18-Substituted Derivatives of Vitamin D: 18-Acetoxy-1α,25-dihydroxyvitamin D_3 and Related Analogues[1a]

David F. Maynard,[1b] Anthony W. Norman,[1c] and William H. Okamura*,[1b]

Department of Chemistry, Division of Biomedical Sciences, and the Department of Biochemistry, University of California, Riverside, California 92521

Received December 23, 1991

The bridged ether **6**, produced via light-induced lead tetraacetate oxidation of Grundmann's alcohol **5**, was transformed in three to four steps to the CD-ring ketones **9b** and **10** which were then transformed via Horner–Wittig coupling to the 18-acetoxy analogues of vitamin D_3, 25-hydroxyvitamin D_3, 1α,25-dihydroxyvitamin D_3, and 1α-hydroxyvitamin D_3 (**4a–d**, respectively). The biological evaluation of **4a–d** in terms of their ability to elicit intestinal calcium absorption and bone calcium mobilization in vivo in the chick is reported. These vitamin D analogues, the first in this steroid series modified at the C-18 position, are nearly devoid of activity. In vitro data for **4a–d** in terms of their ability to bind to the chick intestinal receptor as compared to the natural hormone 1α,25-dihydroxyvitamin D_3, parallels the in vivo data.

Introduction

The metabolic activation of vitamin D_3 (**1**) to 25-hydroxyvitamin D_3 (**2**, 25-OH-D_3) in the liver and then to 1α,25-dihydroxyvitamin D_3 (**3**, 1α,25-$(OH)_2D_3$) in the kidney is now firmly established (Scheme I).[2] Chemical developments in the vitamin D field are of continuing interest because they may be of value in the preparation of analogues with possible applications in the continuing emergence of newly recognized disease states associated with vitamin D metabolism (e.g., cancer chemotherapy[3] and treatment of psoriasis,[4] osteoporosis,[5] and immunosuppressant actions,[6] etc.). While many side-chain, A-ring, and triene modifications of the vitamin D system have been reported,[7] structure–function relationships for CD-ring[8] analogues of vitamin D are rare, probably because of the difficulty in synthetically modifying this portion of the molecule. From the standpoint of developing appropriate biochemical research tools, identification of an analogue for developing a photoaffinity label reagent[9] for characterization of the steroid-binding domain of vitamin D receptor is attractive.[10] CD-ring tethering of photoaffinity label is attractive because it is distal from the A-ring and side-chain hydroxyls considered to be functionally important for the biological action of the hormone 1α,25-$(OH)_2D_3$ or other metabolites. Recently, our laboratory and those of others have directed attention towards modification of the C-11 and/or 9 position of the CD nucleus of vitamin D.[8] It is the purpose of this article to describe a very facile synthesis and characterization of 18-acetoxy derivatives of metabolites **1**, **2**, and **3**, namely **4a**, **4b**, and **4c**, respectively, and also the 18-acetoxy derivative of the pro-drug 1α-hydroxyvitamin D_3, analogue **4d**.

Results and Discussion

Taking advantage of the ready availability of the axial 8β-hydroxy CD-fragment **5** (Scheme II),[11] we determined that photoinduced lead tetraacetate oxidation of **5** according to the procedure of Mihailovic[12] very nicely afforded the tetrahydrofuranyl bridged ether **6**. Particularly diagnostic in the ^{1}H-NMR spectrum of this nonpolar ether is the appearance of an AB pattern at δ 3.68 and 3.72 (J_{AB} = ~8.6 Hz) due to the diastereotopic C_{18} protons and an initially surprising simple pseudo-doublet at δ 4.12 assigned to the equatorial H_8 proton (steroid numbering is used throughout this article). The latter is apparently due to the nearly perpendicular relationship of the vicinal $H_{9\alpha}$ and $H_{14\alpha}$ proton signals to H_8 (J = ~0 Hz). After some trial, it was determined that boron-trifluoride-etherate-mediated ring opening[13] of **6** in acetic anhydride allowed the preparation of diacetate **7** wherein the stereochemistry of C-8 had been inverted. The equatorial orientation of the C-8 acetoxyl group was apparent from the ^{1}H-NMR analysis of **7** wherein the H_8 proton appears at δ 4.90 as an apparent doublet of triplets, indicating the presence of two diaxial splittings (two J = ~10.8 Hz splittings) and one equatorial/axial splitting (J ~4.7 Hz).

Selective saponification of the equatorial 8β-acetoxy of **7** to afford acetoxy alcohol **8** could be effected despite the presence of the primary acetoxyl at C-18, presumably

(1) (a) This is paper 41 in the series Studies of Vitamin D (Calciferol) and Its Analogues. For paper 40, see: Curtin, M. L.; Okamura, W. H. *J. Am. Chem. Soc.* **1991**, *113*, 6958. (b) Department of Chemistry. (c) Division of Biomedical Sciences and the Department of Biochemistry.

(2) For a description of recent advances in the field including leading references, see: (a) Norman, A. W.; Schaeffer, K.; Grigoleit, H.-G.; Herrath, D. V., Eds.; *Vitamin D: Molecular, Cellular and Clinical Endocrinology*; Walter de Gruyter and Co.: Berlin; 1988. (b) Norman, A. W.; Bouillon, R.; Thomasset, M., Eds.; *Vitamin D: Gene Regulation, Structure Function Analysis and Clinical Application*; Walter de Gruyter and Co.: Berlin; 1991.

(3) Honma, Y.; Hozumi, M.; Abe, E.; Konno, K.; Fukushima, M.; Hata, S.; Nishii, Y.; DeLuca, H. F.; Suda, T. *Proc. Natl. Acad. Sci. U.S.A.* **1983**, *80*, 201.

(4) MacLaughlin, J. A.; Gange, W.; Taylor, D.; Smith, E.; Holick, H. F. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 5409.

(5) Nishii, Y.; Abe, J.; Sato, K.; Kobayashi, T.; Okano, T.; Tsugawa, N.; Slatopolsky, E.; Brown, A. J.; Dusso, A.; Raisz, L. G. *Vitamin D: Gene Regulation, Structure Function Analysis and Clinical Application*; (Norman, A. W., Bouillon, R., Thomasset, M., Eds.; Walter de Gruyter and Co.: Berlin, 1991; pp 289–297.

(6) Yukioka, K.; Otani, S.; Matsui-Yuasa, I.; Goto, H.; Morisawa, S.; Okuno, S.; Inaba, M.; Nishizawa, Y.; Morii, H. *Arch. Biochem. Biophys.* **1988**, *260*, 45.

(7) For leading references, see ref 2 as well as the following: Ikekawa, N. *Med. Res. Rev.* **1987**, *7*, 333.

(8) (a) Velluz, L.; Amiard, G. *C.R. Hebd. Seances Acad. Sci.* **1961**, *251*, 603. (b) Revelle, L.; Solan, V.; Londowski, J.; Bollman, S.; Kumar, R. *Biochemistry* **1984**, *23*, 1983. (c) Gibbs, R. A.; Okamura, W. H. *Tetrahedron Lett.* **1987**, *48*, 6021. (d) Okamura, W. H.; Aurrecoechea, J. M.; Gibbs, R. A.; Norman, A. W. *J. Org. Chem.* **1989**, *54*, 4072. (e) Pumar, C.; Aurrecoechea, J. M.; Gibbs, R. A.; Norman, A. W.; Okamura, W. H. *Vitamin D: Molecular, Cellular and Clinical Endocrinology*; Norman, A. W., Schaeffer, K., Grigoleit, H.-G., Herrath, D. v., Eds.; Walter de Gruyter and Co.: Berlin, 1988; pp 54–57. (f) Maestro, M. A.; Sardina, F. J.; Castedo, L.; Mouriño, A. *J. Org. Chem.* **1991**, *56*, 3582. (g) Bouillon, R.; Allewaert, K.; van Leeuwen, J. P. T. M.; Tan, B.-K.; Xiang, D. Z.; De Clercq, P.; Vandewalle, M.; Pols, H. A. P.; Bos, M. P.; Baelen, H. V.; Birkenhäger, J. C. *J. Biol. Chem.* In press.

(9) For a recent review on photoaffinity labeling, see: Schuster, D. I.; Probst, W. C.; Ehrlich, G. K.; Singh, G. *Photochem. Photobiol.* **1989**, *49*, 785.

(10) (a) Ray, R.; Bouillon, R.; van Baelen, H.; Holick, M. F. *Biochemistry* **1991**, *30*, 4809. (b) Ray, R.; Holick, M. F. *Steroids* **1988**, *51*, 623.

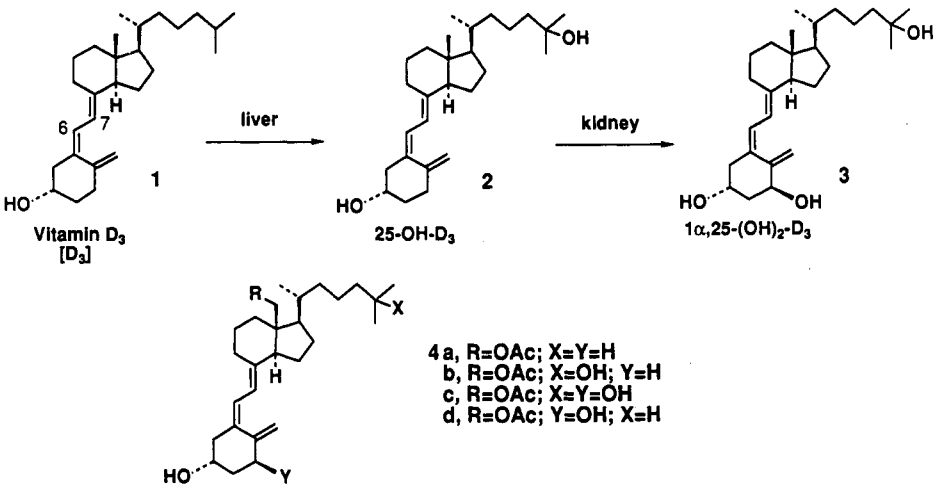
(11) (a) Peterson, P. E.; Leffew, R. L. B.; Jensen, B. L. *J. Org. Chem.* **1986**, *51*, 1948. (b) Inhoffen, H. H.; Quinkert, G.; Siegismund, S.; Kampe, D.; Domagk, G. F. *Chem. Ber.* **1957**, *90*, 664.

(12) Mihailovic, M. L.; Jakovljevic, M.; Cekovic, Z. *Tetrahedron* **1969**, *25*, 2269.

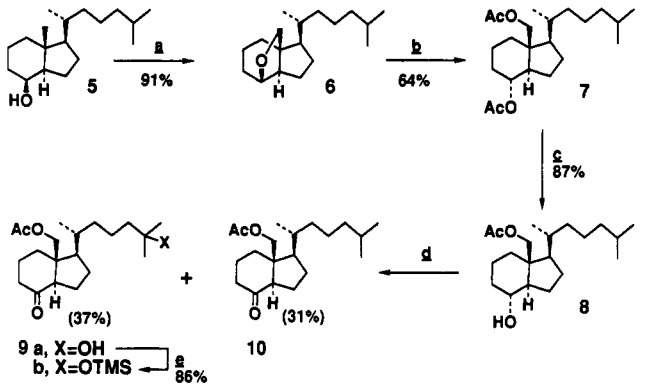
(13) Narayanan, C. R.; Iyer, K. N. *Tetrahedron Lett.* **1964**, 759.

0022-3263/92/1957-3214$03.00/0

Scheme I



Scheme II[a]



[a] Reagents: (a) $Pb(OAc)_4$ (5 equiv), PhH, Py, $h\nu$ (quartz, 450 W, Hanovia med pressure Hg lamp); (b) $BF_3{\cdot}Et_2O$ (20 equiv), Ac_2O, −20 °C and rt (30 min each); (c) K_2CO_3 (2 equiv), MeOH, rt (1 h); (d) $RuCl_3{\cdot}H_2O$ (0.1 equiv), $NaIO_4$ (3.5 equiv), phosphate buffer, CH_3CN–CCl_4; (e) TMS-imidazole, THF, rt (2 h).

because of the sterically congested nature of the primary alcohol ester. Direct ruthenium tetraoxide oxidation[14] of acetoxy alcohol **8** produced a near 1:1 mixture of **9a** and **10** (37% and 31% yields, respectively). Further oxidation of **10** under the same conditions also produced **9a** in about 30% yield. The latter **9a** was then transformed to the side-chain-protected TMS ether **9b**. With both **9b** and **10** in hand it then became a simple matter to couple these CD ketones with the known phosphine oxides **11**[15] and **12**[16] in appropriate combinations. As shown in Scheme III, the various permutations of the coupling of **11** or **12** with **9b** or **10** afforded the various combinations of the hydroxyl-protected vitamin D derivatives **13a–d**. Treatment of each of the latter vitamin D derivatives with tetra-*n*-butylammonium fluoride in THF afforded the four target analogues **4a–d**, respectively.

With analogues **4a–d** in hand, intestinal calcium absorption (ICA) and bone calcium mobilization (BCM) were measured in vivo in comparison to vitamin D_3 (**1**) and $1\alpha,25\text{-}(OH)_2D_3$ (**3**) in the vitamin D deficient chick system previously described.[17] The results in this standard rachitic chick assay can be reported as the percentage of activity observed for ICA and BCM in comparison to standard doses of $1\alpha,25\text{-}(OH)_2D_3$.[18] With respect to the ICA determination, the four analogues **4a**, **4b**, **4c**, and **4d** exhibited only <0.03%, <0.05%, <0.15%, and <0.05%, respectively, of the activity of 100 pmol of $1\alpha,25\text{-}(OH)_2D_3$. Similarly, with respect to the BCM determination, the four analogues **4a**, **4b**, **4c**, and **4d** exhibited only <0.2%, 0.3%, <1.0%, and <0.3%, respectively, of that of 650 pmol of $1\alpha,25\text{-}(OH)_2D_3$. While it is understandable that **4a**, **4b**, and **4d** may exhibit low biological activity because they must presumably be metabolized to **4c** before exerting their biological function, it is surprising that **4c** exhibits so little ICA and BCM activity. In fact, this analogue appears to be one of the least active analogues known which so closely resembles the parent hormone from a structural standpoint (it contains all three important hydroxyls at C-1, C-3, and C-25 and it differs only in the incorporation of an acetoxyl at C-18).

The analogues were evaluated in vitro in terms of their ability to bind to the chick intestinal receptor in comparison to the natural hormone $1\alpha,25\text{-}(OH)_2D_3$ in order to better evaluate whether the C_{18}-OAc renders these analogues exceptionally susceptible to catabolic destruction, thus accounting for their low in vivo biological activity. In this assay,[19] the analogues are evaluated in terms of their relative competitive indices (RCIs) wherein the value for $1\alpha,25\text{-}(OH)_2D_3$ is 100% by definition. The RCI values for **4a**, **4b**, **4c**, and **4d** were 0.00075 ± 0.00017%, 0.0017 ± 0.0005%, 0.036 ± 0.021%, and 0.017 ± 0.010%, respectively. These values are in qualitative agreement with the in vivo results described above. Indeed, the RCI value for **4c** is one of the lowest reported for an analogue which so closely resembles the natural hormone $1\alpha,25\text{-}(OH)_2D_3$.

In summary, **4a–d** are the first 18-substituted analogues of vitamin D to be synthesized and biologically evaluated. Although it would be desirable to evaluate several additional 18-substituted analogues, we tentatively conclude at this juncture that the 18-substituent is deleterious to binding to the chick receptor which is believed to be critical to the in vivo biological function of vitamin D. Although this negative information is very useful in a structure–

(14) (a) Kiegel, J.; Wovkulich, P. M.; Uskoković, M. R. *Tetrahedron Lett.* **1991**, *32*, 6057. We thank Dr. M. Uskoković for a detailed experimental procedure prior to publication. (b) For other ruthenium-catalyzed C–H oxidations, see: Tenaglia, A.; Terranova, E.; Waegell, B. *Tetrahedron Lett.* **1989**, *30*, 5271.

(15) (a) Toh, H. T.; Okamura, W. H. *J. Org. Chem.* **1983**, *48*, 1414. (b) Lythgoe, B.; Nambudiry, M. E. N.; Tideswell, J. *Tetrahedron Lett.* **1977**, 3685.

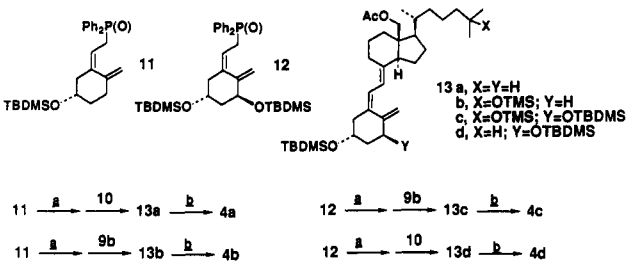
(16) Baggiolini, E. G.; Iacobelli, J. A.; Hennessy, B. M.; Batcho, A. D.; Sereno, J. F.; Uskoković, M. R. *J. Org. Chem.* **1986**, *51*, 3098 and references cited.

(17) Hibberd, K. A.; Norman, A. W. *Biochem. Pharmacol.* **1969**, *18*, 2347.

(18) For a recent example of the expression of in vivo data using this format, see: Figadère, B.; Norman, A. W.; Henry, H. L.; Koeffler, H. P.; Zhou, J.-Y.; Okamura, W. H. *J. Med. Chem.* **1991**, *34*, 2452.

(19) (a) Procsal, D. A.; Okamura, W. H.; Norman, A. W. *J. Biol. Chem.* **1975**, *250*, 8382. (b) Procsal, D. A.; Okamura, W. H.; Norman, A. W. *Am. J. Clin. Nutr.* **1976**, *29*, 1271. (c) Wecksler, W. R.; Norman, A. W. *Methods in Enzymology: Vitamins and Co-Enzymes* **1980**, *67*, 488.

Scheme III[a]



[a] Reagents: (a) *n*-BuLi (1.1 equiv, 1.6 M in hexanes), THF; (b) TBAF (3 equiv, 1 M in THF).

function sense, it is apparent that application of these analogues as affinity probes is unlikely to be fruitful.

Experimental Section[20]

18-Acetoxyvitamin D_3 (4a). A solution of silyl ether **13a** (18 mg, 0.031 mmol) in dry THF (0.6 mL) was placed under argon at room temperature. Tetra-*n*-butylammonium fluoride (0.156 mL, 0.156 mmol, 1 M in THF) was added dropwise. After 12 h, the solvent was partially evaporated and the residue diluted with water (5 mL). The aqueous layer was extracted with ethyl acetate (3 × 5 mL) and then the combined organic layers were washed with water and brine and then dried over Na_2SO_4. The crude residue was chromatographed through a short silica gel column and then further purified by HPLC (50% ethyl acetate/hexanes, 4 mL/min Rainin Dynamax 60 A column) to afford after vacuum drying 10.8 mg (83%) of vitamin **4a** (76% from CD-ring fragment) as a colorless foam. ^{1}H-NMR: ($CDCl_3$) δ 0.86 (6 H, $C_{26,27}$-CH_3, d, $J \sim 6.7$ Hz), 1.01 (3 H, C_{21}-CH_3, d, $J \sim 5.2$ Hz), 2.01 (3 H, Ac, s), 2.56 (1 H, $H_{4\alpha}$, dd, $J \sim 13.0$, 3.3 Hz), 2.86 (1 H, $H_{9\beta}$, dd, $J \sim 11.3$, 2.4 Hz), 3.83 (1 H, H_{18}, d, $J \sim 12.1$ Hz), 3.87 (1 H, $H_{18'}$, d, $J \sim 12.1$ Hz), 3.9–4.0 (1 H, H_3, m), 4.79 (1 H, H_{19E}, d, $J \sim 1.9$ Hz), 5.03 (1 H, H_{19Z}, br s), 6.03 and 6.18 (2 H, $H_{6,7}$, AB pattern, d, $J \sim 11.3$ Hz).

18-Acetoxy-25-hydroxyvitamin D_3 (4b). A solution of silyl ether **13b** (23.1 mg, 0.036 mmol) in dry THF (0.3 mL) was deprotected with tetra-*n*-butylammonium fluoride (0.21 mL, 0.21 mmol, 1 M in THF) and the reaction mixture processed as described for the preparation of **4a**. There was obtained 10 mg (61%) of vitamin **4b** (46% from CD-ring fragment) as a colorless oil. ^{1}H-NMR: ($CDCl_3$) δ 1.03 (3 H, C_{21}-CH_3, d, $J \sim 4.9$ Hz), 1.21 (6 H, $C_{26,27}$-CH_3, s), 2.01 (3 H, Ac, s), 2.56 (1 H, dd, $J \sim 12.9$, 4.3 Hz), 2.86 (1 H, $H_{9\beta}$, br d, $J \sim 12.8$ Hz), 3.83 (1 H, H_{18}, d, $J \sim 12.1$ Hz), 3.88 (1 H, H_{18}, d, $J \sim 12.1$ Hz), 3.9–4.0 (1 H, H_3, m), 4.80 (1 H, H_{19}, br s), 5.17 (1 H, H_{19}, br s), 6.04 and 6.19 (2 H, $H_{6,7}$, AB pattern, d, $J \sim 11.4$ Hz).

18-Acetoxy-1α,25-dihydroxyvitamin D_3 (4c). A solution of silyl ether **13c** (41.7 mg, 0.059 mmol) in THF (0.7 mL) was reacted with tetra-*n*-butylammonium fluoride (0.53 mL, ~0.53 mmol, 1 M in THF) and the reaction mixture processed as described above for **4a**. This afforded 25 mg (91%) of vitamin **4c** (69% from CD-ring fragment) as a colorless oil. ^{1}H-NMR: ($CDCl_3$) δ 1.01 (3 H, C_{21}-CH_3, d, $J \sim 4.0$ Hz), 1.20 (6 H, $C_{26,27}$-CH_3, s), 2.00 (3 H, Ac, s), 2.41 (1 H, d, $J \sim 12.6$ Hz), 2.59 (1 H, dd, $J \sim 13.3$, 2.9 Hz), 2.86 (1 H, $H_{9\beta}$, d, $J \sim 13.0$ Hz), 3.84 (1 H, H_{18}, d, $J \sim 12.0$ Hz), 3.90 (1 H, H_{18}, d, $J \sim 12.0$ Hz), 4.22 (1 H, H_3, m), 4.41 (1 H, H_1, apparent t, $J \sim 5.5$ Hz), 4.97 (1 H, H_{19}, br s), 5.30 (1 H, H_{19}, br s), 6.03 and 6.33 (2 H, $H_{6,7}$, AB pattern, d, $J \sim 11.2$ Hz).

18-Acetoxy-1α-hydroxyvitamin D_3 (4d). A solution of silyl ether **13d** (54 mg, 0.079 mmol) in dry THF (1.5 mL) was treated with tetra-*n*-butylammonium fluoride (0.40 mL, 0.40 mmol, 1 M in THF) and then the reaction mixture worked up and processed as described for **4a**. This afforded 27 mg (75%) of vitamin **4d** (68% from CD-ring fragment) as a colorless oil. ^{1}H-NMR: ($CDCl_3$) δ 0.86 (6 H, $C_{26,27}$-CH_3, d, $J \sim 6.5$ Hz), 1.00 (3 H, C_{21}-CH_3, d, $J \sim 4.3$ Hz), 2.40 (1 H, d, $J \sim 12.6$ Hz), 2.57 (1 H, dd, $J \sim 13.3$, 2.8 Hz), 2.86 (1 H, $H_{9\beta}$, br d, $J \sim 13.0$ Hz), 3.85 (1 H, H_{18}, d, $J \sim 12.1$ Hz), 3.90 (1 H, H_{18}, d, $J \sim 12.1$ Hz), 4.22 (1 H, H_3, m), 4.41 (1 H, H_1, d, $J \sim 5.5$ Hz), 4.97 (1 H, H_{19}, br s), 5.30 (1 H, H_{19}, br s), 6.04 and 6.33 (2 H, $H_{6,7}$, AB pattern, d, $J \sim 11.2$ Hz).

De-*A*,*B*-8β-cholestanol (5). Grundmann's ketone (2.50 g, 9.47 mmol) in 90 mL of dry ether was reduced with $LiAlH_4$ (3.6 g, 94.6 mmol) in 270 mL of dry ether by a method described earlier.[11] There was obtained alcohol **5** as a colorless oil in 96% yield. ^{1}H-NMR: ($CDCl_3$) δ 0.84 (6 H, $C_{26,27}$-CH_3, d, $J \sim 7.6$ Hz), 0.87 (3 H, C_{21}-CH_3, d, $J \sim 7.6$ Hz), 0.90 (3 H, C_{18}-CH_3, s), 4.04 (1 H, $H_{8\alpha}$, m).

De-*A*,*B*-8β,18-epoxycholestane (6). Lead tetraacetate (18.8 g, 43.4 mmol) was added to a solution of de-*A*,*B*-8β-cholestanol (**5**, 2.31 g, 8.69 mmol), pyridine (4.1 mL), and dry benzene (600 mL) in a cylindrical photochemical immersion apparatus equipped with a stir bar and sintered glass nitrogen inlet. The mixture was irradiated for 2.5 h at room temperature with a 450-W Hanovia medium pressure mercury arc lamp. The heterogeneous mixture turns from brown to white during the course of the reaction. The mixture was filtered and the solid residue washed with ether (3 × 100 mL). The crude solution was concentrated and the residue was flashed chromatographed (10% ethyl acetate/hexanes) to yield the 8β,18-ether **6** (2.08 g, 91% yield) as a clear oil. A sample was further purified by HPLC to give a spectroscopically homogeneous sample for spectral analysis. ^{1}H-NMR: ($CDCl_3$) δ 0.84 (6 H, $C_{26,27}$-CH_3, d, $J \sim 6.5$ Hz), 0.87 (3 H, C_{21}-CH_3, d, $J \sim 6.5$ Hz), 3.68 (1 H, H_{18}, br d, $J \sim 8.6$ Hz), 3.72 (1 H, H_{18}, d, $J \sim 8.6$ Hz), 4.12 (1 H, H_8, apparent d, $J \sim 4.2$ Hz).

De-*A*,*B*-8α,18-diacetoxycholestane (7). To the ether **6** (0.13 g, 0.51 mmol) in Ac_2O (10 mL) cooled to −20 °C under an argon atmosphere was added dropwise BF_3·Et_2O (1.2 mL, 1.45 g, 10.2 mmol). The reaction mixture stirred for 30 min at −20 °C and then warmed to room temperature. After an additional 30 min, the mixture was poured into an ice-cooled saturated $NaHCO_3$ solution. The aqueous solution was extracted with ether (3 × 20 mL) and then the combined organic layers were washed with brine (2 × 10 mL), dried over $MgSO_4$, and concentrated. The crude residue was purified by flash chromatography (10% EtOAc/hexanes, silica gel) to afford after vacuum drying the diacetate **7** (0.118 g, 63%) as a colorless oil. ^{1}H-NMR: ($CDCl_3$) δ 0.84 (6 H, $C_{26,27}$-CH_3, d, $J \sim 6.5$ Hz), 0.98 (3 H, C_{21}-CH_3, d, $J \sim 6.0$ Hz), 1.99 (3 H, Ac, s), 2.04 (3 H, Ac, s), 3.95 (1 H, H_{18}, d, $J \sim 11.9$ Hz), 4.14 (1 H, H_{18}, d, $J \sim 11.9$ Hz), 4.90 (1 H, H_8, dt, $J \sim 4.7$ Hz, 10.8 Hz).

De-*A*,*B*-18-acetoxy-8α-cholestanol (8). Diacetate **7** (0.11 g, 0.29 mmol) was added to a suspension of K_2CO_3 (79 mg, 0.57 mmol) in methanol (4 mL). A continuous, slow stream of argon was passed over the reaction mixture using a T tube until the starting material was consumed (TLC). The mixture was then filtered through a short silica gel column and then the filtrate was concentrated. The crude residue was purified by HPLC (Rainin 25 × 1 cm, 50% EtOAc/hexanes) to afford alcohol **8** (85 mg, 73%) as a colorless oil. ^{1}H-NMR: ($CDCl_3$) δ 0.84 (6 H, $C_{26,27}$-CH_3, d, $J \sim 6.6$ Hz), 0.97 (3 H, C_{21}-CH_3, d, $J \sim 6.0$ Hz), 2.02 (3 H, Ac, s), 3.64 (1 H, H_8, dt, $J \sim 4.6$, 10.4 Hz), 3.89 (1 H, H_{18}, d, $J \sim 11.8$ Hz), 4.08 (1 H, H_{18}, d, $J \sim 11.8$ Hz).

De-*A*,*B*-18-acetoxy-25-hydroxy-8-cholestanone (9a) and De-*A*,*B*-18-acetoxy-8-cholestanone (10). To a solution of hydroxy ester **8** (310 mg, 0.85 mmol) in a mixture of carbon tetrachloride (4 mL), acetonitrile (4 mL), and 5 mL of pH 7 aqueous buffer solution (0.05 M in KH_2PO_4 and 0.05 M in NaOH) were added ruthenium(III) chloride hydrate ($RuCl_3$·H_2O, 18 mg, 0.085 mmol) and sodium metaperiodate (651 mg, 2.96 mmol). This heterogeneous mixture was vigorously stirred at 45 °C during which time the reaction turned from black to yellow (after ~15 min) and then back to black after 60 h. The reaction was then quenched with water (10 mL) and the mixture was extracted with dichloromethane (2 × 10 mL). The combined organic layer was dried over Na_2SO_4 and then the concentrated residue was subjected to HPLC (60% EtOAc/hexanes) to afford after vacuum

(20) Spectral and other analytical data are given in the supplementary material. Essential ^{1}H-NMR spectral data are presented in the Experimental Section as well. General experimental procedures are also presented in the supplementary material section and the purity of all new compounds was judged by a combination of HPLC and NMR analyses before mass spectra determination. Satisfactory combustion analyses were obtained for selected compounds and for all new compounds, the level of purity is indicated by the inclusion of copies of ^{1}H-NMR spectra and selected ^{13}C-NMR spectra in the supplementary material. The four vitamins **4a–d** were characterized by NMR, UV, and mass spectral data only due to stability and sample size limitations.

drying 100 mg of ketone **10** (32%, eluted first, less polar) and 119 mg of hydroxy ketone **9a** (37%, eluted second, more polar) as colorless oils. The ketone **10** could also be obtained by oxidation of **8** as described below. ^{1}H-NMR of **9a**: ($CDCl_3$) δ 1.01 (3 H, C_{21}-CH_3, d, $J \sim 6.3$ Hz), 1.20 (6 H, $C_{26,27}$-CH_3, s), 1.96 (3 H, Ac, s), 3.88 (1 H, H_{18}, d, $J \sim 12.0$ Hz), 4.00 (1 H, $H_{18'}$, d, $J \sim 12.0$ Hz). ^{1}H-NMR of **10**: ($CDCl_3$) δ 0.84 (6 H, $C_{26,27}$-CH_3, d, $J \sim 6.4$ Hz), 0.98 (3 H, C_{21}-CH_3, d, $J \sim 6.4$ Hz), 1.95 (3 H, Ac, s), 3.87 (1 H, H_{18}, d, $J \sim 12.0$ Hz), 4.00 (1 H, H_{18}, d, $J \sim 12.0$ Hz).

De-*A*,*B*-18-acetoxy-25-[(trimethylsilyl)oxy]-8-cholestanone (9b). *N*-(Trimethylsilyl)imidazole (14.7 mg, 1.039 mmol) was added dropwise to a stirred solution of hydroxy ketone **9a** (117 mg, 0.347 mmol) in dry THF (4 mL). The reaction mixture was stirred for 1.5 h under argon and then the mixture was directly flash chromatographed through a short column of silica gel (10% ethyl acetate/hexanes). The eluate was concentrated and then the residue was subjected to HPLC (50% ethyl acetate/hexanes) to afford after vacuum drying 122 mg (86%) of the TMS-protected alcohol as a colorless oil. ^{1}H-NMR: ($CDCl_3$) δ 0.09 (9 H, $Si(CH_3)_3$, s), 1.01 (3 H, C_{21}-CH_3, d, $J \sim 6.3$ Hz), 1.19 (6 H, $C_{26,27}$-CH_3, s), 1.97 (3 H, Ac, s), 3.89 (1 H, H_{18}, d, $J \sim 12.0$ Hz), 4.02 (1 H, H_{18}, d, $J \sim 12.0$ Hz).

De-*A*,*B*-18-acetoxy-8-cholestanone (10) by Oxidation of 8. A suspension of pyridinium dichromate (PDC, 0.28 g, 0.74 mmol), pyridinium trifluoroacetate (PTFA, 0.019 g, 0.098 mmol), and dry CH_2Cl_2 (1.2 mL) was prepared and placed under argon. Alcohol **8** (80 mg, 0.25 mmol) in CH_2Cl_2 (1 mL) was added dropwise and the resulting mixture stirred for 4 h. The crude reaction mixture was filtered through a short silica gel column and then the filtrate was concentrated. The crude product was purified by HPLC (Rainin, 25 × 1 cm, 50% EtOAc/hexanes) to yield after vacuum drying ketone **10** (69 mg, 87%) as a clear oil. This material was also obtained as a byproduct in the ruthenium tetraoxide oxidation of **8**.

18-Acetoxyvitamin D_3 *tert*-Butyldimethylsilyl Ether (13a). A solution of *A*-ring phosphine oxide **11** (52 mg, 0.11 mmol) in dry THF (2 mL) was cooled to −78 °C under argon and treated with *n*-butyllithium (0.080 mL, 0.11 mmol, 1.55 M in hexanes). The resulting deep red solution was stirred for 10 min and then treated with a solution of CD-ring ketone **10** (24 mg, 0.075 mmol) in dry THF (0.6 mL).[21] The reaction mixture was stirred for 2 h at −78 °C and then warmed to room temperature. The solvent was partially removed and the remaining residue was dissolved in water (5 mL). The aqueous solution was extracted with ethyl acetate (3 × 5 mL) and the combined organic layer washed with brine, dried over Na_2SO_4, and concentrated. The residue was purified by rapid filtration through a short silica gel column (20% ethyl acetate/hexanes) to afford 31 mg (75%) of the vitamin **13a** of sufficient purity for the next step. ^{1}H-NMR: ($CDCl_3$) δ 0.07 (3 H, Si-CH_3, s), 0.08 (3 H, Si-CH_3, s), 0.86 (6 H, $C_{26,27}$-CH_3, d, $J \sim 7.6$ Hz), 0.89 (9 H, *t*-Bu, s), 1.02 (3 H, C_{21}-CH_3, d, $J \sim 3.6$ Hz), 2.86 (1 H, $H_{9\beta}$, d with fine coupling, $J \sim 12.9$ Hz), 3.7–3.9 (1 H, H_3, m), 3.85 (2 H, $2H_{18}$, br s), 4.74 (1 H, H_{19}, br s), 4.99 (1 H, H_{19}, br s), 6.00 and 6.12 (2 H, $H_{6,7}$, AB pattern, d, $J \sim 11.2$ Hz).

18-Acetoxy-25-[(trimethylsilyl)oxy]vitamin D_3 *tert*-Butyldimethylsilyl Ether (13b). A solution of phosphine oxide **11** (38 mg, 0.084 mmol) in dry THF (2 mL) was treated with *n*-butyllithium (0.054 mL, 0.084 mmol, 1.55 M in hexanes) and then the ketone **9b** (23 mg, 0.056 mmol) in dry THF (0.5 mL) was added as described above for the preparation of **13a**.[21] This afforded 23 mg (63%) of vitamin **13b** of sufficient purity for the next step. ^{1}H-NMR: ($CDCl_3$) δ 0.07 (3 H, $SiCH_3$, s), 0.08 (3 H, $SiCH_3$, s), 0.10 (9 H, TMS), 0.89 (9 H, *t*-Bu, s), 1.03 (3 H, C_{21}-CH_3, d, $J \sim 4.5$ Hz), 1.20 (6 H, $C_{26,27}$-CH_3, s), 2.02 (3 H, Ac, s), 2.86 (1 H, $H_{9\beta}$, br d, $J \sim 12.9$ Hz), 3.7–3.9 (1 H, H_3, m), 3.84 (1 H, H_{18}, d, $J \sim 8.4$ Hz), 3.87 (1 H, H_{18}, d, $J \sim 8.4$ Hz), 4.74 (1 H, H_{19}, br s), 5.00 (1 H, H_{19}, br s), 6.01 and 6.12 (2 H, $H_{6,7}$, AB pattern, d, $J \sim 11.2$ Hz).

18-Acetoxy-25-[(trimethylsilyl)oxy]-1α-[(*tert*-butyldimethylsilyl)oxy]vitamin D_3 *tert*-Butyldimethylsilyl Ether (13c). A solution of A-ring phosphine oxide **12** (122 mg, 0.21 mmol) in dry THF (3 mL) was treated with *n*-butyllithium (0.14 mL, 0.21 mmol, 1.55 M in hexanes) and then with CD-ring ketone **9b** (57 mg, 0.14 mmol) in dry THF (2.2 mL) as described above for the preparation of **13a**.[21] This afforded 81 mg (83%) of the vitamin **13c** of sufficient purity for the next step. ^{1}H-NMR: ($CDCl_3$) δ 0.07 (12 H, SiMe, series of s), 0.10 (9 H, TMS), 0.87 (9 H, *t*-Bu, s), 0.89 (9 H, *t*-Bu, s), 1.03 (3 H, C_{21}-CH_3, d, $J \sim 4.0$ Hz), 1.20 (6 H, $C_{26,27}$-CH_3, s), 2.01 (3 H, Ac, s), 2.87 (1 H, $H_{9\beta}$, d, $J \sim 12.8$ Hz), 3.86 (2 H, $2H_{18}$, s), 4.1–4.3 (1 H, H_3, m), 4.37 (1 H, H_1, apparent t, $J \sim 4.9$ Hz), 4.86 (1 H, H_{19}, d, $J \sim 1.9$ Hz), 5.18 (1 H, H_{19}, br s), 6.03 and 6.19 (2 H, $H_{6,7}$, AB pattern, d, $J \sim 11.1$ Hz).

18-Acetoxy-1α-[(*tert*-butyldimethylsilyl)oxy]vitamin D_3 *tert*-Butyldimethylsilyl Ether (13d). A solution of A-ring phosphine oxide **12** (73 mg, 0.13 mmol) in dry THF (1.9 mL) was treated with *n*-butyllithium (0.083 mL, 0.13 mmol, 1.55 M in hexanes) and then with ketone **10** (27 mg, 0.083 mmol) in dry THF (0.5 mL) as described above for the preparation of **13a**.[21] This afforded 55 mg (96%) of the vitamin **13d** as a foamy residue of sufficient purity for the next step. ^{1}H-NMR: ($CDCl_3$) δ 0.06 (12 H, $SiCH_3$, s), 0.87 (9 H, *t*-Bu, s), 0.89 (9 H, *t*-Bu, s), 0.89 (6 H, $2C_{26,27}$-CH_3, d, $J \sim 7$ Hz), 1.00 (3 H, C_{21}-CH_3, d, $J \sim 4.0$ Hz), 2.00 (3 H, Ac, s), 2.86 (1 H, $H_{9\beta}$, d, $J \sim 13.2$ Hz), 3.85 (2 H, $2H_{18}$, s), 4.1–4.3 (1 H, H_3, m), 4.3–4.4 (1 H, H_1, m), 4.85 (1 H, H_{19}, br s), 5.18 (1 H, H_{19}, br s), 6.02 and 6.19 (2 H, $H_{6,7}$, AB pattern, d, $J \sim 11.0$ Hz).

Biological Evaluation: Intestinal Calcium Absorption and Bone Calcium Mobilization. Intestinal calcium absorption (ICA) and bone calcium mobilization (BCM) were determined in vivo in vitamin D deficient chicks as described previously.[17,18] Twelve hours before assay, the chicks, which had been placed on a zero-calcium diet 48 h before assay, were injected intramuscularly with the vitamin D metabolite or analogue in 0.1 mL of ethanol/1,2-propanediol (1:1, v/v) or with vehicle. At the time of assay, 4.0 mg of $^{40}Ca^{2+}$ + 5 μCi of $^{45}Ca^{2+}$ (New England Nuclear) were placed in the duodenum of the animals anesthetized with ether. After 30 min, the birds were decapitated and the blood collected. The radioactivity content of 0.2 mL of serum was measured in a liquid scintillation counter (Beckman LS8000) to determine the amount of $^{45}Ca^{2+}$ absorbed (which is a measure of ICA). BCM activity was estimated from the increase of total serum calcium as measured by atomic absorption spectrophotometry.

1α,25(OH)$_2$D$_3$ Receptor Steroid Competition Assay. A measure of competitive binding to the chick intestinal 1α,25-$(OH)_2D_3$ receptor was performed by using the hydroxylapatite batch assay.[19] Increasing amounts of nonradioactive 1α,25$(OH)_2D_3$ or analogue were added to a standard amount of [^{3}H]-1α,25-$(OH)_2D_3$ and incubated with chick intestinal cytosol. The relative competitive index (RCI) for the analogues was determined by plotting the percent maximum 1α,25$(OH)_2$-[^{3}H]D_3 bound × 100 on the ordinate versus [competitor]/[1α,25$(OH)_2$-[^{3}H]D_3] on the abscissa. The slope of the line obtained for a particular analogue is divided by the slope of the line obtained for 1α,25$(OH)_2D_3$; multiplication of this value by 100 gives the RCI value. By definition, the RCI for 1α,25$(OH)_2D_3$ is 100.

Acknowledgment. This study was supported by grants from the National Institutes of Health (Grant No. NIH-DK-16595 and NIH-DK-09012). We wish to acknowledge Dr. Milan Uskokovic (Hoffmann-La Roche, Nutley, NJ) and Solvay Duphar B. V. (Weesp, the Netherlands) for providing various starting materials utilized in this study. Ms. June Bishop provided invaluable technical assistance in conducting the biological testing of the analogues. Professor A. Mouriño (University of Santiago de Compostela, Spain) has kindly informed us of related results obtained in his laboratories.

Supplementary Material Available: Spectral and analytical data (selected ^{1}H-NMR, ^{13}C-NMR, MS, HRMS, and other analytical data) (17 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

(21) When this coupling reaction was carried out on smaller scales, it was noted that yields of the vitamin D derivative were lower. We thank Professor G. H. Posner (Johns Hopkins University) for related information.