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13-Methyl-substituted *des*-C,D analogs of (20*S*)-1α,25-dihydroxy-2-methylene-19-norvitamin D_3 (2MD): Synthesis and biological evaluation

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ABSTRACT

Analogs of (20*S*)-1α,25-dihydroxy-2-methylene-19-norvitamin D_3 (**2**, **2MD**), substituted at C-13 but lacking both C and D rings, were prepared in convergent syntheses, starting with the chiral ester **14** and the phosphine oxide **29**. Two of the synthesized vitamins (**11** and **32**) were analogs in which the 13-methyl group constituted a substituent of an unsaturated fragment, that is, C(13)–C(17) double bond, whereas in the two other cases (**12** and **13**), the methyl group belonged to a ternary carbon stereogenic center. The aim of these studies was to further explore extensive modifications in the 'upper' part of the vitamin D skeleton in the hope of finding biologically active analogs of potential therapeutic value.

The commercial (*R*)-(−)-methyl-3-hydroxy-2-methylpropionate (**14**) was converted in six steps to alcohol **18**, the vitamin D side chain fragment. Its subsequent three-step transformation led to aldehyde **20** which was subjected to the Still–Gennari HWE reaction with anion derived from ester **21**. The obtained α,β-unsaturated esters **22** and **23** served as convenient starting compounds to the syntheses of the corresponding chiral acyclic aldehydes, β,γ-unsaturated (**28**) and saturated (**39** and **40**), required for the final Wittig–Horner coupling with the anion of the phosphine oxide **29**. After hydroxyl deprotection, the synthesized vitamin D analogs **11**–**13** and **32** were purified and biologically tested. Only the (13*R*,20*S*)-analog **12** retained substantial, although 30 times lower than 1α,25-$(OH)_2D_3$, binding ability to the full-length rat recombinant vitamin D receptor (VDR). This analog was also very effective in differentiation of HL-60 cells, and it exerted significant transcriptional activity (2 times and 15 times less potent, respectively, as compared to the native hormone). The in vivo tests showed that all synthesized vitamin D analogs were devoid of calcemic activity.

1. Introduction

In addition to its well known, classical role in calcium and phosphorus homeostasis,[1,2] 1α,25-dihydroxyvitamin D_3, [1α,25-$(OH)_2D_3$, calcitriol, **1**; Fig. 1] exhibits a broad spectrum of biological actions. For example, this native hormone possesses significant cellular differentiation activity and a strong immunomodulatory effect.[3,4] Among thousands of structural analogs of calcitriol prepared and biologically tested to date, some exhibit an interesting separation of calcemic and differentiation activities.[5] Considering the fact that strong calcemic activity limits the therapeutic potential of vitamin D compounds in non-classical uses, such selectivity may be of considerable importance.

In the course of our structure–activity studies in the vitamin D field, we discovered one of the most promising structural modifications of the A ring: a 'shift' of the exocyclic methylene moiety from C-10 to C-2. Ten years ago we described the synthesis of 1α,25-dihydroxy-2-methylene-19-norvitamin D analogs which were characterized by significant biological potency, enhanced dramatically in compounds with an 'unnatural' (20*S*)-configuration.[6] Analog **2** (2MD) seemed to be most interesting since it was shown that in ovariectomized rats this compound can produce a significant increase in bone density.[7] 2MD is currently undergoing Phase II clinical testing as a potential drug for osteoporosis. Several compounds related to 2MD (differing mostly in the side chain structure) have been synthesized in our laboratory[8] and some of them displayed a selective pattern of biological activities.[9]

Removal of the C and D rings constitutes another interesting modification of the vitamin D skeleton. First compounds (retiferols **3** and **4**) lacking the C,D-substructure were obtained 13 years

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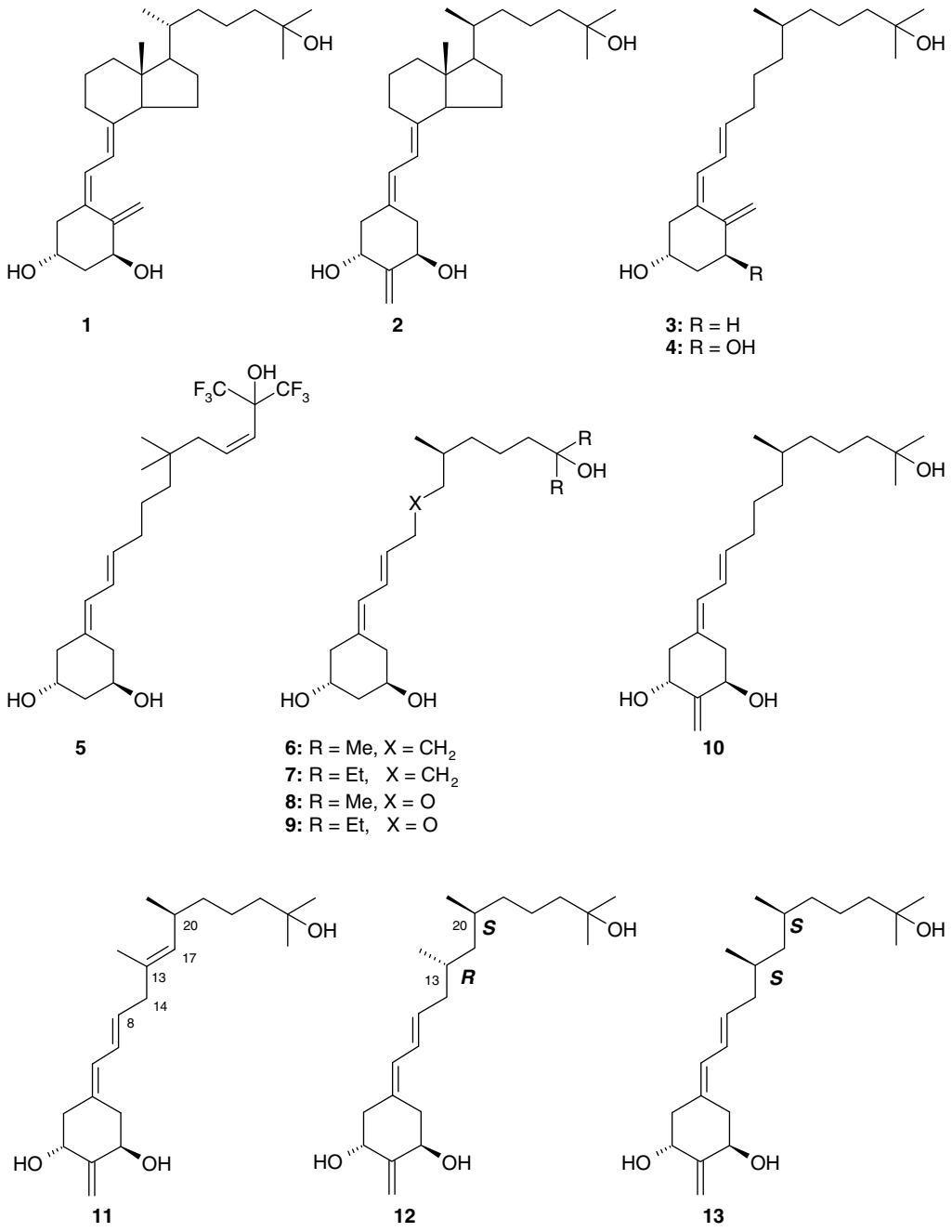


Figure 1. Chemical structures of 1α,25-dihydroxyvitamin D_3 (calcitriol, **1**) and analogs.

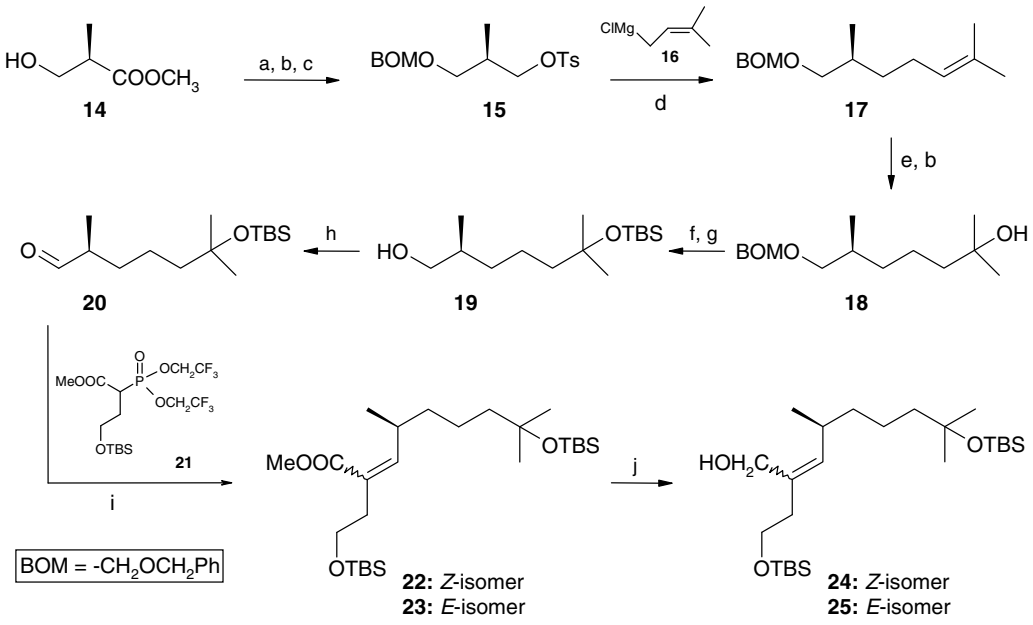
ago.[10] One year later, *des*-C,D vitamins possessing an additional ring E were described[11] and during the following years syntheses of *des*-C,D 19-norvitamin D_3 derivatives (**5**–**9**) were also reported.[12,13] Very recently, we synthesized and biologically tested a *des*-C,D analog of 2MD (**10**), which retained some transcriptional activity and binding affinity to the vitamin D receptor (VDR) albeit decreased by two orders of magnitude in comparison with the analogous vitamins possessing intact C,D rings. Considering that the structure of this analog is characterized by extremely high flexibility, which should allow this molecule to accommodate easily to the active sites of the ligand binding domain (LBD) of the VDR, the poor binding ability of **10** is puzzling. Since it is known that C,D-rings of 2MD are surrounded in its crystalline complex with the LBD of the VDR,[14] by strongly hydrophobic amino acids, insufficient hydrophobic interactions of analog **10** with the receptor are one potential explanation for this result. To verify this hypothesis, we decided to prepare the *des*-C,D vitamins substituted with groups (such as alkyls), which could increase the hydrophobic interaction of the molecule with VDR.

As a continuation of our search for biologically active 2-methylene-19-norvitamin D compounds, we decided to synthesize the analogs **11**–**13** which are characterized by the lack of the C,D-rings and a presence of one methyl group at C-13.

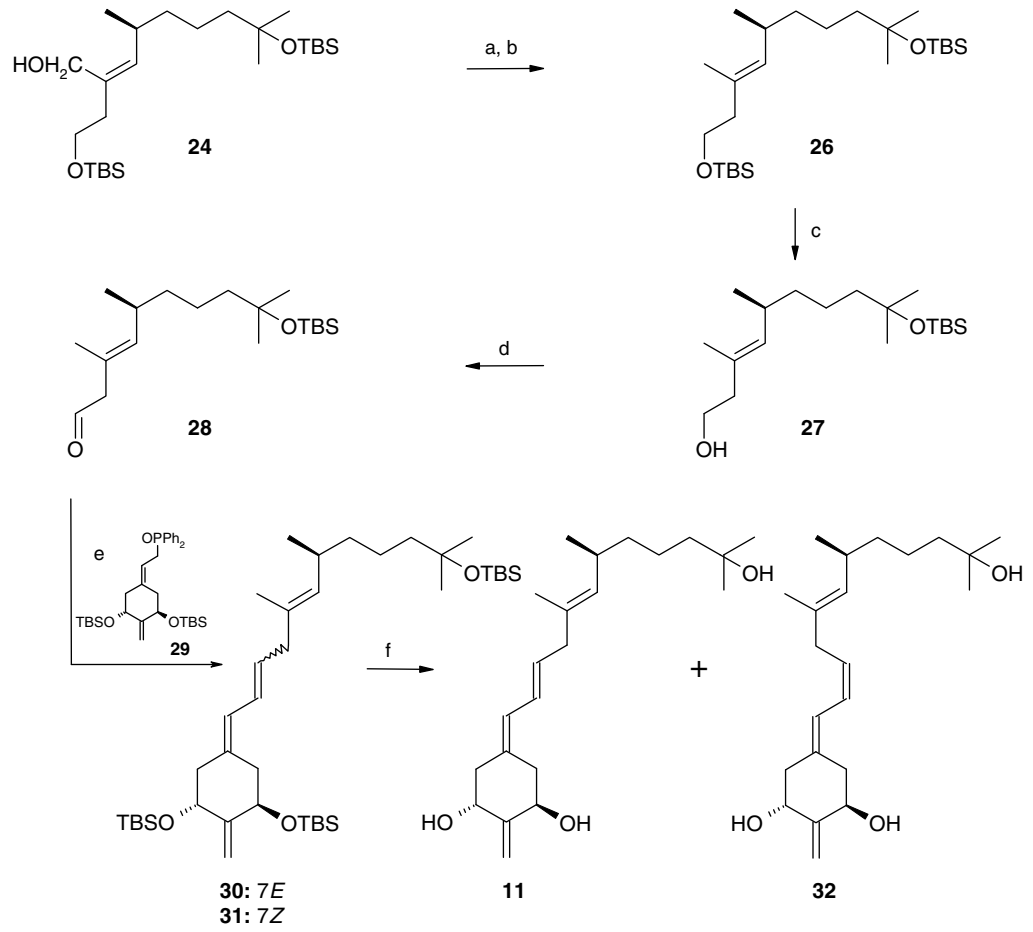
2. Results

2.1. Synthesis

Our synthesis began with commercial (*R*)-(−)-methyl-3-hydroxy-2-methylpropionate (**14**, Scheme 1), and it focused on the prep-



Scheme 1. Synthesis of unsaturated allylic alcohols **24** and **25**. Reagents: (a) $PhCH_2OCH_2Cl$, CH_2Cl_2; (b) $LiAlH_4$, THF; (c) *p*-TsCl, DMAP, Et_3N, CH_2Cl_2; (d) **16**, Li_2CuCl_4, THF; (e) MCPBA, CH_2Cl_2; (f) TBSOTf, CH_2Cl_2; (g) Pd/C, H_2, AcOEt; (h) TPAP, NMO, CH_2Cl_2; (i) **21**, KHMDS, 18-crown-6, THF; (j) DIBALH, CH_2Cl_2/toluene.



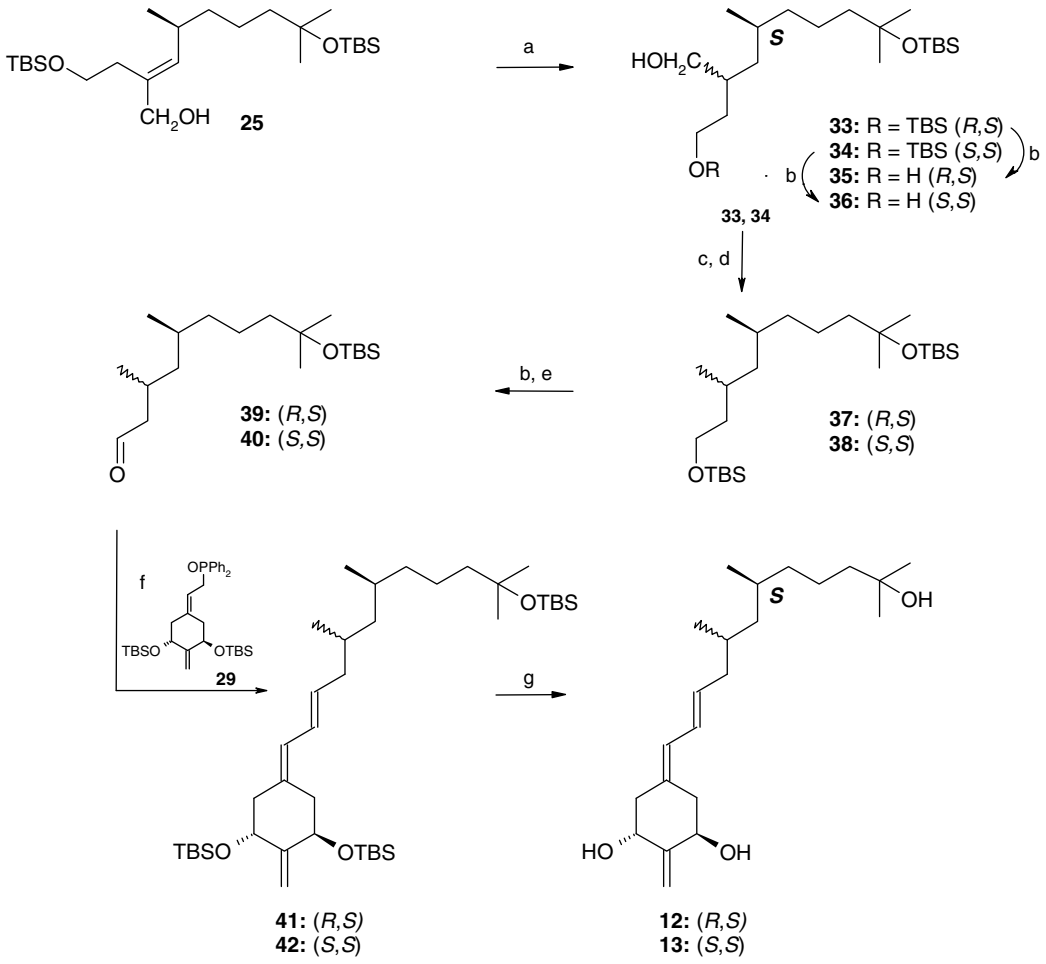
Scheme 2. Synthesis of *des*-C,D vitamins **11** and **32**. Reagents: (a) *p*-TsCl, DMAP, Et_3N CH_2Cl_2; (b) $LiAlH_4$, THF; (c) TBAF, THF; (d) TPAP, NMO, CH_2Cl_2; (e) **29**, PhLi, THF; (f) HF/MeCN, THF; HPLC separation.

aration of the corresponding aldehydes required for Wittig–Horner coupling with the phosphine oxide **29** (Scheme 2), obtained by us previously.[6] Reduction of the BOM-protected hydroxy ester with $LiAlH_4$ and the tosylation of the formed alcohol furnished the tosylate **15** that reacted with Grignard reagent **16** to give an olefinic compound **17**. Epoxidation of the latter compound and the follow-

ing reduction of the formed isomeric epoxides gave alcohol **18**. Its tertiary hydroxy group was protected as TBS ether and then the primary hydroxyl deprotected by hydrogenation yielding hydroxy ether **19**. Subsequent oxidation with PDC provided aldehyde **20** that was treated with the anion derived from phosphono ester **21**, which in turn was prepared from commercial reagents $(F_3CCH_2O)_2POCH_2COOCH_3$ and $Br(CH_2)_2OTBS$. The attempted Still–Gennari HWE olefination reaction proved to be successful – a mixture of *Z*- and *E*-isomeric unsaturated esters **22** and **23** (1.7:1 ratio) was formed in quantitative yield. The assignment of configuration of the products followed from an analysis of their ^1H NMR spectra: signal of olefinic proton in *E*-isomer **23** was deshielded by 0.92 ppm. Since HPLC separation of these geometrical isomers was difficult, they were subjected to the DIBALH reduction and the formed isomeric allylic alcohols **24** and **25** could be easily separated by column chromatography. The prevailing *Z*-isomer **24** was used for the preparation of the β,γ-unsaturated aldehyde **28** (Scheme 2). Thus, tosylation of the allylic alcohol and subsequent reduction of the formed tosylate provided unsaturated diether **26**. Then, primary hydroxyl was selectively deprotected and the alcohol **27** oxidized to the desired aldehyde **28**. Wittig–Horner reaction of this compound with lithium phosphinoxy carbanion derived from the phosphine oxide **29** gave a mixture of protected vitamin D analogs. The main product was identified as 7*E*-isomer **30** contaminated with a minute amount of its 7*Z*-counterpart **31**. Removal of the silyl protecting groups was performed with hydrofluoric acid and the final vitamin D analogs **11** and **32** were separated by HPLC. Value of the vicinal coupling constant (14.9 Hz) between vinylic 7- and 8-protons, found in the ^1H NMR spectra of the main vitamin D products, indicated their *trans*-relationship and allowed to easy assignment of the double bond configurations of compounds **30** and **11**. Unfortunately, the proton magnetic resonance spectrum of the isomeric 7*Z*-isomer **12**, isolated in microgram quantities, was more complicated due to signal overlapping. Therefore, the configurational assignment was based on the literature data (formation of isomeric 7*E*- and 7*Z*-vitamins D was described previously[15–17]) and comparison with analogous vitamins obtained in our laboratory (results to be published).

The allylic alcohol **25** served as a starting compound for the preparation of the saturated aldehydes **39** and **40** (Scheme 3). Hydrogenation of this compound provided the mixture of diastereomeric saturated alcohols **33** and **34**. These products were separated by HPLC and the following synthetic steps were executed separately with both diastereomers. For further characterization of isomers, the corresponding diols **35** and **36** were also obtained. Configuration of the new stereogenic center in the synthesized alcohols was established by a series of chemical transformations (described later) and analysis of circular dichroism (CD) data.

The synthetic path leading to the aldehyde **39** started from tosylation of the (*R*,*S*)-alcohol **33** and the reduction of the tosylate. Selective hydroxyl deprotection in the obtained diether **37** followed by oxidation provided the saturated aldehyde **39** which was subjected to Wittig–Horner coupling with the anion generated from **29**. Removal of the silyl protecting groups in the product **41**



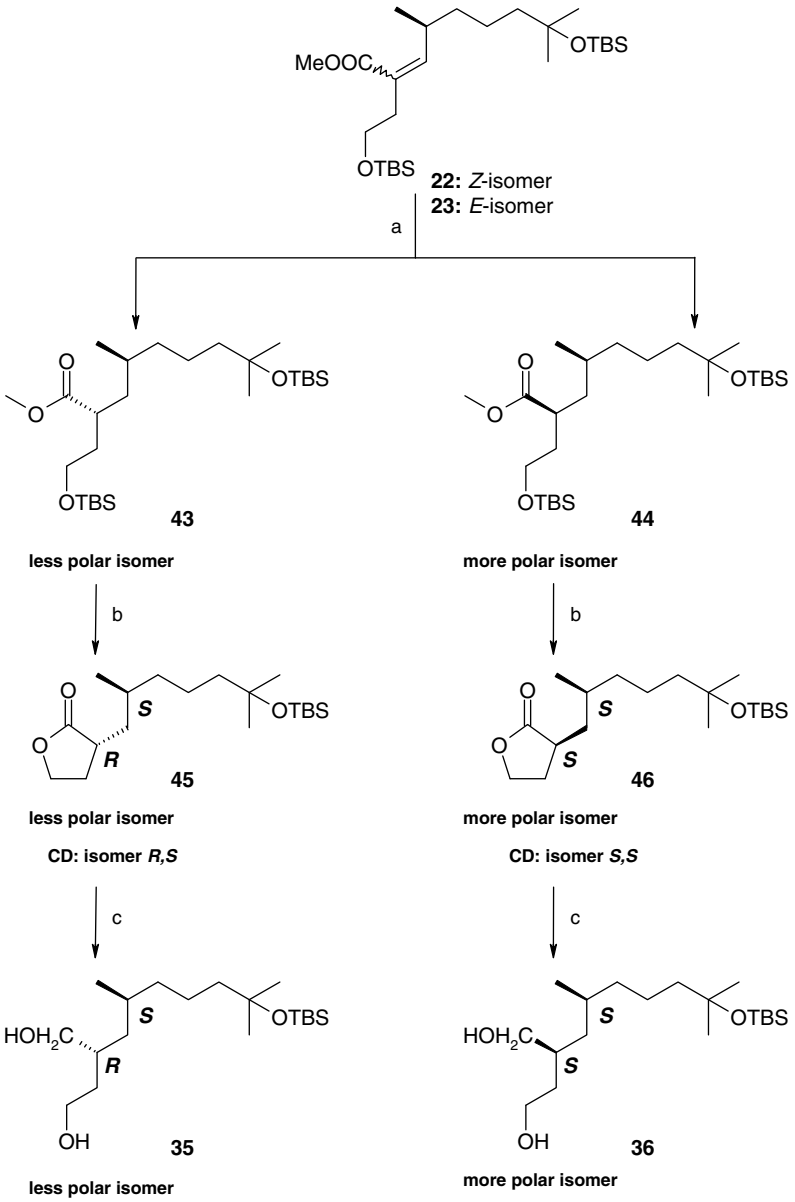
Scheme 3. Synthesis of *des*-C,D vitamins **12** and **13**. Reagents: (a) PtO_2, H_2, MeOH; HPLC separation; (b) TBAF, THF; (c) *p*-TsCl, DMAP, Et_3N, CH_2Cl_2; (d) $LiAlH_4$, THF; (e) TPAP, NMO, CH_2Cl_2; (f) **29**, PhLi, THF; (g) HF/MeCN, THF; HPLC separation.

gave the final (13*R*,20*S*)-vitamin D analog **12**. An analogous synthetic sequence, as the conversion of **33** to **12**, was used for the transformation of the isomeric alcohol **34** into the (13*S*,20*S*)-vitamin D compound **13**.

2.2. Determination of stereochemistry at C-13

As a method allowing us to establish the absolute configurations at the newly created stereogenic centers of the synthesized compounds, we used the CD data of the γ-lactones **45** and **46** (Scheme 4) prepared from the unsaturated esters **22** and **23**. Thus, hydrogenation of these compounds provided a mixture of saturated esters **43** and **44** which were separated by HPLC. The less polar isomer **43** was treated with 10-camphorsulfonic acid and, according to our expectations, it underwent smooth conversion to the corresponding lactone **45**. Analogously, the more polar ester **44** furnished the more polar, stereoisomeric lactone **46**.

The literature data indicate that in the case of γ-lactones a conformation of their penta-membered rings is the determining factor for the sign of the CD bands.[18,19] The 'ring-chirality rule' introduced by Legrand and Bucourt[20] states that a sign of the n–π^* Cotton effect can be directly correlated with a value of the torsion angle O–C(=O)–C_α–C_β (θ, Fig. 2). Thus, a positive (negative) value of angle θ corresponds to a negative (positive) sign of the Cotton effect. Conformation and this angle, in turn, are dependent on the substitution of the lactone ring. It was also nicely demonstrated in our case. We carried out MM^+ force field calculations (HYPERCHEM 7.0, Hypercube, Inc) for the diastereomeric lactones **45** and **46** and, for comparison, their parent compounds with a free tertiary hydroxyl. We then performed conformational searches in every series (described in Section 5) involving rotations of the lactone ring torsions and the torsions from their alkyl substituents. The results of these molecular mechanics studies unequivocally showed that, for the lactones possessing an *R*-configuration of the alkoxyalkyl (or hydroxyalkyl) substituent at C_α, their strongly energetically pre-



Scheme 4. Synthesis of lactones **45** and **46** aimed at establishing of configurations at stereogenic centers in the obtained stereoisomeric compounds. Reagents: (a) PtO_2, H_2, AcOEt; HPLC separation; (b) CAS, MeOH; (c) $LiAlH_4$, THF.

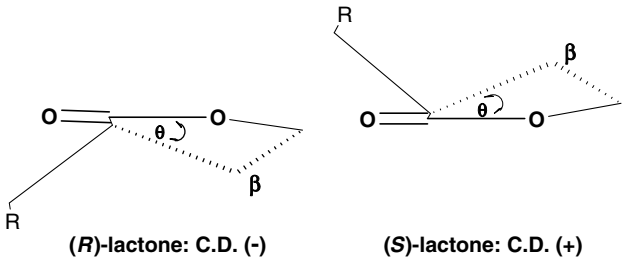


Figure 2. Determination of a sign of the n–π^* Cotton effect for 3-substituted γ-lactones by a 'ring-chirality rule'. The compounds are shown in their energetically preferred ring conformations; hydrogen atoms are omitted for clarity.

ferred ring conformations are characterized by a positive torsional angle θ (ca. 20°), whereas in the case of their counterparts with an *S*-configuration of substituents, the preferred ring conformations have a negative value of this angle (ca. −20°). The steric energy differences (ca. 1.35 kcal/mol) between the global minimum conformations and the lowest energy structures with the 'inverted' rings and the opposite θ values indicate that at room temperature the prevailing conformations amount to more than 90% of the population.

In an effort to corroborate the stereochemical conclusions made on the basis of the 'ring-chirality rule', the ECD spectra of lactones **45** and **46** were calculated by means of the time-dependent density functional theory (TDDFT). For a given molecular conformation, TDDFT provides a detailed picture of the electronic structure, which in turn is the key to a quantitative treatment of chiroptical properties.[21] Thus, comparing the computed CD spectra to the measured ones, we can infer conclusions on the stereochemistry of the lactones studied here. In particular, we can assign the absolute configuration of the crucial C-3 carbon atom for compounds **45** and **46**, which provides an independent test of the 'ring-chirality rule'.

To obtain the simulated CD spectra, the lowest energy conformers of **45** and **46**, calculated by the MM^+ force field, were used (Fig. 2). To reduce calculation times the O-TBDMS group we replaced by OH group. No significant contributions of the O-TBDMS molecular moiety was expected to the overall rotational strengths. Next, the rotatory strength was calculated for each conformer at the B3LYP/6-31++G(D,P) level. As can be seen in Figure 3, the experimental and simulated CD spectra are in an excellent agreement thus providing an independent (conclusive) evidence for the absolute configuration determination. Building on the results of conformational analysis, the experimentally developed 'ring-chirality rule' and the TDDFT calculations we can conclude that the absolute configuration of the synthesized lactones—3*R* for the less polar compound **45** and 3*S* for the isomeric **46** (Fig. 3) is established with high degree of confidence. Each lactone **45** and **46** was then subjected to reduction with $LiAlH_4$ and the isolated products were found identical in all respects with the previously obtained (Scheme 3) diols **35** and **36**.

2.3. Biological evaluation

The described assignment and correlation of configurations allowed us also to assign configurations of the methyl substituents in the vitamin D compounds **12** and **13**. All synthesized *des*-C,D analogs of (20*S*)-1α,25-dihydroxy-2-methylene-19-norvitamin D_3 were tested for their ability to bind rat vitamin D receptor (Table 1). The 7*E*-vitamin **11** with a C(13)–C(17) double bond was 600 times less potent than the natural hormone in displacement of radiolabeled 1α,25-$(OH)_2D_3$ from the receptor protein, and its 7*Z*-isomer **32** was completely devoid of activity. For the (13*R*,20*S*)-isomer **12** more pronounced affinity was detected, albeit still decreased 30 times compared to **1**, whereas the potency of the (13*S*,20*S*)-vitamin D analog **13** proved to be lower by two orders of magnitude in comparison with the native hormone. The next assay constituted measuring the compound ability to induce differentiation of human promyelocyte HL-60 cells into monocytes. Only two of the tested analogs (**12** and **13**) were found active; however, in this case the difference in their potency was dramatic: analog **12** exhibited only twofold decreased activity, whereas isomer **13** proved to be 100 times less active with respect to the parent hormone. A similar pattern of the relative potencies of the *des*-C,D vitamins was discovered when their transcription activity was established, indicated in the 24-hydroxylase (CYP-24) promoter driving luciferase reporter gene system. Thus, analogs **12** and **13** were 15 and 450 times, respectively, less active than 1α,25-$(OH)_2D_3$, whereas the remaining tested compounds **11** and **32** were found inactive in this regard. We tested two *des*-C,D ring analogs, that is, compounds **10** and **13** in vivo for their ability to activate intestinal calcium transport and bone calcium mobilization (serum calcium) (Table 2). Even when tested at very high doses, neither compound had significant activity in raising serum calcium at the expense of one or in stimulating intestinal calcium transport, while 1α,25-$(OH)_2D_3$ (compound **1**) had activity at all dose levels and gave an excellent dose-dependent response.

3. Discussion

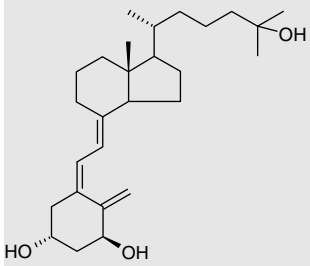
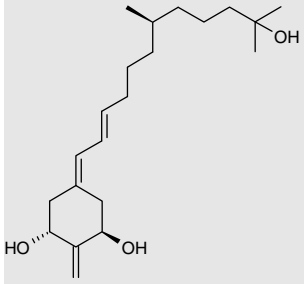
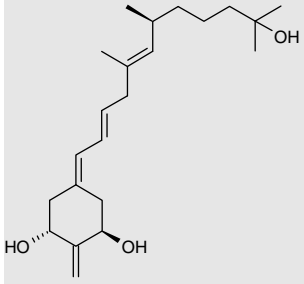
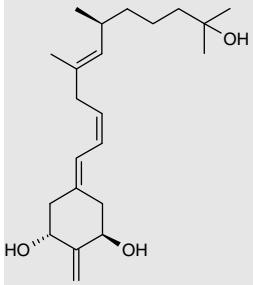
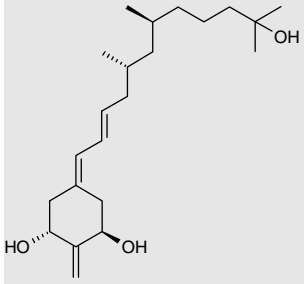
The structure–activity studies in the vitamin D field still attract attention of numerous research laboratories. Among the approximate three thousand analogs of the natural hormone 1α,25-$(OH)_2D_3$ synthesized to date, a vast majority is characterized by an intact hydrindane system of two steroidal C and D rings. Therefore, an examination of the biological effect of removing these two rings seemed to be of significant interest. Considering that the A-ring modification, consisting of a shift of the exocyclic methylene group from C-10 to C-2, exerts a positive influence on the VDR binding and transcriptional activity of the analogs,[6] we decided to synthesize an analog of 2MD lacking its C and D rings. Recently we synthesized compound **10** with such a structure and it turned out that biological activity of this analog is two logs smaller in comparison to the native hormone.[22] In continuation these studies, synthesis of its analogs substituted at C-13 with the methyl group was undertaken and is reported in this paper. Construction of the aliphatic chain, representing the vitamin D side chain (carbons 20–27) and attached carbons 17, 13, 14 and 8, began from the commercially available hydroxy ester **14**. This substrate was efficiently transformed into unsaturated esters **22** and **23** which in turn served as suitable starting compounds for the synthesis of all prepared vitamin D analogs.

A comparison of the in vitro activities of compounds described in this work with the corresponding potencies of the unsubstituted at C-13 analog **10**, obtained by us previously,[22] indicated that an introduction of a double bond between C(13) and C(17) resulted in significant decrease of the compounds' activities, especially drastic in the case of the 7*Z*-isomer **32**. The presence of the additional methyl group as a substituent of the saturated chain proved to be beneficial only in the case of the 13*R*-configuration (analog **12**) at the newly created stereogenic center.

4. Conclusions

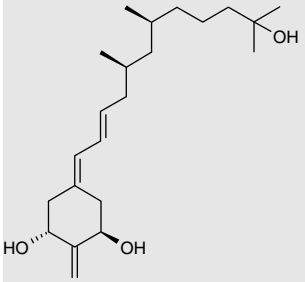
Further structure–activity studies performed on analogs of 2MD lacking the C and D rings showed that such a drastic modification of the basic vitamin D skeleton is promising and can provide compounds of pharmaceutical interest. However, introduction of

Table 1
VDR binding properties,[a] HL-60 differentiating activities,[b] and transcriptional activities of the vitamin D compounds **10–13** and **32**

Compound	Compd no.	VDR binding		HL-60 differentiation		24-OHase 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
	**10**	5.0×10^{-8} M	80	3.0×10^{-7} M	100	5.0×10^{-8} M	250
	**11**	6.0×10^{-8} M	600	$>10^{-6}$ M	>333	$>10^{-7}$ M	>500
	**32**	$>>10^{-6}$ M	>>10000	$>10^{-6}$ M	>333	$>>10^{-7}$ M	>>500
	**12**	3.0×10^{-8}M	30	6.0×10^{-9} M	2.0	3.0×10^{-9} M	15

(*continued on next page*)

Table 1 (*continued*)

Compound	Compd no.	VDR binding		HL-60 differentiation		24-OHase transcription	
		K_i	Ratio	EC_{50}	Ratio	EC_{50}	Ratio
	**13**	1.0×10^{-8} M	100	3.0×10^{-7} M	100	9.0×10^{-8} M	450

[a] Competitive binding of 1α,25-$(OH)_2D_3$ (**1**) and the synthesized vitamin D analogs 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 analogs 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 analogs concentration capable of inducing 50% maturation. The differentiation activity ratio is the average ratio of the analogs ED_{50} to the ED_{50} for 1α,25-$(OH)_2D_3$.

[c] Transcriptional assay in rat osteosarcoma cells stably transfected with a 24-hydroxylase gene reporter plasmid. The ED_{50} values are derived from dose-response curves and represent the analogs concentration capable of increasing the luciferase activity 50%. The lucerifase activity ratio is the average ratio of the analogs ED_{50} to the ED_{50} for 1α,25-$(OH)_2D_3$.

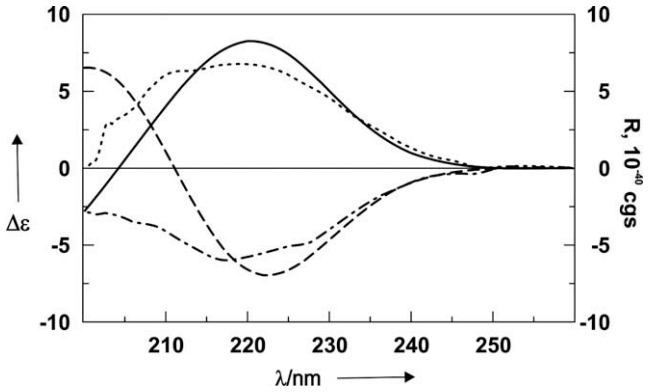


Figure 3. Experimental and simulated CD spectra of lactones **45**: (exp. ·–·–·–), (DFT – – –) and **46**: (exp. ···), (DFT ——).

a rigid moiety, namely the trisubstituted double bond C(13)=C(17), in the long aliphatic fragment consisting of carbons 8, 14, 13, 17 and steroidal side chain, practically abolishes the biological activity of such analogs. Configuration of the methyl substituents at C-13 proved to be of crucial importance: the *R*-substitution significantly enhanced the activity, whereas *S*-alkyl did not seem to play any role. These findings offer clear suggestions regarding the direction of other modifications in the related molecules. It is likely that attaching a longer or branched 13*R*-alkyl substituent will further increase the transcriptional potency and VDR affinity of *des*-C,D vitamin D compounds. Also, the influence of a double alkyl substitution at C-13 on biological activity of the analogs should be examined.

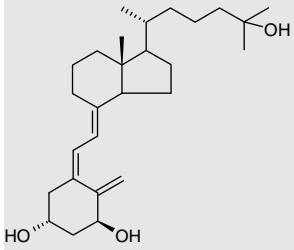
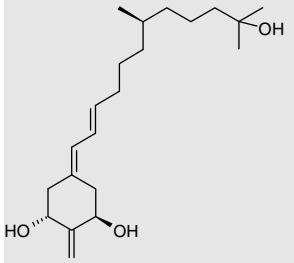
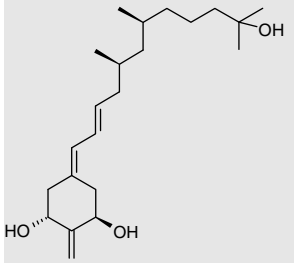
5. Experimental

Chemistry: Ultraviolet (UV) absorption spectra were recorded with a Perkin–Elmer Lambda 3B UV–vis spectrophotometer in ethanol and hexane. Circular dichroism (CD) spectra were recorded between 185 and 300 nm at room temperature with a JASCO J-715 spectropolarimeter in acetonitrile solutions. The solutions with concentrations in the range of 0.8×10^{-4}–1.2×10^{-3} mol/dm^{-3} were examined in cells with the path length of 0.1 or 1 cm. ^{1}H 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 and 100 MHz with a Varian Unity *plus* and Bruker Instrument DMX-400 spectrometers in deuteriochloroform. Chemical shifts (δ) are reported downfield from 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 before use from sodium benzophenone ketyl under argon.

5.1. (*R*)-Toluene-4-sulfonic acid 3-benzyloxymetoxy-2-methyl-propyl ester (15)

To a solution of *R*-(−)-methyl-3-hydroxy-2-methylpropionate (4 mL, 4.26 g, 36 mmol) in anhydrous CH_2Cl_2 (30 mL) was added *N*,*N*-diisopropylethylamine (11.8 mL, 8.75 g, 60 mmol) at room temperature. The mixture was cooled to −78 °C and benzyl chloromethyl ether (5.6 mL, 6.29 g, 40 mmol) was added dropwise via cannula. The cooling bath was removed and the reaction mixture was stirred at room temperature for 16 h. Then tetrabutylammonium iodide (50 mg) and benzyl chloromethyl ether (2 mL, 3.15 g, 20 mmol) was added. The mixture was stirred at room temperature for 3 h, poured into water, and extracted with methylene chloride. The combined organic layers were dried (Na_2SO_4) and concentrated under reduced pressure. The residue was chromatographed on silica gel using hexane/AcOEt (9:1) as an eluent to give (2*R*)-3-benzyloxymetoxy-2-methyl-propionic acid methyl ester (8.29 g, 97%) as a colorless oil; $[\alpha]_D^{24}$ −3 (*c* 0.17, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.19 (3H, d, *J* = 7.1 Hz, CH–C*H*$_3$), 2.77 (1H, m, C*H*–CH$_3$), 3.64 (1H, dd, *J* = 9.4, 5.4 Hz, one of C*H*$_2$–CH), 3.70 (3H, s, CH_3O), 3.78 (1H, dd, *J* = 9.4, 7.8 Hz, one of C*H*$_2$–CH), 4.57 (2H, s, OCH_2O), 4.74 (2H, s, CH_2Ph), 7.29 (1H, m, Ar-H$_{para}$), 7.35 (4H, m, Ar-H$_{ortho,meta}$); ^{13}C NMR (125 MHz) δ 13.91 (CH_3), 39.99 (*C*H–CH$_3$), 51.70 (CH_3O), 69.22 and 69.60 (*C*H$_2$CH and CH$_2$–Ph), 94.50

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

Compound	Compd no.	Bone Ca mobilization and intestinal Ca transport				Intestine (increase in S/M ratio compared to vehicle)
		Dose level		Bone (serum Ca increase compared to vehicle)		
		μg/kg bodyweight	pmol/rat/day	Serum Ca	Serum Ca increase compared to vehicle	
	**1**	0	0	4	—	—
		0.2	87	4.5	0.5	4.2
		0.7	260	5.3	1.3	2.8
		2.1	780	5.9	1.9	4.2
		19	7020	7.9	3.0	5.9
	10	0	0	4.1	—	ND
		1.9	1040	3.9	−0.2	
		5.7	3120	4.2	0.1	
		22.8	12,480	4.3	0.2	
	**13**	0	0	4.3	−0.2	−0.5
		5.5	2340	4.1	−0.1	0.4
		55	23,400	4.2	−0.2	−0.5
		165	70,200	4.2	−0.1	−0.9

[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.

(OCH_2O), 127.63, 127.84 and 128.33 ($Ar_{ortho,meta,para}$), 137.61 (Ar_{ipso}); MS (EI) *m*/*z* (relative intensity) no M^+, 207 (M^+-OCH_3, 2), 131 (34), 120 (64), 91 (100); HRMS (ESI) exact mass calcd for $C_{13}H_{18}O_4Na$ (M^++Na) 261.1103, measured 261.1110.

A solution of the obtained ester (13.3 g, 56 mmol) in anhydrous THF (55 mL) was added dropwise to the suspension of lithium aluminum hydride (3.2 g, 84 mmol) in anhydrous THF (250 mL) at 0 °C. The cooling bath was removed and the reaction was stirred at room temperature for 20 h, quenched with cold water and extracted with AcOEt. The solvents were removed in vacuum and the crude oil was purified by silica gel chromatography using hexane/AcOEt (8:2) as an eluent to afford an oily I-3-benzyloxymethoxy-2-methyl-propan-1-ol (11.1 g, 95%); $[\alpha]_D^{24}$ −3 (*c* 0.17, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.92 (3H, d, *J* = 7.1 Hz, CH–C*H*$_3$), 2.02 (1H, m, C*H*–CH_3), 2.39 (1H, s, OH), 3.54 (1H, dd, *J* = 9.4, 7.6 Hz, one of C*H*$_2$–CH), 3.60 (d, *J* = 9.4 Hz, CH_2OH), 3.65 (1H, dd, *J* = 9.4, 4.8 Hz, one of C*H*$_2$–CH), 4.6 (2H, s, OCH_2O), 4.75 (2H, s, CH_2Ph), 7.30 (1H, m, Ar-H$_{para}$), 7.35 (4H, m, Ar-H$_{ortho,meta}$); ^{13}C NMR (125 MHz) δ 13.61 (CH_3), 35.62 (*C*H–CH_3), 67.19 (CH_2OH), 69.58 (*C*H$_2$CH), 72.38 (CH_2–Ph), 94.79 (OCH_2O), 127.82, 127.90 and 128.49 ($Ar_{ortho,meta,para}$), 137.58 (Ar_{ipso}); MS (EI) *m*/*z* (relative intensity) no M^+, 180 (8), 120 (100), 108 (95), 89 (72); HRMS (ESI) exact mass calcd for $C_{12}H_{18}O_3Na$ (M^++Na) 233.1154, measured 233.1158.

To the mixture of the obtained diol (11.0 g, 50 mmol), DMAP (178 mg, 1.46 mmol) and triethylamine (28 mL, 200 mmol) in anhydrous CH_2Cl_2 (100 mL) was added tosyl chloride (14.25 g, 75 mmol) at 0 °C. The reaction mixture was allowed to warm up to room temperature and the stirring was continued overnight. Then the mixture was diluted with CH_2Cl_2 (100 mL) and it was washed with saturated aq solution of $NaHCO_3$, dried (Na_2SO_4) and concentrated under reduced pressure. The residue was chromatographed on a silica gel using hexane/AcOEt (7:3) as an eluent to give the oily tosylate **15** (18.8 g, 99%); $[\alpha]_D^{24}$ −5 (*c* 0.15, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.94 (3H, d, *J* = 7.1 Hz, CH–C*H*$_3$), 2.09 (1H, m, C*H*–CH_3), 2.42 (3H, s, CH_3Ph), 3.42 (1H, dd, *J* = 9.4, 6.6 Hz, one of C*H*$_2$–CH), 3.47 (1H, dd, *J* = 9.4, 5.1 Hz, one of C*H*$_2$–CH), 3.97 (1H, dd, *J* = 9.4, 5.8 Hz, one of C*H*$_2$–OTs), 4.03 (1H, dd, *J* = 9.4, 5.8 Hz, one of C*H*$_2$–OTs), 4.51 (2H, s, OCH_2O), 4.65 (2H, s, CH_2Ph), 7.30 (7H, br m, Ar-H), 7.78 (2H, d, *J* = 8.2 Hz, Ar-H$_{ortho}$ from tosyl); ^{13}C NMR

(125 MHz) δ 13.58 (CH_3), 21.60 (Ph-CH_3), 33.45 (*C*H–CH_3), 68.61 (*C*H_2CH), 69.27 (CH_2OTs), 71.96 (CH_2–Ph), 94.56 (OCH_2O), 127.68, 127.82, 128.36, 129.75, 132.6, 137.58 and 144.66 (Ar); MS (EI) *m*/*z* (relative intensity) no M^+, 257(M^+–OCH_2Ph, 65), 245 (55), 227 (81), 86 (100); HRMS (ESI) exact mass calcd for $C_{19}H_{24}O_5SNa$ (M^++Na) 387.1242, measured 387.1252.

5.2. (*S*)-1-Benzyloxymethoxy-2,6-dimethyl-hept-5-en (17)

4-Chloro-2-methyl-2-butane (15.5 mL, 14.4 g, 138 mmol) was added dropwise to the stirred magnesium turnings (6.75 g, 225 mmol) in anhydrous THF (465 mL) under argon at 0 °C. The stirring was continued at 0 °C for 1 h. The cooling bath was removed and the mixture was stirred at room temperature for additional 1.5 h. The mixture was then cooled to −78 °C and the formed Grignard reagent **16** was added via cannula to a solution of the tosylate **15** (10 g, 27.5 mmol) in anhydrous THF (70 mL). Then solution of Li_2CuCl_4 [previously prepared from LiCl (1.36 g, 32.1 mmol), $CuCl_2$ (2.17 g, 16.1 mmol) and CH_2Cl_2 (160 mL)] was added. The cooling bath was removed and the reaction was stirred at room temperature for 17 h. The mixture was extracted with CH_2Cl_2., the organic layer was washed with NH_4Cl and $NaHCO_3$, dried (Na_2SO_4) and evaporated. The residue was chromatographed on silica gel using hexane/AcOEt (7:3) as an eluent to give the oily olefin **17** (5.65 g, 78%); $[\alpha]_D^{24}$ +2 (*c* 0.24, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.94 (3H, d, *J* = 6.6 Hz, CH–C*H*$_3$), 1.18 and 1.46 (1H and 1H, each m), 1.60 and 1.68 [3H and 3H, each s, =C(CH_3)$_2$], 1.87 (1H, m, C*H*–CH_3), 2.05 (2H, m, =CCH_2), 3.37 (1H, dd, *J* = 9.4, 6.8 Hz, one of C*H*$_2$–CH), 3.44 (1H, dd, *J* = 9.4, 5.8 Hz, one of C*H*$_2$–CH), 4.60 (2H, s, OCH_2O), 4.76 (2H, s, CH_2Ph), 5.10 (1H, br t, *J* ~ 7 Hz, CH=C), 7.30 (1H, m, Ar-H$_{para}$), 7.34 (4H, m, Ar-H$_{ortho,meta}$); ^{13}C NMR (125 MHz) δ 16.96 (CH-*C*H$_3$), 17.53 (one of *C*H$_3$C=), 25.60 (one of *C*H$_3$C=), 32.92 (*C*H–CH_3), 33.57 (CH_2CH_2CH), 69.27 (*C*H$_2$–Ph), 73.37 (*C*H_2CH), 94.64 (OCH_2O), 124.49 (*C*–CH_3), 127.52, 127.77, 128.28 (Ar$_{ortho,meta,para}$), 137.95 [=*C*(CH_3)$_2$]; MS (EI) *m*/*z* (relative intensity) 262 (M^+, 22), 232.2 (65), 154.1 (100); HRMS (ESI) exact mass calcd for $C_{17}H_{26}O_2Na$ (M^++Na) 285.1830, measured 285.1837.

5.3. (*S*)-7-Benzyloxymethoxy-2,6-dimethyl-heptan-2-ol (18)

Olefin **17** (3.2 g, 12.2 mmol) was dissolved in anhydrous CH_2Cl_2 (60 mL) and $NaHCO_3$ (1.6 g, 18.4 mmol) was added. Then 3-chloroperoxybenzoic acid (60%, 12.8 g, 36.6 mmol) was added at room temperature with stirring. The stirring was continued for 24 h, and the mixture was diluted with ether, shaked with water and 2M NaOH. The organic layer was washed with water and saturated NH_4Cl, dried (Na_2SO_4) and evaporated. The residue was chromatographed on silica gel using hexane/AcOEt (9:1) as an eluent to give the oily mixture of (2*S*)-1-benzyloxymethoxy-2,6-dimethyl-5,6-epoxy-heptanes (2.5 g, 74%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.96 (3H, d, *J* = 6.7 Hz, CH–C*H*$_3$), 1.25 (1H, m), 1.27 and 1.31 [3H and 3H, each s, C(CH_3)$_2$], 1.5–1.7 (3H, br m), 1.79 (1H, m, C*H*–CH_3), 2.73 (1H, m, CH_2C*H*O), 3.45 (2H, br m, C*H*$_2$–CH), 4.60 (2H, s, OCH_2O), 4.76 (2H, s, CH_2Ph), 7.29 (1H, m, Ar-H$_{para}$), 7.34 (4H, d, *J* = 4.3 Hz, Ar-H$_{ortho,meta}$).

To a solution of the formed epoxides (3.05 g, 10.8 mmol) in anhydrous ether (110 mL) at 0 °C was added lithium aluminum hydride (2.0 g, 53 mmol). The cooling bath was removed and the reaction was stirred at room temperature for 18 h. Then the reaction was quenched with cold water and NH_4Cl aq and extracted with CH_2Cl_2. The solvents were removed under reduced pressure and the crude oil was chromatographed on a silica gel using hexane/AcOEt (9:1) as an eluent to give an oily alcohol **18** (2.6 g, 86%); $[\alpha]_D^{24}$ −4 (*c* 0.19, $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.94 (3H, d, *J* = 6.5 Hz, CH–C*H*$_3$), 1.20 [6H, s, (CH_3)$_2$COH], 1.75 (1H, m, C*H*–CH_3), 3.38 (1H, dd, *J* = 10.8, 6.6 Hz, one of C*H*$_2$–CH), 3.46 (1H, dd, *J* = 10.8, 6.0 Hz, one of C*H*$_2$–CH), 4.60 (2H, s, OCH_2O), 4.76 (2H, s, CH_2Ph), ca. 7.3 (5H, m, Ar-H); HRMS (ESI) exact mass calcd for $C_{17}H_{28}O_3Na$ (M^++Na) 303.1936, measured 303.1947.

5.4. (*S*)-6-(*tert*-Butyldimethylsilyloxy)-2,6-dimethyl-heptan-1-ol (19)

To a solution of alcohol **18** (1.08 g, 3.9 mmol) and 2,6-lutidine (0.9 mL, 7.7 mmol) in anhydrous CH_2Cl_2 (21 mL) at 0 °C was dropwise added *tert*-butyldimethylsilyl triflate (1.46 mL, 6.1 mmol). The solution was stirred at 0 °C for 1.5 h and poured into water. The organic layer was separated, the water phase was extracted with CH_2Cl_2. The combined extracts were washed with diluted HCl, dried ($MgSO_4$) and evaporated. The oily residue was chromatographed on silica gel using hexane/AcOEt (9:1) as an eluent to give the oily [(*S*)-6-benzyloxymethoxy-1,1,5-trimetyl-hexyloxy]-*tert*-butyldimethylsilane (1.4 g, 100%); $[\alpha]_D^{24}$ −4 (*c* 0.19, $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.06 [6H, s, Si(CH_3)$_2$], 0.85 (9H, s, Si–*t*-Bu), 0.94 (3H, d, *J* = 6.6 Hz, CH–C*H*$_3$), 1.17 [6H, s, C(CH_3)$_2$], 1.74 (1H, m, C*H*–CH_3), 3.36 (1H, dd, *J* = 9.3, 6.6 Hz, one of OC*H*$_2$–CH), 3.46 (1H, dd, *J* = 9.3, 6.1 Hz, one of OC*H*$_2$CH), 4.60 (2H, s, O–CH_2–O), 4.76 (2H, s, CH_2–Ph), 7.30 (5H, m, Ar-H); ^{13}C NMR (50 MHz) δ −1.84 [Si(CH_3)$_2$], 17.31 (CH–*C*H$_3$), 18.31 [Si*C*(CH_3)$_3$], 21.80 ($CH_2CH_2CH_2$), 26.05 [SiC(*C*H$_3$)$_3$], 29.96 and 30.09 [C(*C*H$_3$)$_2$], 33.69 (*C*H–CH_3), 34.36 (*C*$H_2CH_2CH_2$), 45.49 ($CH_2CH_2CH_2$), 69.43 (*C*H_2–Ph), 73.66 [*C*(CH_3)$_2$], 73.84 (OCH_2CH), 94.98 (OCH_2O), 127.86, 128.11 and 128.62 (Ar$_{ortho,meta,para}$), 138.21 (Ar$_{ipso}$); HRMS (ESI) exact mass calcd for $C_{23}H_{42}O_3SiNa$ (M^++Na) 417.2801, measured 417.2805.

To a solution of the formed diether (0.5 g, 1.27 mmol) in ethyl acetate (25 mL) was added Pd/C (10%, 380 mg) at room temperature. The reaction mixture was hydrogenated for 3 h under the hydrogen pressure of 10 MPa. Then the mixture was filtered and the solvent was evaporated under reduced pressure. The oily residue was applied on a silica Sep-Pak cartridge (5 g) and washed with hexane/AcOEt (9:1) to give an oily alcohol **19** (272 mg, 78%); $[\alpha]_D^{24}$ −5.3 (*c* 0.93, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.06 [6H, s, Si(CH_3)$_2$], 0.85 (9H, s, Si–*t*-Bu), 1.09 (3H, d, *J* = 7.1 Hz, CH–C*H*$_3$), 1.17 [6H, s, C(CH_3)$_2$], 1.63 (1H, m, CH_3C*H*), 3.42 (1H, dd, *J* = 6.6, 10.5 Hz, one of C*H*$_2$OH), 3.51 (1H, dd, *J* = 5.8, 10.5 Hz, one of C*H*$_2$OH); ^{13}C NMR (100 MHz) δ −2.07 [Si(CH_3)$_2$], 16.54 (CH–*C*H$_3$), 18.09 [Si*C*(CH_3)$_3$], 21.56 (CH_2–*C*H_2–CH_2), 25.82 [SiC(*C*H$_3$)$_3$], 29.76 and 29.87 [2 × C(*C*H$_3$)$_2$], 33.69 ($CH_2CH_2CH_2$), 35.78 (*C*H–CH_3), 45.28 (CH_2CH_2*C*H_2), 68.78 (CH_2OH), 73.45 [*C*(CH_3)$_2$]; HRMS (ESI) exact mass calcd for $C_{15}H_{34}O_2SiNa$ (M^++Na) 297.2226, measured 297.2191.

5.5. (*S*)-6-(*tert*-Butyldimethylsilyloxy)-2,6-dimethyl-heptanal (20)

To a solution of NMO (0.3 g, 2.6 mmol) in CH_2Cl_2 (11 mL) were added 4 Å molecular sieves (1.65 g) and the mixture was stirred at room temperature for 15 min. Then was added TPAP (30 mg, 0.08 mmol) and a solution of the alcohol **19** (0.3 g, 1.09 mmol) in CH_2Cl_2 (1.2 mL). The resulted dark mixture was stirred for 30 min, filtered through a silica Sep-Pak (5 g) and evaporated. The oily residue was dissolved in hexane, applied on a silica Sep-Pak cartridge (5 g) and washed with hexane/AcOEt (98:2) to give an oily aldehyde **20** (253 mg, 85%); $[\alpha]_D^{24}$ +14.6 (*c* 0.88, $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.06 [6H, s, Si(CH_3)$_2$], 0.85 (9H, s, Si–*t*-Bu), 0.92 (3H, d, *J* = 6.8 Hz, CH–C*H*$_3$), 1.17 [6H, s, C(CH_3)$_3$], 2.35 (1H, m, CH_3C*H*), 9.62 (1H, d, *J* = 1.9 Hz, CHO); ^{13}C NMR (50 MHz) δ −2.08 [Si(CH_3)$_2$], 13.25 (CH–*C*H$_3$), 18.07 [Si*C*(CH_3)$_2$], 21.58 (CH_2–*C*H_2–CH_2), 25.80 [SiC(*C*H$_3$)$_3$], 29.76 and 29.80 [2 × C(*C*H$_3$)$_2$], 31.02 ($CH_2CH_2CH_2$), 44.96 ($CH_2CH_2CH_2$), 46.34 (*C*H–CH_3), 73.24

[$C(CH_3)_2$], 205.31 (CHO); HRMS (ESI) exact mass calcd for $C_{15}H_{32}O_{2Si}Na$ (M^++Na) 295.2069, measured 295.2090.

5.6. 2-[*P*,*P*-Bis(2′,2′,2′-trifluoroethyl)phosphono]-4-(*tert*-butyldimethylsilyloxy)-butyric acid methyl ester (21)

To a suspension of NaH (60%, 730 mg; washed with hexane) in anhydrous DMF (6.6 mL) at 0 °C was slowly added a solution of $(F_3CCH_2O)_2POCH_2COOCH_3$ (5 g, 15.7 mmol) in anhydrous DMF (6.6 mL). The mixture was stirred at room temperature for 1.5 h and a solution of $Br(CH_2)_2OTBS$ (8.4 mL, 9.3 g, 39.2 mmol) was added in a freshly distilled HMPA (6.8 mL, 39.2 mmol). After stirring at room temperature for 48 h, the reaction mixture was diluted with ethyl acetate and poured into water. The organic phase was separated and the water layer was extracted with ethyl acetate. The combined organic extracts were washed with water, dried ($MgSO_4$) and evaporated. The oily residue was purified by column chromatography on silica gel using hexane/AcOEt (98.5:1.5) as an eluent to give a semicrystalline product **21** (2.3 g, 30%); ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.03 [6H, s, $Si(CH_3)_2$], 0.87 [9H, s, Si–*t*-Bu], 0.92 (3H, d, J = 6.8 Hz, CH–CH_3), 2.08 (1H, m, one of CH_2CH_2CH), 2.21 (1H, m, one of CH_2CH_2CH), 3.39 and 3.44 (1H and 1H, each dd, J = 10.5, 3.5 Hz, PCHC=O), 3.60 and 3.72 (1H and 1H, each m, CH_2OTBS), 3.77 (3H, $COOCH_3$), 4.42 (4H, m, 2 × CH_2OP); HRMS (ESI) exact mass calcd for $C_{15}H_{28}F_6O_6SiP$ ($M+H^+$) 477.1314, measured 477.1297.

5.7. (*Z*)- and (*E*)-(*S*)-8-(*tert*-Butyldimethylsilyloxy)-2-[2′-(*tert*-butyldimethylsilyloxy)ethyl]-4,8-dimethyl-non-2-enoic acid methyl esters (22 and 23)

To a solution of phosphono ester **21** (1 g, 2.12 mmol) and 18-crown-6 (2.5 g, 92.5 mmol) in anhydrous THF (50 mL) at −30 °C was dropwise added KHMDS (0.5 M in toluene, 4.25 mL, 2.12 mmol). After stirring for 15 min the mixture was cooled to −40 °C and a solution of the aldehyde **20** (288 mg, 1.06 mmol) in anhydrous THF (6.3 mL) was added. The mixture was stirred for 2 h at −40 °C, 1 h at −30 °C, 1 h at −20 °C, 1 h at 0 °C and finally for 18 h at room temperature. Saturated NH_4Cl was added and the mixture was extracted with ethyl acetate. The organic phase was dried ($MgSO_4$) and evaporated. The oily residue was purified by column chromatography on silica gel using hexane/AcOEt (95:5) as an eluent to give a mixture of isomeric esters **22** and **23** (1.7:1; 512 mg, 100%); HRMS (ESI) exact mass calcd for $C_{26}H_{54}O_4Si_2Na$ (M^++Na) 509.3458, measured 509.3445; ^{1}H NMR (200 MHz, $CDCl_3$) selected signals of *Z*-isomer **22**: δ 0.97 (3H, d, J = 6.6 Hz, CH–CH_3), 2.44 (2H, m, CH_2C=C), 3.66 (2H, t, J = 7.2 Hz, CH_2OTBS), 3.72 (3H, s, $COOCH_3$), 5.70 (1H, d, J = 10.2 Hz, C=CH); selected signals of *E*-isomer **23**: δ 1.00 (3H, d, J = 6.6 Hz, CH–CH_3), 2.56 (2H, t, J = 7.2 Hz, CH_2C=C), 3.62 (2H, t, J = 7.2 Hz, CH_2OTBS), 3.72 (3H, s, $COOCH_3$), 6.62 (1H, d, J = 10.2 Hz, C=CH).

5.8. (*Z*)- and (*E*)-(*S*)-8-(*tert*-Butyldimethylsilyloxy)-2-[2′-(*tert*-butyldimethylsilyloxy)ethyl]-4,8-dimethyl-non-2-en-1-ol (24 and 25)

To a stirred solution of the isomeric esters **22** and **23** (1.7:1; 250 mg, 0.52 mmol) in toluene/CH_2Cl_2 (2:1, 8.6 mL) was added at −78 °C diisobutylaluminium hydride (1.5 M in toluene, 2.3 mL, 3.3 mmol). Stirring was continued at −78 °C for 1.5 h, and the reaction 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 chromatographed on silica gel using hexane/AcOEt (99:1→95:5) as an eluent: the first product eluted was *Z*-isomer **24** (115 mg) and its *E*-isomer **25** (77.5 mg, overall yield 92%) was obtained in the later fractions.

*Compound **24** (Z-isomer)*: $[\alpha]_D^{24}$ +5.6 (*c* 1.0, $CHCl_3$), ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.04 and 0.08 [6H and 6H, each s, 2 × $Si(CH_3)_2$], 0.84 and 0.90 (9H and 9H, each s, 2 × Si–*t*-Bu), 0.93 (3H, d, J = 6.6 Hz, CH-CH_3), 1.15 and 1.16 [3H and 3H, each s, $OC(CH_3)_2$], 2.30 (2H, t, J = 5.4 Hz, CH_2C=C), 2.43 (1H, m, CH–CH_3), 3.73 (2H, m, CH_2OTBS), 4.12 (2H, m, CH_2OH), 5.08 (1H, d, J = 9.8 Hz, C=CH); ^{13}C NMR (50 MHz) δ −5.32 and −1.89 [$Si(CH_3)_2$], 18.09 and 18.30 [2 × Si$C(CH_3)_3$], 21.61 (CH–CH_3), 22.30 (CH_2), 26.02 and 26.05 [2 × SiC(CH_3)$_3$], 29.89 and 30.11 [$C(CH_3)_2$], 32.16 (*C*H–CH_3), 38.42 (CH_2), 39.19 (*C*H_2C=), 45.29 (CH_2), 61.02 (CH_2OTBS), 64.92 (CH_2OH), 73.66 [$OC(CH_3)_2$], 129.42 (*C*H=C), 136.82 (CH=*C*); HRMS (ESI) exact mass calcd for $C_{25}H_{54}O_3Si_2Na$ (M^++Na) 481.3509, measured 481.3531.

*Compound **25*** (E-isomer): $[\alpha]_D^{24}$ −7.1 (*c* 1.10, $CHCl_3$), ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.04 and 0.08 [6H and 6H, each s, 2 × $Si(CH_3)_2$], 0.83 and 0.90 (each 9H, each s, 2 × Si–*t*-Bu), 0.94 (3H, d, J = 6.6 Hz, CH–CH_3), 1.15 [6H, s, $OC(CH_3)_2$], 2.32 (2H, m, CH_2C=C), 2.42 (1H, m, CH–CH_3), 3.69 (2H, m, CH_2OTBS), 3.98 (2H, m, CH_2OH), 5.25 (1H, d, J = 10.0 Hz, C=CH); ^{13}C NMR (50 MHz) δ −5.47 and −2.09 [$Si(CH_3)_2$], 18.09 and 18.30 [2 × Si$C(CH_3)_3$], 21.07 (CH–CH_3), 22.15 (CH_2–CH_2–CH_2), 25.81 and 25.90 [2 × SiC(*C*H_3)$_3$], 29.71 and 29.93 [$C(CH_3)_2$], 32.24 (*C*H–CH_3), 32.50 (CH_2), 38.08 (*C*H_2C=), 45.18 (CH_2), 63.34 (CH_2OTBS), 68.58 (*C*H_2CO), 73.41 [*C*(CH_3)$_2$], 134.97 (*C*H=C), 136.28 (CH=*C*); HRMS (ESI) exact mass calcd for $C_{25}H_{54}O_3Si_2Na$ (M^++Na) 481.3509, measured 481.3528.

5.9. (*E*)-(*S*)-1,9-Bis-(*tert*-butyldimethylsilyloxy)-3,5,9-trimethyl-dec-3-ene (26)

To a solution of the alcohol **24** (68 mg, 0.14 mmol), Et_3N (65 μL, 0.50 mmol) and catalytic quantity of DMAP in anhydrous CH_2Cl_2 (5 mL) at 0 °C was added solution of TsCl (48 mg, 0.17 mmol) in anhydrous CH_2Cl_2 (2.8 mL). The mixture was stirred at room temperature for 2 h, and at 6 °C for 48 h, and the solvents were removed in vacuo. The residue was redissolved in anhydrous THF (8 mL), cooled to 0 °C and $LiAlH_4$ (230 mg, 6.0 mmol) was then added. After stirring at 6 °C for 18 h, water was added and the mixture was extracted with ethyl acetate. Organic extracts were washed with water, dried ($MgSO_4$) and evaporated. An oily residue was purified on a silica Sep-Pak (2 g). Elution with hexane/AcOEt (98:2) gave an oily diether **26** (37.2 mg, overall yield 57%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.04 and 0.05 [each 6H, each s, 2 × $Si(CH_3)_2$], 0.84 and 0.89 (each 9H, each s, 2 × Si–*t*-Bu), 0.90 (3H, d, J = 6.6 Hz, CH–CH_3), 1.16 [6H, s, $OC(CH_3)_2$], 1.61 (3H, d, J = 1.5 Hz, CH_3–C=C), 2.18 (2H, t, J = 7.2 Hz, CH_2C=C), 2.42 (1H, m, CH–CH_3), 3.65 (2H, t, J = 7.2 Hz, CH_2OTBS), 5.25 (1H, d, J = 10.0 Hz, C=CH); ^{13}C NMR (50 MHz) δ −5.26 and −2.08 [$Si(CH_3)_2$], 16.65 (*C*H_3C=C), 18.08 and 18.34 [2 × Si$C(CH_3)_3$], 21.20 (CH–CH_3), 22.18 (CH_2), 25.83 and 25.96 [2 × SiC(CH_3)$_3$], 29.72 and 29.90 [$C(CH_3)_2$], 32.36 (*C*H–CH_3), 38.36 (CH_2), 43.14 (CH_2), 45.16 (*C*H_2C=), 62.68 (CH_2OTBS), 73.50 [$OC(CH_3)_2$], 127.90 (CH=*C*), 133.39 (*C*H=C); HRMS (ESI) exact mass calcd for $C_{25}H_{54}O_2Si_2Na$ (M^++Na) 465.3560, measured 465.3544.

5.10. (*E*)-(*S*)-9-(*tert*-Butyldimethylsilyloxy)-3,5,9-trimethyl-dec-3-en-1-ol (27)

To a solution of diether **26** (26 mg, 58 μmol) in anhydrous THF (6 mL) was added at room temperature tetrabutylammonium fluoride (1.0 M in THF, 190 μL, 190 μmol). The mixture was stirred under argon at room temperature for 18 h, poured into brine, and extracted with ethyl acetate. 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 an

oily alcohol **27** (19 mg, 100%); $[\alpha]_D^{24}$ +5 (*c* 0.5, $CHCl_3$), ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.04 [6H, s, $Si(CH_3)_2$], 0.84 (9H, s, Si–*t*-Bu), 0.90 (3H, d, *J* = 6.6 Hz CH–CH_3), 1.16 [6H, s, $OC(CH_3)_2$], 1.63 (1H, s, CH_3C=C), 2.23 (2H, t, *J* = 6.2 Hz, CH_2C=C), 2.25 (1H, m, C*H*–CH_3), 5.02 (1H, d, *J* = 9.2 Hz, C=CH); ^{13}C NMR (50 MHz) δ −2.21 [$Si(CH_3)_2$], 15.80 (CH_3C=C), 18.01 [$SiC(CH_3)_3$], 21.16 (CH–CH_3), 22.15 (CH_2), 25.96 [$SiC(CH_3)_3$], 29.60 and 29.75 [$C(CH_3)_2$], 32.43 (*C*H–CH_3), 38.15 (CH_2), 42.59 (CH_2), 45.03 (CH_2C=C), 59.95 (CH_2OH), 73.45 [$OC(CH_3)_2$], 129.36 (CH=*C*), 135.25 (*C*H=C); HRMS (ESI) exact mass calcd for $C_{19}H_{40}O_2SiNa$ (M^++Na) 351.2696, measured 351.2690.

5.11. (*S*)-9-(*tert*-Butyldimethylsilyloxy)-3,5,9-trimethyl-dec-3-enal (28)

To a solution of NMO (22 mg, 120 μmol) in CH_2Cl_2 (0.8 mL) were added 4 Å molecular sieves (120 mg) and the mixture was stirred at room temperature for 15 min. Then was added TPAP (2.7 mg, 7.3 μmol) and a solution of the alcohol **27** (27.5 mg, 80 μmol) in CH_2Cl_2 (35 μL). The resulted dark mixture was stirred for 1 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 (99.8:0.2) to give an unstable β,γ-unsaturated aldehyde **28** (22.3 mg, 83%).

An analytical sample of the product was obtained after HPLC (10 mm × 25 cm Zorbax-Sil column, 4 mL/min) purification using a hexane/ethyl acetate (98:2) solvent system. Pure aldehyde **28** was collected at R_V 32 mL; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.04 [6H, s, $Si(CH_3)_2$], 0.84 (9H, s, Si–*t*-Bu), 0.90 (3H, d, *J* = 6.6 Hz, CH–CH_3), 1.17 [6H, s, $OC(CH_3)_2$], 1.74 (1H, s, CH_3C=C), 2.25 (1H, m, C*H*–CH_3), 3.10 (2H, m, CH_2C=C), 5.24 (1H, d, *J* = 9.8 Hz, C=CH), 9.58 (1H, *J* = 2.2 Hz, CHO).

5.12. (1*R*,3*R*)-5-[(2′*E*,5′*E*)-(*S*)-11′-Hydroxy-5′,7′,11′-trimethyl-dodeca-2′,5′-dienylidene]-2-methylene-cyclohexane-1,3-diol (30) and (1*R*,3*R*)-5-[(2′*Z*,5′*E*)-(*S*)-11′-hydroxy-5′,7′,11′-trimethyl-dodeca-2′,5′-dienylidene]-2-methylene-cyclohexane-1,3-diol (31)

To a solution of phosphine oxide **29** (113 mg, 194 μmol) in anhydrous THF (1.9 mL) at −78 °C was slowly added phenyllithium (1.8 M in cyclohexane, 108 μL, 194 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 **28** (22 mg, 60 μmol) in anhydrous THF (370 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 (99.9:0.1) gave a mixture of protected vitamin D analogs **30** and **31** (15.5 mg, 30%). Main product was identified as (1*R*,3*R*)-1,3-bis-(*tert*-butyldimethylsilyloxy)-5-[(2′*E*,5′*E*)-(*S*)-11′-(*tert*-butyldimethylsilyloxy)-5′,7′,11′-trimethyl-dodeca-2′,5′-dienylidene]-2-methylene-cyclohexane (**30**).

5.12.1. (20*S*)-1α,25-Bis-(tert-butyldimethylsilyloxy)-2-methylene-8(12),14(17)-diseco-13(17)-dehydro-9,11,15,16,18,19-hexanorvitamin D_3 *tert*-butyldimethylsilyl ether (30)

UV (in hexane) λ_{max} 237.5, 244.5, 253.0 nm; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.027, 0.038, 0.052 and 0.063 [each 3H, each s, 4 × $(SiCH_3)_2$], 0.050 [6H, s, $(SiCH_3)_2$], 0.85, 0.87 and 0.89 [each 9H, each s, 3 × Si–*t*-Bu], 0.91 (3H, d, *J* = 6.7 Hz, 21-H_3), 1.16 (6H, s, 26- and 27-H_3), 1.76 (3H, 12-H_3), 2.29–2.48 (3H, br m), 2.72 (2H, d, *J* = 6.9 Hz, 14-H_2), 4.42 (2H, m, 1β- and 3α-H), 4.93 and 4.97 (each 1H, each s, C=CH_2), 5.56 (1H, dt, *J* = 14.9, 6.9 Hz, 8-H), 5.91 (1H, d, *J* = 10.8 Hz, 6-H), 6.25 (1H, dd, *J* = 14.9, 10.8 Hz, 7-H); ^{13}C NMR (100 MHz) δ −5.05, −4.88 and −2.06 [3 × $Si(CH_3)_2$], 16.42 (C-12), 18.11 [3 × $SiC(CH_3)_3$], 21.23 (C-20), 22.24 (C-23), 25.75, 25.82 and 25.86 [3 × $SiC(CH_3)_3$], 29.79 and 30.03 (C-26 and C-27), 32.47 (C-21), 38.42 and 47.20 (C-4 and C-10), 38.88 (C-22), 43.24 (C-24), 45.22 (C-14), 71.73 and 72.30 (C-1 and C-3), 73.32 (C-25), 106.34 (C=CH_2), 127.03 (C-6), 131.47 (C-7), 132.06 (C-8), 132.44 (C-17), 133.28 (C-5), 152.86 (C-2), 160.55 (C-13); HRMS (ESI) exact mass calcd for $C_{14}H_{78}O_3Si_3Na$ (M^++Na) 713.5151, measured 713.5157.

To a solution of protected vitamins **30** and **31** (3.5 mg, 5 μmol) in THF (120 μL) and methanol (120 μL) was added 46% HF/MeCN (1:9, 60 μL) at room temperature. After stirring for 7 h, a saturated $NaHCO_3$ was added. The mixture was extracted with CH_2Cl_2, organic extracts were washed with brine, dried ($MgSO_4$) and evaporated. The residue was first purified on a silica Sep-Pak (2 g). Elution with hexane/ethyl acetate (1:1) gave an oily mixture of deprotected vitamins **11** and **32** (1.2 mg, 70%). Separation of the isomers was achieved by HPLC (9.4 mm × 25 cm Zorbax-Sil column, 4 mL/min) using a hexane/2-propanol (8.5:1.5) solvent system. Pure vitamin D analog **11** was collected at R_V 35 mL, and isomeric compound **32** (37 μg) was collected at R_V 33 mL. Purity of both vitamins was checked by reversed-phase HPLC (9.4 mm × 25 cm, Eclipse XDB-C18 column, 4 mL/min) using a methanol/water (85:15) solvent system. Vitamin D compounds **11** and **32** gave sharp peaks at R_V 21 and 22 mL, respectively.

5.12.2. (20*S*)-1α,25-Dihydroxy-2-methylene-8(12),14(17)-diseco-13(17)-dehydro-9,11,15,16,18,19-hexanorvitamin D_3 (11)

UV (in EtOH) λ_{max} 237.5, 244.5, 253.0 nm; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.92 (3H, d, *J* = 6.7 Hz, 21-H_3), 1.20 (6H, s, 26- and 27-H_3), 1.60 (3H, br s, 12-H_3), 2.27 (1H, dd, *J* = 13.2, 6.6 Hz, 4β-H), 2.35 (1H, dd, *J* = 13.0, 7.6 Hz, 10α-H), 2.56 (1H, dd, *J* = 13.2, 4.2 Hz, 4α-H), 2.73 (1H, dd, *J* = 13.0, 4.2 Hz, 10β-H), 2.75 (2H, d, *J* = 6.9 Hz, 14-H_2), 4.48 (2H, m, 1β- and 3α-H), 4.94 (1H, d, *J* = 9.1 Hz, 17-H), 5.09 and 5.11 (each 1H, each s, C=CH_2); 5.69 (1H, dt, *J* = 14.9, 6.9 Hz, 8-H), 6.04 (1H, d, *J* = 10.8 Hz, 6-H), 6.30 (1H, dd, *J* = 14.9, 10.8 Hz, 7-H); HRMS (ESI) exact mass calcd for $C_{22}H_{36}O_3$ (M^++Na) 371.2562, measured 371.2559.

5.12.3. (7*Z*)-(20*S*)-1α,25-Dihydroxy-2-methylene-8(12),14(17)-diseco-13(17)-dehydro-9,11,15,16,18,19-hexanorvitamin D_3 (32)

UV (in EtOH) λ_{max} 238.5, 245.5 nm; ^{1}H NMR (600 MHz, $CDCl_3$) δ 0.92 (3H, d, *J* = 6.6 Hz, 21-H_3), 1.20 (6H, s, 26- and 27-H_3), 1.61 (3H, br s, 12-H_3), 2.31 (1H, dd, *J* = 13.0, 7.1 Hz, 4β-H), 2.34 (2H, m, 14-H_2), 2.41 (1H, dd, *J* = 13.2, 7.1 Hz, 10α-H), 2.61 (1H, dd, *J* = 13.0, 4.0 Hz, 4α-H), 2.72 (1H, dd, *J* = 13.2, 4.0 Hz, 10β-H), 4.50 (2H, m, 1β- and 3α-H), 4.93 (1H, d, *J* = 9.3 Hz, 17-H), 5.11 (2H, s, C=CH_2), 5.48 (1H, m, 8-H), 6.33 (2H, m, 6- and 7-H).

5.13. (2*R*,4*S*)- and (2*S*,4*S*)-8-(*tert*-Butyldimethylsilyloxy)-2-[2′-(*tert*-butyldimethylsilyloxy)ethyl]-4,8-dimethyl-nonan-1-ol (33 and 34)

To a solution of the alcohol **25** (99 mg, 0.2 mmol mmol) in anhydrous methanol (4 mL) was added PtO_2 (30 mg) at room temperature. The mixture was stirred for 3 days in hydrogen atmosphere, and each day three portions of PtO_2 (30 mg) were added. The mixture was filtered, the solvent evaporated and the oily residue was purified on a silica Sep-Pak (2 g). Elution with hexane/AcOEt (95:5) gave an oily mixture of isomeric alcohols **33** and **34** (1:1.3, 172 mg, 72%). The isomers were separated by HPLC (9.4 mm × 25 cm Zorbax-Sil column, 4 mL/min) using a hexane/ethyl acetate (95:5) solvent system. (2*R*,4*S*)-Alcohol **33** was col-

lected at R_V 45 mL, whereas (2*S*,4*S*)-alcohol **34** was collected at R_V 50 mL.

Compound ***33***: $[\alpha]_D^{24}$ +3.6 (*c* 0.96, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 0.05 and 0.08 [each 6H, each s, 2 × Si(CH_3)$_2$], 0.85 and 0.90 (each 9H, each s, 2 × Si–*t*-Bu), 0.87 (3H, d, *J* = 6.6 Hz, CH–CH_3), 1.17 [6H, s, C(CH_3)$_2$], 1.74 (1H, m, C*H*–CH_3), 3.38 (1H, dd, *J* = 11.0, 7.1 Hz, one of CH_2OH), 3.59 (1H, dd, *J* = 11.0, 3.4 Hz, one of CH_2OH), 3.66 (1H, m, one of CH_2OSiTBS), 3.78 (1H, m, one of CH_2OSiTBS); ^{13}C NMR (50 MHz) δ −5.49 and −2.07 [Si(CH_3)$_2$], 18.09 and 18.21 [Si*C*(CH_3)$_3$], 19.86 (CH–*C*H_3), 21.50 (CH_2–CH_2–CH_2), 25.85 [SiC(*C*H_3)$_3$], 29.78 and 29.78 [2 × C(*C*H_3)$_2$], 30.10 (*C*H–CH_3), 36.39 (CH_2), 37.05 (*C*HCH_2–OH), 37.76 (CH_2), 39.53 (CH_2), 45.30 (CH_2), 61.86 (*C*H_2OTBS), 66.05 (*C*H_2OH), 73.49 [*C*(CH_3)$_2$]; HRMS (ESI) exact mass calcd for $C_{25}H_{56}O_3Si_2Na$ (M^++Na) 483.3666, measured 483.3670.

Compound ***34***: $[\alpha]_D^{24}$ −3.2 (*c* 1.16, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 0.055 and 0.087 [each 6H, each s, 2 × Si(CH_3)$_2$], 0.85 and 0.91 (each 9H, each s, 2 × Si–*t*-Bu), 0.86 (3H, d, *J* = 6.6 Hz, CH–CH_3), 1.17 [6H, s, C(CH_3)$_2$], 3.41 (1H, m, one of CH_2OH), 3.57 (1H, m, one of CH_2OH), 3.65 (1H, m, one of CH_2OSiTBS), 3.78 (1H, m, one of CH_2OSiTBS); ^{13}C NMR (50 MHz) δ −5.50 and −2.07 [Si(CH_3)$_2$], 18.08 and 18.21 [Si*C*(CH_3)$_3$], 19.70 (CH–*C*H_3), 21.55 (CH_2–*C*H_2–CH_2), 25.61 and 25.83 [2 × SiC(*C*H_3)$_3$], 26.90 (*C*H–CH_3), 29.73 and 29.89 [2 × C(*C*H_3)$_2$], 29.98 (*C*H–CH_3), 35.40 (CH_2), 37.24 (*C*HCH_2–OH), 37.76 (CH_2), 39.31 (CH_2), 45.28 (CH_2), 61.92 (*C*H_2OSiTBS), 66.93 (*C*H_2OH), 73.46 [*C*(CH_3)$_2$]; HRMS (ESI) exact mass calcd for $C_{25}H_{56}O_3Si_2Na$ (M^++Na) 483.3666, measured 483.3664.

5.14. (2*R*)-2-[(2′*S*)-6′-(*tert*-Butyldimethylsilyloxy)-2′,6′-dimethyl-heptyl]-butane-1,4-diol (35)

To a solution of diether **33** (15 mg, 33 μmol) in anhydrous THF (3.8 mL) was added at room temperature tetrabutylammonium fluoride (1.0 M in THF, 125 μL, 125 μmol). The mixture was stirred under argon at room temperature for 1.5 h, poured into brine, and extracted with ethyl acetate. Organic extracts were washed with brine, dried ($MgSO_4$), and evaporated. The oily residue was purified on a silica Sep-Pak (0.5 g). Elution with hexane/AcOEt (6:4) gave an oily diol **35** (11 mg, 100%); $[\alpha]_D^{24}$ +11.0 (*c* 0.45, $CHCl_3$); ^1H NMR (200 MHz, $CDCl_3$) δ 0.05 [6H, s, Si(CH_3)$_2$], 0.84 (9H, s, Si–*t*-Bu), 0.89 (3H, d, *J* = 6.4 Hz, CH–CH_3), 1.17 [6H, s, OC(CH_3)$_2$], 3.44 (1H, dd, *J* = 10.7, 7.1 Hz, one of CHCH_2OH), 3.6–3.9 (3H, br m, CH_2CH_2OH and one of CHCH_2OH); ^{13}C NMR (50 MHz) δ −2.05 [Si(CH_3)$_2$], 18.12 [Si*C*(CH_3)$_3$], 19.86 (CH–*C*H_3), 21.51 (CH_2–*C*H_2–CH_2), 25.85 [SiC(*C*H_3)$_3$], 29.78 [C(*C*H_3)$_2$], 30.15 (*C*H–CH_3), 36.62 (CH_2), 36.73 (*C*HCH_2–OH), 37.76 (CH_2), 39.48 (CH_2), 45.29 (CH_2), 61.24 (*C*H_2OH), 66.25 (*C*H_2OH), 73.48 [OC(CH_3)$_2$]; HRMS (ESI) exact mass calcd for $C_{19}H_{42}O_3SiNa$ (M^++Na) 369.2801, measured 369.2798.

5.15. (2*S*)-2-[(2′*S*)-6′-(*tert*-Butyldimethylsilyloxy)-2′,6′-dimethyl-heptyl]-butane-1,4-diol (36)

Deprotection of a primary hydroxyl in isomeric diether **34** (12 mg, 26 μmol) was performed analogously to the process described above for **33**. Pure diol **36** (9 mg, 100%) was eluted from a Sep-Pak cartridge with hexane/ethyl acetate (6:4); $[\alpha]_D^{24}$ +7.0 (*c* 0.35, $CHCl_3$); ^1H NMR (200 MHz, $CDCl_3$) δ 0.06 [6H, s, Si(CH_3)$_2$], 0.84 (9H, s, Si–*t*-Bu), 0.86 (3H, d, *J* = 6.4 Hz, CH–CH_3), 1.17 [6H, s, OC(CH_3)$_2$], 3.46 (1H, dd, *J* = 10.7, 7.1 Hz, CHCH_2OH), 3.6–3.8 (3H, br m, CH_2CH_2OH and one of CHCH_2OH); ^{13}C NMR (50 MHz) δ −2.05 [Si(CH_3)$_2$], 18.11 [Si*C*(CH_3)$_3$], 19.72 (CH–*C*H_3), 21.55 (CH_2CH_2–CH_2), 25.84 [SiC(*C*H_3)$_3$], 29.77 [C(*C*H_3)$_2$], 30.02 (*C*H–CH_3), 35.60 (CH_2), 36.89 (*C*HCH_2–OH), 37.92 (CH_2), 39.30 (CH_2), 45.30 (CH_2), 61.29 (*C*H_2OH), 67.13 (*C*H_2OH), 73.47 [OC(CH_3)$_2$]; HRMS (ESI) exact mass calcd for $C_{19}H_{42}O_3SiNa$ (M^++Na) 369.2801, measured 369.2803.

5.16. (3*R*,5*S*)-1,9-Bis-(*tert*-butyldimethylsilyloxy)-3,5,9-trimethyl-decane (37)

To a solution of the alcohol **33** (32 mg, 70 μmol), Et_3N (38 μL, 290 μmol) and catalytic quantity of DMAP in anhydrous CH_2Cl_2 (155 μL) at 0 °C was added TsCl (30 mg, 106 μmol). The mixture was stirred at room temperature for 18 h, and the solvents were removed in vacuo. The residue was redissolved in anhydrous THF (4 mL), cooled to 0 °C and $LiAlH_4$ (88 mg, 2.35 mmol) was then added. After stirring at 6 °C for 6 h, water was added and the mixture was extracted with ethyl acetate. Organic extracts were washed with water, dried ($MgSO_4$) and evaporated. An oily residue was purified on a silica Sep-Pak (2 g). Elution with hexane/AcOEt (98:2) gave an oily diether **37** (21.4 mg, overall yield 70%); $[\alpha]_D^{24}$ +4.2 (*c* 0.50, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 0.046 and 0.057 [each 6H, each s, 2 × Si(CH_3)$_2$], 0.86 (9H, s, Si–*t*-Bu), 0.90 (9H, s, Si–*t*-Bu and 2 × 3H, CH–CH_3), 1.18 [6H, s, C(CH_3)$_2$], 3.64 (2H, m, CH_2OTBS); ^{13}C NMR (50 MHz) δ −5.24 and −2.05 [Si(CH_3)$_2$], 18.12 and 18.32 [Si*C*(CH_3)$_3$], 19.48 (CH–*C*H_3), 19.58 (CH–*C*H_3), 21.61 (CH_2–*C*H_2–CH_2), 25.86 and 25.98 [2 × SiC(*C*H_3)$_3$], 26.80 (*C*H–CH_3), 29.76 and 29.91 [2 × C(*C*H_3)$_2$], 30.01 (*C*H–CH_3), 38.36 (CH_2), 40.83 (CH_2), 44.91 (CH_2), 45.36 (CH_2), 61.38 (*C*H_2OTBS), 73.57 [*C*(CH_3)$_2$]; HRMS (ESI) exact mass calcd for $C_{25}H_{56}O_2Si_2Na$ (M^++Na) 467.3717, measured 467.3733.

5.17. (3*S*,5*S*)-1,9-Bis-(*tert*-butyldimethylsilyloxy)-3,5,9-trimethyl-decane (38)

Tosylation of the alcohol **34** (34 mg, 74 μmol) and the tosylate reduction were performed analogously to the process described above for the isomeric alcohol **33**. After analogous work-up of the reaction mixture the product was purified on a silica Sep-Pak (2 g). Elution with hexane/AcOEt (98:2) gave an oily diether **38** (23.7 mg, overall yield 72%).

Compound ***38***: $[\alpha]_D^{24}$ −1.1 (*c* 1.15, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 0.055 and 0.066 [each 6H, each s, 2 × Si(CH_3)$_2$], 0.85 and 0.89 (each 9H, each s, 2 × Si–*t*-Bu), 0.82 (3H, d, *J* = 6.4 Hz, CH–CH_3), 0.84 (3H, d, *J* = 6.5 Hz, CH–CH_3), 1.17 [6H, s, C(CH_3)$_2$], 3.64 (2H, m, CH_2OTBS); ^{13}C NMR (50 MHz) δ −5.23 and −2.04 [Si(CH_3)$_2$], 18.11 and 18.33 [Si*C*(CH_3)$_3$], 20.14 (CH–*C*H_3), 20.38 (CH–*C*H_3), 21.60 (CH_2–*C*H_2–CH_2), 25.86 and 25.99 [2 × SiC(*C*H_3)$_3$], 29.82 and 29.87 [2 × C(*C*H_3)$_2$], 30.03 (*C*H–CH_3), 36.40 (CH_2), 37.52 (CH_2), 39.87 (CH_2), 45.39 (CH_2), 61.46 (*C*H_2OTBS), 73.57 [*C*(CH_3)$_2$]; HRMS (ESI) exact mass calcd for $C_{25}H_{56}O_2Si_2Na$ (M^++Na) 467.3717, measured 467.3737.

5.18. (3*R*,5*S*)-9-(*tert*-Butyldimethylsilyloxy)-3,5,9-trimethyl-decanal (39)

To a solution of diether **37** (21.4 mg, 48 μmol) in anhydrous THF (7 mL) was added tetrabutylammonium fluoride (1.0 M w THF, 75 μL, 75 μmol) at room temperature. The mixture was stirred under argon at room temperature for 18 h, poured into brine and extracted with ethyl acetate. The combined organic extracts were washed with brine, dried ($MgSO_4$) and evaporated. The oily product was purified on a silica Sep-Pak (2 g). Elution with hexane/AcOEt (9:1) gave an oily (3*R*,5*S*)-9-(*tert*-butyldimethylsilyloxy)-3,5,9-trimethyl-decan-1-ol (16.5 mg, 100%); $[\alpha]_D^{24}$ +6.6 (*c* 0.34, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 0.06 [6H, s, Si(CH_3)$_2$], 0.86 (9H, s, Si–*t*-Bu), 0.84 (3H, d, *J* = 6.8 Hz, CH–CH_3), 0.87 (3H, d, *J* = 6.8 Hz, CH–CH_3), 1.17 [6H, s, C(CH_3)$_2$], 3.68 (2H, m, CH_2OH); ^{13}C NMR (100 MHz) δ −2.06 [Si(CH_3)$_2$], 18.11 [Si*C*(CH_3)$_3$], 19.43 (CH–*C*H_3), 19.56 (CH–*C*H_3), 21.60 (CH_2–*C*H_2–CH_2), 25.85

[SiC(CH_3)$_3$], 26.91 (*C*H–CH_3), 29.78 and 29.90 [2 × C(CH_3)$_2$], 30.02 (*C*H–CH_3), 38.43 (CH_2), 40.87 (CH_2), 44.91 (CH_2), 45.33 (CH_2), 61.20 (*C*H$_2$OH), 73.54 [*C*(CH_3)$_2$]; HRMS (ESI) exact mass calcd for $C_{19}H_{42}O_2SiNa$ (M^++Na) 453.2852, measured 453.2845.

To a solution of NMO (14 mg, 0.12 mmol) in CH_2Cl_2 (0.5 mL) were added 4 Å molecular sieves (75 mg) and the mixture was stirred at room temperature for 15 min. Then was added TPAP (1 mg, 2.66 μmol) and a solution of the obtained alcohol (16.5 mg, 50 μmol) in CH_2Cl_2 (100 μL). The resulted dark mixture was stirred for 20 min, 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 (98:2) to give an oily aldehyde **39** (11.2 mg, 68%); $[\alpha]_D^{24}$ +4.0 (*c* 0.15, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.06 [6H, s, Si(CH_3)$_2$], 0.85 (9H, s, Si–*t*-Bu), 0.87 (3H, d, *J* = 6.8 Hz, CH–C*H*$_3$), 0.93 (3H, d, *J* = 6.5 Hz, CH–C*H*$_3$), 1.17 [6H, s, C(CH_3)$_2$], 2.11 (1H, m, C*H*–CH_3), 2.24 (1H, ddd, *J* = 15.8, 7.6, 2.2 Hz, one of C*H*$_2$CHO), 2.35 (1H, ddd, *J* = 15.8, 5.7, 2.2 Hz, one of C*H*$_2$CHO), 9.75 (1H, t, *J* = 2.2 Hz, CHO); ^{13}C NMR (100 MHz) δ −2.09 [Si(CH_3)$_2$], 18.08 [Si*C*(CH_3)$_3$], 19.30 (CH–*C*H$_3$), 19.62 (CH–*C*H$_3$), 21.48 (CH_2–*C*H$_2$–CH_2), 25.60 (*C*H–CH_3), 25.82 [SiC(*C*H$_3$)$_3$], 29.94 (*C*H–CH_3), 29.75 and 29.89 [2 × C(CH_3)$_2$], 38.12 (CH_2), 44.53 (CH_2), 45.23 (CH_2), 51.80 (*C*H$_2$CHO), 73.44 [*C*(CH_3)$_2$], 203.16 (CHO); HRMS (ESI) exact mass calcd for $[C_{18}H_{37}O_2Si]^+$ 313.2563, measured 313.2558.

5.19. (3*S*,5*S*)-9-(*tert*-Butyldimethylsilyloxy)-3,5,9-trimethyldecanal (40)

Selective deprotection of the primary hydroxyl group in the diether **38** (27.5 mg, 62 μmol) was performed analogously to the process described above for the isomeric compound **37**. After analogous work-up of the reaction mixture the oily product was purified on a silica Sep-Pak (2 g). Elution with hexane/AcOEt (9:1) gave an oily (3*S*,5*S*)-9-(*tert*-butyldimethylsilyloxy)-3,5,9-trimethyl-decan-1-ol (20.7 mg, 100%); $[\alpha]_D^{24}$ −4.6 (*c* 0.78, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.066 [6H, s, Si(CH_3)$_2$], 0.84 (3H, d, *J* = 6.8 Hz, CH–C*H*$_3$), 0.86 (9H, s, Si–*t*-Bu), 0.87 (3H, d, *J* = 6.8 Hz, CH–C*H*$_3$), 1.17 [6H, s, C(CH_3)$_2$], 3.68 (2H, m, CH_2OH); ^{13}C NMR (100 MHz) δ −2.06 [Si(CH_3)$_2$], 18.10 [Si*C*(CH_3)$_3$], 19.19 (CH–*C*H$_3$), 19.20 (CH–*C*H$_3$), 21.53 (CH_2–*C*H$_2$–CH_2), 25.84 [SiC(*C*H$_3$)$_3$], 26.96 (*C*H–CH_3), 29.78 and 29.87 [2 × C(CH_3)$_2$], 30.01 (*C*H–CH_3), 37.36 (CH_2), 39.90 (CH_2), 44.90 (CH_2), 45.35 (CH_2), 61.25 (*C*H$_2$OH), 73.53 [*C*(CH_3)$_2$]; HRMS (ESI) exact mass calcd for $C_{19}H_{42}O_2SiNa$ (M^++Na) 453.2852, measured 453.2852.

Oxidation of the obtained alcohol (15.5 mg, 47 μmol) was performed analogously to the process described above for the isomeric compound. After analogous work-up of the reaction mixture the oily product was purified on a silica Sep-Pak (2 g). Elution with hexane/AcOEt (98:2) gave an oily aldehyde **40** (15.4 mg, 95%); $[\alpha]_D^{24}$ −10.6 (*c* 0.72, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.06 [6H, s, Si(CH_3)$_2$], 0.85 (9H, s, Si–*t*-Bu), 0.87 (3H, d, *J* = 6.6 Hz, C*H*–CH_3), 0.95 (3H, d, *J* = 6.2 Hz, CH–C*H*$_3$), 1.18 [6H, s, C(CH_3)$_2$], 2.10–2.22 (2H, br m, C*H*–CH_3 and one of C*H*$_2$CHO), 2.39 (1H, m, one of C*H*$_2$CHO), 9.76 (1H, t, *J* = 2.3 Hz, CHO); ^{13}C NMR (100 MHz) δ −2.08 [Si(CH_3)$_2$], 18.09 [Si*C*(CH_3)$_3$], 19.97 (CH–*C*H$_3$), 20.55 (CH–*C*H$_3$), 21.40 (CH_2–*C*H$_2$–CH_2), 25.67 (*C*H–CH_3), 25.82 [SiC(*C*H$_3$)$_3$], 29.77 and 29.88 [2 × C(CH_3)$_2$], 30.01 (*C*H–CH_3), 38.18 (CH_2), 44.80 (CH_2), 45.27 (CH_2), 50.95 (*C*H$_2$CHO), 73.45 [*C*(CH_3)$_2$], 203.20 (CHO); HRMS (ESI) exact mass calcd for $[C_{18}H_{37}O_2Si]^+$ 313.2563, measured 313.2549.

5.20. (1*R*,3*R*)-1,3-Bis-(*tert*-butyldimethylsilyloxy)-5-[(*E*)-(5′*R*,7′*S*)-11′-(*tert*-butyldimethylsilyloxy)-5′,7′,11′-trimethyl-dodec-2′-enylidene]-2-methylene-cyclohexane (41)

To a solution of phosphine oxide **29** (60 mg, 102 μmol) in anhydrous THF (1 mL) at −78 °C was slowly added phenyllithium (1.8 M in cyclohexane, 57 μL, 102 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 **39** (11 mg, 34 μmol) in anhydrous THF (210 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 protected vitamin D analog **41** (2.8 mg, 12%).

5.20.1. (13*R*,20*S*)-1α,25-Bis-(tert-butyldimethylsilyloxy)-2-methylene-8(12),14(17)-diseco-9,11,15,16,18,19-hexanorvitamin D_3 *tert*-butyldimethylsilyl ether (41)

UV (in hexane) λ_{max} 236.0, 243.5, 252.0 nm; ^{1}H NMR (500 MHz, $CDCl_3$; *vitamin D numbering*) δ 0.027, 0.038, 0.064 and 0.066 [each 3H, each s, 4 × Si(CH_3)$_2$], 0.057 [6H, s, ($SiCH_3$)$_2$], 0.82 (3H, d, *J* = 6.4 Hz, 20-H_3), 0.83 (3H, d, *J* = 6.5 Hz, 13-H_3), 0.85, 0.87 and 0.89 [3 × 9H, each s, 3 × Si–*t*-Bu], 1.17 (6H, s, 26- and 27-H_3), 1.92 (1H, m, 21-H), 2.03 (2H, m, 14-H_2), 2.15 (1H, dd, *J* = 13.2, 7.7 Hz, 4β-H), 2.40 (3H, m, 4α-H, 10α- and 10β-H), 4.42 (2H, m, 1β- and 3α-H), 4.93 and 4.96 (each 1H, each s, C=C*H*$_2$), 5.60 (1H, dt, *J* = 14.6, 7.4 Hz, 8-H), 5.90 (1H, d, *J* = 10.8 Hz, 6-H), 6.22 (1H, dd, *J* = 14.6, 10.8 Hz, 7-H); HRMS (ESI) exact mass calcd for $C_{40}H_{80}O_3Si_3Na$ (M^++Na) 715.5313, measured 715.5329.

5.21. (1*R*,3*R*)-1,3-Bis-(*tert*-butyldimethylsilyloxy)-5-[(*E*)-(5′*S*,7′*S*)-11′-(*tert*-butyldimethylsilyloxy)-5′,7′,11′-trimethyl-dodec-2′-enylidene]-2-methylene-cyclohexane (42)

Wittig–Horner reaction of the aldehyde **40** (14.5 mg, 44 μmol) with the anion generated from the phosphine oxide **29** (77 mg, 132 μmol) was performed analogously to the process described above for the isomeric compound **39**. After analogous work-up of the reaction mixture the oily product was purified on a silica Sep-Pak (2 g). Elution with hexane/AcOEt (98:2) gave a protected vitamin D analog **42** (8.8 mg, 30%). This product was further purified by HPLC (9.4 mm × 25 cm Zorbax-Sil column, 4 mL/min) using a hexane/2-propanol (99.9:0.1) solvent system. Pure protected vitamin D analog **42** was collected at R_V 13 mL.

5.21.1. (13*S*,20*S*)-1α,25-Bis-(*tert*-butyldimethylsilyloxy)-2-methylene-8(12),14(17)-diseco-9,11,15,16,18,19-hexanorvitamin D_3 *tert*-butyldimethylsilyl ether (42)

UV (in hexane) λ_{max} 236.0, 243.0, 252.0 nm; ^{1}H NMR (400 MHz, $CDCl_3$; *vitamin D numbering*) δ 0.027 and 0.037 [each 3H, each s, 4 × Si(CH_3)$_2$], 0.059 and 0.064 [each 6H, each s, Si(CH_3)$_2$], 0.85, 0.87 and 0.89 [3 × 9H, each s, 3 × Si–*t*-Bu], 0.85–0.88 (6H, overlapped with Si–*t*-Bu, 13- and 20-H_3), 1.18 (6H, s, 26- and 27-H_3), 1.83 (1H, m, 21-H), 2.39 (4H, m), 4.41 (2H, m, 1β- and 3α-H), 4.93 and 4.96 (each 1H, each s, C=C*H*$_2$), 5.58 (1H, dt, *J* = 14.7, 7.4 Hz, 8-H), 5.90 (1H, d, *J* = 10.7 Hz, 6-H), 6.23 (1H, dd, *J* = 14.7, 10.7 Hz, 7-H); HRMS (ESI) exact mass calcd for $C_{40}H_{80}O_3Si_3Na$ (M^++Na) 715.5313, measured 715.5341.

5.22. (1*R*,3*R*)-5-[(*E*)-(5′*R*,7′*S*)-11′-Hydroxy-5′,7′,11′-trimethyl-dodec-2′-enylidene]-2-methylene-cyclohexane-1,3-diol (12)

To a solution of protected vitamin **41** (2.8 mg, 3 μmol) in THF (1 mL) and acetonitrile (0.5 mL) was added 46% HF/MeCN (1:9, 1 mL) at room temperature. After stirring for 6 h, a saturated $NaHCO_3$ was added. The mixture was extracted with CH_2Cl_2, 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 deprotected vitamin **12** (387 μg, 22%). Purification of this compound was achieved by HPLC

(9.4 mm × 25 cm Zorbax RX-Sil column, 3 mL/min) using a hexane/2-propanol (85:15) solvent system. Vitamin D analog was collected at R_V 25 mL. Further purification was performed by reversed-phase HPLC (9.4 mm × 25 cm, Eclipse XDB-C18 column, 3 mL/min) using a methanol/water (85:15) solvent system. Pure vitamin D compound **12** was collected at R_V 19 mL.

5.22.1. (13*R*,20*S*)-1α,25-dihydroxy-2-methylene-8(12),14(17)-diseco-9,11,15,16,18,19-hexanorvitamin D_3 (12, 13MeLP)

UV (in EtOH) λ_{max} 233.5, 242.0, 251.5 nm; ^{1}H NMR (500 MHz, $CDCl_3$; *vitamin D numbering*) δ 0.83 (3H, d, J = 6.4 Hz, 20-H_3), 0.84 (3H, d, J = 6.4 Hz, 13-H_3), 1.22 (6H, s, 26- and 27-H_3), 2.06 (2H, m, CH_2), 2.26 (1H, dd, J = 13.1, 7.0 Hz, 10α-H), 2.40 (1H, dd, J = 13.2, 7.4 Hz, 4β-H), 2.57 (1H, dd, J = 13.2, 4.1 Hz, 4α-H), 2.70 (1H, dd, J = 13.2, 4.0 Hz, 10β-H), 4.49 (2H, m, 1β- and 3α-H), 5.10 (2H, s, C=CH_2); 5.69 (1H, dt, J = 14.8, 7.3 Hz, 8-H), 6.04 (1H, d, J = 10.8 Hz, 6-H), 6.30 (1H, dd, J = 14.8, 10.8 Hz, 7-H); HRMS (ESI) exact mass calcd for $C_{22}H_{38}O_3Na$ (M^++Na) 373.2719, measured 373.2730.

5.23. (1*R*,3*R*)-5-[(*E*)-(5′*S*,7′*S*)-11′-hydroxy-5′,7′,11′-trimethyl-dodec-2′-enylidene]-2-methylene-cyclohexane-1,3-diol (13)

Deprotection of hydroxyl groups in vitamin D compound **42** (1.7 mg, 2.4 μmol) was performed analogously to the process described above for the isomeric compound **41**. After analogous work-up of the reaction mixture the product was purified on a silica Sep-Pak (0.5 g). Elution with hexane/AcOEt (1:1) gave an oily vitamin D analog **13**. This product was further purified by HPLC (9.4 mm × 25 cm Zorbax RX-Sil column, 4 mL/min) using a hexane/2-propanol (85:15) solvent system. Pure vitamin D analog **13** (343 μg, 40%) was collected at R_V 25 mL.

5.23.1. (13*S*,20*S*)-1α,25-dihydroxy-2-methylene-8(12),14(17)-diseco-9,11,15,16,18,19-hexanorvitamin D_3 (13, 13MeMP)

UV (in EtOH) λ_{max} 236.0, 242.5, 268.0 nm; ^{1}H NMR (500 MHz, $CDCl_3$; *vitamin D numbering*) δ 0.86 (3H, d, J = 6.4 Hz, 20-H_3), 0.87 (3H, d, J = 7.2 Hz, 13-H_3), 1.21 [6H, s, 26- and 27-H_3], 2.26 (1H, dd, J = 13.1, 6.9 Hz, 10α-H), 2.39 (1H, dd, J = 13.2, 7.5 Hz, 4α-H), 2.56 (1H, dd, J = 13.1, 4.0 Hz, 4β-H), 2.71 (1H, dd, J = 13.2, 4.1 Hz, 10β-H), 4.48 (2H, m, 1β- and 3α-H), 5.10 and 5.12 (each 1H, each s, C=CH_2); 5.69 (1H, dt, J = 14.7, 7.4 Hz, 8-H), 6.04 (1H, d, J = 10.8 Hz, 6-H), 6.29 (1H, dd, J = 14.7, 10.8 Hz, 7-H); HRMS (ESI) exact mass calcd for $C_{22}H_{38}O_3Na$ (M^++Na) 373.2719, measured 373.2726.

5.24. (2*R*,4*S*)- and (2*S*,4*S*)-8-(*tert*-Butyldimethylsilyloxy)-2-[2′-(*tert*-butyldimethylsilyloxy)ethyl]-4,8-dimethyl-nonanoic acid methyl esters (43 and 44)

To a stirred solution of the esters **22** and **23** (50 mg, 0.14 mmol) in ethyl acetate (2 mL) was added Pd/C (10%, 25 mg) at room temperature and the mixture was hydrogenated for 4.5 h at 10 MPa. The mixture was filtered, the solvent evaporated and the oily residue was purified on silica Sep-Pak (2 g). Elution with hexane/AcOEt (9:1) gave an oily mixture of products which were separated by HPLC (9.4 mm × 25 cm Zorbax-Sil column, 4 mL/min) using a hexane/ethyl acetate (99:1) solvent system. The isomeric esters **43** (11 mg, 22%) and **44** (10 mg, 20%) were collected at R_V 29 and 30.5 mL, respectively.

Compound ***43***: $[\alpha]_D^{24}$ +1.4 (*c* 1.25, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.036 and 0.054 [each 6H, each s, 2 × $Si(CH_3)_2$], 0.85 and 0.89 (each 9H, each s, 2 × Si–*t*-Bu), 0.88 (3H, d, J = 6.4 Hz, CH–CH_3), 1.16 and 1.17 [each 3H, each s, $OC(CH_3)_2$], 2.65 (1H, m, CHCOOCH$_3$), 3.60 (2H, m, CH_2OTBS), 3.66 (3H, s, $COOCH_3$); ^{13}C NMR (125 MHz) δ −5.24 i −1.85 [$Si(CH_3)_2$], 18.30 and 18.50 [Si$C(CH_3)_3$], 19.43 (CH–CH$_3$), 21.64 (CH_2), 26.05 and 26.12 [2 × $SiC(CH_3)_3$], 29.95 and 30.08 [$C(CH_3)_2$], 31.09 (CH–CH$_3$), 36.09 (CH_2), 36.83 (CH_2), 38.10 (CH_2), 40.13 (*C*–$COOCH_3$), 45.42 (CH_2), 51.53 (OCH_3), 61.17 (CH_2OTBS), 73.69 [O$C(CH_3)_2$], 177.21 (C=O); HRMS (ESI) exact mass calcd for $C_{26}H_{56}O_4Si_2Na$ (M^++Na) 511.3615, measured 511.3596.

Compound ***44***: $[\alpha]_D^{24}$ −1.4 (*c* 0.9, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.034 and 0.057 [each 6H, each s, 2 × $Si(CH_3)_2$], 0.85 and 0.89 (each 9H, each s, 2 × Si–*t*-Bu), ca. 0.88 (3H; overlapped with Si–*t*-Bu; CH–CH_3), 1.17 [6H, s, $OC(CH_3)_2$], 2.61 (1H, m, CHCOOCH$_3$), 3.58 (2H, m, CH_2OTBS), 3.66 (3H, s, $COOCH_3$); ^{13}C NMR (125 MHz) δ −5.19 and −1.85 [$Si(CH_3)_2$], 18.30 and 18.51 [Si$C(CH_3)_3$], 19.87 (CH–CH$_3$), 21.65 (CH_2), 26.08 and 26.16 [2 × $SiC(CH_3)_3$], 30.12 and 30.16 [$C(CH_3)_2$], 30.99 (CH–CH$_3$), 35.45 (CH_2), 36.87 (CH_2), 37.34 (CH_2), 40.09 (*C*–$COOCH_3$), 45.44 (CH_2), 51.60 (OCH_3), 61.27 (CH_2OTBS), 73.68 [O$C(CH_3)_2$], 177.41 (C=O); HRMS (ESI) exact mass calcd for $C_{26}H_{56}O_4Si_2Na$ (M^++Na) 511.3615, measured 511.3606.

5.25. (*R*)-3-[(*S*)-6′-(*tert*-Butyldimethylsilyloxy)-2,6-dimethyl-heptyl]-dihydro-furan-2-one (45)

To a solution of the ester **43** (5 mg, 10 μmol) in MeOH (1.2 mL) was added at room temperature 10-camphorsulfonic acid (20 mg, 86 μmol) and the mixture was stirred for 1 h, poured into brine and extracted with ethyl acetate. The combined organic extracts were washed with brine, dried ($MgSO_4$) and evaporated. The oily product was purified on a silica Sep-Pak (0.5 g). Elution with hexane/AcOEt (95:5) gave an oily lactone **45** (3.3 mg, 85%); $[\alpha]_D^{24}$ −11 (*c* 0.4, $CHCl_3$); CD $\Delta\varepsilon$ (λ_{max}) −0.6 (218.8); ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.06 [6H, s, $Si(CH_3)_2$], 0.85 (9H, s, Si–*t*-Bu), 0.94 (3H, d, J = 7.0 Hz, CH–CH_3), 1.18 [6H, s, $OC(CH_3)_2$], 1.86–1.97 (2H, m, CH–CH$_3$ and one of 4-H_2), 2.41 (1H, m, one of 4-H_2), 2.57 (1H, dq, J = 9.0, 4.5 Hz, 3-H), 4.18 (1H, dt, J = 7.0, 9.0 Hz, one of 5-H_2), 4.35 (1H, dt, J = 3.0, 9.0 Hz, one of 5-H_2); ^{13}C NMR (125 MHz) δ −2.27 [$Si(CH_3)_2$], 18.58 [Si$C(CH_3)_3$], 19.97 (CH–CH$_3$), 21.37 (CH_2), 25.82 [Si$C(CH_3)_3$], 29.57 [$C(CH_3)_2$], 29.83 (CH_2), 30.98 (CH-CH$_3$), 36.42 (CH_2), 38.14 (CH_2), 45.20 (CH-CH_2), 45.26 (CH_2), 66.60 (CH_2O), 73.63 [O$C(CH_3)_2$], 180.10 (C=O); HRMS (ESI) exact mass calcd for $C_{19}H_{38}O_3SiNa$ (M^++Na) 342.2590, measured 342.2599.

5.26. (*S*)-3-[(*S*)-6-(*tert*-Butyldimethylsilyloxy)-2,6-dimethyl-heptyl]-dihydro-furan-2-one (46)

Conversion of the ester **44** (15 mg, 30 μmol) into lactone **46** was performed analogously to the process described above for the isomeric compound **43**. After analogous work-up of the reaction mixture the product was purified on a silica Sep-Pak (0.5 g). Elution with hexane/AcOEt (95:5) gave an oily lactone **46** (9 mg, 86%); $[\alpha]_D^{24}$ +15 (*c* 0.25, $CHCl_3$); CD $\Delta\varepsilon$ (λ_{max}) −0.68 (217.4); ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.06 [6H, s, $Si(CH_3)_2$], 0.85 (9H, s, Si–*t*-Bu), 0.90 (3H, d, J = 7.0 Hz, CH–CH_3), 1.17 [6H, s, $OC(CH_3)_2$], 1.72 (1H, m, CH–CH$_3$), 1.89 and 2.39 (each 1H, each m, 4-H_2), 2.59 (1H, m, 3-H), 4.19 (1H, dt, J = 7.0, 9.5 Hz, one of 5-CH_2), 4.36 (1H, dt, J = 2.5, 9.5 Hz, one of 5-H_2); ^{13}C NMR (125 MHz) δ −2.26 [$Si(CH_3)_2$], 18.31 [Si$C(CH_3)_3$], 18.80 (CH–CH$_3$), 21.75 (CH_2), 26.04 [Si$C(CH_3)_3$], 29.27 (CH_2), 29.95 [$C(CH_3)_2$], 31.16 (CH–CH$_3$), 37.53 (CH_2), 38.30 (CH–CH_2), 45.42 (CH_2), 66.46 (CH_2O), 73.63 [O$C(CH_3)_2$], 180.29 (C=O); HRMS (ESI) exact mass calcd for $C_{19}H_{38}O_3SiNa$ (M^++Na) 342.2590, measured 342.2579.

5.27. Reduction of the lactones 45 and 46 to the diols 35 and 36

5.27.1. (*R*)-2-[(*S*)-6-(*tert*-Butyldimethylsilyloxy)-2,6-dimethyl-heptyl]-1,4-butanodiol (35)

To a suspension of $LiAlH_4$ (50 mg, 1.33 mmol) in anhydrous THF (800 μL) at 0 °C was added solution of the lactone **45** (8 mg,

20 μmol) in THF (200 μL) and the mixture was stirred at 0 °C for 1.5 h. Saturated Na_2SO_4 was then added and the mixture was extracted with ethyl acetate. The combined organic extracts were washed with water, dried ($MgSO_4$) and evaporated. The oily product was purified on a silica Sep-Pak (0.5 g). Elution with hexane/AcOEt (7:3) gave an oily diol **35** (5 mg, 62%).

5.27.2. (*S*)-2-[(*S*)-6-(*tert*-Butyldimethylsilyloxy)-2,6-dimethylheptyl]-1,4-butanodiol (36)

Reduction of the lactone **46** (8 mg, 20 μmol) into diol **36** was performed analogously to the process described above for the isomeric compound **45**. After analogous work-up of the reaction mixture the product was purified on a silica Sep-Pak (0.5 g). Elution with hexane/AcOEt (7:3) gave an oily diol **36** (4 mg, 49%).

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

Purified full-length rat recombinant receptor was prepared as described earlier with a few modifications.[23] 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. Fractions judged 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 $\varepsilon = 18{,}200$ and $\lambda_{max} = 265.0$ nm; the tested 19-norvitamin *des*-C,D compounds: $\varepsilon = 30{,}200$ and $\lambda_{max} = 241.0$ 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 2–3 different occasions.

5.29. 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.[24] 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).

5.30. Transcriptional assay

Transcription activity was measured in ROS 17/2.8 (bone) cells that were stably transfected with a 24-hydroxylase (24Ohase) gene promoter upstream of a luciferase reporter gene.[25] 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 2–3 separate times.

5.31. Computational method

All calculations have been carried out on a simple PC endowed with an AMD AthlonI 64X2 Dual core processor. 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. The procedure used for generation of the respective conformers of the alkoxyalkyl (or hydroxyalkyl) substituents and finding the global minimum structures of the lactones was an analogous to that described previously by us for the vitamin D side chain conformers[26] and involved the Conformational Search module of the HYPERCHEM program. Values of acyclic torsion variation (±10–180°) and ring torsion flexing (±10–70°) were chosen to assure the accuracy of the conformer generation; in all conformational searches the number of iterations was set at the value of 2000.

The calculations of electronic circular dichroism spectra were done for lactones **45** and **46** with OH group instead of O-TBS group. The real minimum energy conformers found by molecular mechanics have been further fully optimized at the DFT/B3LYP/6-31++G(D,P) level as implemented in GAUSSIAN 03 package.[27] To confirm the stability of calculated structures, the frequency calculations were performed at B3LYP/6-311++G(D,P) level. For the stable structures, the rotational strengths were calculated at the B3LYP/6-311++G(D,P) level. The rotatory strengths were calculated using both the length and the velocity representations. The differences between the length and the velocity calculated values of the rotatory strengths were quite small and for this reason only the velocity rotatory strengths were taken into further consideration. The CD were simulated by overlapping Gaussian functions for each transition according to the procedure described by Diedrich and Grimme.[28] No correlation for the medium dielectric constant was implemented.

5.32. In vivo calcemic assays

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 34 h after the last dose. There were 5–6 rats per group.

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