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Efficient Synthesis of Novel 1α-Amino and 3β-Amino Analogues of 1α,25-Dihydroxyvitamin D_3

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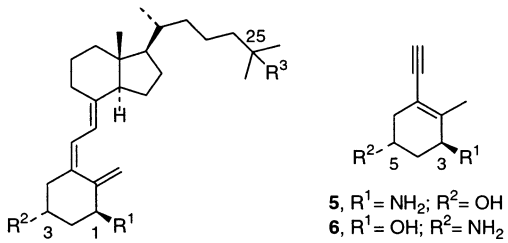
Abstract: Convenient synthetic routes to 1α-amino-25-hydroxyvitamin D_3 (**3**) and 3β-amino-3-deoxy-1α,25-dihydroxyvitamin D_3 (**4**), novel analogues of vitamin D_3 bearing an amino group at the C-1 or C-3 position, have been developed starting from (*S*)-(+)-carvone. Construction of the A-ring fragments was accomplished by selective enzymatic hydrolysis of a diester intermediate and introduction of the amino group under Mitsunobu conditions.

Vitamin D_3 (**1**, Figure 1) and its metabolites, among them 1α,25-dihydroxyvitamin D_3 [calcitriol, **2**, 1α,25-$(OH)_2$-D_3], the hormonally active form, exert control over important physiological processes in the body related to calcium and phosphorus metabolism, cell proliferation and differentiation, and immune reactions.[1] Various derivatives of 1α,25-$(OH)_2$-D_3 have been proposed for the treatment of rickets, osteoporosis, renal osteodystrophy, certain cancers, psoriasis, AIDS, and Alzheimer's disease.[1d,2] Most of the analogues have been developed with the aim of improving the biological profile of the natural hormone for potential therapeutic applications. The biological functions of 1α,25-$(OH)_2$-D_3 are mediated through its nuclear receptor (genomic actions), the nVDR,[3] and by a membrane receptor that modulates rapid nongenomic actions (transcaltachia).[4] Therefore, investigation of the state of the binding domain is important to elucidate the mechanism of the biological action.

The crucial role of the hydroxy groups at C-1 and C-25 for binding to the nVDR and DBP (vitamin D binding protein) proteins has been established.[5] However, structural changes at the 1-position of calcitriol can be made while still maintaining significant biological activity. Inversion of the natural orientation of the 1α-hydroxyl by 1β results in an inhibitor of the nongenomic transcaltachia response.[6] Replacement of a hydroxyl group to fluorine results in paradoxical biological consequences. Thus, 1α-fluoro-25-hydroxyvitamin D_3[7] and 1α-F-25-OH-16-ene-23-yne-D_3[8] abolish all calcemic activity but elicit cell differentiation. In contrast, Paaren et al.[9] report that 1α,25-F_2-D_3 is biologically inert. Furthermore, Posner[10] has shown that 1-hydroxyalkyl-25-hydroxyvitamin D_3 analogues retain calcitriol's antiproliferative activity in murine keratinocytes even though these synthetic homologues are significantly less effective than calcitriol in binding to the 1α,25-$(OH)_2$-D_3 receptor.



5, $R^1 = NH_2$; $R^2 = OH$
6, $R^1 = OH$; $R^2 = NH_2$

1, $R^1 = R^3 = H$; $R^2 = OH$, Vitamin D_3
2, $R^1 = R^2 = R^3 = OH$, 1α,25-Dihydroxyvitamin D_3
3, $R^1 = NH_2$; $R^2 = R^3 = OH$
4, $R^1 = R^3 = OH$; $R^2 = NH_2$

FIGURE 1. Vitamin D_3, 1α,25-$(OH)_2$-D_3, 1α-amino-25-OH-D_3, 3β-amino-3-deoxy-1α,25-$(OH)_2$-D_3, and amino A-ring precursors.

As part of our research program on the development of A-ring modified vitamin D_3 analogues,[11] we describe here the preparation of 1α-amino-25-hydroxyvitamin D_3 (**3**) and 3β-amino-3-deoxy-1α,25-dihydroxyvitamin D_3 (**4**), novel analogues of vitamin D_3 bearing an amino group at the C-1 or C-3 position. It is of interest to know how

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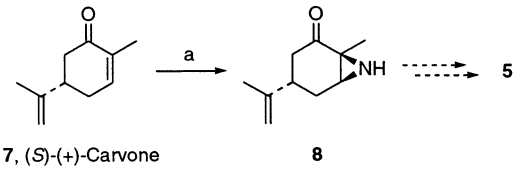
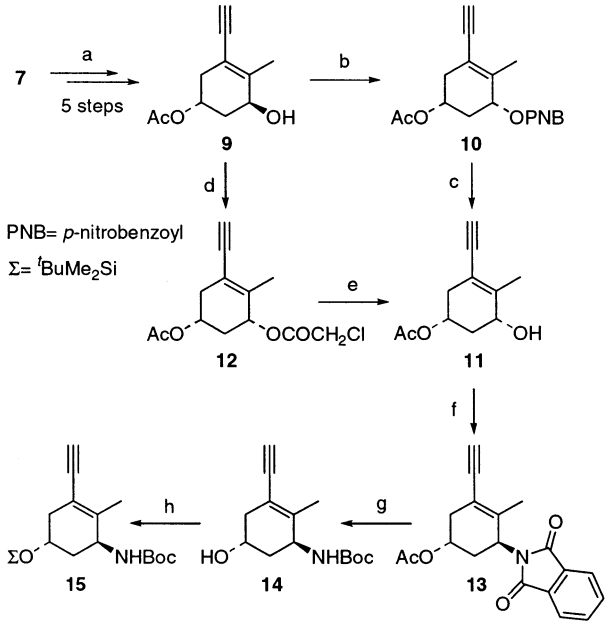
SCHEME 1[a]



[a] Key: (a) Me_2NNH_2, 1,2-epoxypropane, ^{i}PrOH, benzene, 50 → 70 °C, 7 h (41%).

SCHEME 2[a]

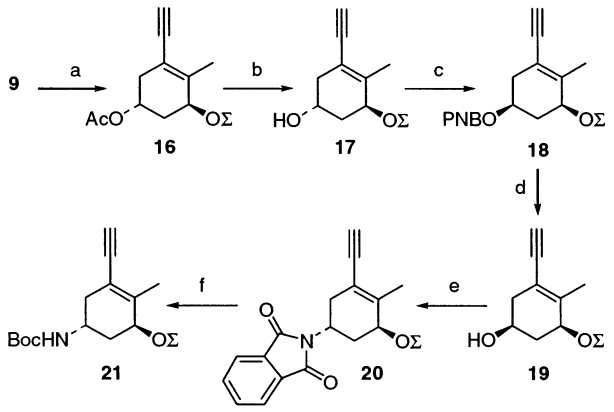


[a] Key: (a) ref 15; (b) *p*-nitrobenzoic acid, PPh_3, DEAD, THF, rt, 2 h (87%); (c) Mg, MeOH, −30 °C, 6 h (56%); (d) $ClCH_2CO_2H$, PPh_3, DEAD, THF, rt, 1.5 h (86%); (e) CVL, 0.1 M KH_2PO_4 (pH 7)/1,4-dioxane (10:2), 30 °C, 5 h (92%); (f) phthalimide, PPh_3, DEAD, THF, rt, 5.5 h, (83%); (g) (i) $MeNH_2$, EtOH, rt, 24 h, then 65 °C, 12 h, (ii) $(Boc)_2O$, $NaHCO_3$ (aq), $CHCl_3$, rt, 24 h (51%); (h) TBDMSCl, imidazole, CH_2Cl_2, rt, 2 h (92%).

the substitution of the 1α- or 3β-hydroxyl group by an amino group can affect the affinities for the VDR and DBP proteins, and consequently their biological properties. The amino function may change the hydrogen bonding properties in the interaction between the receptor and the analogue. Moreover, the different hydrophilic/hydrophobic properties of these new derivatives may provide interesting biological responses in the same way that amino steroids have shown noteworthy pharmacological activity.[12]

The amino derivatives **3** and **4** were synthesized according to the enyne approach, originally developed by Lythgoe.[13] This method, based on the coupling between an A-ring synthon enyne and an enol triflate of the CD-ring/side chain fragment, has become one of the most convenient methods for the synthesis of 1α,25-$(OH)_2$-D_3 analogues. For this, key A-ring precursors **5** and **6** (Figure 1) are required.

SCHEME 3[a]



[a] Key: (a) TBDMSCl, imidazole, CH_2Cl_2, rt, 2 h (91%); (b) MeONa, MeOH, rt, 2 h (quantitative); (c) *p*-nitrobenzoic acid, PPh_3, DEAD, THF, rt, 22 h (84%); (d) MeONa, MeOH, rt, 2 h (quantitative); (e) phthalimide, PPh_3, DEAD, THF, rt, 14 h, (68%); (f) (i) $MeNH_2$, EtOH, rt, 24 h, then 65 °C, 12 h, (ii) $(Boc)_2O$, $NaHCO_3$ (aq), $CHCl_3$, rt, 24 h (83%).

The strategy for the preparation of **5** began with the stereoselective aziridination of (*S*)-(+)-carvone (Scheme 1), in a process similar to the stereoselective epoxidation[14] of (*S*)-(+)-carvone used by Okamura for the synthesis of the natural A-ring synthon.[15] Subsequent modifications, which include the SmI_2−Pd^0-mediated aziridine ring opening, would provide enyne **5**. Although the formation of aziridines from the addition of nitrenes to olefins is a general method,[16] its utility here is limited by the presence of an extra double bond and the nonstereochemical control of the reaction. For this reason, aziridination via Michael addition proves more useful. Among all the methods tested, best results were obtained by direct imination of (*S*)-(+)-carvone with aminimides.[17] After several changes in the reaction conditions, the aziridine **8** was obtained in 41% yield.

An alternative approach to the above-mentioned procedure is a double inversion of the allylic hydroxyl group under Mitsunobu[18] conditions (Scheme 2). We used alcohol **9** as starting material, an intermediate en route to the 1α,25-$(OH)_2$-D_3 A-ring precursor from (*S*)-(+)-carvone described by Okamura et al.[15]

Inversion of configuration at C-3 in **9** using *p*-nitrobenzoic acid (PNBA) afforded *p*-nitrobenzoate ester **10** with total inversion of the configuration and high yield. For the selective deprotection of *p*-nitrobenzoate ester in the presence of acetate ester, we decided to use magnesium methoxide. The usefulness of this mild reagent for selective cleavage of different esters is described in the

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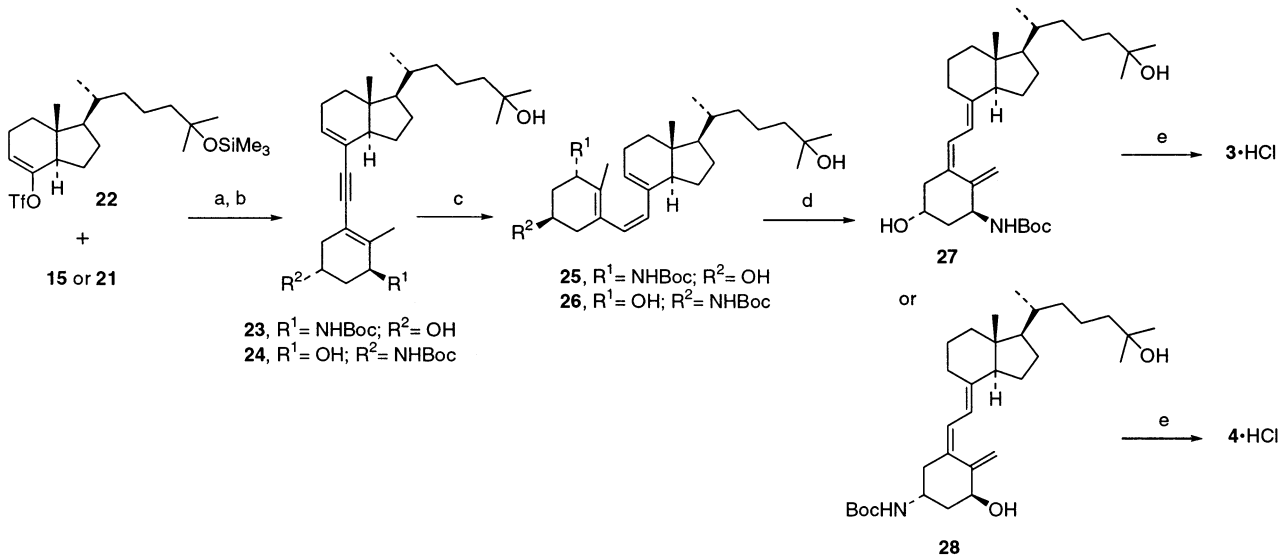
SCHEME 4[a]



[a] Key: (a) $Pd(PPh_3)_2(OAc)_2$, CuI, Et_2NH, DMF, rt, 1 h; (b) Bu_4NF, THF, rt, 12 h (78% for **23** and 91% for **24**, two steps); (c) H_2, Lindlar catalyst, quinoline, MeOH, rt, 20 min; (d) acetone, 80 °C, 4 h (64% for **27** and 72% for **28**, two steps); (e) HCl(g), EtOH, rt, 30 min (72%).

literature.[19] When substrate **10** was subjected to Mg/MeOH conditions, only moderate selectivity in removing PNB with respect to acetate was observed. We investigated several reaction conditions (best results were obtained at −30 °C using 5 equiv of Mg) but the highest yield achieved was 56% after flash chromatography. In addition to the monoalcohol **11**, starting material **10** and the corresponding diol from the deprotection of both esters, were isolated.

An efficient alternative for the selective manipulation of functional groups of similar reactivity is the use of biocatalysts, especially lipases. Applications of enzymes as catalysts in organic synthesis have been enhanced greatly in the past decade.[20] We previously described[11a] how enzymatic hydrolysis of diester **10** takes place with total selectivity, but noted that most of the enzymes we tested led to cleavage of the acetate ester in preference to the *p*-nitrobenzoate. The enzyme probably cannot accommodate a group as large as *p*-nitrobenzoyl in the active site. However, inversion of the allylic alcohol **9** under Mitsunobu conditions using chloroacetic acid, and the subsequent hydrolysis of **12** catalyzed by *Chromobacterium viscosum* lipase (CVL),[21] gave exclusively monoacetate **11**. CVL selectively hydrolyzes the C-3 chloroacetate ester instead of the C-5 acetate. When alcohol **11** was allowed to react with phthalimide in the presence of DEAD and PPh_3, phthalimide derivative **13** was obtained with complete inversion of the configuration. The reaction of **13** with 8 M $MeNH_2$ in EtOH results in complete deprotection of both the hydroxy and the amino groups to give the corresponding amino alcohol, which is N-protected by adding di-*tert*-butyl dicarbonate to the reaction mixture. The resulting product **14** is conveniently protected as a *tert*-butyldimethylsilyl ether.

The synthesis of 3β-amino A-ring fragment **6** is outlined in Scheme 3. First, silylation of alcohol **9** led to **16**, which was saponified using MeONa in MeOH followed by neutralization with ammonium chloride; when acid was used to cleave the ester, concomitant desilylation occurred. Inversion of alcohol **17** and subsequent saponification (as above) gave derivative **19**. Finally, treatment of the latter with phthalimide using the Mitsunobu procedure, deprotection of the amino group with methylamine, and protection as the *N*-Boc derivative afforded A-ring precursor **21**.

Coupling[22] of A-ring synthons **15** and **21** with the CD-triflate **22**, prepared according to the published procedure,[23] using bis(triphenylphosphine)palladium(II) acetate−copper(I) iodide catalyst and desilylation, then produced dienynes **23** and **24**, respectively (Scheme 4). Catalytic hydrogenation of amino alcohols **23** and **24** in deoxygenated MeOH, in the presence of Lindlar catalyst and quinoline, generated previtamins **25** and **26**. Thermolysis of the latter at 80 °C for 4 h followed by deprotection of the amino group with HCl yielded **3** and **4**, which were isolated as their hydrochloride salts.

In conclusion, we have synthesized two novel analogues of 1α,25-$(OH)_2$-D_3, each bearing an amino group in the A-ring fragment. Key features in the synthesis of corresponding A-ring synthons **15** and **21** are the excellent selectivity exhibited by *Chromobacterium viscosum* lipase in the hydrolysis of diester **11** and the direct replacement

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of the hydroxyl group by a phthalimide group with strict inversion of the configuration using Mitsunobu conditions.

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Supporting Information Available: Experimental procedures and complete ^{1}H and ^{13}C NMR spectral data in addition to mp, IR, microanalysis, and MS data for the new compounds. The level of purity is indicated by the inclusion of copies of ^{1}H and ^{13}C NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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