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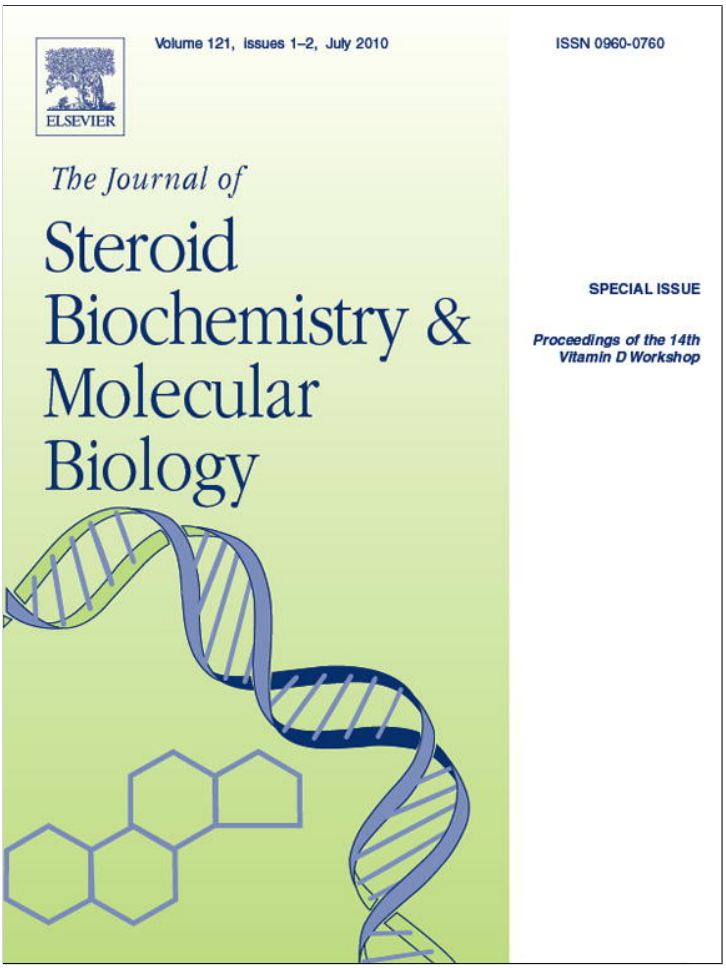

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# Synthesis of 2β-substituted-14-epi-previtamin $D_3$ and testing of its genomic activity☆

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ABSTRACT

2β-Substituted analogs of 14-epi-previtamin $D_3$ were synthesized for the first time by the thermal isomerization of the corresponding 14-epi-vitamin $D_3$ that were available using coupling reaction between the A-ring phosphine oxide derived from a chiral epoxide and CD-ring *cis*-hydrindanone. The VDR binding affinity and transactivation activity of osteocalcin promoter in HOS cells were evaluated, and the new analogs were found to be less active, 0.01–0.18% of VDR binding affinity compared with the natural hormone and $EC_{50}$ 1.0–9.1 nM for transactivation activity, than 14-epi-previtamin $D_3$ with 0.5% (VDR) and $EC_{50}$ 0.46 nM, respectively.



## 1. Introduction

It is well established that vitamin $D_3$ is present in thermal equilibrium with previtamin $D_3$ via [1,7]-sigmatropic rearrangement. In this equilibrium, the vitamin D form (**A**) with the 6-*s-trans* triene structure is more stable and dominant than the 6-*cis* isomer of the previtamin D form (**B**) (Scheme 1). The biologically most active metabolite of vitamin $D_3$, 1α,25$(OH)_2D_3$ (**1**), also contains 5–10% of its previtamin D form, 1α,25$(OH)_2$pre$D_3$ (**pre-1**) at 37 °C in similar equilibrium. The major isomer, the vitamin D form (**A**), has been the focus of therapeutic evaluation rather than the previtamin D form, because previtamin D is easily transformed to vitamin D through thermal equilibrium and is almost impossible to isolate in pure form [1]. While **1** is a ligand of the nuclear vitamin D receptor (VDR), regulates gene transcription, and exhibits various biological responses as a hormone [2], **pre-1** is thought to be a weak ligand of VDR and a poor activator of the above genomic actions [3]; however, **pre-1** has been studied as a responsible compound for rapid responses [4], such as stimulation of intestinal $Ca^{2+}$ transport (transcaltachia), activation of PKC and MAP kinases, and so on, which are called non-genomic actions [5].

Okamura and coworkers reported that the thermal equilibrium ratio between the vitamin D form (**A**) and previtamin D form (**B**) at 80 °C was reversed by epimerizing the CD-ring bridgehead hydrogen of C14 [6]. Briefly, 14-epi-1α,25$(OH)_2$pre$D_3$ (**14-epi-pre-1**) was major and dominant to 14-epi-1α,25$(OH)_2D_3$ (**14-epi-1**), and the former was isolated as a stable single isomer at room temperature. Therefore, we focused on the synthesis of **14-epi-pre-1** analogs with A-ring modification to investigate their more detailed biological properties and potential as therapeutic agents of the previtamin $D_3$ skeleton.

Previously, we found that 2α-alkyl and 2α-(ω-hydroxyalkyl) substitution afforded great enhancements for VDR binding affinity and the subsequent genomic actions [7]. In the preceding paper, we reported the synthesis and biological evaluations of 2α-substituted **14-epi-pre-1** [8]. Here, we prepared analogs with 2β-substitution (**14-epi-pre-1a–c**), because 2β-substitution is known as a important modification for vitamin D derivatization (Scheme 2) [9].

**14-epi-pre-1** could be prepared from **14-epi-1** by thermal isomerization; therefore, we synthesized **14-epi-1** analogs as temporary first targets. The **14-epi-1** analogs were divided into two fragments, CD-ring and A-ring, which were coupled by the Roche coupling method [10]. The CD-ring fragment **2** [6,8] is the known compound, which was obtained by epimerization at H14 in Grundmann's ketone derivative derived from vitamin $D_3$ [11,12]. The A-ring fragments, the phosphine oxides **3a–c**, could be synthesized from dimethyl D-tartrate, and we could introduce various alkyl groups at the 2β-position by nucleophilic epoxide ring-opening reactions [13].

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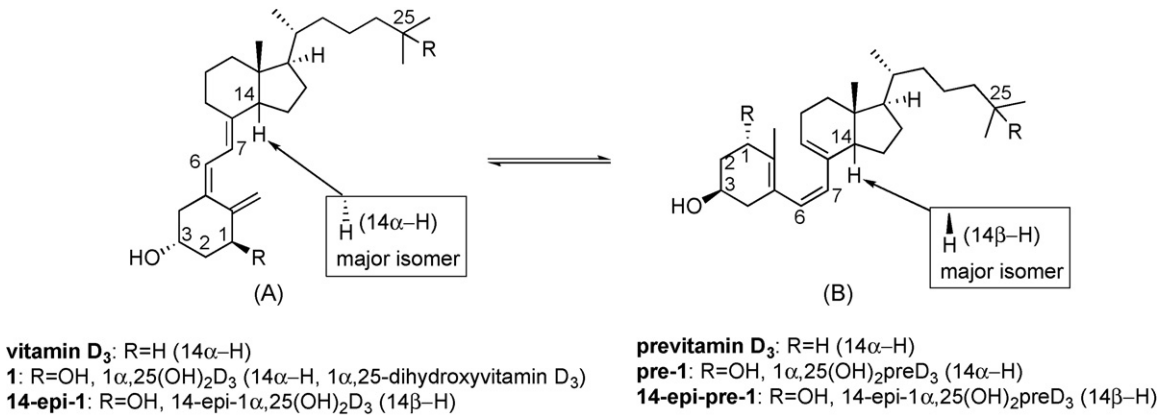
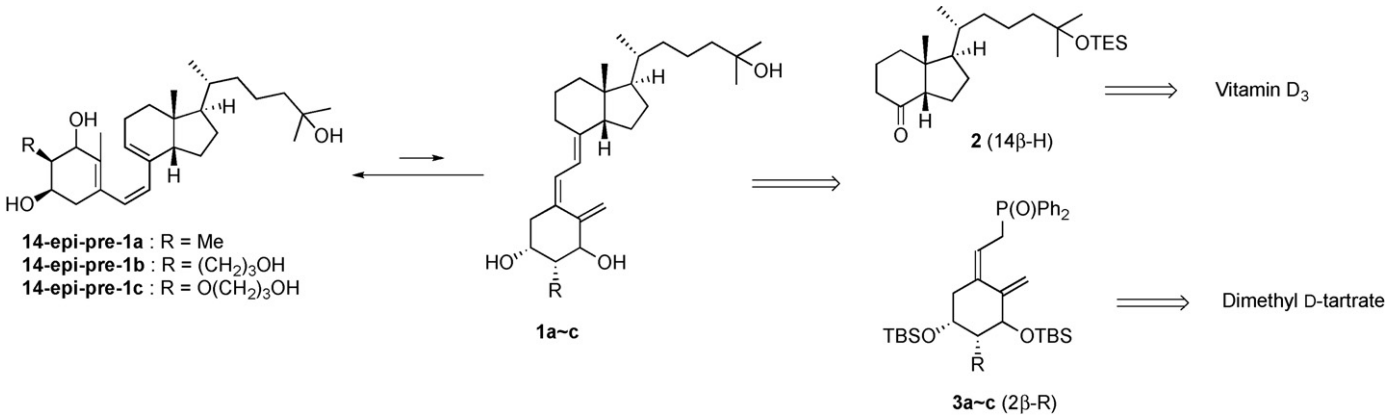


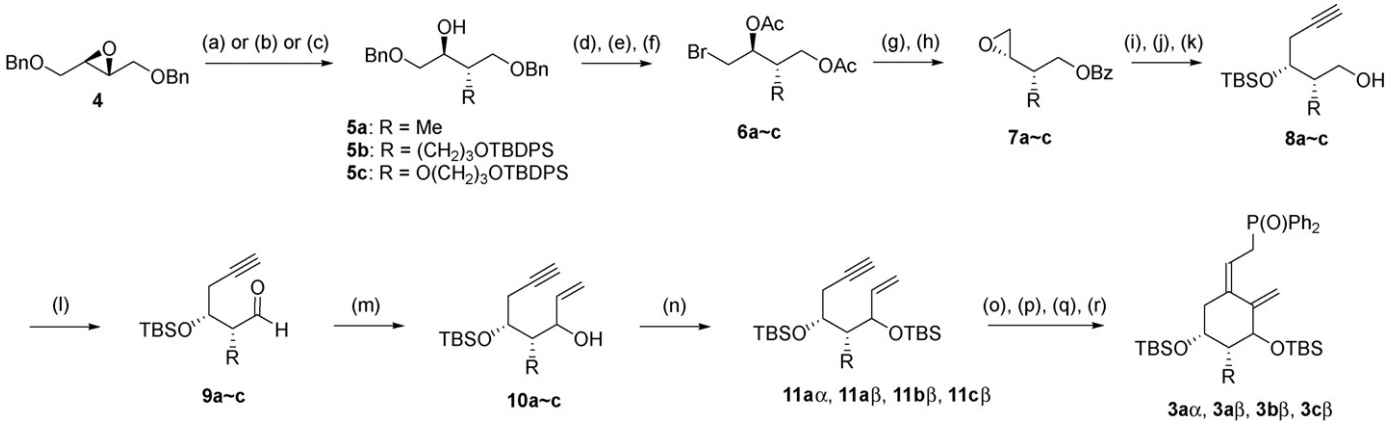

**Scheme 1.** Equilibrium between vitamin $D_3$ and previtamin $D_3$.

## 2. Results and discussion

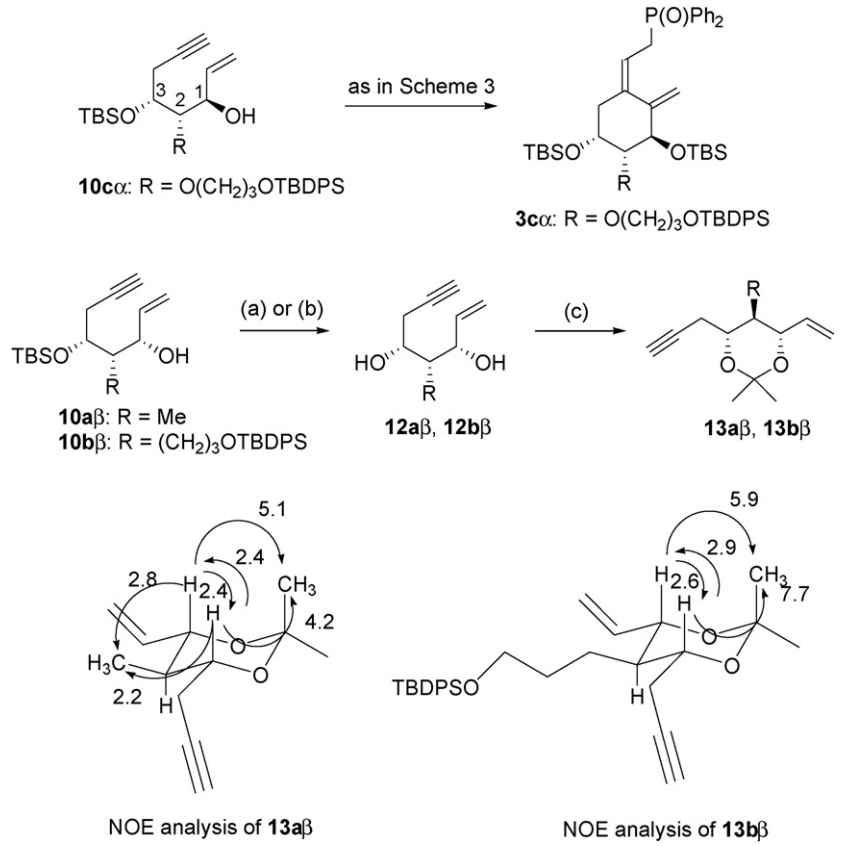
2β-Substituted A-ring fragments (**3a**–**c**) were prepared from the known epoxide **4** derived from dimethyl D-tartrate (Scheme 3) [13,14]. Using the nucleophilic epoxide ring-opening reaction of **4**, three substitutions were introduced as follows: (1) methyl cuprate gave a methyl substitution, (2) an allyl group brought by Grignard reagent was treated with 9-borabicyclo[3,3,1]nonane (9-BBN), and then with $H_2O_2$ to afford a hydroxypropyl substitution, and (3) propylene glycol gave a hydroxypropoxy substitution. After their primary hydroxyls were protected as TBDPS ether, **5a**–**c** were converted into bromide **6a**–**c** by the known procedure [15]. Methanolysis of both acetyl groups under basic conditions led to epoxide formation, and the resultant hydroxyl group was




**Scheme 2.** Retrosynthetic analysis of 2β-substituted 14-epi-1α,25$(OH)_2$pre$D_3$.




**Scheme 3.** Synthesis of the A-ring fragments. Conditions: (a) for **5a** MeLi, CuI, $Et_2O$, 98%; (b) for **5b** (i) allylmagnesium chloride, toluene, (ii) 9-BBN, THF, $H_2O_2$, NaOH, (iii) TBDPSCl, imidazole, DMF, 90% (3 steps); (c) for **5c** (i) propylene glycol, KOtBu, (ii) TBDPSCl, imidazole, DMF, 92% (2 steps); (d) Pd/C, $H_2$, MeOH; (e) $MeC(OMe)_3$, PPTS, $CH_2Cl_2$; (f) AcBr, $CH_2Cl_2$, 60% for **6a**, 52% for **6b**, 55% for **6c** (3 steps); (g) $K_2CO_3$, MeOH; (h) BzCl, $Et_3N$, $CH_2Cl_2$, 80% for **7a**, 95% for **7b**, 83% for **7c** (2 steps); (i) (trimethylsilyl)acetylene, nBuLi, $BF_3{\bullet}OEt_2$, THF; (j) TBSOTf, $iPr_2EtN$, $CH_2Cl_2$; (k) $K_2CO_3$, MeOH, 68% for **8a**, 83% for **8b**, 95% for **8c** (3 steps); (l) $SO_3{\bullet}Py$, $Et_3N$, DMSO, 77% for **9a**, 99% for **9b**, 95% for **9c**; (m) vinylmagnesium chloride, THF, 93% ($\alpha/\beta$ 46/47) for **10a**, 95% ($\alpha/\beta$ 37/58) for **10b**, 73% ($\alpha/\beta$ 15/58) for **10c** (2 steps); (n) TBSOTf, $iPr_2EtN$, $CH_2Cl_2$, 100% for **11aα**, 97% for **11aβ**, 99% for **11bβ**, 99% for **11cβ**; (o) nBuLi, $(CH_2O)_n$, THF; (p) Red-Al, $Et_2O$, then $I_2$, THF; (q) $Pd(PPh_3)_4$, $Et_3N$, MeCN; (r) (i) NCS, $Me_2S$, $CH_2Cl_2$, (ii) nBuLi, $PHPh_2$, THF, then 30% $H_2O_2$, 49% for **3aα**, 57% for **3aβ**, 27% for **3bβ**, 28% for **3cβ** (4 steps).



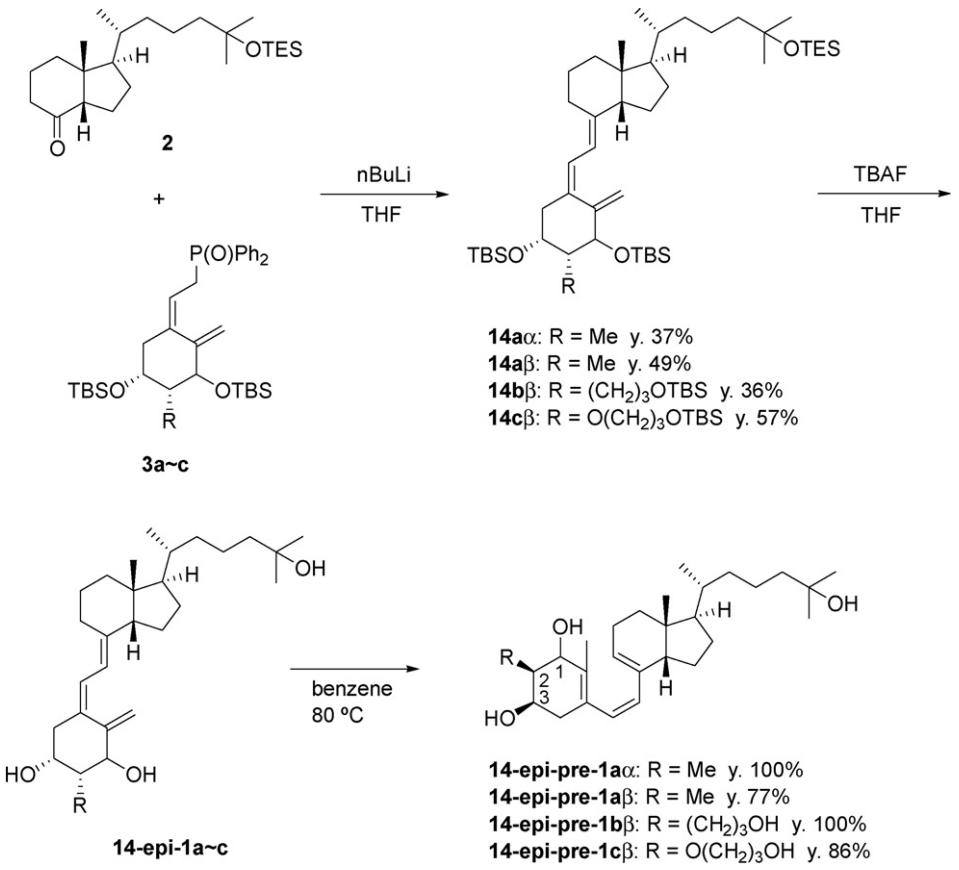

**Scheme 4.** Determination of the stereochemistry of the 1-hydroxy group of **10a–c.** Conditions: (a) for **10aβ**, TBAF, THF, 100%; (b) for **10bβ**, PPTS, EtOH, 60%; (c) dimethoxypropane, PPTS, DMF, 70% for **13aβ**, 90% for **13bβ**.

transformed into benzyl ester **7a–c**. The addition of (trimethylsilyl)acetylene to the epoxide using nBuLi was straightforward, and the generated secondary alcohol was protected as TBS ether, and removal of the terminal TMS group and the benzoyl group gave alkyne **8a–c**. The primary alcohol was oxidized to aldehyde by DMSO and $SO_3$•pyridine complex (**9a–c**), to which the vinyl group was introduced to give a diastereomixture of alcohol **10a–c**. The stereochemistry of the new hydroxy groups is discussed below (Scheme 4), and both isomers of **10a** and the major isomer of **10b** and **10c** were used for further transformation after column chromatography. The hydroxy group of **10a–c** was protected by the TBS group to obtain 2β-substituted enyne **11a–c**. According to the known procedure, enyne **11a–c** were transformed into phosphine oxide in four steps to give **3a–c**, respectively [8,13]. As above, we were able to prepare four A-ring fragments.

As shown in Scheme 4, the minor diastereomer of **10c** (**10cα**) was converted to the phosphine oxide **3cα** by the same strategy as in Scheme 3, and it was identical to the known compound reported by Hatakeyama et al. [13]. Therefore, the stereochemistry of its 1-hydroxy group (steroidal numbering) was found to be α-configuration, and the major diastereomer of **10c** was determined to have the 1β-hydroxy group (**10cβ**). For determination of the stereochemistry in **10a** and **10b**, the TBS groups of the major diastereomers (**10aβ** and **10bβ**) were removed, and the resultant 1,3-dihydroxy groups of **12aβ** and **12bβ** were converted into acetonide **13aβ** and **13bβ**, respectively. NOE analysis is described in Scheme 4, and the stereochemistry of 1,3-dihydroxy groups was determined as *syn*, that is, **10aβ** and **10bβ** had 1β,3β-dihydroxy groups. As above, we found that all of the major diastereomer of **10a–c** had 1β-hydroxy groups.

Using the CD- and A-ring fragments prepared as above, we examined the coupling reaction under basic conditions with nBuLi (Scheme 5) [6,10]. Small excess amounts of the A-ring fragment worked well and we obtained the coupled products **14a–c** in moderate yields. At this point, isomerization to the previtamin D form was seldom observed, probably because TBS groups at the A-ring should have steric hindrance to prevent from reaching the transition state for the [1,7]-sigmatropic hydrogen shift between the vitamin D form and the previtamin D form. Then, all silyl groups in **14a–c** were removed in one step with excess TBAF, and most of the deprotected compounds remained in the vitamin D form (**14-epi-1a–c**), and small amounts of the previtamin D form (**14-epi-pre-1a–c**) were produced under these reaction conditions. However, once they were heated at 80 °C in benzene, isomerization was found to proceed smoothly by $^1$H NMR observation. After 2 h, a large proportion of the vitamin D form had been converted into the previtamin D form, and the isomerization seemed to reach thermal equilibrium, at which the ratio of the compounds was about 5/95 (vitamin D/previtamin D) based on $^1$H NMR studies. Using HPLC, the mixture of both forms was separated, and we were able to obtain **14-epi-pre-1a–c** as pure forms, which were used for further biological studies.

The VDR binding affinity and the osteocalcin promoter transactivation activity of the new compounds were evaluated using chick intestinal VDR and HOS cells, respectively. The results are summarized in Table 1 in comparison with the natural hormone **1** and 14-epi-1α,25(OH)$_2$preD$_3$ (**14-epi-pre-1**), which was synthesized in a similar manner in our laboratory. The new compounds showed lower activity than the natural hormone **1**, and also than




**Scheme 5.** Coupling reaction and synthesis of 2β-substituted 14-epi-1α,25(OH)$_2$preD$_3$.

**Table 1**
Relative binding affinity for chick intestinal VDR and osteocalcin promoter transactivation activity in HOS cells of 2β-substituted 14-epi-1α,25(OH)$_2$preD$_3$.

| Compound | VDR[a] | Osteocalcin transactivation activity ($EC_{50}$, nM) |
|---|---|---|
| **1** | 100 | 0.03 |
| **14-epi-pre-1** | 0.5 | 0.46 |
| **14-epi-pre-1aα** | 0.08 | 1.34 |
| **14-epi-pre-1aβ** | 0.08 | 9.12 |
| **14-epi-pre-1bβ** | 0.18 | 1.01 |
| **14-epi-pre-1cβ** | 0.01 | 1.24 |

[a] The potency of **1** is normalized to 100.

**14-epi-pre-1** regardless of the stereochemistry at the 1-hydroxy group.

## 3. Conclusion

We synthesized 2β-substituted analogs of **14-epi-1** for the first time and were able to isolate these new analogs (**14-epi-pre-1a–c**) after thermal isomerization at 80 °C. We evaluated their VDR binding affinity and transactivation activity of osteocalcin promoter in HOS cells. It was found that 2β-modified analogs of 14-epi-1α,25-dihydroxyprevitamin D$_3$ were considerably less active than the natural hormone (**1**) and than **14-epi-pre-1**, although 2β-modification of **1** afforded important knowledge to the vitamin D SAR studies.

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