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Fluoride-Mediated Elimination of Allyl Sulfones: Application to the Synthesis of a 2,4-Dimethyl-A-ring Vitamin D_3 Analogue

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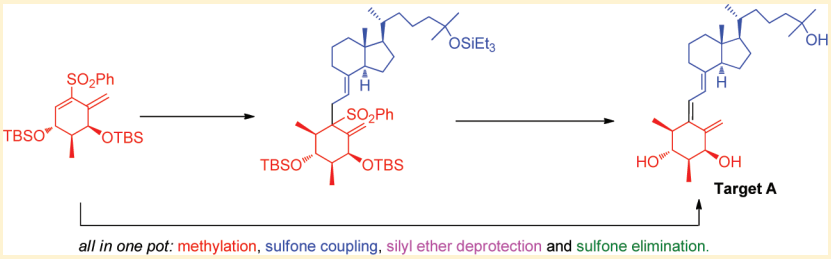
Supporting Information



ABSTRACT: A coupling strategy for the synthesis of 2,4-dimethyl-1α,25(OH)$_2$D$_3$ is achieved which involves methylation of a pro-A ring vinyl sulfone and in situ traping of the allyl sulfonyl anion with a CD ring allyl chloride. TBAF-promoted 1,2-eliminative desulfonylation and concomitant silyl ether deprotection gives the vitamin D_3 analogue.

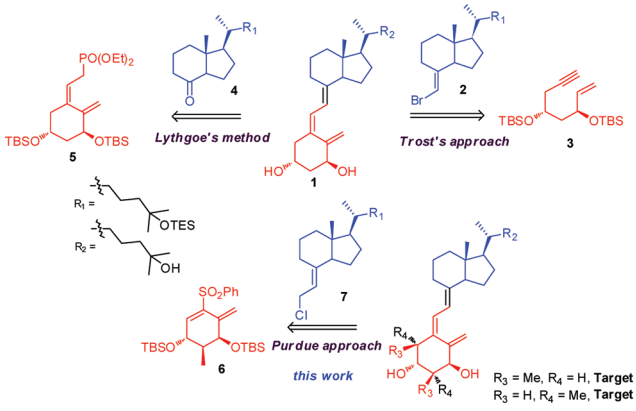
INTRODUCTION

1,25-Dihydroxyvitamin D_3 [1α,25(OH)$_2$D$_3$], the biologically most active metabolite of vitamin D_3, is involved in the regulation of calcium homeostasis and bone metabolism. In addition, it suppresses the growth of numerous human cancer cell lines by inhibiting cell cycle progression and inducing cell death.[1] Several studies have revealed that these biological functions are mediated through binding of 1α,25(OH)$_2$D$_3$ to the vitamin D receptor (VDR),[2] a nuclear protein which belongs to the nuclear receptor superfamily.[3]

More than 2000 analogues of vitamin D_3 have been synthesized because of the potential therapeutic application of 1α,25(OH)$_2$D$_3$ and its analogues in the treatment of cancer and other proliferative diseases. Its properties in modulating cell differentiation, inhibiting cell proliferation, and regulating apoptosis make it an important candidate for the treatment of cancer. 1α,25(OH)$_2$D$_3$ and some of its analogues are on the market or are in clinical trials.[4] Several laboratories over the last three decades have worked on the development of different pathways to generate analogues of 1α,25(OH)$_2$D$_3$. General synthetic approaches for vitamin D can be categorized into three types. The first pathway involves biogenetic photochemical ring-opening of steroid precursors,[5] which is not well-suited for synthesis of analogues. A convergent approach featuring formation of the triene via an ene−yne A-ring synthon was developed by Trost and is widely employed for the analogue synthesis.[6] A classical approach involves coupling a preformed A ring with the CD ring system. The most popular A+CD strategy employs a Horner coupling developed by Lythgoe,[7] although this route suffers due to the number of steps required for preparation of A-ring synthons.

This paper describes a convergent approach for synthesis of a variety of vitamin D analogues bearing A-ring modifications with substitution at the 2- and 4-positions. The synthetic strategy exploits cyclohexenyl sulfone chemistry[8] in combination with a CD ring allyl chloride (Scheme 1). Since the β-chair is deemed responsible for biological activity of 1α,25(OH)$_2$D$_3$,[9] two dimethyl analogues of 1α,25(OH)$_2$D$_3$ were desired for evaluation (targets **A** and **B**), one with a conformationally fixed β-chair and the other with an α-chair similarly locked by 1,3 diaxial methyl interactions.[9]

Scheme 1. Widely Used Approaches for Making 1α,25(OH)$_2$D$_3$ and the Purdue Strategy for Dimethyl 1α,25(OH)$_2$D$_3$



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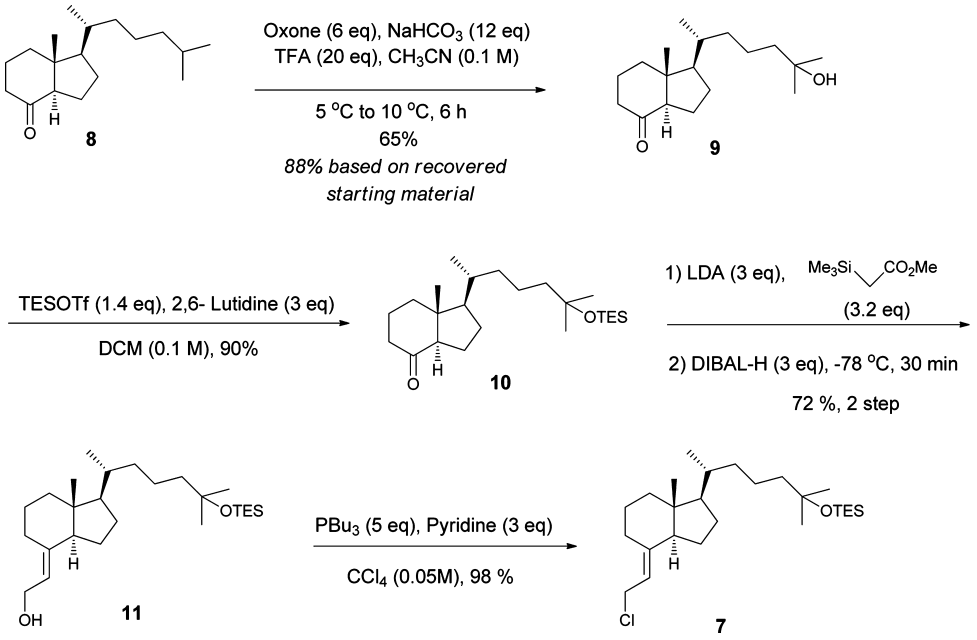
Scheme 2. Synthesis of CD Allyl Chloride 7



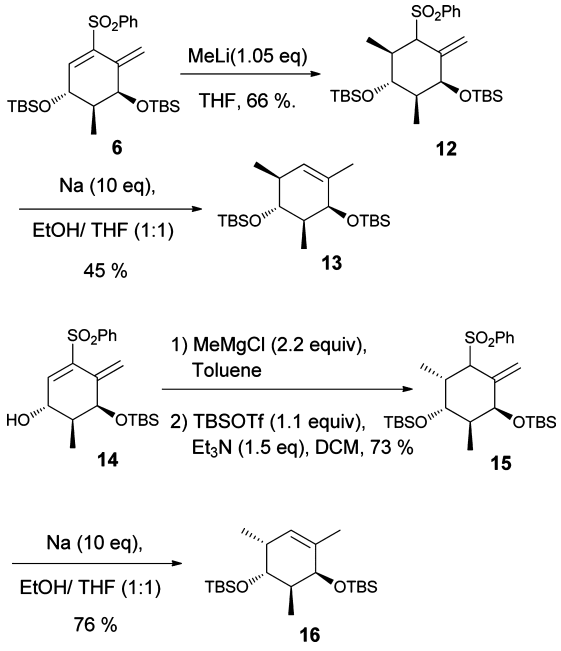
RESULTS AND DISCUSSION

CD segment **7** was synthesized from Grundman's ketone **8**, which was obtained from the known oxidative cleavage of vitamin D (Scheme 2).[10] DMDO is known to effect the C-25 hydroxylation[11] but is slow (48 h), whereas preformed TFDO[12] requires 16 h for reaction and its synthetic preparation and isolation is tedious considering the volatility of this dioxirane. In situ TFDO generated from Oxone and 1,1,1-trifluoroacetone is far more convenient, affording **9** in 6 h at 10 °C. The silyl ether protection of **9** proceeds in high yield, and Peterson olefination of ketone **10**[13] and reduction of vinyl ester using DIBAL-H smoothly provided *E*-allyl alcohol **11**. *E*-Allyl chloride **7** was obtained from alcohol **11** using the Appel reaction.[14]

Conjugate methylation of vinyl sulfone **6**[9] did not proceed using mild reagents (Me_2TiCl, $AlMe_3$, $LiAlMe_4$, MeMgBr, MeMgCl, $MeCeCl_2$) (Scheme 3). However, addition of 1.05 equiv of MeLi to the vinyl sulfone **6** at room temperature gave allyl sulfone **12** in 66% yield.[15] To establish the stereochemistry of the methylation, hydroxy-directed methylation of vinyl sulfone **14**[9] using MeMgCl was also performed.[16] Allyl sulfone **15** was obtained in 73% yield after silyl ether protection.[15] Reductive desulfonylation of allyl sulfones **12** and **15** using sodium in ethanolic THF[17] gave the corresponding functionalized cyclohexene derivatives **13** and **16**, respectively. No common peaks were seen in the NMR spectra of **13** and **16** establishing >95% diastereoselectivity in each of the individual methylation reactions.

To effect the one-pot synthesis of target **A**, the following procedure was established. Vinyl sulfone **6** was treated with 1.05 equiv of MeLi which, as shown above, led to the installation of a methyl group at the 4-position. The allylsulfonyl anion **6a** thus created was treated at room temperature with 1.1 equiv of CD ring allyl chloride **7** to give the coupled product **6b**. In the same pot, 20 equiv of commercial TBAF/THF was added, followed by heating at 60 °C for 6 h. Triene **A** was isolated through this procedure in 25% yield (Scheme 4).

Scheme 3. Methylation of Vinyl Sulfones 6 and 14



There are numerous examples of fluoride-mediated 1,2- and 1,4-eliminations of β-silyl sulfones[18] and β-silyl azides,[19] which proceed via fluorosiliconate intermediates. Similarly, formation of 1,3-dienes and 1,3,5-trienes via Julia-type KO-*t*-Bu elimination of phenylsulfinic acid is also well-known.[20] TBAF and related fluoride source have been used for the various coupling reactions where they act as a source of catalytic base[21−23] and β-elimination of mesylates;[24] Cl, Br, and I using such reagents (KF, CsF, TEAF) also have been reported.[23] It was envisioned that the nucleophilic/basic nature of TBAF could be used for the installation of triene unit and as well as the deprotection of the silyl groups. In the event, using 20 equiv of TBAF led to the β-elimination of phenylsulfinate and thus completed the synthesis of the target **A**.

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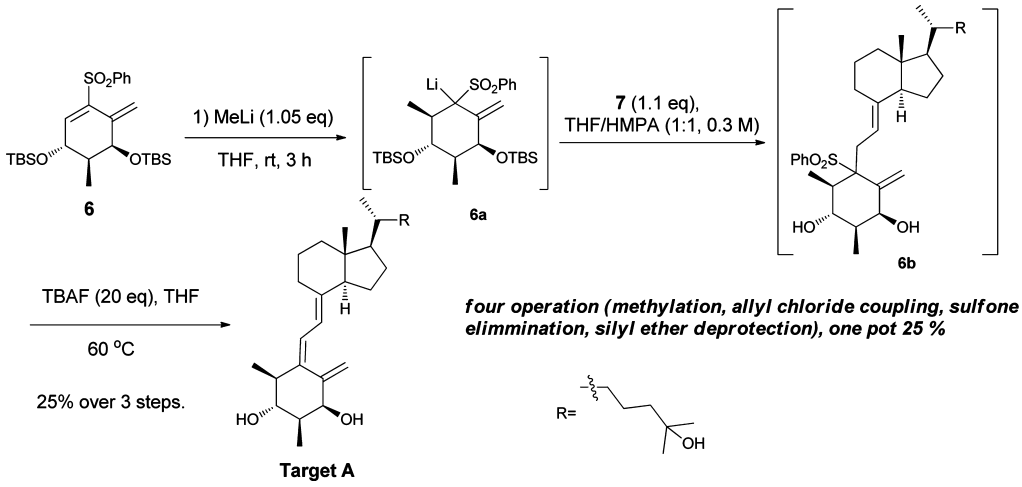
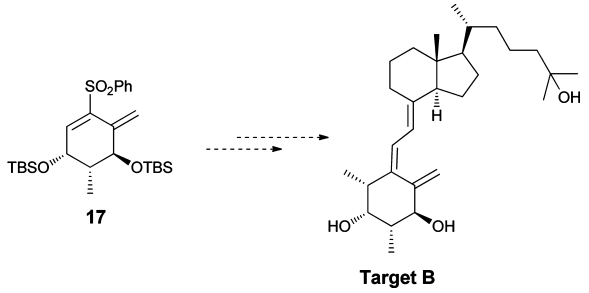
Scheme 4. Synthesis of Target A



Target B could similarly be prepared from A-ring vinyl sulfone **17** following Schemes 3 and 4 (Scheme 5). The synthesis of vinyl sulfone **17** has been previously reported from our group.[9]

Scheme 5. Proposed Synthesis of Target B



The impact target A on the expression of vitamin D-responsive genes in two human colon cancer cell lines was examined: Caco-2 cells and SW480-ADH (Figure 1). These cells respond to 1,25-dihydroxyvitamin D_3 treatment by inducing the expression of various target genes. The most responsive vitamin D regulated gene is for the enzyme CYP24 (a cytochrome P450 family member called 25 hydroxyvitamin D, 24 hydroxylase); this is expressed in both cell lines. In SW480-ADH cells, calcitriol treatment also induces expression of E-cadherin, a tight junction protein that is a marker of cell differentiation. At 1 nM and 10 nM target A was found to be less potent than 1,25 dihydroxyvitamin D_3, but at 100 nM target A had the same activity as $1\alpha,25(OH)_2D_3$ in Caco-2 cell lines and was found to have twice the activity for cell differentiation in SW480-ADH cell lines.

In summary, we have reported a highly convergent synthesis of target A which looks reasonable for the synthesis of Vitamin D_3 analogs with functionalization in the A-ring. Target A was found to be twice as active as $1\alpha,25(OH)_2D_3$ in cell differentiation in SW480-ADH cell lines.

EXPERIMENTAL SECTION

General Methods. Tetrahydrofuran (THF) was distilled from benzophenone ketyl. Sodium sulfate (Na_2SO_4) was anhydrous. Unless otherwise indicated, all reactions were carried out under in a positive pressure of argon in anhydrous solvents and the reaction flasks were fitted with rubber septa for the introduction of substrates and reagents via syringe. Progress of reactions was monitored by thin-layer chromatography (TLC) using silica gel plates. The TLC plates were

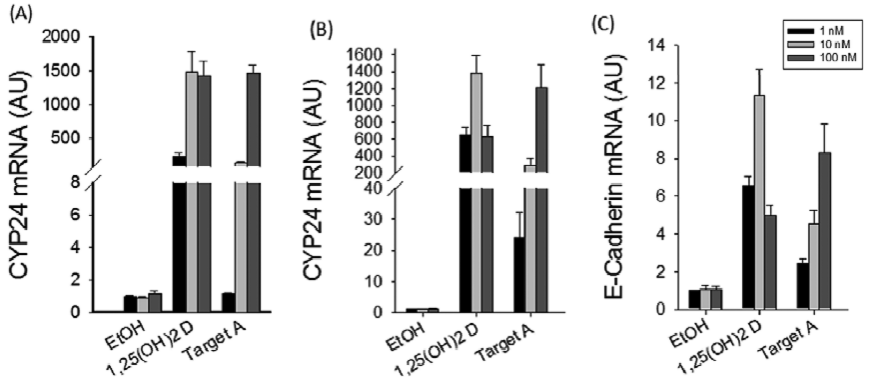


Figure 1. Effect of target A on expression of two vitamin D responsive genes in the human colonic carcinoma cell lines, Caco-2 and SW-480ADH: (A) CYP24 mRNA in Caco-2, (B) CYP24 mRNA in SW480-ADH, (C) E-cadherin mRNA in SW480-ADH. Cells were treated for 6 h with varying doses of vitamin D compounds or with concentration matched ethanol vehicle (EtOH). RNA was isolated and analyzed by real time-PCR. Data are normalized to the expression of the control gene RPLPO. Bars represent the mean ± SEM.

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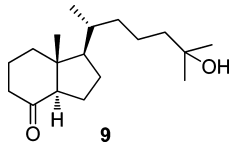
visualized with a UV lamp (254 nm) and/or with TLC visualizing solutions activated with heat. The two commonly employed TLC visualizing solutions were: (i) *p*-anisaldehyde solution (1350 mL of absolute ethanol, 50 mL of concentrated H_2SO_4, 37 mL of *p*-anisaldehyde) and (ii) permanganate solution (weight percents of 1% $KMnO_4$ and 2% Na_2CO_3 in water).

^1H NMR and ^{13}C NMR spectra were recorded on 300−500 MHz spectrometers. The NMR spectra were determined in $CDCl_3$ solution. Peak multiplicities in ^1H NMR spectra, when reported, are abbreviated as s (singlet), d (doublet), t (triplet), m (multiplet), and/or quint (quintet). The mass analyzer instrument was calibrated to a resolution on 10,000 with a 10% valley between peaks using the appropriate perfluorokerosene standard. Electrospray ionization high resolution mass measurements utilized the appropriate polypropylene glycol standards. The mass analyzer used is a double-focusing sector mass spectrometer.

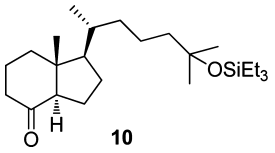
*Preparation of (1R,3aR,7aR)-1-((R)-6-Hydroxy-6-methylheptan-2-yl)-7a-methylhexahydro-1H-inden-4(2H)-one (**9**).*



9

Grundman's ketone **8** (60 mg, 0.23 mmol) was dissolved in 1 mL of CH_3CN/H_2O (10:1) and cooled to 8−10 °C. 1,1,1-Trifluoroacetone (2.3 mmol, 0.2 mL) was added followed by mixture of Oxone (424 mg, 0.69 mmol) and $NaHCO_3$ (116 mg, 1.38 mmol) in small portions over a period of 6 h. The reaction mixture was diluted with 5 mL of water and extracted with dichloromethane (10 mL). The combined extracts were washed with brine, dried over sodium sulfate, filtered, and concentrated under vacuum. The residues were purified on silica gel using ethyl acetate/hexane (1:1) to give the ketone **9** as colorless oil in 65% yield: ^1H NMR ($CDCl_3$, 300 MHz) 2.26 (2H, m), 2.10- 1.09 (17 H, m), 1.039 (6H, s), 0.79 (3H, d, J = 6 Hz), 0.46 (3H, s); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 211.7, 70.2, 61.4, 56.1, 49.5, 43.8, 40.4, 38.4, 35.8, 35.0, 28.9, 28.7, 27.0, 23.6, 20.3, 18.6, 18.2, 12.0; HRMS (EI/CI) calcd for $C_{18}H_{32}O_2$ $(M - H_2O)^+$ 262.2297, found 262.2296.

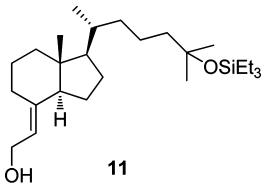
*Preparation of (1R,3aR,7aR)-7a-Methyl-1-((R)-6-methyl-6-((triethylsilyl)oxy)heptan-2-yl)hexahydro-1H-inden-4(2H)-one (**10**).*



10

Ketone **9** (700 mg, 2.5 mmol) was dissolved in CH_2Cl_2 (200 mL) and cooled to −78 °C. 2,6-Lutidine (803 mg, 0.87 mL, 7.5 mmol) was added followed by TESOTf (0.79 mL, 3.5 mmol). The reaction mixture was stirred for 25 min and then quenched by adding water (30 mL). The organic layer was separated and dried over sodium sulfate. The organic layer was filtered and concentrated using a rotovap. The residue was purified on silica gel using 15% ethyl acetate/hexane to give 886 mg (90%) of ketone **10** as a colorless oil: ^1H NMR ($CDCl_3$, 300 MHz) 2.31 (2H, dd, J = 8 Hz, 11 Hz), 2.20−1.16 (25 H, m), 1.09 (6H, s), 0.84 (4H, t, J = 8 Hz), 0.54 (3H, s), 0.42 (6H, q, J = 7 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 211.3, 73.0, 61.7, 56.5, 49.6, 45.2, 40.7, 38.8, 36.0, 35.3, 29.7, 29.5, 27.3, 23.8, 20.4, 18.8, 18.4, 12.1, 6.8, 6.6; HRMS (EI/CI) calculated for $C_{24}H_{46}O_2Si$ (M + H) 395.3345, found 395.3348.

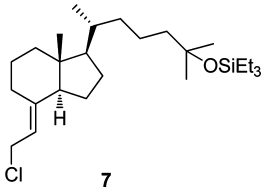
*Preparation of (E)-2-((1R,3aS,7aR)-7a-Methyl-1-((R)-6-methyl-6-((triethylsilyl)oxy)heptan-2-yl)hexahydro-1H-inden-4(2H)-ylidene)-ethanol (**11**).*



11

BuLi (4.76 mmol, 1.9 mL) was added dropwise to a solution of diisopropylamine (4.76 mmol, 0.67 mL) in THF (3 mL) at 0 °C, and the reaction mixture was stirred for 30 min and then cooled to −78 °C. Ethyl (trimethylsilyl)acetate (7.20 mmol, 1.3 mL) was added dropwise, and the resulting yellow solution was allowed to warm to −20 °C gradually over 1 h. The solution was then cooled to −78 °C, ketone **10** (550 mg, 1.40 mmol) dissolved in THF (15 mL) was added, and the solution was brought to room temperature and stirred for 8 h. The reaction was quenched by addition of water (20 mL) and extracted with ethyl acetate (30 mL). The organic layer was separated, washed with brine, and dried over sodium sulfate. The organic layer was filtered and concentrated on a rotovap to give a dark orange oil. The oil was dissolved in toluene (30 mL) and cooled to −78 °C. DIBAL-H (5.6 mmol, 3.7 mL) was added and the reaction mixture stirred for 1.5 h and then quenched by addition of water (20 mL). The solution was diluted with EtOAc (30 mL) and the organic layer separated, washed with brine, and dried over sodium sulfate. The organic layer was filtered, concentrated, and purified on silica gel using EtOAc/hexane (1:1) to give allyl alcohol **11** (425 mg, 72%) as a colorless oil: ^1H NMR ($CDCl_3$, 300 MHz) 5.20 (1H, t, J = 7 Hz), 4.22 (1H, s), 2.61 (1H, m), 2.03−1.87 (3H, m), 1.70−0.92 (28H, m), 0.53 (6H, m); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 143.4, 119.1, 73.3, 58.4, 56.5, 55.5, 45.4, 45.2, 40.3, 36.3, 36.0, 29.9, 29.7, 28.6, 27.5, 23.4, 22.1, 20.7, 18.7, 11.7, 7.0, 6.7; HRMS (CI) calcd for $C_{26}H_{50}O_2Si$ $(M + H - H_2O)$ 405.3553, found 405.3546.

*Preparation of (((R)-6-((1R,3aS,7aR,E)-4-(2-Chloroethylidene)-7a-methyloctahydro-1H-inden-1-yl)-2-methylheptan-2-yl)oxy)-triethylsilane (**7**).*



7

Allyl alcohol **11** (100 mg, 0.24 mmol) was dissolved in CCl_4 (10 mL) and cooled to 0 °C. Pyridine (0.71 mmol, 57 μL) was added followed by PBu_3 (1.2 mmol, 0.3 mL) and the reaction mixture stirred for 2 h. Pentane (10 mL) was then added resulting in the formation of white precipitate which was filtered and filtrate concentrated under vacuum. The residue was purified on silica gel using pentane to give allyl chloride **7** (98%, 103 mg) as a colorless oil: ^1H NMR ($CDCl_3$, 300 MHz) 5.21 (1H, t, J = 8 Hz), 4.13 (1H, d, J = 8 Hz), 2.64 (1H, m)

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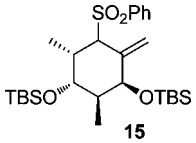
Table 1. Caco-2 Summary

treatment	1 nM	SEM	10 nM	SEM	100 nM	SEM
ethanol	1.00	0.08	0.92	0.10	1.19	0.20
1,25D	233.31	57.10	1479.96	305.84	1417.24	216.53
target A	1.19	0.07	148.63	8.62	1462.80	120.34

2.04−1.19 (22H, m), 0.929 (16H, t, J = 8 Hz), 0.53 (12H, m); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 146.7, 115.9, 73.3, 56.4, 55.5, 45.7, 45.4, 40.5, 40.1, 36.2, 35.9, 29.9, 29.7, 28.4, 27.5, 23.3, 21.9, 20.7, 18.7, 11.6, 7.0, 6.7; HRMS (EI) calcd for $C_{25}H_{46}ClOSi$ (M^+) 425.3006, found 425.3002.

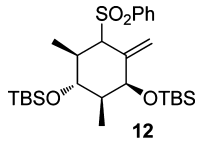
*Preparation of (((1S,2S,3S,4S)-2,4-Dimethyl-6-methylene-5-(phenylsulfonyl)cyclohexane-1,3-diyl)bis(oxy))bis(tert-butyldimethylsilane) (**15**).*



15

Vinyl sulfone **14** (50 mg, 0.13 mmol) was dissolved in toluene (0.2 mL) and cooled to −10 °C. MeMgCl (0.286 mmol, 95 μL), 3 M in THF, was added dropwise and the resulting dark yellow solution warmed to room temperature and stirred for 4 h. The reaction was quenched by addition of water (3 mL) and extracted with ether (10 mL). The organic layer was washed with brine, dried over sodium sulfate, and concentrated to give a colorless residue. The residue was dissolved in CH_2Cl_2 (1 mL) and triethylamine (0.195 mmol, 27 μL) followed by TBSOTf (0.143 mmol, 33 μL) was added and the reaction stirred for 1 h. The reaction mixture was quenched by addition of water (3 mL) and diluted with CH_2Cl_2 (10 mL). The combined extracts were washed with brine, dried over magnesium sulfate, filtered, and concentrated under vacuum. The residue was purified on silica gel using 1:5 ethyl acetate/hexane to give the allyl sulfone **15** in 73% yield (48 mg) as a colorless oil: ^{1}H NMR ($CDCl_3$, 300 MHz) 7.92 (2H, d, J = 6 Hz), 7.51 (3H, m), 4.36 (1H, dd, J = 3 Hz, 9 Hz), 4.22 (1H, d, J = 3 Hz), 3.67 (1H, d, J = 3 Hz), 2.32 (1H, m), 1.77 (1H, m), 0.95 (9H, s), 0.92 (9H, s), 0.91 (3H, d, J = 6 Hz), 0.83 (3H, d, J = 6 Hz), 0.14 (3H, s), 0.12 (3H, s), 0.08 (3H, s), 0.07 (3H, s); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 141.2, 138.5, 132.9, 128.9, 128.1, 121.1, 75.3, 72.5, 70.6, 39.5, 35.5, 26.0, 25.9, 18.3, 18.0, 13.9, 13.5, −4.1, −4.5, −4.6, −4.8; HRMS (ESI) calcd for $C_{27}H_{48}O_4SSi_2$ (M + Na) 547.2710, found 547.2706.

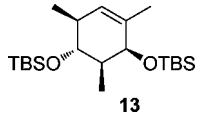
*Preparation of (((1S,2S,3S,4R)-2,4-Dimethyl-6-methylene-5-(phenylsulfonyl)cyclohexane-1,3-diyl)bis(oxy))bis(tert-butyldimethylsilane) (**12**).*



12

Vinyl sulfone **6** (50 mg, 0.098 mmol) was dissolved in THF (0.15 mL), and MeLi (0.103 mmol, 34 μL) was added dropwise at room temperature. The reaction was quenched by addition of water (30 μL) after 3 h and loaded directly on silica gel and eluted with 1:10 ethyl acetate/hexane to give allyl sulfone **12** (34 mg) in 66% yield as a colorless oil: ^{1}H NMR ($CDCl_3$, 300 MHz): 7.81 (2H, d, J = 6 Hz), 7.47 (3H, m), 5.28 (1H, s), 5.24 (1H, s), 4.37 (1H, s), 3.51 (1H, dd, J = 3 Hz, 6 Hz), 2.87 (1H, m), 2.00 (1H, m), 0.97 (9H, s), 0.94 (9H, s), 0.93 (3H, d, J = 6 Hz), 0.79 (3H, d, J = 6 Hz), 0.13 (6H, s), 0.10 (6H, s); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 138.5, 133.1, 129.0, 128.5, 128.4, 117.6, 78.0, 74.4, 68.0, 44.5, 35.1, 25.8, 20.4, 18.1, 11.5, −4.7, −4.9, −5.1; HRMS (ESI) calcd for $C_{27}H_{48}O_4SSi_2$ (M + Na) 547.2710, found 547.2707.

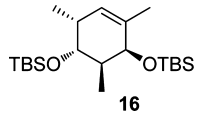
*Reductive Desulfonylation of Allyl Sulfone **12**.*



13

Allyl sulfone **12** (30 mg, 0.057 mmol) was dissolved in THF/EtOH (3 mL, 5:1). The solution was cooled to 0 °C, and Na (13 mg, 0.57 mmol) was added. The reaction mixture was stirred for 8 h at room temperature, and excess sodium was quenched using CH_3OH until the H_2 evolution ceased. The solution was concentrated and loaded on the silica gel column. Flash column chromatography using hexane mixture gave the alkene **13** as a colorless oil in 45% yield (10 mg): ^{1}H NMR ($CDCl_3$, 300 MHz) 5.14 (1H, dd, J = 2 Hz, 2.4 Hz), 4.02 (1H, d, J = 3 Hz), 3.47 (1H, dd, J = 7 Hz, 8 Hz), 2.03(2H, m), 1.70 (3H, s), 1.04 (3H, d, J = 7 Hz), 0.94 (3H, d, J = 7 Hz), 0.91 (18 H, s), 0.08 (6H, s), 0.06 (6H, s); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 134.3, 128.3, 75.6, 73.4, 42.4, 40.4, 26.0, 21.2, 19.6, 18.2, 14.4, −3.7, −3.9; HRMS (CI) calcd for $C_{21}H_{44}O_2Si_2$ (M +) 384.2880, found 384.2882.

*Reductive Desulfonylation of Allyl Sulfone **15**.*



16

Table 2. SW480 ADH Summary

		CYP24			E-cadherin		
compd	dose (nM)	mean	SD	SEM	mean	SD	SEM
ethanol	1	1.00	0.15	0.08	1.00	0.07	0.03
	10	1.03	0.19	0.09	1.07	0.43	0.22
	100	1.12	0.71	0.35	1.07	0.37	0.18
1,25(OH)2 D	1	653.35	92.18	46.09	6.57	0.99	0.50
	10	1380.02	211.28	105.64	11.36	2.65	1.32
	100	634.80	257.35	128.68	4.97	1.16	0.58
target A	1	24.16	8.00	4.00	2.43	0.57	0.28
	10	282.53	86.34	43.17	4.57	1.40	0.70
	100	1214.95	451.17	260.48	8.29	2.61	1.51

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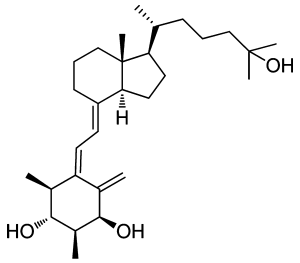
Allyl sulfone **15** (30 mg, 0.057 mmol) was dissolved in THF/EtOH (3 mL, 5:1). The solution was cooled to 0 °C, and Na (13 mg, 0.57 mmol) was added. The reaction mixture was stirred for 8 h at room temperature, and excess sodium was quenched using CH_3OH until the H_2 evolution ceased. The solution was concentrated and loaded on the silica gel column. Flash column chromatography using hexane mixture gave the alkene **16** as colorless oil in 76% yield (16 mg): ^{1}H NMR ($CDCl_3$, 300 MHz) 5.08 (1H, s), 4.34 (1H, s), 3.76 (1H, t, J = 6 Hz), 2.30 (1H, m), 2.03 (1H, m), 1.69 (3H, s), 0.87 (m, 24H), 0.06 (12H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 134.1, 126.0, 74.4, 71.1, 40.2, 32.5, 25.9, 20.7, 18.2, 18.1, 16.2, 12.0, −4.1, −4.5, −4.7; HRMS (CI) calcd for $C_{21}H_{44}O_2Si_2$ (M + H) 384.2880, found 384.2882.

One-Pot Synthesis of (1S,2S,3R,4S,Z)-5-((E)-2-((1R,3aS,7aR)-1-((R)-6-Hydroxy-6-methylheptan-2-yl)-7a-methylhexahydro-1H-inden-4(2H)-ylidene)ethylidene)-2,4-dimethyl-6-methylenecyclohexane-1,3-diol (Target A).



Target A

Vinyl sulfone **6** (30 mg, 0.098 mmol) was dissolved in THF (0.15 mL), and MeLi (0.103 mmol, 34 μL) was added dropwise at room temperature. After the red solution was stirred for 3 h, allyl chloride **7** (0.108 mmol, 47 mg) dissolved in 0.15 mL of HMPA was added and reaction mixture stirred for another 6 h. H_2O (10 μL) and THF (3 mL) were added followed by TBAF (2 mL, 0.98 mmol) and 1 M solution in THF, and the solution was heated to 60 °C. After 8 h of heating, the reaction mixture was diluted with $NaHCO_3$ (1 mL) and extracted with CH_2Cl_2 (10 mL). The combined extracts were washed with brine, dried over sodium sulfate, filtered, and concentrated under vacuum. The residues were purified on silica gel using ethyl acetate/hexane (1:1) to give target A as a colorless oil in 25% yield (9.5 mg): ^{1}H NMR ($CDCl_3$, 400 MHz) 6.46 (1H, d, J = 12 Hz), 6.01 (1H, d, J = 12 Hz), 5.23 (1H, s), 4.89 (1H, s), 4.16 (1H, s), 3.25 (1H, t, J = 8 Hz), 2.84 (1H, dd, J = 12 Hz, 4 Hz), 2.23−0.93 (35H, m), 0.53 (3H, s); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 147.6, 143.4, 137.5, 121.9, 117.2, 113.9, 77.7, 71.1, 56.5, 56.4, 46.1, 45.8, 44.8, 44.3, 40.5, 36.4, 36.1, 29.4, 29.2, 29.1, 27.7, 23.4, 22.2, 20.9, 18.8, 14.2, 13.9, 12.1; HRMS (ESI) calcd for $C_{29}H_{48}O_3$ (M + Na) 467.3501, found 467.3496.

Cell Culture Experiment. Cells were seeded in 12-well dishes (100,000 cells/well for Caco-2; 50000 cells/well for SW480-ADH) and studied when they reached 70−80% confluence (3 days in culture). Cells were treated with 1,25-dihydroxyvitamin D or analogues in high glucose DMEM supplemented with Pen/Strep but without fetal bovine serum (FBS) (Tables 1 and 2). Cell culture conditions have been previously described.[25] Vitamin D compounds were diluted in ethanol and used at three concentrations: 1, 10, and 100 nM. Ethanol control groups were included for all of the three treatments but total ethanol levels in the medium were 0.001, 0.01, and 0.1% for the three treatment groups, respectively. Four replicate wells were used for each of the vitamin D compounds. Six hours after the treatment started, the medium was removed and cells were harvested directly into TriReagent (Molecular Research Center, Inc., Cincinnati, OH). RNA was isolated, converted to cDNA, and analyzed by real-time PCR for mRNA levels for the two target genes using methods previously described.[26] Data were normalized to the expression level of the control gene RPLP0. Primers for human CYP24 and RPLPO have been described previously,[27] and the primers for E-cadherin are as follows: forward, $^{5'}$GATTGCAAATTCCTGCCATT$^{3'}$; reverse, $^{5'}$GCTGGCTCAAGTCAAAGTCC$^{3'}$.

■ ASSOCIATED CONTENT

Supporting Information

^{1}H/ ^{13}C NMR spectra of all new compounds. 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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