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SYNTHESIS AND BIOLOGICAL EVALUATION OF 18-SUBSTITUTED ANALOGS OF 1α,25-DIHYDROXYVITAMIN D_3

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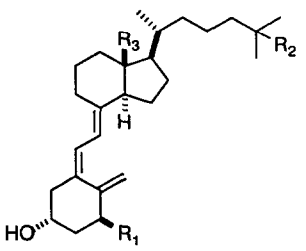
Abstract: The synthesis and biological evaluation of 1α,25-dihydroxy-13-vinyl-18-norvitamin D_3, 1α,25-dihydroxy-18-methylvitamin D_3 and 1α,18,25-trihydroxyvitamin D_3 are described.

One of the most important events in the vitamin D field in the last decade has been the discovery that 1α,25-dihydroxyvitamin D_3 (calcitriol, **1a**), the hormonally active form of vitamin D_3 (**1b**), in addition to its role in calcium homeostasis, also promotes cell differentiation and inhibits cell proliferation.[1] Unfortunately, this hormone cannot be used for the treatment of certain cancers due to its potent calcemic effects. For this reason, there is increasing interest in the synthesis of structurally modified analogs of **1a** which have potent effects on cell differentiation and proliferation but do not cause hypercalcemia. A number of such analogs have already been chosen for clinical evaluation with promising results.[2]

The vitamin D research groups at Riverside[3] and Santiago de Compostela[4] have independently reported the synthesis of new analogs of **1a** modified at C-18 to study their structure-function relationships. We describe here an improved synthetic route to the key lactone **2b,** its use in the preparation of 1α,25-dihydroxy-13-vinyl-18-norvitamin D_3 (**1c**), 1α,25-dihydroxy-18-methylvitamin D_3 (**1d**) and 1α,18,25-trihydroxyvitamin D_3 (**1e**), and the biological behaviour of these three vitamin D_3 analogs.



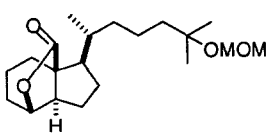
1a, $R_1 = R_2 = OH$, $R_3 = CH_3$

1b, $R_1 = R_2 = H$, $R_3 = CH_3$

1c, $R_1 = R_2 = OH$, $R_3 = CH{=}CH_2$

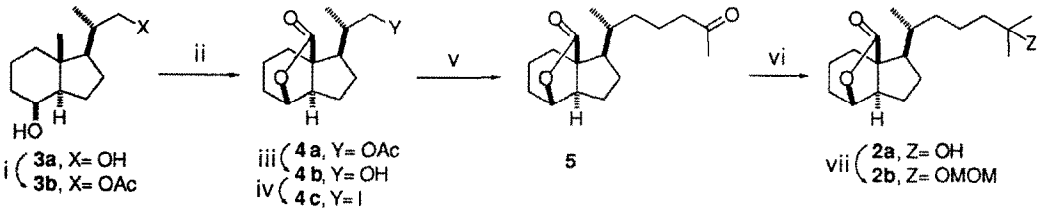
1d, $R_1 = R_2 = OH$, $R_3 = CH_2CH_3$

1e, $R_1 = R_2 = OH$, $R_3 = CH_2OH$



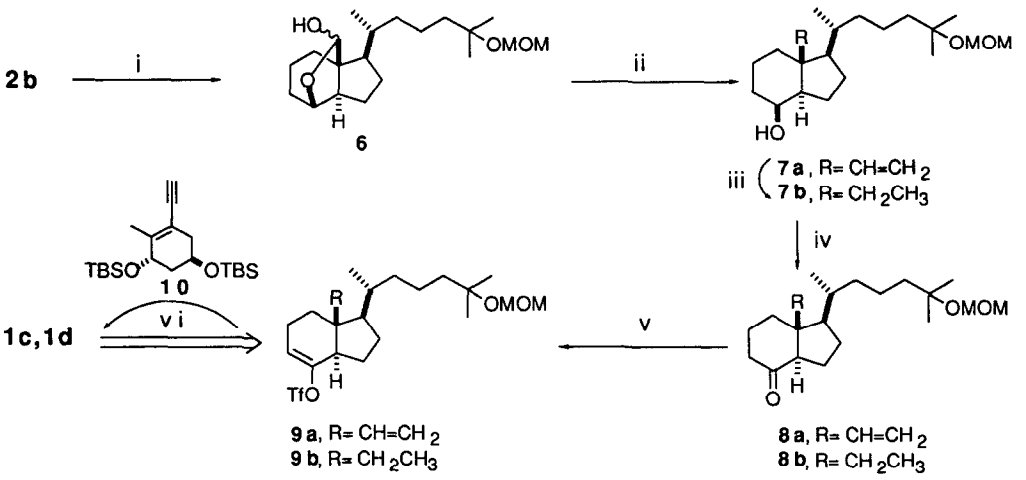
2b

The synthesis of the key lactone **2b** starts with the Inhoffen-Lythgoe diol (**3a**). Selective acetylation of **3a** afforded the monoacetate **3b** (87%).[4] Irradiation of a mixture of **3b**, lead tetraacetate, iodine and $CaCO_3$ in cyclohexane for 3 h at 80°C, followed by work-up and treatment of the resulting mixture with Jones reagent, gave the desired lactone **4a** (72%).[5] Selective saponification of the acetate group (K_2CO_3, MeOH) and subsequent treatment of the resulting alcohol **4b** with Ph_3PI_2 afforded the iodide **4c** (92% yield over the two steps). The iodide **4c** was converted to the ketone **5** by a procedure based on work by Luche and co-workers.[6] Ultrasonically induced conjugate addition of **4c** with methyl vinyl ketone in the presence of Zn and CuI in an aqueous medium, afforded the methyl ketone **5** (85%). Transformation of **5** to the sidechain-protected MOM ether **2b** was accomplished in 85% yield by reaction with methyllithium followed by protection of the resulting alcohol **2a** under standard conditions.



(i) Ac_2O (1 equiv), py, 0 °C, 24 h (87%). (ii) $Pb(OAc)_4$ (5 equiv), $CaCO_3$ (4 equiv), cyclohexane (175 mL/equiv), 80 °C; I_2 (1.3 equiv); **3b**, hv (tungsten lamp, 300 W, reflux, 3 h); work-up (5% $Na_2S_2O_3$, concentration); acetone, 0 °C, CrO_3-H_2SO_4 (Jones reagent), 12 h (72%) (iii) K_2CO_3 (13 equiv), MeOH (15 mL/equiv), r.t., 1 h (95%) (iv) I_2 (1 1 equiv), Ph_3P (1.1 equiv), imidazole (3 equiv), THF, -7 °C, 15 min (96%). (v) Methyl vinyl ketone (5 equiv), Zn (7 equiv), CuI (3 equiv), deoxygenated EtOH-H_2O (7:3, 13 mL/equiv), ultrasound, r.t, 45 min (85%). (vi) MeLi (1 1 equiv), Et_2O (4 mL/equiv), 0 °C, 5 min (87%). (vii) $ClCH_2OCH_3$ (4.4 equiv), *i*-Pr_2NEt (4.3 equiv), CH_2Cl_2 (98%)

With **2b** in hand, we turned to the preparation of the upper fragments **9a** and **9b**. Reduction of the lactone group with diisobutylaluminum hydride afforded the lactol **6** (87%), which upon Wittig olefination with the ylide derived from Ph_3PCH_3Br gave **7a** (95%). Catalytic hydrogenation of **7a** provided **7b** (97%). The alcohols **7a** and **7b** were individually oxidized with pyridinium dichromate to give **8a** (93%) and **8b** (94%) respectively. These ketones were individually converted into the corresponding vinyl triflates **9a** (76%) and **9b** (73%) by treatment with LDA and reaction of the resulting enolates with N-phenyl triflimide. Finally, the desired vitamin D analogs **1c** (53%) and **1d** (56%) were separately synthesized via the dienyne route[6b] (palladium-catalyzed assembly of the enyne **10**[6b,7] with the corresponding vinyl triflates, followed by partial hydrogenation, thermal isomerization and deprotection). The vitamin D analog **1e** was synthesized in 16% overall yield from lactone **2b** as previously described.[4]



(i) Toluene, -78 °C, DIBAL-H (1.4 equiv), 2 h (87%). (ii) Ph_3PCH_3Br (3 equiv), *t*-BuOK (3 equiv), THF, reflux, 12 h, **6**, THF, reflux, 12 h (95%). (iii) H_2/Pt-C, EtOH, 3.5 Psi, r.t., 24 h (97%). (iv) PDC (2.6 equiv), CH_2Cl_2, r.t., 4 h (93% for **8a**, 94% for **8b**). (v) LDA (1.1 equiv), THF, -78 °C, **8a** or **8b** in THF, 2 h; $PhNTf_2$ (1.1 equiv), 15 min, -78 °C→r.t, overnight, (76% for **9a**, 73% for **9b**). (vi) Dienyne route: **10** (1.1 equiv), DMF, Et_3N (2.8 equiv), $(Ph_3P)_2PdCl_2$ (0.04 equiv), 75 °C, 1 h 15 min; hexane, Lindlar, quinoline, H_2 (baloon pressure), r. t., 15 min; isooctane, 100 °C, 5 h; AG 50W-X4 resin, MeOH, r t , 18 h (53% for **1c**, 56 % for **1d**).

The 18-substituted vitamin D analogs **1c**, **1d** and **1e** were tested in *in vitro* assays. Affinity for the intracellular vitamin D receptor (VDR) was determined using the calf thymus VDR kit (Nichols Inst., Calif.). 1α,25-Dihydroxy-18-methylvitamin D_3 has the same high affinity as 1α,25-dihydroxyvitamin D_3. The affinities of 1α,25-dihydroxy-13-vinyl-18-norvitamin D_3 and 1α,18,25-trihydroxyvitamin D_3 are respectively four and fifty times lower than that of 1α,25-dihydroxyvitamin D_3. The capacity to stimulate cell differentiation was measured in terms of nitroblue tetrazolium reducing human leukemia (HL-60) cells.[2d] 1α,25-Dihydroxy-18-methylvitamin D_3, 1α,25-dihydroxy-13-vinyl-18-norvitamin D3 and 1α,18,25-trihydroxyvitamin D_3 are all potent stimulators of HL-60 cell differentiation, though only half as potent as 1α,25-dihydroxyvitamin D_3. Vitamin D binding protein (DBP), the specific carrier for vitamin D and its metabolites in blood, may influence the half-life of vitamin D derivatives in the circulation.[8] 1α,25-Dihydroxy-13-vinyl-18-norvitamin D_3 and 1α,25-dihydroxy-18-methylvitamin D_3 display about the same binding towards human DBP as 1α,25-dihydroxyvitamin D_3, suggesting that they have similar half-lives, *i.e.* they are not metabolized as quickly as calcipotriol, for example.[2c] To establish the calciotropic effect of the vitamin D derivatives, the Caco-2 intestinal cancer cell line was used as a model to measure intestinal calcium transport.[9] 1α,25-Dihydroxy-18-methylvitamin D_3 and 1α,25-dihydroxyvitamin D_3 are both potent stimulators of intestinal calcium transport, whereas 1α,25-dihydroxy-13-vinyl-18-norvitamin D_3 and 1α,18,25-trihydroxyvitamin D_3 are much less effective in this respect.

In conclusion, 1α,25-dihydroxy-13-vinyl-18-norvitamin D_3 and 1α,18,25-trihydroxyvitamin D_3, as potent stimulators of cell differentiation with rather low *in vitro* calciotropic activity, would seem to be promising candidates for further evaluation.

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