7,8-Cis Geometric Isomers of the Steroid Hormone 1α,25-Dihydroxyvitamin D_3[1a]

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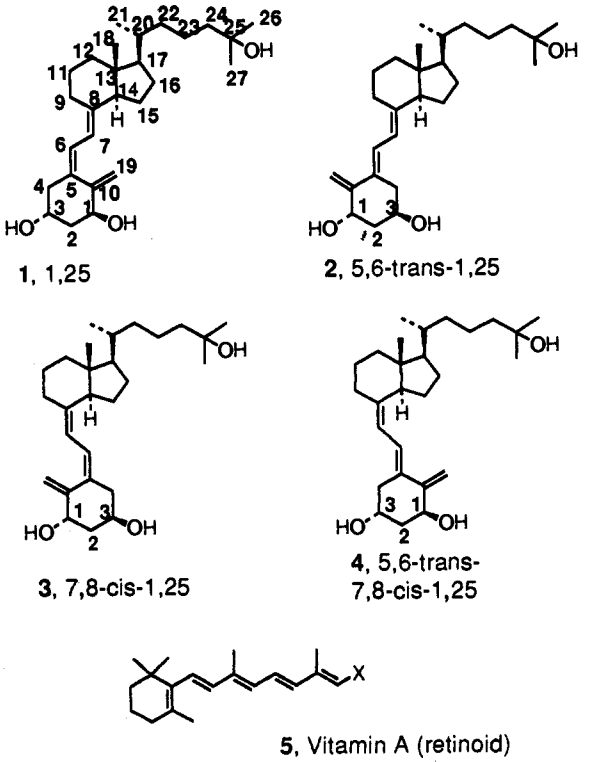
Abstract: The syntheses of the previously unknown, sterically hindered geometric isomers **3** and **4** of the steroid hormone 1α,25-dihydroxyvitamin D_3 (**1**, 1,25) have been achieved for the first time. The stereoselective synthesis of the vinylallene precursor **23a** was achieved by the Inanaga method, Pd(0)–Sm(II)–iPrOH reduction of propargyl benzoate **33**. The latter observation reveals that the Inanaga propargyl ester to allene transformation is in fact stereoselective (~10:1), involving a formal anti-S_N2' displacement of benzoate by hydrogen. Highly stereoselective (50:1, 85% yield) (1,5)-hydrogen shift of vinylallene **23a** to the sterically hindered 7,8-cis isomer of the hormone **1**, namely, **3**, was achieved by the Shibasaki method using (naphthalene)tricarbonylchromium (**22**, (np)$(CO)_3$Cr). By examining this chromium-(0)-mediated isomerization on all four diastereomeric vinylallene analogue systems **6a,b** and **7a,b**, all of which resulted highly selectively (50:1) in hindered 7,8-cis geometric isomers, new stereomechanistic information concerning the Shibasaki type 1,5-shift has also emerged. By cheleotropic addition–extrusion of sulfur dioxide on **3**, there was obtained the final unknown geometric isomer of **1**, 5,6-trans-7,8-cis-1,25 **4**; this result parallels the known transformation of **1** to **2**. Although the vinylallene **23a** can be thermally rearranged via a [1,5]-sigmatropic hydrogen shift to the natural hormone **1**, the yield and selectivity are modest. By contrast, one-way triplet photosensitized isomerism of the now readily available 7,8-cis-isomer **3** results primarily in the natural hormone **1** in good yield and selectivity. *Taken collectively, these observations reveal that the synthesis of all four geometric isomers 1–4 can be achieved from a single precursor, the vinylallene 23a.* Comparative biochemical evaluation, in vitro, of the four geometric isomers in terms of their ability to bind to the chick intestinal receptor reveals that 7,8-cis isomerism (**3** and **4**) significantly suppresses their ability to bind receptor (<2% each versus 100% for the hormone 1,25).

Introduction

Because of the potential of the steroid hormone 1α,25-dihydroxyvitamin D_3 (**1**, 1,25) (Chart 1) in biomedical applications, there is considerable interest in developing a more detailed understanding of the molecular basis of the mode of action of the vitamin D endocrine system through studies of analogues.[2] In this connection, our recent interest has turned to the examination of the biological profile of 1,25 analogues modified in the π-system. Whereas 5,6-trans isomers of many analogues of vitamin D are well-known, including 5,6-*trans*-1α,25-dihydroxyvitamin D_3 (**2**, 5,6-trans-1,25),[3] the corresponding 7,8-cis stereoisomers (e.g., **3** and **4**) are rare. Numerous investigations of the photoisomerization of vitamin D and 5,6-trans vitamin D have been reported, including the particularly detailed study of the photosensitized isomerization of 5,6-trans vitamin D_3 to vitamin D_3 by the Havinga group.[4] Utilization of appropriate photosensitizers in the one-way photoisomerization of 5,6-trans vitamin D to vitamin D has been effective in the development of practical syntheses of 1,25 and various analogues.[5] However, in photochemical experiments, the 7,8-cis isomers have been singularly absent. This is surprising because all 16 geometric isomers of the more complex vitamin A pentaene framework **5** are known, many being obtained by photochemical cis–trans isomerization.[6]

Chart 1



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(1) (a) This is paper 47 in the series Studies of Vitamin D (Calciferol) and Its Analogues. For part 46, see: Midland, M. M.; Plumet, J.; Okamura, W. H. *Biorg. Med. Chem. Lett.* **1993**, *3*, 1799. (b) Department of Chemistry. (c) Department of Biochemistry and the Division of Biomedical Sciences.

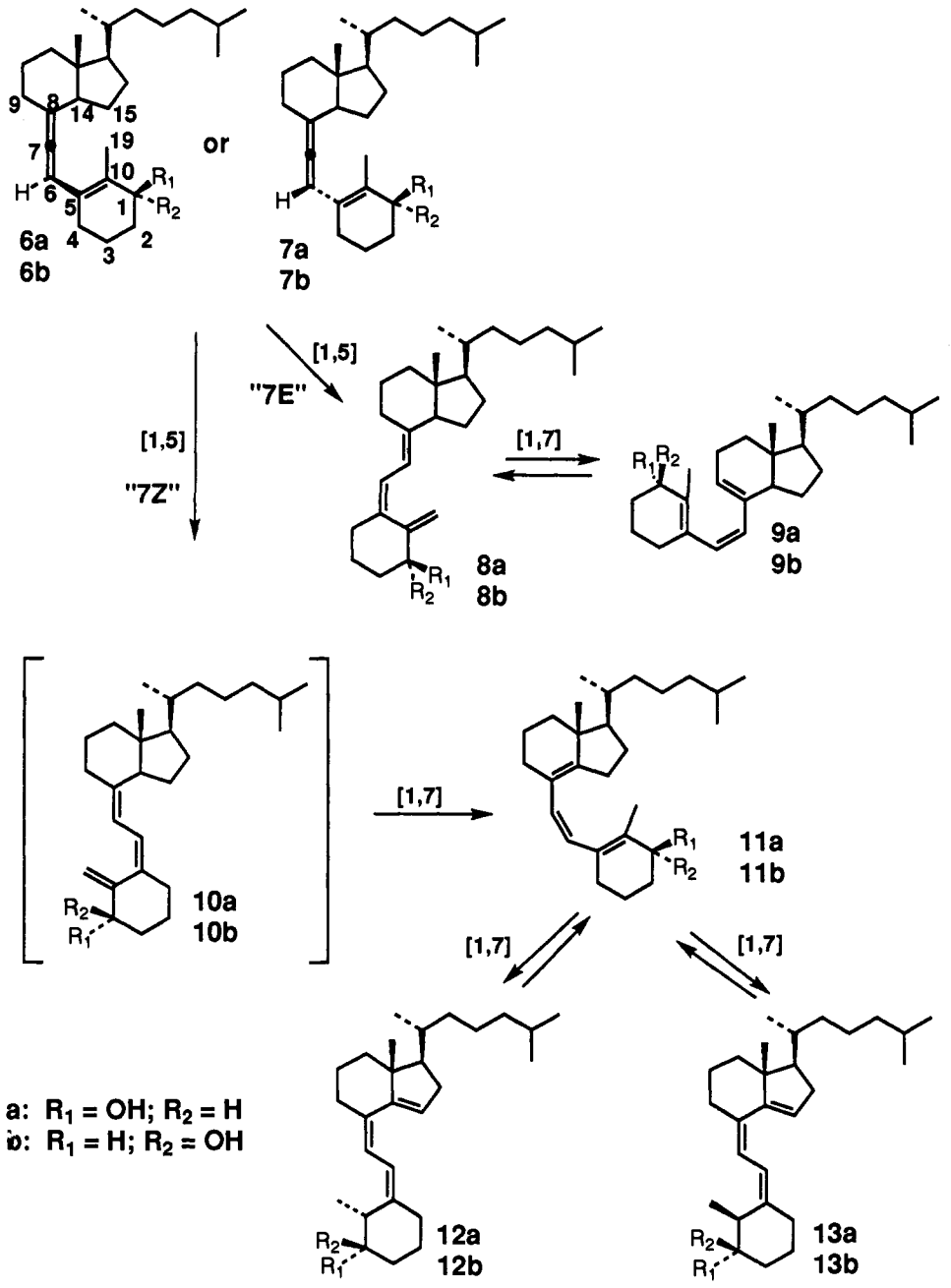
(2) (a) Norman, A. W.; Bouillon, R.; Thomasset, M., Eds. *Vitamin D: Gene Regulation, Structure-Function Analysis and Clinical Application*: Walter de Gruyter and Co.: Berlin, 1991. (b) Norman, A. W. *Vitamin D the Calcium Homeostatic Hormone*; Academic Press: New York, 1979. (c) Reichel, H.; Koeffler, H. P.; Norman, A. W. *New Engl. J. Med.* **1989**, *320*, 980. (d) Ikekawa, N. *Med. Res. Rev.* **1987**, *7*, 333.

(3) To avoid reversal of *Z/E* labels due to group priority changes among vitamin D analogues, it is useful practice to use cis/trans labels with the natural configuration understood to be 5,6-cis-7,8-trans.

(4) (a) Gielen, J. W. J.; Koolstra, R. B.; Jacobs, H. J. C.; Havinga, E. *Recl. Trav. Chem. Pays-Bas* **1980**, *99*, 306. (b) Jacobs, H. J. C.; Gielen, J. W. J.; Havinga, E. In *Vitamin D, Basic Research and Its Clinical Application*; Norman, A. W., Schaefer, K., Herrath, D. v., Grigoleit, H.-G., Eds.; Walter de Gruyter and Co.: Berlin, 1979; p 61. (c) Verloop, A.; Koevoet, A. L.; Havinga, E. *Recl. Trav. Chem. Pays-Bas* **1955**, *74*, 1125.

0002-7863/94/1516-6207$04.50/0

Chart 2



During our investigations of the thermal isomerization of vinylallenes **6** and **7** (Chart 2) as a route to vitamin D analogues, the 7,8-cis vitamin D **10** was proposed as a reactive intermediate leading to the observed products **11–13**.[7] It was established that depending upon the stereochemistry of the allene and the allylic carbinol center (C_1) on the A-ring, [1,5]-sigmatropic hydrogen migration may be favored to proceed via the 7*E* or 7*Z* manifold pathway (Chart 2), leading to varying ratios of **8** and the proposed 7,8-cis-isomer **10**. Unfortunately, the concomitant formation of a triad of interequilibrating triene isomers, **11–13** (from irreversible isomerization of **10**), as well as minor amounts of the previtamin **9** (from reversible isomerization of the vitamin D **8**) makes the vinylallene route of limited practical use as a synthetic approach to the desired vitamin D **8**.

An isomer related to **11**, namely, **14a**, was first synthesized by the Havinga group[8] and later studied by Schnoes and DeLuca in an attempt to utilize **14a** as a thermal precursor of the 7,8-cis-isomer **15a**.[9] However, like our observations of Chart 2, thermolysis of **14a** afforded **16a** and **17a**, but not **15a**.

Mouriño in 1991 reported the first synthesis of a stable 7,8-cis vitamin D compound **15b** (Chart 3),[10] wherein the benzoate ester **19a** was coupled to the lithio anion of the allylphosphine oxide

(5) (a) Choudhry, S. C.; Belica, P. S.; Coffen, D. L.; Focella, A.; Maehr, H.; Manchand, P. S.; Serico, L.; Yang, R. T. *J. Org. Chem.* **1993**, *58*, 1496. (b) Bretting, C.; Calverley, M. J.; Binderup, L. In *Vitamin D: Gene Regulation, Structure-Function Analysis and Clinical Application*; Norman, A. W., Bouillon, R., Thomassset, M., Eds.; Walter de Gruter and Co.: Berlin, 1991; p 159. (c) Kabat, M.; Kiegiel, J.; Cohen, N.; Toth, K.; Wovkulich, P. M.; Uskokovic, M. R. *Tetrahedron Lett.* **1991**, *32*, 2343.

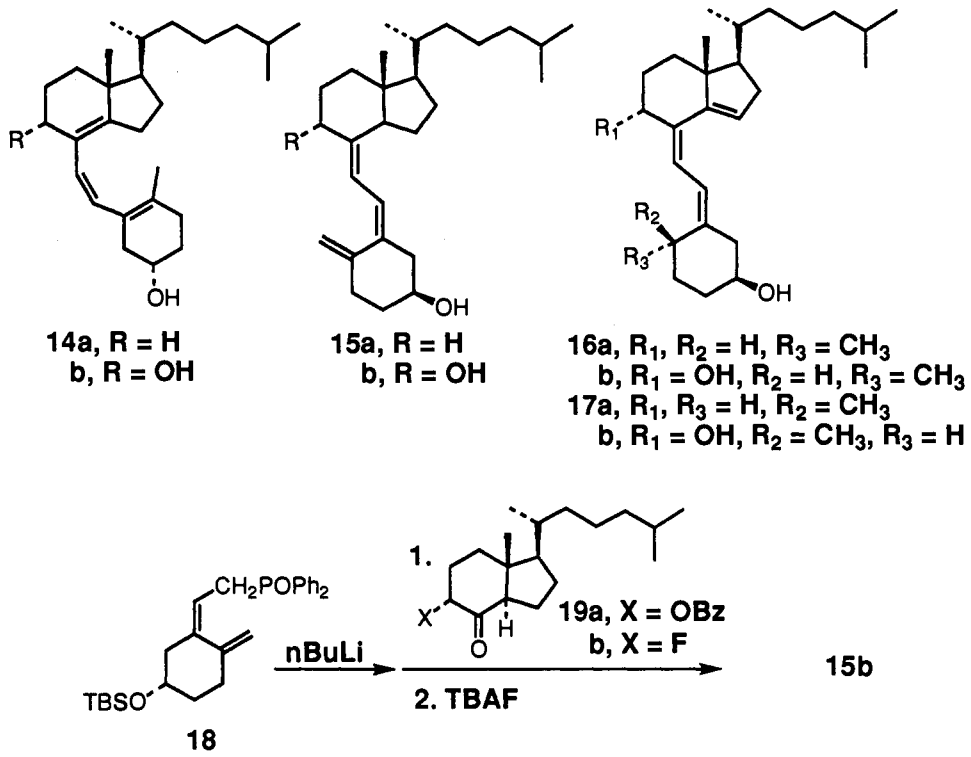
(6) Zhu, Y.; Ganapathy, S.; Trehan, A.; Asato, A. E.; Liu, R. S. H. *Tetrahedron* **1992**, *48*, 10061.

(7) (a) Hammond, M. L.; Mouriño, A.; Okamura, W. H. *J. Am. Chem. Soc.* **1978**, *100*, 4907. (b) Condran, P.; Okamura, W. H. *J. Org. Chem.* **1980**, *45*, 4011. (c) Mouriño, A.; Lewicka-Piekut, S.; Norman, A. W.; Okamura, W. H. *J. Org. Chem.* **1980**, *45*, 4015. (d) Condran, P.; Hammond, M. L.; Mouriño, A.; Okamura, W. H. *J. Am. Chem. Soc.* **1980**, *102*, 6259. (e) Leyes, G. A.; Okamura, W. H. *J. Am. Chem. Soc.* **1982**, *104*, 6099. (f) Okamura, W. H. *Acc. Chem. Res.* **1983**, *16*, 81. (g) Jeganathan, S.; Johnson, A. D.; Kuenzel, E. A.; Norman, A. W.; Okamura, W. H. *J. Org. Chem.* **1984**, *49*, 2152. (h) Barrack, S. A.; Okamura, W. H. *J. Org. Chem.* **1986**, *51*, 3201. (i) Okamura, W. H.; Aurrecoechea, J. M.; Gibbs, R. A.; Norman, A. W. *J. Org. Chem.* **1989**, *54*, 4072.

(8) Verloop, A.; Corts, G. J. B.; Havinga, E. *Recl. Trav. Chim. Pays-Bas* **1960**, *79*, 164.

(9) Onisko, B. L.; Schnoes, H. K.; DeLuca, H. F. *J. Org. Chem.* **1978**, *43*, 3441.

Chart 3



A-ring **18**. The compound obtained following deprotection was exclusively the 7,8-*cis*-9α-hydroxyvitamin D_3 (**15b**), which on heating afforded **14b**, **16b**, and **17b** (analogous to the processes given in Chart 2). Dauben and Greenfield also reported **15b** by a slightly different route,[11] starting from the 9α-fluoro Grundmann's ketone **19b**.

In order to simplify the isomerization behavior of the vinylallenes, we began an evaluation of catalysts for the selective isomerization of vitamin D_3 type vinylallenes (Chart 2). Sodeoka and Shibasaki have used chromium tricarbonyl complexes in the catalytic [1,5]-H migrations of several diene systems.[12] For example, (naphthalene)tricarbonylchromium (**22**, (np)$(CO)_3$Cr) was determined to isomerize the butadiene **20** to the dienamine **21** in high yields with complete geometric selectivity.[12c] In an initial application of this reagent to isomerization of the four diastereomeric vinylallenes **6a,b** and **7a,b**, which comprise all four permutations of the C_1-carbinol and C_6-allene configurations, we observed that **10a,b** was formed stereoselectively in high yield.

This article reports on the details of these model studies and describes the related synthesis of 7,8-cis-1,25 **3**, the first 7,8-cis geometric isomer of a natural vitamin D metabolite. This includes the development of a route to 1,25-vinylallene **23a** and its C_6-epimer **24a** in a stereoselective fashion (~10:1 ratio). Isomerization of the allene via the metal-mediated process leads to 7,8-cis-1,25 **3**. In addition, selective isomerization of **3** leads by one route to the remaining unknown geometric isomer, 5,6-trans-7,8-cis-1,25 **4**, and by another, to 1,25 (**1**) itself.

Results and Discussion

Metal-Mediated Isomerization of Vinylallenes 6a,b and 7a,b. The four diastereomeric vinylallenols **6a**, **6b**, **7a**, and **7b** were prepared as previously described.[7h] Treatment of each vinylallenol in acetone with (np)$(CO)_3$Cr (1.1 equiv),[12] afforded almost complete reaction within 4 h at 38 °C, whereas at 25 °C little reaction occurred. In the absence of (np)$(CO)_3$Cr (38 °C, acetone, 4 h), no isomerization was detectable by ^{1}H-NMR analysis.

Table 1. Chromium-Mediated Isomerization of Vinylallenes

substrate	product (yield)[a]			
6a	**11a** (2%)	**8a** (2%)	**10a** (75%)	
6b	**11b** (5%)		**10b** (81%)	
7a	**11a** (2%)	**8a** (1%)	**10a** (89%)	**7a** (4%)
7b	**11b** (7%)	**8b** (1%)	**10b** (75%)	
23a[b]	**35** (8%)		**3** (86%)	**23a** (7%)

[a] Actual isolated yields, including recovered starting material, are given in parentheses. In several cases, small quantities of minor components could be detected by ^{1}H-NMR analyses, but were not isolated. [b] The starting material consisted of ~10% of the allene epimer **24a**.

The (1*S*,6*S*)-vinylallenol **6a** isomerized to the new 7,8-cis vitamin D isomer **10a** along with two minor products, **8a** and **11a**.[7d,g,h] Besides evidence from spectral data, **10a** was identified on the basis of its thermal isomerization behavior. A sample of 7,8-cis **10a** in C_6D_6, heated at 75 °C in an NMR tube, was monitored by ^{1}H-NMR analysis at appropriate time intervals. Isomerization of **10a** via a [1,7]-hydrogen shift to the known *cis*-isotachysterol **11a** occurred with a $t_{1/2}$ of ~139 min.

Similar results were obtained for vinylallenol **6b** when subjected to the same metal-induced isomerization conditions as for **6a**, except the major product was the C_1 epimeric 7,8-cis isomer **10b**. The latter, like **10a**, isomerized to the *cis*-isotachysterol **11b**, but somewhat more rapidly, with $t_{1/2}$ ~ 81 min at 75 °C. The diastereomeric allenes **7a** and **7b** afforded essentially the same results as **6a** and **6b**.

The results, summarized collectively for the four diastereomeric vinylallenes in Table 1, provide new mechanistic insight into the Shibasaki [1,5]-hydrogen shift (e.g., **20** to **21** in Chart 4) because of the stereochemical features present in the four vinylallenols **6** and **7**. The significant feature of the metal-mediated isomerization **25** to **26** (i.e., **6** or **7** to **10**) shown in Chart 5 is that neither the allene nor carbinol configuration affects the stereochemical course of the isomerization in the same manner as in the complex, thermal process (Chart 2). Very simply, 7,8-cis vitamin **26** is formed in preference to the 7,8-trans isomer with ~50:1 geometric

(10) Maestro, M. A.; Sardina, F. J.; Castedo, L.; Mouriño, A. *J. Org. Chem.* **1991**, *56*, 3582.
(11) Dauben, W. G.; Greenfield, L. J. *J. Org. Chem.* **1992**, *57*, 1597.
(12) (a) Sodeoka, M.; Satoh, S.; Shibasaki, M. *J. Am. Chem. Soc.* **1988**, *110*, 4823. (b) Sodeoka, M.; Yamada, H.; Shibasaki, M. *J. Am. Chem. Soc.* **1990**, *112*, 4906. (c) Yamada, H.; Sodeoka, M.; Shibasaki, M. *J. Org. Chem.* **1991**, *56*, 4569.

Chart 4

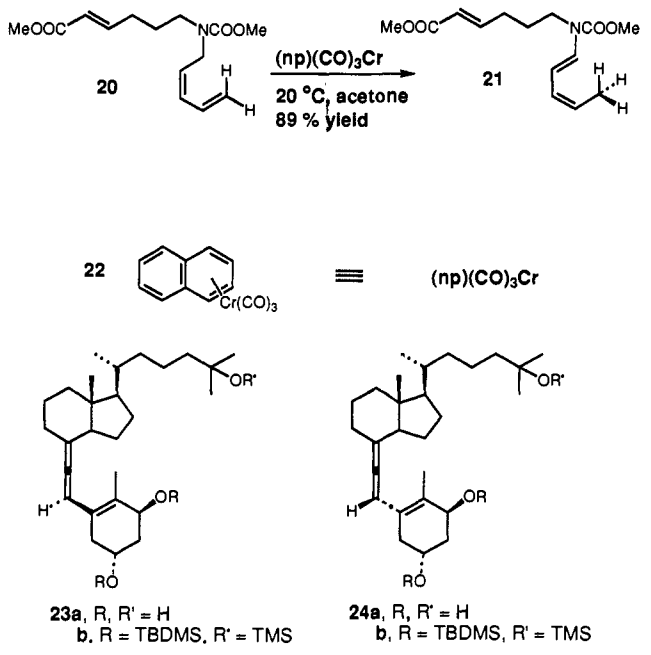
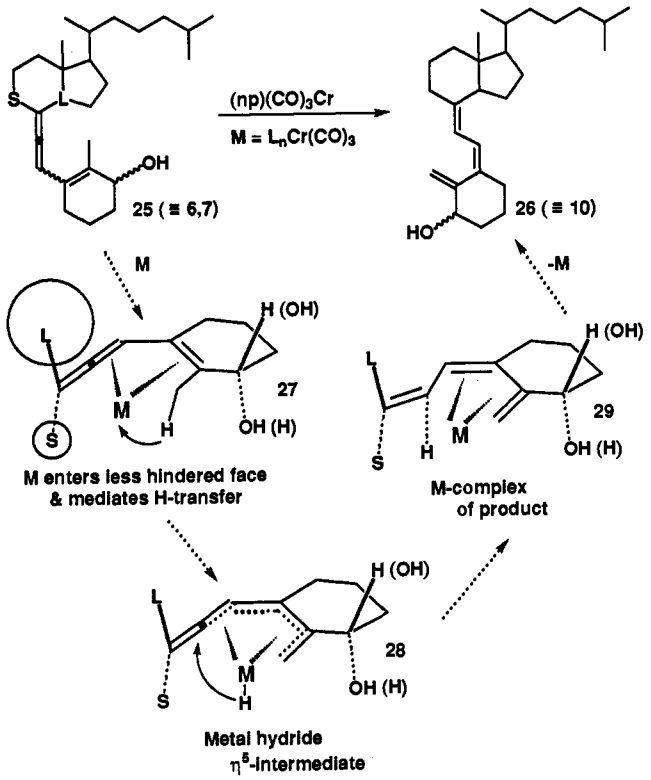


Chart 5



selectivity (7*Z*:7*E* ratio). In contrast, heating **6a** at 100 °C for 10 h was reported to produce a 1.0:3.7 ratio of 7*Z*:7*E* products (i.e., the ratio of **10a** to **8a**), while heating its C_6-epimer **7a** reverses this ratio (3.7 to 1.0 ratio of **10a** to **8a**).[7d,g,h] This sensitivity to stereochemistry was also seen for the similar thermal isomerization of **6b** and **7b**.

For the metal-mediated process **25** to **26**, a simple rationale is presented in the mechanistic scheme given in Chart 5. In the first step, it is envisaged that a chromium(0) species M metalates the vinylallene from its less hindered face to form the η^4 species **27**. Despite the presence of a C_1-hydroxyl, which could in principle direct metal coordination as has been previously observed,[13] the overriding effect is attributed here to a steric effect imparted by the substituents on the allene terminus. The species **27** can then be considered to isomerize to the η^5 intermediate **28** and then to **29**, as has been previously suggested by Shibasaki.[12] Subsequent loss of the chromium(0) species then affords primarily **26**. One final note concerns the conditions used to effect the isomerization, noting that Shibasaki typically used 20 mol % of $(np)(CO)_3Cr$ at 20 °C for 4 h (acetone) in catalyzing the [1,5]-hydrogen shift of **20** to **21**. In this study, the isomerization was conducted at 40 °C using a stoichiometric amount of reagent. The lower reactivity of **25** as compared to the simpler diene systems studied by Shibasaki may simply be of steric origin.

Interestingly, the vicinal ^{1}H-NMR coupling constants between C_6–H and C_7-H for both **10a** and **10b** were determined to be $J \sim 11.5$ Hz, similar to those reported by Condran et al. for analogues with the natural vitamin configuration (**8a** and **8b**; $J \sim 11.4$ Hz).[7d] This implies that the intercyclic diene moiety ($C_5C_6C_7C_8$) is s-transoid and essentially planar as in other analogues and metabolites possessing the natural 5,6-cis-7,8-trans[3] geometric configuration. Furthermore, UV absorption maximum appeared at λ_{max} 257 ± 1 nm, with ϵ values of 20 600 for **10a** and 18 900 for **10b**. These may be compared to the λ_{max} 262 nm, with ϵ values of 16 900 for **8a** and 17 400 for **8b**.[7d] Although it is difficult to anticipate the degree of planarity expected of the intercyclic diene component of the triene system in **10a** and **10b**, it is surprising that their chromophores were not highly twisted about the 6,7 single bond as a result of steric congestion.

1,25-Vinylallenes and Isomerization Studies. The (6*S*)- or (6*R*)-1α,25-$(OH)_2$-vitamin D_3-vinylallenes, **23a** or **24a**, respectively, were prepared from A-ring enyne **30**[14] and the trimethyl silyl ether of 25-OH Grundmann's ketone **31**,[15] as shown in Chart 6. Reaction of a solution of the lithium salt of **30** with silyl ether **31** afforded the propargyl alcohol **32**, which was converted to the benzoate ester **33** (59%) using an *n*-BuLi and benzoyl chloride procedure.[7i] Unreacted propargyl alcohol **32** (27%) was invariably recovered using this procedure. Attempts using copper-based reagents to effect the propargyl ester to allene conversion exemplified by the transformation of **33** to **23**, have been singularly unsuccessful in our vitamin D applications.[7i] However, the method of Inanaga et al.[16] using Sm^{2+}–Pd(0) reagents for the preparation of a variety of substituted allenes proved successful. Addition of propargyl benzoate **33** and $Pd(PPh_3)_4$ in THF to a solution of SmI_2 followed by the addition of a 10-fold molar excess of freshly distilled 2-propanol afforded vinylallenes **23b** and **24b** in a ~10:1 ratio in 76% yield. The anti stereoselective nature of the Inanaga allene synthesis has previously not been reported. The deprotected triols **23a** and **24a** (TBAF, THF) were separated with difficulty by HPLC, but pure triol **23a** could be obtained in this manner for characterization. The triol **24a** was prepared for characterization purposes by photoisomerization whereby a solution of a ~10:1 mixture of vinylallenes **23a** and **24a** in methanol-d_4 (argon) in a quartz NMR tube afforded, after 30 min of UV irradiation (Hanovia medium pressure mercury lamp), a ~50:50 mixture of **23a** and **24a**.[17] HPLC purification gave sufficient amounts of purified **24a** for characterization. Comparison of the ^{1}H-NMR spectra of **23a** and **24a** was made with data reported by van Koeveringe and Lugtenburg in 1976 for similar isomers of vitamin D_3-vinylallenes (identical to **23a** and **24a**, but lacking the allylic and side-chain hydroxyls).[17a] The stereoisomer with the C_{18} methyl ^{1}H-NMR resonance at lower field (δ 0.74) was assigned

(13) Davies, S. G.; Goodfellow, C. L. *J. Organomet. Chem.* **1988**, *340*, 195.

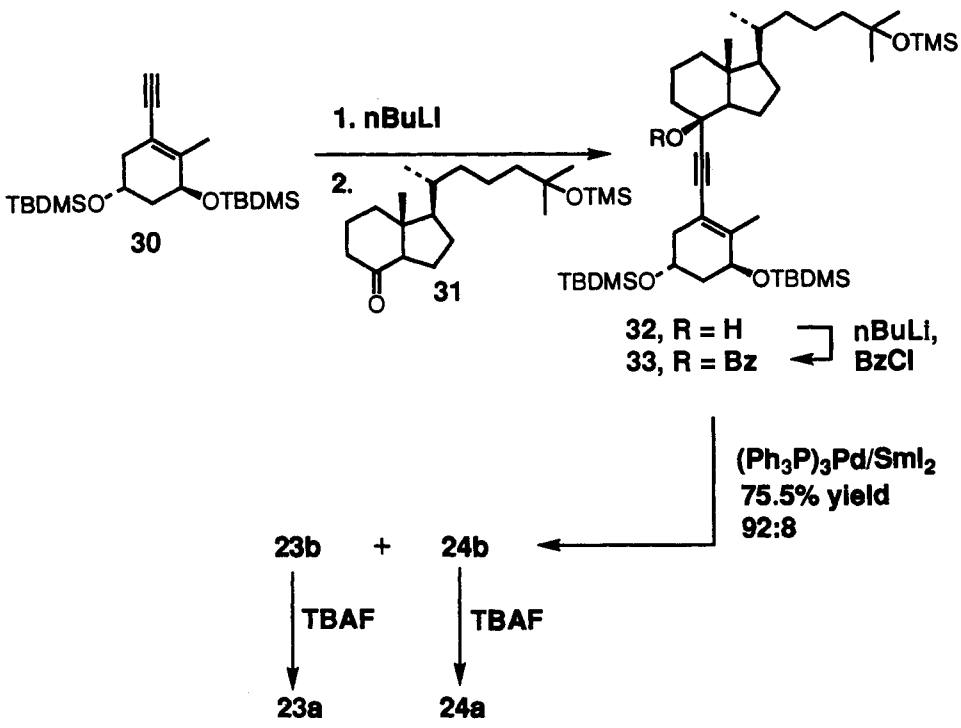
(14) (a) Okamura, W. H.; Aurrecoechea, J. M.; Gibbs, R. A.; Norman, A. W. *J. Org. Chem.* **1989**, *54*, 4072. (b) Castedo, L.; Mascareñas, J. L.; Mouriño, A.; Saradeses, L. A. *Tetrahedron Lett.* **1988**, *29*, 1203.

(15) (a) Kiegiel, J.; Wovkulich, P. M.; Uskokovic, M. R. *Tetrahedron Lett.* **1991**, *32*, 6057. (b) Baggiolini, E. G.; Iacobelli, J. A.; Hennessy, B. M.; Batcho, A. D.; Sereno, J. F.; Uskokovic, M. R. *J. Org. Chem.* **1986**, *51*, 3098.

(16) Tabuchi, T.; Inanaga, J.; Yamaguchi, M. *Tetrahedron Lett.* **1986**, *27*, 5237.

(17) (a) van Koeveringe, J. A.; Lugtenburg, J. *Recl. Trav. Chim. Pays-Bas* **1976**, *95*, 80. (b) Bakker, S. A.; Lugtenburg, J.; Havinga, E. *Recl. Trav. Chim. Pays-Bas* **1972**, *91*, 1459.

Chart 6



as the (6*S*)-vinylallene **23a**, and that with this signal at higher field (δ 0.65) was assigned as the (6*R*)-vinylallene **24a**.

The thermal isomerization of epimerically pure (6*S*)-vinylallene **23a** (isooctane, 100 °C, 14.5 h) afforded a mixture of products (^{1}H-NMR analysis) consisting of ~58% 7*E* products (**1** plus **34**) and 42% 7*Z* products (**35**, **36**, and **37**), as shown in Chart 7. More specifically, ^{1}H-NMR analysis indicated the following distribution of products: hormone **1** (42%), starting **23a** (8%), previtamin **34** (11%), *cis*-isotachysterol **35** (trace), and methyl epimers **36** and **37** (39%) (A ~76–77% total mass balance including ~5% starting material was obtained). The formation of major amounts (~58% **1** + **34**) of 7*E* manifold products (Chart 7) from **23a**, together with the ^{1}H-NMR data, is taken to support its (1*S*,6*S*) configurational assignment (see the discussion concerning the analogous isomerization of **6a** in the Introduction). In a preparative experiment, a yield of ~50% **1** was achieved.

The vinylallene **23a** (usually as a ~10:1 mixture of **23a** and **24a**) was isomerized with (np)$(CO)_3$Cr (1.1 equiv) exactly as described for the 3-deoxy series **6**, **7**, and the results are included in Table 1. The spectral characteristics of the major product, 7,8-cis-1α,25-$(OH)_2$-D_3 (**3**), were similar to those of the natural hormone **1**. The ^{1}H-NMR coupling of the C_6 and C_7 hydrogens (J ~ 11.5) is indicative of a nearly planar transoid relationship of the intercyclic diene component of the triene, just as the natural system **1** and the two 3-deoxy analogues discussed earlier (the 7,8-cis-isomers **10a,b** versus **8a,b**). Furthermore, the UV spectrum of **3** exhibited a λ_{max} at 266 nm (ϵ ~ 15 000), comparable to that of **1**, λ_{max} of 266 nm (ϵ ~ 17 200).[18]

The thermal isomerization of 7,8-cis-1,25 **3** (isooctane, 100 °C, 36 h) reveals that **3** isomerizes irreversibly to **35** and then **35**, **36**, and **37** interequilibrate, all steps involving [1,7]-sigmatropic hydrogen shifts and analogous to earlier studies of the interequilibration between **11**, **12**, and **13**. The equilibrium proportions of **35**, **36**, and **37** (from heating **3**, 100 °C, 36 h) were 13%, 23%, and 64%, respectively (^{1}H-NMR analysis). Heating **37** separately under the same conditions afforded 13%, 26%, and 61% of the same products, verifying that these represent equilibrium proportions of products.

The products **35**, **36**, and **37** were readily separable by HPLC and were identified by comparison of their ^{1}H-NMR spectra to data of related compounds (**11**, **12**, and **13**).[7d,h] The minor constituent, the 1α,25-$(OH)_2$-*cis*-isotachysterol (**35**), could be formed preferentially together with starting material by heating a solution of the 7,8-cis **3** under milder conditions (acetone-d_6, 18 h, 57 °C). After HPLC purification, **35** was isolated in 43% yield. Prolonged warming of **35** at this temperature led to increased formation of the isomers **36** and **37**.

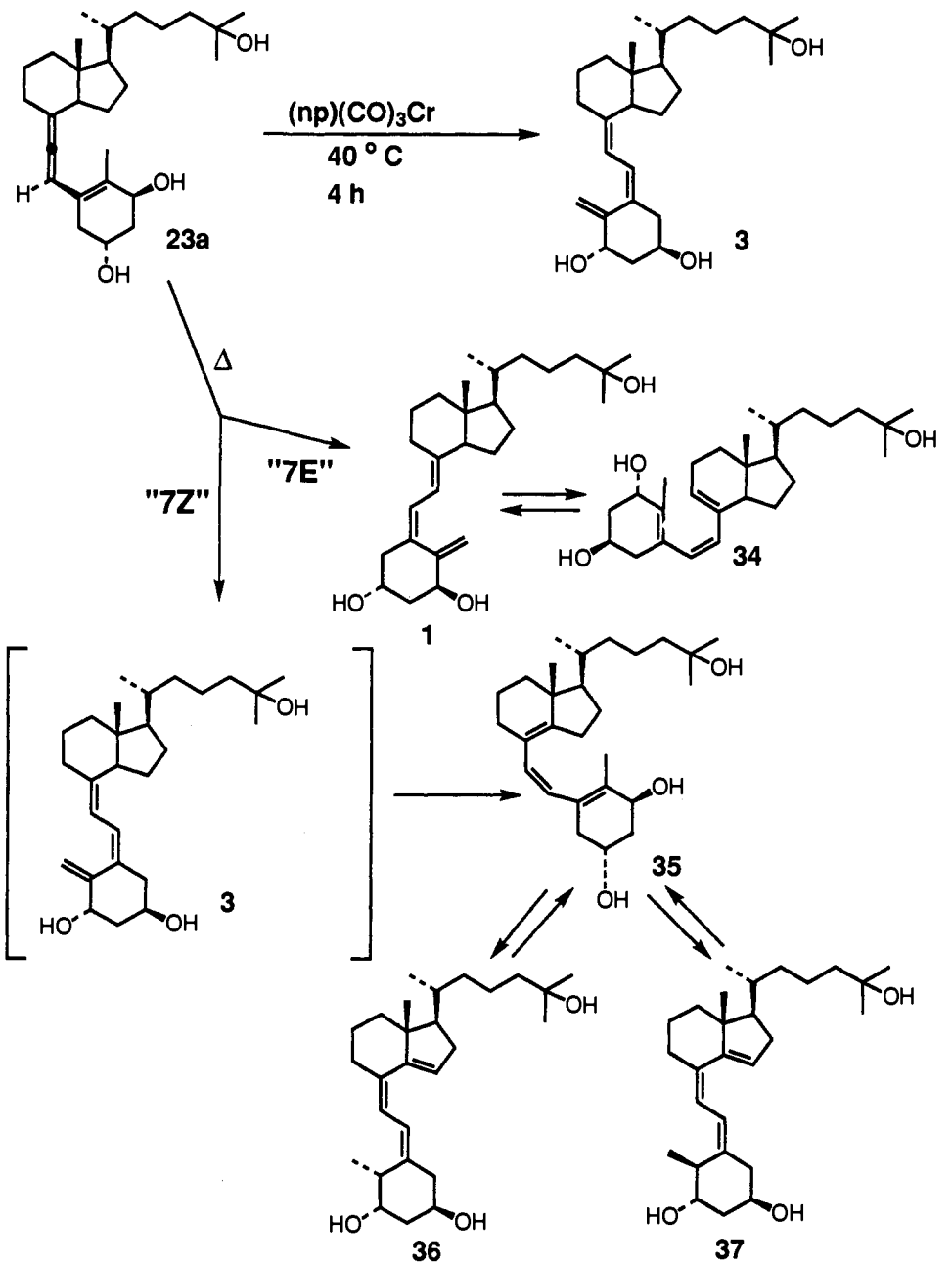
Assignment of the stereochemistry at C_{10} for **36** and **37** was based on a comparison of their ^{1}H-NMR data to those for the analogous compounds in the 3-deoxy-1α-OH-vitamin D_3 series **12a** and **13a**, respectively, reported earlier by our laboratory.[7d,h] The superposition of the chemical shifts for the C_1- and C_3-hydrogens of **36** and **37** in their ^{1}H-NMR spectra did not allow a direct comparison of the splitting patterns of the C_1-H's of these isomers to those of **12a** and **13a**. However, the C_{10}-hydrogens were clearly distinguishable. In the case of **36**, the C_{10}-H appears as an apparent quintet at δ 3.28 (J ~ 6.5 Hz). For **37**, C_{10}-H appears as an apparent quartet at δ 2.98 (J ~ 6.8 Hz). Furthermore, the characteristic AB pattern between the C_6- and C_7-hydrogens had collapsed into a pseudosinglet at δ 6.22 in the case of **36**. For **37**, two doublets were clearly distinguishable at δ 6.12 and 6.31 (J ~ 11.2 Hz). In both these respects, the related diastereomers, **12a** and **13a**, respectively, studied earlier,[7d,h] exhibit similar signals, noting that **12a** and **13a** are the same as **36** and **37** except for the absence of the C_3 and C_{25} hydroxyl groups. Thus, for **12a**, (cf. **36**), the C_{10}-H appears as an apparent quintet at δ 3.21 (J ~ 6 Hz) and the C_6-H and C_7-H appear as a pseudosinglet at δ 6.27 (because of their identical chemical shifts). For **13a** (cf. **37**), the C_{10}-H appears as an apparent quartet at δ 3.03 (J ~ 7 Hz) and the C_6-H and C_7-H appear as an AB pattern at δ 6.11 and 6.25 (J ~ 11.3 Hz). Moreover, at equilibrium (100 °C), the relative proportions of **35**, **36**, and **37** (13%, 23%, and 64%) are similar to those proportions of related derivatives **11a**, **12a**, and **13a** (14%, 36%, and 49%)[7d,h] and related 3β-methyl derivatives **38**, **39**, and **40** (13%, 18%, and 79%),[7e] as summarized in Chart 8. Note that epimeric C_{10}-methyl diastereomers such as **36** and **37** are best viewed as existing in opposite, conformationally locked chair forms with the C_{10}-methyl axially oriented. The preference for an axially oriented C_{10}-methyl in these systems is attributable to $A_{1,3}$-strain.[7d,h,19]

Selective Geometric Isomerization of 7,8-Cis-1,25. Photoisomerization (Hanovia medium pressure Hg lamp, Pyrex) of a

(18) Curtin, M. L.; Okamura, W. H. *J. Am. Chem. Soc.* **1991**, *113*, 6958.

(19) Johnson, F. *Chem. Rev.* **1968**, *68*, 375.

Chart 7



solution of 7,8-cis-1,25 **3** in deuteriated methanol with a 5-fold molar excess of the triplet photosensitizer 9-acetylanthracene resulted in its almost complete isomerization to the hormone **1**.[4a,5] The latter was isolated pure in 67% yield together with small amounts of starting 7,8-cis **3** and 5,6-trans **2**. In a series of control experiments, starting from either pure **1** or pure **3** under otherwise identical conditions, ^{1}H-NMR analysis after >2 h indicated the presence of 92% **1**, 5–6% **2**, 2–3% **3**, and no **4**. The formation of practical amounts of hormone **1** completes a new synthetic scheme of this natural metabolite, effectively utilizing a vinylallene intermediate. This route avoids formation of the various by-products (**34–37**) using the thermal isomerization route. Moreover, it is most interesting that this photochemical route affords a small amount of a 7,8-cis geometric isomer (i.e., **3**) even when pure **1** is utilized as starting material. The formation of a 7,8-cis isomer has never been documented as a photoproduct in any previous photochemical study in the vitamin D series.

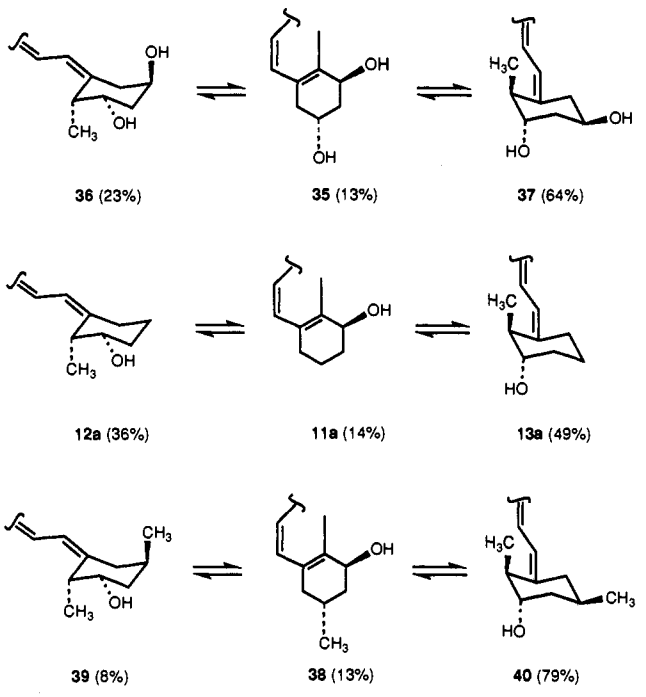
This left only the 5,6-trans-7,8-cis geometric isomer of vitamin D as the only isomer yet to be described. The isomer **4** could be accessed by catalyzed isomerization. Treatment of **3** with a solution of 0.1 mol % I_2 and exposure to ambient fluorescent lighting for 30 min afforded a mixture of four compounds, three already identified and the fourth identified as the final unknown geometric isomer **4** (^{1}H-NMR analysis). From a combination of HPLC separation and NMR analysis, an overall ratio of products was determined as ~8% **1**, 32% **2**, 42% **4**, and 18% **3** (in order of elution from the HPLC), but because of separation problems, only a small amount of pure **4** could be obtained.

An efficient route to 5,6-trans-7,8-cis-isomer **4** was developed through the cheleotropic addition–elimination of sulfur dioxide to 7,8-cis-1,25 **3**. In this route, adducts **40a** and **40b** were produced as a ~1:1 mixture (Chart 9) and then extrusion of sulfur dioxide from the individual diastereomers (**40a** and **40b**) or as a mixture afforded in good yield pure 5,6-trans-7,8-cis-1,25 **4**. The same procedure converts **1** to **2**, as has been previously described.[20]

Biological Comparison of the Geometric Isomers of 1α,25-Dihydroxyvitamin D_3. With the four geometric isomers of the steroid hormones **1** in hand, we have obtained initial information concerning one aspect of their biological profile, namely, their in

(20) (a) Ray, R.; Vicchio, D.; Yergey, A.; Holick, M. F. *Steroids* **1992**, *57*, 142. See also: (b) Yamada, S.; Takayama, H. *Chem. Lett.* **1979**, 583. (c) Reischl, W.; Zbiral, E. *Helv. Chim. Acta* **1979**, *62*, 1763.

Chart 8



vitro ability to bind the chick intestinal receptor. In this assay,[21] analogues are evaluated in terms of their chick intestinal receptor relative competitive indices (RCIs), wherein the value for 1α,25-$(OH)_2$-D_3 is 100 by definition. The RCI values for **2**, **3**, and **4** were 12.8 ± 3.0, 0.82 ± 0.09, and 1.6 ± 0.4, respectively. An examination of the four isomers **1**–**4** in Chart 1 reveals an interesting topological feature of their A-rings. Namely, by a 180° rotation (i.e., geometric isomerization) about either or both the $\Delta^{5,6}$ and $\Delta^{7,8}$ double bonds, the topological orientation of the hydroxyl groups at C_1 and C_3 with respect to the CD-side-chain fragment is retained. For example, in **2**, the C_3 and C_1 hydroxyls in **2** assume pseudo C_1 and C_3 orientations, respectively. That **2** retains the most significant RCI value (~13%) as compared to **3** and **4** may simply reflect the fact that the A-ring of **2** with respect to the CD-side-chain fragment most closely resembles that of the hormone **1** (that is, **2** is related to **1** by simply transposing the $C_{10,19}$ exocyclic methylene from the 1 o'clock to the 11 o'clock orientation). In **3** and **4**, the entire A-ring is shifted to the right in its relationship to the CD-side-chain fragment, thus attenuating their ability to bind to the chick intestinal receptor.

An alternative rationalization is based on the consideration that the hormone **1** binds to receptor via its steroid-like, 6-s-cis conformation **42a** (Chart 10) rather than the extended, 6-s-trans conformation depicted in Chart 1. Note that both **1** and **2** may assume the steroid-like conformation **42a** and **42b**, respectively. By contrast, the new geometric isomers **3** and **4**, by virtue of the presence of the 7*Z* double bond, are incapable of assuming the steroid-like conformation; instead, they can only assume the *cis*-isotachysterol-like (cf. **35**), nonsteroidal orientation **43a** and **43b**, respectively. It remains for future investigations to determine whether steroid-like or extended conformers are pertinent to the chick intestinal receptor binding.

Summary. This article highlights some of the following new contributions. First, 7,8-cis vitamin D analogues can be easily prepared stereoselectively under mild conditions from vinylallenes via chromium-induced [1,5]-hydrogen shifts. This synthesis procedure is likely to be general and should allow the preparation of other previously unknown 7,8-cis analogues of the natural metabolites of vitamin D. It will be possible to examine these previously unknown geometric isomers in terms of their role in the photochemistry and biology of vitamin D. Second, stereomechanistic information has emerged regarding the Inanaga Pd(0)–Sm(II)-induced transformation of propargyl esters to allenes[16] and the Shibasaki chromium(0)-induced [1,5]-hydrogen shift.[12] Third, the allene rearrangement route to vitamin D, coupled with the photosensitized, selective geometric isomerism of the SO_2 cheleotropic addition–elimination process, has been demonstrated to be an effective route to all four geometric isomers of vitamin D from a single intermediate. And fourth, initial biological evaluation of the new geometric isomers in terms of the in vitro chick intestinal receptor binding assay reveals that the 7,8-cis geometry is detrimental to binding. It remains for future studies to establish whether these analogues might be active in other biological target systems recently found to accommodate 1α,25-$(OH)_2$-D_3.[22]

Experimental Section[23]

1α,25-Dihydroxyvitamin D_3 (1) by Thermal Rearrangement of the Vinylallene 23a. A solution of (6*S*)-vinylallene **23a** (8.1 mg, 0.019 mmol) in isooctane (3 mL) was heated at reflux under argon for 14.5 h. The solvent was removed, and the products were analyzed first by NMR spectroscopy and then separated by HPLC (80% EtOAc/hexanes, Rainin Microsorb column, 4 mL/min flow rate) to obtain three fractions of the following composition: fraction I, A (3.3 mg, 41%); fraction II, **B**, **C**, and **D** together (1.2 mg, 15%); mp fraction III, **E** (1.3 mg, 16%). These five substances were identified by their ^{1}H-NMR spectral characteristics; **A**, 1,25-**1**; **B**, starting allene **23a**; **C**, 1,25-previtamin **34**; **D**, the 10α-methyltriene **36**; and **E**, the 10β-methyltriene **37**. **A** and **C** are known compounds; the isolation of **B**, **D**, and **E** is described elsewhere in this Experimental Section. Analysis of the C_{18}-Me region of the ^{1}H-NMR spectrum of the product mixture by the cut-and-weigh method gave the following ratio of products: **A**, 42%; **B**, 8%; **C**, 11%; **D** and **E**, 39%. Overall, the ratio for the 7*E*:7*Z* ((**A** + **C**):(**D** + **E**)) thermal manifold products is ~58:42. ^{1}H-NMR: δ 0.54 (3H, C_{18}-CH_3, s), 0.94 (3H, C_{21}-CH_3, d, J ~ 6.2 Hz), 1.22 (6H, $C_{26,27}$-$2CH_3$, s), 2.32 (1H, 4β, dd, J ~ 13.5 Hz, 6.6 Hz), 2.60 (1H, 4α, dd, J ~ 13.0 Hz, 3.1 Hz), 2.82 (1H, 9β, dd, J ~ 11.8 Hz, 3.4 Hz), 4.2–4.3 (1H, C_3-H, br m, W ~ 17 H), 4.4–4.5 (1H, C_1-H, br m, W ~ 16 Hz), 5.01 (1H, C_{19}-H, br s), 5.33 (1H, C_{19}-H, br s), 6.02 and 6.38 (2H, C_6-H and C_7-H, AB pattern, J ~ 11.2 Hz).

1α,25-Dihydroxyvitamin D_3 (1) by Photosensitized Isomerization. A solution of 7,8-cis-1,25 **3** (4.5 mg, 0.0108 mmol) and 9-acetylanthracene (12.0 mg, 0.0540 mmol) in methanol-d_4 (1 mL) was purged in a Pyrex NMR tube for 1 h with argon. The solution was irradiated through Pyrex with ultraviolet light from a Hanovia 450-W medium pressure lamp while periodically monitoring the course of the reaction by ^{1}H-NMR analysis. After 1.5 h, the solvent was removed, and then the main product obtained by HPLC (80% EtOAc/hexanes, Rainin Microsorb column, 4 mL/min flow rate) was identified as the hormone 1,25-**1** together with a small percentage of the starting 7,8-cis-isomer **3** and the 5,6-trans-isomer **2** (combined yield of 3.2 mg, 82%). Further HPLC purification gave pure **1** (2.6 mg, 67%) as a white foam.

In a separate parallel set of experiments, pure 1,25-**1** and pure 7,8-cis **3** together with sensitizer were photoisomerized under similar conditions (2.2 h total photoreaction time). After HPLC purification (ensuring collection of all trihydroxyvitamin D components), integration of the C_{18}-H signals in the ^{1}H-NMR spectra by the cut-and-weigh method gave the following proportions of the geometric isomers of 1,25: from 1,25-**1**, there was obtained 92% 1,25-**1**, 6% 5,6-trans **2**, 2% 7,8-cis **3**, and no 5,6-trans-7,8-cis **4**; and from 7,8-cis **3**, there was obtained 92% 1,25-**1**, 5% 5,6-trans **2**, 3% 7,8-cis **3**, and no 5,6-trans-7,8-cis **4**.

(21) (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. *Am. J. Clin. Nutr.* **1976**, *29*, 1271. (c) Wecksler, W. R.; Norman, A. W. *Methods Enzymol.*: *Vitam. Co-Enzymes* **1980**, *67*, 488.

(22) (a) Norman, A. W.; Okamura, W. H.; Farach-Carson, M. C.; Allewaert, K.; Branisteanu, D.; Nemere, I.; Muralidharan, K. R.; Bouillon, R. *J. Biol. Chem.* **1993**, *268*, 13811. (b) Norman, A. W.; Bouillon, R.; Farach-Carson, M. C.; Bishop, J. E.; Zhou, L.-X.; Nemere, I.; Zhao, J.; Muralidharan, K. R.; Okamura, W. H. *J. Biol. Chem.* **1993**, *268*, 20022.

(23) Spectral and other analytical data are given in the supplementary material. NMR spectral data in abbreviated form are presented in the Experimental Section as well. General experimental procedures are also presented in the supplementary material. 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. The level of purity is indicated by the inclusion of copies of NMR spectra presented in the supplementary material.

Chart 9

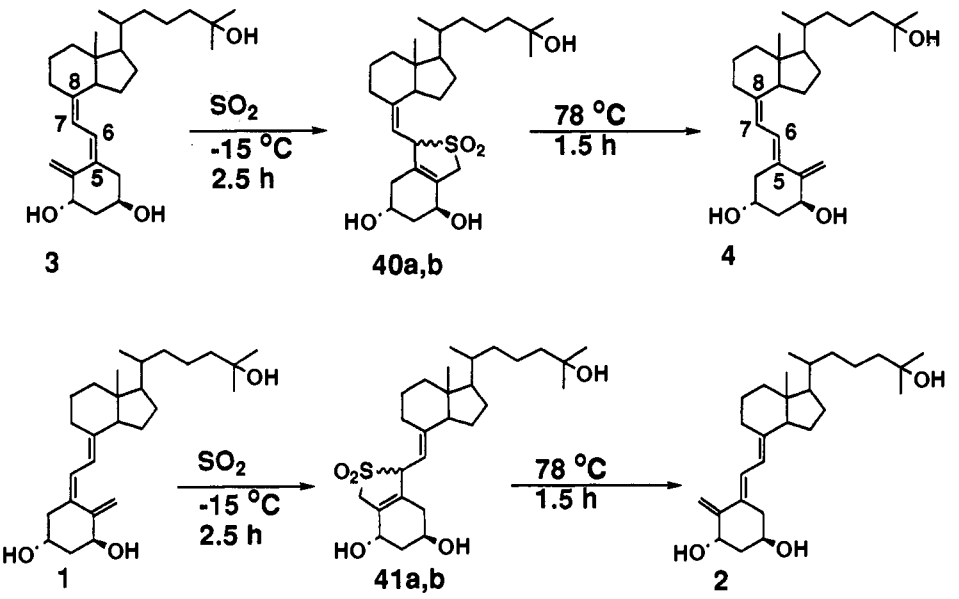
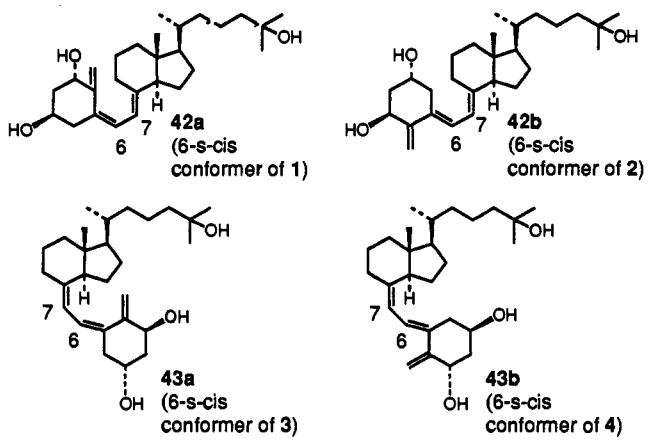


Chart 10



5,6-*trans*-1α,25-Dihydroxyvitamin D_3 (2). The solution of sulfone isomer **A** (**41a**, 6.0 mg, 0.012 mmol) and $NaHCO_3$ (19 mg) in EtOH (10 mL) was flushed with argon for 10 min, and then the mixture was heated at 78 °C for 2.5 h. The solvent was removed, and the crude product, obtained by flash chromatography (silica gel, 80% EtOAc/hexanes), was subjected to HPLC purification (80% EtOAc/hexanes, Rainin Microsorb column, 4 mL/min flow rate) to afford pure 5,6-trans-1,25 **2** (3.4 mg, 65%) as a colorless foam. ^{1}H-NMR: δ 0.56 (3H, C_{19}-CH_3, s), 0.94 (3H, C_{21}-CH_3, d, $J \sim 6.3$ Hz), 1.22 (6H, $C_{26,27}$-$2CH_3$, s), 2.28 (1H, dd, $J \sim 13.2$ Hz, 8.2 Hz), 2.84 (1H, m), 2.89 (1H, m), 4.24 (1H, C_3-H, dddd, $J \sim 4.2$ Hz, 4.2 Hz, 4.2 Hz, 4.2 Hz), 4.50 (1H, C_1-H, br m), 4.98 (1H, C_{19}-H, br s), 5.13 (1H, C_{19}-H, br s), 5.89 and 6.58 (2H, C_6-H and C_7-H, AB pattern, $J \sim 11.6$ Hz). UV: (100% EtOH) λ_{max} 274 nm (ε 16 200), λ_{min} 230 nm (ε 6100).

7,8-*cis*-1α,25-Dihydroxyvitamin D_3 (3). To the vinylallene **23a** (19.7 mg, 0.047 mmol) and (np)$(CO)_3$Cr (**22**, 14.7 mg, 0.0557 mmol) in a 10-mL flask with a stir bar was added 1 mL of acetone (distilled from $CaSO_4$). After deoxygenation of the mixture of four freeze–pump–thaw cycles, the solution was stirred at 40 °C under a positive pressure of argon for 4 h. Acetone was removed under reduced pressure, and the product was purified by flash chromatography (silica gel, 80% EtOAc/hexanes) followed by separation by HPLC (80% EtOAc/hexanes, Rainin Microsorb column, 4.0 mL/min flow rate) to afford three components in the following order of elution: major product **A** (17.0 mg, 86.4%), recovered starting material **B** (1.4 mg, 7.1%), and minor product **C** (1.5 mg, 7.6%). Each purified component was characterized by spectroscopic analysis. Compound **A** was identified as 7,8-cis-1,25 **3**, compound **B** as the starting vinylallene **23a**, and compound **C** as *cis*-isotachysterol **35**. ^{1}H-NMR: δ 0.64 (3H, C_{18}-CH_3, s), 0.95 (3H, C_{21}-CH_3, d, $J \sim 6.4$ Hz), 1.22 (6H, $C_{26,27}$-$2CH_3$, s), 2.24 (1H, dd, $J \sim 12.4$ Hz, 9.0 Hz), 2.55 (1H, dd, $J \sim 12.5$ Hz, 3.4 Hz), 4.17 (1H, C_3-H, dddd, $J \sim 4.2$ Hz, 4.2 Hz, 4.2 Hz, 4.2 Hz), 4.42 (1H, C_1-H, br s), 5.01 (1H, C_{19}-H, br s), 5.32 (1H, C_{19}-H, br s), 6.20 and 6.54 (2H, C_6-H and C_7-H, AB pattern, $J \sim 11.5$ Hz). UV: (100% EtOH) λ_{max} 266 nm (ε 15 000); λ_{min} 228 nm (ε 9300).

5,6-*trans*-7,8-*cis*-1α,25-Dihydroxyvitamin D_3 (4) by Iodine-Catalyzed Cis-Trans Isomerization. To 7,8-cis-isomer **3** (~5.0 mg, 0.012 mmol) was added a solution of 0.1 mol % I_2 in either (1 mL, 9.8×10^{-6} M). The solution was stirred under an argon atmosphere for 30 min, the solvent removed, and the products separated by HPLC. The first separation (80% ethyl acetate/hexanes, Rainin Microsorb column, 4 mL/min flow rate) gave two fractions, each of which was subjected to NMR analysis. Fraction I contained products **A**, **B**, and **C**, and fraction II contained products **B**, **C**, and **D**. By ^{1}H-NMR analysis of the two fractions and their weights, it was estimated that the four products, **A**, **B**, **C**, and **D**, were obtained in an overall ~8:32:42:18 ratio. The four products were identified from the ^{1}H-NMR analysis as the following geometric isomers: **A**, 5,6-cis-7,8-trans **1**; **B**, 5,6-trans-7,8-trans **2**; **C**, 5,6-trans-7,8-cis **4**; and **D**, 5,6-cis-7,8-cis **3**. Repeated purification by HPlC (11% EtOAc/hexanes, Rainin Microsorb column, 6 mL/min flow rate) eventually afforded pure **4** (0.5 mg; colorless, viscous oil), suitable for spectroscopic characterization. ^{1}H-NMR: δ 0.66 (3H, C_{18}-CH_3, s), 0.96 (3H, C_{21}-CH_3, d, $J \sim 6.3$ Hz), 1.22 (6H, $C_{26,27}$-$2CH_3$, s), 2.78 (1H, dd, $J \sim 12.9$ Hz, 2.7 Hz), 4.20-4.28 (1H, C_3-H, m, $W \sim 26$ Hz), 4.45–4.52 (1H, C_1-H, m, $W \sim 23$ Hz), 4.95 (1H, C_{19}-H, br s), 5.05 (1H, C_{19}-H, br s), 6.15 and 6.75 (2H, C_6-H and C_7-H, AB pattern, d, $J \sim 11.8$ Hz). UV: (100% EtOH) λ_{max} 274 nm (ε 17 400); λ_{min} 234 nm (ε 5500).

5,6-*trans*-7,8-*cis*-1α,25-Dihydroxyvitamin D_3 (4) via Sulfur Dioxide Adducts. A solution of sulfone isomer **A** (**40a**, 4.0 mg, 0.0083 mmol) and $NaHCO_3$ (14 mg) in EtOH (5 mL) was flushed with argon for 10 min and then heated at 78 °C for 1.5 h. Solvent was removed, and the crude product, obtained by flash chromatography (silica gel, 80% EtOAc/hexanes), was subjected to HPLC purification (80% EtOAc/hexanes, Rainin Microsorb column, 4 mL/min flow rate) to afford pure 5,6-trans-7,8-cis-1,25 **4** (3.3 mg, 95%) as a colorless, viscous foam. Likewise, treatment of sulfone isomer **B** (**40b**, 3.3 mg, 0.0069 mmol) with $NaHCO_3$ (15 mg) in EtOH (5 mL) followed by workup and purification exactly as above afforded pure **4** (2.5 mg, 86%) as a colorless, viscous foam.

(1*S*,6*S*)-1-Hydroxy-9,10-secocholesta-5(10),6,7-triene (6a) and (1*R*,6*S*)-1-Hydroxy-9,10-secocholesta-5(10),6,7-triene (6b). These compounds have been previously prepared.[7d,g,h]

(1*S*,6*S*)-1-Hydroxy-9,10-secocholesta-5(10),6,7-triene: ^{1}H-NMR δ 0.71 (3H, C_{18}-CH_3, s), 0.86 (6H, $C_{26,27}$-$2CH_3$, d, $J \sim 6.2$ H), 0.93 (3H, C_{21}-CH_3, d, $J \sim 6.4$ Hz), 1.86 (3H, C_{19}-CH_3, br s), 4.03 (1H, C_1-H, br s), 6.15 (1H, C_6-H, dd, $J \sim 4.0$ Hz, 4.0 Hz).

(1*R*,6*S*)-1-Hydroxy-9,10-secocholesta-5(10),6,7-triene: ^{1}H-NMR δ 0.71 (3H, C_{18}-CH_3, s), 0.87 (6H, $C_{26,27}$-$2CH_3$, d, $J \sim 6.2$ Hz), 0.93 (3H, C_{21}-CH_3, d, $J \sim 6.4$ Hz), 1.87 (3H, C_{19}-CH_3, br s), 4.01 (1H, C_1-H, br s), 6.15 (1H, C_6-H, dd, $J \sim 3.8$ Hz, 3.8 Hz).

(1*R*,6*R*)-1-Hydroxy-9,10-secocholesta-5(10),6,7-triene (7a) and (1*S*,6*R*)-1-Hydroxy-9,10-secocholesta-5(10),6,7-triene (7b). These compounds have been previously prepared.[7d,g,h]

(1*S*,6*R*)-1-Hydroxy-9,10-secocholesta-5(10),6,7-triene: ^{1}H-NMR δ

0.65 (3H, C_{18}-CH_3, s), 0.86 (6H, $C_{26,27}$-$2CH_3$, d, $J \sim 6.1$ Hz), 0.92 (3H, C_{21}-CH_3, d, $J \sim 6.4$ Hz), 1.86 (3H, C_{19}-CH_3, br s), 4.01 (1H, C_1-H, br s), 6.13 (1H, C_6-H, dd, $J \sim 3.3$ Hz, 3.3 Hz).

(1*R*,6*R*)-1-Hydroxy-9,10-secocholesta-5(10),6,7-triene: ^{1}H-NMR δ 0.65 (3H, C_{18}-CH_3, s), 0.86 (6H, $C_{26,27}$-$2CH_3$, d, $J \sim 6.1$ Hz), 0.92 (3H, C_{21}-CH_3, d, $J \sim 6.4$ Hz), 1.86 (3H, C_{19}-CH_3, br s), 4.01 (1H, C_1-H, br s), 6.13 (1H, C_6-H, dd, $J \sim 3.3$ Hz, 3.3 Hz).

7,8-*cis*-3-Deoxy-1α-hydroxyvitamin D₃ (10a). To the (1*S*,6*R*)-vinylallenol **7a** (16.5 mg, 0.0430 mmol) and (np)$(CO)_3$Cr (**22**, 12.5 mg, 0.0473 mmol) in a 10-mL flask with a stir bar was added 1 mL of acetone (distilled from $CaSO_4$). After deoxygenation of the solution by four freeze–pump–thaw cycles, the solution was stirred at 38 °C under a positive pressure of argon for 4 h. Acetone was removed under reduced pressure, and the product was purified by flash chromatography (silica gel, 15% EtOAc/hexanes) followed by separation by HPLC (15% EtOAc/hexanes, Rainin Microsorb column, 4.0 mL/min flow rate) to afford four components in the following order of elution: minor product **A** (0.3 mg, 2%), minor product **B** (0.2 mg, 1%), major product **C** (14.7 mg, 89.1%), and starting material **D** (0.6 mg, 4%). Each component was characterized by spectroscopic analysis and/or by direct comparison to known compounds. Compound **A** was identified as (1*S*)-*cis*-isotachysterol (**11a**), compound **B** as 3-deoxy-1α-hydroxyvitamin D_3 (**8a**), compound **C** as 7,8-*cis*-3-deoxy-1α-hydroxyvitamin D_3 (**10a**), and compound **D** as starting vinylallenol (**7a**). Compounds **A** and **B** have been previously reported.[7d,g,h]

Similarly, the (1*S*,6*S*)-vinylallenol **6a** (12.0 mg, 0.0313 mmol) and (np)$(CO)_3$Cr (**22**, 9.4 mg, 0.036 mmol) by the same procedure yielded **A** (0.3 mg, 2%), **B** (0.2 mg, 2%), and **C** (9.0 mg, 75%). No starting (1*S*,6*S*)-vinylallenol **6a** was recovered.

In a control experiment (38 °C, acetone, 4 h) in the absence of catalyst, no isomerization of **7a** (or **6a**, **7b**, and **6b** discussed below) was detectable by ^{1}H-NMR analysis.

As further proof of structure, compound **C** was subjected to thermal sigmatropic rearrangement. To an NMR tube was added compound **C** (4.5 mg, 0.012 mmol) in benzene-d_6 (0.6 mL), and then the tube was heated at 75 °C. At time intervals of 0, 59, 118, 200, 282, 343, and 413 min, the reaction mixture was monitored by NMR analysis. Formation of the thermal product, compound **A**, was observed to occur with a $t_{1/2}$ of ~139 min, by NMR integration. ^{1}H-NMR: δ 0.65 (3H, C_{18}-CH_3, s), 0.87 (6H, $C_{26,27}$-$2CH_3$, d, $J \sim 6.4$ Hz), 0.93 (3H, C_{21}-CH_3, d, $J \sim 6.4$ Hz), 4.19 (1H, C_1-H, dd, $J \sim 3.4$ Hz, 3.4 Hz), 4.92 (1H, C_{19}-H, br s), 5.27 (1H, C_{19}-H, br s), 6.22 and 6.46 (2H, C_6-H and C_7-H, AB pattern, $J \sim 11.5$ Hz). UV: (95% EtOH) λ_{max} 256 nm (ε 18 900); λ_{min} 228 nm (ε 11 000).

7,8-*cis*-3-Deoxy-1β-hydroxyvitamin D₃ (10b). To the (1*R*,6*R*)-vinylallenol **7b** (11.3 mg, 0.0294 mmol) and (np)$(CO)_3$Cr (**22**, 8.8 mg, 0.033 mmol) in a 10-mL flask with a stir bar was added 1 mL of acetone. After deoxygenation of the mixture by four freeze–pump–thaw cycles, the solution was stirred at 38 °C under a positive pressure of argon for 4 h. Acetone was removed under reduced pressure, and the product was purified by flash chromatography (silica gel, 15% EtOAc/hexanes) followed by separation by HPLC (15% EtOAc/hexanes, Rainin Microsorb column, 4.0 mL/min flow rate) to afford three components in the following order of elution: minor product **A** (0.8 mg, 7%), minor product **B** (0.1 mg, 1%), and major product **C** (8.5 mg, 75%). Each purified component was characterized by spectroscopic analysis and, if appropriate, by direct comparison with known compounds. Compound **A** was identified as (1*R*)-*cis*-isotachysterol (**11b**), compound **B** as 3-deoxy-1β-hydroxyvitamin D_3 (**8b**), and compound **C** as 7,8-*cis*-3-deoxy-1β-hydroxyvitamin D_3 (**10b**). Compounds **A** and **B** have been previously reported.[7d,g,h] Compound **C** is new, and no starting **7b** was recovered.

Similarly, the (1*R*,6*S*)-vinylallenol (**6b**) (11.7 mg, 0.0305 mmol) and (np)$(CO)_3$Cr (**22**, 9.1 mg, 0.034 mmol) by the same procedure yielded **A** (0.6 mg, 5%) and **C** (.95 mg, 81%), but no **B** was isolated; in addition, no starting material was recovered.

Compound **C** was subjected to thermal sigmatropic rearrangement as further evidence of structure. To an NMR tube was added compound **C** (4.8 mg, 0.012 mmol) in benzene-d_6 (0.6 mL), and then the NMR tube was heated at 75 °C. At time intervals of 0, 53, 107, 145, 270, and 310 min, the reaction mixture was monitored by NMR analysis. Formation of the thermal product, compound **A**, was observed to occur with a $t_{1/2}$ of ~81 min, by NMR integration. After 145 min, the major thermal rearrangement product of compound **A**, (1*R*,10*R*)-(5*Z*,7*Z*)-9,10-secocholesta-5,7,14-trien-1-ol (**13b**), was also observed. ^{1}H-NMR: δ 0.065 (3H, C_{18}-CH_3, s), 0.87 (6H, $C_{26,27}$-$2CH_3$, d, $J \sim 6.5$ Hz), 0.94 (3H, C_{21}-CH_3, d, $J \sim 6.4$ Hz), 4.10 (1H, C_1-H, br s), 4.94 (1H, C_{19}-H, br s), 5.32 (1H, C_{19}-H, br s), 6.20 and 6.41 (2H, C_6-H and C_7-H, AB pattern, $J \sim 11.4$ Hz). UV: (95% EtOH) λ_{max} 258 nm (ε 20 600); λ_{min} 228 nm (ε 11 200).

(1*S*,3*R*,6*S*)-1,3,25-Trihydroxy-9,10-secocholesta-5(10),6,7-triene (23a). To the vinylallene **23b** (0.1054 g, 0.1469 mmol) was added TBAF (1 M in THF, 1.6 mL, 1.6 mmol). The solution was stirred under an argon atmosphere for 19 h. Water (1 mL) was added and the solution stirred for 30 min. The mixture was extracted with ether (3 × 15 mL), and the ether extracts were washed with brine (1 × 10 mL) and dried ($MgSO_4$). The concentrated residue was subjected to flash chromatography (silica gel, 80% EtOAc/hexanes) followed by HPLC (80% EtOAc/hexanes, Rainin Microsorb column, 4 mL/min flow rate) to afford purified deprotected vinylallene **23a** together with its 6*R*-diastereomer, **24a** (46.1 mg, 75.3% total yield), in a ~92:8 ratio by NMR integration. By shave–recycle HPLC separation, pure **23a** could be obtained and characterized by spectroscopic analysis. ^{1}H-NMR: δ 0.74 (3H, C_{18}-CH_3, s), 0.95 (3H, C_{21}-CH_3, d, $J \sim 6.4$ Hz), 1.22 (6H, $C_{26,27}$-CH_3, s), 1.87 (3H, C_{19}-CH_3, s), 2.29 (1H, br d, $J \sim 13.2$ Hz), 2.62 (1H, br dd, $J \sim 16.5$ Hz, 4.5 Hz), 4.11–4.20 (1H, C_3-H, m, $W \sim 27.8$ Hz), 4.23 (1H, C_1-H, br m, $W \sim 8.6$ Hz), 6.14 (1H, C_6-H, dd, $J \sim 4.1$ Hz, 4.1 Hz). UV: (100% EtOH) λ_{max} 242 nm (ε 24 300), 234 nm (ε 23 500).

(1*S*,3*R*,6*S*)-1,3-Bis[(*tert*-butyldimethylsilyl)oxy]-25-[(trimethylsilyl)-oxy]-9,10-secocholesta-5(10),6,7-triene (23b). Freshly purified 1,2-diiodoethane (412 mg, 1.46 mmol) and samarium metal (286 mg, 1.90 mmol) were dried under vacuum and suspended in 4 mL of THF under an argon atmosphere. This solution was stirred for 2 h until it became deep blue. A solution of propargyl benzoate **33** (477 mg, 0.570 mmol) and $Pd(PPh_3)_4$ (65.8 mg, 0.037 mmol) in 6 mL of THF was added via cannula. Freshly distilled 2-propanol (0.5 mL) was added, and the solution was stirred under a positive argon atmosphere for 14 h. Saturated aqueous Na_2CO_3 (2 mL) was added to quench the reaction. The organic layer was diluted with either, and then the mixture was washed with Na_2CO_3 (3 × 10 mL), dried with $MgSO_4$, and concentrated. The product was purified by flash chromatography (silica gel, 2% EtOAc/hexanes) followed by HPLC (2% EtOAc/hexanes, Rainin Dynamax column, 8 mL/min flow rate) to afford vinylallene **23b** (0.3085 g, 75.5%). The product was identified only by ^{1}H-NMR analysis and immediately deprotected as described below. This material appeared to be more stable as the triol. ^{1}H-NMR: δ 0.06 (6H, Si-$2CH_3$, s), 0.10 (9H, Si-$3CH_3$, s), 0.11 (6H, Si-$2CH_3$, s), 0.73 (3H, C_{21}-CH_3, s), 0.89 (9H, Si-tBu, s), 0.91 (9H, Si-tBu, s), 0.94 (3H, C_{18}-CH_3, d, $J \sim 6.5$ Hz), 1.20 (6H, $C_{26,27}$-CH_3, s), 1.76 (3H, C_{19}-CH_3, s), 4.09–4.13 (1H, C_3-H, m, overlapping C_1-H), 4.17 (1H, C_1-H, br distorted singlet), 6.13 (1H, C_6-H, dd, $J \sim 3.9$ Hz, 3.9 Hz).

(1*S*,3*R*,6*R*)-1,3,25-Trihydroxy-9,10-secocholesta-5(10),6,7-triene (24a). A solution of (6*S*/6*R*)-vinylallenes **23a**, **24a** (2.6 mg, 0.0062 mmol, ~92:8 ratio of 6*S*:6*R*) in methanol-d_4 (1 mL) was prepared in a quartz NMR tube. The solution was saturated with argon for 30 min, and then the NMR tube was capped and then irradiated with ultraviolet light from a Hanovia 450-W medium pressure lamp for 30 min. Integration of the C_{18}-Me signals in the NMR spectrum revealed a ~50:50 mixture of the two isomers. Solvent was removed, and the products were separated by HPLC (11% 2-propanol/hexanes, Rainin Microsorb column, 6 mL/min, flow rate). Taking a front cut of the overlapping peaks gave pure (6*R*)-vinylallene **24a** (0.9 mg, 35%). This product was identified and characterized through spectroscopic analysis. ^{1}H-NMR: δ 0.65 (3H, C_{18}-CH_3, s), 0.94 (3H, C_{12}-CH_3, d, $J \sim 6.4$ Hz), 1.21 (6H, $C_{26,27}$-$2CH_3$, s), 1.87 (3H, C_{19}-CH_3, br s), 2.28 (1H, br d, $J \sim 13.0$ Hz), 2.52 (1H, dd, $J \sim 16.3$ Hz, 5.0 Hz), 4.12 (1H, C_3-H, m, $W \sim 30.0$ Hz, overlapping), 4.20 (1H, C_1-H, br s), 6.10 (1H, C_6-H, dd, $J \sim 3.2$ Hz, 3.2 Hz). UV: (100% EtOH) λ_{max} 242 nm (ε 22 300), 234 nm (ε 22 100).

(3*S*,5*R*)-3,5-Bis[(*tert*-butyldimethylsilyl)oxy]-1-ethynyl-2-methylcyclohex-1-ene (30). This compound, prepared as previously described,[14] was kindly provided by Dr. D. Maynard of this laboratory. ^{1}H-NMR: δ 0.06 (6H, 2Si-CH_3, s), 0.10 (6H, 2Si-CH_3, s), 0.88 (9H, Si-tBu, s), 0.90 (9H, Si-tBu, s), 1.64–1.73 (1H, m), 1.80–1.87 (1H, m), 1.92 (3H, C_2-CH_3, br s), 2.04–2.13 (1H, m), 2.38–2.45 (1H, m), 3.05 (1H, C_2-H, s), 4.05–4.14 (1H, C_5-H, m, $W \sim 26.4$ Hz), 4.20 (1H, C_3-H, dd, $J \sim 3.9$ Hz, 3.9 Hz).

De-A,B-25-[(trimethylsilyl)oxy]-8-cholestanone (31). This material was prepared as previously described.[15] ^{1}H-NMR: δ 0.09 ($3CH_3$-Si, s), 0.63 (3H, C_{18}-CH_3, s), 0.95 (3H, C_{21}-CH_3, d, $J \sim 5.9$ Hz), 1.19 (6H, $C_{26,27}$-$2CH_3$, s), 2.45 (1H, dd, $J \sim 11.5$ Hz, 7.5 Hz).

(1*S*,3*R*,8*S*)-8-Hydroxy-1,3-bis[(*tert*-butyldimethylsilyl)oxy]-25-[(trimethylsilyl)oxy]-9,10-secocholest-5(10)-en-6-yne (32). To A-ring-enyne **30** (483 mg, 1.36 mmol) in dry ether (1.6 mL) under an argon atmosphere at 0 °C was added *n*-BuLi (1.4 mmol, 0.88 mL, 1.6 M in hexanes). The solution was stirred for 1 h at 0 °C, and then the ketone

31 (402 mg, 1.14 mmol) in ether (3 mL) was added dropwise. The solution was stirred at 0 °C for 10 min and then warmed to room temperature. After stirring the mixture for 1 h, brine (1 mL) was added, the mixture was diluted with ether (10 mL), and the aqueous layer was extracted with ether (2 × 10 mL). The combined ether extracts were dried ($MgSO_4$). The residual oil after evaporation was purified by flash chromatography (silica gel, 5% EtOAc/hexanes) followed by HPLC (5% ethyl acetate/hexanes, Rainin Dynamax column, 8 mL/min flow rate) to afford pure product **32** (661 mg, 79% yield). ^{1}H-NMR: δ 0.06 (6H, Si-2CH_3, s), 0.09 (6H, Si-2CH_3, s), 0.10 (9H, Si-3CH_3, s), 0.9–1.0 (24H, series of overlapping signals due to 2 Si-tBu, C_{18}-CH_3 and C_{21}-CH_3), 1.20 (6H, $C_{26,27}$-CH_3, s), 1.87 (3H, C_{19}-CH_3, br s), 4.03–4.12 (1H, C_1-H, m, $W \sim 26.7$ Hz), 4.17 (1H, C_3-H, br s).

(1*S*,3*R*,8*S*)-8-(Benzoyloxy)-1,3-bis[(*tert*-butyldimethylsilyl)oxy]-25-[(trimethylsilyl)oxy]-9,10-secocholest-5(10)-en-6-yne (33). To propargyl alcohol **32** (586 mg, 0.818 mmol) in dry ether (3 mL) at −78 °C under an argon atmosphere was added *n*-BuLi (0.88 mmol, 0.55 mL, 1.6 M in hexanes). The solution was warmed to room temperature, stirred for 2.3 h, and then recooled to −78 °C. Freshly distilled benzoyl chloride (103 μL, 0.883 mmol) was added dropwise. The solution was warmed to room temperature and stirred for 2 h. The reaction was quenched with saturated aqueous $NaHCO_3$ (1 mL) and diluted with ether (20 mL). The organic layer was washed with $NaHCO_3$ (2 × 5 mL) and brine (1 × 5 mL) and dried ($MgSO_4$). The concentrated oil was purified by flash chromatography (silica gel, 2.5% EtOAc/hexanes) followed by HPLC (2.5% EtOAc/hexanes, Rainin Dynamax column, 8 mL/min flow rate) to afford in order of elution pure product **33** (405 mg, 59%) and recovered propargyl alcohol **32** (156 mg, 27%). ^{1}H-NMR: δ 0.05 (6H, Si-2CH_3, s), 0.08 (6H, Si-2CH_3, s), 0.11 (9H, Si-3CH_3, s), 0.87 (9H, Si-tBu, s), 0.88 (9H, Si-tBu, s), 0.93 (3H, C_{21}-CH_3, d, $J \sim 6.5$ Hz), 1.04 (3H, C_{18}-CH_3, s), 1.21 (6H, $C_{26,27}$-CH_3, s), 1.88 (3H, C_{19}-CH_3, s), 2.36 (1H, dd, $J \sim 16.7$ Hz, 4.5 Hz), 3.12 (1H, d, $J \sim 10.1$ Hz), 4.01–4.09 (1H, C_3-H, m, $W \sim 32$ Hz), 4.14 (1H, C_1-H, br s), 7.43 (2H, *m*-Ar, t, $J \sim 7.4$ Hz, 7.7 Hz), 7.55 (1H, *p*-Ar, t, $J \sim 7.3$ Hz), 8.05 (2H, *o*-Ar, d, $J \sim 7.4$ Hz).

1α,25-Dihydroxy-*cis*-isotachysterol (35). To an NMR tube was added 7,8-cis-1,25 **3** (10.4 mg, 0.0250 mmol) in 1 mL of acetone-d_6. The NMR tube was covered with foil, flushed with argon, and then heated at 57 °C. At time intervals of 0, 23, 85, 177, 454, and 1099 min, the reaction mixture was monitored by NMR analysis. Formation of the thermal product **35** was observed to occur with a $t_{1/2}$ of ~1130 min, by NMR integration. No other products were observed. After 18.5 h, the solvent was removed and the product readily separated from starting material by HPLC (80% EtOAc/hexanes, Rainin Microsorb column, 4 mL/min flow rate) to obtain pure **35** (4.5 mg, 43% yield). ^{1}H-NMR: δ 0.88 (3H, C_{18}-CH_3, s), 0.96 (3H, C_{21}-CH_3, d, $J \sim 6.6$ Hz), 1.21 (6H, $C_{26,27}$-2CH_3, s), 1.69 (3H, C_{19}-CH_3, s), 2.52 (1H, dd, $J \sim 16.5$ Hz, 4.2 Hz), 4.07 (1H, C_3-H, m), 4.19 (1H, C_1-H, br s), 5.78 and 5.92 (2H, C_6-H and C_7-H, AB pattern, $J \sim 12.1$ Hz). UV: (100% EtOH) λ_{max} 256 nm (ε 11 000); λ_{min} 230 nm (ε 7500).

(1*S*,3*R*,10*R*)-(5*Z*,7*Z*)-Secocholesta-5,7,14-triene-1,3,25-triol (36) and (1*S*,3*R*,10*S*)-(5*Z*,7*Z*)-9,10-Secocholesta-5,7,14-triene-1,3,25-triol (37). A solution of 7,8-cis-1,25 **3** (10.3 mg, 0.0247 mmol) in isooctane (4 mL) was heated at reflux (100 °C) under argon for 36 h. The solvent was removed, and the products were analyzed first by NMR spectroscopy and then separated by HPLC (80% EtOAc/hexanes, Rainin Microsorb column, 4 mL/min flow rate) to obtain, in order of elution, **A** (2.4 mg, 23%), **B** (0.7 mg, 6.8%), and **C** (5.1 mg, 49%). By NMR integration of the product mixture, the ratio of **A**:**B**:**C** was determined to be 23%:13%:64%. The products were identified by spectroscopic analysis as follows: **A**, (1*S*,3*S*,10*R*)-(5*Z*,7*Z*)-9,10-secocholesta-5,7,14-triene-1,3,25-triol (**36**); **B**, 1α,25-dihydroxy-*cis*-isotachysterol (**35**); and **C**, (1*S*,3*R*,10*S*)-(5*Z*,7*Z*)-9,10-secocholesta-5,7,14-triene-1,3,25-triol (**37**).

In a separate experiment, compound **C** (5.1 mg, 0.012 mmol) in isooctane (5 mL, freshly distilled from $LiAlH_4$) was heated at reflux (100 °C) under argon for 48 h. The solvent was removed, and the products were analyzed. NMR integration indicated the ratio of **A**:**B**:**C** was 26%:13%:61%.

36: ^{1}H-NMR δ 0.86 (3H, C_{18}-CH_3, s), 0.95 (3H, C_{21}-CH_3, d, $J \sim 5.6$ Hz), 1.05 (3H, C_{19}-CH_3, d, $J \sim 7.0$ Hz), 1.23 (6H, $C_{26,27}$-2CH_3, s), 2.40 (1H, ddd, $J \sim 16.2$, Hz, 3.7 Hz, 3.1 Hz), 2.58 (1H, dd, $J \sim 14.4$ Hz, 2.2 Hz), 3.28 (1H, C_{10}-H, dq, $J \sim 6.5$ Hz, 6.5 Hz), 4.04–4.12 (2H, C_1-H and C_3-H, 2 overlapping br m, $W \sim 21$ Hz), 5.49 (1H, C_{15}-H, br s), 6.22 (2H, C_6-H and C_7-H, apparent s); UV (100% EtOH) λ_{max} 274 nm (ε 15 600); λ_{min} 238 nm (ε 4200).

37: ^{1}H-NMR δ 0.87 (3H, C_{18}-CH_3, s), 0.95 (3H, C_{21}-CH_3, d, $J \sim 5.5$ Hz), 1.11 (3H, C_{19}-CH_3, d, $J \sim 7.2$ Hz), 1.23 (6H, $C_{26,27}$-2CH_3, s), 2.98 (1H, C_{10}-H, q, $J \sim 6.8$ Hz), 3.91–3.99 (2H, $C_{1,3}$-2H, 2 overlapping br m, $W \sim 26$ Hz), 5.50 (1H, C_{15}-H, br s), 6.12 and 6.31 (2H, C_6-H and C_7-H, AB pattern, $J \sim 11.2$ Hz); UV (100% EtOH) λ_{max} 272 nm (ε 16 500); λ_{min} 238 nm (ε 5000).

Sulfur Dioxide Adducts A and B of 7,8-*cis*-1α,25-Dihydroxyvitamin D_3 (40a,b). A solution of the 7,8-cis-isomer **3** (15.6 mg, 0.0374 mmol) in dichloromethane (4 mL) was cooled to −15 °C. Sulfur dioxide (5 mL), predried by passage through concentrated sulfuric acid, was condensed into the cooled reaction flask. The solution was stirred for 3 h at −15 °C, and then the mixture was slowly warmed to room temperature, allowing the SO_2 to boil off. The solvent was removed under reduced pressure, and pure product was obtained by HPLC (100% EtOAc, Rainin Microsorb column, 4 mL/min flow rate) as two fractions, **A** (7.2 mg, 40%; colorless, solid residue) and **B** (5.5 mg, 31%; colorless, solid residue). **A** and **B** were identified as the two epimeric sulfone adducts **40a** and **40b**, but absolute stereochemical identification was not attempted.

40a: ^{1}H-NMR δ 0.68 (3H, C_{18}-CH_3, s), 0.96 (3H, C_{21}-CH_3, d, $J \sim 6.2$ Hz), 1.22 (6H, $C_{26,27}$-2CH_3, s), 3.68 (1H, C_{19}-H, d, $J \sim 16.2$ Hz), 3.98 (1H, C_{19}-H, d, $J \sim 16.2$ Hz), 4.24 (1H, C_3-H, dddd, $J \sim 4.3$ Hz, 4.3 Hz, 4.3 Hz, 4.3 Hz), 4.40 (1H, C_1-H, br s), 4.93 and 5.02 (2H, C_6-H and C_7-H, AB pattern, $J \sim 11.2$ Hz).

40b: ^{1}H-NMR δ 0.73 (3H, C_{18}-CH_3, s), 0.95 (3H, C_{21}-CH_3, d, $J \sim 6.4$ Hz), 1.21 (6H, $C_{26,27}$-2CH_3, s), 2.29 (1H, br d, $J \sim 13.1$ Hz), 2.46 (1H, br d, $J \sim 17.5$ Hz), 3.70 (1H, C_{19}-H, d, $J \sim 15.8$ Hz), 4.01 (1H, C_{19}-H, d, $J \sim 15.8$ Hz), 4.23 (1H, C_3-H, m), 4.40 (1H, C_1-H, br s), 4.87 and 4.98 (2H, C_6-H and C_7-H, AB pattern, $J \sim 11.0$ Hz).

Sulfur Dioxide Adducts A and B of 1α,25-Dihydroxyvitamin D_3 (41a,b). A solution of **1** (18.7 mg, 0.0449 mmol) in dichloromethane (5 mL) was cooled to −15 °C. Sulfur dioxide (5 mL), predried by passage through concentrated sulfuric acid, was condensed into the cooled reaction flask. The solution was stirred for 3 h at −15 °C, and then the mixture was slowly warmed to room temperature, allowing the sulfur dioxide to boil off. Solvent removal under reduced pressure followed by HPLC (100% EtOAc, Rainin Microsorb column, 4 mL/min flow rate) afforded two fractions: 6.0 mg, 28%, of a colorless solid residue identified as the sulfone adduct **A** and 6.7 mg, 31%, of a colorless solid residue, identified as a ~1:2 mixture of sulfone adducts **A** and **B**. These compounds have been previously reported.[20]

41a: ^{1}H-NMR δ 0.56 (3H, C_{18}-CH_3, s), 0.94 (3H, C_{21}-CH_3, d, $J \sim 6.0$ Hz), 1.22 (6H, $C_{26,27}$-2CH_3, s), 2.45 (1H, br d, $J \sim 17.6$ Hz), 2.58 (1H, br d, $J \sim 11.9$ Hz), 3.73 (1H, C_{19}-H, d, $J \sim 16.3$ Hz), 4.02 (1H, C_{19}-H, d, $J \sim 16.3$ Hz), 4.26 (1H, C_3-H, m), 4.44 (1H, C_1-H, br s), 4.70 and 4.79 (2H, C_6-H and C_7-H, AB pattern, $J \sim 9.9$ Hz).

41b: ^{1}H-NMR δ 0.64 (3H, C_{19}-CH_3, s), 0.94 (3H, C_{21}-CH_3, d, $J \sim 6.2$ Hz), 1.25 (6H, $C_{26,27}$-2CH_3, s), 3.70 (1H, C_{19}-H, br d, $J \sim 17.5$ Hz), 4.03 (1H, C_{19}-H, br d, $J \sim 16.0$ Hz), 4.20 (1H, C_3-H, m), 4.42 (1H, C_1-H, m), 4.68 and 4.76 (2H, C_6-H and C_7-H, AB pattern, $J \sim 10.4$ Hz).

1α,25-$(OH)_2$-D_3 Chick Intestinal 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.[21] Increasing amounts of nonradioactive 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-$(OH)_2$-[^{3}H]-D_3] bound × 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 (25 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.