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Bioorganic & Medicinal Chemistry 15 (2007) 4193–4202
Bioorganic & Medicinal Chemistry

Synthesis and biological evaluation of new 6-*s*-*cis* locked 1,2,25-trihydroxyprevitamin D_3 analogues

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Received 15 February 2007; revised 15 March 2007; accepted 20 March 2007
Available online 24 March 2007

Dedicated to Professor Miguel Yus on the occasión of his 60th birthday.

Abstract—An efficient synthesis of several diastereomers of 2-hydroxy substituted 1α,25-dihydroxyprevitamin D_3 derivatives was accomplished utilizing a practical route to the A-ring synthon. The biological activity of the analogues was evaluated in vitro. All the synthesized derivatives demonstrated low affinity for the vitamin D receptor and vitamin D-binding protein compared with 1α,25-dihydroxyvitamin D_3, the natural hormone. 1α,2β,25-trihydroxy-19-*nor*-pre-D_3 was the most potent of the analogues in inhibiting proliferation of MCF-7 cells but requires higher EC_{50} concentrations than 1α,25-dihydroxyvitamin D_3.

1. Introduction

1α,25-Dihydroxyvitamin D_3 [**1**, 1α,25-$(OH)_2$-D_3], in addition to its important role in calcium and phosphorus homeostasis and bone mineralization, exerts also cell proliferation and differentiation, and serves as a modulator of the immune system.[1] The plethora of biological actions that are attributable to 1α,25-$(OH)_2$-D_3 suggested wide clinical applications of this metabolite or modified compounds[2] for the treatment of certain cancers, osteoporosis, inflammation, dermatological, and autoimmune diseases.[3]

At the molecular level, 1α,25-$(OH)_2$-D_3 stimulates biological responses both via interaction with nuclear receptors (VDRn) to regulate gene transcription (slow genomic pathway) and via other membrane receptors (VDRm), which generates rapid actions believed to be independent of direct interaction with the genome.[4] Non-genomic biological responses of 1α,25-$(OH)_2$-D_3 have been reported in a variety of systems including stimulation of intestinal Ca^{2+} transport (transcaltachia),[5] opening of chloride channels,[6] and activation of PKC[7] and MAP[8] kinases.

In comparison to other steroid hormones vitamin D_3 and its metabolites are unusually conformationally flexible.[9] This includes the side chain, a *seco* B-ring, and a conformationally active A-ring. These *seco* steroids can undergo a rotation around the 6,7 carbon–carbon single bond which generates a wide array of molecular shapes extending from the 6-*s*-*cis* (steroid-like conformation) to the more stable extended 6-*s*-*trans* conformation. Both conformers are present in slow chemical equilibrium (5–10%) with 1α,25-dihydroxyprevitamin D_3 [**2**, 1α,25-$(OH)_2$-pre-D_3]. Pure pre-1α,25 standing in solution rearranges to 1α,25 with a half-life of 13.5 h at 37 °C, whereas when the C-19 methyl group is deuterated,[10] rearrangement proceeds with a half-life of 81 h. Due to the spontaneous isomerization of previtamin **2** via a [1,7]-sigmatropic hydrogen shift back to the thermally more stable vitamin **1**, biological evaluation of **2** is very difficult.

It has been observed that analogues that are either structurally blocked in the previtamin form[11] or that only

Keywords: Vitamin D_3 analogues; Previtamin D_3; Novel A-ring analogues; Biological evaluation; Calcitriol.
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doi:10.1016/j.bmc.2007.03.058

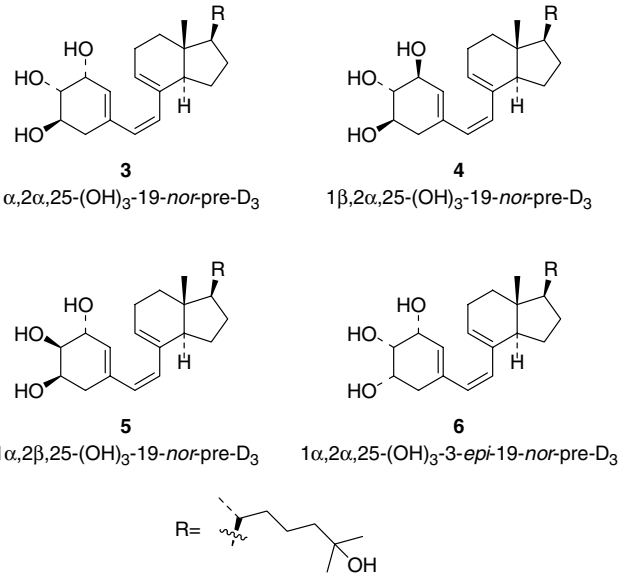
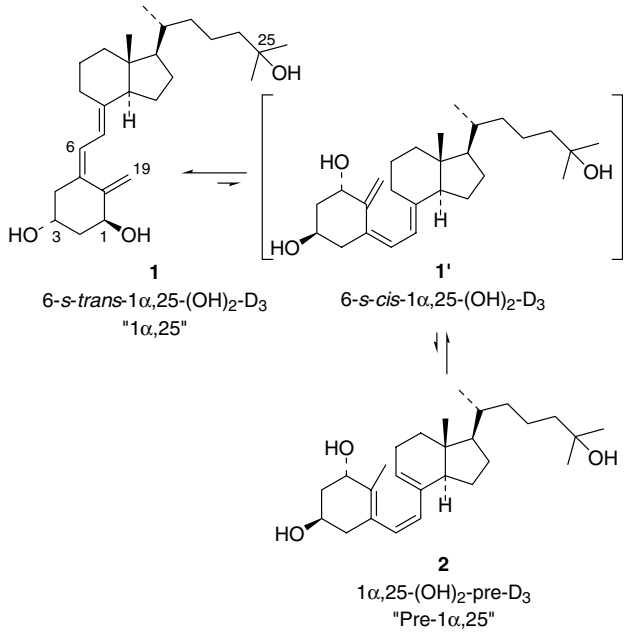


Figure 1. Previtamin D_3 analogues.



Scheme 1. Vitamin–previtamin equilibrium.

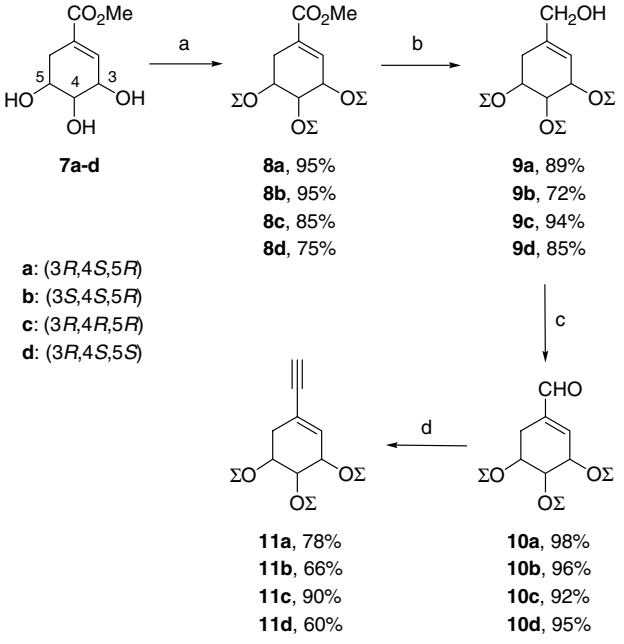
slowly isomerize to the vitamin form[12] are able to fully mimic the membrane actions of 1α,25-$(OH)_2$-D_3 but have little action at the nuclear level with minimal effects on cell proliferation and differentiation. These results suggested that 1α,25-$(OH)_2$-D_3 produces biological responses through two distinct receptors, which recognize totally different ligand shapes. Thus, the genomic actions occur as a consequence of the interaction of a 6-*s-trans* shape of 1α,25-$(OH)_2$-D_3 with its VDRn; meanwhile, the ability of 1α,25-$(OH)_2$-D_3 to generate rapid biological effects requires a 6-*s-cis* shape, which is recognized by a VDRm.[4] In addition, a 9,19-methano-bridged analogue[13] of 1α,25-$(OH)_2$-D_3 and A-ring diastereomers of 1α,25-$(OH)_2$-19-*nor*-pre-D_3[14] have been reported. Recently, the first previtamin structure with genomic activities equivalent to 1α,25-$(OH)_2$-D_3 has been described.[15] This analogue interacted as efficiently as the natural hormone with the VDRn and uses the same contact points within the receptor as did 1α,25-$(OH)_2$-D_3. An important point in this research was the recent disclosure of the detailed structure of the ligand binding of the VDRn.[16]

In order to further probe the less well investigated biological actions of the previtamin form, it is the purpose of this article to describe the synthesis of a new class of locked 1α,25-$(OH)_2$-19-*nor*-previtamin D_3 analogues with a modified A-ring (**3**–**6**, Fig. 1), hence representing a system in which the biological profile of a genuine previtamin form could be assessed. These derivatives are unable to undergo rearrangement to the respective vitamin D form by virtue of the absence of the C-19 methyl group. In addition, results of preliminary biological activities are reported to better understand their mode of action Scheme 1.

2. Results and discussion

For synthesis of the target 2-substituted 19-*nor*-previtamin D_3 derivatives **3**–**6**, we used standard Sonogashira coupling[17] involving the A-ring enyne precursors **11a**–**d** (Scheme 2) with an enol triflate of the CD-ring/side chain fragment.

A-ring synthons **11a**–**d** were obtained starting from methyl shikimate and its epi-isomers. Methyl 3-*epi*,[18] 4-*epi*-,[19] and 5-*epi*-shikimate[20] derivatives **7b**, **c**, and **d** were synthesized as previously reported through simple and efficient approaches. Methyl shikimate (**7a**) was prepared from shikimic acid by treatment with HCl in



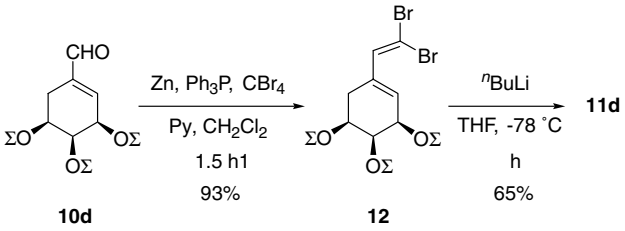
Scheme 2. Reagents and conditions: (a) TBDMS-OTf, 2,6-lutidine, CH_2Cl_2, 0 °C → rt, overnight; (b) DIBALH, toluene, −78 °C, 4 h; (c) PCC, CH_2Cl_2, rt, 5 h; (d) $TMSCHN_2$, ^{n}BuLi, THF, −78 °C → 0 °C, 4 h.

MeOH. Persilylation of **7a–d** with *tert*-butyldimethylsilyl trifuoromethanesulfonate (TBDMS-OTf) and 2,6-lutidine afforded the desired protected silyl ethers **8a–d** in high yields (Scheme 2). Transformation of the ester into the aldehyde was best carried out via a two-step sequence. Thus, reduction of **8a–d** with DIBALH gave the alcohols **9a–d**, which upon oxidation with PCC yielded the aldehydes **10a–d**. Formation of the enynes **11a–d** was accomplished in good yields by reaction with trimethylsilyldiazomethane.

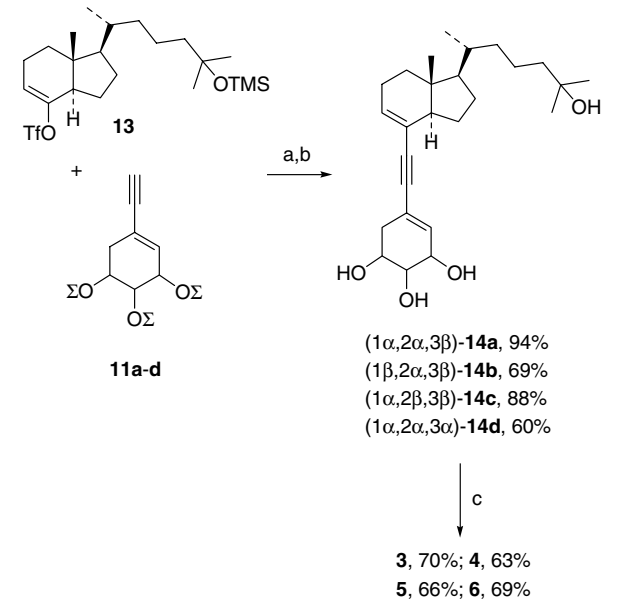
In an attempt to increase the yield of the 5-*epi* enyne derivative **11d**, ethynylation of **10d** was carried out by conversion to the vinyl dibromide **12** and subsequent reaction with ^{n}BuLi.[21] However, this alternative method provides **11d** in a similar overall yield (Scheme 3).

Initially, the protection of **7a–d** was performed with trimethylsilyl chloride (TMSCl). However, the same sequence of reactions for A-ring preparation gave lower yields due to the instability of the TMS group.

Four 6-*s*-*cis* locked previtamin D_3 derivatives were successfully synthesized as outlined in Scheme 4. CD-ring/side chain fragment **13** was prepared starting from



Scheme 3. Alternative synthesis of compound **11d**.



Scheme 4. Reagents and conditions: (a) $Pd(Ph_3P)_2(OAc)_2$, CuI, Et_2NH, DMF, rt, 5 h; (b) TBAF, THF, rt, overnight; (c) H_2, Lindlar cat., quinoline, MeOH, rt, 30 min.

readily available vitamin D_3 following the general procedure described previously.[13,22] A-ring synthons **11a–d** were coupled to protected vinyl triflate **13** in the presence of bis[triphenylphosphine]palladium (II) acetate-copper (I) iodide catalyst and diethylamine in DMF. The resulting silyloxy dienynes were deprotected with TBAF to afford the tetrahydroxydienynes **14a–d** in 60–94% yields. Careful catalytic hydrogenation of **14a–d** in the presence of Lindlar catalyst and quinoline generated previtamins **3–6**.

3. Biological evaluation

We examined the potencies of the previtamin D_3 derivatives **3–6** in terms of their ability to bind to the pig vitamin D receptor (VDR) and human vitamin D-binding protein (hDBP). The results of biological evaluation are summarized in Table 1, in comparison with those of 1α,25-$(OH)_2$-D_3. All the synthesized derivatives have markedly reduced affinity for the VDR. Comparison of the four isomers showed that compounds **3** and **5** possessing the configuration of the natural hormone at C-1 and C-3 positions are more potent than corresponding isomers **4** and **6**. When comparing DBP binding, previtamin analogues exhibited more than 10 times lower affinity than 1α,25-$(OH)_2$-D_3.

In addition, inhibition of MCF-7 breast cancer cell proliferation was measured (Fig. 2). In this study, the rank order of potency was parallel to that of VDR binding. The 2α- and 2β-hydroxy analogues **3** and **5** with 1α,3β configuration exhibited the most potent activity in this series. Thus, 1α,2β,25-$(OH)_3$-19-*nor*-pre-D_3 (**5**) can inhibit the cell proliferation by 70% at a concentration of 10^{-6} M comparable with 1α,25-$(OH)_2$-D_3 but this compound was 17 times less potent than 1α,25-$(OH)_2$-D_3 at the EC_{50} concentration. The 1-epimer as well as 3-epimer showed virtually no inhibition even at the concentration of 10^{-6} M.

The introduction of a 2β-OH group in the 1α,25-$(OH)_2$-19-*nor*-pre-D_3 compound did not alter its biological activity. The binding of compound **5** and 1α,25-$(OH)_2$-19-*nor*-pre-D_3 to VDR (2% and 1%, respectively) and

Table 1. Biological activity of 2-hydroxy derivatives of 1α,25-$(OH)_2$-pre-D_3

Compound	VDR (%)	hDBP (%)	MCF-7 (%)
1α,25-$(OH)_2$-D_3	100	100	100
1α,25-$(OH)_2$-19-*nor*-pre-D_3	1[a]	5[a]	11[a]
3	0.2	4	2
4	<0.1	2	0
5	2	8	6
6	<0.1	7	0

Summary of the in vitro effects of 2-hydroxy analogues of 1α,25-$(OH)_2$-pre-D_3 on receptor binding (VDR), human vitamin D-binding protein (hDBP), and MCF-7 proliferation. The in vitro effect is expressed as percentage activity at EC_{50} in comparison with 1α,25-$(OH)_2$-D_3 (=100% activity).

[a] Data from Refs. 11c and 15.

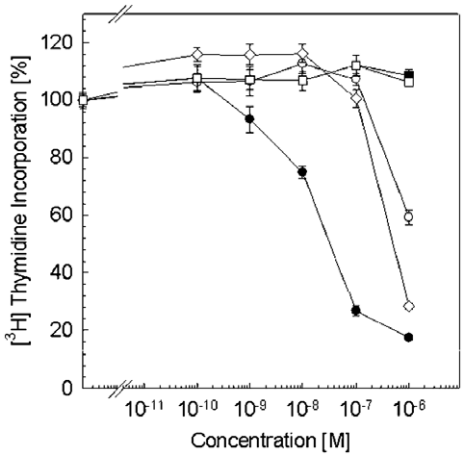


Figure 2. In vitro antiproliferative effects of 2-hydroxy analogues of 1α,25-$(OH)_2$-pre-D_3 on breast cancer MCF-7 cells. 1α,25-$(OH)_2$-D_3 (●); **3** (○); **4** (■); **5** (◇); **6** (□).

hDBP (8% and 5%, respectively) was comparable.[11c,15] Moreover, both compounds did inhibit the MCF-7 cell proliferation more or less with the same potency (6% compound **5** vs 11% 1α,25-$(OH)_2$-19-*nor*-pre-D_3).[15] The introduction of a 2α-OH group in 1α,25-$(OH)_2$-19-*nor*-pre-D_3 decreased the VDR binding affinity five (compound **3**) to ten (compounds **4** and **6**) times as well as its capacity to inhibit the proliferation of MCF-7 cells.

Such as for 1α,25-$(OH)_2$-19-*nor*-pre-D_3,[15] the low biological profile of the studied 19-*nor*-previtamin D_3 analogues is probably due to weak interactions with VDR, vitamin D responsive elements (VDRE), and co-activators.

4. Conclusions

We have described the synthesis and biological evaluation of novel 2-hydroxy substituted 6-*s-cis* locked previtamin D_3 analogues. We investigated the potency in inhibiting MCF-7 cell proliferation and found that derivatives **3** and **5** possessing the natural C-1 and C-3 configuration in the A-ring showed inhibition effects, while analogues **4** and **6** are inactive. These previtamin D_3 derivatives have shown poor binding to VDR and DBP compared with 1α,25-dihydroxyvitamin D_3. Further studies are needed to elucidate fully the activity profiles and modes of action of these analogues.

5. Experimental

5.1. General

Synthesis of **7b**,[18] **c**,[19] **d**,[20] and vinyl triflate **13**[13,22] was previously reported. Unless otherwise specified column chromatography was performed over silica 60 Å (230–400 mesh). HPLC was performed using UV detector and a Varian Dynamax column (microsorb 100–5 Si, 250 × 10 mm).

5.2. Synthesis of 3–6

A flask containing Lindlar catalyst (45 mg) was exposed to a positive pressure of hydrogen gas (balloon). A solution of **14** (15 mg, 0.036 mmol) in MeOH (1.8 mL) and quinoline (130 μL of 0.17 M in hexane, 0.022 mmol) were added. The reaction mixture was stirred vigorously during 30 min. The mixture was filtered on Celite, concentrated, and the crude subjected to flash chromatography using silica 60 Å (32–63 μm) pH 7 (gradient elution with 10–30% acetone/CH_2Cl_2). Further purification by HPLC (Dynamax column, 250 × 10 mm, 4 mL/min, hexane/MeOH/EtOH, 92:4:4 for **3**, **5**, and **6**; 5 mL/min, hexane/MeOH/EtOH, 94:3:3 for **4**) afforded **3**–**6**.

5.2.1. 1α,2α,25-Trihydroxy-19-*nor*-previtamin D_3 (3). Yield 70%. ^{1}H NMR (300.13 MHz, MeOH-d_4): δ 0.79 (s, 3H, Me_{18}), 1.01 (s, 3H, Me_{21}, $^3J_{HH}$ = 6.4 Hz), 1.11 (m, 1H), 1.19 (s, 6H, Me_{26} + Me_{27}), 1.2–1.5 (m, 14H), 1.68 (m, 1H, H_{14}), 1.9–2.3 (m, 6H), 2.68 (dd, 1H, H_{4e}, $|^2J_{HH}|$ = 17.6, $^3J_{HH}$ = 5.4 Hz), 3.51 (m, 1H, H_2), 3.86 (ddd, 1H, H_3, $^3J_{HH}$ = 8.8, 8.8, 5.4 Hz), 4.26 (dd, 1H, H_1, $^3J_{HH}$ = 4.4, 4.4 Hz), 5.43 (sa, 1H, H_9) 5.70 (br s, 1H, H_{10}), 5.8 (d, 1H, H_7, $^3J_{HH}$ = 12.2 Hz), and 5.88 (d, 1H, H_6, $^3J_{HH}$ = 12.2 Hz) ppm; ^{13}C NMR (50.5 MHz, MeOH-d_4): δ 10.0 (C_{18}), 17.9 (C_{21}), 20.5 (CH_2), 23.2 (CH_2), 24.2 (CH_2), 27.8 (CH_2), 27.9 (CH_3), 28.0 (CH_3), 35.8 (CH_2), 36.0 (CH_2), 36.1 (CH), 36.4 (CH_2), 41.9 (C), 43.9 (CH_2), 50.8 (CH), 54.4 (CH), 66.9 (C_3 + C_1), 70.1 (C), 73.1 (C_2), 125.2 (C_9), 128.2 (C_{10}), 129.1 (C_6), 130.6 (C_7), 136.5 (C), and 136.9 (C); (ESI^+, *m/z*) 441 [$(M+Na)^+$, 100%], 442 [$(M+H+Na)^+$, 25]; (EI^+, *m/z*) 418 [M^+, 30%], 400 [$(M-H_2O)^+$, 25], 382 (42), 193 (70), 157 (90), and 69 (100); HRMS (*m/z*) calculated for $C_{26}H_{42}O_4$ (M^+): 418.3078. Found: 418.3072.

5.2.2. 1β,2α,25-Trihydroxy-19-*nor*-previtamin D_3 (4). Yield 63%.^{1}H NMR (400.13 MHz, MeOH-d_4): δ 0.79 (s, 3H, Me_{18}), 1.00 (d, 3H, Me_{21}, $^3J_{HH}$ = 6.4 Hz), 1.09 (m, 1H), 1.18 (s, 6H, Me_{26} + Me_{27}), 1.2–1.6 (m, 14H), 1.65 (dd, 1H, $^3J_{HH}$ = 19.2, 10 Hz), 1.9–2.3 (m, 6H), 2.59 (dd, 1H, H_{4e}, $|^2J_{HH}|$ = 17.2, $^3J_{HH}$ = 5.6 Hz), 3.36 (dd, 1H, H_2, $^3J_{HH}$ = 10, 7.6 Hz), 3.56 (ddd, 1H, H_3, $^3J_{HH}$ = 9.6, 9.6, 5.6 Hz), 4.07 (br s, 1H, H_1), 5.42 (s, 1H, H_9) 5.52 (s, 1H, H_{10}), 5.79 (d, 1H, H_7, $^3J_{HH}$ 12H, and 5.87 (d, 1H, H_6, $^3J_{HH}$ = 12 Hz); ^{13}C NMR (100.6 MHz, MeOH-d_4): δ 10.4 (C_{18}), 17.9 (C_{21}), 20.5 (CH_2), 23.2 (CH_2), 24.2 (CH_2), 27.7 (CH_3), 27.9 (CH_3), 28.0 (CH_2), 36.0 (CH_2), 36.1 (CH), 36.4 (CH_2), 41.9 (C), 43.9 (CH_2), 50.7 (CH), 54.4 (CH), 69.7 (C_3), 70.1 (C), 72.7 (C_1), 77.8 (C_2), 125.3 (C_9), 128.5 (C_6), 129.1 (C_{10}), 130.4 (C_7), 134.6 (C_5), and 136.6 (C_8); (ESI^+, *m/z*) 441 [$(M+Na)^+$, 100%]; 436 [$(M+H_2O)^+$, 85%]; (EI^+, *m/z*) 418 [M^+, 15%], 400 [$(M-H_2O)^+$, 10], 382 [$(M-2H_2O)^+$, 20], 289 (30), 245 (55), 158 (95), 91 (98), and 69 (100). HRMS (*m/z*) calculated for $C_{26}H_{42}O_4$ (M^+): 418.3078. Found: 418.3074.

5.2.3. 1α,2β,25-Trihydroxy-19-*nor*-previtamin D_3 (5). Yield 66%. ^{1}H NMR (400.13 MHz, MeOH-d_4): δ 0.78 (s, 3H, Me_{18}), 1.00 (d, 3H, Me_{21}, $^3J_{HH}$ = 6.8 Hz), 1.09 (m, 1H), 1.18 (s, 6H, Me_{26} + Me_{27}), 1.2–1.5 (m, 14H), 1.65 (m, 1H), 1.9–2.1 (m, 2H), 2.2–2.4 (m, 3H), 2.42

(dd, 1H, H_{4a}, $|^2J_{HH}|$ = 16.8, $^3J_{HH}$ = 5.6 Hz), 2.51 (d, 1H, H_{4e}, $|^2J_{HH}|$ = 16.8 Hz), 3.63 (dd, 1H, H_2, $^3J_{HH}$ = 5.6, 2.4 Hz), 3.98 (ddd, 1H, H_1, $^3J_{HH}$ = 6.4, 4.0, 2.4 Hz), 4.20 (m, 1H, H_3), 5.45 (sa, 1H, H_9) 5.61 (sa, 1H, H_{10}), 5.77 (d, 1H, H_7, $^3J_{HH}$ = 11.6 Hz), and 5.87 (d, 1H, H_6, $^3J_{HH}$ = 12.4 Hz); ^{13}C NMR (100.6 MHz, MeOH-d_4): δ 10.4 (C_{18}), 17.9 (C_{21}), 20.5 (CH_2), 23.0 (CH_2), 24.2 (CH_2), 27.7 (CH_3), 27.9 (CH_3), 28.0 (CH_2), 33.4 (CH_2), 36.0 (CH_2), 36.1 (CH), 36.4 (CH_2), 41.9 (C), 43.9 (CH_2), 50.9 (CH), 54.4 (CH), 67.8 (C_1), 69.8 (C_3), 70.1 (C), 73.9 (C_2), 125.1 (C_9), 127.5 (C_{10}), 129.5 (C_6), 130.2 (C_7), 135.6 (C_5), and 136.5 (C_8); (APCI$^-$, *m/z*) 383 [(M−2H_2O)$^+$, 100%]; (EI$^+$, *m/z*) 418 [M$^+$, 30%], 400 [(M−H_2O)$^+$, 20], 382 [(M−2H_2O)$^+$, 37], 157 (55), 95 (61), 81 (76), and 69 (100); HRMS (*m/z*) calculated for $C_{26}H_{42}O_4$ (M$^+$): 418.3078. Found: 418.3075.

5.2.4. 1α,2α,25-Trihydroxy-3-*epi*-19-*nor*-previtamin D_3 (6). Yield 69%. ^{1}H NMR (400.13 MHz, MeOH-d_4): δ 0.78 (s, 3H, Me_{18}), 1.01 (d, 3H, Me_{21}, $^3J_{HH}$ = 6.4 Hz), 1.09 (m, 1H), 1.19 (s, 6H, Me_{26} + Me_{27}), 1.2–1.5 (m, 14H), 1.70 (ddd, 1H, $^3J_{HH}$ = 8.4, 8.4, 8.4 Hz), 1.9–2.1 (m, 2H), 2.2–2.3 (m, 3H), 2.33 (dd, 1H, H_{4e}, $|^2J_{HH}|$ = 16.8, $^3J_{HH}$ = 5.2 Hz), 2.51 (dd, 1H, H_{4a}, $|^2J_{HH}|$ = 16.8, $^3J_{HH}$ = 9.2 Hz), 3.78 (m, 1H, H_3), 3.90 (s, 1H, H_2), 4.23 (s, 1H, H_1), 5.44 (s, 1H, H_9) 5.54 (s, 1H, H_{10}), 5.78 (d, 1H, H_7, $^3J_{HH}$ = 12.4 Hz), and 5.86 (d, 1H, H_6, $^3J_{HH}$ = 12.4 Hz); ^{13}C NMR (100.6 MHz, MeOH-d_4): δ 10.4 (C_{18}), 17.9 (C_{21}), 21.9 (CH_2), 24.3 (CH_2), 25.6 (CH_2), 29.1 (CH_3), 29.2 (CH_3), 29.4 (CH_2), 33.4 (CH_2), 37.4 (CH_2), 37.6 (CH), 37.8 (CH_2), 43.3 (C), 45.3 (CH_2), 52.4 (CH), 55.8 (CH), 69.7 (C_1), 70.4 (C_3), 71.5 (C_2), 72.2 (C), 126.6 (C_9), 129.4 (C_{10}), 130.8 (C_6), 131.6 (C_7), 136.7 (C_5), and 137.7 (C_8); (ESI$^+$, *m/z*) 441 [(M+Na)$^+$, 100%]; (EI$^+$, *m/z*) 418 [M$^+$, 15%], 400 [(M−H_2O)$^+$, 25], 382 [(M−2H_2O)$^+$, 80], 69 (90), and 55 (100); HRMS (*m/z*) calculated for $C_{26}H_{42}O_4$ (M$^+$): 418.3078. Found: 418.3076.

5.3. Synthesis of 8a–d

2,6-Lutidine (2.6 mL, 22.3 mmol) was added to a suspension of **7** (600 mg, 3.19 mmol) in anhydrous CH_2Cl_2 (3.2 mL). The solution was cooled at 0 °C and TBDMS-OTf (3.7 mL, 15.96 mmol) was added dropwise. The reaction mixture was stirred at room temperature overnight and then poured into water/CH_2Cl_2. The aqueous layer was extracted with CH_2Cl_2, and the combined organic layers were dried over Na_2SO_4 and concentrated. The crude product was subjected to flash chromatography (2% EtOAc/hexane).

5.3.1. Methyl (3*R*,4*S*,5*R*)-3,4,5-tri[(*tert*-butyldimethylsilyl)oxy]cyclohex-1-enecarboxylate (8a). Yield 95%. Mp 42–44 °C; IR (KBr): υ 2948, 2925, 2891, 2854, 1718, and 1651 cm^{-1}; ^{1}H NMR (400.13 MHz, $CDCl_3$): δ 0.08 (s, 12H, 4 Si*Me*), 0.13 (s, 3H, Si*Me*), 0.14 (s, 3H, Si*Me*), 0.86 (s, 9H, SiCMe_3), 0.88 (s, 9H, SiCMe_3), 0.96 (s, 9H, SiCMe_3), 2.18 (dd, 1H, $H_{6e'}$, $|^2J_{HH}|$ = 18.2, $^3J_{HH}$ = 1 Hz), 2.60 (dddd, 1H, $H_{6a'}$, $|^2J_{HH}|$ = 18.2, $^3J_{HH}$ = 3.6, 3, 3 Hz), 3.76 (m, 4H, H_8+H_4), 4.00 (ddd, 1H, H_5, $^3J_{HH}$ = 4.6, 2, 2 Hz), 4.63 (m, 1H, H_3), and 6.69 (s, 1H, H_2); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ −4.7 (Si*Me*), −4.4 (Si*Me*), −4.3 (Si*Me*), 17.9 (Si*C*), 18.1 (Si*C*), 18.5 (Si*C*), 25.7 (SiCMe_3), 25.8 (SiCMe_3), 26.2 (SiCMe_3), 29.5 (C_6), 51.7 (C_8), 68.0 (C_3), 69.8 (C_5), 72.3 (C_4), 126.9 (C_1), 140.4 (C_2), and 167.5 (C_7); (ESI$^+$, *m/z*) 553 [(M+Na)$^+$, 100%].

5.3.2. Methyl (3*S*,4*S*,5*R*)-3,4,5-tri[(*tert*-butyldimethylsilyl)oxy]cyclohex-1-enecarboxylate (8b). Yield 95%. IR (NaCl): υ 2955, 2930, 2890, 2858, 1723, and 1655 cm^{-1}; ^{1}H NMR (400.13 MHz, $CDCl_3$): δ 0.03 (s, 3H, Si*Me*), 0.06 (s, 3H, Si*Me*), 0.09 (s, 3H, Si*Me*), 0.10 (s, 3H, Si*Me*), 0.12 (s, 3H, Si*Me*), 0.13 (s, 3H, Si*Me*), 0.87 (s, 9H, SiCMe_3), 0.88 (s, 9H, SiCMe_3), 0.92 (s, 9H, SiCMe_3), 2.43 (m, 2H, $2H_6$), 3.74 (ddd, 1H, H_4, $^3J_{HH}$ = 4, 1.8, $|^4J_{HH}|$ = 1.8 Hz), 3.77 (s, 3H, H_8), 3.91 (ddd, 1H, H_5, $^3J_{HH}$ = 4, 4, 4 Hz), 4.06 (ddd, 1H, H_3, $^3J_{HH}$ = 2, 2, $|^4J_{HH}|$ = 2 Hz), and 6.78 (ddd, 1H, H_2, $^3J_{HH}$ = 1.2, $|^4J_{HH}|$ = 1.2, 1.2 Hz); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ −4.4 (Si*Me*), −4.3 (Si*Me*), −4.3 (Si*Me*), −2.9 (Si*Me*), 17.9 (Si*C*), 18.2 (Si*C*), 18.2 (Si*C*), 25.7 (SiCMe_3), 25.8 (SiCMe_3), 25.9 (SiCMe_3), 28.8 (C_6), 51.8 (C_8), 68.2 (C_5), 71.7 (C_3), 74.8 (C_4), 126.7 (C_1), 137.7 (C_2), and 167.7 (C_7); (ESI$^+$, *m/z*) 553 [(M+Na)$^+$, 100%], 531 [(M+H_2O)$^+$, 70%].

5.3.3. Methyl (3*R*,4*R*,5*R*)-3,4,5-tri[(*tert*-butyldimethylsilyl)oxy]cyclohex-1-enecarboxylate (8c). Yield 85%. IR (NaCl): υ 2954, 2929, 2892, 2858, and 1725 cm^{-1}. ^{1}H NMR (400.13 MHz, $CDCl_3$): δ 0.07 (s, 3H, Si*Me*), 0.08 (s, 3H, Si*Me*), 0.09 (s, 3H, Si*Me*), 0.10 (s, 3H, Si*Me*), 0.12 (s, 6H, Si*Me*), 0.86 (s, 9H, SiCMe_3), 0.90 (s, 9H, SiCMe_3), 0.91 (s, 9H, SiCMe_3), 2.3–2.5 (m, 2H, H_6), 3.71 (br s, 1H, H_4), 3.77 (s, 3H, H_8), 3.99 (t, 1H, H_5, $^3J_{HH}$ = 6.8, 6.8 Hz), 4.10 (br s, 1H, H_3), and 6.66 (s, 1H, H_2); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ −4.9 (Si*Me*), −4.8 (Si*Me*), −4.7 (Si*Me*), −4.6 (Si*Me*), −4.5 (Si*Me*), −4.4 (Si*Me*), 18.0(Si*C*), 18.1 (Si*C*), 18.3 (Si*C*), 25.8 (SiCMe_3), 26.0 (SiCMe_3), 28.9 (br s C_6),[23] 51.8 (C_8), 67.4 (br s C_5),[23] 71.0 (C_3), 74.9 (C_4), 130.5 (C_1),[23] 135.8 (C_2),[23] and 167.4 (C_7); (ESI$^+$, *m/z*) 553 [(M+Na)$^+$, 100%], 569 [(M+K)$^+$, 5%].

5.3.4. Methyl (3*R*,4*S*,5*S*)-3,4,5-tri[(*tert*-butyldimethylsilyl)oxy]cyclohex-1-enecarboxylate (8d). Yield 75%. Mp 91–93 °C; IR (KBr): υ 2953, 2928, 2892, 2860, 1707, and 1653 cm^{-1}; ^{1}H NMR (300.13 MHz, $CDCl_3$): δ 0.06 (s, 6H, 2Si*Me*), 0.07 (s, 3H, Si*Me*), 0.09 (s, 3H, Si*Me*), 0.11 (s, 3H, Si*Me*), 0.12 (s, 3H, Si*Me*), 0.86 (s, 9H, SiCMe_3), 0.90 (s, 9H, SiCMe_3), 0.93 (s, 9H, SiCMe_3), 2.3–2.5 (m, 2H, $2H_6$), 3.74 (s, 4H, H_5+$3H_8$), 3.90 (br s, 1H, H_4), 4.35 (br s, 1H, H_3), and 6.53 (s, 1H, H_2); ^{13}C NMR (75.5 MHZ, $CDCl_3$): δ −4.7 (Si*Me*), −4.6 (Si*Me*), −4.5 (Si*Me*), −4.4 (Si*Me*), −4.3 (Si*Me*), 18.3 (Si*C*), 18.5 (Si*C*), 18.6 (Si*C*), 25.9 (SiCMe_3), 26.0 (SiCMe_3), 26.1 (SiCMe_3), 30.5 (C_6), 51.8 (C_8), 70.4 (C_5), 71.4 (C_3), 74.6 (C_4), 128.5 (C_1), 140.3 (C_2), and 167.1 (C_7); (ESI$^+$, *m/z*) 553 [(M+Na)$^+$, 100%], 531 [(M+H_2O)$^+$, 70%].

5.4. Synthesis of 9a–d

DIBAL-H (8.26 mL of 1.0 M in toluene, 8.26 mmol) was added dropwise to a solution of **8** (1.46 g,

2.75 mmol) in anhydrous toluene (23 mL) at −78 °C, and the reaction mixture was stirred for 4 h at the same temperature. NH_4Cl (aq.) was added and the mixture was warmed to room temperature, diluted with Et_2O, and filtered through a short column of silica gel, using additional Et_2O to elute the column. The filtrate was concentrated to give a crude sufficiently pure for the next step.

5.4.1. (3*R*,4*S*,5*R*)-3,4,5-Tri[(*tert*-butyldimethylsilyl)oxy]-1-hydroxymethylcyclohex-1-ene (9a). Yield 89%. Mp 74–76 °C; IR (KBr): υ 3430, 2953, 2928, 2894, 2856, and 1643 cm^{-1}; ^1H NMR (400.13 MHz, $CDCl_3$): δ 0.07 (s, 6H, 2Si*Me*), 0.08 (s, 3H, Si*Me*), 0.09 (s, 3H, Si*Me*), 0.11 (s, 6H, 2 Si*Me*), 0.87 (s, 9H, SiCMe_3), 0.88 (s, 9H, SiCMe_3), 0.94 (s, 9H, SiCMe_3), 1.85 (d, 1H, H_{6a}, $|^2J_{HH}|$ = 17.8 Hz), 2.44 (d, 1H, H_{6e}, $|^2J_{HH}|$ = 18.1 Hz), 3.73 (dd, 1H, H_4, $^3J_{HH}$ = 3.8, 3.8 Hz), 4.00 (m, 3H, H_5+2H_7), 4.50 (br s, 1H, H_3), and 5.5 (s, 1H, H_2); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ −4.8 (Si*Me*), −4.7 (Si*Me*), −4.5 (Si*Me*), −4.3 (Si*Me*), −4.2 (Si*Me*), 17.9 (Si*C*), 18.2 (Si*C*), 18.5 (Si*C*), 25.8 (SiCMe_3), 25.9 (SiCMe_3), 26.2 (SiCMe_3), 31.1 (C_6), 66.7 (C_7), 67.8 (C_3), 70.0 (C_5), 73.0 (C_4), 123.9 (C_2), and 135.3 (C_1); (ESI^+, *m*/*z*) 525 $[(M+Na)^+$, 100%].

5.4.2. (3*S*,4*S*,5*R*)-3,4,5-Tri[(*tert*-butyldimethylsilyl)oxy]-1-hydroxymethylcyclohex-1-ene (9b). Yield 72%. Mp 80–81 °C; IR (KBr): υ 3417, 2952, 2928, 2892, 2857, and 1471 cm^{-1}; ^1H NMR (400.13 MHz, $CDCl_3$): δ 0.08 (s, 3H, Si*Me*), 0.10 (s, 3H, Si*Me*), 0.11 (s, 6H, 2Si*Me*), 0.11 (s, 6H, 2Si*Me*), 0.89 (s, 9H, SiCMe_3), 0.90 (s, 9H, SiCMe_3), 0.92 (s, 9H, SiCMe_3), 2.00 (dd, 1H, $H_{6a'}$, $|^2J_{HH}|$ = 16.8, $^3J_{HH}$ = 4.8 Hz), 2.33 (dd, 1H, $H_{6e'}$, $|^2J_{HH}|$ = 16.4, $^3J_{HH}$ = 3.6 Hz), 3.70 (dd, 1H, H_4, $^3J_{HH}$ = 5.2, 2.4 Hz), 3.86 (ddd, 1H, H_5, $^3J_{HH}$ = 4.4, 4.4, 4.4 Hz), 4.03 (s, 4H, H_3+2H_7), and 5.58 (s, 1H, H_2); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ −4.8 (Si*Me*), −4.2 (Si*Me*), −4.2 (Si*Me*), −4.1 (Si*Me*), −4.0 (Si*Me*), −4.0 (Si*Me*), 18.3 (Si*C*), 17.9 (Si*C*), 26.0 (SiCMe_3), 26.1 (SiCMe_3), 26.2 (SiCMe_3), 31.3 (C_6), 66.8 (C_7), 69.1 (C_5), 72.1 (C_3), 75.8 (C_4), 122.9 (C_2), and 135.0 (C_1); (ESI^+, *m*/*z*) 525 $[(M+Na)^+$, 100%].

5.4.3. (3*R*,4*R*,5*R*)-3,4,5-Tri[(*tert*-butyldimethylsilyl)oxy]-1-hydroxymethylcyclohex-1-ene (9c). Yield 94%. Mp 68–70 °C; IR (KBr): υ 3425, 2954, 2929, 2891, 2857, and 1471 cm^{-1}; ^1H NMR (400.13 MHz, $CDCl_3$): δ 0.07 (s, 3H, 2 Si*Me*), 0.08 (s, 3H, Si*Me*), 0.09 (s, 3H, Si*Me*), 0.10 (s, 9H, 3Si*Me*), 0.87 (s, 9H, SiCMe_3), 0.91 (s, 9H, SiCMe_3), 0.91 (s, 9H, SiCMe_3), 1.98 (dd, 1H, $H_{6e'}$, $|^2J_{HH}|$ = 16.4, $^3J_{HH}$ = 5.2 Hz), 2.27 (dd, 1H, $H_{6a'}$, $|^2J_{HH}|$ = 16.4, $^3J_{HH}$ = 9.6 Hz), 3.70 (s, 1H, H_4), 4.0–4.1 (m, 4H, H_3+H_5+2H_7), and 5.52 (br s, 1H, H_2); ^{13}C NMR (100 MHZ, $CDCl_3$): δ −4.8 (Si*Me*), −4.7 (Si*Me*), −4.6 (Si*Me*), −4.5 (Si*Me*), −4.4 (Si*Me*), −4.3 (Si*Me*), 18.2 (Si*C*), 18.3 (Si*C*), 25.8 (SiCMe_3), 25.9 (SiCMe_3), 26.1 (SiCMe_3), 30.8 (C_6),[23] 66.4 (C_7), 67.9 (C_5),[23] 71.5 (C_3), 75.5 (C_4), 120.8 (C_2),[23] and 139.8 (C_1)[23]; (ESI^+, *m*/*z*) 525 $[(M+Na)^+$, 95%]; 541 $[(M+K)^+$, 30%].

5.4.4. (3*R*,4*S*,5*S*)-3,4,5-Tri[(*tert*-butyldimethylsilyl)oxy]-1-hydroxymethylcyclohex-1-ene (9d). Yield 85%. IR (NaCl): υ 3346, 2951, 2929, 2895, 2857, and 1726 cm^{-1}; ^1H NMR (400.13 MHz, $CDCl_3$): δ 0.06 (s, 3H, Si*Me*), 0.07 (s, 3H, Si*Me*), 0.08 (s, 3H, Si*Me*), 0.10 (s, 3H, Si*Me*), 0.10 (s, 3H, Si*Me*), 0.11 (s, 3H, Si*Me*), 0.88 (s, 9H, SiCMe_3), 0.91 (s, 9H, SiCMe_3), 0.93 (s, 9H, SiCMe_3), 1.99 (dd, 1H, $H_{6a'}$, $|^2J_{HH}|$ = 16.4, $^3J_{HH}$ = 5.6 Hz), 2.33 (m, 1H, $H_{6e'}$), 3.78 (ddd, 1H, H_5, $^3J_{HH}$ = 6, 6, 6 Hz), 3.90 (br s, 1H, H_4), 4.01 (br s, 2H, 2H_7), 4.27 (br s, 1H, H_3), and 5.34 (br s, 1H, H_2); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ −4.6 (Si*Me*), −4.4 (Si*Me*), −4.2 (Si*Me*), 18.3 (Si*C*), 18.6 (Si*C*), 18.7 (Si*C*), 26.0 (SiCMe_3), 26.1 (SiCMe_3), 26.3 (SiCMe_3), 31.4 (C_6), 66.1 (C_7), 70.6 (C_5), 71.2 (C_3), 75.4 (C_4), 124.1 (C_2), and 136.6 (C_1); (ESI^+, *m*/*z*) 525 $[(M+Na)^+$, 100%]; 541 $[(M+K)^+$, 10%].

5.5. Synthesis of 10a–d

PCC (1.16 g, 5.38 mmol) was added to a solution of **9** (900 mg, 1.79 mmol) in anhydrous CH_2Cl_2 (18 mL). The reaction mixture was stirred at room temperature for 5 h. Et_2O was added and the gummy residue was filtered through a short column of fluorosil and washed with EtOAc. The filtrate was concentrated to afford **10**, which was sufficiently pure for direct use in the next step. These aldehydes are instable and should be kept in the refrigerator.

5.5.1. (3*R*,4*S*,5*R*)-3,4,5-Tri[(*tert*-butyldimethylsilyl)oxy]-cyclohex-1-enecarbaldehyde (10a). Yield 98%. Mp 47–48 °C; IR (KBr): υ 2953, 2931, 2889, 2852, and 1690 cm^{-1}; ^1H NMR (400.13 MHz, $CDCl_3$): δ 0.06 (s, 3H, Si*Me*), 0.07 (s, 3H, Si*Me*), 0.08 (s, 3H, Si*Me*), 0.09 (s, 3H, Si*Me*), 0.15 (s, 3H, Si*Me*), 0.16 (s, 3H, Si*Me*), 0.84 (s, 9H, SiCMe_3), 0.87 (s, 9H, SiCMe_3), 0.98 (s, 9H, SiCMe_3), 2.17 (d, 1H, $H_{6a'}$, $|^2J_{HH}|$ = 18.2 Hz), 2.48 (dddd, 1H, $H_{6e'}$, $|^2J_{HH}|$ = 18.2, $^3J_{HH}$ = 3, $|^4J_{HH}|$ = 3, 3 Hz), 3.84 (m, 1H, H_4), 4.04 (dd, 1H, H_5, $^3J_{HH}$ = 3, 3 Hz), 4.78 (sa, 1H, H_3), 6.5 (s, 1H, H_2), and 9.47 (s, 1H, H_7); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ −4.9 (Si*Me*), −4.8 (Si*Me*), −4.4 (Si*Me*), 17.9 (Si*C*), 18.0 (Si*C*), 18.5 (Si*C*), 25.6 (SiCMe_3), 25.7 (SiCMe_3), 26.1 (SiCMe_3), 26.7 (C_6), 68.4 (C_3), 69.5 (C_5), 73.0 (C_4), 138.0 (C_1), 151.1 (C_2), and 194.2 (C_7); (ESI^+, *m*/*z*) 523 $[(M+Na)^+$, 100%].

5.5.2. (3*S*,4*S*,5*R*)-3,4,5-Tri[(*tert*-butyldimethylsilyl)oxy]-cyclohex-1-enecarbaldehyde (10b). Yield 96%. Mp 75–77 °C; IR (KBr): υ 2948, 2925, 2889, 2856, and 1683 cm^{-1}; ^1H NMR (400.13 MHz, $CDCl_3$): δ 0.02 (s, 6H, 2Si*Me*), 0.08 (s, 3H, Si*Me*), 0.10 (s, 3H, Si*Me*), 0.13 (s, 6H, Si*Me*), 0.82 (s, 9H, SiCMe_3), 0.85 (s, 9H, SiCMe_3), 0.91 (s, 9H, SiCMe_3), 2.27 (d, 1H, $H_{6a'}$, $|^2J_{HH}|$ = 17.2 Hz), 2.38 (d, 1H, $H_{6e'}$, $|^2J_{HH}|$ = 17.2), 3.81 (s, 1H, H_4), 3.94 (s, 1H, H_5), 4.14 (s, 1H, H_3), 6.53 (s, 1H, H_2), and 9.50 (s, 1H, H_7); ^{13}C NMR (100.6 MHZ, $CDCl_3$): δ −4.9 (Si*Me*), −4.6 (Si*Me*), −4.5 (Si*Me*), −4.4 (Si*Me*), 17.9 (Si*C*), 18.1 (Si*C*), 18.2 (Si*C*), 25.4 (C_6), 25.8 (SiCMe_3), 25.9 (SiCMe_3), 25.9 (SiCMe_3), 67.4 (C_5), 71.7 (C_3), 75.3 (C_4), 136.9 (C_1), 147.9 (C_2), and 194.4 (C_7); (ESI^+, *m*/*z*) 523 $[(M+Na)^+$, 100%]; 501 $[(M+H)^+$, 70%].

5.5.3. (3*R*,4*R*,5*R*)-3,4,5-Tri[(*tert*-butyldimethylsilyl)oxy]-cyclohex-1-enecarbaldehyde (10c). Yield 92%. IR (NaCl): υ 2952, 2930, 2858, and 1694 cm^{-1}; ^1H NMR (400.13 MHz, $CDCl_3$): δ 0.07 (s, 3H, Si*Me*), 0.08 (s, 6H, 2Si*Me*), 0.10 (s, 3H, Si*Me*), 0.14 (s, 6H, 2Si*Me*), 0.85 (s, 9H, SiC*Me*$_3$), 0.90 (s, 9H, SiC*Me*$_3$), 0.92 (s, 9H, SiC*Me*$_3$), 2.29 (d, 1H, $H_{6a'}$, $|^2J_{HH}| = 17.6$, $^3J_{HH} = 8.8$ Hz), 2.40 (dd, 1H, $H_{6e'}$, $|^2J_{HH}| = 17.6$, $^3J_{HH} = 5.3$ Hz), 3.77 (s, 1H, H_4), 4.00 (dd, 1H, H_5, $^3J_{HH} = 6.4$, 5.8 Hz), 4.24 (br s, 1H, H_3), 6.45 (s, 1H, H_2), and 9.51 (s, 1H, H_7); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ −4.9 (Si*Me*), −4.7 (Si*Me*), −4.6 (Si*Me*), −4.5 (Si*Me*), −4.4 (Si*Me*), 18.0 (Si*C*), 18.1 (Si*C*), 18.3 (Si*C*), 25.9 (SiC*Me*$_3$), 26.0 (SiC*Me*$_3$), 26.1 (SiC*Me*$_3$), 26.9 (C_6), 67.5 (C_5),[23] 70.9 (C_3), 75.6 (C_4), 141.7 (C_1),[23] 145.0 (C_2),[23] and 194.1 (C_7)[23]; (ESI^+, *m*/*z*) 523 [$(M+Na)^+$, 13%]; 539 [$(M+Na)^+$, 4%].

5.5.4. (3*R*,4*S*,5*S*)-3,4,5-Tri[(*tert*-butyldimethylsilyl)oxy]-cyclohex-1-enecarbaldehyde (10d). Yield 95%. Mp 92–94 °C; IR (KBr): υ 2951, 2929, 2889, 2857, and 1689 cm^{-1}; ^1H NMR (400.13 MHz, $CDCl_3$): δ 0.08 (s, 6H, 2Si*Me*), 0.09 (s, 3H, Si*Me*), 0.11 (s, 3H, Si*Me*), 0.15 (s, 3H, Si*Me*), 0.16 (s, 3H, Si*Me*), 0.85 (s, 9H, SiC*Me*$_3$), 0.91 (s, 9H, SiC*Me*$_3$), 0.96 (s, 9H, SiC*Me*$_3$), 2.31 (dddd, 1H, $H_{6a'}$, $|^2J_{HH}| = 16.8$, $^3J_{HH} = 10$, $|^4J_{HH}| = 3.2$, 3.2 Hz), 2.41 (dd, 1H, $H_{6e'}$, $|^2J_{HH}| = 17.2$, $^3J_{HH} = 6$ Hz), 3.79 (s, 1H, H_5, $^3J_{HH} = 10$, 6, 1.2 Hz), 3.97 (s, 1H, H_4), 4.46 (br s, 1H, H_3), 6.34 (s, 1H, H_2), and 9.47 (s, 1H, H_7); ^{13}C NMR (100.6 MHZ, $CDCl_3$): δ −4.8 (Si*Me*), −4.7 (Si*Me*), −4.6 (Si*Me*), −4.5 (Si*Me*), −4.4 (Si*Me*), −4.3 (Si*Me*), 18.3 (Si*C*), 18.5 (Si*C*), 18.6 (Si*C*), 25.9 (SiC*Me*$_3$), 26.0 (SiC*Me*$_3$), 26.1 (SiC*Me*$_3$), 27.2 (C_6), 70.3, (C_5), 71.8 (C_3), 75.4 (C_4), 139.4 (C_1), 150.8 (C_2), and 193.2 (C_7); (ESI^+, *m*/*z*) 523 [$(M+Na)^+$, 100%]; 539 [$(M+K)^+$, 10%].

5.6. Synthesis of 11a–d

^{n}BuLi (0.38 mL of 1.6 M in hexane, 0.60 mmol) was added to a solution of $TMSCHN_2$ (0.29 mL of 2.0 M in hexane, 0.57 mmol) at −78 °C. To this solution was added **10** (255 mg, 0.51 mmol) in THF (2 mL). The mixture was stirred and allowed to reach room temperature during 4 h. The reaction mixture was poured into water/Et_2O and the aqueous layer extracted with Et_2O. The combined organic fractions were dried (Na_2SO_4) and concentrated, and the residue purified by flash chromatography using silica 60 Å (32–63 μm) pH 7 (gradient elution with hexane-1% Et_2O/hexane).

5.6.1. (3*R*,4*S*,5*R*)-3,4,5-Tri[(*tert*-butyldimethylsilyl)oxy]-1-ethynylcyclohex-1-ene (11a). Yield 78%. IR (NaCl): υ 3316, 2955, 2930, 2887, 2858, 2099, and 1633 cm^{-1}; $[\alpha]_{Na}^{20} - 52$ (*c* 0.5, $CHCl_3$); ^1H NMR (400.13 MHz, $CDCl_3$): δ 0.08 (s, 6H, 2 Si*Me*), 0.09 (s, 3H, Si*Me*), 0.09 (s, 3H, Si*Me*), 0.11 (s, 6H, 2 Si*Me*), 0.88 (s, 9H, SiC*Me*$_3$), 0.89 (s, 9H, SiC*Me*$_3$), 0.94 (s, 9H, SiC*Me*$_3$), 1.95 (d, 1H, H_{6a}, $|^2J_{HH}| = 17.6$ Hz), 2.55 (dddd, 1H, H_{6e}, $|^2J_{HH}| = 14.7$, $^3J_{HH} = 3.4$, $|^4J_{HH}| = 3.4$, 2.9 Hz), 2.82 (s, 1H, H_8), 3.72 (m, 1H, H_4), 3.94 (m, 1H, H_5), 4.54 (sa, 1H, H_3), and 5.95 (s, 1H, H_2); ^{13}C NMR (100.6 MHZ, $CDCl_3$): δ −4.8 (Si*Me*), −4.7 (Si*Me*), −4.4 (Si*Me*), −4.3 (Si*Me*), 17.9 (Si*C*), 18.1 (Si*C*), 18.4 (Si*C*), 25.7 (SiC*Me*$_3$), 25.8 (SiC*Me*$_3$), 26.1 (SiC*Me*$_3$), 34.2 (C_6), 67.6 (C_3), 69.8 (C_5), 72.3 (C_4), 74.9 (C_8), 84.7 (C_7), 117.0 (C_1), and 137.4 (C_2); (ESI^+, *m*/*z*) 519.2 [$(M+Na)^+$, 90%].

5.6.2. (3*S*,4*S*,5*R*)-3,4,5-Tri[(*tert*-butyldimethylsilyl)oxy]-1-ethynylcyclohex-1-ene (11b). Yield 66%. IR (NaCl): υ 3317, 2956, 2930, 2889, and 2858 cm^{-1}. $[\alpha]_{Na}^{20} + 2$ (*c* 0.9, $CHCl_3$); ^1H NMR (400.13 MHz, $CDCl_3$): δ 0.07 (s, 3H, Si*Me*), 0.08 (s, 3H, Si*Me*), 0.09 (s, 3H, Si*Me*), 0.10 (s, 3H, Si*Me*), 0.11 (s, 3H, Si*Me*), 0.11 (s, 3H, Si*Me*), 0.89 (s, 9H, SiC*Me*$_3$), 0.90 (s, 9H, SiC*Me*$_3$), 0.92 (s, 9H, SiC*Me*$_3$), 2.11 (dd, 1H, $H_{6a'}$, $|^2J_{HH}| = 16.8$, $^3J_{HH} = 4$ Hz), 2.47 (dddd, 1H, $H_{6e'}$, $|^2J_{HH}| = 16.8$, $^3J_{HH} = 4$, $|^4J_{HH}| = 2$, 2 Hz), 2.86 (s, 1H, H_8), 3.69 (dd, 1H, H_4, $^3J_{HH} = 5.2$, 2.8 Hz), 3.84 (ddd, 1H, H_5, $^3J_{HH} = 4.4$, 4.4, 4.4 Hz), 4.00 (br s, 1H, H_3), and 6.03 (s, 1H, H_2); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ −4.9 (Si*Me*), −4.3 (Si*Me*), −4.2 (Si*Me*), −4.1 (Si*Me*), 17.9 (Si*C*), 18.2 (Si*C*), 25.9 (SiC*Me*$_3$), 26.0 (SiC*Me*$_3$), 26.0 (SiC*Me*$_3$), 34.2 (C_6), 68.3 (C_5), 71.6 (C_3), 74.8 (C_4), 75.8 (C_8), 84.9 (C_7), 117.1 (C_1), and 135.5 (C_2); (ESI^+, *m*/*z*) 519 [$(M+Na)^+$, 90%].

5.6.3. (3*R*,4*R*,5*R*)-3,4,5-Tri[(*tert*-butyldimethylsilyl)oxy]-1-ethynylcyclohex-1-ene (11c). Yield 90%. IR (NaCl): υ 3316, 2955, 2929, 2887, 2858, 2090, and 1630 cm^{-1}; $[\alpha]_{Na}^{20} - 70$ (*c* 0.9, $CHCl_3$); ^1H NMR (400.13 MHz, $CDCl_3$): δ 0.08 (s, 3H, Si*Me*), 0.09 (s, 3H, Si*Me*), 0.09 (s, 3H, Si*Me*), 0.10 (s, 3H, Si*Me*), 0.10 (s, 3H, Si*Me*), 0.11 (s, 3H, Si*Me*), 0.88 (s, 9H, SiC*Me*$_3$), 0.91 (s, 9H, SiC*Me*$_3$), 0.92 (s, 9H, SiC*Me*$_3$), 2.13 (dd, 1H, $H_{6e'}$, $|^2J_{HH}| = 16.4$, $^3J_{HH} = 4.7$ Hz), 2.42 (dd, 1H, $H_{6a'}$, $|^2J_{HH}| = 16.4$, $3J_{HH} = 9.4$ Hz), 2.86 (s, 1H, H_8), 3.68 (br s, 1H, H_4), 4.02 (m, 2H, H_3+H_5), and 5.94 (br s, 1H, H_2); ^{13}C NMR (100.6 MHZ, $CDCl_3$): δ −4.8 (Si*Me*), −4.7 (Si*Me*), −4.7 (Si*Me*), −4.6 (Si*Me*), −4.4 (Si*Me*), 18.0 (Si*C*), 18.1 (Si*C*), 18.3 (Si*C*), 25.8 (SiC*Me*$_3$), 26.0 (SiC*Me*$_3$), 34.1 (C_6), 67.1 (C_5), 70.0 (C_3), 71.2 (C_4), 74.8 (C_8), 84.4 (C_7), 121.2 (C_1), and 133.6 (C_2); (ESI^+, *m*/*z*) 519 [$(M+Na)^+$, 30%]; 535 [$(M+K)^+$, 5%].

5.6.4. (3*R*,4*S*,5*S*)-3,4,5-Tri[(*tert*-butyldimethylsilyl)oxy]-1-ethynylcyclohex-1-ene (11d). Yield 60%. Mp 93–95 °C. $[\alpha]_{Na}^{20} - 39$ (*c* 0.7, $CHCl_3$); IR: 3307, 2953, 2928, 2289, and 2857 cm^{-1}; ^1H NMR (400.13 MHz, $CDCl_3$): δ 0.07 (s, 3H, Si*Me*), 0.08 (s, 6H, 2Si*Me*), 0.10 (s, 3H, Si*Me*), 0.10 (s, 3H, Si*Me*), 0.11 (s, 3H, Si*Me*), 0.89 (s, 9H, SiC*Me*$_3$), 0.91 (s, 9H, SiC*Me*$_3$), 0.93 (s, 9H, SiC*Me*$_3$), 2.13 (dd, 1H, $H_{6e'}$, $|^2J_{HH}| = 16$, $^3J_{HH}$ 5.6 Hz), 2.47 (dddd, 1H, $H_{6a'}$, $|^2J_{HH}| = 16.4$, $^3J_{HH} = 10$, $|^4J_{HH}| = 3.2$, 3.2 Hz), 2.86 (s, 1H, H_8), 3.76 (ddd, 1H, H_5, $^3J_{HH} = 10$, 5.6, 1.2 Hz), 3.88 (br s, 1H, H_4), 4.28 (br s, 1H, H_3), and 5.81 (s, 1H, H_2); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ −4.7 (Si*Me*), −4.6 (Si*Me*), −4.6 (Si*Me*), −4.5 (Si*Me*), −4.4 (Si*Me*), 18.3 (Si*C*), 18.5 (Si*C*), 18.6 (Si*C*), 25.9 (SiC*Me*$_3$), 26.2 (SiC*Me*$_3$), 34.5 (C_6), 70.0 (C_5), 71.4 (C_3), 74.5 (C_4), 75.8 (C_8), 83.9 (C_7), 118.4 (C_1), and 137.5 (C_2); (ESI^+, *m*/*z*) 519 [$(M+Na)^+$, 100%].

5.7. Synthesis of 11d from 12

^{n}BuLi (1 mL of 1.6 M in hexane, 1.6 mmol) was added to a solution of **12** (363 mg, 0.55 mmol) in THF (5.5 mL) at −78 °C, and the resulting mixture was stirred during 1 h at the same temperature. The reaction mixture was allowed to reach room temperature and then quenched with NH_4Cl (aqueous). The moisture was extracted with Et_2O, and the combined organic fractions dried (Na_2SO_4) and concentrated. Flash chromatography of the residue using silica 60 Å (32–63 μm) pH 7 (gradient elution with hexane-1% Et_2O/hexane) afforded **11d** in 65% yield.

5.8. Synthesis of (3*R*,4*S*,5*S*)-3,4,5-tri[(*tert*-butyldimethylsilyl)oxy]-1-(2,2-dibromoethenyl)cyclohex-1-ene (12)

CBr_4 (370 mg, 1.11 mmol) was added to a solution of zinc (73 mg, 1.11 mmol) and Ph_3P (280 mg, 1.07 mmol) in CH_2Cl_2 (5.6 mL). The resulting suspension was stirred for 30 min at room temperature. Pyridine (178 μL) and a solution of compound **10d** (89 mg, 0.18 mmol) in CH_2Cl_2 (1.8 mL) were added stepwise to the reaction mixture and stirring was continued for 1 h additional. Work-up was accomplished by dilution of the mixture with Et_2O followed by filtration through a pad of silica gel. The filtrate was concentrated and the crude residue purified by flash chromatography (hexane) to afford **12** in 93% yield. Mp 73–75 °C; IR (KBr): υ 2953, 2921, 2889, and 2857 cm^{-1}; ^{1}H NMR (400.13 MHz, $CDCl_3$): δ 0.03 (s, 3H, Si*Me*), 0.02 (s, 3H, Si*Me*), 0.02 (s, 3H, Si*Me*), −0.01 (s, 3H, Si*Me*), 0.00 (s, 3H, Si*Me*), 0.01 (s, 3H, Si*Me*), 0.78 (s, 9H, SiC*Me*$_3$), 0.81 (s, 9H, SiC*Me*$_3$), 0.83 (s, 9H, SiC*Me*$_3$), 2.19 (dd, 1H, $H_{6e'}$, $|^2J_{HH}|$ = 11.2, $^3J_{HH}$ = 4 Hz), 2.45 (dddd, 1H, $H_{6a'}$, $|^2J_{HH}|$ = 10.8, $^3J_{HH}$ = 6.8, $|^4J_{HH}|$ = 2, 2 Hz), 3.65 (ddd, 1H, H_5, $^3J_{HH}$ = 6.8, 4, 1.2 Hz), 3.78 (s, 1H, H_4), 4.14 (s, 1H, H_3), 5.48 (s, 1H, H_2), and 6.77 (s, 1H, H_7); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ −4.7 (Si*Me*), −4.6 (Si*Me*), −4.6 (Si*Me*), −4.4 (Si*Me*), −4.4 (Si*Me*), −4.3 (Si*Me*), 18.3 (Si*C*), 18.6 (Si*C*), 18.7 (Si*C*), 26.0 (SiC*Me*$_3$), 26.2 (SiC*Me*$_3$), 33.2 (C_6), 70.4 (C_5), 71.4 (C_3), 74.5 (C_4), 87.5 (C_8), 132.9 (C_1), 133.2 (C_2), and 138.2(C_7); (ESI$^+$, *m*/*z*) 679 [($M^{79}Br^{81}Br$+Na)$^+$, 45%]; 681 [($M^{81}Br^{81}Br$+Na)$^+$, 35%], and 677 [($M^{79}Br^{79}Br$+Na)$^+$, 22%].

5.9. Synthesis of 14a–d

CuI (3.5 mg, 0.02 mmol), $(PPh_3)_2Pd(OAc)_2$ (4 mg, 0.005 mmol), and Et_2NH (1.4 mL) were added to a solution of **11** (97 mg, 0.20 mmol) and **13** (73 mg, 0.18 mmol) in DMF (1.4 mL). The reaction was monitored by TLC (hexane). After 5 h, the mixture was poured into water/Et_2O and the aqueous layer extracted with Et_2O. The combined organic fractions were dried (Na_2SO_4) and concentrated. TBAF (1.8 mL of 1.0 M in THF, 1.8 mmol) was added dropwise to a solution of this crude in THF (3.6 mL) at 0 °C and the reaction mixture was stirred overnight at room temperature. The crude residue was poured into water/EtOAc and the aqueous layer extracted with EtOAc. The combined organic fractions were concentrated and subjected to flash chromatography using silica 60 Å (32–63 μm) pH 7 (gradient elution with 30–50% acetone/CH_2Cl_2).

5.9.1. 1α,2α,25-Trihydroxy-6,7-dehydro-19-*nor*-previtamin D$_3$ (14a).

Yield 94%; ^{1}H NMR (300.13 MHz, MeOH-d_4): δ 0.75 (s, 3H, Me_{18}), 1.02 (d, 3H, Me_{21}, $^3J_{HH}$ = 6.4 Hz), 1.12 (m, 1H), 1.20 (s, 6H, Me_{26} + Me_{27}), 1.1–1.6 (m, 14H), 1.82 (m, 1H), 1.9–2.3 (m, 6H), 2.58 (dd, 1H, $H_{4e'}$, $|^2J_{HH}|$ = 17.4, $^3J_{HH}$ = 5.5 Hz), 3.59 (dd, 1H, H_2, $^3J_{HH}$ = 8.6, 4.0 Hz), 3.95 (ddd, 1H, H_3, $^3J_{HH}$ = 7.4, 7.4, 7.4 Hz), 4.29 (dd, 1H, H_1, $^3J_{HH}$ = 4.3, 4.3 Hz), and 5.94 (s, 2H, H_{10} + H_9). ^{13}C NMR (75.5 MHz, MeOH-d_4): δ 10.0 (C_{18}), 17.8 (C_{21}), 20.5 (CH_2), 23.8 (CH_2), 24.6 (CH_2), 27.6 (CH_2), 27.7 (CH_3), 27.9 (CH_3), 35.8 (CH_2), 36.1 (CH), 36.3 (CH_2), 36.4 (CH_2), 41.6 (C), 43.9 (CH_2), 49.9 (CH), 54.7 (CH), 66.4 (C_1 y C_3), 70.1 (C), 72.1 (C_2), 87.7 (C_6), 88.7 (C_7), 121.7 (C_5), 122.8 (C_2), 131.4 (C_9), and 133.4 (C_{10}). (ESI$^+$, *m*/*z*) 439 [(M+Na)$^+$, 100%], 440 [(M+H+Na)$^+$, 35], 455 [(M+K)$^+$, 15], and 417 [(M+H)$^+$, 13]. (EI$^+$, *m*/*z*): 416 [(M^+), 25%], 398 [(M^+-H_2O), 20], 380 [(M^+-2H_2O), 35], 179 (40), 165 (50), 105 (65), 91 (95), 69 (98), and 55 (100). HMRS Calcd for $C_{26}H_{40}O_4$ (M^+) 416.2921. Found 416.2926.

5.9.2. 1β,2α,25-Trihydroxy-6,7-dehydro-19-*nor*-previtamin D$_3$ (14b).

Yield 69%. ^{1}H NMR (400.13 MHz, MeOH-d_4): δ 0.73 (s, 3H, Me_{18}), 0.99 (d, 3H, Me_{21}, $^3J_{HH}$ = 6.8 Hz), 1.11 (m, 1H), 1.18 (s, 6H, Me_{26} + Me_{27}), 1.2–1.5 (m, 14H), 1.78 (ddd, 1H, $^3J_{HH}$ = 6.8, 6.8, 6.8 Hz), 1.9–2.1 (m, 2H), 2.2–2.3 (m, 4H), 2.47 (dd, 1H, H_{4e}, $|^2J_{HH}|$ = 16.4, $^3J_{HH}$ = 5.6 Hz), 3.35 (dd, 1H, H_2, $^3J_{HH}$ = 9.6, 7.6 Hz), 3.64 (ddd, 1H, H_3, $^3J_{HH}$ = 10, 10, 6 Hz), 4.07 (br s, 1H, H_1), 5.74 (dd, 1H, H_{10}, $^3J_{HH}$ = 2.4, $|^4J_{HH}|$ = 2.4 Hz), and 5.93 (s, 1H, H_9); ^{13}C NMR (100.6 MHz, MeOH-d_4): δ 11.4 (C_{18}), 19.2 (C_{21}), 21.9 (CH_2), 25.1 (CH_2), 26.0 (CH_2), 29.0 (CH_2), 29.1 (CH_3), 29.3 (CH_3), 37.2 (CH_2), 37.5 (CH), 37.7 (CH_2), 38.7 (CH_2), 42.9 (C), 45.3 (CH_2), 51.3 (CH), 56.1 (CH), 70.2 (C_3), 71.5 (C), 73.7 (C_1), 78.5 (C_2), 88.5 (C_6), 90.1 (C_7), 120.9 (C_5), 123.6 (C_8), and 134.8 (C_9 + C_{10}); (APCI$^-$, *m*/*z*) 399 [(M−OH)$^+$, 100%]; (EI$^+$, *m*/*z*): 416 [(M^+), 30%], 398 [($M-H_2O$)$^+$, 35], 380 [($M-2H_2O$)$^+$, 40], 165 (55), 129 (65), 91 (80), 69 (90), and 55 (100); HMRS Calcd for $C_{26}H_{40}O_4$ (M^+) 416.2921. Found 416.2918.

5.9.3. 1α,2β,25-Trihydroxy-6,7-dehydro-19-*nor*-previtamin D$_3$ (14c).

Yield 88%. ^{1}H NMR (400.13 MHz, MeOH-d_4): δ 0.77 (s, 3H, Me_{18}), 1.03 (d, 3H, Me_{21}, $^3J_{HH}$ = 8.9 Hz), 1.12 (m, 1H), 1.22 (s, 6H, Me_{26} + Me_{27}), 1.3–1.6 (m, 14H), 1.82 (ddd, 1H, $^3J_{HH}$ = 9, 9, 9 Hz), 1.9–2.1 (m, 2H), 2.2–2.3 (m, 3H), 2.35 (dd, 1H, $H_{4a'}$, $|^2J_{HH}|$ = 17.3, $^3J_{HH}$ = 5.6 Hz), 2.45 (m, 1H, $H_{4e'}$), 3.65 (dd, 1H, H_2, $^3J_{HH}$ = 5.6, 2 Hz), 4.04 (br s, 1H, H_3), 4.24 (br s, 1H, H_1), 5.87 (s, 1H, H_{10}), and 5.95 (s, H, H_9); ^{13}C NMR (100.6 MHz, MeOH-d_4): δ 11.4 (C_{18}), 19.2 (C_{21}), 21.9 (CH_2), 25.2 (CH_2), 26.0 (CH_2), 29.0 (CH_2), 29.1 (CH_3), 29.3 (CH_3), 36.7 (CH_2), 37.2 (CH_2), 37.5 (CH), 37.7 (CH_2), 42.9 (C), 45.2 (CH_2), 51.3 (CH), 56.0 (CH), 68.6 (C_3), 70.6 (C_1) 71.4 (C), 74.8 (C_2), 89.2 (C), 89.8 (C), 122.0 (C), 123.6 (C), 133.1 (C_{10}), and 134.6 (C_9); (ESI$^+$, *m*/*z*) 439 [(M+Na)$^+$, 95%], 855 [(2M+Na)$^+$, 100%]; (EI$^+$, *m*/*z*): 416 [(M^+), 10%], 398 [(M^+-H_2O), 75], 380 [(M^+-2H_2O), 60], 165 (70), 129 (75), 91 (93), 69 (96), and 59 (100); HMRS Calcd for $C_{26}H_{40}O_4$ (M^+) 416.2921. Found 416.2917.

5.9.4. 1α,2α,25-Trihydroxy-6,7-dehydro-3-*epi*-19-*nor*-previtamin D_3 (14d). Yield 60%. ^{1}H NMR (400.13 MHz, MeOH-d_4): δ 0.73 (s, 3H, Me_{18}), 0.99 (d, 3H, Me_{21}, $^3J_{HH}$ = 6.8 Hz), 1.09 (m, 1H), 1.19 (s, 6H, Me_{26} + Me_{27}), 1.2–1.6 (m, 14H), 1.78 (dd, 1H, $^3J_{HH}$ = 17.6, 11.2 Hz), 1.9–2.3 (m, 6H), 2.40 (dddd, 1H, H_{4a}, $|^2J_{HH}|$ = 16.3, $^3J_{HH}$ = 8.8, $|^4J_{HH}|$ = 2.4, 2.4 Hz), 3.84 (ddd, 1H, H_3, $^3J_{HH}$ = 8.4, 5.6, 1.6 Hz), 3.90 (br s, 1H, H_2), 4.24 (br s, 1H, H_1), 5.76 (br s, 1H, H_{10}), and 5.92 (m, 1H, H_9); ^{13}C NMR (100.6 MHz, MeOH-d_4): δ 11.4 (C_{18}), 19.2 (C_{21}), 21.9 (CH_2), 25.2 (CH_2), 26.0 (CH_2), 29.0 (CH_2), 29.1 (CH_3), 29.3 (CH_3), 35.0 (CH_2), 37.2 (CH_2), 37.5 (CH), 37.7 (CH_2), 42.9 (C), 45.2 (CH_2), 51.3 (CH), 56.1 (CH), 69.3 (C_3), 69.6 (C_1), 71.5 (C), 72.0 (C_2), 89.0 (C_6), 89.9 (C_7), 121.7 (C_5), 123.7 (C_8), 133.8 (C_{10}), and 134.5 (C_9); (APCI$^-$, *m*/*z*) 448 [(M+MeOH)$^+$, 100%]; 415 [(M−H)$^+$, 65%]; (EI$^+$, *m*/*z*): 416 [(M$^+$), 30%], 398 [(M−H_2O)$^+$, 40], 380 [(M−2H_2O)$^+$, 65], 165 (73), 91 (98), and 59 (100); HMRS Calcd for $C_{26}H_{40}O_4$ (M$^+$) 416.2921. Found 416.2917.

6. In vitro biological evaluation

6.1. Cell proliferation assay

As a measure of cell proliferation, [^{3}H]thymidine incorporation of breast cancer MCF-7 (ATCC, Rockville, MD) was determined after a 72-h incubation period with various concentrations of 1α,25-$(OH)_2$-D_3, analogues or vehicle as described previously.[24]

6.2. Binding studies

The affinity of 1α,25-$(OH)_2$-D_3 and its analogues to the vitamin D receptor was evaluated by their ability to compete with [^{3}H]-1α,25-$(OH)_2$-D_3 for binding to high speed supernatant from intestinal mucosa homogenates obtained from normal pigs as described previously.[24] The relative affinity of the analogues was calculated from their concentration needed to displace 50% of [^{3}H]-1α,25-$(OH)_2$-D_3 from its receptor compared with the activity of 1α,25-$(OH)_2$-D_3 (assigned a 100% value).

Binding of vitamin D analogues to the human vitamin D binding protein (hDBP) was performed at 4 °C as described previously.[25]

Acknowledgments

Financial support of this work by the Spanish Ministerio de Educación y Ciencia (MEC, Project CTQ-2004-04185) and Principado de Asturias (FICYT, Project IB05-109) is gratefully acknowledged. S.F. thanks MEC for a personal grant (Ramón y Cajal Program). L.S.-A. thanks MEC for a pre-doctoral fellowship.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2007.03.058.

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