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# New 1α,25-dihydroxy Vitamin $D_3$ Analogues with Side Chains Attached to C-18: Synthesis and Biological Activity

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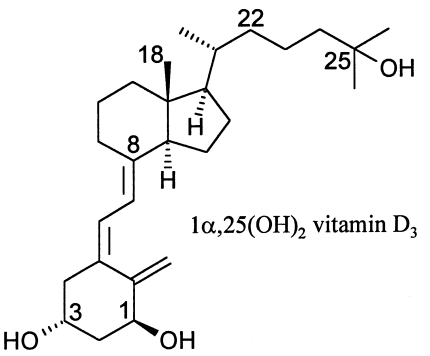
**Abstract**—A new class of analogues of 1α,25-dihydroxy vitamin $D_3$ has been synthesised, in which the side chain (C-23 to C-27) has been removed and where new hydroxylated side chains have been attached to the C-18 methyl group. These analogues show antiproliferative activity in U937 and HaCaT cells comparable to that of 1α,25-dihydroxy vitamin $D_3$. Lack of calcemic activity makes these analogues potentially useful in the treatment of proliferative diseases. © 1998 Published by Elsevier Science Ltd. All rights reserved.

## Introduction

Numerous analogues of 1α,25-dihydroxy vitamin $D_3$ ($1,25(OH)_2$ $D_3$) have been investigated in biological tests in order to explain structure–activity relationships. $1,25(OH)_2$ $D_3$ and its analogues exhibit a multitude of biological activities,[1] including antiproliferative effects and the ability to stimulate cell differentiation, to induce apoptosis, to regulate the immune system and to mobilise calcium. Vitamin $D_3$ compounds are therefore obvious candidates as therapeutic agents in the treatment of immune diseases, bone disorders and hyperproliferative diseases, such as cancer and psoriasis. However, the fact that vitamin $D_3$ compounds affect the calcium homeostasis often leads to induction of hypercalcemia and thus limits the usefulness of such compounds in the clinic. Therefore, the current objective of synthesising analogues of $1,25(OH)_2$ $D_3$ is to develop compounds with potent cellular effects but with reduced effects on the calcium metabolism.[2]

The present work describes our effort to shed some light on the structure–activity relationship of a new class of analogues of $1,25(OH)_2$ $D_3$.[3] The high anti-proliferative activity of 20-epi analogues of $1,25(OH)_2$ $D_3$[2] together




with the calculated preferred orientation of the side chain in these analogues to the 'left'[4] prompted us to test the biological activity of analogues in which the side chain (carbon atoms C-23 to C-27) of $1,25(OH)_2$ $D_3$ has been removed and a variety of new hydroxylated side chains have been attached to C-18. In these compounds, the reach of the side chain hydroxy group would be restricted to a space in or above the plane of the CD-ring system. Furthermore, due to the steric hindrance of the residual 2-propyl group in position 17, the side chain hydroxy group in the new 18-analogues would not be able to reach as far to the 'right' of C-22 as the 25-hydroxy group of $1,25(OH)_2$ $D_3$ is able to. We describe the synthesis and biological activity of these compounds.

Key words: Vitamins; antiproliferative agents.
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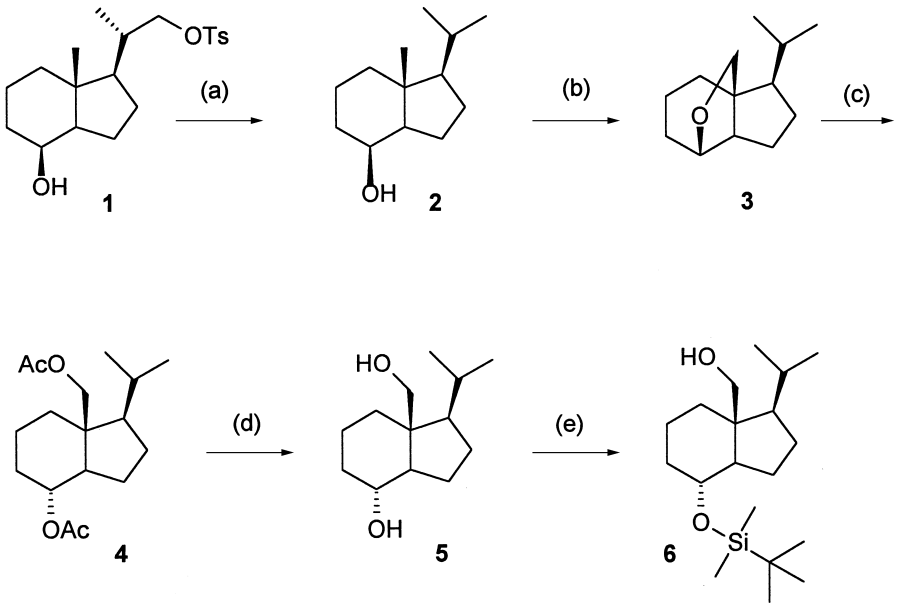



**Scheme 1.** (a) $LiAlH_4$, THF, reflux, 21 h, 93%; (b) $Pb(OAc)_4$, benzene, pyridine, hv, 15 °C, 3.5 h, 88%; (c) $BF_3{\cdot}Et_2O$, $Ac_2O$, −25 to 25 °C, 1 h, 39%; (d) KOH, MeOH, $H_2O$, reflux, 2.5 h, 65%; (e) *tert*-butyldimethylsilyl chloride, imidazole, DMF, 25 °C, 4 h, 94%.

## Results and discussion

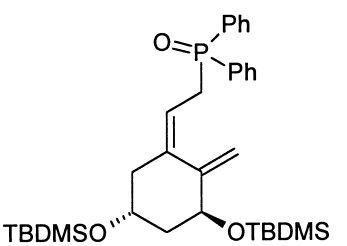
### Synthesis

The synthesis of the new analogues with side chains attached to C-18 is divided into three parts: (1) synthesis of the key CD-ring intermediate **6** (Scheme 1), (2) attachment of the new side chains at C-18 (Schemes 2–4), and (3) attachment of the A-ring followed by removal of protecting groups to give the new analogues **12a–i** (Scheme 2). Chemical yields for compounds **7** to **12** are given in Table 1.

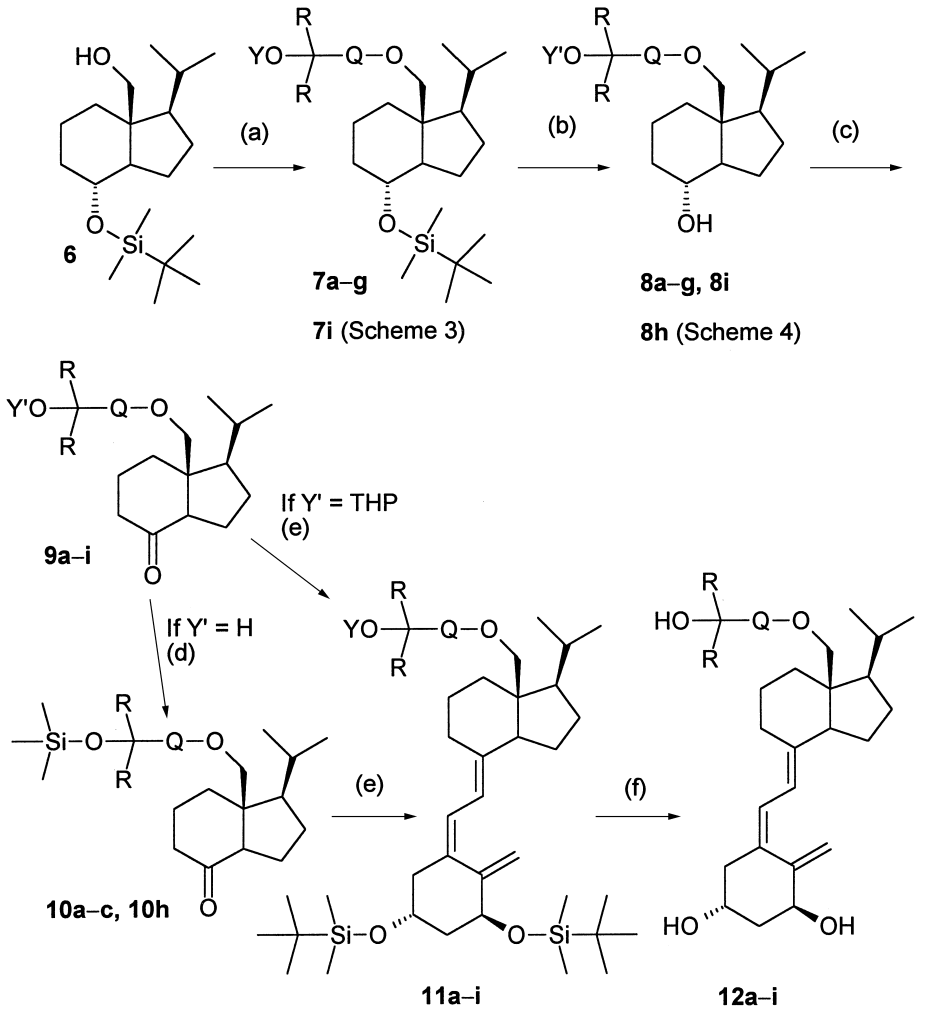
The mono-tosylate **1**[5] was reduced with $LiAlH_4$ to the alcohol **2**, in which the hydroxy group and the 18-methyl group share a 1,3 *trans* diaxial relationship (Scheme 1). Oxidation mediated by lead tetraacetate and UV-light[6,7] produces **3**, in which an ether bridge between C-8 and C-18 has been established. The ether was cleaved with a Lewis acid (boron trifluoride etherate) in acetic anhydride[6] to give the diacetate **4**, where the configuration at C-8 was inverted. Hydrolysis to the diol **5**, followed by mono-silylation completes the synthesis of the key CD-ring intermediate **6** in an overall yield of 20% from **1**.

Compounds **7a–g** were prepared by alkylation of the alcohol at C-18 in compound **6** with a series of hydroxy group protected side chain alkyl bromides (**20a**,[8] **20b**,[9] **20c**,[9] **20d**,[10] **20e**,[10] **20f**,[11] **20g**,[12] see Fig. 1), and potassium hydride/18-crown-6 in THF (Scheme 2). Attempts to alkylate **6** with a preformed alkyl bromide to give **7i** gave elimination of HBr to the conjugated enyne side chain fragment. Thus, the side chain of compound **7i** was attached to **6** in a 5-step sequence (Scheme 3). Similarly, attempts to alkylate **6** with a preformed alkyl bromide to give a compound with the side chain of compound **8h** were unsuccessful. In this case, the side chain was attached in a two-step sequence (Scheme 4).

In order to successfully join the CD-ring moiety with the A-ring phosphine oxide **19**, the deprotected alcohol at C-8 in compounds **8a–i** was oxidised to the corresponding ketone with pyridinium dichromate and any unprotected tertiary alcohol in the side chain was protected as its TMS ether (compounds **10a–c** and **10h**). The tertiary hydroxy group of compounds **9d–g** and **9i** was protected as THP ether. The coupling of the A-with the CD-ring fragments and deprotection of hydroxy groups followed known methods.[13]




**19**



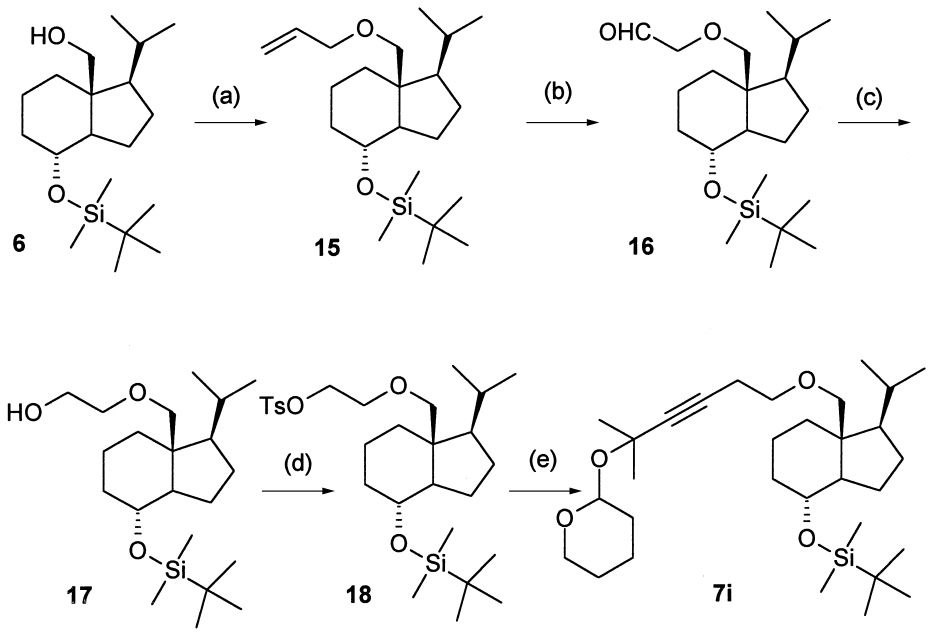

**Scheme 2.** (a) KH, 18-Crown-6, Y-O-$CR_2$-Q-Br (**20a–g**), THF, rt, 20 min; (b) **7a–c**: HF, EtOAc, MeCN, $H_2$, rt, 40 min or **7d–g** and **7i**: TBA fluoride trihydrate, THF, 60 °C, 3 h; (c) pyridinium dichromate, $CH_2Cl_2$, rt, 18 h; (d) **9a–c** and **9h**: trimethylsilyl chloride, ethyldiisopropylamine, $CH_2Cl_2$, rt, 140 min; (e) Ref. 13; (f) HF, EtOAc, MeCN, $H_2O$, rt, 40 min.

## In vitro and in vivo activity

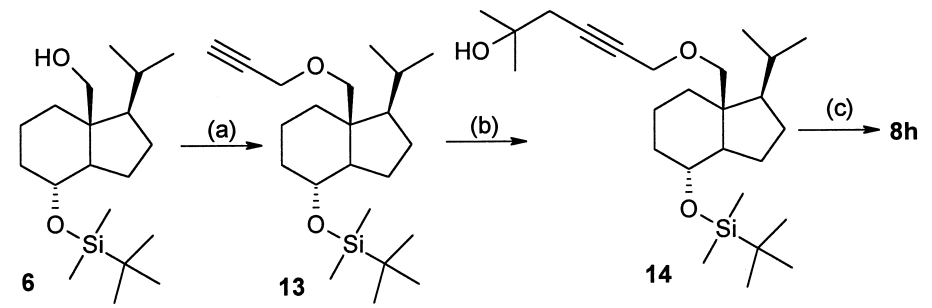
The in vitro potencies of the compounds **12a–i** were investigated by determining their antiproliferative effects in two human cell lines, the U937 lymphoma cell line and the HaCaT keratinocyte cell line. In addition, the binding affinity of the compounds for the vitamin D receptor (VDR) was assessed using isolated VDR's from the intestine of rachitic chickens. Table 2 summarises the results obtained from these in vitro studies. The in vivo calcemic activity of the compounds **12a–i** was determined after 7 days of oral administration to rats (Table 3).

The receptor binding studies were performed by displacement of [$^3$H]-1,25(OH)$_2$ $D_3$ from the receptor by adding increasing concentrations of the test compounds. Displacement of 50% of the bound of [$^3$H]-1,25(OH)$_2$ $D_3$ was obtained with 1,25(OH)$_2$ $D_3$ at $(10.0 \pm 4.5) \times 10^{-11}$ M ($n = 11$). As shown in Table 2, the test compounds bound only weakly to the VDR. The compounds with the most flexible side chains (**12a–c**) displayed the highest binding affinities, although these were still reduced by a factor 3-5 when compared to the parent compound, 1,25(OH)$_2$ $D_3$.

The antiproliferative activity of the test compounds in the HaCaT cell line was assessed by [$^3$H]-thymidine uptake, whereas the activity of the compounds in the U937 cell line was determined by counting the cells. 50% inhibition of the cell growth ($IC_{50}$) was obtained with 1,25(OH)$_2$ $D_3$ at $(4.8 \pm 1.5) \times 10^{-8}$ M ($n = 13$) and




**Scheme 3.** (a) KH, 18-Crown-6,3-bromoprop-1-ene, THF, rt, 80 min, 87%; (b) ozone, $CH_2Cl_2$, MeOH, −70 °C, 30 min, 100%; (c) $NaBH_4$, THF, MeOH, 0 °C, 35 min, 76%; (d) TsCl, pyridine, 0 °C, 4 h, 59%; (e) H-C≡C-$CMe_2$OTHP, *n*-BuLi, dioxane, 90 °C, 48 h, 55%.




**Scheme 4.** (a) *tert*-BuOK, 18-Crown-6, THF, 3-bromoprop-1-yne, rt, 19 h, 50%; (b) *n*-BuLi, THF, $BF_3{\cdot}Et_2O$, 2,2-dimethyloxirane, −78 °C to rt, 1 h, 45%; (c) HF, EtOAc, MeCN, $H_2O$, rt, 40 min, 85%.

$(3.4 \pm 2.3) \times 10^{-8}$ M ($n = 12$) in the HaCaT and in the U937 cell line, respectively.

Interestingly, the compounds with the highest binding affinity for the VDR (**12a–c**) also appeared to be the most potent analogues with respect to their effects on cell proliferation, but the difference in antiproliferative activity between the nine analogues (**12a–i**) is quite small (within one order of magnitude). This comparable antiproliferative activity of these analogues of 1,25$(OH)_2$ $D_3$ together with the limited range of the C-18 side chain point to a binding site for the side chain hydroxy group onto the VDR on the same side of the CD ring system as the C-18 methyl group but not far to the right of the 2-propyl group at C-17 (cf. Ref. 14).

Table 3 shows the mean urinary excretion of calcium and the serum calcium levels after 7 days of oral administration of the test compounds to rats. The data clearly show that all the compounds **12a–i** exerted only weak effects on the calcium metabolism in vivo and were at least 20 times less calcemic than 1,25$(OH)_2$ $D_3$.

## Conclusion

The synthesis of 1α,25-dihydroxy vitamin $D_3$ analogues, in which the side chain from C-23 to C-27 was removed and in which new side chains were attached to the C-18 methyl group have succesfully been carried out. These analogues show a weak binding to the VDR and an

**Table 1.** Chemical yields

| Compound Y/Y′ Chemical yield (%) | | | | | | R | Q |
|---|---|---|---|---|---|---|---|
| **7a** | **8a** | **9a** | **10a** | **11a** | **12a** | Me | $(CH_2)_3$ |
| TMS | H | H | | TMS | | | |
| 72 | 55 | 78 | 80 | 80 | 66 | | |
| **7b** | **8b** | **9b** | **10b** | **11b** | **12b** | Et | $(CH_2)_3$ |
| TMS | H | H | | TMS | | | |
| 66 | 73 | 81 | 65 | 59 | 75 | | |
| **7c** | **8c** | **9c** | **10c** | **11c** | **12c** | Me | $(CH_2)_4$ |
| TMS | H | H | | TMS | | | |
| 64 | 79 | 80 | 61 | 59 | 67 | | |
| **7d** | **8d** | **9d** | | **11d** | **12d** | Me | *m*-$C_6H_4CH_2$ |
| THP | THP | THP | | THP | | | |
| 95 | 87 | 79 | | 61 | 54 | | |
| **7e** | **8e** | **9e** | | **11e** | **12e** | Et | *m*-$C_6H_4CH_2$ |
| THP | THP | THP | | THP | | | |
| 80 | 75 | 74 | | 43 | 60 | | |
| **7f** | **8f** | **9f** | | **11f** | **12f** | Me | $C=CCH_2$ |
| THP | THP | THP | | THP | | | |
| 65 | 77 | 67 | | 41 | 48 | | |
| **7g** | **8g** | **9g** | | **11g** | **12g** | Et | $C=CCH_2$ |
| THP | THP | THP | | THP | | | |
| 73 | 86 | 74 | | 13 | 69 | | |
| | **8h** | **9h** | **10h** | **11h** | **12h** | Me | $CH_2C=CCH_2$ |
| | H | H | | TMS | | | |
| | 85 | 81 | 34 | 70 | 50 | | |
| **7i** | **8i** | **9i** | | **11i** | **12i** | Me | $C=C(CH_2)_2$ |
| H | THP | THP | | THP | | | |
| 55 | 62 | 60 | | 40 | 57 | | |

antiproliferative activity comparable to that of 1α, 25-dihydroxy vitamin $D_3$. This activity indicates a binding site for the side chain hydroxy group to the vitamin D receptor in or above the plane of the CD-ring system. Lack of calcemic activity makes these analogues potentially useful in the treatment of proliferative diseases.

## Experimental

### General

$^1H$ and $^{13}C$ NMR spectra were recorded in $CDCl_3$ on a Bruker AM300 spectrometer and chemical shifts are reported in ppm downfield from tetramethylsilane (s, d, t, m and b for singlet, doublet, triplet, multiplet and broad, respectively). Mass spectra were recorded on an Autospec Micromass spectrometer (EI spectra) and a Quattro II Micromass spectrometer (ES spectra). For column chromatography Merck silica gel 60 (40–63 μm) was used. Organic extracts were dried with magnesium sulfate before evaporation in vacuo on a rotary evaporator.

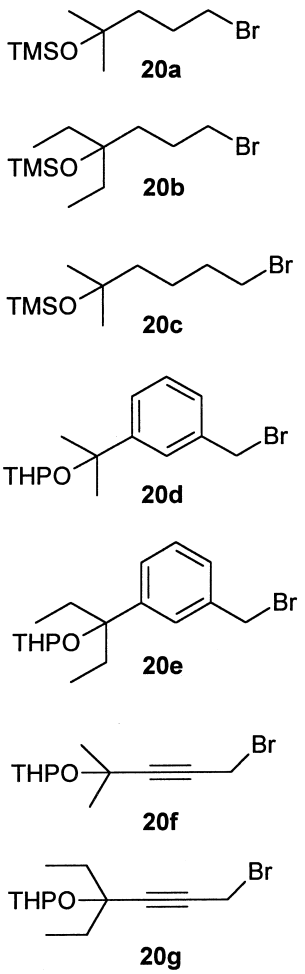
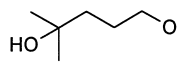
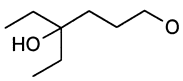
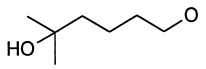
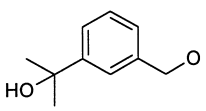
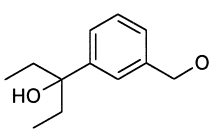
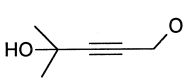
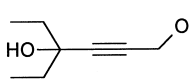
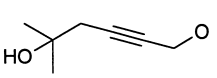
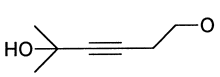



**Figure 1.** Hydroxy group protected side chain alkyl bromides **20a–g**. TMS = trimethylsilyl and THP-tetrahydropyran-2-yl.

Ozone was produced on a Fisher ozone generator model 500.

**20-Methyl-de-*A,B*-pregnan-(8*S*)-ol (2).** A solution of 20*S*-(4-methylphenylsulfonyloxymethyl)-de-*A,B*-pregnan-(8*S*)-ol (**1**)[5] (44.0 g, 120 mmol) in dry THF (450 mL) was refluxed under argon with $LiAlH_4$ (8.5 g, 223 mmol) for 21 h. After cooling the mixture was quenched with drops of water and filtered through filter aid. The filtrate was mixed with water (1.0 L) and ether (0.5 L). The organic phase was washed with water (0.5 L) and brine (0.5 L), and removal of solvent gave compound **2** as a colourless oil (22.0 g, 93%). An analytical sample was obtained by chromatography with EtOAc/petroleum ether (1/2). $^1H$ NMR δ 0.84 (d, 3H), 0.91 (d, 3H), 0.93 (s, 3H), 0.90–1.92 (m, 13H), 1.98 (m, 1H), 4.07 (m, 1H). $^{13}C$ NMR δ 69.4, 58.8, 52.7, 41.9, 40.3, 33.6, 30.6, 27.4, 23.1, 22.6, 22.4, 17.5, 13.7. MS ($EI^+$) *m/z* 196.2 ($M^+$).

**Table 2.** In vitro biological activities

| Compound | Side chain | VDR binding[a] | Inhibition of proliferation HaCaT[b] | Inhibition of proliferation U937[c] |
|---|---|---|---|---|
| **12a** |  | 0.2× | 0.5×[d] | 2× |
| **12b** |  | 0.3× | 2×[e] | 1× |
| **12c** |  | 0.2× | 2× | 3× |
| **12d** |  | < 0.02× | 0.5× | < 0.5× |
| **12e** |  | < 0.04× | 0.4× | 0.3× |
| **12f** |  | 0.02× | < 0.4× | 0.3× |
| **12g** |  | 0.07× | 0.8× | 0.4× |
| **12h** |  | 0.02× | 0.9× | 0.4× |
| **12i** |  | 0.02× | 0.8× | 0.4× |

[a]The binding affinity for the vitamin D receptor (VDR) of each compound was measured ($n=3$) relative to 1,25$(OH)_2$ $D_3$: $IC_{50}=1.00\times10^{-10}$ M (coefficient of variation (CV) = 45%, $n=11$).
[b]The inhibitory effect of each compound was measured ($n=2$) relative to 1,25$(OH)_2$ $D_3$: $IC_{50}=4.8\times10^{-8}$ M (CV = 31%, $n=13$).
[c]The inhibitory effect of each compound was measured ($n=3$) relative to 1,25$(OH)_2$ $D_3$: $IC_{50}=3.4\times10^{-8}$ M (CV = 68%, $n=12$).
[d]$n=1$.
[e]$n=3$.

**8*S*,18-Epoxy-20-methyl-de-*A*,*B*-pregnane (3).** (cf. Ref. 6). A mixture of compound **2** (1.40 g, 7.1 mmol), pyridine (3.5 mL) and lead tetraacetate (15.9 g, 35.8 mmol) in benzene (375 mL) in an atmosphere of argon was irradiated with UV-light from a Hanau TG 700 W mercury lamp at 15 °C for 3.5 h. The reaction mixture was filtered, the precipitate was washed with ether (3×100 mL) and the solvent was removed from the combined filtrates. The residue was mixed with EtOAc/petroleum ether (1/3), filtered and chromatographed with EtOAc/petroleum ether 1/3 to give compound **3** (1.22 g, 88%). $^1$H NMR δ 0.85 (d, 3H), 0.91 (d, 3H), 0.90–2.10 (m, 13H), 3.70 (m, 2H), 4.14 (d, 1H). $^{13}$C NMR δ 78.9, 70.9, 57.9, 54.2, 53.4, 37.1, 32.4, 32.4, 28.5, 24.9, 22.9, 22.5, 18.9.

**8*R*,18-Diacetoxy-20-methyl-de-*A*,*B*-pregnane (4).** Boron trifluoride etherate (90 mL) was added dropwise under argon and at −25 to −20 °C to a solution of compound

**Table 3.** Calcemic activity in normal rats[a]

| Compound | Dose μg/kg/day oral 7x | Calcium in urine μmol/day mean ± SD | p-value[b] | Calcium in serum mmol/L mean ± SD | p-value[b] |
|---|---|---|---|---|---|
| **12a** | 1.0 | 24.8 ± 9.9 | ns | 2.93 ± 0.04 | ns |
| | 10 | 37.8 ± 14.6 | $p < 0.01$ | 2.96 ± 0.15 | ns |
| $1,25(OH)_2$ $D_3$ | 0.5 | 111.9 ± 49.3 | $p < 0.001$ | 3.11 ± 0.08 | $p < 0.02$ |
| Control | — | 25.9 ± 4.1 | — | 2.91 ± 0.03 | — |
| **12b** | 1.0 | 17.5 ± 6.0 | ns | 2.76 ± 0.17 | ns |
| | 10 | 20.7 ± 7.1 | $p < 0.001$ | 2.78 ± 0.10 | ns |
| $1,25(OH)_2$ $D_3$ | 0.5 | 57.2 ± 37.4 | $p < 0.001$ | 2.87 ± 0.08 | ns |
| Control | — | 16.5 ± 6.7 | — | 2.70 ± 0.10 | — |
| **12c** | 1.0 | 20.5 ± 7.0 | ns | 2.91 ± 0.20 | ns |
| | 10 | 34.1 ± 13.6 | $p < 0.01$ | 2.71 ± 0.06 | ns |
| $1,25(OH)_2$ $D_3$ | 0.5 | 152.7 ± 39.8 | $p < 0.001$ | 3.03 ± 0.14 | $p < 0.05$ |
| Control | — | 20.2 ± 6.0 | — | 2.69 ± 0.12 | — |
| **12d** | 10 | 33.5 ± 12.8 | $p < 0.05$ | 2.73 ± 0.15 | ns |
| | 100 | 24.5 ± 8.9 | ns | 3.00 ± 0.04 | $p < 0.01$ |
| $1,25(OH)_2$ $D_3$ | 1.0 | 155.7 ± 58.6 | $p < 0.001$ | 2.88 ± 0.22 | ns |
| Control | — | 24.2 ± 8.4 | — | 2.61 ± 0.12 | — |
| **12e** | 10 | 31.4 ± 10.0 | $p < 0.001$ | 2.87 ± 0.08 | $p < 0.05$ |
| | 100 | 31.8 ± 20.7 | ns | 2.71 ± 0.13 | ns |
| $1,25(OH)_2$ $D_3$ | 1.0 | 155.7 ± 58.6 | $p < 0.001$ | 2.88 ± 0.22 | ns |
| Control | — | 24.2 ± 8.4 | — | 2.61 ± 0.12 | — |
| **12f** | 10 | 25.9 ± 13.0 | ns | 2.79 ± 0.02 | ns |
| | 100 | 17.9 ± 8.0 | $p < 0.01$ | 2.75 ± 0.13 | ns |
| $1,25(OH)_2$ $D_3$ | 0.5 | 93.3 ± 37.1 | $p < 0.001$ | 3.04 ± 0.05 | ns |
| Control | — | 29.4 ± 9.6 | — | 2.81 ± 0.17 | — |
| **12g** | 1.0 | 24.1 ± 10.2 | ns | 2.88 ± 0.13 | ns |
| | 10 | 27.4 ± 15.8 | ns | 2.87 ± 0.14 | ns |
| $1,25(OH)_2$ $D_3$ | 0.5 | 152.7 ± 39.8 | $p < 0.01$ | 3.03 ± 0.14 | $p < 0.05$ |
| Control | — | 20.2 ± 6.0 | — | 2.69 ± 0.12 | — |
| **12h** | 1.0 | 25.4 ± 6.7 | $p < 0.02$ | 3.07 ± 0.22 | ns |
| | 10 | 17.3 ± 6.3 | ns | 3.06 ± 0.06 | $p < 0.02$ |
| $1,25(OH)_2$ $D_3$ | 0.5 | 80.3 ± 32.6 | $p < 0.001$ | 3.04 ± 0.02 | $p < 0.01$ |
| Control | — | 19.7 ± 4.5 | — | 2.85 ± 0.06 | — |
| **12i** | 10 | 22.7 ± 6.6 | ns | 1.98 ± 0.02 | $p < 0.01$ |
| | 100 | 17.5 ± 3.7 | ns | 1.71 ± 0.06 | $p < 0.001$ |
| $1,25(OH)_2$ $D_3$ | 0.5 | 112.7 ± 29.7 | $p < 0.001$ | 2.37 ± 0.45 | ns |
| Control | — | 19.6 ± 5.2 | — | 2.23 ± 0.06 | — |

[a]For experimental details, see text.
[b]ns: not significant.

**3** (8.0 g, 41.2 mmol) in acetic anhydride (500 mL). Stirring was continued for 45 min at −20 °C and for 1 h at rt. The mixture was poured into ice cold saturated aqueous sodium hydrogencarbonate (1 L). After stirring for 1 h the aqueous phase was extracted with ether (3×300 mL). The combined ether phases were washed with saturated aqueous sodium hydrogencarbonate (6×200 mL), water (500 mL) and brine (250 mL), and were dried and evaporated. Chromatography with EtOAc/petroleum ether (1/10 to 1/4) gave compound **4** (4.7 g, 39%). $^1$H NMR δ 0.85 (d, 3H), 1.02 (d, 3H), 2.01 (s, 3H), 2.06 (s, 3H), 0.90–2.35 (m, 13H), 3.97 (d, 1H), 4.16 (d, 1H), 4.90 (m, 1H). $^{13}$C NMR δ 171.0, 170.6, 73.0, 62.2, 58.8, 53.9, 47.8, 34.5, 32.0, 31.2, 27.7, 23.4, 23.4, 22.8, 21.5, 21.2, 21.0. MS (ES$^+$) *m/z* 297 ([M + H]$^+$).

**20-Methyl-de-*A*,*B*-pregnane-8*R*,18-diol (5).** Compound **4** (4.7 g, 15.9 mmol) was dissolved in methanol (50 mL) and a 10% (w/v) solution of potassium hydroxide in methanol (30 mL) was added. After reflux for 2.5 h the reaction mixture was filtered through silica gel. The filtrate was evaporated and chromatographed with EtOAc/petroleum ether (2/1) to give compound **5** (2.2 g, 65%). Melting point 100–101 °C (from chloroform).

$^1$H NMR δ 0.88 (d, 3H), 1.04 (d, 3H), 0.90–2.10 (m, 14H), 2.33 (m, 1H), 3.62 (m, 2H), 3.67 (m, 1H). $^{13}$C NMR δ 70.6, 60.5, 58.9, 56.9, 49.2, 35.8, 34.1, 31.4, 28.0, 23.4, 23.4, 23.2, 21.9. MS (EI$^+$) *m*/*z* 212.1 (M$^+$).

**8*R*-*tert*-Butyldimethylsilyloxy-20-methyl-de-*A*,*B*-pregnan-18-ol (6).** Compound **5** (2.2 g, 10.4 mmol), imidazole (1.8 g, 26.4 mmol) and *tert*-butyldimethylsilyl chloride (1.7 g, 11.3 mmol) was stirred in dry DMF (95 mL) at rt for 4 h. The reaction mixture was partitioned between water (200 mL) and ether (200 mL). The organic phase was washed with water (100 mL) and brine (100 mL) and evaporated. The residue was chromatographed with EtOAc/petroleum ether (1/3) to give compound **6** (3.2 g, 94%). $^1$H NMR δ 0.01 (s, 3H), 0.02 (s, 3H), 0.85 (s, 9H), 0.85 (d, 3H), 1.02 (d, 3H), 0.90–1.98 (m, 13H), 2.29 (m, 1H), 3.52–3.70 (m, 3H). $^{13}$C NMR δ 71.0, 60.5, 58.7, 56.7, 48.9, 36.2, 33.8, 31.3, 27.6, 25.7, 24.1, 23.2, 23.0, 21.8, −4.4, −4.9. MS (EI$^+$) *m*/*z* 326.2 (M$^+$).

**The alkylation of compound 6 to compound 7g is representative for the series of reactions leading from 6 to 7a–g**

**Alkylation of compound 6 to compound 7g.** Compound **6** (0.234 g, 0.72 mmol) and 1-bromo-4-ethyl-4-(tetrahydropyran-2-yloxy)-hex-2-yne (**20g**) (0.42 g, 1.45 mmol) were dissolved in dry THF (5 mL) under argon and potassium hydride (0.22 mL of a 20% oil emulsion, 1.1 mmol) was added. After 5 min a solution of 18-crown-6 (0.19 g, 0.72 mmol) in dry THF (1.5 mL) was added. After stirring for 20 min at rt the reaction mixture was partitioned between water (50 mL) and ether (50 mL). The ether phase was washed with brine (25 mL) and the residue after evaporation of the solvent was chromatographed with ether/petroleum ether (1/10) to give the desired compound **7g** (1/1 mixture of two THP isomers). Yield: 0.28 g (73%). $^1$H NMR δ 0.01 (s, 3H), 0.02 (s, 3H), 0.81 (d, 3H), 0.86 (s, 9H), 0.90–1.95 (m, 31H), 2.27 (m, 1H), 3.26 (dd, 1H), 3.46 (m, 2H), 3.64 (m 1H), 3.93 (m, 1H), 4.13 (m, 2H), 5.03 (m, 1H). $^{13}$C NMR δ 95.7, 95.7, 86.5, 86.4, 82.4, 82.4, 78.3, 71.2, 67.5, 67.5, 63.1, 63.1, 58.9, 58.3, 56.8, 48.1, 36.2, 34.5, 32.5, 31.9, 31.8, 31.7, 31.7, 31.0, 27.7, 25.7, 25.2, 24.2, 23.1, 22.9, 21.6, 20.2, 20.2, 17.9, 8.6, 8.6, 8.3, 8.3, −4.4, −4.9.

**8*R*-*tert*-Butyldimethylsilyloxy-20-methyl-18-(prop-2-yn-1-yloxy)-de-*A*,*B*-pregnane (13).** A solution of compound **6** (0.446 g, 1.37 mmol) , 18-crown-6 (0.36 g, 1.36 mmol) and potassium *tert*-butoxide (0.31 g, 2.8 mmol) in dry THF (12 mL) was stirred for 10 min and 3-bromoprop-1-yne (0.32 g, 2.7 mmol) was added, and after stirring for 1 h 3-bromoprop-1-yne (0.64 g, 5.4 mmol) and potassium *tert*-butoxide (0.62 g, 5.5 mmol) were finally added. After stirring for 19 h ether was added and the reaction mixture was washed with water (40 mL) and brine (40 mL) and concentrated. Chromatography with ether/petroleum ether (1/20) as eluant gave compound **13** (0.25 g, 50%). $^1$H NMR δ 4.06 (d, 2H), 3.66 (dt, 1H), 3.42 (d, 1H), 3.29 (d, 1H), 2.39 (t, 1H), 2.24 (m, 1H), 1.95–0.85 (m, 12H), 0.96 (d, 3H), 0.86 (s, 9H), 0.82 (d, 3H), 0.029 (s, 3H), 0.024 (s, 3H). $^{13}$C NMR δ 80.0, 73.8, 71.1, 68.2, 58.9, 58.2, 56.9, 48.2, 36.3, 34.8, 31.0, 27.8, 25.7, 24.2, 23.2, 22.8, 21.7, 18.0, −4.3, −4.8.

**8*R*-*tert*-Butyldimethylsilyloxy-20-methyl-18-(5-hydroxy-5-methyl-hex-2-yn-1-yloxy)-de-*A*,*B*-pregnane (14).** *n*-Butyl lithium (0.47 mL, 1.5 M in hexane) was slowly added under argon to a solution of compound **13** (0.25 g, 0.69 mmol) in dry THF at −78 °C. The dark solution was stirred at this temperature for 40 min and boron trifluoride diethyletherate (0.100 mL, 0.79 mmol) was added. After stirring for 10 min 2,2-dimethyloxirane (0.060 g, 0.83 mmol) in dry THF (2 mL) was added and the mixture was stirred for 1 h at rt. Saturated aq ammonium chloride (10 mL) was added and the mixture was extracted with EtOAc (2×30 mL). The organic phase was washed with saturated sodium hydrogencarbonate (30 mL), water (30 mL) and brine (30 mL) and concentrated. Chromatography with ether/petroleum ether (1/2) gave **14** (0.137 g, 45%). $^1$H NMR δ 4.08 (t, 2H), 3.64 (dt, 1H), 3.46 (d, 1H), 3.27 (d, 1H), 2.40 (t, 2H), 2.25 (m, 1H), 1.95–0.85 (m, 13H), 1.31 (s, 6H), 0.96 (d, 3H), 0.85 (s, 9H), 0.81 (d, 3H), 0.02 (s, 6H). $^{13}$C NMR δ 82.6, 79.4, 71.2, 69.8, 67.8, 58.9, 58.5, 56.8, 48.2, 36.2, 34.7, 34.3, 31.0, 28.4, 27.7, 25.7, 24.2, 23.1, 22.9, 21.7, 18.0, −4.3, −4.8.

**8*R*-*tert*-Butyldimethylsilyloxy-20-methyl-18-(prop-2-en-1-yloxy)-de-*A*,*B*-pregnane (15).** Potassium hydride (3.14 mL, 20% emulsion in oil) was added to a solution of compound **6** (0.945 g, 2.9 mmol) and 18-Crown-6 (0.767 g, 2.9 mmol) in dry THF (9 mL) at −78 °C under argon. Allylbromide (2.7 g, 22 mmol) in THF (3 mL) was added and the mixture was stirred for at rt for 80 min. Ether (150 mL) was added and the mixture was quenched and washed with water (100 mL). The organic phase was washed with water (100 mL) and brine (100 mL) and concentrated. Chromatography with ether/petroleum ether (2/98) gave **15**. Yield: 0.92 g (87%). $^1$H NMR ($CDCl_3$) δ 5.86 (m, 1H), 5.24 (m, 1H), 5.12 (m, 1H), 3.86 (m, 2H), 3.69 (dt, 1H), 3.34 (d, 1H), 3.21 (d, 1H), 2.28 (m, 1H), 1.95–0.90 (m, 12H), 0.98 (d, 3H), 0.86 (s, 9H), 0.82 (d, 3H), 0.024 (s, 3H), 0.018 (s, 3H). $^{13}$C NMR ($CDCl_3$) δ 135.1, 115.5, 72.1, 71.2, 68.7, 58.9, 56.9, 48.3, 36.3, 35.1, 31.0, 27.8, 25.7, 24.2, 23.2, 22.9, 22.0, 18.0, −4.3, −4.8.

**8*R*-*tert*-Butyldimethylsilyloxy-20-methyl-18-(carbonylmethoxy)-de-*A*,*B*-pregnane (16).** Ozone was passed through a solution of compound **15** (0.90 g, 2.45 mmol) in a mixture of dichloromethane (60 mL) and methanol

(20 mL) at −70 °C for 30 min until no more starting compound **15** could be detected (TLC, ether/petroleum ether, 1/10). Triphenylphosphine (0.8 g, 3.0 mmol) was added and the mixture was stirred at −70 °C for 30 min. The solvent was removed and the residue was chromatographed with ether/petroleum ether (1/4) followed by ether as eluant to give compound **16** (0.90 g, 100%). $^1$H NMR δ 9.72 (t, 1H), 3.95 (t, 2H), 3.63 (dt, 1H), 3.47 (d, 1H), 3.31 (d, 1H), 2.33 (m, 1H), 1.95–0.90 (m, 12H), 1.00 (d, 3H), 0.87 (s, 9H), 0.83 (d, 3H), 0.024 (s, 3H), 0.014 (s, 3H). $^{13}$C NMR δ 201.2, 76.7, 71.0, 70.3, 58.8, 56.7, 48.5, 36.2, 34.6, 31.3, 27.7, 25.7, 24.2, 23.2, 22.8, 21.8, 17.9, −4.3, −4.8.

**8*R*-*tert*-Butyldimethylsilyloxy-20-methyl-18-(2-hydroxy-ethoxy)-de-*A*,*B*-pregnane (17).** Sodium borohydride (0.43 g, 11.4 mmol) was added to an ice cold solution of compound **16** (0.90 g, 2.45 mmol) in a mixture of THF (20 mL) and methanol (40 mL). After stirring for 35 min the mixture was evaporated and the residue was chromatographed with ether/petroleum ether (1/7) followed by ether to give compound **17** (0.69 g, 76%). $^1$H NMR δ 3.68 (m, 2H), 3.65 (dt, 1H), 3.45 (m, 2H), 3.39 (d, 1H), 3.25 (d, 1H), 2.28 (m, 1H), 1.95–0.80 (m, 13H), 0.99 (d, 3H), 0.86 (s, 9H), 0.82 (d, 3H), 0.022 (s, 3H), 0.014 (s, 3H). $^{13}$C NMR δ 72.1, 71.1, 69.2, 61.8, 58.8, 56.7, 48.4, 36.2, 34.6, 31.3, 27.8, 25.7, 24.2, 23.1, 22.8, 21.8, 17.9, −4.3, −4.8.

**8*R*-*tert*-Butyldimethylsilyloxy-20-methyl-18-[2-(4-toluene-sulfonyloxy)-ethoxy]-de-*A*,*B*-pregnane (18).** 4-Toluene-sulfonyl chloride (0.70 g, 3.7 mmol) was added to an ice cold solution of **17** (0.69 g, 1.86 mmol) in pyridine (7.5 mL). After stirring for 4 h at 0 °C ether (40 mL) was added and the mixture was washed with water (2×40 mL) and brine (40 mL) and concentrated. Chromatography with ether/petroleum ether (1/4) to give compound **18** (0.57 g, 59%). $^1$H NMR δ 7.77 (d, 2H), 7.32 (d, 2H), 4.11 (m, 2H), 3.58 (dt, 1H), 3.52 (m, 2H), 3.31 (d, 1H), 3.16 (d, 1H), 2.42 (s, 3H), 2.15 (m, 1H), 1.95-0.90 (m, 14H), 0.89 (d, 3H), 0.84 (s, 9H), 0.77 (d, 3H), 0.007 (s, 3H), 0.003 (s, 3H). $^{13}$C NMR δ 144.5, 132.9, 129.6, 127.7, 71.1, 69.4, 69.0, 68.4, 58.7, 56.6, 48.3, 36.2, 34.5, 31.0, 27.7, 25.7, 24.2, 23.1, 22.7, 21.7, 21.4, 17.9, −4.4, −4.8.

**8*R*-*tert*-Butyldimethylsilyloxy-20-methyl-18-[5-hydroxy-5-methylhex-3-yn-1-yloxy]-de-*A*,*B*-pregnane (7i).** *n*-Butyl lithium (1.4 mL, 1.5 M in hexane) was added to a solution of 3-(tetrahydropyral-2-yloxy)-3-methylbut-1-yne (0.37 g, 2.2 mmol) in dry dioxane (6 mL) at −5 °C. After stirring for 30 min at 5 °C and 1 h at rt a solution of **18** (0.28 g, 0.53 mmol) in dry dioxane (3 mL) was added. The mixture was stirred at 90 °C for 48 h. The reaction mixture was partitioned between saturated aq. sodium hydrogencarbonate (25 mL) and ethyl acetate (25 mL). The aq phase was extracted with ethyl acetate (25 mL) and the combined organic phases were washed with brine and concentrated. The residue was chromatographed with ether/petroleum ether (1/10) to give the desired compound **7i**: 0.152 g (55%). This compound was not pure and was used in the next step without further purification.

### The desilylation of compound 7g to compound 8g is representative for the series of reactions leading from 7d–g and 7i to 8d–g and 8i, respectively

**Desilylation of compound 7g with TBA fluoride.** Compound **7g** (0.250 g, 0.47 mmol) and tetra-*n*-butylammonium fluoride trihydrate (0.445 g, 1.4 mmol) in THF (5 mL) was stirred at 60 °C for 3 h. Saturated aq sodium hydrogen carbonate was (25 mL) added and the mixture was extracted with EtOAc (50 mL). The organic phase was washed with water (25 mL) and brine (25 mL) and concentrated. Chromatography with EtOAc/petroleum ether (1/1) as eluant gave the desired compound **8g** (1/1 mixture of two isomers). Yield: 0.170 g (86%). $^1$H NMR δ 0.84 (d, 3H), 0.90–2.10 (m, 32H), 3.29 (m, 1H), 3.34 (d, 1H), 3.48 (m, 2H), 3.73 (m, 1H), 3.94 (m, 1H), 4.13 (m, 2H), 5.06 (m, 1H). $^{13}$C NMR δ 95.8, 86.7, 82.7, 82.6, 78.5, 77.2, 70.8, 70.7, 67.9, 67.8, 63.2, 59.0, 58.6, 57.2, 48.5, 35.8, 34.9, 32.7, 32.1, 31.9, 31.1, 28.2, 28.2, 25.5, 23.5, 23.5, 23.4, 23.0, 21.9, 20.3, 20.3, 8.8, 8.5.

### The desilylation of compound 7c to compound 8c is representative for the series of reactions leading from 7a–c and 14 to 8a–c and 8h, respectively

**Desilylation of compound 7c with hydrogen fluoride.** A mixture of compound **7c** (0.380 g, 0.73 mmol) in EtOAc (2.5 mL), acetonitrile (8.5 mL) and a 5% (w/v) solution of hydrofluoric acid in acetonitrile/water (7/1, v/v, 7.5 mL) was stirred for 40 min at rt EtOAc (50 mL) was added and the mixture was washed with saturated aq. sodium hydrogen carbonate (50 mL), water (50 mL), brine (25 mL) and concentrated. Chromatography with EtOAc as eluant gave the desired compound **8c**. Yield: 0.189 g (79%). $^1$H NMR δ 0.84 (d, 3H), 1.00 (d, 3H), 1.21 (s, 6H), 0.85–2.10 (m, 20H), 3.31 (m, 1H), 3.20 (d, 1H), 3.31 (m, 3H), 3.72 (m, 1H). $^{13}$C NMR δ 71.2, 71.0, 70.8, 69.0, 59.0, 57.1, 48.7, 43.7, 35.9, 35.2, 31.2, 30.3, 29.2, 29.2, 28.3, 23.5, 23.4, 23.1, 22.1, 21.2.

### The oxidation of compound 8c to compound 9c is representative for the series of reactions leading from 8a–h to 9a–h

### Oxidation of compound 8c with PDC

A solution of compound **8c** (0.180 g, 0.55 mmol) and pyridinium dichromate (0.621 g, 1.65 mmol) in dichloromethane (15 mL) was stirred at rt for 18 h. Dichloro-

methane (100 mL) was added and the mixture was washed with saturated aq. sodium hydrogencarbonate (100 mL), water (100 mL) and brine (50 mL) and concentrated. The residue was chromatographed with EtOAc (compounds **9d–g** use EtOAc/petroleum ether, 1/3) as eluant to give the desired ketone **9c**. Yield: 0.142 g (80%). $^1$H NMR δ 0.86 (d, 3H), 1.03 (d, 3H), 1.20 (s, 3H), 1.21 (s, 3H), 1.10–2.40 (m, 19H), 2.45 (m, 1H), 3.12 (d, 1H), 3.28 (m, 3H). $^{13}$C NMR δ 211.5, 71.3, 70.8, 70.0, 60.4, 58.6, 52.7, 43.4, 40.5, 36.2, 30.9, 30.0, 29.3, 29.2, 27.8, 23.9, 23.2, 23.0, 20.8, 19.5.

**The silylation of compound 9c to compound 10c is representative for the series of reactions leading from 9a–c and 9h to 10a–c and 10h, respectively**

**Silylation of side chain hydroxy group in compound 9c.** A solution of compound **9c** (0.132 g, 0.41 mmol), *N*-ethyldiisopropylamine (0.106 g, 0.82 mmol) and trimethylsilyl chloride (0.089 g, 0.82 mmol) in dichloromethane (4 mL) was stirred at rt for 140 min. Dichloromethane (30 mL) was added and the mixture was diluted with phosphate buffer (30 mL, pH 6.5). The organic phase was washed with brine (30 mL) and concentrated. Chromatography with EtOAc/petroleum ether (1/4) as eluant gave the desired TMS-ether **10c**. Yield: 0.100 g (61%). $^1$H NMR δ 0.08 (s, 9H), 0.84 (d, 3H), 1.02 (d, 3H), 1.18 (s, 6H), 1.10–2.40 (m, 18H), 2.47 (m, 1H), 3.06 (d, 1H), 3.24 (m, 3H). $^{13}$C NMR δ 211.3, 73.8, 71.4, 69.2, 60.3, 58.4, 52.6, 44.5, 40.4, 35.6, 30.8, 29.9, 29.6, 29.6, 27.5, 23.7, 23.0, 22.9, 20.9, 19.2, 2.4.

**Coupling of the phosphine oxide 19 with CD-ring ketone 9d–g, 9i, 10a–c and 10h.** The method described in Ref. 13 was used. Yields for compounds **11a–i** are given in Table 1.

**Deprotection of compounds 11a-i with hydrogen fluoride.** The procedure for deprotection of compounds **7a–c** was used. Yields for compounds **12a–i** are given in Table 1.

**12a**: $^1$H NMR δ 6.38 (d, 1H), 6.04 (d, 1H), 5.30 (bs, 1H), 4.39 (m, 1H), 4.21 (m, 1H), 3.30 (m, 2H), 3.14 (s, 2H), 2.84 (bd, 1H), 2.55 (dd, 1H), 2.45–1.05 (m, 22H), 1.21 (s, 3H), 1.20 (s, 3H), 1.01 (s, 3H), 0.86 (s, 3H). $^{13}$C NMR δ 147.3,142.4, 133.2, 124.4, 117.1, 111.9, 71.8, 71.0, 70.4, 69.2, 66.5, 58.6, 55.5, 49.2, 45.3, 42.6, 40.8, 35.9, 31.2, 29.1, 28.8, 28.8, 27.9, 24.8, 23.3, 23.1, 22.2. MS (EI$^+$) *m*/*z* 446.2 (M$^+$).

**12b**: $^1$H NMR δ 0.85 (d, 3H), 1.03 (d, 3H), 1.20 (s, 6H), 1.00–2.15 (m, 22H), 2.31 (dd, 1H), 2.44 (m, 1H), 2.61 (dd, 1H), 2.85 (m, 1H), 3.08 (s, 2H), 3.27 (t, 2H), 4.23 (m, 1H), 4.43 (m, 1H), 4.99 (m, 1H), 5.32 (m, 1H), 6.00 (d, 1H), 6.39 (d, 1H). 13C NMR d 147.6, 143.2, 133.1, 125.0, 117.3, 112.0, 71.1, 71.0, 68.7, 66.8, 58.9, 55.6, 49.4, 45.4, 43.7, 42.9, 35.8, 31.5, 30.4, 29.2, 29.1, 29.0, 27.9, 23.5, 23.3, 23.3, 22.4, 21.2. MS (EI$^+$) *m*/*z* 460.3 (M$^+$).

**12c**: $^1$H NMR δ 0.85 (m, 9H), 1.03 (d, 3H), 1.00–2.20 (m, 24H), 2.30 (dd, 1H), 2.38 (m, 1H), 2.61 (dd, 1H), 2.86 (m, 1H), 3.12 (s, 2H), 3.29 (m, 2H), 4.24 (m, 1H), 4.42 (m, 1H), 4.98 (m, 1H), 5.30 (m, 1H), 6.03 (d, 1H), 6.38 (d, 1H). $^{13}$C NMR δ 147.6, 142.8, 133.3, 124.8, 117.3, 112.1, 74.4, 72.0, 71.2, 69.3, 66.8, 58.9, 55.7, 49.5, 45.5, 42.8, 36.1, 35.4, 31.4, 30.9, 30.9, 29.0, 28.1, 24.1, 23.6, 23.3, 23.3, 22.4, 7.9, 7.8. MS (EI$^+$) *m*/*z* 474.3 (M$^+$).

**12d**: $^1$H NMR δ 0.85 (d, 3H), 1.07 (d, 3H), 1.57 (s, 6H), 0.80–2.15 (m, 16H), 2.30 (dd, 1H), 2.52 (m, 1H), 2.60 (dd, 1H), 2.85 (m, 1H), 3.18 (m, 2H), 4.23 (m, 1H), 4.40 (m, 3H), 4.94 (m, 1H), 5.28 (m, 1H), 5.98 (d, 1H), 6.36 (d, 1H), 7.16 (d, 1H), 7.29 (t, 1H), 7.38 (d, 1H), 7.42 (s, 1H). $^{13}$C NMR δ 149.1, 147.5, 143.0, 139.0, 133.2, 128.1, 125.5, 124.9, 123.2, 123.2, 117.3, 112.1, 77.2, 73.6, 71.1,68.5, 66.8, 58.9, 55.7, 49.5, 45.4, 42.8, 35.7, 31.7, 31.6, 31.5, 29.1, 27.8, 23.6, 23.4, 23.3, 22.3. MS (EI$^+$) *m*/*z* 494.3 (M$^+$).

**12e**: $^1$H NMR δ 0.74 (t, 3H), 0.75 (t, 3H), 0.84 (d, 3H), 1.06 (d, 3H), 1.00–2.15 (m, 20H), 2.31 (dd, 1H), 2.52 (m, 1H), 2.60 (dd, 1H), 2.84 (m, 1H), 3.17 (m, 2H), 4.24 (m, 1H), 4.40 (m, 2H), 4.42 (m, 1H), 4.95 (m, 1H), 5.30 (m, 1H), 5.99 (d, 1H), 6.36 (d, 1H), 7.10–7.35 (m, 4H). $^{13}$C NMR δ 147.6, 145.7, 143.0, 138.7, 133.2, 127.8, 127.3, 124.9, 124.4, 124.4, 117.3, 112.0, 77.2, 73.7, 71.0, 68.4, 66.8, 58.9, 55.7, 49.4, 45.4, 42.9, 35.7, 34.9, 34.9, 31.5, 29.0, 27.8, 23.5, 23.4, 23.3, 22.3, 7.8. MS (EI$^+$) *m*/*z* 522.3 (M$^+$).

**12f**: $^1$H NMR δ 6.40 (d, 1H), 6.05 (d, 1H), 5.31 (bs, 1H), 4.99 (bs, 1H), 4.43 (m, 1H), 4.21 (m, 1H), 4.11 (d, 1H), 3.92 (d, 1H), 3.32 (d, 1H), 3.06 (d, 1H), 2.87 (bd, 1H), 2.65 (bs, 1H), 2.60 (dd, 1H), 2.42 (m, 1H), 2.30 (dd, 1H), 2.15–1.05 (m,15H), 1.49 (s, 3H), 1.47 (s, 3H), 1.01 (d, 3H), 0.85 (d, 3H). $^{13}$C NMR δ 147.3, 143.1, 133.5, 124.7, 117.1, 112.6, 91.1, 78.4, 71.7, 67.3, 66.6, 65.0, 58.7, 58.4, 55.8, 49.2, 45.5, 42.7, 35.4, 31.6, 31.4, 31.1, 29.1, 27.8, 23.5, 23.2, 22.1. MS (EI$^+$) *m*/*z* 442.2 (M$^+$).

**12g**: $^1$H NMR δ 0.85 (d, 3H), 1.00 (t, 6H), 1.00 (d, 3H), 1.00–2.25 (m, 20H), 2.30 (dd, 1H), 2.43 (m, 1H), 2.61 (dd, 1H), 2.87 (m, 1H), 3.10 (d, 1H), 3.30 (d, 1H), 4.09 (m, 2H), 4.23 (m, 1H), 4.43 (m, 1H), 5.00 (m, 1H), 5.32 (m, 1H), 6.03 (d, 1H), 6.39 (d, 1H). $^{13}$C NMR δ 147.4, 143.0, 133.4, 124.8, 117.2, 112.3, 88.8, 80.7, 72.1, 71.4, 67.4, 66.7, 58.8, 58.4, 55.7, 49.1, 45.5, 42.9, 35.3, 34.4, 34.1, 31.4, 29.0, 27.7, 23.4, 23.2, 23.2, 22.2, 8.6, 8.6. MS (EI$^+$) *m*/*z* 470.3 (M$^+$).

**12h**: $^1$H NMR δ 6.38 (d, 1H), 6.02 (d, 1H), 5.31 (bs, 1H), 4.98 (bs, 1H), 4.41 (m, 1H), 4.23 (m, 1H), 4.06 (m, 2H),

3.27 (d, 1H), 3.10 (d, 1H), 2.77 (bd, 1H), 2.60 (dd, 1H), 2.43 (bd, 1H), 2.37 (bt, 2H), 2.30 (dd, 1H), 2.15–1.05 (m, 16H), 1.29 (s, 6H), 1.01 (d, 3H), 0.84 (d, 3H). $^{13}$C NMR δ 147.4, 142.9, 133.2, 124.9, 117.3, 112.4, 82.8, 79.6, 71.4, 70.0, 67.6, 66.7, 58.8, 58.7, 55.7, 49.1, 45.5, 42.9, 35.3, 34.4, 31.5, 29.0, 28.6, 27.7, 23.4, 23.3, 23.2, 22.2. MS ($EI^+$) $m/z$ 456.2 ($M^+$).

**12i**: $^1$H NMR δ 6.38 (d, 1H), 6.01 (d, 1H), 5.32 (bs, 1H), 4.99 (bs, 1H), 4.43 (m, 1H), 4.23 (m, 1H), 3.37 (bt, 2H), 3.14 (ABq, 2H), 2.85 (bd, 1H), 2.59 (dd, 1H), 2.53 (m, 1H), 2.38 (t, 2H), 2.30 (dd, 1H), 2.05–1.00 (m, 16H), 1.47 (s, 6H), 1.02 (d,3H), 0.85 (d.3H). $^{13}$C NMR δ 147.5, 143.0, 133.3, 124.8, 117.3, 112.3, 85.7, 79.9, 71.1, 69.7, 69.0, 66.8, 65.2, 58.8, 55.6, 49.4, 45.4, 42.8, 35.7, 31.6, 31.5, 29.0, 28.0, 23.5, 23.3, 23.3, 22.4, 20.1. MS ($EI^+$) $m/z$ 456.2 ($M^+$).

**Vitamin D receptor binding affinity.**[15] The binding affinity of the compounds for the vitamin D receptor was assessed by displacement of bound $^3$H-labelled 1,25-$(OH)_2$ $D_3$ from isolated receptors obtained from intestinal epithelium of rachitic chickens (Amersham Denmark Aps, Birkerd, DK). For each compound, three separate experiments were performed. Results are shown in Table 2.

**HaCaT proliferation test.**[15] HaCaT human hyperproliferating skin cells[16] were incubated in the presence of increasing concentrations of the test compounds. After 120 h of incubation, the DNA synthesis was determined by incorporation of $^3$H-labelled thymidine. Each sample was tested in quadruplicate, and two separate experiments per compound were performed. The molar concentration resulting in 50% inhibition of DNA synthesis ($IC_{50}$) was calculated from a dose response curve. Results are shown in Table 2.

**U937 proliferation test.**[15] U937 human monocytic tumour cells (ATCC, Rockville, MD) were incubated in the presence of increasing concentrations of the test compounds for 96 h. At the end of the incubation period, the cells were counted and the molar concentration resulting in 50% inhibition of cell growth ($IC_{50}$) was calculated from a dose response curve. For each compound, three separate experiments were performed. Results are shown in Table 2.

**Calcemic activity in vivo.**[15] The effect of the compounds on the calcium metabolism in vivo was determined after oral administration for 7 days to rats. Urine was collected daily, and blood was collected by cardiac puncture at day 7. Calcium levels in urine and serum were determined by complex formation with *o*-cresolphthalein, using a Hitachi 911 autoanalyser (Boehringer Mannheim, Kvistgaard, DK). The urinary excretion of calcium was calculated from days 3–7 (steady state conditions) and expressed in mmol/day (mean ± SD). Serum calcium was measured on day 7 and expressed in mmol/L (mean ± SD). Statistical analysis was carried out using Student's t-test. Results are shown in Table 3.

**Calculations.** In all in vitro experiments 1,25$(OH)_2$ $D_3$ served as reference. The results were calculated using the actual value of 1,25$(OH)_2$ $D_3$ in each separate experiment.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