of the mice in the MES test. Neurological impairment was measured in mice using the horizontal screen (tox) text.[14] Previously trained mice were dosed with the compound and then placed individually on top of a square (13 × 13 cm) wire screen (no. 4 mesh), which was mounted on a vertical rod. The rod was then rotated 180°, and the number of mice that returned to the top of the screen within 1 min was determined. To determine $ED_{50}$ values, a dose-response curve was determined at the time of peak anticonvulsant activity with at least three to four doses and 12 mice per dose. The $TD_{50}$ is the estimated dose that impaired 50% of the mice.

**Acknowledgment.** We thank E. E. Beedle, Dennis Thompson, and R. Lawson (Lilly Research Laboratories) for their expert technical assistance.

**Registry No.** **3a**, 133873-19-5; **3b**, 133873-25-3; **3c**, 133873-26-4; **3d**, 133873-20-8; **3e**, 133873-27-5; **3f**, 133873-22-0; **3g**, 133873-28-6; **3h**, 133873-29-7; **3i**, 133873-30-0; **3j**, 133873-31-1; **3k**, 133873-32-2; **3l**, 133873-33-3; **3m**, 133873-34-4; **3n**, 133873-35-5; **3o**, 133873-36-6; **3p**, 133873-37-7; **3q**, 133873-38-8; **3r**, 133873-39-9; **3s**, 133873-23-1; **3t**, 117422-80-7; **3u**, 133873-40-2; **3v**, 133873-41-3; **3w**, 133873-24-2; **3x**, 133887-00-0; **3y** (diastereomer 1), 133873-42-4; **3y** (diastereomer 2), 133886-68-7; **3z**, 133873-43-5; **5**, 124421-42-7; **6a**, 133873-08-2; **6b**, 133873-09-3; **6c**, 133873-10-6; **6d**, 133873-11-7; **6e**, 133873-12-8; **6g**, 133873-13-9; **6h**, 133873-14-0; **6k**, 133873-15-1; **6m**, 133873-16-2; **6n**, 133873-17-3; **7**, 133873-18-4; **8** (diastereomer 1), 133873-45-7; **8** (diastereomer 2), 133873-46-8; $PhNH_2$, 62-53-3; $PhCH_2NH_2$, 100-46-9; $PhNHNH_2$, 100-63-0; $H_2NNHCOOCH_2Ph$, 5331-43-1; NaOPh, 139-02-6; MeSH, 74-93-1; EtSH, 75-08-1; PhSH, 108-98-5; (±)-$AcNHCH(NHCH_2Ph)CONHCH_2Ph$, 133873-44-6; morpholine, 110-91-8; 3-aminopyrazole, 1820-80-0; isoxazolidine hydrobromide, 111780-15-5.

# Arocalciferols: Synthesis and Biological Evaluation of Aromatic Side-Chain Analogues of 1α,25-Dihydroxyvitamin $D_3$[1a]

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Aromatic side-chain analogues (arocalciferols **6–9**) of the steroid hormone 1α,25-dihydroxyvitamin $D_3$ (**1**) were synthesized and biologically evaluated. The analogues were prepared by coupling the vitamin D A-ring enyne **14** with the appropriate enol triflate of a modified CD steroid fragment of the type **22**. The resulting dienyne **23** was then transformed in three steps to the vitamin D analogues **6–9**. Biological evaluation of these analogues have provided information concerning side-chain topographical effects on in vivo and in vitro activity.

## Introduction

The steroid hormone 1α,25-dihydroxyvitamin $D_3$ [**1**, $1,25(OH)_2D_3$], the physiologically active form of vitamin D (calciferol), is considered to be one of the most potent stimulators of calcitropic effects (intestinal calcium absorption, ICA, and bone calcium mobilization, BCM).[2] Besides its traditional role as a hormone in calcium homeostasis,[3] $1,25(OH)_2D_3$ induces differentiation and affects cellular proliferation, indicating its possible use in the treatment of certain cancers and skin disorders.[4-6] There has accordingly been an increased interest in the further development of $1,25(OH)_2D_3$ analogues for these new therapeutic purposes.

The clinical utility of $1,25(OH)_2D_3$ is limited because therapeutically effective doses induce hypercalcemia.[7] This has led investigators toward the development of an analogue with a more useful therapeutic index, specifically directed toward analogues with high cell differentiating ability and low calcitropic action. A series of remarkably diverse side-chain analogues of $1,25(OH)_2D_3$ exhibiting promising therapeutic indices have in fact been reported

(1) (a) Paper 39 in the series Studies of Vitamin D (Calciferol) and Its Analogues. For paper 38, see: Enas, J. D.; Shen, G.-Y.; Okamura, W. H. *J. Am. Chem. Soc.* **1991**, *113*, 3873. (b) Department of Chemistry, University of California, Riverside (UCR). (c) Division of Biomedical Sciences, UCR. (d) Department of Biochemistry, UCR. (e) Department of Medicine, Cedars-Sinai Medical Center, University of California, Los Angeles.

(2) (a) Norman, A. W. *Vitamin D, the Calcium Homeostatic Steroid Hormone*; Academic Press: New York, 1979. (b) For a description of recent advances in the field including leading references, see: Norman, A. W., Schaeffer, K., Grigoleit, H.-G., Herrath, D. V., Eds.; *Vitamin D: Molecular, Cellular and Clinical Endocrinology*; Walter de Gruyter and Co.: Berlin, 1988. (c) Pardo, R.; Santelli, M. *Bull. Chim. Soc. Fr.* **1985**, 98. (d) Jones, G. (guest editor). *Steroids* **1987**, *49*, 1-196. (e) Ikekawa, N. *Med. Res. Rev.* **1987**, *7*, 333.

(3) (a) Minghetti, P. P.; Norman, A. W. *FASEB J.* **1988**, *2*, 3043. (b) Carson-Jurica, M. A.; Schrader, W. T.; O'Malley, B. W. *Endocrine Rev.* **1990**, *11*, 201. (c) The amino acid sequence of the human $1,25(OH)_2D_3$ receptor has been determined: Baker, A. R.; MacDonnell, D. P.; Hughes, M.; Crisp, T. M.; Mangelsdorf, D. J.; Haussler, M. R.; Pike, J. W.; Shine, J.; O'Malley, B. W. *Proc. Natl. Acad. Sci. U.S.A.* **1988** *85*, 3294.

(4) (4) Differentiation of leukemia cells: (a) Honma, Y.; Hozumi, M.; Abe, E.; Konno, K.; Fukushima, M.; Hata, S.; Nishii, Y.; DeLuca, H. F.; Suda, T. *Proc. Natl. Acad. Sci. U.S.A.* **1983**, *80*, 201. (b) Koeffler H. P.; Amatruda, T.; Ikekawa, N.; Kobayashi, Y.; DeLuca, H. F. *Cancer Res.* **1984**, *4*, 5624. (c) Munker, R.; Norman, A. W.; Koeffler, H. P. *J. Clinical Invest.* **1986**, *78*, 424. Differentiation of skin cells (psoriasis treatment). (d) MacLaughlin, J. A.; Gange, W.; Taylor, D.; Smith, E.; Holick, M. F. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 5409. (e) Morimoto, S.; Onishi, T.; Imanaka, S.; Yukawa, H.; Kosuka, T.; Kitano, Y.; Yoshikawa, Y. *Calcif. Tissue. Int.* **1986**, *38*, 119.

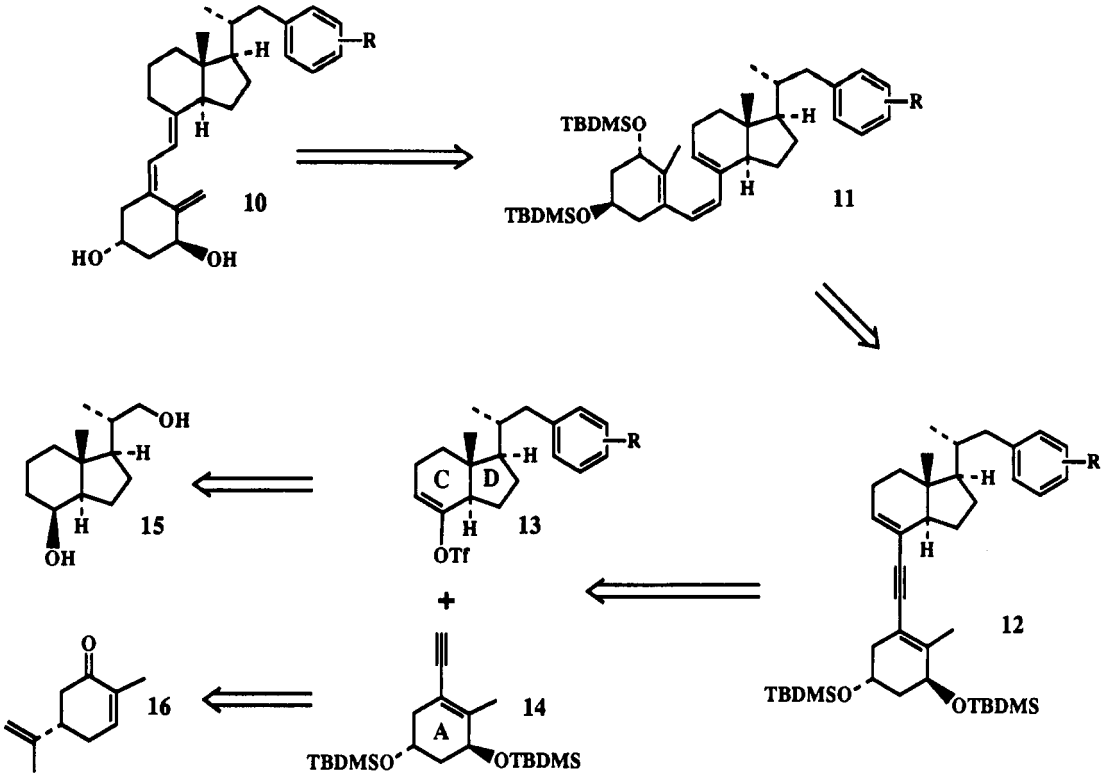
(5) (a) Abe, E.; Miyaura, C.; Sakagami, H.; Takeda, M.; Konno, K.; Yamazaki, T.; Yoshiki, S.; Suda, T. *Proc. Natl. Acad. Sci. U.S.A.* **1981**, *78*, 4990. (b) Colston, K.; Colston, M. J.; Feldman, D. *Endocrinology* **1981**, *108*, 1083.

(6) Shiina, Y.; Abe, E.; Miyaura, C.; Tanaka, H.; Yamada, S.; Ohnori, M.; Nakayama, K.; Takayama, H.; Matsuyama, I.; Nishii, Y.; DeLuca, H. F.; Suda, T. *Arch. Biochem. Biophys.* **1983**, *220*, 90-94. (b) Shiina, Y.; Miyaura, C.; Tanaka, H.; Abe, E.; Yamada, S.; Yamamoto, K.; Inoe, E.; Takayama, H.; Matsumaya, I.; Nishii, Y.; Suda, T. *J. Med. Chem.* **1985**, *28*, 1153-1158.

(7) Koeffler, H. P.; Hirji, K.; Itri, L.; The Southern California Leukemia Group *Cancer Treat. Rep.* **1985**, *69*, 1399.

0022-2623/91/1834-2452$02.50/0 

Scheme I




and they include 1α,25(*S*),26-trihydroxy-22,23-didehydrovitamin $D_3$ (**2**),[8] 16,17,23,23,24,24-hexadehydro-1α,25-dihydroxyvitamin $D_3$ (**3**),[9] 1α,25-dihydroxy-22-oxavitamin $D_3$ (**4**),[10] and 1α,24(*R*)-dihydroxy-22,23,26,27-tetradehydrovitamin $D_3$ (**5**).[11] One facet of the mode of action of these steroids leading to cellular differentiation is thought to entail their binding to an intracellular receptor followed by a genomic response characteristic of other steroid hormones.[3] The diversity of side-chain structural units in analogues **2–5** in comparison to the native hormone $1,25(OH)_2D_3$ makes difficult an assessment of intelligible structure–activity correlations needed in developing an understanding in their mode of action and possibly in the design of yet more effective analogues.

Unlike the other more classical, mammalian steroid hormones, which bear truncated side chains (progesterone, cortisol and aldosterone) or no side chain at all (estradiol and testosterone), $1,25(OH)_2D_3$ is unique because it possesses the fully intact, conformationally flexible eight-carbon side chain characteristic of cholesterol itself. As exemplified by the analogues **2–5**, the side-chain structure is clearly a crucial factor in the development of structure–function concepts in this area. The side chain of $1,25(OH)_2D_3$ may assume any number of conformational orientations and it is hardly possible to predict at this juncture how $1,25(OH)_2D_3$ binds to its putative receptor. We have accordingly been prompted to direct our efforts toward the synthesis of side-chain analogues of $1,25(OH)_2D_3$ possessing more rigid structural units. In order to develop a better understanding of the structure–activity correlation as a function of the spatial positioning of the 25-hydroxyl moiety and the remainder of the molecule, we have synthesized new vitamin D analogues possessing a rigid side chain in the form of an aromatic ring. By analogy with a family of retinoid (vitamin A) analogues known as the arotinoids,[12] we refer to this class of compounds as the arocalciferols (**6–9** as shown in Chart I). In addition, the results of in vitro and in vivo biological evaluation of these analogues are described.

## Results and Discussion

**General Synthetic Approach.** The synthesis of the arocalciferols **6–9** (depicted as general structure **10**) follows the general route shown in Scheme I developed by Lythgoe as modified by Mouriño and Castedo.[13] This route has become particularly efficacious because of the ready availability of the Inhoffen–Lythgoe diol **15** (the CD fragment)[14] and the A-ring enyne **14**.[15,16] Our newly developed synthesis of **14** from (*S*)-carvone (**16**) now renders this route particularly efficacious.[16]

(8) Wovkulich, P. M.; Batcho, A. D.; Baggiolini, E. G.; Boris, A.; Truitt, G.; Uskokovic, M. R. *Vitamin D: Chemical, Biochemical and Clinical Update*; Norman, A. W., Schaefer, K., Grigoleit, H.-G., Herrath, D. v., Eds.; W. de Gruyter: Berlin, 1985; p 755.

(9) Zhou, J. Y.; Norman, A. W.; Collins, E.; Lubbert, M.; Uskokovic, M. R.; Koeffler, H. P. *Blood* **1989**, *74*, 82.

(10) Abe, J.; Morikawa, M.; Miyamoto, K.; Kaiho, S.; Fukushima, M.; Miyaura, C.; Abe, E.; Suda, T.; Nishii, Y. *FEBS Lett.* **1987**, *226*, 58.

(11) Calverley, M. J. *Tetrahedron* **1987**, *43*, 4609.

(12) Bollag, W. *Cancer Chemother. Pharmacol.* **1981**, *7*, 27.

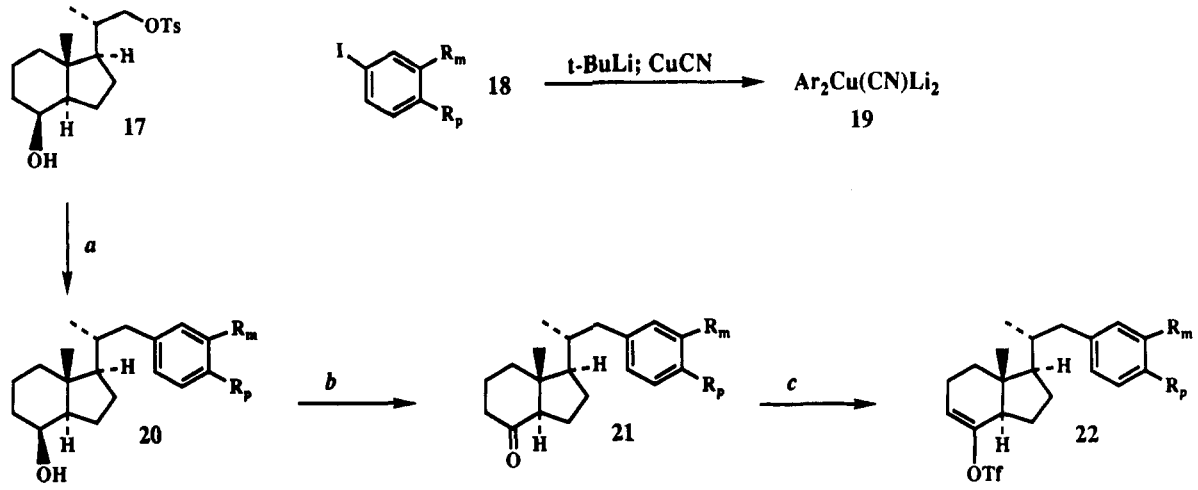
(13) (a) Castedo, L.; Mouriño, A.; Sarandeses, L. A. *Tetrahedron Lett.* **1986**, *27*, 1523. (b) Cacchi, S.; Morera, E.; Ortar, G. *Synthesis* **1986**, 320.

(14) (a) Lythgoe, B.; Roberts, D. A.; Waterhouse, I. *J. Chem. Soc. Perkin Trans. I* **1977**, 2608. (b) Leyes, G. A.; Okamura, W. H. *J. Am. Chem. Soc.* **1982**, *104*, 6099. (c) Leyes, G. A. Ph.D. Dissertation, University of California, Riverside, CA, 1981. (d) Sardina, F. J.; Mourino, A.; Castedo, L. *J. Org. Chem.* **1986**, *51*, 1264. (e) Barrack, S. A.; Gibbs, R. A.; Okamura, W. H. *J. Org. Chem.* **1988**, *53*, 1790.

(15) (a) Harrison, R. G.; Lythgoe, B.; Wright, P. W. *J. Chem. Soc., Perkin Trans. I* **1974**, 2654. (b) Castedo, L.; Mascarenas, J. L.; Mourino, A. *Tetrahedron Lett.* **1987**, *28*, 2099. (c) Baggiolini, E. G.; Hennessy, B. M.; Iacobelli, J. M.; Uskokovic, M. R. *Tetrahedron Lett.* **1987**, *28*, 2095. (d) Desmaele, D.; Tanier, S. *Tetrahedron Lett.* **1985**, *26*, 4941.

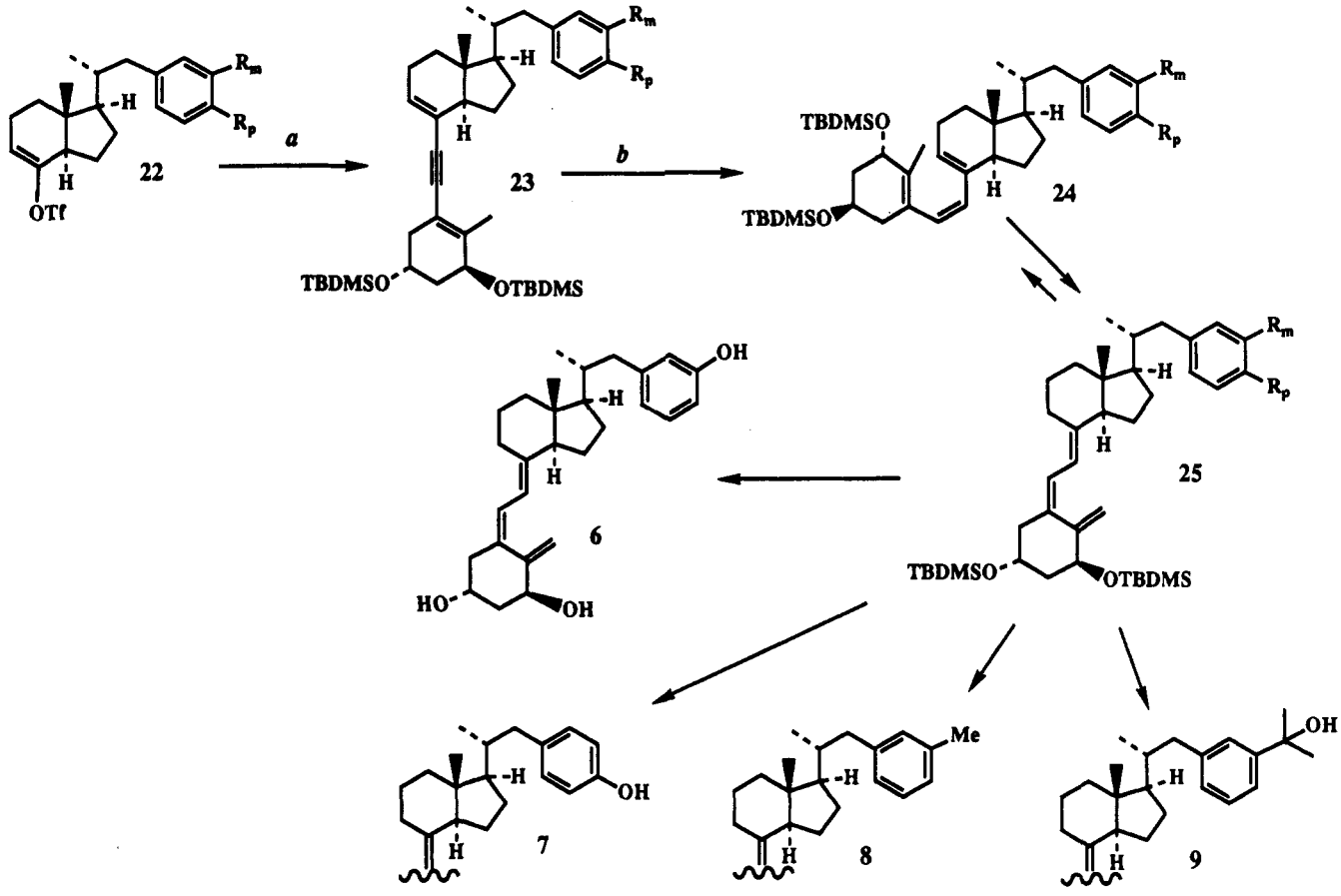
(16) Okamura, W. H.; Aurrecoechea, J. M.; Gibbs, R. A.; Norman, A. W. *J. Org. Chem.* **1989**, *54*, 4072.

**Scheme II**[a,b]




[a] (a) $R_m$ = OTBDMS, $R_p$ = H; (b) $R_m$ = H, $R_p$ = OTBDMS; (c) $R_m$ = Me, $R_p$ = H; (d) $R_m$ = -$CMe_2$(OTMS), $R_p$ = H. [b] Reagents: (a) 2 equiv of $Ar_2Cu(CN)Li_2$ (19), 65 °C, 20 h; (b) PDC, $CH_2Cl_2$, room temperature, 20 h; (c) LDA, THF, −50 °C, 8 h; $PhN(Tf)_2$, 0 °C, 12 h.

**Scheme III**[a,b]




[a] (a) $R_m$ = OTBDMS, $R_p$ = H; (b) $R_m$ = H, $R_p$ = OTBDMS; (c) $R_m$ = Me, $R_p$ = H; (d) $R_m$ = -$CMe_2$(OTMS), $R_p$ = H. [b] Reagents: (a) 14, $Pd(PPh_3)_2Cl_2$, CuI, $Et_2NH$, DMF, room temperature, 2 h; (b) $H_2$, Lindlar cat., EtOAc, room temperature, 1 h; isooctane, 98 °C, 2 h; TBAF, THF, room temperature, 12 h.

**Synthesis of the Arocalciferols 6–9.** The preparation of the requisite CD fragments **13** is outlined in Scheme II. The known tosylate **17**, readily prepared from the Inhoffen–Lythygoe diol **15**,[14] was reacted directly with an excess of the Lipshutz-type higher order mixed cuprate **19**[17] to afford the CD alcohols **20**. The latter was then transformed in two steps to the enol triflates **22** (general structure **13**).[18] The overall yield of **22a**, **22b**, **22c**, and **22d** (three steps from **17**) was 57%, 34%, 32%, and 30% respectively.

Scheme III depicts the remaining steps of the sequence leading to the arocalciferols **6–9**. The A-ring enyne **14** was coupled with each of the CD enol triflates **22** to afford their respective protected dienynes **23**.[19] Without isolation of

(17) Lipshutz, B. H.; Wilhelm, R. S. *J. Am. Chem. Soc.* **1981**, *103*, 7672.

(18) For a review on the preparation of triflates, see: Stang, P. J.; Hanack, M.; Subramanian, L. R. *Synthesis* **1980**, 85.

**Table I.** Effect of Vitamin D Analogues on Intestinal Calcium Absorption, Bone Calcium Mobilization in the Rachitic Chick, and Relative Competitive Indices

| lab code | name | intestinal $Ca^{2+}$ absorption (ICA) ratio[a] $[1,25(OH)_2D_3]$/ [analogue dose] × 100 | bone $Ca^{2+}$ mobilization (BCM) ratio[a] $[1,25(OH)_2D_3]$/ [analogue dose] × 100 | relative competitive index[b] (RCI) chick int. | HL-60 | $RCI_{DBP\text{-}1,25\text{-}D}$ |
|---|---|---|---|---|---|---|
| 1 (C) | $1,25(OH)_2D_3$ | 100 | 100 | 100 | 100 | 100 |
| 7 (DF) | *p*-hydroxyphenyl analogue | 0.04 | 0.08 | 5 ± 1 | 12 | 1980 |
| 6 (DE) | *m*-hydroxyphenyl analogue | 0.28 | 1.0 | 28 ± 1 | 27 | 980 |
| 9 (EV) | *m*-(dimethylhydroxymethyl)phenyl analogue | 30.7 | 7.7 | 62 ± 6 | 32 ± 6 | 2 |
| 8 (ET) | *m*-methylphenyl analogue | 0.31 | 0.06 | 1.4 ± 2 | 3.5 ± 4 | ND |

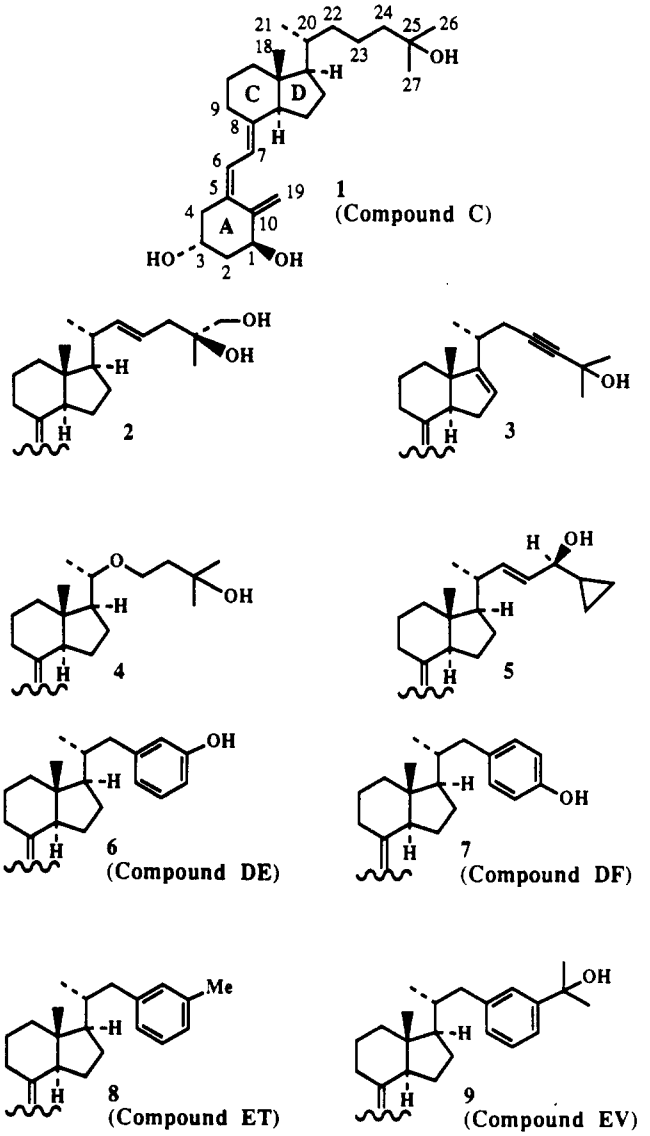
[a] A range of doses for each analogue and the standard $1,25(OH)_2D_3$ was administered (see Experimental Section) so as to achieve a standard curve of ICA or BCM responses. The dose of each analogue required to achieve either an ICA or BCM response equivalent to that of 100 pmol of $1,25(OH)_2D_3$ was determined and the indicated ratios were calculated and are reported in this table. [b] Standard deviations are given for those that are the average of three determinations; in their absence, the RCI value was measured only once.

intermediates, each of the dienynes **23** was subjected to semihydrogenation, thermolysis, and then deprotection as shown to afford the arocalciferols **6–9**. The overall yield of **6**, **7**, **8**, and **9** (4 steps from **22** or **14**) was 51%, 27%, 26%, and 30%, respectively. The limiting factor in the latter sequence is the capriciousness of the semihydrogenation step (**23** to **24**), not an uncommon observation for this type of transformation. However, it should be noted that the incorporation of this alkyne to *cis*-alkene transformation at a late stage in the synthesis provides the possibility of isotope label incorporation, an important factor in the study of biomolecules. The palladium-catalyzed cross-coupling procedure[19] leading to **23** typically occurs in at least 80% yield without extensive optimization studies.

The analogues **6–9** were characterized by $^1H$ NMR[20] and UV spectroscopy and by mass spectrometry. In their UV spectra (ethanol), calciferols typically exhibit a $\lambda_{max}$ in the region near 264 nm with an extinction coefficient ($\epsilon$) of ~18 800.[21] The arocalciferols **6**, **7**, **8**, and **9** exhibited $\lambda_{max}$ ($\epsilon$) values of 268 (20 600), 266 (20 000), 266 (19 200), and 266 nm (19 500), respectively. The latter values, because of the superposition of the aromatic ring chromophore, are, not unexpectedly, shifted from the typical chromophore of the calciferols with unperturbed chromophores.[21] In the mass spectrum of vitamin D, the major characteristic fragment includes as a base peak the lower half of the molecule resulting from $C_{7,8}$ cleavage together with fragmentation peaks resulting from $H_2O$ loss from this dominant fragment.[22] For the arocalciferols **6–9**, as well as for $1,25(OH)_2D_3$ (**1**), which possesses an identical A ring, a base peak at $m/z$ 152 or 134 (152 less $H_2O$) should be expected. Although all four arocalciferols exhibited peaks at these two nominal masses, they do not dominate the mass spectral fragmentation pattern as they do for **1**. Before spectral characterization and biological assay, each of the calciferols was subjected to HPLC purification to homogeneity and the analogues and the unsaturated precursors were routinely stored under argon in solution (ether or ethanol) or as a neat residue in a low temperature freezer.

**Biological Studies.** It should be noted that letter codes have been assigned in these laboratories to all vitamin D compounds as they are biologically evaluated as a convenience to our collaborators. Thus, letter codes [**6**, DE; **7**, DF; **8**, ET; and **9** EV] as defined in Chart I will also be used in much of the ensuing discussion. The results of in vivo biological evaluation of the four arocalciferol analogues in the standard rachitic chick assay are given in Table I.[23] The results are reported as the percentage of activity ob-

**Chart I**




(19) (a) Sonogashira, K.; Tohda, Y.; Hagihara, N. *Tetrahedron Lett.* **1975**, 4470. (b) See also, ref 13b.

(20) See, for leading references: (a) Wing, R. M.; Okamura, W. H.; Rego, A.; Pirio, M. R.; Norman, A. W. *J. Am. Chem. Soc.* **1975**, *97*, 4980. (b) Helmer, B.; Schnoes, H. K.; De Luca, H. F. *Arch. Biochem. Biophys.* **1985**, *241*, 608.

(21) Baggiolini, E. G.; Iacobelli, J. A.; Hennessy, B. M.; Batcho, A. D.; Sereno, J. F.; Uskokovic, M. R. *J. Org. Chem.* **1986**, *51*, 3098.

(22) For leading references, see: Okamura, W. H.; Hammond, M. L.; Jacobs, H. J. C.; Thuijl, J. v. *Tetrahedron Lett.* **1976**, 4807.

(23) (a) Hibberd, K. A.; Norman, A. W. *Biochem. Pharmacol.* **1969**, *18*, 2347. (b) Wilhelm, F.; Dauben, W. G.; Kohler, B.; Roesle, A.; Norman, A. W. *Arch. Biochem. Biophys.* **1984**, *233*, 127.

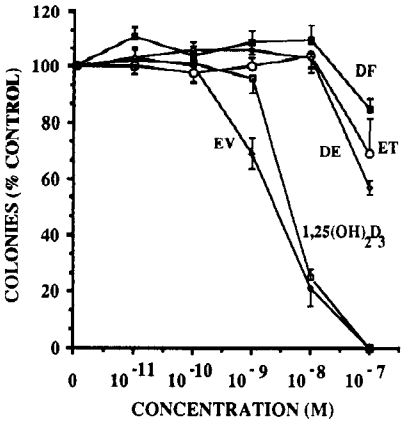



**Figure 1.** Dose–response of vitamin D analogues on clonal proliferation of leukemic cells from HL-60 line. Results are expressed as a percent of control plates not exposed to vitamin D compounds. (Control cultures contain a mean of 123 colonies ± 12 SE). Each point represents a mean of three experiments with triplicates dishes: C (1, $1,25(OH)_2D_3$), EV (9), ET (8), DE (6), DF (7).

served for both ICA and BCM in relation to a standard dose of 100 pmol of $1,25(OH)_2D_3$. Only analogue EV (9) displayed significant activity, 31% for ICA and 6% for BCM; the other analogues had ICA and BCM values that were significantly lower, i.e. ≤0.37.

Table I also summarizes the relative competitive index (RCI) values for binding to the chick intestinal receptor, the human HL-60 cell receptor, and the human plasma D-binding protein.[24] With respect to the $1,25(OH)_2D_3$ receptors (chick and HL-60), all four analogues had a readily measurable level of affinity; the rank ordered RCI for both the chick intestinal $1,25(OH)_2D_3$ and HL-60 receptors was EV > DE > DF > ET (9 > 6 > 7 > 8).

It is apparent that the structural modifications incorporated into analogues EV (9) and DE (6) were better able to satisfy the ligand binding properties of the $1,25(OH)_2D_3$ receptor than those provided by analogues DF (7) and ET (8). It is understandable that analogue ET (8) should have the lowest RCI since it possesses no side-chain hydroxyl group. It is noteworthy that the presence of the side-chain aromatic group particularly in the meta orientation of the hydroxyl groups, as in analogues EV (9) and DE (6), is well tolerated by the $1,25(OH)_2D_3$ receptor.

Table I also reports the relative ability of the four analogues to compete with tritiated $1,25(OH)_2D_3$ for binding to the human plasma D-binding protein (DBP).[25] It is known that the ligand binding domain of the DBP is different from that of receptors for $1,25(OH)_2D_3$; thus the optimal ligand for DBP is $25(OH)D_3$ while for the $1,25(OH)_2D_3$ receptor it is $1,25(OH)_2D_3$. The $RCI_{DBP}$ values in Table I are normalized such that the 0.15 value for $1,25(OH)_2D_3$ [in relation to the RCI = 100 value for $25(OH)D_3$] is set to 100. The $RCI_{DBP\text{-}1,25\text{-}D}$ results for the four analogues fell into two significantly different groups. The hydroxyphenyl side-chain analogues DE (6) and DF (7) had $RCI_{DBP\text{-}1,25\text{-}D}$ 10–20× greater than that of $1,25(OH)_2D_3$ while the (dimethylhydroxymethyl)phenyl analogue EV (9) had an $RCI_{DBP\text{-}1,25\text{-}D}$ only 2% that of $1,25(OH)_2D_3$. The reason for these dramatic differences is not known and will be the subject of future evaluation.

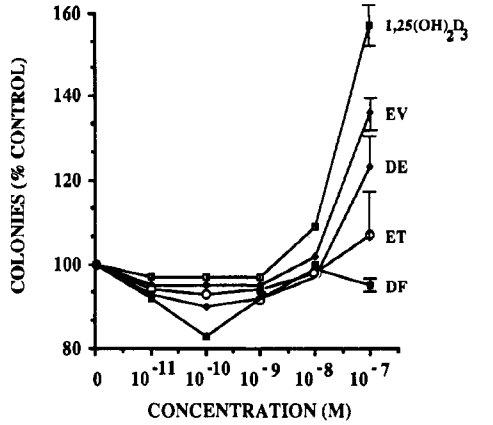



**Figure 2.** Dose–response of vitamin D analogues on the clonal growth of myeloid stem cells from normal individuals. Human bone marrow mononuclear cells were cultured in soft agar; colonies were counted on day 10 and each point represents the mean number of colonies per triplicate cultures performed three times. (Control cultures contained a mean of 74 ± 11 SE). Results are expressed as a percent of control cells not exposed to vitamin D compound: C (1, $1,25(OH)_2D_3$), EV (9), ET (8), DE (6), DF (7).

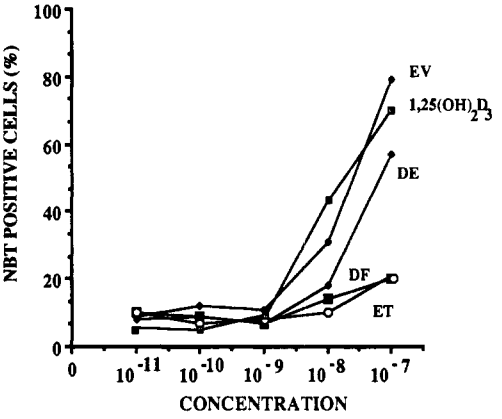



**Figure 3.** Dose–response of vitamin D analogues on differentiation of HL-60 cells. Results are expressed as a percentage of HL-60 cells that reduce nitroblue tetrazolium (NBT). Each point represents the mean of three experiments: C (1, $1,25(OH)_2D_3$), EV (9), ET (8), DE (6), DF (7).

Dose–response effects of the various vitamin D analogues on the clonogenic proliferation of HL-60 promyelocytes are shown in Figure 1.[26] The most potent analogue was EV (9) which inhibited 50% growth ($ED_{50}$) at $3 \times 10^{-8}$ M. The other vitamin $D_3$ analogues DE (6), ET (8), and DF (7) were less potent and did not achieve an $ED_{50}$.

Normal human myeloid clonogenic cells (GM-CFC) were slightly stimulated in their clonal growth by $1,25(OH)_2D_3$ and EV (9) and to a lesser extent by DE (6) (Figure 2). At $10^{-7}$ M, enhancement of clonal growth was about 50% and 40% for $1,25(OH)_2D_3$ and EV (9), respectively. In the absence of GM-CSF (granulocyte–monocyte–colony stimulating factor), none of the analogues was able to sustain clonal growth of GM-CFC (data not shown).

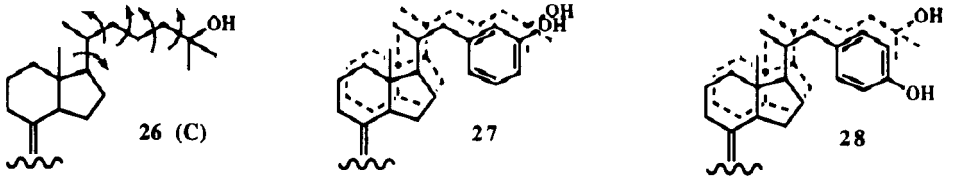
The HL-60 cells differentiate toward macrophages when cultured in the presence of $1,25(OH)_2D_3$.[26] A marker of this cellular differentiation is the ability to reduce nitroblue

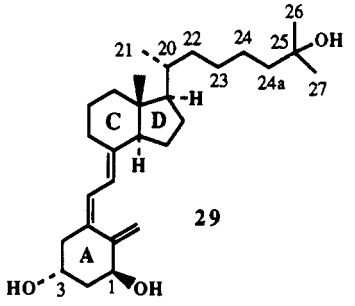
(24) (a) Procsal, D. A.; Okamura, W. H.; Norman, A. W. *J. Biol. Chem.* **1975**, *250*, 8382. (b) Procsal, D. A.; Okamura, W. H.; Norman, A. W. *Am. J. Clinical Nutrition.* **1976** *29*, 1271. (c) Wecksler, W. R.; Norman, A. W. *Methods in Enzymology: Vitamins and Co-Enzymes* **1980**, *67*, 488.

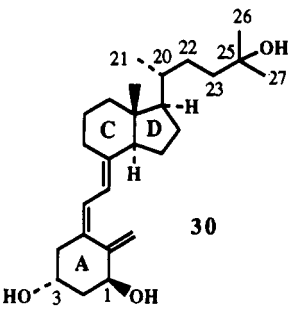
(25) Norman, A. W.; Roberts, P. A. *Methods in Enzymology: Vitamins and Co-Enzymes* **1980**, *67*, 473.

(26) (a) See ref 4c. (b) Mangelsdorf, D. J.; Koeffler, H. P.; Donaldson, C. A.; Pike, J. W.; Haussler, M. R. *J. Cell Biol.* **1984**, *89*, 391. (c) Collins, S. J.; Gallo, R. C.; Gallagher, R. E. *Nature (London)* **1977**, *270*, 347. (d) Tanaka, H.; Abe, C.; Miyaura, T.; Kuribayashi, K.; Konno, K.; Nishii, Y.; Suda, T. *Biochem. J.* **1984**, *204*, 713.

**Chart II**




tetrazolium (NBT). We examined the ability of the $1,25(OH)_2D_3$ analogues to induce differentiation of the HL-60 cells (Figure 3). The most potent inducer of differentiation was $1,25(OH)_2D_3$ ($ED_{50} = 1 \times 10^{-8}$ M) (data not shown). EV (**9**) and DE (**6**) had $ED_{50}$ of $3 \times 10^{-8}$ M and $1 \times 10^{-7}$ M, respectively, while DF (**7**) and ET (**8**) had almost no effect on differentiation.

In this study of several novel $1,25(OH)_2D_3$ analogues EV (**9**) had nearly the same potency as $1,25(OH)_2D_3$ in the ability either to induce differentiation or to inhibit proliferation of HL-60 leukemic cells. Nevertheless, this compound was not toxic to the proliferation of normal human myeloid stem cells; in fact, it was slightly stimulatory to their proliferation. We[26b] and others[26d] have previously shown that the HL-60 cells have about 4000 receptors for $1,25(OH)_2D_3$ per cell with a $K_d$ for the ligand in the range of $5 \times 10^{-9}$ M. Further studies showed that the ability of the $1,25(OH)_2D_3$ analogues to bind to $1,25(OH)_2D_3$ receptors in general paralleled their ability to induce differentiation of HL-60 cells, which is consistent with these compounds, including presumably EV (**9**) to mediate their effects through the $1,25(OH)_2D_3$ receptors.[26b,d]

The natural hormone $1,25(OH)_2D_3$ and all of the new analogues investigated possess identical A, seco-B triene, and CD stereostructural features. We found that a major modification of the side chain by insertion of a phenyl ring does not alter the potency of the analogue EV (**9**) to affect growth and differentiation of hematopoietic cells as compared to $1,25(OH)_2D_3$. However, truncating the aliphatic arm by two carbons decreases activity [DE (**6**) and DF(**7**)]. Of interest, the potency was slightly greater when the hydroxyl group of C-22 was in the meta position [DE (**6**)] as compared to the para position [DF (**7**)]. The reason for this is evaluated further below. Also of interest is the observation that DE (**6**) was 400-fold less potent than $1,25(OH)_2D_3$ in ICA and 100-fold less potent than $1,25(OH)_2D_3$ in BCM. In contrast, EV (**9**) was 3-fold less active than $1,25(OH)_2D_3$ in ICA activity and 13-fold less active than $1,25(OH)_2D_3$ in BCM activity. These results suggest that it may be possible to administer higher doses of DE (**6**) than EV (**9**) without causing hypercalcemia and, therefore, therapeutic/toxicity ratios of these two analogues might be comparable.

None of the arocalciferols inhibited the 1-hydroxylase (Table II),[27] indicating that the phenyl group probably

**Table II.** Effect of Arocalciferols on 25-OH-$D_3$-1-hydroxylase Activity

| lab code[a] | name | 25-OH-$D_3$-1-hydroxylase activity: pmol/10 min[b] | 25-OH-$D_3$-1-hydroxylase activity: % control |
|---|---|---|---|
| | | 17.4 ± 1.2 | |
| 1 (C) | $1,25(OH)_2D_3$ | 2.1 ± 0.2 | 12 |
| 7 (DF) | *p*-hydroxyphenyl analogue | 18.1 ± 0.9 | 104 |
| 6 (DE) | *m*-hydroxyphenyl analogue | 16.9 ± 0.8 | 97 |
| 9 (EV) | *m*-(dimethylhydroxymethyl)-phenyl analogue | 17.2 ± 0.4 | 99 |
| 8 (ET) | *m*-methylphenyl analogue | 16.7 ± 1.1 | 96 |

[a] Mean ± SD ($n = 4$). [b] All compounds were added to the reaction mixture at a final concentration of $10^{-6}$ M.

renders the side chain too bulky to be accommodated by the active site of cytochrome P4501. It is interesting to note that of some 20 vitamin D analogues that have been tested for 1-hydroxylase inhibition, this is the first group to be nearly completely inactive in this regard.

**Structure-Activity Relationships.** As indicated earlier, there are a multitude of conformational orientations accessible to the side chain of $1,25(OH)_2D_3$ as depicted schematically by **26** (Chart II). If one considers all of the possible skew orientations (~60° dihedral angles about the five single bonds indicated in **26**),[28] 172 reasonable conformational permutations of the side are feasible. Energy minimization of this set reveals that 41, 11, and 7 of these conformations are within 3, 2, and 1 kcal/mol, respectively, of the conformer with the lowest energy. It is not worthwhile to depict these conformers here, but suffice it to say that the actual conformational orientation of the side chain of $1,25(OH)_2D_3$ bound to its receptor is hardly clarified with this kind of analysis. Although the analysis represents a convenient bookkeeping of lower energy conformational orientations of the side chain, at this stage any one of these topographical orientations, and quite possibly others (e.g., even partially eclipsed side-chain orientations), may represent the specific

(27) (a) Henry, H. L.; Amdahl, L. D. *J. Steroid Biochem.* **1984**, *20*, 645. See also: (b) Henry, H. L. *J. Biol. Chem.* **1979**, *254*, 2722.

(28) We are grateful to Dr. J. A. Palenzuela for carrying out these computations using PC Model (Serena Software) and related programs.

manner in which the side chain of $1,25(OH)_2D_3$ bound to receptor is oriented.

Accordingly, a considerably more simplistic view (27 and 28) can be envisaged. The study was directed toward examining the meta isomer DE (6) in order to ask the question whether its hydroxyl group could mimic the side chain of $1,25(OH)_2D_3$ in its extended form as depicted in the overlayed structure 27. As reference, compounds for biological comparison to DE (6), namely, DF (7) and ET (8), were also synthesized. As shown in overlay 28, the *p*-hydroxy substrate DE (6) would possess its hydroxyl shifted to a pseudo 26-position with respect to the extended side chain of $1,25(OH)_2D_3$. The toluyl analogue ET (8), which is spatially similar to DE (6) (except that the aromatic hydroxyl is replaced by a methyl group), was prepared to assess the ability of the aromatic ring alone to influence the ability of this new family of analogues to bind to the receptor for $1,25(OH)_2D_3$. Finally, the analogue EV (9) bearing a tertiary hydroxyl group moiety identical with the side chain end group in $1,25(OH)_2D_3$ was studied. Even though the side chain in EV (9) is more analogous to 24a-homo-$1,25(OH)_2D_3$ (29),[29] it was reasoned that its side chain might more closely resemble that of $1,25(OH)_2D_3$ because of the presence of the three $sp^2$ carbons in the side chain of EV (9). That is, contrary to the repsentation of the schematic overlay in 27, the shortening of the side chain as a consequence of the shorter bond lengths due to the $sp^2$ centers may result in attenuated binding and in vivo activity. We have already shown that 24-nor-$1,25(OH)_2D_3$ (30)[30] has much diminished activity compared to $1,25(OH)_2D_3$ and even when compared to 24a-homo-$1,25(OH)_2D_3$ (29).[28] Thus, the analogue EV (9), the most active of the new compounds studied, may be considered to possess a side-chain-length intermediate between $1,25-(OH)_2D_3$ and 29, whereas DE (6), the second most active substrate examined, may be considered to possess a side-chain-length intermediate between $1,25-(OH)_2D_3$ and the least active nor analogue 30.

## Experimental Section

**General.** The $^1H$ nuclear magnetic resonance spectra ($^1H$ NMR) were obtained on a 300-MHz GE QE-300 spectrometer with deuteriochloroform ($CDCl_3$, Aldrich 99.8% D) as solvent and internal standard (tetramethylsilane was also used). The chemical shifts are given in $\delta$ values and the coupling constants ($J$) in hertz (Hz). The $^{13}C$ nuclear magnetic resonance ($^{13}C$ NMR) spectra were obtained on a 75.5-MHz GE QE-300 spectrometer with $CDCl_3$ as solvent and internal standard. Chemical shifts are given in $\delta$ values. Infrared spectra were obtained on a Perkin-Elmer 283 grating spectrophotometer, using 0.1-mm NaCl plates with carbon tetrachloride ($CCl_4$) as solvent. Ultraviolet spectra were obtained on a HP 8451A diode array UV-vis spectrophotometer with 95% ethanol (EtOH) as solvent. Mass spectra (MS) were obtained on VG-ZAB or VG-7070 instruments.

All experiments involving air- and/or moisture-sensitive materials were carried out under a nitrogen or argon atmosphere, which was dried prior to use by passage through a column of KOH layered with $CaSO_4$. Tetrahydrofuran, ether, and benzene were distilled from sodium benzophenone ketyl immediately prior to use. Hexanes was distilled from $CaH_2$. High pressure liquid chromatography (HPLC) was performed on a Waters 6000A or 510 pump with a R401 refractive index detector. Flash chromatography was performed on silica gel (Sigma, 230–400 mesh). Thin layer chromatography (TLC) was run on a plastic plate precoated with silica gel (Brinkman, 0.25 mm) and developed by spraying with a ~15% ethanol solution of phosphomolybdic acid.

**22-(*m*-Hydroxyphenyl)-23,24,25,26,27-pentanor-1α-hydroxyvitamin $D_3$ (6, DE).** Dienyne **23a** (26 mg, 0.034 mmol) in 16 mL of EtOAc, 52 mg of Lindlar catalyst, and quinoline (52 μL, 0.107 M in hexanes) were stirred for 1 h at room temperature under a positive pressure of hydrogen. The mixture was passed through a pad of diatomaceous earth and then the filtrate was evaporated to dryness. The residue in isooctane (14 mL) was refluxed for 2 h. The solvent was evaporated, and to the residue were added 0.95 mL of THF and 0.23 mL of a solution of tetrabutylammonium fluoride (1 M in THF). After stirring the mixture at room temperature for 12 h, 2 mL of a saturated solution of NaCl was added. The mixture was extracted four times with EtOAc and the combined organic extracts were dried ($MgSO_4$) and then concentrated to dryness. After filtration of the residue through a pad of silica gel (EtOAc), HPLC purification (Rainin Dynamax, 1 × 25 cm, 8 μm, 4 mL/min, 100% EtOAc) afforded 8.3 mg (63%) of the vitamin D **6** as a colorless, amorphous solid: $^1H$ NMR $\delta$ 0.58 (3 H, $C_{18}$-Me, s), 0.83 (3 H, $C_{21}$-Me, d, $J$ = 6.3 Hz), 2.32 (1 H, dd, $J$ = 6.6 Hz, 13.2 Hz), 2.61 (1 H, dd, $J$ = 1.5 Hz, 13.5 Hz), 2.84 (1 H, apparent dt, $J$ = 2.1 Hz, 12.9 Hz; this signal most likely consists of two doublets both with $J$ = 12.9 Hz assignable to $H_{9\beta}$ and probably one of the two $H_{22}$ protons), 4.24 (1 H, $H_3$, br s), 4.44 (1 H, $H_1$, br s), 4.60 (1 H, ArOH, br s), 5.02 (1 H, $H_{19}$, s), 5.34 (1 H, $H_{19}$, s), 6.04 (1 H, $H_7$, d, $J$ = 11.4 Hz), 6.39 (1 H, $H_6$, d, $J$ = 11.4 Hz), 6.63 (1 H, $ArH_2$, s), 6.64 (1 H, Ar, d, $J$ = 7.5 Hz), 6.71 (1 H, Ar, d, $J$ = 7.5 Hz), 7.13 (1 H, $ArH_5$, t, $J$ = 7.5 Hz); UV (95% EtOH) $\lambda_{max}$ 268 nm ($\epsilon$ 20600); HRMS, $m/z$ 422.2839 (calcd for $C_{28}H_{38}O_3$, 422.2821); MS, $m/z$ 422 (10, M), 404 (base), 386 (12), 363 (3), 349 (2), 334 (2), 315 (4), 297 (6), 269 (10), 251 (8), 227 (6), 195 (9), 159 (15), 155 (12), 152 (7), 134 (31), 107 (85), 91 (34), 79 (25), 67 (16), 55 (23).

**22-(*p*-Hydroxyphenyl)-23,24,25,26,27-pentanor-1α-hydroxyvitamin $D_3$ (7, DF).** A mixture of dienyne **23b** (0.019 g, 0.025 mmol) in ethyl acetate (11 mL), quinoline (0.17 M in hexanes, 0.040 mL, 0.042 mmol), and Lindlar's catalyst (0.040 g) was stirred under an atmosphere of hydrogen for 1 h. After filtration of the mixture through a short pad of silica gel and concentration, the crude residue was purified by HPLC (Rainin Dynamax, 1.0 × 25 cm, 8-μm silica gel column, 0.4% EtOAc/hexanes). The inseparable previtamin and vitamin mixture was dissolved in isooctane (7 mL) and heated to reflux for 2 h, following which the solvent was removed. The residue was dissolved in THF (0.5 mL) and tetrabutylammonium fluoride (1 M in THF, 0.117 mL, 0.117 mmol) was added at room temperature. The solution was stirred at 20 °C for 12 h. A saturated solution of NaCl (1 mL) was added and then the mixture was extracted with ethyl acetate (4 × 2 mL). The combined organic extracts were dried ($MgSO_4$) and then concentrated to dryness. The crude material, after passage through a short pad of silica gel (EtOAc), was purified by HPLC (Rainin Dynamax 1.0 × 25 cm, 8 μm, 100% EtOAc) to afford the vitamin **7** (3.6 mg, 34%) as an amorphous, white solid: $^1H$ NMR $\delta$ 0.57 (3 H, $C_{18}$-Me, s), 0.81 (3 H, $C_{21}$-Me, d, $J$ = 6.6 Hz), 2.33 (1 H, dd, $J$ = 13.5 Hz, 6.6 Hz), 2.61 (1 H, dd, $J$ = 13.5 Hz, 2.7 Hz), 2.82 (2 H, apparent dd, $J$ = 13.5 Hz, 2.4 Hz; this signal most likely consists of overlapping doublets assignable to $H_{9\beta}$ and probably one of the $H_{22}$ protons), 4.24 (1 H, $H_3$, m), 4.44 (1 H, $H_1$, m), 5.01 (1 H, $H_{19}$, s), 5.34 (1 H, $H_{19}$, s), 6.03 (1 H, $H_7$, d $J$ = 11.1 Hz), 6.38 (1 H, $H_6$, d, $J$ = 11.1 Hz), 6.74 (2 H, $ArH_{3,5}$, d, $J$ = 8.3 Hz), 6.99 (2 H, $ArH_{2,6}$, d, $J$ = 8.3 Hz); UV (absolute EtOH) $\lambda_{max}$ 266 nm ($\epsilon$ 20000); HRMS $m/z$ 422.2824 (calcd for $C_{28}H_{38}O_3$, 422.2821); MS, $m/z$ 422 (19, M), 404 (15), 386 (25), 363 (8), 348 (8), 320 (3), 297 (9), 279 (5), 241 (6), 223 (7), 197 (12), 157 (16), 155 (12), 152 (3), 134 (32), 107 (base), 95 (14), 81 (13), 71 (14), 57 (15), 55 (26).

**22-*m*-Toluyl-23,24,25,26,27-pentanor-1α-hydroxyvitamin $D_3$ (8, ET).** Dienyne **23c** (0.020 g, 0.0309 mmol) was dissolved

(29) (a) Perlman, K.; Kutner, A.; Prahl, J.; Smith, C.; Inaba, M.; Schnoes, H. K.; DeLuca, H. F. *Biochemistry* **1990**, *29*, 190. (b) Ostrem, V. K.; Tanaka, Y.; Prahl, J.; DeLuca, H. F.; Ikekawa, N. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 2610. (c) Sai, H.; Takatsuto, S.; Ikekawa, N.; Tanaka, Y.; DeLuca, H. F. *Chem. Pharm. Bull* **1986**, *34*, 4508. (d) Ostrem, V. K.; Lau, W. F.; Lee, S. H.; Perlman, K.; Prahl, J.; Schnoes, H. K.; DeLuca, H. F.; Ikekawa, N. *J. Biol. Chem.* **1987**, *262*, 14164. See also: (e) Kutner, A.; Perlman, K. L.; Lago, A.; Sicinski, R. R.; Schnoes, H. K.; DeLuca, H. F. *J. Org. Chem.* **1988**, *53*, 3450. (f) Johnson, R. L.; Carey, S. C.; Norman, A. W.; Okamura, W. H. *J. Med. Chem.* **1977**, *20*, 5.

(30) (a) Reference 29d. See also: (b) Mouriño, A.; Blair, P.; Wecksler, W.; Johnson, R. L.; Norman, A. W.; Okamura, W. H. *J. Med. Chem.* **1978**, *21*, 1025.

in 16 mL of EtOAc, and 50 μL of a solution of quinoline (0.17 M in hexanes) and then Lindlar catalyst (50 mg) were introduced. The mixture was stirred for 1 h under a positive pressure of hydrogen at room temperature and then the mixture was filtered through a short column of silica gel. After concentration of the filtrate, the crude residue was purified by HPLC (Rainin-Dynamax, 1.0 × 25 cm, 8 μm, 0.5% EtOAc/hexanes, 4 mL/min) to afford 0.015 g of the crude vitamin and previtamin mixture. The latter dissolved in 10.5 mL of isooctane was heated at reflux for 2 h. After evaporation of the solvent, the crude residue was dissolved in 0.7 mL of dry THF and 0.17 mL of a solution 1 M of tetrabutylammonium fluoride, and the mixture was stirred at room temperature for 12 h (protected from light). A saturated solution of NaCl (2 mL) was added and then the mixture was extracted with EtOAc. The combined extracts were dried ($MgSO_4$) and then concentrated to dryness. After passing the residue through a short column of silica gel (EtOAc), purification by HPLC (Rainin Dynamax, 1.0 × 25 cm, 8 μm, 100% EtOAc, 4 mL/min) afforded 4.1 mg (32%) of the vitamin 8 as a colorless, amorphous solid: $^1$H NMR δ 0.58 (3 H, $C_{18}$-Me, s), 0.82 (3 H, $C_{21}$-Me, d, $J$ = 6.3 Hz), 2.33 (3 H, ArMe, s), 2.60 (1 H, d, $J$ = 15 Hz), 2.85 (2 H, apparent t, $J$ = 12 Hz; this signal most likely consists of two doublets assignable to $H_{9\beta}$ and probably one of the two $H_{22}$ protons), 4.24 (1 H, $H_3$, m), 4.44 (1 H, $H_1$, m), 5.02 (1 H, $H_{19}$, s), 5.34 (1 H, $H_{19}$, s), 6.04 (1 H, $H_7$, d, $J$ = 11.1 Hz), 6.39 (1 H, $H_6$, d, $J$ = 11.1 Hz), 6.93 (1 H, Ar, d, $J$ = 7.2 Hz), 6.95 (1 H, $ArH_2$, s), 6.98 (1 H, Ar, d, $J$ = 7.8 Hz), 7.15 (1 H, $ArH_5$, t, $J$ = 7.5 Hz); UV (absolute EtOH) $\lambda_{max}$ 266 nm (ε 19 200); HRMS, $m/z$ 420.3002 (calcd for $C_{29}H_{40}O_2$, 420,3028); MS, $m/z$ 420 (11, M), 403 (11), 402 (25), 385 (34), 384 (base), 369 (5), 349 (5), 333 (5), 319 (7), 297 (7), 273 (15), 251 (16), 226 (11), 209 (16), 195 (15), 155 (35), 152 (20), 134 (36), 119 (16), 105 (74), 93 (10), 81 (10), 69 (6), 55 (9).

**22-[3-(1′-Methyl-1′-hydroxyethyl)phenyl]-23,24,25,26,27-pentanor-1α-hydroxyvitamin $D_3$ (9, EV).** Dienyne **23d** (0.020 g, 0.026 mmol) was dissolved in 13 mL of EtOAc, and 42 μL of a solution of quinoline (0.17 M in hexanes) and then 42 mg of Lindlar catalyst were added. The mixture was stirred for 1 h under a positive pressure of hydrogen at room temperature and then filtered through a short column of silica gel. After concentration the filtrate, the crude residue was purified by flash chromagraphy (1% EtOAc/hexanes) to afford 17 mg of the mixture of vitamin and previtamin. This mixture was added to 10 mL of isooctane and the solution was heated at reflux for 2 h. After evaporation of solvent, the crude product was dissolved in 0.7 mL of dry THF and 0.17 mL of a THF solution 1 M of tetrabutylammonium fluoride. The mixture was stirred at room temperature for 12 h (protected from the light) and then 2 mL of a saturated solution of NaCl was added. The mixture was extracted with EtOAc and then the combined organic extracts were dried over $MgSO_4$ and concentrated. After passing the residue through a short column of silica gel, the crude product was purified by HPLC (Rainin Dynamax, 1.0 × 25 cm, 8 μm, 100% EtOAc, 4 mL/min) to afford 3.9 mg (32%) of the vitamin **9** as a white, amorphous solid: $^1$H NMR δ 0.58 (3 H, $C_{18}$-Me, s), 0.82 (3 H, $C_{21}$-Me, d, $J$ = 6.6 Hz), 1.55 and 1.58 (3 H and 3 H, diastereotopic $Me_2C$, two s), 2.32 (1 H, dd, $J$ = 6.3 Hz, 13.2 Hz), 2.61 (1 H, dd, $J$ = 2.7 Hz, 13.2 Hz), 2.83 (1 H, br d, $J$ = 12.6 Hz), 2.93 (1 H, dd, $J$ = 2.4 Hz, 13.2 Hz), 4.23 (1 H, $H_3$, m), 4.44 (1 H, $H_1$, m), 5.02 (1 H, $H_{19}$, br s), 5.34 (1 H, $H_{19}$, br s), 6.04 (1 H, $H_7$, d, $J$ = 11.1 Hz), 6.39 (1 H, $H_6$, d, $J$ = 11.1 Hz), 7.02 (1 H, Ar, d, $J$ = 6.9 Hz), 7.26 (3 H, Ar, m) [The signals at δ 2.82 and 2.93 are probably assignable to $H_{9\beta}$ and one of the $H_{22}$ protons, respectively, or vice versa]; UV (95% EtOH) $\lambda_{max}$ 266 nm (ε 19 500); HRMS, $m/z$ 464.3307 (calcd for $C_{31}H_{44}O_3$, 464.3290); MS, $m/z$ 464 (14, M), 446 (33), 428 (55), 410 (base), 384 (10), 369 (5), 341 (5), 313 (7), 297 (11), 277 (10), 251 (20), 225 (12), 209 (24), 195 (16), 171 (18), 155 (19), 152 (7), 134 (18), 131 (27), 105 (25), 95 (12), 81 (9), 69 (5), 59 (5).

**1-Iodo-3-[(*tert*-butyldimethylsilyl)oxy]benzene (18a).** *m*-Iodophenol (2.20 g, 10 mmol) was placed in a 100-mL flask equipped with a magnetic stir bar. *tert*-Butyldimethylsilyl chloride (3.00 g, 20 mmol) and imidazole (3.15 g, 46 mmol) were added with 50 mL of dry DMF (distilled with benzene and then from BaO) and then the mixture was stirred at room temperature for 12 h (protected from the light). Crushed ice (50 mL) was added to the reaction solution and then the mixture was extracted with pentane (3 × 20 mL). The combined extracts were washed with brine and then dried over $MgSO_4$. After concentrating the solution, the resulting residue was purified by flash chromatography (100% hexanes, silica gel column 15 × 5 cm) to afford 3.12 g (94%) of silyl ether **18a** as a spectroscopically homogeneous ($^1$H and $^{13}$C NMR analysis), colorless liquid: $^1$H NMR δ 0.21 (6 H, $Me_2Si$, s), 0.99 (9 H, *t*-Bu, s), 6.81 (1 H, $H_4$, d with fine structure, $J$ = 9 Hz), 6.95 (1 H, $H_5$, t, $J$ = 9 Hz), 7.23 (1 H, $H_2$, s), 7.29 (1 H, $H_6$, d, $J$ = 9 Hz); $^{13}$C NMR δ −3.9, 18.7, 26.2, 94.7, 120.1, 130.0, 131.0, 131.2, 156.9; IR ($CCl_4$) ν 3080 (w), 3030 (w), 2970 (s), 1585 (s), 1470 (s), 1270 (s) cm$^{-1}$; HRMS, $m/z$ 334.0250 (calcd for $C_{12}H_{19}OISi$, 334.0250); MS, $m/z$ 334 (34, M), 278 (24), 277 (base, M − *t*-Bu), 263 (2), 247 (2), 203 (2), 185 (5), 150 (23), 135 (16), 121 (2), 91 (3), 73 (11), 57 (4).

**1-Iodo-4-[(*tert*-butyldimethylsilyl)oxy]benzene (18b).** *p*-Iodophenol (2.20 g, 10 mmol) was placed in a 100-mL flask equipped with magnetic stir bar and then *tert*-butyldimethylsilyl chloride (3 g, 20 mmol) and imidazole (3.15 g, 46 mmol) were added with 50 mL of dry DMF. The mixture was stirred at room temperature for 12 h (protected from light). Crushed ice (50 mL) was added to the reaction mixture and then the mixture was extracted with pentane (3 × 20 mL). The combined extracts were washed with brine and then dried over $MgSO_4$ and concentrated. Purification of the residue by flash chromatograph (100% hexanes, silica gel column, 15 × 5 cm) gave 3.28 g (98%) of silyl ether **18b** as a colorless liquid. The spectroscopically homogeneous material ($^1$H and $^{13}$C NMR analysis) was used without further purification: $^1$H NMR δ 0.19 (6 H, $Me_2Si$, s), 0.98 (9 H, *t*-Bu, s), 6.61 and 7.51 (2 H and 2 H, $H_{3,5}$ and $H_{2,6}$, d, $J$ = 9 Hz and d, $J$ = 9 Hz); $^{13}$C NMR δ −3.9, 18.8, 26.2, 84.3, 123.1, 138.9, 156.2; IR ($CCl_4$) ν 3080 (w), 2940 (s), 1590 (m), 1480 (s), 1260 (s), 900 (s) cm$^{-1}$; HRMS, $m/z$ 334.0240 (calcd for $C_{12}H_{19}OISi$, 334.0250); MS, $m/z$ 334 (45, M), 278 (29), 277 (base, M − *t*-Bu), 247 (2), 203 (3), 192 (3), 177 (2), 150 (39), 135 (22), 121 (2), 91 (4), 73 (7), 59 (4).

**3-[1′-Methyl-1′-(trimethylsilyloxy)ethyl]iodobenzene (18d).** To a stirred solution of 3-iodobenzoic acid (0.54 g, 2.20 mmol) in THF (15 mL) at 0 °C was added methylmagnesium bromide (3 M in ether, 5 mL, 8.70 mmol) dropwise via a syringe. After being stirred for 2 h at 0 °C, the reaction solution was quenched with water. The mixture was extracted with ether and then the combined ether extracts were washed with water, dried ($MgSO_4$), and then concentrated to dryness. The crude, dry residue (mainly the methyl ketone accompanied by 5–10% of tertiary alcohol) was dissolved in THF (15 mL) and then cooled to 0 °C. Methylmagnesium bromide (3 M in aqueous ether, 2.5 mL, 4.35 mmol) was added and the mixture stirred at 0 °C for 1 h. After quenching the reaction mixture as above and working up of the crude dried product, the latter was dissolved in $CH_2Cl_2$ (15 mL) and 1-(trimethylsilyl)imidazole (0.6 mL, 4 mmol) was added at room temperature. The solution was stirred at 20 °C for 12 h and then water was added. The mixture was extracted with $CH_2Cl_2$ (3 × 5 mL) and the combined organic extracts were washed with brine and then dried over $MgSO_4$. After evaporation of the solvent, the crude product was purified by flash chromatography (silica gel, 2% EtOAc/hexanes) to afford the silyl ether **18d** (0.36 g, 50% overall yield from 3-iodobenzoic acid) as a colorless oil: $^1$H NMR δ 0.16 (9 H, $Me_3Si$, s), 1.59 (6 H, $Me_2C$, s), 7.08 (1 H, $H_5$, t, $J$ = 7.5 Hz), 7.43 (1 H, $H_4$, d, $J$ = 7.5 Hz), 7.58 (1 H, $H_6$, d, $J$ = 7.5 Hz), 7.84 (1 H, $H_2$, s); $^{13}$C NMR δ 2.7, 32.7, 75.0, 94.6, 124.3, 130.0, 134.3, 135.6, 152.9; IR ($CCl_4$) ν 2950 (m), 1590 (s), 1480 (s), 1260 (s), 900 (s) cm$^{-1}$; HRMS, $m/z$ 334.0251 (calcd for $C_{12}H_{19}OISi$, 334.0250); MS, $m/z$ 334 (2, M), 320 (16), 319 (base), 245 (2), 192 (2), 177 (3), 131 (3), 117 (1), 75 (54), 73 (42).

**22-[m-[(*tert*-Butyldimethylsilyl)oxy]phenyl]-de-*A*,*B*-24-norcholan-8β-ol (20a).** A solution of 1-iodo-3-[(*tert*-butyldimethylsilyl)oxy]benzene (**18a**, 0.668 g, 2 mmol) in 10 mL of dry THF was stirred at −78 °C and *tert*-butyllithium (2.35 mL, 4 mmol, 1.7 M solution in pentanes) was added. The solution was allowed to warm to room temperature for 1 h and then recooled to −78 °C. Cuprous cyanide (0.089 g, 1 mmol) was added to the aryllithium solution via a solid addition funnel. The solution was warmed to room temperature for another 1 h and then the hydroxy tosylate **17** (0.183 g, 0.5 mmol in 2 mL of dry THF and 1 mL washing) was added via cannula at room temperature. The mixture was heated to reflux for 24 h and then the reaction was

quenched at room temperature with a saturated solution of $NH_4Cl$. The organic layer was extracted with ether, washed with brine, and dried over $MgSO_4$. After concentration, the resulting crude residue was purified by flash chromatography and then by HPLC (Rainin Dynamax, 1.0 × 25 cm, 8 μm, 10% EtOAc/hexanes, 4 mL/min) to afford 0.159 g of the alcohol **20a** (79%) as a colorless, viscous oil: $^1H$ NMR δ 0.18 (6 H, $Me_2Si$, s), 0.79 (3 H, $C_{21}$-Me, d, $J$ = 6.6 Hz), 0.97 (3 H, $C_{18}$-Me, s), 0.98 (9 H, $t$-Bu, s), 2.84 (1 H, dd, $J$ = 13.2 Hz, 2.7 Hz), 4.09 (1 H, $H_8$, br s), 6.61 (1 H, $ArH_2$, s), 6.65 (1 H, $ArH_4$, d, $J$ = 8.1 Hz), 6.73 (1 H, $ArH_6$, d, $J$ = 7.5 Hz), 7.11 (1 H, $ArH_5$, t, $J$ = 7.5 Hz); $^{13}C$ NMR δ −3.8, 14.2, 18.1, 18.8 23.3, 26.4, 28.5, 34.2, 38.7, 41.0, 42.6, 43.1, 53.3, 57.7, 69.9, 117.8, 121.9, 123.1, 129.4, 143.7, 155.9; HRMS, $m/z$ 402.2967 (calcd for $C_{25}H_{42}O_2Si$, 402.2954); MS, $m/z$ 402 (42, M), 384 (5), 369 (2), 345 (3), 327 (4), 275 (2), 263 (2), 249 (4), 235 (8), 223 (20), 222 (base), 209 (15), 195 (16), 182 (11), 181 (66), 163 (22), 151 (2), 135 (10), 121 (4), 109 (6), 95 (12), 81 (12), 69 (4), 57 (3).

**22-[*p*-[(*tert*-Butyldimethylsilyl)oxy]phenyl]-de-*A*,*B*-24-norcholan-8β-ol (20b).** 1-Iodo-4-[(*tert*-butyldimethylsilyl)-oxy]benzene (**18b**, 0.668 g, 2 mmol) in 10 mL of dry THF was placed in a 25-mL flask and cooled to −78 °C under argon. *tert*-Butyllithium (2.35 mL, 4 mmol, 1.7 M) was then added dropwise via a syringe. After being stirred for 15 min, the solution was warmed to 0 °C over 1 h and then recooled to −78 °C. Cuprous cyanide (0.089 g, 1 mmol) was then added at once and the solution was warmed to room temperature over 1 h. The cuprate solution was cooled to 0 °C and the tosylate **17** (0.183 g, 0.5 mmol) in 3 mL of dry THF was added via cannula. The mixture was refluxed for 20 h and, after cooling to room temperature, 10 mL of a saturated solution of $NH_4Cl$ was added. The mixture was extracted with ether and then the organic extract was washed with brine and dried over $MgSO_4$. After filtration and concentration, the resulting residue was purified by flash chromatography (10% EtOAc/hexanes, 15 × 0.5 cm silica gel column) to give 0.131 g (71%) of the alcohol **20b** as a colorless, viscous liquid. This material, which exhibited satisfactory $^1H$ and $^{13}C$ NMR spectra, was oxidized to the corresponding ketone **21b** without further characterization: $^1H$ NMR δ 0.18 (6 H, $Me_2Si$, s), 0.78 (3 H, $C_{21}$-$CH_3$, d, $J$ = 6 Hz), 0.96 (3 H, $C_{18}$-Me, s), 0.98 (9 H, $t$-Bu, s), 2.82 (1 H, dd, $J$ = 12 Hz, 3 Hz), 4.09 (1 H, $H_8$, br s), 6.74 and 6.97 (2 H and 2 H, $ArH_{3,5}$ and $ArH_{2,6}$, d, $J$ = 6 Hz, and d, $J$ = 6 Hz); $^{13}C$ NMR δ −4.4, 13.6, 17.4 18.0, 18.2, 22.6, 25.7, 27.8, 33.6, 38.2, 40.3, 41.7, 42.0, 52.6, 57.0, 69.4, 119.5, 130.1, 134.2, 153.4.

**22-*m*-Toluyl-de-*A*,*B*-24-norcholan-8β-ol (20c).** 3-Iodotoluene (**18c**, 3.5 g, 16 mmol) in THF (80 mL) was cooled to −78 °C and *tert*-butyllithium (1.7 M in hexanes, 18.8 mL, 32 mmol) was introduced dropwise (20 min) via syringe. The solution was warmed to room temperature and, after stirring for 1 h, it was cooled to −30 °C. Cuprous cyanide (0.716 g, 8 mmol) was added to the *m*-toluyllithium solution with a solid addition funnel. After the mixture was stirred for 30 min at −30 °C, the solution was warmed to room temperature for 1 h and then cooled again to −30 °C. The tosylate **17** (1.464 g, 4 mmol in 2 mL of THF) was introduced via cannula. The reaction solution was then heated to reflux in THF for 12 h and quenched at room temperature with a saturated solution of aqueous $NH_4Cl$ (40 mL). The mixture was extracted with ether (3 × 20 mL) and the combined extracts were washed with brine and dried over $MgSO_4$. After evaporation of the solvent, the crude residue was purified by flash chromatography (silica gel, 10% EtOAc/hexanes, 20 cm × 2) to afford the alcohol **20c** (0.856 g, 75%) as a colorless, viscous oil: $^1H$ NMR δ 0.84 (3 H, $C_{21}$-$CH_3$, d, $J$ = 6.3 Hz), 1.01 (3 H, $C_{18}$-$CH_3$, s), 2.37 (3 H, $ArCH_3$, s), 2.91 (1 H, dd, $J$ = 12.9 Hz, 1.5 Hz), 4.13 (1 H, $H_8$, br s), 6.90–7.06 (3 H, $ArH_{2,4,6}$, m), 7.19 (1 H, $ArH_5$, t, $J$ = 7.5 Hz); $^{13}C$ NMR δ 13.8, 17.7, 18.4, 21.7, 22.9, 28.1, 33.9, 38.4, 40.6, 42.3, 42.8, 53.0, 57.5, 69.6, 126.5, 126.7, 128.1, 130.4, 137.7, 141.8; HRMS, $m/z$ 286.2297 (calcd for $C_{20}H_{30}O$, 286.2297); MS, $m/z$ 286 (32, M), 268 (6), 253 (5), 227 (5), 214 (5), 199 (5), 181 (32), 164 (18), 163 (base), 147 (9), 136 (11), 135 (35), 111 (74), 95 (18), 81 (18), 69 (5), 55 (6).

**22-[3-[1′-Methyl-1′-[(trimethylsilyl)oxy]ethyl]phenyl]-de-*A*,*B*-24-norcholan-8β-ol (20d).** To a solution of the iodide **18d** (2 g, 6 mmol) in 30 mL of THF cooled to −78 °C was added 7 mL of a 1.7 M solution of $t$-BuLi (12 mmol) in pentanes via syringe. After being stirred 1 h at −78 °C, the solution was warmed to 0 °C for 15 min and then cooled again to −78 °C. Cuprous cyanide (0.269 g, 3 mmol) was added via a solid additional funnel. After 30 min at −78 °C, the solution was warmed to room temperature for 30 min and cooled again to −78 °C. After the addition of 0.520 g (1.4 mmol) of the tosylate **17**, the cooling bath was removed and the solution was heated at reflux for 36 h. The reaction was then quenched at room temperature with a saturated solution of $NH_4Cl$ and the mixture was extracted with ether. The combined ether extracts were washed with brine and then dried over $MgSO_4$. After evaporation of the solvent, the crude residue was purified by flash chromatography (10% EtOAc/hexanes) to afford 0.492 g (88%) of the alcohol **20d** as a colorless, viscous oil: $^1H$ NMR δ 0.09 (9 H, $Me_3Si$, s), 0.82 (3 H, $C_{21}$-Me, d, $J$ = 6.3 Hz), 0.99 (3 H, $C_{18}$-Me, s), 1.56 and 1.60 (3 H and 3 H, diastereotopic $Me_2C$, two s), 2.93 (1 H, dd, $J$ = 13.2 Hz, 2.7 Hz), 4.12 (1 H, $H_8$, br s), 7.00 (1 H, Ar, d, $J$ = 6.3 Hz), 7.24 (3 H, Ar, m); $^{13}C$ NMR δ 2.6, 13.8, 17.7, 18.4, 22.9, 28.1, 32.6, 32.9, 33.8, 38.4, 40.5, 42.3, 43.0, 52.9, 57.3, 69.6, 75.4, 122.1, 126.2, 127.6, 127.7, 141.3, 149.9; IR ($CCl_4$) ν 3640 (OH, w), 2980 (s), 1600 (w), 1440 (m), 1380 (m), 1260 (s), 1180 (s), 1080 (s), 910 (m) 840 (s) $cm^{-1}$; HRMS, $m/z$ 402.2966 (calcd for $C_{25}H_{42}O_2Si$, 402.2954); MS, $m/z$ 402 (4, M), 388 (32), 387 (base), 369 (4), 294 (1), 269 (2), 163 (2), 135 (2), 96 (1), 82 (2), 75 (1), 73 (1).

**22-[*m*-[(*tert*-Butyldimethylsilyl)oxy]phenyl]-de-*A*,*B*-24-norcholan-8-one (21a).** A solution of the alcohol **20a** (0.385 g, 0.95 mmol) in 5 mL of $CH_2Cl_2$ (distilled from $CaH_2$) was transferred via cannula to a magnetically stirred suspension of pyridinium dichromate (1.07 g, 2.85 mmol) and pyridinium trifluoroacetate (0.072 g, 0.38 mmol) in 5 mL of $CH_2Cl_2$ under argon. The reaction mixture was then stirred for 10 h at room temperature. The resulting black mixture was passed through a fritted glass funnel containing a slurry of diatomaceous earth covered by a slurry of silica gel. The solvent was removed from the filtrate to give a brown oil, which was purified by flash chromatography (5% EtOAc/hexanes, 15 × 2 cm silica gel column) to afford 0.372 g (94%) of ketone **21a** as colorless, viscous oil: $^1H$ NMR δ 0.18 (6 H, $Me_2Si$, s), 0.67 (3 H, $C_{18}$-Me, s), 0.84 (3 H, $C_{21}$-Me, d, $J$ = 6 Hz), 0.98 (9 H, $t$-Bu, s), 2.47 (1 H, dd, $J$ = 9 Hz, 6 Hz), 2.84 (1 H, dd, $J$ = 3 Hz, 12 Hz), 6.61 (1 H, $ArH_2$, s), 6.66 (1 H, $ArH_4$, d, $J$ = 9 Hz), 6.72 (1 H, $ArH_6$, d, $J$ = 9 Hz), 7.12 (1 H, $ArH_5$, t, $J$ = 9 Hz); $^{13}C$ NMR δ −3.8, 13.1, 18.8, 18.9, 19.8, 24.6, 26.3, 28.7, 38.8, 39.5, 41.5, 43.0, 50.5, 57.5, 62.6, 118.0, 121.8, 123.0, 129.5, 143.2, 155.9, 212.5; IR ($CCl_4$) ν 2980 (C–H, s), 1720 (C=O, s), 1605 (m), 1590 (m), 1280 (s), 840 (s) $cm^{-1}$; HRMS; $m/z$ 400.2810 (calcd for $C_{25}H_{40}O_2Si$, 400.2798); MS, $m/z$ 400 (28, M), 385 (2), 343 (30, M − $t$-Bu), 287 (2), 261 (6), 247 (10), 222 (40), 181 (82), 161 (32), 136 (11), 135 (base), 109 (13), 95 (14), 81 (16), 69 (8), 55 (10).

**22-[*p*-[(*tert*-Butyldimethylsilyl)oxy]phenyl]-de-*A*,*B*-24-norcholan-8-one (21b).** The alcohol **20b** (0.080 g, 0.2 mmol) dissolved in $CH_2Cl_2$ (1 mL distilled from $CaH_2$) was added via cannula (with 1 mL washing) to a magnetically stirred suspension of pyridinium dichromate (0.225 g, 0.6 mmol) and pyridinium trifluoroacetate (0.015 g, 0.08 mmol) in $CH_2Cl_2$ (2 mL) under argon. The reaction mixture was then stirred for 10 h at room temperature. The resulting black mixture was passed through a filtered glass funnel containing a slurry of diatomaceous earth covered by a slurry of silica gel. The filtrate was concentrated to give a brown oil, which was purified by flash chromatography (5% EtOAc/hexanes, 15 × 0.5 cm silica gel column) to give 0.075 g (94%) of ketone **21b** as a colorless, viscous oil: $^1H$ NMR δ 0.18 (6 H, $Me_2Si$, s), 0.66 (3 H, $C_{18}$-Me, s), 0.82 (3 H, $C_{21}$-Me, d, $J$ = 6 Hz), 0.97 (9 H, $t$-Bu, s), 2.47 (1 H, dd, $J$ = 6 Hz, 12 Hz), 2.82 (1 H, dd, $J$ = 12 Hz, 3 Hz), 6.97 and 6.73 (2 H and 2 H, $ArH_{3,5}$ and $ArH_{2,6}$, d, $J$ = 9 Hz, and d, $J$ = 9 Hz); $^{13}C$ NMR δ −3.9, 13.1, 18.7, 18.8, 19.8, 24.6, 26.3, 28.7, 39.0, 39.5, 41.5, 42.2, 50.6, 57.5, 62.6, 120.2, 130.7, 134.3, 154.1, 212.6; IR ($CCl_4$) ν 3030 (w), 2980 (s), 1720 (CO, s), 1610 (w), 1510 (m), 1260 (s), 915 (s), 840 (m) $cm^{-1}$; HRMS, $m/z$ 400.2810 (calcd for $C_{25}H_{40}O_2Si$, 400.2798); MS, $m/z$ 400 (19, M), 343 (8, M − $t$-Bu), 221 (base), 207 (3), 181 (9), 165 (9), 149 (3), 135 (7), 109 (2), 95 (3), 73 (10), 59 (4).

**22-*m*-Toluyl-de-*A*,*B*-24-norcholan-8-one (21c).** The alcohol **20c** (0.858 g, 3 mmol) was dissolved in $CH_2Cl_2$ (25 mL distilled from $CaH_2$) and then added via cannula to a magnetically stirred suspension of pyridinium dichromate (4.5 g, 12 mmol) and pyridinium trifluoroacetate (0.386 g, 2 mmol) in 25 mL of dry $CH_2Cl_2$ under argon. The reaction mixture was then stirred for 10 h at room temperature. The resulting black mixture was passed

through a fritted glass funnel containing a slurry of diatomaceous earth covered with a slurry of silica gel. The solvent was removed to give a brown oil, which was purified by flash chromatography (5% EtOAc/hexanes, 15 × 4 cm silica gel column) to afford 0.787 g (93%) of ketone **21c** as a colorless, viscous oil: $^1$H NMR $\delta$ 0.70 (3 H, $C_{18}$-Me, s), 0.88 (3 H, $C_{21}$-Me, d, $J$ = 6.6 Hz), 2.35 (3 H, Ar-Me, s), 2.49 (1 H, dd, $J$ = 7.5 Hz, 11.4 Hz), 2.90 (1 H, dd, $J$ = 2.1 Hz, 13.2 Hz), 6.95 (1 H, $ArH_6$ or $ArH_4$, d, $J$ = 7.8 Hz), 6.97 (1 H, $H_2$, s), 7.02 (1 H, $ArH_4$ or $ArH_6$, d, $J$ = 7.5 Hz), 7.18 (1 H, $ArH_5$, t, $J$ = 7.5 Hz); $^{13}$C NMR $\delta$ 12.8, 18.6, 19.5, 21.7, 24.3, 28.4, 38.6, 39.1, 41.2, 42.7, 50.2, 57.3, 62.3, 126.6, 126.7, 128.2, 130.3, 137.8, 141.3, 212.0; IR ($CCl_4$) $\nu$ 2970 (s), 1715 (C=O, s) cm$^{-1}$; HRMS, $m/z$ 284.2145 (calcd for $C_{20}H_{28}O$, 284.2145); MS, $m/z$ 286 (10, M + 2), 285 (13, M + 1), 284 (44, M), 269 (5), 241 (5), 223 (5), 211 (5), 179 (base), 163 (74), 135 (56), 105 (82), 95 (35), 81 (61), 69 (13), 55 (10).

**22-[3-[1′-Methyl-1′-[(trimethylsilyl)oxy]ethyl]phenyl]-de-*A*,*B*-24-norcholan-8-one (21d).** The alcohol **20d** (0.46 g, 1.14 mmol) dissolved in 1 mL of $CH_2Cl_2$ was added via cannula (with 1 mL of washing) to a magnetically stirred suspension of pyridinium dichromate (1.5 g, 4 mmol) and pyridinium trifluoroacetate (0.12 g, 0.7 mmol) in $CH_2Cl_2$ (16 mL) under argon. The reaction mixture was then stirred for 10 h at room temperature. The resulting dark solution was passed through a filtered glass funnel containing a slurry of diatomaceous earth covered with a slurry of silica gel. The solvent was removed to give a brown oil, which was purified by HPLC (Rainin Dynamax, 1.0 × 25 cm, 8 $\mu$m, 10% EtOAc/hexanes, 4 mL/min) to afford 0.22 g of the ketone **21d** (50%) as a colorless, viscous oil: $^1$H NMR $\delta$ 0.09 (9 H, $Me_3Si$, s), 0.70 (3 H, $C_{18}$-Me, s), 0.87 (3 H, $C_{21}$-Me, d, $J$ = 6.3 Hz), 1.59 and 1.60 (3 H and 3H, diastereotopic $Me_2C$, two s), 2.50 (1 H, dd, $J$ = 7.5 Hz, 12.4 Hz), 2.92 (1 H, dd, $J$ = 2.7 Hz, 13.2 Hz), 7.00 (1 H, Ar, d, $J$ = 6.9 Hz), 7.15–7.30 (3 H, Ar, m); $^{13}$C NMR $\delta$ 2.6, 12.8, 18.6, 19.5, 24.2, 28.4, 32.6, 32.9, 38.5, 39.1, 41.2, 42.9, 50.2, 57.2, 62.3, 75.4, 122.3, 126.2, 127.5, 127.8, 140.8, 150.0, 212.1; HRMS (CI, $CH_4$), $m/z$ 399.2728 ((M − 1) instead of (M + 1) because an unusual loss of $H_2$) calcd for $C_{25}H_{40}O_2Si$ − 1, 399.2719); MS (EI, 20 eV), $m/z$ 400 (<1, M), 386 (31), 385 (base), 339 (2), 310 (5), 295 (13), 277 (1), 179 (4), 161 (7), 135 (13), 105 (2), 95 (2), 75 (10).

**22-[*m*-[(*tert*-Butyldimethylsilyl)oxy]phenyl]-de-*A*,*B*-24-norchol-8-enyl Trifluoromethanesulfonate (22a).** Lithium diisopropylamide (LDA) was prepared by treating diisopropylamine (0.258 mL, 1.85 mmol) with *n*-BuLi (1.16 mL, 1.85 mmol, 1.60 M in hexanes) in 2.05 mL of dry THF at 0 °C. After being stirred for 15 min, the LDA solution was cooled to −30 °C and the ketone **21a** (0.37 g, 0.92 mmol) in 0.24 mL of dry THF was added dropwise via cannula. After being stirred 8 h at −30 °C, the enolate solution was cooled to −78 °C and *N*-phenyl-*N*-[(trifluoromethyl)sulfonyl]trifluoromethanesulfonamide (0.66 g, 1.85 mmol) in 0.81 mL of THF added. The reaction solution was transferred to a bath maintained at 0 °C and stirring was continued for 15 h. The reaction was quenched with water and then the solution was transferred to a separatory funnel and washed successively with a solution 10% $H_2SO_4$, $H_2O$, saturated $NaHCO_3$, $H_2O$, and saturated brine. The organic solution was dried ($MgSO_4$), filtered, and then concentrated. The resulting residue was purified by flash chromatography (5% EtOAc/hexanes, 15 × 2 cm silica gel column) to afford 0.326 g (77%) of the triflate **22a** as a colorless, viscous oil: $^1$H NMR $\delta$ 0.22 (6 H, $Me_2Si$, s), 0.83 (3 H, $C_{18}$-Me, s), 0.87 (3 H, $C_{21}$-Me, d, $J$ = 6.3 Hz), 1.02 (9 H, *t*-Bu, s), 2.86 (1 H, dd, $J$ = 2.4 Hz, 13.5 Hz), 5.60 (1 H, $H_9$, m), 6.65 (1 H, $ArH_2$, s), 6.69 (1 H, $ArH_6$, d, $J$ = 8.1 Hz), 6.76 (1 H, $ArH_4$, d, $J$ = 7.5 Hz), 7.15 (1 H, $ArH_5$, t, $J$ = 7.8 Hz); $^{13}$C NMR $\delta$ −3.8, 11.9, 18.8, 22.2, 24.4, 26.3, 29.5, 35.3, 39.2, 43.0, 45.9, 50.8, 55.2, 116.7, 118.0, 121.8, 123.0, 129.5, 143.2, 150.4, 156.0; IR ($CCl_4$) $\nu$ 3040 (w), 2980 (s), 1610 (m), 1590 (m), 1420 (s), 1215 (s), 1150 (s) cm$^{-1}$; HRMS, $m/z$ 532.2287 (calcd for $C_{26}H_{39}O_4SSiF_3$, 532.2290); MS, $m/z$ 533 (21, M + 1), 532 (65, M), 475 (8, M − *t*-Bu), 367 (5), 341 (32), 313 (68), 275 (3), 222 (60), 191 (base), 164 (41), 163 (16), 135 (77), 107 (21), 91 (32), 73 (39), 69 (36), 67 (22), 59 (13), 57 (25).

**22-[*p*-[(*tert*-Butyldimethylsilyl)oxy]phenyl]-de-*A*,*B*-24-norchol-8-enyl Trifluoromethanesulfonate (22b).** Lithium diisopropylamide was prepared by treating diisopropylamine (0.17 mL, 1.2 mmol) with *n*-BuLi (0.75 mL, 1.2 mmol, 1.60 M in hexanes) in 1.35 mL of dry THF at 0 °C. After being stirred for 15 min, the solution was cooled to −30 °C and the ketone **21b** (0.20 g, 0.5 mmol) in 0.13 mL of THF was added dropwise via cannula. After being stirred 4 h at −30 °C, the enolate solution was cooled to −78 °C and *N*-phenyl-*N*-[(trifluoromethyl)sulfonyl]trifluoromethanesulfonamide (0.393 g, 1.1 mmol) in 0.55 mL of THF added. The reaction solution was transferred to a bath maintained at 0 °C and stirring was continued for 15 h. The reaction was quenched with water and then the solution was transferred to a separatory funnel and washed with 10% $H_2SO_4$, $H_2O$, saturated $NaHCO_3$, $H_2O$, and saturated brine. The organic solution was dried ($MgSO_4$), filtered, and then concentrated. The residue was purified by flash chromatography (5% EtOAc/hexanes, 15 × 0.5 cm, silica gel column) to afford 0.135 g (51%) of the triflate **22b** as a colorless, viscous liquid: $^1$H NMR $\delta$ 0.19 (6 H, $Me_2Si$, s), 0.80 (3 H, $C_{18}$-Me, s), 0.83 (3 H, $C_{21}$-Me, d, $J$ = 6 Hz), 0.99 (9 H, *t*-Bu, s), 2.82 (1 H, dd, $J$ = 12 Hz, ~3 Hz), 5.58 (1 H, $H_9$, m), 6.98 and 6.75 (2 H and 2 H, $ArH_{3,5}$ and $ArH_{2,6}$, d, $J$ = 8.5 Hz, and d, $J$ = 8.5 Hz); $^{13}$C NMR $\delta$ −3.8, 11.9, 18.7, 22.2, 24.4, 26.3, 29.5, 35.3, 39.4, 42.3, 45.9, 50.8, 55.1, 116.7, 120.2, 130.7, 134.2, 150.4, 154.2; IR ($CCl_4$) $\nu$ 3030 (w), 2960 (s), 1610 (w), 1510 (m), 1425 (s), 1250 (s), 1215 (s), 1150 (s), 910 (s) cm$^{-1}$; HRMS, $m/z$ 532.2261 (calcd for $C_{26}H_{39}O_4F_3SSi$, 532.2290); MS, $m/z$ 532 (32, M), 341 (17), 313 (23), 249 (2), 221 (base), 191 (9), 165 (12), 135 (8), 105 (7), 91 (11), 73 (33), 69 (13), 57 (10).

**22-*m*-Toluyl-de-*A*,*B*-24-norchol-8-enyl Trifluoromethanesulfonate (22c).** Diisopropylamine (0.117 mL, 0.84 mmol) in THF (0.92 mL) was cooled to 0 °C and *n*-butyllithium (1.6 M in hexanes, 0.52 mL, 0.84 mmol) was added dropwise via a syringe. After 15 min of stirring at 0 °C, the solution was cooled to −30 °C and the CD ketone **21c** (0.20 g, 0.70 mmol in 0.22 mL of THF) was added via cannula. After 8 h at −30 °C, *N*-phenyl-*N*-[(trifluoromethyl)sulfonyl]trifluoromethanesulfonamide (0.224 g, 0.76 mmol, in 0.36 mL of THF) was added via cannula. The solution was warmed to 0 °C and stirred for 12 h. Water was added and the mixture was extracted with ether. The combined extracts were washed with a solution of 10% HCl and a saturated solution of $NaHCO_3$ and brine and then dried over $MgSO_4$. After evaporation of the solvent, the crude residue was purified by flash chromatography (silica gel, 100% hexanes) to afford 0.135 g (46%) of the triflate **22c** as a colorless, viscous liquid: $^1$H NMR $\delta$ 0.81 (3 H, $C_{18}$-Me, s), 0.84 (3 H, $C_{21}$-Me, d, $J$ = 6.3 Hz), 2.35 (3 H, Ar-Me, s), 2.53 (1 H, m), 2.88 (1 H, dd, $J$ = 13.2 Hz, 2.4 Hz), 5.60 (1 H, $H_9$, narrow m), 6.95 (1 H, $ArH_4$, d, $J$ = 7.5 Hz), 6.96 (1 H, $ArH_2$, s), 7.14 (1 H, $ArH_6$, d, $J$ = 7.5 Hz), 7.18 (1 H, $ArH_5$, t, $J$ = 7.2 Hz); $^{13}$C NMR $\delta$ 11.9, 18.8, 22.0, 22.2, 24.4, 29.5, 35.3, 39.4, 43.1, 45.9, 50.8, 55.2, 77.8, 116.7, 126.9, 127.0, 128.5, 130.7, 138.2, 141.6, 150.3; IR ($CCl_4$) $\nu$ 2980 (m), 1610 (w), 1420 (s), 1250 (m), 1140 (s), 900 (m) cm$^{-1}$; HRMS, $m/z$ 416.1640 (calcd for $C_{21}H_{27}O_3F_3S$, 416.1640); MS, $m/z$ 416 (18, M), 402 (3), 310 (3), 281 (4), 255 (3), 241 (3), 221 (6), 177 (3), 161 (52), 133 (8), 106 (base), 91 (4), 81 (3), 69 (10), 55 (9).

**22-[3-[1′-Methyl-1′-[(trimethylsilyl)oxy]ethyl]phenyl]-de-*A*,*B*-24-norchol-8-enyl Trifluoromethanesulfonate (22d).** Diisopropylamine (0.072 mL, 0.5 mmol) in THF (0.7 mL) was stirred at 0 °C and *n*-BuLi was added (0.32 mL, 0.5 mmol, 1.6 M solution). After 15 min at 0 °C, the solution was cooled to −78 °C and ketone **21d** (0.055 g, 0.13 mmol in 0.2 mL THF) was added. The mixture was stirred at −78 °C for 8 h and then *N*-phenyl-*N*-[(trifluoromethyl)sulfonyl]trifluoromethanesulfonamide (0.178 g, 0.5 mmol, in 0.25 mL of THF) was added. The reaction mixture was allowed to warm to room temperature and stirred for 12 h. Water was added and the mixture was extracted with ether. The combined etheral extracts were washed successively with a 10% solution of HCl, a saturated solution of $NaHCO_3$, and brine and then dried ($MgSO_4$). The solvent was evaporated and the crude residue was filtered over a pad of silica gel (5% EtOAc/hexanes). Purification of the resulting material by HPLC (Rainin Dynamax, 1.0 × 25 cm, 8 $\mu$m, 4 mL/min, 5% EtOAc/hexanes) afforded 50 mg (69%) of trilfate **22d** as a colorless, viscous oil: $^1$H NMR $\delta$ 0.09 (9 H, $Me_3Si$, s), 0.81 (3 H, $C_{18}$-Me, s), 0.86 (3 H, $C_{21}$-Me, d $J$ = 6.3 Hz), 1.59 (6 H, $CMe_2$, s, br), 2.90 (1 H, d, $J$ = 13.2 Hz), 5.60 (1 H, $H_9$, m), 7.00 (1 H, Ar, d, $J$ = 6.6 Hz), 7.19–7.27 (3 H, Ar, m); $^{13}$C NMR $\delta$ 2.3, 11.4, 18.3, 21.6, 23.8, 29.0, 32.3, 32.6, 34.7, 38.8, 42.7, 45.3, 50.2, 54.5, 75.1, 116.1, 122.1, 125.9, 127.3, 127.6, 140.5, 149.79, 149.80; HRMS, $m/z$ 532.2283 (cacld for $C_{26}H_{39}O_4F_3SiS$, 532.2290); MS, $m/z$ 532 (<1, M), 537 (base), 442 (72), 367 (3), 348 (2), 292 (11), 255 (7), 229 (5), 197 (3), 161 (45), 132

(76), 131 (26), 105 (11), 91 (3), 75 (37), 55 (5).

**1α,3β-Bis[(*tert*-butyldimethylsilyl)oxy]-22-[*m*-[(*tert*-butyldimethylsilyl)oxy]phenyl]-24-nor-9,10-secochola-5(10),8-dien-6-yne (23a).** The A-ring fragment **14** (0.077 g, 0.14 mmol) and 0.060 g (0.16 mmol) of the CD ring triflate **22a** were dissolved in 0.6 mL of dry DMF. Bis(triphenylphosphine)palladium dichloride complex ($Pd(PPh_3)Cl_2$, 3 mg) and diethylamine (0.076 mL, 0.55 mmol) were then introduced. The mixture was heated to 80 °C for 5 h, and then after cooling to room temperature, water was added and the mixture was extracted with ether. The combined ether extracts were washed successively with a solution 10% HCl, a solution of saturated $NaHCO_3$, and brine. After drying ($MgSO_4$) and concentrating the solution, the crude residue was passed through a short column of silica gel (1% EtOAc/hexanes) and then purified by HPLC (Rainin Dynamax-60A column, 0.4% EtOAc/hexanes, 8 mL/min) to afford 86 mg (81%) of the dienyne **23a** as a colorless residue: $^1H$ NMR δ 0.09 (6 H, $Me_2Si$, s), 0.12 (6 H, $Me_2Si$, s), 0.21 (6 H, $Me_2Si$, s), 0.76 (3 H, $C_{18}$-Me, s), 0.86 (3 H, $C_{21}$-Me, d, $J$ = 6.3 Hz), 0.91 (9 H, *t*-Bu, s), 0.92 (9 H, *t*-Bu, s), 1.00 (9 H, *t*-Bu, s), 1.93 (3 H, $C_{19}$-Me, s), 2.43 (1 H, dd, $J$ = 3.6 Hz, 16.2 Hz), 2.87 (1 H, dd, $J$ = 2.1 Hz, 13.2 Hz), 4.12 (1 H, $H_1$, m), 4.22 (1 H, $H_1$, m), 6.00 (1 H, $H_9$, m), 6.64 (1 H, $ArH_2$, s), 6.68 (1 H, Ar, d, $J$ = 8.4 Hz), 6.76 (1 H, Ar, d, $J$ = 7.5 Hz), 7.13 (1 H, $ArH_5$, t, $J$ = 7.8 Hz); $^{13}C$ NMR δ −4.5, −4.4, −4.3, −4.1, −4.0, 11.3, 18.3, 18.4, 18.6, 19.4, 24.5, 25.4, 26.0, 26.1, 26.2, 28.9, 36.1, 39.1, 40.1, 41.5, 42.2, 42.8, 50.5, 55.3, 64.4, 70.3, 88.4, 92.6, 115.7, 117.5, 121.5, 122.6, 122.7, 129.1, 133.5, 140.7, 143.3, 155.6; HRMS, $m/z$ 762.5303 (calcd for $C_{46}H_{78}O_3Si_3$, 762.5259); MS, $m/z$ 762 (2, M), 623 (25), 631 (57), 630 (base), 628 (11), 574 (10), 499 (18), 498 (41), 441 (6), 407 (2), 381 (2), 355 (2), 324 (19), 277 (11), 268 (10), 249 (11), 222 (32), 193 (4), 165 (4), 132 (3), 105 (3), 75 (52), 56 (2).

**1α,3β-Bis[(*tert*-butyldimethylsilyl)oxy]-22-[*p*-[(*tert*-butyldimethylsilyl)oxy]phenyl]-24-nor-9,10-secochola-5(10),8-dien-6-yne (23b).** The CD-ring triflate **22b** (0.053 g, 0.1 mmol) and the A-ring **14** (0.046 g, 0.12 mmol) were dissolved under argon in 0.4 mL of dry DMF (distilled from benzene and then from BaO). Diethylamine (0.054 mL, 0.39 mmol) and bis(triphenylphosphine)palladium dichloride (2 mg, $Pd(PPh_3)_2Cl_2$) were added and the mixture was heated at 80 °C for 4.5 h. The solution was cooled and then diluted with ether. The organic layer was separated and washed with a solution 10% HCl, a saturated solution of $NaHCO_3$, and then brine. After drying ($MgSO_4$) and concentrating, the residue was purified by HPLC (Rainin Dynamax-60A column, 0.4% EtOAc/hexanes, 8 mL/min) to afford 0.061 g (80%) of the dienyne **23b** as a colorless, residual oil: $^1H$ NMR δ 0.08 (6 H, $Me_2Si$, s), 0.12 (6 H, $Me_2Si$, s) 0.20 (6 H, $Me_2Si$, s), 0.75 (3 H, $C_{18}$-Me, s), 0.84 (3 H, $C_{21}$-Me, d, $J$ = 6.0 Hz), 0.91 (9 H, *t*-Bu, s), 0.92 (9 H, *t*-Bu, s), 0.99 (9 H, *t*-Bu, s), 1.93 (3 H, $C_{19}$-Me, s), 2.43 (1 H, dd, $J$ = 3.6 Hz, 16.2 Hz), 2.85 (1 H, dd, $J$ = 2.1 Hz, 13.2 Hz), 4.13 (1 H, $H_3$, m), 4.21 (1 H, $H_1$, br s), 5.99 (1 H, $H_9$, m), 6.76 (2 H, $ArH_{3,5}$, d, $J$ = 8.4 Hz), 7.00 (2 H, $ArH_{2,6}$, d, $J$ = 8.1 Hz); $^{13}C$ NMR δ −4.8, −4.7, −4.6, −4.4, −4.3, 11.1, 18.0, 18.2, 19.2, 24.3, 25.2, 25.7, 25.8, 25.9, 28.6, 35.8, 39.1, 39.8, 41.3, 41.8, 42.0, 50.2, 55.1, 64.2, 70.0, 88.2, 92.4, 115.5, 119.6, 122.5, 130.3, 133.3, 134.1, 140.4, 153.5; HRMS, $m/z$ 762.5289 (calcd for $C_{46}H_{78}O_3Si_3$, 762.5259); MS, $m/z$ 762 (2, M), 632 (18), 631 (43), 630 (78), 574 (6), 500 (11), 499 (30), 498 (73), 441 (3), 409 (2), 277 (8), 249 (8), 222 (22), 221 (base), 195 (2), 165 (19), 132 (6), 105 (3), 75 (93), 56 (3).

**1α,3β-Bis[(*tert*-butyldimethylsilyl)oxy]-22-*m*-toluyl-24-nor-9,10-secochola-5(10),8-dien-6-yne (23c).** The triflate **22c** (0.076 g, 0.183 mmol) and 0.076 g (0.200 mmol) of the A-ring fragment **14** were dissolved in 1 mL of dry DMF. Bis(triphenylphosphine)palladium diacetate ($Pd(PPh_3)_2(OAc)_2$, 5 mg, 0.007 mmol) and 4.8 mg of cuprous iodide (0.025 mmol) and then 1 mL of diethylamine (9.6 mmol) were added. The mixture was stirred at room temperature for 2 h, water was added, and the organic layer was extracted with ether. The combined ether extracts were then washed with brine, dried over $MgSO_4$, and concentrated. The crude residue was passed through a short column of silica gel (1% EtOAc/hexanes) and then further purified by HPLC (Rainin Dynamax, 1.0 × 25 cm, 8 μm, 0.5% EtOAc/hexanes, 4 mL/min) to afford 0.43 g of the chromatographically homogeneous dienyne **23c** (80%) as a colorless residue: $^1H$ NMR δ 0.09 (6 H, $Me_2Si$, s), 0.12 (6 H, $Me_2Si$, s), 0.76 (3 H, $C_{18}$-Me, s), 0.86 (3 H, $C_{21}$-Me, d, $J$ = 6.3 Hz), 0.91 (9 H, *t*-Bu, s), 0.92 (9 H, *t*-Bu, s), 1.94 (3 H, $C_{19}$-Me, s), 2.35 (3 H, Ar-Me, s), 2.45 (1 H, dd, $J$ = 2.7 Hz, 16.5 Hz), 2.90 (1 H, dd, $J$ = 12.9 Hz, 1.2 Hz), 4.12 (1 H, $H_3$, br m), 4.22 (1 H, $H_1$, br s), 6.00 (1 H, $H_9$, m), 6.85–7.05 (3 H, Ar, m), 7.18 (1 H, Ar-$H_5$, t, $J$ = 7.5 Hz); $^{13}C$ NMR δ −4.79, −4.75, −4.69, −4.6, −4.3, 11.1, 18.0, 18.1, 18.3, 19.2, 19.3, 21.4, 24.3, 25.1, 25.80, 25.84, 25.9, 28.7, 35.8, 38.97, 39.03, 39.8, 41.3, 42.0, 42.6, 50.2, 55.2, 64.1, 64.2, 69.7, 70.1, 88.2, 92.4, 115.5, 122.5, 126.3, 126.4, 127.9, 130.1, 133.3, 137.5, 140.4, 141.5; HRMS (CI, methane), $m/z$ 647.4668 (calcd for $C_{41}H_{67}O_2Si_2$, MH, 647.4680); MS (CI, methane), $m/z$ 648 (11, MH + 1), 647 (21, MH), 646 (10, M), 631 (22), 589 (14), 555 (6), 514 (base), 489 (3), 458 (6), 423 (4), 393 (39), 355 (2), 322 (43), 282 (17), 250 (5), 223 (2), 189 (5), 133 (12), 115 (21), 95 (4), 75 (67), 57 (20).

**1α,3β-Bis[(*tert*-butyldimethylsilyl)oxy]-22-[3-[1′-methyl-1′-[(trimethylsilyl)oxy]ethyl]phenyl]-24-nor-9,10-secochola-5(10),8-dien-6-yne (23d).** CD-ring triflate **22d** (0.032 g, 0.06 mmol) and A-ring enyne **14** (0.025 g, 0.06 mmol) were stirred in DMF (0.4 mL) in the presence of 1.5 mg of $Pd(PPh_3)_2(OAc)_2$, 1 mg of cuprous iodide, and 0.4 mL of $Et_2NH$. After stirring the mixture for 2 h at room temperature, water was added, and the mixture was extracted with ether. The combined ether extracts were washed with a 10% solution of HCl, a saturated solution of $NaHCO_3$, and brine. After drying ($MgSO_4$), the solvent was evaporated and the residue was filtered through a pad of silica gel (1% EtOAc–hexanes). The crude dienyne **23d** was purified by HPLC (Rainin Dynamax, 1.0 × 25 cm, 8 μm, 0.5% EtOAc/hexanes, 4 mL/min) to afford 42 mg (93%) of dienyne as a chromatographically homogeneous, colorless oil: $^1H$ NMR δ 0.09 (12 H, 4MeSi, s), 0.11 (9 H, 3MeSi, s), 0.76 (3 H, $C_{18}$-Me, s), 0.86 (3 H, $C_{21}$-Me, d, $J$ = 6.6 Hz), 0.90 (9 H, *t*-Bu, s), 0.91 (9 H, *t*-Bu, s), 1.59 and 1.60 (3 H and 3 H, diastereotopic $Me_2C$, two s), 1.93 (3 H, $C_{19}$-Me, s), 2.43 (1 H, dd, $J$ = 2.7 Hz, 15.9 Hz), 2.93 (1 H, dd, $J$ = 2.1 Hz, 13.2 Hz), 4.11 (1 H, $H_3$, br m), 4.21 (1 H, $H_1$, br s), 5.99 (1 H, $H_9$, m), 7.01 (1 H, Ar, d, $J$ = 6.6 Hz), 7.24 (3 H, Ar, m); $^{13}C$ NMR δ −4.8, −4.7, −4.6, −4.3, 2.3, 11.1, 14.1, 18.0, 18.1, 18.3, 19.2, 22.7, 24.3, 25.2, 25.8, 25.9, 26.0, 28.7, 31.6, 32.3, 32.7, 35.8, 39.0, 39.8, 41.3, 42.0, 42.8, 50.2, 55.1, 64.2, 70.0, 75.2, 88.2, 92.4, 115.5, 121.9, 122.5, 126.0, 127.3, 127.5, 133.3, 140.4, 140.9, 149.7; HRMS, $m/z$ 762.5207 (calcd for $C_{46}H_{78}O_3Si_3$, 762.5259); MS, $m/z$ 762 (2, M), 747 (4), 705 (2), 633 (5), 632 (18), 631 (44), 630 (78), 574 (5), 541 (10), 540 (18), 494 (9), 438 (2), 408 (13), 362 (3), 308 (2), 277 (4), 249 (4), 207 (4), 131 (20), 75 (base), 73 (37).

**Assays of Intestinal Calcium Absorption and Bone Calcium Mobilization.** Intestinal calcium absorption (ICA) and bone calcium mobilization (BCM) were measured in vivo, in the vitamin D deficient chick model system as described previously.[23] Twelve hours before assay, the chickens, which had been placed on a zero-calcium diet 48 h before assay, were injected intramuscularly with the vitamin metabolite or analogue dissolved in 0.1 mL of ethanol/1,2-propanediol (1:1, v/v) or with vehicle. At the time of assay, 4.0 mg of $^{40}Ca^{2+}$ + 5 μCi of $^{45}Ca^{2+}$ (New England Nuclear) were placed in the duodenum of the birds lightly anesthetized with ether. After 30 min, the birds were decapitated and the blood was collected. The radioactivity content of 0.2 mL of serum was measured in a liquid scintillation counter (Beckman LS8000) to determine the amount of $^{45}Ca^{2+}$ absorbed (which is a measure of ICA). BCM activity was estimated from the increase of total serum calcium concentration, as determined by atomic absorption spectrophotometry.

**1,25(OH)$_2$D$_3$ Receptor and D-Binding Protein Steroid Competition Assays.** The assay of competitive binding to the chick intestinal or HL-60 cell 1,25(OH)$_2$D$_3$ receptor was performed by using the hydroxylapatite batch assay.[24c] Increasing amounts of unlabeled 1,25(OH)$_2$D$_3$ or analogue were added to a constant amount of [$^3$H]-1,25(OH)$_2$D$_3$ and incubated with chick intestinal cytosol or HL-60 cells. The relative competitive index (RCI) for the analogues was calculated by plotting the percent maximum 1,25(OH)$_2$-[$^3$H]D$_3$ bound × 100 on the ordinate versus [competitor]/[1,25(OH)$_2$-[$^3$H]D$_3$] on the abscissa. The slope of the line obtained for the analogue is divided by the slope of the line obtained for 1,25(OH)$_2$D$_3$; multiplication of this value by 100 results in the RCI. By definition, the RCI for 1,25(OH)$_2$D$_3$ is 100.

The assay of competitive binding to the plasma transport protein (DBP) for vitamin D seco steroids, the D-binding protein, was carried out according to the general procedure of Roberts and

Norman.[25] In this assay, separation of bound-from-free steroid is effected by utilization of charcoal-coated dextran. The human DBP protein used in this assay was purchased from Sigma Chemical Co. (St. Louis). The resulting data are developed as described above so that a $RCI_{DBP}$ was calculated. By definition the $RCI_{DBP}$ for 25-hydroxyvitamin $D_3$ [25(OH)$D_3$] is 100 and the observed $RCI_{DBP}$ for 1,25(OH)$_2D_3$ is 0.15. Under some circumstances a normalized $RCI_{DBP\text{-}1,25\text{-}D}$ is utilized where the $RCI_{DBP}$ for 1,25(OH)$_2D_3$ is set to 100; under these conditions the $RCI_{DBP\text{-}1,25\text{-}D}$ for 25(OH)$D_3$ is 66,700.

**Colony Formation in Soft Agar and Analysis of Differentiation.** HL-60 cells were used in the tissue culture experiments; they are promelocytes from a patient with acute myeloid leukemia.[26c] The cells were cultured in tissue culture flasks (Lux Miles Laboratories, Inc., Naperville, IL) in alpha medium (Flow Laboratories, Inc., McLean, VA) with 10% fetal calf serum (FCS, Irvine Scientific, Santa Ana, CA). Only cells in logarithmic growth were used for plating experiments.

Bone marrow was obtained from healthy volunteers by aspiration after written consent was obtained. The mononuclear cells were isolated by centrifugation on Ficoll-Hypaque gradients, washed twice in 1 × phosphate-buffered saline (PBS), and suspended in alpha medium containing 10% FCS.[26a]

The vitamin D compounds used in the tissue culture experiments were dissolved in absolute ethanol at $10^{-3}$ M to create a stock solution, which was stored at −20 °C and protected from light. Dilutions of the stock solution were made in alpha medium without FCS. The maximum concentration of ethanol in the culture (0.1%) did not influence either cell growth or differentiation.

The scheme of analysis of vitamin D analogues is shown in Figure 2. Cells were plated in Lux culture dishes in a two-layer soft agar system according to previously described methods.[26a] The bottom layer contained 0.5% agar, and the top layer contained 0.3% (Difco Laboratories, Inc., Detroit, MI). The culture medium was alpha medium. For each layer, the stock agar solutions were mixed with prewarmed alpha medium containing 28.6% FCS and 1 mL was carefully pipetted into each culture dish. This mixture became semisolid at room temperature within 20 min. Compounds to be tested were mixed into the bottom layer. The colony-stimulating factor (CSF) was added to the bottom layer of cultures containing KG-1 cells. Recombinant GM-CSF (200 pM, maximally stimulating concentration, data not shown) was used as a source of CSF (20, generous gift of S. Clark, Genetics Institute, Boston MA). The cells to be tested were mixed with the top layer. Cell concentrations were $2 \times 10^3$/per plate for HL-60 leukemic cells and $2 \times 10^5$/per plate for normal bone marrow cells. Cultures were placed in a humidified atmosphere, 5% $CO_2$ at 37 °C for 10–12 days. All experiments contained three dishes per experimental point using control plates with either no CSF or only CSF. Each experiment was performed at least twice. Colonies (≥40 cells) were scored with an inverted microscope.

Induction of differentiation was measured by reduction of nitroblue tetrazolium (NBT).[26a] The cells were grown in liquid culture with alpha medium, 10% FCS for 6 days in humidified 7.0% $CO_2$ atmosphere at 37 °C. For NBT, cell suspension ($2 \times 10^5$ mL) was mixed with an equal volume of solution containing 1.25 mg/mL NBT (Sigma Chemical Co., St. Louis, MO), 17 mg/mL bovine serum albumin, and 1 μg/mL 12-O-tetradecanoylphorbol 13-acetate (Miles Laboratories) for 30 min at 37 °C. The cells were washed in PBS, cytocentrifuged, fixed in methanol for 5 min, and stained with gram sufranin for 10 min.

**Inhibition of 1α-Hydroxylase.** For kidney 1-hydroxylase assays, mitochondria were isolated from a 10% (in 0.25 M sucrose) homogenate of kidney tissue from 3–4-week-old vitamin D deficient chicks by standard differential centrifugation as described previously.[27] Assays of 25-OH-$D_3$-1-hydroxylase activity were carried out in a final volume of 2 mL containing 25 mM Tris-Cl, 75 mM sucrose, 10 mM malate, 5 mM $MgCl_2$, $5 \times 10^{-8}$ M 25-OH-[26,27-$^3$H]-vitamin $D_3$ (40 mCi/mmol), and 2.5–3.0 mL of mitochondrial protein. The vitamin D analogue of interest was added in ethanol just prior to the initiation of the reaction with substrate. Incubations were at 37 °C for 10 min and were terminated by the addition of $CHCl_3$/methanol (1:2, v/v).

Lipids were extracted by a modification of the method of Bligh and Dyer[31] and samples were prepared for chromatography on a 10 μm silica Radial-Pak cartridge. Radioactivity associated with specific metabolites of [$^3$H]-25-OH-$D_3$ was quantitated either by collection of individual fractions and subsequent liquid scintillation counting or by flow-through detection of radioactivity with a Beckman Model 171 flow-through detector. The percent of total radioactivity converted to [$^3$H]-1,25(OH)$_2D_3$ was determined and converted to pmol/10 min based on the known specific activity of the starting substrate.

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**Supplementary Material Available:** $^1$H NMR and selected $^{13}$C NMR spectra of all new compounds (22 pages). Ordering information is given on any current masthead page.

(31) Bligh, E. G.; Dyer, W. J. *Can. J. Biochem. Physiol.* **1959**, *37*, 911.