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26-Desmethyl-2-methylene-22-ene-19-nor-1α,25-dihydroxyvitamin D_3 compounds selectively active on intestine

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ABSTRACT

Six new analogs of 2-methylene-19-nor-1α,25-dihydroxyvitamin D_3, **6**–**7** and **8a**,**b**–**9a**,**b**, have been synthesized. All compounds are characterized by a *trans* double bond located in the side chain between C-22 and C-23. While compounds **6** and **7** possess C-26 and C-27 methyls, compounds **8a**,**b** and **9a**,**b** lack one of these groups. A Lythgoe-based synthesis, employing the Wittig–Horner reaction was used for these preparations. Two different types of $\Delta^{22}E$-25-hydroxy Grundmann's ketone, having either only one stereogenic center located at position C-20 (**20** and **21**), or two stereogenic centers located at 20- and 25-positions (**24a**,**b**–**25a**,**b**) were obtained by a multi-step procedure from commercial vitamin D_2. The introduction of a double bond at C-22 appeared to lower biological activity *in vitro* and *in vivo*. Further removal of a 26-methyl in these analogs had little effect on receptor binding, HL-60 differentiation and CYP24A expression but markedly diminished or eliminated *in vivo* activity on bone calcium mobilization while retaining activity on intestinal calcium transport.

1. Introduction

1α,25-Dihydroxyvitamin D_3, [1α,25$(OH)_2D_3$] (**1**) is perhaps the central regulator of calcium homeostasis [1,2]. In addition, 1α,25$(OH)_2D_3$ plays a role in controlling differentiation and growth of a variety of cells and may play a significant role in the activity of B and T cells [3–8]. The biological responses to 1α,25$(OH)_2D_3$ are mediated by the vitamin D receptor (VDR), which is a member of the nuclear receptor superfamily. It acts as a ligand-dependent gene transcription factor [1]. 1α,25$(OH)_2D_3$ and its analogs have significant therapeutic potential in the treatment of osteoporosis, vitamin D-resistant rickets, secondary hyperparathyroidism, psoriasis, and renal osteodystrophy [1]. However, use of 1α,25$(OH)_2D_3$ itself is limited because it induces significant hypercalcemia. A number of 1α,25$(OH)_2D_3$ analogs have therefore been synthesized, and some of them have been shown to have low calcemic activity [9] (Fig. 1). Two of these analogs, 19-nor-1α,25-$(OH)_2D_2$ (paricalcitol, Zemplar) (**2**) and 1α-$(OH)D_2$ (doxercalciferol, Hectorol) (**3**) have been developed and used to treat secondary hyperpathyroidism (SH) [3,10].

In our continuing effort to identify vitamin D_3 hormone analogs with selective biological activity, we have recently given focus to the synthesis and characterization of 2-substituted 19-nor derivatives with various side chain modifications. This endeavor has yielded several tissue-selective compounds with therapeutic potential [3,11], among them **2MD** (**4**) (Fig. 1), one of the most promising analogs [12]. This analog is at least 30-fold more effective than 1α,25$(OH)_2D_3$ in stimulating osteoblast-mediated bone calcium mobilization while being approximately equally potent in supporting intestinal calcium transport [13].

A very recent addition to our ongoing structure–activity relationship studies has been the development of 2-methylene-19,26-dinor-1α,25-dihydroxyvitamin D_3 analogs [14]. Indeed, the results of our biological studies revealed that removing only one of the two methyl groups at C-25 and maintaining the 25-hydroxy group is an effective method of weakening calcemic activity [14]. In general, (25*R*)-hydroxy analogs exhibit more efficacy, measured both *in vitro* and *in vivo*, than (25*S*) diastereoisomers, with the (20*S*,25*R*)-2-methylene-19,26-dinor-1α,25$(OH)_2D_3$ analog **5** (Fig. 1), being the most potent of the new series [14]. We have now prepared two new 2-methylene-$\Delta^{22}E$-19-nor-1α,25$(OH)_2D_3$ compounds **6** (**20*R***) and **7** (**20*S***) (Fig. 1), which are characterized by the presence of a double bond between C-22 and C-23 in the side chain, as in vitamin D2 analogs **2** and **3** (Fig. 1). Then, to probe whether combining the introduction of a double bond at C-22 with the absence of one of the two methyl groups at C-25 (as in 2-methylene-19,26-dinor-1α,25-dihydroxyvitamin D_3 compound **5** (Fig. 1), might improve tissue selectivity while reducing the calcemic activity, we also synthesized four new 2-methylene-Δ^{22}

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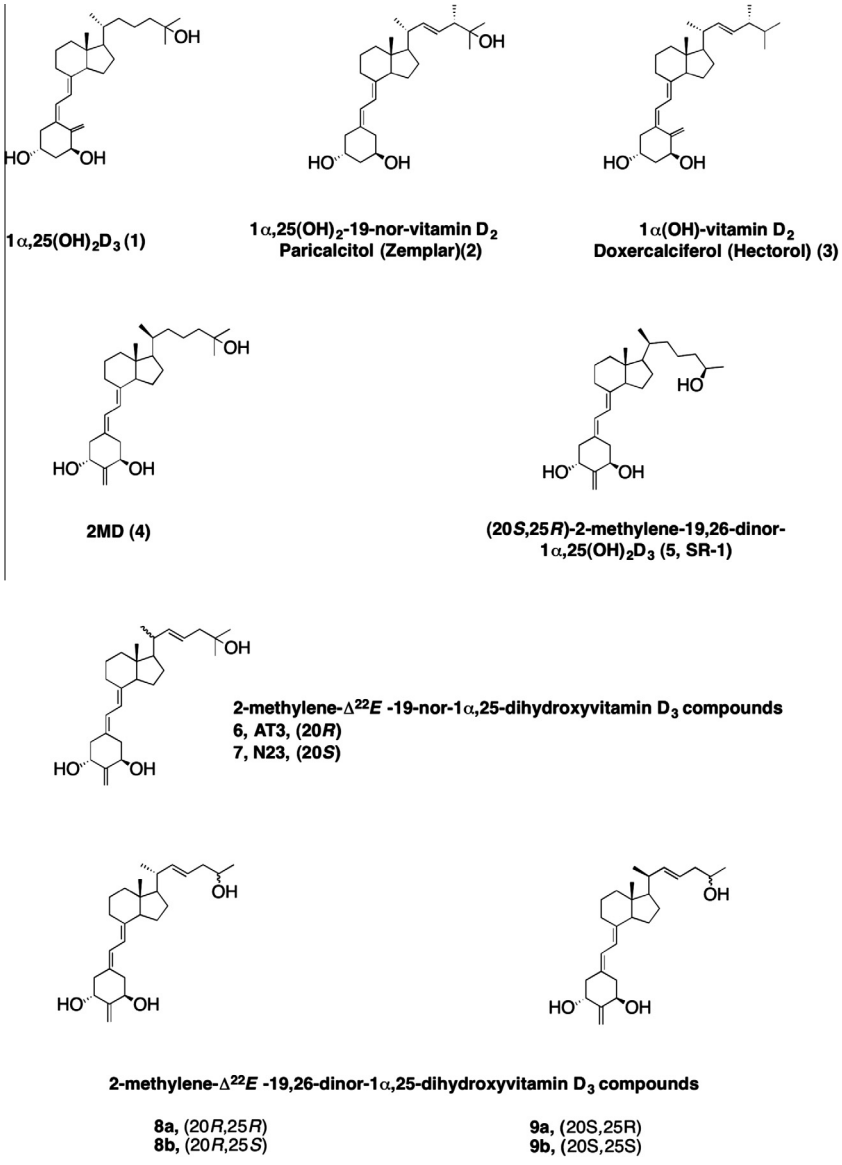


Fig. 1. Chemical structures of 1α,25-dihydroxyvitamin D_3 (calcitriol, **1**) and its analogs.

E-19,26-dinor-1α,25$(OH)_2D_3$ compounds (**8a**, **8b**, **9a**, **9b**; Fig. 1). Structurally all these six new 2-methylene-19-nor-vitamin D analogs have a hydroxyl substituent attached to C-25 in the side chain, and a *trans* double bond located between C-22 and C-23 in the side chain ($\Delta^{22}E$).

In addition, in compounds **8a**,**b**–**9a**,**b** one of the two methyl groups normally located at C-25 in the side chain has been replaced with a hydrogen atom (26-nor). Therefore, the side chains of these last four compounds have two stereogenic centers located at the 20- and 25-positions, and all the four possible 2-methylene-$\Delta^{22}E$-19,26-dinor-1α,25$(OH)_2D_3$ stereoisomers **8a** (20*R*,25*R*), **8b** (20*R*,25*S*), **9a** (20*S*,25*R*), and **9b** (20*S*,25*S*) (Fig. 1) are described.

2. Experimental methods

2.1. General

Optical rotations were measured in chloroform or methanol using a Perkin–Elmer model 343 polarimeter at 22 °C. Ultraviolet (UV) absorption spectra were recorded with a Perkin–Elmer Lambda 3B UV–Vis spectrophotometer in ethanol. ^{1}H nuclear magnetic resonance (NMR) spectra were recorded in deuteriochloroform, or acetone-d_6, at 400 and 500 MHz with Bruker Instruments DMX-400 and DMX-500 Avance console spectrometers. ^{13}C NMR spectra were recorded in deuteriochloroform, at 100 and 125 MHz with the same Bruker Instruments. Chemical shifts (δ) in parts per million are quoted relative to internal Me_4Si (δ 0.00). Electron impact (EI) mass spectra were obtained with a Micromass AutoSpec (Beverly, MA) instrument. HPLC was performed on a Waters Associates liquid chromatograph equipped with a model 6000A solvent delivery system, model U6K Universal injector, and model 486 tunable absorbance detector. THF was freshly distilled before use from sodium benzophenone ketyl under argon. A designation "(volume + volume)", which appears in general procedures, refers to an original volume plus a rinse volume.

Both final vitamin D analogues synthesized by us gave single sharp peaks on HPLC, and they were judged at least 99.5% pure. The purity and identity of the synthesized vitamins were additionally confirmed by inspection of their ^{1}H NMR, ^{13}C NMR, UV absorption, and high-resolution mass spectra.

2.2. Synthesis of compounds

2.2.1. General procedure for the synthesis of compounds **13**, **14**, **16a**, **16b**, **17a**, **17b**

To a stirred suspension of the phosphonium salt **12** or **15a**–**b** (3.0 equiv) [14] in anhydrous THF (5 mL), *n*-butyllithium (6.0 equiv) was added at −20 °C. The solution was stirred at −20 °C for 1 h and it turned deep orange. A pre-cooled solution of aldehyde **10** or **11** (1 equiv) [14] in anhydrous THF (1 + 1 mL) was added and the reaction mixture was stirred at −20 °C for 4 h and at room temperature for 18 h. The reaction was quenched with water and the mixture was extracted with ethyl acetate. Combined organic phases were washed with brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by column chromatography on silica (5–10% ethyl acetate/hexane) to give the product **13**, **14**, **16a**, **16b**, **17a**, **17b**.

2.2.2. (8S,20R)-Des-A,B-8-benzoyloxy-20-[4′-hydroxy-4′-methyl-pent-(1′E)-en-yl]-pregnane (**13**)

According to a general procedure the pure product **13** (67 mg, 47% yield) was obtained from the aldehyde **10** (117 mg, 0.37 mmol), the phosphonium iodide **12** (476 mg, 1.11 mmol) and *n*-butyllithium (1.95 M, 1.14 mL, 2.22 mmol). $[\alpha]_D^{24}$ = +87.8° (*c* 2.75, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.06 (2H, m, *o*-H_{Bz}), 7.55 (1H, m, *p*-H_{Bz}), 7.44 (2H, m, *m*-H_{Bz}), 5.41 (1H, s, 8α-H), 5.39 (2H, m, 22-H and 23-H), 1.19 (6H, s, 26,27-H_6), 1.07 (3H, s, 18-H_3), 1.06 (3H, d, *J* = 6.7 Hz, 21-H_3); ^{13}C NMR (100 MHz) δ 166.40, 141.29, 132.64, 130.80, 129.48, 128.29, 122.80, 72.11, 70.46, 55.93, 51.60, 46.79, 41.79, 40.00, 39.77, 30.45, 28.97, 27.69, 22.61, 20.55, 17.96, 13.70; exact mass calculated for $C_{25}H_{36}O_3Na$ (MNa^+) 407.2562, found 407.2548.

2.2.3. (8S,20S)-Des-A,B-8-benzoyloxy-20-[4′-hydroxy-4′-methyl-pent-(1′E)-en-yl]-pregnane (**14**)

According to a general procedure the pure product **14** (52 mg, 45% yield) was obtained from the aldehyde **11** (93 mg, 0.30 mmol), the phosphonium iodide **12** (476 mg, 1.11 mmol) and *n*-butyllithium (1.61 M, 1.38 mL, 2.22 mmol). $[\alpha]_D^{24}$ = −25.1° (*c* 2.5, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.04 (2H, m, *o*-H_{Bz}), 7.55 (1H, m, *p*-H_{Bz}), 7.44 (2H, m, *m*-H_{Bz}), 5.42 (3H, m, 8α-H, 22-H, 23-H), 1.22 (6H, s, 26,27-H_6), 1.04 (3H, s, 18-H_3), 0.94 (3H, d, *J* = 6.6 Hz, 21-H_3); ^{13}C NMR (125 MHz) δ 166.41, 141.34, 132.64, 130.83, 129.50, 128.29, 122.86, 72.06, 70.68, 56.30, 51.46, 46.92, 41.91, 40.23, 39.33, 30.57, 29.12, 29.11, 26.83, 22.49, 21.57, 17.78, 13.80; exact mass calculated for $C_{25}H_{36}O_3Na$ (MNa^+) 407.2562, found 407.2561.

2.2.4. (8S,20R)-Des-A,B-8-benzoyloxy-20-[(4′R)-hydroxy-pent-(1′E)-en-yl]-pregnane (**16a**)

According to a general procedure the pure product **16a** (47 mg, 49% yield) was obtained from the aldehyde **10** (81 mg, 0.26 mmol), the phosphonium iodide **15a** (361 mg, 0.78 mmol) and *n*-butyllithium (1.6 M, 980 μL, 1.56 mmol). $[\alpha]_D^{24}$ = +69.6° (*c* 1.3, $CHCl_3$); ^{1}H NMR (500 MHz, acetone-d_6) δ 8.05 (2H, m, *o*-H_{Bz}), 7.62 (1H, m, *p*-H_{Bz}), 7.52 (2H, m, *m*-H_{Bz}), 5.41 (1H, dt, *J* = 15.4, 7.0 Hz, 23-H) 5.38 (1H, d, *J* = 1.8 Hz, 8α-H), 5.31 (1H, dd, *J* = 15.4, 8.4 Hz, 22-H), 3.72 (1H, m, 25-H), 3.37 (1H, d, *J* = 4.0 Hz, OH) 1.102 (3H, d, *J* = 6.4 Hz, 27-H_3), 1.096 (3H, s, 18-H_3), 1.05 (3H, d, *J* = 6.6 Hz, 21-H_3); ^{13}C NMR (100 MHz) δ 166.44, 140.80, 132.66, 130.84, 129.51, 128.32, 123.25, 72.14, 67.20, 55.97, 51.64, 42.37, 41.84, 39.91, 39.80, 30.49, 27.58, 22.57, 22.57, 20.59, 17.99, 13.72; exact mass calcd for $C_{24}H_{34}O_3$ (M^+) 370.2508, found 370.2503.

2.2.5. (8S,20R)-Des-A,B-8-benzoyloxy-20-[(4′S)-hydroxy-pent-(1′E)-en-yl]-pregnane (**16b**)

According to a general procedure the pure product **16b** (42 mg, 52% yield) was obtained from the aldehyde **10** (70 mg, 0.22 mmol), the phosphonium iodide **15b** (310 mg, 0.67 mmol) and *n*-butyllithium (1.6 M, 840 μL, 1.34 mmol). $[\alpha]_D^{24}$ = +98.7° (*c* 1.75, $CHCl_3$); ^{1}H NMR (500 MHz, acetone-d_6) δ 8.05 (2H, m, *o*-H_{Bz}), 7.63 (1H, m, *p*-H_{Bz}), 7.52 (2H, m, *m*-H_{Bz}), 5.42 (1H, dt, *J* = 15.2, 7.0 Hz, 23-H) 5.38 (1H, d, *J* = 2.5 Hz, 8α-H), 5.32 (1H, dd, *J* = 15.2, 8.5 Hz, 22-H), 3.72 (1H, m, 25-H), 3.32 (1H, d, *J* = 4.4 Hz, OH) 1.102 (3H, d, *J* = 6.1 Hz, 27-H_3), 1.096 (3H, s, 18-H_3), 1.05 (3H, d, *J* = 6.6 Hz, 21-H_3); ^{13}C NMR (100 MHz) δ 166.43, 140.86, 132.66, 130.82, 129.50, 128.32, 123.42, 72.12, 67.15, 55.87, 51.63, 42.48, 41.81, 39.93, 39.79, 30.47, 27.65, 22.59, 22.48, 20.47, 17.98, 13.72; exact mass calcd for $C_{24}H_{34}O_3$ (M^+) 370.2508, found 370.2491.

2.2.6. (8S,20S)-Des-A,B-8-benzoyloxy-20-[(4′R)-hydroxy-pent-(1′E)-en-yl]-pregnane (**17a**)

According to a general procedure the pure product **17a** (39 mg, 48% yield) was obtained from the aldehyde **11** (70 mg, 0.22 mmol), the phosphonium iodide **15a** (221 mg, 0.66 mmol) and *n*-butyllithium (1.6 M, 720 μL, 1.15 mmol). $[\alpha]_D^{24}$ = −28.8° (*c* 0.8, $CHCl_3$); ^{1}H NMR (500 MHz, acetone-d_6) δ 8.05 (2H, m, *o*-H_{Bz}), 7.63 (1H, m, *p*-H_{Bz}), 7.52 (2H, m, *m*-H_{Bz}), 5.46 (1H, dt, *J* = 15.4, 6.9 Hz, 23-H) 5.38 (1H, s, 8α-H), 5.36 (1H, dd, *J* = 15.4, 8.5 Hz, 22-H), 3.76 (1H, m, 25-H), 3.49 (1H, d, *J* = 4.0 Hz, OH) 1.13 (3H, d, *J* = 6.2 Hz, 27-H_3), 1.07 (3H, s, 18-H_3), 0.92 (3H, d, *J* = 6.7 Hz, 21-H_3); ^{13}C NMR (100 MHz) δ 166.45, 140.74, 132.67, 130.86, 129.53, 128.32, 123.33, 72.08, 67.70, 56.33, 51.48, 42.46, 41.94, 40.16, 39.48, 30.60, 26.86, 22.74, 22.50, 21.46, 17.81, 13.89; exact mass calcd for $C_{24}H_{34}O_3Na$ (MNa^+) 393.2406, found 393.2407

2.2.7. (8S,20S)-Des-A,B-8-benzoyloxy-20-[(4′S)-hydroxy-pent-(1′E)-en-yl]-pregnane (**17b**)

According to a general procedure the pure product **17b** (37 mg, 50% yield) was obtained from the aldehyde **11** (65 mg, 0.2 mmol), the phosphonium iodide **15b** (201 mg, 0.6 mmol) and *n*-butyllithium (1.6 M, 560 μL, 0.9 mmol). $[\alpha]_D^{24}$ = −11.4° (*c* 1.4, $CHCl_3$); ^{1}H NMR (500 MHz, acetone-d_6) δ 8.04 (2H, m, *o*-H_{Bz}), 7.63 (1H, m, *p*-H_{Bz}), 7.52 (2H, m, *m*-H_{Bz}), 5.46 (1H, dt, *J* = 15.4, 6.8 Hz, 23-H) 5.39 (1H, s, 8α-H), 5.35 (1H, dd, *J* = 15.4, 6.3 Hz, 22-H), 3.78 (1H, m, 25-H), 3.40 (1H, d, *J* = 4.2 Hz, OH) 1.13 (3H, d, *J* = 6.2 Hz, 27-H_3), 1.07 (3H, s, 18-H_3), 0.93 (3H, d, *J* = 6.7 Hz, 21-H_3); ^{13}C NMR (100 MHz) δ 166.45, 141.11, 132.66, 130.87, 129.53, 128.32, 123.41, 72.09, 67.23, 56.34, 51.47, 42.56, 41.95, 40.15, 39.37, 30.59, 26.80, 22.73, 22.49, 21.56, 17.83, 13.85; exact mass calcd for $C_{24}H_{34}O_3Na$ (MNa^+) 393.2406, found 393.2410.

2.2.8. General procedure for the synthesis of compounds **18**, **19**, **22a**, **22b**, **23a**, **23b**

To a stirred solution of the alcohol **13**, **14**, **16a**, **16b**, **17a** or **17b** (1.0 equiv) and 2,6-lutidine (3.5 eq.) in anhydrous methylene chloride (3 mL), *tert*-butyldimethylsilyl trifluoromethane-sulfonate (1.8 equiv) was added at −20 °C. The reaction mixture was stirred at 0 °C for 1 h. It was quenched with water and extracted with methylene chloride. Combined organic phases were washed with brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (3% ethyl acetate/hexane) to give the product **18**, **19**, **22a**, **22b**, **23a**, **23b**.

2.2.9. (8S,20R)-Des-A,B-8-benzoyloxy-20-[4′-(tert-butyldimethylsilyloxy)-4′-methyl-pent-(1′E)-en-yl]-pregnane (**18**)

According to a general procedure the pure product **18** (67 mg, 96% yield) was obtained from the alcohol **13**. $[\alpha]_D$ +62.9 (*c* 3.35, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.06 (2H, m, *o*-H_{Bz}), 7.55 (1H, m, *p*-H_{Bz}), 7.44 (2H, m, *m*-H_{Bz}), 5.41 (1H, d, *J* = 2.3 Hz, 8α-H), 5.38 (1H, m, 23-H), 5.24 (1H, dd, *J* = 15.4, 8.4 Hz, 22-H), 1.15 (6H, d, *J* = 2.0 Hz, 26,27-H_6), 1.07 (3H, s, 18-H_3), 1.04 (3H, d, *J* = 6.6 Hz, 21-H_3), 0.86 (9H, s, Si-*t*-Bu), 0.06 (6H, s, $SiMe_2$); ^{13}C NMR (100 MHz) δ 166.44, 139.08, 132.65, 130.90, 129.53, 128.32,

124.31, 73.67, 72.20, 56.26, 51.69, 48.33, 41.82, 39.97, 39.84, 30.54, 29.74, 29.40, 27.66, 25.81, 22.66, 20.57, 18.03, 18.03, 13.72, -2.05; exact mass calculated for $C_{31}H_{50}O_3SiNa$ (MNa^+) 521.3427, found 521.3422.

*2.2.10. (8S,20S)-Des-A,B-8-benzoyloxy-20-[4′-(tert-butyldimethylsilyloxy)-4′-methyl-pent-(1′E)-en-yl]-pregnane (**19**)*

According to a general procedure the pure product **19** (65 mg, 93% yield) was obtained from the alcohol **14**. $[\alpha]_D$ −21.2 (*c* 4.95, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 8.05 (2H, m, *o*-H_{Bz}), 7.54 (1H, m, *p*-H_{Bz}), 7.43 (2H, m, *m*-H_{Bz}), 5.41 (2H, m, 8α-H and 23-H), 5.29 (1H, dd, *J* = 15.4, 9.1 Hz, 22-H), 1.18 (6H, d, *J* = 4.5 Hz, 26,27-H_6), 1.04 (3H, s, 18-H_3), 0.93 (3H, d, *J* = 6.6 Hz, 21-H_3), 0.87 (9H, s, Si-*t*-Bu), 0.08 (6H, s, $SiMe_2$); ^{13}C NMR (125 MHz) δ 166.43, 139.10, 132.62, 130.91, 129.53, 128.30, 124.39, 73.72, 72.14, 56.44, 51.52, 48.29, 41.94, 40.30, 39.28, 30.63, 29.76, 29.65, 26.88, 25.83, 22.56, 21.53, 18.04, 17.82, 13.68, −2.04; exact mass calculated for $C_{31}H_{50}O_3SiNa$ (MNa^+) 521.3427, found 521.3450.

*2.2.11. (8S,20R)-Des-A,B-8-benzoyloxy-20-[(4′R)-(tert-butyldimethylsilyl)oxy-pent-(1′E)-en-yl]-pregnane (**22a**)*

According to a general procedure the pure product **22a** (30 mg, 78% yield) was obtained from the alcohol **16a**. $[\alpha]_D$ +53.8 (*c* 1.1, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$) δ 8.05 (2H, m, *o*-H_{Bz}), 7.55 (1H, m, *p*-H_{Bz}), 7.44 (2H, m, *m*-H_{Bz}), 5.41 (1H, s, 8α-H), 5.35–5.26 (2H, m, 22-H and 23-H), 3.81 (1H, m, 25-H), 1.12 (3H, d, *J* = 6.0 Hz, 27-H_3), 1.06 (3H, s, 18-H_3), 1.03 (3H, d, *J* = 6.6 Hz, 21-H_3), 0.88 (9H, s, Si-*t*-Bu), 0.06 (6H, s, $SiMe_2$); ^{13}C NMR (125 MHz) δ 166.86, 138.78, 132.56, 131.11, 129.77, 128.50, 124.63, 72.62, 69.13, 56.51, 51.96, 43.16, 42.15, 39.87, 30.53, 27.54, 26.08, 23.53, 22.88, 22.60, 18.45, 18.36, 18.05, 13.98, −4.32, −4.45.

*2.2.12. (8S,20R)-Des-A,B-8-benzoyloxy-20-[(4′S)-(tert-butyldimethylsilyl)oxy-pent-(1′E)-en-yl]-pregnane (**22b**)*

According to a general procedure the pure product **22b** (37 mg, 95% yield) was obtained from the alcohol **16b**. $[\alpha]_D$ +54.1 (*c* 1.2, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$) δ 8.05 (2H, m, *o*-H_{Bz}), 7.55 (1H, m, *p*-H_{Bz}), 7.44 (2H, m, *m*-H_{Bz}), 5.41 (1H, s, 8α-H), 5.35–5.26 (2H, m, 22-H and 23-H), 3.78 (1H, m, 25-H), 1.10 (3H, d, *J* = 6.0 Hz, 27-H_3), 1.07 (3H, s, 18-H_3), 1.03 (3H, d, *J* = 6.6 Hz, 21-H_3), 0.89 (9H, s, Si-*t*-Bu), 0.05 (6H, s, $SiMe_2$); ^{13}C NMR (125 MHz) δ 166.70, 138.93, 132.88, 131.13, 129.77, 128.55, 124.44, 72.44, 69.23, 56.47, 51.92, 43.15, 42.05, 39.99, 30.77, 27.74, 26.12, 23.45, 22.85, 22.63, 18.40, 18.26, 18.04, 13.96, −4.32, −4.45.

*2.2.13. (8S,20S)-Des-A,B-8-benzoyloxy-20-[(4′R)-(tert-butyldimethylsilyl)oxy-pent-(1′E)-en-yl]-pregnane (**23a**)*

According to a general procedure the pure product **23a** (24 mg, 84% yield) was obtained from the alcohol **17a**. ^1H NMR (400 MHz, $CDCl_3$) δ 8.05 (2H, m, *o*-H_{Bz}), 7.54 (1H, m, *p*-H_{Bz}), 7.42 (2H, m, *m*-H_{Bz}), 5.41 (1H, s, 8α-H), 5.40–5.20 (2H, m, 22-H and 23-H), 3.78 (1H, m, 25-H), 1.11 (3H, d, *J* = 6.0 Hz, 27-H_3), 1.02 (3H, s, 18-H_3) 0.88 (9H, s, Si-*t*-Bu), 0.82 (3H, d, *J* = 6.5 Hz, 21-H_3), 0.04 (6H, s, $SiMe_2$); ^{13}C NMR (100 MHz) δ 166.52, 138.87, 132.66, 130.90, 129.55, 128.33, 124.17, 72.15, 68.74, 56.38, 52.18, 42.89, 41.88, 40.08, 34.86, 30.61, 26.98, 25.80, 23.67, 22.68, 18.61, 18.48, 18.03, 13.78, −4.47, −4.75.

*2.2.14. (8S,20S)-Des-A,B-8-benzoyloxy-20-[(4′S)-(tert-butyldimethylsilyl)oxy-pent-(1′E)-en-yl]-pregnane (**23b**)*

According to a general procedure the pure product **23b** (35 mg, 89% yield) was obtained from the alcohol **17b**. ^1H NMR (600 MHz, $CDCl_3$) δ 8.05 (2H, m, *o*-H_{Bz}), 7.56 (1H, m, *p*-H_{Bz}), 7.45 (2H, m, *m*-H_{Bz}), 5.45 (1H, s, 8α-H), 5.33–5.24 (2H, m, 22-H and 23-H), 3.80 (1H, m, 25-H), 1.18 (3H, d, *J* = 6.0 Hz, 27-H_3), 1.05 (3H, s, 18-H_3), 0.95 (3H, d, *J* = 6.6 Hz, 21-H_3), 0.88 (9H, s, Si-*t*-Bu), 0.051 (6H, s, $SiMe_2$); ^{13}C NMR (125 MHz) δ 166.47, 139.55, 132.85, 131.11, 129.73, 128.52, 124.42, 72.40, 69.17, 56.67, 51.26, 43.25, 42.15, 40.22, 39.40, 30.77, 26.81, 26.12, 23.45, 22.85, 19.42, 18.26, 18.04, 13.96, −4.32, −4.45.

*2.2.15. General procedure for the synthesis of compounds **20**, **21**, **24a**, **24b**, **25a**, **25b***

To a stirred solution of the benzoate **18**, **19**, **22a**, **22b**, **23a** or **23b** in anhydrous ethanol (10 mL), a solution of sodium hydroxide in anhydrous ethanol (2.5 M, 2 mL) was added. The reaction mixture was refluxed for 18 h. It was cooled to room temperature, neutralized with 5% aqueous solution of HCl and extracted with methylene chloride. Combined organic phases were washed with a saturated aqueous $NaHCO_3$ solution, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (5–10% ethyl acetate/hexane) to give the alcohol. Pyridinium dichromate (5 equiv) was added to a solution of the alcohol (1 equiv) and pyridinium *p*-toluenesulfonate (0.3 equiv) in anhydrous methylene chloride (5 mL). The resulting suspension was stirred at room temperature for 3 h. The reaction mixture was filtered through a Waters silica Sep-Pak cartridge (5 g) that was further washed with hexane/ethyl acetate (8:2). After removal of solvents the ketone **20**, **21**, **24a**, **24b**, **25a**, or **25b** was obtained.

*2.2.16. (20R)-Des-A,B-20-[4′-(tert-butyldimethylsilyloxy)-4′-methyl-pent-(1′E)-en-yl]-pregnan-8-one (**20**)*

According to a general procedure the pure product **20** (22 mg, 93% yield) was obtained from the benzoate **18** in two steps. $[\alpha]_D$ −5.8 (*c* 1.1, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 5.40 (1H, ddd, *J* = 15.3, 7.8, 7.1 Hz, 23-H), 5.25 (1H, dd, *J* = 15.3, 8.4 Hz, 22-H), 2.45 (1H, dd, *J* = 11.2, 7.6 Hz), 1.16 (6H, s, 26,27-H_6), 1.05 (3H, d, *J* = 6.6 Hz, 21-H_3), 0.86 (9H, s, Si-*t*-Bu), 0.66 (3H, s, 18-H_3), 0.06 (6H, s, $SiMe_2$); ^{13}C NMR (100 MHz) δ 212.01, 138.45, 124.87, 73.63, 62.03, 56.48, 49.77, 48.30, 40.96, 39.86, 38.84, 29.76, 29.42, 27.87, 25.80, 24.06, 20.76, 19.06, 18.04, 12.66, −2.05; exact mass calculated for $C_{24}H_{44}O_2SiNa$ (MNa^+) 415.3008, found 415.3022.

*2.2.17. (20S)-Des-A,B-20-[4′-(tert-butyldimethylsilyloxy)-4′-methyl-pent-(1′E)-en-yl]-pregnan-8-one (**21**)*

According to a general procedure the pure product **21** (27 mg, 92% yield) was obtained from the benzoate **19** in two steps. $[\alpha]_D$ −39.3 (*c* 1.35, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 5.42 (1H, ddd, *J* = 15.4, 8.2, 7.0 Hz, 23-H), 5.28 (1H, dd, *J* = 15.4, 9.1 Hz, 22-H), 2.42 (1H, dd, *J* = 11.4, 7.6 Hz), 1.17 (6H, s, 26,27-H_6), 0.94 (3H, d, *J* = 6.6 Hz, 21-H_3), 0.86 (9H, s, Si-*t*-Bu), 0.61 (3H, s, 18-H_3), 0.069 and 0.065 (each 3H, each s, each $SiMe_2$); ^{13}C NMR (100 MHz) δ 212.10, 138.69, 124.97, 73.65, 61.91, 56.57, 50.03, 48.23, 41.03, 40.44, 38.28, 29.79, 29.62, 27.21 (t), 25.79, 23.93, 21.52, 18.97, 18.03, 12.49, −2.06; exact mass calculated for $C_{24}H_{44}O_2SiNa$ (MNa^+) 415.3008, found 415.3018.

*2.2.18. (20R)-Des-A,B-20-[(4′R)-(tert-butyldimethylsilyl)oxy-pent-(1′E)-en-yl]-pregnan-8-one (**24a**)*

According to a general procedure the pure product **24a** (9 mg, 61% yield) was obtained from the benzoate **20a** in two steps. ^1H NMR (400 MHz, $CDCl_3$) δ 5.38–5.23 (2H, m, 22-H and 23-H), 3.79 (1H, m, 25-H), 2.44 (1H, m), 1.07 (3H, d, *J* = 6.6 Hz, 27-H_3), 0.95 (3H, d, *J* = 6.6 Hz, 21-H_3), 0.89 (9H, s, Si-*t*-Bu), 0.67 (3H, s, 18-H_3), 0.046 (6H, s, $SiMe_2$); ^{13}C NMR (100 MHz) δ 211.97, 138.20, 124.71, 68.97, 62.08, 56.51, 49.78, 42.90, 40.87, 39.67, 38.85, 27.73, 25.84, 24.09, 23.17, 20.70, 19.07, 18.11, 12.68, −4.52, −4.68; exact mass calcd for $C_{23}H_{42}O_2Si$ Na (MNa^+)401.2852, found 401.2847.

*2.2.19. (20R)-Des-A,B-20-[(4′S)-(tert-butyldimethylsilyl)oxy-pent-(1′E)-en-yl]-pregnan-8-one (**24b**)*

According to a general procedure the pure product **24b** (16 mg, 81% yield) was obtained from the benzoate **22b** in two steps. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.34–5.20 (2H, m, 22-H and 23-H), 3.74 (1H, m, 25-H), 2.41 (1H, dd, J = 11.5, 7.6 Hz), 1.05 (3H, d, J = 6.1 Hz, 27-H_3), 0.99 (3H, d, J = 6.6 Hz, 21-H_3), 0.84 (9H, s, Si-*t*-Bu), 0.61 (3H, s, 18-H_3), 0.043 (6H, s, $SiMe_2$); ^{13}C NMR (100 MHz) δ 211.97, 138.10, 124.74, 68.92, 62.02, 56.46, 49.76, 42.89, 40.95, 39.69, 38.85, 27.73, 25.88, 24.05, 23.23, 20.61, 19.03, 18.17, 12.68, −4.54, −4.69; exact mass calcd for $C_{23}H_{42}O_2Si$ Na $(MNa)^+$ 401.2852, found 401.2845.

*2.2.20. (20S)-Des-A,B-20-[(4′R)-(tert-butyldimethylsilyl)oxy-pent-(1′E)-en-yl]-pregnan-8-one (**25a**)*

According to a general procedure the pure product **25a** (7 mg, 67% yield) was obtained from the benzoate **23a** in two steps. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.35–5.22 (2H, m, 22-H and 23-H), 3.74 (1H, m, 25-H), 2.41 (1H, dd, J = 11.5, 7.6 Hz), 1.13 (3H, d, J = 6.1 Hz, 27-H_3), 0.89 (9H, s, Si-*t*-Bu), 0.84 (3H, d, J = 5.9 Hz, 21-H_3), 0.63 (3H, s, 18-H_3), 0.053 (6H, s, $SiMe_2$); ^{13}C NMR (100 MHz) δ 212.13, 139.12, 124.44, 68.66, 62.22, 56.49, 50.04, 42.66, 41.05, 40.18, 33.85, 27.13, 25.89, 24.03, 23.78, 21.61, 18.93, 18.16, 12.70, −4.38, −4.70; exact mass calculated for $C_{23}H_{42}O_2SiNa$ $(MNa)^+$ 401.2852, found 401.2848.

*2.2.21. (20S)-Des-A,B-20-[(4′S)-(tert-butyldimethylsilyl)oxy-pent-(1′E)-en-yl]-pregnan-8-one (**25b**)*

According to a general procedure the pure product **25b** (10 mg, 67% yield) was obtained from the benzoate **23b**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.35–5.22 (2H, m, 22-H and 23-H), 3.76 (1H, m, 25-H), 2.41 (1H, dd, J = 11.5, 7.6 Hz), 1.15 (3H, d, J = 6.1 Hz, 27-H_3), 1.01 (3H, s, 18-H_3), 0.88 (3H, d, J = 6.6 Hz, 21-H_3), 0.84 (9H, s, Si-*t*-Bu), 0.052 (6H, s, $SiMe_2$); ^{13}C NMR (100 MHz) δ 211.97, 139.10, 124.44, 68.72, 62.32, 56.46, 51.76, 42.89, 41.15, 40.19, 33.85, 27.73, 25.88, 24.05, 23.60, 20.61, 19.03 18.27, 12.68, −4.54, −4.69; exact mass calculated for $C_{23}H_{42}O_2Si$ Na $(MNa)^+$ 401.2852, found 401.2848.

*2.2.22. General procedure for the synthesis of compounds **27**, **28**, **29a**, **29b**, **30a**, **30b***

To a stirred solution of the phosphine oxide **26** (3.7 equiv) [15] in anhydrous THF (500 μL), a solution of phenyllithium (1.8 M in di-*n*-butylether, 1.2 equiv) was added at −20 °C under argon. The mixture was stirred for 30 min and then cooled to −78 °C. A pre-cooled solution of the Grundmann's type ketone **20**, **21**, **24a**, **24b**, **25a** or **26b** (1 equiv) in anhydrous THF (200 + 100 μL) was added *via* cannula and the reaction mixture was stirred for 4 h at −78 °C. Then the reaction mixture was stirred at 4 °C for 19 h. Ethyl acetate (20 mL) was added and the organic phase was washed with brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified on a Waters silica Sep-Pak cartridge (0–2% ethyl acetate/hexane) to give the protected vitamin D compound **27**, **28**, **29a**, **29b**, **30a** or **30b**.

*2.2.23. (20R)-1a-[(tert-Butyldimethylsilyl)oxy]-2-methylene-25-[(tert-butyldimethyl-silyl)oxy]-19-nor-22(E)-ene-vitamin D_3 tert-butyldimethylsilyl ether (**27**)*

According to a general procedure the pure protected analog **27** (35.06 mg, 83% yield) was obtained from the phosphine oxide **26** (62 mg, 107 μmol), PhLi (1.8 M in di-*n*-butylether, 60 μL, 114 μmol) and the ketone **20** (22 mg, 56 μmol). UV (in hexane) λ_{max} 262.5, 253.0, 245.0 nm; ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.22 and 5.84 (each 1H, each d, J = 11.2 Hz, 6- and 7-H), 5.38 (1H, ddd, J = 15.3, 7.7, 7.2 Hz, 23-H), 5.26 (1H, dd, J = 15.3, 8.5 Hz, 22-H), 4.97 and 4.92 (each 1H, each s, =CH_2), 4.43 (2H, m, 1β- and 3α-H), 2.83 (1H, dm, J = 12.5 Hz, 9b-H), 2.51 (1H, dd, J = 13.2, 5.9 Hz, 10α-H), 2.46 (1H, dd, J = 12.6, 4.3 Hz, 4α-H), 2.33 (1H, dd, J = 13.2, 2.7 Hz, 10β-H), 2.18 (1H, dd, J = 12.6, 8.4 Hz, 4β-H), 1.165 (6H, s, 26,27-H_6), 1.02 (3H, d, J = 6.6 Hz, 21-H_3), 0.897 (9H, s, Si-*t*-Bu), 0.867 (9H, s, Si-*t*-Bu), 0.863 (9H, s, Si-*t*-Bu), 0.562 (3H, s, 18-H_3), 0.081 (3H, s, SiMe), 0.071 (6H, s, 2× SiMe), 0.068 (3H, s, SiMe), 0.050 (3H, s, SiMe), 0.027 (3H, s, SiMe); ^{13}C NMR (125 MHz) d 152.97, 141.15, 139.34, 132.76, 124.25, 122.40, 116.14, 106.26, 73.75, 72.51, 71.64, 56.36, 48.37, 47.60, 45.60, 40.49, 38.56, 29.79, 29.42, 28.74, 28.05, 25.84, 25.78, 23.43, 22.23, 20.88, 18.25, 18.17, 18.06, 12.28, −2.02, −4.86, −5.10; exact mass calculated for $C_{45}H_{84}O_3Si_3Na$ (MNa^+) 779.5626, found 779.5652.

*2.2.24. (20S)-1a-[(tert-Butyldimethylsilyl)oxy]-2-methylene-25-[(tert-butyldimethyl-silyl)oxy]-19-nor-22(E)-ene-vitamin D_3 tert-butyldimethylsilyl ether (**28**)*

According to a general procedure the pure protected analog **28** (39.54 mg, 79% yield) was obtained from the phosphine oxide **26** (67 mg, 115 μmol), PhLi (1.8 M in di-*n*-butylether, 75 μL, 137 μmol) and the ketone **21** (26 mg, 66 μmol). UV (in hexane) λ_{max} 262.5, 253.0, 245.0 nm; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.21 and 5.83 (each 1H, each d, J = 11.1 Hz, 6- and 7-H), 5.38 (1H, ddd, J = 15.4, 8.4, 6.8 Hz, 23-H), 5.29 (1H, dd, J = 15.4, 8.7 Hz, 22-H), 4.97 and 4.92 (each 1H, each s, =CH_2), 4.42 (2H, m, 1β- and 3α-H), 2.81 (1H, dm, J = 13.1 Hz, 9β-H), 2.52 (1H, dd, J = 13.2, 5.8 Hz, 10α-H), 2.46 (1H, dd, J = 12.7, 4.3 Hz, 4α-H), 2.33 (1H, dd, J = 13.2, 2.4 Hz, 10β-H), 2.17 (1H, dd, J = 12.7, 8.4 Hz, 4β-H), 1.16 (6H, s, 26,27-H_6), 0.93 (3H, d, J = 6.4 Hz, 21-H_3), 0.895 (9H, s, Si-*t*-Bu), 0.865 (9H, s, Si-*t*-Bu), 0.855 (9H, s, Si-*t*-Bu), 0.518 (3H, s, 18-H_3), 0.077 (3H, s, SiMe), 0.066 (9H, s, 3× SiMe), 0.047 (3H, s, SiMe), 0.025 (3H, s, SiMe); ^{13}C NMR (100 MHz) δ 152.99, 141.32, 139.40, 132.63, 124.25, 122.42, 116.00, 106.24, 73.78, 72.53, 71.63, 56.67, 56.20, 48.29, 47.61, 45.76, 40.91, 39.86, 38.55, 29.83, 29.62, 28.77, 27.41, 25.84, 25.78, 23.23, 22.11, 21.57, 18.25, 18.16, 18.07, 12.11, -2.04, −4.86, −4.90, −5.10; exact mass calculated for $C_{45}H_{84}O_3Si_3Na$ (MNa^+) 779.5626, found 779.5651.

*2.2.25. (20R,25R)-1α-[(tert-Butyldimethylsilyl)oxy]-2-methylene-25-[(tert-butyldimethyl-silyl)oxy]-19,26-dinor-22-(E)-ene-vitamin D_3 tert-butyldimethylsilyl ether (**29a**)*

According to a general procedure the pure protected analog **29a** (8 mg, 48% yield) was obtained from the phosphine oxide **26** (41 mg, 70 μmol), PhLi (1.8 M in di-*n*-butylether, 48 μL, 86 μmol) and the ketone **24a** (9 mg, 23 μmol). For analytical purpose a sample of the protected vitamin **29a** was further purified by HPLC (9.4 × 250 mm Zorbax Sil column, 4 mL/min, hexane/2-propanol (99.9:0.1) solvent system, R_t = 3.70 min); UV (in hexane) λ_{max} 263.1, 253.2, 244.3 nm; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.23 and 5.83 (each 1H, each d, J = 11.7 Hz, 6- and 7-H), 5.40–5.24 (2H, m, 22-H and 23-H), 4.97 and 4.93 (each 1H, each s, =CH_2), 4.40 (2H, m, 1β- and 3α-H), 3.78 (1H, m, 25-H), 2.78 (1H, dm, J = 12.1 Hz, 9β-H), 2.52 (1H, dd, J = 13.5, 6.1 Hz, 10α-H), 2.48 (1H, dd, J = 12.7, 4.5 Hz, 4α-H), 2.34 (1H, dd, J = 13.5, 6.3 Hz, 10β-H), 2.18 (1H, dd, J = 12.7, 8.6 Hz, 4β-H), 1.11 (3H, d, J = 6.3 Hz, 27-H_3), 0.96 (3H, d, J = 6.3 Hz, 21-H_3), 0.897 (9H, s, Si-*t*-Bu), 0.895 (9H, s, Si-*t*-Bu), 0.867 (9H, s, Si-*t*-Bu), 0.56 (3H, s, 18-H_3), 0.081 (3H, s, SiMe), 0.067 (3H, s, SiMe), 0.055 (9H, s, 3xSiMe), 0.027 (3H, s, SiMe); ^{13}C NMR (100 MHz) δ 152.96, 141.13, 138.98, 132.76, 124.74, 122.40, 116.09, 106.25, 72.54, 71.63, 68.73, 56.68, 56.33, 47.59, 45.57, 38.55, 36.13, 35.98, 28.76, 27.73, 26.11, 25.85, 25.72, 23.62, 23.46, 22.18, 20.70, 18.77, 18.25, 18.17, 12.24, −4.34, −4.62, −4.85, −4.87, −5.10; exact mass calculated for $C_{44}H_{82}O_3Si_3$-Na $(MNa)^+$ 765.5470, found 765.5456.

*2.2.26. (20R,25S)-1α-[(tert-Butyldimethylsilyl)oxy]-2-methylene-25-[(tert-butyldimethyl-silyl)oxy]-19,26-dinor-22-(E)-ene-vitamin D_3 tert-butyldimethylsilyl ether (**29b**)*

According to a general procedure the pure protected analog **29b** (15 mg, 48% yield) was obtained from the phosphine oxide **26** (73 mg, 127 μmol), PhLi (1.8 M in di-*n*-butylether, 85 μL, 180 μmol) and the ketone **24b** (16 mg, 42 μmol). For analytical purpose a sample of the protected vitamin **29b** was further purified by HPLC (9.4×250 mm Zorbax Sil column, 4 mL/min, hexane/2-propanol (99.9:0.1) solvent system, R_t = 3.80 min); UV (in hexane) λ_{max} 263.1, 253.2, 244.3 nm; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.22 and 5.83 (each 1H, each d, J = 11.2 Hz, 6- and 7-H), 5.38–5.27 (2H, m, 22-H and 23-H), 4.96 and 4.90 (each 1H, each s, =CH_2), 4.43 (2H, m, 1β- and 3α-H), 3.78 (1H, m, 25-H), 2.82 (1H, dm, J = 11.8 Hz, 9β-H), 2.52 (1H, dd, J = 13.1, 5.9 Hz, 10α-H), 2.47 (1H, dd, J = 12.6, 4.3 Hz, 4α-H), 2.33 (1H, dd, J = 13.1, 2.3 Hz, 10β-H), 2.17 (1H, dd, J = 12.6, 8.7 Hz, 4β-H), 1.11 (3H, d, J = 6.0 Hz, 27-H_3), 1.02 (3H, d, J = 6.5 Hz, 21-H_3), 0.897 (9H, s, Si-*t*-Bu), 0.895 (9H, s, Si-*t*-Bu), 0.867 (9H, s, Si-*t*-Bu), 0.56 (3H, s, 18-H_3), 0.081 (3H, s, SiMe), 0.067 (3H, s, SiMe), 0.055 (9H, s, 3× SiMe), 0.027 (3H, s, SiMe); ^{13}C NMR (100 MHz) δ 152.96, 141.13, 138.98, 132.76, 124.74, 122.40, 116.09, 106.25, 72.54, 71.63, 68.73, 56.33, 47.59, 45.57, 42.97, 40.47, 38.55, 36.13, 35.98, 28.76, 27.73, 26.11, 25.85, 25.22, 23.42, 23.30, 22.18, 20.70, 18.25, 18.17, 12.24, −4.34, −4.62, −4.87, −5.10; exact mass calcd for $C_{44}H_{82}O_3Si_3Na$ $(MNa)^+$ 765.5470, found 765.5439.

*2.2.27. (20S,25R)-1α-[(tert-Butyldimethylsilyl)oxy]-2-methylene-25-[(tert-butyldimethyl-silyl)oxy]-19,26-dinor-22-(E)-ene-vitamin D_3 tert-butyldimethylsilyl ether (**30a**)*

According to a general procedure the pure product **30a** (6 mg, 48% yield) was obtained from the phosphine oxide **26** (25 mg, 43 μmol), PhLi (1.8 M in di-*n*-butylether, 34 μL, 61 μmol) and the ketone **25a** (6.5 mg, 17 μmol). For analytical purpose a sample of the protected vitamin **30a** was further purified by HPLC (9.4 × 250 mm Zorbax Sil column, 4 mL/min, hexane/2-propanol (99.9:0.1) solvent system, R_t = 3.70 min): UV (in hexane) λ_{max} 262.6, 253.0, 244.8 nm; ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.22 and 5.84 (each 1H, each d, J = 11.2 Hz, 6- and 7-H), 5.38–5.27 (2H, m, 22-H and 23-H), 4.97 and 4.91 (each 1H, each s, =CH_2), 4.43 (2H, m, 1β- and 3α-H), 3.77 (1H, m, 25-H), 2.83 (1H, dm, J = 12.6 Hz, 9β-H), 2.52 (1H, dd, J = 13.2, 6.0 Hz, 10α-H), 2.46 (1H, dd, J = 12.6, 4.5 Hz, 4α-H), 2.33 (1H, dd, J = 13.2, 2.9 Hz, 10β-H), 2.18 (1H, dd, J = 12.6, 8.3 Hz, 4β-H), 1.12 (3H, d, J = 6.0 Hz, 27-H_3), 0.898 (9H, s, Si-*t*-Bu), 0.892 (9H, s, Si-*t*-Bu), 0.867 (9H, s, Si-*t*-Bu), 0.84 (3H, d, J = 6.5 Hz, 21-H_3), 0.54 (3H, s, 18-H_3), 0.082 (3H, s, SiMe), 0.067 (3H, s, SiMe), 0.052 (9H, s, 3× SiMe), 0.027 (3H, s, SiMe); ^{13}C NMR (125 MHz) δ 152.98, 141.22, 138.98, 132.74, 124.74, 122.40, 116.11, 106.25, 72.53, 71.65, 68.74, 56.62, 56.19, 47.61, 45.67, 38.57, 36.13, 35.92, 28.76, 27.37, 26.13, 25.84, 25.78, 23.67, 23.45, 22.32, 20.80, 18.76, 18.25, 18.17, 12.23, −4.38, −4.71, −4.87, −5.09; exact mass calculated for $C_{44}H_{82}O_3Si_3Na$ $(MNa)^+$ 765.5468, found 765.5461.

*2.2.28. (20S,25S)-1α-[(tert-Butyldimethylsilyl)oxy]-2-methylene-25-[(tert-butyldimethyl-silyl)oxy]-19,26-dinor-22-(E)-ene-vitamin D_3 tert-butyldimethylsilyl (**30b**)*

According to a general procedure the pure product **30b** (10 mg, 46% yield) was obtained from the phosphine oxide **26** (52 mg, 89 μmol), PhLi (1.8 M in di-*n*-butylether, 61 μL, 110 μmol) and the ketone **25b** (9 mg, 24 μmol). For analytical purpose a sample of the protected vitamin **30b** was further purified by HPLC (9.4 × 250 mm Zorbax Sil column, 4 mL/min, hexane/2-propanol (99.9:0.1) solvent system, R_t = 3.51 min): UV (in hexane) λ_{max} 263.1, 253.2, 244.3 nm; ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.22 and 5.83 (each 1H, each d, J = 11.2 Hz, 6- and 7-H), 5.38–5.27 (2H, m, 22-H and 23-H), 4.96 and 4.90 (each 1H, each s, =CH_2), 4.43 (2H, m, 1β- and 3α-H), 3.78 (1H, m, 25-H), 2.85 (1H, dm, J = 12.6 Hz, 9β-H), 2.52 (1H, dd, J = 13.2, 6.0 Hz, 10α-H), 2.47 (1H, dd, J = 12.6, 4.5 Hz, 4α-H), 2.33 (1H, dd, J = 13.2, 2.9 Hz, 10β-H), 2.18 (1H, dd, J = 12.6, 8.5 Hz, 4β-H)1.11 (3H, d, J = 6.0 Hz, 27-H_3), 1.02 (3H, d, J = 6.5 Hz, 21-H_3), 0.898 (9H, s, Si-*t*-Bu), 0.895 (9H, s, Si-*t*-Bu), 0.867 (9H, s, Si-*t*-Bu), 0.52 (3H, s, 18-H_3), 0.082 (3H, s, SiMe), 0.067 (3H, s, SiMe), 0.055 (9H, s, 3× SiMe), 0.027 (3H, s, SiMe); ^{13}C NMR (125 MHz) δ 152.96, 141.13, 138.98, 132.76, 124.74, 122.40, 116.09, 106.25, 72.54, 71.63, 68.73, 56.63, 56.29, 47.61, 45.67, 40.61, 40.24, 38.55, 36.13, 35.98, 28.76, 27.73, 25.93, 25.85, 25.78, 23.89, 23.45, 22.33, 22.22, 18.77, 18.25, 18.17, 12.06, −4.37, −4.66, −4.86, −5.09; exact mass calculated for $C_{44}H_{82}O_3Si_3Na$ $(MNa)^+$ 765.5468, found 765.5461.

*2.2.29. General procedure for the synthesis of compounds **6**, **7**, **8a**, **8b**, **9a**, **9b***

To a solution of the protected vitamin **27**, **28**, **29a**, **29b**, **30a** or **30b** in THF (2 mL) and acetonitrile (2 mL), a solution of aqueous 48% HF in acetonitrile (1:9 ratio, 2 mL) was added at 0 °C and the resulting mixture was stirred at room temperature for 6 h. The reaction was quenched with a saturated aqueous $NaHCO_3$ solution and extracted with ethyl acetate. Combined organic phases were washed with brine, dried over anhydrous Na_2SO_4, concentrated under reduced pressure. The residue was purified on a Waters silica Sep-Pak cartridge (10–30% ethyl acetate/hexane) to give the crude products. Final purification of the vitamin D compounds was performed by straight phase HPLC (15% 2-propanol/hexane; 4 mL/min; 9.4 mm × 25 cm Zorbax Sil column), and/or by reversed-phase HPLC (15% water/methanol; 3 mL/min; 9.4 mm × 25 cm Zorbax Eclipse XDB-C18 column) to give the analytically pure 19,26-dinorvitamin D analogs **6**, **7**, **8a**, **8b**, **9a** or **9b**.

*2.2.30. 2-Methylene-19-nor-22(E)-ene-1α,25-dihydroxyvitamin D_3 (**6**)*

According to a general procedure the pure 2-methylene analog **6** (12.24 mg, 64% yield) was obtained from the protected vitamin **27** (35.05 mg, 46 μmol). The vitamin **6** was further purified by straight phase HPLC [R_t = 6.66 min.] and then by reverse phase HPLC [R_t = 11.53 min.], as it's described in a general procedure. UV (in EtOH) λ_{max} 261.5, 252.5, 244.5 nm; ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.36 and 5.88 (1H and 1H, each d, J = 11.2 Hz, 6-H and 7-H), 5.39 (2H, m, 22,23-H_2), 5.11 and 5.09 (each 1H, each s, =CH_2), 4.48 (2H, m, 1β- and 3α-H), 2.85 (1H, dd, J = 12.8, 4.3 Hz, 10β-H), 2.82 (1H, br d, J = 11.9 Hz, 9β-H), 2.57 (1H, dd, J = 13.3, 3.2 Hz, 4α-H), 2.33 (1H, dd, J = 13.3, 6.0 Hz, 4β-H), 2.29 (1H, dd, J = 12.8, 8.6 Hz, 10α-H), 1.20 (6H, s, 26,27-H_6), 1.04 (3H, d, J = 6.6 Hz, 21-H_3), 0.570 (3H, s, 18-H_3); ^{13}C NMR (125 MHz) δ 151.95, 143.13, 141.60, 130.53, 124.15, 122.74, 115.37, 107.72, 71.78, 70.62, 70.55, 56.33, 56.06, 46.84, 45.75, 45.69, 40.54, 40.30, 38.13, 29.02, 28.90, 28.04, 23.44, 22.28, 20.86, 12.30; exact mass calculated for $C_{27}H_{42}O_3$ (M^+) 414.3134, found 414.3135.

*2.2.31. (20S,22E)-2-Methylene-19-nor-22-ene-1α,25-dihydroxyvitamin D_3 (**7**)*

According to a general procedure the pure 2-methylene analog **7** (12.74 mg, 59% yield) was obtained from the protected vitamin **28** (39.44 mg, 52 μmol). The vitamin **7** was further purified by straight phase HPLC [R_t = 6.46 min] and then by reverse phase HPLC [R_t = 10.19 min], as it's described in a general procedure. UV (in EtOH) λ_{max} 261.0, 252.0, 244.5 nm; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.35 and 5.88 (1H and 1H, each d, J = 11.2 Hz, 6- and 7-H), 5.44 (2H, m, 22-H and 23-H), 5.11 and 5.09 (each 1H, each s, =CH_2), 4.48 (2H, m, 1β- and 3α-H), 2.84 (1H, dd, J = 13.3, 4.4 Hz, 10β-H), 2.80 (1H, br d, J = 14.2 Hz, 9β-H), 2.56 (1H, dd, J = 13.4, 3.6 Hz, 4α-H), 2.32 (1H, dd, J = 13.4, 6.0 Hz, 4β-H), 2.28 (1H, dd, J = 13.3, 8.4 Hz, 10α-H), 1.20 (6H, d, J = 1.2 Hz, 26,27-H_6), 0.95

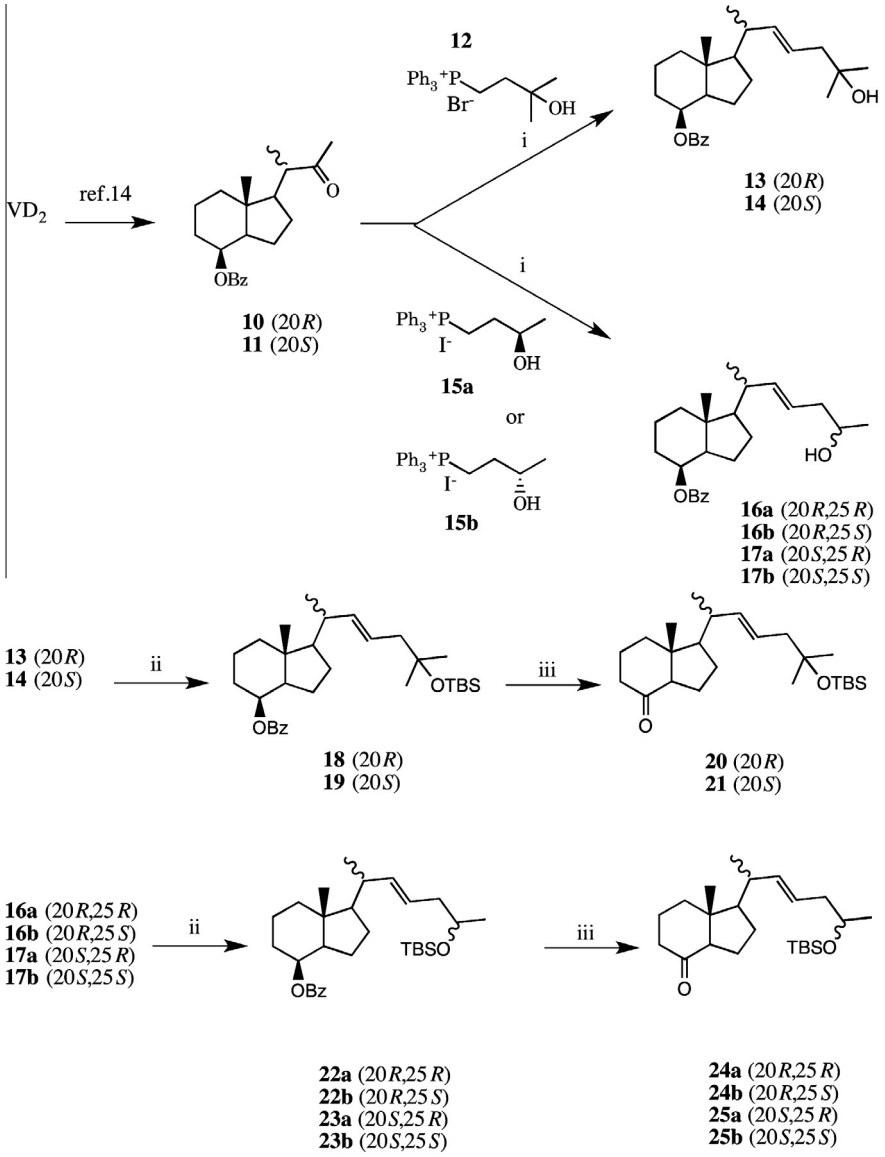
(3H, d, J = 6.6 Hz, 21-H_3), 0.528 (3H, s, 18-H_3); ^{13}C NMR (100 MHz) δ 151.96, 143.27, 141.71, 130.45, 124.14, 122.64, 115.26, 107.69, 71.76, 70.75, 70.60, 56.54, 56.13, 46.90, 45.80, 45.74, 40.72, 39.80, 38.11, 29.11, 29.05, 28.91, 27.26, 23.24, 22.09, 21.61, 12.28; exact mass calculated for $C_{27}H_{42}O_3$ (M^+) 414.3134, found 414.3142.

2.2.32. (20R,25R)-2-Methylene-19,26-dinor-22-(E)-ene-1α,25-dihydroxyvitamin D_3 (**8a**)

According to a general procedure the pure 2-methylene analog **8a** (1.6 mg, 44% yield) was obtained from the protected vitamin **29a** (7 mg, 9 μmol). The final compound **8a** was purified by reverse-phase HPLC (R_t = 13.7 min) as it's described in a general procedure. UV (in EtOH) λ_{max} 261.4, 252.4, 244.4 nm; ^{1}H NMR (900 MHz, $CDCl_3$) δ 6.35 and 5.87 (1H and 1H, each d, J = 10.8 Hz, 6- and 7-H), 5.40–5.38 (1H, m, 22-H), 5.34–5.31 (1H, m, 23-H), 5.10 and 5.08 (each 1H, each s, =CH_2), 4.47 (2H, m, 1β- and 3α-H), 3.78 (1H, m, 25-H), 2.84 (1H, dd, J = 13.3, 5.4 Hz, 10β-H), 2.81 (1H, br d, J = 13.5 Hz, 9β-H), 2.56 (1H, dd, J = 13.5, 3.6 Hz, 4α-H), 2.32 (1H, dd, J = 13.5, 5.4 Hz, 4β-H), 2.28 (1H, dd, J = 13.3, 8.1 Hz, 10α-H), 1.17 (3H, d, J = 6.3 Hz, 27-H_3), 1.03 (3H, d, J = 6.3 Hz, 21-H_3), 0.55 (3H, s, 18-H_3); exact mass calcd for $C_{26}H_{40}ONa$ (MNa^+) 423.2875, found 423.2873.

2.2.33. (20R,25S)-2-Methylene-19,26-dinor-22-(E)-ene-1α,25-dihydroxyvitamin D_3 (**8b**)

According to a general procedure the pure 2-methylene analog **8b** (4 mg, 54% yield) was obtained from the protected vitamin **29b** (15 mg, 34 μmol). The final compound **8b** was purified by straight-phase HPLC (R_t = 9.3 min) and then by reverse-phase HPLC (R_t = 12.9 min) as it's described in a general procedure. UV (in EtOH) λ_{max} 262.1, 252.6, 244.1 nm; ^{1}H NMR (800 MHz, $CDCl_3$) δ 6.35 and 5.88 (1H and 1H, each d, J = 11.2 Hz, 6- and 7-H), 5.41–5.32 (2H, m, 22-H and 23-H), 5.11 and 5.09 (each 1H, each s, =CH_2), 4.47 (2H, m, 1β- and 3α-H), 3.75 (1H, m, 25-H), 2.83 (1H, dd, J = 13.3, 4.5 Hz, 10β-H), 2.81 (1H, br d, J = 13.2 Hz, 9β-H), 2.57 (1H, dd, J = 13.4, 3.7 Hz, 4α-H), 2.33 (1H, dd, J = 13.4, 6.1 Hz, 4β-H), 2.29 (1H, dd, J = 13.3, 8.3 Hz, 10α-H),1.19 (3H, d, J = 6.2 Hz, 27-H_3), 1.03 (3H, d, J = 6.4 Hz, 21-H_3), 0.55(3H, s, 18-H_3); exact mass calculated for $C_{26}H_{40}O_3Na^+$ (MNa^+) 423.2875, found 423.2874.



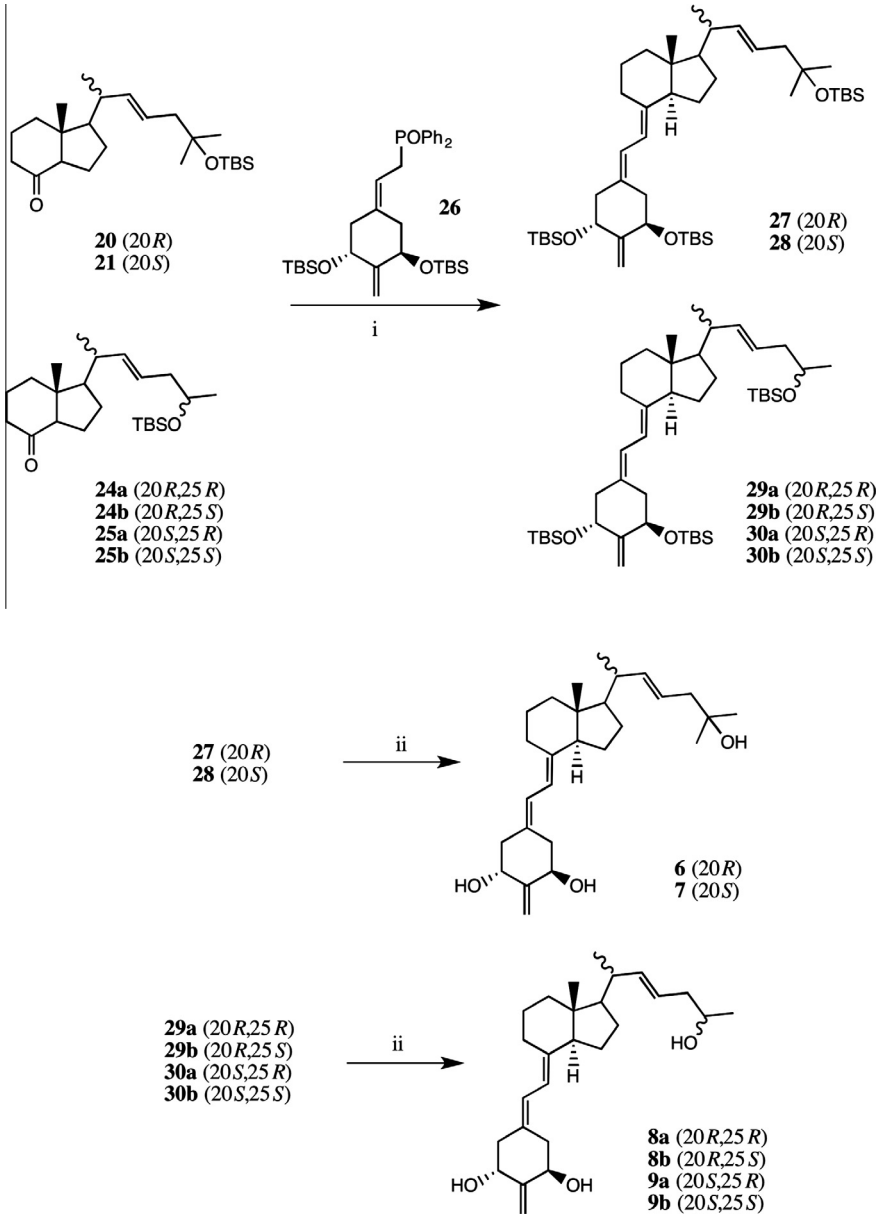
Scheme 1. Reagents: (i) *n*-*Bu*Li, THF; (ii) TBSOTf,2,6-lutine, CH_2CL_2; (iii) (1) KOH, EtOH; (2) PDC CH_2CL_2.

*2.2.34. (20S,25R)-2-Methylene-19,26-dinor-22-(E)-ene-1α,25-dihydroxyvitamin D_3 (**9a**)*

According to a general procedure the pure 2-methylene analog **9a** (1 mg, 43% yield) was obtained from the protected vitamin **30a** (4.5 mg, 6 μmol). The final compound **9a** was purified by reverse-phase HPLC (R_t = 11.8 min) as it's described in a general procedure. UV (in EtOH) λ_{max} 262.1, 252.6, 244.1 nm; ^{1}H NMR (900 MHz, $CDCl_3$) δ 6.28 and 5.81 (each 1H, each d, J = 11.7 Hz, 6- and 7-H), 5.38–5.25 (2H, m, 22-H and 23-H), 5.04 and 5.02 (each 1H, each s, $=CH_2$), 4.40 (2H, m, 1β- and 3α-H), 3.76 (1H, m, 25-H), 2.78 (1H, dd, J = 13.1, 4.5 Hz, 10β-H), 2.73 (1H, br d, J = 13.5 Hz, 9β-H), 2.51 (1H, dd, J = 13.5, 4.5 Hz, 4α-H), 2.27 (1H, dd, J = 13.5, 6.3 Hz, 4β-H), 2.22 (1H, dd, J = 13.1, 8.1 Hz, 10α-H), 1.11 (3H, d, J = 6.3 Hz, 27-H_3), 0.87 (3H, d, J = 6.3 Hz, 21-H_3), 0.45 (3H, s, 18-H_3); exact mass calculated for $C_{26}H_{40}O_3Na^+$ (MNa^+) 423.2875, found 423.2881.

*2.2.35. (20S,25S)-2-Methylene-19,26-dinor-22-(E)-ene-1α,25-dihydroxyvitamin D_3 (**9b**)*

According to a general procedure the pure 2-methylene analog **9b** (1.3 mg, 36% yield) was obtained from the protected vitamin **30b** (7 mg, 9 μmol). The final compound **9b** was purified by straight-phase HPLC (R_t = 9.3 min) and then by reverse-phase HPLC (R_t = 11.1 min) as it's described in a general procedure. UV (in EtOH) λ_{max} 262.1, 252.6, 244.1 nm; ^{1}H NMR (800 MHz, $CDCl_3$) δ 6.35 and 5.87 (1H and 1H, each d, J = 11.2 Hz, 6- and 7-H), 5.45–5.42 (1H, m, 22-H), 5.34–5.30 (1H, m, 23-H), 5.11 and 5.09 (each 1H, each s, $=CH_2$), 4.48 (2H, m, 1β- and 3α-H), 3.78 (1H, m, 25-H), 2.84 (1H, dd, J = 12.8, 4.8 Hz, 10β-H), 2.80 (1H, br d, J = 12.8 Hz, 9β-H), 2.57 (1H, dd, J = 12.8, 2.5 Hz, 4α-H), 2.32 (1H, dd, J = 12.8, 6.4 Hz, 4β-H), 2.29 (1H, dd, J = 12.8, 8.0 Hz, 10α-H), 1.19 (3H, d, J = 6.4 Hz, 27-H_3), 0.95 (3H, d, J = 6.4 Hz, 21-H_3), 0.52 (3H, s, 18-H_3); exact mass calcd for $C_{26}H_{40}O_3Na$ $(MNa)^+$ 423.2875, found 423.2870.



Scheme 2. Reagents: (i) PhLi, (ii) aq. HF, THF, MeCN.

Table 1
VDR binding properties,[a] HL-60 differentiating activities,[b] and transcriptional activities[c] of the vitamin D analogs **6–7**; **8a,b–9a,b**.

Compound	Compd no.	VDR binding[a]		HL-60 differentiation[b]		CYP24A1 transcription[c]	
		K_i (M)	Ratio	EC_{50} (M)	Ratio	EC_{50} (M)	Ratio
1α,25-$(OH)_2D_3$	1	1×10^{-10}	1	3×10^{-9}	1	2×10^{-10}	1
19-Nor-1α,25-dihydroxyvitaminD_2 (Zemplar)	2	1×10^{-10}	1	4×10^{-9}	1.3	3×10^{-10}	1.5
(20*S*)-2-Methylene-19-nor-1α,25-$(OH)_2D_3$ (2MD)	4	1×10^{-10}	1	8×10^{-11}	0.027	7×10^{-12}	0.035
(20*S*,25*R*)-2-Methylene-19,26-dinor-1α,25-$(OH)_2D_3$ (SR1)	5	9×10^{-11}	0.9	9×10^{-11}	0.03	1×10^{-11}	0.05
(20*R*)-2-Methylene-Δ^{22}*E*-19-nor-1α,25-$(OH)_2D_3$ (AT3)	6	6×10^{-11}	0.6	2×10^{-10}	0.07	2×10^{-11}	0.1
(20*S*)-2-methylene-Δ^{22}*E*-19-nor-1α,25-$(OH)_2D_3$ (N23)	7	6×10^{-11}	0.6	2×10^{-10}	0.07	2×10^{-11}	0.1
(20*R*,25*R*)-2-Methylene-Δ^{22}*E*-19,26-dinor-1α,25-dihydroxyvitamin D_3	8a	9×10^{-11}	0.9	2×10^{-10}	0.07	2×10^{-11}	0.1
(20*R*,25*S*)-2-Methylene-Δ^{22}*E*-19,26-dinor-1α,25-$(OH)_2D_3$	8b	3×10^{-10}	3	1×10^{-9}	0.33	1×10^{-10}	0.5
(20*S*,25*R*)-2-Methylene-Δ^{22}*E*-19,26-Dinor-1α,25-$(OH)_2D_3$	9a	8×10^{-11}	0.8	8×10^{-10}	0.27	1×10^{-10}	0.5
(20*S*,25*S*)-2-Methylene-Δ^{22}*E*-19,26-dinor-1α,25-$(OH)_2D_3$	9b	2×10^{-10}	2	1×10^{-9}	0.33	1×10^{-10}	0.5

[a] Competitive binding of 1α,25-$(OH)_2D_3$ (**1**) and the synthesized vitamin D analogs to the full-length recombinant rat vitamin D receptor. The K_i values are derived from dose–response curves and represent the inhibition constant when radiolabeled 1α,25-$(OH)_2D_3$ is present at 1 nM and a K_d of 0.2 nM is used. The binding ratio is the average ratio of the analog K_i to the K_i for 1α,25-$(OH)_2D_3$.

[b] Induction of differentiation of HL-60 promyelocytes to monocytes by 1α,25-$(OH)_2D_3$ and the synthesized vitamin D analogs. Differentiation state was determined by measuring the percentage of cells reducing nitro blue tetrazolium (NBT). The ED_{50} values are derived from dose–response curves and represent the analog concentration capable of inducing 50% maturation. The differentiation activity ratio is the average ratio of the analog ED_{50} to the ED_{50} for 1α,25-$(OH)_2D_3$.

[c] Transcriptional assay in rat osteosarcoma cells stably transfected with a CYP24A1 gene reporter plasmid. The ED_{50} values are derived from dose–response curves and represent the analog concentration capable of increasing the luciferase activity by 50%. The luciferase activity ratio is the average ratio of the ED_{50} for the analog to the ED_{50} for 1α,25-$(OH)_2D_3$. All the experiments were carried out in duplicate on at least two different occasions.

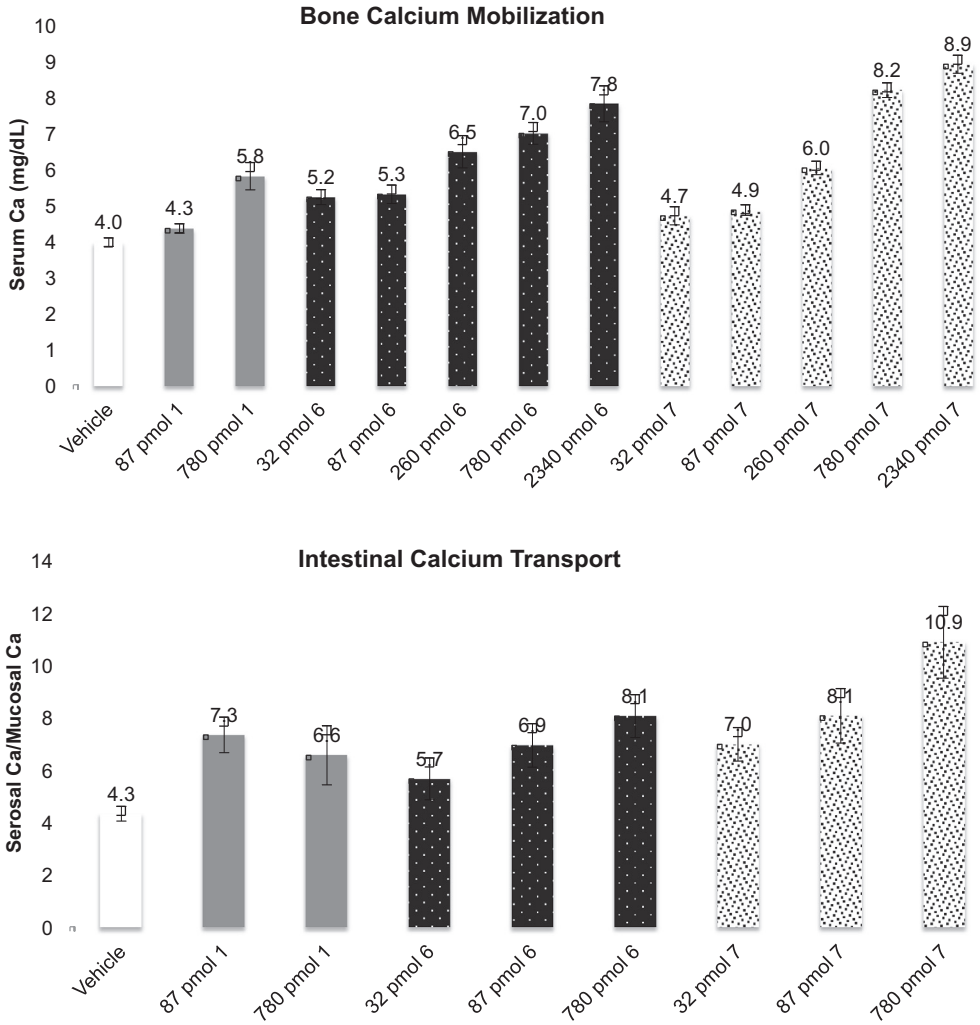


Fig. 2. Effects of 1,25$(OH)_2D_3$ (**1**) and compounds **6–7** on bone calcium mobilization and intestinal calcium transport (structures are shown in Figure 1).

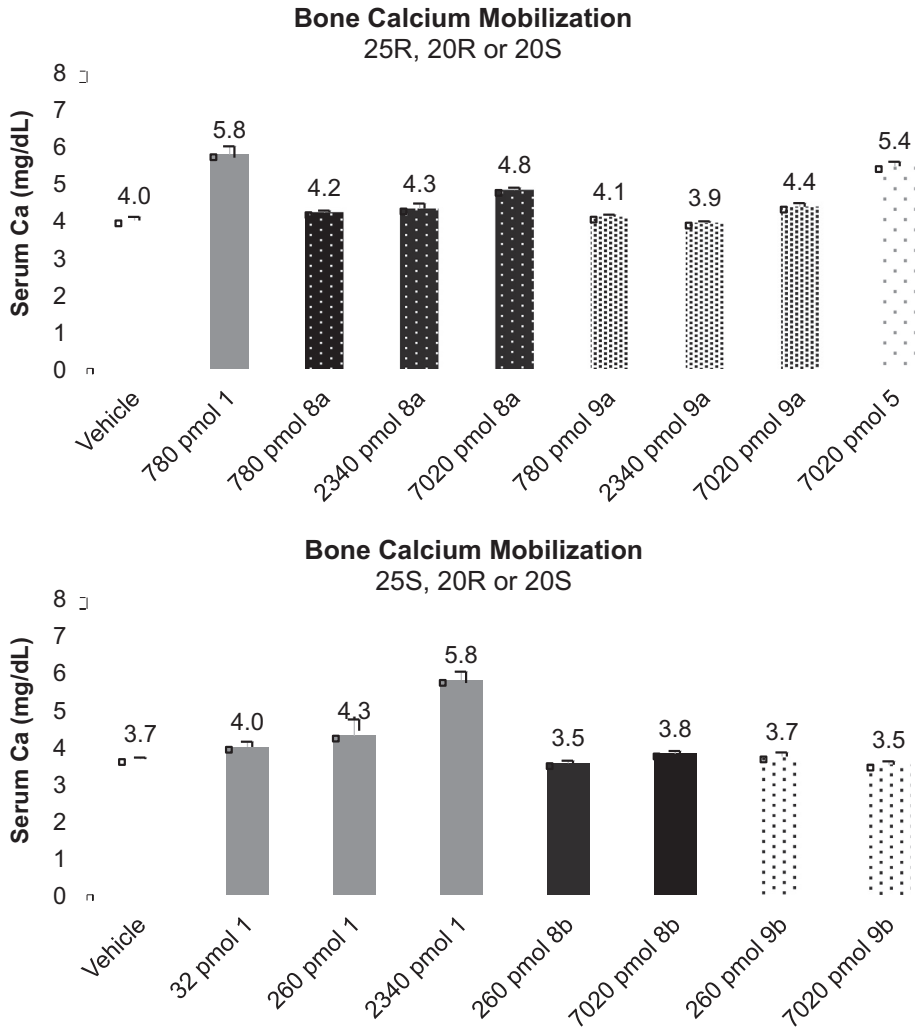


Fig. 3. Total serum calcium levels reflecting the ability of each analog to release bone calcium stores. Compounds 1, 5, 8a, 8b, 9a and 9b are shown in Figure 1.

2.3. Biological studies

2.3.1. In vitro studies

VDR binding, HL-60 differentiation and 24-hydroxylase transcription assays were performed as previously described [16,17].

2.3.2. In vivo studies

Bone calcium mobilization and intestinal calcium transport were performed as previously described [16,17]. Briefly, weanling rats were made vitamin D-deficient by housing under lighting conditions that block vitamin D production in the skin. In addition, the animals were fed a diet devoid of vitamin D. Experimental compounds were administered intraperitoneally once per day for four consecutive days. Twenty-four hours after the last dose was given, the blood was collected, and everted gut sacs were prepared. Calcium transport was measured ex vivo and bone calcium mobilization was carried out as previously described [16,17]. There were 5–6 animals in each group. The control animals received vehicle only, while positive control animals received the indicated dose of 1,25-$(OH)_2D_3$ in the vehicle.

3. Results and discussion

3.1. Chemistry

The synthesis strategy of the new 2-methylene-$\Delta^{22}E$-19-nor-1α,25$(OH)_2D_3$ compounds **6** (**20*R***) and **7** (**20*S***) and 2-methylene-$\Delta^{22}E$-19,26-dinor-1α,25$(OH)_2D_3$ compounds **8a**,**b**–**9a**,**b** was based on the Wittig–Horner olefination reaction [18] between Grundmann-type ketones (**20**–**21**; **24a**,**b**–**25a**,**b**) and the phosphine oxide **26** (Scheme 2). The A-ring fragment **26** was prepared according to the published procedure [15] whereas the syntheses of the necessary $\Delta^{22}E$-25-hydroxy C,D-ring ketones (**20**–**21**; **24a**,**b**–**25a**,**b**) are presented in Scheme 1. As recently reported by us [14], the Wittig reaction between the C,D-ring aldehydes **10** and **11**, previously prepared in our laboratory from commercial vitamin D_2 [19], and either the hydroxyphosphonium bromide **12** [20] or the hydroxyphosphonium iodides **15a** and **15b**, easily prepared in our laboratory from commercially available (*S*)- and (*R*)-1,3-butanediols [14,20], efficiently provided only the olefinic products with the *E*-geometry of the introduced double bond **13**–**14** and **16a**,**b**–**17a**,**b**, respectively [14,20]. Then, after protection of the tertiary hydroxyl groups as *tert*-butyldimethylsilyl ethers **18**–**19** and **22a**,**b**–**23a**,**b**, the removal of the benzoyl group under basic conditions gave the secondary alcohols, which were immediately subjected to oxidation with PDC affording the Grundmann ketones **20**–**21** and **24a**,**b**–**25a**,**b** in very good yields. As outlined in Scheme 2, each of the six $\Delta^{22}E$-25-hydroxy Grundmann ketones (**20**–**21**, **17a**,**b**–**18a**,**b**) was coupled with the anion, generated from phosphine oxide **26** and phenyllithium, affording the corresponding six protected 19-norvitamin D analogs (**27**–**28**, **29a**,**b**–**30a**,**b**). Then, after silyl groups removal using hydrofluoric acid the final 2-methylene-$\Delta^{22}E$-19-nor-1α,25$(OH)_2D_3$ (**6**–**7**) and 2-methylene-

$\Delta^{22}E$-19,26-dinor-1α,25(OH)$_2$D$_3$ (**8a**,**b**–**9a**,**b**) compounds were complete.

3.2. Biological activity

All compounds bound the receptor with very similar affinities. Only one of the analogs, compound **8b** (20*R*, 25*S*), had a slightly lower affinity (Table 1). The two 2-methylene-$\Delta^{22}E$-19-nor-1α,25(OH)$_2$D$_3$ compounds **6** and **7** exhibited approximately 10 times higher HL-60 differentiation activity as compared to the natural hormone **1** and 20 times higher than19-nor-1α,25-dihydroxyvitaminD$_2$ **2**. The 25*R* isomers **8a** and **9a** displayed higher cell differentiation activity as compared to the corresponding 25*S* isomers **8b** and **9b**, with isomer **8a** being the most potent of this series, having about 10 times more HL-60 differentiation potency as compared to the natural hormone **1**. As shown in Table 1, the 25*S* isomers **8b** and **9b** are equally potent in inducing cell differentiation and their efficacy is 3 times higher than that of the natural hormone **1** and 4 times higher than that of 19-nor-1α,25-dihydroxyvitaminD$_2$ **2**. Compound **8a** (20*R*, 25*R*) is the most potent of the 2-methylene-$\Delta^{22}E$-19,26-dinor-1α,25(OH)$_2$D$_3$ analogs, and its potency is comparable to that of **5**, **6** and **7**. The pattern of potencies in *in vitro* transcription assays are shown (Table 1). Similar to that observed in the HL60 cell differentiation assays, compounds **6**–**7**, and the (20*R*,25*R*) compound **8a**, express the highest transcriptional potency. They are more potent than both **2** and 1α,25(OH)$_2$D$_3$ (**1**), but less active than 2MD (**4**) and **5**. As shown in Table 1, the transcriptional activity of the 20*R*,25*R* isomer **8a** is about 20 times that of the corresponding 20*S*,25*R* isomer **9a**. Usually the 20-epimerization increases the transcriptional activity, and this result constitutes an exception to that pattern. On the other hand, the 20-epimerization does not affect the transcriptional activity of the 25*S* isomers **8b** and **9b**, whose activity is comparable to that of the natural hormone (**1**).

It is unclear why compounds **4** and **5** have more than 10X the activity of 1α,25(OH)$_2$D$_3$ in causing differentiation of HL-60 cells and CYP24A1 transcription while having similar activity in binding to the VDR. The former assays involve a cell culture assay in which 5% serum is present. The vitamin D binding protein (DBP) in serum binds 1α,25(OH)$_2$D$_3$ reducing the free 1α,25(OH)$_2$D$_3$. The compounds with a 20S configuration are bound poorly by the DBP. Thus, compounds **4**, **5**, **7**, **9a**, and **9b** would have a much higher free concentration resulting in high activity. Because this unexpected high activity did not occur in *in vivo* assays (Figs. 2–4), its

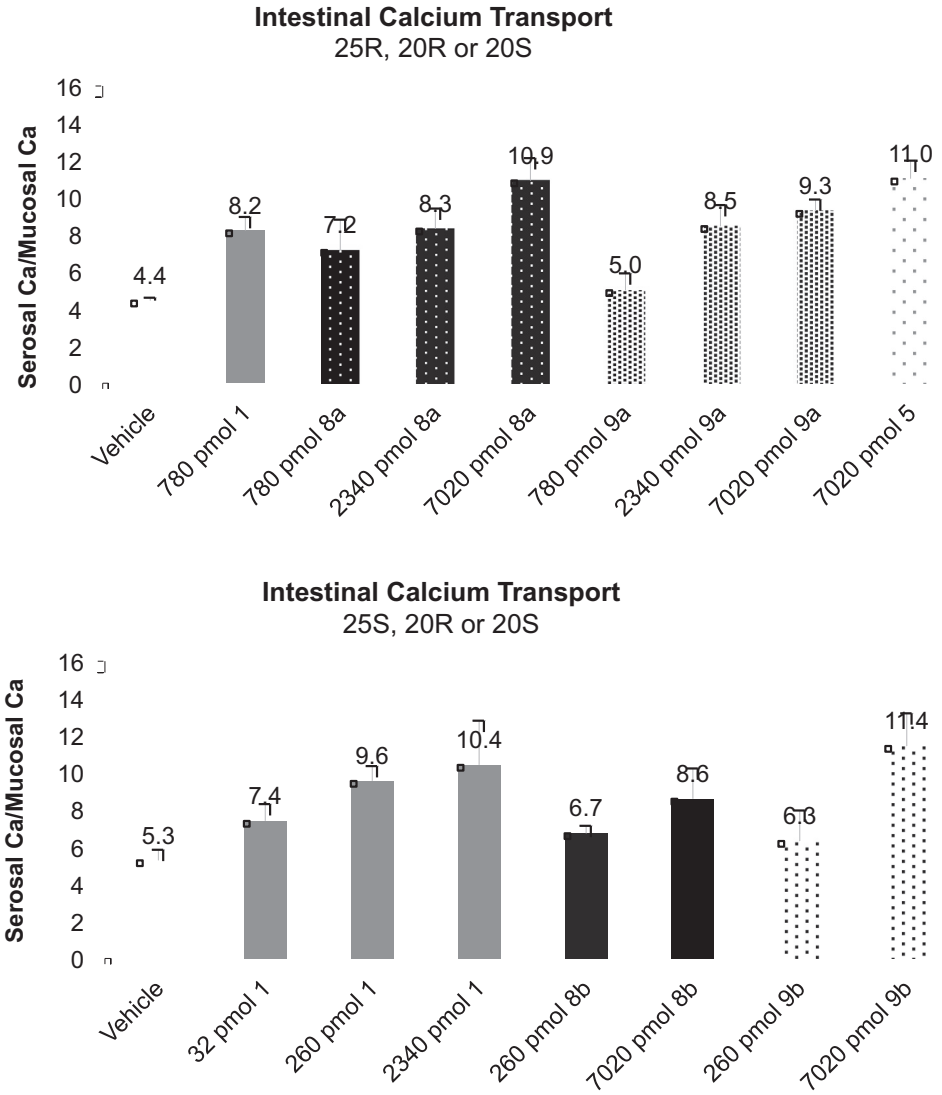


Fig. 4. *In vivo* intestinal calcium transport compared to the native hormone (**1**). Compounds 1, 5, 8a, 8b, 9a and 9b are shown in Figure 1.

significance is unlikely. Nevertheless, activity in HL-60 may represent anti-cancer activity, which is not assessed by the *in vivo* assays used in the present study.

In the present series[14], removal of the 26-methyl group has little impact on receptor binding (compounds **4** and **5**) and slightly reduces HL-60 differentiation (compounds **4** and **7**). A combination of a double bond at carbon 22 with removal of the 26-methyl results in a compound with one log less *in vitro* potency (compare compounds **4** and **9a**).

In vivo biological activities of compounds **6** and **7** are shown in Fig. 2. Consistent with the *in vitro* results, these two analogs show increased potency in bone calcium mobilization compared to the native hormone. However, introduction of a *trans* double bond between C-22 and C-23 resulted in significantly decreased activity in bone compared to 2MD (260 pmol 2MD will raise serum calcium by 5.3 mg/dL [12] compared to 2.5 mg/dL for compound **6** and 2.0 mg/dL for compound **7**). As shown in Figs. 3 and 4, removal of the 26-methyl group from 2-methylene-Δ^{22}*E*-19-nor-1α,25(OH)$_2$D$_3$ compounds **6** and **7** selectively reduced *in vivo* activity. In fact, while all the new 26-nor analogs **8a**,**b–9a**,**b** have virtually no bone calcium mobilization activity *in vivo* (Fig. 3) but retain calcium transport activity in the intestine (**1**) (Fig. 4). As shown in Fig. 3 the 25*S* isomers **8b** and **9b** are 200$\times$ times less potent on bone than 1α,25(OH)$_2$D$_3$ (**1**), and the 25*R* isomers **8a** and **9a** are at least 30 times less active in mobilizing calcium from bone. Thus, the *in vivo* results reaffirm the potency profile observed *in vitro*: coupling a double bond at C-22 with 26-methyl removal results in analogs with significantly lower potencies.

4. Conclusion

Removing the 26-methyl group from 2-methylene-22-ene-19-nor-1α,25-dihydroxyvitamin D$_3$ results in compounds that are selectively active on intestinal calcium transport. Additionally this activity is increased by a 20*S* configuration.

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