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13,13-Dimethyl-*des*-C,D analogues of (20*S*)-1α,25-dihydroxy-2-methylene-19-norvitamin D_3 (2MD): Total synthesis, docking to the VDR, and biological evaluation

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ABSTRACT

As a continuation of our studies focused on the vitamin D compounds lacking the C,D-hydrindane system, 13,13-dimethyl-*des*-C,D analogues of (20*S*)-1α,25-dihydroxy-2-methylene-19-norvitamin D_3 (**2**, 2MD) were prepared by total synthesis. The known cyclohexanone **30**, a precursor of the desired A-ring phosphine oxide **11**, was synthesized starting with the keto acetal **13**, whereas the aldehyde **12**, constituting an acyclic 'upper' building block, was obtained from the isomeric esters **34**, prepared previously in our laboratory.

The commercial 1,4-cyclohexanedione monoethylene ketal (**13**) was enantioselectively α-hydroxylated utilizing the α-aminoxylation process catalyzed by L-proline, and the introduced hydroxy group was protected as a TBS, TPDPS, and SEM ether. Then the keto group in the obtained compounds **15–17** was methylenated and the allylic hydroxylation was performed with selenium dioxide and pyridine N-oxide. After separation of the isomers, the newly introduced hydroxy group was protected and the ketal group hydrolyzed to yield the corresponding protected (3*R*,5*R*)-3,5-dihydroxycyclohexanones **30–32**. The esters **34**, starting compounds for the C,D-fragment **12**, were first α-methylated, then reduced and the resulted primary alcohols **36** were deoxygenated using the Barton-McCombie protocol. Primary hydroxy group in the obtained diether **38** was deprotected and oxidized to furnish the aldehyde **12**. The Wittig–Horner coupling of the latter with the anion of the phosphine oxide **11**, followed by hydroxyl deprotection furnished two isomeric 13,13-dimethyl-*des*-C,D analogues of 2MD (compounds **10** and **42**) differing in configuration of their 7,8-double bond. Pure vitamin D analogues were isolated by HPLC and their biological activity was examined. The in vitro tests indicated that, compared to the analogue **7**, unsubstituted at C-13, the synthesized vitamin D analogue **10** showed markedly improved VDR binding ability, significantly enhanced HL-60 differentiation activity as well as increased transcriptional potency. Docking simulations provided a rational explanation for the observed binding affinity of these ligands to the VDR. Biological in vivo tests proved that *des*-C,D compound **10** retained some intestinal activity. Its geometrical isomer **42** was devoid of any biological activity.

1. Introduction

1α,25-Dihydroxyvitamin D_3, [1α,25-$(OH)_2D_3$, calcitriol, **1**; Fig. 1] exerts a broad range of biological activities, many of which are not directly related with its classical action in maintaining calcium and phosphorus homeostasis.[1–3] Several observations have implicated its role as a promoter of cellular differentiation, as well as immunomodulatory agent.[4–6] These observations have long inspired many research groups to undertake synthesis of the vitamin D compounds characterized by selective biological potencies.[7] More than three thousand calcitriol analogues have been prepared and tested to date but there is continuing interest in such structure–activity studies.[8] Of particular importance are the analogues demonstrating suppressed calcemic activity due to their potential pharmaceutical use as anticancer agents which do not induce hypercalcemia. One of the most promising A-ring modification of the vitamin D molecule seems to be a 'shift' of the exomethylene moiety originally placed at C-10 to the carbon 2. In 1998, we reported a synthesis of the first analogues in this series,[9] the most interesting one being 2MD (**2**),[10–12] and since then many other 2-methylene-19-norvitamin D compounds differing in the side

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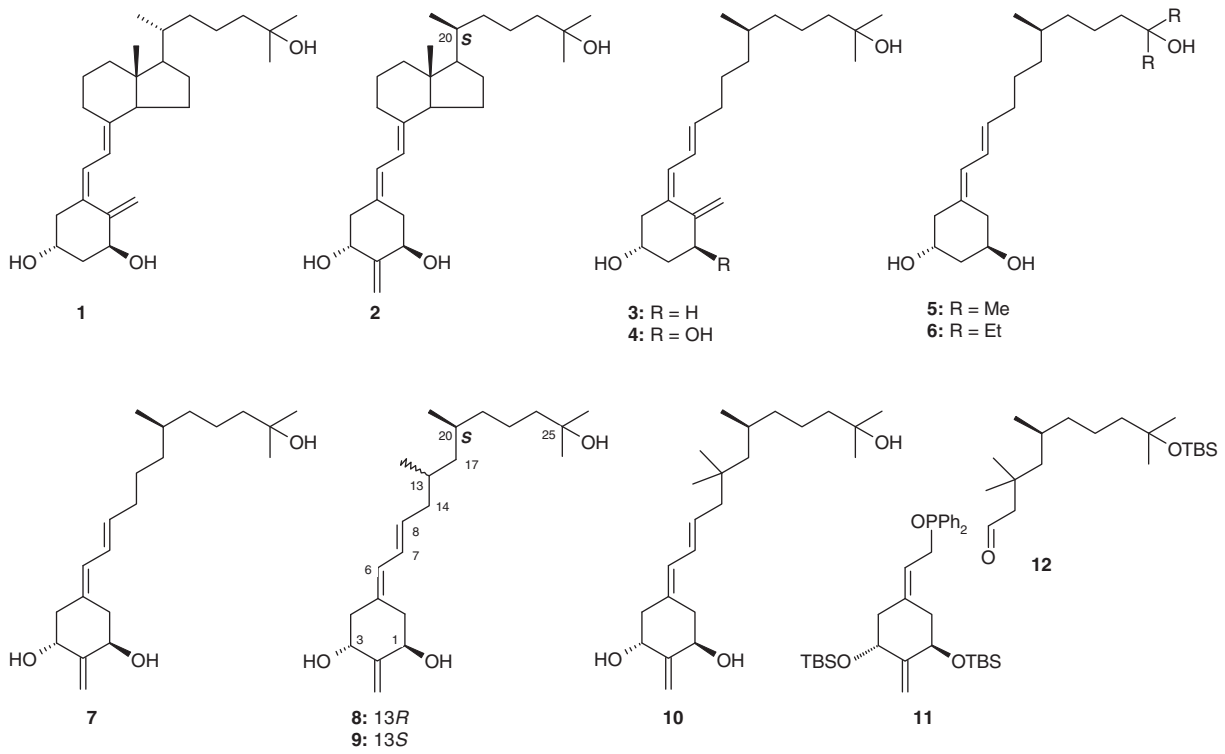


Figure 1. Chemical structures of 1α,25-dihydroxyvitamin D_3 (calcitriol, **1**) and 2MD (**2**), their *des*-C,D analogues and the building blocks for synthesis of **10**.

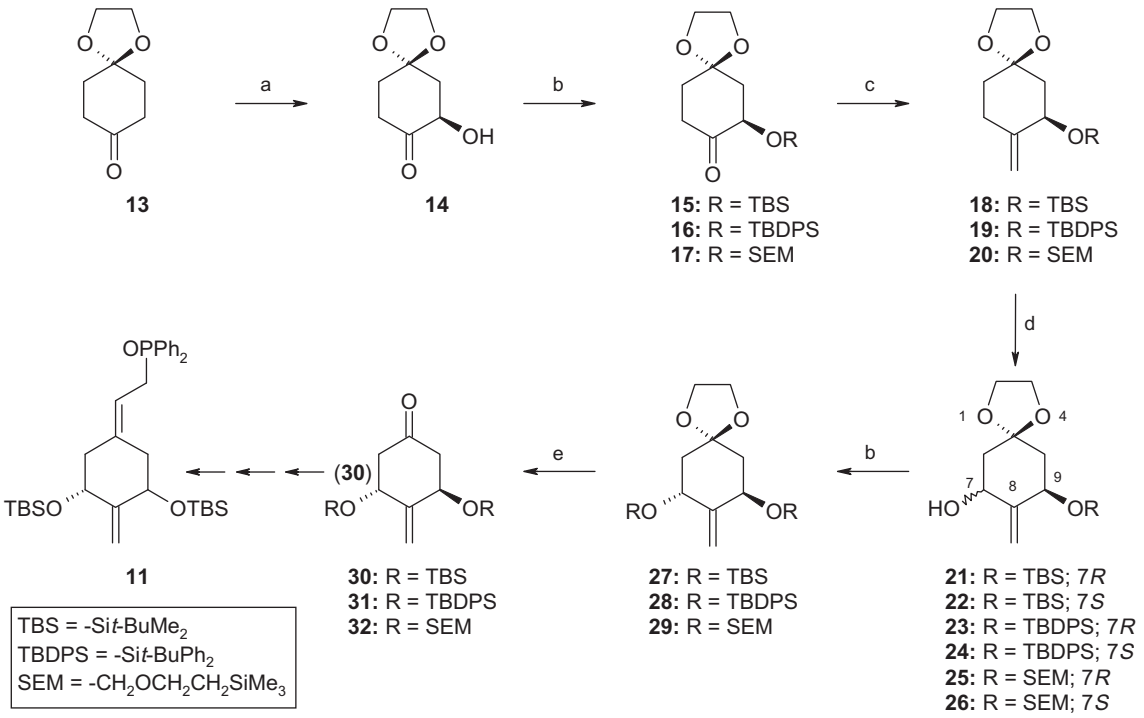
chain structure have been obtained in our and other laboratories, [13–22] some of them showing disparate biological actions. Continuing these studies, we have recently turned our attention to more drastic modification of the vitamin D skeleton, that is, removal of the C and D rings. First vitamin D compounds lacking both these rings (retiferols **3** and **4**) were described in 1995,[23] another *des*-C,D analogues (including 19-norvitamins **5** and **6**) were synthesized later.[24,25] Recently, we synthesized and biologically tested a *des*-C,D analogue of 2MD (**7**) and its derivatives **8** and **9** methylated at C-13.[26,27] All these compounds were practically devoid of any calcemic activity. However, they showed binding ability to the full-length rat recombinant vitamin D receptor (VDR), although ca. 2 orders of magnitude lower than the natural hormone **1**. Compared to the analogue 7, the (13*R*,20*S*)-methylated compound **8** exhibited significantly enhanced HL-60 differentiation activity and increased transcriptional activity.[27] Taking into account these observations and the crystallographic data indicating that the *seco*-B, C and D rings of 2MD are surrounded in its complex with the ligand binding domain (LBD) of the VDR by strongly hydrophobic amino acids,[28] we have decided to synthesize homologous *des*-C,D vitamin **10**, carrying two methyl groups attached to C-13. The results of molecular modeling of its complex with rVDR, described later in this work, seemed to support our expectations that such alkyl substituents could advantageously increase the hydrophobic interactions of the analogue with the LBD.

This paper is an extension of our structure–activity studies directed to the 19-norvitamin D compounds lacking the C,D-rings and describes the total synthesis and biological potency of 13, 13-dimethyl-*des*-C,D analogues of (20*S*)-1α,25-dihydroxy-2-methylene-19-norvitamin D_3 (2MD). Since the strategy of our synthesis was based on the Wittig–Horner coupling of the phosphine oxide **11** with the aldehyde **12**, we have developed the procedures for the preparation of both these fragments.

2. Results and discussion

2.1. Synthesis

As a starting compound for the synthesis of the A-ring fragment **11** prepared by us previously by transformations of the natural (1*R*,3*R*,4*S*,5*R*)-quinic acid,[9] we used the commercially available 1,4-cyclohexanedione monoethylene ketal (**13**, Scheme 1). Its conversion to the chiral hydroxyketone **14** was accomplished by utilization of the asymmetric α-aminoxylation process elaborated by Cordova[29] and Hayashi.[30] We employed slightly altered reaction conditions using slow addition of 1.75 molar excess of the nitrosobenzene to the ketone **13**, chloroform as a solvent and larger catalyst loading (40 mol % of L-proline). The reaction was carried out at 4 °C, then the mixture was allowed to warm up to the ambient temperature and the aqueous workup was performed. The procedure applied by us resulted in the hydrolysis of the initially formed labile α-aminoxylated ketone and allowed to isolate the desired compound **14** by column chromatography on silica. Despite many attempts, we did not succeed in improving the yield (44%) of the product which was obtained in 96% ee. Then, in order to test the usefulness of the different hydroxy protecting groups for the planned synthesis, we obtained three silylated compounds **15–17**. Final confirmation of *R*-configuration of the stereogenic center present in these products followed from a single-crystal X-ray analysis of TPS-protected compound **16**, the outcomes of which are shown in Figure 2 as well as in Section 4. Then all cyclohexanones **15–17** were subjected to the Wittig reaction with an ylide generated from methyltriphenylphosphonium bromide and *n*-butyllithium. The obtained compounds **18–20** were hydroxylated at the allylic position by treatment with selenium dioxide in the presence of pyridine N-oxide.[31] Our numerous attempts did not result in the increased yield of the desired products with the hydroxy



Scheme 1. Synthesis of the A-ring fragments. Reagents: (a) nitrosobenzene, L-proline, $CHCl_3$; (b) TBSCl, imidazole, DMF (for **15**); TPSCl, imidazole, DMF (for **16**); SEMCl, DEA, CH_2Cl_2 (for **17**); (c) $CH_3(Ph_3P)Br$, *n*-BuLi, THF; (d) SeO_2, pyridine N-oxide, dioxane; HPLC separation; (e) $FeCl_3 \times 6H_2O$, CH_2Cl_2.

group introduced *trans* with respect to the silyloxy substituent. In the case of compounds with TBS- and TPS-protecting groups, the allylic hydroxylation process provided the mixture of 7*R*- and 7*S*-hydroxylated products in the ratio of ca. 2:1 and the yields only slightly exceeding 50%, whereas for the SEM-protected compound **20** the reaction outcome was disappointing. After chromatographical separation of the desired isomers, the 7*R*-alcohols **21**, **23** and **25** were silylated and in the formed protected compounds **27–29** hydrolysis of the ketal moiety was effected by the treatment with the ferric chloride on silica. With the notable exception of the TBS-protected compound **30**, the other respective cyclohexanones **31** and **32** were obtained in excellent and good yield, respectively. The above described five-step transformation of **14** to these final compounds shows that hydroxy protection with the most stable TPS group is the most advantageous, providing cyclohexanone **31** in total yield of 22%. The structures of the synthesized compounds were assigned on the basis of their ^1H and ^{13}C NMR spectra. The proton resonance spectroscopy was also useful for their conformational analysis. Thus, comparison of the magnitudes of vicinal coupling constants in the allylic ethers **18–29** with the respective data reported for cyclohexanol protons ($J_{ax,ax}$ = 11.1 Hz, $J_{eq,eq}$ = 2.7 Hz),[32] allowed us to establish that, in the case of compounds **18–26**, conformational equilibrium of the cyclohexane ring is significantly shifted towards the form possessing the silyloxy group occupying an equatorial position (Fig. 3a–c), whereas both chair forms are apparently equally populated for the corresponding diprotected derivatives **27–29** (Fig. 3d).

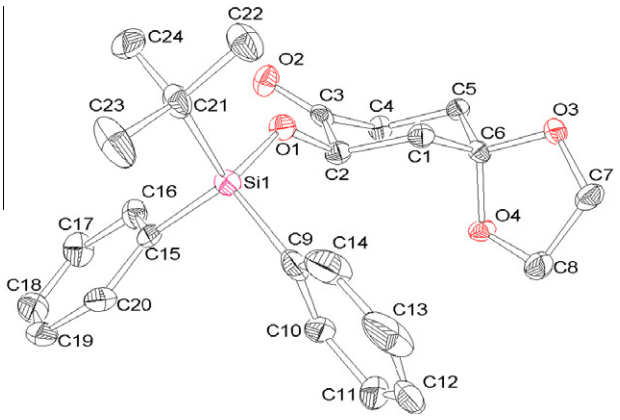


Figure 2. The ORTEP drawing resulting from the single-crystal X-ray analysis of compound **16**.

All chiral ketones **30–32**, characterized by *C*2 symmetry, represent useful synthons for the preparation of different 2-methylene-19-norvitamin D compounds by means of Julia coupling with the corresponding C,D-ring sulphones.[33,34] They can be also converted to the respective allylic phosphine oxides, the A-ring intermediates suitable for the Wittig–Horner olefination process; transformation of the TBS-protected ketone **30** to the phosphine oxide **11** has been already achieved in our laboratory.[9]

For the synthesis of the 'upper' fragment **12**, we used epimeric esters **34**, obtained by us previously from commercial (*R*)-(−)-methyl-3-hydroxy-2-methylpropionate (**33**, Scheme 2),[27] and the following synthetic steps were carried out with such a diastereomeric mixture. Alkylation process of the carbanions, generated from these esters by LDA, resulted in the introduction of a methyl substituent at the α position to the carbomethoxy group. DIBALH reduction of the formed methylated esters **35** provided the alcohols **36** which were converted to imidazole-1-carbothioic acid esters **37**. These, in turn, were subjected to tributylstannane-catalyzed deoxygenation to give the diether **38** possessing *gem*-dimethyl group. Selective deprotection of primary hydroxy group in this compound furnished the alcohol **39** which was oxidized to the desired aldehyde **12**. Wittig–Horner coupling of this compound with lithium phosphinoxy carbanion, generated from the phosphine oxide **11** and phenyllithium, gave the expected mixture of protected vitamin D analogues **40** and **41** which, after deprotection with hydrogen

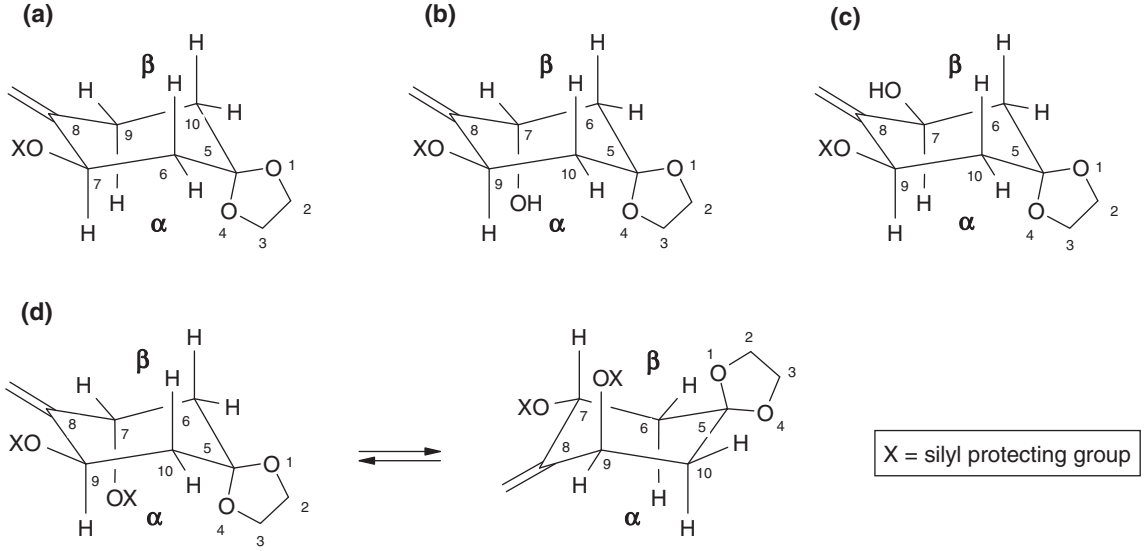


Figure 3. Preferred conformations of the synthesized compounds: (a) **18**–**20**, (b) **21**, **23** and **25**, (c) **22**, **24** and **26**; (d) conformational equilibrium in cyclohexane ring of compounds **27**–**29**.

fluoride, afforded 13,13-dimethyl-*des*-C,D analogues of (20*S*)-1α,25-dihydroxy-2-methylene-19-norvitamin D_3. Analysis of the vicinal coupling constants ($J_{7,8}$) in the ^{1}H NMR spectra of the purified products **10** and **42** (14.7 and 9.6 Hz, respectively) allowed their configurational assignment as 7*E*- and 7*Z*-isomers. The spectral properties of the final products exhibited close similarity to these found for the respective geometric isomers of *des*-C,D vitamins obtained by us previously.[27]

2.2. Biological evaluation

The synthesized *des*-C,D vitamin D analogues were tested for their affinity to rat vitamin D receptor (Table 1). It turned out that dimethylated compound **10** was characterized by substantial binding ability, exceeding 25 times that of compound **7** unsubstituted at C-13 and being only 20 times less active than the natural hormone **1**. The analogous improvement of biological activity in vitro was also observed in the other tests of the analogue **10**. Thus, compared to **7**, this compound exhibited also an increased ability (by a factor of 50 and 25, respectively) to differentiate human promyelocyte HL-60 cells and to induce transcription of the *Cyp24a1* luciferase reporter gene system. The geometrical 7*Z*-isomer **42** proved to be only few times more active than **7** in the VDR binding and transcriptional assays.

The interesting results of the above described tests encouraged us to examine also the in vivo activity of the prepared *des*-C,D analogues of 2MD. The results of these studies, performed on vitamin D-deficient rats, are presented in Table 2. They indicate that compound **10** shows some minor intestinal calcium transport activity, especially when used in high doses, being inactive in mobilizing calcium from bone. The isomer **42** was completely devoid of the calcemic activity.

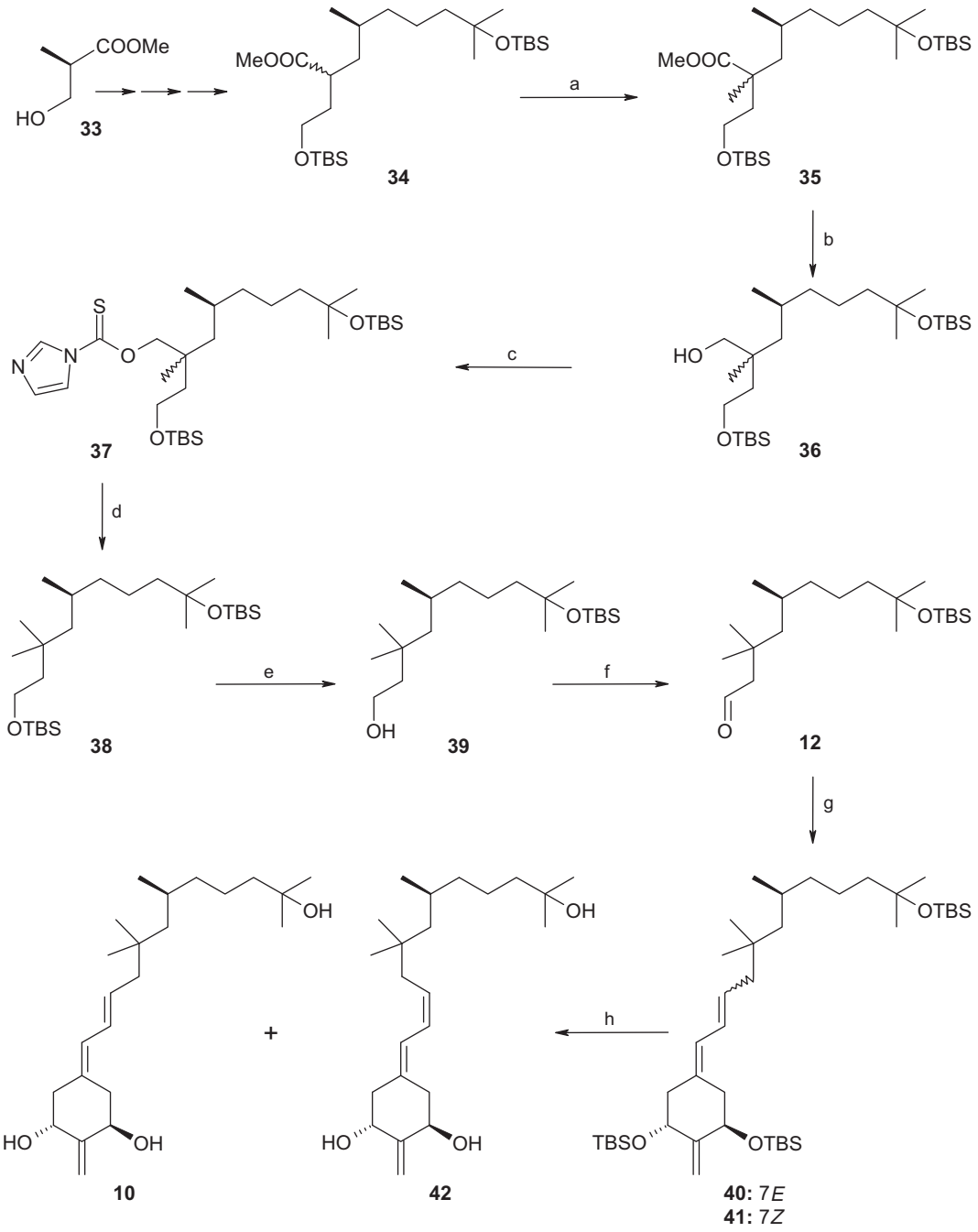
2.3. Molecular modeling and docking experiments

In order to better understand why the synthesized compound **10** still preserves its fundamental biological functions, such as VDR binding and transcription (Table 1), we modeled VDR complexes with two *des*-C,D vitamin D analogues. Since all the reported X-ray structures of VDR were obtained for the holoVDR deletion mutants and, moreover, all rVDR complexes were cocrystallized with DRIP coactivator moiety,[28] we decided to dock our new ligands **7** and **10** into the modeled full-length [118–423] ligand binding pocket (LBP) of the rat vitamin D receptor.[35] While analyzing the calculated complexes, we took into consideration not only the lowest energy criterion but also the positioning of an unique tryptophan residue in respect to the ligand intercyclic 5,7-diene moiety.

Tryptophan is of special importance for the receptor functions, as it appears just once in the VDR sequence and occupies the center of its LBP. Mutation experiments revealed that this residue is essential for ligand binding and transcription of genes controlled by VDR.[36] Our NMR studies confirmed that in aqueous solutions,[37] analogueously as in the crystal state,[28,38,39] the position of tryptophan in the ligand–VDR complexes is preserved, even in the case of vitamin D analogues which drastically differ in their biological activities.

In the rVDR-**7** and rVDR-**10** complexes, tryptophan lies parallel to the ligand 5,7-diene fragment, with its indole ring positioned at a distance of 5.1 Å and 5.0 Å from the C(7)═C(8) bond, respectively. For comparison, in the crystalline hVDR-**1** and rVDR-DRIP-**2** complexes such distances equal 4.0 Å and 3.7 Å, respectively.[28,38] Figures 4 and 5 show that *des*-C,D vitamin D analogue **10** anchors the VDR cavity in a similar fashion to 1α,25-$(OH)_2D_3$ in hVDR-**1**. Interestingly, the conformations of the 'side chain' (C-20 to C-27) of the analogue **10** and **7** closely resemble those of **1** and **2**, respectively, in their crystalline complexes with the receptor. In both the modeled rVDR-**7** and rVDR-**10** complexes exist five and four hydrogen bonds (Table 3), respectively, from the six hydrogen bonds found in the crystalline hVDR-**1**.[38]

Recently, we showed that not only the presence of a methyl group at C-13 in the *des*-C,D analogues of 2MD but also the configuration of a carbon atom bearing a methyl substituent is important for biological functions.[27] The results presented here show that an introduction of the second methyl group at C-13 results in a substantial increase of the binding ability of the synthesized analogue **10** and, moreover, the appearance of intestinal calcemic activity (ICA). It can be assumed that one methyl substituent attached to C-13 in compound **10** is an equivalent of the angular 13β-methyl group present in the natural hormone **1**, while the second methyl mimics its methylene C(12)H_2 group. According to the modeled rVDR-**10** complex, these two methyl substituents at C-13



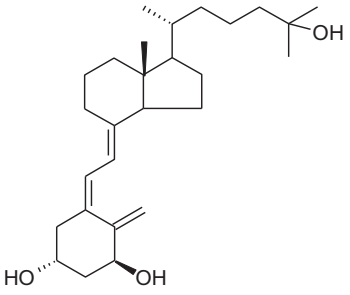
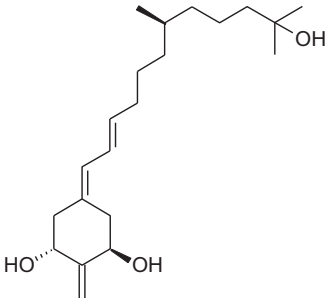
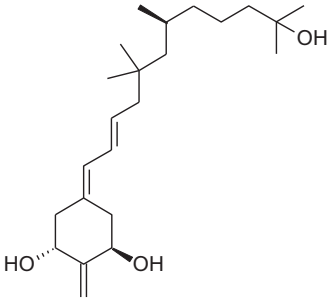
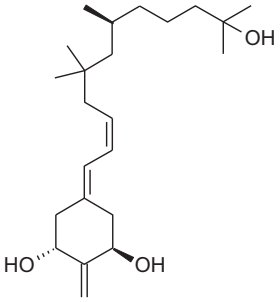
Scheme 2. Synthesis of *des*-C,D vitamins **10** and **42**. Reagents: (a) MeI, LDA, THF; (b) DIBALH, toluene/THF, CH_2Cl_2; (c) TCDI, THF; (d) Bu_3SnH, AIBN, toluene; (e) TBAF, THF; (f) TPAP, NMO, CH_2Cl_2; (g) **11**, PhLi, THF; (h) HF/MeCN, THF; HPLC separation.

efficiently contact (at distances of 2.4–4.6 Å) five hydrophobic VDR residues: L226, L229, V230, I267 and V296 (Table 3) of great biological importance.[36]

It has recently been reported that introduction of the 12β-CH_3 substituent into the 1α,25-$(OH)_2D_3$ causes a fourfold increase of the binding ability of the analogue, while introduction of 12α-CH_3 has the opposite effect.[40] It has been also found that the substitution of C-12 with the hydroxy group impairs the binding activity of this new analogue to the receptor by two orders of magnitude.[40] Inspection of the modeled rVDR-**10** revealed that five hydrophobic contacts to the ligand's methyl substituents at C-13 are preserved, out of six contacts to C(12)H_2 and 13β-CH_3 groups present in hVDR-**1** (Table 3). It is highly probable that these hydrophobic interactions are responsible for increased binding affinity of the analogue **10** in comparison with its counterparts possessing only one methyl substituent at C-13.[27]

It has been proved that irrespective of the structure modification vitamin D analogues anchor to the LBP of VDR in a similar fashion to 1α,25-$(OH)_2D_3$. The side chain flexibility allows the different compounds to adapt to the binding cavity and to create the anchoring hydrogen bonds as well as hydrophobic contacts. Thus, the 25-hydroxy group is always positioned between histidines 301 and 393, whereas the terminal methyl substituents at C-25 contact similar residues (L233, V230, L400, L410 and V414) as the analogous methyl groups in hVDR-**1** complex.[28,39] According to our docking experiments, all of these contacts are also preserved

Table 1
VDR binding properties,[a] HL-60 differentiating activities,[b] and transcriptional activities[c] of the vitamin D compounds **10**, **18** and **19**

Compound	Compound no.	VDR binding		HL-60 differentiation		*Cyp24a1* transcription	
		K_i	Ratio	EC_{50}	Ratio	EC_{50}	Ratio
	**1**	1.0×10^{-10} M	1	3.0×10^{-9} M	1	2.0×10^{-10} M	1
	**7**	5.0×10^{-8} M	500	3.0×10^{-7} M	100	5.0×10^{-8} M	250
	**10**	2.0×10^{-9} M	20	6.0×10^{-9} M	2	2.0×10^{-9} M	10
	**42**	1.0×10^{-8} M	100	ND	ND	3.0×10^{-8} M	150

[a] Competitive binding of 1α,25-$(OH)_2D_3$ (**1**) and the synthesized vitamin D analogues to the full-length recombinant rat vitamin D receptor. The experiments were carried out in duplicate on two different occasions. The K_i values are derived from dose–response curves and represent the inhibition constant when radiolabeled 1α,25-$(OH)_2D_3$ is present at 1 nM and a K_d of 0.2 nM is used. The binding ratio is the average ratio of the analogue K_i to the K_i for 1α,25-$(OH)_2D_3$.

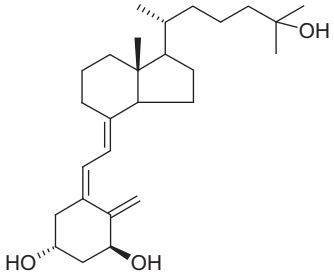
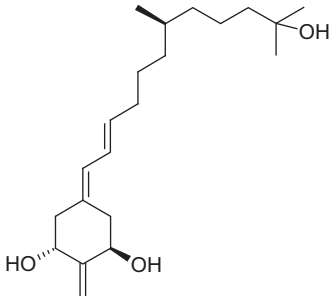
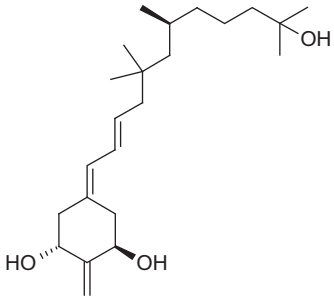
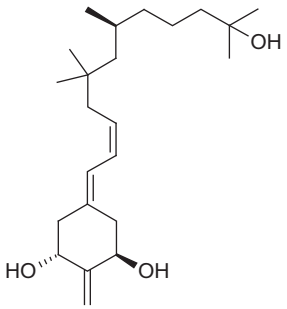
[b] Induction of differentiation of HL-60 promyelocytes to monocytes by 1α,25-$(OH)_2D_3$ and the synthesized vitamin D analogues. Differentiation state was determined by measuring the percentage of cells reducing nitro blue tetrazolium (NBT). The experiment was repeated in duplicate two times. The ED_{50} values are derived from dose–response curves and represent the analogue concentrations capable of inducing 50% maturation. The differentiation activity ratio is the average ratio of the analogue ED_{50} to the ED_{50} for 1α,25-$(OH)_2D_3$.

[c] Transcriptional assay in rat osteosarcoma cells stably transfected with a *Cyp24a1* reporter plasmid. The ED_{50} values are derived from dose–response curves and represent the analogue concentration capable of increasing the luciferase activity by 50%. The lucerifase activity ratio is the average ratio of the analogue ED_{50} to the ED_{50} for 1α,25-$(OH)_2D_3$.

in the modeled rVDR complexes accommodating *des*-C,D compounds (Table 3). It is worth mentioning that in the case of the rVDR-**10** complex, three contacts between terminal CH_3 groups and L400 (2.2 Å), L410 (2.2 Å) and V414 (2.5 Å), are even stronger than in the hVDR-**1** complex, where the corresponding distances are 3.2 Å, 3.1 Å and 2.8 Å, respectively. The findings described above could explain why the binding affinity of compound **10** to VDR is only 20 times lower in comparison with the vitamin D hormone **1**.

A fundamental biological action of 1α,25-$(OH)_2D_3$ and its analogues is the activation of the transcription factor, that is, vitamin D receptor, regulating over 60 genes associated with calcium/phosphorous homeostasis, immune responses, cellular growth, cell differentiation or apoptosis.[41] Analogues with

Table 2
Support of intestinal calcium transport and bone calcium mobilization by *des*-C,D analogues of (20*S*)-1α,25-dihydroxy-2-methylene-19-norvitamin D_3 (**2**) in vitamin D-deficient rats on a low-calcium diet[a]

Compound	Compound No.	Bone Ca mobilization and intestinal Ca transport				
		Dose level		Bone		Intestine
		μg/kg body weight	pmol/rat/day	Serum Ca (mg/dL)	Serum Ca increase compared to vehicle[b]	Calcium levels (increase in S/M ratio compared to vehicle[b])
	**1**	0	0	4.0	–	–
		0.2	87	4.5	0.5 ± 0.1	4.2 ± 0.9
		0.7	260	5.3	1.3 ± 0.3	2.8 ± 0.7
		2.1	780	5.9	1.9 ± 0.4	4.2 ± 0.8
		19	7020	7.9	3.9 ± 0.2	5.9 ± 0.7
	**7**	0	0	4.1	–	
		1.9	1040	3.9	−0.2 ± 0.2	
		5.7	3120	4.2	0.1 ± 0.1	ND
		22.8	12480	4.3	0.2 ± 0.1	
	**10**	0	0	3.7	–	–
		2.1	780	3.9	0.2 ± 0.2	0.0 ± 0.8
		6.5	2340	4.3	0.6 ± 0.2	0.4 ± 0.5
		19	7020	4.1	0.4 ± 0.3	1.0 ± 1.2
		97	35100	4.0	0.3 ± 0.2	2.6 ± 2.5
	**42**	0	0	3.7	–	–
		6.5	2340	3.9	0.2 ± 0.1	0.1 ± 0.8

[a] Weanling male rats were maintained on a 0.47% Ca diet for 1 week and then switched to a low-calcium diet containing 0.02% Ca for an additional 3 weeks. During the last week, they were dosed daily with the appropriate vitamin D compound for 7 consecutive days. All doses were administered intraperitoneally in 0.1 ml propylene glycol/ethanol (95:5). Controls received the vehicle. Determinations were made 24 h after the last dose. There were 5–6 rats per group.
[b] Average ± SEM are shown (mg/dL), vehicle = vehicle treated rats.

'unnatural' 20*S*-configuration (called 20-*epi*-) reveal much higher activity than calcitriol in transcription, antiproliferation and differentiation.[41] Broad mutational experiments indicate 22 amino acids deeply involved in VDR transcription controlled by 1α,25-$(OH)_2D_3$, its 20-*epi* analogue (MC1288) and 2MD.[36] The configuration of the 20-methyl group in compound **10** is the same as in 2MD, however, this analogue adapts its side chain to the VDR pocket like the natural hormone (Fig. 5a). Therefore, we can expect that common amino acids (Y143, D144, Y147, F150, L223, **L226**, L229, S233, I234, I264, **I267**, R270, W282, Y291, **V296**, L305, H393, Y397, **L400**, **L410**, **V414**, F418),[36] responsible for transcriptional activity of 1α,25-$(OH)_2D_3$, MC1288 and 2MD, could also influence this function of analogue **10**; comparison of the VDR binding pockets accommodating **1** and **10** revealed that six marked in bold residues have closer contacts to the *des*-C,D ligand than to the hormone (Table 3). These stronger interactions occur between: (a) methyl group at C-13 (mimicking 13β-CH_3 in **1**) and L226 and I267; (b) 20-CH_3 and conserved residue V296; (c) terminal methyl groups at C-25 and L400, L410, and V414. All these residues either influence the holding of active VDR conformation[36] or they are known in the nuclear

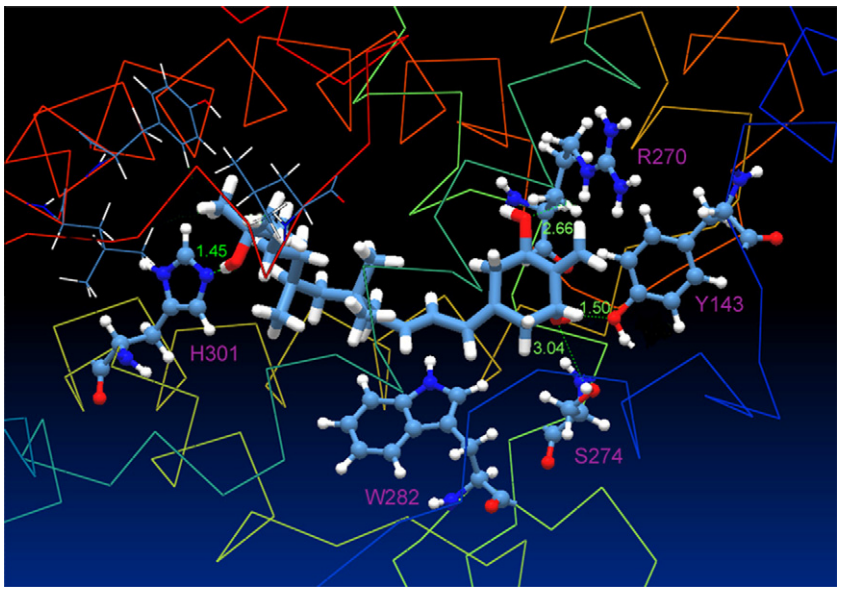


Figure 4. View of the three-dimensional structure of ligand binding cavity of the rat VDR with the docked vitamin D analogue **10** (FlexiDock, TRIPOS). The four amino acids (Tyr 143, Ser 274, Arg 270, and His 301) forming the shortest hydrogen bonds (the Å distances are marked in green) with the ligand are depicted; also the Trp 282 residue is shown.

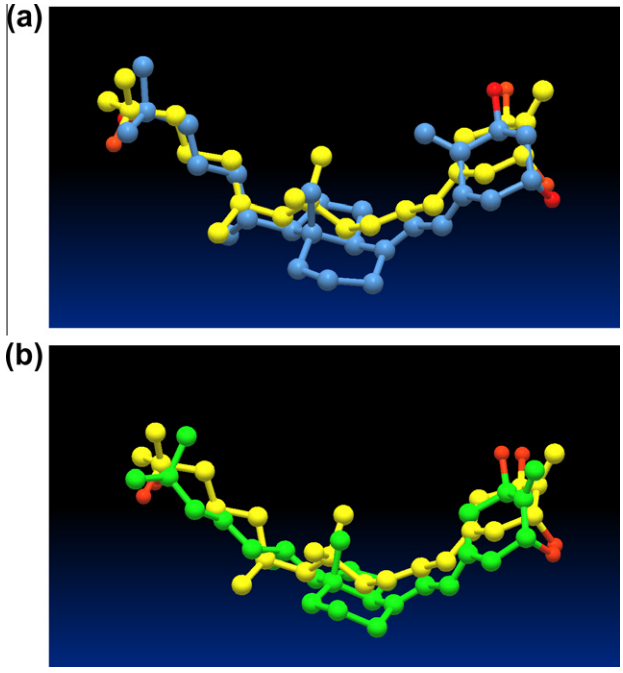


Figure 5. Overlaid structures (ball-and-stick representation; hydrogen atoms are omitted for clarity) of the analogue **10** (yellow) docked to LBD of rVDR (FlexiDock, TRIPOS) and: (a) natural hormone 1α,25-$(OH)_2D_3$ (blue) found in its crystalline complex hVDR-**1**;[38] (b) 2MD (green) found in its crystalline complex rVDR-DRIP-**2**.[28]

receptor family as amino acids interacting with various coactivators. Thus, I267 interacts with transcription factors (TFs and TAFs), whereas L400, L410 and V414, constituting the AF-2 surface, presumably interact with p-160 or DRIP comodulators.[42] Inspection of binary (hVDR-**1**) and ternary (rVDR-DRIP-**1**) complexes showed that DRIP moves L400 closer to the terminal methyl groups of the natural hormone **1** by 0.4 Å. The facts discussed above suggest that lack of hydrophobic interactions to the hydrindane C,D rings in the synthesized compound **10** can be compensated for by the presence of strong contacts of the receptor residues to the ligand methyl groups situated at C-13, C-20 and C-25. This effect could be responsible for only a slight decrease of transcription (10 times) and differentiation (2 times) activities of the analogue **10** in comparison with 1α,25-$(OH)_2D_3$.

3. Conclusions

The preparation of the *des*-C,D vitamin D analogues described in this paper constitutes a continuation of our previous synthetic and biological studies concerning this class of compounds. The A-ring building block was synthesized from the simple precursor, achiral cyclohexanedione derivative **13**. The upper, acyclic C,D-fragment was obtained from the esters **34** which, in turn, were previously prepared by us from the simple, commercially available precursor **33**. Therefore, the described convergent route to the 13,13-dimethyl-*des*-C,D analogues of (20*S*)-1α,25-dihydroxy-2-methylene-19-norvitamin D_3 (2MD) can be considered a total synthesis of these vitamins. Continuation of the structure-activity studies of *des*-C,D vitamin D compounds is of significant importance. Despite the fact that only few of them have been described to date, the available biological data seemed to indicate that such analogues, although lacking the hydrindane moiety, can retain some biological action. According to our expectations, the presence of two methyl substituents at C-13, mimicking $C(12)H_2$ and angular 13β-methyl group, in the molecule of the obtained vitamin D analogue **10** appeared to be beneficial for its biological potency. The results of the tests performed for this compound show that introduction of such *gem*-dimethyl moiety resulted in a significant increase of its VDR binding ability and transcriptional activity. In these both in vitro assays, the dimethylated analogue **10** was found 25 times more active than the compound **7** unsubstituted at C-13, that was obtained in our laboratory previously. The above described docking experiments provide a possible explanation of this phenomena.

The studies on the analogues of 2MD lacking hydrindane fragment, as described herein, prove that such a substantial modification of the vitamin D structure does not abolish the biological activity of the analogues. Moreover, evaluation of the effects of

Table 3
Specific contacts between vitamin D compounds and VDR[a]

Contact	Neighbor	Atom	Ligand						
			1	Atom	**2**	Atom	**7**	Atom	**10**
1α-OH	S233(*237*)	HG	2.3	HG	2.5	HG	4.3	HG	4.2
	S233(*237*)	OG	2.8	OG	2.8	OG	4.8	OG	4.7
1α-OH	R270(*274*)	HH11	1.9	HH11	1.9	HE	2.8	HE	2.7
	R270(*274*)	NH1	2.9	NH1	2.9	NE	3.3	NE	3.2
3β-OH	Y143(*143*)	OH	2.0	OH	2.8	OH	1.6	OH	1.5
	Y143(*143*)	OH	2.8	OH	2.7	OH	2.5	OH	2.4
3β-OH	S274(*278*)	HG	2.6	OG	2.1	HG	3.0	HG	2.8
	S274(*278*)	OG	2.9	OG	3.0	OG	3.3	OG	3.3
25-OH	H301(*305*)	NE2	3.0	NE2	1.8	NE2	3.2	NE2	1.5
	H301(*305*)	NE2	2.8	NE2	2.7	NE2	3.2	NE2	2.4
25-OH	H393(*397*)	NE2	3.4	NE2	3.2	NE2	1.5	NE2	4.2
	H393(*397*)	NE2	2.8	NE2	2.8	NE2	2.4	NE2	4.6
$C(12)H_2$ or	L226(*230*)	HD11	2.4	HD11	2.5	HD12	2.9	HD21	2.4
$[C(13)H_2$ or 13-$CH_3]$[b]	L226(*230*)	CD1	4.3	CD1	4.3	CD2	5.0	CD2	4.1
$C(12)H_2$ or	V296(*300*)	HG23	2.4	HG23	2.0	HG23	4.3	HG23	3.7
$[C(13)H_2$ or 13-$CH_3]$[b]	V296(*300*)	CG2	3.9	CG2	3.8	CG2	5.7	CG2	5.7
$C(18)H_3$	L226(*230*)	HD23	3.0	HD23	4.1	HD12	2.9	HD21	2.4
$[C(13)H_2$ or 13-$CH_3]$[b]	L226(*230*)	CD2	4.7	CD2	5.7	CD2	5.0	CD2	4.1
$C(18)H_3$	L229(*233*)	HD21	2.7	HD21	3.5	HD12	5.0	HD21	4.6
$[C(13)H_2$ or 13-$CH_3]$[b]	L229(*233*)	CD2	4.5	CD2	5.0	CD2	6.8	CD2	5.7
$C(18)H_3$	V230(*234*)	HG23	2.6	HG23	2.4	HD12	2.9	HG23	2.2
$[C(13)H_2$ or 13-$CH_3]$[b]	V230(*234*)	CG2	3.6	CG2	3.7	CG2	4.5	CG2	3.9
$C(18)H_3$	I267(*271*)	HG22	3.3	HG22	2.6	HG22	3.5	HG22	2.4
$[C(13)H_2$ or 13-$CH_3]$[b]	I267(*271*)	CG2	5.0	CG2	4.1	CG2	5.2	CG2	3.7
$C(21)H_3$	I264(*268*)	HD12	3.6	HD12	2.6	HD12	2.3		None[c]
	I264(*268*)	CD1	5.5	CD1	3.9	CD1	3.8		
$C(21)H_3$	M268(*272*)	HE3	3.0	HE3	2.5	HE1	3.8		None[c]
	M268(*272*)	CE	4.8	CE	4.0	CE	5.5		
$C(21)H_3$	V296(*300*)	HG12	2.9	HG12	3.9	HG12	4.3	HG11	2.1
	V296(*300*)	CG1	4.3	CG1	5.4	CG1	5.9	CG1	3.7
$C(21)H_3$	L305(*309*)	HD22	2.7	HD22	2.5	HD21	2.8	HD21	4.2
	L305(*309*)	CD2	3.8	CD2	4.1	CD2	3.8	CD2	5.3
$C(21)H_3$	L309(*313*)	HD13	2.8	HD23	3.0	HD13	3.7	HD13	4.5
	L309(*313*)	CD1	4.3	CD2	4.5	CD1	5.0	CD1	5.7
$C(26/27)H_3$	L223(*227*)	HD12	2.4	HD13	2.7	HD22	4.1	HD22	3.6
	L223(*227*)	C26-CD1	3.6	C26-CD1	3.6	C26-CD2	5.3	C26-CD2	5.0
$C(26/27)H_3$	V230(*234*)	HG12	3.4	HG12	2.5	HG12	2.8	HG21	3.5
	V230(*234*)	C27-CG1	4.9	C27-CG1	4.0	C27-CG1	4.8	C26-CG2	5.0
$C(26/27)H_3$	L400(*404*)	HD22	3.2	HD12	2.4	HD11	3.3	HD13	2.2
	L400(*404*)	C26-CD2	4.5	C26-CD1	4.0	C26-CD1	4.5	C27-CD1	3.9
$C(26/27)H_3$	L410(*414*)	HD23	3.1	HD23	3.4	HD22	3.0	HD22	2.2
	L410(*414*)	C27-CD2	4.7	C26-CD2	4.4	C26-CD2	3.9	C26-CD2	3.6
$C(26/27)H_3$	V414(*418*)	HG12	3.1	HG12	3.3	HG21	2.8	HG22	2.5
	V414(*418*)	C27-CG1	4.0	C27-CG1	4.5	C27-CG2	3.9	C26-CG2	4.3

[a] All residues are numbered according to the rVDR sequence;[47] the atom naming is in agreement with pdb format. The Table lists amino acids surrounding methyl groups (up to 3.5 Å) of 1α,25-$(OH)_2D_3$ (**1**) in its crystalline complex hVDR-**1**;[39] hydrophobic contacts between hVDR and hydrogen atoms attached to C-12 are also shown. The shortest distances between hydrogens involved in hydrophobic interactions are given; in the case of hydrophilic interactions the distances are given between hydrogens and heavy atoms (N, O) being the hydrogen-bond acceptors. The distances between heavy atoms are underlined. Energy of modeled hVDR-**1** is −75 kcal/mol, rVDR-**7** is −56 kcal/mol and rVDR-**10** is −58 kcal/mol.

[b] Two hydrogens at C-13 in the analogue **7** and two methyl groups attached to C-13 in the compound **10** mimic $C(12)H_2$ and $C(18)H_3$ groups in the hormone **1** and 2MD (**2**).

[c] Hydrophobic contacts longer than 6.0 Å are not considered.

the work done in this area already resulted in the vitamin D compounds of potential pharmaceutical application and indicated further changes of the vitamin D molecule which might be of interest. Thus, the results of molecular modeling suggest that introduction of a larger alkyl substituent at C-13 and modification of substitution at C-20 can increase VDR binding of the related *des*-C,D vitamin D analogues. In this paper, we present a total synthesis of *des*-C,D analogues of 2MD, which will also allow the preparation of a series of related analogues to study the structure–activity relationships and further optimize these compounds for therapeutic use. A negligible calcemic potency observed in this class of compounds combined with their relatively high transcriptional and binding activities allow to consider them as substitutes of the vitamin D hormone in its pleiotropic actions on many chronic diseases, especially in the cases when the development of hypercalcemia is an important factor.

4. Experimental

4.1. Chemistry

Ultraviolet (UV) absorption spectra were recorded with a Perkin–Elmer Lambda 3B UV–VIS spectrophotometer in ethanol and hexane. ^1H nuclear magnetic resonance (NMR) spectra were recorded at 200, 400 and 500 MHz with Varian Unity *plus* spectrometer as well as Bruker Instruments DMX-400 and DMX-500 Avance console spectrometers in deuteriochloroform. ^{13}C nuclear magnetic resonance (NMR) spectra were recorded at 50, 100 and

125 MHz with a Varian Unity *plus* and Bruker Instrument DMX-400 spectrometers in deuteriochloroform. Chemical shifts (δ) are reported downfield to internal Me_4Si (δ 0.00). Electron impact (EI) mass spectra were obtained with a Micromass AutoSpec (Beverly, MA) instrument. High-performance liquid chromatography (HPLC) was performed on a Waters Associates liquid chromatograph equipped with a Model 6000A solvent delivery system, a Model U6K Universal injector, and a Model 486 tunable absorbance detector. THF was freshly distilled prior to use from sodium benzophenone ketyl under argon.

The starting mixture (ca. 1:1) of (2*R*,4*S*)- and (2*S*,4*S*)-8-[(*tert*-butyldimethylsilyl)oxy]-2-[2′-[(*tert*-butyldimethylsilyl)oxy]ethyl]-4,8-dimethyl-nonanoic acid methyl esters (**34**) was obtained from commercial *R*-(−)-methyl 3-hydroxy-2-methylpropionate (**33**) as described previously.[27]

4.1.1. (*R*)-7-Hydroxy-1,4-dioxa-spiro[4.5]decan-8-one (14)

To a stirred solution of 1,4-cyclohexanedione monoethylene ketal (**13**; 3.00 g, 19.23 mmol) and L-proline (0.89 g, 7.73 mmol) in $CHCl_3$ (10 mL) at 4 °C, a solution of nitrosobenzene (3.60 g, 33.65 mmol) in $CHCl_3$ (16 mL) was added slowly over 24 h with a syringe pump. The mixture was stirred at room temperature for additional 2 h and the reaction was poured into cold brine. The organic layer was separated and the aqueous layer was extracted with ethyl acetate, dried ($MgSO_4$) and concentrated in vacuum. The oily residue was chromatographed on silica gel (0.5→20% ethyl acetate/hexane gradient) to give the α-hydroxy ketone **14** as an oil (1.45 g, 44%). Purity of the product was checked by HPLC (4.6 mm × 25 cm Chiralcell OD-H column, 1.5 mL/min) using a hexane/2-propanol (99:1) solvent system; it was found to have an enantiomeric excess (ee) higher than 96% (R_V = 8.0 mL; for the *S*-enantiomer R_V = 6.0 mL); $[\alpha]_D^{24}$ +27 (*c* 0.65, $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.85 (1H, t, *J* = 12.4 Hz, 6β-H), 2.05 (2H, m, 10-H_2), 2.50 (2H, br m, 6α- and 9β-H), 2.70 (1H, dt, *J* = 6.8, 13.2 Hz, 9α-H), 3.46 (1H, s, OH), 4.03 (4H, m, O–CH_2CH_2–O), 4.38 (1H, dd, *J* = 12.4, 6.8 Hz, 7α-H); HRMS (ESI) exact mass calcd for $C_8H_{12}O_4Na$ (M^++Na) 195.0633, measured 195.0628.

4.1.2. (*R*)-7-[(*tert*-Butyldimethylsilyl)oxy]-1,4-dioxa-spiro[4.5]decan-8-one (15)

To a solution of alcohol **14** (605 mg, 3.5 mmol) and imidazole (880 mg, 12.8 mmol) in anhydrous DMF (3.1 mL) at 0 °C was added *tert*-butyldimethylsilyl chloride (840 mg, 5.05 mmol). The solution was stirred at 0 °C for 3 h, then at room temperature for 30 min, and it was poured into brine. The mixture was extracted with ethyl acetate, and the combined organic layers were washed with brine, dried ($MgSO_4$) and evaporated. The oily residue was chromatographed on silica gel using a hexane/ethyl acetate (9:1) as an eluent to give the silylated compound **15** as an oil (882 mg, 90%); $[\alpha]_D^{24}$ +22 (*c* 1.1, $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.03 and 0.14 [3H and 3H, each s, $Si(CH_3)_2$], 0.89 (9H, s, Si-*t*-Bu), 1.8–2.0 (2H, br m, 10-H_2), 2.05 (1H, t, *J* ∼ 12.5 Hz, 6β-H), 2.29 (1H, ddd, *J* = 12.9, 6.8, 3.2 Hz, 6α-H), 2.37 (1H, ddd, *J* = 14.3, 4.9, 2.7 Hz, 9β-H), 2.59 (1H, ddt, *J* = 6.9, 1.0, 14.3 Hz, 9α-H), 3.9–4.1 (4H, m, O-CH_2CH_2-O), 4.42 (1H, ddd, *J* = 12.2, 6.8, 1.0 Hz, 7α-H); ^{13}C NMR (50 MHz) δ −5.3 and −4.4 [$Si(CH_3)_2$], 18.6 [Si*C*$(CH_3)_3$], 26.0 [SiC(*C*$H_3)_3$], 34.4 (10-C), 35.7 (9-C), 44.5 (6-C), 64.9 (O–CH_2CH_2–O), 73.7 (7-C), 107.8 (5-C), 208.4 (C=O); HRMS (ESI) exact mass calcd for $C_{14}H_{26}O_4SiNa$ (M^++Na) 309.1498, measured 309.1495.

4.1.3. (*R*)-7-[(*tert*-Butyldiphenylsilyl)oxy]-1,4-dioxa-spiro[4.5]decan-8-one (16)

tert-Butyldiphenylsilyl chloride (3.55 mL, 3.75 g, 13.65 mmol) was added to a solution of the α-hydroxy ketone **14** (1.60 g, 13.65 mmol) and imidazole (2.32 g, 33.9 mmol) in anhydrous DMF (9 mL). The mixture was stirred at room temperature for 18 h, then it was poured into brine and extracted with hexane. The combined organic phases were dried ($MgSO_4$) and concentrated under reduced pressure. Column chromatography on silica gel (1→4% hexane/ethyl acetate gradient) provided the protected compound **16** (3.35 g, 88%) as a white solid that was recrystallized from hexane to afford colorless needles; mp = 77.8–79.9 °C; $[\alpha]_D^{24}$ +17 (*c* 1.04, $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.10 (9H, s, Si-*t*-Bu), 1.8–2.1 (4H, br m, 6- and 10-H_2), 2.35 (2H, m, 9-H_2), 3.62 and 3.82 (1H and 3H, each m, O–CH_2CH_2–O), 4.40 (1H, dd, *J* = 11.8, 7.6 Hz, 7α-H), 7.38 (6H, m, Ar-H), 7.67 (4H, m, Ar-H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 19.4 [Si*C*$(CH_3)_3$], 27.1 [SiC(*C*$H_3)_3$], 34.7 (10-C), 35.9 (9-C), 43.8 (6-C), 64.5 and 64.7 (O–CH_2CH_2–O), 73.8 (7-C), 107.6 (5-C), 127.7, 127.8, 129.8, 129.9, 133.3, 134.1, 136.0 and 136.1 (Ar-C), 207.7 (C=O); HRMS (ESI) exact mass calcd for $C_{24}H_{30}O_4SiNa$ (M^++Na) 433.1811, measured 433.1822.

4.1.4. (*R*)-7-[2′-(Trimethylsilyl)ethoxymethoxy]-1,4-dioxa-spiro[4.5]decan-8-one (17)

To a solution of the hydroxy ketone **14** (330 mg, 1.9 mmol) in anhydrous CH_2Cl_2 (1 mL) at room temperature was added *N*,*N*-diisopropylethylamine (1.7 mL) and 2-(trimethylsilyl)ethoxymethyl chloride (680 μL, 3.8 mmol). The solution was stirred for 3 h and it was poured into 2% HCl. The organic layer was separated and the aqueous layer was extracted with CH_2Cl_2. The combined organic layers were washed with diluted $NaHCO_3$, dried ($MgSO_4$) and evaporated. The oily residue was chromatographed on silica gel using hexane/ethyl acetate (97:3) as an eluent to give the silyl ether **17** as an oil (505 mg, 87%); $[\alpha]_D^{24}$ +67 (*c* 0.5, $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.02 [9H, s, $Si(CH_3)_3$], 0.89 [2H, t, *J* = 8.4 Hz, *C*H_2–$Si(CH_3)_3$], 1.9–2.05 (2H, br m, 10-H_2), 2.00 (1H, t, *J* ∼ 12.5 Hz, 6β-H), 2.28–2.45 (2H, br m, 6α- and 9β-H), 2.62 (1H, ddt, *J* = 7.0, 0.6, 14.2 Hz, 9α-H), 3.64 (2H, m, O–C*H*$_2$–CH_2–Si), 3.9–4.1 (4H, m, O–CH_2CH_2–O), 4.44 (1H, ddd, *J* = 12.2, 5.8, 0.6 Hz, 7α-H), 4.72 and 4.76 (1H and 1H, each d, *J* = 12.2 Hz, O–CH_2–O); ^{13}C NMR (50 MHz) δ −1.3 [$Si(CH_3)_3$], 18.2 [*C*H_2–$Si(CH_3)_3$], 34.7 (10-C), 36.2 (9-C), 42.1 (6-C), 64.9 and 65.0 (O–CH_2CH_2–O), 65.7 (O-*C*H_2-CH_2-Si), 75.9 (7-C), 94.2 (O-CH_2-O), 107.6 (5-C), 208.0 (C=O); HRMS (ESI) exact mass calcd for $C_{14}H_{26}O_5SiNa$ (M^++Na) 325.1447, measured 325.1447.

4.1.5. (*R*)-7-[(*tert*-Butyldimethylsilyl)oxy]-8-methylene-1,4-dioxa-spiro[4.5]decane (18)

To the methyltriphenylphosphonium bromide (1.46 g, 4.1 mmol) in anhydrous THF (16 mL) under argon at 0 °C was slowly added *n*-BuLi (2.5 M in hexane, 3.3 mL; 8.3 mmol) with stirring. After 5 min the next portion of the bromide (1.46 g, 4.1 mmol) was added and the mixture was stirred at 0 °C for 10 min and at room temperature for 20 min. The red-orange mixture was then cooled to −78 °C and ca. 2/3 of the solution was transferred to a solution of the ketone **15** (860 mg, 3 mmol) in anhydrous THF (3 mL). After 4 h of stirring a brine containing 1% HCl (24 mL) was added followed by ethyl acetate (18 mL), benzene (7 mL), diethyl ether (7 mL), $NaHCO_3$ (7 mL) and water (7 mL) and the mixture was vigorously stirred for 20 h. The organic layer was separated and the aqueous layer was extracted with ethyl acetate. The combined organic layers were washed with brine, dried ($MgSO_4$) and evaporated. The oily residue was chromatographed on silica gel using a hexane/ethyl acetate (95:5) as an eluent to give the compound **18** as an oil (628 mg, 73%); $[\alpha]_D^{24}$ +5 (*c* 1.2, $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.07 [6H, s, $Si(CH_3)_2$], 0.92 (9H, s, Si-*t*-Bu), 1.53 (1H, dt, *J* = 5.6, 13.1 Hz, 10β-H), 1.61 (1H, t, *J* ∼ 12 Hz, 6β-H), 1.77 (1H, ddt, *J* = 13.1, 4.6, 2,7 Hz, 10α-H), 2.04 (1H, ddd, *J* = 12.3, 5.4, 2.7 Hz, 6α-H), 2.20 (1H, br dt, *J* ∼ 4.5, ∼13.5 Hz, 9α-H), 2.34 (1H, ddd, *J* = 13.6, 5.6, 2.7 Hz, 9β-H), 3.9–4.0 (4H, m,

O-CH_2CH_2-O), 4.22 (1H, br dd, J = 11.3, 5.4 Hz, 7α-H), 4.79 and 5.04 (1H and 1H, each br s, C=CH_2); ^{13}C NMR (50 MHz) δ −4.81 and −4.76 [Si(CH_3)$_2$], 18.5 [Si*C*(CH_3)$_3$], 26.1 [SiC(*C*H_3)$_3$], 29.8 (9-C), 35.8 (10-C), 45.8 (6-C), 64.5 and 64.7 (O-CH_2CH_2-O), 70.5 (7-C), 106.2 (C=*C*H_2), 109.5 (5-C), 149.5 (*C*=CH_2); HRMS (ESI) exact mass calcd for $C_{15}H_{28}O_3SiNa$ (M^++Na) 307.1703, measured 307.1705.

4.1.6. (*R*)-7-[(*tert*-Butyldiphenylsilyl)oxy]-8-methylene-1,4-dioxa-spiro[4.5]decane (19)

The Wittig reaction of ketone **16** (2.85 g, 6.93 mmol) in anhydrous THF was performed as described above for compound **15**. The reaction mixture was stirred at −78 °C for 4 h and then at room temperature for 1 h. Then it was treated as described above and the product was purified by flash chromatography on silica gel. Elution with hexane/ethyl acetate (97:3) gave pure 8-methylene compound **19** (2.83 g, 97%) as a colorless oil; $[\alpha]_D^{24}$ +3 (*c* 1.16, $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.01 (9H, s, Si-*t*-Bu), 1.44 (1H, t, $J \sim 12$ Hz, 6β-H), 1.54–1.72 (3H, br m, 6α-H and 10-H_2), 2.12 (1H, br dt, J = 4.9, ~13.2 Hz, 9α-H), 2.33 (1H, ddd, J = 13.7, 4.9, 2.7 Hz, 9β-H), 3.36 and 3.70 (1H and 3H, each m, O-CH_2CH_2-O), 4.30 (1H, dd, J = 11.0, 5.0 Hz, 7α-H), 4.88 and 5.31 (1H and 1H, each br s, =CH_2), 7.35 (6H, m, Ar-H), 7.70 (4H, m, Ar-H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 19.5 [Si*C*(CH_3)$_3$], 22.9 [SiC(*C*H_3)$_3$], 30.0 (9-C), 36.3 (10-C), 44.5 (6-C), 64.1 and 64.3 (O-CH_2CH_2-O), 71.2 (7-C), 106.6 (C=*C*H_2), 109.2 (5-C), 127.8, 129.78, 129.84, 134.1, 134.8, 136.0 and 136.2 (Ar-C), 149.3 (*C*=CH_2); HRMS (ESI) exact mass calcd for $C_{25}H_{32}O_3SiNa$ (M^++Na) 431.2018, measured 431.2000.

4.1.7. (*R*)-8-Methylene-7-[2′-(trimethylsilyl)ethoxymethoxy]-1,4-dioxa-spiro[4.5]decane (20)

The Wittig reaction of ketone **17** (0.72 g, 2.38 mmol) in anhydrous THF was performed as described above for compound **15**. The reaction mixture was stirred at −78 °C for 4 h. Then it was treated as described above and the product was purified by flash chromatography on silica gel. Elution with hexane/ethyl acetate (9:1) gave the pure 8-methylene compound **20** (521 mg, 73%) as a colorless oil; $[\alpha]_D^{24}$ +30 (*c* 0.75, $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.02 [9H, s, Si(CH_3)$_3$], 0.93 [2H, t, J = 8.4 Hz, C*H*$_2$-Si(CH_3)$_3$], 1.55–1.8 (3H, br m, 10-H_2 and 6β-H), 2.11 (1H, ddd, J = 12.6, 5.0, 2.0 Hz, 6α-H), 2.20 (1H, br dt, $J \sim 5$, ~13 Hz, 9α-H), 2.36 (1H, dt, J = 13.6, 4.9 Hz, 9β-H), 3.94 (2H, m, O-C*H*$_2$-CH_2-Si), 3.95 (4H, m, O-CH_2CH_2-O), 4.22 (1H, br dd, J = 10.0, 5.0 Hz, 7α-H), 4.69 and 4.73 (1H and 1H, each d, J = 13.4, Hz, O-CH_2-O), 4.83 and 4.97 (1H and 1H, each br s, C=CH_2); ^{13}C NMR (50 MHz) δ −1.2 [Si(CH_3)$_3$], 18.3 [*C*H$_2$-Si(CH_3)$_3$], 29.7 (9-C), 36.0 (10-C), 42.8 (6-C), 64.8 and 64.5 (O-CH_2CH_2-O), 65.3 (O-*C*H_2-CH_2-Si), 74.5 (7-C), 93.4 (O-CH_2-O), 109.1 (5-C), 107.7 (C=CH_2), 146.8 (*C*=CH_2); HRMS (ESI) exact mass calcd for $C_{15}H_{28}O_4SiNa$ (M^++Na) 323.1655, measured 323.1655.

4.1.8. (7*R*,9*R*)- and (7*S*,9*R*)-9-[(*tert*-Butyldimethylsilyl)oxy]-8-methylene-1,4-dioxa-spiro[4.5]decan-7-ols (21 and 22)

To a solution of allylic ether **18** (600 mg, 2.11 mmol) in freshly distilled dioxane (17 mL) was added pyridine N-oxide (798 mg, 8.38 mmol) and SeO_2 (282 mg, 2.52 mmol). The mixture was heated at 90 °C for 2 h with stirring. Then it was poured into water and extracted with ethyl acetate. The organic layer was washed with saturated $NaHCO_3$, dried ($MgSO_4$) and evaporated. The residue was subjected to column chromatography on silica gel. Elution with hexane/ethyl acetate (85:15) gave an oily mixture of isomeric alcohols **21** and **22** (325 mg, 51%; ratio of 2:1). Separation of both isomers was achieved by HPLC (9.4 mm × 25 cm, Zorbax RX-Sil column, 4 mL/min) using a hexane/2-propanol (9:1) solvent system. The main product **21** (214 mg) was collected at R_V 25.0 mL and the 7*S*-isomer **22** (109 mg) at R_V 25.5 mL.

Compound ***21***: $[\alpha]_D^{24}$ −36 (*c* 1.55, $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.10 [6H, s, Si(CH_3)$_2$], 0.93 (9H, s, Si-*t*-Bu), 1.67 (1H, t, $J \sim 12$ Hz, 10β-H), 1.84 (1H, dd, J = 14.1, 3.9 Hz, 6β-H), 2.05 (1H, dt, J = 14.1, 2.8 Hz, 6α-H), 2.11 (1H, ddd, J = 12.2, 5.1, 2.8 Hz, 10α-H), 4.01 (4H, m, O-CH_2CH_2-O), 4.44 (1H, br t, $J \sim 3.2$ Hz, 7β-H), 4.61 (1H, ddt, J = 11.5, 5.1, 1.8 Hz, 9α-H), 5.01 and 5.16 (1H and 1H, each t, $J \sim 2$ Hz, C=CH_2); ^{13}C NMR (50 MHz) δ −4.78 and −4.81 [Si(CH_3)$_2$], 18.5 [Si*C*(CH_3)$_3$], 26.0 [SiC(*C*H_3)$_3$], 40.9 (10-C), 45.8 (6-C), 64.3 and 65.1 (O-CH_2CH_2-O), 67.2 (7-C), 72.2 (9-C), 109.1 (C=*C*H_2), 109.8 (5-C), 150.6 (*C*=CH_2); HRMS (ESI) exact mass calcd for $C_{15}H_{28}O_4SiNa$ (M^++Na) 323.1655, measured 323.1654.

Compound ***22*** : $[\alpha]_D^{24}$ −2 (*c* 2.15, $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.08 (6H, s, Si(CH_3)$_2$], 0.92 (9H, s, Si-*t*-Bu), 1.59 (1H, t, $J \sim 12$ Hz, 6β-H), 1.67 (1H, t, $J \sim 12$ Hz, 10β-H), 2.00 (1H, ddd, J = 12.4, 5.4, 2.4 Hz, 10α-H), 2.15 (1H, ddd, J = 12.1, 5.0, 2.6 Hz, 6α-H), 3.98 (4H, m, O-CH_2CH_2-O), 4.2–4.3 (2H, m, 7α- and 9α-H), 5.11 and 5.21 (1H and 1H, each narr m, C=CH_2); ^{13}C NMR (50 MHz) δ −4.88 and −4.81 [Si(CH_3)$_2$], 18.5 [Si*C*(CH_3)$_3$], 26.0 [SiC(*C*H_3)$_3$], 44.7 (10-C), 45.4 (6-C), 64.5 and 64.8 (O-CH_2CH_2-O), 68.4 (7-C), 68.9 (9-C), 103.5 (C=*C*H_2), 107.3 (5-C), 152.8 (*C*=CH_2); HRMS (ESI) exact mass calcd for $C_{15}H_{28}O_4SiNa$ (M^++Na) 323.1655, measured 323.1655.

4.1.9. (7*R*,9*R*)- and (7*S*,9*R*)-9-[(*tert*-Butyldiphenylsilyl)oxy]-8-methylene-1,4-dioxa-spiro[4.5]decan-7-ols (23 and 24)

The hydroxylation of allylic ether **19** (167 mg, 0.41 mmol) was performed as described above for compound **18**. The products were purified and separated by column chromatography on silica gel. Elution with hexane/ethyl acetate (8:2) gave an oily mixture of alcohols **23** and **24** (93 mg, 53%; ratio of 2:1). Separation of the isomers was achieved by rechromatography using CH_2Cl_2 as an eluent. Purity of compounds was confirmed by HPLC (9.4 mm × 25 cm, Zorbax RX-Sil column, 4 mL/min) using a hexane/2-propanol (93:7) solvent system. The main product **23** was collected at R_V 27 mL and the 7*S*-isomer **24** at R_V 33 mL.

Compound ***23*** : $[\alpha]_D^{24}$ −23 (*c* 1.1, $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.01 (9H, s, Si-*t*-Bu), 1.48 (1H, br t, $J \sim 12$ Hz, 10β-H), 1.70 (1H, ddd, J = 12.4, 5.1, 1.7 Hz, 10α-H), 1.89 (2H, m, 6-H_2), 3.42 and 3.72 (1H and 3H, each m, O-CH_2CH_2-O), 4.44 (1H, t, J = 3.3 Hz, 7β-H), 4.67 (1H, dm, J = 11.2 Hz, 9α-H), 5.09 (1H, t, J = 1.8 Hz, one of C=CH_2), 5.40 (1H, t, J = 2.1 Hz, one of C=CH_2), 7.39 (6H, m, Ar-H), 7.69 (4H, m, Ar-H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 19.5 [Si*C*(CH_3)$_3$], 27.2 [SiC(*C*H_3)$_3$], 41.6 (6-C), 44.53 (10-C), 64.0 and 64.6 (O-CH_2CH_2-O), 68.2 (7-C), 72.1 (9-C), 109.2 (C=*C*H_2), 109.4 (5-C), 127.8, 129.9, 135.9 and 136.1 (Ar-C), 150.6 (*C*=CH_2); HRMS (ESI) exact mass calcd for $C_{25}H_{32}O_4SiNa$ (M^++Na) 447.1968, measured 447.1977.

Compound ***24***: $[\alpha]_D^{24}$ +9 (*c* 1.0, $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.07 (9H, s, Si-*t*-Bu), 1.48 (1H, dd, J = 12.4, 10.7 Hz, 10β-H), 1.61 (2H, br m, 6β- and 10α-H), 2.03 (1H, ddd, J = 12.0, 5.1, 2.2 Hz, 6α-H), 3.39 and 3.73 (1H and 3H, each m, O-CH_2CH_2-O), 4.17 (1H, dd, J = 11.0, 5.1 Hz, 7α-H), 4.29 (1H, dd, J = 10.7, 5.2 Hz, 9α-H), 5.19 and 5.45 (1H and 1H, each br d, J = 1.7 Hz, C=CH_2), 7.38 (6H, m, Ar-H), 7.67 (4H, m, Ar-H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 19.5 [Si*C*(CH_3)$_3$], 27.2 [SiC(*C*H_3)$_3$], 44.0 (6-C), 45.1 (10-C), 64.27 and 64.33 (O-CH_2CH_2-O), 68.5 (7-C), 68.7 (9-C), 104.0 (C=CH_2), 107.0 (5-C), 127.8, 129.7, 135.9 and 136.2 (Ar-C), 152.5 (*C*=CH_2); HRMS (ESI) exact mass calcd for $C_{25}H_{32}O_4SiNa$ (M^++Na) 447.1968, measured 447.1960.

4.1.10. (7*R*,9*R*)- and (7*S*,9*R*)-8-Methylene-7-[2′-(trimethylsilyl) ethoxymethoxy]-1,4-dioxa-spiro[4.5]decan-7-ols (25 and 26)

The hydroxylation of allylic ether **20** (521 mg, 1.7 mmol) in dioxane was performed as described above for compound **18**. The crude products were subjected to column chromatography on silica gel. Elution with hexane/ethyl acetate (95:5→80:20) gave pure

alcohol **25** (96 mg, 18%) and its isomer **26** (50 mg, 9%) as colorless oils.

Compound ***25*** : $[\alpha]_D^{24}$ −12 (*c* 1.20, $CHCl_3$); ^1H NMR (200 MHz, $CDCl_3$) δ 0.02 [9H, s, $Si(CH_3)_3$], 0.94 [2H, t, *J* = 8.6 Hz, CH_2-$Si(CH_3)_3$], 1.75 (1H, dd, *J* = 12.8, 9.8 Hz, 10β-H), 1.96 (2H, m, 6-H_2), 2.19 (1H, br dd, *J* = 12.8, 5.0 Hz, 10α-H), 3.64 (2H, m, O-CH_2-CH_2-Si), 4.02 (4H, br s, O-CH_2CH_2-O), 4.45 (1H, m, 7β-H), 4.54 (1H, br dd, *J* = 9.8, 5.0 Hz, 9α-H), 4.73 and 4.78 (1H and 1H, each d, *J* = 10.6 Hz, O-CH_2-O), 5.10 (1H, br s, one of C=CH_2), 5.13 (1H, t, *J* = 1.8 Hz, one of C=CH_2); ^{13}C NMR (50 MHz) δ −1.2 [$Si(CH_3)_3$], 18.3 [CH_2-$Si(CH_3)_3$], 42.0 (10-C), 42.8 (6-C), 64.7 and 64.9 (O-CH_2CH_2-O), 65.5 (O-CH_2-CH_2-Si), 71.2 (9-C), 72.0 (7-C), 93.5 (O-CH_2-O), 109.5 (C=CH_2), 109.5 (5-C), 148.2 (*C*=CH_2); HRMS (ESI) exact mass calcd for $C_{15}H_{28}O_5SiNa$ (M^++Na) 339.1604, measured 339.1601.

Compound ***26****:* ^1H NMR (500 MHz, $CDCl_3$) δ 0.02 [9H, s, $Si(CH_3)_3$], 0.90 [2H, t, *J* = 8.6 Hz, CH_2-$Si(CH_3)_3$], 1.78 (2H, br m, 6β- and 10β-H), 2.10 (2H, br m, 6α- and 10α-H), 3.71 (2H, m, O-CH_2-CH_2-Si), 3.99 (4H, m, O-CH_2CH_2-O), 4.28 (2H, m, 7α- and 9α-H), 4.75 (2H, s, O-CH_2-O), 5.17 and 5.19 (1H and 1H, each d, *J* = 1.5 Hz, C=CH_2); HRMS (ESI) exact mass calcd for $C_{15}H_{28}O_5SiNa$ (M^++Na) 339.1604, measured 339.1610.

4.1.11. (7*R*,9*R*)-7,9-Bis-[(*tert*-Butyldimethylsilyl)oxy]-8-methylene-1,4-dioxa-spiro[4.5]decane (27)

Protection of the hydroxy group in compound **21** (60 mg, 0.2 mmol) was performed as described above for conversion of alcohol **14** into the ether **15**. The crude product was subjected to column chromatography on silica gel. Elution with hexane/ethyl acetate (98:2) gave the protected compound **27** (60 mg, 72%); $[\alpha]_D^{24}$ −12 (*c* 1.24, $CHCl_3$); ^1H NMR (200 MHz, $CDCl_3$) δ 0.04 and 0.06 [each s, each 6H, 2 × $Si(CH_3)_2$], 0.89 (18H, s, 2 × Si-*t*-Bu), 1.83 (4H, m, *J* ∼ 12 Hz, 6- and 10-H_2), 3.92 (4H, m, O-CH_2CH_2-O), 4.55 (2H, dd, *J* = 6.6, 4.6 Hz, 7α- and 9β-H), 4.97 (2H, s, C=CH_2); ^{13}C NMR (50 MHz) δ −4.9 and −4.7 [$Si(CH_3)_2$], 18.3 [Si*C*$(CH_3)_3$], 25.9 [SiC(CH_3)$_3$], 44.4 (6- and 10-C), 64.2 (O-CH_2CH_2-O), 69.9 (7- and 9-C), 107.2 (C=*C*H_2), 109.2 (5-C), 152.0 (*C*=CH_2); HRMS (ESI) exact mass calcd for $C_{21}H_{42}O_4Si_2Na$ (M^++Na) 437.2519, measured 437.2500.

4.1.12. (7*R*,9*R*)-7,9-Bis-[(*tert*-butyldiphenylsilyl)oxy]-8-methylene-1,4-dioxa-spiro[4.5]decane (28)

Protection of the hydroxy group in compound **23** (32 mg, 0.072 mmol) was performed as described above for conversion of alcohol **14** into the ether **16**. The crude product was subjected to column chromatography on silica gel. Elution with hexane/ethyl acetate (98:2) gave the protected compound **28** as an oil (43 mg, 88%); $[\alpha]_D^{24}$ −0.07 (*c* 1.14, $CHCl_3$); ^1H NMR (200 MHz, $CDCl_3$) δ 1.035 (18H, s, 2 × Si-*t*-Bu), 1.56 (2H, dd, *J* = 13.3, 7.1 Hz, 6α- and 10β-H), 1.67 (2H, dd, *JJ* = 13.3, 4.4 Hz, 6β- and 10α-H), 3.53 and 3.65 (2H and 2H, each m, O-CH_2CH_2-O), 4.69 (2H, dd, *J* = 7.1, 4.4 Hz, 7β- and 9α-H), 5.02 (2H, s, C=CH_2), 7.33 (12H, m, Ar-H), 7.76 (8H, m, Ar-H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 19.51 [Si*C*$(CH_3)_3$], 27.13 [SiC(CH_3)$_3$], 44.04 (6-C, 10-C), 64.04 (O-CH_2CH_2-O), 70.74 (7-C, 9-C), 107.98 (5-C), 108.96 (C=*C*H_2), 129.69, 127.63, 134.08, 134.78 and 136.14 (Ar-C), 151,00 (*C*=CH_2); HRMS (ESI) exact mass calcd for $C_{41}H_{50}O_4Si_2Na$ (M^++Na) 685.3145, measured 685.3126.

4.1.13. (7*R*,9*R*)-7,9-Bis-[2′-(trimethylsilyl)ethoxymethoxy]-8-methylene-1,4-dioxa-spiro[4.5]decane (29)

Protection of the hydroxy group in compound **25** (96 mg, 0.3 mmol) was performed as described above for conversion of alcohol **14** into the ether **17**. The crude product was purified by column chromatography on silica gel. Elution with hexane/ethyl acetate (95:5) gave the compound **32** as an oil (104 mg, 75%); $[\alpha]_D^{24}$ +22 (*c* 0.20, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 0.02 (18H, s, 6 × Si-CH_3), 0.93 [4H, m, 2 × CH_2-$Si(CH_3)_3$], 1.85 (2H, ddd, *J* = 13.5, 7.0, 1.5 Hz, 6α- and 10β-H), 2.06 (2H, ddd, *J* = 13.5, 4.5, 1.5 Hz, 6β- and 10α-H), 3.56 and 3.72 (2H and 2H, each m, 2 × O-CH_2-CH_2-Si), 3.99 (4H, m, O-CH_2CH_2-O), 4.43 (2H, dd, *J* = 7.0, 4.5 Hz, 7β- and 9α-H), 4.70 and 4.71 (2H and 2H, each d, *J* = 17.0 Hz, 2 × O-CH_2-O), 5.18 (2H, s, C=CH_2); ^{13}C NMR (125 MHz) δ −1.2 [$Si(CH_3)_3$], 18.3 [CH_2-$Si(CH_3)_3$], 42.0 (CH_2, 6- and 10-C), 64.7 (O-CH_2CH_2-O), 65.3 (O-CH_2-CH_2-Si), 72.9 (7- and 9-C), 92.7 (O-CH_2-O), 109.0 (5-C), 111.6 (C=*C*H_2), 145.2 (*C*=CH_2); HRMS (ESI) exact mass calcd for $C_{21}H_{42}O_6Si_2Na$ (M^++Na) 469.2418, measured 469.2413.

4.1.14. (3*R*,5*R*)-3,5-Bis-[(*tert*-butyldimethylsilyl)oxy]-4-methylene-cyclohexanone (30)

To a solution of the ketal **27** (60 mg, 0.14 mmol) in anhydrous CH_2Cl_2 (2.3 mL) was added $FeCl_3 \times 6H_2O$ (143 mg, 0.37 mmol). After 20 min of stirring at room temperature the mixture was poured into saturated $NaHCO_3$. The organic layer was separated and the aqueous layer was extracted with CH_2Cl_2. The combined organic layers were washed with water, dried (Na_2SO_4) and evaporated. The oily residue was chromatographed on silica gel using hexane/ethyl acetate (99:1) as an eluent to give the ketone **30** as an oil (9 mg, 17%) which was identical in all respects with an authentic sample;[9] $[\alpha]_D^{24}$ −58 (*c* 1.0, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 0.05 and 0.07 [6H and 6H, each s, 2 × $Si(CH_3)_2$], 0.88 (18H, s, 2 × Si-*t*-Bu), 2.45 (2H, ddd, *J* = 14.5, 7.0, 1.5 Hz, 2β- and 6α-H), 2.64 (2H, ddd, *J* = 14.5, 4.5, 1.5 Hz, 2α- and 6β-H), 4.69 (2H, dd, *J*=7.0, 4.5 Hz, 3α- and 5β-H), 5.16 (2H, s, C=CH_2); ^{13}C NMR (125 MHz) δ −4.8 and −4.7 [$Si(CH_3)_2$], 18.3 [Si*C*$(CH_3)_3$], 25.9 [SiC(CH_3)$_3$], 51.7 (2- and 6-C), 70.3 (3- and 5-C), 108.8 (C=CH_2,), 150.3 (*C*=CH_2), 207.3 (C=O); HRMS (ESI) exact mass calcd for $C_{19}H_{38}O_3Si_2Na$ (M^++Na) 393.2257, measured 393.2254.

4.1.15. (3*R*,5*R*)-3,5-Bis-[(*tert*-butyldiphenylsilyl)oxy]-4-methylene-cyclohexanone (31)

Deprotection of the keto group in the ketal **28** (56 mg, 0.084 mmol) was performed as described above for conversion of ketal **27** into the cyclohexanone **30**. The crude product was purified by column chromatography on silica gel using hexane/ethyl acetate (99:1) as an eluent to give the ketone **31** as an oil (50 mg, 96%); $[\alpha]_D^{24}$ +8 (*c* 1.3, $CHCl_3$); ^1H NMR (200 MHz, $CDCl_3$) δ 0.989 (18H, s, 2 × Si-*t*-Bu), 2.36 (2H, dd, *J* = 14.6, 6.6 Hz, 2β- and 6α-H), 2.46 (2H, dd, *J* = 14.6, 4.9 Hz, 2α- and 6β-H), 4.78 (2H, dd, *J* = 6.6, 4.9 Hz, 3α- and 5β-H), 5.18 (2H, s, C=CH_2), 7.35 (12H, m, Ar-H), 7.61 (8H, m, Ar-H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 19.40 [Si*C*$(CH_3)_3$], 27.06 [SiC(CH_3)$_3$], 51.27 (2-C, 6-C), 71.55 (3-C, 5-C), 109.59 (C=*C*H_2), 127.90, 130.11, 133.64 and 135.95 (Ar-C), 149.17 (*C*=CH_2), 206.82 (*C*=O); HRMS (ESI) exact mass calcd for $C_{39}H_{46}O_3Si_2Na$ (M^++Na) 641.2883, measured 641.2899.

4.1.16. (3*R*,5*R*)-3,5-Bis-[2′-(trimethylsilyl)ethoxymethoxy]-4-methylene-cyclohexanone (32)

Deprotection of the keto group in the ketal **29** (104 mg, 0.23 mmol) was performed as described above for conversion of ketal **27** into the cyclohexanone **30**. The crude product was purified by column chromatography on silica gel using hexane/ethyl acetate (95:5) as an eluent to give the ketone **32** as an oil (65 mg, 69%); $[\alpha]_D^{24}$ +51 (*c* 1.0, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 0.016 (18H, s, 6 × Si-CH_3), 0.92 [4H, m, 2 × CH_2-$Si(CH_3)_3$], 2.55 (2H, ddd, *J* = 14.5, 7.0, 1.5 Hz, 2β- and 6α-H), 2.75 (2H, ddd, *J* = 14.5, 5.0, 1.5 Hz, 2α- and 6β-H), 3.53 and 3.69 (2H and 2H, each m, 2 × O-CH_2-CH_2-Si), 4.56 (2H, dd, *J* = 7.0, 5.0 Hz, 3α- and 5β-H), 4.66 (4H, s, 2 × O-CH_2-O), 5.35 (2H, s, C=CH_2); ^{13}C NMR (125 MHz) δ −1.4 [$Si(CH_3)_3$], 18.1 [CH_2-$Si(CH_3)_3$], 48.3 (CH_2,

2- and 6-C), 65.5 (2 × O-CH_2-CH_2), 72.7 (3- and 5-C), 92.3 and 92.5 (2 × O-CH_2-O), 113.0 (=CH_2), 143.9 (=CH_2), 206.3 (C=O); HRMS (ESI) exact mass calcd for $C_{19}H_{38}O_5Si_2Na$ (M^++Na) 425.2156, measured 425.2157.

4.1.17. (2*R*,4*S*)- and (2*S*,4*S*)-8-[(*tert*-Butyldimethylsilyl)oxy]-2-[2′-[(*tert*-butyldimethylsilyl)oxy]ethyl]-2,4,8-trimethyl-nonanoic acid methyl esters (35)

To a solution of diisopropylamine (85 μL, 0.56 mmol) in anhydrous THF (0.5 mL) was added *n*-BuLi (1.6 M in cyclohexane, 350 μL, 0.56 mmol) under argon at −20 °C. The mixture was stirred for 20 min at −20 °C and then cooled to −78 °C. To this LDA solution was added a solution of the esters **34** (70 mg, 0.14 mmol) in anhydrous THF (240 μL) and the mixture was stirred for 40 min. Then a solution of MeI (54 μL, 0.84 mmol) in freshly distilled HMPA (0.2 mL) was added. Stirring was continued at −78 °C for 3 h, then the mixture was allowed to warm up to the room temperature and stirred for 17 h. The mixture was poured into water and extracted with ethyl acetate. The combined organic layers were washed with water, dried ($MgSO_4$) and evaporated. The oily residue was purified on a silica Sep-Pak (2 g). Elution with hexane/AcOEt (9:1) gave the esters **35** as an oil (62 mg, 86%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.03 and 0.05 [6H and 6H, each s, 2 × Si(CH_3)$_2$], 0.77 (3H, d, J = 6.0 Hz, CH_3CH), 0.85 and 0.88 (9H and 9H, each s, 2 × Si-*t*-Bu), 1.16 (3H, s, CH_3CCOOCH$_3$), 1.17 [6H, s, OC(CH_3)$_2$], 3.58 (2H, m, CH_2OTBS), 3.64 (3H, s, COOCH_3); HRMS (ESI) exact mass calcd for $C_{27}H_{59}O_4Si_2$ (M+H$^+$) 503.3952, measured 503.3958.

4.1.18. (2*R*,4*S*)- and (2*S*,4*S*)-8-[(*tert*-Butyldimethylsilyl)oxy]-2-[2′-[(*tert*-butyldimethylsilyl)oxy]ethyl]-2,4,8-trimethyl-nonan-1-ols (36)

To a stirred solution of the esters **35** (32 mg, 0.06 mmol) in toluene/CH_2Cl_2 (2:1, 1 mL) was added at −78 °C diisobutylaluminium hydride (1.5 M in toluene, 0.27 mL, 0.39 mmol). Stirring was continued at −78 °C for 3 h, and the reaction mixture was quenched by addition of 2 M potassium sodium tartrate and diluted HCl. The mixture was extracted with ethyl acetate, the organic extracts were washed with water, dried ($MgSO_4$) and evaporated. The oily residue was purified on a silica Sep-Pak (2 g). Elution with hexane/AcOEt (95:5) gave the alcohols **36** as an oil (31 mg, 100%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.06 and 0.09 [6H and 6H, each s, 2 × Si(CH_3)$_2$], 0.93 (3H, d, J = 6.8 Hz, CH_3CH), 0.88 (3H, s, CH_3CCH$_2$O), 0.85 and 0.90 (9H and 9H, each s, 2 × Si-*t*-Bu), 1.17 [6H, s, OC(CH_3)$_2$], 3.30 and 3.62 (1H and 1H, each m, CH_2OH), 3.58 (2H, m, CH_2OTBS); HRMS (ESI) exact mass calcd for $C_{26}H_{58}O_3NaSi_2$ (M^++Na) 497.3822, measured 497.3829.

4.1.19. Imidazole-1-carbothioic acid *O*-[(2*R*,4*S*)- and (2*S*,4*S*)-8-[(*tert*-butyldimethylsilyl)oxy]-2-[2′-[(*tert*-butyldimethylsilyl)oxy]ethyl]-2,4,8-trimethyl-nonyl] esters (37)

To a solution of the alcohols **36** (60 mg, 0.13 mmol) in anhydrous THF (5 mL) was added 1,1′-thiocarbonyldiimidazole (150 mg, 0.84 mmol). The mixture was stirred at 75 °C for 6 h and at room temperature for 16 h. Solvent was evaporated and the oily residue was purified on a silica Sep-Pak (2 g). Elution with hexane/AcOEt (95:5) gave the thioesters **37** as an oil (70 mg, 94%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.05 and 0.06 [6H and 6H, each s, 2 × Si(CH_3)$_2$], 0.93 (3H, d, J = 6.6 Hz, CH_3CH), 0.84 and 0.87 (9H and 9H, each s, 2 × Si-*t*-Bu), 1.07 (3H, s, CH_3CCH$_2$O), 1.16 [6H, s, OC(CH_3)$_2$], 1.28 (2H, t, J = 7.2 Hz), 3.72 (2H, t, J = 6.6 Hz, CH_2OTBS), 4.44 (2H, m, CH_2OCS), 7.05, 7.63 and 8.35 (each 1H, each s, Im-H); ^{13}C NMR (125 MHz) δ −5.4 and −2.1 [Si(CH_3)$_2$], 18.2 [SiC(CH_3)$_3$], 21.7 ($CH_2CH_2CH_2$), 22.4 (CH_3CH), 22.8 (CH_3CCH$_2$O), 25.8 and 25.9 [SiC(CH_3)$_3$], 28.3 (CH_3CH), 29.7 and 29.8 [OC(CH_3)$_2$], 37.0 (CH_3CCH$_2$O), 40.1 (CH_2), 40.3 (CH_2), 45.2 (CH_2), 45.3 (CH_2), 59.3 (CH_2OTBS), 73.4 [OC(CH_3)$_2$], 117.6, 130.8 and 184.1 (Im); HRMS (ESI) exact mass calcd for $C_{30}H_{60}O_3N_2SNaSi_2$ (M^++Na) 607.3761, measured 607.3761.

4.1.20. (*S*)-1,9-Bis-[(*tert*-butyldimethylsilyl)oxy]-3,3,5,9-tetramethyl-decane (38)

To a refluxing solution of thioesters **37** (60 mg, 0.1 mmol) and AIBN (2.5 mg, 0.015 mmol) in anhydrous toluene (3 mL) was dropwise added Bu_3SnH (54 μL, 0.2 mmol) during 1 h. The mixture was stirred at 120 °C for 2 h and for 17 h at room temperature. Solvents were evaporated and the oily residue was applied on a silica Sep-Pak (2 g). Elution with hexane/AcOEt (99.8:0.2) gave the diether **38** as an oil (22.7 mg, 48%); $[\alpha]_D^{24}$ −0.8 (*c* 1.1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.05 and 0.06 [6H and 6H, each s, 2 × Si(CH_3)$_2$], 0.85 and 0.89 (9H and 9H, each s, 2 × Si-*t*-Bu), 0.88 and 0.90 [3H and 3H, each s, C(CH_3)$_2$], 0.91 (3H, d, J = 6.6 Hz, CH_3CH), 1.17 [6H, s, OC(CH_3)$_2$], 1.48 (2H, t, J = 7.7 Hz), 3.66 (2H, t, J = 7.7 Hz, CH_2OTBS); ^{13}C NMR (100 MHz) δ −5.2 and −2.1 [Si(CH_3)$_2$], 18.1 and 18.3 [SiC(CH_3)$_3$], 21.8 ($CH_2CH_2CH_2$), 22.7 (CH_3CH), 25.8 and 26.0 [SiC(CH_3)$_3$], 27.73 and 27.77 [C(CH_3)$_2$], 28.8 (CH_3CH), 29.8 and 29.9 [OC(CH_3)$_2$], 32.9 [C(CH_3)$_2$], 40.3 (CH_2), 45.2 (CH_2), 45.3 (CH_2), 50.1 (CH_2), 60.2 (CH_2OTBS), 73.6 [OC(CH_3)$_2$]; HRMS (ESI) exact mass calcd for $C_{26}H_{58}O_2NaSi_2$ (M^++Na) 481.3873, measured 481.3856.

4.1.21. (*S*)-9-[(*tert*-Butyldimethylsilyl)oxy]-3,3,5,9-tetramethyl-decan-1-ol (39)

To a solution of the diether **38** (30 mg, 65 μmol) in anhydrous THF (10 mL) was added tetrabutylammonium fluoride (1.0 M in THF, 130 μL, 130 μmol). The mixture was stirred under argon at room temperature for 18 h, poured into brine and extracted with ethyl acetate. The organic extracts were washed with brine, dried ($MgSO_4$) and evaporated. The oily residue was purified on a silica Sep-Pak (2 g). Elution with hexane/AcOEt (9:1) gave the alcohol **39** as an oil (23 mg, 100%); $[\alpha]_D^{24}$ −0.6 (*c* 1.15, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.05 [6H, s, Si(CH_3)$_2$], 0.85 (9H, s, Si-*t*-Bu), 0.90 [6H, s, C(CH_3)$_2$], 0.91 (3H, d, J = 6.6 Hz, CH_3CH), 1.17 [6H, s, OC(CH_3)$_2$], 3.70 (2H, t, J = 7.7 Hz, CH_2OH); ^{13}C NMR (100 MHz) δ −2.1 [Si(CH_3)$_2$], 18.1 [SiC(CH_3)$_3$], 21.0 (CH_3CH), 21.8 ($CH_2CH_2CH_2$), 25.8 [SiC(CH_3)$_3$], 27.7 and 27.8 [C(CH_3)$_2$], 28.8 (CH_3CH), 29.8 [OC(CH_3)$_2$], 33.0 [C(CH_3)$_2$], 40.2 (CH_2), 45.28 (CH_2), 45.33 (CH_2), 50.0 (CH_2), 59.9 (CH_2OH), 73.5 [OC(CH_3)$_2$]; HRMS (ES) exact mass calcd for $C_{20}H_{44}O_2NaSi$ (M^++Na) 367.3008, measured 367.3000.

4.1.22. (*S*)-9-[(*tert*-Butyldimethylsilyl)oxy]-3,3,5,9-tetramethyl-decanal (12)

To a solution of NMO (19 mg, 0.16 mmol) in CH_2Cl_2 (0.7 mL) were added 4 Å molecular sieves (100 mg) and the mixture was stirred at room temperature for 15 min. Then TPAP (1.4 mg, 3.72 μmol) was added followed by a solution of the alcohol **39** (23 mg, 67 μmol) in CH_2Cl_2 (0.2 mL). The resulted dark mixture was stirred for 2 h, filtered through a silica Sep-Pak (2 g) and evaporated. The oily residue was dissolved in hexane, applied on a silica Sep-Pak cartridge (2 g) and washed with hexane/AcOEt (99:1) to give the aldehyde **12** as an oil (16 mg, 70%); $[\alpha]_D^{24}$ −0.6 (*c* 0.80, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.06 [6H, s, Si(CH_3)$_2$], 0.85 (9H, s, Si-*t*-Bu), 0.93 (3H, d, J = 6.6 Hz, CH_3CH), 1.06 [6H, s, C(CH_3)$_2$], 1.17 [6H, s, OC(CH_3)$_2$], 2.26 (2H, m, CH_2CHO), 9.85 (1H, t, J = 3.1 Hz, CHO); ^{13}C NMR (100 MHz) δ −2.1 [Si(CH_3)$_2$], 18.1 [SiC(CH_3)$_3$], 21.7 (CH_3CH), 22.5 ($CH_2CH_2CH_2$), 25.8 [SiC(CH_3)$_3$], 27.6 and 29.8 [OC(CH_3)$_2$], 28.9 (CH_3CH), 34.2 [C(CH_3)$_2$], 40.0 (CH_2), 45.22 (CH_2), 50.2 (CH_2), 55.6 (CH_2OH), 73.4 [OC(CH_3)$_2$], 203.9 (CHO); HRMS (ES) exact mass calcd for $C_{20}H_{42}O_2NaSi$ (M^++Na) 365.2852, measured 365.2840.

4.1.23. (1*R*,3*R*)-1,3-Bis-[(*tert*-butyldimethylsilyl)oxy]-5-[(*E*)-(*S*)-11′-[(*tert*-butyldimethylsilyl)oxy]-5′,5′,7′,11′-tetramethyl-dodec-2′-enylidene]-2-methylene-cyclohexane (40) and (1*R*,3*R*)-1,3-bis-[(*tert*-butyldimethylsilyl)oxy]-5-[(*Z*)-(*S*)-11′-[(*tert*-butyldimethylsilyl)oxy]-5′,5′,7′,11′-tetramethyl-dodec-2′-enylidene]-2-methylene-cyclohexane (41)

To a solution of phosphine oxide **11** (83 mg, 141 μmol) in anhydrous THF (0.8 mL) at −78 °C was slowly added phenyllithium (1.8 M in cyclohexane, 75 μL, 141 μmol) under argon with stirring. The solution turned deep orange. The mixture was stirred at −78 °C for 20 min and a precooled (−78 °C) solution of the aldehyde **12** (16 mg, 47 μmol) in anhydrous THF (300 μL) was slowly added. The mixture was stirred at −78 °C under argon for 3 h and at 6 °C for 16 h. Ethyl acetate and water were added, and the organic phase was separated, washed with brine, dried ($MgSO_4$) and evaporated. The oily residue was purified on a silica Sep-Pak cartridge (2 g). Elution with hexane/AcOEt (99.8:0.2) gave a mixture of the isomeric protected vitamin D analogues **40** and **41** (17 mg, 52%; ratio of ca. 18:1).

4.1.24. (20S)-1α,25-Bis-[(*tert*-butyldimethylsilyl)oxy]-2-methylene-8(12),14(17)-diseco-9,11,15,16,19-pentanorvitamin D_3 *tert*-butyldimethylsilyl ether (40, major isomer). UV (hexane) λ_{max} 237.0, 244.0, 253.5 nm; ^{1}H NMR (400 MHz, $CDCl_3$; vitamin D numbering) δ 0.027 and 0.037 [3H and 3H, each s, 2 × $Si(CH_3)_2$], 0.058 and 0.064 [6H and 6H, each s, 2 × $(SiCH_3)_2$], 0.85, 0.87 and 0.89 (3 × 9H, each s, 3 × Si-*t*-Bu), 0.85–0.91 (9H, 21- and 13-Me_2; overlapped with Si-*t*-Bu), 1.17 (6H, s, 26- and 27-H_3), 1.96 (1H, m, 20-H), 2.15 (1H, dd, *J* = 12.5, 8.0 Hz, 4β-H), 2.40 (3H, m, 4α-, 10α- and 10β-H), 4.42 (2H, m, 1β- and 3α-H), 4.93 and 4.96 (1H and 1H, each s, C=CH_2), 5.64 (1H, dt, *J* = 14.7, 7.6 Hz, 8-H), 5.92 (1H, d, *J* = 10.8 Hz, 6-H), 6.21 (1H, dd, *J* = 14.7, 10.8 Hz, 7-H); ^{13}C NMR (100 MHz) δ −5.0, −4.9 and −2.1 [3 × $Si(CH_3)_2$], 18.11, 18.14 and 18.23 [3 × $SiC(CH_3)_3$], 21.9 (C-20), 22.8 (C-23), 25.7, 25.8 and 25.9 [3 × $SiC(CH_3)_3$], 27.36 and 27.40 (13-Me_2), 28.9 (C-13), 29.9 (C-26 and C-27), 34.0 (C-21), 39.0 (C-22), 40.3 and 45.4 (C-4 and C-10), 46.7 (C-24), 47.2 (C-14), 49.6 (C-17), 71.8 and 72.3 (C-1 and C-3), 73.5 (C-25), 106.3 (C=CH_2), 127.2 (C-6), 128.2 (C-7), 130.2 (C-8), 132.9 (C-5), 152.8 (C-2); HRMS (ES) exact mass calcd for $C_{41}H_{82}O_3Si_3Na$ (M^++Na) 729.5470, measured 729.5437.

4.1.25. (1R,3R)-5-[(E)-(S)-11′-Hydroxy-5′,5′,7′,11′-tetramethyl-dodec-2′-enylidene]-2-metylene-cyclohexane-1,3-diol (10) and (1R,3R)-5-[(Z)-(S)-11′-hydroxy-5′,5′,7′,11′-tetramethyl-dodec-2′-enylidene]-2-metylene-cyclohexane-1,3-diol (42). To a solution of protected vitamins **40** and **41** (17 mg, 24 μmol) in THF (3 mL) and acetonitrile (1 mL) was added MeCN/46% HF (9:1, 4 mL) at room temperature. After stirring for 4 h, a saturated $NaHCO_3$ was added. The mixture was extracted with CH_2Cl_2, the organic extracts were washed with brine, dried ($MgSO_4$) and evaporated. The residue was first purified on a silica Sep-Pak (0.5 g). Elution with hexane/ethyl acetate (1:1) gave a mixture of deprotected vitamins **10** and **42** (5.2 mg, 63%). Separation of both isomers was achieved by reversed-phase HPLC (9.4 mm × 25 cm, Eclipse XDB-C18 column, 3 mL/min) using a methanol/water (85:15) solvent system. Vitamin D analogue **10** (4.9 mg) was collected at R_V 30.8 mL and the 7*Z*-isomer **42** (280 μg) at R_V 32.0 mL.

4.1.26. (20S)-1α,25-Dihydroxy-2-methylene-8(12),14(17)-diseco-9,11,15,16,19-pentanorvitamin D_3 (10). UV (EtOH) λ_{max} 238.5, 244.0, 254.0 nm; ^{1}H NMR (400 MHz, $CDCl_3$; vitamin D numbering) δ 0.87 (6H, s, 13-Me_2), 0.91 (3H, d, *J* = 6.6 Hz, 21-H_3), 1.21 (6H, s, 26- and 27-H_3), 2.26 (1H, dd, *J* = 13.2, 6.9 Hz, 4β-H), 2.38 (1H, dd, *J* = 13.3, 7.4 Hz, 10α-H), 2.56 (1H, dd, *JJ* = 13.2, 4.3 Hz, 4α-H), 2.71 (1H, dd, *J* = 13.3, 4.2 Hz, 10β-H), 4.48 (2H, m, 1β- and 3α-H), 5.09 (2H, s, C=CH_2), 5.71 (1H, dt, *J* = 14.7, 7.6 Hz, 8-H), 6.05 (1H, d, *J* = 10.8 Hz, 6-H), 6.27 (1H, dd, *J* = 14.7, 10.8 Hz, 7-H); ^{13}C NMR (100 MHz) δ 21.9 (C-23), 22.5 (C-21), 27.5 and 27.7 (13-Me_2), 28.7 (C-13), 29.3 (C-26 and C-27), 34.5 (C-20), 38.1 (C-22), 40.1 and 45.5 (C-4 and C-10), 44.2 (C-24), 46.3 (C-14), 49.4 (C-17), 71.0 (C-25), 71.1 and 71.4 (C-1 and C-3), 107.9 (C=CH_2), 127.4 (C-6), 128.8 (C-7), 131.1 (C-8), 132.1 (C-5), 151.8 (C-2); HRMS (ESI) exact mass calcd for $C_{23}H_{40}O_3Na$ (M^++Na) 387.2875, measured 387.2859.

4.1.27. (7Z)-(20S)-1α,25-Dihydroxy-2-methylene-8(12),14(17)-diseco-9,11,15,16,19-pentanorvitamin D_3 (42). UV (EtOH) λ_{max} 233.5, 242.0, 251.5 nm; ^{1}H NMR (500 MHz, $CDCl_3$; vitamin D numbering) δ 0.89 and 0.90 (3H and 3H, each s, 13-Me_2), 0.91 (3H, d, *J* = 6.6 Hz, 21-H_3), 1.21 (6H, s, 26- and 27-H_3), 2.08 and 2.12 (1H and 1H, each dd, *J* = 13.2, 7.5 Hz, 14-H_2), 2.32 (1H, dd, *J* = 13.1, 6.7 Hz, 4β-H), 2.39 (1H, dd, *J* = 13.2, 7.6 Hz, 10α-H), 2.60 (1H, dd, *J* = 13.1, 4.2 Hz, 4α-H), 2.74 (1H, dd, *J* = 13.2, 4.2 Hz, 10β-H), 4.49 (2H, m, 1β- and 3α-H), 5.11 (2H, s, C=CH_2), 5.56 (1H, dt, *J* = 9.6, 7.5 Hz, 8-H), 6.33 (2H, m, 6- and 7-H); HRMS (ESI) exact mass calcd for $C_{23}H_{40}O_3Na$ (M^++Na) 387.2875, measured 387.2876.

4.2. Measurement of binding to the full-length rat recombinant vitamin D receptor (VDR)

A purified full-length rat recombinant receptor was prepared as described earlier[43] with some minor modifications. The entire coding region for the rat VDR was inserted into the p29 plasmid including the flexible insertion region (residues 165–211). During the purification of the full-length receptor, the eluate from the metal affinity column was dialyzed against the same buffer but at a pH of 8.0 instead of 7.0 and 50 mM sodium phosphate was used instead of 20 mM. The size of the SP-Sepharose Fast Flow column was slightly different—1.5 × 17 cm and the salt gradient used for elution of the VDR from this column linearly increased from 0 to 0.8 M phosphate buffer over a total volume of 300 mL. The fractions judged to be pure by SDS-PAGE were combined and dialyzed against 25 mM EPPS at pH 8.5, containing 50 mM NaCl and 0.02% NaN_3. Following dialysis, the protein was concentrated by ultracentrifugation to approximately 1.4 mg/mL. Aliquots of the purified protein were flash-frozen in liquid nitrogen and stored at −80 °C until use. On the day of each binding assay, the protein was diluted in $TEDK_{50}$ (50 mM Tris, 1.5 mM EDTA, pH 7.4, 5 mM DTT, 150 mM KCl) with 0.1% CHAPS detergent. The receptor protein and ligand concentration were optimized such that no more than 20% of the added radiolabeled ligand was bound to the receptor. Unlabeled ligands were dissolved in ethanol and the concentrations determined using UV spectrophotometry [1α,25$(OH)_2D_3$: molar extinction coefficient ε = 18,200 and λ_{max} = 265.0 nm; the tested 19-norvitamin *des*-C,D compounds: ε = 30,200 and λ_{max} = 241.0 nm]. Radiolabeled ligand [^{3}H-1α,25$(OH)_2D_3$, ~159 Ci/mmol] was added in ethanol at a final concentration of 1 nM. Radiolabeled and unlabeled ligands were added to 100 μL of the diluted protein at a final ethanol concentration of ⩽10%, mixed and incubated overnight on ice to reach binding equilibrium. The following day, 100 μL of hydroxylapatite slurry (50%) was added to each tube and mixed at 10-min intervals for 30 min. The hydroxylapatite was collected by centrifugation and then washed three times with Tris-EDTA buffer (50 mM Tris, 1.5 mM EDTA, pH 7.4) containing 0.5% Titron X-100. After the final wash, the pellets were transferred to scintillation vials containing 4 mL of Biosafe II scintillation cocktail, mixed and placed in a scintillation counter. Total binding was determined from the tubes containing only radiolabeled ligand.

The displacement experiments were carried out in duplicate on two-three different occasions.

4.3. Measurement of cellular differentiation

Human promyelocytic leukemia (HL-60) cells were grown in RPMI-1640 medium containing 10% fetal bovine serum at 37 °C in the presence of 5% CO_2. HL-60 cells were plated at 1.2×10^5 cells/plate. Eighteen hours after plating, cells in duplicate were treated with the compound tested so that the final concentration of ethanol was less than 0.2%. Four days later, the cells were harvested and a nitro blue tetrazolium (NBT) reduction assay was performed. The percentage of differentiated cells was determined by counting a total of 200 cells and recording the number that contained intracellular black-blue formazan deposits.[44] The experiment was repeated 2–3 times and the results are reported as the mean. Verification of differentiation to monocytic cells was determined by measuring phagocytic activity (data not shown).

4.4. Transcriptional assay

Transcription activity was measured in ROS 17/2.8 (bone) cells that were stably transfected with a *Cyp24a1* promoter upstream of a luciferase reporter gene.[45] Cells were given a range of doses. Sixteen hours after dosing, the cells were harvested and luciferase activities were measured using a luminometer. Each experiment was performed in duplicate two-three separate times.

4.5. Docking studies

Docking simulations were performed by FlexiDock software from Tripos.[46] The receptor was kept immobile while internal rotations around ligand single bonds (except those creating ring A) were allowed. FlexiDock requires an approximate starting position of the ligand to be provided. Several simulations, of 100,000 steps each, were performed with various initial positions of the ligand in the LBP that was always described with the same set of amino acids, lining the binding pocket in the crystalline hVDR-**1**.[38] The *des*-C,D analogues **7** and **10** were docked in *s-trans* conformation of their C(5)═C(6)–C(7)═C(8) diene system and in the A-ring β-chair form with equatorially oriented 1α-hydroxy groups. Such conformation of vitamin D analogues is always present in holoVDR crystals. For final consideration only the lowest energy complexes were selected, possessing tryptophan oriented parallel to the ligand 5,7-diene moiety. The results of docking experiments are presented in Table 3. All amino acids from human VDR discussed in this paper are recalculated according to the rVDR sequence.[47]

Comparison of vitamin D positions in the ligand-binding cavities of the modeled (rVDR-**10**) and crystalline (hVDR-**1** and rVDR-DRIP-**2**) complexes is depicted in Figure 5. Superimpositions of complexes were performed in three steps using the BioShell package,[48,49] a multipurpose suite for bioinformatic and biophysical calculations. Alignment of the VDR chains differing in length was the first step of procedure. In the second step, the optimal transformation (rotation matrix and translation vector) was established between compared pairs of Cα atoms. Finally, the ligand molecule (**7** or **10**) has been transferred from holo-rVDR to the LBP of the crystalline complexes hVDR-**1**[38] and rVDR-DRIP-**2**.[28]

4.6. Computational method

The calculation of optimized geometries and steric energies was done using the algorithm from the MM^+ HyperChem (release 7.0) software package (Hypercube, Inc.). MM^+ is an all-atom force field based on the MM2 functional form.

4.7. Crystallographic studies

4.7.1. Crystal data (for compound 16)

$C_{24}H_{30}O_4Si$, $M = 410.57$, $T = 90(2)$ K, orthorhombic, space group $P2_12_12_1$, $Z = 8$, $a = 9.2588(5)$, $b = 20.4594(13)$, $c = 23.2702$ Å, $\alpha\beta\gamma = 90°$, $V = 4408.1(4)$ Å^3, $D_x = 1.237$ g cm^{-3}, 32858 unique data ($2\theta_{max} = 26.50°$), 8966 with $I > 2.0\sigma(I)$, $R = 0.0888$, $Rw = 0.1045$, $S = 1.016$.

4.7.2. Structure determination

The data were collected using the Bruker Kappa APEX II Ultra controlled by APEXII software,[50] equipped with MoKα rotating anode X-ray source ($\lambda = 0.71073$ Å, 50.0 kV, 22.0 mA) monochromatized by multi-layer optics and APEX-II CCD detector. The experiments were carried out at 90 K using the Oxford Cryostream cooling device. The crystal was mounted on a cactus needle with a droplet of Pantone-N oil and immediately cooled. Indexing, integration and initial scaling were performed with SAINT[51] and SADABS[52] software (Bruker, 2008).

The crystal was positioned at 40 mm from the CCD camera. 1545 frames were measured at 0.5° intervals with a counting time of 20–30 s.

The structures were solved by the direct method approach using the SHELXS-97[53] program and refined with the SHELXL-97.[54] Multi-scan absorption corrections have been applied in the scaling procedure.

The refinement was based on F^2 for all reflections except for those with negative intensities. Weighted R factors wR and all goodness-of-fit S values were based on F^2, whereas conventional R factors were based on the amplitudes, with F set to zero for negative F^2. The $F_0^2 > 2\sigma(F_0^2)$ criterion was applied only for R factors calculation and was not relevant to the choice of reflections for the refinement. The R factors based on F^2 are for all structures about twice as large as those based on F. The hydrogen atoms were located in idealized geometrical positions, except for hydrogen atoms in the solvent molecule. Scattering factors were taken from Tables 4.2.6.8 and 6.1.1.4 from the International Tables for Crystallography.[55]

Crystallographic data for the structure reported in this paper have been deposited at the Cambridge Crystallographic Data Centre with the deposition number CCDC-844267.

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References and notes

1. DeLuca, H. F. *Nutr. Rev.* **2008**, *66*, 573.
2. *Vitamin D Second Edition*; Feldman, D., Pike, J. W., Glorieux, F. H., Eds.; Elsevier Academic Press: Burlington, 2005.
3. *Vitamin D Endocrine System: Structural, Biological, Genetic and Clinical Aspects*; Norman, A. W., Bouillon, R., Thomasset, M., Eds.; University of California Riverside: Riverside, 2000.
4. Brown, A. J.; Slatoplosky, E. *Mol. Aspects Med.* **2008**, *29*, 433.
5. Abe, E.; Miyaura, C.; Sakagami, H.; Takeda, M.; Konno, K.; Yamazaki, T.; Yoshiki, S.; Suda, T. *Proc. Natl. Acad. Sci. U.S.A.* **1981**, *78*, 4990.
6. Ostrem, V. K.; DeLuca, H. F. *Steroids* **1987**, *49*, 73.
7. Zhu, G.-D.; Okamura, W. H. *Chem. Rev.* **1995**, *95*, 2457.
8. Bouillon, R.; Okamura, W. H.; Norman, A. W. *Endocr. Rev.* **1995**, *16*, 200.
9. Sicinski, R. R.; Prahl, J. M.; Smith, C. M.; DeLuca, H. F. *J. Med. Chem.* **1998**, *41*, 4662.

10. Plum, L. A.; Fitzpatrick, L. A.; Ma, X.; Binkley, N. C.; Zella, J. B.; Clagett-Dame, M.; DeLuca, H. F. *Osteoporos. Int.* **2006**, *17*, 704.
11. Ke, H. Z.; Qi, H.; Crawford, D. T.; Simmons, H. A.; Xu, G.; Li, M.; Plum, L.; Clagett-Dame, M.; DeLuca, H. F.; Thompson, D. D.; Brown, T. A. *J. Bone Miner. Res.* **2005**, *20*, 1742.
12. Shevde, N. K.; Plum, L. A.; Clagett-Dame, M.; Yamamoto, H.; Pike, J. W.; DeLuca, H. F. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 13487.
13. Sibilska, I.; Barycka, K. M.; Sicinski, R. R.; Plum, L. A.; DeLuca, H. F. *J. Steroid Biochem. Mol. Biol.* **2010**, *121*, 51.
14. Grzywacz, P.; Chiellini, G.; Plum, L. A.; Clagett-Dame, M.; DeLuca, H. F. *J. Med. Chem.* **2010**, *53*, 8642.
15. Barycki, R.; Sicinski, R. R.; Plum, L. A.; Grzywacz, P.; Clagett-Dame, M.; DeLuca, H. F. *Bioorg. Med. Chem.* **2009**, *17*, 7658.
16. Chiellini, G.; Grzywacz, P.; Plum, L. A.; Barycki, R.; Clagett-Dame, M.; DeLuca, H. F. *Bioorg. Med. Chem.* **2008**, *16*, 8563.
17. Yoshimoto, N.; Inaba, Y.; Yamada, S.; Makishima, M.; Shimizu, M.; Yamamoto, K. *Bioorg. Med. Chem.* **2008**, *16*, 457.
18. Grzywacz, P.; Plum, L. A.; Sicinski, R. R.; Clagett-Dame, M.; DeLuca, H. F. *Arch. Biochem. Biophys.* **2007**, *460*, 274.
19. Igarashi, M.; Yoshimoto, N.; Yamamoto, K.; Shimizu, M.; Ishizawa, M.; Makishima, M.; DeLuca, H. F.; Yamada, S. *Arch. Biochem. Biophys.* **2007**, *460*, 240.
20. Vanhooke, J. L.; Tadi, B. P.; Benning, M. M.; Plum, L. A.; DeLuca, H. F. *Arch. Biochem. Biophys.* **2007**, *460*, 161.
21. Sato, M.; Nakamichi, Y.; Nakamura, M.; Sato, N.; Ninomiya, T.; Muto, A.; Nakamura, H.; Ozawa, H.; Iwasaki, Y.; Kobayashi, E.; Shimizu, M.; DeLuca, H. F.; Takahashi, N.; Udagawa, N. *Bone* **2007**, *40*, 293.
22. Sicinski, R. R. *Polish J. Chem.* **2006**, *80*, 573.
23. Kutner, A.; Zhao, H.; Fitak, H.; Wilson, S. R. *Bioorg. Chem.* **1995**, *23*, 22.
24. Sabbe, K.; D'Hallewyn, C.; De Clercq, P.; Vandewalle, M. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1697.
25. Barbiel, P.; Bauer, F.; Mohr, P.; Muller, M.; Pirson, W. Cyclohexanodiol derivatives, WO 99/43646, 1999.
26. Plonska-Ocypa, K.; Grzywacz, P.; Sicinski, R. R.; Plum, L. A.; DeLuca, H. F. *J. Steroid Biochem. Mol. Biol.* **2007**, *103*, 298.
27. Plonska-Ocypa, K.; Sicinski, R. R.; Plum, L. A.; Grzywacz, P.; Frelek, J.; Clagett-Dame, M.; DeLuca, H. F. *Bioorg. Med. Chem.* **2009**, *17*, 1747.
28. Vanhooke, J. L.; Benning, M. M.; Bauer, C. B.; Pike, J. W.; DeLuca, H. F. *Biochemistry* **2004**, *43*, 4101.
29. Bøgevig, A.; Sunden, H.; Cordova, A. *Angew. Chem., Int. Ed.* **2004**, *43*, 1109.
30. Hayashi, Y.; Yamaguchi, J.; Sumiya, T.; Hibino, K.; Shoji, M. *J. Org. Chem.* **2004**, *69*, 5966.
31. Parker, K. A.; Dermatakis, A. *J. Org. Chem.* **1997**, *62*, 6692.
32. Anet, F. A. L. *J. Am. Chem. Soc.* **1962**, *84*, 1053.
33. Glebocka, A.; Sicinski, R. R.; Plum, L. A.; Clagett-Dame, M.; DeLuca, H. F. *J. Med. Chem.* **2006**, *49*, 2909.
34. Ono, K.; Yoshida, A.; Saito, M.; Fujishima, T.; Honzawa, S.; Suhara, Y.; Kishimoto, S.; Sugiura, T.; Waku, K.; Takayama, H.; Kittaka, A. *J. Org. Chem.* **2003**, *68*, 7407.
35. Sicinski, R. R.; Rotkiewicz, P.; Kolinski, A.; Sicinska, W.; Prahl, J. M.; Smith, C. M.; DeLuca, H. F. *J. Med. Chem.* **2002**, *45*, 3366.
36. Yamada, S.; Yamamoto, K. *Curr. Top. Med. Chem.* **2006**, *6*, 1255.
37. Sicinska, W.; Westler, W. M.; DeLuca, H. F. *Proteins* **2005**, *61*, 461.
38. Rochel, N.; Moras, D. *Curr. Top. Med. Chem.* **2006**, *6*, 1229.
39. Tocchini-Valentini, G. D.; Rochel, N.; Wurtz, J. M.; Mitschler, A.; Moras, D. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 5491.
40. Gonzales-Avion, X.; Mourino, A. *Org. Lett.* **2003**, *13*, 2291.
41. Yamada, S.; Shimizu, M.; Yamamoto, K. *Med. Res. Rev.* **2003**, *23*, 89.
42. Sicinska, W.; Kurcinski, M. *J. Steroid Biochem. Mol. Biol.* **2010**, *121*, 34.
43. Martin, D. L.; DeLuca, H. F. *Am. J. Physiol.* **1969**, *216*, 1351.
44. Ostrem, V. K.; Lau, W. F.; Lee, S. H.; Perlman, K.; Prahl, J.; Schnoes, H. K.; DeLuca, H. F.; Ikekawa, N. *J. Biol. Chem.* **1987**, *262*, 14164.
45. Arbour, N. C.; Ross, T. K.; Zierold, C.; Prahl, J. M.; DeLuca, H. F. *Anal. Biochem.* **1998**, *255*, 148.
46. SYBYL *Modeling Program (7.1 ed.) and* SYBYL-X *1.2 (linux_os2x)*; Tripos Inc.: St. Louis, MO.
47. Sicinska, W.; Rotkiewicz, P. *J. Steroid Biochem. Mol. Biol.* **2009**, *113*, 253.
48. Gront, D.; Kolinski, A. *Bioinformatics* **2008**, *24*, 584.
49. Gront, D.; Kolinski, A. *Bioinformatics* **2006**, *22*, 621.
50. APEXII-2008 (v 1.0) Bruker Nonius, 2007.
51. SAINT V7.34A Bruker Nonius, 2007.
52. SADABS-2008/1 Bruker Nonius Area Detector Scaling and Absorption Correction, 2008.
53. Sheldrick, G. M. *Acta Crystallogr., Sect. A* **1990**, *46*, 467.
54. Sheldrick, G. M. SHELXL*93. Program for the Refinement of Crystal Structures*; Univ. of Göttingen: Germany.
55. *International Tables for Crystallography*; Wilson, A. J. C., Ed.; Kluwer: Dordrecht, 1992; Vol. C.