the time necessary to assure complete formation of the diazo ketone. At this time, the excess of diazomethane was destroyed with AcOH, and the solution was concentrated under reduced pressure (below 40 °C) and diluted with AcOEt (200 cm^3). The organic layers were washed successively with water, saturated aq. $NaHCO_3$, and water, dried on anhydrous Na_2SO_4, and evaporated. The residue was flash chromatographed (eluting with hexane–AcOEt, 60 : 40, v/v) to afford (*S*)-2-*benzyloxycarbonylamino*-6-*diazo*-5-*oxohexanoic acid tert-butyl ester* (9.44 g, 88%) as an oil (Found: C, 59.9; H, 6.3; N, 11.7. $C_{18}H_{23}N_3O_5$ requires C, 59.8; H, 6.4; N, 11.6%); $[\alpha]_D$ +16.6 (*c* 2 in $CHCl_3$); δ_H (500 MHz; $CDCl_3$) 1.43 [9 H, s, $C(CH_3)_3$], 1.94 (1 H, dddd, *J* 5.5, 8.5, 8.5 and 14.0, 3-Ha), 2.16 (1 H, m, 3-Hb), 2.27–2.44 (2 H, overlapping, 4-Ha and 4-Hb), 4.22 (1 H, ddd, *J* 4.5, 7.5 and 8.5, 2-H), 5.05–5.12 (2 H, AB system, OCH_2Ph), 5.20 (1 H, br s, 6-H), 5.41 (1 H, d, *J* 7.5, NH), 7.28–7.37 (5 H, Ph).

(ii) Preparation of (*S*)-2-benzyloxycarbonylamino-6-bromo-5-oxohexanoic acid *tert*-butyl ester 4a. To a solution of (*S*)-2-benzyloxycarbonylamino-6-diazo-5-oxohexanoic acid *tert*-butyl ester (9.0 g, 24.9 mmol) in THF (80 cm^3) containing Thymol Blue (5 mg) was added dropwise a solution of HBr in AcOH (33%) under stirring at −5 °C. As soon as the solution turned violet, saturated aq. $NaHCO_3$ was added followed by AcOEt (150 cm^3) and the resulting solution was washed with water (150 cm^3) and dried on anhydrous Na_2SO_4. Evaporation of the mixture under reduced pressure afforded a residue, which was purified by flash chromatography (eluting with hexane–AcOEt, 80 : 20, v/v) to afford (*S*)-2-*benzyloxycarbonylamino*-6-*bromo*-5-*oxohexanoic acid tert-butyl ester* **4a** (6.6 g, 64%) as an oil (Found: C, 52.0; H, 5.8; N, 3.5. $C_{18}H_{24}BrNO_5$ requires C, 52.2; H, 5.8; N, 3.4%); $[\alpha]_D$ −0.3 (*c* 1, $CHCl_3$); TLC (hexane–AcOEt; 60 : 40; v/v; R_f 0.55); δ_H ($CDCl_3$) 1.46 [9 H, s, $C(CH_3)_3$], 1.89 (1 H, m, 3-Ha), 2.20 (1 H, m, 3-Hb), 2.67 (1 H, ddd, *J* 5.5, 8.5, 18.0, 4-Ha), 2.75 (1H, ddd, *J* 7.0, 8.5, 18.0, 4-Hb), 3.87 (2 H, s, 6-H_2), 4.24 (1 H, ddd, *J* 4.5, 8.0, 8.5, 2-H), 5.06–5.14 (2 H, AB system, OCH_2Ph), 5.37 (1 H, d, *J* 8.0, NH), 7.31–7.38 (5 H, Ph).

(*S*)-6-Bromo-2-*tert*-butoxycarbonylamino-5-oxohexanoic acid *tert*-butyl ester 4b

(i) Preparation of (*S*)-2-*tert*-butoxycarbonylamino-6-diazo-5-oxohexanoic acid *tert*-butyl ester. Starting with a solution of (*S*)-2-(*tert*-butoxycarbonylamino)pentanedioic acid 1-*tert*-butyl ester (10 g, 33.0 mmol; Aldrich) and following the procedure described above for the preparation of the analogue **4a**, (*S*)-*2-tert-butoxycarbonylamino*-6-*diazo*-5-*oxohexanoic acid*

tert-butyl ester was first obtained (8.52 g, 79%) as a pure oil after flash chromatography eluting with hexane–AcOEt (60 : 40, v/v) (Found: C, 54.8; H, 7.6; N, 12.7. $C_{15}H_{25}N_3O_5$ requires C, 55.0; H, 7.7; N, 12.8%); $[\alpha]_D$ +11.4 (*c* 1 in $CHCl_3$); δ_H (500 MHz; $CDCl_3$) 1.45 [9 H, s, $C(CH_3)_3$], 1.47 [9 H, s, $C(CH_3)_3$], 1.88 (1 H, m, 3-Ha), 2.20 (1 H, m, 3-Hb), 2.32–2.45 (2 H, overlapping, together 4-H_2), 4.17 (1 H, m, 2-H), 5.09 (1 H, d, *J* 5.0, NH), 5.20 (1 H, br s, 6-H).

(ii) Preparation of (*S*)-6-bromo-2-*tert*-butoxycarbonylamino-5-oxohexanoic acid *tert*-butyl ester 4b. The obtained (*S*)-2-*tert*-butoxycarbonylamino-6-diazo-5-oxohexanoic acid *tert*-butyl ester (8.0 g, 24.4 mmol) was treated with a solution of HBr in AcOH (33%) as described above for the benzyloxycarbonyl analogue and afforded (*S*)-6-bromo-2-*tert*-butoxycarbonylamino-5-oxohexanoic acid *tert*-butyl ester **4b** (6.03 g, 65%) as a pure oil after flash chromatography eluting with hexane–AcOEt (80 : 20, v/v) (Found: C, 47.5; H, 7.1; N, 3.7. $C_{15}H_{26}BrNO_5$ requires C, 47.4; H, 6.9; N, 3.7%); TLC (hexane–AcOEt, 50 : 50, v/v; R_f 0.66); $[\alpha]_D$ −1.08 (*c* 1, $CHCl_3$) (lit.,[9] −0.88); δ_H ($CDCl_3$) 1.45 [9 H, s, $C(CH_3)_3$], 1.47 [9 H, s, $C(CH_3)_3$], 1.86 (1 H, m, 3-Ha), 2.19 (1 H, m, 3-Hb), 2.67 (1 H, ddd, *J* 6.0, 8.5, 18.0, 4-Ha), 2.74 (1 H, ddd, *J* 7.5, 8.5, 18.0, 4-Hb), 3.89 (2 H, s, 6-H_2), 4.19 (1 H, ddd, *J* 4.5, 8.0, 8.5, 2-H), 5.06 (1 H, d, *J* 8.0, NH).

General procedure for selecting the best conditions for the preparation of (*S,S*)-1-benzyl-4-[2-benzyloxycarbonylamino-2-(*tert*-butoxycarbonyl)ethyl]-5-[3-benzyloxycarbonylamino-3-(*tert*-butoxycarbonyl)propyl]pyridinium-3-olate 6b starting from benzylamine and bromo ketone 4b (Table 1)

To a solution of benzylamine (5.9 mg, 0.055 mmol) and the bromo ketone, **4b** (50 mg, 0.13 mmol) in CH_3CN (2 cm^3) was added anhydrous K_2CO_3 (27 mg, 0.19 mmol) and the mixture was stirred under nitrogen for 8 h. At this time the disappearance of the starting bromo ketone was monitored (TLC), and, in experiments 4 and 5 (Table 1), the mixture was directly diluted with a new solvent: MeOH (2 cm^3, entry 4) or THF (2 cm^3, entry 5). In the other cases, the solid K_2CO_3 was filtered off, the solvent evaporated off, and the crude residue **5b** was recovered with MeOH (2 cm^3, entries 1–3) or THF (2 cm^3, entries 6–8). In all cases naphthalene (11 mg) was added to the reaction mixture (as internal standard) followed by the appropriate base: K_2CO_3 (27 mg, 0.19 mmol, entries 1, 4–6), TMG (19.6 mg, 0.17 mmol, entries 2 and 8) or DBU (25.8 mg, 0.17 mmol, entries 3 and 7).

Each resulting mixture was stirred at 25 °C under a oxygen atmosphere for the appropriate time (Table 1), with monitoring of the formation of title compound **6b** by HPLC.

Application of the general procedure selected for the preparation of 1,4,5-trisubstituted pyridinium-3-olate nucleus (6a–d)

To a solution containing benzylamine or allylamine (4 mmol) and the appropriate bromo ketone **4a** or **4b** (8.8 mmol) in CH_3CN (80 cm^3) was added anhydrous K_2CO_3 (1.85 g, 13.2 mmol) and the mixture was stirred under nitrogen for 8 h. At this time the disappearance of the starting bromo ketone was monitored (TLC), the solid K_2CO_3 was filtered off on a pad of Celite, and the solvent was evaporated off under reduced pressure. The residue (**5a**–**d**) was then dissolved in MeOH (80 cm^3) and TMG (1.5 cm^3, 12 mmol) was added to the mixture, which was vigorously stirred at 25 °C under an oxygen atmosphere for 6 h. At this time, the solvent was removed under reduced pressure and the residue was purified by rapid chromatography.

(*S,S*)-1-Allyl-4-[2-benzyloxycarbonylamino-2-(*tert*-butoxycarbonyl)ethyl]-5-[3-benzyloxycarbonylamino-3-(*tert*-butoxycarbonyl)propyl]pyridinium-3-olate 6a

The general procedure was used with allylamine and bromo ketone **4a** to give, after chromatographic purification (eluting with AcOEt–MeOH, 90 : 10, v/v), the *pyridiniumolate* **6a** (1.83 g, 65%) as an oil (Found: C, 66.4; H, 6.8; N, 6.1. $C_{39}H_{49}N_3O_9$ requires C, 66.6; H, 7.0; N, 6.0%); $[\alpha]_D$ +2.3 (*c* 1, $CHCl_3$); δ_H ($CDCl_3$) 1.37 [9 H, s, $C(CH_3)_3$], 1.43 [9 H, s, $C(CH_3)_3$], 1.89 (1 H, m), 2.02 (1 H, m), 2.71 (2 H, m), 2.96 (1 H, m), 3.31 (1 H, m), 4.20 (1 H, m, NCHCO), 4.27 (1 H, m, NCHCO), 4.56 (2 H, d, *J* 5.5, $N^+CH_2CH{=}CH_2$], 4.93–5.10 (4 H, 2 × OCH_2Ph), 5.36 (1 H, dd, *J* 16.9, <1, $N^+CH_2CH{=}CHH$), 5.40 (1 H, dd, *J* 10.1, <1, $N^+CH_2CH{=}CHH$), 5.62 (2 H, m, 2 × NH), 5.91 (1 H, m, $N^+CH_2CH{=}$), 7.08 (1 H, br s, pyridinium ring proton), 7.23–7.33 (10 H, Ph), 7.71 (1 H, br s, pyridinium ring proton).

(*S,S*)-1-Benzyl-4-[2-*tert*-butoxycarbonylamino-2-(*tert*-butoxycarbonyl)ethyl]-5-[3-*tert*-butoxycarbonylamino-3-(*tert*-butoxycarbonyl)propyl]pyridinium-3-olate 6b

The general procedure was used with benzylamine and bromo ketone **4b** to give, after chromatographic purification (eluting with CH_2Cl_2–MeOH, 100 : 5, v/v), the *pyridiniumolate* **6b** (1.81 g, 66%) as an oil (Found: C, 64.7; H, 8.0; N, 6.3. $C_{37}H_{55}N_3O_9$ requires C, 64.8; H, 8.1; N, 6.1%); $[\alpha]_D$ +4.2 (*c* 1, $CHCl_3$); δ_H ($CDCl_3$) 1.34 [9 H, s, $C(CH_3)_3$], 1.37 [9 H, s, $C(CH_3)_3$], 1.43 (18 H, s, 2 × $C(CH_3)_3$], 1.83 (1 H, m), 1.94 (1 H, m), 2.68 (2 H, m), 2.89 (1 H, m), 3.30 (1 H, m), 4.13 (2 H, overlapping, 2 × NCHCO), 5.16 (2 H, s, N^+CH_2Ph), 5.19 (2 H, m, 2 × NH), 7.20 (1 H, br s, pyridinium ring proton), 7.31–7.39 (5 H, Ph), 7.60 (1 H, br s, pyridinium ring proton).

(*S,S*)-1-Allyl-4-[2-*tert*-butoxycarbonylamino-2-(*tert*-butoxycarbonyl)ethyl]-5-[3-*tert*-butoxycarbonylamino-3-(*tert*-butoxycarbonyl)propyl]pyridinium-3-olate 6c

The general procedure was used with allylamine and bromo ketone **4b** to give, after chromatographic purification (eluting with CH_2Cl_2–MeOH, 100 : 2, v/v), the *pyridiniumolate* **6c** (1.60 g, 63%) as an oil (Found: C, 62.1; H, 8.4; N, 6.8. $C_{33}H_{53}N_3O_9$ requires C, 62.3; H, 8.4; N, 6.6%); $[\alpha]_D$ +1.8 (*c* 1, $CHCl_3$); δ_H ($CDCl_3$) 1.34 [9 H, s, $C(CH_3)_3$], 1.38 [9 H, s, $C(CH_3)_3$], 1.43 (18 H, s, 2 × $C(CH_3)_3$], 1.84 (1 H, m), 1.94 (1 H, m), 2.70 (2 H, m), 2.92 (1 H, m), 3.31 (1 H, m), 4.14 (2 H, overlapping, 2 × NCHCO), 4.59 (2 H, d, *J* 5.6, $N^+CH_2CH{=}CH_2$), 5.21 (2 H, m, 2 × NH), 5.38 (1 H, dd, *J* 17.0, <1, $N^+CH_2CH{=}CHH$), 5.42 (1 H, dd, *J* 9.8, <1, $N^+CH_2CH{=}CHH$), 5.91 (1 H, m, $N^+CH_2CH{=}$), 7.20 (1 H, br s, pyridinium ring proton), 7.60 (1 H, br s, pyridinium ring proton).

(*S,S*)-1-Benzyl-4-[2-benzyloxycarbonylamino-2-(*tert*-butoxycarbonyl)ethyl]-5-[3-benzyloxycarbonylamino-3-(*tert*-butoxycarbonyl)propyl]pyridinium-3-olate 6d

The general procedure was used with benzylamine and bromo ketone **4a** to give, after chromatographic purification (eluting with AcOEt–MeOH, 100 : 15, v/v), the *pyridiniumolate* **6d** (1.96 g, 65%) as an oil (Found: C, 68.6; H, 6.7; N, 5.5. $C_{43}H_{51}N_3O_9$ requires C, 68.5; H, 6.8; N, 5.6%); $[\alpha]_D$ +4.1 (*c* 1 in $CHCl_3$); δ_H ($CDCl_3$) 1.34 [9 H, s, $C(CH_3)_3$], 1.42 [9 H, s, $C(CH_3)_3$], 1.85 (1 H, m), 1.98 (1 H, m), 2.67 (2 H, m), 2.91 (1 H, m), 3.31 (1 H, m), 4.19 (2 H, overlapping, 2 × NCHCO), 4.90–5.09 (6 H, 2 × OCH_2Ph and N^+CH_2Ph), 5.57 (2 H, m, 2 × NH), 7.07 (1 H, br s, pyridinium ring proton), 7.22–7.36 (15 H, Ph), 7.56 (1 H, br s, pyridinium ring proton).

(*S,S*)-4-[2-Benzyloxycarbonylamino-2-(*tert*-butoxycarbonyl)-ethyl]-5-[3-benzyloxycarbonylamino-3-(*tert*-butoxycarbonyl)-propyl]-3-hydroxypyridine 7a

(i) Using Pd^0 chemistry. To a solution of 1-allyl-4,5-disubstituted pyridinium-3-olate derivative **6a** (1.41 g, 2 mmol) and 2-mercaptobenzoic acid (1.54 g, 10 mmol) in CH_2Cl_2 (150 cm^3), was added tetrakis(triphenylphosphine)palladium(0) (121 mg, 0.1 mmol). The resulting orange solution was stirred

for 1.5 h at room temperature under argon. Then the solution was washed with a saturated aq. $NaHCO_3$ (100 cm^3) and the aqueous phase was extracted with CH_2Cl_2 (2 × 20 cm^3). The combined organic phases were dried over anhydrous Na_2SO_4 and the solvent was evaporated off under reduced pressure to afford, after column chromatography (eluting with CH_2Cl_2–AcOEt, 40 : 60, v/v), the desired 3-hydroxypyridine **7a** (1.07 g, 81%) as a glass (Found: C, 64.9; H, 6.8; N, 6.2. $C_{36}H_{45}N_3O_9$ requires C, 65.1; H, 6.8; N, 6.3%); mp 71–80 °C; $[a]_D$ +1.4 (*c* 1, $CHCl_3$) (lit.,[11] +1.2); δ_H ($CDCl_3$) 1.31 [9 H, s, $C(CH_3)_3$], 1.46 [9 H, s, $C(CH_3)_3$], 1.88 (1 H, m), 2.01 (1 H, m), 2.69 (2 H, m), 3.09 (2 H, m), 4.30 (1 H, m, NCHCO), 4.44 (1 H, m, NCHCO), 4.94–5.14 (4 H, 2 × OCH_2Ph), 5.82 (2 H, m, 2 × NH), 7.20–7.34 (10 H, Ph), 7.88 (1 H, br s, pyridinium ring proton), 8.08 (1 H, br s, pyridinium ring proton).

(ii) Using titanium(IV) chemistry. To a solution of 1-allyl-4,5-disubstituted pyridinium-3-olate derivative **6a** (1.41 g, 2 mmol) in diethyl ether (7.0 cm^3) and titanium(IV) isopropoxide (2.94 cm^3, 10 mmol), cooled to −45 °C, was added isopropylmagnesium chloride (10.0 cm^3 of a 2 M solution in diethyl ether, 20 mmol) and the mixture was stirred for 1 h at −45 °C. At this time, the brown mixture was poured into ice-cold aq. 1 M HCl (15.5 cm^3) and the organic phase was separated, and washed successively with water, saturated aq. $NaHCO_3$, and water. Then the organic layer was dried over anhydrous Na_2SO_4 and the solvent was evaporated off under reduced pressure to afford, after column chromatography (eluting with CH_2Cl_2–AcOEt, 40 : 60, v/v), the desired 3-hydroxypyridine **7a** (1.00 g, 76%) as glassy soild, identical in all respects with that described above.

(*S,S*)-4-[2-*tert*-Butoxycarbonylamino-2-(*tert*-butoxycarbonyl)-ethyl]-5-[3-*tert*-butoxycarbonylamino-3-(*tert*-butoxycarbonyl)-propyl]-3-hydroxypyridine 7b

(i) From the benzyl derivative 6b. The pyridinium derivative **6b** (0.68 g, 1.0 mmol) was dissolved in methanol (100 cm^3) and hydrogenated in the presence of 10% Pd/C (150 mg) for 1 h. After filtration on a pad of Celite, the solvent was evaporated off to afford a residue, which, after column chromatography (eluting with CH_2Cl_2–AcOEt, 40 : 60, v/v), afforded the 3-hydroxypyridine **7b** (0.51 g, 85%) as a solid (Found: C, 60.6; H, 8.2; N, 7.0. $C_{30}H_{49}N_3O_9$ requires C, 60.5; H, 8.3; N, 7.0%); mp 85–88 °C (lit.,[9] 86–89 °C) $[a]_D$ −12.8 (*c* 1 in MeOH); δ_H ($CDCl_3$) 1.43 [18 H, s, 2 × $C(CH_3)_3$], 1.46 [18 H, s, 2 × $C(CH_3)_3$], 1.82 (1 H, m), 2.02 (1 H, m), 2.70 (2 H, m), 3.06 (2 H, m), 4.21 (1 H, m, NCHCO), 4.36 (1 H, m, NCHCO), 5.25 (1 H, br s, NH), 5.59 (1 H, br s, NH), 7.90 (1 H, br s, pyridinium ring proton), 8.12 (1 H, br s, pyridinium ring proton).

(ii) From the allyl derivative 6c. To a solution of 1-allyl-4,5-disubstituted pyridinium-3-olate derivative **6c** (1.27 g, 2 mmol) and 2-mercaptobenzoic acid (1.54 g, 10 mmol) in CH_2Cl_2 (150 cm^3) was added tetrakis(triphenylphosphine)palladium(0) (121 mg, 0.1 mmol). The resulting orange solution was stirred under argon at room temperature for 1.5 h. Then the solution was washed with saturated aq. $NaHCO_3$ (100 cm^3) and the aqueous phase was extracted with CH_2Cl_2 (2 × 20 cm^3). The combined organic phases were dried over anhydrous Na_2SO_4 and the solvent was evaporated off under reduced pressure to afford, after column chromatography (eluting with CH_2Cl_2–AcOEt, 40 : 60, v/v), the desired 3-hydroxypyridine **7b** (0.94 g, 79%) identical in all aspects with that reported above.

(*S,S*)-4-[2-Amino-2-carboxyethyl]-5-[3-amino-3-carboxypropyl-]-3-hydroxypyridine 3

(i) From the benzyl derivative 6d. The benzyl derivative **6d** (0.75 g, 1 mmol), dissolved in MeOH (150 cm^3), was hydrogenated in the presence of 10% Pd/C (150 mg) for 12 h at room temperature and atmospheric pressure. After filtration to remove the catalyst and evaporation of the MeOH under reduced pressure, the residue was dissolved in TFA–water (10 cm^3, 95 : 5, v/v) and the resulting solution was stirred at room temperature for 2 h. Evaporation of the mixture afforded the hydroxypyridine **3**[9] (0.226 g, 73%) as a glass which resisted all efforts at crystallisation (Found: C, 50.7; H, 6.1; N, 14.7. $C_{12}H_{17}N_3O_5$ requires C, 50.9; H, 6.05; N, 14.8%); δ_H (D_2O) 2.03 (2 H, m), 2.73 (1 H, m), 2.83 (1 H, m), 3.22 (2 H, m), 3.80 (1 H, dd, *J* 5.8, 5.8, NCHCO), 4.00 (1 H, dd, *J* 6.7, 7.9, NCHCO), 8.01 (1 H, br s, pyridinium ring proton), 8.04 (1 H, br s, pyridinium ring proton).

(ii) From the pyridine derivative 7a. The pyridine derivative **7a** (1.33 g, 2.0 mmol) was dissolved in methanol (200 cm^3) and hydrogenated in the presence of 10% Pd/C (300 mg) for 12 h at room temperature and atmospheric pressure. After filtration to remove the catalyst and evaporation of the MeOH under reduced pressure, the residue was dissolved in TFA–water (20 cm^3, 95 : 5, v/v) and the resulting solution was stirred at room temperature for 2 h. Evaporation of the mixture afforded the hydroxypyridine **3** (0.439 g, 71%) identical in all aspects with the compound reported above.

(iii) From the pyridine derivative 7b. The pyridine derivative **7b** (1.19 g, 2.0 mmol) was dissolved in TFA–water (20 cm^3; 95 : 5, v/v) and the resulting solution was stirred at room temperature for 2 h. Evaporation of the mixture afforded the hydroxypyridine **3** (0.482 g, 78%) identical in all aspects with the compound reported above.

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