Two Novel Allenic Side Chain Analogues of 1α,25-Dihydroxyvitamin D_3[1a]

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Two new analogues of the steroid hormone 1α,25-dihydroxyvitamin D_3 (1) in which allenic functionality has been incorporated into the side chain have been synthesized. Both the 22*S* and 22*R* allene analogues (**2a** and **2b**) were each separately synthesized in nine steps from a common precursor **9**. While both analogues exhibited significant binding to the 1α,25-dihydroxyvitamin D_3 chick intestinal receptor, they showed low in vivo activity in stimulating intestinal calcium absorption in the chick.

Introduction

Analogues of 1α,25-dihydroxyvitamin D_3[2] (**1**, 1α,25-$(OH)_2$-D_3), which differ from the natural hormone only in the structure of the side chain, are of particular interest as potential cancer chemopreventive agents. Those analogues which exhibit high cell-differentiating ability and antiproliferative effects but low calcitropic activity are of greatest interest.[3,4] Analogues which incorporate rigid functionality in the side chain may also serve as useful probes of the conformational requirements of the side chain necessary for expression of biological activity. In this connection, we recently reported[5] the synthesis and biological activity of a series of analogues of 1α,25-$(OH)_2$-D_3 in which rigid functionality in the form of an aromatic ring had been incorporated into the side chain.

Here we wish to report the synthesis of two new epimeric analogues of 1α,25-$(OH)_2$-D_3, namely (22*S*)-**2a** and (22*R*)-**2b**, in which allenic functionality has been incorporated into the side chain. The presence of the axially stereogenic allenic units imparts a novel 180° twist in the side-chain orientation. It was intriguing to determine whether this stereochemical change would be translated into subtle, informative differences in the biological profile of the analogues.

Results and Discussion

Synthesis. Both allenic side chain analogues were prepared using the convergent synthetic strategy (Scheme I) first reported by Lythgoe[6] and later modified by others.[7-9] Our recently developed concise synthesis of the enantiomerically pure A-ring enyne **5**[9,10] from (*S*)-(+)-carvone renders this a particularly attractive synthetic route. Both the *R* and *S* allene CD-ring fragments **4a** and **4b** were prepared starting from the readily available Inhoffen-Lythgoe diol **6**.[11] Treatment of **6** with *p*-toluenesulfonyl chloride afforded the known tosylate **7**[11] which was then benzoylated to give **8**. Using a slight modification of Mouriño's reported procedure[12] for the oxidation of **8**, the aldehyde **9** was prepared in the absence of any C-20 epimer as determined by ^{1}H- and ^{13}C-NMR analysis. Treatment of **9** with the lithiated derivative of 2-methyl-3-butyn-2-ol *tert*-butyldimethylsilyl ether afforded a 2:1 diastereomeric mixture of propargylic alcohols **10a** (22*R*) and **10b** (22*S*). The major "Cram" diastereomer **10a** and the less polar "minor" diastereomer **10b** were separated by flash silica chromatography. Oxidation of the diastereomeric mixture of propargylic alcohols with PDC to the corresponding ketone **10c** followed by reduction with the complex $LiAlH_4$/(−)-*N*-methylephedrine/3,5-dimethylphenol[13] afforded a 11:1 ratio (91% combined yield) of **10a** to **10b**. This reagent is known to give predominantly the 22*R* isomer in the reduction of other propargylic alcohols in related vitamin D and steroid side chain systems.[12,14]

Treatment of **10a** with phenylsulfenyl chloride (NEt_3, ether, −78 °C) afforded exclusively the (22*R*)-allene sulfoxide **11a** via stereospecific [2,3]-sigmatropic rearrangement of the intermediate phenylsulfenate ester.[15] Similarly, the (22*S*)-allene sulfoxide **11b** was obtained exclusively from propargylic alcohol **10b**. In both cases a small percentage of a sulfoxide diastereomer could be observed. However, the diastereomeric sulfoxides (referred to as **11a**/**11a'** or **11b**/**11b'** in the Experimental Section) of each allene were found to react identically in the following desulfurization step. Individual reduction of the allene

(1) (a) This is paper 43 in the series Studies of Vitamin D (Calciferol) and Its Analogues. For part 42, see: Lee, A. S.; Norman, A. W.; Okamura, W. H. *J. Org. Chem.*, in press. (b) Department of Chemistry. (c) Division of Biomedical Sciences and the Department of Biochemistry.

(2) For a review of the chemistry and/or biochemistry of vitamin D, see: (a) Norman, A. W. *Vitamin D the Calcium Homeostatic Hormone*; Academic Press: New York, 1979. (b) DeLuca, H. F.; Paaren, H. E.; Schnoes, H. K. *Top. Curr. Chem.* **1979**, *83*, 1. (c) Georghiou, P. E. *Chem. Soc. Rev.* **1977**, *6*, 83. (d) Fieser, L. F.; Fieser, M. *Steroids*; Reinhold: New York, 1959. (e) Pardo, R.; Santelli, M. *Bull Chim. Soc. Fr.* **1985**, 98. (f) Jones, G. (guest Ed.) *Steroids* **1987**, *49*, 1. (g) Ikekawa, N. *Med. Res. Rev.* **1987**, *7*, 333. (h) Norman, A. W., Bouillon, R., Thomasset, M., Eds. *Vitamin D: Gene Regulation, Structure Function Analysis and Clinical Application*; Walter de Gruyter and Co.: Berlin, 1991.

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(7) (a) Castedo, L.; Mouriño, A.; Sarandeses, L. A. *Tetrahedron Lett.* **1986**, *27*, 1523. (b) Castedo, L.; Mascareñas, J. L.; Mouriño, A.; Sarandeses, L. A. *Tetrahedron Lett.* **1988**, *29*, 1203. (c) Cacchi, S.; Morera, E.; Ortar, G. *Synthesis* **1986**, 320.

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(9) (a) Okamura, W. H.; Aurrecoechea, J. M.; Gibbs, R. A.; Norman, A. W. *J. Org. Chem.* **1989**, *54*, 4072. (b) Curtin, M. L.; Okamura, W. H. *J. Am. Chem. Soc.* **1991**, *113*, 6958.

(10) For previous syntheses of **5**, see: (a) Reference 6a. (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.

(11) (a) Lythgoe, B.; Roberts, D. A.; Waterhouse, I. *J. Chem. Soc., Perkin Trans. 1* **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) See also ref 8.

(12) See ref 7a. Improvements on this procedure will soon be reported (Mouriño, A. Personal communication).

(13) (a) Vigneron, J.-P.; Bloy, V. *Tetrahedron Lett.* **1979**, 2683. (b) Vigneron, J.-P.; Jacquet, I. *Tetrahedron* **1976**, *32*, 939.

(14) Sardina, F. J.; Mouriño, A.; Sarandeses, L. A. *Tetrahedron Lett.* **1983**, *24*, 4477.

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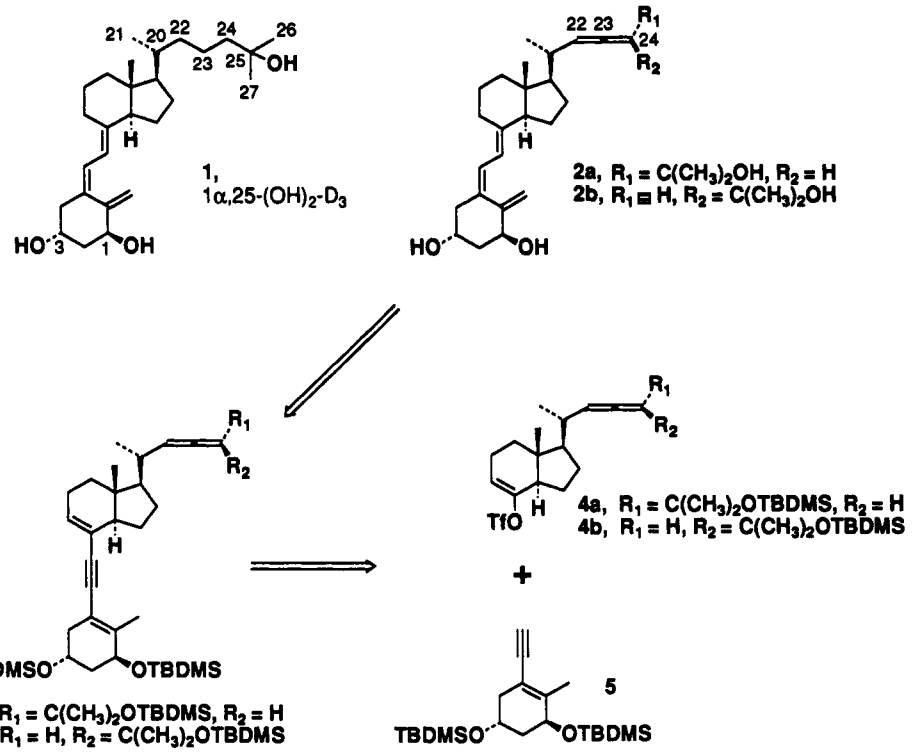
Scheme I



sulfoxides (**11a**/**11a**′ and **11b**/**11b**′ to **12a** and **12b**, respectively) with retention of configuration was achieved by treatment with *t*-BuLi (6 equiv, ether) in the presence of an in situ proton source (MeOH, 3 equiv) according to the procedure recently reported by this laboratory.[16] In this case excess *t*-BuLi was employed to compensate for reagent consumed in the simultaneous deprotection to the alcohol of the secondary benzoyloxy group under the reaction conditions. The conditions were tailored to minimize formation of side products suspected to originate from deprotonation of the allenic protons H_{22} or H_{24}, which seemed to be followed by more deep-seated rearrangement reactions. To summarize, the absolute configurations of the allenic moieties in **12a** and **12b** (and their subsequent transformation products leading ultimately to **2a** and **2b**, respectively) follow from the C-22 absolute configurations assigned to **10a** and **10b** and to two known stereospecific processes.[15,16] The first is the known stereochemical course of the intramolecular transfer of a central chiral element of a propargyl alcohol to an axial chiral element of an allene via the sulfenate ester–sulfoxide [2,3]-sigmatropic rearrangement (cf. **10a** to **11a** and **10b** to **11b**).[15] The second is the known retention of configuration characteristic of the method used for reduction of allenic sulfoxides (cf. **11a** to **12a** and **11b** to **12b**).[16]

The alcohols **12a**/**12b** were oxidized with PDC to ketones **13a**/**13b** followed by treatment with LHMDS to generate the kinetic enolates which were trapped with *N*-phenyltrifluoromethanesulfonimide to afford the required enol triflates **4a**/**4b**. When LDA was used as the base in this reaction none of the desired enol triflate was obtained. Again it was suspected that competing deprotonation of the allene was leading to other products, which were not examined further.

Coupling of the A-ring enyne **5** with the CD-ring enol triflates **4a**/**4b** was effected using palladium catalyst [$Pd(PPh_3)_2(OAc)_2$] with copper cocatalyst (CuI) under mild conditions (DMF, Et_2NH, room temperature) according to the method of Mouriño.[12] Careful Lindlar hydrogenation of the resulting dienynes **3a**/**3b** afforded the trienes **14a**/**14b** without reduction of the allene functionality. Thermally induced (~100 °C, isooctane) [1,7]-sigmatropic H-shift followed by deprotection of the hydroxyl groups (TBAF, THF, room temperature) afforded the pure *S*- and *R*-allene vitamin analogues **2a**/**2b** after HPLC purification.

Biological Evaluation. With analogues **2a** (analogue HQ[17]) and **2b** (analogue HR[17]) in hand a determination of their relative abilities to mediate intestinal calcium absorption (ICA) and bone calcium mobilization (BCM) was carried out in our vitamin D deficient (–D) chick bioassay system as previously described.[18] Each of these assays includes a negative control (response of –D chicks) and positive controls of 3.25 nmol of vitamin D_3 administered 48 h before assay as well as series of graded, increasing doses of either 1α,25-$(OH)_2$-D_3 (usually in the range of 65–1300 pmol) or the test allene analogue administered 14 h before assay. In order to compare the ICA and BCM activity of the allene analogues to that of 1α,25-$(OH)_2$-D_3, a plot of log dose vs either ICA or BCM was constructed. The doses of analogue required to achieve a response equivalent to 100 pmol of 1α,25-$(OH)_2$-D_3 was determined.

(15) (a) Haces, A.; van Kruchten, E. M. G. A.; Okamura, W. H. *Isr. J. Chem.* **1985**, *26*, 140. (b) Haces, A.; van Kruchten, E. M. G. A.; Okamura, W. H. *Tetrahedron Lett.* **1982**, *23*, 2707. For some earlier reports, see the following: (c) Gibbs, R. A.; Okamura, W. H. *J. Am. Chem. Soc.* **1988**, *110*, 4062. (d) Okamura, W. H.; Peter, R.; Reischl, W. *J. Am. Chem. Soc.* **1985**, *107*, 1034. (e) Braverman, S.; Stabinsky, Y. *Isr. J. Chem.* **1967**, *5*, 125. (f) Smith, G.; Stirling, C. J. M. *J. Chem. Soc. C* **1971**, 1530. (g) Cinquini, M.; Colonna, S.; Cozzi, F.; Stirling, C. J. M. *J. Chem. Soc., Perkin Trans. 1* **1976**, 2061.

(16) (a) Theobald, P. G.; Okamura, W. H. *J. Org. Chem.* **1990**, *55*, 741. (b) See also: Theobald, P. G.; Okamura, W. H. *Tetrahedron Lett.* **1987**, *28*, 6565. (c) For some earlier reports, see refs 15a and 15b as well as the following: Neef, G.; Eder, U.; Seeger, A. *Tetrahedron Lett.* **1980**, *21*, 903.

(17) The code letters HQ and HR for **2a** and **2b**, respectively, are used in the Riverside laboratories for a comprehensive data base of analogues and their biological activities currently under development.

(18) (a) Hibberd, K. A.; Norman, A. W. *Biochem. Pharm.* **1969**, *18*, 2347. (b) Norman, A. W.; Wong, R. G. *J. Nutrition* **1972**, *102*, 1709. (c) For a recent description of biological evaluations of a related series of side chain analogues, see ref 5.

Thus, if 100 pmol of an analogue were required to produce an ICA response equivalent to 100 pmol of the natural steroid hormone, 1α,25-$(OH)_2$-D_3, then it would be stated to have 100% activity; likewise if a 1000 pmol dose of the analogue were required, then its activity would be only 10% that of 1α,25-$(OH)_2$-D_3. Thus, the allene **2a** (analogue HQ) was found to have an ICA activity of <2.4% and BCM activity of 10%, while allene **2b** (analogue HR) had activities of 2.4% and 35%, respectively.

The analogues were also evaluated in terms of their ability to bind to the chick intestinal 1α,25-$(OH)_2$-D_3 receptor, under in vitro conditions. In this steroid competition assay the relative competitive index (RCI)[19] is determined. By definition the RCI of 1α,25-$(OH)_2$-D_3 is set to 100%. Allene **2a** (analogue HQ) was found to have a RCI of 21% ± 3% while the RCI of allene **2b** (analogue HR) was 52% ± 2%. Thus, these diastereomeric allenes exhibit greater than 2-fold differential binding to the chick intestinal receptor and, as has been noted with some other 1α,25-$(OH)_2$-D_3 side-chain analogues biologically assayed by these laboratories,[20] including the enyne analogue **16** and the cyclopropyl analogue **17** (see the comparison with **1**), both allenes bind better to the chick intestinal receptor than would be predicted on the basis of their in vivo biological activity assays (ICA). It is also interesting that their activity in bone (BCM) is greater than that in the intestine (ICA). While the physiological basis for these differences is not known, it may well prove that these allenes could by virtue of their reasonable affinity for the 1α,25-$(OH)_2$-D_3 receptor combined with their low ICA activity have some clinical utility.

Experimental Section[21]

(22*S*)-1α,25-Dihydroxy-22,23,23,24-tetradehydrovitamin D_3 (2a). A solution of previtamin **14a** (12.0 mg, 15.9 mmol) in isooctane (8.0 mL) was refluxed (~100 °C) under an argon atmosphere for 2.4 h. The solvent was removed under vacuum to afford a colorless residue, which was determined to be an 88:12 inseparable mixture of vitamin **15a** and previtamin **14a**. A solution of this mixture in THF (1.0 mL) was treated with tetrabutylammonium fluoride (275 μL, 1.0 M in THF, 0.275 mmol) at room temperature for 15 h, protected from the light. The reaction was quenched by the addition of brine (2 mL), and the mixture was extracted with ethyl acetate (4 × 2 mL). The combined organic extracts were dried ($MgSO_4$) and concentrated and the crude product passed through a short pad of silica gel. Purification was effected by HPLC (Rainin Dynamax, 1 × 25 cm, 8 μm, 4 mL/min, 100% ethyl acetate) to afford after drying 4.7 mg (71%) of the vitamin **2a** as a viscous colorless oil. ^{1}H-NMR: δ 0.57 (3 H, C_{18}-Me, s), 1.08 (3 H, C_{21}-Me, d, J = 6.6 Hz), 1.34 (6 H, $C_{26,27}$-$2CH_3$, s), 2.32 (1 H, $H_{4\beta}$, dd, J = 13.2, 6.0 Hz), 2.60 (1 H, $H_{4\alpha}$, dd, J = 13.2, 3.0 Hz), 2.83 (1 H, $H_{9\beta}$, dd, J = 11.7, 3.0 Hz), 4.23 (1 H, H_3, m, W = 20 Hz), 4.43 (1 H, H_1, m, W = 12 Hz), 5.00 (1 H, H_{19Z}, narrow m), 5.33 (1 H, H_{19E}, narrow m), 5.28–5.35 (2 H, H_{22} and H_{24}, m, partially obscured by H_{19E}), 6.02 and 6.38 (2 H, H_6 and H_7, AB pattern, J = 11.2 Hz).

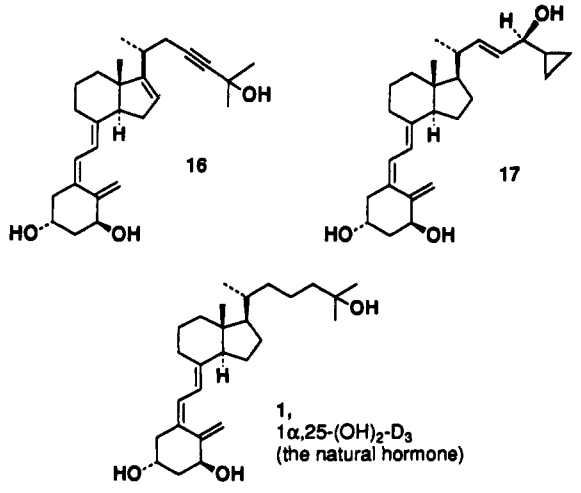
(22*R*)-1α,25-Dihydroxy-22,23,23,24-tetradehydrovitamin D_3 (2b). A solution of previtamin **14b** (15 mg, 19.9 mmol) in isooctane (10 mL) was refluxed (~100 °C) for 2 h under an argon atmosphere. The solvent was removed under vacuum to give a colorless residue, which after HPLC separation (Rainin Dynamax, 0.1% ethyl acetate/hexanes) afforded a 9:1 mixture of vitamin **15b** and previtamin **14b**. The mixture was dissolved in THF (1 mL) and treated with tetrabutylammonium fluoride (273 μL, 1.0 M in THF, 0.273 mmol) at room temperature for 15 h, protected from the light. The reaction was quenched by the addition of brine (2 mL), and then the mixture was extracted with ethyl acetate (4 × 2.0 mL). The combined organic extracts were dried ($MgSO_4$), filtered, and concentrated. Purification was effected by short column flash chromatography (silica gel, 100% ethyl acetate) followed by HPLC separation (Rainin Dynamax, 100% ethyl acetate) to afford after vacuum drying vitamin **2b** (5.4 mg, 66%) as a colorless foam. ^{1}H-NMR: δ 0.57 (3 H, C_{18}-Me, s), 1.09 (3 H, C_{21}-Me, d, J = 6.6 Hz), 1.34 (6 H, $C_{26,27}$-$2CH_3$, s), 2.32 (1 H, $H_{4\beta}$, dd, J = 13.2, 6.0 Hz), 2.60 (1 H, $H_{4\alpha}$, dd, J = 13.2, 3.0 Hz), 2.83 (1 H, $H_{9\beta}$, dd, J = 12.0, 3.0 Hz), 4.23 (1 H, H_3, m, W = 20 Hz), 4.43 (1 H, H_1, m, W = 12 Hz), 5.00 (1 H, H_{19Z}, s), 5.33 (1 H, H_{19E}, s), 5.26–5.35 (2 H, H_{22} and H_{24}, m, partially obscured by H_{19E}), 6.02 and 6.38 (2 H, H_6 and H_7, AB pattern, J = 11.2 Hz).

(22*S*)-1α,25-Bis[(*tert*-butyldimethylsilyl)oxy]-6,7,22,23,23,24-hexadehydroprevitamin D_3 *tert*-Butyldimethylsilyl Ether (3a). Bis(triphenylphosphine)palladium(II) acetate (5.0 mg, 6.7 mmol) and copper(I) iodide (4.8 mg, 25.2 mmol) were added at ambient temperature to a mixture of enol triflate **4a** (54.8 mg, 0.105 mmol), enyne **5** (48.0 mg, 0.126 mmol) in DMF (1.0 mL), and diethylamine (1.0 mL) under an argon atmosphere. The mixture was stirred at room temperature for 2.5 h after which time ether (10 mL) was added and the mixture washed with brine (3 × 10 mL). The organic layer was dried ($MgSO_4$), filtered, and concentrated to afford a dark brown residue. The crude product was passed down a short silica gel column (15% ethyl acetate/hexanes) followed by HPLC separation (Rainin Dynamax, 1.0 × 25 cm, 8 μm, 1% ethyl acetate/hexanes) to afford, after drying, spectroscopically homogeneous dienyne **3a** (59 mg, 75%) as a colorless oil. ^{1}H-NMR: δ 0.06 (6 H, $SiMe_2$, s), 0.07 (6 H, $Si\text{-}Me_2$, s), 0.09 (6 H, $SiMe_2$), 0.72 (3 H, C_{18}-Me, s), 0.85 (9 H, *t*-Bu, s), 0.88 (9 H, *t*-Bu, s), 0.89 (9 H, *t*-Bu, s), 1.09 (3 H, C_{21}-Me, d, J = 6.6 Hz), 1.30 (3 H, $C_{26,27}$-CH_3, s), 1.31 (3 H, $C_{26,27}$-CH_3, s), 1.90 (3 H, C_{19}-Me, br s), 4.09 (1 H, H_3, broad m, W = 15 Hz), 4.19 (1 H, H_1, m), 5.18 (1 H, H_{22}, dd, J = 6.6, 6.6 Hz), 5.28 (1 H, H_{24}, dd, J = 6.6, 1.8 Hz), 5.97 (1 H, H_9, narrow m).

(22*R*)-1α,25-Bis[(*tert*-butyldimethylsilyl)oxy]-6,7,22,23,23,24-hexadehydroprevitamin D_3 *tert*-Butyldimethylsilyl Ether (3b). Bis(triphenylphosphine)palladium(II) acetate (6.0 mg, 8.1 mmol) and copper(I) iodide (5.8 mg, 30.4 mmol) were added at ambient temperature to a mixture of enol triflate **4b** (64 mg, 0.123 mmol), enyne **5** (56 mg, 0.147 mmol) in DMF (1.2 mL), and diethylamine (1.2 mL) under an argon atmosphere. The mixture was stirred at room temperature for 2.5

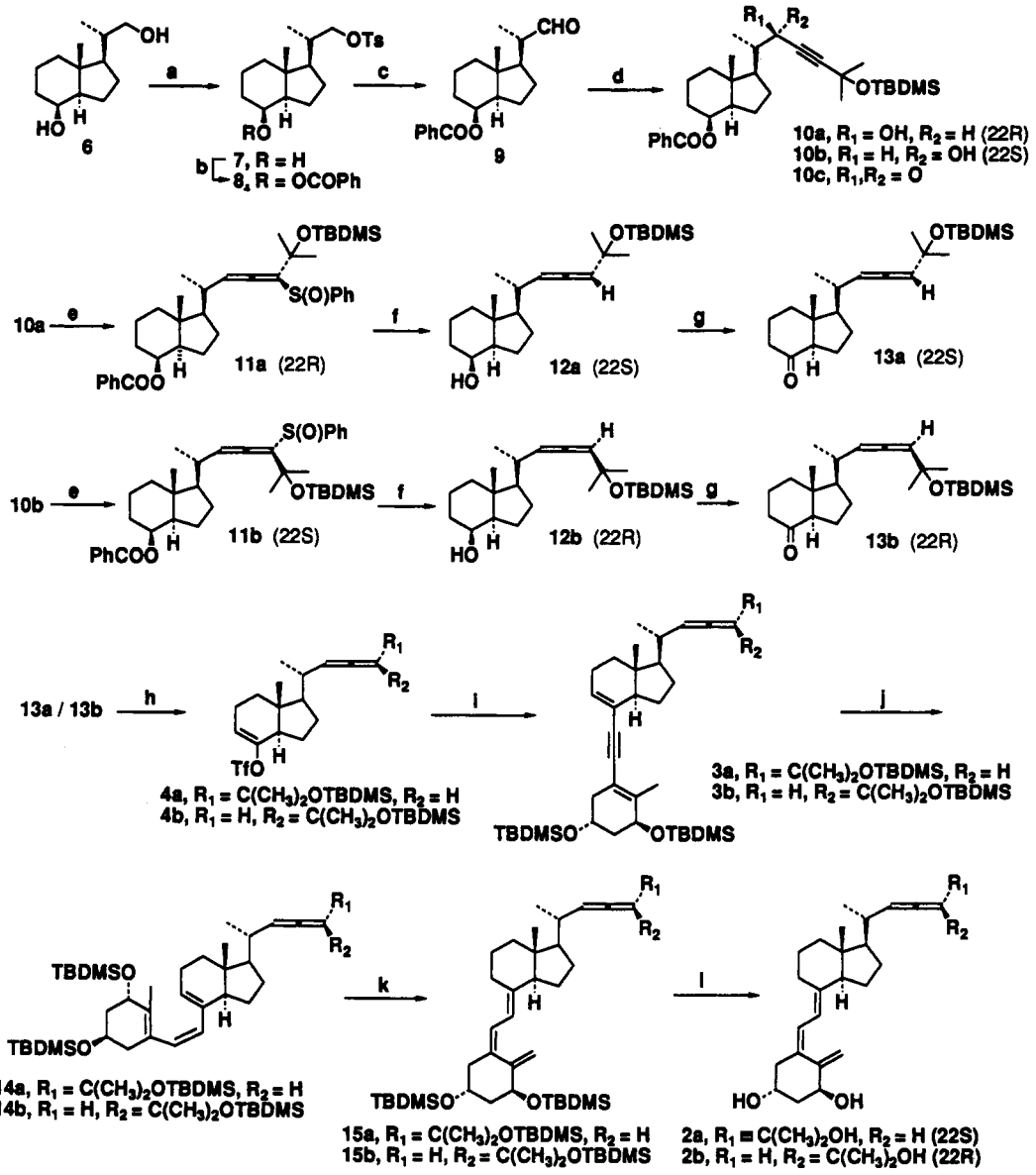
(19) (a) Procsal, D. A.; Okamura, W. H.; Norman, A. W. *J. Biol. Chem.* **1975**, *250*, 8382. (b) Procsal, D. A.; Okamura, W. H.; Norman, A. W. *Am. J. Clinical Nutr.* **1976**, *29*, 1271. (c) Wecksler, W. R.; Norman, A. W. *Methods in Enzymology: Vitamins and Co-Enzymes* **1980**, *67*, 488.

(20) The analogues **16** and **17** (as compared to **1**, 1α,25-dihydroxyvitamin D_3) exhibited little in vivo ICA and BCM, but bound well to the chick intestinal receptor. See the following: (a) Zhou, J.-Y.; Norman, A. W.; Lubbert, M.; Collins, E. D.; Uskokovic, M. R.; Koeffler, H. P. *Blood* **1989**, *74*, 82. (b) Zhou, J.-Y.; Norman, A. W.; Chen, D.-L.; Sun, G.-W.; Uskokovic, M. R.; Koeffler, H. P. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 3929.



(21) Spectral and other analytical data are given in the supplementary material. Essential ^{1}H-NMR spectral data are presented in the Experimental Section as well. General experimental procedures are also presented in the supplementary material, and the purity of all new compounds were judged by a combination of HPLC and ^{1}H- and ^{13}C-NMR analysis before mass spectral determination. For all new compounds, the level of purity is indicated by the inclusion of copies of ^{1}H-NMR spectra and selected ^{13}C-NMR spectra in the supplementary material.

Scheme II[a]



[a] Reagents: (a) TsCl, pyridine, 90%; (b) PhCOCl, pyridine, 93%; (c) DMSO, *s*-collidine, 140 °C, 65%; (d) $LiCCC(CH_3)_2OTBDMS$, THF, 72% (2:1 mixture, (*R*)-**10a**/(*S*)-**10b**); (e) PhSCl, NEt_3, ether, −78 °C, 78–90%; (f) *t*-BuLi, MeOH, ether, −78 °C, 10 min, 42–50%; (g) PDC, PTA, CH_2Cl_2, 75–80%; (h) LHMDS, THF, −78 °C; $PhNTf_2$, −78 to 0 °C, 15 h, 80–85%; (i) A-ring enyne **5**, $Pd(PPh_3)_2(OAc)_2$, CuI, Et_2NH, DMF, rt, 2 h, 75–93%; (j) H_2-Lindlar, quinoline, hexanes, 70–81%; (k) reflux isooctane, 12 h; (l) TBAF, THF, rt, 15 h, 68–71%.

h after which ether (10 mL) was added and the mixture washed with brine (3 × 10 mL). The organic layers was dried ($MgSO_4$), filtered, and concentrated to afford a dark brown residue. Purification was effected by short-path flash chromatography (silica gel, 15% ethyl acetate/hexanes) followed by HPLC separation (Rainin Dynamax, 1.0 × 25 cm, 8 μm, 1% ethyl acetate/hexanes) to afford, after drying, spectroscopically homogeneous dienyne **3b** (86 mg, 93%) as a colorless oil. ^{1}H-NMR: δ 0.06 (6 H, $SiMe_2$, s), 0.07 (6 H, $SiMe_2$, s), 0.09 (6 H, $SiMe_2$, s), 0.72 (3 H, C_{18}-Me, s), 0.85 (9 H, *t*-Bu, s), 0.88 (9 H, *t*-Bu, s), 0.89 (9 H, *t*-Bu, s), 1.09 (3 H, C_{21}-Me, d, J = 6.6 Hz), 1.29 (3 H, $C_{26,27}$-CH_3, s), 1.30 (3 H, $C_{26,27}$-CH_3, s), 1.89 (3 H, C_{19}-Me, br s), 4.1 (1 H, H_3, br m), 4.19 (1 H, H_1, m), 5.15 (1 H, H_{22}, dd, J = 6.6 Hz, 6.6 Hz), 5.27 (1 H, H_{24}, dd, J = 6.6 Hz, 1.8 Hz), 5.97 (1 H, H_9, narrow m).

(22*S*)-25-[(*tert*-Butyldimethylsilyl)oxy]-de-*A*,*B*-cholesta-8,22,23-trien-8-yl Trifluoromethanesulfonate (4a). A solution of LHMDS (0.314 mL, 1.0 M in THF, 0.314 mmol) was added to a solution of ketone **13a** (49.0 mg, 0.126 mmol) in THF (2.0 mL) at −78 °C under an argon atmosphere. The mixture was stirred for 30 min and then transferred to an ice bath with stirring for a further 6 h. After the mixture was recooled to −78 °C a solution of $PhN(Tf)_2$ (112 mg, 0.314 mmol) in THF (1.0 mL) was added via cannula. After the solution was stirred for 10 min the reaction vessel was transferred to an ice bath and stirred for 15 h (warming to room temperature after 4–5 h). The reaction mixture was quenched with saturated NH_4Cl solution (5 mL) and ether (5 mL), and the layers were separated. The aqueous layer was extracted with ether (2 × 10 mL), the combined organic extracts were washed with saturated $NaHCO_3$ and brine and dried ($MgSO_4$), and the solvent was evaporated. Purification was effected by short column flash chromatography (silica gel, 15% ethyl acetate/hexanes) followed by HPLC (Rainin Dynamax, 1.0 × 25 cm, 8 μm, 4% ethyl acetate/hexanes) to afford after vacuum drying, the enol triflate **4a** (55 mg, 83%) as a colorless, viscous liquid. ^{1}H-NMR: δ 0.07 (6 H, $SiMe_2$, s), 0.79 (3 H, C_{18}-Me, s), 0.85 (9 H, *t*-Bu, s), 1.08 (3 H, C_{21}-Me, d, J = 6.6 Hz), 1.30 (3 H, $C_{26,27}$-CH_3, s), 1.31 (3 H, $C_{26,27}$-CH_3, s), 5.17 (1 H, H_{22}, dd, J = 6.6, 6.6 Hz), 5.30 (1 H, H_{24}, dd, J = 6.6, 1.8 Hz), 5.57 (1 H, H_9, ddd, J = 3.3, 3.3, 3.3 Hz).

(22*R*)-25-[(*tert*-Butyldimethylsilyl)oxy]-de-*A*,*B*-cholesta-8,22,23-trien-8-yl Trifluoromethanesulfonate (4b). A solution of LHMDS (0.393 mL, 1.0 M in THF, 0.393 mmol) was added to a cold solution (−78 °C) of ketone **13b** (61 mg, 0.157

mmol) in THF (3.0 mL) under an argon atmosphere. The mixture was stirred for 30 min at −78 °C and then transferred to an ice bath and stirring continued for 6 h. After the mixture was recooled to −78 °C, a solution of $PhN(Tf)_2$ (0.140 g, 0.393 mmol) in THF (1.0 mL) was added via cannula. After the solution was stirred for 10 min the reaction vessel was transferred to an ice bath and the mixture was stirred for 15 h (warming to room temperature after 4–5 h). The reaction mixture was quenched with saturated NH_4Cl solution (5 mL) and ether (5 mL), and the layers were separated. The aqueous layer was extracted with ether (3 × 10 mL), and the combined organic extracts were washed with saturated $NaHCO_3$ and brine, dried ($MgSO_4$), filtered, and concentrated. Purification was effected by short column flash chromatography (silica gel, 15% ethyl acetate/hexanes) followed by HPLC separation (Rainin Dynamax, 1.0 × 25 cm, 8 μm, 4 mL/min, 4% ethyl acetate/hexanes) to afford after drying the enol tiflate **4b** (70 mg, 85%) as a colorless oil. ^{1}H-NMR: δ 0.07 (6 H, $SiMe_2$, s), 0.78 (3 H, C_{18}-Me, s), 0.85 (9 H, *t*-Bu, s), 1.09 (3 H, C_{21}-Me, d, J = 6.6 Hz), 1.29 (3 H, $C_{26,27}$-CH_3, s), 1.30 (3 H, $C_{26,27}$-CH_3, s), 5.13 (1 H, H_{22}, dd, J = 6.6, 6.6 Hz), 5.28 (1 H, H_{24}, dd, J = 6.0, 1.8 Hz), 5.58 (1 H, H_9, ddd, J = 3.3, 3.3, 3.3 Hz).

22-Oxo-de-*A*,*B*-23,24-dinorcholan-8β-yl Benzoate (9). A solution of the benzoyloxy tosylate 8[11d] (4.40 g, 9.36 mmol) and *s*-collidine (Kugelrohr distilled) in DMSO (50 mL, previously heated to 140 °C under a strong stream of argon and allowed to cool back to room temperature) was heated to 140 °C under an argon atmosphere for 15 min. The mixture was cooled rapidly (water bath) and water (50 mL) added. The mixture was extracted immediately with ethyl acetate (3 × 75 mL), washed with brine, and dried ($MgSO_4$) and solvent evaporated to give a pale yellow oily residue. The crude product was purified by flash silica chromatography (7.5–10% ethyl acetate/hexanes) to afford the aldehyde (1.90 g, 65%). It was found that the literature preparation[11d] resulted in the formation of the desired aldehyde **9** contaminated by 10–26% of its inseparable C_{20} epimer. ^{1}H-NMR: δ 1.09 (3 H, C_{18}-Me, s), 1.14 (3 H, C_{21}-Me, d, J = 6.9 Hz), 2.39 (1 H, C_{20}-H, m), 5.43 (1 H, C_8-H, br s), 7.44 (2 H, Ar-H, m), 7.56 (1 H, Ar-H, m), 8.04 (2 H, Ar-H, m), 9.59 (1 H, CHO, d, J = 3.0 Hz).

(22*R*)-25-[(*tert*-Butyldimethylsilyl)oxy]-22-hydroxy-de-*A*,*B*-cholest-23-yn-8β-yl Benzoate (10a; Major, More Polar) and Its (22*S*)-Epimer 10b (Minor, Less Polar). Method A. To a solution of 2-methyl-3-butyn-2-ol *tert*-butyldimethylsilyl ether (1.81 g, 9.20 mmol) in THF (70 mL) at 0 °C under an argon atmosphere was added *n*-BuLi (1.6 M in hexanes, 5.35 mL, 0.56 mmol). After being stirred for 20 min the solution was cooled to −78 °C and a solution of the aldehyde **9** (1.92 g, 6.11 mmol) in THF (20 mL) was added slowly via cannula. Stirring was continued at −78 °C for 40 min, after which the reaction was quenched with saturated NH_4Cl solution and the reaction mixture allowed to warm to room temperature. The organic layer was separated, and the remaining aqueous layer was extracted with ether. The combined organic extracts were dried ($MgSO_4$), filtered, and concentrated to afford a crude 2:1 mixture of the diastereomers **10a** (major, more polar) and **10b** (minor, less polar). The diastereomers were separated by flash silica chromatography (gradient elution 7.5% to 12% ethyl acetate/hexanes) to afford 0.80 g of the higher R_f diastereomer **10b** (minor, less polar) and 1.51 g of the lower R_f diastereomer **10a** (major, more polar) (combined yield 72%). Both isomers after vacuum drying could be obtained as spectroscopically and chromatographically homogeneous materials. **10a**. ^{1}H-NMR: δ 0.15 (6 H, Me_2Si, s), 0.86 (9 H, *t*-Bu, s), 1.06 (3 H, C_{18}-Me, s), 1.13 (3 H, C_{21}-Me, d, J = 6.3 Hz), 1.46 (6 H, $C_{26,27}$-$2CH_3$, s), 4.50 (1 H, H_{22}, dd, J = 6.3, 1.2 Hz), 5.4 (1 H, H_8, broad s), 7.44 (2 H, ArH, m), 7.56 (1 H, ArH, m), 8.05 (2 H, ArH, d, J = 7.5 Hz). **10b**. ^{1}H-NMR: δ 0.17 (6 H, Me_2Si, s), 0.87 (9 H, *t*-Bu, s), 1.07 (3 H, C_{18}-Me, s), 1.07 (3 H, C_{21}-Me, d, J = 6.3 Hz), 1.47 (6 H, $C_{26,27}$-$2CH_3$, s), 4.47 (1 H, H_{22}, m), 5.4 (1 H, H_8, broad s), 7.45 (2 H, ArH, m), 7.56 (1 H, ArH, m), 8.05 (2 H, ArH, d, J = 7.5 Hz).

Method B (via Reduction of Ketone 10c). A solution of (−)-*N*-methylephedrine (74 mg, 0.41 mmol) in ether (2.5 mL) was added slowly (15 min) at room temperature to a solution of lithium aluminum hydride (412 μL, 1.0 M in THF, 0.41 mmol) and the mixture stirred for 30 min. A solution of 3,5-dimethylphenol (101 mg, 0.82 mmol) in ether (2 mL) was added over a period of 10 min and stirring continued for 2 h. The resulting mixture was cooled to −15 °C, and a solution of ketone **10c** (105 mg, 0.21 mmol) in ether (1.5 mL) was added over 10 min. After being stirred at −15 °C for 30 min the mixture was quenched with 5% NaOH (5 mL) and ether (5 mL), and the layers were separated. The aqueous layer was extracted with ether, and the combined organic extracts were washed with 5% HCl, 5% NaOH, and water. The organic layer was dried ($MgSO_4$), filtered, and concentrated to afford a crude 11:1 mixture of the 22*R* and 22*S* diastereomers **10a** (more polar) and **10b** (less polar). The diastereomers were separated by flash silica chromatography (7.5% to 12% ethyl acetate/hexanes) to afford 88 mg of **10a** and 8 mg of **10b** (combined yield 91%).

25-[(*tert*-Butyldimethylsilyl)oxy]-22-oxo-de-*A*,*B*-cholest-23-yn-8β-yl Benzoate (10c). To a suspension of PDC (481 mg, 1.28 mmol) in CH_2Cl_2 (3 mL) was added a solution of a mixture of epimeric propargylic alcohol **10a** and **10b** (262 mg, 0.51 mmol) in CH_2Cl_2 (3 mL + 2 mL washings) followed by pyridinium trifluoroacetate (39 mg, 0.20 mmol). The mixture was stirred at room temperature for 8 h. The reaction mixture was passed through a column of Celite (eluted with 3 × 10 mL of CH_2Cl_2) and then concentrated under reduced pressure. The dark brown residue was extracted with hexanes and then concentrated. Purification was effected by flash silica chromatography (10% ethyl acetate/hexanes) to afford, after drying, spectroscopically pure ketone **10c** (230 mg, 88%) as a colorless oil. ^{1}H-NMR: δ 0.19 (6 H, Me_2Si, s), 0.88 (9 H, *t*-Bu, s), 1.07 (3 H, C_{18}-Me, s), 1.24 (3 H, C_{21}-Me, d, J = 6.9 Hz), 1.53 (6 H, $C_{26,27}$-$2CH_3$, s), 2.58 (1 H, H_{20}, dq, J = 10.5, 6.3 Hz), 5.43 (1 H, H_8, br s), 7.45 (2 H, ArH, t, J = 7.5 Hz), 7.56 (1 H, ArH, t, J = 7.5 Hz), 8.04 (2 H, ArH, m).

(22*R*)-25-[(*tert*-Butyldimethylsilyl)oxy]-24-(phenylsulfinyl)-de-*A*,*B*-cholesta-22,23-dien-8β-yl Benzoate (11a). A solution of freshly generated phenylsulfenyl chloride in CCl_4 (2.7 M, 170 mL, 0.45 mmol) was added via syringe to a cooled solution (−78 °C) of the propargylic alcohol **10a** (major, more polar alcohol epimer; 0.202 g, 0.394 mmol) and freshly distilled triethylamine (110 mL, 0.79 mmol) in ether (5 mL). The solution was stirred for 2 h and then allowed to warm to room temperature followed by quenching with water (5 mL). The organic layer was separated and the remaining aqueous layer extracted with ether (3 × 10 mL). The combined organic extracts were washed with saturated $NaHCO_3$ solution, dried ($MgSO_4$), filtered, and then concentrated under reduced pressure. Flash silica chromatography afforded the major phenyl sulfoxide diastereomer **11a** (0.21 g, 86%) and a slower moving diastereomer **11a′** (10 mg, 4%). Upon storing this mixture at room temperature for several weeks, an approximate 1:1 ratio of **11a**:**11a′** was obtained. This equilibration is characteristic of the epimerization process at sulfur characteristic of related allenic sulfoxides occurring via two consecutive [2,3]-sigmatropic shifts.[22] **11a**. ^{1}H-NMR: δ 0.15 (3 H, MeSi, s), 0.20 (3 H, MeSi, s), 0.88 (3 H, C_{21}-Me, d, J = 6.6 Hz), 0.94 (9 H, *t*-Bu, s), 0.99 (3 H, C_{18}-Me, s), 1.43 (3 H, $C_{26,27}$-CH_3, s), 1.66 (3 H, $C_{26,27}$-CH_3, s), 5.15 (1 H, H_{22}, d, J = 8.1 Hz), 5.40 (1 H, $H_{8\alpha}$, broad s), 7.4 (5 H, ArH, m), 7.5 (3 H, ArH, m), 8.0 (2 H, ArH, m). **11a′**. ^{1}H-NMR: δ 0.17 (3 H, MeSi, s), 0.20 (3 H, MeSi, s), 0.56 (3 H, C_{21}-Me, d, J = 6.6 Hz), 0.93 (9 H, C_{18}-Me, s), 0.95 (9 H, *t*-Bu, s), 1.48 (3 H, $C_{26,27}$-CH_3, s), 1.63 (3 H, $C_{26,27}$-CH_3, s), 5.4 (1 H, $H_{8\alpha}$, broad s), 5.59 (1 H, H_{22}, d, J = 7.5 Hz), 7.45 (5 H, ArH, m), 7.52 (1 H, ArH, m), 7.65 (2 H, ArH, m), 8.02 (2 H, ArH, m).

(22*S*)-25-[(*tert*-Butyldimethylsilyl)oxy]-24-(phenylsulfinyl)-de-*A*,*B*-cholesta-22,23-dien-8β-yl Benzoate (11b). A solution of freshly generated phenylsulfenyl chloride in CCl_4 (0.955 mL, 2.1 M, 1.98 mmol) was added via syringe to a cold solution (−78 °C) of the propargylic alcohol **10b** (0.88 g, 1.72 mmol) and freshly distilled triethylamine (0.478 mL, 3.44 mmol) in ether (20 mL) under an argon atmosphere. The solution was stirred for 2 h and then allowed to warm to room temperature followed by quenching with water (15 mL). The organic layer was separated and the remaining aqueous layer extracted with ether (3 × 15 mL). The combined organic extracts were washed with saturated $NaHCO_3$, dried ($MgSO_4$), filtered, and concentrated. Flash silica

(22) For example, see the discussion and footnote 10 of the following paper: Shen, G.-Y.; Tapia, R.; Okamura, W. H. *J. Am. Chem. Soc.* **1987**, *109*, 7499.

chromatography (7.5–10% ethyl acetate/hexanes) afforded 0.78 g (73%) of the major phenylsulfoxide **11b** and 53 mg (5%) of the minor diastereomer **11b'**. Both diastereomers interconvert to a 1:1 mixture when left at 5 °C for several weeks.[22] Only the major, less polar epimer was spectroscopically characterized. **11b.** ^{1}H-NMR: δ 0.14 (3 H, SiMe, s), 0.20 (3 H, SiMe, s), 0.93 (9 H, *t*-Bu, s), 1.00 (3 H, C_{18}-Me, s), 1.04 (3 H, C_{21}-Me, d, J = 6.6 Hz), 1.40 (3 H, $C_{26,27}$-CH_3, s), 1.65 (3 H, $C_{26,27}$-CH_3, s), 5.35 (1 H, H_{22}, d, J = 6.6 Hz), 5.4 (1 H, H_8, broad s), 7.4 (5 H, ArH, m), 7.6 (3 H, ArH, m), 8.0 (2 H, ArH, d, J = 7.5 Hz). Only the ^{1}H-NMR spectrum of the mixture was obtained to reveal the presence of **11b'** (data not reported).

(22*S*)-25-[(*tert*-Butyldimethylsilyl)oxy]-de-*A*,*B*-cholesta-22,23-dien-8β-ol (12a). A solution of *tert*-butyllithium (1.70 M in pentane, 345 μL, 0.59 mmol) was added to a solution of the allene sulfoxide **11a** (61 mg, 0.098 mmol; the reduction of the major, less polar sulfoxide is described here, but analogous results were obtained by reduction of the more polar diastereomer or a mixture of both) and anhydrous methanol (15 μL, 0.37 mmol) in ether (2 mL) under an argon atmosphere at −78 °C. After being stirred at −78 °C for 10 min, the reaction mixture was quenched with methanol (0.5 mL) and then allowed to warm to room temperature. A saturated solution of $NaHCO_3$ (4 mL) and ether (4 mL) was added to the solution, and the layers were separated. The aqueous layer was extracted with ether (3 × 4 mL), and the combined organic extracts were dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The residue was subjected to flash silica chromatography (gradient elution, 7–10% ethyl acetate/hexanes) to afford, after drying, 19.5 mg (50%) of the spectroscopically pure allene **12a** as a colorless oil. ^{1}H-NMR: δ 0.07 (6 H, $SiMe_2$, s), 0.85 (9 H, *t*-Bu, s), 0.96 (3 H, C_{18}-Me, s), 1.04 (3 H, C_{21}-Me, d, J = 6.6 Hz), 1.29 (3 H, $C_{26,27}$-CH_3, s), 1.31 (3 H, $C_{26,27}$-CH_3, s), 4.1 (1 H, $H_{8\alpha}$, broad s), 5.16 (1 H, H_{22}, dd, J = 6.6 Hz, 6.6 Hz), 5.27 (1 H, H_{24}, dd, J = 6.6 Hz, 1.8 Hz).

(22*R*)-25-[(*tert*-Butyldimethylsilyl)oxy]-de-*A*,*B*-cholesta-22,23-dien-8β-ol (12b). A solution of *tert*-butyllithium (345 μL, 1.70 M in pentane, 0.59 mmol) was added to a solution of allene sulfoxide **11b** (60 mg, 97 mmol; the reduction of the major, less polar sulfoxide is described here, but the analogous results were obtained by reduction of the mixture of diastereomers **11b** and **11b'**) and anhydrous methanol (14 μL, 0.35 mmol) in ether (2 mL) at −78 °C under an argon atmosphere. After being stirred at −78 °C for 10 min, the reaction mixture was quenched with methanol (0.75 mL) and then allowed to warm to room temperature. Saturated $NaHCO_3$ (4 mL) and ether (4 mL) were added and the mixture stirred for 5 min after which the layers were separated and the aqueous layer extracted with ether (3 × 4 mL). The combined organic extracts were dried ($MgSO_4$), filtered, and concentrated. The residue was subjected to flash silica chromatography (gradient elution: 7.5 to 10%, ethyl acetate/hexanes) to afford, after drying, the spectroscopically pure allene **12b** (16.5 mg, 42%) as a colorless oil. ^{1}H-NMR: δ 0.07 (6 H, $SiMe_2$, s), 0.85 (9 H, *t*-Bu, s), 0.96 (3 H, C_{18}-Me, s), 1.05 (3 H, C_{21}-Me, d, J = 6.6 Hz), 1.29 (3 H, $C_{26,27}$-CH_3, s), 1.30 (3 H, $C_{26,27}$-CH_3, s), 4.1 (1 H, H_8, broad s), 5.13 (1 H, H_{22}, dd, J = 6.6, 6.6 Hz), 5.26 (1 H, H_{24}, dd, J = 6.3, 1.8 Hz).

(22*S*)-25-[(*tert*-Butyldimethylsilyl)oxy]-de-*A*,*B*-cholesta-22,23-dien-8-one (13a). To a suspension of PDC (240 mg, 0.63 mmol) in CH_2Cl_2 (3 mL) was added a solution of alcohol **12a** (82 mg, 0.21 mmol) in CH_2Cl_2 (3 mL + 2 mL washings) followed by pyridinium trifluoroacetate (20 mg, 0.10 mmol). The solution was stirred at room temperature for 5.5 h. The reaction mixture was passed through a column of Celite (eluted with 3 × 8 mL of CH_2Cl_2) and then concentrated under reduced pressure. Purification was effected by short column flash chromatography (silica gel, 15% ethyl acetate/hexanes) followed by HPLC (Rainin Dynamax, 1.0 × 25 cm, 8 μm, 7.5% EtOAc/hexanes, 4 mL/min) to afford, after drying, 63 mg (80%) of the spectroscopically pure ketone **13a** as a colorless oil. ^{1}H-NMR: δ 0.07 (6 H, $SiMe_2$, s), 0.66 (3 H, C_{18}-Me, s), 0.85 (9 H, *t*-Bu, s), 1.10 (3 H, C_{21}-Me, d, J = 6.6 Hz), 1.30 (3 H, $C_{26,27}$-CH_3, s), 1.31 (3 H, $C_{26,27}$-CH_3, s), 5.18 (1 H, H_{22}, dd, J = 6.9 Hz, 6.9 Hz), 5.29 (1 H, H_{24}, dd, J = 6.0, 2.1 Hz).

(22*R*)-25-[(*tert*-Butyldimethylsilyl)oxy]-de-*A*,*B*-cholesta-22,23-dien-8-one (13b). To a suspension of PDC (280 mg, 0.74 mmol) in CH_2Cl_2 (3 mL) was added a solution of alcohol **12b** (97 mg, 0.25 mmol) in CH_2Cl_2 (3 mL + 2 mL washings) followed by pyridinium trifluoroacetate (21 mg, 0.11 mmol). The solution was stirred at room temperature for 6 h after which the mixture was filtered through a short pad of Celite (eluted with CH_2Cl_2, 3 × 8 mL) and concentrated. Purification was effected by short column flash chromatography (silica gel, 15% ethyl acetate/hexanes) followed by HPLC separation (Rainin Dynamax, 1.0 × 25 cm, 8 μm, 4 mL/min, 7.5% ethyl acetate/hexanes) to afford the spectroscopically pure ketone **13b** (72 mg, 75%) as a colorless oil. ^{1}H-NMR: δ 0.07 (6 H, $SiMe_2$, s), 0.66 (3 H, C_{18}-Me, s), 0.85 (9 H, *t*-Bu, s), 1.10 (3 H, C_{21}-Me, d, 6.9 Hz), 1.29 (3 H, $C_{26,27}$-CH_3, s), 1.31 (3 H, $C_{26,27}$-CH_3, s), 5.14 (1 H, H_{22}, dd, J = 6.6, 6.6 Hz), 5.28 (1 H, H_{24}, dd, J = 6.3, 1.8 Hz).

(22*S*)-1α,25-Bis[(*tert*-butyldimethylsilyl)oxy]-22,23,23,24-tetradehydroprevitamin D_3 *tert*-Butyldimethylsilyl Ether (14a). A mixture of dienyne **13a** (10.0 mg, 0.013 mmol), quinoline (75 μL, 0.17 M in hexanes, 0.013 mmol), and Lindlar catalyst (21 mg) in hexanes (3.5 mL) was stirred under an atmosphere of hydrogen for 1 h. The mixture was filtered through a short pad of silica gel and the residue concentrated to afford a colorless oil. The crude product was purified by HPLC (Rainin Dynamax, 1.0 × 25 cm, 8 μm, 0.1% ethyl acetate/hexanes) to afford, after vacuum drying, the spectroscopically pure previtamin **14a** (8.0 mg, 81%) as a colorless oil. ^{1}H-NMR: δ 0.05 (3 H, SiMe, s), 0.06 (3 H, SiMe, s), 0.07 (6 H, $SiMe_2$, s), 0.09 (6 H, $SiMe_2$, s), 0.71 (3 H, C_{18}-Me, s), 0.85 (9 H, *t*-Bu, s), 0.886 (9 H, *t*-Bu, s), 0.895 (9 H, *t*-Bu, s), 1.09 (3 H, C_{21}-Me, d, J = 6.6 Hz), 1.30 (3 H, $C_{26,27}$-Me, s), 1.31 (3 H, $C_{26,27}$-Me, s), 1.65 (1 H, C_{19}-Me, br s), 4.01–4.10 (1 H, H_3, m), 4.11 (1 H, H_1, br s), 5.17 (1 H, H_{22}, dd, J = 6.9, 6.9 Hz), 5.27 (1 H, H_{24}, dd, J = 6.6, 1.8 Hz), 5.55 (1 H, H_9, narrow m), 5.73 and 5.88 (2 H, H_6 and H_7, AB pattern, J = 12.0 Hz).

(22*R*)-1α,25-Bis[(*tert*-butyldimethylsilyl)oxy]-22,23,23,24-tetradehydroprevitamin D_3 *tert*-Butyldimethylsilyl Ether (14b). A mixture of dienyne **13b** (10.0 mg, 0.013 mmol), quinoline (80 μL, 0.17 M in hexanes, 0.013 mmol), and Lindlar catalyst (20 mg) in hexanes (3.0 mL) was stirred under an atmosphere of hydrogen for 40 min. The mixture was filtered through a short pad of silica gel and the residue concentrated to afford, after drying, a colorless oil. HPLC separation (Rainin Dynamax, 1.0 × 25 cm, 8 μm, 0.1% ethyl acetate/hexanes) afforded the spectroscopically pure previtamin **14b** (7.0 mg, 70%) as a colorless oil. ^{1}H-NMR: δ 0.05 (3 H, SiMe, s), 0.06 (3 H, SiMe, s), 0.07 (6 H, $SiMe_2$, s), 0.09 (6 H, $SiMe_2$, s), 0.71 (3 H, C_{18}-Me, s), 0.85 (9 H, *t*-Bu, s), 0.886 (9 H, *t*-Bu, s), 0.894 (9 H, *t*-Bu, s), 1.09 (3 H, C_{21}-Me, d, J = 6.6 Hz), 1.29 (3 H, $C_{26,27}$-CH_3, s), 1.31 (3 H, $C_{26,27}$-CH_3, s), 1.65 (3 H, C_{19}-Me, broad s), 4.01–4.10 (1 H, H_3, m), 4.11 (1 H, H_1, broad s), 5.14 (1 H, H_{22}, dd, J = 6.6, 6.6 Hz), 5.27 (1 H, H_{24}, dd, J = 6.6, 2.1 Hz), 5.54 (1 H, H_9, narrow m), 5.72 and 5.90 (2 H, H_6 and H_7, AB pattern, J = 12.0 Hz).

Biological Evaluation: Intestinal Calcium Absorption and Bone Calcium Mobilization. Intestinal calcium absorption (ICA) and bone calcium mobilization (BCM) were determined in vivo in vitamin D deficient chicks as described previously.[18] Twelve hours before assay, the chicks which had been placed on a zero-calcium diet 48 h before assay were injected intramuscularly with vitamin D metabolite or analogue 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 animals anesthetized with ether. After 30 min, the birds were decapitated and the blood collected. The radioactivity content of 2.0 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 as measured by atomic absorption spectrophotometry.

1α,25-$(OH)_2$-D_3 Receptor Steroid Competition Assay. A measure of competitive binding to the chick intestinal 1α,25-$(OH)_2$-D_3 receptor was performed by using the hydroxylapatite batch assay.[19] Increasing amounts of labeled 1α,25-$(OH)_2$-D_3 or analogue were added to a standard amount of [^{3}H]-1α,25-$(OH)_2$-D_3 and incubated with chick intestinal cytosol. The relative competitive index (RCI) for the analogues was determined by plotting the percent maximum 1α,25-$(OH)_2$-D_3 bound x 100 on the ordinate versus [competitor/1α,25-$(OH)_2$-[^{3}H]D_3] on the abscissa. The slope of the line obtained for a particular analogue is divided by

the slope of the line obtained for 1α,25-$(OH)_2$-D_3; multiplication of this value by 100 gives the RCI value. By definition, the RCI for 1α,25-$(OH)_2$-D_3 is 100.

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Supplementary Material Available: Spectral and analytical data (21 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

An Intramolecular Diels–Alder Approach to the Cis Ring Fused Isomer of the 25-Hydroxy Vitamin D_2 Grundmann Ketone

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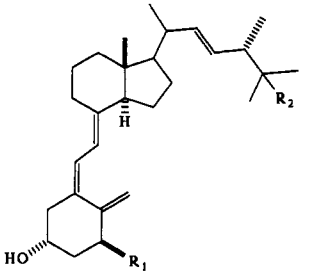
Stereospecific Claisen and intramolecular Diels–Alder reactions of chiral ester synthon **4** results in conversion of a single asymmetric center of commercially available ester **4** to a vitamin D synthon, the C/D cis Grundmann ketone **3**. The addition of propenyllithium to aldehyde **9** was dominated by a chelated anti-Cram transition state and yielded the threo isomer **12a** as the major product. The stereochemistry of **12a** was determined by X-ray crystallography. Conversion of benzyl-protected erythro isomer **11b** to its dimethylacryloyl ester followed by ester enolate Claisen rearrangement led to the C17 and C20 stereochemistry of vitamin D. Addition of a pentadienyl anion to aldehyde **15** gave a tetraene, **17**, which underwent an intramolecular Diels–Alder reaction to produce compound **18**. Removal of the C16 hydroxyl and hydrolysis gave only the cis-fused isomer of **3**.

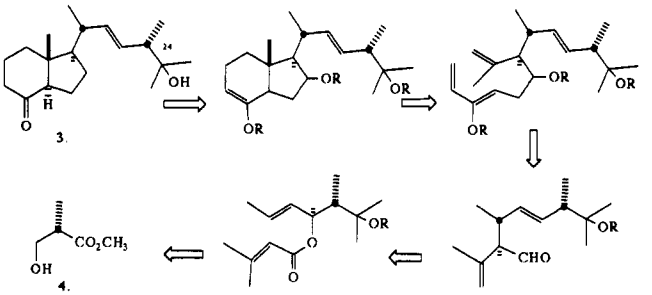
Recently there has been a resurgence of interest concerning vitamin D_2 and its hydroxylated metabolites **1** and **2**.[1] The tranditional activity of the vitamin D hormone in calcium homeostasis has been broadened by the discovery of its role in normal cell differentiation and the immune system. For example, recent work on the stim-



1. R_1 = H, R_2 = OH
2. R_1 = R_2 = OH

ulation of macrophages[2] has lead to the topical use of vitamin D analogs in the treatment of psoriasis.[3] New

Scheme I



analogs have been discovered with incredible immunosupresive activity[4] (one million times greater than cyclosporin A!). As part of a program of developing new synthetic methodology we have investigated a diastereoselective synthesis of the cis-fused isomer of 25-hydroxy Grundmann ketone **3**, using the intramolecular Diels–Alder reaction. The trans ketone **3** is a key building block used for the synthesis of vitamin D compounds by many groups.[5,6]

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