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Enzymatic Desymmetrization of 19-*nor*-Vitamin D_3 A-Ring Synthon Precursor: Synthesis, Structure Elucidation, and Biological Activity of 1α,25-Dihydroxy-3-*epi*-19-*nor*-vitamin D_3 and 1β,25-Dihydroxy-19-*nor*-vitamin D_3

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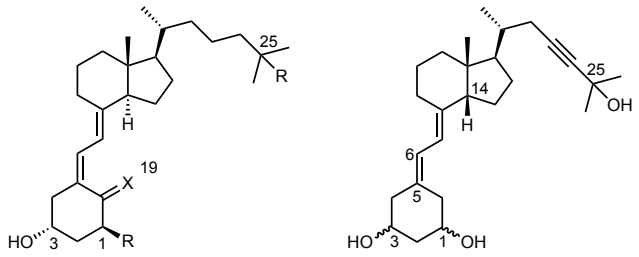
Abstract. In a search for novel vitamin D derivatives of potential therapeutic value, structurally simple but synthetically challenging A-ring epimers of the *19-nor*-Calcitriol [19-*nor*-1α,25-$(OH)_2$-D_3] at C1 and C3 were efficiently synthesized. Both analogues (1-*epi*- and 3-*epi*-19-*nor*-Calcitriol) were obtained through a convergent synthesis starting from *cis,cis*-1,3,5-cyclohexanetriol and the protected 25-hydroxy Grundmann´s ketone. After Julia-Kocienski coupling of the corresponding C,D-ring/side chain sulfone fragment with the A-ring ketone moiety, both vitamin D analogues were isolated. The critical point was how to determine the structural configuration of both diastereoisomers since similar ^{1}H NMR spectra were observed. For that, a biocatalytic approach was crucial in the synthesis of orthogonally protected derivatives. NMR spectroscopy allows the unambiguous identification of these compounds and as a result the structural elucidation of the desired vitamin D diastereomeric analogues. Affinity studies demonstrated that these 1,25-19-*nor* analogues have a very low affinity for the vitamin D receptor compared with 1α,25-dihydroxyvitamin D_3 or 1α,25-dihydroxy-19-*nor*-vitamin D_3. In addition, these analogues have a lower binding affinity for the human vitamin D binding protein than the natural hormone. *In vitro* cell culture studies revealed that synthesized analogues were less active than 1α,25-dihydroxyvitamin D_3 in inhibiting cell proliferation.

Keywords: Enzymatic desymmetrization; Vitamin D; 19-*nor* analogues; A-Ring modified analogues; Calcitriol

Introduction

Due to its huge biological importance, 1α,25-$(OH)_2$-D_3 (**2**, Calcitriol, Figure 1), the hormonally active form of vitamin D_3 (**1**), was considered as a candidate for therapeutic usage. However, side effects like hypercalcemia and hypercalciuria hobble its pharmaceutical interest.[1]



1, X= CH_2, R= H; Vitamin D_3
2, X= CH_2, R= OH; 1α,25-$(OH)_2$-D_3
3, X= H_2, R= OH; 1α,25-$(OH)_2$-19-*nor*-D_3
4, TX522 (1α,3β)
5, 3-*epi*-TX522 (1α,3α)
6, 1-*epi*-TX522 (1β,3β)

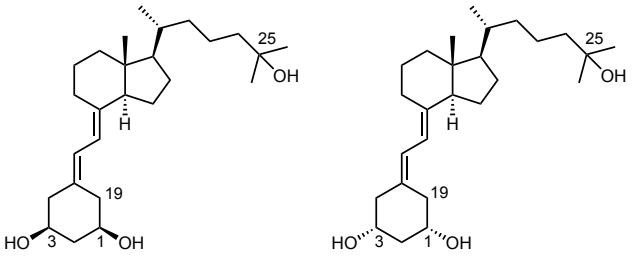
Figure 1. Vitamin D and 19-*nor*-analogues.

For this reason, research related to vitamin D and especially focused at Calcitriol, has provided synthetic routes and biological activities for over 3,000 new analogues. Nowadays, the emphasis is on obtaining molecules with enhanced molecular activity as well as reduced calcemic effects.[2] Simple modifications on the structure of the natural hormone led to interesting analogues. There are several examples where the chirality importance is reflected because of stereoisomers of the same analogue having remarkably different biological profiles. For example, the stereochemistry of C1 and C3 has a strong impact on binding to the vitamin D receptor (VDR) and also to the vitamin D binding protein (DBP). Inversion of the natural orientation of the 3β-hydroxyl and 1α-hydroxyl resulted in a 4- to 90-fold reduction in VDR binding. However, inversion of the

1α-hydroxyl to the 1β-orientation results in a 65.7-fold increase in DBP binding with respect to 1α,25-$(OH)_2$-D_3, illustrating that the optimal ligand for DBP is 25-OH-D_3. Intriguingly, 1β,25-$(OH)_2$-D_3 was found to be a specific inhibitor of the nongenomic responses of 1α,25-$(OH)_2$-D_3 such as transcaltachia and Ca^{2+} uptake, making it the first analogue that displays antagonist properties at the receptor level.[3]

It is noteworthy that the removal of the 19-exomethylene function is beneficial in itself. Thus, 1α,25-dihydroxy-19-*nor*-vitamin D_3 (**3**) shows minor calcemic effects while retaining good cell-differentiating properties.[4] In an attempt to carry out the preparation of the corresponding C1 and C3 diastereoisomers of 1α,25-$(OH)_2$-14-*epi*-19-*nor*-D_3 analogues, Wu *et al.*[5] described the preparation of 1-*epi* and 3-*epi* derivatives of TX522 (**4**–**6**, Figure 1) through a convergent process whose key step involves the coupling between an aldehyde of A-ring derivative and an allyl bromide CD-ring fragment. The main drawback of this route is the low selectivity obtained in the solvolysis of the coupling reaction product (cyclovitamin intermediate). It proceeds through an intermediate with two rotamers in equilibrium around the 5,6-bond. This disadvantage along with the challenging identification of both isomers has been a barrier to expand the methodology to the synthesis of 1-*epi* or 3-*epi* derivatives of 1α,25-$(OH)_2$-19-*nor*-D_3.

With this goal in mind, here we report a practical approach for the preparation of the synthetically elusive epimers C3 and C1 of 1α,25-$(OH)_2$-19-*nor*-D_3 (**7** and **8**, respectively, Figure 2).



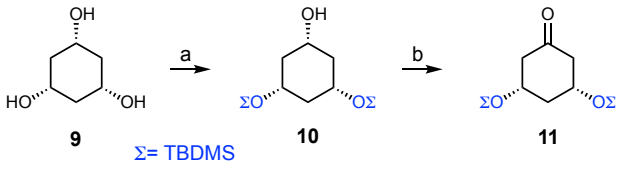
7, 1α,25-$(OH)_2$-3-*epi*-19-*nor*-D_3 **8**, 1β,25-$(OH)_2$-19-*nor*-D_3

Figure 2. C1 and C3 epimers of 1α,25-$(OH)_2$-19-*nor*-D_3.

Results and Discussion

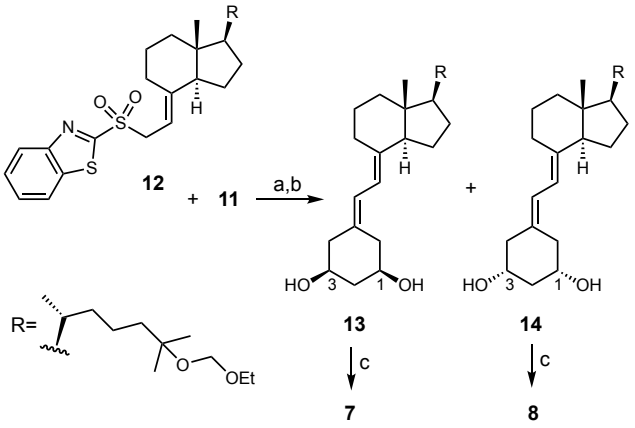
Chemistry

The convergent construction of the vitamin D skeleton is based on an approach previously reported by Kittaka.[6] The synthesis of compounds **7** and **8** was envisaged using a Julia-Kociensky olefination to construct the diene unit between the A-ring and the CD-ring/side chain fragment, which was previously reported starting from Grundmann´s ketone.[7] The synthesis of A-ring synthon **11** was developed as shows in Scheme 1. Treatment of *cis*,*cis*-1,3,5-cyclohexanetriol (**9**) with Et_3N, and *tert*-butyldimethylsilyl chloride (TBDMSCl) at –20 ºC led to the corresponding di-silyl protected derivative **10**, which was next treated with Dess-Martin periodinane reagent to achieve the ketone **11**.



Scheme 1. Preparation of symmetric A-ring synthon **11**. *Reaction conditions*: (a) TBDMSCl, Et_3N, THF, −20 ºC, 2 h and then 16 h at r.t. (43%); (b) Dess-Martin reagent, CH_2Cl_2, r.t., 30 min (97%).

Convergent coupling reaction between **11** and the lithium enolate of sulfone **12** (Scheme 2) took place with 51% yield, giving an equimolecular diastereomeric mixture, which was hardly separated by HPLC. For this reason, the mixture was treated with tetrabutylammonium fluoride (TBAF), which resulted in silyl ether deprotection, affording the isomers **13** and **14**. These compounds were easily separated by semipreparative HPLC in an equimolar ratio (see Figure S1, Supporting Information).

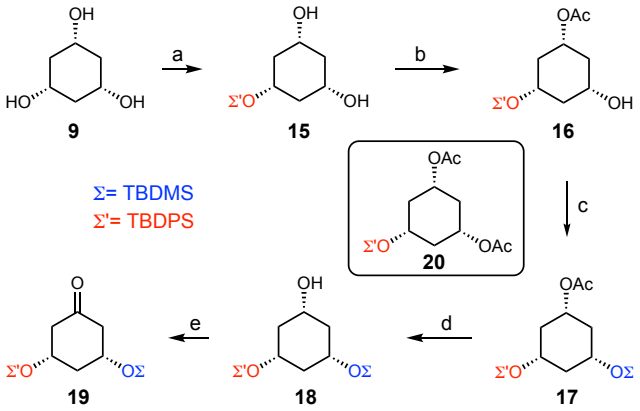


Scheme 2. Synthesis of 1-*epi* and 3-*epi* 19-*nor*-vitamin D_3 analogues. *Reaction conditions*: (a) LHMDS, THF, −78 ºC to −50 ºC, 7 h (51%); (b) TBAF, THF, r.t., 2 h (85%); (c) EtOH sat HCl, r.t., 30 min (88% for **7** and 91% for **8**).

Structure determination from conventional techniques was challenging due to the structural similarity of both isomers. Accordingly, an extensive NMR study was performed using a high resolution NMR spectrometer. Despite the fact of using a 600 MHz apparatus, we were not able to distinguish the diastereomers **13** and **14** by NMR methods (see Figure S2, Supporting Information).

To deduce unequivocally the correct stereochemistry of both analogues, our efforts were focused on the design of a new route for the synthesis of an A-ring synthon in which orthogonal protection of hydroxyl groups would be the key to solve the

problem of the structural determination (Scheme 3). For this purpose, we take into account the pioneering work of Wirz and co-workers,[8] who carried out the acetylation of corresponding mono-TBDMS-protected derivative of **9** catalyzed by QLM lipase to afford the asymmetrically monoacylated compound in quantitative yield and >99% ee. In our case, to facilitate the analysis of the enzymatic reaction by HPLC, we used as silyl ether the TBDPS (UV visible) instead the TBDMS group.



Scheme 3. Preparation of orthogonally protected A-ring synthon **19**. *Reaction conditions*: (a) TBDPSCl, Et_3N, NaH, THF, 45 ºC, 48 h (85%); (b) Lipase, vinyl acetate, solvent, 30 ºC; (c) TBDMSCl, Imidazole, CH_2Cl_2, r.t., 3 h (94%); (d) MeONa, MeOH, r.t., 4 h (95%); (e) Dess-Martin reagent, CH_2Cl_2, r.t., 1 h (97%).

Thus, triol **9** was mono-TBDPS-protected by treatment with *tert*-butyldiphenylsilyl chloride (TBDPSCl), Et_3N, and NaH in THF at 40 ºC to afford the diol **15** in 85% yield.[9] At this point, two different easily available lipases were tested for the asymmetric acetylation of *meso*-diol **15** in vinyl acetate: *Candida antarctica* lipase type B (CAL-B, Novozyme 435) and *Pseudomonas cepacia* lipase (PSL-IM). The choice of these enzymes was based on the excellent results that they showed in enzymatic resolution of secondary alcohols via enantioselective acylation or hydrolysis of their esters.[10]

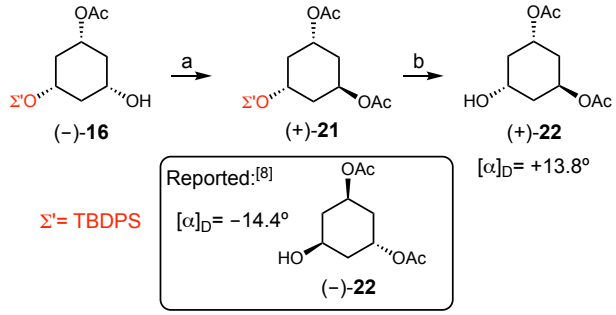
Table 1. Lipase catalyzed acylation of **15**.[a)]

	Enzyme[b)]	Solvent	t (h)	**15** (%)[b)]	**16** (%)[b)]	**16** ee (%)[b)]	**20** (%)[b)]
1	-	THF	12	>99	-	-	-
2	-	Toluene	12	>99	-	-	-
3	-	TBME	12	>99	-	-	-
4	CAL-B	THF	12	-	92	>99	8
5	CAL-B	Toluene	12	9	85	98	6
6	CAL-B	TBME	10	-	90	97	10
7	PSL-IM	THF	12	74	24	85	2
8	PSL-IM	Toluene	4	-	>99	>99	-
9	PSL-IM	TBME	4	-	>99	>99	-

[a)] Ratio **15**:enzyme (1:0.5, p/p), with vinyl acetate at 30 ºC.
[b)] Determined by chiral HPLC.

Enzymatic catalysis in non-aqueous media significantly extends the conventional aqueous-based biocatalysis.[11] The effect of three different organic solvents [THF, *tert*-butylmethyl ether (TBME), and toluene] on the process was evaluated. The reactions were carried out at 30 ºC using 5 equiv. of vinyl acetate. *Candida antarctica* lipase B (CAL-B), showed very high enantioselectivity in all solvents (entries 4−6, Table 1) but different regioselectivities: total conversion was achieved with THF and TBME, after 12 and 10 h, respectively, but the diacetylated product **20** was also obtained. Using toluene as solvent, the reaction was slower and after 12 h starting material was recovered.

On the other hand, when the acylation process of **15** was examined with *Pseudomonas cepacia* lipase as catalyst and THF as solvent, lower reaction rate and enantioselectivity were observed (entry 7, Table 1). On the contrary, excellent results were obtained using toluene and TBME (entries 8−9, Table 1). Thus, 99% conversion was achieved after 4 h of reaction and enantiopure (−)-**16** was obtained (see Figure S3, Supporting Information).

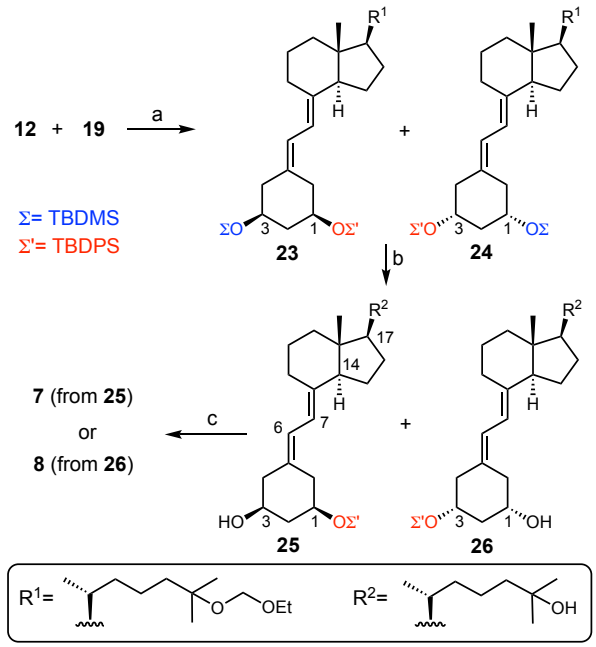


Scheme 4. Synthesis of enantiomerically pure diacetate **22**. *Reaction conditions*: (a) Ph_3P, DIAD, AcOH, THF, r.t., 4 h (72%); (b) TBAF, THF, r.t., 3 h (79%).

Both enzymes showed the same stereochemical preference. For the assignment of the absolute configurations of **16**, a sequence of chemical transformations were carried out to convert the optically pure compound (−)-**16** into the previously described[8] (+)-1,3-diacetoxy-5-hydroxycyclohexane **22** (Scheme 4). Inversion of the free hydroxyl group in **16** under Mitsunobu conditions furnished **21**. Subsequent removal of the silyl ether with TBAF afforded the corresponding diacylated diol (+)-**22**. The comparison of the specific rotation of this compound to that described in the literature led us to determine the (1*R*,3*S*,5*S*)-absolute configuration for the product (−)-**16** obtained from the enzymatic acylation.[8]

The synthesis of the A-ring synthon was completed with three additional steps (Scheme 3). The treatment of **16** with TBDMSCl and imidazole led us to achieve the compound **17**, which bears orthogonal protection for its three hydroxyl groups. Sequentially, the removal of acetyl group in basic conditions afforded the alcohol **18**, which was treated with Dess-Martin

periodinane reagent to give the final A-ring synthon **19**. The absolute configuration was maintained in all steps, as it was verified by quiral HPLC analysis of compound **18** (see Figure S4, Supporting Information).



Scheme 5. Synthesis of 19-*nor*-vitamin D analogues. *Reaction conditions*: (a) LHMDS, THF, −78 ºC to −50 ºC, 7 h (63%); (b) $Bi(OTf)_3$, CH_2Cl_2, 0 ºC, 2 h (75%); (c) EtOH sat HCl, r.t., 30 min (89%).

A-Ring modified 19-*nor*-vitamin D_3 analogues were synthesized through the Julia-modified coupling reaction as illustrated in Scheme 5. Reaction of ketone **19** with the lithium enolate of sulfone **12** gave a mixture of diastereomers **23** and **24**. The separation of **23** and **24** by semipreparative HPLC was not possible due to their low polarity, which was later modulated through hydroxyl group deprotection.

On the other hand, the hydroxyl groups must be differentiated from each other for stereochemical determination. Therefore, different conditions were tested to carry out the selective deprotection of TBDMS *vs* TBDPS group. The best result was achieved using a Lewis acid, $Bi(OTf)_3$, in an organic solvent. The removal of the ethoxymethyl protecting group in the side chain took place simultaneously, providing a 1.37:1 diastereomeric mixture of analogues **25** and **26** that were successfully separated by HPLC. At this stage, we called diastereomer compounds A and B according to the rate of elution in the HPLC chromatogram (see Figure S5, Supporting Information). The identification of the synthesized analogues was performed by NMR. The protons at C6 and C7 in the ^{1}H NMR spectrum, and also the signals corresponding to the carbons C14 and C17 could be identified by monodimensional DEPT-135, DEPT-90 and bidimensional ^{13}C-^{1}H HMBC analysis (see pp S55-S60, Supporting Information). At this point, the 1D NMR selective NOE experiments were the key to undoubtedly complete the structure elucidation for both isomers. In the case of compound A, the selective irradiation of H7 allowed us to identify H10 (Figure 3). This signal was also showed in the 1D NMR selective NOE experiment on the multiplet at 4.08 ppm. In this second experiment we could also observe NOE signals with the *ortho* aromatic protons of the TBDPS group. This proved that the signal at 4.08 ppm belonged to the proton adjacent to OTBDPS and that this silyl ether was in the position C1. Moreover, this aromatic signal and H7 were close enough to exhibit a NOE enhancement. These data led us to identify the isomer A with the structure of **25**. This assignment could be verified analyzing the selective NOE experiment on H3 and H6, where we were able to observe the signals of H4.

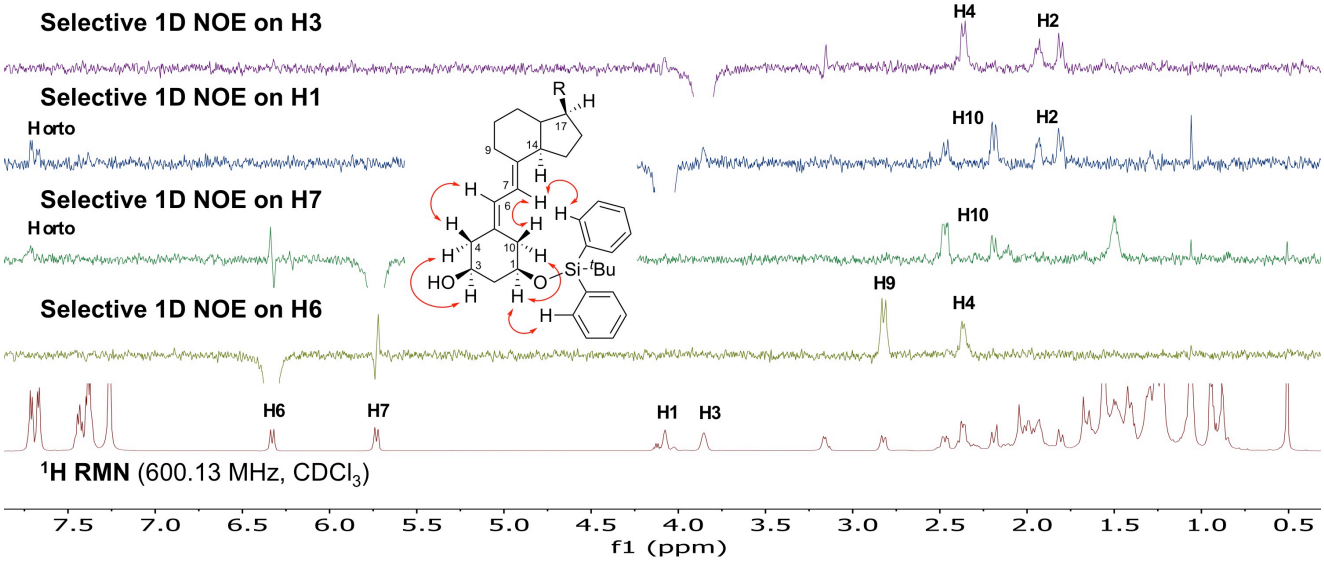


Figure 3. Selective 1D NOE experiments on compound A.

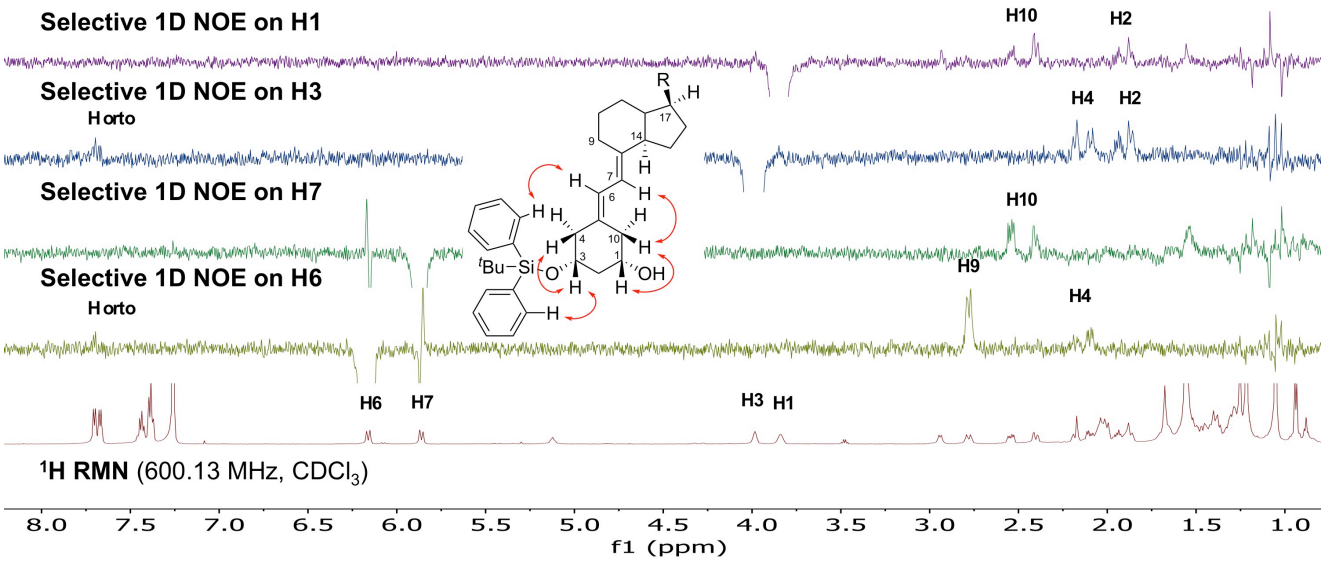


Figure 4. Selective 1D NOE experiments on compound B.

The same argument could be applied for the structure elucidation of compound B (Figure 4). The *ortho* aromatic protons exhibited NOE enhancement with H6, which in turn transferred nuclear spin polarization through transverse relaxation with H4. This effect was also observed between H4 and the proton in alpha position to OTBDPS group. These data ratified the assignment of compound B with the structure of **26**. In this case, the anisotropy effect of the aromatic ring made possible to observe the *ortho* protons when H6 was irradiated.

After successfully complete structure elucidation of the isomers **25** and **26**, the TBDPS group was removed with a saturated solution of HCl in EtOH, giving the final compounds **7** and **8**, respectively.

Biological Evaluation

The biological activity of the newly synthesized analogues was tested *in vitro*, and the results are summarized in Table 2 and Figures 5-8.

Table 2. Biological activities of 19-*nor* analogues of 1α,25-$(OH)_2$-D_3.[a)]

Compound	VDR (%)	hDBP (%)	MFC-7 (%)	HL 60 (%)
1α,25-$(OH)_2$-D_3	100	100	100	100
19-*nor*-1α,25-$(OH)_2$-D_3	100	10	116	170
7	1.2	14	18	10
8	0.4	3	12	6

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

The affinity of compounds **7** and **8** for pig mucosa cytosol VDR and for human vitamin D binding protein (hDBP) was evaluated in comparison to the natural hormone and its 19-*nor* derivative. In addition, inhibition of MCF-7 breast cancer and HL60 leukemia cell proliferation by 1α,25-$(OH)_2$-D_3, 1α,25-$(OH)_2$-19-*nor*-D_3, and analogues **7** and **8** was measured.

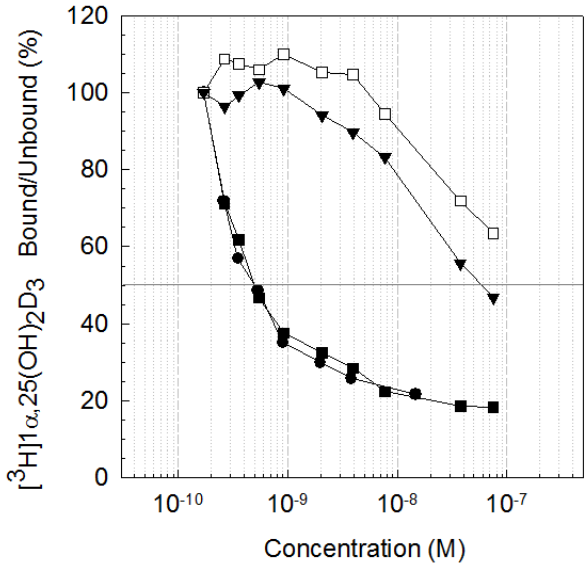


Figure 5. Affinity of 1α,25-$(OH)_2$-D_3 and 19-*nor* analogues for pig vitamin D receptor (VDR). Notes: 1α,25-$(OH)_2$-D_3 (●);1α,25-$(OH)_2$-19-*nor*-D_3 (■); **7** (▼); **8** (□).

The 1α,25-$(OH)_2$-19-*nor*-D_3 analogue, possesing the same configuration of the natural hormone at C-1 and C-3 positions, has the same potency in terms of its ability to bind to the pig VDR, but analogues **7** and **8** have markedly reduced affinity for the VDR (Table 2 and Figure 5).

When comparing hDBP binding among the three 1,25-19-*nor* analogues, compound **7** exhibited the highest affinity, which was still seven times lower than that of 1α,25-$(OH)_2$-D_3 (Table 2 and Figure 6).

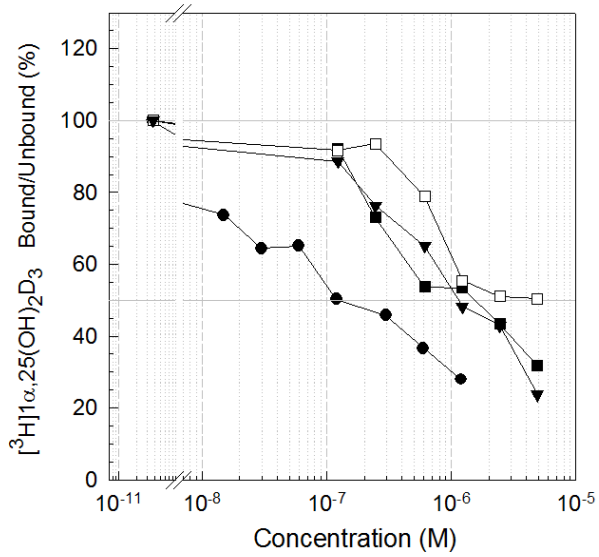


Figure 6. Affinity of 1α,25-$(OH)_2$-D_3 and 19-*nor* analogues for human vitamin D binding protein (hDBP). Notes: 1α,25-$(OH)_2$-D_3 (●);1α,25-$(OH)_2$-19-*nor*-D_3 (■); **7** (▼); **8** (□).

As a measure of the antiproliferative activity of 1,25-19-*nor* analogues, [^{3}H]thymidine incorporation of MCF-7 breast cancer cells and HL 60 leukemia cells was measured after a 72 h incubation period with various concentrations of 1α,25-$(OH)_2$-D_3, analogues or ethanol. The most active compound is the 1α,25-$(OH)_2$-19-*nor*-D_3, which is slightly more active than the natural hormone. Analogues **7** and **8** showed 6-10 and 8-17 fold lower activities than 1α,25, respectively, on MCF-7 and HL 60 cells (Table 1 and Figures 7 and 8).

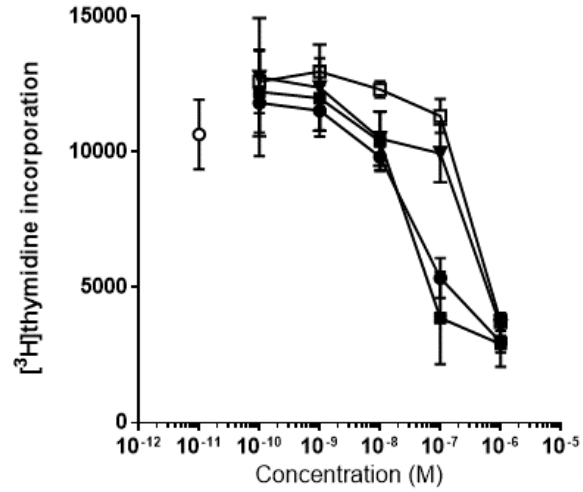


Figure 7. In vitro antiproliferative effects of 1α,25-$(OH)_2$-D_3 and 19-*nor* analogues on breast cancer MCF-7 cells. Notes: Vehicle (○); 1α,25-$(OH)_2$-D_3 (●);1α,25-$(OH)_2$-19-*nor*-D_3 (■); **7** (▼); **8** (□).

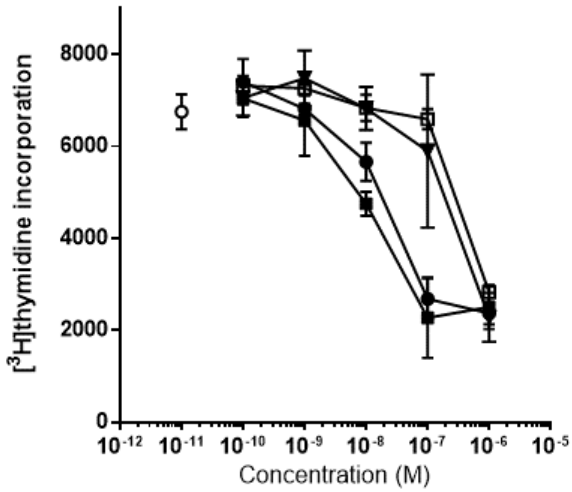


Figure 8. *In vitro* antiproliferative effects of 1α,25-$(OH)_2$-D_3 and 19-*nor* analogues on promyelocytic HL 60 leukemia cells. Notes: Vehicle (○); 1α,25-$(OH)_2$-D_3 (●);1α,25-$(OH)_2$-19-*nor*-D_3 (■); **7** (▼); **8** (□).

These findings prove the key role of the stereochemistry in the biological activity and this would be reflected in different pharmacokinetic profiles of theses analogues.

Conclusion

The synthesis of A-ring diastereomers of 1α,25-$(OH)_2$-19-*nor*-D_3 has been successfully accomplished. For the construction of the diene unit, a strategy based on a Julia-Kocienski olefination between the sulfone **12** and the cyclohexanone derivative **11** was employed. *cis*,*cis*-1,3,5-Cyclohexanetriol, a commercial available compound with the appropriate stereochemistry, was used as starting material for the A-ring precursor. To elucidate the structure of the 19-*nor* analogues, an alternative synthesis was carried out through orthogonally protected derivatives. It is noteworthy the importance of the biocatalytic desymmetrization process of **15** to give different chemical nature to the A-ring hydroxyl groups. This step was successfully performed with *Pseudomonas cepacia* lipase as catalyst, vinyl acetate as acylating reagent, and toluene or TBME as solvents. The preparation of these 19-*nor*-analogues has not been described previously, probably due to the complexity in structure elucidation of the diastereoisomers. The structure of both compounds was established by selective NOE NMR spectroscopy, homonuclear (COSY, TOCSY) and heteronuclear (HSQC, HMBC) correlations. Biological assays on 1,25-19-*nor* analogues **7** and **8** have shown poor binding to VDR, whereas 1α,25-$(OH)_2$-19-*nor*-D_3 has the same affinity as the natural hormone. All three 1,25-19-*nor* analogues have a lower affinity for hDBP than 1α,25-$(OH)_2$-D_3. The most active compound in the inhibition of MCF-7 cell proliferation and HL 60 cell

differentiation was 1α,25-$(OH)_2$-19-*nor*-D_3, even slightly higher than 1α,25.

Experimental Section

General Considerations. All reagents were at highest commercial quality and used without further purification. All non-aqueous reactions were carried out under argon atmosphere in anhydrous, freshly destilled, solvents. *Candida antarctica* lipase type B (CAL-B, Novozyme 435, immobilized by adsorption in Lewatit, 9120 PLU/g) was kindly donated by Novozyme. *Pseudomonas cepacia* lipase (Lipase PS "Amano IM", immobilized on diatomaceous earth, 943 U/g) was purchased from Amano Enzyme. Reactions were monitored by TLC carried out using UV light as visualizing agent and/or spraying a 5% aqueous sulphuric acid solution containing cerium(IV) sulphate (1%) and molybdophosphoric acid (2.5%). Flash chromatography was performed using silica gel 60 (230–400 mesh). ^{1}H, ^{13}C NMR, and DEPT were obtained using 300.13, 400.13 or 600.13 MHz apparatus for ^{1}H, and 75.5, 90.61 or 150.90 MHz for ^{13}C. The same spectrometers were used for the acquisition of ^{1}H-^{1}H homonuclear (COSY, TOCSY, and NOESY) and ^{1}H-^{13}C heteronuclear (HSQC and HMBC) correlations. Optical rotations were recorded on a polarimeter, and values are reported as follows: $[\alpha]_\lambda^T$ (c: g/100 mL, solvent). IR spectra were recorded as thin films on NaCl plates or KBr pellets on an Infrared FT spectrophotometer. Mass spectra (MS) and high resolution mass spectra (HRMS) were recorded on a mass spectrometer under electron spray ionization (ESI) or atmospheric pressure chemical ionization (APCI) conditions.

1α,25-Dihydroxy-3-*epi*-19-*nor*-vitamin D_3 (7). To a solution of **13** (13.86 mg, 0.03 mmol) or **25** (19.29 mg, 0.03 mmol) in EtOH (622 μL), a saturated solution of HCl in EtOH (7.6 mL) was added. The reaction was stirred for 30 min. Then, the solvent was removed, the mixture was extracted with a saturated aqueous solution of $NaHCO_3$ and Et_2O, and the organic extract was washed with brine and dried (Na_2SO_4). The residue was purified by column chromatography using 80% EtOAc/hexane as eluent. Rf: 0.2 (90% EtOAc/hexane); ^{1}H NMR (600.13 MHz, $CDCl_3$): δ 0.54 (s, 3H, *Me*$_{18}$), 0.84 (m, 1H), 0.93 (d, 3H, *Me*$_{21}$, *J* 6.4 Hz), 1.21 (s, 6H, *Me*$_{26}$ + *Me*$_{27}$), 1.25–2.10 (several m), 2.25 (dd, 1H, H_{4ax}, *J* 13.2, 7.1 Hz), 2.40 (d, 1H, H_{10ax}, *J* 13.1, 7.1 Hz), 2.48 (dd, 1H, H_{4eq}, *J* 13.7, 3.6 Hz), 2.59 (dd, 1H, H_{10eq}, *J* 13.5, 3.5 Hz), 2.81 (dd, 1H, H_{9eq}, *J* 12.4, 4.0 Hz), 3.90 (m, 1H, H_1 + H_3), 5.85 (d, 1H, H_7, *J* 11.2 Hz), 6.31 (d, 1H, H_6, *J* 11.3 Hz) ppm; ^{13}C NMR (150.5 MHz, $CDCl_3$): δ 12.2 (*Me*$_{18}$), 19.0 (*Me*$_{21}$), 21.0 (CH_2), 22.4 (CH_2), 23.6 (CH_2), 27.8 (CH_2), 29.0 (CH_2), 29.3 and 29.5 (*Me*$_{26}$ + *Me*$_{27}$), 36.2 (CH, C_{20}), 36.5 (CH_2), 37.0 (CH_2), 40.6 (CH, C_2), 41.4 (CH_2), 44.6 (CH_2), 45.3 (CH_2), 45.9 (C, C_{13}), 56.5 and 56.7 (2CH, C_{14} and C_{17}), 68.7 and 69.0 (2*C*H, C_1 + C_3), 71.3 (C, C_{25}), 115.6 (CH, C_7), 124.2 (CH, C_6), 130.1, and 142.9 (2*C*H, C_5 + C_8) ppm; MS (ESI^+, *m/z*): 405 [$(M+H)^+$, 100%].

1β,25-Dihydroxy-19-*nor*-vitamin D_3 (8). For the synthesis of this derivative, we employed the same procedure as **7** but starting from **14** or **26.** Rf: 0.2 (90% EtOAc/hexane); ^{1}H NMR (600.13 MHz, $CDCl_3$): δ 0.55 (s, 3H, *Me*$_{18}$), 0.88 (m, 1H), 0.93 (d, 3H, *Me*$_{21}$, *J* 6.5 Hz), 1.21 (s, 6H, *Me*$_{26}$ + *Me*$_{27}$), 1.25–2.07 (several m), 2.30 (dd, 1H, H_{4ax}, *J* 13.4, 6.0 Hz), 2.47 (apparent d, 2H, H_{4eq}+ H_{10eq}, *J* 13.6 Hz), 2.56 (dd, 1H, H_{10ax}, *J* 13.7, 6.1 Hz), 2.81(dd, 1H, H_{9eq}, *J* 11.7, 4.1Hz), 4.00 (m, 2H, H_1 + H_3), 5.86 (d, 1H, H_7, *J* 11.2 Hz), 6.34 (d, 1H, H_6, *J* 11.2 Hz) ppm; ^{13}C NMR (150.5 MHz, $CDCl_3$): δ 12.2 (*Me*$_{18}$), 19.0 (*Me*$_{21}$), 21.0 (CH_2), 22.4 (CH_2), 23.6 (CH_2), 27.8 (CH_2), 29.1 (CH_2), 29.4 and 29.5 (*Me*$_{26}$ + *Me*$_{27}$), 36.3 (CH, C_{20}), 36.5 (CH_2), 36.8 (CH_2), 40.4 (*C*H, C_2), 41.0 (CH_2), 44.6 (CH_2), 44.8 (CH_2), 45.9 (C, C_{13}), 56.4 and 56.6 (2CH, C_{14} + C_{17}), 68.8 and 69.0 (2*C*H, C_1 + C_3), 71.3 (C, C_{25}), 115.5 (CH, C_7), 124.2 (*C*H, C_6), 130.1 and 142.9 (2*C*H, C_5 + C_8) ppm; MS (ESI^+, *m/z*): 405 [$(M+H)^+$, 100%].

(1*r*,3*R*,5*S*)-3,5-bis(*tert*-Butyldimethylsilyloxy)cyclohexanol (10). To a solution of **9** (1.0 g, 7.57 mmol) in anhydrous THF (30 mL) at −20 ºC, were successively added anhydrous Et_3N (2.1 mL, 15.14 mmol) and *tert*-butyldimethylsilyl chloride (2.3 g, 15.14 mmol). The reaction was stirred at this temperature for 2 h and then 16 h at r.t. The solvent was evaporated and the crude was purified by column chromatography (30% Et_2O/hexane) to give the compound **10** as a white solid in 43% yield; Rf: 0.4 (30% Et_2O/hexane); mp 78-80 °C; IR (KBr): ν 3269, 2943, 2928, 1471, 1463, 1378 cm^{-1}; ^{1}H NMR (300.13 MHz, $CDCl_3$): δ 0.05 (s, 12H, Si*Me*), 0.87 (s, 18H, SiC*Me*$_3$), 1.32 (m, 3H, H_{2ax} + H_{4ax} + H_{6ax}), 1.72 (s, 2H, OH), 1.99 (m, 1H, H_{6eq}), 2.11 (m, 2H, H_{2eq}+ H_{4eq}), 3.57 (m, 3H, H_1 + H_3 + H_5) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$): δ −4.6 (Si*Me*), −4.5 (Si*Me*), 18.3 (Si*C*), 26.0 (CH_3, SiC*Me*$_3$), 44.8 (C_2 + C_6), 45.1 (C_4), 66.2 (C_1), 66.7 (C_3 + C_5) ppm; MS ($APCI^+$, *m/z*): 361 [$(M+H)^+$, 25%], 383 [$(M+Na)^+$, 100%].

(3*R*,5*S*)-3,5-bis(*tert*-Butyldimethyllsilyloxy)cyclohexanone (11). To a solution of **10** (295 mg, 0.82 mmol) in anhydrous CH_2Cl_2 (8.2 mL), Dess-Martin reagent was added (381 mg, 0.898 mmol). The mixture was stirred at r.t. for 30 min. Then, it was diluted with Et_2O (9.5 mL) and a mixture of saturated aqueous $Na_2S_2O_3$ and $NaHCO_3$ solution (1:1, v/v, 9.5 mL), obtaining a white precipitate that disappeared after 10 min. The mixture was extracted with Et_2O. The combined organic fractions were dried (Na_2SO_4), filtered and concentrated. Purification of the crude by column chromatography (5% Et_2O/hexane) gave **11** as a white solid in 97% yield; Rf: 0.4 (10% Et_2O/hexane); mp: 62-64 °C; IR (KBr): ν 2953, 2928, 2856, 1712, 1471, 1463, 1389 cm^{-1}; ^{1}H NMR (300.13 MHz, $CDCl_3$): δ −0.04 (s, 6H, Si*Me*), 0.04 (s, 6H, Si*Me*), 0.86 (s, 18H, SiC*Me*$_3$), 1.76 (m, 1H, H_{4ax}), 2.25 (m, 1H, H_{4eq}), 2.23 (dd, 2H, H_{2ax} + H_{6ax}, *J* 13.7, 11.2 Hz), 2.53 (dd, 2H, *J* 13.7, 3.9 Hz, H_{2eq} + H_{6eq}), 3.78 (m, 2H, H_3 + H_5) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$): δ −4.6 (Si*Me*), 18.1 (Si*C*), 25.9 (CH_3, SiC*Me*$_3$), 45.2 (C_4), 51.0 (C_2 + C_6), 66.0 (C_3 + C_5), 207 (C=O) ppm; MS (ESI^+, *m/z*): 359 [$(M+H)^+$, 5%], 383 [$(M+Na)^+$, 100%].

Procedure for the synthesis of 13 and 14. To a solution of **12** (167 mg, 0.30 mmol) in anhydrous THF (1.1 mL) at −78 ºC was added dropwise LHMDS (305 μL, 1.0 M in THF, 0.30 mmol), resulting in a deep red colour solution. The mixture was stirred at the same temperature for 2 h, and then, a solution of the corresponding ketone **11** (63 mg, 0.19 mmol) in anhydrous THF (1.2 mL) was added dropwise to the mixture via cannula transfer. After being stirred at −50 ºC during 5 h, the reaction mixture was poured into a saturated aqueous solution of NH_4Cl and extracted with Et_2O. The combined organic fractions were dried (Na_2SO_4), filtered, and concentrated to give a crude that was purified by column chromatography (4% Et_2O/hexane) to give an equimolecular diastereomeric mixture in 51% yield. Then, TBAF (725 μL, 1 M in THF, 0.70 mmol) was added dropwise to the epimeric mixture (100 mg, 0.10 mmol) in anhydrous THF (1.5 mL) at 0 ºC in darkness. After 5 min, the mixture was stirred at r.t. for 2 h. Then, solvent was evaporated and the mixture was extracted with EtOAc. The combined organic fractions were dried (Na_2SO_4), concentrated, and the residue purified by column chromatography (60% Et_2O/hexane). The diastereoisomers were isolated by preparative HPLC (SunFire® C18 Column, 100 Å, 5 □m, 10 mm x 250 mm). Conditions: 4.0 mL/min, 10% ^{i}PrOH/hexane.

25-Ethoxymethyloxy-1α-hydroxy-3-*epi*-19-*nor*-vitamin D_3 (13). Rf: 0.2 (60% EtOAc/hexane). IR (NaCl): ν 3367, 2943, 2872, 1463, 1380 cm^{-1}; ^{1}H NMR (600.13 MHz, $CDCl_3$): δ 0.54 (s, 3H, *Me*$_{18}$), 0.92 (d, 3H, *Me*$_{21}$, *J* 6.5 Hz), 1.02 (m, 1H, H_{22}), 1.20 (t, 3H, $H_{3'}$, *J* 4.0 Hz), 1.21 (s, 6H, *Me*$_{26}$ + *Me*$_{27}$), 1.28 (m, 4H, H_{17} + H_{12} + H_{16}), 1.39 (m, 5H, H_{20} + $1H_{22}$ + $1H_{24}$ + H_{23}), 1.51 (m, 6H, $2H_{15}$ + $1H_{24}$ + 3H), 1.66 (m, 3H, $1H_9$ + $2H_{11}$), 1.78 (m, 1H, H_{2ax}), 1.88 (m, 1H),

1.99 (dd, 3H, H_{12}, *J* 11.4, 8.1 Hz), 2.06 (dt, 1H, H_{2eq}, *J* 12.9, 3.3 Hz), 2.24 (dd, 2H, H_{4ax} + OH, 13.2, 7.1 Hz), 2.39 (dd, 1H, H_{10ax}, 13.5, 7.2 Hz), 2.48 (dd, 1H, H_{4ax}, *J* 14.0, 3.2 Hz), 2.48 (dd, 1H, H_{4eq}, *J* 13.3, 3.6 Hz), 2.59 (dd, 1H, H_{10eq}, *J* 13.5, 3.5 Hz), 2.80 (m, 1H, H_9), 3.61 (q, 2H, *J* 7.1 Hz, $H_{2'}$), 3.90 (m, 2H, H_3 + H_1), 4.75 (s, 2H, $H_{1'}$), 5.85 (d, 1H, H_7, *J* 11.3 Hz), 6.31 (d, 1H, H_6, *J* 11.2 Hz) ppm; ^{13}C NMR (150.5 MHz, $CDCl_3$): δ 12.2 (*Me*$_{18}$), 15.3 (*Me*$_{3'}$), 18.9 (*Me*$_{21}$), 20.7 (CH_2, C_{23}), 22.4 (CH_2, C_{15}), 23.6 (CH_2, C_{11}), 26.5 y 26.6 (*Me* $_{26}$ + *Me*$_{27}$) 27.8 (CH_2, C_{16}), 29.0 (CH_2, C_9), 36.3 (CH, C_{20}), 36.6 (CH_2, C_{22}), 37.0 (CH_2, C_{10}), 40.6 (CH_2, C_{12}), 41.4 (CH_2, C_2), 42.4 (CH_2, C_{24}), 45.3 (CH_2, C_4), 45.9 (C, C_{13}), 56.4 (CH, C_{14}), 56.7 (CH, C_{17}), 63.1 (CH_2, $C_{2'}$), 68.7 (CH, C_3), 68.9 (CH, C_1), 76.4 (C, C_{25}), 89.6 (CH_2, $C_{1'}$), 115.5 (CH, C_7), 123.8 (CH, C_6), 130.3 (C, C_5), 142.9 (C, C_8) ppm; MS (ESI^+, *m/z*): 485 [$(M+Na)^+$, 50%].

25-Ethoxymethyloxy-1β-hydroxy-19-*nor*-vitamin D$_3$ (14). Rf: 0.2 (60% EtOAc/hexane). IR (NaCl): ν 3368, 2943, 2872, 1458, 1348 cm^{-1}; ^{1}H NMR (600.13 MHz, $CDCl_3$): δ 0.54 (s, 3H, *Me*$_{18}$), 0.93 (d, 3H, *Me*$_{21,}$ *J* 6.5 Hz), 1.03 (dd, 1H, H_{22}, *J* 20.0, 9.7 Hz), 1.20 (t, 3H, $H_{3'}$, *J* 4.0 Hz,), 1.21 (s, 6H, *Me*$_{26}$ + *Me*$_{27}$), 1.29 (m, 4H, H_{17} + 2H_{12} + H_{16}), 1.40 (m, 4H, H_{20} + 1H_{22} + 1H_{24} + 1H_{23}), 1.50 (m, 5H, H_{15} + 1H_{24}+ 2H), 1.66 (m, 3H, 1H_9 + 2H_{11}), 1.86 (m, 2H, 1H_2 + 1H_{16}), 1.99 (m, 3H, H_2 + H_{14}), 2.29 (dd, 1H, H_4, *J* 13.4, 6.2 Hz), 2.34 (s, 1H, OH), 2.47 (dd, 1H, H_{10}, *J* 14.0, 3.2 Hz), 2.48 (dd, 1H, H_{10}, *J* 12.6, 2.7 Hz), 2.56 (dd, 1H, H_{10}, *J* 13.8, 6.1 Hz), 2.81 (dd, 1H, H_9, *J* 13.6, 5.3 Hz), 3.61 (q, 2H, *J* 7.1 Hz, $H_{2'}$), 4.00 (m, 2H, H_3 + H_1), 4.75 (s, 2H, $H_{1'}$), 5.86 (d, 1H, H_7, *J* 11.3 Hz), 6.33 (d, 1H, H_6, *J* 11.3 Hz) ppm; ^{13}C NMR (150.5 MHz, $CDCl_3$): δ 12.2 (*Me*$_{18}$), 15.3 (*Me*$_{3'}$), 18.9 (*Me*$_{21}$), 20.6 (CH_2, C_{23}), 22.4 (CH_2, C_{15}), 23.7 (CH_2, C_{11}), 26.5 and 26.6 (*Me*$_{26}$ + *Me* $_{27}$) 27.8 (CH_2, C_{16}), 29.1 (CH_2, C_9), 36.3 (CH, C_{20}), 36.6 (CH_2, C_{22}), 36.8 (CH_2, C_{10}), 40.4 (CH_2, C_2), 40.6 (CH_2, C_{12}), 42.4 (CH_2, C_{24}), 45.2 (CH_2, C_4), 45.9 (C, C_{13}), 56.4 (CH, C_{14}), 56.7 (CH, C_{17}), 63.1 (CH_2, $C_{2'}$). 68.8 (CH, C_3), 69.0 (CH, C_1), 76.4 (C, C_{25}), 89.6 (CH_2, $C_{1'}$), 115.5 (CH, C_7), 124.2 (CH, C_6), 130.1 (C, C_5), 142.9 (C, C_8) ppm; MS (ESI^+, *m/z*): 387 [$(M-OCH_2OEt)^+$, 100%], 399 [$(M-2OH-Et)^+$, 100%], 463 [$(M+H)^+$, 75%], 485 [$(M+Na)^+$, 50%].

(1*R*,3*S*,5*s*)-5-(*tert*-Butyldiphenylsilyloxy)cyclohexane-1,3-diol (15). To a solution of **9** (1 g, 7.18 mmol) in anhydrous THF (20 mL) at r.t. were successively added TBDPSCl (2.3 mL, 8.62 mmol) and anhydrous Et_3N (1.2 mL, 8.62 mmol). After being stirred at this temperature for 30 min, NaH was added (60% w/w in mineral oil, 372 mg, 9.35 mmol). When the solution stopped bubbling, the mixture was heated to 45 ºC for 48 h. After this time, it was cooled to 0 ºC and filtered over Celite®. Solvents were evaporated and the residue purified by column chromatography (EtOAc as eluent) to give the diol **15** in 85% yield. Rf: 0.4 (80% EtOAc/hexane); mp: 94-96 °C; IR (KBr): ν 3324, 3052, 2939, 2860, 1961, 1904, 1826, 1475 cm^{-1}; ^{1}H NMR (300.13 MHz, $CDCl_3$): δ 1.06 (s, 9H, SiC*Me*$_3$), 1.30 (m, 1H, H_{2ax}), 1.44 (dt, 2H, H_{4ax} + H_{6ax}, *J* 17.6, 8.8 Hz), 2.09 (m, 3H, H_{2eq} + H_{4eq} +H_{6eq}), 2.24 (s, 2H, OH), 3.46 (ddd, 2H, H_1 + H_3, *J* 14.3, 10.2, 3.9 Hz), 3.67 (m, 1H, H_5), 7.40 (m, 6H, H_{meta} + H_{para}), 7.68 (dd, 4H, H_{ortho}, *J* 7.7, 1.6 Hz) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 19.2 (Si*C*), 27.0 (CH_3, SiC*Me*$_3$), 43.4 (CH_2, C_2), 43.6 (2CH_2, C_4 + C_6), 65.9 (2CH, C_1 + C_3), 67.5 (CH, C_5), 127.8 (4CH, C_{meta}), 129.8 (2CH, C_{para}), 134.0 (2C, C_{ipso}), 135.8 (4CH, C_{ortho}) ppm; MS (ESI^+, *m/z*): 371 [$(M+H)^+$, 100%]; HRMS (ESI^+, m/z): calcd for $C_{22}H_{30}NaO_3Si$ [$(M+Na)^+$]: 393.1856, found: 393.1860.

(1*R*,3*S*,5*S*)-3-(*tert*-Butyldiphenylsilyloxy)-5-hydroxycyclohexyl acetate (16). *Enzymatic reactions:* A mixture of compound **15** (540 mg, 1.46 mmol), lipase (270 mg) and vinyl acetate (672 µL, 7.29 mmol) in the anhydrous solvent (0.2 M, 7.3 mL) was shaken at 30 ºC and 250 rpm. The progress of the reaction was analyzed by TLC (50% EtOAc/hexane) until the achievement of the complete conversion (4-12 h). Then, the enzyme was removed by filtration and washed with CH_2Cl_2. The crude residue was purified by column chromatography (20-40% EtOAc/hexane as gradient eluent) to give **16** in 99% yield. *Racemic derivative (for the HPLC analyses)*: To a solution of **15** (100 mg, 0.27 mmol) in anhydrous CH_2Cl_2 (2.7 mL) at 0 ºC, Et_3N (63 µL, 0.46 mmol), DMAP (4 mg, 0.033 mmol) and acetic anhydride (38 µL, 0.40 mmol, dropwise) were successively added. The reaction was stirred at r.t. for 2 h and then the solvent was evaporated and the residue purified by column chromatography (20-40% EtOAc/hexane as gradient eluent) to give the racemic derivative **16** (65% yield). Rf: 0.5 (40% EtOAc/hexane); IR (NaCl): ν 3425, 3070, 3048, 2953, 2858, 1961, 1902, 1825, 1737, 1472, 1427 cm^{-1}; $[\alpha]_D^{20}$= −12 (*c* 1.0, $CHCl_3$); ^{1}H NMR (300.13 MHz, $CDCl_3$): δ 1.05 (s, 9H,SiC*Me*$_3$), 1.24-1.67 (m, 4H, H_{2ax} + H_{4ax} +H_{6ax}+ OH), 2.01 (s, 3H, *Me*, OAc), 2.10 (m, 3H, H_{2eq} + H_{4eq} + H_{6eq}), 3.50 (m, 1H, H_5), 3.69 (m, 1H, H_3), 4.56 (m, 1H, H_1), 7.41 (m, 6H, H_{meta} + H_{para}), 7.65 (dd, 4H, H_{ortho}, *J* 7.7, 1.6 Hz) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 19.2 (Si*C*), 21.4 (*Me*, OAc), 27.0 (CH_3, SiC*Me*$_3$), 39.9 (CH_2), 40.0 (CH_2), 43.7 (CH_2), 65.6 (CH, C_5), 67.1 (CH, C_3), 67.9 (CH, C_1), 127.8 (4CH, C_{meta}), 129.9 (2CH, C_{para}), 133.9 (C, C_{ipso}), 134.0 (C, C_{ipso}), 135.9 (4CH, C_{ortho}), 170.5 (C=O) ppm; MS (ESI^+, *m/z*): 413 [$(M+H)^+$, 100%]; HRMS (ESI^+, m/z): calcd for $C_{24}H_{32}NaO_4Si$ [$(M+Na)^+$]: 435.1962, found: 435.1983.

(1*R*,3*R*,5*S*)-3-(*tert*-Butyldiphenylsilyloxy)-5-(*tert*-butyldimethylsilyloxy)cyclohexyl acetate (17). To a solution of **16** (500 mg, 1.21 mmol) in anhydrous CH_2Cl_2 (4.8 mL) at 0 ºC, were added imidazole (215 mg, 3.15 mmol) and *tert*-butyldimethylsilyl chloride (439 mg, 2.91 mmol). Afterwards, the reaction was stirred at r.t. for 3 h. Then, solvent was evaporated and the residue purified by column chromatography (10% Et_2O/hexane as eluent) to give **17** in 94% yield. Rf: 0.5 (10% EtOAc/hexane); IR (NaCl): ν 3425, 3070, 3048, 2953, 2858, 1961, 1902, 1825, 1737, 1472, 1427 cm^{-1}; $[\alpha]_D^{20}$= □3 (*c* 1.0, $CHCl_3$); ^{1}H NMR (300.13 MHz, $CDCl_3$): δ −0.04 (s, 3H, Si*Me*), −0.03 (s, 3H, Si*Me*), 0.85 (s, 9H, SiC*Me*$_3$, TBDMS) 1.11 (s, 9H, SiC*Me*$_3$, TBDPS), 1.36 (m, 2H, H_{2ax} + H_{6ax}), 1.51 (dd, 1H, H_{4ax}, *J* 21.8, 10.3 Hz), 1.98 (m, 1H, H_{4eq}), 2.00 (s, 6H, *Me*, OAc), 2.09 (m, 1H, H_{2eq}), 2.22 (m, 1H, H_{6eq}) 3.40 (m, 1H, H_3), 3.65 (m, 1H, H_5), 4.57 (m, 2H, H_1), 7.41 (m, 6H, H_{meta} + H_{para}), 7.65 (dd, 4H, H_{ortho}, *J* 7.8, 1.6 Hz) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$): δ −4.7 (Si*Me*), −4.6 (Si*Me*), 18.1 (Si*C*), 19.2 (Si*C*), 21.3 (*Me*, OAc), 25.9 (SiC*Me*$_3$, TBDMS), 27.0 (SiC*Me*$_3$, TBDPS), 40.6 (CH_2, C_6), 40.9 (CH_2, C_2), 45.0 (CH_2, C_4), 66.1 (CH, C_3), 67.2 (CH, C_5), 68.0 (CH, C_1), 127.6 (2CH, C_{meta}), 127.7 (2CH, C_{meta}) 129.8 (2CH, C_{para}), 134.2 (2C, C_{ipso}), 134.4 (2C, C_{ipso}), 135.8 (2CH, C_{ortho}), 135.9 (2CH, C_{ortho}), 170.2 (C=O) ppm; MS (ESI^+, *m/z*): 527 [$(M+H)^+$, 100%].

(1*R*,3*R*,5*S*)-3-(*tert*-Butyldiphenylsilyloxy)-5-(*tert*-butyldimethylsilyloxy)cyclohexan-1-ol (18). To a solution of **17** (520 mg, 0.99 mmol) in anhydrous MeOH (4.8 mL), MeONa (140 mg, 2.47 mmol) was added, showing a progressive cloudiness. After being stirred at r.t. for 4 h, solid ammonium chloride was added until the medium was neutralized. Then, the solvent was evaporated, the residue was dissolved in EtOAc and it was filtered to remove the salts. The crude was purified by column chromatography (20% Et_2O/hexane as eluent) to give **18** in 95% yield; Rf: 0.4 (20% Et_2O /hexane); IR (NaCl): ν 3349, 3071, 3049, 2951, 2857, 1958, 1887, 1822, 1471, 1427 cm^{-1}; $[\alpha]_D^{20}$= +9 (*c* 1.0, $CHCl_3$); ^{1}H NMR (300.13 MHz, $CDCl_3$): δ −0.05 (s, 6H, Si*Me*), 0.84 (s, 9H, SiC*Me*$_3$, TBDMS) 1.07 (s, 9H, SiC*Me*$_3$, TBDPS), 1.27 (m, 1H), 1.38 (m, 2H), 1.59 (s, 1H, OH), 1.92 (m, 1H), 2.05 (m, 1H), 2.15 (m, 1H), 3.38 (m, 2H, H_1 + H_5), 3.58 (m, 1H, H_3), 7.41 (m, 6H, H_{meta} + H_{para}), 7.69 (ddd, 4H, H_{ortho}, *J* 7.9, 4.5, 1.7 Hz) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$): δ −4.7 (Si*Me*), −4.6 (Si*Me*), 18.3 (Si*C*), 19.2 (Si*C*), 26.0 (SiC*Me*$_3$, TBDMS), 27.1 (SiC*Me*$_3$, TBDPS), 44.5 (CH_2), 44.7 (CH_2), 44.8 (CH_2), 66.1 and 66.4 (2CH, C_1 + C_5), 67.3 (CH, C_3), 127.7 (4CH, C_{meta}), 129.76 (CH, C_{para}), 129.81 (CH, C_{para}), 134.31 (C, C_{ipso}), 134.35 (C, C_{ipso}), 135.86 (2CH, C_{ortho}), 135.88 (2CH, C_{ortho}) ppm; MS (ESI^+, *m/z*): 485 [$(M+H)^+$, 100%].

(3*S*,5*R*)-3-(*tert*-Butyldiphenylsilyloxy)-5-(*tert*-butyldimethylsilyloxy)cyclohexanone (19). To a solution of **18** (452 mg, 0.93 mmol) in anhydrous CH_2Cl_2 (9.0 mL), Dess-Martin reagent was added (448 mg, 1.02 mmol). The mixture was stirred at r.t. for 1 h and then, it was diluted with Et_2O (9.5 mL) and a mixture of saturated aqueous $Na_2S_2O_3$ and $NaHCO_3$ solution (1:1, v/v, 9.5 mL), obtaining a white precipitate that disappeared after 10 min. After that, the mixture was extracted with Et_2O. The combined organic fractions were dried (Na_2SO_4), filtered and concentrated. Purification of the crude by column chromatography (10% Et_2O/hexane) gave **19** in 97% yield; Rf: 0.7 (20% Et_2O/hexane); IR (KBr): ν 3064, 3049, 2951, 2890, 1958, 1894, 1822, 1717, 1467, 1427 cm^{-1}; $[\alpha]_D^{20}$= −1 (*c* 1.0, $CHCl_3$); ^1H NMR (300.13 MHz, $CDCl_3$): δ −0.03 (s, 6H, Si*Me*), 0.86 (s, 9H, SiC*Me*$_3$, TBDMS) 1.11 (s, 9H, SiC*Me*$_3$, TBDPS), 1.84 (m, 1H, H_{4ax}), 2.17 (m, 1H, H_{4eq}), 2.35 (dd, 1H, H_{6ax}, *J* 13.8, 11.1 Hz), 2.50 (dt, 2H, H_{2ax} + H_{6eq}, *J* 14.0, 7.0 Hz), 2.63 (dd, 1H, H_{2eq}, *J* 14.1, 5.1 Hz), 3.59 (m, 1H, H_5), 3.84 (m, 1H, H_3), 7.42 (m, 6H, H_{meta} + H_{para}) and 7.70 (ddd, 4H, H_{ortho}, *J* 16.8, 7.6, 1.5 Hz) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$): δ −4.9 (Si*Me*), −4.8 (Si*Me*), 18.1 (*C*Si), 19.1 (Si*C*), 25.8 (SiC*Me*$_3$, TBDMS), 27.0 (SiC*Me*$_3$, TBDPS), 44.7 (CH_2, C_4), 50.6 (CH_2, C_2), 50.9 (CH_2, C_6), 65.6 (CH, C_5), 66,6 (CH, C_3), 127.8 (4CH, C_{meta}), 130.0 (2CH, C_{para}),133.5 (C, C_{ipso}), 133.8 (C, C_{ipso}), 135.7 (2CH, C_{ortho}), 135.8 (2CH, C_{ortho}), 207.1 (C=O) ppm; MS (ESI^+, *m/z*): 483 [$(M+H)^+$, 100%].

(1*R*,3*S*,5*s*)-5-(*tert*-Butyldiphenylsilyloxy)cyclohexan-1,3-diyl diacetate (20). By-product of the enzymatic reaction. Rf: 0.8 (40% EtOAc/hexane); IR (NaCl): ν 3071, 3049, 2954, 2930, 2857, 1959, 1887, 1738, 1471, 1427 cm^{-1}; ^1H NMR (300.13 MHz, $CDCl_3$): δ 1.05 (s, 9H, SiC*Me*$_3$), 1.34 (q, 1H, H_{2ax}, *J* 11.6 Hz), 1.48 (c, 2H, H_{4ax} + H_{6ax}, *J* 11.7 Hz), 2.00 (s, 6H, *Me*, OAc), 2.19 (m, 3H, H_{2eq} + H_{4eq} +H_{6eq}), 3.67 (m, 1H, H_5), 4.53 (m, 2H, H_1 + H_3), 7.41 (m, 6H, H_{meta} + H_{para}), 7.65 (dd, 4H, H_{ortho}, *J* 7.8, 1.6 Hz) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 19.2 (Si*C*), 21.2 (*Me*, OAc), 27.0 (CH_3, *Me*$_2$CSi), 36.5 (CH_2, C_2), 40.5 (CH_2, C_4 + C_6), 66.7 (CH, C_5), 67.3 (CH, C_1 + C_3), 127.8 (4CH, C_{meta}), 129.9 (2CH, C_{para}), 133.8 (2C, C_{ipso}), 135.8 (4CH, C_{ortho}), 170.1 (C=O) ppm; MS (ESI^+, *m/z*): 455 [$(M+H)^+$, 100%].

(1*R*,3*R*)-5-(*tert*-Butyldiphenylsilyloxy)cyclohexan-1,3-diyl diacetate (21). To a solution of (−)-**16** (60 mg, 0.15 mmol) in anhydrous THF (250 µL), were successively added PPh_3 (57 mg, 0.22 mmol), DIAD (42 µL, 0.22 mmol, dropwise) and acetic acid (13 µL, 0.22 mmol, dropwise). After being stirred at r.t. for 4 h, the solvent was evaporated, and the residue purified by column chromatography (20% Et_2O/hexane) to give the diacetate **21** (72%). Rf: 0.2 (20% Et_2O/hexane); IR (NaCl): ν 3071, 3049, 2958, 2857, 1961, 1892, 1741, 1734, 1471, 1427 cm^{-1}; $[\alpha]_D^{20}$= +1 (*c* 1.0, $CHCl_3$); ^1H NMR (300.13 MHz, $CDCl_3$): δ 1.05 (s, 9H, SiC*Me*$_3$), 1.50 (m, 3H, H_{2ax} + H_{4ax} + H_{6ax}), 1.80 (s, 3H, *Me*, OAc). 1.86 (m, 1H, H_{6eq}), 1.96 (m, 1H, H_{2eq}), 2.02 (s, 3H, *Me*C=O), 2.26 (m, 1H, H_{4eq}), 3.90 (m, 1H, H_5), 4.88 (m, 1H, H_3), 5.14 (m, 1H, H_1) 7.39 (m, 6H, H_{meta} + H_{para}), 7.70 (dd, 4H, H_{ortho}, *J* 7.7, 1.6 Hz) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 19.2 (Si*C*), 21.2 (*Me*, OAc), 21.4 (*Me*, OAc), 27.0 (SiC*Me*$_3$), 34.9 (CH_2, C_2), 38.6 (CH_2, C_6), 40.5 (CH_2, C_4), 66.4 (CH, C_5), 68.0 (CH, C_3), 68.7 (CH, C_1), 127.7 (2CH, C_{meta}), 127.8 (2CH, C_{meta}), 129.82 (CH, C_{para}), 129.83 (CH, C_{para}), 134.0 (C, C_{ipso}), 134.1 (C, C_{ipso}), 135.8 (2CH, C_{ortho}),135.9 (2CH, C_{ortho}), 170.0 (C=O), 170.4 (C=O) ppm; MS (ESI^+, *m/z*): 455 [$(M+H)^+$, 100%].

(1*S*,3*S*)-5-Hydroxycyclohexan-1,3-diyl diacetate (22). TBAF (219 µL, 0.22 mmol, 1.0 M in THF) was added dropwise to a solution of silyl ether **21** (40 mg, 0.09 mmol) in anhydrous THF (437 µL) at 0 ºC in darkness. The reaction was stirred at r.t. until TLC (60% Et_2O/hexane) shows complete consumption of starting material (3 h). Then, solvent was evaporated and the mixture was extracted with EtOAc. The combined organic fractions were dried (Na_2SO_4) and concentrated under vacuum. The crude was purified by column chromatography (30% EtOAc/hexane) to give **22** in 79% yield. Rf: 0.3 (30% AcOEt/hexane); IR (NaCl): ν 3444, 2958, 2865, 1738, 1734, 1716, 1435, 1372, 1256, 1238 cm^{-1}; $[\alpha]_D^{20}$= +13.8 (*c* 1.0, $CHCl_3$); ^1H NMR (300.13 MHz, $CDCl_3$): δ 1.53 (m, 3H, H_{2ax} + H_{4ax} + H_{6ax}), 1.96 (s, 1H, OH), 2.05 (s, 3H, *Me*, OAc), 2.06 (s, 3H, *Me*, OAc), 2.08 (m, 2H), 2.28 (m, 1H), 4.03 (m, 1H, H_5), 5.05 and 5.28 (2m, 2H, H_1 + H_3) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 21.3 (*Me*, OAc), 21.4 (*Me*, OAc), 34.9 (CH_2), 38.3 (CH_2), 40.0 (CH_2), 65.2 (CH, C_5), 68.2 and 68.5 (2CH, C_3 + C_1), 170.3 (C=O), 170.4 (C=O) ppm; MS (ESI^+, *m/z*): 217 [$(M+H)^+$, 100%].

Procedure for the synthesis of 25 and 26. To a solution of **12** (300 mg, 0.55 mmol) in anhydrous THF (1.8 mL) at −78 ºC was added dropwise LHMDS (520 µL, 1.0 M in THF, 0.52 mmol), resulting in a deep red color solution. The mixture was stirred at the same temperature for 2 h and then, a solution of the corresponding ketone **19** (264 mg, 0.55 mmol) in anhydrous THF (1.2 mL) was added dropwise via cannula transfer. After being stirred at −50 ºC during 5 h, the reaction was poured into a saturated aqueous solution of NH_4Cl and extracted with Et_2O. The combined organic fractions were dried (Na_2SO_4), filtered, and concentrated to give a crude that was purified by column chromatography (4% Et_2O/hexane). Then, the resulting mixture (269 mg, 0.33 mmol) was dissolved in anhydrous CH_2Cl_2 (3.3 mL), $Bi(OTf)_3$ (238 mg, 0.36 mmol) was added and the mixture was stirred at 0 ºC for 2 h. The reaction was quenched by adding a saturated aqueous solution of $NaHCO_3$. Next, it was diluted with CH_2Cl_2 and filtered over Celite®. Solvents were evaporated under vacuum and the residue purified by column chromatography (50% Et_2O/hexane). The diastereoisomers were isolated by preparative HPLC (SunFire® C18 Column**,** 100 Å, 5 □m**,** 10 mm x 250 mm). Conditions: 5.0 mL/min, 5% ^{i}PrOH/hexane.

1α-(*tert*-Butyldiphenylsilyloxy)-25-hydroxy-3-*epi*-19-*nor*-vitamin D$_3$ (25). Rf: 0.4 (70% Et_2O/hexane); ^1H NMR (600.13 MHz, $CDCl_3$): δ 0.51 (s, 3H, *Me*$_{18}$), 0.88 (m, 1H), 0.94 (d, 3H, *Me*$_{21}$, *J* 6.4 Hz), 1.06 (s, 9H, SiC*Me*$_3$), 1.23 (s, 6H, *Me*$_{26}$ + *Me*$_{27}$), 1.25–2.07 (several m, 19H), 2.19 (d, 1H, H_{10eq}, *J* 13.8 Hz), 2.37 (dd, 2H, H_4, *J* 10.2, 4.4 Hz), 2.47 (dd, 1H, H_{10ax}, *J* 13.7, 5.7 Hz), 2.82 (m, 1H, H_9), 3.16 (d, 1H, *J* 8.6 Hz), 3.85 (br s, 1H, H_3), 4.08 (br s, 1H, H_1), 5.73 (d, 1H, H_7, *J* 11.2 Hz), 6.33 (d, 1H, H_6, *J* 11.3 Hz), 7.38 (q, 4H, H_{meta}, *J* 7.1 Hz), 7.43 (m, 2H, H_{para}), 7.67 (d, 2H, *J* 6.6 Hz,), 7.71 (d, 2H, *J* 7.71 Hz) ppm; ^{13}C NMR (150.5 MHz, $CDCl_3$): δ 12.3 (*Me*$_{18}$), 18.9 (*Me*$_{21}$), 19.4 (*C*Si), 21.0 (CH_2), 22.5 (CH_2), 23.6 (CH_2), 27.2 (SiC*Me*$_3$), 27.8 (CH_2), 29.0 (CH_2) 29.3 y 29.5 (*Me*$_{26}$ + *Me*$_{27}$), 36.3 (CH, C_{20}), 36.5 (CH_2), 36.6 (CH_2), 40.7 (CH_2, C_2), 40.5 (CH_2), 44.6 (CH_2), 45.5 (CH_2), 45.9 (C, C_{13}), 56.4 and 56.7 (2CH, C_{14} + C_{17}), 68.9 (CH, C_3), 70.6 (CH, C_1), 71.5 (C, C_{25}), 115.8 (CH, C_7), 124.0 (CH, C_6), 127.75 and 127.83 (2CH, C_{meta}), 129.90 and 130.02 (2CH, C_{para}), 130.6 (C_5), 133.4 and 133.7 (2C, C_{ipso}) 135.97 and 136.06 (2CH, C_{ortho}), 142.3 (C_8) ppm.

3-(*tert*-Butyldiphenylsilyl)-1β,25-dihydroxy-19-*nor*-vitamin D$_3$ (26). Rf: 0.4 (70% Et_2O/hexane); ^1H NMR (600.13 MHz, $CDCl_3$): δ 0.53 (s, 3H, *Me*$_{18}$), 0.88 (m, 1H), 0.94 (d, 3H, *Me*$_{21}$, *J* 6.4 Hz), 1.05 (s, 9H, SiC*Me*$_3$), 1.22 (s, 6H, *Me*$_{26}$ + *Me*$_{27}$), 1.25–2.07 (several m, 20 H), 2.18 (m, 1H, H_{4eq}), 2.40 (dd, 1H, H_{10eq}, *J* 13.4, 3.3 Hz), 2.54 (dd, 1H, H_{10ax}, *J* 13.5, 6.3 Hz), 2.78 (dd, 1H, H_{9eq}, *J* 14.2, 4.4 Hz), 2.94 (d, 1H, H_{2eq}, *J* 9.1 Hz), 3.84 (br s, 1H, H_1), 3.99 (m, 1H, H_3), 5.14 (br s, 1H, OH), 5.86 (d, 1H, H_7, *J* 11.2 Hz), 6.16 (d, 1H, H_6, *J* 11.2 Hz), 7.38 (m, 4H, H_{meta}), 7.43 (m, 2H, H_{para}), 7.67 (d, 2H, *J* 7.9 Hz,), 7.70 (d, 2H, *J* 6.7 Hz) ppm; ^{13}C NMR (150.5 MHz, $CDCl_3$): δ 12.2 (*Me*$_{18}$), 18.9 (*Me*$_{21}$), 19.2 (*C*Si), 21.0 (CH_2), 22.4 (CH_2), 23.6 (CH_2), 27.2 (SiC*Me*$_3$), 27.9 (CH_2), 29.0 (CH_2), 29.4 and 29.5 (*Me*$_{26}$ + *Me*$_{27}$), 36.3 (CH, C_{20}), 36.6 (CH_2), 37.1 (CH_2), 40.4 (CH_2), 40.6 (CH_2), 44.5 (CH_2), 45.2 (CH_2), 45.9 (C, C_{13}), 56.4 and 56.6 (2CH, C_{14} + C_{17}), 68.7 (CH, C_3), 71.0 (CH, C_1), 71.3 (C, C_{25}), 115.9 (CH, C_7), 124.2 (CH, C_6), 127.78 and 127.81 (2CH, C_{meta}), 129.90 and 129.95 (2CH, C_{para}),

130.5 (C_5), 133.7 and 134.0 (2C, C_{ipso}) 136.00 and 136.09 (2CH, C_{ortho}), 142.3 (C_8) 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.[12] 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.[13] [^{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.[12]

Cell differentiation assays. Differentiation of promyeolocytic HL-60 leukemia cells (ATCC) was measured by the nitro blue tetrazolium (NBT) reduction assay after a 72 h incubation period in the presence of 1α,25-$(OH)_2$-D_3, analogues or vehicle.[12]

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