the same day prior to use by using standard techniques.

Instrumentation and Data-Handling Procedures. Cyclic and linear-sweep voltammetry were performed on a JAS Instrument Systems J-1600-B potentiostat driven by a Hewlett-Packard 3314A function generator. After passing a sample through a Stanford Research Systems Model SR640 dual-channel low-pass filter, the data were recorded on a Nicolet Model 310 digital oscilloscope with 12-bit resolution. The oscilloscope and function generator were controlled by an IBM AT compatible personal computer via an IEEE interface. The current-potential curves were collected at selected trigger intervals to reduce periodic noise,[23] and 20 curves were averaged before treatment with a frequency domain low-pass digital filter and numerical differentiation.

Cyclic Voltammetry Measurements. A standard three-electrode one-compartment cell was used for all kinetic measurements. Positive feedback IR compensation was used to minimize the effects of uncompensated solution resistance. Reference electrodes were $Ag/AgNO_3$ (0.01 M) in acetonitrile constructed in the manner described by Moe.[24] The working electrodes, 0.2-0.8-mm Pt, were prepared by sealing wire in glass and polishing to a planar surface as described previously.[25] The working electrodes were cleaned before each series of measurements with a fine polishing powder (Struers, OP-Alumina Suspension) and wiped with a soft cloth. The cell was immersed in a water bath controlled to 25 ± 0.2 °C.

Kinetic Measurements. Rate constants were obtained by comparing derivative cyclic voltammetry[26] data to theoretical data obtained by digital simulation.[27] The reactions were studied under second-order conditions with use of solutions containing substrate (1.0 mM) and nucleophile (2.0 mM). Rate constants were evaluated at several different sweep rates (ν), giving a range of values for the derivative peak ratio (R'_1)[26] by using eq 19 in which the constant depends upon R'_1. For example, when R'_1 is 0.500, the constant is equal to 79.2 when the substrate concentration is 1.0 mM.

$$\log k(\mathrm{M/s}) = (\text{constant})(F/RT)\nu \qquad (19)$$

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(23) Lasson, E.; Parker, V. D. *Anal. Chem.* **1990**, *62*, 412.
(24) Moe, N. S. *Anal. Chem.* **1974**, *46*, 968.
(25) Lines, R.; Parker, V. D. *Acta Chem. Scand.* **1977**, *B31*, 369.
(26) Parker, V. D. *Electroanal. Chem.* **1986**, *14*, Chapter 1.
(27) Feldberg, S. W. *Electroanal. Chem.* **1969**, *3*, Chapter 3.

1α,25-Dihydroxyprevitamin D_3: Synthesis of the 9,14,19,19,19-Pentadeuterio Derivative and a Kinetic Study of Its [1,7]-Sigmatropic Shift to 1α,25-Dihydroxyvitamin D_3[1]

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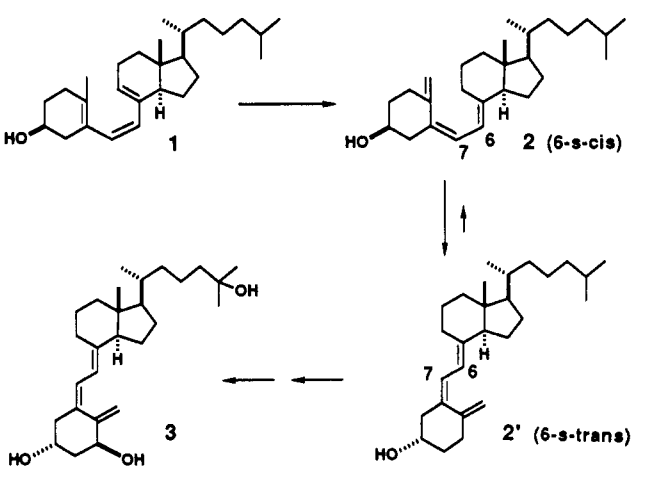
Abstract: The hormonally active steroid 1α,25-dihydroxyvitamin D_3 (**3**) exists in equilibrium with its previtamin form **5**. In an attempt to further understand the significance of this previtamin and previtamin D's in general, the pentadeuterio analogue of **5** was synthesized. Accordingly, 9,14,19,19,19-pentadeuterio-1α,25-dihydroxyprevitamin D_3 (**6**) was prepared from the readily available, optically active synthons (*S*)-(+)-carvone (**10**) and the Inhoffen-Lythgoe diol (**9**). A general method was developed to regioselectively deuteriate β-methyl-α,β-unsaturated aldehydes via their Schiff bases and was used in the synthesis to convert aldehyde **16a** to its deuteriated analogue **16b**. Thermolysis of **6** afforded 9,9,14,19,19-pentadeuterio-1α,25-dihydroxyvitamin D_3 (**24**). A kinetic investigation of the [1,7]-sigmatropic hydrogen (deuterium) shifts which convert previtamins **5** and **6** to vitamins **3** and **24**, respectively, revealed that at 25 °C the process proceeds with a relatively normal primary kinetic isotope effect, k_H/k_D, of 5.5.

Introduction

It is now well-established that a key step in the primary metabolic pathway leading to the physiologically active form of vitamin D, namely 1α,25-dihydroxyvitamin D_3 (**3**),[2] is the transformation of previtamin D_3 (**1**) to vitamin D_3 (**2** and **2'**) (Scheme I). This metabolic conversion, at least formally a [1,7]-sigmatropic hydrogen shift, has been well-studied in solution.

Scheme I



The intermediacy of the previtamin D_2 in the conversion of provitamin D_2 (ergosterol) to vitamin D_2 was first demonstrated

(1) This is paper 40 in the following series: Studies on Vitamin D (Calciferol) and Its Analogues. For paper 39, see: Figadere, B.; Norman, A. W.; Henry, H. L.; Koeffler, H. P.; Zhou, J.-Y.; Okamura, W. H. *J. Med. Chem.*, in press.

(2) (a) For general reviews of the biochemistry of vitamin D, see: Norman, A. W. *Vitamin D, the Calcium Homeostatic Steroid Hormone*; Academic Press: New York, 1979. De Luca, H. F.; Paaren, H. E.; Schnoes, H. K. *Top. Curr. Chem.* **1979**, *83*, 1. (b) For general reviews of the chemistry of vitamin D, see: Georghiou, P. E. *Chem. Soc. Rev.* **1977**, *6*, 83. Fieser, L. F.; Fieser, M. *Steroids*; Reinhold: New York, 1959. For papers related to the metabolic 7-dehydrocholesterol-previtamin D_3 and previtamin D_3-vitamin D_3 conversions, see: (c) 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. (d) 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. (e) Holick, M. F. *J. Invest. Dermatol.* **1981**, *76*, 51.

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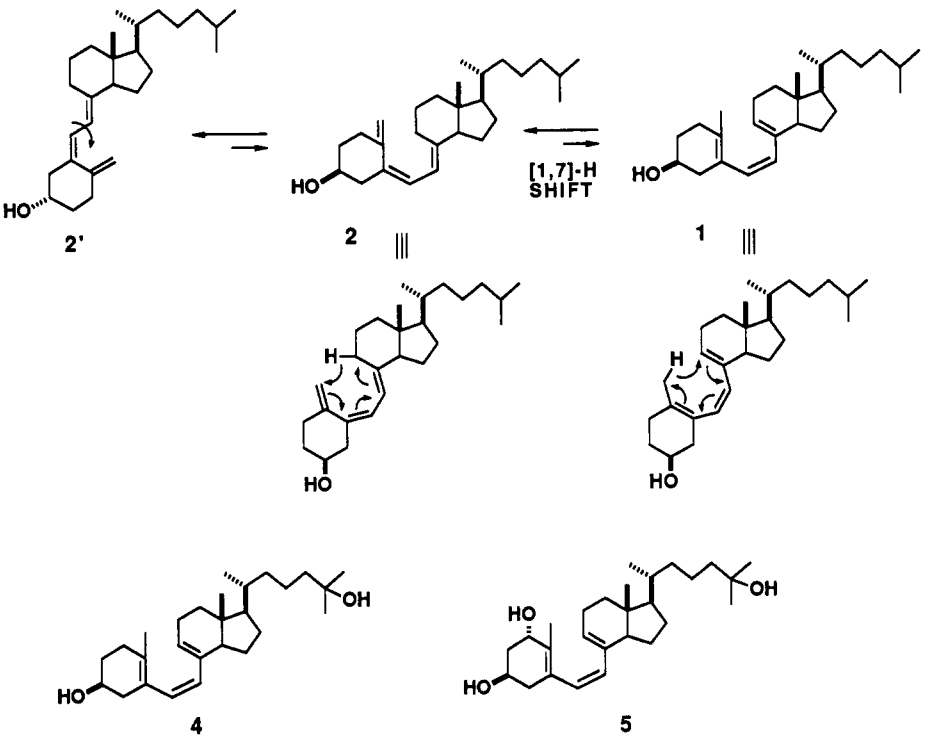
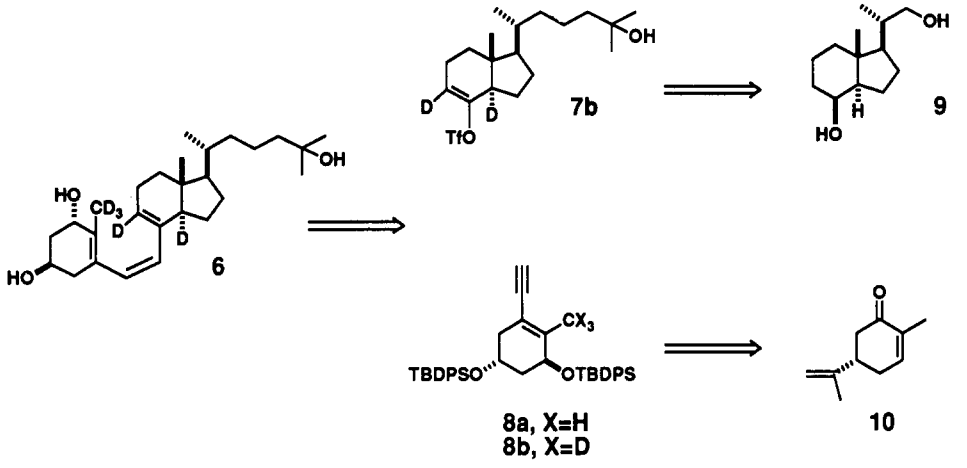
Scheme II



Scheme III



by Velluz and co-workers in 1949.[3] In this and subsequent studies the rate of the previtamin–vitamin conversion of the dinitrobenzoate derivative of previtamin D_2 was evaluated.[4,5] Kinetic investigations of the conversion of natural previtamin D_3 (**1**) to vitamin D_3 (**2** and **2'**) were subsequently reported in 1961 by Rappoldt and co-workers.[6] They determined that the activation energy and entropy for this process were 19.5 kcal/mol and 20.4 cal/mol K, respectively, at 25 °C.

The intramolecular nature of this reaction was demonstrated by Akthar and Gibbons through the use of C-19 tritium-labeled previtamin D_3.[7] While the predicted antarafaciality[8] of this specific transformation has not been established, work from this laboratory has demonstrated such antarafaciality for the [1,7]-hydrogen shifts of several isomeric *cis*-isotachysterols.[9] In addition, extensive work has been completed by several laboratories to evaluate the conformational features of previtamin D_3 and vitamin D_3.[10,11] In 1979, Mazur and co-workers synthesized

(3) (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.

(4) (a) Verloop, A. Ph.D. Thesis, State University Leiden, 1956; p 44. (b) Verloop, A.; Koevoet, A. L.; Havinga, E. *Recl. Trav. Chim. Pays-Bas* **1957**, *76*, 689. (c) Schlatmann, J. L. M. A.; Pot, J.; Havinga, E. *Recl. Trav. Chim. Pays-Bas* **1964**, *83*, 1173.

(5) Legrand, M.; Mathieu, J. *Compt. Rend. Seances Acad. Sci.* **1957**, *245*, 2502.

(6) (a) Hanewald, K. H.; Rappoldt, M. P.; Roborgh, J. R. *Recl. Trav. Chim. Pays-Bas* **1961**, *80*, 1003. (b) For a kinetic study of this transformation in liquid–crystalline solvents, see: Cassis, E. G., Jr.; Weiss, R. G. *Photochem. Photobiol.* **1982**, *35*, 439. (c) For a kinetic study of this transformation in synthetic phosolipid bilayers, see: Yamamoto, J. K.; Borch, R. F. *Biochemistry* **1985**, *24*, 3338.

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

(8) (a) Woodward, R. B.; Hoffmann, R. *J. Am. Chem. Soc.* **1965**, *87*, 2511. (b) For a general review of [1,j]-sigmatropic shifts, see: Spangler, C. W. *Chem. Rev.* **1976**, *76*, 187.

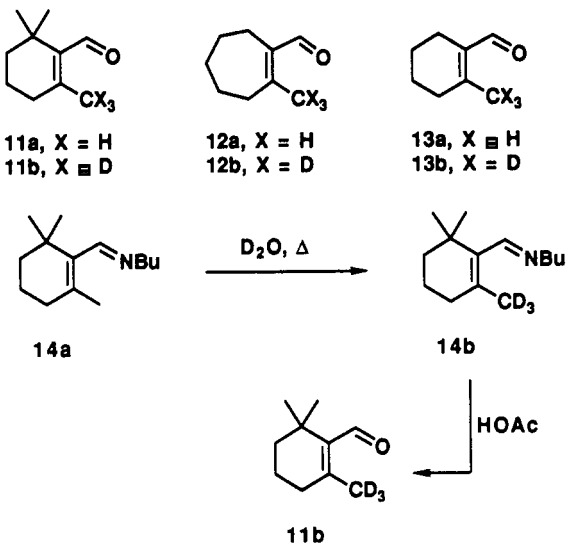
(9) (a) Hoeger, C. A.; Okamura, W. H. *J. Am. Chem. Soc.* **1985**, *107*, 268. (b) Hoeger, C. A.; Okamura, W. H. *J. Am. Chem. Soc.* **1987**, *109*, 4690.

(10) (a) Wing, R. M.; Okamura, W. H.; Rego, A.; Pirio, M. R.; Norman, A. W. *J. Am. Chem. Soc.* **1975**, *97*, 4980. (b) Wing, R. M.; Okamura, W. H.; Pirio, M. R.; Sine, S. M.; Norman, A. W. *Science* **1974**, *186*, 939.

19,19-dideuteriovitamin D_3 and reported that the transformation of previtamin D_3 to vitamin D_3 occurs with a large primary deuterium kinetic isotope effect (k_H/k_D).[12] In a later investigation from this laboratory, it was found that 1-hydroxylated-3-deoxyprevitamin D_3 deuteriated at the C_9 and C_{19} positions afforded a primary isotope effect of ~6 upon conversion to its vitamin form.[13] Our interest in C_9–C_{19} deuteriated analogues of previtamin D_3 arises from the notion that because the [1,7]-hydrogen shift is reversible, all vitamin D's should be in equilibrium with their previtamin forms (Scheme II). Accordingly, previtamins **1**, **4**, and **5** should be present in vivo with their vitamin D forms. It has been shown that a heavy isotope at C_9 and C_{19} will attenuate the rate of the [1,7]-sigmatropic shift and thus facilitate handling of the thermally unstable previtamins.[12-14] The synthesis of a 9,19,19,19-deuteriated analogue of each of the previtamins would allow for the evaluation of each of their biological profiles. Furthermore, such analogues may have practical applications as "slow release" forms of the highly potent and potentially toxic steroid hormone **3**. This paper thus describes the synthesis and quantitative evaluation of the rearrangement of 9,14,19,19,19-pentadeuterio-1α,25-dihydroxyprevitamin D_3 (**6**). A quantitative assessment of the conversion of the labeled and unlabeled previtamins to their vitamin D forms would provide information concerning the temperature dependence of the primary deuterium kinetic isotope effects for [1,7]-sigmatropic hydrogen shifts for which there is presently only a limited amount of information.[15]

The overall synthetic plan which was used in this study is outlined in Scheme III. Previtamin **6** was envisaged to result from CD fragment **7b** and A-ring fragment **8b**. This kind of approach was first used by Lythgoe and co-workers[16] and later modified to that shown in Scheme III by a number of other research groups including our own.[17,18] It was known that deuteriated CD fragment **7b** could be made from the readily available and optically active Inhoffen–Lythgoe diol (**9**) via a method modified in our laboratory. Furthermore, it was anticipated that A-ring fragment **8b** could originate from commercially available (*S*)-(+)-carvone (**10**). The conversion of carvone (**10**) to enyne **8b** was accomplished through the development of a general method for regiospecifically deuteriating the methyl groups of β-methyl-α,β-unsaturated aldehydes.

Scheme IV



Results and Discussion

Synthetic Studies. The synthesis of labeled A-ring fragment **8b** was achieved starting from protio analogue enyne **8a**,[19] which has recently been shown to be obtainable in ~15-g quantities from (*S*)-(+)-carvone in just 3 weeks.

A method was needed to selectively deuteriate the methyl group of enyne **8a**. It was believed that this could be accomplished via the aldehyde form of this enyne with a method investigated in this laboratory[20] in which it was found that heating a sample of β-cyclocitral (**11a**) in the presence of deuterium oxide in a sealed tube at temperatures above 100 °C afforded high deuterium incorporation at the vinyl methyl group of this aldehyde (Scheme IV).[21] The mechanism is thought to involve a [1,5]-sigmatropic

(11) (a) Dauben, W. G.; Funhoff, D. J. H. *J. Org. Chem.* **1988**, *53*, 5070. (b) Dauben, W. G.; Funhoff, D. J. H. *J. Org. Chem.* **1988**, *53*, 5376. (c) Delaroff, V.; Rathle, P.; Legrand, M. *Bull. Soc. Chim. Fr.* **1963**, 1739. (d) La Mar, G. N.; Budd, D. L. *J. Am. Chem. Soc.* **1974**, *96*, 7317. (e) Kotovych, G.; Aarts, G. H. M.; Bock, K. *Can. J. Chem.* **1980**, *58*, 1206. (f) Kotowych, G.; Nakashima, T. T.; Green, M. K.; Aarts, G. H. M. *Can. J. Chem.* **1980**, *58*, 45. (g) Hodgkin, D. C.; Rimmer, B. M.; Dunitz, J. D.; Trueblood, K. N. *J. Chem. Soc.* **1963**, 4945. (h) Knobler, C.; Romers, C.; Braun, P. B.; Hornstra, J. *Acta Crystallogr., Sect. B* **1972**, *28*, 2097. (i) Hull, S. E.; Leban, I.; Main, P.; White, P. S.; Woolfson, M. M. *Acta Crystallogr. Sect. B* **1976**, *32*, 2374. (j) Ikekawa, N.; Eguchi, T.; Hirano, Y.; Tanaka, Y.; DeLuca, H. F.; Itai, A.; Iitaka, Y. *J. Chem. Soc. Chem. Commun.* **1981**, 1157. (k) Trinh, T.; Ryan, R. C.; Simon, G. L.; Calabrese, J. C.; Dahl, L. F.; DeLuca, H. F. *J. Chem. Soc., Perkin Trans. II* **1977**, 393. (l) Trinh, T.; DeLuca, H. F.; Dahl, L. F. *Vitamin D: Biochemical, Chemical and Clinical Aspects Related to Calcium Metabolism*; Norman, A. W., Schaefer, K., Coburn, J. W., DeLuca, H. F., Fraser, D., Grigoleit, H. G., Herrath, D. v., Eds.; Walter de Gruyter & Co.: West Berlin, 1977; pp 927–936. (m) Berman, E.; Friedman, N.; Mazur, Y.; Sheves, M.; Zaretskii, Z. V. I. *Vitamin D: Basic Research and Its Clinical Application*; Norman, A. W., Schaefer, K., Herrath, D. v., Grigoleit, H.-G., Coburn, J. W., DeLuca, H. F., Mawer, E. B., Suda, T., Eds.; Walter de Gruyter & Co.: West Berlin, 1979; pp 65–72. (n) Helmer, B.; Schnoes, H. K.; DeLuca, H. F. *Archives Biochem. Biophys.* **1985**, *241*, 608. (o) Tsukida, K.; Akutsu, K.; Saiki, K. *J. Nutr. Sci. Vitaminol.* **1975**, *21*, 411. (p) Eguchi, T.; Ikekawa, N. *Bioorg. Chem.* **1990**, *18*, 19. (q) See, also: Moriarty, R., to be published in the proceedings of the 8th Workshop on Vitamin D, July 5–10, 1991, Paris, France.

(12) Sheves, M.; Berman, E.; Mazur, Y.; Zaretskii, Z. V. I. *J. Am. Chem. Soc.* **1979**, *101*, 1882. Professor Mazur has kindly informed us that the k_H/k_D value of ~45 originally reported (cf. ref 13) is in error.

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

(14) (a) Melander, L.; Saunders, W. H., Jr. *Reaction Rates of Isotopic Molecules*; Wiley: New York, 1980; pp 140–147. See, also: (b) Westheimer, F. H. *Chem. Rev.* **1961**, *61*, 265. (c) More O'Ferrall, R. A. *J. Chem. Soc. (B)* **1970**, 785. (d) Wolfsberg, M. *Acc. Chem. Res.* **1972**, *5*, 225. (e) Kwart, H. *Acc. Chem. Res.* **1982**, *15*, 401.

(15) For other k_H/k_D temperature dependence studies, see: (a) Baldwin, J. E.; Reddy, V. P. *J. Am. Chem. Soc.* **1987**, *109*, 8051. (b) Baldwin, J. E.; Reddy, V. P. *J. Org. Chem.* **1988**, *53*, 1129. (c) Baldwin, J. E.; Reddy, V. P. *J. Am. Chem. Soc.* **1988**, *110*, 8223. (d) Palenzuela, J. A.; Elnagar, H. Y.; Okamura, W. H. *J. Am. Chem. Soc.* **1989**, *111*, 1770.

(16) (a) Harrison, R. G.; Lythgoe, B.; Wright, P. W. *J. Chem. Soc., Perkin Trans. I* **1974**, 2654. (b) Dawson, T. M.; Dixon, J.; Littlewood, P. S.; Lythgoe, B.; Saksena, A. K. *J. Chem. Soc. (C)* **1971**, 2960. (c) Lythgoe, B. *Chem. Soc. Rev.* **1980**, *9*, 449.

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

(18) For previous syntheses of 1α,25-$(OH)_2$-D_3, see: (a) Semmler, E. J.; Holick, M. F.; Schnoes, H. K.; DeLuca, H. F. *Tetrahedron Lett.* **1972**, 4147. (b) Rubio-Lightbourn, J.; Morisaki, M.; Ikekawa, N. *Chem. Pharm. Bull.* **1973**, *21*, 1854. (c) Morisaki, M.; Rubio-Lightbourn, J.; Ikekawa, N.; Takeshita, T. *Chem. Pharm. Bull.* **1973**, *21*, 2568. (d) Barton, D. H. R.; Hesse, R. H.; Pechet, M. M.; Rizzardo, E. *J. Chem. Soc., Chem. Commun.* **1974**, 203. (e) Fürst, A.; Labler, L.; Meier, W. *Helv. Chim. Acta* **1981**, *64*, 1870. (f) Sato, T.; Yamauchi, H.; Ogata, Y.; Tsujii, M.; Kunii, T.; Kagei, K.; Toyoshima, S.; Kobayashi, T. *Chem. Pharm. Bull.* **1978**, *26*, 2933. (g) Ochi, K.; Matsunaga, I.; Nagano, H.; Fukushima, M.; Shindo, M.; Kaneko, C.; Ishikawa, M.; DeLuca, H. F. *J. Chem. Soc., Perkin Trans. I* **1979**, 165. (h) Paaren, H. E.; DeLuca, H. F.; Schnoes, H. K. *J. Org. Chem.* **1980**, *45*, 3253. (i) Fürst, A.; Labler, L.; Meier, W. *Helv. Chim. Acta* **1982**, *65*, 1499. (j) Vanmaele, L.; De Clercq, P. J.; Vandewalle, M. *Tetrahedron* **1985**, *41*, 141. (k) Pardo, R.; Santelli, M. *Bull. Soc. Chim. Fr.* **1985**, 98. (l) Baggiolini, E. G.; Iacobelli, J. A.; Hennessy, B. M.; Batcho, A. D.; Sereno, J. F.; Uskokovic, M. R. *J. Org. Chem.* **1986**, *51*, 3098. (m) Andrews, D. R.; Barton, D. H. R.; Hesse, R. H.; Pechet, M. M. *J. Org. Chem.* **1986**, *51*, 4819. (n) Mascareñas, J. L.; Sarandeses, L. A.; Castedo, L.; Mouriño, A., manuscript submitted for publication. (o) Hatakeyama, S.; Sugawara, K.; Numata, H.; Takano, S. *J. Org. Chem.* **1991**, *56*, 461.

(19) (a) Aurrecoechea, J. M.; Okamura, W. H. *Tetrahedron Lett.* **1987**, *28*, 4947. (b) Okamura, W. H.; Aurrecoechea, J. M.; Gibbs, R. A.; Norman, A. W. *J. Org. Chem.* **1989**, *54*, 4072.

(20) Okamura, W. H.; Palenzuela, J. A., unpublished results. This general approach was called to our attention first by Dr. Peter Schiess (University of Basel, Switzerland).

shift of the methyl hydrogens to the proximal carbonyl oxygen. Exchange of the proton of the resulting enol with a deuteron from D_2O and subsequent [1,5]-deuterium migration back to the terminal double bond would afford a labeled methyl group after three cycles. The synthetic utility of this reaction was exemplified by the deuteriation of aldehyde **12a** in excellent yield with high label incorporation.[22]

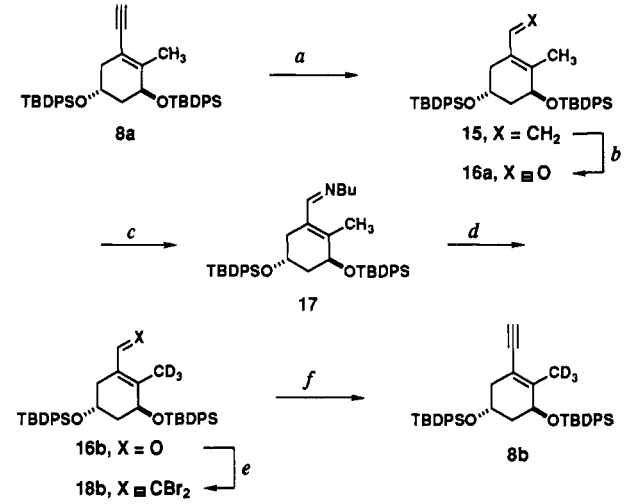
The problem with this method arose from the experimental observation that this exchange was extremely slow for six-membered rings not possessing a *gem*-dimethyl group adjacent to the carboxyaldehyde moiety. This may be due to the presence of the adjacent *gem*-dimethyl group, which could increase the s-cis conformer population of the aldehyde required for the [1,5]-sigmatropic hydrogen shift. In the absence of the *gem*-dimethyl group, the s-trans conformer, which cannot undergo a [1,5]-shift, could predominate to a much larger degree and thus attenuate the [1,5]-sigmatropic shift. As an example of this effect, it was determined that treatment of aldehyde **11a** with D_2O at 110 °C for 68 h afforded ~78% deuterium incorporation, while treatment of aldehyde **13a**, which lacks the *gem*-dimethyl group, with D_2O at 110 °C for 68 h gave only ~25% incorporation.

It was hoped that this problem could be overcome by the use of the Schiff bases of the aldehydes involved. While it was not clear how a terminal nitrogen would affect the [1,5]-sigmatropic shift presumably involved in this process, it was known from previous work in this laboratory that dienimines readily undergo another pericyclization reaction, six-electron electrocyclization, to afford dihydropyridines.[23]

Accordingly, imine **14a**, available from the treatment of β-cyclocitral (**11a**) with butylamine in the presence of 4Å molecular sieves, was treated with deuterium oxide in refluxing benzene (80 °C) for 32 h to afford, after hydrolysis with 1 M acetic acid, aldehyde **11b** with ~73% deuterium incorporation (300 MHz ^1H NMR analysis). By comparison, treatment of the parent aldehyde **11a** under the same conditions for 81 h gave only ~13% conversion to labeled aldehyde. It should be mentioned that the Schiff base resulting from the use of aniline gave similar results but was more difficult to hydrolyze and the amine was harder to separate from the deuteriated aldehyde. In other attempts to improve this method it was found that the *N,N*-dimethylhydrazone of aldehyde **11a** did not undergo exchange, while imines possessing a β- or γ-*N,N*-dimethylamino group did not give improved exchange. The decreased rate of exchange observed for the hydrazone case may be attributed to decreased resonance stabilization or increased lone pair repulsion (an α-effect) upon isomerization to the putative enamine-like intermediate resulting from a [1,5]-shift.

With this information in hand it was now necessary to examine the deuterium exchange of the Schiff base of the aldehyde corresponding to enyne **8a**. Accordingly, hydrogenation of **8a** in the presence of Lindlar catalyst afforded the desired diene **15**, which was then oxidatively cleaved with catalytic osmium tetraoxide and an excess of sodium periodate to give aldehyde **16a** (Scheme V). Schiff base formation as above afforded imine **17** in near quantitative yield. Imine **17** (as the robust (*tert*-butyldiphenyl)silyl, TBDPS,[24] protected A-ring) was determined to undergo clean exchange to afford labeled aldehyde in excellent yields. It was also found that acidic workup of the labeled imine was not necessary as silica gel could effect complete hydrolysis. On a preparative scale imine **17** was treated with D_2O in benzene in a sealed tube at 150 °C for 3 h. To ensure complete exchange

Scheme V[a]



[a] Reagents: (a) H_2, Lindlar catalyst (86%); (b) OsO_4, $NaIO_4$ (68%); (c) $BuNH_2$, 4Å molecular sieves (95%); (d) D_2O, C_6H_6, 150 °C (3 × 3h cycles); SiO_2 (84%); (e) PPh_3, CBr_4, Zn (99%); (f) BuLi (91%).

the D_2O was replaced, and this cycle was repeated two more times to afford after flash chromatography aldehyde **16b** in 84% yield with >97% deuterium incorporation (300-MHz ^1H NMR analysis). It should be mentioned that the exchange reaction could be run at 120 °C (3 × 12 h cycles) and that the higher temperature (150 °C) was selected only for convenience, and a more detailed quantitative investigation was not pursued.

Mass spectral analysis of the deuterium content was better determined on a later synthetic intermediate because initial indications were that this aldehyde undergoes exchange with residual protons in the mass spectrometer (see Experimental Section). Concerning the rate of exchange of the imine versus its parent aldehyde, separate samples of imine **17** and free aldehyde **16a** were heated at 120 °C in the presence of D_2O for 12 h in sealed ampules. After the usual silica gel hydrolysis and isolation, ^1H NMR analysis indicated that while the imine sample had undergone ~51% deuterium exchange, the free aldehyde sample had less than 3% deuterium incorporation.

The proposed mechanism for the imine deuteriation is believed to begin with the thermally induced [1,5]-sigmatropic hydrogen shift of imine **17** which after proton/deuteron exchange and a second [1,5]-shift would afford a monolabeled imine. Two more of these cycles and hydrolysis would give aldehyde **16b**. This mechanism is supported by the fact that only the methyl hydrogens are observed to exchange. No deuterium incorporation was found at the C_1 allylic position which is not in the correct cisoid orientation for a [1,5]-shift.

Completion of the labeled A-ring fragment synthesis involved the Corey–Fuchs procedure[25] wherein treatment of **16b** with the preformed dibromo Wittig reagent resulting from a mixture of carbon tetrabromide, triphenylphosphine, and zinc dust afforded dibromide **18b**. Treatment of **18b** with *n*-butyllithium gave labeled enyne **8b** in excellent yield. Mass spectral analysis of this compound indicated 93% d_3 and 7% d_2 species although ^1H NMR analysis indicated a higher level of label incorporation (>97%).

The synthesis of labeled CD fragment **7b** (Scheme III) began with the Inhoffen–Lythgoe diol (**9**) which is available in multigram quantities in this laboratory from the oxidative degradation of vitamin D_2. With a multistep method initially developed by Lythgoe[26] and later modified in this laboratory,[17c,27] diol **9** was converted to ketone **19a** in 74% overall yield. Deuteriation of

(21) For a nonselective deuterium incorporation of this type, see: Dawson, M. I.; Hobbs, P. D.; Chan, R. L.-S.; Chao, W.-R. *J. Med. Chem.* **1981**, *24*, 1214. See, also: Chauhan, Y. S.; Chandraratna, R. A. S.; Miller, D. A.; Kondrat, R. W.; Reischl, W.; Okamura, W. H. *J. Am. Chem. Soc.* **1985**, *107*, 1028.

(22) (a) Enas, J. D. Ph.D. Thesis, University of California, Riverside, CA, March 1990. (b) Enas, J. D.; Palenzuela, J. A.; Okamura, W. H. *J. Am. Chem. Soc.* **1991**, *113*, 1355. (c) See, also: Enas, J. D.; Shen, G.-Y.; Okamura, W. H. *J. Am. Chem. Soc.* **1991**, *113*, 3873.

(23) (a) Okamura, W. H.; de Lera, A. R.; Reischl, W. *J. Am. Chem. Soc.* **1988**, *110*, 4462. (b) de Lera, A. R.; Reischl, W.; Okamura, W. H. *J. Am. Chem. Soc.* **1989**, *111*, 4051.

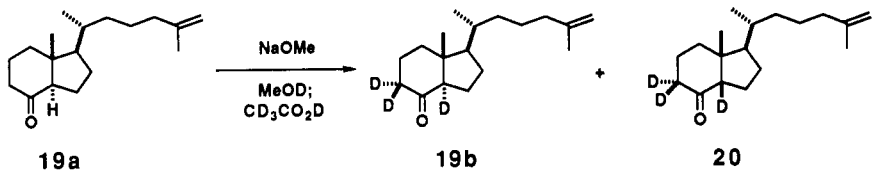
(24) Hanessian, S.; Lavallee, P. *Can. J. Chem.* **1975**, *53*, 2975.

(25) (a) Corey, E. J.; Fuchs, P. L. *Tetrahedron Lett.* **1972**, 3769. (b) Castedo, L.; Mascareñas, J. L.; Mouriño, A. *Tetrahedron Lett.* **1987**, *28*, 2099. (c) Hijfte, L. V.; Kolb, M.; Witz, P. *Tetrahedron Lett.* **1989**, *30*, 3655.

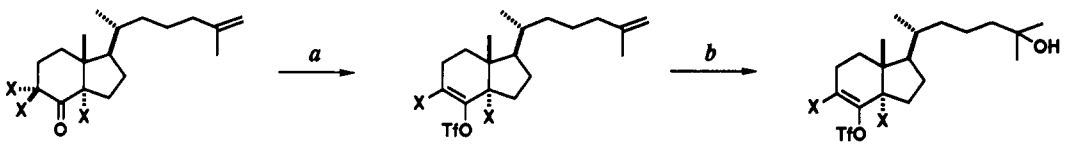
(26) Lythgoe, B.; Roberts, D. A.; Waterhouse, I. *J. Chem. Soc., Perkin Trans. 1* **1977**, 2608.

(27) Leyes, G. A. Ph.D. Thesis, University of California, Riverside, CA, December 1981.

Scheme VI



Scheme VII[a]



[a] (a) LDA, −78 °C; $PhNTf_2$ (**21a**, 83%; **21b**, 81%); (b) $Hg(OAc)_2$; $NaBH_4$, NaOH (**7a**, 91%; **7b**, 87%).

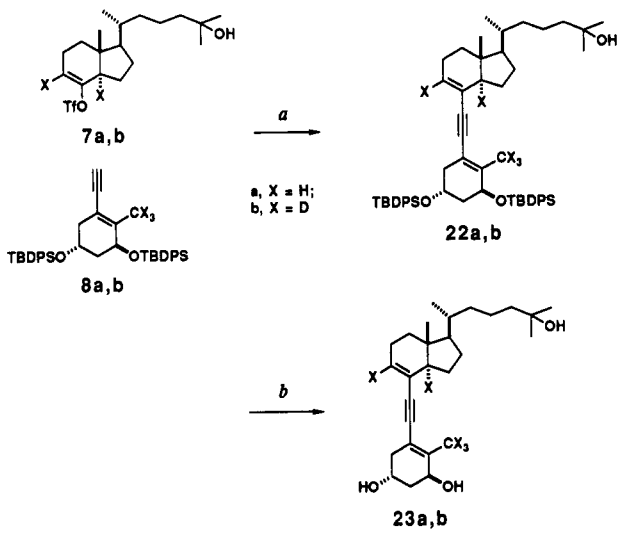
ketone **19a** (Scheme VI) was accomplished with the procedure of Dawson and co-workers[21] later adapted in this laboratory.[13] This method involved base-catalyzed deuterium–hydrogen exchange with sodium methoxide/methanol-d_4 with an acetic acid-d_4 quench (three cycles). However, this procedure incorporates deuterium not only in the desired 9-position but also at the 14-position along with epimerization at the same 14-position. Thus, a mixture of trideuterioketone **19b** and its C_{14} epimer **20** were obtained (1:3.6 ratio; 96% yield), and the isomers were subjected to preparative HPLC separation. The undesired epimer **20** could be recycled under the same conditions. Mass spectral analysis of **19b** indicated in a typical case a deuterium content of 97% d_3 and 3% d_2 species, while the ^1H NMR analysis indicated complete exchange (>98% d_3). The syntheses of both the labeled and unlabeled CD fragments were completed as follows. Treatment of ketone **19a,b** with lithium diisopropylamine (LDA) at −78 °C afforded the kinetic enolate which was trapped with *N*-phenyltrifluoromethanesulfonamide to give enol triflate **21a,b** in good yield (Scheme VII). In a modification of previous vitamin D syntheses of this laboratory, it was found that oxymercuration/demercuration of unsaturated enol triflate **21a,b** could be accomplished in a highly efficient fashion.[28]

The CD and A-ring fragments, **7a,b** and **8a,b**, respectively, were coupled with the mild procedure of Ortar and co-workers[29] in which bis[triphenylphosphine]palladium(II) acetate–copper(I) iodide catalyst in a dimethylformamide/diethylamine mixture was used to afford diprotected enyne **22a,b** in good yield (Scheme VIII). Deprotection using tetrabutylammonium fluoride afforded polar and unstable triol **23a,b**.

Hydrogenation of triol **23a,b** could be achieved in methanol in the presence of Lindlar catalyst and quinoline poison followed by HPLC purification (Scheme IX). In this manner, overreduction could be kept to a minimum, and isolated yields of previtamin were generally good. As expected, thermolysis of previtamins **5** and **6** at 80 °C afforded the corresponding vitamins **3** and **24** (**3**, 68%; **24**, 72%). The spectral data of the protio previtamin was in good agreement with the recent assignments of Vandewalle,[18j] while the data for the unlabeled vitamin was in accord with the assignments reported by Wing,[10] Vandewalle,[18j] and Hesse.[18m]

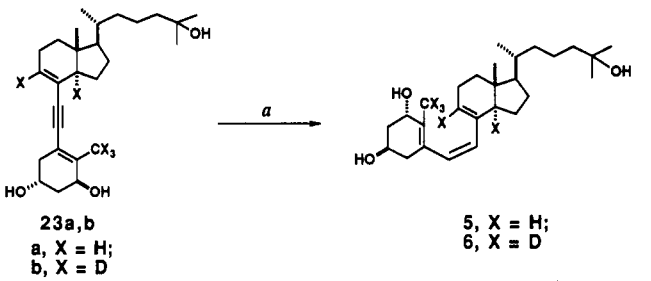
NMR analysis at 300 MHz of labeled previtamin **6** revealed that the signal at δ 1.76 (assigned to the C_{19}–Me) was essentially absent, while the δ 5.50 signal (assigned to H_9) could not be detected even at high spectrum amplitude. Furthermore, analysis of the spectrum of deuteriovitamin **24** showed that the signals at δ 2.82 ($H_{9\beta}$), 5.00 (H_{19Z}), and 5.30 (H_{19E}) could not be discerned again even at high spectrum amplitude. In this manner, the abundance of all four of the important labels ($3D_{19}$ and D_9 on previtamin **6**; D_9 and $2D_{19}$ on vitamin **24**) could be directly

Scheme VIII[a]



[a] (a) $(PPh_3)_2Pd(OAc)_2$, CuI, Et_2NH, room temperature (**22a**, 84%; **22b**, 81%); (b) TBAF, THF, room temperature (**23a**, 70%; **23b**, 83%).

Scheme IX[a]



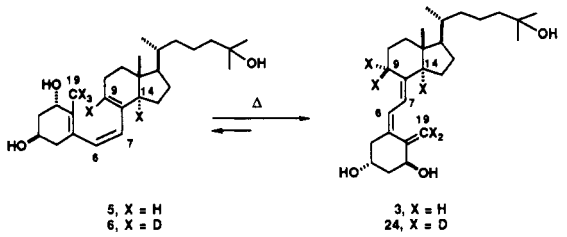
[a] (a) H_2, Lindlar catalyst, quinoline, MeOH, 20 min (**5**, 90%; **6**, 70%).

evaluated by ^1H NMR analysis. The following deuterium content by mass spectral analysis was obtained for vitamin **24**: 96% d_5, 4% d_4, and no d_3, d_2, d_1, or d_0 species.

Kinetic Studies. With the unlabeled and labeled previtamins **5** and **6** in hand, a kinetic study of their [1,7]-hydrogen shifts was carried out to evaluate the primary kinetic isotope effect (k_H/k_D) for their rearrangements at various temperatures. This would quantify the stability of labeled previtamin toward the [1,7]-shift to its vitamin form in comparison to its unlabeled analogue. The studies were performed with ^1H NMR monitoring in a manner similar to that previously described.[30] Previtamins **5** and **6** proved

(28) Brown, H. C.; Geoghegan, P. J., Jr. *J. Org. Chem.* **1970**, *35*, 1844.
(29) Cacchi, S.; Morera, E.; Ortar, G. *Synthesis* **1986**, 320.
(30) Curtin, M. L.; Okamura, W. H. *J. Org. Chem.* **1990**, *55*, 5278.

Scheme X



5, X = H
6, X = D

3, X = H
24, X = D

Table I. Summary of Kinetic Data for **5** and **6**

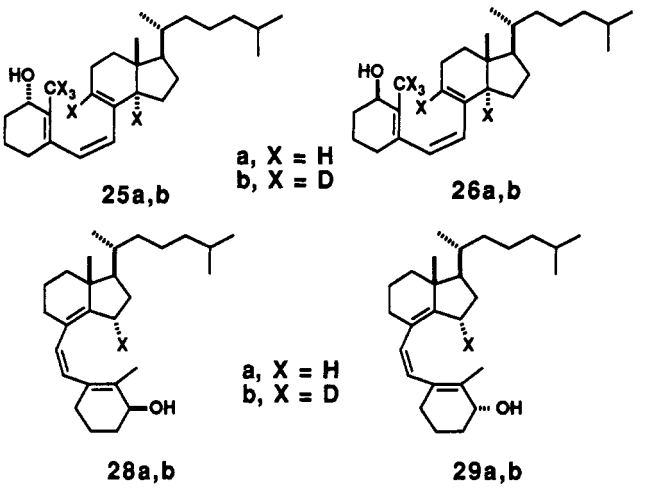
substrate	T,[a] °C	$k^b \times 10^4$, s^{-1}	K_{eq}[c]
5	44.2	0.281 (±0.015)	14.4 (±0.7)
	56.2	0.832 (±0.023)	11.1 (±0.6)
	68.1	2.23 (±0.13)	9.0 (±0.5)
	80.3	5.63 (±0.29)	7.3 (±0.4)
6	60.2	0.161 (±0.005)	10.0 (±0.5)
	70.3	0.387 (±0.005)	8.1 (±0.4)
	80.3	0.731 (±0.040)	7.3 (±0.4)
	92.3	1.64 (±0.09)	4.3 (±0.2)

[a] ±0.2 °C. [b] The sample size was ~20 mg per 1 mL of acetone. The errors are maximum errors (absolute deviations from the mean). [c] Vitamin/previtamin ratio at equilibrium.

far too insoluble in hydrocarbon solvent (e.g., benzene-d_6) so that acetone-d_6 was used instead. The rate of cyclization was determined by monitoring the previtamin/vitamin ratio in the probe of a QE-300 NMR spectrometer calibrated at the desired temperatures. Integration of the H_6 or H_7 signal of previtamin versus the H_6 or H_7 signal of vitamin (steroid numbering for **5**, **6**, **3**, and **24** as in Scheme X) in the ^1H NMR spectrum could be used to quantify the relative amounts of previtamin and vitamin. With the irreversible, first-order kinetic rate law (plotting ln [mole fraction of previtamin] versus time [s]; followed to 30–50% conversion), the rate constants for the forward reactions were obtained (see Experimental Section). In addition, monitoring these reactions for >10 half-lives allowed for the determination of the equilibrium constants at the various temperatures (Table I). Activation parameters (80 °C) for the [1,7]-shifts of previtamins **5** and **6** observed over a >30 °C temperature range are summarized in Table II.

From a qualitative standpoint, it is worth noting the ease with which the deuteriated previtamin **6** can be purified, uncontaminated (^1H NMR analysis) by the corresponding vitamin structure **24**. By contrast, even when precautions are taken to maintain unlabeled previtamin **5** at <0 °C, the kinetic samples of **5** are already contaminated by detectable amounts of the vitamin **3**. Because of the need to purify vitamins by HPLC at ambient temperatures, it is hard to avoid vitamin contamination in the unlabeled case, but this does not affect the kinetic results. From a quantitative standpoint, the rate constant for the [1,7]-sigmatropic shift of unlabeled previtamin **5** to vitamin **3** at 80 °C was calculated to be 5.63×10^{-4} s^{-1} which corresponds to a half-life of ~21 min. The rate constant for the [1,7]-shift of labeled previtamin **6** to vitamin **24** at this same temperature was determined to be 0.73×10^{-4} s^{-1} which represented a half-life of ~158 min. These two rate constants indicated that the k_H/k_D at 80 °C was ~7.5. These results can be compared with the results from a number of previous studies mentioned earlier. The calculated kinetic isotope effect from this study (~7.5) is normal and varies little from that of the [1,7]-hydrogen shifts of previtamin **25** (6.06),[13] previtamin **26** (6.13),[13] triene **28** (4.0),[9] and triene **29** (2.6)[9] (Chart I). Furthermore, the rate constants (at 80 °C) for the [1,7]-shifts of previtamins **5** and **6** (5.63×10^{-4} s^{-1} and 0.73×10^{-4} s^{-1}) are only modestly different from those of **25a,b** (7.67×10^{-4} s^{-1} and 1.25×10^{-4} s^{-1}), **26a,b** (6.20×10^{-4} s^{-1} and 1.02×10^{-4} s^{-1}), and **1** (4.62×10^{-4} s^{-1}).[6] These similarities attest to the validity of the method of kinetic analysis utilized in this and previous studies from this laboratory.

Chart I



25a,b — a, X = H; b, X = D — 26a,b

28a,b — a, X = H; b, X = D — 29a,b

As shown in Table II, the activation parameters for previtamin **5** are very similar to those previously reported for the isomerization of several other previtamins while those of **6** are somewhat different. It can be extrapolated that the k_H/k_D for this system is 7.5 at 80.0 °C, 6.0 at 37 °C, and 5.5 at 25 °C thus indicating a small decrease in isotope effect with decreasing temperature. To the extent that this data represent suitable criteria,[14] the values for ΔE_a, 1.2 kcal/mol, and A_H/A_D, 1.3, appear to indicate a linear and symmetrical hydrogen transfer with little tunneling contribution or temperature dependence.[14e]

Summary

The pentadeuterio 1α,25-$(OH)_2$-pre-D_3 **6** along with its unlabeled form **5** has been synthesized in an efficient manner with high label incorporation from the readily available and optically active synthons (*S*)-(+)-carvone (**10**) and Inhoffen–Lythgoe diol (**9**). To accomplish this, a general method has been developed by which the methyl groups of β-methyl-α,β-unsaturated aldehydes can be selectively deuteriated via their Schiff bases as exemplified by the transformation of aldehyde **16a** to its deuteriated analogue **16b**. In addition, the efficacious oxymercuration/demercuration of intermediate enol triflate **21a,b** has improved the efficiency of the overall synthetic scheme.

Kinetic studies have for the first time determined the half-life of the irreversible, first-order [1,7]-sigmatropic hydrogen shift of 1α,25-(OH_2)-pre-D_3 to 1α,25-$(OH)_2$-D_3 (d_0 and d_5) at various temperatures. Furthermore, it has been shown that at 80.0 °C the [1,7]-shift occurs with a relatively normal primary kinetic isotope effect, k_H/k_D, of 7.5 and that the previtamin exists in equilibrium with its vitamin form in a ratio of 12:88. Activation

Table II. Activation Parameters[a] for the Previtamin D–Vitamin D Transformation

substrate	E_a[b]	log A[c]	$\Delta G^{\ddagger}$[b]	$\Delta H^{\ddagger}$[b]	$\Delta S^{\ddagger}$[d]
5	18.5 (±0.1)	8.2 (±0.04)	26.1 (±0.1)	17.8 (±0.09)	−23.3 (±0.1)
6	17.3 (±0.6)	6.6 (±0.2)	27.5 (±0.9)	16.6 (±0.6)	−30.8 (±1.0)
25[e]	18.8 (±0.1)	8.5 (±0.03)	25.9 (±0.1)	18.1 (±0.1)	−22.2 (±0.1)
26[e]	19.1 (±0.5)	8.6 (±0.2)	26.1 (±0.7)	18.4 (±0.5)	−21.7 (±0.6)
30[f]	19.1 (±0.5)	8.5 (±0.2)	26.3 (±0.7)	18.4 (±0.5)	−22.2 (±0.6)

[a] At 80.0 °C. Standard deviations are given in parentheses. The sample size was ~20 mg per 1 mL of acetone. The rate constants were determined over the temperature range 44.2–80.3 °C (±0.2 °C) for **5** and 60.2–92.3 °C (±0.2 °C) for **6**. [b] kcal/mol. [c] A in s^{-1}. [d] cal/mol K. [e] Data at 80.0 °C from ref 12. [f] Data at 80.0 °C from ref 6.

parameters for the process were determined, and the data reveal the k_H/k_D is only modestly temperature dependent.

Experimental Section[31]

1α,25-Dihydroxyvitamin D_3 (3). A solution of previtamin **5** (44 mg, 0.11 mmol) in acetone-d_6 (1 mL) was placed in a tube equipped with a high-pressure seal screw cap. After the solution was subjected to three freeze–thaw cycles under vacuum, the sealed, argon-flushed tube was placed in a thermostated constant temperature bath set at 80.3 °C. After 3 h the tube was removed and allowed to cool, and the previtamin/vitamin product distribution was immediately estimated by ^{1}H NMR analysis. The sample was then concentrated under vacuum, and the resulting oil was purified by HPLC (50% ethyl acetate/hexanes; 5 mL/min; Rainin Dynamax 60A column) to afford, in order of elution, 1α,25-dihydroxyvitamin D_3 (**3**, 26 mg, retention time ~14 min) and 1α,25-dihydroxyprevitamin D_3 (**5**, 4 mg, retention time ~22 min). The combined yield was 68%, and the previtamin/vitamin equilibrium ratio was determined to be 12/88 (by ^{1}H NMR analysis, 12/88; by HPLC integration, 11/89; by isolated weight, 13/87).

Vitamin **3** was isolated after recrystallization with ethyl acetate/benzene as a white solid (mp 101–104 °C; lit.[18f] mp 100–103 °C): $[\alpha]_D^{25}$ +45.0° (*c* 0.44, EtOH); lit.[18j] $[\alpha]_D$ +47.9° (*c* 0.5, EtOH). The spectral data for this substance were in accord with the recent assignments reported by Wing[10a] ($CDCl_3$), Vandewalle[18j] ($CDCl_3$), and Hesse[18m] (acetone-d_6): ^{1}H NMR ($CDCl_3$) δ 0.54 (3 H, $C_{18}CH_3$, s), 0.93 (3 H, $C_{21}CH_3$, d, J ~ 6.3 Hz), 1.21 (6 H, C_{26} and $C_{27}2CH_3$, s), 2.31 (1 H, 4β, dd, J ~ 13.4 Hz, 6.5 Hz), 2.59 (1 H, 4α, dd, J ~ 13.4 Hz, 3.2 Hz), 2.82 (1 H, 9β, dd, J ~ 11.7 Hz, 3.3 Hz), 4.2–4.3 (1 H, H_3, complex m, W ~ 20.1 Hz), 4.4–4.5 (1 H, H_1, m, W ~ 11.7 Hz), 4.95–5.05 (1 H, H_{19Z}, narrow m), 5.30–5.35 (1 H, H_{19E}, narrow m), 6.01 and 6.37 (2 H, H_6 and H_7, AB pattern, J ~ 11.4 Hz).

For the kinetic studies, a solution of previtamin **5** (20 mg, 0.05 mmol) in acetone-d_6 (1 mL) was placed in a 7″ × 5 mm NMR tube and then subjected to three freeze–thaw cycles under vacuum. The tube was then sealed under vacuum and placed in an NMR probe calibrated at the various temperatures. After sample equilibration to the desired temperature, the ^{1}H NMR spectra were recorded at regular time intervals, and there was revealed the presence of both previtamin **5** and vitamin **3**. The rate of reaction was monitored by following the disappearance of the δ 5.93 signal (H_6) of previtamin **5** versus the appearance of the δ 6.09 signal (H_7) of vitamin **3**. Another signal of previtamin **5** [δ 5.75 (H_7)] was also monitored versus another signal of vitamin **3** [δ 6.29 (H_6)] to calculate the irreversible first-order rate constant. The calculated rate constants determined by either integration method showed good agreement (±3%). In a separate experiment, the thermal rearrangement of samples of vitamin **3** were followed by ^{1}H NMR at various temperatures until complete equilibration had occurred. Further details of the equilibrium and kinetic investigations are presented in the supplementary material.

1α,25-Dihydroxyprevitamin D_3 (5). A stirred mixture of dienyne **23a** (61 mg, 0.15 mmol), Lindlar catalyst (70 mg, Aldrich), and quinoline (700 μL, 0.17 M solution in hexanes, 0.12 mmol) in methanol (5.0 mL) was exposed to hydrogen gas for 20 min. Filtration through a 0.5-μm PTFE membrane (Millipore Millex-SR filter unit, catalog no. SLSRO25NS) and concentration afforded a residual oil which was subjected to HPLC purification (80% ethyl acetate/hexanes, 3 mL/min, Rainin Dynamax 60A column) to afford 56 mg (90%) of previtamin **5** as a spectrally homogeneous, colorless oil which decomposes rapidly when neat: $[\alpha]_D^{25}$ −52.7° (*c* 1.1, EtOH). The spectral data for this substance were in agreement with the recent assignments of Vandewalle:[18j] ^{1}H NMR ($CDCl_3$) δ 0.69 (3 H, $C_{18}CH_3$, s), 0.95 (3 H, $C_{21}CH_3$, d, J ~ 6.6 Hz), 1.21 (6 H, C_{26} and $C_{27}2CH_3$, s), 1.76 (3 H, C_{19}, br s), 2.49 (1 H, dd, J ~ 16.5 Hz, 4.2 Hz), 4.0–4.1 (1 H, H_3, complex m, W ~ 28 Hz), 4.15–4.25 (1 H, H_1, m), 5.45–5.55 (1 H, H_9, m), 5.77 and 5.90 (2 H, H_6 and H_7, AB pattern, J ~ 12.2 Hz).

1α,25-Dihydroxy-9,14,19,19,19-pentadeuterioprevitamin D_3 (6). A stirred mixture of dienyne **23b** (40 mg, 0.10 mmol), Lindlar catalyst (46 mg, Aldrich), and quinoline (460 μL, 0.17 M solution in hexanes, 0.08 mmol) in methanol (5.0 mL) was exposed to hydrogen gas for 20 min. Filtration through a 0.5-μm PTFE membrane (Millipore Millex-SR filter unit, catalog no. SLSRO25NS) and concentration afforded a residual oil which was subjected to HPLC purification (80% ethyl acetate/hexanes, 3 mL/min, Rainin Dynamax 60A column) to afford 28 mg (70%) of previtamin **6** as a spectrally homogeneous, colorless oil.

25-Hydroxy-de-*A*,*B*-cholest-8-en-8-yl Trifluoromethanesulfonate (7a). To enol triflate **21a** (1.04 g, 2.63 mmol) under nitrogen in a 250-mL flask was added THF (30 mL) and H_2O (10 mL). To the resulting solution at room temperature was added mercuric acetate (0.92 g, 2.89 mmol) in one portion, and the orange solution was stirred for 1 h. To the reaction mixture was added 6 M aqueous NaOH (10 mL) followed by 0.5 M $NaBH_4$ in 3 M aqueous NaOH (15 mL) with a water bath being used to maintain room temperature. The gray mixture was stirred an additional 30 min, diluted with ether (40 mL), and washed with saturated $NaHCO_3$ and brine (40 mL each). The resulting organic layer was dried ($MgSO_4$), filtered, and concentrated to afford after flash chromatography (silica gel, 5 × 15 cm, 18% ethyl acetate/hexanes) 987 mg (91%) of hydroxy triflate **7a** as a clear, viscous liquid which was used without further purification: $[\alpha]^{25}_D$ 20.7° (*c* 1.9, $CHCl_3$). An analytical sample was prepared by HPLC purification (20% ethyl acetate/hexanes; 4 mL/min; Rainin Dynamax 60A column): ^{1}H NMR ($CDCl_3$) δ 0.75 (3 H, $C_{18}CH_3$, s), 0.94 (3 H, $C_{21}CH_3$, d, J ~ 6.3 Hz), 1.21 (6 H, C_{26} and C_{27}, $2CH_3$, s), 5.56 (1 H, H_9, ddd, J ~ 3.4 Hz, 3.4 Hz, 3.4 Hz).

9,14-Dideuterio-25-hydroxy-de-*A*,*B*-cholest-8-en-8-yl Trifluoromethanesulfonate (7b). To enol triflate **21b** (2.38 g, 5.98 mmol) under nitrogen in a 250-mL flask was added THF (90 mL) and H_2O (30 mL). To the resulting solution at room temperature was added mercuric acetate (2.10 g, 6.58 mmol) in one portion, and the orange solution was stirred for 2 h. To the reaction mixture with water bath cooling was added 6 M aqueous NaOH (15 mL) followed by 0.5 M $NaBH_4$ in 3 M aqueous NaOH (20 mL). The gray mixture was stirred an additional 30 min, diluted with ether (40 mL), and washed with saturated $NaHCO_3$ and brine (40 mL each). The organic layer was dried ($MgSO_4$), filtered, and concentrated to afford after flash chromatography (silica gel, 5 × 15 cm, 15% ethyl acetate/hexanes) 2.15 g (87%) of hydroxy triflate **7b** as a clear, viscous liquid which was spectrally homogeneous and used without further purification.

(3*S*,5*R*)-3,5-Bis[((*tert*-butyldiphenyl)silyl)oxy]-1-ethynyl-2-methylcyclohex-1-ene (8a). Freshly purified 1,2-diiodoethane (29.3 g, 104 mmol) was dissolved to dry THF (100 mL), and the solution was added via cannula to a stirred suspension of samarium metal (21.5 g, 143 mmol) in THF (100 mL) under an argon atmosphere. An exothermic reaction took place, and an ice bath was used to keep the reaction from refluxing. Stirring of the resulting deep blue SmI_2 solution was continued at room temperature for 1.5 h at which time a solution of (1*S*,2*R*,4*S*,6*S*)-2,4-diacetoxy-2-ethynyl-1-methyl-7-oxabicyclo[4.1.0]heptane (11.2 g, 44 mmol) and $Pd(PPh_3)_4$ (1.2 g, 1 mmol; Aldrich) in dry THF (100 mL) was added via cannula. The solution was stirred at room temperature for 8 h and then evaporated to a volume of ~100 mL which was then poured into water (200 mL). After stirring for 5 min the color changed to brown-green, and the mixture was extracted with ethyl acetate (4 × 100 mL). After evaporation the resulting residue was dissolved in diethyl ether (200 mL) and filtered. Evaporation afforded an acetate which was dissolved in methanol (50 mL), cooled to 0 °C, and treated with 0.2 M sodium methoxide in methanol (200 mL). After stirring for 4 h at 0 °C, the solution was acidified with Dowex 50X4-400 resin (200–400 mesh). After removal of the resin by filtration, the solution was evaporated, and the residue was purified by flash chromatography (silica gel, 5 × 15 cm, ethyl acetate) to give 4.5 g of the deprotected diol. The diol was then treated with *tert*-butylchlorodiphenylsilane (34.2 mL, 36.1 g, 131 mmol) and imidazole (17.9 g, 263 mmol) in DMF (200 mL) at room temperature for 16 h protected from light. An ice–water slurry (150 mL) was added to the solution, and stirring was continued for 0.5 h. The solution was extracted with diethyl ether (4 × 100 mL), and the combined ether extracts were washed with brine, dried ($MgSO_4$), and evaporated. Flash chromatography of the crude product [silica gel, 10 × 15 cm; hexanes (1 L), 2% ethyl acetate/hexanes (1 L), 3% ethyl acetate/hexanes (2 L)] afforded 8.6 g of product and 16.3 g of product contaminated by a silyl byproduct. This mixture was chromatographed in the same fashion as above to afford an additional 5.9 g of protected enyne **8a** (14.6 g total, 53% from diacetate) as a spectrally homogeneous oil: $[\alpha]^{25}_D$ −40.3° (*c* 3.0, $CHCl_3$). An analytical sample was prepared by HPLC purification (0.25% ethyl acetate/hexanes; 4 mL/min; Whatman Partisil M9 column): ^{1}H NMR ($CDCl_3$) δ 0.97 (18 H, 2Si-*t*-Bu, s), 1.79 (3 H, C_2CH_3,

(31) Spectral and other analytical data, along with a detailed description of the kinetic studies, are presented in the supplementary material section. ^{1}H NMR spectral data of unlabeled (d_0) compounds in abbreviated form are presented in the Experimental Section as well. All experiments involving air- and/or moisture-sensitive materials were carried out under a nitrogen or argon atmosphere, which was dried prior to use by passage through a column of KOH layered with $CaSO_4$. Tetrahydrofuran, ether, and benzene were distilled from sodium benzophenone ketyl immediately prior to use. Hexanes were distilled from CaH_2. Unless otherwise indicated for workup procedures, organic solutions were dried over $MgSO_4$, filtered, and then finally concentrated on a rotary evaporator at reduced pressure. 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.

br s), 3.00 (1 H, sp-CH, s), 4.1–4.3 (1 H, H_5, m, $W \sim 22$ Hz), 4.37 (1 H, H_3, dd, $J \sim 4.5$ Hz, 4.5 Hz), 7.25–7.45 (12 H, m), 7.55–7.70 (8 H, m).

In the second procedure, to a solution of dibromide **18a** (75 mg, 0.10 mmol) in ether (5 mL) cooled to −78 °C was added *n*-BuLi (0.13 mL, 0.21 mmol, 1.6 M in hexanes), 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 saturated NH_4Cl (20 mL). The mixture was extracted with ether (25 mL), and then the combined organic layers were washed with brine, dried ($MgSO_4$), and concentrated. Flash chromatography (silica gel, 1 × 15 cm, 3% ethyl acetate/hexanes) of the residue afforded 53 mg (84%) of enyne **8a** as an oil.

(3*S*,5*R*)-3,5-Bis[((*tert*-butyldiphenyl)silyl)oxy]-1-ethynyl-2-(trideuteriomethyl)cyclohex-1-ene (8b). To a solution of dibromide **18b** (520 mg, 0.66 mmol) in ether (20 mL) cooled to −78 °C was added *n*-BuLi (0.78 mL, 1.25 mmol, 1.6 M in hexanes), 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 saturated NH_4Cl (20 mL). The mixture was extracted with ether (2 × 25 mL), and then the combined organic layers were washed with brine, dried ($MgSO_4$), and concentrated. Flash chromatography (silica gel, 2 × 15 cm, 3% ethyl acetate/hexanes) of the residue afforded 378 mg (91%) of deuterated enyne **8b** as a yellow oil which was used without further purification. An analytical sample was prepared by HPLC purification (0.25% ethyl acetate/hexanes; 5 mL/min; Whatman Partisil M9 column).

(3*S*,5*R*)-3,5-Bis[((*tert*-butyldiphenyl)silyl)oxy]-1-ethenyl-2-methylcyclohex-1-ene (15). A stirred solution of enyne **8a** (500 mg, 0.81 mmol), quinoline solution (5 mL; 100 μL of quinoline in 20 mL hexanes), Lindlar catalyst (500 mg), and 5 mL of hexanes was exposed to a slightly positive pressure of hydrogen gas at room temperature for 15 min. The reaction mixture was then passed through a short silica gel plug (5 × 3 cm; 50 mL 10% ethyl acetate/hexanes) and evaporated to afford 451 mg (90%) of diene **15** as a spectrally homogeneous oil: $[\alpha]^{25}_D$ −54.1° (*c* 2.9, $CHCl_3$). In five trials, the yields obtained ranged from 81% to 90%, with an average yield of 86%. An analytical sample was prepared by HPLC purification (0.25% ethyl acetate/hexanes; 4 mL/min; Whatman Partisil M9 column): ^{1}H NMR ($CDCl_3$) δ 0.97 and 1.02 (18 H, two s), 1.61 (3 H, br s), 2.32 (1 H, dd, $J \sim 16.5$ Hz, 3.9 Hz), 4.2–4.4 (2 H, two overlapping m), 4.95 and 5.00 (2 H, two narrow m), 6.66 (1 H, m).

(3*S*,5*R*)-3,5-Bis[((*tert*-butyldiphenyl)silyl)oxy]-1-formyl-2-methylcyclohex-1-ene (16a). To a solution of diene **15** (1.3 g, 2.1 mmol) in 20 mL of 1:1 THF/H_2O was added 1 mL of a 1% aqueous solution of osmium tetraoxide. After stirring at room temperature for 20 min, sodium periodate (1.1 g, 5.2 mmol) was added in one portion. After 18 h, an additional 1 mL of 1% osmium tetraoxide solution was added. After stirring for a total of 46 h, water (10 mL) was added, and the mixture was washed with 10% sodium bisulfite solution (2 × 25 mL), dried ($MgSO_4$), and concentrated. The crude product was purified by flash chromatography (silica gel, 3 × 15 cm, 4% ethyl acetate/hexanes) to give 808 mg (64%) of aldehyde **16a** as a spectrally homogeneous oil. An analytical sample was prepared by HPLC purification (6% ethyl acetate/hexanes; 4 mL/min; Whatman Partisil M9 column): ^{1}H NMR ($CDCl_3$) δ 0.93 and 1.00 (18 H, 2*t*-Bu, two s), 2.08 (3 H, CH_3, br s), 2.16 (1 H, $H_{6\alpha}$ or $H_{6\beta}$, m), 2.29 (1 H, $H_{6\alpha}$ or $H_{6\beta}$, m), 4.20 (1 H, H_5, dddd, $J \sim 4.8$ Hz, 4.8 Hz, 4.8 Hz, 4.8 Hz), 4.55 (1 H, H_3, dd, $J \sim 5.9$ Hz, 5.9 Hz), 7.2–7.7 (20 H, m), 10.09 (1 H, CHO, s).

(3*S*,5*R*)-3,5-Bis[((*tert*-butyldiphenyl)silyl)oxy]-1-formyl-2-(trideuteriomethyl)cyclohex-1-ene (16b). A solution of imine **17** (580 mg, 0.84 mmol) and D_2O (2 mL; 99.9 atom % D; Aldrich Chemical Co.) in benzene (5 mL; freshly distilled from Na/benzophenone) was placed in an ampoule with a screw cap seal. The sealed, argon-flushed ampoule was placed in an oil bath and heated at 150 °C for 3 h. After cooling the ampoule, the aqueous phase of the solution was removed by pipet and replaced with 2 mL of fresh D_2O. Heating was then continued at 150 °C for an additional 3 h. After the replacement/heating cycle was repeated for the second time (total of 9 h at 150 °C), brine (10 mL) was added, and the organic layer was dried ($MgSO_4$), filtered, and concentrated to afford a 4:1 imine/aldehyde mixture. Flash chromatography (silica gel, 3 × 15 cm, 4% ethyl acetate/hexanes) gave 451 mg (84%) of deuterated aldehyde **16b** as a spectrally homogeneous oil. An analytical sample was prepared by HPLC purification (6% ethyl acetate/hexanes; 4 mL/min; Whatman Partisil M9 column). Although mass spectral analysis indicated only 79% d_3, 17% d_2, and 4% d_1, integration of the region δ 2.0–2.5 in the ^{1}H NMR indicated >97% exchange. In agreement with this, mass spectral analysis of a later synthetic intermediate, enyne **8b**, indicated 93% d_3, 7% d_2, 0% d_1, and 0% d_0. It is suspected that the deuterated aldehyde undergoes exchange with residual protons in the mass spectrometer. Concerning the rate of exchange, for comparative purposes separate samples of imine **17** and free aldehyde **16a** (40 mg each) were heated at 120 °C in the presence of 1 mL of D_2O and 1 mL of C_6D_6 for 12 h in sealed ampoules. Usual isolation and ^{1}H NMR analysis indicated that while the imine sample had undergone ~51% deuterium exchange, the free aldehyde sample had less than 3% deuterium incorporation.

(3′*S*,5′*R*)-*N*-[1-[3′,5′-Bis[((*tert*-butyldiphenyl)silyl)oxy]-2′-methylcyclohexen-1′-yl]methylidene]butylamine (17). To a stirred solution of aldehyde **16a** (479 mg, 0.76 mmol) in ether (10 mL) over 4Å molecular sieves was added *n*-butylamine (0.75 mL, 7.57 mmol) in one portion. The resulting mixture was stirred at room temperature for 12 h at which time it was filtered through a 0.5-μm PTFE membrane (Millipore Millex-SR filter unit, catalog no. SLSRO25NS) and concentrated to afford 481 mg (92%) of imine **17** as a spectrally homogeneous oil which was used without further purification: $[\alpha]^{25}_D$ −40.0° (*c* 1.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.93 (3 H, t, $J \sim 7.4$ Hz), 0.94 and 0.97 (18 H, two s), 1.31 (2 H, sextet, $J \sim 7.4$ Hz), 1.57 (2 H, quintet, $J \sim 7.2$ Hz), 1.8 (2 H, m), 1.80 (3 H, br s), 2.28 (1 H, dd, $J \sim 17.4$ Hz, 4.8 Hz), 2.49 (1 H, br d, $J \sim 17.4$ Hz), 3.45 (2 H, t, $J \sim 7.1$ Hz), 4.2–4.3 (1 H, m), 4.45 (1 H, dd, $J \sim 5.3$ Hz, 5.3 Hz), 7.2–7.5 (12 H, m), 7.5–7.7 (8 H, m), 8.27 (1 H, s).

(3*S*,5*R*)-3,5-Bis[((*tert*-butyldiphenyl)silyl)oxy]-1-(2,2-dibromoethenyl)-2-methylcyclohex-1-ene (18a). To a solution of zinc dust (93 mg, 1.42 mmol) and triphenylphosphine (373 mg, 1.42 mmol, recrystallized from ether) in CH_2Cl_2 (10 mL) was added CBr_4 (472 mg, 1.42 mmol) in CH_2Cl_2 (5 mL) via cannula at room temperature. The resulting suspension was stirred for 12 h at which time aldehyde **16a** (174 mg, 0.28 mmol) in CH_2Cl_2 (5 mL) was added via cannula. The resulting mixture was stirred for 3 h at which time the reaction was complete (TLC). Workup was accomplished by dilution of the mixture with pentane (30 mL), filtration through Celite to remove the insoluble material, and evaporation of the solvent. The insoluble material left in the reaction flask was subjected to additional cycles (3×) of CH_2Cl_2 extraction and pentane precipitation to remove any remaining olefinic product. After concentration of the pentane extract, the resulting oil was taken up in hexanes and passed through a short column of silica gel (2 × 7 cm) with 10% ethyl acetate/hexanes (30 mL) to afford, after evaporation of solvent, the dibromoolefin **18a** (198 mg, 90%) as a colorless oil which was used in the next step without further purification: $[\alpha]^{25}_D$ −51.8° (*c* 1.4, $CHCl_3$). An analytical sample was prepared by HPLC purification (0.25% ethyl acetate/hexanes; 4 mL/min; Whatman Partisil M9 column): ^{1}H NMR ($CDCl_3$) δ 0.95 and 1.00 (18 H, two s), 1.44 (3 H, s), 1.97 (1 H, dt, $J \sim 12.9$ Hz, 3.6 Hz), 2.21 (1 H, dd, $J \sim 16.7$ Hz, 2.6 Hz), 4.2–4.3 (2 H, m), 6.80 (1 H, s), 7.3–7.5 (12 H, m), 7.6–7.7 (8 H, m).

(3*S*,5*R*)-3,5-Bis[((*tert*-butyldiphenyl)silyl)oxy]-1-(2,2-dibromoethenyl)-2-(trideuteriomethyl)cyclohex-1-ene (18b). To a solution of zinc dust (225 mg, 3.43 mmol) and triphenylphosphine (900 mg, 3.43 mmol, recrystallized from ether) in CH_2Cl_2 (25 mL) was added CBr_4 (1.14 g, 3.43 mmol) in CH_2Cl_2 (5 mL) via cannula at room temperature. The resulting suspension was stirred for 23 h at which time aldehyde **16b** (363 mg, 0.57 mmol) in CH_2Cl_2 (5 mL) was added via cannula. The resulting mixture was stirred for 1 h at which time the reaction was complete (TLC). Workup was accomplished by dilution of the mixture with pentane (50 mL), filtration through Celite to remove the insoluble material, and evaporation of the solvent. The insoluble material left in the reaction flask was subjected to additional cycles (3×) of CH_2Cl_2 extraction and pentane precipitation to remove any remaining olefinic product. After concentration of the pentane extract, the resulting oil was taken up in hexanes and passed through a short column of silica gel (2 × 7 cm) with 10% ethyl acetate/hexanes (100 mL) to afford, after evaporation of solvent, the dibromoolefin **18b** (449 mg, 99%) as a colorless oil which was used in the next step without further purification. An analytical sample was prepared by HPLC purification (0.25% ethyl acetate/hexanes; 4 mL/min; Whatman Partisil M9 column).

De-*A*,*B*-25-cholesten-8-one (19a). The ketone **19a** was obtained in 94% yield as a colorless oil with a known procedure:[17c,27] ^{1}H NMR ($CDCl_3$) δ 0.64 (3 H, s), 0.96 (3 H, d, $J \sim 5.7$ Hz), 1.71 (3 H, s), 2.3 (2 H, m), 2.44 (1 H, dd, $J \sim 11.6$ Hz, 7.7 Hz), 4.67 (2 H, two narrow signals).

9,9,14-Trideuterio-de-*A*,*B*-25-cholesten-8-one (19b). To a solution of 1.0 M NaOMe prepared from 422 mg (18.4 mmol) of Na and methanol-*O*-d_1 (MeOD, 18 mL; Aldrich, 99.5 atom % d) was added ketone **19a** (4.02 g, 15.3 mmol) in MeOD (5 mL) at room temperature under an argon atmosphere. The orange solution was allowed to stir for 48 h at which time it was cooled to 0 °C, quenched with acetic acid-d_4 (2.1 mL, 36.8 mmol; Aldrich, 99.5 atom % d), and finally diluted with water. The crude deuterated ketone was extracted with hexanes (3 × 20 mL), and the combined organic layers were washed with saturated $NaHCO_3$ and brine (25 mL each), dried ($MgSO_4$), filtered, and concentrated. The

product was redissolved in MeOD (5 mL) and added to 18 mL of a fresh solution of NaOMe in MeOD prepared as before. The second exchange was allowed to proceed for 85 h and then quenched with acetic acid-d_4 (2.1 mL, 36.8 mmol). The ketone was isolated as before, and a third exchange was carried out for 69 h and worked up exactly as before. After concentration, 3.92 g (96%) of product was obtained as a mixture of deuterated ketone **19b** and its C_{14} epimer **20**. The mixture was subjected to HPLC purification (10% ethyl acetate/hexanes; 8 mL/min; Whatman Partisil M20 column) to afford the *epi*-ketone **20** (less polar, eluted first) and the desired ketone **19b** (more polar, eluted second) in a 3.6:1 ratio. The ^{1}H NMR spectrum (300 MHz) of **19b** exhibited no apparent proton signals between δ 2.20 and δ 2.44 ppm.

In a second procedure directed toward recycling the already deuterated unwanted isomer, *epi*-ketone-d_3 **20** (1.71 g, 6.4 mmol) was dissolved in MeOD (5 mL) and added to 8 mL of a fresh solution of 1.0 M NaOMe in MeOD prepared from 178 mg (7.7 mmol) of Na and MeOD. The solution was stirred for 48 h at which time it was cooled to 0 °C, quenched with acetic acid-d_4 (0.87 mL, 15.4 mmol), and diluted with water (10 mL). The crude reaction mixture was extracted with hexanes (3 × 20 mL), and then the combined organic layers were washed with saturated $NaHCO_3$ and brine (25 mL each), dried ($MgSO_4$), and filtered. Concentration afforded 1.70 g (99%) of a 3.6:1 mixture of less polar *epi*-ketone **20** and more polar ketone **19b**, which was purified by HPLC as above.

De-*A*,*B*-cholesta-8,25-dien-8-yl Trifluoromethanesulfonate (21a). The triflate **21a** was obtained in 83% yield as a colorless oil with a known procedure:[17c] ^{1}H NMR ($CDCl_3$) δ 0.77 (3 H, s), 0.95 (3 H, d, $J \sim 6.3$ Hz), 1.71 (3 H, s), 2.0 (4 H, m), 2.2–2.4 (2 H, m), 2.4–2.5 (1 H, m), 4.68 (2 H, two narrow signals), 5.57 (1 H, ddd, $J \sim 3.4$ Hz, 3.4 Hz, 3.4 Hz).

9,14-Dideuterio-de-*A*,*B*-cholesta-8,25-dien-8-yl Trifluoromethanesulfonate (21b). Lithium diisopropylamide was prepared by treating diisopropylamine (0.49 mL, 3.50 mmol) with *n*-BuLi (2.19 mL, 3.50 mmol, 1.60 M in hexanes) in 10 mL of dry THF at 0 °C. After being stirred for 15 min, the solution was cooled to −78 °C, and ketone **19b** (773 mg, 2.91 mmol) in 5 mL of THF was added dropwise via cannula. After being stirred for 15 min, the enolate solution was warmed to room temperature over 50 min and then recooled to −78 °C. *N*-Phenyltrifluoromethanesulfonimide (1.25 g, 3.50 mmol) was dissolved in 4 mL of THF and added to the enolate solution at −78 °C via cannula. The reaction mixture was allowed to warm to room temperature over 5 h and stirred for an additional 10 h at which time the solution was transferred to a separatory funnel and washed with saturated NH_4Cl, saturated $NaHCO_3$, and brine (25 mL each). The extract was dried with $MgSO_4$, filtered, and concentrated. Purification was achieved by flash chromatography (5 × 15 cm, 100% hexanes; sample suspended on silica gel prior to addition to the column) to afford 937 mg (81%) of spectrally homogeneous enol triflate **21b** which was used without further purification.

1α-[((*tert*-Butyldiphenyl)silyl)oxy]-6,7-dehydro-25-hydroxyprevitamin D_3 (*tert*-Butyldiphenyl)silyl Ether (22a). To a mixture of enol triflate **7a** (1.11 g, 2.68 mmol) and enyne **8a** (1.86 g, 2.96 mmol) in diethylamine (6 mL) and dimethylformamide (6 mL) was added CuI (0.05 g, 0.27 mmol) and bis[triphenylphosphine]palladium(II) acetate (0.06 g, 0.08 mmol). The dark brown reaction mixture was stirred at room temperature for 1.5 h under argon. Ether (30 mL) was added, and the mixture was washed with brine (2 × 30 mL), dried ($MgSO_4$), filtered, and concentrated. The crude oil was chromatographed (silica gel, 5 × 15 cm, 15% ethyl acetate/hexanes) to afford 2.01 g (84%) of dienyne **22a** as a viscous oil which was used without further purification: $[\alpha]^{25}_D$ −80.0° (*c* 1.7, $CHCl_3$). An analytical sample was prepared by HPLC purification (15% ethyl acetate/hexanes; 4 mL/min; Rainin Dynamax 60A column): ^{1}H NMR ($CDCl_3$) δ 0.70 (3 H, $C_{18}CH_3$, s), 0.96 and 0.98 (21 H, 2*t*-Bu and C_{21}Me, two s), 1.23 (6 H, C_{26} and $C_{27}2CH_3$, s), 1.74 (3 H, $C_{19}CH_3$, br s), 4.1–4.3 (1 H, H_3, br m, $W \sim 15$ Hz), 4.3–4.4 (1 H, H_1, m), 5.9–6.0 (1 H, H_9, narrow m), 7.25–7.45 (12 H, aromatic m), 7.55–7.65 (8 H, aromatic m).

1α-[((*tert*-Butyldiphenyl)silyl)oxy]-6,7-dehydro-25-hydroxy-9,14,19,19,19-pentadeuterioprevitamin D_3 (*tert*-Butyldiphenyl)silyl Ether (22b). To a mixture of enol triflate **7b** (0.94 g, 2.27 mmol) and enyne **8b** (1.44 g, 2.27 mmol) in diethylamine (6 mL) and dimethylformamide (6 mL) was added CuI (0.04 g, 0.23 mmol) and bis[triphenylphosphine]palladium(II) acetate (0.05 g, 0.07 mmol). The dark brown reaction mixture was stirred at room temperature for 2.5 h under argon. Ether (30 mL) was added, and the mixture was washed with brine (2 × 30 mL), dried ($MgSO_4$), filtered, and concentrated. The crude oil was chromatographed (silica gel, 5 × 15 cm, 15% ethyl acetate/hexanes) to afford 1.64 g (81%) of dienyne **22b** as a viscous oil which was used without further purification. A sample for spectral analysis was prepared by HPLC purification (15% ethyl acetate/hexanes; 4 mL/min; Rainin Dynamax 60A column).

1α,25-Dihydroxy-6,7-dehydroprevitamin D_3 (23a). To dienyne **22a** (2.01 g, 2.25 mmol) under argon was added tetrabutylammonium fluoride (11.3 mL, 1.0 M solution in tetrahydrofuran, 11.3 mmol) by syringe. The resulting mixture was stirred at room temperature in the dark for 21 h at which time it was diluted with ethyl acetate (40 mL) and washed with brine (2 × 20 mL). The aqueous layer was washed with ethyl acetate (2 × 20 mL), and the combined organic layers were dried ($MgSO_4$), filtered, and concentrated to afford crude triol as a yellow oil. Flash chromatography (silica gel, 3 × 15 cm, 90% ethyl acetate/hexanes, sample suspended on silica prior to chromatography) gave 650 mg (70%) of triol **23a** as a white solid (mp 144–145 °C) which was used without further purification: $[\alpha]^{25}_D$ −23.0° (*c* 0.1, MeOH). An analytical sample was prepared by HPLC purification (15% isopropyl alcohol/hexanes; 4 mL/min; Rainin Dynamax 60A column): ^{1}H NMR ($CDCl_3$) δ 0.69 (3 H, $C_{18}CH_3$, s), 0.95 (3 H, $C_{21}CH_3$, d, $J \sim 6.6$ Hz), 1.21 (6 H, C_{26} and $C_{27}2CH_3$, s), 1.98 (3 H, $C_{19}CH_3$, br s), 2.55 (1 H, dd, $J \sim 16.7$ Hz, 4.1 Hz), 4.0–4.2 (1 H, H_3, complex m, $W \sim 27$ Hz), 4.2–4.3 (1 H, H_1, narrow m), 5.95–6.05 (1 H, H_9, narrow m).

1α,25-Dihydroxy-6,7-dehydro-9,14,19,19,19-pentadeuterioprevitamin D_3 (23b). To dienyne **22b** (1.01 g, 1.13 mmol) under argon was added tetrabutylammonium fluoride (11.3 mL, 1.0 M solution in tetrahydrofuran, 11.3 mmol) by syringe. The resulting mixture was stirred at room temperature in the dark for 33 h at which time it was diluted with ethyl acetate (30 mL) and washed with brine (2 × 20 mL). The aqueous layer was washed with ethyl acetate (2 × 20 mL), and the combined organic layers were dried ($MgSO_4$), filtered, and concentrated to afford crude triol as a yellow oil. Flash chromatography (silica gel, 3 × 15 cm, 90% ethyl acetate/hexanes, sample suspended on silica prior to chromatography) gave 393 mg (83%) of deuterated triol **23b** as a white solid (mp 142–144 °C) which was used without further purification. A sample for spectral analysis was prepared by HPLC purification (15% isopropyl alcohol/hexanes; 4 mL/min; Rainin Dynamax 60A column).

1α,25-Dihydroxy-9,14,19,19,19-pentadeuteriovitamin D_3 (24). A solution of previtamin **6** (25 mg, 0.06 mmol) in acetone-d_6 (1 mL) was placed in a tube equipped with a high-pressure seal screw cap. After the solution was subjected to three freeze–thaw cycles under vacuum, the sealed, argon-flushed tube was placed in a thermostated constant temperature bath set at 80.3 °C. After 12 h the tube was removed and allowed to cool, and the previtamin/vitamin product distribution was immediately estimated by ^{1}H NMR analysis. The sample was then concentrated under vacuum, and the resulting oil was purified by HPLC (50% ethyl acetate/hexanes; 5 mL/min; Rainin Dynamax 60A column) to afford, in order of elution, 1α,25-dihydroxy-9,9,14,19,19-pentadeuteriovitamin D_3 (**24**, 16 mg, retention time ~15 min) and 1α,25-dihydroxy-9,9,14,19,19-pentadeuterioprevitamin D_3 (**6**, 2 mg, retention time ~23 min). The combined yield was 72%, and the previtamin/vitamin equilibrium ratio was 12/88 (by ^{1}H NMR analysis, 12/88; by isolated weight, 11/89). Pentadeuteriovitamin **24** was isolated after recrystallization with ethyl acetate/benzene as a white solid (mp 100–103 °C).

For the kinetic studies, a solution of previtamin **6** (20 mg, 0.05 mmol) in acetone-d_6 (1 mL) was placed in a 7″ × 5 mm NMR tube and then subjected to three freeze–thaw cycles under vacuum. The tube was then sealed under vacuum and placed in an NMR probe calibrated at various temperatures. After sample equilibration to the desired temperature, the ^{1}H NMR spectra were recorded at regular time intervals and revealed the presence of both previtamin **6** and vitamin **24**. The rate of reaction was monitored by following the disappearance of the δ 5.93 signal (H_6) of previtamin **6** versus the appearance of the δ 6.09 signal (H_7) of vitamin **24**. Another signal of previtamin **6** [δ 5.75 (H_7)] was also monitored versus another signal of vitamin **24** [δ 6.29 (H_6)] to calculate the irreversible first-order rate constant. The calculated rate constants determined by either integration method showed good agreement (±4%). In separate experiments, the thermal rearrangement of samples of vitamin **24** were followed by ^{1}H NMR at various temperatures until complete equilibration had occurred. Further details of the equilibrium and kinetic investigations are presented in the supplementary material.

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Supplementary Material Available: Spectral data for all new compounds and general experimental details (26 pages). Ordering information is given on any current masthead page.