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Total Synthesis of 25-Hydroxy-16,23*E*-diene Vitamin D_3 and 1α,25-Dihydroxy-16,23*E*-diene Vitamin D_3: Separation of Genomic and Nongenomic Vitamin D Activities

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Abstract—Separation of genomic and nongenomic vitamin D activities was achieved by structural modification of 1,25-dihydroxy vitamin D_3 by introduction of 16 and 23*E* double bonds. The modified compound **3**, lacking a 1α-hydroxy group, exhibits only nongenomic activity. Its 1α-hydroxy relative **4** expresses fully both genomic and nongenomic activities. A total synthesis of analogues **3** and **4** is described.

Introduction

The nuclear 1,25-dihydroxy vitamin D_3 (**1**) receptor (VDR) is a member of the steroid–thyroid receptor superfamily that regulates various gene transcriptions. These gene transcriptions are usually mediated by a heterodimer of VDR and a 9-*cis*-retinoic acid receptor (RXR) which targets sequence-specific DNA binding domains, vitamin D receptor-responsive elements (VDRE).[1,2] **1** causes numerous transcriptional responses[3] including the differentiation of intestine, bone, and skin cells, and the differentiation and inhibition of proliferation of various tumor cells. In target organs involved in calcium homeostasis, such as intestine, **1** initiates gene transcription of proteins (such as calbinidin D_{28} and calbinidin D_9) which are thought to be involved in transepithelial calcium absorption. In bone, **1** is responsible for increased gene transcriptions leading to bone matrix proteins such as osteocalcin,[4,5] osteoponin,[6,7] and collagen[8,9] type 1 produced by osteoblasts in the process of bone formation. The **1** receptor (VDR) in osteoblasts affects both the process of bone formation and bone resorption thus altering bone turnover. Although **1** has long been recognized as a calcitropic hormone that potentiates long-term changes resulting from gene activation, recent studies have shown that physiological concentrations of **1** also cause rapid effects such as acute increases of calcium influx into osteoblasts cells.[10] The rapid increases in intracellular calcium stimulated by **1** in mouse osteoblast primary cultures is blocked by nitrendipine and varapamil, indicating that a specific class of channels called 'L-type calcium channels' is involved.[10,11] **1** rapidly activates a similar calcium influx in cardiac muscle[12] and myoblasts[13] as well as both calcium influx and internal calcium release in liver.[14] Multiple signal transduction pathways are stimulated in the CaCo2 intestinal cell line including calcium influx, internal calcium release, phosphoinositol turnover,[15] and tyrosine kinase phosphorylation.[16]

Key words: Vitamin D_3 analogues; genomic effect; nongenomic effect; calcium channel; transcriptional effect.
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Results

Separation of genomic and nongenomic vitamin D activities

After the original observation of the rapid effects of **1** on perfused chick duodenum,[17] multiple vitamin D_3 analogues have also been tested for transepithelial calcium transport (transcaltachia).[18,19] Several compounds, which have a low potency (less than 1%) for activation

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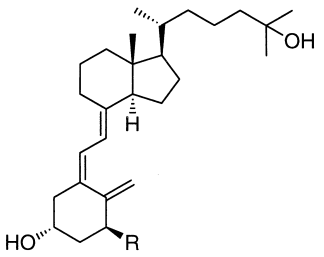
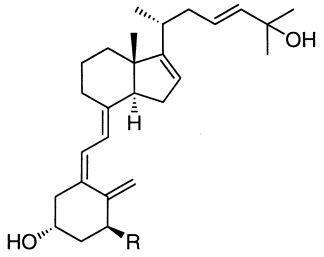


1 R=OH [1,25(OH)$_2$D$_3$]
2 R=H [25(OH)D$_3$]



3 R=H, [25(OH)-16,23*E*-diene D$_3$]
4 R=OH, [1,25(OH)$_2$-16,23*E*-diene D$_3$]

Chart 1.

of genomic events, are found to stimulate this process.[19] These compounds also stimulate rapid uptake of [$^{45}Ca^{2+}$] into ROS cells[20] or activate plasma membrane calcium influx through Ca^{2+} channels[21,22] suggesting the same rapidly acting nongenomic receptor may be present in both bone and intestine. It is generally thought that the 1α-hydroxy group is critical for affinity on VDR. Alterations in the CD-ring side chain in the absence of the 1α-hydroxy group, however, contribute to an increased stimulation of calcium current via calcium channels, despite that their binding to VDR is insignificant.

For example, the analogue 25(OH)-16,23*E*-diene D$_3$ (**3**) (Chart) potently stimulates calcium influx with a K 1/2 greater than 0.05 nM, compared to **1** which has K 1/2 of 0.5 nM, but the analogue **3** does not stimulate transcription by VDR.[23] From the data for **1** and **3**, it is not clear what the effect of 1α-hydroxy group is with respect to nongenomic activity. We have examined, therefore, the nongenomic effects of the addition of a 1α-hydroxy group to **3**, i.e., 1,25(OH)$_2$-16,23*E*-diene D$_3$ (**4**). At the same time, we looked at the effects of the insertion of 16- and 23*E*-double bonds in **1** on the genomic function, by measuring the production of osteocalcin in ROS 17/2.8 cells.

Comparison of calcium channel potentiation by **3** and its 1α-hydroxy analogue **4** indicates that the 1α-hydroxy function diminishes affinity for calcium channel potentiation (Table 1) only slightly. In comparison to the activity of 25(OH)D$_3$ (**2**) and **1**, the 16,23*E*-diene structural modification does contribute significantly to calcium channel potentiation.

Osteocalcin biosynthesis by **1** and **4** was almost identical, but **3** was much less active, indicating the significance of the 1α-hydroxyl group influence on this genomic transcriptional event (Table 2). Thus, at physiological concentrations (10^{-10} M), **3** exhibits only the nongenomic rapid effects of **1** cleanly without any significant genomic effects mediated by VDR.

Total synthesis of 3 and 4

Recently, we described a convergent synthesis of **1** and **2** in which 25-hydroxy Grundmann–Windaus ketone was coupled with a cyclopropane–acetylenic precursor of the A ring **5**.[24,25] Two asymmetric routes elaborated in our laboratory, starting from cyclopentenone (**7**), provided **5** in multigram quantities. For the synthesis of title compounds **3** and **4**, we investigated a similar coupling of the A-ring precursor **5** (Scheme 1) with a suitably functionalized 25-hydroxy-16-ene-23-yne C/D-ring **6**. We anticipated that the synthetic precursor of compound **6** would be the olefin **8**, which was obtained from Hajos ketone **9**.[26]

The *ene* reaction between olefin **8**[27] and aldehyde **10**[28] produced, in the presence of dimethylaluminum chloride, an epimeric 4:1 mixture of propargylic alcohols **11** in quantitative yield (Scheme 2).[29] The natural configuration at C-20 in product **11** was secured by the *Z*-configuration of the ethylidene double bond of **8**. The stereogenic center at C-22 was inconsequential since the hydroxyl group was removed by two-step procedure developed by Barton et al.[30] Toward this end, compounds **11** were transformed into the respective mixture of phenylthiocarbonyl derivatives **12** that on treatment with tri-*n*-butyltin hydride yielded compound **13**. Hydrolysis of the acetate group and deprotection of the 25-hydroxyl group provided diol **14**, which was oxidized with PCC to give hydroxy ketone **15**. Protection of the 25-hydroxyl group of **15** with TMS-imidazole produced the C/D ring precursor **6**.

In the next stage, we performed the coupling of A-ring precursor **5** and the C/D fragment **6** (Scheme 3). Treatment of compound **5** with *n*-BuLi produced its acetylide, which reacted with ketone **6** to give the main framework **16**. Deprotection of the 25-hydroxyl group with TBAF provided diol **17**. Compound **17**, the structure of which consists of two propargylic alcohols,

Table 1. Potentiation of calcium channels by vitamin D_3 in ROS cells. The agent 25(OH)-16,23*E*-diene D_3 (**3**) shows very potent effect (mean mV ± SE) on calcium channels at 0.05 nM and maximal effects at 0.5 nM. Likewise, the agent 1,25$(OH)_2$-16,23*E*-diene D_3 (**4**) is nearly as potent at 0.05 nM, suggesting that addition of the 1α group does not inhibit affinity for calcium channel activation

Vitamin D analogue	Concn				
	0.05 nM	0.1 nM	0.5 nM	5 nM	50 nM
25(OH)D_3			1.6 ± 1.16[a]	4.7 ± 3.7[a]	14.0 ± 3.6[a]
1,25$(OH)_2D_3$	1.78 ± 0.82[c]		5.73 ± 1.33[a]	9.79 ± 0.19[c]	10.17 ± 0.54[c]
25(OH)-16,23*E*-diene D_3	6.05 ± 0.4[c]	9.31 ± 0.4[b]	10.53 ± 0.3[a]		
1,25$(OH)_2$-16,23E-diene D_3	5.17 ± 2.98[a]	8.36 ± 0.23[b]	10.42 ± 1.07[b]	10.78 ± 1.34[b]	

[a] Number of patches equals 3.
[b] Number of patches equals 4.
[c] Number of patches equals 5.

served as an essential intermediate for the synthesis of both vitamin D_3 analogs **3** and **4**. In the synthesis of **3**, compound **17** was reduced with $LiAlH_4$ in THF to give the product **18** bearing two allylic alcohols. To generate the vitamin D_3 triene, solvolysis of the cyclopropane-allylic alcohol moiety in **18** was carried out analogously to the way we described earlier,[24,25] i.e., by the treatment with a catalytic amount of acid (*p*-TsOH) in dioxane–water. The obtained mixture of **3** and the corresponding 5,6-*cis*/*trans*-isomer was photolysed with a mercury lamp in the presence of 9-acetoxyanthracene as photosensitizer, to give the desired compound **3** as a single product.

Table 2. Concentration dependence of osteocalcin secretion by vitamin D_3 analogs. As previously reported, 1,25$(OH)_2D_3$ (**1**) stimulates osteocalcin secretion above the control value. The analogue 1,25$(OH)_2$-16,23*E*-diene D_3 (**4**) shows an equally potent secretion but 25(OH)-16,23*E*-diene D_3 (**3**) does not show secretion above control until concentration of 1 nM

Vitamin D analogue	Concn (nM)	Osteocalcin secretion[a]
Control		11 ± 0.6[b]
1,25$(OH)_2D_3$	0.001	20 ± 2
	0.01	51 ± 7
	0.1	71 ± 10.5
	1	78 ± 11
	10	70 ± 17
25(OH)-16,23*E*-diene D_3	0.001	12 ± 0.5
	0.01	13 ± 0.4
	0.1	12 ± 0.2
	1	19 ± 0.4
	10	21 ± 0.8
1,25$(OH)_2$-16,23*E*-diene D_3	0.001	18 ± 1.6
	0.01	49 ± 4.6
	0.1	78 ± 9.4
	1	79 ± 7.4
	10	72 ± 6.3

[a] Mean value ± SE
[b] ng/mg of protein/24 h

For the synthesis of compound **4**, we needed to introduce a hydroxyl group with the *alpha* configuration at C-1 carbon. For this purpose, a two-step procedure was applied which consisted of the oxidation of **17** at the allylic position with selenium dioxide. The obtained mixture of 1-ketone and 1α-alcohol was then reduced with Super-Hydride®. The reduction of 1-ketone was fully stereospecific (none of the epimeric alcohol was detected). Subsequently, the reduction of **19** with $LiAlH_4$ provided bis allylic alcohols **20**. This product was subjected to solvolysis and photolytic isomerization of thus-obtained 5,6-*cis*/*trans* mixture to produce **4** as a single compound.

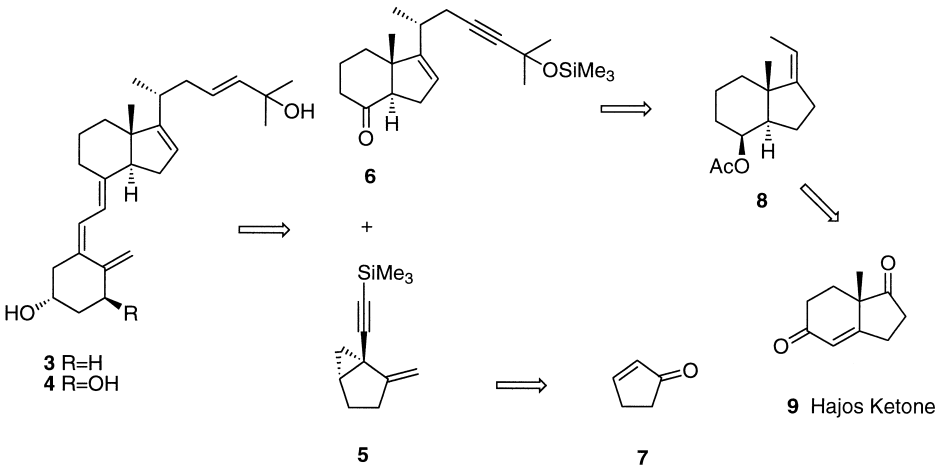
Conclusion

Introduction of the 16- and 23*E*-bonds into 25-hydroxy vitamin D_3 structure produced **3** which at physiological concentrations exhibits only nongenomic rapid effects characteristic of **1**, cleanly, without significant genomic effect mediated by VDR. In addition, the 1α group added to this molecule does not alter the sensitivity of the nongenomic effect of **3**.

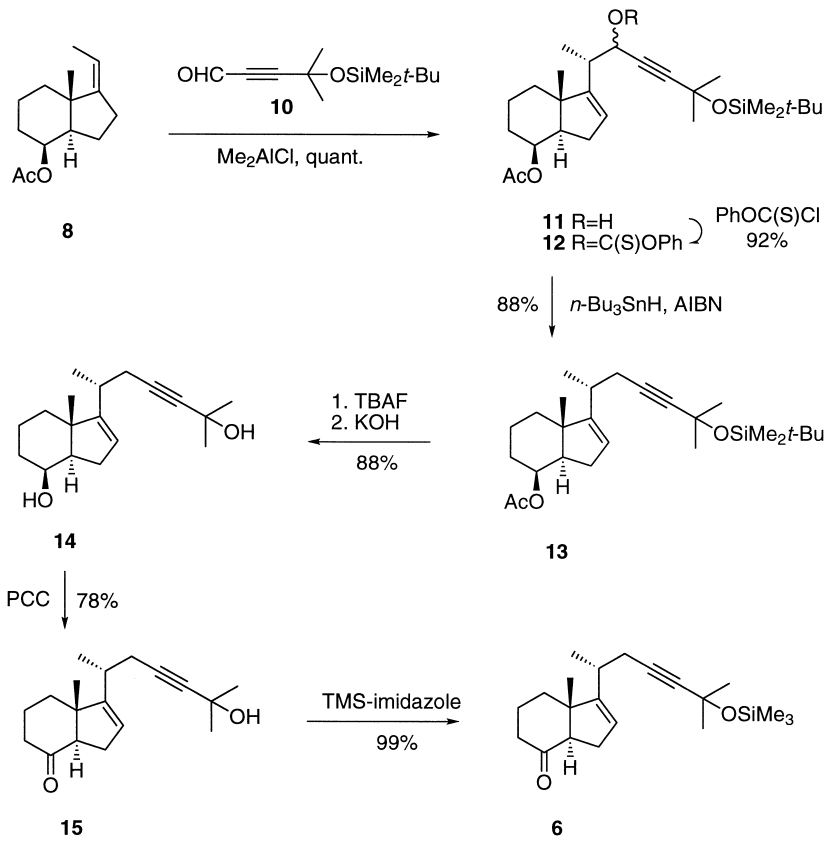
Experimental

Materials and methods

Cell culture. ROS 17/2.8 cells were grown in 5% CO_2 at 37 °C in Hamm's F12 medium (Life Technologies, Inc.) containing 5% heat-inactivated fetal calf serum (Life Technologies) and 0.1 mg/mL kanamycin buffered with 25 mM $NaHCO_3$ and 14 mM Hepes using NaOH to adjust pH to 7.4. For patch clamp experiments, cells



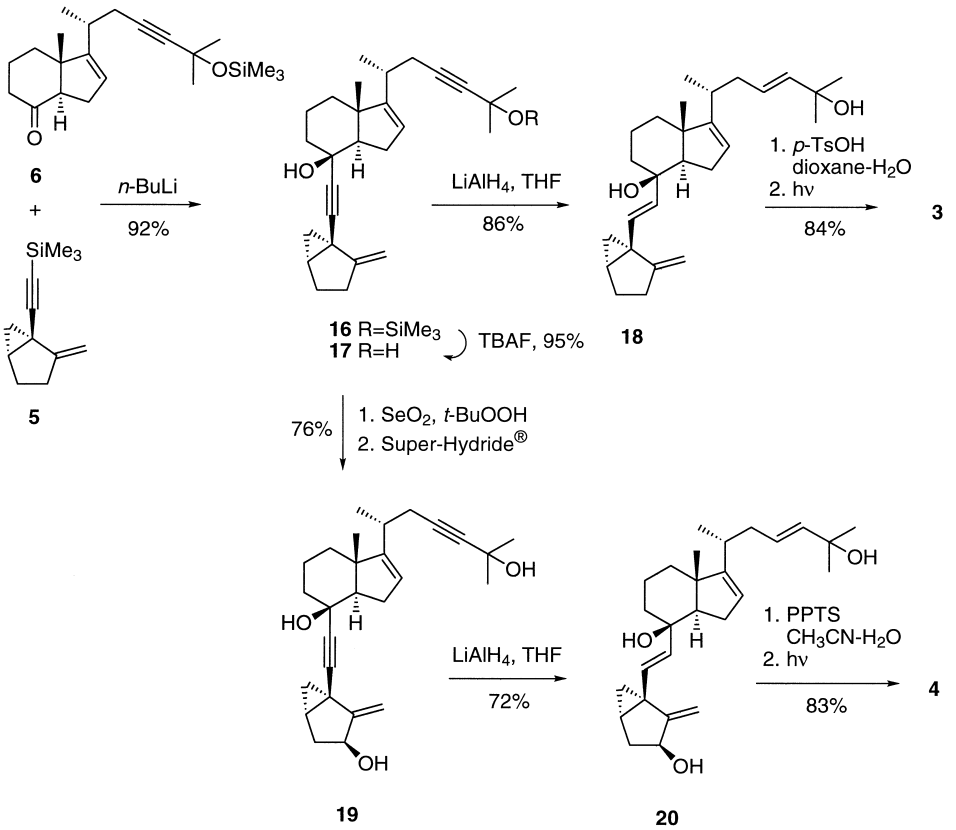
Scheme 1.



Scheme 2.

were seeded at low density as single cells onto pieces of coverslips. Patch clamp experiments were performed within 24 h after plating, on single cells after overnight incubation in 1% charcoal-treated serum. The data in each table are compiled from one seeding of cells from which control and test experiments were alternated on the same day.

Solutions. The perforated-patch recording technique was used for measuring inward barium currents under



Scheme 3.

voltage clamp conditions. The composition of the pipette solution was 100 mM KOH, 150 mM Hepes, 20 mM EGTA, 2 mM $CaCl_2$, 2 mM $MgCl_2$, 10 mM K_2HPO_4 buffered to pH 7.4 with KOH and osmolarity adjusted to 290 mosm/kg using K-Hepes. Amphotericin B, an antibiotic that created small nonselective pores in membrane to allow ion flow, was added to this solution at a final concentration of 240 mg/mL, and then the mixture was added to the tip of the pipette. The pipette was then backfilled with pipette saline solution onto the antibiotic saline mixture. The composition of the initial external solution was 140 mM NaCl, 5 mM KCl, 20 mM Hepes buffered to pH 7.4 with NaOH, and the osmolarity adjusted to 290 mosm with Na-Hepes. After whole cell currents were established, a solution which consisted of 115 mM $BaCl_2$ and 20 mM Hepes buffered to pH 7.4 with tetraethylammonium hydroxide, was added to the initial external solution so the final concentration of barium was 20 mM. Barium was used as a current carrier for two reasons. Barium current through L-type channels is known to be larger than calcium currents, and barium inhibits the activation of potassium channels. External tetraethylammonium hydroxide was used as a potassium channel blocker. Hepes, a nonpermeant anion, was used to eliminate inward currents via anion conductance. Using these conditions the inward barium current is stimulated by BayK 8644, completely blocked by nitrendipine, and displays the voltage-gating characteristics indicative of L-type calcium channels.

Current measurement. A pipette was placed to the surface of cell, and then gentle suction was applied until a tight seal of about 10 Gohm was formed. After about 3–10 min, the amphotericin B diffused into the cell membrane under the patch pipette causing the capacitance to increase, at which time the experiment was initiated. The cell membrane potential was held at −70 mV; 10 mV step pulses were applied for 300 ms between −60 and +50 mV with 2 s interval between pulses. Currents were monitored on an EPC-7 patch clamp amplifier (List Electronics, Darmstadt, Germany) and visualized on a Nicolet digital oscilloscope (Nicolet Instrument, Madison, WI) after filtering at 1 kHz through a Bessel filter (Frequency Devices, Haverhill, MA). Test substances (vitamin D_3 metabolites) were dissolved in a bath solution with 10% ethanol then added to the bath solution by pipette. The test substances were diluted

into the bath in a 1 to 100 ratio giving a final dilution of ethanol of 1/1000. Controls for addition of ethanol did not cause left shift or changes in the amplitude of calcium channel currents. It took about 30 s to add test substances. After the initiation of drug addition, the first currents were recorded within 30 s, and the data were sampled once per min afterwards. The data, which were recorded just after adding the samples, showed ca. 80% left shift. The data at 1.5 min showed a maximal steady-state value. Data were stored and analyzed on computer (IBM-PC compatible 386, AST) using the P-clamp software version 5.5 (Axon instruments, Foster City, CA). At the end of each experiment BayK 8644 (1 μM) was added to determine the maximal current through the L-type calcium channels and to standardize the data between experiments. This amount of BayK 8644 was previously shown in control cells to cause a maximal increase in barium current and maximal left shift in barium current. This concentration of BayK 8644 usually caused large currents in the range of −20 mV; thus, because of the rather high resistance of the amphotericin patch, the clamp was not totally effective in this voltage range. BayK 8644 was used at the end of each experiment to ensure that after three sequential steroid additions the maximal barium currents could be stimulated.

The peak of the current–voltage relationship was estimated by drawing a line joining the three largest current amplitudes. Using an equation that weights the slope of the line on either side of the maximal measured negative current, the peak current was estimated. Thus, the peak current was estimated by equation $\{(a-b)/(a-b+a-c)\} \times 10\,\text{mV} + e$, where a is the maximal current measured by the depolarization protocol; b is the current measured 10 mV more positive and c is the current measured 10 mV more negative than maximal current. e is the difference between the voltage depolarization causing the maximal measured current and 0 mV. All data are represented as mean ± SE. For statistical analyses, the Student's test was used, and $p < 0.05$ was recognized as statistically significant.

Osteocalcin secretion. Hanks' buffered salt solution (pH 7.4) twice and incubated with a mixture of Ham's F12 medium/DMEM (1/1) containing 2% fetal bovine serum and 50 μg of ascorbic acid per mL for 24 h. The incubation was begun by replacing the medium with the same medium plus 0.01 μM menadione, vitamin K_3 (Sigma), and vitamin D metabolites. The incubation was stopped at the appropriate time by removing the medium and storing it at −20 °C. Osteocalcin in the medium was measured by radioimmunoassay. Specific antibodies to rat osteocalcin or bovine osteocalcin that cross-react with human material were purchased from Biomedical Technologies (Stoughton, MA).

General

Melting points were measured in open capillary tubes and are uncorrected. ^{1}H NMR spectra were obtained in $CDCl_3$ at 200 or 400 MHz. Unless otherwise noted, reactions were conducted under an atmospheric pressure of dry argon. Organic extracts were dried over anhydrous $MgSO_4$ and filtered prior to removal of the solvent under reduced pressure on rotary evaporator (bath temperature 35 °C). Chromatography was performed on 230–400 mesh EM silica gel 60.

[3aS-[3(1*R),3aα,7α,7aβ]]-7-(acetoxy)-[3-[[(1,1-dimethylethyl)dimethylsilyl]oxy]-3-methyl-1-butynyl]-3a,4,5,6,7,7a-hexahydro-β,3a-dimethyl-1H-indene-3-ethanol (mixture of epimers) (11).** To the solution of [3a*R*-(1*Z*,3aα,4β,7aβ]-1-ethylidene-octahydro-7a-methyl-1H-inden-4-ol acetate (**8**) (2.0 g, 9.0 mmol) and 4-methyl-4-*tert*-butyldimethylsilyloxy-butynal (**10**) (2.25 g, 9.0 mmol) in anhydrous dichloromethane (5 mL), cooled at −78 °C, was added over a 10 min period dimethylaluminum chloride (20 mL, 1 M solution, 20 mmol). After 2 h, the reaction by TLC was only half complete; therefore, additional aldehyde **10** (2.25 g, 9 mmol) and dimethylaluminum chloride (20 mL, 1 M solution, 20 mmol) were added. After 1 h additional stirring, the reaction mixture was poured into potassium bicarbonate (600 mL, 2 N solution) and extracted with ethyl acetate (4×100 mL). The extracts were combined, washed with water and brine, dried, filtered, and evaporated to dryness. Flash chromatography on a silica gel column, eluting first with methylene chloride and then with hexanes/ethyl acetate (3/1), gave the epimeric mixture **11** (4.16 g, 100% yield) which was separated by HPLC with hexanes/ethyl acetate (10/1). **Major epimer** (3.25 g, 78% yield); mp 47–48 °C; $[\alpha]_D^{25} + 7.2°$ (*c* 0.56, EtOH); ^{1}H NMR ($CDCl_3$) δ 0.15 (s, 3H), 0.16 (s, 3H), 0.86 (s, 9H), 1.07 (s, 3H), 1.14 (d, *J* 6.9 Hz, 3H), 1.44 (s, 6H), 2.05 (s, 3H), 2.70 (p, *J* 6.4 Hz, 1H), 4.43 (t, *J* 6.3 Hz, 1H), 5.21 (brs, 1H), 5.68 (brs, 1H); Anal. calcd for $C_{26}H_{44}O_4Si$: C, 69.59; H, 9.88. Found: C, 69.85; H, 9.74. **Minor epimer** (0.754 g, 18% yield); mp 77–79 °C; $[\alpha]_D^{25} + 18.1°$ (*c* 0.52, EtOH); ^{1}H NMR ($CDCl_3$) δ 0.17 (2s, 6H), 0.87 (s, 9H), 1.03 (s, 3H), 1.14 (d, *J* 6.9 Hz, 3H), 1.47 (s, 6H), 2.05 (s, 3H), 2.37 (p, *J* 6.4 Hz, 1H), 4.40 (dd, *J* 3.7 and 7.8 Hz, 1H), 5.21 (brs, 1H), 5.54 (brs, 1H); Anal. calcd for $C_{26}H_{44}O_4Si$: C, 69.59; H, 9.88. Found: C, 69.42; H, 10.15.

[3a*S*-[3(1*R),3aα,7α,7aβ]]-thiocarbonic acid O-[1[1-[7-(acetoxy)-3a,4,5,6,7,7a-hexahydro-3a-methyl-1H-inden-3-yl]ethyl]-4-[[[(1,1-dimethylethyl)dimethylsilyl]oxy]4-methyl-2-pentynyl] O-phenyl ester (mixture of epimers) (12).** To the solution of major epimer **11** (448 mg, 1.0 mmol) in anhydrous dichloromethane (10 mL) was added pyridine (0.5 mL) and phenyl chlorothionoformate (0.533 mL, 4.0 mmol). The reaction mixture was then

stirred at rt for 3 h, quenched with methanol, and after 15 min diluted with water (200 mL). After extraction with ethyl acetate, the extracts were washed with 2 N HCl, water, brine, 2 N potassium bicarbonate, water, and brine, dried, filtered and evaporated to dryness. Purification was performed by flash chromatography on a silica gel column with dichloromethane/hexanes (3/1) to give compound **12** (538 mg, 92% yield), a colorless oil. $[\alpha]_D^{25} + 69.6°$ (*c* 0.23, EtOH); ^{1}H NMR ($CDCl_3$) δ 0.17 (s, 3H), 0.18 (s, 3H), 0.86 (s, 9H), 1.06 (s, 3H), 1.20 (d, *J* 7.1 Hz, 3H), 1.75 (s, 6H), 2.05 (s, 3H), 2.68 (p, *J* 6.6 Hz, 1H), 5.22 (brs, 1H), 5.62 (brs, 1H), 5.89 (d, *J* 6.8.0 Hz, 1H), 7.09 (d, *J* 8.0 Hz, 2H), 7.30 (t, *J* 8.0 Hz, 1H), 7.42 (t, *J* 8.0 Hz, 2H); Anal. calcd for $C_{33}H_{48}O_5SSi$: C, 67.77; H, 8.27. Found: C, 67.81; H, 8.24.

[3a*S*-[3(*S),3aα,7α,7aβ]]-3-[5-[[[(1,1-dimethylethyl)dimethylsilyl]oxy]-1,5-dimethyl-3-hexyl]-3a,4,5,6,7,7a-hexahydro-3a-methyl-1H-inden-7-ol acetate (13).** A solution of compound **12** (223 mg, 0.38 mmol) in toluene (15 mL) was added dropwise over 1.5 h period to a preheated (120 °C) mixture of AIBN (5 mg) and tri-*n*-butyltin hydride (5 g) under argon. The temperature was maintained at 120° for 15 min. The reaction mixture was cooled to rt and the toluene was removed under reduced pressure. The residue was subjected to flash chromatography with hexane/ethyl acetate (10/1) and the title product was further purified by HPLC with hexane/ethyl acetate (10/1) to afford amorphous **13** (146 mg, 88% yield). IR ($CHCl_3$) 1730 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.13 (s, 6H), 0.85 (s, 9H), 1.00 (s, 3H), 1.09 (m, 3H), 1.41 (s, 6H), 2.03 (s, 3H), 5.20 (brs, 1H), 5.36 (brs, 1H); Anal. calcd for $C_{27}H_{48}O_3Si$: C, 72.26; H, 10.78. Found C, 72.45; H, 10.91.

[3a*S*-[3(*S),3aα,7α,7aβ]]-3a,4,5,6,7,7a-hexahydro-3-(5-hydroxy-1,5-dimethy-3-hexynyl)-3a-methyl-1H-inden-7-ol (14).** To a solution of compound **13** (283 mg, 0.65 mmol) in THF (5 mL) was added tetrabutylammonium fluoride (2 mL, 1 M in THF, 2 mmol) and the reaction mixture was stirred overnight at rt. Then potassium hydroxide (700 mg), ethanol (2 mL), and water (2 mL) were added and the reaction mixture was heated 3 h at 80 °C. After dilution with water, the reaction mixture was extracted with ethyl acetate. The extract was washed with water, dried, filtered, and evaporated to dryness to give crude product (245 mg). Further purification by HPLC eluting with hexanes/ethyl acetate (1/1) afforded **14** (160 mg, 88% yield). Mp 107–109 °C (dichloromethane–hexane); $[\alpha]_D^{25} + 25.0°$ (*c* 0.2, EtOH); ^{1}H NMR ($CDCl_3$) δ 1.08 (s, 3H), 1.09 (d, *J* 8.0 Hz, 3H), 1.79 (s, 6H), 4.19 (brs, 1H), 5.39 (brs, 1H); Anal. calcd for $C_{18}H_{28}O_2$: C, 78.21; H, 10.21. Found: C, 78.07; H, 10.22.

[3a*R*-[1(*R),3aα,7aβ]]-3,3a,5,6,7,7a-hexahydro-1-(5-hydroxy-1,5-dimethy-3-hexynyl)-7a-methyl-4H-inden-4-one (15).** To a solution of compound **14** (720 mg, 2.6 mmol) in dry methylene chloride (44 mL) was added anhydrous sodium acetate (1.59 g, 19.4 mmol) and pyridium chlorochromate (PCC) (3.18 g, 10.5 mmol). The mixture was stirred at rt for 2 h. Additional PCC (1.59 g, 5.27 mmol) was then added and stirring was continued for another 2 h. After this time, 2-propanol (6 mL) was introduced and 15 min later, the mixture was diluted with water and extracted with ether/ethyl acetate (1/1). The organic phase was washed with water, 1 N aqueous H_2SO_4, saturated aqueous $NaHCO_3$, and brine. After drying, the solution was evaporated and the residue chromatographed on silica gel. Eluting with ethyl acetate/hexanes (1/1) yielded compound **15** (560 mg, 78% yield), a colorless glass. $[\alpha]_D^{25} + 35.3°$ (*c* 0.36, $CHCl_3$); IR ($CHCl_3$) 3600, 2225, 1709 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.84 (s, 3H), 1.15 (d, *J* 8.0 Hz, 3H), 1.46 (s, 6H), 2.44 (m, 1H), 2.85 (m, 1H), 5.36 (brs, 1H); MS (*m*/*e*) 274 (M^+).

[3a*R*-[1(*R),3aα,7aβ]]-1-[1,5-dimethyl-5-[(trimethylsilyl)oxy]-3-hexynyl]-3,3a,5,6,7,7a-hexahydro-7a-methyl-4H-inden-4-one (6).** To a solution of compound **15** (552 mg, 2.01 mmol) in dry dichloromethane (70 mL) was added 1-(trimethylsilyl)imidazole (2.00 g, 14.2 mmol). The mixture was stirred at rt for 17 h and then was quenched with water (22 mL). After stirring for an additional 20 min, the mixture was extracted with ethyl acetate and the organic phase was washed with water and saturated brine, dried, and evaporated to dryness. The residue was chromatographed on silica gel eluting with ethyl acetate/hexanes (1/4) to give compound **6** (693 mg, 99% yield): $[\alpha]_D^{25} + 29.5°$ (*c* 0.20, $CHCl_3$); IR ($CHCl_3$) 1710, 842 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.16 (s, 9H), 0.84 (s, 3H), 1.14 (d, *J* 8.0 Hz, 3H), 1.43 (s, 6H), 1.77 (m, 1H), 1.91 (m, 1H), 2.84 (m, 1H), 5.34 (brs, 1H); MS (*m*/*e*) 346 (M^+).

[3a*S*-[3(*S),3aα,7α,7(1S*,5S*),7aβ]]-3-[1,5-dimethyl-5-[(trimethylsilyl)oxy]3-hexynyl]-3a,4,5,6,7,7a-hexahydro-3a-methyl-7-[(2-methylenebicyclo[3.1.0]hex-1-yl)ethynyl]-1H-inden-7-ol (16).** A cold solution (−30 °C) of compound **5** (306 mg, 1.61 mmol) in THF (15 mL) was treated dropwise over 10 min with *n*-BuLi (1.0 mL, 1.6 M in hexane, 1.6 mmol). After the addition was complete, the cooling bath was removed and the mixture was heated at 45 °C for 15 min. Then the solution of acetylide was cooled to −78 °C and a solution of ketone **6** (372 mg, 1.08 mmol) in THF (3 mL) was added dropwise. The reaction mixture was stirred for 15 min and then was allowed to warm to rt over ca. 30 min. Water was added, followed by extraction with ethyl acetate. The extract was washed with water and brine, dried, filtered, and the solvent was removed under reduced pressure. The crude product was purified by column chromatography on silica gel eluting with hexanes/ether (96/4) to give crude **16** (453 mg, 92% yield), as a colorless oil, contaminated with unreacted ketone **6** (ca. 7%). Without

further purification, crude compound **16** was used in the next step. ^{1}H NMR ($CDCl_3$) δ 0.17 (s, 9H), 1.01 (s, 3H), 1.08 (d, *J* 6.0 Hz, 3H), 1.44 (s, 6H), 4.87 (s, 1H), 5.11 (s, 1H), 5.39 (s, 1H).

[3a*S*-[3(*S),3aα,7α,7(1*S**,5*S**),7aβ]]-3a,4,5,6,7,7a-hexahydro-3-(5-hydroxy-1,5-dimethyl-3-hexynyl)-3a-methyl-7-[(2-methylenebicyclo[3.1.0]hex-1-yl)ethynyl]-1H-inden-7-ol (17).** To a solution of crude compound **16** (453 mg, 0.97 mmol) in THF (12 mL), tetra-*n*-butylammonium fluoride (1.5 mL, 1 M in THF, 1.50 mmol) was added. The solution was stirred for 1 h at rt, whereupon it was taken up by ethyl acetate, washed with water and brine, then dried. The solvent was removed under reduced pressure. The residue was purified by chromatography on silica gel while eluting with hexanes/ethyl acetate (9/1) to yield compound **17** (363 mg, 95% yield) as a foam. $[\alpha]_D^{25}$ −48.1° (*c* 0.32, CH_2Cl_2); IR ($CHCl_3$) 3600, 2231, 1653 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.02 (s, 3H), 1.08 (d, *J* 6.1 Hz, 3H), 1.48 (s, 6H), 4.88 (s, 1H), 5.12 (s, 1H), 5.40 (s, 1H); MS (*m*/*e*) 375 (9%), 357 (12), 154 (100), 136 (84), 115 (91); HRMS for $C_{27}H_{36}O_2$ (M^+) calcd, 392.2715; found, 392.2707. Anal. calcd for $C_{27}H_{36}O_2$: C, 82.61; H, 9.24. Found: C, 82.96; H, 9.40.

[3a*S*-[3(1*S,3*E*),3aα,7α,7[*E*(1*R**,5*S**),7aβ,]]-3a,4,5,6,7,7a-hexahydro-3-(5-hydroxy-1,5-dimethyl-3-hexenyl)-3a-methyl-7-[2-(2-methylenebicyclo[3.1.0]hex-1-yl)ethenyl]-1H-inden-7-ol (18).** To a solution of compound **17** (60 mg, 0.15 mmol) in THF (3 mL) was added $LiAlH_4$ (20 mg, 0.53 mmol) and the reaction mixture was stirred at 55 °C for 5 h. After cooling to rt, saturated NH_4Cl (0.2 mL) was carefully added, and the reaction mixture was extracted with ethyl acetate. The extract was washed with water and brine, then dried. After evaporation of solvent the residue was purified by chromatography on silica gel eluting with hexanes/ethyl acetate (8/2) to afford compound **18** (52 mg, 86% yield) as a colorless oil. $[\alpha]_D^{25}$ 16.1 (*c* 0.41, CH_2Cl_2); IR ($CHCl_3$) 3601 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.83 (t, *J* 4.6 Hz, 1H), 0.98 (d, *J* 6.4 Hz, 1H), 1.03 (s, 3H), 1.48 (s, 6H), 4.76 (s, 1H), 4.81 (s, 1H), 5.30 (s, 1H), 5.45 (d, *J* 14.8 Hz, 1H), 5.54–5.66 (m, 2H), 6.02 (d, *J* 14.8 Hz, 1H).

(3β,5*Z*,7*E*,23*E*)-9,10-Secocholesta-5,7,10(19),16,23-pentaene-3,25-diol (3). To a solution of compound **18** (43 mg, 0.11 mmol) in dioxane/water (8/2, 3 mL), *p*-TsOH (catalytic amount, 1 mg) was added. The reaction mixture was stirred at 60 °C under argon for 2 h. The solution was extracted with ethyl acetate and the organic phase was washed with water and brine; then dried. The solvent was evaporated to dryness and the residue was passed through silica gel while eluting with hexanes/ethyl acetate (8/2). Fractions containing mixtures of *cis*/*trans*-isomers were combined and the solvents were removed. The residue was then dissolved in *t*-BuOMe (3 mL) and photolyzed in a quartz tube under argon with 450 W low-pressure mercury lamp and uranium filter in the presence of 9-acetoxyanthracene (1 mg). After 45 min, the solvent was removed and the residue was purified by column chromatography eluting with hexanes/ethyl acetate (8/2) to give compound **3** (36 mg, 84% yield) as a glass. $[\alpha]_D^{25}$ 62.5 (*c* 0.2, MeOH); IR ($CHCl_3$) 3605, 1637 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.67 (s, 3H), 1.00 (s, 3H), 1.28 (s, 6H), 2.56 (m, 1H), 2.80 (m, 1H), 3.95 (brs, 1H), 4.82 (s, 1H), 4.98 (s, 1H), 5.24 (s, 1H), 5.41–5.56 (m, 2H), 6.04 (d, *J* 12.0 Hz, 1H), 6.16 (d, *J* 12.0 Hz, 1H); MS (*m*/*e*) 396 (M^+, 4%), 378 (12), 245 (5), 158 (19), 136 (19), 118 (23), 105 (14), 91 (27); HRMS: for $C_{27}H_{40}O_2$ (M^+) calcd, 396.3028; found, 396.3020.

(1α,3α,5α)-3,5-cyclo-9,10-secocholesta-10(19),16-dien-6,23-diyne-1,8,25-triol (19). A solution of compound **17** (42 mg, 0.10 mmol) in dichloromethane (5 mL) was treated at +4 °C with *t*-butyl hydroperoxide (107 μL, 3 M in isooctane, 0.32 mmol) and selenium dioxide (4 mg, 0.03 mmol). The reaction mixture was monitored by TLC (hexanes/ethyl acetate (1/1)). After 48 h, all the starting material disappeared and the mixture of products (1-alcohol and 1-ketone) was extracted with ethyl ether and the extracts washed with water. The extract was dried and the solvents were removed under reduced pressure. The residue was dissolved in THF (3 mL) and treated at −60 °C with Super-Hydride® (0.2 mL, 1 M in THF, 0.2 mmol). After 15 min, the icebath was removed and the solution was extracted with ethyl acetate, the extracts was washed with water and dried, and the solvent was evaporated under reduced pressure. The residue was purified by chromatography on silica gel eluting with hexanes/ethyl acetate (7/3) to give compound **19** (32 mg, 76% yield) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 0.90 (t, *J* 5.0 Hz, 1H), 1.02 (s, 3H), 1.08 (d, *J* 6.0 Hz, 3H), 1.48 (s, 6H), 4.17 (t, *J* 8.0 Hz, 1H), 5.17 (d, *J* 2.0 Hz, 1H), 5.30 (d, *J* 2.0 Hz, 1H), 5.40 (s, 1H); MS (*m*/*e*) 408 (M+, 1%), 390 (1), 375 (4), 347 (3), 265 (7), 199 (35), 159 (34), 115 (43), 105 (58), 91 (88), 55 (57), 43 (100); HRMS: for $C_{27}H_{36}O_3$ calcd, 408.2664; found 408.73.

(1α,3α,5α,6*E*,23*E*)-3,5-cyclo-9,10-secocholesta-6,10(19),16,23-tetraene-1,8,25-triol (20). A stirred solution of compound **19** (22 mg, 0.054 mmol) in THF (4 mL) was treated with $LiAlH_4$ (15 mg, 0.40 mmol) and the reaction mixture was heated at 65 °C for 16 h. Then it was cooled to 0 °C and carefully treated with saturated NH_4Cl solution, whereupon it was dissolved in ethyl acetate, washed with water, dried and filtered. The solvent was removed under reduced pressure and the oily residue was purified by passing through a short path of silica gel eluting with hexanes/ethyl acetate (6/4) to afford compound **20** (16 mg, 72% yield) as a colorless

oil. ^{1}H NMR ($CDCl_3$) δ 0.72 (t, *J* 4.8 Hz, 1H), 0.99 (d, *J* 6.2.0 Hz, 3H), 1.03 (s, 3H), 1. 29 (s, 6H), 4.26 (q, *J* 8.2 Hz, 1H), 4.98 (d, *J* 2.0 Hz, 1H), 5.10 (d, *J* 2.0 Hz, 1H), 5.31 (s, 1H), 5.48 (d, *J* 15.6 Hz, 1H), 5.55–5.63 (m, 2H), 6.04 (d, *J* 15.6 Hz, 1H).

(1α,3β,5*Z*,7*E*,23*E*)-9,10-secocholesta-5,7,10(19),16,23-pentaene-1,3,25-triol (4). A solution of compound **20** (12 mg, 0.012 mmol) and pyridinum *p*-toluenesulfonate (PPTS) (1 mg) in acetonitrile/water (7/3, 3 mL) was stirred under argon at 65 °C for 3 h. Then the reaction mixture was dissolved in ethyl acetate and washed with sodium bicarbonate solution, then dried. After evaporation of the solvent, an isomeric mixture of 5,6-*cis*/*trans* compounds (12 mg) was obtained. ^{1}H NMR ($CDCl_3$) δ 0.68 and 0.70 (2s, 3H), 1.02 (d, *J* 6.3 Hz, 3H), 1.30 (s, 6 H), 4.25 (m, 1 H), 4.49 (m, 1 H), 4.99, 5.02, 5.14, 5.34 (4s), 5.55–5.59 (m), 5.99 and 6.58 (2d, *J* 11.7 Hz, =CH– of 5,6-*trans*-compound), 6.11 and 6.38 (2d, *J* 10.8 Hz, =CH– of 5,6- *cis* compound). The mixture of 5,6-*cis*/*trans* compounds was subsequently irradiated for 30 min in *t*-BuOMe (3 mL), in the presence of 9-acetoxyanthracene (1 mg), with a 450 W low-pressure mercury lamp through a uranium filter. The solvent was removed and the residue was purified by passing through a short path of silica gel. Elution with hexanes/ethyl acetate (7/3) afforded pure compound **4** (10 mg, 83% yield) as a colorless glass. IR ($CHCl_3$) 3605, 1652 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.68 (s, 3H), 1.02 (d, *J* 6.3 Hz, 3H), 1.30 (s, 6H), 2.54–2.68 (m, 1H), 2.76–2.88 (m, 1H), 4.25 (m, 1H,), 4.45 (m, 1H), 5.02 (s, 1H), 5.33 and 5.34 (two overlapped s, 2H), 5.55–5.68 (m, 2H), 6.11 (d, *J* 11.1 Hz, 1H), 6.38 (d, *J* 11.1 Hz, 1H); MS (*m*/*e*) 394 (8%), 376 (9), 358 (14), 343 (7), 105 (100).

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