Studies on the Introduction of a Photoreactive Aryldiazirine Group into the Vitamin D Skeleton[‡]

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Dedicated to the memory of Prof. Angel Alberola

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The synthesis of a vitamin D analog with a diazirinyl benzylidene fragment replacing the side chain shows that diazirinylarylation of the upper vitamin D unit does not prevent the use of the convergent Lythgoe–Hoffman La Roche route to photoreactive analogs of potential value for photoaffinity labeling. The key step towards the diazirinylarylated upper fragment is the simultaneous oxidation of the hydroxyl and diaziridinyl groups of intermediate **10**.
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Introduction

Therapeutic interest in calcitriol (**1a**, 1α,25-dihydroxyvitamin D_3; Figure 1), which is the hormonally active form of vitamin D_3, and in some of its analogs, arises from their ability to control abnormal cellular processes by modulating cell differentiation, inhibiting cell proliferation and regulating apoptosis.[2] Several of these compounds, including calcitriol itself, are already marketed or are undergoing clinical trials for the treatment of skin disease and cancer.[2f,2g] Research is currently focused on the development of calcitriol analogs that have high antiproliferative activity against a broad spectrum of cancer cells while lacking significant calcemic effects.[2] The rational design of such analogs depends on a detailed knowledge of the mode of action of the parent hormone in its multiple physiological roles, including anti-cancer and immunoregulatory effects.[3] Currently it is known that these activities involve specific binding of calcitriol to vitamin D receptor (VDR),[4] heterodimerization of VDR with retinoid X receptor (RXR) and binding of VDR-calcitriol-RXR complex to a vitamin D response element (VDRE) in DNA, which is followed by transcription of the associated gene.[2] An understanding of the three-dimensional structures of VDR, vitamin D binding protein (DBP)[5] and the corresponding protein-ligand complexes is of crucial importance for gaining new insights into the mode of action of calcitriol. The tertiary structures of VDR and DBP have been studied by means of site-specific mutations,[6] affinity-photoaffinity labeling,[7] fluorescence measurements,[8] X-ray diffractometry,[9] and modeling.[9b,9c,10]

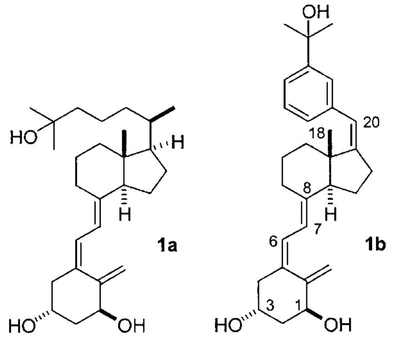

Figure 1. Structures of calcitriol (**1a**) and an aromatic side chain analog (**1b**)

Photoaffinity labeling technique (PAL) has been used to explore the ligand binding sites of a wide range of biomolecules, including steroids, peptide hormones and retinal. In this technique, a photosensitive functional group is activated photochemically to generate highly reactive intermediates such as nitrenes or carbenes, which form stable covalent bonds with proteins.[11] PAL studies of proteins related to vitamin D have used the triene system[12] of calcitriol itself and a 4-azido-2-nitro-phenyl moiety[7] attached to calcitriol through its 3-hydroxy group, but have not employed the aryltrifluoromethyldiazirinyl system, which has been reported to be superior in many cases to classical azido probes.[11,13,14] This, and the fact that replacement of a fragment of calcitriol by an aromatic ring can give rise to

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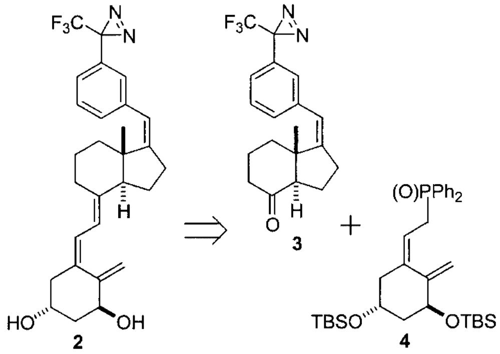
vitamin D analogs that bind significantly to VDR and/or have differentiation-promoting or antiproliferative effects, but with reduced calcemic activity,[15] has led us to consider the use of aryldiazirinyl units to prepare calcitriol-like PAL reagents. We describe here the synthesis of the first such vitamin D arylaziridine analog **2**, in which the side chain of the aromatic ring of analog **1b** (Scheme 1) has been replaced by a trifluoromethyldiazirinyl fragment. Compound **2** was chosen as our target because **1b** stimulates cell differentiation to the same degree as calcitriol itself, but with less calcemic effects.[16]



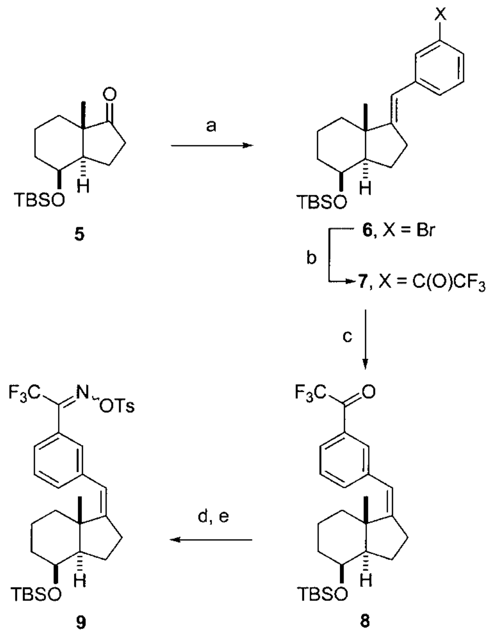
Scheme 1. Retrosynthesis of the diazirinylarylated vitamin D analog **2**

Results and Discussion

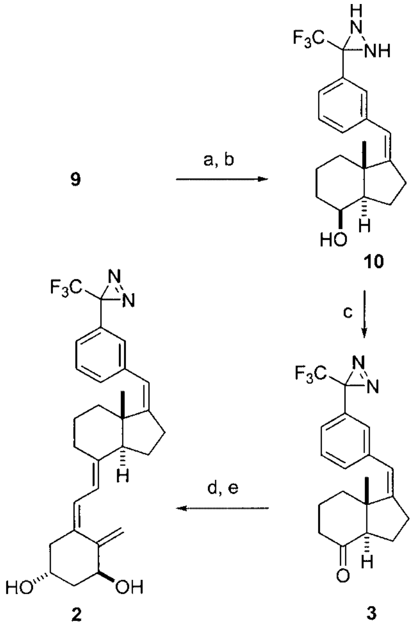
Our plan for the synthesis of vitamin D analog **2** was to use the convergent Lythgoe−Hoffman La Roche approach to the vitamin D triene system.[2a,2c] Key elements of the synthesis include the formation of the diazirine-bearing fragment **3** and its coupling with the lithium anion of phosphane oxide **4** (Scheme 1). The first of these stages began with the preparation of *O*-tosyloxime **9** from ketone **5**[17] (Scheme 2).

Ketone **5** was converted, as previously described,[16] to an approximately 4:1 mixture of bromide **6** and its *Z* diastereoisomer. Lithiation of these bromides (*n*BuLi, THF), followed by treatment with methyl trifluoroacetate, led to an 86% yield of trifluoroketones **7** in the same isomer ratio. Irradiation of a solution of this mixture in THF (450 W, Hanovia medium pressure-Hg lamp, Pyrex, 90 min) inverted the isomer ratio, giving an approximately 4:1 ratio of **8** and **7**, the stereochemistry of which was established by ^{1}H NMR NOE experiments and by comparison of their ^{1}H NMR spectra with those of similar compounds.[18] *O*-tosyloxime **9** was formed by treatment of **8** with hydroxylamine, followed by conventional tosylation of the resulting oxime (82% from **8**).[19]

Scheme 3 shows the formation of the diazirine-bearing upper fragment **3** and its coupling to vitamin D ring A in the form of phosphane oxide **4**. Reaction of *O*-tosyloxime **9** with liquid ammonia, followed by removal of the silyl protecting group, produced the diaziridine **10** in excellent yield.[20] Treatment of alcohol **10** with pyridinium dichromate (PDC) resulted in the oxidation of both the hydroxyl and diaziridine groups, giving the desired diazirine **3** in 87% yield.[21]



Scheme 2. a: Diethyl (3-bromobenzyl)phosphonate, KH, THF, Δ (69%); b: *n*BuLi, THF, CF$_3$COOMe (86%); c: hν, THF (75%); d: H$_2$NOH·HCl, py, EtOH, Δ (90%); e: *p*-TsCl, Et$_3$N, DMAP, CH$_2$Cl$_2$ (91%)



Scheme 3. a: NH$_3$, Et$_2$O, −78 °C (99%); b: HF aq., CH$_3$CN (99%); c: PDC, CH$_2$Cl$_2$ (87%); d: **4**, THF, *n*BuLi, −78 °C; then **3**, −78 °C to room temp. (97%); e: TBAF, THF (99%)

Finally, it was gratifying to observe that the diazirine ring survived the Wittig−Horner reaction used to introduce the vitamin D triene system[13d,19] during the coupling of ketone **3** to the anion of phosphane oxide **4**.[22] Removal of the *tert*-butyldimethylsilyl (TBS) groups from the resulting protected vitamin D compound with tetra-*n*-butylammonium fluoride (TBAF),[23] gave the desired calcitriol analog **2** (96% yield from **3**).

In summary, the first synthesis of a diazirine-bearing photoactivable calcitriol analog has been achieved and should be easily generalizable to related analogs. The synthesis is concise (nine steps, 30% yield from ketone **5**), and starts from commercially available vitamin D_2. Diazirinylarylation of the upper vitamin D fragment does not prevent the use of the mild, convergent Lythgoe Wittig−Horner approach.

Experimental Section

General Methods. All reactions involving oxygen- or moisture-sensitive compounds were carried out under a dry argon atmosphere. Reaction temperatures refer to external bath temperatures. All dry solvents were distilled under argon immediately prior to use. Tetrahydrofuran (THF) was distilled from Na/benzophenone; dichloromethane (CH_2Cl_2) was distilled from P_2O_5; absolute ethanol was distilled from Mg/I_2; pyridine was distilled from KOH and CaH_2. 4-(Dimethylamine)pyridine (DMAP) was purchased from Aldrich and used without further purification. Liquid reagents or solutions of reagents were added by syringe or cannula. Photochemical reactions were performed in a Pyrex glass reactor using a Hanovia lamp (450 W) of medium mercury pressure. Organic extracts were dried over anhydrous Na_2SO_4, filtered and concentrated using a rotary evaporator at aspirator pressure (20−30 Torr). Reactions were monitored by thin-layer chromatography (TLC) using aluminum-backed Merck 60 silica gel plates (0.2 mm thickness); the chromatograms were visualized first with ultraviolet light (254 nm) and then by immersion in a solution of phosphomolybdic acid in MeOH (5%), followed by heating. Flash column chromatography was performed with Merck 60 (230−400 mesh) silica gel. All NMR spectra were measured with solutions in $CDCl_3$ unless otherwise stated. Chemical shifts are reported on the δ scale (ppm) downfield from tetramethylsilane (δ = 0.0 ppm) using the residual solvent signal at δ = 7.26 ppm (^1H) or δ = 77.0 ppm (^{13}C) as internal standard; coupling constants are reported in Hz. Distorsionless Enhancement by Polarization Transfer (DEPT) was used to assign carbon types. Mass spectra were obtained using electron-impact ionization at 70 eV. Melting points were measured in open capillary tubes and are uncorrected.

8β-*tert*-Butyldimethylsilyloxy-17(*E*/*Z*)-{1-[3-(2,2,2-trifluoroacetyl)-phenyl]methylidene}de-A,B-androstane (7): A solution of *n*BuLi (2.35 M in hexanes, 0.67 mL, 1.58 mmol) was added dropwise with a syringe to a solution of bromide **6** (625 mg, 1.44 mmol; 1:4 *E*:*Z* mixture) in dry THF (10 mL) at −78 °C. The resulting pale yellow solution was stirred at −78 °C for 45 min. Freshly distilled $MeOC(O)CF_3$ (434 μL, 4.32 mmol) was then added slowly. The solution was stirred at −78 °C for 1.5 h and then warmed to room temp. over 40 min. The reaction was quenched with NH_4Cl/H_2O (1:4). The mixture was extracted with EtOAc and the combined organic phases were washed with brine, dried, filtered, and concentrated in vacuo. The residue was purified by flash chromatography (100% hexanes) to give 558 mg of **7** [86%, R_f = 0.63 (10% EtOAc/hexanes), yellow oil]. The mixture of *Z* and *E* isomers was used directly for the next reaction. ^1H NMR ($CDCl_3$, 250 MHz): δ = 0.01 (s, 3 H, MeSi), 0.03 (s, 3 H, MeSi), 0.05 (s, 6 H, MeSi), 0.89 (s, 9 H, *t*BuSi *Z*), 0.91 (s, 9 H, *t*BuSi *E*), 1.13 (s, 3 H, Me-18 *E*), 1.27 (s, 3 H, Me-18 *Z*), 2.39 (m, 1 H, H-16 *Z*),2.48 (m, 1 H, H-16 *E*), 2.68 (m, 1 H, H-16 *Z*), 2.81 (m, 1 H, H-16 *E*), 4.06 (m, 1 H, H-8 *Z*), 4.14 (m, 1 H, H-8 *Z*), 6.06 (s, 1 H, H-20 *E*), 6.19 (s, 1 H, H-20 *Z*), 7.47 (t, *J* = 7.7 Hz, 1 H, H-Ar),7.49 (m, 3 H, H-Ar), 7.64 (d, *J* = 7.7 Hz, 1 H, H-Ar), 7.85 (d, *J* = 7.8 Hz, 1 H, H-Ar),7.87 (s, 1 H, H-Ar), 8.02 (s, 1 H, H-Ar) ppm. ^{13}C NMR ($CDCl_3$, 62.83 MHz): δ = −5.2 (CH_3 *Z*), −5.2 (CH_3 *E*), −4.9 (CH_3 *Z*), −4.9 (CH_3 *E*), 17.5 (CH_2 *Z*), 17.6 (CH_2 *E*), 18.0 (C *Z*), 18.0 (C *E*), 20.6 (CH_3 *Z*), 21.6 (CH_3 *E*), 23.0 (CH_2 *Z*), 24.1 (CH_2 *E*), 25.8 (3 CH_3 *E*/*Z*), 28.4 (CH_2 *E*), 31.2 (CH_2 *Z*), 34.2 (CH_2 *Z*), 34.6 (CH_2 *E*), 36.8 (CH_2 *E*), 37.4 (CH_2 *Z*), 44.7 (C *Z*), 45.4 (C *E*), 50.5 (CH *E*), 52.5 (CH *Z*), 69.2 (CH *E*), 69.7 (CH *Z*), 115.0 (CH *E*), 116.8 (CF_3 *E*/*Z*, $J_{C,F}$ = 291.6 Hz), 117.1 (CH *Z*), 126.9 (CH *E*), 127.6 (CH *Z*), 128.1 (CH *Z*), 128.8 (CH *E*), 129.2 (C *Z*), 129.5 (CH *E*), 129.9 (C *E*), 130.9 (CH *Z*), 135.0 (CH *E*), 136.4 (CH *Z*), 139.9 (C *E*), 140.1 (C *Z*), 156.6 (C *Z*), 158.9 (C *E*), 180.6 (C=O *E*/*Z*, q, $J_{C,F}$ = 34.8 Hz).

8β-*tert*-Butyldimethylsilyloxy-17(*Z*)-{1-[3-(2,2,2-trifluoroacetyl)-phenyl]methylidene}de-A,B-androstane (8). A solution of **7** (200 mg, 0.442 mmol, 4:1 *E*:*Z*) in dry THF (30 mL) was irradiated for 90 min and then concentrated. The residue, which consisted of a 4:1 *Z*:*E* mixture, was purified by flash chromatography (1% EtOAc/hexanes) to give 198 mg of **8** (99%). MS (FAB): *m*/*z* (%) = 453 (20) [MH^+], 451 (40) [$M^+ - H$], 395 (40) [$M^+ - C(CH_3)_3$], 383 (100) [$M^+ - CF_3$], 345 (37), 319 (95) [M^+ − OTBS], 251 (87) [M^+ − OTBS − CF_3]. HRMS (calcd. for $C_{25}H_{36}F_3O_2Si$): 453.2437; found 453.2439.

8β-*tert*-Butyldimethylsilyloxy-17(*Z*)-{1-[3-(2,2,2-trifluoro-1-hydroxyiminoethyl)phenyl]methylidene}de-A,B-androstane (precursor of 9): $H_2NOH{\cdot}HCl$ (74 mg, 1.06 mmol) was added to a solution of **8** (319 mg, 0.71 mmol, *Z*:*E*:4:1) in dry pyridine (8 mL) and dry EtOH (4 mL) and the resulting mixture was refluxed. After 4 h, H_2O was added and the aqueous layer was extracted with Et_2O. The combined organic fractions were washed with brine, dried, filtered and concentrated in vacuo. The residue was purified by flash chromatography (20% CH_2Cl_2/hexanes) to give 297 mg of the precursor of **9** [90%, R_f (*Z*) = 0.35 (10% EtOAc/hexanes), 4:1 *Z*:*E*]. This oxime was purified by successive flash chromatography, taking the head tubes at each stage. ^1H NMR ($CDCl_3$, 250 MHz): δ = −0.06 (s, 3 H, MeSi), −0.05 (s, 3 H, MeSi), 0.82 (s, 9 H, *t*BuSi), 1.17 (s, 3 H, Me-18), 2.58 (m, 1 H), 3.99 (m, 1 H, H-8), 6.12 (s, 1 H, H-20), 7.26 (m, 4 H, H-Ar), 8.92 (broad s, 1 H, H-Ar) ppm. ^{13}C NMR ($CDCl_3$, 62.83 MHz): δ = −5.1 (CH_3), −4.8 (CH_3), 17.5 (CH_2), 18.0 (C), 20.5 (CH_3), 23.0 (CH_2), 25.8 (3 CH_3), 31.1 (CH_2), 34.2 (CH_2), 37.1 (CH_2), 44.6 (C), 52.5 (CH), 69.7 (CH), 117.7 (CH), 120.6 (CF_3, q, $J_{C,F}$ = 274.9 Hz), 125.0 (C), 125.9 (CH), 127.6 (CH), 129.6 (CH), 131.4 (CH), 139.5 (C), 147.9 (C, q, $J_{C,F}$ = 32.3 Hz, C-CF_3), 155.7 (C) ppm. MS: *m*/*z* (%) = 467 (3) [M^+], 410 (13) [$M^+ - C(CH_3)_3$], 335 (26) [M^+ − OTBS], 75 (100). MS (FAB): *m*/*z* (%) = 468 (100) [MH^+], 452 (85) [M^+ − OH], 410 (20) [$M^+ - C(CH_3)_3$], 336 (75) [M^+ − OTBS]. HRMS (calcd. for $C_{25}H_{37}F_3NO_2Si$: 468.2546; found 468.2542.

8β-*tert*-Butyldimethylsilyloxy-17(*Z*)-{1-[3-(2,2,2-trifluoro-1-tosyloxyiminoethyl) phenyl]methylidene}-de-A,B-androstane (9). Dry Et_3N (111 μL, 0.79 mmol) and DMAP (1.3 mg, 0.01 mmol) were added to a solution of the previous oxime (247 mg, 0.53 mmol) in dry CH_2Cl_2 (12 mL). The resulting solution was stirred at room temp. for 45 min and then *p*-TsCl (152 mg, 0.79 mmol) was added. After 16 h of stirring in the dark the reaction was quenched with citric acid (0.2 M) and the aqueous phase was extracted with Et_2O. The combined organic fractions were washed with H_2O, dried, filtered and concentrated in vacuo. The residue was purified by flash chromatography (10% CH_2Cl_2/hexanes) to give 300 mg of pure **9** [91%, R_f = 0.45 (8% EtOAc/hexanes), colorless oil]. ^1H NMR ($CDCl_3$, 250 MHz) [*O*-Ts (*major*)]: δ = 0.00 (s, 3 H, MeSi), 0.01 (s, 3 H, MeSi), 0.88 (s, 9 H, *t*BuSi), 1.20 (s, 3 H, Me-18), 2.34 (m, 1 H), 2.43 (s, 3 H, Ar-*Me*), 2.61 (m, 1 H), 4.04 (m, 1 H, H-8), 6.11 (s, 1 H, H-20), 7.28 (m, 6 H, H-Ar), 7.87 (d, J = 8.3 Hz, 2 H, H-Ar) ppm. ^{13}C NMR ($CDCl_3$, 62.83 MHz) [*O*-Ts (*major*)]: δ = −5.2 (CH_3), −4.8 (CH_3), 17.5 (CH_2), 18.0 (C), 20.6 (CH_3), 21.7 (CH_3), 23.0 (CH_2), 25.8 (3 CH_3), 31.0 (CH_2), 34.2 (CH_2), 37.3 (CH_2), 44.6 (C), 52.5 (CH), 69.6 (CH), 116.6 (CF_3, $J_{C,F}$ = 385.0 Hz), 117.3 (CH), 126.2 (CH), 126.8 (C), 127.7 (CH), 129.1 (CH), 129.6 (CH), 129.8 (CH), 131.6 (C), 132.7 (CH), 139.7 (C), 145.8 (C), 154.4 (C, q, $J_{C,F}$ = 33.4 Hz), 156.4 (C) ppm. MS: *m*/*z* (%) = 490 (10) [M^+ − TBS], 450 (32), 318 (59) [M^+ − Ts, − OTBS], 133 (100). HRMS (calcd. for $C_{25}H_{37}F_3NO_2Si$): 468.2546; found 468.2542.

^1H NMR ($CDCl_3$, 250 MHz) [*O*-Ts (*minor*)]: δ = 0.00 (s, 3 H, MeSi), 0.01 (s, 3 H, MeSi), 0.89 (s, 9 H, *t*BuSi), 1.21 (s, 3 H, Me-18), 2.43 (s, 3 H, Ar-*Me*), 2.63 (m, 1 H), 4.04 (m, 1 H), 6.13 (s, 1 H, H-20), 7.30 (d, J = 8.2 Hz, 2 H, H-Ar), 7.35 (m, 4 H, H-Ar), 7.85 (d, J = 8.3 Hz, 2 H, H-Ar) ppm. ^{13}C NMR ($CDCl_3$, 62.83 MHz) [*O*-Ts (*minor*)]: δ = −5.2 (CH_3), −4.9 (CH_3), 17.5 (CH_2), 18.0 (C), 20.7 (CH_3), 21.7 (CH_3), 23.0 (CH_2), 25.8 (3 CH_3), 31.0 (CH_2), 34.2 (CH_2), 37.2 (CH_2), 44.6 (C), 52.5 (CH), 69.7 (CH),117.3 (CH),119.6 (CF_3, q, $J_{C,F}$ = 277.7 Hz), 123.8 (C), 125.6 (CH),127.9 (CH),129.1 (CH),129.2 (CH), 129.8 (CH),131.4 (C), 132.3 (CH),139.8 (C), 146.0 (C),154.3 (C, q, $J_{C,F}$ = 33.4 Hz), 156.1 (C).

8β-*tert*-Butyldimethylsilyloxy-17(*Z*)-{1-[3-[(trifluoromethyl)-1,2-diaziran-3-yl]phenyl]methylidene}de-A,B-androstane (precursor of 10): Liquid NH_3 (2 mL) was added dropwise with a syringe to a solution of tosylate **9** (400 mg, 0.643 mmol) in dry Et_2O (30 mL) at−78 °C. The resulting solution was stirred at −78 °C for 2 h and then warmed to room temp. over 10 h before the reaction was quenched with H_2O (30 mL). The mixture was extracted with Et_2O and the combined organic fractions were dried, filtered, and concentrated in vacuo. The residue was purified by flash chromatography (3% EtOAc/hexanes) to give 300 mg of pure diaziridine [99%, R_f = 0.36 (8% EtOAc/hexanes), colorless oil]. ^1H NMR ($CDCl_3$, 250 MHz): δ = −0.01 (s, 3 H, MeSi), 0.00 (s, 3 H, MeSi), 0.87 (s, 9 H, *t*BuSi), 1.23 (s, 3 H, Me-18), 2.20 (broad s, 1 H, NH), 2.31 (m, 1 H), 2.62 (m, 1 H), 2.73 (broad s, 1 H, NH), 4.03 (s, 1 H),6.15 (s, 1 H, H-20), 7.23 (m, 2 H, H-Ar), 7.39 (m, 2 H, H-Ar) ppm. ^{13}C NMR ($CDCl_3$, 62.83 MHz): δ = −5.1 (CH_3), −4.8 (CH_3), 17.5 (CH_2), 18.0 (C), 20.5 (CH_3), 20.7 (CH_3), 23.0 (CH_2), 25.8 (3 CH_3), 31.1 (CH_2), 34.2 (CH_2), 37.2 (CH_2), 37.3 (CH_2), 44.5 (C), 52.6 (CH), 58.0 (C, q, *C*-CF_3, J = 35.7 Hz), 69.7 (CH), 117.8 (CH), 123.6 (C, q, CF_3, J = 278.6 Hz), 125.4 (CH), 127.7 (CH), 129.1 (CH), 130.7 (C), 131.0 (C), 131.1 (CH), 139.6 (C), 155.5 (C), 155.6 (C) ppm. MS (FAB): *m*/*z* (%) = 467 (100) [MH^+], 452 (47) [M^+ − N], 409 (17) [M^+ − $C(CH_3)_3$], 335 (60) [M^+ − OTBS], 318 (15), 239 (21). HRMS (calcd. for $C_{25}H_{38}F_3N_2OSi$): 467.2706; found 467.2701.

17(*Z*)-{1-[3-[(Trifluoromethyl)-1,2-diaziran-3-yl]phenyl]methylidene}de-A,B-androstan-8β-ol (10): An aqueous solution of HF (48%, 50 drops) was added dropwise to a solution of the previous diaziridine (200 mg, 0.429 mmol) in acetonitrile (10 mL). The resulting heterogeneous mixture was stirred at room temp. for 2 h, and then treated with saturated aqueous $NaHCO_3$ and the aqueous portion was extracted with Et_2O. The combined organic fractions were dried, filtered, and concentrated in vacuo. Flash chromatography (10−40% Et_2O/hexanes) afforded 150 mg of **10** [99%, R_f = 0.28 (40% Et_2O/hexanes), colorless oil]. ^1H NMR ($CDCl_3$, 250 MHz): δ = 1.19 (s, 3 H, Me-18), 2.18 (d, J = 8.69 Hz, 1 H, NH), 2.30 (m, 1 H), 2.61 (m, 1 H), 2.72 (d, J = 8.69 Hz, 1 H, NH), 4.04 (m, 1 H, H-8), 6.12 (s, 1 H, H-20), 7.23 (m, 4 H, H-Ar) ppm. ^{13}C NMR ($CDCl_3$, 62.83 MHz): δ = 17.2 (CH_2), 20.2 (CH_3), 22.5 (CH_2), 30.9 (CH_2), 33.5 (CH_2), 37.2 (CH_2), 44.2 (C), 52.1 (CH), 58.0 (C, q, *C*-CF_3, J = 35.8 Hz), 69.4 (CH), 118.2 (CH), 123.5 (C, q, CF_3, J = 278.4 Hz), 125.5 (CH), 127.7 (CH), 127.8 (CH), 129.0 (CH), 130.8 (C), 130.9 (CH), 131.0 (CH), 139.3 (C), 139.4 (C), 154.7 (C), 154.8 (C) ppm. MS: *m*/*z* (%) = 353 (100) [MH^+], 352 (19) [M^+], 335 (72.5) [MH^+ − H_2O], 147 (81). MS (FAB): *m*/*z* (%) = 353 (100) [MH^+], 335 (31) [M^+ − H_2O], 237 (65). HRMS (calcd. for $C_{19}H_{24}F_3N_2O$): 353.1841; found 353.1842.

17(*Z*)-{1-[3-[(Trifluoromethyl)-3*H*-1,2-diaziren-3-yl]phenyl]methylidene}de-A,B-androstan-8-one (3): PDC (279 mg, 0.74 mmol) was added to a solution of **10** (130 mg, 0.37 mmol) in CH_2Cl_2 (13 mL). After stirring for 9 h the resulting suspension was filtered through celite and concentrated. The resulting residue was purified by flash chromatography (10% Et_2O/hexanes) to afford 112 mg of pure **3** [87% (2 steps), R_f = 0.72 (40% Et_2O/hexanes), colorless oil]. ^1H NMR ($CDCl_3$, 250 MHz): δ = 0.88 (s, 3 H, Me-18), 2.48 (m, 3 H), 6.23 (s, 1 H, H-20), 6.92 (m, 2 H, H-Ar), 7.18 (m, 2 H, H-Ar) ppm. ^{13}C NMR ($CDCl_3$, 62.83 MHz): δ = 19.3 (CH_2), 19.3 (CH_3), 23.4 (CH_2), 28.3 (C, $J_{C,F}$ = 40.2 Hz, *C*-CF_3), 30.7 (CH_2), 35.3 (CH_2), 40.7 (CH_2), 124.1 (CH), 50.6 (C), 61.3 (CH), 119.8 (CH), 122.1 (C, $J_{C,F}$ = 274.7 Hz, CF_3), 127.2 (CH), 128.2 (CH), 128.5 (C), 130.3 (CH), 139.0 (C), 152.7 (C), 210.9 (C=O) ppm. MS (FAB): *m*/*z* (%) = 371 (8) [M^+ + Na], 349 (100) [MH^+], 320 (15) [M^+ − N_2H_2]. HRMS (calcd. for $C_{19}H_{20}F_3N_2O$): 349.1528; found 349.1527.

(17*Z*)-1α-*tert*-Butyldimethylsilyloxy-20-[3-[(trifluoromethyl)-3*H*-1,2-diaziren-3-yl]phenyl]-17,20-didehydro-21,22,23,24,25,26,27-heptanorvitamin D_3 *tert*-Butyldimethylsilyl Ether (precursor of 2): A solution of *n*BuLi (2.25 M, 0.22 mL, 0.50 mmol) was added dropwise by syringe to a solution of the phosphane oxide **4** (325 mg, 0.558 mmol) in THF (9 mL) at −78 °C. The resulting deep red solution was stirred at −78 °C for 1.5 h and a solution of ketone **3** (93 mg, 0.267 mmol) in THF (5 mL) was then added slowly. The red solution was stirred in the dark at −78 °C for 4.5 h and then warmed to −40 °C over 2 h. The reaction was quenched with H_2O. The mixture was extracted with Et_2O and the combined organic fractions were washed with brine, dried, filtered, and concentrated in vacuo. The residue was purified by flash chromatography (2−80% Et_2O/hexanes) to give 184 mg of pure protected analog [97%, R_f = 0.81 (1% Et_2O/hexanes), colorless oil]. ^1H NMR (CD_2Cl_2, 250 MHz): δ = 0.09 (s, 6 H, Me_2Si), 0.12 (s, 6 H, Me_2Si), 0.91 (s, 9 H, *t*BuSi), 0.92 (s, 3 H, Me-18), 0.93 (s, 9 H, *t*BuSi), 4.22 (m, 1 H), 4.43 (m, 1 H), 4.91 (broad s, 1 H), 5.25 (broad s, 1 H), 6.27, 6.14 (AB, J = 11.3 Hz, 2 H), 6.31 (s, 1 H, H-20), 6.99 (d, J = 7.00 Hz, 1 H, H-Ar), 7.08 (s, 1 H, H-Ar), 7.28 (m, 2 H, H-Ar) ppm. ^{13}C NMR (CD_2Cl_2, 62.83 MHz): δ = −4.7 (CH_3), −4.5 (CH_3), −4.4 (CH_3), −4.3 (CH_3), 18.6 (C), 18.7 (C), 19.0 (CH_3), 22.9 (CH_2), 23.7 (CH_2), 26.2 (6 CH_3), 29.0 (C, q, $J_{C,F}$ = 40.3 Hz, *C*-CF_3), 29.2 (CH_2), 32.1 (CH_2), 37.4 (CH_2), 45.5 (CH_2), 46.6 (CH_2), 47.8 (C), 56.9 (CH), 68.1 (CH), 72.6 (CH), 111.7 (CH_2), 119.4 (CH),119.8 (CH), 122.9 (C, q, $J_{C,F}$ = 274.7 Hz, CF_3), 123.5 (CH), 124.3 (CH), 127.9 (CH), 128.6 (C), 128.8 (CH), 131.1 (CH), 136.4 (C), 140.4 (C), 140.4 (C), 149.1 (C), 155.8 (C).

(17*Z*)-1α-20-[3-[(Trifluoromethyl)-3*H*-1,2-diaziren-3-yl]phenyl]-17,20-didehydro-21,22,23,24,25,26,27-heptanorvitamin D_3 (2): A solution of TBAF (1.11 M in THF, 3.7 mL, 4.13 mmol) was added by syringe to a solution of the protected analog (140 mg, 0.196 mmol) in THF (2 mL). After stirring at room temp. for 6 h in the dark, a solution of NH_4Cl was added and the resulting mixture was extracted with Et_2O. The combined organic extracts were dried, filtered, and concentrated in vacuo to give a residue that was purified by flash chromatography (50−100% Et_2O/hexanes) to afford 94 mg of pure vitamin D analog **2** [99%, $R_f = 0.35$ (50% EtOAc/hexanes), pale yellow solid]. ^1H NMR (CD_2Cl_2, 250 MHz): $\delta = 0.80$ (s, 3 H, Me-18), 4.08 (m, 1 H), 4.30 (m, 1 H), 4.89 (1 H, broad s), 5.22 (broad s, 1 H), 6.20 (s, 1 H, H-20), 6.23, 6.01 (AB, $J = 11.1$ Hz, 2 H), 6.88 (d, $J = 7.7$ Hz, 1 H, H-Ar), 6.96 (s, 1 H, H-Ar), 7.20 (m, 2 H, H-Ar) ppm. ^{13}C NMR (CD_2Cl_2, 62.83 MHz): $\delta = 18.7$ (CH_3), 22.8 (CH_2), 23.6 (CH_2), 28.8 (C, q, $J_{C,F} = 40.3$ Hz, C-CF_3), 29.2 (CH_2), 31.8 (CH_2), 37.1 (CH_2), 43.3 (CH_2), 45.6 (CH_2), 47.7 (C), 56.7 (CH), 67.1 (CH), 71.0 (CH), 111.9 (CH_2), 118.5 (CH), 119.7 (CH), 122.7 (C, q, $J_{C,F} = 274.7$ Hz, CF_3), 124.2 (CH), 124.6 (CH), 127.7 (CH), 128.5 (C), 128.6 (CH), 130.9 (CH), 134.7 (C), 140.2 (C), 141.9 (C),148.4 (C), 155.5 (C). UV (2-propanol): λ_{max} = 260 nm, 364 nm. $C_{28}H_{31}F_3N_2O_2$ (484.6): calcd. C 69.39, H 6.45, N 5.78; found C 69.20, H 7.17, N 5.36.

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