Total synthesis of deoxypyridinoline, a biochemical marker of collagen turnover

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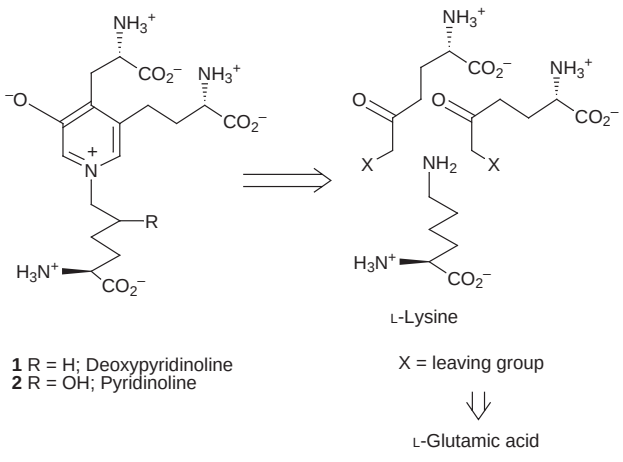
A convergent synthesis of deoxypyridinoline is described which involves the assembly of a suitably polysubstituted 3-hydroxypyridinium ring starting with fully protected L-lysine and L-glutamic acid.

The paper describes the synthesis[1] and complete characterization of the optically pure deoxypyridinoline **1**. This compound and pyridinoline **2** represent two crosslinks of the mature form of collagen and, at present, are the most effective biochemical markers of collagen turnover correlated with diseases such as osteoporosis, bone cancer and arthropathies.[2]

Compounds **1** and **2** were isolated from bones after several purification steps, but despite the various efforts devoted to improve their extraction, the yields are currently very poor.[3] Although in only small amounts, deoxypyridinoline **1** and pyridinoline **2** have recently been obtained in high purity from bones,[4] and their individual UV molar extinction coefficient values have been established. These values are necessary for the correct standardization of the analytical techniques normally used in clinical determinations in human urine. On the other hand it is still impossible to have a definitive validation of these parameters determined from synthetic compounds using the only multistep synthesis of the deoxypyridinoline **1** (of unspecified diastereomeric purity) and of the pyridinoline **2** (as mixture of epimers at the hydroxylated alkyl carbon) so far reported.[5] This synthesis is accomplished starting with a single fully-protected synthetic L-amino acid, prepared according to the Schoelkopf's approach. However, the enantiomeric purity of this starting building block as well as the physico-chemical parameters (UV molar extinction coefficients, optical rotations, elemental analyses) of the final compounds **1** and **2** were unreported.

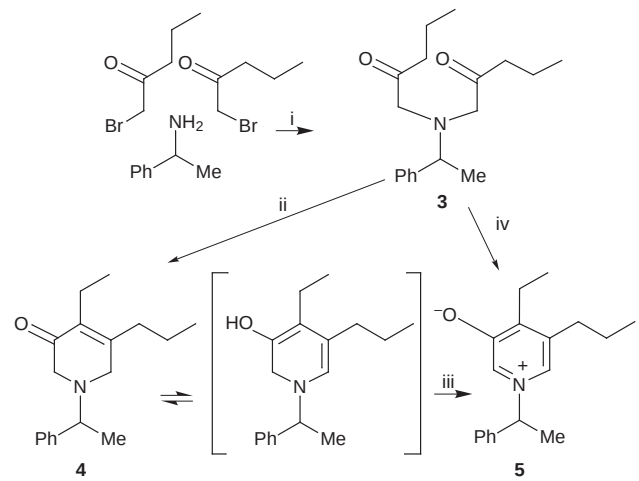
In our work we decided to start from enantiomerically pure and easily available natural L-α-amino acids, in order to avoid the formation of complex diastereomeric mixtures in a possible convergent synthesis of **1**. In particular, a strategic disconnection of the pyridinium compound **1** suggested its assembly from an L-lysine residue and from two identical fragments derivable from L-glutamic acid (Scheme 1).



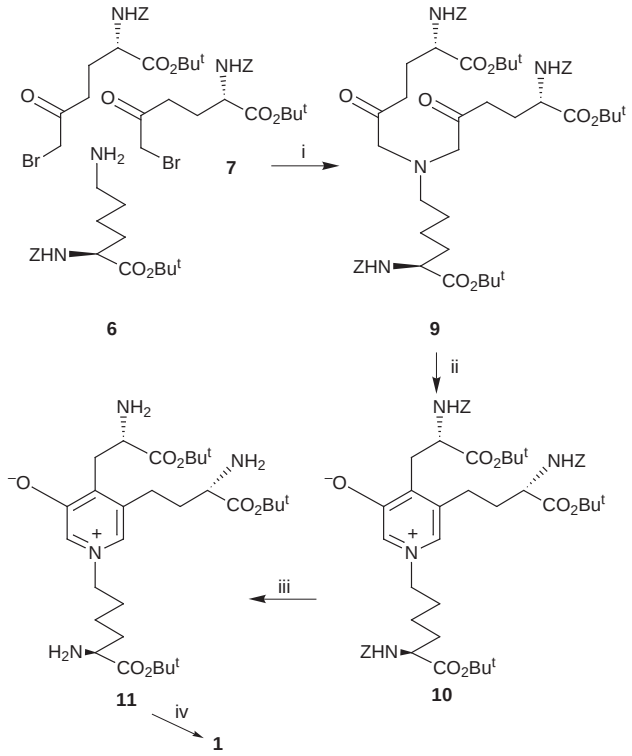
Scheme 1

With this in mind, the possibility of constructing the pyridine ring of **1** was first studied using a simpler model lacking the stereocenters and the amino acidic functionalities (Scheme 2). Easily available[6] 1-bromopentan-2-one was reacted with (*R*)-1-phenylethylamine to afford, using the best conditions (K_2CO_3, MeCN, 12 h, 25 °C), the diketoamine **3** in very high yield (80%).† Initially, various unsuccessful attempts were made to promote in the same reaction both the formation of the ketoamine **3** and its condensation to give the ketone **4**, by modulating the reaction conditions (reaction times, temperatures and/or addition of appropriate crown ethers). Subsequently, the internal condensation was attempted with the isolated and purified compound **3**. Surprisingly, treatment of amine **3** with K_2CO_3 in MeOH afforded (70% yield) a single fluorescent product, to which the structure **5**‡ could be assigned. Formation of compound **5** shows that, contrary to our expectations, the aldolic condensation occurred, followed by the oxidation of the possible intermediate cyclic ketone **4**.

The ketone **4** could be isolated and completely characterized when the reaction was performed under complete exclusion of oxygen. This compound was transformed into the pyridinium salt **5** when it was dissolved in MeOH and stirred in the presence of air and K_2CO_3. Compound **5** was also obtained, in a one-pot reaction, by mixing 1-bromopentan-2-one and (*R*)-1-phenylethylamine in an open vessel containing MeCN and K_2CO_3. When the complete formation of the ketoamine **3** was monitored, the addition of MeOH leads to the formation of the desired pyridinium salt **5**. Similar spontaneous oxidations were also recently observed[7] in other syntheses of pyridinium derivatives, but it was always impossible to map the mechanism of their formation. In our case, the isolation of compound **4** under anaerobic conditions and the observation that no tetrahydro derivative was detectable in the final reaction mixture made it possible to establish that oxygen, and not a disproportionation reaction, is responsible for the formation of pyridinium salt **5**.



Scheme 2 *Reagents and conditions*: i, K_2CO_3, MeCN, room temp., 80%; ii, K_2CO_3, MeOH, room temp., 85%; iii, K_2CO_3, air, MeOH, room temp., 81%; iv, K_2CO_3, air, MeOH, room temp., 70%.



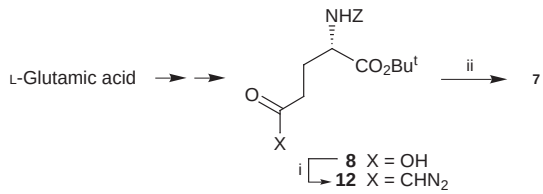
Scheme 3 *Reagents and conditions*: i, K_2CO_3, MeCN, room temp., 78%; ii, K_2CO_3, air, MeOH, room temp., 73%; iii, H_2, Pd/C, MeOH, room temp., 94%; iv, TFA, room temp., 72%.

The possibility of extending the method used for the synthesis of the simple model compound **5** to the more complex deoxypyridinoline **1** was then demonstrated by reacting under similar conditions (Scheme 3) the known[8] protected L-lysine **6** with the bromo ketone **7**§ obtained in two steps from *N*-(benzyloxycarbonyl)-L-glutamic acid 1-*tert*-butyl ester[9] **8** (Scheme 4).

Thus, the reaction in MeCN afforded the amine **9** in good yield [78% yield; an oil, $[\alpha]_D^{20}$ −0.8, $[\alpha]_{365}^{20}$ +3.1, ($CHCl_3$, 1% solution]. This amine is rather unstable in solution (decomposes in a few days) but it was transformed into the protected deoxypyridinoline **10**¶ when it was dissolved in MeOH containing K_2CO_3 and stirred in the presence of air.

Regeneration of the three protected amino groups of **10** by hydrogenolysis afforded the ester **11**‖ which, by treatment with TFA, easily gave deoxypyridinoline **1** as monotrifluoracetate salt in an epimerization-free process.[10] This salt of deoxypyridinoline **1** crystallized from aq. EtOH as a white monohydrate which was completely characterized.** Its UV molar extinction coefficients proved higher (10–20%) than those reported for the corresponding tetrachloride dihydrate salt.[4] The same difference was observed for the corresponding tetrachloride salt of **1**, which in our hands crystallized as monohydrate.

The preparation of the bromo derivative **7**, which required the activation of the ω-carboxylic group of the protected glutamic acid **8** and its substitution for an α-bromo ketone *via* an intermediate α-diazo ketone, merits comment. These transformations, well-established in the case of the shorter aspartic derivative,[11] were in our case complicated by the easy concurrent cyclisation to the known[12] (*S*)-5-(1,1-dimethylethoxy)carbonyl-1-benzyloxycarbonyl-2-pyrrolidone, cyclisation of which was not allowed in the case of the aspartic acid derivative. This cyclisation occurs during the activation of the ω-carboxylic group as an acid chloride (using $SOCl_2$ it is the only compound formed) or as mixed anhydride (using $ClCO_2Et$[13] it forms in 40% yield). Only when performing the activation with $ClCO_2Bn$ does the diazo ketone **12** [oil; $[\alpha]_D^{25}$ +16.6 ($CHCl_3$, 2% solution)] form in quantitative yield, avoiding the formation of cyclic pyroglutamate.



Scheme 4 Reagents and conditions: i, $ClCO_2Bn$, *N*-methylmorpholine, THF, −20 °C, then CH_2N_2, −20 °C → 0 °C, 88%; ii, HBr, AcOH, 0 °C, 64%.

Further work is currently underway to accomplish the synthesis of pyridinoline **2** by a similar convergent assembly of the pyridine nucleus starting from three L-glutamic units.

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Notes and references

† All new compounds gave correct elemental C, H, N and Cl analyses.

‡ *Selected data* for **5**: oil; $[\alpha]_D^{20}$ +3.3, $[\alpha]_{436}^{20}$ −13.3 ($CHCl_3$, *c* 1); δ_H(500 MHz, $CDCl_3$ 8.17 (1H, br s, pyridinium ring proton), 7.04 (1H, br s, pyridinium ring proton), 5.40 (1H, q, *J* 7.0, NC*H*CH_3), 1.96 (3H, d, *J* 7.0 Hz, NCHCH_3), 1.16 (3H, t, *J* 7.0, pyr-CH_2C*H*$_3$), 0.92 (3H, t, *J* 7.0, pyr-CH_2CH_2C*H*$_3$).

§ *Selected data* for **7**: oil; $[\alpha]_D^{20}$ −0.3, $[\alpha]_{365}^{20}$ −14.8 ($CHCl_3$, *c* 1); δ_H(500 MHz, $CDCl_3$) 4.24 [1H, ddd, *J* 8.5, 8.0, 4.5, C*H*(NHZ)CO_2Bu^t], 3.87 (2H, s, BrC*H*$_2$CO), 2.75 (1H, ddd, *J* 18.0, 8.5, 7.0, $BrCH_2COCHH$), 2.67 (1H, ddd, *J* 18.0, 8.5, 5.5, $BrCH_2COCHH$).

¶ *Selected data* for **10**: oil; $[\alpha]_D^{20}$ +5.3, $[\alpha]_{436}^{20}$ +29.2 ($CHCl_3$, *c* 1); δ_H(500 MHz, $CDCl_3$) 8.09 (1H, br s, pyridinium ring proton), 6.90 (1H, br s, pyridinium ring proton).

‖ *Selected data* for **11**: oil; $[\alpha]_D^{20}$ +32.5 ($CHCl_3$, *c* 1); δ_H(500 MHz, CD_3OD) 7.57 (1H, br s, pyridinium ring proton), 7.48 (1H, br s, pyridinium ring proton).

** *Selected data* for **1** monotrifluoroacetate monohydrate ($C_{18}H_{29}N_4O_7 \cdot CF_3CO_2^- \cdot H_2O$): $[\alpha]_D^{20}$ +33.0 ($CHCl_3$, *c* 0.98); λ_{max}(HCl 0.1 M)/nm (ε/M^{-1} cm^{-1}), 239 (3850), 293 (6480); λ_{max}(50 mM phosphate buffer, pH 7.5)/nm (ε/M^{-1} cm^{-1}) 252 (3660), 324 (6100); δ_H(500 MHz, D_2O) 8.69 (1H, br s, pyridinium ring proton), 8.62 (1H, br s, pyridinium ring proton), 4.92 (2H, t, *J* 6.5, $CH_2CH_2N^+$), 4.54 [1H, t, *J* 7.0, C*H*(NH_3^+)COO^-], 4.32 [1H, t, *J* 5.0 Hz, C*H*(NH_3^+)COO^-], 4.16 [1H, t, *J* 6.0, C*H*(NH_3^+)COO^-]; δ_C(D_2O) 175.1, 174.5, 173.6, 163.6 (q, CF_3*C*OO), 156.8, 142.1, 141.9, 136.0, 129.7, 117.5 (q, *C*F_3COO), 61.8, 55.2, 54.9, 53.6, 31.4, 30.7, 30.5, 28.6, 26.4, 21.9.

1 Presented at the 24th *Convegno Nazionale, Divisione di Chimica Organica*, Salerno (Italy) September 21–25, 1997.

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