Novel 1α,25-Dihydroxyvitamin D 3 Analogues with the Side Chain at C12

ARTICLE *in* JOURNAL OF MEDICINAL CHEMISTRY · APRIL 2006

Impact Factor: 5.45 · DOI: 10.1021/jm049016g · Source: PubMed

Articles

Novel 1α,25-Dihydroxyvitamin D_3 Analogues with the Side Chain at C12

Xosé C. González-Avión,[†] Antonio Mouriño,[†] Natacha Rochel,*[,‡] and Dino Moras[‡]

Departamento de Química Orgánica y Unidad Asociada al Consejo Superior de Investigaciones Científicas, Universidad de Santiago de Compostela, 15782 Santiago de Compostela, Spain, and Département de Biologie et de Génomique Structurales, Institut de Génétique et de Biologie Moléculaire et Cellulaire, Centre National de la Recherche Scientifique/Institut National de la Santé et de la Recherche Médicale/ Université Louis Pasteur, Parc d'Innovation BP10142, 67404 Illkirch Cedex, France

Received December 2, 2004

The plethora of actions of 1α,25$(OH)_2D_3$ in various systems suggested wide clinical applications of vitamin D nuclear receptor (VDR) ligands in treatments of inflammation, dermatological indication, osteoporosis, cancers, and autoimmune diseases. More than 3000 vitamin D analogues have been synthesized in order to reduce the calcemic side effects while maintaining the transactivation potency of the natural ligand. In light of the crystal structures of the vitamin D nuclear receptor (VDR), novel analogues of the hormone 1α,25-$(OH)_2D_3$ with side chains attached to C-12 were synthesized via the convergent Wittig−Horner approach. Among the compounds studied, the analogue **2b** showed the highest binding affinity for VDR and was the most potent at inducing VDR transcriptional activity in a transient transfection assay (20% of the transactivation activity of the natural ligand).

Introduction

The biologically active form of vitamin D_3 (cholecalciferol; **1a** in Figure 1) is its dihydroxy derivative, the steroid hormone 1α,25-dihydroxyvitamin D_3 [1α,25$(OH)_2D_3$,[a] calcitriol; **1b**]. The first action of this hormone to be discovered was its contribution to bone maintenance through control of calcium and phosphate metabolism.[1] Subsequently it has been found to participate in the regulation of the cell cycle, affecting cell proliferation, differentiation, and apoptosis, and to have immunosuppressive effects.[2] These roles have suggested that calcitriol could be used not only for treatment of bone disorders such as osteoporosis or renal osteodystrophy, but also to treat leukemia, cancer of the breast, colon, and prostate gland, psoriasis, autoimmune diseases, and graft rejection.[3,4] However, the calcemic effects of excess calcitriol (bone resorption, hypercalcemia, and calcification of soft tissue) limit its use for these therapeutic purposes. As a result, analogues have been sought that have less intense calcemic effects without appreciable loss of other activities. To date, more than 3000 calcitriol analogues have been synthesized, but few are of clinical interest.[5,6]

Rational design of new pharmacologically useful calcitriol analogues must be based on precise understanding of the mechanisms of action of the native hormone. It is known that most of its actions are mediated by a specific nuclear receptor,[7] the vitamin D receptor (VDR), which upon binding calcitriol undergoes a series of events leading to the activation of the transcription of target genes through the association with the retinoid X nuclear receptor (RXR), coactivators of the p160 family (SRC-1),[8] cointegrators that remodel chromatin (CBP),[9] and mediator complexes that recruit RNA polymerase (DRIP/TRAP).[10] In previous work we determined the crystal structures of several complexes in which calcitriol or agonist analogues are bound to a modified human VDR ligand binding domain (hVDRΔ LBD) that has the same ligand-binding, RXR-associating, and transactivation capacities as those of wild-type hVDR.[11−14] We report here the synthesis of three new calcitriol analogues designed in the light of these crystal structures, **2a**−**c**, together with the results of preliminary evaluation of their molecular biological properties. The hope that the new ligands may display novel interaction patterns of pharmacological interest rests on their side chains being located at C12 rather than at C17, the position of the side chain in the natural hormone and in most existing analogues.

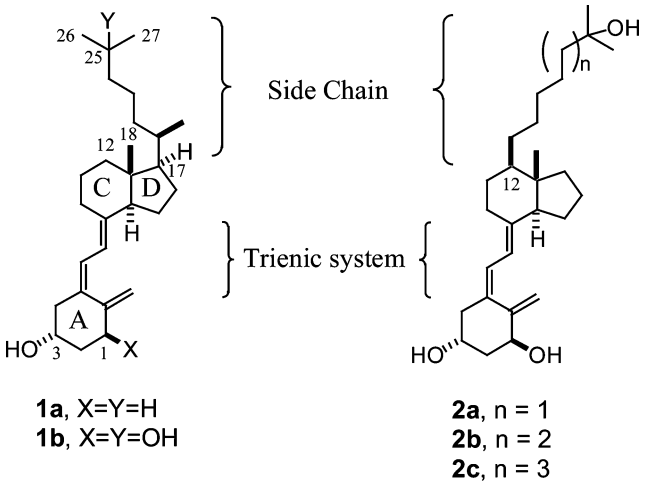


Figure 1. Chemical formulas of vitamin D_3 (**1a**, cholecalciferol), the steroid hormone 1α,25-dihydroxyvitamin D_3 [**1b**, 1α,25$(OH)_2D_3$, calcitriol], and the new analogues **2a**−**c**.

Results and Discussion

Design. The C25-hydroxyl group of calcitriol is essential for its high-affinity binding to hVDR, since it forms hydrogen bonds with hVDR histidines 305 and 397.[11] Inspection of the cal-

* Corresponding author: tel (33) 3 88 65 33 50; fax (33) 3 88 65 32 76; e-mail rochel@igbmc.u-strasbg.fr.

[†] Universidad de Santiago de Compostela.

[‡] CNRS/INSERM/Université Louis Pasteur.

[a] Abbreviations: NR, nuclear receptor; VDR, vitamin D NR; LBD, ligand binding domain; 1α,25$(OH)_2$-D_3, 1alpha,25-dihydroxyvitamin D_3.

10.1021/jm049016g CCC: $33.50
Published on Web 02/15/2006

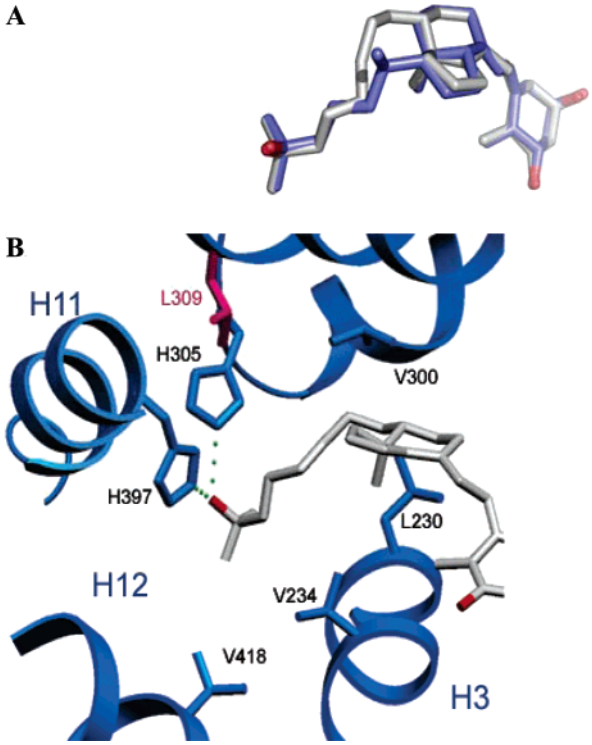
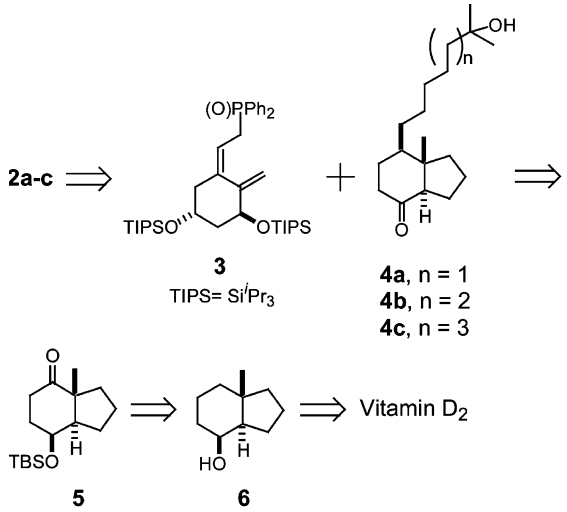


Figure 2. Docking of **2b** into the LBP of hVDRΔ. (A) Possible conformation for the side chain of **2b** (gray) superimposed with the active conformation of 1α,25(OH)$_2$D$_3$ (blue). (B) VDR−**2b** complex. The view is restricted to the region of the protein (H3, H6, H11, and H12) contacting the aliphatic side chain. Only residues closer than 4.0 Å are shown. The ligand is shown in stick representation with carbon and oxygen atoms in gray and red, respectively. The hydrogen bonds formed by the 25-OH group of **2b** are shown as green dashed lines.

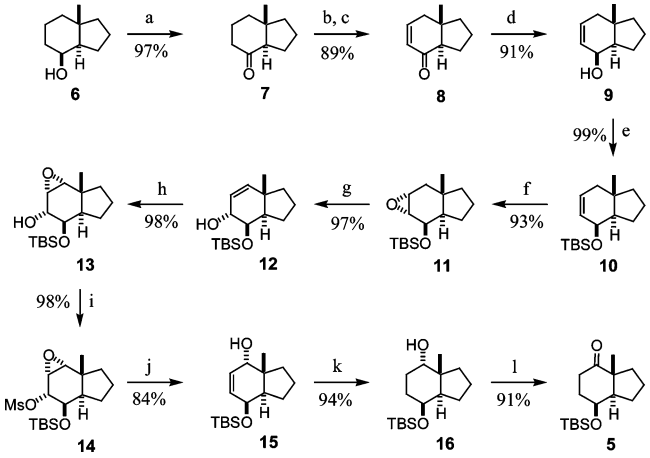
citriol−hVDRΔ LBD complex mentioned above showed that only side chains attached to the "northern" positions of the calcitriol CD system (C17, C18, and C12) would be able to interact with these histidines via a terminal hydroxyl group. Since analogues with side chains at C17 have already been extensively investigated and the effects of moving the side chain to C18 have also been examined,[15,16] we decided to explore the possibilities of placing the side chain at C12*β*. According to our calculations, a C12-borne hydroxy-terminated side chain must contain seven carbon atoms between C12 and terminal 25-OH groups (standard C−C distances equal to 1.5−1.6 Å for sp^3 carbons) in order to fit into the VDR ligand-binding pocket, considered in our design that both C12 and the terminal 25-OH occupy the same locations, relative to the VDR, as in the VDR bound to the natural hormone. Imposing the additional requirement that the side chain should terminate in the same dimethylhydroxymethyl group as calcitriol, we therefore set out to prepare compound **2b** (Figures 1 and 2), in which the side chain is seven carbons long, and compounds **2a** and **2c**, in which it has one less and one more carbon, respectively. Interactions of the **2b** side chain with the protein (Figure 2) are similar to those of 1α,25(OH)$_2$D$_3$ except with Leu309 and decreased interactions with H305 and H397.

Synthesis. Our strategy for the synthesis of analogues **2a**−**c** was based on the convergent Wittig−Horner approach,[17] which is based on the coupling of the anion of compound **3** (the phosphine oxide of ring A) with ketones **4a**−**c**, which comprise the CD ring system and a C12-borne side chain (Scheme 1). Ketones **4a**−**c** would be obtained by attaching the appropriate side chain to the C12-ketone **5**, which would be prepared from

Scheme 1. Retrosynthetic Analysis of Analogues **2a**−**c**



Scheme 2. Synthesis of Ketone **5** from the Known Alcohol **6**[a]



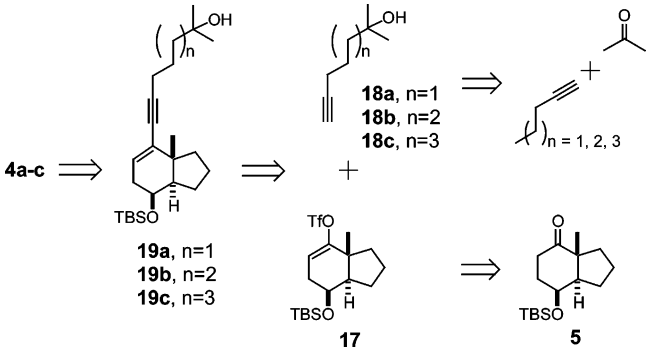
[a] Reagents and conditions: (a) PDC, CH_2Cl_2 (97%). (b) LDA, THF; TMSCl. (c) $Pd(OAc)_2$, CH_3CN (89%, two steps). (d) DIBAL-H, THF (91%). (e) TBSCl, imidazole, DMF (99%). (f) *m*-CPBA, CH_2Cl_2 (93%). (g) $LiNEt_2$, HMPA, Et_2O (97%). (h) *m*-CPBA, CH_2Cl_2 (98%). (i) MsCl, Et_3N, CH_2Cl_2 (98%). (j) Na/naphthalene, THF (84%). (k) H_2, 10% Pd/C, EtOAc (94%). (l) PDC, CH_2Cl_2 (91%).

the known alcohol **6** (itself obtained from commercially available vitamin D$_2$)[16,18] by methods recently developed in our laboratory.[19]

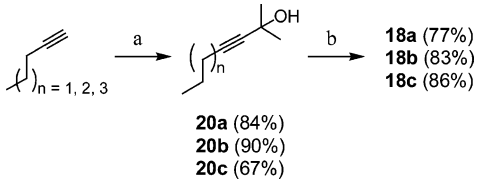
The preparation of compound **5** (Scheme 2) began with oxidation of **6** followed by α,*β*-unsaturation of the resulting ketone **7** by Saegusa's methodology.[20] Reduction of the resulting enone **8** provided the alcohol **9**, which was protected as the silyl ether derivative **10**. Epoxidation of **10** from the less-hindered face afforded **11**, which was opened with freshly prepared lithium diethylamine,[21] to afford the allylic alcohol **12** in excellent yield. Epoxidation of **12** followed by mesylation afforded the epoxymesylate **14**, which was treated with soldium naphthalenide in dry THF[22] to afford allylic alcohol **15** (81% yield from its isomer **12**). Finally, standard catalytic hydrogenation of **15** to **16**, followed by oxidation of the C12 hydroxyl group, afforded **5** in 49% overall yield from **6** (12 steps).

Ketones **4** were prepared by palladium-catalyzed coupling between the appropriate side chains, in terminal alkyne form (**18**), and compound **17**, the enol triflate of **5** (Scheme 3). The required alkynes were prepared straightforwardly by metalation of 1-pentyne, 1-hexyne, and 1-heptyne with *n*-hexyllithium in dry THF, followed by trapping of the resulting alkynyl carban-

Scheme 3. Retrosynthesis of Ketones **4a**−**c**

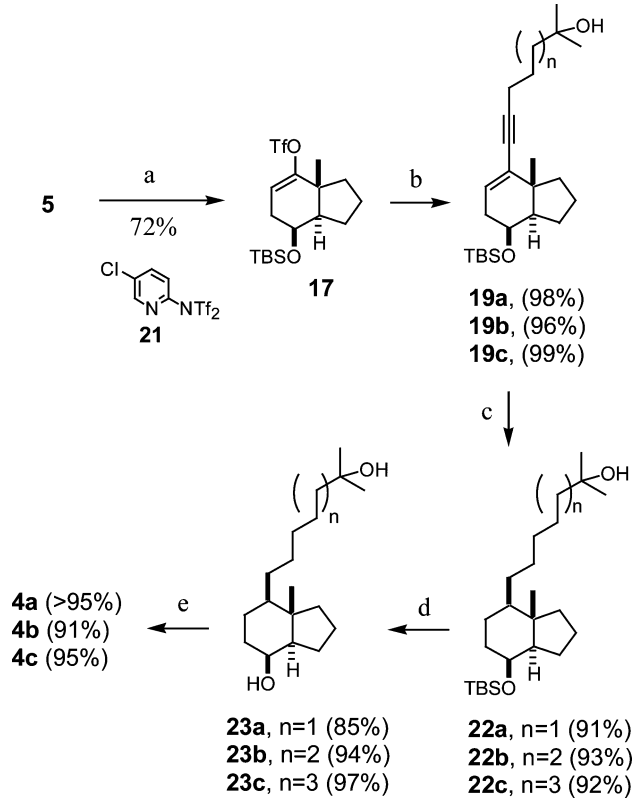


Scheme 4. Synthesis of Side-Chain Building Blocks **18a**−**c**[a]



[a] Reagents and conditions: (a) ^{n}HexLi, THF; acetone (67−90%). (b) KH, 1,3-diaminopropane (77−86%).

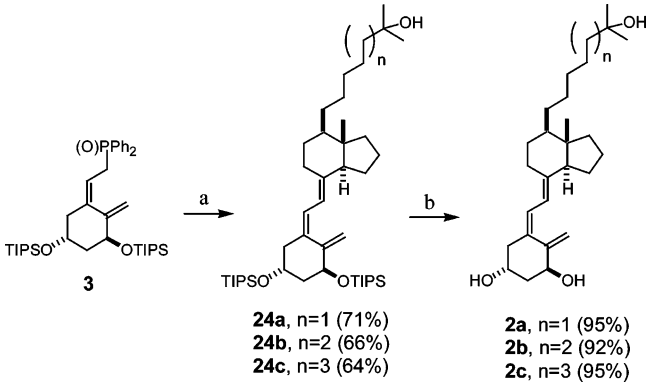
Scheme 5. Synthesis of Ketones **4a**−**c**[a]



[a] Reagents and conditions: (a) LDA, THF; **21** (72%). (b) **18a**−**c**, CuI, $(Ph_3P)_2PdCl_2$, Et_2NH. (c) H_2, 10% Pd/C, EtOAc. (d) TBAF, THF. (e) PDC, CH_2Cl_2.

ions with acetone (Scheme 4). Treatment of the resulting internal alkynes **20a**−**c** with KAPA base[23] (potassium 3-aminopropylamide) caused triple-bond migration to the terminal positions they have in the desired products **18**. Enol triflate **17** was prepared by treating ketone **5** with freshly prepared LDA and reaction of the resulting lithium enolate with triflimide **21** (Scheme 5). Various sets of reaction conditions were then evaluated for the key coupling reaction between the enoltriflate **17** and alkynes **18**. Best yields of the desired products **19** (>96%) were obtained when catalytic amounts of copper iodide and bis(triphenylphosphine)palladium(II) chloride were sequentially added to a mixture of **17** and the appropriate alkyne **18** in dry diethylamine at 0 °C. Because of their lability, enynes **19** were immediately hydrogenated (10% Pd/C, EtOAc) to compounds **22**, which after desilylation and standard oxidation of the resulting alcohols **23** afforded ketones **4a**−**c**.

Scheme 6. Synthesis of Analogues **2a**−**c**[a]



[a] Reagents and conditions: (a) ^{n}BuLi, THF, −78 °C; **4a**−**c**. (b) TBAF, THF.

Finally, Wittig−Horner coupling of the anion of phosphine oxide **3** with ketones **4a**−**c**, followed by desilylation of the protected analogues **24a**−**c**, afforded the target compounds **2a**, **2b**, and **2c** in overall yields of 19%, 16%, and 19%, respectively, from known alcohol **6** (Schemes 6 and 1).

Biological Activity. The strength of the interaction of **2a**, **2b**, and **2c** for hVDRΔ LBD, was evaluated by titration and competition experiments with electrospray ionization mass spectrometry (ESI-MS), a technique that allows characterization of noncovalent interactions[24] and is accordingly increasingly being used to study ligand binding.[25−30] This methodology is used to evaluate electrostatic and H-bond specific contacts involved in the binding of a ligand to a receptor. Changes in electrostatic and H-bond contacts upon chemical modification of the ligand are directly reflected in the ESI-MS data, which allows a qualitative classification of ligands. This qualitative classification obtained by ESI-MS is similar to that obtained by classical competition experiments.[14,26−30] ESI-MS titration experiments were performed for hVDRΔ LBD binding with **2a**, **2b**, **2c**, 1α,25$(OH)_2D_3$, and as negative control 9-*cis*-retinoic acid, ligand for the retinoid NRs with no known affinity for VDR. The dominant species were found to be $[M + 12H]^{12+}$ and $[M + 11H]^{11+}$ charged states of the monomeric hVDRΔ LBD. All analogues, **2a**, **2b**, and **2c**, were shown by ESI-MS to bind to VDR LBD. Figure 3 shows the ESI mass spectra obtained after addition of a 3-fold molar excess of **2b** to hVDRΔ. ESI mass spectra for **2a**, **2c**, 1α,25$(OH)_2D_3$, and 9-*cis*-retinoic acid are shown in Figure S3 in the Supporting Information. The ligand with the highest affinity is shown as the species with the highest relative abundance. The relative abundances of liganded hVDRΔ are the following: 95%, 69%, 71%, and 60% of complexes in the presence of 1α,25$(OH)_2D_3$, **2a**, **2b**, and **2c**, respectively. Increasing the incubation time from 15 min to 24 h does not change the relative abundance values. The relative affinity of hVDRΔ for the ligand is in the order **2c** < **2a** < **2b** ≪ 1α,25$(OH)_2D_3$. Competition experiments (Supporting Information for **2b**) confirmed the titration experiments results. Together these results suggest a gradual increase in the affinity of hVDRΔ for **2c**, **2a**, and **2b**.

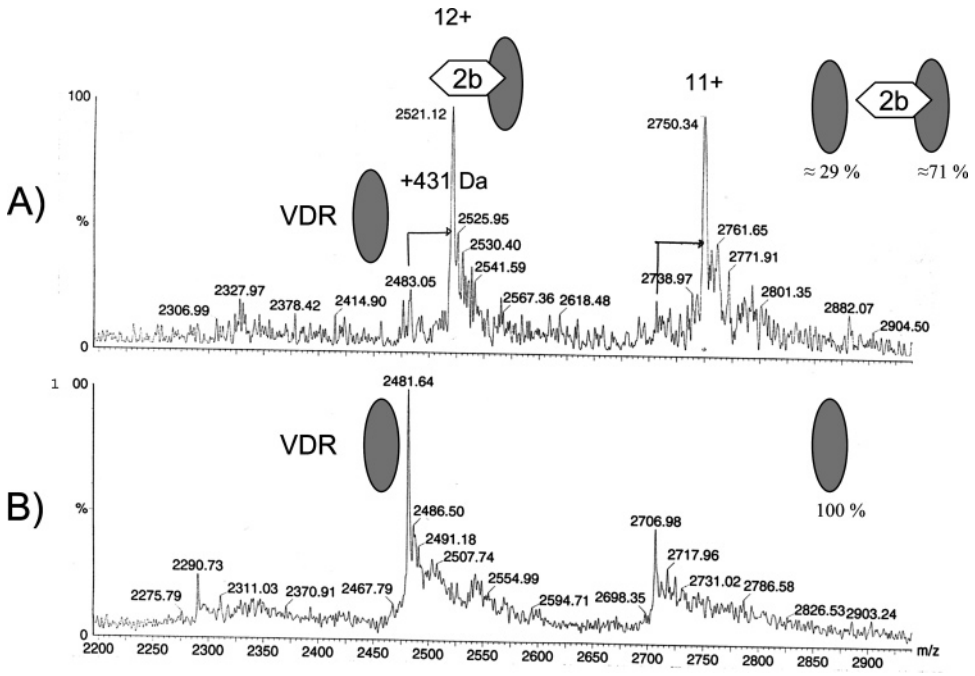


Figure 3. ESI mass spectra of hVDR LBD. The mass spectra were acquired at 20 V. (A) After addition of 3 molar equiv of **2b** (MM = 431 Da). 71% of the detected species correspond to the hVDRΔ−**2b** complex and 29% are related to unliganded VDR. (B) Before any ligand.

The functional activity of compounds **2a**−**c** was evaluated in a transient transfection assay in COS-7 cells transfected with luciferase reporter plasmid and an expression vector for a Gal4 DBD−hVDRΔ LBD fusion protein. All ligands have low to very low transactivation properties. At a concentration of 10^{-7} M, the transactivation activities of **2a**−**c** were only 8%, 20%, and 6%, respectively, compared with that of calcitriol (Figure 4A). This low response may reflect the difficulty for the C12-side chain analogues to reach the H305 and H397 contact points due to the influence of C18. Figure 4B shows the dose−response curve of the **2b** analogue with 1α,25$(OH)_2D_3$ as reference. At 10^{-5} M **2b** had the same transactivation activity as the natural ligand. The agonist behavior of **2b** was confirmed by obtaining, under standard conditions, small crystals of hVDRΔ LBD−**2b** complex that were isomorphous to hVDRΔ LBD−1α,25-$(OH)_2D_3$.[10−12] The finding that increasing the concentration of **2b** from 10^{-7} to 10^{-5} M increases its relative transactivation activity from 17% to 100% might be due either to a partial stabilization of an inactive VDR conformation or to a faster catabolic degradation in vivo than that of calcitriol (sufficiently faster to reduce its activity at 10^{-7} M but not so fast as to achieve any significant reduction at 10^{-5} M). Both mechanisms might allow **2b** to be of pharmacological utility.

Conclusion. Analysis of the crystal structure of hVDRΔ LBD−calcitriol complex led to the design and synthesis of novel calcitriol analogues bearing a side chain at C12 instead of C17 (compounds **2a**−**c**). The affinities of these analogues for hVDRΔ LBD were 60−71% that of calcitriol, and at a concentration of 10^{-7} M their abilities to activate a Gal4 DBD−hVDRΔ LBD construct ranged from 6% to 20% that of calcitriol, with the **2b** analogue being the most active.

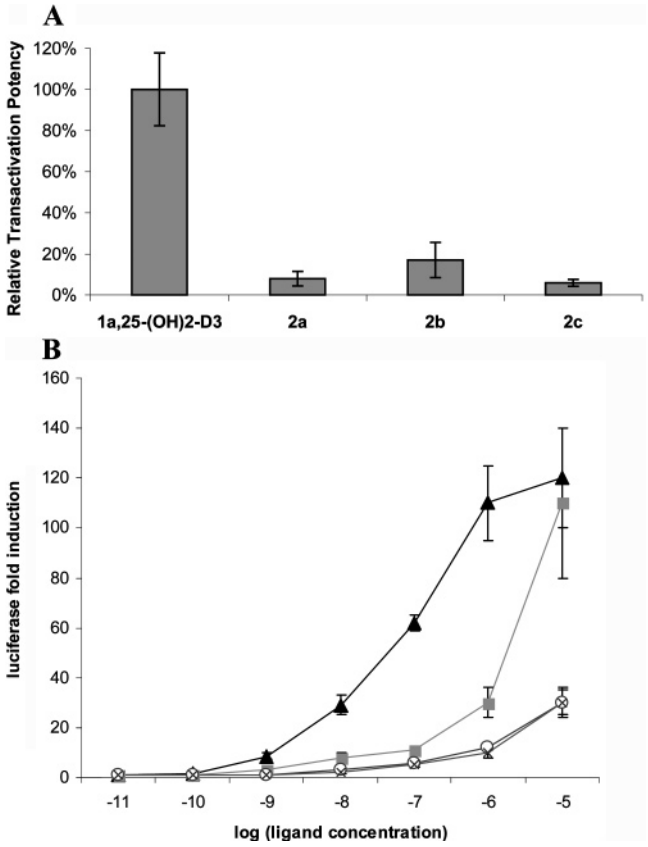


Figure 4. (A) Transactivation of a luciferase reporter gene induced by 10^{-7} M concentrations of **2a**−**c**, as percentages of that induced by calcitriol. (B) Dose−response curves for **2a** (○), 1α,25$(OH)_2D_3$ (▲), **2c** (×), and **2b** (■). COS cells were transiently transfected with a Gal4-VDR expression plasmid and 17m5-TATA-Luc reporter. Cells were treated with either vehicle or the indicated concentration of the various compounds.

Experimental Section

des-*A*,*B*-Androstan-8-one (7). Pyridinium dichromate (15.48 g, 41.19 mmol) was added to a solution of **6** (1.58 g, 10.2 mmol) in dry CH_2Cl_2 (40 mL). The mixture was stirred for 8 h at room temperature (RT) and filtered through a layer of silica gel. The solids were washed with Et_2O and the solution was concentrated. The residue was purified by flash chromatography on silica gel (4% EtOAc/hexanes) to afford **7** [1.55 g, 39.95 mmol, 97%, R_f = 0.4 (15% EtOAc/hexanes), colorless oil].

des-*A,B*-Androst-9(11)-en-8-one (8). A lithium diisopropylamide solution was prepared by the addition of ^{n}BuLi (8.69 mL, 21.29 mmol, 2.20 M solution in hexanes) to dry diisopropylamine (3.20 mL, 22.9 mmol) at −78 °C. The stirred mixture was allowed to warm until the formation of LDA as semisolid slurry. After cooling at −78 °C, the LDA slurry was dissolved with dry THF (60 mL) and a solution of **7** (2.49 g, 16.38 mmol) in dry THF (50 mL) was then added dropwise. The mixture was allowed to warm to RT and was stirred for 1 h. The enolate was trapped by the addition of dry chlorotrimethylsilane (3.11 mL, 24.57 mmol) at −78 °C. The mixture was allowed to warm to 0 °C for 2.5 h and the reaction was quenched with aqueous $NaHCO_3$ (60 mL). The aqueous layer was extracted with Et_2O (2 × 50 mL) and the combined organic solution was dried, filtered, and concentrated *in a vacuum*. The residue was dissolved in dry CH_3CN (100 mL), and palladium(II) acetate (3.86 g, 17.2 mmol) was added. After the mixture was stirred overnight at RT, the formation of a palladium mirror was observed. The solids were removed by filtration through a layer of silica gel and washed with Et_2O (50 mL). Aqueous $NaHCO_3$ (100 mL) was added to the organic solution, and the aqueous layer was extracted with Et_2O (3 × 80 mL). The combined organic solution was dried, filtered, and concentrated in vacuo. The residue was purified by flash chromatography on silica gel (8% EtOAc/hexanes) to give **8** [2.19 g, 14.58 mmol, 89% in two steps, R_f = 0.35 (15% EtOAc/hexanes), colorless oil].

des-*A,B*-Androst-9(11)-en-8β-ol (9). Diisobutylaluminum hydride (21.1 mL, 21.1 mmol, 1 M solution in hexanes) was added dropwise to a stirred solution of **8** (2.11 g, 14.1 mmol) in dry THF (80 mL) at −78 °C. The reaction mixture was stirred for 15 min and quenched by the successive addition of water (50 mL) and HCl (200 mL, 5% aqueous solution). The aqueous layer was extracted with Et_2O (3 × 100 mL) and the combined organic solution was washed with aqueous $NaHCO_3$ (100 mL), dried, filtered, and concentrated in vacuo. The residue was purified by flash chromatography on silica gel (9% EtOAc/hexanes) to give **9** [1.95 g, 12.8 mmol, 91%, R_f = 0.4 (20% EtOAc/hexanes), colorless oil]. Due to its volatility, high-vacuum drying is not recommended for **9**.

8β-*tert*-Butyldimethylsilyloxy-des-*A,B*-androst-9(11)-ene (10). Imidazole (3.42 g, 50.3 mmol) and *tert*-butyldimethylsilyl chloride (6.06 g, 40.23 mmol) were successively added to a solution of **9** (1.53 g, 10.1 mmol) in dry DMF. The mixture was stirred overnight at room temperature. Brine (100 mL) was added and the aqueous layer was extracted with hexanes (3 × 50 mL). The combined organic solution was dried, filtered, and concentrated in vacuo. The residue was purified by flash chromatography on silica gel (hexanes) to yield **10** [2.66 g, 10.0 mmol, 99%, R_f = 0.8 (hexanes), colorless oil].

8β-*tert*-Butyldimethylsilyloxy-des-*A,B*-9α,11α-epoxyandrostane (11). 3-Chloroperoxybenzoic acid (4.87 g, 27.7 mmol) was added in portions to a stirred solution of **10** (2.61 g, 9.81 mmol) in dry CH_2Cl_2 (100 mL) at 0 °C. The mixture was stirred in the dark for 2 h at 0 °C and for 3 h at room temperature. The reaction was quenched by the addition of $Na_2S_2O_4$ (80 mL, saturated aqueous solution). The resulting mixture was vigorously shaken and the aqueous layer was extracted with CH_2Cl_2 (3 × 50 mL). The combined organic solution was dried, filtered, and concentrated in vacuo. The residue was purified by flash chromatography on silica gel (3% Et_2O/hexanes) to afford **11** [2.58 g, 9.12 mmol, 93%, R_f = 0.3 (hexanes), colorless oil].

8β-*tert*-Butyldimethylsilyloxy-des-*A,B*-androst-11-en-9α-ol (12). A lithium diethylamide solution was prepared by the addition of ^{n}BuLi (17.9 mL, 44.7 mmol, 2.5 M solution) to a stirred solution of Et_2NH (5.87 mL, 53.9 mmol) in dry Et_2O (20 mL) at −40 °C. After 20 min, a solution of **11** (2.53 g, 8.98 mmol) in dry Et_2O (25 mL) and dry HMPA (12.0 mL, 69.0 mmol) were sequentially added. The mixture was stirred for 13 h at RT, and the reaction was quenched with a few drops of aqueous NH_4Cl and HCl (100 mL, 2% aqueous solution). The aqueous layer was extracted with Et_2O (3 × 50 mL) and the combined organic solution was dried, filtered, and concentrated in vacuo. The residue was purified by flash chromatography on silica gel (7% EtOAc/hexanes) to give **12** [2.45 g, 8.71 mmol, 97%, R_f = 0.55 (20% EtOAc/hexanes), white solid (mp 34 °C)].

8β-*tert*-Butyldimethylsilyloxy-des-*A,B*-11α,12α-epoxyandrostan-9α-ol (13). 3-Chloroperoxybenzoic acid (3.05 g, 17.6 mmol) was added in portions to a solution of **12** (1.66 g, 5.88 mmol) in dry CH_2Cl_2 (40 mL) at 0 °C. The mixture was stirred in the dark for 2 h at RT. The reaction was quenched by the addition of aqueous $Na_2S_2O_4$ (40 mL). The resulting mixture was vigorously shaken and the aqueous layer was extracted with CH_2Cl_2 (2 × 30 mL). The combined organic solution was washed with $NaHCO_3$ (50 mL, saturated aqueous solution), dried, filtered, and concentrated in vacuo. The residue was purified by flash chromatography on silica gel (6% EtOAc/hexanes) to give **13** [1.71 g, 5.76 mmol, 98%, R_f = 0.5 (20% EtOAc/hexanes), white solid (mp 64 °C)].

8β-*tert*-Butyldimethylsilyloxy-des-*A,B*-11α,12α-epoxyandrostan-9α-ylmethanesulfonate (14). Dry Et_3N (1.01 mL, 7.27 mmol) and dry methanesulfonyl chloride (0.52 mL, 6.7 mmol) were successively added to a solution of **13** (1.67 g, 5.59 mmol) in dry CH_2Cl_2 (15 mL) at −10 °C. After the mixture was stirred for 1 h, the reaction was quenched with water (25 mL). The aqueous layer was extracted with CH_2Cl_2 (2 × 25 mL) and the combined organic solution was dried, filtered, and concentrated in vacuo to give crude **14** [2.10 g, 5.55 mmol, 99%, R_f = 0.5 (20% EtOAc/hexanes), same R_f as **13**, colorless oil]. Crude **14** was submitted to the next reaction without further purification.

8β-*tert*-Butyldimethylsilyloxy-des-*A,B*-androst-9(11)-en-12α-ol (15). A 3 M sodium naphthalenide solution was prepared by the addition of a solution of naphthalene (7.89 g, 60 mmol) in dry THF (20 mL) to Na (1.38 g, 60 mmol, small pieces) at RT. After being stirred overnight, the resulting deep blue solution (18.2 mL, 54.5 mmol) was added to a solution of crude **14** (2.05 g, 5.45 mmol) in dry THF (25 mL) at −10 °C. The mixture was stirred for 20 min and the reaction was quenched by the careful addition of H_2O (50 mL). The aqueous layer was extracted with EtOAc (3 × 30 mL) and the combined organic solution was dried, filtered, and concentrated in vacuo. The residue was purified by flash chromatography on silica gel (7% EtOAc/hexanes) to afford **15** [1.30 g, 4.58 mmol, 84%, R_f = 0.4 (20% EtOAc/hexanes), white solid (mp 113 °C)].

8β-*tert*-Butyldimethylsilyloxy-des-*A,B*-androstan-12α-ol (16). A catalytic amount of 10% palladium on active carbon (0.050 g) was suspended in a solution of **15** (1.244 g, 4.41 mmol) in EtOAc (60 mL). The mixture was stirred under hydrogen atmosphere (balloon pressure) for 2 h at RT. The solids were removed by filtration through a short layer of silica gel, and the product was eluted with Et_2O. After concentration in vacuo, the residue was purified by flash chromatography on silica gel (7% EtOAc/hexanes) to afford **16** [1.18 g, 4.15 mmol, 94%, R_f = 0.5 (20% EtOAc/hexanes), white solid (mp 73 °C)].

8β-*tert*-Butyldimethylsilyloxy-des-*A,B*-androstan-12-one (5). Pyridinium dichromate (5.90 g, 15.7 mmol) was added to a solution of **16** (1.11 g, 3.91 mmol) in CH_2Cl_2 (60 mL). After the mixture was stirred for 36 h at RT, the solids were removed by filtration through a layer of silica gel and washed with Et_2O. The solution was concentrated in vacuo and the resulting residue was purified by flash chromatography on silica gel (5% EtOAc/hexanes) to afford **5** [1.00 g, 3.56 mmol, 91%, R_f = 0.6 (20% EtOAc/hexanes), white solid (mp 32 °C)].

2-Methyl-3-heptyn-2-ol (20a). ^{n}Hexyllithium (35.7 mL, 80.0 mmol, 2.24 M solution in hexanes) was added to a stirred solution of 1-pentyne (7.88 mL, 80.0 mmol) in dry THF (100 mL) at −78 °C. After the mixture was stirred for 1 h at RT, acetone (6.17 mL, 84.0 mmol) was added at −78 °C. The mixture was allowed to warm to RT for 3 h and the reaction was quenched with aqueous NH_4Cl (100 mL). The aqueous layer was extracted with Et_2O (3 × 70 mL) and the combined organic solution was dried, filtered, and concentrated in vacuo. The residue was purified by flash chromatography on silica gel (9% EtOAc/hexanes) to give **20a** [8.51 g, 67.2 mmol, 84%, R_f = 0.65 (25% EtOAc/hexanes), colorless oil].

2-Methyl-3-octyn-2-ol (20b). See **20a** for reaction procedure. Reagents: ^{n}hexyllithium (35.7 mL, 80.0 mmol, 2.24 M solution in hexanes), 1-hexyne (9.00 mL, 80.0 mmol), and dry THF (100 mL). Product: **20b** [10.1 g, 72.0 mmol, 90%, R_f = 0.65 (25% EtOAc/hexanes), colorless oil].

2-Methyl-3-nonyn-2-ol. (20c). See **20a** for reaction procedure. Reagents: ^{n}hexyllithium (35.7 mL, 80.0 mmol, 2.24 M solution in hexanes), 1-heptyne (10.5 mL, 80.0 mmol), and dry THF (100 mL). Product: **20c** [8.30 g, 53.6 mmol, 67%, R_f = 0.65 (25% EtOAc/hexanes), colorless oil].

2-Methyl-6-heptyn-2-ol (18a). A suspension of KH in mineral oil was washed with dry hexane (3 × 40 mL) under an argon atmosphere and dried under high vacuum to give KH (9.06 g, 225 mmol) as a gray powder. Dry 1,3-diaminopropane (120 mL) was added at 0 °C and the mixture was stirred for 3 h at this temperature and then overnight at room temperature. Caution must be taken due to hydrogen evolution during the first 3 h. The above solution (40 mL, 75 mmol) was added to neat **20a** (3.15 g, 25.0 mmol) at room temperature. After being stirred for 20 h, the mixture was cautiously poured over crushed ice/NaCl. NH_4Cl (150 mL, saturated aqueous solution) was added and the aqueous layer was extracted with Et_2O (6 × 40 mL). The combined organic solution was dried, filtered, and concentrated in vacuo. The residue was purified by flash chromatography on silica gel (8−12% EtOAc/hexanes) to give **18a** [2.44 g, 19.25 mmol, 77%, R_f = 0.55 (25% EtOAc/hexanes), colorless oil].

2-Methyl-7-octyn-2-ol (18b). See **18a** for experimental procedure. Reagents: 40 mL (75 mmol approximately) of KH solution and **20b** (3.51 g, 25.0 mmol). Product: **18b** [2.91 g, 20.8 mmol, 83%, R_f = 0.55 (25% EtOAc/hexanes), colorless oil].

2-Methyl-8-nonyn-2-ol. (18c). See **18a** for experimental procedure. Reagents: 40 mL (75 mmol approximately) of KH solution and **20c** (3.73 g, 24.2 mmol). Product: **18c** [3.22 g, 20.9 mmol, 86%, R_f = 0.55 (25% EtOAc/hexanes), colorless oil].

8*β*-*tert*-Butyldimethylsilyloxy-des-*A*,*B*-androst-11-en-12-yl-trifluoromethanesulfonate (17). A lithium diisopropylamide solution was prepared by the addition of ^{n}hexyllithium (1.03 mL, 2.46 mmol, 2.38 M solution in hexanes) to neat $^i Pr_2NH$ (0.367 mL, 2.65 mmol) at −78 °C. The cooling bath was removed and the temperature was allowed to warm until the formation of LDA as semisolid slurry. After cooling again at −78 °C the solid was dissovled in THF (8 mL). A solution of **5** (0.534 g, 1.89 mmol) in dry THF (8 mL) was added by cannula. The mixture was stirred for 4 h at RT and the enolate was trapped with a solution of the triflimide **21** (1.49 g, 3.78 mmol) in dry THF (8 mL) at −30 °C. The mixture was allowed to warm to RT and then stirred for 2 days. Water (50 mL) was added and the aqueous layer was extracted with Et_2O (3 × 40 mL). The combined organic solution was dried, filtered, and concentrated in vacuo. The residue was purified by flash chromatography on silica gel (20% CH_2Cl_2/hexanes and 8% EtOAc/hexanes) to give **17** [0.564 g, 1.36 mmol, 72%, R_f = 0.6 (2% EtOAc/hexanes), white solid (mp 41 °C)] and 0.139 g of recovered starting material (26%).

20(17→12)-*abeo*-8*β*-*tert*-Butyldimethylsilyloxy-des-*A*,*B*-24-homo-21-norcholest-11-en-20(22)-yn-25-ol (19a). A catalytic amount of CuI (2 mg) and of $(Ph_3P)_2PdCl_2$ (2 mg) were sequentially added to a mixture of **17** (0.065 g, 0.157 mmol) and **18a** (0.059 g, 0.471 mmol) in dry Et_2NH (4 mL) at 0 °C. The mixture was stirred at 0 °C for 1 h and at RT for 1 h. NH_4Cl (10 mL, saturated aqueous solution) was added and the aqueous layer was extracted with Et_2O (3 × 10 mL). The combined organic solution was dried, filtered, and concentrated in vacuo. The residue was purified by flash chromatography (8% EtOAc/hexanes) to give **19a** [0.060 g, 0.154 mmol, 98%, R_f = 0.6 (25% EtOAc/hexanes), colorless oil]. Compound **19a** decomposes in contact with air at RT. It must be stored at −20 °C under argon.

20(17→12)-*abeo*-8*β*-*tert*-Butyldimethylsilyloxy-des-*A*,*B*-24-dihomo-21-norcholest-11-en-20(22)-yn-25-ol (19b). See **19a** for experimental procedure. Reagents: CuI (5 mg), bis(triphenylphosphine)palladium(II) chloride (5 mg), **17** (0.236 g, 0.570 mmol), **18b** (0.239 g, 1.71 mmol), and dry Et_2NH (12 mL). Product: **19b** [0.222 g, 0.547 mmol, 96%, R_f = 0.6 (25% EtOAc/hexanes), colorless oil].

20(17→12)-*abeo*-8*β*-*tert*-Butyldimethylsilyloxy-des-*A*,*B*-24-trihomo-21-norcholest-11-en-20(22)-yn-25-ol (19c). See **19a** for experimental procedure. Reagents: CuI (5 mg), bis(triphenylphosphine)palladium(II) chloride (5 mg), **17** (0.194 g, 0.462 mmol), **18c** (0.216 g, 1.41 mmol), and dry Et_2NH (12 mL). Product: **19c** [0.193 g, 0.457 mmol, 99%, R_f = 0.6 (25% EtOAc/hexanes), colorless oil].

(17→12*β*)-*abeo*-8*β*-*tert*-Butyldimethylsilyloxy-des-*A*,*B*-24-homo-21-norcholestan-25-ol (22a). A catalytic amount of 10% palladium on active carbon (5 mg) was suspended in a solution of **19a** (0.060 g, 0.144 mmol) in EtOAc (5 mL). The mixture was stirred under hydrogen atmosphere (balloon pressure) for 16 h at RT. The solids were removed by filtration through a layer of silica gel, and the product was eluted with Et_2O. The solution was concentrated in vacuo and the residue was purified by flash chromatography on silica gel (10% EtOAc/hexanes) to afford **22a** [0.055 g, 0.131 mmol, of 91%, R_f = 0.5 (20% EtOAc/hexanes), colorless oil].

(17→12*β*)-*abeo*-8*β*-*tert*-Butyldimethylsilyloxy-des-*A*,*B*-24-dihomo-21-norcholestan-25-ol.(22b). See **22a** for experimental procedure. Reagents: 10% palladium on active carbon (10 mg), **19b** (0.204 g, 0.504 mmol), and EtOAc (10 mL). Product: **22b** [0.193 g, 0.469 mmol, 93%, R_f = 0.5 (20% EtOAc/hexanes), colorless oil].

(17→12*β*)-*abeo*-8*β*-*tert*-Butyldimethylsilyloxy-des-*A*,*B*-24-trihomo-21-norcholestan-25-ol (22c). See **22a** for experimental procedure. Reagents: 10% palladium on active carbon (10 mg), **19c** (0.177 g, 0.504 mmol), and EtOAc (10 mL). Product **22c** [0.165 g, 0.463 mmol, 92%, R_f = 0.5 (20% EtOAc/hexanes), colorless oil].

20(17→12*β*)-*abeo*-des-*A*,*B*-24-Homo-21-norcholestan-8*β*,25-diol (23a). Tetrabutylammonium fluoride trihydrate (0.644 g, 2.04 mmol) was added to a solution of **22a** (0.054 g, 0.136 mmol) in dry THF (5 mL). The stirred mixture was refluxed for 2 days. Water (15 mL) was added and the aqueous layer was extracted with Et_2O (3 × 10 mL). The combined organic solution was dried, filtered, and concentrated in vacuo. The residue was purified by flash column chromatography on silica gel (22% EtOAc/hexanes) to give **23a** [0.032 g, 0.116 mmol, 85%, R_f = 0.35 (30% EtOAc/hexanes), colorless oil] and recovered **22a** (0.008 g, 15%).

20(17→12*β*)-*abeo*-des-*A*,*B*-24-Dihomo-21-norcholestan-8*β*,25-diol (23b). See **23a** for experimental procedure. Reagents: Tetrabutylammonium fluoride trihydrate (1.14 g, 3.63 mmol), **22b** (0.149 g, 0.363 mmol), and dry THF (3 mL). Product: **23b** [0.101 g, 0.341 mmol, 94%, R_f = 0.35 (30% EtOAc/hexanes), colorless oil].

20(17→12*β*)-*abeo*-des-*A*,*B*-24-Trihomo-21-norcholestan-8*β*,-25-diol (23c). See **23a** for experimental procedure. Reagents: Tetrabutylammonium fluoride trihydrate (0.921 g, 2.92 mmol), **22c** (0.124 g, 0.292 mmol), and dry THF (3 mL). Product: **23c** [0.088 g, 0.283 mmol, 97%, R_f = 0.35 (30% EtOAc/hexanes), colorless oil].

20(17→12*β*)-*abeo*-25-Hydroxy-des-*A*,*B*-24-homo-21-norcholestan-8-one (4a). Pyridinium dichromate (0.140 g, 0.374 mmol) was added to a solution of **23a** (0.035 g, 0.125 mmol) in dry CH_2Cl_2 (4 mL). The mixture was stirred in the dark for 5 h at RT. The reaction was monitored by TLC (double elution with 3:3:7 EtOAc/CH_2Cl_2/hexanes). The solids were removed by filtration through a layer of silica gel and the product was eluted with Et_2O. The solution was concentrated in vacuo and the residue was purified by flash chromatography on silica gel (20% EtOAc/hexanes) to afford **4a** [0.034 g, 0.123 mmol, >95%, R_f = 0.35 (30% EtOAc/hexanes), colorless oil].

20(17→12*β*)-*abeo*-25-Hydroxy-des-*A*,*B*-24-dihomo-21-norcholestan-8-one (4b). See **4a** for experimental procedure. Reagents: Pyridinium dichromate (0.208 g, 0.553 mmol), **23b** (0.082 g, 0.277 mmol), and dry CH_2Cl_2 (6 mL). Product: **4b** [0.074 g, 0.252 mmol, 91%, R_f = 0.45 (40% EtOAc/hexanes), colorless oil].

20(17→12*β*)-*abeo*-25-Hydroxy-des-*A*,*B*-24-trihomo-21-norcholestan-8-one (4c). See **4a** for experimental procedure. Reagents: Pyridinium dichromate (0.169 g, 0.450 mmol), **23c** (0.070

g, 0.225 mmol), and dry CH_2Cl_2 (5 mL). Product: **4c** [0.066 g, 0.214 mmol, 95%, $R_f = 0.45$ (40% EtOAc/hexanes), colorless oil].

20(17→12*β*)-*abeo*-1α-Triisopropylsilyloxy-25-hydroxy-24-homo-21-norvitamin D_3 Triisopropylsilyl Ether (24a). ^{n}BuLi (0.312 mL, 0.449 mmol, 1.44 M solution in hexanes) was added dropwise to a solution of dry **3** (0.333, 0.499 mmol) in THF (3 mL) at −78 °C. The resulting deep red solution was stirred at −78 °C for 1 h followed by the slow addition of a solution of the ketone **4a** (0.035 g, 0.125 mmol) in dry THF (3 mL). The mixture was stirred in the dark for 3 h at −78 °C and for 4 h at −40 °C. The reaction was quenched with H_2O (15 mL) and the aqueous layer was extracted with Et_2O (3 × 10 mL). The combined organic solution was dried, filtered, and concentrated in vacuo. The residue was purified by flash column chromatography on silica gel (15% EtOAc/hexanes) to afford **24a** [0.065 g, 0.089 mmol, 71%, $R_f = 0.7$ (30% EtOAc/hexanes), colorless oil].

20(17→12*β*)-*abeo*-1α-Triisopropylsilyloxy-25-hydroxy-24-dihomo-21-norvitamin D_3 Triisopropylsilyl Ether (24b). See **24a** for experimental procedure. Reagents: ^{n}BuLi (0.46 mL, 0.66 mmol, 1.44 M solution in hexanes), **3** (0.490, 0.735 mmol), dry THF (4.5 mL), and **4b** (0.054 g, 0.184 mmol) in dry THF (4 mL). Product: **24b** [0.090 g, 0.121 mmol, 66%, $R_f = 0.65$ (30% EtOAc/hexanes), colorless oil].

20(17→12*β*)-*abeo*-1α-Triisopropylsilyloxy-25-hydroxy-24-trihomo-21-norvitamin D_3 Triisopropylsilyl Ether (24c). See **24a** for experimental procedure. Reagents: ^{n}BuLi (0.41 mL, 0.59 mmol, 1.44 M solution in hexanes), **3** (0.441, 0.661 mmol), THF (4 mL), and **4c** (0.054 g, 0.184 mmol) in dry THF (4 mL). Product: **24c** [0.080 g, 0.118 mmol, 64%, $R_f = 0.65$ (30% EtOAc/hexanes), colorless oil].

20(17→12*β*)-*abeo*-1α,25-Dihydroxy-24-homo-21-norvitamin D_3 (2a). Tetrabutylammonium fluoride trihydrate (0.052 g, 0.165 mmol) was added to a solution of **24a** (0.036 g, 0.049 mmol) in dry THF (1.5 mL). The mixture was stirred in the dark for 20 h at room temperature. Aqueous NH_4Cl (10 mL) was added and the aqueous layer was extracted with Et_2O (3 × 8 mL). The combined organic solution was dried, filtered, and concentrated in vacuo. The residue was purified by flash column chromatography on silica gel (60−65% EtOAc/hexanes) and by reverse-phase chromatography (RP 18, 18% H_2O/MeOH) to give **2a** [0.020 g, 0.048 mmol, >95%, $R_f = 0.5$ (EtOAc), white solid].

20(17→12*β*)-*abeo*-1α,25-Dihydroxy-24-dihomo-21-norvitamin D_3 (2b). See **2a** for experimental procedure. Reagents: Tetrabutylammonium fluoride trihydrate (0.068 g, 0.215 mmol), **24b** (0.040 g, 0.054 mmol), and dry THF (1.5 mL). Product: **2b** [0.022 g, 0.050 mmol, 92%, $R_f = 0.5$ (EtOAc), white solid].

20(17→12*β*)-*abeo*-1α,25-Dihydroxy-24-trihomo-21-norvitamin D_3 (2c). See **2a** for experimental procedure. Reagents: Tetrabutylammonium fluoride trihydrate (0.095 g, 0.301 mmol), **24c** (0.057 g, 0.075 mmol), and dry THF (2 mL). Product: **2c** [0.032 g, 0.074 mmol, >95%, $R_f = 0.5$ (EtOAc), colorless oil].

Expression, Purification, and Crystallization of hVDRΔ. The LBD of the human VDR (residues 118−427 Δ166−216) was cloned in pET28b expression vector, to obtain an N-terminal hexahistidine-tagged fusion protein, and overproduced in *Escherichia coli* BL21 (DE3) strain. Cells were grown in LB medium and subsequently induced for 6 h at 20 °C with 1 mM isopropyl thio-*β*-D-galactoside. The purification included a metal affinity chromatography step on a cobalt-chelating resin. After tag removal by thrombin digestion, the protein was further purified by gel filtration. The final protein buffer was 10 mM Tris, pH 7.5, 100 mM NaCl, and 5 mM dithiothreitol. Purity and homogeneity were assessed by sodium dodecyl sulfate (SDS) and native polyacrylamide gel electrophoresis (PAGE) and denaturant and native electrospray ionization mass spectrometry. Crystallization trials of the different complexes were performed at 4 °C by vapor diffusion in hanging drops with 0.1 M 2-(*N*-morpholino)ethanesulfonic acid (Mes), pH 6.0, and 1.4 M ammonium sulfate as precipitant agent.

Electrospray Ionization Mass Spectrometry. The hVDRΔ LBD was concentrated to about 2 mg/mL, and dialyzed extensively against 50 mM ammonium acetate at pH = 6.5 by use of Centricon-10 concentrators (Amicon), and diluted at a final concentration of 10^{-5} M. Experiments were performed on an electrospray ionization time-of-flight mass spectrometer (LCT, Micromass) with continuously infusion of the sample into the ion source at a flow rate of 4 μL/min by a Harward Model 11 syringe pump (Harward Apparatus). All ligands were dissolved in ethanol at 10^{-2} M. Ligand (0.6 μL) was added to 40 μL of protein solution (2.4 nmol) to maintain an ethanol level below 1.5%. Titration experiments were done by adding 3 molar equiv of ligands to protein solution, followed by 15 min of incubation at 4 °C. Competition experiments were performed by adding an equimolar mixture of two ligands (each in 3-fold molar excess) to the protein, followed by 15 min of incubation. To prevent dissociation in the gas phase during the ionization and desorption process, the cone voltage was optimized to 10−20 V. Mass data were acquired in the positive ion mode on a mass range of 1000−5000 *m*/*z*. Calibration of the instrument was performed with the multiply charged ions produced by a separate injection of horse heart myoglobin diluted to 2 μM in 1:1 water/acetonitrile (v/v) acidified with 1% (v/v) formic acid. The relative abundance of the different species present on ESI mass spectra was measured from their respective peak intensities. All experiments were reproduced at least three times.

Cell Culture, Transfection, and Transactivation Assays. COS cells (SV40-transformed African Green monkey kidney) were maintained in Dulbecco's modified Eagle's medium supplemented with 5% dextran-coated charcoal-stripped foetal bovine serum and gentamycin. Transient transfection assays were carried out in 24-well plates (10^5 cells/well) by a standard calcium phosphate coprecipitation technique as described previously.[11] Cells were cotransfected with precipitates containing 250 ng of receptor expression vectors PXJ440 VDRΔ (118−427 Δ166−216), 2 μg of reporter gene 5 × 17m-TATA-Luc(luciferase), 2 μg of an internal control recombinant expressing *β*-galatosidase pCH110lacZ (Pharmacia), and completed to 20 μg with carrier DNA ppSK$^+$. Cells were treated with 10^{-11} to 10^{-5} M 1α,25$(OH)_2D_3$, analogues, or ethanol vehicle. Thirty-six hours following transfection, cells extracts were assayed for luciferase and *β*-galactosidase activity. Luciferase values were normalized to *β*-galactosidase activity. The data were expressed as luciferase *x*-fold induction values, where the value 1 is assigned to the normalized luciferase activity of blank cultures.

Acknowledgment. We thank H. Nierengarten for mass spectrometry analysis. We are grateful to the DIGICYT (Spain, projects SAF 2001-3187 and 2004-01885) and CNRS, INSERM, Hôpital Universitaire de Strasbourg, and Ministère de la Recherche et de la Technologie for financial support. This work benefits from the technical platform of structural genomics supported by the Genopole and SPINE programs. We thank J.P. van de Velde, J. Zorgdrager, and M. Sno (Solvay Pharmaceuticals BV, Weesp, The Netherlands) for the gift of starting materials. X.C.G.-A. thanks the Spanish Ministry of Science and Technology for an FPI fellowship.

Supporting Information Available: Experimental data, NMR and analytical data, and ESI MS data. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Chen, T. C.; Castillo, L.; Korycka-Dahl, M.; DeLuca, H. F. Role of vitamin D metabolites in phosphate transport of rat intestine. *J. Nutr.* **1974**, *104*, 1056−1060.
(2) Holick, M. F., Ed. *Vitamin D: Physiology, Molecular Biology and Clinical Applications*;Humana Press: Totowa, NJ, 1999.
(3) Bouillon, R.; Okamura, W. H.; Norman, A. W. Structure−function relationships in the vitamin D endocrine system. *Endocr. Rev.* **1995**, *16*, 200−257.
(4) Beckman, M. J.; Deluca, H. F. Modern view of vitamin D3 and its medicinal uses. *Prog. Med. Chem.* **1998**, *35*, 1−56.
(5) Stein, M. S.; Wark, J. D. An update on the therapeutic potential of vitamin D analogues. *Expert Opin. Investig. Drugs* **2003**, *12* (5), 825−840.
(6) Carlberg, C.; Mouriño, A. New Vitamin D Receptor Ligands. *Expert Opin. Ther. Patents* **2003**, *13*, 761−772.

(7) Mangelsdorf, D. J.; Thummel, C.; Beato, M.; Herrlich, P.; Schutz, G.; Umesono, K.; Blumberg, B.; Kastner, P.; Mark, M.; Chambon, P.; et al. The nuclear receptor superfamily: the second decade. *Cell* **1995**, *83*, 835−839.
(8) Kalkhoven, E.; Valentine, J. E.; Heery, D. M.; Parker, M. G. Isoforms of steroid receptor co-activator 1 differ in their ability to potentiate transcription by the oestrogen receptor. *EMBO J.* **1998**, *17*, 232−243.
(9) Kamei, Y.; Xu, L.; Heinzel, T.; Torchia, J.; Kurokawa, R.; Gloss, B.; Lin, S. C.; Heyman, R. A.; Rose, D. W.; Glass, C. K.; Rosenfeld, M. G. A CBP integrator complex mediates transcriptional activation and AP-1 inhibition by nuclear receptors. *Cell* **1996**, *85*, 403−414.
(10) Rachez, C.; Lemon, B. D.; Suldan, Z.; Bromleigh, V.; Gamble, M.; Naar, A. M.; Erdjument-Bromage, H.; Tempst, P.; Freedman, L. P. Ligand-dependent transcription activation by nuclear receptors requires the DRIP complex. *Nature* **1999**, *398*, 824−828.
(11) Rochel, N.; Wurtz, J. M.; Mitschler, A.; Klaholz, B.; Moras, D. The crystal structure of the nuclear receptor for vitamin D bound to its natural ligand. *Mol. Cell* **2000**, *5*, 173−179.
(12) Tocchini-Valentini, G.; Rochel, N.; Wurtz, J. M.; Mitschler, A.; Moras, D. Crystal structures of the vitamin D receptor complexed to superagonist 20-epi ligands. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 5491−5496.
(13) Tocchini-Valentini, G.; Rochel, N.; Wurtz, J. M.; Moras, D. Crystal structures of the vitamin D nuclear receptor liganded with the vitamin D side chain analogues calcipotriol and seocalcitol, receptor agonists of clinical importance. Insights into a structural basis for the switching of calcipotriol to a receptor antagonist by further side chain modification. *J. Med. Chem.* **2004**, *47*, 1956−1961.
(14) Rochel, N.; Tocchini-Valentini, G.; Egea, P. F.; Juntunen, K.; Garnier, J. M.; Vihko, P.; Moras, D. Functional and structural characterization of the insertion region in the ligand binding domain of the vitamin D nuclear receptor. *Eur. J. Biochem.* **2001**, *268*, 971−979.
(15) Cornella, I.; Pérez, J.; Mouriño, A.; Sarandeses, L. A. Synthesis of new 18-substituted analogues of calcitriol using a photochemical remote functionalization. *J. Org. Chem.* **2002**, *67*, 4707−4714.
(16) Moman, E.; Nicoletti, D.; Mouriño. Synthesis of novel analogues of 1α,25-dihydroxyvitamin D_3 with side chains at C-18. *J. Org. Chem.* **2004**, *69*, 4615−4625.
(17) Kabat, M. M.; Radinov, R. The practical synthesis of vitamin D analogues: a challenge for process research. *Curr. Opin. Drug Discovery Dev.* **2001**, *4*, 808−833.
(18) Fernández, B.; Martínez Pérez, J. A.; Granja, J. R.; Castedo, L.; Mouriño, A. Synthesis of Hydrindan Derivatives Related to Vitamin D. *J. Org. Chem.* **1992**, *57*, 3173−3178.
(19) González-Avión, X. C.; Mouriño, A. Functionalization at C-12 of 1α,25-Dihydroxyvitamin D3 Strongly Modulates the Affinity for the Vitamin D Receptor (VDR). *Org. Lett.* **2003**, *5*, 2291−2293.
(20) Ito, Y.; Hirao, T.; Saegusa, T. Synthesis of α,β-unsaturated carbonyl compounds by palladium(II)-catalyzed dehydrosilylation of silyl enol ethers. *J. Org. Chem.* **1978**, *43*, 1011−1013.
(21) Fiaud, J. C.; Legros, J. Y. New method for the classification of nucleophiles in the palladium-catalyzed substitution of allylic acetates. *J. Org. Chem.* **1987**, *52*, 1907.
(22) Bannai, K.; Takana, T.; Okamura, N.; Hazato, A.; Sugiura, S.; Mananabe, K.; Tomimori, K.; Kurozumi, S. Improved synthesis of isocarbacyclin using regioselective alkylation of allylic alcohols. *Tetrahedron Lett.* **1986**, *27*, 6353.
(23) Brown, A. C.; Yamashita, A. The acetylene zipper. An exceptionally facile "contrathermodynamic" multipositional isomerization of alkynes with potassium 3-aminopropylamide. *J. Am. Chem. Soc.* **1975**, *97*, 891−892.
(24) Loo, J. A. Studying noncovalent protein complexes by electrospray ionization mass spectrometry. *Mass Spectrosc. Rev.* **1997**, *16*, 1−23.
(25) Ayed, A.; Krutchinsky, A. N.; Ens, W.; Standing, K. G.; Duckworth, H. W. Quantitative evaluation of protein−protein and ligand−protein equilibria of a large allosteric enzyme by electrospray ionization time-of-flight mass spectrometry. *Rapid Commun. Mass Spectrom.* **1998**, *12*, 339−344.
(26) Greschik, H.; Wurtz, J. M.; Sanglier, S.; Bourguet, W.; van Dorsselaer, A.; Moras, D.; Renaud, J. P. Structural and functional evidence for ligand-independent transcriptional activation by the estrogen-related receptor 3. *Mol. Cell.* **2002**, *9*, 303−313.
(27) Sanglier, S.; Bourguet, W.; Germain, P.; Chavant, V.; Moras, D,; Gronemeyer, H.; Potier, N.; Van Dorsselaer, A. Monitoring ligand-mediated nuclear receptor−coregulator interactions by noncovalent mass spectrometry. *Eur. J. Biochem.* **2004**, *271*, 4958−4967.
(28) Bitsch, F.; Aichholz, R.; Kallen, J.; Geisse, S.; Fournier, B.; Schlaeppi, J. M. Identification of natural ligands of retinoic acid receptor-related orphan receptor alpha ligand-binding domain expressed in Sf9 cells—a mass spectrometry approach. *Anal. Biochem.* **2003**, *323*, 139−149.
(29) Rogniaux, H.; Van Dorsselaer, A.; Barth, P.; Biellmann, J. F.; Barbanton, J.; van Zandt M.; Chevrier, B.; Howard, E.; Mitschler, A.; Potier, N.; Urzhumtseva, L.; Moras, D.; Podjarny, A. Binding of aldose reductase inhibitors: correlation of crystallographic and mass spectrometric studies. *J. Am. Soc. Mass Spectrom.* **1999**, *10*, 635−647.
(30) Lengqvist, J.; Alvelius, G.; Jornvall, H.; Sjovall, J.; Perlmann, T.; Griffiths, W. J. Electrospray Mass Spectrometry for the Direct Accurate Mass Measurement of Ligands in Complex With the Retinoid X Receptor alpha Ligand Binding Domain. *J. Am. Soc. Mass Spectrom.* **2005**, *16*, 1631−1640.

JM049016G