

Synthesis of (1 α)-1,25-Dihydroxyvitamin D₃ with a β -Positioned Seven-Carbon Side Chain at C(12)

by **Diego M. Carballa^a**, **Antonio Rumbo^a**, **Mercedes Torneiro^a**, **Miguel Maestro^b**, and **Antonio Mouriño^{*a}**

^a) Departamento de Química Orgánica y Unidad Asociada al CSIC, Universidad de Santiago de Compostela, ES-15782 Santiago de Compostela

(phone: +34 600942435; fax: +34 981595012; e-mail: antonio.mourino@usc.es)

^b) Departamento de Química Fundamental, Universidad de A Coruña, ES-15701 A Coruña

Dedicated to Prof. Dr. *Dieter Seebach* on the occasion of his 75th birthday

A convergent synthesis of an analogue of (1 α)-1,25-dihydroxyvitamin D₃ (**1b**) with a C₇ side chain at C(12), *i.e.*, of **5** (*Fig.*), is described. A key step of the synthesis is the assembly of the triene system by a Pd^{II}-catalyzed ring closure of an enol triflate ('bottom' fragment) followed by coupling of the resulting Pd^{II} intermediate with an alkenylboronate ('upper' fragment) (*Scheme 2*). The synthetic strategy allows isotopic labelling at the end of the synthesis.

Introduction. – Vitamin D₃¹⁾ (**1a**; *Fig.*), before eliciting its biological function, must be dihydroxylated to (1 α)-1,25-dihydroxyvitamin D₃ (1 α ,25(OH)₂D₃, 1,25D; **1b**), which is considered the hormonally active form of vitamin D₃ [1]. This hormone interacts with the vitamin D nuclear receptor (VDR) [2][3] to form a ligand–VDR complex, which binds to the retinoid X receptor (RXR). The resulting heterodimer interacts with the vitamin D response elements of the DNA to induce important biological functions such as regulation of the mineral metabolism, cell differentiation, cell proliferation, cell growth, apoptosis, and the immune system [1][4]. The fact that the VDR has been found in more than 30 target tissues and cell tumors has led to the consideration that 1 α ,25(OH)₂D₃ is involved in a wider array of biological functions including cancer prevention [1][4][5]. However, the clinical application of 1 α ,25(OH)₂D₃ has been limited by its secondary hypercalcemic effects [6][7]. Efforts to develop vitamin D analogues with strong cell-differentiating ability and low calcemic action have led to the synthesis of more than 3000 vitamin D analogues, but only a few have found clinical applications [8–11]. Most of the vitamin D analogues synthesized to date are modified at the side chain [5][12], some of them with rigid units [13], and others with longer [12][14] or shorter side chains [12][15]. Vitamin D analogues with substituents at the C ring [16], D ring [17], C(18) [18], triene system [19], or A ring [20] or without the C or D ring or both rings [21] have also been developed (for reviews on the synthesis of vitamin D analogues, see [22]).

¹⁾ Trivial atom numbering; for systematic names, see *Exper. Part*.

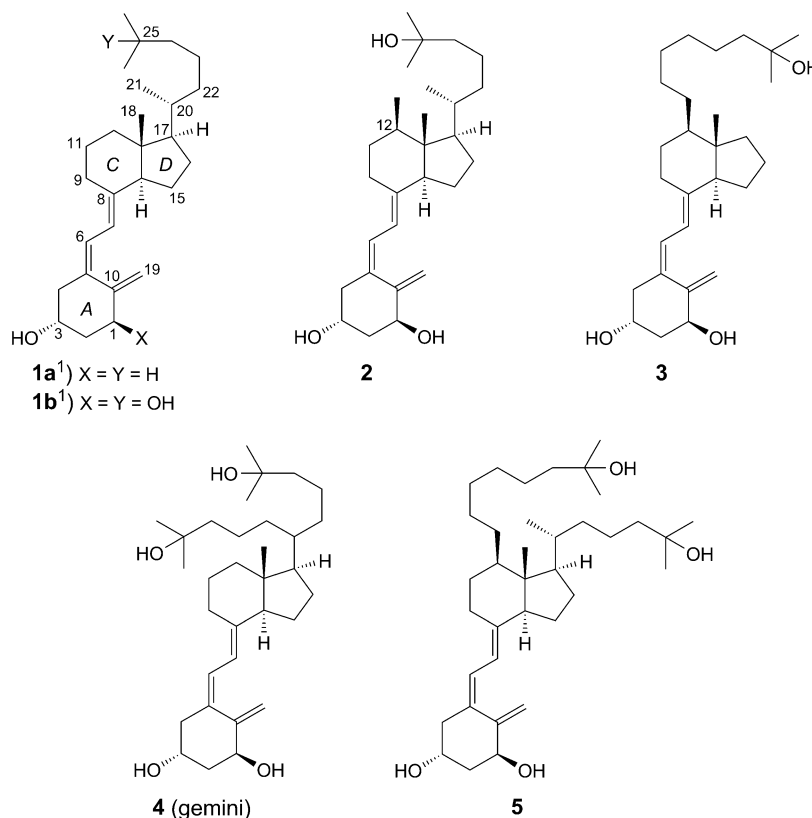
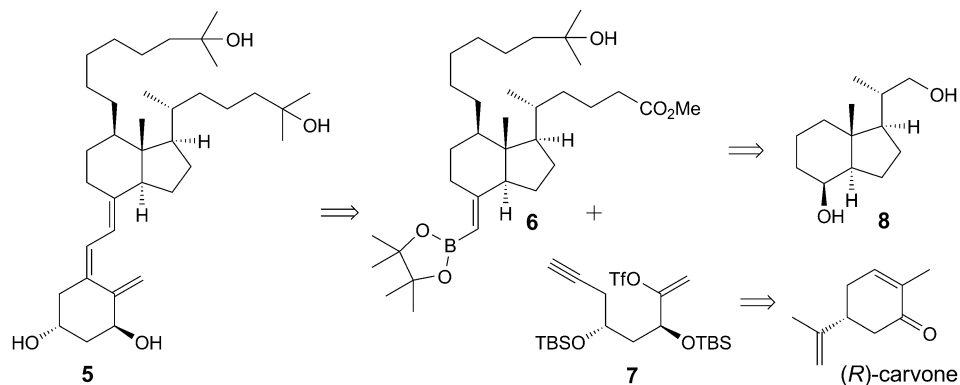


Figure. Vitamin D₃ (**1a**), (1α)-1,25-dihydroxyvitamin D₃ (**1b**), and analogues **2–5**

Recently, *Dino Moras* and co-workers published the crystal structure of an engineered ligand-binding domain of the vitamin D receptor (VDR-LBD) that lacks a flexible insertion domain between helices H1 and H3; the mutant VDR bound to 1α,25(OH)₂D₃ (VDR–1,25D complex) exhibited similar conformation, transactivation ability, and biophysical properties than the wild-type counterpart [23]. The crystal structure of the VDR–1,25D complex shows the H-bonding nature of the interactions between each of the three OH groups of the ligand with the mutant vitamin D receptor (1α-OH with both Ser-237 and Arg-274, 3β-OH with both Tyr-143 and Ser-278, and 25-OH with both His-305 and His-397). We have recently utilized the structural features of *Moras*' crystallographic structure of the VDR–1,25D complex to design active vitamin D analogues [17b] [24]. For example, we synthesized the 12β-methyl analogue **2**, which binds strongly to the VDR [16b], and the analogue **3**, which lacks the natural side chain at C(17) and binds significantly to the VDR [16c]. Inspired by the interesting biological profile of compounds **2** and **3** and the potent biological activity of 'gemini' analogue **4**, a vitamin D analogue with two side chains [25], and related compounds [25], we designed and synthesized the new analogue **5**, which possesses the normal side chain of 1α,25(OH)₂D₃ and a hydroxylated C₇ side chain at C(12).

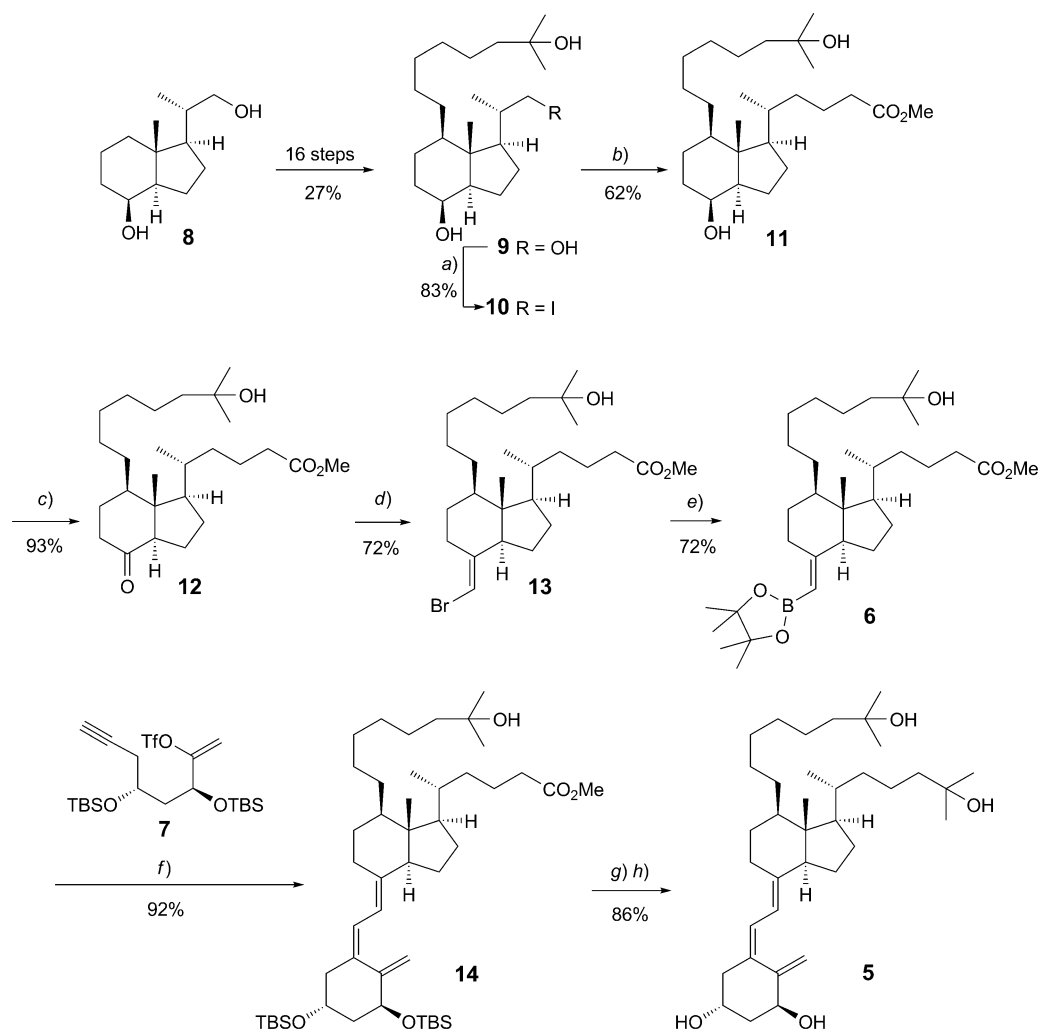
Results. – The synthetic plan for the synthesis of the target vitamin D₃ analogue **5** is depicted in *Scheme 1* and involves the construction of the triene system by stereoselective Pd-catalyzed cyclization of the enol triflate **7** followed by *Suzuki–Miyaura* coupling of the resulting Pd^{II} intermediate with the cyclic alkenylboronate **6** according to a methodology recently developed in this laboratory [26]. The boronate **6** was constructed in a linear fashion from the *Inhoffen–Lythgoe* diol **8**, which is usually prepared by degradation of vitamin D₂.

Scheme 1. *Synthetic Plan for the Target (1 α)-1,25-Dihydroxyvitamin D₃ Analogue 5*. TBS = ^tBuMe₂Si.



The synthesis of the target compound **5** is outlined in *Scheme 2*. The required triol **9** [27] was prepared in 27% overall yield (16 steps) from *Inhoffen–Lythgoe* diol **8** following known methods [28] [16b,c]. Transformation of triol **9** into iodo derivative **10** was selectively accomplished with iodine and triphenylphosphine (83% yield). Ni⁰-Catalyzed oxidative addition [29] of iodo derivative **10** to methyl acrylate provided methyl ester **11** (62% yield), which was oxidized with *Dess–Martin* periodinane to keto derivative **12** (92%). Conversion of **12** to bromomethylene derivative **13** was accomplished in 72% yield by a *Wittig* reaction with the ylide Ph₃P=CHBr, generated from (Ph₃PCH₂Br)Br and ^tBuOK following a modification of the *Trost* procedure [26]. The upper-fragment cyclic boronate **6** was prepared in 72% yield by *Miyaura* borylation [30] of **13** with bis(pinacolato)diborane (=4,4,4',4',5,5,5',5'-octamethyl-2,2'-bi-1,3,2-dioxaborolane) in the presence of [1,1'-bis(diphenylphosphino- κ p)ferrocene]dichloropalladium(II)–dichloromethane complex as the catalyst and tricyclohexylphosphine as ligand. The triene system was installed in 92% yield by treatment of alkenylboronate **6** with an equimolar amount of enol triflate **7** in the presence of a catalytic amount of [PdCl₂(Ph₃P)₂] and K₃PO₄ in H₂O/THF. Finally, treatment of the resulting methyl ester **14** with methylmagnesium bromide followed by removal of the protecting groups gave the desired analogue **5** in 86% yield (20% overall yield from triol **9**, 8 steps).

Conclusions. – An efficient convergent synthesis of **5**, an analogue of (1 α)-1,25-dihydroxyvitamin D₃ bearing a C₇ side chain at C(12), was developed. A key step of the synthesis was the Pd-catalyzed assembly of the triene system in the presence of OH and ester functionalities in aqueous medium. The synthetic strategy allows isotopic

Scheme 2. *Synthesis of the Target Analogue 5*. TBS = tBuMe_2Si .

a) I_2 , Ph_3P , 1*H*-imidazole, THF, $-20^\circ \rightarrow \text{r.t.}$ b) Zn, $\text{NiCl}_2 \cdot 6 \text{H}_2\text{O}$, py, $\text{CH}_2=\text{CHCOOMe}$, r.t. c) Dess–Martin periodinane, CH_2Cl_2 . d) $(\text{Ph}_3\text{PCH}_2\text{Br})\text{Br}$, tBuOK , toluene, ultrasounds, $-17 \rightarrow 0^\circ$; then **12**. e) $[\text{PdCl}_2(\text{dppf})] \cdot \text{CH}_2\text{Cl}_2$, Cy_3P , Pin_2B_2 , AcOK, DMSO, 80° . f) **7**, $[\text{PdCl}_2(\text{Ph}_3\text{P})_2]$, K_3PO_4 , THF, H_2O . g) MeMgBr , Et_2O , 0° . h) Bu_4NF , THF.

labelling at the end of the synthesis. Biological testing of the new vitamin D analogue is in progress in our laboratory.

We thank the Spanish Ministry of Education and Innovation (projects SAF2007-67205 and SAF2010-15291) for financial support and the *Center of Supercomputing of Galicia (CESGA)* for allocating computer time. We thank *Dishman-Netherlands BV* for a generous gift of vitamin D₂ used for the preparation of the *Inhoffen–Lythgoe* diol, and Dr. X. C. González–Avión and Dr. X. Pérez for preliminary experiments.

Experimental Part

General. Pyridinium dichromate (PDC) was prepared following Corey's procedure [31]. $(\text{Ph}_3\text{PCH}_2\text{Br})\text{Br}$ was prepared as reported [32]. Zinc was activated according to Amarego's indications [33]. All reactions involving oxygen- or moisture-sensitive compounds were carried out under Ar. Reaction temp. referred to external bath temp. All solvents were distilled under Ar immediately prior to use: THF and Et_2O from Na/benzophenone, toluene from Na, CH_2Cl_2 from P_2O_5 , pyridine from CaH_2 , and DMSO from CaH_2 and stored over activated 4 Å molecular sieves. Acetone/dry ice baths were used for reactions at low temperature. Alternatively, acetone baths were cooled with a CC-100 Cryocool-immersion cooler, provided with a temp. regulator. Sonications were carried out in a 120–240 W, 35 kHz ultrasonic cleaning bath. Org. extracts were dried over anhyd. Na_2SO_4 and concentrated with a rotary evaporator at aspirator pressure (20–30 Torr). TLC: aluminium-backed Merck-60 silica gel plates (0.2 mm thickness); visualization under UV light at 254 nm and by immersion of the plate in a soln. containing either *p*-anisaldehyde (2.5%), acetic acid (1%), and sulfuric acid (3.4%) in 95% ethanol or a soln. of ceric ammonium nitrate (0.5 g) and ammonium molybdate (4.8 g) in H_2O (100 ml) and H_2SO_4 (5.6 ml) followed by heating with a hot gun. Flash column chromatography (FC): Merck silica gel (230–400 mesh). IR Spectra: Bruker spectrometer, model IFS-66V FT-IR; $\tilde{\nu}$ in cm^{-1} . NMR Spectra: Bruker-DPX 250-MHz spectrometer, unless otherwise stated; CDCl_3 solns.; chemical shifts δ in ppm downfield from Me_4Si ($=0.0$ ppm) with the residual solvent signal at $\delta(\text{H})$ 7.26 (*s* in CDCl_3) and $\delta(\text{C})$ 77.0 (*t* in CDCl_3) as internal standard, coupling constants *J* in Hz; distortionless enhancement by polarization transfer (DEPT) for the assignment of C types. Low- and high-resolution (HR) MS: Micromass Instruments Autospec (EI) and Bruker-Microtof spectrometer (ESI-TOF); in *m/z* (rel. %).

($8\beta,12\beta$)-12-(7-Hydroxy-7-methyloctyl)-22-iodo-de-A,B-23,24-dinorcholan-8-ol (= (3*R*,3*aR*,4*R*,7*S*,7*aR*)-Octahydro-3-[(1*S*)-2-iodo-2-methylethyl]- α,α -3*a*-trimethyl-1*H*-indene-4-heptanol; **10**). Triphenylphosphine (272 mg, 1.04 mmol) and 1*H*-imidazole (127 mg, 1.86 mmol) were successively added to a soln. of **9** (147 mg, 0.41 mmol) in THF (10 ml). The mixture was cooled to -20° , and I_2 (208 mg, 0.82 mmol) was added in five portions each 15 min. After 15 min, the mixture was removed from the cooling bath and stirred for 30 min at r.t. The reaction was quenched by slow addition of sat. NaHCO_3 soln. (15 ml) and sat. $\text{Na}_2\text{S}_2\text{O}_3$ soln. (15 ml). The aq. layer was extracted with AcOEt (3×25 ml), the combined org. layer dried and concentrated, and the residue purified by FC (SiO_2 (1×8 cm), 10–20% AcOEt/hexanes): **10** (159 mg, 83%). Colorless oil. R_f 0.55 (50% AcOEt/hexanes). IR (film): 3413 (br., OH), 2930s (CH), 2860s (CH). ^1H -NMR (250 MHz): 4.01 (br. *d*, $J=1.9$, H-C(8)); 3.48 (*dd*, $J=2, 9.5$, 1 H-C(22)); 2.89 (*dd*, $J=9.5, 9.5$, 1 H-C(22)); 1.19 (*s*, Me_2COH); 1.11 (*d*, $J=6.7$, Me(21)); 0.86 (*s*, Me(18)). ^{13}C -NMR (62.9 MHz): 71 (COH); 68.7 (CH(8)); 56.6 (CH); 53 (CH); 49.4 (CH); 45.4 (C(13)); 43.9 (CH₂); 36.9 (CH); 34 (CH₂); 31.4 (CH₂); 30.1 (CH₂); 29.9 (CH₂); 29.2 (Me₂COH); 28.2 (CH₂); 24.3 (CH₂); 23.6 (CH₂); 23.5 (Me); 22.2 (CH₂); 20.2 (CH₂); 14.8 (CH₂(22)); 11.3 (Me). EI-MS: 446 (19, $[M - \text{H}_2\text{O}]^+$), 428 (27, $[M - 2 \text{H}_2\text{O}]^+$), 319 (20, $[M - \text{H}_2\text{O} - \text{I}]^+$). HR-EI-MS: 446.2066 ($\text{C}_{22}\text{H}_{39}\text{IO}^+$; calc. 446.2046).

Methyl ($8\beta,12\beta$)-8-Hydroxy-12-(7-hydroxy-7-methyloctyl)-de-A,B-cholan-24-oate (= Methyl ($\delta R,1R,3aR,4S,7R,7aR$)-Octahydro-4-hydroxy-7-(7-hydroxy-7-methyloctyl)- $\delta,7a$ -dimethyl-1*H*-indene-1-pentanoate; **11**). Freshly distilled methyl acrylate (330 μl , 3.66 mmol) and $\text{NiCl}_2 \cdot 6 \text{H}_2\text{O}$ (196 mg, 0.82 mmol) were successively added to a suspension of activated Zn (239 mg, 3.66 mmol) in pyridine (6 ml). The mixture was heated at 60° for 2 h. The red mixture was allowed to warm to r.t., and a soln. of **10** (85 mg, 0.18 mmol) in pyridine (2 ml) was added dropwise *via* cannula. After 20 min, AcOEt (10 ml) was added, and the mixture was filtered through a layer of silica gel. The solids were washed with AcOEt (3×15 ml). The resulting filtrate was successively washed with 10% aq. HCl soln. (3×30 ml) and sat. NaHCO_3 soln. (2×30 ml), dried, and concentrated and the residue purified by FC (SiO_2 (1×8 cm), 15% AcOEt/hexanes): **11** (48 mg, 62%). Colorless oil. R_f (60% AcOEt/hexanes) 0.50. IR (film): 3429 (br., OH), 2930s (CH), 2862s (CH), 1740s (C=O). ^1H -NMR (250 MHz): 3.98 (br. *s*, H-C(8)); 3.66 (*s*, CO_2Me); 1.20 (*s*, Me_2COH); 0.92 (*d*, $J=6.8$, Me(21)); 0.82 (*s*, Me(18)). ^{13}C -NMR (62.9 MHz): 174.3 (C(25)); 71 (COH); 69.2 (CH(8)); 57.4 (CH); 53.7 (CH); 51.5 (Me); 49.9 (CH); 45.4 (C(13)); 44 (CH₂); 34.5 (CH₂); 34 (CH₂); 33.3 (CH₂); 32.9 (CH); 31.3 (CH₂); 30.2 (CH₂); 30 (CH₂); 29.2 (2 Me); 28.2 (CH₂);

24.3 (CH₂); 24 (CH₂); 23.5 (CH₂); 22.6 (CH₂); 22.2 (Me); 20.9 (CH₂); 11.3 (Me). EI-MS: 406 (13, [M – H₂O]⁺), 388 (47, [M – 2 H₂O]⁺). HR-EI-MS: 406.3432 (C₂₆H₄₆O₃⁺; calc. 406.3447).

Methyl (12β)-12-(7-Hydroxy-7-methyloctyl)-8-oxo-de-A,B-cholan-24-oate (= *Methyl (δR,1R,3aR,7R,7aR)-Octahydro-7-(7-hydroxy-7-methyloctyl)-δ,7a-dimethyl-4-oxo-1H-indene-1-pentanoate*; **12**). Dess–Martin periodinane (120 mg, 0.283 mmol) was added to a soln. of **11** (93 mg, 0.218 mmol) in CH₂Cl₂ (6 ml). The mixture was stirred for 1 h in the absence of light. The mixture was filtered through a layer of silica gel. The solids were washed with AcOEt (3 × 15 ml), and the resulting filtrate was concentrated. The residue was purified by FC (SiO₂ (1.5 × 5 cm), 20% AcOEt/hexanes): **12** (86 mg, 93%). Colorless oil. *R*_f (45% AcOEt/hexanes) 0.25. IR (film): 3508 (br., OH), 2956s (CH), 2931s (CH), 2860s (CH), 1739s (C=O), 1717s (C=O). ¹H-NMR (250 MHz): 3.65 (s, CO₂Me); 1.20 (s, Me₂COH); 0.94 (d, *J* = 6.8, Me(21)); 0.57 (s, Me(18)). ¹³C-NMR (62.9 MHz): 212.5 (C(8)); 174.1 (C(25)); 71 (COH); 62.2 (CH); 56.9 (CH); 52.4 (C(13)); 51.5 (MeO); 49 (CH); 43.9 (CH₂); 40.4 (CH₂); 34.3 (CH₂); 33 (CH); 32.6 (CH₂); 30.8 (CH₂); 30.1 (CH₂); 29.9 (CH₂); 29.6 (CH₂); 29.2 (Me₂COH); 28.2 (CH₂); 24.3 (CH₂); 23.9 (CH₂); 21.8 (Me); 21.2 (CH₂); 19.3 (CH₂); 10.1 (Me). EI-MS: 404 (71, [M – H₂O]⁺), 386 (7, [M – 2 H₂O]⁺). HR-EI-MS: 404.3284 (C₂₆H₄₄O₃⁺; calc. 404.3290).

Methyl (8E,12β)-8-(Bromomethylene)-12-(7-hydroxy-7-methyloctyl)-de-A,B-cholan-24-oate (= *Methyl (δR,1R,3aR,4E,7R,7aR)-4-(Bromomethylene)octahydro-7-(7-hydroxy-7-methyloctyl)-δ,7a-dimethyl-1H-indene-1-pentanoate*; **13**). A suspension of (Ph₃PCH₂Br)Br (544 mg, 1.25 mmol) in toluene (9 ml) was prepared by sonication for 30 min. After cooling to –17°, 1M ^tBuOK in THF (1.23 ml, 1.23 mmol) was added, and the resulting mixture was stirred for 3 h. A soln. of **12** (66 mg, 0.156 mmol) in toluene (6 ml) previously cooled to 0° was added *via* cannula. The mixture was stirred for 2 h at –17° and 3 h at r.t. The reaction was quenched by addition of sat. NH₄Cl soln. (1 ml), and the mixture was filtered through a layer of silica gel. The solids were washed with AcOEt (3 × 15 ml), and the filtrate was concentrated. The residue was purified by FC (SiO₂ (2 × 6 cm), 15% AcOEt/hexanes): **13** (56 mg, 72%). Colorless oil. *R*_f (40% AcOEt/hexanes) 0.51. IR (film): 3457 (br., OH), 3084_w (=CH), 2954s (CH), 2931s (CH), 2861s (CH), 1741s (C=O), 1632_w (C=C). ¹H-NMR (250 MHz): 5.61 (s, H–C(7)); 3.65 (s, CO₂Me); 1.20 (s, Me₂COH); 0.92 (d, *J* = 6.8, Me(21)); 0.46 (s, Me(18)). ¹³C-NMR (62.9 MHz): 174.2 (C(25)); 144.8 (C(8)); 97 (CH(7)); 71 (COH); 56.7 (CH); 56.1 (CH); 51.5 (MeO); 49.5 (CH); 48.8 (C(13)); 44 (CH₂); 34.4 (CH₂); 33.6 (CH); 32.6 (CH₂); 31.2 (CH₂); 31 (CH₂); 30.2 (CH₂); 29.2 (Me₂COH); 28.3 (CH₂); 28.1 (CH₂); 24.3 (CH₂); 23.9 (CH₂); 22.3 (CH₂); 21.9 (Me); 21.3 (CH₂); 9.6 (Me). EI-MS: 480 (4, [M – H₂O]⁺), 401 (100, [M – H₂O – Br]⁺). HR-EI-MS: 480.2596 (C₂₇H₄₅BrO₃⁺; calc. 480.2306).

Methyl (8E,12β)-12-(7-Hydroxy-7-methyloctyl)-8-[(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)methylene]-de-A,B-cholan-24-oate (= *Methyl (δR,1R,3aS,4E,7R,7aR)-Octahydro-7-(7-hydroxy-7-methyloctyl)-δ,7a-dimethyl-4-[(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)methylene]-1H-indene-1-pentanoate*; **6**). Cy₃P (2 mg, 0.007 mmol) and [PdCl₂(dppf)]·CH₂Cl₂ (3 mg, 0.003 mmol) were dissolved in DMSO (2 ml), and the mixture was stirred for 25 min. A soln. of **13** (56 mg, 0.112 mmol) in DMSO (2 ml), KOAc (33 mg, 0.336 mmol), and Pin₂B₂ (57 mg, 0.224 mmol) were successively added. The mixture was heated to 80° for 3 h and then cooled to r.t. The reaction was quenched by addition of H₂O (15 ml). The aq. layer was extracted with AcOEt (4 × 25 ml), the combined org. layer dried and concentrated, and the residue purified by FC (SiO₂ (2 × 5 cm), 10–15% AcOEt/hexanes): **6** (44 mg, 72%). Colorless oil. *R*_f (40% AcOEt/hexanes) 0.48. IR (film): 3515 (br., OH), 2931s (CH), 2861s (CH), 1742s (C=O), 1640s (C=C). ¹H-NMR (250 MHz): 4.88 (s, H–C(7)); 3.65 (s, CO₂Me); 1.25 (s, 2 Me₂COB); 1.20 (s, Me₂COH); 0.92 (d, *J* = 6.8, Me(21)); 0.45 (s, Me(18)). ¹³C-NMR (62.9 MHz): 174.3 (C(25)); 166 (C(8)); 82.5 (COB); 71 (COH); 58.8 (CH); 57.1 (CH); 51.4 (MeO); 50 (CH); 49.4 (C(13)); 44 (CH₂); 34.5 (CH₂); 33.6 (CH); 33.2 (CH₂); 32.6 (CH₂); 31.3 (CH₂); 30.2 (CH₂); 30.1 (CH₂); 30 (CH₂); 29.2 (Me₂COH); 28.2 (CH₂); 24.9 (1 Me₂COB); 24.8 (1 Me₂COB); 24.3 (CH₂); 24 (CH₂); 22.6 (CH₂); 21.9 (CH₃); 21.1 (CH₂); 9.8 (CH₃). EI-MS: 546 (3, *M*), 528 (13, [M – H₂O]⁺), 399 (100, [M – C₆H₁₆BO₃]⁺). HR-EI-MS: 546.4464 (C₃₃H₅₀BO₃⁺; calc. 546.4456).

(1α,12β)-3-O-[(tert-Butyl)dimethylsilyl]-1-[(tert-Butyl)dimethylsilyloxy]-12-(7-hydroxy-7-methyloctyl)-24-(methoxycarbonyl)-25,26,27-trinorvitamin D₃ (= *Methyl (δR,1R,3aS,4E,7R,7aR)-4-[(2Z)-2-[(3S,5R)-3,5-bis[(1,1-dimethylethyl)dimethylsilyloxy]-2-methylenecyclohexylidene]ethylidene]octahydro-7-(7-hydroxy-7-methyloctyl)-δ,7a-dimethyl-1H-indene-1-pentanoate*; **14**). A 2M aq. K₃PO₄ soln.

(0.9 ml, 1.8 mmol) and $[\text{PdCl}_2(\text{PPh}_3)_2]$ (2 mg, 0.003 mmol) were successively added to a soln. of **6** (36 mg, 0.066 mmol) and **7** (40 mg, 0.077 mmol) in THF (2 ml). The mixture protected from light was vigorously stirred for 1 h. Then H_2O (1 ml) was added, and the aq. layer was extracted with AcOEt (3×10 ml). The combined org. layer was dried and concentrated and the residue purified by FC (SiO_2 (2×6 cm), 8% AcOEt/hexanes): **14** (48 mg, 92%). Colorless oil. R_f (30% AcOEt/hexanes) 0.54. $^1\text{H-NMR}$ (250 MHz): 6.21 (*d*, $J = 11.2$, H–C(6)); 6.00 (*d*, $J = 11.2$, H–C(7)); 5.18 (*s*, 1 H–C(19)); 4.85 (*s*, 1 H–C(19)); 4.37 (*m*, H–C(1)); 4.19 (*m*, H–C(3)); 3.65 (*s*, CO_2Me); 1.21 (*s*, Me_2COH); 0.98–0.77 (*m*, Me(21), 2 Me_3CSi); 0.44 (*s*, Me(18)); 0.05 (overlapped *s*, 2 Me_2Si). $^{13}\text{C-NMR}$ (62.9 MHz): 174.3 (C(25)); 148.4 (C); 140.7 (C); 134.9 (C); 123.2 (CH); 117.7 (CH); 111 (CH₂); 71.8 (CH); 71.1 (COH); 67.5 (CH); 57.2 (CH); 56.9 (CH); 51.4 (MeO); 50.1 (CH); 49.1 (C(13)); 45.9 (CH₂); 44.8 (CH₂); 44 (CH₂); 34.5 (CH₂); 33.7 (CH); 32.6 (CH₂); 31.4 (CH₂); 30.2 (CH₂); 30 (CH₂); 29.7 (CH₂); 29.2 (Me_2COH); 28.8 (CH₂); 28.2 (CH₂); 25.8 (2 Me_3CSi); 24.4 (CH₂); 24 (CH₂); 22.4 (CH₂); 21.9 (Me); 21.4 (CH₂); 18.2 (CSi); 18.1 (CSi); 9.7 (Me); – 4.7 (1 Me_2Si); – 4.8 (1 MeSi); – 5.1 (1 MeSi).

(1 α ,12 β)-1,25-Dihydroxy-12-(7-hydroxy-7-methyloctyl)vitamin D₃ (= (1*R*,3*S*,5*Z*)-4-Methylene-5-[(2*E*)-2-[(1*R*,3*aS*,7*R*,7*aR*)-octahydro-1-[(1*R*)-5-hydroxy-1,5-dimethylhexyl]-7-(7-hydroxy-7-methyloctyl)-7*a*-methyl-4*H*-inden-4-ylidene]ethylidene]cyclohexane-1,3-diol; **5**). A 3*M* MeMgBr soln. in Et₂O (102 μl , 0.306 mmol) was added dropwise to a cooled (-78°) soln. of **14** (40 mg, 0.051 mmol) in Et₂O (4 ml). After 15 min, the cooling bath was removed, and the mixture was stirred at r.t. for 1 h in the absence of light. The reaction was quenched at 0° by slow addition of sat. NH_4Cl soln. (10 ml). The aq. layer was extracted with tBuOMe (3×10 ml). The combined org. layer was dried and concentrated to give a colorless oil (39 mg, 0.049 mmol; R_f (30% AcOEt/hexanes) 0.25. $^1\text{H-NMR}$ (250 MHz, CD_2Cl_2): 6.25 (*d*, $J = 11.2$, H–C(6)); 6.02 (*d*, $J = 11.2$, H–C(7)); 5.19 (*s*, 1 H–C(19)); 4.85 (*s*, 1 H–C(19)); 4.41 (*m*, H–C(1)); 4.16 (*m*, H–C(3)); 1.17 (*s*, 2 Me_2COH); 0.96–0.83 (*m*, Me(21), 2 Me_3CSi); 0.47 (*s*, Me(18)); 0.05 (*m*, 2 Me_2Si). This colorless oil was dissolved in THF (4 ml) and treated with 1*M* Bu₄N in THF (600 μl , 0.6 mmol). After stirring for 20 h in the dark, the reaction was quenched by addition of sat. NH_4Cl soln. (15 ml). The aq. layer was extracted with AcOEt (3×15 ml), the combined org. layer dried and concentrated, and the residue purified by FC (SiO_2 (2×6 cm), 60–90% AcOEt/hexanes): **5** (25 mg, 86%). White foam. R_f (90% AcOEt/hexanes) 0.18. IR (CHCl_3): 3366 (br., OH), 2928s (CH), 2856s (CH), 1648w (C=C). $^1\text{H-NMR}$ (400 MHz, CD_2Cl_2): 6.34 (*d*, $J = 11.3$, H–C(6)); 6.00 (*d*, $J = 11.3$, H–C(7)); 5.29 (*s*, 1 H–C(19)); 4.95 (*s*, 1 H–C(19)); 4.37 (*dd*, $J = 4.4, 7.5$, H–C(1)); 4.15 (*ddd*, $J = 3.6, 3.6, 10.3$, H–C(3)); 2.81 (*dd*, $J = 3.8, 13.4$, H–C(9)); 2.55 (*dd*, $J = 3.3, 13.4$, 1 H–C(4)); 2.26 (*dd*, $J = 6.6, 13.4$, 1 H–C(4)); 1.16 (overlapped *s*, Me_2COH , Me(26)/Me(27)); 0.93 (*d*, $J = 6.8$, Me(21)); 0.48 (*s*, Me(18)). $^{13}\text{C-NMR}$ (100 MHz, CD_2Cl_2): 148.6 (C(10)); 143.4 (C(8)); 133.9 (C(5)); 125.2 (CH(6)); 117.4 (CH(7)); 111.9 (CH₂(19)); 71.3 (COH, C(25)); 71.3 (CH(1)); 67.3 (CH(3)); 57.9 (CH); 57.7 (CH); 50.7 (CH); 49.9 (C(13)); 45.9 (CH₂(4)); 45 (CH₂); 44.6 (CH₂); 43.5 (CH₂); 34.4 (CH); 34.1 (CH₂); 32 (CH₂); 30.8 (CH₂); 30.6 (CH₂); 30.3 (CH₂); 30 (CH₂); 29.6 (Me_2COH); 29.6 (Me(26), Me(27)); 28.8 (CH₂); 24.9 (CH₂); 23.8 (CH₂); 23.1 (CH₂); 22.3 (Me(21)); 21.9 (CH₂); 10.2 (Me(18)). ESI-TOF-MS: 581 (100, $[\text{M} + \text{Na}]^+$), 541 (0.3, $[\text{M} - \text{OH}]^+$), 523 (24, $[\text{M} - \text{OH} - \text{H}_2\text{O}]^+$). HR-ESI-TOF-MS: 581.4538 ($\text{C}_{36}\text{H}_{62}\text{NaO}_4^+$; calc. 581.4540).

REFERENCES

- [1] ‘Proceedings of the 14th Workshop on Vitamin D’, Eds. R. Bouillon, A. W. Norman, J. R. Pasqualini, *J. Steroid Biochem. Mol. Biol.* **2010**, *121*, 1–478, and the previous 13 volumes in this series.
- [2] A. Chawla, J. J. Repa, R. M. Evans, D. J. Mangelsdorf, *Science (Washington, DC, U.S.)* **2001**, *294*, 1866.
- [3] S. A. Kliewer, K. Umesono, D. J. Mangelsdorf, R. M. Evans, *Nature (London, U.K.)* **1992**, *355*, 446.
- [4] ‘Vitamin D’, 3rd edn., Eds. D. Feldman, J. W. Pike, J. S. Adams, Academic Press, New York, 2011.
- [5] R. Bouillon, W. H. Okamura, A. W. Norman, *Endocrine Rev.* **1995**, *16*, 200.
- [6] G. Eelen, C. Gysemans, L. Verlinden, E. Vanoirbeek, P. De Clercq, D. Van Haver, C. Mathieu, R. Bouillon, A. Verstuyf, *Curr. Med. Chem.* **2007**, *14*, 1893.
- [7] R. Vieth, *Bone* **1990**, *11*, 267.

- [8] M. M. Kabat, R. Radinov, *Curr. Opin. Drug Discovery Dev.* **2001**, *4*, 808.
- [9] C. Carlberg, A. Mouriño, *Expert Opin. Ther. Pat.* **2003**, *13*, 761.
- [10] M. S. Stein, D. L. Wark, *Expert Opin. Invest. Drugs* **2003**, *12*, 825.
- [11] K. K. Debb, D. L. Trump, C. S. Johnson, *Nat. Rev. Cancer* **2007**, *7*, 684.
- [12] C. Calberg, F. Molnár, A. Mouriño, *Expert Opin. Ther. Pat.* **2012**, *22*, 417.
- [13] S. Hourai, L. C. Rodrigues, P. Antony, B. Reina-San-Martin, F. Ciesielski, B. Magnier, K. Schoonjans, A. Mouriño, N. Rochel, D. Moras, *Chem. Biol.* **2008**, *15*, 383; R. Riveiros, A. Rumbo, L. A. Sarandeses, A. Mouriño, *J. Org. Chem.* **2007**, *72*, 5477; X. Pérez-García, A. Rumbo, M. J. Larriba, P. Ordóñez, A. Muñoz, A. Mouriño, *Org. Lett.* **2003**, *5*, 4033; A. Fernández-Gacio, C. Vitale, A. Mouriño, *J. Org. Chem.* **2000**, *65*, 6978; J. A. Martínez-Pérez, L. Sarandeses, J. Granja, J. A. Palenzuela, A. Mouriño, *Tetrahedron Lett.* **1998**, *39*, 4725.
- [14] Y. Inaba, K. Yamamoto, N. Yoshimoto, M. Matsunawa, S. Uno, S. Yamada, M. Makishima, *Mol. Pharmacol.* **2007**, *71*, 1298.
- [15] A. Glebocka, R. Sicinski, L. Plum, H. F. DeLuca, *J. Med. Chem.* **2011**, *54*, 6832.
- [16] a) R. Bouillon, K. Allewaert, J. P. T. M. Van Leeuwen, B. K. Tan, P. De Clercq, M. Vandewalle, H. A. P. Pols, M. P. Bos, H. Van Baelen, J. C. Birkenhäger, *J. Biol. Chem.* **1992**, *267*, 3044; b) X.-C. González-Avion, A. Mouriño, *Org. Lett.* **2003**, *5*, 2291; c) X. C. González-Avión, A. Mouriño, N. Rochel, D. Moras, *J. Med. Chem.* **2006**, *49*, 1509.
- [17] a) K. Shindo, G. Kumagai, M. Takano, D. Sawada, N. Saito, H. Saito, S. Kakuda, K. Takagi, E. Ochiai, K. Horie, M. Takimoto-Kamimura, S. Ishizuka, K. Takenouchi, A. Kittaka, *Org. Lett.* **2011**, *13*, 2852; b) S. Eduardo-Canosa, R. Fraga, R. Sigüeiro, M. Marco, N. Rochel, D. Moras, A. Mouriño, *J. Steroid Biochem. Mol. Biol.* **2010**, *121*, 7.
- [18] L. Verlinden, A. Verstuyf, G. Eelen, R. Bouillon, P. Ordóñez-Morán, M. J. Larriba, A. Muñoz, N. Rochel, Y. Sato, D. Moras, M. Maestro, S. Seoane, F. Domínguez, S. Eduardo-Canosa, D. Nicoletti, E. Moman, A. Mouriño, *ChemMedChem* **2011**, *6*, 788, and refs. cit. therein.
- [19] K. Sokolowska, A. Mouriño, R. R. Sicinski, L. A. Plum, H. F. DeLuca, *J. Steroid Biochem. Mol. Biol.* **2010**, *121*, 29, and refs. cit. therein.
- [20] N. Saito, A. Kittaka, *ChemBioChem* **2006**, *7*, 1478; D. Sawada, T. Katayama, Y. Tsukuda, N. Saito, H. Saito, K. Takagi, E. Ochiai, S. Ishizuka, K. Takenouchi, A. Kittaka, *Tetrahedron* **2010**, *66*, 5407, and refs. cit. therein; R. R. Sicinski, A. Glebocka, L. A. Plum, H. F. DeLuca, *J. Med. Chem.* **2007**, *50*, 6154.
- [21] G. Eelen, N. Valle, Y. Sato, N. Rochel, I. Verlinden, P. De Clercq, D. Moras, R. Bouillon, A. Muñoz, A. Verstuyf, *Chem. Biol.* **2008**, *15*, 1029; G. Eelen, I. Verlinden, R. Bouillon, P. De Clercq, A. Muñoz, A. Verstuyf, *J. Steroid Biochem. Mol. Biol.* **2010**, *121*, 417.
- [22] G. D. Zhu, W. H. Okamura, *Chem. Rev.* **1995**, *95*, 1877; S. Krause, H.-G. Schmalz, in 'Organic Synthesis Highlights IV', Ed. H.-G. Schmalz, Wiley-VCH, Weinheim, 2000; A. S. Chapelon, D. Moraléda, R. Rodríguez, C. Ollivier, M. Santelli, *Tetrahedron* **2007**, *63*, 11511.
- [23] N. Rochel, J. M. Wurtz, A. Mitschler, B. Klaholz, D. Moras, *Mol. Cell* **2000**, *5*, 173; Protein Data Bank Accession Number: 1DB1.
- [24] R. Fraga, F. Zacconi, F. Sussman, P. Ordóñez-Morán, A. Muñoz, T. Huet, F. Molnár, D. Moras, N. Rochel, M. Maestro, A. Mouriño, *Chem. – Eur. J.* **2012**, *18*, 603, and refs. cit. therein.
- [25] H. J. Lee, S. Paul, N. Atalla, P. E. Thomas, X. Lin, I. Yang, B. Buckley, G. Lu, X. Zheng, Y. R. Lou, A. H. Conney, H. Maehr, L. Adorini, M. Uskokovic, N. Suh, *Cancer Prev. Res.* **2008**, *1*, 476; H. Maehr, H. J. Lee, B. Perry, N. Suh, M. R. Uskokovic, *J. Med. Chem.* **2009**, *52*, 5505.
- [26] P. Gogoi, R. Sigüeiro, S. Eduardo, A. Mouriño, *Chem. – Eur. J.* **2010**, *16*, 1432.
- [27] D. Carballa. Ph.D. Thesis, Universidad de Santiago, 2012.
- [28] T. Doi, I. Hijikuro, T. Takahashi, *J. Am. Chem. Soc.* **1999**, *121*, 6749.
- [29] R. Sustmann, P. Hopp, P. Holl, *Tetrahedron Lett.* **1989**, *30*, 689; B. Schönecker, D. Walther, R. Prousa, E. Schrötter, H. Schick, P. Droscher, R. Fischer, D. Walther, M. Reichenbacher, S. Gliesing, 'Proceedings of the 8th Workshop on Vitamin D', Eds. A. W. Norman, R. Bouillon, M. Thomasset, Walter de Gruyter, Berlin–New York, 1991, pp. 212; Y. J. Chen, L. J. Gao, I. Murad, A. Verstuyf, L. Verlinden, C. Verboven, R. Bouillon, D. Viterbo, M. Milanesio, D. Van Haver, M. Vandewalle, P. J. De Clercq, *Org. Biomol. Chem.* **2003**, *1*, 257; M. A. Regueira, S. Samanta, P. J. Malloy, P. Ordóñez-

- Morán, D. Resende, F. Sussman, A. Muñoz, A. Mouriño, D. Feldman, M. Torneiro, *J. Med. Chem.* **2011**, *54*, 3950.
- [30] T. Ishiyama, K. Ishida, N. Miyauro, *Tetrahedron* **2001**, *57*, 9813.
- [31] E. J. Corey, G. Schmidt, *Tetrahedron Lett.* **1979**, *20*, 399.
- [32] G. Vassilikogiannakis, M. Hatzimarinaki, M. Orfanopoulos, *J. Org. Chem.* **2000**, *65*, 8180.
- [33] W. L. F. Amarego, C. L. L. Chai, 'Purification of Laboratory Chemicals', 5th edn., Elsevier, New York, 2003.

Received August 8, 2012