RESEARCH ARTICLE doi.org/10.1002/adsc.202201158

Convergent Approaches and Biological Activity of C1 and/or C3 Mono or Diamino Derivatives of 1α,25-Dihydroxy-19-*nor*-vitamin D_3. Key Enzymatic Desymmetrization of A-Ring Synthon Precursor

Tania González-García,[a, b] Annemieke Verstuyf,[c] Lieve Verlinden,[c] Susana Fernández,[a,*] and Miguel Ferrero[a,*]

[a] Departamento de Química Orgánica e Inorgánica, Universidad de Oviedo, 33006-Oviedo (Asturias), Spain
Phone: (+34)-985-102-984 (S. F.) or (+34)-985-105-013 (M. F.)
E-mail: fernandezgsusana@uniovi.es; mferrero@uniovi.es
[b] Current address: FAES FARMA, 48940-Leioa (Vizcaya), Spain
[c] Laboratorium voor Experimentele Geneeskunde en Endocrinologie, Katholieke Universiteit Leuven, Gasthuisberg, B-3000 Leuven, Belgium

Manuscript received: October 19, 2022; Revised manuscript received: December 19, 2022;
Version of record online: January 18, 2023

Supporting information for this article is available on the WWW under https://doi.org/10.1002/adsc.202201158

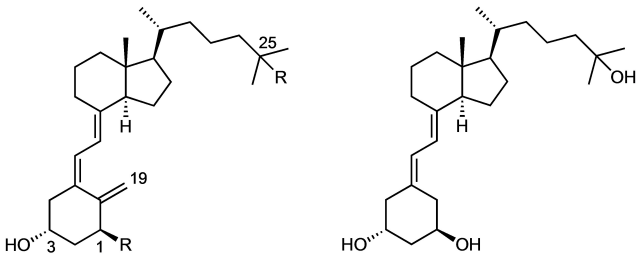
Abstract: Three novel 19-*nor*-1α,25-dihydroxyvitamin D_3 derivatives modified at C1 and/or C3 with an amino group were synthesized to study the influence of the substitution of one or both hydroxyl groups of A-ring by amino groups on the affinity for vitamin D receptor (VDR) and vitamin D binding protein (hDBP), and also on the antiproliferative activity. The A-ring precursors were prepared from *cis*,*cis*-1,3,5-cyclohexanetriol applying a desymmetrization reaction of a prochiral 1,3-diol catalyzed by *Pseudomonas cepacia* lipase as a key step. The structural elucidation of diastereoisomers were unambiguously assigned by NMR spectroscopy.

Keywords: 19-*nor*-vitamin D_3 analogs; biocatalysis; enzymatic desymmetrization; A-ring amino derivatives of calcitriol

Introduction

The biologically active metabolite of vitamin D_3 (**1**, Figure 1), known as Calcitriol [**2**, 1α,25-dihydroxyvitamin D_3, 1α,25-$(OH)_2$-D_3], regulates bone formation, calcium, and phosphate homeostasis,[1] but it also is a potent differentiator and growth inhibitor of several types of cancer cells.[2]

To show the latter actions, supraphysiological doses are required resulting in calcemic effects, which limit the use of this natural hormone.[3] In order to overcome this limitation, many structural modified analogs of the 1α,25-$(OH)_2$-D_3 have been synthesized.[4] Among the changes, A-ring modified 19-*nor* derivatives, which lack the methylene group at C19, are particularly attractive, since show selective activities, induce potent



1, R= H; Vitamin D_3
2, R= OH; 1α,25-$(OH)_2$-D_3
3, 1α,25-$(OH)_2$-19-*nor*-D_3

Figure 1. Structures of vitamin D_3, its hormone and 1α,25-$(OH)_2$-19-*nor*-D_3.

differentiation and have a very low or absent bone calcitropic activity,[5] in addition of increased stability due to absence of conjugated triene. Thus, 1α,25-dihydroxy-19-*nor*-vitamin D_3 (**3**) shows minor calcemic effects while retaining good cell-differentiating properties.[6] Single modifications on the structure of the natural hormone, such as stereochemistry change of C1 and C3, has a strong impact on activities of the resulting new analogs.[7] On the other hand, Medicinal Chemists often seek out for drug candidate development in chiral amines due to their inherent properties related with biologically relevant hydrogen bonding capabilities.[8]

Most of new derivatives are hybrids, this implies that more than one modification in one or more parts within the molecule are present.

Despite the large number of derivatives of vitamin D_3, only a few analogs have amino groups in their structures. The first amino vitamin D derivatives synthesized were 3α- or 3β-amino-3-deoxyvitamin D_3 (**4**, Figure 2).[9]

C2β-Amino[10] **5** or C2α/β-*N,(N)*-(di)substituted[11] **6** vitamin D compounds were also reported, since substituents at C2 increase the biological activity of these derivatives. Our research group has described the preparation of mono or diamino 1α,25-$(OH)_2$-D_3 analogs **7** with several configurations at C1 and C3.[12] The only analogs **8** described with modifications in the side chain were patented by Hesse *et al.*,[13] in which C25-hydroxyl was replaced by amino groups. A-Ring monoamino derivatives **9** and **10**[14] represent the only examples of 19-*nor*-D_3 analogs with an amino group in their structure, both endocyclic and exocyclic, respectively. Some of these derivatives have shown interesting biological properties.

Taking into account all these precedents and our research focused on the preparation of A-ring modified analogs,[15] we now describe several new derivatives (**11**–**15**) produced by replacing one or both hydroxyl groups of the A-ring of 1α,25-dihydroxy-19-*nor*-vitamin D_3 by amino groups and to change the configurations on C1 and C3 stereocenters (Figure 3). In addition to the synthesis, we also report the biological activities.

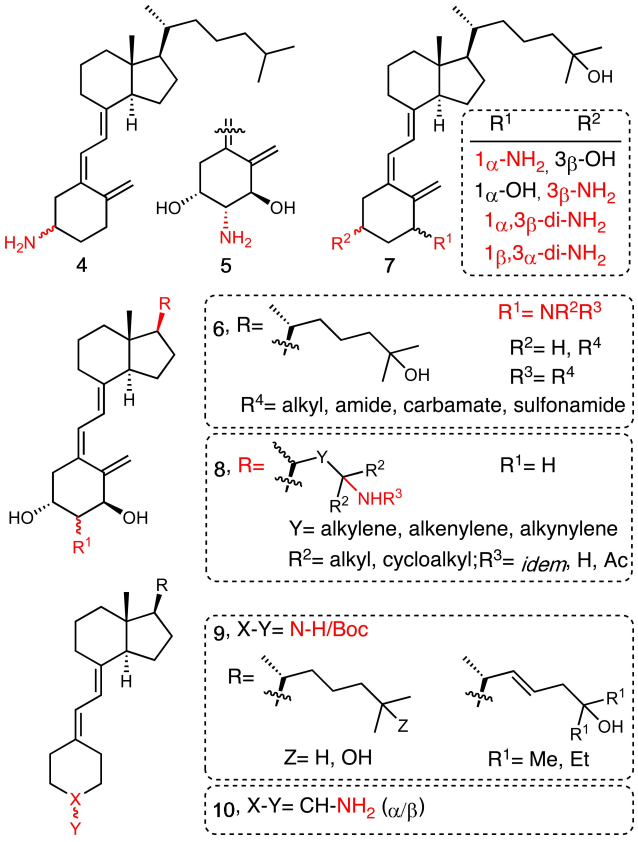


Figure 2. Amino derivatives of vitamin D_3 and 19-*nor*-D_3.

Results and Discussion

Chemistry

The synthetic strategy involves the coupling between a ketone A-ring precursor and the sulfone of the CD ring-side chain fragment to construct a diene using the modified Julia olefination previously reported by Kittaka.[16]

A-ring synthons were obtained starting from *cis*,*cis*-1,3,5-cyclohexanetriol. Thus, triol **16** was monoprotected with *tert*-butyldimethylsilyl chloride (TBSCl), Et_3N, and NaH in THF to afford silyl ether **17** in 77% yield (Scheme 1). Tosylation of **17** gave **18**, which was treated with tetrabutylamoniumfluoride (TBAF) to provide **19**. Transformation of tosyl groups to inverted amines by reaction with NaN_3 in DMF at 70 °C yield **20**. Best results were achieved by direct conversion in mild reaction conditions of diol **17** to diazide compound **21** by treatment with Ph_3P, DIAD and diphenylphosphorylazide (DPPA) under Mitsunobu method. After silyl deprotection, this approach provides the diazide **20** in 54% yield (instead 30% yield) from triol **16**. Next, compound **20** was treated with Dess Martin

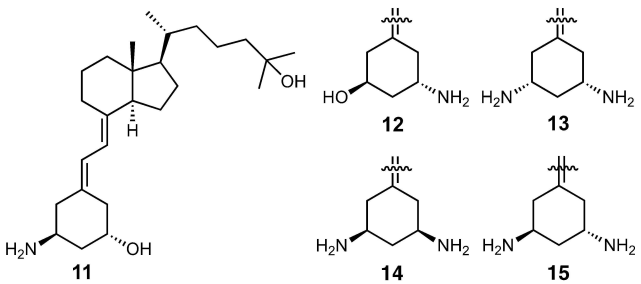
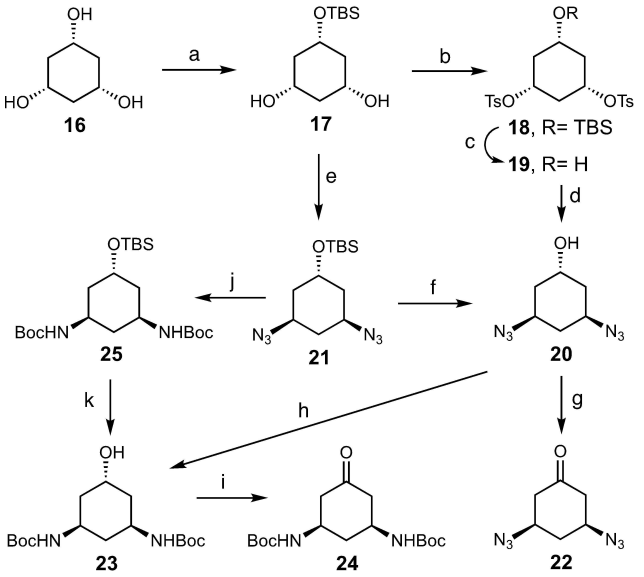


Figure 3. Target C1 and C3 epimers of amino/diamino 19-*nor*-vitamin D_3 analogs.

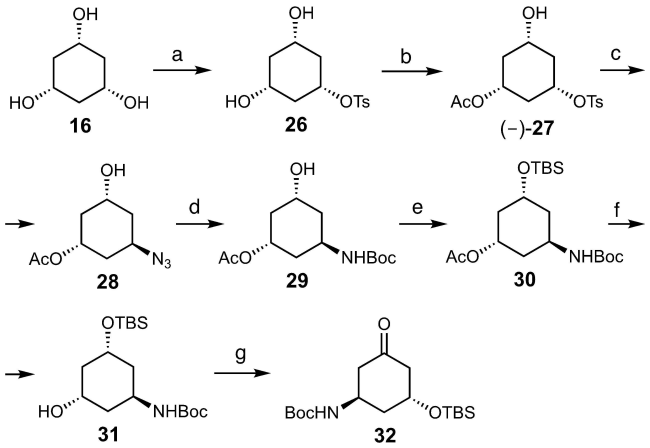


Scheme 1. Synthesis of *cis*-diamino A-ring precursor **24**. Reagents and conditions: a) TBSCl, Et_3N, NaH, THF, rt to 45 °C, 19.5 h, 77%; b) TsCl, Py, 0 °C, 48 h, 90%; c) TBAF, THF, rt, 3 h, 71%; d) NaN_3, DMF, 70 °C, 4 h, 60%; e) Ph_3P, DIAD, $(PhO)_2P(O)N_3$, THF, 0 °C to rt, 12.5 h, 83%; f) TBAF, THF, 0 °C, 2 h, 85%; g) Dess-Martin, CH_2Cl_2, rt, traces; h) Me_3P, Boc_2O, NaOH 1 M,THF, rt, overnight, 63% or Pd/C, H_2, Boc_2O, EtOAc, rt, 18.5 h, 93%; i) Dess-Martin, NH_4Cl, CH_2Cl_2, rt, 30 min, 80% or TEMPO, KBr, NaClO, HCl, CH_2Cl_2, rt, 2 h, 90%; j) Pd/C, H_2, Boc_2O, EtOAc, rt, 18.5 h, 53%; k) TBAF, THF, rt, 10 h, 82%.

periodinane. However, the desired ketone **22** was obtained as traces since the major product of the reaction is the α,β-unsaturated ketone resulted from the elimination of an azide group. As an alternative sequence, diazide **20** was transformed to *N*-Boc compound **23** by the Staundinger reaction using the optimized conditions reported by Vilarrasa et al.[17] Thus, treatment of **20** with Me_3P in the presence of NaOH 1 M and Boc_2O afforded **23** in 63% yield. Better yield was observed by hydrogenation of **20** in the presence of Pd/C catalyst followed by *insitu N*-protection with Boc_2O, affording **23** in excellent yield (93%). On the other hand, transformation of diazide **21** to *N*-Boc compound **25** and subsequent silyl deprotection provides **23** in lower yield. Oxidation of alcohol **23** to ketone **24** was performed by two methods. First, using the stable nitroxyl radical, 2,2,6,6-tetramethyl-1-piperidinyloxy (TEMPO), with KBr and NaClO, compound **24** was obtained in 90% yield. Controlled addition of NaClO and HCl is required to maintain the pH of the reaction medium at 6.5–7.5. In addition, reaction of **23** with Dess Martin periodinane give place to **24** in 80% yield. Due to its simplicity, this is the method of choice for scaling reactions.

To synthesised the aminoalcohol A-ring precursor of 19-*nor*-vitamin D_3 analogs **11** and **12**, *cis*,*cis*-1,3,5-cyclohexanetriol was monotosylated by treatment with 1 equivalent of BuLi in THF–Py followed by addition of TsCl to led **26** in 70% yield (Scheme 2).

Next, asymmetric acylation of *meso*-diol **26** was attempted exploiting the potential of enzymes in desymmetrization processes. A similar process has been described in the literature using *Pseudomonas fluorescens* lipase (SAM II). The drawback of this reaction was that the final product was not completely enantiopure and five days of reaction were required.[18] In the present study, two commercial immobilized lipases were tested: *Candida antarctica* lipase type B (CAL–B, Novozyme 435) and *Pseudomonas cepacia* lipase (PSL-IM). The reactions were carried out with vinyl acetate at 30 °C and THF, *tert*-butylmethyl ether (TBME) or toluene as solvent (Table 1). In the absence of biocatalyst, the acylation of **26** is not observed in any of the solvents used after 24 h at 30 °C.



Scheme 2. Synthesis of amino alcohol A-ring precursor **32**. Reagents and conditions: a) *n*BuLi, Py, then TsCl, THF, 45 °C to rt, 15.5 h, 70%; b) Enzyme, vinyl acetate, solvent, 30 °C (see details within text and Table 1); c) NaN_3, DMF, 70 °C, 2 h, 89%; d) Pd/C, H_2, Boc_2O, EtOAc, rt, 18.5 h, 85%; e) TBSCl, Imidazole, CH_2Cl_2, rt, overnight, 90%; f) K_2CO_3, MeOH, rt, 2 h, 85%; g) Dess-Martin, NH_4Cl, CH_2Cl_2, rt, 30 min, 97%.

Table 1. Enzymatic desymmetrization of **26**.[a]

Enzyme	Solvent	t (h)	**26** (%)	**27** (%)	**33** (%)	**27** ee (%)
CAL-B	THF	8	7	51	42	>99
CAL-B	Toluene	8	2	67	31	97
CAL-B	TBME	5	–	>99	–	63
PSL-IM	THF	24	66	34	–	>99
PSL-IM	Toluene	17	2	98	–	>99
PSL-IM	TBME	8	7	93	–	95

[a] Ratio **26**:Enzyme (1:0.5, w/w), 0.2 M substrate concentration, with vinyl acetate at 30 °C. All percentages were determined by chiral HPLC.

Acetylation of **26** in the presence of CAL–B takes place in short reaction times. In THF or toluene, monoacetate **27** is obtained in excellent enantiomeric excess. However, diacetate **33** (Figure 4) is also isolated in these conditions. Although the use of TBME prevents the formation of the diacetylated compound, the desired compound **27** is obtained in low enantiomeric excess. On the other hand, when the acetylation reaction was examined with *Pseudomonas cepacia* lipase, excellent results were observed. Higher conversions were achieved in toluene and TBME, giving place exclusively to monoacetate **27** in >99% y 95% enantiomeric excess, respectively. Thus, treatment of **26** with vinyl acetate, toluene, and PSL-IM at 30 °C afforded after 17 h enantiopure compound **27** (determined by chiral HPLC, see Supplementary Information) in 95% yield.

Diacetate formation in the acylation reaction catalyzed by CAL–B has been observed in other cyclic diols (in a lesser extent, 6–10%), although high ee was obtained with the three solvents. In case of PSL-IM catalyzed process also lower reactivity was observed in THF.[19]

For the assignment of the absolute configurations of **27**, transformation into the previously described compound (−)-**34**[20] is carried out (Figure 4). For that, tosylated (−)-**27** was treated with cesium acetate in the presence of 18-crown-6 in toluene at 60 °C to afford the inverted acetate (−)-**34** in 75% yield after 32 h.

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 (−)-**27** obtained from the enzymatic acetylation.

Tosylated **27** was transformed to azide **28** by the treatment with NaN_3, and the later was converted directly to *N*-Boc compound **29**. Protection of the free alcohol as silyl ether and deprotection of the acetyl group conducted to **31**. Finally, oxidation of alcohol with Dess-Martin periodinane afforded the A-ring precursor **32** in excellent yield.

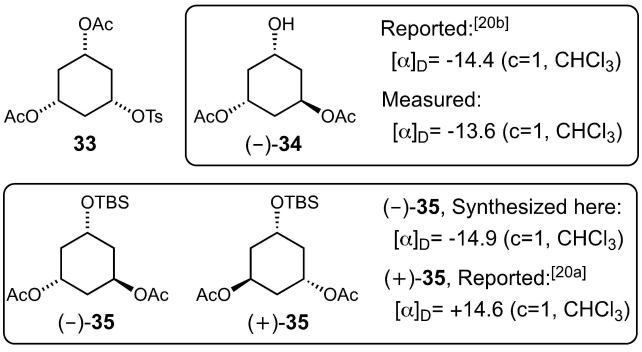
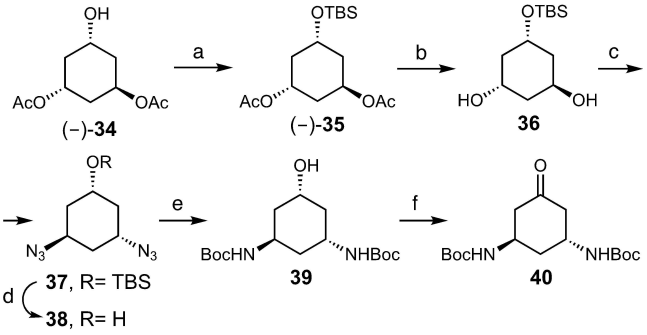


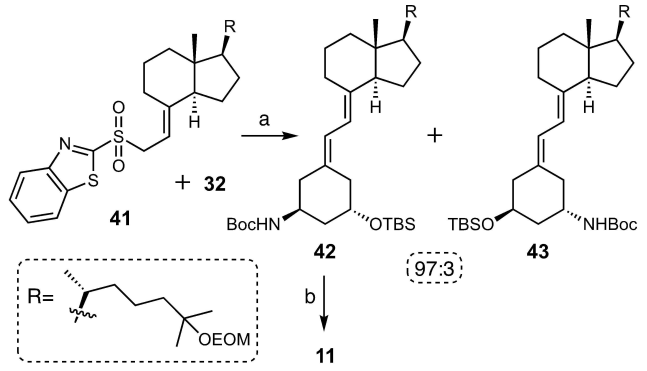
Figure 4. Several A-ring precursors of 19-*nor*-vitamin D_3 analogs.

A-ring precursor *trans*-diamino-**40** were obtained starting from derivative (−)-**34**, which was protected in high yield to the silyl ether (−)-**35** (Scheme 3). To corroborate the absolute configuration of (−)-**27**, comparation of the specific rotation of (−)-**35** was done with the alpha-value of its enantiomer (+)-**35** described in the literature. That is, we were performed a double checked with two different known compounds: (−)-**34** and (+)-**35** (Figure 4). Subsequent saponification of the acetate ester with MeONa gives place to **36**. Transformation of **36** into **40** was performed in a similar manner as above described for **24**.

C1 or C3 amino derivatives of 1α,25-dihydroxy-19-*nor*-vitamin D_3 were synthesized as illustrated in Scheme 4. Reaction of ketone **32** with the lithium enolate of sulfone **41**[21] takes place with excellent diastereoselectivity, giving rise mainly to one of the two possible diastereoisomers (ratio 97:3), which is isolated after semipreparative HPLC. The structure



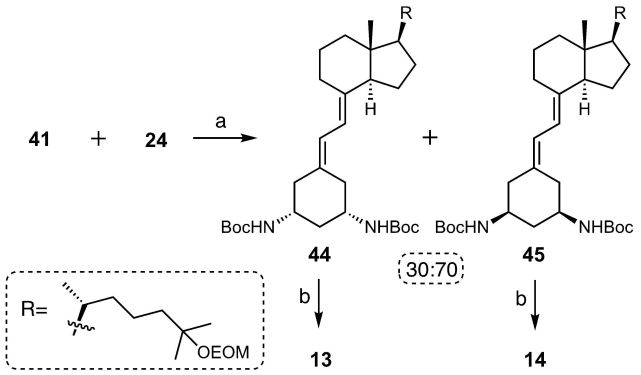
Scheme 3. Synthesis of *trans*-diamino A-ring precursor **40**. Reagents and conditions: a) TBSCl, Imidazol, CH_2Cl_2, rt, overnight, 95%; b) MeONa, MeOH, rt, 2 h, 88%; c) Ph_3P, DIAD, $(PhO)_2P(O)N_3$, THF, 0 °C to rt, 12.5 h, 75%; d) TBAF, THF, 0 °C, 2 h, 81%; e) Pd/C, H_2, Boc_2O, EtOAc, rt, 18.5 h, 95%; f) Dess-Martin, NH_4Cl, CH_2Cl_2, rt, 30 min, 87%.



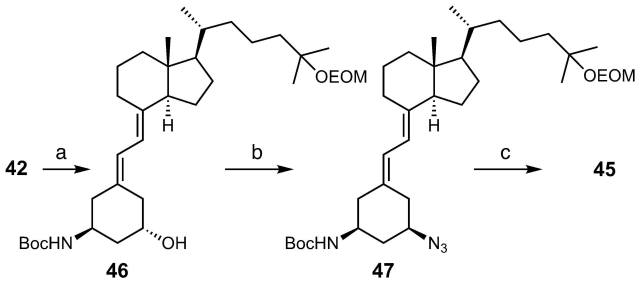
Scheme 4. Synthesis of amino alcohol analogs of 19-*nor*-vitamin D_3 **11** and **12**. Reagents and conditions: a) LHMDS, THF, −78 °C to rt, 7 h, 59%; b) EtOH sat. HCl, rt, 1 h, 87%.

identification of this diastereoisomer was determined as follows.

The protons at C1 and C3 are identified, but not assigned, by two-dimensional ^{13}C–^{1}H HSQC analysis, considering that carbon in adjacent position to a carbamate group is upfield with respect to one adjacent to the OTBS group. The signals corresponding to the carbons at C14 and C17 (56.3 and 56.7 ppm) are identified by one-dimensional DEPT-135, DEPT-90, and two-dimensional ^{13}C–^{1}H HMBC analysis. A correlation cross-peak between the signal at 56.7 ppm and one of the alkenyl protons allows assignment of H6 and H7, in addition to C14 and C17. At this point, 1D NMR selective NOE experiments were performed. Selective irradiation of H7 leads to identification of H10. This signal was also observed in the irradiation of the broad singlet at 3.85 ppm, previously assigned as the proton adjacent to the OTBS group, determining its position in C1 and identifying this diastereoisomer with the structure of **42**.



Scheme 5. Synthesis of *cis*-diamino derivatives of 19-*nor*-vitamin D_3 **13** and **14**. Reagents and conditions: a) LHMDS, THF, −78 °C to rt, 7 h, 47%; b) EtOH sat. HCl, rt, 1 h, 90% (**13**) and 92% (**14**).



Scheme 6. Synthesis of *cis*-diamino analog **45** from amino alcohol protected derivative **42**. Reagents and conditions: a) TBAF, THF, 0 °C, 2 h, 64%; b) Ph_3P, DIAD, $(PhO)_2P(O)N_3$, THF, 0 °C to rt, 12.5 h, 62%; c) Me_3P, Boc_2O, NaOH 1 M, THF, rt, overnight, 55%.

Cleavage of the protecting groups in **42** was carried out with a saturated solution of HCl in EtOH to afford 19-*nor*-vitamin D_3 analog **11**.

Similarly, coupling of *cis*-diamino ketone **24** with the lithium enolate of sulfone **41** gave a mixture of **44** and **45** in a 30:70 ratio (t_R of minor isomer 42.4 min, t_R major isomer 44.3 min; Kromasil 60 Å, 7 µm, Si 250 × 20 mm, 3% ^{i}PrOH/hexane, 7 mL/min). Both diastereoisomers were separated by semipreparative HPLC, but structure elucidation was not possible by NMR spectroscopy (Scheme 5).

Thus, C3-amino derivative **42** was transformed to the (1*R*,3*S*)-diamino diastereoisomer by selective deprotection of the silyl ether group, substitution of hydroxyl group in **46** by azide with simultaneous inversion of the configuration using the Mitsunobu method, and subsequent treatment with Me_3P in the presence of Boc_2O (Scheme 6).

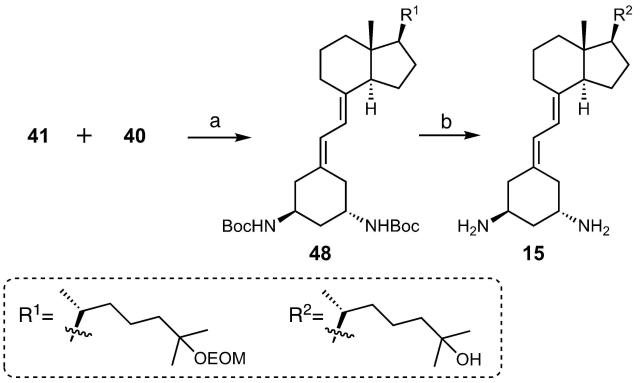
Comparison of the HPLC chromatograms of the diastereoisomeric mixture obtained after the coupling reaction and the isomer synthesized from **42** allows the identification of (1*R*,3*S*)-**45** as the major diastereoisomer obtained in the coupling process.

Treatment of **44** and **45** with a saturated solution of HCl in EtOH provides 19-*nor*-vitamin D_3 analogs **13** and **14**, respectively.

Finally, synthesis of the *trans*-diamino analog **15** was carried out in the same manner by coupling of ketone **40** and the lithium enolate of sulfone **41** (Scheme 7).

Biological Evaluation

We evaluated in vitro the affinity of compounds **11**, **13**, **14**, and **15** for pig mucosa cytosol vitamin D receptor (VDR) and for human vitamin D binding protein (hDBP) in comparison to the natural hormone. These compounds do not have the ability to access the VDR binding pocket. With respect to their ability to bind the



Scheme 7. Synthesis of *trans*-diamino analog of 19-*nor*-vitamin D_3 **15**. Reagents and conditions: a) LHMDS, THF, −78 °C to rt, 7 h, 63%; b) EtOH sat. HCl, rt, 1 h, 87%.

hDBP protein, only the analog carrying a 1β-hydroxyl substituent (**11**) showed 10% of activity compared to 1α,25-$(OH)_2$-D_3 (Figure 5).

None of the synthesized analogues were able to inhibit the proliferation of MCF-7 breast cancer cells.

Conclusion

We have synthesized 19-*nor*-1α,25-dihydroxyvitamin D_3 analogs with amino functionalization at C1 and/or C3 in the A-ring via a Julia olefination to construct the diene system. *cis*,*cis*-1,3,5-Cyclohexanetriol, a commercial available compound with the appropriate stereochemistry, was employed as starting material for A-ring precursors. Key feature of these approaches is the enzymatic desymmetrization reaction of a prochiral 1,3-diol catalyzed by *Pseudomonas cepacia* lipase in high yield and enantioselectivity. The transformation of alcohol to inverted amine was achieved by activation of the hydroxyl group as tosylate followed by reaction with NaN_3 or by direct introduction of the amino group with total inversion of the configuration under Mitsunobu conditions using diphenylphosphorylazide as nucleophile. The structural elucidation of the analogs has been established by one-dimensional (^{1}H, ^{13}C, DEPT-90, DEPT-135), two-dimensional (^{13}C–^{1}H HMBC, ^{13}C–^{1}H HSQC), and selective NOE NMR spectroscopy. Alternative syntheses were also carried out to assign through indirect evidence the structure of the *cis*-diamino diastereoisomers. Biological evaluation of these analogs reveals that only the aminoalcohol derivative with a 1β-hydroxyl exhibits a slight binding capacity to the hDBP transporter protein, although the required concentration for half maximal binding (EC_{50}) is 10 times higher than that of the natural hormone. Unfortunately, the analogs were not able to bind to the VDR and demonstrated no antiproliferative activity (data not shown, see Supplementary Material).

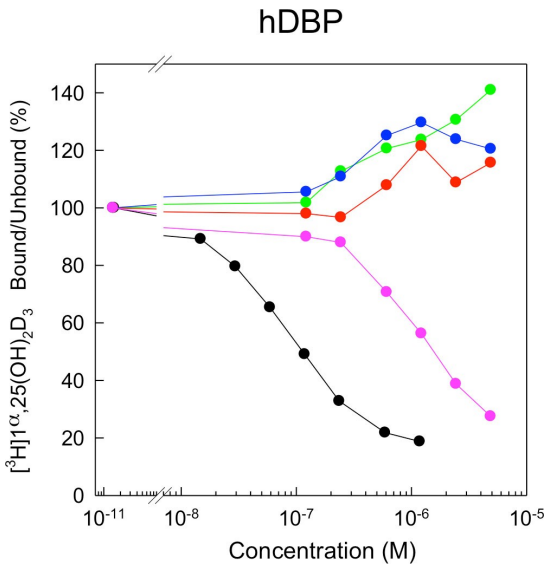


Figure 5. Affinity of amino/diamino 19-*nor*-vitamin D_3 analogs for human vitamin D binding protein (hDBP). Notes: 1α,25-$(OH)_2$-D_3 (●); **11** (●); **13** (●); **14** (●); **15** (●).

Experimental Section

General. *Candida antarctica* lipase type B (CAL–B, Novozyme 435, immobilized by adsorption in Lewatit, 9120 PLU/g) was purchased from Novozymes. *Pseudomonas cepacia* lipase (Lipase PS "Amano IM", immobilized on diatomaceous earth, 943 U/g) was purchased from Amano Enzyme. The structure of the synthesized compounds was determined by NMR spectroscopy. The signals of the ^{1}H and ^{13}C NMR spectra of the new compounds are fully assigned on the basis of ^{1}H and ^{13}C chemical shifts, proton coupling constants, and two-dimensional ^{1}H–^{1}H (COSY) and ^{1}H–^{13}C spectra (HSQC and HMBC). Full NMR data for new compounds are available in the Supporting Information. The level of purity is indicated by the inclusion of copies of ^{1}H, ^{13}C, DEPT and 2D NMR spectra. Optical rotations were recorded on a Perkin-Elmer 241 polarimeter, and values are reported as follows: $[\alpha]_\lambda^T$ (c: g/100 mL, solvent).

3α-Amino-1β,25-dihydroxy-3-deoxy-19-*nor*-vitamin D_3 (11). A solution of HCl/EtOH (5.6 mL), generated by bubbled HCl(g) in EtOH, was added to a solution of **42** (13.5 mg, 0.02 mmol) in EtOH (455 μL). After being stirred at rt for 1 h, solvents were concentrated, the residue was poured in saturated aqueous $NaHCO_3$ solution, and extracted with Et_2O. The combined organic layers were dried and concentrated. The crude was purified by column chromatography (2% NH_4OH/MeOH) to afford **11** as a colorless oil (87% yield). R_f: 0.2 (2% NH_4OH/MeOH). HRMS (ESI$^+$, *m/z*): Calcd. for $C_{26}H_{46}NO_2$ [(M + H)$^+$]: 404.3523. Found: 404.3497; Calcd. for $C_{26}H_{45}NNaO_2$ [(M + Na)$^+$]: 426.3343. Found: 426.3328.

1β,3β-Diamino-25-hydroxy-3-deoxy-19-*nor*-vitamin D_3 (13). Same procedure as **11** but starting from **44**. Column chromatography (5% NH_4OH/MeOH). Colorless oil (90% yield). R_f: 0.3 (5% NH_4OH/MeOH). MS (ESI$^+$, *m/z*): 403 [(M + H)$^+$, 100%]. HRMS (ESI$^+$, *m/z*): Calcd. for $C_{26}H_{47}N_2O$ [(M + H)$^+$]: 403.3683. Found: 403.3700.

1α,3α-Diamino-25-hydroxy-3-deoxy-19-*nor*-vitamin D_3 (14). Same procedure as **11** but starting from **45**. Column chromatography (5% NH_4OH/MeOH). Colorless oil (92% yield). R_f: 0.3 (5% NH_4OH/MeOH). MS (ESI$^+$, *m/z*): 403 [(M + H)$^+$, 100%]. HRMS (ESI$^+$, *m/z*): Calcd. for $C_{26}H_{47}N_2O$ [(M + H)$^+$]: 403.3683. Found: 403.3677.

1β,3α-Diamino-25-hydroxy-3-deoxy-19-*nor*-vitamin D_3 (15). Same procedure as **11** but starting from **48**. Column chromatography (5% NH_4OH/MeOH). Colorless oil (87% yield). R_f: 0.3 (5% NH_4OH/MeOH). MS (ESI$^+$, *m/z*): 403 [(M + H)$^+$, 100%]. HRMS (ESI$^+$, *m/z*): Calcd. for $C_{26}H_{47}N_2O$ [(M + H)$^+$]: 403.3683. Found: 403.3659; Calcd. for $C_{26}H_{46}N_2NaO$ [(M + Na)$^+$]: 425.3502. Found: 425.3482.

(1*R*,3*S*,5*s*)-5-((*tert*-Butyldimethylsilyl)oxy)cyclohexane-1,3-diol (17). To a solution of **16** (2 g, 15.14 mmol) in anhydrous THF (40 mL) at rt were successively added Et_3N (2.3 mL, 16.64 mmol) and TBSCl (2.51 g, 16.64 mmol). After being

stirred at this temperature for 1.5 h, NaH (60% w/w in mineral oil, 790 mg, 16.64 mmol) was added. When the solution stopped bubbling, the mixture was heated at 45 °C for 18 h. Next, it was cooled to 10 °C and filtered on Celite®. Solvents were concentrated and the residue was ground in the presence of hexane (20 mL) to give **17** as a white solid (77% yield). Mp: 122–125 °C. R_f: 0.6 (5% MeOH/EtOAc). HRMS (ESI$^+$, *m/z*): Calcd. for $C_{12}H_{27}O_3Si$ [(M + H)$^+$]: 247.1724. Found: 247.1735.

(1*R*,3*S*,5*r*)-5-((*tert*-Butyldimethylsilyl)oxy)cyclohexane-1,3-diyl bis(*p*-toluensulfonate) (18). To a solution of **17** (2.46 g, 10 mmol) in anhydrous pyridine (8.7 mL) at 0 °C was added freshly recrystallized TsCl (6.86 g, 36 mmol). The solution changes from colorless to light pink and is stirred for 48 h at 0 °C. Next, it was poured into water and extracted with EtOAc. The combined organic layers were concentrated to ~ 5 mL, water was added (20 mL), and allowed to precipitate overnight. After filtration, compound **18** is obtained as a white solid (90% yield). Mp: 112–114 °C. R_f: 0.6 (30% EtOAc/hexane). MS (ESI$^+$, *m/z*): 577 [(M + Na)$^+$, 100%].

(1*R*,3*S*,5*r*)-5-Hydroxycyclohexane-1,3-diyl bis(*p*-toluensulfonate) (19). TBAF (17.1 mL, 1 M in THF, 17.1 mmol) was added to a solution of **18** (3.8 g, 6.85 mmol) in anhydrous THF (30 mL), and the reaction was stirred for 3 h at rt. Then, 10 mL of aqueous saturated solution of $NaHCO_3$ was added and the mixture was extracted with EtOAc. The combined organic layers were dried and concentrated. The residue was purified by column chromatography (40% EtOAc/hexane) to afford **19** as a light pink solid (71% yield). Mp: 127–129 °C. R_f: 0.2 (40% EtOAc/hexane). MS (ESI$^+$, *m/z*): 463 [(M + Na)$^+$, 100%].

(1*r*,3*R*,5*S*)-3,5-Diazidocyclohexane-1-ol (20). *From **19***: NaN_3 (3.18 g, 48.9 mmol) was added to a stirred solution of **19** (2.15 g, 4.89 mmol) in anhydrous DMF (35 mL). The reaction was stirred for 4 h at 70 °C. Then, solvents were concentrated, EtOAc was added, the mixture was filtered on Celite®, washed with MeOH, and concentrated to leave a residue which was purified by column chromatography (35% Et_2O/hexane) to give **20** as a yellow oil (60% yield). *From **21***: TBAF (0.84 mL, 1 M in THF, 0.84 mmol) was added dropwise to a solution of **21** (100 mg, 0.34 mmol) in anhydrous THF (3.4 mL) at 0 °C, and the reaction was stirred at this temperature for 2 h. Then, solvents were concentrated, the residue was poured in water, and extracted with EtOAc. The combined organic layers were dried and concentrated. The residue was purified by column chromatography (35% EtOAc/hexane) to afford **20** as a yellow oil (85% yield). R_f: 0.4 (50% Et_2O/hexane). MS (ESI$^+$, *m/z*): 183 [(M + H)$^+$, 100%].

***tert*-Butyl(((1*r*,3*R*,5*S*)-3,5-diazidocyclohexyl)oxy)-dimethylsilane (21)**. Diisopropilazodicarboxilate (1.20 mL, 5.86 mmol) was added dropwise to a stirred solution of **17** (555 mg, 2.25 mmol) and Ph_3P (1.48 g, 5.62 mmol) in anhydrous THF (6.4 mL) at 0 °C. The mixture was stirred for 30 min at this temperature and then, diphenylphosphoryl azide (1.60 mL, 5.86 mmol) was added. Stirring continues vigorously a rt for 12 h. Solvent was removed to leave a residue, which was purified by column chromatography (2% Et_2O/hexane) to afford **21** as a yellow oil (83% yield). R_f: 0.6 (10% Et_2O/hexane). MS (ESI$^+$, *m/z*): 296 [M$^+$, 100%].

Di-*tert*-butyl ((1*R*,3*S*,5*r*)-5-hydroxycyclohexane-1,3-diyl)dicarbamate (23). A flask containing **20** (256 mg, 1.41 mmol) and Pd/C (105 mg) was exposed to a positive pressure of hydrogen gas (balloon). Anhydrous and deoxygenated EtOAc (5.6 mL) was added. The mixture was stirred vigorously for 30 min and then, Boc_2O (971 µL, 4.23 mmol) was added. After 18 h of stirring, the mixture was filtered on Celite®, washed with MeOH, and concentrated to afford a residue which was purified by column chromatography (40% EtOAc/hexane) to afford **23** as a white solid (93% yield). Mp: 235–242 °C (decompose). R_f: 0.6 (75% EtOAc/hexane). MS (ESI$^+$, *m/z*): 353 [(M + Na)$^+$, 100%].

Di-*tert*-butyl ((1*R*,3*S*)-5-oxocyclohexane-1,3-diyl)-dicarbamate (24). To a solution of **23** (132 mg, 0.4 mmol) in anhydrous CH_2Cl_2 (4 mL), NH_4Cl (44 mg, 0.80 mmol) and Dess-Martin reagent (193 mg, 0.44 mmol) were added. The mixture was stirred at rt for 30 min. Then, it was added a mixture of saturated aqueous $Na_2S_2O_3$ and $NaHCO_3$ solutions (1:1, v/v, 9.5 mL), and extracted with CH_2Cl_2. The combined organic layers were dried and concentrated. The residue was purified by column chromatography packed with deactivated alumina (30% EtOAc/hexane) to afford **24** as a white solid (80% yield). Mp: 209–210 °C (decompose). R_f: 0.6 (50% EtOAc/hexane). MS (ESI$^+$, *m/z*): 351 [(M + Na)$^+$, 100%]. HRMS (ESI$^+$, *m/z*): Calcd. for $C_{16}H_{28}N_2Na_5O_5$ [(M + Na)$^+$]: 351.1890. Found: 351.1857.

Di-*tert*-butyl ((1*R*,3*S*,5*r*)-5-((*tert*-butyldimethylsilyl)-oxy)cyclohexane-1,3-diyl)dicarbamate (25). Same procedure as **23** but starting from **21**. Column chromatography (15% EtOAc/hexane). White solid (53% yield). R_f: 0.2 (20% EtOAc/hexane). MS (ESI$^+$, *m/z*): 445 [(M + H)$^+$, 100%].

(1*s*,3*R*,5*S*)-3,5-Dihydroxycyclohexyl 4-methylbenzene-sulfonate (26). ^{n}BuLi (4.73 mL, 1.6 M in hexane, 7.57 mmol) was added to a solution of **16** in anhydrous THF (6.4 mL), and the mixture is stirred at 45 °C for 10 min. Then, anhydrous pyridine (2.8 mL) was added, and the color of the solution turns orange. After stirring 30 min at this temperature, the mixture was cooled to 0 °C and freshly recrystallized TsCl (1.44 g, 7.71 mmol) was added. The reaction was allowed to reach rt and stirred for 15 h. Then, the mixture was poured in water and extracted with EtOAc. The combined organic layers were dried and concentrated. The residue was purified by column chromatography (50% EtOAc/hexane-EtOAc-15% ^{i}PrOH/EtOAc as gradient eluent) to afford **26** as a white solid (70% yield). Mp: 94–97 °C. R_f: 0.4 (5% MeOH/EtOAc). MS (ESI$^+$, *m/z*): 309 [(M + Na)$^+$, 100%]. HRMS (ESI$^+$, *m/z*): Calcd. for $C_{13}H_{18}NaO_5S$ [(M + Na)$^+$]: 309.0767. Found: 309.0759; Calcd. for $C_{26}H_{36}NaO_{10}S_2$ [(2 M + Na)$^+$]: 595.1642. Found: 595.1626.

(1*R*,3*S*,5*S*)-3-Hydroxy-5-(*p*-toluensulfonate)cyclohexyl acetate (−)-(27). In a standard procedure, anhydrous solvent freshly distilled on standard drying agent (10 mL) was added to an Erlenmeyer flask that contain compound **26** (583 mg, 2.04 mmol), lipase (ratio **26**:CAL–B is 1:0.5, w/w) and vinyl acetate (940 µL, 10.2 mmol). The mixture is stirred at 30 °C and 250 rpm (reaction times are indicated in Table 1). Then, the enzyme was filtered off, washed with CH_2Cl_2, and the solvents concentrated. The residue was purified by column chromatography (20–50% EtOAc/hexane as gradient eluent) to afford (−)-**27** as a yellow oil (95% yield, > 99% ee, PSL-IM, toluene). R_f:

0.6 (60% EtOAc/hexane). $[\alpha]_D^{20}$ =-14 (c = 1, $CHCl_3$). HRMS (ESI$^+$, *m/z*): Calcd. for $C_{15}H_{21}O_6S$ [(M + H)$^+$]: 329.1053. Found: 329.1043; Calcd. for $C_{15}H_{20}O_6SNa$ [(M + Na)$^+$]: 351.0873. Found: 351.0886. Chiral HPLC retention times (see Supporting Information): 17.0 min for (−)-**27** and 20.6 min (+)-**27**.

(1*S*,3*R*,5*R*)-3-Azido-5-hydroxycyclohexyl acetate (28). NaN_3 (1.30 g, 19.9 mmol) was added to a stirred solution of **27** (1.31 g, 3.98 mmol) in anhydrous DMF (32 mL). The reaction was stirred for 2 h at 70 °C and then solvent was concentrated. The residue was poured into water and extracted with EtOAc. The combined organic layers were dried and concentrated to leave a crude which was purified by column chromatography (50% EtOAc/hexane) to give **28** as a yellow oil (89% yield). R_f: 0.5 (60% EtOAc/hexane). $[\alpha]_D^{20}$ = +1.6 (c = 1.6, $CHCl_3$). MS (ESI$^+$, *m/z*): 222 [(M + Na)$^+$, 100%]. HRMS (ESI$^+$, *m/z*): Calcd. for $C_8H_{13}N_3NaO_3$ [(M + Na)$^+$]: 222.0849. Found: 222.0844.

(1*S*,3*R*,5*R*)-3-((*tert*-Butoxycarbonyl)amino)-5-hydroxycyclohexyl acetate (29). A flask containing **28** (360 mg, 1.81 mmol) and Pd/C (134 mg) was exposed to a positive pressure of hydrogen gas (balloon). Anhydrous and deoxygenated EtOAc (7.2 mL) was added. The mixture was stirred vigorously for 30 min and then, Boc_2O (623 µL, 2.72 mmol) was added. After 18 h of stirring, the mixture was filtered on Celite®, washed with MeOH, and concentrated to afford a residue which was purified by column chromatography (40% EtOAc/hexane) to afford **29** as a white foam solid (85% yield). R_f: 0.4 (70% EtOAc/hexane). MS (ESI$^+$, *m/z*): 296 [(M + Na)$^+$, 100%]. HRMS (ESI$^+$, *m/z*): Calcd. for $C_{13}H_{24}NO_5$ [(M + H)$^+$]: 274.1649. Found: 274.1637; Calcd. for $C_{13}H_{23}NNaO_5$ [(M + Na)$^+$]: 296.1468. Found: 296.1481.

(1*S*,3*S*,5*R*)-3-((*tert*-Butoxycarbonyl)amino)-5-((*tert*-butyldimethylsilyl)oxy)cyclohexyl acetate (30). Imidazole (151 mg, 2.22 mmol) and TBSCl (309 mg, 2.05 mmol) was added to a solution of **29** (467 mg, 1.71 mmol) in anhydrous CH_2Cl_2 (6.8 mL). Afterwards, the reaction was stirred at rt overnight. Solvents were concentrated and the residue purified by column chromatography (15% EtOAc/hexane) to afford **30** as a colorless oil (90% yield). R_f: 0.3 (20% EtOAc/hexane). MS (ESI$^+$, *m/z*): 388 [(M + H)$^+$, 100%], 272 [(M-TBDMS)$^+$, 98%]. HRMS (ESI$^+$, *m/z*): Calcd. for $C_{19}H_{38}NO_5Si$ [(M + H)$^+$]: 388.2514. Found: 388.2508; Calcd. for $C_{19}H_{37}NNaO_5Si$ [(M + Na)$^+$]: 410.2333. Found: 410.2333.

***tert*-Butyl ((1*S*,3*R*,5*S*)-3-((*tert*-butyldimethylsilyl)oxy)-5-hydroxycyclohexyl)carbamate (31)**. To a solution of **30** (500 mg, 1.29 mmol) in anhydrous MeOH (8.6 mL), K_2CO_3 (165 mg, 1.29 mmol) was added. After being stirred at rt for 2 h, a saturated aqueous NH_4Cl solution was added until neutral pH and extracted with EtOAc. The combined organic layers were dried and concentrated. The residue was purified by column chromatography (35% EtOAc/hexane) to afford **31** as a colorless oil (85% yield). R_f: 0.4 (45% EtOAc/hexane). MS (ESI$^+$, *m/z*): 346 [(M + H)$^+$, 100%], 368 [(M + H)$^+$, 50%]. HRMS (ESI$^+$, *m/z*): Calcd. for $C_{17}H_{36}NO_4Si$ [(M + H)$^+$]: 346.2408. Found: 346.2391; Calcd. for $C_{17}H_{35}NNaO_5Si$ [(M + Na)$^+$]: 368.2228. Found: 368.2237.

***tert*-Butyl ((1*R*,3*R*)-3-((*tert*-butyldimethylsilyl)oxy)-5-oxocyclohexyl)carbamate (32)**. Same procedure as **24** but starting from **31**. Column chromatography (20% EtOAc/hexane). Colorless oil (97% yield). R_f: 0.6 (45% EtOAc/hexane). HRMS (ESI$^+$, *m/z*): Calcd. for $C_{17}H_{33}NNaO_4Si$ [(M + Na)$^+$]: 366.2071. Found: 366.2071.

(1*R*,3*R*)-5-Hydroxycyclohexane-1,3-diyl diacetate (34). To a solution of (−)-**27** (1.67 g, 5.14 mmol) in anhydrous toluene (47 mL), 18-crown-6 (4.07 mg, 15.41 mmol) and cesium acetate (3.02 g, 15.41 mmol) were added. The mixture is stirred for 32 h at 60 °C, poured in water, and extracted with EtOAc. The combined organic layers were dried and concentrated. The residue was purified by column chromatography (40–50% EtOAc/hexane as gradient eluent) to afford (−)-**34** as a colorless oil (75% yield). R_f: 0.3 (30% EtOAc/hexane). $[\alpha]_D^{20}$ =-13.6 (c = 1, $CHCl_3$). MS (ESI$^+$, *m/z*): 217 [(M + H)$^+$, 100%].

(1*S*,3*S*)-5-((*tert*-Butyldimethylsilyl)oxy)cyclohexane-1,3-diyl diacetate (35). Same procedure as **30** but starting from **34**. Colorless oil (95% yield). R_f: 0.4 (20% EtOAc/hexane). $[\alpha]_D^{20}$ =-14.9 (c = 1, $CHCl_3$). MS (ESI$^+$, *m/z*): 271 [(M-OAc)$^+$, 100%], 331 [(M + H)$^+$, 70%]. HRMS (ESI$^+$, *m/z*): Calcd. for $C_{16}H_{30}NaO_5Si$ [(M + Na)$^+$]: 353.1764. Found: 353.1755.

(1*S*,3*S*)-5-((*tert*-Butyldimethylsilyl)oxy)cyclohexane-1,3-diol (36). NaOMe (918 mg, 16.14 mmol) was added to a solution of **35** (1.07 g, 3.23 mmol) in anhydrous MeOH (32 mL). After being stirred at rt for 2 h, solid NH_4Cl was added until neutral pH. Solvents were concentrated and the residue filtered in a short column chromatography (65% EtOAc/hexane) to afford **36** as a white solid (88% yield). Mp: 112–114 °C. R_f: 0.3 (70% EtOAc/hexane). HRMS (ESI$^+$, *m/z*): Calcd. for $C_{12}H_{26}NaO_3Si$ [(M + Na)$^+$]: 269.1543. Found: 269.1529.

***tert*-Butyl(((3*R*,5*R*)-3,5-diazidocyclohexyl)oxy)-dimethylsilane (37)**. Same procedure as **21** but starting from **36**. Column chromatography (5% Et_2O/hexane). Yellow oil (75% yield). R_f: 0.5 (10% Et_2O/hexane). MS (ESI$^+$, *m/z*): 296 [M$^+$, 100%], 297 [(M + H)$^+$, 70%].

(3*R*,5*R*)-3,5-Diazidocyclohexan-1-ol (38). Same procedure as **20** from **21** but starting from **37**. Column chromatography (40% Et_2O/hexane). Yellow oil (81% yield). R_f: 0.4 (50% Et_2O/Hexane). MS (ESI$^+$, *m/z*): 183 [(M + H)$^+$, 100%].

Di-*tert*-butyl ((1*R*,3*R*)-5-hydroxycyclohexane-1,3-diyl)-dicarbamate (39). Same procedure as **23** but starting from **38**. White solid (95% yield). R_f: 0.3 (40% EtOAc/hexane). MS (ESI$^+$, *m/z*): 353 [(M + Na)$^+$, 100%].

Di-*tert*-butyl ((1*R*,3*R*)-5-oxocyclohexane-1,3-diyl)-dicarbamate (40). Same procedure as **24** but starting from **39**. Column chromatography (20% EtOAc/hexane). White solid (87% yield). Mp: 215–225 °C (decompose). R_f: 0.6 (40% EtOAc/hexane). MS (ESI$^+$, *m/z*): 351 [(M + Na)$^+$, 100%].

Procedure for the synthesis of 42 and 43. To a solution of **41** (167 mg, 0.30 mmol) in anhydrous THF (1.1 mL) at −78 °C was added dropwise LHMDS (289 µL, 1.0 M in THF, 0.29 mmol), resulting in a deep red color solution. The mixture was stirred at the same temperature for 2 h, and then, was added dropwise via cannula transfer to a solution of the corresponding ketone **32** (103.1 mg, 0.30 mmol) in anhydrous THF (2.0 mL).

A color change from red to yellow is observed. The reaction is allowed to reach rt for 5 h. Then, it was poured into a saturated aqueous solution of NH_4Cl and extracted with Et_2O. The combined organic fractions were dried and concentrated to give a crude that was purified by column chromatography (15% Et_2O/hexane) to give a 97:3 mixture of diastereosiomers in 59% yield. The major diastereoisomer was isolated by preparative HPLC (Kromasil 60 Å, 7 µm, Si 250×20 mm, 1% ^{i}PrOH/hexane, 5 mL/min). t_R (min) **42** (73.5).

1β-((*tert*-Butyldimethylsilyl)oxy)-3α-((*tert*-butoxycarbonyl)amino)-25-ethoxymethyloxy-3-deoxy-19-*nor*-vitamin D_3 (42). Colorless oil. R_f: 0.8 (60% EtOAc/hexane). MS (ESI$^+$, *m/z*): 544 [(M-OTBDMS)$^+$, 100%], 699 [(M+Na)$^+$, 15%]. HRMS (ESI$^+$, *m/z*): Calcd. for $C_{40}H_{73}NNaO_6Si$ [(M+Na)$^+$]: 698.5150. Found: 698.5146.

Procedure for the synthesis of 44 and 45. Same procedure as synthesis of **42** and **43** but starting from ketone **24**. The crude was purified by column chromatography (40% Et_2O/hexane) to give a 30:70 mixture of diastereosiomers in 47% yield. Both isomers were separated by preparative HPLC (Kromasil 60 Å, 7 µm, Si 250×20 mm, 3% ^{i}PrOH/hexane, 7 mL/min). t_R (min) **44** (42.4); **45** (44.3).

*Synthesis of **45** from **47**.* To a solution of **47** (8 mg, 0.014 mmol) in THF (200 µL) was added successively an aqueous solution of 1 M NaOH (30 µL), trimethylphosphine (41 µL, 0.041 mmol) and Boc_2O (9 µL, 0.041 mmol) resulting in a slightly yellow solution. After being vigorously stirred at rt overnight, the residue was extracted with EtOAc. The combined organic layers were dried and concentrated to leave a crude which was purified by column chromatography (60% Et_2O/hexane) to afford **45** as a colorless oil (55% yield).

1β,3β-Bis((*tert*-butoxycarbonyl)amino)-25-ethoxymethyloxy-3-deoxy-19-*nor*-vitamin D_3 (44). Colorless oil. R_f: 0.5 (60% EtOAc/hexane). HRMS (ESI$^+$, *m/z*): Calcd. for $C_{39}H_{68}N_2O_6Si$ [(M+Na)$^+$]: 683.4975. Found: 683.5005.

1α,3α-Bis((*tert*-butoxycarbonyl)amino)-25-ethoxymethyloxy-3-deoxy-19-*nor*-vitamin D_3 (45). Colorless oil. R_f: 0.5 (60% EtOAc/hexane). HRMS (ESI$^+$, *m/z*): Calcd. for $C_{39}H_{68}N_2O_6Si$ [(M+Na)$^+$]: 683.4975. Found: 683.4996.

3α-((*tert*-Butoxycarbonyl)amino)-25-ethoxymethyloxy-1β-hydroxy-3-deoxy-19-*nor*-vitamin D_3 (46). Same procedure as **20** from **21** but starting from **42**. Column chromatography (20% EtOAc/hexane). Colorless oil (64% yield). R_f: 0.7 (40% EtOAc/hexane). MS (ESI$^+$, *m/z*): 562 [(M+H)$^+$, 20%], 1124 [(2 M+H)$^+$, 70%].

1α-Azido-3α-((*tert*-butoxycarbonyl)amino)-25-ethoxymethyloxy-3-deoxy-19-*nor*-vitamin D_3 (47). Same procedure as **21** but starting from **46**. Column chromatography (10% Et_2O/hexane). Colorless oil (62% yield). R_f: 0.7 (30% EtOAc/hexane). MS (ESI$^+$, *m/z*): 609 [(M+Na)$^+$, 85%], 625 [(M+K)$^+$, 100%]. HRMS (ESI$^+$, *m/z*): Calcd. for $C_{39}H_{68}N_2O_6Na$ [(M+Na)$^+$]: 609.4356. Found: 698.4327.

1β,3α-Bis((*tert*-butoxycarbonyl)amino)-25-ethoxymethyloxy-3-deoxy-19-*nor*-vitamin D_3 (48). Same procedure as synthesis of **42** and **43** but starting from ketone **40**. The crude was purified by column chromatography (40% Et_2O/hexane) to give **48** as a colorless oil (63% yield). R_f: 0.5 (60% EtOAc/hexane). HRMS (ESI$^+$, *m/z*): Calcd. for $C_{39}H_{68}N_2O_6Na$ [(M+Na)$^+$]: 683.475. Found: 698.5146.

In vitro Biological Evaluation

Affinity for VDR. The affinity of 1α,25-$(OH)_2$-D_3 and its analogs 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.[22] The relative affinity of the analogs 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 analogs to hDBP was performed at 4 °C as described previously.[23] [^{3}H]1α,25-$(OH)_2$-D_3 and 1α,25-$(OH)_2$-D_3 or its analogs 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, analogs or vehicle as described previously.[22]

Acknowledgements

Financial support by Principado de Asturias (Project SV-PA-21-AYUD-2021-51542) is gratefully acknowledged. We also thank Dr. I. Merino (NMR Facility, Universidad de Oviedo) for her assistance in the NMR study.

References

[1] J. C. Fleet, Regulation of Intestinal Calcium and Phosphate Absorption, in *Vitamin D*, *4th ed.*,*Vol. 1* (Ed.: D. Feldman), Academic Press, London, **2018**, pp. 329–342.

[2] a) S. Samuel, M. D. Sitrin, *Nutr. Rev.* **2008**, *66*, S116–124; b) M. T. Cantorna, Y. Zhu, M. Froicu, A. Wittke, *Am. J. Clin. Nutr.* **2004**, *80*, 1717S–1720S.

[3] a) C. Leyssens, L. Verlinden, A. Verstuyf, *Endocr.-Relat. Cancer* **2013**, *20*, R31–R47; b) T. C. Chen, M. F. Holick, *Trends Endocrinol. Metab.* **2003**, *14*, 423–430.

[4] a) S. Fernández, A. Hernández-Martín, T. González-García, M. Ferrero, *Curr. Top. Med. Chem.* **2014**, *14*, 2424–2445; b) A. Glebocka, G. Chiellini, *Arch. Biochem. Biophys.* **2012**, *523*, 48–57; c) E. S. Agoston, M. A. Hatcher, T. W. Kensler, G. H. Posner, *Anti-Cancer Agents Med. Chem.* **2006**, *6*, 53–71; d) N. Saito, S. Honzawa, A. Kittaka, *Curr. Top. Med. Chem.* **2006**, *6*, 1273–1288; e) L. Binderup, E. Binderup, W. O. Godtfredsen, A. M. Kissmeyer, In *Vitamin D*, 2nd ed. (Eds. D. Feldman, J. W. Pike, F. H. Glorieux), Academic Press, London, UK, **2005**, Chapter 2, pp. 1489–1510; f) S.

Peleg, G. H. Posner, *Curr. Topics Med. Chem.* **2003**, *3*, 1555–1572; g) G.-D. Zhu, W. H. Okamura, *Chem. Rev.* **1995**, *95*, 1877–1952.
[5] S. Fernández, M. Ferrero, *Pharmaceuticals* **2020**, *13*, 159.
[6] a) S. Yang, C. Smith, H. F. DeLuca, *Biochim. Biophys. Acta* **1993**, *1158*, 279–286; b) K. L. Perlman, R. E. Swenson, H. E. Paaren, H. K. Schnoes, H. F. DeLuca, *Tetrahedron Lett.* **1991**, *32*, 7663–7666; c) K. L. Perlman, R. R. Sicinski, H. K. Schnoes, H. F. DeLuca, *Tetrahedron Lett.* **1990**, *31*, 1823–1824.
[7] a) T. González-García, A. Verstuyf, L. Verlinden, S. Fernández, M. Ferrero, *Adv. Synth. Catal.* **2018**, *360*, 2762–2772; b) L. Sánchez-Abella, S. Fernández, A. Verstuyf, L. Verlinden, M. Ferrero, V. Gotor, *Bioorg. Med. Chem.* **2007**, *15*, 4193–4202; c) M. Díaz, M. Ferrero, S. Fernández, V. Gotor, *J. Org. Chem.* **2000**, *65*, 5647–5652; d) M. Díaz, M. Ferrero, S. Fernández, V. Gotor, *Tetrahedron Lett.* **2000**, *41*, 775–779; e) A. W. Norman, R. Bouillon, M. C. Farach-Carson, J. E. Bishop, L.-X. Zhou, I. Nemere, J. Zhao, K. R. Muralidharan, W. H. Okamura, *J. Biol. Chem.* **1993**, *268*, 20022–20030; f) K. R. Muralidharan, A. R. De Lera, S. D. Isaeff, A. W. Norman, W. H. Okamura, *J. Org. Chem.* **1993**, *58*, 1895–1899.
[8] T. C. Nugent, *Chiral Amine Synthesis. Methods, Developments and Applications*, Wiley-VCH: Weinheim, **2010**.
[9] H. Loibner, E. Zbiral, *Tetrahedron* **1978**, *34*, 713–716.
[10] K. Miyamoto, N. Kubodera, K. Ochi, I. Matsunaga, E. Murayama, *Eur. Pat. Appl.* (1986) EP 184206.
[11] J. Abe, Y. Nagai, R. Higashikuni, K. Iida, T. Hirokawa, H. Nagai, K. Kominato, T. Tsuchida, M. Hirata, M. Inada, C. Miyaura, K. Nagasawa, *Org. Biomol. Chem.* **2012**, *10*, 7826–7839.
[12] a) D. Oves, S. Fernández, M. Ferrero, R. Bouillon, A. Verstuyf, V. Gotor, *Bioorg. Med. Chem.* **2006**, *14*, 928–937; b) D. Oves, M. Ferrero, S. Fernández, V. Gotor, *J. Org. Chem.* **2003**, *68*, 1154–1157.
[13] R. H. Hesse, S. K. S. Setty, M. Ramgopal, WO95/16672, 1994.
[14] J. Yoon, J. Kim, H. Park, E. Kim, K. Ahn, S. Yoon, C. Cho, B. Kim, Y. Lee, *Int. J. Oncol.* **2008**, *32*, 387–396.
[15] a) A. Hernández-Martín, S. Fernández, A. Verstuyf, L. Verlinden, M. Ferrero, *Eur. J. Org. Chem.* **2017**, 504–513; b) T. González-García, S. Fernández, E. Lubian, F. Mancin, M. Ferrero, *RSC Adv.* **2016**, *6*, 31840–31849; c) A. Hernández-Martín, T. González-García, M. Lawlor, L. Preston, V. Gotor, S. Fernández, M. Ferrero, *Bioorg. Med. Chem.* **2013**, *21*, 7779–7789; d) L. Sánchez-Abella, S. Fernández, A. Verstuyf, L. Verlinden, V. Gotor, M. Ferrero, *J. Med. Chem.* **2009**, *52*, 6158–6162; e) L. Sánchez-Abella, S. Fernández, A. Verstuyf, L. Verlinden, V. Gotor, M. Ferrero, *Bioorg. Med. Chem.* **2008**, *16*, 10244–10250; f) D. Oves, S. Fernández, L. Verlinden, R. Bouillon, A. Verstuyf, M. Ferrero, V. Gotor, *Bioorg. Med. Chem.* **2006**, *14*, 7512–7519; g) V. Gotor-Fernández, S. Fernández, M. Ferrero, V. Gotor, R. Bouillon, A. Verstuyf, *Bioorg. Med. Chem.* **2004**, *12*, 5443–5451.
[16] K. Ono, A. Yoshida, N. Saito, T. Fujishima, S. Honzawa, Y. Suhara, S. Kishimoto, T. Sugiura, K. Waku, H. Takayama, A. Kittaka, *J. Org. Chem.* **2003**, *68*, 7407–7415.
[17] X. Ariza, O. Pineda, F. Urpi, J. Vilarrasa, *Tetrahedron Lett.* **2001**, *42*, 4995–4999.
[18] P. Huang, K. Sabbe, M. Pottie, M. Vandewalle, *Tetrahedron Lett.* **1995**, *36*, 8299–8302.
[19] T. González-García, A. Verstuyf, L. Verlinden, S. Fernández, M. Ferrero, *Adv. Synth. Catal.* **2018**, *360*, 2762–2772.
[20] a) H. Hilpert, B. Wirz, *Tetrahedron* **2001**, *57*, 681–694; b) B. Wirz, H. Iding, H. Hilpert, *Tetrahedron: Asymmetry* **2000**, *11*, 4171–4178.
[21] L. Sánchez-Abella, S. Fernández, A. Verstuyf, L. Verlinden, V. Gotor, M. Ferrero, *J. Med. Chem.* **2009**, *52*, 6158–6162.
[22] A. Verstuyf, L. Verlinden, H. Van Baelen, K. Sabbe, C. D'Halleweyn, P. De Clercq, M. Vandewalle, R. Bouillon, *J. Bone Miner. Res.* **1998**, *13*, 549–558.
[23] R. Bouillon, H. Van Baelen, P. De Moor, *J. Steroid Biochem.* **1980**, *13*, 1029–1034.