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Calcitriol derivatives with two different side chains at C-20. V. Potent Inhibitors of Mammary Carcinogenesis and Inducers of Leukemia Differentiation

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Abstract

Calcitriol is implicated in many cellular functions including cellular growth and differentiation thus explaining its anti-tumor effects. It was shown that gemini, the calcitriol derivative containing two side chain at C20, is also active in gene transcription with enhanced anti-tumor activity. We have now further optimized both the A-ring and the two side chains. The chemical structures of the resulting 18 geminis were correlated with biological activities. Those containing the 1α -fluoro A-ring are the least active. Those featuring 23-yne and 23(E) side-chains are generally more active in human breast cancer-cell growth inhibition and human leukemia-cell differentiation induction than their 23(Z) counterparts. Based on these evaluations we selected as lead compound a 20(R) gemini, related to calcitriol in terms of it's A-ring, where one side chain was modified by introduction of a 23-yne function and replacement of the geminal methyl groups with trifluoromethyl groups, the other created by extension of C21 with a 3-hydroxy-3-trideuteromethyl-4,4,4-trideutero-butyl moiety.

Introduction

Gemini is the cumulative term for vitamin D analogs with two side chains emanating at C-20. In the first generation of this compound category, both side chains were identical as in 1a. The arrangement comprises the unaltered chain of $1\alpha,25(OH)_2D_3$ (1) and a duplicate thereof as an extension of the methyl group $21.^1$ From a historical viewpoint it was argued that 20-epi-1 exhibited transcriptional activities that were some 200-5000 times higher than the 'natural' counterpart; 2,3,4 therefore, a biologically active derivative featuring two side chains was a tenable proposition. The presence of two side chains in one molecule raised the prospect of enhanced gene transactivation but, on the other hand, it dimmed the ability for such a compound to function as a ligand as it might overwhelm the space allotment provided by the VDR with concomitant loss of receptor affinity. Even if binding would occur, there was no guarantee for induction of the specific conformational change in the receptor essential for transcriptional activity.

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A number of important discoveries were made in subsequent studies. Most significantly, gene transcription of gemini was demonstrated ^{1,6} and the ligand binding pocket was shown to be sufficiently malleable to allow the ligand to determine the conformation of the LBD suitable for its accommodation. ⁷ The ligand-dependent conformation of the LBD was investigated and access to a channel that can accommodate the second side chain of gemini was discovered. ⁸ Molecular dynamics simulations, likewise, confirmed that the additional side chain, tantamount to a 25% increase in volume, could be domiciliated by minor reorganizations of some 30 amino acids and major realignments of several others, without disturbing the overall structure of the LBD. ^{9,10} In this arrangement, one of the gemini side chains assumes the same position as the one of the natural ligand 1, while the other has actually two spatial opportunities for residence. ¹¹

It is further perceived that two distinct receptors, the nuclear VDR and the membrane VDR, recognize different shapes of the conformationally flexible 1 to elicit gene transcriptions and rapid signal transduction pathways, respectively. ¹² If the LBD is, to a certain extent, indiscriminate toward ligands of variegated chemical make-up, the prospect of designing specifically targeted ligands exists ⁷ and, with the resulting conformational changes, targeted biochemical responses can be envisioned.

Further to the classical role of 1α,25(OH)₂D₃, a multitude of previously unexpected physiological and biochemical activities have come to light. Most prominent are the anti-tumor effects based on antiproliferation, prodifferentiation and reduced angiogenesis. In view of the apparent VDR's promiscuity toward ligands, we incorporated into the gemini side-chains those features that were previously established for the 'one-side-chain' analogs as enhancements of biological activities. These augmentations are, at least in part, due to the prevention, or retardation, of biological degradation, most notably the degradative cascade initiated by 24hydroxylation, and include replacement of the geminal methyl groups with trifluoromethyl entities and introduction of C23-unsaturation. This 24-hydroxylation, occurring preferentially at the pro-R side chain of 1a, 13 entails higher drug dosages to increase blood calcium levels and to suppress INF-γ release in MLR. ¹⁴,15 Thus, the resulting new generation gemini maintain the side chain of 1 but feature a different second chain. In view of the physiological precariousness of vitamin D analogs, including gemini, we argued that a gemini's half-live could be extended by replacing the geminal methyl groups at the calcitriol side chain with trideuteromethyl moieties. 16 Such a modification was expected to alter, neither the propensity of this arm to assume the customary position within the VDR, nor the kinetically controlled binding affinity, but contemporaneously, to prolong the intra-cellular life span due to increased lipophilicity and bond strengths.. This lemma, together with the assurance of side-chain

similarity to the natural hormone, led to the expectation that gemini with deuterated methyl groups to be superior drugs in an in vivo environment. Fortunately, this proposition was subsequently substantiated^{1,17-20} and is corroborated in the present study comprising 18 derivatives of **1a** as shown in Scheme 1.

Results and Discussion

Chemical Syntheses

The first part of the syntheses, illustrated in Schemes 2 and 3 is concerned with the preparation of the so-called CD-ring synthones and commences with the previously described methyl ester 5²¹ which was treated with trideuteromethyl magnesium bromide in ether to afford alcohol 6. An ene-reaction with paraformaldehyde and dimethylaluminum chloride in DCM gave the pair of diols 7E and 7Z. Although this pair of epimers was readily separated by flash chromatography, it was hydrogenated as a mixture to furnish 8S and 8R, again easily separable by preparative HPLC. These two intermediates are the starting points for the entire cascade of the 'S' and 'R' series of the CD-ring systems. Subsequent TEMPO/chloramine-T mediated oxidations led to the aldehydes 9, which were homologated to the alkynes 10.²² Conversion of the tertiary hydroxyl group to the TMS ether, prior to deprotonation with butyllithium and condensation with hexafluoracetone, gave higher yields of the diols 11S-a and 11R-a when compared to the use of the unprotected counterparts. Deprotection with fluorosilicic acid²³ furnished the triols 11S-b and 11R-b. The stereochemical assignments of the epimeric alcohols 8a and 8b, obtained after chromatographic separation (Scheme 2), was confirmed by a crystallographic analysis of 11S-b whose ORTEP rendition is depicted in Figure 1. Subsequent oxidation with PDC in DCM gave 12S-a and 12R-a and a following silylation of the tertiary hydroxyl groups led to the ketones 12S-b and 12R-b, respectively. The trimethylsilyloxy groups at the gem-trifluoromethyl positions, however, are of limited stability and partially lost upon purification of the reaction mixtures.

The intermediate triols **11S-b** and **11R-b** served as starting materials for the preparation of the alkene series of CD-ring synthones as shown in Scheme 3. Reduction with sodium bis(2-methoxyethoxy)aluminum hydride led to the (*E*)-alkene-triols **13S** and **13R**, whereas catalytic hydrogenation with Lindlar catalyst furnished the (*Z*)-alkene-triols **15S** and **15R**. The members of both pairs were oxidized with PDC to the ketones, and then further protected by silylation to lead to the (*E*)-alkene-ketones **14S-b** and **14R-b** and the (*Z*)-alkene-ketones **16S-b** and **16-b**, respectively. The previous sequence, comprising oxidation at C8 and silylation of the tertiary hydroxyl groups, converted the triol pairs **13** and **15** to the protected ketone pairs **14S-b**/**14R-b** and **16S-b**/**16R-b** which served as suitable coupling partners with the A-ring synthones.

The second phase of the synthesis included the coupling reactions of the CD-ring moieties with three different A-ring synthones as depicted in Scheme 4. The three A-rings, in the form of the allyldiphenylphosphane oxides 17,²⁴⁻²⁶ 18,²⁷⁻²⁹ and 19³⁰⁻³² were used in Wittig-Horner reactions with the six CD-ring units 12S-b, 14S-b, 16S-b and their (*R*)-counterparts which, after deprotection with TBAF, afforded 18 combinations as final products ranging from 2S-1 to 4R-3 (Scheme 4). The crystal structure of one of them (2R-2) is shown in Figure 2. While the crystal structure of 11S-b (Figure 1) corroborated the configurational assignments in the 20-S series, the structure of 2R-2 authenticates the stereochemical veracity of the 20(*R*) series.

Biological Studies

It became apparent that the side-chain modification, the resulting configurational duality at C20 and the nature of the A-ring exert different biological responses. In a recent study with non-deuterated gemini, the analog of **2S-1** was preferred and suggested gemini to modulate the expression and activity of proteins associated in cancer-cell proliferation by inhibition of

the Akt-mTOR pathway.³³ The effect, generated by introduction of deuterium, was underscored when 2S-2 was compared with the analogous, non-deuterated, epimeric pair **2S-2** and **2R-2** in the mouse MC-26 colon cancer model. ¹⁹ While the non-deuterated analog of 2R-2 exerted little effect, the epimeric non-deuterated analog of 2S-2 caused a significant reduction of colon tumor growth and tumor weight when administered at equal concentrations. The replacement of the hydrogen atom at the geminal methyl groups with deuterium, as in 2S-2, enhanced the antitumor effect ten-fold. This remarkable C20 epimeric effect was also observed in the alteration of VDR-dependent monocytic differentiation in human leukemia cells, ³⁴ and in the present study of MCF10CA1a cell proliferation, but, surprisingly, it was largely unpronounced in the gemini series containing the 23-yne side chain. Different antitumor activities among epimers are somewhat remarkable as it is generally contended that they assert their antiproliferative activity as ligands inside the LBD of the VDR. If a certain epimer of a given pair is established as a superior ligand capable to engender an antitumor effect, one would expect this bias to persevere in all antitumor responses. In view of the huge difference in antiproliferative effects between 1 and epi-1, as a result of disparate LBD conformations, different antineoplastic responses of two C20-epimeric geminis are not surprising. The observation that the partners of such an epimeric pair actually function differently, much like tailor-made entities for very specific antiproliferative activities, is unexpected and warrants further exploration.

We now report the effects of gemini on cell proliferation of MCF10CA1a human breast cancer cells and on differentiation of NB4 human promyelocytic leukemia cells. The current study centers on the gemini pairs $\bf 2$, $\bf 3$ and $\bf 4$, formal derivatives of $\bf 1$ ($\bf 2S$, $\bf 3S$ and $\bf 4S$, left column in Scheme 1) and 20-epi- $\bf 1$ ($\bf 2R$, $\bf 3R$ and $\bf 4R$, right column in Scheme 1), respectively. They feature the two traditional six-carbon chains of gemini ($\bf 1a$); the significant differences in a comparison to $\bf 1a$, however, are manifold, i.e., the inequality of the two chains, the replacement of the geminal methyl groups of calcitriol's "natural chain' by deuteromethyl moieties and the transmutation of the other to incorporate geminal trifluoromethyl groups, and $\bf 23$ -yne, $\bf 23$ ($\bf E$) and $\bf 23$ ($\bf Z$)-ene unsaturations. To continue the theme of pharmacophoric periodicity, we also commuted the "natural" 1-hydroxy A-ring part to 19-nor and $\bf 1\alpha$ -fluoro analogs. In the designations used, the first letter refers to the unsaturation of the side chain, e.g., $\bf 2$ for 23-yne, $\bf 3$ for $\bf 23$ ($\bf E$)-ene and $\bf 4$ for $\bf 23$ ($\bf Z$)-ene. The symbols $\bf R$ and $\bf S$ refer to the absolute configuration at C20 and the extensions -1, -2, -3 refer to the A-ring characteristics, i.e., 1-hydroxy or "normal", 19-nor, and $\bf 1\alpha$ -fluoro arrangements, respectively. As per example, $\bf 2S$ - $\bf 2$ has an alkyne side chain, the configuration at C20 is ($\bf S$), and the A-ring portion is of the 19-nor type.

Gemini-type compounds were shown to be more active in inhibiting cell proliferation of breast-cancer cells than **1**,^{35,36} and in BMP/Smad-specific signaling in human breast epithelial cells. ³⁷ In preclinical animal studies **2S-2** and **2R-2** prevented estrogen receptor (ER)-positive mammary tumorigenesis induced by N-methyl-N-nitrosourea in rats.^{36,15} In addition, **2R-2** exhibited significant tumor-growth suppression in the ER-negative MCF10DCIS.com xenograft model without hypercalcemic toxicity.³⁸ A mode of action study suggested the inhibitory effect of **2R-2** to be associated with an increase of cyclin-dependent kinase inhibitor p21 and the insulin-like growth-factor binding-protein 3 in both animal models. Spina *et al* also reported that **2S-2** significantly reduced tumor growth of mouse colorectal cancer cells in BALB/c mice⁹ and inhibited the invasive spread of the tumor into the surrounding muscle.¹⁷

We first studied the inhibitory effect of gemini on MCF10CA1a cell proliferation. The biological activities in cell growth inhibition are compared in terms of IC $_{50}$ values and illustrated in Table 1. Most gemini are superior inhibitors of cell growth when compared to 1, the naturally occurring 1α ,25(OH) $_2$ D $_3$, and significant differences ensue as a result of changes in A-rings, C20 configuration, and side-chain unsaturation. Those containing the 1α -fluoro A-rings (rows 4, 7, and 10) exerted relatively weak activities; in general, gemini

featuring an alkyne side-chain (rows 2-4) or the 23(*E*) function (rows 5-7), inhibited MCF10CA1a cell proliferation most effectively.

In view of the well-known ability of vitamin D as an inducer of cell differentiation, we investigated the gemini's potential as differentiation inducers in NB4 human leukemia cells by measuring the induction of monocyte cell-surface markers CD11b and CD14. In general, gemini induced NB4 cell-differentiation stronger than 1. Conforming to the trend observed in growth inhibition of breast cancer cells, the 1α -fluoro analogs were less active in inducing cell-differentiation markers. Those containing the 1-hydroxy A-rings and the 19-nor A-rings exerted similar effects. The 23(Z)-ene side chains were generally less active than those with the 23-yne and 23(E)-ene chains, as illustrated in Figure 5. While the C20 epimeric effect was not perceivable among members containing the 23-yne or 23(E)-ene side-chain, the differentiation was markedly enhanced in the 20R-series of those containing the 23(Z)-ene chain (4R-1, 4R-2, 4R-3 versus their S-counterparts).

Conclusion

In summary, we have synthesized 18 closely related gemini. The structural features incorporated into these molecules comprise established lead optimizations including side-chain unsaturations, geminal trifluoromethyl and trideuteromethyl functions, A-ring variations and duality due to C20 diastereotopicity. The resulting plexus affords an insight into the diverse structural requirements for specific biological activities. Compounds related to 1-fluoro-1desoxy-calcitriol were less active than those containing the A-ring of calcitriol or 19-norcalcitriol based on all parameters evaluated. As judged on the basis of IC₅₀ values in MCF10CA1a human breast cancer cells, compound containing the 23-yne and 23(E)-ene sidechains were more potent than those featuring the Z-alkene chains. Members of the 23-yne category showed little differences between the C20 epimers; members of the 23(E)-ene category, however, were generally more active in the 20S-series while those in the 23(Z)-ene category were more active in the 20R-series. This preference of the 20S configuration in the 23(E)-ene category is reflected in the extraordinary activity of 3S-2 where the E-alkene chain gives rise to a compound that surpasses the alkyne counterpart. The activity profile associated with the nature of the A-rings was generally upheld in the study of induction potentials of monocyte cell-surface markers CD11b and CD14. The C20 epimeric effect was only discernible among members containing the 23(Z) side chain where the 20(R) epimers were profoundly more active. Most significantly; however, the 20S epimers were more active than the 20R counterparts in the colon cancer models while the C20 epimeric effect among members containing the 23-vne side chain was unpronounced in the present study of MCF10CA1a cell proliferation. Guided by the notion that this C20 epimeric effect was minimally expressed in gemini containing the 23-yne side chain, we focused our attention on a member of that category with the hope to select a compound with a broader spectrum of anticancer activities.

Experimental Section

 1 H-, 13 C-, and 19 F-NMR spectra were recorded at on a Varian 400 MHz spectrometer. The relatively long relaxation times of 13 C at the trideuteromethyl positions and the multiplicity due to their spin coupling to deuterium renders these 13 C resonances difficult to observe. Mass spectra were recorded on Waters ZQ (low resolution) and Brucker APEX II spectrometers (high resolution). Crystallographic data were obtained on a Brucker-Nonius Kappa CCD diffractometer. TLC was performed on silica gel GF $_{254}$ plates (Merck), flash chromatography employed 40-65 μ silica gel and for preparative column chromatography 15-20 μ silica gel was used. The trimethylsilyl groups at the gem-trifluoromethyl carbinol position in the ketones 12, 14, and 16 are unstable and cause small losses of the TMS groups during purification. Further losses of the TMS groups occur during the quench of the coupling reactions with the A-rings, and after extractive and chromatographic work-up these losses are almost complete

so that the TBAF treatments commence with partially deprotected products. Progress of deprotection of the final condensation products was monitored by TLC using 1:19 and 1:9 EtOAc - hexane; 1:1 EtOAc - hexane and EtOAc were the mobile phases for the detection of the final materials. Detection was by UV light and/or phosphomolybdic acid spray. Preparative HPLC was performed on a $2\times18"$ 15-20 μ silica YMC column using a flow rate of 100 mL/min and RI-detection. All final materials were of at least 95% purity; the major contaminants of non-crystalline materials were limited to solvent traces. Elemental analyses of the final materials are supplied in the Supporting Information. Solutions were dried with sodium sulfate unless specified otherwise.

6-[(1R,3aR,4S,7aR)-4-(tert-Butyl-dimethyl-silanyloxy)-7a-methyl-octahydroinden-1-yl]-1,1,1-trideutero-2-trideuteromethyl-hept-6-en-2-ol (6)—A solution of ester 5 (18.92 g, 47.94 mmol) in ether (330 mL) was cooled in an ice-bath and a 1 M solution of deuteromethylmagnesium iodide in ether (130 mL) was added dropwise. Stirring was continued at ambient temperature for 2 h, the soln was cooled again in an ice bath and a satd ammonium chloride solution (100 mL) was added dropwise followed by water (150 mL) to dissolve the white precipitate present. The aqueous layer was re-extracted with ether (30 mL) and the combined ether layers were washed with 1:4 satd ammonium chloride solution - water (100 mL) then dried (MgSO₄), filtered and evaporated. The residue was flashchromatographed on silica gel using hexane → 1:19 EtOAc - hexane as mobile phase to afford 6 as a colorless oil (19.2 g, 100 %); TLC (1:3 EtOAc - hexane) $R_f 0.58$; $[\alpha]_D^{28} + 22.1$ (c 0.4, methanol); ¹H NMR (400 MHz, CDCl₃) δ 0.01 (br s, 3 H), 0.02 (br s, 3 H), 0.80 (s, 3 H), 0.89 (s, 9 H), 1.17 (td, J=13.5, 3.4 Hz, 1 H), 1.32 - 1.87 (m, 15 H), 1.97 - 2.09 (m, 3 H), 3.99 - 4.05 (m, 1 H), 4.78 (s, 1 H), 4.89 (s, 1 H); LRMS-EI(+) m?z 400 (12, M+), 382 (80, M-H₂O), 382 (42, M-H₂O-CH₃); HRMS-ES(+) calcd for C₂₄H₄₀D₆O₂Si (M+H)1⁺: 401.3717, found 401.3713; calcd for $C_{24}H_{40}D_6O_2Si$ (M+Na)1+ 423.3536, found 423.3535.

(E)-3-[(1R,3aR,4S,7aR)-4-(tert-Butyldimethyl-silanyloxy)-7a-methyl-octahydroinden-1-yl]-8,8,8-trideutero-7-trideuteromethyl-oct-3-ene-1,7-diol (7E)—A mixture of 6 (19.2 g, 48 mmol), paraformaldehyde (1.72 g, 57 mmol) and DCM (160 mL) was stirred under nitrogen for 10 min, cooled to -20 \square , and a 1 M dimethylaluminum chloride solution in hexane (122 mL) was added dropwise within 15 min. The mixture was the stirred in an ice bath for 1 h to complete the reaction (TLC, 1:1 EtOAc - hexane), cooled again to -20 □, water (125 mL) was added dropwise causing the temperature to rise to 10 °C, then transferred to a separatory funnel with ether washes $(2\times30 \text{ mL})$. The milky soln was allowed to separate; the aqueous layer was extracted with ether (3×100 mL), the combined ether layers were washed with dilute pH 7 phosphate buffer (50 mL), dried and evaporated to an oily residue. This material was chromatographed on a silica gel column using $1:9 \rightarrow 1:4 \rightarrow 1:1$ EtOAc - hexane to yield 7E as major geometric isomer that was further purified by fractional crystallization (13.34 g). A second chromatography of the mixture fractions and the mother liquors gave an additional 1.67 g of crystalline product (total yield 72.6%). The E-geometry for **7E** was assigned by default based on the ¹H NMR NOE difference observed for the **7Z** isomer; TLC (1:1 EtOAc - hexane) Rf 0.27; $[\alpha]_D^{30}$ +14.4 (c 0.5, methanol); ¹H NMR (400 MHz, CDCl₃) δ 0.01 (s, 3 H), 0.02 (s, 3 H), 0.79 (s, 3 H), 0.89 (s, 9 H), 1.12 (td, J=12.6, 3.3 Hz, 1 H), 1.31 -1.49 (m, 6 H), 1.53 (t, J=8.1 Hz, 2 H), 1.58 - 1.84 (m, 6 H), 2.03 (t, J=9.2 Hz, 1 H), 2.11 - 2.28 (m, 3 H), 2.54 (dt, J=13.3, 7.5 Hz, 1 H), 3.61 (t, J=7.0 Hz, 2 H), 3.98 - 4.06 (m, 1 H), 5.37 (t, *J*=7.0 Hz, 1 H); LRMS-EI(+) *m*?*z* 430 (5, M+), 412 (62, M-H₂O), 394 (78, M-H₂O-CD₃); HRMS-ES(+) calcd for C₂₅H₄₂D₆O₃Si: 431.3822 (M+H), found 431.3818; Anal. Calcd for C₂₅H₄₂D₆O₃Si: C, 69.71; found C, 69.70.

(Z)-3-[(1R,3aR,4S,7aR)-4-(*tert*-Butyldimethyl-silanyloxy)-7a-methyl-octahydro-inden-1-yl]-8,8,8-trideutero-7-trideuteromethyl-oct-3-ene-1,7-diol (7Z)—The

chromatographic band preceeding the major one, as described above, was rechromatographed to give the Z-isomer (1.2 g); TLC (1:1 EtOAc - hexane) $R_f\,0.36;\, [\alpha]_D^{24}$ -43.6 (c 0.3, methanol); 1H NMR (300 MHz, CDCl_3) $\delta\,0.00$ (s, 3 H), 0.01 (s, 3 H), 0.86 (s, 3 H), 0.88 (s, 9 H), 1.09 (td, J=12.6, 3.7 Hz, 1 H), 1.29 - 1.86 (m, 14 H), 1.98 - 2.43 (m, 4 H), 2.53 (t, J=9.0 Hz, 1 H), 3.40 - 3.53 (m, 1 H), 3.57 - 3.70 (m, 1 H), 3.99 - 4.08 (m, 1 H), 5.35 (dd, J=8.2, 5.7 Hz, 1 H); LRMS-EI(+) $m/z\,$ 430 (9, M+), 412 (92, M-H_2O); 13 C NMR (300 MHz, CDCl_3) $\delta\,$ 0.01, 0.38, 21.01, 22.76, 23.17, 28.71, 28.80, 28.93, 30.96, 39.75, 43.28, 44.37, 49.00, 51.36, 56.66, 57.50, 67.81, 74.38, 75.82, 81.72, 82.14, 82.58, 136.31, 139.81; HRMS-ES(+) calcd for $C_{25}H_{42}D_6O_3Si:\,$ 453.3641(M+Na)1+, found 453.3640. Anal. Calcd for $C_{25}H_{42}D_6O_3Si:\,$ C, 69.71; found C, 69.64.

(S)-3-[(1R,3aR,4S,7aR)-4-(tert-Butyl-dimethyl-silanyloxy)-7a-methyl-octahydroinden-1-yl]-8,8,8-trideutero-7-trideuteromethyl-octane-1,7-diol (8S)-**047-094-4-5** A mixture of **7E** and **7Z** (16.59 g, 38.5 mmol), 5% PtO₂-C (2.2 g) and EtOAc (250 mL) was hydrogenated at hydrostatic pressure (500 mm) overnight; the catalyst was filtered off (Celite), washed with EtOAc and the filtrate and washes evaporated to a thick syrupy material (16.34 g) then pre-purified on a silica gel column (50×130 mm) using $1:4 \rightarrow 1:1$ EtOAc - hexane. The resulting pre-purified epimeric mixture (14.6 g) was resolved by preparative HPLC using 2:1 EtOAc - hexane as mobile phase and operating at 100 mL/min in 9 consecutive runs. The peak with the elution maximum of 3.4 L was evaporated to give **8S** as a sticky syrup (total quantity of 6.45 g). TLC (1:1 EtOAc - hexane) $R_f 0.28$; $[\alpha]_D^{32} + 34.8$ (c 0.83, MeOH); ¹H NMR (400 MHz, CDCl₃) δ -0.03 (s, 3 H), -0.02 (s, 3 H), 0.86 (s, 9 H), 0.89 (s, 3 H), 1.00 - 1.85 (m, 22 H), 1.93 (d, J=12.1 Hz, 1 H), 3.53 - 3.67 (m, 2 H), 3.94 - 4.02 (m, 1 H); ¹³C NMR (400 MHz, CDCl₃) δ -5.21, -4.83, 13.87, 17.62, 17.96, 19.74, 22.82, 25.75, 26.57, 31.63, 34.09, 34.39, 35.98, 40.44, 42.15, 44.21, 52.98, 53.45, 60.44, 69.32, 70.67; LRMS-EI(+) m/z 414 (18, M-H₂O)⁺, 396 (21, M-H₂O-CD₃); HRMS-ES(+) calcd for C₂₅H₄₄D₆O₃Si: 455.3798 (M+Na)1⁺, found 455.3795

(*R*)-3-[(1*R*,3a*R*,4*S*,7a*R*)-4-(tert-Butyl-dimethyl-silanyloxy)-7a-methyl-octahydro-inden-1-yl]-8,8,8-trideutero-7-trideuteromethyl-octane-1,7-diol (8*R*)—The peak of the chromatograms described above, with the elution maximum of 4.2 L, was also evaporated to give 8*R* (total quantity of 6.24 g); TLC (1:1 EtOAc - hexane) R_f 0.25; $[\alpha]_D^{30}$ +34.1 (c 0.57, MeOH); 1 H NMR (400 MHz, CDCl₃) δ -0.01 (s, 3 H), 0.00 (s, 3 H), 0.88 (s, 9 H), 0.91 (s, 3 H), 1.13 (td, J=12.8, 3.4 Hz, 1 H), 1.20 - 1.62 (m, 17 H), 1.62 - 1.72 (m, 2 H), 1.72 - 1.84 (m, 2 H), 1.84 - 1.91 (m, 1 H), 3.56 - 3.71 (m, 2 H), 3.99 (br s, 1 H); LRMS-ES(+) m/z 415 (100, M-17)+, HRMS-ES(+) calcd for $C_{25}H_{44}D_6O_3Si$: 455.3798 [M+Na]1+, found 455.3797.

(S)-3-[(1*R*,3a*R*,4S,7a*R*)-4-(tert-Butyl-dimethyl-silanyloxy)-7a-methyl-octahydro-inden-1-yl]-8,8,8-trideutero-7-hydroxy-7-trideuteromethyl-octanal (9*S*)—A

mixture of **8***S* (1.13 g, 2.61 mmol), DCM (20 mL), TEMPO (84 mg), and tetrabutylammonium chloride hydrate (146 mg), dissolved in 0.5 M potassium hydrogen carbonate soln, was stirred at ambient temperature and chloramine-T (1.46 g) was added and vigorous stirring continued for 1.5 h. Production of the aldehyde was followed by TLC (1:1 EtOAc - hexane, R_f 0.84). The mixture was diluted with DCM (50 mL); the organic layer was washed with water (25 mL), dried (sodium sulfate) and evaporated. The resulting residue was triturated with hexane and the suspension filtered onto a silica gel column. Elution with 1:39 \rightarrow 1:9 \rightarrow 1:4 EtOAc - hexane furnished pure **9***S* (1.05 g, 93.4 %); ¹H NMR (400 MHz, CDCl₃) δ 0.00 (s, 3 H), 0.01 (s, 3 H), 0.89 (s, 9 H), 0.95 (s, 3 H), 1.08 - 1.19 (m, 1 H), 1.19 - 1.47 (m, 13 H), 1.51 - 1.61 (m, 1 H), 1.62 - 1.72 (m, 1 H), 1.72 - 1.87 (m, 3 H), 1.91 - 2.04 (m, 1 H), 2.42 (ddd, *J*=16.7, 7.7, 3.0 Hz, 1 H), 2.59 (ddd, *J*=16.7, 4.3, 1.8 Hz, 1 H), 3.98 - 4.02 (m, 1 H), 9.78 (dd, *J*=3.0, 1.8 Hz, 1 H); LRMS-EI(+) *m/z* 355 (73, M-H₂O-C₄H₉); 354 (100, M-HOD-C₄H₉); HRMS-ES(+) calcd for C₂₅H₄₂D₆O₃Si: 453.3641 (M+Na)1⁺, found 453.3649.

(R)-3-[(1R,3aR,4S,7aR)-4-(tert-Butyl-dimethyl-silanyloxy)-7a-methyl-octahydroinden-1-yl]-8,8,8-trideutero-7-hydroxy-7-trideuteromethyl-octanal (9R)—To a soln of diol 8R (6.24 g, 14.4 mmol) in DCM (110 mL) was added 0.5 M aqueous potassium hydrogen carbonate soln containing tetrabutylammonium chloride hydrate and TEMPO (0.44 g, 2.8 mmol). The 2-phase system was stirred vigorously and chloramine-T (8.0 g, 28.6 mmol) was added. The conversion to the aldehyde 9R was complete after one has monitored by TLC (1:1 EtOAc - hexane, R_f 0.84). The mixture was diluted with DCM (100 mL) and the organic layer washed with water (50 mL), dried and evaporated. The resulting residue was triturated with hexane; the resulting suspension filtered onto a silica gel column then eluted with 1:39 and 1:4 EtOAc - hexane. The fractions containing 9R were pooled, evaporated, and taken up in methanol (55 mL) for the next step; ${}^{1}H$ NMR (400 MHz, CDCl₃) δ -0.01 (s, 3 H), 0.01 (s, 3 H), 0.88 (s, 9 H), 0.95 (s, 3 H), 1.15 (td, J=12.7, 3.1 Hz, 1 H), 1.21 - 1.45 (m, 12 H), 1.45 -1.85 (m, 5 H), 1.90 (ddd, J=12.3, 3.3, 3.1 Hz, 1 H), 2.06 (br s, 1 H), 2.31 (ddd, J=16.3, 7.5, 3.0 Hz, 1 H), 2.44 (ddd, J=16.3, 4.5, 1.8 Hz, 1 H), 3.97 - 4.02 (m, 1 H), 9.78 (dd, J=3.0, 1.8 Hz, 1 H); LRMS-EI(+) m/z 430 (7, M⁺), 412 (30, M-CD₃), 394 (79, M-CD₃-H₂O), 393 (95, M-CD₃-H CD₃-HOD); HRMS-ES(+) calcd for C₂₅H₄₂D₆O₃Si: 431.3822 (M+H)1, found 431.2916.

(S)-6-[(1R,3aR,4S,7aR)-4-(tert-Butyl-dimethyl-silanyloxy)-7a-methyl-octahydroinden-1-vI]-1,1,1-trideutero-2-trideuteromethyl-non-8-yn-2-ol (10S-a)—A stirred soln of 9S (1.35 g, 3.13 mmol), methanol (20 mL), and dimethyl diazomethylphosphonate (0.725 g, 4.83 mmol) was cooled in an ice bath and powdered potassium carbonate (0.705 mg, 5.1 mmol) was added. The mixture was stirred in the ice bath for 30 min then at room temperature for 6 h. Reaction progress was monitored by TLC (1:4 EtOAc - hexane, Rf 0.19 \rightarrow 0.42). The mixture was equilibrated with hexane (80 mL) and water (40 mL), the aqueous layer was re-extracted with hexane (20 mL) and the combined extracts were washed with water (30 mL) and brine (10 mL), dried and evaporated. The resulting oily residue was chromatographed on a silica gel column using hexane \rightarrow 1:19 \rightarrow 1:9 EtOAc - hexane to lead to **10S-a** (1.14 g, 85%) as a colorless oil; $[\alpha]_D^{29} + 33.3^{\circ}$ (c 0.49, methanol); ¹H NMR (300 MHz, CDCl₃) δ 0.00 (s, 3 H), 0.02 (s, 3 H), 0.89 (s, 9 H), 0.91 (br s, 3 H), 1.15 - 1.90 (m, 20 H), 1.93 (t, J=2.2 Hz, 1 H), 2.22 - 2.46 (m, 2 H), 4.00 (br s, 1 H); 13 C NMR (300 MHz, CDCl₃) δ -5.18, -4.80, 13.83, 17.63, 17.99, 20.57, 20.78, 22.84, 25.76, 26.83, 31.83, 34.08, 34.32, 37.92, 40.13, 41.94, 44.06, 52.57, 52.85, 69.33, 69.63, 70.67, 82.88; LRMS-EI(+) m/z 351 (45, M-H₂O-C₄H₉); 295 (4, M-C₄H₉); HRMS-ES(+) calcd for C₂₆H₄₂D₆O₂Si: 449.3692 (M+Na)1+, found 449.3696.

(1*R*,3a*R*,4*S*,7a*R*)-4-(tert-Butyl-dimethyl-silanyloxy)-7a-methyl-1-((S)-6,6,6-trideutero-1-prop-2-ynyl-5-trideuteromethyl-5-trimethylsilanyloxy-hexyl)-octahydro-indene (10*S*-b)—To a soln of 10*S*-a (1.25 g, 2.93 mmol) in cyclohexane (20 mL) was added TMS-imidazole (2.0 mL, 13.6 mmol) and the mixture was allowed to stand overnight. The suspension was filtered and chromatographed on silica gel; the column was eluted with hexane \rightarrow 1:100 \rightarrow 1: 79 EtOAc - hexane to furnish 10*R*-a; 1.28 g, 87.6%; TLC (1:19, EtOAc - hexane) R_f 0.68; ¹H NMR (300 MHz, CDCl₃) δ 0.00 (s, 3 H), 0.01 (s, 3 H), 0.11 (s, 9 H), 0.89 (s, 9 H), 0.91 (s, 3 H), 1.11 - 1.95 (m, 20 H), 2.28 - 2.41 (m, 2 H), 4.00 (br s, 1 H); ¹³C NMR (300 MHz, CDCl₃) δ -5.14, -4.76, 2.65, 13.89, 17.69, 18.04, 20.58, 20.86, 22.91, 25.82, 26.86, 31.84, 34.39, 37.95, 40.17, 42.00, 44.87, 52.68, 52.91, 69.42, 69.54, 73.71, 83.02; LRMS-EI(+) m/z 367 (22, M-OTBS), 351 (28, M-C₄H₉-TMSOH); HRMS-EI(+) calcd for C₂₉H₅₀D₆O₃Si₂: 498.4189 (M⁺); found 498.4203, calcd for C₂₉H₅₀D₆O₃Si₂: 480.3772 (M-CD₃)⁺, found 480.3788.

(*R*)-6-[(1R,3aR,4S,7aR)-4-(tert-Butyl-dimethyl-silanyloxy)-7a-methyl-octahydro-inden-1-yl]-1,1,1-trideutero-2-trideuteromethyl-non-8-yn-2-ol (10R-a)—The stirred methanolic soln of 9R obtained as described above was cooled in an ice bath and

dimethyl diazomethylphosphonate (3.24 g, 21.6 mmol) was added followed by powdered potassium carbonate (3.0 g, 21.6 mmol). The cooling bath was removed after 1 h and stirring continued for an additional 5 h. Reaction progress was monitored by TLC (1:1 EtOAc - hexane, $R_f\,0.72\to0.86$). The mixture was equilibrated with hexane (300 mL) and water (100 mL), the aqueous layer re-extracted with hexane (2×50 mL) and all extracts were evaporated. For analysis, a small portion was dissolved in hexane and chromatographed using hexane \to 1:19 \to 1: 9 EtOAc - hexane to furnish pure **10R-a**; 1H NMR (400 MHz, CDCl₃) δ 0.00 (s, 3 H), 0.01 (s, 3 H), 0.89 (s, 9 H), 0.92 (s, 3 H), 1.13 - 1.77 (m, 17 H), 1.77 - 1.95 (m, 4 H), 2.10 - 2.18 (m, 1 H), 2.30 - 2.37 (m, 1 H), 3.95 - 4.06 (m, 1 H); LRMS-EI(+) m/z 408 (100, M+H-H₂O); HRMS-ES(+) calcd for $C_{26}H_{42}D_{6}O_{2}Si$: 449.3692 (M+Na)1+, found 449.3692. The material was used without further purification in the next step.

(1*R*,3a*R*,4*S*,7a*R*)-4-(tert-Butyl-dimethyl-silanyloxy)-7a-methyl-1-((*R*)-6,6,6-trideutero-1-prop-2-ynyl-5-trideuteromethyl-5-trimethylsilanyloxy-hexyl)-octahydro-indene (10*R*-b)—The bulk of 10*R*-a, together with the purified material described above, was dissolved in cyclohexane (80 mL) and TMS-imidazole (8 mL). After 4 h the suspension was filtered onto a silica gel column, together with hexane washes, and the column was eluted with hexane \rightarrow 1:79 EtOAc - hexane to give 10*R*-b as a colorless oil (5.41 g, 75.3% from diol 8*R*); ¹H NMR (400 MHz, CDCl₃) δ -0.01 (s, 3 H), 0.00 (s, 3 H), 0.10 (s, 9 H), 0.88 (s, 9 H), 0.91 (s, 3 H), 1.12 - 1.45 (m, 11 H), 1.46 - 1.62 (m, 4 H), 1.62 - 1.72 (m, 1 H), 1.72 - 1.86 (m, 2 H), 1.88 (t, J=2.5 Hz, 1 H), 1.92 (dt, J=12.3, 2.8 Hz, 1 H), 2.12 (ddd, J=16.9, 5.3, 2.6 Hz, 1 H), 2.32 (dt, J=16.9, 3.0 Hz, 1 H), 3.93 - 4.03 (m, 1 H); ¹³C NMR (300 MHz, CDCl₃) δ -5.10, -4.73, 2.65, 14.05, 17.75, 18.07, 20.74, 22.95, 25.86, 27.04, 31.52, 34.50, 38.63, 40.63, 42.11, 44.96, 53.03, 53.12, 68.93, 69.52, 73.72, 83.02; LRMS-ES(+) *m/z* 409 (35, M-TMSO), 295 (100, M-TMSO-TBS+H); HRMS-ES(+) calcd for C₂₉H₅₀D₆O₃Si₂: 521.4087 (M+Na)1⁺, found 521.4088.

(*S*)-6-[(1*R*,3a*R*,4*S*,7a*R*)-4-(tert-Butyl-dimethyl-silanyloxy)-7a-methyl-octahydro-inden-1-yl]-11,11,11-trideutero-1,1,1-trifluoro-10-trideuteromethyl-2-trifluoromethyl-10-trimethylsilyloxy-undec-3-yn-2-ol (11S-a)—The condensation of 10S-b (1.034 g, 2.07 mmol) with hexafluoroacetone was repeated as described below for the synthesis of 11R-a. The crude product was purified by chromatography with hexane → 1:200 → 1:100 → 1:79 EtOAc - hexane as mobile phases to give 11S-a as a colorless oil, 1.276 g, 92.7%, 1 H NMR (400 MHz, CDCl₃) δ -0.007 (s, 3 H), -0.009 (s, 3 H), 0.11 (s, 9 H), 0.88 (s, 9 H), 0.91 (s, 3 H), 1.15 - 1.50 (m, 14 H), 1.63 - 1.89 (m, 4 H), 2.41 (dd, J=17.4, 5.3 Hz, 1 H), 2.49 (m, J=17.4, 4.0 Hz, 1 H), 3.49 (br s, 1 H), 3.96 - 4.03 (m, 1 H); 13 C NMR (300 MHz, CDCl₃) δ -5.15, -4.77, 2.58, 13.87, 14.14, 17.68, 18.04, 20.60, 20.89, 22.87, 25.81, 27.00, 31.62, 32.11, 34.30, 37.92, 40.18, 41.97, 44.66, 52.88, 53.09, 69.32, 70.47, 71.24, 73.95, 91.11, 121.13; LRMS-EI(+) m/z 664 (3, M+), 646 (48, M-H₂O), 607 (29, M-C₄H₉); HRMS-ES(+) calcd for C₃₂H₅₀D₆O₃Si₂F6: 687.3941 (M+Na)1+, found 687.3937.

(S)-11,11,11-Trideutero-1,1,1-trifluoro-6-((1R,3aR,4S,7aR)-4-hydroxy-7a-methyl-octahydro-inden-1-yl]-10-trideuteromethyl-2-trifluoromethyl-undec-3-yne-2,10-diol (11S-b)—A stirred soln of 11S-a (0.406 g, 0.61 mmol) in acetonitrile (6 mL) was cooled in an ice bath and 25% aqueous fluorosilicic acid was added. Stirring was continued at ambient temperature for 4h. The mixture was equilibrated with EtOAc (40 mL) and water (15 mL), the organic layer was washed with water (2×15 m) and 2:1 brine - satd sodium hydrogen carbonate soln (10 mL) then dried and evaporated. The residue was chromatographed using 1:4 \rightarrow 1:2 EtOAc - hexane as mobile phases to afford 11S-b as a syrup that crystallized when treated with DCM - hexane. This material was recrystallized from the same solvent; TLC (1:19 methanol - DCM) R_f 0.25; (1:1 EtOAc - hexane) R_f 0.68; mp 132-3 °C; $[\alpha]_D$ ²⁵ +15.6 (c 0.72, MeOH); ¹H NMR (400 MHz, CDCl₃) δ 0.94 (s, 3 H), 1.09 - 1.55 (m, 16 H), 1.60 - 1.71

(m, 1 H), 1.74 - 1.96 (m, 4 H), 2.32 (dd, J=17.4, 7.0 Hz, 1 H), 2.55 (dd, J=17.4, 4.3 Hz, 1 H), 4.08 (br s, 1 H), 5.60 (s, 1 H); 13 C NMR (400 MHz, CDCl₃) δ 13.60, 17.31, 20.07, 21.42, 22.26, 26.95, 32.15, 33.17, 38.46, 40.06, 41.77, 43.15, 52.45, 53.06, 69.28, 69.50, 70.61, 71.03, 71.91, 89.95, 121.41; LRMS-ES(+) m/z 478 (15, M+), 477 (100 M-H); HRMS-ES(+) calcd for C₂₃H₂₈D₆O₃F6: 501.2681 (M+Na)1⁺, found 501.2680; Anal. Calcd for C₂₃H₂₈D₆F₆O₃: C, 57.73; found 57.87; Roentgen diffraction analysis (Figure 1): C₂₃H₃₄F₆O₃, orthorhombic, space group P2₁2₁2₁ with unit cell dimensions of a = 6.8335(14), b = 18.386(4), c = 18.871 (4) Å, volume 2371.0(8) Å³ (Z = 4)

(R)-6-[(1R,3aR,4S,7aR)-4-(tert-Butyl-dimethyl-silanyloxy)-7a-methyl-octahydroinden-1-yl]-11,11,11-trideutero-1,1,1-trifluoro-10-trideuteromethyl-2trifluoromethyl-10-trimethylsilyloxy-undec-3-yn-2-ol (11 R-a)—A 3-neck flask equipped with a magnetic stirrer, an addition funnel in the center surrounded by a dry-ice bath and topped by a dry-ice condenser with a hexafluoroacetone inlet, a Claisen adapter with nitrogen sweep connected to the exhaust slot of the hood and a rubber septum, was charged with 10R-b (5.41 g, 10.8 mmol) and THF (31.13 g). The soln was immersed into a dry-ice/ acetone bath and a 1.6 M butyllithium soln in hexane (9 mL) was added dropwise over an 8min period. Immediately after the addition the dry-ice condenser and the dry-ice bath surrounding the addition funnel were filled dry-ice and, after stirring for 5 min, hexafluoroacetone was condensed in. The condensate, ca. 1 mL, was then added to the reactor. The starting material was no longer detectable after 1.5 h (TLC, 1:19 EtOAc - hexane). The system was flushed with nitrogen then pH 7 phosphate buffer (20 mL) was added, the stirred mixture allowed to reach room temperature, further diluted with hexane (50 mL) and transferred to a separatory funnel with a hexane rinse (25 mL). The organic layer was washed with brine (15 mL), dried, and evaporated. From this oily residue most of a self-condensation product derived from hexafluoroacetone could be removed by high-vacuum distillation at a bath temperature of 30-40 \square . For analysis, a small aliquot was purified on a silica gel column using hexane \rightarrow 1:200 \rightarrow 1:100 \rightarrow 1:79 EtOAc - hexane, to furnish pure **11***R***-a**, TLC (cyclohexane, $R_f 0.14$); ¹H NMR (400 MHz, CDCl₃) δ -0.01 (s, 3 H), 0.00 (s, 3 H), 0.10 (s, 6 H), 0.88 (s, 9 H), 0.92 (s, 3 H), 1.13 (td, J=12.9, 3.0 Hz, 1 H), 1.18 - 1.46 (m, 15 H), 1.48 -1.72 (m, 8 H), 1.72 - 1.87 (m, 3 H), 1.91 (d, J = 12.1 Hz, 1 H), 2.22 (dd, J = 17.4, 6.0 Hz, 1 H),2.42 (dd, *J*=17.4, 3.7 Hz, 1 H), 3.33 (br s, 1 H), 3.96 - 4.03 (m, 1 H); LRMS (ES(-) *m/z* 628 $(38, M-H_2O-CD_3)$; HRMS-ES(+) calcd for $C_{32}H_{50}D_6F_6O_3Si>_2$: $687.3941 (M+Na)1^+$, found 687.3928.

(R)-11,11,11-Trideutero-1,1,1-trifluoro-6-((1R,3aR,4S,7aR)-4-hydroxy-7amethyl-octahydro-inden-1-yl]-10-trideuteromethyl-2-trifluoromethyl-undec-3yne-2,10-diol (11R-b)—The bulk of the crude 11R-a, together with the purified material described above, was dissolved in acetonitrile (100 mL), cooled in an ice bath and a 25% aqueous fluorosilicic acid solution (24 mL) was added. The mixture was stirred in the ice bath for 5 min then at room temperature for 5 h. The mixture was equilibrated with EtOAc (200 mL) and water (50 mL), the organic phase was washed with water (2×50 mL) then 2:1 brine - satd sodium hydrogen carbonate (50 mL), dried and evaporated. This material was charged to a silica gel column and eluted with $1:4 \rightarrow 1:2$ EtOAc - hexane to yield 11R-b as a colorless foam, 5.1 g, 98.6% from the acetylene **10***R***-b**; TLC (1:1, EtOAc - hexane, R_f 0.43); $[\alpha]_D^{26}$ +6.1 (c 0.70, MeOH); ¹H NMR (400 MHz, CDCl₃) δ 0.92 (s, 3 H), 1.09 - 1.70 (m, 13 H), 1.70 -1.89 (m, 3 H), 1.93 (d, J=12.7 Hz, 1 H), 2.18 (dd, J=17.2, 6.3 Hz, 1 H), 2.41 (dd, J=17.2, 3.3 Hz, 1 H), 3.38 (br s, 3 H), 4.08 (br s, 1 H); ¹³C NMR (400 MHz, CDCl₃) δ 13.60, 17.31, 20.07, 21.42, 22.26, 26.95, 32.15, 33.17, 38.46, 40.06, 41.77, 43.15, 52.45, 53.06, 69.28, 69.50, 70.61,71.03, 71.91, 89.95, 121.41; 19 F NMR (376.31 MHz, CDCl₃) δ -78.12 (br s); HRMS-EI(+) calcd for $C_{23}H_{28}D_6O_3F_6$: 501.2681 (M+Na)1+; found 501.2679.

(1*R*,3a*R*,7a*R*)-7a-Methyl-1-[(*S*)-6,6-trifluoro-5-hydroxy-1-(5,5,5-trideutero-4-hydroxy-4-trideuteromethyl-pentyl)-5-trifluoromethyl-hex-3ynyl]-octahydro-inden-4-one (12S-a)—To a stirred soln of 11S-b (0.239 mg, 0.500 mmol) in DCM (6.5 g) was added, in succession, Celite (0.43 g) and PDC (0.83 g, 2.20 mmol). The suspension was diluted with ether (15 mL) after 7 h, stirred for 5 min and applied to a silica gel plug (2.5 g). The eluate and ether washes were combined and evaporated to a colorless oil which was allowed to crystallize. Recrystallization from DCM - hexane gave pure 12S-a, 0.23 g, 96.5 %; mp 176-177 \Box ; [α] $_D^{26}$ - 7.7 (c 0.44, MeOH); 1 H NMR (300 MHz, CDCl₃) δ 0.64 (s, 3 H), 1.58 (s, 13 H), 1.83 - 2.11 (m, 4 H), 2.15 - 2.33 (m, 2 H), 2.39 (dd, *J*=17.6, 5.9 Hz, 1 H), 2.48 (dd, *J*=11.4, 7.7 Hz, 1 H), 2.57 (dd, *J*=17.6, 3.7 Hz, 1 H), 5.67 (br s, 1 H); 19 F NMR (376.31 MHz, CDCl₃) δ -78.28 br s; LRMS(EI)(+) m/z 476 (7, M+); HRMS-EI calcd for C₂₃H₂₆D₆O₃F₆: 499.2524 (M+Na)1⁺, found 499.2527. Anal. Calcd for C₂₃H₂₆D₆O₃F₆: C, 57.97; found C, 57.92.

(1R,3aR,7aR)-7a-Methyl-1-[(S)-6,6,6-trifluoro-1-(5,5,5-trideutero-4-trideuteromethyl-4-trimethylsilanyloxy-pentyl)-5-trifluoromethyl-5-trimethylsilanyloxy-hex-3-ynyl]-octahydro-inden-4-one (12S-b)—To a suspension of the ketone 12S-a (0.22 g) in ether (3 mL) was added TMS-imidazole (0.3 mL) whereupon a soln was obtained. TLC (1:19 methanol - DCM and 1:39 EtOAc - hexane) confirmed the absence of educt and the conversion to 12S-b after 1.5 h. The resulting suspension was filtered onto a flash column which was then eluted with hexane \rightarrow 1:39 EtOAc - hexane eluting 12S-b (0.229 g, 80%); 1 H NMR (300 MHz, CDCl₃) δ 0.11 (s, 9 H), 0.29 (s, 9 H), 0.65 (s, 3 H), 1.05 - 1.48 (m, 8 H), 1.63 - 2.14 (m, 8 H), 2.14 - 2.40 (m, 2 H), 2.51 (br s, 3 H); LRMS(EI)(+) m/z 602 (68, M-CD₃); HRMS-EI(+) calcd for $C_{29}H_{42}D_6O_3Si_2F_6$: 643.3315 (M+Na)1+, found 643.3333. Continued elution with 1:19 \rightarrow 1:4 EtOAc - hexane gave the corresponding mono-TMS ether (0.023 g, 9 %); 1 H NMR (300 MHz, CDCl₃) δ 0.12 (s, 9 H), 0.65 (s, 3 H), 1.58 (s, 14 H), 2.12 - 2.61 (m, 7 H), 3.57 (br s, 1 H).

(1*R*,3a*R*,7a*R*)-7a-Methyl-1-[(*R*)-6,6,6-trifluoro-5-hydroxy-1-(5,5,5-trideutero-4-hydroxy-4-trideuteromethyl-pentyl)-5-trifluoromethyl-hex-3-ynyl]-octahydro-inden-4-one (12*R*-a)—To a stirred soln of 11*R*-b (0.330 g, 0.69 mmol) in DCM (9 g) was added, in succession, Celite (06 g) and PDC (1.16 g, 3 mmol). The mixture was stirred for 14 h, diluted with ether (15 mL) stirred for 5 min and applied to a silica gel G column. Elution was completed with 1:9 EtOAc - DCM, the effluent containing the ketone was evaporated, the residue taken up in DCM, diluted with hexane and allowed to crystallize, 0.24 g (73 %); mp 156-7 \Box ; TLC (1:4, 2-PrOH - cyclohexane) R_f 0.80; mp 156-7 \Box ; [α]_D²³ -15.3 (c 0.43, MeOH); H NMR (400 MHz, CDCl₃) δ 0.65 (s, 3 H), 1.24 - 1.82 (m, 13 H), 1.82 - 1.98 (m, 2 H), 1.98 - 2.11 (m, 2 H), 2.17 - 2.35 (m, 3 H), 2.44 - 2.52 (m, 2 H), 5.81 (br s, 1 H); ¹³C NMR (400 MHz, CDCl₃) δ 12.71, 19.02, 20.06, 21.95, 24.02, 27.44, 32.91, 38.65, 38.81, 40.89, 42.67, 49.74, 53.53, 61.85, 70.79, 70.87, 71.26, 72.17, 89.99, 121.20, 211.47; ¹⁹F NMR (376.31 MHz, CDCl₃) δ -78.35 (br m); LRMS(ES)(-) m/z 475 (100, M-H); HRMS-ES(+) calcd for C₂₃H₂₆D₆O₃F₆ 499.2524 (M+Na)1+; found 499.2520. Anal. Calcd for C₂₃H₂₆D₆O₃F₆: C, 57.97; found C, 57.77.

(1R,3aR,7aR)-7a-Methyl-1-[(R)-6,6,6-trifluoro-1-(5,5,5-trideutero-4-trideuteromethyl-4-trimethylsilanyloxy-pentyl)-5-trifluoromethyl-5-trimethylsilanyloxy-hex-3-ynyl]-octahydro-inden-4-one (12R-b)—To a suspension of 12R-a (0.22 g, 0.46 mmol) in cyclohexane (3 mL) was added TMS-imidazole (0.30 mL, 2 mmol) to form a solution which was stored overnight and then filtered onto a silica gel column. Elution with hexane \rightarrow 1:79 \rightarrow 1:39 \rightarrow 1:19 EtOAc - hexane gave 12R-b as a colorless oil, 0.26 g (91 %), TLC (1:39 EtOAc - hexane) R_f 0.11; ¹H NMR (400 MHz, CDCl₃) δ 0.11 (s, 9 H), 0.29 (s, 9 H), 0.67 (s, 3 H), 1.17 - 1.50 (m, 6 H), 1.57 - 1.68 (m, 4 H), 1.71 - 1.84 (m, 2 H),

1.84 - 1.99 (m, 2 H), 1.99 - 2.14 (m, 2 H), 2.20 - 2.36 (m, 3 H), 2.43 - 2.53 (m, 2 H); ^{19}F NMR (376.31 MHz, CDCl₃) δ -8 2.41(s); LRMS(ES)(+) m/z 531 (100, M-OTMS); HRMS-EI(+) calcd for $C_{29}H_{42}D_6O_3Si_2F_6$: 643.3315 (M+Na)1⁺, found 643.3311.

(S)-11,11,11-Trideutereo-1,1,1-trifluoro-6-((1R,3aR,4S,7aR)-4-hydroxy-7amethyl-octahydro-indene-1-yl)-10-trideuteromethyl-2-trifluoromethyl-undec-3 (E)-ene-2,10 diol (13S)—A 3-neck flask equipped with a magnetic stirrer, an addition funnel, a Claisen adapter with nitrogen sweep and a rubber septum, was charged with a 3.5 M soln of sodium bis(2-methoxyethoxy)aluminum hydride in toluene and THF (50 mL) and cooled to -30 \(\pi\). To this soln was added a soln of triol **11S-b** (1.1 g, 2.3 mmol) in THF (45 mL) dropwise below $-20 \square$ and the mixture was allowed to warm to $0 \square$ within 25 min then maintained at that temperature for 3 h. The mixture was diluted with ether (50 mL), refrigerated overnight, filtered through Celite and the filtrate was evaporated. The residue was chromatographed on a silica gel column with $1:4 \rightarrow 1:3 \rightarrow 1:2$ EtOAc - hexane. The pooled product fractions were evaporated and the residue allowed to crystallize from DCM - hexane, to afford **13S**, 1.03 g (93 %); TLC (1:9 methanol - DCM) R_f 0.69; mp 141 \Box ; $[\alpha]_D^{30}$ +10.55 ° (c 0.44, methanol); H NMR (400 MHz, CDCl₃) δ 0.96 (s, 3 H), 1.10 - 1.64 (m, 16 H), 1.75 -1.89 (m, 3 H), 1.94 (d, *J*=12.8 Hz, 1 H), 2.07 - 2.18 (m, 1 H), 2.48 (dt, *J*=14.3, 4.7 Hz, 1 H), 4.03 - 4.10 (m, 1 H), 5.58 (d, *J*=15.6 Hz, 1 H), 6.28 (ddd, *J*=15.6, 7.6, 7.5 Hz, 1 H); ¹³C NMR $(400 \text{ MHz}, \text{CDCl}_3) \ \delta \ 13.66, \ 17.40, \ 19.77, \ 22.36, \ 26.85, \ 31.91, \ 33.51, \ 35.23, \ 38.91, \ 40.32,$ 41.89, 43.34, 52.53, 53.52, 69.29, 71.34, 76.13, 118.92, 122.29, 138.59; ¹⁹F-NMR (376.31 MHz) δF , q, ${}^{4}J_{F,F} = 8.78$ Hz, -77.80, 3F, q, ${}^{4}J_{F,F} = 9.40$ Hz; LRMS-ES(-) m/z 480 (20, M) 479 (39, M-H); HRMS-ES(+) found 503.2838 (M+Na)1⁺, calcd for C₂₃H₃₀D₆O₃F₆: 503.2837; Anal. Calcd for C₂₃H₃₀D₆O₃F₆: C, 57.48, found 57.38.

(*R*)-11,11,11-Trideutereo-1,1,1-trifluoro-6-((1*R*,3a*R*,4S,7a*R*)-4-hydroxy-7a-methyl-octahydro-indene-1-yl)-10-trideuteromethyl-2-trifluoromethyl-undec-3 (*E*)-ene-2,10 diol (13R)—The triol 13R was prepared as described for the isomer 13S and was obtained as a white powder (88.6 % yield); $[\alpha]_D^{28} + 17.3$ ° (c 0.22, methanol); H NMR (400 MHz, CDCl₃) δ 0.95 (s, 3 H), 1.13 - 1.66 (m, 17 H), 1.75 - 1.91 (m, 3 H), 1.90 - 2.09 (m, 2 H), 2.37 (dt, *J*=14.7, 5.1 Hz, 1 H), 3.79 (br s, 1 H), 4.05 - 4.10 (m, 1 H), 5.60 (d, *J*=15.6 Hz, 1 H), 6.29 (ddd, *J*=15.3, 8.8, 6.3 Hz, 1 H); 13 C NMR (400 MHz, CDCl₃) δ 13.78, 17.55, 19.82, 22.45, 27.11, 31.65, 33.54, 35.06, 39.24, 40.23, 42.03, 43.35, 52.58, 53.31, 69.35, 71.35, 76.08, 118.87, 122.23, 138.43; 19 F-NMR (376.31 MHz) δ -78.08, 3F, q, 4 J_{F,F} = 9.53 Hz, -77.87, 3F, q, 4 J_{F,F} = 9.28 Hz; LRMS-ES(+) *m/z* 463 (100, M-OH), 445 (25, M-OH-H₂O); HRMS-ES(+) found 503.2835 (M+Na)1+, calcd for C₂₃H₃₀D₆O₃F₆: 503.2837; Anal. Calcd for C₂₃H₃₀D₆O₃F₆: C, 57.48. Found 57.38.

(1R,3aR,7aR)-7a-Methyl-1-[(S)-6,6-6-trifluoro-5-hydroxy-1-(5,5,5-trideutero-4-hydroxy-4-trideuteromethyl-pentyl)-5-trifluoromethyl-hex-3-enyl]-octahydro-inden-4-one (14S-a)—The triol 13S was oxidized as described for the preparation of the alkyne analog 12S-a to obtain crystalline 14S-a from DCM - hexane (96 % yield); TLC (1:9 MeOH - DCM) Rf 0.75; $[\alpha]_D^{31}$ -9.5 ° 1 (c 0.45, methanol); H NMR (400 MHz, CDCl₃) δ 0.64 (s, 3 H), 1.11 - 1.44 (m, 7 H), 1.45 - 1.96 (m, 8 H), 1.97 - 2.08 (m, 2 H), 2.12 - 2.33 (m, 3 H), 2.45 (dd, J=11.4, 7.6 Hz, 2 H), 5.06 (br s, 1 H), 5.59 (d, J=15.4 Hz, 1 H), 6.30 (ddd, J=15.4, 7.6, 7.5 Hz, 1 H); 13 C NMR (400 MHz, CDCl₃) δ 12.56, 18.86, 19.69, 23.96, 26.94, 31.64, 34.94, 38.73, 38.97, 40.76, 43.47, 49.88, 53.37, 61.80, 71.33, 75.83, 119.50, 122.47, 137.71, 212.42; 19 F-NMR (376.31 MHz) δ -77.83, 3F, q, 4 J_{F,F} = 8.27 Hz, -77.73, 3F, q, 4 J_{F,F} = 7.53 Hz; LRMS-ES(-) m/z 523 (100, M+HCOO⁻), 477 (30, M-H); HRMS-ES(+) calcd for C₂₃H₂₈D₆O₃F₆: 501.2681 (M+Na)1⁺, found 501.2685; Anal. Calcd for C₂₃H₂₈D₆O₃F₆: C, 57.13; found C, 57.08.

(1R,3aR,7aR)-7a-Methyl-1-[(S)-6,6,6-trifluoro-1-(5,5,5-trideutero-4-trideuteromethyl-4-trimethylsilanyloxy-pentyl)-5-trifluoromethyl-5-trimethylsilanyloxy-hex-3(E)-enyl]-octahydro-inden-4-one (14S-b)—The ketonediol 14S-a was silylated as described for the preparation of the disilyl ether 12R-b to give 14S-b; 1 H NMR (400 MHz, CDCl₃) δ 0.09 (s, 9 H), 0.22 (s, 9 H), 0.67 (s, 3 H), 1.08 - 1.46 (m, 7 H), 1.46 - 2.10 (m, 9 H), 2.16 - 2.43 (m, 4 H), 2.46 (dd, J=11.3, 7.7 Hz, 1 H), 5.56 (d, J=15.4 Hz, 1 H), 6.15 (dt, J=15.4, 7.3 Hz, 1 H).

(1R,3aR,7aR)-7a-Methyl-1-[(R)-6,6,6-trifluoro-5-hydroxy-1-(5,5,5-trideutero-4-hydroxy-4-trideuteromethyl-pentyl)-5-trifluoromethyl-hex-3(E)-enyl]-octahydro-inden-4-one (14R-a)—To a stirred suspension of triol 13R (1.68 g), DCM (26 g) and Celite (1.6 g) was added PDC (2.60 g, 6.64 mmol) and the mixture stirred for 5.5 h, then diluted with ether (30 mL) and applied to a silica gel-G plug (10.9 g). The column was washed exhaustively with 1:9 EtOAc - ether; the fractions containing the ketone were evaporated and the residue crystallized from DCM-hexane; TLC (1:9 methanol - DCM) R_f 0.75, [α]_D³¹ -4.1 ° 1 (c 0.32, methanol); H NMR (400 MHz, CDCl₃) δ 0.65 (s, 3 H), 1.22 - 1.69 (m, 12 H), 1.69 - 1.81 (m, 1 H), 1.81 - 1.97 (m, 2 H), 1.97 - 2.12 (m, 3 H), 2.16 - 2.42 (m, 3 H), 2.46 (dd, J=11.5, 7.7 Hz, 1 H), 4.27 (br s, 1 H), 5.61 (d, J=15.4 Hz, 1 H), 6.29 (ddd, J=15.4, 8.7, 6.2 Hz, 1 H); ¹³C NMR (400 MHz, CDCl₃) δ 12.71, 19.00, 19.74, 24.08, 27.34, 31.75, 34.91, 38.77, 39.37, 40.90, 43.30, 49.93, 53.35, 61.86, 71.24, 75.80, 119.19, 122.24, 137.92, 211.76; ¹⁹F-NMR (376.31 MHz) δ -77.84, 3F, q, ⁴J_{F,F} = 8.91 Hz, -77.99, 3F, q, ⁴J_{F,F} = 11.92 Hz; LRMS-ES(+) m/z 479 (100, M+H); HRMS-ES(+) calcd for C₂₃H₂₈D₆O₃F₆: 479.2862 (M +H)1+; found 479.2861; Anal. Calcd for C₂₃H₂₈D₆O₃F₆: C, 57.73, found 57.90.

(1R,3aR,7aR)-7a-Methyl-1-[(R)-6,6,6-trifluoro-1-(5,5,5-trideutero-4-trideuteromethyl-4-trimethylsilanyloxy-pentyl)-5-trifluoromethyl-5-trimethylsilanyloxy-hex-3(E)-enyl]-octahydro-inden-4-one (14R-b)—The silylation of 14R-a was repeated as described for the preparation of the disilyl ether 12R-b to give 14R-b; $^1{\rm H}$ NMR (400 MHz, CDCl₃) δ 0.10 (s, 9 H), 0.22 (s, 9 H), 0.66 (s, 3 H), 1.10 - 1.46 (m, 7 H), 1.46 - 2.12 (m, 9 H), 2.17 - 2.43 (m, 4 H), 2.45 (dd, J=11.6, 7.6 Hz, 1 H), 5.55 (d, J=15.6 Hz, 1 H), 6.13 (m, 1 H); $^{19}{\rm F}$ -NMR (376.31 MHz) δ -77.84, 3F, q, $^{4}{\rm J}_{{\rm F},{\rm F}}$ = 9.41 Hz, -76.48, 3F, q, $^{4}{\rm J}_{{\rm F},{\rm F}}$ = 9.41 Hz.

(S)-11,11,11-Trideutereo-1,1,1-trifluoro-6-((1R,3aR,4S,7aR)-4-hydroxy-7amethyl-octahydro-indene-1-yl)-10-trideuteromethyl-2-trifluoromethyl-undec-3 (**Z**)-ene-2,10 diol (15**S**)—A suspension comprising the triol 11**S-b** (1.00 g, 2.09 mmol), hexane (25 mL), EtOAc (10 mL) and 5% Pd-CaCO₃ containing 3.5 % Pb (0.3 g, Degussa) was stirred under hydrogen, near hydrostatic balance, for 30 min and then filtered. The suspension was filtered, the filtrate was evaporated to a white foam (1.03 g) and further purified by chromatography on a silica gel using $1:4 \rightarrow 1:3 \rightarrow 1:2$ EtOAc - hexane to afford 15S in the form of white solids after evaporation of a soln in methyl formate (0.99 g, 98.5%); TLC (1:1, EtOAc - hexane) $R_f 0.75$; [α] $_D^{29} + 6.70$ ° (c 0.49, methanol); 1H NMR (400 MHz, CDCl₃) δ 0.93 (s, 3 H), 1.16 (td, *J*=13.4, 3.4 Hz, 1 H), 1.21 - 1.63 (m, 15 H), 1.69 - 1.96 (m, 4 H), 2.52 -2.70 (m, 2 H), 4.02 - 4.10 (m, 1 H), 5.42 (d, J=12.4 Hz, 1 H), 5.95 (ddd, J=12.4, 7.4, 7.2 Hz, 1 H); ¹³C NMR (400 MHz, CDCl₃) δ 13.59, 17.39, 18.34, 22.30, 26.51, 29.71, 31.38, 33.36, 39.14, 40.07, 43.50, 52.42, 52.52, 69.42, 71.71, 116.99, 122.90, 141.95; ¹⁹F-NMR (376.31 MHz) δ -77.76, 3F, q, ${}^{4}J_{E,F}$ = 9.78 Hz, -78.25, 3F, q, ${}^{4}J_{E,F}$ = 9.78 Hz.; LRMS-ES(-) m/z 480 (20, M) 479 (100, M-H); HRMS-ES(+) calcd for $C_{23}H_{30}D_6O_3F_6$: $503.2837 (M+Na)1^+$, found 503.2837.

(*R*)-11,11,11-Trideutereo-1,1,1-trifluoro-6-((1*R*,3a*R*,4*S*,7a*R*)-4-hydroxy-7a-methyl-octahydro-indene-1-yl)-10-trideuteromethyl-2-trifluoromethyl-undec-3

(*Z*)-ene-2,10 diol (15*R*)—The hydrogenation with Lindlar catalyst was repeated with 11*R*-b as described above to yield 15*R* as white solids; $[\alpha]_D^{29}$ +16.9 ° (c 0.34, methanol); ¹H NMR (400 MHz, CDCl₃) δ 0.93 (s, 3 H), 1.10 - 1.25 (m, 1 H), 1.26 - 1.64 (m, 16 H), 1.72 - 1.89 (m, 3 H), 1.93 (d, *J*=13.0 Hz, 1 H), 2.39 - 2.50 (m, 1 H), 2.51 - 2.63 (m, 1 H), 4.07 (br s, 1 H), 5.36 - 5.44 (m, 2 H), 5.96 (ddd, *J*=12.1, 7.9, 6.4 Hz, 1 H); ¹³C NMR (400 MHz, CDCl₃) δ 13.71, 17.55, 18.60, 22.40, 26.96, 29.89, 31.28, 33.42, 39.62, 40.04, 43.52, 52.39, 52.58, 69.43, 71.65, 116.92, 122.87, 141.90; ¹⁹F-NMR (376.31 MHz) δ -77.82, 3F, q, ⁴J_{F,F} = 9.91 Hz, -78.23, 3F, q, ⁴J_{F,F} = 9.90 Hz.; LRMS-ES(-) *m/z* 480 (20, M) 479 (100, M-H); HRMS-ES(+) calcd for C₂₃H₃₀D₆O₃F₆: 503.2837 (M+Na)1+, found 503.2834.

(1R,3aR,7aR)-7a-Methyl-1-[(S)-6,6,6-trifluoro-5-hydroxy-1-(5,5,5-trideutero-4hydroxy-4-trideuteromethyl-pentyl)-5-trifluoromethyl-hex-3(Z)-enyl]octahydro-inden-4-one (16S-a)—A solution of triol 15S (0.957 g, 2 mmol) in DCM (27.3 g) was stirred and a mixture of pyridinium dichromate (2.65 g, 7 mmol) and Celite (1.64 g) was added. The oxidation was complete after 7 h as judged by TLC (1:9 MeOH - DCM, R_f $0.68 \rightarrow 0.75$). The suspension was diluted with ether (30 mL) and filtered through silica gel G. Filtrate and ether washed were combined and evaporated to an oil which was flashchromatographed using $1:6 \rightarrow 1:4 \rightarrow 1:3$ EtOAc - hexane as mobile phases to afford **16S-a** as a white solid, 0.74 g (77.3 %); TLC (1:19 MeOH - DCM, R_f 0.48; 1:4 EtOAc - hexane R_f 0.07); $[\alpha]_D^{34} + 13.2$ ° (c 0.38, methanol); ¹ H NMR (400 MHz, CDCl₃) δ 0.63 (s, 3 H), 1.16 - 1.45 (m, 7 H), 1.45 - 1.79 (m, 6 H), 1.78 - 1.94 (m, 2 H), 1.94 - 2.08 (m, 2 H), 2.14 - 2.33 (m, 2 H), 2.45 (dd, *J*=11.4, 7.6 Hz, 1 H), 2.65 (t, *J*=6.4 Hz, 2 H), 5.46 (d, *J*=12.2 Hz, 1 H), 5.54 (br s, 1 H), 5.96 (dt, *J*=12.2, 7.2 Hz, 1 H); ¹³C NMR (400 MHz, CDCl₃) δ 12.50, 18.39, 18.86, 23.99, 26.70, 29.88, 31.35, 38.63, 39.35, 40.82, 43.39, 49.83, 52.63, 61.84, 71.63, 77.07, 117.49, 122.93, 141.40, 212.30. ¹⁹F-NMR (376.31 MHz) δ -77.73, 3F, q, ⁴J_{F,F} = 9.91 Hz, -78.22, 3F, q, ${}^{4}J_{E,F} = 9.91 \text{ Hz.}$; LRMS-ES(-) m/z 478 (15, M) 477 (90, M-H); HRMS-ES(+) calcd for C₂₃H₂₈D₆O₃F₆: 479.2862 (M+H)1⁺, found 479.2863.

(1R,3aR,7aR)-7a-Methyl-1-[(S)-6,6-trifluoro-1-(5,5,5-trideutero-4-trideuteromethyl-4-trimethylsilanyloxy-pentyl)-5-trifluoromethyl-5-trimethylsilanyloxy-hex-3(Z)-enyl]-octahydro-inden-4-one (16S-b)—The ketone-diol 16S-a (0.74 g, 1.55 mmol) was silylated as described for the preparation of the disilyl ether 12R-b to give 16S-b (0.864 g, 90%); TLC (1:9 EtOAc - hexane) Rf 0.43. This material was divided into 3 nearly equal parts and reserved for the syntheses of 4S-1, 4S-2, and 4S-3 described below.

(1R,3aR,7aR)-7a-Methyl-1-[(R)-6,6,6-trifluoro-5-hydroxy-1-(5,5,5-trideutero-4-hydroxy-4-trideuteromethyl-pentyl)-5-trifluoromethyl-hex-3(Z)-enyl]-octahydro-inden-4-one (16R-a)—The triol 15R (0.90 g, 1.87 mmol) was oxidized as described for the preparation of the ketone analog 16S-a to afford, after chromatography, a white foam (0.82 g, 91%); $^1\mathrm{H}$ NMR (400 MHz, CDCl₃) δ 0.62 (s, 3 H), 1.30 (br s, 7 H), 1.44 - 1.79 (m, 6 H), 1.79 - 1.96 (m, 2 H), 2.02 (d, J=9.8 Hz, 2 H), 2.12 - 2.32 (m, 2 H), 2.45 (dd, J=11.4, 7.8 Hz, 2 H), 2.59 (ddd, J=16.3, 8.3, 8.2 Hz, 1 H), 5.42 (d, J=12.2 Hz, 1 H), 5.54 (br s, 1 H), 5.94 (ddd, J=12.2, 8.0, 6.3 Hz, 1 H); $^{13}\mathrm{C}$ NMR (400 MHz, CDCl₃) δ 12.62, 18.65, 18.94, 24.09, 27.18, 29.87, 31.38, 38.57, 39.75, 40.83, 43.47, 50.03, 52.58, 61.88, 71.54, 77.23, 117.21, 122.83, 141.35, 212.31; $^{19}\mathrm{F}$ -NMR (376.31 MHz) δ -77.81, 3F, q, $^{4}\mathrm{J}_{\mathrm{F,F}}$ = 9.78 Hz, -78.20, 3F, q, $^{4}\mathrm{J}_{\mathrm{F,F}}$ = 9.91 Hz.; LR MS-ES(-) m/z 478 (18, M) 477 (100, M-H); HRMS-ES(+) calcd for C₂₃H₂₈D₆O₃F₆: 501.2681 (M+Na)1+, found 501.2682.

(1R,3aR,7aR)-7a-Methyl-1-[(R)-6,6,6-trifluoro-1-(5,5,5-trideutero-4-trideuteromethyl-4-trimethylsilanyloxy-pentyl)-5-trifluoromethyl-5-trimethylsilanyloxy-hex-3(Z)-enyl]-octahydro-inden-4-one (16R-b)—The ketone-

diol **16R-a** (0.82 g) was silylated as described for the preparation of the disilyl ether **12R-b** to give **16R-b** (1.03 g, 97%); TLC (1:4 and 1:39 EtOAc - hexane) R_f 0.71 and 0.08, respectively. This material was divided into 3 nearly equal parts and reserved for the syntheses of **4R-1**, **4R-2**, and **4R-3** described below.

(20S)-1a,25-dihydroxy-21-(3-hydroxy-3-trifluoromethyl-4,4,4-trifluoro-but-1ynyl)-26,26,26,27,27,27-hexadeutero-cholecalciferol (2S-1)—A 3-neck flask equipped with a magnetic stirrer, thermometer, a Claisen adapter with addition funnel, nitrogen sweep and a rubber septum, was charged with 17 (0.2973 g, 0.52 mmol) and THF (2 mL). The flask was cooled to -75 °C and a 1.6 M butyllithium soln in hexane (0.325 mL, 0.52 mmol) was added dropwise over a 10 min period, followed by a soln of 12S-b (0.1337 g, 0.215 mmol) in THF, at the same rate. Although no starting ketone was detectable after 4 h, the condensation product was a mixture of tri- and tetra-silyl ethers with R_f values of 0.30 and 0.57, respectively (TLC, 1:19 EtOAc - hexane). The reaction was quenched by the addition of a satd ammonium chloride soln (5 mL) at -65 °C, allowed to warm to 10 °C, and then distributed between hexane (35 mL) and water (10 mL). The organic layer was washed with 5:2 brine - pH 7 buffer (1 M, 7 mL), dried and evaporated. At this stage only the trisilyl ether was detectable. This material was chromatographed on a flash column, using hexane $\rightarrow 1:72 \rightarrow 1:39 \rightarrow 1:19$ EtOAc - hexane as mobile phases to yield the protected **2S-1** as colorless residue (0.1561 g). This residue was taken up in pentane, filtered, evaporated to a white foam and dried at 0.5 torr for 1 h, 0.1503 g, ¹H NMR (400 MHz, CDCl₃) δ 0.06 (s, 6 H), 0.06 (s, 6 H), 0.11 (s, 9 H), 0.53 (s, 3 H), 0.87 (s, 18 H), 1.14 - 1.53 (m, 13 H), 1.62 - 1.81 (m, 3 H), 1.82 - 1.95 (m, 3 H), 2.01 (t, J=9.7 Hz, 1 H), 2.22 (dd, J=13.1, 7.4 Hz, 1 H), 2.37 - 2.56 (m, 3 H), 2.83 (d, J=15.8 Hz, 1 H), 3.45 (s, 1 H), 4.13 - 4.23 (m, 1 H), 4.34 - 4.40 (m, 1 H), 4.86 (d, J=2.1 Hz, 1 H), 5.18 (d, J=1.5 Hz, 1 H), 6.02 (d, *J*=11.3 Hz, 1 H), 6.23 (d, *J*=11.3 Hz, 1 H); LRMS-ES(-) *m/z* 958(18, M-H+HCOOH), 856 (100, M-C₄H₉); HRMS-ES(+) calcd for $C_{47}H_{74}D_6O_4Si_3F_6$: 913.5718 (M+H)1⁺, found 913.5723. This material was dissolved in a 1M TBAF soln in THF (3 mL) and allowed to stand for 45 h then diluted with brine (5 mL), stirred for 5 min and transferred to a separatory funnel with EtOAc (40 mL) and water (8 mL). The aqueous phase was extracted once with EtOAc (10 mL) and the combined layers were washed with water (5×10 mL) and brine (10 mL) then dried and evaporated (0.1261 g). The resulting residue was chromatographed using $1:1 \rightarrow 2:1$ \rightarrow 4:1 EtOAc - hexane as mobile phases. The product fractions were pooled and evaporated (0.100 g). This residue was taken up in methyl formate, filtered and transferred to a 5-mL flask then evaporated to yield 2S-1 as a solid, white foam that was dried at 0.5 torr for 2 h; UV λ_{max} (ϵ) 212 (14801), 264 (16493) nm; [α]_D²⁹ +16.9° (c 0.37, methanol); ¹H NMR (400 MHz, CDCl₃) δ 0.53 (s, 3 H), 1.21 - 1.63 (m, 12 H), 1.63 - 1.77 (m, 2 H), 1.80 - 2.13 (m, 8 H), 2.26 - 2.42 (m, 2 H), 2.52 (dd, *J*=17.7, 2.8 Hz, 1 H), 2.57 (dd, *J*=14.3, 2.6 Hz, 1 H), 2.75 - 2.89 (m, 1 H), 3.75 (s, 1 H), 4.14 - 4.25 (m, 1 H), 4.42 (dd, *J*=6.9, 4.2 Hz, 1 H), 4.98 (s, 1 H), 5.32 (s, 1 H), 6.01 (d, J=11.2 Hz, 1 H), 6.23 (br s, 1 H), 6.35 (d, J=11.2 Hz, 1 H); 13 C NMR (400 MHz, CDCl₃) δ 12.18, 20.19, 21.71, 22.17, 23.58, 27.42, 29.01, 32.95, 38.73, 40.10, 42.64, 43.13, 45.08, 45.64, 53.19, 56.14, 66.84, 70.78, 71.15, 71.94, 89.89, 111.88, 117.22, 121.35, 124.75, 133.08, 142.42, 147.30; 19 F-NMR (376.31 MHz) δ -78.28, 6F, s; LRMS-EI(+) m/z 612 (4, M), 594 (40, M-H₂O); 594(39, M-2H₂O), 558 (100, M-3H₂O); HRMS-ES(+) calcd for $C_{32}H_{38}D_6O_4F_6$: 635.3412 (M+Na)1+, found 35.3407.

(20R)-1 α ,25-dihydroxy-21-(3-hydroxy-3-trideuteromethyl-4,4,4-trideuterobutyl)-26,26,26,27,27,27-hexafluoro-23-yne-cholecalciferol (2R-1)—A 2-neck

flask, equipped with magnetic stirrer, thermometer and a Claisen adapter containing a nitrogen sweep and rubber septum, was charged with diphenylphosphine oxide **17** (0.465 g) and THF (3.5 mL). The solution was cooled to -70 °C and a 1.6 M solution of butyl lithium in hexane (0.50 mL) was added dropwise over a 10 min period. To this red soln was added, via syringe, dropwise, within 20 min, ketone **12R-b** (0.2895 g, 0.466 mmol) dissolved in THF (3 mL).

After 5 h the mixture was allowed to warm to -35 °C and a satd ammonium chloride soln (10 mL) was added. The mixture was allowed to warm to 10 °C and was then distributed between hexane (60 mL) and enough water (5 mL) to dissolve all salts. The extract was washed with 5:1 brine - pH 7 buffer (10 ml) then dried and evaporated. The resulting residue (0.71 g) was flash-chromatographed using hexane $\rightarrow 1:72 \rightarrow 1:39$ EtOAc - hexane as mobile phases to give a mixture (0.38 g) of the tetrasilyl (TLC 1:19, EtOAc - hexane, R_f 0.76) and the trisilyl ether which lacks the silyl function at the gem-trifluoromethyl-carbinol site (TLC 1:19, EtOAc hexane, R_f 0.33). This mixture was dissolved in a 1 M soln of TBAF (4 mL) and allowed to remain at room temperature in the dark for 45 h. The soln was diluted with brine (5 mL) and equilibrated with EtOAc (40 mL) and water (10 mL). The aqueous phase was extracted once with EtOAc (10 mL) and the combined extracts were washed with water (5×10 mL) and brine (10 mL) then dried and evaporated. The residue was flash-chromatographed using $1:4 \rightarrow 1:2$ \rightarrow 1:1 \rightarrow 2:1 EtOAc - hexane \rightarrow EtOAc as mobile phases. The product fractions were pooled, filtered and evaporated, then and co-evaporated twice from hexane to furnish a residue (0.25 g) that was taken up in methyl formate and allowed to crystallize. The suspension was concentrated, diluted with pentane and refrigerated. The ML was decanted from the crystalline **2R-1**, 0.198 g, 69.3%; UV λ_{max} (ϵ) 211 (17454), 265 (18463) nm; [α]_D²⁹ +13.2° (c 0.25, methanol); ¹H NMR (400 MHz, DMSO- d_6) δ 0.51 (s, 3 H), 1.09 - 1.69 (m, 15 H), 1.73 - 1.87 (m, 2 H), 1.87 - 1.99 (m, 2 H), 2.16 (dd, J=13.5, 5.4 Hz, 1 H), 2.26 (d, J=16.9 Hz, 1 H), 2.35(d, J=11.7 Hz, 1 H), 2.42 (d, J=16.9 Hz, 1 H), 2.49 (s, 1 H), 2.73 - 2.83 (m, 1 H), 3.92 - 4.02 (m, 1 H), 4.05 (s, 1 H), 4.13 - 4.23 (m, 1 H), 4.56 (d, J=3.6 Hz, 1 H), 4.75 (d, J=1.6 Hz, 1 H),4.86 (d, *J*=4.6 Hz, 1 H), 5.22 (br s, 1 H), 5.98 (d, *J*=11.1 Hz, 1 H), 6.18 (d, *J*=11.1 Hz, 1 H), 8.93 (s, 1 H); 13 C NMR (400 MHz, DMSO- d_6) δ 11.89, 20.17, 20.51, 21.82, 23.14, 26.71, 28.38, 31.52, 38.76, 39.82, 43.13, 43.83, 44.94, 45.13, 52.29, 55.75, 65.07, 68.40, 68.50, 70.20, 70.40, 89.59, 109.89, 117.60, 121.47, 122.37, 135.87, 139.64, 149.33; ¹⁹F-NMR (376.31 MHz) δ -76.77, HZ, 6F, s; LRMS-ES(-) m/z 611 (100, M-1); HRMS-ES(+) calcd for $C_{32}H_{38}D_6O_4F_6$: 635.3412 (M+Na)1+, found 635.3412.

(20S)-1a,25-dihydroxy-21-(3-hydroxy-3-trifluoromethyl-4,4,4-trifluoro-but-1vnyl)-26,26,26,27,27,27-hexadeutero-19-nor-cholecalciferol (2S-2)—A 3-neck flask, equipped with magnetic stirrer, thermometer and a Claisen adapter containing a nitrogen sweep and rubber septum, was charged with diphenylphosphine oxide 18 (0.2973 g, 0.52 mmol) and THF (2 mL) freshly distilled from sodium benzophenone ketyl. The solution was cooled to -70 °C and a 1.6 M solution of butyllithium in hexane (0.325 mL, 0.52 mmol) was added dropwise over a 10 min period. To this soln was added, via syringe, dropwise over a 10 min period, a soln of 12S-b (0.1271 g) in a soln of THF (2 mL). Starting material (TLC 1:4 EtOAc - hexane) was no longer observed after 4 h and a mixture of tri- and tetra-silylated condensation products was present (TLC 1:19 EtOAc - hexane). The mixture was allowed to warm to -55 °C, a satd ammonium chloride soln (5 mL) was added, and then allowed to warm further to 10 °C. The resulting mixture was equilibrated with hexane (35 mL) and water (10 mL), the aqueous layer was re-extracted once with hexane (15 mL). The combined extract, now containing mostly the trisilyl ether, was washed with 5:2 brine / pH 7 buffer, then dried and evaporated and chromatographed using hexane $\rightarrow 1:72 \rightarrow 1:39$ EtOAc - hexane as mobile phases. The product fractions were pooled and evaporated to give a colorless oil (0.1737 g) that was taken up in pentane, filtered and evaporated. The resulting white foam was dried at 0.5 torr and represents the trisilyl ether of 2S-2; ${}^{1}H$ NMR (400 MHz, CDCl₃) δ 0.05 (s, 9 H), 0.06 (br s, 3 H), 0.11 (s, 9 H), 0.54 (s, 3 H), 0.87 (d, J=6.0 Hz, 18 H), 1.17 - 1.54 (m, 13 H), 1.57 - 1.74 (m, 3 H), 1.74 - 1.83 (m, 1 H), 1.84 - 1.96 (m, 2 H), 2.03 (t, J=9.6 Hz, 1 H), 2.10(dd, J=12.9, 8.0 Hz, 1 H), 2.21 - 2.31 (m, 1 H), 2.33 - 2.57 (m, 4 H), 2.82 (m, J=2.3 Hz, 1 H), 3.45 (s, 1 H), 3.99 - 4.16 (m, 2 H), 5.82 (d, J=11.1 Hz, 1 H), 6.16 (d, J=11.1 Hz, 1 H). The trisilyl ether obtained as described above was dissolved in a 1 M soln of TBAF (3 mL) and allowed to remain in the dark for 38 h. The mixture was diluted with brine (5 mL), stirred for

5 min and equilibrated with EtOAc (40 mL) and water (8 mL). The aqueous phase was extracted once with EtOAc (10 mL) and the combined extracts were washed with water (5×10 mL) and brine (10 mL), dried and evaporated. The residue was chromatographed using 1:1 \rightarrow 2:1 \rightarrow 4:1 EtOAc - hexane \rightarrow EtOAc as mobile phases. The product fractions were pooled and evaporated. The residue (0.1038 g) was taken up in methyl formate, filtered and evaporated to afford **2S-2** as a white, solid foam, dried at 0.5 torr (0.1009 g); UV λ_{max} (ϵ) 242 (33349), 251(39366), 260 (26943) nm (methanol); $[\alpha]_D^{29}$ +46.0° (c 0.55, methanol); 1 H NMR (400 MHz, CDCl₃) δ 0.55 (s, 3 H), 1.22 - 1.48 (m, 9 H), 1.48 - 1.62 (m, 4 H), 1.61 - 1.76 (m, 4 H), 1.74 - 1.85 (m, 1 H), 1.85 - 2.09 (m, 5 H), 2.17 - 2.27 (m, 2 H), 2.35 (dd, J=17.3, 7.0 Hz, 1 H), 2.48 (dd, J=13.3, 2.9 Hz, 1 H), 2.57 (dd, J=17.3, 3.8 Hz, 1 H), 2.74 (dd, J=13.3, 3.8 Hz, 1 H), 2.81 (m, 1 H), 4.04 (br s, 1 H), 4.12 (br s, 1 H), 5.61 (br s, 1 H), 5.86 (d, J=11.3 Hz, 1 H), 6.31 (d, J=11.3 Hz, 1 H); I¹³C NMR (400 MHz, CDCl₃) δ 12.29, 20.13, 22.03, 22.18, 23.48, 27.45, 28.86, 33.23, 37.19, 38.66, 40.16, 42.13, 42.89, 44.61, 45.54, 53.40, 56.11, 67.23, 67.43, 71.04, 72.15, 90.22, 115.48, 121.26, 123.65, 131.31, 142.30; LRMS-ES(-) m/z 599 (100, M-H); HRMS-ES(+) calcd for C₃₁H₃₈D₆O₄F₆: 623.3412 (M+Na)1+, found 623.3414.

(20R)-1a,25-dihydroxy-21-(3-hydroxy-3-trideuteromethyl-4,4,4-trideuterobutyl)-26,26,26,27,27,27-hexafluoro-23-yne-19-nor-cholecalciferol (2R-2)—The diphenylphosphine oxide 18 (0.4222 g, 0.74 mmol) was deprotonated with a 1.6 M solution of butyllithium in hexane and allowed to react with the ketone **12R-b** (0.257 g, 0.414 mmol) as described for the preparation of 2S-1. The condensation product (0.31 g) was purified as described for the epimer 2S-2, and then dissolved in a 1 M soln of TBAF (5 mL). After 18 h the temperature was increased and maintained at 30 °C for 16 h. The deprotected product was isolated and purified by chromatography as described for the epimer 2S-2. The resulting colorless syrup (0.20 g) was crystallized from methyl formate, 0.15 g (60% from ketone 12R**b**), mp 165-167 °C, UV λ_{max} (ϵ) 243 (35935), 251(41886), 261 (228440) nm (methanol); $[\alpha]_D^{30} + 58.4^{\circ}$ (c 0.31, methanol); ¹H NMR (400 MHz, DMSO- d_6) δ 0.52 (s, 3 H), 1.13 - 1.73 (m, 17 H), 1.79 - 2.11 (m, 5 H), 2.23 - 2.32 (m, 2 H), 2.39 - 2.48 (m, 2 H), 2.70 - 2.83 (m, 1 H), 3.74 - 3.84 (m, 1 H), 3.84 - 3.93 (m, 1 H), 4.05 (s, 1 H), 4.39 (d, *J*=3.6 Hz, 1 H), 4.49 (d, J=3.6 Hz, 1 H), 5.81 (d, J=11.1 Hz, 1 H), 6.08 (d, J=11.1 Hz, 1 H), 8.94 (br s, 1 H); ¹³C NMR $(400 \text{ MHz}, DMSO-d_6) \delta 12.01, 20.17, 21.72, 23.01, 26.79, 28.22, 31.48, 36.95, 38.79, 39.82,$ 42.23, 43.81, 44.99, 52.26, 55.71, 65.28, 65.55, 68.38, 70.19, 70.39, 89.58, 116.04, 120.81, 121.46, 134.70, 139.02; ¹⁹F-NMR (376.31 MHz) δ -76.76, HZ, 6F, s; LRMS-ES(-) m/z 599 (30, M-H); HRMS-ES(+) calcd for $C_{31}H_{38}D_6O_4F_6$: 623.3412 (M+Na)1+, found 623.3410; Roentgen diffraction analysis (Figure 2): C₃₁H₄₄F₆O₄, orthorhombic, space group P2₁2₁2₁ with unit cell dimensions of a = 7.3400(15), b = 16.6070(3), c = 25.9300(5) Å, volume 3160.9 (11) $Å^3$ (Z = 4).

(20S)- 1α -fluoro-21-(3-hydroxy-3-trifluoromethyl-4,4,4-trifluoro-but-1-ynyl)-25-hydroxy-26,26,26,27,27,27-hexadeutero-cholecalciferol (2S-3)—The

diphenylphosphine oxide **19** (0.286 g, 0.61 mmol) was deprotonated with a 1.6 M solution of butyllithium in hexane and allowed to react with the ketone **12S-b** (0.159 g, 0.256 mmol) and the resulting condensation product was purified as described for **2S-2**; the residue was dissolved in a 1 M soln of TBAF (3 mL) and allowed to stand for 24 h at ambient temperature. The deprotected product was isolated and purified by chromatography as described for **2S-2** to afford **2S-3** as a white solid foam (75 mg, 47% from **12S-b**); UV λ_{max} (ϵ) 210 (14386), 242 (14376), 270 (14177) nm (methanol); [α]_D²³ +27.4° (c 0.25, methanol); ¹H NMR (400 MHz, DMSO- d_6) δ 0.50 (s, 3 H), 1.03 - 2.01 (m, 20 H), 2.05 - 2.20 (m, 2 H), 2.43 - 2.56 (m, 2 H), 2.84 (d, J=12.4 Hz, 1 H), 3.83 - 3.99 (m, 1 H), 4.05 (s, 1 H), 4.86 (d, J=4.5 Hz, 1 H), 4.99 (s, 1 H), 5.15 (d, J=48.4 Hz, 1 H), 5.39 (br s, 1 H), 5.94 (d, J=11.2 Hz, 1 H), 6.37 (d, J=11.2 Hz, 1 H), 8.93 (s, 1 H); ¹³C NMR (400 MHz, DMSO- d_6) δ 11.73, 20.30, 20.36, 21.63, 23.11, 26.75, 28.39, 31.93, 38.16, 40.70, 43.79, 44.88, 45.12, 52.22, 55.59, 64.52, 68.41, 7.45, 70.82, 89.41,

91.29, 92.96, 115.55, 117.09, 121.57, 124.16, 133.12, 141.75, 143.19; 19 F-NMR (376.31 MHz) δ -76.64 Hz, 6F, br m; LRMS-ES(-) m/z 613 (100, M-H); HRMS-ES(+) calcd for $C_{32}H_{37}D_6O_3F_7$: 637.3369 (M+Na)1⁺, found 637.3362.

(20R)-1 α -fluoro-21-(3-hydroxy-3-trideuteromethyl-4,4,4-trideutero-butyl)-25-hydroxy-26,26,26,27,27,27-hexafluoro-23-yne-cholecalciferol (2R-3). The diphenylphosphine oxide **19** (0.3042 g, 0.646 mmol) was deprotonated with a 1.6 M solution of butyllithium in hexane and allowed to react with the ketone **12R-b** (0.159 g, 0.61 mmol) and the condensation product was purified as described for **2S-2**. The resulting material (0.24 g) was dissolved in a 1 M soln of TBAF (3 mL) and allowed to stand for 24 h at ambient temperature. The deprotected product was isolated (0.17 g) and purified by chromatography as described to afford **2R-3** as a white, solid foam (0.1552 g, 41% from ketone; UV λ_{max} (ϵ) 209 (15954), 242(14652), 270 (14328) nm (methanol); $[\alpha]_D^{29}$ +34.3° (ϵ 0.24, methanol); 1H NMR (400 MHz, CDCl₃) δ 0.55 (s, 3 H), 1.16 - 1.79 (m, 16 H), 1.80 - 2.07 (m, 5 H), 2.12 - 2.26 (m, 2 H), 2.30 (dd, J=12.9, 8.0 Hz, 2 H), 2.44 (dd, J=17.3, 3.2 Hz, 1 H), 2.62 (dd, J=13.1, 2.9 Hz, 1 H), 2.76 - 2.89 (m, 1 H), 4.16 - 4.27 (m, 1 H), 5.13 (ddd, J=49.6, 6.0, 3.6 Hz, 1 H), 5.10 (s, 1 H), 5.39 (br s, 1 H), 6.02 (d, J=11.2 Hz, 1 H), 6.39 (d, J=11.2 Hz, 1 H); ^{19}F -NMR (376.31 MHz) δ -78.36 Hz, 6F, br m; LRMS-ES(-) m/z 659 (35, M+HCOOH-H), 613 (100, M-H); HRMS-ES(+) calcd for $C_{32}H_{37}D_6O_3F_7$: 637.3369 (M+Na)1+, found 637.3365.

(20S)-1α,25-dihydroxy-21-(3-hydroxy-3-trifluoromethyl-4,4,4-trifluoro-but-1(E)-enyl)-26,26,26,27,27,27-hexadeutero-cholecalciferol (3S-1)—The

diphenylphosphine oxide 17 (0.3042 g, 0.522 mmol) was deprotonated with a 1.6 M solution of butyllithium in hexane, allowed to react with the ketone 12R-b (0.3296 g, 0.529 mmol) and the condensation product was purified as described for 2S-2; the resulting material (0.51 g) was dissolved in a 1 M soln of TBAF (5 mL) and allowed to stand for 41 h at ambient temperature. The deprotected material was isolated (0.17 g) and purified by chromatography as described to afford 3S-1 as a white, solid foam (0.2844 g, 87% from ketone); UV $\lambda_{max}(\epsilon)$ $213 (16297), 266 (17812) \text{ nm (methanol)}; [\alpha]_D^{30} + 9.9^{\circ} (c 0.30, \text{methanol}); {}^{1}H \text{ NMR } (400 \text{ MHz}, 10.00); {}^{1}$ DMSO- d_6) δ 0.53 (s, 3 H), 1.03 - 1.56 (m, 13 H), 1.56 - 1.72 (m, 3 H), 1.72 - 1.85 (m, 2 H), 1.87 - 2.03 (m, 2 H), 2.17 (dd, *J*=13.5, 5.2 Hz, 1 H), 2.20 - 2.29 (m, 1 H), 2.36 (d, *J*=11.5 Hz, 2 H), 2.80 (d, *J*=10.2 Hz, 1 H), 3.98 (br s, 1 H), 4.02 (s, 1 H), 4.19 (br s, 1 H), 4.55 (d, *J*=3.4 Hz, 1 H), 4.76 (br s, 1 H), 4.87 (d, J=4.5 Hz, 1 H), 5.23 (br s, 1 H), 5.61 (d, J=15.3 Hz, 1 H), 5.99 (d, *J*=11.0 Hz, 1 H), 6.20 (d, *J*=11.0 Hz, 1 H), 6.27 (ddd, *J*=15.1, 7.4, 7.1 Hz, 1 H), 8.03 (s, 1 H); ¹³C NMR (400 MHz, DMSO-*d*₆) δ 11.85, 19.26, 21.76, 23.17, 26.66, 28.39, 31.06, 33.31, 38.98, 43.13, 44.12, 44.94, 45.32, 52.20, 55.62, 65.10, 68.41, 68.53, 75.51, 109.97, 117.66, 119.31, 122.49, 122.74, 135.95, 137.55, 139.92, 149.52; 19 F-NMR (376.31 MHz) δ -75.85 Hz, 6F, m;); LRMS-ES(-) m/z 613 (40, M-H); HRMS-ES(+) calcd for $C_{32}H_{40}D_6O_4F_6$: 637.3569 (M+Na)1⁺, found 637.3569.

(20R)-1 α ,25-dihydroxy-21-(3-hydroxy-3-trideuteromethyl-4,4,4-trideuterobutyl)-26,26,26,27,27,27-hexafluoro-23(E)-ene-cholecalciferol (3R-1)—The

diphenylphosphine oxide **17** (0.3546 g, 0.608 mmol) was deprotonated with a 1.6 M solution of butyllithium in hexane and allowed to react with the ketone **12R-b** (0. 0.2639 g, 0.424 mmol) as described. The condensation product was purified as described for **2S-2**; the resulting material was dissolved in a 1 M soln of TBAF (5 mL) and allowed to stand for 41 h at ambient temperature. The deprotected material was isolated and purified by chromatography as described to afford **3S-1** as a white, solid foam (0.1842 g, 70% from ketone **14R-b**; UV λ_{max} (ϵ) 205 (14284), 211 (15019), 265 (16299) nm (methanol); [α]_D³⁰ +14.1° (c 0.20, methanol); ¹H NMR (400 MHz, DMSO- d_6) δ 0.52 (s, 3 H), 1.10 - 1.57 (m, 13 H), 1.57 - 1.71 (m, 3 H), 1.73 - 1.85 (m, 2 H), 1.90 (d, J=9.6 Hz, 1 H), 1.96 (t, J=9.4 Hz, 1 H), 2.01 - 2.12 (m, 1 H), 2.16 (dd, J=13.4, 5.5 Hz, 1 H), 2.22 - 2.32 (m, 1 H), 2.36 (d, J=11.7 Hz, 1 H), 2.79 (m, J=9.6 Hz, 1 H), 3.98 (br s, 1 H), 4.03 (s, 1 H), 4.15 - 4.26 (m, 1 H), 4.56 (d, J=3.2 Hz, 1 H),

4.76 (d, J=1.9 Hz, 1 H), 4.87 (d, J=4.7 Hz, 1 H), 5.23 (d, J=1.1 Hz, 1 H), 5.61 (d, J=15.6 Hz, 1 H), 5.99 (d, J=11.3 Hz, 1 H), 6.19 (d, J=11.3 Hz, 1 H), 6.22 - 6.32 (m, 1 H), 8.03 (br s, 1 H); LRMS-ES(-) m/z 613 (35, M-H); HRMS-ES(+) calcd for $C_{32}H_{40}D_6O_4F_6$: 637.3569 (M+Na) 1^+ , found 637.3570.

(20S)-1 α ,25-dihydroxy-21-(3-hydroxy-3-trifluoromethyl-4,4,4-trifluoro-but-1(E)-enyl)-26,26,27,27,27-hexadeutero-19-nor-cholecalciferol (3S-2)—The

diphenylphosphine oxide **18** (0.5340 g, 0.936 mmol) was deprotonated with a 1.6 M solution of butyllithium in hexane, allowed to react with the ketone **14S-b** (0.3498 g, 0.561 mmol), and the condensation product was purified as described for **2S-2**; the resulting partially desilylated material (0.48 g) was dissolved in a 1 M soln of TBAF (5 mL) and allowed to stand for 63 h at ambient temperature. The deprotected material was isolated and purified by chromatography as described to afford **3S-2** as a white, solid foam (0.2862 g, 84%); UV λ_{max} (ϵ) 243 (34631), 251(40591), 261 (27593) nm (methanol); $[\alpha]_D^{28}$ +42.3° (c 0.25, methanol); ¹H NMR (400 MHz, DMSO- d_6) δ 0.53 (s, 3 H), 1.01 - 1.72 (m, 17 H), 1.72 - 2.12 (m, 5 H), 2.15 - 2.31 (m, 2 H), 2.31 - 2.47 (m, 2 H), 2.66 - 2.83 (m, 1 H), 3.76 - 3.95 (m, 2 H), 4.04 (s, 1 H), 4.40 (d, J=3.5 Hz, 1 H), 4.50 (d, J=3.5 Hz, 1 H), 5.61 (d, J=15.2 Hz, 1 H), 5.81 (d, J=11.0 Hz, 1 H), 6.08 (d, J=11.0 Hz, 1 H), 6.28 (ddd, J=15.2, 7.4, 7.1 Hz, 1 H), 8.03 (s, 1 H); ¹³C NMR (400 MHz, DMSO- d_6) δ 11.98, 19.26, 21.66, 23.04, 26.72, 28.23, 31.06, 33.29, 36.98, 39.01, 42.23, 44.15, 44.61, 45.19, 52.20, 55.59, 65.32, 65.59, 68.43, 75.51, 116.12, 119.31, 120.95, 122.74, 134.76, 137.58, 139.34; LRMS-ES(-) m/z 601 (30, M-H); HRMS-ES(+) calcd for $C_{31}H_{40}D_6O_4F_6$: 625.3569 (M+Na)1+, found 625.3572.

(20R)-1α,25-dihydroxy-21-(3-hydroxy-3-trideuteromethyl-4,4,4-trideutero-butyl)-26,26,26,27,27,27-hexafluoro-23(E)-ene-19-nor-cholecalciferol (3R-2)—

The diphenylphosphine oxide 18 (0.244 g, 0.427 mmol) was deprotonated with a 1.6 M solution of butyllithium in hexane and allowed to react with the ketone **14R-b** (0.152 g, 0.244 mmol). The condensation product was purified as described for 2S-2; the resulting partially desilylated material was dissolved in a 1 M soln of TBAF (3 mL) and allowed to stand for 50 h at ambient temperature. The deprotected material was isolated and purified by chromatography as described to afford **3R-2** which was crystallized from methyl formate (90 mg, 0.1493 mmol, 61% from ketone **14R-b**); UV λ_{max} (ϵ) 243 (33111), 251(35071), 261 (26178) nm (methanol); $[\alpha]_D^{28}$ +44.2° (c 0.22, methanol); ¹H NMR (400 MHz, DMSO- d_6) δ 0.52 (s, 3 H), 1.10 - 1.73 (m, 17 H), 1.76 - 2.14 (m, 6 H), 2.16 - 2.33 (m, 2 H), 2.43 (d, J=10.2 Hz, 1 H), 2.75 (d, J=10.0 Hz, 1 Hz, 1 H), 2.75 (d, J=10.0 Hz, 1 Hz, 1Hz, 1 H), 3.73 - 3.84 (m, 1 H), 3.84 - 3.94 (m, 1 H), 4.04 (s, 1 H), 4.40 (d, J=3.7 Hz, 1 H), 4.50(d, J=3.7 Hz, 1 H), 5.62 (d, J=15.2 Hz, 1 H), 5.80 (d, J=11.1 Hz, 1 H), 6.08 (d, J=11.1 Hz, 1 H), 6.26 (ddd, J=15.2, 7.5, 7.4 Hz, 1 H), 8.03 (s, 1 H); ¹³C NMR (400 MHz, DMSO- d_6) δ 11.99, 19.34, 21.79, 23.09, 26.87, 28.27, 30.92, 33.39, 36.97, 42.24, 44.03, 44.63, 45.19, 52.10, 55.65, 65.31, 65.58, 68.43, 75.47, 116.00, 119.13, 120.86, 122.62, 134.62, 137.55, 139.21; LRMS-ES(-) m/z 601 (34, M-H); HRMS-ES(+) calcd for $C_{31}H_{40}D_6O_4F_6$; 625.3569, found 625.3575.

(20S)-1 α -fluoro-25-hydroxy-21-(3-hydroxy-3-trifluoromethyl-4,4,4-trifluorobut-1(E)-enyl)-26,26,26,27,27,27-hexadeutero-cholecalciferol (3S-3)—The

diphenylphosphine oxide **19** (0.35 g, 0.743 mmol)) was deprotonated with a 1.6 M solution of butyllithium in hexane and allowed to react with the ketone **14S-b** (0.295 g, 0.474 mmol). The condensation product was purified as described for **2S-2**; the resulting partially desilylated material was dissolved in a 1 M soln of TBAF (4 mL) and allowed to stand for 25 h at ambient temperature. The deprotected material was isolated and purified by chromatography as described to afford a crystalline residue. This material was co-evaporated twice from hexane, then taken up in methyl formate, filtered, concentrated to a volume of ca. 1-2 mL, warmed to dissolve most crystals then allowed to crystallize, first at room temperature, then refrigerator.

The mother liquor was decanted, the solids washed with pentane then dried to give **3S-3** (0.14 g, 48 %); mp 148-50 °C; UV λ_{max} (ϵ) 212 (16206), 242(15668), 271 (15186) nm (methanol); $[\alpha]_D^{28}$ +30.72° (c 0.23, methanol); 1 H NMR (400 MHz, DMSO- d_6) δ 0.52 (s, 3 H), 1.03 - 1.58 (m, 13 H), 1.59 - 1.85 (m, 4 H), 1.92 (d, J=11.5 Hz, 1 H), 1.99 (t, J=9.2 Hz, 1 H), 2.05 - 2.18 (m, 2 H), 2.18 - 2.29 (m, 1 H), 2.29 - 2.42 (m, 1 H), 2.52 - 2.56 (m, 1 H), 2.83 (d, J=12.6 Hz, 1 H), 3.82 - 3.98 (m, 1 H), 4.02 (s, 1 H), 4.86 (d, J=4.3 Hz, 1 H), 5.00 (s, 1 H), 5.15 (d, J=49.7 Hz, 1 H), 5.39 (br s, 1 H), 5.61 (d, J=15.6 Hz, 1 H), 5.95 (d, J=11.1 Hz, 1 H), 6.20 - 6.33 (m, 1 H), 6.37 (d, J=11.1 Hz, 1 H), 8.03 (s, 1 H); 13 C NMR (400 MHz, DMSO- d_6) δ 11.89, 19.27, 21.62, 23.09, 26.63, 28.38, 31.05, 33.24, 38.94, 40.70, 44.11, 44.88, 45.40, 52.14, 55.55, 64.52, 68.40, 75.50, 91.28, 92.94, 15.51, 117.02, 119.30, 122.73, 124.17, 133.05, 137.52, 141.91, 143.21; 19 F-NMR (376.31 MHz) δ -75.86 Hz, 6F, m; LRMS-ES(-) m/z 661 (100, M +HCOO⁻), 615 (10, M-H); HRMS-ES(+) calcd for $C_{32}H_{39}D_6O_3F_7$: 639.3526 [M+Na]1⁺, found 639.3515.

(20R)-1α-fluoro-25-hydroxy-21-(3-hydroxy-3-trideuteromethyl-4,4,4-trideuterobutyl)-26,26,26,27,27,27-hexafluoro-23(E)-ene-cholecalciferol (3R-3)—The diphenylphosphine oxide **19** (0.374 g, 0.795 mmol) was deprotonated with a 1.6 M solution

diphenylphosphine oxide 19 (0.374 g, 0.795 mmol) was deprotonated with a 1.6 M solution of butyllithium in hexane and allowed to react with the ketone **14R-b** (0.262 g, 0.421 mmol). The condensation product was purified as described for 2S-2; the resulting partially desilvlated material was dissolved in a 1 M soln of TBAF (3mL) and allowed to stand for 24 h at ambient temperature. The deprotected material was isolated and purified by chromatography as described to afford 3R-3 as a white, solid foam (0.153 g, 59 %; UV λ_{max} (ϵ) 209 (23061), 241 (15755), 270 nm (14829); $[\alpha]_D^{28}$ +29.2° (c 0.26, methanol); ¹H NMR (400 MHz, CDCl₃) δ 0.55 (s, 3 H), 1.17 - 1.78 (m, 16 H), 1.78 - 2.13 (m, 7 H), 2.12 - 2.42 (m, 3 H), 2.61 (dd, J=13.1, 3.1 Hz, 1 H), 2.77 - 2.87 (m, 1 H), 4.20 (ddd, J=7.4, 3.8, 3.7 Hz, 1 H), 5.12 (ddd, J=49.7, 6.1, 3.5 Hz, 1 H), 5.10 (s, 1 H), 5.38 (br s, 1 H), 5.58 (d, J=15.4 Hz, 1 H), 6.02 (d, J=11.1 Hz, 1 H), 6.27 (ddd, *J*=15.4, 8.5, 6.5 Hz, 1 H), 6.39 (d, *J*=11.1 Hz, 1 H); ¹³C NMR (400 MHz, CDCl₃) δ 12.21, 19.70, 22.12, 23.56, 27.44, 29.05, 31.70, 34.83, 39.95, 40.26, 40.67, 43.50, 44.93, 45.95, 53.08, 56.26, 66.50, 71.40, 75.83, 90.89, 92.60, 115.23, 117.14, 119.07, 122.42, 125.43, 131.48, 138.09, 142.84, 143.00; 19 F NMR (376.31 MHz) δ -77.80, 3F, q, 4 J_{F,F} = 7.90 Hz, -77.88, 3F, q, ${}^{4}J_{E,F} = 7.80 \text{ Hz.}$; LRMS-ES(-) m/z 661 (100, M+HCOO-), 615 (20, M-H); $HRMS-ES(+) \ calcd \ for \ C_{32}H_{39}D_6O_3F_7: \ 639.3526 \ [M+Na]1^+, \ found \ 639.3523.$

(20S)-1 α ,25-dihydroxy-21-(3-hydroxy-3-trifluoromethyl-4,4,4-trifluoro-but-1(Z)-enyl)-26,26,26,27,27,27-hexadeutero-cholecalciferol (4S-1)—The

diphenylphosphine oxide 17 (0.497 g, 0.853 mmol) was deprotonated with a 1.6 M solution of butyllithium in hexane and allowed to react with the ketone 16S-b (0.288 g, 0.462 mmol). The condensation product was purified as described for 2S-2; the resulting partially desilylated material was dissolved in a 1 M soln of TBAF (5 mL) and allowed to stand for 19 h at ambient temperature. The deprotected material was isolated and purified by chromatography as described to afford **4S-1** as a white, solid foam (0.238 g, 84 %); UV λ_{max} (ϵ) 210 (66396), 264 (16769) nm; $[\alpha]_D^{28} + 29.2^{\circ}$ (c 0.26, methanol); ¹H NMR (400 MHz, DMSO- d_6) δ 0.52 (s, 3) H), 1.09 - 1.37 (m, 8 H), 1.37 - 1.58 (m, 5 H), 1.58 - 1.70 (m, 3 H), 1.74 - 1.86 (m, 2 H), 1.90 -2.03 (m, 2 H), 2.17 (dd, J=13.4, 5.1 Hz, 1 H), 2.32 - 2.47 (m, 2 H), 2.71 - 2.86 (m, 2 H), 3.99(br s, 1 H), 4.02 (s, 1 H), 4.19 (br s, 1 H), 4.55 (d, *J*=2.1 Hz, 1 H), 4.76 (br s, 1 H), 4.87 (d, J=4.3 Hz, 1 H), 5.23 (br s, 1 H), 5.42 (d, J=11.5 Hz, 1 H), 5.96 - 6.10 (m, 2 H), 6.19 (d, J=11.5 HzHz, 1 H), 8.01 (s, 1 H); ¹³C NMR (400 MHz, DMSO-d₆) δ 11.80, 19.27, 21.79, 23.17, 26.34, 28.41, 29.44, 31.43, 43.12, 44.33, 45.23, 52.30, 55.61, 65.08, 68.49, 76.78, 109.94, 116.77, 117.57, 122.50, 123.02, 135.88, 140.02, 141.37, 149.52; ¹⁹F-NMR (376.31 MHz) δ -75.87 Hz, 6F, m; LRMS-ES(-) m/z 659 (85, M+HCOO⁻), 613 (100, M-H); HRMS-ES(+) calcd for C₃₂H₄₀D₆O₄F₆: 637.3569 [M+Na]1⁺, found 637.3574.

(20R)-1 α ,25-dihydroxy-21-(3-hydroxy-3-trideuteromethyl-4,4,4-trideuterobutyl)-26,26,26,27,27,27-hexafluoro-23(Z)-ene-cholecalciferol (4R-1)—The

diphenylphosphine oxide 17 (0.429 g, 0.736 mmol) was deprotonated with a 1.6 M solution of butyllithium in hexane and allowed to react with the ketone **16S-b** (0.3518 g, 0.565 mmol). The condensation product was purified as described for 2S-2; the resulting partially desilylated material was dissolved in a 1 M soln of TBAF (4 mL) and allowed to stand for 32 h at ambient temperature. The deprotected material was isolated and purified by chromatography as described to afford **4R-1** as a white, solid foam (0.250 g, 72%); UV λ_{max} (ϵ) 212 (14818), 266 (17067) nm; $[\alpha]_D^{28} + 18.1 \text{ (c} = 0.29 \text{ %, methanol)}$; ¹H NMR (400 MHz, DMSO- d_6) δ 0.52 (s, 3 H), 1.12 - 1.58 (m, 13 H), 1.58 - 1.70 (m, 3 H), 1.70 - 1.84 (m, 2 H), 1.89 (d, *J*=11.1 Hz, 1 H), 1.93 - 2.04 (m, 1 H), 2.17 (dd, *J*=13.4, 5.1 Hz, 1 H), 2.31 - 2.45 (m, 2 H), 2.53 - 2.62 (m, 1 H), 2.80 (d, *J*=10.2 Hz, 1 H), 3.97 (br s, 1 H), 4.02 (s, 1 H), 4.18 (br s, 1 H), 4.55 (d, *J*=3.4 Hz, 1 H), 4.76 (br s, 1 H), 4.87 (d, *J*=4.5 Hz, 1 H), 5.23 (br s, 1 H), 5.41 (d, *J*=11.9 Hz, 1 H), 5.99 (d, *J*=11.1 Hz, 1 H), 6.02 - 6.11 (m, 1 H), 6.19 (d, *J*=11.1 Hz, 1 H), 7.98 (s, 1 H); ¹³C NMR (400 MHz, DMSO- d_6) δ 11.99, 19.39, 21.80, 23.20, 26.48, 28.40, 29.39, 31.39, 43.12, 44.17, 44.91, 45.35, 52.23, 55.55, 65.04, 68.47, 76.73, 109.85, 116.69, 117.49, 122.37, 122.89, 135.74, 139.85, 141.22, 149.33; ¹⁹F-NMR (376.31 MHz) δ - 75.83 Hz, 6F, m; LRMS-ES(-) m/z 659 (30, M+HCOOH-H⁻), 613 (100, M-H); HRMS-ES(+) calcd for C $^{32}H_{40}D_6O_4F_6$: 637.3569 [M+Na]1+, found 637.3562.

(20S)-1α,25-dihydroxy-21-(3-hydroxy-3-trifluoromethyl-4,4,4-trifluoro-but-1(Z)-enyl)-26,26,27,27,27-hexadeutero-19-nor-cholecalciferol (4S-2)—The

diphenylphosphine oxide **18** (0.4436, 0.778 mmol) was deprotonated with a 1.6 M solution of butyllithium in hexane and allowed to react with the ketone **16S-b** (0.2869 g, 0.460 mmol) and the condensation product was purified as described for **2S-2**; the resulting partially desilylated material was dissolved in a 1 M soln of TBAF (5 mL) and allowed to stand for 32 h at ambient temperature. The deprotected material was isolated and purified by chromatography as described to afford **4S-2** as a white, solid foam (0.243 g, 87.6 %); UV λ_{max} (ϵ) 243 (34246), 252 (39955), 261 (27120) nm; $[\alpha]_D^{28}$ +31 (ϵ = 0.31 %, methanol); ¹H NMR (400 MHz, DMSO- d_6) δ 0.52 (s, 3 H), 1.07 - 1.72 (m, 17 H), 1.72 - 2.15 (m, 5 H), 2.26 (d, J=11.3 Hz, 1 H), 2.32 - 2.47 (m, 2 H), 2.70 - 2.83 (m, 2 H), 3.80 (br s, 1 H), 3.87 (br s, 1 H), 4.02 (s, 1 H), 4.38 (d, J=3.6 Hz, 1 H), 4.49 (d, J=3.6 Hz, 1 H), 5.42 (d, J=11.9 Hz, 1 H), 5.80 (d, J=11.1 Hz, 1 H), 5.98 - 6.13 (m, 2 H), 8.01 (s, 1 H); ¹³C NMR (400 MHz, DMSO- d_6) δ 11.91, 19.25, 21.69, 23.02, 26.40, 28.24, 29.43, 31.43, 36.96, 42.21, 44.34, 45.07, 52.31, 55.56, 65.29, 65.57, 68.47, 76.76, 116.02, 116.75, 120.94, 123.02, 134.69, 139.40, 141.38; ¹⁹F-NMR (376.31 MHz) δ -75.85 Hz, 6F, m; LRMS-ES(-) m/z 647 (55, M+HCOOH-H⁻), 602 (28, M), 601 (100, M-H); HRMS-ES(+) calcd for C₃₁H₄₀D₆O₄F₆: 625.3569 [M+Na]1⁺, found 625.3576.

The diphenylphosphine oxide **18** (0.5078 g, 0.889 mmol) was deprotonated with a 1.6 M solution of butyllithium in hexane and allowed to react with the ketone **16R-b** (0.3298 g, 0.529 mmol). The condensation product was purified as described for **2S-2**; the resulting partially desilylated material was dissolved in a 1 M soln of TBAF (4 mL) and allowed to stand for 48.5 h at ambient temperature. The deprotected material was isolated and purified by chromatography as described to afford **4R-2** as a white, solid foam (0.2446 g, 76.7 %); UV λ_{max} (ϵ) 243 (32530), 252 (38012), 261 (25710) nm; [α]_D²⁵ +51.4° (ϵ = 0.33 %, methanol); ¹H NMR (400 MHz, DMSO- d_6) δ 0.52 (s, 3 H), 1.13 - 1.72 (m, 17 H), 1.80 (br s, 1 H), 1.89 (d, J=11.3 Hz, 1 H), 1.93 - 2.12 (m, 3 H), 2.27 (d, J=11.1 Hz, 1 H), 2.32 - 2.48 (m, 2 H), 2.53 - 2.62 (m, 1 H), 2.69 - 2.81 (m, 1 H), 3.76 - 3.84 (m, 1 H), 3.88 (br s, 1 H), 4.02 (s, 1 H), 4.38 (d, J=3.5 Hz, 1 H), 4.48 (d, J=3.5 Hz, 1 H), 5.42 (d, J=11.9 Hz, 1 H), 5.80 (d, J=11.1 Hz, 1 H), 6.01 - 6.11 (m, 2 H), 7.99 (s, 1 H); ¹³C NMR (400 MHz, DMSO- d_6) δ 12.11, 19.42,

21.71, 23.06, 26.55, 28.25, 29.40, 31.39, 36.95, 39.57, 42.23, 44.17, 45.20, 52.25, 55.51, 65.27, 65.54, 68.44, 76.73, 115.94, 116.70, 120.83, 122.92, 134.56, 139.25, 141.22; 19 F-NMR (376.31 MHz) δ -75.82 Hz, 6F, m; LRMS-ES(-) m/z 647 (30, M+HCOO⁻), 602 (25, M), 601 (100, M-H); HRMS-ES(+) calcd for $C_{31}H_{40}D_6O_4F_6$; 625.3569 [M+Na]1⁺, found 625.3583.

(20S)-1α-fluoro-25-hydroxy-21-(3-hydroxy-3-trifluoromethyl-4,4,4-trifluorobut-1(Z)-enyl)-26,26,26,27,27,27-hexadeutero-cholecalciferol (4S-3)—The diphenylphosphine oxide 19 (0.3557 g, 0.756 mmol) was deprotonated with a 1.6 M solution of butyllithium in hexane and allowed to react with the ketone **16S-b** (0.2700 g, 0.433 mmol) and the condensation product was purified as described for 2S-2; the resulting partially desilylated material was dissolved in a 1 M soln of TBAF (5 mL) and allowed to stand for 24.5 h at ambient temperature. The deprotected material was isolated and purified by chromatography as described to afford 4S-3 as a white, solid foam (0.1714 g, 64 %); UV λ_{max} (ϵ) 209 (13713), 243 (13893), 271 (13673) nm; [α]_D²⁵ +16.8° (c = 0.20 %, methanol); ¹H NMR (400 MHz, DMSO- d_6) δ 0.51 (s, 3 H), 1.06 - 1.88 (m, 18 H), 1.88 - 2.04 (m, 2 H), 2.04 - 2.21 (m, 2 H), 2.33 - 2.46 (m, 1 H), 2.70 - 2.88 (m, 2 H), 3.83 - 3.97 (m, 1 H), 4.01 (s, 1 H), 4.86 (d, *J*=4.3 Hz, 1 H), 5.00 (s, 1 H), 5.15 (d, *J*=49.9 Hz, 1 H), 5.33 - 5.46 (m, 2 H), 5.95 (d, *J*=11.1 Hz, 1 H), 6.04 (ddd, *J*=12.1, 6.4, 6.2 Hz, 1 H), 6.37 (d, *J*=11.1 Hz, 1 H), 8.01 (s, 1 H); 13 C NMR (400 MHz, DMSO- d_6) δ 11.83, 19.27, 21.66, 23.09, 26.34, 28.42, 29.39, 31.42, 40.70, 44.31, 44.87, 45.31, 52.26, 55.55, 64.52, 68.46, 76.77, 91.27, 92.94, 115.47, 116.77, 116.94, 123.01, 133.00, 141.34, 141.99, 143.23; $^{19}\text{F-NMR}$ (376.31 MHz) δ -75.85 Hz, 6F, m; LRMS-ES(-) m/z 661 (55, M+HCOOH-H⁻), 616 (20, M), 615 (100, M-H); HRMS-ES(+) calcd for $C_{32}H_{39}D_6O_3F_7$: 639.3526 [M+Na]1+, found 639.3523.

(20R)-1α-fluoro-25-hydroxy-21-(3-hydroxy-3-trideuteromethyl-4,4,4-trideuterobutyl)-26,26,26,27,27,27-hexafluoro-23(Z)-ene-cholecalciferol (4R-3)—The diphenylphosphine oxide 19 (0.5632 g, 1.20 mmol) was deprotonated with a 1.6 M solution of butyllithium in hexane and allowed to react with the ketone **16R-b** (0.3354 g, 0.538 mmol) and the condensation product was purified as described for 2S-2; the resulting partially desilvlated material was dissolved in a 1 M soln of TBAF (4 mL) and allowed to stand for 23 h at ambient temperature. The deprotected material was isolated and purified by chromatography as described to afford 4R-3 as a white solid that was crystallized from methyl formate (0.285 g, 86 %); UV $\lambda_{\text{max}}(\epsilon)$ 208 (17000), 242 (16068), 270 (15615) nm; $[\alpha]_D^{25}$ +36.2° $(c = 0.31 \text{ %, methanol}); {}^{1}\text{H NMR } (400 \text{ MHz, DMSO-} d_{6}) \delta 0.51 \text{ (s, 3 H), } 1.12 - 1.58 \text{ (m, 13)}$ H), 1.58 - 1.84 (m, 4 H), 1.90 (d, *J*=11.3 Hz, 1 H), 2.00 (t, *J*=9.2 Hz, 1 H), 2.04 - 2.20 (m, 2 H), 2.32 - 2.46 (m, 1 H), 2.52 - 2.61 (m, 2 H), 2.83 (d, *J*=12.6 Hz, 1 H), 3.82 - 3.95 (m, 1 H), 4.02 (s, 1 H), 4.86 (d, J=4.3 Hz, 1 H), 4.99 (s, 1 H), 5.14 (ddd, J=50.1, 5.1, 3.2 Hz, 1 H), 5.37 - 5.44 (m, 2 H), 5.94 (d, *J*=11.3 Hz, 1 H), 6.00 - 6.10 (m, 1 H), 6.37 (d, *J*=11.3 Hz, 1 H), 7.98 (s, 1 H); ¹³C NMR (400 MHz, DMSO-*d*₆) δ 12.03, 19.37, 21.69, 23.13, 26.49, 28.41, 29.39, 31.37, 40.70, 44.17, 44.87, 45.42, 52.19, 55.49, 64.50, 68.44, 76.73, 91.19, 92.86, 115.35, 116.70, 116.88, 122.90, 124.07, 132.88, 141.22, 141.82, 143.07; 19 F-NMR (376.31 MHz) δ -75.83 Hz, 6F, m, -170.48 Hz, 1F, m); LRMS-ES(-) m/z 661 (20, M+HCOO⁻), 616 (30, M), 615 (100, M-H); HRMS-ES(+) calcd for C₃₂H₃₉D₆O₃F₇: 639.3526 [M+Na]1⁺, found 639.3522.

Cell Culture—The MCF10CA1a human breast epithelial cell line was developed and provided by Dr. Fred Miller's group at the Karmanos Cancer Institute (Detroit, MI). The cells were maintained in DMEM/F12 medium supplemented with 5 % horse serum, 1% penicillin/streptomycin and 1% HEPES solution at 37 °C, 5% CO₂. The cells were passaged every 3-4 days. Human promyelocytic leukemia NB4 cells (Dr. Ethan Dmitrovsky, Dartmouth Medical School, Hanover, NH) were grown in suspension culture at 37 °C with 5% CO₂ in RPMI 1640 (Sigma, St. Louis, MO) with 10% heat-activated, defined iron-supplemented bovine calf serum

(Hyclone, Logan, UT). The NB4 cells were passaged two to three times a week to maintain log-phase growth.

Determination of cell proliferation by [3H]thymidine Uptake Assay—The

MCF10CA1a human breast epithelial cells (10,000 cells/well in a 24-well plate) were incubated with compounds in DMEM/F12 medium supplemented with 5% horse serum, 1% penicillin/streptomycin and 1% HEPES solution for 3 days. One μ Ci of [³H]thymidine was added to each well 3 h before the harvest. The cells were washed with PBS and precipitated with 10% trichloroacetic acid for 10 min. The cells were solubilized with the NaOH solution containing the salmon sperm DNA and the radioactivity of [³H] thymidine incorporated to the cells was analyzed with a liquid scintillation counter (Beckman Coulter, Fullerton, CA).

Determination of Differentiation Markers—The NB4 cells were incubated with test compounds at 1 nM concentrations for 48 h. Aliquots of 1×10^6 cells were washed twice with PBS, and then incubated with 0.5 μ l MY4-RD1 and 0.5 μ l Mo1-FITC (Coulter, Miami, FL) to analyze the expression of differentiated cell surface markers CD14 and CD11b. The cells were suspended in 0.5 mL of PBS and analyzed with an Epics Profile II instrument (Coulter Electronics, Hialeah, FL).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

VDR, vitamin-D receptor

LBD, ligand binding domain

INF, interferon

MLR, mixed lymphocyte reaction

DCM, dichloromethane

THF, tetrahydrofuran

TBAF, tetrabutylammonium fluoride

TMS, trimethylsilyl

TBSO, t-butyl-dimethylsilyloxy

TEMPO, 2,2,6,6-tetramethyl-1-piperidinyloxy, free radical

PDC, pyridinium dichromate

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2S-1: R¹=CH₂, R²= OH

2S-2: R¹=H₂, R²= OH

2S-3: R¹=CH₂, R²= F

2R-1: R¹=CH₂, R²= OH

2R-2: $R^1 = H_2$, $R^2 = OH$

2R-3: $R^1 = CH_2$, $R^2 = F$

3S-1: R¹=CH₂, R²= OH

3S-2: $R^1 = H_2$, $R^2 = OH$

3S-3: R¹=CH₂, R²= F

$$F_3C$$
 HO
 F_3C
 H
 D_3C
 OH
 R^1
 R^2

3R-1: R¹=CH₂, R²= OH

3R-2: $R^1 = H_2$, $R^2 = OH$

3R-3: $R^1 = CH_2$, $R^2 = F$

$$F_3C$$
 OH CF_3 H D_3C OH R^1 R^2

4S-1: R¹=CH₂, R²= OH

4S-2: R¹=H₂, R²= OH

4S-3: R¹=CH₂, R²= F

4R-1: R¹=CH₂, R²= OH

4R-2: $R^1 = H_2$, $R^2 = OH$

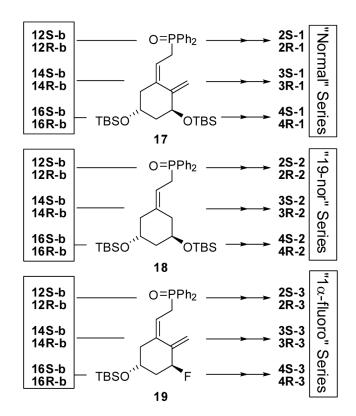
4R-3: R¹=CH₂, R²= F

Scheme 1. Summary of synthetic targets

Reagents and conditions: (a) CD₃MgI, ether (b) (CH₂O)_n, Me₂AlCl, (c) Chromatography, (d) PtO₂-C, EtOAc, (e) TEMPO, chloramine-T, DCM, buffer, (f) N₂CHP(O)(OMe)₂, MeOH, K₂CO₃, (g) TMS-imidazole, cyclohexane, (h) BuLi, THF, hexafluoroacetone, (i) fluorosilicic acid, AcCN, (j) PDC, DCM.

Scheme 3.

Reagents and conditions: (a) sodium bis(2-methoxyethoxy)aluminum hydride, toluene, (b) Lindlar catalyst, H₂, EtOH, (c) PDC, DCM, (d) TMS-imidazole, THF



Scheme 4. Assembly of target compounds 2S-1 to 4R-3

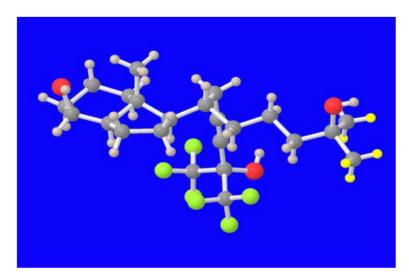


Figure 1. ORTEP drawing of 11S-b

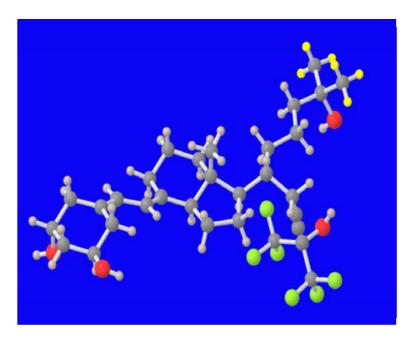


Figure 2. ORTEP drawing of 2R-2

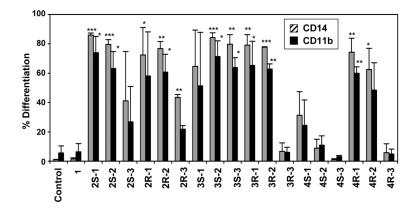


Figure 5. Differentiation induction of NB4 leukemia cell line by analysis of differentiated cell surface markers CD14 and CD11b. Two independent experiments were performed and combined, and values shown are averages \pm S.D. Statistical significances between 1 and the geminis were evaluated using Student's t-test (* p<0.05, ** p<0.01, *** p<0.001).

Table 1

 IC_{50} values of Gemini vitamin D analogs in MCF10CA1a human breast cancer cells. MCF10CA1a cells were incubated with gemini at concentrations of 0.001, 0.01, 0.1, 1, 10 nM in 5% horse serum DMEM/F12 medium for 3 days. IC_{50} values (Table 1) were determined using TableCurve 2D® software (Ver. 5.01, Systat)

1 3.3 0.062 2R-1 0.053 2S-2 0.12 2R-2 0.19 2S-3 0.56 2R-3 1.6 3S-1 0.33 3R-1 0.13 3S-2 0.008 3R-2 0.23 3S-3 0.18 3R-3 2.3 4S-1 1.6 4R-1 0.56	Compound	IC ₅₀ (nM)	Compound	IC ₅₀ (nM)
2S-2 0.12 2R-2 0.19 2S-3 0.56 2R-3 1.6 3S-1 0.33 3R-1 0.13 3S-2 0.008 3R-2 0.23 3S-3 0.18 3R-3 2.3 4S-1 1.6 4R-1 0.56	1	3.3		
2S-3 0.56 2R-3 1.6 3S-1 0.33 3R-1 0.13 3S-2 0.008 3R-2 0.23 3S-3 0.18 3R-3 2.3 4S-1 1.6 4R-1 0.56	2S-1	0.062	2R-1	0.053
3S-1 0.33 3R-1 0.13 3S-2 0.008 3R-2 0.23 3S-3 0.18 3R-3 2.3 4S-1 1.6 4R-1 0.56	2S-2	0.12	2R-2	0.19
3S-2 0.008 3R-2 0.23 3S-3 0.18 3R-3 2.3 4S-1 1.6 4R-1 0.56	2S-3	0.56	2R-3	1.6
3S-3 0.18 3R-3 2.3 4S-1 1.6 4R-1 0.56	3S-1	0.33	3R-1	0.13
4S-1 1.6 4R-1 0.56	38-2	0.008	3R-2	0.23
	38-3	0.18	3R-3	2.3
40.4	4S-1	1.6	4R-1	0.56
48-2 3.2 4R-2 0.43	4S-2	3.2	4R-2	0.43
4S-3 >10 4R-3 6.9			4R-3	