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TETRAHEDRON: *ASYMMETRY*

Versatile synthesis of (+)-deoxypyridinoline, a biochemical marker for diagnosis of osteoporosis

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Abstract

A versatile chiral synthesis of the bone collagen cross-link, (+)-deoxypyridinoline (Dpd, **1**) was described starting from a 3-hydroxypyridine derivative (**2**) via sequential introduction of three amino acid chains followed by hydrolysis. The key synthon **2**, was prepared from vitamin B_6 (**6**). © 1999 Published by Elsevier Science Ltd. All rights reserved.

Osteoporosis is a degenerative bone disease, which affects the aged population, particularly postmenopausal women.[1] The current methods for diagnosis of osteoporosis involve histomorphometry and densitometry of bones.[2] The efforts for prevention of this metabolic bone disease as well as for development of effective antiresorptive therapy, have increased the search for more reliable and noninvasive biochemical markers.[3] In recent years, the pyridinium cross-link, deoxypyridinoline (Dpd, **1**)[4] (Fig. 1) has attracted considerable attention due its clinical utility.[5–7] Thus, Dpd (**1**) is needed for use as calibrators, controls, clinical reference standards, and for immunoassay development. Currently, it is isolated in a very low yield from bones (sheep, ox, turkey, etc.) by 6–9 M HCl hydrolysis at 110°C, a process that could affect the integrity of the stereocenters in Dpd.[8] Therefore, Dpd (**1**) became an attractive synthetic target due to its novel structural features and practical applications, since its analogs are imperative for diagnosis and therapy management of osteoporosis and other bone diseases. The reported syntheses of Dpd (**1**) involved construction of a substituted pyridine ring from amino acid components utilizing aldol chemistry.[9] In this paper we present a novel and conceptually different approach for (+)-Dpd (**1**) starting from a 3-hydroxypyridine derivative (**2**).

In this new strategy, the (+)-Dpd (**1**) (Fig. 1) was constructed from a 3-hydroxypyridine derivative (**2**) via introduction of three amino acid chains sequentially, which provides diverse flexibility for preparation of a variety of its analogs. The 4- and 5-amino acid chains in (+)-**1** were introduced by utilizing (2*R*)-(−)-2,5-dihydro-3,6-dimethoxy-2-isopropylpyrazine (Schollkopf's reagent, **3**)[10] and Wittig reagent (*R*)-(−)-**4**,[11] respectively, and the lysine chain at the 1-position of the pyridine ring was installed via quaternization

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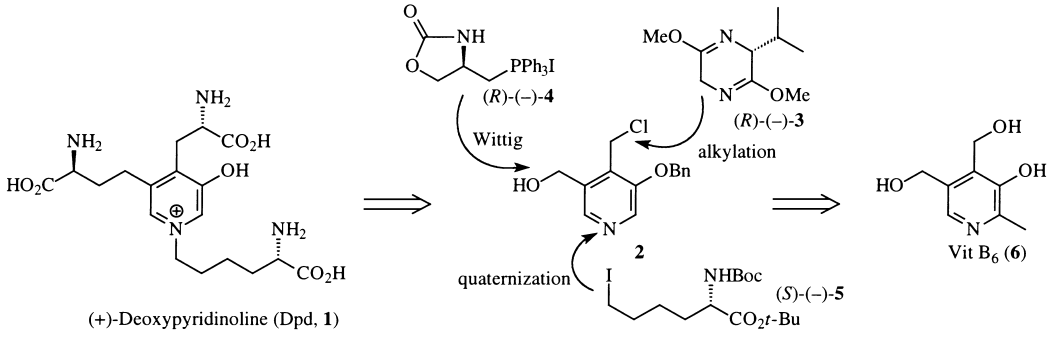
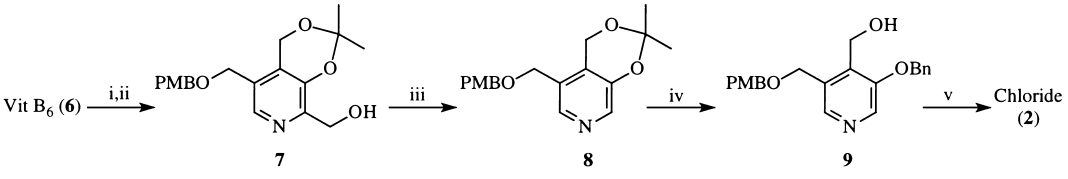


Figure 1.

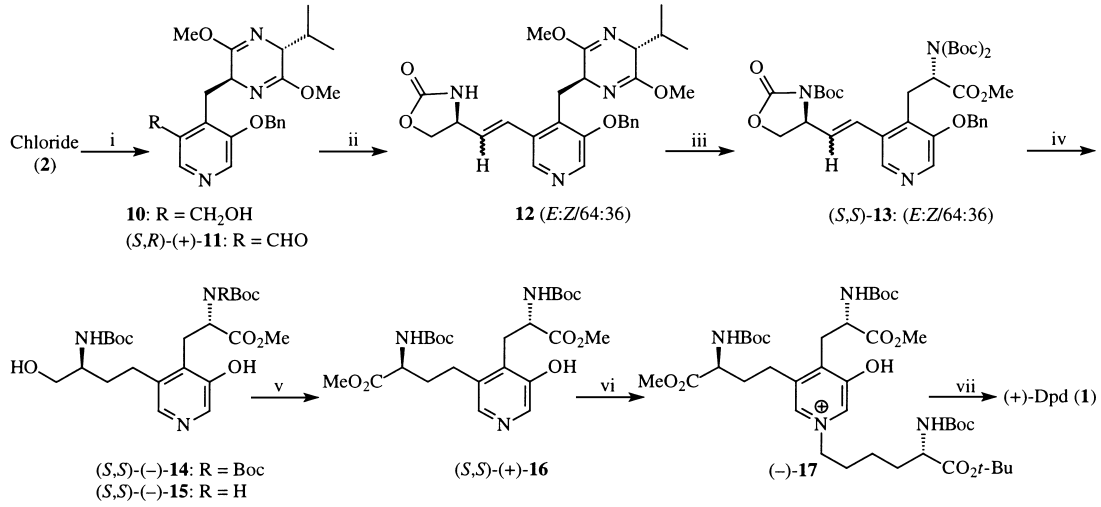
with iodide (*S*)-(−)-**5**. The key synthon, chloride **2**, was envisioned from an inexpensive vitamin B_6 (**6**). Thus, the 2-methyl group in B_6 (**6**) was functionalized (Scheme 1) to the corresponding hydroxy methyl, after protecting its native hydroxyl groups,[12] to give **7**, which upon oxidation and decarboxylation gave 2-norpyridoxine derivative **8**.[13] The acetonide group in **8** was hydrolyzed and the 3-hydroxy group was selectively protected as the benzyl ether using benzyldimethyl phenylammonium chloride[14] to afford **9**. The hydroxyl group in **9** was then converted to the chloride and the *p*-methoxybenzyl group hydrolyzed using 0.5N HCl to give the desired chloride **2** in 58% yield.



Scheme 1. Reagents and conditions. (i) a. Korytnyk et al.[12]; b. NaH, PMBCl, DMF, 0°C–rt, 15 h, 83%; (ii) a. *m*-CPBA, CH_2Cl_2, rt, 2 h; b. TFAA, CH_2Cl_2, 0°C–rt, 5 h, MeOH, 86%; (iii) a. MnO_2, $CHCl_3$, rt, 21 h; b. Ag_2O, KOH, EtOH, rt, 1 h, then xylene, reflux, 25 min, 71%; (iv) a. PPTS, EtOH, reflux, 3 days 71%; b. $PhNMe_2BnCl$, EtONa, EtOH, −78°C, then xylene, reflux, 4 h, 30%; (v) $SOCl_2$, benzene, 0°C–reflux, 0.5 h then 0.5N HCl, reflux, 15 min, 58%

With gram quantities of chloride **2** in hand, we proceeded to the sequential introduction of amino acid chains present in (+)-Dpd (**1**). Thus, alkylation (Scheme 2) of (*R*)-(−)-**3** with the chloride **2** using *n*-BuLi at −78°C, gave a diastereomeric mixture of **10** [(*S*,*R*:*S*,*S*)/69:31] in 86% yield. Our attempts to separate the isomeric mixture (*S*,*R*/*S*,*S*)-**10** by a variety of techniques, including preparative HPLC, were unsuccessful. However, to our delight, the corresponding diastereomeric mixture of aldehydes **11**, which was obtained by oxidation of (*S*,*R*/*S*,*S*)-**10** with MnO_2, was easily separated by silica gel column chromatography (40% EtOAc in hexanes) to afford the desired major isomer, (*S*,*R*)-(−)-**11**, in 62% yield and >98% de. Wittig reaction of aldehyde (*S*,*R*)-(−)-**11** with the ylide, which was generated from (*R*)-(−)-**4** using *n*-BuLi in THF at −78°C, gave the olefin **12** in good yield (72%) as a mixture of *E*:*Z* isomers (ratio, 64:36). Hydrolysis of the dihydropyrazine ring in **12** was carried out using 0.5N HCl in MeCN, and the protection of the resulting free amine using $(Boc)_2O$ in MeCN afforded the tri-Boc compound (*S*,*S*)-**13** (*E*:*Z*/64:36) in 56% overall yield. The (*S*,*S*)-**13** was subjected to hydrolysis with cesium carbonate,[15] and then hydrogenated over 10% Pd/C in methanol to afford (*S*,*S*)-(−)-**14** in good overall yield. Concerned with the sensitivity of the bis-(*tert*-butoxycarbonyl)amino group in (*S*,*S*)-(−)-**14** for subsequent oxidation and quaternization steps, it was therefore selectively hydrolyzed with trifluoroacetic acid[16] in CH_2Cl_2 to give (*S*,*S*)-(−)-**15** in 69% yield. Oxidation of (*S*,*S*)-(−)-**15** with Jones reagent[17] followed by esterification afforded (*S*,*S*)-(+)-**16** in 55% yield as a colorless solid {$[\alpha]_D^{20}$ +17.3 (c 0.59, $CHCl_3$)}.

The 3-hydroxypyridine derivative (*S*,*S*)-(+)-**16**, requires only elaboration of the lysine chain at the



Scheme 2. Reagents and conditions. (i) a. (*R*)-(−)-**3**, *n*-BuLi, THF, −78°C, 5 h, 86%, [(*S*,*R*:*S*,*S*)/69:31]; b. MnO_2, $CHCl_3$, rt, 20 h, 62%, de: >98%; (ii) a. (*R*)-(−)-**4**, *n*-BuLi, THF, −78°C, 4 h, 72%, (*E*:*Z*/64:36); (iii) 0.5N HCl, MeCN, rt, 45 min then $(Boc)_2O$, DMAP, Et_3N, MeCN, rt, 6 h, 56%; (iv) a. Cs_2CO_3, MeOH, rt, 2.5 h, 63%; b. 10% Pd/C, MeOH, H_2, HCl, 20 psi, 4 h, 78%; c. TFA, CH_2Cl_2, rt, 26 h, 69%; (v) Jones reagent, acetone, rt, 40 min, then $TMSCHN_2$, benzene, MeOH, rt, 15 min, 55%; (vi) (*S*)-(−)-**5**, anhydrous 1,4-dioxane, reflux, 4 h, 27%; (vii) LiOH, THF–water, rt, 1 h, then TFA–water (ratio, 9.5:0.5), rt, 1 h, 84%

1-position for completion of Dpd (**1**) synthesis. Thus, quaternization of (*S*,*S*)-(+)-**16** with (*S*)-(−)-*tert*-butyl-[(2-*tert*-butoxycarbonyl)amino]-6-iodohexanoate (**5**)[9,16a] was carried out in refluxing 1,4-dioxane for 4 h, and the product was purified by preparative reverse phase HPLC to afford pyridinium compound (−)-**17** in 27% yield. Finally, alkaline hydrolysis of the methyl esters in (−)-**17** using lithium hydroxide in THF–water, followed by removal of the Boc and *t*-butyl protective groups with TFA–water (9.5:0.5 ratio) and purification by preparative reverse phase HPLC afforded (+)-Dpd (**1**) in 84% yield as its TFA salt {$[\alpha]_D^{20}$ +36.2 (c 0.54, MeOH); lit.[9a] $[\alpha]_D^{20}$ +31.6 (c 0.25, MeOH)}.

In summary, a versatile synthesis of (+)-deoxypyridinoline (**1**), a biochemical marker for the diagnosis of osteoporosis was developed from 3-hydroxypyridine derivative (**2**). The method is amenable for preparation of variety of Dpd analogs and immunoreagents useful for diagnosis and treatment of osteoporosis.

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