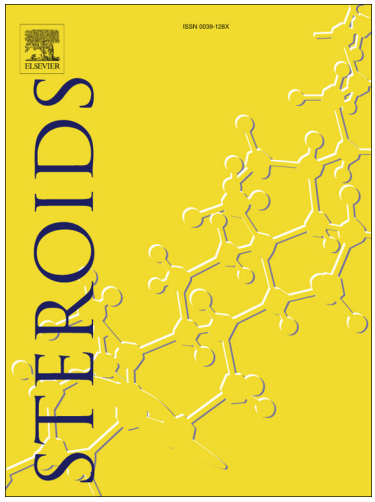

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Synthesis of novel vitamin D_3 analog with an additional ring annulated to A and *seco*-B rings

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Abstract

A simple method for the synthesis of yet unknown 5*E*-vitamin D_3 analogs with an additional six-membered ring connecting C-6 and C-19 was developed. Ring-closing metathesis (RCM) was used for efficient formation thereof from the corresponding 5*E*-isomers of 6-alkenyl vitamin D_3 compounds which in turn were prepared from the 6-oxo-3,5-cyclovitamin D_3. Reinvestigation of the Grignard reactions of this latter compound as well as the following acid-catalyzed cycloreversions showed discrepancies with the literature data describing the course of such processes.

Keywords: Vitamin D analogs; 5*E*-Vitamin D_3, B-*seco*-Steroids; 3,5-Cyclovitamin D_3; Ring-closing metathesis

1. Introduction

Since a long time, there is an established view that vitamin D_3 (**1**, Figure 1) undergoes two-step activation in the living organisms [1] by two sequential enzymatic hydroxylations occurring in the liver [1], and then in the kidney [2,3], resulting in the introduction of 25- and 1α-hydroxyl groups, respectively. The final product of these transformations, 1α,25-dihydroxyvitamin D_3 [**2**, 1α,25-$(OH)_2D_3$, calcitriol] is commonly considered as biologically active, hormonal form responsible for all actions associated with calcium and phosphorus homeostasis [4]. In addition to its well-known, classical role in mineral metabolism and bone growth, calcitriol exerts pleiotropic actions in multiple organs and tissues [5-7]. Of particular interest are its antiproliferative functions, which have stimulated numerous synthetic efforts to obtain the analogs characterized by selective biological activities [8]. Taking into account the potential risk of hypercalcemia, the main goal of these studies was to obtain low-calcemic compounds possessing elevated antiproliferative and differentiation activity against various types of malignant cells [9]. Among thousands of calcitriol analogs synthesized to the date and characterized by a wide array of structural changes [10], modifications of the intercyclic 5,7-diene fragment were introduced only into several compounds [11-15]. We have recently described the synthesis and biological evaluation of the series of 6-substituted analogs of 1α,25-dihydroxyvitamin D_3 and 1α,25-dihydroxy-19-norvitamin D_3 [16,17]. Particularly noteworthy is one of them, 1α,25-dihydroxy-6-methylvitamin D_3 (**3**), that binds the vitamin D receptor (VDR) very effectively. Taking into consideration the tendency of this analog to rearrange to its previtamin D form [16], we decided to direct our continued structure-activity studies to the respective 5*E*-isomers of 6-substituted vitamin D_3 compounds in which such a thermal sigmatropic reaction could not take place.

The 5*E*-configuration "transforms" the natural 3β-hydroxyl group into a *pseudo*-1α-OH group, that plays a crucial role in binding VDR. It has been established that an analog of the natural hormone, (5*E*)-1α,25-dihydroxyvitamin D_3 (**4**), in which the exocyclic methylene group is transposed, compared to **2**, from the right side to the left side of ring A, is still characterized by a good affinity to VDR [18]. Thus, the presence of the C(10)=C(19) methylene group, playing a role of a substituent at C-4, decreased the binding affinity of such modified analog *ca*. 8-fold; even a smaller difference in VDR affinities between the 5Z- and "unnatural" 5*E*-isomers (only 2-fold) was found in the case of 1α,25-dihydroxyvitamin D_2 [19]. Interestingly, analog **4** was less calcemic than **2** [18] but exerted similar inhibition of clonal proliferation of several tumor cells [20]. It was also established that configurational 5*Z*→5*E* modification combined with some other structural changes in the vitamin D molecule could result in analogs having unique activity profiles. (5*E*)-16-Dehydro-calcitriol (**5**) may serve as an example, being at least 40-fold less calcemic than **2**, nevertheless exerting antiproliferative activities against cancer cell lines (HL-60, MCF-7 and LNCaP) greater by two orders of magnitude [20]. Studies on the A-ring stereoisomers of 2-methyl-1α,25-dihydroxyvitamin D_3 proved that decreased VDR binding affinity of some 5*E*-isomers, especially of those characterized by side chain alterations, does not exclude their substantial cell differentiation-inducing potency [21].

Here, we would like to present the results of our work concerning the synthesis of the 6-substituted 5*E*- and 5Z-vitamin D_3 acetates **6**-**11**, as well as of the analog **12**, possessing an additional ring connecting C-6 and C-19.

2. Experimental

2.1. General

All reactions involving oxygen- or moisture-sensitive compounds were carried out under dry argon atmosphere. Reaction temperatures refer to external bath temperatures. Tetrahydrofuran (THF) was distilled from Na/benzophenone, whereas diethyl ether (Et_2O), dichloromethane (CH_2Cl_2) and toluene were distilled from P_2O_5. Liquid reagents or solutions of reagents were added *via* a syringe or a cannula. Reactions were monitored by thin-layer chromatography (TLC) using aluminum-backed MERCK 60 silica gel plates (0.2 mm thickness); the chromatograms were visualized first with ultraviolet light (254 nm) and then by immersion in a cerium-molybdenum solution [10 g $Ce(SO_4)_2 \times 4\ H_2O$, 25 g phosphomolybdic acid, 60 mL H_2SO_4 and 940 mL H_2O), followed by heating. Flash column chromatography was performed using MERCK Silica Gel 60 (230-400 mesh). High-performance liquid chromatography (HPLC) was performed on a Waters Associates liquid chromatograph equipped with a Model 486 tunable absorbance detector or Shimadzu UFLS liquid chromatograph equipped with SPD-20A tunable absorbance detector. Ultraviolet spectra were obtained on a Perkin-Elmer Lambda 3B UV-VIS spectrophotometer in 100% ethanol (EtOH).

All nuclear magnetic resonance spectra were recorded using Varian Unity plus 200 MHz, and Bruker DMX 500, using solutions in $CDCl_3$ and Me_4Si (δ 0.00) as an internal standard. To describe the signals in ^1H NMR spectra, the following abbreviations were used: s - singlet, d - doublet, t - triplet, q - quartet, b - broad, narr. – narrow, *w*/2 = half-width. High resolution mass spectra were recorded on LCT (TOF) or Mass Quattro LC spectrometers.

The starting 6-oxo-3,5-cyclovitamin D_3 (**13**) was prepared according to the published procedure [22].

*2.1.1. (6S)- and (6R)-6-Allyl-3,5-cyclovitamin D_3 (**14** and **15**)*

Allylmagnesium bromide (1 M in THF; 0.28 mL, 0.28 mmol) was added to a solution of the keto cyclovitamin **13** (44 mg, 0.115 mmol) in anhydrous THF (1 mL) and the mixture was stirred at room temperature for 1 h under argon. The reaction was quenched by addition of water, extracted with ethyl acetate, dried ($MgSO_4$), and evaporated. The residue was purified by flash chromatography on silica gel. Elution with hexane/Et_2O (9:1) gave a mixture of epimeric 6-hydroxy cyclovitamins **14** and **15** (ratio of 2:1; 47 mg, 97%) as a colorless oil.

14 and **15**: ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.537 and 0.585 (2H and 1H, each s, 18-H_3), 0.647 (1H, t, J = 4.4 Hz, one of 4-H_2), 0.868 (6H, d, J = 6.7 Hz, 26- and 27-H_3), 0.913 (3H, d, J = 6.2 Hz, 21-H_3), 3.19 and 3.28 (0.67H and 0.33H, each br d, J ~ 10 Hz), 4.9-5.2 (4H, m, 3'-H_2 and 19-H_2), 5.16 and 5.42 (0.67H and 0.33H, each br s, 7-H), 5.84 (1H, ddt, J = 17.5, 12.0, 7.4 Hz, 2'-H); HRMS (ESI) exact mass calculated for $C_{30}H_{48}ONa$ $(M + Na)^+$ 447.3603, measured 447.3609.

*2.1.2. (6S)- and (6R)-6-(But-3'-enyl)-3,5-cyclovitamin D_3 (**16** and **17**)*

4-Bromo-1-butene (0.38 mL, 3.60 mmol) was dropwise added to a vigorously stirred mixture of magnesium turnings (2.72 g, 3.60 mmol) in anhydrous THF (25 mL) under argon. When magnesium was dissolved, the resulted solution of the Grignard reagent was transferred to a solution of the keto cyclovitamin **13** (300 mg, 0.79 mmol) in anhydrous THF (10 mL) and the mixture was stirred at room temperature for 2.5 h. The reaction was quenched by addition of water and extracted with ethyl acetate, dried ($MgSO_4$), and evaporated. The residue was purified

by flash chromatography on silica gel. Elution with hexane/Et_2O (9:1) gave a mixture of isomers **16** and **17** (ratio of 5.5:1; 96%) as a colorless oil.

16 and **17**: ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.553 and 0.590 (2.54H and 0.46H, each s, 18-H_3), 0.641 (1H, br t, *J* = 4.4 Hz, one of 4-H_2), 0.851 and 0.883 (3H and 3H, each d, *J* = 6.6 Hz, 26- and 27-H_3), 0.918 (3H, d, *J* = 6.2 Hz, 21-H_3), 3.14 and 3.31 (0.85H and 0.15H, each br d, *J* ~ 10 Hz), 4.86-5.06 (4H, m, 4'-H_2 and 19-H_2), 5.18 and 5.36 (0.85H and 0.15H, each br s, 7-H), 5.84 (1H, ddt, *J* = 16.8, 10.1, 6.6 Hz, 3'-H); HRMS (ESI) exact mass calculated for $C_{31}H_{50}ONa$ $(M + Na)^+$ 461.3759, measured 461.3763.

*2.1.3. (6S)- and (6R)-6-(Pent-4'-enyl)-3,5-cyclovitamin D_3 (**18** and **19**)*

Reaction of the keto cyclovitamin **13** with 4-pentenylmagnesium bromide was carried out as described above for the preparation of **16** and **17**. The crude products were purified by flash chromatography on silica gel. Elution with hexane/Et_2O (9:1) gave epimeric alcohols **18** and **19** (ratio of 6.1:1; 97%) as a colorless oil.

18 and **19**: ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.545 and 0.584 (2.58H and 0.42, each s, 18-H_3), 0.625 (1H, br t, *J* = 4.4 Hz, one of 4-H_2), 0.863 (6H, d, *J* = 6.6 Hz, 26- and 27-H_3), 0.913 (3H, d, *J* = 6.0 Hz, 21-H_3), 3.13 and 3.25 (0.86H and 0.14H, each br d, *J* ~ 10 Hz), 4.86-5.06 (4H, m, 5'-H_2 and 19-H_2), 5.16 and 5.35 (0.86H and 0.14H, each br s, 7-H), 5.80 (1H, ddt, *J* = 17.0, 10.2, 6.5 Hz, 4'-H); HRMS (ESI) exact mass calculated for $C_{32}H_{52}ONa$ $(M + Na)^+$ 475.3916, measured 475.3932.

*2.1.4. (5E)- and (5Z)-6-Allyl-vitamin D_3 acetate (**6** and **7**)*

A solution of the cyclovitamins **14** and **15** (45 mg, 0.102 mmol) in glacial acetic acid (1.7 mL) was heated at 60 °C for 25 min, cooled to room temperature, quenched by slow addition of

ice-cold aqueous solution of $NaHCO_3$ and extracted with Et_2O. The organic layers were washed with saturated $NaHCO_3$ and brine, dried ($MgSO_4$), and evaporated. The residue was purified by flash chromatography on silica gel. Elution with hexane/Et_2O (98:2) gave an oily mixture of isomeric vitamins **6** and **7** (ratio of 1.5:1; 48 mg, 98%) which were separated by HPLC (9.4 mm × 25 cm Zorbax-Sil column, 4 mL/min) using hexane/ethyl acetate (99:1). The vitamins **6** and **7** were collected at Rv 35 mL and Rv 47 mL, respectively.

6: $[\alpha]^{24}_D$ +42° (*c* 0.38, $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.581 (3H, s, 18-H_3), 0.868 and 0.872 (3H and 3H, each d, *J* = 6.6 Hz, 26- and 27-H_3), 0.920 (3H, d, *J* = 6.0 Hz, 21-H_3), 2.01 (3H, s, $OCOCH_3$), 2.59 (1H, dd, *J* = 13.4, 4.2 Hz, 4α-H), 2.98 (2H, m, 1'-H_2), 4.81 (1H, d, *J* = 2.6 Hz, one of 19-H_2), 4.88 (1H, m, 3α-H), 4.90-5.02 (3H, m, 3'-H_2 and one of 19-H_2), 5.28 (1H, br s, 7-H), 5.74 (1H, ddt, *J* = 16.8, 10.2, 6.5 Hz, 2'-H); ^{13}C NMR (50 MHz) δ 12.32 (C-18), 19.18 (C-21), 21.53 ($OCO\underline{C}H_3$), 22.83, 23.16 (C-26 and C-27), 24.24, 28.01, 28.35, 30.24, 32.97, 33.42, 33.62, 33.80, 36.50, 38.14, 39.82, 40.63, 44.95, 55.88, 56.93, 72.21, 111.02, 114.40, 120.22, 132.03, 133.96, 139.12, 142.47, 147.09, 170.97 ($O\underline{C}OCH_3$); HRMS (ESI) exact mass calculated for $C_{32}H_{50}O_2Na$ $(M + Na)^+$ 489.3709, measured 489.3703.

7: $[\alpha]^{24}_D$ +26° (*c* 0.29, $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.534 (3H, s, 18-H_3), 0.866 (6H, d, *J* = 6.6 Hz, 26- and 27-H_3), 0.907 (3H, d, *J* = 6.2 Hz, 21-H_3), 2.03 (3H, s, $OCOCH_3$), 2.59 (1H, dd, *J* = 14.0, 4.0 Hz, 4α-H), 2.86 (2H, m, 1'-H_2), 4.70 and 4.87 (1H and 1H, each br d, *J* = 2.4 Hz, 19-H_2), 4.90-5.05 (3H, m, 3α- and 3'-H_2), 5.42 (1H, br s, 7-H), 5.74 (1H, ddt, *J* = 16.8, 10.2, 6.5 Hz, 2'-H); ^{13}C NMR (50 MHz) δ 12.34 (C-18), 19.04 (C-21), 21.60 ($OCO\underline{C}H_3$), 22.73, 22.98 and 23.05 (C-26 and C-27), 24.08, 27.89, 28.19, 30.28, 32.05, 32.43, 32.80, 33.10, 36.07, 36.31, 39.69, 40.70, 46.23, 55.75, 56.73, 72.28, 112.96, 114.57, 121.95, 131.71, 133.59, 139.45,

139.58, 146.72, 170.76 (O<u>C</u>OCH$_3$); HRMS (ESI) exact mass calculated for $C_{32}H_{50}O_2Na$ (M + Na)$^+$ 489.3709, measured 489.3705.

*2.1.5. (5E)- and (5Z)-6-(But-3'-enyl)-vitamin D$_3$ acetate (**8** and **9**)*

Solvolysis of the isomeric compounds **16** and **17** with glacial acetic acid was performed as described above for the cyclovitamins **14** and **15**. The crude products were purified by flash chromatography on silica. Elution with hexane/Et$_2$O (98:2) gave a mixture of vitamins **8** and **9** as a colorless oil (ratio of 2.5:1; 99% yield). Isomeric products were separated by HPLC (9.4 mm × 25 cm Zorbax-Sil column, 4 mL/min) using hexane/ethyl acetate (99:1). The vitamins **8** and **9** were collected at Rv 31 mL and Rv 38 mL, respectively.

8: $[\alpha]^{24}_D$ +39^o (*c* 1.30, CHCl$_3$); ^{1}H NMR (200 MHz, CDCl$_3$) δ 0.598 (3H, s, 18-H$_3$), 0.867 and 0.870 (3H and 3H, each d, *J* = 6.6 Hz, 26- and 27-H$_3$), 0.925 (3H, d, *J* = 6.0 Hz, 21-H$_3$), 2.00 (3H, s, OCOCH$_3$), 2.60 (1H, dd, *J* = 13.0, 3.6 Hz, 4α-H), 4.74 (1H, d, *J* = 2.4 Hz, one of 19-H$_2$), 4.86 (1H, tt, *J* ~ 8 and 4 Hz, 3α-H), 4.91 (1H, ddd, *J* = 10.1, 1.9, 1.2 Hz, 4'-H$_{(E)}$), 4.95 (1H, d, *J* = 2.4 Hz, one of 19-H$_2$), 4.97 (1H, ddt, *J* = 16.9, 1.9, 1.6 Hz, 4'-H$_{(Z)}$), 5.28 (1H, br s, 7-H), 5.79 (1H, ddt, *J* = 16.9, 10.1, 6.6 Hz, 3'-H); ^{13}C NMR (50 MHz) δ 12.29 (C-18), 19.06 (C-21), 21.57 (OCO<u>C</u>H$_3$), 22.64, 22.78, 23.05 (C-26 and C-27), 24.12, 27.89, 28.22, 30.12, 32.85, 33.30, 33.49, 33.67, 36.38, 38.01, 39.71, 40.58, 45.52, 55.75, 56.79, 72.07, 111.04, 114.25, 120.13, 131.73, 133.80, 139.14, 142.39, 147.15, 170.64 (O<u>C</u>OCH$_3$); HRMS (ESI) exact mass calculated for $C_{33}H_{52}O_2Na$ (M + Na)$^+$ 503.3865, measured 503.3866.

9: $[\alpha]^{24}_D$ +27^o (*c* 0.58, CHCl$_3$); ^{1}H NMR (200 MHz, CDCl$_3$) δ 0.540 (3H, s, 18-H$_3$), 0.868 (6H, d, *J* = 6.6 Hz, 26- and 27-H$_3$), 0.911 (3H, d, *J* = 6.2 Hz, 21-H$_3$), 2.05 (3H, s, OCOCH$_3$), 2.66 (1H, dd, *J* = 13.5, 3.5 Hz, 4α-H), 4.67 (1H, d, *J* = 2.4 Hz, one of 19-H$_2$), 4.86 (1H, br d, *J* ~ 10 Hz, 4'-

$H_{(E)}$), ca. 4.9 (1H, m, 3α-H; overlapped), 4.97 (1H, d, *J* = 2.4 Hz, one of 19-H_2), 5.00 (1H, br dd, *J* = 16.9, 1.8 Hz, 4'-$H_{(Z)}$), 5.38 (1H, br s, 7-H), 5.82 (1H, ddt, *J* = 16.9, 10.3, 6.5 Hz, 3'-H); ^{13}C NMR (50 MHz) δ 12.40 (C-18), 19.07 (C-21), 21.62 (OCO<u>C</u>H_3), 22.69, 22.79, 23.04 and 23.09 (C-26 and C-27), 24.12, 27.93, 28.23, 30.32, 32.10, 32.48, 32.85, 33.14, 36.11, 36.36, 39.73, 40.75, 46.28, 55.79, 56.77, 72.47, 112.95, 114.63, 121.89,131.75, 133.33, 138.91, 139.71, 146.70, 170.72 (O<u>C</u>OCH_3); HRMS (ESI) exact mass calculated for $C_{33}H_{52}O_2Na$ $(M + Na)^+$ 503.3865, measured 503.3872.

*2.1.6. (5E)- and (5Z)-6-(Pent-3'-enyl)-vitamin D_3 acetate D_3 (**10** and **11**)*

Glacial acetic acid solvolysis of the isomeric compounds **18** and **19** was performed as described above for the cyclovitamins **14** and **15**. The crude products were purified by flash chromatography on silica gel. Elution with hexane/Et_2O (98:2) gave a mixture of vitamins **10** and **11** (ratio of 3:1; 99% yield) as a colorless oil. Isomeric products were separated by HPLC (9.4 mm × 25 cm Zorbax-Sil column, 4 mL/min) using the hexane/ethyl acetate (99:1). The vitamins **10** and **11** were collected at Rv 31 mL and Rv 37 mL, respectively.

10: $[\alpha]^{24}_D$ +69° (*c* 1.06, $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.590 (3H, s, 18-H_3), 0.869 and 0.873 (3H and 3H, each d, *J* = 6.6 Hz, 26- and 27-H_3), 0.922 (3H, d, *J* = 6.2 Hz, 21-H_3), 2.00 (3H, s, O<u>C</u>OCH_3), 2.59 (1H, dd, *J* = 12.8, 4.0 Hz, 4α-H), 4.72 (1H, d, *J* = 2.4 Hz, one of 19-H_2), 4.86 (1H, tt, *J* ~ 8 and 4 Hz, 3α-H), 4.92 (1H, ddd, *J* = 10.1, 2.2. 1.2 Hz, 5'-$H_{(E)}$), 4.94 (1H, one of 19-H_2, overlapped), 4.97 (1H, ddt, *J* = 17.2, 2.2, 1.4 Hz, 5'-$H_{(Z)}$), 5.28 (1H, br s, 7-H), 5.79 (1H, ddt, *J* = 17.2, 10.1, 6.6 Hz, 4'-H); ^{13}C NMR (50 MHz) δ 12.38 (C-18), 19.19 (C-21), 21.71 (OCO<u>C</u>H_3), 22.90 and 23.17 (C-26 and C-27), 24.17, 24.84, 25.41, 28.02 (C-25), 30.04, 33.67, 36.52, 39.83, 40.59, 45.53, 55.00, 55.74, 72.11, 110.95, 114.43, 120.31, 131.35, 134.37, 139.23,

142.13, 147.27, 171.53 (O<u>C</u>OCH$_3$); HRMS (ESI) exact mass calculated for $C_{34}H_{54}O_2Na$ (M + Na)$^+$ 517.4022, measured 517.4009.

11: $[\alpha]^{24}_D$ +51^o (*c* 0.36, $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.537 (3H, s, 18-H$_3$), 0.866 and 0.868 (6H, each d, *J* = 6.6 Hz, 26- and 27-H$_3$), 0.911 (3H, d, *J* = 6.2 Hz, 21-H$_3$), 2.04 (3H, s, OCOCH$_3$), 2.62 (1H, dd, *J* = 13.0, 3.4 Hz, 4α-H), 4.67 and 4.85 (1H and 1H, each br d, *J* = 2.4 Hz, 19-H$_2$), ca. 4.9 (1H, m, 3α-H, overlapped), 4.94 (1H, ddd, *J* = 10.1, 2.1, 1.2 Hz, 5'-H$_{(E)}$), 4.99 (1H, ddt, *J* = 17.1, 2.1, 1.6 Hz, 5'-H$_{(Z)}$), 5.37 (1H, s, 7-H), 5.80 (1H, ddt, *J* = 17.1, 10.1, 6.5 Hz, 4'-H); ^{13}C NMR (50 MHz) δ 12.39 (C-18), 19.07 (C-21), 21.63 (OCO<u>C</u>H$_3$), 22.71, 22.79, 23.05 and 23.10 (C-26 and C-27), 24.12, 27.85, 27.93, 28.23, 30.28, 31.99, 32.44, 32.98, 33.92, 36.00, 36.37, 39.73, 40.75, 46.18, 55.78, 56.77, 72.37, 112.85, 114.59, 122.08, 131.48, 133.93, 139.14, 139.48, 146.84, 170.77 (O<u>C</u>OCH$_3$); HRMS (ESI) exact mass calculated for $C_{34}H_{54}O_2Na$ (M + Na)$^+$ 517.4022, measured 517.4012.

*2.1.7. Isomerization of vitamin **11** to its geometrical E-isomer **10***

A solution of vitamin **11** (10 mg, 0.019 mmol) in Et_2O (10 mL) containing a catalytic amount of iodine (0.2 mg, 0.002 mmol) was kept under diffuse daylight. The ratio (1:5.3) of the equilibrium concentration of **11** and its *E*-isomer **10** was achieved after 1 h.

*2.1.8. Thermal isomerization of vitamin **11** to its previtamin form 20*

A solution of vitamin **11** (20 mg, 0.039 mmol) in hexane (5 mL) was heated at 40 °C for 6 h. The ratio of vitamin **11** to its previtamin form **20** was determined by HPLC as 1.5:1 after 30 min and 1:2.2 after 3 h. The equilibrium was reached after 6 h and the ratio of **11**:**20** was established as 1:5.2, respectively. Isomeric products were separated by HPLC (9.4 mm × 25 cm Zorbax-Sil

column, 4 mL/min) using the hexane/ethyl acetate (99:1). The compounds **20** and **11** were collected at Rv 34 mL and Rv 37 mL, respectively.

20: ^{1}H NMR (500 MHz, $CDCl_3$, 23 °C) δ 0.669 (3H, br s, 18-H_3), 0.865 and 0.870 (3H and 3H, each d, *J* = 6.5 Hz, 26- and 27-H_3), 0.934 (3H, d, *J* = 6.5 Hz, 21-H_3), 1.55 (3H, br s, 19-H_3), 2.03 (3H, s, $OCOCH_3$), 2.59 (1H, dd, *J* = 12.8, 4.0 Hz, 4α-H), 4.90 (0.5H, narr m, 3α-H), 4.92 (1H, ddd, *J* = 10.5 Hz, 5'-$H_{(E)}$), 5.00 (1H, dm, *J* = 17.3 Hz, 5'-$H_{(Z)}$), 5.05 (0.5H, narr m, 3α-H), 5.44 and 5.53 (0.5H and 0.5H, each narr m, 9-H), 5.53 (1H, s, 7-H), 5.80 (1H, ddt, *J* = 17.3, 10.5, 6.5 Hz, 4'-H); ^{13}C NMR (125 MHz) δ 171.51 and 171.48 ($O\underline{C}OCH_3$), 139.12, 135.68, 135.54, 127.38, 125.29, 125.05, 122.77, 122.17, 114.65, 102.55, 99.83, 91.24, 83.68, 77.48, 77.23, 76.98, 71.06, 70.09, 63.17, 58.25, 54.82, 53.08, 51.49, 51.07, 42.39, 39.74, 37.87, 37.74, 36.49, 36.42, 35.15, 34.27, 33.76, 29.94, 29.77, 28.59, 28.25, 27.50, 27.27, 25.25, 24.12, 23.90, 23.05 and 22.79(C-26 and C-27), 21.73 ($OCO\underline{C}H_3$), 20.45, 19.01 (C-21), 14.35 (C-19), 11.39 (C-18).

20: ^{1}H NMR (500 MHz, $CDCl_3$, - 23 °C) δ 0.649 and 0.659 (1.5H and 1.5H, each s, 18-H_3), 0.857 and 0.861 (3H and 3H, each d, *J* = 6.5 Hz, 26- and 27-H_3), 0.926 (3H, d, *J* = 6.5 Hz, 21-H_3), 1.54 and 1.56 (1.5H and 1.5H, each s, 19-H_3), 2.07 (3H, s, $OCOCH_3$), 2.28 (1H, br d, *J* = 17.5 Hz, 4α-H), 4.87 (0.5H, narr m, 3α-H), 4.97 (1H, dd, *J* = 10.0, 2.5 Hz, 5'-$H_{(E)}$), 5.02 (1H, dm, *J* = 17.0 Hz, 5'-$H_{(Z)}$), 5.07 (0.5H, narr m, 3α-H), 5.44 (0.5H, narr m, 9-H), 5.52 (1H, s, 7-H), 5.54 (0.5H, narr m, 9-H), 5.82 (1H, ddt, *J* = 17.0, 10.0, 6.5 Hz, 4'-H);

20: ^{1}H NMR (500 MHz, $CDCl_3$, 55 °C) δ 0.678 (3H, s, 18-H_3), 0.868 and 0.873 (3H and 3H, each d, J = 6.5 Hz, 26- and 27-H_3), 0.939 (3H, d, J = 6.5 Hz, 21-H_3), 1.55 (1H, br s, 19-H_3), 2.01 (3H, s, $OCOCH_3$), 2.30 (1H, m, 4α-H), 4.94 (1H, dm, J = 10.0 Hz, 5'-$H_{(E)}$), 4.99 (1H, dm, J = 17.0 Hz, 5'-$H_{(Z)}$), 5.01–4.92 (1H, m, 3α-H overlapped with 5'-$H_{(Z)}$ and 5'-$H_{(E)}$), 5.48 (1H, br s, 9-H), 5.54 (1H, s, 7-H), 5.80 (1H, ddt, J = 17.0, 10.0, 6.5 Hz, 4'-H); HRMS (ESI) exact mass calculated for $C_{34}H_{54}O_2Na$ $(M + Na)^+$ 517.4022, measured 517.4016.

*2.1.9. (5E)-1α-Hydroxy- and (5E)-1β-hydroxy-(6-pent-4'-enyl)-vitamin D_3 acetate (**21** and **22**)*

tert-Butyl hydroperoxide (5.5 M in decane; 0.123 mmol, 0.022 mL) was added to a mixture of SeO_2 (8.6 mg, 0.078 mmol) in anhydrous CH_2Cl_2 (1 mL) and stirred vigorously at room temperature for 30 min. The mixture was cooled to 15 °C and a solution of **10** (10.2 mg, 0.021 mmol) in anhydrous CH_2Cl_2 (1 mL) was dropwise added. After 4 h of stirring at room temperature, the reaction was quenched by slow addition of aqueous 10% solution of NaOH (10 mL) and extracted with Et_2O. The organic layers were washed with 10% aqueous solution of NaOH, then with brine, dried ($MgSO_4$), and evaporated. The residue was purified by flash chromatography on silica gel. Elution with hexane/ethyl acetate (8:2) gave the mixture of the vitamins **21** and **22** (ratio of 2.1:1; 7.2 mg, 68%). The isomeric vitamins were purified and separated by HPLC (9.4 mm × 25 cm Zorbax-Sil column, 4 mL/min) using hexane/ethyl acetate (85:15). The 1β-hydroxy compound **22** and its 1α-hydroxy isomer **21** were collected at Rv 51 mL and Rv 56 mL, respectively.

21: ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.596 (3H, s, 18-H_3), 0.870 and 0.874 (3H and 3H, each d, J = 6.6 Hz, 26- and 27-H_3), 0.924 (3H, d, J = 6.0 Hz, 21-H_3), 1.99 (3H, s, $OCOCH_3$), 4.39 (1H, m, 1β-H), 4.90 (1H, narr m, one of 19-H_2), 4.92 (1H, br dd, J = 10.3, 2.1 Hz, 5'-$H_{(E)}$), 4.98 (1H, ddt, J = 17.1, 2.1, 1.6 Hz, 5'-$H_{(Z)}$), 5.13 (1H, m, 3α-H), 5.21 (1H, t, J = 1.6 Hz, one of 19-H_2), 5.27 (1H, br s, 7-H), 5.79 (1H, ddt, J = 17.1, 10.3, 6.6 Hz, 4'-H); HRMS (ESI) exact mass calculated for $C_{34}H_{54}O_3Na$ $(M + Na)^+$ 533.3971, measured 533.3983.

22: ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.593 (3H, s, 18-H_3), 0.870 and 0.874 (3H and 3H, each d, J = 6.6 Hz, 26- and 27-H_3), 0.925 (3H, d, J = 6.0 Hz, 21-H_3), 2.02 (3H, s, $OCOCH_3$), 2.62 (1H, dd, J = 13.2, 4.0 Hz, 4α-H), 4.17 (1H, m, 1α-H), 4.90 (2H, narr m, 3α-H and one of 19-H_2), 4.92 (1H,

br dd, *J* = 10.5, 2.0 Hz, 5'-$H_{(E)}$), 4.97 (1H, ddt, *J* = 16.9, 2.0, 1.6 Hz, 5'-$H_{(Z)}$), 5.23 (1H, t, *J* = 1.6 Hz, one of 19-H_2), 5.29 (1H, br s, 7-H), 5.78 (1H, ddt, *J* = 16.9, 10.5, 6.6 Hz, 4'-H); HRMS (ESI) exact mass calculated for $C_{34}H_{54}O_3Na$ $(M + Na)^+$ 533.3971, measured 533.3984.

*2.1.10. 6,19-Dimethylene-vitamin D_3 acetate (**23**)*

(a) The 2nd generation Grubbs catalyst (7.2 mg, 0.008 mmol) was added to a solution of the vitamin **8** (40 mg, 0.008 mmol) in anhydrous toluene (5 mL) at room temperature. Then the mixture was stirred at 70 °C for 2 h, cooled to room temperature and applied on a silica Sep-Pak (2 g). The product was eluted with hexane/ethyl acetate (95:5) and it was further purified by HPLC (9.4 mm × 25 cm Zorbax-Sil column, 4 mL/min) using hexane/ethyl acetate (99.5:0.5). The tetracyclic vitamin **23** (31 mg, 83%) was collected at Rv 55 mL.

(b) Metathesis reaction of the vitamin **10** was performed in the presence of the 2nd generation Grubbs catalyst and carried out as described above for the vitamin **8**. The crude product was purified on a silica Sep-Pak and then by HPLC (9.4 mm × 25 cm Zorbax-Sil column, 4 mL/min) using hexane/ethyl acetate (99.5:0.5) to give the vitamin **23** in 46% yield.

23: UV (in EtOH) λ_{max} 282 nm; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.586 (3H, s, 18-H_3), 0.870 and 0.874 (3H and 3H, each d, *J* = 6.7 Hz, 26- and 27-H_3), 0.927 (3H, d, *J* = 6.5 Hz, 21-H_3), 2.03 (3H, s, $OCOCH_3$), 2.64 (1H, dd, *J* = 15.0, 4.5 Hz, 4α-H), 4.93 (1H, tt, *J* = 8.5, 4.4 Hz, 3α-H), 5.51 (1H, narr m, 19-H), 5.53 (1H, s, 7-H); ^{13}C NMR (50 MHz) δ 12.19 (C-18), 19.10 (C-21), 21.67 ($OCOCH_3$), 22.71, 22.79, 22.90, 23.05, 23.51, 24.15, 27.92, 28.25, 28.46, 29.63, 29.85, 30.96, 33.14, 36.40, 39.74, 40.68, 45.79, 56.12, 56.84, 71.52, 119.56, 120.47, 126.54, 131.74, 134.50, 141.73, 170.84 ($OCOCH_3$); HRMS (ESI) exact mass calculated for $C_{31}H_{48}O_2Na$ $(M + Na)^+$ 475.3552, measured 475.3570.

*2.1.11. 6,19-Dimethylene-vitamin D_3 (**12**)*

Lithium aluminum hydride (2.6 mg, 0.069 mmol) was added to a stirred solution of **23** (4.2 mg, 0.009 mmol) in anhydrous THF (1 mL) at -40°C under argon. The mixture was warmed up to 0°C during 1 h and stirred at this temperature for next 3 h. The excess of the reagent was decomposed with saturated aqueous Na_2SO_4, and the mixture was extracted with ethyl acetate. The organic layers were washed with brine, dried ($MgSO_4$), and evaporated. The oily residue was applied on a silica Sep-Pak (2 g). Elution with hexane/ethyl acetate (9:1) gave the compound **12** that was further purified by HPLC (9.4 mm × 25 cm Zorbax-Sil column, 4 mL/min) using hexane/ethyl acetate (85:15). The deprotected vitamin **12** (3.6 mg, 98%) was collected at Rv 26 mL. The purity of the final compound was confirmed by reversed-phase HPLC (9.4 mm × 25 cm Zorbax-C18 column, 4 mL/min) using methanol as a solvent: the tetracyclic vitamin D analog **12** was eluted as a single sharp peak at Rv 64 mL.

12: UV (in EtOH) λ_{max} 285 nm (ε 19 900); ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.601 (3H, s, 18-H_3), 0.871 and 0.876 (3H and 3H, each d, *J* = 6.5 Hz, 26- and 27-H_3), 0.930 (3H, d, *J* = 6.5 Hz, 21-H_3), 2.69 (1H, br dd, *J* ~ 15, 4 Hz, 4α-H), 3.87 (1H, tt, *J* = 8.5, 4.2 Hz, 3α-H), 5.52 (1H, narr m, 19-H), 5.59 (1H, s, 7-H); ^{13}C NMR (50 MHz) δ 12.21 (C-18), 19.10 (C-21), 22.71, 22.82, 22.89, 23.07, 23.53, 24.18, 27.92, 28.22, 28.54, 29.63, 29.89, 31.00, 33.16, 36.42, 39.83, 40.59, 45.80, 56.11, 56.82, 71.41, 119.54, 120.41, 126.42, 131.43, 135.64, 145.71; HRMS (ESI) exact mass calculated for $C_{29}H_{46}ONa$ $(M + Na)^+$ 433.3446, measured 433.3452.

3. Results and discussion

We chose 6-oxo-3,5-cyclovitamin D_3 (**13**, Scheme 1) as a starting material, which was obtained from vitamin D_3 (**1**) using the procedure described by Mazur [22]. Treatment of this

compound with the respective Grignard reagent, derived from allyl, 3-butenyl or 4-pentenyl bromide, resulted in the formation of 2:1, 5.5:1 and 6.1:1 mixtures of 6*S*- and 6*R*-hydroxy-3,5-cyclovitamins: **14** and **15**, **16** and **17**, **18** and **19**, respectively. The structures of the obtained cyclovitamin D products were established on the basis of their spectral data and mechanistic rationale.

Since an attack of nucleophiles at a carbonyl center should occur from the less hindered side of a molecule, we decided to use molecular modeling studies to determine the stereochemistry of the major product. The calculations of optimized geometry and steric energies of the ketone **13**, carried out using PCModel (v9.0, Serena Software) program, led to two low-energy conformers (**A** and **B**) differing in steric energy by only 0.13 kcal/mol (Figure 2). Careful inspection of the conformer **A** structure indicates that hydrogens at C-4 and C-9 hinder the access of nucleophiles to the 6-carbonyl group from the *si* face of the molecule. In the conformation **B**, the presence of a bulky exomethylene group at C-10 also shields the *si* face, while attack from *re* face, as in the previous case, seems to be easier. Thus, results of molecular modeling of the ketone **13** suggest that approach of RMgX from *re* face should be preferred, especially for the bulky Grignard reagents, resulting in formation of 6*S*-hydroxy cyclovitamins.

In the next acetolysis step, the mixtures of epimeric alcohols **14** and **15**, **16** and **17**, **18** and **19** were used, and the corresponding pairs of 5*E*- and 5*Z*-vitamin D_3 acetates **6** and **7**, **8** and **9**, **10** and **11** (Scheme 2) were obtained in the ratio of 1.5:1, 2.5:1 and 3:1, respectively. In order to establish the double bond stereochemistry of the obtained compounds, the ^{1}H NOE difference experiments were performed for isomers **10** and **11**. The irradiation of 7-H (5.37 ppm) in the 5*Z*-analog **11** resulted in the expected enhancement (2.6%) of the signal (4.67 ppm) derived from the vinylic 19-$H_{(Z)}$. In agreement with expectations, the analogous experiment performed for 5*E*-

isomeric compound **10** (irradiated 7-H signal at 5.28 ppm) did not change the intensity of the signals of protons from 10-exomethylene group.

Earlier, Ray and collaborators reported that acidic solvolysis of the analogous epimeric mixture of 6-hydroxy-3,5-cyclovitamin D_3 compounds gave predominantly 5*Z*-vitamin D_3 derivatives [13]. However, the results of our studies suggest that configurations at C-6 assigned by these Authors for 3,5-cyclovitamins and, consequently, configurations of the triene products, resulting from their cycloreversion processes, are incorrect.[1]

Literature data indicate that cycloreversion step may occur according to mixed S_N1'/S_N2' mechanism [23]. In the latter case, the protonated hydroxyl group of 6-alkylated 6*R*-cyclovitamin leaves simultaneously to nucleophilic attack at 3β-position (Scheme 3). A necessary condition for S_N2' process, resulting in the stereoselective formation of 5*Z*-vitamin, is *anti*-relationship of C(3)-C(5) with respect to C(6)-OH (energetically preferred in the studied compounds). On the contrary, such a nucleophilic substitution of the 6*S*-cyclovitamin should provide exclusively the 5*E*-product. However, due to the fact that in the lowest energy conformation, C(3)-C(5) and C(6)-OH bonds of the 6*S*-epimer are in the *syn*-relationship (*anti*-orientation is less stable by *ca*. 2 kcal/mol), S_N2' mechanism can be less favored. Alternatively, if 6-hydroxyl group dissociates from the cyclovitamin compound (S_N1' mechanism) during the solvolysis, the formed carbocation may react with a solvent molecule in a nonstereospecific fashion, yielding 6*R*- or 6*S*-cyclovitamin or it may produce the corresponding vitamin product. Taking into account the ratios of the 5,7,10(19)-trienes, obtained by us from acetolysis of the respective mixtures of 6*S*- and 6*R*-cyclovitamins, and assuming that 6*R*-cyclovitamins formed almost exclusively 5*Z*-trienes, it can be calculated that the majority (ca. 7/8) of 6*S*-cyclovitamins were converted to the isomeric 5*E*-products. These

[1] Although the paper [13] indicates (6*S*)-hydroxy-3,5-cyclovitamins D_3 as the major products of reaction of 6-ketone **13** with Grignard reagents, the structures of these compounds shown in the included figures have the opposite 6*R*-configuration.

findings, being in agreement with the mechanism of cycloreversion of 6-substituted alcohols proposed by DeLuca [23] and Mazur [24], prove the highly stereospecific nature of the investigated process and support our configurational assignment of cyclovitamins. If 6*R*-cyclovitamins were the prevailing products of Grignard reaction of the ketone **13**, their acetolysis would more likely result in a higher content of the 5*Z*-vitamins. However, in order to unequivocally establish the configurations of the isomeric trienes formed by the cycloreversion reaction, two of them (**10** and **11**) were chosen as the representative products and subjected to additional experiments.

At first, isomerization of vitamin **11** to its geometrical isomer **10** (ratio of the equilibrium concentrations of 1:5.3, respectively) was achieved (Scheme 4), induced by visible light and catalytic amount of iodine. It is well known, that reversible iodine-catalyzed isomerization of vitamin D compounds strongly favors *E*-isomers [25].

Also, thermal isomerization of vitamin **11** to its previtamin form **20** (ratio of the equilibrium concentrations of 1:5.2, respectively) was accomplished by its heating in 40 °C solution. It is evident that such reversible thermal sigmatropic [1,7]-hydrogen shift may occur only between 5*Z*-vitamins and their previtamin D counterparts. Interestingly, the anomaly in the proton NMR spectra of the triene **20** was detected indicating its conformational isomerism and existence of two rotamers in approximately equal populations (Scheme 4). Thus, under normal temperature conditions the signal of 3α-H (and also the olefinic 9-H) appeared as two broad peaks; signals of methyl groups at C-10 and C-13 were significantly broadened. At higher temperature (55 ^{o}C) the separated peaks coalesced to the single signals whereas at low temperature (-23 ^{o}C) the signal splitting of the above mentioned methine signals was more intense, and each of the methyl signals appeared as two singlets.

Finally, the correctness of our structural assignment was confirmed by comparison of the

reaction of 5*E*- (**10**) and 5Z-vitamin (**11**) with oxidizing system of selenium dioxide and *tert*-butyl hydroperoxide [26]. In the case of the isomer **10**, such oxidation led to the epimeric mixture of 1-hydroxylated vitamins **21** and **22** (ratio of 2.1:1; Scheme 5), that was in a full agreement with the reported reactivity of 5*E*-vitamin D compounds, efficiently undergoing allylic oxidation [27]. On the contrary, the same reaction conditions applied to the analog **11** resulted in formation of a complex mixture of products, that was consistent with the literature data indicating that, in the case of the 5*Z*-vitamin D compounds, concomitant overoxidation and isomerization processes drastically reduce the yields of the desired 1α-hydroxylated derivatives [23].

The strategy of forming a bridge connecting C-6 and C-19 was based on the ring closing metathesis (RCM) of 5*E*-vitamin D_3 analogs [28]. The reaction was carried out in toluene in the presence of the 2nd generation Grubbs catalyst. As a result of cyclization of compound **6**, we expected to obtain the analog possessing an additional 5-membered ring. Since, despite many introduced modifications of the reaction conditions, we were unable to obtain the pure product in reasonable yield, we decided to synthesize the homologous compound having the less-strained 6-membered ring. For this purpose, we used 5*E*-vitamin D_3 acetate with 3'-butenyl substituent at C-6 (**8**), that after the RCM reaction, followed by deprotection of hydroxyl group in the formed tetracyclic product **23** (Scheme 6), provided the desired target vitamin D_3 analog **12**. In agreement with the assigned structure, the ^{1}H NMR spectrum of the latter compound showed the presence of two olefinic protons at C-7 and C-19 (singlet at 5.59 ppm and a narrow multiplet at 5.52 ppm) and a triplet of triplets at 3.87 ppm, originating from the 3α-H. Since the observed vicinal coupling constant $J_{3\alpha,4\beta}$ was 8.5 Hz, the prevailing A-ring conformer should have an equatorially oriented hydroxyl; molecular modeling fully supported this conclusion. In the calculated lowest-energy conformation of compound **12**, its ring A, condensed with the cyclohexadiene unit, preferentially

assumes the half-chair conformation with an equatorial hydroxyl group, four neighboring atoms (C-1, C-10, C-5 and C-4) lying in one plane, and two other (C-2 and C-3) located above and below that plane, respectively.

We decided to extend our studies by using also the homologous compound **10** as a substrate for the ring closure process. However, as a result of cyclization, instead of the expected product possessing an additional 7-membered ring, we obtained the analog **23** again, albeit in lower yield. Its formation is obviously the result of a double bond isomerization and subsequent ring closing metathesis with the liberation of propene. Such alternative reaction course is known [29,30] and it has been attributed to the presence of ruthenium hydride complexes in the reaction matrix [31,32].

4. Conclusions

We have established a convenient route for the preparation of vitamin D_3 analogs possessing an additional 6-membered ring, by applying the ring-closing metathesis (RCM) as a method of its construction. Additionally, in the search for the suitable precursors of 6-substituted 5*E*-vitamin D_3 structure, we reexamined the method described in literature and involving the reaction of 6-oxo-3,5-cyclovitamin D_3 with Grignard reagents, followed by acid-catalyzed cycloreversion of the formed tertiary 6-alcohols. Contrary to the literature data, the desired 5*E*-isomers were prevailing products of this two-step reaction sequence. Our findings may be useful for the design of novel B-*seco* steroids possessing the additional rings sharing the carbon atoms with A and B rings.

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Figure legends

Figure 1. Chemical structures of 25-dihydroxyvitamin D_3 (**1**), 1α,25-dihydroxyvitamin D_3 (calcitriol, **2**) and their analogs.

Figure 2. Stereomodels of the ketone **13** in its low-energy conformations **A** and **B** (viewed from the *re* face of carbonyl moiety).

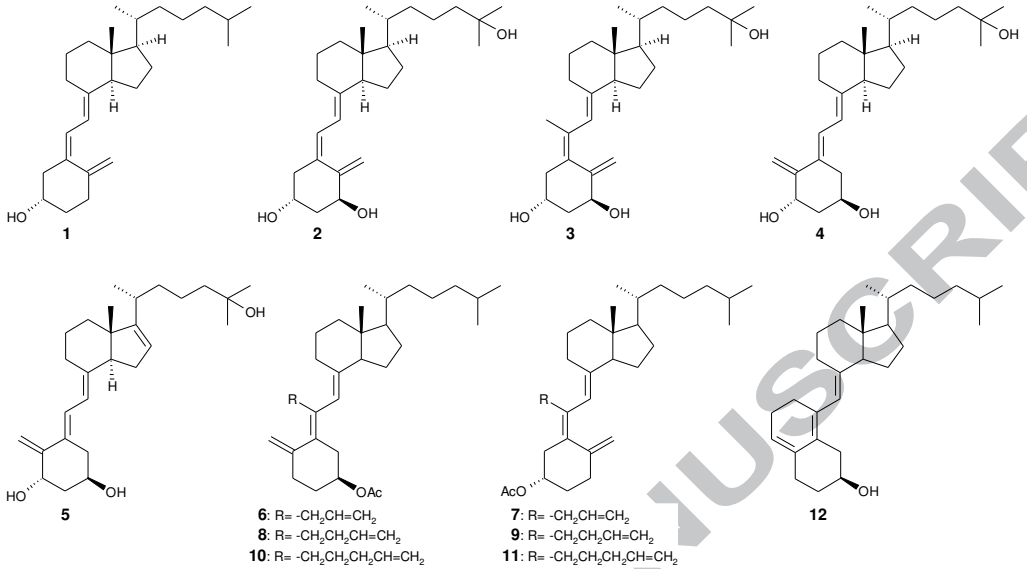


Figure 1

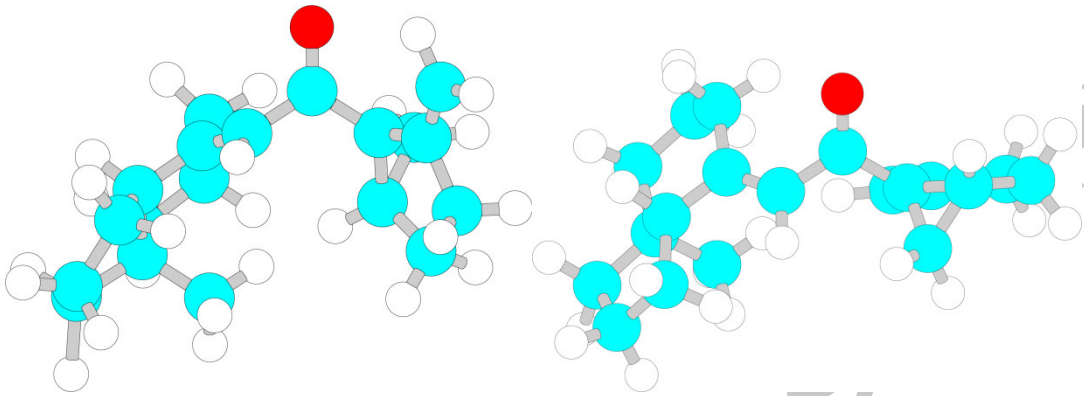
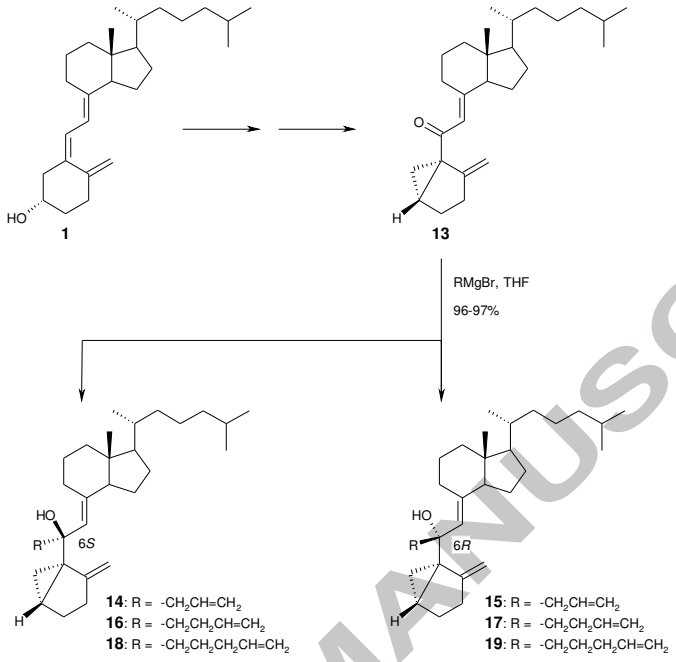
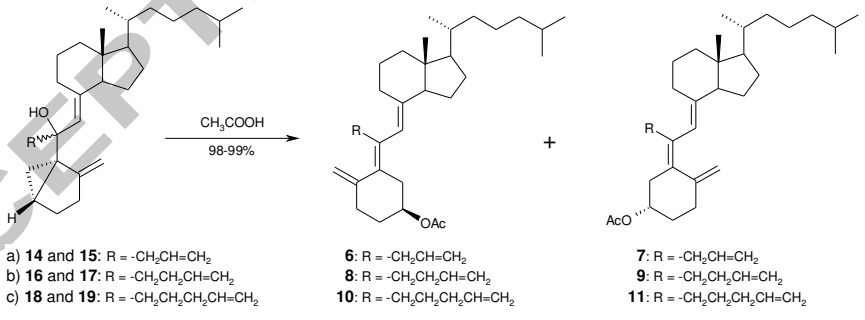


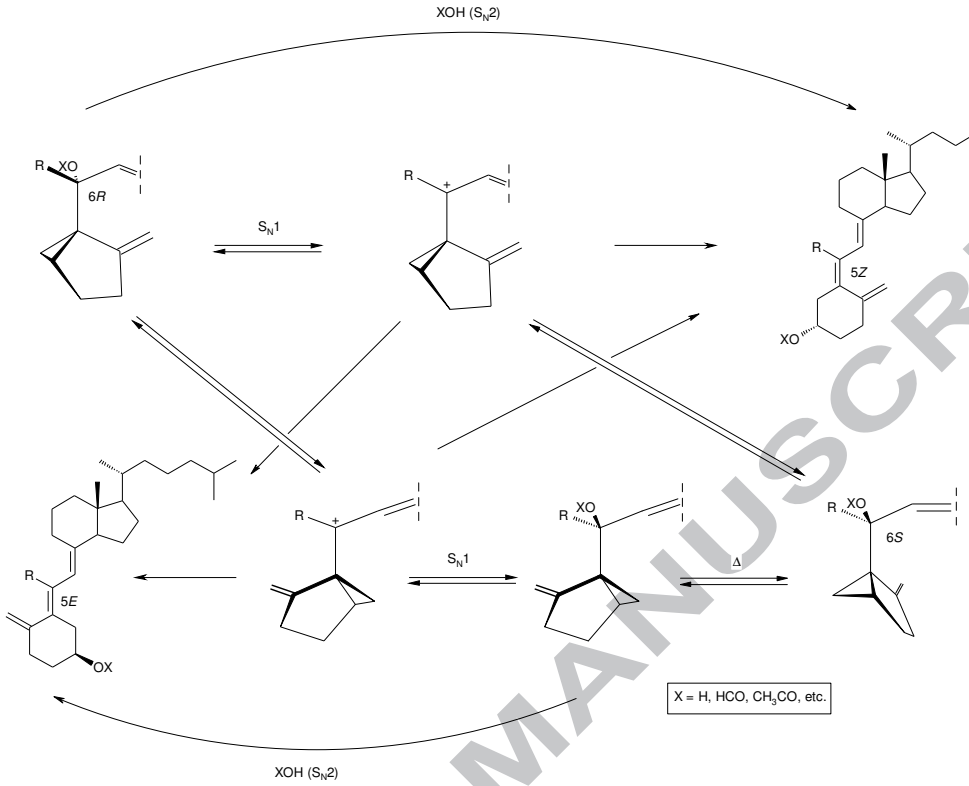
Figure 2



Scheme 1



Scheme 2



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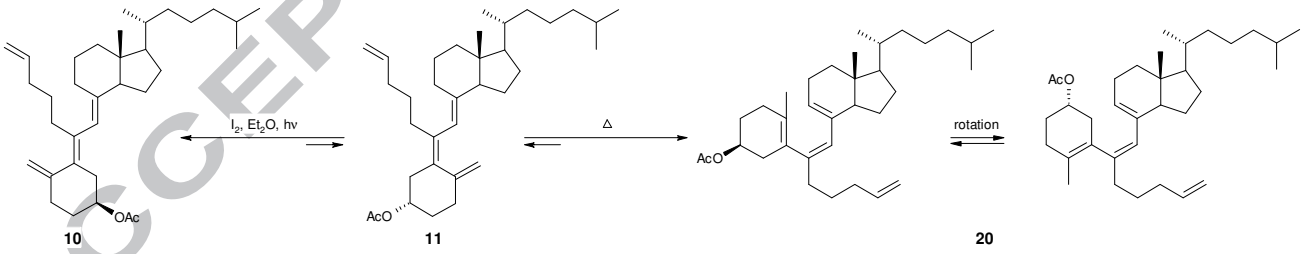

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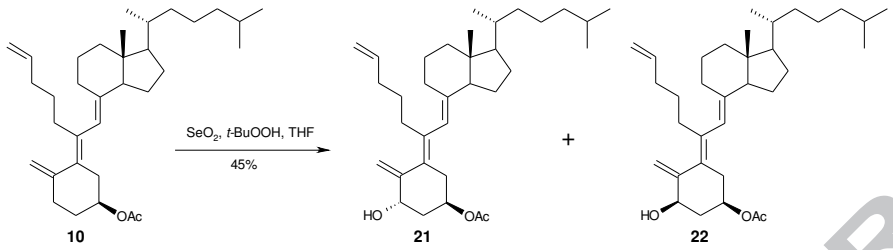
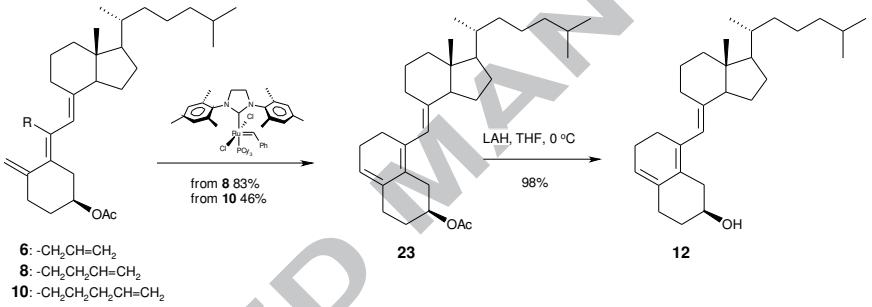


10 21 22

Scheme 5



6: -$CH_2CH{=}CH_2$
8: -$CH_2CH_2CH{=}CH_2$
10: -$CH_2CH_2CH_2CH{=}CH_2$

23 12

Scheme 6

1. Grignard reaction of 6-oxo-3,5-cyclovitamin D_3 is reinvestigated

2. The mechanism of acid-catalyzed cycloreversion of 6-substituted 3,5-cyclovitamin D compounds is discussed

3. The synthesis of new 5*E*-vitamin D analogs is described, possessing dimethylene bridge connecting C-6 and C-19