Organic Process Research & Development **2002**, *6*, 246–255

A Stereospecific Synthesis of 24(*S*)-Hydroxyvitamin D_2, a Prodrug for 1α,24(*S*)-Dihydroxyvitamin D_2

Lisa D. Coutts,[†] William B. Geiss,[†] Brian T. Gregg,[†] Mark A. Helle,[†] Chi-Hsin R. King,[†] Zinovy Itov,[†] Mary E. Mateo,[†] Harold Meckler,*[,†] Mark W. Zettler,[†,§] and Joyce C. Knutson[‡]

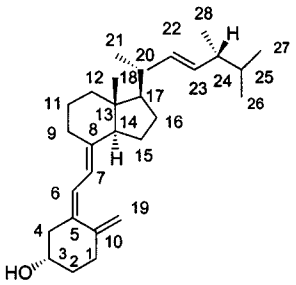
Chemical Development Department, Albany Molecular Research, Inc., P.O. Box 15098, 21 Corporate Circle, Albany, New York 12212-5098, U.S.A., and Bone Care International, Inc. Suite 300, 1600 Aspen Commons, Middleton, Wisconsin 53562, U.S.A.

Abstract:

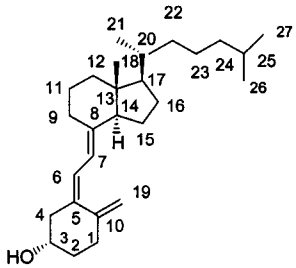
This contribution describes the first stereospecific synthesis of 24(*S*)-hydroxyvitamin D_2 (1), a metabolite of vitamin D_2. This metabolite acts as a prodrug for 1α,24(*S*)-dihydroxyvitamin D_2 (2), which is under development for treatment of various diseases characterized by cellular hyperproliferation. The key step of the synthesis involves the Wittig−Horner olefination of (*S*)-2,3-dimethyl-2-triethysilyloxybutyraldehyde (17) and a vitamin D_2 phosphine oxide derivative (22). The synthesis of the requisite aldehyde started with the commercially available L-(+)-valine and was completed in seven steps. The vitamin D_2 phosphine oxide derivative was synthesized in seven steps starting from vitamin D_2.

Introduction

Vitamin D_2 [(3β,5*Z*,7*E*,22*E*)-9,10-secoergosta-5,7,10(19),-22-tetraen-3-ol; ergocalciferol, **18**][1] is one member of the vitamin D family of compounds that have long been known for their importance in bone and mineral metabolism. For example, the vitamin D compounds serve a critical role in maintaining serum calcium levels through stimulation of intestinal calcium absorption, bone remodeling, and control of parathyroid hormone production.



Vitamin D_2



Vitamin D_3

In the 1970s, DeLuca and co-workers discovered that vitamin D_3 [(3β,5*Z*,7*E*)-9,10-secocholesta-5,7,10(19)-trien-3-ol; cholecalciferol] or vitamin D_2 (**18**) must be hydroxylated at C-1 and C-25 to form the 1α,25-dihydroxyvitamin D derivative before it would exert its biological effects.[2,3] This discovery led to the development of dihydroxylated vitamin D compounds for the treatment of bone depletive disorders. These active forms of vitamin D mediate their effects on calcium homeostasis through stereospecific, high affinity binding to a nuclear receptor protein. These specific receptors for 1α,25-dihydroxyvitamin D_3 were subsequently found in cells of diverse organs not involved in calcium homeostasis,[4] suggesting a role for active vitamin D in processes beyond those of mineral metabolism. 1α,25-Dihydroxyvitamin D compounds decreased cell proliferation and increased differentiation of a variety of cultured cells.[5a,b] These data presaged the use of vitamin D analogues for the treatment of hyperproliferative diseases such as cancer and psoriasis.[6a,b]

The activated vitamin D class of compounds can produce dangerously elevated blood calcium levels due to their inherent calcemic activity. Because of this toxicity, 1α,25-dihydroxyvitamin D_3 can only be administrated at dosages that are modestly beneficial in preventing or treating loss of bone or bone mineral content.[7] From the consideration of the diverse biological actions and their potential as therapeutic agents, vitamin D compounds have been sought that affect proliferation and differentiation but that have less calcemic activity than the known compounds.

Two areas that have been pursued in an effort to identify novel vitamin D compounds with lowered calcemic activity are (1) placement of the hydroxyl group in the side chain on C-24 rather than on C-25 and (2) the use of prodrugs. Vitamin D compounds which possess hydroxyl groups on C-1 and C-24 are reported to have less calcemic activity than 1α,25-dihydroxyvitamin D_3.[8a,b] Prodrugs of vitamin D compounds are desired because they are biologically inactive as

* To whom correspondence should be addressed. Telephone: (518) 464-0279. Fax: (518) 464-0289. E-mail: hmeckler@albmolecular.com..

[†] Albany Molecular Research, Inc.

[‡] Bone Care International, Inc.

[§] Current address: Dow AgroSciences, 9330 Zionsville Rd., Indianapolis IN 46268-1053.

(1) The vitamin D numbering system is derived from the parent steroid numbering system and is illustrated by the number structures in Figure 1.

(2) Holick, M. F.; Schnoes, H. K.; DeLuca, H. F. *Proc. Natl. Acad. Sci. U.S.A.* **1971**, *68*, 803.

(3) Jones, G.; Schnoes, H. K.; DeLuca, H. F. *Biochemistry* **1975**, *14*, 1250.

(4) Stumpf, W. E.; Sar, M.; Reid, F. A.; Tanaka, Y.; DeLuca, H. F. *Science* **1979**, *206*, 1188.

(5) (a) Hosomi, J.; Hosoi, J.; Abe, E.; Suda, T.; Kuroki, T. *Endocrinology* **1983**, *113*, 1950. (b) Skowronski, R. J.; Peehl, D. M.; Feldman, D. *Endocrinology* **1995**, *136*, 20.

(6) (a) Kragballe, K. *J. Cell. Biochem.* **1992**, *49*, 46. (b) Colston, K. W.; Berger, U.; Coombers, R. C. *Lancet* **1989**, *1*, 188.

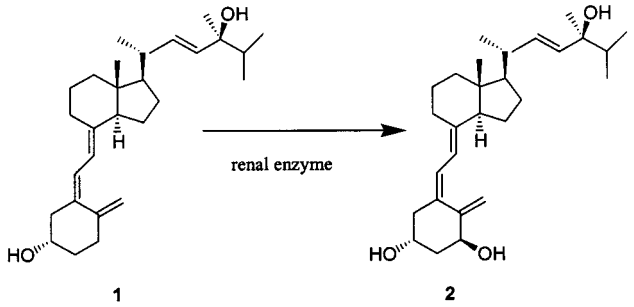
(7) Ott, S. M.; Chesnut, C. H. *Ann. Intern. Med.* **1989**, *110*, 267.

(8) (a) Binderup, L. *Pharmacol. Toxicol.* **1993**, *72*, 240. (b) Knutson, J. C.; LeVan, L. W.; Valliere, C. R.; Bishop, C. W. *Biochem. Pharmacol.* **1997**, *53*, 829.

Published on Web 04/04/2002

10.1021/op010229e CCC: $22.00

Scheme 1. **Biological conversion of 24(*S*)-hydroxyvitamin D_2 (1) to 1α,24(*S*)-dihydroxyvitamin D_2 (2)**



administered but are metabolized to active compounds in vivo. Two prodrugs in the vitamin D field are today marketed as pharmaceuticals.[9a,b]

With 24(*S*)-hydroxyvitamin D_2 (**1**), the precursor to 1α,-24(*S*)-dihydroxyvitamin D_2 (**2**), these two areas of interest converge. 24(*S*)-Hydroxyvitamin D_2 (**1**) is hydroxylated in vivo at the 1α-position to become **2**, the active form of vitamin D_2 (Scheme 1).[10] As a prodrug, **1** does not bind to the intestinal vitamin D receptors that mediate intestinal calcium absorption. This property results in reduced hypercalcemia compared with that from similar dosing of the active vitamin D compounds, such as 1α,25-dihydroxyvitamin D_3.

Although metabolic production of 24-hydroxyvitamin D_2 or 1α,24-dihydroxyvitamin D_2 has been reported in several species,[11a−c] few chemical syntheses are known. Strugnell and co-workers[12a] described the synthesis of a diastereomeric 24(*R*)- and 24(*S*)-hydroxyvitamin D_2 mixture (**10**) and the subsequent conversion of this mixture to the corresponding diastereomeric mixture of 1α,24(*R*)- and 1α,24(*S*)-dihydroxyvitamin D_2 (Scheme 2). Their synthesis started with ergosterol, **3**, that was acetylated and reacted with 4-phenyl-1,2,4-triazoline-3,5-dione to afford **5**. The side chain double bond was cleaved with ozone to afford aldehyde **6**. An aldol condensation of aldehyde **6** with the anion of methyl isopropyl ketone followed by an acid-catalyzed dehydration afforded the α,*β*-unsaturated ketone **7**. Reaction with excess methylmagnesium bromide afforded **8** which was deprotected with lithium aluminum hydride to produce 24(*R*)- and 24-(*S*)-hydroxyergosterol, (**9**). Intermediate **9** was irradiated and thermally converted by known methods to afford the diastereomeric mixture, 24(*R*)- and 24(*S*)-hydroxyvitamin D_2 (**10**).[13]

Six additional steps, following the procedure reported by Paaren and co-workers, introduced a hydroxyl group on C-1.[12b] These investigators then separated C-24 epimers by reversed phase semipreparative HPLC chromatography. One epimer, the later-eluting HPLC peak, was crystallized, and the absolute stereochemistry of the C-24 center was determined to be the 24(*R*) by a single-crystal X-ray study. The assignment of the earlier-eluting HPLC peak as the 24(*S*)-hydroxy epimer was then made by deduction. The structure of the biologically produced dihydroxylated metabolite was conclusively assigned on the basis of an HPLC coelution study with the laboratory-prepared and -isolated 1α,24(*S*)-dihydroxyvitamin D_2. This study established the identity of the oxidized biological metabolites of vitamin D_2 as 24(*S*)-hydroxyvitamin D_2 (**1**) and 1α,24(*S*)-dihydroxyvitamin D_2 (**2**) (Scheme 1).

Since the above synthesis produced an almost equal diastereomeric mixture[13] of 24(*R*)- and 24(*S*)-hydroxyvitamin D_2 and required a late-stage chromatographic separation, it was evident at the start of this project that a stereospecific synthesis was needed to prepare larger quantities of the desired 24(*S*)-hydroxyvitamin D_2. This report discusses our approach to the stereospecific synthesis of 24(*S*)-hydroxyvitamin D_2.

Results and Discussion

Our strategy for the stereoselective synthesis of 24(*S*)-hydroxyvitamin D_2 (**1**) involved a Wittig−Horner coupling reaction between two key intermediates, (*S*)-2,3-dimethyl-2-triethylsilyloxybutyraldehyde (**17**) and a suitably protected vitamin D phosphine oxide derivative (**22**). The preparation of (*S*)-2,3-dimethyl-2-triethylsilyloxybutyraldehyde (**17**) from commercially available L-(+)-valine (**11**) using a modification of a Seebach protocol[14] is outlined in Scheme 3.

At the inception of this project, (*S*)-2-hydroxyisovaleric acid (**12**) was only commercially available in small laboratory quantities, and its relatively high cost raised a barrier to scale-up synthesis. Instead, L-(+)-valine (**11**) was converted to (*S*)-2-hydroxyisovaleric acid (**12**) in 56% yield.[15] The acid-catalyzed condensation of **12** with pivaldehyde in hexanes produced the crude dioxolane (**13**) as a mixture of *cis*/*trans* isomers in a 23:1 ratio.[16] Attempts to remove the undesired *trans* isomer by a short-path distillation produced a less desirable 5:1 *cis*/*trans* mixture. It was found that the crude

(9) (a) Holick, M. F.; Semmler, E. J.; Schnoes, H. K.; DeLuca, H. F. *Science* **1973**, *180*, 190. (b) Lam, H. Y. P.; Schnoes, H. K.; DeLuca, H. F. *Science* **1974**, *186*, 1038.

(10) Unpublished results from Bone Care International, Inc.

(11) (a) Jones, G.; Schnoes, H. K.; LeVan, L.; DeLuca, H. F. *Arch. Biochem. Biophys.* **1980**, *202*, 450. (b) Mawer, E. B.; Jones, G.; Davies, M.; Still, P. E.; Byford, V.; Schroeder, N. J.; Makin, H. L. J.; Bishop, C. W.; Knutson, J. C. *J. Clin. Endocrinol. Metab.* **1998**, *83*, 2156. (c) Horst, R. L.; Koszewski, N. J.; Reinhardt, T. A. *Biochemistry* **1990**, *29*, 578.

(12) (a) Strugnell, S.; Byford, V.; Makin, H. L. J.; Moriarty, R. M.; Gilardi, R.; LeVan, L. W.; Knutson, J. C.; Bishop, C. W.; Jones, G. *Biochem. J.* **1995**, *310*, 233. (b) Paaren, H. E.; DeLuca, H. F.; Schnoes, H. K. *J. Org. Chem.* **1980**, *45*, 3253.

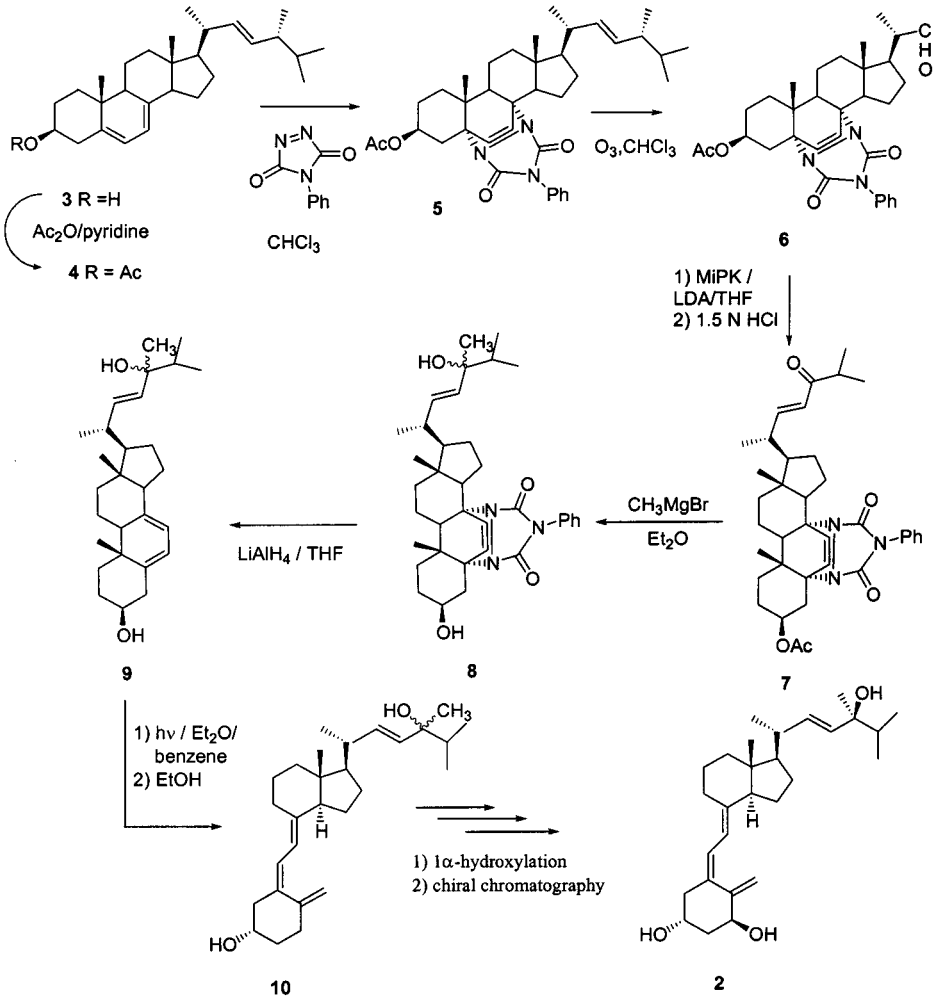
(13) The ratio of the *R* to the *S* diastereomers of 24-hydroxyvitamin D_2 prepared in ref 11a was not reported in that reference. When the mixture of diastereomers of 1α,24-dihydroxyvitamin D_2 was prepared by this procedure, analysis by HPLC in the laboratories of Bone Care International, Inc., yielded the following results: Lot no. 1, Epimer 1 (24-(*S*)): 47.2% and Epimer 2 (24-(*R*)): 52.8%; Lot no. 2, Epimer 1 (24-(*S*)): 43.1% and Epimer 2 (24-(*R*)): 56.9%. These results indicate that the chiral vitamin D skeleton induced virtually no diastereoselectivity of Grignard addition to the prochiral C24 carbonyl group.[10]

(14) Seebach, D.; Naef, R.; Calderari, G. *Tetrahedron* **1984**, *40*, 1313.

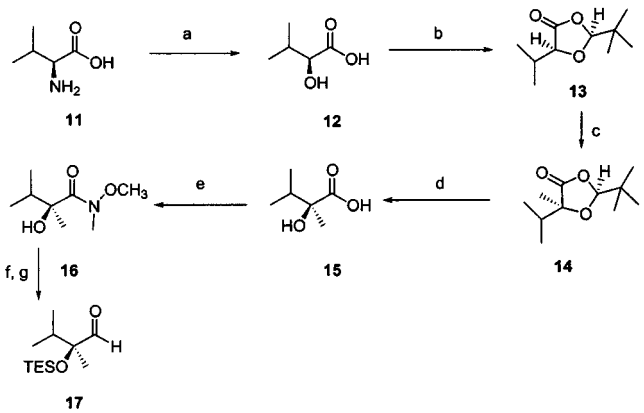
(15) Donkor, I. O.; Zheng, X.; Han, J.; Miller, D. D. *Chirality* **2000**, *12*, 551; Gonzalez, I.; Jou, G.; Caba, J. M.; Albericio, F.; Lloyd-Williams, P.; Giralt, E. *J. Chem. Soc., Perkin Trans. 1* **1996**, 1427; Burgess, K.; Li, W. *Tetrahedron Lett.* **1995**, *36*, 2725.

(16) Due to the very small magnitude of the optical rotation and lack of any usable chromophore above 215 nm in the UV spectra of intermediates **13** through **17**, our ability to chromatographically determine the optical activity or % ee of these intermediates were extremely limited. Following the NMR procedures described by Seebach and co-workers,[14] we were able to determine (within the limits of proton and carbon NMR analysis) that the crude 13 was a 23:1 *cis*/*trans* mixture, the crystallized **13** was exclusively the *cis* isomer and that the *cis*:*trans* ratio of the methyl group to the proton in **14** was 27: 1.

Scheme 2. **Strugnell preparation of diastereomeric 24-hydroxyvitamin D_2's and conversion to optically active 1α,24(*S*)-dihydroxyvitamin D_2**



Scheme 3. **Synthesis of (S)-2,3-dimethyl-2-triethylsilyloxybutyraldehyde (17)[a]**



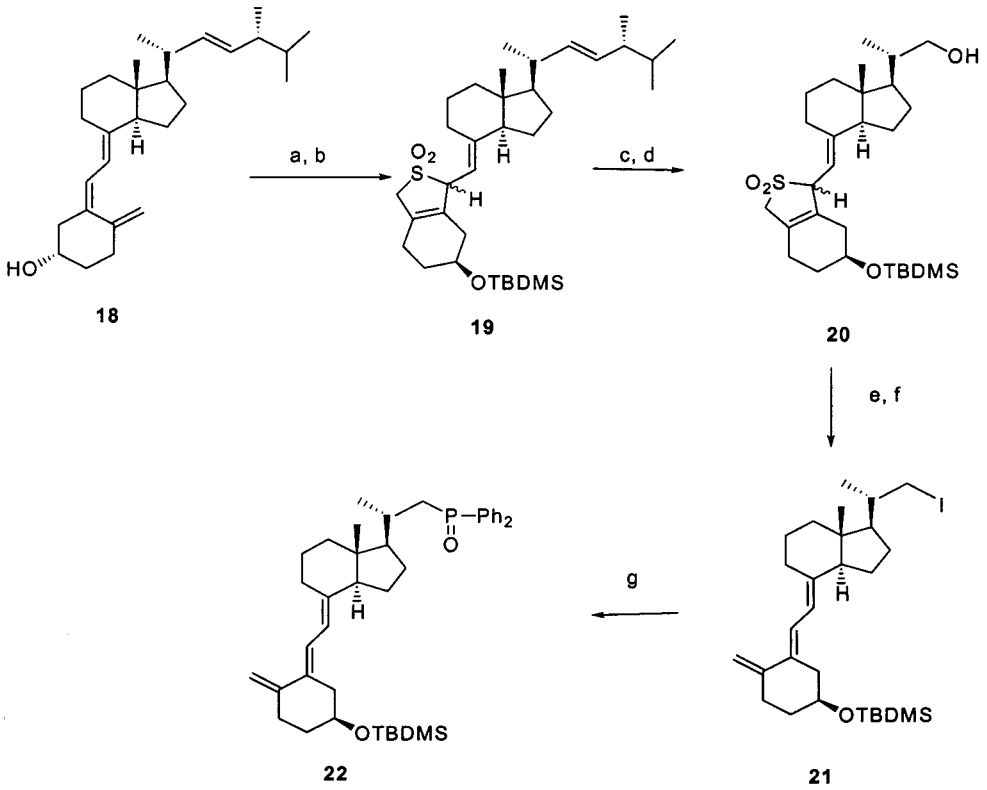
[a] Reagents: (a) $NaNO_2$, H_2SO_4, H_2O, 56%; (b) pivaldehyde, hexanes, *p*-TsOH, 82%; (c) i. KHMDS, THF; ii. CH_3I, THF 78% of 96.5% *cis*-isomer; (d) KOH, CH_3OH, H_2O, reflux, 94%; (e) CDI, imidazole, DMAP, CH_2Cl_2, *N*,*O*-dimethylhydroxylamine hydrochloride, 94%; (f) TESCl, imidazole, DMF, 72%; (g) DIBAL-H, THF, −60 °C, 74%.

dioxolane **13** could be purified by crystallization from an ether/pentane or hexanes solution to provide exclusively the *cis*-isomer of **13**. This crystallization−purification was incorporated into the scale-up efforts to produce *cis*-**13** in 82% yield. Dioxolane **13** was deprotonated and stereoselectively alkylated with methyl iodide to give 5-methyldioxolane, **14**, in 78% yield. The *cis*:*trans* ratio of the newly incorporated methyl group (δ 1.36) to the acetal proton (δ 5.13) in the proton NMR was 27:1 (96.5% of the desired *cis*-isomer). This was further confirmed by examination of the carbon NMR spectrum which showed the intensities of two acetal carbons (δ 106.8 and δ 108.7 ppm) from the corresponding *cis*- and *trans*-isomers were also in a 96:4 ratio. The hydrolysis of compound **14** to acid **15**, utilizing potassium hydroxide in a water/methanol mixture, proceeded in excellent yield.[17] The conversion of **15** to its corresponding Weinreb amide **16** proceeded in 94% yield by the sequential addition of 1,1-carbonyldiimidazole, imidazole, *N*,*O*-dimethylhydroxylamine hydrochloride and 4-(dimethylamino)-pyridine in methylene chloride.

Reduction of the unprotected Weinreb amide using controlled amounts of the classical reducing agents under low-temperature conditions ($LiAlH_4$, $Li(tBuO)_3AlH$, $Li(MeO)_3AlH$, $LiBEt_3H$, $Na[(MeOEtO)_2AlH_2]$, and $(i\text{-}Bu)_2AlH$) led to a number of synthetic difficulties (reactions ranging from no reduction to over-reduction to the diol and mixtures of products).[18] Diisobutylaluminum hydride (DIBAL-H) in

(17) Fráter, G.; Müller, U.; Günther, W. *Tetrahedron Lett.* **1981**, *22*, 4221.
(18) Unpublished results, Albany Molecular Research, Inc.

Scheme 4. **Synthesis of the vitamin D phosphine oxide (22)**[a]



[a] Reagents: (a) SO_2, CH_2Cl_2, −78 °C; (b) TBDMSCl, imidazole, CH_2Cl_2; (c) O_3, CH_3OH, NaOAc, AcOH, −25 °C; (d) $NaBH_4$, CH_2Cl_2; (e) PPh_3, imidazole, CH_2Cl_2, I_2; (f) $NaHCO_3$, 95% EtOH, 51% from **18**; (g) i. $LiPPh_2$, THF, −78 °C; ii. 10% H_2O_2, 83%.

a toluene/THF mixture at −60 °C led to the best conversion to the desired α-hydroxyaldehyde. While this α-hydroxyaldehyde was stable at −78 °C, it readily decomposed as it approached ambient temperature. Protection of the 2-hydroxy group of **16** was proposed to both stabilize the aldehyde and eliminate the need for excess base in the Wittig−Horner coupling with the appropriately functionalized and protected vitamin D skeleton. The ideal protecting group would need to be sufficiently robust to survive the coupling sequence, but labile enough for removal once the entire vitamin D_2 skeleton was assembled (preferably under the same conditions as the removal of the protecting groups from the vitamin D portion of the molecule).

In Evans' total synthesis of the macrolide antibiotic cytovaricin,[19] the authors were successful in carrying out a Wittig−Horner coupling on an α-triethylsilyloxyaldehyde. Using this information, **16** was silylated with triethylsilyl chloride[19] to yield the corresponding α-triethylsilyloxyhydroxamide in 72% yield. The reduction of the protected Weinreb amide with diisobutylaluminum hydride in a toluene/THF mixture afforded (*S*)-2,3-dimethyl-2-triethylsilyloxybutyraldehyde (**17**) in 74% yield. The reduction worked best by the slow addition of 2 equiv of DIBAL-H in toluene to a chilled solution of **16** in anhydrous THF at a temperature between −42 and −20 °C. A nonaqueous quench with potassium tartrate afforded the best isolation yield of **17**.

The synthesis of vitamin D phosphine oxide (**22**) is based on the Barton−Hesse strategy[20] in which the iodide **21** was prepared by following modifications to the procedure reported by Manchand and co-workers (Scheme 4).[21] Treatment of ergocalciferol (vitamin D_2, **18**) with SO_2 provided the known, thermally unstable mixture of C-6/C-19 epimeric SO_2 adducts which were then silylated to afford the C-3 protected mixture **19** in quantitative yield. Ozonolysis of **19** was carried out in the reported dual-solvent system of methanol and methylene chloride (1:3) at −25 °C, but the yields were found to be unreliable. Tsuji and Ishikawa reported that the addition of a catalytic amount of a metal halide (0.1 equiv of $CaCl_2$, $MgCl_2$, $AlCl_3$, $ZnCl_2$, or $TiCl_4$) to an ozonolysis reaction allowed the reaction to be run between −10 and 3 °C.[22] They speculated as to the possible reasons that these additives acted as an ozonolysis stabilizer. In our case, **19** (or the ozonolysis products derived from **19**) was not particularly stable in the presence of any of the additives utilized by Tsuji and Ishikawa. We found that the addition of 1 equiv each of sodium acetate and acetic acid to the reaction mixture resulted in a more consistent reaction yield. We do not know if this result is due to the stabilizing salt effect reported by Tsuji and Ishikawa or to the sodium acetate/acetic acid pH buffering effect, but this modification has worked in other ozonolysis reactions of pH-sensitive materials.[18] Reductive workup of the ozonide with sodium

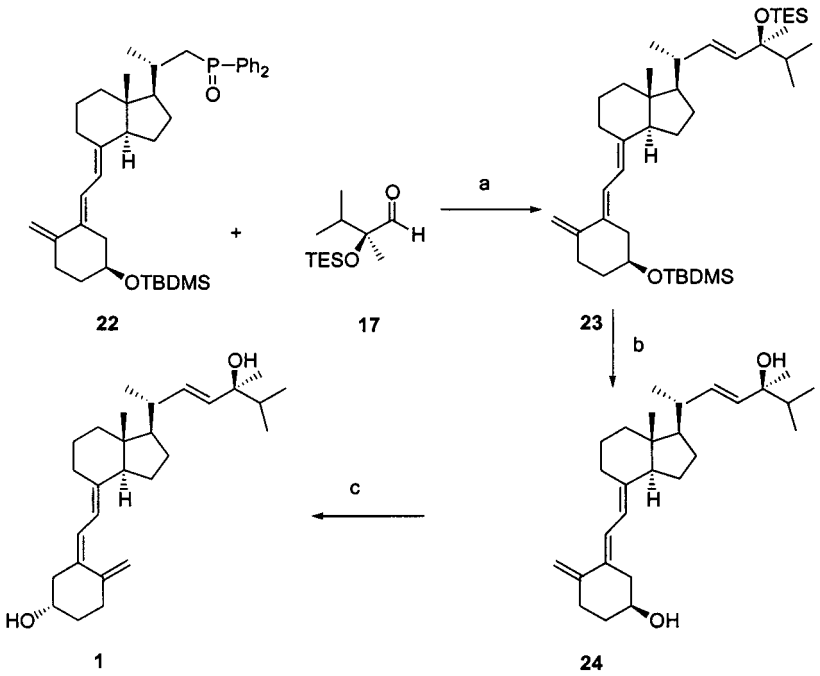
(19) Evans, D. A.; Kaldor, S. W.; Jones, T. K.; Clardy, J.; Stout, T. *J. Am. Chem. Soc.* **1990**, *112*, 7001.

(20) Andrews, D. R.; Barton, D. H. R.; Hesse, R. H.; Pechet, M. M. *J. Org. Chem*. **1986**, *51*, 4819.

(21) Manchand, P. S.; Yiannikouros, G. P.; Belica, P. S.; Madan, P. *J. Org. Chem*. **1995**, *60*, 6574.

(22) Tsuji, K.; Ishikawa, H. *Synth. Commun*. **1997**, *27*, 595.

Scheme 5. **Completion of the synthesis of 24(*S*)-hydroxyvitamin D_2[a]**



[a] Reagents (a) i. *n*-BuLi, THF, −75 °C; ii. *t*-BuOK, THF; (b) TBAF, THF, 31% over 2 steps; (c) i. 9-acetylanthracene, hν, 366 nm, CH_3OH, 80%; ii. recrystallization, methyl formate, 83%.

borohydride gave crude alcohol(s) **20**, which were then iodinated (I_2/PPh_3/imidazole). The diastereomeric mixture of primary iodides was subjected to a thermal SO_2 extrusion, in refluxing 95% ethanol, to yield the C-3 protected vitamin D iodide **21** in 51% yield over six steps. It was found that the thermal retro Diels−Alder reaction proceeded in the highest isolated yield when it was buffered with solid sodium bicarbonate. Iodide **21** was converted to phosphine oxide **22** in 83% yield by treatment with lithium diphenylphosphide, followed by oxidative workup with hydrogen peroxide.[23] During the oxidative workup, the diphenylphosphine intermediate reacted instantaneously with 10% hydrogen peroxide solution. It was extremely important to keep the oxidative workup time to a minimum, since prolonged exposure (e.g., overnight) of **22** to the hydrogen peroxide solution resulted in extensive decomposition of the vitamin D skeleton.

The coupling reaction of the optically active aldehyde **17** and phosphine oxide derivative **22** and the completion of the synthesis are depicted in Scheme 5. The coupling was undertaken with *n*-butyllithium in THF to afford the hydroxyphosphine oxide adduct which was treated with potassium *tert*-butoxide to afford exclusively the desired 22(*E*)-olefin **23**. Initially, the coupling reaction was performed by using 2.0 equiv of **17**, which was not recoverable, for each equivalent of **22**. After a series of small-scale trial reactions, the optimum scale-up conditions were found to be 1.3 equiv of **17** and 2.0 equiv of *n*-butyllithium in anhydrous THF at −75 °C. The crude condensation product was carried into the elimination step without purification. Both hydroxyl groups in **23** were deprotected with tetrabutylammonium fluoride in THF to afford *trans*-24(*S*)-hydroxyvitamin D_2 (**24**) in 31% yield over two steps.[24] HPLC analysis of the isolated **24** indicated that the material was 85.8% of the desired *trans*-isomer and 5.1% of the corresponding *cis*-24(*S*)-hydroxyvitamin D_2 (**1**), which was carried as a minor component from the C-6/C-19 SO_2 retro Diels−Alder reaction. This was confirmed by a detailed examination of the proton NMR spectrum that showed the presence of two isomers of the central triene system.

In the final step, **24** was subjected to photoisomerization to the *cis*-24(*S*)-hydroxyvitamin D_2 (**1**). This triplet-sensitized photoisomerization is well-known for the conversion of *trans*- to *cis*-vitamin D compounds.[25] This reaction was performed in methanol at 5−10 °C, using 9-acetylanthracene as a sensitizer and was irradiated with a 366 nm light source (a 400 W Hanovia lamp through a canary-yellow uranium quartz filter).[21,26] At the beginning of our development work, poor yields (ca. 20%) of **1** were obtained. The observation of polymeric byproducts that settled out of the methanol solution, suggested that the degradation of **1** was due to prolonged photolysis. To circumvent this problem, the photoisomerization of **24** was closely monitored. Proton NMR analysis of the vinyl protons indicated that the reaction achieved the highest percent conversion after 1 h. The crude photolysis reaction mixture was concentrated under reduced pressure and purified by silica gel column chromatography (mainly to remove the sensitizer), affording crude **1** as a light-yellow solid in 80% yield. A crystallization study of **1** was conducted, and methyl formate was found to give the best recovery of high-quality **1**. Recrystallization of the column-

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(24) In the vitamin D nomenclature, *trans*- and *cis*- refer to the configuration of the C5−C6 double bond.

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purified **1** from methyl formate gave pure **1** in 83% recovery. The purity of **1** was determined to be 99.22% by HPLC analysis (area normalization). The undesired diastereomer, 24(*R*)-hydroxyvitamin D_2, was also detected (0.78%). This identification was confirmed by co-injection with a sample of the 24(*R*)- and 24(*S*)-hydroxyvitamin D2 mixture.

Conclusions

This report describes the first stereoselective synthesis of the oxidized vitamin D_2 prodrug, 24(*S*)-hydroxyvitamin D_2 (**1**). Many of the reaction conditions were modified to process larger quantities of material, and several significant process improvements were made. Of particular note are the synthesis of aldehyde derivative **17**, starting from L-(+)-valine and the conditions used for its coupling with a protected vitamin D subunit **22** using the Wittig−Horner coupling reaction. This reaction allowed introduction of the chiral side chain under conditions amenable to larger-scale preparation. While additional process development and optimization of specific steps will be required before further scale-up, the improvements reported herein allowed for the first stereospecific synthesis of **1** from commercial vitamin D_2 and the preparation of materials for further biological testing.

Experimental Section

All nonaqueous reactions were performed under an atmosphere of dry nitrogen. Reagents purchased from commercial sources were used as received, unless noted otherwise. Anhydrous tetrahydrofuran was either purchased or obtained by the distillation in the presence of sodium metal and benzophenone ketyl. Proton and carbon nuclear magnetic resonance spectra were obtained on either a Bruker AC 300 NMR spectrometer at 300 MHz for proton spectra and 75 MHz for carbon spectra, or on a Brucker AMX 500 spectrometer at 500 MHz for proton spectra and 125 MHz for carbon spectra. Tetramethylsilane was used as an internal reference. Infrared spectra were recorded with a Perkin-Elmer Spectrum 1000 spectrophotometer. Mass spectra analyses were performed on a Shimadzu QP-5000 GC−MS (CI). Optical rotations were measured with a Perkin-Elmer 243B Polarimeter in a 1-cm cell. HPLC chiral purities were obtained on a Spectra-Physics HPLC system with a Chiralpak AD column (4.6 mm × 250 mm, Daicel Chemical Industries, Ltd.), with a mobile phase of a 60:40 mixture of hexanes and ethanol containing 0.2% ethylamine at a flow rate of 0.1 mL/min, and UV detection at 254 nm. HPLC chemical purities were obtained on a Spectra-Physics HPLC system with a Waters Nova-Pak C-18, 4 μm column (3.9 mm × 150 mm), with a mobile phase of a 60:40 mixture of acetonitrile and water at a flow rate of 1.0 mL/min, and UV detection at 265 nm. Thin-layer chromatography (TLC) was performed using 1 in. × 3 in. Whatman 60A (0.025 mm thick) silica gel plates. TLC plates were visualized by observation under UV lamp or by staining with saturated ceric ammonium sulfate solution in 50% aqueous sulfuric acid, or with commercial phosphomolybdic acid. Melting points were obtained by differential scanning calorimetry using a Mettler Toledo Star 80 DSC, calibrated on an indium standard. Quantitative Technologies, Inc. of Whitehouse, NJ, performed the elemental analyses.

Preparation of (*S*)-(+)-Hydroxyisovaleric Acid (12). A 12-L, three-neck, round-bottom flask equipped with an overhead stirrer, pressure-equalizing addition funnel, and a thermometer was charged with L-(+)-valine (710 g, 6.1 mol). Water (3 L) was added, affording a white suspension. The stirrer was started, and concentrated sulfuric acid (314 g, 3.2 mol) was added slowly, affording a clear solution. Ice (2 kg) was added to the solution, cooling the reaction mixture to below 5 °C. Cooling was aided by the use of an external ice bath. A solution of sodium nitrite (530 g, 6.2 mol) in water (2 L) was added slowly, while maintaining the reaction temperature below 5 °C. When the addition of sodium nitrite was complete, the stirred solution was allowed to warm to ambient temperature slowly, overnight. The pH of the reaction mixture was adjusted to 3−4 by the slow addition of solid sodium bicarbonate, and this solution was extracted with ethyl acetate (3 × 2 L). The combined organic extracts were dried over magnesium sulfate, clarified, and concentrated. The residue was recrystallized from an ethyl acetate/hexanes (3:1) mixture to afford a 30% yield (212 g) of **12** as a white solid. The mother liquors were concentrated, and the residue was recrystallized from an ethyl acetate/hexanes (3:1) mixture to afford an additional 26% (186 g) of **12**. The total yield of **12** was 56%. $[\alpha]^{20}_D$ +13.5° (*c* 1, $CHCl_3$); IR (KBr) 3425, 2971 and 1711 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 4.16 (d, 1H, J = 3.5 Hz), 2.17 (m, 1H), 1.07 (d, 3H, J = 6.7 Hz), and 0.93 (d, 3H, J = 7.0 Hz) ppm.

Preparation of (2*S*,5*S*)-2-(*tert*-Butyl)-5-isopropyl-1,3-dioxolan-4-one (13). To a 2-L, one-necked, round-bottom flask equipped with a magnetic stirrer, Dean−Stark trap, and reflux condenser was charged **12** (97 g, 0.82 mol) and hexanes (800 mL). The stirrer was started, and trimethylacetaldehyde (100 g, 1.16 mol) and *p*-toluenesulfonic acid (1 g) were added. The reaction mixture was heated under reflux until approximately 15 mL of water was collected. Heating was discontinued, and the colorless solution was allowed to cool to room temperature. The reaction mixture was poured into saturated aqueous sodium bicarbonate solution (400 mL), and the layers were mixed well. The layers were separated, and the aqueous layer was extracted with ethyl acetate (400 mL). The combined organics were dried over anhydrous magnesium sulfate and filtered, and the solvent was removed in vacuo to afford a colorless oil (151 g) which consisted of a 23:1 mixture of diastereomers, by NMR analysis. Crystallization from hexanes afforded an 82% yield (126 g) of **13** as white crystals: $[\alpha]^{24}_D$ −0.84° (*c* 2.9, $CHCl_3$); IR (KBr) 1792 cm^{-1}; ^1H (300 MHz, $CDCl_3$) δ 5.09 (d, 1H, J = 1.2 Hz), 4.09 (dd, 1H, J = 3.7 Hz), 2.19 (m, 1H), 1.11 (d, 3H, J = 7.1 Hz), 1.01 (d, 3H, J = 7.1 Hz), and 0.99 ppm (s, 9H). Anal. Calcd for $C_{10}H_{18}O_3$: C, 64.47; H, 9.76. Found: C, 64.32; H, 9.48.

Preparation of (2*S*,5*R*)-2-(*t*ert-Butyl)-5-methyl-5-isopropyl-1,3-dioxolan-4-one (14). To a 12-L, three-neck, round-bottom flask equipped with an overhead mechanical stirrer, nitrogen bubbler, pressure-equalizing addition funnel, and a low-temperature thermocouple was charged dry THF

(3.5 L). To this was added a commercial solution of potassium hexamethyldisilazide (0.5 M solution in toluene, 1.6 L). The resulting solution was cooled to −78 °C, and a solution of compound **13** (126 g, 0.67 mol) in dry THF (400 mL) was added in one portion. The resulting yellow solution was allowed to stir for 45 min, at which point methyl iodide (139 g, 0.96 mol) was added over 0.5 h. The reaction mixture was allowed to slowly warm to −30 °C over 3.5 h. After this time, the reaction was quenched with saturated aqueous ammonium chloride solution (2 L) and extracted with methyl *tert*-butyl ether (2 × 2 L). The combined organic extracts were dried over anhydrous magnesium sulfate and filtered through a pad of diatomaceous earth. The filtrate was concentrated under reduced pressure to afford the crude product as an orange oil. This oil was dissolved in ethyl acetate (200 mL) and filtered and rinsed through a plug of silica gel (to remove the baseline impurities). The solvent was removed under reduced pressure, providing a clear orange oil (141 g). Crystallization from hexanes afforded a 78% yield (104 g) of **14** as pale yellow crystals: $[\alpha]^{25}_{D}$ +2.2° (*c* 1, $CHCl_3$); IR (neat) 1797 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 5.13 (s, 1H), 2.02 (m, 1H), 1.36 (s, 3H), 1.05 (d, 3H, $J = 6.7$ Hz), 1.00 (d, 3H, $J = 6.3$ Hz), and 0.99 (s, 9H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.5, 106.8, 82.1, 34.0, 23.7, 17.4, 16.8, and 16.0 ppm. Anal. Calcd for $C_{11}H_{21}O_3$: C, 65.62; H, 10.54. Found: C, 65.96; H, 10.27.

Preparation of (*S*)-(+)-2,3-Dimethyl-2-hydroxybutyric Acid (15). To a 1-L, round-bottom flask equipped with a magnetic stir bar and reflux condenser was charged a mixture of **14** (94 g, 0.47 mol), methanol (450 mL), and water (100 mL). The stir bar was started and potassium hydroxide pellets (48 g, 0.85 mol) were added. After most of the solids had dissolved, the reaction was heated to reflux for 30 min. The mixture was cooled to room temperature and concentrated under reduced pressure to afford a milky suspension. This mixture was diluted with water (100 mL), cooled to 0 °C, and acidified with concentrated hydrochloric acid (15 mL) to pH ≈ 6. Water (100 mL) was added and the mixture extracted with ethyl acetate (3 × 400 mL). The combined organic extracts were washed with water (600 mL) and saturated aqueous sodium chloride solution (600 mL) and dried over anhydrous magnesium sulfate and filtered. The filtrate was concentrated under reduced pressure to afford a pale-yellow oil, which on drying in vacuo afforded a 94% yield (59 g) of **15** as a light-yellow crystalline solid: ^1H NMR (300 MHz, $CDCl_3$) δ 1.99 (m, 1H), 1.41 (s, 3H), 0.97 (d, 3H, $J = 7.0$ Hz), and 0.90 (d, 3H, $J = 6.9$ Hz) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 180.4, 76.9, 35.4, 23.3, 17.2, and 15.7 ppm. Anal. Calcd for $C_6H_{12}O_3$: C, 54.52; H, 9.17. Found: C, 54.84; H, 9.48.

Preparation of (2*S*)-(+)-*N*-Methoxy-2,3-dimethyl-2-hydroxybutyramide (16). To a 3-L, two-necked round-bottom flask equipped with a magnetic stir bar, a thermometer, and a nitrogen bubbler was charged compound **15** (59 g, 0.44 mol) and dry methylene chloride (880 mL). The stir bar was started, and the solution was cooled to 0 °C, at which point, 1,1-carbonyldiimidazole (87 g, 0.54 mol) was added in 5-g portions. The yellow solution was gradually warmed to room temperature and stirred under a nitrogen atmosphere overnight. To this reaction mixture was sequentially added imidazole (60 g, 0.88 mol), 4-(dimethylamino)pyridine (1.6 g, 0.01 mol), and *N*,*O*-dimethylhydroxylamine hydrochloride (53 g, 0.54 mol). The solution was stirred overnight, and then the resulting mixture was washed with 2 N aqueous hydrochloric acid (2 × 600 mL), water (800 mL), and saturated aqueous sodium chloride solution (800 mL). The organic layer was dried over anhydrous magnesium sulfate and filtered. The solvent was removed under reduced pressure to provide a 94% yield (73 g) of **16** as a yellow oil: $[\alpha]^{20}_{D}$ −2.9° (*c* 1, $CHCl_3$); IR (neat) 1739, 1637 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 3.71 (s, 3H), 3.28 (s, 3H), 2.03 (m, 1H), 1.42 (s, 3H), 0.99 (d, 3H, $J = 6.8$ Hz), and 0.77 (d, 3H, $J = 6.8$ Hz) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 177.4, 76.8, 60.6, 60.4, 36.0, 33.6, 33.4, 23.1, 17.2, and 15.7 ppm. Anal. Calcd for $C_8H_{17}NO_3$: C, 54.82; H, 9.80; N, 7.99. Found: C, 54.63; H, 9.83; N, 7.92.

Preparation of (2*S*)-(+)-2,3-Dimethyl-2-triethylsilyloxybutyraldehyde (17). To a magnetically stirred solution of **16** (25 g, 0.14 mol) in *N*,*N*-dimethylformamide (400 mL) in a 1-L, round-bottom flask under a nitrogen atmosphere was added imidazole (20 g, 0.29 mol), followed by triethylsilyl chloride (24.1 g, 0.16 mol). The resulting solution was allowed to stir overnight, under a nitrogen atmosphere. The reaction was then diluted with methyl *tert*-butyl ether (800 mL) and washed with water (600 mL). The aqueous layer was extracted with methyl *tert*-butyl ether (2 × 400 mL). The combined organic extracts were washed with saturated aqueous sodium chloride solution (2 × 600 mL), dried over anhydrous magnesium sulfate, and filtered. The filtrate was evaporated under reduced pressure to provide the crude protected **16** as a yellow oil (45 g). Column chromatography (silica gel column, 10% ethyl acetate in hexanes) afforded 29 g (72% yield) of protected **16** as a pale-yellow oil: $[\alpha]^{20}_{D}$ −18.7° (*c* 1, $CHCl_3$); IR (neat) 1664 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 3.68 (s, 3H), 3.35 (s, 3H), 1.39 (s, 3H), 0.85−1.08 (m, 15H), and 0.61 (m, 6H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.5, 81.6, 60.0, 35.7, 35.2, 21.6, 17.0, 16.9, 7.1, 6.7, 6.5, and 5.8 ppm. Anal. Calcd for $C_{14}H_{31}NO_3Si$: C, 58.07; H, 10.81; N, 4.84. Found: C, 58.02; H, 11.14; N 4.27.

To a 5-L, three-neck, round-bottom flask equipped with a mechanical stirrer, thermocouple, pressure-equalizing addition funnel, and nitrogen bubbler was added a solution of the protected **16** (66 g, 0.23 mol) in dry THF (2 L). The stirred reaction mixture was cooled to −60 °C and maintained under an atmosphere of dry nitrogen. Diisobutylaluminum hydride (1.0 M solution in toluene, 460 mL, 0.46 mol) was transferred to the addition funnel and slowly added to the reaction solution over 20 min. The resulting solution was stirred for 3 h until TLC analysis (silica gel plates eluted with 20% ethyl acetate in hexanes) indicated that starting material was no longer present. At this point, potassium tartrate (108 g) was added, the cooling bath was removed, and the resulting white slurry was stirred overnight at ambient temperature. The reaction mixture was evaporated under reduced pressure to approximately one-quarter of the original

volume. The mixture was then diluted with ethyl acetate (1 L), and diatomaceous earth was added. The resulting thick slurry was vacuum-filtered through a silica gel pad. The filtrate was evaporated under reduced pressure to afford crude **17** as a yellow oil (48 g). Silica gel column chromatography (5% ethyl acetate in hexanes) provided a 74% yield of **17** (40 g) as a colorless oil: IR (neat) 2797, 1735 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 9.57 (s, 1H), 1.84 (m, 1H), 1.21 (s, 3H), 0.96 (t, 3H, $J = 7.8$ Hz), 0.91 (d, 3H, $J = 1.2$ Hz), 0.88 (d, 3H, $J = 1.4$ Hz), and 0.61 (q, 6H, $J = 7.8$ Hz) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ 205.6, 82.3, 35.6, 19.5, 16.6, 16.5, 6.8, and 6.6 ppm. Anal. Calcd for $C_{12}H_{26}O_2Si$: C, 62.53; H, 11.39. Found: C, 62.85; H, 11.28.

Preparation of SO_2-Adduct of (3*S*)-*tert*-Butyldimethylsiloxy-9,10-secoergosta-5,7-(*E*),10(19),22(*E*)-tetraene (19). Sulfur dioxide (approximately 300 mL) was condensed at −78 °C into a 2-L, three-neck, round-bottom flask equipped with a dry ice condenser, low-temperature thermocouple, pressure-equalizing addition funnel, and a mechanical stirrer. The stirrer was started, and a solution of ergocalciferol (vitamin D_2, **18**; 198 g, 0.5 mol) in methylene chloride (500 mL) was added in one portion. This produced a bright-yellow reaction mixture, which progressively turned to a red color. The flask was then attached to two sequential gas scrubber systems (using 15 M aqueous sodium hydroxide solution), and the reaction was allowed to gradually warm to −10 °C over 3 h. At this point, the remaining sulfur dioxide and the methylene chloride solvent was removed under reduced pressure to afford the Diels−Alder adduct as a crude, pink foam. This foam was dissolved in fresh methylene chloride (700 mL) and cooled in an ice bath, with stirring, to 5 °C. To this solution was added imidazole (44 g, 0.65 mol), producing an orange solution that was stirred for 15 min, at which time, *tert*-butyldimethylsilyl chloride (98 g, 0.65 mol) was added, producing a milky, yellow suspension. The suspension was gradually warmed to room temperature where it was stirred for 17 h. The reaction was filtered through a pad of diatomaceous earth, and the residue was washed with methylene chloride (2 × 400 mL). The combined filtrates and washings were washed with saturated aqueous sodium chloride solution (2 × 500 mL), dried over anhydrous magnesium sulfate, and filtered. The filtrate was evaporated in vacuo to afford **19** (288 g) as a pale-yellow foam. This material was used in the next step without purification.

Preparation of SO_2-Adduct of (3*S*)-*tert*-Butyldimethylsilyloxy-(20*S*)-hydroxymethyl-9,10-secopregna-5(*Z*),7-(*E*),10(19)-triene (20). To a 12-L, three-neck, round-bottom flask equipped with a gas bubbler, mechanical stirrer, thermocouple, and a reflux condenser was charged a solution of **19** (288 g, 0.5 mol) in methylene chloride (2.9 L) and methanol (1.1 L), sodium acetate (41 g, 0.5 mol), and acetic acid (29 mL, 0.5 mol). The stirrer was started, and the mixture was cooled to −25 °C. Ozone (generated from air, using a Griffin ozone generator) was bubbled through the solution for 4.5 h or until TLC analysis (silica gel plates, 20% ethyl acetate in hexanes) indicated no further change. The reaction mixture was purged with nitrogen for 15 min, and sodium borohydride (69 g, 1.81 mol) was added in 10−15-g portions over 1 h. The resulting mixture was stirred for 1.5 h at room temperature. At this point, 0.5 N aqueous hydrochloric acid solution (2.9 L) was slowly added, and the mixture was extracted with hexanes (3.5 L). The combined organic extracts were washed with saturated aqueous sodium chloride solution (2 × 4 L), dried over anhydrous magnesium sulfate, and filtered. The filtrate was evaporated under reduced pressure to afford 274 g of **20** (a mixture of isomers) as a yellow foam that was used without purification in the next step.

Preparation of (3*S*)-*tert*-Butyldimethylsilyloxy-(20*S*)-iodomethyl-9,10-secopregna-5(*Z*),7(*E*),10(19)-triene (21). A 12-L, three-neck, round-bottom flask equipped with a mechanical stirrer, thermocouple, nitrogen bubbler, and an addition funnel was charged with imidazole (204 g, 2.99 mol), triphenylphosphine (300 g, 1.14 mol), and methylene chloride (2.5 L). The resulting solution was cooled to −2 °C, and iodine (290 g, 1.15 mol) was added. The resulting mixture was stirred for 15 min, and a solution of **20** (274 g, 0.50 mol) in methylene chloride (1.3 L) was added over 35 min. The resulting orange mixture was allowed to warm to room temperature where it was stirred for 3 h. The reaction mixture was filtered, and the filtrate was washed successively with 2% aqueous sodium sulfite solution (2 L), 0.1 N aqueous hydrochloric acid solution (1.5 L), and saturated aqueous sodium chloride solution (1.5 L). The organic extracts were then dried over anhydrous magnesium sulfate and filtered. The solvent was removed under reduced pressure to afford a yellow semisolid that was dissolved in methyl *tert*-butyl ether (4 L) producing a white precipitate (triphenylphosphine oxide). The solution was filtered, and the filtrate was evaporated under reduced pressure to afford 320 g of the SO_2-protected iodide as a yellow oil contaminated with triphenylphosphine oxide. This material was used in the next step without purification.

A 12-L, three-neck flask equipped with a reflux condenser, mechanical stirrer, and a stopper was charged with the SO_2-protected iodide (308 g), sodium bicarbonate (309 g, 3.7 mol), and 95% ethanol (5 L). The resulting suspension was heated under reflux for 2 h or until TLC analysis (silica gel plates, 2% ethyl acetate in hexanes) indicated that no starting material remained. The reaction mixture was cooled to room temperature and filtered, and the filtrate was concentrated under reduced pressure. The crude residue was dissolved in methyl *tert*-butyl ether (3 L) and washed with water (5 L). The aqueous layer was back-extracted with methyl *tert*-butyl ether (3 L). The combined extracts were dried over anhydrous magnesium sulfate and filtered through a pad of diatomaceous earth. The filtrate was evaporated under reduced pressure to afford crude **21** (277 g) as a yellow foam. Silica gel column chromatography (1% ethyl acetate in hexanes) provided compound **21** as a white solid (93.7 g). The impure fractions were combined and rechromatographed to afford an additional crop of iodide **21** (46 g). A total of 139.7 g of **21** was obtained. The overall yield for the conversion of **18** to **21** was 51%. ^1H NMR (300 MHz, $CDCl_3$) δ 6.45 (d, 1H, $J = 11.5$ Hz), 5.82 (d, 1H, $J = 11.5$ Hz), 4.92 (s, 1H), 4.65 (s, 1H), 3.85 (br s, 1H), 3.35 (dd,

1H, $J = 7.0$, 3.0 Hz), 3.19 (dd, 1H, $J = 7.0$, 3.0 Hz), 1.04 (d, 3H, $J = 6.5$ Hz), 0.59 (s, 3H), 0.85 (s, 9H), and 0.05 (s, 6H) ppm. Compound **21** was photolytically unstable and was carried on directly to the next step.

Preparation of (3*S*)-*tert*-Butyldimethylsilyloxy-(20*S*)-(diphenylphosphonium)-9,10-secopregna-5(*Z*),7(*E*),10(19)-triene (22). To a 5-L, three-neck, round-bottom flask equipped with a mechanical stirrer, thermocouple, nitrogen bubbler, and two pressure-equalizing addition funnels was charged diphenylphosphine (41 g, 0.22 mol) and dry THF (570 mL). The stirrer was started, and the solution was cooled to −78 °C. To one addition funnel was charged *n*-butyllithium (2.5 M solution in hexanes, 90 mL, 0.23 mol), and this was slowly added to the cooled solution, producing a reddish-orange mixture that was stirred at −78 °C for 45 min. A solution of compound **21** (94 g, 0.17 mol) in dry THF (570 mL) was transferred to the second addition funnel, and this solution was added to the reaction mixture over 20 min. The resulting pale-yellow solution was stirred for 45 min at −78 °C and then gradually warmed to ambient temperature where it was stirred for 3 h. The reaction mixture was diluted with methyl *tert*-butyl ether (4 L) and washed with saturated aqueous ammonium chloride solution (2 L). The organic layer was gently washed with 10% hydrogen peroxide solution (3 × 1 L). The organic layer was then washed with saturated aqueous sodium chloride solution (2 × 1.5 L), dried over anhydrous magnesium sulfate, and clarified. The yellow filtrate was evaporated under reduced pressure to afford the crude product as a yellow oil (140 g). Silica gel column chromatography (5% ethyl acetate in hexanes) provided an 83% yield of compound **22** (88 g) as a yellow oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.76 (m, 4H), 7.48 (m, 6H), 6.45 (d, 1H, $J = 12.5$ Hz), 5.82 (d, 1H, $J = 12.3$ Hz), 4.92 (s, 1H), 4.64 (s, 1H), 3.84 (m, 1H), 2.84 (dd, 1H, $J = 4.1$, 12.7 Hz), 2.63 (dd, 1H, $J = 3.7$, 12.4 Hz), 2.43 (br m, 3H), 2.30−1.78 (b m, 9H), 1.75−1.40 (br m, 9H), 1.15−1.10 (m, 4H), 0.89 (s, 9H), 0.48 (s, 3H), and 0.15 (s, 6H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 149.9, 136.5, 131.4, 130.9, 130.8, 130.6, 130.5, 128.6, 128.4, 121.2, 119.9, 118.1, 116.3, 112.0, 107.6, 70.5, 69.4, 58.2, 58.1, 56.5, 56.4, 46.8, 46.0, 40.4, 37.6, 36.4, 35.5, 35.2, 32.7, 32.1, 31.6, 31.2, 28.9, 27.9, 26.0, 25.9, 23.4, 22.6, 22.1, 21.3, 18.1, and 11.9 ppm.

Preparation of the Bis-silyl-protected *trans*-24(*S*)-Hydroxyvitamin D_2 (23). To a 3-L, three-neck, round-bottom flask equipped with a mechanical stirrer, thermocouple, addition funnel, and nitrogen bubbler was charged a solution of compound **22** (47.1 g, 74.9 mmol) in dry THF (700 mL). The solution was cooled to −75 °C, and *n*-butyllithium (2.5 M solution in hexanes, 60 mL, 150 mmol) was added, producing a red solution that was stirred for 45 min. At this point, a solution of compound **17** (22.3 g, 96.8 mmol) in THF (100 mL) was added over 20 min. This solution was stirred at −75 °C for 1 h, producing a yellow solution. The solution was warmed to 0 °C over 1.5 h at which point ethyl acetate (800 mL) was added. The reaction solution was washed with saturated aqueous ammonium chloride solution (800 mL), water (800 mL), and saturated aqueous sodium chloride solution (800 mL). The organic layer was dried over anhydrous magnesium sulfate and clarified, and the solvents were evaporated under reduced pressure to afford a yellow oil (72 g). This oil was dissolved in dry THF (1.3 L) and transferred to a dry, 3-L, three-neck, round-bottom flask equipped with a mechanical stirrer, thermocouple, nitrogen bubbler, and a rubber septum. The reaction was cooled to −12 °C, and solid potassium *t*-butoxide (70 g, 62.4 mmol) was added, producing an orange reaction mixture. The reaction mixture was allowed to stir at this temperature for 2.5 h at which point it was diluted with ethyl acetate (1.4 L) and washed successively with 0.01 N aqueous hydrochloric acid solution (2 × 1 L), water (1 L), and saturated aqueous sodium chloride solution (1 L). The organic extracts were dried over anhydrous magnesium sulfate and clarified, and the solvent was evaporated under reduced pressure, affording 58 g of crude compound **23** as a yellow oil. This oil was used directly in the next step without purification.

Preparation of *trans*-24(*S*)-Hydroxyvitamin D_2 (24). To a 3-L, three-neck, round-bottom flask equipped with a mechanical stirrer, a rubber septum, and an addition funnel was charged a solution of compound **23** (48 g, 74.0 mmol) in dry THF (1 L). The stirrer was started, and the solution was cooled to 0 °C at which point tetrabutylammonium fluoride (1.0 M solution in THF, 500 mL, 500 mmol) was slowly added. The resulting dark-colored solution stirred at 0 °C for 1 h and slowly warmed to ambient temperature where it was stirred for 48 h. The reaction mixture was diluted with water (1.5 L) and extracted with ethyl acetate (2 × 1 L). The combined organic extracts were washed with 0.01 N aqueous hydrochloric acid solution (1 L) and saturated aqueous sodium chloride solution (2 × 1.5 L), dried over anhydrous magnesium sulfate, and clarified. The filtrate was concentrated under reduced pressure to afford crude **24** as an orange oil (58.5 g). Silica gel column chromatography (20% ethyl acetate in hexanes) afforded **24** as a white foam in an overall yield of 31% from **22** (9.5 g). By HPLC analysis, this material consisted of 5.1% of the *cis*-isomer and 85.8% of the *trans*-isomer ($t_R = 19.4$ and 21.4 min, respectively) and 9.1% of related minor impurities. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.55 (major, *trans*-isomer, d, 1H, $J = 12.1$ Hz), 6.23 (minor, *cis*-isomer, d, 1H, $J = 11.3$ Hz), 6.03 (minor, *cis*-isomer, d, 1H, $J = 11.2$ Hz), 5.87 (major, *trans*-isomer, d, 1H, $J = 12.4$ Hz), 5.45 (d, 1H, $J = 4.8$ Hz), 5.44 (d, 1H, $J = 2.7$ Hz), 5.04 (minor, *cis*-isomer, d, 1H, $J = 2.0$), 4.96 (major, *trans*-isomer, d, 1H, $J = 2.0$ Hz), 4.81 (minor, *cis*-isomer, d, 1H, $J = 2.3$), 4.69 (major, *trans*-isomer, d, 1H, $J = 2.4$ Hz), 3.90 (m, 1H), 2.88 (dd, 1H, $J = 4.7$, 12.7 Hz), 2.40−2.55 (m, 1H), 1.92−2.30 (m, 6H), 1.45−1.80 (m, 8H), 1.22−1.44 (m, 6H), 1.21 (s, 3H), 1.03 (d, 3H, $J = 6.7$ Hz), 0.91 (d, 3H, $J = 4.0$ Hz), and 0.60 ppm (s, 3H); CI MS *m*/*z* 412 $[M]^+$.

Preparation of 24(*S*)-Hydroxyvitamin D_2 (1). To a 4-L, water-jacketed, Pyrex photoreactor was charged a solution of compound **24** (9.5 g, 23 mmol) and 9-acetylanthracene (1.2 g, 6 mmol) in methanol (4 L). The stirrer was started, and the resulting solution was cooled to 7 °C and purged with nitrogen for 1.5 h. It was then irradiated using a 400

W Hanovia Lamp through a canary-yellow uranium quartz filter for 1 h. An aliquot (20 mL) was removed and concentrated to dryness under reduced pressure. The ^{1}H NMR spectrum of the crude residue showed the isomerization to be complete. The remainder of the solvent was then concentrated to dryness to afford the crude **1** as a yellow oil (12.1 g). A second photoisomerization reaction was carried out using the same protocol on a second batch of **24** (7.2 g, 17.4 mmol) and 9-acetylanthracene (1.5 g, 7 mmol) in methanol (4 L) to afford additional crude **1** as a yellow oil (8.1 g). Silica gel column chromatography on the combined lots (20% ethyl acetate in hexanes) afforded **1** (13.3 g, 80% yield from **24**) as a white solid. Recrystallization from methyl formate afforded 9.9 g of **1** as white crystals. A second crop of crystals, obtained on concentration of the mother liquor, afforded an additional 1.1 g of **1** for an 83% recovery from chromatographed **1** (11.0 g): mp 135−137 °C (DSC); $[\alpha]^{24}_D$ +123.7° (*c* 1.0, EtOH); IR (KBr) 3422, 2940, 2873, 1458 and 1371 cm^{-1}; UV λ_{max} 264.5, 213.0 nm; ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.23 (d, 1H, J = 11.3 Hz), 6.03 (d, 1H, J = 11.2 Hz), 5.45 (d, 1H, J = 4.8 Hz), 5.44 (d, 1H, J = 2.7 Hz), 5.04 (d, 1H, J = 2.0 Hz), 4.81 (d, 1H, J = 2.3 Hz), 3.95 (m, 1H), 2.82 (dd, 1H, J = 4.5, 12.7 Hz), 2.57 (dd, 1H, J = 3.6, 13.1 Hz), 1.90−2.41 (m, 8H), 1.44−1.94 (m, 12H), 1.22−1.39 (m, 2H), 1.21 (s, 3H), 1.03 (d, 3H, J = 6.7 Hz), 0.89 (d, 6H, J = 3.3 Hz), and 0.56 (s, 3H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 145.3, 142.1, 135.4, 134.9, 133.4, 122.5, 117.8, 112.5, 75.1, 69.3, 56.5, 56.4, 46.1, 46.0, 40.6, 40.4, 38.3, 35.3, 32.1, 29.1, 28.1, 25.2, 23.7, 22.4, 21.1, 17.7, 17.4, and 12.4 ppm; CI MS m/z 412 $[M]^+$. By HPLC analysis, this material was 99.22% of 24(*S*)-hydroxyvitamin D_2 and 0.78% of 24(*R*)-hydroxyvitamin D_2. Anal. Calcd for $C_{28}H_{44}O_2$: C, 81.50; H, 10.75. Found: C, 81.53; H, 10.62.

Acknowledgment

We thank Dr. William Boulanger for helpful discussions and assistance with the photoreactor system and Drs. Farahnaz Mohammadi and Donald Kuhla for assistance with preparation of this manuscript. We thank Bone Care International, Inc. for permission to publish this unit of work.

Received for review September 13, 2001.

OP010229E