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# Synthesis and in vitro evaluation of side chain-unsaturated analogs of 24a,24b-dihomo-1,25-dihydroxycholecalciferol

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*A synthesis and an in vitro evaluation of side chain-unsaturated analogs **3** and **4** of 24a,24b-dihomo-1,25-dihydroxycholecalciferol (**1**) are described. Novel $C_{23a,24}$-vitamin D synthons (sulfone **10** and aldehyde **11**) were used for the synthesis of analog **4** and for the efficient preparation of the parent compound **1**. The synthetic approach developed allows the use of easily available side chain fragments, such as oxirane **12** or Wittig reagent **15** for the preparation of compound **1** and analog **4**, respectively. Introduction of a 24a*E *double bond results in a selective, 1000-fold increase in the binding affinity of analog **4** for the vitamin D receptor, compared to the affinity of **1**, whereas the affinity of **4** for the vitamin D-binding protein and the activity in stimulating the differentiation of human promyelocytic leukemia HL-60 cells remained largely unchanged.* (*Steroids* **62**:546–553, 1997) © *1997 by Elsevier Science Inc.*

**Keywords:** vitamin D analogs; vitamin D-binding protein; vitamin D receptor; cell differentiation; calcium activity

## Introduction

Structure-activity studies[1] revealed that elongation of the side chain of 1,25-dihydroxycholecalciferol [1,25-$(OH)_2D_3$] by one carbon unit[2] improves the cell differentiation activity by one order of magnitude. Side chain-elongated analogs at both C-24 and C-26 showed an eight-fold increase[3] in inducing maturation of human promyelocytic leukemia HL-60 cells. Further elongation of the side chain caused even more profound changes[4] in the activity of an analog of 1,25-$(OH)_2D_3$. 24-Dihomo analog **1** (Figure 1) provided the first evidence[5] that the cell differentiation and calcemic activities of 1,25-$(OH)_2D_3$ can be separated by means of synthetic modifications. This finding has greatly stimulated the search for other low calcemic analogs of 1,25-$(OH)_2D_3$ with retained or increased cell differentiation activity.[6–8] It has also encouraged us to develop methods[9] for the efficient preparation of compound **1**. Due to its unique activity profile, compound **1** became a target molecule for several synthetic approaches. A convergent strategy was developed[10] for the preparation of **1** and for other side chain-modified analogs. The key step of this synthesis involved the coupling of a $C_{22}$-vitamin D synthon with its corresponding side chain fragment. This way, compound **1** was first obtained[11] by alkylation of $C_{22}$-vitamin D tosylate with the deprotonated $C_6$-sulfone of the side chain. Another $C_{22}$-vitamin D synthon[12] was also used in a selenoacetal approach to **1**. In our improved procedure,[13] compound **1** (PRI-1890) was obtained from a $C_{24}$-vitamin D synthon derived directly from the natural C-24 cholanoic acid. Compound **1** was used as a reference[14] in evaluating the affinity ratio of a series of analogs of 1,25-$(OH)_2D_3$ for the intracellular vitamin D receptor (VDR) and for the blood vitamin D-binding protein (DBP). A classical synthetic route[15] was also employed to prepare compound **1** from the steroid $C_{24}$-synthon derived from cholenic acid and from $C_{22}$-*i*-steroid, originated from stigmasterol. Activity of compound **1** was not significantly affected[5] by the insertion of a 22*E* double bond (analog **2**), as in the side chain of vitamin $D_2$. Analog **3** (PRI-2168) was synthesized[16] to test our hypothesis that transposition of a *trans* double bond from the natural C-22 to the metabolically involved C-24 position might change substantially the activity of **1**. Furthermore, to develop this concept, we designed analog **4** (PRI-1901) with a 24a*E* double bond, located in the vicinity of the biologically important C-25 center.

In this paper, we report a synthesis of analog **4** from the C-24a synthon and a practical preparation of the lead compound **1**, as well as an in vitro evaluation of analogs **3** and **4**.

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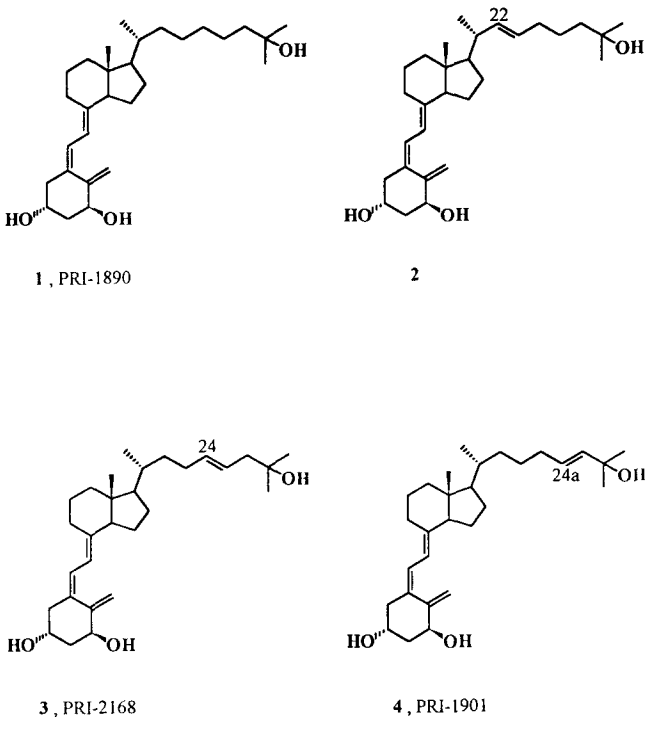



**Figure 1** 24a,24b-Dihomo-1,25-dihydroxycholecalciferol (**1**) and its side chain-unsaturated analogs **2**, **3**, and **4**.

## Experimental

### *Affinity for intracellular vitamin D receptor*

Vitamin D compounds **1**, **3**, and **4** were dissolved in ethanol in concentrations ranging from $10^{-13}$ to $10^{-7}$ M. The affinity towards the specific intracellular VDR was determined with Nichol's radioreceptor assay (Nichol's Institute, San Juan Capistrano, California, USA) by measuring the displacement of 1,25-(OH)$_2$-[26,27-$^3$H$_3$]D$_3$, which is specifically bound to the calf thymus VDR,[17] with the compound tested.

### *Affinity for blood DBP*

Human DBP was purified from the total human serum.[18,19] DBP was incubated with 1,25-(OH)$_2$[26,27-$^3$H$_3$]D$_3$ and with 1,25-(OH)$_2$D$_3$ or with the vitamin D compound investigated. Vitamin D compounds were dissolved in ethanol in concentrations ranging from $10^{-11}$ to $2.5 \times 10^{-6}$ M. 1,25-(OH)$_2$[26,27-$^3$H$_3$]D$_3$ and 1,25-(OH)$_2$D$_3$ or their analogs (in ethanol) were added to glass tubes and incubated with DBP at 4°C for 3 h. Phase separation was then obtained by the addition of cold dextran-coated charcoal. The percentage of bound to unbound 1,25-(OH)$_2$[26,27-$^3$H$_3$]D$_3$ was calculated.

### *Human promyelocytic cell differentiation*

Vitamin D compounds were dissolved in ethanol in concentrations ranging from $10^{-12}$ to $10^{-6}$ M and tested for their ability to induce the differentiation[2,3] of the human promyelocytic leukemia cell line HL-60. HL-60 cells were maintained in a continuous suspension culture in a humidified atmosphere of 5% $CO_2$ in air at 37°C. Under these conditions, the doubling time of cells was approximately 30 h. The cells were incubated for 30 min with nitro blue tetrazolium (NBT; Sigma Chemical Company, St. Louis, Missouri, USA), and the resulting black formazan deposits were measured with a hemocytometer. The percentage of NBT-reducing cells was established as a measure for cell differentiation.

### *Intestinal calcium transport and renal calcium reabsorption*

Transepithelial calcium transport and renal calcium reabsorption were characterized in a model of differentiated human colon adenocarcinoma cell line Caco-2[20,21] in culture exhibiting structural and biochemical characteristics of mature enterocytes. Vitamin D-induced influx of $^{45}Ca^{2+}$ was measured in monolayers of Caco-2 cells grown in 24-well plates at 37°C. The cells were preincubated with the vitamin D compounds in concentrations ranging from $10^{-9}$ to $10^{-5}$M and incubated with 0.7 mM $^{45}Ca^{2+}$ in buffer solution for 30 min. After this, the reaction was stopped, cells were washed and solubilized, and $^{45}Ca^{2+}$ and protein content were measured. The vitamin D-induced $Ca^{2+}$ influx was expressed in nmol of $^{45}Ca^{2+}$/mg of protein/30 min and corrected for the concentration-driven $Ca^{2+}$ influx. Renal calcium reabsorption was measured in primary cultures of monolayers of rabbit kidney cells. The cells were isolated by immunodissection of renal connecting tubules with the aid of monoclonal antibodies. The cell monolayers were first preincubated with 1,25-(OH)$_2$D$_3$ or with the vitamin D analogs in concentrations ranging from $10^{-9}$ to $10^{-5}$ M. To establish the renal $Ca^{2+}$ reabsorption, the monolayers were incubated with $^{45}Ca^{2+}$ at 37°C for 2 h. $Ca^{2+}$ reabsorption was measured in nmol/cm$^2$/h and corrected for the concentration-dependent $Ca^{2+}$ reabsorption. Measurements were done in triplicate.

### *Synthesis*

Vitamin D synthon **5** was obtained form Infarm (Warsaw, Poland) by the previously described methods.[22,23] Sodium amalgam was obtained in this laboratory by the method adopted from Organic Syntheses. Tetrahydrofuran (THF) was distilled over sodium benzophenone ketyl. IR spectra were recorded on a Perkin-Elmer Corp. (Norwalk, Connecticut, USA) model 1725X FT-IR spectrophotometer as films of oily substances or $CHCl_3$ solutions. Ultraviolet (UV) spectra were taken on a Shimadzu (Tokyo, Japan) model 160A UV-VIS spectrophotometer in the solvents indicated. $^1$H NMR and $^{13}$C NMR spectra were recorded at 200 MHz on a Varian (San Fernando, California, USA) Gemini 2000 spectrometer, at 400 MHz on a Bruker (Billerica, Massachusetts, USA) AM 400 spectrometer, and at 500 MHz on a Bruker AM 500 spectrometer in the solvents indicated, downfield from the internal tetramethyl-silane. Electron impact mass spectra (EIMS) were recorded on a Finnigan MAT model 8200 spectrometer. Differential scanning calorimetry was done on a Perkin-Elmer model DSC 7 instrument. Column flash chromatography was performed on silica gel Si 60 (230- to 400-mesh; Merck, Darmstadt, Germany) and on LiChroprep RP-18 (25–40 μm; Merck). High-performance liquid chromatography was done using a Knauer Instrument (Bad Homburg, Germany) model 64, a Hibar Si 60 column, 5 μm, 4 × 25 cm (Merck), and a Si 100 column, 10 μm, 10 × 25 cm and 22 × 25 cm (Solvay Duphar B.V., Weesp, The Netherlands).

### *Preparation of methyl ester of (5Z,7E)-(1S,3R)-1,3-dihydroxy-23a-homo-9,10-secochola-5,7,10(19)-trien-24-oic acid (6)*

A solution of KOH in methanol (0.1 N, 108 ml) was added to the solution of 1.8 g (3.9 mmol) of ester **5** in 108 ml of THF. The mixture was stirred at RT for 3 h and was neutralized with 10 ml of 1 N HCl. Removal of solvents and extraction with ethyl acetate gave 1.57 g (96%) of dihydroxyester **6** as a colorless oil: IR ($CHCl_3$) 3450, 2950, 2890, 1705 cm$^{-1}$, $^1$H NMR ($CDCl_3$) δ 0.54

(3H, s, 18-$CH_3$), 0.94 (3H, d, $J$ = 5 Hz, 21-$CH_3$), 3.66 (3H, s, -$COOCH_3$), 4.17 (1H, m, 3-H), 4.40 (1H, m, 1-H), 4.99 (1H, br s, 19Z-H), 5.32 (1H, br s, 19*E*-H), 6.04 (1H, br d, $J$ = 11 Hz, 7-H), 6.35 (1H, br d, J = 11 Hz, 6-H).

### *Preparation of methyl ester of (5Z, 7E)-(1S, 3R)-1,3-bis[(t-butyldimethylsilyl)oxy]-23a-homo-9,10-secochola-5,7,10(19)-trien-24-oic acid (7)*

Imidazole (Im; 1.3 g, 22.8 mmol) and *t*-butyldimethylsilyl chloride (TBDMSCl; 1.3 g, 8.7 mmol) were added to a solution of 1.5 g (3.6 mmol) of dihydroxyester **6** in 10 ml of dimethylformamide (DMF). The mixture was stirred at RT for 2.5 h. Extraction with hexane/ethyl acetate (4:1) and gel filtration gave 2.15 g (93%) of disilylated ester **7** as a colorless oil: UV (hexane) $\gamma_{max}$ 264.8 nm; IR ($CHCl_3$) 2950, 2860, 1705 $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$ 0.06 (12H, br s, Si-$CH_3$) 0.53 (3H, s, 18-$CH_3$), 0.87 (21H, br s, Si-C-$CH_3$, 21-$CH_3$), 3.66 (3H, s, -$COOCH_3$), 4.17 (1H, m, 3-H), 4.40 (1H, m, 1-H), 4.86 (1H, br s, $J$ = 1.8 Hz, 19Z-H), 5.18 (1H, br s, $J$ = 1.8 Hz, 19*E*-H), 6.02 (1H, br d, $J$ = 11 Hz, 7-H), 6.35 (1H, br d, $J$ = 11 Hz, 6-H).

### *Preparation of (5Z, 7E)-(1S, 3R)-1,3-bis[(t-butyldimethylsilyl)oxy]-23a-homo-9,10-secochola-5,7,10(19)-trien-24-ol (8)*

A solution of ester **7** (2 g, 3.1 mmol) in 5 ml of THF was added under argon, with stirring, to a suspension of 212 mg (5.5 mmol) of $LiAlH_4$ in 10 ml of anhydrous THF at RT. The mixture was stirred at RT for 30 min and cooled to 0°C. Ethyl ether extraction and celite filtration gave 1.75 g (91%) of alcohol **8** as a colorless foam: UV (EtOH) $\gamma_{max}$ 263.6 nm; IR ($CHCl_3$) 3463, 2949, 2856, 1644 $cm^{-1}$, $^1H$ NMR ($CDCl_3$) $\delta$ 0.05 (12H, br s, Si-$CH_3$), 0.5 (3H, s, 18-$CH_3$), 0.85 (21H, br s, Si-C-$CH_3$, 21-$CH_3$), 3.60 (2H, t, $J$ = 6.5 Hz, 24-$CH_2$), 4.14 (1H, m, 3-H), 433 (1H, m, 1-H), 4.84 (1H, br s, $J$ = 2.3 Hz, 19*E*-H), 5.15 (1H, br s, $J$ = 2.3 Hz, 19Z-H), 5.98 (1H, d, $J$ = 11.1 Hz, 7-H), 6.21 (1H, d, $J$ = 11.1 Hz, 6-H); $^{13}C$ NMR ($CDCl_3$) $\delta$ −5.10, −4.81, −4.70 (Si-$CH_3$), 11.94 (C-18), 18.11, 18.20 (Si-C-$CH_3$), 18.77 (C-21), 22.12 (C-15), 22.26 (C-23), 23.48 (C-11), 25.79, 25.83 (S-C-$CH_3$), 27.69 (C-16), 28.86 (C-9), 33.16 (C-23a), 35.65 (C-22), 36.04 (C-20), 40.60 (C-12), 44.79 (C-2), 45.75 (C-13), 46.01 (C-4), 56.32 (C-14), 56.44 (C-17), 62.92 (C-24), 67.52 (C-3), 72.07 (C-1), 111.2 (C-19), 117.84 (C-7), 123.15 (C-6), 134.91 (C-5), 141.02 (C-8), 148.27 (C-10); EIMS m/z (relative intensity) 616 ($M^+$, 7.5), 601 (2), 485 (6.5), 353 (2.5), 246 (100); high-resolution mass spectra (HRMS) calculated for $C_{37}H_{68}O_3Si_2$: 616.4707; found: 616.4709.

### *Preparation of (5Z, 7E)-(1S, 3R)-1,3-bis[(t-butyldimethylosilyl)oxy]-24-(p-toluenesulfonyl-oxy)-23a-homo-9,10-secochola-5,7,10(19)-trien (9)*

Triethylamine (TEA; 3.7 ml, 26.6 mmol), *p*-toluenesulfonyl chloride (595 mg, 3.12 mmol), and *N,N*-dimethylaminopyridine (DMAP; 10 mg) were added under argon to the solution of 1.19 g (1.9 mmol) of alcohol **8** in 11 ml of $CH_2Cl_2$. The mixture was stirred at RT under argon for 16 h. The solution was cooled to 5°C, and 20 ml of $CH_2Cl_2$, 20 ml of $H_2O$, and 200 mg of solid $NaHCO_3$ were added. Stirring was continued for 1 h. Extraction with methylene chloride gave 1.6 g of an oily residue. Silica gel chromatography (32 g; hexane/ethyl acetate, 20:1) gave 1.3 g (87%) of tosylate **9** as a colorless foam: UV (EtOH) $\gamma_{max}$ 262.8 nm; IR ($CHCl_3$) 2952, 2857, 1649, 1600, 1360, 1175, 1076 $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$ 0.06 (12H, s, Si-$CH_3$), 0.50 (3H, s, 18-$CH_3$), 0.87 (21H, br s, Si-C-$CH_3$, 21-$CH_3$), 2.45 (3H, s, Ph-$CH_3$), 4.02 (2H, t, $J$ = 6,4 Hz, 24-$CH_2$), 4.19 (1H, m, 3-H), 4.37 (1H, m, 1-H), 4.86 (1H, br s, $J$ = 2.5 Hz, 19*E*-H), 5.18 (1H, br s, $J$ = 2.2 Hz, 19Z-H), 6.00 (1H, d, $J$ = 11.3 Hz, 7-H), 6.23 (1H, d, $J$ = 11.3 Hz, 6-H), 7.34 and 7.80 (2H and 2H, each m, Ar-H); EIMS *m/z* (relative intensity) 770 ($M^+$,90), 755 (8), 638 (60), 248 (90), 75 (100), 73 (90); HRMS calculated for $C_{44}H_{74}O_5Si_2S$: 770.4796; found: 770.4796.

### *Preparation of (5Z, 7E)-(1S, 3R)-1,3-bis[(t-butyldimethylsilyl)oxy]-24-phenylsulfonyl-23a-homo-9,10-secochola-5,7,10(19)-trien (10)*

Lithium carbonate (321.4 mg, 4.35 mmol) and 377.8 mg (4.35 mmol) of LiBr were added under argon, to a solution of 1.12 g (1.45 mmol) of tosylate **9** in 37 ml of DMF. The suspension was stirred at 80°C for 1 h. Sodium salt of benzenesulfinic acid (1.904 g, 11.5 mmol) was added, and the mixture was stirred at 80°C for 2 h. Extraction with ethyl acetate and silica gel chromatography (22 g; hexane/ethyl acetate, 20:1) gave 750 mg (66%) of sulfone **10** as a colorless foam: UV (EtOH) $\gamma_{max}$ 264.6 nm; IR ($CHCl_3$) 2952, 2857, 1620, 1471, 1307, 1147, 1087, 836 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 500 MHz) $\delta$ 0.06 (12H, br s, Si-$CH_3$), 0.50 (3H, s, 18-$CH_3$), 0.87 (21H, ds, Si-C-$CH_3$, 21-$CH_3$), 2.21 (1H, dd, $J$ = 7.5 Hz, $J$ = 13.3 Hz, 4$\beta$-H), 2.44 (1H, dd, $J$ = 3.8 Hz, $J$ = 13.2 Hz, 4$\alpha$-H), 2.81 (1H, dd, $J$ = 4.1 Hz, $J$ = 12.4 Hz, 9$\beta$-H), 3.08 (1H, m, 24-$CH_2$), 4.19 (1H, m, 3-H), 4.37 (1H, m, 1-H), 4.86 (1H, br s, $J$ = 2.4 Hz, 19*E*-H), 5.18 (1H, br s, $J$ = 2.4 Hz, 19Z-H), 6.01 (1H, d, $J$ = 11.0 Hz, 7-H), 6.24 (1H, d, $J$ = 11.2 Hz, 6-H), 7.37 and 7.57 (2H and 2H, each m, Ar-H), 7.65 (1H, m, Ar-H); EIMS *m/z* (relative intensity) 740 ($M^+$, 28), 725 (5), 608 (75), 248 (95), 75 (75), 73 (100); HRMS calculated for $C_{43}H_{72}O_4Si_2S$: 740.46897, found: 740.46897.

### *Preparation of (5Z, 7E)-(1S, 3R)-1,3-bis[(t-butyldimethylsilyl)oxy]-23a-homo-9,10-secochola-5,7,10(19)-trien-24-al (11)*

A solution of diisobutylaluminum hydride (DIBAl-H) in toluene (1 M, 750 $\mu$l, 0.75 mmol) was added dropwise under argon to a solution of 500 mg (0.77 mmol) of disilylated ester **7** in 2.5 ml of toluene at −70°C. The mixture was stirred at −65°C for 3 h. Celite filtration and silica gel chromatography gave 330 mg (69%) of aldehyde **11** as a colorless oil: UV (hexane) $\gamma_{max}$ 264.6 nm; IR ($CHCl_3$) 2930, 1722, 1645 $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$ 0.05 (12H, br s, Si-$CH_3$) 0.51 (3H, s, 18-$CH_3$), 0.86 (21H, br s, Si-C-$CH_3$, 21-$CH_3$), 4.2 (1H, m, 3-H), 4.3 (1H, m, 1-H), 4.84 (1H, br s, $J$ = 2.3 Hz, 19Z-H), 5.15 (1H, br s, $J$ = 2.3 Hz, 19*E*-H), 6.02 (1H, br d, $J$ = 11 Hz, 7-H), 6.35 (1H, br d, $J$ = 11 Hz, 6-H), 9.74 (1H, t, $J$ = 1.8 Hz, CHO); $^{13}C$ NMR ($CDCl_3$) $\delta$ −5.09, −4.80, −4.69 (Si-$CH_3$), 11.94 (C-18), 18.15, 18.22 (Si-C-$CH_3$), 18.69 (C-21), 22.11 (C-15), 22.65 (C-23), 23.46 (C-11), 25.79, 25.84 (Si-C-$CH_3$), 27.68 (C-16), 28.84 (C-9), 35.39 (C-22), 35.97 (C-20), 40.56 (C-12), 44.22 (C-23a), 44.78 (C-2), 45.75 (C-13), 46.01 (C-4), 56.19 (C-14), 56.28 (C-17), 67.49 (C-3), 72.04 (C-1), 111.22 (C-19), 117.70 (C-7), 123.12 (C-6), 134.99 (C-5), 140.91 (C-8), 148.25 (C-10), 202.98 (C-24); EIMS *m/z* (relative intensity) 614 ($M^+$), 482 (100), 248 (95); HRMS calculated for $C_{37}H_{66}O_3Si_2$: 614.4546; found: 614.4551. Alcohol **8** (50 mg, 11%) was also isolated, as a by-product.

### *Preparation of (5Z, 7E)-(1S, 3R)-1,3-bis[(t-butyldimethylsilyl)oxy]-24a-phenylsulfonyl-24a,24b-dihomo-9,10-secocholesta-5,7,10(19)-trien-25-ol (13)*

A solution of *n*-butyllithium in hexane (1.3 M, 623 $\mu$l, 0.8 mmol) was added to the solution of 200 mg (0.27 mmol) of sulfone **10** in 3 ml of THF, under argon, at −73°C (in the presence of 1,10-

phenanthroline as an indicator). The mixture was stirred at −73°C for 40 min and then at −20°C for 30 min. The mixture was cooled to −60°C, and hexamethlphosphoric triamide (HMPT; 300 μl, 1.72 mmol) was added. Stirring was continued at −60°C for 20 min. The mixture was cooled to −73°C, and 1,1-dimethylepoxyethane (277 μl, 2.7 mmol) was added. The mixture was warmed up to −20°C, within 40 min. Ethyl acetate extraction and silica gel chromatography (10 g, hexane/ethyl acetate, 10:1) gave 107 mg (49%) of hydroxysulfone **13** as a colorless foam: UV (EtOH) $\gamma_{max}$ 265.0 nm, $\gamma_{min}$ 231.2 nm; IR ($CHCl_3$) 3503, 2095, 2856, 1471, 1376, 1298 cm$^{-1}$, $^1$H NMR ($CDCl_3$, 500 MHz) δ 0.06 (12H, br s, Si-$CH_3$), 0.47 and 0.48 (3H each, ds, 18-$CH_3$), 0.87 (21H, m, Si-C-$CH_3$, 21-$CH_3$), 1.21 (6H, s, 26,27-$CH_3$), 2.21 (1H, dd, $J$ = 7.7 Hz, $J$ = 12.8 Hz, 4β-H), 2.47 (1H, dd, $J$ = 13.0 Hz, $J$ = 3.2 Hz, 4α-H), 2.83 (1H, dd, $J$ = 12.3 Hz, $J$ = 3.7 Hz, 9β-H), 3.26 (1H, m, 24a-H), 4.19 (1H, m, 3-H), 4.37 (1H, m, 1-H), 4.86 (1H, t, $J$ = 2.1 Hz, 19*E*-H), 5.18 (1H, t, $J$ = 2.3 Hz, 19*Z*-H), 6.03 (1H, d, $J$ = 11.3 Hz, 7-H), 6.25 (1H, d, $J$ = 11.1 Hz, 6-H), 7.58 and 7.93 (2H each, m, Ar-H), 7.66 (1H, m, Ar-H); EIMS *m/z* (relative intensity) 812 (M$^+$, 25), 797 (5), 680 (65), 623 (10), 368 (10), 248 (100), 59 (75); HRMS calculated for $C_{47}H_{80}O_5Si_2S$: 812.5265; found: 812.5265.

### *Preparation of 24a,24b-dihomo-1,25-dihydroxycholecalciferol (1)*

Powdered anhydrous $Na_2HPO_4$ (500 mg) was added under argon to a solution of 269.5 mg (0.33 mmol) of hydroxysulfone **13** in 10 ml of MeOH saturated with $Na_2HPO_4$, and the suspension was stirred at RT for 20 min. Sodium amalagam (5%, 3 g) was added, and the mixture was stirred for 4 h. Extraction with hexane-ethyl acetate, Celite filtration, and silica gel chromatography (10 g; hexane/ethyl acetate, 10:1) gave 158.6 mg (72%) of disilylated triol **14** as a colorless foam. This was dissolved in 4 ml of THF, and 377.5 mg (1.2 mmol) of tetrabutylammonium fluoride was added. The mixture was stirred at 50°C for 5 h. Extraction with ethyl acetate and silica gel chromatography (5 g; benzene/acetone/methanol, 10:1:1) gave 78.4 mg (75%) of triol **1** as a colorless foam: UV (2-propanol/hexane) $\gamma_{max}$ 264.6 nm, $\gamma_{min}$ 232.4 nm; IR ($CHCl_3$) 3609, 3432, 2935, 2863, 1467, 1376, 1147, 1058, 949, 698 cm$^{-1}$; $^1$H NMR ($CDCl_3$, 500 MHz) δ 0.55 (3H, s, 18-$CH_3$), 0.92 (3H, d, $J$ = 6.4 Hz, 21-$CH_3$), 1.2 (6H, s, 26,27-$CH_3$), 2.35 (1H, dd, $J$ = 13.3 Hz, $J$ = 6.3 Hz, 4β-H), 2.63 (1H, dd, $J$ = 13.5 Hz, $J$ = 3.5 Hz, 4α-H), 2.85 (1H, dd, $J$ = 12.0, $J$ = 3.8 Hz, 9β-H), 4.23 (1H, m, 3-H), 4.43 (1H, m, 1-H), 5.0 (1H, br s, $J$ = 1.2 Hz, 19*Z*-H), 5.32 (1H, br s, $J$ = 1.5 Hz, 19*E*-H), 6.04 (1H, d, $J$ = 11.3 Hz, 7-H), 6.4 (1H, d, $J$ = 11.3 Hz, 6-H); EIMS *m/z* (relative intensity) 444 (M$^+$, 6), 426 (100), 408 (29), 393 (15), 251 (15), 135 (35), 134 (47), 59 (65); HRMS calculated for $C_{29}H_{48}O_3$: 444.3603; found: 444.3603.

### *Preparation of ethyl ester of (5Z, 7E)-(1S, 3R)-(23bE)-1,3-bis[(t-butyldimethylsilyl)oxy]-23a,23b,23c-trihomo-9,10-secochola-5,7,10(19),23b-tetraen-24-oic acid (16)*

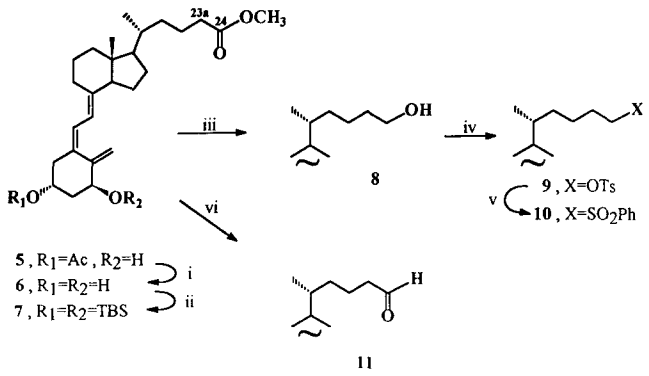
A solution of aldehyde **11** (89 mg, 0.145 mmol) in 2 ml of THF was added, under argon, to the solution of 70 mg (0.2 mmol) of ethoxycarbonylmethylenetriphenylphosphorane (**15**) in 2 ml of THF under argon at RT. The mixture was stirred for 16 h. Silica gel chromatography gave 102 mg (91% yield) of ester **16** as a colorless oil: UV (hexane) $\gamma_{max}$ 264.6 nm; IR ($CHCl_3$) 2951, 1708, 1652 cm$^{-1}$; $^1$H NMR ($CDCl_3$) δ 0.049 (12H, br s, Si-$CH_3$), 0.52 (3H, s, 18-$CH_3$), 0.89 (21H, br s, Si-C-$CH_3$, 21-$CH_3$), 1.27 (3H, t, $J$ = 7.1 Hz, -$CH_2CH_3$), 4.2 (1H, m, 3-H), 4.3 (1H, m, 1-H), 4.84 (1H, br s, $J$ = 2.4 Hz, 19*Z*-H), 5.15 (1H, br s, $J$ = 2.4 Hz, 19*E*-H), 5.78 (1H, d t, $J$ = 15.7 Hz, $J$ = 1.4 Hz, 23b-H), 6.02 (1H, br d, $J$ = 11 Hz, 7-H), 6.35 (1H, br d, $J$ = 11 Hz, 6-H), 6.90 (1H, d t, $J$ = 15.8 Hz, $J$ = 7.2 Hz, 23c-H); $^{13}$C NMR ($CDCl_3$) δ −5.05, −4.77, −4.66 (Si-$CH_3$), 11.98 (C-18), 14.27 ($CH_2$-$\underline{C}H_3$), 18.13, 18.2 (Si-$\underline{C}$-$CH_3$), 18.79 (C-21), 22.17 (C-15), 23.51 (C-11), 24.76 (C-23), 25.84 (Si-C-$CH_3$), 27.69 (C-16), 28.89 (C-9), 35.51 (C-22), 35.98 (C-20), 40.68 (C-12), 44.91 (C-2), 45.82 (C-13), 46.10 (C-4), 56.38 (C-14), 56.52 (C-17), 60.06 (-$\underline{C}H_2$-$CH_3$), 67.58 (C-3), 72.12 (C-1), 111.16 (C-19), 117.97 (C-7), 121.31 (C-6), 123.17 (C-23b), 135.07 (C-5), 140.93 (C-8), 148.44 (C-10), 149.37 (C-23a), 166.74 (C-24).

### *Preparation of (5Z,7E)-(1S,3R)-(24a)-24a,24b-dihomo-9,10-secocholesta-5,7,10(19),24a-tetraen-1,3,25-triol (4)*

A solution of ester **16** (60 mg, 0.088 mmol) in 1 ml of THF was added under argon at RT to the solution of methylmagnesium bromide in ethyl ether (3M, 0.1 ml, 0.3 mmol) diluted with 0.75 ml of THF. The mixture was stirred for 24 h. Silica gel chromatography gave 31.3 mg (54%) of alcohol **17** as a colorless oil. This was diluted with 1 ml of THF, and it was added, under argon, to a solution of 315 mg (1 mmol) of tetrabutylammonium fluoride in 1 ml of THF. The mixture was stirred at RT for 16 h. Extraction with ethyl acetate and silica gel chromatography gave 19.3 mg (94% yield) of triol **4** as a colorless oil: m.p. (from acetone, determined by DSC) 139.15°C; UV (EtOH) $\gamma_{max}$ 263.8 nm; $^1$H NMR ($CDCl_3$) δ 0.54 (3H, s, 18-$CH_3$), 0.92 (3H, d, $J$ = 3 Hz, 21-$CH_3$), 1.31 (6H, s, 26,27-$CH_3$), 4.2 (1H, m, 3-H), 4.4 (1H, m, 1-H), 5.0 (1H, br s, 19*Z*-H), 5.3 (1H, br s, 19*E*-H), 5.61 (2H, d, 24a-H, 24b-H), 6.02 (br d, $J$ = 11 Hz, 7-H), 6.35 (br d, J = 11 Hz, 6-H); $^{13}$C NMR ($CDCl_3$) δ 12.01 (C-18), 18.85 (C-21), 22.28 (C-23), 23.61 (C-15), 25.81 (C-11), 27.62 (C-16), 29.10 (C-9), 29.87 (C-26,27), 32.54 (C-24), 35.38 (C-22), 35.93 (C-20), 40.50 (C-12), 42.89 (C-2), 45.29 (C-4), 45.93 (C-13), 56.37 (C-17), 56.52 (C-14), 66.87 (C-3), 70.67 (C-25), 70.85 (C-1), 111.78 (C-19), 117.02 (C-7), 125.01 (C-6), 127.41 (C-24a), 132.86 (C-5), 137.89 (C-24b), 143.24 (C-8), 147.66 (C-10); EIMS *m/z* (relative intensity) 442 (M$^+$, 39), 424 (M$^+$ -$H_2O$, 95), 406 (M$^+$ -2$H_2O$, 93), 388 (M$^+$ -3$H_2O$, 100), 285 (100); HRMS calculated for $C_{29}H_{46}O$: 442.3447; found: 442.3440.

### *Preparation of 2-methyl-3-(phenylsulfonyl)-2-[(triethylsilyl)oxy]propane (20)*

Sodium thiophenoxide (3g, 22.7 mmol) was added to a solution of 1.4 ml (13.6 mmol) of 1,1-dimethylepoxyethane in 7 ml of DMF. The mixture was stirred at 50°C for 5 h, and it was left to stir overnight at RT. Extraction with methylene chloride and silica gel filtration (50 g, 70- to 230-mesh) gave 1.47 g (59% yield) of sulfide **18** as a colorless oil: IR (film) 3401, 3059, 2973, 2928, 2873, 1666, 1584, 1480, 1439, 1377 cm$^{-1}$, EIMS *m/z* (relative intensity) 182 (M$^+$, 10), 167 (5), 149 (8), 124 (100), 109 (10), 59 (100). A suspension of magnesium monoperoxyphthalate (6.86 g, 13.9 mmol) was added with cooling on an ice bath to a solution of sulfide **18** (1.47 g, 8.11 mmol) in 4.5 ml of methylene chloride. The mixture was stirred overnight at RT. Celite filtration, washing out with 5% sodium sulfite, and silica gel chromatography (20 g, 70-230 mesh) gave 1.4 g (81% yield) of sulfone **19** as a colorless solid: $^1$H NMR ($CDCl_3$) δ 1.46 (6H, s, $CH_3$), 3.32 (2H, s, $CH_2$), 7.6, 7.9 (3H and 2H each, m, Ar-H); EIMS *m/z* (relative intensity) 214 (M$^+$, 3), 199 (90), 156 (70), 141 (80), 77 (10), 59 (60). Triethylsilyl chloride (TESCl; 0.4 ml, 2.89 mmol) and imidazole (357 mg, 5.25 mmol) were added to a solution of 562.8 mg (2.62 mmol) of sulfone **19** in 4 ml of methylene chloride. The mixture was stirred at RT for 16 h. Extraction with methylene chloride and

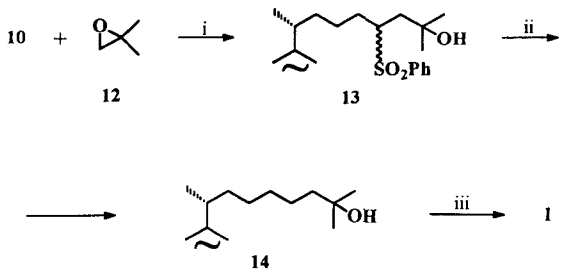


**Scheme 1** Synthesis of $C_{23a,24}$-vitamin D synthons **10** and **11**. Reagents and conditions: (i) KOH, MeOH, RT, 3 h; (ii) TBDMSCl, Im, DMF, RT, 2.5 h; (iii) $LiAlH_4$, THF, RT, 30 min; (iv) TsCl, DMAP, TEA, $CH_2Cl_2$, RT, 16 h; (v) $Li_2CO_3$, LiBr, DMF, 80°C, 1 h; $PhSO_2Na$, 80°C, 2 h; (vi) DIBAL-H, $PhCH_3$, −65°C, 3h.

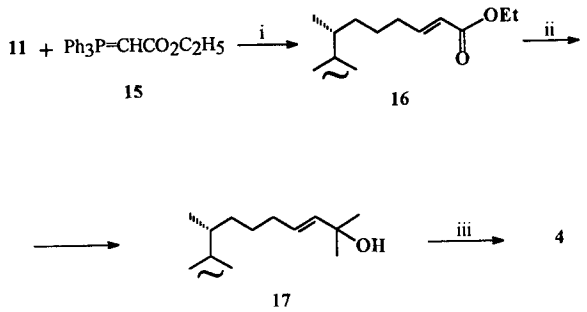
silica gel chromatography (10 g, 70 to 230-mesh) gave protected sulfone **20** as a colorless oil: IR (film) 2966, 2874, 1446, 1320, 1150, 1043, 746 $cm^{-1}$; $^1H$ NMR ($CDCl_3$) δ 0.47 (6H, q, $J$ = 7.5 Hz, Si-$CH_2$-), 0.86 (9H, t, $J$ = 7.5 Hz, Si-$CH_2$-$CH_3$-), 1.5 (6H, s, 1-$CH_3$, 2-$CH_3$), 3.25 (2H, s), 7.59 (3H, m, Ar-H), 7.92 (2H, m, Ar-H); EIMS *m/z* (relative intensity) 299 (100), 241 (40), 181 (10) 143 (10), 115 (15), 103 (10), 75 (25).

## Results and discussion

Our synthesis of analogs **1** and **4** represents a further development of the general convergent synthesis[11] involving vitamin D synthons as key intermediates for the preparation of side chain- modified analogs of 1,25-$(OH)_2D_3$. In the method described here, we use $C_{23a,24}$-vitamin D synthons **10** and **11** (Scheme 1, numbering system of carbon atoms based on cholanoic acid) for the preparation of analogs **1** and **4**, respectively. Synthons **10** and **11** were obtained by known procedures[24] from the intermediate 23a-homo $C_{24}$ ester **5**.[25] Our selecting of ester **5** as the key intermediate allows the use of commercially available side chain fragments for the preparation of analogs **1** and **4**, as well as for other side chain-modified compounds. Ester **5** might also be used as a precursor of both 1,25-$(OH)_2D_3$ and its tritiated analog.[26,27] Lithium aluminum hydride reduction of ester **7** provided alcohol **8** in 91% yield. This alcohol was reacted with *p*-toluenesulfonyl chloridein the presence of catalytic amount of *N,N*-dimethylaminopyridine, and it afforded to-



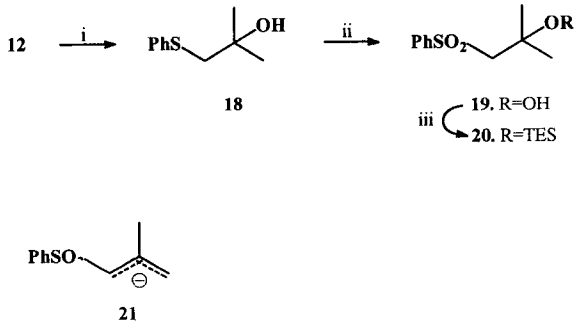
**Scheme 2** Synthesis of 24a,24b-dihomo-1,25-$(OH)_2D_3$ (**1**). Reagents and conditions: (i) *n*-BuLi, THF, HMPT, −20°C, 40 min; (ii) 5% Na/Hg, $Na_2HPO_4$, MeOH, RT, 4 h; (iii) [*n*-Bu]$_4$NF, THF, 50°C, 5 h.



**Scheme 3** Synthesis of (24a*E*)-24a-dehydro-24a,24b-dihomo-1,25-$(OH)_2D_3$ (**4**). Reagents and conditions: (i) THF, RT, 16 h; (ii) MeMgBr, THF, RT, 24 h; (iii) [*n*-Bu]$_4$NF, THF, RT, 16 h.

sylate **9** in 87% yield. Sulfone moiety at C-24 was introduced in 66% yield by treating of tosylate **9** with sodium salt of benzenesulfinic acid in DMF. Aldehyde **11** was obtained directly from ester **7** by the reduction with diisobutylaluminum hydride at −70°C in 69% yield. Deprotonation of sulfone **10** (Scheme 2, cholesterol-type numbering system) with *n*-butyllithium and coupling with oxirane **12**[28] afforded the mixture of diastereomeric $C_{24a}$ sulfones **13** in 49% yield. Sodium amalgam desulfonylation in buffered methanol and fluoride ion promoted desilylation provided analog **1** in 18% yield from ester **7**. Analog **1** obtained by the present method was found to be identical in all respects to the previously obtained[13] authentic sample.

In our approach to analog **4**, we used Wittig olefination (Scheme 3). This method was previously described[29] to give *cis*-olefines by the coupling of steroid C-22 aldehyde with an alkyl-triphenylphosphonium bromide. For our Wittig reaction, we selected an alkoxycarbonylmethylenetriphenylphosphorane **15** as a side chain equivalent that should provide the desired *trans*-olefin.[30,31] α,β-Unsaturated ester **16** was obtained from aldehyde **11** and Wittig reagent **15** in 91% yield with no detectable amount of 24a*Z* isomer. The 24a*E* geometry was assigned to **16** based on the value of vicinal coupling constant of the olefinic protons ($^3J_{24a,24b}$ = 15.7 Hz). It might be assumed that the 24a*E* geometry is retained in the course of the final Grignard reaction. Ester **16** was treated with methylmagnesium bromide and gave, after desilylation, analog **4** in 32% overall yield from starting ester **7**. Initially, we attempted to obtain analog **4** with the 24*E* double bond by Julia-Paris olefination of aldehyde



**Scheme 4** Synthesis of protected sulfone **20**. Reagents and conditions: (i) PhSNa, DMF, 50°C, 5 h; (ii) magnesium monoperoxyphthalate, $CH_2Cl_2$, RT; TESCl, Im, $CH_2Cl_2$, RT, 16 h.

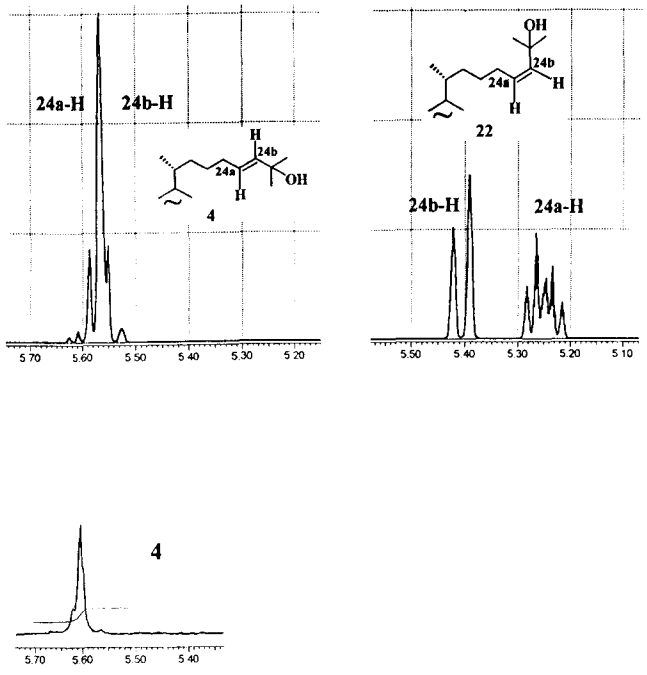



**Figure 2** $^1$H NMR spectra of analog **4** and its 24aZ isomer **22**, showing the signals of side chain olefinic protons (a) Calculated spectrum of **4**; (b) calculated spectrum of **22**; (c) experimental spectrum of **4**. Spectra calculated[32] and recorded at 400 MHz in $CDCl_3$, downfield from the internal tetramethylsilane. Line width of 0.6 was selected for the calculated spectra.

**11** with the side chain sulfone **20** (Scheme 4). Sulfide **18** was obtained by a ring opening of oxirane **12** with sodium thiophenoxide in 59% yield. This sulfide was oxidized to sulfone **19** with magnesium monoperoxyphthalate in 81% yield. Sulfone **20** was obtained by final protection of tertiary hydroxyl with triethylsilyl chloride. Unfortunately, the carbanion generated from sulfone **20** with *n*-butyllithium was not stable enough for coupling with aldehyde **11**. After we completed our experiments, it was reported[32] that deprotonated sulfone **19** undergoes a facile elimination to form unsaturated sulfone **21**. This might also explain the instability of silylated sulfone **20**.

Having only one isomer at C-24a of analog **4** available, it is not possible to unambiguously assign the geometry of the double bond in the side chain. Our tentative assignment of 24a*E* geometry in analog **4** was based on calculated and experimental spectroscopic data. Signals originated from C-24a and C-24b protons in the $^1$H NMR spectrum of **4** gave a multiplet with the coupling constant $^3J_{24a,24b}$ difficult to be precisely measured. However, the estimated value was lower than 10 Hz, and it fell within the range characteristic for $^3J_{ab}(cis)$. It is known that electronegative substituents attached directly to the olefinic carbon reduce significantly both vicinal coupling constants.[33] Also, the sterically favored antiperiplanar orientation of C-25 hydroxyl might increase the effect of the lower-than-usual value of $^3J_{ab}$. To solve this problem, we used the recently developed software[34] to calculate spectral parameters in $^1$H and $^{13}$C NMR. Chemical shifts of 24a and 24b protons were very different in the $^1$H NMR spectra of **4** and **22** (Fig. 2 a and b). The calculated coupling constants $^3J_{24a,24b}$ for **4** and **22** were 15.6 and 12.2 Hz, respectively. The signal originated from 24a-H and 24b-H in the experimental spectrum of **4** was almost identical (in terms of chemical shift and coupling constant) with that in the calculated one for the 24a*E* isomer. Calculated chemical shifts of both 24 and 25 carbons ($\alpha$ to the double bond) in the $^{13}$C NMR spectrum of **4** also correlated very well with the experimental values, and they were significantly different from the calculated chemical shifts for **22** (Table 1).

A previously obtained[16] analog **3** and a new analog **4** were evaluated in vitro for their affinity to vitamin D proteins and for their activity in stimulating cell differentiation, as well as intestinal calcium transport and renal calcium reabsorption. Compound **1** and 1,25-(OH)$_2$D$_3$ were used as references, and the data obtained were compared with those reported[5] for analog **2** (Table 2). Both compound **1** and analog **2** were described previously as noncalcemic vitamins D with the HL-60 activity one order of magnitude higher than that of 1,25-(OH)$_2$D$_3$. The values in Table 2 indicate how much lower the activity of an analog is compared to that of 1,25-(OH)$_2$D$_3$. One of the most striking findings is that the presence of an allylic hydroxyl at C-25 increased the affinity of analog **4** for VDR of one order of magnitude, as compared to 1,25-(OH)$_2$D$_3$, and over three orders of magnitude, as compared to the parent compound **1** and a close analog **3.** Thus, the change in the activity profile we expected previously[16] for analog **3** with the single unsaturation in the side chain at C-24 occurred now for analog **4** with the unsaturation at C-24a. The allylic double bond in analog **4** being somewhat more electronegative, compared to the presence of sp$^3$ carbons near the allylic hydroxyl, increased the hydrogen-bonding capability of a hydroxyl group, thus enhancing its ability to bind more effectively to the receptor. In a previously reported an in vivo experiment,[5] compound **1** and analog **2** showed an at least 10 times lower activity in stimulation of an intestinal calcium transport than that of 1,25-(OH)$_2$D$_3$. However, in an in vitro study reported here, both compounds showed the activity comparable to that of 1,25-(OH)$_2$D$_3$. Work on other side

**Table 1** Chemical shifts (in ppm) of diagnostic carbons in $^{13}$C NMR spectra of **4** and in calculated spectra of **4** and **22**

| Compound | C-24 | C-24a | C-24b | C-25 |
|---|---|---|---|---|
| **4** | 32.54 | 127.41 | 137.89 | 70.67 |
| **4**[a] | 32.25 ± 3.6 | 126.82 ± 4.9 | 139.61 ± 5.1 | 71.48 ± 2.4 |
| **22**[a] | 26.35 ± 3.0 | 126.82 ± 4.9 | 139.61 ± 5.1 | 65.08 ± 4.0 |

[a] Spectrum calculated at 400 MHz in $CDCl_3$.

**Table 2** Relative affinity of side chain unsaturated analogs **2, 3,** and **4** of 24a,24b-dihomo-1,25-$(OH)_2D_3$ (**1**) for VDR and the blood DBP and their relative activity in cell differentiation and relative calcemic effects

| Compound and code | VDR[a] | DBP[b] | HL-60[c] | Calcemic effects | |
|---|---|---|---|---|---|
| | | | | Intestinal Ca transport[d] | Renal Ca reabsorption[e] |
| 1,25-$(OH)_2D_3$ | 1 | 1 | 1 | 1 | 1 |
| **1**, PRI-1890 | 140 | 130 | 1.7 | 1 | 0.9 |
| **2**[f] | 30[g] | — | 0.1 | 10[h] | — |
| **3**, PRI-2168 | 130 | 50 | 4 | −0.4 | 1 |
| **4**, PRI-1901 | 0.1 | 30 | 1 | 1 | 1 |

[a] Affinity for calf thymus intracellular VDR determined with Nichol's kit, expressed as the $EC_{50}$ for the analog/$EC_{50}$ for 1,25-$(OH)_2D_3$; data from three separate experiments, each done in duplicate. The $EC_{50}$ is calculated from the displacement curve of 1,25-$(OH)_2$[26,27-$^3H_3$]$D_3$ determined in concentrations ranging from $10^{-13}$ to $10^{-7}$ M. The lower the value, the higher the VDR affinity.
[b] Affinity for human serum DBP purified by Sepharose 4B affinity chromatography[18] and expressed as the $EC_{50}$ for the analog/$EC_{50}$ for 1,25-$(OH)_2D_3$; data from two separate experiments, each done in duplicate. The $EC_{50}$ is the concentration at which 50% of the 1,25-$(OH)_2$[26,27-$^3H_3$]$D_3$ is displaced from the DBP. High values indicate low DBP affinities.
[c] Differentiation of HL-60 cells[2,3] measured by the percentage of NBT-reducing cells and expressed as the $EC_{50}$ for the analog/$EC_{50}$ for 1,25-$(OH)_2D$. A low value indicates a high differentiation activity.
[d] Increase in $CA^{2+}$ transport measured in an in vitro model[20,21] of the human colon adenocarcinoma cell line Caco-2, calculated as a ratio of analog/1,25-$(OH)_2D_3$ (0.639 nmol/mg of protein/30 min at $10^{-7}$ M).
[e] Increase in $^{45}Ca^{2+}$ reabsorption in monolayers of a rabbit renal tubule primary cell culture, calculated as a ratio of analog/1,25-$(OH)_2D_3$ (39.2 nmol/cm$^2$/h at $10^{-7}$ M).
[f] Synthesis of and activity reported previously.[5]
[g] Affinity for VDR from rat intestinal and HL-60 cell extracts,[5] data from two separate experiments, each done in duplicate.
[h] Intestinal calcium transport determined in vitamin D-deficient rats.[5]

chain-unsaturated analogs of **1** is under way in these laboratories.

## Acknowledgments

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