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Difluoromethyl Analogs of the Natural Hormone 1 α ,25-Dihydroxyvitamin D₃: Design, Synthesis, and Preliminary Biological Evaluation

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Abstract

Three new vitamin D analogs **3-5** incorporating a –CHF₂ group as an –OH surrogate have been prepared. Two of these new analogs (**3** and **5**) are strongly antiproliferative toward murine keratinocytes and are approximately 50 times less calciuric in vivo than the natural hormone calcitriol. The transcriptional activity of the 25-CHF₂ analog **3** is higher than that of the 1-CHF₂ analog **4**.

Keywords

Fluorinated D analogs; growth inhibition; low-calcemic; protease digestion assay; VDR-binding

1. Introduction

The natural hormone 1 α ,25-dihydroxyvitamin D₃ (calcitriol, **1**) helps maintain good health in humans. Chemotherapeutic use of this natural hormone at supraphysiological levels, however, is often harmful, causing bone calcium depletion and ultimately calcium excretion [1,2]. Therefore, medicinal organic chemists have synthesized many calcitriol analogs, aiming to maximize desirable antiproliferative activity and to minimize undesirable calcemic activity [3,4]. Several such analogs are now drugs on the market to cure patients suffering from osteoporosis, psoriasis, or renal dysfunction [4]. We have reported on calcitriol hybrid analog QW-1624F₂-2 (**2**) in which the two fluorine atoms on carbon-24 were placed there intentionally to prevent cytochrome CYP-24 hydroxylation (catabolism) at that site [5,6]. This rationally designed highly antiproliferative but low-calcemic analog has recently been shown to prevent mouse skin tumorigenesis [7] and to diminish the growth of human neuroblastoma in mouse

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xenografts [8]. Here we report on two new analogs **3** and **4** of calcitriol (**1**) in which one of the OH groups of the natural hormone is replaced by a difluoromethyl group and also on new hybrid analog **5** carrying a 1-CHF₂ group as well as a newly discovered potentiating 23-oxa-25-ketone side chain. We chose to incorporate a difluoromethyl group because this group is a very good surrogate for an hydroxyl group in terms of H-bonding ability [9,10].

2. Results and Discussion

2.1. Chemistry

Synthesis of the desired 1-CHF₂ A-ring phosphine oxide (±)-**16** was accomplished as shown in Scheme 1. Two noteworthy features of this scheme are as follows: 1) chameleon diene 3-bromo-2-pyrone **7** cycloadded to acrolein regioselectively and stereoselectively to give bicyclic lactone aldehyde (±)-**9**, and 2) DAST difluorination of this aldehyde gave the corresponding gem-difluoro compound (±)-**10** in high yield. The following nine chemical steps proceeded according to our established protocol [11] to give the target 1-CHF₂ A-ring phosphine oxide (±)-**16** without destroying the 1-CHF₂ functionality. Horner-Wadsworth-Emmons (HWE) coupling of this A-ring phosphine (±)-**16** with C-8 ketone **18** proceeded smoothly to give the new analog (+)-**4** (Scheme 1). HWE coupling of the same 1-CHF₂ A-ring phosphine oxide (±)-**16** regiospecifically at C-8 of the 8,25-diketone **21** produced the 23-oxa hybrid analog (+)-**5** (Scheme 2). For biological comparison, the corresponding non-fluorinated 1α-OH-23-oxa-25-ketone analog (+)-**6** was prepared as well (Scheme 2).

2.2. Biology

Using our standard in vitro murine keratinocyte assay [11], we determined that 25-CHF₂ analog **3** was similar to calcitriol (**1**) in antiproliferative activity (Fig. 1) but that 1α-CHF₂ analog **4** was less antiproliferative than calcitriol (**1**) (data not shown). These results suggest that the presence of the natural 1α-OH group (as in analog **3**) is more important for strong antiproliferative activity than the presence of the natural 25-OH (as in analog **4**). Docking each ligand into a 12 angstrom flexible site model of the wild type vitamin D receptor (VDR) binding site gave a lowest energy conformation of analog **3** with an apparent association energy almost identical to that of calcitriol (-37.0 kJ/mol versus -37.7) with only minor perturbations to protein side chain conformations. Analog **4** has a calculated association energy 3.2 kJ/mol weaker than calcitriol and required residues in the vicinity of the A-ring to undergo large conformational changes in order to be accommodated by the receptor binding pocket. These docking results are consistent with 1α-OH-25-CHF₂ analog **3** being more antiproliferative than 1α-CHF₂-25-OH analog **4**.

To promote antiproliferative activity, we designed hybrid analog **5** incorporating a new potentiating 23-oxa-25-ketone side chain along with the calcemia-ablating (see below) 1α-CHF₂ group. The antiproliferative activity of hybrid analog **5** is similar to that of natural calcitriol (**1**). (Fig. 1)

VDR-mediated transcriptional activities of these new analogs were tested in CV1 cells co-transfected with the human VDR expression plasmid and a reporter gene containing the human osteocalcin VDRE attached to the thymidine kinase promoter and the growth hormone gene [12]. We found that 25-CHF₂ analog **3** was 10 times less potent than calcitriol (**1**) in the transcription assay (ED₅₀ = 8 nM compared with ED₅₀ of 0.8 nM for calcitriol) but that 1α-CHF₂ analog **4** was much less potent than calcitriol (ED₅₀ = 350 nM). These results suggest that the presence of the natural 1α-OH group (as in analog **3**) is more important for strong transcriptional activity than the presence of the natural 25-OH (as in analog **4**).

To determine if the loss of transcriptional activity found in analog **4** could be rescued, we tested the hybrid analog **5** which combined the potentiating 23-oxa-25-ketone side chain along with the 1 α -CHF₂ group. The transcriptional potency of hybrid analog **5** was four times greater than that of analog **4** (ED₅₀ = 80 nM), although this analog was still significantly less potent than calcitriol.

To determine the relationship between transcription potency and affinity for the vitamin D receptor, we performed competitive binding assays (HAP assays) using extracts from COS-1 cells transfected with the human VDR expression plasmid as previously described [12]. Interestingly, these assays did not reflect transcriptional potencies or antiproliferative activities, since all of the analogs had a similar reduction in their ability to bind the human VDR, with an IC₅₀ of 75 nM (analog **3**), 40 nM (analog **4**) and 20 nM (analog **5**), compared to IC₅₀ of 1.2 nM for calcitriol. However, similar discrepancies between affinity for the VDR and transcriptional potency have been previously described and explained by demonstrating that the mode of interaction of analogs with the VDR with respect to the amino acid residues that are used as contact points has a greater impact on transcriptional potency than binding affinity [13]. An additional explanation could involve metabolic changes of the analogs in the cultured cells that increase or decrease their transcriptional activities.

Using our standard mouse protocol to determine calciuria levels [14], we determined that the non-fluorinated 1 α -OH-23-oxa-25-ketone analog **6** has significant calciuric activity. Thus, analog **6** at 10 μ g/kg had roughly double the calciuric activity of calcitriol at 0.5 μ g/kg. The corresponding 1 α -CHF₂ analog **5** is substantially less calciuric than the natural hormone calcitriol (**1**) (Fig. 2). Thus, treatment of rats with a low, submaximal dose (0.5 μ g/kg) of calcitriol produced the same extent of calciuria as treatment of rats with a 50-fold higher dose (25 μ g/kg) of analog **5** (Fig. 2). Likewise, the 25-CHF₂ analog **3** is approximately 50 times less calciuric than calcitriol (**1**) (Fig. 2). Clearly, analogs **3** and **5** are distinguished from calcitriol by exhibiting both high antiproliferative activity and low calcemic activity.

3. Conclusions

The hydroxyl group surrogate -CHF₂ can be placed at the 25-position of the natural hormone calcitriol (**1**) without significantly changing in vitro antiproliferative activity. However, replacing the natural 1 α -OH group by a 1 α -CHF₂ group diminishes antiproliferative potency. Restoring antiproliferative potency was achieved by incorporating a potentiating 23-oxa-25-ketone side chain; such hybrid analog **5** is almost as antiproliferative in vitro as calcitriol (**1**) while being substantially less calciuric than calcitriol (**1**) in vivo and significantly less calciuric than the corresponding non-fluorinated 23-oxa-25-ketone analog **6**. The difluorinated analogs **3-5** should be useful probes for exploring the molecular biology of vitamin D action.

4. Experimental [11]

Bromobicyclic lactone ($\pm$)-**9**

A 25 mL hydrolysis tube was charged with 182.4 mg of 3-bromo-2-pyrone **7** (1.04 mmol), 619.12 μ L of acrolein (**8**, 8.34 mmol), a catalytic amount of barium carbonate, and 10 mL of CH₂Cl₂. The reaction mixture was sealed under nitrogen and warmed to 75 °C for 95h with constant stirring. A stream of nitrogen was then blown over the reaction mixture to remove the acrolein, the mixture was diluted with CH₂Cl₂/Et₂O (ca. 1:1) and passed through a plug of Celite. The crude product was concentrated in vacuo, and then purified by column chromatography (25% EtOAc/hexanes) to give 156.5 mg (65%) of bromobicyclic lactone **9** as a colorless oil: ¹H NMR (400 MHz, CDCl₃) δ 9.82 (d, *J* = 1.2 Hz, 1H), 6.51 (d, *J* = 4.4 Hz, 1H), 6.50 (d, *J* = 2.4 Hz, 1H), 5.33-5.30 (m, 1H), 3.14 (dd, *J* = 9.6, 3.2 Hz, 1H), 2.49 (dt, *J* = 13.6, 5.6 Hz, 1H), 2.31 (dq, *J* = 13.6, 1.6 Hz, 1H).

Difluorinated bromobicyclic lactone ($\pm$)-10

To a solution of bromobicyclic lactone **9** (13.7 mg, 0.059 mmol) in anhydrous CH_2Cl_2 (3.0 mL) was added 19.59 μL of DAST (0.15 mmol) at rt. The reaction mixture was allowed to stir overnight at rt. Water (1 mL) was added, the mixture extracted with CH_2Cl_2 (2×20 mL), washed with sat. NaHCO_3 , dried over MgSO_4 , concentrated in vacuo, and then purified by column chromatography (25% $\rightarrow$ 30% $\rightarrow$ 50% EtOAc/hexanes) to give 12.5 mg (83%) of the product **10** as a colorless oil: ^1H NMR (300 MHz, CDCl_3) δ 6.47-6.42 (m, 2H), 6.26 (tt, J = 54.6, 1.2 Hz, 1H), 5.32 (s, 1H), 2.75-2.60 (m, 1H), 2.52 (dt, J = 18.4, 5.6 Hz, 1H), 2.07 (ddt, J = 16.8, 5.6, 1.6 Hz, 1H); ^{19}F NMR (282 MHz, CDCl_3) δ -120.44 (ddd, J = 290.46, 53.58, 2.82 Hz, 1F), -127.18 (ddd, J = 290.46, 28.20, 2.82 Hz, 1F); IR (neat, cm^{-1}) 1766, 1690, 1378, 1355, 1284, 1172, 1149, 1120, 1085, 1073, 1026, 990, 973, 885, 708, 697.

Difluorinated methyl ester ($\pm$)-11

A solution of the bicyclic lactone **10** (299.4 mg, 1.18 mmol), tributyltin hydride (0.45 mL, 1.66 mmol) and 2,2-azobisisobutyronitrile (38.75 mg, 0.24 mmol) in benzene (10 mL) was refluxed for 3h. After the reaction mixture was cooled to rt and the solvent was removed, the residue was taken up in ether (10 mL) and treated with DBU (1.3 mmol). The resulting mixture was stirred for 15 min and the resulting precipitate was removed by filtration through Celite. The mixture was concentrated in vacuo, and then purified by column chromatography (10% $\rightarrow$ 20% $\rightarrow$ 30% EtOAc/hexanes) to give 117.4 mg (57%) of the debrominated product as a colorless oil. To a solution of the debrominated lactone (116.9 mg, 0.67 mmol) in 6 mL of MeOH and CH_2Cl_2 (1:1, each 3 mL) was added a solution of sodium methoxide (prepared freshly with 38.60 mg of Na and 1.5 mL of MeOH) at -78 $^\circ\text{C}$. The cold bath was removed and the resulting mixture was allowed to warm to rt and stir for 4 h. Water (1 mL) was added, the mixture extracted with CH_2Cl_2 (2×20 mL), washed with sat. ammonium chloride, dried over MgSO_4 , concentrated in vacuo, and then purified by column chromatography (25% $\rightarrow$ 50% EtOAc/hexanes) to give 127.3 mg (92%) of the product **11** as a colorless oil: ^1H NMR (300 MHz, CDCl_3) δ 7.03 (s, 1H), 6.04 (dt, J = 56.1, 1.8 Hz, 1H), 4.09 (brs, 1H), 3.67 (s, 3H), 3.35 (brs, 1H), 3.12 (d, J = 29.4 Hz, 1H), 2.55 (dt, J = 19.5, 5.1 Hz, 1H), 2.20 (d, J = 13.5 Hz, 1H), 2.09 (dd, J = 19.2, 7.8 Hz, 1H), 1.59-1.49 (m, 1H); ^{13}C NMR (75 MHz, CDCl_3) δ 166.40, 142.53, 125.40 (dd, J = 8.25, 1.5 Hz), 116.02 (t, J = 242.25 Hz), 62.90 (d, J = 3.75 Hz), 51.71, 38.38 (dd, J = 21.0, 20.25 Hz), 34.21, 28.69 (dd, J = 4.5, 1.5 Hz); ^{19}F NMR (282 MHz, CDCl_3) δ -121.76 (ddd, J = 276.36, 56.40, 5.64 Hz, 1F), -124.68 (ddd, J = 276.36, 56.40, 28.2 Hz, 1F); IR (neat, cm^{-1}) 3390, 3001, 2943, 1705, 1643, 1443, 1255, 1090, 749.

Allylic alcohol ($\pm$)-12

To a solution of difluorinated methyl ester **11** (156.3 mg, 0.76 mmol) in CH_2Cl_2 (10 mL) was added 114.78 μL of 2,6-lutidine (0.99 mmol), and 208.84 μL of TBSOTf (0.91 mmol) dropwise at 0 $^\circ\text{C}$. The reaction mixture was allowed to stir for 0.5 h at the same temperature. Water (2.0 mL) was added, the mixture was extracted with CH_2Cl_2 (2×30 mL), washed with brine, dried over MgSO_4 , concentrated in vacuo, and then purified by column chromatography (10% EtOAc/hexanes) to give 201.85 mg (83%) of the desired methyl ester as a colorless oil. To a solution of the methyl ester (201.8 mg, 0.63 mmol) in THF (5 mL) at -78 $^\circ\text{C}$ was slowly added 1.26 mL of diisobutylaluminum hydride (1.89 mmol, 25 wt% (1.5 M) solution in toluene). The reaction mixture was allowed to warm to rt and stir for 1 h. The reaction was quenched with sat. sodium potassium tartrate, extracted with CH_2Cl_2 (2×30 mL), washed with brine, dried over MgSO_4 , concentrated in vacuo, and then purified by column chromatography (30% EtOAc/hexanes) to give 164.50 mg (89%) of the desired allylic alcohol **12** as a colorless oil. Without further characterization, it was carried on to the next step.

Ethyl ester (E,Z)-13

A 25 mL hydrolysis tube containing a solution of the allylic alcohol **12** (164.5 mg, 0.56 mmol), 1-(phenylsulfinyl)-2,2,2-triethoxyethane (418.85 mg, 1.46 mmol), and 2,4,6-trimethyl benzoic acid (5 mg) in CH_2Cl_2 (5 mL) was heated to 110 °C for 20 h. After the reaction mixture was cooled to rt, water (2 mL) was added. The mixture was extracted with CH_2Cl_2 (2×30 mL), washed with brine, dried over MgSO_4 , concentrated in vacuo, and then purified by column chromatography (5% EtOAc/hexanes) to give 158.18 mg (78 %) of the desired ethyl ester **13** as a colorless oil. ^1H NMR analysis showed the geometric ratio of (Z)-isomer:(E)-isomer was 1.5 : 1. Ethyl ester (E)-**13**: ^1H NMR (300 MHz, CDCl_3) δ 7.26 (s, 1H), 5.92 (s, 1H), 5.81 (dt, $J = 56.4, 4.5$ Hz, 1H), 5.29 (s, 1H), 4.97 (s, 1H), 4.20-4.13 (m, 3H), 3.12 (dd, $J = 15.3, 3.0$ Hz, 1H), 3.01 (dt, $J = 15.0, 6.0$ Hz, 2H), 1.89 (t, $J = 5.0$ Hz, 2H), 1.28 (t, $J = 7.2$ Hz, 3H), 0.86 (s, 9H), 0.07 (s, 6H); ^{19}F NMR (282 MHz, CDCl_3) δ -120.35 (ddd, $J = 287.64, 56.40, 11.28$ Hz, 1F), -123.76 (ddd, $J = 279.18, 56.40, 16.92$ Hz, 1F). Ethyl ester (Z)-**13**: ^1H NMR (300 MHz, CDCl_3) δ 5.88 (dt, $J = 56.1, 4.2$ Hz, 1H), 5.64 (d, $J = 0.6$ Hz, 1H), 5.12 (s, 1H), 5.09 (s, 1H), 4.13-4.06 (m, 3H), 3.03-2.90 (m, 1H), 2.47 (dd, $J = 12.6, 4.2$ Hz, 1H), 2.26 (dd, $J = 12.6, 7.8$ Hz, 1H), 2.03 (dt, $J = 13.5, 5.1$ Hz, 1H), 1.84-1.75 (m, 1H), 1.22 (t, $J = 1.22$ Hz, 3H), 0.86 (s, 9H), 0.05 (s, 6H); ^{13}C NMR (75 MHz, CDCl_3) δ 165.95, 152.14, 140.92 (dd, $J = 6.0, 3.75$ Hz), 117.68, 116.75 (t, $J = 241.5$ Hz), 115.32, 67.46 (d, $J = 1.5$ Hz), 59.98, 46.39, 45.34 (dd, $J = 21.75, 20.25$ Hz), 34.17 (t, $J = 3.75$ Hz), 25.68, 18.02, 13.89, -4.89; ^{19}F NMR (282 MHz, CDCl_3) δ -119.09 (ddd, $J = 279.18, 56.40, 14.1$ Hz, 1F), -124.04 (ddd, $J = 279.18, 56.40, 16.92$ Hz, 1F); IR (neat, cm^{-1}) 2955, 2920, 2895, 2860, 1725, 1637, 1466, 1366, 1255, 1220, 1178, 1096, 1055, 1037, 1008, 837, 780.

Photoisomerization to ethyl ester 14

A borosilicate test tube was charged with 131.4 mg of (Z)- and (E)-**13** (0.36 mmol, 1.5:1), 10 mg of 9-fluorenone-4-carboxylic acid, and 10 mL of tert-butyl methyl ether. The test tube was sealed with a rubber septum and placed in a solution of 2M sodium orthovanadate and irradiated with a medium pressure mercury arc lamp for 40 h. On disappearance of the less polar (E)-**13** by TLC analysis, concentration of the reaction followed by ^1H NMR analysis suggested > 20:1 ratio in favor of the (Z)-isomer. The residue was purified by column chromatography (5% EtOAc/hexanes) to give 107.75 mg (82%) of the desired (Z)-**14** as a colorless oil.

(±)-1α-CHF₂ A ring phosphine oxide 16

To a solution of dienolate **14** (175.0 mg, 0.49 mmol) in CH_2Cl_2 (10 mL) was added dropwise 0.81 mL of diisobutyl aluminum hydride (1.21 mmol, 1.5 M solution in toluene) at -78 °C. The reaction was allowed to stir at -78 °C for 2h, at which time TLC analysis showed starting materials were present. A second portion of diisobutyl aluminum hydride (1.0 eq) was added -78 °C and then the reaction was stirred for 1h. The mixture was quenched with 1.0 mL of 2N sodium potassium tartrate solution, extracted with CH_2Cl_2 (2×30 mL), washed with brine, dried over MgSO_4 , concentrated in vacuo, and then purified by column chromatography (20% EtOAc/hexanes) to give 142.22 mg (92 %) of the desired allylic alcohol **15** as a colorless oil. To a solution of N-chlorosuccinimide (207.30 mg, 1.55 mmol) in CH_2Cl_2 (3 mL) at 0 °C was slowly added dimethyl sulfide (0.11 mL, 1.55 mmol). The resulting white cloudy solution was stirred for 15 min at 0 °C and then cooled to -20 °C and treated with a solution of the allylic alcohol **15** (154.5 mg, 0.49 mmol) in CH_2Cl_2 (2 mL). The reaction was allowed to stir 2h. Water (1 mL) was added, the reaction mixture was warmed to rt and stir for 15 min, and then extracted with CH_2Cl_2 (2×30 mL), washed with brine, dried over MgSO_4 , concentrated in vacuo, and then purified by column chromatography (10% EtOAc/hexanes) to give 151.19 mg (93%) of the desired allylic chloride as a colorless oil. To a stirring solution of diphenyl phosphine (390.47 μL , 2.24 mmol) in anhydrous THF (3 mL) at 0 °C was added 1.40 mL of nBuLi (2.24 mmol, 1.6 M in hexanes). The resulting deep orange/red solution was slowly added

to a solution of allylic chloride (151.2 mg, 0.45 mmol) in THF (5 mL) at -78°C via cannula. Once the deep orange color persisted in the reaction flask, addition was stopped. After 30 min stirring -78°C , TLC analysis confirmed the full consumption of the starting allylic chloride. Water (1 mL) was added and the reaction was allowed to warm to rt. THF was removed by concentration in vacuo before the addition of CH_2Cl_2 (4.5 mL) to the reaction mixture slurry in water. Water (2 mL) and 3 mL of hydrogen peroxide (30% solution in water) were added and the reaction was stirred vigorously at rt for 15h. The reaction mixture was extracted with CH_2Cl_2 (3×20 mL), washed with brine, dried over MgSO_4 , concentrated in vacuo, and then purified by column chromatography (25% EtOAc/hexanes) to give 137.60 mg (61%) of the desired A ring phosphine oxide **16** as a foaming solid: ^1H NMR (300 MHz, CDCl_3) δ 7.79-7.66 (m, 4H), 7.55-7.43 (m, 6H), 5.46 (d, $J = 7.2$ Hz, 1H), 5.33 (dt, $J = 56.1, 6.0$ Hz, 1H), 5.11 (s, 1H), 4.99 (s, 1H), 3.83-3.76 (m, 1H), 3.29-3.17 (m, 2H), 2.78-2.64 (m, 1H), 2.42 (d, $J = 12.9$ Hz, 1H), 2.17 (t, $J = 9.6$ Hz, 1H), 1.94-1.84 (m, 2H), 1.75-1.65 (m, 1H), 0.83 (s, 9H), 0.015 (s, 3H), 0.011 (s, 3H); ^{13}C NMR (75 MHz, CDCl_3) δ 140.67 (d, $J = 12.0$ Hz), 132.95 (d, $J = 4.5$ Hz), 131.81 (d, $J = 3.0$ Hz), 131.72 (d, $J = 3.0$ Hz), 131.64 (d, $J = 3.0$ Hz), 131.09 (d, $J = 9.0$ Hz), 130.91 (d, $J = 3.75$ Hz), 130.78 (d, $J = 3.0$ Hz), 128.55 (d, $J = 7.5$ Hz), 128.40 (d, $J = 7.5$ Hz), 116.28 (t, $J = 242.25$ Hz), 115.85, 115.44, 115.33, 66.85 (d, $J = 2.25$ Hz), 46.10 (d, $J = 1.5$ Hz), 45.75 (t, $J = 21.0$ Hz), 35.91 (d, $J = 4.5$ Hz), 31.46, 30.53, 25.63, 17.92, -4.92, -4.99; ^{19}F NMR (282 MHz, CDCl_3) δ -117.95 (ddd, $J = 279.18, 56.40, 14.1$ Hz, 1F), -123.81 (ddd, $J = 279.18, 56.40, 14.10$ Hz, 1F); IR (neat, cm^{-1}) 3401, 3060, 2954, 2930, 295, 2860, 1972, 1901, 1825, 1725, 1637, 1584, 1472, 1437, 1390, 1360, 1255, 11778, 1102, 1072, 1049, 837, 779, 732, 696, 550, 514; HRMS m/z ($\text{M} + \text{Na}$) calcd 525.2161 for $\text{C}_{28}\text{H}_{37}\text{F}_2\text{O}_2\text{PSiNa}^+$, found 525.2142.

(Trimethylsilyl)oxy C,D ring ketone **18**

To a solution of tert-alcohol **17** [15] (40.2 mg, 0.14 mmol) in CH_2Cl_2 (5 mL) was added dropwise 42.06 μL of 1-(trimethylsilyl)imidazole (0.29 mmol) at rt. The reaction mixture was allowed to stir overnight at rt. The reaction was concentrated in vacuo, and then purified by column chromatography (25% EtOAc/hexanes) to give 46.25 mg (92%) of the desired ketone **18** as a colorless oil: ^1H NMR (400 MHz, CDCl_3) δ 2.44 (dd, $J = 11.6, 7.6$ Hz, 1H), 2.30-2.19 (m, 2H), 2.12 (ddd, $J = 13.2, 4.4, 2.4$ Hz, 1H), 2.03-1.95 (m, 1H), 1.94-1.84 (m, 2H), 1.77-1.66 (m, 1H), 1.57 (dd, $J = 13.2, 5.2$ Hz, 1H), 1.54-1.48 (m, 1H), 1.44-1.29 (m, 8H), 1.19 (s, 6H), 1.04-1.02 (m, 1H), 0.95 (d, $J = 6.4$ Hz, 3H), 0.63 (s, 3H), 0.09 (s, 9H).

1 α -CHF₂-25-OH analog (+)-**4**

A solution of 79.03 mg (0.16 mmol) of racemic phosphine oxide **16** in 2.0 mL of anhydrous THF was cooled to -78°C and treated with 98.27 μL (0.16 mmol, 1.6 M in hexanes) of *n*-BuLi under argon atmosphere. The mixture turned red and was stirred for 15 min at -78°C . To the solution was added dropwise a precooled (-78°C) solution of the C,D-ring ketone **18** (46.2 mg, 0.13 mmol) in 1.0 mL of anhydrous THF at -78°C . The deep red solution was allowed to stir for 5 h at -78°C . The reaction was quenched by adding 1.0 mL of pH 7 buffer, then warmed to room temperature, extracted with EtOAc (20 mL $\times$ 2), washed with brine, dried over MgSO_4 , concentrated in vacuo, and then purified by column chromatography (10% EtOAc/hexanes) to afford 51.5 mg (62%) of the coupled product as a colorless oil. The coupled product (51.5 mg, 0.081 mmol) was dissolved in 5 mL of anhydrous THF, and to this solution was added 0.32 mL (0.32 mmol) of a 1.0 M solution of TBAF in THF at rt. The reaction was run in darkness for 1 d, then extracted with EtOAc (20 mL $\times$ 2), washed with brine, dried over MgSO_4 , concentrated in vacuo, and then purified by column chromatography (20% $\rightarrow$ 25% EtOAc/hexanes) to give 32.9 mg (90%) of a mixture of two diastereomers as a colorless oil. The diastereomeric ratio was 3:1, which was confirmed by ^1H NMR and HPLC. The diastereomers were separated by Reverse Phase HPLC (C-18 Luna semipreparative column, 63% $\text{CH}_3\text{CN}/\text{H}_2\text{O}$, 3.0 mL/min) to afford 16.1 mg (49%) of **4** (1 α , 3 β , t_R 74 min) and 4.93 mg

(15%) of the other isomer (1 β , 3 α , t_R 71 min) as colorless oils. **4**: $[\alpha]_D^{24} +50.2$ (c 0.14, CHCl₃); ¹H NMR (300 MHz, CDCl₃) δ 6.31 (d, J = 11.1 Hz, 1H), 5.95 (d, J = 11.1 Hz, 1H), 5.76 (dt, J = 56.4, 5.4 Hz, 1H), 5.21 (s, 1H), 5.05 (s, 1H), 4.09-4.03 (m, 1H), 2.90-2.80 (m, 1H), 2.79 (d, J = 11.7 Hz, 1H), 2.61 (dd, J = 13.2, 3.9 Hz, 1H), 2.28 (dd, J = 13.2, 8.7 Hz, 1H), 2.10 (dt, J = 13.2, 4.5 Hz, 1H), 2.02-2-1.94 (m, 3H), 1.88-1.76 (m, 2H), 1.69-1.54 (m, 4H), 1.50-1.33 (m, 8H), 1.31-1.25 (m, 2H), 1.21 (s, 6H), 1.09-1.03 (m, 1H), 0.92 (d, J = 6.6 Hz, 3H), 0.52 (s, 3H); ¹³C NMR (75 MHz, CDCl₃) δ 143.50, 133.31, 124.07, 119.54, 117.04, 116.70 (t, J = 242.21 Hz), 115.98, 115.08, 71.13, 66.66, 56.55, 56.33, 45.94, 45.75 (t, J = 20.51 Hz), 45.71, 44.41, 40.51, 36.41, 36.10, 29.37, 29.19, 29.13, 27.67, 23.58, 22.12, 20.81, 18.82; ¹⁹F NMR (282 MHz, CDCl₃) δ -119.25 (ddd, J = 279.18, 56.40, 11.28 Hz, 1F), -123.87 (ddd, J = 279.18, 59.22, 14.10 Hz, 1F); IR (neat, cm⁻¹) 3377, 2942, 2860, 1713, 1648, 1466, 1372, 1266, 1243, 1149, 1050, 1002, 908, 732; UV (MeOH) λ_{max} 263 nm (ϵ 7827); HRMS m/z (M + Na) calcd 505.3099 for C₂₈H₄₄F₂O₄Na⁺, found 505.3122.

8-Keto-23(Oxa)-25-(O)-26-TB 21

To ice-cooled hexanes-washed KH (120 mg of a 30% suspension in mineral oil, 0.9 mmol) was added a solution of **19** (compound **19** was prepared analogously to the TES ether in [11]) (110 mg, 0.30) in THF (2.5 mL). The mixture was stirred at rt for 45 min until a light yellow color developed. Tosylate **20** [16] (240 mg, 0.9 mmol) and Bu₄Ni (6 mg, 0.02 mmol) were added to the reaction flask and the mixture was stirred at rt for 14 h. The reaction was quenched by addition of water and extracted with CH₂Cl₂ (3 $\times$ 5 mL). The combined organic extracts were dried over MgSO₄, filtered, concentrated, and purified by column chromatography (15% ethyl acetate in hexanes) to afford 100 mg of crude allylic ether as a colorless oil in 72% yield. Through a solution of crude allylic ether (70 mg, 0.17 mmol) in CH₂Cl₂ (15 mL) at -78 °C was bubbled O₃ until a deep blue color developed and persisted (approximately 20 min). The solution was subsequently flushed with O₂ for 15 min until the blue color faded. The colorless solution was cooled to 0 °C and dimethyl sulfide (151 μ L, 2.06 mmol) was added. The solution was allowed to warm slowly to rt and stir overnight. The solution was concentrated and subjected to column chromatography (10% ethyl acetate in hexanes) to afford 34 mg of the corresponding C-25 ketone. To a solution of the crude material in CH₃CN was added HF (49%, 300 μ L, 7.3 mmol) and the solution was stirred at rt 14 h. The mixture was quenched with a saturated solution of NaHCO₃ and extracted with ethyl acetate (3 $\times$ 10 mL). The combined organic extracts were rinsed with brine, dried over MgSO₄, filtered, concentrated, and purified by column chromatography (10-25% ethyl acetate in hexanes) to afford 13 mg of 8-OH-25-ketone as a colorless oil in 27% yield for the 2 steps. A solution of the C-8 alcohol (12.5 mg, 0.04 mmol) in CH₂Cl₂ (2 mL) was cannulated into a reaction vessel equipped with PDC (40 mg, 0.11 mmol) and oven dried Celite (40 mg). After stirring overnight the mixture was filtered, the filtrate concentrated in vacuo, and purified by column chromatography (10-25% ethyl acetate in hexanes) to afford 12 mg of **21** as a colorless oil in 95% yield: $[\alpha]_D^{23} +3.4$ (c 0.675, CHCl₃); ¹H NMR (CDCl₃, 400 MHz) δ 4.28 (m, 1H), 4.07 (m, 1H), 3.38 (dd, J = 8.8, 3.6 Hz, 1H), 3.27 (dd, J = 8.8, 6.8 Hz, 1H), 2.45 (dd, J = 11.6, 7.6 Hz, 1H), 1.88-1.20 (m, 11H), 1.16 (s, 9H), 1.15 (d, J = 6.4 Hz, 3H), 0.65 (s, 3H); ¹³C NMR (CDCl₃, 100 MHz) δ 212.08, 211.91, 76.54, 72.03, 61.66, 53.24, 49.90, 42.85, 40.94, 38.77, 36.60, 27.06, 26.26, 24.01, 19.16, 17.52, 12.48; IR (neat, cm⁻¹) 3402 (w), 2960 (s), 2975 (s), 1715 (s), 1478 (m), 1426 (w), 1368 (m), 1308 (w), 1240 (m), 1220 (m), 1179 (w), 1150 (m), 1056 (m), 1001 (m), 942 (w), 837 (w), 720 (w); HRMS m/z (M + Na) calcd 331.2244 for C₁₉H₃₂O₃Na⁺, found 331.2245.

KSP-BCS-1 α -CHF₂-23Oxa25(O)TB (+)-5

Racemic phosphine oxide **16** and CD-ring ketone **21** were separately azeotropically dried with anhydrous benzene (4 $\times$ 5 mL) on a rotary evaporator and held under vacuum (ca. 0.1 mm Hg) for at least 48 h prior to use. A flame-dried 10 mL recovery flask equipped with a magnetic stir bar and an Ar balloon was charged with phosphine oxide ($\pm$)-**16** (82 mg, 0.16 mmol). The

reagent was dissolved in 2.0 mL freshly distilled THF and cooled to -78°C . To this solution *n*-BuLi (113 μL , 0.17 mmol, 1.60 M solution in hexanes) was added drop-wise over several minutes during which time a deep red color developed and persisted. This mixture was allowed to stir at -78°C for an additional 10 min. Meanwhile, a flame-dried 10 mL flask containing CD-ring ketone **21** (20 mg, 0.065 mmol) was dissolved in 0.75 mL of freshly distilled THF and cooled to -78°C . The solution of CD-ring ketone was transferred dropwise into the flask containing the phosphine oxide anion at -78°C via cannula over several minutes. After the addition was complete, the deep red color persisted and the mixture was allowed to stir at -78°C for 3.5 h. Upon observation of a light yellow color, the reaction was quenched with 5 mL of pH 7 buffer and allowed to warm to room temperature. The mixture was extracted with ethyl acetate (3 $\times$ 15 mL). The combined organic extracts were washed with water and brine, dried over MgSO_4 , and filtered. The filtrate was concentrated in vacuo to give crude product that was purified by column chromatography (5-50% ethyl acetate in hexanes) affording 16.0 mg of product **15** in a 43% yield. The protected analog was dissolved in THF (2 mL) and to the solution was added TBAF (70 μL , 1.0 M solution in THF). The solution was stirred for 16 h and then quenched with water, extracted with dichloromethane (3 $\times$ 5 mL), rinsed with brine, dried over MgSO_4 , and concentrated in vacuo. Column chromatography (50% ethyl acetate in hexanes with 1% NEt_3) afforded 12 mg of a 4:1 diastereomeric mixture of analogs **5** as an oil in 93% yield. Separation of diastereomers by HPLC (phenomenex Luna 5 μ C18 250 $\times$ 10 mm eluted with 71:29 $\text{CH}_3\text{CN}/\text{H}_2\text{O}$ t_{R} = 56.8 min) afforded 4.0 mg pure **5**: $[\alpha]_{\text{D}}^{24} +50.2$ (c 0.14, CHCl_3); ^1H NMR (CDCl_3 , 400 MHz) δ 6.31 (d, J = 11.2 Hz, 1H), 5.96 (d, J = 11.2 Hz, 1H), 5.77 (m, 1H), 5.34 (m, 1H), 5.21 (m, 1H), 5.05 (m, 1H), 4.29 (d, J = 2.8 Hz, 2H), 4.06 (m, 1H), 3.41 (dd, J = 8.8, 3.2 Hz, 1H), 3.21 (dd, J = 8.8, 7.6 Hz, 1H), 2.85 (m, 2H), 2.62 (dd, J = 12.4, 4.4 Hz, 1H), 2.29 (m, 1H), 2.24 (m, 1H), 2.05-1.20 (m, 13H), 1.16 (s, 9H), 1.09 (d, J = 6.8 Hz, 3H), 0.54 (s, 3H); ^{13}C NMR (CDCl_3 , 100 MHz) δ 212.13, 143.30, 140.96, 133.38, 124.01, 117.07, 115.98, 77.21, 76.84, 72.14, 66.65, 56.00, 53.14, 46.00, 45.75, 45.68, 42.87, 40.28, 37.20, 34.22, 29.70, 29.09, 27.22, 26.29, 23.51, 22.19, 17.57, 11.89; ^{19}F NMR (CDCl_3 , 280 MHz) δ -118.30 (d, J = 274 Hz, 1F), -123.91 (d, J = 274 Hz, 1F); IR (neat, cm^{-1}) 3371 (br), 2957 (s), 2918 (s), 2849 (s), 1722 (s), 1666 (w), 1462 (m), 1377 (w), 1261 (m), 1093 (m), 1056 (s), 915 (w), 800 (m), 730 (w); UV (MeOH) λ_{max} 265 nm (ϵ 15,164); HRMS m/z (M^+) calcd 478.32585 for $\text{C}_{29}\text{H}_{44}\text{F}_2\text{O}_3$, found 478.32532.

KSP-23(Oxa)-25-(O)-26-TB (+)-6

Enantiomerically pure phosphine oxide (-)-**22** [17] and CD-ring ketone **21**, were separately azeotropically dried with anhydrous benzene (4 $\times$ 5 mL) on a rotary evaporator and held under vacuum (ca. 0.1 mm Hg) for at least 48 hours prior to use.

A flame-dried 10 mL recovery flask equipped with a magnetic stir bar and an Ar balloon was charged with phosphine oxide (-)-**22** (60 mg, 0.10 mmol). The reagent was dissolved in 2.0 mL freshly distilled THF and cooled to -78°C . To this solution *n*-BuLi (67 μL , 0.10 mmol, 1.60 M solution in hexanes) was added drop-wise over several minutes during which time a deep red color developed and persisted. This mixture was allowed to stir at -78°C for an additional 10 min. Meanwhile, a flame-dried 10 mL flask containing CD-ring ketone **21** (12.0 mg, 0.04 mmol) was dissolved in 0.75 mL of freshly distilled THF and cooled to -78°C . The solution of CD-ring ketone was transferred drop-wise into the flask containing the phosphine oxide anion at -78°C via cannula over several minutes. After the addition was complete, the deep red color persisted and the mixture was allowed to stir at -78°C for 6 h. Upon observation of a light yellow color, the reaction was quenched with 5 mL of pH 7 buffer and allowed to warm to room temperature. The mixture was extracted with ethyl acetate (3 $\times$ 15 mL). The combined organic extracts were washed with water and brine, dried over MgSO_4 , and filtered. The filtrate was concentrated *in vacuo* to give crude product that was purified by column chromatography (5-50% ethyl acetate in hexanes) affording 13.0 mg of product in a 50% yield.

The protected analog was dissolved in CH₃CN (2 mL) and HF (49%, 15 μ L) was added. After stirring 1 h the reaction was quenched with a saturated solution of NaHCO₃ and extracted with ethyl acetate (3 $\times$ 10 mL), dried over MgSO₄, and filtered. The filtrate was concentrated *in vacuo* to give the crude product that was purified by column chromatography (50–75% ethyl acetate in hexanes with 1% NEt₃) to afford 6 mg of analog **6** as an oil (40% yield for 2 steps). [α]_D^{24.4} + 11.2 (c 0.275, CHCl₃); IR (neat, cm⁻¹) 3422 (s), 1718 (m), 177 (w), 1654 (m), 1636 (m), 1419 (w), 1260 (w), 1056 (m), 754 (w); ¹H NMR (CDCl₃, 400 MHz) δ 6.38 (d, 1H, J = 11.2 Hz), 6.02 (d, 1H, J = 11.2 Hz), 5.33 (m, 1H), 4.99 (m, 1H), 4.29 (d, 2H, J = 2.4 Hz), 4.23 (m, 1H), 3.42 (dd, 1H, J = 8.8, 3.2 Hz), 3.21 (dd, 1H, J = 8.8, 7.6 Hz), 2.82 (m, 1H), 2.59 (m, 1H), 2.31 (m, 1H), 2.05–1.08 (m, 16H), 1.16 (s, 9H), 1.09 (d, 3H, J = 6.8 Hz), 0.58 (s, 3H); ¹³C NMR (CDCl₃, 400 MHz) δ 212.18, 147.63, 143.02, 132.96, 124.94, 117.06, 111.76, 76.85, 72.13, 70.80, 66.84, 56.03, 53.15, 46.00, 45.24, 42.85, 40.28, 37.21, 29.06, 28.91, 27.21, 26.28, 23.53, 22.36, 17.56, 12.02; HRMS: calcd for C₂₈H₄₄O₄Na⁺ [M+Na]: 467.3132, found 467.3162. UV (MeOH) λ_{max} 265 nm (ϵ 9614).

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Figure 1.

Effect of Vitamin D3 Analogs on Calcium Levels in Rat Urine

Figure 2.

Scheme 1.

Scheme 2.

Calitriol (1)

QW-1624F₂-2 (2)

Structures 1-6.