Thermal [1,7]-Sigmatropic Shift of Previtamin D_3 to Vitamin D_3: Synthesis and Study of Pentadeuterio Derivatives[1]

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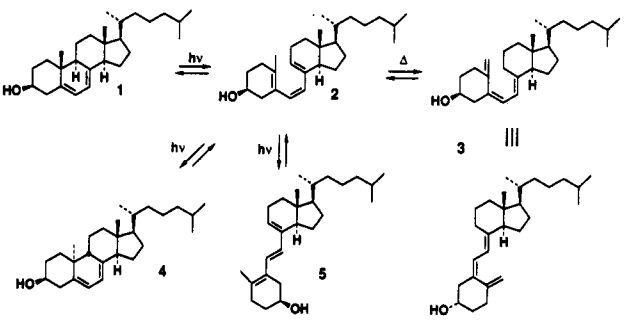
Specifically pentadeuteriated previtamin D_3 **11** has been synthesized to impart thermal stability to the otherwise labile material. A 12-step synthesis of the trideuteriated A-ring **14b** from *p*-methoxyphenol was developed and employed the addition of (methyl-d_3)magnesium iodide to either the keto thiomethylene intermediate **23** or the keto dioxane **27**. The enantiomerically pure trideuterio A-ring (−)-**14b** was then coupled with the deuteriated CD fragment **13b** followed by hydrogenation to afford pentadeuteriated previtamin D_3 **11**. A primary deuterium kinetic isotope effect (KIE) study of the [1,7]-sigmatropic hydrogen migration in the conversion of previtamin D_3 to vitamin D_3 indicated a more "normal" primary deuterium isotope effect (as compared to a previously reported literature value of ~45). At 80 °C, a k_H/k_D for the previtamin D_3 to vitamin D_3 isomerization was determined to be ~6.2. At 25 °C, this [1,7]-sigmatropic hydrogen migration proceeds with a k_H/k_D of ~11.4. The reversible, first-order [1,7]-sigmatropic hydrogen shift of previtamin D_3 to D_3, determined over the temperature range 60.1–85.5 °C is characterized by the following activation parameters: log A^H = 8.8 and E_a^H = 19.6 kcal/mol. Deuteriated pre-D_3, which rearranges over this temperature range, is characterized by the activation parameters log A^D = 9.5 and E_a^D = 21.9 kcal/mol.

Introduction

One of the continuing goals of this laboratory is to develop a detailed understanding at the molecular level of the biochemical mode of action of vitamin D_3 (**3**) by synthesizing analogues designed for the purpose of obtaining mechanistic information.[2] The chemical developments could lead to substrates which may serve as useful biochemical research tools. It is now widely recognized that vitamin D_3 is produced in the skin as a result of ultraviolet irradiation (Scheme I).[3,4] The metabolic pathway formally incorporates two classical pericyclic processes. The first involves a photochemical, conrotatory electrocyclic ring opening of 7-dehydrocholesterol (**1**) leading to previtamin D_3 (**2**). This photochemical process gives rise to other photoisomers[3] including lumisterol (**4**) and tachysterol (**5**).

The second involves transformation of previtamin D_3 (**2**), the primary photoproduct of the irradiation of

Scheme I



7-dehydrocholesterol (**1**), to vitamin D_3 (**3**) via a formal [1,7]-sigmatropic hydrogen shift wherein the thermal equilibrium favors the latter.[3b] It was once believed that **1** was converted directly to **3** by photolysis. The intermediacy of the previtamin[4] was demonstrated in 1949 by Velluz who coined the term "previtamin D",[4a,b] and this area was further developed in the Netherlands by the Havinga group.[3,4d-e] In 1977, Holick[4f-i] studied the photometabolism of [3α-^{3}H]-7-dehydrocholesterol in the skin of rats and identified the major photolytic product as previtamin D_3 (**2**), seemingly demonstrating the in vivo intermediacy of the latter. The kinetics and thermodynamics of this isomerization for the specific case of previtamin D_3 has been studied in detail by Hanewald et al.[5a] and others.[5b,c] The equilibrium ratio of previtamin to vitamin is temperature dependent and the reaction follows reversible, first-order kinetics. The intramolecular nature of this thermal process has been established through the work of Havinga, Akhtar, and others.[3-6] The antarafacial stereochemistry of the [1,7]-sigmatropic process

(1) This is paper 44 in the series Studies of Vitamin D (Calciferol) and Its Analogues. For publication 43, see: Craig, A. S.; Norman, A. W.; Okamura, W. H. *J. Org. Chem.* **1992**, *57*, 4374.

(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 editor) *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.

(3) For a review of the photochemistry of vitamin D and its isomers, see: (a) Jacobs, H. J. C.; Havinga, E. *Adv. Photochem.* **1979**, *11*, 305. (b) Havinga, E. *Experientia* **1973**, *29*, 1181.

(4) (a) Velluz, L.; Amiard, G.; Petit, A. *Bull. Soc. Chim. Fr.* **1949**, *16*, 501. (b) Velluz, L.; Amiard, G.; Goffinet, B. *Bull Soc. Chim. Fr.* **1955**, *22*, 1341. (c) Legrand, M.; Mathieu, J. *C. R. Hebd. Seances Acad. Sci.* **1957**, *245*, 2502. (d) Schlatmann, J. L. M. A.; Pot, J.; Havinga, E. *Recl. Rev. Trav. Chim. Pays-Bas* **1964**, *83*, 1173. (e) Verloop, A.; Koevoet, A. L.; Havinga, E. *Recl. Trav. Chim. Pays-Bas* **1957**, *76*, 689. (f) Holick, M. F.; Frommer, J. E.; McNeill, S. C.; Richtand, N. M.; Henley, J. W.; Potts, J. T., Jr. *Biochem. Biophys. Res. Commun.* **1977**, *76*, 107. (g) Holick, M. F.; MacLaughlin, J. A.; Clark, M. B.; Holick, S. A.; Potts, J. T., Jr.; Anderson, R. R.; Blank, I. H.; Parrish, J. A.; Elias, P. *Science* **1980**, *210*, 203. (h) Holick, M. F. *J. Invest. Dermatol.* **1981**, *76*, 51. (i) MacLaughlin, J. A.; Anderson, R. R.; Holick, M. F. *Science* **1982**, *216*, 1001.

(5) (a) Hanewald, K. H.; Rappoldt, M. P.; Roborgh, J. R. *Recl. Trav. Chim. Pays-Bas* **1961**, *80*, 1003. (b) Yamamoto, J. K.; Borch, R. F. *Biochemistry 1985, 24*, 3338. (c) Cassis, E. G., Jr.; Weiss, R. G. *Photochem. Photobiol.* **1982**, *35*, 439.

(6) (a) Akhtar, M.; Gibbons, C. J. *Tetrahedron Lett.* **1965**, 509. (b) Akhtar, M.; Gibbons, C. J. *J. Chem. Soc.* **1965**, 5964.

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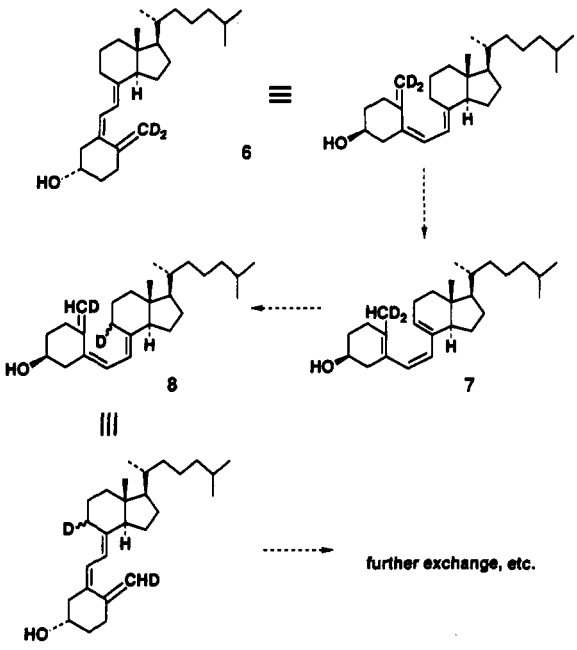
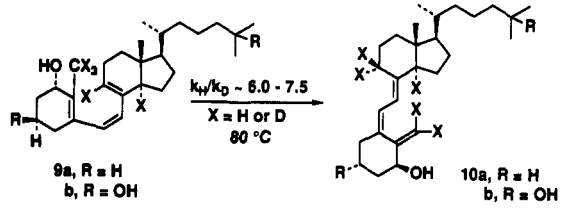
Scheme II



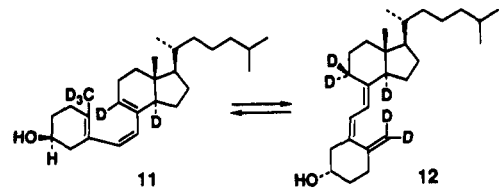
Scheme III



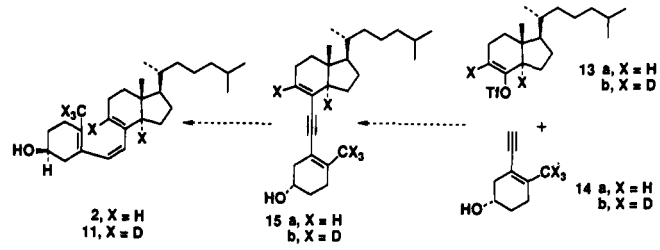
predicted by Woodward and Hoffmann[7] was demonstrated recently using a deuterium-labeled analog of vitamin D.[8]

The first primary KIE (kinetic isotope effect)[9] study of the [1,7]-sigmatropic hydrogen shift of previtamin D_3 to vitamin D_3 was carried out by Mazur and co-workers in 1979.[10] They reported that the interconversion of deuterium-labeled previtamin D_3 occurs with an exceptionally large primary deuterium KIE of ~45. They prepared C-19 dideuteriated D_3 (**6**) and followed the kinetics of the [1,7]-sigmatropic hydrogen shift at 80 °C (Scheme II). Their data was reportedly a reflection of the rate of transfer of deuterium from C_{19} of labeled previtamin D_3 **7** or another C_{19}-labeled species (generated in situ) to the vitamin form. The primary KIE value of ~45 for the transformation **7** to **8** is now considered to be in error.[10] In more recent studies (Scheme III),[11] a "normal" primary deuterium KIE (~6.0) was observed for the [1,7]-sigmatropic hydrogen migration at 80 °C in the isomerization of d_5-labeled 3-deoxy-1α-hydroxyprevitamin D_3 (**9a**) to the corresponding vitamin **10a** as shown in Scheme III. In the most recent investigation, the rearrangement of analogously labeled

Scheme IV



Scheme V



1α,25-dihydroxyprevitamin D_3-d_5 (**9b**) to 1α,25-dihydroxyvitamin D_3-d_5 (**10b**) was studied, and a similar primary KIE value of ~7.5 (80 °C) was also determined.[12]

It is the purpose of this article to describe a more direct study than that described earlier[10] of the transformation of the parent previtamin D_3 to vitamin D_3 in labeled form (i.e., **11** to **12**, respectively in Scheme IV) through appropriate synthetic and kinetic investigations in complete parallel to our recent studies of the rearrangement of **9** to **10**.[11,12]

Results and Discussion

Synthesis Studies. The plan for preparing the target 9,14,19,19,19-pentadeuteriated-previtamin D_3 (**11**) resembles a Lythgoe type route as modified by several groups including our own for synthesizing various vitamin D_3 analogues.[13] The initial approach (Scheme V) entailed the palladium-catalyzed coupling of enol triflate **13** and A-ring enynol **14** (as a racemic mixture) to afford dienynol **15** as a mixture of potentially separable diastereomers. Studies revealed the impracticality of achieving the separation of diastereomeric mixtures (epimeric at C-3) of **15** or later intermediates. Thus, this required the preparation of enantiomerically pure **14** in the ultimately successful synthesis. Lindlar hydrogenation of the desired coupled intermediate **15** with the correct stereochemistry at the 3-position was anticipated to afford the desired previtamin **11** (or **2**). This route also allows, in principle, radiolabel incorporation at the very last stage of the synthesis (i.e., in the Lindlar hydrogenation step using tritium gas) should biochemical needs arise.

The CD-ring triflate **13a** was prepared by a known procedure from Grundmann's ketone **16a**[11,12] as shown in Scheme VI. For preparing the 9,14-dideuterio triflate **13b**, the deuterium label could be introduced by base-catalyzed deuterium–hydrogen exchange (3 cycles) of the precursor ketone **16a**.[11] (Steroid numbering is used unless otherwise indicated.) This introduces deuterium not only in the desired 9-position, but also at the 14-position along with epimerization at the same 14-position. A mixture of

(7) (a) Woodward, R. B.; Hoffmann, R. *J. Am. Chem. Soc.* **1965**, *87*, 2511. (b) Spangler, C. W. *Chem. Rev.* **1976**, *76*, 187.

(8) (a) Hoeger, C. A.; Okamura, W. H. *J. Am. Chem. Soc.* **1985**, *107*, 268. (b) Hoeger, C. A.; Johnston, A. D.; Okamura, W. H. *J. Am. Chem. Soc.* **1987**, *109*, 4690.

(9) (a) Melander, L.; Saunders, W. H., Jr. *Reaction Rates of Isotopic Molecules*; Wiley: New York, 1980; pp 144, 156. For a review of the magnitude of the primary deuterium KIE, see: (b) Westheimer, F. H. *Chem. Rev.* **1961**, *61*, 265. (c) Wolfsberg, M. *Acc. Chem. Res.* **1972**, *5*, 225.

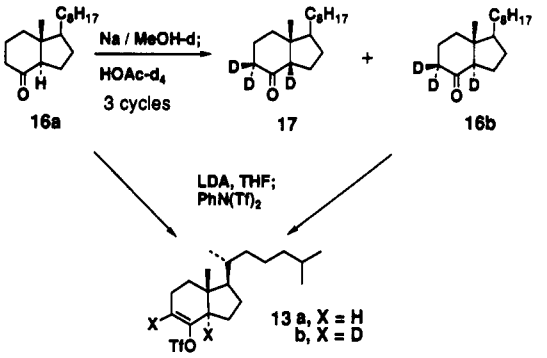
(10) Sheves, M.; Berman, E.; Mazur, Y.; Zaretskii, Z. V. I. *J. Am. Chem. Soc.* **1979**, *101*, 1882. We acknowledge Dr. Y. Mazur and Dr. M. Sheves for helpful information on this matter.

(11) Okamura, W. H.; Hoeger, C. A.; Miller, K. J.; Reischl, W. *J. Am. Chem. Soc.* **1988**, *110*, 973.

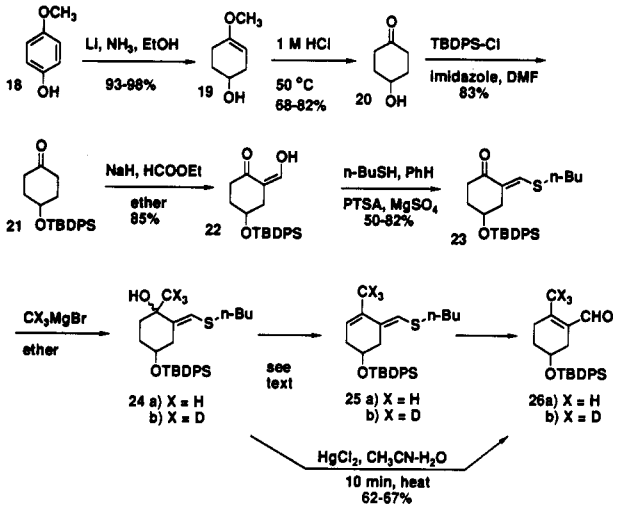
(12) Curtin, M. L.; Okamura, W. H. *J. Am. Chem. Soc.* **1991**, *113*, 6958.

(13) (a) Castedo, L.; Mouriño, A.; Sarandeses, L. A. *Tetrahedron Lett.* **1986**, *27*, 1523. (b) Barrack, S. A.; Gibbs, R. A.; Okamura, W. H. *J. Org. Chem.* **1988**, *53*, 1790. (c) Castedo, L.; Mascareñas, J. L.; Mouriño, A.; Sarandeses, L. A. *Tetrahedron Lett.* **1988**, *29*, 1203.

Scheme VI



Scheme VII



trideuterio Grundmann's ketone **16b** and its C_{14} epimer **17** was obtained (~1:2.1 ratio), and the isomers were subjected to chromatographic separation. The desired deuteriated ketone **16b** was transformed to the corresponding dideuteriated triflate **13b** in a manner similar to that of the protio analogue. Protio analogues of all the deuteriated species employed in this study were prepared side-by-side for the purpose of analytical and spectral comparison. Mass spectral deuterium analysis of the labeled triflate **13b** ranged from 96% to 98.5% d_2 and 4% to 1.5% d_1 with no d_0 species. The ^{1}H-NMR signal at δ 5.58 assigned to H_9 for the protio triflate was completely absent in the corresponding deuteriated triflate. The lower deuterium incorporation may occur at the 14-position (since this methine hydrogen being tertiary should be less acidic and hence exchanged more slowly than the secondary H-9 protons in **16a**). The H_{14} proton ^{1}H-NMR signal of **16a** and **13a** is overlapped with other signals so it could not be ascertained whether this was the case.

For preparing the A-ring component **14**, we envisaged a classical resolution approach utilizing well-known 4-oxygenated cyclohexanone intermediates. As depicted in Scheme VII, the hydroxycyclohexanone **20** was obtained via Birch reduction of *p*-methoxyphenol (**18**) followed by acid hydrolysis. The procedure of Radlick as modified by Marshall was followed[14] and the keto–alcohol **20** was then transformed to the crystalline *tert*-butyldiphenylsilyl (TBDPS) ether **21**.[15] Formylation of the keto silyl ether **21** to **22** was accomplished under standard reaction conditions.[16] Protection of the formyl ketone as the (*n*-butylthio)methylene[17] ketone **23** proceeded in a good yield when freshly purchased *n*-butanethiol was used.

The commercially available CD_3MgBr ((methyl-d_3)-magnesium iodide, 99+ atom % D, 1.0 M solution in ether) was utilized in this study as the source of the labeled C-19 of the target molecule. The addition of methylmagnesium iodide to the keto enol thioether **23** occurs in a 1,2-manner, and after workup, the resulting unstable hydroxy enol thioether intermediate **24a** was found to convert cleanly to the diene sulfide **25a** upon standing. In the labeled compound **25b**, the ^{1}H-NMR signal at δ 1.72 (assigned to the vinylic methyl in the protio analogue **25a**) was not detectable.

In attempts to effect the hydrolysis of the relatively stable diene sulfide **25** to corresponding vinyl aldehyde **26**, several alternative conditions were explored[18] with disappointing results.[19] Hydrolysis in aqueous acetonitrile with mercuric chloride,[20] a procedure developed by Corey for hydrolysis of cyclic vinyl sulfides, gave somewhat better results. The yield however never exceeded 40%. The hydrolysis was slow, and the higher temperatures and long reaction times needed may have led to deterioration of starting material or product, including partial hydrolysis of the silyl protecting group.

It became apparent that direct hydrolysis of the labile intermediate **24** might give better results. However, as indicated above, **24** easily loses water to afford **25**. It is noteworthy that mercuric chloride hydrolysis of this unstable intermediate **24** in the presence of $CaCO_3$ slightly improved the yield in small-scale reactions.[21] Under the standard conditions ($HgCl_2$–$CaCO_3$/CH_3CN–H_2O; warm) the diene sulfide **25** is inevitably formed and was found to be stable even when heated for several hours. It was determined that the use of Corey's conditions without added $CaCO_3$, a rapid workup procedure, and a short hydrolysis reaction time were key to obtaining satisfactory yields. Thus, the labeled and unlabeled vinyl aldehydes, **26b** and **26a**, respectively, were obtained in significantly higher yields under these new conditions (62% and 67%, respectively). Another procedure based on the 1,3-dioxane **27** (Scheme VIII) has emerged as a somewhat improved procedure for conversion of **22** to **26a,b**.[22] The ^{1}H-NMR signal at δ 2.12, assigned to the vinylic methyl in the protio enal **26a**, is essentially absent in the deuterio analogue **26b**. Mass spectral deuterium analysis of the labeled aldehyde afforded the following deuterium content: 98.6% d_3 and 1.4% d_2 and no d_1 or d_0 species.

It should also be noted that the recently reported method for the synthesis of 2-alkylated 1-cyclohexenecarboxal-

(14) (a) Jones, E. R. H.; Sondheimer, F. *J. Chem. Soc.* **1949**, 615. (b) Radlick, P.; Crawford, H. T. *J. Org. Chem.* **1972**, *37*, 1669. (c) Marshall, J. A.; Flynn, G. A. *Synth. Commun.* **1979**, *9*, 123.

(15) Hanessian, S.; Lavallee, P. *Can. J. Chem.* **1975**, *53*, 2975.

(16) Eaton, P. E.; Job, P. G. *Synthesis* **1983**, 796.

(17) (a) Ireland, R. E.; Marshall, J. A. *J. Org. Chem.* **1962**, *27*, 1615. (b) Bernstein, P. R. *Tetrahedron Lett.* **1979**, 1015.

(18) (a) Woods, G. F.; Griswold, P. H., Jr.; Armbrecht, B. H.; Blumenthal, D. I.; Plapinger, R. *J. Am. Chem. Soc.* **1949**, *71*, 2028. (b) Ho, T.-L.; Wong, C. M. *Synthesis* **1972**, 561. (c) Stahl, I. *Synthesis* **1981**, 135. (d) Corey, E. J.; Hase, T. *Tetrahedron Lett.* **1975**, 3267. (e) Fuji, K.; Ichikawa, K.; Fujita, E. *Tetrahedron Lett.* **1978**, 3561. (f) Ho, T.-L.; Ho, H. C.; Wong, C. M. *J. Chem. Soc., Chem. Commun.* **1972**, 791. (g) For a review, see: Gröbel, B.-T.; Seebach, D. *Synthesis* **1977**, 357.

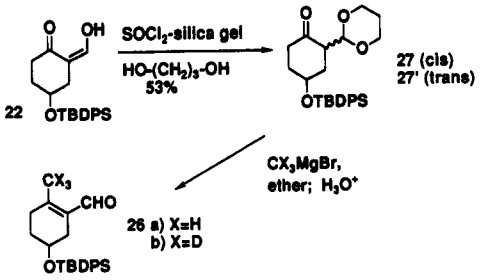
(19) For more complete details, see: Elnagar, H. Y. Ph.D. Dissertation, University of California, Riverside, Riverside, CA, 1989.

(20) Corey, E. J.; Shulman, J. I. *J. Org. Chem.* **1970**, *35*, 777.

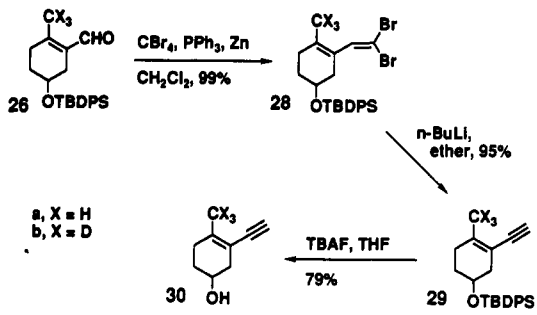
(21) See for example: (a) Corey, E. J.; Noyori, R. *Tetrahedron Lett.* **1970**, 311. (b) Fieser, L. F.; Fieser, M. *Reagents Org. Synth.* **1967**, *1*, 654; **1969**, *2*, 182.

(22) Kamitori, Y.; Hojo, M.; Masuda, R.; Kimura, T.; Yoshida, T. *J. Org.* **1986**, *51*, 1427.

Scheme VIII



Scheme IX

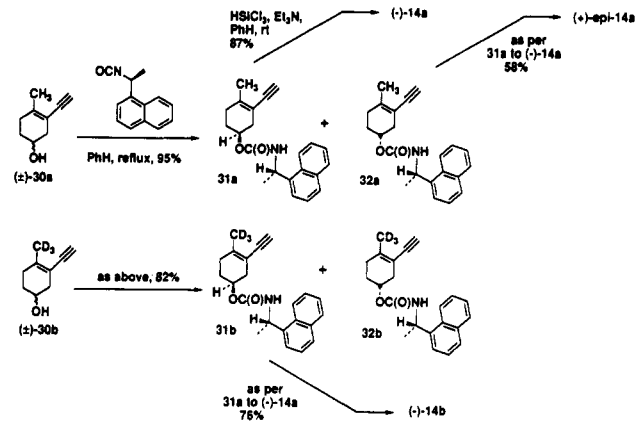


dehydes[23] by Lugtenburg and co-workers was unsuccessfully pursued. Although the latter procedure was used successfully in this laboratory for the synthesis of a related 7-membered-ring vinyl aldehyde,[24] disappointing results were obtained when applied to the keto silyl ether **21**.

The Corey–Fuchs procedure[25] was used for the transformation of the vinyl aldehyde **26** (X = H or D) to the corresponding enyne **29** (X = H or D). The dibromide **28** (X = H or D) was prepared in excellent yield upon treatment of **26** with CBr_4–PPh_3/Zn in dichloromethane (Scheme IX). The reaction of the dibromide with *n*-BuLi led to the enyne **29** also in excellent yield. Finally, treatment of **29** with fluoride ion resulted in the smooth removal of the *tert*-butyldiphenylsilyl ether group.[15] Thus, the racemic labeled A-ring **30** was obtained in ten steps starting from the commercially available *p*-methoxyphenol.

The racemic enynol **30a** (as well as the labeled counterpart **30b**) was coupled with (*S*)-naphthyl isocyanate (in benzene at reflux for 3 days), allowing for the preparation of pure (1*S*,1′*S*) and (1*R*,1′*S*) carbamates **31a** and **32a** (Scheme X). The resulting diastereomeric carbamate mixture was subjected in each case to HPLC chromatographic separation, and the base-line separated carbamates eluted in the following order: (1*S*,1′*S*) isomer **31a** followed by (1*R*,1′*S*) isomer **32a**. The stereochemical assignments for **31a** and **32a** are based upon an empirical rule governing elution orders of these carbamates as developed by Pirkle.[26] Furthermore, their assignment as the (1*S*,1′*S*) and (1*R*,1′*S*) isomers was supported by correlation of their corresponding alcohols to known compounds. The (1*S*,1′*S*) carbamate **31a** was deprotected

Scheme X



with trichlorosilane[27] in the presence of Et_3N in benzene to afford the alcohol (−)-**14a**. This same alcohol (−)-**14a** has been previously reported[28] by Lythgoe and co-workers using a different route. In a similar manner, (1*R*,1′*S*) carbamate **32a** was deprotected to afford the alcohol (+)-*epi*-**14a**, thus confirming the stereochemical assignments of the carbamate precursors. Based on the base line separation of the carbamate precursors and the assumed retention in the carbamate ester hydrolysis,[27,29] the desired alcohol (−)-**14a** (as well as its epimer (+)-*epi*-**14a**) is assumed to be configurationally pure despite the discrepancy in specific rotation with that reported by Lythgoe.[28]

In a similar fashion the deuterium labeled (±)-alcohol **30b** was coupled with the same (*S*)-naphthylethyl isocyanate to afford the (1*S*,1′*S*) and (1*R*,1′*S*) carbamates **31b** and **32b**, respectively. The desired deuteriated (1*S*,1′*S*) isomer **31b** was deprotected with trichlorosilane to afford the deuterium labeled alcohol (−)-**14b**. Again, the latter alcohol (−)-**14b** is assumed to be configurationally pure based on the base-line separation of the carbamate precursors and the assumed stereochemical retention in the carbamate hydrolysis step.

The ^{1}H-NMR signal at δ 1.90 assigned to the vinylic methyl of the (−)-alcohol **14a** is absent in the deuteriated analogue (−)-alcohol **14b**. The ^{13}C-NMR signal at δ 21.5 assigned to the same vinylic methyl carbon of the protio alcohol is essentially absent in the deuteriated counterpart. With the optically active A-ring fragment in hand, its coupling with the CD fragment was expected to afford the dienynol, the immediate precursor to target previtamin (Scheme V). The (−)-alcohol **14a** was coupled to the CD fragment **13a** to afford the corresponding (−)-dienynol **15a** (X = H).[30,31] Similarly, the deuteriated (−)-alcohol **14b** was coupled to the deuteriated CD fragment **13b** to afford in ≥70% yield the (−)-dienynol compound **15b** (X = D). The ^{1}H-NMR signals at δ 5.93 and 1.88 assigned to H_9 and the C_{19}-Me, respectively, of the protio (−)-dienynol **15a** (X = H) were essentially absent in the labeled (−)-dienynol **15b** (X = D).

Hydrogenation (in the presence of Lindlar catalyst and quinoline) of (−)-dienynol **15a** (X = H) and the pentadeuterio analogue **15b** (X = D) afforded (+)-previtamin

(23) Courtin, J. M. L.; Verhagen, L.; Biesheuvel, P. L.; Lugtenburg, J.; van der Bend, R. L.; van Dam, K. *Recl. Trav. Chim. Pays-Bas* **1987**, *106*, 112.

(24) (a) Enas, J. D.; Palenzuela, J. A.; Okamura, W. H. *J. Am. Chem. Soc.* **1991**, *113*, 1355. (b) Enas, J. D.; Shen, G.-Y.; Okamura, W. H. *J. Am. Chem. Soc.* **1991**, *113*, 3873.

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Table I. Summary of Kinetic Data for the Previtamin D_3 to Vitamin D_3 Transformation

substrate	T, °C	$k \times 10^4$ (s^{-1})[a]	K_{eq}[b]
previtamin D_3 (d_0)	60.1 (±0.10)	0.972 (±0.03)	5.37 (±0.41)
	69.35 (±0.15)	2.12 (±0.08)	4.53 (±0.35)
	74.35 (±0.15)	3.34 (±0.17)	4.17 (±0.32)
	79.9 (±0.20)	5.02 (±0.37)	3.82 (±0.28)
	85.5 (±0.15)	7.91 (±0.33)	3.51 (±0.25)
previtamin D_3 (d_5)	60.5 (±0.10)	0.132 (±0.034)	5.42 (±0.17)
	69.7 (±0.15)	0.328 (±0.096)	4.66 (±0.19)
	74.1 (±0.15)	0.483 (±0.128)	4.36 (±0.23)
	80.4 (±0.15)	0.886 (±0.643)	3.99 (±0.25)
	85.5 (±0.15)	1.29 (±0.374)	3.72 (±0.28)

[a] Sample concentration: $c_p = 2.550 \times 10^{-3}$ g/100 mL, $c_D = 1.775 \times 10^{-3}$ g/100 mL, sample size: 2.5 mL/ampoule. The errors are absolute deviations from the mean. [b] Vitamin/previtamin ratio at equilibrium; the errors are absolute deviations from the mean. The equilibrations were conducted by starting independently from both the vitamin and previtamin.

D_3 **2** (X = H) and the (+)-pentadeuterioprevitamin D_3 **11** (X = D), respectively. The relatively modest yield (44% and 47% for previtamin D_3 and pentadeuterio previtamin D_3, respectively) seems to be due in part to the instability of the intermediate dienynols **15**. The semihydrogenation yield of **15b** was however improved to 92% in later experiments. Upon standing, even at −80 °C, additional polar fractions (by HPLC, 15% EtOAc/hexanes) are formed with a subsequent decrease of the dienynol fraction. These dienynols are very sensitive to air wherein the mass spectrum of the dienynols show peaks at M + 16 and M + 17, indicating the uptake of oxygen. The ^{1}H-NMR signals at δ 5.48 and 1.63 assigned to H_9 and the C_{19}-Me of previtamin D_3[30] (**2**) were essentially absent for the pentadeuterio previtamin **11** (X = D). The signal due to H_{14} could not be clearly discerned because of overlapping signals. When comparing the ^{13}C-NMR spectra of the labeled and unlabeled previtamins, the signals at δ 124.5 (C_9), 50.8 (C_{14}), and 19.7 (C_{19}) are reduced to low-intensity multiplets for the former.

Thus, the pentadeuterio previtamin D_3 **11** has been synthesized diastereomerically pure in 16 steps starting from the available *p*-methoxyphenol (**18**) and Grundmann's ketone (**16a**). The spectral data for the labeled previtamin **11** are consistent with those for the natural, unlabeled previtamin **2** reported only recently in detail by Dauben[32a,b] and earlier by Lythgoe and co-workers.[32c]

Kinetic Studies. With the unlabeled (**2**) and labeled previtamins (**11**) in hand, a kinetic study of their [1,7]-sigmatropic hydrogen shifts was carried out according to the UV analytical method developed by Hanewald et al.[5] Assuming a reversible, first-order kinetic rate law and following the reaction to 8.5–13 half-lives, with separate determination of the equilibrium constants for the previtamin D_3–vitamin D_3 interconversion over the same temperature range, the results summarized in Tables I and II were obtained. The rate constants and the vitamin/previtamin ratio at equilibrium measured for the rearrangement of the protio and deuterio previtamin D_3 to the corresponding vitamin D_3 are summarized in Table I. The rate constant for the [1,7]-hydrogen migration of the unlabeled previtamin **2** at 80 °C was calculated to be 5.14×10^{-4} s^{-1}, which is comparable to the 5.6×10^{-4} s^{-1} value calculated for the analogous isomerization of 1α,25-dihydroxyprevitamin D_3 (**9b**) and only modestly different from the 4.3×10^{-4} s^{-1} value determined by Hanewald et al. for the same rearrangement.[5]

The activation parameters (Table II) for the previtamin D_3–vitamin D_3 rearrangement bear resemblance to the recently reported rearrangement of simple trienes[33] from the laboratory of Baldwin and other vitamin D related systems from this laboratory.[11,12]

The k_H/k_D value of 6.2 at 80 °C reflects a difference of ~107 min in half-lives of the isomerization of the protio and deuterio previtamins. When extrapolated to 25 °C, the k_H/k_D from this study is 11.4 at 25 °C, indicating a greater temperature dependence as compared to the same transformation of **9b** to the hormonally active analogue **10b**.[12] To the extent that the data presented herein represent suitable criteria, the differences in activation parameters for the labeled and unlabeled materials appear to indicate a linear and symmetrical H-transfer with a significant tunneling contribution.[33] At 25 °C, the [1,7]-process of **2** to **11** proceeds with a k_H/k_D value (11.4) more than twice that observed (5.5) for the isomerization of 1α,25-dihydroxyprevitamin D_3 to 1α,25-dihydroxyvitamin D_3. The origin of this effect is unclear since such differences in k_H/k_D ratios in seemingly related molecules are not exceptional.[33,34] However, the presence of systematic experimental error cannot be ruled out. Nevertheless, the kinetic results described in this article clearly reveal that the previtamin D_3 to vitamin D_3 [1,7]-sigmatropic hydrogen shift is characterized by a reasonably "normal" primary KIE, not the extraordinarily large value of ~45 reported previously.[10,11]

The availability of the thermally more stable previtamin D_3-d_5 (**11**), whose rate of rearrangement to vitamin D_3 is suppressed by a kinetic isotope effect, will also make it possible to more unambiguously evaluate the intrinsic biological properties of previtamin D_3. Such studies are in progress.

Experimental Section[35]

(3*S*)-(+)-(6*Z*)-9,10-Secocholesta-5(10),6,8-trien-3-ol (2, Previtamin D_3). A stirred mixture of (3*S*)-(−)-dienynol **15a** (4.5 mg, 0.01 mmol), Lindlar catalyst (50 mg), and quinoline (50 μL, 0.17 M solution in hexanes, 0.01 mmol) in ethyl acetate (5.0 mL) was exposed to hydrogen gas for 45 min. Filtration through Celite and concentration afforded a residual oil, which was subjected to HPLC (Whatman Partisil M9 column, 30% EtOAc/hexanes) to afford 2.0 mg (44%) of previtamin D_3 (**2**), $[\alpha]_D$ +22 (*c* 0.08, $CHCl_3$). This substance has been previously reported by Velluz[4a,b] and later by Lythgoe:[32c] $[\alpha]_D^{18}$ +40 (*c* 1%, benzene). The previtamin synthesized in this study was identical (^{1}H-NMR spectrum, retention time, optical rotation) with the natural previtamin (obtained by refluxing vitamin D_3 in isooctane for 2 h followed by cooling to −78 °C and HPLC separation from the vitamin, Whatman Partisil M9 column, 30% EtOAc/hexanes).

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(35) Spectral and other analytical data are given in the supplementary material. ^{1}H-NMR spectral data of unlabeled (d_0) compounds in abbreviated form are presented in the Experimental Section as well. General experimental procedures are also presented in the supplementary material section. 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. Satisfactory combustion analyses were also obtained for selected compounds. For other new compounds, the level of purity is indicated by the inclusion of copies of NMR spectra presented in the supplementary material.

Table II. Activation Parameters[a] for the Previtamin–Vitamin Transformation (80 °C)

Substrate	E_a[b]	log A[c]	$\Delta G^{\ddagger}$[b]	$\Delta H^{\ddagger}$[b]	$\Delta S^{\ddagger}$[d]
1,25-pre D_3 (d_0)[e]	18.5 (±0.1)	8.2 (±0.04)	26.1 (±0.1)	17.8 (±0.09)	−23.3 (±0.1)
1,25-pre D_3 (d_5)[e]	17.3 (±0.6)	6.6 (±0.2)	27.5 (±0.9)	16.6 (±0.6)	−30.8 (±1.0)
(1*S*)-pre D_3[f]	18.8 (±0.1)	8.5 (±0.03)	25.9 (±0.1)	18.1 (±0.1)	−22.2 (±0.1)
(1*R*)-pre D_3[f]	19.1 (±0.5)	8.6 (±0.2)	26.1 (±0.7)	18.4 (±0.5)	−21.7 (±0.06)
pre D_3[g]	19.1 (±0.5)	8.5 (±0.2)	26.2 (±0.7)	18.4 (±0.5)	−22.0 (±0.6)
pre D_3 (d_0)[a]	19.6 (±0.2)	8.8 (±0.1)	26.1 (±0.3)	18.9 (±0.2)	−20.4 (±0.2)
pre D_3 (d_5)[a]	21.9 (±0.4)	9.5 (±0.2)	27.4 (±0.5)	21.2 (±0.4)	−17.5 (±0.3)

[a] At 80 °C. Standard deviations are given in parentheses. The sample size was 2.5 mL/ampoule, $c_P = 2.550 \times 10^{-3}$ g/100 mL, $c_D = 1.775 \times 10^{-3}$ g/100 mL. The rate constants were determined over the temperature range 60.1–85.5 °C for previtamin D_3–d_0 and 60.5–85.5 °C for previtamin D_3–d_5. [b] kcal/mol. [c] A in s^{-1}. [d] cal/mol K. [e] Data at 80 °C from ref 12. [f] Data at 80.0 °C from ref 11a. [g] Data at 80 °C from ref 5.

In a typical HPLC separation of the thermal mixture, previtamin D_3 (~33% yield) and vitamin D_3 (~67% yield) eluted at 17 and 24 min, respectively (2.5 mL/min flow rate; Whatman Partisil M9 column, 30% EtOAc/hexanes). The actual equilibrium value of previtamin D_3 to vitamin D_3 at 100 °C was measured quantitatively as ~22:78. The spectral data for this substance are compatible with the recent assignments reported by Dauben:[32a,b] ^{1}H NMR ($CDCl_3$) δ 0.70 (3 H, C_{18}-Me, s), 0.86 (6 H, C_{26} and C_{27}-2Me, overlapping d, $J \sim 6.5$ Hz), 0.93 (3 H, C_{21}-Me, d, $J \sim 6.4$ Hz), 1.63 (3 H, C_{19}-Me, br s), 2.40 (1 H, $H_{4\alpha}$, d, $J \sim 14.6$ Hz), 3.87 (1 H, H_3, narrow m), 5.48 (1 H, H_9, narrow m), 5.67 (1 H, H_7, d, $J \sim 12.1$ Hz), 5.93 (1 H, H_6, d, $J \sim 12.1$ Hz).

(6*Z*)-9,10-Secocholesta-5(10),6,8-trien-3α- and -3β-ol (3-Epiprevitamin D_3, the C-3 Epimer of 2, and Previtamin D_3, 2, Respectively). A stirred diastereomeric mixture of dienynol **15a** (as a mixture of C_3-epimers, 17.0 mg, 0.04 mmol), Lindlar catalyst (90.0 mg), and quinoline (200 μL, 0.17 M solution in hexanes, 0.03 mmol) in hexanes (5.0 mL) was exposed to hydrogen gas for 1.5 h. Filtration through Celite and concentration afforded a residual oil which was subjected to HPLC (Whatman Partisil M9 column, 30% EtOAc/hexanes) to afford 4.2 mg (25%) of previtamin D_3 as a pair of inseparable 3α- and 3β-epimers. No further attempt was made to optimize this reaction since separation was not successful.

(3*S*)-(+)-(6*Z*)-9,14,19,19,19-Pentadeuterio-9,10-secocholesta-5(10),6,8-trien-3-ol (Previtamin D_3-d_5, 11). The pentadeuteriated previtamin D_3 11 was prepared in the same manner as the protio analogue. A mixture of pentadeuteriated dienynol **15b** (3.0 mg, 0.008 mmol), Lindlar catalyst (26.0 mg), and quinoline (200 μL, 0.17 M solution in hexanes, 0.03 mmol) in ethyl acetate (5.0 mL) was exposed to hydrogen gas for 2 h. Workup similar to the protio analogue **2** afforded after HPLC (Whatman Partisil M9 column, 15% EtOAc/hexanes) the desired pentadeuteriated previtamin D_3 11 (1.4 mg, 47%, $[\alpha]_D$ +20 (*c* 0.13, $CHCl_3$)).

De-*A*,*B*-cholest-8-en-8-yl Trifluoromethanesulfonate (13a). Lithium diisopropylamide (LDA) was prepared by addition of *n*-butyllithium (1.1 mL, 1.60 M in hexanes, 1.8 mmol) to a solution of diisopropylamine (0.30 mL, 2.1 mmol) in THF (3.0 mL) at 0 °C. The mixture was stirred for 20 min, and then the LDA solution was cooled to −78 °C. A solution of Grundmann's ketone **16a** (370 mg, 1.40 mmol) in THF (6.0 mL) was added dropwise to the LDA solution. After the addition was completed, the reaction mixture was allowed to warm to 0 °C and left to stand for 70 min and then cooled again to −78 °C. To the enolate solution was added a solution of *N*-phenyltrifluoromethanesulfonimide (536 mg, 1.50 mmol) in THF (5.0 mL), and the reaction mixture was allowed to warm to 0 °C and left to stir for 2 h followed by an additional 18 h at room temperature. The resulting golden-colored solution was quenched with ammonium chloride solution, and the separated organic layer was washed with saturated sodium bicarbonate and brine. The dried solution (Na_2SO_4) was concentrated, and the residue was subjected to flash chromatography (silica gel, hexanes) to afford after vacuum drying, 336 mg (61% yield) of the triflate **13a** as a colorless oil. This material was sufficiently pure (^{1}H-NMR spectrum) for use in the next step: ^{1}H NMR ($CDCl_3$) δ 0.76 (3 H, C_{18}-CH_3, s), 0.87 (6 H, $C_{26,27}$-2CH_3, d, $J \sim 6.4$ Hz), 0.94 (3 H, C_{21}-CH_3, d, $J \sim 6.4$ Hz), 5.58 (1 H, H_9, ddd, $J \sim 3.4, 3.4, 3.4$ Hz).

9,14-Dideuterio-de-*A*,*B*-cholest-8-en-8-yl Trifluoromethanesulfonate (13b). This compound was prepared in essentially the same manner as the protio analogue **13a** except that the trideuteriated Grundmann's ketone **16b** (264 mg, 0.98 mmol) was employed to afford 285 mg (73%) of the dideuteriated triflate **13b**.

(1*S*)-(−)-3-Ethynyl-4-methylcyclohex-3-en-1-ol (14a). To a solution of the (1*S*,1′*S*)-carbamate (less polar isomer) **31a** (95.0 mg, 0.29 mmol) and triethylamine (162 mL, 1.16 mmol) in benzene (3.0 mL) was added trichlorosilane (88.0 μL, 0.87 mmol) dropwise. The solution was stirred at room temperature for 2 days under a nitrogen atmosphere. The reaction mixture was diluted with ether (20 mL) and washed with ammonium chloride solution (50 mL). The aqueous layer was extracted with ether (2 × 40 mL), and the combined organic layers were dried ($MgSO_4$) and concentrated. The resulting residue was filtered through a short silica gel column (30% EtOAc/hexanes) and then dried. HPLC chromatographic purification (Whatman Partisil M9 column, 30% EtOAc/hexanes) afforded 34 mg (87%) of the pure (by HPLC analysis and by ^{1}H- and ^{13}C-NMR spectral analysis), optically active carbinol **14a** ($[\alpha]_D$ −57.7 (*c* 0.31, $CHCl_3$)). This alcohol has been previously reported by Lythgoe:[28] $[\alpha]^{35}_D$ −65 ($CHCl_3$). Based on the base-line separation of the precursor diastereomeric carbamate esters used in their preparative HPLC separation (see the experiment describing the preparation and separation of the carbamate esters), and the assumed retention in the carbamate ester hydrolysis, the alcohol (−)-**14a** is assumed to be configurationally pure despite the discrepancy in our specific rotation and that reported by Lythgoe.[28]

(1*R*)-(+)-3-Ethynyl-4-methylcyclohex-3-en-1-ol [(+)-*epi*-14a]. To a solution of the (1*R*,1′*S*)-carbamate (more polar isomer) **32a** (180 mg, 0.54 mmol) and triethylamine (310 μL, 2.22 mmol) in benzene (5.0 mL) was added trichlorosilane (170 μL, 1.68 mmol) dropwise. The solution was stirred at room temperature for 2 days under a nitrogen atmosphere. The reaction mixture was diluted with ether (20 mL) and washed with ammonium chloride solution (50 mL). The aqueous layer was extracted with ether (2 × 30 mL), and the combined organic layers were dried ($MgSO_4$) and concentrated. The residue was filtered through a short silica gel column (30% EtOAc/hexanes) and then dried. HPLC chromatographic purification (Whatman Partisil M9 column, 30% EtOAc/hexanes) afforded 42.0 mg (58%) of the pure (by HPLC analysis and ^{1}H- and ^{13}C-NMR spectral analysis), optically active alcohol (+)-*epi*-**14a** ($[\alpha]_D$ +53.3 (*c* 0.20, $CHCl_3$)). The enantiomer of this alcohol has been reported by Lythgoe,[28] $[\alpha]^{35}_D$ −65 ($CHCl_3$). Based on the base-line separation of the precursor diastereomeric carbamate esters used in their preparative HPLC separation (see the experiment describing the preparation and separation of the carbamate esters), and the assumed retention in the carbamate ester hydrolysis, the alcohol (+)-*epi*-**14a** is assumed to be configurationally pure despite the discrepancy in our specific rotation and that reported by Lythgoe.[28]

(1*S*)-(−)-3-Ethynyl-4-(trideuteriomethyl)cyclohex-3-en-1-ol (14b). The optically active deuteriated enynol **14b** was prepared in the same manner as the unlabeled (−)-**14a** except that trideuteriated (1*S*,1′*S*)-carbamate **31b** (152 mg, 0.45 mmol) was used to afford 48 mg (76%) of alcohol (−)-**14b** ($[\alpha]_D$ −59.8 (*c* 0.44, $CHCl_3$)). Based on the base-line separation of the precursor diastereomeric carbamate esters used in their preparative HPLC separation (see the experiment describing the preparation and separation of the carbamate esters), and the assumed retention in the carbamate ester hydrolysis, the alcohol (−)-**14b** is assumed to be configurationally pure despite the

discrepancy in our specific rotation and that reported by Lythgoe.[28]

9,10-Secocholesta-5(10),8-dien-6-yn-3-ol (C_3 Diastereomeric Mixture of 15a). To a mixture of triflate **13a** (80.0 mg, 0.20 mmol) and racemic enynol **30a** (38.0 mg, 0.28 mmol) in diethylamine (1.0 mL) and dimethylformamide (1.0 mL) was added CuI (4.8 mg, 0.025 mmol) and bis[triphenylphosphine]-palladium(II) acetate (5.0 mg 0.007 mmol). The reaction mixture was stirred at room temperature for 1.5 h under argon. Ether (25 mL) was added to the reaction mixture, and then the mixture was washed with water (3 × 10 mL), dried (Na_2SO_4), and concentrated. Chromatographic purification (Whatman Partisil, M-9 column, 30% EtOAc/hexanes) afforded 67 mg (88%) of the C-3 diastereomeric mixture of dienynol **15a** as a colorless, viscous oil.

(−)-9,10-Secocholesta-5(10),8-dien-6-yn-3-ol (15a). This diastereomerically pure dienynol **15a** was prepared in the same manner as the epimeric dienynol except the optically active enynol (−)-**14a** (11.0 mg, 0.08 mmol) was coupled to the triflate **13a** (32.0 mg, 0.08 mmol) to afford after HPLC purification (Whatman Partisil M9 column, 15% EtOAc/hexanes) 18 mg (60%) of the desired dienynol (−)-**15a** ($[\alpha]_D$ −2.7 (*c* 0.74, $CHCl_3$)). This substance has been reported by Lythgoe group,[32b] $[\alpha]_D$ −8 ($CHCl_3$). The ^{1}H-NMR spectrum of this diastereomerically pure material was hardly distinguishable from the C_3 epimeric alcohol mixture except for some very minor sharpening of peaks in the steroid envelope region (δ 0.5–2.5 region): ^{1}H NMR ($CDCl_3$) δ 0.70 (3 H, C_{18}-CH_3, s,), 0.87 (6 H, $C_{26,27}$-$2CH_3$, d, J ~ 6.6 Hz), 0.93 (3 H, C_{21}-CH_3, d, J ~ 6.3 Hz), 1.88 (3 H, C_{19}-CH_3, s), 3.94 (1 H, H_3, m), 5.93 (1 H, H_9, narrow m).

(−)-9,14,19,19,19-Pentadeuterio-9,10-secocholesta-5(10),8-dien-6-yn-3-ol (15b). The diastereomerically pure pentadeuterio dienynol (−)-**15b** was prepared in the same manner as the protio analogue. The enantiomerically pure trideuterio enynol (−)-**14b** (24.0 mg, 0.17 mmol) was coupled to the dideuterio triflate **13b** (61.0 mg, 0.15 mmol) to afford after HPLC purification (Whatman Partisil M9 column, 15% EtOAc/hexanes) 41 mg (70%) of the unstable dienynol-d_5 (−)-**15b** sufficiently pure (^{1}H-NMR) for the next step. The ^{1}H-NMR spectrum of this material was similar to that of the unlabeled material, the most significant difference being the absence of the δ 1.88 and 5.94 signals assigned to the C_{19} vinylic methyl and H_9, respectively.

9,9,14-Trideuterio-de-*A,B*-cholestan-8-one (16b and 17). The procedure of Dawson et al.[11b] adapted by K. Miller of this laboratory[11a] for deuteriation was followed. To a solution of 0.12 M NaOMe prepared from 280 mg (12.2 mmol) of Na and methanol-*O*-d_1 (MeOD, 10 mL) was added Grundmann's ketone **16a** (2.79 g, 10.5 mmol) in MeOD (5.0 mL) at room temperature under a nitrogen atmosphere. The orange solution was allowed to stir for 48 h. The solution was cooled to 0 °C, quenched with acetic acid-d_4 (1.5 mL, 26.6 mmol; Aldrich, 99.5 atom % *d*), and finally diluted with water (20 mL). The crude deuteriated ketone was extracted with hexanes (2 × 70 mL), and then the organic extract was washed with brine, dried ($MgSO_4$), and concentrated. The product was redissolved in MeOD (5 mL) and added to 10 mL of a fresh solution of NaOMe in MeOD prepared as before. The second exchange was allowed to proceed for 75 h and then quenched with acetic acid-d_4 (1.5 mL, 26.6 mmol). The ketone was isolated as before. A third exchange was carried out for 72 h and worked up exactly as before. After concentration, 2.41 g (86%) of product was obtained as mixture of deuteriated Grundmann's ketone **16b** and its C_{14} epimer **17**. The mixture was subjected to HPLC purification (Whatman Partisil column, 10% EtOAc/hexanes) to afford the *epi*-ketone **17** (less polar, eluted first) and the desired ketone **16b** (more polar, eluted second) in a 2.1:1 ratio. The ^{1}H-NMR spectrum (300 MHz) of **16b** exhibited no apparent proton signals at δ 2.20 and 2.40 ppm (less than 2% unlabeled signals by ^{1}H-NMR integration after expansion).

4-Methoxy-3-cyclohexen-1-ol (19). The procedure of Radlick[14b] as modified by Marshall[14c] was followed. Into a 1-L three-necked round-bottomed flask cooled with a dry ice bath and equipped with a mechanical stirrer and a cold finger was distilled 450 mL of ammonia (dried by passage through a potassium hydroxide pellet tower). To the stirred solution was added, over a period of 0.5 h, 15.0 g (2.16 mol) of hexane washed lithium wire cut into 0.5-in. pieces. A bronze pool of the liquified metal formed beneath the blue ammonia solution as addition was completed. A solution of *p*-methoxyphenol **18** (20.5 g, 165 mmol) in ether (100 mL) was added via a 125-mL addition funnel over a period of 0.5 h. Throughout the reaction a nitrogen atmosphere was maintained. After addition of the methoxyphenol was complete, absolute ethanol (20 mL) was added, and the solution was stirred and maintained below −33 °C for 1 h. Ethanol was periodically added every 20 min (9 × 20 mL). A total of 200 mL of ethanol was used until the blue color was discharged. Solid ammonium chloride (60.0 g, 1.12 mmol) was carefully added, and the ammonia was allowed to evaporate overnight. The contents of the flask were transferred with water (0.5 L) into 2-L separatory funnel and extracted with chloroform (6 × 200 mL). The combined extracts were dried (Na_2SO_4) and concentrated to afford 19.7 g (93%) of the enol ether **19**: ^{1}H NMR ($CDCl_3$) δ 1.6–2.5 (6 H, $C_{2,5,6}$-methylenes, m), 1.86 (1 H, hydroxy, br, s), 3.48 (3 H, C_4-methoxy, s), 3.93 (1 H, H_1, m), 4.45 (1 H, H_3, t, J ~ 3.7 Hz).

4-Hydroxycyclohexanone (20). To the enol ether **19** (19.7 g, 154 mmol) was added 1 M HCl (25 mL), and then the mixture was heated at 50 °C for 45 min. To the cooled aqueous solution was added saturated sodium bicarbonate–sodium chloride solution (50 mL, 1:1), and then the mixture was extracted with chloroform (5 × 100 mL). The combined organic extracts were dried (Na_2SO_4) and concentrated to afford 14.4 g (82%) of the desired hydroxy ketone **20** as a light brown oil. The hydroxy ketone was further purified by distillation (bp 130 °C, 1.0 mm; lit.[14b] bp 93 °C, 0.3 mm): ^{1}H-NMR ($CDCl_3$) δ 2.01 (4 H, $C_{2,6}$-methylenes, m), 2.32 and 2.61 (4 H, $C_{3,5}$-methylenes, 2 m), 4.20 (1 H, H_4, pseudo-septet, J ~ 3.5 Hz).

4-((*tert*-Butyldiphenylsilyl)oxy)cyclohexanone (21). To a solution of imidazole (970 mg, 14.2 mmol) and *tert*-butylchlorodiphenylsilane (3.40 g, 12.3 mmol) in dry *N,N*-dimethylformamide (DMF, 10 mL; dried by distillation from BaO) was added a solution of 4-hydroxycyclohexanone (**20**, 1.08 g, 9.46 mmol) in DMF (5.0 mL). After the mixture was left to stand at room temperature for 5 h under a nitrogen atmosphere, the reaction mixture was poured into a beaker containing 50 mL of water. After the mixture was extracted with ether (2 × 70 mL), the organic layer was dried (Na_2SO_4) and concentrated, and then the residue was recrystallized from hexanes (mp 104–105 °C). A total of 2.76 g (83%) of the desired product **21** was obtained: ^{1}H NMR ($CDCl_3$) δ 1.11 (9 H, t-Bu, s), 1.72–2.22 (4 H, 2 H_2 and 2 H_6, 2 m), 2.21 (2 H, axial H_3 and H_5, dt, J ~ 14.5 Hz, 5.3 Hz), 2.75 (2 H, equatorial H_3 and H_5, m), 4.16 (1 H, H_4, pseudoseptet, J ~ 2.6 Hz), 7.3–7.7 (10 H, aromatic, 2 m).

4-((*tert*-Butyldiphenylsilyl)oxy)-2-((*n*-butylthio)methylidene)cyclohexanone (23). To a suspension of NaH (1.36 g, 34.0 mmol, 60% dispersion in mineral oil) in ether (50 mL) was added a solution of silyloxy ketone **21** (6.71 g, 19.0 mmol) in ether (10 mL) followed by ethyl formate (3.30 mL, 40.8 mmol). The reaction mixture was left to stand at room temperature for 4.5 h under a nitrogen atmosphere. Water (100 mL) was added to dissolve the sodium salt, and the organic layer was separated and discarded. The aqueous layer was acidified with HCl (10.0 mL, 2.0 M), and a white solid immediately precipitated. The precipitate was extracted with ether (3 × 75 mL), and then the ether extract was dried (Na_2SO_4) and concentrated to afford 6.17 g (85%) of the crude keto aldehyde **22**. A portion of the keto aldehyde **22** without further purification or characterization was then transformed directly to the corresponding thioether **23**. To a mixture of the crude keto aldehyde **22** (4.67 g, 12.2 mmol) in benzene (50 mL), *p*-toluenesulfonic acid (PTSA, 50 mg, 0.26 mmol), and magnesium sulfate (1.46 g, 12.2 mmol) was added 1-butanethiol (2.00 mL, 18.7 mmol) at room temperature, and the mixture was stirred overnight. The reaction mixture was diluted with hexanes (100 mL), and then the mixture was washed with sodium bicarbonate solution then with brine. After the hexane solution was dried ($MgSO_4$), solvent evaporation followed by vacuum drying afforded 4.55 g (82%) of the oily product **23**, sufficiently pure (^{1}H-NMR spectrum) for use in the next step. A sample of crude product was subjected to HPLC purification (15% EtOAc/hexanes, Whatman Partisil M9 column) for spectroscopic characterization: ^{1}H NMR ($CDCl_3$) δ 0.93 (3 H, C_4-Me, t, J ~ 7.3 Hz), 1.05 (9 H, *tert*-butyl, s), 1.42 (2 H,

C_3-methylene, apparent sextet, $J \sim 7.4$ Hz), 1.66 (2 H, C_2-methylene, apparent quintet, $J \sim 7.4$ Hz), 1.78–2.03 (2 H, C_5-methylene, 2 m), 2.27 (1 H, first H_6, ddd, $J \sim 18.2, 6.0, 6.0$ Hz), 2.40 (1 H, first H_3, doublet with fine structure, $J \sim 16.6$ Hz), 2.52 (1 H, second H_3, dd, $J \sim 16.6, 5.3$ Hz), 2.72 (1 H, second H_6, ddd, $J \sim 18.2, 9.6, 6.0$ Hz), 2.84 (2 H, C_1-methylene, apparent t, $J \sim 7.4$ Hz), 4.17 (1 H, H_4, br m), 7.3–7.7 (11 H, 10 aromatic + H_7, 2 m).

1-((*tert*-Butyldiphenylsilyl)oxy)-4-methyl-3-((*n*-butylthio)methylidene)cyclohex-4-ene (25a). To a solution of ketone **23** (255 mg, 0.78 mmol) in dry ether (20 mL) cooled to −78 °C was added dropwise methylmagnesium iodide (3.0 M solution in diethyl ether, 520 μL, 1.78 mmol). After the addition was complete, the cooling bath was removed and the reaction mixture was left to stand for an additional 60 min and then quenched with ammonium chloride (10 mL). The aqueous layer was separated and extracted with ether (100 mL). The combined organic layers were washed with brine, dried ($MgSO_4$), and condensed to afford 293 mg (83%) of diene **25a**, sufficiently pure for use in its conversion to enal **26a**. A sample was purified by HPLC (Whatman Partisil M9, 10% EtOAc–hexanes) for spectroscopic characterization of the material: ^{1}H NMR ($CDCl_3$) δ 0.93 (3 H, C_4-Me, t, $J \sim 7.3$ Hz), 1.07 (9 H, *tert*-butyl, s), 1.36–1.66 (4 H, C_2- and C_3-methylenes, 2 m), 1.73 (3 H, C_4-Me, br s), 2.18 (2 H, C_6 methylene, br m), 2.26 (1 H, first H_2, ddd, $J \sim 14.7, 10.5, 1.7$ Hz), 2.69 (2 H, C_1-methylene, apparent t, $J \sim 7.3$ Hz), 2.73 (1 H, second H_2, overlapped with $H_{2'}$; dd, $J \sim 14.7, 4.2$ Hz), 3.94 (1 H, H_1, m), 5.29 (1 H, H_5, t, $J \sim 3.9$ Hz), 5.84 (1 H, H_7, br s), 7.31–7.72 (10 H, aromatic, 2 m).

1-((*tert*-Butyldiphenylsilyl)oxy)-4-(trideuteriomethyl)-3-((*n*-butylthio)methylidene)cyclohex-4-ene (25b). This compound was prepared in essentially the same manner as the protio analogue **25a** except that (methyl-d_3)magnesium iodide (Aldrich, 99+ atom % D, 1.0 M solution in diethyl ether) was employed starting from ketone **23** (423 mg, 0.93 mmol) to afford 384 mg (91%) of the trideuteriated diene **25b**.

5-((*tert*-Butyldiphenylsilyl)oxy)-2-methylcyclohex-1-enecarboxaldehyde (26a). Method A. Diene sulfide **25a** (1.97 g, 4.38 mmol) in acetonitrile (50 mL) was added dropwise to a refluxing solution of mercuric chloride (2.38 g, 8.76 mmol) in acetonitrile–water (100 mL, 70:30 ratio). As addition continued a pink color appeared and then a white solid started to precipitate. Heating was continued for 2 h, and then the reaction mixture was cooled and filtered through Celite. The precipitate was washed with chloroform (150 mL) and the latter combined with the initial filtrate. The combined solution was washed with sodium bicarbonate (~100 mL) and then brine. The organic layer was dried ($MgSO_4$) and concentrated, and then the residue was subjected to filtration through a short column (silica gel; 15% EtOAc/hexanes). After concentration, the vacuum-dried residue was subjected to HPLC purification (Whatman Partisil M9 column, 15% EtOAc/hexanes) to afford 651 mg (39%) of **26a** as an oil: ^{1}H NMR ($CDCl_3$) δ 1.04 (9 H, *t*-Bu, s), 1.63 (2 H, 2 H_4, pseudo q, $J \sim 6.1$ Hz), 2.12 (3 H, C_2-Me, s), 2.08 (1 H, first H_3, obscurred by CH_3 group, m; in the labeled material, the presence of a ddd, $J \sim 19.4, 6.6, 6.6$ Hz, is revealed), 2.25 (1 H, first H_6, dd, $J \sim 17.5$ and 6.2 Hz), 2.33–2.48 (2 H, second H_3 and second H_6, overlapping m), 4.00 (1 H, H_5, pseudoquintet, $J \sim 5.4$ Hz), 7.3–7.7 (10 H, aromatic, 2 m), 10.09 (1 H, aldehydic, s).

Method B. To a solution of ketone **23** (1.07 g, 2.36 mmol) in ether (50 mL) cooled to −78 °C under a nitrogen atmosphere was added dropwise a solution of MeMgI (3.0 M in diethyl ether, 1.60 mL, 4.80 mmol). Stirring was continued for 10 min, the cooling bath was removed, and stirring was continued for an additional 1 h. The reaction mixture was cooled again to −78 °C and then quenched with water (50 mL). The organic layer was separated, and the solid aqueous layer was extracted with ether (30 mL). The combined organic layers were washed with brine (60 mL) and then concentrated. The residue was redissolved in acetonitrile (160 mL), and mercuric chloride (950 mg, 3.50 mmol) in water (40 mL) was added dropwise. The flask was connected to a condenser, and the mixture was heated at reflux for 10 min. The reaction mixture was cooled, filtered through Celite, and as in method A, the precipitate was washed with chloroform (70 mL). The combined organic solution was washed with sodium bicarbonate (2 × 50 mL), dried ($MgSO_4$), and concentrated under reduced pressure. After passage of the residue through a short silica gel column (15% EtOAc/hexanes), the eluate was concentrated and then the residue was vacuum dried. HPLC purification (Whatman Partisil M9 column, 15% EtOAc/hexanes) afforded 600 mg (67%) of the oily, colorless aldehyde **26a**, sufficiently pure for spectral characterization and for use in the next step.

5-((*tert*-Butyldiphenylsilyl)oxy)-2-(trideuteriomethyl)-cyclohex-1-enecarboxaldehyde (26b). Method A. The trideuteriated diene sulfide **25b** (1.49 g, 3.27 mmol) was hydrolyzed in the same manner as the unlabeled diene sulfide **25a** to afford 437 mg (35%) of the trideuteriated aldehyde **26b** as a colorless oil.

Method B. As in the unlabeled case, to a solution of the ketone **23** (879 mg, 1.94 mmol), in ether (45 mL) at −78 °C was added (methyl-d_3)magnesium iodide (Aldrich, 99 atom % D, 1.0 M solution in diethyl ether, 3.90 mL, 3.90 mmol). Workup was identical to that described for the preparation of the unlabeled compound. The crude reaction mixture after concentration was redissolved in acetonitrile (160 mL), mercuric chloride (800 mg, 2.94 mmol) in water (40 mL) was added, and then the mixture was heated under reflux for 10 min to afford after workup and HPLC purification (Whatman Partisil M9 Column, 15% EtOAc/hexanes), 461 mg (62%) of the trideuteriated aldehyde **26b** as a colorless oil. Later studies revealed that method B may be capricious.

Method C. To a stirred solution of the diastereomeric ketones **27**/**27′** (570 mg, 1.30 mmol) in ether (50 mL) at 0 °C was added (methyl-d_3)magnesium iodide (7.80 mL, 7.80 mmol, Aldrich, 99 atom % D, 1.0 M solution in ether). After stirring was continued for 10 min, the cooling bath was removed and stirring was continued for an additional 1 h. The reaction was quenched with saturated NH_4Cl (50 mL). After extraction of the aqueous layer with ether, the combined organic layers were dried ($MgSO_4$) and concentrated to give a complex mixture of diastereomeric tertiary alcohols. This crude product in acetone (20 mL) was heated with concentrated HCl (1.0 mL, 12 mmol). After the mixture was stirred for 30 min, saturated $NaHCO_3$ (10 mL) and ether (50 mL) were added. The organic layer was washed twice with water (30 mL), dried ($MgSO_4$), and concentrated. The crude product was purified by flash chromatography (silica gel; 15% EtOAc/hexanes, 2.5 × 30 cm) to afford 278 mg (0.73 mmol, 56%) of aldehyde **26b** as a spectrally homogeneous oil.

(2*S,4*S**)-4-(*tert*-Butyldiphenylsiloxy)-2-[2′-(1′,3′-dioxanyl)]cyclohexane (27, cis) and (2*S**,4*R**)-4-(*tert*-Butyldiphenylsiloxy)-2-[2′-(1′,3′-dioxanyl)]cyclohexane (27′, trans).** To a well-stirred slurry of silica gel (20 g) in CH_2Cl_2 (40 mL) was added dropwise $SOCl_2$ (20 g) at room temperature. Evolution of copious amounts of HCl and SO_2 occurred instantaneously. After stirring for another 1 h, the solvent was removed under reduced pressure, and the $SOCl_2$–SiO_2 thus prepared was used in the following experiment.[22] To a suspension of $SOCl_2$–SiO_2 (0.53 g) and 1,3-propanediol (0.30 mL, 1.9 mmol) in dry benzene (25 mL) was added a solution of formyl ketone **22** (1.00 g, 2.63 mmol) in benzene (5 mL). After being stirred at room temperature for 16 h, the suspension was filtered and Et_2O (50 mL) was added. The organic phase was extracted with water (2 × 50 mL), dried ($MgSO_4$), and concentrated to afford 1.14 g of the crude product. Flash chromatography (silica gel, 10% EtOAc/hexanes, 2.5 × 30 cm) gave a mixture of dioxane diastereomers **27** (cis) and **27′** (trans) (611 mg total, 53%).

The diastereomeric mixture was subjected to HPLC separation (4.7 mL/min; 15% ethyl acetate/hexanes, Rainin Dynamax 60A column). The base line separated diastereomeric dioxanes eluted in the following order: (2*S**,4*S**) isomer **27** (8 min), (2*S**,4*R**) isomer **27′** (9 min) (ratio ~1/2). After treatment of 50 mg of this 1:2 mixture with 2 mL of 0.087 M solution of MeONa in MeOH for 24 h the ratio of the two diastereomers changed to ((2*S**,4*S**):(2*S**,4*R**) ~3:1). **27** (cis): ^{1}H NMR ($CDCl_3$) δ 1.09 (9 H, *t*-Bu, s), 1.35 (1 H, m), 1.50–2.10 (4 H, m), 2.17–2.32 (2 H, m), 2.78 (1 H, ddd, $J \sim 6.0, 14.0, 14.0$ Hz), 3.11 (1 H, C_2-H_{ax}, ddd, $J_{ax,ax} \sim 12.0, 5.0, 6.0$ Hz), 3.79 (2 H, m), 4.06 (2 H, ddd, $J \sim 6.0, 13.0, 13.0$ Hz), 4.25 (1 H, C_4-H_{ax}, m), 5.00 (1 H, $C_{2'}$-H, d, $J \sim 5.0$ Hz), 7.30–7.75 (10 H, aromatic, 2 m). **27′** (trans): ^{1}H NMR ($CDCl_3$) δ 1.07 (9 H, *t*-Bu, s), 1.30 (1 H, m), 1.65–2.50 (8 H, m), 3.72–3.85 (2 H, m), 4.00–4.15 (3 H, C_4-H and two other protons, m), 5.07 (1 H, $C_{2'}$-H, d, $J \sim 3.0$ Hz), 7.35–7.72 (10 H, aromatic, 2 m).

1-((*tert*-Butyldiphenylsilyl)oxy)-3-(2,2-dibromoethenyl)-4-methylcyclohex-3-ene (28a). To a solution of zinc dust (327 mg, 5.0 mmol) and triphenylphosphine (1.31 g, 5.0 mmol, recrystallized from ether) in methylene chloride (25 mL) was added CBr_4 (1.66 g, 5.0 mmol) in methylene chloride (5 mL) at room temperature. The resulting suspension was stirred for 25 h under a nitrogen atmosphere. The aldehyde **26a** (315 mg, 0.83 mmol) in CH_2Cl_2 (5 mL) was added to the reaction mixture, and stirring was continued for an additional 5 h (followed by TLC, 10% EtOAc/hexanes). Workup was accomplished by dilution of the mixture with pentane (50 mL) followed by filtration of the resulting mixture through Celite to remove the insoluble material and evaporation of the pentane/dichloromethane. The insoluble material was subjected to additional CH_2Cl_2 (2×) extraction and pentane precipitation to remove any remaining olefinic product. The methylene chloride–pentane mixture after an additional Celite filtration was concentrated, and the residue was redissolved in 10% EtOAc/hexane (10 mL) and filtered through a short silica gel column to remove any remaining insoluble material. After solvent evaporation the dibromide **28a** (443 mg) was obtained in essentially quantitative amounts as a colorless oil. The vacuum dried, unstable material was used directly for spectral characterization and for the next step: ^{1}H NMR ($CDCl_3$) δ 1.05 (9 H, *t*-Bu, s), 1.58 (3 H, C_4-CH_3, br s), 3.94 (1 H, H_1, m), 6.84 (1 H, vinyl H, br s), 7.3–7.7 (10 H, aromatic 2 m).

1-((*tert*-Butyldiphenylsilyl)oxy)-3-(2,2-dibromoethenyl)-4-(trideuteriomethyl)cyclohex-3-ene (28b). This compound was prepared in the same manner as the unlabeled **28a** except that trideuteriated aldehyde **26b** (436 mg, 1.14 mmol) was used to afford 573 mg (93%) of product.

1-((*tert*-Butyldiphenylsilyl)oxy)-3-ethynyl-4-methylcyclohex-3-ene (29a). To a solution of dibromide **28a** (439 mg, 0.82 mmol) in ether (20 mL) cooled to −78 °C was added *n*-BuLi (1.60 M in hexanes, 1.30 mL, 1.80 mmol), and the resulting mixture was stirred for 10 min. After removing the cold bath, the mixture was stirred for an additional 1 h, and then the reaction was quenched with ammonium chloride solution (20 mL). The mixture was extracted with ether (3 × 25 mL), and then the combined organic layers were washed with brine, dried (Na_2SO_4), and concentrated. Flash chromatography (silica gel, 10% EtOAc/hexane) of the residue afforded 293 mg (95%) of the enyne **29a**, which was sufficiently pure for use in the next step. The sample for spectral characterization was purified by HPLC (Whatman Partisil M9 column, 5% EtOAc–hexanes): ^{1}H NMR ($CDCl_3$) δ 1.10 (9 H, *tert*-butyl, s), 1.89 (3 H, C_4-Me, s), 3.01 (1 H, acetylenic H, s), 3.97 (1 H, H_1, m), 7.4–7.8 (10 H, aromatic, 2 m).

1-((*tert*-Butyldiphenylsilyl)oxy)-3-ethynyl-4-(trideuteriomethyl)cyclohex-3-ene (29b). A solution of trideuteriated dibromide **28b** (294 mg, 0.54 mmol) in THF (30 mL) was treated with *n*-BuLi (1.6 M in hexanes, 1.00 mL, 1.60 mmol) in a similar manner as the unlabeled compound **28a** to give the corresponding trideuteriated enyne **29b** in essentially quantitative yield (208 mg).

(±)-3-Ethynyl-4-methylcyclohex-3-en-1-ol (30a). To a solution of the silyl ether **29a** (324 mg, 0.86 mmol) in tetrahydrofuran (15 mL) was added tetrabutylammonium fluoride (1.1 M in THF, 2.30 mL, 2.60 mmol) under a nitrogen atmosphere. The mixture was stirred for 3 h at room temperature and then diluted with ether (40 mL) and washed successively with brine (15 mL) and water (20 mL). The organic layer was dried ($MgSO_4$) and concentrated. Chromatographic purification (Whatman Partisil, 30% EtOAc/hexanes) afforded 92 mg (79%) of the alcohol **30a** as an oil (lit.[28] mp 33.5–34.5 °C) sufficiently pure for use in the next step: ^{1}H NMR ($CDCl_3$) δ 1.90 (3 H, C_4-CH_3, s; overlaps a br multiplet), 3.03 (1 H, acetylenic H, s), 3.97 (1 H, H_1, m).

(±)-3-Ethynyl-4-(trideuteriomethyl)cyclohex-3-en-1-ol (30b). This compound was prepared in the same manner as the unlabeled **30a** except that trideuteriated compound **29b** (674 mg, 1.78 mmol) was used to afford 188 mg (77%) of **30b**.

(1*S*,1′*S*)- (31a, Less Polar) and (1*R*,1′*S*)-3-Ethynyl-4-methylcyclohex-3-en-1-yl *N*-[1′-(1-Naphthyl)ethyl]carbamate (32a, More Polar). The procedure of Pirkle[26] was followed. A mixture of racemic alcohol **30a** (355 mg, 2.61 mmol) and (*S*)-(+)-1-(1-naphthyl)ethyl isocyanate (860 mg, 4.36 mmol) in benzene (10 mL) was refluxed under a nitrogen atmosphere for 70 h. After cooling of the reaction mixture and then removal of solvent under reduced pressure, the resulting carbamate mixture was subjected to HPLC chromatographic separation (flow rate, 7 mL/min; 20% EtOAc/hexanes, Whatman Partisil 10 magnum 20/50 column). The base line separated, diastereomeric carbamates eluted in the following order: (1*S*,1′*S*) isomer **31a** (46 min) followed by the (1*R*,1′*S*) isomer **32a** (52 min). There was obtained 396 mg (46%) of the desired isomer **31a** (1*S*,1′*S*, mp 147–148 °C, recrystallized from hexanes) and 428 mg (49%) of the more polar diastereomer **32a** (1*R*,1′*S*, mp 114–115 °C, recrystallized from 5% EtOAc/hexanes). **31a**: ^{1}H NMR ($CDCl_3$) δ 1.65 (3 H, C_2-Me, d, $J \sim 6.3$ Hz), 1.91 (3 H, C_4-Me, br s), 3.03 (1 H, acetylenic, s), 4.98–5.09 (2 H, H_1 and N-H, br m), 5.67 (1 H, $H_{1'}$, br s), 7.4–8.2 (7 H, aromatic, three m). (C_6D_6, 100 °C) δ 1.31 (3 H, C_2-Me, d, $J \sim 6.6$ Hz), 1.74 (3 H, C_4-Me, br s), 2.80 (1 H, acetylenic, s), 4.50 (1 H, N-H, br s), 4.99 (1 H, H_1, apparent br s), 5.63 (1 H, $H_{1'}$, m), 7.00–8.13 (7 H, naphthyl-H, 3 m). **32a**: ^{1}H NMR ($CDCl_3$) δ 1.65 (3 H, C_2-Me, d, $J \sim 5.2$ Hz), 1.89 (3 H, C_4-Me, br s), 3.00 (1 H, acetylenic, s), 4.96 (2 H, H_1 and N-H, br m), 5.64 (1 H, $H_{1'}$, br s), 7.4–8.1 (7 H, aromatic, 3 m); (C_6D_6, 100 °C) δ 1.33 (3 H, C_2-Me, d, $J \sim 6.7$ Hz), 1.73 (3 H, C_4-Me, br s), 2.78 (1 H, acetylenic, s), 4.58 (1 H, N-H, br s), 4.97 (1 H, H_1, apparent quintet, $J \sim 5.4$ Hz), 5.59 (1 H, $H_{1'}$, m), 7.15–8.09 (7 H, naphthyl-H, 3 m).

(1*S*,1′*S*)- (31b, Less Polar) and (1*R*,1′*S*)-3-Ethynyl-4-(trideuteriomethyl)cyclohex-3-en-1-yl *N*-[1′-(1-Naphthyl)ethyl]carbamate (32b, More Polar). The trideuteriated carbamates (1*S*,1′*S*) **31b** and (1*R*,1′*S*) **32b** were prepared in the same manner as the unlabeled carbamates **31a** and **32a**. Following the procedure of Pirkle[26] and starting with the trideuteriated racemic alcohol **30b** (195 mg, 1.40 mmol) there was obtained the desired labeled (1*S*,1′*S*) isomer **31b** (185 mg, 39%) and the (1*R*,1′*S*) isomer **32b** (203 mg, 43%), respectively.

General Procedure for the Kinetic Studies. 1. Preparation of the Stock Solutions. a. Unlabeled Compounds. For the kinetic studies, the previtamin D_3 (abbreviated P) solutions were prepared under argon in the following manner. Crystalline vitamin D_3 (abbreviated D, 980.7 mg, 2.53 mmol) was dissolved in isooctane (12 mL) and heated for 2 h at reflux. The reaction vessel was covered with aluminum foil to avoid side reactions induced by light. After the equilibration, the solution was flash cooled to −78 °C, warmed to room temperature, and filtered (0.5-μm PTFE membrane, Millipore Millex-SR filter). To obtain pure P, the ~78/22 D/P mixture was subjected to HPLC separation (Dynamax Macro HPLC silica column; injection with ~65.4 mg/10 μL samples, t_P = 16 min, t_D = 22 min; solvent, 25% ethyl acetate/hexanes). The separated P solution was collected at 0 °C, and all runs were combined. The solvent was evaporated, and the P slightly contaminated with its rearrangement product D was diluted with ethyl acetate (2–3 mL).

A small portion (~0.7 mL) of the latter solution was exposed to HPLC again. The P fraction was collected in a flask cooled to −78 °C and was quickly warmed to room temperature within 5 min (and a stop watch started, t = 0) since it was expected that rearrangement would start approximately within this time period. The sample was concentrated as rapidly as possible. The material thus obtained (127.5 mg) was redissolved in absolute ethanol to afford 100 mL of solution. A portion of this solution (10 mL) was further diluted with ethanol to give a stock solution of 500 mL ($c = 2.550 \times 10^{-3}$ g/100 mL). The absorption spectra were recorded on a UV spectrophotometer [HP 8452A diode array spectrophotometer utilizing a HP Chem Station or HP 89531A UV/vis operating software], and the absorption value was determined at 260 nm (the UV maximum of P) and also at 266 nm. The readings were recorded after 100, 190, 270, and 380 min to give a calibration curve which made it possible to extrapolate the absorptions to t = 0 min (as defined above). The absorption calculated for t = 0 min (266 nm, 0.4375) can simply be transformed into the extinction value $E^{1\%/1cm}$ of pure P by dividing the absorption at t = 0 min by the concentration of the solution given in g/100 mL. The $E^{1\%/1cm}$ so calculated is then called E_P (171.57), the extinction of pure previtamin D_3. The definition

of $E^{1\%/1cm}$, abbreviated $E^{1\%}$, is described elsewhere,[36] wherein $E^{1\%} = A/cb$ where A = absorption, c = concentration in grams/100 mL, b = path length in centimeters.

A solution of vitamin D_3 (D) was prepared in a similar fashion. Crystalline commercially available D (35.5 mg, which does not appear to contain any P unless allowed to stand in solution) was dissolved in absolute ethanol (10 mL). An aliquot (1 mL) has further diluted to 100 mL to give the needed stock solution ($c = 1.775 \times 10^{-3}$ g/100 mL). The absorption spectra was recorded as quickly as possible with a UV spectrometer (time between preparation and examination of the solution was <10 min) and the absorption value determined at 266 nm (0.6605), the maximum of the curve. $E^{1\%/1cm}$ could be calculated again by dividing the absorption value by the concentration given in g/100 mL. The $E^{1\%/1cm}$ value so calculated is called E_D (372.11), the extinction of pure D.

b. Labeled Compounds. After Lindlar semihydrogenation of the dienyne, the labeled P was separated by HPLC (Whatman Partisil M10, 25% ethyl acetate/hexanes, t_P = 17 min). The solution of labeled P was collected and evaporated as quickly as possible to give pure labeled P (11.37 mg) which was dissolved in 500 mL of absolute ethanol ($c = 2.277 \times 10^{-3}$ g/100 mL). The absorption value at 266 nm was found to be 0.3683, and the E_P value was calculated to be 161.78. Note that because of the primary kinetic deuterium isotope effect, this labeled material is considerably more easily handled than the unlabeled substrate.

Labeled D was obtained by equilibration of labeled P using a procedure similar to that described above. The mixture was subjected to HPLC separation (Whatman Partisil M10, 25% ethyl acetate/hexanes, t_D = 24 min). The solution of labeled D was collected and evaporated as quickly as possible to give pure labeled vitamin D_3 (3.6 mg), which was dissolved in ethanol (100 mL). A portion of this solution (60 mL) was further diluted with ethanol to give the stock solution ($c = 2.160\ 10^{-3}$ g/100 mL), for which UV analysis afforded an absorption value at 266 nm of 0.7857. The E_D was calculated to be 363.75.

2. Preparation of the Samples and the Kinetic Runs. From the stock solutions, the samples for the kinetic investigation were prepared as follows. (Every kinetic run incorporates the data from 15 to 20 samples drawn from the heating bath at appropriate time intervals.)

Prior to use, the ampoules (5 mL) were washed (1 × NaOH, 1 × $NaHCO_3$, 2 × H_2O) and dried overnight at 120 °C. After sealing the ampoules with rubber septa, they were flushed with argon, filled with 2.5 mL of the stock solution, and put through three freeze–thaw cycles under vacuum. The 15–20 ampoules for each kinetic run were then sealed with a sharp flame and, after brief equilibration to room temperature, collectively placed in a thermostated constant temperature bath (Fisher Isotemp Immersion Circulator with bath, Model 730-13 equipped with a Fluke 52 K/J Thermometer) set at the appropriate temperature. In order to equilibrate the samples to the desired temperature, a dead time of 3 min was determined. At regular time intervals, an ampoule was removed from the constant temperature bath and cooled immediately to −78 °C. All samples were stored in a freezer (−70 °C) until the run was completed and the samples could be analyzed collectively. The UV spectra were recorded for each sample twice (two different cells, Fisher, cat. no. 14 384 914B, 1 scan per cell, scan time 25 s, room temperature), and the absorbance value was measured at 266 nm. E values (no sub- or superscript) at time t were then calculated wherein the concentration values (c) were those of the stock solutions given above.

3. Kinetic Calculations. The kinetic data (E vs t) for the labeled and unlabeled P/D interconversion was determined in ethanol using UV spectrometry. The analysis of the kinetic data was accomplished according to a paper published by Hanewald and co-workers.[5a] Measurements were made at five different temperatures between 85 and 60 °C. Each temperature is represented by three independent runs. Every run covers a time range of ~13 or ~8.5 half-lives of the unlabeled and labeled previtamin, respectively. The absorption values (average for the two different 1-cm quartz cells employed) at 266 nm obtained for each run (for 15–20 ampoules, respectively) were plotted as absorption (A) against the heating time (t, s) to show the change of P to D until equilibration.

$$\text{previtamin } D_3 \text{ (P)} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \text{vitamin } D_3 \text{ (D)} \quad (1)$$

The first order rate constants of the reactions between P and D are defined by the following rate law:

$$k = k_1 + k_2 = \frac{1}{t} \ln \frac{c_e - c_o}{c_e - c} \quad (2)$$

where c_o is the concentration of D at t = 0 seconds, c_e is the concentration of D at equilibrium, c is its concentration at time t, and a (the stock solution concentration given above) is the total concentration of P and D. Thus the previtamin concentration is $a - c$ at time t. It can be shown that concentrations can be replaced by the absorption values to give the equivalent equation:

$$k = k_1 + k_2 = \frac{1}{t} \ln \frac{A_e - A_o}{A_e - A} \quad (3)$$

where A_o is the measured absorption value of the first sample in every kinetic run for t = 0 seconds; A_e is the absorption value at equilibrium; and A is the absorption value at time, t. In a plot of ln ($A_e - A_o/A_e - A$) versus the time (s), the slope of the line is k, the sum of k_1 and k_2. To calculate the two rate constants the following equations are needed:

$$A = [(a - c)/a]A_P + (c/a)A_D \text{ or } E = [(a - c)/a]E_P + (c/a)E_D \quad (4)$$

$$c = [a/(A_D - A_P)](A - A_P) \text{ or } c = [a/(E_o - E_P)](E - E_P)$$

As mentioned earlier, the concentrations (g/100 mL) can be replaced by absorption values using these relationships because every absorption value is the sum of the spectra of P and D. To render kinetic data of experiments using slightly different concentrations comparable, the absorptions can further be transformed into $E^{1\%/1cm}$ values as described above. With this information in hand the values for k_1 and k_2 are described as follows:

$$k_1 = (C_e/a)k = [(E_e - E_P)/(E_D - E_P)]k$$

$$k_2 = [(a - C_e)/a]k = [(E_D - E_e)/(E_D - E_P)]k \quad (5)$$

where k, E_D, and E_P were determined as described above. E_e could be determined by averaging the E_e values obtained in the kinetic runs at infinite time, starting from the previtamin or the vitamin. Instead of determining the E_e values from each kinetic run because the values seemed somewhat scattered, they were determined in an independent experiment. Samples of the stock solutions of previtamin and vitamin respectively were taken and heated to equilibrium at five different temperatures. A plot of the absorptions (or ln A) found at 266 nm, respectively, versus the temperature (or $1/T$) gives a straight in both cases, so that the equilibrium extinctions (E_e) for the kinetic runs (different temperatures) could be extrapolated to the appropriate temperature. With k_1 and k_2 in hand the equilibrium constant K_{eq} can be calculated as follows:

$$K_{eq} = \frac{k_1}{k_2} \quad (6)$$

The activation parameters were computed from an Arrhenius plot of the natural logarithm of the rate constants for the P to D conversion (k_1) versus the reciprocal of the absolute temperature. Table I and II in the next give a summary of the data.

4. k_H/k_D Calculations. The kinetic isotope effect k_H/k_D was calculated from the equations obtained for k_1:

unlabeled previtamin D_3

$$\ln k_1 = -9861.047\ 712\ 322\ 4/T + 20.348\ 761\ 632\ 848$$

(36) Silverstein, R. M.; Bassler, G. C.; Morill, T. C. *Spectrometric Identification of Organic Compounds*, 4th ed.; John Wiley: New York, 1981; p 307.

labeled previtamin D_3

$$\ln k_1 = -11\,013.108\,298\,279/T + 21.782\,253\,134\,787$$

The k_H/k_D was determined to be 6.2 at 80 °C and 11.4 at 25 °C.

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Supplementary Material Available: Spectral data for all new compounds, general experimental details, and descriptions of the kinetic studies (30 pages). This material is contained in 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.