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Synthesis and Biological Evaluation of 1α,25-Dihydroxyvitamin D_3 Analogues with a Long Side Chain at C12 and Short C17 Side Chains†

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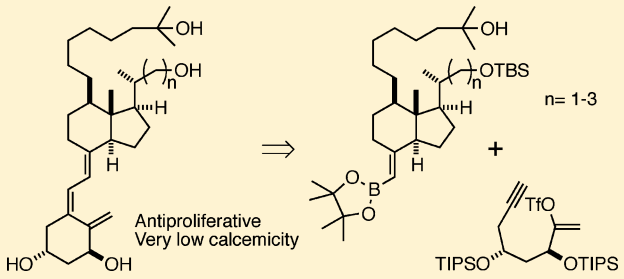
Supporting Information

ABSTRACT: Structure-guided optimization was used to design new analogues of 1α,25-dihydroxyvitamin D_3 bearing the main side chain at C12 and a shorter second hydroxylated chain at C17. The new compounds **5a–c** were efficiently synthesized from ketone **9** (which is readily accessible from the Inhoffen–Lythgoe diol) with overall yields of 15%, 6%, and 3% for **5a**, **5b**, and **5c**, respectively. The triene system was introduced by the Pd-catalyzed tandem cyclization–Suzuki coupling method. The new analogues were assayed against human colon and breast cancer cell lines and in mice. All new vitamin D_3 analogues bound less strongly to the VDR than 1α,25-dihydroxyvitamin D_3 but had similar antiproliferative, pro-differentiating, and transcriptional activity as the native hormone. In vivo, the three analogues had markedly low calcemic effects.



INTRODUCTION

The active form of vitamin D_3, the hormone 1α,25-dihydroxyvitamin D_3 (1α,25-$(OH)_2D_3$, 1,25D, **1**, also known as calcitriol, Figure 1) participates in numerous biological processes. In addition to its classical role in mineral homeostasis and bone mineralization, this hormone promotes cell differentiation, inhibits proliferation and is involved in the regulation of the immune system, among other activities.[1] Because of these "nonclassical" actions, calcitriol and its analogues have attracted considerable interest as potential drugs for the treatment of hyperproliferative diseases and immune disorders.[2] Nevertheless, their clinical application is severely hampered by their side effects: potent hypercalcemia and increased bone resorption.[3] The challenge to medicinal chemists is to develop analogues with selective properties.[4]

Calcitriol exerts most of its biological functions by regulating the transcription of target genes through interaction with a specific nuclear receptor, the vitamin D receptor (VDR).[5] The VDR is a transcription factor that binds to the gene promoter region as a heterodimer with the retinoid X receptor (RXR). Binding to calcitriol or other agonist ligands induces a conformational change of the VDR, in which helix 12 closes the ligand-binding pocket by a mouse-trap-like mechanism.[6] This conformational shift promotes the release of corepressor proteins and the recruitment of coactivator proteins, leading to activation of gene transcription.[1b,7] In addition, 1α,25-$(OH)_2D_3$ exerts rapid, transcription-independent (nongenomic) regulatory actions on ion channels, kinases, phosphatases, and phospholipases that are mediated by VDR and perhaps other still not well-characterized receptors.[8] Recent findings show that 1,25D mediates nongenomic effects via binding to the alternative ligand-binding pocket, a ligand binding site of the VDR overlapping the well-characterized genomic pocket.[9]

A key for understanding the molecular mode of action of the hormone and hence for rational design of analogues was the elucidation by Moras et al. in 2000 of the crystal structure of the ligand binding domain (LBD) of the human VDR forming a complex with its natural ligand 1,25D.[10] Since then, around 50 structures of agonist or superagonist calcitriol analogues complexed with human, rat, or zebrafish VDR LBDs have been determined.[11]

We recently designed and synthesized new active analogues of 1α,25-$(OH)_2D_3$ on the basis of simulation studies of their docking in the human VDR(LBD).[12] In silico, all these new analogues bind significantly to the Moras VDR(LBD).[13] Among these vitamin D analogues exhibiting interesting biological profile are compounds **2a–c**,[14] which have

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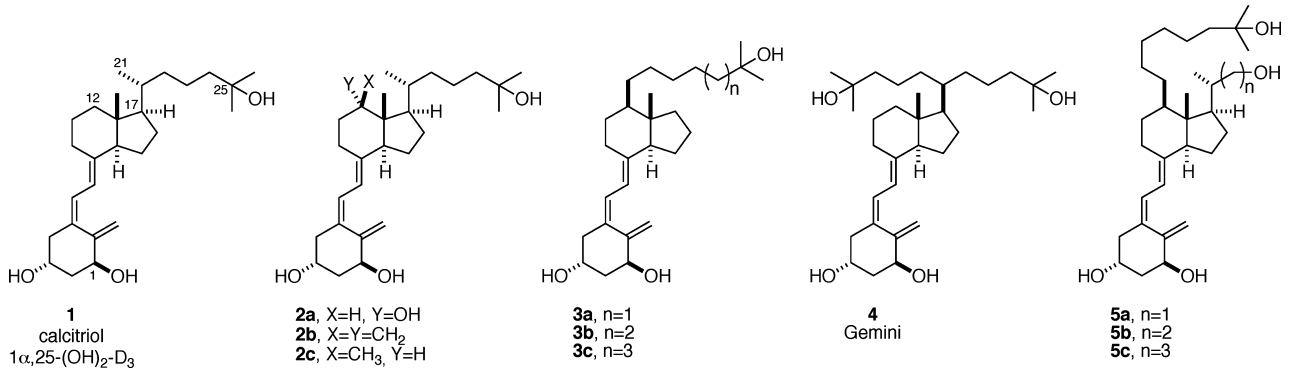

Figure 1. Structure of 1α,25-dihydroxyvitamin D_3 and analogues **2**–**5**.

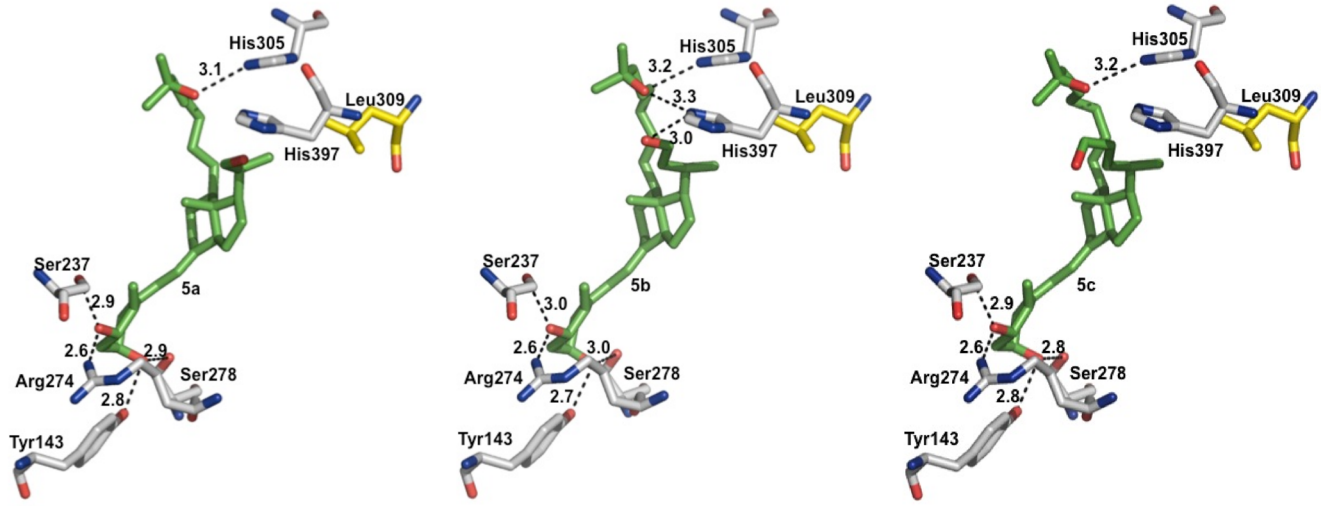

Figure 2. Predicted structures of the complexes of analogues **5a** (left), **5b** (center), and **5c** (right) with the Moras VDR(LBD). Ligand conformation and interactions with Leu309 and with hydrogen-bond forming residues are shown. Distances are measured in angstroms. Nitrogens are depicted in blue, oxygens in red, and carbons in green (analogues **5**), yellow (Leu309), or gray (hydrogen bond forming residues). Hydrogens are omitted.

substituents at C12 and compounds **3a**–**c**[12c] in which the side chain has been moved to C12 (Figure 1). In particular, the VDR binding affinity of **2c** is 4.4 times higher than that of 1,25D, and the affinities of analogues **3a**–**c** for the VDR are 60–71% as compared with the natural hormone 1,25D (100%), analogue **3b** being the most active in transactivation studies (20% compared with 1,25D). We have accordingly used **3b** as the starting point for the development of new analogues in this series with improved biological potency. Inspired by the interesting biological profile of Gemini, a 1,25D analogue with two hydroxylated side chains (**4**, Figure 1),[15] and related compounds,[16] we developed the new analogues **5a**–**c** (Figure 1), which like **3b** bear a 7C-long side chain at C12 and also have a second shorter hydroxylated chain at C17. We describe here their design on the basis of docking studies, their syntheses, and their biological evaluation.

RESULTS AND DISCUSSION

Design. The new vitamin D_3 analogues **5a**–**c** were designed on the basis of computational docking studies using the LBD derived from the X-ray crystal structure of Moras VDR(LBD)-1,25D complex and the biological activity of analogues **3**. In previous docking studies, we found that the rings and triene system of analogue **3b** adopt the same conformation as the natural hormone 1,25D in the binding pocket. Interactions of the **3b** side chain with the protein are similar to those of 1,25D except with Leu309 and decreased interactions with His305 and His397.[12c] The observation of a free space around position C17, originated by the removal of the natural side chain in **3b**, led us to envisage that the agonist conformation of the ligand–VDR complex might receive additional stabilization from the attachment of a second small side chain at C17. Structural studies of several superagonist analogues of 1,25D in complex with the VDR have shown their superagonism to be due to additional stabilization of helix H12 and stronger interactions with transcription coactivators.[17,18] Accordingly, we modified **3b** as analogues **5a**–**c**, in which C17 bears the Inhoffen–Lythgoe diol side chain fragment or a higher homologue. In contrast to **3b**, the three new analogues have a 21-methyl group as 1,25D for interaction with Leu309, which plays a key role in ligand-mediated protein folding and therefore in the maintenance of the active conformation of the VDR,[19] and they each also hold an extra hydroxyl group for additional polar contacts with the receptor. Docking calculations show that analogues **5a**–**c** form stronger complexes with Moras VDR(LBD) than the natural ligand 1,25D in the order: **5b** > **5c** > **5a**. In all the complexes (Figure 2), the hydrophobic interaction of the 21-methyl group with the side chain of the Leu309 amino acid residue is restored, the ring A hydroxyl groups form hydrogen bonds with the same amino acid residues as the natural ligand,[10] and His305 is within hydrogen bonding distance of

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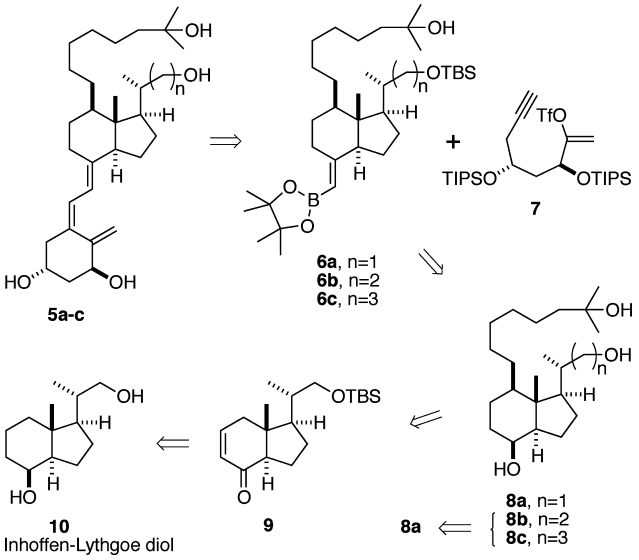
the hydroxyl group of the longer side chain. Only in the complex with **5b**, the His397 forms a hydrogen bond with the hydroxyl group of the longer C12 side chain and it also forms a hydrogen bond with the hydroxyl group of the shorter (C17) side chain. Thus **5b** interacts best in terms of both energy and the number of hydrogen bonds with the receptor. It is worth noting that this theoretical hydrogen bonding pattern of analogue **5b** is similar to that observed in the crystallographic structures of Gemini[20] and the superagonist Gemini72[17e] analogues, in complex with a wild-type zebrafish VDR(LBD). We have previously found that 1,25D analogues that bind significantly in silico are also biologically active.[12] The promising docking results for **5a–c** accordingly encouraged us to pursue their synthesis to further study the structure–activity relationships of vitamin D analogues.

Synthesis. Our strategy for the synthesis of analogues **5a–c** was based on the convergent method recently developed in our group (Scheme 1).[21] In this approach, the triene system is

Scheme 1. Retrosynthetic Analysis of Analogues 5a, 5b, and 5c



efficiently constructed by a stereoselective Pd-catalyzed intramolecular cyclization of an enol triflate (A-ring precursor **7**) followed by a Suzuki–Miyaura coupling of the resulting Pd(II)-intermediate with an alkenyl boronic ester (CD–side chain fragments **6a–c**). Boronates **6** would be accessed by manipulation of the secondary hydroxyl group of triols **8a–c**. Triol **8a**, precursor of the elongated triols **8b–c**, would be prepared from the Inhoffen–Lythgoe diol (**10**) employing methodology developed in our laboratories.[12c,14,22]

The key triol intermediate **8a** was prepared from the Inhoffen–Lythgoe diol (**10**) through the known unsaturated ketone **9**[23] (Scheme 2). Reduction of ketone **9** with DIBAL-H followed by protection of the resulting allylic alcohol **11** with *tert*-butyldimethylsilyl chloride provided the silyl ether **12**, which upon epoxidation from the less hindered face with *m*-chloroperbenzoic acid gave epoxide **13** (82% over the three steps). Opening of epoxide **13** with lithium diethylamide followed by hydroxyl-directed epoxidation of the resulting allylic alcohol **14** furnished the epoxide **15**, which was converted to mesylate **16** (91% over the three steps). Treatment of **16** with sodium naphthalene provided allylic alcohol **17**, which was converted to ketone **19** by catalytic hydrogenation and pyridinium dichromate oxidation (82% over the three steps). Ketone **19** was transformed into the enoltriflate **21** in 84% yield by deprotonation with LDA and trapping of the resulting enolate with Comins triflimide (**20**). The desired triol **8a** was finally prepared in 82% yield by a three-step sequence: palladium-catalyzed coupling of **21** with alkyne **22**,[14] catalytic hydrogenation of the resulting enyne **23**, and deprotection of **24** (13 steps from **9**, 44% overall yield). The structure and stereochemistry of triol **8a** was confirmed by X-ray crystallography (see Supporting Information).

With the parent triol **8a** at hand, we proceeded to the preparation of triols **8b** and **8c**, precursors of analogues **5b** and **5c**, respectively. Elongation of the residual side chain at C17 of **8a** (Scheme 3) to **8b** was accomplished in 46% yield by a three-step sequence: selective tosylation of the primary hydroxyl group, S_N2 displacement of the tosylate **25a** with potassium cyanide, and reduction of the nitrile **26a** with diisobutylaluminum hydride. A second homologation following the same procedure provided triol **8c** in 46% yield via tosylate **25b** and nitrile **26b**.[24]

Completion of the synthesis of analogues **5a–c** is illustrated in Scheme 4. Selective silylation of the primary hydroxyl group of triols **8a–c** followed by oxidation of the secondary hydroxyl group afforded ketones **28a–c**, which were converted to the desired alkenyl bromides **29a–c** by Wittig reaction with ylide $Ph_3P{=}CHBr$.[25] The upper boronates **6a–c** were prepared by Miyaura borylation[26] of **29a–c** with bis(pinacolato)diboron in the presence of [1,1′-bis(diphenylphosphino)ferrocene]-dicloro-palladium(II)–dichloromethane complex as catalyst and tricyclohexylphosphine as ligand. Finally, the triene system was installed by treatment of **6a–c** with an equimolar amount of enol triflate **7**[21] in the presence of catalytic $PdCl_2(PPh_3)_2$ and K_3PO_4 in H_2O/THF. Removal of the protecting groups gave the desired analogues **5a–c** in good yields (overall yields from **8a–c**: 34% for **5a**, 27% for **5b**, and 29% for **5c**).

Biological Assays. The biological activity of analogues **5a**, **5b**, and **5c** was first evaluated in intact human SW480-ADH colon cancer cells. All three compounds have a prodifferentiating action on these cells comparable to that of 1,25D, inducing at 10^{-7} M the formation of compact epithelioid islands of highly adherent cells (Figure 3). This effect was partially evident at 10^{-8} M in the case of 1,25D but not of the three analogues. Accordingly, the three compounds induced the expression of E-cadherin, the key intercellular adhesion protein in epithelial cells that behaves as an invasion suppressor, similarly to 1,25D at 10^{-7} M although with less potency at 10^{-9} M (Figure 4A).[27] As phosphatidylinositol-5-phosphate 4-kinase type II β (PIP4K2B)-mediated $PI(4,5)P_2$ signaling has been reported to be important to E-cadherin induction by 1,25D in colon cancer cells,[28] we studied the expression of this enzyme. However, the cellular level of *PIP4K2B* RNA did not change following treatment with 1,25D or analogues **5a–c** in SW480-ADH cells as measured by quantitative RT-PCR (Supporting Information Figure 2S).

As for E-cadherin, the three analogues increased the level of the tumor suppressor protein Cystatin D[29] with slightly less potency than $1\alpha,25\text{-}(OH)_2D_3$ at low concentrations (Figure 4A). We also studied the effect of the analogues **5a–c** on the expression of c-MYC oncogene, a strong inducer of cell proliferation that is repressed by 1,25D by direct and indirect mechanisms in several cell types.[27,30] Again, all three

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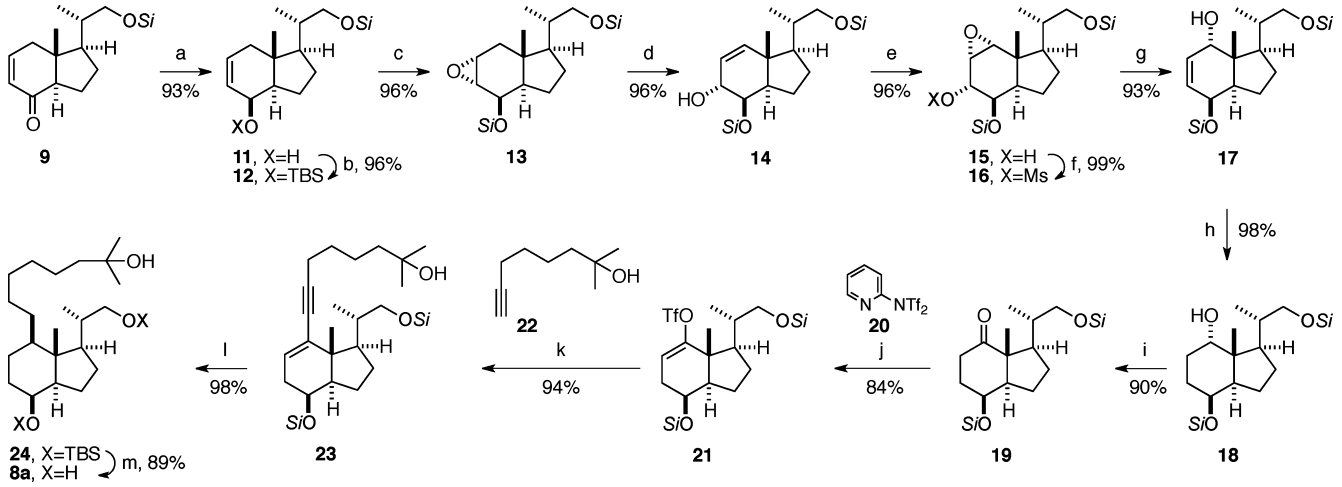
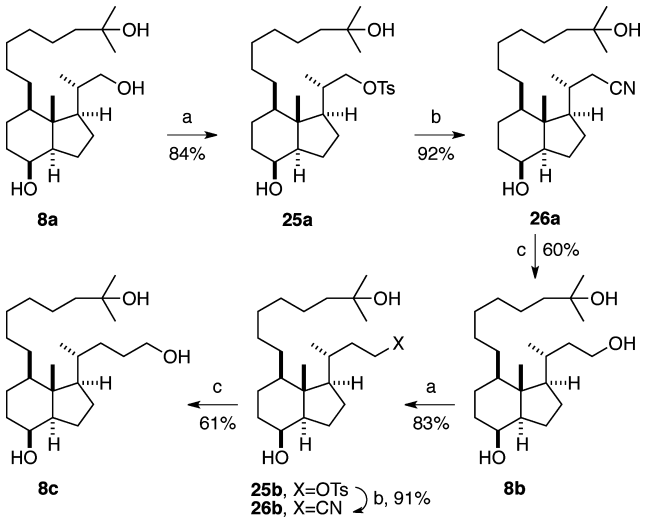
Scheme 2. Synthesis of Triol 8a[a]



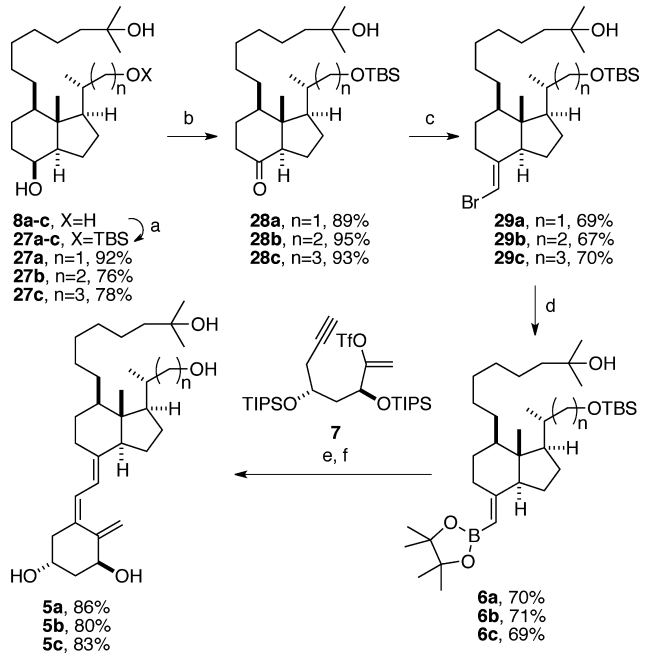
[a]Reagents and conditions: (a) DIBAL-H, THF, −78 °C; (b) TBSCl, Im, DMF; (c) *m*-CPBA, CH_2Cl_2, 0 °C; (d) $LiNEt_2$, HMPA, Et_2O; (e) *m*-CPBA, CH_2Cl_2, 0 °C; (f) MsCl, Et_3N, CH_2Cl_2, −20 °C; (g) Na/naphthalene, THF, 0 °C; (h) H_2, 10% Pd−C, EtOAc; (i) PDC, CH_2Cl_2; (j) LDA, THF, −78 °C; **20**, −78 °C → 0 °C; (k) **22**, $PdCl_2(Ph_3P)_2$, CuI, Et_2NH; (l) H_2, 10% Pd−C, EtOAc; (m) TBAF, THF, Δ. *Si*=TBS=Si(*t*-Bu)Me_2.

Scheme 3. Synthesis of Triols 8b and 8c[a]



[a]Reagents and conditions: (a) TsCl, Py, 4 °C; (b) KCN, DMSO, 90 °C; (c) DIBAL-H, CH_2Cl_2, −5 °C, HCl 3 M/Et_2O, DIBAL-H, THF, −78 °C.

Scheme 4. Synthesis of Analogues 5a−c[a]



[a]Reagents and conditions: (a) TBSCl, Im, DMF; (b) PDC, CH_2Cl_2; (c) $(Ph_3PCH_2Br)Br$, KO*t*-Bu, toluene, ultrasounds, −17 → 0 °C; then **28**; (d) $PdCl_2(dppf)\cdot CH_2Cl_2$, PCy_3, Pin_2B_2, KOAc, DMSO, 80 °C; (e) **7**, $PdCl_2(PPh_3)_2$, K_3PO_4, THF, H_2O; (f) TBAF, THF.

compounds showed comparable gene regulatory activity than the natural hormone (Figure 4A).

Cell proliferation was evaluated in the MCF-7 breast cancer cells using the MTT assay. Compound **5a** at 10^{-7} M significantly ($P < 0.001$) decreased cell proliferation, similarly to 1,25D, with respect to untreated cells. Compounds **5b** and **5c** were also able to statistically ($P < 0.05$) decrease cell proliferation in relation to control cells (Figure 4B).

Together, the data of the biological evaluation of the analogues show that **5a−c** share the mechanism of action of 1α,25-$(OH)_2D_3$ in human cancer cells, albeit with slightly less potency at low concentrations. The upregulation of E-cadherin and Cystatin D expression and the prodifferentiation and antiproliferative effects indicate a protective action against cancer.

We next tested by competitive binding assay[31] the biological ability of the analogues **5a−c** to bind VDR, as compared to the natural hormone **1**. The VDR binding affinity of compounds **5a−c** is approximately 10 times less than that of the 1,25D (Figure 5A).

The ability of the analogues to induce transcriptional activation of a vitamin D target gene was evaluated by transfecting MCF-7 cells with the pCYP24A1-Luc vector. The compounds **5a−c** and the natural hormone **1** induced a strong dose-dependent activation of the VDRE, as measured by luciferase activity. However, all analogues were less potent to activate the reporter gene transcription than 1,25D (Figure 5B).

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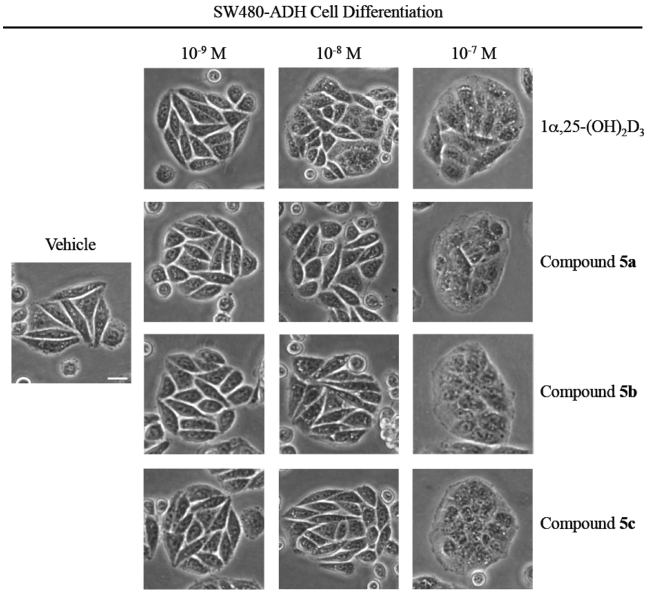

Figure 3. Activity of analogues **5a**, **5b**, and **5c** in human colon cancer cells. Phase-contrast micrographs showing the induction by analogues **5a**–**c** of a differentiated adhesive epithelial phenotype in human SW480-ADH colon cancer cells. The cells were treated with analogues **5a**–**c** or 1,25D at the indicated concentrations or with vehicle for 48 h. A representative experiment is shown. Bar, 15 μm.

Calcium serum levels were evaluated in mice injected intraperitoneally with compounds **5a**–**c**, as well as with the natural ligand **1** (Figure 6). Our data indicated that administration of analogues **5a**, **5b**, and **5c** to mice did not significantly raise calcium levels, as compared with vehicle-treated mice. However, the natural hormone significantly ($P < 0.001$) increases calcemia, in comparison with control mice. No significant changes in body weight were observed in mice after three weeks of treatment with compounds **1** or **5**.

When comparing the docking ranking of the analogues with the measured VDR binding and transcriptional and calcemic potencies, a general tendency is not observed (Table 1). Compound **5b,** which shows the best in silico binding, is also the analogue with the higher in vitro affinity for the VDR, but it shows the lower transcriptional potency and the highest calcemic activity. Although the analogues **5a**–**c** are only slightly less potent than the natural hormone **1** in the transactivation assays, they showed markedly lower calcemic effects in vivo.

CONCLUSIONS

On the basis of the biological profile of 1α,25-$(OH)_2D_3$ analogues with susbtituents at C12 (**2a**–**c** and **3a**–**c**) and docking simulations in the binding domain of the nuclear vitamin D receptor, we have designed three new analogues (**5a**–**c**) of 1α,25-$(OH)_2D_3$ bearing the side chain at C12 and a second short hydroxylated chain of different length at C17 that replaces the natural side chain. These compounds were readily synthesized from the Inhofen–Lythgoe diol via the α,β-unsaturated ketone **9** in 15% (19 steps, **5a**), 6% (22 steps, **5b**), and 3% (25 steps, **5c**) global yields from **9**. The triene system was efficiently introduced by Pd(II)-catalyzed coupling of the enol-triflate **7** (A-ring fragment) with boronates **6a**–**c** (CD–side chain fragments). The new analogues **5** are more potent in terms of biological activity than the parent analogues **3** lacking substitution at C17. Thus, in spite of the fact that analogues **5a**, **5b**, and **5c** showed reduced VDR binding compared to hormone **1**, in vitro biological assays demonstrated that all compounds have pro-differentiating, antiproliferative, and transcriptional actions comparable to that of 1α,25-$(OH)_2D_3$. Interestingly, the three analogues showed markedly lower calcemic effects in vivo than the natural hormone **1**. The antiproliferative properties of compounds **5a**–**c** coupled to its very low calcemic effects make these new vitamin D analogues of potential clinical interest.

EXPERIMENTAL SECTION

Docking Procedure for Structure-Guided Design. The docking process for the ligands **5a**–**c** was similar to the one previously described for other vitamin D analogues.[12f] The binding affinity was determined by the difference in energy between the complex and its components (protein and ligand) for the top 200 poses of every ligand. The calculated values (kcal/mol) of the binding energy promediated for the 200 better-bound conformations of each ligand were −93.2 for the natural hormone **1**, −96.7 for analogue **5a**, −105.6 for analogue **5b**, and −104.2 for analogue **5c**.

Chemistry. *General Methods and Materials.* All reactions involving oxygen or moisture sensitive compounds were carried out under argon (L-50) atmosphere. Reaction temperatures refer to external bath temperatures. All solvents were distilled under argon immediately prior to use. THF and Et_2O were distilled from Na/benzophenone, toluene was distilled from Na, CH_2Cl_2 was distilled from P_2O_5, and pyridine, Et_3N, Et_2NH, and *i*-Pr_2NH were distilled from CaH_2. DMF was stored over activated 4 Å molecular sieves. DMSO and HMPA were distilled from CaH_2 and stored over activated 4 Å molecular sieves. 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 rotary evaporator at aspirator pressure (20–30 mmHg). Reactions were monitored by thin-layer chromatography (TLC) using aluminum-backed MERCK 60 silica gel plates (0.2 mm thickness). After visualization under ultraviolet light at 254 nm, the plates were developed by immersion in a solution containing either a mixture of *p*-anisaldehyde (2.5%), acetic acid (1%), and sulfuric acid (3.4%) in 95% ethanol or a solution of ceric ammonium nitrate (0.5 g) and ammonium molybdate (4.8 g) in H_2O (100 mL) and H_2SO_4 (5.6 mL) followed by heating with a hot gun. Flash column chromatography was performed with Merk silica gel (230–400 mesh). NMR spectra were recorded in $CDCl_3$ or methanol-d_4 solutions on a Bruker AMX 500 MHz, Varian Inova 400 MHz and Bruker DPX 250 MHz. 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 = 3.31 ppm (^{1}H, q, methanol-d_4), δ = 77.0 ppm (^{13}C, t, $CDCl_3$), or δ = 49.0 ppm (^{13}C, hp, methanol-d_4) as internal standard; coupling constants are reported in Hz. Distortionless enhancement by polarization transfer (DEPT) was used to assign carbon types. Melting points (mp) were measured in a Büchi apparatus and are uncorrected. Infrared (IR) spectra were recorded on a Bruker spectrometer, model IFS-66 V FT-IR. Low (MS) and high resolution mass spectra (HRMS) were performed in a Micromas Instruments Autospec spectrometer for (CI) and (FAB) while (ESI-TOF) was performed in Bruker-Microtof spectrometer. Elementary analysis (EA) were recorded on element analyzer FISONS, model EA 1108. Optical rotations were measured at 25 °C on a Jasco, model DIP-370. UV spectra were recorded on a HP spectrophotometer, model 8452A. HPLC purifications were performed on a Shimadzu preparative liquid chromatograph, model LC-8A, equipped with a UV absorbance detector using a HPLC Phenomenex-Luna silica column (⌀ 250 mm × 10 mm). Analogues **5a**–**c** have a purity of >95% (HPLC).

Chemicals. CuI was purified following Kauffman's indications.[32] Pyridinium dichromate (PDC) was prepared following Corey's

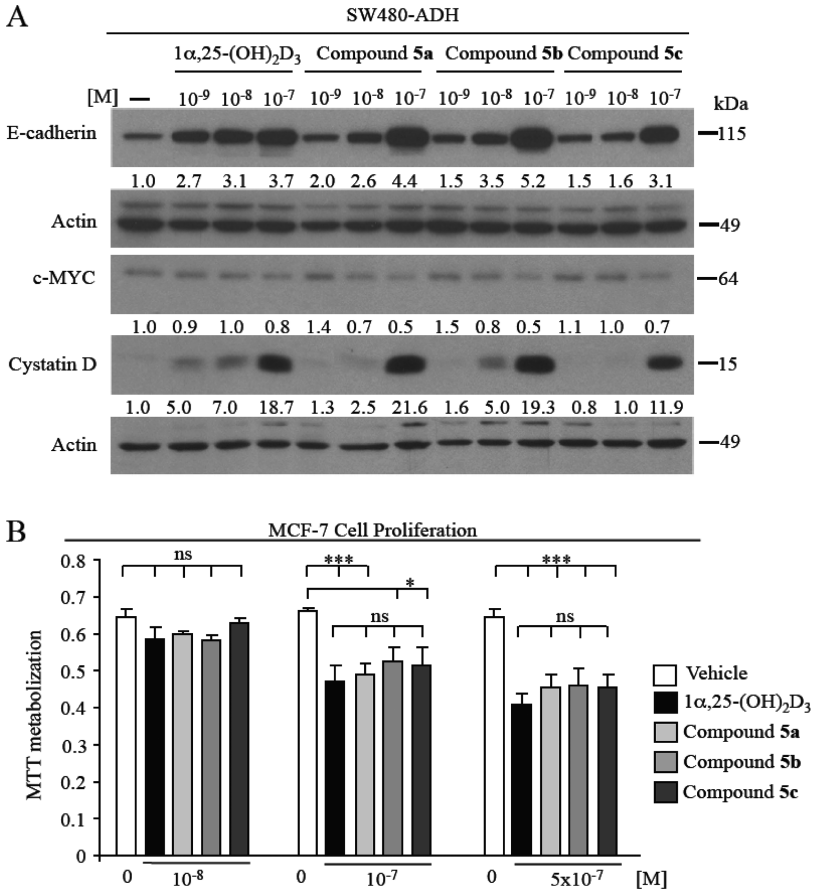

Figure 4. Activity of analogues **5a**, **5b**, and **5c** in human colon and breast cancer cells. (A) Regulation of target genes. Western blot analysis of the induction of E-cadherin and Cystatin D and of the repression of c-MYC proteins at 48 h of treatment of SW480-ADH cells with each compound (10^{-9} to 10^{-7} M). Actin was used as loading control. Numbers correspond to mean values of fold-change obtained in two experiments. (B) Cell proliferation in human MCF-7 breast adenocarcinoma cells. Cells were treated with vehicle or analogues **5a**, **5b**, and **5c** or 1,25D at concentrations of 10^{-8}, 10^{-7}, or 5×10^{-7} M for 48 h, and then a MTT assay was carried out. Each point represents the average of three individual experiments. Error bars represent standard deviation (SD). * $P < 0.05$, ***$P < 0.001$.

procedure.[33] *N*-(2-Pyridyl)triflimide and $(Ph_3PCH_2Br)Br$ were prepared as reported.[34,35] *m*-Chloroperbenzoic acid (*m*-CPBA) was purified according to Perrin's indications.[36]

*22-(tert-Butyldimethylsilyloxy)-de-A,B-23,24-dinorchol-9-en-8β-ol (**11**).* A solution of diisobutyl-aluminum hydride in CH_2Cl_2 (9 mL, 1 M, 9 mmol) was added dropwise to a solution of **9**[23] (1.939 g, 6.01 mmol) in THF (30 mL) at −78 °C. The reaction mixture was allowed to warm to rt. After 30 min, the reaction was quenched by slowly addition of saturated NaCl at 0 °C. 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, 4 cm × 10 cm, 4% EtOAc−hexanes) to afford **11** [1.808 g, 5.57 mmol, 93%, R_f = 0.40 (20% EtOAc−hexanes), colorless oil, $[\alpha]_D$ = +101.5 (c = 1.0, $CHCl_3$)]. ^{1}H NMR ($CDCl_3$, 250 MHz): 5.91−5.68 (2H, m, H-9 and H-11), 4.11 (1H, broad s, H-8), 3.58 (1H, dd, J_1 = 3.5, J_2 = 9.6, H-22), 3.28 (1H, dd, J_1 = 7.2, J_2 = 9.6, H-22), 0.95 (3H, d, J = 6.4, H-21), 0.87 (9H, s, Me_3C-Si), 0.81 (3H, s, H-18), 0.01 (6H, s, Me_2Si). ^{13}C NMR ($CDCl_3$, 63 MHz): 129.6 (CH), 128.3 (CH), 67.6 (CH_2, C-22), 66.2 (CH, C-8), 52.9 (CH), 50.0 (CH), 41.8 (CH_2), 39.8 (C, C-13), 38.4 (CH), 26.8 (CH_2), 25.9 (3 × CH_3, Me_3C-Si), 21.2 (CH_2), 18.2 (C, C-Si), 16.5 (CH_3), 13.4 (CH_3), −5.5 (2 × CH_3, Me_2Si). IR (film, cm^{-1}): 3380 (ν_{O-H}), 3022 (ν_{C-H}), 2957 (ν_{C-H}), 2929 (ν_{C-H}). MS ([ESI-TOF]$^+$, m/z, %): 347 ([M + Na]$^+$, 100), 307 ([M − OH]$^+$, 6). HRMS: [ESI-TOF]$^+$, calcd for $[C_{19}H_{36}O_2SiNa]^+$, 347.2377; found, 347.2354.

*8β,22-Bis(tert-butyldimethylsilyloxy)-de-A,B-23,24-dinorchol-9-ene (**12**).* Imidazole (876 mg, 12.87 mmol) and TBSCl (1.47 g, 9.75 mmol) were successively added to a solution of **11** (1.266 g, 3.90 mmol) in DMF (35 mL). After 48 h, the reaction was quenched by addition of saturated NaCl (30 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, 3 cm × 8 cm, hexanes) to afford **12** [1.648 g, 3.76 mmol, 96%, R_f = 0.95 (10% EtOAc−hexanes), colorless oil, $[\alpha]_D$ = +143.9 (c = 1.9, $CHCl_3$)]. ^{1}H NMR ($CDCl_3$, 250 MHz): 5.84−5.64 (2H, m, H-9 and H-11), 4.15 (1H, broad s, H-8), 3.64 (1H, dd, J_1 = 2.9, J_2 = 9.4, H-22), 3.35 (1H, dd, J_1 = 7.1, J_2 = 9.4, H-22), 1.03 (3H, d, J = 6.5, H-21), 0.95 (9H, s, Me_3C-Si), 0.94 (9H, s, Me_3C-Si), 0.88 (3H, s, H-18), 0.14−0.03 (12H, m, 2 × Me_2Si). ^{13}C NMR ($CDCl_3$, 63 MHz): 129.1 (CH), 128.4 (CH), 67.8 (CH_2, C-22), 66.3 (CH, C-8), 53.3 (CH), 50.7 (CH), 42.4 (CH_2), 40.2 (C, C-13), 38.7 (CH), 27.2 (CH_2), 26.1 (3 × CH_3, Me_3C-Si), 25.9 (3 × CH_3, Me_3C-Si), 22.0 (CH_2), 18.4 (C, C-Si), 18.1 (C, C-Si), 16.8 (CH_3), 13.6 (CH_3), −4.3 (CH_3, MeSi), −5.0 (CH_3, MeSi), −5.3 (CH_3, MeSi), −5.3 (CH_3, MeSi). IR (film, cm^{-1}): 3022 (ν_{C-H}), 2957 (ν_{C-H}), 2929 (ν_{C-H}). MS ([ESI-TOF]$^+$, m/z, %): 461 ([M + Na]$^+$, 4), 381 ([M − *t*-Bu]$^+$, 41). HRMS: [ESI-TOF]$^+$, calcd for $[C_{25}H_{50}O_2Si_2Na]^+$, 461.3242; found, 461.3256.

*8β,22-Bis(tert-butyldimethylsilyloxy)-de-A,B-23,24-dinor-9α,11α-epoxycholane (**13**).* *m*-Chloroperbenzoic acid (4.05 g, 23.49 mmol) was added in three portions (each 15 min) to a solution of **12** (3.68 g, 8.39 mmol) in CH_2Cl_2 (80 mL) at 0 °C. The mixture was stirred in the dark at 0 °C for 1.5 h and then allowed to warm to rt for 2.5 h. The reaction was quenched at 0 °C by slow addition of saturated $Na_2S_2O_3$ (50 mL). The aqueous layer was extracted with CH_2Cl_2 (3 × 50 mL). The combined organic layer was washed with saturated $NaHCO_3$ (2 ×

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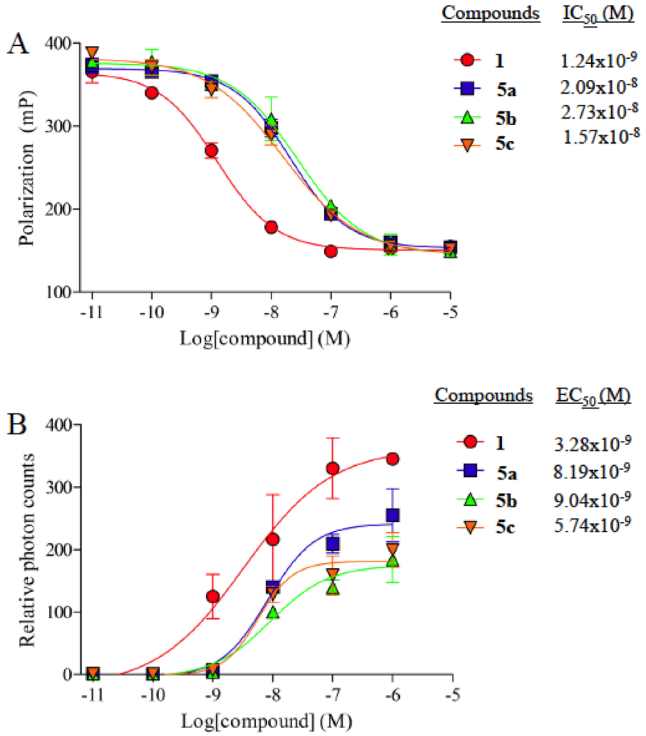

Figure 5. Vitamin D receptor binding and transactivation activities. (A) Competitive binding of 1α,25-$(OH)_2D_3$ (**1**) and the synthesized vitamin D_3 analogues **5a**–**c** to the full-length human VDR. The experiments were carried out in duplicate on two different occasions. IC_{50} values are derived from dose–response curves and represent the measure of 50% inhibition of polarization of a compound. (B) MCF-7 cells were transfected with a 24-hydroxylase gene reporter vector and then treated with 10^{-11} to 10^{-6} M concentrations of 1,25D or analogues **5a**–**c** for 48 h. The luciferase activity was then measured. The EC_{50} values are derived from dose–response curves and represent the analogue concentration capable of increasing the luciferase activity by 50%. All of the experiments were carried out in duplicate on at least two different occasions. Error bars represent standard deviation (SD).

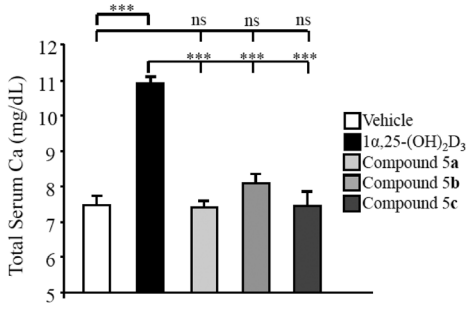

Figure 6. Calcium levels in mice treated with the natural hormone 1,25D and compounds **5a**–**c**. Six mice per group were treated with 0.3 μg/kg of compounds **5**, 1,25D, or vehicle every other day during 3 weeks, and calcium levels were measured on day 21. Error bars represent standard deviation (SD). ***$P < 0.001$.

30 mL), dried, filtrated, and concentrated. The residue was purified by flash chromatography (SiO_2, 6 cm × 8 cm, hexanes) to afford **13** [3.65 g, 8.02 mmol, 96%, R_f = 0.75 (6% EtOAc–hexanes), white solid, mp 47–49 °C (EtOAc–hexanes), $[\alpha]_D$ = +42.0 (c = 1.4, $CHCl_3$)]. ^{1}H NMR ($CDCl_3$, 500 MHz): 4.20 (1H, broad d, J = 2.2, H-8), 3.54 (1H, dd, J_1 = 3.2, J_2 = 9.6, H-22), 3.25 (1H, dd, J_1 = 7.1, J_2 = 9.6, H-22), 3.16 (1H, dd, J_1 = 3.3, J_2 = 5.8, H-11), 3.07 (1H, d, J = 3.3, H-9), 2.13 (1H, dd, J_1 = 5.8, J_2 = 15.0, H-12), 1.49–1.40 (1H, m, H-20), 0.95 (3H, d, J = 6.6, H-21), 0.90 (9H, s, Me_3C-Si), 0.87 (9H, s, Me_3C-Si), 0.83 (3H, s, H-18), 0.09 (3H, s, MeSi), 0.04 (3H, s, MeSi), 0.01 (6H, s, Me_2Si). ^{13}C NMR ($CDCl_3$, 125 MHz): 67.5 (CH_2, C-22), 67.2 (CH, C-8), 56.3 (CH, C-9), 52.7 (CH), 52.3 (CH, C-11), 44.6 (CH), 40.0 (C, C-13), 40.0 (CH_2, C-12), 38.3 (CH, C-20), 26.9 (CH_2), 25.9 (3 × CH_3, Me_3C-Si), 25.8 (3 × CH_3, Me_3C-Si), 21.1 (CH_2), 18.3 (C, C-Si), 18.0 (C, C-Si), 16.7 (CH_3, C-21), 16.1 (CH_3, C-18), −4.7 (CH_3, MeSi), −5.2 (CH_3, MeSi), −5.4 (CH_3, MeSi), −5.5 (CH_3, MeSi). IR (neat, cm^{-1}): 2956 (ν_{C-H}), 2929 (ν_{C-H}). MS ([ESI-TOF]$^+$, m/z, %): 477 ([M + Na]$^+$, 65), 455 ([M − H_2O − TBSOH]$^+$, 14). EA: calculated for [$C_{25}H_{50}O_3Si_2$], C (66.02), H (11.08); found, C (65.94), H (11.37).

*8β,22-Bis(tert-butyldimethylsilyloxy)-de-A,B-23,24-dinorchol-11-en-9α-ol (**14**).* A solution of lithium diethylamide was prepared by dropwise addition of a solution of *n*-BuLi in hexanes (18.7 mL, 2.25 M, 42.0 mmol) to diethylamine (5.1 mL, 49.0 mmol) at −40 °C. The white semisolid slurry formed was melted at rt, then cooled to −40 °C and dissolved in Et_2O (22.5 mL). After warm to rt, a solution of **13** (3.18 g, 6.99 mmol) in Et_2O (50 mL) and HMPA (8.5 mL, 49.0 mmol) were successively added via cannula. After 15 h, the reaction was quenched by the slowly addition of a few drops of saturated NH_4Cl and aqueous HCl (100 mL, 2%). The aqueous layer was extracted with MTBE (3 × 50 mL). The combined organic layer was dried, filtered, and concentrated. The residue was purified by flash chromatography (SiO_2, 6 cm × 12 cm, 2% EtOAc–hexanes) to afford **14** [3.06 g, 6.73 mmol, 96%, R_f = 0.33 (10% EtOAc–hexanes), white solid, mp 95–97 °C (EtOAc–hexanes), $[\alpha]_D$ = −4.5 (c = 0.7, $CHCl_3$)]. ^{1}H NMR ($CDCl_3$, 250 MHz): 6.42 (1H, d, J = 10.1, H-12), 5.56 (1H, dd, J_1 = 3.6, J_2 = 10.1, H-11), 4.06–3.92 (2H, m, H-8 and H-9), 3.58 (1H, dd, J_1 = 3.2, J_2 = 9.6, H-22), 3.28 (1H, dd, J_1 = 7.1, J_2 = 9.6, H-22), 1.07 (3H, d, J = 6.4, H-21), 0.96 (3H, s, H-18), 0.88 (18H, overlapped s, 2 × Me_3C-Si), 0.08 (3H, s, MeSi), 0.04 (3H, s, MeSi), 0.02 (6H, s, Me_2Si). ^{13}C NMR ($CDCl_3$, 63 MHz): 142.2 (CH), 125.7 (CH), 75.0 (CH), 72.8 (CH), 67.5 (CH_2, C-22), 48.1 (2 × CH), 43.2 (C, C-13), 38.9 (CH), 28.1 (CH_2), 25.9 (3 × CH_3, Me_3C-Si), 25.8 (3 × CH_3, Me_3C-Si), 21.9 (CH_2), 18.3 (C, C-Si), 18.0 (C, C−Si), 17.0 (CH_3), 16.4 (CH_3), −4.9 (CH_3, MeSi), −5.2 (CH_3, MeSi), −5.4 (2 × CH_3, Me_2Si). IR (neat, cm^{-1}): 3315 (ν_{O-H}), 2955 (ν_{C-H}), 2929 (ν_{C-H}). MS ([ESI-TOF]$^+$, m/z, %): 477 ([M + Na]$^+$, 100), 437 ([M − OH]$^+$, 48). EA: calcd for [$C_{25}H_{50}O_3Si_2$], C (66.02), H (11.08); found, C (65.59), H (11.23).

*8β,22-Bis(tert-butyldimethylsilyloxy)-de-A,B-23,24-dinor-11α,12α-epoxycholan-9α-ol (**15**).* *m*-Chloroperbenzoic acid (1.374 g, 7.96 mmol) was added in three portions (each 30 min) to a solution of **14** (1.81 g, 3.98 mmol) in CH_2Cl_2 (40 mL) at 0 °C. The cooling bath was removed, and the mixture was stirred in the dark for 6 h. The reaction was quenched at 0 °C by the slowly addition of saturated $Na_2S_2O_3$ (30 mL). The aqueous layer was extracted with CH_2Cl_2 (3 × 50 mL). The combined organic layer was washed with saturated $NaHCO_3$ (2 × 30 mL), saturated NaCl (30 mL), dried, filtered, and concentrated. The residue was purified by flash chromatography (SiO_2, 4 cm × 8 cm, 5% EtOAc–hexanes) to afford **15** [1.799 g, 3.82 mmol, 96%, R_f = 0.53 (10% EtOAc–hexanes), white solid, mp 115–118 °C (EtOAc–hexanes), $[\alpha]_D$ = +7.8 (c = 1.0, $CHCl_3$)]. ^{1}H NMR ($CDCl_3$, 500 MHz): 3.89 (1H, d, J = 4.1, H-9), 3.62–3.58 (2H, m, H-8 and H-22), 3.53 (1H, d, J = 4.1, H-12), 3.37 (1H, t, J = 4.2, H-11), 3.28 (1H, dd, J_1 = 7.4, J_2 = 9.5, H-22), 1.09 (3H, d, J = 6.3, H-21), 1.00 (3H, s, H-18), 0.89 (9H, s, Me_3C-Si), 0.88 (9H, s, Me_3C-Si), 0.07 (3H, s, MeSi), 0.03 (6H, s, Me_2Si), 0.02 (3H, s, MeSi). ^{13}C NMR ($CDCl_3$, 125 MHz): 74.1 (CH, C-8), 72.0 (CH, C-9), 67.4 (CH_2, C-22), 62.7 (CH, C-12), 54.5 (CH, C-11), 47.4 (CH), 42.0 (C, C-13), 41.4 (CH), 38.3 (CH), 26.3 (CH_2), 25.9 (3 × CH_3, Me_3C-Si), 25.8 (3 × CH_3, Me_3C-Si), 21.4 (CH_2), 18.3 (C, C-Si), 17.9 (C, C-Si), 17.0 (CH_3, C-21), 13.7 (CH_3, C-18), −4.9 (CH_3, MeSi), −5.2 (CH_3, MeSi), −5.4 (CH_3, MeSi), −5.4 (CH_3, MeSi). IR (neat, cm^{-1}): 3345 (ν_{O-H}), 2956 (ν_{C-H}), 2929 (ν_{C-H}). MS ([ESI-TOF]$^+$, m/z, %): 493 ([M + Na]$^+$, 100), 453 ([M − OH]$^+$, 7). HRMS: [ESI-TOF]$^+$, calcd for [$C_{25}H_{50}O_4Si_2Na$]$^+$, 493.3140; found, 493.3147.

*8β,22-Bis(tert-butyldimethylsilyloxy)-de-A,B-23,24-dinor-11α,12α-epoxycholan-9α-yl methanesulfonate (**16**).* Dry triethylamine (1.138 mL, 8.16 mmol) was added to a solution of **15** (1.923 g, 4.08 mmol) in CH_2Cl_2 (25 mL) at −20 °C. After 15 min,

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Table 1. VDR Binding Properties, Transcriptional Activities, and Calcemic Effects of the Natural Hormone 1α,25-$(OH)_2D_3$ (1), and 5a, 5b, and 5c Vitamin D Analogues[a]

		VDR binding		transcriptional activity		calcemic activity
compd	in silico binding energy kcal/mol	IC_{50} (M)	%	EC_{50}(M)	%	%
1	−93.2	1.24×10^{-9}	100	3.28×10^{-9}	100	100
5a	−96.7	2.09×10^{-8}	17	8.19×10^{-9}	40	0
5b	−105.6	2.73×10^{-8}	22	9.04×10^{-9}	36	18
5c	−104.2	1.57×10^{-8}	13	5.74×10^{-9}	57	1

[a]The in silico binding energy refers to the calculated energy difference between the **1**/**5**−VDR complex and the free protein and ligand **1**/**5** and is promediated for the 200 better-bound conformations of the ligand. The VDR binding and transcriptional activity is expressed as percentage activity at IC_{50} or EC_{50}, respectively, in comparison with 1α,25-$(OH)_2D_3$ (**1**, 100% activity). Calcemic activity is expressed as percentage of the calcium increase in mice treated with the different compounds in relation with untreated mice, considering 100% 1α,25-$(OH)_2D_3$ treated-mice.

methanesulfonyl chloride (635 μL, 8.16 mmol) was added. The mixture was stirred for 1 h. The reaction was quenched by the slow addition of H_2O (25 mL). The aqueous layer was extracted with CH_2Cl_2 (3 × 30 mL). The combined organic layer was dried, filtered, and concentrated to give **16** [2.22 g, 4.04 mmol, 99%, R_f = 0.53 (10% EtOAc−hexanes), white solid, mp 99−101 °C (EtOAc−hexanes), $[\alpha]_D$ = −20.9 (c = 1.1, $CHCl_3$)] that was used in the next reaction without further purification. ^{1}H NMR ($CDCl_3$, 250 MHz): 4.93 (1H, d, J = 3.8, H-9), 3.82 (1H, broad d, J = 2.3, H-8), 3.60 (1H, dd, J_1 = 2.0, J_2 = 9.4, H-22), 3.54−3.42 (2H, m, H-11 and H-12), 3.28 (1H, dd, J_1 = 6.7, J_2 = 9.4, H-22), 3.14 (3H, s, MeS), 1.09 (3H, d, J = 5.6, H-21), 1.01 (3H, s, H-18), 0.89 (9H, s, Me_3C-Si), 0.88 (9H, s, Me_3C-Si), 0.12 (3H, s, MeSi), 0.03 (9H, overlapped s, Me_2Si and MeSi). ^{13}C NMR ($CDCl_3$, 63 MHz): 81.9 (CH, C-9), 71.8 (CH, C-8), 67.3 (CH_2, C-22), 61.1 (CH), 51.2 (CH), 47.4 (CH), 42.2 (CH), 41.7 (C, C-13), 39.3 (CH_3, MeS), 38.2 (CH), 26.2 (CH_2), 25.9 (3 × CH_3, Me_3C-Si), 25.7 (3 × CH_3, Me_3C-Si), 21.1 (CH_2), 18.3 (C, C-Si), 17.9 (C, C-Si), 17.0 (CH_3), 13.8 (CH_3), −4.8 (2 × CH_3, Me_2Si), −5.4 (2 × CH_3, Me_2Si). IR (neat, cm^{-1}): 2956 (ν_{C-H}), 2929 (ν_{C-H}), 1208 ($\nu_{O=S=O}$). MS ([ESI-TOF]$^+$, m/z, %): 571 ([M + Na]$^+$, 100), 475 ([M − O − t-Bu]$^+$, 15). HRMS: [ESI-TOF]$^+$, calcd for $[C_{26}H_{52}O_6SSi_2Na]^+$, 571.2915; found, 571.2921.

*8β,22-Bis(tert-butyldimethylsilyloxy)-de-A,B-23,24-dinorchol-9-en-12α-ol (**17**).* A solution of naphthalene (5.23 g, 40.8 mmol) in dry THF (13 mL) was added via cannula to freshly cut sodium pieces (938 mg, 40.8 mmol). After 18 h, the resulting deep-blue mixture was added via cannula to a solution of **16** (2.22 g, 4.04 mmol) in THF (25 mL) at 0 °C. The mixture was stirred for 3 h at 0 °C. The reaction was quenched by slowly addition of H_2O (50 mL). The aqueous layer was extracted with EtOAc (3 × 60 mL). The combined organic layer was dried, filtered, and concentrated. The residue was purified by flash chromatography (SiO_2, 6 cm × 10 cm, 3% EtOAc−hexanes) to afford **17** [1.715 g, 3.78 mmol, 93%, R_f = 0.45 (15% EtOAc−hexanes), white solid, mp 75−78 °C (EtOAc−hexanes), $[\alpha]_D$ = +177.9 (c = 0.6, $CHCl_3$)]. ^{1}H NMR ($CDCl_3$, 250 MHz): 5.91−5.83 (2H, m, H-9 and H-11), 4.07 (1H, t, J = 3.9, H-8), 4.00 (1H, d, J = 4.5, H-12), 3.63 (1H, dd, J_1 = 4.3, J_2 = 9.8, H-22), 3.28 (1H, dd, J_1 = 7.4, J_2 = 9.8, H-22), 1.02 (3H, d, J = 6.6, H-21), 0.89 (9H, s, Me_3Si-C), 0.87 (9H, s, Me_3C-Si), 0.04 (9H, overlapped s, MeSi and Me_2Si), 0.02 (3H, s, MeSi). ^{13}C NMR ($CDCl_3$, 63 MHz): 132.0 (CH), 129.2 (CH), 71.7 (CH), 67.8 (CH_2, C-22), 65.7 (CH), 44.9 (C, C-13), 44.1 (CH), 43.4 (CH), 38.3 (CH), 26.1 (CH_2), 26.0 (3 × CH_3, Me_3C−Si), 25.8 (3 × CH_3, Me_3C-Si), 21.0 (CH_2), 18.4 (C, C-Si), 18.0 (C, C-Si), 15.4 (CH_3), 13.9 (CH_3), −4.4 (CH_3, MeSi), −5.2 (CH_3, MeSi), −5.3 (2 × CH_3, Me_2Si). IR (neat, cm^{-1}): 3318 (ν_{O-H}), 2956 (ν_{C-H}), 2929 (ν_{C-H}). MS ([ESI-TOF]$^+$, m/z, %): 477 ([M + Na]$^+$, 100), 437 ([M − OH]$^+$, 4). HRMS: [ESI-TOF]$^+$, calcd for $[C_{25}H_{50}O_3Si_2Na]^+$, 477.3191; found, 477.3193.

*8β,22-Bis(tert-butyldimethylsilyloxy)-de-A,B-23,24-dinorcholan-12α-ol (**18**).* Pd/C (38 mg, 10%) was added to a solution of **17** (1.249 g, 2.75 mmol) in EtOAc (50 mL). The mixture was degassed under reduced pressure and refilled with H_2 for three times. The reaction mixture was stirred under H_2 atmosphere (balloon pressure) for 48 h. The mixture was filtered through a layer of silica gel. The solids were washed with EtOAc (3 × 15 mL), and the resulting filtrate was concentrated to give **18** [1.237 g, 2.71 mmol, 98%, R_f = 0.78 (15% EtOAc−hexanes), white solid, mp 51−54 °C (EtOAc−hexanes), $[\alpha]_D$ = +46.1 (c = 1.4, $CHCl_3$)], which was used in the next reaction without further purification. ^{1}H NMR ($CDCl_3$, 250 MHz): 3.96 (2H, overlapped broad s, H-8 and H-12), 3.58 (1H, dd, J_1 = 3.8, J_2 = 9.6, H-22), 3.28 (1H, dd, J_1 = 7.1, J_2 = 9.6, H-22), 1.01 (3H, d, J = 6.4, H-21), 0.95 (3H, s, H-18), 0.88 (18H, overlapped s, 2 × Me_3C-Si), 0.02 (6H, s, Me_2Si), 0.00 (3H, s, MeSi), −0.01 (3H, s, MeSi). ^{13}C NMR ($CDCl_3$, 63 MHz): 73.1 (CH), 69.2 (CH), 67.6 (CH_2, C-22), 45.9 (C, C-13), 45.0 (CH), 44.2 (CH), 38.2 (CH), 28.2 (CH_2), 25.9 (CH_2), 25.9 (3 × CH_3, Me_3C-Si), 25.8 (3 × CH_3, Me_3C-Si), 24.5 (CH_2), 22.2 (CH_2), 18.3 (C, C-Si), 17.9 (C, C-Si), 15.7 (CH_3), 14.7 (CH_3), −4.9 (CH_3, MeSi), −5.2 (CH_3, MeSi), −5.4 (2 × CH_3, Me_2Si). IR (neat, cm^{-1}): 3403 (ν_{O-H}), 2956 (ν_{C-H}), 2929 (ν_{C-H}). MS ([ESI-TOF]$^+$, m/z, %): 479 ([M + Na]$^+$, 100), 439 ([M − OH]$^+$, 13). HRMS: [ESI-TOF]$^+$, calcd for $[C_{25}H_{52}O_3Si_2Na]^+$, 479.3347; found, 479.3343.

*8β,22-Bis(tert-butyldimethylsilyloxy)-de-A,B-23,24-dinorcholan-12-one (**19**).* Pyridinium dichromate (2.547 g, 6.77 mmol) was added to a solution of **18** (1.237 g, 2.71 mmol) in CH_2Cl_2 (40 mL). The reaction mixture, protected from light, was stirred for 36 h. The mixture was filtered through a layer of silica gel. The solids were washed with MTBE (3 × 40 mL), and the resulting filtrate was concentrated. The residue was purified by flash chromatography (SiO_2, 3 cm × 10 cm, 2% EtOAc−hexanes) to afford **19** [1.114 g, 2.45 mmol, 90%, R_f = 0.66 (10% EtOAc−hexanes), colorless oil, $[\alpha]_D$ = +88.8 (c = 1.6, $CHCl_3$)]. ^{1}H NMR ($CDCl_3$, 250 MHz): 4.02 (1H, broad d, J = 1.8, H-8), 3.61 (1H, dd, J_1 = 3.5, J_2 = 9.5, H-22), 3.30 (1H, dd, J_1 = 7.8, J_2 = 9.5, H-22), 3.19−2.93 (1H, m, H-11), 1.31 (3H, s, H-18), 0.95−0.89 (12H, m, H-21 and Me_3C−Si), 0.88 (9H, s, Me_3C-Si), 0.06 (3H, s, MeSi), 0.04 (3H, s, MeSi), 0.02 (6H, s, Me_2Si). ^{13}C NMR ($CDCl_3$, 63 MHz): 215.6 (C, C-12), 68.2 (CH, C-8), 67.7 (CH_2, C-22), 57.2 (C, C-13), 57.0 (CH), 43.9 (CH), 39.1 (CH), 37.8 (CH_2), 34.9 (CH_2), 26.5 (CH_2), 26.0 (3 × CH_3, Me_3C-Si), 25.8 (3 × CH_3, Me_3C-Si), 22.9 (CH_2), 18.3 (C, C-Si), 18.0 (C, C-Si), 17.4 (CH_3), 13.3 (CH_3), −4.8 (CH_3, MeSi), −5.1 (CH_3, MeSi), −5.4 (2 × CH_3, Me_2Si). IR (film, cm^{-1}): 2955 (ν_{C-H}), 2929 (ν_{C-H}), 2857 (ν_{C-H}), 1712 ($\nu_{C=O}$). MS ([CI]$^+$, m/z, %): 455 ([M + H]$^+$, 81), 439 ([M − Me]$^+$, 75), 397 ([M − t-Bu], 60), 323 ([M − TBSO]$^+$, 100). HRMS: [CI]$^+$, calcd for $[C_{25}H_{51}O_2Si_2]^+$, 455.3377; found, 455.3365.

*8β,22-Bis(tert-butyldimethylsilyloxy)-de-A,B-23,24-dinorchol-11-en-12-yl trifluoromethanesulfonate (**21**).* A solution of lithium diisopropylamide was prepared by dropwise addition of solution of n-BuLi in hexanes (1.62 mL, 2.27 M, 3.67 mmol) to diisopropylamine (397 mg, 3.92 mmol) at −78 °C. The mixture was stirred at rt until formation of a semisolid slurry. The slurry was cooled to −78 °C and dissolved in THF (16 mL). A solution of **19** (1.114 g, 2.45 mmol) in THF (30 mL) was added via cannula. The mixture was stirred at rt for 4 h and then cooled to −78 °C. A solution of triflimide **20** (1.924 g, 4.90 mmol) in THF (20 mL) was added. The mixture was allowed to warm to rt overnight. The reaction was quenched by addition of saturated NaCl (40 mL). The aqueous layer was extracted with MTBE (3 × 40 mL), and the combined organic layer was dried, filtered, and concentrated. The residue was purified by flash chromatography (SiO_2,

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3 cm × 8 cm, 20% CH_2Cl_2–hexanes and 10% EtOAc–hexanes) to afford **21** [1.208 g, 2.06 mmol, 84%, R_f = 0.69 (5% EtOAc–hexanes), colorless oil, $[\alpha]_D$ = +25.6 (c = 1.2, $CHCl_3$)]. ^{1}H NMR ($CDCl_3$, 250 MHz): 5.53 (1H, t, J = 3.9, H-11), 4.12 (1H, broad d, J = 6.1, H-8), 3.66 (1H, dd, J_1 = 4.1, J_2 = 9.7, H-22), 3.41 (1H, dd, J_1 = 7.0, J_2 = 9.7, H-22), 2.66 (1H, ddd, J_1 = 3.7, J_2 = 6.2, J_3 = 19.0, H-9), 2.32 (1H, dd, J_1 = 4.2, J_2 = 19.0, H-9), 1.26 (3H, s, H-18), 1.04 (3H, d, J = 6.9, H-21), 0.90 (18H, overlapped s, 2 × Me_3C-Si), 0.06 (3H, s, MeSi), 0.04 (9H, overlapped s, MeSi and Me_2Si). ^{13}C NMR ($CDCl_3$, 63 MHz): 157.3 (C, C-12), 118.4 (C, q, J = 319, CF_3), 112.6 (CH, C-11), 66.5 (CH_2, C-22), 65.8 (CH, C-8), 53.8 (CH), 50.7 (CH), 45.9 (C, C-13), 36.4 (CH_2), 34.9 (CH), 25.9 (3 × CH_3, Me_3C-Si), 25.7 (3 × CH_3, Me_3C-Si), 23.3 (CH_2), 22.2 (CH_2), 19.5 (CH_3), 18.2 (C, C-Si), 17.9 (C, C-Si), 15.8 (CH_3), −4.8 (CH_3, MeSi), −5.2 (CH_3, MeSi), −5.5 (2 × CH_3, Me_2Si). IR (film, cm^{-1}): 2956 (ν_{C-H}), 2931 (ν_{C-H}), 2886 (ν_{C-H}), 2859 (ν_{C-H}), 1666 ($\nu_{C=C}$). MS ([CI]$^+$, m/z, %): 587 ([M + H]$^+$, 18), 455 ([M − TBSO]$^+$, 20), 305 ([M − TBSO − TfOH]$^+$, 86). HRMS: [CI]$^+$, calcd for $[C_{26}H_{50}O_5F_3SSi_2]^+$, 587.2870; found, 587.2864.

*8β,22-Bis(tert-butyldimethylsilyloxy)-12-(7-hydroxy-7-methyloct-1-ynyl)-de-A,B-23,24-dinorchol-11-ene (**23**).* CuI (60 mg, 0.31 mmol) and $PdCl_2(PPh_3)_2$ (60 mg, 0.08 mmol) were successively added to a solution of **21** (1.00 g, 1.71 mmol) and **22**[14] (1.20 g, 8.56 mmol) in Et_2NH (100 mL) at 0 °C. The reaction mixture was stirred for 1 h at 0 °C and 12 h at rt. The reaction was quenched by slowly addition of saturated NH_4Cl (100 mL). Aqueous layer was extracted with MTBE (3 × 50 mL). The combined organic layer was dried, filtered, and concentrated. The residue was purified by flash chromatography (SiO_2, 4.5 cm × 9 cm, 10% EtOAc–hexanes) to afford **23** [925 mg, 1.60 mmol, 94%, R_f = 0.62 (30% EtOAc–hexanes), colorless oil, $[\alpha]_D$ = +28.2 (c = 1.1, $CHCl_3$)]. ^{1}H NMR ($CDCl_3$, 250 MHz): 5.80–5.64 (1H, m, H-11), 4.08 (1H, broad d, J = 6.0, H-8), 3.70 (1H, dd, J_1 = 3.1, J_2 = 9.1, H-22), 3.30 (1H, t, J = 9.1, H-22), 1.18 (6H, s, Me_2C-OH), 1.06 (3H, s, H-18), 1.00 (3H, d, J = 6.8, H-21), 0.86 (9H, s, Me_3C-Si), 0.84 (9H, s, Me_3C-Si), 0.01 (6H, s, Me_2Si), −0.02 (6H, s, Me_2Si). ^{13}C NMR ($CDCl_3$, 63 MHz): 132.1 (C, C-12), 131.9 (CH, C-11), 89.5 (C), 80.1 (C), 70.8 (C, C-OH), 66.6 (CH, C-8), 66.3 (CH_2, C-22), 51.5 (CH), 51.5 (CH), 44.8 (C, C-13), 43.3 (CH_2), 37.1 (CH_2), 33.8 (CH), 29.1 (2 × CH_3, Me_2C-OH), 25.9 (3 × CH_3, Me_3C-Si), 25.7 (3 × CH_3, Me_3C-Si), 23.8 (CH_2), 21.8 (CH_2), 21.5 (CH_2), 20.2 (CH_3), 19.4 (2 × CH_2), 18.1 (C, C-Si), 17.9 (C, C–Si), 17.1 (CH_3), −4.8 (CH_3, MeSi), −5.2 (CH_3, MeSi), −5.3 (CH_3, MeSi), −5.3 (CH_3, MeSi). IR (film, cm^{-1}): 3368 (ν_{O-H}), 2955 (ν_{C-H}), 2930 (ν_{C-H}), 2884 (ν_{C-H}), 2858 (ν_{C-H}). MS ([CI]$^+$, m/z, %): 577 ([M + H]$^+$, 9), 559 ([M − OH]$^+$, 28), 445 ([M − TBSO]$^+$, 14), 427 ([M − OH − TBSOH]$^+$, 51), 295 ([M − OH − 2TBSOH]$^+$, 100). HRMS: [CI]$^+$, calcd for $[C_{34}H_{65}O_3Si_2]^+$, 577.4472; found, 577.4468.

*8β,22-Bis(tert-butyldimethylsilyloxy)-12β-(7-hydroxy-7-methyloctyl)-de-A,B-23,24-dinorcholane (**24**).* Pd/C (240 mg, 10%) was added to a solution of enyne **23** (1.89 g, 3.27 mmol) in EtOAc (75 mL). The mixture was degassed under reduced pressure and refilled with H_2 for three times. The reaction mixture was stirred under H_2 atmosphere (balloon pressure) for 72 h. The mixture was filtered through a layer of silica gel. The solids were washed with EtOAc (3 × 20 mL), and the resulting filtrate was concentrated. The residue was purified by flash chromatography (SiO_2, 4 cm × 8 cm, 4% EtOAc–hexanes) to afford **24** [1.88 g, 3.22 mmol, 98%, R_f = 0.70 (30% EtOAc–hexanes), colorless oil, $[\alpha]_D$ = +37.4 (c = 1.0, $CHCl_3$)]. ^{1}H NMR ($CDCl_3$, 250 MHz): 3.92 (1H, broad d, J = 1.7, H-8), 3.69 (1H, dd, J_1 = 4.2, J_2 = 9.5, H-22), 3.32 (1H, dd, J_1 = 8.4, J_2 = 9.5, H-22), 1.19 (6H, s, Me_2C-OH), 0.96 (3H, d, J = 6.7, H-21), 0.88 (9H, s, Me_3C-Si), 0.87 (9H, s, Me_3C-Si), 0.81 (3H, s, H-18), 0.02 (6H, s, Me_2Si), −0.01 (3H, s, MeSi), −0.02 (3H, s, MeSi). ^{13}C NMR ($CDCl_3$, 63 MHz): 71.0 (C, C-OH), 69.0 (CH, C-8), 66.5 (CH_2, C-22), 56.6 (CH), 53.8 (CH), 49.4 (CH), 45.5 (C, C-13), 44.0 (CH_2), 34.9 (CH_2), 34.9 (CH), 31.4 (CH_2), 30.2 (CH_2), 30.1 (CH_2), 29.2 (2 × CH_3, Me_2C-OH), 28.3 (CH_2), 26.0 (3 × CH_3, Me_3C-Si), 25.8 (3 × CH_3, Me_3C-Si), 24.4 (CH_2), 23.7 (CH_2), 22.5 (CH_2), 21.2 (CH_2), 20.5 (CH_3), 18.2 (C, C–Si), 18.0 (C, C-Si), 11.0 (CH_3), −4.8 (CH_3, MeSi), −5.1 (CH_3, MeSi), −5.3 (2 × CH_3, Me_2Si). IR (film, cm^{-1}): 3362 (ν_{O-H}), 2954 (ν_{C-H}), 2930 (ν_{C-H}), 2857 (ν_{C-H}). MS ([CI]$^+$, m/z, %): 583 ([M + H]$^+$, 4), 565 ([M − OH]$^+$, 20), 451 ([M − TBSO]$^+$, 3), 433 ([M − OH − TBSOH]$^+$, 34), 301 ([M − OH − 2TBSOH]$^+$, 100). HRMS: [CI]$^+$, calcd for $[C_{34}H_{71}O_3Si_2]^+$, 583.4942; found, 583.4916.

*12β-(7-Hydroxy-7-methyloctyl)-de-A,B-23,24-dinorcholan-8β,22-diol (**8a**).* A solution of TBAF in THF (8.25 mL, 1 M, 8.25 mmol) was added to a solution of **24** (320 mg, 0.548 mmol) in THF (10 mL). The reaction mixture was heated at 55 °C for 7 days. The reaction was quenched by addition of saturated NH_4Cl (30 mL). Aqueous layer was extracted with EtOAc (3 × 20 mL). The combined organic layer was dried, filtered, and concentrated. The residue was purified by flash chromatography (SiO_2, 2 cm × 15 cm, 40% EtOAc–hexanes) to afford **8a** [172 mg, 0.485 mmol, 89%, R_f = 0.30 (70% EtOAc–hexanes), white solid, mp 114–118 °C (Et_2O–hexanes), $[\alpha]_D$ = +30.6 (c = 1.7, $CHCl_3$)]. ^{1}H NMR ($CDCl_3$, 250 MHz): 4.01 (1H, broad d, J = 2.6, H-8), 3.76 (1H, dd, J_1 = 3.9, J_2 = 10.3, H-22), 3.36 (1H, dd, J_1 = 8.2, J_2 = 10.3, H-22), 1.19 (6H, s, Me_2C-OH), 1.03 (3H, d, J = 6.9, H-21), 0.86 (3H, s, H-18). ^{13}C NMR ($CDCl_3$, 63 MHz): 70.9 (C, C-OH), 68.7 (CH, C-8), 66.3 (CH_2, C-22), 56.1 (CH), 53.2 (CH), 49.3 (CH), 45.2 (C, C-13), 43.8 (CH_2), 34.9 (CH), 33.9 (CH_2), 31.2 (CH_2), 30.1 (CH_2), 29.9 (CH_2), 29.0 (2 × CH_3, Me_2C-OH), 28.1 (CH_2), 24.2 (CH_2), 23.4 (CH_2), 22.2 (CH_2), 21.1 (CH_2), 20.3 (CH_3), 10.8 (CH_3). IR (film, cm^{-1}): 3384 (ν_{O-H}), 2932 (ν_{C-H}), 2862 (ν_{C-H}). MS ([CI]$^+$, m/z, %): 337 ([M − OH]$^+$, 8), 319 ([M − OH − H_2O]$^+$, 77), 301 ([M − OH − 2H_2O]$^+$, 100). HRMS: [CI]$^+$, calcd for $[C_{22}H_{39}O]^+$, 319.3001; found, 319.3008.

*22-(tert-Butyldimethylsilyloxy)-12β-(7-hydroxy-7-methyloctyl)-de-A,B-23,24-dinorcholan-8β-ol (**27a**).* Imidazole (65 mg, 0.958 mmol) and TBSCl (116 mg, 0.766 mmol) were successively added to a solution of **8a** (170 mg, 0.479 mmol) in DMF (7 mL) at 0 °C. After 10 h at rt, the reaction was quenched by addition of saturated NaCl (35 mL). The aqueous layer was extracted with EtOAc (3 × 20 mL). The combined organic layer was dried, filtered, and concentrated. The residue was purified by flash chromatography (SiO_2, 2 cm × 8 cm, 30% EtOAc–hexanes) to afford **27a** [206 mg, 0.44 mmol, 92%, R_f = 0.60 (60% EtOAc–hexanes), colorless oil, $[\alpha]_D$ = +25.8 (c = 0.6, $CHCl_3$)]. ^{1}H NMR ($CDCl_3$, 250 MHz): 3.98 (1H, broad d, J = 1.2, H-8), 3.65 (1H, dd, J_1 = 4.3, J_2 = 9.6, H-22), 3.32 (1H, dd, J_1 = 7.7, J_2 = 9.6, H-22), 1.18 (6H, s, Me_2C-OH), 0.96 (3H, d, J = 6.8, H-21), 0.86 (9H, s, Me_3C-Si), 0.83 (3H, s, H-18), 0.05 (6H, s, Me_2Si). ^{13}C NMR ($CDCl_3$, 63 MHz): 70.9 (C, C-OH), 68.9 (CH, C-8), 66.5 (CH_2, C-22), 56.3 (CH), 53.3 (CH), 49.2 (CH), 45.2 (C, C-13), 44.0 (CH_2), 34.8 (CH), 34.0 (CH_2), 31.3 (CH_2), 30.2 (CH_2), 30.0 (CH_2), 29.1 (2 × CH_3, Me_2C-OH), 28.2 (CH_2), 25.9 (3 × CH_3, Me_3C-Si), 24.3 (CH_2), 23.5 (CH_2), 22.1 (CH_2), 21.1 (CH_2), 20.5 (CH_3), 18.2 (C, C-Si), 10.8 (CH_3), −5.4 (2 × CH_3, Me_2Si). IR (film, cm^{-1}): 3399 (ν_{O-H}), 2930 (ν_{C-H}), 2858 (ν_{C-H}). MS ([FAB]$^+$, m/z, %): 469 ([M + H]$^+$, 63), 451 ([M − OH]$^+$, 52). HRMS: [FAB]$^+$, calcd for $[C_{28}H_{57}O_3Si]^+$, 469.4077; found, 469.4086.

*22-(tert-Butyldimethylsilyloxy)-12β-(7-hydroxy-7-methyloctyl)-de-A,B-23,24-dinorcholan-8-one (**28a**).* Pyridinium dichromate (400 mg, 1.069 mmol) was added to a solution of **27a** (167 mg, 0.356 mmol) in CH_2Cl_2 (10 mL). The reaction mixture was protected from the light and stirred for 2 h at rt. The mixture was filtered through a layer of silica gel. The solids were washed with MTBE (3 × 40 mL), and the resulting filtrate was concentrated. The residue was purified by flash chromatography (SiO_2, 3 cm × 6 cm, 30% EtOAc–hexanes) to afford ketone **28a** [150 mg, 0.321 mmol, 89%, R_f = 0.48 (40% EtOAc–hexanes), colorless oil]. ^{1}H NMR ($CDCl_3$, 250 MHz): 3.60 (1H, dd, J_1 = 4.5, J_2 = 9.7, H-22), 3.32 (1H, dd, J_1 = 7.6, J_2 = 9.7, H-22), 1.16 (6H, s, Me_2C–OH), 0.97 (3H, d, J = 6.9, H-21), 0.84 (9H, s, Me_3C-Si), 0.56 (3H, s, H-18), −0.02 (6H, s, Me_2Si). ^{13}C NMR ($CDCl_3$, 63 MHz): 212.4 (C, C-8), 70.8 (C, C-OH), 66.0 (CH_2, C-22), 61.8 (CH), 56.0 (CH), 52.2 (C, C-13), 48.3 (CH), 43.8 (CH_2), 40.3 (CH_2), 35.2 (CH), 30.7 (CH_2), 30.1 (CH_2), 29.8 (CH_2), 29.6 (CH_2), 29.1 (2 × CH_3, Me_2C-OH), 28.2 (CH_2), 25.8 (3 × CH_3, Me_3C-Si), 24.2 (CH_2), 21.6 (CH_2), 20.1 (CH_3), 18.7 (CH_2), 18.2 (C, C-Si), 9.8 (CH_3), −5.4 (2 × CH_3, Me_2Si). IR ($CHCl_3$, cm^{-1}): 3459 (ν_{O-H}), 2956 (ν_{C-H}), 2930 (ν_{C-H}), 2884 (ν_{C-H}), 1717 ($\nu_{C=O}$). MS ([CI]$^+$, $m/$

z, %): 349 ([M − OH]$^+$, 20), 317 ([M − OH − TBSOH]$^+$, 100). HRMS: [CI]$^+$, calcd for [$C_{28}H_{53}O_2Si$]$^+$, 349.3815; found, 349.3821.

*(E)-8-(Bromomethylene)-22-(tert-butyldimethylsilyloxy)-12β-(7-hydroxy-7-methyloctyl)-de-A,B-24,23-dinorcholane (**29a**).* A suspension of (Ph_3PCH_2Br)Br (1.121 g, 2.57 mmol) in toluene (18 mL) was prepared by sonication for 30 min. After cooling at −17 °C, a solution of KO*t*-Bu in THF (2.5 mL, 1 M, 2.5 mmol) was added and the resulting mixture was stirred for 3 h. A solution of ketone **28a** (150 mg, 0.32 mmol) in toluene (12 mL) previously cooled to 0 °C was added via cannula. The mixture was stirred for 2 h at −17 °C and 3 h at rt. The reaction was quenched by addition of saturated NH_4Cl (1 mL), and the mixture was filtered through a layer of silica gel. The solids were washed with EtOAc (3 × 15 mL), and the filtrate was concentrated. The residue was purified by flash chromatography (SiO_2, 3 cm × 8 cm, 30% EtOAc−hexanes) to afford **29a** [121 mg, 0.22 mmol, 69%, R_f = 0.50 (20% EtOAc−hexanes), colorless oil]. ^{1}H NMR ($CDCl_3$, 250 MHz): 5.63 (1H, s, H-7), 3.67 (1H, dd, J_1 = 4.2, J_2 = 9.6, H-22), 3.44−3.30 (1H, m, H-22), 1.20 (6H, s, Me_2C-OH), 0.99 (3H, d, *J* = 6.9, H-21), 0.88 (9H, s, Me_3C-Si), 0.50 (3H, s, H-18), 0.02 (6H, s, Me_2Si). ^{13}C NMR ($CDCl_3$, 63 MHz): 145.3 (C, C-8), 97.6 (CH, C-7), 71.4 (C, C-OH), 66.5 (CH_2, C-22), 56.9 (CH), 55.6 (CH), 49.4 (CH), 49.1 (C, C-13), 44.4 (CH_2), 36.3 (CH), 31.6 (CH_2), 31.6 (CH_2), 30.6 (CH_2), 30.4 (CH_2), 29.6 (2 × CH_3, Me_2C-OH), 28.8 (CH_2), 28.6 (CH_2), 26.4 (3 × CH_3, Me_3C-Si), 24.8 (CH_2), 22.2 (CH_2), 22.0 (CH_2), 20.5 (CH_3), 18.7 (C, C-Si), 9.8 (CH_3), −4.9 (2 × CH_3, Me_2Si). IR (film, cm^{-1}): 3369 (ν_{O-H}), 3085 ($\nu_{=C-H}$), 2953 (ν_{C-H}), 2930 (ν_{C-H}), 2857 (ν_{C-H}), 1632 ($\nu_{C=C}$). MS ([CI]$^+$, *m*/*z*, %): 525 ([M − OH]$^+$, 3), 393 ([M − OH − TBSOH]$^+$, 31), 313 ([M − $C_6H_{18}BrO_2Si$]$^+$, 100). HRMS: [CI]$^+$, calcd for [$C_{29}H_{54}BrOSi$]$^+$, 525.3127; found, 525.3134.

*(E)-22-(tert-Butyldimethylsilyloxy)-12β-(7-hydroxy-7-methyloctyl)-8-[(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)methylene]-de-A,B-24,23-dinorcholane (**6a**).* PCy_3 (4 mg, 0.013 mmol) and $PdCl_2$(dppf)·CH_2Cl_2 (5 mg, 0.006 mmol) were dissolved in DMSO (2 mL), and the mixture was stirred for 25 min. A solution of **29a** (118 mg, 0.22 mmol) in DMSO (2 mL), KOAc (64 mg, 0.65 mmol), and Pin_2B_2 (110 mg, 0.43 mmol) were successively added. The mixture was heated to 80 °C for 3 h and then cooled to rt. The reaction was quenched by addition of H_2O (15 mL). The aqueous layer was extracted with EtOAc (4 × 20 mL). The combined organic layer was dried, filtered, and concentrated. The residue was purified by flash chromatography (SiO_2, 3 cm × 10 cm, 5−10% EtOAc−hexanes) to afford **6a** [74 mg, 0.15 mmol, 70%, R_f = 0.40 (20% EtOAc−hexanes), colorless oil, [α]$_D$ = +44.7 (*c* = 0.2, $CHCl_3$)]. ^{1}H NMR ($CDCl_3$, 250 MHz): 4.90 (1H, s, H-7), 3.69 (1H, dd, J_1 = 4.1, J_2 = 9.6, H-22), 3.35 (1H, dd, J_1 = 8.3, J_2 = 9.6, H-22), 1.25 (12H, s, 2 × Me_2C-OB), 1.20 (6H, s, Me_2C-OH), 0.99 (3H, d, *J* = 6.9, H-21), 0.88 (9H, s, Me_3C-Si), 0.50 (3H, s, H-18), 0.02 (6H, s, Me_2Si). ^{13}C NMR ($CDCl_3$, 63 MHz): 166.5 (C, C-8), 82.5 (2 × C, 2 × C-OB), 71.0 (C, C-OH), 66.2 (CH_2, C-22), 58.5 (CH), 56.2 (CH), 49.5 (CH), 49.3 (CH, C-13), 43.9 (CH_2), 36.0 (CH), 33.2 (CH_2), 31.3 (CH_2), 30.2 (2 × CH_2), 30.0 (CH_2), 29.2 (2 × CH_3, Me_2C-OH), 28.3 (CH_2), 26.0 (3 × CH_3, Me_3C-Si), 24.9 (2 × CH_3, Me_2C-OB), 24.8 (2 × CH_3, Me_2C-OB), 24.3 (CH_2), 22.1(CH_2), 21.4 (CH_2), 20.1 (CH_3), 18.3 (C, C-Si), 9.7 (CH_3), −5.3 (2 × CH_3, Me_2Si). IR (film, cm^{-1}): 3385 (ν_{O-H}), 2954 (ν_{C-H}), 2930 (ν_{C-H}), 2857 (ν_{C-H}), 1640 ($\nu_{C=C}$). MS ([CI]$^+$, *m*/*z*, %): 573 ([M − OH]$^+$, 10), 441 ([M − OH − TBSOH]$^+$, 100), 315 ([M − $C_{12}H_{28}BO_4Si$]$^+$, 20). HRMS: [CI]$^+$, calcd for [$C_{35}H_{66}BO_3Si$]$^+$, 573.4874; found, 573.4878.

*1α,22-Dihydroxy-12β-(7-hydroxy-7-methyloctyl)-23,24,25,26,27-pentanorvitamin D_3 (**5a**).* An aqueous solution of K_3PO_4 (1.65 mL, 2 M) and $PdCl_2(PPh_3)_2$ (4 mg, 0.006 mmol) were successively added to a solution of **6a** (70 mg, 0.118 mmol) and **7**[21] (83 mg, 0.138 mmol) in THF (4 mL). The reaction mixture protected from light was vigorously stirred for 1 h. Then H_2O (10 mL) was added, and the aqueous layer was extracted with Et_2O (3 × 15 mL). The combined organic layer was dried, filtered, and concentrated. The residue was dissolved in THF (7 mL) and a solution of TBAF in THF (835 μL, 1 M, 0.835 mmol) was added. After 6 h in the dark, the reaction was quenched by addition of saturated NH_4Cl (15 mL). 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, 60−95% EtOAc−hexanes) to give **5a** [50 mg, 0.102 mmol, 86%, R_f = 0.18 (90% EtOAc−hexanes), white solid, mp 85−88 °C (Et_2O−hexanes), [α]$_D$ = −25.6 (*c* = 0.9, EtOH 96%)]. Further purification by HPLC (Phenomenex-LUNA 5 μ Silica(2) column, 10 mm × 250 mm, 20% *i*-PrOH−hexanes) gave an analytically pure (>95%) sample which was used for biological assays. ^{1}H NMR (CD_3OD, 500 MHz): 6.31 (1H, d, *J* = 11.2, H-6), 6.09 (1H, d, *J* = 11.2, H-7), 5.29 (1H, s, H-19), 4.90 (1H, s, H-19), 4.36 (1H, t, *J* = 5.9, H-1), 4.16−4.10 (1H, m, H-3), 3.76 (1H, dd, J_1 = 3.4, J_2 = 10.4, H-22), 3.30−3.24 (1H, m, H-22), 2.87 (1H, dd, J_1 = 2.9, J_2 = 13.9, H-9), 2.52 (1H, dd, J_1 = 3.4, J_2 = 13.4, H-4), 2.26 (1H, dd, J_1 = 6.8, J_2 = 13.4, H-4), 2.13−2.04 (1H, m, H-20), 1.17 (6H, s, Me_2C-OH), 1.06 (3H, d, *J* = 6.8, H-21), 0.54 (3H, s, H-18). ^{13}C NMR (CD_3OD, 125 MHz): 149.8 (C, C-10), 142.1 (C, C-8), 135.7 (C, C-5), 124.9 (CH, C-6), 118.8 (CH, C-7), 112.0 (CH_2, C-19), 71.4 (C, C-OH), 71.4 (CH, C-1), 67.4 (CH, C-3), 66.4 (CH_2, C-22), 58.2 (CH), 57.1 (CH), 51.4 (CH), 50.3 (C, C-13), 46.1 (CH_2, C-4), 44.9 (CH_2), 43.7 (CH_2, C-2), 37.5 (CH), 32.5 (CH_2), 31.4 (CH_2), 31.1 (CH_2), 30.5 (CH_2), 30.0 (CH_2, C-9), 29.4 (CH_2), 29.2 (CH_3, MeC-OH), 29.1 (CH_3, MeC-OH), 25.4 (CH_2), 23.3 (CH_2), 22.4 (CH_2), 20.6 (CH_3, C-21), 10.1 (CH_3, C-18). IR (KBr, cm^{-1}): 3403 (ν_{O-H}), 2930 (ν_{C-H}), 2861 (ν_{C-H}), 1635 ($\nu_{C=C}$). MS ([ESI-TOF]$^+$, *m*/*z*, %): 511 ([M + Na]$^+$, 100), 453 ([M − OH − H_2O]$^+$, 28). HRMS: [ESI-TOF]$^+$, calcd for [$C_{31}H_{52}O_4Na$]$^+$, 511.3758; found, 511.3764. UV (96% EtOH): λ_{max} = 264 nm, λ_{min} = 230 nm.

*8β-Hydroxy-12β-(7-hydroxy-7-methyloctyl)-de-A,B-23,24-dinorcholan-22-yl p-toluenesulfonate (**25a**).* *p*-Toluenesulfonyl chloride (138 mg, 0.724 mmol) was added to a solution of **8a** (214 mg, 0.604 mmol) in pyridine (8 mL) at 0 °C. The mixture at 0 °C was stirred for 2 h and then was kept at 4 °C for 22 h. The reaction was quenched by addition of ice and saturated NaCl (10 mL). The aqueous layer was extracted with MTBE (3 × 20 mL), and the combined organic layer was washed with aqueous HCl (3 × 25 mL, 5%), saturated $NaHCO_3$ (3 × 25 mL), and saturated NaCl (2 × 20 mL), dried, filtered, and concentrated. The residue was purified by flash chromatography (SiO_2, 3 cm × 10 cm, 30−60% EtOAc−hexanes) to afford **25a** [258 mg, 0.507 mmol, 84%, R_f = 0.58 (70% EtOAc−hexanes), colorless oil, [α]$_D$ = +28.4 (*c* = 0.5, $CHCl_3$)]. ^{1}H NMR ($CDCl_3$, 250 MHz): 7.76 (2H, d, *J* = 8.1, H-Ar), 7.32 (2H, d, *J* = 8.1, H-Ar), 4.10 (1H, dd, J_1 = 3.7, J_2 = 9.1, H-22), 3.97 (1H, broad d, *J* = 2.0, H-8), 3.75 (1H, t, *J* = 9.1, H-22), 2.43 (3H, s, Me-Ar), 1.19 (6H, s, Me_2C-OH), 0.96 (3H, d, *J* = 6.8, H-21), 0.70 (3H, s, H-18). ^{13}C NMR ($CDCl_3$, 63 MHz): 144.6 (C, Ar-SO_2), 133.0 (C), 129.7 (2 × CH, Ar-H), 127.8 (2 × CH, Ar-H), 74.4 (CH_2, C-22), 71.0 (C, C-OH), 68.6 (CH, C-8), 55.3 (CH), 53.0 (CH), 49.2 (CH), 45.2 (C, C-13), 43.9 (CH_2), 33.9 (CH_2), 32.1 (CH), 31.2 (CH_2), 30.1 (CH_2), 29.9 (CH_2), 29.1 (2 × CH_3, Me_2C-OH), 28.1 (CH_2), 24.2 (CH_2), 23.3 (CH_2), 22.0 (CH_2), 21.6 (CH_3, Me-Ar), 21.1 (CH_2), 20.1 (CH_3), 10.7 (CH_3). IR (film, cm^{-1}): 3549 (ν_{O-H}), 3433 (ν_{O-H}), 2930 (ν_{C-H}), 2858 (ν_{C-H}), 1176 ($\nu_{O=S=O}$). MS ([ESI-TOF]$^+$, *m*/*z*, %): 531 ([M + Na]$^+$, 100), 491 ([M − OH]$^+$, 22). HRMS: [ESI-TOF]$^+$, calcd for [$C_{29}H_{48}O_5SNa$]$^+$, 531.3106; found, 531.3115.

*8β-Hydroxy-12β-(7-hydroxy-7-methyloctyl)-de-A,B-23,24-dinorcholane-22-carbonitrile (**26a**).* Potassium cyanide (128 mg, 1.97 mmol) was added to a solution of **25a** (500 mg, 0.983 mmol) in DMSO (15 mL). The mixture was stirred at 90 °C for 2 h and then cooled to rt. The reaction was quenched by addition of H_2O (50 mL), and the aqueous layer was extracted with EtOAc (4 × 40 mL). The combined organic layer was dried, filtered, and concentrated. The residue was purified by flash chromatography (SiO_2, 3 cm × 5 cm, 20−50% EtOAc−hexanes) to afford **26a** [328 mg, 0.902 mmol, 92%, R_f = 0.50 (70% EtOAc−hexanes), colorless oil, [α]$_D$ = +25.9 (*c* = 0.2, $CHCl_3$)]. ^{1}H NMR ($CDCl_3$, 250 MHz): 4.01 (1H, broad d, *J* = 2.1, H-8), 2.47 (1H, dd, J_1 = 3.3, J_2 = 16.1, H-22), 2.37−2.27 (1H, m, H-22), 1.20 (6H, s, Me_2C-OH), 1.15 (3H, d, *J* = 6.7, H-21), 0.84 (3H, s, H-18). ^{13}C NMR ($CDCl_3$, 63 MHz): 120.1 (C, C≡N), 71.0 (C, C-OH), 68.6 (CH, C-8), 55.8 (CH), 53.4 (CH), 49.2 (CH), 45.5 (C, C-13), 43.9 (CH_2), 34.0 (CH_2), 31.6 (CH_2), 30.4 (CH), 30.2 (CH_2), 30.0

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(CH_2), 29.2 (2 × CH_3, Me_2C-OH), 28.2 (CH_2), 24.3 (CH_2), 23.4 (CH_2), 22.6 (CH_3), 22.0 (CH_2), 22.0 (CH_2), 21.2 (CH_2), 11.0 (CH_3). IR (film, cm^{-1}): 3450 (ν_{O-H}), 2931 (ν_{C-H}), 2875 (ν_{C-H}), 2247 ($\nu_{C\equiv N}$). MS ($[CI]^+$, m/z, %): 346 ($[M - OH]^+$, 52), 328 ($[M - OH - H_2O]^+$, 100). HRMS: $[CI]^+$, calcd for $[C_{23}H_{40}NO]^+$, 346.3110; found, 346.3110.

*12β-(7-Hydroxy-7-methyloctyl)-de-A,B-24-norcholan-8β,23-diol (**8b**).* A solution of diisobutylaluminium hydride in CH_2Cl_2 (2.72 mL, 1 M, 2.72 mmol) was diluted in CH_2Cl_2 (3 mL) and cooled to −5 °C. A solution of **26a** (283 mg, 0.778 mmol) in CH_2Cl_2 (4 mL) was added. The mixture was vigorously stirred at −15 °C for 2 h and then allowed to warm to 0 °C for 1 h. A suspension of aqueous HCl (10 mL, 3 M) in Et_2O (10 mL) was added, and the mixture was stirred for 2 h at 5 °C. The aqueous layer was extracted with CH_2Cl_2 (3 × 30 mL). The combined organic layer was washed with saturated NaCl (3 × 20 mL), dried, filtered, and concentrated. The residue was dissolved in THF (10 mL). After cooling at −78 °C, a solution of diisobutylaluminium hydride in CH_2Cl_2 (2.72 mL, 1 M, 2.72 mmol) was added. After 2 h, the reaction was quenched by slowly addition of aqueous HCl (20 mL, 10%) at 0 °C. The aqueous layer was extracted with EtOAc (3 × 30 mL), and the combined organic layer was dried, filtered, and concentrated. The residue was purified by flash chromatography (SiO_2, 3 cm × 7.5 cm, 40−50% EtOAc−hexanes) to afford **8b** [171 mg, 0.464 mmol, 60%, R_f = 0.30 (70% EtOAc−hexanes), white foam, $[\alpha]_D$ = +24.9 (c = 0.7, $CHCl_3$)]. ^1H NMR ($CDCl_3$, 250 MHz): 3.98 (1H, broad s, H-8), 3.71−3.45 (2H, m, H-23), 1.16 (6H, s, Me_2C-OH), 0.89 (3H, d, J = 6.8, H-21), 0.83 (3H, s, H-18). ^{13}C NMR ($CDCl_3$, 63 MHz): 71.0 (C, C-OH), 68.9 (CH, C-8), 62.0 (CH_2, C-23), 57.2 (CH), 53.7 (CH), 49.8 (CH), 45.4 (C, C-13), 43.9 (CH_2), 36.7 (CH_2), 33.9 (CH_2), 31.3 (CH_2), 30.1 (CH_2), 29.9 (CH_2), 29.3 (CH), 29.1 (2 × CH_3, Me_2C-OH), 28.1 (CH_2), 24.2 (CH_2), 23.5 (CH_2), 22.5 (CH_2), 22.5 (CH_3), 21.0 (CH_2), 11.9 (CH_3). IR (film, cm^{-1}): 3365 (ν_{O-H}), 2930 (ν_{C-H}), 2863 (ν_{C-H}). MS ($[FAB]^+$, m/z, %): 351 ($[M - OH]^+$, 70), 433 ($[M - OH - H_2O]^+$, 100). HRMS: $[FAB]^+$, calcd for $[C_{23}H_{43}O_2]^+$, 351.3263; found, 351.3256.

*23-(tert-Butyldimethylsilyloxy)-12β-(7-hydroxy-7-methyloctyl)-de-A,B-24-norcholan-8β-ol (**27b**).* See **27a** for reaction procedure. Reagents: Imidazole (50 mg, 0.738 mmol), TBSCl (72 mg, 0.477 mmol), **8b** (160 mg, 0.434 mmol), and DMF (10 mL). Product: **27b** [160 mg, 0.33 mmol, 76%, R_f = 0.78 (70% EtOAc−hexanes), colorless oil, $[\alpha]_D$ = +21.5 (c = 0.2, $CHCl_3$)]. ^1H NMR ($CDCl_3$, 250 MHz): 3.98 (1H, broad s, H-8), 3.71−3.43 (2H, m, H-23), 1.15 (6H, s, Me_2C-OH), 0.92−0.74 (15H, m, H-18, H-21 and Me_3C-Si), 0.00 (6H, s, Me_2Si). ^{13}C NMR ($CDCl_3$, 63 MHz): 70.8 (C, C-OH), 69.0 (CH, C-8), 62.0 (CH_2, C-23), 57.1 (CH), 53.7 (CH), 50.1 (CH), 45.5 (C, C-13), 43.9 (CH_2), 36.7 (CH_2), 34.0 (CH_2), 31.1 (CH_2), 30.2 (CH_2), 29.9 (CH_2), 29.1 (2 × CH_3, Me_2C-OH), 28.8 (CH), 28.1 (CH_2), 25.9 (3 × CH_3, Me_3C-Si), 24.3 (CH_2), 23.3 (CH_2), 22.7 (CH_2), 22.3 (CH_3), 20.8 (CH_2), 18.2 (C-Si), 11.4 (CH_3), −5.4 (2 × CH_3, Me_2Si). IR ($CHCl_3$, cm^{-1}): 3402 (ν_{O-H}), 2929 (ν_{C-H}), 2858 (ν_{C-H}). MS ($[CI]^+$, m/z, %): 465 ($[M - OH]^+$, 19), 333 ($[M - TBSO - H_2O]^+$, 36), 315 ($[M - TBSO - 2H_2O]^+$, 100). HRMS: $[CI]^+$, calcd for $[C_{29}H_{57}O_2Si]^+$, 465.4128; found, 465.4124.

*23-(tert-Butyldimethylsilyloxy)-12β-(7-hydroxy-7-methyloctyl)-de-A,B-24-norcholan-8-one (**28b**).* See **28a** for reaction procedure. Reagents: pyridinium dichromate (362 mg, 0.963 mmol), **27b** (155 mg, 0.321 mmol), and CH_2Cl_2 (10 mL). Product: ketone **28b** [146 mg, 0.304 mmol, 95%, R_f = 0.46 (40% EtOAc−hexanes), colorless oil]. ^1H NMR ($CDCl_3$, 250 MHz): 3.67−3.43 (2H, m, H-23), 1.17 (6H, s, Me_2C-OH), 0.90 (3H, d, J = 6.9, H-21), 0.85 (9H, s, Me_3C-Si), 0.58 (3H, s, H-18), 0.00 (6H, s, Me_2Si). ^{13}C NMR ($CDCl_3$, 63 MHz): 212.4 (C, C-8), 70.7 (C, C−OH), 62.2 (CH), 61.6 (CH_2, C-23), 56.5 (CH), 52.5 (C, C-13), 49.1 (CH), 43.8 (CH_2), 40.3 (CH_2), 36.0 (CH_2), 30.6 (CH_2), 30.1 (CH_2), 29.8 (CH_2), 29.4 (CH_2), 29.1 (2 × CH_3, Me_2C-OH), 28.7 (CH), 28.1 (CH_2), 25.9 (3 × CH_3, Me_3C-Si), 24.2 (CH_2), 21.8 (CH_3), 21.1 (CH_2), 19.4 (CH_2), 18.1 (C, C-Si), 10.0 (CH_3), −5.5 (2 × CH_3, Me_2Si). IR ($CHCl_3$, cm^{-1}): 3467 (ν_{O-H}), 2958 (ν_{C-H}), 2930 (ν_{C-H}), 2858 (ν_{C-H}), 1718 ($\nu_{C=O}$). MS ($[CI]^+$, m/z, %): 463 ($[M - OH]^+$, 58), 405 ($[M - OH - t\text{-}Bu]^+$, 48), 331 ($[M - OH - TBSOH]^+$, 94). HRMS: $[CI]^+$, calcd for $[C_{29}H_{55}O_2Si]^+$, 463.3971; found, 463.3986.

*(E)-8-(Bromomethylene)-23-(tert-butyldimethylsilyloxy)-12β-(7-hydroxy-7-methyloctyl)-de-A,B-24-norcholane (**29b**).* See **29a** for reaction procedure. Reagents: $(Ph_3PCH_2Br)Br$ (689 mg, 1.58 mmol) in toluene (18 mL), KOt-Bu in THF (1.56 mL, 1 M, 1.56 mmol), and ketone **28b** (95 mg, 0.19 mmol) in toluene (5 mL). Product: **29b** [74 mg, 0.13 mmol, 67%, R_f = 0.43 (20% EtOAc−hexanes), colorless oil]. ^1H NMR ($CDCl_3$, 250 MHz): 5.62 (1H, s, H-7), 3.76−3.49 (2H, m, H-23), 1.20 (6H, s, Me_2C-OH), 0.91 (3H, d, J = 6.9, H-21), 0.88 (9H, s, Me_3C-Si), 0.51 (3H, s, H-18), 0.03 (6H, s, Me_2Si). ^{13}C NMR ($CDCl_3$, 63 MHz): 144.9 (C, C-8), 96.9 (CH, C-7), 71.0 (C, C-OH), 62.1 (CH_2, C-23), 56.8 (CH), 55.8 (CH), 49.8 (CH), 48.9 (C, C-13), 44.0 (CH_2), 36.2 (CH_2), 31.1 (2 × CH_2), 30.2 (CH_2), 30.0 (CH_2), 29.6 (CH), 29.2 (2 × CH_3, Me_2C-OH), 28.2 (CH_2), 28.1 (CH_2), 26.0 (3 × CH_3, Me_3C-Si), 24.3 (CH_2), 22.5 (CH_2), 22.0 (CH_3), 21.4 (CH_2), 18.3 (C, C-Si), 9.7 (CH_3), −5.3 (2 × CH_3, Me_2Si). IR (film, cm^{-1}): 3369 (ν_{O-H}), 3084 ($\nu_{=C-H}$), 2954 (ν_{C-H}), 2930 (ν_{C-H}), 2858 (ν_{C-H}), 1632 ($\nu_{C=C}$). MS ($[CI]^+$, m/z, %): 539 ($[M- OH]^+$, 14), 407 ($[M - OH - TBSOH]^+$, 27), 327 ($[M - C_6H_{18}BrO_2Si]^+$, 100). HRMS: $[CI]^+$, calcd for $[C_{30}H_{56}BrOSi]^+$, 539.3284; found, 539.3275.

*(E)-23-(tert-Butyldimethylsilyloxy)-12β-(7-hydroxy-7-methyloctyl)-8-[(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)methylene]-de-A,B-24-norcholane (**6b**).* See **6a** for reaction procedure. Reagents: PCy_3 (3 mg, 0.011 mmol) and $PdCl_2(dppf)\cdot CH_2Cl_2$ (5 mg, 0.005 mmol) in DMSO (2 mL), **29b** (103 mg, 0.18 mmol) in DMSO (2 mL), KOAc (54 mg, 0.55 mmol), and Pin_2B_2 (91 mg, 0.36 mmol). Product: **6b** [79 mg, 0.13 mmol, 71%, R_f = 0.30 (10% EtOAc−hexanes), colorless oil, $[\alpha]_D$ = +46.1 (c = 0.2, $CHCl_3$)]. ^1H NMR ($CDCl_3$, 250 MHz): 4.88 (1H, s, H-7), 3.74−3.41 (2H, m, H-23), 1.24 (12H, s, 2 × Me_2C-OB), 1.19 (6H, s, Me_2C-OH), 0.90 (3H, d, J = 6.9, H-21), 0.87 (9H, s, Me_3C-Si), 0.49 (3H, s, H-18), 0.02 (6H, s, Me_2Si). ^{13}C NMR ($CDCl_3$, 63 MHz): 166.0 (C, C-8), 82.4 (2 × C, 2 × C-OB), 71.0 (C, C-OH), 62.2 (CH_2, C-23), 58.9 (CH), 56.8 (CH), 50.2 (CH), 49.5 (C, C-13), 43.9 (CH_2), 36.2 (CH_2), 33.2 (CH_2), 31.2 (CH_2), 30.2 (CH_2), 30.0 (2 × CH_2), 29.6 (CH), 29.2 (2 × CH_3, Me_2C-OH), 28.2 (CH_2), 26.0 (3 × CH_3, Me_3C-Si), 24.9 (2 × CH_3, Me_2C-OB), 24.7 (2 × CH_3, Me_2C-OB), 24.3 (CH_2), 22.7 (CH_2), 22.0 (CH_3), 21.2 (CH_2), 18.3 (C, C−Si), 10.0 (CH_3), −5.3 (2 × CH_3, Me_2Si). IR (film, cm^{-1}): 3390 (ν_{O-H}), 2955 (ν_{C-H}), 2930 (ν_{C-H}), 2858 (ν_{C-H}), 1640 ($\nu_{C=C}$). MS ($[CI]^+$, m/z, %): 587 ($[M - OH]^+$, 32), 455 ($[M - OH - TBSOH]^+$, 100), 329 ($[M - C_{12}H_{28}BO_4Si]^+$, 25). HRMS: $[CI]^+$, calcd for $[C_{36}H_{68}BO_3Si]^+$, 587.5028; found, 587.5031.

*1α,23-Dihydroxy-12β-(7-hydroxy-7-methyloctyl)-24,25,26,27-tetranorvitamin D_3 (**5b**).* See **5a** for reaction procedure. Reagents: aqueous solution of K_3PO_4 (1.6 mL, 2 M), $PdCl_2(PPh_3)_2$ (4 mg, 0.006 mmol), **6b** (68 mg, 0.112 mmol), 7 (79 mg, 0.132 mmol), and THF (4 mL). Then THF (7 mL) and TBAF in THF (840 μL, 1 M, 0.840 mmol). Product: **5b** [45 mg, 0.089 mmol, 80%, R_f = 0.20 (90% EtOAc−hexanes), white solid, mp 87−91 °C (Et_2O−hexanes), $[\alpha]_D$ = −30.0 (c = 1.2, EtOH 96%)]. ^1H NMR ($CDCl_3$, 400 MHz): 6.34 (1H, d, J = 11.2, H-6), 6.00 (1H, d, J = 11.2, H-7), 5.31 (1H, s, H-19), 4.98 (1H, s, H-19), 4.41 (1H, dd, J_1 = 4.3, J_2 = 7.7, H-1), 4.26−4.15 (1H, m, H-3), 3.69 (1H, ddd, J_1 = 4.8, J_2 = 8.8, J_3 = 10.2, H-23), 3.58 (1H, dt, J_1 = 7.63, J_2 = 10.2, H-23), 2.80 (1H, dd, J_1 = 3.9, J_2 = 13.4, H-9), 2.52 (1H, dd, J_1 = 3.2, J_2 = 13.3, H-4), 2.30 (1H, dd, J_1 = 6.8, J_2 = 13.3, H-4), 1.20 (6H, s, Me_2C-OH), 0.94 (3H, d, J = 6.8, H-21), 0.49 (3H, s, H-18). ^{13}C NMR ($CDCl_3$, 100 MHz): 147.6 (C, C-10), 142.6 (C, C-8), 133.0 (C, C-5), 124.9 (CH, C-6), 116.9 (CH, C-7), 111.7 (CH_2, C-19), 71.1 (C, C-OH), 70.7 (CH, C-1), 66.7 (CH, C-3), 62.2 (CH_2, C-23), 57.3 (CH), 56.7 (CH), 50.1 (CH), 49.3 (C, C-13), 45.2 (CH_2, C-4), 43.9 (CH_2), 42.8 (CH_2, C-2), 36.1 (CH_2), 31.4 (CH_2), 30.2 (CH_2), 30.1 (CH), 29.9 (CH_2), 29.3 (CH_2), 29.2 (2 × CH_3, Me_2C-OH), 29.0 (CH_2, C-9), 28.2 (CH_2), 24.3 (CH_2), 22.6 (CH_2), 22.1 (CH_3, C-21), 21.5 (CH_2), 10.0 (CH_3, C-18). IR (KBr, cm^{-1}): 3396 (ν_{O-H}), 2930 (ν_{C-H}), 2871 (ν_{C-H}), 1649 ($\nu_{C=C}$). MS ([ESI-TOF]$^+$, m/z, %): 525 ($[M + Na]^+$, 100), 467 ($[M - OH - H_2O]^+$, 58). HRMS: [ESI-TOF]$^+$, calcd for $[C_{32}H_{54}O_4Na]^+$, 525.3914; found, 525.3913. UV (96% EtOH): λ_{max} = 264 nm, λ_{min} = 230 nm.

*8β-Hydroxy-12β-(7-hydroxy-7-methyloctyl)-de-A,B-24-norcholan-23-yl p-toluenesulfonate (**25b**).* See **25a** for reaction procedure. Reagents: *p*-toluenesulfonyl chloride (168 mg, 0.879 mmol), **8b** (216 mg, 0.586 mmol), and pyridine (15 mL). Product: **25b** [255 mg, 0.488 mmol, 83%, R_f = 0.60 (70% EtOAc−hexanes), colorless oil]. ^{1}H NMR ($CDCl_3$, 250 MHz): 7.74 (2H, d, *J* = 8.2, H-Ar), 7.30 (2H, d, *J* = 8.2, H-Ar), 4.18−3.87 (3H, m, H-8 and H-23), 2.40 (3H, s, Me-Ar), 1.16 (6H, s, Me_2C-OH), 0.80 (3H, d, *J* = 6.8, H-21), 0.74 (3H, s, H-18). ^{13}C NMR ($CDCl_3$, 63 MHz): 144.5 (C, Ar-SO_2), 133.0 (C), 129.7 (2 × CH, Ar-H), 127.7 (2 × CH, Ar-H), 70.8 (C, C-OH), 69.8 (CH_2, C-23), 68.7 (CH, C-8), 56.8 (CH), 53.5 (CH), 49.6 (CH), 45.4 (C, C-13), 43.8 (CH_2), 33.9 (CH_2), 32.5 (CH_2), 31.2 (CH_2), 30.1 (CH_2), 29.8 (CH_2), 29.0 (2 × CH_3, Me_2C-OH), 28.9 (CH), 28.1 (CH_2), 24.2 (CH_2), 23.8 (CH_2), 22.3 (CH_2), 21.8 (CH_3, Me-Ar), 21.5 (CH_3), 20.8 (CH_2), 11.2 (CH_3). IR (film, cm^{-1}): 3545 (ν_{O-H}), 3425 (ν_{O-H}), 2930 (ν_{C-H}), 2860 (ν_{C-H}), 1176 ($\nu_{O=S=O}$). MS ([FAB]$^+$, *m*/*z*, %): 545 ([M + Na]$^+$, 4), 487 ([M − OH − H_2O]$^+$, 25), 315 ([M − TsO − 2H_2O]$^+$, 31). HRMS: [FAB]$^+$, calcd for [$C_{30}H_{50}O_5SNa$]$^+$, 545.3277; found, 545.3271.

*8β-Hydroxy-12β-(7-hydroxy-7-methyloctyl)-de-A,B-24-norcholane-23-carbonitrile (**26b**).* See **26a** for reaction procedure. Reagents: potassium cyanide (159 mg, 2.44 mmol), **25b** (255 mg, 0.488 mmol), and DMSO (10 mL). Product: **26b** [167 mg, 0.442 mmol, 91%, R_f = 0.50 (70% EtOAc−hexanes), colorless oil]. ^{1}H NMR ($CDCl_3$, 250 MHz): 3.96 (1H, broad s, H-8), 2.39−2.16 (2H, m, H-23), 1.14 (6H, s, Me_2C−OH), 0.90 (3H, d, *J* = 6.8, H-21), 0.82 (3H, s, H-18). ^{13}C NMR ($CDCl_3$, 63 MHz): 119.9 (C, C≡N), 70.9 (C, C-OH), 68.7 (CH, C-8), 57.0 (CH), 53.6 (CH), 49.7 (CH), 45.6 (C, C-13), 43.9 (CH_2), 34.0 (CH_2), 32.3 (CH), 31.4 (CH_2), 30.2 (CH_2), 29.9 (CH_2), 29.2 (CH_2), 29.1 (2 × CH_3, Me_2C-OH), 28.2 (CH_2), 24.3 (CH_2), 23.5 (CH_2), 22.4 (CH_2), 21.4 (CH_3), 20.8 (CH_2), 15.9 (CH_2), 11.4 (CH_3). IR (film, cm^{-1}): 3445 (ν_{O-H}), 2931 (ν_{C-H}), 2873 (ν_{C-H}), 2247 ($\nu_{C\equiv N}$). MS ([CI]$^+$, *m*/*z*, %): 377 ([M]$^+$, 7), 360 ([M − OH]$^+$, 48), 342 ([M − OH − H_2O]$^+$, 100). HRMS: [CI]$^+$, calcd for [$C_{24}H_{42}NO$]$^+$, 360.3266; found, 360.3269.

*12β-(7-Hydroxy-7-methyloctyl)-de-A,B-cholan-8β,24-diol (**8c**).* See **8b** for reaction procedure. Reagents: diisobutylaluminium hydride in CH_2Cl_2 (2.01 mL, 1 M, 2.01 mmol) and CH_2Cl_2 (5 mL), **26b** (152 mg, 0.402 mmol) in CH_2Cl_2 (5 mL) and aqueous HCl (10 mL, 3 M) in Et_2O (10 mL), then THF (6 mL) and diisobutylaluminium hydride in CH_2Cl_2 (2.01 mL, 1 M, 2.01 mmol). Product: **8c** [95 mg, 0.248 mmol, 61%, R_f = 0.38 (70% EtOAc−hexanes), white foam, $[\alpha]_D$ = +38.4 (*c* = 0.2, $CHCl_3$)]. ^{1}H NMR ($CDCl_3$, 250 MHz): 4.01 (1H, broad d, *J* = 1.4, H-8), 3.71−3.51 (2H, m, H-24), 1.19 (6H, s, Me_2C-OH), 0.91 (3H, d, *J* = 6.8, H-21), 0.83 (3H, s, H-18). ^{13}C NMR ($CDCl_3$, 63 MHz): 71.0 (C, C-OH), 69.0 (CH, C-8), 63.1 (CH_2, C-24), 57.5 (CH), 53.6 (CH), 49.8 (CH), 45.4 (C, C-13), 43.9 (CH_2), 33.9 (CH_2), 32.8 (CH), 31.7 (CH_2), 31.3 (CH_2), 30.1 (CH_2), 29.9 (CH_2), 29.6 (CH_2), 29.1 (2 × CH_3, Me_2C-OH), 28.1 (CH_2), 24.3 (CH_2), 23.5 (CH_2), 22.5 (CH_2), 22.3 (CH_3), 20.8 (CH_2), 11.3 (CH_3). IR (film, cm^{-1}): 3335 (ν_{O-H}), 2932 (ν_{C-H}), 2864 (ν_{C-H}). MS ([FAB]$^+$, *m*/*z*, %): 365 ([M − OH]$^+$, 53), 342 ([M − OH − H_2O]$^+$, 100). HRMS: [FAB]$^+$, calcd for [$C_{24}H_{45}O_2$]$^+$, 365.3420; found, 365.3422.

*24-(tert-Butyldimethylsilyloxy)-12β-(7-hydroxy-7-methyloctyl)-de-A,B-cholan-8β-ol (**27c**).* See **27a** for reaction procedure. Reagents: imidazole (30 mg, 0.441 mmol), TBSCl (30 mg, 0.199 mmol), **8c** (35 mg, 0.091 mmol), and DMF (10 mL). Product: **27c** [35 mg, 0.07 mmol, 78%, R_f = 0.78 (70% EtOAc−hexanes), colorless oil]. ^{1}H NMR ($CDCl_3$, 250 MHz): 4.01 (1H, broad d, *J* = 1.2, H-8), 3.64−3.52 (2H, m, H-24), 1.20 (6H, s, Me_2C-OH), 0.93−0.82 (15H, m, H-18, H-21 and Me_3C-Si), 0.04 (6H, s, Me_2Si). ^{13}C NMR ($CDCl_3$, 63 MHz): 71.0 (C, C-OH), 69.1 (CH, C-8), 63.5 (CH_2, C-24), 57.6 (CH), 53.8 (CH), 49.9 (CH), 45.4 (C, C-13), 44.0 (CH_2), 34.0 (CH_2), 32.8 (CH), 32.0 (CH_2), 31.3 (CH_2), 30.2 (CH_2), 30.0 (CH_2), 29.6 (CH_2), 29.1 (2 × CH_3, Me_2C-OH), 28.2 (CH_2), 25.9 (3 × CH_3, Me_3C-Si), 24.3 (CH_2), 23.5 (CH_2), 22.6 (CH_2), 22.3 (CH_3), 20.8 (CH_2), 18.3 (C, C-Si), 11.3 (CH_3), −5.3 (2 × CH_3, Me_2Si). IR (film, cm^{-1}): 3400 (ν_{O-H}), 2930 (ν_{C-H}), 2858 (ν_{C-H}). MS ([CI]$^+$, *m*/*z*, %): 479 ([M − OH]$^+$, 53), 461 ([M − OH − H_2O]$^+$, 18), 329 ([M − 2H_2O − TBSO], 100). HRMS: [CI]$^+$, calcd for [$C_{30}H_{59}O_2Si$]$^+$, 479.4284; found, 479.4291.

*24-(tert-Butyldimethylsilyloxy)-12β-(7-hydroxy-7-methyloctyl)-de-A,B-cholan-8-one (**28c**).* See **28a** for reaction procedure. Reagents: pyridinium dichromate (198 mg, 0.525 mmol), **27c** (87 mg, 0.175 mmol), and CH_2Cl_2 (8 mL). Product: ketone **28c** [80 mg, 0.162 mmol, 93%, R_f = 0.62 (60% EtOAc−hexanes), colorless oil]. ^{1}H NMR ($CDCl_3$, 250 MHz): 3.61−3.48 (2H, m, H-24), 1.17 (6H, s, Me_2C-OH), 0.91 (3H, d, *J* = 6.8, H-21), 0.84 (9H, s, Me_3C-Si), 0.58 (3H, s, H-18), 0.00 (6H, s, Me_2Si). ^{13}C NMR ($CDCl_3$, 63 MHz): 212.5 (C, C-8), 70.8 (C, C-OH), 63.3 (CH_2, C-24), 62.2 (CH), 57.1 (CH), 52.4 (C, C-13), 48.9 (CH), 43.9 (CH_2), 40.4 (CH_2), 33.0 (CH), 31.8 (CH_2), 30.7 (CH_2), 30.1 (CH_2), 29.8 (CH_2), 29.6 (CH_2), 29.1 (2 × CH_3, Me_2C-OH), 29.0 (CH_2), 28.2 (CH_2), 25.8 (3 × CH_3, Me_3C-Si), 24.2 (CH_2), 21.9 (CH_3), 21.1 (CH_2), 19.3 (CH_2), 18.2 (C, C-Si), 10.1 (CH_3), −5.3 (2 × CH_3, Me_2Si). IR (film, cm^{-1}): 3460 (ν_{O-H}), 2955 (ν_{C-H}), 2930 (ν_{C-H}), 2858 (ν_{C-H}), 1717 ($\nu_{C=O}$). MS ([CI]$^+$, *m*/*z*, %): 477 ([M − OH]$^+$, 25), 419 ([M − OH − *t*-Bu]$^+$, 33), 327 ([M − 2H_2O − TBSO]$^+$, 100). HRMS: [CI]$^+$, calcd for [$C_{30}H_{57}O_2Si$]$^+$: 477.4128; found: 477.4131.

*(E)-8-(Bromomethylene)-24-(tert-butyldimethylsilyloxy)-12β-(7-hydroxy-7-methyloctyl)-de-A,B-cholane (**29c**).* See **29a** for reaction procedure. Reagents: (Ph_3PCH_2Br)Br (782 mg, 1.79 mmol) in toluene (12 mL), KO*t*-Bu in THF (1.76 mL, 1 M, 1.76 mmol), ketone **28c** (111 mg, 0.22 mmol) in toluene (6 mL). Product: **29c** [90 mg, 0.16 mmol, 70%, R_f = 0.52 (20% EtOAc−hexanes), colorless oil]. ^{1}H NMR ($CDCl_3$, 250 MHz): 5.61 (1H, s, H-7), 3.61−3.48 (2H, m, H-24), 1.20 (6H, s, Me_2C-OH), 0.92 (3H, d, *J* = 6.9, H-21), 0.88 (9H, s, Me_3C-Si), 0.49 (3H, s, H-18), 0.03 (6H, s, Me_2Si). ^{13}C NMR ($CDCl_3$, 63 MHz): 144.9 (C, C-8), 97.0 (CH, C-7), 71.0 (C, C-OH), 63.5 (CH_2, C-24), 56.7 (CH), 56.3 (CH), 49.5 (CH), 48.7 (C, C-13), 44.0 (CH_2), 33.6 (CH), 32.0 (CH_2), 31.2 (CH_2), 31.1 (CH_2), 30.2 (CH_2), 30.0 (CH_2), 29.2 (2 × CH_3, Me_2C-OH), 29.0 (CH_2), 28.3 (CH_2), 28.2 (CH_2), 25.9 (3 × CH_3, Me_3C-Si), 24.3 (CH_2), 22.3 (CH_2), 22.0 (CH_3), 21.2 (CH_2), 18.3 (C, C−Si), 9.6 (CH_3), −5.3 (2 × CH_3, Me_2Si). IR (film, cm^{-1}): 3369 (ν_{O-H}), 3084 ($\nu_{=C-H}$), 2953 (ν_{C-H}), 2930 (ν_{C-H}), 2858 (ν_{C-H}), 1632 ($\nu_{C=C}$). MS ([CI]$^+$, *m*/*z*, %): 553 ([M − OH]$^+$, 36), 421 ([M − OH − TBSOH]$^+$, 42), 341 ([M − $C_6H_{18}BrO_2Si$]$^+$, 100). HRMS: [CI]$^+$, calcd for [$C_{31}H_{58}BrOSi$]$^+$, 553.3440; found, 553.3444.

*(E)-24-(tert-Butyldimethylsilyloxy)-12β-(7-hydroxy-7-methyloctyl)-8-[(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)methylene]-de-A,B-cholane (**6c**).* See **6a** for reaction procedure. Reagents: PCy_3 (2 mg, 0.008 mmol) and $PdCl_2$(dppf)·CH_2Cl_2 (4 mg, 0.004 mmol) in DMSO (2 mL), **29c** (82 mg, 0.14 mmol) in DMSO (2 mL), KOAc (41 mg, 0.42 mmol), and Pin_2B_2 (71 mg, 0.28 mmol). Product: **6c** [61 mg, 0.10 mmol, 69%, R_f = 0.28 (15% EtOAc−hexanes), colorless oil]. ^{1}H NMR ($CDCl_3$, 250 MHz): 4.89 (1H, s, H-7), 3.62−3.52 (2H, m, H-24), 1.25 (12H, s, 2 × Me_2C-BO), 1.20 (6H, s, Me_2C-OH), 0.94−0.85 (12H, m, H-21 and Me_3C-Si), 0.47 (3H, s, H-18), 0.03 (6H, s, Me_2Si). ^{13}C NMR ($CDCl_3$, 63 MHz): 166.1 (C, C-8), 82.5 (2 × C, 2 × C-OB), 71.0 (C, C-OH), 63.6 (CH_2, C-24), 57.4 (CH), 56.3 (CH), 50.0 (CH), 49.4 (C, C-13), 44.0 (CH_2), 33.7 (CH), 33.2 (CH_2), 32.0 (CH_2), 31.3 (CH_2), 30.2 (2 × CH_2), 30.0 (CH_2), 29.2 (2 × CH_3, Me_2C-OH), 29.0 (CH_2), 28.2 (CH_2), 25.9 (3 × CH_3, Me_3C-Si), 24.9 (2 × CH_3, Me_2C-OB), 24.8 (2 × CH_3, Me_2C-OB), 24.3 (CH_2), 22.5 (CH_2), 22.0 (CH_3), 21.0 (CH_2), 18.3 (C, C-Si), 9.9 (CH_3), −5.3 (2 × CH_3, Me_2Si). IR (film, cm^{-1}): 3369 (ν_{O-H}), 2953 (ν_{C-H}), 2930 (ν_{C-H}), 2858 (ν_{C-H}), 1640 ($\nu_{C=C}$). MS ([CI]$^+$, *m*/*z*, %): 601 ([M − OH]$^+$, 71), 469 ([M − OH− TBSOH]$^+$, 100), 343 ([M − $C_{12}H_{28}BO_4Si$]$^+$, 29). HRMS: [CI]$^+$, calcd for [$C_{37}H_{70}BO_3Si$]$^+$, 601.5187; found, 601.5197.

*1α,24-Dihydroxy-12β-(7-hydroxy-7-methyloctyl)-25,26,27-trinorvitamin D_3 (**5c**).* See **5a** for reaction procedure. Reagents: aqueous solution of K_3PO_4 (1.2 mL, 2 M), $PdCl_2(PPh_3)_2$ (3 mg, 0.004 mmol), **6c** (54 mg, 0.087 mmol), **7** (61 mg, 0.102 mmol), and THF (3 mL). Then THF (6 mL) and TBAF in THF (560 μL, 1 M, 0.560 mmol). Product: **5c** [37 mg, 0.072 mmol, 83%, R_f = 0.18 (90% EtOAc−hexanes), white solid, mp 88−92 °C (Et_2O−hexanes), $[\alpha]_D$ = −20.4 (*c* = 0.5, EtOH 96%)]. ^{1}H NMR ($CDCl_3$, 400 MHz): 6.34 (1H, d, *J* =

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11.2, H-6), 6.01 (1H, d, J = 11.2, H-7), 5.32 (1H, s, H-19), 4.98 (1H, s, H-19), 4.42 (1H, dd, J_1 = 4.2, J_2 = 7.7, H-1), 4.24−4.18 (1H, m, H-3), 3.61 (2H, td, J_1 = 2.2, J_2 = 6.5, H-24), 2.80 (1H, dd, J_1 = 3.8, J_2 = 13.3, H-9), 2.58 (1H, dd, J_1 = 2.9, J_2 = 13.2, H-4), 2.30 (1H, dd, J_1 = 6.5, J_2 = 13.2, H-4), 1.21 (6H, s, Me_2C-OH), 0.94 (3H, d, J = 6.8, H-21), 0.48 (3H, s, H-18). ^{13}C NMR ($CDCl_3$, 100 MHz): 147.6 (C, C-10), 142.7 (C, C-8), 133.0 (C, C-5), 124.9 (CH, C-6), 116.9 (CH, C-7), 111.7 (CH_2, C-19), 71.1 (C, C-OH), 70.7 (CH, C-1), 66.8 (CH, C-3), 63.3 (CH_2, C-24), 57.2 (CH), 57.0 (CH), 50.1 (CH), 49.3 (C, C-13), 45.2 (CH_2, C-4), 44.0 (CH_2), 42.8 (CH_2, C-2), 33.8 (CH), 31.8 (CH_2), 31.4 (CH_2), 30.2 (CH_2), 30.0 (CH_2), 29.7 (CH_2), 29.4 (CH_2), 29.2 (2 × CH_3, Me_2C-OH), 29.0 (CH_2, C-9), 28.2 (CH_2), 24.3 (CH_2), 22.5 (CH_2), 22.0 (CH_3, C-21), 21.3 (CH_2), 9.9 (CH_3, C-18). IR (KBr, cm^{-1}): 3388 (ν_{O-H}), 2930 (ν_{C-H}), 2861 (ν_{C-H}), 1632 ($\nu_{C=C}$). MS ([ESI-TOF]$^+$, m/z, %): 539 ([M + Na]$^+$, 100), 481 ([M − OH − H_2O]$^+$, 24). HRMS: [ESI-TOF]$^+$, calcd for [$C_{33}H_{56}O_4Na$]$^+$, 539.4071; found, 539.4069. UV (96% EtOH): λ_{max} = 264 nm, λ_{min} = 230 nm.

Functional Studies. *Cell Culture.* Human MCF-7 breast adenocarcinoma and SW480-ADH colon cancer cells were grown in DMEM and RPMI media, respectively, supplemented with 10% FBS, 100 U/mL penicillin, 100 U/mL streptomycin, and 2 mM L-glutamine (all from Invitrogen, Paisley, UK), in air-CO_2 (95:5) atmosphere a 37 °C. Confluent cells were washed twice with phosphate-buffered saline (PBS) and harvested by a brief incubation with trypsin−EDTA solution (Sigma Aldrich St. Louis, USA) in PBS. Treatments with 1α,25-$(OH)_2D_3$ (**1**), or compounds **5a−c** were carried out using medium supplemented with charcoal-treated FCS to remove liposoluble hormones. Control cells were treated with the corresponding vehicle.

MTT Metabolization. Cell proliferation experiments were carried out using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assays, where MTT is reduced to purple formazan by the mitochondria of living cells. Increase in cell number is detected by augmented MTT metabolization. MCF-7 cells were plated at a 5 × 10^4 cells per well in 24-well plates. Twenty-four hours klater, the cells were treated with compounds **5a−c** at concentrations of 10, 100, or 500 nM during 48 h. MTT (0.5 μg/μL) was added to each well, and the mixture was incubated for 1 h. The medium was then removed, and DMSO (500 μL) was added to each well. Absorbance of samples was measured at 570 nm in a Mithras LB 940 from Berthold Technologies (Bad Wildbad, Germany).

Luciferase Reporter Assays. Cells were cultured as described above. Then 12−24 h before transfection, 2 × 10^5 cells per well were seeded in 24-well plates and allowed to attach overnight. Cells were then transfected using JetPEI transfection reagent (PolyPlus Transfection, Illrich, France) following the manufacturer's guidelines. Transfections were performed in triplicate using 1 μg of pCYP24A1-Luc plasmid (kindly provided by Dr. Aranda, Instituto de Investigaciones Biomédicas Alberto Sols, Madrid). This vector encoding the luciferase gene under control of a consensus vitamin D response element (24-hydroxylase promoter, CYP24A1), and it is very responsive to the 1α,25-$(OH)_2D_3$ treatment. After incubation for 24 h in DMEM supplemented with 10% of charcoal-stripped FCS, culture medium was replaced to phenol red free DMEM containing 10% FCS for 24 h with each compound (**1**, **5a−c**) at several concentrations (1 × 10^{-11} to 1 × 10^{-6} M). Cells were then treated during 10 min with luciferin potassiumsalt (100 mg/L) (Regis Technologies, Morton Grove, IL), and bioluminescence images acquired with the In Vivo Imaging System (IVIS, Caliper Life Sciences, Alameda, CA, USA), quantified as total photon counts, and processed by Living Image software (Caliper Life Sciences). The EC_{50} values are derived from dose−response curves and represent the analogue concentration capable of increasing the luciferase activity by 50%. The luciferase activity ratio is the average ratio of the EC_{50} for the analogue to the EC_{50} for 1α,25-$(OH)_2D_3$. All experiments were carried out in duplicate on at least two different occasions.

Human VDR Binding Assay. Binding affinity to VDR was evaluated using a 1α,25-$(OH)_2D_3$ assay kit under manufacturer conditions (Polarscreen Vitamin D receptor competitor assay, Red, catalogue no. PV4569, Invitrogen). This kit is a fluorescence polarization (FP)-based competition assay that provides a sensitive and robust method for high-throughput screening of potential vitamin D receptor ligands. VDR is added to a fluorescent VDR ligand to form a receptor/tracer complex resulting in a high polarization value. This complex is then added to individual test compounds in microwell plates. Competitors will displace the tracer from the complex, causing the fluorescent ligand to tumble more rapidly during its fluorescence lifetime and resulting in a low polarization value. The polarized fluorescence was measured in a 384-well black plate during 200 ms/well using a Mithras LB 940 (Berthold Technologies). All compounds (**1**, **5a−c**) were evaluated within the range from 10^{-11} M to 10^{-5} M. IC_{50} values were calculated using average of measured values. The activity of each compound is also shown as percentage, in which the activity of the natural hormone 1α,25-$(OH)_2D_3$ was normalized to 100%.

Western Blotting. Western blotting was performed as previously described.[27] Protein concentration was measured using the Bio-Rad DC protein assay kit. Analysis of cell lysates was performed by electrophoresis in SDS gels and protein transfer to Immobilon P membranes (Millipore Corp., Billerica, MA, USA). The membranes were incubated with the appropriate primary and secondary horseradish peroxidase-conjugated antibodies, and the antibody binding was visualized using the ECL detection system (Amersham−GE Healthcare, Barcelona, Spain). We used rabbit polyclonal antibodies generated against Cystatin D,[29] mouse monoclonal antibodies against E-cadherin (BD Transduction Laboratories, San Diego, CA, USA), c-MYC (Santa Cruz Biotechnology, Heidelberg, Germany), and goat polyclonal antibody against β-actin (Santa Cruz Biotechnology).

Quantitative RT-PCR. Total RNA was extracted from cultured cells using NucleoSpin miRNA extraction kit (Macherey-Nagel) and retrotranscribed using the iScript cDNA Synthesis Kit (Bio-Rad). The quantitative PCR reaction was performed in a CFX384 real-time PCR detection system (Bio-Rad) using the TaqMan Universal Master Mix (Applied Biosystems) and TaqMan probes for *PIP4K2B* (Hs01552176_m1) and *RPLP0* (Hs99999902_m1) (both from Applied Biosystems). Thermal cycling consisted of an initial denaturing step at 95 °C for 10 min and 40 cycles of denaturing at 95 °C for 15 s and annealing and elongation at 60 °C for 30 s. RNA expression values were normalized versus the housekeeping gene *RPLP0* (ribosomal protein, large, P0) using the comparative C_T method. All measurements were performed in triplicate.

Serum Calcium Quantitation and Weight Measure. All animal studies were approved by the University of Santiago de Compostela Ethics Committee for Animal Experiments. Female CD-1 mice (age matched, between 6 and 8 weeks) were obtained from Charles River Laboratories (L'Arbresle, France). The compounds (**1**, **5a−c**) were dissolved in sesame oil and administered intraperitoneally (0.3 μg/kg) every other day for three weeks. Calcium measurement was determined a day after the last dose using the QuantiChom Calcium Assay Kit (BioAssay Systems, Hayward, CA, USA) following the manufacturer's guidelines. Weight of mice was carried out every other day for a week.

Statistical Analysis. Each experiment was performed at least three times. Values are expressed as means ± SD. Means were compared by unpaired t-tests or one-way ANOVA with the Tukey−Kramer multiple comparison for post hoc comparisons. Statistical significance is taken to be indicated by * $P < 0.05$, *** $P < 0.001$. Dose−response curves for the luciferase reporter assay and competitive VDR binding, as well as calculation of EC_{50} and IC_{50} values, were performed using GraphPad Prism 5 software (San Diego, CA, USA).

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Data from docking calculations, RT-PCR data, NMR spectra for the new compounds, X-ray structure and data of triol **8a**, and HPLC traces of vitamins **5a−c**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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DEDICATION

[†]Dedicated to Prof. Milan Uskokovic.

ABBREVIATIONS USED

CI, chemical ionization; 1,25D, 1α,25-dihydroxyvitamin D_3; DEPT, distortionless enhancement by polarization transfer; DIBAL-H, diisobutylaluminium hydride; dppf, 1,1′-bis-(diphenylphosphino)ferrocene; ESI-TOF, electrospray ionization-time-of-flight; FAB, fast atom bombardment; HMPA, hexamethylphosphoramide; Im, imidazole; LBD, ligand binding domain; LDA, lithium diisopropylamide; Ms, methylsulfonyl; 1α,25-$(OH)_2D_3$, 1α,25-dihydroxyvitamin D_3; PDC, pyridinium dichromate; Pin, pinacolato; RXR, retinoic X receptor; TBS, *tert*-butyldimethylsilyl; Tf, trifluoromethanesulfonyl; VDR, vitamin D receptor; MCF-7, human adenocarcinoma breast cancer cell line; SW480-ADH, human colon cancer cell line

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