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Bioorganic & Medicinal Chemistry Letters 15 (2005) 1451–1455
Bioorganic & Medicinal Chemistry Letters

New derivatives of 1α,25-dihydroxy-19-norvitamin D_3 with two substituents at C-2: synthesis and biological activity

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Received 29 November 2004; accepted 29 December 2004

Abstract—To examine the effect of 2,2-disubstitution on the biological activities of 19-norvitamin D analogs, novel 2,2-disubstituted-(20*R*)- and (20*S*)-1α,25-dihydroxy-19-norvitamin D_3 analogs were prepared and their biological activities were studied. All the synthesized analogs possessing hydrophobic 2α-substituents were more active than the corresponding 2β-isomers both in binding to the vitamin D receptor and in activating gene transcription. The 2α-methyl-2β-hydroxy analog **9b** was found to have markedly higher transcriptional activity (32-fold) than the natural ligand **1a**, although the two had the same binding affinity to the vitamin D receptor. To our knowledge, this analog is among the most potent of 19-norvitamin D analogs.

1α,25-Dihydroxyvitamin D_3 (1α,25-$(OH)_2D_3$) **1a**, in addition to its well known crucial role in calcium and phosphorus metabolism, bone mineralization, and the prevention of rickets, regulates the growth, differentiation, and function of several cell types, both normal and malignant.[1] It has also been shown to serve as a modulator of the immune system. 1α,25-$(OH)_2D_3$ is a secosteroid hormone that functions through a nuclear receptor, the vitamin D receptor (VDR). This VDR is a ligand-dependent transcription factor and is believed to act by binding as a retinoid X receptor (RXR)/VDR heterodimer to vitamin D-responsive elements found in the promoter of target genes.[1]

1α,25-Dihydroxy-19-norvitamin D_3 **2a**, which lacks the 10(19)-exomethylene of **1a**, has low calcemic activity, but still has the same cell differentiating ability as **1a**.[2,3] Recently, much attention has been focused on the discovery of A-ring-modified 19-norvitamin D analogs. Introduction of a substituent into the C-2 position of 19-norvitamin D has been shown to confer increased affinity for the VDR, strong calcemic activity, as well as high HL-60 differentiating activity.[4–7] Recently the crystal structures of the ligand binding domain (LBD) of the rat VDR complexed with 2-substituted 19-norvitamin D analogs have been disclosed,[8] and in these complexes the ligands are docked in the ligand binding pocket in a manner similar to the natural hormone **1a** in the human VDR.[9] We have developed a novel and efficient method for preparation of the A-ring synthons of 19-norvitamin D_3 from D-glucose, and synthesized a number of 2-substituted 19-norvitamin D_3 analogs.[10] From the structure–activity relationship (SAR) of the 2-functionalized 19-norvitamin D_3 analogs, we showed that (20*S*)-1α,25-dihydroxy-2β-hydroxyethoxy-19-norvitamin D_3 had the strongest binding affinity for the VDR (five times more active than **1a**) among the known 19-norvitamin D analogs and significantly enhanced transcriptional activity (30 times more active than **1a**).[11,12] In the course of investigating the SAR of the 19-nor analogs, specifically the effect of the substituent at C-2 of the 19-norvitamin D_3 analog, we were interested to examine the introduction of a second substituent at C-2 that might have additional biologic effects due to ligand–protein interactions among the substituents. Furthermore, the

Keywords: 2,2-Disubstituted 19-norvitamian D; Synthesis; Vitamin D receptor.
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doi:10.1016/j.bmcl.2004.12.090

limited number of 2,2-disubstituted 19-norvitamin D analogs have been synthesized up to now.[13] Here we report the design, synthesis, biological activities, and docking properties of a new class of (20*R*)- and (20*S*)-19-norvitamin D analogs **3**–**12** with two different substituents at the C-2 position (Fig. 1).

For synthesis of the target 2,2-disubstituted 19-norvitmain D_3 analogs **3**–**12**, we used a Wittig–Horner coupling approach[14] involving the A-ring phosphine oxide **24** with the 25-hydroxy Grundmann's ketones possessing a natural 20*R*- (**25**, **26**) and an unnatural 20*S*- (20-*epi*, **27**) configuration (Fig. 2).

First, we prepared the A-ring synthon **24** with a 4,4-epoxy moiety, as outlined in Figure 2. The cyclohexanone derivative **13**, bearing three silyl-protected hydroxyl groups, was synthesized from D-glucose in 26% yield.[10] Reduction of **13** with $NaBH_4$ yielded a 1-hydroxy compound **14** (sugar numbering) as an approximate 2:1 diastereomeric mixture, which was treated with benzyl bromide to afford a benzyl ether **15**. The trimethylsilyl protecting group of **15** was selectively hydrolyzed by treatment with a mixture of aqueous acetic acid in THF to give a 4-hydroxy compound **16**. Oxidation of **16** proceeded quantitatively under Swern conditions to give the 4-keto derivatives **17** as a single compound. The Wittig reaction of **17** with methyltriphenylphosphonium bromide afforded a 4-methylene product **18** in excellent yield. Epoxidation of **18** with *m*-chloroperbenzoic acid, followed by Pd/C-catalyzed hydrogenolysis of the benzyl ether **19** proceeded quantitatively to provide a spiro-oxirane **20** as an approximate 3:1 mixture of diastereomers, which, upon Swern oxidation, gave a corresponding 1-keto compound **21** as a single product.

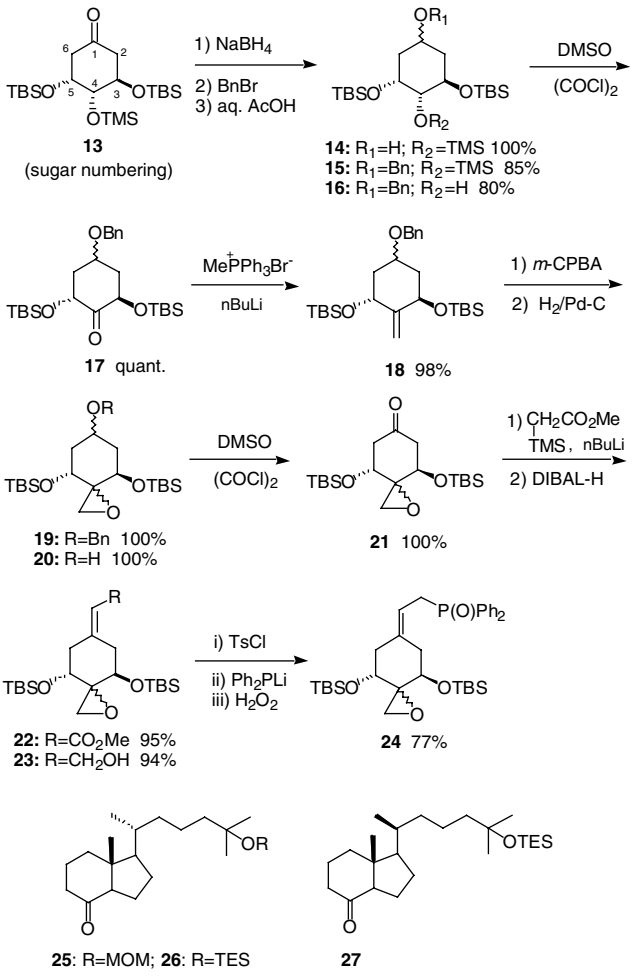

Figure 2. Synthetic scheme of the A-ring phosphine oxide with the epoxy group.

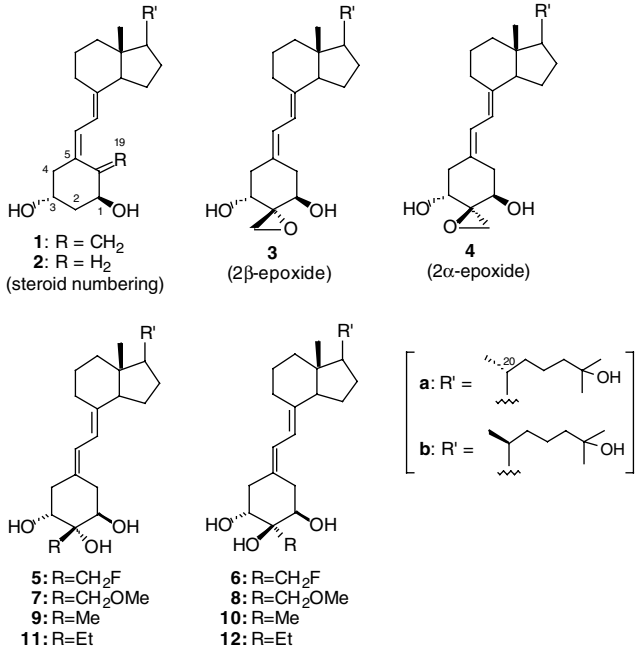

Figure 1. Structures of the active vitamin D_3 and its 19-norvitamin D analogs.

Peterson olefination of **21** with methyl (trimethylsilyl)acetate afforded an allylic ester **22** in an approximate 3:1 mixture of diastereomers due to the newly generated double bond isomerism. The allylic ester **22** was reduced with $(i\text{-Bu})_2AlH$ to give an allylic alcohol **23**. Transformation of **23** into the A-ring phosphine oxide **24** was achieved in a one-pot process according to the procedures described by DeLuca and co-workers.[5]

Protected 25-hydroxy Grundmann's ketones **25**–**27** with a 20*R*- or unnatural 20*S*-configuration were prepared starting from the readily available vitamin D_3 or vitamin D_2 following the general procedures described previously (Fig. 2).[15–18]

Ten 2,2-disubstituted 19-norvitamin D_3 analogs **3a**–**12a** were successfully synthesized according to the sequence shown in Figure 3. The key intermediates **28** (ca. 5:1) and **29** (ca. 3:1) as a mixture of diastereomers were obtained by condensation of the A-ring epoxide **24** with the C/D-ring ketones **25** and **26**, respectively. Deprotection of **28** with $n\text{Bu}_4\text{NF}$ yielded the diastereomeric pairs of **3a** and **4a**, together with fluorinated analogs **5a** and **6a** resulting from a nucleophilic ring-opening reaction with fluoride anion. A methoxymethyl protected oxirane

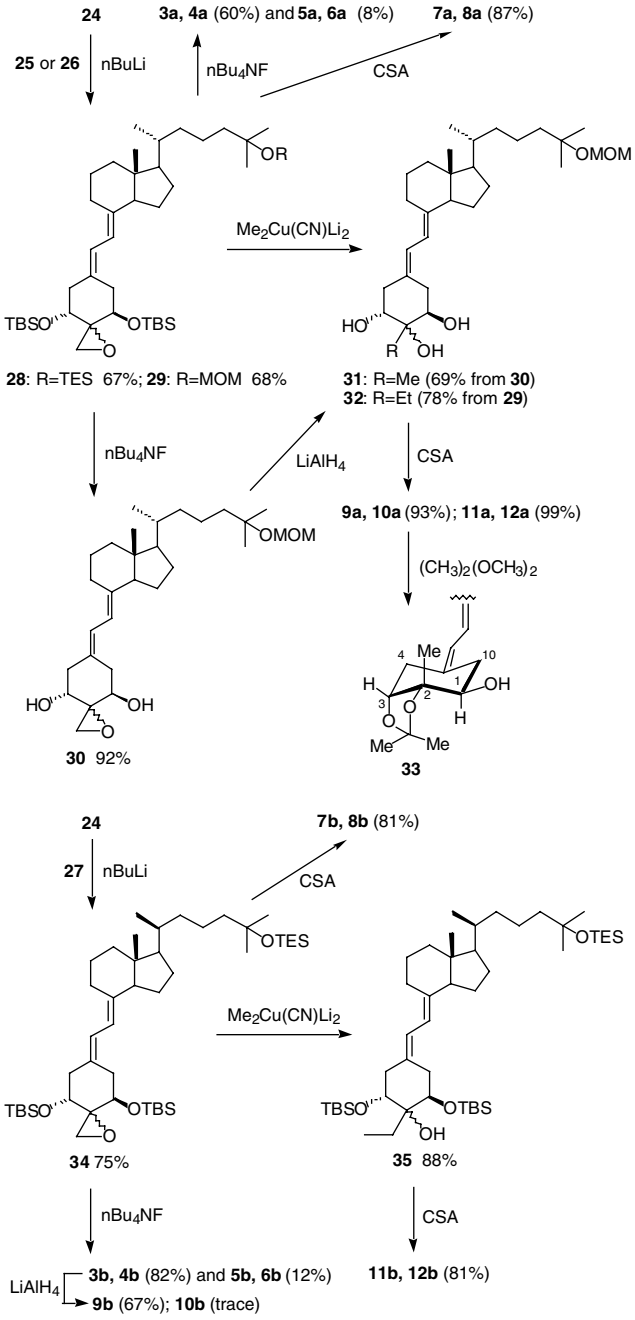

Figure 3. Synthetic scheme of 2,2-disubstituted 19-norvitamin D_3 analogs (**3**–**12**) with natural and unnatural C(20)-configuration.

29 was treated with camphor sulfonic acid (CSA) in MeOH, yielding the 2-methoxymethylated analogs **7a** and **8a**. The isomeric 2-methyl-19-nor analogs **9a** and **10a** were obtained by reductive ring cleavage of **30**, followed by removal of the methoxymethyl group of **31** with CSA. The nucleophilic oxirane cleavage reaction of **29** with higher-order mixed organocuprate $Me_2Cu(CN)Li_2$[19,20] proceeded smoothly to afford a ring-opened product **32** in good yield, which after deprotection with CSA, gave **11a** and **12a**. All C(2)-epimeric pairs (**3a**–**12a**) were separated by HPLC. The stereochemistry at C-2 of 2,2-disubstituted 19-norvitamin D analogs was assigned by phase-sensitive 2D NOESY NMR analysis. The major 2-methyl-2-hydroxy-19-nor analog **9a** was converted to a corresponding acetonide **33**. In **33**, an important correlation cross-peak was observed between 2α-Me and 3α-H, but not between 1β-H and 2α-Me. An NOE was also observed between 2α-Me and 10α-H. These correlations clearly demonstrate the structure for **33** as indicated in Figure 3. In the minor product **10a**, a cross-peak was observed between 2β-Me and 1β-H or 4β-H or 10β-H, and the 2α-configuration of the hydroxyl substituent was assigned. These findings indicate that the major isomers of the synthetic intermediates **28** and **29** have the 2β-epoxy functionality.

It is well known that vitamin D analogs with the 20*S*-configuration have enhanced VDR binding affinity with respect to their 20*R* counterparts.[21] We synthesized 2,2-disubstituted 19-norvitamin D_3 analogs **3b**–**12b** with the unnatural (20*S*)-side chain by analogous procedures as described above (Fig. 3). Coupling of **24** with the protected (20*S*)-25-hydroxy C/D-ring ketone **27** yielded **34** as an approximate 3:1 isomeric mixture. Treatment of **34** with nBu$_4$NF gave oxirane derivatives **3b** and **4b**, together with fluorinated products **5b** and **6b**, whereas treatment of **34** with CSA in MeOH afforded the methoxymethylated analogs **7b** and **8b**. $LiAlH_4$ reduction of **3b** and **4b**, followed by hydrolysis, provided **9b** and **10b** (trace). Epoxide **34** was reacted with $Me_2Cu(CN)Li_2$ to yield **35**, which was treated with CSA to afford **11b** and **12b**.

We evaluated the potencies of the 2,2-disubstituted 19-norvitamin D_3 analogs (**3a**,**b**–**12a**,**b**) to bind to the bovine thymus VDR and to activate the gene in a transient transcription assay in COS-7 cells, as described previously.[22,23] Table 1 summarizes the results (Table 1). 2-Methyl-2-hydroxy-19-norvitamin D_3 analogs **9a** and **9b** have higher transcriptional activities (Fig. 4), and the largest difference in activity among the tested compounds was found between the 2α-methyl compound **9a** and the 2β-methyl isomer **10a**. Other analogs **5a**–**12a**, except for the 2-methyl analogs, show comparable efficacy, whereas the difference in activity between the

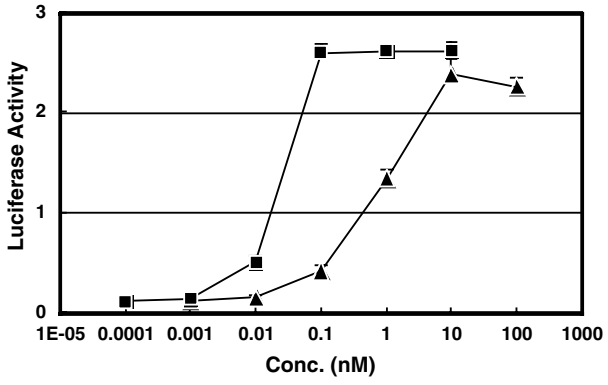

Figure 4. Dose–response behavior of the most potent analog (**9b**) with VDR in cellular reporter gene assays. Data represent the mean and S.E. of at least three independent experiments for 1α,25-$(OH)_2D_3$ (triangle) or **9b** (square). 1α,25-$(OH)_2D_3$: ED_{50} = 0.7 nM; **9b**: ED_{50} = 0.022 nM.

Table 1. Relative VDR affinity and transcriptional activity of 2,2-disubstituted 19-norvitamin D_3 analogs[a]

Compounds	VDR affinity	Transcription
1a	100	100
3a	4	5[c]
4a	3	4[c]
3b	50	89[c]
4b	20	18[c]
5a	2	59[b]
6a	0.02	14[b]
5b	60	213[b]
6b	ND	46[b]
7a	4	48[b]
8a	0.1	8[b]
7b	20	176[b]
8b	10	39[b]
9a	30 (21[d])	280[c]
10a	0.1 (2.6[d])	6[c]
9b	100 (23[d])	3200[c]
10b	ND (18[d])	ND
11a	2 (83[e])	67[b]
12a	0.05 (1[e])	10[b]
11b	5 (200[e])	92[b]
12b	0.1 (17[e])	90[b]

ND: Not determined.

[a] Activities are presented as % effect of **1a**.

[b] Results are expressed as % activity at 10^{-8} M or 10^{-9} M in comparison with **1a**.

[c] Activity was assessed in terms of ED_{50}.

[d] VDR affinity of the corresponding 2-Me-1α,25-dihydroxy-19-norvitamin D_3.[5]

[e] VDR affinity of the corresponding 2-Et-1α,25-dihydroxy-19-norvitamin D_3.[6]

two isomers is small. The replacement of hydrogen by fluorine (CH_3/CH_2F transposition) causes markedly reduced affinity for the VDR and transcriptional activity. These results suggest that the CH_2F substituent has a close steric relationship and biological similarity with methoxymethyl and ethyl groups. When comparing receptor binding and transcriptional activities of C-2 isomeric pairs of 19-norvitamin D_3 analogs, hydrophobic substituents above the A-ring and hydrophilic substituents below the A-ring show a good effect, and this arrangement of two substituents at the C-2 position is complementary to the amino acid residues of the LBP. In the unveiled three-dimensional structure of VDR LBD, the hydrophobic amino acid residues (L233, F150, and the phenyl ring of Y236) lie above the A-ring of **1a**, while the hydrophilic residues (R274, S275, and Y143) lie below it.[9,24,25] When compared to the parent 2-methyl- and 2-ethyl-19-nor derivatives in terms of binding affinity to the VDR,[5,6] the corresponding 2-methyl-2-hydroxy analogs **9** and **10** show a 1.5–3-fold increase in potency, while the 2-ethyl-2-hydroxy-derivatives **11** and **12** have markedly reduced potency. 2,2-Disubstituted analogs with the 20*S*-configuration proved to be more active than the corresponding isomers with 20*R*-stereochemistry in terms of both receptor binding and transcriptional activity. These results are consistent with the findings reported previously.[21]

Docking studies of **9a** and **10a** using the docking software FlexX (Tripos, St. Louis) show that a hydrophobic phenyl ring of F150 contacts the newly introduced 2α-methyl group in **9a**, whereas hydrophilic R274 forms a hydrogen bond with the 2β-hydroxyl moiety. Thus, the A-ring is stabilized in the VDR LBP by both hydrophilic and hydrophobic interactions. In isomer **10a** with a 2α-hydroxyl group, none of the hydrophobic and hydrogen bonding interactions seen in **9a** are observed.

In conclusion, we have described the synthesis and biological evaluation of novel 2,2-disubstituted 19-norvitamin D_3 analogs.[26] 2,2-Disubstituted analogs have significant biological activities, in particular the 2α-methyl-2β-hydroxy-19-nor analog **9b** is characterized by an extremely high ability to activate gene transcription, and to our knowledge this is among the most potent of 19-norvitamin D analogs. We expect that novel 2,2-disubstituted 19-norvitamin D_3 analogs will have a much broader spectrum of activities. Full details of the synthesis and results of broad biological evaluation will be reported in due course.

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26. The structures of all new compounds were confirmed based on the ^{1}H and ^{19}F NMR, mass, and UV spectra. The stereochemistry of the new compounds was established from the 2D NOESY spectra.