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11-Fluoro-1α-hydroxyvitamin D3: The Quest for Experimental Evidence of the Folded Vitamin D Conformation

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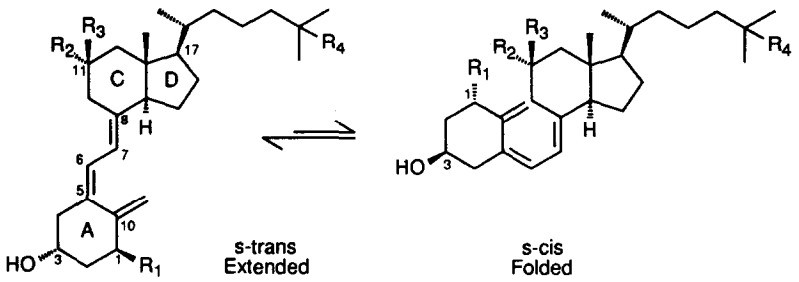
Dedicated to Professor Léon Ghosez on the occasion of his 60th birthday.

Key Words: 11-Fluoro-1α-hydroxyvitamin D3; FAR; DAST; conformational analysis; MM2.

Abstract: *In order to investigate the folded vitamin D conformation in solution, 11-fluorinated vitamin D3 derivatives have been synthesized by fluorination of the corresponding 11-hydroxy derivatives using FAR and DAST. From ^{1}H-NMR analysis no conclusive evidence could be drawn for the presence of the folded form in solution.*

1α,25-Dihydroxyvitamin D3 (1α,25-(OH)2-D3, **3**, Scheme 1), the hormonally active form of cholecalciferol (vitamin D3, **1**), is responsible for controling intestinal calcium absorption (ICA) and bone calcium mobilization (BCM).[1] The discovery that it is also involved in cell differentiation and proliferation and may play a role in the immune system, has stimulated in recent years an impressive search for analogues with a potential therapeutic value.[2,3]



compd	R_1	R_2	R_3	R_4
1	H	H	H	H
1a	H	F	H	H
1b	H	H	F	H
2	OH	H	H	H
2a	OH	F	H	H
2b	OH	H	F	H
3	OH	H	H	OH

Scheme 1

In spite of numerous structure-function analyses,[4] precise information about the active topology of 1α,25-(OH)$_2$-D$_3$ at the time it interacts with its receptor(s) is still lacking. This is primarily due to the flexible nature of the molecule.[5] In this context the vitamin D structure can be viewed as a central CD ring system, to which are connected two conformationally independent flexible entities, the upper side chain and the lower part consisting of the A ring and the connecting diene (so-called seco B ring). Several vitamin D structures have been investigated by X-ray diffraction;[6] they only reveal, however, a static image of the molecule. The side chain, with its five rotatable C-C bonds, is more appropriately analyzed through force field calculations.[5,7] As for the A ring, NMR solution studies have established a dynamic equilibrium between nearly equimolar populations of two chair conformations.[8] More recent force field calculations and ^{1}H-NMR LIS experiments on vitamin D$_3$ have shown that two intermediate diplanar conformations, referred to as half-chair or twist forms, should also be taken into account.[9]

Rotation about the C6-C7 single bond must be facile. Indeed, the known vitamin-previtamin D equilibration requires vitamin D to assume the s-cis or folded geometry.[10,11] The preferred conformation of the diene moiety as revealed by X-ray diffraction[6] and ^{1}H NMR,[12] however, is the s-trans or extended geometry. Quite surprisingly, recent calculations by the group of Wilson indicated the s-cis conformation in which the A ring is folded over the top of the C ring to be the global energy minimum.[13] In this steroid-like conformation the 1α-OH group is in proximity of the C11 position, a functional area known to be of critical importance to glucocorticoids. So far, however, no experimental evidence has been obtained for the presence of the folded geometry in solution.[8f,9a] An attractive proposal, therefore, would consist in placing a suitable substituent on C11 to interact with the 1α-OH group in order to increase the population of the folded conformers up to a detectable level. Being only slightly more bulky than a H atom, thus modifying the vitamin D structure only to a small extent, and known to give hydrogen bonding,[14] a F substituent seemed ideal for this purpose. In the present paper we describe, therefore, 11-fluorinated vitamin D$_3$ derivatives that were synthesized with the aim of inducing the folded conformation via intramolecular hydrogen bonding between the 1-OH group and the 11-F substituent.

MOLECULAR MODELING

Molecular model examination of the folded conformation reveals that such a hydrogen bond would involve an axial 11β-F substituent on the C ring and an axially oriented 1α-OH group on the A ring, with the torsional angle C2-C1-O-H close to 180°. In order to confirm this working hypothesis, force field calculations were performed on models for 1α-OH-D$_3$ (**2**), 11α-F-1α-OH-D$_3$ (**2a**) and 11β-F-1α-OH-D$_3$ (**2b**).

The molecular mechanics calculations were carried out using MM2(91), including Allinger's latest MM2 force field together with a π-system treatment (former MMP2).[15] To simplify the calculations, the vitamin D$_3$ side chain, considered to play no role in the present investigation, was substituted by a methyl group. Thus, so far as modeling is concerned, the cited derivatives refer to the corresponding model compound with a methyl group on C17.

The s-trans or extended (E) and s-cis or folded (F) geometries of the diene moiety were each combined with four conformations of the A ring, two chair (C) and two diplanar or twist (T) conformations in which the 3-OH adopts either the equatorial (e) or axial (a) position. Using a (+) or (-) to refer to the sign of the torsional angle at C5-C6-C7-C8, eight conformations were considered. Table 1 shows the result of the MM2(91) minimizations using starting geometries with a torsional angle C5-C6-C7-C8 of 180° (E) or +/-60° (F), C2-C1-O-H of 180° and C2-C3-O-H of 60°.

From these results we derive the following conclusions: (i) in our hands, the (+)EC(e) form or classical s-trans geometry corresponds to the global energy minimum in **2** and **2a**; (ii) the folded (-)FC(e) and (-)FT(a) forms,

Table 1. Relative Steric Energies E (kJ mol^{-1}) and 1O···11F Distances d (pm) of the Calculated Vitamin D Conformations[a]

compd	2		2a		2b	
conform	E	d	E	d	E	d
(+)EC(e)	0.00		0.00		4.02	
(-)ET(e)	1.93		1.80		5.86	
(-)EC(a)	2.93		2.55		6.86	
(+)ET(a)	3.10		2.76		7.16	
(-)FC(e)	5.10		4.98	322	0.00	287
(-)FT(a)	6.36		7.82		3.01	275
(+)FT(e)	7.07		6.49		11.63	
(+)FC(a)	8.62		7.66		13.56	

[a] Program MM2(91); values of distances d > 400 pm omitted.

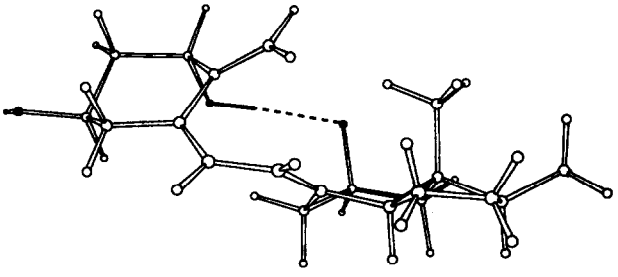


Figure 1. Preferred (-)FC(e) conformation of 11β-F Derivative **2b**

Table 2. Calculated Conformations of Vitamin D Derivative **2b**[a]

	torsional angle (°)			
conform	(-)FC(e)	(-)FT(a)	(+)EC(e)	(-)ET(e)
5-6-7-8	- 61.1	- 58.2	176.4	- 171.7
6-5-10-19	- 32.9	- 33.5	- 46.3	40.6
10-1-2-3	- 50.3	42.2	- 50.9	- 43.6
1-2-3-4	61.0	- 66.2	58.8	69.5
2-3-4-5	- 54.7	41.3	- 54.8	- 42.1
3-4-5-10	40.3	4.9	46.2	- 7.7
4-5-10-1	- 32.2	- 29.1	- 41.7	33.6
5-10-1-2	36.5	4.3	42.8	- 6.2
2-1-O-H	- 168.9	- 158.3	- 152.6	- 165.6

[a] Program MM2(91).

however, become the preferred conformations in the 11β-F derivative **2b**, with calculated 1O···11F distances of 287 pm and 275 pm, respectively, in line with the presence of a OH···F hydrogen bond[14] (Figure 1). The relevant torsional angles of the folded (-)FC(e) and (-)FT(a) forms, and of the extended (+)EC(e) and (-)ET(e) forms for comparison, are listed in Table 2. The result also indicates that, as in the case of vitamin D_3,[9] the A-ring diplanar or twist forms are real minima with steric energies comparable to the corresponding chair forms.

For derivative **2b** four additional folded conformations were calculated in which the C ring was in the boat form (equatorial position of the 11β-F substituent). Upon minimization no OH···F hydrogen bond was found and the steric energies were calculated to be more than 10 kJ mol^{-1} higher then for the (-)FC(e) minimal energy conformer (C-ring chair form), indicating a negligible participation in the conformational mixture.

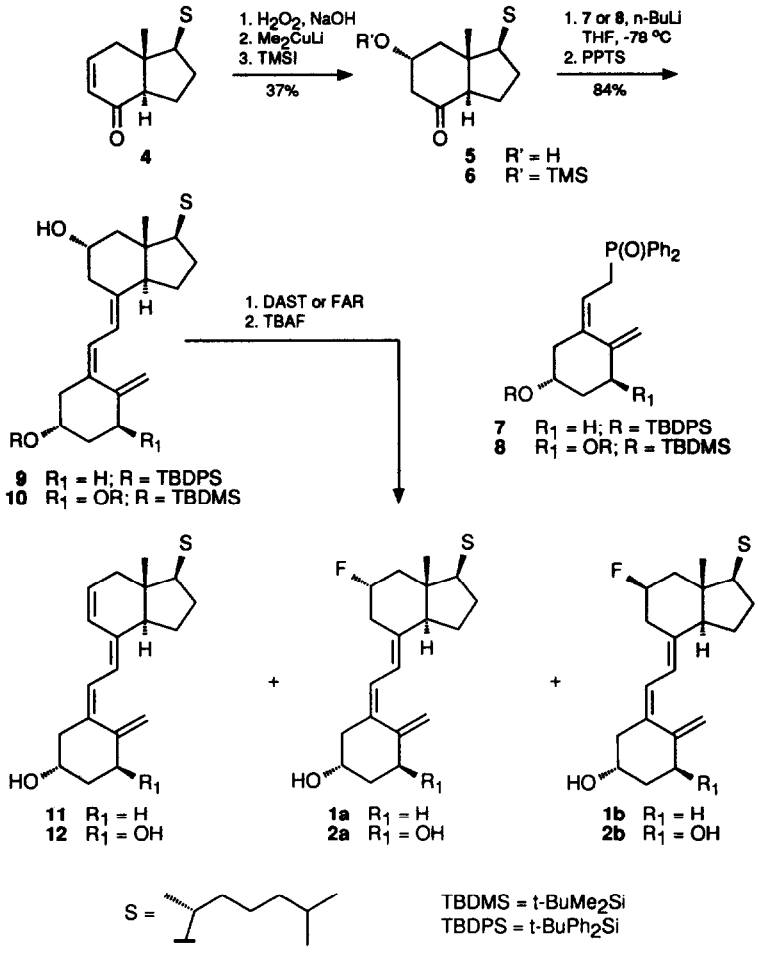
To have a more quantitative idea of the distribution between the extended and folded geometries, one must know the contribution of all the possible conformations to the equilibrium mixture, including the rotamers of the 1-OH and 3-OH groups. Therefore, 3 x 3 rotamers (torsional angles C2-C1-O-H and C2-C3-O-H of 60^0, -60^0 and 180^0) were generated for each of the above eight conformations of the 11β-F derivative **2b** and all 72 starting geometries were minimized using MM2(91), resulting in 32 extended and 26 folded unique conformations. From their relative steric energies, according to a Boltzmann distribution at 298 K, the E/F ratio was calculated to be 31:69 in favour of the folded geometries. In comparison, the same type of calculation carried out for 1α-OH-D_3 (**2**) gave an E/F ratio of 88:12.

SYNTHESIS

Our synthetic efforts towards the 11-fluorinated vitamin D derivatives are summarized in Scheme 2. Epoxidation of enone **4**, readily available from Grundmann's ketone,[16] gave the α-epoxide[17] which was reductively opened with lithium dimethylcuprate to yield the 11α-OH intermediate **5**. After protection of the OH group using 1-(trimethylsilyl)imidazole, ether **6** was subjected to the Wittig-Horner conditions with the known phosphine oxides **7** and **8**,[18] resulting in the corresponding trienes in high yield. Selective cleavage of the TMS ether with pyridinium *p*-toluenesulfonate (PPTS) in dichloromethane afforded the A-ring protected alcohols **9** and **10**, respectively (yield over 90%).[19]

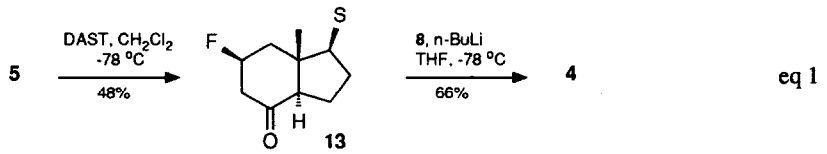
Treatment of **9** with (diethylamino)sulfur trifluoride (DAST)[20] in dichloromethane at -78 °C resulted in a complex reaction mixture which was first desilylated using tetrabutylammonium fluoride (TBAF) in tetrahydrofuran and then carefully separated by chromatography (column on silica gel, followed by twice HPLC). This gave in 23% combined yield the elimination product 9,11-dehydrovitamin D_3 (**11**)[21] and a single 11-fluorinated derivative (ratio 3:2, respectively). The latter was shown by ^{1}H NMR to correspond to the α-isomer **1a**, i.e. the 11β-hydrogen has a large sum of vicinal coupling constants J_{vic} of 32 Hz (axial position) and the angular Me group appears as a singlet at the same position as in **1** and **2** (~0.55 ppm). No formation of the β-isomer **1b** could be detected. The retention of configuration observed during this process is probably due to homoallylic participation by the Δ7,8 bond.[22]

For the purpose of synthesizing the 11β-fluorinated 1α-OH-D_3 derivative **2b**, it became clear that an alternative fluorinating agent would be necessary. N-(2-chloro-1,1,2-trifluoroethyl)diethylamine (FAR)[23] has been shown to substitute hydroxyl groups with less formation of elimination products.[23b] Therefore, alcohol **10** was treated with FAR in dichloromethane at 0 °C for 1 h and subsequently with tetrabutylammonium fluoride in tetrahydrofuran, leading to a 1:3:1 mixture of the 9,11-dehydro elimination product **12**,[24] the α-isomer **2a** and the desired β-isomer **2b**. The configuration of the latter was established by ^{1}H NMR: the 11α-hydrogen shows a small J_{vic} of 12 Hz (equatorial position) and the angular Me resonance, due to a [1,3]-syndiaxial interaction with the 11β-F substituent, is shifted downfield of the normal position to 0.69 ppm and appears as a doublet with a



Scheme 2

coupling of 2.6 Hz. Long-range coupling between syndiaxially oriented F substituents and Me groups is well documented in the steroid field and is very diagnostic in configurational assignments.[25]



eq 1

In the above experiments our attempts to displace the 11α-OH group by fluorine were carried out on the preformed full vitamin D skeleton, rather then on the hydroxy intermediate **5**. Indeed, we had found that, although **5** could be converted into the 11β-F compound **13** in 48% yield using DAST, the subsequent Wittig-Horner coupling of **13** with the anion derived from the phosphine oxide **8** only led to elimination to give back enone **4** (eq 1).

Table 3. ^{1}H NMR Spectral Parameters of Vitamin D_3 Derivatives[a]

comp		H6	H7	H19(E)	H19(Z)	H11	H1β	H3	H9β	H4α	H12β	H1β	H4β	H1α
1	i.	6.25	6.03	5.05	4.82			3.94	2.82	2.56		2.39	2.28	2.17
	ii.	ABd	ABd	dt	d			dddd	brd	dd		ddd	dd	ddd
	iii.	11.2	11.2	2.5	2.5			7.6	12.0	13.2		13.9	13.2	13.9
				1.2				7.5		3.6		7.5	7.5	8.6
								3.8				5.0		4.5
								3.6						
1a	i.	6.22	6.12	5.06	4.80	4.72		3.96	3.28	2.59	2.48	2.41	2.31	2.17
	ii.	ABd	ABd	t	d	dtt		brs	dt	dd	dt	ddd	dd	ddd
	iii.	11.2	11.3	1.2	2.5	48.8		Δ16	12.9	13.2	11.7	13.2	13.2	14.4
						11.0			4.2	3.2	5.4	7.6	7.7	8.5
						5.0						4.7		4.7
2	i.	6.38	6.02	5.33	5.00		4.43	4.22	2.82	2.60			2.31	
	ii.	ABd	ABd	t	brs		dd	tt	m	dd			dd	
	iii.	11.3	11.3	1.6			8.0	6.9		13.4			13.4	
							4.4	3.7		3.5			6.5	
2a	i.	6.36	6.11	5.34	4.98	4.72	4.45	4.25	3.28	2.61	2.48		2.34	
	ii.	ABd	ABd	t	t	dtt	brm	brm	dt	dd	dt		dd	
	iii.	11.2	11.4	1.6	1.4	48.8			12.9	13.5	11.6		13.6	
						10.5			4.3	3.2	5.3		6.4	
						5.6								
2b	i.	6.36	6.20	5.34	5.02	4.99	4.43	4.24	3.19	2.60	2.44		2.32	
	ii.	ABd	ABd	t	brs	dqu	brm	brm	t	dd	tt		dd	
	iii.	11.2	11.2	1.6		44.6			15.7	13.8	14.8		13.5	
						2.8				3.2	2.0		6.4	

[a] 360 or 500 MHz in $CDCl_3$. Chemical shift δ (ppm)(i), multiplicity (ii) and coupling constants J (Hz)(iii). See Experimental for remaining data of **1a**, **2a** and **2b**. For ^{1}H-NMR spectral data of **1** and **2**, see also reference 8.

RESULTS AND DISCUSSION

The ^{1}H-NMR spectral parameters of the fluorinated vitamin D_3 derivatives in $CDCl_3$ as solvent are listed in Table 3. The non-fluorinated vitamin D_3 (**1**) and 1α-OH-D_3 (**2**) are included for comparison.[8] Of particular interest to the present investigation is the vicinal coupling constant $^3J_{6,7}$ of the diene moiety, the value of which should be a measure for the E/F ratio of the different derivatives in solution. All entries in Table 3, including the 11β-F derivative **2b**, show a large $^3J_{6,7}$ coupling of 11.2-11.4 Hz, reflecting an almost exclusive s-trans or extended geometry. No change was observed when the spectrum of derivative **2b** was recorded using a $7x10^{-5}$ M solution in $CDCl_3$, a concentration for which possible competition of intermolecular hydrogen bonding can certainly be excluded.

We preferred the $^3J_{6,7}$ coupling constant as a sensitive measure of the occurence of the folded conformation in **2b** because, even in the case of a small population of folded conformers, its value should be considerably less than the normally observed 11.2 Hz. Indeed, the calculated (-)FC(e) and (-)FT(a) forms show an average torsional angle H6-C6-C7-H7 of ±50° and according to the relevant Karplus relation (=CH-HC=)[26] should have a $^3J_{6,7}$ of 2.0 Hz. Hence for an E/F ratio of 31:69, as computed above, an experimental value as low as 4.7 Hz is expected. If the folded conformers would make up only 10% of the population, a notable decrease to 9.8 Hz should still be observed, which is not the case. The high precentage of folded conformers calculated for **2b**, which is apparently the result of a OH···F interaction found by MM2(91) in the (-)FC(e) and (-)FT(a) forms *in vacuo*, is likely to decrease when the influence of solvent is taken into account. Especially the use of polar solvents, necessary to keep 11-F-1α-OH-D_3 in solution, is expected to favour the extended conformation of the vitamin, as we observe.

Several fluorinated vitamin D derivatives have been reported to exhibit interesting biological activities.[27] Due to the absence of a 25-OH group, however, the biological activity of our vitamin D derivatives has not been investigated.

EXPERIMENTAL

Modeling.

All MM2(91) calculations were performed on a Digital MicroVAX II. In order to calculate the 11-fluorinated derivatives with MM2(91), a torsional parameter was needed for the atom sequence F-C(sp^3)-C(sp^3)-C(sp^2=C) which was not available from the program. For this we used the parameter as supplied by the MacroModel program of Still[28] (V1 = 0, V2 = 0 and V3 = 0.35 kcal mol^{-1}). We consider the use of this low-quality value (A3 parameter) in the calculations of the strained CD-ring part of the molecule to have little or no influence on the modeling results in terms of E/F ratios.

Synthesis.

All reactions involving air and/or moisture sensitive materials were conducted under atmospheres of dry Ar or N_2. All solvents were purified before use. Diethyl ether (Et_2O) and tetrahydrofuran (THF) were distilled from sodium benzophenone ketyl, CH_2Cl_2 was distilled from phosphorous pentoxide.

Analytical and preparative thin-layer chromatography (TLC) employed glass plates precoated (0.25-mm layer) with silica gel 60 F_{254} (E. Merck). Flash chromatography was performed using E. Merck silica gel 60 (70-235 or 230-400 mesh). High-performance liquid chromatography (HPLC) was performed with a Kontron model 420 or Knauer model 64 HPLC pump in combination with a Sicon Analytic LCD201 or Knauer differential re-

fractometer. Unless otherwise noted, separations were carried out on 25-cm x 1.0-cm and 25-cm x 2.2-cm Bio-Sil D 90-10 silica colums, particle size 10 μm.

^{1}H-NMR spectra were recorded on Bruker AM-360 and AM-500 spectrometers in $CDCl_3$ (Aldrich 99% D) as solvent, unless otherwise noted. Chemical shifts are reported in parts per million (ppm) downfield from TMS using residual $CHCl_3$ (7.27 ppm) as an internal standard. Infrared (IR) spectra were recorded on a Beckman IR-4230 or a Perkin-Elmer 1600 series FT-IR spectrometer using KBr plates with film or solution in CH_2Cl_2. Ultraviolet (UV) spectra were obtained on a Perkin-Elmer Lambda 3 UV-VIS spectrophotometer in MeOH as solvent. Mass spectra (MS) were recorded on a Finnigan 4000 or HP-5988 spectrometer at 70 eV. Optical rotations were determined on a Perkin-Elmer model 241 polarimeter.

De-A,B-11α-hydroxycholestan-8-one (5).

To a suspension of CuI (1.17 g, 6.0 mmol, dried under vacuum with heating) in dry Et_2O (30 ml) was added MeLi (1.5 M solution in *n*-hexane, 8.2 ml, 12.3 mmol) at 0 °C. De-A,B-9,11-epoxycholestan-8-one[17] (0.852 g, 3.06 mmol) in dry Et_2O (16 ml) was then added dropwise to the formed Me_2CuLi solution. After 5 min the reaction was quenched via addition of aqueous NH_4Cl (30 ml) and 25% aqueous NH_4OH (8 ml). The inorganic phase was extracted with Et_2O (100 ml) and the combined organic layers were dried over anhydrous $MgSO_4$. Following flash chromatography the product was further purified by HPLC (*n*-hexane/EtOAc 35:65) to give 0.55 g (64%) of **5** as colourless crystals; R_f 0.23 (*n*-hexane/EtOAc 35:65); mp 64 °C; $[\alpha]_D^{20}$ +18.5 (c = 1.0, $CHCl_3$); IR (film) 3388, 2956, 1714, 1467, 1384 cm^{-1}; ^{1}H NMR (360 MHz, $CDCl_3$) 4.25 (m, 1 H), 2.70 (ddd, J = 13.5, 5.9, 0.95 Hz, 1 H), 2.52 (m, 2 H), 2.33 (ddd, J = 13.4, 10.48, 1.2 Hz, 1 H), 0.97 (d, J = 6.3 Hz, 3 H), 0.87 (d, J = 6.6 Hz, 3 H), 0.85 (d, J = 6.5 Hz, 3 H), 0.62 (s, 3 H); MS *m/z* 280 (M^+), 239, 43.

De-A,B-11α-trimethylsilyloxycholestan-8-one (6).

To a solution of alcohol **5** (0.202 g, 0.185 mmol) in dry CH_2Cl_2 (10 ml) was added 1-(trimethylsilyl)imidazole (TMSI, 0.202 g, 1.44 mmol) at room temperature. The reaction mixture was stirred for 2 h and was then immediately chromatographed through silica gel (*n*-hexane/EtOAc 15:1). Further purification by HPLC (*n*-hexane/EtOAc 10:1) afforded 0.245 g (96%) of **6** as a colourless oil; R_f 0.65 (*n*-hexane/EtOAc 9:1); IR (film) 2959, 1721, 1469, 1383, 1251, 1160, 1107, 1076 cm^{-1}; ^{1}H NMR (360 MHz, $CDCl_3$) δ 4.17 (m, Δ = 32.3 Hz, 1 H), 2.59 (dd, J = 13.8, 5.8 Hz, 1 H), 2.51 (dd, J = 11.7, 7.7 Hz, 1 H), 2.35 (m, 2 H), 1.95 (m, 1 H), 1.65 (m, 1 H), 0.96 (d, J = 6.0 Hz, 3 H), 0.87 (d, J = 6.7 Hz, 3 H), 0.86 (d, J = 6.7 Hz, 3 H), 0.65 (s, 3 H), 0.13 (s, 9 H); MS *m/z* 354 (M^+), 352, 337, 262, 221, 73. Anal. calcd for $C_{21}H_{40}O_2Si$: C, 71.59; H, 11.36. Found: C, 72.04; H, 11.33.

A-Ring Protected 11α-Hydroxyvitamin D_3 Derivatives 9 and 10.

To a stirred solution of the A-ring phosphine oxide **7** or **8**[18] (0.56 mmol) in dry THF (3.0 ml) at -78 °C under argon was added *n*-butyllithium (2.5 M in *n*-hexane, 0.56 mmol, 224 μl). The resulting red solution was stirred at -78 °C for 2 h and ketone **6** (98 mg, 0.28 mmol) in THF (1 ml) was added dropwise. Stirring was continued for 40 min and the still red solution was allowed to come to room temperature. The reaction mixture was filtered through silica gel and the product was purified by HPLC (*n*-hexane/EtOAc 40:1) to give the corresponding 11α-trimethylsilyloxyvitamin D_3 derivative in 90% and 92% yield, respectively. The compound was dissolved in CH_2Cl_2, PPTS was added and the solution was stirred at room temperature for 1 h. The reaction mixture was filtered through silica gel and the product was purified by HPLC (*n*-hexane/EtOAc) to afford the alcohol **9** or **10**, respectively.

Data of **9**: yield 94%; white solid; R_f 0.26 (*n*-hexane/EtOAc 6:1); IR (film) 3448, 2954, 1635, 1477, 1265 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) 7.68 (m, 4 H), 7.43 (m, 2 H), 7.38 (m, 4 H), 6.07 (ABd, J = 11.3 Hz, 1 H), 6.04

(ABd, J = 11.3 Hz, 1 H), 4.99 (t, J = 1.2 Hz, 1 H), 4.74 (d, J = 2.4 Hz, 1 H), 3.92 (m, 1 H), 3.85 (m, 1 H), 3.11 (dd, J = 13.1, 5.4 Hz, 1 H), 2.41 (m, 1 H), 2.35 (m, 1 H), 2.30 (m, 1 H), 2.06-1.92 (m, 3 H), 1.80-1.60 (m, 2 H), 1.50-1.15 (m, 15 H), 1.05 (s, 9 H), 0.87 (d, J = 6.7 Hz, 3 H), 0.86 (d, J = 7.0 Hz, 3 H), 0.85 (d, J = 7.0 Hz, 3 H), 0.55 (s, 3 H); MS *m/z* 638 (M^+), 621, 564, 317, 199.
Data of **10**: yield 91%; R_f 0.43 (*n*-hexane/EtOAc 5:1); IR (film) 3449, 2954, 1636, 1428, 1265 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 6.26 (d, J = 11.2 Hz, 1 H), 6.08 (d, J = 11.3 Hz, 1 H), 5.19 (dd, J = 2.4, 1.0 Hz, 1 H), 4.86 (d, 2.3 Hz, 1 H), 4.38 (m, 1 H), 4.19 (tt, J = 7.2, 3.5 Hz, 1 H), 3.90 (m, J = 33 Hz, 1H), 3.16 (dd, J = 13.0, 5.1 Hz, 1 H), 2.45 (dd, J = 13.3, 3.8 Hz, 1 H), 2.36 (dd, J = 12.0, 4.6 Hz, 1 H), 2.23 (dd, J = 13.0, 5.0 Hz, 1 H), 2.04 (dd, J = 11.9, 7.0 Hz, 1 H), 1.93 (m, 1 H), 1.84 (m, 1 H), 1.79 (m, 1 H), 1.72 (t, J = 11.7 Hz, 1 H), 1.55-1.10 (m, 14 H), 0.94 (d, 6.2 Hz, 3 H), 0.88 (s, 9 H), 0.87 (s, 9 H), 0.87 (d, J = 6.6 Hz, 3 H), 0.85 (d, J = 6.6 Hz, 3 H), 0.57 (s, 3 H), 0.06 (s, 6 H), 0.05 (s, 6 H); MS *m/z* 644 (M^+), 627, 512, 455, 248, 73.

11α-Fluorovitamin D_3 (1a) and 9,11-dehydrovitamin D_3 (11).
To a solution of (diethylamino)sulfur trifluoride (DAST, 10 mg, 0.063 mmol) in CH_2Cl_2 (2 ml) was added alcohol **9** (40 mg, 0.063 mmol) in CH_2Cl_2 (1 ml) at -78 °C. The reaction mixture was stirred for 1 h and then quenched by the addition of H_2O (2 ml). The aqueous layer was extracted with CH_2Cl_2, the combined extracts were washed with H_2O and dried over anhydrous $MgSO_4$. After concentration under reduced pressure the residue was chromatographed on silica gel (*n*-hexane/EtOAc 40:1). The isolated mixture of fluorinated and elimination products was dissolved in THF (1.5 ml) and treated with (n-Bu)$_4$NF (TBAF, 1 M solution in THF, 0.36 ml, 0.36 mmol) at room temperature for 20 h. The reaction mixture was filtered through a short column of silica gel and separated by HPLC (*n*-hexane/EtOAc 4:1, and again with CH_2Cl_2). 11α-Fluorovitamin D_3 (**1a**, 3.0 mg) and 9,11-dehydrovitamin D_3 (**11**, 2.0 mg) were obtained in 23% combined yield.
Data of **1a**: R_f 0.32 (*n*-hexane/EtOAc 4:1); IR (film) 3417, 2953, 1633, 1467, 1379, 1162, 1049, 962, 907 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 1.95 (m, 3 H), 1.69 (m, 1 H), 1.35 (m, 4 H), 1.15 (m, 2 H), 0.95 (d, J = 5.8 Hz, 3 H), 0.87 (d, J = 6.7 Hz, 3 H), 0.86 (d, J = 6.7 Hz, 3 H), 0.56 (s, 3 H) (see Table 3 for signals above δ 2.1); MS *m/z* 402 (M^+), 382, 335, 43.

11α-Fluoro-1α-hydroxyvitamin D_3 (2a), 11β-Fluoro-1α-hydroxyvitamin D3 (2b) and A-ring protected 1α-hydroxy-9,11-dehydrovitamin D_3 (12).

(i) *Fluorination of 10 using DAST.* Essentially the same procedure as described for the fluorination of **9** was followed. The crude reaction mixture was purified by column chromatography using n-hexane/EtOAc 50:1. After treatment with TBAF in THF and filtration through silica gel, the residual material was separated by HPLC (*n*-hexane/EtOAc 1:2) affording a mixture of **2a** and **12** (10.3 mg, 1:5 ratio) in 15% combined yield.

(ii) *Fluorination of 10 using FAR.* To a solution of alcohol **10** (50 mg, 0.078 mmol) in CH_2Cl_2 (3 ml) was added N-(2-chloro-1,1,2-trifluoroethyl)diethylamine (FAR, 44 mg, 0.023 mmol) by syringe at 0 °C. The reaction mixture was stirred at 0 °C for 1 h and was then added to a precooled saturated $NaHCO_3$ solution. The mixture was extracted with Et_2O, the combined extracts were washed with H_2O and dried over anhydrous $MgSO_4$. After concentration under reduced pressure the residual material was separated on silica gel (*n*-hexane/EtOAc 200:1) yielding a mixture of A-ring protected **2a**, **2b** and **12** (36 mg, 1:3:1 ratio) in 71% combined yield. Pure protected **2a** and **12** were obtained by preparative TLC (20-cm x 20-cm x 0.25-mm plates, eluent *n*-hexane/CH_2Cl_2 3:1, 5 mg each plate), but **2b** remained contaminated by **2a**. As described for the synthesis of **1a**, deprotection with TBAF

in THF afforded **2a** and **2b** (contaminated with **2a**) in 95% yield. Pure **2b** was finally obtained by HPLC (column RSil CN, particle size 10 µm, eluent *n*-hexane/i-PrOH/acetonitrile 93:6:1).

Data of A-ring protected **12**: R_f 0.41 (*n*-hexane/CH_2Cl_2 3:1); ^1H NMR (500 MHz, $CDCl_3$) δ 6.59 (dd, J = 10.1, 2.0 Hz, 1 H), 6.37 (d, J = 11.6 Hz, 1 H), 6.09 (d, J = 11.7 Hz, 1 H), 5.69 (m, 1 H), 5.20 (dd, J = 2.3, 0.9 Hz, 1 H), 4.89 (d, J = 2.4 Hz, 1 H), 4.38 (m, 1 H), 4.18 (m, 1 H), 2.47 (dd, J = 13.1, 3.7 Hz, 1 H), 2.36-2.15 (m, 6 H), 2.10-1.65 (m, 4 H), 1.55-1.10 (m, 9 H), 0.93 (d, J = 6.5 Hz, 3 H), 0.89 (s, 9 H), 0.87 (s, 9 H), 0.86 (d, J = 6.6 Hz, 3 H), 0.84 (d, J = 6.6 Hz, 3 H), 0.54 (s, 3 H), 0.05 (s, 6 H), 0.05 (s, 6 H).

Data of **2a**: R_f 0.39 (*n*-hexane/EtOAc 1:2); IR (film) 3420, 2957, 1630, 1463, 1387, 1107, 1053, 937, 867 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 2.03 (m, 1 H), 1.95 (m, 3 H), 1.60-1.15 (m, 14 H), 0.94 (d, J = 5.8 Hz, 3 H), 0.87 (d, J = 6.6 Hz, 3 H), 0.86 (d, J = 6.6 Hz, 3 H), 0.55 (s, 3 H) (see Table 3 for signals above δ 2.1); MS *m/z* 418 (M^+), 398, 331, 311, 43.

Data of **2b**: R_f 0.39 (*n*-hexane/EtOAc 1:2); ^1H NMR (500 MHz, $CDCl_3$) δ 2.03 (m, 3 H), 1.91 (m, 2 H), 1.60 - 1.00 (m, 13 H), 0.93 (d, J = 6.4 Hz, 3 H), 0.87 (d, J = 6.6 Hz, 3 H), 0.86 (d, J = 6.6 Hz, 3 H), 0.69 (d, J = 2.6 Hz, 3 H) (see Table 3 for signals above δ 2.1).

De-A,B-11β-fluorocholestan-8-one (13).

Fluorination of alcohol **5** using DAST was carried out by essentially the same procedure as described for alcohol **9**. The crude material was purified by HPLC (*n*-pentane/EtOAc 65:35) affording **13** in 48% yield as a colourless oil; R_f 0.20 (*n*-hexane/EtOAc 9:1); $[\alpha]_D^{20}$ -6.4 (c = 0.875, $CHCl_3$); IR (film) 2956, 1720, 1467, 1384 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 5.28 (ddt, J = 47.7, 5.3, 2.1 Hz, 1 H), 2.65 (dddd, J = 16.9, 3.9, 1,9, 0.9 Hz, 1 H), 2.48 (ddd, J = 17.1, 5.3, 0.8 Hz, 1 H), 2.46 (dd, J = 11.7, 7.2 Hz, 1 H), 0.97 (d, J = 6.0 Hz, 3 H), 0.88 (d, 6.5 Hz, 3 H), 0.86 (d, J = 6.5 Hz, 3 H), 0.79 (d, J = 2.8 Hz, 3 H); MS *m/z* 282 (M^+) 267, 221, 170, 43.

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