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4,4-Difluoro-1α,25-Dihydroxyvitamin D_3 : Analog to Probe A-Ring Conformation in Vitamin D-Receptor Complex

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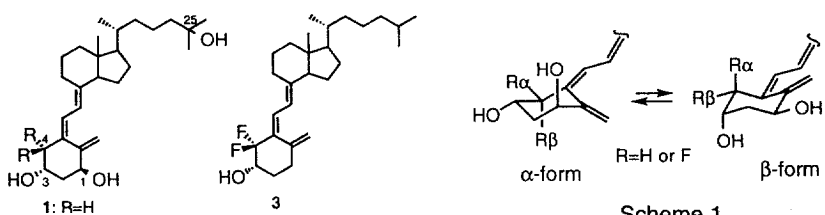
Abstract: 4,4-Difluoro-1α,25-dihydroxyvitamin D_3 was synthesized from ergosterol and analysis of its ^{19}F NMR showed it to be a useful probe to analyze the receptor-bound A-ring conformation of vitamin D.

Keywords: conformation; fluorine and compounds; NMR; vitamins

Biological responses mediated by 1α,25-$(OH)_2D_3$ **1** are regulated at the level of gene expression by binding of the vitamin D receptor (VDR)-ligand complex to the target gene [1]. It is now well documented that the transactivation function of members of a nuclear receptor super family is highly dependent on the conformation of a small C-terminal part of the receptor's ligand binding domain (AF2) [2] and three-dimensional structure of the ligand is critical to determine the conformation of AF2. Because of the flexible nature of vitamin D that can adopt a number of conformations around the side chain, seco-B-ring and A-ring, we are focusing our primary attention on the conformation-function relationship of vitamin D. A series of studies using rationally designed conformationally-restricted analogs have led us to propose the active side chain conformation of vitamin D [3]. To directly investigate the A-ring conformation binding to the VDR, we proposed the use of ^{19}F NMR with fluorinated vitamin D as a probe and have been synthesizing various fluorinated A-ring analogs [4]. The A-ring has been known to adopt two chair conformations (Scheme 1), the α- and β-form, according to ^1H NMR and X-ray analysis [5], but it has not been known which conformation is responsible for VDR binding. By monitoring the signal of fluorine substituents on the A-ring, we can analyze the conformation of the A-ring in the vitamin D-VDR complex without interference from proton signals.



Scheme 1

This paper reports the successful synthesis of 4,4-difluorovitamin D analogs **2** and **3** as suitable probes

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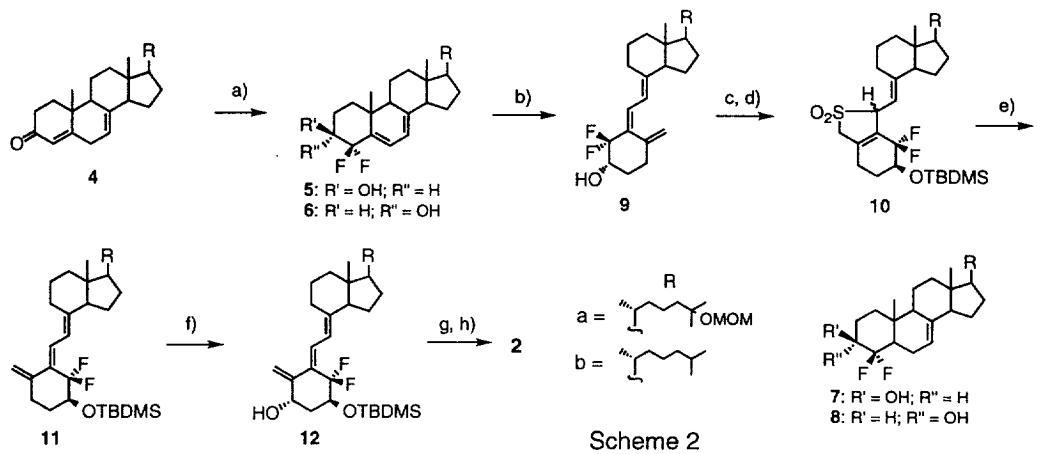
designed for the ^{19}F NMR study. The low-temperature ^{19}F NMR spectrum of **2** showed two well separated frozen conformations indicating **2** to be a useful probe to analyze the VDR-bound A-ring conformation of vitamin D.

4,4-Difluorovitamin D **2** was synthesized starting with an enone **4a** which was constructed from ergosterol (Scheme 2). Electrophilic fluorination of **4a** under thermodynamic conditions yielded exclusively 4,4-difluorinated 3-ketone, which upon reduction with $NaBH_4$ gave the desired 3β-OH compound **5a** as the major (62%) product, together with a 3α-OH isomer **6a** as a minor product (10%) [6]. Fluoroprovitamin D **6a** was converted to 4,4-difluorovitamin D_3 **9a** by photochemical means as usual. A 1α-hydroxyl group was introduced by Hesse's method [7] via a 5*Z*-isomer **11a**, which was produced selectively via SO_2-adduct **10a** [8]. Oxidation of **11a** with selenium oxide yielded 1α-hydroxylated product **12a** as the major product (33%), together with its C-1 epimer (9%). Dye-sensitized photo-isomerization and removal of the protecting groups gave 4,4-difluoro-1α,25-dihydroxyvitamin D_3 **2** which showed unusually long wavelength absorption maximum in the UV spectrum (λmax 271 nm) [9]. Analogous 4,4-difluorovitamin D **3** was synthesized similarly from an enone **4b** derived from 7-dehydrocholesterol [9].



Scheme 2

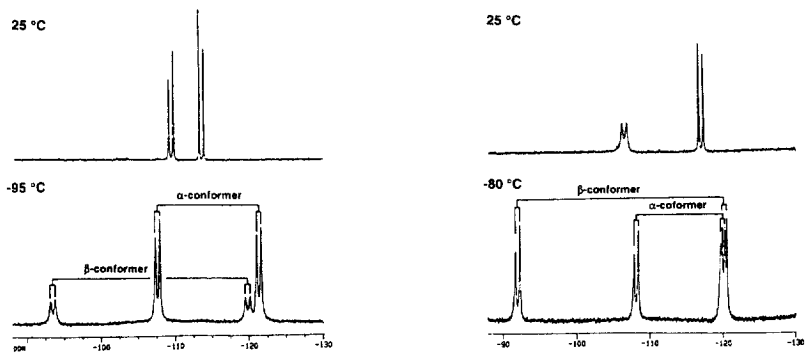
Conditions: a) $(PhSO_2)_2NF$, ^tBuOK, THF,-30 °C; $NaBH_4$, EtOH, r.t., 62 % for **5a**, 10 % for **6a**; b) hν, Hg lamp, PhH-EtOH, 0 °C, 52 %; EtOH, r.t., 52 %; c) liq. SO_2, reflux, 75 %; d) TBDMSOTf, Et_3N, Tol, -20 °C to r.t., 41 %; e) octane, 100 °C, 76 %; f) SeO_2, NMO, MeOH-CH_2Cl_2, reflux, 33 %; g) hν, halogen lamp, anthracene, PhH-EtOH, 0 °C, 98 %; h) CSA, MeOH, r.t., 90 %.

In the ^{19}F NMR spectra of both 4,4-difluorovitamins **2** and **3**, two distinct conformers were observed at low temperature. The spectrum of **3**, which lacks a 1α-hydroxyl group, showed two fluorine signals, 4β-F at δ -113.6 (dd, J = 232, 12 Hz) and 4α-F at δ -109.5 ppm (d, J = 232 Hz) at 25 ℃ (Fig. 1a) [10]. At -95 ℃, these peaks become separated into two pairs of doublets in an approximately 8:2 ratio: δ -121.1, -108.1 (each d, J = 226 Hz) and -119.8, -92.9 (each d, J = 236 Hz); 8:2 (Fig. 1a). We assigned the major component to the α-conformer and the minor to the β-conformer on the basis of the ^1H NMR of **3** at -95 ℃ [6, 11]. There is a smaller fluorine chemical shift difference in the α-conformer (13.1 ppm) and a larger chemical shift difference in the β-conformer (26.9 ppm).

Two A-ring conformers were also well separated in the ^{19}F NMR spectra of 1α-hydroxylated fluorovitamin D **2** at even higher temperature (-80 ℃) (Fig. 1b), the ratio of the two conformers being approximately 1:1. Compared with the spectrum of **3**, one pair of doublets [δ -120.2 and -92.0 (each d, J =

240 Hz)] with a larger chemical shift difference (28.2 ppm) was assigned to the β-conformer and the other [δ -119.9 and -108.2 (each d, J = 230 Hz)] with a smaller difference (11.7 ppm) to the α-conformer. The large conformer-based fluorine chemical shift difference will help clarify which A-ring conformer is involved in the VDR-ligand complex [12].

Figure. 1 **a)** ^{19}F NMR (CD_2Cl_2-CD_3OD) of **3** **b)** ^{19}F NMR (CD_2Cl_2-CD_3OD) of **2**



Dynamic ^{1}H NMR studies have also been conducted on **2**, and the energy barrier for A-ring flipping was estimated to be 9.8 kcal mol^{-1} using the modified Eyring equation [13], which is slightly larger than that of **1** (9.5 kcal mol^{-1}) [14], and smaller than that of the 4,4-dimethylvitamin D analog (11.0 kcal mol^{-1}) [15]. The observed increase in the energy barrier for **2** compared to **1** is probably due to steric congestion both between the two fluorine atoms and the protons at C-2 and C-6.

Binding affinity of fluorovitamin D **2** for VDR was evaluated using bovine thymus VDR. Though the affinity of **2** was considerably small (about 1%) relative to the natural ligand **1**, the VDR-bound form is estimated to be still exclusive (B/F: ca. 10^9) on the basis of Kd of **1**.

In conclusion, we have observed, for the first time, two conformers of the vitamin D A-ring in the ^{19}F NMR using newly synthesized 4,4-difluoro-1,25-dihydroxyvitamin D_3 **2**. ^{19}F NMR study of the VDR-ligand complex is progressing through the use of this compound.

References and Notes

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[9] All new compounds displayed satisfactory spectroscopic data. Low-temperature NMR spectra were taken in CD_2Cl_2-CD_3OD (2:1; v/v). ^{19}F NMR chemical shifts were reported in ppm with respect to trifluorotoluene as an external standard (δ -63 ppm). The compounds **7a** and **8a** were prepared by hydrogenolysis (H_2, PtO_2) of **5a** and **6a**, respectively.
5a: ^1H NMR ($CDCl_3$) δ: 3.79 (1 H, m, 3-H). ^{19}F NMR ($CDCl_3$) δ: -116.5 (d, *J* = 244 Hz, 4α-F); -98.1 (dd, *J* = 244 and 15 Hz, 4β-F). **6a**: ^1H NMR ($CDCl_3$) δ: 4.01 (1 H, m, 3-H). ^{19}F NMR ($CDCl_3$) δ: -115.0 (d, *J* = 260 Hz, 4α-F); 82.6 (d, *J* = 260 Hz, 4β-F). **7a**: ^{19}F NMR ($CDCl_3$) δ: -126.4 (ddd, *J* = 238, 28, 21 Hz, 4β-F); -108.5 (d, *J* = 238 Hz, 4α-F). **8a**: ^{19}F NMR ($CDCl_3$) δ: -112.1 (dd, *J* = 252, 27 Hz, 4β-F); -106.9 (d, *J* = 252 Hz, 4α-F). **3**: ^1H NMR (CD_2Cl_2-CD_3OD) δ: 0.51 (3 H, s, 18-H); 0.82 (6 H, d, *J* = 5.8 Hz, 26, 27-H); 0.89 (3 H, d, *J* = 6.0 Hz, 21-H); 3.85 (1 H, m, 3-H); 4.91 and 5.18 (each 1 H, s, 19-H); 6.06 and 6.87 (each 1 H, d, *J* = 11.5 Hz, 7, 6-H). ^{19}F NMR (CD_2Cl_2-CD_3OD) δ: -113.6 (dd, *J* = 232 & 12 Hz); -109.5 (d, *J* = 232 Hz). UV λmax (95 % EtOH): 270 nm (ε 19400). **2**: ^1H NMR (CD_2Cl_2-CD_3OD) δ: 0.53 (3 H, s, 18-H); 0.91 (3 H, d, *J* = 6.4 Hz, 21-H); 1.14 (6 H, s, 26, 27-H); 4.10 (1 H, m, 3-H); 4.35 (1 H, m, 1-H); 5.08 and 5.44 (each 1 H, s, 19-H); 6.08 and 6.97 (each 1 H, d, *J* = 11.4 Hz, 7, 6-H). ^{19}F NMR (CD_2Cl_2-CD_3OD,) δ: -115.1 (d, *J* = 235 Hz); -104.5 (broad d, *J* = 200 Hz). MS m/z (%): 452 (M^+, 17); 434 (34); 323 (56); 305 (18); 135 (100). UV λmax (95 % EtOH): 271 nm.

[10] The signals in the ^{19}F NMR spectra of **2** and **3** were assigned on the basis of the spectra of rigid difluorosteroid derivatives **5** - **8**: In the spectra of **5** and **6**, which have both an α-OH group and an α-double bond, the fluorine signals appear in the following order of increasing shielding: (1) axial F with an *anti*-parallel OH and a parallel π-bond orbital (-82.6), (2) axial with a *cis*-OH and a parallel π-bond orbital (-98.1), (3) equatorial with a *cis*-OH and an orthogonal π-orbital (-115.0), and (4) equatorial with a trans-OH and an orthogonal π-orbital (-116.5), while in the spectra of **7** and **8**, which have only an α-OH group, the order is: (1) equatorial F with a *cis*-OH (-106.9), (2) equatorial with a *trans*-OH (-108.5), (3) axial with an *anti*-parallel α-OH (-112.1), and (4) axial with a *cis*-OH (-126.4). Thus, the signals in the spectra shown in Fig. 1b can be assigned as follows: the lowest signal (-92.9) to 4α-F and its partner (-119.8) to 4β-F in the β-form; the highest signal (-121.2) to 4α-F and its partner (-108.1) to 4β-F in the α-form: a) Bovey FA, Anderson EW, Hood FP, Kornegay RL. J. Chem. Phys. 1964;40:3099-3109; b) Franklin NC, Feltkamp H. Angew. Chem. Int. Ed. Engl. 1965;4:774-783.

[11] The two conformers in **3** can be distinguished by the signals of the proton at C-3 (δ 3.72 and 3.90; an 8:2 ratio), the major signal in higher field being attributed to the axial C-3 proton in the α-conformation and the minor in lower field to the equatorial C-3 proton in the β-conformation.

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