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Synthesis and Biological Evaluation of 1 α ,25-Dihydroxyvitamin D₃ Analogues with a Long Side Chain at C12 and Short C17 Side Chains[†]

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(5) Supporting Information

ABSTRACT: Structure-guided optimization was used to design new analogues of 1α ,25-dihydroxyvitamin D₃ 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 Pdcatalyzed tandem cyclization–Suzuki coupling method. The new analogues were assayed against human colon and breast cancer cell lines and in mice. All new vitamin D₃ analogues bound less



strongly to the VDR than 1α ,25-dihydroxyvitamin D₃ 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 ,25dihydroxyvitamin D_3 (1α ,25-(OH)₂ D_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)₂D₃ 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)₂D₃ 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 D₃ 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 His 397.^{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 21methyl 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 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 Gemini²⁰ 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





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_N^2 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 Ph₃P=CHBr.²⁵ The upper boronates 6a-c were prepared by Miyaura borylation²⁶ 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 PdCl₂(PPh₃)₂ and K_3PO_4 in H₂O/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)P₂ 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 1α ,25-(OH)₂D₃ 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

Scheme 2. Synthesis of Triol 8a^a



^{*a*}Reagents and conditions: (a) DIBAL-H, THF, -78 °C; (b) TBSCl, Im, DMF; (c) *m*-CPBA, CH₂Cl₂, 0 °C; (d) LiNEt₂, HMPA, Et₂O; (e) *m*-CPBA, CH₂Cl₂, 0 °C; (f) MsCl, Et₃N, CH₂Cl₂, -20 °C; (g) Na/naphthalene, THF, 0 °C; (h) H₂, 10% Pd-C, EtOAc; (i) PDC, CH₂Cl₂; (j) LDA, THF, -78 °C; **20**, -78 °C $\rightarrow 0$ °C; (k) **22**, PdCl₂(Ph₃P)₂, CuI, Et₂NH; (l) H₂, 10% Pd-C, EtOAc; (m) TBAF, THF, Δ . *Si*=TBS=Si(*t*-Bu)Me₂.



^aReagents and conditions: (a) TsCl, Py, 4 °C; (b) KCN, DMSO, 90 °C; (c) DIBAL-H, CH_2Cl_2 , -5 °C, HCl 3 M/Et₂O, DIBAL-H, THF, -78 °C.

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)₂D₃ 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

Scheme 4. Synthesis of Analogues $5a-c^a$



^aReagents and conditions: (a) TBSCl, Im, DMF; (b) PDC, CH_2Cl_2 ; (c) (Ph₃PCH₂Br)Br, KOt-Bu, toluene, ultrasounds, $-17 \rightarrow 0$ °C; then **28**; (d) PdCl₂(dppf)·CH₂Cl₂, PCy₃, Pin₂B₂, KOAc, DMSO, 80 °C; (e) 7, PdCl₂(PPh₃)₂, K₃PO₄, THF, H₂O; (f) TBAF, THF.

natural hormone 1. The VDR binding affinity of compounds 5a-c is approximately 10 times less than that of the 1,25D (Figure SA).

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).



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 μ m.

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)₂D₃ analogues with subtituents 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)₂D₃ 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)₂D₃. 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 Et₂O were distilled from Na/ benzophenone, toluene was distilled from Na, CH₂Cl₂ was distilled from P2O5, and pyridine, Et3N, Et2NH, and i-Pr2NH were distilled from CaH₂. DMF was stored over activated 4 Å molecular sieves. DMSO and HMPA were distilled from CaH2 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 Na₂SO₄, 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 H₂O (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 CDCl₃ 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 ($\delta = 0.0$ ppm) using the residual solvent signal at $\delta = 7.26$ ppm (¹H, CDCl₃), $\delta = 3.31$ ppm (¹H, q, methanol- d_4), $\delta = 77.0$ ppm (¹³C, t, CDCl₃), or $\delta = 49.0$ ppm (¹³C, 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 $^{\circ}\!C$ 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 (ø 250 mm × 10 mm). Analogues 5a-c have a purity of >95% (HPLC).

Chemicals. Cul 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 $(10^{-9} \text{ to } 10^{-7} \text{ M})$. 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 (Ph₃PCH₂Br)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-8βol (11). A solution of diisobutyl-aluminum hydride in CH₂Cl₂ (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 $^\circ\text{C}.$ The aqueous layer was extracted with EtOAc $(3 \times 25 \text{ mL})$. The combined organic layer was dried, filtered, and concentrated. The residue was purified by flash chromatography (SiO₂, 4 cm \times 10 cm, 4% EtOAc-hexanes) to afford 11 [1.808 g, 5.57 mmol, 93%, $R_f = 0.40$ (20% EtOAc-hexanes), colorless oil, $[\alpha]_{D} = +101.5 (c = 1.0, CHCl_{3})]$. ¹H NMR (CDCl₃, 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, Me₃C-Si), 0.81 (3H, s, H-18), 0.01 (6H, s, Me₂Si). ¹³C NMR (CDCl₃, 63 MHz): 129.6 (CH), 128.3 (CH), 67.6 (CH₂, C-22), 66.2 (CH, C-8), 52.9 (CH), 50.0 (CH), 41.8 (CH₂), 39.8 (C, C-13), 38.4 (CH), 26.8 (CH₂), 25.9 (3 × CH₃, Me₃C-Si), 21.2 (CH₂), 18.2 (C, C-Si), 16.5 (CH₃), 13.4 (CH₃), $-5.5 (2 \times CH_3, Me_2Si)$. 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 [C₁₉H₃₆O₂SiNa]⁺, 347.2377; found, 347.2354.

 8β ,22-Bis(tert-butyldimethylsilyloxy)-de-A,B-23,24-dinorchol-9ene (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 $(3 \times 15 \text{ mL})$. The combined organic layer was dried, filtered, and concentrated. The residue was purified by flash chromatography (SiO₂, 3 cm \times 8 cm, hexanes) to afford 12 [1.648 g, 3.76 mmol, 96%, $R_{\rm f}$ = 0.95 (10% EtOAc–hexanes), colorless oil, $[\alpha]_{\rm D} = +143.9 \ (c = 1.9, \ {\rm CHCl}_3)].$ ¹H NMR (CDCl₃, 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 \times Me₂Si). ¹³C NMR (CDCl₃, 63 MHz): 129.1 (CH), 128.4 (CH), 67.8 (CH₂, C-22), 66.3 (CH, C-8), 53.3 (CH), 50.7 (CH), 42.4 (CH₂), 40.2 (C, C-13), 38.7 (CH), 27.2 (CH₂), 26.1 ($3 \times CH_3$, Me₃C-Si), 25.9 ($3 \times CH_3$, Me₃C-Si), 22.0 (CH₂), 18.4 (C, C-Si), 18.1 (C, C-Si), 16.8 (CH₃), 13.6 (CH₃), -4.3 (CH₃, MeSi), -5.0 (CH₃, MeSi), -5.3 (CH₃, MeSi), -5.3 (CH₃, 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,11αepoxycholane (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 CH₂Cl₂ (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 Na₂S₂O₃ (50 mL). The aqueous layer was extracted with CH₂Cl₂ (3 × 50 mL). The combined organic layer was washed with saturated NaHCO₃ (2 ×



Figure 5. Vitamin D receptor binding and transactivation activities. (A) Competitive binding of $1\alpha_2 25$ -(OH)₂D₃ (1) and the synthesized vitamin D₃ analogues **5a**-**c** to the full-length human VDR. The experiments were carried out in duplicate on two different occasions. IC₅₀ 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 EC₅₀ 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 μ g/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.

s, H-18), 0.09 (3H, s, MeSi), 0.04 (3H, s, MeSi), 0.01 (6H, s, Me₂Si). ¹³C NMR (CDCl₃, 125 MHz): 67.5 (CH₂, 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 (CH₂, C-12), 38.3 (CH, C-20), 26.9 (CH₂), 25.9 (3 × CH₃, Me₃C-Si), 25.8 (3 × CH₃, Me₃C-Si), 21.1 (CH₂), 18.3 (C, C-Si), 18.0 (C, C-Si), 16.7 (CH₃, C-21), 16.1 (CH₃, C-18), -4.7 (CH₃, MeSi), -5.2 (CH₃, MeSi), -5.4 (CH₃, MeSi), -5.5 (CH₃, MeSi). IR (neat, cm⁻¹): 2956 (ν _{C-H}), 2929 (ν _{C-H}). MS ([ESI-TOF]⁺, *m/z*, %): 477 ([M + Na]⁺, 65), 455 ([M - H₂O - TBSOH]⁺, 14). EA: calculated for [C₂₅H₅₀O₃Si₂], C (66.02), H (11.08); found, C (65.94), H (11.37).

8,22-Bis(tert-butyldimethylsilyloxy)-de-A,B-23,24-dinorchol-11en-9 α -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 Et₂O (22.5 mL). After warm to rt, a solution of 13 (3.18 g, 6.99 mmol) in Et₂O (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 NH₄Cl 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 (SiO₂, 6 cm \times 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, $CHCl_{2}$]. ¹H NMR (CDCl₂, 250 MHz): 6.42 (1H, d, J = 10.1, H-12), 5.56 (1H, dd, J₁ = 3.6, J₂ = 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 × Me₃C-Si), 0.08 (3H, s, MeSi), 0.04 (3H, s, MeSi), 0.02 (6H, s, Me₂Si). ¹³C NMR (CDCl₃, 63 MHz): 142.2 (CH), 125.7 (CH), 75.0 (CH), 72.8 (CH), 67.5 (CH₂, C-22), 48.1 (2 × CH), 43.2 (C, C-13), 38.9 (CH), 28.1 (CH₂), 25.9 ($3 \times CH_3$, Me₃C-Si), 25.8 (3× CH₃, Me₃C-Si), 21.9 (CH₂), 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 $(2 \times$ CH_3 , Me_2Si). IR (neat, cm^{-1}): 3315 (ν_{O-H}), 2955 (ν_{C-H}), 2929 $(\nu_{\rm C-H})$. 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 NaHCO₃ (2 \times 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, CHCl₃)]. ¹H NMR (CDCl₃, 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, Me₃C-Si), 0.88 (9H, s, Me₃C-Si), 0.07 (3H, s, MeSi), 0.03 (6H, s, Me₂Si), 0.02 (3H, s, MeSi). ¹³C NMR (CDCl₃, 125 MHz): 74.1 (CH, C-8), 72.0 (CH, C-9), 67.4 (CH₂, 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 (CH₂), 25.9 (3 × CH₃, Me₃C-Si), 25.8 (3 × CH₃, Me₃C-Si), 21.4 (CH₂), 18.3 (C, C-Si), 17.9 (C, C-Si), 17.0 (CH₃, C-21), 13.7 (CH₃, C-18), -4.9 (CH₃, MeSi), -5.2 (CH₃, MeSi), -5.4 $(CH_3, MeSi)$, -5.4 $(CH_3, MeSi)$. IR (neat, cm⁻¹): 3345 (ν_{O-H}) , 2956 $(\nu_{\rm C-H})$, 2929 $(\nu_{\rm C-H})$. MS ([ESI-TOF]⁺, m/z, %): 493 ([M + Na]⁺, 100), 453 ([M - OH]⁺, 7). HRMS: [ESI-TOF]⁺, calcd for [C₂₅H₅₀O₄Si₂Na]⁺, 493.3140; found, 493.3147.

 8β , 22-Bis(tert-butyldimethylsilyloxy)-de-A, B-23, 24-dinor-11 α , 12 α -epoxycholan-9 α -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 CH₂Cl₂ (25 mL) at -20 °C. After 15 min,

Table 1. VDR Binding Properties, Transcriptional Activities, and Calcemic Effects of the Natural Hormone 1	.α,25-(OH)	$_{2}D_{3}(1)$	t),
and Sa, Sb, and Sc 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 IC₅₀ or EC₅₀, respectively, in comparison with 1α ,25-(OH)₂D₃ (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)₂D₃ 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 H2O (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), $[\alpha]_{\rm D} = -20.9 \ (c = 1.1, \text{ CHCl}_3)]$ that was used in the next reaction without further purification. ¹H NMR (CDCl₃, 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, J₂ = 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, Me₃C-Si), 0.88 (9H, s, Me₃C-Si), 0.12 (3H, s, MeSi), 0.03 (9H, overlapped s, Me₂Si and MeSi). ¹³C NMR (CDCl₃, 63 MHz): 81.9 (CH, C-9), 71.8 (CH, C-8), 67.3 (CH₂, C-22), 61.1 (CH), 51.2 (CH), 47.4 (CH), 42.2 (CH), 41.7 (C, C-13), 39.3 (CH₃, MeS), 38.2 (CH), 26.2 (CH₂), 25.9 ($3 \times CH_3$, Me₃C-Si), 25.7 (3 × CH₃, Me₃C-Si), 21.1 (CH₂), 18.3 (C, C-Si), 17.9 (C, C-Si), 17.0 (CH₃), 13.8 (CH₃), -4.8 (2 × CH₃, Me₂Si), -5.4 (2 × CH₃, Me₂Si). 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-9en-12 α -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 H₂O (50 mL). The aqueous layer was extracted with EtOAc $(3 \times 60 \text{ mL})$. The combined organic layer was dried, filtered, and concentrated. The residue was purified by flash chromatography (SiO₂, 6 cm \times 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), $[\alpha]_{\rm D} = +177.9$ (c = 0.6, CHCl₃)]. ¹H NMR (CDCl₃, 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 $(1H, dd, J_1 = 4.3, J_2 = 9.8, H-22), 3.28 (1H, dd, J_1 = 7.4, J_2 = 9.8, H-22)$ 22), 1.02 (3H, d, J = 6.6, H-21), 0.89 (9H, s, Me₃Si-C), 0.87 (9H, s, Me₃C-Si), 0.04 (9H, overlapped s, MeSi and Me₂Si), 0.02 (3H, s, MeSi). ¹³C NMR (CDCl₃, 63 MHz): 132.0 (CH), 129.2 (CH), 71.7 (CH), 67.8 (CH₂, C-22), 65.7 (CH), 44.9 (C, C-13), 44.1 (CH), 43.4 (CH), 38.3 (CH), 26.1 (CH₂), 26.0 ($3 \times$ CH₃, Me₃C–Si), 25.8 ($3 \times$ CH₃, Me₃C-Si), 21.0 (CH₂), 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 $(2 \times$ CH₃, Me₂Si). 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-12 α -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 H₂ for three times. The reaction mixture was stirred under H₂ atmosphere (balloon pressure) for 48 h. The mixture was filtered through a layer of silica gel. The solids were washed with EtOAc (3 \times 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), $[\alpha]_{\rm D}$ = +46.1 (c = 1.4, CHCl₃)], which was used in the next reaction without further purification. ¹H NMR (CDCl₃, 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 × Me₃C-Si), 0.02 (6H, s, Me₂Si), 0.00 (3H, s, MeSi), -0.01 (3H, s, MeSi). ¹³C NMR (CDCl₃, 63 MHz): 73.1 (CH), 69.2 (CH), 67.6 (CH₂, C-22), 45.9 (C, C-13), 45.0 (CH), 44.2 (CH), 38.2 (CH), 28.2 (CH₂), 25.9 (CH₂), 25.9 (3 × CH_{3} , $Me_{3}C-Si$), 25.8 (3 × CH_{3} , $Me_{3}C-Si$), 24.5 (CH_{2}), 22.2 (CH_{2}), 18.3 (C, C-Si), 17.9 (C, C-Si), 15.7 (CH₃), 14.7 (CH₃), -4.9 (CH₃, MeSi), -5.2 (CH₃, MeSi), -5.4 (2 × CH₃, Me₂Si). IR (neat, cm⁻¹): 3403 ($\nu_{\text{O-H}}$), 2956 ($\nu_{\text{C-H}}$), 2929 ($\nu_{\text{C-H}}$). MS ([ESI-TOF]⁺, m/z, %): 479 ($[M + Na]^+$, 100), 439 ($[M - OH]^+$, 13). HRMS: $[ESI-TOF]^+$, calcd for [C25H52O3Si2Na]+, 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 \times 40 mL), and the resulting filtrate was concentrated. The residue was purified by flash chromatography (SiO₂, 3 cm × 10 cm, 2% EtOAc-hexanes) to afford 19 [1.114 g, 2.45 mmol, 90%, $R_{\rm f}$ = 0.66 (10% EtOAc-hexanes), colorless oil, $[\alpha]_{\rm D}$ = +88.8 (*c* = 1.6, CHCl₃)]. ¹H NMR (CDCl₃, 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 Me₃C-Si), 0.88 (9H, s, Me₃C-Si), 0.06 (3H, s, MeSi), 0.04 (3H, s, MeSi), 0.02 (6H, s, Me₂Si). ¹³C NMR (CDCl₃, 63 MHz): 215.6 (C, C-12), 68.2 (CH, C-8), 67.7 (CH₂, C-22), 57.2 (C, C-13), 57.0 (CH), 43.9 (CH), 39.1 (CH), 37.8 (CH₂), 34.9 (CH_2) , 26.5 (CH_2) , 26.0 $(3 \times CH_3, Me_3C-Si)$, 25.8 $(3 \times CH_3, Me_3C-Si)$ Si), 22.9 (CH₂), 18.3 (C, C-Si), 18.0 (C, C-Si), 17.4 (CH₃), 13.3 (CH_3) , -4.8 $(CH_3$, MeSi), -5.1 $(CH_3$, MeSi), -5.4 $(2 \times CH_3)$ Me₂Si). IR (film, cm⁻¹): 2955 (ν_{C-H}), 2929 (ν_{C-H}), 2857 (ν_{C-H}), 1712 $(\nu_{C=0})$. 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 [C₂₅H₅₁O₂Si₂]⁺, 455.3377; found, 455.3365.

8β,22-Bis(tert-butyldimethylsilyloxy)-de-A,B-23,24-dinorchol-11en-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 (SiO₂) 3 cm \times 8 cm, 20% CH₂Cl₂-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, CHCl₃)]. ¹H NMR (CDCl₃, 250 MHz): 5.53 (1H, t, I = 3.9, H-11), 4.12 (1H, broad d, I = 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-10) 21), 0.90 (18H, overlapped s, 2 × Me₃C-Si), 0.06 (3H, s, MeSi), 0.04 (9H, overlapped s, MeSi and Me₂Si). ¹³C NMR (CDCl₃, 63 MHz): 157.3 (C, C-12), 118.4 (C, q, J = 319, CF₃), 112.6 (CH, C-11), 66.5 (CH₂, C-22), 65.8 (CH, C-8), 53.8 (CH), 50.7 (CH), 45.9 (C, C-13), 36.4 (CH₂), 34.9 (CH), 25.9 (3 × CH₃, Me₃C-Si), 25.7 (3 × CH₃, Me₃C-Si), 23.3 (CH₂), 22.2 (CH₂), 19.5 (CH₃), 18.2 (C, C-Si), 17.9 (C, C-Si), 15.8 (CH₃), -4.8 (CH₃, MeSi), -5.2 (CH₃, MeSi), -5.5 (2 × CH₃, Me₂Si). IR (film, cm⁻¹): 2956 (ν_{C-H}), 2931 (ν_{C-H}), 2886 $(\nu_{\rm C-H})$, 2859 $(\nu_{\rm C-H})$, 1666 $(\nu_{\rm C=C})$. MS ($[CI]^+$, m/z, %): 587 ($[M + 10^{-4}]$ H]⁺, 18), 455 ([M – TBSO]⁺, 20), 305 ([M – TBSO – TfOH]⁺, 86). HRMS: [CI]⁺, calcd for [C₂₆H₅₀O₅F₃SSi₂]⁺, 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 PdCl₂(PPh₃)₂ (60 mg, 0.08 mmol) were successively added to a solution of 21 (1.00 g, 1.71 mmol) and 22¹⁴ (1.20 g, 8.56 mmol) in Et₂NH (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 NH₄Cl (100 mL). Aqueous layer was extracted with MTBE (3 \times 50 mL). The combined organic layer was dried, filtered, and concentrated. The residue was purified by flash chromatography (SiO₂, 4.5 cm \times 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, CHCl₃)]. ¹H NMR (CDCl₃, 250 MHz): 5.80-5.64 (1H, m, H-11), 4.08 (1H, broad d, J = 6.0, H-8), 3.70 (1H, dd, J₁ = 3.1, J₂ = 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, Me₃C-Si), 0.84 (9H, s, Me₃C-Si), 0.01 (6H, s, Me₂Si), -0.02 (6H, s, Me₂Si). ¹³C NMR (CDCl₃, 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 (CH₂, C-22), 51.5 (CH), 51.5 (CH), 44.8 (C, C-13), 43.3 (CH₂), 37.1 (CH₂), 33.8 (CH), 29.1 (2 × CH₃, Me₂C-OH), 25.9 (3 × CH_{31} Me₃C-Si), 25.7 (3 × CH₃₁ Me₃C-Si), 23.8 (CH₂), 21.8 (CH₂), 21.5 (CH₂), 20.2 (CH₃), 19.4 (2 × CH₂), 18.1 (C, C-Si), 17.9 (C, C-Si), 17.1 (CH₃), -4.8 (CH₃, MeSi), -5.2 (CH₃, MeSi), -5.3 (CH₃, MeSi), -5.3 (CH₃, MeSi). IR (film, cm⁻¹): 3368 (ν_{O-H}), 2955 (ν_{C-H}), 2930 ($\nu_{\rm C-H}$), 2884 ($\nu_{\rm C-H}$), 2858 ($\nu_{\rm C-H}$). 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 H₂ for three times. The reaction mixture was stirred under H₂ 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 (SiO₂, 4 cm \times 8 cm, 4% EtOAc-hexanes) to afford 24 [1.88 g, 3.22 mmol, 98%, $R_{\rm f}$ = 0.70 (30% EtOAc-hexanes), colorless oil, $[\alpha]_{D} = +37.4$ (c = 1.0, CHCl₃)]. ¹H NMR (CDCl₃, 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, J₁ = 8.4, J₂ = 9.5, H-22), 1.19 (6H, s, Me₂C-OH), 0.96 (3H, d, J = 6.7, H-21), 0.88 (9H, s, Me₃C-Si), 0.87 (9H, s, Me₃C-Si), 0.81 (3H, s, H-18), 0.02 (6H, s, Me₂Si), -0.01 (3H, s, MeSi), -0.02 (3H, s, MeSi). ¹³C NMR (CDCl₃, 63 MHz): 71.0 (C, C-OH), 69.0 (CH, C-8), 66.5 (CH₂, C-22), 56.6 (CH), 53.8 (CH), 49.4 (CH), 45.5 (C, C-13), 44.0 (CH₂), 34.9 (CH₂), 34.9 (CH), 31.4 (CH_2) , 30.2 (CH_2) , 30.1 (CH_2) , 29.2 $(2 \times CH_3, Me_2C-OH)$, 28.3 (CH_2) , 26.0 (3 × CH₃, Me₃C-Si), 25.8 (3 × CH₃, Me₃C-Si), 24.4 (CH₂), 23.7 (CH₂), 22.5 (CH₂), 21.2 (CH₂), 20.5 (CH₃), 18.2 (C, C-Si), 18.0 (C, C-Si), 11.0 (CH₃), -4.8 (CH₃, MeSi), -5.1 (CH₃, MeSi), -5.3 (2 × CH₃, Me₂Si). IR (film, cm⁻¹): 3362 (ν_{O-H}), 2954

 $\begin{array}{l} (\nu_{\rm C-H}), \, 2930 \, (\nu_{\rm C-H}), \, 2857 \, (\nu_{\rm C-H}). \ {\rm MS} \, ([{\rm CI}]^+, \, m/z, \, \%): \, 583 \, ([{\rm M} + {\rm H}]^+, \, 4), \, 565 \, ([{\rm M} - {\rm OH}]^+, \, 20), \, 451 \, ([{\rm M} - {\rm TBSO}]^+, \, 3), \, 433 \, ([{\rm M} - {\rm OH} - {\rm TBSOH}]^+, \, 34), \, 301 \, ([{\rm M} - {\rm OH} - {\rm 2TBSOH}]^+, \, 100). \ {\rm HRMS:} \ [{\rm CI}]^+, \, {\rm calcd} \ {\rm for} \, [{\rm C}_{34}{\rm H}_{71}{\rm O}_{3}{\rm Si}_{2}]^+, \, 583.4942; \ {\rm found}, \, 583.4916. \end{array}$

12β-(7-Hydroxy-7-methyloctyl)-de-A,B-23,24-dinorcholan-8β,22diol (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 NH₄Cl (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 (SiO₂, 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 (Et₂O–hexanes), $[\alpha]_{D} = +30.6$ (*c* = 1.7, CHCl₃)]. ¹H NMR (CDCl₃, 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, Me₂C-OH), 1.03 (3H, d, J = 6.9, H-21), 0.86 (3H, s, H-18). ¹³C NMR (CDCl₃, 63 MHz): 70.9 (C, C-OH), 68.7 (CH, C-8), 66.3 (CH₂, C-22), 56.1 (CH), 53.2 (CH), 49.3 (CH), 45.2 (C, C-13), 43.8 (CH₂), 34.9 (CH), 33.9 (CH₂), 31.2 (CH₂), 30.1 (CH_2) , 29.9 (CH_2) , 29.0 $(2 \times CH_3, Me_2C-OH)$, 28.1 (CH_2) , 24.2 (CH₂), 23.4 (CH₂), 22.2 (CH₂), 21.1 (CH₂), 20.3 (CH₃), 10.8 (CH₃). 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 - H₂O]⁺, 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 (SiO₂, 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, CHCl₃)]. ¹H NMR (CDCl₃, 250 MHz): 3.98 (1H, broad d, J = 1.2, H-8), 3.65 (1H, dd, J₁ = 4.3, J₂ = 9.6, H-22), 3.32 (1H, dd, J₁ = 7.7, J₂ = 9.6, H-22), 1.18 (6H, s, Me₂C-OH), 0.96 (3H, d, J = 6.8, H-21), 0.86 (9H, s, Me₃C-Si), 0.83 (3H, s, H-18), 0.05 (6H, s, Me₂Si). ¹³C NMR (CDCl₃, 63 MHz): 70.9 (C, C-OH), 68.9 (CH, C-8), 66.5 (CH₂, C-22), 56.3 (CH), 53.3 (CH), 49.2 (CH), 45.2 (C, C-13), 44.0 (CH₂), 34.8 (CH), 34.0 (CH₂), 31.3 (CH₂), 30.2 (CH₂), 30.0 (CH₂), 29.1 (2 × CH₃, Me₂C-OH), 28.2 (CH₂), 25.9 (3 × CH₃, Me₃C-Si), 24.3 (CH₂), 23.5 (CH₂), 22.1 (CH₂), 21.1 (CH₂), 20.5 (CH_3) , 18.2 (C, C-Si), 10.8 (CH_3) , -5.4 (2 × CH₃, Me₂Si). IR (film, cm⁻¹): 3399 ($\nu_{\rm O-H}$), 2930 ($\nu_{\rm C-H}$), 2858 ($\nu_{\rm C-H}$). MS ([FAB]⁺, m/z, %): 469 ($[M + H]^+$, 63), 451 ($[M - OH]^+$, 52). HRMS: $[FAB]^+$, calcd for [C₂₈H₅₇O₃Si]⁺, 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 CH₂Cl₂ (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 $(3 \times 40 \text{ mL})$, and the resulting filtrate was concentrated. The residue was purified by flash chromatography (SiO₂, 3 cm \times 6 cm, 30% EtOAc-hexanes) to afford ketone 28a [150 mg, 0.321 mmol, 89%, R_f = 0.48 (40% EtOAchexanes), colorless oil]. ¹H NMR (CDCl₃, 250 MHz): 3.60 (1H, dd, J₁ = 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, Me₂C-OH), 0.97 (3H, d, J = 6.9, H-21), 0.84 (9H, s, Me₃C-Si), 0.56 (3H, s, H-18), -0.02 (6H, s, $\rm Me_2Si).$ ^{13}C NMR (CDCl_3, 63 MHz): 212.4 (C, C-8), 70.8 (C, C-OH), 66.0 (CH₂, C-22), 61.8 (CH), 56.0 (CH), 52.2 (C, C-13), 48.3 (CH), 43.8 (CH₂), 40.3 (CH₂), 35.2 (CH), 30.7 (CH₂), 30.1 (CH₂), 29.8 (CH₂), 29.6 (CH₂), 29.1 (2 × CH₃, Me₂C-OH), 28.2 (CH₂), 25.8 (3 × CH₃, Me₃C-Si), 24.2 (CH₂), 21.6 (CH₂), 20.1 (CH₃), 18.7 (CH₂), 18.2 (C, C-Si), 9.8 (CH₃), -5.4 (2 × CH₃, Me₂Si). IR (CHCl₃, cm⁻¹): 3459 (ν_{O-H}), 2956 (ν_{C-H}) , 2930 (ν_{C-H}) , 2884 (ν_{C-H}) , 1717 (ν_{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)-12 β -(7hydroxy-7-methyloctyl)-de-A,B-24,23-dinorcholane (29a). A suspension of (Ph₃PCH₂Br)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 KOt-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 NH₄Cl (1 mL), and the mixture was filtered through a layer of silica gel. The solids were washed with EtOAc (3 \times 15 mL), and the filtrate was concentrated. The residue was purified by flash chromatography (SiO₂, 3 cm \times 8 cm, 30% EtOAc-hexanes) to afford 29a [121 mg, 0.22 mmol, 69%, $R_{\rm f}$ = 0.50 (20% EtOAc-hexanes), colorless oil]. ¹H NMR $(CDCl_3, 250 \text{ MHz})$: 5.63 (1H, s, H-7), 3.67 (1H, dd, $J_1 = 4.2, J_2 = 9.6, J_2 =$ H-22), 3.44-3.30 (1H, m, H-22), 1.20 (6H, s, Me₂C-OH), 0.99 (3H, d, J = 6.9, H-21), 0.88 (9H, s, Me₃C-Si), 0.50 (3H, s, H-18), 0.02 (6H, s, Me₂Si). ¹³C NMR (CDCl₃, 63 MHz): 145.3 (C, C-8), 97.6 (CH, C-7), 71.4 (C, C-OH), 66.5 (CH₂, C-22), 56.9 (CH), 55.6 (CH), 49.4 (CH), 49.1 (C, C-13), 44.4 (CH₂), 36.3 (CH), 31.6 (CH₂), 31.6 (CH_2) , 30.6 (CH_2) , 30.4 (CH_2) , 29.6 $(2 \times CH_3, Me_2C-OH)$, 28.8 (CH₂), 28.6 (CH₂), 26.4 (3 × CH₃, Me₃C-Si), 24.8 (CH₂), 22.2 (CH₂), 22.0 (CH₂), 20.5 (CH₃), 18.7 (C, C-Si), 9.8 (CH₃), -4.9 (2 × CH₃, Me₂Si). IR (film, cm⁻¹): 3369 (ν_{O-H}), 3085 ($\nu_{=C-H}$), 2953 $(\nu_{\rm C-H})$, 2930 $(\nu_{\rm C-H})$, 2857 $(\nu_{\rm C-H})$, 1632 $(\nu_{\rm C=C})$. MS ([CI]⁺, m/z, %): 525 ([M – OH]⁺, 3), 393 ([M – OH – TBSOH]⁺, 31), 313 ([M - C₆H₁₈BrO₂Si]⁺, 100). HRMS: [CI]⁺, calcd for [C₂₉H₅₄BrOSi]⁺ 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). PCy3 (4 mg, 0.013 mmol) and PdCl₂(dppf)·CH₂Cl₂ (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 Pin₂B₂ (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 H₂O (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 (SiO₂, 3 cm \times 10 cm, 5–10% EtOAc-hexanes) to afford **6a** [74 mg, 0.15 mmol, 70%, $R_f = 0.40$ (20% EtOAc-hexanes), colorless oil, $[\alpha]_{D} = +44.7$ (*c* = 0.2, CHCl₃)]. ¹H NMR (CDCl₃, 250 MHz): 4.90 (1H, s, H-7), 3.69 (1H, dd, J₁ = 4.1, J₂ = 9.6, H-22), 3.35 (1H, dd, $J_1 = 8.3$, $J_2 = 9.6$, H-22), 1.25 (12H, s, 2 × Me₂C-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, Me₂Si). ¹³C NMR (CDCl₃, 63 MHz): 166.5 (C, C-8), 82.5 (2 × C, 2 × C-OB), 71.0 (C, C-OH), 66.2 (CH₂, 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 $(2 \times CH_2)$, 30.0 (CH₂), 29.2 (2 × CH₃, Me₂C-OH), 28.3 (CH₂), 26.0 (3 × CH₃, Me₃C-Si), 24.9 (2 × CH₃, Me₂C-OB), 24.8 (2 × CH₃, Me₂C-OB), 24.3 (CH₂), 22.1(CH₂), 21.4 (CH₂), 20.1 (CH₃), 18.3 (C, C-Si), 9.7 (CH_3) , -5.3 (2 × CH₃, Me₂Si). IR (film, cm⁻¹): 3385 (ν_{O-H}), 2954 $(\nu_{\rm C-H})$, 2930 $(\nu_{\rm C-H})$, 2857 $(\nu_{\rm C-H})$, 1640 $(\nu_{\rm C=C})$. 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)-23,24,25,26,27pentanorvitamin D_3 (**5a**). An aqueous solution of K_3PO_4 (1.65 mL, 2 M) and PdCl₂(PPh₃)₂ (4 mg, 0.006 mmol) were successively added to a solution of **6a** (70 mg, 0.118 mmol) and 7²¹ (83 mg, 0.138 mmol) in THF (4 mL). The reaction mixture protected from light was vigorously stirred for 1 h. Then H₂O (10 mL) was added, and the aqueous layer was extracted with Et₂O (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 NH₄Cl (15 mL). The aqueous layer

was extracted with EtOAc $(3 \times 15 \text{ mL})$. The combined organic layer was dried, filtered, and concentrated. The residue was purified by flash chromatography (SiO₂, 2 cm \times 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 (Et₂O–hexanes), $[\alpha]_{D} = -25.6$ (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. ¹H NMR (CD₃OD, 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, J₁ = 3.4, J₂ = 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), 2.26 (1H, dd, $J_1 = 6.8$, $J_2 = 13.4$, H-4), 2.26 (1H, dd, $J_1 = 6.8$, $J_2 = 13.4$, H-4), 2.26 (1H, dd, $J_1 = 6.8$, $J_2 = 13.4$, H-4), 2.26 (1H, dd, $J_1 = 6.8$, $J_2 = 13.4$, H-4), 2.26 (1H, dd, $J_1 = 6.8$, $J_2 = 13.4$, H-4), 2.26 (1H, dd, $J_1 = 6.8$, $J_2 = 13.4$, H-4), 2.26 (1H, dd, $J_1 = 6.8$, $J_2 = 13.4$, H-4), 2.26 (1H, dd, $J_1 = 6.8$, $J_2 = 13.4$, H-4), 2.26 (1H, dd, $J_1 = 6.8$, $J_2 = 13.4$, H-4), 2.26 (1H, dd, $J_1 = 6.8$, $J_2 = 13.4$, H-4), 2.26 (1H, dd, $J_1 = 6.8$, $J_2 = 13.4$, H-4), 2.26 (1H, dd, $J_1 = 6.8$, $J_2 = 13.4$, H-4), 2.26 (1H, dd, $J_1 = 6.8$, $J_2 = 13.4$, H-4), 2.26 (1H, dd, $J_1 = 6.8$, $J_2 = 13.4$, H-4), 2.26 (1H, dd, $J_1 = 6.8$, $J_2 = 13.4$, H-4), 2.26 (1H, dd, $J_1 = 6.8$, $J_2 = 13.4$, H-4), 2.26 (1H, dd, $J_1 = 6.8$, $J_2 = 13.4$, H-4), 2.26 (1H, dd, J_1 = 6.8, $J_2 = 13.4$, H-4), 2.26 (1H, dd, J_1 = 6.8, $J_2 = 13.4$, H-4), 2.26 (1H, dd, J_1 = 6.8, $J_2 = 13.4$, H-4), 2.26 (1H, dd, J_1 = 6.8, $J_2 = 13.4$, H-4), 2.26 (1H, dd, J_1 = 6.8, $J_2 = 13.4$, H-4), 2.26 (1H, dd, J_1 = 6.8, $J_2 = 13.4$, H-4), 2.26 (1H, dd, J_1 = 6.8, $J_2 = 13.4$, H-4), 2.26 (1H, dd, J_1 = 6.8, $J_2 = 13.4$, H-4), 2.26 (1H, dd, J_1 = 6.8, $J_2 = 13.4$, H-4), 2.26 (1H, dd, J_1 = 6.8, $J_2 = 13.4$, H-4), 2.26 (1H, dd, J_1 = 6.8, $J_2 = 13.4$, H-4), 2.26 (1H, dd, J_1 = 6.8, $J_2 = 13.4$, H-4), 2.26 (1H, dd, J_1 = 6.8, $J_2 = 13.4$, H-4), 2.26 (1H, dd, J_1 = 6.8, $J_2 = 13.4$, H-4), 2.26 (1H, dd, J_1 = 6.8, $J_1 = 13.4$, $J_2 = 13.4$, $J_2 = 13.4$, $J_1 = 13.4$, $J_2 = 13.4$, $J_2 = 13.4$, $J_2 = 13.4$, $J_1 = 13.4$, $J_2 = 13.4$, $J_2 = 13.4$, $J_1 = 13.4$, $J_2 = 13.4$, $J_2 = 13.4$, $J_2 = 13.4$, $J_1 = 13.4$, $J_2 = 13.4$, $J_2 = 13.4$, $J_1 = 13.4$, $J_2 = 13.4$, J13.4, H-4), 2.13-2.04 (1H, m, H-20), 1.17 (6H, s, Me₂C-OH), 1.06 (3H, d, J = 6.8, H-21), 0.54 (3H, s, H-18).¹³C NMR (CD₃OD, 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 (CH₂, C-19), 71.4 (C, C-OH), 71.4 (CH, C-1), 67.4 (CH, C-3), 66.4 (CH₂, C-22), 58.2 (CH), 57.1 (CH), 51.4 (CH), 50.3 (C, C-13), 46.1 (CH₂, C-4), 44.9 (CH₂), 43.7 (CH₂) C-2), 37.5 (CH), 32.5 (CH₂), 31.4 (CH₂), 31.1 (CH₂), 30.5 (CH₂), 30.0 (CH₂, C-9), 29.4 (CH₂), 29.2 (CH₃, MeC-OH), 29.1 (CH₃, MeC-OH), 25.4 (CH₂), 23.3 (CH₂), 22.4 (CH₂), 20.6 (CH₃, C-21), 10.1 (CH₃, C-18). IR (KBr, cm⁻¹): 3403 (ν_{O-H}), 2930 (ν_{C-H}), 2861 $(\nu_{\rm C-H})$, 1635 $(\nu_{\rm C=C})$. 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): $\lambda_{max} =$ 264 nm, $\lambda_{\min} = 230$ 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 $^\circ C$ for 22 h. The reaction was quenched by addition of ice and saturated NaCl (10 mL). The aqueous layer was extracted with MTBE (3 \times 20 mL), and the combined organic layer was washed with aqueous HCl $(3 \times 25 \text{ mL},$ 5%), saturated NaHCO₃ (3 \times 25 mL), and saturated NaCl (2 \times 20 mL), dried, filtered, and concentrated. The residue was purified by flash chromatography (SiO2, 3 cm × 10 cm, 30-60% EtOAchexanes) to afford $\mathbf{25a}$ [258 mg, 0.507 mmol, 84%, $R_{\rm f}$ = 0.58 (70% EtOAc-hexanes), colorless oil, $[\alpha]_D = +28.4$ (c = 0.5, CHCl₃)]. ¹H NMR (CDCl₃, 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, Me₂C-OH), 0.96 (3H, d, J = 6.8, H-21), 0.70 (3H, s, H-18). ¹³C NMR (CDCl₃, 63 MHz): 144.6 (C, Ar-SO₂), 133.0 (C), 129.7 (2 \times CH, Ar-H), 127.8 (2 × CH, Ar-H), 74.4 (CH₂, 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 (CH₂), 33.9 (CH₂), 32.1 (CH), 31.2 (CH₂), 30.1 (CH₂), 29.9 (CH₂), 29.1 (2 × CH₃, Me₂C-OH), 28.1 (CH₂), 24.2 (CH₂), 23.3 (CH₂), 22.0 (CH₂), 21.6 (CH₃, Me-Ar), 21.1 (CH₂), 20.1 (CH₃), 10.7 (CH₃). 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 [C₂₉H₄₈O₅SNa]⁺, 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 (SiO₂, $3 \text{ cm} \times 5 \text{ cm}$, 20– 50% EtOAc–hexanes) to afford 26a [328 mg, 0.902 mmol, 92%, $R_{\rm f}$ = 0.50 (70% EtOAc-hexanes), colorless oil, $[\alpha]_{D} = +25.9$ (c = 0.2, CHCl₃)]. ¹H NMR (CDCl₃, 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, Me₂C-OH), 1.15 (3H, d, J = 6.7, H-21), 0.84 (3H, s, H-18). ¹³C NMR (CDCl₃, 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 (CH₂), 34.0 (CH₂), 31.6 (CH₂), 30.4 (CH), 30.2 (CH₂), 30.0 (CH₂), 29.2 (2 × CH₃, Me₂C-OH), 28.2 (CH₂), 24.3 (CH₂), 23.4 (CH₂), 22.6 (CH₃), 22.0 (CH₂), 22.0 (CH₂), 21.2 (CH₂), 11.0 (CH₃). IR (film, cm⁻¹): 3450 (ν_{O-H}), 2931 (ν_{C-H}), 2875 (ν_{C-H}), 2247 ($\nu_{C=N}$). MS ([CI]⁺, m/z, %): 346 ([M – OH]⁺, 52), 328 ([M – OH – H₂O]⁺, 100). HRMS: [CI]⁺, calcd for [C₂₃H₄₀NO]⁺, 346.3110; found, 346.3110.

 12β -(7-Hydroxy-7-methyloctyl)-de-A,B-24-norcholan-8 β ,23-diol (8b). A solution of diisobutylaluminium hydride in CH_2Cl_2 (2.72 mL, 1 M, 2.72 mmol) was diluted in CH₂Cl₂ (3 mL) and cooled to -5 °C. A solution of 26a (283 mg, 0.778 mmol) in CH₂Cl₂ (4 mL) was added. The mixture was vigorously stirred at -15 °C for 2 h and then allowed to warm to 0 $^\circ\text{C}$ for 1 h. A suspension of aqueous HCl (10 mL, 3 M) in Et₂O (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 CH₂Cl₂ (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 $(3 \times 30 \text{ mL})$, and the combined organic layer was dried, filtered, and concentrated. The residue was purified by flash chromatography (SiO₂, 3 cm \times 7.5 cm, 40–50% EtOAc-hexanes) to afford 8b [171 mg, 0.464 mmol, 60%, R_f = 0.30 (70% EtOAchexanes), white foam, $[\alpha]_D = +24.9$ (c = 0.7, CHCl₃)]. ¹H NMR (CDCl₃, 250 MHz): 3.98 (1H, broad s, H-8), 3.71-3.45 (2H, m, H-23), 1.16 (6H, s, Me₂C-OH), 0.89 (3H, d, J = 6.8, H-21), 0.83 (3H, s, H-18). ¹³C NMR (CDCl₃, 63 MHz): 71.0 (C, C-OH), 68.9 (CH, C-8), 62.0 (CH₂, C-23), 57.2 (CH), 53.7 (CH), 49.8 (CH), 45.4 (C, C-13), 43.9 (CH₂), 36.7 (CH₂), 33.9 (CH₂), 31.3 (CH₂), 30.1 (CH₂), 29.9 (CH₂), 29.3 (CH), 29.1 (2 × CH₃, Me₂C-OH), 28.1 (CH₂), 24.2 (CH₂), 23.5 (CH₂), 22.5 (CH₂), 22.5 (CH₃), 21.0 (CH₂), 11.9 (CH₃). IR (film, cm⁻¹): 3365 (ν_{O-H}), 2930 (ν_{C-H}), 2863 (ν_{C-H}). MS $([FAB]^+, m/z, \%): 351 ([M - OH]^+, 70), 433 ([M - OH - H_2O]^+, 100)$ 100). HRMS: [FAB]⁺, calcd for [C₂₃H₄₃O₂]⁺, 351.3263; found, 351.3256.

23-(tert-Butyldimethylsilyloxy)-12β-(7-hydroxy-7-methyloctyl)de-A,B-24-norcholan-8^β-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, $[\alpha]_{D}^{T} = +21.5$ (c = 0.2, CHCl₃)]. ¹H NMR (CDCl₃, 250 MHz): 3.98 (1H, broad s, H-8), 3.71-3.43 (2H, m, H-23), 1.15 (6H, s, Me₂C-OH), 0.92-0.74 (15H, m, H-18, H-21 and Me₃C-Si), 0.00 (6H, s, Me₂Si). ¹³C NMR (CDCl₃, 63 MHz): 70.8 (C, C-OH), 69.0 (CH, C-8), 62.0 (CH₂, C-23), 57.1 (CH), 53.7 (CH), 50.1 (CH), 45.5 (C, C-13), 43.9 (CH₂), 36.7 (CH₂), 34.0 (CH₂), 31.1 (CH₂), 30.2 (CH₂), 29.9 (CH₂), 29.1 (2 × CH₃, Me₂C-OH), 28.8 (CH), 28.1 (CH₂), 25.9 $(3 \times CH_3, Me_3C-Si)$, 24.3 (CH₂), 23.3 (CH₂), 22.7 (CH₂), 22.3 (CH_3) , 20.8 (CH_2) , 18.2 (C-Si), 11.4 (CH_3) , -5.4 $(2 \times CH_3, Me_2Si)$. IR (CHCl₃, 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 – H₂O]⁺, 36), 315 ([M - TBSO - 2H₂O]⁺, 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 CH₂Cl₂ (10 mL). Product: ketone 28b [146 mg, 0.304 mmol, 95%, $R_f = 0.46$ (40% EtOAc-hexanes), colorless oil]. ¹H NMR (CDCl₃, 250 MHz): 3.67-3.43 (2H, m, H-23), 1.17 (6H, s, Me₂C-OH), 0.90 (3H, d, J = 6.9, H-21), 0.85 (9H, s, Me₃C-Si), 0.58 (3H, s, H-18), 0.00 (6H, s, Me₂Si). ¹³C NMR (CDCl₃, 63 MHz): 212.4 (C, C-8), 70.7 (C, C-OH), 62.2 (CH), 61.6 (CH₂, C-23), 56.5 (CH), 52.5 (C, C-13), 49.1 (CH), 43.8 (CH₂), 40.3 (CH₂), 36.0 (CH_2) , 30.6 (CH_2) , 30.1 (CH_2) , 29.8 (CH_2) , 29.4 (CH_2) , 29.1 $(2 \times$ CH₃, Me₂C-OH), 28.7 (CH), 28.1 (CH₂), 25.9 (3 × CH₃, Me₃C-Si), 24.2 (CH₂), 21.8 (CH₃), 21.1 (CH₂), 19.4 (CH₂), 18.1 (C, C-Si), 10.0 (CH₃), -5.5 (2 × CH₃, Me₂Si). IR (CHCl₃, cm⁻¹): 3467 (ν_{O-H}), 2958 ($\nu_{\rm C-H}$), 2930 ($\nu_{\rm C-H}$), 2858 ($\nu_{\rm C-H}$), 1718 ($\nu_{\rm C=0}$). MS ([CI]⁺, m/ z_{1} %): 463 ([M - OH]⁺, 58), 405 ([M - OH - t-Bu]⁺, 48), 331 ([M

- OH - TBSOH]⁺, 94). HRMS: [CI]⁺, calcd for $[C_{29}H_{58}O_2Si]^+$, 463.3971; found, 463.3986.

(E)-8-(Bromomethylene)-23-(tert-butyldimethylsilyloxy)-12 β -(7hydroxy-7-methyloctyl)-de-A,B-24-norcholane (29b). See 29a for reaction procedure. Reagents: (Ph₂PCH₂Br)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 (CDCl₃, 250 MHz): 5.62 (1H, s, H-7), 3.76-3.49 (2H, m, H-23), 1.20 (6H, s, Me₂C-OH), 0.91 (3H, d, J = 6.9, H-21), 0.88 (9H, s, Me₃C-Si), 0.51 (3H, s, H-18), 0.03 (6H, s, Me₂Si). ¹³C NMR (CDCl₃, 63 MHz): 144.9 (C, C-8), 96.9 (CH, C-7), 71.0 (C, C-OH), 62.1 (CH₂, C-23), 56.8 (CH), 55.8 (CH), 49.8 (CH), 48.9 (C, C-13), 44.0 (CH₂), 36.2 (CH₂), 31.1 (2 × CH₂), 30.2 (CH₂), 30.0 (CH₂), 29.6 (CH), 29.2 (2 × CH₃, Me₂C-OH), 28.2 (CH₂), 28.1 (CH₂), 26.0 $(3 \times CH_3, Me_3C-Si)$, 24.3 (CH₂), 22.5 (CH₂), 22.0 (CH₃), 21.4 (CH_2) , 18.3 (C, C-Si), 9.7 (CH₃), -5.3 (2 × CH₃, Me₂Si). IR (film, cm⁻¹): 3369 ($\nu_{\rm O-H}$), 3084 ($\nu_{\rm =C-H}$), 2954 ($\nu_{\rm C-H}$), 2930 ($\nu_{\rm C-H}$), 2858 $(\nu_{\rm C-H})$, 1632 $(\nu_{\rm C=C})$. 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 [C₃₀H₅₆BrOSi]⁺, 539.3284; found, 539.3275.

(E)-23-(tert-Butyldimethylsilyloxy)-12 β -(7-hydroxy-7-methyloctyl)-8-[(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)methylene]-de-Á,B-24-norcholane (6b). See 6a for reaction procedure. Reagents: PCy₃ (3 mg, 0.011 mmol) and PdCl₂(dppf)·CH₂Cl₂ (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 Pin₂B₂ (91 mg, 0.36 mmol). Product: **6b** [79 mg, 0.13 mmol, 71%, $R_f = 0.30$ (10% EtOAchexanes), colorless oil, $[\alpha]_D = +46.1$ (c = 0.2, CHCl₃)]. ¹H NMR (CDCl₃, 250 MHz): 4.88 (1H, s, H-7), 3.74-3.41 (2H, m, H-23), 1.24 $(12H, s, 2 \times Me_2C-OB)$, 1.19 (6H, s, Me₂C-OH), 0.90 (3H, d, J = 6.9, H-21), 0.87 (9H, s, Me₃C-Si), 0.49 (3H, s, H-18), 0.02 (6H, s, Me₂Si). 13 C NMR (CDCl₃, 63 MHz): 166.0 (C, C-8), 82.4 (2 × C, 2 × C-OB), 71.0 (C, C-OH), 62.2 (CH₂, C-23), 58.9 (CH), 56.8 (CH), 50.2 (CH), 49.5 (C, C-13), 43.9 (CH₂), 36.2 (CH₂), 33.2 (CH₂), 31.2 (CH_2) , 30.2 (CH_2) , 30.0 $(2 \times CH_2)$, 29.6 (CH), 29.2 $(2 \times CH_3)$ Me_2C-OH), 28.2 (CH₂), 26.0 (3 × CH₃, Me₃C-Si), 24.9 (2 × CH₃) Me₂C-OB), 24.7 (2 × CH₃, Me₂C-OB), 24.3 (CH₂), 22.7 (CH₂), 22.0 (CH_3) , 21.2 (CH_2) , 18.3 $(C_1 C-Si)$, 10.0 (CH_3) , -5.3 $(2 \times CH_3)$ Me₂Si). 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 [C₃₆H₆₈BO₃Si]⁺, 587.5028; found, 587.5031.

 1α ,23-Dihydroxy-12 β -(7-hydroxy-7-methyloctyl)-24,25,26,27-tetranorvitamin D_3 (5b). See 5a for reaction procedure. Reagents: aqueous solution of K₃PO₄ (1.6 mL, 2 M), PdCl₂(PPh₃)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 μ L, 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 (Et₂O-hexanes), $[\alpha]_{\rm D}$ = -30.0 (c = 1.2, EtOH 96%)]. ¹H NMR (CDCl₃, 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, Me₂C-OH), 0.94 (3H, d, J = 6.8, H-21), 0.49 (3H, s, H-18). ¹³C NMR (CDCl₃, 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 (CH₂, C-19), 71.1 (C, C-OH), 70.7 (CH, C-1), 66.7 (CH, C-3), 62.2 (CH₂, C-23), 57.3 (CH), 56.7 (CH), 50.1 (CH), 49.3 (C, C-13), 45.2 (CH₂, C-4), 43.9 (CH₂), 42.8 (CH₂, C-2), 36.1 (CH₂), 31.4 (CH₂), 30.2 (CH₂), 30.1 (CH), 29.9 (CH₂), 29.3 (CH₂), 29.2 ($2 \times CH_3$, Me₂C-OH), 29.0 (CH₂, C-9), 28.2 (CH₂), 24.3 (CH₂), 22.6 (CH₂), 22.1 (CH₃, C-21), 21.5 (CH₂), 10.0 (CH₃, C-18). IR (KBr, cm⁻¹): 3396 ($\nu_{\rm O-H}$), 2930 ($\nu_{\rm C-H}$), 2871 ($\nu_{\rm C-H}$), 1649 ($\nu_{\rm C=C}$). MS ([ESI-TOF]⁺, m/z, %): 525 ([M + Na]⁺, 100), 467 ([M - OH - H₂O]⁺, 58). HRMS: [ESI-TOF]⁺, calcd for [C₃₂H₅₄O₄Na]⁺, 525.3914; found, 525.3913. UV (96% EtOH): $\lambda_{max} = 264$ nm, $\lambda_{min} = 230$ 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]. ¹H NMR (CDCl₃, 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 $(6H, s, Me_2C-OH), 0.80 (3H, d, J = 6.8, H-21), 0.74 (3H, s, H-18).$ ¹³C NMR (CDCl₃, 63 MHz): 144.5 (C, Ar-SO₂), 133.0 (C), 129.7 (2 × CH, Ar-H), 127.7 (2 × CH, Ar-H), 70.8 (C, C-OH), 69.8 (CH₂, C-23), 68.7 (CH, C-8), 56.8 (CH), 53.5 (CH), 49.6 (CH), 45.4 (C, C-13), 43.8 (CH₂), 33.9 (CH₂), 32.5 (CH₂), 31.2 (CH₂), 30.1 (CH₂), 29.8 (CH₂), 29.0 (2 × CH₃, Me₂C-OH), 28.9 (CH), 28.1 (CH₂), 24.2 (CH₂), 23.8 (CH₂), 22.3 (CH₂), 21.8 (CH₃, Me-Ar), 21.5 (CH₃), 20.8 (CH₂), 11.2 (CH₃). IR (film, cm⁻¹): 3545 (ν_{O-H}), 3425 (ν_{O-H}), 2930 $(\nu_{\rm C-H})$, 2860 $(\nu_{\rm C-H})$, 1176 $(\nu_{\rm O=S=O})$. MS ([FAB]⁺, m/z, %): 545 $([M + Na]^+, 4), 487 ([M - OH - H_2O]^+, 25), 315 ([M - T_sO - H_2O]^+, 25))$ 2H,O]⁺, 31). HRMS: [FAB]⁺, calcd for [C₃₀H₅₀O₅SNa]⁺, 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]. ¹H NMR (CDCl₂, 250 MHz): 3.96 (1H, broad s, H-8), 2.39-2.16 (2H, m, H-23), 1.14 (6H, s, Me₂C-OH), 0.90 (3H, d, J = 6.8, H-21), 0.82 (3H, s, H-18). ¹³C NMR (CDCl₃, 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 (CH₂), 34.0 (CH₂), 32.3 (CH), 31.4 (CH₂), 30.2 (CH₂), 29.9 (CH₂), 29.2 (CH₂), 29.1 (2 × CH₃, Me₂C-OH), 28.2 (CH₂), 24.3 (CH₂), 23.5 (CH₂), 22.4 (CH₂), 21.4 (CH₃), 20.8 (CH₂), 15.9 (CH₂), 11.4 (CH₃). 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 [C₂₄H₄₂NO]⁺, 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 CH₂Cl₂ (2.01 mL, 1 M, 2.01 mmol) and CH₂Cl₂ (5 mL), 26b (152 mg, 0.402 mmol) in CH₂Cl₂ (5 mL) and aqueous HCl (10 mL, 3 M) in Et₂O (10 mL), then THF (6 mL) and diisobutylaluminium hydride in CH₂Cl₂ (2.01 mL, 1 M, 2.01 mmol). Product: 8c [95 mg, 0.248 mmol, 61%, $R_{\rm f} = 0.38$ (70% EtOAc-hexanes), white foam, $[\alpha]_{\rm D} =$ +38.4 (c = 0.2, CHCl₃)]. ¹H NMR (CDCl₃, 250 MHz): 4.01 (1H, broad d, J = 1.4, H-8), 3.71-3.51 (2H, m, H-24), 1.19 (6H, s, Me₂C-OH), 0.91 (3H, d, J = 6.8, H-21), 0.83 (3H, s, H-18). ¹³C NMR (CDCl₃, 63 MHz): 71.0 (C, C-OH), 69.0 (CH, C-8), 63.1 (CH₂, C-24), 57.5 (CH), 53.6 (CH), 49.8 (CH), 45.4 (C, C-13), 43.9 (CH₂), 33.9 (CH₂), 32.8 (CH), 31.7 (CH₂), 31.3 (CH₂), 30.1 (CH₂), 29.9 (CH₂), 29.6 (CH₂), 29.1 (2 × CH₃, Me₂C-OH), 28.1 (CH₂), 24.3 (CH₂), 23.5 (CH₂), 22.5 (CH₂), 22.3 (CH₃), 20.8 (CH₂), 11.3 (CH₃). 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-8\beta-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]. ¹H NMR (CDCl₃, 250 MHz): 4.01 (1H, broad d, J = 1.2, H-8), 3.64-3.52 (2H, m, H-24), 1.20 (6H, s, Me₂C-OH), 0.93-0.82 (15H, m, H-18, H-21 and Me₃C-Si), 0.04 (6H, s, Me₂Si). ¹³C NMR (CDCl₃, 63 MHz): 71.0 (C, C-OH), 69.1 (CH, C-8), 63.5 (CH₂, C-24), 57.6 (CH), 53.8 (CH), 49.9 (CH), 45.4 (C, C-13), 44.0 (CH₂), 34.0 (CH₂), 32.8 (CH), 32.0 (CH₂), 31.3 (CH₂), 30.2 (CH₂), 30.0 (CH₂), 29.6 (CH₂), 29.1 (2 × CH₃, Me₂C-OH), 28.2 (CH₂), 25.9 (3 × CH₃, Me₃C-Si), 24.3 (CH₂), 23.5 (CH₂), 22.6 (CH₂), 22.3 (CH₃), 20.8 (CH₂), 18.3 (C, C-Si), 11.3 (CH₃), -5.3 (2 × CH₃, Me₂Si). IR (film, cm⁻¹): 3400 $(\nu_{\rm O-H})$, 2930 $(\nu_{\rm C-H})$, 2858 $(\nu_{\rm C-H})$. MS ([CI]⁺, m/z, %): 479 ([M - $OH^{+}_{,}$ 53), 461 ([M - OH - H₂O]⁺, 18), 329 ([M - 2H₂O -

TBSO], 100). HRMS: [CI]⁺, calcd for [C₃₀H₅₉O₂Si]⁺, 479.4284; found, 479.4291.

24-(tert-Butyldimethylsilyloxy)-12 β -(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 CH₂Cl₂ (8 mL). Product: ketone 28c [80 mg, 0.162 mmol, 93%, $R_f = 0.62$ (60% EtOAc-hexanes), colorless oil]. ¹H NMR (CDCl₃, 250 MHz): 3.61-3.48 (2H, m, H-24), 1.17 (6H, s, Me₂C-OH), 0.91 (3H, d, I = 6.8, H-21), 0.84 (9H, s, Me₂C-Si), 0.58 (3H, s, H-18), 0.00 (6H, s, Me₂Si). ¹³C NMR (CDCl₃, 63 MHz): 212.5 (C, C-8), 70.8 (C, C-OH), 63.3 (CH₂, C-24), 62.2 (CH), 57.1 (CH), 52.4 (C, C-13), 48.9 (CH), 43.9 (CH₂), 40.4 (CH₂), 33.0 (CH), 31.8 (CH₂), 30.7 (CH₂), 30.1 (CH₂), 29.8 (CH₂), 29.6 (CH₂), 29.1 (2 × CH₃, Me₂C-OH), 29.0 (CH₂), 28.2 (CH₂), 25.8 (3 × CH₃, Me₃C-Si), 24.2 (CH₂), 21.9 (CH₃), 21.1 (CH₂), 19.3 (CH₂), 18.2 (C, C-Si), 10.1 (CH_3) , -5.3 (2 × CH₃, Me₂Si). IR (film, cm⁻¹): 3460 (ν_{O-H}), 2955 $(\nu_{\rm C-H})$, 2930 $(\nu_{\rm C-H})$, 2858 $(\nu_{\rm C-H})$, 1717 $(\nu_{\rm C=O})$. 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)-12 β -(7hydroxy-7-methyloctyl)-de-A,B-cholane (29c). See 29a for reaction procedure. Reagents: (Ph₃PCH₂Br)Br (782 mg, 1.79 mmol) in toluene (12 mL), KOt-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]. ¹H NMR (CDCl₃, 250 MHz): 5.61 (1H, s, H-7), 3.61-3.48 (2H, m, H-24), 1.20 (6H, s, Me₂C-OH), 0.92 (3H, d, J = 6.9, H-21), 0.88 (9H, s, Me₃C-Si), 0.49 (3H, s, H-18), 0.03 (6H, s, Me₂Si). ¹³C NMR (CDCl₃, 63 MHz): 144.9 (C, C-8), 97.0 (CH, C-7), 71.0 (C, C-OH), 63.5 (CH₂, C-24), 56.7 (CH), 56.3 (CH), 49.5 (CH), 48.7 (C, C-13), 44.0 (CH₂), 33.6 (CH), 32.0 (CH₂), 31.2 (CH₂), 31.1 (CH₂), 30.2 (CH₂), 30.0 (CH₂), 29.2 (2 × CH₃, Me₂C-OH), 29.0 (CH₂), 28.3 (CH₂), 28.2 (CH₂), 25.9 (3 × CH₃, Me₃C-Si), 24.3 (CH₂), 22.3 (CH₂), 22.0 (CH₃), 21.2 (CH₂), 18.3 (C, C-Si), 9.6 (CH₃), -5.3 (2 × CH₃, Me₂Si). 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 -C₆H₁₈BrO₂Si]⁺, 100). HRMS: [CI]⁺, calcd for [C₃₁H₅₈BrOSi]⁺, 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: PCy₃ (2 mg, 0.008 mmol) and PdCl₂(dppf)·CH₂Cl₂ (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 Pin₂B₂ (71 mg, 0.28 mmol). Product: 6c [61 mg, 0.10 mmol, 69%, $R_f = 0.28$ (15% EtOAc-hexanes), colorless oil]. ¹H NMR (CDCl₃, 250 MHz): 4.89 (1H, s, H-7), 3.62–3.52 (2H, m, H-24), 1.25 (12H, s, 2 × Me₂C-BO), 1.20 (6H, s, Me₂C-OH), 0.94-0.85 (12H, m, H-21 and Me₃C-Si), 0.47 (3H, s, H-18), 0.03 (6H, s, Me₂Si). ¹³C NMR (CDCl₃, 63 MHz): 166.1 (C, C-8), 82.5 ($2 \times C$, 2 × C-OB), 71.0 (C, C-OH), 63.6 (CH₂, C-24), 57.4 (CH), 56.3 (CH), 50.0 (CH), 49.4 (C, C-13), 44.0 (CH₂), 33.7 (CH), 33.2 (CH₂), 32.0 (CH_2) , 31.3 (CH_2) , 30.2 $(2 \times CH_2)$, 30.0 (CH_2) , 29.2 $(2 \times CH_3)$ Me₂C-OH), 29.0 (CH₂), 28.2 (CH₂), 25.9 (3 × CH₃, Me₃C-Si), 24.9 $(2 \times CH_3, Me_2C-OB)$, 24.8 $(2 \times CH_3, Me_2C-OB)$, 24.3 (CH_2) , 22.5 (CH₂), 22.0 (CH₃), 21.0 (CH₂), 18.3 (C, C-Si), 9.9 (CH₃), -5.3 (2 × CH₃, Me₂Si). 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 – C₁₂H₂₈BO₄Si]⁺, 29). HRMS: [CI]⁺, calcd for [C₃₇H₇₀BO₃Si]⁺, 601.5187; found, 601.5197.

1α,24-Dihydroxy-12β-(7-hydroxy-7-methyloctyl)-25,26,27-trinorvitamin D₃ (**5c**). See **5a** for reaction procedure. Reagents: aqueous solution of K₃PO₄ (1.2 mL, 2 M), PdCl₂(PPh₃)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% EtOAchexanes), white solid, mp 88–92 °C (Et₂O-hexanes), [α]_D = -20.4 (c= 0.5, EtOH 96%)]. ¹H NMR (CDCl₃, 400 MHz): 6.34 (1H, d, J =

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, J₁ = 4.2, J₂ = 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), 2.30 (1H, dd, $J_1 = 6.5$, $J_2 = 13.2$, H-4), 2.30 (1H, dd, $J_1 = 6.5$, $J_2 = 13.2$, H-4), 2.30 (1H, dd, $J_1 = 6.5$, $J_2 = 13.2$, H-4), 2.30 (1H, dd, $J_1 = 6.5$, $J_2 = 13.2$, H-4), 2.30 (1H, dd, $J_1 = 6.5$, $J_2 = 13.2$, H-4), 2.30 (1H, dd, $J_1 = 6.5$, $J_2 = 13.2$, H-4), 2.30 (1H, dd, $J_1 = 6.5$, $J_2 = 13.2$, H-4), 2.30 (1H, dd, $J_1 = 6.5$, $J_2 = 13.2$, H-4), 2.30 (1H, dd, $J_1 = 6.5$, $J_2 = 13.2$, H-4), 2.30 (1H, dd, $J_1 = 6.5$, $J_2 = 13.2$, H-4), 2.30 (1H, dd, $J_1 = 6.5$, $J_2 = 13.2$, H-4), 2.30 (1H, dd, $J_1 = 6.5$, $J_2 = 13.2$, H-4), 2.30 (1H, dd, $J_1 = 6.5$, $J_2 = 13.2$, H-4), 2.30 (1H, dd, $J_2 = 6.5$, $J_3 = 13.2$, H-4), 2.30 (1H, dd, $J_1 = 6.5$, $J_2 = 13.2$, H-4), 2.30 (1H, dd, $J_1 = 6.5$, $J_2 = 13.2$, H-4), 2.30 (1H, dd, $J_1 = 6.5$, $J_2 = 13.2$, H-4), 2.30 (1H, dd, J_2 = 6.5, $J_2 = 13.2$, H-4), 2.30 (1H, dd, J_1 = 6.5, $J_2 = 13.2$, H-4), 2.30 (1H, dd, J_1 = 6.5, $J_2 = 13.2$, H-4), 2.30 (1H, dd, J_1 = 6.5, $J_2 = 13.2$, H-4), 2.30 (1H, dd, J_1 = 6.5, $J_2 = 13.2$, H-4), 2.30 (1H, dd, J_1 = 6.5, $J_2 = 13.2$, H-4), 2.30 (1H, dd, J_1 = 6.5, $J_2 = 13.2$, H-4), 2.30 (1H, dd, J_1 = 6.5, $J_2 = 13.2$, H-4), 2.30 (1H, dd, J_1 = 6.5, $J_2 = 13.2$, H-4), 2.30 (1H, dd, J_1 = 6.5, $J_2 = 13.2$, H-4), 2.30 (1H, dd, J_1 = 6.5, $J_2 = 13.2$, H-4), 2.30 (1H, dd, J_1 = 6.5, $J_2 = 13.2$, H-4), 2.30 (1H, dd, J_1 = 6.5, $J_2 = 13.2$, H-4), 2.30 (1H, dd, J_1 = 6.5, $J_2 = 13.2$, J_2 13.2, H-4), 1.21 (6H, s, Me₂C-OH), 0.94 (3H, d, J = 6.8, H-21), 0.48 (3H, s, H-18). ¹³C NMR (CDCl₃, 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 (CH₂, C-19), 71.1 (C, C-OH), 70.7 (CH, C-1), 66.8 (CH, C-3), 63.3 (CH₂, C-24), 57.2 (CH), 57.0 (CH), 50.1 (CH), 49.3 (C, C-13), 45.2 (CH₂, C-4), 44.0 (CH₂), 42.8 (CH₂, C-2), 33.8 (CH), 31.8 (CH₂), 31.4 (CH₂), 30.2 (CH₂), 30.0 (CH₂), 29.7 (CH₂), 29.4 (CH₂), 29.2 (2 \times CH₃, Me₂C-OH), 29.0 (CH₂, C-9), 28.2 (CH₂), 24.3 (CH₂), 22.5 (CH₂), 22.0 (CH₃, C-21), 21.3 (CH₂), 9.9 (CH₃, C-18). IR (KBr, cm⁻¹): 3388 ($\nu_{\rm O-H}$), 2930 ($\nu_{\rm C-H}$), 2861 ($\nu_{\rm C-H}$), 1632 ($\nu_{\rm C=C}$). MS $([ESI-TOF]^+, m/z, \%): 539 ([M + Na]^+, 100), 481 ([M - OH -$ H₂O]⁺, 24). HRMS: [ESI-TOF]⁺, calcd for [C₃₃H₅₆O₄Na]⁺, 539.4071; found, 539.4069. UV (96% EtOH): $\lambda_{max} = 264$ nm, $\lambda_{min} = 230$ 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-CO₂ (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)₂D₃ (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 $\mu g/\mu L$) 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 μ g 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 (24hydroxylase promoter, CYP24A1), and it is very responsive to the $1\alpha_{2}$ 25-(OH)₂D₃ 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 $1 \times$ 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 EC₅₀ 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 $1\alpha,\!25$ (OH)₂D₃. 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)₂D₃ 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. IC₅₀ 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 $1\alpha_225$ -(OH)₂D₃ 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 $C_{\rm T}$ 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 μ g/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 \pm 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 EC₅₀ and IC₅₀ values, were performed using GraphPad Prism 5 software (San Diego, CA, USA).

ASSOCIATED CONTENT

S 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, 1 α ,25-dihydroxyvitamin D₃; 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)₂D₃, 1α ,25-dihydroxyvitamin D₃; 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

REFERENCES

(1) (a) Norman, A. W.; Bouillon, R.; Adamski, J. Proceedings of the 14th Workshop on Vitamin D. *J. Steroid Biochem. Mol. Biol.* **2010**, *121*, 1–478. and the previous 13 volumes in this series. (b) *Vitamin D*, 3rd ed.; Feldman, D.; Pike, J. W.; Adams, J. S.; Eds.; Academic Press: New York, 2011. (c) Jones, G.; Strugnell, S. A.; DeLuca, H. F. Current Understanding of the Molecular Actions of Vitamin D. *Physiol. Rev.* **1998**, *78*, 1193–1231.

(2) (a) Bouillon, R.; Okamura, W. H.; Norman, A. W. Structure– Function Relationships in the Vitamin D Endocrine System. *Endocr. Rev.* 1995, *16*, 200–257. (b) Beckman, M. J.; DeLuca, H. F. Modern View of Vitamin D₃ and its Medicinal Uses. *Prog. Med. Chem.* 1998, *35*, 1–56. (c) von Essen, M. R.; Kongsbak, M.; Schjerling, P.; Olgaard, K.; Odum, N.; Geisler, C. Vitamin D Controls T Cell Antigen Receptor Signaling and Activation of Human T Cells. *Nature Immunol.* 2010, *11*, 344–349. (d) Campbell, M. J.; Adorini, L. The Vitamin D Receptor as a Therapeutic Target. *Expert Opin. Ther. Targets* 2006, *10*, 735–748. (3) Eelen, G.; Gysemans, C.; Verlinden, L.; Vanoirbeek, E.; De Clercq, P.; Van Haver, D.; Mathieu, C.; Bouillon, R.; Verstuyf, A. Mechanism and Potential of the Growth-Inhibitory Actions of Vitamin D and Analogues. *Curr. Med. Chem.* 2007, *14*, 1893–1910.

(4) (a) Stein, M. S.; Wark, J. D. An Update on the Therapeutic Potential of Vitamin D Analogues. *Expert Opin. Invest. Drugs* 2003, 12, 825–840. (b) Kabat, M. M.; Radinov, R. The Practical Synthesis of Vitamin D Analogues: A Challenge for Process Research. Curr. Opin. Drug Discov. Dev. 2001, 4, 808–833. (c) Carlberg, C.; Mouriño, A. New Vitamin D Receptor Ligands. Expert Opin. Ther. Pat. 2003, 13, 761–772. (d) Nagpal, S.; Na, S.; Rathnachalam, R. Noncalcemic Actions of Vitamin D Receptor Ligands. Endocr. Rev. 2005, 26, 662– 687. (e) Carlberg, C.; Molnár, F. Current Status of Vitamin D Signaling and its Therapeutic Applications. Curr. Top. Med. Chem. 2012, 12, 528–547.

(5) (a) Vitamin D: Molecular Biology, Physiology, and Clinical Applications; Holick, M. F., Ed.; Humana: Totowa, NJ, 1999. (b) Prüfer, K.; Racz, A.; Lin, G. C.; Barsony, J. Dimerization with Retinoid X Receptors Promotes Nuclear Localization and Subnuclear Targeting of Vitamin D Receptors. J. Biol. Chem. 2000, 275, 41114–41123. (c) Jurutka, P. W.; Whitfield, G. K.; Hsieh, J.-C.; Thompson, P. D.; Haussler, C. A.; Haussler, M. R. Molecular Nature of the Vitamin D Receptor and its Role in Regulation of Gene Expression. Rev. Endocr. Metab. Disord. 2001, 2, 203–216. (d) Christakos, S.; Dhawan, P.; Benn, B.; Porta, A.; Hediger, M.; Oh, G. T.; Jeung, E.-B.; Zhong, Y.; Ajibade, D.; Dhawan, K.; Joshi, S. Vitamin D—Molecular Mechanism of Action. Ann. N. Y. Acad. Sci. 2007, 1116, 340–348.

(6) (a) Moras, D.; Gronemeyer, H. The Nuclear Receptor Ligand-Binding Domain: Structure and Function. *Curr. Opin. Cell Biol.* **1998**, *10*, 384–391. (b) Huang, P.; Chandra, V.; Rastinejad, F. Structural Overview of the Nuclear Receptor Superfamily: Insights into Physiology and Therapeutics. *Annu. Rev. Physiol.* **2010**, *72*, 247–272. (7) (a) Robyr, D.; Wolffe, A.; Wahli, W. Nuclear Hormone Receptor Coregulators in Action: Diversity for Shared Tasks. *Mol. Endocrinol.* **2000**, *14*, 329–347. (b) Kim, S.; Shevde, N. K.; Pike, J. W. 1,25-Dihydroxyvitamin D₃ Stimulates Cyclic Vitamin D Receptor/Retinoid X Receptor DNA-Binding, Co-activator Recruitment, and Histone Acetylation in Intact Osteoblasts. *J. Bone Miner. Res.* **2005**, *20*, 305– 317. (c) Carlberg, C.; Molnár, F. Current Status of Vitamin D Signaling and its Therapeutic Applications. *Curr. Top. Med. Chem.* **2012**, *12*, 528–547.

(8) Ordóñez-Morán, P.; Muñoz, A. Nuclear Receptors: Genomic and Non-Genomic Effects Converge. *Cell Cycle* **2009**, *8*, 1675–1680.

(9) Haussler, M. R.; Jurutka, P. W.; Mizwicki, M.; Norman, A. W. Vitamin D Receptor (VDR)-Mediated Actions of 1α ,25(OH)₂Vitamin D₃: Genomic and Non-Genomic Mechanisms. *Best Pract. Res., Clin. Endocrinol. Metab.* **2011**, *25*, 543–559.

(10) PDB ID: 1DB1. Rochel, N.; Wurtz, J. M.; Mitschler, A.; Klaholz, B.; Moras, D. The Crystal Structure of the Nuclear Receptor for Vitamin D Bound to its Natural Ligand. *Mol. Cell* **2000**, *5*, 173–179. (11) (a) Carlberg, C.; Molnár, F.; Mouriño, A. Vitamin D Receptor Ligands: The Impact of Crystal Structures. *Expert Opin. Ther. Pat.* **2012**, *22*, 417–435. (b) Berman, H. M.; Henrick, K.; Nakamura, H. Announcing the Worldwide Protein Data Bank. *Nature Struct. Biol.* **2003**, *10*, 980–980, www.wwpdb.org.

(12) (a) Pérez-García, X.; Rumbo, A.; Larriba, M. J.; Ordóñez, P.; Muñoz, A.; Mouriño, A. The First Locked Side-Chain Analogues of Calcitriol (1 α ,25-Dihydroxyvitamin D₃) Induce Vitamin D Receptor Transcriptional Activity. Org. Lett. 2003, 5, 4033-4036. (b) Rochel, N.; Hourai, S.; Pérez-García, X.; Rumbo, A.; Mouriño, A.; Moras, D. Crystal Structure of the Vitamin D Nuclear Receptor Ligand Binding Domain in Complex with a Locked Side Chain Analogue of Calcitriol. Arch. Biochem. Biophys. 2007, 460, 172-176. (c) González-Avión, X. C.; Mouriño, A.; Rochel, N.; Moras, D. Novel 1*a*,25-Dihydroxyvitamin D₃ Analogues with the Side Chain at C12. J. Med. Chem. 2006, 49, 1509-1516. (d) Hourai, S.; Rodrigues, L. C.; Antony, P.; Reina-San-Martin, B.; Ciesielski, F.; Magnier, B. C.; Schoonjans, K.; Mouriño, A.; Rochel, N.; Moras, D. Structure-Based Design of a Superagonist Ligand for the Vitamin D Nuclear Receptor. Chem. Biol. 2008, 15, 383-392. (e) Antony, P.; Sigüeiro, R.; Huet, T.; Sato, Y.; Ramalanjaona, N.; Rodrigues, L. C.; Mouriño, A.; Moras, D.; Rochel, N. Structure-Function Relationships and Crystal Structures of the Vitamin D Receptor Bound 2α -Methyl-(20S,23S)- and 2α -Methyl-(20S,23R)-epoxymethano-1a,25-dihydroxyvitamin D₃. J. Med. Chem. 2010, 53, 1159-1171. (f) Regueira, M. A.; Samanta, S.; Malloy, P. J.; Ordóñez-Morán, P.; Resende, D.; Sussman, F.; Muñoz, A.;

Mouriño, A.; Feldman, D.; Torneiro, M. Synthesis and Biological Evaluation of 1 α ,25-Dihydroxyvitamin D₃ Analogues Hydroxymethylated at C-26. J. Med. Chem. **2011**, 54, 3950–3962. (g) Verlinden, L.; Verstuyf, A.; Eelen, G.; Bouillon, R.; Ordóñez-Morán, P.; Larriba, M. J.; Muñoz, A.; Rochel, N.; Sato, Y; Moras, D.; Maestro, M.; Seoane, S.; Domínguez, F.; Eduardo-Canosa, S.; Nicoletti, D.; Moman, E.; Mouriño, A. Synthesis, Structure, and Biological Activity of des-Side Chain Analogues of 1 α ,25-Dihydroxyvitamin D₃ with Substituents at C18. ChemMedChem **2011**, 6, 788–793. (h) Fraga, R.; Zacconi, F.; Sussman, F.; Ordóñez-Morán, P.; Muñoz, A.; Huet, T.; Molnar, F.; Moras, D.; Rochel, N.; Maestro, M.; Mouriño, A. Design, Synthesis, Evaluation, and Structure of Vitamin D Analogues with Furan Side Chains. Chem.—Eur. J. **2012**, 18, 603–612.

(13) Eduardo-Canosa, S.; Fraga, R.; Sigüeiro, R.; Marco, M.; Rochel, N.; Moras, D.; Mouriño, A. Design and Synthesis of Active Vitamin D Analogues. J. Steroid. Biochem. Mol. Biol. 2010, 121, 7–12.

(14) González-Avión, X. C.; Mouriño, A. Functionalization at C-12 of 1α ,25-Dihydroxyvitamin D₃ Strongly Modulates the Affinity for the Vitamin D Receptor (VDR). *Org. Lett.* **2003**, *5*, 2291–2293.

(15) Norman, A. W.; Manchand, P. S.; Uskokovic, M. R.; Okamura, W. H.; Takeuchi, J. A.; Bishop, J. E.; Hisatake, J.-I.; Koeffler, H. P.; Peleg, S. Characterization of a Novel Analogue of 1α ,25(OH)₂-Vitamin D₃ with Two Side Chains: Interaction with its Nuclear Receptor and Cellular Actions. *J. Med. Chem.* **2000**, *43*, 2719–2730.

(16) (a) Lee, H. J.; Paul, S.; Atalla, N.; Thomas, P. E.; Lin, X. J.; Yang, I.; Buckley, B.; Lu, G.; Zheng, X.; Lou, Y. R.; Conney, A. H.; Maehr, H.; Adorini, L.; Uskokovic, M.; Suh, N. Gemini Vitamin D Analogues Inhibit Estrogen Receptor-Posistive and Estrogen Receptor-Negative Mammary Tumorigenesis without Hypercalcemic Toxicity. *Cancer Prev. Res.* **2008**, *1*, 476–484. (b) Maehr, H.; Lee, H. J.; Perry, B.; Suh, N.; Uskokovic, M. R. Calcitriol Derivatives with Two Different Side Chains at C-20. V. Potent Inhibitors of Mammary Carcinogenesis and Inducers of Leukemia Differentiation. *J. Med. Chem.* **2009**, *52*, 5505–5519.

(17) (a) Tocchini-Valentini, G.; Rochel, N.; Wurtz, J. M.; Mitschler, A.; Moras, D. Crystal Structures of the Vitamin D Receptor Complexed to Superagonist 20-epi Ligands. Proc. Natl. Acad. Sci. U. S. A. 2001, 98, 5491-5496. (PDB entries 1IE8 and 1IE9). (b) Eelen, G.; Verlinden, L.; Rochel, N.; Claessens, F.; De Clercq, P.; Vandewalle, M.; Tocchini-Valentini, G.; Moras, D.; Bouillon, R.; Verstuyf, A. Superagonistic Action of 14-Epi-analogues of 1,25-Dihydroxyvitamin D Explained by Vitamin D Receptor-Coactivator Interaction. Mol. Pharmacol. 2005, 67, 1566-1573. (PDB entry 1TXI). (c) Eelen, G.; Valle, N.; Sato, Y.; Rochel, N.; Verlinden, L.; De Clercq, P.; Moras, D.; Bouillon, R.; Muñoz, A.; Verstuyf, A. Superagonistic Fluorinated Vitamin D₃ Analogues Stabilize Helix 12 of the Vitamin D Receptor. Chem. Biol. 2008, 15, 1029-1034. (PDB entry 3DR1). (d) Shimizu, M.; Miyamoto, Y.; Takaku, H.; Matsuo, M.; Nakabayashi, M.; Masuno, H.; Udagawa, N.; DeLuca, H. F.; Ikura, T.; Ito, N. 2-Substituted-16ene-22-thia-1alpha,25-dihydroxy-26,27-dimethyl-19-norvitamin D₃ Analogues: Synthesis, Biological Evaluation, and Crystal Structure. Bioorg. Med. Chem. 2008, 16, 6949-6964. (PDB entries 2ZL9, 2ZLA, and 2ZLC). (e) Huet, T.; Maehr, H.; Lee, H. J.; Uskokovic, M. R.; Suh, N.; Moras, D.; Rochel, N. Structure-Function Study of Gemini Derivatives with Two Different Side Chains at C-20, Gemini-0072 and Gemini-0097. MedChemComm 2011, 2, 424-429 (PDB entries 301D and 301E).

(18) (a) Yamada, S.; Shimizu, M.; Yamamoto, K. Structure–Function Relationships of Vitamin D Including Ligand Recognition by the Vitamin D Receptor. *Med. Res. Rev.* **2003**, *23*, 89–115. (b) Yamamoto, K.; Inaba, Y.; Yoshimoto, N.; Choi, M.; DeLuca, H. F.; Yamada, S. 22-Alkyl-20-*epi*-1 α ,25-dihydroxyvitamin D₃ Compounds of Superagonistic Activity: Syntheses, Biological Activities and Interaction with the Receptor. *J. Med. Chem.* **2007**, *50*, 932–939.

(19) Yamamoto, K.; Abe, D.; Yoshimoto, N.; Choi, M.; Yamagishi, K.; Tokiwa, H.; Shimizu, M.; Makishima, M.; Yamada, S. Vitamin D Receptor: Ligand Recognition and Allosteric Network. *J. Med. Chem.* **2006**, *49*, 1313–1324. (20) PDB ID: 2HCD. Ciesielski, F.; Rochel, N.; Moras, D. Adaptability of the Vitamin D Nuclear Receptor to the Synthetic Ligand Gemini: Remodelling the LBP with One Side Chain Rotation. *J. Steroid Biochem. Mol. Biol.* **2007**, *103*, 235–242.

(21) Gogoi, P.; Sigüeiro, R.; Eduardo, S.; Mouriño, A. An Expeditious Route to 1α ,25-Dihydroxyvitamin D₃ and Its Analogues by an Aqueous Tandem Palladium-Catalyzed A-Ring Closure and Suzuki Coupling to the C/D Unit. *Chem.—Eur. J.* **2009**, *16*, 1432–1435.

(22) Torneiro, M.; Fall, Y.; Castedo, A.; Mouriño, A. An Efficient Route to 1α ,25-Dihydroxyvitamin D₃ Functionalized at C-11. *Tetrahedron Lett.* **1992**, 33, 105–108.

(23) Doi, T.; Hijikuro, I.; Takahashi, T. An Efficient Solid-Phase Synthesis of the Vitamin D_3 System. J. Am. Chem. Soc. 1999, 121, 6749–6750.

(24) Attempts to prepare nitrile **26b** directly from tosylate **25a** by displacement with the α -anion of acetonitrile were unsuccessful.

(25) (a) Trost, B. M.; Dumas, J.; Villa, M. New Strategies for the Synthesis of Vitamin D Metabolites via Palladium-Catalyzed Reactions. *J. Am. Chem. Soc.* **1992**, *114*, 9836–9845. (b) For an improved preparation of this ylide, see ref 21.

(26) Ishiyama, T.; Ishida, K.; Miyaura, N. Synthesis of Pinacol Arylboronates via Cross-Coupling Reaction of Bis(pinacolato)diboron with Chloroarenes Catalyzed by Palladium(0)–Tricyclohexylphosphine Complexes. *Tetrahedron* **2001**, *57*, 9813–9816.

(27) Pálmer, H. G.; González-Sancho, J. M.; Espada, J.; Berciano, M. T.; Puig, I.; Baulida, J.; Quintanilla, M.; Cano, A.; de Herreros, A. G.; Lafarga, M.; Muñoz, A. Vitamin D_3 Promotes the Differentiation of Colon Carcinoma Cells by the Induction of E-Cadherin and the Inhibition of β -Catenin Signaling. J. Cell Biol. **2001**, 154, 369–388.

(28) Kouchi, Z.; Fujiwara, Y.; Yamaguchi, H.; Nakamura, Y.; Fukami, K. Phosphatidylinositol 5-Phosphate 4-Kinase Type II Beta is Required for Vitamin D Receptor-Dependent E-Cadherin Expression in SW480 Cells. *Biochem. Biophys. Res. Commun.* **2011**, *408*, 523–529.

(29) Álvarez-Díaz, S.; Valle, N.; García, J. M.; Peña, C.; Freije, J. M.; Quesada, V.; Astudillo, A.; Bonilla, F.; Lopez-Otín, C.; Muñoz, A. Cystatin D is a Candidate Tumour Suppressor Gene Induced by Vitamin D in Human Colon Cancer Cells. J. Clin. Invest. 2009, 119, 2343–2358.

(30) Rohan, J. N.; Weigel, N. L. 1α ,25-Dihydroxyvitamin D₃ Reduces c-Myc Expression, Inhibiting Proliferation and Causing G1 Accumulation in C4–2 Prostate Cancer Cells. *Endocrinology* **2009**, *150*, 2046–2054.

(31) Shindo, K.; Kumagai, G.; Takano, M.; Sawada, D.; Saito, N.; Saito, H.; Kakuda, S.; Takagi, K.; Ochiai, E.; Horie, K.; Takimoto-Kamimura, M.; Ishizuka, S.; Takenouchi, K.; Kittaka, A. New C15-Substituted Active Vitamin D₃. *Org. Lett.* **2011**, *13*, 2852–2855.

(32) Kauffmann, G. B.; Teler, L. A. Tetrakis[iodo(tri-*n*-butylphosphine)-copper(I)] and Iodo-(2,2'-bipyridine)-(tri-*n*-butylphosphine)-copper(I). *Inorg. Synth.* **1963**, *7*, 9–12.

(33) Corey, E. J.; Schmidt, G. Useful Procedures for the Oxidation of Alcohols Involving Pyridinium Dichromate in Aprotic Media. *Tetrahedron Lett.* **1979**, *20*, 399–402.

(34) Comins, D. L.; Dehghani, A.; Foti, C. J.; Joseph, S. P. Pyridine-Derived Triflating Reagents: N-(2-Pyridyl)triflimide and N-(5-Chloro-2-pyridyl)triflimide. *Org. Synth.* **1997**, *74*, 77–83.

(35) Vassilikogiannakis, G.; Hatzimarinaki, M.; Orfanopoulos, M. Mechanism of the [2 + 2] Photocycloaddition of Fullerene C60 with Styrenes. *J. Org. Chem.* **2000**, *65*, 8180–8187.

(36) Perrin, D. D.; Amarego, W. L. F. Purification of Laboratory Chemicals; Pergamon Press: Oxford, 1988.