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Synthesis and Biological Evaluation of 1α,25-Dihydroxyvitamin D_3 Analogues Hydroxymethylated at C-26†

María A. Regueira,[‡] Shaonly Samanta,[§] Peter J. Malloy,[§] Paloma Ordóñez-Morán,[∥] Diana Resende,[‡] Fredy Sussman,[‡] Alberto Muñoz,[∥] Antonio Mouriño,[‡] David Feldman,[§] and Mercedes Torneiro*[,‡]

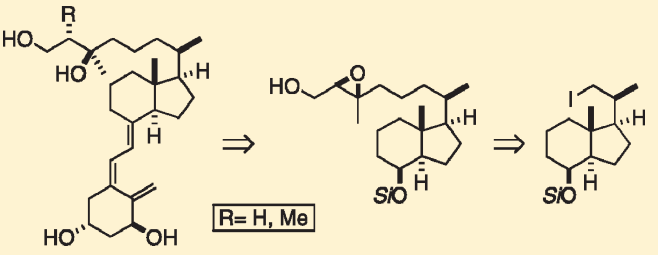
[‡]Departamento de Química Orgánica y Unidad Asociada al CSIC, Universidad de Santiago de Compostela, 15782, Santiago de Compostela, Spain

[§]Department of Medicine, Stanford University School of Medicine, Stanford, California 94305-5103, United States

[∥]Instituto de Investigaciones Biomédicas "Alberto Sols", CSIC-UAM, 28029 Madrid, Spain

Supporting Information

ABSTRACT: We designed by docking and synthesized two novel analogues of 1α,25-dihydroxyvitamin D_3 hydroxymethylated at C-26 (**2** and **3**). The syntheses were carried out by the convergent Wittig−Horner approach via epoxide **12a** as a common key intermediate. The antiproliferative and transactivation potency of the compounds was evaluated in colon and breast cancer cell lines. The analogues showed a similar but reduced activity compared to 1,25$(OH)_2D_3$. Analogue **3** was more potent than analogue **2**, and in some assays it exhibited potency similar to that of the natural ligand.



INTRODUCTION

1α,25-Dihydroxyvitamin D_3 [calcitriol, 1,25$(OH)_2D_3$, 1,25D, **1**, Figure 1], the hormonally active form of vitamin D_3, plays a central role in calcium homeostasis and bone mineralization and is also implicated in other significant extraskeletal biological processes ranging from cell differentiation and proliferation to modulation of the immune response.[1] As a consequence of its potent biological activity, calcitriol finds clinical application in the treatment of osteodystrophy due to renal failure, rickets, osteoporosis, and psoriasis.[2] Calcitriol is a hormonal ligand that exerts its activity by binding to a specific nuclear receptor, the vitamin D receptor (VDR),[2,3] a transcription factor that exerts its biological functions by regulating target gene expression. The formation of the hormone−receptor complex is the first step to activate or repress the transcription of target genes. Binding to the hormone induces a conformational change in the VDR that enables the complex to dimerize with the retinoic X receptor (RXR), followed by anchoring of the heterodimer to the response element of target genes and recruitment of co-regulatory factors to carry out gene transcription.[4]

The participation of calcitriol in the regulation of the cell cycle, together with its immunosuppressive effects, has suggested that the hormone could be used not only for treatment of bone disorders but also to treat multiple diseases including leukemia, cancer (breast, colon, prostate), psoriasis, autoimmune diseases, and graft rejection.[5] However, hypercalcemic side effects limit its use as a therapeutic drug for clinical application. As a result, a great deal of attention is being focused on the synthesis of vitamin D analogues with selective properties but few as yet have progressed past the pre-clinical stage stage.[6] Most of these analogues are modified at the side chain, such as calcipotriol[7] (MC903, Dovonex, Leo Pharmaceuticals, Denmark, Figure 1), used topically for the treatment of psoriasis,[8] or seocalcitol[9] (EB1089, Leo Pharmaceuticals, Denmark, Figure 1), a potent inhibitor of cell proliferation.[10]

On the basis of docking studies using the crystal structure of an engineered ligand binding domain of the VDR [VDR(Moras)],[11] we have recently designed and synthesized new active analogues of 1,25D.[12] All of these new analogues bind significantly in silico to VDR(Moras).[13] Attracted by the potent biological activities of a few 1,25D analogues modified at the side chain[7−10,12d] and to further study the correlation between in silico ligand design with biological activity, we designed by docking two new 1,25D analogues hydroxymethylated at C-26 (**2** and **3**, Figure 2). Here we describe their synthesis and biological properties.

RESULTS AND DISCUSSION

Design. The design of the new vitamin D_3 analogues **2** and **3** was based on the X-ray diffraction structures of the human VDR ligand binding domain in complex with 1,25D,[11] and some superagonists or agonists of clinical relevance modified at the side chain (Figure 1).[14] These structures show the conservation of the VDR structure and the adaptation of the ligands to the binding pocket.[15] Whereas the A, seco-B, and CD rings adopt

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similar conformations and the same interactions with the VDR in all complexes, the corresponding side chains follow different pathways to anchor the 25-hydroxyl group to the same residues (His305 and His397). Therefore, the aliphatic side chains establish different contacts with the ligand binding pocket (LBP) in each case. The superagonistic action of the 20-epi-analogues MC1288 and KH1060 has been attributed to the establishment of more and stronger contact points with the LBP in comparison to the hormone. The complexes of seocalcitol and KH1060, analogues with additional methyl groups at positions C-26 and C-27, present structural disorder of those methyl groups in the crystals. This is indicative of their ability to adopt different conformations and suggests the availability of additional space near those positions that could be occupied for improved potency and functional activity. On the basis of these observations, we hypothesized that modification of the natural hormone by the introduction of an hydroxymethyl group at the pro-*R* methyl group C-26, in the vicinity of the anchoring 25-hydroxyl group, would provide analogue **2** (Figure 2) with the ability to establish additional interactions with the ligand binding pocket and, ultimately, would lead to modulation of the biological responses.

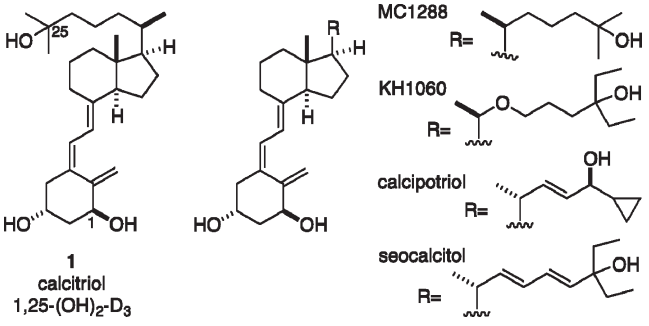


Figure 1. Structures of 1α,25-dihydroxyvitamin D_3 and some analogues modified at the side chain.

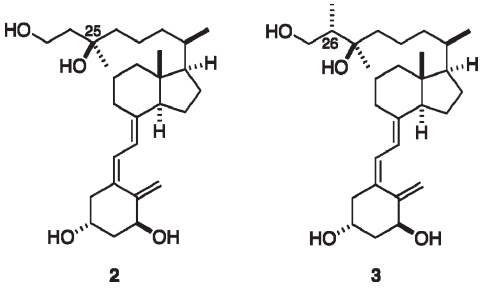
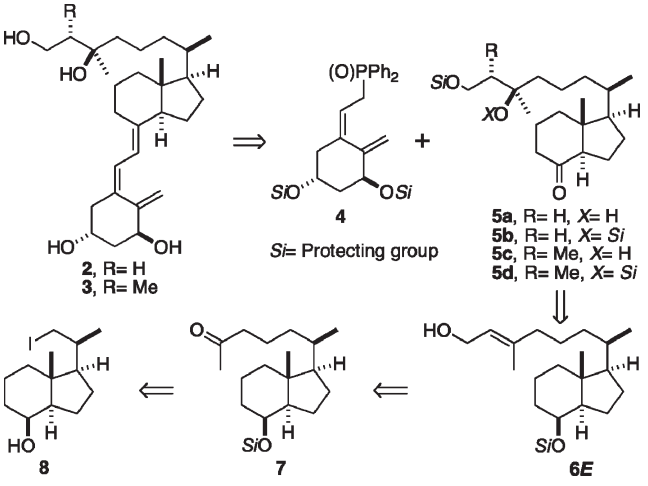


Figure 2. Structures of analogues **2** and **3**.

Docking calculations using the VDR(Moras)[11] revealed that analogue **2** binds with lower affinity to the VDR than the natural ligand **1**. The new hydroxyl group displaces the original 25-hydroxyl group from its hydrogen bond contact with the His397, causing a decrease in the affinity for the VDR (Figure 3). Interestingly, the introduction of an additional methyl group at C-26 in analogue **3** greatly increases its affinity for the VDR, binding to the receptor slightly better than the natural hormone. The additional methyl group at C-26 lies in a hydrophobic pocket near Leu227 (Figure 3). In complex with VDR the side chain of analogue **3** adopts a conformation that differs from that of the natural hormone, although the 25-hydroxyl group still forms hydrogen bonds with His305 and His397. In previous work we found in silico that analogues in general with moderate affinity for the VDR are biologically active.[12] Therefore, compound **3** might exhibit agonist activity with selective actions compared to the natural hormone. In order to test the biological effect of the C-26 hydroxymethyl and methyl substituents, we synthesized both analogues **2** and **3**.

Synthesis. The synthesis of compounds **2** and **3** was based on Lythgoe's methodology[16] by Wittig–Horner coupling of ketones **5** and the anion of known phosphine oxide **4** (Scheme 1). The key steps on the route to ketones **5** are the stereoselective epoxidation of allylic alcohol **6*E*** and opening of the resulting epoxide. The choice of the *E*-stereoisomer of allylic alcohol **6** is based on the superior yields of the asymmetric Sharpless epoxidation of *E*-olefins than for *Z*-isomers.[17] Allylic alcohol

Scheme 1. Retrosynthetic Analysis of Analogues 2 and 3



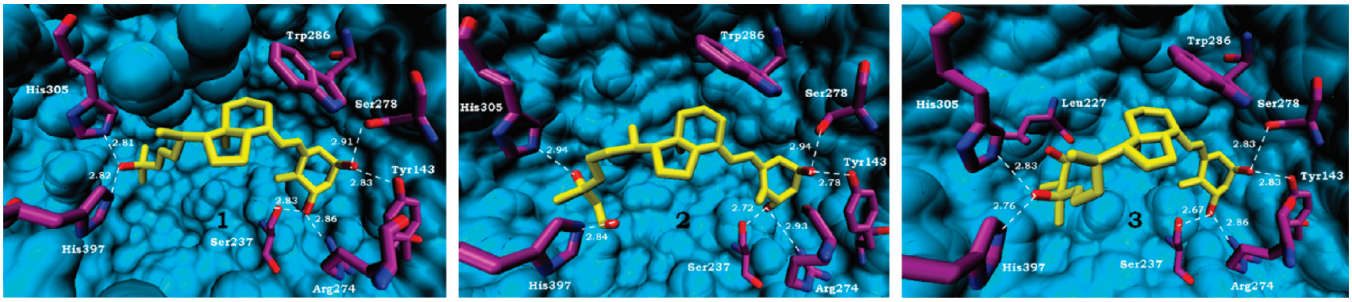


Figure 3. Structure of the complex VDR–calcitriol (left).[11] Predicted structures of the complexes of the VDR with analogues **2** (center) and **3** (right).

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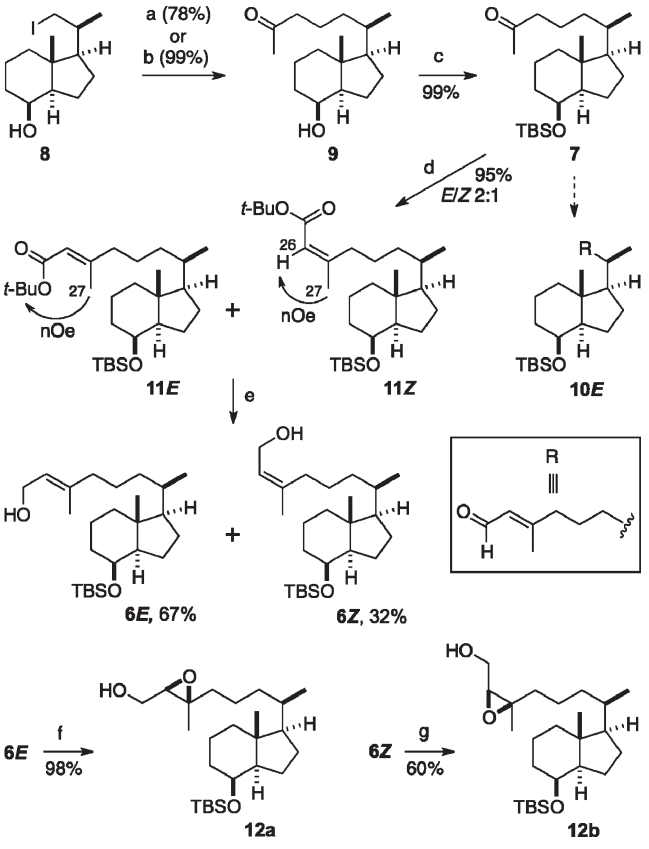
6E would result from elongation of the side chain of ketone **7**, which would be prepared from the known iodide **8** employing methodology developed in these laboratories.

The synthesis began with iodide **8** (Scheme 2), which was easily prepared from commercial vitamin D_2 in two steps.[18] The zinc−copper induced conjugate addition of iodide **8** to methyl vinyl ketone under sonochemical aqueous conditions[18] afforded ketone **9** in 78% yield. Alternatively, ketone **9** was prepared in quantitative yield under Sustmann's conditions[19] by reaction of **8** with methyl vinyl ketone using Zn and $NiCl_2$ in pyridine. Protection of the hydroxyl group of **9** with TBSCl afforded ketone **7** quantitatively.

Scheme 2. Synthesis of Allylic Alcohols 6 and Allylic Epoxides 12[a]



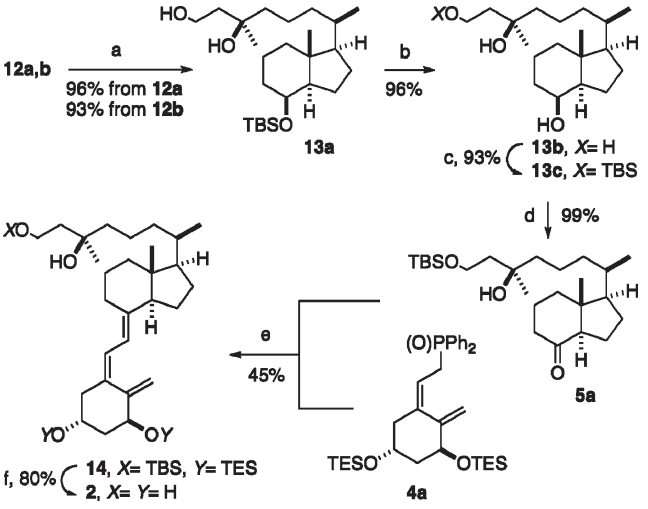
[a] Reagents and conditions: (a) $CH_2CHCOMe$, Zn, CuI, EtOH/H_2O, ultrasound; (b) $CH_2CHCOMe$, Zn, $NiCl_2 \cdot 6H_2O$, Py; (c) TBSCl, Im, DMF; (d) $(EtO)_2P(O)CH_2COO(t\text{-}Bu)$, K-*t*-BuO, *t*-BuOH; (e) DIBAL-H, THF (99%); (f) D-(−)-DIPT, *t*-BuO_2H, Ti(*i*-PrO)$_4$, CH_2Cl_2, 4 Å molecular sieves; (g) L-(+)-DIPT, *t*-BuO_2H, Ti(*i*-PrO)$_4$, CH_2Cl_2, 4 Å molecular sieves.

We envisioned the formation of the allylic alcohol **6E** via the α,β-unsaturated aldehyde **10E**, which would be accessed following Dauben's methodology,[20] a strategy that relies on an oxidative rearrangement of a tertiary allylic alcohol. When ketone **7** was treated with allylmagnesium bromide, followed by oxidation with PDC, we surprisingly found that the *Z* aldehyde was obtained as the major isomer instead of the target compound **10E**.[21] Those unexpected results led us to try a second route to the desired alcohol **6E**. The Wadsworth−Horner−Emmons reaction of ketone **7** with *tert*-buthyl diethylphosphonoacetate using potassium *tert*-butoxide as base gave α,β-unsaturated ester **11** as a mixture of *E* and *Z* isomers (about 2:1)[22] in 95% yield. The mixture of α,β-unsaturated esthers **11E** and **11Z** was reduced with DIBAL-H to afford allylic alcohols **6E** and **6Z** that could be separated by column chromatography. The isolated yields of the *E* and *Z* isomers, 67% and 32%, respectively, are in agreement with the stereoselectivity in the previous step. The proposed structures for **6E** and **6Z** are supported by NMR and NOESY data.

The next step in the synthetic strategy of the desired analogues involves the stereoselective introduction of the C-25 hydroxyl group. To reach this goal, both allylic alcohols **6E** and **6Z** were submitted to catalytic asymmetric Sharpless epoxidation[23] using opposite chiral auxiliaries[24] to give the epoxy alcohols **12a** and **12b** in 98% and 60% yields, respectively.[17]

We next focused our attention on the preparation of analogue **2** (Scheme 3). Epimeric compounds **12a** and **12b** were submitted to epoxide opening with Red-Al to deliver, in both cases, the diol

Scheme 3. Synthesis of Vitamin D_3 Analogue 2[a]



[a] Reagents and conditions: (a) Red-Al, THF, −78 °C; (b) TBAF, THF, 55 °C; (c) TBSCl, Im, DMF; (d) PDC, CH_2Cl_2; (e) **4a**, *n*-BuLi, THF, −78 °C, then **5a**; (f) TBAF, THF, 0 °C.

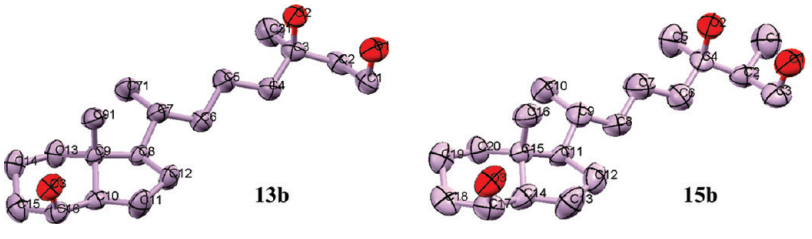


Figure 4. X-ray crystal structure of triols **13b** and **15b** (mercury representation, hydrogens omitted for clarity).

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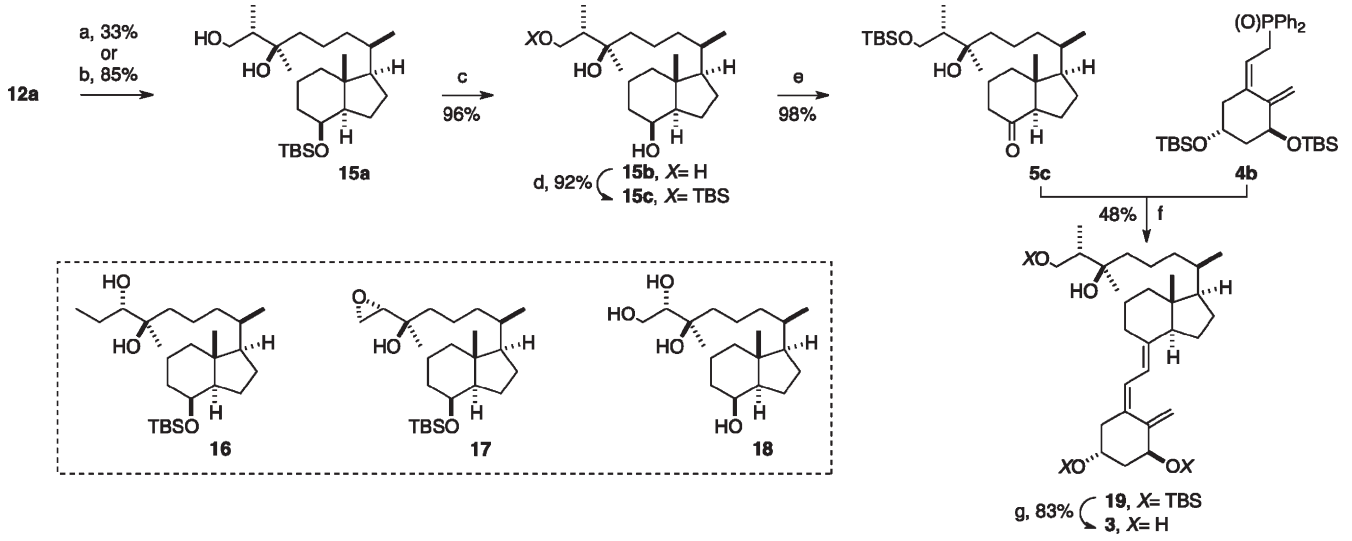
Scheme 4. Synthesis of Analogue 3[a]



[a] Reagents and conditions: (a) MeMgBr, CuI, Et_2O, −45 °C; (b) $Me_2CuLi \cdot CNLi$, Et_2O, −78 °C to room temp; (c) TBAF, THF, 55 °C; (d) TBSCl, Im, DMF; (e) PDC, CH_2Cl_2; (f) **4b**, *n*-BuLi, THF, −78 °C; **5c**; (g) TBAF, THF, 0 °C.

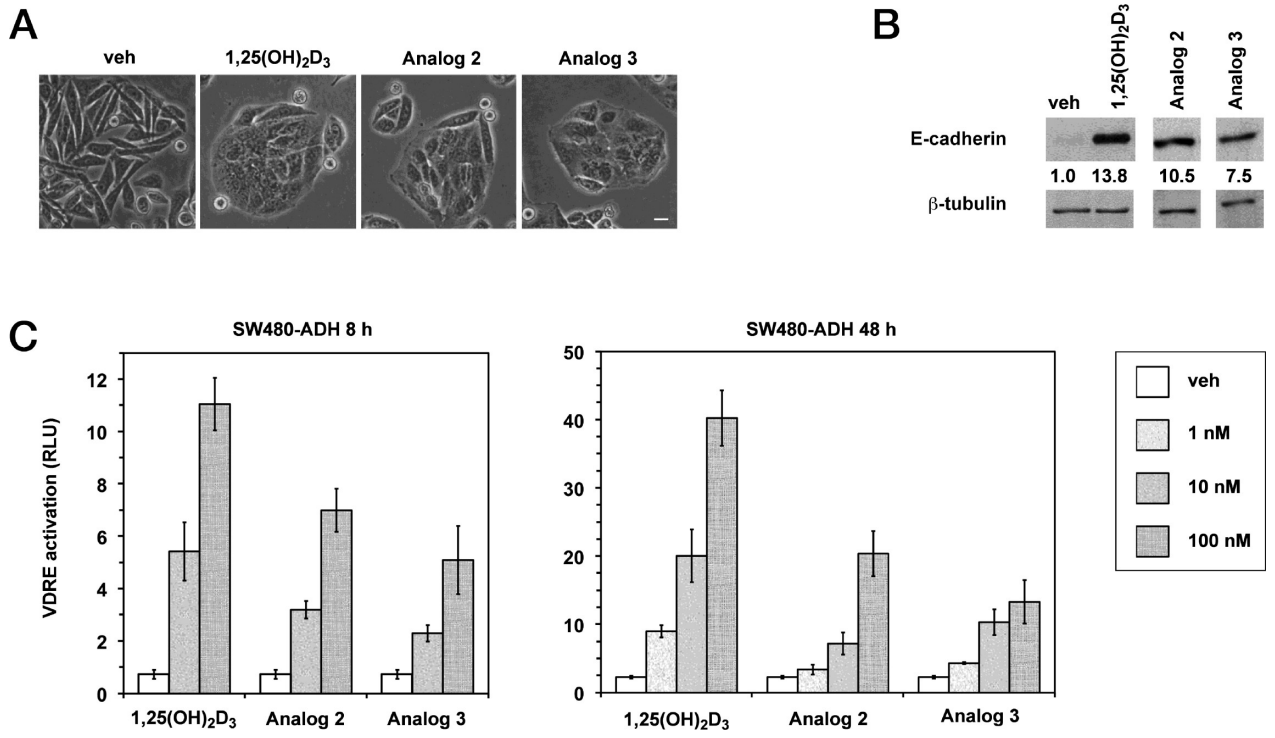


Figure 5. Activity of analogues **2** and **3** in colon cancer cells. (A) Phase-contrast micrographs showing the induction by analogues **2** and **3** of a differentiated adhesive epithelial phenotype in human SW480-ADH colon cancer cells. The cells were treated with 10^{-7} M analogue **2** or **3** or $1,25(OH)_2D_3$ or with vehicle for 48 h. A representative experiment is shown: bar, 10 μm. (B) Induction of E-cadherin expression: Western blot analysis of the expression of E-cadherin and β-tubulin (loading control) at 48 h of treatment of SW480-ADH cells with 10^{-7} M of each compound. Numbers correspond to mean values of fold-increase obtained in two experiments. (C) Activation of VDR transcriptional activity: dose-dependent activation of a consensus VDRE by $1,25(OH)_2D_3$ or analogue **2** or **3** at 8 h (left) or 48 h (right) of treatment of SW480-ADH cells, mean ± SD (n = 3).

13a in good yields. Subsequent deprotection with TBAF of the secondary hydroxyl group produced in 96% yield the triol **13b**, whose structure and relative stereochemistry were secured by X-ray crystallography (Figure 4). Ketone **5a** was prepared from **13b** by selective protection of the primary hydroxyl group with TBSCl, followed by oxidation of the secondary hydroxyl group with PDC (92% yield for the two steps). We decided to try the Wittig−Horner coupling with unmasked hydroxy ketone **5a**.[25,26] Indeed, the reaction of ketone **5a** with the anion of the phosphine oxide **4a**, generated by treatment with *n*-BuLi, took place in

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moderate yield to stereoselectively deliver the partially protected vitamin D **14** (45%, 81% based on recovered starting material). Finally, deprotection of **14** with TBAF in THF gave in 80% yield the desired vitamin D_3 analogue **2**, whose structure was fully characterized by ^{1}H NMR, ^{13}C NMR, UV, and mass spectral data.

With the parent analogue **2** at hand, we proceeded to the synthesis of the methylated compound **3** from epoxide **12a**. We planned to introduce the methyl group at C-26 via regioselective and stereospecific cleavage of the epoxide by a cuprate. Treatment of **12a** with methylmagnesium bromide in the presence of catalytic copper iodide in Et_2O gave the desired diol **15a** in low yield, as an inseparable 1:1 mixture with its structural isomer **16** (combined yield of 66%).[27] To circumvent the problems associated with the deprotonation of the primary hydroxyl group of **12a**, we decided to reverse the order of the operations and remove the TBS group of **12a** and selectively protect the primary hydroxyl group before carrying out the cuprate opening of the epoxide. Surprisingly, when **12a** was treated with hydrated tetrabutylamonium fluoride, tetraol **18**, resulting from simultaneous deprotection and opening of the epoxide by water, was obtained as the single reaction product. The structure of **18** was confirmed by X-ray crystallography (see Supporting Information). Those results led us to test a different cuprate for the introduction of the methyl group. The higher order cyanocuprate,[28] prepared from methylithium (2 equiv) and copper cyanide, reacted smoothly at the less hindered carbon of the epoxide **12a**, affording the diol **15a** in good yield, in the presence of the unprotected primary hydroxyl group, probably by intramolecular attack of the alkoxy cuprate to the epoxide.

The key ketone **5c**, required for the coupling to the lower part of the vitamin D, was prepared by a three-step sequence involving removal of the TBS group of **15a** with TBAF, selective protection of the primary hydroxyl as TBS ether (TBSCl, imidazole), and oxidation of the secondary hydroxyl group with PDC to produce the ketone **5c** in 87% yield over the three steps. The stereochemistry of the 26-methyl group was confirmed by X-ray crystallography of the intermediate triol **15b** (Figure 4). Completion of the synthesis was realized, similarly as for analogue **2**, by building the trienic system via stereoselective Wittig−Horner coupling of **5c** with phosphine oxide **4b** to give the TBS-triprotected compound **19** in 48% yield (83% based on recovered starting material), followed by desilylation with TBAF to deliver the desired analogue **3** in 83% yield (Scheme 4). The chemical structure of **3** was fully characterized by ^{1}H NMR, ^{13}C NMR, UV, and mass spectral data.

Biological Assays. The biological activity of analogues **2** and **3** were studied in vivo in intact human SW480-ADH colon cancer cells. Both analogues have a pro-differentiating action on these cells comparable to that of 1,25D, inducing the formation of compact epithelioid islands of highly adherent cells (Figure 5A). Accordingly, analogues **2** and **3** induced the expression of E-cadherin, the key intercellular adhesion protein in epithelial cells, although with less potency than 1,25D (Figure 5B). The ability of analogues **2** and **3** to activate the transcriptional activity of VDR was studied by transfecting SW480-ADH cells with a plasmid encoding the luciferase gene under the control of a consensus vitamin D response element (VDRE). At both 8 and 48 h post-transfection, analogue **2** or **3** strongly induced in a dose-dependent manner the activation of the VDRE as measured by estimating the luciferase activity. However their potency was lower (roughly, 1 order of magnitude) than that of 1,25D (Figure 5C). In conclusion regarding activity in human colon

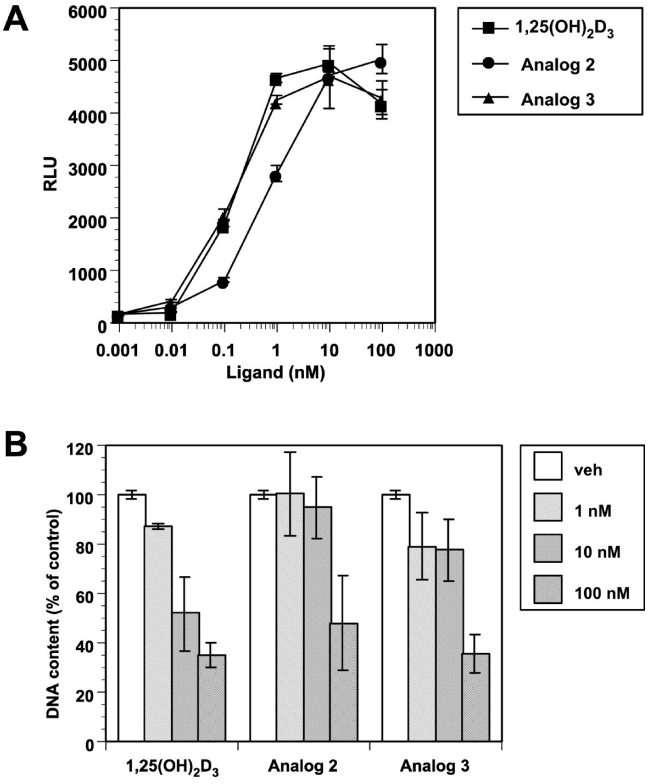

Figure 6. Effects of analogues on transactivation and cell growth. (A) Transactivation of 24-hydroxylase-luciferase reporter in COS-7 cells. COS-7 cells were transfected with WT VDR expression vector and a 24-hydroxylase reporter and then treated with graded concentrations of $1,25(OH)_2D_3$ or analogues **2** and **3** for 24 h. The luciferase activity was then measured. Error bars represent ± SD. (B) Effects of $1,25(OH)_2D_3$ and analogues on MCF-7 cell growth. MCF-7 cells were grown in six-well plates for 6 days in DMEM F12 with 5% FBS and treated with graded concentrations of $1,25(OH)_2D_3$ or analogues **2** and **3**. Control samples were treated with vehicle (0.1% ethanol). Fresh medium and ligands were replaced every other day. DNA levels are expressed as percent of control, which was 99.96 ± 1.7 μg DNA/well. Each point represents the average of three individual experiments. Error bars represent ±SD.

cancer cells, analogues **2** and **3** show a similar but reduced biological activity compared to 1,25D.

The transactivation potency of the analogues was also examined in COS-7 using a 24-hydroxylase promoter, a classic target gene for 1,25D. As shown in Figure 6A, in transactivation assays using this promoter−luciferase reporter construct, the potency of analogue **2** was approximately 2- to 3-fold lower than 1,25D while analogue **3** exhibited a potency similar to that of the natural ligand. The antiproliferative activity of the analogues was also examined in the MCF-7 human breast cancer cell line. As shown in Figure 6B, 1,25D and the analogues caused a dose-dependent inhibition in the cell growth. 1,25D had a modest inhibitory effect on cell growth at 1 nM (∼ 15%) but induced significant growth inhibition at 10 nM (∼50%) and 100 nM (∼66%) doses. At 100 nM, analogues **2** and **3** induced ∼53% and 65% growth inhibition, respectively. 1,25D was a more potent inhibitor of cell growth than the analogues, with order of potency being 1,25D > **3** > **2**.

CONCLUSIONS

On the basis of the crystal structures of the vitamin D nuclear receptor, novel calcitriol analogues substituted with a

hydroxymethyl group at C-26 were designed and synthesized in 11 steps each and in 19% (analogue **2**) and 18% (analogue **3**) yields from readily available iodide **8**. Synthetic key features were the stereoselective formation of allylic alcohol **6*E*** by Wadsworth−Horner−Emmons reaction followed by reduction and its transformation into epoxy alcohol **12a** via catalytic asymmetric Sharpless epoxidation.

The biological activity of the new analogues was first studied in SW480-ADH colon cancer cells. Both compounds show prodifferentiating and transcriptional activities similar to those of 1,25D, but their potency was roughly 1 order of magnitude lower than that of 1,25D. In transactivation assays using COS-7 the potency of analogue **2** was approximately 2- to 3-fold lower than 1,25D while analogue **3** exhibited a potency similar to the natural ligand. The antiproliferative activity of the analogues was also examined in the MCF-7 human breast cancer cell line. At 100 nM, analogues **2** and **3** induced ∼53% and 65% growth inhibition, respectively, whereas the natural ligand 1,25D induced 66%. As hypothesized, we found a correlation beween docking design and biological activity for compounds **2** and **3**.

EXPERIMENTAL SECTION

General Methods and Materials. All reactions involving oxygen or moisture sensitive compounds were carried out under a dry argon (L-50) atmosphere. Reaction temperatures refer to external bath temperatures. All dry solvents were distilled under argon immediately prior to use. THF and Et_2O were distilled from sodium benzophenone. CH_2Cl_2 was distilled over P_2O_5. MeOH and *t*-BuOH were distilled from Mg/I_2, and pyridine was distilled from CaH_2. DMF (Merck) was stored over activated 4 Å molecular sieves. CuI was purified by the method of Kauffman,[29] and Zn was activated as recommended in the book by Perrin.[30] Acetone−dry ice baths were used for reactions at low temperature. Alternatively, acetone baths were cooled with a CryoCool immersion cooler, provided with a temperature regulator. Sonications were carried out in a 120−240 W, 35 kHz ultrasonic cleaning bath. Organic extracts were dried over anhydrous Na_2SO_4, filtered, and concentrated using a rotary evaporator at aspirator pressure (20−30 mmHg). Liquid-phase reactions were monitored by thin-layer chromatography (TLC) using aluminum-backed silica gel plates (0.2 mm thickness). The chromatograms were visualized with ultraviolet light (254 nm) and then by staining with a solution of phosphomolybdic acid in EtOH (5%). Flash column chromatography was performed with silica gel (230−400 mesh). ^{1}H NMR (500 MHz) and ^{13}C NMR (126 MHz) spectra were measured with solutions in $CDCl_3$, CD_2Cl_2, or CD_3OD. Chemical shifts are reported on the δ scale (ppm) downfield from tetramethylsilane (δ = 0.0 ppm) using the residual solvent signal at δ = 7.26 ppm (^{1}H, $CDCl_3$), δ = 5.30 ppm (^{1}H, t, CD_2Cl_2), δ = 3.31 ppm (^{1}H, q, CD_3OD), δ = 77.0 ppm (^{13}C, $CDCl_3$), δ = 49.0 ppm (^{13}C, hp, CD_3OD), or δ = 53.7 ppm (^{13}C, q, CD_2Cl_2) as internal standard. Coupling constants are reported in Hz. Distortionless enhancement by polarization transfer (DEPT) was used to assign carbon types. Infrared (IR) spectra were recorded on KBr windows for the solids and CsI windows for the liquids. LRMS were recorded at 75 eV (EI+) or 60 eV (CI+). Analogues **2** and **3** have a purity of >95% (HPLC).

Docking Procedure for Structure-Guided Design. The docking process for the ligands **1**−**3** was similar to the one previously described in a study devoted to predicting the activity of a wide variety of vitamin D analogues.[31,32] The binding affinity was determined by the difference in energy between the complex and its components (protein and ligand) for the top 50 poses of every ligand. Since analogues **2** and **3** differ in polarity with the natural hormone **1**, a desolvation component was introduced in the binding free energy. The desolvation free energies were calculated by the generalized Born with a simple switching function (GBSW), an algorithm that approximates analytically the solutions of the Poisson−Boltzmann equation.[33] The value of the protein dielectric constant was set to 2.[34] The calculated values (kcal/mol) of the binding energy promediated for the 50 better bound conformations of each ligand were −62.0 for the natural hormone **1**, −52.7 for analogue **2**, and −63.0 for analogue **3**.

8*β*-Hydroxy-de-*A*,*B*-27-norcholestan-25-one (9). *Method a.* A solution of iodide **8**[35] (0.5 g, 1.55 mmol) in EtOH/H_2O (7:3, 25 mL, previously deoxygenated) was sonicated for 15 min. Methyl vinyl ketone (0.4 mL, 4.66 mmol, vacuum-distilled) was added. After sonication for 15 min, CuI (0.442 g, 2.33 mmol) and Zn (0.709 g, 10.9 mmol) were successively added. The reaction mixture was sonicated in periods of 30 min during 5 h. Then EtOAc (15 mL) was added, and the mixture was sonicated for another 15 min. Filtration through a short path of Celite and washing of the solids with EtOAc (100 mL) gave a solution that was washed with saturated NaCl (50 mL), dried, filtered, and concentrated. The residue was purified by flash chromatography (SiO_2, 2 cm × 15 cm, 10% EtOAc/hexanes), affording **9**[36] [0.324 g, 1.21 mmol, 78%, R_f = 0.25 (20% EtOAc/hexanes), yellow oil]. ^{1}H NMR ($CDCl_3$, 500 MHz): 4.06 (1H, m, H-8), 2.38 (2H, m, H-24), 2.13 (3H, s, H-26), 0.92 (3H, s, H-18), 0.90 (3H, d, J = 6.6 Hz, H-21). ^{13}C NMR ($CDCl_3$, 126 MHz): 209.2 (C, C-25), 68.7 (CH, C-8), 56.0 (CH, C-17), 52.3 (CH, C-14), 43.9 (CH_2, C-24), 41.5 (C, C-13), 40.1 (CH_2, C-9), 34.9 (CH_2, C-12), 34.8 (CH, C-20), 33.3 (CH_2, C-22), 29.5 (CH_3, C-26), 26.9 (CH_2, C-16), 22.2 (CH_2, C-15), 20.0 (CH_2, C-23), 18.1 (CH_3, C-21), 17.2 (CH_2, C-11), 13.2 (CH_3, C-18). IR ($CHCl_3$, cm^{-1}): 3492 (ν_{OH}), 1712 ($\nu_{C=O}$). MS ([EI]$^+$, *m*/*z*, %): 266 ([M]$^+$, 1), 251 ([M − CH_3]$^+$, 3), 135 ([M − H_2O − $C_7H_{13}O$]$^+$, 12), 111 ([M − $C_{10}H_{19}O$]$^+$, 100). EA: calculated for [$C_{17}H_{30}O_2$], C (76.64), H (11.35); found, C (76.37), H (11.02).

Method b. Methyl vinyl ketone (0.509 mL, 25.6 mmol, vacuum-distilled) and $NiCl_2 \cdot 6H_2O$ (0.67 g, 5.14 mmol) were successively added to a suspension of Zn (1.66 g, 25.6 mmol) in Py (40 mL). The mixture was heated at 65 °C during 2 h and then allowed to reach room temperature. A solution of **8** (1.10 g, 3.42 mmol) in Py (5 mL) was added via cannula. After being stirred for 30 min, the mixture was diluted with EtOAc (20 mL) and filtered through Celite. The solids were washed with EtOAc (20 mL), and the filtrate was washed with HCl (2 × 25 mL, 5%) and saturated $NaHCO_3$ (30 mL). The organic phase was dried, filtered, and concentrated. The residue was purified by flash chromatography (SiO_2, 3 cm × 13 cm, 10% EtOAc/hexanes) to afford **9**[36] [0.898 g, 3.37 mmol, 99%, R_f = 0.25, (20% EtOAc/hexanes), yellow oil].

8*β*-[(*tert*-Butyldimethylsilyl)oxy]-de-*A*,*B*-27-norcholestan-25-one (7). Imidazole (1.53 g, 22.5 mmol) and TBSCl (1.69 g, 11.3 mmol) were successively added to a solution of **9** (2.00 g, 7.51 mmol) in dry DMF (30 mL). The reaction mixture was stirred for 4 h. The mixture was diluted with hexane (20 mL) and NaCl (40 mL). The aqueous phase was extracted with hexanes (3 × 25 mL), and the combined organic phase was dried, filtered, and concentrated. The residue was purified by flash chromatography (SiO_2, 3 cm × 13 cm, 1% EtOAc/hexanes) to give **7** [2.86 g, 7.51 mmol, 99%, R_f = 0.75 (20% EtOAc/hexanes), yellow oil]. ^{1}H NMR ($CDCl_3$, 500 MHz): 3.96 (1H, m, H-8), 2.34 (2H, m, H-24), 2.09 (3H, s, H-26), 0.87 (3H, d, J = 6.3 Hz, H-21), 0.86 (9H, s, Me_3CSi], 0.85 (3H, s, H-18), −0.02 (3H, s, MeSi), −0.04 (3H, s, MeSi). ^{13}C NMR ($CDCl_3$, 126 MHz): 209.3 (C, C-25), 69.4 (CH, C-8), 58.5 (CH, C-17), 53.0 (CH, C-14), 44.2 (CH_2, C-24), 42.1 (C, C-13), 40.7 (CH_2, C-9), 35.3 (CH_2, C-12), 35.1 (CH, C-20), 34.4 (CH_2, C-22), 29.8 (CH_3, C-26), 27.3 (CH_2, C-16), 25.8 (3 × CH_3, Me_3CSi), 23.0 (CH_2, C-15), 20.3 (CH_2, C-23), 18.5 (CH_3, C-21), 18.0 (C, C−Si), 17.6 (CH_2, C-11), 11.2 (CH_3, C-18), −4.8 (CH_3, MeSi), −5.2 (CH_3, MeSi). IR (film, cm^{-1}): 1718 ($\nu_{C=O}$). MS ([FAB]$^+$, *m*/*z*, %): 381 ([M + H]$^+$, 12), 323 ([M − *t*-Bu]$^+$, 18), 265 ([M − TBS]$^+$, 9), 247 ([M −

TBSOH]$^+$, 78), 135 ([M − TBSOH − $C_7H_{14}O$]$^+$, 100). EA: calculated for [$C_{23}H_{44}O_2Si$], C (72.57), H (11.65); found, C (72.85), H (11.33).

8β-[(*tert*-Butyldimethylsilyl)oxy]-26-(*tert*-butoxycarbonyl)-de-*A*,*B*-colest-25(26)-ene (11). *tert*-Butyl diethylphosphonoacetate (0.951 mL, 4.05 mmol) and K-*t*-BuO (0.455 g, 4.05 mmol) were successively added to a solution of ketone **7** (0.514 g, 1.35 mmol) in dry *t*-BuOH (20 mL). The mixture was refluxed for 2 h and then concentrated. The residue was dissolved in H_2O (20 mL), and the aqueous layer was extracted with Et_2O (3 × 15 mL). The organic combined layer was dried, filtered, and concentrated. The residue was purified by flash chromatography (SiO_2, 3 cm × 12 cm, 1% EtOAc/hexanes) to afford **11** as a 2:1 mixture of *E*/*Z* isomers [0.612 g, 1.27 mmol, 95%, R_f = 0.75 (5% EtOAc/hexanes), colorless oil]. ^{1}H NMR ($CDCl_3$, 500 MHz, 2:1 *E*/*Z* mixture): 5.56 (0.7H, m, *J* = 1.2 Hz, H-26*E*), 5.54 (0.3H, m, H-26*Z*), 3.97 (1H, m, H-8), 2.09 (2.1H, d, *J* = 1.2 Hz, H-27*E*), 1.81 (0.9H, d, *J* = 1.0 Hz, H-27*Z*), 1.45 (6.3H, s, $(CH_3)_3COE$), 1.45 (2.7H, s, $(CH_3)_3COZ$), 0.9 (3H, s, H-18), 0.88 (3H, d, *J* = 6.5 Hz, H-21), 0.87 (9H, s, Me_3CSi), −0.01 (3H, s, MeSi), −0.02 (3H, s, MeSi). ^{13}C NMR ($CDCl_3$, 126 MHz, 2:1 *E*/*Z* mixture): 166.4 (C, COO*E*), 165.8 (C, COO*Z*), 158.6 (C, C-25*E*), 158.3 (C, C-25*Z*), 118.0 (CH, C-26*Z*), 117.1 (CH, C-26*E*), 79.2 (C, $Me_3\underline{C}$-O), 69.5 (CH, C-8), 56.8 (CH, C-17*Z*), 56.6 (CH, C-17*E*), 53.1 (CH, C-14), 42.1 (C, C-13), 41.3 (CH_2, C-9), 40.7 (CH_2, C-12), 35.7 (CH_2-24*Z*), 35.4 (CH_2, C-24*E*), 35.2 (CH, C-20*Z*), 35.0 (CH, C-20*E*), 34.5 (CH_2, C-22*E*), 33.3 (CH_2, C-22*Z*), 28.3 (3xCH_3, Me_3CO), 27.3 (CH_2, C-16), 25.8 (3 × CH_3, Me_3CSi), 23.8 (CH_2, C-15), 23.0 (CH_2, C-23), 18.6 (CH_3, C-21), 18.6 (CH_3, C-27*Z*), 18.4 (CH_3, C-27*E*), 18.0 (C, C-Si), 17.7 (CH_2, C-11), 13.7 (CH_3, C-18), −4.8 (CH_3, MeSi), −5.2 (CH_3, MeSi). IR (film, cm^{-1}): 1712 ($\nu_{C=O}$). MS ([CI]$^+$, *m*/*z*, %): 479 ([M + H]$^+$, 39), 421 ([M − *t*-Bu]$^+$, 62), 405 ([M − *t*-BuO]$^+$, 77), 377 ([M *t*-BuCOO]$^+$, 31), 363 ([M − TBS]$^+$, 39), 347 ([M − TBSO]$^+$, 77), 135 ([M − TBSO − $C_{13}H_{24}O_2$]$^+$, 100). EA: calculated for [$C_{29}H_{54}O_3Si$], C (72.74), H (11.37); found, C (72.49), H (11.45).

(25*E*)-8β-[(*tert*-Butyldimethylsilyl)oxy]-26-(hydroxymethyl)-de-*A*,*B*-colest-25(26)-ene (6*E*) and (25*Z*)-8β-[(*tert*-Butyldimethylsilyl)oxy]-26-(hydroxymethyl)-de-*A*,*B*-colest-25(26)-ene (6*Z*). A solution of DIBAL-H in THF (5.22 mL, 5.22 mmol, 1 M) was added to a stirred solution of **11** (1.00 g, 2.08 mmol) in dry THF (40 mL) at −78 °C. The mixture was allowed to reach room temperature and concentrated. The residue was dissolved in Et_2O (30 mL), and the organic layer was washed with HCl (30 mL, 10%). The aqueous layer was extracted with Et_2O (3 × 15 mL), and the combined organic layer was dried, filtered, and concentrated. The residue was purified by flash chromatography (SiO_2, 4 cm × 20 cm, 1% EtOAc/hexanes) to afford **6*E*** [0.571 g, 1.40 mmol, 67%, R_f = 0.32 (20% EtOAc/hexanes), colorless oil] and **6*Z*** [0.280 g, 0.680 mmol, 32%, R_f = 0.40 (20% EtOAc/hexanes), white solid, mp 68−69 °C (EtOAc)].

6*E*. ^{1}H NMR ($CDCl_3$, 500 MHz): 5.40 (1H, t, *J* = 6.9 Hz, H-26), 4.14 (2H, d, *J* = 6.9 Hz, $C\underline{H}_2OH$), 3.99 (1H, m, H-8), 1.66 (3H, s, H-27), 0.90 (3H, s, H-18), 0.89 (3H, d, *J* = 6.3 Hz, H-21), 0.88 (9H, s, Me_3CSi), 0.00 (3H, s, MeSi), −0.01 (3H, s, MeSi). ^{13}C NMR ($CDCl_3$, 126 MHz): 140.3 (C, C-25), 123.1 (CH, C-26), 69.5 (CH, C-8), 59.4 (CH_2, $\underline{C}H_2OH$), 56.8 (CH, C-17), 53.1 (CH, C-14), 42.1 (C, C-13), 40.8 (CH_2, C-9), 40.0 (CH_2, C-24), 35.4 (CH_2, C-12), 35.2 (CH, C-20), 34.5 (CH_2, C-22), 27.3 (CH_2, C-16), 25.8 (3 × CH_3, Me_3CSi), 24.1 (CH_2, C-23), 23.1 (CH_2, C-15), 18.7 (CH_3, C-21), 18.1 (C, C−Si), 17.7 (CH_2, C-11), 16.2 (CH_3, C-27), 13.7 (CH_3, C-18), −4.8 (CH_3, MeSi), −5.1 (CH_3, MeSi). IR (film, cm^{-1}): 3339 (ν_{OH}). MS ([CI]$^+$, *m*/*z* %): 409 ([M + H]$^+$, 11), 277 ([M − TBSO]$^+$, 28), 259 ([M − TBSO − H_2O]$^+$, 99). EA: calculated for [$C_{25}H_{48}O_2Si$], C (73.46), H (11.84); found, C (73.69), H (11.92).

6*Z*. ^{1}H NMR ($CDCl_3$, 500 MHz): 5.40 (1H, t, *J* = 7.0 Hz, H-26), 4.11 (2H, d, *J* = 7.0 Hz, $C\underline{H}_2OH$), 3.99 (1H, m, H-8), 1.72 (3H, d, *J* = 0.9 Hz, H-27), 0.90 (3H, s, H-18), 0.88 (9H, s, Me_3CSi), 0.87 (3H, d, *J* = 6.7 Hz, H-21), 0.00 (3H, s, MeSi), −0.01 (3H, s, MeSi). ^{13}C NMR ($CDCl_3$, 126 MHz): 140.5 (C, C-25), 123.9 (CH, C-26), 69.5 (CH, C-8), 59.1 (CH_2, $\underline{C}H_2OH$), 56.7 (CH, C-17), 53.0 (CH, C-14), 42.1 (C, C-13), 40.7 (CH_2, C-9), 35.6 (CH_2, C-12), 35.1 (CH, C-20), 34.4 (CH_2, C-22), 32.3 (CH_2, C-24), 27.3 (CH_2, C-16), 25.8 (3 × CH_3, Me_3CSi), 24.7 (CH_2, C-23), 23.4 (CH_3, C-27), 23.0 (CH_2, C-15), 18.6 (CH_3, C-21), 18.0 (C, C-Si), 17.6 (CH_2, C-11), 13.7 (CH_3, C-18), −4.8 (CH_3, MeSi), −5.1 (CH_3, MeSi). IR (KBr, cm^{-1}): 3327 (ν_{OH}). MS ([CI]$^+$, *m*/*z*, %): 409 ([M + H]$^+$, 12), 391 ([M − OH]$^+$, 83), 351 ([M − C_3H_5O]$^+$, 12), 259 ([M − TBSO − H_2O]$^+$, 100). EA: calculated for [$C_{25}H_{48}O_2Si$], C (73.46), H (11.84); found, C (73.62), H (11.94).

(25*R*,26*R*)-8β-[(*tert*-Butyldimethylsilyl)oxy]-25,26-epoxy-26-(hydroxymethyl)-de-*A*,*B*-colestane (12a). Ti(*i*-PrO)$_4$ (0.018 mL, 0.061 mmol) and *t*-BuOOH (0.366 mL, 1.83 mmol) were successively added to a mixture of D-(−)-DIPT (0.019 mL, 0.089 mmol) and molecular sieves (4 Å) in dry CH_2Cl_2 (40 mL) at −10 °C. After 10 min, the mixture was cooled to −20 °C and a solution of compound **6*E*** (0.500 g, 1.22 mmol) in CH_2Cl_2 (2 mL) was added via cannula. After 2 h the mixture was allowed to reach 0 °C. Distilled H_2O (8 mL) and a solution of NaOH (30%) saturated with NaCl (25 mL) were successively added. After the mixture was stirred for 10 min the aqueous layer was extracted with CH_2Cl_2 (3 × 25 mL) and the combined organic layer was dried, filtered, and concentrated. The residue was purified by flash chromatography (SiO_2, 3 cm × 13 cm, 5% EtOAc/hexanes) to provide **12a** [0.510 g, 1.20 mmol, 98%, R_f = 0.20 (20% EtOAc/hexanes), glassy solid]. ^{1}H NMR ($CDCl_3$, 500 MHz): 3.95 (1H, m, H-8), 3.78 (1H, dd, J_1 = 12.2 Hz, J_2 = 3.3 Hz, $C\underline{H}_2OH$), 3.62 (1H, dd, J_1 = 12.2 Hz, J_2 = 6.8 Hz, $C\underline{H}_2OH$), 2.93 (1H, dd, J_1 = 6.8 Hz, J_2 = 4.0 Hz, H-26), 1.24 (3H, s, H-27), 0.86 (3H, s, H-18), 0.86 (3H, d, *J* = 6.4 Hz, H-21), 0.85 (9H, s, Me_3CSi), −0.03 (3H, s, MeSi), −0.05 (3H, s, MeSi). ^{13}C NMR ($CDCl_3$, 126 MHz): 69.3 (CH, C-8), 63.1 (CH, C-26), 61.4 (C, C-25), 61.3 (CH_2, $\underline{C}H_2OH$), 56.6 (CH, C-17), 52.9 (CH, C-14), 42.0 (C, C-13), 40.6 (CH_2, C-9), 38.8 (CH_2, C-24), 35.6 (CH_2, C-12), 35.1 (CH, C-20), 34.4 (CH_2, C-22), 27.2 (CH_2, C-16), 25.7 (3 × CH_3, Me_3CSi), 22.9 (CH_2, C-15), 21.4 (CH_2, C-23), 18.5 (CH_3, C-27), 17.9 (C, C−Si), 17.6 (CH_2, C-11), 16.7 (CH_3, C-21), 13.8 (CH_3, C-18), −4.9 (CH_3, MeSi), −5.2 (CH_3, MeSi). IR (KBr, cm^{-1}): 3413 (ν_{OH} hidroxilo). MS ([CI]$^+$, *m*/*z*, %): 425 ([M + H]$^+$, 6), 407 ([M − OH]$^+$, 9), 393 ([M − CH_3O]$^+$, 18), 379 ([M − C_2H_5O]$^+$, 21), 337 ([M − $C_4H_9O_2$]$^+$, 29), 323 ([M − TBSO]$^+$, 10), 293 ([M − $C_7H_{15}O_2$]$^+$, 163 ([M − TBSO − $C_7H_{14}O_2$]$^+$, 68), 135 ([M − TBSO − $C_9H_{18}O_2$]$^+$, 100). EA: calculated for [$C_{25}H_{48}O_3Si$], C (70.70), H (11.39); found, C (70.35), H (11.06).

(25*R*,26*S*)-8β-[(*tert*-Butyldimethylsilyl)oxy]-25,26-epoxy-26-(hydroxymethyl)-de-*A*,*B*-colestane (12b). Ti(*i*-PrO)$_4$ (0.008 mL, 0.029 mmol) and *t*-BuOOH (0.171 mL, 0.855 mmol) were successively added to a mixture of L-(+)-DIPT (0.009 mL, 0.042 mmol) and molecular sieves (4 Å) in dry CH_2Cl_2 (15 mL) at −10 °C. After 10 min the mixture was cooled to −20 °C, and a solution of **6*Z*** (0.233 g, 0.570 mmol) in CH_2Cl_2 (2 mL) was added via cannula. The mixture was stirred for 4 h and then allowed to reach 0 °C. Distilled H_2O (4 mL) was added, and the mixture was allowed to reach room temperature. The reaction mixture was treated with a solution of NaOH (30%) saturated with NaCl (15 mL) and then stirred for 10 min until the separation of two layers. The aqueous layer was extracted with CH_2Cl_2 (3 × 15 mL), and the combined organic layer was dried, filtered, and concentrated. The residue was purified by flash chromatography (SiO_2, 2 cm × 13 cm, 5% EtOAc/hexanes) to provide **12b** [0.145 g, 0.341 mmol, 60%, R_f = 0.20 (20% EtOAc/hexanes), colorless oil]. ^{1}H NMR ($CDCl_3$, 500 MHz): 3.98 (1H, m, H-8), 3.83 (1H, m, $C\underline{H}_2OH$), 3.64 (1H, dd, J_1 = 12.1 Hz, J_2 = 7.0 Hz, $C\underline{H}_2OH$), 2.95 (1H, dd, J_1 = 7.0 Hz, J_2 = 4.1 Hz, H-26), 1.31 (3H, s, H-27), 0.90 (3H, s, H-18), 0.89 (3H, d, *J* = 6.4 Hz, H-21), 0.88 (9H, s, Me_3CSi), 0.00 (3H, s, MeSi), −0.01 (3H, s, MeSi). ^{13}C NMR ($CDCl_3$, 126 MHz): 69.4 (CH, C-8), 64.2 (CH, C-26), 61.8 (C, C-25), 61.2 (CH_2, $\underline{C}H_2OH$), 56.6 (CH, C-17), 53.0 (CH, C-14), 42.1 (C, C-13), 40.7 (CH_2,

C-9), 38.9 (CH_2, C-24), 35.9 (CH_2, C-12), 35.2 (CH, C-20), 34.4 (CH_2, C-22), 27.3 (CH_2 C-16), 25.8 (3 × CH_3, Me_3CSi), 23.0 (CH_2, C-15), 21.4 (CH_2, C-23), 18.6 (CH_3, C-27), 18.0 (C, C−Si), 17.7 (CH_2, C-11), 16.7 (CH_3, 21), C-13.7 (CH_3, C-18), −4.8 (CH_3, MeSi), −5.1 (CH_3, MeSi). IR (film, cm^{-1}): 3439 (ν_{OH}). MS ([CI]$^+$, *m/z*, %): 425 ([M + H]$^+$, 30), 349 ([M − *t*-Bu − H_2O]$^+$, 44), 293 ([M − TBSO]$^+$, 94), 275 ([M − TBSO − H_2O]$^+$, 100), 257 ([M − TBSO − 2H_2O]$^+$, 98). EA: calculated for [$C_{25}H_{48}O_3Si$], C (70.70), H (11.39); found, C (70.43), H (11.73).

(25*R*)-8β-[(*tert*-Butyldimethylsilyl)oxy]-26-(hydroxymethyl)-de-*A*,*B*-colestan-25-ol (13a), from 12a. A solution of Red-Al in toluene (0.283 mL, 0.926 mmol, 65%) was added via a syringe (provided with a thick needle) to a solution of **12a** (0.328 g, 0.772 mmol) in dry THF (40 mL) at −78 °C. The mixture was stirred for 2 h and then concentrated. The residue was dissolved in Et_2O (30 mL). The organic layer was washed with HCl (20 mL, 10%). The aqueous layer was extracted with Et_2O (3 × 15 mL). The combined organic layer was dried, filtered, and concentrated. The residue was purified by flash chromatography (SiO_2, 2 cm × 10 cm, 15% EtOAc/hexanes) to provide **13a** [0.317 g, 0.742 mmol, 96%, R_f = 0.38 (40% EtOAc/hexanes), white solid, mp 69−70 °C (EtOAc)]. ^{1}H NMR ($CDCl_3$, 500 MHz): 3.98 (1H, m, H-8), 3.87 (2H, m, CH_2OH), 1.23 (3H, s, H-27), 0.90 (3H, s, H-18), 0.89 (3H, d, *J* = 6.4 Hz, H-21), 0.88 (9H, s, Me_3C−Si), 0.00 (3H, s, MeSi), −0.02 (3H, s, MeSi). ^{13}C NMR ($CDCl_3$, 126 MHz): 74.0 (C, C-25), 69.5 (CH, C-8), 59.9 (CH_2, CH_2OH), 56.8 (CH, C-17), 53.1 (CH, C-14), 43.2 (CH_2, C-26), 42.1 (C, C-13), 41.4 (CH_2, C-24), 40.7 (CH_2, C-9), 36.3 (CH_2, C-12), 35.2 (CH, C-20), 34.5 (CH_2, C-22), 27.3 (CH_2, C-16), 26.7 (CH_3, C-27), 25.8 (3 × CH_3, Me_3CSi), 23.1 (CH_2, C-15), 20.4 (CH_2, C-23), 18.6 (CH_3, C-21), 18.0 (C, C−Si), 17.7 (CH_2, C-11), 13.7 (CH_3,C-18), −4.8 (CH_3, MeSi), −5.2 (CH_3, MeSi). IR (film, cm^{-1}): 3347 (ν_{OH}). MS ([CI]$^+$, *m/z*, %): 409([M − OH]$^+$, 46), 277 ([M − TBSO − H_2O]$^+$, 77), 259 ([M − TBSO − 2H_2O]$^+$, 71), 163 ([M − TBSO − $C_7H_{16}O_2$]$^+$, 71), 135 ([M − TBSO, $C_9H_{20}O_2$]$^+$, 100). EA: calculated for [$C_{25}H_{50}O_3Si$], C (70.36), H (11.81); found, C (69.96), H (11.86).

13a, from 12b. A solution of Red-Al in toluene (0.172 mL, 0.565 mmol, 65%) was added via a syringe (provided with a thick needle) to a solution of **12b** (0.100 g, 0.235 mmol) in dry THF (25 mL) at −78 °C. The mixture was stirred for 4 h and then concentrated. The residue was dissolved in Et_2O (20 mL). The organic layer was washed with HCl (15 mL, 10%), and the aqueous layer was extracted with Et_2O (3 × 15 mL). The combined organic layer was dried, filtered, and concentrated. The residue was purified by flash chromatography (SiO_2, 1.5 cm × 10 cm, 15% EtOAc/hexanes) to give **13a** [0.096 g, 0.218 mmol, 93%, R_f = 0.38 (40% EtOAc/hexanes), white solid, mp 69−70 °C (EtOAc)].

(25*R*)-26-(Hydroxymethyl)-de-*A*,*B*-colestan-8β,25-diol (13b). A solution of TBAF·3H_2O in THF (10.5 mL, 10.5 mmol, 1M) was added dropwise to a solution of **13a** (0.561 g, 1.30 mmol) in dry THF (50 mL). The mixture was heated at 55 °C for 7 days. The mixture was concentrated, and the residue was dissolved in EtOAc (30 mL). The organic layer was washed with HCl (40 mL, 10%), and the aqueous layer was extracted with EtOAc (3 × 25 mL). The combined organic layer was dried, filtered, and concentrated. The residue was purified by flash chromatography (SiO_2, 2.5 cm × 13 cm, 30% EtOAc/hexanes) to provide **13b** [0.398 g, 1.27 mmol, 96%, R_f = 0.30 (EtOAc), white solid, mp 118−119 °C (EtOAc)]. ^{1}H NMR ($CDCl_3$, 500 MHz): 4.04 (1H, m, H-8), 3.84 (2H, m, CH_2OH), 1.21 (3H, s, H-27), 0.90 (3H, s, H-18), 0.88 (3H, d, *J* = 6.5 Hz, H-21). ^{13}C NMR ($CDCl_3$, 126 MHz): 73.9 (C, C-25), 69.3 (CH, C −8), 59.8 (CH_2, CH_2OH), 56.6 (CH, C-17), 52.6 (CH, C-14), 43.1 (CH_2, C-26), 41.8 (C, C-13), 41.3 (CH_2, C-24), 40.4 (CH_2, C-9), 36.2 (CH_2, C-12), 35.2 (CH, C-20), 33.5 (CH_2, C-22), 27.1 (CH_2, C-16), 26.7 (CH_3, C-27), 22.5 (CH_2, C-15), 20.4 (CH_2, C-23), 18.5 (CH_3, C-21), 17.4 (CH_2, C-11), 13.7 (CH_3, C-18). IR (KBr, cm^{-1}): 3371 (ν_{OH}). MS ([CI]$^+$, *m/z*, %): 295 ([M − OH]$^+$, 29), 277 ([M − H_2O − OH]$^+$, 100). EA: calculated for [$C_{19}H_{36}O_3$], C (73.03), H (11.61); found, C (73.29), H (11.89).

(25*R*)-26-[((*tert*-Buthyldimethylsilyl)oxy)methyl]-de-*A*,*B*-colestan-8β,25-diol (13c). Imidazol (0.131 g, 1.92 mmol) and TBSCl (0.145 g, 0.960 mmol) were successively added to a solution of **13b** (0.200 g, 0.640 mmol) in dry DMF (25 mL). The resulting solution was stirred at room temperature for 1 h. A saturated solution of NaCl (20 mL) was added. The mixture was extracted with hexanes (3 × 15 mL), and the combined organic layer was dried, filtered, and concentrated. The residue was purified by flash chromatography (SiO_2, 3 cm × 13 cm, 20% EtOAc/hexanes) to afford **13c** [0.253 g, 0.593 mmol, 93%, R_f = 0.75 (50% EtOAc/hexanes), colorless oil]. ^{1}H NMR ($CDCl_3$, 500 MHz): 3.97 (1H, m, H-8), 3.81 (2H, m, CH_2OTBS), 1.11 (3H, s, H-27), 0.85 (3H, s, H-18), 0.83 (3H, d, *J* = 6.7 Hz, H-21), 0.83 (9H, s, Me_3CSi), 0.01 (6H, Me_2Si). ^{13}C NMR ($CDCl_3$, 126 MHz): 72.5 (C, C-25), 68.9 (CH, C-8), 50.5 (CH_2, CH_2OTBS), 56.5 (CH, C-17), 52.5 (CH, C-14), 42.7 (CH_2, C-26), 41.7 (C, C-13), 41.2 (CH_2, C-24), 40.3 (CH_2, C-9), 36.2 (CH_2, C-12), 35.0 (CH, C-20), 33.5 (CH_2, C-22), 27.0 (CH_2, C-16), 26.3 (CH_2, C-15), 25.8 (3 × CH_3, Me_3CSi), 22.4 (CH_3, C-27), 20.2 (CH_2, C-23), 18.6 (CH_3, C-21), 18.0 (C, C−Si), 17.3 (CH_2, C-11), 13.4 (CH_3, C-18), −5.7 (2 × CH_3, Me_2Si). IR (film, cm^{-1}): 3458 (ν_{OH}). MS ([CI]$^+$, *m/z* %): 427 ([M + H]$^+$, 29), 409 ([M − OH]$^+$, 93), 391 ([M − H_2O − OH]$^+$, 84), 277 ([M − TBSO − H_2O]$^+$, 86), 259 ([M − TBSO − 2H_2O]$^+$, 100). EA: calculated for [$C_{25}H_{50}O_3Si$], C (70.36), H (11.81); found, C (70.06), H (12.02).

(25*R*)-26-[((*tert*-Butyldimethylsilyl)oxy)methyl]-25-hydroxy-de-*A*,*B*-colestan-8-one (5a). PDC (0.264 g, 0.703 mmol) was added to a solution of alcohol **13c** (0.100 g, 0.234 mmol) in dry CH_2Cl_2 (20 mL) protected from light. After being stirred for 30 min, the mixture was filtered through a bed of Celite. The Celite was washed with EtOAc (3 × 25 mL), and the combined filtrate was concentrated. The residue was purified by flash chromatography (SiO_2, 2 × 10 cm, 20% EtOAc/hexanes) to give **5a** [0.100 g, 0.234 mmol, 99%, R_f = 0.75 (50% EtOAc/hexanes), colorless oil]. ^{1}H NMR ($CDCl_3$, 400 MHz): 3.86 (2H, m, CH_2OTBS), 3.73 (1H, broad s, OH), 2.41 (1H, dd, J_1 = 11.6 Hz, J_2 = 7.5 Hz, H-14), 1.16 (3H, s, H-27), 0.93 (3H, d, *J* = 6.0 Hz, H-21), 0.87 (9H, s, Me_3CSi), 0.60 (3H, s, H-18), 0.05 (6H, s, Me_2Si). ^{13}C NMR ($CDCl_3$, 101 MHz): 211.9 (C, C-8), 72.6 (C, C-25), 61.9 (CH, C-17), 60.7 (CH_2. CH_2OTBS), 56.6 (CH, C-14), 49.9 (C, C-13), 42.8 (CH_2, C-26), 41.3 (CH_2, C-24), 40.9 (CH_2, C-9), 38.9 (CH_2, C-12), 36.3 (CH_2, C-22), 35.4 (CH, C-20), 27.4 (CH_2, C-16), 26.4 (CH_3, C-27), 25.8 (3 × CH_3, Me_3CSi), 24.0 (CH_2, C-15), 20.2 (CH_2, C-11), 19.0 (CH_2, C-23), 18.7 (CH_3, C-21), 18.0 (C, C-Si), 12.4 (CH_3, C-18), −5.7 (2 × CH_3, Me_2Si). IR (film, cm^{-1}): 3512 (ν_{OH}), 1714 ($\nu_{C=O}$). MS ([CI]$^+$, *m/z*, %): 425 ([M + H]$^+$, 10), 407 ([M − OH]$^+$, 100), 275 ([M − H_2O − TBSO]$^+$, 21). EA: calculated for [$C_{25}H_{48}O_3Si$], C (70.70), H (11.39); found, C (70.56), H (11.68).

(25*R*)-26-[((*tert*-Butyldimethylsilyl)oxy)methyl]-25-hydroxy-1α-[(triethylsilyl)oxy]vitamin D_3 Triethylsilyl Ether (14). A solution of *n*-BuLi in hexane (0.277 mL, 0.682 mmol, 2.5M) was added dropwise via syringe to a cooled (−78 °C) solution of phosphine oxide **4a**[37] (0.412 g, 0.706 mmol) in dry THF (15 mL). The appearance of an intense red coloration was observed. After 1 h, a solution of ketone **5a** (0.100 g, 0.235 mmol) in dry THF (10 mL) was added via cannula. The reaction mixture was protected from light and stirred at −78 °C for 5 h. A saturated solution of NaCl (10 mL) was added, and the mixture was allowed to reach room temperature. The mixture was extracted with EtOAc (3 × 15 mL), and the combined organic layer was dried, filtered, and concentrated. The residue was purified by flash chromatography (SiO_2, 2 cm × 13 cm, 5% EtOAc/hexanes) to give **14** [0.083 g, 0.106 mmol, 45%, R_f = 0.60 (10% EtOAc/hexanes), colorless oil], recovered **5a** (0.045 g, 0.103 mmol, 44%), and excess **4a** (0.312 g). ^{1}H NMR ($CDCl_3$, 500 MHz): 6.24 (1H, d, *J* = 11.3 Hz, H-6), 6.04 (1H, d, *J* = 11.3

Hz, H-7), 5.20 (1H, dd, J_1 = 2.4 Hz, J_2 = 0.9 Hz, H-19), 4.88 (1H, d, J = 2.4 Hz, H-19), 4.39 (1H, dd, J_1 = 6.9 Hz, J_2 = 3.6 Hz, H-1), 4.19 (1H, m, H-3), 3.89 (2H, m, CH_2OTBS), 3.73 (1H, broad s, OH), 1.19 (3H, s, H-27), 0.95 [18H, t, J = 8.1 Hz, 2 × (CH_3CH_2)$_3$Si], 0.94 (3H, d, J = 6.8 Hz, H-21), 0.90 (9H, s, Me_3CSi), 0.59 [12H, cd, J_1 = 7.9 Hz, J_2 = 3.9 Hz, 2 × (CH_3CH_2)$_3$Si], 0.53 (3H, s, H-18), 0.08 (6H, s, Me_2Si). ^{13}C NMR ($CDCl_3$, 126 MHz): 148.3 (C, C-10), 141.2 (C, C-8), 134.9 (C, C-5), 123.2 (CH, C-6), 117.8 (CH, C-7), 111.2 (CH_2, C-19), 72.7 (C, C-25), 71.6 (CH, C-1), 67.2 (CH, C-3), 60.8 (CH_2, CH_2OTBS), 56.6 (CH, C-17), 56.3 (CH, C-14), 46.0 (CH_2, C-26), 45.8 (C, C-13), 45.0 (CH_2, C-4), 42.9 (CH_2, C-2), 41.2 (CH_2, C-24), 40.6 (CH_2, C-9), 36.5 (CH_2, C-12), 36.0 (CH, C-20), 28.9 (CH_2, C-22), 27.7 (CH_2, C-16), 26.4 (CH_3, C-27), 25.8 (3 × CH_3, Me_3CSi), 23.6 (CH_2, C-15), 22.1 (CH_2, C-23), 20.5 (CH_2, C-11), 18.8 (CH_3, C-21), 18.0 (C, C−Si), 11.9 (CH_3, C-18), 6.8 (6xCH_2, CH_3CH_2Si), 4.8 (6 × CH_3, CH_3CH_2Si), −5.7 (2 × CH_3, Me_2Si). MS (m/z [ESI-TOSI]$^+$, %): 789 ([M + H]$^+$, 4), 771 ([M − OH]$^+$, 2), 657 ([M − TBSO]$^+$, 7), 639 ([M − H_2O − TBSO]$^+$, 5), 525 ([M − TESO − TESOH]$^+$, 7), 515 ([M − TBSO − $C_9H_{18}O$]$^+$, 13), 507([M − OH − 2TESOH]$^+$, 6), 393 ([M − TBSO − 2TESOH]$^+$, 9), 375 ([M − H_2O− TBSO − 2TESOH]$^+$, 4). HRMS [ESI-TOF]$^+$: calculated for [$C_{46}H_{89}O_4Si_3$]$^+$ ([M + H]$^+$), 789.6063; found, 789.6063.

(25*R*)-1α,25-Dihydroxy-26-(hydroxymethyl)vitamin D_3 (2). A solution of TBAF·3H_2O in THF (0.170 mL, 0.170 mmol, 1M) was added via syringe to a solution of alcohol **14** (0.033 g, 0.042 mmol) in dry THF (10 mL) at 0 °C. The mixture was stirred for 30 min, and then saturated NaCl (10 mL) was added. The aqueous layer was extracted with EtOAc (3 × 8 mL), and the combined organic layer was dried, filtered, and concentrated. The residue was purified by normal phase HPLC (Phenomenex-LUNA SiO_2 column, 25 cm × 1 cm, 90% EtOAc/hexane) to give **2** [0.015 g, 0.034 mmol, 80%, R_f = 0.15 (EtOAc), white solid, mp 163−165 °C (EtOAc), >95% purity (NMR, HPLC)]. ^{1}H NMR (CD_2Cl_2, 500 MHz): 6.35 (1H, d, J = 11.3 Hz, H-6), 6.00 (1H, d, J = 11.3 Hz, H-7), 5.28 (1H, m, H-19), 4.95 (1H, m, H-19), 4.36 (1H, dd, J_1 = 6.9 Hz, J_2 = 3.6 Hz, H-1), 4.15 (1H, m, H-3), 3.80 (2H, m, CH_2OH), 1.19 (3H, s, H-27), 0.92 (3H, d, J = 6.5 Hz, H-21), 0.53 (3H, s, H-18). ^{13}C NMR (CD_2Cl_2, 126 MHz): 148.3 (C, C-10), 143.2 (C, C-8), 133.8 (C, C-5), 124.8 (CH, C-6), 117.4 (CH, C-7), 111.8 (CH_2, C-19), 74.0 (C, C-25), 70.9 (CH, C-1), 67.1 (CH, C-3), 60.1 (CH_2, CH_2OH), 56.8 (CH, C-17), 56.6 (CH, C-14), 46.2 (C, C-13), 45.7 (CH_2, C-26), 43.6 (CH_2, C-4), 43.3 (CH_2, C-2), 42.0 (CH_2, C-24), 40.8 (CH_2, C-9), 36.8 (CH_2, C-12), 36.4 (CH, 20), 30.0 (CH_2, C-22), 27.9 (CH_2, C-16), 26.9 (CH_3, C-27), 23.9 (CH_2, C-15), 22.6 (CH_2, C-23), 20.8 (CH_2, C-11), 18.8 (CH_3, C-21), 12.1 (CH_3, C-18). MS ([ESI-TOF]$^+$, m/z, %): 469 ([M + Na]$^+$, 66), 437 ([M + Na-CH_3OH]$^+$, 39), 427 ([M−OH]$^+$, 15), 413 ([M − CH_3O]$^+$, 11), 377 ([M + Na−H_2O−C_4H_9OH]$^+$, 19), 301 ([M + Na − 2H_2O − $C_7H_{16}O_2$]$^+$, 100). HRMS [ESI-TOF]$^+$: calculated for [$C_{28}H_{46}NaO_4$]$^+$ ([M + Na]$^+$), 469.3288; found, 469.3288. UV (EtOH): λ_{max}= 264 nm (ε = 1.8 × 10^4), λ_{min} = 232 nm.

((25*R*,26*S*)-8β-[(*tert*-Butyldimethylsilyl)oxy]-26-(hydroxymethyl)-26-methyl-de-*A*,*B*-cholestan-25-ol (15a). *Method a.* A solution of MeMgBr in THF (0.777 mL, 2.33 mmol, 3M) was added via syringe to a suspension of CuI (0.012 g, 0.063 mmol) in dry Et_2O (5 mL) at −45 °C. The mixture was stirred for 30 min, and then epoxide **12a** (0.090 g, 0.212 mmol) was added via cannula. The mixture was allowed to reach room temperature and stirred for 8 h. After the mixture was cooled at −78 °C, saturated NH_4Cl (10 mL) was added. The mixture was stirred for 15 min and then extracted with Et_2O (3 × 15 mL). The combined organic layer was dried, filtered, and concentrated. The residue was purified by flash chromatography (SiO_2, 3 cm × 13 cm, 10% EtOAc/hexanes) to give **17** [0.010 g, 0.023 mmol, 11%, R_f = 0.70 (30% EtOAc/hexanes), yellow oil] and a 1:1 mixture of **15a** and **16** [0.050 g, 0.113 mmol, 65%, R_f = 0.5 (30% EtOAc/hexanes), yellow oil].

Epoxide **17**. ^{1}H NMR ($CDCl_3$, 500 MHz): 4.32 (1H, t, J = 5.7 Hz, H-26a), 4.04 (1H, dd, J_1 = 12.6 Hz, J_2 = 4.9 Hz, H-26), 3.99 (1H, m, H-8), 3.96 (1H, dd, J_1 = 12.6 Hz, J_2 = 7.0 Hz, H-26), 1.25 (3H, s, H-27), 0.91 (3H, s, H-18), 0.90 (3H, d, J = 6.3 Hz, H-21), 0.89 (9H, s, Me_3CSi), 0.01 (3H, s, MeSi), −0.01 (3H, s, MeSi). ^{13}C NMR ($CDCl_3$, 126 MHz): 75.2 (C, C-25), 69.5 (CH, C-8), 66.5 (CH_2, C-26), 56.7 (CH, C-26a), 53.1 (CH, C-17), 48.2 (CH, C-14), 42.2 (C, C-13), 41.4 (CH_2, C-24), 40.7 (CH_2, C-9), 36.1 (CH_2, C-12), 35.1 (CH, C-20), 34.5 (CH_2, C-22), 27.3 (CH_2, C-16), 26.1 (CH_3, C-27), 25.8 (3 × CH_3, Me_3CSi), 23.1 (CH_2, C-15), 19.9 (CH_2, C-23), 18.7 (CH_3, C-21), 18.0 (C, C−Si), 17.7 (CH_2, C-11), 13.7 (CH_3, C-18), −4.8 (CH_3, MeSi), −5.1 (CH_3, MeSi). MS ([CI]$^+$, m/z, %): 425 ([M + H]$^+$, 20), 349 ([M − t-Bu − H_2O]$^+$, 57), 293 ([M − TBSO]$^+$, 58), 257 ([M − TBSO − 2H_2O]$^+$, 100). HRMS [CI]$^+$: calculated for: [$C_{25}H_{48}O_3Si$]$^+$ ([M]$^+$): 424.3373; found, 424.3373.

15a and **16** (1:1 mixture). ^{1}H NMR ($CDCl_3$, 500 MHz): 3.98 (1H, s, H-8), 3.80 (0.5H, c, J = 6.4 Hz, H-26-**16**), 3.74 (0.5H, m, CH_2OH-**15a**), 3.64 (0.5H, m, CH_2OH-**15a**), 1.24 (1.5H, s, H-27-**16**), 1.13 (1.5H, s, H-27-**15a**), 0.90 (3H, s, H-18), 0.88 (3H, d, J = 6.4 Hz, H-21), 0.87 (9H, s, Me_3CSi), 0.79 (3H, d, J = 7.1 Hz, CH_3CH-26-**15a**, CH_3CH_2CH-26-**16**), −0.01 (3H, s, MeSi), −0.02 (3H, s, MeSi). ^{13}C NMR ($CDCl_3$, 126 MHz): 76.4 (C, C-25), 74.2 (CH, C-26-**16**) 69.4 (CH, C-8), 66.0 (CH_2, CH_2OH-**15a**), 56.9 (CH, C-17-**16**), 56.7 (CH, C-17-**15a**), 53.0 (CH, C-14), 42.3 (CH_2, C-24), 42.1 (C, C-13), 41.2 (CH, C-26-**15a**), 40.7 (CH_2, C-9), 36.3 (CH_2, C-22-**15a**), 36.1 (CH_2, C-22-**16**), 35.2 (CH, C-20), 34.4 (CH_2, C-12), 29.7 (CH_2, CH_3CH_2-**16**), 27.3 (CH_2, C-16), 26.2 (CH_3, C-27), 25.8 (3 × CH_3, Me_3CSi), 23.0 (CH_2, C-15), 22.4 (CH_3, CH_3C-26), 19.2 (CH_2, C-23), 18.6 (CH_3, C-21), 18.0 (C, C-Si), 17.6 (CH_2, C-11), 16.8 (CH_3, CH_3CH_2CH-26-**16**), 13.7 (CH_3, C-18), 12.8 (CH_3, CH_3CH-26-**15a**), −4.8 (CH_3, MeSi), −5.2 (CH_3, MeSi).

Method b. A solution of MeLi in Et_2O (22.7 mL, 36.25 mmol, 1.6 M) was added dropwise via syringe to a cooled suspension (−78 °C) of CuCN (1.61 g, 18.04 mmol) in Et_2O (5 mL). The mixture was stirred for 30 min, and then **12a** (0.700 g, 1.64 mmol) was added via cannula. The mixture was allowed to warm to room temperature. After being stirred for 24 h, the mixture was cooled to −78 °C, and saturated NH_4Cl (10 mL) was added. The mixture was stirred for 15 min and then was extracted with Et_2O (3 × 15 mL). The combined organic layer was dried, filtered, and concentrated. The residue was purified by flash chromatography (SiO_2, 3 cm × 12 cm, 10% EtOAc/hexanes) to provide **15a** [0.614 g, 1.39 mmol, 85%, R_f = 0.65 (50% EtOAc/hexanes), yellow oil]. ^{1}H NMR ($CDCl_3$, 500 MHz): 3.97 (1H, m, H-8), 3.72 (1H, s, CH_2OH), 3.64 (1H, s, CH_2OH), 1.12 (3H, s, H-27), 0.89 (3H, s, H-18), 0.88 (3H, d, J = 6.5 Hz, H-21), 0.87 (9H, s, Me_3CSi), 0.79 (3H, d, J = 6.9 Hz, CH_3CH-26), −0.01 (3H, s, MeSi), −0.02 (3H, s, MeSi). ^{13}C NMR ($CDCl_3$, 126 MHz): 76.4 (C, C-25), 69.4 (CH, C-8), 66.0 (CH_2, CH_2OH), 56.7 (CH, C-17), 53.0 (CH, C-14), 42.3 (CH_2, C-24), 42.1 (C, C-13), 41.2 (CH, C-26), 40.7 (CH_2, C-9), 36.3 (CH_2, C-12), 35.2 (CH, C-20), 34.4 (CH_2, C-22), 27.3 (CH_2, C-16), 25.8 (3 × CH_3, Me_3CSi), 23.0 (CH_2, C-15), 22.4 (CH_3-27), 19.2 (CH_2, C-23), 18.6 (CH_3, C-21), 18.0 (C, C−Si), 17.6 (CH_2, C-11), 13.7 (CH_3, CH_3CH-26), 12.8 (CH_3, C-18), −4.8 (CH_3, MeSi), −5.2 (CH_3, MeSi). IR (film, cm^{-1}): 3332 (ν_{OH}). MS ([CI]$^+$, m/z, %): 423 ([M − OH]$^+$, 63), 381 ([M − C_3H_7O]$^+$, 11), 323 ([M − $C_6H_{13}O_2$]$^+$, 11), 291 ([M − H_2O − TBSO]$^+$, 100). EA: calculated for [$C_{26}H_{52}O_3Si$], C (70.85), H (11.89); found, C (70.49), H (12.23).

(25*R*,26*S*)-26-(Hydroxymethyl)-26-methyl-de-*A*,*B*-cholestan-8β,25-diol (15b). A solution of TBAF·3H_2O in THF (4.53 mL, 4.53 mmol, 1M) was added via syringe to a solution of diol **15a** (0.500 g, 1.13 mmol) in dry THF (50 mL), and the mixture was heated at 55 °C for 7 days. The reaction mixture was concentrated, and the residue was dissolved in EtOAc (30 mL). The solution was washed with HCl (40 mL, 10%), and the aqueous layer was extracted with EtOAc (3 × 25 mL). The combined organic layer was dried, filtered, and

concentrated. The crude was purified by flash chromatography (SiO_2, 2.5 cm × 13 cm, 30% EtOAc/hexanes) to afford **15b** [0.352 g, 1.10 mmol, 96%, R_f = 0.15 (50% EtOAc/hexanes), white solid, mp 125−126 °C (EtOAc)].

(25*R*,26*S*)-26-[((*tert*-Butyldimethylsilyl)oxy)methyl]-26-methyl-de-*A*,*B*-cholestan-8β,25-diol (15c). Imidazole (0.106 g, 1.56 mmol) and TBSCl (0.118 g, 0.781 mmol) were successively added to a solution of **15b** (0.170 g, 0.521 mmol) in dry DMF (25 mL). The mixture was stirred at room temperature for 1 h. The reaction was quenched by addition of saturated NaCl (20 mL). The aqueous layer was extracted with hexanes (3 × 15 mL). The combined organic layer was dried, filtered, and concentrated. The residue was purified by flash chromatography (SiO_2, 2.5 cm × 13 cm, 20% EtOAc/hexanes) to give **15c** [0.211 g, 0.479 mmol, 92%, R_f = 0.80 (50% EtOAc/hexanes), colorless oil]. ^{1}H NMR ($CDCl_3$, 500 MHz): 3.99 (1H, m, H-8), 3.66 (1H, dd, J_1 = 10.0 Hz, J_2 = 4.6 Hz, CH_2OTBS), 3.60 (1H, dd, J_1 = 10.0 Hz, J_2 = 3.5 Hz, CH_2OTBS), 1.03 (3H, s, H-27), 0.86 (3H, s, H-18), 0.85 (3H, d, J = 6.4 Hz, H-21), 0.84 (9H, s, Me_3CSi), 0.74 (3H, d, J = 6.9 Hz, $\underline{CH_3}CH$-26), 0.03 (6H, Me_2Si). ^{13}C NMR ($CDCl_3$, 126 MHz): 74.7 (C, C-25), 69.2 (CH, C-8), 66.8 (CH_2, CH_2OTBS), 56.6 (CH, C-17), 52.6 (CH, C-14), 41.9 (CH_2, C-24), 41.8 (C, C-13), 41.0 (CH, C-26), 40.4 (CH_2, C-9), 36.4 (CH_2, C-12), 35.3 (CH, C-20), 33.6 (CH_2, C-22), 27.2 (CH_2, C-16), 25.8 (3 × CH_3, Me_3CSi), 22.6 (CH_2, C-15), 22.5 (CH_3, C-27), 19.4 (CH_2, C-23), 18.6 (CH_3, C-21), 18.0 (C, C-Si), 17.5 (CH_2, C-11), 13.5 (CH_3, $\underline{CH_3}CH$-26), 12.6 (CH_3, C-18), −5.7 (CH_3, MeSi), −5.8 (CH_3, MeSi). IR (film, cm^{-1}): 3453 (ν_{OH} hydroxyl). MS ($[CI]^+$, m/z, %): 423 $[M - OH]^+$, 50), 405 $[M - H_2O - OH]^+$, 43), 291 $[M - H_2O - TBSO]^+$, 93), 273 $[M - 2H_2O - TBSO]^+$, 100). EA: calculated for $[C_{26}H_{52}O_3Si]$, C (70.85), H (11.89); found, C (71.07), H (12.15).

(25*R*,26*S*)-26-[((*tert*-Butyldimethylsilyl)oxy)methyl]-25-hydroxy-26-methyl-de-*A*,*B*-cholestan-8-one (5c). PDC (0.204 g, 0.545 mmol) was added to a solution of **15c** (0.082 g, 0.182 mmol) in dry CH_2Cl_2 (15 mL). The reaction mixture was protected from light and stirred at room temperature for 30 min. The mixture was filtered through a bed of Celite, and the solids were washed with EtOAc (3 × 25 mL). The filtrate was concentrated. The residue was purified by flash chromatography (SiO_2, 2 cm × 12 cm, 20% EtOAc/hexanes) to give **5c** [0.080 g, 0.178 mmol, 98%, R_f = 0.80 (40% EtOAc/hexanes), colorless oil]. ^{1}H NMR ($CDCl_3$, 400 MHz): 4.15 (1H, s, OH), 3.68 (1H, dd, J_1 = 10.1 Hz, J_2 = 4.6 Hz, CH_2OTBS), 3.68 (1H, t, J = 9.3 Hz, CH_2OTBS), 2.39 (1H, dd, J_1 = 11.5 Hz, J_2 = 7.5 Hz, H-14), 2.19 (2H, m, H-9), 1.05 (3H, s, H-27), 0.92 (3H, d, J = 6.3 Hz, H-21), 0.85 (9H, s, Me_3CSi), 0.74 (3H, d, J = 7.0 Hz, $\underline{CH_3}CH$-26), 0.58 (3H, s, H-18), 0.04 (6H, s, Me_2Si). ^{13}C NMR ($CDCl_3$, 101 MHz): 212.0 (C, C-8), 74.7 (C, C-25), 66.8 (CH_2, CH_2OTBS), 61.9 (CH, C-17), 56.5 (CH, C-14), 49.9 (C, C-13), 41.9 (CH_2, C-24), 41.1 (CH, C-26), 40.9 (CH_2, C-9), 38.9 (CH_2, C-12), 36.4 (CH_2, C-22), 35.5 (CH, C-20), 27.4 (CH_2, C-16), 25.8 (3 × CH_3, Me_3CSi), 24.0 (CH_2, C-15), 22.3 (CH_3, C-27), 19.3 (CH_2, C-23), 19.0 (CH_2, C-11), 18.7 (CH_3, C-21), 18.0 (C, C−Si), 12.5 (CH_3, C-18), 12.4 (CH_3, CH_3CH-26), −5.7 (CH_3, MeSi), −5.8 (CH_3, MeSi). IR (film, cm^{-1}): 3495 (ν_{OH}), 1470 ($\nu_{C=O}$). MS ($[CI]^+$, m/z, %): 439 ($[M + H]^+$, 5), 421 ($[M - OH]^+$, 37), 289 ($[M - H_2O - TBSO]^+$, 100). EA: calculated for $[C_{26}H_{50}O_3Si]$, C (71.17), H (11.49); found, C (71.35), H (11.76).

(25*R*,26*S*)-1α-[(*tert*-Butyldimethylsilyl)oxy]-26-[((*tert*-butyldimethylsilyl)oxy)methyl]-25-hydroxy-26-methylvitamin D_3 *tert*-Butyldimethylsilyl Ether (19). A solution of *n*-BuLi in hexane (0.560 mL, 0.896 mmol, 1.6M) was added dropwise via syringe to a −78 °C-cooled solution of **4b**[36] (0.580 g, 0.995 mmol) in dry THF (10 mL). The appearance of an intense red coloration was observed, and after 1 h, a solution of ketone **5c** (0.080 g, 0.182 mmol) in THF (2 mL) was added via cannula. The mixture was protected from light and stirred at −78 °C for 6 h. Saturated NaCl (10 mL) was added. The mixture was warmed to room temperature, and the aqueous layer was extracted with EtOAc (3 × 15 mL). The combined organic layer was dried, filtered, and concentrated. The residue was purified by flash chromatography (SiO_2, 2 cm × 10 cm, 5% EtOAc/hexanes) to afford **19** [0.070 g, 0.087 mmol, 48%, R_f = 0.60 (10% EtOAc/hexanes), colorless oil] and recover unreacted **5c** (0.034 g, 0.077 mmol, 42%) and excess **4b** (0.420 g). ^{1}H NMR (CD_2Cl_2, 500 MHz): 6.26 (1H, d, J = 11.0 Hz, H-6), 6.04 (1H, d, J = 11.0 Hz, H-7), 5.21 (1H, d, J = 2.1 Hz, H-19), 4.86 (1H, d, J = 2.1 Hz, H-19), 4.39 (1H, m, H-1), 4.18 (1H, m, H-3), 3.66 (1H, dd, J_1 = 10.5 Hz, J_2 = 7.9 Hz, CH_2OTBS), 3.71 (1H, dd, J_1 = 10.5 Hz, J_2 = 4.8 Hz, CH_2OTBS), 1.05 (3H, s, H-27), 0.92 (3H, d, J = 6.4 Hz, H-21), 0.89 (27H, s, 3 × Me_3CSi), 0.79 (3H, d, J = 7.0 Hz, $\underline{CH_3}CH$-26), 0.54 (3H, s, H-18), 0.08 (18H, s, 3 × Me_2Si). ^{13}C NMR (CD_2Cl_2, 126 MHz): 148.8 (C, C-10), 141.8 (C, C-8), 135.4 (C, C-5), 123.4 (CH, C-6), 118.1 (CH, C-7), 111.4 (CH_2, C-19), 74.8 (C, C-25), 71.9 (CH, C-1), 67.6 (CH, C-3), 67.0 (CH_2, CH_2OTBS), 56.9 (CH, C-17), 56.7 (CH, C-14), 46.3 (CH_2, C-4), 46.1 (C, C-13), 45.4 (CH_2, C-2), 42.3 (CH_2, C-24), 41.6 (CH, C-26), 40.9 (CH_2, C-9), 36.9 (CH_2, C-12), 36.4 (CH, C-20), 31.5 (CH_3, $\underline{CH_3}CH$-26), 30.0 (CH_3, C-27), 29.2 (CH_2, C-22), 28.0 (CH_2, C-16), 25.9 (9 × CH_3, 3 × Me_3Si), 23.9 (CH_2, C-15), 22.4 (CH_2, C-23), 19.9 (CH_2, C-11), 19.0 (CH_3, C-21), 18.3 (C, C−Si), 12.0 (CH_3, C-18), −4.9 (3 × CH_3, 3 × MeSi), −5.5 (3 × CH_3, 3 × MeSi). MS ($[ESI\text{-}TOF]^+$, m/z, %): 671 ($[M - TBSOH]^+$, 6), 587 ($[M - TBSOH - C_5H_8O]^+$, 53), 478 ($[M - TBSOH - C_3H_8O]^+$, 15), 421 ($[M - 2TBSOH - C_7H_{17}O]^+$, 81). HRMS $[ESI\text{-}TOF]^+$: calculated for $[C_{47}H_{90}O_4Si_3]^+$ ($[M]^+$), 802.6133; found, 802.6130.

(25*R*,26*S*)-1α,25-Dihydroxy-26-(hydroxymethyl)-26-methyl-vitamin D_3 (3). A solution of TBAF·$3H_2O$ in THF (0.159 mL, 0.159 mmol, 1M) was added to a solution of **19** (0.032 g, 0.040 mmol) in dry THF (10 mL). The mixture was stirred for 20 h at 0 °C in the dark, and then saturated NaCl (10 mL) was added. The mixture was warmed to room temperature, and the aqueous layer was extracted with EtOAc (3 × 10 mL). The combined organic layer was dried, filtered, and concentrated. The residue was purified by HPLC (Phenomenex-LUNA SiO_2 column, 25 cm × 1 cm, 90% EtOAc/hexanes) to give the analogue **3** [0.015 g, 0.033 mmol, 83%, R_f = 0.20 (EtOAc), white solid, mp 160−162 °C (EtOAc), >95% purity (NMR, HPLC)]. ^{1}H NMR (CD_3OD, 500 MHz): 6.37 (1H, d, J = 11.1 Hz, H-6), 6.14 (1H, d, J = 11.1 Hz, H-7), 5.34 (1H, dd, J_1 = 2.1 Hz, J_2 = 1.1 Hz, H-19), 4.95 (1H, dd, J_1 = 2.1 Hz, J_2 = 0.7 Hz, H-19), 4.40 (1H, t, J_1 = 5.8 Hz, H-1), 4.18 (1H, m, H-3), 3.83 (2H, dd, J_1 = 10.6 Hz, J_2 = 5.9 Hz $\underline{CH_2}OH$), 1.14 (3H, s, H-27), 1.00 (3H, d, J = 6.4 Hz, H-21), 0.98 (3H, d, J = 7.4 Hz, $\underline{CH_3}CH$-26), 0.62 (3H, s, H-18). ^{13}C NMR (CD_3OD, 126 MHz): 149.9 (C, C-10), 142.6 (C, C-8), 135.7 (C, C-5), 124.9 (CH, C-6), 119.0 (CH, C-7), 112.0 (CH_2, C-19), 75.9 (C, C-25), 71.5 (CH, C-1), 67.4 (CH, C-3), 65.5 (CH_2, CH_2OH), 58.0 (CH, C-17), 57.6 (CH, C-14), 47.0 (C, C-13), 46.2 (CH_2, C-4), 43.9 (CH, C-24), 43.7 (CH_2, C-2), 42.6 (CH_2, C-26), 41.9 (CH_2, C-9), 37.8 (CH_2, C-12), 37.5 (CH, C-20), 30.7 (CH_3, C-27), 30.0 (CH_2, C-22), 28.8 (CH_2, C-16), 24.1 (CH_2, C-15), 23.3 (CH_2, C-23), 23.2 (CH_3, CH_3C-26), 20.5 (CH_2, C-11), 19.4 (CH_3, C-21), 12.4 (CH_3, C-18). MS ($[ESI\text{-}TOF]^+$, m/z, %): 483 ($[M + Na]^+$, 100), 413 ($[M + Na - H_2O - CH_3OH]^+$, 27). HRMS $[ESI\text{-}TOF]^+$: calculated for $[C_{29}H_{48}O_4Na]^+$ ($[M + Na]^+$), 483.3428; found, 483.3422. UV (EtOH): λ_{max} = 264 nm ($\varepsilon = 1.8 \times 10^4$), λ_{min} = 232 nm.

Functional Studies. Cell Culture and Treatment of Colon Cancer Cells. Human SW480-ADH colon cancer cells were grown in RPMI medium supplemented with 10% FCS, GlutaMAX I, 100 U/mL penicillin, and 100 μg/mL streptomycin (all from Invitrogen, Paisley, U.K.). Treatment with 1,25D or the analogues **2** and **3** was carried out using medium supplemented with charcoal-treated FCS to remove liposoluble hormones. Control cells were treated with the corresponding vehicle concentration.

Western Blotting. Western blotting was performed as previously described.[38] Antibodies used are mouse monoclonal anti-E-cadherin (Transduction Laboratories, San José, CA, U.S.) and anti-β-tubulin

(Sigma, St Louis, MO, U.S.). Blots were developed using the ECL detection system (G.E. Healthcare-Amersham).

Transactivation Assays of Colon Cancer Cells. SW480-ADH cells were transfected in triplicate 24-well plates using JetPEI transfection reagent (PolyPlus Transfection, Illrich, France) following the manufacturer's guidelines. The 4×VDRE-DR3-tk-LUC construct containing four copies in tandem of a consensus DR3 response element for vitamin D cloned upstream of the herpes virus simplex thymidine kinase gene promoter and the luciferase reporter gene was provided by Prof. C. Carlberg, University of Luxembourg, Luxembourg. After 48 h of incubation in the presence or absence of the indicated concentrations of each compound, firefly luciferase (LUC) and *Renilla reniformes* luciferase (RLUC) activities were measured using the dual luciferase reporter assay system (Promega, Madison, WI, U.S.). LUC activity was normalized by RLUC activity.

Transactivation Assays of COS-7 Cells. COS-7 African green monkey kidney cells were grown to 60−80% confluence in 12-well tissue culture plates in DMEM containing 10% bovine growth serum (Hyclone, Logan, UT). Cells were transfected with 62.5 ng of WT or mutant VDR expression plasmid and 125 ng of rat 24-hydroxylase promoter VDRE-luciferase plasmid using Polyfect (Qiagen, Valencia, CA). Following a 16 h transfection, the cells were incubated in DMEM containing 1% bovine growth serum with or without 1,25D or analogues for 24 h. The cells were then lysed in 250 μL of reporter lysis buffer reagent (Promega). Aliquots were assayed for luciferase activities using the dual luciferase assay (Promega) and a Turner Design luminometer (Turner Design, Sunnyvale, CA).

Growth Inhibition Assays of Breast Cancer Cells. MCF-7 human breast cancer cells (ATCC, Rockville, MD) were grown in DMEM F12 medium containing 5% fetal bovine serum (FBS, HyClone) at 37 °C in a humidified atmosphere with 5% CO_2. For growth inhibition assays MCF-7 cells were seeded in six-well tissue culture plates at an initial density of 100 000 cells/well in 3 mL of DMEM F12 with 5% FBS. Twenty-four hours later, fresh medium was added and cells were treated in triplicate over the next 6 days with either 0.1% ethanol vehicle or graded doses of 1,25D or the various analogues. Fresh medium and ligands were replenished every other day. At the end of the treatment, cell growth was assessed by measuring the DNA content of attained cell mass using the Burton assay.[39]

■ ASSOCIATED CONTENT

Supporting Information. NMR spectra for the new compounds, X-ray diffraction structure of tetraol **18**, and HPLC chromatograms for analogues **2** and **3**. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Phone: +34 881 814 224. Fax: +34 981 595 012. E-mail: mercedes.torneiro@usc.es.

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DEDICATION

†Dedicated to Prof. Julio Alvarez-Builla on the occasion of his 65th birthday.

■ ABBREVIATIONS USED

1,25D, 1α,25-dihydroxyvitamin D_3; DEPT, distortionless enhancement by polarization transfer; DIBAL-H, diisobutylaluminum hydride; DIPT, diisopropyl tartrate; DR, direct repeat; ECL, enhanced chemiluminescence; GBSW, generalized Born with a simple switching function; Im, imidazole; LBP, ligand binding pocket; LUC, firefly luciferase; NOESY, nuclear Overhauser effect spectroscopy; $1,25(OH)_2D_3$, 1α,25-dihydroxyvitamin D_3; Red-Al, sodium bis(2-methoxyethoxy)aluminum hydride; RLU, relative luciferase units; RLUC, *Renilla reniformes* luciferase; RXR, retinoic X receptor; VDR, vitamin D receptor; VDRE, vitamin D response element; veh, vehicle; WT, wild type

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