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1α,25-Dihydroxyvitamin D$_3$ Hybrid Analogs with Structural Changes at Both the A-Ring and the C,D-Ring Side-chain

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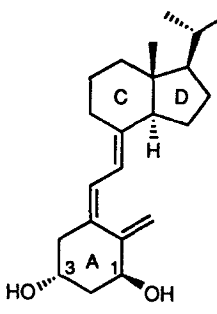
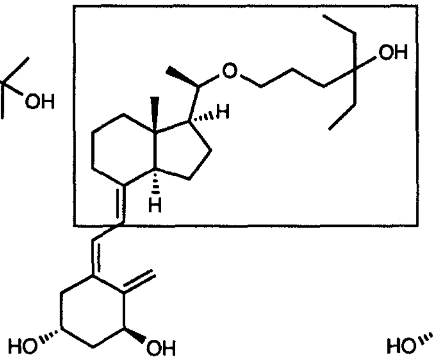
Summary: Calcitriol analogs **5**, designed to combine **two** remote structural changes each of which separately produces a sharply different biological profile, have biological activities that are a **blending** of the effects of each structural change.

Besides regulating phosphorus metabolism, intestinal calcium absorption and bone calcium mobilization, the hormonally active metabolite 1α,25-dihydroxyvitamin D$_3$ (calcitriol, **1**) promotes cell differentiation, inhibits cell proliferation[1] and affects the human immune system.[2] Calcitriol and some synthetic vitamin D$_3$ derivatives (deltanoids) have been used recently in practical, clinical chemotherapy of such diverse human illnesses as osteoporosis, cancer and skin disorders such as psoriasis.[1] A major international objective is to prepare vitamin D$_3$ analogs as new drugs in which calcemic activity is effectively separated from cell growth regulation and differentiation, and toward this goal several hundred deltanoids have been synthesized, mainly with structural variations on the C,D-ring side-chain. For example, incorporating an extra oxygen atom at the 22-position (22-oxa-calcitriol) leads to a significant increase in anticancer potency[3,4] as does side-chain homologation[5,6] and inversion of stereochemistry at the 20-position. Combining[7] these side-chain modifications produces two analogs [KH 1060, **2**[8] and EB 1089, **3**[9]) that have extraordinarily enhanced abilities to induce cancer cell differentiation and inhibit the mixed lymphocyte reaction.[8] Likewise, 20-epi-23-oxa-calcitriol has recently been shown to have highly potent anticancer activities.[10] Recently we showed that deltanoids (–)-**4a** and (+)-**4b**, with hydroxymethyl groups at the 1-position in the A-ring, are antiproliferative but with very low VDR-binding affinity and with virtually no calcemic effect.[11,12] It was not clear, however, whether a **hybrid** deltanoid **combining** a potentiating side-chain modification (*e.g.* **2**) with a potentiating A-ring modification (*e.g.* **4**) would exhibit **blended** biological activities or whether the effect of only one of these structural changes would partially or completely dominate its biological profile. Therefore, as an initial probe into this question, we have prepared and have begun to evaluate deltanoids **5** as structural **hybrids** of side-chain analog **2** and A-ring analogs **4**.

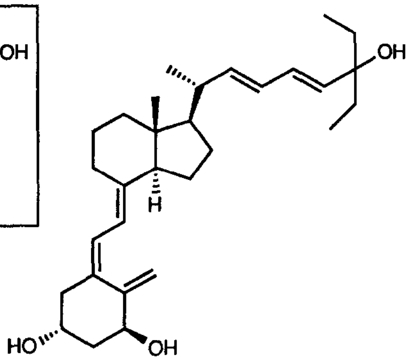
Synthesis of diastereomeric hybrid analogs (–)-**5a** and (+)-**5b** was achieved by convergent coupling[11,12] of the racemic A-ring phosphine oxide (±)-**6**, as its conjugate base, with the enantiomerically pure C,D-ring ketone (–)-**7** carrying the 20-epi-22-oxa side-chain[13] characteristic of KH-1060 (**2**, eq. 1). Separation of diastereomers **5a** and **5b** by HPLC gave enantiomerically pure hybrid deltanoids (–)-**5a** and (+)-**5b**,[17] with the C,D-ring chiron (–)-**7** used in this process as a resolving agent.[12c] Tentative assignment of A-ring stereochemistry to these two deltanoids was made primarily by comparing the characteristic portions of their ^1H NMR spectra[17] with those of 1-hydroxymethyl analogs (–)-**4a** and (+)-**4b**[11a] and secondarily by comparing signs of optical rotation.



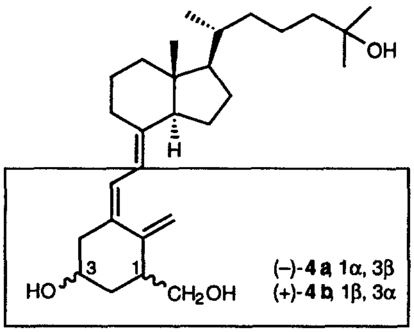
(–)-**1**, Calcitriol



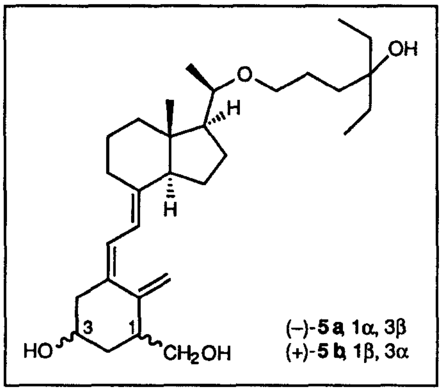
2, KH-1060



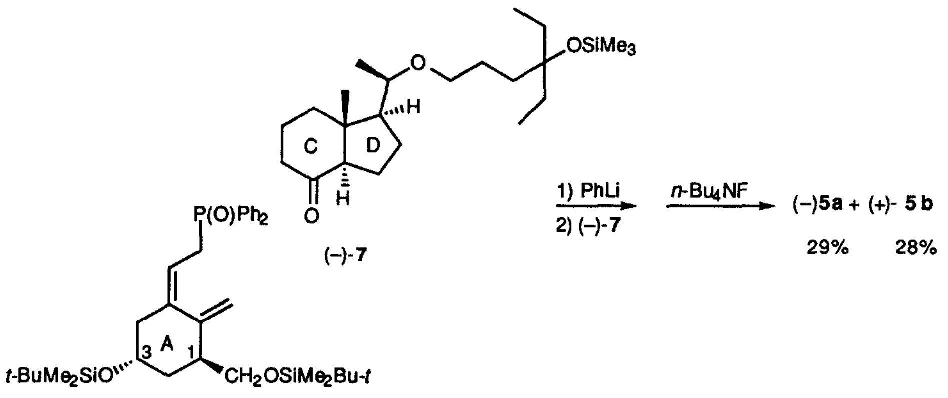
3, EB-1089



(–)-**4a** 1α, 3β
(+)-**4b** 1β, 3α



(–)-**5a** 1α, 3β
(+)-**5b** 1β, 3α



(±)-**6**

$\xrightarrow[\text{2) (–)-7}]{\text{1) PhLi}} \xrightarrow{n\text{-Bu}_4\text{NF}}$ (–)**5a** + (+)- **5b** (1)

29% 28%

Preliminary biological evaluations produced the following results: (1) in inhibiting proliferation of murine keratinocytes using our previously described protocol,[11a] hybrid analog (+)-**5b** is at least as potent as calcitriol (**1**) even at physiologically relevant nanomolar levels, and also (+)-**5b** is considerably more potent than its disastereomer (–)-**5a** (data not shown) and than singly-modified analog (+)-**4b** (see Fig. 1);[11a] (2) the same trend in antiproliferative potencies is observed also in human leukemic RWLeu-4 cells;[12b] (3) in competitive binding to the calf thymus vitamin D receptor (VDR), doubly-modified analogs **5**, like the singly-modified analogs **4** but unlike KH-1060 (**2**),[8] are less than 10^{-3} as effective as calcitriol (**1**);[18] and (4) in rat osteosarcoma cells, hybrid deltanoid (+)-**5b** is comparable to calcitriol (**1**) but much more potent than its diastereomer (–)-**5a** and than singly-modified analogs **4** at the nanomolar level in stimulating calcium currents *via* an instantaneous, non-genomic process.[19] Thus, the 1-hydroxymethyl group A-ring modification of analog (+)-**5b** seems to cause low VDR binding, whereas the KH-1060 C,D-ring side-chain modification of this analog (+)-**5b** seems to cause powerful antiproliferative and calcium channel-opening effects. **These initial findings, therefore, show for the first time that two remote structural modifications of the calcitriol skeleton can produce a hybrid analog like (+)-5b having blended and powerful biological activities.**

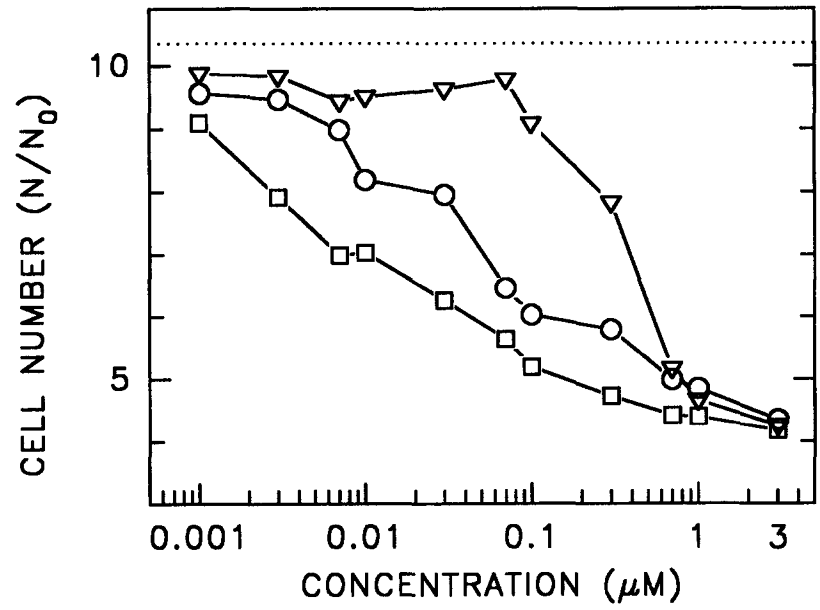


Fig. 1 Growth inhibition of murine keratinocyte cell line PE according to the protocol in ref. 11a.

In conclusion, a major 1992 review[20] of structure-activity relationships (SAR's), concluded that "any loss of activity relative to 1α,25-dihydroxyvitamin D_3 conferred by these [A-ring] modifications probably could be more than offset by appropriate side-chain modifications, and **the scope for fine-tuning is enormous.**" Our results with hybrid analog (+)-**5b** support this hypothesis and, therefore, encourage preparation and evaluation of other hybrid deltanoids. Broad biological screening of hybrid deltanoid (+)-**5b** is currently underway to delineate its full range of activities. Results of such studies will be reported in due course.

Acknowledgment

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12. For synthesis and biological activity of related 1-substituted deltanoids, see (a) Posner, G.H.; Dai, H. *Bioorg. Med. Chem. Lett.*, **1993**, *3*, 1829-1834; (b) Posner, G.H.; Guyton, K.Z.; Kensler, T.W.; Barsony, J.; Lieberman, M.E.; Reddy, G.S.; Clark, J.W.; Wankadiya, K.-F.; Tserng, K.-Y. *ibid.*, **1993**, *3*, 1835-1840; (c) Posner, G.H.; Dai, H.; Afarinkia, K.; Murthy, N.M.; Guyton, K.Z.; Kensler, T.W. *J. Org. Chem.*, **1993**, *58*, 7209-7215. For a recent review on vitamin D syntheses, see Dai, H.; Posner, G.H. *Synthesis*, **1994**, 1383-1398.

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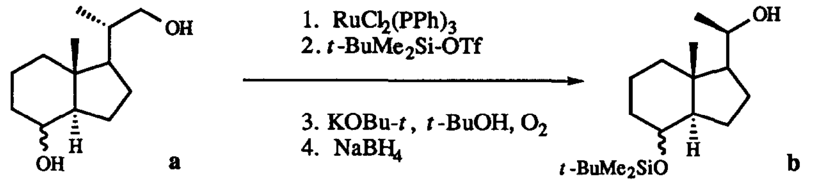

The following four transformations were used as modifications of the Wilson procedure to prepare alcohol **b**:

A flame-dried 50 mL round bottomed flask was charged with 910 mg (0.94 mmol, 0.8 equiv.) of $RuCl_2(PPh_3)_3$, 319.4 mg (3.8 mmol) of $NaHCO_3$ and 20 mL of benzene. Diol **a** (223.1 mg, 1.1 mmol) was sequentially taken up in a total of 60 mL of benzene and added portionwise to the stirring $RuCl_2(PPh_3)_3$ solution. The reaction mixture was stirred at room temperature for approximately 5 days. The benzene was removed by rotary evaporation and the resulting dark green solid was washed with Et_2O (7 X 10 mL). The Et_2O washings were collected and quickly passed through a plug of silica gel. Due to its instability, the hydroxy aldehyde product was typically carried on without further purification. Removal of all traces of ruthenium related compound(s) (i.e. hydridochlorotris(triphenylphosphine)ruthenium), visually identifiable as insoluble dark green solids, required proper purification by silica gel column chromatography (10% EtOAc/ hexane) and afforded 133.9 mg (0.64 mmol, 60%) of the hydroxy aldehyde product as a yellow oil (slowly decomposing, evidenced by TLC and ^1H NMR) (R*f* = 0.45, 50% EtOAc/ hexane), ^1H NMR ($CDCl_3$) δ 9.55 (d, *J* =3.2Hz, 1H), 4.08 (m, 1H), 2.34 (m, 1H), 1.92-1.1 (m, 13H), 1.08 (d, *J* = 6.8 Hz, 3H), 0.96 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 205.06, 68.80, 51.88, 51.44, 49.06, 42.31, 40.10, 33.55, 26.05, 22.78, 17.32, 13.83, 13.23; FT-IR 4214, 3617, 3020, 2940, 2873, 2715, 1720, 1474, 1458, 1446, 1374 cm^{-1}. Larger scale reactions were less successful.

The aforementioned hydroxy aldehyde (133.9 mg, 0.64 mmol) was dissolved in 4 mL of DMF and cooled to 0°C. To this solution was added 0.18 mL (1.5 mmol) of 2,6-lutidine followed by 0.35 mL (1.5 mmol) of *tert* butyldimethylsilyl trifluoromethanesulfonate (*t* -BDMS-OTf). The progress of the reaction was monitored closely by TLC until the reaction was complete (ca. 2 h, 0°C). The reaction mixture was quenched with approximately 40 mL of water, extracted with Et_2O (3 X 25 mL), the organic portion was dried over $MgSO_4$, filtered, concentrated by rotary evaporation and immediately purified by silica gel chromatography (100% hexane) to afford 181.9 mg (0.56 mmol , 87%) of O-silylated aldehyde as a light yellow oil (R*f* = 0.48, 15% EtOAc/ hexane). Spectroscopic data correspondsto those previously reported.[14] Due to the instability of this O-silylated aldehyde (as evidenced by TLC and ^1H NMR), storage at -20°C did not exceed a 12 h period.

O_2 was bubbled through a solution of KO-*t* -Bu (2.7 mL, 2.7 mmol) in 6 mL of dry *t* -BuOH (freshly distilled from CaH_2) for 10-15 min. A solution of the O-silylated aldehyde (181.9 mg, 0.56 mmol) in 2.0 mL of *t* -BuOH was added and O_2 was bubbled through the solution for an additional 10 min followed by N_2 for 15 min. The solution was quenched with H_2O, extracted with Et_2O (3 X 35 mL), dried over $MgSO_4$, filtered, concentrated, and chromatographed on a silica gel column (100% hexane) to afford 94.5 mg (0.30 mmol, 80%) of the desired O-silylated ketone as a white solid (Rf = 0.8, 20% EtOAc/ hexane); m.p. 33-34 °C (lit. ref.[14] m.p. 34-35°C), with physical and spectroscopic properties corresponding to those reported.[14]

A flame-dried 25 mL round bottomed flask was charged with 94.5 mg (0.30 mmol) of the O-silylated ketone, dissolved in 5 mL of anhydrous MeOH, and cooled to 0°C. Solid sodium borohydride (57.0 mg, 1.5 mmol, 5 equiv.) was added portionwise to the solution until the disappearance of all starting material was observed by TLC. The reaction mixture was quenched with water, extracted with Et_2O (3 X 25 mL), dried over $MgSO_4$, filtered, concentrated, and purified by silica gel column chromatography (10% EtOAc/ hexane) to afford 64.8 mg of the desired alcohol (20R)-epimer **b** and 13.5 mg of the alcohol (20S)-epimer (4.8:1) both as light yellow oils in 83% total yield ; alcohol (20R)-**b** : ^1H NMR ($CDCl_3$) δ 4.01 (m, 1H), 3.74 (m, 1H), 2.02-1.16 (m, 13H), 1.12 (d, *J* = 6.4 Hz, 3H), 1.0 (bs, 3H), 0.88 (s, 9H), 0.01 and -0.007 (2s, 6H); ^{13}C NMR ($CDCl_3$) δ 70.19, 69.16, 59.15, 52.60, 41.95, 40.93, 34.42, 25.82, 24.75, 23.35, 23.25, 17.54, 14.36, -4.78, -5.17; FT-IR 3475, 2925, 2975, 2852, 1763, 1487, 1375 cm^{-1} ; alcohol (20S): ^1H NMR ($CDCl_3$) δ 4.02 (m, 1H), 3.68 (m, 1H), 2.02-1.16 (m, 13H), 1.25 (s, 3H), 1.20 (d, *J* = 6.0 Hz, 3H), 0.88 (s, 9H), 0.012 and 0.003 (2s, 6H). Stereochemical assignments were made by comparing ^1H NMR chemical shift and coupling constant data reported in the literature for similar compounds [see ref.15 for assignment of (20S)-7 alcohol ; see ref.16 for assignment of (20R)-7 alcohol].

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17. Coupling *via* eq. (1) was achieved as follows:

Racemic phosphine oxide **6** and C-D ring ketone (-)-**7** were separately azeotropically dried three times with freshly distilled benzene and held under vacumn (0.05 mm Hg) for 24 h immediately prior to use. Racemic phosphine oxide (±)-**6** (124.5 mg, 0.21 mmol) was dissolved in 2 mL freshly distilled anhydrous THF and cooled to -78°C under an Ar atmosphere. To this was added 0.155 mL (0.229 mmol, 1.1 equiv.) of PhLi (1.48 M in THF) dropwise over 2-3 min during which time a deep red-orange color developed and persisted. The mixture was allowed to stir an addition 7-8 min at -78°C at which time a precooled (-78°C) solution of C-D ring ketone (-)-**7** (55 mg, 0.14 mmol) dissolved in 1 mL freshly distilled anhyrous THF was added dropwise *via* cannula. The deep red-orange solution was stirred in the dark for approximately 3.0 h during which time (periodically checked visually) it was observed turning progressively lighter in color until it reached a light yellow color. Upon observation of the light yellow color, the reaction mixture was immediately quenched at -78°C with 0.3 mL of 2N sodium potassium tartrate followed by addition of dilute aqueous potassium carbonate. After warming to room temperature, the reaction was extracted with EtOAc (3 X 20 mL), the organic portion was dried over $MgSO_4$, filtered, concentrated, and purified quickly by silica gel column chromatography (7% EtOAc/ hexane) to afford 101.0 mg (0.13 mmol) of the crude coupled product in 93% yield based on C-D ring (-)-**7**. This was immediately placed in a flame-dried 25 mL round bottomed flask and dissolved in 5 mL of freshly distilled anhydrous THF under argon. To this was added 463.3 mg (1.7 mmol, 13 equiv.) of solid n-Bu_4NF and 273.0 mg of dry 3 angstrom molecular sieves. The mixture was stirred at room temperature for approximately 12 h in the dark. The solvent was evaporated and the mixture was carefully purified by silica gel column chromatography (silica gel slurry: 99% EtOAc/ 1% Et_3N; eluent: 100% EtOAc) to afford 39 mg (0.082mmol, 63%) of a mixture of two diastereomers [(-)-**5a**] and [(+)-**5b**] slightly contaminated with impurities (as evidenced by TLC and ^1H NMR). The mixture of diastereomers was subjected to HPLC separation (gradient elution: H_2O 30% (15 min), 20% (15 min), 30% (1 min)/ CH_3CN; reverse phase; C_{18} column, semi-prep, flow rate 1mL/min-2mL/min, retention times: [(-)-**5a**] 29.51 min, [(+)-**5b**] 31.46 min) to give pure diastereomers in 29% and 28% yields, respectively. (-)-**5a:** $[\alpha]^{32}D$ -140° ($c = 1.1 \times 10^{-3}$ g/ml, MeOH); ^1H NMR ($CDCl_3$) δ 6.32 (d, $J = 11.2$ Hz, 1H), 5.93 (d, $J =11.2$ Hz, 1H), 5.17 (d, $J = 2$Hz, 1H), 5.01 (d, $J = 2.0$ Hz, 1H), 4.0-3.88 (m, 1H), 3.6-3.5 (m, 2H), 3.30-3.18 (m, 2H), 2.85-2.77 (m, 1H), 2.67-2.56 (m, 1H), 2.29-2.22 (m, 1H), 2.18-2.12 (m, 1H), 1.08 (d, $J = 6.0$ Hz, 3H), 0.84 (dt, $J = 2.0$, 9.6 Hz, 6H), 0.55 (s, 3H); ^{13}C ((CD_3OD) δ 147.61, 142.53, 136.52, 123.92, 118.96, 114.06, 79.70, 75.24, 69.98, 67.39, 64.66, 58.28, 57.09, 47.40, 46.96, 46.50, 41.82, 37.61, 36.15, 31.70, 31.64, 30.06, 26.14, 25.39, 24.66, 23.56, 18.69, 12.98, 8,17, 8.10; UV (MeOH) λ_{max} 264 nm (ε 16,000); HRMS, calcd for $C_{30}H_{50}O_4$ 474.3709, found 474.3706.

(+)-**5b:** $[\alpha]^{32}D$ +4.0 ° ($c = 1.0 \times 10^{-3}$ g/ml, MeOH); ^1H NMR ($CDCl_3$) δ 6.32 (d, $J = 11.2$ Hz, 1H), 5.92 (d, $J =11.6$ Hz, 1H), 5.15 (dd, $J = 2.1$, 1.0 Hz, 1H), 4.99 (d, $J = 2.0$ Hz, 1H), 4.03-3.97 (m, 1H), 3.63-3.52 (m, 2H), 3.30-3.17 (m, 2H), 2.83-2.78 (m, 1H), 2.65-2.56 (m, 1H), 2.30-2.22 (m, 1H), 2.15 (m, 1H), 1.08 (d, $J = 6.0$ Hz, 3H), 0.84 (dt, $J = 2.0$, 9.6 Hz, 6H), 0.52 (s, 3H); ^{13}C (CD_3OD) δ 147.68, 142.72, 136.59, 123.86, 118.91, 113.79, 79.70, 75.25, 69.98, 67.43, 64.64, 58.23, 57.04, 47.39, 46.92, 46.38, 41.85, 37.56, 36.15, 31.70 31.63, 30.07, 26.21, 25.38, 24.54, 23.45, 18.69, 12.96, 8.17, 8.10; UV (MeOH) λ_{max} 264 nm (ε 13,300); HRMS, calcd for $C_{30}H_{50}O_4$ 474.3709, found 474.3711.

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