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Novel side chain analogs of 1α,25-dihydroxyvitamin D_3: design and synthesis of the 21,24-methano derivatives

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Abstract

The syntheses of the new 21,24-methano derivatives of 1α,25-dihydroxyvitamin D_3 [*viz.* 1(*S*),3(*R*)-dihydroxy-17(*R*)-(1′,4′-*cis*-(4′-(1′-hydroxy-1′-methylethyl)-*cyclo*-hexyl))-9,10-*seco*-androsta-5(*Z*),7(*E*),10(19)-triene (**MC 2108**) and its (1′,4′-*trans*)-isomer (**MC 2110**)] are described. The key step is the establishment, by Diels-Alder reaction on a CD-ring side chain diene intermediate prepared from vitamin D_2, of a 1,4-disubstituted cyclohexene moiety in the side chain. Hydrogenation to a 1:1 mixture of *cis* and *trans* cyclohexane derivatives and separation of the two series at a stage prior to the standard Horner-Wittig coupling with the (Hoffmann-La Roche) ring-A building block were other important steps in the syntheses of the target analogs. The relative configurations of intermediates were assigned by NMR spectroscopy. **MC 2108** and **MC 2110** are of interest as conformationally locked side chain derivatives to probe the receptor interactions of not only the parent vitamin D hormone but also its biologically active symmetrical 'double side chain' analog [21-(3′-hydroxy-3′-methylbutyl)-9,10-*seco*-cholesta-5(*Z*),7(*E*),10(19)-triene-1(*S*),3(*R*),25-triol (**MC 2100**)], 'both' side chains of which can formally be traced out in the new analogs. The preferred conformations, inferred from an analysis of ^{13}C-NMR characteristics, notably the chemical shift of C-17 in a series of analogs, to have the tertiary alcohol (1′-hydroxy-1′-methylethyl) substituent equatorial on the cyclohexane chair, are confirmed by molecular modeling.

Keywords: Sterol; Vitamin D analogs; Side chain conformation; Diels-Alder reaction; ^{13}C-NMR spectroscopy; Molecular modeling

1. Introduction

1.1. Background and design

Hydrogen bonding interaction of the of both the ring-A (1α-*OH*) and the side chain (25-*OH*) hydroxyl groups of 1α,25-dihydroxyvitamin D_3 (**1**) with their respective sites in the ligand binding domain (LBD) of the vitamin D receptor protein (VDR) is the paradigm for hormonal activation (by the ligand-induced conformational change) of the VDR prior to its initiating the cascade of events leading to regulation of vitamin D related gene expression and protein synthesis [1]. A feature characteristic to the vitamin D hormone (as compared to other members of the steroid hormone superfamily) is the long, conformationally flexible sterol side chain (the C-17 substituent on ring-D) to which the liganding atom is attached. The discovery of the increased biologic potency associated with the 20-epimer (**2, MC 1288**) of 1α,25-dihydroxyvitamin D_3 or the 20-epimers of other analogs [2–4] prompted both theoretical studies using molecular mechanics based conformational analysis [5] and an X-ray study [6], revealing that the side chain backbone shows a different directional preference in the unnatural epi-series. Other series have been similarly analyzed by molecular modeling, and a correlation with biologic results was achieved [7]. Furthermore, analogs have been designed in which particular regions of space (for the isolated molecule) can be energetically excluded or made accessible to the side chain hydroxyl, and the correlation with biologic potency was remarkable [8]. Thus the concept of an 'active' conformation for the side chain of the vitamin D hormone is emerging. On the other hand, limited protease digestion studies [9,10] (in which the bound ligands stabilize different fragments of the receptor) suggest that the side chains of the 20-normal and 20-epi compounds have different contact sites within the LBD, and that a unique location for the hydroxyl group is therefore not a requirement for receptor activation. Indeed there is evidence that the 'double side chain' analog (**3**) interacts differently from either **1** or **2** [11].

The double side chain modification to 1α,25-dihydroxyvitamin D_3 was presented independently by the Hoff-

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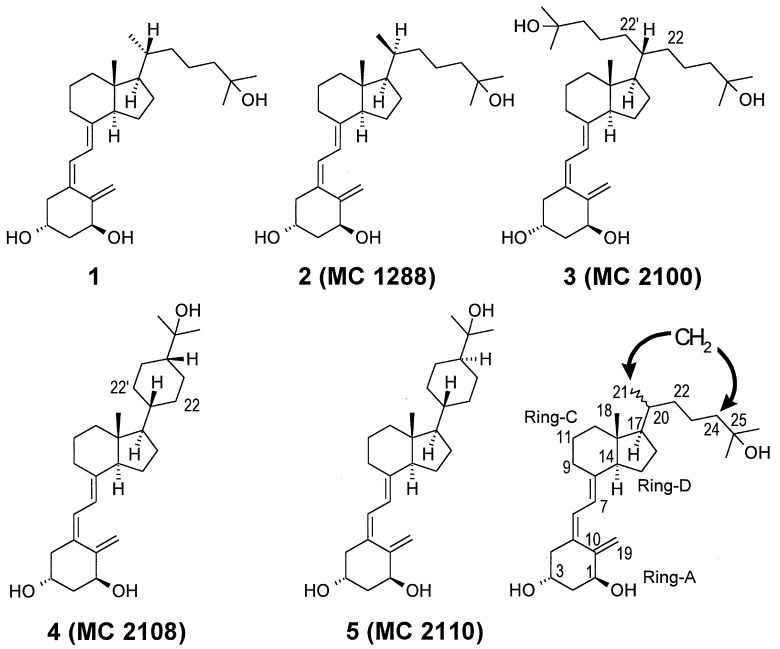


Fig. 1.

mann-La Roche (Nutley, NJ) and the Leo (Denmark)/Polish Academy of Sciences, Warsaw, groups at the 10th Workshop on Vitamin D (Strasbourg, France) [11,12]. This compound (**3,** Leo code **MC 2100**) binds to the VDR, is active in functional assays, and furthermore is a substrate for vitamin D side chain metabolism enzymes [13]. In order to investigate the effects of restricting the conformational freedom of the side chains, we have formally tied them back into a ring. The synthesis and properties of the simplest 're-converging' double side chain analogs (**4, MC 2108** and **5, MC 2110**),[1] containing, as a first attempt essentially to lock the locus of the 25-*O*, the strain-free cyclohexane moiety, form the subject of this communication. Of course, these compounds can also be considered as the *21,24-methano bridged* (Fig. 1) derivatives of 1α,25-dihydroxyvitamin D_3 (and its 20-epimer) and thus represent two conformationally locked, 'snapshot' versions of side chain geometries accessible to both the normal and epi parents (**1** and **2**) as probes for receptor interactions.

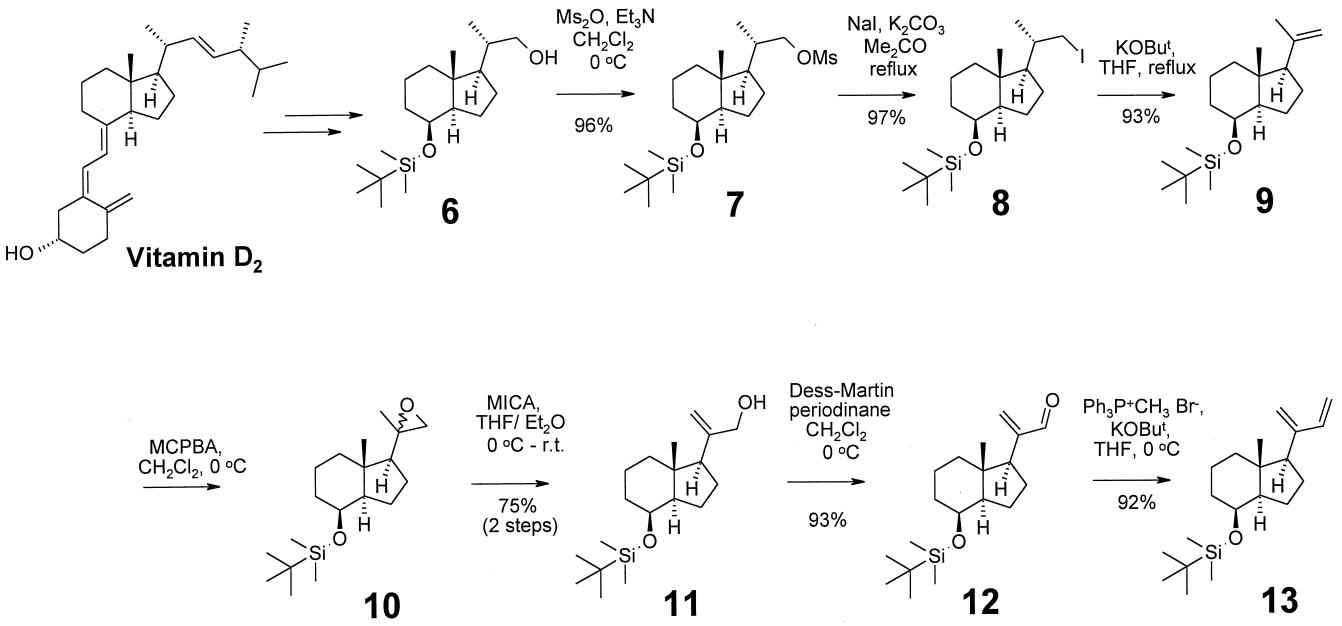
2. Synthesis and discussion

The synthetic strategy involves generation of the cyclohexane ring *via* hydrogenation of the Diels-Alder adduct of the requisite side chain diene with methyl acrylate. Since neither the cyclo-addition nor the reduction was anticipated to be feasible on the intact triene system of vitamin D analog key intermediates [2,3], we elected to perform these reactions on CD-ring intermediates. Thus, the monosilylated diol (**6**) [14] [prepared from the corresponding diol, obtained [15] by degradation of vitamin D_2, by selective desilylation (Bu_4N^+ F^-/THF; room temperature) of the bis-TBS ether] was converted *via* the mesylate (**7**) to the iodide (**8**), which (unlike **7**) smoothly underwent elimination of HI by treatment with *t*-butoxide to the olefin (**9**) (Scheme 1). A small amount (5%) of the *t*-butyl ether by-product formed in this reaction was easily removed by chromatography. Essentially quantitative epoxidation with *m*-chloroperbenzoic acid to a 2:1 20*S*:20*R*[2] mixture of the epoxides (**10**) followed by rearrangement of these with magnesium isopropyl-cyclohexylamide (MICA) afforded a single allylic alcohol (**11**). Oxidation with the Dess-Martin periodinane produced the conjugated aldehyde (**12**), which on Wittig olefination with methylenetriphenylphosphorane gave the key intermediate (**13**).

Diels-Alder reaction of **13** with methyl acrylate (7 molar equiv.) in the presence of anhydrous aluminum chloride (0.5 molar equiv.) cleanly generated the 1,4-adducts (**14a** and **14b**), epimeric at C-24, with none of the 1,3-regio-isomers

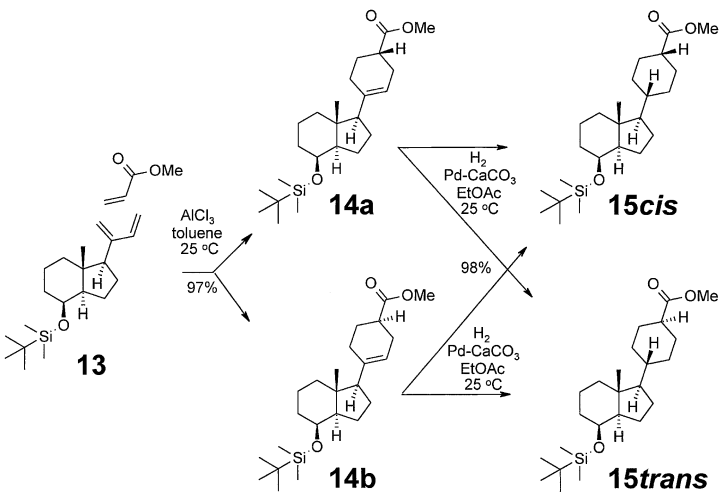
[1] Note that the projection (up or down) of the C-20 hydrogen is arbitrary in drawing **4** or **5;** it is the relative projections of the 20-*H* and the 24-*H* (neither C-20 nor C-24 is chiral) that distinguish the compounds. The carbon atoms of the 'normal' and 'epi' side chain residues in both double and reconverging side chains are conveniently numbered for identification as indicated on structures **3** and **4.** For convenience the numbering system of Fig. 1 is retained in the discussion of synthetic intermediates even when they lack the full C_{27} 9,10-seco-cholestane framework of vitamin D_3.

[2] The configuration assignments were made by comparison with the product of a stereoselective (but less efficient) synthesis of either isomer by (a) reaction of the 20-ketone obtained by ozonolysis of **9** with dimethylsulphonium methylide (*S*-isomer) or (b) α-bromination of the same 20-ketone followed by reaction with MeMgBr (*R*-isomer), invoking in both cases the well known Felkin-Anh selectivity of kinetic nucleophilic addition to the 20-ketopregnane system.



Scheme 1.

detectable by NMR spectroscopy (Scheme 2). The ^{1}H-NMR spectrum of the product showed two sharp singlets (δ 0.76 and δ 0.74 ppm) for the angular methyls (C-18) of **14** in a 9:11 ratio (height), but it has not been possible to assign the structures at this point. Recrystallization of the mixture only inverted the ratio, but provided a 1:4 mixture in the mother liquor. This was however of no consequence, since catalytic hydrogenation (over palladium) of the cyclohexenes gave a precisely 1:1 mixture of the 1,4-*cis* and 1,4-*trans* disubstituted cyclohexanes **15*cis*** and **15*trans*** (the only two possible stereoisomers), *irrespective of the ratio of starting materials.* The ratio and relative configurations in these two products were readily determined by the integration and shape of the 24-*H* signals in the proton-NMR spectrum, in particular the diagnostic *trans*-diaxial proton-proton [24-*H*,23(23′)-*H*] coupling constants (12.3 Hz) which can only be associated with the 1,4-diequatorial substitution pattern of **15*trans*** (Fig. 2). Partial equilibration of the mixture by treatment with KOBu^{t} in methanol under reflux resulted in an increased proportion of the more stable **15*trans,*** thus confirming the assignment, but since both isomers of the title analogs were required for biologic study, the 1:1 mixture (if separable) was ideal. It transpired that separation of the series was best deferred to a later stage in the synthesis (**15*cis*** and **15*trans*** ran very closely on TLC), so this mixture was carried through the sequence **15** → **16**→ **17** → **18,** involving reaction with methyl Grignard, desilylation with HF, and Dess-Martin oxidation, as shown in Scheme 3. A slight kinetic resolution of the mixture of esters was observed under the first step of this sequence: A very small



Scheme 2.

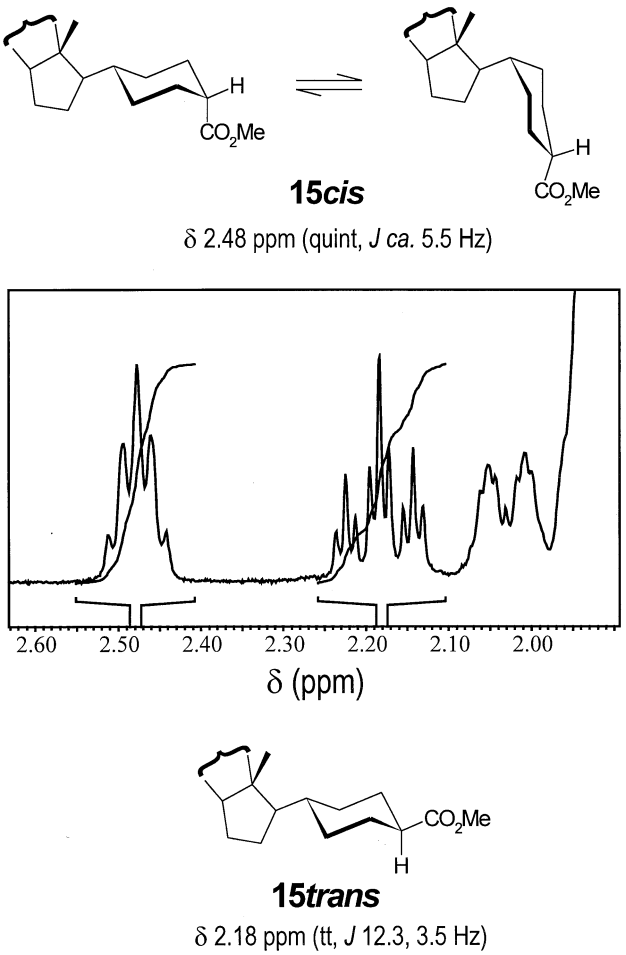


Fig. 2. Partial proton-NMR spectrum (300 MHz, $CDCl_3$, Me_4Si) of the mixture of compounds **15.** The respective protons on C-24 are indicated in partial structures showing the preferred chair conformation of **15*trans*** and the two chair conformations of **15*cis*.**

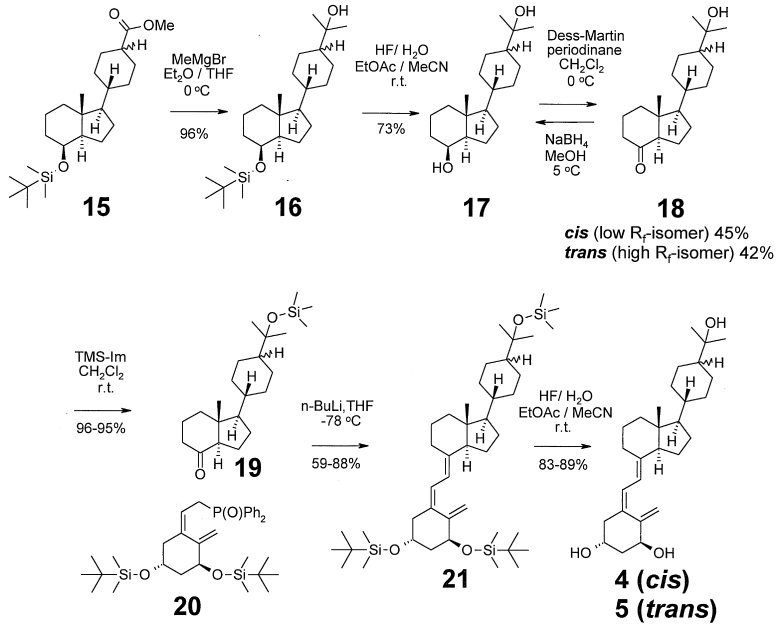
amount (about 1%) of 'unreacted starting material' was recovered during the purification process and shown by NMR to consist of essentially pure *cis* isomer **15*cis*.** Resubmission of this compound to the Grignard reaction under the original conditions then gave a reference sample of **16*cis*.** The ketones **18** (providing the optimum difference in TLC R_f-values) were isolated in isomerically pure form from the final mixture by chromatography. Samples of **18*cis*** (low R_f-isomer) and **18*trans*** (high R_f-isomer) were individually reduced back to reference samples of their respective precursor alcohols **17** with borohydride as shown in order to correlate their configurations with **15.** Thus, the NMR spectra of **17*cis*** and the previously obtained sample of **16*cis*** showed the expected correspondence (see Table 1, discussed below), and desilylation of **16*cis*** did indeed give **17*cis*.**

The syntheses of the target compounds **4** and **5** were completed in separate sequences by protection of the *tert*-alcohol function in **18** as the trimethylsilyl ether prior to the Horner-Wittig coupling of **19** with the A-ring building block (**20**) [16,17] used in the Hoffmann-La Roche protocol. The relatively low yield (59%) of **21(*cis*)** encountered in the *cis* series was partly due to an adventitious excess of *n*-butyl-lithium, which resulted in the consumption of some of the ketone to give a substantial amount of the *n*-butyl carbinol as a single by-product. The final step was deprotection of the three alcohol groups by desilylation of **21** with HF to give 21,24-methano-1α,25-dihydroxyvitamin D_3 (**4** and **5**).[3]

A useful feature noticed in the ^{13}C-NMR spectra of the cyclohexyl-containing compounds described in this communication (Scheme 3) is the sensitivity of the chemical shift (δ_C) of C-17 to the conformation of the side chain ring whilst at the same time being relatively insensitive to the nature of ring-C substitution (in contradistinction to the C-14 signal, which shows the opposite dependence). Relevant data are collected in Table 1. It is apparent that all compounds in the *trans* series show a C-17 δ-value corresponding closely, though being slightly higher, to that observed for the 20-normal side chain compounds such as **1.** The value for the 20-epi isomer (**2**) (and cognate compounds) is on the other hand slightly, but consistently, lower. The truncated side chain compound **22** [18] has the highest δ_C value, providing a comparison standard in which γ-substituents, with their associated magnetic shielding effect, are not present. Taken alone, these results would not have attracted attention, but the comparison with the *cis* series is dramatic and merits comment. Thus, whereas there is a rather small deviation from the *trans* compound C-17 δ value (0.7 ppm to high field or 1.7 ppm to low field for the vitamin D analog series) in the examples discussed so far, the deviation between *cis* and *trans* isomers is 9 ppm (for the tertiary alcohol derivatives). These data are consistent

[3] **MC 2108** (**4**): M.p. 191–192°C (from methyl formate); UV (EtOH): λ_{max} 264 nm (ϵ 17 500); NMR ($CDCl_3$, Me_4Si): δ_H (500 MHz) (*J* in Hz) 0.55 (3H, s, 18-*H*$_3$), 1.18 (6H, s, 26-*H*$_3$, 27-*H*$_3$), 2.32 (H, dd, *J* 6.5 13.4, 4β-*H*), 2.60 (H, dd, *J* 3 13.4; 4α-*H*), 2.84 (H, bd, *J* 12.4, 9β-*H*), 4.23 (H, m, 3-*H*), 4.43 (H, m, 1-*H*), 5.01 (H, bs, 19*E*-*H*), 5.33 (H, bs, 19*Z*-*H*), 6.02 (H, d, J 11.3, 7-*H*), 6.38 (H, d, *J* 11.3, 6-*H*) ppm; δ_C (125.8 MHz) ($CDCl_3$ = 76.8) 11.5 (C-18), 21.5 and 21.7 (C-23, C-23′), 21.9 (C-11), 23.3 (C-15), 26.6 (C-27 and C-26), 27.3 (C-16), 28.7 (C-9), 29.2 and 29.8 (C-22, C-22′), 35.3 (C-20), 40.0 (C-12), 42.4 (C-2), 44.8 (C-4), 45.6 (C-13), 47.7 (C-17), 49.0 (C-24), 56.3 (C-14), 66.2 (C-3), 70.3 (C-1), 72.7 (C-25), 111.5 (C-19), 116.9 (C-7), 124.35 (C-6), 133.1 (C-5), 142.5 (C-8), 147.35 (C-10) ppm; MS: Calcd. for $C_{28}H_{44}O_3$ (M^+) 428.3290. Found 410.3320, Calcd. for $C_{28}H_{42}O_2$ (M^+-H_2O) 410.3185. Found 410.3193.

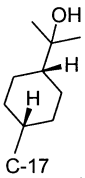
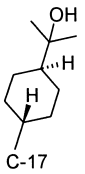
MC 2110 (**5**): M.p. 122–124°C (from methyl formate); UV (EtOH): λ_{max} 264 nm (ϵ 17 500); NMR ($CDCl_3$, Me_4Si): δ_H 0.54 (3H, s, 18-*H*$_3$), 0.82–1.05 (4H, m, 22-*H*, 22′-*H*, 23-*H*, 23′-*H*), 1.14 (6H, s, 26-*H*$_3$, 27-*H*$_3$), 2.31 (1H, dd, *J* 6.5 13.4, 4β-*H*), 2.56 (1H, dd, *J* 3.1 13.5, 4α-*H*), 2.83 (1H, bd, *J* 13.3, 9β-*H*), 4.21 (1H, m, 3-*H*), 4.42 (1H, m, 1-*H*), 4.99 (1H, bs, 19*E*-*H*), 5.32 (1H, bs, 19*Z*-*H*), 6.04 (1H, d, *J* 11.3, 7-*H*), 6.35 (1H, d, *J* 11.3, 6-*H*) ppm; δ_C (125.8 MHz) ($CDCl_3$ = 76.8) 11.8 (C-18), 21.9 (C-11), 23.3 (C-15), 26.4 (C-26 and C-27), 26.95 and 27.0 (C-23,C-23′), 27.1 (C-16), 28.8 (C-9), 32.2 and 32.5 (C-22 and C-22′), 40.3 (C-12), 40.8 (C-20), 42.3 (C-2), 44.8 (C-4), 45.6 (C-13), 48.6 (C-24), 56.1 (C-14), 56.7 (C-17), 66.2 (C-3), 70.3 (C-1), 72.6 (C-25), 111.6 (C-19), 116.85 (C-7), 124.4 (C-6), 133.0 (C-5), 142.6 (C-8), 147.3 (C-10); MS: Calcd. for $C_{28}H_{44}O_3$ (M^+) 428.3290. Found 428.3289.



Scheme 3.

with the C20-C17 bond essentially adopting the axial conformation in the *cis* compounds, C-17 experiencing thereby two γ-gauche interactions [19], while the bulky tertiary alcohol substituent claims the equatorial conformation on the cyclohexane chair. In the 1,4-diequatorial situation of the *trans* series, these gauche interactions are removed, and

Table 1
Selected ^{13}C-NMR data (75.5 or 125.8 MHz, solvent $CDCl_3$) in ppm, relative to solvent signal set to 76.8 ppm

Compound	Side chain	δ_C C-17	**15,16**	δ_C C-14		Vitamin D analog
				17	**18**	
15*cis*	(cis-ester)	52.9	52.9			
16*cis*		47.8	53.1			
17*cis*		47.7		52.6		
18*cis*		48.1			62.0	
4		47.7				56.3
(All)		(47.8 ± 0.3)				
15*trans*	(trans-ester)	56.9	52.9			
16*trans*		57.1	52.9			
17*trans*		56.9		52.4		
18*trans*		56.9			61.8	
5		56.7				56.1
(All)		(56.9 ± 0.2)				
1	normal	56.4				56.2
2	epi	56.0				56.2
3	double	52.8				56.1
22		58.4				56.1

Note that for compounds **1, 2,** and **3,** the corresponding δ-values are identical in their respective 25-desoxy (25,25′-didesoxy) derivatives (formulae not shown). Compound **22:** 23,24,25,26,27-pentanor-1α-hydroxyvitamin D_3.

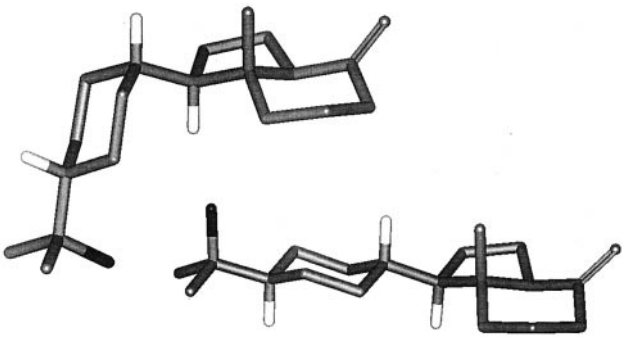


Fig. 3. Energy minimized (PCMODEL) partial structures of MC 2108 (**4**) (upper) and MC 2110 (**5**), wherein the C-7 appendage of the intact molecules has been replaced by a hydrogen atom in order to facilitate the computations (cf. Ref. [5]). For clarity only hydrogens (white atoms) on carbons 17, 20, and 24 are depicted (black atoms: oxygens on C-25).

the chemical shift approaches that observed in **22.** Molecular modeling studies nicely confirm these features in the preferred conformations of the molecules (see Fig. 3).[4] In the case of the *ester derivative*, **15*cis,*** the lesser steric requirement of the methoxycarbonyl substituent makes for a more equal time-averaged distribution of conformers, in accord with the intermediate coupling constant (ca. 5.5 Hz) noted in the ^{1}H-NMR spectrum (Fig. 2), and here the C-17 δ value is also intermediate (Table 1). In **15*trans,*** the substituents do not have to vie for the equatorial position, consistent with the top-of-the-range *trans*-diaxial value for the coupling constants 24-*H*,23(23′)-*H* already mentioned.

As a final observation, it is interesting to note that the C-17 δ value typical for the *double* side chain analogues (e.g. **3**) is *also intermediate* between the two extremes presented in **4** and **5.** This suggests that the *gauche* 20,22-conformation is significantly populated by one of the two side chains at any given time in the dynamic situation. Modeling studies on **3** [or rather on its 25,25′-didesoxy-derivative (NB comment in the Table), a prodrug form of **3,** where intramolecular hydrogen bonding is ruled out] (unpublished) actually point to the global energy minimum with one side chain extended (in fact the normal side chain) and the other (epi) gauche (with only a slight energy difference to the switched situation). Indeed exactly this mixed conformation scenario is dramatically demonstrated in the X-ray crystal structure [12,20] of a 23′-oxa double side chain derivative [12,21]. In the same context, it is interesting to recall the molecular modeling studies [5] on **1** and **2** that reveal the gauche conformation of the (single) side chain in each case as the global minimum. The slightly lower C-17 δ-value drawn attention to in Table 1 for **2** relative to **1** is thus tentatively explained by a proposing a higher proportion (albeit still very minor) of the gauche side chain conformer in solution, reflecting the more congested epi situation.

Preliminary in vitro biologic tests indicate that the *cis* and *trans* isomers of 21,24-methano-1α,25-dihydroxyvitamin D_3 (**4** and **5**) are markedly less potent than the parent compounds **1, 2,** and **3** indicating that an agonistic LBD interaction of the side chain hydroxyl group is essentially 'locked out' in the new analogs,[5] rendering them uninteresting for further biologic study. On the other hand, we can conclude that the NMR spectroscopic data for these locked, extreme, side chain conformations in the cyclohexane analogs are useful in so far as they throw new light on the solution conformations of flexible, open conformation, active ligands, including 1α,25-dihydroxyvitamin D_3 itself.

[4] PCMODEL: Serena Software, Bloomington, Indiana, USA; Website: http://serenasoft.com

[5] This negative result is with hindsight perhaps not surprising in the light of the recently published crystal structure of the LBD with its bound natural ligand [22], revealing an active side chain conformation of **1** seemingly not readily accessible to **4** or **5.**

Acknowledgments

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