VOLUME 72, NUMBER 15 JOC Articles JULY 20, 2007

Synthesis and Conformational Analysis of 17α,21-Cyclo-22-Unsaturated Analogues of Calcitriol†

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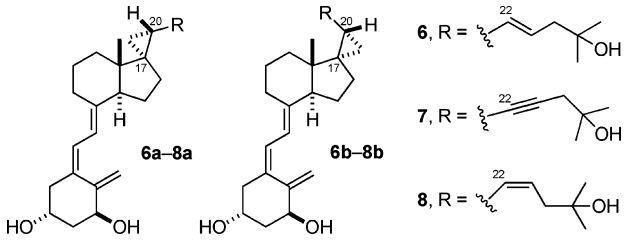
Received December 8, 2006



Six new calcitriol analogues, conformationally restricted at their side chain by the introduction of both a cyclopropane ring at C17−C20 and a double or triple bond at C22, were synthesized using the Wittig−Horner approach to construct the triene system. The six CD-ring and side-chain bearing fragments were prepared from ketone **14** by a divergent route to generate both series of epimers at C20, followed by stereoselective cyclopropanation. The (*E*)-alkenyl side chain was synthesized by means of a Wittig reaction. The alkynyl side chain was prepared by Corey−Fuchs homologation, followed by alkylation. The (*Z*)-alkenyl side chain was prepared from the previous alkyne by partial hydrogenation. The 20-*epi* analogues bind more strongly to VDR than the corresponding analogues with the C20 natural stereochemistry. These results can be reasoned by conformational analysis and hydrophobic interactions with the VDR ligand-binding domain.

Introduction

Calcitriol [1α,25-dihydroxyvitamin D_3, 1α,25-$(OH)_2$-D_3, **1a**, Figure 1], the hormonally active form of vitamin D_3, is produced by enzymatic C1-hydroxylation of 25-hydroxyvitamin D_3 [25-OH-D_3, **1b**], which, borne by the vitamin D binding protein (DBP), is the major circulating form of vitamin D.[1] Calcitriol presents a broad spectrum of biological functions which includes roles in the regulation of calcium and phosphorus metabolism, in the promotion of cell differentiation, in the inhibition of the proliferation of various types of malignant cells, and in the immune response.[2] Its best-understood mechanism of action involves its binding intracellularly to the vitamin D receptor (VDR), a member of the nuclear receptor superfamily.[3]

Because the therapeutic utility of calcitriol in the treatment of cancer and psoriasis is limited by its potent calcemic effects,

† Dedicated to Professors Joan Bosch and Joaquín Plumet on the occasion of their 60th birthdays.

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10.1021/jo0625195 CCC: $37.00
Published on Web 03/03/2007

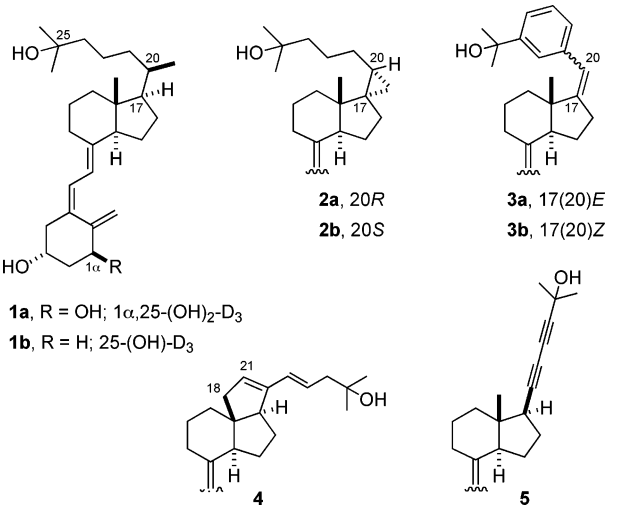

FIGURE 1. Calcitriol (**1a**), 25-OH−D_3 (**1b**), and previously described analogues with restricted C25-OH position or fixed side chain orientation (**2**−**5**).

a multitude of vitamin D analogues have been synthesized to search for derivatives showing higher anti-proliferative activity and much less calcemic activity than those found in the natural hormone.[4] Most of these vitamin D analogues are modified at the side chain. With a few exceptions,[5] modifications of other regions of the molecule produce compounds that bind poorly to VDR, even though they may bind well to the vitamin D binding protein (DBP).[6] Additionally, conformational studies of analogues with modified side chains,[7] together with crystallographic results,[8] suggest that activity depends, inter alia, on the ability to adopt a conformation in which the C25-OH is able to form hydrogen bonds with a specific region of the VDR (His305 and His397).

A few years ago, we initiated a program for the development of calcitriol analogues with selective biological properties by restricting the mobility and flexibility of the side chain.[9] We prepared calcitriol analogues showing a restricted C25-OH position and fixed side-chain orientation by introducing: (i) a double bond or cyclopropane ring at C17−C20, as in **2** and **3** (Figure 1),[9a,b] (ii) an additional ring at C18−C21 (**4**),[9c] or (iii) multiple unsaturations at the side chain (**5**).[9d] These studies afforded some analogues with significant biological activity, such as the induction of the transcriptional activity of VDR in human colon cancer cells[9d] and also give information about the bioactive side chain conformation of the natural hormone in the binding pocket. Following with this program, herein we describe the synthesis, preliminary biological evaluation, and conformational analysis of the more tightly restricted analogues, **6**, **7**, and **8**, which feature both a cyclopropane ring at C17−C20 and a double or triple bond at C22 (Scheme 1).

Results and Discussion

Chemical Synthesis.[10] The synthesis of compounds **6a**, **7a**, and **8a** (C20 stereochemistry corresponding to 20*R*-natural configuration) and their 20-epimers, **6b**, **7b**, and **8b** (C20 stereochemistry corresponding to 20*S*-*epi* configuration), was envisaged using the convergent Wittig−Horner coupling[11] between ketones **9a**/**b**−**11a**/**b**, which contain the CD-ring and side chain fragment, and phosphine oxide **12**,[12] which provides the 1α-hydroxylated A-ring (Scheme 1). For the preparation of ketones **9a**/**b**, **10a**/**b**, and **11a**/**b** we planned a divergent synthetic strategy starting from known ketone **14**.[13]

In previous work, the C17−C20 cyclopropyl moiety was introduced in poor yield by direct Simmons−Smith cyclopropanation or photochemical methods. Better results were obtained, although under harsh conditions, by dichloropropanation followed by removal of the chlorine atoms.[9a] In this case the construction of the C17−C20 cyclopropyl fragment was introduced by hydroxyl-directed cyclopropanation of an allylic alcohol containing the C17−C20 double bond under mild conditions. Starting from ketone **14**, stereoselective Horner−Wadsworth−Emmons reaction with the anion of triethyl phosphonoacetate afforded the (*E*)-α,*β*-unsaturated ester **15** in 97% yield (Scheme 2) as the only stereoisomer isolated from the reaction mixture. The synthesis of **a**-series compounds was followed by reduction of ester **15** with DIBALH to the (*E*)-allylic alcohol **16** (97% yield), and further stereoselective hydroxyl-directed cyclopropanation of **16**, by treatment with CH_2I_2 and Et_2Zn.[14] The alcohol **13a** was obtained in 77% yield as the only isomer isolated from the reaction mixture. This high stereoselectivity

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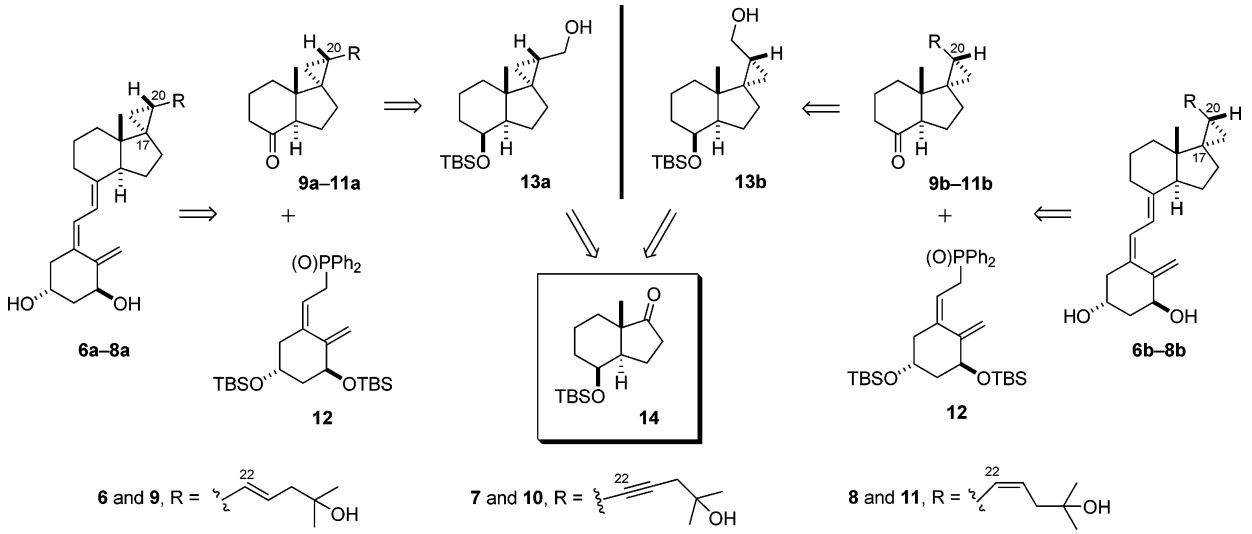
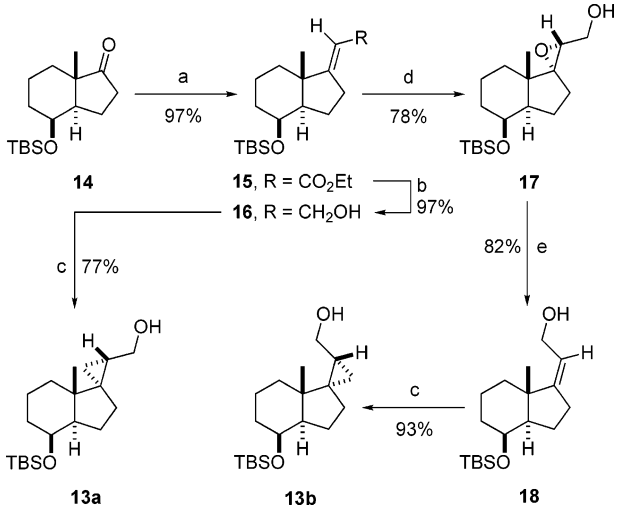
SCHEME 1. Retrosynthetic Analysis

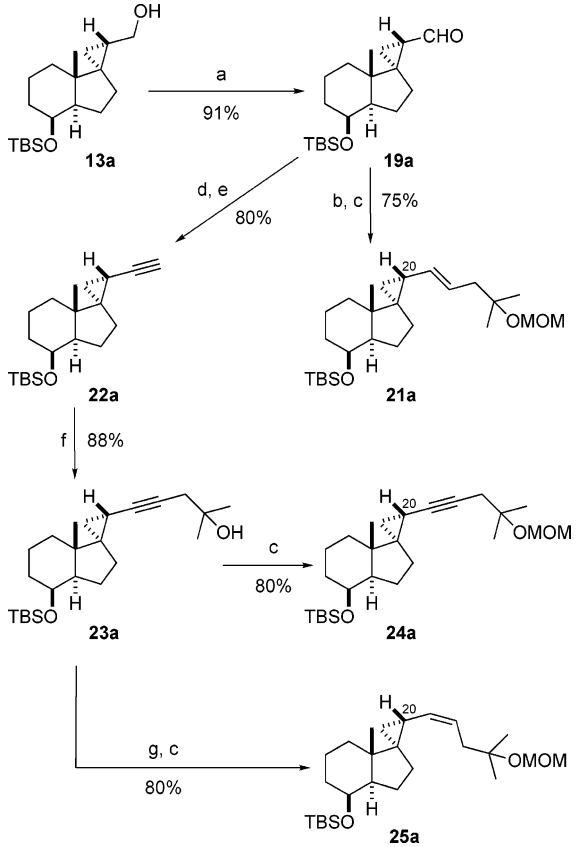


SCHEME 2. Synthesis of Allylic Alcohols 13a and 13b[a]



[a] (a) $(EtO)_2P(O)CH_2CO_2Et$, NaOEt, EtOH, reflux. (b) DIBALH, toluene, −78 °C. (c) CH_2I_2, Et_2Zn, toluene, −78 °C to rt. (d) *t*-BuOOH, $VO(acac)_2$, toluene, −20 °C. (e) $LiPPh_2$, THF, 0 °C, then MeI, rt.

SCHEME 3. Synthesis of Compounds 21a, 24a, and 25a[a]



[a] (a) DMSO, $(COCl)_2$, CH_2Cl_2, then Et_3N, rt. (b) $Ph_3P^+CH_2CH_2C(Me)_2OH$, Br^- (**20**), MeLi, THF, −20 °C. (c) MOMCl, *i*-Pr_2NEt, CH_2Cl_2, 0 °C. (d) CBr_4, Ph_3P, Zn, py, CH_2Cl_2. (e) *n*-BuLi, THF, −78 °C. (f) *n*-BuLi, THF, −78 °C, then BF_3•OEt_2, 1,2-isobutylene oxide, −78 °C to rt. (g) H_2, Lindlar, hexanes, rt.

can be explained on the basis of the steric hindrance of the steroidal β-face.

For the synthesis of compounds with 20-*epi* configuration (**b** series) we considered the same synthetic sequence using the (*Z*)-isomer of the allylic alcohol **16**. Thus, the isomerization of the C17−C20 double bond was performed by stereoselective epoxidation of **16** [*t*-BuOOH, $VO(acac)_2$, 78%] and subsequent treatment of the epoxide **17** with $LiPPh_2$ followed by MeI.[15] The (*Z*)-allylic alcohol **18** was converted to **13b** by hydroxyl-directed stereoselective cyclopropanation with CH_2I_2 and Et_2Zn in 93% yield. Remarkably, both the epoxidation and the cyclopropanation occur, with complete stereoselectivity, as in the case of the previous compound, **13a**.

The synthesis of the CD-ring and side chain bearing fragments with C20-natural stereochemistry (**21a**, **24a**, and **25a**) from cyclopropylcarbinol **13a** is depicted in Scheme 3. Swern oxidation of **13a** gave aldehyde **19a** in 91% yield. Following the Salmond procedure,[16] reaction of **19a** with the ylide generated from phosphonium salt **20**[17] (Scheme 3) by treatment with MeLi, yielded a mixture of alkenols 22(*E*):22(*Z*) in 7.5:1

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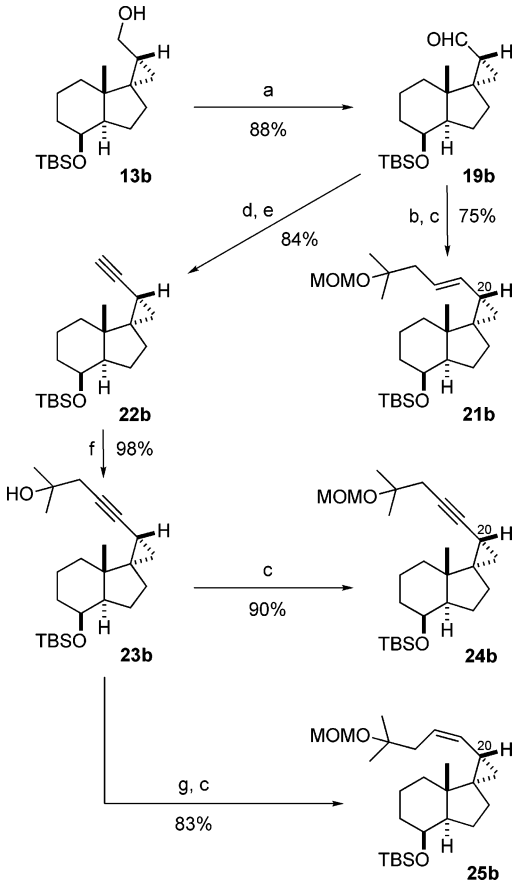
SCHEME 4. Synthesis of 20-*epi* Compounds 21b, 24b, and 25b[a]



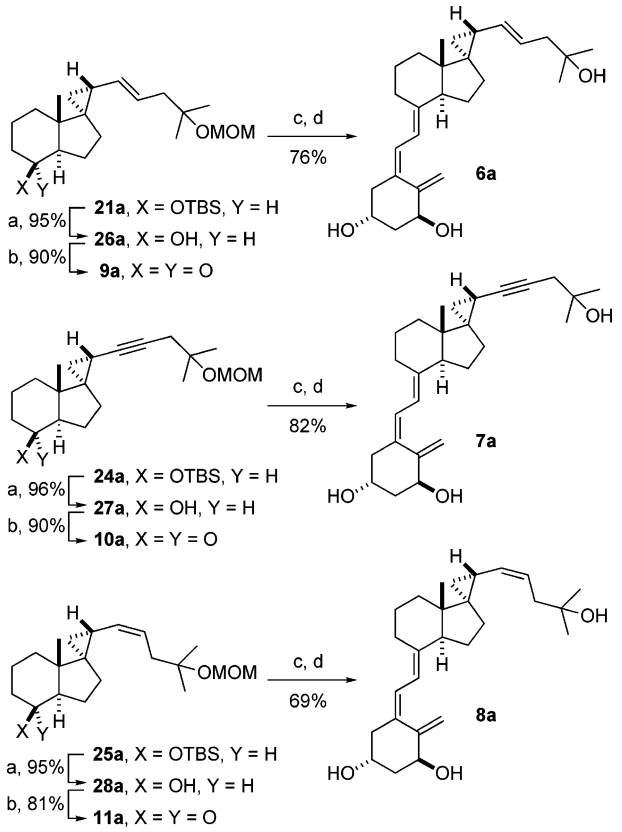
[a] (a) DMSO, $(COCl)_2$, CH_2Cl_2, then Et_3N, rt. (b) $Ph_3P^+CH_2CH_2C(Me)_2OH$, Br^- (**20**), MeLi, THF, −20 °C. (c) MOMCl, *i*-Pr_2NEt, CH_2Cl_2, 0 °C. (d) CBr_4, Ph_3P, Zn, py, CH_2Cl_2. (e) *n*-BuLi, THF, −78 °C. (f) *n*-BuLi, THF, −78 °C, then BF_3•OEt_2, 1,2-isobutylene oxide, −78 °C to rt. (g) H_2, Lindlar, hexanes, rt.

ratio. Pure 22(*E*)-stereoisomer was obtained after purification by HPLC. The 22(*E*) stereochemistry can be explained on the basis of an internal Schlosser "*trans*-selective Wittig" mechanism.[16] Finally, protection of the C25 hydroxyl group as an MOM ether, afforded the desired compound **21a** in 75% yield (two steps).

The synthesis of both the alkynyl and the (*Z*)-alkenyl side chains, necessary for the preparation of **7** and **8**, started with Corey−Fuchs homologation[18] of **19a** to form the alkyne **22a** in 80% yield. Subsequent alkylation of the acetylide, generated from **22a** with 1,2-isobutylene oxide in the presence of a Lewis acid such as BF_3•OEt_2, afforded the alkynol **23a** in good yield (70%, three steps from **19a**). Finally, protection of the C25 hydroxyl group in **23a** as MOM ether, produced compound **24a** in 80% yield. Partial hydrogenation of **23a** with Lindlar catalyst followed by protection of the hydroxyl group as MOM ether, gave **25a** in 80% yield (two steps).

The 20-*epi* precursors, **21b**, **24b**, and **25b,** were prepared from **13b** in a similar synthetic sequence to that of their C20 epimers, as depicted in Scheme 4. In this case, the Wittig reaction under Salmond conditions[16] afforded alkene **21b** as a mixture of

SCHEME 5. Synthesis of Vitamin D_3 Analogues 6a, 7a, and 8a[a]



[a] (a) TBAF, THF, reflux. (b) PDC, PPTS cat., CH_2Cl_2, rt. (c) **12**, *n*-BuLi, THF, −78 °C. (d) TBAF, THF, rt, then AG 50W-X4, MeOH, rt.

isomers in 8:1 ratio (*E*:*Z*). This mixture was used to prepare analogue **6b**, which was separated from the corresponding isomer by HPLC. The Corey−Fuchs homologation of **19b** and acetylide alkylation occurred in 82% yield (three steps), and the 25-MOM protected 20-*epi* compounds **24b** and **25b** were obtained in good yields (90 and 83%, respectively, from **23b**).

The synthesis of calcitriol analogues **6a/b**, **7a/b**, and **8a/b** was accomplished from their precursors **21a/b**, **24a/b**, and **25a/b**, respectively, following a common five-step synthetic sequence consisting in (i) deprotection of C8 hydroxyl group by treatment with TBAF, (ii) further oxidation of the resulting C8 alcohol with PDC, and (iii) Wittig−Horner reaction with the anion derived from the phosphine oxide **12**. Finally, the resulting protected calcitriol analogues were deprotected by (iv) treatment with TBAF to remove the silyl protecting groups at C1 and C3, and (v) treatment with AG 50W-X4 resin to further deprotect the C25 hydroxyl group (Schemes 5 and 6). After this synthetic sequence, vitamin D analogues **6a**, **7a**, and **8a** were synthesized in 10−13 steps and 20−32% overall yield from **14** (Scheme 5), and the 20-epimers **6b**, **7b**, and **8b** in 12−15 steps and 25% overall yield from **14** (Scheme 6).

Biological Evaluation. The biological evaluation of the vitamin D_3 analogues **6a/b**, **7a/b**, and **8a/b** includes: (a) binding affinity for the calf thymus VDR,[19] (b) binding affinity for the human vitamin D binding protein (hDBP),[20] and (c) cell-

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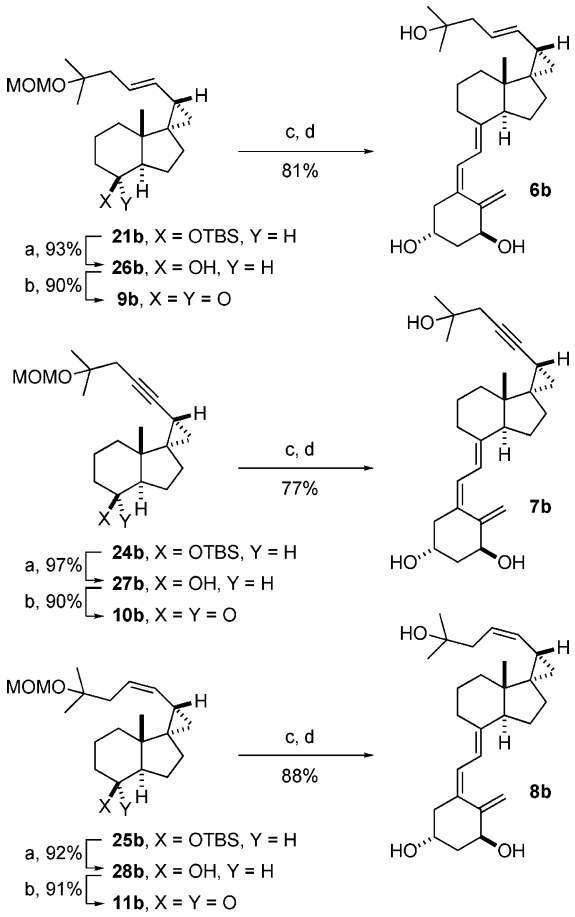
SCHEME 6. Synthesis of 20-*epi* Vitamin D_3 Analogues 6b, 7b, and 8b[a]



[a] (a) TBAF, THF, reflux. (b) PDC, PPTS cat., CH_2Cl_2, rt. (c) **12**, *n*-BuLi, THF, −78 °C. (d) TBAF, THF, rt, then AG 50W-X4, MeOH, rt.

differentiating activity in vitro on HL-60 human leukaemia cell lines.[21] Results are given in Table 1. None of the new compounds showed significant affinity for DBP in comparison with the natural hormone. The most active were **8b** (19%) and **6a** (14%). The other compounds do not bind significantly to

TABLE 1. Biological Activities of Compounds 6, 7, and 8[a]

	binding		cell differentiation
compound	VDR (calf thymus)	DBP (human)	HL-60
1	100	100	100
6a	4	14	1.5
7a	17	0	10
8a	116	2	3
6b	75	2	50
7b	0.8	0	0.6
8b	176	19	50

[a] Activities of the vitamin D_3 analogues are presented as relative values with respect to 1α,25-$(OH)_2$-D_3 (**1**, 100%).

DBP. For VDR binding, the best value was exhibited by the 22(*Z*) compounds **8b** (176%) and **8a** (116%), followed by the 20-*epi*-22(*E*) compound **6b** (75%) and **7a** (17%). None of the others show significant binding to VDR. The side chain olefinic compounds **6b** and **8b** with the 20*S* (20-*epi*) configuration at C20, which bind well to VDR, also induce a significant cell-differentiating activity (50%). The other compounds were ≤10% active compared to that of 1α,25-$(OH)_2$-D_3 (100% activity).

Conformational Analysis. To gain insight into the conformational-function relationships of the new vitamin D_3 analogues, we explored the conformational profile of their side chains. The low-energy side chain conformations are represented in the form of dot maps which reflect the sites most accessible by the tertiary hydroxyl groups. Figure 2 shows the superimposition of structures of 25-OH-D_3 and 1α,25-$(OH)_2$-D_3 bound to DBP[8c] and VDR-LBD,[8a] respectively, maintaining the corresponding CD ring systems as closely as possible. The dot maps corresponding to conformational minima were superimposed atop of the CD hydrindane moiety.

None of the side chains reach the C25-OH group of 25-OH-D_3 bound to DBP explaining the negligible or low binding of the new compounds to DBP. The side chains of **6a**, **7a**, and **7b** are also too conformationally restricted to reach the C25-OH group of 1α,25-$(OH)_2$-D_3 bound to VDR-LBD. However **6b**, **8a**, and **8b** can place their side chain hydroxyl groups suitably for VDR binding. The dot maps of **8a** (20*R* natural stereochemistry) indicate that the C25-OH group is located toward the right

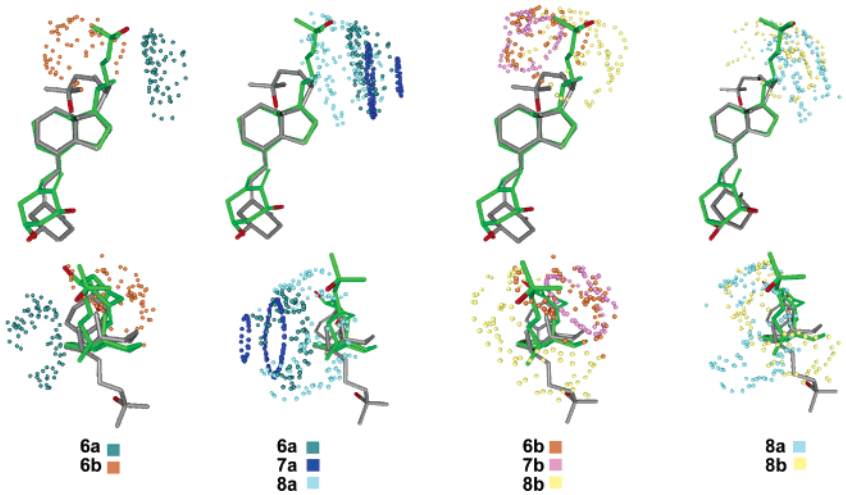


FIGURE 2. Structures of DBP−bound 25-OH-D_3 (gray) and VDR-LBD−bound 1α,25-$(OH)_2$-D_3 (green), with their CD systems superimposed. The dot maps are superimposed atop the CD hydrindane moiety and identify an occupancy volume for the location of the side chain hydroxyl group of the new vitamin D_3 analogues **6**, **7**, and **8**.

side with respect to the 1α,25-$(OH)_2$-D_3 side chain. For the C20 epimers **6b** and **8b**, the C25-OH groups are located toward the left side of the side chain of the natural hormone. This observations suggest that the greater activity of the 20-*epi* compounds in the VDR binding and HL-60 cell differentiation assays, may have been due to the paths of the corresponding side chains in the VDR-LBD region in agreement with the results obtained by Dino Moras and co-workers[22] on 20-*epi* superagonists MC1288 and KH1060. The 20-*epi* analogues **6b** and **8b** interfere hydrophobically stronger with the amino acid residues located at the right side of the VDR-LBD than the analogues with the natural stereochemistry at C20.

Conclusions

In conclusion, we synthesized six new calcitriol analogues conformationally restricted at their side chain by the introduction of both a cyclopropane ring at C17−C20 and a double or triple bond at C22, using a divergent route to generate both epimers at C20, starting from ketone **14**. Further studies on the conformational analysis and biological tests show that compounds **6a**/**b**, **7a**/**b**, and **8a**/**b** do not bind to vitamin D binding protein (DBP), apparently because their conformationally restricted C17 side chains are unable to reach the DBP-bound 25-OH-D_3 binding region. Compounds **6a**, **7a**, and **8b** also fail to bind to the vitamin D receptor (VDR), which in the case of the alkynes **7a** and **7b** may be due to the high rigidity of their side chains and to the impossibility of rotation about C17−C20. However, the remaining three compounds bind to VDR with affinities that increase in the order **6b**(75) < **8a**(116) < **8b**(176) (numbers in parentheses are percentages of the affinity of calcitriol for VDR). The 20-*epi* compounds **6b** and **8b** bind to VDR better than to their counterparts **6a** and **8a.** This result is attributable to their C20 stereochemistry, allowing them to lie in the VDR ligand-binding pocket in such a way as to be able to form strong interactions with hydrophobic amino acids. The fact that **8a** binds well to VDR but does not inhibit the growth of HL-60 cells suggests that it may be an antagonist or non-agonist. The compounds **8a** and **8b** bind best to VDR. 20-*Epi* compounds induce higher VDR affinities and HL-60 cell differentiation activities than the corresponding compounds with the natural stereochemistry at C20. Finally, the fact that the VDR-active compounds do not bind to DBP suggests that their local administration for treatment of antiproliferative disease would not be accompanied by side effects requiring their transport in the bloodstream, such as hypercalcemia.

Experimental Section

(17*E*)-(8β)-8-[(*tert*-Butyldimethylsilyl)oxy]-des-*A*,*B*-pregn-17-(20)-en-21-oic acid ethyl ester (15). A solution of NaOEt in EtOH (106 mmol, 65 mL) was added to a mixture of the ketone **14** (3 g, 10.63 mmol) and $(EtO)_2P(O)CH_2CO_2Et$ (53.17 mmol, 10.6 mL, freshly distilled). The mixture was refluxed for 12 h and then concentrated to small volume. H_2O (60 mL) and HOAc were added till pH = 5. The resulting mixture was extracted with Et_2O (3 × 50 mL). The combined organic phase was dried, filtered, and concentrated. The residue was purified by flash chromatography (5% EtOAc/hexanes) to afford **15** [3.6 g, 97%, R_f = 0.4 (5% EtOAc/hexanes), colorless oil]. ^{1}H NMR δ 5.47 (t, J = 2.4 Hz, 1 H), 4.13 (m, 3 H), 2.81 (m, 2 H), 1.26 (t, J = 7.1 Hz, 3 H), 1.05 (s, 3 H), 0.88 (s, 9 H), 0.02 (s, 6 H); ^{13}C NMR δ 177.1 (C), 167.6 (C), 107.5 (CH), 69.1 (CH), 59.4 (CH_2), 50.4 (CH_3), 45.7 (C), 36.0 (CH_2), 34.4 (CH_2), 29.6 (CH_2), 25.7 (3 × CH_3), 23.4 (CH_2), 21.1 (CH_3), 18.0 (C), 17.5 (CH_2), 14.4 (CH), −4.9 (CH_3), −5.2 (CH_3); MS (EI) m/z 352 (M^+, 6), 337 ($M^+ - CH_3$, 3), 307 ($M^+ - C_2H_5O$, 5), 295 (100); HRMS (EI) calcd for $C_{20}H_{36}O_3Si$ 352.2434 (M^+), found 352.2432.

(17*E*)-(8β)-8-[(*tert*-Butyldimethylsilyl)oxy]-des-*A*,*B*-pregn-17-(20)-en-21-ol (16). A solution of DIBALH in heptane (4.1 mL, 3.7 mmol, 1 M) was added slowly to a solution of **15** (0.65 g, 1.85 mmol) in dry toluene (20 mL) at −78 °C. The reaction mixture was allowed to reach rt for 14 h. The reaction was quenched by addition of an aqueous solution of HCl (10%, 40 mL). The aqueous phase was extracted with Et_2O (2 × 25 mL). The combined organic phase was washed with a saturated solution of $NaHCO_3$ (50 mL) and a saturated solution of NaCl (50 mL), dried, filtered, and concentrated. The residue was purified by flash chromatography (10% EtOAc/hexanes) to afford **16** [0.56 g, 97%,R_f = 0.3 (20% EtOAc/hexanes), colorless oil]. ^{1}H NMR δ 5.16 (m, 1 H), 4.07 (m, 3 H), 2.29 (m, 2 H), 1.00 (s, 3 H), 0.88 (s, 9 H), 0.02 (s, 3 H), 0.01 (s, 3 H); ^{13}C NMR δ 156.6 (C), 114.3 (CH), 69.2 (CH), 60.2 (CH_2), 50.9 (CH), 43.5 (C), 36.5 (CH_2), 34.6 (CH_2), 25.8 (3 × CH_3), 25.3 (CH_2), 23.3 (CH_2), 21.5 (CH_3), 18.0 (CH_2), 17.5 (C), −4.9 (CH_3),−5.2 (CH_3); MS (FAB) m/z 333 (M^+ + Na, 19), 310 (M^+, 3), 309 (M^+ − H, 14), 293 (100); HRMS (FAB) calcd for $C_{18}H_{34}O_2NaSi$ 333.2226 (M^+ + Na), found 333.2226.

(8β)-(20*S*)-8-[(*tert*-Butyldimethylsilyl)oxy]-des-*A*,*B*-17α,21-cyclo-23,24-dinorcholan-22-ol (13a). A solution of Et_2Zn in toluene (30.3 mL, 1 M) was slowly added to a solution of **16** (1.88 g, 6.06 mmol) and CH_2I_2 (2.44 mL, 30.3 mmol, filtered through Al_2O_3), in dry toluene (50 mL) at −78 °C. The reaction was allowed to reach rt for 5 h and then quenched by the addition of an aqueous solution of HCl (5%, 50 mL). The aqueous phase was extracted with Et_2O (3 × 40 mL), and the combined organic phase was dried, filtered, and concentrated. The residue was purified by flash chromatography (4% EtOAc/hexanes) to give **13a** [1.51 g, 77%, R_f = 0.4 (20% EtOAc/hexanes), white solid, mp 75−78 °C]. ^{1}H NMR δ 4.06 (m, 1 H), 3.61 (m, 1 H), 3.48 (m, 1 H), 2.04 (m, 1 H), 1.00 (s, 3 H), 0.89 (s, 9 H), 0.02 (s, 3 H), 0.01 (s, 3 H); ^{13}C NMR δ 69.0 (CH), 65.2 (CH_2), 51.4 (CH), 40.4 (C), 37.1 (C), 34.7 (CH_2), 33.7 (CH_2), 28.3 (CH_2), 25.8 (3 × CH_3), 23.5 (CH_2), 19.6 (CH), 19.4 (CH_3), 18.0 (C), 17.1 (CH_2), 16.5 (CH_2), −4.8 (CH_3), −5.2 (CH_3); MS (EI) m/z 309 ($M^+ - CH_3$, 1), 209 (100); HRMS (EI) calcd for $C_{19}H_{36}O_2Si$ 324.2485 (M^+), found 324.2483.

(8β)-(20*R*)-8-[(*tert*-Butyldimethylsilyl)oxy]-des-*A*,*B*-17α,20α-epoxypregnan-21-ol (17). A solution of *t*-BuOOH in decane (0.350 mL, 1.77 mmol, 5 M) was slowly added to a solution of **16** (0.5 g, 1.61 mmol) and a catalytic amount of $VO(acac)_2$ in toluene (7.5 mL) at −20 °C. The reaction was stirred at the same temperature for 24 h and then quenched by the addition of a saturated solution of $NaHCO_3$ (10 mL). The aqueous phase was extracted with Et_2O (3 × 10 mL), and the organic phase was dried, filtered, and concentrated. The residue was purified by flash chromatography (10% EtOAc/hexanes, 1% of Et_3N) to afford **17** [0.41 g, 78%, R_f = 0.3 (30% EtOAc/hexanes), colorless oil]. ^{1}H NMR δ 4.10 (m, 1 H), 3.82 (dd, J = 12.2 and 3.5 Hz, 1 H), 3.55 (dd, J = 12.2 and 7.0 Hz, 1 H), 3.02 (dd, J = 7.0 and 3.5 Hz, 1 H), 2.15 (m, 1 H), 1.01 (s, 3 H), 0.88 (s, 9 H), 0.02 (s, 3 H), 0.01 (s, 3 H); ^{13}C NMR δ 74.6 (C), 69.0 (CH), 62.7 (CH_2), 57.6 (CH), 49.2 (CH), 41.4 (C), 34.3 (CH_2), 30.8 (CH_2), 26.8 (CH_2), 25.7 (3 × CH_3), 22.6 (CH_2), 18.5 (CH_3), 18.0 (C), 16.5 (CH_2), −4.8 (CH_3), −5.2 (CH_3); MS (EI) m/z 326 (M^+, 1), 311 ($M^+ - CH_3$, 1),

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269 ($M^+ - C_4H_9$, 30), 75 (100); HRMS (EI) calcd for $C_{14}H_{25}O_3Si$ 269.1573 ($M^+ - C_4H_9$), found 269.1574.

(17*Z*)-(8*β*)-8-[(*tert*-Butyldimethylsilyl)oxy]-des-*A*,*B*-pregn-17-(20)-en-21-ol (18). A solution of *n*-BuLi in hexanes (1.2 mL, 3.0 mmol, 2.5 M) was slowly added to a solution of Ph_2PH (0.49 mL, 2.83 mmol) in dry THF (2 mL) at 0 °C. The resulting red mixture was protected from light and stirred for 4 h. A solution of **17** (0.350 g, 1.07 mmol) in dry THF (3 mL) was added, and the resulting mixture was stirred for 1 h. MeI (0.31 mL, 5.1 mmol, filtered through Al_2O_3) was added, and the mixture was stirred for 4 h at rt. The reaction was quenched by the addition of H_2O. The white suspension was filtered and the solids were washed with Et_2O (4 × 10 mL). The organic solution was dried, filtered, and concentrated. The residue was purified by flash chromatography (10% EtOAc/hexanes) to give **18** [0.273 g, 82%, $R_f = 0.7$ (30% EtOAc/hexanes), white solid, mp 53−55 °C]. ^{1}H NMR δ 5.23 (dd, $J = 7.3$ and 7.1 Hz, 1 H), 4.29 (dd, $J = 12.2$ and 7.3 Hz, 1 H), 4.14 (dd, $J = 12.2$ and 7.1 Hz, 1 H), 4.07 (m, 1 H), 2.45 (m, 1 H), 2.20 (m, 2 H), 1.12 (s, 3 H), 0.89 (s, 9 H), 0.02 (s, 3 H), 0.01 (s, 3 H); ^{13}C NMR δ 155.0 (C), 117.9 (CH), 69.6 (CH), 58.7 (CH_2), 52.3 (CH), 44.6 (C), 38.1 (CH_2), 34.2 (CH_2), 30.6 (CH_2), 25.8 (3 × CH_3), 23.5 (CH_2), 20.5 (CH_3), 18.0 (C), 17.8 (CH_2), −4.8 (CH_3), −5.1 (CH_3); MS (EI) *m*/*z* 310 (M^+, 1), 295 ($M^+ - CH_3$, 1), 75 (100); HRMS (EI) calcd for $C_{18}H_{34}O_2Si$ 310.2328 (M^+), found 310.2333.

(8*β*)-(20*S*)-8-[(*tert*-Butyldimethylsilyl)oxy]-des-*A*,*B*-17α,21-cyclo-23,24-dinorcholan-22-al (19a). Dry DMSO (0.253 mL, 3.57 mmol) was slowly added to a solution of oxalyl chloride (0.160 mL, 1.79 mmol) in dry CH_2Cl_2 (20 mL) at −78 °C. The solution was stirred for 15 min. A solution of **13a** (0.455 g, 1.41 mmol) in dry CH_2Cl_2 (9 mL) was added. The mixture was stirred at −78 °C for 0.5 h, and Et_3N (2 mL, 14.1 mmol) was added. The mixture was allowed to reach rt for 7 h. The reaction was quenched by the addition of H_2O (40 mL) and CH_2Cl_2 (40 mL). The organic phase was washed with brine (40 mL), dried, filtered, and concentrated. The residue was purified by flash chromatography (5% EtOAc/hexanes) to afford **19a** [0.413 g, 91%, $R_f = 0.6$ (20% EtOAc/hexanes), white solid, mp 120−123 °C]. ^{1}H NMR δ 9.08 (d, $J =$ 6.3 Hz, 1 H), 4.08 (m, 1 H), 2.04 (m, 1 H), 1.02 (s, 3 H), 0.88 (s, 9 H), 0.02 (s, 3 H), 0.01 (s, 3 H); ^{13}C NMR δ 202.0 (CH), 68.8 (CH), 50.5 (CH), 44.5 (C), 41.6 (C), 34.5 (CH_2), 33.6 (CH_2), 30.9 (CH), 28.8 (CH_2), 25.8 (3 × CH_3), 23.5 (CH_2), 21.2 (CH_2), 19.3 (CH_3), 18.0 (C), 17.0 (CH_2), −4.8 (CH_3), −5.2 (CH_3); MS (EI) *m*/*z* 323 ($M^+ + H$, 7), 322 (M^+, 12), 307 ($M^+ - CH_3$, 34), 209 (100); HRMS (EI) calcd for $C_{19}H_{34}O_2Si$ 322.2328 (M^+), found 322.2316.

(22*E*)-(8*β*)-(20*R*)-8-[(*tert*-Butyldimethylsilyl)oxy]-25-[(methoxymethyl)oxy]-des-*A*,*B*-17α,21-cyclocholest-22-ene (21a). A solution of MeLi in Et_2O (0.81 mL, 1.3 mmol, 1.6 M) was added to a solution of the phosphonium salt $Ph_3P^+CH_2CH_2CMe_2OH\ Br^-$ (**20**, 0.28 g, 0.65 mmol) in THF (5 mL) at −20 °C. The red mixture was stirred for 1.5 h at −20 °C and 8 h at rt. After cooling to −30 °C, a solution of **19a** (0.100 g, 0.33 mmol) in THF (4 mL) was slowly added. After stirring for 12 h, the reaction was quenched with H_2O (10 mL). The mixture was filtered through celite. The filtrate was extracted with Et_2O, and the resulting organic layer was washed with brine, dried, filtered, and concentrated. The residue was purified by flash chromatography (10% EtOAc/hexanes) to give a mixture of isomers (*E*:*Z* = 7.5:1), which were separated by HPLC (Phenomenex, Zorbax Silica, SP/6885B; 2% *i*-PrOH/hexanes; 2 mL/min) to afford (22*E*)-(8*β*)-(20*R*)-8-[(*tert*-butyldimethylsilyl)oxy]-des-*A*,*B*-17α,21-cyclocholest-22-en-25-ol [0.105 g, 82%, $R_f = 0.4$ (15% EtOAc/hexanes), colorless oil]. ^{1}H NMR δ 5.50 (dt, $J = 15.2$ and 7.5 Hz, 1 H), 5.17 (dd, $J = 15.2$ and 8.8 Hz, 1 H), 4.05 (m, 1 H), 2.15 (d, $J = 7.5$ Hz, 2 H), 1.98 (m, 1 H), 1.19 (s, 6 H), 0.96 (s, 3 H), 0.88 (s, 9 H), 0.11 (dd, $J = 5.0$ Hz, 1 H), 0.01 (s, 3 H), 0.00 (s, 3 H); ^{13}C NMR δ 137.0 (CH), 123.4 (CH), 70.3 (C), 69.1 (CH), 51.5 (CH), 46.9 (CH_2), 41.0 (C), 38.9 (C), 34.6 (CH_2), 33.8 (CH_2), 29.0 (CH_2), 29.0 (CH_3), 28.9 (CH_3), 25.7 (3 × CH_3), 23.5 (CH_2), 20.6 (CH), 19.6 (CH_2), 18.9 (CH_3), 18.0 (C), 17.1 (CH_2), −4.8 (CH_3), −5.1 (CH_3); MS (FAB) *m*/*z* 393 ($M^+ + H$, 4), 392 (M^+, 4), 391 ($M^+ - H$, 8), 209 (100); HRMS (FAB) calcd for $C_{24}H_{43}O_2Si$ 391.3032 ($M^+ - H$), found 391.3028.

To a solution of the above alcohol (500 mg, 1.28 mmol) was added MOMCl (0.32 mL, 3.8 mmol) and *i*-Pr_2NEt (0.67 mL, 3.8 mmol) in CH_2Cl_2 (25 mL) at 0 °C. The reaction mixture was stirred for 24 h. The reaction was quenched by the addition of ice and an aqueous solution of HCl (10%). The resulting mixture was extracted with CH_2Cl_2 and the resulting organic layer was washed with H_2O, dried, filtered, and concentrated. The residue was purified by flash chromatography (5% EtOAc/hexanes) to give **21a** [513 mg, 92%, $R_f = 0.7$ (15% EtOAc/hexanes), colorless oil]. ^{1}H NMR δ 5.47 (dt, $J = 15.2$ and 7.3 Hz, 1 H), 5.10 (dd, $J = 15.2$ and 8.7 Hz, 1 H), 4.72 (s, 2 H), 4.05 (m, 1 H), 3.36 (s, 3 H), 2.21 (d, $J = 7.3$ Hz, 2 H), 1.99 (m, 1 H), 1.19 (s, 6 H), 0.95 (s, 3 H), 0.88 (s, 9 H), 0.08 (br d, $J = 4.8$ Hz, 1 H), 0.01 (s, 3 H), 0.00 (s, 3 H); ^{13}C NMR δ 135.3 (CH), 124.1 (CH), 91.0 (CH_2), 76.4 (C), 69.1 (CH), 55.0 (CH_3), 51.5 (CH), 45.1 (CH_2), 41.0 (C), 38.7 (C), 34.7 (CH_2), 33.8 (CH_2), 28.9 (CH_2), 26.1 (2 × CH_3), 25.8 (3 × CH_3), 23.5 (CH_2), 20.6 (CH), 19.4 (CH_2), 18.8 (CH_3), 18.0 (C), 17.1 (CH_2), −4.8 (CH_3), −5.2 (CH_3); MS (FAB) *m*/*z* 435 ($M^+ - H$, 8), 405 ($M^+ - CH_3O$, 7), 209 (89), 109 (100); HRMS (FAB) calcd for $C_{25}H_{45}O_2Si$ 405.3189 ($M^+ - CH_3O$), found 405.3192.

(8*β*)-(20*S*)-8-[(*tert*-Butyldimethylsilyl)oxy]-des-*A*,*B*-17α,21-cyclo-24-norchol-22-yne (22a). A solution of aldehyde **19a** (0.485 g, 1.58 mmol) in CH_2Cl_2 (16 mL) and pyridine (1.3 mL, 16.3 mmol) were successively added to a mixture of Zn (0.633 g, 9.7 mmol), CBr_4 (3.2 g, 9.7 mmol), and Ph_3P (2.54 g, 9.7 mmol) in CH_2Cl_2 (30 mL). The reaction mixture was stirred for 5 h. The reaction was quenched by the addition of Et_2O (50 mL). The suspension was filtered through celite and the solids were washed with Et_2O. The organic layer was concentrated and the residue was purified by flash chromatography (hexanes) to afford (8*β*)-(20*R*)-8-[(*tert*-butyldimethylsilyl)oxy]-des-*A*,*B*-17α,21-cyclo-23,23-dibromo-24-norchol-22-ene [714 mg, 94%, $R_f = 0.8$ (5% EtOAc/hexanes), white solid, mp 52−54 °C]. ^{1}H NMR δ 5.94 (d, $J = 9.3$ Hz, 1 H), 4.07 (m, 1 H), 2.04 (m, 1 H), 1.03 (s, 3 H), 0.89 (s, 9 H), 0.32 (br d, $J =$ 5.0 Hz, 1 H), 0.02 (s, 3 H), 0.01 (s, 3 H); ^{13}C NMR δ 140.7 (CH), 84.7 (C), 68.9 (CH), 51.2 (CH), 40.9 (C), 40.4 (C), 34.5 (CH_2), 33.8 (CH_2), 29.4 (CH_2), 25.8 (3 × CH_3), 23.5 (CH_2), 22.4 (CH), 21.0 (CH_2), 19.6 (CH_3), 18.0 (C), 17.0 (CH_2), −4.8 (CH_3), −5.2 (CH_3); MS (EI) *m*/*z* 476 (M^+, 2), 463 (41), 421 (80), 209 (100); HRMS (EI) calcd for $C_{20}H_{34}OSiBr_2$ 476.0746 (M^+), found 476.0750.

A solution of *n*-BuLi in hexanes (0.45 mL, 0.96 mmol, 2.1 M) was slowly added to a solution of the above dibromide (0.139 g, 0.29 mmol) in THF (10 mL), at −78 °C. After stirring for 0.5 h, the reaction was quenched with MeOH. The mixture was concentrated and diluted with brine. The mixture was extracted with Et_2O (25 mL). The organic phase was dried, filtered, and concentrated. The residue was purified by flash chromatography (hexanes) to give **22a** [790 mg, 85%, $R_f = 0.5$ (hexanes), white solid, mp 62−64 °C]. ^{1}H NMR δ 4.06 (s, 1 H), 2.27 (m, 1 H), 1.83 (s, 1 H), 1.02 (s, 3 H), 0.89 (s, 9 H), 0.38 (br s, 1 H), 0.02 (s, 6 H); ^{13}C NMR δ 86.3 (C), 69.1 (CH), 65.4 (CH), 52.2 (CH), 40.5 (C), 39.2 (C), 34.5 (CH_2), 33.9 (CH_2), 29.9 (CH_2), 25.8 (3 × CH_3), 23.4 (CH_2), 21.0 (CH_2), 19.3 (CH_3), 18.0 (C), 17.0 (CH_2), 6.5 (CH), −4.8 (CH_3), −5.2 (CH_3); MS (EI) *m*/*z* 303 ($M^+ - CH_3$, 1), 261 (5), 209 (100); HRMS (EI) calcd for $C_{20}H_{34}OSi$ 318.2379 (M^+), found 318.2367.

(8*β*)-(20*S*)-8-[(*tert*-Butyldimethylsilyl)oxy]-des-*A*,*B*-17α,21-cyclocholest-22-yn-25-ol (23a). A solution of *n*-BuLi in hexanes (1 mL, 2.26 mmol, 2.27 M) was slowly added to a solution of **22a** (0.654 g, 2.05 mmol) in THF (30 mL) at −78 °C. After 30 min, $BF_3{\cdot}OEt_2$ (1.26 mL, 10.2 mmol) and a solution of isobutylene oxide (0.92 mL, 10.3 mmol) in THF (12 mL) were successively added. After stirring for 6 h at rt, the reaction was quenched with a few drops of MeOH. The mixture was extracted with Et_2O (100 mL).

The organic phase was washed with $NaHCO_3$ (50 mL) and brine (50 mL), dried, filtered, and concentrated. The residue was purified by flash chromatography (10% EtOAc/hexanes) to afford **23a** [703 mg, 88%, $R_f = 0.4$ (15% EtOAc/hexanes), colorless oil]. ^{1}H NMR δ 4.06 (s, 1 H), 2.33 (d, $J = 1.2$ Hz, 2 H), 2.20 (m, 1 H), 1.27 (s, 6 H), 1.01 (s, 3 H), 0.89 (s, 9 H), 0.30 (br d, $J = 3.2$ Hz, 1 H), 0.02 (s, 6 H); ^{13}C NMR δ 85.2 (C), 73.6 (C), 69.8 (C), 69.1 (CH), 52.1 (CH), 40.5 (C), 39.2 (C), 34.7 (CH_2), 34.5 (CH_2), 33.9 (CH_2), 30.3 (CH_2), 28.5 (2 × CH_3), 25.8 (3 × CH_3), 23.4 (CH_2), 21.1 (CH_2), 19.3 (CH_3), 18.0 (C), 17.0 (CH_2), 6.8 (CH), −4.8 (CH_3), −5.2 (CH_3); MS (EI) *m*/*z* 390 (M^+, 1), 375 ($M^+ - CH_3$, 9), 209 (100); HRMS (EI) calcd for $C_{24}H_{42}O_2Si$ 390.2954 (M^+), found 390.2950.

(8*β*)-(20*S*)-8-[(*tert*-Butyldimethylsilyl)oxy]-25-[(methoxymethyl)-oxy]-des-A,B-17α,21-cyclocholest-22-yne (24a). MOMCl (0.25 mL, 3.3 mmol) and *i*-Pr_2NEt (0.6 mL, 3.3 mmol) were added to a solution of **23a** (433 mg, 1.11 mmol) in CH_2Cl_2 (10 mL) at 0 °C. After stirring for 24 h, the reaction was quenched with ice and an aqueous solution of HCl (10%). The resulting mixture was extracted with CH_2Cl_2 (15 mL). The organic phase was washed with H_2O, dried, filtered, and concentrated. The residue was purified by flash chromatography (5% EtOAc/hexanes) to give **24a** [330 mg, 92%, $R_f = 0.7$ (15% EtOAc/hexanes), colorless oil]. ^{1}H NMR δ 4.72 (s, 2 H), 4.04 (m, 1 H), 3.35 (s, 3 H), 2.38 (d, $J = 1.3$ Hz, 2 H), 2.22 (m, 1 H), 1.29 (s, 6 H), 0.99 (s, 3 H), 0.88 (s, 9 H), 0.26 (br d, $J = 3.5$ Hz, 1 H), 0.00 (s, 6 H); ^{13}C NMR δ 91.2 (CH_2), 83.6 (C), 76.0 (C), 74.2 (C), 69.1 (CH), 55.1 (CH_3), 52.1 (CH), 40.4 (C), 39.1 (C), 34.5 (CH_2), 33.9 (CH_2), 32.5 (CH_2), 30.1 (CH_2), 25.9 (2 × CH_3), 25.8 (3 × CH_3), 23.4 (CH_2), 21.0 (CH_2), 19.2 (CH_3), 18.0 (C), 17.0 (CH_2), 6.9 (CH), −4.8 (CH_3), −5.2 (CH_3); MS (EI) *m*/*z* 419 ($M^+ - CH_3$, 3), 403 ($M^+ - CH_3O$, 8), 209 (100); HRMS (EI) calcd for $C_{26}H_{46}NaO_3Si$ 457.3114 (M^+), found 457.3108.

(22*Z*)-(8*β*)-(20*R*)-8-[(*tert*-Butyldimethylsilyl)oxy]-25-[(meth-oxymethyl)oxy]-des-*A*,*B*-17α,21-cyclocholest-22-ene (25a). A mixture of quinoline (0.018 g, 0.14 mmol), Lindlar catalyst (0.050 g), and **23a** (0.070 g, 0.18 mmol) in dry hexane (70 mL) was hydrogenated for 30 min (balloon pressure). The mixture was filtered through a short pad of celite. Concentration gave a residue which was purified by flash chromatography (5% EtOAc/hexanes) to afford (22*Z*)-(8*β*)-(20*R*)-8-[(*tert*-butyldimethylsilyl)oxy]-des-*A*,*B*-17α,21-cyclocholest-22-en-25-ol [61 mg, 87%, $R_f = 0.4$ (15% EtOAc/hexanes), colorless oil]. ^{1}H NMR δ 5.42 (dt, $J = 10.9$ and 7.5 Hz, 1 H), 5.14 (dd, $J = 10.8$ and 9.9 Hz, 1 H), 4.06 (m, 1 H), 2.33 (d, $J = 7.7$ Hz, 2 H), 2.03 (m, 1 H), 1.29 (s, 6 H), 0.98 (s, 3 H), 0.88 (s, 9 H), 0.10 (br d, $J = 4.6$ Hz, 1 H), 0.02 (s, 6 H); ^{13}C NMR δ 135.7 (CH), 122.9 (CH), 71.2 (C), 69.1 (CH), 51.4 (CH), 41.4 (CH_2), 41.0 (C), 39.4 (C), 34.7 (CH_2), 34.0 (CH_2), 29.2 (CH_3), 29.0 (CH_3), 28.9 (CH_2), 25.8 (3 × CH_3), 23.5 (CH_2), 20.9 (CH_2), 19.3 (CH_3), 18.0 (C), 17.1 (CH_2), 16.5 (CH), −4.8 (CH_3), −5.2 (CH_3); MS (FAB) *m*/*z* 393 ($M^+ + H$, 24), 392 (M^+, 27), 391 ($M^+ - H$, 67), 377 ($M^+ - CH_3$, 11), 375 (75), 265 (100); HRMS (FAB) calcd for $C_{24}H_{44}O_2Si$ 392.3116 (M^+), found 392.3092.

Protection of the above alcohol, following the same experimental procedure as for **24a**, afforded **25a** [92%, $R_f = 0.7$ (15% EtOAc/hexanes), colorless oil]. ^{1}H NMR δ 5.39 (dt, $J = 10.9$ and 7.5 Hz, 1 H), 5.06 (dd, $J = 10.9$ and 9.8 Hz, 1 H), 4.74 (s, 2 H), 4.06 (m, 1 H), 3.37 (s, 3 H), 2.37 (d, $J = 7.4$ Hz, 2 H), 2.01 (m, 1 H), 1.24 (s, 6 H), 0.97 (s, 3 H), 0.88 (s, 9 H), 0.07 (br d, $J = 4.8$ Hz, 1 H), 0.01 (s, 3 H), 0.01 (s, 3 H); ^{13}C NMR δ 133.9 (CH), 123.6 (CH), 91.1 (CH_2), 76.7 (C), 69.1 (CH), 55.0 (CH_3), 51.4 (CH), 41.0 (C), 39.5 (CH_2), 39.2 (C), 34.7 (CH_2), 34.0 (CH_2), 28.8 (CH_2), 26.3 (CH_3), 26.1 (CH_3), 25.8 (3 × CH_3), 23.5 (CH_2), 20.7 (CH_2), 19.4 (CH_3), 18.0 (C), 17.1 (CH_2), 16.5 (CH), −4.8 (CH_3), −5.2 (CH_3); MS (FAB) *m*/*z* 435 ($M^+ - H$, 7), 405 ($M^+ - CH_3O$, 8), 109 (100); HRMS (FAB) calcd for $C_{25}H_{45}O_2Si$ 405.3189 ($M^+ - CH_3O$), found 405.3190.

(22*E*)-(8*β*)-(20*R*)-25-[(Methoxymethyl)oxy]-des-*A*,*B*-17α,21-cyclocholest-22-en-8-ol (26a). A solution of *n*-Bu_4NF in THF (9 mL, 9 mmol, 1 M) was added to a solution of **21a** (400 mg, 0.92 mmol) in THF (15 mL). The mixture was refluxed for 12 h and then treated with saturated aqueous $NaHCO_3$ (5 mL). The aqueous phase was extracted with Et_2O (30 mL), and the combined organic phase was dried, filtered, and concentrated. The residue was purified by flash chromatography (5% EtOAc/hexanes) to give **26a** [280 mg, 95%, $R_f = 0.4$ (25% EtOAc/hexanes), colorless oil]. ^{1}H NMR δ 5.45 (dt, $J = 15.2$ and 7.2 Hz, 1 H), 5.07 (dd, $J = 15.3$ and 8.7 Hz, 1 H), 4.69 (s, 2 H), 4.09 (m, 1 H), 3.33 (s, 3 H), 2.19 (d, $J = 7.2$ Hz, 2 H), 2.00 (m, 1 H), 1.16 (s, 6 H), 0.96 (s, 3 H), 0.08 (br d, $J = 5.0$ Hz, 1 H); ^{13}C NMR δ 134.8 (CH), 124.3 (CH), 90.9 (CH_2), 76.3 (C), 68.8 (CH), 54.9 (CH_3), 51.0 (CH), 45.0 (CH_2), 40.6 (C), 38.6 (C), 33.7 (CH_2), 33.5 (CH_2), 28.7 (CH_2), 26.0 (2 × CH_3), 22.9 (CH_2), 20.5 (CH), 19.3 (CH_2), 18.4 (CH_3), 16.8 (CH_2); MS (FAB) *m*/*z* 345 ($M^+ + Na$, 4), 291 ($M^+ - CH_3O$, 14), 109 (100); HRMS (FAB) calcd for $C_{20}H_{34}O_3Na$ 345.2406 ($M^+ + Na$), found 345.2399.

(22*E*)-(20*R*)-25-[(Methoxymethyl)oxy]-des-*A*,*B*-17α,21-cyclo-cholest-22-en-8-one (9a). Pyridinium dichromate (450 mg, 1.20 mmol) and pyridinium *p*-toluenesulfonate (25 mg) were added to a solution of **26a** (227 mg, 0.71 mmol) in CH_2Cl_2 (10 mL). After 8 h, Et_2O (40 mL) was added and the resulting mixture was filtered through Celite. Concentration after washing the solids with Et_2O (3 × 40 mL) gave a residue which was purified by flash chromatography (10% EtOAc/hexanes) to afford **9a** [203 mg, 90%, $R_f = 0.5$ (25% EtOAc/hexanes), colorless oil]. ^{1}H NMR δ 5.44 (dt, $J = 15.2$ and 7.5 Hz, 1 H), 5.06 (dd, $J = 15.2$ and 8.6 Hz, 1 H), 4.68 (s, 2 H), 3.32 (s, 3 H), 2.62 (dd, $J = 11.1$ and 7.4 Hz, 1 H), 2.18 (d, $J = 7.2$ Hz, 2 H), 1.15 (s, 6 H), 0.67 (s, 3 H), 0.20 (br d, $J = 5.2$ Hz, 1 H); ^{13}C NMR δ 211.4 (C), 133.9 (CH), 125.1 (CH), 90.9 (CH_2), 76.2 (C), 60.2 (CH), 54.9 (CH_3), 48.5 (C), 45.0 (CH_2), 40.9 (CH_2), 37.8 (C), 32.8 (CH_2), 28.7 (CH_2), 26.0 (2 × CH_3), 23.1 (CH_2), 21.2 (CH), 19.8 (CH_2), 19.0 (CH_2), 16.9 (CH_3); MS (FAB) *m*/*z* 343 ($M^+ + Na$, 6), 289 ($M^+ - CH_3O$, 31), 150 (100); HRMS (FAB) calcd for $C_{20}H_{32}O_3Na$ 343.2249 ($M^+ + Na$), found 343.2236.

(5*Z*,7*E*,22*E*)-(1*S*,3*R*,20*R*)-17α,21-cyclo-9,10-secocholesta-5,7,-10(19),22-tetraene-1,3,25-triol (6a). A solution of *n*-BuLi in hexanes (0.200 mL, 0.45 mmol, 2.26 M) was added dropwise to a solution of **12** (283 mg, 0.49 mmol) 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 **9a** (82 mg, 0.26 mmol) in THF (3 mL). The red solution was stirred in the dark at −78 °C for 3 h and then warmed to −40 °C over 2 h. The reaction was quenched with H_2O (5 mL). The mixture was extracted with Et_2O (30 mL) and the organic phase was washed with brine, dried, filtered, and concentrated. The residue was purified by flash chromatography (2% EtOAc/hexanes) to give (5*Z*,7*E*,22*E*)-(1*S*,3*R*,20*R*)-1,3-di-[(*tert*-butyldimethylsilyl)oxy]-25-[(methoxymethyl)oxy]-17α,21-cyclo-9,-10-secocholesta-5,7,10(19),22-tetraene [150 mg, 86%, (22*E*:22*Z* = 8:1), $R_f = 0.7$ (5% EtOAc/hexanes), colorless oil]. ^{1}H NMR δ 6.24 and 6.06 (2 d, AB system, $J = 11.1$ Hz, 2 H), 5.46 (dt, $J = 15.2$ and 7.5 Hz, 1 H), 5.19 (br s, 1 H), 5.12 (dd, $J = 15.2$ and 8.6 Hz, 1 H), 4.88 (br s, 1 H), 4.72 (s, 2 H), 4.38 (m, 1 H), 4.19 (m, 1 H), 2.84 (m, 1 H), 2.23 (d, $J = 7.3$ Hz, 2 H), 1.20 (s, 6 H), 0.88 (s, 18 H), 0.59 (s, 3 H), 0.16 (d, $J = 5.0$ Hz, 1 H), 0.06 (s, 12 H); ^{13}C NMR δ 148.3 (C), 140.8 (C), 135.2 (C), 135.0 (CH), 124.3 (CH), 123.1 (CH), 117.8 (CH), 111.2 (CH_2), 91.0 (CH_2), 76.4 (C), 72.0 (CH), 67.5 (CH), 55.0 (CH_3), 54.6 (CH), 46.0 (CH_2), 45.1 (CH_2), 44.8 (CH_2), 44.5 (C) 38.0 (C), 34.3 (CH_2), 29.0 (CH_2), 28.8 (CH_2), 26.1 (CH_3), 25.8, 25.8 (6 × CH_3), 23.0 (CH_2), 22.8 (CH_2), 22.6 (C), 21.1 (CH), 19.4 (CH_2), 18.2 (C), 18.1 (C), 16.7 (CH_3), −4.7 (CH_3), −4.7 (CH_3), −4.8 (CH_3), −5.1 (CH_3); MS (FAB) *m*/*z* 685 ($M^+ + H$, 12), 684 (M^+, 10), 653 ($M^+ - CH_3O$, 9), 133 (100); HRMS (FAB) calcd for $C_{40}H_{69}O_3Si_2$ 653.4785 ($M^+ - CH_3O$), found 653.4787.

A solution of *n*-Bu_4F in THF (2.5 mL, 2.5mmol, 1 M) was added via syringe to a solution of the protected vitamin D analogue (85 mg, 0.12 mmol) in THF (3 mL). After stirring in the dark at rt for 12 h, a saturated solution of NH_4Cl (10 mL) was added. The

resulting mixture was extracted with Et_2O (3 × 15 mL). The resulting organic phase was dried, filtered, and concentrated. The residue was dissolved in deoxygenated MeOH (3 mL) and treated with cationic resin AG 50W-X4 (1 g, prewashed with MeOH). The mixture was stirred at rt for 5 h in the dark. Filtration and concentration gave a residue which was purified by flash chromatography (60% EtOAc/hexanes) to afford vitamin D analogue **6a** [45 mg, 88%, $R_f = 0.4$ (80% EtOAc/hexanes), white solid]. ^{1}H NMR (CD_3OD) δ 6.34 and 6.14 (2 d, AB system, $J = 11.1$ Hz, 2 H), 5.52 (dt, $J = 15.2$ and 7.5 Hz, 1 H), 5.31 (br s, 1 H), 5.13 (dd, $J = 15.2$ and 8.5 Hz, 1 H), 4.92 (m, 1 H), 4.37 (m, 1 H), 4.14 (m, 1 H), 1.16 (s, 6 H), 0.64 (s, 3 H), 0.19 (br d, $J = 5.0$ Hz, 1 H); ^{13}C NMR (CD_3OD) δ 149.7 (C), 142.4 (C), 136.1 (CH), 135.9 (C), 125.9 (CH), 124.8 (CH), 119.0 (CH), 112.1 (CH_2), 71.5 (CH), 71.5 (C), 67.4 (CH), 55.9 (CH), 48.1 (CH_2), 46.1 (C), 45.7 (CH_2), 43.7 (CH_2), 39.0 (C), 35.5 (CH_2), 30.1 (CH_2), 30.0 (CH_2), 29.0 (CH_3), 28.9 (CH_3), 24.1 (CH_2), 24.0 (CH_2), 22.2 (CH), 20.0 (CH_2), 17.2 (CH_3); MS (FAB) *m*/*z* 413 ($M^+ + H$, 4), 412 (M^+, 8), 394 ($M^+ - H_2O$, 8), 286 (22), 137 (100); HRMS (FAB) calcd for $C_{27}H_{40}O_3$ 412.2977 (M^+), found 412.2971.

Acknowledgment. This work was supported by the Spanish MEC (Projects SAF2001-3187 and SAF2004-1885). R.R. and A.R. thank the Xunta de Galicia and MEC for the respective "Isidro Parga Pondal" and "Ramón y Cajal" contracts. We also thank CESGA for computing time, Solvay Pharmaceuticals BV (Weesp, The Netherlands) for the gift of starting materials and Dr. Y. Fall for his previous contributions to this project. The cover was designed by Rita Sigüeiro.

Supporting Information Available: General experimental methods and experimental procedures for the preparation of compounds **13b**, **19b**, **21b**, **22b**, **23b**, **24b**, **25b**, **27a**, **10a**, **7a**, **28a**, **11a**, **8a**, **26b**, **9b**, **6b**, **27b**, **10b**, **7b**, **28b**, **11b**, and **8b**, including spectroscopic and analytical data. Copies of NMR spectra for compounds prepared. This material is available free of charge via the Internet at http://pubs.acs.org.

JO0625195