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Synthesis of 9-Alkylated Calcitriol and Two 1α,25-Dihydroxy-9-methylene-10,19-dihydrovitamin D_3 Analogues with a Non-natural Triene System by Thermal Sigmatropic Rearrangements

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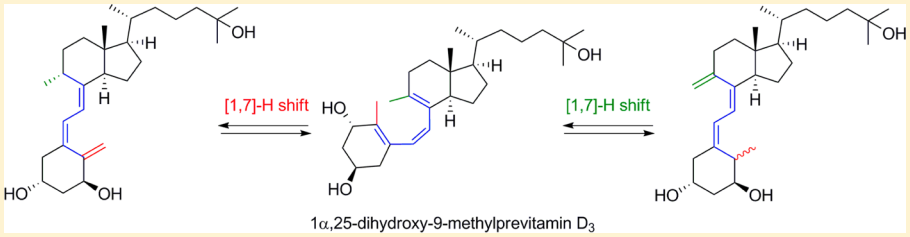
Supporting Information



ABSTRACT: 1α,25-$(OH)_2$-9α-Methylvitamin D_3 (**4**), the first known analogue of the natural hormone 1α,25-$(OH)_2D_3$ (**3**) with an alkyl substituent at C-9, and two 1α,25-$(OH)_2$-9-methylene-10,19-dihydrovitamin D_3 analogues (**7** and **8**) with an unprecedented non-natural triene system were synthesized by thermal isomerization of 1α,25-$(OH)_2$-9-methylprevitamin D_3 (**6**). Three alternative approaches (Sonogashira, Stille, or stereoselective dehydration of a tertiary propargyl alcohol) have been successfully used to construct the dienyne precursors of previtamin **6** possessing two methyl groups capable of participating in the [1,7]-sigmatropic hydrogen shift.

INTRODUCTION

1α,25-Dihydroxyvitamin D_3 [calcitriol, 1α,25-$(OH)_2D_3$ (**3**); Figure 1] is the most potent vitamin D_3 (**2**) metabolite, a hormonal form responsible for calcium and phosphorus homeostasis.[1] Its genomic actions are mediated through the vitamin D receptor (VDR),[2,3] a member of the nuclear receptor superfamily,[4] whose established presence in more than 30 tissues[5] indicates that 1α,25-$(OH)_2D_3$ may also be involved in a broad array of physiological processes not yet known. A metabolic precursor of **2** is isomeric previtamin D_3 (**1**), which in turn is produced in the skin by UV irradiation of 7-dehydrocholesterol.[6] For a conversion of previtamin D_3 to vitamin D_3, the temperature of the human body is sufficient; at room temperature an equilibrium **1** ⇆ **2** (8:92) is reached after few days ($t_{1/2}$ = 70 h).[7] This thermally induced process of crucial biological importance represents an elegant example of [1,7]-sigmatropic hydrogen shift defined by Woodward and Hoffmann.[8] Kinetic and thermodynamic aspects of such pericyclic process have attracted chemists' attention for more than a half of century.[7,9] The intramolecular nature of this hydrogen shift was established long ago[10] and supported by studies involving 19-substituted vitamin D analogues.[11] Antarafacial stereochemistry of the thermal [1,7]-hydrogen shift, as predicted by Woodward–Hoffmann rules,[8,12] was demonstrated by the Okamura group in their studies on *cis*-isotachysterol analogues.[13] To achieve this goal, the authors designed the conjugated 1-hydroxy-9,10-*seco*-cholesta-5-(10),6,8(14)-trienes labeled with deuterium at the C-15 position.

The discovery of calcitriol and its role in living organisms, extending far beyond the classical regulation of calcium and phosphorus levels, stimulated studies directed to the synthesis of its analogues with modified biological activity.[14] Among more than 3000 such compounds synthesized to date, only very few of them were characterized by substitution at C-9. Thus, in 1991, Mouriño and co-workers reported the preparation of the 9α-hydroxy analogue of calcitriol with a different configuration of the intercyclic diene moiety,[15] and in the more recent Japanese patent, several 9α-alkylated 19-norvitamin D compounds were described.[16] Therefore, as an extension of the structure–activity studies carried out in our laboratories, we designed new homologues of calcitriol methylated at C-9 (**4** and **5**). Their precursor, previtamin D compound **6**, would possess a conjugated hexatriene moiety, symmetrically substituted with two methyl groups at the terminal carbons C-9 and C-10. Examination of the thermal rearrangement of such an unsaturated system seemed to be an attractive endeavor because two competitive [1,7]-hydrogen shifts were envisioned, occurring from both methyl substituents located at the terminal carbons of the hexatriene moiety. The first process, involving methyl located at C-10, would result in the formation of the

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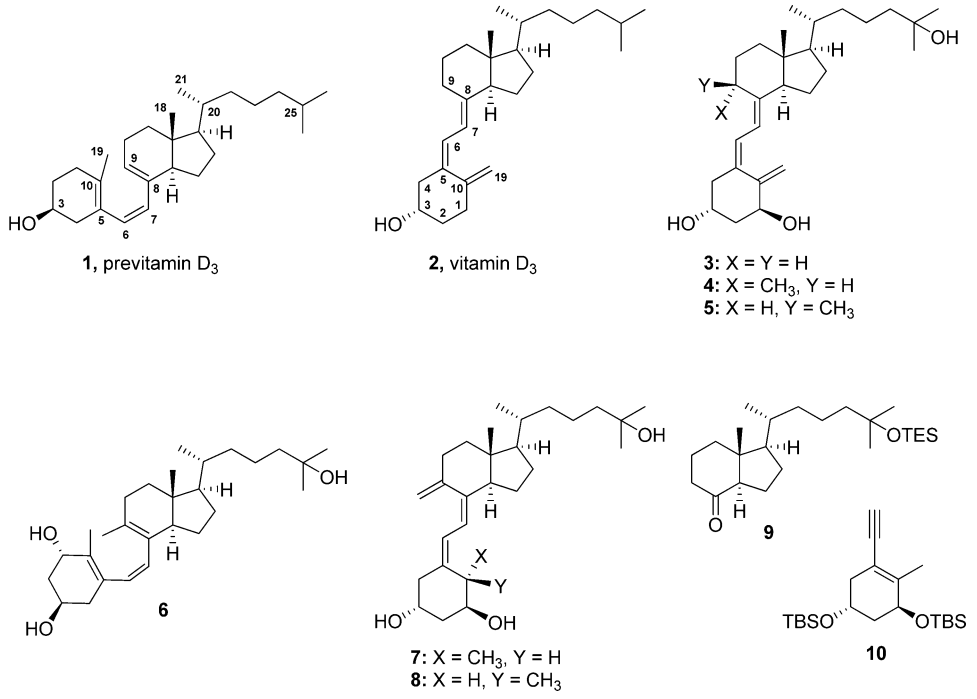
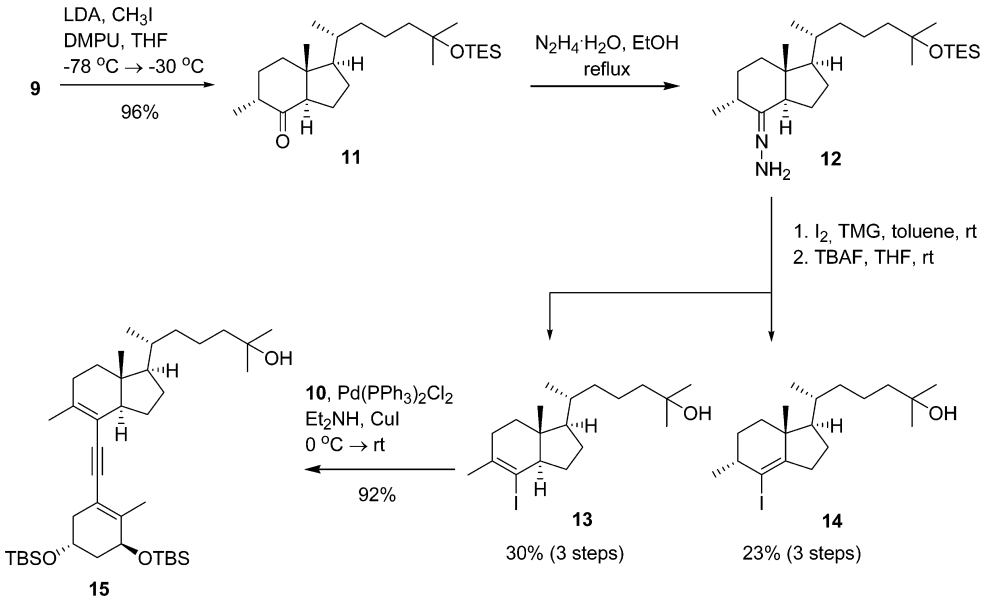


Figure 1. Structures of the 1α,25-$(OH)_2D_3$, its metabolic precursors, the synthesized vitamin D_3 analogues, and their synthetic precursors.

Scheme 1. Synthesis of Dienyne 15 via Sonogashira Coupling



target 9-methyl calcitriol analogues **4** and **5** whereas the alternative sigmatropic shift of hydrogen from the methyl attached to C-9 would produce 9-methylene-10,19-dihydrovitamins **7** and **8**. These latter compounds would be characterized by a unique triene system.

RESULTS AND DISCUSSION

We have chosen two known compounds, namely the protected 25-hydroxy Grundmann ketone **9**[17]and the enyne **10**,[18] as suitable substrates for the synthesis of the analogues **4**, **5**, **7**, and **8**. Since a strategy of our synthesis involved Sonogashira coupling, we first transformed the starting hydrindanone **9** into vinyl iodide **13** (Scheme 1). α-Methylation of **9** was achieved in almost quantitative yield by its treatment with LDA in the presence of DMPU, followed by addition of MeI.[16] The stereochemistry of the methylated ketone **11** was easily determined by analysis of its ^{1}H NMR spectrum and molecular modeling (PCModel, v9.0, Serena Software). Spin decoupling experiment performed with the multiplet derived from the methine proton at C-9 (δ 2.43) allowed for measuring vicinal coupling constants (6.3 and 2.1 Hz) indicating its equatorial disposition (the calculated values for **11**: $J_{9\beta,11\beta}$ = 6.8 Hz, $J_{9\beta,11\alpha}$ = 1.2 Hz) and, consequently, axial α-orientation of the introduced methyl group.

The following conversion of the ketone **11** into vinyl iodide was performed by application of the Barton's procedure.[19] The intermediate hydrazones were reacted with iodine, and the formed isomeric vinyl iodides, after hydroxyl deprotection, were

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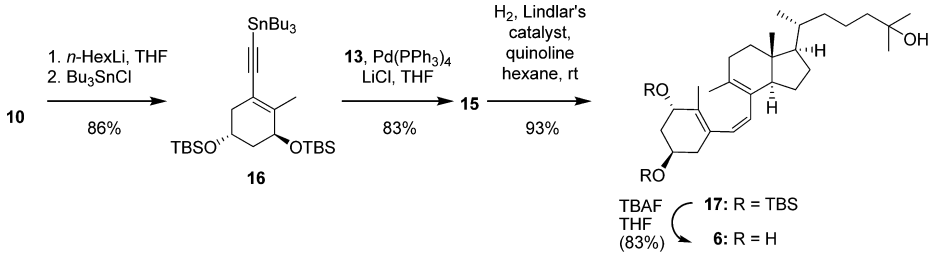
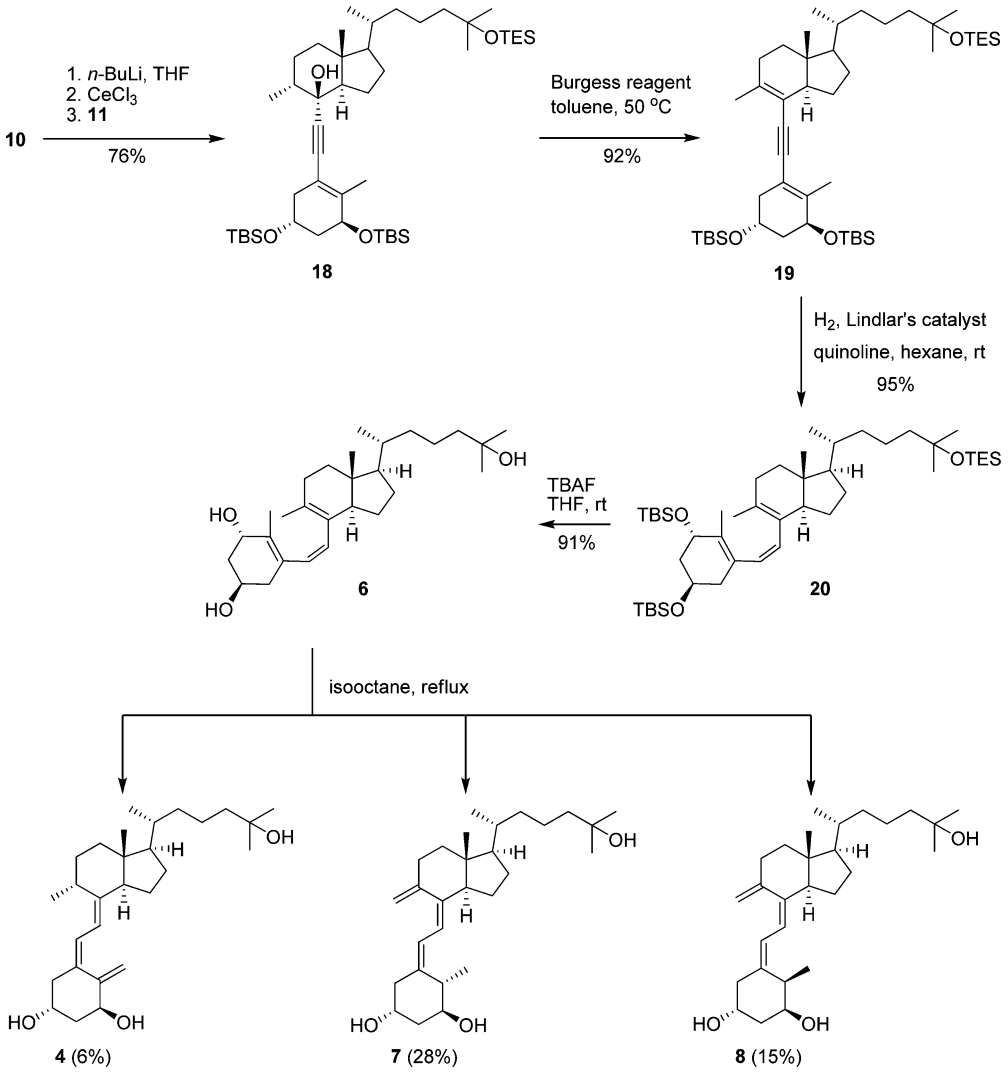
Scheme 2. Synthesis of 1α,25-Dihydroxy-9-methylprevitamin D_3 (6) via Stille Coupling



Scheme 3. Synthesis of 1α,25-Dihydroxy-9-methylprevitamin D_3 (6) via Acetylide Addition to Hydrindanone and Its Thermal Isomerization Process



effectively separated by reversed-phase HPLC. The desired prevailing isomer **13** was then coupled to A-ring synthon **10** in the presence of bis[triphenylphosphine]palladium(II) chloride-copper(I) iodide catalyst and diethylamine.[20] The dienyne **15** was obtained in excellent yield using a 5-fold molar excess of the starting enyne **10**.[21] We also explored an alternative approach[22] to dienyne **15** to avoid the use of an excess of A-ring fragment. Thus, treatment of tributyltin derivative **16** (Scheme 2), prepared from enyne **10**, with an equimolar amount of the iodide **13** provided dienyne **15** in good yield. The resulting dienyne **15** was subsequently semihydrogenated in the presence of Lindlar catalyst and quinoline to afford the triene **17** which was deprotected to previtamin D **6**. The UV spectrum of the latter compound shows an absorption maximum at 248 nm, that differs considerably from those of previtamins unsubstituted at C-9 (λ_{max} 261 nm). The observed blue shift in the UV spectrum can be attributed, as in the case of 6-methylprevitamin D_3 described by Sheves and Mazur,[23] to the increased deviation from planarity of the hexatriene chromophore, caused by steric interaction of its additional alkyl substituent. Interestingly, the UV spectrum of **6** is similar to that reported for the 6-methylated analogue (λ_{max} 247 nm).[23]

In an attempt to improve the yield of previtamin D compound **6**, a new synthetic method was developed. Thus,

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the methylated Grundmann ketone **11** was treated with an anion of the enyne **10** to afford the enynol **18** (Scheme 3), which underwent *syn*-elimination upon reaction with the Burgess reagent[24] to provide the desired dienyne **19** in high yield. The stereochemical outcome of this process confirmed an attack of an acetylide anion on ketone **11** from the α-side despite the presence of 9α-methyl substituent. The observed chemical shift of the angular methyl group at C-13 (δ 0.93) in **18** is similar to that observed in the analogous 8β-hydroxy-*des*-A,B-steroid.[25] Compound **19** was then successfully converted to the desired previtamin D **6** using the method described above.

With the 9-methylprevitamin D compound **6** in hand, we proceeded to study its thermal sigmatropic behavior. The thermal isomerization was carried out in refluxing isooctane, and its progress was carefully monitored by HPLC. The equilibrium state was reached after approximately 14 h when no further significant change in the product composition was observed. HPLC separation provided the starting previtamin **6** (40%) and three isomeric products **4**, **7**, and **8** in 6%, 28%, and 15% yield, respectively. The C(9)-epimer of **4**, another theoretically possible reaction product, was not found in the equilibrium mixture. This observation was not surprising, because the presence of equatorial 9β-methyl substituent in the isomeric compound **5** would result in its strong $A^{(1,3)}$-strain interaction with the vinyl hydrogen at C-6.[26,27] HPLC monitoring of the thermal reaction indicated that isomer **7** formed faster than **8**, and after prolonged heating, this compound was still the prevailing product. Thus, hydrogen from the 9-methyl group migrates to C-10 preferentially in a *syn* manner to the 1α-hydroxyl group. A similar *syn*-directing effect was observed by Okamura during the studies on thermal rearrangement of isotachysterol derivatives.[13] Not unexpectedly, the UV spectra of compounds **7** and **8**, possessing "the rotated" triene chromophore, were almost identical to the respective spectra of the vitamin D hormone **3** and its currently synthesized 9α-methylated homologue **4**.

The structures of the synthesized compounds **4**, **7**, and **8** were tentatively assigned on the basis of ^{1}H NMR data. In the case of vitamin **4**, multiplicity of the H-9 signal at 3.07 ppm (approx quintet, $J \approx 7$ Hz) and strong NOE between this proton and vinylic H-6 (δ 6.38) confirmed its equatorial β-orientation and, consequently, the presence of the 9α-methyl group. The proton coupling network in isomeric products **7** and **8** supported the expected preponderance of A-ring chair conformers in which 10-methyl substituents must be axially oriented in order to avoid strong allylic interactions.[26,27] Therefore, an axial disposition of H-4β in **7** (δ 2.31), and H-1β in **8** (δ 4.05), deduced from their vicinal coupling constants, was sufficient to establish configurations at C-10 in these epimeric compounds; comparison with the spectra of related 10,19-dihydrovitamins[28] also supported this assignment.

The biological evaluation of the new synthesized compounds is underway. The results of preliminary in vitro testing reveal that a presence of the 9α-methyl group results in significantly reduced VDR binding activity and HL-60 differentiation potency of homologue **4** in comparison to 1α,25-$(OH)_2D_3$.

CONCLUSIONS

We designed and synthesized the first 9-alkylated derivative of calcitriol (**4**) and two 1α,25-dihydroxy-9-methylene-10,19-dihydrovitamin D_3 analogues (**7** and **8**) possessing an unprecedented non-natural triene system. A key feature of the synthesis involves the generation of the natural and non-natural vitamin D triene moieties from a common previtamin D compound by thermal sigmatropic [1,7]-H shifts. The required previtamin D **6** was synthesized through three alternative approaches: Sonogashira, Stille, and stereoselective dehydration of a propargylic alcohol by Burgess reagent.

EXPERIMENTAL SECTION

(1*R*,3a*R*,5*R*,7a*R*)-1-[(*R*)-1′,5′-Dimethyl-5′-[(triethylsilyl)oxy]-hexyl]-5,7a-dimethyloctahydroinden-4-one (11). A solution of ketone **9** (400 mg, 1.01 mmol) in anhydrous THF (5 mL) was added dropwise to a solution of LDA (2.0 M in THF/heptane/ethylbenzene; 1.01 mL, 2.02 mmol) in THF (3 mL) under argon at −78 °C, followed by addition of DMPU (95 μL, 0.8 μmol). The solution was warmed to −30 °C and stirred at this temperature for 1 h. Then the resulting mixture was cooled to −78 °C, and methyl iodide (4.05 mmol, 0.25 mL) was added. The reaction mixture was allowed to reach −30 °C over 1 h, stirred at this temperature for 3 h, and then quenched by addition of water. The product was extracted with ether, and the combined organic extracts were washed with brine, dried ($MgSO_4$), and evaporated in rotary evaporator. The remaining oily residue was purified by flash chromatography. Elution with hexane/Et_2O (98:2) gave the methylated ketone **11** (396 mg, 96%) as a colorless oil: $[\alpha]^{24}_D$ −29.3 (*c* 2.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.562 (6H, br q, J = 8.0 Hz, 3 × $SiCH_2$), 0.650 (3H, s, CH_3-7a), ca. 0.93 (3H, d, $J \approx$ 6.5 Hz, CH_3-1′), 0.945 (9H, t, J = 8.0 Hz, 3 × $SiCH_2CH_3$), 1.183 (3H, d, J = 7.2 Hz, CH_3-5), 1.189 (6H, s, CH_3-5′ and H_3-6′), 2.43 (1H, m, H-5), 2.66 (1H, dd, J = 11.0, 7.6 Hz, H-3a); ^{13}C NMR (125 MHz, $CDCl_3$) δ 7.0, 7.3, 13.3, 18.7, 18.8, 19.2, 20.9, 27.8, 30.0, 30.2, 30.5, 35.3, 35.7, 36.4, 44.0, 45.6, 49.8, 56.9, 57.5, 73.6, 216.3; HRMS (ESI) exact mass calcd for $C_{25}H_{48}O_2SiNa$ $(M + Na)^+$ 431.3321, found 431.3346.

(*R*)-6-[(1′*R*,3a′*R*,7a′*R*)-4-Iodo-5′,7a′-dimethyl-2′,3′,3a′,6′,7′,7a′-hexahydro-1*H*-inden-1-yl)-2-methylheptan-2-ol (13) and (*R*)-6-[(1′*R*,5′*R*,7a′*R*)-4-Iodo-5′,7a′-dimethyl-2′,3′,5′,6′,7′,7a′-hexahydro-1*H*-inden-1-yl)-2-methylheptan-2-ol (14). A solution of ketone **11** (300 mg, 0.73 mmol) in EtOH (1 mL) was added to a solution of hydrazine (80% in water; 40 μL, 0.81 mmol) in EtOH (0.2 mL). The reaction mixture was refluxed for 2 h, and then the solvent was evaporated under vacuum. The residue was dissolved in ether, and the resulting solution was washed with water, dried ($MgSO_4$), and concentrated. The oily product **12** was dried in vacuo for 2 h and then dissolved in toluene (5 mL). To this solution was added tetramethylguanidine (0.32 mL, 2.57 mmol), followed by a solution of iodine (196 mg, 1.54 mmol) in toluene (7 mL). The mixture was stirred at room temperature for 2 h and then quenched by dropwise addition of 5% aq HCl, poured into saturated $Na_2S_2O_3$ solution, and extracted with ether. The combined organic extracts were dried ($MgSO_4$) and concentrated to give a residue which was applied on a silica Sep-Pak (5 g) and eluted with hexane/Et_2O (95:5). The crude product (200 mg) was dissolved in anhydrous THF (10 mL), and TBAF (1.0 M in THF; 0.70 mL, 0.70 mmol) was added at room temperature. The reaction mixture was stirred overnight and quenched by addition of brine, extracted with ether, dried ($MgSO_4$), and concentrated. The residue was applied on a silica Sep-Pak (5 g) and eluted with hexane/Et_2O (98:2) to give a mixture of isomeric iodides **13** and **14**. Final separation of the obtained mixture was achieved by HPLC (9.4 × 250 mm Agilent Eclipse XDB-C18 column, 4 mL/min) using a methanol/water (93:7) solvent system. The vinyl iodide **14** was collected at Rv 51 mL (68 mg, 23% after three steps) and the isomeric iodide **13** at Rv 56 mL (89 mg, 30% after three steps).

13: $[\alpha]^{24}_D$ −5.2 (*c* 2.0, $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.76 (3H, s, CH_3-7a′), 0.93 (3H, d, J = 6.4 Hz, H_3-7), 1.22 (6H, s, H_3-1 and CH_3-2), 1.81 (3H, d, J = 2.4 Hz, CH_3-5′), 2.22 (1H, m), 2.46 (1H, m); ^{13}C NMR (50 MHz, $CDCl_3$) δ 11.3, 18.8, 20.8, 27.1, 28.2, 29.2, 29.4, 31.3, 32.3, 36.0, 36.3, 36.5, 44.3, 44.4, 55.5, 56.9, 71.1, 100.6, 137.2; HRMS (ESI) exact mass calcd for $C_{19}H_{33}OINa$ $(M + Na)^+$ 427.1474, found 427.1452.

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14: $[\alpha]^{24}_{D}$ +42.3 (*c* 1.7, $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.93 (3H, s, CH_3-7a′), ca. 0.95 (3H, d, $J \approx 6$ Hz, H_3-7), 1.16 (3H, d, J = 7.0 Hz, CH_3-5′), 1.22 (6H, s, H_3-1 and CH_3-2); ^{13}C NMR (50 MHz, $CDCl_3$) δ 18.3, 18.9, 20.8, 21.8, 26.4, 28.0, 29.4, 29.6, 32.6, 35.1, 35.9, 36.1, 40.7, 44.5, 47.9, 57.0, 71.2, 103.9, 153.9; HRMS (ESI) exact mass calcd for $C_{19}H_{33}OINa$ $(M + Na)^+$ 427.1474, found 427.1458.

1α,3β-Bis[(*tert*-butyldimethylsilyl)oxy]-25-hydroxy-9-methyl-9,10-secocholesta-5(10),8-dien-6-yne (15). (a) CuI (6 mg, 0.03 mmol) and $Pd(PPh_3)_2Cl_2$ (6 mg, 0.01 mmol) were added to a solution of the iodide **13** (63 mg, 0.16 mmol) and enyne **10** (237 mg, 0.62 mmol) in dry Et_2NH (7 mL) at 0 °C. The mixture (protected from light) was stirred at 0 °C for 1 h and then at room temperature for 3 h. A saturated solution of NH_4Cl was added, and the mixture was extracted with ether. The combined organic extracts were dried ($MgSO_4$) and concentrated to give an oily residue which was purified by flash chromatography on silica gel. Elution with hexane/Et_2O (95:5) afforded dienyne **15** (94 mg, 92%) as a colorless oil: $[\alpha]^{24}_{D}$ −24.8 (*c* 1.0, $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.07 and 0.10 (6H and 6H, each s, 4 × $SiCH_3$), 0.66 (3H, s, H_3-18), 0.89 and 0.90 (9H and 9H, each s, 2 × Si-*t*-Bu), 0.95 (3H, d, J = 7.4 Hz, H_3-21), 1.22 (6H, s, H_3-26 and H_3-27), 1.87 and 1.92 (3H and 3H, each br s, H_3-19 and CH_3-9), 4.11 (1H, br m, H-3α), 4.20 (1H, narr m, H-1β); ^{13}C NMR (50 MHz, $CDCl_3$) δ −4.8, −4.7, −4.6, −4.3, 11.2, 18.0, 18.2, 18.7, 19.2, 20.8, 21.1, 24.8, 25.8, 25.9, 28.5, 29.2, 29.4, 30.8, 36.2, 36.3, 36.4, 40.0, 41.4, 42.1, 44.4, 50.6, 54.7, 64.3, 70.1, 71.1, 91.7, 92.4, 115.9, 116.7, 139.5, 140.6; HRMS (ESI) exact mass calcd for $C_{40}H_{72}O_3Si_2Na$ $(M + Na)^+$ 679.4918, found 679.4899.

(b) *n*-Hexyllithium (2.3 M in hexane; 0.34 mL, 0.79 mmol) was added slowly to a stirred solution of enyne **10** (200 mg, 0.52 mmol) in THF (4 mL) at −78 °C. After 1 h, freshly distilled tri-*n*-butyltin chloride (0.24 mL, 0.84 mmol) was added. The mixture was stirred at −78 °C for 15 min and then at room temperature for 2 h. The reaction was quenched by addition of water, diluted with ether, and washed with saturated $NaHCO_3$ solution. The organic phase was dried ($MgSO_4$), concentrated, and dried in vacuum. The crude product **16** (260 mg, 0.39 mmol) was dissolved in the solution of the iodide **13** (157 mg, 0.39 mmol) in THF (8 mL). $Pd(PPh_3)_4$ (8 mg, 0.01 mmol) and LiCl (127 mg, 2.99 mmol, dried in vacuum) were added, and the mixture was refluxed in the dark for 3 h, poured into water, and extracted with ether. The organic extracts were dried ($MgSO_4$) and concentrated. The residue was purified by flash chromatography on silica gel. Elution with hexane/ethyl acetate (98:2) gave the dienyne **15** (211 mg, 83%) as a colorless oil.

1α-[(*tert*-Butyldimethylsilyl)oxy]-25-hydroxy-9-methylprevitamin D_3 *tert*-Butyldimethylsilyl Ether (17). Lindlar catalyst (70 mg) and a 0.5% (v/v) solution of quinoline in hexane (0.12 mL) were added to a solution of dienyne **15** (63 mg, 0.096 mmol) in hexane (10 mL). The mixture was stirred under a hydrogen atmosphere for 4.5 h until all starting material disappeared (TLC control). Formation of a slightly less polar product was observed. The reaction mixture was filtered through Celite, and the filtrate was concentrated. The residue was purified by flash chromatography on silica gel. Elution with hexane/Et_2O (95:5) furnished previtamin **17** (51 mg, 93%): $[\alpha]^{24}_{D}$ +2.6 (*c* 1.3, $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.04, 0.05, and 0.10 (3H, 3H and 6H, each s, 4 × $SiCH_3$), 0.69 (3H, s, H_3-18), 0.88 and 0.89 (9H and 9H, each s, 2 × Si-*t*-Bu), 0.95 (3H, d, J = 6.4 Hz, H_3-21), 1.22 (6H, s, H_3-26 and H_3-27), 1.44 and 1.65 (3H and 3H, each br s, CH_3-9 and H_3-19), 4.05 (1H, br m, H-3α), 4.13 (1H, narr m, H-1β), 5.75 and 6.00 (1H and 1H, each d, J = 12.2 Hz, H-7 and H-6); ^{13}C NMR (50 MHz, $CDCl_3$) δ −4.8, −4.7, −4.6, −4.2, 11.7, 17.6, 18.0, 18.2, 18.8, 19.7, 20.9, 24.2, 25.9, 26.0, 28.8, 29.2, 29.3, 31.2, 36.2, 36.4, 36.6, 38.7, 41.8, 42.3, 44.4, 51.7, 54.4, 64.9, 71.1, 71.6, 129.1, 129.1, 129.3, 130.6, 131.0, 131.2; UV (EtOH) λ_{max} = 248 nm (ε = 10400); HRMS (ESI) exact mass calcd for $C_{40}H_{74}O_3Si_2Na$ $(M + Na)^+$ 681.5074, found 681.5093.

1α,25-Dihydroxy-9-methylprevitamin D_3 (6). Tetrabutylammonium fluoride (1.0 M in THF; 2.34 mL, 2.34 mmol) was added to a solution of the protected previtamin D analogue **17** (51 mg, 0.078 mmol) in anhydrous THF (5 mL). The reaction mixture was stirred overnight and quenched by addition of brine, extracted with ethyl acetate, dried ($MgSO_4$), and concentrated. The residue was purified by HPLC (10 mm × 25 cm Phenomenex Luna Silica column, 4 mL/min) using a hexane/2-propanol (8:2) solvent system. The previtamin D compound **6** was collected at Rv 50 mL (28 mg, 83%): $[\alpha]^{24}_{D}$ +3.2 (*c* 0.9, $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.71 (3H, s, H_3-18), 0.95 (3H, d, J = 6.6 Hz, H_3-21), 1.22 (6H, s, H_3-26 and H_3-27), 1.44 and 1.77 (3H and 3H, each br s, CH_3-9 and H_3-19), 4.02 (1H, br m, H-3α), 4.22 (1H, narr m, H-1β), 5.80 and 6.05 (1H and 1H, each d, J = 12.2 Hz, H-7 and H-6); ^{13}C NMR (50 MHz, $CDCl_3$) δ 11.5, 17.0, 18.7, 19.8, 20.8, 24.1, 28.8, 29.2, 29.4, 31.1, 36.1, 36.4, 36.6, 38.0, 40.6, 42.3, 44.4, 51.8, 54.4, 64.3, 70.9, 71.1, 128.6, 129.2, 130.1, 130.8, 130.9, 131.7; UV (EtOH) λ_{max} = 248 nm (ε =10500); HRMS (ESI) exact mass calcd for $C_{28}H_{46}O_3Na$ $(M + Na)^+$ 453.3345, found 453.3344.

1α,3β-Bis[(*tert*-butyldimethylsilyl)oxy]-9α-methyl-25-[(triethylsilyl)oxy]-9,10-secocholest-5(10)-en-6-yn-8β-ol (18). *n*-Butyllithium (1.56 M in hexane; 556 μL, 0.87 mmol) was added dropwise to a stirred solution of enyne **10** (300 mg, 0.79 mmol) in anhydrous THF (4 mL) at −78 °C. After 5 min, $CeCl_3$ (214 mg, 0.87 mmol) was added, and the resulting suspension was stirred at −78 °C for 30 min. Then a solution of ketone **11** (290 mg, 0.71 mmol) in THF (4 mL) was added, and the mixture was stirred for 15 min and warmed to room temperature. The reaction was quenched after 1 h by addition of saturated solution of NH_4Cl. The resulting mixture was extracted with Et_2O, and the combined organic extracts were washed with brine, dried ($MgSO_4$), and concentrated. The residue was purified by flash chromatography on silica gel. Elution with hexane/Et_2O (98:2) gave enynol **18** (473 mg, 76%) as a colorless oil: $[\alpha]^{24}_{D}$ −33.8 (*c* 2.0, $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$) δ 0.07 and 0.10 (6H and 6H, each s, 4 × $SiCH_3$), 0.57 (6H, q, J = 7.1 Hz, 3 × $SiCH_2$), 0.89 and 0.90 (9H and 9H, each s, 2 × Si-*t*-Bu), ca. 0.90 (3H, d, $J \approx 6.5$ Hz, H_3-21), 0.93 (3H, s, H_3-18), 0.95 (9H, t, J = 7.1 Hz, 3 × $SiCH_2CH_3$), 1.15 (3H, d, J = 7.4 Hz, CH_3-9α), 1.19 (6H, s, H_3-26 and H_3-27), 1.89 (3H, br s, H_3-19), 2.38 (1H, dd, J = 16.1, 5.0 Hz), 4.08 (1H, br m, H-3α), 4.18 (1H, narr m, H-1β); ^{13}C NMR (62 MHz, $CDCl_3$) δ −4.8, −4.7, −4.6, −4.3, 6.9, 7.2, 13.3, 17.6, 18.1, 18.2, 18.4, 18.8, 20.8, 21.1, 24.7, 25.9, 26.0, 26.8, 29.9, 30.1, 34.5, 35.4, 36.2, 39.5, 39.8, 41.3, 43.0, 45.6, 50.7, 57.1, 64.2, 70.0, 73.5, 74.2, 84.6, 95.5, 115.0, 141.1; HRMS (ESI) exact mass calcd for $C_{46}H_{88}O_4Si_3Na$ $(M + Na)^+$ 811.5888, found 811.5866.

1α,3β-Bis[(*tert*-butyldimethylsilyl)oxy]-9-methyl-25-[(triethylsilyl)oxy]-9,10-secocholesta-5(10),8-dien-6-yne (19). A solution of alcohol **18** (100 mg, 0.127 mmol) in toluene (8 mL) was added dropwise to a solution of methyl *N*-(triethylammoniumsulfonyl)carbamate (36 mg, 0.152 mmol) in anhydrous toluene (5 mL). Next, the reaction mixture was heated to 50 °C and stirred at this temperature for 30 min. Then the mixture was poured into brine and extracted with ether. The combined organic extracts were dried ($MgSO_4$) and evaporated to give a residue which was purified by flash chromatography on silica gel. Elution with hexane/Et_2O (98:2) gave dienyne **19** (90 mg, 92%): $[\alpha]^{24}_{D}$ −30.7 (*c* 1.1, $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.07 and 0.10 (6H and 6H, each s, 4 × $SiCH_3$), 0.56 (6H, q, J = 7.8 Hz, 3 × $SiCH_2$), 0.66 (3H, s, H_3-18), 0.889 and 0.894 (9H and 9H, each s, 2 × Si-*t*-Bu), 0.93 (3H, d, J = 7.4 Hz, H_3-21), 0.94 (9H, t, J = 7.8 Hz, 3 × $SiCH_2CH_3$), 1.19 (6H, s, H_3-26 and H_3-27), 1.87 and 1.92 (3H and 3H, each br s, CH_3-9 and H_3-19), 4.08 (1H, br m, H-3α), 4.20 (1H, narr m, H-1β); ^{13}C NMR (50 MHz, $CDCl_3$) δ −4.6, −4.5, −4.4, −4.1, 7.0, 7.3, 11.3, 18.2, 18.4, 18.9, 19.4, 21.0, 21.3, 25.0, 26.0, 26.1, 28.7, 30.0, 30.2, 31.0, 36.4, 36.5, 36.6, 40.2, 41.6, 42.2, 45.7, 50.8, 55.0, 64.5, 70.3, 73.6, 91.9, 92.5, 116.1, 116.9, 139.7, 140.84; HRMS (ESI) exact mass calcd for $C_{46}H_{86}O_3Si_3Na$ $(M + Na)^+$ 793.5783, found 793.5764.

1α-[(*tert*-Butyldimethylsilyl)oxy]-9-methyl-25-[(triethylsilyl)oxy]-previtamin D_3 *tert*-Butyldimethylsilyl Ether (20). Previtamin **20** was obtained by hydrogenation of the dienyne **19** performed analogously to the process described above for the preparation of **17**. The product was purified by flash chromatography (hexane) on silica gel to give previtamin **20** (86 mg, 95%) as a colorless oil: $[\alpha]^{24}_{D}$ + 2.6 (*c* 1.1, $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.04, 0.05, and 0.10 (3H, 3H and 6H, each s, 4 × $SiCH_3$), 0.56 (6H, q, J = 7.6 Hz, 3 × $SiCH_2$), 0.70 (3H, s, H_3-18), 0.88 and 0.89 (9H and 9H, each s, 2 × Si-

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t-Bu), ca. 0.89 (3H, H_3-21, overlapped), 0.95 (9H, t, J = 7.6 Hz, 3 × $SiCH_2CH_3$), 1.19 (6H, s, H_3-26 and H_3-27), 1.43 and 1.65 (3H and 3H, each br s, CH_3-9 and H_3-19), 4.05 (1H, br m, H-3α), 4.13 (1H, narr m, H-1β), 5.75 and 6.00 (1H and 1H, each d, J = 12.2 Hz, H-7 and H-6); ^{13}C NMR (50 MHz, $CDCl_3$) δ −4.6, −4.5, −4.4, −4.0, 7.0, 7.3, 11.8, 17.7, 18.2, 18.4, 18.9, 19.8, 21.0, 24.3, 26.0, 26.2, 28.9, 30.0, 30.2, 31.3, 36.4, 36.6, 36.8, 38.9, 42.0, 42.4, 45.7, 51.9, 54.7, 65.1, 71.8, 73.7, 129.2, 129.2, 129.5, 130.8, 131.2, 131.3; UV (EtOH) λ_{max} = 246 nm (ε = 10400); HRMS (ESI) exact mass calcd for $C_{46}H_{88}O_3Si_3Na$ $(M + Na)^+$ 795.5939, found 795.5959.

1α,25-Dihydroxy-9α-methylvitamin D_3 (4), 1α,25-Dihydroxy-9-methylene-10(*S*),19-dihydrovitamin D_3 (7), and 1α,25-Dihydroxy-9-methylene-10(*R*),19-dihydrovitamin D_3 (8). Protected from light, a solution of previtamin **6** (28 mg, 0.065 mmol) in isooctane (16 mL) was refluxed for 14 h and then concentrated. The resulting mixture of isomeric compounds was separated by HPLC (9.4 mm × 25 cm Eclipse XDB-C18 column, 4 mL/min) using a methanol/water (86:14) solvent system. The 10α-methyl compound **8** was collected at Rv 32 mL (4.2 mg, 15%), 10β-methyl compound **7** at Rv 36 mL (7.8 mg, 28%), 9α-methylcalcitriol (**4**) at Rv 46 mL (1.7 mg, 6%), and the unreacted previtamin **6** at Rv 48 mL (11.2 mg, 40%).

1α,25-Dihydroxy-9α-methylvitamin D_3 (4). Final purification of the vitamin D compound **4** was achieved by HPLC (10 mm × 25 cm Phenomenex Luna silica column, 4 mL/min) using a hexane/2-propanol (8:2) solvent system. The vitamin D compound **4** was collected at Rv 50 mL: $[\alpha]^{24}_D$ −19.4 (*c* 0.5, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.536 (3H, s, H_3-18), 0.941 (3H, d, J = 6.4 Hz, H_3-21), 1.076 (3H, d, J = 7.0 Hz, CH_3-9α), 1.218 (6H, s, H_3-26 and H_3-27), 2.32 (1H, dd, J = 12.9, 6.5 Hz, H-4β), 2.60 (1H, dd, J = 13.0, 3.2 Hz, H-4α), 3.07 (1H, approx quintet, $J \approx$ 7 Hz, H-9β), 4.23 and 4.44 (1H and 1H, each m, H-3α and H-1β), 5.01 and 5.33 (1H and 1H, each br s, H_2-19), 5.96 and 6.38 (1H and 1H, each dd, J = 11.2, 1.6 Hz, H-7 and H-6); ^{13}C NMR (125 MHz, $CDCl_3$) δ 11.5, 18.8, 19.9, 20.8, 22.6, 27.6, 29.2, 29.3, 29.4, 30.1, 35.7, 36.1, 36.4, 42.8, 44.4, 45.3, 45.9, 50.8, 56.5, 66.9, 70.8, 71.2, 111.9, 117.1, 124.7, 132.9, 147.4, 147.6; UV (EtOH) λ_{max} = 265 nm (ε = 17500); HRMS (ESI) exact mass calcd for $C_{28}H_{46}O_3Na$ $(M + Na)^+$ 453.3345, found 453.3346.

1α,25-Dihydroxy-9-methylene-10(*S*),19-dihydrovitamin D_3 (7). Final purification of compound **7** was achieved by HPLC (10 mm × 25 cm Phenomenex Luna silica column, 4 mL/min) using a hexane/2-propanol (8:2) solvent system. The vitamin D compound **7** was collected at Rv 32 mL: $[\alpha]^{24}_D$ +72.8 (*c* 0.9, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.637 (3H, s, H_3-18), 0.938 (3H, d, J = 6.6 Hz, H_3-21), 1.107 (3H, d, J = 7.6 Hz, H_3-19), 1.217 (6H, s, H_3-26 and H_3-27), 2.24 (1H, ddd, J = 14.2, 4.7, 2.5 Hz, H-11α), 2.31 (1H, br t, J = 13.2 Hz, H-4β), ca. 2.36 (1H, H-11β, overlapped), 2.38 (1H, dd, J = 13.2, 5.7 Hz, H-4α), 3.02 (1H, br q, J = 7.5 Hz, H-10α), 3.94 (1H, narr m, H-1β), 3.96 (1H, br m, H-3α), 4.77 and 5.01 [1H and 1H, each t, J = 2.5 Hz, C(9)=$CH_{(Z)}$ and C(9)=$CH_{(E)}$], 5.91 (1H, dd, J = 11.5, 1.8 Hz, H-7), 6.48 (1H, d, J = 11.5 Hz, H-6); ^{13}C NMR (125 MHz, $CDCl_3$) δ 12.1, 17.2, 19.0, 21.1, 22.6, 28.5, 29.6, 29.6, 33.1, 36.3, 36.6, 37.6, 38.0, 40.8, 42.1, 44.6, 45.8, 56.2, 57.2, 67.6, 71.3, 73.4, 112.5, 116.6, 126.1, 136.6, 144.1, 146.9; UV (EtOH) λ_{max} = 262 nm (ε = 17400); HRMS (ESI) exact mass calcd for $C_{28}H_{46}O_3Na$ $(M + Na)^+$ 453.3345, found 453.3352.

1α,25-Dihydroxy-9-methylene-10(*R*),19-dihydrovitamin D_3 (8). Final purification of vitamin **8** was achieved by HPLC (10 mm × 25 cm Phenomenex Luna silica column, 4 mL/min) using a hexane/2-propanol (8:2) solvent system. The vitamin D compound **8** was collected at Rv 35 mL: $[\alpha]^{24}_D$ +118.2 (*c* 0.7, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.645 (3H, s, H_3-18), 0.941 (3H, d, J = 6.6 Hz, H_3-21), 1.060 (3H, d, J = 7.4 Hz, H_3-19), 1.219 (6H, s, H_3-26 and H_3-27), 2.24 (1H, ddd, J = 14.0, 4.7, 2.5 Hz, H-11α), 2.36 (1H, br m, H-11β), 2.58 (1H, br d, $J \approx$ 15 Hz, H-4α), 3.27 (1H, m, H-10β), 4.05 (1H, dt, J = 12.2, 5.2 Hz, H-1β), 4.12 (1H, narr m, H-3α), 4.77 and 5.01 [1H and 1H, each t, J = 2.4 Hz, C(9)=$CH_{(Z)}$ and C(9)=$CH_{(E)}$], 5.95 and 6.34 (1H and 1H, each dd, J = 11.3, 1.6 Hz, H-7 and H-6); ^{13}C NMR (125 MHz, $CDCl_3$) δ 10.3, 11.9, 19.0, 21.1, 22.6, 28.6, 29.5, 29.6, 33.1, 36.3, 36.4, 36.6, 37.9, 39.1, 40.8, 44.6, 45.8, 56.2, 57.2, 67.5, 68.1, 71.3, 112.6, 116.5, 125.5, 137.0, 144.0, 146.9; UV (EtOH) λ_{max} = 264 nm (ε = 16300); HRMS (ESI) exact mass calcd for $C_{28}H_{46}O_3Na$ $(M + Na)^+$ 453.3345, found 453.3332.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

General experimental details, spectra (^{1}H and ^{13}C NMR) of all new compounds, and HPLC chromatograms. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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