this dimer, complex **5** was prepared in a zinc reduction using an analogous procedure as described for **2**. Experimental details will be reported in a separate manuscript.
[27] A more electron-deficient rhodium center might also accelerate insertion reactions at Rh^{III} intermediates, which would result in increased activity.
[28] The formation of an intermediate with the same characteristics as **7** was also observed in a reaction of 1-phenylpropanal with **6**. At this point an alternative structure for **7** can not be excluded (for example, the branched isomer as in **3′**).
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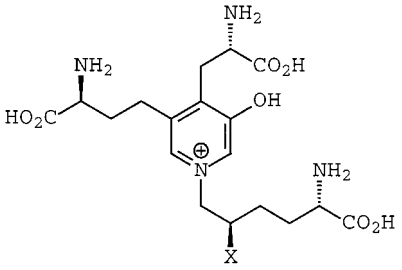
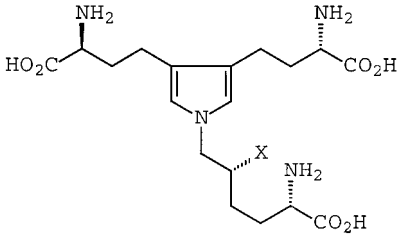


(+)-**1**: X=OH, pyridinoline
(+)-**3**: X=H, deoxypyridinoline



2: X=OH, pyrrololine
(+)-**4**: X=H, deoxypyrrololine

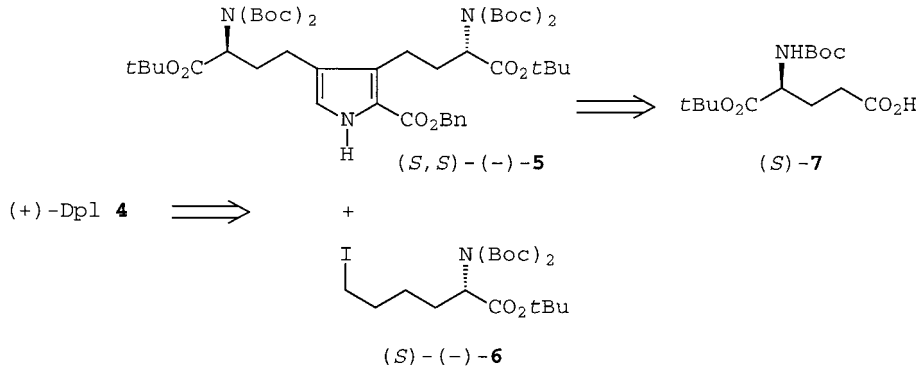
Total Synthesis of (+)-Deoxypyrrololine: A Potential Biochemical Marker for Diagnosis of Osteoporosis

Maciej Adamczyk,* Donald D. Johnson, and Rajarathnam E. Reddy

Osteoporosis is a crippling degenerative bone disease that affects the aged population, particularly postmenopausal women.[1] This metabolic disease is a consequence of an inbalance in the bone renewal process, which occurs when bone resorption exceeds bone formation. The current methods for diagnosis of osteoporosis involve analysis of bone based on histomorphometry and densitometric measurements.[2] The efforts for prevention of this bone disease, as well as to develop an effective antiresorptive therapy, have increased the search for reliable and noninvasive biochemical markers of bone resorption.[1c, 3] The traditional markers of bone resorption, for example urinary calcium[4] and hydroxyproline,[5] lack clinical sensitivity and specificity for diagnosis of osteoporosis.

In recent years, the pyridinium cross-links (+)-pyridinoline (Pyd, **1**)[6] and (+)-deoxypyridinoline (Dpd, **3**)[7] have gained much attention owing to their potential clinical utility in the diagnosis of osteoporosis and other bone diseases.[8–10] In 1981 Scott et al.[11] postulated the existence of pyrrole cross-links pyrrololine (Pyl, **2**) and deoxypyrrololine (Dpl, **4**)[12] in various tissues. Subsequent studies by several other groups have provided further convincing evidence for the existence of pyrrole cross-links.[13, 14] Unfortunately, the attempts to isolate cross-links **2** and **4** have not been successful so far.[3a,d] It was proposed that **2** and **4** are formed from natural (2*S*,5*R*)-hydroxylysine and (*S*)-lysine present in collagen by a lysyl oxidase mediated enzymatic process, in an analogous fashion to the pyridinium cross-links **1** and **3**.[3d, 13c,d,f] Here we report the first synthesis of the pyrrole cross-link (+)-deoxypyrrololine (Dpl, **4**) from (4*S*)-5-(*tert*-butoxy)-4-[(*tert*-butoxycarbonyl)amino]-5-oxo-pentanoic acid (**7**).

The synthesis of **4** involves alkylation of the pyrrole derivative (*S*,*S*)-(−)-**5** with iodide (*S*)-(−)-**6**, followed by hydrolysis of the protecting groups and removal of the 2-carboxybenzyl ester moiety (Scheme 1). It was envisioned that the key intermediate (*S*,*S*)-(−)-**5** could be prepared from the α-acetoxynitro compound **13** (see Scheme 2) and benzyl isocyanoacetate (**14**)[15, 16] by a base-promoted condensation and cyclization process. Compound **13** could be obtained from **7**.



Scheme 1. Retrosynthesis of (+)-deoxypyrrololine (Dpl, **4**). Bn = benzyl, Boc = *tert*-butyloxycarbonyl.

Accordingly, the commercially available (*S*)-**7** was converted into the aldehyde (*S*)-(−)-**8**,[17] which was then reduced

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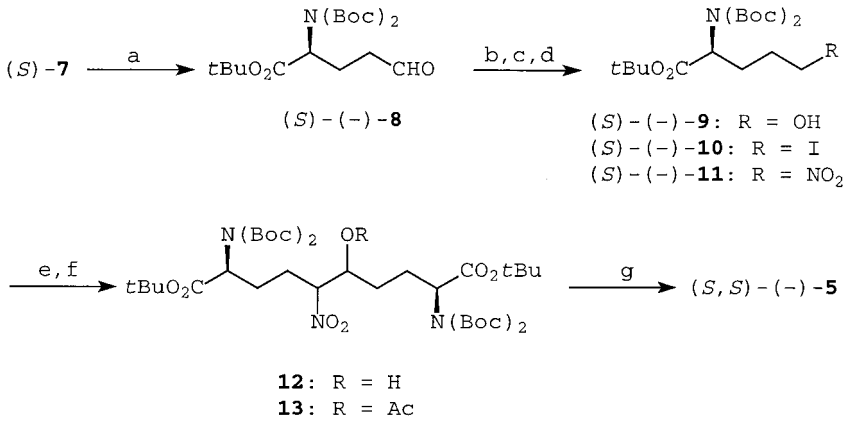
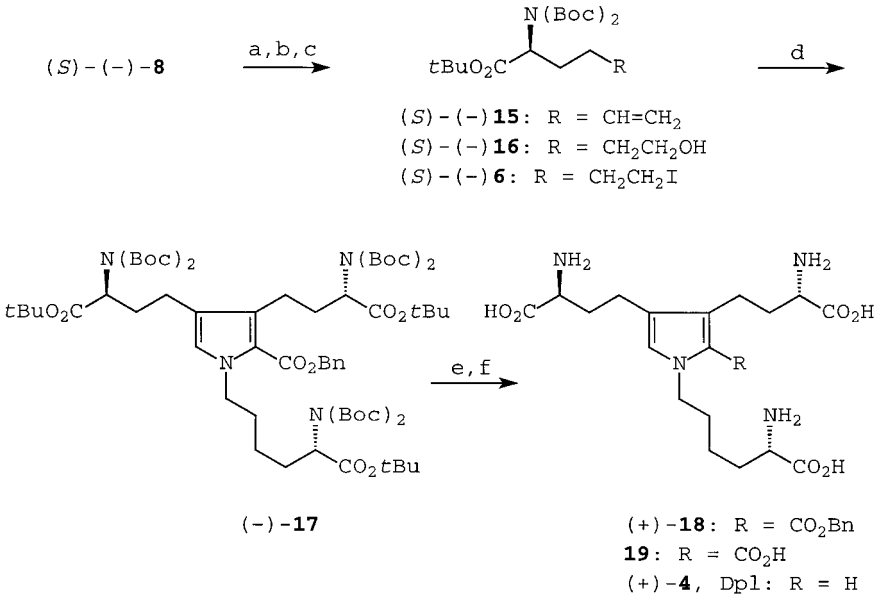


Scheme 2. Synthesis of (*S*,*S*)-(−)-**5**. Reagents and conditions: a) See ref. [17]; b) $NaBH_4$ (1.0 equiv), MeOH, 0 °C, 45 min, 95 %; c) I_2 (1.5 equiv), PPh_3 (1.6 equiv), imidazole (2.0 equiv), THF, 23 °C, 1.5 h, 94 %; d) $NaNO_2$ (2.0 equiv), DMF, 23 °C, 30 min, 55 %; e) (*S*)-(−)-**8** (1.1 equiv), DMAP, (4.0 equiv), CH_2Cl_2, 23 °C, 8 d, 91 %; f) Ac_2O (1.5 equiv), DMAP (0.1 equiv), THF, 23 °C, 2 h, 96 %; g) BnO_2CCH_2NC (**14**, 1.3 equiv), DBU (2.5 equiv), THF, 0 – 23 °C, 5 h, 57 %. DBU = 1,8-diazabicyclo[5.4.0]undec-7-ene, DMAP = 4-dimethylaminopyridine.

with $NaBH_4$ in MeOH to the alcohol (*S*)-(−)-**9** in 95 % yield (Scheme 2).[18] The hydroxyl group in (*S*)-(−)-**9** was converted into the iodide to provide (*S*)-(−)-**10**, which was subsequently treated with sodium nitrite in DMF[19] to afford the corresponding nitro compound (*S*)-(−)-**11** in 55 % yield. Condensation of (*S*)-(−)-**11** with aldehyde (*S*)-(−)-**8** (Henry reaction)[20] in the presence of 4-dimethylaminopyridine in CH_2Cl_2 gave α-hydroxynitro compound **12** in 91 % yield as a mixture of diastereomers (1:1 ratio). Since the two new chiral centers (the locations of the NO_2 and OH groups) in **12** are eventually eliminated during the pyrrole ring formation, the diastereomeric mixture of **12** was converted into the corresponding acetate derivative **13** by treatment with Ac_2O in THF in almost quantitative yield (96 %). Finally, condensation of the diastereomeric mixture of α-acetoxynitro compound **13** with benzyl isocyanoacetate (**14**)[15, 16] in the presence of DBU in THF at 0 °C to room temperature, followed by column chromatography on silica gel, gave the key intermediate (*S*,*S*)-(−)-**5** in 57 % yield ($[\alpha]_D^{23} = -14.9$ ($c = 1.37$, MeOH)).

The next step in the synthesis of (+)-**4** was installation of lysine chain at the pyrrole nitrogen atom in (*S*,*S*)-(−)-**5** by alkylation with **6**. The iodide (*S*)-(−)-**6** was prepared from aldehyde (*S*)-(−)-**8** in three steps (Scheme 3). First, the (*S*)-(−)-**8** was extended by a methylene group to the corresponding olefin (*S*)-(−)-**15** by Wittig reaction,[17] which upon hydroboration afforded the alcohol (*S*)-(−)-**16** in excellent yield (87 %). The hydroxyl group in (*S*)-(−)-**16** was then converted to provide the desired iodide (*S*)-(−)-**6** in 92 % yield using I_2, PPh_3, and imidazole in THF. Treatment of (*S*,*S*)-(−)-**5** with 1.0 equivalent of *t*BuOK in THF and subsequent reaction with 2.0 equivalents of (*S*)-(−)-**6** in the presence of [18]crown-6[21] at room temperature afforded the *N*-alkylated pyrrole derivative (−)-**17** in 42 % yield after purification by column chromatography on silica gel.[22] The derivative (−)-**17** has the required amino acid chains at the 1-,3-, and 4-positions of the pyrrole ring. The synthesis of (+)-Dpl (**4**) would only require unmasking the protecting groups and removal of the benzyloxycarbonyl group at the 2-position of pyrrole ring. Thus, (−)-**17** was first treated with trifluoroacetic acid and water,[9a] and the mixture obtained was purified by preparative reverse-phase HPLC to afford (+)-**18** in 79 % yield. The benzyl group in (+)-**18** was removed by hydrogenation over 10 % Pd/C in MeOH to give the acid **19**, which without purification was decarboxylated with trifluoroacetic acid.[23] Concentration of the crude reaction mixture followed by purification by preparative reverse-phase HPLC and lyophilization afforded **4**[24] in 39 % yield as a pale pink powder ($[\alpha]_D^{23} = +20.6$ ($c = 0.17$, H_2O)).



Scheme 3. Synthesis of (+)-**4**. Reagents and conditions: $MePPh_3Br$ (2.0 equiv), *n*BuLi (2.0 equiv), THF 0 °C, 45 min, 61 %; b) $B_2H_6 \cdot$ THF (1.3 equiv), THF, 0 → 23 °C, 17 h, 87 %; c) I_2 (1.5 equiv), PPh_3 (1.6 equiv), imidazole (2.0 equiv), THF, 23 °C, 1 h, 92 %; d) (*S*,*S*)-(−)-**5**, *t*BuOK (1.0 equiv), [18]crown-6 (0.1 equiv), THF, 23 °C, 7 h, 42 %; e) TFA/water (95/5), 23 °C, 2 h, 79 %; f) 10 % Pd/C, H_2, MeOH, 23 °C, 1 h, then TFA, 23 °C, 15 min, 39 %. TFA = trifluoroacetic acid.

In summary a general and convergent total synthesis of (+)-deoxypyrrololine (Dpl, **4**), a pyrrole cross-link of collagen, has been achieved utilizing a commercially available chiral starting material (**7**). The method is currently being applied to the synthesis of pyrrololine (**2**) and for the preparation of

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various immunocomponents (immunogens, antibodies, and probes) which are imperative for development of assays for diagnosis and treatment of bone diseases.

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[22] (−)-**17**: $R_f = 0.42$ (silica gel, EtOAc/hexanes 2/8); $[\alpha]_D^{28} = -6.8$ ($c = 2.0$, MeOH); analytical reverse-phase HPLC (Waters, Rcm, μBondapak C18 column, 10 μm, 8 × 10 mm; MeCN/0.1 % aq. trifluoroacetic acid 95/5, 2.0 mL min^{-1}, detection at 215 nm): $R_t = 8.7$ min (97 %); ^{1}H NMR (300 MHz, $CDCl_3$, 23 °C, TMS): $\delta = 7.43 - 7.26$ (m, 5H, Ar-H), 6.58 (s, 1H, pyrrole-H), 5.26 (q, 2H, $J = 48.3$, 12.3 Hz, Ar-CH_2), 4.72 – 4.61 (m, 3H, 3 × N-C*H*-CO_2tBu), 4.11 (t, 2H, $J = 8.1$ Hz, pyrrole-N-CH_2), 2.82 – 2.60 (m, 2H), 2.44 – 1.24 (m, 12H), 1.49 (s, 18H), 1.48 (s, 18H), 1.47 (s, 18H), 1.44 (s, 9H), 1.43 (s, 9H), 1.42 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$, 23 °C, TMS): $\delta = 169.8$, 169.7, 169.6, 161.0, 152.5, 152.4, 136.6, 132.2, 128.5, 127.9, 126.5, 121.4, 118.2, 82.7, 82.5, 82.4, 81.1, 81.0, 77.2, 65.3, 59.3, 58.8, 58.3, 49.7, 31.7, 31.1, 29.2, 29.1, 28.0, 27.9, 23.8, 23.1, 21.3; ESI-MS (m/z %): 1319 (100) $[M+NH_4]^+$; HR-MS (FAB): calcd for $C_{68}H_{109}N_4O_{20}$: 1323.7427 $[M+Na]^+$, found: 1323.7449.

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[24] (+)-Dpl (**4**): $[\alpha]_D^{20} = +20.6$ ($c = 0.17$, H_2O); analytical reverse-phase HPLC (Waters, Rcm, μBondapak C18 column, 10 μm, 8 × 10 mm; MeCN/0.1 % aq. trifluoroacetic acid 4/96, 2.0 mL min^{-1}, detection at 215 nm): $R_t = 5.54$ min (96 %); ^{1}H NMR (300 MHz, CD_3OD, 23 °C, TMS): $\delta = 6.51$ (s, 2H, pyrrole-H), 3.92 – 3.78 (m, 5H, 3 × N-CH-CO_2H and pyrrole-N-CH_2), 2.64 – 2.52 (m, 4H), 2.20 – 1.98 (m, 4H), 1.94 – 1.82 (m, 2H), 1.82 – 1.70 (m, 2H), 1.48 – 1.38 (m, 2H); ^{13}C NMR (75 MHz, D_2O (0.5 mL + MeCN (2.0 μL), 23 °C): $\delta = 173.7$, 120.0, 119.7 (CN), 54.1, 54.0, 49.0, 31.6, 30.8, 30.1, 22.1, 20.7, 1.5 (CH_3); ESI-MS (m/z %): 399 (100) $[MH^+]$; HR-MS (FAB): calcd for $C_{18}H_{31}N_4O_6$: 399.2244 $[MH^+]$, found: 399.2253.

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