COMMUNICATION

A new modular synthesis of deoxypyridinoline, a primary reference material for monitoring bone metabolism

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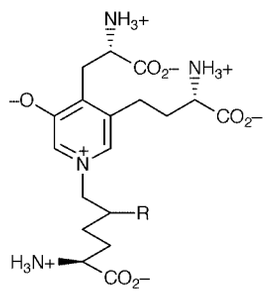
A new modular synthesis of deoxypyridinoline is described which assembles first the 4,5-disubstituted 3-hydroxypyridine ring and then inserts the L-lysine side chain at the pyridine nitrogen.

Pyridinoline **1** and deoxypyridinoline **2** are two crosslinks of the mature form of collagen and, at present, are considered

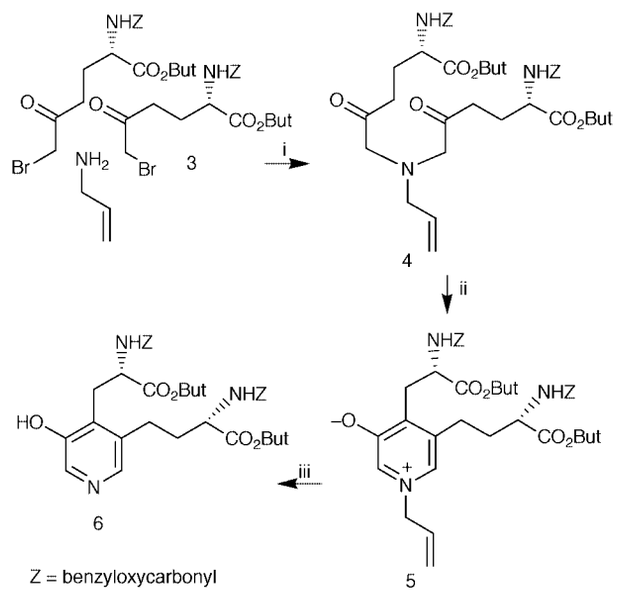


1 R = OH; Pyridinoline
2 R = H; Deoxypyridinoline

the most effective biochemical markers for the assessment of bone resorption correlated with diseases such as osteoporosis, bone cancer and arthropathies.[1] Thus these collagen crosslinks are measured routinely by using different HPLC assays as diagnostic or prognostic tools or for monitoring therapy.[1,2] Compounds **1** and **2** were isolated from bone and converted completely to monochloride trihydrochloride salts by S. P. Robins *et al.*[3] who assessed the composition, the purity, and some physicochemical properties (UV molar absorptivity, high field ^{1}H-NMR, mass spectra, elemental analysis, including the chloride counterions and HPLC behaviour) of the isolated compounds. As a consequence, many analytical methods (physicochemical or immunological)[4,5] have been set up for the detection of pyridinoline **1** and of deoxypyridinoline **2** in human urine using reference standards calibrated on the base of UV molar absorptivity values reported by the Robins group.[3]

In a recent paper[6] we have reported the first synthesis of deoxypyridinoline **2** in significant amounts which have allowed the preparation of two salts of this compound, the monotrifluoroacetate monohydrate and the chloride trihydrochloride monohydrate. This has permitted the first comparison of the physicochemical properties of these synthetic salts with those of the natural pyridinoline. All physicochemical properties were confirmed but minor discrepancies were evidenced concerning the UV molar absorptive values which were proved higher (10–20%) than those reported for the chloride trichloride dihydrate obtained from natural sources.[3] In a different synthesis,[7] published at the same time as our paper, various physicochemical characteristics of deoxypyridinoline were given, but those necessary for the standardisation of secondary analytical standards (elemental analysis of the salt, UV molar absorptivity) were not reported. In a previous synthesis[8] no effort was devoted to defining the stereochemistry and the composition of the isolated salt of deoxypyridinoline.

In this paper we report a new synthesis of deoxypyridinoline **2** and its properties.† The synthesis of the deoxypyridinoline was performed by constructing first the 3-hydroxypyridine ring, lacking the lysine side chain, and then introducing the substituent at the pyridine nitrogen. The pyridine ring was constructed (Scheme 1) by reaction of allylamine with the

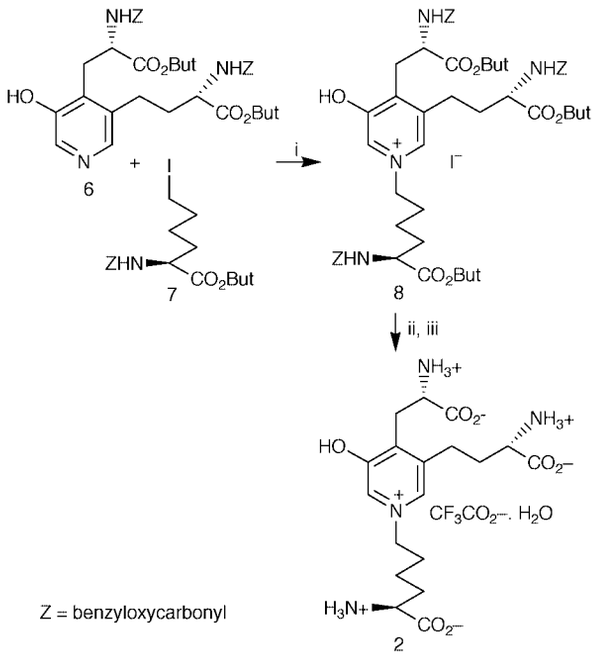


Z = benzyloxycarbonyl

Scheme 1 *Reagents and conditions:* i, K_2CO_3, MeCN, room temp.; ii, DBU, air, THF, room temp., 41% from **3**; iii, Ti(O^{i}Pr)$_4$ (5 equiv.), ^{i}PrMgBr (10 equiv.), Et_2O, −45 °C, 1 h then HCl (1 M), 76%.

bromoketone **3**, obtained from *N*-(benzoyloxycarbonyl)-L-glutamic acid *tert*-butyl ester.[6] The reaction occurs in MeCN saturated with K_2CO_3 and affords the relatively unstable amine **4** which was not isolated but was transformed into the protected 3-hydroxy-4,5-substituted pyridinium derivative **5**‡ by evaporation of the solvent (MeCN) under reduced pressure and dissolution of the crude residue into THF containing DBU.

This part of the synthesis represents an evolution of our method[6] for preparing the 3-hydroxy-4,5-substituted pyridinium ring. It shows various elements of novelty such as the use of allylamine as a temporary chemical auxiliary for the introduction of the pyridine nitrogen atom and the deallylation of compound **5** to afford the substituted 3-hydroxypyridine **6**.§ This was obtained by extending to a nitrogen atom the *C*-deallylation promoted by (η^2-propene)Ti(O-i-Pr)$_4$. This titanium(II) compound is a convenient deallylating agent of 2-allylmalonic esters[9] but allows also the deallylation of compound **5** in conditions which well tolerate the presence of the protective groups of its amino acidic moiety. In the successive step, the alkylation of the pyridine nitrogen (Scheme 2) was performed using the iodide **7**, obtained[10,11] from the known[6] 6-amino-2-benzyloxycarbonylaminohexanoic acid *tert*-butyl



Scheme 2 *Reagents and conditions:* i, MeCN, reflux, 4 h, 76%; ii, H_2, Pd/C, MeOH, room temp., 93%; iii, TFA, room temp., 72%.

ester by simple chemistry.¶ In the best conditions found, the alkylation of **6** to afford compound **8** was performed in MeCN. Protected deoxypyridinoline **8** was isolated (in 76% yield) in a form sufficiently pure‖ for the subsequent regeneration of the protected functions (hydrogenolysis followed by treatment with TFA).[6] Thus the trifluoroacetate monohydrate of deoxypyridinoline **2** was isolated** with the identical water content and physicochemical properties to those previously observed.[6] This monohydrate salt, when dissolved in excess hydrochloric acid, forms a chloride trichloride which is recovered by elimination of the solvent under reduced pressure and may be crystallised from aqueous ethanol.[7]

The isolated salt was then used for the determination of the optical rotation and of the UV molar absorptivity values. These were in complete agreement with those previously observed by us and essentially confirm those reported by Robins *et al.*[3] even if UV absorptivity is still a little higher (10–20%) than that observed for deoxypyridinoline purified from bone.

In conclusion we report a new method for preparing deoxypyridinoline **2** which appears more flexible than that we previously reported and should permit the synthesis also of pyridinoline **1** and other congeners, with a shorter or a longer amino acid at the pyridinic nitrogen. These are useful internal standards[12] for detecting deoxypyridinoline by various analytical procedures (using HPLC or mass spectrometry).

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Notes and references

† All new compounds gave elemental C, H, N and Cl analyses that were within 0.3% of the theoretical values. Deoxypyridinoline chloride trichloride was obtained from bone[3] as the dihydrate, probably due to the use of a different procedure for drying the salt.

‡ *Selected data* for **5**: oil; $[\alpha]_D^{20}$ +2.3 (*c* 1 in $CHCl_3$); δ_H (500 MHz, $CDCl_3$) 7.71 (1H, br s, pyridinium ring proton), 7.33–7.23 (10H, phenylic protons), 7.08 (1H, br s, pyridinium ring proton), 5.91 (1H, m, N^+CH_2CH=), 5.62 (2H, m, 2 × N*H*), 5.40 (1H, dd, *J* 10.1, < 1, N^+CH_2-CH=C*H*H), 5.36 (1H, dd, *J* 16.9, < 1, N^+CH_2CH=CH*H*), 5.10–4.93 (4H, 2 × OCH_2Ph), 4.56 (2H, d, *J* 5.5, N^+CH_2CH=CH_2), 4.27 (1H, m, NC*H*CO), 4.20 (1H, m, NC*H*CO), 3.31 (1H, m), 2.96 (1H, m), 2.71 (2H, m), 2.02 (1H, m), 1.89 (1H, m), 1.43 (9H, s, $C(CH_3)_3$), 1.37 (9H, s, $C(CH_3)_3$).

§ *Selected data* for **6**: glassy; mp 71–80 °C; $[\alpha]_D^{20}$ +1.2 (*c* 1 in $CHCl_3$); δ_H (500 MHz, $CDCl_3$) 8.08 (1H, br s, pyridinium ring proton), 7.88 (1H, br s, pyridinium ring proton), 7.34–7.20 (10H, phenylic protons), 5.82 (2H, m, 2 × N*H*), 5.14–4.94 (4H, 2 × OCH_2Ph), 4.44 (1H, m, NC*H*CO), 4.30 (1H, m, NC*H*CO), 3.09 (2H, m), 2.69 (2H, m), 2.01 (1H, m), 1.42 (1H, m), 1.46 (9H, s, $C(CH_3)_3$), 1.31 (9H, s, $C(CH_3)_3$).

¶ From this L-lysine derivative, the iodide **7** was obtained by diazotisation with $NaNO_2$ in aqueous AcOH[10] and iodination with triphenylphosphyne–iodine–imidazole.[7,11] *Selected data* for **7**: oil; $[\alpha]_D^{20}$ +11.0 (*c* 2.6 in *CHCl*$_3$); δ_H (500 MHz, $CDCl_3$) 7.40–7.28 (5H, phenylic protons), 5.33 (1H, d, *J* 7.4, N*H*), 5.09 (1H, A part of AB system, OC*H*HPh), 5.07 (1H, B part of AB system, OCH*H*Ph), 4.23 (1H, m, NC*H*CO), 3.14 (2H, t, *J* 6.7, CH_2I), 1.87–1.75 (3H, overlapping), 1.64 (1H, m), 1.48–1.40 (2H, overlapping), 1.45 (9H, s, $C(CH_3)_3$).

‖ *Selected data* for **8**: oil; $[\alpha]_D^{20}$ +5.1 (*c* 1 in $CHCl_3$); δ_H (500 MHz, $CDCl_3$) 8.09 (1H, br s, pyridinium ring proton), 7.40–7.20 (15H, phenylic protons), 6.90 (1H, br s, pyridinium ring proton), 5.61 (1H, d, *J* 7.0, N*H*), 4.93 (2H, m, 2 × N*H*), 5.16–5.05 (6H, overlapping, 3 × OCH_2Ph), 4.24–4.14 (3H, overlapping, 3 × NC*H*CO), 3.87 (2H, m, N^+CH_2), 3.37 (1H, m), 2.88 (1H, dd, *J* 12.0, 3.0), 2.68 (2H, m), 2.08 (1H, m), 1.92–1.80 (4H, overlapping), 1.65 (1H, m), 1.45, 1.42, 1.40 (3 × 9H, 3 × s, 3 × $C(CH_3)_3$), 1.35 (2H, m).

** *Selected data* for **2** chloride trichloride monohydrate. Found: C, 37.61; H, 6.20; Cl, 24.40; N, 9.51. $C_{18}H_{34}Cl_4N_4O_8$ requires: C, 37.51; H, 5.95; Cl, 24.61; N, 9.72%. $[\alpha]_D^{20}$ +32.1 (*c* 0.9 in D_2O); λ_{max} (HCl 0.1 M)/nm 239 (ε/dm^3 mol^{-1} cm^{-1} 3820), 293 (6460); λ_{max} (50 mM phosphate buffer, pH 7.5)/nm 252 (ε/dm^3 mol^{-1} cm^{-1} 3640), 324 (6080). All other characteristics of this compound and of the corresponding monotrifluoroacetate monohydrate were completely identical to those reported for this salt in a previous paper.[6]

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