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Design and synthesis of active vitamin D analogs☆

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ABSTRACT

A review of the design and synthesis of structural analogs of the vitamin D hormone recently investigated in our laboratories, and the first report on a new class of vitamin D analogs characterized by an aromatic D-ring, is described.

1. Introduction

1α,25-Dihydroxyvitamin D_3 [**1**, 1α,25-$(OH)_2$-D_3, Fig. 1], the hormonally active metabolite of the seco-steroid vitamin D_3, interacts with the vitamin D nuclear receptor (VDR) [1] to initiate a cascade of events ultimately to control mineral homeostasis and a multitude of cellular processes including differentiation, anti-proliferation, growth, apoptosis, angiogenesis and immunomodulation [2]. The discovery of the VDR in more than 30 tissues including skin, brain, heart, pancreas, kidney, intestine, colon, prostate, ovary, and breast [3] has led to the targeting of VDR as a possible therapy for diseases such as cancer, psoriasis, rickets, renal osteodystrophy, and autoimmunity (multiple sclerosis, rheumatoid arthritis, inflammatory diseases, and type I diabetes) [4,5]. The natural VDR ligand, 1α,25-$(OH)_2$-D_3 (or its prodrug 1α-(OH)-D_3), is an established clinical treatment for renal osteodystrophy and various types of rickets [6] but the investigation of pharmacological doses for treatment of a wide variety of other diseases, including breast and prostate cancers, autoimmune diseases, psoriasis, and osteoporosis [7] has been limited by the parallel induction of hypercalcemic effects [2c,8]. More than 3000 vitamin D_3 analogs have been synthesized in recent years in an attempt to find therapeutic agents with low calcemic activity, but only a few have reached advanced clinical trials [3b,9]. A notable example is calcipotriol (Dovonex/Daivonex LEO) on the market since 1991 for the topical treatment of psoriasis [10].

The development of new compounds like calcipotriol took place in an era when design was essentially based on systematic structural variations. In the 21st century there has been real progress in the rational design of new analogs of 1α,25-$(OH)_2$-D_3 with selective biological functions as potential drugs.

2. Structure–function relationships

Our understanding of the structure–function relationships of vitamin D has emerged during the last 40 years. The investigation of the topological orientations of the 1α-OH group and the C-17 side chain and the nature of the triene system has been of particular importance (Fig. 1).

In 1974, Okamura et al. established a relationship between the molecular topology and biological function for the natural hormone 1α,25-$(OH)_2$-D_3 and other 1α-hydroxylated analogs. The Riverside group proposed that 1α,25-$(OH)_2$-D_3 exerts its biological functions through the A-ring chair conformation possessing the C1-OH group equatorially oriented [11]. This conclusion was based on the finding that dihydrotachysterol$_3$ (DHT_3), one of the four 10,19-dihydrovitamins D_3, was the most biologically active stereoisomer. DHT_3 was shown by NMR spectroscopy to contain the highest population of the chair conformer with the 3β- or pseudo-1α-hydroxyl group equatorially oriented [12]. On the basis of crystal structures, Suwinska and Kutner have also suggested the same β-chair of 1α,25$(OH)_2D_3$ as the active conformer [13].

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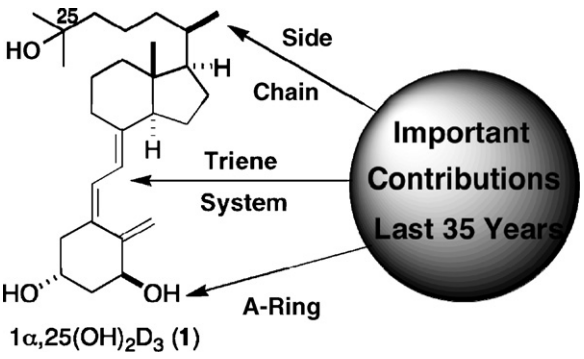

Fig. 1. 1α-Hydroxyl group (A-ring), triene system and side-chain.

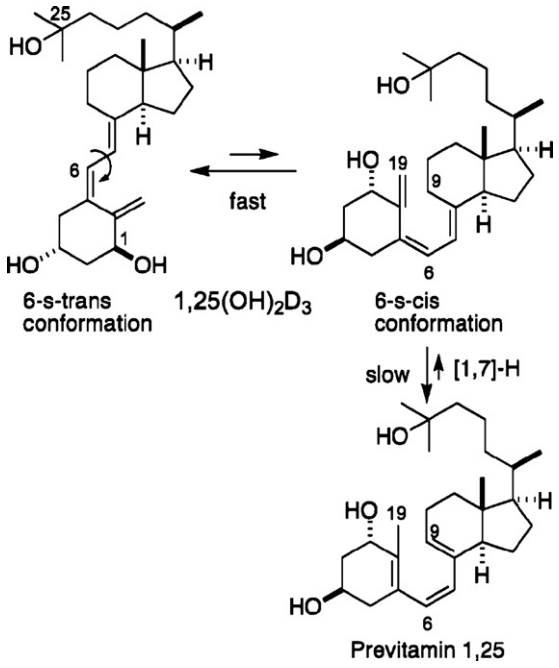

Fig. 2. [1,7]-sigmatropic hydrogen shift to produce 1α,25-dihydroxyprevitamin D_3.

With regard to the nature of the vitamin D triene system, the s-*cis* conformation is required for equilibration with the corresponding pre-1α,25$(OH)_2D_3$ form via a [1,7]-H sigmatropic shift (by hydrogen migration from C-9 to C-19) (Fig. 2). Studies carried out in Wisconsin, Belgium and Santiago on the corresponding 19-nor derivatives of the 1α,25-$(OH)_2$-D_3 forms, which cannot equilibrate due to the lack of the 19-carbon (Fig. 3), demonstrated that the pre-1α,25-$(OH)_2$-D_3 form does not induce genomic biological actions, while the corresponding vitamin D form does. This result indicates that the natural hormone induces genomic responses through the

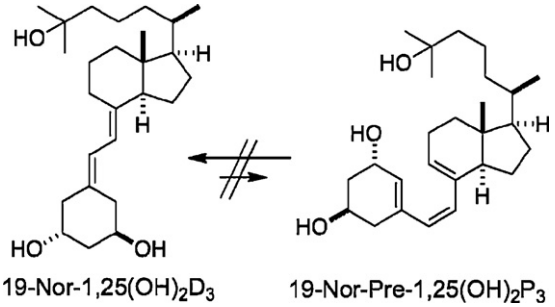

Fig. 3. The Vitamin D-Previtamin D equilibrium is forbidden for 19-nor-1α,25$(OH)_2D_3$.

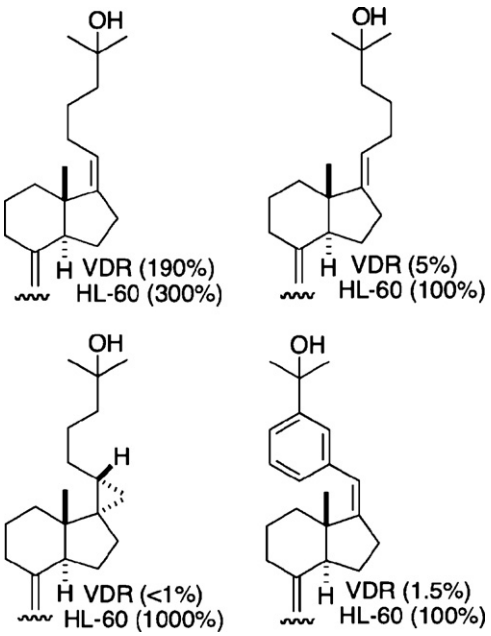

Fig. 4. Four 1α,25$(OH)_2D_3$ analogs with locked units at C17-C20.

vitamin D form, although no conclusion was established at this time about the roles on the biological action of the 6-s-*trans* and the 6-*cis* conformations [14].

The groups led by Okamura and Midland at Riverside and Yamada in Tokyo [15] used conformational analysis of the side chain of the natural hormone and their analogs to understand conformation–function relationships [3b,16]. They constructed dot maps to illustrate the volume in space occupied by the side chain and location for the 25-hydroxyl group. However, this volume was too large to define the precise orientation of the side chain and location of the 25-OH group in the binding pocket. This concept was refined by Okamura's group to vitamin D analogs bearing side chains with rigid fragments in the form of aromatic or allenic units to reduce the active space volume [16]. In order to define a smaller occupancy volume to understand the topology of the bioactive side-chain conformation of 1α,25$(OH)_2D_3$, the Santiago group synthesized a series of partially rigid side-chain analogs which incorporate rigid units closer to the rigid D-ring (Fig. 4) [17a,b]. The conformationally locked units used were a double bond or a cyclopropane ring at C17–C20 [17a], or a double bond at C17–C20 conjugated with an aromatic ring [17b]. Interestingly, all four compounds induced HL-60 cell differentiation with at least the same potency as the natural hormone. However, three of these compounds did not show any significant competitive binding *in vitro* to the calf thymus VDR showing once again [17c] that there is clearly no correlation between binding and biological activity. The outcome of these studies together with simple plastic molecular models was to indicate that the bioactive 25-hydroxyl group of 1α,25-$(OH)_2$-D_3 should adopt a "Northern" orientation [18].

Soon afterwards, the Strasbourg group published the crystal structure of an engineered ligand binding domain of the vitamin D receptor (VDR LBD) that lacks a flexible insertion domain between helices H1 and H3. The mutant VDR bound to 1α,25$(OH)_2D_3$ [VDR(Moras)-1,25 complex] exhibits similar conformation, transactivation ability, and biophysical properties than the wild-type counterpart [19]. The crystal structure of VDR(Moras)-1,25 complex shows the hydrogen bonding nature of the interactions between each of the three hydroxyl groups of the ligand with the mutant vitamin D receptor (1α-OH with both Ser-237 and Arg-274, 3β-OH and both Tyr-143 and Ser-278, and 25-OH and both His-305

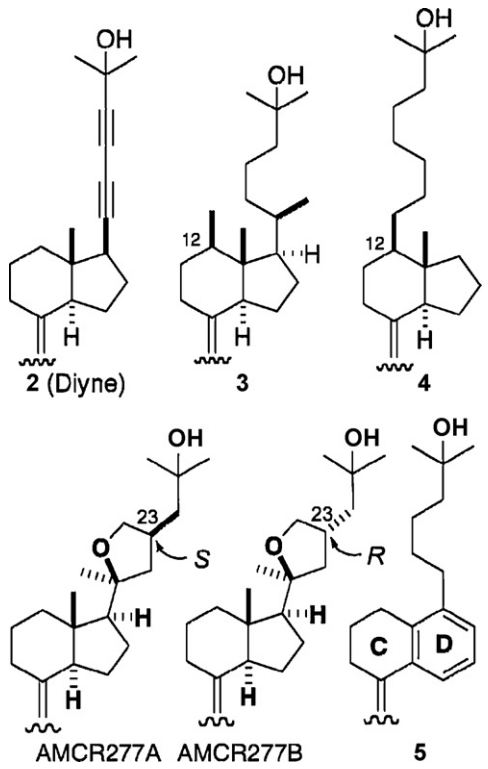

Fig. 5. Binding to VDR and biological activity of vitamin D analogs in comparison with 1α,25(OH)$_2$D$_3$ (100%). Analog **2**: VDR (15%), Transactivation (1250%, 10^{-9} M). Analog **3**: VDR (440%), Transactivation (108%). Analog **4**: VDR (70%), Transactivation (17%, 10^{-7} M, 100%, 10^{-5} M). **AMCR277A**: Transactivation (1200%, 10^{-10} M). **AMCR277B**: Transactivation (100%).

and His-397). The structural details of the binding pocket allowed for the first time the rational design of new active vitamin D analogs.

3. Rational design of active vitamin D analogs

Our continued interest in the synthesis of 1α,25(OH)$_2$D$_3$ side-chain analogs with a high degree of rigidity to define the topography of the 25-OH group that induces transcription led us to use the crystallographic structure of the complex VDR(Moras)-1,25 to design diyne **2** (Fig. 5), which incorporates two adjacent triple bonds at the side chain. The synthesis of this analog was disclosed independently by the Santiago group [20a] and by Martin Calverley at LEO-Pharma [20b], who reported a binding affinity of 11% relative to the natural hormone for the bovine thymus VDR. Its biological evaluation was carried out by the Muñoz group at Madrid and crystallographic studies were accomplished in Moras' Laboratory [20c]. The diyne **2** induced transactivation 12.5 times higher than the natural hormone. The crystallographic structure of the complex VDR(Moras)-**2** gives important structural information on the bioactive conformation of the side chain of the natural hormone.

The observation of a "hole" near C-12 of 1α,25(OH)$_2$D$_3$ in the crystalline complex with VDR(Moras), led us to explore for the first time the biological behavior of new 1α,25(OH)$_2$D$_3$ analogs with substituents at this position. We first synthesized 12β-methyl-1α,25(OH)$_2$D$_3$ (**3**) (Fig. 5). The binding affinity of analog **3** is 4.4 times higher than that of 1α,25(OH)$_2$D$_3$. The interesting biological profile of analog **3**, led us to explore the biological properties of new analogs with hydroxylated side chains at C12. Our docking studies into the binding pocket of the complex VDR(Moras)-1,25 showed that the hydroxyl group on the seven-carbon side-chain analog **4** is optimally placed for interaction with histidines H305 and H397. The binding affinity of **4** for VDR(Moras) was about 70% and the relative transactivation was 17% (10^{-7} M) or 100% (10^{-5} M) [21].

The superimposition of the three crystal structures corresponding to the complexes of VDR(Moras) with 1α,25(OH)$_2$D$_3$ and superagonists MC1288 and KH1060 indicates that the side chains of the three compounds in the binding pocket follow different pathways to reach the hydroxyl bearing carbon at a common point. The observation of the side-chain conformations suggested that a new analog with a tetrahydrofurane unit at the side chain might also behave as a new superagonist. Fig. 5 shows the structure of the proposed new superagonist AMCR277A and its epimer at C23 AMCR277B [22]. Superimposition of the crystal structures of VDR(Moras) complexed to AMCR277A and AMCR277B indicates that side chain of the first analog is closer to Val-418 residue. Considering that this amino-acid residue is important for transcription [19], the analog AMCR277A might be expected to be more active than AMCR277B. Remarkably, isomer AMCR277A induces transactivation of human VDR 12 times more efficiently than the natural hormone at concentrations of 10^{-10} M, confirming its predicted superagonistic nature. Isomer AMCR277B shows the same activity as the natural hormone [22].

4. Design and synthesis of a new class of vitamin D analogs with a benzene D-ring

The observation of a tryptophane residue (Trp-286) in the proximity of the D-ring of 1α,25(OH)$_2$D$_3$ in crystal complex

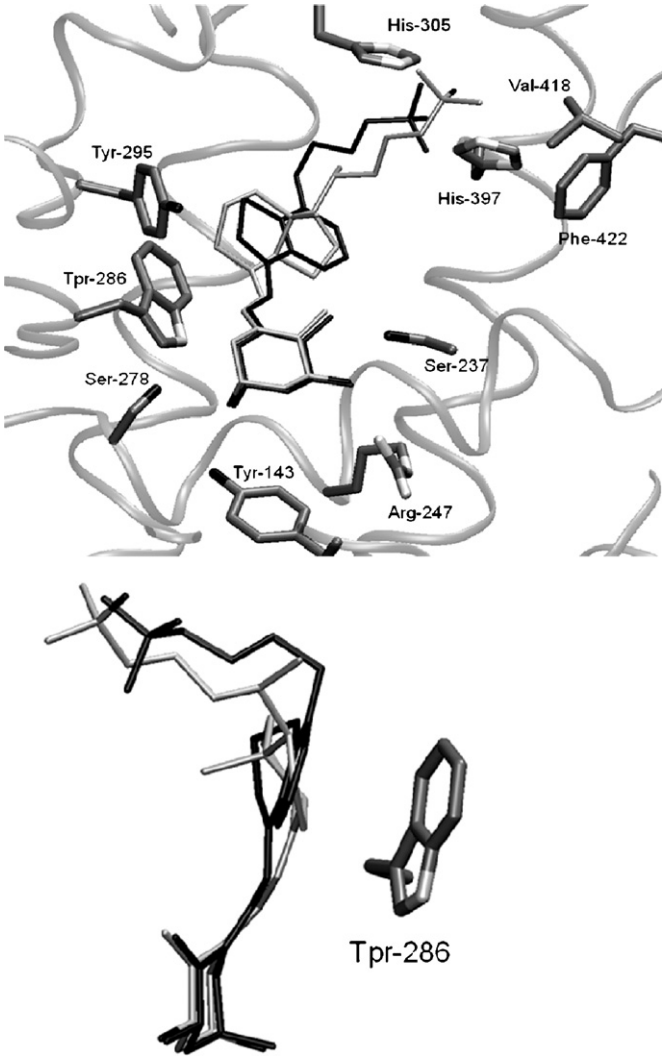

Fig. 6. Docking (Gold) of **5** into VDR(Moras)-1α,25(OH)$_2$D$_3$ crystal complex.

VDR(Moras)–1α,25(OH)$_2$D$_3$ led us to consider the possibility of developing a new class of vitamin D analogs with aromatic D-ring, for example **5** (Fig. 5). With this idea in mind, we decided to carry out docking studies of the new analog **5** into the VDR(Moras)–1α,25(OH)$_2$D$_3$ crystal complex. The low energy conformation shown in Fig. 6 fits reasonably well in the binding pocket although the side chain and CD moieties are slightly shifted with respect to those of 1α,25(OH)$_2$D$_3$. The CD fragment gets close to Tpr286 adopting a parallel orientation.

Attempts to synthesize analog **5** using Lythgoe's Wittig–Horner approach [23], Lythgoe's dienyne route [24], and Okamura's vinyallene approach [25] were unsuccessful. We recently disclosed a convergent strategy to vitamin D analogs in which the ring-A and triene unit are constructed by one-pot Pd-catalyzed tandem cyclization–Negishi coupling process involving an alkenyl zinc intermediate and a vinyl triflate [26]. On the basis this strategy, we envisioned that the triene system of our protected target vitamin D analog **5** could be formed using vinyl triflate **6** and alkenyl zinc **7** (Fig. 7).

Our attempts to synthesize **5** started with the known bromide **11** [27], which was coupled with EtO$_2$C(CH$_2$)$_4$ZnBr·LiCl in the presence of catalytic Pd(0) by the method of Knochel et al. [28] to produce **10**. The ketoester **10** was then converted to triflate **9** in 43% yield by the three steps sequence: olefination, enol formation and triflation. The resulting enol triflate **9** was converted to iodide **8** in 32% yield by the sequence: methylation, stannylation and iodination. The desired Zn intermediate **7** was prepared in the usual way the successive additions of *t*-BuLi and dry ZnBr$_2$ to **8**. Unfortunately, addition of a mixture of A-ring enol triflate **6** and Pd(0) to **7** in THF gave the olefin corresponding to the protonated product. Replacement of Et$_3$Si– (TES) protecting group by MeOCH$_2$– (MOM) only gave the corresponding homodimer in 85% yield. We cannot explain

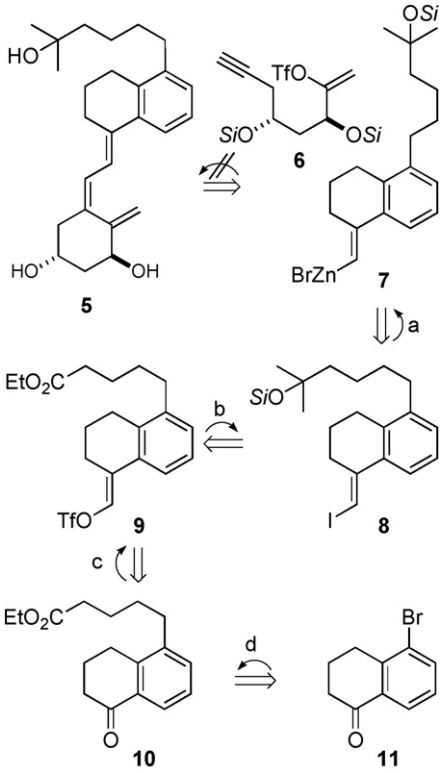

Fig. 7. Pd-catalyzed cyclization-coupling strategy. Attempts to synthesize the target analog **5**. (d) EtO$_2$C(CH$_2$)$_4$ZnBr.LiCl, Pd$_2$(dba)$_3$, (*t*-Bu)$_3$P, THF (83%). (c) Ph$_3$PCH=CHOMe, THF, (79%); HCO$_2$H (69%); Tf$_2$O, Et$_3$N, CH$_2$Cl$_2$ (79%). (b) MeLi, THF, −78 °C (71%); Et$_3$SiOTf (90%); (Me$_3$Sn)$_2$, (Ph$_3$P)$_4$Pd, LiCl, THF, △; I$_2$, Et$_2$O (50%, 2 steps).

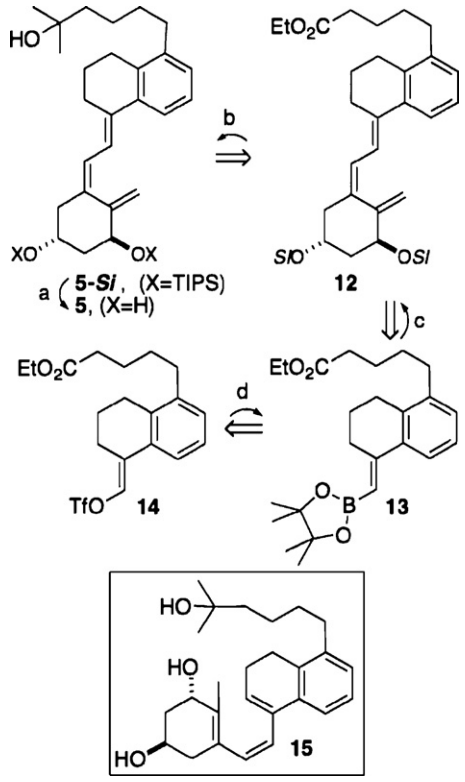

Fig. 8. Pd-catalyzed cyclization-coupling strategy. Synthesis of **5**. (d) PinH, (dppf)PdCl$_2$, Et$_3$N, dioxane, 75 °C (68%). (c) Enol triflate **13a**, (Ph$_3$P)$_2$PdCl$_2$, K$_3$PO$_4$ (2M), THF (55%). (b) MeLi, THF, −78 °C (88%). (a) aq. HF-48%, CH$_3$CN, CH$_2$Cl$_2$ (78%).

the preference of the coupling reaction for the homodimer rather than the vitamin D triene. The unexpected formation of the homodimer led us to consider the use of boron chemistry instead of Zn chemistry for the construction of the vitamin D triene of our target compound **5** (Fig. 8).

In the new synthetic plan (Fig. 8), the palladium catalyzed cyclization of enol triflate **6**, precursor of the A-ring fragment of vitamin D analogs, followed by Suzuki coupling of the resulting palladium intermediate with alkenyl boronic ester **13** would generate the desired vitamin D triene in one-pot process. Pd(0)-catalyzed reaction of enol triflate **14** with pinacolborane gave boronate **13** in 68% yield. In the key step, treatment of a THF solution of enol triflate **6**, boronate **13** and aqueous K$_3$PO$_4$ (2 M) with a catalytic amount of bis-triphenylphospine palladium (II) dichloride, afforded the desired vitamin D ester **12** in 55% yield. Methylation of **12** with methyllithium afforded the protected analog **5**-*Si* in 88% yield [29]. Finally desilylation of **5**-*Si* using aqueous hydrogen fluoride provided the desired analog **5** in 88% yield. Unexpectedly, this analog equilibrates largely to its previtamin D form **15** on standing in CDCl$_3$ (ratio **5**/**15** 1:2 after 120 h at room temperature as determined by ^{1}H NMR). Biological testing of **5** and the synthesis of the corresponding 19-nor derivative are in progress.

In summary, docking studies provide a rapid *in silico* method for "screening" large numbers of proposed analogs. The challenges now are to be able to synthesize many of the good candidates and to perform biological testing on them. We feel that our new route to the vitamin D-ring system at least goes a long way towards meeting the chemical challenge.

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the phosphine oxide (A-ring fragment); (b) the multistep preparation of the phosphine oxide required for coupling with the upper ketone (CD-side chain fragment), and (c) the low yield obtained in the formation of the triene unit when some C-ring or A-ring substituted fragments are used. For details, see:
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[29] **5**-*Si*: 1H NMR (250 MHz): 7.40 (1H, d, *J* = 7.1 Hz, H-15), 7.13–6.93 (3H, m), 6.34 (1H, d, *J* = 11.3 Hz, H-6), 5.38 (1H, s, H-19E), 4.98 (1H, s, H-19Z), 4.61 (1H, m, H-1), 4.37 (1H, m, H-3), 2.80–1.39 (19H, m), 1.23 (6H, s), 1.12–1.03 (42H, m). 13C NMR (62.89 MHz): 149.1 (C), 140.2 (C), 138.7 (C), 137.1 (C), 135.6 (C), 134.7 (C), 127.3 (CH), 125.5 (CH), 123.9 (CH), 122.1 (CH), 120.6 (CH), 111.6 (CH_2), 71.6 (CH), 71.0 (C), 67.8 (CH), 46.3 (CH_2), 45.2 (CH_2), 43.8 (CH_2), 33.3 (CH_2), 30.9 (CH_2), 29.3 (2× CH_3), 26.6 (CH_2), 26.4 (CH_2), 24.5 (CH_2), 23.1 (CH_2), 18.1 (12× CH_3), 12.4 (3× CH), 12.3 (3× CH). MSLR: *m*/*z* ([IQ]+, %):709 ($[M+H]^+$, 32), 708 ($[M]^+$, 33), 691 ($[M-OH]^+$, 37), 517 ($[M-H_2O\text{-}OTIPS]^+$, 100). 5: 1H NMR (500 MHz): 7.40 (1H, d, *J* = 7.7 Hz), 7.08 (1H, t, *J* = 7.7 Hz), 7.02 (1H, d, *J* = 7.1 Hz), 6.92 (1H, d, *J* = 11.4 Hz), 6.49 (1H, d, *J* = 11.4 Hz), 5.40 (1H, s), 5.08 (1H, s), 4.50 (1H, m), 4.27 (1H, m), 2.76–2.57 (7H, m), 2.39 (1H, m), 2.01 (2H, t, *J* = 5.6 Hz), 1.87 (2H, m), 1.63–1.42 (7H, m), 1.22 (6H, s).Compound **5** equilibrates with its previtamin D form on standing in CDCl3. Ratio **5**/**15** by 1H NMR (250 MHz): 48 h (1:1.3); 72 h (1:1.5); 96 h (1:1.8); 120 h(1:2).