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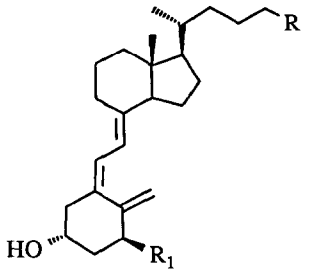
25-PHOSPHORUS ANALOGS OF VITAMIN D_3

William G. Dauben,*[a] Richard R. Ollmann, Jr.,[a] Angelika S. Funhoff,[a] Suzanne S. Leung,[a]
Anthony W. Norman[b] and June E. Bishop[b]

a: *Department of Chemistry, University of California, Berkeley, Berkeley, California 94720.*
b: *Division of Biomedical Sciences, University of California, Riverside, Riverside, California 92521*

Summary: The syntheses 1α-hydroxy-25-oxo-25-phosphavitamin D_3, 25-oxo-25-phospha-26,27-dioxa-26,27-dimethylvitamin D_3, and 1α-hydroxy-25-oxo-25-phospha-26,27-dioxa-26,27-dimethylvitamin D_3 are reported. The biological activity of 25-oxo-25-phosphavitamin D_3 and the above compounds are reported.

Vitamin D_3 (**1**) is metabolized to 25-hydroxyvitamin D_3 (25-OHD_3; **2**) and then to 1α,25-dihydroxyvitamin D_3 (1α,25-$(OH)_2D_3$; **3**). Compound **3**, acting as a steroid hormone, controls calcium and phosphorus homeostasis in higher organisms through intestinal calcium absorption (ICA) and bone calcium mobilization (BCM).[1] This hormone induces differentiation of leukemia cells to macrophages,[2] is effective



1	$R = CH(CH_3)_2$	$R_1 = H$
2	$C(OH)(CH_3)_2$	H
3	$C(OH)(CH_3)_2$	OH
4	$N(CH_3)_2$	H
5	$PO(CH_3)_2$	H
6	$PO(CH_3)_2$	OH
7	$PO(OCH_3)_2$	H
8	$PO(OCH_3)_2$	OH

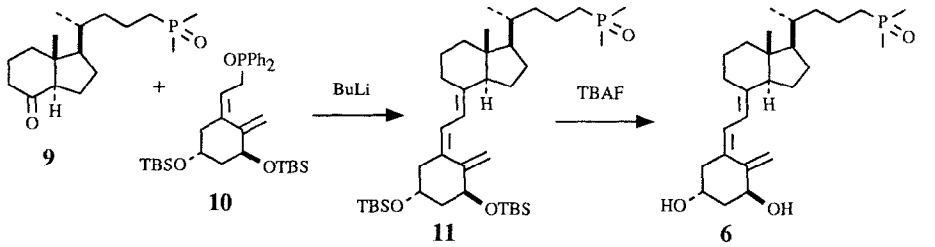
in the treatment of psoriasis,[3] and recently has been reported to be of interest in the treatment of many biological disorders.[4]

Few analogs of vitamin D_3 which possess heteroatoms in place of carbon atoms at position C-25 have been synthesized. DeLuca and coworkers have synthesized 25-azavitamin D_3 (**4**)[5] and it was found to inhibit vitamin D activity by competing for the biological site of 25 hydroxylation.[6] The synthesis of the first phosphorus-containing analog of vitamin D, 25-oxo-25-phosphavitamin D_3 (**5**) has been reported from this laboratory.[7] The syntheses of hydroxylated and phosphonate analogs of **5**, as well as their biological testing data, are reported in this communication.

The phosphine was introduced into the 25-position of vitamin D because it was anticipated that the

heteroatom at the 25-position could inhibit 25-hydroxylation as was the case for compound **4**. It was also anticipated that, if hydrogen-bonding to the oxygen of the hydroxyl group is significant in the binding of 25-hydroxyvitamin D to the biologically active site, then the P=O containing analogs should bind stronger at this site. If hydrogen-bonding from the active site to the hydrogen of the hydroxy group in 25-hydroxyvitamin D is significant, then it should be expected that the phosphorus analogs will not bind as efficiently to 25-hydroxyvitamin D receptor sites.

The introduction of the polar phosphorus functionalities into vitamin D_3 should alter the solubility and transport properties of the new analogs. These features becomes important in the use of vitamin D in the treatment of cancer. If a vitamin D analog can be designed that retains the ability to induce differentiation of cancer cells and is only capable of reaching a target organ, problems with toxic effects of vitamin D, such as hypercalcemia, could be avoided.[2] The 25-phosphorus analogs of vitamin D_3 should be worthy of study from this stand point.

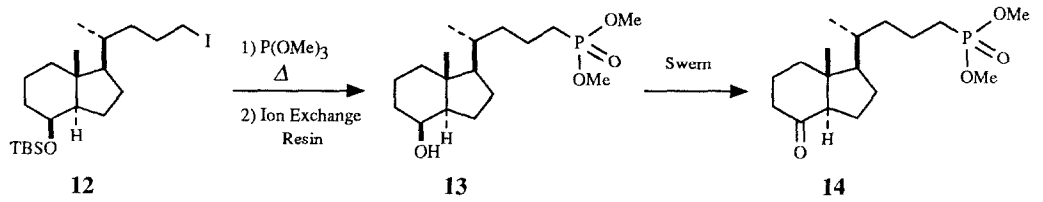


The synthesis of the 1α-hydroxy-derivative of **5** involves condensation of **9** with the proper hydroxylated A-ring fragment **10**.[8] The synthesis of **10** followed the published procedure except, for the introduction of the 3β-acetate via the Baeyer-Villiger oxidation, a Criegee rearrangement[9] was used.

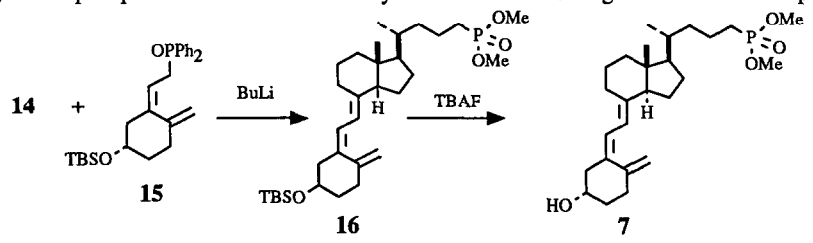
With the proper A-ring piece in hand, the anion of **10** was allowed to condense with **9** to give **11** in 95% yield. Compound **9** was found to be, in this experiment, hygroscopic and it was necessary to azeotropically distill benzene/water from **9** prior to use in order to achieve acceptable yields. Deprotection of **11** using TBAF gave compound **6** in 83% yield.[10]

The CD-ring primary iodide **12** that was generated in the synthesis of **5**[7] was the ideal intermediate for the syntheses of phosphonates **7** and **8.** Iodide **12** was allowed to react with trimethylphosphite, under refluxing conditions, to give the phosphonate as a white, crystalline solid in 26% yield. Initial attempts to remove the silyl ether protecting group with TBAF provided complex product mixtures. Treatment of the phosphonate with freshly activated AG-50W-X4 cationic exchange resin[11,12] in methanol gave alcohol **13** as light yellow crystals in 98% yield.

Compound **13** was oxidized, under Swern conditions,[13] to give CD-ring ketone **14** in 86% yield. This compound was handled with care to avoid epimerization of the ring juncture hydrogen, as with the CD-ring ketone **9**.

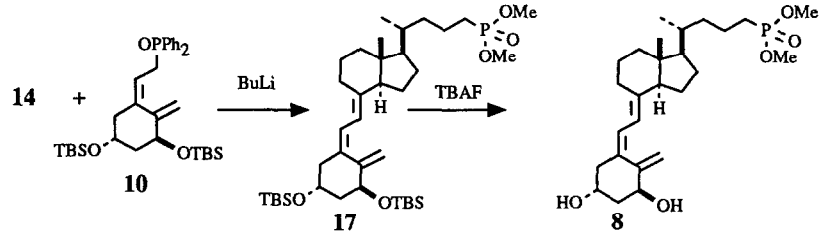


Again using the Lythgoe coupling, ketone **14** was condensed with the anion of **15** to give the protected form of compound **16** in 74% yield. The silyl ether group was removed from **16** by allowing it to react with TBAF to give the phosphonate vitamin **7** in 67% yield.[14] It is interesting to note that the deprotection of **16**



could be affected with fluoride ion in the presence of a phosphonate group while attempts to deprotect **12** with fluoride ion failed. The reason for this is probably that the rate of cleavage of the silyl ether of **16** is much faster than the silyl ether of **12**. While both silyl ethers are of secondary alcohols on six-membered rings, the silyl ether of **12** is located in a sterically congested area, whereas the silyl ether of **16** is more accessible to the attack by fluoride ion.

In a manner that parallels the synthesis of **7**, the anion of **10** was condensed with **14** to give the protected vitamin derivative **17** in 83% yield. Deprotection of this compound gave compound **8** in 83% yield.[15]



The four phosphorus analogs of vitamin D_3 were analyzed for biological activity. Each analog showed slight vitamin D-like activity. In general, the phosphonates were more active than the phosphine oxides and the 1α-hydroxylated compounds were more active that the non-1α-hydroxylated analogs. The analog showing the highest activity was compound **8**. In comparing **8** to 1α,25-dihydroxyvitamin D_3, it possesses a Relative Competitive Index (RCI)[16] of 7% in binding, under *in vitro* conditions, to chick intestinal receptor; and in an *in vivo* assay conducted on vitamin D-deficient chicks,[17] intestinal calcium absorption (ICA) of 2%; and bone calcium mobilization (BCM) of 1%. Both 1α-hydroxyl derivatives showed only weak activity on inhibiting the clonogenic proliferation of HL-60 promyelocytes and almost no activity on promoting the differentiation of HL-60 cells.[18]

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10. **6** glassy solid; $[\alpha]_D$ = +33.1° (CH_2Cl_2, *c* 0.95); UV (EtOH) λ_{max} 265 (ϵ 14700), 212 (ϵ 12700) nm; IR (film) 3380, 2940, 2890, 1650, 1305, 1235, 1060 cm^{-1}; ^{1}H-NMR (500 MHz, CD_2Cl_2) δ 6.34 (d, 1 H, J = 11.3 Hz), 6.02 (d, 1 H, J = 11.3 Hz), 5.29 (m, 1 H), 4.95 (m, 1 H), 4.36 (dd, 1 H, J = 4.3, 7.9 Hz), 4.15 (m, 1 H), 2.83 (m, 1 H), 2.54 (m, 1 H), 2.26 (dd, 1 H, J = 6.5, 13.3 Hz), 2.01-1.46 (m, 16 H), 1.42 (d, 6 H, J = 12.5 Hz), 1.36-1.16 (m, 6 H), 0.95 (d, 3 H, J = 6.5 Hz), 0.55 (s, 3 H); ^{31}P-NMR (18.8 MHz, CD_2Cl_2) δ 43.90.
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14. **7** clear oil; $[\alpha]_D$ = +27.0° (CH_2Cl_2, *c* 0.73); UV (EtOH) λ_{max} 264 (ϵ 14700) nm; IR (film) 3400, 2950, 2890, 1640, 1233, 1060, 1035 cm^{-1}; ^{1}H-NMR (250 MHz, d_6-acetone) δ 6.25 (d, 1 H, J = 11.2 Hz), 6.08 (d, 1 H, J = 11.2 Hz), 5.03 (m, 1 H), 4.75 (m, 1 H), 3.78 (m, 1 H), 3.64 (d, 6 H, J = 10.7 Hz), 2.91-1.04 (m, 26 H), 0.97 (d, 3 H, J = 6.0 Hz), 0.58 (s, 3 H); ^{31}P-NMR (18.8 MHz, d_6-acetone) δ 35.56.
15. **8** clear oil; $[\alpha]_D$ = +38.3° (CH_2Cl_2, *c* 0.98); UV λ_{max} 264 (ϵ 18100), 211 (ϵ 17000) nm; IR (film) 3390, 2944, 2890, 1645, 1235, 1054, 1030 cm^{-1}; ^{1}H-NMR (250 MHz, d_6-acetone) δ 6.30 (d, 1 H, J = 11.1 Hz), 6.10 (d, 1 H, J = 11.1 Hz), 5.32 (m, 1 H), 4.87 (m, 1 H), 3.69 (d, 6 H, J = 10.7 Hz), 2.51 (m, 1 H), 2.29 (m, 1 H), 2.06-1.20 (m, 22 H), 0.97 (d, 3 H, J = 6.0 Hz), 0.92-0.90 (m, 1 H), 0.59 (s, 3 H); ^{31}P-NMR (18.8 MHz, d_6-acetone) δ 35.69.
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