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

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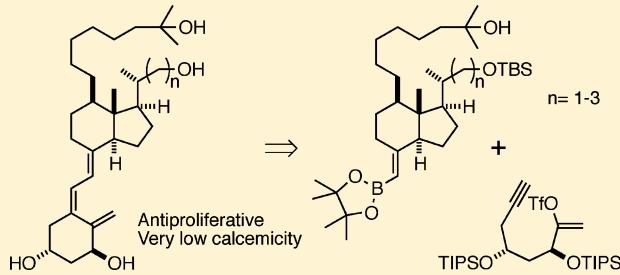
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¹² Supporting Information

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 Pd-catalyzed tandem cyclization–Suzuki coupling method. The new analogues were assayed against human colon and breast cancer cell lines and in mice. All new vitamin 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 D₃, the hormone 1α ,25-dihydroxyvitamin D₃ (1α ,25-(OH)₂D₃, 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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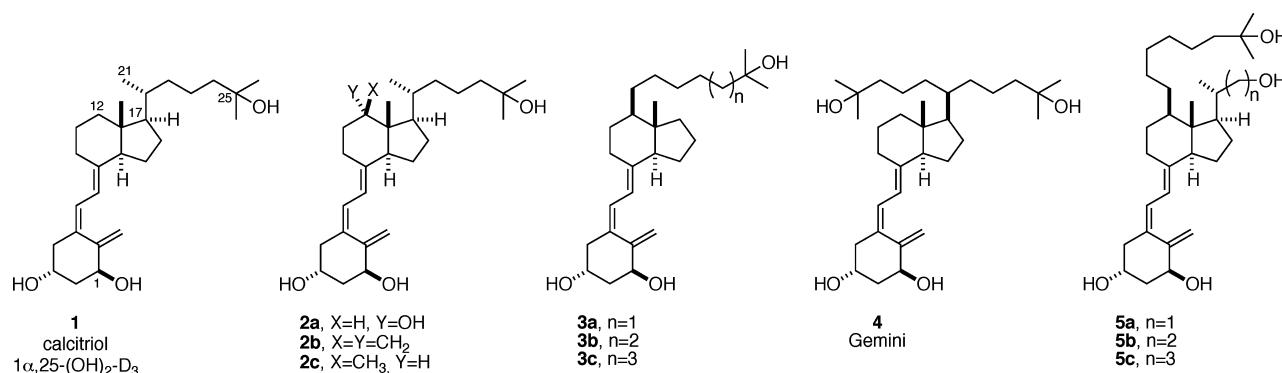


Figure 1. Structure of $1\alpha,25$ -dihydroxyvitamin D_3 and analogues **2–5**.

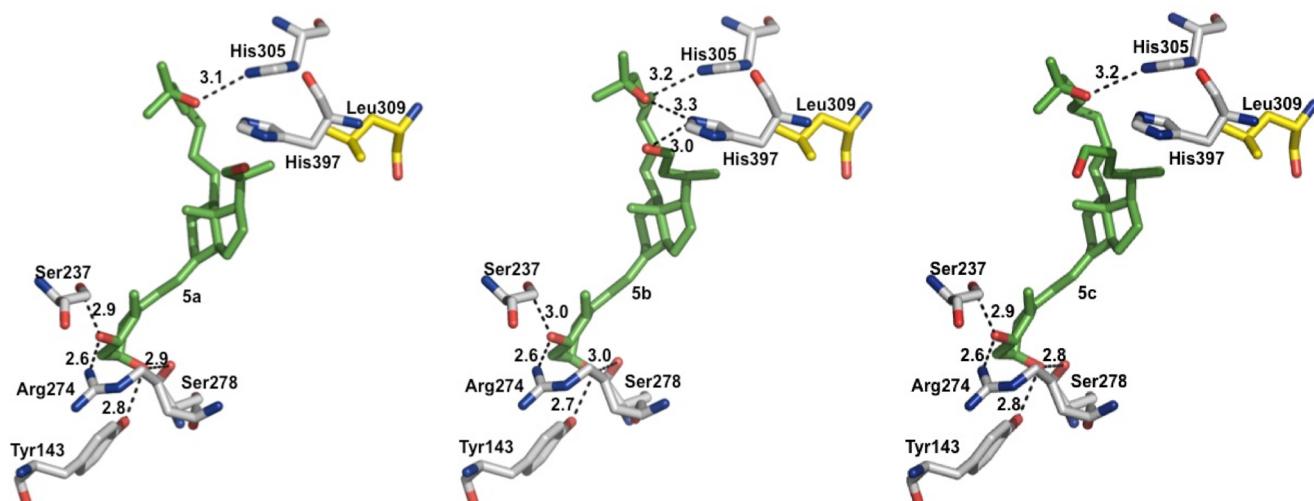


Figure 2. Predicted structures of the complexes of analogues **5a** (left), **5b** (center), and **5c** (right) with the Moras VDR(LBD). Ligand conformation and interactions with Leu309 and with hydrogen-bond forming residues are shown. Distances are measured in angstroms. Nitrogens are depicted in blue,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

72 substituents at C12 and compounds **3a–c**^{12c} in which the side
 73 chain has been moved to C12 (Figure 1). In particular, the
 74 VDR binding affinity of **2c** is 4.4 times higher than that of
 75 1,25D, and the affinities of analogues **3a–c** for the VDR are
 76 60–71% as compared with the natural hormone 1,25D (100%),
 77 analogue **3b** being the most active in transactivation studies
 78 (20% compared with 1,25D). We have accordingly used **3b** as
 79 the starting point for the development of new analogues in this
 80 series with improved biological potency. Inspired by the
 81 interesting biological profile of Gemini, a 1,25D analogue with
 82 two hydroxylated side chains (**4**, Figure 1),¹⁵ and related
 83 compounds,¹⁶ we developed the new analogues **5a–c** (Figure
 84 1), which like **3b** bear a 7C-long side chain at C12 and also
 85 have a second shorter hydroxylated chain at C17. We describe
 86 here their design on the basis of docking studies, their
 87 syntheses, and their biological evaluation.

RESULTS AND DISCUSSION

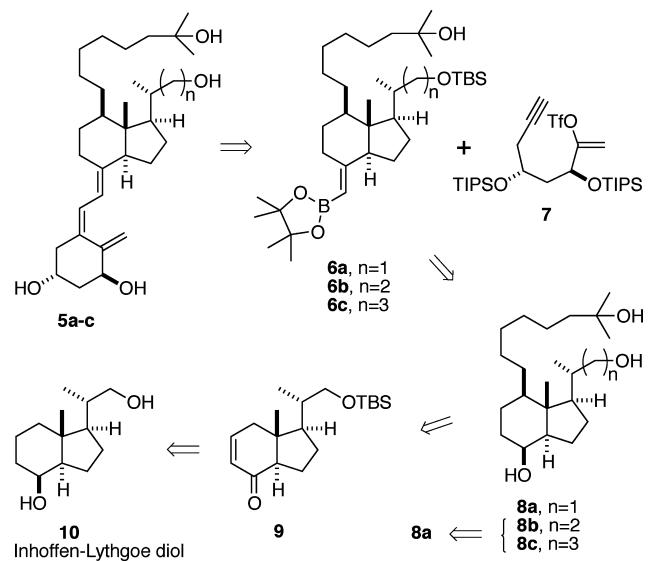
88 **Design.** The new vitamin D₃ analogues **5a–c** were designed
 89 on the basis of computational docking studies using the LBD
 90 derived from the X-ray crystal structure of Moras VDR(LBD)-
 91 1,25D complex and the biological activity of analogues **3**. In
 92 previous docking studies, we found that the rings and triene
 93 system of analogue **3b** adopt the same conformation as the
 94 natural hormone 1,25D in the binding pocket. Interactions of
 95

the **3b** side chain with the protein are similar to those of 1,25D⁹⁶ except with Leu309 and decreased interactions with His305 and⁹⁷ His397.^{12c} The observation of a free space around position⁹⁸ C17, originated by the removal of the natural side chain in **3b**,⁹⁹ led us to envisage that the agonist conformation of the ligand—¹⁰⁰ VDR complex might receive additional stabilization from the¹⁰¹ attachment of a second small side chain at C17. Structural¹⁰² studies of several superagonist analogues of 1,25D in complex¹⁰³ with the VDR have shown their superagonism to be due to¹⁰⁴ additional stabilization of helix H12 and stronger interactions¹⁰⁵ with transcription coactivators.^{17,18} Accordingly, we modified¹⁰⁶ **3b** as analogues **5a–c**, in which C17 bears the Inhoffen—¹⁰⁷ Lythgoe diol side chain fragment or a higher homologue¹⁰⁸ (Figure 2). In contrast to **3b**, the three new analogues have a¹⁰⁹ f2 21-methyl group as 1,25D for interaction with Leu309, which¹¹⁰ plays a key role in ligand-mediated protein folding and¹¹¹ therefore in the maintenance of the active conformation of¹¹² the VDR,¹⁹ and they each also hold an extra hydroxyl group for¹¹³ additional polar contacts with the receptor. Docking calcu-¹¹⁴ lations show that analogues **5a–c** form stronger complexes with¹¹⁵ Moras VDR(LBD) than the natural ligand 1,25D in the order:¹¹⁶ **5b** > **5c** > **5a**. In all the complexes, the hydrophobic interaction¹¹⁷ of the 21-methyl group with the side chain of the Leu309¹¹⁸ amino acid residue is restored, the ring A hydroxyl groups form¹¹⁹ hydrogen bonds with the same amino acid residues as the¹²⁰ natural ligand,¹⁰ and His305 is within hydrogen bonding¹²¹

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 Gemini¹⁸ and the superagonist Gemini72^{17e,20} analogues, in complex with a wild-type zebrafish VDR(LBD). We have previously found that 1,25D analogues that bind significantly in silico are also biologically active.¹² The promising docking results for **5a–c** accordingly encouraged us to pursue their synthesis to further study the structure–activity relationships of vitamin D analogues.

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

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



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

The key triol intermediate **8a** was prepared from the Inhoffen–Lythgoe diol (**10**) through the known unsaturated ketone **9**²³ (Scheme 2). Reduction of ketone **9** with DIBAL-H followed by protection of the resulting allylic alcohol **11** with *tert*-butyldimethylsilyl chloride provided the silyl ether **12**, which upon epoxidation from the less hindered face with *m*-chloroperbenzoic acid gave epoxide **13** (82% over the three steps). Opening of epoxide **13** with lithium diethylamide followed by hydroxyl-directed epoxidation of the resulting allylic alcohol **14** furnished the epoxide **15**, which was converted to mesylate **16** (91% over the three steps). Treatment of **16** with sodium naphthalene provided allylic

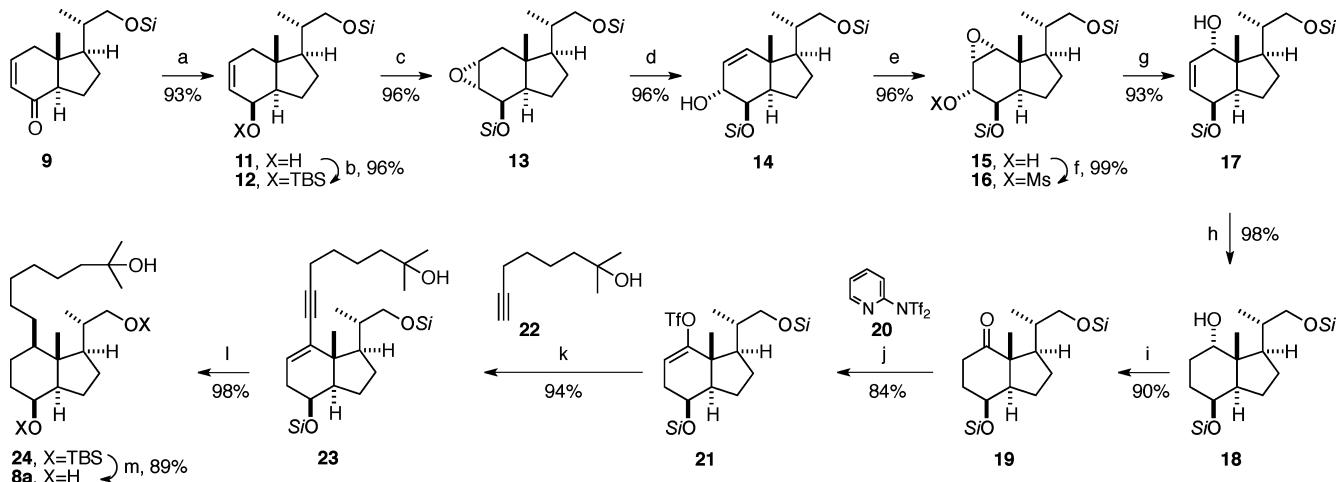
alcohol **17**, which was converted to ketone **19** by catalytic hydrogenation and pyridinium dichromate oxidation (82% over the three steps). Ketone **19** was transformed into the enoltriflate **21** in 84% yield by deprotonation with LDA and trapping of the resulting enolate with Comins triflimide (**20**). The desired triol **8a** was finally prepared in 82% yield by a three-step sequence: palladium-catalyzed coupling of **21** with alkyne **22**,¹⁴ catalytic hydrogenation of the resulting enyne **23**, and deprotection of **24** (13 steps from **9**, 44% overall yield). The structure and stereochemistry of triol **8a** was confirmed by X-ray crystallography (see Supporting Information).

With the parent triol **8a** at hand, we proceeded to the preparation of triols **8b** and **8c**, precursors of analogues **5b** and **5c**, respectively. Elongation of the residual side chain at C17 of **8a** (Scheme 3) to **8b** was accomplished in 46% yield by a three-step sequence: selective tosylation of the primary hydroxyl group, *S*_N2 displacement of the tosylate **25a** with potassium cyanide, and reduction of the nitrile **26a** with diisobutylaluminum hydride. A second homologation following the same procedure provided triol **8c** in 46% yield via tosylate **25b** and nitrile **26b**.²⁴

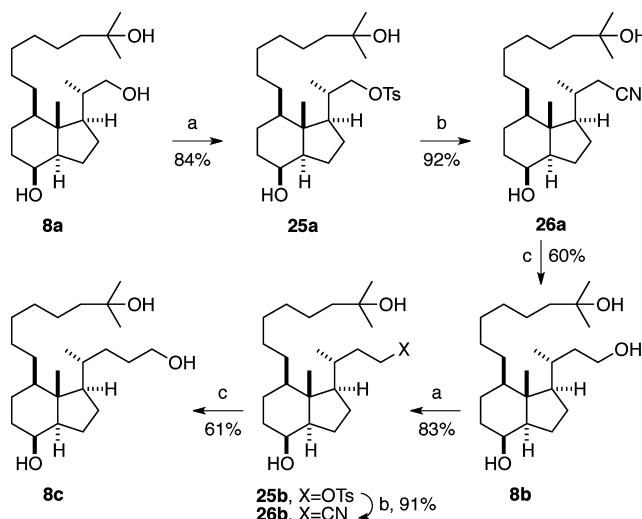
Completion of the synthesis of analogues **5a–c** is illustrated in Scheme 4. Selective silylation of the primary hydroxyl group of triols **8a–c** followed by oxidation of the secondary hydroxyl group afforded ketones **28a–c**, which were converted to the desired alkenyl bromides **29a–c** by Wittig reaction with ylide **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]-dichloro-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 **K₃PO₄** 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 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 D²⁹ with slightly less potency than $1\alpha,25$ -(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

^aReagents and conditions: (a) DIBAL-H, THF, -78 °C; (b) TBSCl, Im, DMF; (c) *m*-CPBA, CH₂Cl₂, 0 °C; (d) LiN(Et₂)₂, 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 → 0 °C; (k) 22, PdCl₂(Ph₃P)₂, CuI, Et₂NH; (l) H₂, 10% Pd-C, EtOAc; (m) TBAF, THF, Δ. Si=TBSSi(t-Bu)Me₂.

Scheme 3. Synthesis of Triols 8b and 8c^a

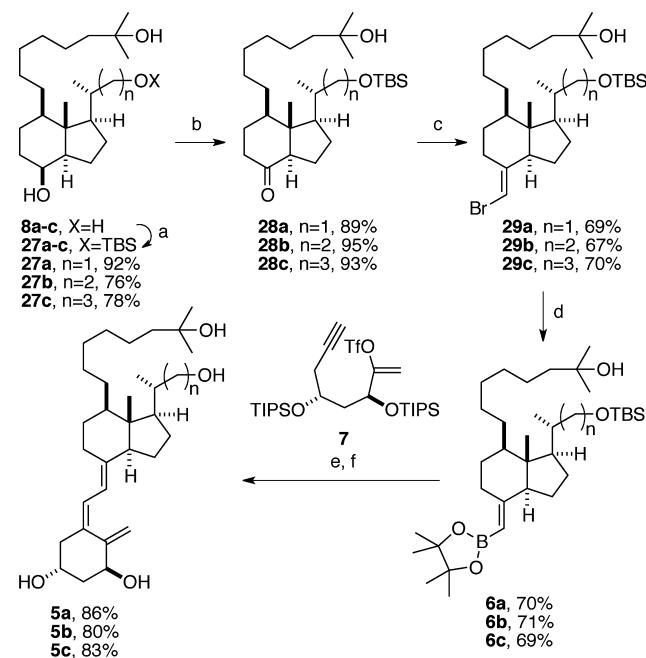
^aReagents and conditions: (a) TsCl, Py, 4 °C; (b) KCN, DMSO, 90 °C; (c) DIBAL-H, CH₂Cl₂, -5 °C, HCl 3 M/Et₂O, DIBAL-H, THF, -78 °C.

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

225 Cell proliferation was evaluated in the MCF-7 breast cancer
226 cells using the MTT assay. Compound 5a at 10⁻⁷ M
227 significantly ($P < 0.001$) decreased cell proliferation, similarly
228 to 1,25D, with respect to untreated cells. Compounds 5b and
229 5c were also able to statistically ($P < 0.05$) decrease cell
230 proliferation in relation to control cells (Figure 4B).

231 Together, the data of the biological evaluation of the
232 analogues show that 5a–c share the mechanism of action of
233 1 α ,25-(OH)₂D₃ in human cancer cells, albeit with slightly less
234 potency at low concentrations. The upregulation of E-cadherin
235 and Cystatin D expression and the prodifferentiation and
236 antiproliferative effects indicate a protective action against
237 cancer.

238 We next tested by competitive binding assay³¹ the biological
239 ability of the analogues 5a–c to bind VDR, as compared to the

Scheme 4. Synthesis of Analogs 5a–c^a

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

240 natural hormone 1. The VDR binding affinity of compounds 240
241 5a–c is approximately 10 times less than that of the 1,25D 241
(Figure 5A). 242 f5

243 The ability of the analogues to induce transcriptional 243
244 activation of a vitamin D target gene was evaluated by 244
245 transfecting MCF-7 cells with the pCYP24A1-Luc vector. The 245
246 compounds 5a–c and the natural hormone 1 induced a strong 246
247 dose-dependent activation of the VDRE, as measured by 247
248 luciferase activity. However, all analogues were less potent to 248
249 activate the reporter gene transcription than 1,25D (Figure 5B). 249

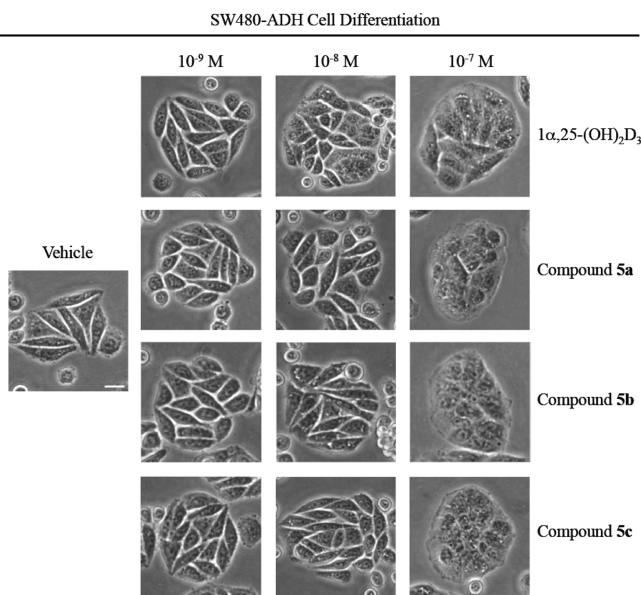


Figure 3. Activity of analogues **5a**, **5b**, and **5c** in human colon cancer cells. Phase-contrast micrographs showing the induction by analogues **5a**–**c** of a differentiated adhesive epithelial phenotype in human SW480-ADH colon cancer cells. The cells were treated with analogues **5a**–**c** 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 1 α ,25-(OH)₂D₃ analogues with substituents 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 1 α ,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 1 α ,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. Sonication was 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 H₂SO₄ (5.6 mL) followed by heating with a hot gun. Flash column chromatography was performed with Merck 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 °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 (ϕ 250 mm × 10 mm). Analogues **5a**–**c** have a purity of >95% (HPLC).

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

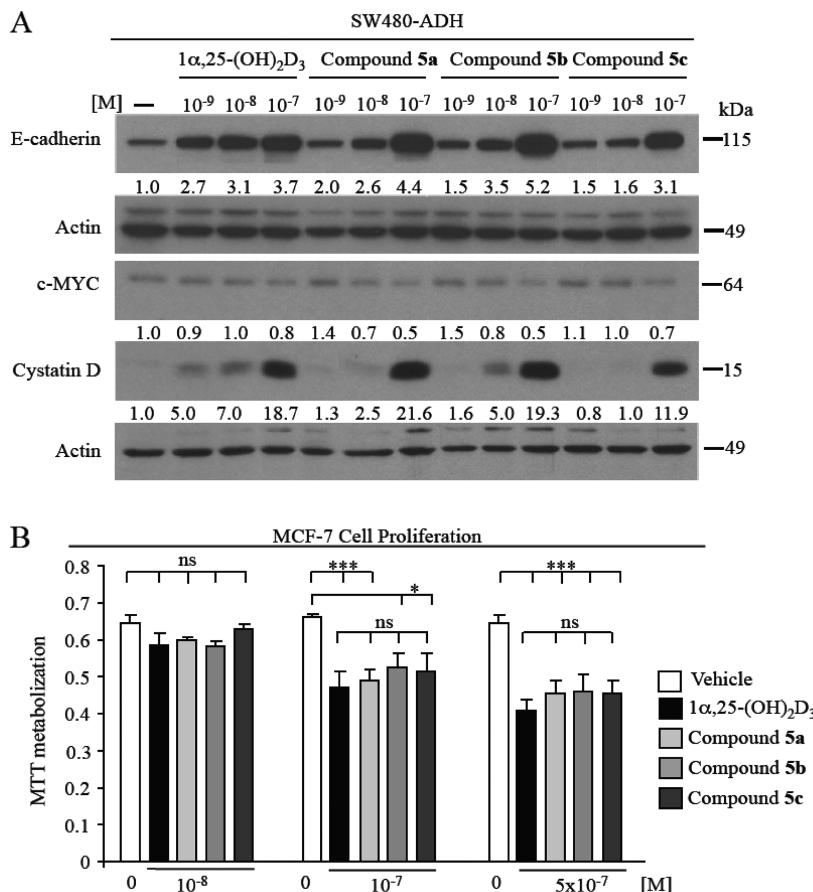


Figure 4. Activity of analogues 5a, 5b, and 5c in human colon and breast cancer cells. (A) Regulation of target genes. Western blot analysis of the induction of E-cadherin and Cystatin D and of the repression of c-MYC proteins at 48 h of treatment of SW480-ADH cells with each compound (10^{-9} to 10^{-7}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

procedure.³³ ($\text{Ph}_3\text{PCH}_2\text{Br}$)Br and *N*-(2-pyridyl)triflimide 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-8 β -ol (11). A solution of diisobutyl-aluminum hydride in CH_2Cl_2 (9 mL, 1 M, 9 mmol) was added dropwise to a solution of 9²³ (1.939 g, 6.01 mmol) in THF (30 mL) at -78°C . The reaction mixture was allowed to warm to rt. After 30 min, the reaction was quenched by slowly addition of saturated NaCl at 0 °C. The aqueous layer was extracted with EtOAc (3 × 25 mL). The combined organic layer was dried, filtered, and concentrated. The residue was purified by flash chromatography (SiO_2 , 4 cm × 10 cm, 4% EtOAc–hexanes) to afford 11 [1.808 g, 5.57 mmol, 93%, $R_f = 0.40$ (20% EtOAc–hexanes), colorless oil, $[\alpha]_D = +101.5$ ($c = 1.0, \text{CHCl}_3$)]. ¹H NMR (CDCl_3 , 250 MHz): 5.91–5.68 (2H, m, H-9 and H-11), 4.11 (1H, broad s, H-8), 3.58 (1H, dd, $J_1 = 3.5, J_2 = 9.6$, H-22), 3.28 (1H, dd, $J_1 = 7.2, J_2 = 9.6$, H-22), 0.95 (3H, d, $J = 6.4$, H-21), 0.87 (9H, s, $\text{Me}_3\text{C-Si}$), 0.81 (3H, s, H-18), 0.01 (6H, s, Me_2Si). ¹³C NMR (CDCl_3 , 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, $\text{Me}_3\text{C-Si}$), 21.2 (CH₂), 18.2 (C, C-Si), 16.5 (CH₃), 13.4 (CH₃), 5.5 (2 × CH₃, Me_2Si). IR (film, cm^{-1}): 3380 ($\nu_{\text{O-H}}$), 3022 ($\nu_{\text{C-H}}$), 2957 ($\nu_{\text{C-H}}$), 2929 ($\nu_{\text{C-H}}$). MS ([ESI-TOF]⁺, m/z , %): 347 ([M + Na]⁺, 100), 307 ([M – OH]⁺, 6). HRMS: [ESI-TOF]⁺, calcd for [C₁₉H₃₆O₂SiNa]⁺, 347.2377; found, 347.2354.

8 β ,22-Bis(*tert*-butyldimethylsilyloxy)-de-*A,B*-23,24-dinorchol-9-ene (12). Imidazole (876 mg, 12.87 mmol) and TBSCl (1.47 g, 9.75 mmol) were successively added to a solution of 11 (1.266 g, 3.90

mmol) in DMF (35 mL). After 48 h, the reaction was quenched by addition of saturated NaCl (30 mL). The aqueous layer was extracted with hexanes (3 × 15 mL). The combined organic layer was dried, filtered, and concentrated. The residue was purified by flash chromatography (SiO_2 , 3 cm × 8 cm, hexanes) to afford 12 [1.648 g, 3.76 mmol, 96%, $R_f = 0.95$ (10% EtOAc–hexanes), colorless oil, $[\alpha]_D = +143.9$ ($c = 1.9, \text{CHCl}_3$)]. ¹H NMR (CDCl_3 , 250 MHz): 5.84–5.64 (2H, m, H-9 and H-11), 4.15 (1H, broad s, H-8), 3.64 (1H, dd, $J_1 = 2.9, J_2 = 9.4$, H-22), 3.35 (1H, dd, $J_1 = 7.1, J_2 = 9.4$, H-22), 1.03 (3H, d, $J = 6.5$, H-21), 0.95 (9H, s, $\text{Me}_3\text{C-Si}$), 0.94 (9H, s, $\text{Me}_3\text{C-Si}$), 0.88 (3H, s, H-18), 0.14–0.03 (12H, m, 2 × Me_2Si). ¹³C NMR (CDCl_3 , 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 × CH₃, $\text{Me}_3\text{C-Si}$), 25.9 (3 × CH₃, $\text{Me}_3\text{C-Si}$), 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 ($\nu_{\text{C-H}}$), 2957 ($\nu_{\text{C-H}}$), 2929 ($\nu_{\text{C-H}}$). MS ([ESI-TOF]⁺, m/z , %): 461 ([M + Na]⁺, 4), 381 ([M – t-Bu]⁺, 41). HRMS: [ESI-TOF]⁺, calcd for [C₂₅H₅₀O₂Si₂Na]⁺, 461.3242; found, 461.3256.

8 β ,22-Bis(*tert*-butyldimethylsilyloxy)-de-*A,B*-23,24-dinor-9 α ,11 α -epoxycholane (13). *m*-Chloroperbenzoic acid (4.05 g, 23.49 mmol) was added in three portions (each 15 min) to a solution of 12 (3.68 g, 8.39 mmol) in CH_2Cl_2 (80 mL) at 0 °C. The mixture was stirred in the dark at 0 °C for 1.5 h and then allowed to warm to rt for 2.5 h. The reaction was quenched at 0 °C by slow addition of saturated $\text{Na}_2\text{S}_2\text{O}_3$ (50 mL). The aqueous layer was extracted with CH_2Cl_2 (3 × 50 mL). The combined organic layer was washed with saturated NaHCO_3 (2 × 405 mL).

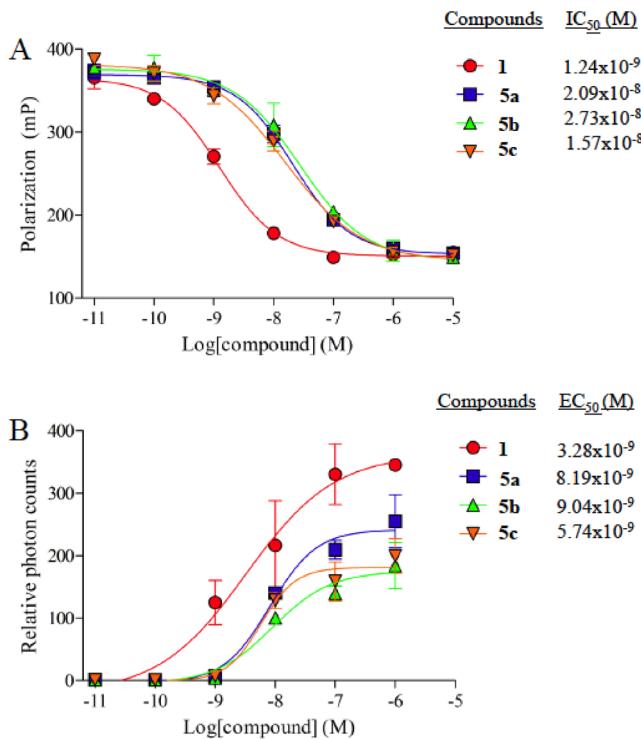


Figure 5. Vitamin D receptor binding and transactivation activities. (A) Competitive binding of $1\alpha,25-(OH)_2D_3$ (**1**) and the synthesized vitamin D₃ analogues **5a–c** to the full-length human VDR. The experiments were carried out in duplicate on two different occasions. IC_{50} values are derived from dose–response curves and represent the measure of 50% inhibition of polarization of a compound. (B) MCF-7 cells were transfected with a 24-hydroxylase gene reporter vector and then treated with 10^{-11} to 10^{-6} M concentrations of $1,25D$ or analogues **5a–c** for 48 h. The luciferase activity was then measured. The EC_{50} values are derived from dose–response curves and represent the analogue concentration capable of increasing the luciferase activity by 50%. All of the experiments were carried out in duplicate on at least two different occasions. Error bars represent standard deviation (SD).

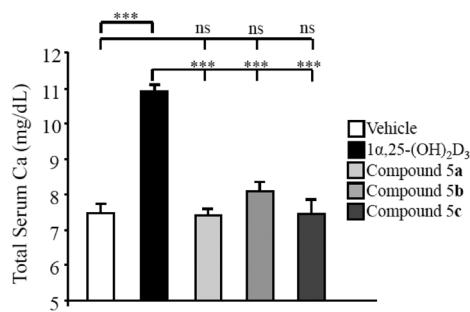


Figure 6. Calcium levels in mice treated with the natural hormone $1,25D$ and compounds **5a–c**. Six mice per group were treated with 0.3 μ g/kg of compounds **5**, $1,25D$, or vehicle every other day during 3 weeks, and calcium levels were measured on day 21. Error bars represent standard deviation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

s, H-18), 0.09 (3H, s, MeSi), 0.04 (3H, s, MeSi), 0.01 (6H, s, Me_2Si). ¹³C NMR ($CDCl_3$, 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 \times CH₃, Me_3C-Si), 25.8 (3 \times CH₃, Me_3C-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 (423 ([M + Na]⁺, 65), 455 ([M - H₂O - TBSOH]⁺, 14)). EA: calculated for $[C_{25}H_{50}O_3Si_2]$, C (66.02), H (11.08); found, C (65.94), H (11.37).

8 $\beta,22$ -Bis(tert-butylidimethylsilyloxy)-de-A,B-23,24-dinorchol-11-en-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 Et_2O (22.5 mL). After warm to rt, a solution of **13** (3.18 g, 6.99 mmol) in Et_2O (50 mL) and HMPA (8.5 mL, 49.0 mmol) were successively added via cannula. After 15 h, the reaction was quenched by the slowly addition of a few drops of saturated NH_4Cl and aqueous HCl (100 mL, 2%). The aqueous layer was extracted with MTBE (3 \times 50 mL). The combined organic layer was dried, filtered, and concentrated. The residue was purified by flash chromatography (SiO_2 , 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, $CHCl_3$)]. ¹H NMR ($CDCl_3$, 250 MHz): 6.42 (1H, d, J = 10.1, H-12), 5.56 (1H, dd, J_1 = 3.6, J_2 = 10.1, H-11), 4.06–3.92 (2H, m, H-8 and H-9), 3.58 (1H, dd, J_1 = 3.2, J_2 = 9.6, H-22), 3.28 (1H, dd, J_1 = 7.1, J_2 = 9.6, H-22), 1.07 (3H, d, J = 6.4, H-21), 0.96 (3H, s, H-18), 0.88 (18H, overlapped s, 2 \times Me_3C-Si), 0.08 (3H, s, MeSi), 0.04 (3H, s, MeSi), 0.02 (6H, s, Me_2Si). ¹³C NMR ($CDCl_3$, 63 MHz): 142.2 (CH), 125.7 (CH), 75.0 (CH), 72.8 (CH), 67.5 (CH₂, C-22), 48.1 (2 \times CH), 43.2 (C, C-13), 38.9 (CH), 28.1 (CH₂), 25.9 (3 \times CH₃, Me_3C-Si), 25.8 (3 \times CH₃, Me_3C-Si), 21.9 (CH₂), 18.3 (C, C-Si), 18.0 (C, C-Si), 17.0 (CH₃), 16.4 (CH₃), -4.9 (CH₃, MeSi), -5.2 (CH₃, MeSi), -5.4 (2 \times CH₃, MeSi). IR (neat, cm^{-1}): 3315 (ν_{O-H}), 2955 (ν_{C-H}), 2929 (451 (ν_{C-H})). MS ([ESI-TOF]⁺, m/z, %): 477 ([M + Na]⁺, 100), 437 ([M - OH]⁺, 48). EA: calcd for $[C_{25}H_{50}O_3Si_2]$, C (66.02), H (11.08); found, C (65.59), H (11.23).

8 $\beta,22$ -Bis(tert-butylidimethylsilyloxy)-de-A,B-23,24-dinorchol-11 $\alpha,12\alpha$ -epoxycholan-9 α -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 \times 50 mL). The combined organic layer was washed with saturated $NaHCO_3$ (2 \times 30 mL), saturated NaCl (30 mL), dried, filtered, and concentrated. The residue was purified by flash chromatography (SiO_2 , 4 cm \times 8 cm, 5% EtOAc–hexanes) to afford **15** [1.799 g, 3.82 mmol, 96%, R_f = 0.53 (10% EtOAc–hexanes), white solid, mp 115–118 °C (EtOAc–hexanes), $[\alpha]_D$ = +7.8 (c = 1.0, $CHCl_3$)]. ¹H NMR ($CDCl_3$, 500 MHz): 3.89 (1H, d, J = 4.1, H-9), 3.62–3.58 (2H, m, H-8 and H-22), 3.53 (1H, d, J = 4.1, H-12), 3.37 (1H, dd, J_1 = 4.2, J_2 = 4.2, H-11), 3.28 (1H, dd, J_1 = 7.4, J_2 = 9.5, H-22), 1.09 (3H, d, J = 6.3, H-21), 1.00 (3H, s, H-18), 0.89 (9H, s, Me_3C-Si), 0.88 (9H, s, Me_3C-Si), 0.07 (3H, s, MeSi), 0.03 (6H, s, Me_2Si), 0.02 (3H, s, MeSi). ¹³C NMR ($CDCl_3$, 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 \times CH₃, Me_3C-Si), 25.8 (3 \times CH₃, Me_3C-Si), 21.4 (CH₃), 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 (CH₃, MeSi), -5.4 (CH₃, MeSi). IR (neat, cm^{-1}): 3345 (ν_{O-H}), 2956 (478 (ν_{C-H})), 2929 (ν_{C-H})). MS ([ESI-TOF]⁺, m/z, %): 493 ([M + Na]⁺, 100), 453 ([M - OH]⁺, 7). HRMS: [ESI-TOF]⁺, calcd for $[C_{25}H_{48}O_4Si_2Na]^+$, 493.3140; found, 493.3147.

8 $\beta,22$ -Bis(tert-butylidimethylsilyloxy)-de-A,B-23,24-dinorchol-11 $\alpha,12\alpha$ -epoxycholan-9 α -yl methane-sulfonate (16**).** Dry triethyl-amine (1.138 mL, 8.16 mmol) was added to a solution of **15** (1.923 g, 4.08 mmol) in CH_2Cl_2 (25 mL) at -20 °C. After 15 min,

30 mL), dried, filtrated, and concentrated. The residue was purified by flash chromatography (SiO_2 , 6 cm \times 8 cm, hexanes) to afford **13** [3.65 g, 8.02 mmol, 96%, R_f = 0.75 (6% EtOAc–hexanes), white solid, mp 47–49 °C (EtOAc–hexanes), $[\alpha]_D$ = +42.0 (c = 1.4, $CHCl_3$)]. ¹H NMR ($CDCl_3$, 500 MHz): 4.20 (1H, broad d, J = 2.2, H-8), 3.54 (1H, dd, J_1 = 3.2, J_2 = 9.6, H-22), 3.25 (1H, dd, J_1 = 7.1, J_2 = 9.6, H-22), 3.16 (1H, dd, J_1 = 3.5, J_2 = 5.7, H-11), 3.07 (1H, d, J = 3.0, H-9), 2.13 (1H, dd, J_1 = 6.0, J_2 = 15.0, H-12), 1.49–1.40 (1H, m, H-20), 0.95 (3H, d, J = 6.6, H-21), 0.90 (9H, s, Me_3C-Si), 0.87 (9H, s, Me_3C-Si), 0.83 (3H,

Table 1. VDR Binding Properties, Transcriptional Activities, and Calcemic Effects of the Natural Hormone $1\alpha,25\text{-}(\text{OH})_2\text{D}_3$ (**1**), and **5a**, **5b**, and **5c** Vitamin D Analogs^a

compd	in silico binding energy kcal/mol	VDR binding		transcriptional activity		calcemic activity	
		IC ₅₀ (M)	%	EC ₅₀ (M)	%	%	%
1	-93.2	1.24×10^{-9}	100	3.28×10^{-9}	100	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	

^aThe in silico binding energy refers to the calculated energy difference between the **1**/**5a–c**–VDR complex and the free protein and ligand **1**/**5a–c** 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 $1\alpha,25\text{-}(\text{OH})_2\text{D}_3$ (**1**, 100% activity). Calcemic activity is expressed as percentage of the calcium increase in mice treated with the different compounds in relation with untreated mice, considering 100% $1\alpha,25\text{-}(\text{OH})_2\text{D}_3$ treated-mice.

methanesulfonyl chloride (635 μL , 8.16 mmol) was added. The mixture was stirred for 1 h. The reaction was quenched by the slow addition of H₂O (25 mL). The aqueous layer was extracted with CH₂Cl₂ (3 \times 30 mL). The combined organic layer was dried, filtered, and concentrated to give **16** [2.22 g, 4.04 mmol, 99%, R_f = 0.53 (10% EtOAc–hexanes), white solid, mp 99–101 °C (EtOAc–hexanes), $[\alpha]_D = -20.9$ ($c = 1.1$, CHCl₃)] 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, J₁ = 2.0, J₂ = 9.4, H-22), 3.54–3.42 (2H, m, H-11 and H-12), 3.28 (1H, dd, J₁ = 6.7, J₂ = 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, Me₃Si 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 CH₃, Me₃C-Si), 25.7 (3 \times 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 \times CH₃, Me₂Si), -5.4 (2 \times CH₃, Me₂Si). IR (neat, cm⁻¹): 2956 ($\nu_{\text{C-H}}$), 2929 ($\nu_{\text{C-H}}$), 1208 ($\nu_{\text{O-S=O}}$). MS ([ESI-TOF]⁺, m/z, %): 571 ([M + Na]⁺, 100), 475 ([M – O – t-Bu]⁺, 15). HRMS: [ESI-TOF]⁺, calcd for [C₂₆H₅₂O₆SSi₂Na]⁺, 571.2915; found, 571.2921.

8 β ,22-Bis(tert-butylidimethylsilyloxy)-de-A,B-23,24-dinorchol-9-en-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 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), $[\alpha]_D = +177.9$ ($c = 0.6$, CHCl₃)]. ¹H NMR (CDCl₃, 250 MHz): 5.91–5.83 (2H, m, H-9 and H-11), 4.07 (1H, dd, J₁ = 3.9, J₂ = 3.9, H-8), 4.00 (1H, d, J = 4.5, H-12), 3.63 (1H, dd, J₁ = 4.3, J₂ = 9.8, H-22), 3.28 (1H, dd, J₁ = 7.4, J₂ = 9.8, H-22), 1.02 (3H, d, J = 6.6, H-21), 0.89 (9H, s, Me₃C-Si), 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 (CH₃), 13.9 (CH₃), -4.4 (CH₃, MeSi), -5.2 (CH₃, MeSi), -5.3 (2 \times CH₃, Me₂Si). IR (neat, cm⁻¹): 3318 ($\nu_{\text{O-H}}$), 2956 ($\nu_{\text{C-H}}$), 2929 ($\nu_{\text{C-H}}$). MS ([ESI-TOF]⁺, m/z, %): 477 ([M + Na]⁺, 100), 437 ([M – OH]⁺, 4). HRMS: [ESI-TOF]⁺, calcd for [C₂₅H₅₀O₃Si₂Na]⁺, 477.3191; found, 477.3193.

8 β ,22-Bis(tert-butylidimethylsilyloxy)-de-A,B-23,24-dinorchol-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), $[\alpha]_D = +46.1$ ($c = 1.4$, 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, J₁ = 3.8, J₂ = 9.6, H-22), 3.28 (1H, dd, J₁ = 7.1, J₂ = 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 \times 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 \times CH₃, Me₃C-Si), 25.8 (3 \times CH₃, Me₃C-Si), 24.5 (CH₂), 22.2 (CH₂), 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 \times 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.

8 β ,22-Bis(tert-butylidimethylsilyloxy)-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 CH₂Cl₂ (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 \times 10 cm, 2% EtOAc–hexanes) to afford **19** [1.114 g, 2.45 mmol, 90%, R_f = 0.66 (10% EtOAc–hexanes), colorless oil, $[\alpha]_D = +88.8$ ($c = 1.6$, CHCl₃)]. ¹H NMR (CDCl₃, 250 MHz): 4.02 (1H, broad d, J = 1.8, H-8), 3.61 (1H, dd, J₁ = 3.5, J₂ = 9.5, H-22), 3.30 (1H, dd, J₁ = 7.8, J₂ = 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 (CH₂), 26.5 (CH₂), 26.0 (3 \times CH₃, Me₃C-Si), 25.8 (3 \times CH₃, Me₃C-Si), 22.9 (CH₂), 18.3 (C, C-Si), 18.0 (C, C-Si), 17.4 (CH₃), 13.3 (CH₃), -4.8 (CH₃, MeSi), -5.1 (CH₃, MeSi), -5.4 (2 \times CH₃, Me₂Si). IR (film, cm⁻¹): 2955 ($\nu_{\text{C-H}}$), 2929 ($\nu_{\text{C-H}}$), 2857 ($\nu_{\text{C-H}}$), 1712 ($\nu_{\text{C=O}}$). MS ([ESI-TOF]⁺, m/z, %): 455 ([M + H]⁺, 81), 439 ([M – Me]⁺, 75), 397 ([M – t-Bu]⁺, 60), 323 ([M – TBSO]⁺, 100). HRMS: calcd for [C₂₅H₅₀O₃Si₂]⁺, 455.3377; found, 455.3365.

8 β ,22-Bis(tert-butylidimethylsilyloxy)-de-A,B-23,24-dinorchol-11-en-12-yl trifluoromethanesulfonate (21**).**

A solution of lithium diisopropylamide was prepared by dropwise addition of solution of n-BuLi in hexanes (1.62 mL, 2.27 M, 3.67 mmol) to diisopropylamine (397 mg, 3.92 mmol) at -78 °C. The mixture was stirred at rt until formation of a semisolid slurry. The slurry was cooled to -78 °C and dissolved in THF (16 mL). A solution of **19** (1.114 g, 2.45 mmol) in THF (30 mL) was added via cannula. The mixture was stirred at rt for 4 h and then cooled to -78 °C. A solution of triflimide **20** (1.924 g, 4.90 mmol) in THF (20 mL) was added. The mixture was allowed to warm to rt overnight. The reaction was quenched by addition of saturated NaCl (40 mL). The aqueous layer was extracted with MTBE (3 \times 40 mL), and the combined organic layer was dried, filtered, and concentrated. The residue was purified by flash chromatography (SiO₂,

594 3 cm × 8 cm, 20% CH_2Cl_2 –hexanes and 10% EtOAc–hexanes) to
 595 afford **21** [1.208 g, 2.06 mmol, 84%, $R_f = 0.69$ (5% EtOAc–hexanes),
 596 colorless oil, $[\alpha]_D = +25.6$ ($c = 1.2$, CHCl_3)]. ^1H NMR (CDCl_3 , 250
 597 MHz): 5.53 (1H, dd, $J_1 = 3.9$, $J_2 = 3.9$, H-11), 4.12 (1H, broad d, $J =$
 598 6.1, H-8), 3.66 (1H, dd, $J_1 = 4.1$, $J_2 = 9.7$, H-22), 3.41 (1H, dd, $J_1 = 7.0$,
 599 $J_2 = 9.7$, H-22), 2.66 (1H, ddd, $J_1 = 3.7$, $J_2 = 6.2$, $J_3 = 18.9$, H-9), 2.32
 600 (1H, dd, $J_1 = 4.2$, $J_2 = 19.0$, H-9), 1.26 (3H, s, H-18), 1.04 (3H, d, $J =$
 601 6.9, H-21), 0.90 (18H, overlapped s, 2 × $\text{Me}_3\text{C-Si}$), 0.06 (3H, s,
 602 MeSi), 0.04 (9H, overlapped s, MeSi and Me_3Si). ^{13}C NMR (CDCl_3 ,
 603 63 MHz): 157.3 (C, C-12), 118.4 (C, q, $J = 319$, CF_3), 112.6 (CH, C-
 604 11), 66.5 (CH_2 , C-22), 65.8 (CH, C-8), 53.8 (CH), 50.7 (CH), 45.9
 605 (C, C-13), 36.4 (CH_2), 34.9 (CH), 25.9 (3 × CH_3 , $\text{Me}_3\text{C-Si}$), 25.7 (3
 606 × CH_3 , $\text{Me}_3\text{C-Si}$), 23.3 (CH_2), 22.2 (CH_2), 19.5 (CH_3), 18.2 (C, C-
 607 Si), 17.9 (C, C-Si), 15.8 (CH_3), –4.8 (CH_3 , MeSi), –5.2 (CH_3 ,
 608 MeSi), –5.5 (2 × CH_3 , Me_2Si). IR (film, cm^{-1}): 2956 ($\nu_{\text{C-H}}$, 2931
 609 ($\nu_{\text{C-H}}$), 2886 ($\nu_{\text{C-H}}$), 2859 ($\nu_{\text{C-H}}$), 1666 ($\nu_{\text{C=C}}$). MS ([$\text{Cl}]^+, m/z$,
 610 %): 587 ([M + H]⁺, 18), 455 ([M – TBSO]⁺, 20), 305 ([M – TBSO
 611 – TfOH]⁺, 86). HRMS: [$\text{Cl}]^+$, calcd for $[\text{C}_{26}\text{H}_{50}\text{O}_5\text{F}_3\text{SSi}_2]^+$,
 612 587.2870; found, 587.2864.

613 *8β,22-Bis(tert-butylidemethylsilyloxy)-12-(7-hydroxy-7-methyloct-1-ynyl)-de-A,B-23,24-dinorchol-11-ene* (**23**). CuI (60 mg, 0.31 mmol) and $\text{PdCl}_2(\text{PPh}_3)_2$ (60 mg, 0.08 mmol) were successively added to a solution of **21** (1.00 g, 1.71 mmol) and **22**¹⁴ (1.20 g, 8.56 mmol) in Et_2NH (100 mL) at 0 °C. The reaction mixture was stirred for 1 h at 0 °C and 12 h at rt. The reaction was quenched by slowly addition of saturated NH_4Cl (100 mL). Aqueous layer was extracted with MTBE (3 × 50 mL). The combined organic layer was dried, filtered, and concentrated. The residue was purified by flash chromatography (SiO_2 , 4.5 cm × 9 cm, 10% EtOAc–hexanes) to afford **23** [925 mg, 1.60 mmol, 94%, $R_f = 0.62$ (30% EtOAc–hexanes), colorless oil, $[\alpha]_D = +28.2$ ($c = 1.1$, CHCl_3)]. ^1H NMR (CDCl_3 , 250 MHz): 5.80–5.64 (1H, m, H-11), 4.08 (1H, broad d, $J = 6.0$, H-8), 3.70 (1H, dd, $J_1 = 3.1$, $J_2 = 9.1$, H-22), 3.30 (1H, dd, $J_1 = 9.1$, $J_2 = 9.1$, H-22), 1.18 (6H, s, $\text{Me}_2\text{C-OH}$), 1.06 (3H, s, H-18), 1.00 (3H, d, $J = 6.8$, H-21), 0.86 (9H, s, $\text{Me}_3\text{C-Si}$), 0.84 (9H, s, MeSi), 0.01 (6H, s, Me_2Si), –0.02 (6H, s, Me_2Si). ^{13}C NMR (CDCl_3 , 63 MHz): 132.1 (C, C-12), 131.9 (CH, C-11), 89.5 (C), 80.1 (C), 70.8 (C, C-OH), 66.6 (CH, C-8), 66.3 (CH_2 , C-22), 51.5 (CH), 51.5 (CH), 44.8 (C, C-13), 43.3 (CH_2), 37.1 (CH), 33.8 (CH), 29.1 (2 × CH_3 , $\text{Me}_2\text{C-OH}$), 25.9 (3 × CH_3 , $\text{Me}_3\text{C-Si}$), 25.7 (3 × CH_3 , $\text{Me}_3\text{C-Si}$), 23.8 (CH), 21.8 (CH), 21.5 (CH_2), 20.2 (CH), 19.4 (2 × CH_2), 18.1 (C, C-Si), 17.9 (C, C-Si), 17.1 (CH), –4.8 (CH, MeSi), –5.2 (CH, MeSi), –5.3 (CH, MeSi), –5.3 (CH, MeSi). IR (film, cm^{-1}): 3368 ($\nu_{\text{O-H}}$, 2955 ($\nu_{\text{C-H}}$), 2930 ($\nu_{\text{C-H}}$), 2884 ($\nu_{\text{C-H}}$), 2858 ($\nu_{\text{C-H}}$)). MS ([$\text{Cl}]^+, m/z$, %): 577 ([M + H]⁺, 9), 559 ([M – OH]⁺, 28), 445 ([M – TBSO]⁺, 14), 427 ([M – OH – TBSO]⁺, 51), 295 ([M – OH – 2TBSO]⁺, 100). HRMS: [$\text{Cl}]^+$, calcd for $[\text{C}_{34}\text{H}_{65}\text{O}_3\text{Si}_2]^+$, 577.4472; found, 577.4468.

642 *8β,22-Bis(tert-butylidemethylsilyloxy)-12β-(7-hydroxy-7-methyloctyl)-de-A,B-23,24-dinorcholane* (**24**). Pd/C (240 mg, 10%) was added to a solution of enyne **23** (1.89 g, 3.27 mmol) in EtOAc (75 mL). The mixture was degassed under reduced pressure and refilled with H_2 for three times. The reaction mixture was stirred under H_2 atmosphere (balloon pressure) for 72 h. The mixture was filtered through a layer of silica gel. The solids were washed with EtOAc (3 × 20 mL), and the resulting filtrate was concentrated. The residue was purified by flash chromatography (SiO_2 , 4 cm × 8 cm, 4% EtOAc–hexanes) to afford **24** [1.88 g, 3.22 mmol, 98%, $R_f = 0.70$ (30% EtOAc–hexanes), colorless oil, $[\alpha]_D = +37.4$ ($c = 1.0$, CHCl_3)]. ^1H NMR (CDCl_3 , 250 MHz): 3.92 (1H, broad d, $J = 1.7$, H-8), 3.69 (1H, dd, $J_1 = 4.2$, $J_2 = 9.5$, H-22), 3.32 (1H, dd, $J_1 = 8.4$, $J_2 = 9.5$, H-22), 1.19 (6H, s, $\text{Me}_2\text{C-OH}$), 0.96 (3H, d, $J = 6.7$, H-21), 0.88 (9H, s, MeSi), 0.87 (9H, s, $\text{Me}_3\text{C-Si}$), 0.81 (3H, s, H-18), 0.02 (6H, s, Me_2Si), –0.01 (3H, MeSi), –0.02 (3H, s, MeSi). ^{13}C NMR (CDCl_3 , 63 MHz): 71.0 (C, C-OH), 69.0 (CH, C-8), 66.5 (CH_2 , C-22), 56.6 (CH), 53.8 (CH), 49.4 (CH), 45.5 (C, C-13), 44.0 (CH), 34.9 (CH_2), 34.9 (CH), 31.4 (CH_2), 30.2 (CH_2), 30.1 (CH_2), 29.2 (2 × CH_3 , $\text{Me}_2\text{C-OH}$), 28.3 (CH), 26.0 (3 × CH_3 , $\text{Me}_3\text{C-Si}$), 25.8 (3 × CH_3 , $\text{Me}_3\text{C-Si}$), 24.4 (CH), 23.7 (CH_2), 22.5 (CH_2), 21.2 (CH), 20.5 (CH), 18.2 (C, C-Si), 18.0 (C, C-Si), 11.0 (CH), –4.8 (CH, MeSi), –5.1 (CH, MeSi), –5.3 (2 × CH_3 ,

Me₂Si). IR (film, cm^{-1}): 3362 ($\nu_{\text{O-H}}$), 2954 ($\nu_{\text{C-H}}$), 2930 ($\nu_{\text{C-H}}$), 664 2857 ($\nu_{\text{C-H}}$). MS ([$\text{Cl}]^+, m/z$, %): 583 ([M + H]⁺, 4), 565 ([M – 665 OH]⁺, 20), 451 ([M – TBSO]⁺, 3), 433 ([M – OH – TBSO]⁺, 34), 666 301 ([M – OH – 2TBSO]⁺, 100). HRMS: [$\text{Cl}]^+$, calcd for $[\text{C}_{34}\text{H}_{71}\text{O}_3\text{Si}_2]^+$, 583.4942; found, 583.4916.

12β-(7-Hydroxy-7-methyloctyl)-de-A,B-23,24-dinorcholan-8β,22-diol (**8a**). A solution of TBAF in THF (8.25 mL, 1 M, 8.25 mmol) was added to a solution of **24** (320 mg, 0.548 mmol) in THF (10 mL). The reaction mixture was heated at 55 °C for 7 days. The reaction was quenched by addition of saturated NH_4Cl (30 mL). Aqueous layer was extracted with EtOAc (3 × 20 mL). The combined organic layer was dried, filtered, and concentrated. The residue was purified by flash chromatography (SiO_2 , 2 cm × 15 cm, 40% EtOAc–hexanes) to afford **8a** [172 mg, 0.485 mmol, 89%, $R_f = 0.30$ (70% EtOAc–hexanes), white solid, mp 114–118 °C (Et₂O–hexanes), $[\alpha]_D = +30.6$ ($c = 1.7$, CHCl₃)]. ^1H NMR (CDCl_3 , 250 MHz): 4.01 (1H, broad d, $J = 2.6$, H-8), 3.76 (1H, dd, $J_1 = 3.9$, $J_2 = 10.3$, H-22), 3.36 (1H, dd, $J_1 = 8.2$, $J_2 = 10.3$, H-22), 1.19 (6H, s, $\text{Me}_2\text{C-OH}$), 1.03 (3H, d, $J = 6.9$, H-21), 0.86 (3H, s, H-18). ^{13}C NMR (CDCl_3 , 63 MHz): 70.9 (C, C-OH), 68.7 (CH, C-8), 66.3 (CH_2 , C-22), 56.1 (CH), 53.2 (CH), 49.3 (CH), 45.2 (CH, C-13), 43.8 (CH), 33.9 (CH), 31.2 (CH), 30.1 (CH), 29.9 (CH), 29.0 (2 × CH_3 , $\text{Me}_2\text{C-OH}$), 28.1 (CH), 24.2 (CH), 23.4 (CH), 22.2 (CH), 21.1 (CH), 20.3 (CH), 10.8 (CH). IR (film, cm^{-1}): 3384 ($\nu_{\text{O-H}}$), 2932 ($\nu_{\text{C-H}}$), 2862 ($\nu_{\text{C-H}}$). MS ([$\text{Cl}]^+, m/z$, %): 337 ([M – OH]⁺, 8), 319 ([M – OH – H₂O]⁺, 77), 301 ([M – OH – 2H₂O]⁺, 100). HRMS: [$\text{Cl}]^+$, calcd for $[\text{C}_{22}\text{H}_{39}\text{O}]^+$, 319.3001; found, 319.3008.

22-(tert-Butylidemethylsilyloxy)-12β-(7-hydroxy-7-methyloctyl)-de-A,B-23,24-dinorcholan-8β-ol (**27a**). Imidazol (65 mg, 0.958 mmol) and TBSCl (116 mg, 0.766 mmol) were successively added to a solution of **8a** (170 mg, 0.479 mmol) in DMF (7 mL) at 0 °C. After 10 h at rt, the reaction was quenched by addition of saturated NaCl (35 mL). The aqueous layer was extracted with EtOAc (3 × 20 mL). The combined organic layer was dried, filtered, and concentrated. The residue was purified by flash chromatography (SiO_2 , 2 cm × 8 cm, 30% EtOAc–hexanes) to afford **27a** [206 mg, 0.44 mmol, 92%, $R_f = 0.60$ (60% EtOAc–hexanes), colorless oil, $[\alpha]_D = +25.8$ ($c = 0.6$, CHCl₃)]. ^1H NMR (CDCl_3 , 250 MHz): 3.98 (1H, broad d, $J = 1.2$, H-8), 3.65 (1H, dd, $J_1 = 4.3$, $J_2 = 9.6$, H-22), 3.32 (1H, dd, $J_1 = 7.7$, $J_2 = 9.6$, H-22), 1.18 (6H, s, $\text{Me}_2\text{C-OH}$), 0.96 (3H, d, $J = 6.8$, H-21), 0.86 (9H, s, $\text{Me}_3\text{C-Si}$), 0.83 (3H, s, H-18), 0.05 (6H, s, Me_2Si). ^{13}C NMR (CDCl_3 , 63 MHz): 70.9 (C, C-OH), 68.9 (CH, C-8), 66.5 (CH_2 , C-22), 56.3 (CH), 53.3 (CH), 49.2 (CH), 45.2 (C, C-13), 44.0 (CH), 34.8 (CH), 34.0 (CH), 31.3 (CH), 30.2 (CH), 30.0 (CH), 29.1 (2 × CH_3 , $\text{Me}_2\text{C-OH}$), 28.2 (CH), 25.9 (3 × CH_3 , $\text{Me}_3\text{C-Si}$), 24.3 (CH), 23.5 (CH), 22.1 (CH), 21.1 (CH), 20.5 (CH), 18.2 (C, C-Si), 10.8 (CH), –5.4 (2 × CH_3 , Me_2Si). IR (film, cm^{-1}): 3399 ($\nu_{\text{O-H}}$), 2930 ($\nu_{\text{C-H}}$), 2858 ($\nu_{\text{C-H}}$). MS ([$\text{FAB}]^+, m/z$, %): 469 ([M + H]⁺, 63), 451 ([M – OH]⁺, 52). HRMS: [$\text{FAB}]^+$, calcd for $[\text{C}_{28}\text{H}_{57}\text{O}_3\text{Si}]^+$, 469.4077; found, 469.4086.

22-(tert-Butylidemethylsilyloxy)-12β-(7-hydroxy-7-methyloctyl)-de-A,B-23,24-dinorcholan-8-one (**28a**). Pyridinium dichromate (400 mg, 1.069 mmol) was added to a solution of **27a** (167 mg, 0.356 mmol) in CH_2Cl_2 (10 mL). The reaction mixture was protected from the light and stirred for 2 h at rt. The mixture was filtered through a layer of silica gel. The solids were washed with MTBE (3 × 40 mL), and the resulting filtrate was concentrated. The residue was purified by flash chromatography (SiO_2 , 3 cm × 6 cm, 30% EtOAc–hexanes) to afford ketone **28a** [150 mg, 0.321 mmol, 89%, $R_f = 0.48$ (40% EtOAc–hexanes), colorless oil]. ^1H NMR (CDCl_3 , 250 MHz): 3.60 (1H, dd, $J_1 = 4.5$, $J_2 = 9.7$, H-22), 3.32 (1H, dd, $J_1 = 7.6$, $J_2 = 9.7$, H-22), 1.16 (6H, s, $\text{Me}_2\text{C-OH}$), 0.97 (3H, d, $J = 6.9$, H-21), 0.84 (9H, s, $\text{Me}_3\text{C-Si}$), 0.56 (3H, s, H-18), –0.02 (6H, s, Me_2Si). ^{13}C NMR (CDCl_3 , 63 MHz): 212.4 (C, C-8), 70.8 (C, C-OH), 66.0 (CH, C-22), 61.8 (CH), 56.0 (CH), 52.2 (C, C-13), 48.3 (CH), 43.8 (CH), 40.3 (CH), 35.2 (CH), 30.7 (CH), 30.1 (CH), 29.8 (CH), 29.6 (CH), 29.1 (2 × CH_3 , $\text{Me}_2\text{C-OH}$), 28.2 (CH), 25.8 (3 × CH_3 , $\text{Me}_3\text{C-Si}$), 24.2 (CH), 21.6 (CH), 20.1 (CH), 18.7 (CH), 18.2 (C, C-Si), 9.8 (CH), –5.4 (2 × CH_3 , Me_2Si). IR (CHCl_3 , cm^{-1}): 3459 ($\nu_{\text{O-H}}$), 2956 ($\nu_{\text{C-H}}$), 2930 ($\nu_{\text{C-H}}$), 2884 ($\nu_{\text{C-H}}$), 1717 ($\nu_{\text{C=O}}$). MS ([$\text{Cl}]^+, m/z$, %): 469 ([M + H]⁺, 63), 451 ([M – OH]⁺, 52). HRMS: [$\text{FAB}]^+$, calcd for $[\text{C}_{28}\text{H}_{57}\text{O}_3\text{Si}]^+$, 469.4077; found, 469.4086.

⁷³⁴ z , %): 349 ($[M - OH]^+$, 20), 317 ($[M - OH - TBSOH]^+$, 100).
⁷³⁵ HRMS: $[Cl]^+$, calcd for $[C_{28}H_{53}O_2Si]^+$, 349.3815; found, 349.3821.
⁷³⁶ (*E*)-8-(Bromomethylene)-22-(*tert*-butyldimethylsilyloxy)-12*β*-(7-
⁷³⁷ hydroxy-7-methyloctyl)-de-*A,B*-24,23-dinorcholane (**29a**). A suspen-
⁷³⁸ sion of (Ph_3PCH_2Br) Br (1.121 g, 2.57 mmol) in toluene (18 mL) was
⁷³⁹ prepared by sonication for 30 min. After cooling at $-17\text{ }^\circ\text{C}$, a solution
⁷⁴⁰ of $KOT-Bu$ in THF (2.5 mL, 1 M, 2.5 mmol) was added and the
⁷⁴¹ resulting mixture was stirred for 3 h. A solution of ketone **28a** (150
⁷⁴² mg, 0.32 mmol) in toluene (12 mL) previously cooled to $0\text{ }^\circ\text{C}$ was
⁷⁴³ added via cannula. The mixture was stirred for 2 h at $-17\text{ }^\circ\text{C}$ and 3 h
⁷⁴⁴ at rt. The reaction was quenched by addition of saturated NH_4Cl (1
⁷⁴⁵ mL), and the mixture was filtered through a layer of silica gel. The
⁷⁴⁶ solids were washed with EtOAc (3 \times 15 mL), and the filtrate was
⁷⁴⁷ concentrated. The residue was purified by flash chromatography (SiO_2 ,
⁷⁴⁸ 3 cm \times 8 cm, 30% EtOAc–hexanes) to afford **29a** [121 mg, 0.22
⁷⁴⁹ mmol, 69%, $R_f = 0.50$ (20% EtOAc–hexanes), colorless oil]. ¹H NMR
⁷⁵⁰ ($CDCl_3$, 250 MHz): 5.63 (1H, s, H-7), 3.67 (1H, dd, $J_1 = 4.2$, $J_2 = 9.6$,
⁷⁵¹ H-22), 3.44–3.30 (1H, m, H-22), 1.20 (6H, s, Me_2C-OH), 0.99 (3H,
⁷⁵² d, $J = 6.9$, H-21), 0.88 (9H, s, Me_3C-Si), 0.50 (3H, s, H-18), 0.02 (6H,
⁷⁵³ s, Me_2Si). ¹³C NMR ($CDCl_3$, 63 MHz): 145.3 (C, C-8), 97.6 (CH, C-
⁷⁵⁴ 7), 71.4 (C, C-OH), 66.5 (CH₂, C-22), 56.9 (CH), 55.6 (CH), 49.4
⁷⁵⁵ (CH), 49.1 (C, C-13), 44.4 (CH₂), 36.3 (CH), 31.6 (CH₂), 31.6
⁷⁵⁶ (CH₂), 30.6 (CH₂), 30.4 (CH₂), 29.6 (2 \times CH₃, Me_2C-OH), 28.8
⁷⁵⁷ (CH₂), 28.6 (CH₂), 26.4 (3 \times CH₃, Me_3C-Si), 24.8 (CH₂), 22.2
⁷⁵⁸ (CH₂), 22.0 (CH₂), 20.5 (CH₃), 18.7 (C, C-Si), 9.8 (CH₃), –4.9 (2 \times
⁷⁵⁹ CH₃, Me_2Si). IR (film, cm^{-1}): 3369 (ν_{O-H}), 3085 (ν_{C-H}), 2953
⁷⁶⁰ (ν_{C-H}), 2930 (ν_{C-H}), 2857 (ν_{C-H}), 1632 ($\nu_{C=C}$). MS ($[Cl]^+$, m/z ,
⁷⁶¹ %): 525 ($[M - OH]^+$, 3), 393 ($[M - OH - TBSOH]^+$, 31), 313 ($[M$
⁷⁶² – $C_6H_{18}BrO_2Si]^+$, 100). HRMS: $[Cl]^+$, calcd for $[C_{29}H_{54}BrOSi]^+$,
⁷⁶³ 525.3127; found, 525.3134.

⁷⁶⁴ (*E*)-22-(*tert*-Butyldimethylsilyloxy)-12*β*-(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(dpfpf)·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 $80\text{ }^\circ\text{C}$ 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 \times 20 mL). The combined organic layer was dried, filtered, and concentrated. The residue was purified by flash chromatography (SiO_2 , 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, $[\alpha]_D = +44.7$ ($c = 0.2$, $CHCl_3$)]. ¹H NMR ($CDCl_3$, 250 MHz): 4.90 (1H, s, H-7), 3.69 (1H, dd, $J_1 = 4.1$, $J_2 = 9.6$, H-22), 3.35 (1H, dd, $J_1 = 8.3$, $J_2 = 9.6$, H-22), 1.25 (12H, s, 2 \times Me_2C-OH), 1.20 (6H, s, Me_2C-OH), 0.99 (3H, d, $J = 6.9$, H-21), 0.88 (9H, s, Me_3C-Si), 0.50 (3H, s, H-18), 0.02 (6H, s, Me_2Si). ¹³C NMR ($CDCl_3$, 63 MHz): 166.5 (C, C-8), 82.5 (2 \times C, 2 \times C-OH), 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 (CH₂), 36.0 (CH), 33.2 (CH₂), 31.3 (CH₂), 30.2 (2 \times CH₂), 30.0 (CH₂), 29.2 (2 \times CH₃, Me_2C-OH), 28.3 (CH₂), 26.0 (3 \times CH₃, Me_3C-Si), 24.9 (2 \times CH₃, Me_2C-OH), 24.8 (2 \times CH₃, Me_2C-OH), 24.3 (CH₂), 22.1(CH₂), 21.4 (CH₂), 20.1 (CH₃), 18.3 (C, C-Si), 9.7 (CH₃), –5.3 (2 \times CH₃, Me_2Si). IR (film, cm^{-1}): 3385 (ν_{O-H}), 2954 (ν_{C-H}), 2930 (ν_{C-H}), 2857 (ν_{C-H}), 1640 ($\nu_{C=C}$). MS ($[Cl]^+$, m/z , %): 573 ($[M - OH]^+$, 10), 441 ($[M - OH - TBSOH]^+$, 100), 315 ($[M - C_{12}H_{28}BO_4Si]^+$, 20). HRMS: $[Cl]^+$, calcd for $[C_{33}H_{66}BO_3Si]^+$, 573.4874; found, 573.4878.

⁷⁹³ 1*α*,22-Dihydroxy-12*β*-(7-hydroxy-7-methyloctyl)-23,24,25,26,27-⁷⁹⁴ pentanorvitamin D₃ (**5a**). An aqueous solution of K₃PO₄ (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 \times 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 μ L, 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 mL). The combined organic layer was dried, filtered, and concentrated. The residue was purified by flash chromatography (SiO_2 , 2 cm \times 10 cm, 60–95% EtOAc–hexanes) to 805 give **5a** [50 mg, 0.102 mmol, 86%, $R_f = 0.18$ (90% EtOAc–hexanes), 807 white solid, mp 85–88 $^\circ\text{C}$ (Et₂O–hexanes), $[\alpha]_D = -25.6$ ($c = 0.9$, 808 EtOH 96%)]. Further purification by HPLC (Phenomenex-LUNA 5 μ Silica(2) column, 10 mm \times 250 mm, 20% *i*-PrOH–hexanes) gave an analytically pure (>95%) sample which was used for biological assays. ⁸¹¹ ¹H NMR (CD_3OD , 500 MHz): 6.31 (1H, d, $J = 11.2$, H-6), 6.09 (1H, d, $J = 11.2$, H-7), 5.29 (1H, s, H-19), 4.90 (1H, s, H-19), 4.36 (1H, dd, $J_1 = 5.9$, $J_2 = 5.9$, H-1), 4.13 (1H, ddd, $J_1 = 5.3$, $J_2 = 5.3$, $J_3 = 10.4$, H-3), 3.76 (1H, dd, $J_1 = 3.4$, $J_2 = 10.4$, H-22), 3.30–3.24 (1H, m, H-22), 2.87 (1H, dd, $J_1 = 2.9$, $J_2 = 13.9$, H-9), 2.52 (1H, dd, $J_1 = 3.4$, $J_2 = 13.4$, H-4), 2.26 (1H, dd, $J_1 = 6.8$, $J_2 = 13.4$, H-4), 2.13–2.04 (1H, m, H-20), 1.17 (6H, s, Me_2C-OH), 1.06 (3H, d, $J = 6.8$, H-21), 0.54 (3H, s, H-18). ¹³C NMR (CD_3OD , 125 MHz): 149.8 (C, C-10), 142.1 (C, C-8), 135.7 (C, C-5), 124.9 (CH, C-6), 118.8 (CH, C-7), 112.0 (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 (ν_{C-H}), 1635 (ν_{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): $\lambda_{max} = 264\text{ nm}$, $\lambda_{min} = 230\text{ nm}$.

⁸*β*-Hydroxy-12*β*-(7-hydroxy-7-methyloctyl)-de-*A,B*-23,24-di-norcho-⁸³¹ lan-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 $0\text{ }^\circ\text{C}$. The mixture at $0\text{ }^\circ\text{C}$ was stirred for 2 h and then was kept at $4\text{ }^\circ\text{C}$ 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 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 (SiO_2 , 3 cm \times 10 cm, 30–60% EtOAc–hexanes) to afford **25a** [258 mg, 0.507 mmol, 84%, $R_f = 0.58$ (70% EtOAc–hexanes), colorless oil, $[\alpha]_D = +28.4$ ($c = 0.5$, $CHCl_3$)]. ¹H NMR ($CDCl_3$, 250 MHz): 7.76 (2H, d, $J = 8.1$, H-Ar), 7.32 (2H, d, $J = 8.1$, H-Ar), 4.10 (1H, dd, $J_1 = 3.7$, $J_2 = 9.1$, H-22), 3.97 (1H, broad d, $J = 2.0$, H-8), 3.75 (1H, dd, $J_1 = 9.1$, $J_2 = 9.1$, H-22), 2.43 (3H, s, Me-Ar), 1.19 (6H, s, Me_2C-OH), 0.96 (3H, d, $J = 6.8$, H-21), 0.70 (3H, s, H-18). ¹³C NMR ($CDCl_3$, 63 MHz): 144.6 (C, Ar-SO₂), 133.0 (C), 129.7 (2 \times CH, Ar-H), 127.8 (2 \times 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 \times CH₃, Me_2C-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 $[C_{29}H_{48}O_5Na]^+$, 531.3106; found, 531.3115.

⁸*β*-Hydroxy-12*β*-(7-hydroxy-7-methyloctyl)-de-*A,B*-23,24-di-norcho-⁸⁵⁸ lane-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 $90\text{ }^\circ\text{C}$ for 2 h and then cooled to rt. The reaction was quenched by addition of H₂O (50 mL), and the aqueous layer was extracted with EtOAc (4 \times 40 mL). The combined organic layer was dried, filtered, and concentrated. The residue was purified by flash chromatography (SiO_2 , 3 cm \times 5 cm, 20–50% EtOAc–hexanes) to afford **26a** [328 mg, 0.902 mmol, 92%, $R_f = 0.50$ (70% EtOAc–hexanes), colorless oil, $[\alpha]_D = +25.9$ ($c = 0.2$, $CHCl_3$)]. ¹H NMR ($CDCl_3$, 250 MHz): 4.01 (1H, broad d, $J = 2.1$, H-8), 2.47 (1H, dd, $J_1 = 3.3$, $J_2 = 16.1$, H-22), 2.37–2.27 (1H, m, H-22), 1.20 (6H, s, Me_2C-OH), 1.15 (3H, d, $J = 6.7$, H-21), 0.84 (3H, s, H-18). ¹³C NMR ($CDCl_3$, 63 MHz): 120.1 (C, C≡N), 71.0 (C, C-OH), 68.6 (CH, C-8), 55.8 (CH), 53.4 (CH), 49.2 (CH), 45.5 (C, C-13), 43.9 (CH₂), 34.0 (CH₂), 31.6 (CH₂), 30.4 (CH), 30.2 (CH₂), 30.0

874 (CH_2), 29.2 (2 \times CH_3 , $\text{Me}_2\text{C-OH}$), 28.2 (CH_2), 24.3 (CH_2), 23.4
 875 (CH_2), 22.6 (CH_3), 22.0 (CH_2), 22.0 (CH_2), 21.2 (CH_2), 11.0 (CH_3).
 876 IR (film, cm^{-1}): 3450 ($\nu_{\text{O-H}}$), 2931 ($\nu_{\text{C-H}}$), 2875 ($\nu_{\text{C-H}}$), 2247
 877 ($\nu_{\text{C}\equiv\text{N}}$). MS ([$\text{Cl}]^+, m/z, \%$): 346 ([$\text{M}-\text{OH}]^+, 52), 328 ([$\text{M}-\text{OH}$
 878 - $\text{H}_2\text{O}]^+, 100). HRMS: [$\text{Cl}]^+$, calcd for $[\text{C}_{23}\text{H}_{40}\text{NO}]^+$, 346.3110;
 879 found, 346.3110.$$

880 *12\beta*-(7-Hydroxy-7-methyloctyl)-de-A,B-24-norcholan-8\beta,23-diol
 881 (**8b**). A solution of diisobutylaluminium hydride in CH_2Cl_2 (2.72 mL,
 882 1 M, 2.72 mmol) was diluted in CH_2Cl_2 (3 mL) and cooled to -5 °C.
 883 A solution of **26a** (283 mg, 0.778 mmol) in CH_2Cl_2 (4 mL) was
 884 added. The mixture was vigorously stirred at -15 °C for 2 h and then
 885 allowed to warm to 0 °C for 1 h. A suspension of aqueous HCl (10
 886 mL, 3 M) in Et_2O (10 mL) was added, and the mixture was stirred for
 887 2 h at 5 °C. The aqueous layer was extracted with CH_2Cl_2 (3 \times 30
 888 mL). The combined organic layer was washed with saturated NaCl (3
 889 \times 20 mL), dried, filtered, and concentrated. The residue was dissolved
 890 in THF (10 mL). After cooling at -78 °C, a solution of
 891 diisobutylaluminium hydride in CH_2Cl_2 (2.72 mL, 1 M, 2.72 mmol)
 892 was added. After 2 h, the reaction was quenched by slowly addition of
 893 aqueous HCl (20 mL, 10%) at 0 °C. The aqueous layer was extracted
 894 with EtOAc (3 \times 30 mL), and the combined organic layer was dried,
 895 filtered, and concentrated. The residue was purified by flash
 896 chromatography (SiO_2 , 3 cm \times 7.5 cm, 40–50% EtOAc–hexanes)
 897 to afford **8b** [171 mg, 0.464 mmol, 60%, $R_f = 0.30$ (70% EtOAc–
 898 hexanes), white foam, $[\alpha]_D = +24.9$ ($c = 0.7$, CHCl_3)]. ^1H NMR
 899 (CDCl_3 , 250 MHz): 3.98 (1H, broad s, H-8), 3.71–3.45 (2H, m, H-
 900 23), 1.16 (6H, s, $\text{Me}_2\text{C-OH}$), 0.89 (3H, d, $J = 6.8$, H-21), 0.83 (3H, s,
 901 H-18). ^{13}C NMR (CDCl_3 , 63 MHz): 71.0 (C, C-OH), 68.9 (CH, C-
 902 8), 62.0 (CH_2 , C-23), 57.2 (CH), 53.7 (CH), 49.8 (CH), 45.4 (C, C-
 903 13), 43.9 (CH₂), 36.7 (CH₂), 33.9 (CH₂), 31.3 (CH₂), 30.1 (CH₂),
 904 29.9 (CH₂), 29.3 (CH), 29.1 (2 \times CH_3 , $\text{Me}_2\text{C-OH}$), 28.1 (CH₂), 24.2
 905 (CH₂), 23.5 (CH₂), 22.5 (CH₂), 22.5 (CH₃), 21.0 (CH₂), 11.9 (CH₃).
 906 IR (film, cm^{-1}): 3365 ($\nu_{\text{O-H}}$), 2930 ($\nu_{\text{C-H}}$), 2863 ($\nu_{\text{C-H}}$). MS
 907 ([$\text{FAB}]^+, m/z, \%$): 351 ([$\text{M}-\text{OH}]^+, 70), 433 ([$\text{M}-\text{OH}-\text{H}_2\text{O}]^+,
 908 100). HRMS: [$\text{FAB}]^+$, calcd for $[\text{C}_{23}\text{H}_{43}\text{O}_2]^+$, 351.3263; found,
 909 351.3256.$$

910 *23*-(tert-Butyldimethylsilyloxy)-12\beta-(7-hydroxy-7-methyloctyl)-
 911 de-A,B-24-norcholan-8\beta-ol (**27b**). See **27a** for reaction procedure.
 912 Reagents: Imidazole (50 mg, 0.738 mmol), TBSCl (72 mg, 0.477
 913 mmol), **8b** (160 mg, 0.434 mmol), and DMF (10 mL). Product: **27b**
 914 [160 mg, 0.33 mmol, 76%, $R_f = 0.78$ (70% EtOAc–hexanes), colorless
 915 oil, $[\alpha]_D = +21.5$ ($c = 0.2$, CHCl_3)]. ^1H NMR (CDCl_3 , 250 MHz):
 916 3.98 (1H, broad s, H-8), 3.71–3.43 (2H, m, H-23), 1.15 (6H, s, $\text{Me}_2\text{C-}$
 917 OH), 0.92–0.74 (15H, m, H-18, H-21 and $\text{Me}_3\text{C-Si}$), 0.00 (6H, s,
 918 Me_2Si). ^{13}C NMR (CDCl_3 , 63 MHz): 70.8 (C, C-OH), 69.0 (CH, C-
 919 8), 62.0 (CH_2 , C-23), 57.1 (CH), 53.7 (CH), 50.1 (CH), 45.5 (C, C-
 920 13), 43.9 (CH₂), 36.7 (CH₂), 34.0 (CH₂), 31.1 (CH₂), 30.2 (CH₂),
 921 29.9 (CH₂), 29.1 (2 \times CH_3 , $\text{Me}_2\text{C-OH}$), 28.8 (CH), 28.1 (CH₂), 25.9
 922 (3 \times CH_3 , $\text{Me}_3\text{C-Si}$), 24.3 (CH₂), 23.3 (CH₂), 22.7 (CH₂), 22.3
 923 (CH₃), 20.8 (CH₂), 18.2 (C-Si), 11.4 (CH₃), -5.4 (2 \times CH_3 , Me_2Si).
 924 IR (CHCl_3 , cm^{-1}): 3402 ($\nu_{\text{O-H}}$), 2929 ($\nu_{\text{C-H}}$), 2858 ($\nu_{\text{C-H}}$). MS
 925 ([$\text{Cl}]^+, m/z, \%$): 465 ([$\text{M}-\text{OH}]^+, 19), 333 ([$\text{M}-\text{TBSO}-\text{H}_2\text{O}]^+,
 926 36), 315 ([$\text{M}-\text{TBSO}-2\text{H}_2\text{O}]^+, 100). HRMS: [$\text{Cl}]^+$, calcd for
 927 $[\text{C}_{29}\text{H}_{57}\text{O}_2\text{Si}]^+$, 465.4128; found, 465.4124.$$$

928 *23*-(tert-Butyldimethylsilyloxy)-12\beta-(7-hydroxy-7-methyloctyl)-
 929 de-A,B-24-norcholan-8-one (**28b**). See **28a** for reaction procedure.
 930 Reagents: pyridinium dichromate (362 mg, 0.963 mmol), **27b** (155
 931 mg, 0.321 mmol), and CH_2Cl_2 (10 mL). Product: ketone **28b** [146
 932 mg, 0.304 mmol, 95%, $R_f = 0.46$ (40% EtOAc–hexanes), colorless oil].
 933 ^1H NMR (CDCl_3 , 250 MHz): 3.67–3.43 (2H, m, H-23), 1.17 (6H, s,
 934 $\text{Me}_2\text{C-OH}$), 0.90 (3H, d, $J = 6.9$, H-21), 0.85 (9H, s, $\text{Me}_3\text{C-Si}$), 0.58
 935 (3H, s, H-18), 0.00 (6H, s, Me_2Si). ^{13}C NMR (CDCl_3 , 63 MHz):
 936 212.4 (C, C-8), 70.7 (C, C-OH), 62.2 (CH), 61.6 (CH₂, C-23), 56.5
 937 (CH), 52.5 (C, C-13), 49.1 (CH), 43.8 (CH₂), 40.3 (CH₂), 36.0
 938 (CH₂), 30.6 (CH₂), 30.1 (CH₂), 29.8 (CH₂), 29.4 (CH₂), 29.1 (2 \times
 939 CH_3 , $\text{Me}_2\text{C-OH}$), 28.7 (CH), 28.1 (CH₂), 25.9 (3 \times CH_3 , $\text{Me}_3\text{C-Si}$),
 940 24.2 (CH₂), 21.8 (CH₃), 21.1 (CH₂), 19.4 (CH₂), 18.1 (C, C-Si), 10.0
 941 (CH₃), -5.5 (2 \times CH_3 , Me_2Si). IR (CHCl_3 , cm^{-1}): 3467 ($\nu_{\text{O-H}}$,
 942 2958 ($\nu_{\text{C-H}}$), 2930 ($\nu_{\text{C-H}}$), 2858 ($\nu_{\text{C-H}}$), 1718 ($\nu_{\text{C=O}}$). MS ([$\text{Cl}]^+, m/
 943 z, %]: 463 ([$\text{M}-\text{OH}]^+, 58), 405 ([$\text{M}-\text{OH}-t\text{-Bu}]^+, 48), 331 ([$\text{M}$$$$

- OH - TBSO] $^+$, 94). HRMS: [$\text{Cl}]^+$, calcd for $[\text{C}_{29}\text{H}_{55}\text{O}_2\text{Si}]^+$, 944
 463.3971; found, 463.3986. 945

(E)-8-(Bromomethylene)-23-(tert-butyldimethylsilyloxy)-12\beta-(7-
 946 hydroxy-7-methyloctyl)-de-A,B-24-norcholan (**29b**). See **29a** for 947
 reaction procedure. Reagents: ($\text{Ph}_3\text{PCH}_2\text{Br}$)Br (689 mg, 1.58 mmol) 948
 in toluene (18 mL), KOt-Bu in THF (1.56 mL, 1 M, 1.56 mmol), and 949
 ketone **28b** (95 mg, 0.19 mmol) in toluene (5 mL). Product: **29b** [74 950
 mg, 0.13 mmol, 67%, $R_f = 0.43$ (20% EtOAc–hexanes), colorless oil]. 951
 ^1H NMR (CDCl_3 , 250 MHz): 5.62 (1H, s, H-7), 3.76–3.49 (2H, m, 952
 H-23), 1.20 (6H, s, $\text{Me}_2\text{C-OH}$), 0.91 (3H, d, $J = 6.9$, H-21), 0.88 (9H, 953
 s, $\text{Me}_3\text{C-Si}$), 0.51 (3H, s, H-18), 0.03 (6H, s, Me_2Si). ^{13}C NMR 954
 (CDCl_3 , 63 MHz): 144.9 (C, C-8), 96.9 (CH, C-7), 71.0 (C, C-OH), 955
 62.1 (CH_2 , C-23), 56.8 (CH), 55.8 (CH), 49.8 (CH), 48.9 (C, C-13), 956
 44.0 (CH₂), 36.2 (CH₂), 31.1 (2 \times CH₂), 30.2 (CH₂), 30.0 (CH₂), 957
 29.6 (CH), 29.2 (2 \times CH₃, $\text{Me}_2\text{C-OH}$), 28.2 (CH₂), 28.1 (CH₂), 26.0 958
 (3 \times CH₃, $\text{Me}_3\text{C-Si}$), 24.3 (CH₂), 22.5 (CH₂), 22.0 (CH₃), 21.4 959
 (CH₂), 18.3 (C, C-Si), 9.7 (CH₃), -5.3 (2 \times CH₃, Me_2Si). IR (film, 960
 cm^{-1}): 3369 ($\nu_{\text{O-H}}$), 3084 ($\nu_{\text{C-H}}$), 2954 ($\nu_{\text{C-H}}$), 2930 ($\nu_{\text{C-H}}$), 2858 961
 ($\nu_{\text{C-H}}$), 1632 ($\nu_{\text{C=C}}$). MS ([$\text{Cl}]^+, m/z, \%$): 539 ([$\text{M}-\text{OH}]^+, 14), 407 962
 ([$\text{M}-\text{OH}-\text{TBSO}]^+, 27), 327 ([$\text{M}-\text{C}_6\text{H}_{18}\text{BrO}_2\text{Si}]^+, 100). 963
 HRMS: [$\text{Cl}]^+$, calcd for $[\text{C}_{30}\text{H}_{56}\text{BrO}_2\text{Si}]^+$, 539.3284; found, 539.3275. 964$$$

(E)-23-(tert-Butyldimethylsilyloxy)-12\beta-(7-hydroxy-7-methyloc- 965
 tyl)-8-[(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)methylene]-de- 966
 A,B-24-norcholan (**6b**). See **6a** for reaction procedure. Reagents: 967
 PCy_3 (3 mg, 0.011 mmol) and $\text{PdCl}_2(\text{dpf})\cdot\text{CH}_2\text{Cl}_2$ (5 mg, 0.005 968
 mmol) in DMSO (2 mL), **29b** (103 mg, 0.18 mmol) in DMSO (2 969
 mL), KOAc (54 mg, 0.55 mmol), and Pin₂B₂ (91 mg, 0.36 mmol). 970
 Product: **6b** [79 mg, 0.13 mmol, 71%, $R_f = 0.30$ (10% EtOAc– 971
 hexanes), colorless oil, $[\alpha]_D = +46.1$ ($c = 0.2$, CHCl_3)]. ^1H NMR 972
 (CDCl_3 , 250 MHz): 4.88 (1H, s, H-7), 3.74–3.41 (2H, m, H-23), 1.24 973
 (12H, s, 2 \times $\text{Me}_2\text{C-OB}$), 1.19 (6H, s, $\text{Me}_2\text{C-OH}$), 0.90 (3H, d, $J = 6.9$, 974
 H-21), 0.87 (9H, s, $\text{Me}_3\text{C-Si}$), 0.49 (3H, s, H-18), 0.02 (6H, s, Me_2Si). 975
 ^{13}C NMR (CDCl_3 , 63 MHz): 166.0 (C, C-8), 82.4 (2 \times C, 2 \times C- 976
 OB), 71.0 (C, C-OH), 62.2 (CH₂, C-23), 58.9 (CH), 56.8 (CH), 50.2 977
 (CH), 49.5 (C, C-13), 43.9 (CH₂), 36.2 (CH₂), 33.2 (CH₂), 31.2 978
 (CH₂), 30.2 (CH₂), 30.0 (2 \times CH₂), 29.6 (CH), 29.2 (2 \times CH₃, 979
 $\text{Me}_2\text{C-OH}$), 28.2 (CH₂), 26.0 (3 \times CH₃, $\text{Me}_3\text{C-Si}$), 24.9 (2 \times CH₃, 980
 $\text{Me}_2\text{C-OB}$), 24.7 (2 \times CH₃, $\text{Me}_2\text{C-OB}$), 24.3 (CH₂), 22.7 (CH₂), 22.0 981
 (CH₃), 21.2 (CH₂), 18.3 (C, C-Si), 10.0 (CH₃), -5.3 (2 \times CH₃, 982
 Me_2Si). IR (film, cm^{-1}): 3390 ($\nu_{\text{O-H}}$), 2955 ($\nu_{\text{C-H}}$), 2930 ($\nu_{\text{C-H}}$), 983
 2858 ($\nu_{\text{C-H}}$), 1640 ($\nu_{\text{C=C}}$). MS ([$\text{Cl}]^+, m/z, \%$): 587 ([$\text{M}-\text{OH}]^+, 984
 32), 455 ([$\text{M}-\text{OH}-\text{TBSO}]^+, 100), 329 ([$\text{M}-\text{C}_{12}\text{H}_{28}\text{BO}_4\text{Si}]^+, 985
 25). HRMS: [$\text{Cl}]^+$, calcd for $[\text{C}_{36}\text{H}_{68}\text{BO}_3\text{Si}]^+$, 587.5028; found, 986
 587.5031. 987$$$

1*α,23-Dihydroxy-12\beta*-(7-hydroxy-7-methyloctyl)-24,25,26,27-tet- 988
 ranorvitamin D₃ (**5b**). See **5a** for reaction procedure. Reagents: 989
 aqueous solution of K₃PO₄ (1.6 mL, 2 M), PdCl₂(PPh₃)₂ (4 mg, 0.006 990
 mmol), **6b** (68 mg, 0.112 mmol), 7 (79 mg, 0.132 mmol), and THF 991
 (4 mL). Then THF (7 mL) and TBAF in THF (840 μL , 1 M, 0.840 992
 mmol). Product: **5b** [45 mg, 0.089 mmol, 80%, $R_f = 0.20$ (90% 993
 EtOAc–hexanes), white solid, mp 87–91 °C (Et₂O–hexanes), $[\alpha]_D = 994$
 -30.0 ($c = 1.2$, EtOH 96%). ^1H NMR (CDCl_3 , 400 MHz): 6.34 (1H, 995
 d, $J = 11.2$, H-6), 6.00 (1H, d, $J = 11.2$, H-7), 5.31 (1H, s, H-19), 4.98 996
 (1H, s, H-19), 4.41 (1H, dd, $J_1 = 4.3$, $J_2 = 7.7$, H-1), 4.26–4.15 (1H, 997
 m, H-3), 3.69 (1H, ddd, $J_1 = 4.8$, $J_2 = 8.8$, $J_3 = 10.1$, H-23), 3.28 (1H, 998
 ddd, $J_1 = 7.6$, $J_2 = 7.6$, $J_3 = 10.3$, H-23), 2.80 (1H, dd, $J_1 = 3.9$, $J_2 = 13.4$, 999
 H-9), 2.52 (1H, dd, $J_1 = 3.2$, $J_2 = 13.3$, H-4), 2.30 (1H, dd, $J_1 = 6.8$, $J_2 = 1000$
 13.3, H-4), 1.20 (6H, s, $\text{Me}_2\text{C-OH}$), 0.94 (3H, d, $J = 6.8$, H-21), 0.49 (1001
 3H, s, H-18). ^{13}C NMR (CDCl_3 , 100 MHz): 147.6 (C, C-10), 142.6 (1002
 C, C-8), 133.0 (C, C-5), 124.9 (CH, C-6), 116.9 (CH, C-7), 111.7 (1003
 CH₂, C-19), 71.1 (C, C-OH), 70.7 (CH, C-1), 66.7 (CH, C-3), 62.2 (1004
 CH₂, C-23), 57.3 (CH), 56.7 (CH), 50.1 (CH), 49.3 (C, C-13), 45.2 (1005
 CH₂, C-4), 43.9 (CH₂), 42.8 (CH₂, C-2), 36.1 (CH₂), 31.4 (CH₂), 1006
 30.2 (CH₂), 30.1 (CH), 29.9 (CH₂), 29.3 (CH₂), 29.2 (2 \times CH₃, 1007
 $\text{Me}_2\text{C-OH}$), 29.0 (CH₂, C-9), 28.2 (CH₂), 24.3 (CH₂), 22.6 (CH₂), 1008
 22.1 (CH₃, C-21), 21.5 (CH₂), 10.0 (CH₃, C-18). IR (KBr, cm^{-1}): 1009
 3396 ($\nu_{\text{O-H}}$), 2930 ($\nu_{\text{C-H}}$), 2871 ($\nu_{\text{C-H}}$), 1649 ($\nu_{\text{C=C}}$). MS ([$\text{ESI-TOF}]^+, m/z, \%$): 525 ([$\text{M}+\text{Na}]^+, 100), 467 ([$\text{M}-\text{OH}-\text{H}_2\text{O}]^+, 1011
 58). HRMS: [$\text{ESI-TOF}]^+$, calcd for $[\text{C}_{32}\text{H}_{54}\text{O}_4\text{Na}]^+$, 525.3914; found, 1012
 525.3913. UV (96% EtOH): $\lambda_{\text{max}} = 264$ nm, $\lambda_{\text{min}} = 230$ nm. 1013$$

1014 *8β-Hydroxy-12β-(7-hydroxy-7-methyloctyl)-de-A,B-24-norcho-*
 1015 *lan-23-yl p-toluenesulfonate (25b)*. See 25a for reaction procedure.
 1016 Reagents: *p*-toluenesulfonyl chloride (168 mg, 0.879 mmol), **8b** (216
 1017 mg, 0.586 mmol), and pyridine (15 mL). Product: **25b** [255 mg, 0.488
 1018 mmol, 83%, $R_f = 0.60$ (70% EtOAc–hexanes), colorless oil]. ^1H NMR
 1019 (CDCl_3 , 250 MHz): 7.74 (2H, d, $J = 8.2$, H-Ar), 7.30 (2H, d, $J = 8.2$,
 1020 H-Ar), 4.18–3.87 (3H, m, H-8 and H-23), 2.40 (3H, s, Me-Ar), 1.16
 1021 (6H, s, $\text{Me}_2\text{C-OH}$), 0.80 (3H, d, $J = 6.8$, H-21), 0.74 (3H, s, H-18).
 1022 ^{13}C NMR (CDCl_3 , 63 MHz): 144.5 (C, Ar-SO₂), 133.0 (C), 129.7 (2
 1023 × CH, Ar-H), 127.7 (2 × CH, Ar-H), 70.8 (C, C-OH), 69.8 (CH₂, C-
 1024 23), 68.7 (CH, C-8), 56.8 (CH), 53.5 (CH), 49.6 (CH), 45.4 (C, C-
 1025 13), 43.8 (CH₂), 33.9 (CH₂), 32.5 (CH₂), 31.2 (CH₂), 30.1 (CH₂),
 1026 29.8 (CH₂), 29.0 (2 × CH₃, $\text{Me}_2\text{C-OH}$), 28.9 (CH), 28.1 (CH₂), 24.2
 1027 (CH₂), 23.8 (CH₂), 22.3 (CH₂), 21.8 (CH₃, Me-Ar), 21.5 (CH₃), 20.8
 1028 (CH₂), 11.2 (CH₃). IR (film, cm^{-1}): 3545 ($\nu_{\text{O-H}}$), 3425 ($\nu_{\text{O-H}}$), 2930
 1029 ($\nu_{\text{C-H}}$), 2860 ($\nu_{\text{C-H}}$), 1176 ($\nu_{\text{O=S=O}}$). MS ([FAB]⁺, m/z , %): 545
 1030 ([M + Na]⁺, 4), 487 ([M – OH – H₂O]⁺, 25), 315 ([M – TsO –
 1031 2H₂O]⁺, 31). HRMS: [FAB]⁺, calcd for [C₃₀H₅₀O₅SnA]⁺, 545.3277;
 1032 found, 545.3271.

1033 *8β-Hydroxy-12β-(7-hydroxy-7-methyloctyl)-de-A,B-24-norcho-*
 1034 *lane-23-carbonitrile (26b)*. See 26a for reaction procedure. Reagents:
 1035 potassium cyanide (159 mg, 2.44 mmol), **25b** (255 mg, 0.488 mmol),
 1036 and DMSO (10 mL). Product: **26b** [167 mg, 0.442 mmol, 91%, $R_f =$
 1037 0.50 (70% EtOAc–hexanes), colorless oil]. ^1H NMR (CDCl_3 , 250
 1038 MHz): 3.96 (1H, broad s, H-8), 2.39–2.16 (2H, m, H-23), 1.14 (6H,
 1039 s, $\text{Me}_2\text{C-OH}$), 0.90 (3H, d, $J = 6.8$, H-21), 0.82 (3H, s, H-18). ^{13}C
 1040 NMR (CDCl_3 , 63 MHz): 119.9 (C, C≡N), 70.9 (C, C-OH), 68.7
 1041 (CH, C-8), 57.0 (CH), 53.6 (CH), 49.7 (CH), 45.6 (C, C-13), 43.9
 1042 (CH₂), 34.0 (CH₂), 32.3 (CH), 31.4 (CH₂), 30.2 (CH₂), 29.9 (CH₂),
 1043 29.2 (CH₂), 29.1 (2 × CH₃, $\text{Me}_2\text{C-OH}$), 28.2 (CH₂), 24.3 (CH₂),
 1044 23.5 (CH₂), 22.4 (CH₂), 21.4 (CH₃), 20.8 (CH₂), 15.9 (CH₂), 11.4
 1045 (CH₃). IR (film, cm^{-1}): 3445 ($\nu_{\text{O-H}}$), 2931 ($\nu_{\text{C-H}}$), 2873 ($\nu_{\text{C-H}}$),
 1046 2247 ($\nu_{\text{C≡N}}$). MS ([CI]⁺, m/z , %): 377 ([M]⁺, 7), 360 ([M – OH]⁺,
 1047 48), 342 ([M – OH – H₂O]⁺, 100). HRMS: [CI]⁺, calcd for
 1048 [C₂₄H₄₂NO]⁺, 360.3266; found, 360.3269.

1049 *12β-(7-Hydroxy-7-methyloctyl)-de-A,B-cholan-8β,24-diol (8c)*.
 1050 See 8b for reaction procedure. Reagents: diisobutylaluminum hydride
 1051 in CH_2Cl_2 (2.01 mL, 1 M, 2.01 mmol) and CH_2Cl_2 (5 mL), **26b** (152
 1052 mg, 0.402 mmol) in CH_2Cl_2 (5 mL) and aqueous HCl (10 mL, 3 M)
 1053 in Et_2O (10 mL), then THF (6 mL) and diisobutylaluminum hydride
 1054 in CH_2Cl_2 (2.01 mL, 1 M, 2.01 mmol). Product: **8c** [95 mg, 0.248
 1055 mmol, 61%, $R_f = 0.38$ (70% EtOAc–hexanes), white foam, $[\alpha]_D =$
 1056 +38.4 ($c = 0.2$, CHCl_3)]. ^1H NMR (CDCl_3 , 250 MHz): 4.01 (1H,
 1057 broad d, $J = 1.4$, H-8), 3.71–3.51 (2H, m, H-24), 1.19 (6H, s, $\text{Me}_2\text{C-}$
 1058 OH), 0.91 (3H, d, $J = 6.8$, H-21), 0.83 (3H, s, H-18). ^{13}C NMR
 1059 (CDCl_3 , 63 MHz): 71.0 (C, C-OH), 69.0 (CH, C-8), 63.1 (CH₂, C-
 1060 24), 57.5 (CH), 53.6 (CH), 49.8 (CH), 45.4 (C, C-13), 43.9 (CH₂),
 1061 33.9 (CH₂), 32.8 (CH), 31.7 (CH₂), 31.3 (CH₂), 30.1 (CH₂), 29.9
 1062 (CH₂), 29.6 (CH₂), 29.1 (2 × CH₃, $\text{Me}_2\text{C-OH}$), 28.1 (CH₂), 24.3
 1063 (CH₂), 23.5 (CH₂), 22.5 (CH₂), 22.3 (CH₃), 20.8 (CH₂), 11.3 (CH₃).
 1064 IR (film, cm^{-1}): 3335 ($\nu_{\text{O-H}}$), 2932 ($\nu_{\text{C-H}}$), 2864 ($\nu_{\text{C-H}}$). MS
 1065 ([FAB]⁺, m/z , %): 365 ([M – OH]⁺, 53), 342 ([M – OH – H₂O]⁺,
 1066 100). HRMS: [FAB]⁺, calcd for [C₂₄H₄₅O₂]⁺, 365.3420; found,
 1067 365.3422.

1068 *24-(tert-Butyldimethylsilyloxy)-12β-(7-hydroxy-7-methyloctyl)-*
 1069 *de-A,B-cholan-8β-ol (27c)*. See 27a for reaction procedure. Reagents:
 1070 imidazol (30 mg, 0.441 mmol), TBSCl (30 mg, 0.199 mmol), **8c** (35
 1071 mg, 0.091 mmol), and DMF (10 mL). Product: **27c** [35 mg, 0.07
 1072 mmol, 78%, $R_f = 0.78$ (70% EtOAc–hexanes), colorless oil]. ^1H NMR
 1073 (CDCl_3 , 250 MHz): 4.01 (1H, broad d, $J = 1.2$, H-8), 3.64–3.52 (2H,
 1074 m, H-24), 1.20 (6H, s, $\text{Me}_2\text{C-OH}$), 0.93–0.82 (15H, m, H-18, H-21
 1075 and $\text{Me}_3\text{C-Si}$), 0.04 (6H, s, Me_2Si). ^{13}C NMR (CDCl_3 , 63 MHz): 71.0
 1076 (C, C-OH), 69.1 (CH, C-8), 63.5 (CH₂, C-24), 57.6 (CH), 53.8
 1077 (CH), 49.9 (CH), 45.4 (C, C-13), 44.0 (CH₂), 34.0 (CH₂), 32.8
 1078 (CH), 32.0 (CH₂), 31.3 (CH₂), 30.2 (CH₂), 30.0 (CH₂), 29.6 (CH₂),
 1079 29.1 (2 × CH₃, $\text{Me}_2\text{C-OH}$), 28.2 (CH₂), 25.9 (3 × CH₃, $\text{Me}_3\text{C-Si}$),
 1080 24.3 (CH₂), 23.5 (CH₂), 22.6 (CH₂), 22.3 (CH₃), 20.8 (CH₂), 18.3
 1081 (C, C-Si), 11.3 (CH₃), –5.3 (2 × CH₃, Me_2Si). IR (film, cm^{-1}): 3400
 1082 ($\nu_{\text{O-H}}$), 2930 ($\nu_{\text{C-H}}$), 2858 ($\nu_{\text{C-H}}$). MS ([CI]⁺, m/z , %): 479 ([M –
 1083 OH]⁺, 53), 461 ([M – OH – H₂O]⁺, 18), 329 ([M – 2H₂O –

TBSO][–], 100). HRMS: [CI]⁺, calcd for [C₃₀H₅₉O₂Si]⁺, 479.4284; 1084
 found, 479.4291. 1085

1086 *24-(tert-Butyldimethylsilyloxy)-12β-(7-hydroxy-7-methyloctyl)-*
 1087 *de-A,B-cholan-8-one (28c)*. See 28a for reaction procedure. Reagents: 1087
 1088 pyridinium dichromate (198 mg, 0.525 mmol), **27c** (87 mg, 0.175 1088
 1089 mmol), and CH_2Cl_2 (8 mL). Product: ketone **28c** [80 mg, 0.162 1089
 1090 (mmol, 93%, $R_f = 0.62$ (60% EtOAc–hexanes), colorless oil]. ^1H NMR 1090
 1091 (CDCl_3 , 250 MHz): 3.61–3.48 (2H, m, H-24), 1.17 (6H, s, $\text{Me}_2\text{C-}$ 1091
 1092 OH), 0.91 (3H, d, $J = 6.8$, H-21), 0.84 (9H, s, $\text{Me}_3\text{C-Si}$), 0.58 (3H, s, 1092
 1093 H-18), 0.00 (6H, Me_2Si). ^{13}C NMR (CDCl_3 , 63 MHz): 212.5 (C, C- 1093
 1094 8), 70.8 (C, C-OH), 63.3 (CH₂, C-24), 62.2 (CH), 57.1 (CH), 52.4 1094
 1095 (C, C-13), 48.9 (CH), 43.9 (CH₂), 40.4 (CH₂), 33.0 (CH), 31.8 1095
 1096 (CH₂), 30.7 (CH₂), 30.1 (CH₂), 29.8 (CH₂), 29.6 (CH₂), 29.1 (2 × 1096
 1097 CH₃, $\text{Me}_2\text{C-OH}$), 29.0 (CH₂), 28.2 (CH₂), 25.8 (3 × CH₃, $\text{Me}_3\text{C-Si}$), 1097
 1098 24.2 (CH₂), 21.9 (CH₃), 21.1 (CH₂), 19.3 (CH₂), 18.2 (C, C-Si), 10.1 1098
 1099 (CH₃), –5.3 (2 × CH₃, Me_2Si). IR (film, cm^{-1}): 3460 ($\nu_{\text{O-H}}$), 2955 1099
 1099 ($\nu_{\text{C-H}}$), 2930 ($\nu_{\text{C-H}}$), 2858 ($\nu_{\text{C-H}}$), 1717 ($\nu_{\text{C=O}}$). MS ([CI]⁺, m/z , 1100
 1100 %): 477 ([M – OH]⁺, 25), 419 ([M – OH – t-Bu]⁺, 33), 327 ([M – 1101
 2H₂O – TBSO]⁺, 100). HRMS: [CI]⁺, calcd for [C₃₀H₅₈O₂Si]⁺: 1102
 477.4128; found: 477.4131. 1103

1104 *(E)-8-(Bromomethylene)-24-(tert-butylidemethylsilyloxy)-12β-(7-*
 1104 *hydroxy-7-methyloctyl)-de-A,B-cholane (29c)*. See 29a for reaction 1104
 1105 procedure. Reagents: (Ph₃PCH₂Br)Br (782 mg, 1.79 mmol) in 1106
 1106 toluene (12 mL), KOT-Bu in THF (1.76 mL, 1 M, 1.76 mmol), ketone 1107
 1107 **28c** (111 mg, 0.22 mmol) in toluene (6 mL). Product: **29c** [90 mg, 1108
 0.16 mmol, 70%, $R_f = 0.52$ (20% EtOAc–hexanes), colorless oil]. ^1H 1108
 1109 NMR (CDCl_3 , 250 MHz): 5.61 (1H, s, H-7), 3.61–3.48 (2H, m, H- 1110
 1110 24), 1.20 (6H, s, $\text{Me}_2\text{C-OH}$), 0.92 (3H, d, $J = 6.9$, H-21), 0.88 (9H, s, 1111
 1111 $\text{Me}_3\text{C-Si}$), 0.49 (3H, s, H-18), 0.03 (6H, Me_2Si). ^{13}C NMR (CDCl_3 , 1112
 1112 63 MHz): 144.9 (C, C-8), 97.0 (CH, C-7), 71.0 (C, C-OH), 63.5 1113
 1113 (CH₂, C-24), 56.7 (CH), 56.3 (CH), 49.5 (CH), 48.7 (C, C-13), 44.0 1114
 1114 (CH₂), 33.6 (CH), 32.0 (CH₂), 31.2 (CH₂), 31.1 (CH₂), 30.2 (CH₂), 1115
 30.0 (CH₂), 29.2 (2 × CH₃, $\text{Me}_2\text{C-OH}$), 29.0 (CH₂), 28.3 (CH₂), 1116
 28.2 (CH₂), 25.9 (3 × CH₃, $\text{Me}_3\text{C-Si}$), 24.3 (CH₂), 22.3 (CH₂), 22.0 1117
 1117 (CH₃), 21.2 (CH₂), 18.3 (C, C-Si), 9.6 (CH₃), –5.3 (2 × CH₃, 1118
 1118 Me_2Si). IR (film, cm^{-1}): 3369 ($\nu_{\text{O-H}}$), 3084 ($\nu_{\text{C-H}}$), 2953 ($\nu_{\text{C-H}}$), 1119
 2930 ($\nu_{\text{C-H}}$), 2858 ($\nu_{\text{C-H}}$), 1632 ($\nu_{\text{C=C}}$). MS ([CI]⁺, m/z , %): 553 1120
 1120 ([M – OH]⁺, 36), 421 ([M – OH – TBSOH]⁺, 42), 341 ([M – 1121
 C₆H₁₈BrO₂Si]⁺, 100). HRMS: [CI]⁺, calcd for [C₃₁H₅₈BrOSi]⁺, 1122
 553.3440; found, 553.3444. 1123

1124 *(E)-24-(tert-Butyldimethylsilyloxy)-12β-(7-hydroxy-7-methyloc-*
 1125 *tyl)-8-[4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)methylene]-de-*
 1126 *A,B-cholane (6c)*. See 6a for reaction procedure. Reagents: PCy₃ (2 1126
 1126 mg, 0.008 mmol) and PdCl₂(dpdpf)· CH_2Cl_2 (4 mg, 0.004 mmol) in 1127
 1127 DMSO (2 mL), **29c** (82 mg, 0.14 mmol) in DMSO (2 mL), KOAc 1128
 41 mg, 0.42 mmol), and Pin₂B₂ (71 mg, 0.28 mmol). Product: **6c** [61 1129
 1129 mg, 0.10 mmol, 69%, $R_f = 0.28$ (15% EtOAc–hexanes), colorless oil]. 1130
 1130 ^1H NMR (CDCl_3 , 250 MHz): 4.89 (1H, s, H-7), 3.62–3.52 (2H, m, 1131
 1131 H-24), 1.25 (12H, s, 2 × $\text{Me}_2\text{C-BO}$), 1.20 (6H, s, $\text{Me}_2\text{C-OH}$), 0.94– 1132
 0.85 (12H, m, H-21 and $\text{Me}_3\text{C-Si}$), 0.47 (3H, s, H-18), 0.03 (6H, 1133
 1133 Me_2Si). ^{13}C NMR (CDCl_3 , 63 MHz): 166.1 (C, C-8), 82.5 (2 × C, 2 1134
 1134 × C-OB), 71.0 (C, C-OH), 63.6 (CH₂, C-24), 57.4 (CH), 56.3 (CH), 1135
 50.0 (CH), 49.4 (C, C-13), 44.0 (CH₂), 33.7 (CH), 33.2 (CH₂), 32.0 1136
 (CH₂), 31.3 (CH₂), 30.2 (2 × CH₂), 30.0 (CH₂), 29.2 (2 × CH₃, 1137
 1137 $\text{Me}_2\text{C-OH}$), 29.0 (CH₂), 28.2 (CH₂), 25.9 (3 × CH₃, $\text{Me}_3\text{C-Si}$), 24.9 1138
 1138 (2 × CH₃, $\text{Me}_2\text{C-OB}$), 24.8 (2 × CH₃, $\text{Me}_2\text{C-OB}$), 24.3 (CH₂), 22.5 1139
 1139 (CH₂), 22.0 (CH₃), 21.0 (CH₂), 18.3 (C, C-Si), 9.9 (CH₃), –5.3 (2 × 1140
 1140 CH₃, Me_2Si). IR (film, cm^{-1}): 3369 ($\nu_{\text{O-H}}$), 2953 ($\nu_{\text{C-H}}$), 2930 1141
 1141 ($\nu_{\text{C-H}}$), 2858 ($\nu_{\text{C-H}}$), 1640 ($\nu_{\text{C=C}}$). MS ([CI]⁺, m/z , %): 601 ([M – 1142
 OH]⁺, 71), 469 ([M – OH – TBSOH]⁺, 100), 343 ([M – 1143
 C₁₂H₂₈BO₄Si]⁺, 29). HRMS: [CI]⁺, calcd for [C₃₇H₇₀BO₃Si]⁺, 1144
 601.5187; found, 601.5197. 1145

1146 *1α,24-Dihydroxy-12β-(7-hydroxy-7-methyloctyl)-25,26,27-trinor-*
 1147 *vitamin D₃ (5c)*. See 5a for reaction procedure. Reagents: aqueous 1147
 1148 solution of K₃PO₄ (1.2 mL, 2 M), PdCl₂(PPh₃)₂ (3 mg, 0.004 mmol), 1148
 1149 **6c** (54 mg, 0.087 mmol), 7 (61 mg, 0.102 mmol), and THF (3 mL). 1149
 Then THF (6 mL) and TBAF in THF (560 μL , 1 M, 0.560 mmol). 1150
 Product: **5c** [37 mg, 0.072 mmol, 83%, $R_f = 0.18$ (90% EtOAc– 1151
 hexanes), white solid, mp 88–92 °C (Et₂O–hexanes), $[\alpha]_D = -20.4$ (c 1152
 = 0.5, EtOH 96%)]. ^1H NMR (CDCl_3 , 400 MHz): 6.34 (1H, d, $J =$ 1153

1154 11.2, H-6), 6.01 (1H, d, J = 11.2, H-7), 5.32 (1H, s, H-19), 4.98 (1H, s, 1155 H-19), 4.42 (1H, dd, J_1 = 4.2, J_2 = 7.7, H-1), 4.21 (1H, dddd, J_1 = 3.5, 1156 J_2 = 3.5, J_3 = 6.7, J_4 = 6.7, H-3), 3.61 (2H, ddd, J_1 = 2.2, J_2 = 6.5, J_3 = 1157 6.5, H-24), 2.80 (1H, dd, J_1 = 3.8, J_2 = 13.3, H-9), 2.58 (1H, dd, J_1 = 1158 2.9, J_2 = 13.2, H-4), 2.30 (1H, dd, J_1 = 6.5, J_2 = 13.2, H-4), 1.21 (6H, s, 1159 Me₂C-OH), 0.94 (3H, d, J = 6.8, H-21), 0.48 (3H, s, H-18). ¹³C NMR 1160 (CDCl₃, 100 MHz): 147.6 (C, C-10), 142.7 (C, C-8), 133.0 (C, C-5), 1161 124.9 (CH, C-6), 116.9 (CH, C-7), 111.7 (CH₂, C-19), 71.1 (C, C- 1162 OH), 70.7 (CH, C-1), 66.8 (CH, C-3), 63.3 (CH₂, C-24), 57.2 (CH), 1163 57.0 (CH), 50.1 (CH), 49.3 (C, C-13), 45.2 (CH₂, C-4), 44.0 (CH₂), 1164 42.8 (CH₂, C-2), 33.8 (CH), 31.8 (CH₂), 31.4 (CH₂), 30.2 (CH₂), 1165 30.0 (CH₂), 29.7 (CH₂), 29.4 (CH₂), 29.2 (2 × CH₃, Me₂C-OH), 1166 29.0 (CH₂, C-9), 28.2 (CH₂), 24.3 (CH₂), 22.5 (CH₂), 22.0 (CH₃, C- 1167 21), 21.3 (CH₂), 9.9 (CH₃, C-18). IR (KBr, cm⁻¹): 3388 (ν_{O-H}), 2930 1168 (ν_{C-H}), 2861 (ν_{C-H}), 1632 ($\nu_{C=C}$). MS ([ESI-TOF]⁺, *m/z*, %): 539 1169 ([M + Na]⁺, 100), 481 ([M - OH - H₂O]⁺, 24). HRMS: [ESI- 1170 TOF]⁺, calcd for [C₃₃H₅₆O₄Na]⁺, 539.4071; found, 539.4069. UV 1171 (96% EtOH): λ_{max} = 264 nm, λ_{min} = 230 nm.

1172 **Functional Studies. Cell Culture.** Human MCF-7 breast 1173 adenocarcinoma and SW480-ADH colon cancer cells were grown in 1174 DMEM and RPMI media, respectively, supplemented with 10% FBS, 1175 100 U/mL penicillin, 100 U/mL penicillin, 100 U/mL streptomycin, 1176 and 2 mM L-glutamine (all from Invitrogen, Paisley, UK), in air-CO₂ 1177 (95:5) atmosphere at 37 °C. Confluent cells were washed twice with 1178 phosphate-buffered saline (PBS) and harvested by a brief incubation 1179 with trypsin-EDTA solution (Sigma Aldrich St. Louis, USA) in PBS. 1180 Treatments with 1 α ,25-(OH)₂D₃ (1), or compounds 5a–c were 1181 carried out using medium supplemented with charcoal-treated FCS to 1182 remove liposoluble hormones. Control cells were treated with the 1183 corresponding vehicle.

1184 **MTT Metabolization.** Cell proliferation experiments were carried 1185 out using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bro- 1186 mide (MTT) assays, where MTT is reduced to purple formazan by 1187 the mitochondria of living cells. Increase in cell number is detected by 1188 augmented MTT metabolism. MCF-7 cells were plated at a 5 × 10⁴ 1189 cells per well in 24-well plates. Twenty-four hours later, the cells were 1190 treated with compounds 5a–c at concentrations of 10, 100, or 500 nM 1191 during 48 h. MTT (0.5 µg/µL) was added to each well, and the 1192 mixture was incubated for 1 h. The medium was then removed, and 1193 DMSO (500 µL) was added to each well. Absorbance of samples was 1194 measured at 570 nm in a Mithras LB 940 from Berthold Technologies 1195 (Bad Wildbad, Germany).

1196 **Luciferase Reporter Assays.** Cells were cultured as described above. 1197 Then 12–24 h before transfection, 2 × 10⁵ cells per well were seeded 1198 in 24-well plates and allowed to attach overnight. Cells were then 1199 transfected using JetPEI transfection reagent (PolyPlus Transfection, 1200 Illkirch, France) following the manufacturer's guidelines. Transfections 1201 were performed in triplicate using 1 µg of pCYP24A1-Luc plasmid 1202 (kindly provided by Dr. Aranda, Instituto de Investigaciones 1203 Biomédicas Alberto Sols, Madrid). This vector encoding the luciferase 1204 gene under control of a consensus vitamin D response element (24- 1205 hydroxylase promoter, CYP24A1), and it is very responsive to the 1206 1 α ,25-(OH)₂D₃ treatment. After incubation for 24 h in DMEM 1207 supplemented with 10% of charcoal-stripped FCS, culture medium was 1208 replaced to phenol red free DMEM containing 10% FCS for 24 h with 1209 each compound (1, 5a–c) at several concentrations (1 × 10⁻¹¹ to 1 × 1210 10⁻⁶ M). Cells were then treated during 10 min with luciferin 1211 potassium salt (100 mg/L) (Regis Technologies, Morton Grove, IL), 1212 and bioluminescence images acquired with the In Vivo Imaging 1213 System (IVIS, Caliper Life Sciences, Alameda, CA, USA), quantified as 1214 total photon counts, and processed by Living Image software (Caliper 1215 Life Sciences). The EC₅₀ values are derived from dose-response 1216 curves and represent the analogue concentration capable of increasing 1217 the luciferase activity by 50%. The luciferase activity ratio is the 1218 average ratio of the EC₅₀ for the analogue to the EC₅₀ for 1 α ,25- 1219 (OH)₂D₃. All experiments were carried out in duplicate on at least two 1220 different occasions.

1221 **Human VDR Binding Assay.** Binding affinity to VDR was evaluated 1222 using a 1 α ,25-(OH)₂D₃ assay kit under manufacturer conditions 1223 (PolarScreen Vitamin D receptor competitor assay, Red, catalogue no.

PV4569, Invitrogen). This kit is a fluorescence polarization (FP)-based 1224 competition assay that provides a sensitive and robust method for 1225 high-throughput screening of potential vitamin D receptor ligands. 1226 VDR is added to a fluorescent VDR ligand to form a receptor/tracer 1227 complex resulting in a high polarization value. This complex is then 1228 added to individual test compounds in microwell plates. Competitors 1229 will displace the tracer from the complex, causing the fluorescent 1230 ligand to tumble more rapidly during its fluorescence lifetime and 1231 resulting in a low polarization value. The polarized fluorescence was 1232 measured in a 384-well black plate during 200 ms/well using a Mithras 1233 LB 940 (Berthold Technologies). All compounds (1, 5a–c) were 1234 evaluated within the range from 10⁻¹¹ M to 10⁻⁵ M. IC₅₀ values were 1235 calculated using average of measured values. The activity of each 1236 compound is also shown as percentage, in which the activity of the 1237 natural hormone 1 α ,25-(OH)₂D₃ was normalized to 100%. 1238

1239 **Western Blotting.** Western blotting was performed as previously 1240 described.²⁷ Protein concentration was measured using the Bio-Rad 1241 DC protein assay kit. Analysis of cell lysates was performed by 1242 electrophoresis in SDS gels and protein transfer to Immobilon P 1243 membranes (Millipore Corp., Billerica, MA, USA). The membranes 1244 were incubated with the appropriate primary and secondary 1244 horseradish peroxidase-conjugated antibodies, and the antibody 1245 binding was visualized using the ECL detection system (Amer- 1246 sham—GE Healthcare, Barcelona, Spain). We used rabbit polyclonal 1247 antibodies generated against Cystatin D,²⁹ mouse monoclonal 1248 antibodies against E-cadherin (BD Transduction Laboratories, San 1249 Diego, CA, USA), c-MYC (Santa Cruz Biotechnology, Heidelberg, 1250 Germany), and goat polyclonal antibody against β -actin (Santa Cruz 1251 Biotechnology).

1252 **Quantitative RT-PCR.** Total RNA was extracted from cultured cells 1253 using NucleoSpin miRNA extraction kit (Macherey-Nagel) and 1254 retrotranscribed using the iScript cDNA Synthesis Kit (Bio-Rad). 1255 The quantitative PCR reaction was performed in a CFX384 real-time 1256 PCR detection system (Bio-Rad) using the TaqMan Universal Master 1257 Mix (Applied Biosystems) and TaqMan probes for PIP4K2B 1258 (Hs01552176_m1) and RPLP0 (Hs99999902_m1) (both from 1259 Applied Biosystems). Thermal cycling consisted of an initial 1260 denaturing step at 95 °C for 10 min and 40 cycles of denaturing at 1261 95 °C for 15 s and annealing and elongation at 60 °C for 30 s. RNA 1262 expression values were normalized versus the housekeeping gene 1263 RPLP0 (ribosomal protein, large, P0) using the comparative CT 1264 method. All measurements were performed in triplicate. 1265

1266 **Serum Calcium Quantitation and Weight Measure.** All animal 1267 studies were approved by the University of Santiago de Compostela 1268 Ethics Committee for Animal Experiments. Female CD-1 mice (age 1269 matched, between 6 and 8 weeks) were obtained from Charles River 1270 Laboratories (L'Arbresle, France). The compounds (1, 5a–c) were 1271 dissolved in sesame oil and administered intraperitoneally (0.3 µg/kg) 1272 every other day for three weeks. Calcium measurement was 1273 determined a day after the last dose using the QuantiChom Calcium 1274 Assay Kit (BioAssay Systems, Hayward, CA, USA) following the 1274 manufacturer's guidelines. Weight of mice was carried out every other 1275 day for a week. 1276

1277 **Statistical Analysis.** Each experiment was performed at least three 1278 times. Values are expressed as means ± SD. Means were compared by 1279 unpaired t-tests or one-way ANOVA with the Tukey-Kramer multiple 1280 comparison for post hoc comparisons. Statistical significance is taken 1280 to be indicated by * $P < 0.05$, *** $P < 0.001$. Dose-response curves 1281 for the luciferase reporter assay and competitive VDR binding, as well 1282 as calculation of EC₅₀ and IC₅₀ values, were performed using 1283 GraphPad Prism 5 software (San Diego, CA, USA). 1284

ASSOCIATED CONTENT

Supporting Information

1285 Data from docking calculations, RT-PCR data, NMR spectra 1287 for the new compounds, X-ray structure and data of triol 8a, 1288 and HPLC traces of vitamins 5a–c. This material is available 1289 free of charge via the Internet at <http://pubs.acs.org>. 1290

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1299 Notes

1300 The authors declare no competing financial interest.

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1312 ■ DEDICATION

1313 †Dedicated to Prof. Milan Uskokovic.

1314 ■ ABBREVIATIONS USED

1315 CI, chemical ionization; 1,25D, 1 α ,25-dihydroxyvitamin D₃;
 1316 DEPT, distortionless enhancement by polarization transfer;
 1317 DIBAL-H, diisobutylaluminum hydride; dppf, 1,1'-bis-
 1318 (diphenylphosphino)ferrocene; ESI-TOF, electrospray ioniza-
 1319 tion-time-of-flight; FAB, fast atom bombardment; HMPA,
 1320 hexamethylphosphoramide; Im, imidazole; LBD, ligand binding
 1321 domain; LDA, lithium diisopropylamide; Ms, methylsulfonyl;
 1322 1 α ,25-(OH)₂D₃, 1 α ,25-dihydroxyvitamin D₃; PDC, pyridinium
 1323 dichromate; Pin, pinacolato; RXR, retinoid X receptor; TBS,
 1324 tert-butyltrimethylsilyl; Tf, trifluoromethanesulfonyl; VDR,
 1325 vitamin D receptor; MCF-7, human adenocarcinoma breast
 1326 cancer cell line; SW480-ADH, human colon cancer cell line

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