# Synthesis and biological activities of 8(14)a-homocalcitriol

**Andreas Steinmeyer, Günter Neef, Gerald Kirsch, Katica Schwarz, Petra Rach, Martin Haberey, and Ruth Thieroff-Ekerdt**

*Research Laboratories of Schering AG, Berlin, Germany*

*8(14)a-Homocalcitriol was synthesized and tested for its biologic activities. It exhibited a vitamin D agonist activity profile. The compound was bound to the pig intestinal receptor with an affinity slightly less than calcitriol, showed the same potency in inducing HL 60 cell differentiation and inhibition of keratinocyte proliferation as calcitriol, and was found to be approximately 10-fold less potent in inducing hypercalcemia and hypercalciuria after a single injection in normal rats.* (*Steroids* **57**:447–452, 1992)

**Keywords:** vitamin D; convergent synthesis; seven-membered C ring, calcitriol agonist; sterols

## Introduction

1α,25-Dihydroxyvitamin $D_3$ (calcitriol), the biologically active metabolite of vitamin $D_3$, plays an important role in mediating calcium and phosphorous homeostasis. Recently it has been found that the hormonally active form of the vitamin exerts potent effects on proliferation and differentiation of certain cell types.[1–4] This discovery has enormously stimulated efforts in vitamin D research, which may lead to the development of new drugs against disease like psoriasis[5,6] or cancer.[7,8]

Meanwhile, a number of structurally modified calcitriol derivatives have been synthesized. Some of them are claimed to show drastically reduced influence in calcium and phosphorous metabolism while maintaining strong regulatory effects in cell proliferation and differentiation.[9,10]

At present most published routes to such analogs refer to variations of the vitamin D side chain.[11,12] In our contribution to this area we wish to report the synthesis of a new calcitriol derivative **1** bearing a seven-membered C ring (Scheme 1).

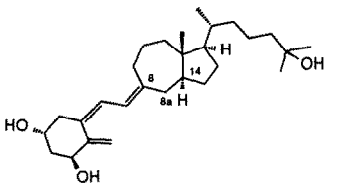
## Experimental

### *Chemistry*

Our approach is based on the Lythgoe synthesis of cholecalciferol[13] using a convergent strategy (Scheme 2). As the starting material for the "northern" part of the molecule we chose the known ketone **2**,[14] which could be prepared according to the literature. Reaction with trimethylsulfonium iodide in the presence of potassium *tert*-butylate yielded a diasteriomeric mixture of the spiro epoxides **3** and **4** (1 : 2 ratio), which were separated by chromatography. Subsequently, these oxiranes were converted to amino alcohols **5** and **6** by an aminolysis reaction. Ring enlargement could finally be achieved using classical Tiffeneau-Demyanov conditions.[15] Either of the two amino alcohols was converted to a 1 : 4 mixture of the regioisomeric ketones **7** and **8**, which easily could be silylated at the side chain alcohol function after chromatographical separation, yielding **9** and **10**.

Verification of the structures was achieved by an alternative regioselective synthesis of **9** based on a Simmons-Smith reaction[16] at the kinetically controlled silyl enol ether of ketone **2** and subsequent radical cleavage of the cyclopropyl ring with $FeCl_3$/pyridine.[17,18] Dehydrochlorination and hydrogenation of the double bond gave ketone **9**.

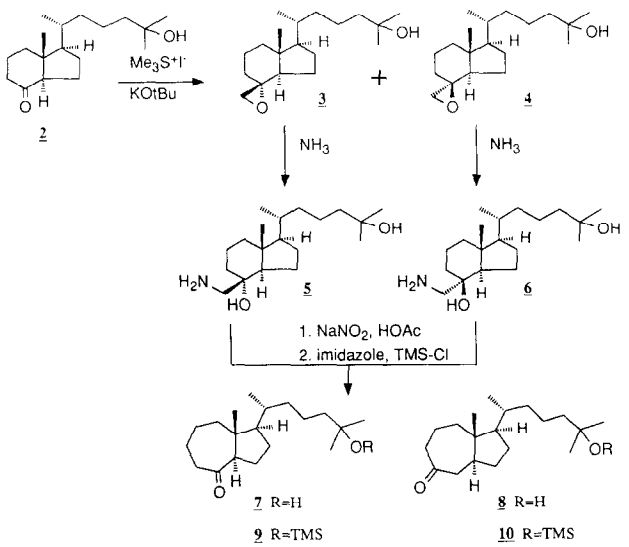
After deprotection, Wittig-Horner reaction of the deprotonated allylic phosphine oxide **11**[14] and ketone **10** yielded 8(14)a-homocalcitriol **1** (ZK150123, Scheme 3). Unfortunately the identical coupling reaction using ketone **9** failed completely, probably due to steroid reasons. Nevertheless, an approach to a potentially interesting new calcitriol analog was established.




8(14)a-homocalcitriol 1

**Scheme 1**

Address reprint requests to Dr. Andreas Steinmeyer at the Research Laboratories of Schering AG, Müllerstraße 170-178, D-1000 Berlin 65, Germany.
Received November 21, 1991; accepted April 28, 1992.



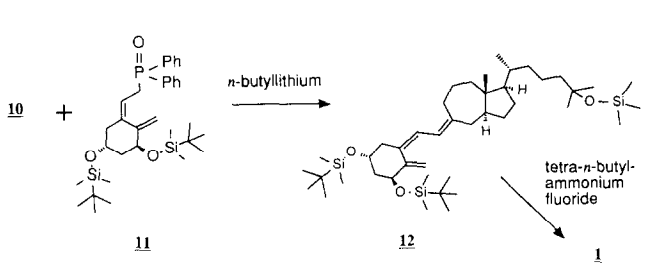

**Scheme 2**

## *General remarks*

$^1$H Nuclear magnetic resonance (NMR) spectra: General Electric QE 300 (tetramethylsilane as internal standard); infrared (IR) spectra: Perkin-Elmer PE 621; UV spectra: Perkin-Elmer Lambda 3; mass spectra (MS): Finnigan TSQ 700; and optical rotation: Perkin-Elmer polarimeter 141.

Tetrahydrofuran and diethyl ether were distilled over sodium/benzophenone prior to use. All other solvents were purchased as p.a. (pro analysi) quality and were dried over molecular sieves. All reactions were run under positive argon pressure. Unless noted otherwise, "usual work-up" indicates quenching of the reaction mixture with sodium chloride solution, extraction with ethyl acetate, washing of the organic layer with ether sodium bicarbonate solution or dilute hydrochloric acid and sodium chloride solution, drying over sodium sulfate, and evaporation of the solvent. Purification of crude materials was performed by chromatography on silicagel using ethyl acetate/hexane as eluents.

**[1*R*-[1$\alpha$(*R**),3a$\beta$,4$\beta$,7a$\alpha$]]-6-(Octahydro-7a-methylspiro[4*H*-indene-4,2'-oxiran]-1-yl)-2-methyl-2-heptanol 3 and [1*R*-[1$\alpha$(*R**),3a$\beta$,4$\alpha$,7a$\alpha$]]-6-(Octahydro-7a-methylspiro[4*H*-indene-4,2'-oxiran]-1-yl-2-methyl-2-heptanol 4.** Trimethylsulfonium iodide (5.01 g, 25 mmol) was added to a solution of **2** (4.7 g, 17 mmol) in 107 ml *N,N*-dimethylformamide. At 3 C the mixture was treated portionwise with potassium *tert*-butylate (3.11 g, 27 mmol) and stirred for 1 hour at ambient temperature. After usual work-up and purification, 1.46 g (34%) **3** and 3.0 g (64%) **4** were isolated separately as colorless oils.




**Scheme 3**

Compound **3:** IR (film): 3,440, 2,040 cm$^{-1}$ NMR (300 MHz, $CDCl_3$): $\delta$ = 0.82 ppm (d, 3H), 0.92 (s, 3H), 1.12 (s, 6H), 2.41 (d, 1H), 2.52 (d, 1H). MS (EI) m/z: 294 ($M^+$, 10), 277 (58), 259 (18), 58 (5), 35 (100). $[\alpha]_D^{20}$ = +42.7 (c = 0.515, $CHCl_3$). Analysis calculated for $C_{19}H_{34}O_2$: C, 77.50; H, 11.64. Found: C, 77.64; H, 11.80.

Compound **4:** IR (KBr): 3,480, 3,044 cm$^{-1}$. NMR (300 MHz, $CDCl_3$): $\delta$ = 0.78 ppm (s, 3H), 0.88 (d, 3H), 1.14 (s, 3H), 2.28 (d, 1H), 2.42 (d, 1H). MS (CI) m/z: 294 ($M^+$, 22), 277 (100), 259 (70), 135 (32), 35 (89). $[\alpha]_D^{20}$ = +50.4 (c = 0.525, $CHCl_3$). Analysis calculated for $C_{19}H_{34}O_2$: C, 77.50; H, 11.64. Found: C, 77.59; H, 11.69.

**[1*R*-[1$\alpha$(*R**),3a$\beta$,4$\beta$,7a$\alpha$]]-4-(Aminomethyl)octahydro-7a-methyl-1-(1,5-dimethyl-5-hydroxyhexyl)-1*H*-inden-4-ol 5.** Compound **3** (1.4 g, 5 mmol) and 20 ml ammonium hydroxide (33%) in 40 ml ethanol were stirred for 7 hours at 100 C under nitrogen pressure (30 bar). The solution was filtered through Celite, evaporated to dryness, and purified, yielding 1.4 g (90%) **5** as a colorless oil. IR (neat): 3,380, 2,950, 1,730, 1,540 cm$^{-1}$. NMR (300 MHz, $CDCl_3$): $\delta$ = 0.88 ppm (s, 3H), 0.95 (s, 3H), 1.22 (s, 6H), 2.58 (brs, 2H). MS (EI) m/z: 293 ($M^+$-$H_2O$, 1), 263 (8), 151 (13), 126 (13), 71 (100). $[\alpha]_D^{20}$ = +24.7 (c = 0.525, $CHCl_3$). Analysis calculated for $C_{19}H_{37}O_2N$: C, 73.26; H, 11.97; N, 4.50. Found: C, 73.21; H, 11.83; N 4.43.

**[1*R*-[1$\alpha$(*R**),3a$\beta$,4$\alpha$,7a$\alpha$]]-4-(Aminomethyl)octahydro-7a-methyl-1-(1,5-dimethyl-5-hydroxyhexyl)-1*H*-iden-4-ol 6.** Compound **4** (2.94 g, 10 mmol) was subjected to the previously described conditions (compound **5**), giving 2.8 g (90%) **6** as a colorless oil. IR (KBr): 3,420, 3,370, 3,310, 2,940, 1,610 cm$^{-1}$. NMR (300 MHz, $CDCl_3$): $\delta$ = 0.92 ppm (d, 3H), 0.95 (s, 3H), 1.22 (s, 6H), 2.75 (brs, 2H). MS (EI) m/z: 293 ($M^+$-$H_2O$, 1), 263 (7), 189 (5), 71 (100). $[\alpha]_D^{20}$ = +37.1 (c = 0.525, $CHCl_3$). Analysis calculated for $C_{19}H_{37}O_2N$: C, 73.26, H, 11.97; N, 4.50. Found: C, 73.31; H, 12.03, N, 4.49.

**[1*R*-[1$\alpha$(*R**),3a$\beta$,8a$\alpha$]]-Octahydro-1-(5-hydroxy-1,5-dimethylhexyl)-8a-methyl-4(1*H*)-azulenone 7 and [1*R*-[1$\alpha$(*R**),3a$\beta$,8a$\alpha$]]-Octahydro-1-(5-hydroxy-1,5-dimethylhexyl)-8a-methyl-5(1*H*)-azulenone 8.** A solution of sodium nitrite (1.10 g, 16 mmol) in 6 ml water was added dropwise at 3 C to a solution of **5** (1.4 g, 4.5 mmol) in 13.5 ml acetic acid over 30 minutes. The mixture was stirred for 4 hours at ambient temperature and was worked up as usual. After purification 90 mg (7%) **7** and 360 mg (27%) **8** were isolated as colorless oils. Using compound **6** as the starting material in this reaction gave the identical products in a similar yield.

Compound **7:** IR ($CHCl_3$): 3,443, 2,965, 1,687 cm$^{-1}$. NMR (300 MHz, $CDCl_3$): $\delta$ = 0.63 ppm (s, 3H), 0.96 (d, 3H), 1.43 (s, 3H), 2.98 (t, 1H). MS (EI) m/z: 276 ($M^+$-$H_2O$, 32), 261 (13), 165 (33), 125 (66), 55 (100). $[\alpha]_D^{20}$ = −37.9 (c = 0.245, $CHCl_3$). Analysis calculated for $C_{19}H_{34}O_2$: C, 77.50; H, 11.64. Found: C, 77.61; H, 11.70.

Compound **8:** IR (KBr): 3,340, 1,700 cm$^{-1}$. NMR (300 MHz, $CDCl_3$): $\delta$ = 0.61 (s, 3H), 0.91 (d, 3H), 1.12 (s, 3H). MS (EI) m/z: 276 ($M^+$-$H_2O$, 13), 261 (7), 192 (23), 165 (26), 87 (57), 59 (100). $[\alpha]_D^{20}$ = +10.05 (c = 0.530, $CHCl_3$). Analysis calculated for $C_{19}H_{34}O_2$: C, 77.50; H, 11.64. Found: C, 77.41; H, 11.66.

**[1*R*-[1$\alpha$(*R**),3a$\beta$,8a$\alpha$]]-Octahydro-8a-methyl-1-[1,5-dimethyl-5-[(trimethylsilyl)oxyl]hexyl]-4-(1*H*)-azulenone 9.** A solution of **7** (290 mg, 1 mmol) in 25 ml diethyl ether was treated with imidazole (272 mg, 4 mmol) and trimethylchlorosilane (216 mg, 2 mmol) and stirred for 3 hours at ambient temperature. The usual work-up and purification yielded 250 mg (70%) **9** as a

colorless oil. IR ($CHCl_3$): 2,966, 1,687, 1,382, 1,261, 1,042 $cm^{-1}$. NMR (300 MHz, $CDCl_3$): $\delta$ = 0.10 ppm (s, 9H), 0.64 (s, 3H), 0.95 (d, 3H), 1.20 (s, 3H), 3.40 (t, 1H). MS (EI) m/z: 351 ($M^+$-Me, 5), 308 (8), 131 (100), 73 (63). $[\alpha]_D^{20}$ = −29.7 (c = 0.525, $CHCl_3$). Analysis calculated for $C_{22}H_{42}O_2Si$: C, 72.07; H, 11.55. Found: C, 71.99; H, 11.51.

**[1*R*-[1α(*R**),3aβ,8aα]]-Octahydro-8a-methyl-1-[1,5-dimethyl-5-[(trimethylsilyl)oxy]-hexyl]-5(1*H*)-azulenone 10.** Compound **8** (1.05 g, 3.5 mmol) was subjected to the previously described conditions (compound **9**), yielding 980 mg (76%) **10** as a colorless oil. IR (neat): 2,880, 1,700, 1,360, 1,250, 1,040 $cm^{-1}$. NMR (300 MHz, $CDCl_3$): $\delta$ = 0.10 ppm (s, 9H) 0.67 (s, 3H), 0.97 (d, 3H), 1.21 (s, 6H). MS (EI) m/z: 351 ($M^+$-Me, 3), 307 (4), 276 (9), 131 (100), 59 (97). $[\alpha]_D^{20}$ = +72.1 (c = 0.510, $CHCl_3$). Analysis calculated for $C_{22}H_{42}O_2Si$: C, 72.07; H, 11.55. Found: C, 72.00; H, 11.46.

**(5*Z*,7*E*)-(1*S*,3*R*)-8(14)a-Homo-9,10-secocholesta-5,7,10(19)-triene-1,3,25-triol[8(14)a-Homocalcitriol] 1 (ZK 150123).** (*3S-trans*)-[2-[3,5-Bis[[dimethyl(1,1-dimethylethyl)silyl]oxy]-2-methylenecyclohexylidene]-ethyl]diphenylphosphine oxide (520 mg, 0.89 mmol) was dissolved in 25 ml tetrahydrofuran and treated at −78 C with *n*-butyllithium (0.56 ml, 0.89 mmol, 1.6 M in hexane) and subsequently **10** (366 mg, 1 mmol) in 6 ml tetrahydrofuran was slowly added. After 2 hours of stirring at −78 C the mixture was quenched with 15 ml of a 1 : 1 mixture of 1 M potassium bicarbonate and 1 M sodium potassium tartrate solution. The usual work-up and purification yielded 80 mg (12%) **11** as a colorless oil, which was heated at 50 C with tetra-*n*-butylammonium fluoride trihydrate (81 mg, 0.26 mmol) in 2 ml tetrahydrofuran for 30 minutes. Usual work-up and purification gave 20 mg (42%) **1** as a colorless foam. IR (KBr): 3,400, 2,940, 1,470, 1,380, 1,055 $cm^{-1}$. NMR (300 MHz, $CDCl_3$): $\delta$ = 0.77 ppm (s, 3H), 0.86 (d, 3H), 1.13 (s, 6H), 4.13 (brs, 1H), 4.38 (brs, 1H), 4.92 (d, 1H), 5.27 (d, 1H), 6.02 (d, 1H), 6.22 (d, 1H). MS (EI) m/z: 430 ($M^+$, 3), 412 (30), 265 (12), 159 (28), 147 (37), 133 (42), 107 (96), 81 (100). UV ($CH_3OH$): $\lambda_{max}$ = 260.8 nm, $\lambda_{min}$ = 226.0 nm. $[\alpha]_D^{20}$ = +32.1 (c = 0.330, $CHCl_3$). Analysis calculated for $C_{28}H_{46}O_3$: C, 78.09; H, 10.77. Found: C, 78.14; H, 10.81.

## *Biologic methods and materials*

**Chemicals.** 1α,25-Dihydroxy-[26,27-methyl-$^3$H]cholecalciferol (180 Ci/mmol), 25-hydroxy-[26,27-methyl-$^3$H]cholecalciferol (174 Ci/mmol), and [methyl-$^3$H]thymidine (40 Ci/mmol) were purchased from Amersham Buchler (Braunschweig, Germany). 1,25-$(OH)_2$-vitamin $D_3$ and 25-OH-vitamin $D_3$ were from Solvay Duphar B.V. (Weesp, The Netherlands).

**Receptor-binding affinity.** A vitamin D receptor preparation was made from the mucosa of normal pig intestine as described.[19] Briefly, mucosa was homogenized in hypotonic TED buffer (Tris-HCl 50 mmol/l, EDTA 1.5 mmol/L, dithiothreitol 5 mmol/l) and centrifuged. The pellet was homogenized in TEDK buffer (TED + 0.3 mol/l KCl) and cleared by centrifugation to yield the receptor preparation.

Receptor-binding affinity of ZK 150123 **(1)** was assessed by displacement of [$^3$H]-1,25-$(OH)_2$-vitamin $D_3$ from the receptor preparation. To 250 μl of the receptor preparation (1 mg/ml protein) was added 10 μl each of a gradually diluted ethanolic solution of 1,25-$(OH)_2$-vitamin $D_3$ or ZK 150123 and 10 μl (0.025 μCi) [$^3$H]-1,25-$(OH)_2$-vitamin $D_3$; the solution was incubated for 1 hour at 4 C. Bound [$^3$H]-1,25-$(OH)_2$-vitamin $D_3$ was determined after removal of free tracer by the addition of 250 μl 1% dextran-coated charcoal, 20 minutes' incubation at 4 C, and centrifugation. Relative-binding affinity (RBA) was calculated from the displacement curves according to the equation RBA = $IC_{50}$ 1,25-$(OH)_2D_3$ × 100/$IC_{50}$ ZK 150123, $IC_{50}$ being the concentration at which 50% displacement of the tracer was achieved.

**Affinity for vitamin D-binding protein.** Purified human vitamin D-binding protein (DBP) was a gift from R. Bouillon (Leuven, Belgium). Affinity for DBP was assessed by displacement of [$^3$H]-25-OH-vitamin $D_3$. Vitamin D-binding protein (1.25 mg/ml phosphate-buffered saline) was diluted 1,000-fold in phosphate-buffered saline + 1% ovalbumin, and 500 μl added to 10 μl gradually dissolved solutions of 25-OH-vitamin $D_3$, 1,25-$(OH)_2$-vitamin $D_3$ and ZK 150123, and 40 μl [$^3$H]-25-OH-vitamin $D_3$. After incubation for 60 minutes at 4 C, 500 μl of 1% dextran-coated charcoal was added; the solution was incubated for 40 minutes at 4 C, and bound and free [$^3$H]-25-OH-vitamin $D_3$ were separated by centrifugation. Relative binding affinity was calculated from the displacement curves according to the equation RBA = $IC_{50}$ 25 − OH-$D_3$ × 100/$IC_{50}$ ZK 150123.

**HL 60 cell differentiation.** Human promyelocytic HL 60 cells were obtained from the American Type Culture Collection and grown in RPMI 1640 with 10% heat-inactivated fetal calf serum (FCS) at 37 C in a humidified atmosphere at 5% $CO_2$ in air. For incubation with 1,25-$(OH)_2$-vitamin $D_3$ or ZK 150123, cells were seeded at a density of 2 × $10^5$ cells/well in 96-well microtiter plates (Flow) in 200 μl RPMI 1640 without phenol red with 10% FCS. Compounds were gradually diluted in ethanol and to the cells, final ethanol concentration not exceeding 0.1%. Controls were incubated with ethanol only. After incubation for 96 hours at 37 C, 100 μl of an NBT-TPA solution (final concentrations: 1 mg/ml NBT and 2 × $10^{-7}$ mol/l TPA) was added to the cells and incubated for 2 hours at 37 C. The adhering cells were then fixed with methanol for 15 minutes at room temperature and air dried. Formation for formazan by adhering cells was quantified[20] by solution of the intracellularly precipitated dye in 100 μl KOH (2 mol/L) and 100 μl DMSO/well and reading the optical density on an ELISA plate reader (SLT) at 570 nm.

**Keratinocyte proliferation.** Keratinocyte cultures from neonatal mouse keratinocytes were established as previously published,[21] with slight modifications. Briefly, mice were killed by decapitation; the whole skin was removed and incubated in Dispase overnight at 4 C. Single cell suspensions from the peeled-off epidermis were prepared by trypsinization, and 4 × $10^5$ cells/$cm^2$ were seeded in 24-well plates in M 199 with 10% FCS. After 24 hours, medium was switched to serum-free M 199 and compounds were added as ethanolic solutions in serial dilution (final ethanol concentration, 1%). Proliferation was assessed 24 hours later by a 4-hour pulse of [$^3$H]thymidine and determination in proteinase K-digested cells[22] of acid-insoluble radioactivity and DNA.[23]

**Hypercalcemic and hypercalciuric activity in vivo.** Calcitriol (2 μg/kg) or ZK 150123 (20 μg/kg) was injected subcutaneously in 500 μl ethanol/0.9% sodium chloride (40/60 v/v) to normal female Wistar rats (150 to 170 g) housed in metabolism cages with free access to a normal diet (Altromin) and water. Urine was collected for a period of 0 to 16 hours, then the animals were fed 1 mmol calcium in 5 ml 6.5% Klucell (hydroxypropylcellulose) and urine was collected for a second period of 16 to 22 hours. Urine volume of each fraction was measured for calculating absolute calcium excretion. After 22 hours animals were killed by decapitation and blood was collected. The calcium concentration in serum and urine was determined by complex formation with 2-methylphenolphthalein.

## Results

The binding affinity to the pig intestine vitamin D receptor of ZK 150123 was slightly reduced compared with 1,25-$(OH)_2$-vitamin $D_3$ (RBA = 40) (Figure 1). Binding affinity of ZK 150123 to the major transport protein of vitamin D metabolites, DBP, was substantially reduced (RBA = 0.4) compared with 25-OH-vitamin $D_3$ (Figure 2). Relative to 1,25-$(OH)_2$-vitamin $D_3$, the binding affinity of ZK 150123 was found to be only reduced 4.3-fold.

ZK 150123 was equally active as 1,25-$(OH)_2$-vitamin $D_3$ in inducing differentiation of HL 60 cells (Figure 3); 50% of the maximal NBT reduction was achieved with a concentration of $5 \times 10^{-9}$ mol/L for 1,25-$(OH)_2$-vitamin $D_3$ and $5.6 \times 10^{-9}$ mol/L for ZK 150123. Maximal NBT reduction under these experimental conditions usually was found to be equivalent to 90% differentiated cells, as estimated in parallel by their CD 14 expression.

In keratinocytes, ZK 150123 also was equally active as 1,25-$(OH)_2$-vitamin $D_3$ in inhibiting cell proliferation (Figure 4). In mouse keratinocyte cultures, thymidine incorporation into DNA was inhibited by 50% at a concentration of $5.5 \times 10^{-10}$ mol/L of 1,25-$(OH)_2$-vitamin $D_3$ and $4.4 \times 10^{-10}$ mol/L for ZK 150123. Absolute DNA concentration per well was not reduced considerably by 1,25-$(OH)_2$-vitamin $D_3$ and ZK 150123 in the concentration range tested (DNA concentration at $10^{-6}$ mol/L, 1,25-$(OH)_2$-vitamin $D_3$ and ZK 150123 being 97% and 91% of solvent control, respectively).

In vivo the potency of ZK 150123 to induce acute hypercalcemia and hypercalciuria (Table 1) is reduced approximately 10-fold compared with 1,25-$(OH)_2$-vitamin $D_3$. ZK 150123 at a subcutaneous dose of 20 μg/kg induced a slight hypercalcemia (increase from 2.6 mmol/L [control rats] to 2.8 mmol/L). This was somewhat lower than the increase in serum calcium concen-

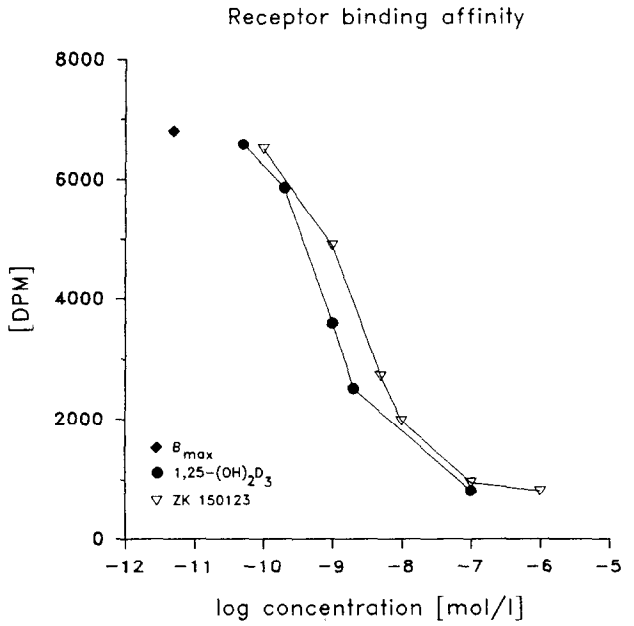



**Figure 1** Displacement of [$^3$H]-1,25-$(OH)_2D_3$ from pig intestine vitamin D receptor preparation by increasing concentrations of 1,25-$(OH)_2D_3$ and ZK 150123. Nonlinear regression analysis of binding data was performed according to the Rodbard model ($B_{max}$: binding of tracer in the absence of ligand, 11% of total tracer).

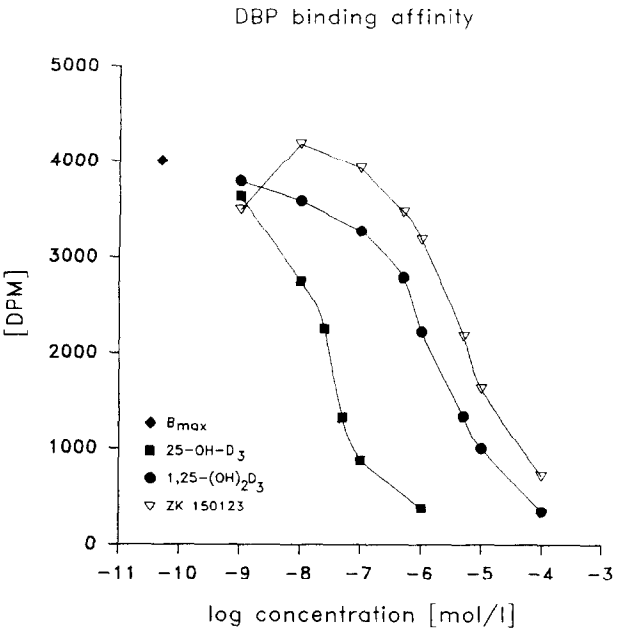



**Figure 2** Displacement of [$^3$H]-1,25-$(OH)_2D_3$ from purified human DBP by increasing concentrations of 25-OH-$D_3$, 1,25-$(OH)_2D_3$, and ZK 150123. Nonlinear regression analysis of binding data was performed according to the Rodbard model ($B_{max}$: binding of tracer in the absence of ligand, 8% of total tracer).

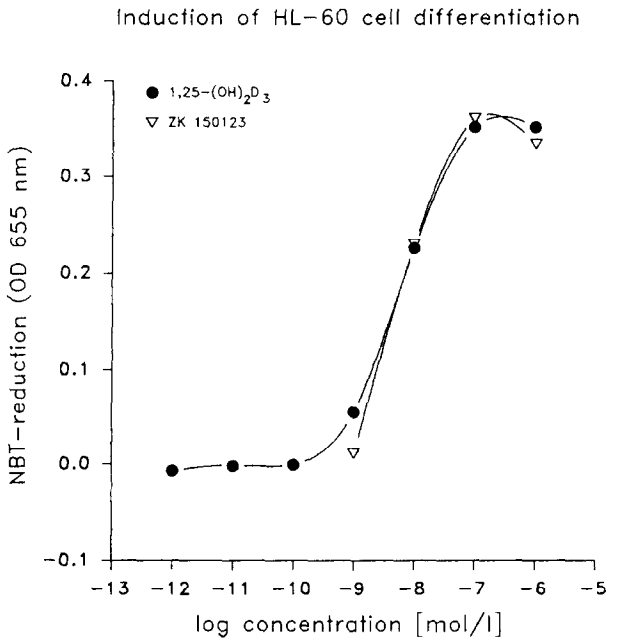



**Figure 3** Dose-response curves of the induction of differentiation of HL 60 cells by 1,25)$_2D_3$ and ZK 150123.

**Table 1** Induction of hypercalcemia and hypercalciuria 22 hours after subcutaneous injection of 2 μg/kg 1,25-$(OH)_2$-vitamin $D_3$ and 20 μg/kg ZK 150123 to normal rats

| | Solvent | 1,25-$(OH)_2$-$D_3$ (2 μg/kg) | ZK 150123 (20 μg/kg) |
|---|---|---|---|
| Serum calcium concentration (mmol/L) | 2.6 (2.6/2.9) | 3.0[a] (2.9/3.0) | 2.8[a] (2.8/2.9) |
| Urinary calcium 0 to 16 hours after injection (μmol/animal) | 10.6 (5.1/24.2) | 52.0[a] (40.5/75.5) | 68.9[a] (53.8/93.1) |
| Urinary calcium 16 to 22 hours after injection (μmol/animal) | 57.2 (52.8/61.4) | 72.2[a] (64.4/94.1) | 89.3[a] (67.9/102.7) |

Data are expressed as median values (25/75 quantile; n = 10).
[a]Significant to solvent control (α = 0.05, Mann Whitney U test).

tration produced by application of 2 μg/kg 1,25-$(OH)_2$-vitamin $D_3$ (3.0 mmol/L). Calcium excretion in urine was significantly elevated in the early (0 to 16 hours) collection period and further increased within the second collection period (16 to 22 hours). Roughly the same increase in calcium excretion was found after application of a 10-fold lower dose (2 μg/kg) of 1,25-$(OH)_2$-vitamin $D_3$.

## Discussion

ZK 150123, a C-ring homolog of 1,25-$(OH)_2$-vitamin $D_3$, has been synthesized to investigate the consequences of chemical variation in the C ring with regard to vitamin D receptor affinity, biologic activity, and potential selectivity of action. ZK 150123 was shown to bind to the classic vitamin D receptor with only slightly reduced affinity and to exert biologic effects both on growth modulation in vitro and on calcium metabolism in vivo with identical or slightly reduced potency compared with 1,25-$(OH)_2$-vitamin $D_3$. Besides the effect of C-ring homologation, the triene structure characteristic for 1,25-$(OH)_2$-vitamin $D_3$, and thereby the link between the A and C rings, is displaced by one methylene group. Surprisingly, this variation does not result in considerable loss of biologic activity characteristic for the hormonal form of vitamin D.

There was no evidence for selectivity of action on either cell differentiation induction or calcium mobilizing activity. Further effects, such as the influence on bone cells, lymphocytes, and macrophages, were not investigated. Other variations in the C ring already known comprise substitutions at C-11.[24] Small alkyl substituents give rise to strong agonistic activity in vitro (receptor affinity, cell differentiation potential). On the other hand, the introduction of sterically demanding or polar groups results in a remarkable decrease of biologic activity.

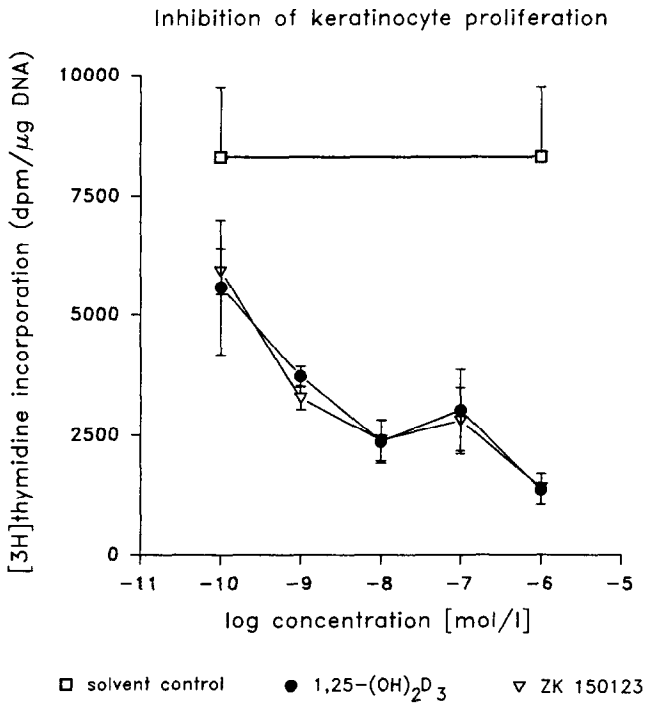



**Figure 4** Dose-response curves for the inhibition of [$^3$H]thymidine incorporation into DNA in mouse keratinocyte cultures of 1,25-$(OH)_2D_3$ and ZK 150123.

## References


1. Reichel H, Koeffler HP, Norman AW (1989). The role of the vitamin D endocrine system in health and disease. *N Engl J Med* **320**:980–991.
2. Mangelsdorf DJ, Koeffler HP, Donaldson CA, Pike JW, Haussler MR (1984). 1,25-Dihydroxyvitamin $D_3$ induced differentiation in a human promyelocytic leukemia cell line (HL-60): receptor-mediated maturation to macrophage-like cells. *J Cell Biol* **98**:391–398.
3. Abe E, Miyaura C, Sakagami H, Takeda M, Konno K, Yamazaki T, Yoshiki S, Suda T (1981). Differentiation of mouse myeloid leukemia cells induced by 1α,25-dihydroxyvitamin $D_3$. *Proc Natl Acad Sci USA* **78**:4990–4994.
4. Smith EL, Walworth NC, Holick MF (1986). Effect of 1,25-dihydroxyvitamin $D_3$ on morphologic and biochemical differentiation of cultured human epidermal keratinocytes grown in serum-free conditions. *J Invest Dermatol* **86**:709–714.
5. Morimoto S, Onishi T, Imanaka S, Yukawa H, Kozuka T, Kitano Y, Yoshikawa K, Kumahara Y (1986). Topical administration of 1,25-dihydroxyvitamin $D_3$ for psoriasis: report of five cases. *Calcif Tissue Int* **38**:119–122.

6. Kato T, Roguko M, Terui T, Tagami H (1986). Successful treatment of psoriasis with topical application of active vitamin $D_3$ analogue, 1α,24-dihydroxycholecalciferol. *Br J Dermatol* **115:**431–433.
7. Eisman JA, Barkla DH, Tutton PJM (1987). Suppression of in vivo growth of human cancer solid tumor xenografts by 1,25-dihydroxyvitamin $D_3$. *Cancer Res* **47:**21–25.
8. Frampton RJ, Omond SA, Eisman JA (1983). Inhibition of human cancer cell growth by 1,25-dihydroxyvitamin $D_3$ metabolites. *Cancer Res* **43:**4443–4447.
9. Binderup L, Bramm E (1988). Effects of a novel vitamin D analogue MC 903 on cell proliferation and differentiation in vitro and on calcium metabolism in vivo. *Biochem Pharmacol* **37:**889–895.
10. Abe J, Morikawa M, Miyamoto K, Kaiho S, Fukushima M, Miyaura C, Abe E, Suda T, Nishii Y (1987). Synthetic analogues of vitamin $D_3$ with an oxygen atom in the side chain skeleton. *FEBS Lett* **226:**58–62.
11. Calverley MJ (1987). Synthesis of MC 903, a biologically active vitamin D metabolite analogue. *Tetrahedron* **43:** 4609–4619.
12. Murayama E, Miyamoto K, Kubodera N, Mori T, Matsunaga I (1986). Synthetic studies of vitamin $D_3$ analogues VIII. Synthesis of 22-oxavitamin $D_3$ analogues. *Chem Pharm Bull (Tokyo)* **34:** 4410–4413.
13. Harrison RG, Lythgoe B, Wright PW (1974). Calciferol and its relatives. Part XVIII. Total synthesis of 1α-hydroxyvitamin $D_3$. *J Chem Soc Perkin I* 2654–2657.
14. Baggiolini EG, Iacobelli JA, Hennessy BM, Batcho AD, Sereno JF, Uskokovic MR (1986). Stereocontrolled total synthesis of 1α,25-dihydroxycholecalciferol and 1α,25-dihydroxyergocalciferol. *J Org Chem* **51:**3098–3108.
15. Krow GR (1987). One carbon ring expansions of bridged bicyclic ketones. *Tetrahedron* **43:**3–38 (and references therein).
16. Simmons HE, Cairns TL, Vladuchick SA, Honess CM (1973). Cyclopropanes from unsaturated compounds, methylene iodide, and zinc-copper couple. In: Dauben WG (ed.), *Organic Reactions*, vol. 20. Wiley, New York, pp. 1–131.
17. Ito Y, Fujii S, Saegusa T (1976). Reaction of 1-silyloxybicyclo[n.1.0]alkanes with $Fe^{III}Cl_3$. A facile synthesis of 2-cycloalkenones via ring enlargement of cyclic ketones. *J Org Chem* **41:**2073–2074.
18. Denis JM, Girard C, Conia JM (1972). Improved Simmons-Smith reactions. *Synthesis* 549–551.
19. Dame MC, Pierce EA, DeLuca HF (1985). Identification of the porcine intestinal 1,25-dihydroxyvitamin $D_3$ receptor on sodium dodecyl sulfate/polyacrylamide gels by renaturation and immunoblotting. *Proc Natl Acad Sci USA* **82:**7825–7829.
20. Rook GAW, Steele J, Umar S, Dockrell HM (1985). A simple method for the solubilisation of reduced NBT, and its use as a colorimetric assay for activation of human macrophages by γ-interferon. *J Immunol Methods* **82:**161–167.
21. Yuspa SH, Harris CC (1974). Altered differentiation of mouse epidermal cells treated with retinyl acetate in vitro. *Exp Cell Res* **86:**95–105.
22. Gross-Bellard M, Oudet P, Chambon P (1973). Isolation of high-molecular-weight DNA from mammalian cells. *Eur J Biochem* **36:**32–38.
23. Kapuscinski J, Skoczylas B (1977). Simple and rapid fluorometric method for DNA microassay. *Anal Biochem* **83:**252–257.
24. Bouillon R, De Clercq PJ, Eliard P, Vandewalle M (1989). Nouveaux dérivés de lavitamine D: applications thérapeutiques et aux dosages des métabolites de la vitamine D. *Eur Pat* 0 341 158 A2.