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Conformationally Restricted Hybrid Analogues of the Hormone 1α,25-Dihydroxyvitamin D_3: Design, Synthesis, and Biological Evaluation

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Abstract—Four new conformationally restricted hybrid analogues of the hormone 1α-25-dihydroxyvitamin D_3 (1,25D3) have been synthesized in a convergent manner by combining enantiomerically pure C,D-ring ketones (−)-**15** and (−)-**17** with racemic 1-hydroxymethyl A-ring phosphine oxide (±)-**18**. Parent hybrid analogue **6**, which combines the calcemia-inactivating 1β-hydroxymethyl A-ring modification with the antiproliferation- activating 20-epi-22-oxa-25-hydroxydiethyl C,D-ring side chain modification, is comparable in potency to 1,25D3 at the low nM level in inhibiting proliferation in a wide assortment of malignant cell lines in vitro with extremely low calcemic activity in vivo. Surprisingly, both conformationally restricted analogues of **6** (**8b** and **9b**), which incorporate rigidifying units at their 25-hydroxyl side chain termini, retained the desirable antiproliferative, transcriptional, and calcemic activities of the parent compound.

Introduction

In addition to its 'classical' physiological role in the regulation of calcium and phosphorus homeostasis,[1] the hormonally active metabolite of vitamin D_3, 1α,25-dihydroxyvitamin D_3 (1,25D3, **1**) is known to contribute significantly to the growth and differentiation of a variety of cell types (e.g., keratinocytes from epidermal precursors,[2] osteoclasts from mononuclear precursors[3]). At supraphysiological concentrations, the growth regulatory abilities of 1,25D3 have proven effective at suppressing proliferation and inducing differentiation in psoriatic cells[4] and in a variety of malignant cell lines both in vitro and in vivo.[5] The therapeutic usefulness of 1,25D3 when administered at pharmacological doses to treat such hyperproliferative diseases as cancer or psoriasis is limited, however, by the causation of toxic hypercalcemia that results from its potent effects on calcium metabolism.[6]

Since the molecular modes of action mediating 1,25D3's diverse physiological effects are not yet well defined,[7] rationale for the design of analogues of 1,25D3 in which the calcium regulating activity is effectively separated from the regulation of cell growth has been largely based on information obtained from structure–activity relationships (SARs).[8] These studies attempt to define the influence of specific structural unit modifications on 1,25D3's various biological activities. The high conformational flexibility of 1,25D3, however, suggests that its diverse spectrum of biological activities may in part be induced by different topologies (shapes) of the hormone.[8,9] One reasonable hypothesis is that different forms of 1,25D3 interact selectively with different proteins (e.g., receptors, transcription factors) ultimately resulting in the variety of biological responses observed.[9] Many of 1,25D3's biological activities result from transcriptional modifications initiated by the hormone binding to the nuclear vitamin D receptor (VDR). The selectivity in transcriptional responses observed with many of the structural analogues is thought to be the result of ligand induction of distinct conformational changes in the VDR dimerization interfaces that regulate the selection of dimerization partners such as

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nuclear vitamin D response elements (VDRE), co-activators, and other transcriptional effectors.[7] If this is the case, conformation–activity relationships, particularly of 1,25D3 analogues that have already demonstrated a promising separation between antiproliferative and calcemic activities, would strongly augment the information obtained from SARs for the design of analogues with selective physiological action (Fig. 1).

The majority of structural modifications capable of substantially enhancing the antiproliferative potency of 1,25D3 appear at the steriodal D-ring side chain. Outstanding examples of beneficial individual modifications include the following: (1) methyl homologation at C-26 and C-27, (2) homologation at C-24, (3) incorporation of an oxygen atom at C-22, (4) inversion of stereochemistry at C-20.[8] Side-chain analogues of 1,25D3 that incorporate rigid, conformationally restricting structural units tend to show moderate antiproliferative activities with low calcemic activities. Outstanding examples include side-chain aromatic analogues (arocalciferols),[10] 23-yne analogues (e.g., Hoffmann-La Roche's Ro-23-7553[11]) and Leo Pharmaceutical Company's 22,24-diene analogue EB 1089 **3**.[12,13] Leo Pharmaceutical's analogue KH 1060 **2**,[14,15] which incorporates many of the aforementioned structural side-chain modifications, has extraordinarily enhanced abilities to induce differentiation and inhibit proliferation relative to 1,25D3 in a variety of skin and malignant cell lines, but retains undesirable calcemic activity. When these antiproliferation activating structural modifications were incorporated into the side chain of anticalcemic 1β-(hydroxymethyl) A ring analogue **4**,[16] a dramatic enhancement of antiproliferative activity was observed with no increase in calcemic activity.[17] Extraordinarily, hybrid analogue **6** was equipotent to 1,25D3 at the low nM level in inhibiting proliferation in human malignant cell lines SK Br3 (breast cell carcinoma), HL-60 (myeloid leukemia cells);[17] murine malignant cell lines B-16 (malignant melanoma), EMT-6 (breast cell carcinoma), and RENCA (renal cell carcinoma).[18] Conversely, hybrid analogue **5**, differing only in relative stereochemistry at the 1 and 3 positions [i.e., **5** (1α,3β) versus **6** (1β,3α)], showed no significant antiproliferative activity, even in the μM range. Curiously, hybrid analogue **6** also demonstrated a marked increase in vitamin D receptor (VDR) mediated transcriptional activity relative to **4** which was not associated with increased binding affinity to the VDR.[19]

The side-chain terminus, via the 25-hydroxyl moiety, is thought to play a crucial role in the binding of 1,25D3 to the VDR and in eliciting its biological effects.[20] Inversion of stereochemistry at C-20 is thought to bias the conformation of the side chain such that the terminal 25-hydroxyl moiety resides primarily on the 'northwest' portion of the molecule (i.e., above the C-ring).[21,22] In a conformation–activity study performed by the researchers at Leo Pharmaceuticals, compound **7**, in which the KH 1060 side chain was sterically restricted over the 'northwest' portion of the molecule, showed diminished antiproliferative activity with respect to the parent compound but enhanced activity with respect to the natural hormone 1,25D3.[23] The biological activity was thought to suggest the presence of a 25-hydroxyl moiety binding site to the northwest of the C,D-ring system. As previously indicated, several structural modifications made directly at the side chain terminus encasing the critical 25-hydroxyl group (i.e., one carbon homologation at C-24, C-26, and C-27) in KH 1060 and hybrid analogues **5** and **6** are known to potentiate the antiproliferative effects of 1,25D3. Although it is evident that these structural modifications increase the length and hydrophobicity of the side chain, it is not clear what role, if any, the conformation of this extended terminus plays on the biological profile of analogues that incorporate it. To evaluate this, we have designed, synthesized, and biologically evaluated two sets of

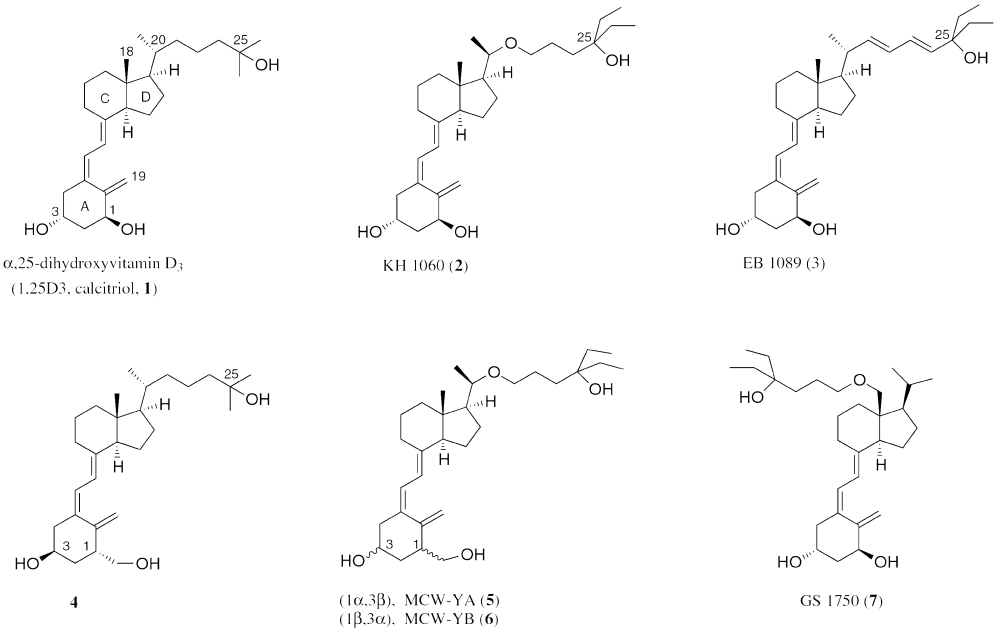


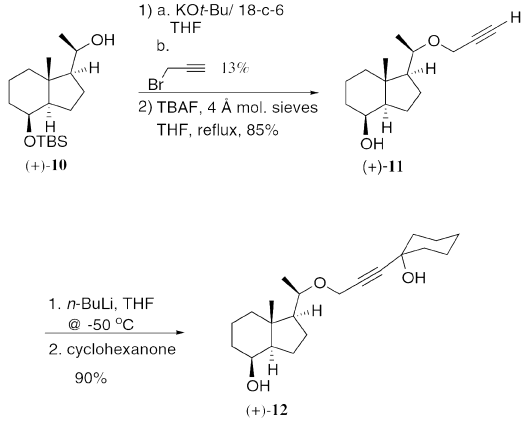
Figure 1. Structure of 1α, 25-dihydroxyvitamin D_3 and its analogues.

conformationally restricted analogues of **5** and **6**, analogues **8** and **9**. Analogues **8** and **9** both incorporate the floppy terminal C-25 diethyl groups into a conformationally rigid cyclohexyl ring. Analogue **9** further restricts conformational flexibility at the side-chain terminus by incorporating a rigidifying alkyne unit between C-24 and C-24a.

Results

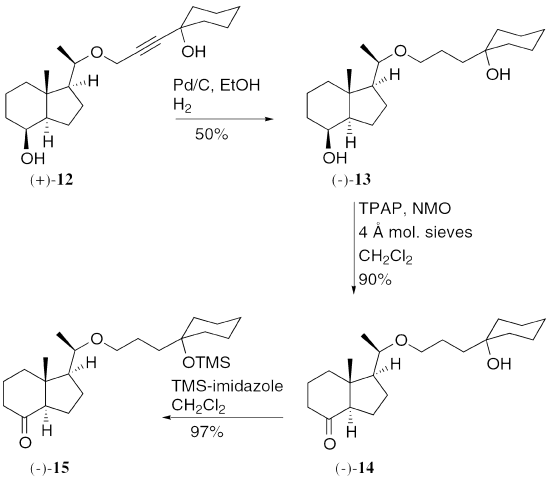
Chemistry

We have prepared enantiomerically pure C,D-ring ketones (−)-**15** and (−)-**17** for Horner–Wadsworth–Emmons (HWE) coupling with racemic 1-(hydroxymethyl) A-ring phosphine oxide (±)-**18** in a convergent approach to conformationally restricted hybrid analogues **8** and **9**. A retrosynthetic analysis for ketones (−)-**15** and (−)-**17** suggests diol (+)-**12** as a suitable intermediate for both. As shown in Scheme 1, 20-epi alcohol (+)-**10**, prepared according to literature methods from commercial vitamin D_2,[17] was converted into its propargyl ether derivative (+)-**11** by alkylation of the potassium alkoxide with propargyl bromide (13% yield, 80% recovered alcohol, ca. 54% over 11 runs) followed by fluoride promoted desilylation of the C-8 alcohol in 85% yield. Formation of the bis-lithiated derivative of (+)-**11** (2.5 eq *n*-BuLi, THF, −50 °C) followed by condensation with cyclohexanone and an aqueous work up gave the desired key intermediate diol (+)-**12** in 90% yield. When the reaction was run at −78 °C, the bis-lithiated species precipitated out of solution and the condensation yields dropped to 60%. Attempts to desilylate the C-8 secondary alcohol after condensation gave very poor yields (20–30%) of diol (+)-**12**. Initial attempts to couple a pre-assembled primary tosylate side chain to the 20-epi alcohol (+)-**10** via a Williamson ether coupling reaction gave no desired product with 70% recovery of (+)-**10** and complete consumption of side chain.



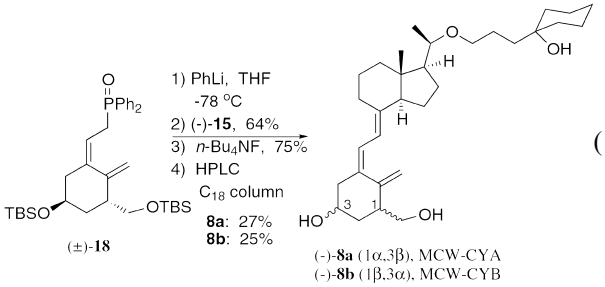
Scheme 1.

Transformation of diol (+)-**12** into saturated *O*-silylated C,D-ring ketone (−)-**15** proceeded smoothly in three steps. Catalytic hydrogenation (Pd/C) of (+)-**12** in ethanol provided fully saturated diol (−)-**13** in 50% yield. Oxidation of the C-8 hydroxyl group by tetrapropylammonium perruthenate (TPAP) and *N*-methylmorpholine *N*-oxide (NMO) in the presence of molecular sieves (MS) provided ketone (−)-**14** in 90% yield. Silyl protection of the tertiary alcohol in preparation for HWE coupling was effected with 1-(trimethylsilyl)-imidazole (TMS-imid.) in CH_2Cl_2 to afford the *O*-silylated product (−)-**15** in 97% yield (Scheme 2).



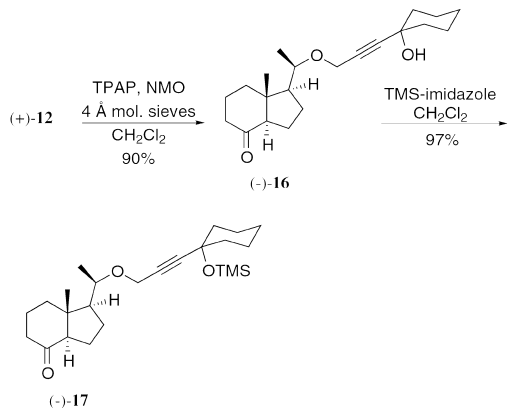
Scheme 2.

Convergent HWE coupling of enantiomerically pure (−)-**15** with racemic 1-(hydroxymethyl) A ring phosphine oxide (±)-**18** (64%), followed by fluoride-promoted desilylation (75%), gave diastereomeric secosteriods (−)-**8a** (1α,3β) and (−)-**8b** (1β,3α) that were easily separated by C-18 reverse-phase HPLC. The absolute stereochemistry at C-1 and C-3 in these diastereomers was tentatively assigned primarily by comparing characteristic portions of their 400 MHz ^{1}H NMR spectra and optical rotation data with those of the corresponding 1-(hydroxymethyl) analogues (see Table 1).[16,17]



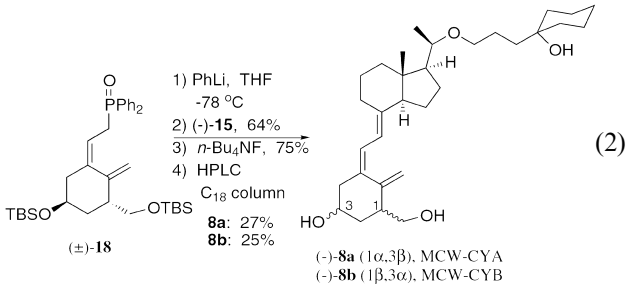
(1)

Elaboration of diol (+)-**12** into O-silylated unsaturated C,D-ring ketone (−)-**17** proceeded smoothly in two steps. Diol (+)-**12** underwent TPAP oxidation at the C-8 position to afford unsaturated ketone (−)-**16** in 90% yield which was silylated at the tertiary C-25 alcohol to provide saturated, *O*-silylated ketone (−)-**17** in 90% yield (Scheme 3).



Scheme 3.

Convergent HWE coupling of enantiomerically pure (−)-**17** with racemic 1-(hydroxymethyl) A ring phosphine oxide (±)-**18** (68%), followed by fluoride-promoted desilylation (70%) gave diastereomeric *seco*-steriods (−)-**9a** (1α,3β) and (−)-**9b** (1β,3α) that were easily separated by C-18 reverse-phase HPLC and characterized as described above (see Table 1).



(2)

Table 1. Characteristics of hybrid analogues

Analogue	^{1}H NMR (δ)			$[\alpha]_D^{25}$(°)
	C-18	C-19a	C-19b	
5	0.55	5.16	5.01	−140
6	0.53	5.15	4.99	+4.0
8a	0.55	5.16	5.01	−149
8b	0.52	5.14	4.98	−1.3
9a	0.62	5.17	5.02	−182
9b	0.59	5.15	4.99	−5.0

Biology

Conformationally restricted analogues **8** and **9**, hybrid analogue **6**, and the natural hormone 1,25D3 (**1**) were all evaluated for in vitro antiproliferative activity in murine B16 malignant melanoma cells using our previously described protocol.[19] As predicted based on previous studies of diastereomeric sets of hybrid analogues, the diastereomers with the 1α,3β stereochemistry (i.e., **8a** and **9a**) were much less potent inhibitors of cellular proliferation than their diastereomeric counterparts bearing the 1β,3α stereochemistry (i.e., **6**, **8b**, and **9b**). Surprisingly, despite the conformation restrictions imposed on the side-chain termini of both **8b** and **9b**, the desirable antiproliferative activities of these analogues were similar to those of the parent hybrid compound **6** at drug concentrations ranging from 1 to 1000 nM (Fig. 2). Even at physiologically relevant 1 and 10 nM concentrations, conformationally restricted hybrids **8b** and **9b**, like hybrid **6**, were at least as potent antiproliferative agents as 1,25D3 (**1**). The average standard deviation for the data points shown in Figure 1 ($n=3$ for each) was ±10% of control.

To further characterize their biological profiles, the in vitro VDR-mediated transcriptional activities of conformationally restricted analogues **8b** and **9b** were tested in rat osteosarcoma ROS 17/2.8 cells using our previously described protocol.[19] Reminiscent of the antiproliferative activity trends, the transcriptional potencies of both **8b** and **9b** paralleled those of parent compound **6** at drug concentrations ranging from 0.01 to 100 nM (Fig. 3). Both the saturated analogue **8b** ($ED_{50}=2\times10^{-10}$ M) and unsaturated analogue **9b** ($ED_{50}=1\times10^{-10}$ M), like **6** ($ED_{50}=1\times10^{-10}$ M) were at least as transcriptionally potent as 1,25D3 ($ED_{50}=3\times10^{-10}$ M).

Having established that conformationally restricted hybrid analogues **8b** and **9b** have antiproliferation and VDR-mediated transcriptional potencies paralleling those of parent hydrid **6**, we were interested in evaluating their calcemic activities in vivo. In contrast to the natural hormone 1,25D3, which produced marked elevations in blood ionized calcium levels in female Bl-6 mice after 7 days of treatment at doses of 1 and 10 μg/kg/day, both **8b** and **9b**, like **6**, produced no calcium elevation above controls even at 100 μg/kg/day, a dose which was lethal for 1,25D3 (Fig. 4). In addition, the dose dependent weight loss seen with 1,25D3 was not observed with any of these hybrid analogues (data not shown).

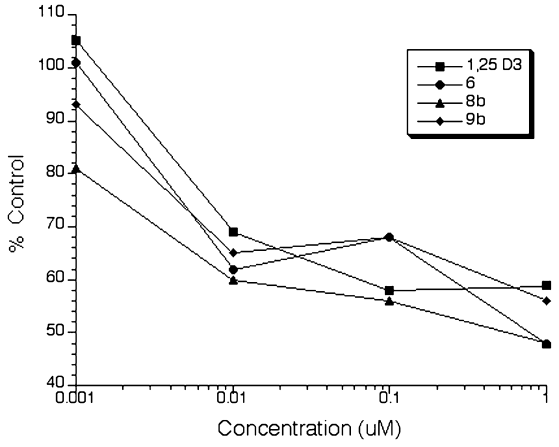


Figure 2. Dose–response effects of 1,25D3 and its analogues on murine B16 malignant melanoma cell proliferation. The average standard deviation for data points ($n=3$ for each) was ±10% of control.

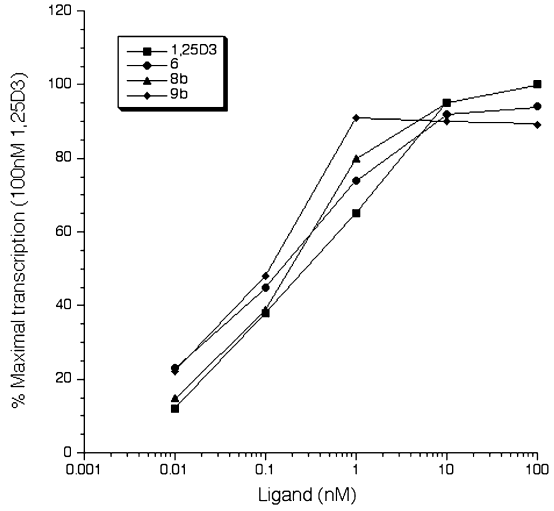


Figure 3. Transcriptional activation in rat osteosarcoma ROS, 17/2.8 cells in reponse to treatment with 1,25D3, **6**, and conformationally restricted hybrid analogues **8b** and **9b**.

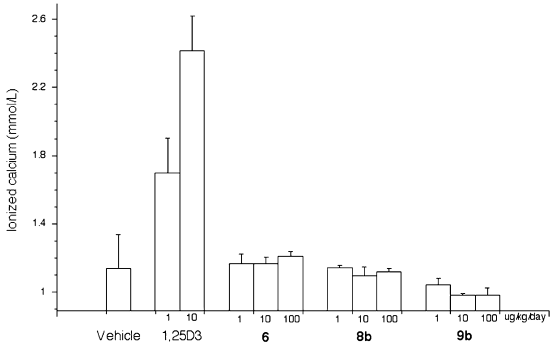


Figure 4. Ionized calcium levels in murine blood following 7 days of intraperitoneal injection of 1,25D3, **6**, and conformationally restricted hybrid analogues **8b** and **9b** at 1, 10, and 100 ug/kg/day. Values represent the mean ± standard deviation for three animals in each group.

Discussion

Hybrid analogues **8b** and **9b**, in which conformationally rigidifying units have been incorporated into their side-chain terminus, displayed a highly favorable biological profile similar to that of parent hybrid analogue **6**. The similarity in antiproliferative, transcriptional, and calcemic activities of **9b** and **6** are especially noteworthy since the presence of an acetylenic unit in **9b**'s side-chain terminus severely distorts the topology of the terminus away from one that could be adopted by **6** via σ bond rotation. On the basis of these preliminary studies, it appears as if the topology of the side-chain terminus, thought to be crucial for the analogue's binding to the VDR and eliciting a biological response, does not measurably influence the selective biological activities of hybrid analogues of 1,25D3 described in this study. These findings may prove significant in the future design of clinically relevant 1,25D3 analogues. For example, the bioavailabilty of compounds like **6** has been shown to be limited by metabolic hydroxylation at C-24 by the renal P-450 enzyme 24-hydroxylase. Incorporation of the acetylenic unit between C-24 and C-24a might interfere with this metabolic hydroxylation and thus increase an analogue's in vivo half-life without, as we have demonstrated herein, diminishing the analogue's desired biological activities.

Conclusion

Four new conformationally restricted hybrid analogues of the hormone 1α-25-dihydroxyvitamin D3 (1,25D3) have been synthesized in a convergent manner by combining enantiomerically pure C,D-ring ketones (−)-**15** and (−)-**17** with racemic 1-hydroxymethyl A-ring phosphine oxide (±)-**18**. Both conformationally restricted analogues of **6** (**8b** and **9b**), which incorporate rigidifying units at their 25-hydroxyl side-chain termini, retained the desirable antiproliferative, transcriptional, and calcemic activities of the parent compound. These findings may have pharmacologically favorable metabolic implications for the future design of clinically relevant 1,25D3 analogues.

Experimental

General

Tetrahydrofuran (THF) and diethyl ether (Et_2O) were distilled from benzophenone ketyl prior to use. Methylene chloride (CH_2Cl_2) and triethylamine (NEt_3) were distilled from calcium hydride prior to use. Commercially available anhydrous solvents were used in other instances. All reagents were purchased from Aldrich Chemical Co. (Milwaukee, WI, USA) and were used as received without further purification. FT-IR and UV spectra were recorded using a Perkin-Elmer Model 1600 FT-IR spectrophotometer and a Beckman Du-70 spectrophotometer, respectively. The ^1H and ^{13}C NMR spectra were recorded on a Varian XL-400 spectrometer operating at 400 and 100 MHz, respectively. Chemical shifts are expressed in parts per million downfield from tetramethylsilane. Unless otherwise indicated, all reactions were run under an Ar atmosphere. The purity of products was judged to be at least 95% on the basis of their chromatographic homogeneity.

Acetylenic alcohol (+)-11. To a solution of 1.22 g (3.91 mmol) of alcohol (+)-**10** dissolved in 15.0 mL of anhydrous THF was added 5.3 mL (5.3 mmol) of potassium *t*-butoxide (1.0 M solution in THF). The mixture was allowed to stir for 1 h at which time 1.4 g (5.3 mmol) of 18-crown-6 dissolved in 5.0 mL of anhydrous THF was added via cannula. The mixture was allowed to stir for an additional hour at which time 0.9 mL of propargyl bromide (80 wt.% solution in toluene) was added dropwise via syringe. Immediately upon addition of the propargyl bromide, a deep purple color developed and persisted. The mixture was allowed to stir for 12 h at which time it was quenched with 10.0 mL of water, extracted with EtOAc (3×, 50 mL), dried over $MgSO_4$, filtered, concentrated, and purified by silica gel chromatography (10% EtOAc/hexanes) to afford 182.0 mg (0.52 mmol) of propargylic ether in 13% yield and 972.0 mg (3.11 mmol) of recovered alcohol (+)-**10** in 80%

yield. This procedure was repeated 11 times to afford 589 mg (1.68 mmol) of the desired propargylic ether in a calculated 54% yield. An oven-dried 50.0 mL round bottomed flask equipped with a magnetic stirring bar was charged with 695 mg (1.98 mmol) of propargylic ether, 30.0 mL of anhydrous THF, 700.0 mg of activated 4 Å powdered molecular sieves (MS), and 1.04 g (4.0 mmol) of solid tetrabutylammonium fluoride hydrate (n-Bu_4NF, TBAF). The reaction mixture was refluxed for 2 days during which time additional TBAF (2.44g, 9.3 mmol) and molecular sieves (1.1 g) were added. The reaction mixture was cooled to room temperature, concentrated by rotary evaporation, and purified by silica gel chromatography (10% EtOAc/hexanes) to afford 368.0 mg (1.6 mmol) of acetylenic alcohol (+)-**11** in 80% yield: $[\alpha]_D^{28}$ +1.1° (c 2.7, EtOAc); ^{1}H NMR ($CDCl_3$) δ 4.17 (dd, J=2.4, 15.6 Hz, 1H), 4.07 (m, 1H), 4.06 (dd, J=2.4, 15.6 Hz, 1H), 3.54 (m, 1H), 2.37 (t, J=2.4 Hz), 2.13 (m, 1H), 1.07 (d, J=5.6 Hz, 3H), 0.98 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 80.46, 76.21, 73.67, 69.18, 56.74, 54.64, 52.05, 41.69, 40.01, 33.78, 24.66, 22.55, 17.63, 17.34, 14.05, IR ($CHCl_3$, cm^{-1}) 3619, 3307, 3008, 2935, 2872, 1456; HRMS m/z ($M^+NH_4^+$) calcd 254.2120 for $C_{15}H_{24}O_2{\cdot}NH_4^+$, found 254.2123.

Acetylenic diol (+)-12. An oven dried 25 mL round bottomed flask equipped with a magnetic stirring bar was charged with 198 mg (0.84 mmol) of acetylenic alcohol (+)-**11** and 10 mL of anhydrous THF. The solution was cooled to −50 °C and 1.3 mL (2.08 mmol) of n-BuLi (1.6 M solution in hexanes) was added. The solution was allowed to stir for 30 min at which time 0.2 mL (1.9 mmol) of cyclohexanone was added via syringe. Additional n-BuLi (1.2 mL, 1.9 mmol) followed by additional cyclohexanone (0.2 mL, 1.9 mmol) was added to the solution until the disappearance of all starting material was observed by thin layer chromatography (TLC). The reaction mixture was quenched at −50 °C with water, allowed to slowly warm up to room temperature, extracted with EtOAc (3×, 50 mL), dried over $MgSO_4$, filtered, concentrated, and purified by silica gel chromatography (30% EtOAc/hexanes) to yield 250 mg (0.75 mmol) of the desired acetylenic diol (+)-**12** in 90% yield: $[\alpha]_D^{28}$ +1.6° (c 0.88, EtOAc); ^{1}H NMR ($CDCl_3$) δ, 4.24 (d, J=15.6 Hz, 1H), 4.11 (d, J=16.0 Hz, 1H), 4.08 (m, 1H), 3.56 (m, 1H), 2.16 (m, 1H), 1.07 (d, J=6.0 Hz, 3H), 1.01 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 89.26, 80.76, 76.03, 69.21, 68.62, 56.74, 54.96, 52.04, 41.71, 40.05, 39.81, 39.74, 33.78, 25.12, 24.71, 23.14, 22.59, 17.70, 17.34, 14.15; IR ($CHCl_3$, cm^{-1}) 3597, 3007, 2938, 2863, 1451, 1375; HRMS m/z ($M^+NH_4^+$) calcd 352.2852 for $C_{21}H_{34}O_3{\cdot}NH_4^+$, found 352.2847.

Saturated diol (−)-13. To a 50.0 mL oven dried flask charged with 90.0 mg (0.27 mmol) of acetylenic diol (+)-**12** was added 10.0 mL of EtOH. The solution was purged with argon under reduced pressure (0.01 mm Hg) followed by rapid addition of 40.0 mg of Pd/C (Pd 10%) in 2.0 mL of EtOH via pippette. The hetrogeneous mixture was stirred over a hydrogen atmosphere at room temperature for 12 h at which time it was filtered through a silica gel plug (100% EtOH). The filtrate was evaporated and the residue was purified by silica gel chromatography (30% EtOAc/hexanes) to afford 47.0 mg (0.14 mmol) of saturated diol (−)-**13** in 52% yield: $[\alpha]_D^{28}$ −9.3° (c 1.2, EtOAc); ^{1}H NMR ($CDCl_3$) δ 4.08 (m, 1H), 3.56 (m, 1H), 3.26 (m, 2H), 2.20 (bs, 1H), 2.12 (m, 1H), 1.07 (d, J=6.0 Hz, 3H), 0.94 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 77.61, 70.72, 69.22, 68.83, 56.84, 52.06, 41.67, 40.21, 37.98, 37.17, 33.76, 25.89, 24.72, 23.78, 22.63, 22.34, 22.25, 18.10, 17.35, 14.27; IR ($CHCl_3$, cm^{-1}) 3378, 2935, 2870, 1455, 1374; HRMS m/z (M^+) calcd 338.2821 for $C_{21}H_{38}O_3$, found 338.2826.

Saturated ketone (−)-14. Solid tetrapropylammonium perruthenate (TPAP, 5.0 mg, 0.01 mmol) was added in one portion to a stirring mixture of diol (−)-**13** (47.0 mg, 0.14 mmol), 4-methyl morpholine N-oxide (NMO, 41.0 mg, 0.35 mmol), and 4 Å MS (100.0 mg) in 10.0 mL of anhyd CH_2Cl_2. Upon completion, the reaction mixture was diluted with a 50% EtOAc/hexanes solution and filtered through a silica gel plug. The filtrate was evaporated and the residue was purified by silica gel chromatography (30% EtOAc/hexanes) to afford 45.0 mg (0.13 mmol) of saturated ketone (−)-**14** in 93% yield: $[\alpha]_D^{28}$ −42.0° (c 0.81, EtOAc); ^{1}H NMR ($CDCl_3$) δ 3.57 (m, 1H), 3.24 (m, 2H), 2.46 (m, 1H), 1.1 (d, J=6.0 Hz, 3H), 0.64 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 212.09, 77.55, 70.81, 68.90, 61.43, 56.76, 49.86, 41.15, 38.88, 37.85, 37.32, 25.85, 25.02, 24.16, 23.75, 22.31, 22.25, 19.33, 18.18, 13.04; IR ($CHCl_3$, cm^{-1}) 3392, 2937, 2876, 2859, 1705, 1451, 1381; HRMS m/z (M^+) calcd 336.2664 for $C_{21}H_{36}O_3$, found 336.2669.

***O*-Silylated saturated ketone (−)-15.** An oven dried 25.0 mL round bottomed flask was charged with 45.0 mg (0.13 mmol) of tertiary alcohol (−)-**14** and 10.0 mL of anhydrous CH_2Cl_2. The reagent 1-(trimethylsilyl)-imidazole (TMS-imid., 0.11 mL, 0.71 mmol) was added dropwise via syringe. The mixture was stirred at room temperature for 12 h, quenched with 2.5 mL of H_2O, extracted with EtOAc (3×, 25.0 mL), dried over $MgSO_4$, filtered, concentrated and purified by silica gel chromatography (15% EtOAc/hexanes) to afford 51.0 mg (0.12 mmol) of O-silylated saturated ketone (−)-**15** in 92% yield: $[\alpha]_D^{28}$ −37.0° (c 0.87, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 3.58 (m, 1H), 3.24 (m, 1H), 3.14 (m, 1H), 2.46 (m, 1H), 1.09 (d, J=6.0 Hz, 3H), 0.65 (s, 3H), 0.10 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 212.17, 77.50, 75.40, 68.97, 61.52, 56.84, 49.93, 41.18, 38.94, 38.21, 38.17, 25.91, 25.07, 24.22, 24.15, 22.71, 19.39, 18.26, 12.95, 2.72; IR ($CHCl_3$, cm^{-1}) 2938, 2876, 2859, 1705; HRMS m/z (M^+H^+) calcd 409.3138 for $C_{24}H_{44}O_3Si{\cdot}H^+$, found 409.3141.

Acetylenic ketone (−)-16. Solid tetrapropylammonium perruthenate (TPAP, 10.0 mg, 0.03 mmol) was added in one portion to a stirring mixture of diol (+)-**12** (90.0 mg, 0.27 mmol), 4-methylmorpholine N-oxide (NMO, 63.0 mg, 0.54 mmol), and activated 4 Å MS (120.0 mg) in 14.0 mL of anhyd CH_2Cl_2. Upon completion, the reaction mixture was diluted with a solution of 50% EtOAc/hexanes and filtered through a silica gel plug. The filtrate was evaporated and the residue was purified by silica gel chromatography (30% EtOAc/hexanes) to

afford 83.0 mg (0.25 mmol) of acetylenic ketone (−)-**16** in 92% yield: $[\alpha]_D^{28}$ −28.3° (*c* 1.47, EtOAc); ^{1}H NMR ($CDCl_3$) δ 4.26 (d, *J* = 15.6 Hz, 1H), 4.12 (d, *J* = 15.6 Hz, 1H), 3.54 (m, 1H), 2.96 (m, 1H), 1.10 (d, *J* = 6.0 Hz, 3H), 0.70 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 212.04, 89.49, 80.44, 75.83, 68.60, 61.38, 56.62, 54.98, 49.86, 41.14, 39.80, 39.74, 38.73, 25.08, 24.99, 24.17, 23.12, 19.28, 17.68, 12.90; IR ($CHCl_3$, cm^{-1}) 3595, 3009, 2938, 2876, 2859, 1705, 1452, 1378; HRMS *m*/*z* (M^+) calcd 332.2351 for $C_{21}H_{32}O_3$, found 332.2356.

***O*-Silylated acetylenic C-8-ketone (−)-17.** An oven dried 25.0 mL round bottomed flask was charged with 68.0 mg (0.20 mmol) of alcohol (−)-**16** and 10.0 mL of anhyd CH_2Cl_2. The reagent 1-(trimethylsilyl)-imidazole (TMS-imid., 0.2 mL, 1.4 mmol) was added dropwise via syringe. The mixture was stirred at room temperature for 12 h, quenched with 5.0 mL water, extracted with EtOAc (3×, 25.0 mL), dried over $MgSO_4$, filtered, concentrated, and purified by silica gel chromatography (10% EtOAc/hexanes) to afford 74.0 mg (0.18 mmol) of the desired *O*-silylated product (−)-**17** in 90% yield: $[\alpha]_D^{28}$ −28.6° (*c* .58, EtOAc); ^{1}H NMR ($CDCl_3$) δ 4.27 (d, *J* = 16.0 Hz, 1H), 4.11 (d, *J* = 15.6 Hz, 1H), 3.54 (m, 1H), 2.46 (m, 1H), 1.1 (d, *J* = 5.6 Hz, 3H), 0.70 (s, 3H), 0.16 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 212.02, 89.88, 81.38, 75.80, 69.89, 61.40, 56.62, 55.04, 49.88, 41.16, 41.01, 38.75, 25.19, 25.03, 24.15, 23.02, 19.30, 17.58, 12.88, 1.99; IR ($CHCl_3$, cm^{-1}) 3003, 2938, 2858, 1705, 1451, 1378; HRMS *m*/*z* (M^+) calcd 404.2747 for $C_{24}H_{40}O_3Si$, found 404.2756.

Saturated hybrid analogues (−)-8a and (−)-8b. Racemic phosphine oxide A-ring (±)-**18** and C,D-ring ketone (−)-**15** were each azeotropically dried three times with anhydrous benzene and held under vacuum for 24 h immediately prior to use. Racemic phosphine oxide (±)-**18** (74.0 mg, 0.12 mmol) was dissolved in 1.0 mL of anhydrous THF (freshly distilled over benzophenone ketyl) and cooled to −78 °C under an argon atmosphere. To this was added 97.0 μL (0.17 mmol) of PhLi (1.8 M soln in cyclohexanes) dropwise during which time a deep red/orange color developed and persisted. The mixture was allowed to stir an additional 5 min at −78 °C at which time a pre-cooled (−78 °C) solution of C,D-ring ketone (−)-**15** (51.0 mg, 0.12 mmol) dissolved in 1.0 mL of anhydrous THF was transfered dropwise via cannula. The deep-red/orange solution was stirred in the dark for approximately 5 h during which time the color faded to light yellow. The reaction mixture was quenched at −78 °C with 2.0 mL of 2 N sodium potassium tartrate solution followed by addition of 1.0 mL of dilute aqueous potassium carbonate. The reaction mixture was allowed to warm to room temperature at which time it was extracted with EtOAc (3×, 25.0 mL), dried over $MgSO_4$, filtered, concentrated, and purified by silica gel chromatography (0.1% NEt_3, 10% EtOAc, hexanes) to afford 63.0 mg (0.08 mmol) of the coupled product in 64% yield. The coupled product was immediately placed in an oven dried 25.0 mL flask and dissolved in 5.0 mL of anhyd THF with 20.0 μL of NEt_3 under Ar. To this solution was added 364.0 mg (1.4 mmol) of TBAF and 100.0 mg of activated 4 Å MS. The reaction mixture was stirred at room temperature for 12 h in the dark. The solvent was evaporated and the mixture was purified by silica gel chromatography (1% NEt_3/EtOAc) to afford 28.0 mg (0.06 mmol) of a mixture of two desilylated diastereomers (−)-**8a** and (−)-**8b** in 75% yield. The mixture of diastereomers was seperated using reverse-phase HPLC (C-18 semipreparative column, 27% H_2O/CH_3CN, 2.0 mL/min) to give (−)-**8a** (1α,3β, t_R 25.43 min, 27% yield), (−)-**8b** (1β,3α, t_R 27.96 min, 25% yield) and 20% was left as a mixture: (−)-**8a:** $[\alpha]_D^{28}$ −149.0° (*c* 0.47, MeOH); ^{1}H NMR ($CDCl_3$) δ 6.32 (d, *J* = 10.8 Hz, 1H), 5.93 (d, *J* = 11.6 Hz, 1H), 5.16 (m, 1H), 5.01 (m, 1H), 3.95 (m, 1H), 3.55 (m, 3H), 3.25 (m, 2H), 2.81 (m, 1H), 2.62 (m, 2H), 2.25 (m, 1H), 1.08 (d, *J* = 6.0 Hz, 3H), 0.55 (s, 3H); ^{13}C NMR (CD_3OD) δ 147.79, 142.69, 136.71, 124.09, 119.14, 114.22, 79.87, 72.12, 70.24, 67.54, 64.82, 58.42, 57.24, 47.55, 47.12, 46.65, 41.96, 38.58, 38.32, 37.75, 30.20, 27.21, 26.28, 25.09, 24.81, 23.71, 23.49, 18.85, 13.13; UV (MeOH) λ_{max} 264 nm (ε 14 250); HRMS *m*/*z* (M^+) calcd 486.3709 for $C_{31}H_{50}O_4$, found 486.3718. (−)-**8b**: $[\alpha]_D^{28}$ −1.3° (*c* 0.47, MeOH); ^{1}H NMR ($CDCl_3$) δ 6.32 (d, *J* = 11.2 Hz, 1H), 5.92 (d, *J* = 11.2 Hz, 1H), 5.14 (m, 1H), 4.98 (m, 1H), 4.00 (m, 1H), 3.58 (m, 3H), 3.24 (m, 2H), 2.82 (m, 1H), 2.61 (m, 2H), 2.26 (m, 1H), 1.08 (d, *J* = 6.0 Hz, 3H), 0.52 (s, 3H); ^{13}C NMR (CD_3OD) δ 147.85, 142.87, 136.77, 124.03, 119.09, 113.95, 79.86, 72.12, 70.23, 67.58, 64.79, 58.37, 57.19, 47.54, 47.07, 46.53, 41.98, 38.58, 38.32, 37.70, 30.21, 27.20, 26.34, 25.08, 24.68, 23.57, 23.48, 18.84, 13.10; UV (MeOH) λ_{max} 263 nm (ε 16 371). HRMS *m*/*z* (M^+) calcd 486.3709 for $C_{31}H_{50}O_4$, found 486.3714.

Unsaturated hybrid analogues (−)-9a and (−)-9b. Racemic phosphine oxide A-ring (±)-**18** and C,D-ring ketone (−)-**17** were each azeotropically dried three times with anhydrous benzene and held under vacuum for 24 h immediately prior to use. Racemic phosphine oxide (±)-**18** (74.0 mg, 0.12 mmol) was dissolved in 1.0 mL of anhyd THF (freshly distilled over benzophenone ketyl) and cooled to −78 °C under an argon atmosphere. To this was added 88.0 μL (0.15 mmol) of PhLi (1.8 M soln in cyclohexanes) dropwise during which time a deep red/orange color developed and persisted. The mixture was allowed to stir an additional 5 min at −78 °C at which time a pre-cooled (−78 °C) solution of C,D-ring ketone (−)-**17** (61.4 mg, 0.15 mmol) dissolved in 1.0 mL of anhydrous THF was transferred dropwise via cannula. The deep red/orange solution was stirred in the dark for approximately 6 h during which time the color faded to light yellow. The reaction mixture was quenched at −78 °C with 2.0 mL of 2 N sodium potassium tartrate solution followed by addition of 1.0 mL of dilute aqueous potassium carbonate. The reaction mixture was allowed to warm to room temperature at which time it was extracted with EtOAc (3×, 25.0 mL), dried over $MgSO_4$, filtered, concentrated, and purified by silica gel chromatography (0.1% NEt_3, 10% EtOAc, hexanes) to afford 67.0 mg (0.08 mmol) of the coupled product in 68% yield. The coupled product was immediately placed in an oven dried 25.0 mL flask and dissolved in 5.0 mL of anhyd THF with 20.0 μL of NEt_3 under Ar. To this solution was added 374.0 mg (1.43

mmol) of TBAF and 100.0 mg of activated 4 Å MS. The reaction mixture was stirred at room temperature for 12 h in the dark. The solvent was evaporated and the mixture was purified by silica gel chromatography (1% NEt_3/EtOAc) to afford 29.0 mg (0.06 mmol) of a mixture of two desilylated diastereomers (−)-**9a** and (−)-**9b** in 70% yield. The mixture of diastereomers was seperated using reverse-phase HPLC (C-18 semipreparative column, 27% H_2O/CH_3CN, 2.0 mL/min) to give (−)-**9a** (1α,3β, t_R 16.49 min, 20% yield), and (−)-**9b** (1β,3α, t_R 18.22 min, 35% yield) and 20% was left as a mixture: (−)-**9a**: $[\alpha]_D^{28}$ −182.0° (*c* 0.56, MeOH); ^{1}H NMR ($CDCl_3$) δ 6.33 (d, *J* = 11.2 Hz, 1H), 5.94 (d, *J* = 11.2 Hz, 1H), 5.17 (m, 1H), 5.02 (m, 1H), 4.25 (d, *J* = 15.6 Hz, 1H), 4.11 (d, *J* = 15.6 Hz, 1H), 3.96 (m, 1H), 3.55 (m, 3H), 2.82 (m, 1H), 2.62 (m, 2H), 1.09 (d, *J* = 6.0 Hz, 3H), 0.62 (s, 3H); ^{13}C NMR (CD_3OD) δ 147.81, 142.72, 136.72, 124.08, 119.12, 114.19, 90.75, 81.55, 78.35, 69.24, 67.56, 64.82, 58.30, 57.17, 56.06, 47.54, 47.14, 46.62, 41.72, 40.97, 40.93, 37.77, 30.22, 26.54, 26.19, 24.86, 24.47, 23.62, 18.39, 13.19; UV (MeOH) λ_{max} 264 nm (ε 18 905); HRMS *m*/*z* (M^+) calcd 482.3396 for $C_{31}H_{46}O_4$, found 482.3404. (−)-**9b**: $[\alpha]_D^{28}$ −5.0° (*c* 0.43, MeOH); ^{1}H NMR ($CDCl_3$) δ 6.32 (d, *J* = 11.2 Hz, 1H), 5.92 (d, *J* = 11.6 Hz, 1H), 5.15 (m, 1H), 4.99 (m, 1H), 4.25 (d, *J* = 16.0 Hz, 1H), 4.11 (d, *J* = 15.6 Hz, 1H), 4.00 (m, 1H), 3.56 (m, 3H), 2.82 (m, 1H), 2.61 (m, 2H), 1.09 (d, *J* = 6.0 Hz, 3H), 0.59 (s, 3H); ^{13}C NMR (CD_3OD) δ 147.86, 142.91, 136.77, 124.04, 119.08, 113.96, 90.74, 81.55, 78.34, 69.23, 67.58, 64.80, 58.25, 57.12, 56.05, 47.54, 47.09, 46.53, 41.75, 40.97, 40.92, 37.71, 30.23, 26.53, 26.25, 24.73, 24.46, 23.50, 18.40, 13.17. UV (MeOH) λ_{max} 263 nm (ε 19 143). HRMS *m*/*z* (M^+) calcd 482.3396 for $C_{31}H_{46}O_4$, found 482.3399.

Determination of serum calcium levels

Female C57 Bl-6 mice (*n* = 3) received daily intraperitoneal injections of one of four compounds (1,25D3, **6**, **8b**, and **9b**) at 1, 10, or 100 μg/kg in 100 μL of vehicle (80% propylene glycol/20% 0.05 M phosphate buffered saline). Mice were monitored daily for weight loss as an indicator of drug toxicity; 1,25D3 at 100 μg/kg/day was fatally toxic by day 4. At day 7, mice were anesthetized with intraperitoneal injection of 200 μL of a stock solution containing 25 mg/mL ketamine hydrochloride, 2.5 mg/mL xylazine, and 14.25% ethanol all diluted 1:3 in 0.9% sodium chloride solution. Each blood sample was collected via cardiac puncture into a heparanized gastight syringe which was immediately sealed and placed on ice. Samples were then quantitatively analyzed within 2.5 h for ionized calcium content using a Chiron 865 instrument in the Critical Care Lab at Johns Hopkins Hospital. Results are expressed as the mean ± SD from three animals in each group. The pH values of all samples were determined concomitantly with the same instrument and were within normal limits.

Supplementary Material

Proton and ^{13}C NMR spectra for compounds **11**–**17** and **8**–**9** are available.

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