Development of Analogues of 1α,25-Dihydroxyvitamin D_3 with Biased Side Chain Orientation: Methylated Des-C,D-Homo Analogues

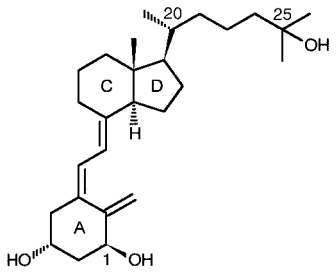
Stefan Gabriëls,[a] Dirk Van Haver,[a] Maurits Vandewalle,[a] Pierre De Clercq,*[a] Annemieke Verstuyf,[b] and Roger Bouillon[b]

Abstract: The discovery that 1α,25-dihydroxyvitamin D_3 is effective in the inhibition of cellular proliferation and in the induction of cellular differentiation has led to a search for analogues in which these activities and the classical calcemic activity of this hormone are separated. In this context, the synthesis and biological evaluation are reported of the three stereoisomeric CD-ring modified structural analogues in order to enforce a particular and different orientation of the 25-hydroxylated side chain. Comparison of the results of the biological evaluation and conformational analysis of the side chain suggests one defined and "active" geometry.

Keywords: structure – activity relationships · terpenoids · vitamin D_3

Introduction

The last decade has witnessed a very active search for analogues of 1α,25-dihydroxyvitamin D_3 (**1**, further abbreviated as 1,25$(OH)_2D_3$), the hormonally active form of vitamin D.[1] Next to its classical calciotropic activity,[2] 1,25$(OH)_2D_3$ (**1**) has been shown to possess immunosuppressive activity,[3] to inhibit cellular proliferation, and induce cellular differentiation.[4] Its therapeutic value in the treatment of certain cancers and skin diseases is, however, limited since effective doses provoke calcemic side effects such as hypercalcemia, hypercaliuria, and bone decalcification. This has stimulated the development of analogues of the natural hormone in which the calcemic activity and the antiproliferative and/or prodifferentiating activities are separated.[5] In this context various successful structural modifications have already been introduced, such as 19-*nor*,[6] 22-oxa,[7] 23-yne,[8] 20-*epi* (**2**),[9] 1-hydroxymethyl[10] derivatives, and combinations thereof. Whereas the above modifications are located in the flexible parts of the molecule, that is the side chain and the A-ring, our laboratory has focussed on the development of analogues in recent years that vary in the structure of the central CD-ring system.[11] A typical example is analogue **3**, the structure of which is characterized by the absence of the six-membered C-ring and by the presence of an enlarged D-ring, that is a formal 8,9-*seco*-9,11-bis-*nor*-15-*homo* derivative.[11f] For the sake of simplicity and clarity we will further use "6D analogues" as descriptive term. Furthermore, the carbon atoms of the modified vitamin D skeleton will be referred to according to the conventional steroid numbering.



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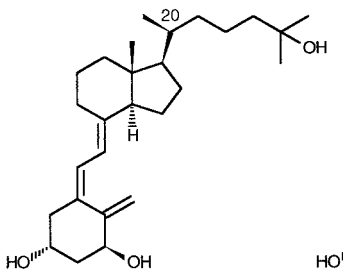

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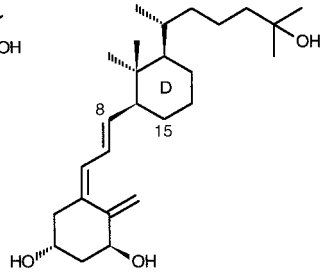

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The comparison of some relevant biological activities for compounds **1**–**3** shown in Table 1 illustrates the possibility of discrimination of the various actions of vitamin D. It is hereby striking how with similar affinities for the vitamin D receptor (VDR), the 20-epimer **2** is several orders of magnitude more potent than the natural hormone **1** both in prodifferentiating and calcemic activity,[12] whereas 6D analogue **3** is much less calcemic. The higher activity of **2** compared with **1** can be associated with different orientations of the side chain (see below).

Table 1. Selected biological activities of **1**–**3**.[a]

	Binding		Differentiation	Calcium
Entry	VDR(pig)	DBP (human)	HL-60	Ca serum (mice)
1	100	100	100	100
2	88	0.2	3000	800
3	125	80	90	4

[a] The activities are presented as relative values, the reference value of $1,25(OH)_2D_3$ (**1**) being defined as 100%. Further details about the methodology are given in the Experimental Section.

Results and Discussion

The present study fits into a project aiming at the development of analogues possessing side chains with biased spatial orientations. In particular 6D-analogues **4** are presented here so that, depending on the relative orientation of the methyl substituents at C13 and C16, the side chain at C17 would adopt different and distinct conformations. The three selected configurations **a**, **b**, and **c** constrain the mobility in the segment C17-C20-C22 in a way that is further illustrated in Figure 1. For each of these configurations the preferred conformation(s) is (are) shown on the basis of: 1) a chair conformation for the six-membered D-ring possessing the two larger substituents on C14 and C17 in equatorial position; 2) the four-carbon C17-C20-C22-C23 chain adopting conformations in which steric interactions are minimized, in particular in which *syn*-pentane interactions are avoided.[13] Since all segments, cyclic and acyclic, are considered to adopt minimum energy staggered conformations, relevant geometries can be generated on a diammond lattice (shown as dotted lines).[14] Black circles further indicate positions on the lattice that, if occupied by a carbon atom of the backbone chain, that is C22 in relation to the (C17, C20)-bond rotation and C23 (shown as R) in relation to the (C20, C22)-bond rotation, would generate a destabilizing *syn*-pentane interaction; the penalty of occupying such position has been evaluated at 7–9 kJ mol^{-1}.[15] On this exclusion basis, and referring to the torsion angles C13-C17-C20-C22/C17-C20-C22-C23, the preferred side chain conformations correspond in the **a**-series to a (g^+/a)-conformation,[16] in the **b**-series to a (a/a)- or (a/g^+)-conformation, and in the **c**-series to a (g^-/a)- or (g^-/g^-)-conformation, where a, g^+, and g^- designate the three possible staggered geometries that correspond to backbone dihedral angles of 180°, +60°, and −60°, respectively. The reason why these three particular side chain orientations were selected will become clear in the Discussion.

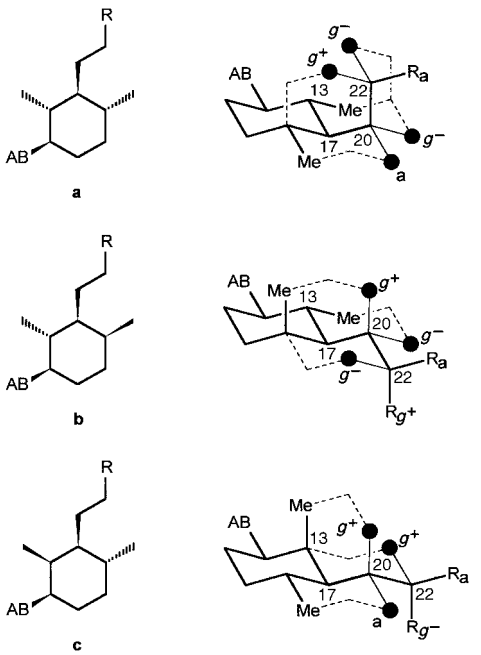
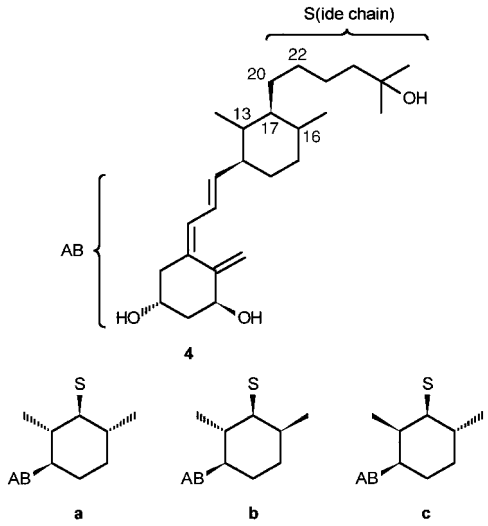
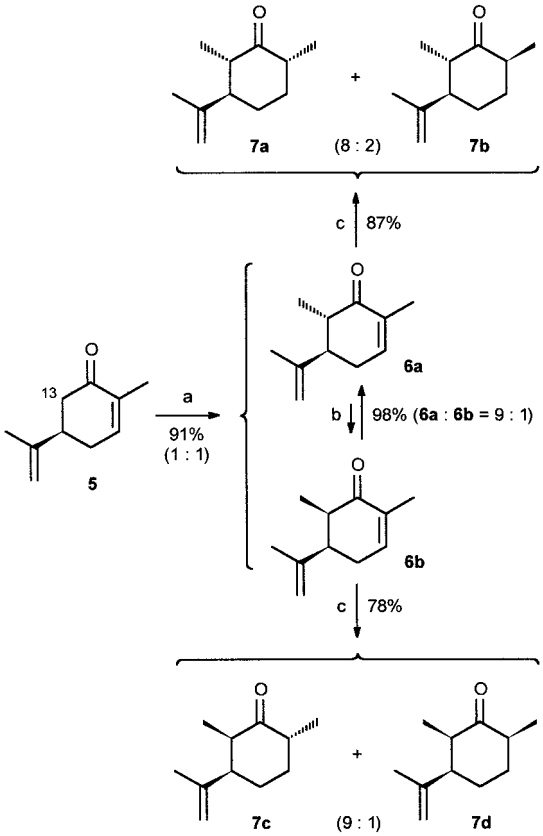


Figure 1. Preferred orientation of the side chain in the three series **a**, **b**, and **c** following the analysis of diamond-lattice type conformations in the segment C13-C17-C20-C22; black circles correspond to positions that, if occupied by a backbone carbon atom, would generate a *syn*-pentane interaction.



Synthesis: The enantioselective preparation of analogues **4a**, **4b**, and **4c** is outlined in the Schemes 1, 3, 5, and 6. In Scheme 1 the conversion of the chiral pool monoterpene (*R*)-(−)-carvone (**5**) into the three cyclohexanone derivatives **7a**, **7b**, and **7c** is described in which the desired stereochemistry at C13, C14, and C16 is established. The introduction of the ethoxycarbonylmethyl substituent (cf. **13**), which will further serve as a handle for the construction of the desired side chain, with concomittant formation of the required C17 stereocenter, is outlined in Scheme 3.

The final conversion of esters **13** into the corresponding analogues **4a**, **4b**, and **4c**, following a common sequence in the three series **a**, **b**, and **c**, is shown in Schemes 5 and 6. Key-

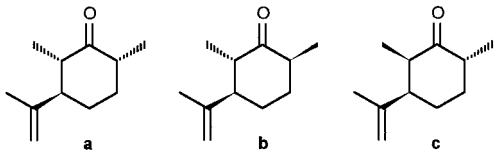


Scheme 1. Synthesis of diastereomeric ketones **7a**–**c**. a) LDA, THF; MeI, DMPU. b) NaOMe, MeOH/THF. c) $Na_2S_2O_4$, Adogen 464, $NaHCO_3$, toluene/H_2O.

features of the latter sequence involve i) the attachment of the remaining portion of the side chain through nucleophilic alkynyl substitution followed by hydrogenation of the triple bond (cf. **18** to **20** in Scheme 5), and ii) the attachment of the *seco*-B,A-ring part (cf. **25**) in a Horner–Wittig reaction of aldehyde **24** (Scheme 6).[17] We further note that out of the eleven steps of this sequence (**13** to **4**), six (!) are somehow involved in the conversion of the 2-propenyl group into the required aldehyde function. These steps became necessary as a consequence of: i) the deliberate choice of introducing the side chain via the above alkynyl pathway since thus 22-yne analogues also become available; ii) the unexpected observation that the Horner coupling failed at the methyl ketone stage (cf. **21**).

The introduction of the methyl group at C13 involves a classical alkylation reaction on (*R*)-(−)-carvone (**5**) which led to a separable 1:1 mixture of **6a** and **6b**.[18] The configurational assignment of both isomers at this stage follows from the observation that isomerization in base (sodium methoxide)[18b] leads to an equilibrium mixture in favor of the more stable isomer **6a** (**6a**:**6b** = 9:1).[19] Further conjugate reduction of **6a** and **6b** with sodium dithionite under phase-transfer conditions led to mixtures of ketones **7** in which the diastereomers **7a** and **7c**, respectively, predominate.[20] The confirmation of the configuration of the three required ketones **7a**, **7b**, and **7c** convincingly follows from inspection of the ^{1}H NMR spectral data; the most relevant of which are summarized in Table 2. The relative disposition of the two

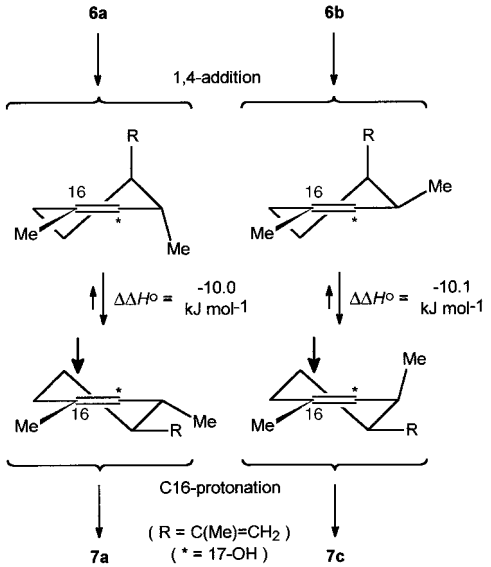
Table 2. Relevant ^{1}H NMR spectral data of diastereomeric ketones **7a**, **7b**, and **7c**.[a]



	H13				H16			
Entry	δ	J(13,14)	J(13,Me)	ΣJ_{exptl}	δ	J(16,15)	J(16,Me)	ΣJ_{exptl}
7a	2.37	12.1	6.5	29.6	2.42	12.8, 5.6	6.4	37.1
7b	2.57	10.3	6.7	29.2	2.57	[b]	7.2	30.3
7c	2.70	≈5	7.3	26.5	2.59	12.7, 6.4	6.4	38.2

[a] Determined by double irradiation of methyl groups located on C13 and C16; coupling constants [Hz]. [b] Could not be determined due to overlapping signals.

methyl groups in the three series involving chair cyclohexanones with the large 2-propenyl group in equatorial position is substantiated by the magnitude of the vicinal coupling constants (individual or as a sum) of H13 and H16, with the most indicative coupling with the methyl substituent (> 7.0 Hz for an axial Me, < 7.0 Hz for an equatorial Me).[21] Presumably, the preferred formation of ketones **7a** (from **6a**) and **7c** (from **6b**) is the result of kinetic control.[22] In particular proton addition to the enol (or enolate anion) that is obtained upon the conjugate hydride addition determines the configuration at C16. In both cases the stereoelectronically preferred perpendicular addition would occur from the β-face. Indeed, as shown in Scheme 2, in the case of **6a** unhindred β-protonation at C16 can occur on the preferred half-chair enol conformation; in the case of **6b** perpendicular protonation on the more stable half-chair enol conformation would also occur from the β-face. In any event, the followed reaction pathway allows for obtaining the *three* required cyclohexanones in sufficient amount for further conversion to the desired analogues.



Scheme 2. Possible explanation for the preferred formation of **7a** and **7c** upon conjugate reduction of **6a** and **6b**.

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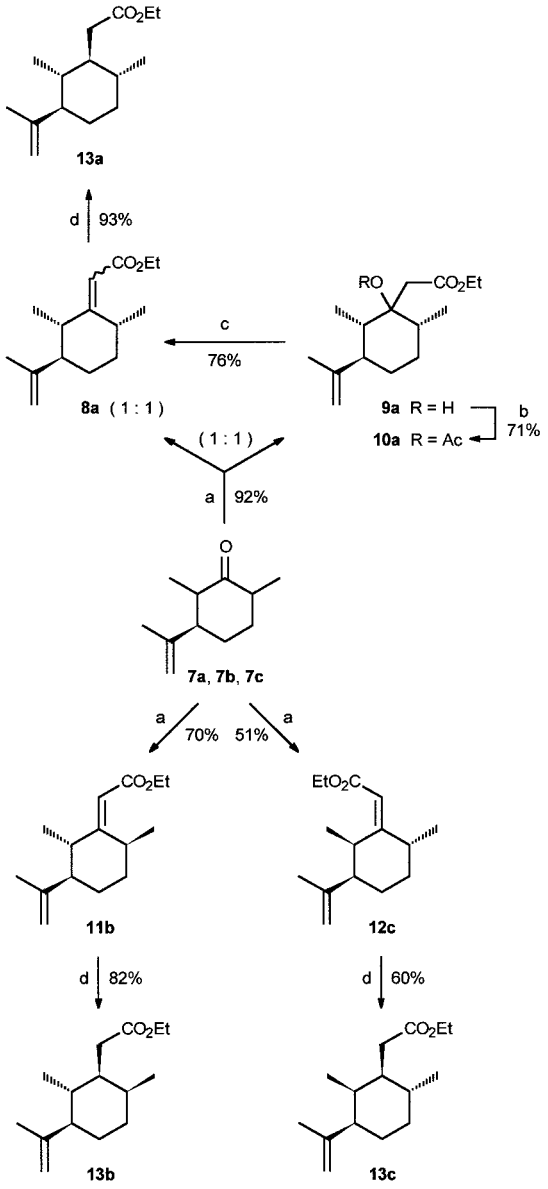
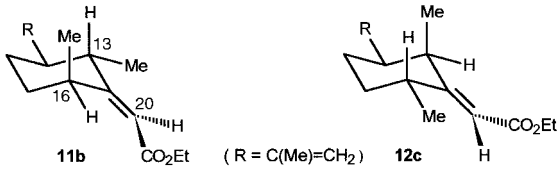
The introduction of the ethoxycarbonylmethyl substituent with the required configuration at C17 (cf. **13**) is performed in two stages (Scheme 3): Attachment of the two-carbon chain as α,β-unsaturated ester (**8a**, **11b**, and **12c**, respectively),



Scheme 3. Synthesis of diastereomeric esters **13a–c**. a) Ethoxyethynyl-MgBr, toluene/THF; dil. H_2SO_4, THF. b) AcCl, $PhNMe_2$, $CHCl_3$. c) KO*t*Bu, *t*BuOH. d) Li, liq. NH_3, *t*BuOH, Et_2O.

followed by dissolving metal reduction to afford esters **13a**, **13b** and **13c**, respectively.[23] Whereas the direct Peterson olefination proved unsuccessful,[24] a two-stage process involving addition of the Grignard derivative derived from ethoxyethyne, followed by sulfuric acid hydrolysis, afforded the required unsaturated derivatives.[25] In the case of **7a** this reaction led to a separable 1:1 mixture of the desired unsaturated ester **8a** (as an unseparable *E*,*Z*-mixture) and the tertiary alcohol **9a**. Elimination of the latter through subsequent formation of the corresponding acetate **10a** led to a further portion of **8a**.[26] In contrast to the **a**-series, reaction of **7b** and **7c** led directly to the desired unsaturated ester derivatives in a single isomeric form, that is *E*-**11b** and *Z*-**12c**. Again the configurational assignment of both compounds rests on the detailed analysis of the relevant ^{1}H NMR spectral data shown in Table 3.[27] It is symptomatic how in both cases the ester group has lodged itself in the open space that is available through the axial arrangement of one of the methyl groups.

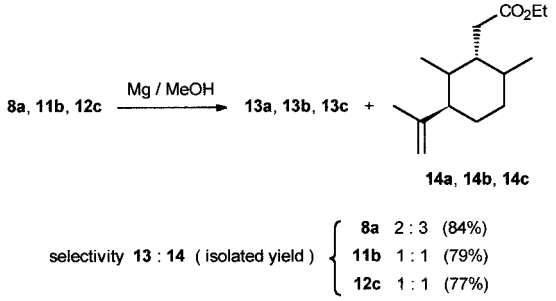
Table 3. Relevant ^{1}H NMR spectral data of diastereomeric unsaturated esters **11b** and **12c**.[a]



	H13			H16		
Entry	δ	J(13,Me)	ΣJ_{exptl}	δ	J(16,Me)	ΣJ_{exptl}
11b	2.42	6.5	30	4.16	7.2	[b]
12c	4.39	7.3	26	2.44	6.5	37

[a] Determined through double irradiation of the methyl group located on C13 and C16; coupling constants [Hz]. [b] Could not be determined due to overlapping signals ($COOCH_2CH_3$).

The subsequent conjugate reduction of the α,β-unsaturated esters **13** proved quite instructive. Indeed, whereas in each series the lithium/liquid ammonia reduction led selectively to the more stable diastereomer, that is **13a**, **13b**, and **13c**, the magnesium in methanol reduction afforded a mixture of **13** and **14** in each case as the two possible stereoisomers at C17 (Scheme 4). The latter observations are in line with the result



Scheme 4. Conjugate reduction of unsaturated esters **8a**, **11b**, and **12c** with magnesium in methanol.

that has been obtained in the past upon reduction of a similar substrate.[23] This repeated lack of stereoselectivity somehow throws a shadow on the general usefulness of the magnesium/methanol conjugate reduction reaction of α,β-unsaturated esters.[28] On the other hand, the availability of the C17 isomeric esters **14** in the three series **a**, **b**, and **c**, even as inseparable mixtures with the corresponding isomers **13**, turned out to be very useful in the context of the structural determination of **13a**, **13b**, and **13c**. Careful analysis of the ^{1}H COSY NMR spectra of the mixtures of **13** and **14** allowed the distinction between the two C17 isomeric series, in particular on the basis of the magnitude of the sum of the vicinal

coupling constant values of H17, which turns out to be larger in the isomers **13** which are characterized by the preferred *cis*-diequatorial orientation of the two larger substituents at C14 an C17 (Figure 2). The similarity between the signals observed for the CH_2COOEt moiety in the **b**- and **c**-series is striking and fully in accord with the pseudo-enantiomeric relationship that one may identify between the two series.

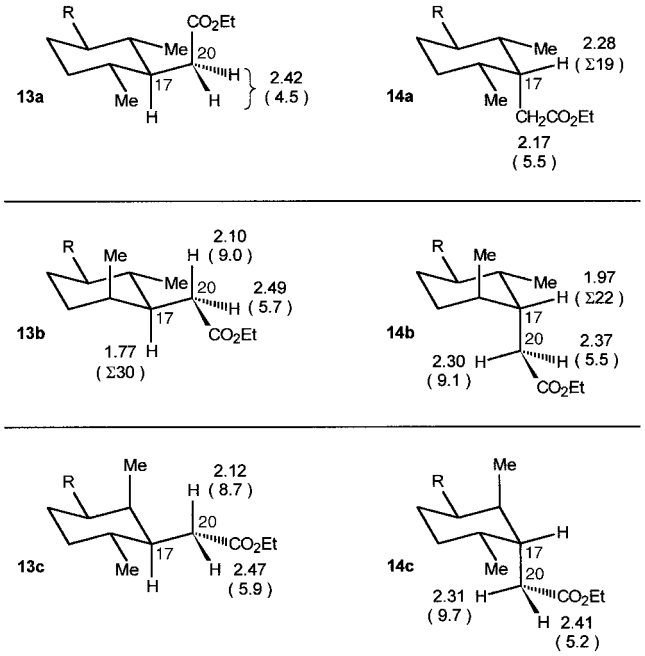
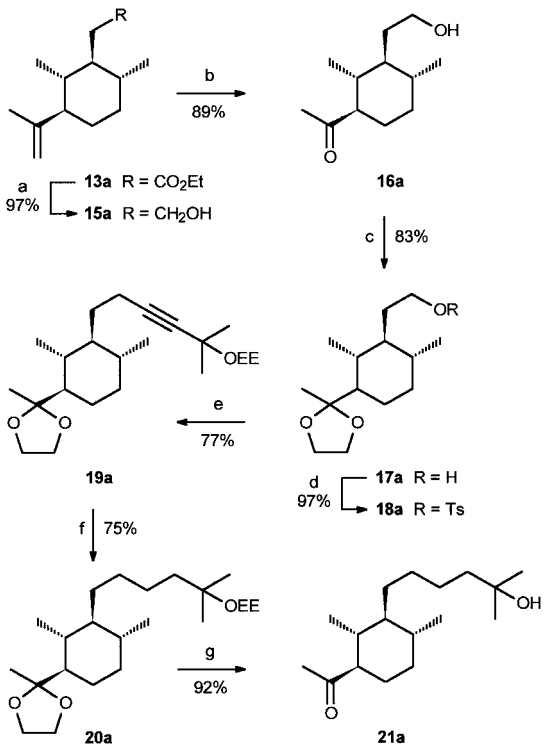


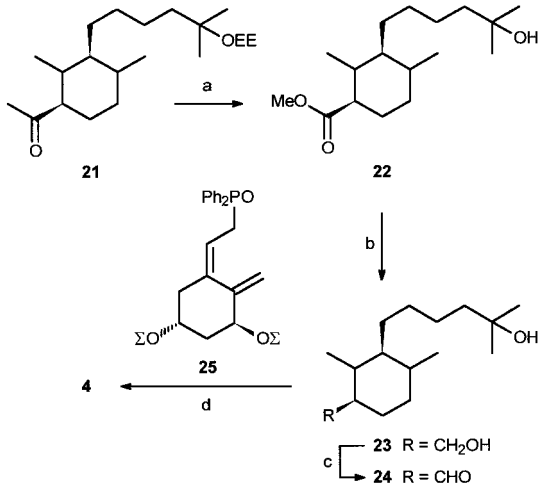
Figure 2. Relevant ^{1}H NMR spectral data of the diastereomeric esters **13a–c** and **14a–c**: chemical shifts (δ) and vicinal coupling constant values in parentheses [Hz]; assignments aided by H,H-COSY. R = isopropenyl.

The sequence followed for the further conversion of esters **13a**, **13b**, and **13c** into the corresponding ketones **21a**, **21b**, and **21c** is described in detail for the **a**-series (Scheme 5). After reduction of ester **13a** to the corresponding alcohol **15a**, the oxidative cleavage (ozone in dichloromethane/methanol)[29] of the propenyl double bond led to ketone **16a**. After protection of the carbonyl through acetalisation to **17a** and conversion of the alcohol to the corresponding tosylate **18a**, substitution with the sodium salt of 2-methyl-3-butyn-2-ol, protected as ethoxyethyl ether, afforded alkyne **19a** in good yields.[30] Catalytic hydrogenation to **20a** followed by acid hydrolysis led to methyl ketone **21a**.

The final conversion of the methyl ketones **21a**, **21b**, and **21c** into the vitamin D analogues **4a**, **4b**, and **4c** is outlined in Scheme 6. The transformation of ketone **21** into the required aldehyde **24** for subsequent attachment of the *seco*-B,A-ring portion of the vitamin D molecule involved three steps: 1) the electrochemical oxidation of **21** into ester **22**,[31] 2) lithium aluminumhydride reduction to **23**, and 3) oxidation with pyridinium chlorochromate supported on alumina to **24**.[32] The first step is the electrochemical variant of the classical bromoform reaction: two platinum electrodes are immersed in a solution of ketone **21** and sodium bromide as the electrolyte in methanol and submitted to a 20–30 V tension. For reasons that remain unclear, in the **a**-series the yield was



Scheme 5. Synthesis of ketone **21a**. a) LAH, THF. b) O_3, CH_2Cl_2/MeOH, $-40\,^\circ$C. c) $HO(CH)_2OH$, $HC(OMe)_3$, *p*TsOH. d) *p*TsCl, CH_2Cl_2, Et_3N, DMAP. e) NaH, DMSO; OEE-protected 2-methyl-3-butyn-2-ol, DMSO. f) 5% Rh/Al_2O_3, H_2, EtOAc. g) PPTS, acetone/H_2O.



Scheme 6. Synthesis of vitamin D analogues **4a**, **4b**, and **4c**. a) NaBr, MeOH, Pt-electrode, 20–30 V. b) LAH, THF. c) PCC, CH_2Cl_2. d) *n*BuLi, **25**, THF; TBAF.

deceivingly low: 28% for **22a**, compared with 46% for **22b**, and 76% for **22c**. As shown in Table 4 analysis of the signal observed for H14 in the ^{1}H NMR spectrum of the ester derivatives **22** allows for the confirmation of the configurational assignment in the series of three. Finally, treatment of the aldehydes **24a**, **24b**, and **24c** with the anion derived from the known phosphine oxide **25**,[33] followed by in situ silylether deprotection with tetrabutylammonium fluoride (TBAF), led to the analogues **4a**, **4b**, and **4c** in fair yields.

Following the above sequence isomerization at C14 is in principle possible at several stages (cf. formation of **16**, **17**, **21**,

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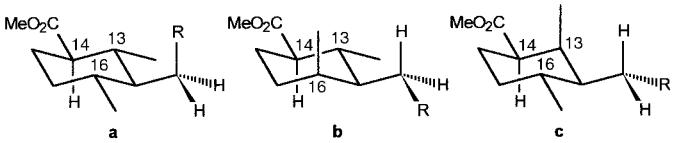
22, **24**, and even **4**). Nevertheless it is assumed that, at least from **21** onward, the more stable diastereomer will predominate. As expected, calculations performed on the methyl ester **22** indicate the shown (14*R*)-stereoisomer to be the more stable epimer in each series.[34] This is further substantiated by the ^{1}H NMR spectral data shown in Table 4.

Table 4. Relevant ^{1}H NMR spectral data of diastereomeric esters **22a–c** (R = 4-hydroxy-4-methylpentyl).[a]



	H14			H13		H16	
Entry	δ	m	J_{vic}	δ(Me)	J(H13,Me)	δ(Me)	J(H16,Me)
22a	2.03	td	11.6, 11.6, 3.1	0.81[b]	6.4	0.89[b]	6.4
22b	2.00	td	11.5, 11.5, 3.9	0.82	6.3	0.85	7.1
22c	2.41	dt	11.0, 4.8, 4.19	0.69	7.1	0.85	6.4

[a] Coupling constants in Hz. [b] Data of the methyl ketone **21a**.

Biological evaluation: The biological evaluation of analogues **4a–c** includes the determination of 1) the binding affinity for the porcine intestinal VDR, 2) the antiproliferative activity in vitro on breast cancer MCF-7 cell, 3) the cell-differentiating activity in vitro on a leukemic HL-60 cell line, and 4) the calcemic activity in vivo in vitamin D-replete normal NMRI mice. Results are shown in Table 5.

Table 5. Selected biological activities of **4a–c**.[a]

	Binding	Cell differentiation and proliferation		Calcium
Entry	VDR(pig)	HL-60	MCF-7	Ca serum (mice)
1	100	100	100	100
4a	20	90	200	4
4b	2	9	30	< 0.25
4c	2	8	70	< 0.25

[a] The activities are presented as relative values, the reference value of 1,25(OH)$_2$D$_3$ (**1**) being defined as 100%. Further details about the methodology are given in the Experimental Section.

The 6D-analogue **4a** displayed 20% of the VDR affinity compared with 1,25(OH)$_2$D$_3$ (100% binding, Table 5). The two epimers **4b** and **4c** demonstrated only 2% of the affinity for the VDR. The 6D-analogue **4a** has equipotent prodifferentiating activity when compared with the activity of 1,25(OH)$_2$D$_3$ on HL-60 cells while this analogue was two times more potent in the inhibition of the proliferation of MCF-7 cells. Epimers **4b** and **4c** were less potent in the inhibition of the MCF-7 cell proliferation or the stimulation of the HL-60 cell differentiation compared with 1,25(OH)$_2$D$_3$ whereas both analogues had poor calcemic effects in vivo (< 0.25% compared with 1,25(OH)$_2$D$_3$).

Conformational analysis: Vitamin D activity is normally expressed via a genomic pathway:[35] The hormone binds with the intracellular vitamin D receptor (VDR) so as to regulate gene transcription and synthesis of new proteins that are more directly responsible for the biological response. In view of this mechanism the geometry of the VDR-1,25(OH)$_2$D$_3$ complex is crucial. Until recently,[36] no precise information about the active shape of the hormone was known because of problems encountered in obtaining a detailed crystal structure of the liganded nuclear receptor (see below).[37] Therefore indirect means had to be developed. The determination of the active shape of the vitamin D hormone is intrinsically difficult because of its flexible nature. Indeed, flexible ligands are known to undergo substantial distortions away from their preferred geometry in order to achieve optimal binding with the receptor.[38] Since a greater preorganization of the active geometry is expected to result in enhanced binding, it is logical to conceive analogues that are characterized by reduced mobility in the flexible parts of the molecule. With regard to the side chain this may consist in constraining one or several of the rotatable carbon–carbon bonds along the C17–C25 chain. Whereas this goal can be achieved by unsaturated moieties within the side chain,[8] a more subtle way may consist in changing the substitution pattern in this part of the molecule. The present work has been performed in this particular context.

Central in this and related work is the use of conformational maps to describe the preferred geometries of the side chain. The concept of dot maps in this area was first introduced by Okamura and Midland.[39] In this approach force field calculations are performed so as to generate within a given energy window all possible local minimum energy conformations that the side chain may adopt. The orientation in space of each found conformation is further defined by a dot that corresponds to the position of the 25-oxygen atom in that particular conformation. Subsequently, volume maps have been used in our laboratory in order to optimize the visualization aspect of the procedure.[40] Volume maps corresponding to the side chain conformations of **4a–c** are presented in Figure 3. Note that these have been generated for model derivatives that lack the A-ring. Three different views are shown: next to the classical top and front views, a view analogous to the one used in Figure 1 has been included. From inspection of Figure 1 it is readily apparent that with each configuration **a**, **b**, and **c** fit a specific and different side chain orientation. To a fair extent the latter also correspond nicely with those deduced via the exclusion procedure that is illustrated in Figure 1. Prior to this work four conformationally restricted 22-methyl substituted analogues of 1α,25(OH)$_2$D$_3$ (**1**), diastereomeric at C20 and C22, have been studied by Yamada et al., with the aim to study the three-dimensional structure of vitamin D that is involved in the binding to the receptor.[41] Interestingly, one among those was found to possess a markedly higher binding affinity for the VDR; moreover, the same diastereomer was substantially more potent than the natural hormone **1** in inducing differentiation of HL-60 cells. In a more recent study dealing with structure–function relationships related to the orientation of the side chain, the same group identified particular regions in which the preferred orientation of the 25-hydroxy group would correspond to the increased cell-differentiating

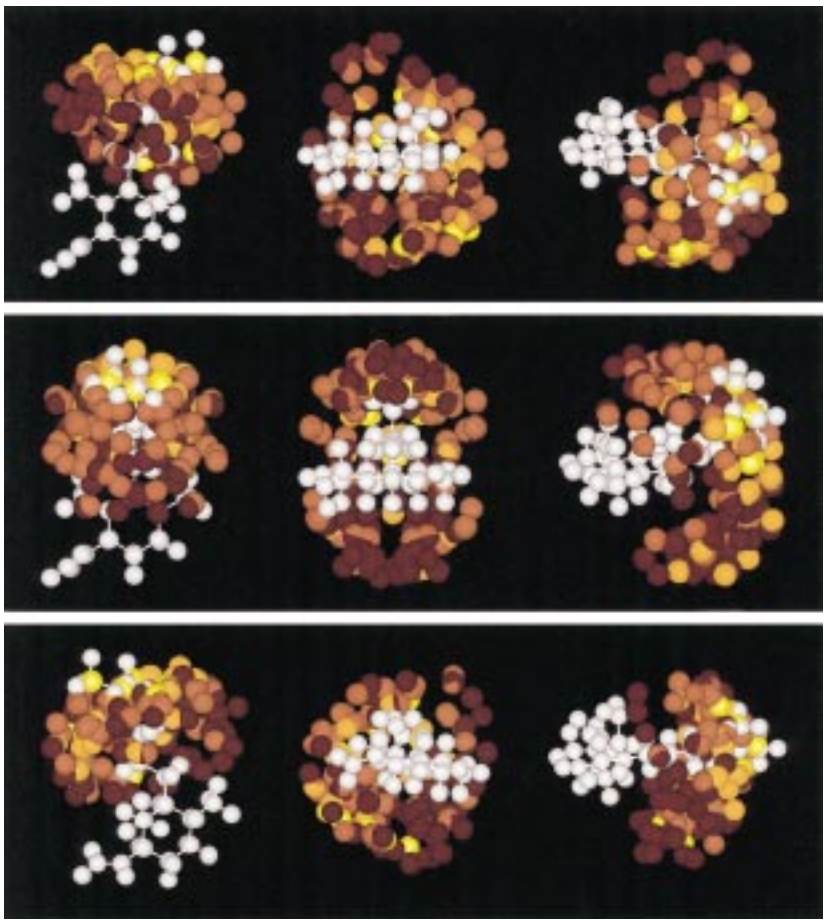


Figure 3. Volume maps representing the conformational behavior of the side chain of truncated models of 6D analogues **4a**, **4b**, and **4c** (middle, top and bottom line, respectively) in three different viewing directions: top (left), front (middle), and side (right). Colored spheres indicate positions of O25; total energy window: 20 kJ mol^{-1}.

potency.[42] Following this active space group concept five main regions designated as A, G, EG, EA, and F were identified. With this study a correlation was proposed between the cell-differentiating potency of numerous analogues and the conformational space occupied by the vitamin D side chain. As an important result it was observed that the preferred side chain orientations of almost all potent analogues were distributed among the EA region. These different regions are visualized in Figure 4. Note how within the same representation are combined the side chain mappings of 1α,25(OH)$_2$D$_3$ (yellow) and 20-*epimer* **2** (blue).

Further a superposition with the so-called relative activity volume (red) is shown in this representation. The latter is generated so that, among a pair of epimeric analogues possessing very different activities (ideally a very active and an inactive compound), a sphere is created that contains the lowest possible mole fraction of the side chain conformations of the less active analogue, but at the same time contains the highest possible mole fraction of the conformations of the more active of the pair.[43] The relative activity volume shown was generated on the basis of the conformational profiles of the most and least active diastereomers among the four 22-methyl substituted analogues developed by Yamada. The applied procedure has been described in detail.[40] Also included in Figure 4 is the location of the 25-hydroxy group (green) of the conformation of the natural ligand when bound to the receptor. This important information has become recently available through the high-resolution crystal structure of the complex between the VDR ligand binding domain and 1,25(OH)$_2$D$_3$.[36] This study shows the three-dimensional arrangement of the ligand-binding pocket around 1,25(OH)$_2$D$_3$ and reveals in particular that the elongated ligand occupies only 56% of the accessible volume of the VDR cavity. Obviously, the results of these different conformational studies shown in Figure 4 are overlaid so as to allow for a relative spatial comparison.

Finally, the side chain mappings corresponding to the analogues **4a**, **4b**, and **4c** following Yamada's view are shown in Figure 5, also including the above relative activity volume. The occupation of this volume by the 25-hydroxy group of the

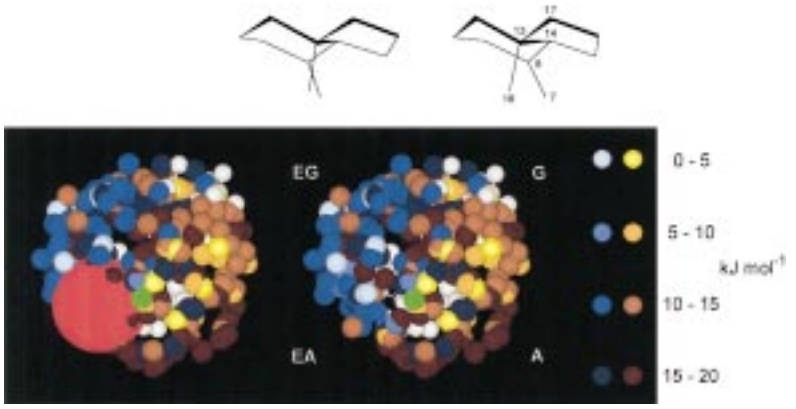


Figure 4. Volume maps representing the conformational behavior of the side chain of 1,25(OH)$_2$D$_3$ (**1**) (yellow) and its 20-epimer (**2**) (cyan) with the indicated regions A, G, EG, EA, and F following ref. [41]; the viewing direction is given in the stereoscopic view. Relative activity volume (red) following ref. [40]. Position of 25-oxygen in receptor-bound conformation (green) according to ref. [36].

0947-6539/01/0702-0526 $ 17.50+.50/0 *Chem. Eur. J.* **2001**, *7*, No. 2

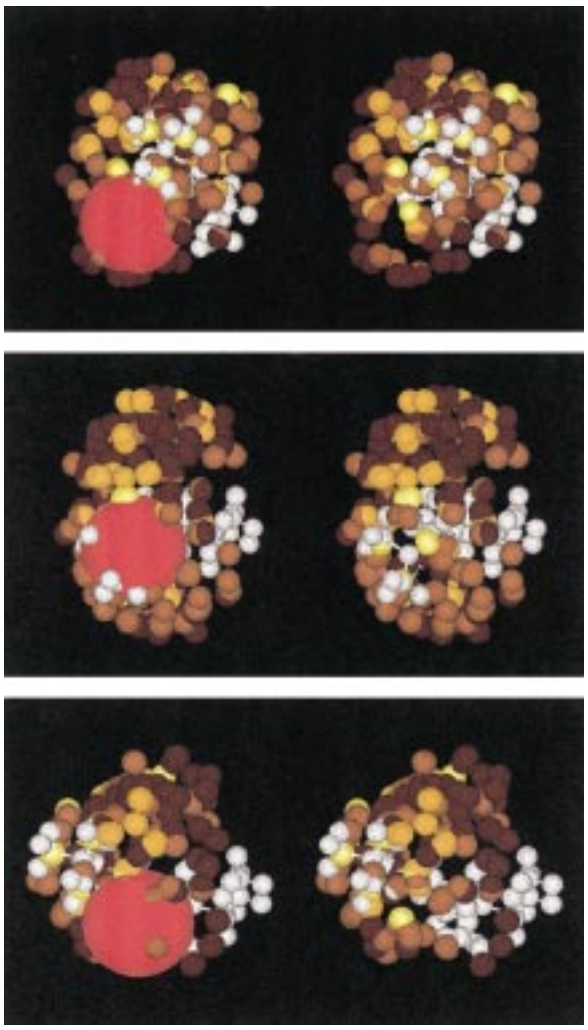


Figure 5. Volume maps representing the conformational behavior of the side chain of 6D analogues **4a** (middle), **4b** (top), and **4c** (bottom) with inclusion of the relative activity volume (red) according to ref. [40]; direction of view similar to the one used in Figure 4.

side chain was calculated to correspond to 63%, 6%, and 5% for **4a**, **4b**, and **4c**. Interestingly the biological activity of the 6D-analogue **3** is quite similar to the one observed for **4a**, in particular the differentiation of HL-60 cells and calcemic activity are identical.[11g] Furthermore, the conformational profile of the side chain **3** is analogous to the one calculated for **4a**; in particular the occupation of the same relative activity volume by the 25-hydroxy group of **3** corresponds to 69%. This further confirms the existence of a (qualitative) correlation between side chain orientation and cell-differentiating potency.

The structure of **4** is peculiar in that, when compared with $1,25(OH)_2D_3$, it lacks several atoms such as one methyl group at C13, the methyl group at C20, and a substantial part of the C-ring and possesses two extra carbon atoms in the D-ring. Yet, these important structural changes in the central part of the molecule do not prevent binding to the receptor. Whereas these changes may be responsible for the observed diminished calcemic activity, it is interesting to note how the cell differentiation activity can, in a first approximation, be regulated by the orientation of the side chain. These observations certainly warrant the continuation of the search for analogues that would feature an even more pronounced discrimination in activity.

Experimental Section

Synthesis: Thin-layer chromatography was performed on Merck silica gel 60F-254 TLC plates. All products were purified by flash chromatography (Merck silica gel 60F254) or HPLC: Waters 4000, Kontron 420/422. $[\alpha]_D^{20}$ ($CHCl_3$): Perkin–Elmer 241. IR (NaBr): Perkin–Elmer 1600 series. ^1H NMR ($CDCl_3$): 500 MHz, Bruker AN-500 (internal TMS as reference). ^{13}C NMR ($CDCl_3$): 50 MHz, Varian Gemini-200 (with DEPT program). MS: Finnigan 4000 or Hewlett–Packard 5988A.

Conjugate reduction of (5*R*,6*S*)-2,6-dimethyl-5-isopropenyl-2-cyclohexenone (6a) and (5*R*,6*R*)-2,6-dimethyl-5-isopropenyl-2-cyclohexenone (6b): A mixture of cyclohexenone **6a** (2.91 g, 17.72 mmol), sodium bicarbonate (26.8 g), and phase-transfer catalyst (Adogen 464, 0.96 g, 5.31 mmol) in toluene (220 mL) and water (220 mL) was stirred vigorously under nitrogen. Sodium dithionite (27.76 g, 159.45 mmol) was added and the mixture heated under reflux for 2 h. After cooling, the aqueous layer was separated and extracted with diethyl ether. The combined organic extracts were washed with water, dried, and the solvent removed in vacuo. After column chromatography on silica gel and further separation by HPLC (hexane/diethyl ether 85:15) ketones **7a** (2.35 g, 80%) and **7b** (0.52 g, 18%) were obtained as colorless oils. In exactly the same way the reduction of cyclohexenone **6b** afforded ketone **7c** (70% isolated yield).

Compound 7a: $R_f = 0.43$ (pentane/acetone 98:2); $[\alpha]_D^{20} = -2.5$ ($c = 0.85$, $CHCl_3$); IR (NaBr): $\tilde{\nu} = 2927, 1712, 1644, 1454, 1376, 1317, 1180, 1132, 980, 946, 892\ cm^{-1}$; ^1H NMR (500 MHz, $CDCl_3$): $\delta = 4.78$ (m, 1H), 4.75 (m, 1H), 2.42 (ddq, 1H, $J = 12.8$, 5.6, 6.4 Hz), 2.37 (dq, 1H, $J = 12.1$ Hz, 6.5 Hz), ≈2.08 (m, 2H), 1.85 (qd, 1H, $J = 12.9$, 3.8 Hz), 1.73 (*A*Bq, 1H, $J = 13.5$, 3.4 Hz), 1.70 (s, 3H), 1.38 (A*B*q, 1H, $J = 13.1$, 3.8 Hz), 1.02 (d, 3H, $J = 6.4$ Hz), 0.90 (d, 3H, $J = 6.5$ Hz); ^{13}C NMR/DEPT (50 MHz, $CDCl_3$): $\delta =$ 214.0 (C=O), 146.2 (C=), 112.1 (CH_2=), 55.6 (CH), 47.5 (CH), 45.1 (CH), 35.5 (CH_2), 31.4 (CH_2), 18.0 (CH_3), 14.6 (CH_3), 11.9 (CH_3); MS: *m*/*z*: (%): 166 $[M]^+$, 164 (100), 149 (32), 135 (23), 121 (28), 107 (37), 91 (43), 77 (27), 69 (46), 43 (64).

Compound 7b: $R_f = 0.40$ (pentane/acetone 98:2); $[\alpha]_D^{20} = +92.7$ ($c = 1.03$, $CHCl_3$); IR (NaBr): $\tilde{\nu} = 2933, 1708, 1646, 1456, 1374, 1231, 959, 892\ cm^{-1}$; ^1H NMR (500 MHz, $CDCl_3$): $\delta = 4.79$ (m, 1H), 4.72 (br s, 1H), 2.57 (dq, 1H, $J = 10.3$, 6.7 Hz), 2.57 (m, 1H), 2.13 (td, 1H, $J = 10.2$, 4.0 Hz), ≈1.90 (m, 2H), 1.73–1.57 (m, 2H), 1.71 (s, 3H), 1.18 (d, 3H, $J = 7.2$ Hz), 0.96 (d, 3H, $J = 6.7$ Hz); ^{13}C NMR/DEPT (50 MHz, $CDCl_3$): $\delta = 216.4$ (C=O), 145.9 (C=), 111.8 (CH_2=), 53.1 (CH), 43.4 (CH), 43.1 (CH), 31.0 (CH_2), 25.0 (CH_2), 18.4 (CH_3), 16.4 (CH_3), 12.7 (CH_3); MS: *m*/*z* (%): 166 (35) $[M]^+$, 151 (15), 139 (1), 137 (10), 123 (23), 109 (54), 95 (43), 81 (100), 67 (81), 55 (79), 41 (84).

Compound 7c: $R_f = 0.39$ (pentane/acetone 98:2); $[\alpha]_D^{20} = -1.2$ ($c = 1.07$, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): $\delta = 4.90$ (s, 1H), 4.73 (s, 1H), 2.70 (m, 1H, $\Sigma J = 26.5$ Hz), 2.59 (ddq, 1H, $J = 12.7$, 6.4, 6.4 Hz), 2.39 (m, 1H), 2.07 (m, 1H), 1.90 (qd, 1H, $J = 13.0$, 3.7 Hz), 1.68 (m, 1H), 1.67 (s, 3H), 1.30 (qd, 1H, $J = 13.0$, 3.7 Hz), 1.02 (d, 3H, $J = 6.5$ Hz), 0.95 (d, 3H, $J = 7.3$ Hz); ^{13}C NMR/DEPT (50 MHz, $CDCl_3$): $\delta = 216.7$ (C=O), 145.2 (C=), 111.2 (CH_2=), 48.3 (CH), 46.7 (CH), 40.0 (CH), 34.3 (CH_2), 23.7 (CH_2), 22.1 (CH_3), 14.5 (CH_3), 11.8 (CH_3).

Ethyl [(2*S*,3*R*,6*R*)-2,6-dimethyl-3-isopropenylcyclohexylidene]acetate (8a): Ethoxyethyne (0.33 mL, 2.35 mmol) and toluene (2 mL) were added 0 °C to a solution of ethylmagnesium bromide (1.98 mL, 1.99 mmol) in tetrahydrofuran (3 mL). After raising the temperature to 60 °C a solution of ketone **7a** (0.3 g, 1.80 mmol) in toluene (0.8 mmol) was added; the reaction mixture was further heated under reflux for 2 h and then poured into ice-water. The organic phase was separated after addition of ammonium chloride and the aqueous phase was extracted with diethyl ether. The organic phases were combined, washed with water, and concentrated in vacuo. The obtained residue was further dissolved in tetrahydrofuran (42 mL) and treated with 10% sulfuric acid (0.9 mL). After stirring for 10 h, the solution was concentrated in vacuo and the residue extracted with diethyl ether. The combined ether phases were washed with water and

dried ($MgSO_4$). After filtration and concentration in vacuo, the residue was purified by column chromatography and HPLC (pentane/acetone 98:2 to 95:5) to afford ester **8a** (0.189 g, 44%) and alcohol **9a** (0.221 g, 48%).

Compound 8a (inseparable mixture of *E*/*Z* 55:45): $R_f = 0.33$ (pentane/diethyl ether 98:2); IR (NaBr): $\tilde{\nu} = 2934, 1713, 1631, 1454, 1383, 1221, 1195, 1166, 1150, 1099, 1037, 891, 869\ cm^{-1}$; ^1H NMR (500 MHz, $CDCl_3$): $\delta = 5.68$ (s, 1H), 5.65 (s, 1H), 4.78 (m, 2H), 4.70 (m, 2H); MS: *m*/*z* (%): 236 (3) $[M]^+$, 221 (4), 207 (3), 193 (75), 165 (30), 154 (31), 121 (38), 107 (34), 81 (46), 77 (46), 41 (100); $C_{15}H_{24}O_2$ (236.35): calcd C 76.2, H 10.2; found C 76.15, H 10.23.

Compound 9a: $R_f = 0.26$ (pentane/diethyl ether 92:8); $[\alpha]_D^{20} = +22.2$ ($c = 1.19$, $CHCl_3$); IR (NaBr): $\tilde{\nu} = 3550, 2927, 1715, 1645, 1443, 1374, 1301, 1197, 1096, 1032, 966, 888\ cm^{-1}$; ^1H NMR (500 MHz, $CDCl_3$): $\delta = 4.71$ (br s, 1H), 4.70 (br s, 1H), ≈4.14 (m, 2H), 2.57 (s, 2H), 2.12 (td, 1H, $J = 11.8, 3.5$ Hz), 1.61 (s, 3H), 1.60–1.35 (m, 7H), 1.27 (t, 3H, $J = 7.1$ Hz), 0.95 (d, 3H, $J = 6.6$ Hz), 0.86 (d, 3H, $J = 6.7$ Hz); ^{13}C NMR/DEPT (50 MHz, $CDCl_3$): $\delta =$ 172.5 (C=O), 149.0 (C=), 111.2 (CH_2=), 74.6 (C–O), 60.4 (CH_2–O), 47.7 (CH), 41.3 (CH), 40.8 (CH_2), 39.7 (CH), 31.8 (CH_2), 30.0 (CH_2), 18.4 (CH_3), 16.0 (CH_3), 14.1 (CH_3), 12.5 (CH_3); MS: *m*/*z* (%): 254 (3) $[M]^+$, 236 (15), 208 (8), 197 (19), 170 (23), 144 (44), 123 (26), 109 (36), 82 (56), 69 (84), 55 (100); $C_{15}H_{26}O_3$ (254.37): calcd C 70.8, H 10.3; found C 70.72, H 10.28.

Compound 10a: A solution of alcohol **9a** (0.336 g, 1.32 mmol), *N*,*N*-dimethylaniline (8.6 mL, 68.12 mmol), and acetyl chloride (1.86 mL, 26.22 mmol) in chloroform (13 mL) was heated at reflux for 24 h. The resulting solution was diluted with water and acidified with hydrochloric acid. After extraction with diethyl ether, the organic phase was dried ($MgSO_4$), filtered, and concentrated in vacuo. Column chromatography and HPLC (pentane/acetone 99:1) afforded acetate **10a** (0.279 g, 71%). $R_f = 0.27$ (pentane/acetone 99:1); $[\alpha]_D^{20} = +28.3$ ($c = 1.16$, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): $\delta = 4.70$ (m, 2H), 4.12 (m, 2H), 3.54 (*AB*, 1H, $J =$ 15.1 Hz), 3.44 (A*B*, 1H, $J = 15.1$ Hz), 2.15 (td, 1H, $J = 11.6, 3.6$ Hz), 2.04 (s, 3H), 1.93 (m, 1H), 1.84 (dq, 1H, $J = 11.3, 6.7$ Hz), 1.60 (s, 3H), 1.56–1.40 (m, 4H), 1.24 (t, 3H, $J = 7.1$ Hz), 1.02 (d, 3H, $J = 6.8$ Hz), 0.90 (d, 3H, $J =$ 6.7 Hz); ^{13}C NMR/DEPT (50 MHz, $CDCl_3$): $\delta = 170.5$ (C=O), 148.4 (C=), 111.4 (CH_2=), 88.0 (C-O), 60.1 (CH_2-O), 47.8 (CH), 40.6 (CH), 39.1 (CH), 37.9 (CH_2), 31.5 (CH_2), 39.9 (CH_2), 22.2 (CH_3), 18.1 (CH_3), 17.2 (CH_3), 14.1 (CH_3), 13.6 (CH_3); $C_{17}H_{28}O_4$ (296.40): calcd C 68.9, H 9.5; found C 69.01, H 9.46.

Conversion of 10a to 8a: A solution of the acetate **10a** (0.241 g, 0.813 mmol) in a 1M solution of potassium *tert*-butoxide in *tert*-butanol (1 mL) was stirred for 24 h at 25°C. After extraction with diethyl ether, the organic phase was dried ($MgSO_4$), filtered, and concentrated in vacuo. HPLC (pentane/diethyl ether 98:2) afforded **8a** (0.146 g, 76%).

Ethyl [(*E*,2*S*,3*R*,6*S*)-2,6-dimethyl-3-isopropenylcyclohexylidene]acetate (11b): Treatment of ketone **7b** (0.80 g) with the Grignard derivative derived from ethoxyethyne, followed by acid hydrolysis, as described for the conversion of **7a** to **8a**, afforded **11b** (70%). $R_f = 0.36$ (pentane/diethyl ether 98:2); $[\alpha]_D^{20} = -12.1$ ($c = 0.97$, $CHCl_3$); IR (NaBr): $\tilde{\nu} = 2931, 1716, 1639, 1458, 1376, 1300, 1243, 1186, 1157, 1038, 890\ cm^{-1}$; ^1H NMR (500 MHz, $CDCl_3$): $\delta = 5.59$ (s, 1H), 4.73–4.70 (m, 2H), 4.16 (m, 1H), 4.15 (m, 2H), 2.42 (m, 1H), ≈1.82 (m, 2H), 1.70 (s, 3H), 1.65–1.42 (m, 3H), 1.29 (t, 3H, $J = 7.1$ Hz), 1.16 (d, 3H, $J = 7.2$ Hz), 0.92 (d, 3H, $J = 6.5$ Hz); ^{13}C NMR/DEPT (50 MHz, $CDCl_3$): $\delta = 170.2$ (C=O), 167.0 (C=), 148.0 (C=), 111.5 (CH_2=), 111.3 (CH=), 59.5 (CH_2-O), 55.4 (CH), 35.3 (CH), 32.5 (CH_2), 30.8 (CH), 26.3 (CH_2), 18.5 (CH_3), 18.2 (CH_3), 15.3 (CH_3), 14.3 (CH_3); MS: *m*/*z* (%): 236 (3) $[M]^+$, 221 (5), 193 (90), 191 (19), 165 (21), 147 (24), 133 (26), 121 (28), 107 (35), 81 (48), 67 (46), 41 (100); $C_{15}H_{24}O_2$ (236.35): calcd C 76.2, H 10.2; found C 76.09, H 10.17.

Ethyl [(*Z*,2*R*,3*R*,6*R*)-2,6-dimethyl-3-isopropenylcyclohexylidene]acetate (12c): Treatment of ketone **7c** (0.50 g) with the Grignard derivative derived from ethoxyethyne, followed by acid hydrolysis, as described for the conversion of **7a** to **8a**, afforded **12c** (51%). $R_f = 0.33$ (pentane/diethyl ether 99:1); $[\alpha]_D^{20} = -78.7$ ($c = 1.14$, $CHCl_3$); IR (NaBr): $\tilde{\nu} = 2933, 1716, 1637, 1458, 1374, 1201, 1152, 1037, 889\ cm^{-1}$; ^1H NMR (500 MHz, $CDCl_3$): $\delta = 5.58$ (s, 1H), 4.83 (m, 1H), 4.66 (s, 1H), 4.39 (qd, 1H, $J = 7.1, 4.4$ Hz), 4.16 (m, 2H), 2.44 (1H, $\Sigma J = 37$ Hz), 2.08 (m, 1H), 1.91 (dq, 1H, $J = 12.8, 3.0$ Hz), 1.78 (qd, 1H, $J = 13.1, 3.9$ Hz), 1.76 (s, 3H), 1.56 (m, 1H), 1.29 (t, 3H, $J = 7.2$ Hz), 1.11 (qd, 1H, $J = 12.8, 3.8$ Hz), 1.03 (d, 3H, $J = 6.5$ Hz), 0.87 (d, 3H, $J = 7.3$ Hz); ^{13}C NMR/DEPT (50 MHz, $CDCl_3$): $\delta = 170.8$ (C=O), 167.2 (C=), 147.3 (C=), 110.5 (CH=), 110.1 (CH_2=), 59.6 (CH_2-O), 48.0 (CH), 36.4 (CH_2), 33.3 (CH), 32.6 (CH), 24.1 (CH_2), 22.4 (CH_3), 18.2 (CH_3), 14.4 (CH_3), 13.0 (CH_3); MS: *m*/*z* (%): 236 (3) $[M]^+$, 221 (9), 207 (3), 193 (65), 165 (20), 154 (100), 121 (49), 107 (60), 81 (56), 69 (56), 41 (83).

Metal reduction of the α,β-unsaturated esters 8a, 11b, and 12c with lithium in liquid ammonia: A solution of ester **8a** (0.435 g, 1.84 mmol) in diethyl ether (11 mL) and *tert*-butanol (0.14 mL) was added dropwise to a solution of lithium (0.037 g, 5.34 mmol) in liquid ammonia (100 mL, distilled from sodium in the presence of $FeCl_3$). After stirring for 20 min the reaction mixture was quenched with ammonium chloride. After further stirring for 30 min, the ammonia was evaporated and the residue extracted with diethyl ether. The organic phase was dried ($MgSO_4$) and concentrated in vacuo. Column chromatography and HPLC (hexane/ethyl acetate 96:4) afforded ester **13a** (0.408 g, 93%). In the same manner unsaturated esters **11b** (0.746 g) and **12c** (0.650 g) afforded pure **13b** (82%) and **13c** (68%), respectively.

Compound 13a: $R_f = 0.37$ (pentane/diethyl ether 98:2); $[\alpha]_D^{20} = +18.3$ ($c = 1.00$, $CHCl_3$); IR (NaBr): $\tilde{\nu} = 2924, 1732, 1450, 1373, 1157, 1039, 885\ cm^{-1}$; ^1H NMR (500 MHz, $CDCl_3$): $\delta = 4.68$ (m, 2H), 4.12 (m, 2H), 2.42 (d, 2H, $J = 4.5$ Hz), ≈1.70 (m, 2H), 1.61 (s, 3H), 1.55–1.18 (m, 5H), 1.25 (t, 3H, $J = 7.1$ Hz), 1.10 (m, 1H), 0.93 (d, 3H, $J = 6.4$ Hz), 0.83 (d, 3H, $J = 6.4$ Hz); ^{13}C NMR/DEPT (50 MHz, $CDCl_3$): $\delta = 173.6$ (C=O), 149.4 (C=), 110.7 (CH_2=),60.0 (CH_2-O), 53.6 (CH), 47.5 (CH), 38.1 (CH), 36.6 (CH), 36.1 (CH_2), 35.5 (CH_2), 32.0 (CH_2), 20.6 (CH_3), 18.7 (CH_3), 17.3 (CH_3), 14.2 (CH_3); MS: *m*/*z* (%): 238 (12) $[M]^+$, 223 (3), 195 (28), 192 (5), 164 (9), 150 (80), 135 (38), 109 (56), 95 (75), 82 (98), 67 (77), 41 (100); $C_{15}H_{26}O_2$ (238.37): calcd C 75.6, H 11.0; found C 75.57, H 10.91.

Compound 13b: $R_f = 0.33$ (pentane/diethyl ether 98:2); $[\alpha]_D^{20} = +35.8$ ($c = 0.93$, $CHCl_3$); IR (NaBr): $\tilde{\nu} = 2929, 1737, 1644, 1452, 1382, 1253, 1207, 1160, 1126, 1034, 888\ cm^{-1}$; ^1H NMR (500 MHz, $CDCl_3$): $\delta =$ ≈4.69 (m, 2H), 4.12 (m, 2H), 2.49 (dd, 1H, $J = 14.8, 5.7$ Hz), 2.10 (dd, 1H, $J = 14.8, 9.0$ Hz), 1.87 (m, 1H), 1.77 (m, 1H, $\Sigma J = 30$ Hz), 1.80–1.30 (m, 6H), 1.63 (s, 3H), 1.25 (t, 3H, $J = 7.1$ Hz), 0.90 (d, 3H, $J = 7.2$ Hz), 0.75 (d, 3H, $J = 6.5$ Hz); ^{13}C NMR/DEPT (50 MHz, $CDCl_3$): $\delta = 173.9$ (C=O), 149.2 (C=), 110.9 (CH_2=), 60.1 (CH_2-O), 53.9 (CH), 43.7 (CH), 37.5 (CH_2), 32.9 (CH_2), 32.4 (CH), 31.2 (CH), 26.2 (CH_2), 18.7 (CH_3), 16.9 (CH_3), 14.2 (CH_3), 12.6 (CH_3); MS: *m*/*z* (%): 238 (23) $[M]^+$, 209 (3), 195 (35), 175 (12), 150 (100), 127 (68), 109 (62), 95 (96), 67 (70), 41 (74); $C_{15}H_{26}O_2$ (238.37): calcd C 75.6, H 11.0; found C 75.62, H 11.02.

Compound 13c: $R_f = 0.31$ (pentane/diethyl ether 98:2); $[\alpha]_D^{20} = -60.5$ ($c = 1.36$, $CHCl_3$); IR (NaBr): $\tilde{\nu} = 2926, 1736, 1644, 1444, 1373, 1334, 1302, 1270, 1251, 1189, 1159, 1126, 1032, 887\ cm^{-1}$; ^1H NMR (500 MHz, $CDCl_3$): $\delta =$ 4.77 (m, 1H), 4.57 (s, 1H), 4.16 (q, 2H, $J = 7.1$ Hz), 2.47 (dd, 1H, $J = 14.9, 5.9$ Hz), 2.12 (dd, 1H, $J = 14.9, 8.7$ Hz), 2.00 (m, 2H), 1.77–1.00 (m, 6H), 1.67 (s, 3H), 1.25 (t, 3H, $J = 7.1$ Hz), 0.84 (d, 3H, $J = 6.5$ Hz), 0.61 (d, 3H, $J = 7.1$ Hz); ^{13}C NMR/DEPT (50 MHz, $CDCl_3$): $\delta = 174.0$ (C=O), 148.0 (C=), 109.5 (CH_2=), 60.2 (CH_2-O), 48.2 (CH), 45.3 (CH), 37.6 (CH_2), 35.8 (CH_2), 33.5 (CH), 30.7 (CH), 24.1 (CH_2), 22.4 (CH_3), 19.0 (CH_3), 14.3 (CH_3), 6.9 (CH_3); MS: *m*/*z* (%): 238 (13) $[M]^+$, 208 (1), 195 (32), 169 (13), 150 (79), 135 (36), 109 (92), 82 (98), 67 (100), 55 (46); $C_{15}H_{26}O_2$ (238.37): calcd C 75.6, H 11.0; found C 75.43, H 11.19.

Reduction of α,β-unsaturated esters 8a, 11b, and 12c: Magnesium powder (0.012 g, 0.494 mmol) was added to a solution of the ester **8a** (0.050 g, 0.212 mmol) in dry methanol (2 mL). After consumption of the magnesium (ca. 45 min) a second portion was added. This process was repeated twice (total amount Mg: 0.048 g, 1.976 mmol). After cooling of the mixture, acetic acid (0.23 mL) was added, followed by water (1 mL). The reaction mixture was extracted with diethyl ether. The combined organic phases were washed with water and dried ($MgSO_4$). Column chromatography and HPLC (hexane/ethyl acetate 97:3) afforded a mixture of **13a** and **14a** (8:3, 84%). In the same way the reduction of **11b** (0.050 g) and **12c** (0.050 g) afforded mixtures of **13b** and **14b** (1:1, 79%) and **13c** and **14c** (1:1, 77%), respectively.

Relevant spectral data for ***14a*** (as mixture with **13a**): ^1H NMR (500 MHz, $CDCl_3$): $\delta = 4.68$ (m, 2H), 4.12 (m, 2H), 2.28 (quintet, 1H, $J = 4.5$ Hz), 2.17 (d, 2H, $J = 5.5$ Hz), 1.61 (s, 3H), 1.25 (t, 3H, $J = 7.2$ Hz), 0.86 (d, 3H, $J =$ 6.9 Hz), 0.75 (d, 3H, $J = 6.7$ Hz).

For ***14b*** (as a mixture with **13b**): ^1H NMR (500 MHz, $CDCl_3$): $\delta = 4.70$ (m, 2H), 4.12 (m, 2H), 2.37 (*AB*d, 1H, $J = 15.2, 5.4$ Hz), 2.30 (A*B*d, 1H, $J =$ 15.2, 9.1 Hz), 1.63 (s, 3H), 1.25 (t, 3H, $J = 7.2$ Hz), 1.04 (d, 3H, $J = 7.1$ Hz), 0.77 (d, 3H, $J = 7.2$ Hz).

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For ***14c*** (as a mixture with **13c**): ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 4.77$ (m, 1H), 4.57 (m, 1H), 4.14 (m, 2H), 2.42 (*A*Bd, 1H, $J = 14.8$, 5.2 Hz), 2.32 (A*B*d, 1H, $J = 14.8$, 9.7 Hz), 1.66 (s, 3H), 1.25 (t, 3H, $J = 7.1$ Hz), 0.84 (d, 3H, $J = 6.5$ Hz), 0.80 (d, 3H, $J = 7.2$ Hz).

2-[(1*S*,2*R*,3*R*,6*R*)-3-Isopropenyl-2,6-dimethylcyclohexyl] ethanol (15a): A solution of ester **13a** (0.354 g, 1.48 mmol) in THF (2 mL) was added at 0 °C to a solution of lithium aluminumhydride (0.113 g, 2.98 mmol) in tetrahydrofuran (3 mL). After stirring for 3 h at 25 °C, the reaction mixture was quenched at 0 °C by the successive addition of water (0.113 mL), 15% aqueous hydroxide solution (0.113 mL), and water (0.340 mL). After filtration over a silica gel pad, the filtrate was concentrated in vacuo. Column chromatography and HPLC (pentane/acetone 9:1) afforded alcohol **15a** (0.283 g, 97%). $R_f = 0.33$ (pentane/acetone 9:1); $[\alpha]_D^{20} = +15.4$, ($c = 1.04$, $CHCl_3$); IR (NaBr): $\tilde{\nu} = 3336$, 2921, 1447, 1376, 1042, 886 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = \approx 4.67$ (m, 2H), 3.64 (t, 2H, $J = 7.9$ Hz), 1.76 (td, 2H, $J = 8.0$, 3.7 Hz), ≈1.68 (m, 2H), 1.61 (s, 3H), 1.53 (dq, 1H, $J = 13.0$, 3.4 Hz), 1.35 (qd, 1H, $J = 12.8$, 3.5 Hz), 1.30–1.15 (m, 3H), 1.04 (qd, 1H, $J = 12.6$, 3.5 Hz), 0.94 (d, 3H, $J = 6.5$ Hz), 0.83 (d, 3H, $J = 6.4$ Hz), 0.66 (tt, 1H, $J = 10.6$, 3.6 Hz); ^{13}C NMR/DEPT (50 MHz, $CDCl_3$): $\delta = 149.5$ (C=), 110.6 (CH_2=), 60.7 (CH_2-O), 53.9 (CH), 47.4 (CH), 37.6 (CH), 36.2 (CH), 35.7 (CH_2), 33.2 (CH_2), 32.1 (CH_2), 20.6 (CH_3), 18.7 (CH_3), 17.3 (CH_3); MS: *m*/*z* (%): 196 (4) $[M]^+$, 181 (5), 163 (1), 153 (13), 137 (9), 135 (6), 121 (7), 109 (31), 95 (42), 82 (100), 67 (43), 55 (39), 41 (35); $C_{13}H_{24}O$ (196.33): calcd C 79.6, H 12.3; found C 79.52, H 12.37.

1-[(1*R*,2*R*,3*S*,4*R*)-3-(2-Hydroxyethyl)-2,4-dimethylcyclohexyl]ethanone (16a): A stream of ozone was passed through a solution of alkene **15a** (0.050 g, 0.255 mmol) in dichloromethane (1.4 mL) and methanol (1.1 mL) at −40 °C until persistance of a blue color. After passing of nitrogen through the solution dimethylsulfide (0.112 mL, 1.528 mL) was added dropwise at −20 °C and the resulting mixture was stirred overnight at 25 °C. After concentration in vacuo, the residue was dissolved in ether and the organic phase washed with water and brine. After drying ($MgSO_4$) and concentration in vacuo purification of the residue by column chromatography and HPLC (pentane/acetone 84:16) afforded ketone **16a** (45 mg, 89%). M.p. 42 °C; $R_f = 0.29$ (pentane/acetone 84:16); $[\alpha]_D^{20} = -0.3$ ($c = 1.26$, $CHCl_3$); IR (NaBr): $\tilde{\nu} = 3406$, 2924, 1704, 1448, 1362, 1246, 1168, 1042 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 3.64$ (br t, 2H, $J = 7$ Hz), 2.16 (m, 1H), 2.13 (s, 3H), 1.75–1.00 (m, 9H), 0.94 (d, 3H, $J = 6.4$ Hz), 0.86 (d, 3H, $J = 6.4$ Hz), 0.70 (tt, 1H, $J = 10.8$, 3.7 Hz); ^{13}C NMR/DEPT (50 MHz, $CDCl_3$): $\delta = 213.5$ (C=O), 60.4 (CH_2-O), 59.4 (CH), 46.6 (CH), 36.8 (CH), 35.7 (CH), 35.0 (CH_2), 32.4 (CH_2), 29.4 (CH_2), 29.3 (CH_3), 20.4 (CH_3), 17.9 (CH_3); MS: *m*/*z* (%): 198 (5) $[M]^+$, 180 (3), 165 (15), 153 (3), 137 (13), 109 (30), 95 (52), 81 (58), 69 (43), 43 (100); $C_{12}H_{22}O_2$ (198.31): calcd C 72.68, H 11.18; found C 72.63, H 11.16.

2-[(1*S*,2*R*,3*R*,6*R*)-2,6-Dimethyl-3-(2-methyl-[1,3]-dioxolan-2-yl)cyclohexyl]ethanol (17a): A solution of the methylketone **16a** (0.330 g, 1.664 mmol), trimethylorthoformate (0.330 mL, 3.013 mmol), and *p*-toluenesulfonic acid (0.017 g, 0.089 mmol) in 1,2-ethanediol (0.985 mL, 17.654 mmol) was stirred at 25 °C for 24 h. The reaction mixture was quenched with an aqueous solution of sodium bicarbonate. After stirring for a further 5 min the mixture was extracted with diethyl ether. The combined extracts were concentrated in vacuo. Column chromatography and HPLC (pentane/acetone 83:47) afforded acetal **17a** (0.335 g, 83%). M.p. 30 °C; $R_f = 0.38$ (pentane/acetone 85:15); $[\alpha]_D^{20} = -2.1$ ($c = 1.06$, $CHCl_3$); IR (NaBr): $\tilde{\nu} = 3307$, 2880, 1591, 1478, 1455, 1379, 1304, 1210, 1120, 1078, 1038, 950 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 3.97$ (q, 1H, $J = 6.5$ Hz), 3.90 (dt, 1H, $J = 6.8$, 7.2 Hz), 3.84 (m, 2H), 3.62 (m, 2H), 1.96 (dq, 1H, $J = 12.6$, 3.1 Hz), ≈1.74 (m, 3H), 1.24–1.14 (m, 5H), 1.22 (s, 3H), 1.07 (d, 3H, $J = 6.0$ Hz), 1.00 (m, 1H), 0.93 (d, 3H, $J = 6.4$ Hz), 0.62 (tt, 1H, $J = 10.2$, 3.6 Hz); ^{13}C NMR/DEPT (50 MHz, $CDCl_3$): $\delta = 112.3$ (O-C-O), 64.2 (CH_2-O), 63.1 (CH_2-O), 60.8 (CH_2-O), 51.1 (CH), 47.7 (CH), 38.0 (CH), 36.5 (CH), 35.5 (2 × CH_2), 28.4 (CH_2), 20.8 (CH_3), 19.3 (CH_3), 17.5 (CH_3); MS: *m*/*z* (%): 227 (1), 165 (1), 135 (1), 99 (2), 87 (100), 67 (3), 43 (19); $C_{14}H_{26}O_3$ (242.36): calcd C 69.4, H 10.8; found C 69.07, H 10.98.

2-[(1*S*,2*R*,3*R*,6*R*)-2,6-Dimethyl-3-(2-methyl-[1,3]-dioxolan-2-yl)cyclohexyl]ethyl toluene-4-sulfonate (18a): Triethylamine (0.13 mL, 0.957 mmol), a solution of *p*-toluenesulfonyl chloride (0.091 g, 0.479 mmol) in dichloromethane (0.5 mL) and a trace amount of 4-dimethylaminopyridine were added at 0 °C to a solution of alcohol **17a** (0.059 g, 0.239 mmol) in dichloromethane (1.7 mL). After stirring for 20 h at 25 °C the reaction mixture was concentrated to half its volume and filtered. The filtrate was further concentrated and the residue purified by column chromatography and HPLC (pentane/acetone 9:1) to afford tosylate **18a** (0.092 g, 97%). $R_f = 0.36$ (pentane/acetone 9:1); $[\alpha]_D^{20} = +2.9$ ($c = 0.98$, $CHCl_3$); IR (NaBr): $\tilde{\nu} = 2879$, 1456, 1362, 1177, 1097, 1040, 954, 815, 668 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 7.77$ (d, 2H, $J = 8.2$ Hz), 7.37 (d, 2H, $J = 8.1$ Hz), 4.00 (t, 2H, $J = 7.9$ Hz), 3.93 (q, 1H, $J = 6.5$ Hz), 3.85 (m, 1H), 3.80 (m, 2H), 2.43 (s, 3H), 1.92 (m, 1H), 1.85–0.90 (m, 8H), 1.15 (s, 3H), 0.92 (d, 3H, $J = 6.3$ Hz), 0.80 (d, 3H, $J = 6.4$ Hz), 0.60 (tt, 1H, $J = 10.6$, 3.5 Hz); ^{13}C NMR/DEPT (50 MHz, $CDCl_3$): $\delta = 144.6$ (Ar-C), 133.2 (Ar-C), 129.7 (2 × Ar-CH), 127.9 (2 × Ar-CH), 112.0 (O-C-O), 68.7 (CH_2-O), 64.2 (CH_2-O), 63.0 (CH_2-O), 51.1 (CH), 47.4 (CH), 37.6 (CH), 36.0 (CH), 35.3 (CH_2), 28.9 (CH_2), 28.3 (CH_2), 21.5 (CH_3), 20.5 (CH_3), 19.2 (CH_3), 17.3 (CH_3); MS: *m*/*z* (%): 381 (1), 155 (1), 87 (100), 43 (13); $C_{21}H_{32}O_5S$ (396.54): calcd C 63.6, H 8.1; found C 63.45, H 8.32.

2-[(1*S*,2*R*,3*R*,4*R*)-3-[5-(1-Ethoxyethoxy)-5-methylhex-3-ynyl]-2,4-dimethylcyclohexyl-2-methyl-[1,3]-dioxolane (19a): A suspension of sodium hydride (60% dispersion in mineral oil, 0.079 g, 1.967 mmol) in dimethylsulfoxide (2 mL) was stirred for 90 min at 65 °C. The resulting mixture was cooled, and treated with 3-(1-ethoxyethoxy)-3-methyl-1-butyne (0.310 g, 1.967 mmol) and after 5 min with a solution of tosylate **18a** (0.078 g, 0.197 mmol) in dimethylsulfoxide (0.3 mL) and tetrahydrofuran (0.3 mL). After stirring for 2 h, the reaction mixture was poured into an ice-cold saturated solution of ammonium chloride. After extraction with ether, the organic phase was dried ($MgSO_4$) and concentrated in vacuo. Purification of the residue by column chromatography and HPLC (pentane/acetone 97:3) afforded alkyne **19a** (0.058 g, 77%). $R_f = 0.31$ (pentane/acetone 97:3); IR (NaBr): $\tilde{\nu} = 2981$, 1456, 1378, 1250, 1158, 1117, 1079, 1040, 976 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 5.08$ (q, 1H, $J = 5.1$ Hz), 3.95 (q, 1H, $J = 6.6$ Hz), 3.88 (m, 1H), 3.82 (m, 2H), 3.68 (dq, 1H, $J = 9.2$, 7.1 Hz), 3.48 (dq, 1H, $J = 9.3$, 7.1 Hz), 2.10 (t, 2H, $J = 8.3$ Hz), 1.95 (m, 1H), 1.75–1.00 (m, 8H), 1.47 (s, 3H), 1.40 (s, 3H), 1.31 (d, 3H, $J = 5.2$ Hz), 1.20 (s, 3H), 1.17 (t, 3H, $J = 7.1$ Hz), 1.07 (d, 3H, $J = 6.1$ Hz), 0.89 (d, 3H, $J = 6.4$ Hz), 0.70 (tt, 1H, $J = 10.2$, 3.1 Hz); ^{13}C NMR/DEPT (50 MHz, $CDCl_3$): $\delta = 112.2$ (O-C-O), 96.2 (O-CH-O), 85.2 (C≡), 82.2 (C≡), 70.2 (C-O), 65.2 (CH_2-O), 63.0 (CH_2-O), 60.7 (CH_2-O), 51.0 (CH), 49.5 (CH), 36.7 (CH), 35.4 (CH_2), 35.0 (CH), 30.7 (CH_3), 30.3 (CH_3), 28.5 (CH_2), 28.4 (CH_2), 22.4 (CH_3), 20.7 (CH_3), 19.2 (CH_3), 17.3 (CH_3), 15.3 (CH_3), 14.3 (CH_3); MS: *m*/*z* (%): 365 (1), 307 (2), 229 (2), 203 (1), 87 (100), 73 (16).

2-[(1*S*,2*R*,3*S*,4*R*)-3-[5-(1-Ethoxyethoxy)-5-methylhexyl]-2,4-dimethylcyclohexyl-2-methyl-[1,3]-dioxolane (20a): 5% Rhodium on aluminum oxide (0.048 g) was added to a solution of alkyne **19a** (0.053 g, 0.139 mmol) in ethyl acetate (5.3 mL). The suspension was hydrogenated, filtered through a short pad of silica gel and the filtrate concentrated in vacuo. The residue was purified by column chromatography and HPLC (pentane/acetone 98:2) to afford **20a** (0.040 g, 75%). $R_f = 0.31$ (pentane/acetone 98:2); IR (NaBr): $\tilde{\nu} = 2936$, 1446, 1378, 1207, 1123, 1087, 1034, 973, 865 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 4.87$ (q, 1H, $J = 5.2$ Hz), 3.95 (q, 1H, $J = 6.6$ Hz), 3.89 (q, 1H, $J = 6.8$ Hz), 3.83 (m, 2H), 3.53 (dq, 1H, $J = 8.8$, 7.1 Hz), 3.45 (dq, 1H, $J = 8.8$, 7.1 Hz), 1.94 (m, 1H), 1.69 (m, 1H), 1.35–1.00 (m, 13H), 1.26 (d, 3H, $J = 5.3$ Hz), 1.21 (s, 3H), 1.19 (s, 3H), 1.17 (s, 3H), 1.16 (t, 3H, $J = 6.9$ Hz), 1.00 (d, 3H, $J = 6.0$ Hz), 0.86 (d, 3H, $J = 6.4$ Hz), 0.65 (tt, 1H, $J = 10.0$ Hz); ^{13}C NMR/DEPT (50 MHz, $CDCl_3$): $\delta = 112.5$ (O-C-O), 93.6 (O-CH-O), 75.7 (C-O), 64.3 (CH_2-O), 63.1 (CH_2-O), 58.7 (CH_2-O), 51.1 (CH), 50.1 (CH), 42.2 (CH_2), 36.8 (CH), 35.7 (CH_2), 35.1 (CH), 28.9 (CH_2), 28.7 (CH_2), 26.5 (CH_3), 26.3 (CH_3), 25.0 (CH_2), 24.9 (CH_2), 22.0 (CH_3), 20.8 (CH_3), 19.2 (CH_3), 17.3 (CH_3), 15.5 (CH_3); MS: *m*/*z* (%): 369 (1), 295 (5), 233 (1), 131 (5), 109 (3), 87 (100), 73 (48), 43 (22); $C_{23}H_{44}O_4$ (384.60): calcd C 71.8, H 11.5; found C 71.66, H 11.94.

1-[(1*R*,2*R*,3*S*,4*R*)-3-[5-Hydroxy-5-methylhexyl)-2,4-dimethylcyclohexyl]-ethanone (21a): A solution of acetal **20a** (0.039 g, 0.101 mmol) in acetone (3.9 mL) containing a trace amount of pyridinium *p*-toluenesulfonate and a few drops of water was heated at reflux for 20 h. After cooling, the solution was concentrated in vacuo, the residue was dissolved in diethyl ether and washed with a saturated sodium bicarbonate solution and brine. After drying ($MgSO_4$) and concentration the residue was purified by column chromatography and HPLC (pentane/acetone 85:15) to afford **21a** (0.025 g, 92%). $R_f = 0.45$ (pentane/acetone 86:14); $[\alpha]_D^{20} = +5.5$ ($c = 1.05$, $CHCl_3$); IR (NaBr): $\tilde{\nu} = 3421$, 2931, 1707, 1458, 1376, 1205, 1170, 904 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 2.17$ (m, 1H), 2.12 (s, 3H), 1.74 (m, 2H), 1.61–1.20 (m, 12H), 1.20 (s, 2 × 3H), 1.20 (s, 3H), 1.04 (qd, 1H, $J = 13.2$, 3.5 Hz), 0.89 (d, 3H, $J = 6.5$ Hz), 0.81 (d, 3H, $J = 6.4$ Hz), 0.71 (tt, 1H, $J =$

10.7, 3.5 Hz); ^{13}C NMR/DEPT (50 MHz, $CDCl_3$): $\delta = 213.7$ (C=O), 71.0 (C-O), 59.5 (CH), 48.8 (CH), 43.9 (CH_2), 35.6 (CH), 35.1 (CH_2), 34.3 (CH), 29.5 (CH_2), 29.3 ($3 \times CH_3$), 28.1 (CH_2), 25.2 (CH_2), 24.4 (CH_2), 20.3 (CH_3), 17.7 (CH_3); MS: *m/z* (%): 250 (5), 232 (2), 207 (9), 177 (3), 152 (6), 137 (6), 109 (26), 95 (28), 69 (32), 43 (100); $C_{17}H_{32}O_2$ (268.44): calcd C 76.1, H 12.0; found C 75.18, H 12.31. In a similar procedure compounds **21b** and **21c** were obtained.

Compound 21b: $R_f = 0.40$ (pentane/acetone 87:13); $[\alpha]_D^{20} = +39.2$ ($c = 0.76$, $CHCl_3$); IR (NaBr): $\tilde{\nu} = 2933$, 1705 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): $\delta =$ 2.12 (m, 1H), 2.12 (s, 3H), 1.93 (m, 1H), 1.68 – 0.94 (m, 15H), 1.20 (s, 3H), 1.20 (s, 3H), 0.83 (d, 3H, $J = 7.2$ Hz), 0.78 (d, 3H, $J = 6.4$ Hz); ^{13}C NMR/DEPT (50 MHz, $CDCl_3$): $\delta = 213.2$ (C=O), 84.8 (C≡), 82.7 (C≡), 65.1 (C-O), 59.1 (CH), 48.2 (CH), 35.4 (CH), 34.8 (CH_2), 34.2 (CH), 31.5 ($2 \times CH_3$), 29.3 (CH_3, CH_2), 27.3 (CH_2), 20.2 (CH_3), 17.8 (CH_3), 14.0 (CH_2); MS: *m/z* (%): 249 (10), 203 (3), 161 (5), 133 (8), 107 (11), 95 (17), 43 (100).

Compound 21c: $R_f = 0.36$ (pentane/acetone 85:15); $[\alpha]_D^{20} = -105.2$ ($c =$ 1.05, $CHCl_3$); IR (NaBr): $\tilde{\nu} = 3431$, 2938, 1705, 1464, 1377, 1356, 1197, 905 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 2.40$ (dt, 1H, $J = 11.5$, 4.1 Hz), 2.34 (m, 1H), 2.12 (s, 3H), 1.70 (dq, 1H, $J = 13.2$, 3.5 Hz), 1.65 – 0.10 (m, 13H), 1.22 (s, 3H), 1.22 (s, 3H), 0.92 (qd, 1H, $J = 12.8$, 5.2 Hz), 0.85 (d, 3H, $J = 6.4$ Hz), 0.62 (d, 3H, $J = 7.1$ Hz); ^{13}C NMR/DEPT (50 MHz, $CDCl_3$): $\delta = 211.8$ (C=O), 70.8 (C-O), 56.0 (CH), 47.7 (CH), 43.8 (CH_2), 35.0 (CH_2), 31.7 (CH), 30.6 (CH), 29.9 (CH_2), 29.2 ($2 \times CH_3$), 28.1 (CH_3), 27.4 (CH_2), 24.7 (CH_2), 21.0 (CH_2), 20.2 (CH_3), 7.7 (CH_3); MS: *m/z* (%): 250 (3), 235 (2), 207 (9), 195 (3), 165 (3), 152 (5), 123 (9), 109 (17), 87 (64), 69 (45), 43 (100).

Electrochemical oxidation of ketones 21a, 21b, and 21c: The electrochemical oxidation was performed in an undivided cell equipped with two platinum electrodes under magnetic stirring at 0 °C (external cooling). A solution of methyl ketone **21a** (0.090 g, 0.335 mmol) and sodium bromide (0.069 g, 0.671 mmol) in methanol (3 mL) was brought under a tension of 20 – 30 V for 45 min. The reaction mixture was concentrated in vacuo and extracted with diethyl ether. The combined organic phases were washed with water, dried ($MgSO_4$) and concentrated in vacuo. Column chromatography and HPLC (pentane/acetone 88:12) afforded methyl ester **22a** (0.027 g, 28%). Similarly methyl ketones **21b** (0.042 g) and **21c** (0.100 g) led to the corresponding esters **22b** (46%) and **22c** (76%).

Compound 22a: $R_f = 0.31$ (pentane/acetone 9:1); $[\alpha]_D^{20} = -13.9$ ($c = 0.64$, $CHCl_3$); IR (NaBr): $\tilde{\nu} = 3395$, 2932, 1737, 1456, 1380, 1256, 1195, 1141 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 3.67$ (s, 3H), 2.03 (td, 1H, $J = 11.6$, 3.1 Hz), 1.81 (dq, 1H, $J = 12.9$, 3.2 Hz), 1.71 (dq, 1H, $J = 13.3$, 3.2 Hz), 1.65 – 0.95 (m, 13H), 1.2 (s, 3H), 1.20 (s, 3H), 0.89 (m, 6H), 0.71 (tt, 1H, $J =$ 10.7 Hz); ^{13}C NMR (50 MHz, $CDCl_3$): $\delta = 176.9$, 70.9, 51.8, 51.2, 48.6, 43.8, 36.1, 34.7, 34.0, 29.9, 29.7, 29.1, 28.1, 25.0, 24.2, 20.2, 17.7; MS: *m/z* (%): 269 (15), 266 (29), 253 (13), 237 (19), 226 (58), 219 (10), 206 (38), 191 (33), 169 (18), 151 (18), 137 (41), 126 (38), 123 (20), 109 (92), 95 (41), 81 (37), 69 (47), 59 (100), 43 (48).

Compound 22b: $R_f = 0.25$ (pentane/acetone 9:1); $[\alpha]_D^{20} = +14.2$ ($c = 0.90$, $CHCl_3$); IR (NaBr): $\tilde{\nu} = 3417$, 2935, 1738, 1454, 1381, 1257, 1196, 1168, 1144, 1021, 909 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 3.67$ (s, 3H), 2.00 (td, 1H, $J = 11.5$, 3.9 Hz), 1.93 (m, 1H), 1.70 – 0.90 (m, 15H), 1.21 (s, 2×3H), 0.85 (d, 3H, $J = 7.1$ Hz), 0.82 (d, 3H, $J = 6.3$ Hz); ^{13}C NMR/DEPT (50 MHz, $CDCl_3$): $\delta =$ 17ss7.0 (C=O), 71.0 (C-O), 52.2 (CH), 51.3 (CH_3-O), 45.2 (CH), 44.0 (CH_2), 32.7 (CH), 32.5 (CH_2), 29.8 (CH_2), 29.2 ($2 \times CH_3$), 28.7 (CH), 27.4 (CH_2), 24.7 (CH_2), 24.2 (CH_2), 18.0 (CH_3), 12.1 (CH_3); MS: *m/z* (%): 266 (3), 226 (5), 206 (6), 167 (5), 137 (7), 109 (30), 95 (16), 59 (100).

Compound 22c: $R_f = 0.27$ (pentane/acetone 9:1); $[\alpha]_D^{20} = -51.0$ ($c = 0.82$, $CHCl_3$); IR (NaBr): $\tilde{\nu} = 3413$, 2936, 1735, 1458, 1379, 1204, 1133, 1021, 908 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 3.67$ (s, 3H), 2.41 (dt, 1H, $J =$ 11.0, 4.8 Hz), 2.30 (m, 1H, $\Sigma J = 28$ Hz), 1.75 – 0.90 (m, 15H), 1.21 (s, 3H), 1.21 (s, 3H), 0.85 (d, 3H, $J = 6.4$ Hz), 0.69 (d, 3H, $J = 7.1$ Hz); ^{13}C NMR/DEPT (50 MHz, $CDCl_3$): $\delta = 175.8$ (C=O), 71.0 (C-O), 51.4 (CH_3-O), 48.0 (CH), 47.3 (CH), 44.0 (CH_2), 35.2 (CH_2), 32.1 (CH), 30.5 (CH), 30.0 (CH_2), 29.2 ($2 \times CH_3$), 27.4 (CH_2), 24.8 (CH_2), 21.9 (CH_2), 20.2 (CH_3), 8.1 (CH_3); MS: *m/z* (%): 266 (8), 237 (3), 226 (6), 191 (12), 170 (3), 167 (9), 137 (15), 109 (28), 95 (23), 81 (37), 59 (100).

Conversion of esters 22a, 22b, and 22c into the corresponding aldehydes 24a, 24b, and 24c: Esters **22a**, **22b**, and **22c** were reduced with lithium aluminumhydride in tetrahydrofuran to the corresponding alcohols, as described for the conversion of ester **13a** into primary alcohol **15a**, to afford alcohols **23a** (99%), **23b** (52%), and **23c** (41%), respectively. A suspension of alcohol **23a** (5.5 mg, 0.022 mmol) and pyridinium chlorochromate on aluminum oxide (0.070 g, 0.327 mmol) in dichloromethane (0.55 mL) was stirred for 5 h. After filtration and washing of the precipitate with diethyl ether, the ether phase was concentrated in vacuo and the residue purified by column chromatography and HPLC (pentane/acetone 9:1) to afford aldehyde **24a** (3 mg, 55%). In the same way were obtained **24b** (66%) and **24c** (55%).

Compound 24a: $R_f = 0.27$ (pentane/acetone 88:12); IR (NaBr): $\tilde{\nu} = 2930$, 1718 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 9.51$ (d, 1H, $J = 4.1$ Hz), 1.98 (tt, 1H, $J = 11.6$ Hz), 1.80 – 0.80 (m, 15H), 1.21 (s, 3H), 1.21 (s, 3H), 0.90 (d, 3H, $J = 6.5$ Hz), 0.76 (tt, 1H, $J = 10.6$, 3.4 Hz).

Compound 24b: $R_f = 0.24$ (pentane/acetone 9:1); IR (NaBr): $\tilde{\nu} = 2933$, 1726 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 9.51$ (d, 1H, $J = 4.0$ Hz), 1.95 (m, 2H), 1.70 – 1.05 (m, 15H), 1.20 (s, 3H), 1.20 (s, 3H), 0.89 (d, 3H, $J =$ 6.4 Hz), 0.84 (d, 3H, $J = 7.2$ Hz); ^{13}C NMR/DEPT (50 MHz, $CDCl_3$): $\delta =$ 205.4 (C=O), 71.0 (C-O), 57.8 (CH), 44.9 (CH), 44.0 (CH_2), 30.1 (CH), 29.5 (CH_2), 29.3 ($2 \times CH_3$), 28.4 (CH), 27.5 (CH_2), 24.7 (CH_2), 20.5 (CH_2), 18.2 (CH_3), 12.2 (CH_3); MS: *m/z* (%): 237 (3), 191 (3), 179 (1), 153 (3), 137 (5), 125 (5), 109 (27), 81 (17), 59 (100), 43 (31).

Compound 24c: $R_f = 0.31$ (pentane/acetone 9:1); IR (NaBr): $\tilde{\nu} = 3398$, 2934, 1723, 1462, 1377, 1159, 908 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): $\delta =$ 9.67 (s, 1H), 2.45 (m, 1H, $\Sigma J = 28$ Hz), 2.29 (dt, 1H, $J = 12.6$, 3.6 Hz), 1.74 (m, 2H), 1.60 – 1.05 (m, 12H), 1.22 (s, 3H), 1.22 (s, 3H), 0.97 (qd, 1H, $J =$ 12.8, 3.4 Hz), 0.87 (d, 3H, $J = 7.0$ Hz), 0.70 (d, 3H, $J = 7.1$ Hz); MS: *m/z* (%): 236 (3), 207 (2), 191 (2), 165 (3), 139 (3), 123 (5), 95 (21), 69 (15), 59 (100).

Wittig – Horner coupling reaction of aldehydes 24a, 24b, and 24c with phosphine oxide 25: *n*-Butyllithium (0.041 mL, 2.5 M hexanes, 0.104 mmol) was added dropwise at −78 °C to a solution of phosphine oxide **25** (0.060 g, 0.104 mmol) in dry tetrahydrofuran (0.42 mL). After stirring for 20 min a solution of aldehyde **24a** (0.004 g, 0.016 mmol) in tetrahydrofuran (1.05 mL) was added very slowly to the deep red reaction mixture. After stirring for 3 h at −78 °C, the reaction mixture was warmed slowly to 0 °C. After stirring for a few minutes exposed to air (the reaction mixture turned to yellow), the mixture was directly loaded on a silica gel column (pentane/ether 7:3). The crude product was dissolved in tetrahydrofuran (1.75 mL) and treated with tetrabutylammonium fluoride (0.35 mL, 1 M THF). After stirring overnight at 25 °C, the mixture was concentrated in vacuo and the residue purified by column chromatography and HPLC (pentane/acetone 7:3) to afford analogue **4a** (4.6 mg, 57%). In the same way aldehydes **24b** and **24c** were converted into analogues **4b** (57%) and **4c** (49%), respectively.

Compound 4a: $R_f = 0.29$ (pentane/acetone 7:3); IR (NaBr): $\tilde{\nu} = 3395$, 2929, 1450, 1378, 1048, 970, 908, 735 cm^{-1}; UV (MeOH): $\lambda_{max} = 209$, 250 nm; ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 6.33$ (dd, 1H, $J = 15.2$, 10.8 Hz), 6.03 (d, 1H, $J = 10.7$ Hz), 5.52 (dd, 1H, $J = 15.3$, 8.9 Hz), 5.30 (s, 1H), 5.00 (s, 1H), 4.44 (m, 1H), 4.21 (m, 1H), 2.57 (dd, 1H, $J = 13.1$, 9.8 Hz), 2.26 (dd, 1H, $J = 13.1$, 7.5 Hz), 2.00 – 0.95 (m, 20H), 1.20 (s, 3H), 1.20 (s, 3H), 0.87 (d, 3H, $J = 6.4$ Hz), 0.82 (d, 3H, $J = 6.4$ Hz), 0.67 (m, 1H); MS: *m/z* (%): 372 (3), 351 (21), 319 (3), 303 (9), 273 (3), 225 (7), 199 (45), 183 (19), 135 (36), 105 (36), 77 (55), 43 (100).

Compound 4b: $R_f = 0.30$ (pentane/acetone 7:3); IR (NaBr): $\tilde{\nu} = 3354$, 2912 cm^{-1}; UV (MeOH): $\lambda_{max} = 209$, 248 nm; ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 6.32$ (dd, 1H, $J = 15.2$, 10.9 Hz), 6.03 (d, 1H, $J = 10.8$ Hz), 5.53 (dd, 1H, $J = 15.2$, 9.0 Hz), 5.31 (s, 1H), 4.99 (s, 1H), 4.43 (br s, 1H), 4.21 (br s, 1H), 2.57 (dd, 1H, $J = 13.4$, 3.6 Hz), 2.26 (dd, 1H, $J = 13.0$, 7.4 Hz), 2.00 – 0.90 (m, 21H), 1.21 (s, 3H), 1.21 (s, 3H), 0.81 (d, 3H, $J = 7.2$ Hz), 0.82 (d, 3H, $J =$ 6.0 Hz); MS: *m/z* (%): 372 (5), 354 (3), 336 (3), 255 (1), 191 (3), 166 (9), 148 (66), 109 (44), 95 (50), 43 (100).

Compound 4c: $R_f = 0.25$ (pentane/acetone 7:3); IR (NaBr): $\tilde{\nu} = 3339$, 2930, 1455, 1380, 1057, 976, 908 cm^{-1}; UV (MeOH): $\lambda_{max} = 207$, 247 nm; ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 6.34$ (dd, 1H, $J = 15.3$, 10.8 Hz), 6.05 (d, 1H, $J =$ 10.8 Hz), 5.74 (dd, 1H, $J = 15.4$, 6.7 Hz), 5.31 (br s, 1H), 5.01 (br s, 1H), 4.44 (m, 1H), 4.22 (m, 1H), 2.57 (dd, 1H, $J = 13.3$, 3.4 Hz), 2.27 (dd, 1H, $J =$ 13.3, 6.9 Hz), 2.14 (m, 1H), 2.00 – 0.95 (m, 18H), 1.85 (m, 1H), 1.66 (dq, 1H, $J = 13.1$, 3.4 Hz), 1.21 (s, 3H), 1.21 (s, 3H), 0.84 (d, 3H, $J = 6.4$ Hz), 0.66 (d, 3H, $J = 7.1$ Hz); MS: *m/z* (%): 372 (5), 354 (2), 314 (1), 278 (1), 255 (1), 223 (1), 207 (3), 166 (12), 148 (50), 109 (51), 91 (48), 43 (100).

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Conformational analysis and molecular modeling: Conformational analysis of the side chain of compounds **4a**, **4b**, and **4c** was carried out using the MacroModel molecular modeling program of Still[44] run on a Digital VAXstation 4000–90A or SiliconGraphics Octane. Molecular mechanics calculations were carried out on model compounds in which the A-ring and diene system up to C6 were replaced by a H atom. Rotations with 60° increments were applied to the rotatable C–C bonds of the side chain, while the 25-OH was rotated with increments of 120°. The starting conformations thus generated were minimized using the MM2* force field implementation of MacroModel and the conformations within 20 kJ mol^{-1} of the minimum energy form were retained. Using a PC computer program all conformations of each compound were then overlaid using C13 as common origin ($x, y, z = 0$), C14 was positioned in the y,z plane ($x = 0$) and the 18-position was made to coincide with the positive y axis ($x, z = 0$). A line drawing was generated of the minimum energy conformation and the position of O25 in each of the local energy minima within the given energy window was represented by a ball to obtain the volume maps shown in Figures 3, 4, and 5.

Biological evaluation: Compound **2** (MC 1288) was a kind gift of L. Binderup (Leo Pharmaceuticals, Ballerup, Denmark).

Binding studies: The affinity of the 6D-analogues of 1,25$(OH)_2D_3$ to the vitamin D receptor was evaluated in their ability to compete with [^{3}H]1,25$(OH)_2D_3$ for binding to high speed supernatant from intestinal mucosa homogenates obtained from normal pigs as described previously.[11g] The relative affinity of the analogues was calculated from their concentration needed to displace 50% of [^{3}H]1,25$(OH)_2D_3$ from its receptor compared with the activity of 1,25$(OH)_2D_3$.

MCF-7 proliferation assay: The human breast carcinoma (MCF-7) cell line was obtained from the American Tissue Culture Company (Rockville, MD). The antiproliferative activity of 1,25$(OH)_2D_3$ or 6D analogues on MCF-7 cells was assessed by evaluating [^{3}H]thymidine incorporation. Cells were seeded in 96-well plates (7500 cells per well) and 1 μCi [methyl-^{3}H]thymidine (ICN Biomedicals, Costa Mesa, CA) was added 72 h after the initiation of treatment. Cells were semi-automatically harvested after an additional 6 h of incubation on filter plates only retaining incorporated thymidine (GF/C Filter and Filtermate Universal Harvester, Packard Instrument, Meriden, CT). Counting was performed using a microplate scintillation counter (Topcount, Packard).

Differentiation of HL-60 cells: The human promyelocytic leukemia cell line (HL-60) was obtained from the American Tissue Culture Company (Rockville, MD). HL-60 cells were seeded at 4×10^4 cells mL^{-1} in 25 cm^2 Falcon tissue chambers using RPMI 1640 medium supplemented with 20% fetal calf serum (Sera-Lab, W. Sussex, UK) and gentamycin (50 μg mL^{-1}; Gibco, Roskilde, Denmark). 1,25$(OH)_2D_3$, analogues, or vehicle were added the day after plating. After 4 d of culture, differentiation was measured by using the NBT reduction assay as described previously.[45]

In vivo studies: NMRI mice were obtained from the Proefdierencentrum of Leuven (Belgium) and fed on a vitamin D-replete diet (0.2% calcium, 1% phosphate, 2000 U vitamin D kg^{-1}; Hope Farms, Woerden, The Netherlands). The hypercalcemic effect of the 6D analogues was tested in NMRI mice by daily subcutaneous injection of 1,25$(OH)_2D_3$, its analogues or the solvent during seven consecutive days, using serum calcium concentration as parameter.

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