A-Ring Modified 2-Hydroxyethylidene Previtamin D_3 Analogues. Synthesis and Biological Evaluation[‡]

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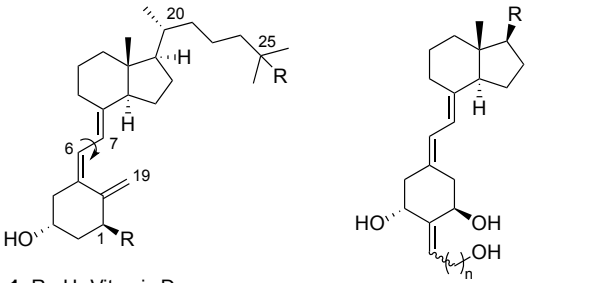
Abstract: To investigate the biological profile of the previtamin form of vitamin D, we have synthesized new analogues of 19-*nor*-1α,25-dihydroxyprevitamin D_3 bearing a 2-hydroxyethylidene moiety at the A-ring. The target compounds were prepared by convergent synthesis employing the Sonogashira coupling between an A-ring enyne synthon and a CD-ring/side chain vinyl triflate. We have demonstrated the versatility of shikimic acid as starting material for the synthesis of the A-ring precursors. The binding affinity to Vitamin D Receptor (VDR) and Vitamin D Binding Protein (DBP), and MCF-7 cell antiproliferative activity have been evaluated.

Introduction

Over the last three decades, more than 3,000 analogues of vitamin D_3 (**1**, Figure 1) have been synthesized because of the therapeutic applications of 1α,25-dihydroxyvitamin D_3 (**2**), the hormonally active form of vitamin D_3. In addition to its classical role in the regulation of calcium homeostasis and bone metabolism,[1] this hormone and its derivatives are associated with the inhibition of cell growth, the stimulation of cell differentiation, and the regulation of the immune system, among other activities.[2] Several laboratories have worked on the development of clinically useful vitamin D_3 drugs for a diverse set of disease indications. As a result, more than nine analogues are currently on the market in the United States, Japan, or Europe for clinical uses, with many more in development.[3]

The crystal structures of vitamin D_3 derivatives with different biological activities in complex with Vitamin D receptor (VDR) do not exhibit significant changes, suggesting the adaptability of the vitamin D_3 ligand along with the potential flexibility of the protein.[4] Several modifications in the vitamin D_3 skeleton have been reported.[5] 19-*nor*-Vitamin D_3 analogues (**3**), in which the exomethylene group at C-19 was removed, have received much attention as compounds characterized by dissociation of cell differentiation and calcemic activities.[6] In this context, introduction of substituents at the C-2 position of the A-ring causes changes in the biological profile compared with the parent 19-*nor*-vitamin D_3.[7] Among them, the group (2*E*)-hydroxyethylidene have been described to give place to stronger VDR affinity and ligand-dependent transcriptional activity compared with the natural 1α,25-dihydroxyvitamin D_3.[8] It was found that the incorporation of the (2*E*)-hydroxyethylidene substituent in 19-*nor*-vitamin D derivatives with a 22,24-diene, 22-oxa, or 16-ene-22-thia-26,27-dimethyl modifications showed higher potency in the transcriptional assay.[9] Furthermore, analogues bearing 20*S*-configuration presented much higher ability to induce osteoclast formation and inhibition of the expression of the dendritic cells maturation marker CD86 than their corresponding 20*R*-counterpart.[10]



1, R= H, Vitamin D_3
2, R= OH, 1α,25-$(OH)_2$- D_3

n= 1-2, R= 20*R* and 20*S* side chains

3, 2-hydroxyalkylidene-19-*nor*-D_3 analogues

Figure 1. Chemical structures of vitamin D_3 (**1**), 1α,25-dihydroxyvitamin D_3 (**2**), and 19-*nor* analogues (**3**).

The hormone 1α,25-dihydroxyvitamin D_3 exists in thermal equilibrium (5-10%) with 1α,25-dihydroxyprevitamin D_3.[11] Most of the developed analogues are focused in the vitamin D form due to the spontaneous isomerization of 1α,25-$(OH)_2$-pre-D_3 (**4**) to the more stable 1α,25-$(OH)_2$-D_3 (**2**) via [1,7]-sigmatropic hydrogen shift (Scheme 1). However, scarce examples of previtamin D analogues are described in the literature. These include the four A-ring diastereoisomers of 19-*nor*-1α,25-$(OH)_2$-pre-D_3,[12] a 9,19-methano-bridged analogue of 1α,25-$(OH)_2$-D_3,[13] and a series of 2-substituted derivatives of 14-*epi*-previtamin D_3,[14] in which the 14-*epi*-1α,25-$(OH)_2$-pre-D_3 form was major and dominant to 14-*epi*-1α,25-$(OH)_2$-D_3. In our laboratory, we have synthesized conformationally locked previtamin D_3 derivatives with a 2-hydroxy, 2β-(3'-hydroxypropoxy), or a 2β,3β-epoxy group.[15] To the best of our knowledge, the unique reported previtamin D analogue with activities equivalent to 1α,25-$(OH)_2$-D_3 is characterized by the presence of a *trans*-fused decalin CD-ring system.[16] This

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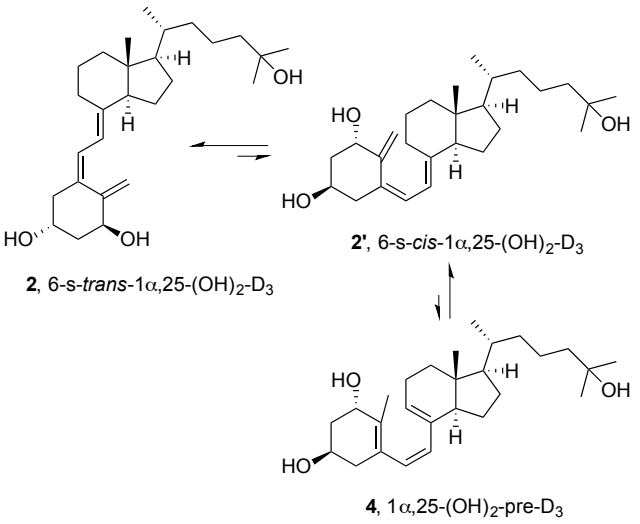
analogue, developed by Bouillon and coworkers, interacts as efficiently as the natural hormone with the VDR and uses the same contact points within the receptor as 1α,25-$(OH)_2$-D_3. Challenges remain in the design of new analogs and the evaluation of its biological actions in order to investigate structure-activity relationships.



Scheme 1. Equilibrium of 1α,25-dihydroxyvitamin D_3 (**2**) and 1α,25-dihydroxyprevitamin D_3 (**4**).

On the basis of the above mentioned considerations and our ongoing interest in the preparation and biological evaluation of vitamin D_3 derivatives,[17] herein, we report the synthesis and biological activities of a series of 19-*nor*-previtamin D_3 analogues (**5**, **6**, and **7**, Figure 2) that possess the hydroxyethylidene function in different positions of the A-ring. The synthetic strategy involves the coupling of a dienyne precursor of the A-ring with an enol triflate of the CD-ring/side chain fragment.

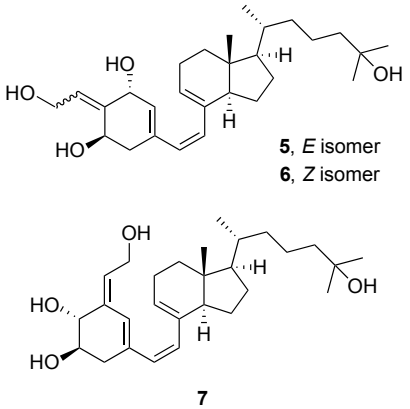
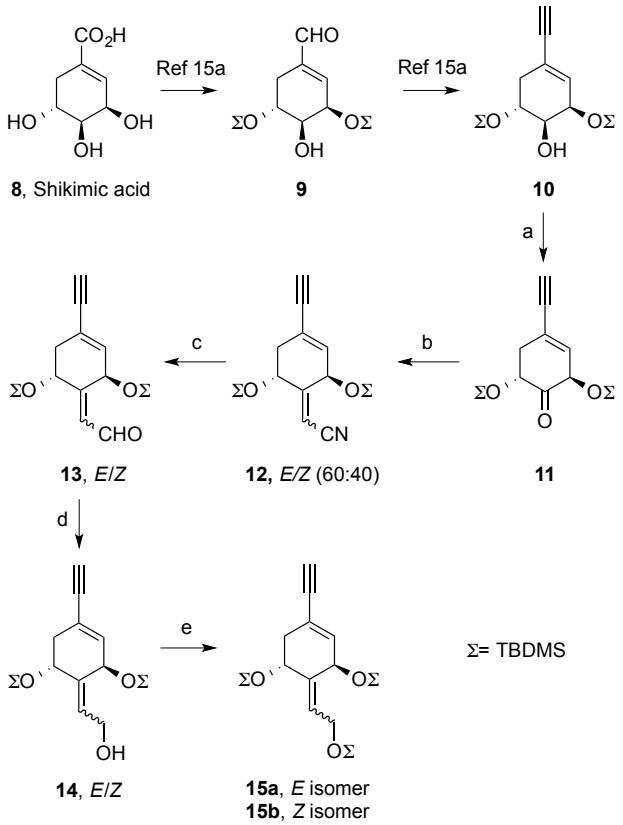


Figure 2. Targeted hydroxyalkyliden-19-*nor*-previtamin D_3 analogues.

Results and Discussion

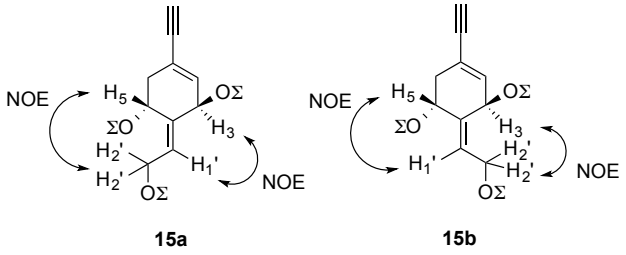
Chemistry. The A-ring precursors were synthesized starting from commercially available shikimic acid (**8**, Scheme 2). The selective protection of the 3- and 5-hydroxy groups and the reduction of the ester to aldehyde, as previously described,[15a] generated compound **9**. Transformation of the latter into the enyne **10** was accomplished with a 56% yield by reaction with trimethylsilyldiazomethane. For the introduction of the hydroxyethylidene function, the free OH group in C-4 was oxidized with Dess-Martin periodinane reagent, affording ketone **11** as a single product in the TLC. This compound showed to be unstable to standard purification by column chromatography, being isolated with a 78% yield (neutral silica gel).



Scheme 2. Reagents and conditions: a) DMP, CH_2Cl_2, rt, overnight, 78%; b) $(EtO)_2P(O)CH_2CN$, ^{n}BuLi, THF, –40 °C, 1 h, 82%; c) DIBAL-H, toluene, –78 °C, 1 h, 65%; d) $NaBH_4$, EtOH, 0 °C, 45 min, 78%; e) TBDMSCl, imidazole, CH_2Cl_2, 0 °C to rt, 1 h, 97%.

Cyanomethylation of **11** with the ylide generated from diethyl(cyanomethyl)phosphonate and *n*-butyllithium gave derivative **12** as an approximately 60:40 mixture of *E* and *Z* isomers and 82% yield. Both isomers were reduced with diisobutylaluminium hydride followed by sodium borohydride to afford 2-(2-hydroxyethylidene) derivative **14**. Protection of the free hydroxy group of **14** with *tert*-butyldimethylsilyl chloride yielded the desired A-ring synthons **15a** (*E* isomer) and **15b** (*Z*

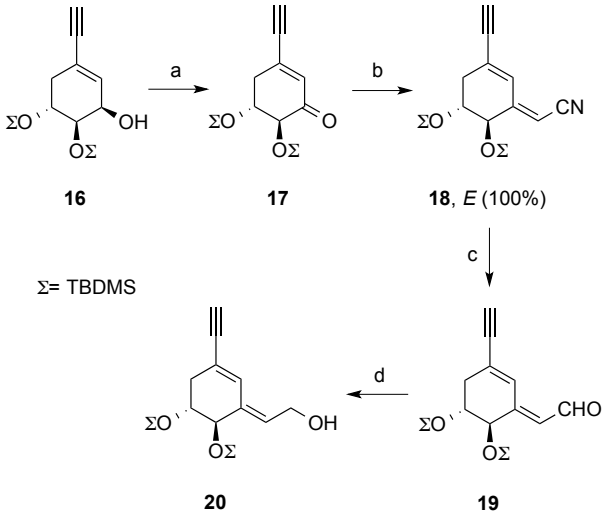
isomer), which could be separated by semipreparative HPLC in excellent yield. Configuration of the ethylidene unit was unambiguously determined by 2D NOESY spectroscopy experiments (Figure 3). In **15a**, a correlation cross peak was observed between allylic proton H-3 and vinyl proton H-1'. In addition, NOE was detected between H-5 and H-2'. From these results, the stereochemistry of the C-2 substituent of **15a** were determined to be *E*. On the other hand, derivative **15b** showed a cross peak between H-3 and H-2', which confirmed a *Z* configuration. A weak NOE connectivity between H-5 and H-1' corroborated the assigned stereochemistry for **15b**.



15a **15b**

Figure 3. NOE connectivities for **15a** and **15b**.

In the scale-up preparation of compound **10**, migration of the *O*-silyl group was occasionally observed, giving a mixture of 3,5- and 4,5-disilyl ethers. This migration occurs typically under basic conditions and proceeds intramolecularly through a pentacoordinate silicon intermediate.[18] Therefore, we postulate that, since the ^{n}BuLi used in the transformation of **9** to **10** also contains LiOH to a greater or lesser extent, depending on the ^{n}BuLi batch, different ratio of regioisomers from 99:1 to 1:1 (**10**:**16**) was observed. Both derivatives could be isolated by semipreparative HPLC chromatography for characterization.

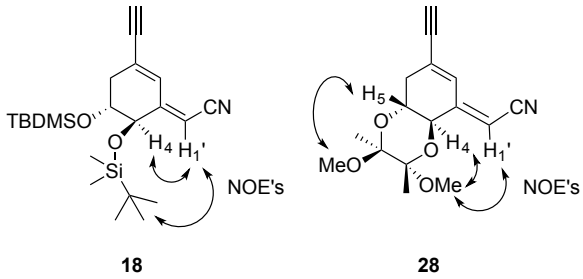


16 **17** **18**, *E* (100%) **20** **19**

Scheme 3. Reagents and conditions: a) MnO_2, CH_2Cl_2, rt, 16 h, 86%; b) $(EtO)_2P(O)CH_2CN$, ^{n}BuLi, THF, –40 °C, 2.5 h, 89%; c) DIBAL-H, toluene, –78 °C, 1 h, 85%; d) $NaBH_4$, EtOH, 0 °C, 45 min, 65%.

For a practical separation of the unwanted isomer **16**, a selective oxidation of the allylic alcohol in C-3 was performed with manganese dioxide to provide the ketone **17**, which could be separated from the alcohol **10** via standard column chromatography. With the compound **17** in hand, we explored the synthesis of the A-ring precursor having a 2-hydroxyethylidene function attached to the allylic position (Scheme 3). Thus, the Wittig-Horner reaction of compound **17** with diethyl(cyanomethyl)phosphonate produced selectively **18** as the only isomer.

The stereochemistry of the 2-hydroxyethylidene moiety of **18** was determined by analysis of 2D NOESY spectrum. NOE was observed between H-1' and H-4, while a correlation cross peak was detected between H-1' and the *tert*-butyl of the TBDMS group (Figure 4), so this isomer must have the *E* configuration. Next, derivative **18** was treated with DIBAL-H, and the resulting aldehyde **19** was transformed into the alcohol **20** by treatment with $NaBH_4$.

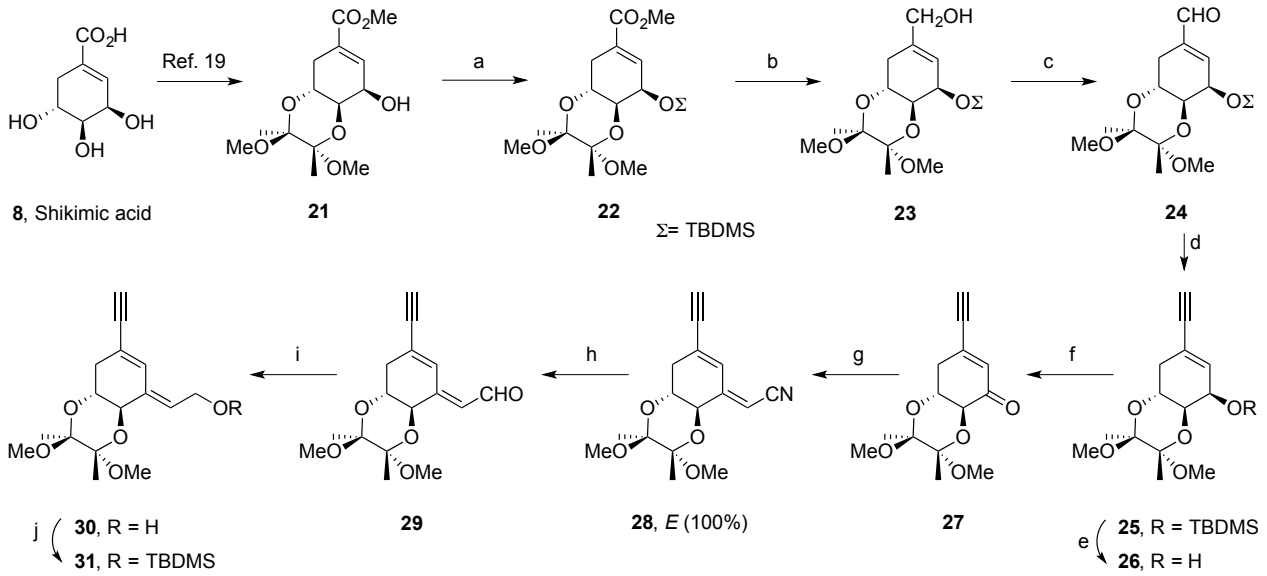


18 **28**

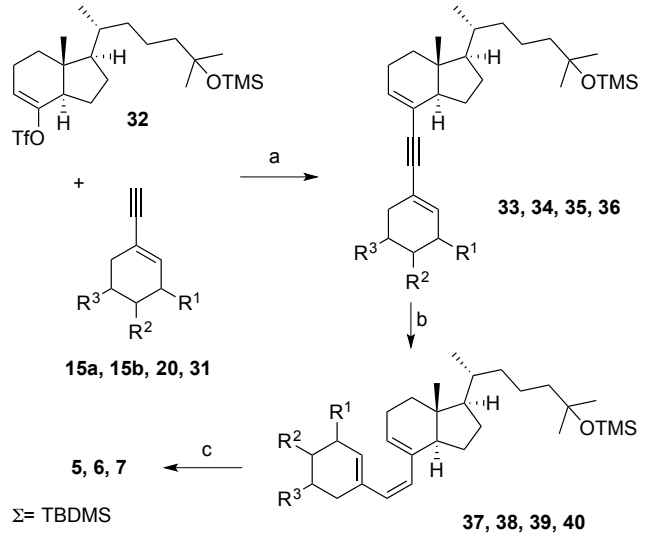
Figure 4. NOE connectivities for **18** and **28**.

We also explored an alternative pathway to the C-3 functionalized A-ring precursor from shikimic acid using our reported protocol[19] for the one-pot selective protection of the *trans*-1,2-diol. The resulting alcohol **21** was protected as the *tert*-butyldimethylsilyl ether, and the ester **22** was transformed into the aldehyde **24** by reduction with DIBAL-H and subsequent oxidation of the alcohol **23** with Dess-Martin periodinane. Treatment of **24** with trimethylsilyldiazomethane provided **25** in 65% yield. Removal of the silyl protecting group followed by oxidation of the hydroxy group gave the corresponding keto compound **27**. The latter underwent cyanomethylation with diethyl(cyanomethyl)phosphonate to afford exclusively 2-hydroxyethylidene derivative **28**. The structure of **28** was proven by NOESY experiments (Figure 4). The cross peaks observed between H-4/H-5 and the OMe groups of the bisacetal allowed the assignment of the OMe groups. The NOE detected between H-1' and the OMe that correlated with H-4 supported the *E* stereochemistry. Transformation of **28** into **31** was carried out utilizing the same sequence of reactions as described in Scheme 2 for **15**.

The synthesis of previtamin D_3 analogues was carried out by Sonogashira reaction as outlined in Scheme 5. Coupling of A-ring synthons **15a**, **15b**, **20**, and **31** with the CD-ring/side chain triflate **32**[13,20] in the presence of bis(triphenylphosphine)-



Scheme 4. Reagents and conditions: a) TBDMSCl, imidazole, CH_2Cl_2, 0 °C to rt, 16 h, 94%; b) DIBAL-H, Et_2O, –78 °C, 4 h, 85%; c) DMP, CH_2Cl_2, rt, 2 h, 94%; d) $TMSCHN_2$, ^{n}BuLi, THF, –78 °C to rt, 1 h, 65%; e) TBAF, THF, 0 °C to rt, 4 h, 95%; f) from **26**, DMP, CH_2Cl_2, rt, 2 h, 91%; g) $(EtO)_2P(O)CH_2CN$, ^{n}BuLi, THF, –40 °C, 4 h, 80%; h) DIBAL-H, toluene, –78 °C, 1 h, 88%; i) $NaBH_4$, EtOH, 0 °C, 45 min, 75%; j) TBDMSCl, imidazole, CH_2Cl_2, 0 °C to rt, 1 h, 80%.



15a, 33, 37: R^1= (*R*)-OΣ, R^2= (*E*)-=$CHCH_2O\Sigma$, R^3= (*R*)-OΣ
15b, 34, 38: R^1= (*R*)-OΣ, R^2= (*Z*)-=$CHCH_2O\Sigma$, R^3= (*R*)-OΣ
20, 35, 39: R^1= (*E*)-=$CHCH_2OH$, R^2= (*R*)-OΣ, R^3= (*R*)-OΣ
31, 36, 40: R^1= (*E*)-=$CHCH_2O\Sigma$, R^2R^3= 2,3-dimethoxybutanebisacetal

Sheme 5. Reagents and conditions: a) $(PPh_3)_2Pd(OAc)_2$, CuI, Et_2NH, DMF, 2 h, 70% for **33**, 90% for **34**, 65% for **35**, 60% for **36**; b) H_2, Lindlar catalyst, quinoline, hexane, 4 h, 90% for **38**, 60% for **37**, 90% for **38**, 70% for **39**, 75% for **40**; c) TBAF, THF, 0 °C to rt, 4-18 h, 90% for **5**, 60% for **6**, 85% for **7**.

palladium (II) acetate-copper (I) iodide catalyst yielded the dienynes **33**, **34**, **35**, and **36**, respectively. Next, catalytic hydrogenation with Lindlar catalyst generated derivatives **37**, **38**, **39**, and **40**. Deprotection of the silyloxy groups with TBAF afforded the desired previtamins **5**, **6**, and **7**. Several reagents were tested for the removal of the bisacetal protecting group in **40**. Among them, trifluoroacetic acid, piridinium *p*-toluensulphonate, trimethylsilylbromide and iron (III) chloride. However, the previtamin derivative showed to be unstable under these conditions, not being possible the access to **7** through this route.

Table 1. Biological activities of A-ring modified 2-hydroxyethylidene previtamin D_3 analogues.[a]

Compound	VDR (%)	hDBP (%)	MCF-7 (%)
1α,25-$(OH)_2$-D_3	100	100	100
(2*E*)-hydroxyethylidene-19-*nor*-1α,25-$(OH)_2$-D_3	200[b]	---	---
5	0	3	4
7	0	2	0

[a] Values are expressed as percentages of activity EC_{50} concentration relative to 1α,25-$(OH)_2$-D_3 (100%). [b] Reference 8.

Biological Evaluation. The biological activity of the newly synthesized analogues was tested *in vitro*, and the results are summarized in Table 1 and Figures 5-7.

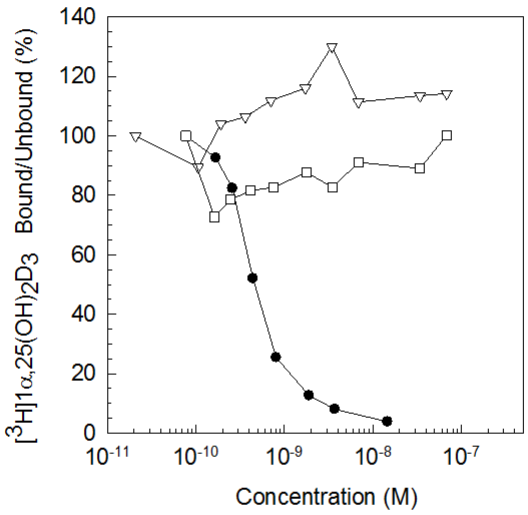


Figure 5. Affinity of 1α,25-$(OH)_2$-D_3 and 2-hydroxyethylidene previtamin D_3 analogues of 1α,25-$(OH)_2$-pre-D_3 for pig vitamin D receptor (VDR). Notes: 1α,25-$(OH)_2$-D_3 (●); **5** (∇); **7** (□).

For this purpose we evaluated the affinity to pig mucosa cytosol VDR and to human Vitamin D Binding Protein (hDBP). Next we tested the potency of both compounds to inhibit the proliferation of human breast adenocarcinoma MCF-7 cells. Modification by the (2*E*)-hydroxyethylidene group at 19-*nor*-1α,25-$(OH)_2$-pre-D_3 eliminated the affinity to VDR with respect to (2*E*)-hydroxy-ethylidene-19-*nor*-1α,25-$(OH)_2$-D_3, which was about two-fold as active as the natural hormone.[8]

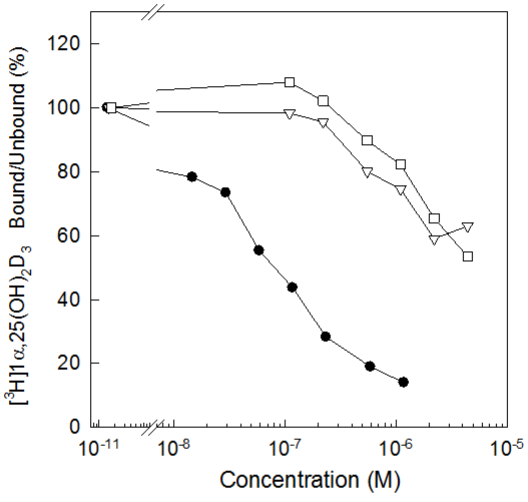


Figure 6. Affinity of 1α,25-$(OH)_2$-D_3 and 2-hydroxyethylidene previtamin D_3 analogues of 1α,25-$(OH)_2$-pre-D_3 for human vitamin D binding protein (hDBP). Notes: 1α,25-$(OH)_2$-D_3 (●); **5** (∇); **7** (□).

These results indicate that 6-s-*cis* conformer **5** do not have the ability as the 6-s-*trans* isomer to access the VDR binding pocket.[21] Compound **7**, possessing a (*E*)-hydroxyethylidene group instead of the 1α-hydroxyl group, was also not binding the VDR (Figure 5). A similar pattern of relative activities of both derivatives was found in the ability to bind the hDBP protein. Derivatives **5** and **7** showed a significantly reduced affinity compared to 1α,25-$(OH)_2$-D_3 (Figure 6). The (2*E*)-hydroxyethylidene previtamin D_3 analogue **5** was 25 times less potent than 1α,25-$(OH)_2$-D_3 to inhibit the proliferation of MCF-7 cells. Derivative **7** with the modification at C-1 demonstrated no effects on the MCF-7 cell proliferation (Figure 7).

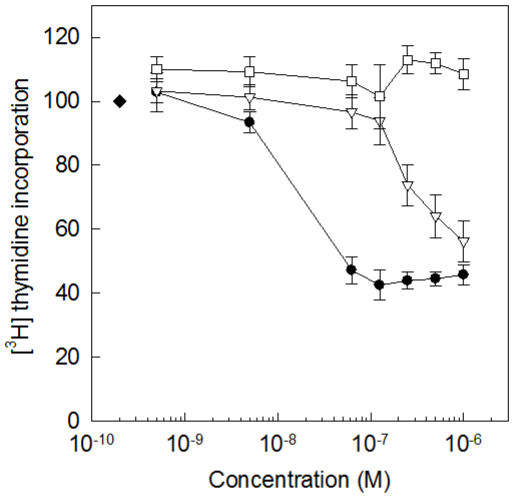


Figure 7. In vitro antiproliferative effects of 1α,25-$(OH)_2$-D_3 and 2-hydroxyethylidene previtamin D_3 analogues of 1α,25-$(OH)_2$-pre-D_3 on breast cancer MCF-7 cells. Notes: Vehicle (◆); 1α,25-$(OH)_2$-D_3 (●) ; **5** (∇); **7** (□).

Conclusions

In the present study, we have described the synthesis of new locked previtamin D derivatives with a hydroxyethylidene moiety at the A-ring. These novel target compounds have been synthesized in order to investigate important structure-activity features. All analogues were successfully obtained through convergent synthesis, starting from shikimic acid, for the preparation of the A-ring precursors, and the vinyl triflate of the CD-ring/side chain fragment. The hydroxyethylidene group was introduced by a Wittig-Horner reaction. NOESY spectroscopy experiments were used to assign the stereochemistry of the compounds. Their biological activity profiles were assessed in terms of affinity for the VDR and DBP, and effects on MCF-7 cell proliferation. The C-2 substituted 6-s-*cis* analogue **5** showed no affinity for the VDR but demonstrated very low affinity to hDBP and some inhibitory effect on MCF-7 cell proliferation, meanwhile analogue with a hydroxyethylidene group at the 1-position as in compound **7** was unable to bind VDR and devoid of *in vitro* antiproliferative activity on MCF-7 cells.

Experimental Section

General

All reagents were bought from Aldrich at highest commercial quality and used without further purification. All non-aqueous reactions were carried out under anhydrous conditions in dry, freshly distilled solvents. Reactions were monitored by TLC carried out on 0.25 mm Merck silica gel plates (60F-254) using UV light as visualizing agent and/or acidic aqueous permanganate. Flash chromatography was performed using silica gel 60 (230–400 mesh). Melting points were taken on samples in open capillary tubes and are uncorrected. IR spectra were recorded as thin films on NaCl plates on an Infrared FT spectrophotometer (Agilent or Perkin Elmer). NMR spectra of ^{1}H, ^{13}C and DEPT were obtained using Bruker 300.13, 400.13 or 600.13 MHz for ^{1}H, and 75.5, 90.61 MHz or 150.90 for ^{13}C. The same spectrometers were used for the acquisition of ^{1}H-^{1}H homonuclear (COSY and NOESY) and ^{1}H-^{13}C heteronuclear (HSQC and HMBC) correlations. Mass spectra (MS) and high resolution mass spectra (HRMS) were recorded, respectively, on a Hewlett-Packard 1100 and Bruker MicrOTOF-Q mass spectrometers under electrospray ionization (ESI) conditions.

Compounds **9**,[15a] **10**,[15a] **21**,[19] **22**,[22] and **23**[22] have been previously described. Full analytical data for new compounds are described in the Supporting Information.

(*E*)-2-(2-Hydroxyethyliden)-19-*nor*-1α,25-dihydroxyprevitamin D$_3$ (5). To a stirred solution of **37** (11.5 mg, 0.013 mmol) in anhydrous THF (270 µL) at 0 ºC and in darkness, was added dropwise TBAF (117 µL, 1 M in THF, 0.117 mmol). After 5 min, the ice bath was removed and the reaction was stirred at room temperature overnight. Next, water was added and the mixture was extracted with EtOAc. The resulting organic layer was dried over Na_2SO_4 and evaporated to dryness. The residue was purified by column chromatography on silica gel using 100% EtOAc to afford **5** (5.4 mg, 90% yield) as a white solid. ^{1}H-NMR (400.13 MHz, acetone-d_6): δ 0.75 (s, 3H, *Me*$_{18}$), 0.99 (d, 3H, *Me*$_{21}$, *J* 6.4 Hz), 1.15 (s, 6H, *Me*$_{26}$+*Me*$_{27}$), 0.84-2.65 (several m), 3.11 (br s, 1H, *OH*), 3.65 (br s, 1H, *OH*), 3.89 (br s, 1H, *OH*), 4.02 (br s, 1H, *OH*), 4.22 (m, 2H, H$_{2'}$), 4.87 (s, 2H, H$_1$+H$_3$), 5.37 (s, 1H, H$_9$) and 5.72-5.87 (several m, 4H, H$_{1'}$, H$_{10}$, H$_7$ and H$_6$) ppm; HRMS (ESI$^+$, m/z): 467.3126 (Calcd for $C_{28}H_{44}NaO_4$: 467.3132).

(*Z*)-2-(2-Hydroxyethyliden)-19-*nor*-1α,25-dihydroxyprevitamin D$_3$ (6). A procedure analogous to that described for the synthesis of **5**, starting from **38** (17 mg, 0.020 mmol), afforded **6** (5.3 mg, 60% yield) as a white solid (eluent gradient for column chromatography: 90% EtOAc/hexanes - 2% MeOH/EtOAc). ^{1}H-NMR (400.13 MHz, acetone-d_6): δ 0.79 (s, 3H, *Me*$_{18}$), 0.99 (d, 3H, *Me*$_{21}$, *J* 6.5 Hz), 1.15 (s, 6H, *Me*$_{26}$+*Me*$_{27}$), 0.84-2.66 (several m), 2.63 (dd, 1H, H$_4$, *J* 16.1, 5.4 Hz), 3.10 (s, 1H, *OH*), 3.64 (t, 1H, *OH*, *J* 5.5 Hz), 3.93 (d, 1H, *OH*, *J* 5.4 Hz), 4.02 (d, 1H, *OH*, *J* 5.4 Hz), 4.22 (apparent t, 2H, H$_{2'}$, *J* 5.3 Hz), 4.42 (m, 1H, H$_3$), 4.97 (t, 1H, H$_1$, *J* 4.2 Hz), 5.38 (s, 1H, H$_9$), 5.73-5.94 (several m, 4H, H$_{1'}$, H$_{10}$, H$_7$ and H$_6$) ppm; HRMS (ESI$^+$, m/z): 467.3142 (Calcd for $C_{28}H_{44}NaO_4$: 467.3132).

(*E*)-1-(2-Hydroxyethyliden)-19-*nor*-2α,25-dihydroxyprevitamin D$_3$ (7). A procedure analogous to that described for the synthesis of **5**, starting from **39** (6 mg, 0.008 mmol), afforded **7** (3 mg, 85% yield) as a white solid (eluent for column chromatography: 100% EtOAc). The reaction was monitored by TLC (20% Et_2O/hexanes, 3-4 h). ^{1}H-NMR (600.13 MHz, acetone-d_6): δ 0.79 (s, 3H, *Me*$_{18}$), 0.82-2.31 (several m), 0.99 (d, 3H, *Me*$_{21}$, *J* 6.6 Hz), 1.15 (s, 6H, *Me*$_{26}$+*Me*$_{27}$), 2.36 (dd, 1H, H$_{4ax}$, *J* 17.5, 9.1 Hz), 2.65 (dd, 1H, H$_{4ec}$, *J* 17.1, 5.1 Hz), 3.09 (s, 1H, *OH*), 3.49 (s, 1H, *OH*), 3.60 (apparent t, 1H, H$_5$, *J* 5.5 Hz), 3.92 (br s, 2H, *OH*), 4.08 (br s, 1H, H$_4$), 4.23 (m, H, H$_{2'}$), 4.30 (m, H, H$_{2'}$), 5.42 (s, 1H, H$_9$), 5.79 (d, 1H, H$_7$, *J* 11.8 Hz), 5.85 (t, 1H, H$_{1'}$, *J* 6.8 Hz), 5.94 (d, 1H, H$_6$, *J* 12.2 Hz), 6.42 (s, 1H, H$_{10}$) ppm; HRMS (ESI$^+$, m/z): 467.3157 (Calcd for $C_{28}H_{44}NaO_4$: 467.3132).

(3*R*,5*R*)-3,5-Di[(*tert*-butyldimethylsilyl)oxy]-1-ethynyl-4-oxo-1-cyclohexene (11). To a stirred solution of **10** (185 mg, 0.484 mmol) in anhydrous CH_2Cl_2 (3.2 mL) was added Dess-Martin periodinane (308 mg, 0.726 mmol). The resulted suspension was stirred overnight at room temperature. The solution was then diluted with Et_2O (6 mL) and a 1:1 mixture (v/v, 6 mL) of saturated aqueous $Na_2S_2O_3$ and saturated $NaHCO_3$, generating a white suspension which turned clear after ten minutes. After extraction of the aqueous layer with Et_2O, the resulting organic layer was dried (Na_2SO_4) and evaporated to dryness. The residue was purified by flash chromatography on silica gel 60 Å (32-63 µm) pH 7 using 1-2% Et_2O/hexanes to afford ketone **11** (144 mg, 78%) as a colorless thick oil. ^{1}H-NMR (300.13 MHz, $CDCl_3$): δ 0.08 (s, 3H, Si*Me*), 0.10 (s, 6H, Si*Me*$_2$), 0.12 (s, 3H, Si*Me*), 0.90 (s, 9H, SiC*Me*$_3$), 0.91 (s, 9H, SiC*Me*$_3$), 2.60 (ddt, 1H, H$_6$, *J* 17.5, 6.5, 1.2 Hz), 2.82 (ddt, 1H, H$_6$, *J* 17.4, 5.7, 1.4 Hz), 2.94 (s, 1H, H$_8$), 4.55 (dd, 1H, H$_5$, *J* 6.5, 5.7 Hz), 4.78 (apparent d, 1H, H$_3$, *J* 3.8 Hz) and 6.08 (m, 1H, H$_2$) ppm; MS (ESI$^+$, m/z): 419 [(M+K)$^+$, 100%], 403 [(M+Na)$^+$, 60%].

(*E*)- and (*Z*)-(3*R*,5*R*)-3,5-Di[(*tert*-butyldimethylsilyl)oxy]-4-cyanomethylidene-1-ethynyl-1-cyclohexene (12). To a stirred solution of diethyl (cyanomethyl)phosphonate (419 mg, 2.367 mmol) in anhydrous THF (10 mL) at -40 ºC was added ^{n}BuLi (1.3 mL, 1.6 M in hexanes, 2.129 mmol). The mixture was stirred for 15-20 min after which time a solution of **11** (450 mg, 1.183 mmol) in anhydrous THF (10 mL) was added dropwise. The resulting deep red solution was stirred at -40 ºC for additionally 1 h, after which the mixture was quenched with saturated NH_4Cl and extracted with EtOAc. The organic layer was dried over Na_2SO_4, filtered and concentrated under vacuum. The residue was purified by flash chromatography on silica gel using 1-3% Et_2O/hexanes to afford **12** (392 mg, 82%) as a white solid mixture of diastereoisomers *E*/*Z* in a ratio 60:40. ^{1}H-NMR (300.13 MHz, $CDCl_3$): *(E-isomer)* δ 0.09-0.17 (several s, 12H, 4Si*Me*), 0.89-0.94 (several s, 18H, 2SiC*Me*$_3$), 2.42 (d, 1H, H$_6$, *J* 17.9 Hz), 2.60 (m, 1H, H$_6$), 2.89 (s, 1H, H$_8$), 5.06 (dd, 1H, H$_5$, *J* 3.3, 2.3 Hz), 5.20 (sa, 1H, H$_3$), 5.46 (d, 1H, H$_{1'}$, *J* 2.0 Hz) and 5.94 (sa, 1H, H$_2$) ppm; *(Z-isomer)* δ 0.09-0.17 (several s, 12H, 4Si*Me*), 0.89-0.94 (several s, 18H, 2SiC*Me*$_3$), 2.21 (ddd, 1H, H$_{6ax}$, *J* 15.6, 9.5, 1.6 Hz), 2.66 (dd, 1H, H$_{6ec}$, *J* 16.6, 6.2 Hz), 2.94 (s, 1H, H$_8$), 4.77 (m, 1H, H$_5$), 5.12 (d, 1H, H$_3$, *J* 5.0 Hz), 5.58 (d, 1H, H$_{1'}$, *J* 1.8 Hz) and 6.08 (dd, 1H, H$_2$, *J* 4.7, 2.2 Hz) ppm; MS (ESI$^+$, m/z): 426 [(M+Na)$^+$, 100%].

(*E*)- and (*Z*)-(3*R*,5*R*)-3,5-Di[(*tert*-butyldimethylsilyl)oxy]-1-ethynyl-4-formylmethylidene-1-cyclohexene (13). To a stirred solution of **11** (50 mg, 0.123 mmol) in anhydrous toluene (1.2 mL) at -78 ºC was added dropwise DIBAL-H (370 µL, 1 M in toluene, 0.370 mmol). The mixture was stirred at this temperature for 1 h and diluted with hexane. The solution was loaded directly on top of a silica gel column and eluted with 2-3% Et_2O/hexanes, to give **13** (33 mg, 65%) as a white solid (*E*/*Z* 60:40). ^{1}H-NMR (300.13 MHz, $CDCl_3$): *(E-isomer)* δ 0.06-0.13 (several s, 12H, 4Si*Me*), 0.88-0.94 (several s, 18H, 2SiC*Me*$_3$), 2.47 (dd, 1H, H$_6$, *J* 17.6, 3.6 Hz), 2.63 (m, 1H, H$_6$), 2.89 (s, 1H, H$_8$), 5.21 (br s, 1H, H$_3$), 5.51 (apparent t, 1H, H$_5$, *J* 3.8 Hz), 6.02 (br s, 1H, H$_2$), 6.12 (m, 1H, H$_{1'}$) and 10.17 (d, 1H, H$_{2'}$, *J* 7.3 Hz) ppm; *(Z-isomer)* δ 0.06-0.13 (several s, 12H, 4Si*Me*), 0.88-0.94 (several s, 18H, 2SiC*Me*$_3$), 2.27 (ddd, 1H, H$_{6ax}$, *J* 16.8, 9.5, 2.4 Hz), 2.68 (m, 1H, H$_{6ec}$), 2.94 (s, 1H, H$_8$), 4.81 (ddd, 1H, H$_5$, *J* 9.2, 6.4, 1.5 Hz), 5.65 (d, 1H, H$_3$, *J* 5.1 Hz), 6.12 (m, 1H, H$_2$), 6.25 (dd, 1H, H$_{1'}$, *J* 7.8, 1.5 Hz) and 10.11 (d, 1H, H$_{2'}$, *J* 7.8 Hz) ppm; MS (ESI$^+$, m/z): 429 [(M+Na)$^+$, 100%].

(*E*)- and (*Z*)-(3*R*,5*R*)-3,5-Di[(*tert*-butyldimethylsilyl)oxy]-1-ethynyl-4-(2-hydroxyethylidene)-1-cyclohexene (14). To a stirred solution of the

aldehyde **13** (117.8 mg, 0.290 mmol) in EtOH (3.6 mL) at 0 ºC was added portionwise $NaBH_4$ (11 mg, 0.290 mmol). After being stirred for 45 min at this temperature, some drops of cold water were added and the mixture was extracted with EtOAc. The resulting organic layer was dried (Na_2SO_4), filtered and concentrated under vacuum. The residue was purified by flash chromatography on silica gel using 10% EtOAc/hexanes to give **14** (92 mg, 78%) as a colorless oil (*E*/*Z* 60:40). ^{1}H-NMR (300.13 MHz, $CDCl_3$): *(E-isomer)* δ 0.07-0.12 (several s, 12H, 4Si*Me*), 0.87-0.94 (several s, 18H, 2SiC*Me*$_3$), 2.33 (dd, 1H, H_6, *J* 17.3, 4.1 Hz), 2.47 (m, 1H, H_6), 2.84 (s, 1H, H_8), 4.17 (dd, 1H, $H_{2'}$, *J* 12.9, 6.7 Hz), 4.32 (dd, 1H, $H_{2'}$, *J* 13.0, 6.8 Hz), 4.91 (apparent t, 1H, H_5, *J* 4.1 Hz), 4.97 (br s, 1H, H_3), 5.73 (apparent t, 1H, $H_{1'}$, *J* 6.7 Hz) and 6.05 (br s, 1H, H_2) ppm; *(Z-isomer)* δ 0.07-0.12 (several s, 12H, 4Si*Me*), 0.87-0.94 (several s, 18H, 2SiC*Me*$_3$), 2.16 (m, 1H, H_6), 2.55 (dd, 1H, H_6, *J* 16.7, 5.9 Hz), 2.89 (s, 1H, H_8), 4.26 (d, 2H, $H_{2'}$, *J* 6.9 Hz), 4.60 (m, 1H, H_5), 5.01 (d, 1H, H_3, *J* 4.9 Hz), 5.85 (t, 1H, $H_{1'}$, *J* 7.1 Hz) and 6.05 (br s, 1H, H_2) ppm. MS (ESI$^+$, m/z): 431 [(M+Na)$^+$, 100%].

(*E*)- and (*Z*)-(3*R*,5*R*)-3,5-Di[(*tert*-butyldimethylsilyl)oxy]-4-[2-(*tert*-butyldimethylsilyl)oxyethylidene]-1-ethynyl-1-cyclohexene (15a and 15b). To a stirred solution of **14** (123 mg, 0.301 mmol) in anhydrous CH_2Cl_2 (1.5 mL) at 0 ºC were added imidazole (27 mg, 0.391 mmol) and TBSCl (54 mg, 0.361 mmol). After addition, the ice bath was removed and the reaction was stirred for 1 h. Afterwards, water was added and the mixture was extracted with CH_2Cl_2. The resulting organic layer was washed with brine, dried over Na_2SO_4, filtered and concentrated under vacuum. The resulting residue was purified by flash chromatography on silica gel using 1% Et_2O/hexanes to obtain **15** (152 mg, 97% yield) as a mixture of diastereoisomers. This mixture was further purified by HPLC chromatography (Spherisorb W, 5 µm, 250 × 20 mm, 0.05% ^{i}PrOH/hexanes, 4 mL min^{-1}) to give **15a** (64.6 mg) and **15b** (56.3 mg), both colorless oils. **15a** (*E-isomer*): ^{1}H-NMR (400.13 MHz, $CDCl_3$): δ 0.05 (s, 3H, Si*Me*), 0.06 (s, 3H, Si*Me*), 0.07 (s, 3H, Si*Me*), 0.08 (s, 3H, Si*Me*), 0.10 (s, 3H, Si*Me*), 0.11 (s, 3H, Si*Me*), 0.88 (s, 9H, SiC*Me*$_3$), 0.90 (s, 9H, SiC*Me*$_3$), 0.94 (s, 9H, SiC*Me*$_3$), 2.29 (dt, 1H, H_6, *J* 17.3, 1.6 Hz), 2.44 (ddd, 1H, H_6, *J* 17.3, 6.3, 2.7 Hz), 2.83 (s, 1H, H_8), 4.23 (ddd, 1H, $H_{2'}$, *J* 13.1, 5.3, 1.4 Hz), 4.37 (ddd, 1H, $H_{2'}$, *J* 13.1, 7.2, 1.0 Hz), 4.90 (apparent t, 1H, H_5, *J* 3.5 Hz), 5.03 (br s, 1H, H_3), 5.60 (ddd, 1H, $H_{1'}$, *J* 7.1, 5.4, 1.7 Hz) and 6.05 (br s, 1H, H_2) ppm; MS (ESI$^+$, m/z): 545 [(M+Na)$^+$, 100%]; HRMS (ESI$^+$, m/z): 545.3292 (Calcd for $C_{28}H_{54}NaO_3Si_3$: 545.3273). **15b** (*Z-isomer*): ^{1}H-NMR (400.13 MHz, $CDCl_3$): δ 0.062 (s, 3H, Si*Me*), 0.066 (s, 3H, Si*Me*), 0.069 (s, 3H, Si*Me*), 0.081 (s, 3H, Si*Me*), 0.084 (s, 3H, Si*Me*), 0.10 (s, 3H, Si*Me*), 0.87 (s, 9H, SiC*Me*$_3$), 0.90 (s, 9H, SiC*Me*$_3$), 0.93 (s, 9H, SiC*Me*$_3$), 2.15 (ddd, 1H, H_{6ax}, *J* 16.5, 9.7, 2.3 Hz), 2.53 (dd, 1H, H_{6ec}, *J* 16.7, 5.9 Hz), 2.88 (s, 1H, H_8), 4.29 (dd, 2H, $H_{2'}$, *J* 6.6, 1.4 Hz), 4.58 (ddd, 1H, H_5, *J* 9.3, 5.9, 1.3 Hz), 4.95 (d, 1H, H_3, *J* 5.0 Hz), 5.74 (dt, 1H, $H_{1'}$, *J* 6.5, 1.6 Hz) and 6.06 (dd, 1H, H_2, *J* 4.8, 2.3 Hz) ppm; MS (ESI$^+$, m/z): 545 [(M+Na)$^+$, 100%]; HRMS (ESI$^+$, m/z): 545.3263 (Calcd for $C_{28}H_{54}NaO_3Si_3$: 545.3273).

(3*R*,4*R*,5*R*)-4,5-Di[(*tert*-butyldimethylsilyl)oxy]-1-ethynyl-3-hydroxy-1-cyclohexene (16). To a stirred solution of $TMSCHN_2$ (1.12 mL, 2.0 M in hexanes, 2.250 mmol) in anhydrous THF (8 mL) at -78 ºC was added ^{n}BuLi (1.53 mL, 1.6 M in hexanes, 2.455 mmol). After 15 min at this temperature, a solution of **9** (790 mg, 2.045 mmol) in anhydrous THF (6.2 mL) was added to this mixture and stirred at -78 ºC for additional 5 min. Afterwards, the reaction was further stirred gaining room temperature over 16 h. The reaction is then stopped by addition of water (2 mL). THF is then evaporated and the resulting aqueous phase is extracted with Et_2O/H_2O. The combined organic fractions were washed with brine, dried (Na_2SO_4) and concentrated to give a crude, that after purification by flash chromatography on silica gel (5% EtOAc/hexanes) affords a 1:1 mixture of isomers **10** and **16** (442 mg, 56%). The two compounds are separated by semi-preparative HPLC (Kromasil 250 x 20 mm, 5% EtOAc/hexanes, 7 mL·min^{-1}) giving **10** (217 mg, 28%) and **16** (211 mg, 27%). White solid; ^{1}H-NMR (300.13 MHz, $CDCl_3$): δ 0.07 (s, 3H, Si*Me*), 0.08 (s, 3H, Si*Me*), 0.12 (s, 3H, Si*Me*), 0.13 (s, 3H, Si*Me*), 0.88 (s, 9H, SiC*Me*$_3$), 0.91 (s, 9H, SiC*Me*$_3$), 2.03 (d, 1H, H_{6ax}, *J* 17.4 Hz), 2.27 (d, 1H, *OH*, *J* 9.7 Hz), 2.50 (d, 1H, H_{6ec}, *J* 17.6 Hz), 2.85 (s, 1H, H_8), 3.78 (m, 1H, H_4), 3.99 (m, 1H, H_5), 4.28 (d, 1H, H_3, *J* 7.9 Hz) and 6.04 (s, 1H, H_2) ppm; MS (ESI$^+$, m/z): 405 [(M+Na)$^+$, 100%].

(4*S*,5*R*)-4,5-Di[(*tert*-butyldimethylsilyl)oxy]-1-ethynyl-3-oxo-1-cyclohexene (17). To a stirred solution of **16** (103 mg, 0.269 mmol) in anhydrous CH_2Cl_2 (2.7 mL) was added MnO_2 (234 mg, 2.695 mmol). The resulting suspension was stirred at room temperature overnight and filtered afterwards over Celite. The filtrate was concentrated under vacuum and the resulting residue was purified by flash chromatography with a 1.5% EtOAc/hexanes to afford **17** (88 mg, 86% yield) as a white solid. ^{1}H-NMR (300.13 MHz, $CDCl_3$): δ 0.079 (s, 3H, Si*Me*), 0.082 (s, 6H, 2Si*Me*), 0.10 (s, 3H, Si*Me*), 0.87 (s, 9H, SiC*Me*$_3$), 0.91 (s, 9H, SiC*Me*$_3$), 2.44 (ddd, 1H, H_6, *J* 18.0, 5.8, 1.7 Hz), 2.81 (ddd, 1H, H_6, *J* 18.0, 4.0, 1.5 Hz), 3.51 (s, 1H, H_8), 3.90 (d, 1H, H_4, *J* 7.2 Hz), 4.05 (ddd, 1H, H_5, *J* 7.1, 5.9, 4.1 Hz) and 6.22 (s, 1H, H_2) ppm; MS (ESI$^+$, m/z): 381 [(M+H)$^+$, 100%], 403 [(M+Na)$^+$, 75%].

(*E*)-(4*R*,5*R*)-4,5-Di[(*tert*-butyldimethylsilyl)oxy]-3-cyanomethylidene-1-ethynyl-1-cyclohexene (18). A procedure analogous to that described for the synthesis of **12**, starting from **17** (85 mg, 0.224 mmol), afforded **18** (80 mg, 89% yield) after 2.5 h as a yellow solid and a single diastereoisomer (eluent for column chromatography: 1.5% EtOAc/hexanes). ^{1}H-NMR (300.13 MHz, $CDCl_3$): δ 0.06 (s, 3H, Si*Me*), 0.07 (s, 3H, Si*Me*), 0.08 (s, 3H, Si*Me*), 0.12 (s, 3H, Si*Me*), 0.87 (s, 9H, SiC*Me*$_3$), 0.90 (s, 9H, SiC*Me*$_3$), 2.31 (dd, 1H, H_6, *J* 18.0, 5.4 Hz), 2.69 (dd, 1H, H_6, *J* 18.1, 3.0 Hz), 3.26 (s, 1H, H_8), 3.85 (m, 1H, H_5), 4.03 (dd, 1H, H_4, *J* 6.7, 0.9 Hz), 5.29 (s, 1H, $H_{1'}$) and 6.91 (s, 1H, H_2) ppm; ^{13}C-NMR (75.5 MHz, $CDCl_3$): δ -4.7 (Si*Me*), -4.5 (Si*Me*), -4.1 (2Si*Me*), 18.1 (Si*C*), 18.2 (Si*C*), 26.0 (2SiC*Me*$_3$), 37.0 (C_6), 70.2 (C_5), 73.4 (C_4), 82.7 (C_8), 83.8 (C_7), 95.6 ($C_{1'}$), 116.5 (CN), 126.5 (C_1), 129.7 (C_2) and 157.1 (C_3) ppm.

(*E*)-(4*R*,5*R*)-4,5-Di[(*tert*-butyldimethylsilyl)oxy]-1-ethynyl-3-formylmethylidene-1-cyclohexene (19). A procedure analogous to that described for the synthesis of **13**, starting from **18** (93 mg, 0.231 mmol), afforded **19** (80 mg, 85% yield) as a yellow oil (eluent for column chromatography: 5-10% EtOAc/hexanes). ^{1}H-NMR (300.13 MHz, $CDCl_3$): δ 0.04 (s, 3H, Si*Me*), 0.06 (s, 3H, Si*Me*), 0.07 (s, 3H, Si*Me*), 0.11 (s, 3H, Si*Me*), 0.84 (s, 9H, SiC*Me*$_3$), 0.88 (s, 9H, SiC*Me*$_3$), 2.29 (dd, 1H, H_6, *J* 18.0, 3.8 Hz), 2.75 (apparent dt, 1H, H_6, *J* 18.1, 3.0 Hz), 3.25 (s, 1H, H_8), 3.93 (apparent dt, 1H, H_5, *J* 5.5, 3.8 Hz), 4.02 (d, 1H, H_4, *J* 5.5 Hz), 5.87 (d, 1H, $H_{1'}$, *J* 8.0 Hz), 7.35 (s, 1H, H_2) and 10.18 (d, 1H, $H_{2'}$, *J* 8.0 Hz) ppm; MS (ESI$^+$, m/z): 407 [(M+H)$^+$, 45%]; HRMS (ESI$^+$, m/z): 429.2256 (Calcd for $C_{22}H_{38}NaO_3Si_2$: 429.2252).

(*E*)-(4*R*,5*R*)-4,5-Di[(*tert*-butyldimethylsilyl)oxy]-1-ethynyl-3-(2-hydroxyethylidene)-1-cyclohexene (20). A procedure analogous to that described for the synthesis of **14**, starting from **19** (65 mg, 0.160 mmol), afforded **20** (43 mg, 65% yield) as a yellow thick oil (eluent for column chromatography: 10% EtOAc/hexanes). ^{1}H-NMR (300.13 MHz, $CDCl_3$): δ 0.04 (s, 3H, Si*Me*), 0.05 (s, 3H, Si*Me*), 0.06 (s, 3H, Si*Me*), 0.10 (s, 3H, Si*Me*), 0.85 (s, 9H, SiC*Me*$_3$), 0.87 (s, 9H, SiC*Me*$_3$), 2.16 (dd, 1H, H_6, *J* 17.7, 3.2 Hz), 2.67 (dt, 1H, H_6, *J* 17.7, 2.8 Hz), 3.03 (s, 1H, H_8), 3.86 (apparent dt, 1H, H_5, *J* 5.3, 3.6 Hz), 3.92 (d, 1H, H_4, *J* 5.3 Hz), 4.33 (d, 2H, $H_{2'}$, *J* 6.9 Hz), 5.65 (t, 1H, $H_{1'}$, *J* 6.9 Hz) and 6.70 (s, 1H, H_2) ppm; MS (ESI$^+$, m/z): 447 [(M+K)$^+$, 100%]; HRMS (ESI$^+$, m/z): 431.2360 (Calcd for $C_{22}H_{40}NaO_3Si_2$: 431.2408).

(3*R*,4*S*,5*R*)-3-[(*tert*-Butyldimethylsilyl)oxy]-1-formyl-4,5-(2,3-dimethoxybutan-2,3-dioxy)-1-cyclohexene (24). A procedure analogous to that described for the synthesis of **11**, starting from **23** (170 mg, 0.438 mmol), afforded **24** (159 mg, 94% yield) after 2 h as a white solid (eluent for column chromatography: 10% EtOAc/hexanes). ^{1}H-NMR (300.13 MHz, $CDCl_3$): δ 0.11 (s, 3H, Si*Me*), 0.13 (s, 3H, Si*Me*), 0.89 (s, 9H, SiC*Me*$_3$), 1.27 (s, 3H, *Me*), 1.29 (s, 3H, *Me*), 2.07 (ddd, 1H, H_{6ax}, *J* 17.0, 10.3, 1.9 Hz), 2.73 (dd, 1H, H_{6ec}, *J* 17.5, 6.0 Hz), 3.21 (s, 3H, *OMe*), 3.24 (s, 3H, *OMe*), 3.52 (dd, 1H, H_4, *J* 10.8, 3.8 Hz), 4.08 (apparent td, 1H, H_5, *J* 10.6, 6.0 Hz), 4.44 (apparent t, 1H, H_3, *J* 4.4 Hz), 6.57 (dd, 1H, H_2, *J* 4.8, 2.2 Hz) and 9.51 (s, 1H, H_7) ppm; MS (ESI$^+$, m/z): 355 [(M-OCH_3)$^+$, 100%], 409 [(M+Na)$^+$, 65%].

(3*R*,4*S*,5*R*)-3-[(*tert*-Butyldimethylsilyl)oxy]-1-ethynyl-4,5-(2,3-dimethoxybutan-2,3-dioxy)-1-cyclohexene (25). To a stirred solution of $TMSCHN_2$ (346 µL, 2.0 M in hexanes, 0.692 mmol) in anhydrous THF (2.5 mL) at -78 ºC was added ^{n}BuLi (472 µL, 1.6 M in hexanes, 0.755 mmol). After 15 min at this temperature, a solution of **24** (243 mg, 0.629 mmol) in anhydrous THF (2.5 mL) was added to this mixture and stirred at -78 ºC for additional 5 min. Afterwards, the cooling bath was removed and the reaction was let to reach room temperature for over 1 h. Then, the reaction was poured into H_2O/Et_2O and the aqueous layer extracted with Et_2O. The combined organic fractions were washed with brine, dried (Na_2SO_4) and concentrated, and the residue was purified by flash chromatography on silica gel (5% EtOAc/hexanes) to afford **25** (156 mg, 65%) as a white solid. ^{1}H-NMR (300.13 MHz, $CDCl_3$): δ 0.08 (s, 3H, Si*Me*), 0.11 (s, 3H, Si*Me*), 0.89 (s, 9H, SiC*Me*$_3$), 1.28 (s, 3H, *Me*), 1.29 (s, 3H, *Me*), 2.26 (ddd, 1H, H_{6ax}, *J* 17.0, 10.3, 2.4 Hz), 2.49 (dd, 1H, H_{6ec}, *J* 17.1, 6.1 Hz), 2.87 (s, 1H, H_8), 3.23 (s, 3H, *OMe*), 3.25 (s, 3H, *OMe*), 3.48 (dd, 1H, H_4, *J* 10.8, 3.8 Hz), 4.14 (apparent td, 1H, H_5, *J* 10.5, 6.2 Hz), 4.22 (apparent t, 1H, H_3, *J* 4.6 Hz) and 6.07 (dd, 1H, H_2, *J* 5.1, 2.1 Hz) ppm; MS (ESI$^+$, m/z): 351 [(M-OCH_3)$^+$, 100%], 405 [(M+Na)$^+$, 80%].

(3*R*,4*S*,5*R*)-1-Ethynyl-3-hydroxy-4,5-(2,3-dimethoxybutan-2,3-dioxy)-1-cyclohexene (26). To a stirred solution of silyl ether **25** (120 mg, 0.314 mmol) in anhydrous THF (6 mL) at 0 ºC and in darkness, was added dropwise TBAF (785 µL, 1 M in THF, 0.785 mmol). After 10 min at this temperature, the ice bath was removed and the reaction was stirred at room temperature until complete consumption of the starting material (monitored by TLC 10% EtOAc/hexanes, 2-3 h). The reaction was stopped by addition of water followed by extraction with EtOAc. The resulting organic layer was washed with brine, dried over Na_2SO_4 and concentrated. The residue was purified by flash chromatography on silica gel (25% EtOAc/hexanes) to afford **26** (80 mg, 95%) as a white semisolid. ^{1}H-NMR (300.13 MHz, $CDCl_3$): δ 1.29 (s, 3H, *Me*), 1.33 (s, 3H, *Me*), 2.30 (ddd, 1H, H_{6ax}, *J* 17.0, 10.4, 2.6 Hz), 2.49 (dd, 1H, H_{6ec}, *J* 17.1, 5.9 Hz), 2.77 (br s, 1H, *OH*), 2.91 (s, 1H, H_8), 3.25 (s, 3H, *OMe*), 3.26 (s, 3H, *OMe*), 3.60 (dd, 1H, H_4, *J* 10.8, 4.2 Hz), 4.12 (apparent td, 1H, H_5, *J* 10.6, 6.0 Hz), 4.28 (apparent t, 1H, H_3, *J* 4.7 Hz) and 6.18 (dd, 1H, H_2, *J* 5.4, 2.5 Hz) ppm; MS (ESI$^+$, m/z): 291 [(M+Na)$^+$, 100%].

(4*S*,5*R*)-1-Ethynyl-4,5-(2,3-dimethoxybutan-2,3-dioxy)-3-oxo-1-cyclohexene (27). A procedure analogous to that described for the synthesis of **11**, starting from **26** (75 mg, 0.280 mmol), afforded **27** (68 mg, 91% yield) after 2 h as a white solid (eluent for column chromatography: 20% EtOAc/hexanes). ^{1}H-NMR (300.13 MHz, $CDCl_3$): δ 1.32 (s, 3H, *Me*), 1.41 (s, 3H, *Me*), 2.72 (m, 2H, H_6), 3.25 (s, 3H, *OMe*), 3.30 (s, 3H, *OMe*), 3.59 (s, 1H, H_8), 4.11 (m, 1H, H_5), 4.27 (d, 1H, H_4, *J* 11.5 Hz) and 6.29 (d, 1H, H_2, *J* 1.3 Hz) ppm; MS (ESI$^+$, m/z): 289 [(M+Na)$^+$, 100%].

(*E*)-(4*R*,5*R*)-3-Cyanomethylidene-1-ethynyl-4,5-(2,3-dimethoxybutan-2,3-dioxy)-1-cyclohexene (28). A procedure analogous to that described for the synthesis of **12**, starting from **27** (60 mg, 0.225 mmol), afforded **28** (52 mg, 80% yield) after 4 h as a yellow solid and a single diastereoisomer (eluent for column chromatography: 10% EtOAc/hexanes). ^{1}H-NMR (400.13 MHz, $CDCl_3$): δ 1.33 (s, 3H, *Me*), 1.38 (s, 3H, *Me*), 2.53 (ddd, 1H, H_{6ax}, *J* 17.8, 10.3, 1.9 Hz), 2.61 (dd, 1H, H_{6ec}, *J* 17.6, 6.0 Hz), 3.26 (s, 3H, *OMe*), 3.27 (s, 3H, *OMe*), 3.31 (s, 1H, H_8), 3.85 (apparent td, 1H, H_5, *J* 10.5, 6.0 Hz), 4.30 (dd, 1H, H_4, *J* 10.8, 2.2 Hz), 5.57 (s, 1H, H_{15}) and 6.91 (d, 1H, H_2, *J* 2.4 Hz) ppm; MS (ESI$^+$, m/z): 312 [(M+Na)$^+$, 100%].

(*E*)-(4*R*,5*R*)-1-Ethynyl-3-formylmethylidene-4,5-(2,3-dimethoxybutan-2,3-dioxy)-1-cyclohexene (29). A procedure analogous to that described for the synthesis of **13**, starting from **28** (169 mg, 0.586 mmol), afforded **29** (151 mg, 88% yield) as a yellow solid (eluent for column chromatography: 20% EtOAc/hexanes). ^{1}H-NMR (300.13 MHz, $CDCl_3$): δ 1.33 (s, 3H, *Me*), 1.38 (s, 3H, *Me*), 2.52 (ddd, 1H, H_{6ax}, *J* 17.8, 10.5, 2.5 Hz), 2.63 (dd, 1H, H_{6ec}, *J* 17.6, 6.0 Hz), 3.26 (s, 3H, *OMe*), 3.27 (s, 3H, *OMe*), 3.29 (s, 1H, H_8), 3.92 (apparent td, 1H, H_5, *J* 10.6, 6.0 Hz), 4.33 (dd, 1H, H_4, *J* 10.7, 2.1 Hz), 6.28 (d, 1H, H_{15}, *J* 7.6 Hz), 7.39 (d, 1H, H_2, *J* 2.1 Hz) and 10.14 (d, 1H, *CHO*, *J* 7.6 Hz) ppm; MS (ESI$^+$, m/z): 315 [(M+Na)$^+$, 100%].

(*E*)-(4*R*,5*R*)-1-Ethynyl-3-(2-hydroxyethylidene)-4,5-(2,3-dimethoxybutan-2,3-dioxy)-1-cyclohexene (30). A procedure analogous to that described for the synthesis of **14**, starting from **29** (85 mg, 0.291 mmol), afforded **30** (64 mg, 75% yield) as a yellowish semisolid of low stability (eluent for column chromatography: 30% EtOAc/hexanes). ^{1}H-NMR (400.13 MHz, $CDCl_3$): δ 1.33 (s, 3H, *Me*), 1.39 (s, 3H, *Me*), 2.49 (m, 2H, H_6), 3.08 (s, 1H, H_8), 3.26 (s, 3H, *OMe*), 3.29 (s, 3H, *OMe*), 3.81 (ddd, 1H, H_5, *J* 10.6, 9.5, 7.0 Hz), 4.20 (d, 1H, H_4, *J* 10.6 Hz), 4.30 (dd, 1H, H_{16}, *J* 13.5, 6.0 Hz), 4.38 (dd, 1H, H_{16}, *J* 12.9, 7.6 Hz), 6.01 (apparent t, 1H, H_{15}, *J* 6.5 Hz) and 6.74 (s, 1H, H_2) ppm.

(*E*)-(4*R*,5*R*)-3-[2-(*tert*-Butyldimethylsilyl)oxyethylidene]-1-ethynyl-4,5-(2,3-dimethoxybutan-2,3-dioxy)-1-cyclohexene (31). A procedure analogous to that described for the synthesis of **15**, starting from **30** (113 mg, 0.384 mmol), afforded **31** (126 mg, 80% yield) as a thick colorless oil (eluent for column chromatography: 5% EtOAc/hexanes). ^{1}H-NMR (300.13 MHz, $CDCl_3$): δ 0.077 (s, 3H, Si*Me*), 0.080 (s, 3H, Si*Me*), 0.91 (s, 9H, SiC*Me*$_3$), 1.32 (s, 3H, *Me*), 1.38 (s, 3H, *Me*), 2.46 (apparent d, 2H, H_6, *J* 7.9 Hz), 3.04 (s, 1H, H_8), 3.25 (s, 3H, *OMe*), 3.28 (s, 3H, *OMe*), 3.79 (m, 1H, H_5), 4.17 (dd, 1H, H_4, *J* 10.6, 2.1 Hz), 4.27 (ddd, 1H, H_{16}, *J* 13.8, 5.2, 2.2 Hz), 4.45 (ddd, 1H, H_{16}, *J* 13.8, 7.7, 1.6 Hz), 5.91 (apparent td, 1H, H_{15}, *J* 6.7, 1.2 Hz) and 6.73 (s, 1H, H_2) ppm; HRMS (ESI$^+$, m/z): 431.2233 (Calcd for $C_{22}H_{36}NaO_5Si$: 431.2224).

General procedure for the synthesis of 33-36.

To a stirred solution of vinyl triflate **32** (27 mg, 0.056 mmol) and the corresponding A-ring precursor **15a**, **15b, 20** or **31** (0.071 mmol) in deoxygenated anhydrous DMF (490 µL), were added CuI (1.2 mg, 0.006 mmol), $(Ph_3P)_2Pd(OAc)_2$ (1.4 mg, 0.002 mmol) and deoxygenated anhydrous Et_2NH (490 µL). After 2 h stirring at room temperature, water was added and the mixture was extracted with Et_2O. The resulting organic layer was dried over Na_2SO_4, filtered and concentrated under vacuum to give a residue that was purified by flash chromatography on silica gel (eluents: 1% Et_2O/hexanes for **33-34**; 10% Et_2O/hexanes for **35**; and 2% EtOAc/hexanes for **36**).

(*E*)-(1*R*,3*R*)-1,3-Di[(*tert*-butyldimethylsilyl)oxy]-2-[2-(*tert*-butyldimethylsilyl)oxyethylidene]-6,7-dehydro-25-trimethylsilyloxy-19-*nor*-previtamin D_3 (33). Colorless oil, 70% yield; ^{1}H-NMR (300.13 MHz, $CDCl_3$): δ 0.05 (s, 3H, Si*Me*), 0.06 (s, 3H, Si*Me*), 0.07 (s, 3H, Si*Me*), 0.08 (s, 3H, Si*Me*), 0.10 (s, 3H, Si*Me*), 0.107 (s, 3H, Si*Me*), 0.113 (s, 9H,

TMS), 0.70 (s, 3H, *Me*$_{18}$), 0.88 (s, 9H, SiC*Me*$_3$), 0.90 (s, 9H, SiC*Me*$_3$), 0.94 (s, 9H, SiC*Me*$_3$), 1.21 (s, 6H, *Me*$_{26}$+*Me*$_{27}$), 0.88-2.47 (several m), 4.23 (ddd, 1H, H$_{2'}$, *J* 13.1, 5.2, 1.3 Hz), 4.39 (ddd, 1H, H$_{2'}$, *J* 13.1, 7.2, 0.9 Hz), 4.88 (t, 1H, H$_3$, *J* 3.6 Hz), 5.01 (s, 1H, H$_1$), 5.58 (m, 1H, H$_{1'}$), 5.89 (s, 1H, H$_{10}$) and 5.96 (d, 1H, H$_9$, *J* 3.0 Hz) ppm; MS (ESI$^+$, m/z): 725 [(M-OTBS)$^+$, 100%]; HRMS (ESI$^+$, m/z): 879.5942 (Calcd for $C_{49}H_{92}NaO_4Si_4$: 879.5965).

(*Z*)-(1*R*,3*R*)-1,3-Di[(*tert*-butyldimethylsilyl)oxy]-2-[2-(*tert*-butyldimethylsilyl)oxyethylidene]-6,7-dehydro-25-trimethylsilyloxy-19-*nor*-previtamin D$_3$ (34). Colorless oil, 90% yield; ^{1}H-NMR (300.13 MHz, $CDCl_3$): δ 0.06 (s, 9H, 3Si*Me*), 0.08 (s, 6H, 2Si*Me*), 0.09 (s, 3H, Si*Me*), 0.11 (s, 9H, *TMS*), 0.70 (s, 3H, *Me*$_{18}$), 0.86 (s, 9H, SiC*Me*$_3$), 0.90 (s, 9H, SiC*Me*$_3$), 0.93 (s, 9H, SiC*Me*$_3$), 0.95 (d, 3H, *Me*$_{21}$, *J* 6.6 Hz), 1.21 (s, 6H, *Me*$_{26}$+*Me*$_{27}$), 1.22-2.26 (several m), 2.51 (dd, 1H, H$_4$, *J* 16.7, 6.0 Hz), 4.29 (dd, 2H, H$_{2'}$, *J* 6.6, 1.4 Hz), 4.59 (dd, 1H, H$_3$, *J* 8.7, 6.9 Hz), 4.94 (d, 1H, H$_1$, *J* 5.3 Hz), 5.72 (dd, 1H, H$_{1'}$, *J* 6.6, 4.9 Hz), 5.89 (dd, 1H, H$_{10}$, *J* 5.0, 2.3 Hz) and 5.99 (d, 1H, H$_9$, *J* 3.0 Hz) ppm; MS (ESI$^+$, m/z): 725 [(M-OTBS)$^+$, 100%]; HRMS (ESI$^+$, m/z): 879.5920 (Calcd for $C_{49}H_{92}NaO_4Si_4$: 879.5965).

(*E*)-(2*R*,3*R*)-2,3-Di[(*tert*-butyldimethylsilyl)oxy]-6,7-dehydro-1-(2-hydroxyethylidene)-25-trimethylsilyloxy-19-*nor*-previtamin D$_3$ (35). Yellow thick oil, 65% yield; ^{1}H-NMR (400.13 MHz, $CDCl_3$): δ 0.04 (s, 3H, Si*Me*), 0.05 (s, 3H, Si*Me*), 0.06 (s, 3H, Si*Me*), 0.10 (s, 3H, Si*Me*), 0.11 (s, 9H, *TMS*), 0.72 (s, 3H, *Me*$_{18}$), 0.85 (s, 9H, SiC*Me*$_3$), 0.88 (s, 9H, SiC*Me*$_3$), 0.96 (d, 3H, *Me*$_{21}$, $^3J_{HH}$ 6.5 Hz), 0.98-2.30 (several m), 1.21 (s, 6H, *Me*$_{26}$+*Me*$_{27}$), 2.16 (dd, 1H, H$_4$, *J* 17.3, 3.6 Hz), 2.67 (d, 1H, H$_4$, *J* 16.8 Hz), 3.85 (m, 1H, H$_3$), 3.91 (d, 1H, H$_2$, *J* 5.4 Hz), 4.32 (m, 2H, H$_{2'}$), 5.61 (t, 1H, H$_{1'}$, *J* 7.0 Hz), 6.01 (d, 1H, H$_9$, *J* 3.0 Hz) and 6.55 (s, 1H, H$_{10}$) ppm; MS (APCI, m/z): 725 [(M - OH)$^+$, 100%].

(*E*)-(2*R*,3*R*)-1-[2-(*tert*-Butyldimethylsilyl)oxyethylidene]-6,7-dehydro-2,3-(2,3-dimethoxybutan-2,3-dioxy)-25-trimethylsilyloxy-19-*nor*-previtamin D$_3$ (36). Colorless thick oil, 60% yield; ^{1}H-NMR (600.13 MHz, acetone-d_6): δ 0.097 (s, 3H, Si*Me*), 0.101 (s, 12H, Si*Me*+*TMS*), 0.73 (s, 3H, *Me*$_{18}$), 0.92 (s, 9H, SiC*Me*$_3$), 1.00 (d, 3H, *Me*$_{21}$, *J* 6.6 Hz), 1.220, 1.222 (2s, 6H, *Me*$_{26}$ and *Me*$_{27}$), 1.26 (s, 3H, *Me*), 1.32 (s, 3H, *Me*), 0.91-2.38 (several m), 2.41 (dd, 1H, H$_4$, *J* 16.9, 5.8 Hz), 3.21 (s, 3H, *OMe*), 3.23 (s, 3H, *OMe*), 3.70 (apparent td, 1H, H$_3$, *J* 10.5, 5.9 Hz), 4.08 (d, 1H, H$_2$, *J* 10.7 Hz), 4.38 (ddd, 1H, H$_{2'}$, *J* 13.8, 6.1, 1.6 Hz), 4.47 (ddd, 1H, H$_{2'}$, *J* 13.8, 6.9, 1.7 Hz), 5.82 (apparent t, 1H, H$_{2'}$, *J* 6.4 Hz), 5.97 (apparent q, 1H, H$_9$, *J* 3.6 Hz) and 6.64 (d, 1H, H$_2$, *J* 1.8 Hz) ppm.

General procedure for the synthesis of 37-40.

A round bottom flask containing Lindlar catalyst (30 mg), was exposed to a positive pressure of hydrogen gas (balloon). Then, a solution of the corresponding dienyne **33-36** (0.024 mmol) in hexanes (11 mL) and quinoline (30 μL 0.5% v/v in hexanes, 0.001 mmol) were added. The reaction was stirred vigorously during 4 h after which was filtered on Celite, whasing with a mixture of hexanes/Et_2O. The filtrate was concentrated and the crude was purified by flash chromatography on silica gel (eluents: 1% Et_2O/hexanes for **37-38**; 15% Et_2O/hexanes for **39**; and 4% Et_2O/hexanes for **40**).

(*E*)-(1*R*,3*R*)-1,3-Di[(*tert*-butyldimethylsilyl)oxy]-2-[2-(*tert*-butyldimethylsilyl)oxyethylidene]-25-trimethylsilyloxy-19-*nor*-previtamin D$_3$ (37). Colorless oil, 60% yield; ^{1}H-NMR (300.13 MHz, $CDCl_3$): δ 0.04 (s, 3H, Si*Me*), 0.06 (s, 6H, 2Si*Me*), 0.07 (s, 3H, Si*Me*), 0.10 (s, 3H, Si*Me*), 0.105 (s, 3H, Si*Me*), 0.113 (s, 3H, *TMS*), 0.71 (s, 3H, *Me*$_{18}$), 0.88 (s, 9H, SiC*Me*$_3$), 0.90 (s, 9H, SiC*Me*$_3$), 0.95 (s, 9H, SiC*Me*$_3$), 1.21 (s, 6H, *Me*$_{26}$+*Me*$_{27}$), 0.87-2.42 (several m), 2.47 (dd, 1H, H$_4$, *J* 17.2, 3.9 Hz), 4.26 (dd, 1H, H$_{2'}$, *J* 12.2, 5.2 Hz), 4.45 (dd, 1H, H$_{2'}$, *J* 12.6, 7.2), 4.86 (t, 1H, H$_3$, *J* 3.9 Hz), 4.96 (s, 1H, H$_1$), 5.42 (s, 1H, H$_9$), 5.54 (t, 1H, H$_{1'}$, *J* 5.3 Hz), 5.62 (s, 1H, H$_{10}$), 5.71 (d, 1H, H$_7$, *J* 11.9 Hz) and 5.83 (d, 1H, H$_6$, *J* 12.6 Hz) ppm; MS (APCI$^+$, m/z): 727 [(M-OTBDMS)$^+$, 100%]; HRMS (ESI$^+$, m/z): 881.6107 (Calcd for $C_{49}H_{94}NaO_4Si_4$: 881.6121).

(*Z*)-(1*R*,3*R*)-1,3-Di[(*tert*-butyldimethylsilyl)oxy]-2-[2-(*tert*-butyldimethylsilyl)oxyethylidene]-25-trimethylsilyloxy-19-*nor*-previtamin D$_3$ (38). Colorless oil, 90% yield; ^{1}H-NMR (400.13 MHz, $CDCl_3$): δ 0.06 (s, 6H, 2Si*Me*), 0.07 (s, 6H, 2Si*Me*), 0.08 (s, 3H, Si*Me*), 0.09 (s, 3H, Si*Me*), 0.12 (s, 3H, *TMS*), 0.74 (s, 3H, *Me*$_{18}$), 0.87 (s, 9H, SiC*Me*$_3$), 0.90 (s, 9H, SiC*Me*$_3$), 0.93 (s, 9H, SiC*Me*$_3$), 1.21 (s, 6H, *Me*$_{26}$+*Me*$_{27}$), 0.87-2.29 (several m), 2.53 (dd, 1H, H$_4$, *J* 16.3, 5.6 Hz), 4.31 (s, 2H, H$_{2'}$), 4.53 (dd, 1H, H$_3$, *J* 8.2, 6.1 Hz), 4.94 (d, 1H, H$_1$, *J* 4.7 Hz), 5.41 (s, 1H, H$_9$), 5.61 (d, 1H, H$_{10}$, *J* 3.4 Hz), 5.71 (m, 2H, H$_{1'}$+H$_7$) and 5.83 (d, 1H, H$_6$, *J* 12.2 Hz) ppm; MS (APCI$^+$, m/z): 727 [(M-OTBDMS)$^+$, 100%]; HRMS (ESI$^+$, m/z): 881.6099 (Calcd for $C_{49}H_{94}NaO_4Si_4$: 881.6121).

(*E*)-(2*R*,3*R*)-2,3-Di[(*tert*-butyldimethylsilyl)oxy]-1-(2-hydroxyethylidene)-25-trimethylsilyloxy-19-*nor*-previtamin D$_3$ (39). Yellow oil, 70% yield; ^{1}H-NMR (300.13 MHz, $CDCl_3$): δ 0.03 (s, 3H, Si*Me*), 0.05 (s, 6H, 2Si*Me*), 0.08 (s, 3H, Si*Me*), 0.11 (s, 9H, *TMS*), 0.73 (s, 3H, *Me*$_{18}$), 0.85 (s, 9H, SiC*Me*$_3$), 0.90 (s, 9H, SiC*Me*$_3$), 0.81-2.78 (several m), 0.97 (d, 3H, *Me*$_{21}$, *J* 6.5 Hz), 1.21 (s, 6H, *Me*$_{26}$+*Me*$_{27}$), 3.83 (m, 1H, H$_3$), 3.93 (d, 1H, H$_2$, *J* 5.7 Hz), 4.31 (m, 2H, H$_{2'}$), 5.44 (s, 1H, H$_9$), 5.62 (t, 1H, H$_{1'}$, *J* 7.0 Hz), 5.78 (d, 1H, H$_7$, *J* 12.0 Hz), 5.88 (d, 1H, H$_6$, *J* 12.2 Hz) and 6.34 (s, 1H, H$_{10}$) ppm.

(*E*)-(2*R*,3*R*)-1-[2-(*tert*-Butyldimethylsilyl)oxyethylidene]-2,3-(2,3-dimethoxybutan-2,3-dioxy)-25-trimethylsilyloxy-19-*nor*-previtamin D$_3$ (40). Yellow oil, 75% yield; ^{1}H-NMR (600.13 MHz, acetone-d_6): δ 0.091 (s, 3H, Si*Me*), 0.093 (s, 3H, Si*Me*), 0.10 (s, 9H, *TMS*), 0.79 (s, 3H, *Me*$_{18}$), 0.91 (s, 9H, SiC*Me*$_3$), 1.00 (d, 3H, *Me*$_{21}$, *J* 6.6 Hz), 1.215, 1.217 (2s, 6H, *Me*$_{26}$ and *Me*$_{27}$), 1.25 (s, 3H, *Me*), 1.31 (s, 3H, *Me*), 0.86-2.42 (several m), 2.55 (dd, 1H, H$_4$, *J* 16.8, 5.4 Hz), 3.18 (s, 3H, *OMe*), 3.24 (s, 3H, *OMe*), 3.62 (apparent td, 1H, H$_3$, *J* 10.6, 5.4 Hz), 4.09 (dd, 1H, H$_2$, *J* 10.4, 1.6 Hz), 4.37 (ddd, 1H, H$_{2'}$, *J* 13.5, 6.1, 1.9 Hz), 4.47 (ddd, 1H, H$_{2'}$, *J* 13.4, 7.0, 1.7 Hz), 5.46 (br s, 1H, H$_9$), 5.77 (apparent t, 1H, H$_{2'}$, *J* 5.6 Hz), 5.84 (d, 1H, H$_7$, *J* 11.9 Hz), 5.95 (d, 1H, H$_6$, *J* 12.2 Hz) and 6.45 (s, 1H, H$_2$) ppm.

***In vitro* Biological Evaluation**

Affinity for VDR. 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.[23] 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).

Affinity for DBP. Binding of vitamin D metabolites and analogues to hDBP was performed at 4 °C as described previously.[24] [^{3}H]1α,25-(OH)$_2$-D$_3$ and 1α,25-(OH)$_2$-D$_3$ or its analogues were incubated with hDBP (0.18 μM) in a final volume of 1 ml (0.01 M Tris-HCl buffer and 0.154 M NaCl, pH 7.4) for 3 h at 4 °C. Phase separation was then obtained by the addition of 0.5 ml of cold dextran-coated charcoal.

Cell proliferation assays. As a measure of cell proliferation, [^{3}H]-thymidine incorporation of breast cancer MCF-7 cells (ATCC) was

determined after a 72 h incubation period with various concentrations of 1α,25-$(OH)_2$-D_3, analogues or vehicle as described previously.[23]

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Keywords: previtamin D analogues • A-ring modified • biological assays

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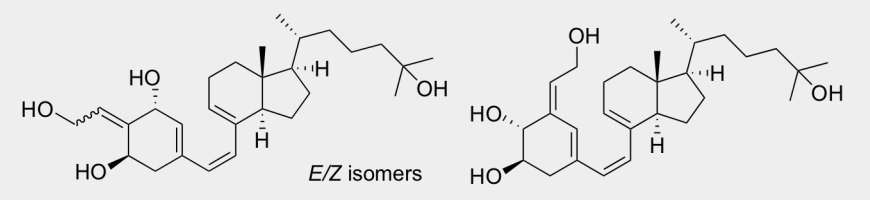

The synthesis of previtamin D_3 analogues possessing a 2-hydroxyethylidene function in the A-ring is described. The strategy involves coupling of a dienyne precursor of the A-ring from shikimic acid with an enol triflate of the CD-ring/side chain fragment. The binding affinity to Vitamin D Receptor (VDR) and Vitamin D Binding Protein (DBP), and MCF-7 cell antiproliferative activity is discussed to examine the effect of the 6-s-*cis* conformer on the biological profile.

Vitamin D analogues

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