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Synthesis and Testing of 2α-Modified 1α,25-Dihydroxyvitamin D_3 Analogues with a Double Side Chain: Marked Cell Differentiation Activity

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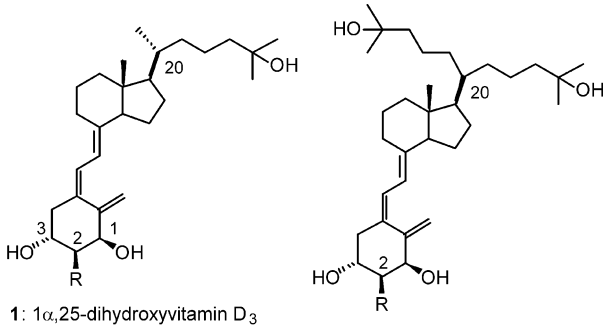
Abstract—The 2α-methyl-, 2α-(3-hydroxypropyl)-, and 2α-(3-hydroxypropoxy)-derivatives of the 'double side chain' analogue of 1α,25-dihydroxyvitamin D_3 were synthesized using Trost A-ring/CD-ring connective strategy. Regarding the requisite A-ring building blocks, a new, high yield and stereoselective route to the 2α-methyl compound starting from D-glucose was developed. All three new analogues showed potent HL-60 cancer cell differentiation activity.

The *seco*-steroid hormone 1α,25-dihydroxyvitamin D_3 (**1**) is the most potent natural metabolite of vitamin D_3 and regulates primarily calcium and phosphorus homeostasis, as well as proliferation and differentiation of cells.[1] Some synthetic analogues of **1** are clinically used in the treatment of bone diseases, secondary hyperparathyroidism, and psoriasis.[2] Most of the biological actions of **1** are mediated through its specific receptor, the vitamin D receptor (VDR), which is a member of the nuclear receptor superfamily acting as a ligand-dependent transcription factor with coactivators.[3,4] Recently, we have found several structural modifications on the A-ring of **1** that increase the binding affinity for VDR. Thus introduction of the motifs shown in structures **1a–c** [2α-methyl (**1a**),[5,6] 2α-(3-hydroxypropyl) (**1b**),[7,8] or 2α-(3-hydroxypropoxy) (**1c**)[9] groups] resulted in ca. 2- to 4-fold higher binding affinities for the bovine thymus VDR relative to the natural hormone (**1**).[5–9] Moreover, the combination of these 2α-modification on the A-ring with a known potentiating side chain modification, that is 20-*epi*,[10] showed an additive effect on VDR binding. The highest in affinity in this series so far achieved was with 2α-methyl-20-*epi*-1α,25-dihydroxybitamin D_3, which exhibits 12-fold higher affinity for VDR than **1**.[6,11,12] Very recently, the intriguing new 'double side chain' modification was introduced [1α,25-dihydroxy-21-(3-hydroxy-3-methylbutyl)vitamin D_3, **2**] to probe further role of the 20-*epi* and normal side chain interactions with VDR (Fig. 1).[13]



1: 1α,25-dihydroxyvitamin D_3 (R = H)
1a: R = Me
1b: R = $(CH_2)_3OH$
1c: R = $O(CH_2)_3OH$

2: double side chain (R = H)
2a: R = Me
2b: R = $(CH_2)_3OH$
2c: R = $O(CH_2)_3OH$

Figure 1. Structures of 1α,25-dihydroxyvitamin D_3 (**1**), 'double side chain' (**2**), and their 2α-substituted analogues **1a–c** and **2a–c**.

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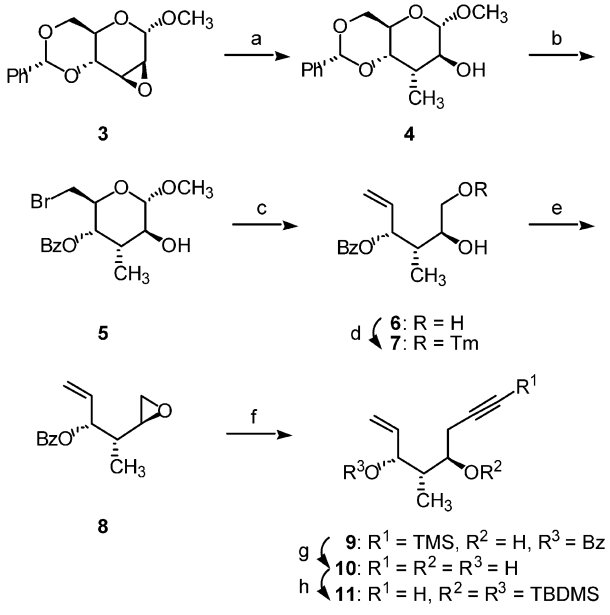
Profiles of the biological activities of **2** are interesting:[13] it was significantly more potent than **1** in gene transcriptional activity and in inhibition of clonal growth of HL-60, MCF7, and LNCaP, which possess VDR. Reported binding affinity of **2** for chick intestinal VDR was 38% as compared to the natural hormone (100%);[13] therefore, introduction of the positive motif into the 2α position of 'double side chain' **2** would give us much information on the structure–activity relationships of 1α,25-dihydroxyvitamin D_3 analogues and developing potential therapeutic agents.

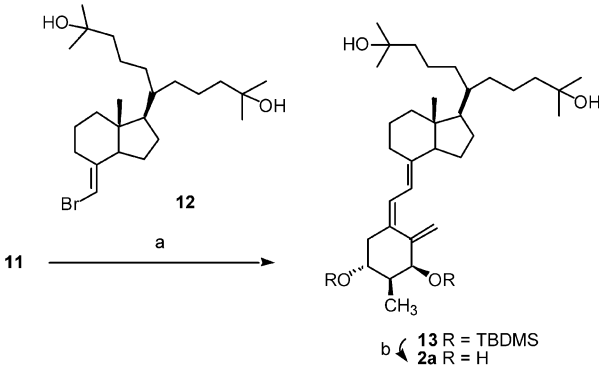
For synthesizing 2α-substituted vitamin D_3 (**2a**–**c**), we utilized Trost's convergent method[14] with some modifications.[5–9] In the case of the 2α-methyl analogue **2a**, the A-ring precursor enyne **11** was prepared from the known crystalline epoxide **3**,[9] which is readily available from methyl α-D-glucoside,[15] instead of the previous non-diastereoselective synthesis from methyl (*S*)-3-hydroxy-2-methylpropionate.[5,6] As shown in Scheme 1, introduction of the methyl group at the C-3 position of **3** was carried out with the regiospecific ring opening using Grignard reagent.[16] The configurations of C2, C3, and C4 on resulting **4** satisfy required 3β, 2α, and 1α stereochemistries of **2a**, respectively, and the following steps are based on the synthesis of the A-ring precursor enyne **15**.[9] Treatment of benzylidene acetal **4** with NBS gave bromide **5** in 85% yield.

Pyranose ring opening of **5** with activated zinc powder in the presence of $NaBH_3CN$ afforded the diol **6** in 75% yield. The primary hydroxyl group of **6** was selectively sulfonylated and then converted to epoxide **8** with LiHMDS. After introduction of the acetylene unit to **8**, the terminal TMS and the benzoyl groups of **9** were removed under basic conditions in 95% yield. Finally, protection of the secondary hydroxyls provided the desired enyne **11** in high yield.



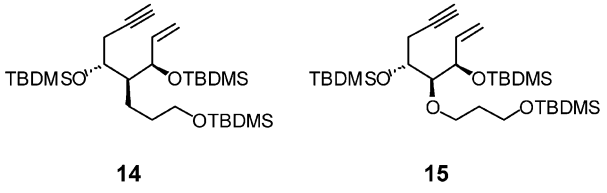
Scheme 1. Conditions and yields: (a) 3 M MeMgCl in THF, ether, 80 °C, 7 days, 75%; (b) NBS, $BaCO_3$, CCl_4, reflux, 35 min, 85%; (c) Zn powder, $NaBH_3CN$, 1-propanol–H_2O (10:1), 95 °C, 45 min, and then $NaBH_4$, rt, 75%; (d) TmCl, DMAP, CH_2Cl_2, 0 °C–rt, 84%; (e) LiHMDS, THF, −78 °C–rt, 90%; (f) TMSCCH, BuLi, BF_3OEt_2, THF, −78 °C, 91%; (g) K_2CO_3, MeOH, 95%; (h) TBDMSOTf, 2,6-lutidine, CH_2Cl_2, 0 °C, 89%.

Bromoolefin of the CD-ring part **12** was prepared from the 'double side chain' ketone.[13,17] Palladium-catalyzed coupling[14] of **11** with **12**, followed by deprotection of the TBDMS groups gave the desired 2α-methyl analogue with 'double side chain', **2a** (Scheme 2).[18]



Scheme 2. Conditions and yields: (a) cat. $(Ph_3P)_4Pd$, Et_3N–toluene (3:1), reflux; (b) Bu_4NF, THF, 12% for two steps.

The other enynes **14** and **15** were also synthesized by our reported methods,[8,9] and they were coupled with bromoolefin **12** by the same procedure to yield 2α-(3-hydroxypropyl)- and 2α-(3-hydroxypropoxy) derivatives **2b** and **2c**, respectively.[19,20]



The receptor binding potency of **2** and **2a**–**c** was evaluated using bovine thymus VDR,[21] and the results are summarized in Table 1. Introduction of three motifs into the 2α-position increases binding affinity for the VDR when compared to the original structure of **2**. Each group at the 2α-position in **2a**–**c** seems to be effective to stabilize the ligand–VDR complex.[6,8,9]

Table 1. Relative binding affinity for bovine thymus VDR and HL-60 cell differentiation activity of **2** and the novel 2α-substituted vitamin D_3 analogues **2a**–**c** with 'double side chain'

Compd	VDR[a]	Relative differentiation activity[a,b]
1	100	100
2	24	1060
2a	53	3840
2b	57	1280
2c	36	1920

[a]The potency of **1** is normalized to 100.
[b]Data are mean of three separate experiments.

Next, we examined ability of **2** and **2a–c** to induce differentiation of HL-60 cells, which are human promyelocytic leukemia cells, by NBT reduction method.[22] As shown in Table 1, the potencies of these compounds in inducing HL-60 cell differentiation were markedly higher than that of **1** (ca. 11∼38 times more potent than **1**), and the rank order of the potency was **2a** > **2c** > **2b** > **2**. These data indicate that the 2α-substituted 'double side chain' 1α,25-dihydroxyvitamin D_3 analogues (**2a–c**) have a considerably potent ability to activate VDR like **2** as compared to the natural hormone **1**.[23]

In conclusion, we have introduced the positive motifs, 2α-methyl, 2α-(3-hydroxypropyl), and 2α-(3-hydroxypropoxy) substituents, into the biologically active vitamin D_3 analogue of 'double side chain', **2**. The A-ring precursor of the 2α-methyl derivative **2a**[5] was stereoselectively synthesized from chiral template **3**. Binding affinity for bovine thymus VDR was evaluated and found that **2a–c** possess higher potency of binding than that of the parent analogue **2**. All three analogues **2a–c** have shown considerably higher differentiation activity in HL-60 cells than the natural hormone **1**. Further detailed studies of biological activities of these analogues are currently in progress in our laboratories.

Acknowledgements

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17. To a solution of bromomethyltriphenylphosphonium bromide (596 mg, 1.37 mmol) in THF (15 mL) was added 1.0 M NaHMDS in THF solution (1.28 mL) at −78 °C under Ar. The mixture was stirred at −78 °C for 1 h, and then the ketone[13] with the 'double side chain' (100 mg, 0.273 mmol) in THF (5 mL) was added dropwise at −78 °C. After the reaction mixture was stirred at room temperature, the solution was diluted with hexane (100 mL). The resulting solution was filtered through Celite followed by concentration of the filtrate. The resin was purified with silica gel column chromatography (hexane/EtOAc 5:1) to give bromoolefin **12** (82 mg, 68% yield) as a colorless oil. Data for bromoolefin **12**: $[\alpha]_D^{20}$ +41.4 (*c* 0.77, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 0.56 (3H, s), 1.22 (12H, s), 1.81–1.87 (1H, m), 1.92–1.94 (1H, m), 1.96–2.01 (1H, m), 2.86–2.89 (1H, m), 5.65 (1H, t, J = 1.6 Hz) ; ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.0, 19.8, 20.1, 21.9, 22.6, 27.0, 29.25, 29.36, 29.39, 30.9, 31.1, 31.3, 39.3, 39.6, 44.4, 44.5, 45.6, 52.3, 55.9, 71.0, 97.5, 145.1; HREIMS calcd for $C_{24}H_{43}O_2{}^{79}Br$ (M^+) 442.2447, found 442.2447.
18. Enyne **11** (20.0 mg, 54.3 μmol) and vinyl bromide **12** (25.0 mg, 56.5 μmol) were dissolved in TEA/toluene (3:1, 2.0 mL), and tetrakis(triphenylphosphine)-palladium (0) (100 mg, 86.5 μmol) was added. After 15 min of stirring at room temperature, the resultant yellow solution was refluxed for 2 h. The reaction mixture was filtered through a silica gel pad. Concentration followed by preparative thin-layer chromatography on silica gel (20% EtOAc in hexane) gave crude **13** as white solids, which was used in the next step without further purification. To a cooled (0 °C) and stirred solution of crude **13** in THF (2.0 mL) was added 1.0 M tetrabutylammonium fluoride in THF (0.5 mL). After 1 h stirring at 0 °C, the reaction mixture was allowed to warm to room temperature and stirred for further 12 h. The resultant solution was diluted with EtOAc (10 mL), and washed with brine (3×1 mL). The aqueous layer was extracted with EtOAc (3×2 mL), and the combined organic layer was dried over Na_2SO_4. Filtration and concentration followed by preparative thin-layer chromatography on silica gel (10% MeOH in CH_2Cl_2) gave (2.2 mg, 12% yield in two steps) of 2α-methyl analogue **2a** as a white solid. Data for 2α-methyl analogue **2a**: $[\alpha]_D^{20}$ +32.4 (*c*, 0.034, $CHCl_3$); UV (EtOH) λ_{max} 267 nm, λ_{min} 227 nm; ^1H NMR (400 MHz, $CDCl_3$) δ 0.53 (3H, s), 1.22 (12H, s), 1.90–1.94 (2H, m), 2.23 (1H, dd, J = 8.0, 13.7 Hz), 2.67 (1H, dd, J = 4.1, 13.7 Hz), 2.84

(1H, m), 3.84 (1H, dt, $J=4.1$, 8.0 Hz), 4.30 (1H, d, $J=3.3$ Hz), 5.01 (1H, d, $J=1.9$ Hz), 5.28 (1H, bs), 6.01 (1H, d, $J=11.2$ Hz), 6.38 (1H, d, $J=11.2$ Hz) ; ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.2, 12.5, 13.6, 19.9, 20.2, 22.1, 23.6, 27.1, 29.1, 29.2, 29.3, 29.4, 29.7, 31.4, 39.4, 40.2, 43.5, 44.2, 44.4, 46.0, 52.2, 53.0, 56.3, 71.0, 71.7, 75.5, 76.8, 77.0, 77.2, 113.2, 117.4, 125.5, 133.1, 143.1, 146.6; HREIMS calcd for $C_{33}H_{56}O_4$ (M^+) 516.5179, found 516.4174.

19. Enyne **14** (20.0 mg, 41.4 μmol) was converted to the 2α-hydroxypropyl analogue (**2b**, 4.1 mg, 18% yield in two steps) as a white solid according to the procedure described above for **2a**. Data for **2b**: $[\alpha]_D^{20}$ +14.3 (*c*, 0.11, $CHCl_3$); UV (EtOH) λ_{max} 269 nm, λ_{min} 227 nm; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.53 (3H, s), 1.21 (12H, s), 2.00 (1H, dd, $J=4.4$, 7.5 Hz), 2.24 (1H, dd, $J=9.0$, 13.4 Hz), 2.68 (1H, dd, $J=4.7$, 13.4 Hz), 2.83 (1H, m), 3.37 (1H, dd, $J=3.3$, 7.4 Hz), 3.76–3.91 (5H, m), 4.05 (1H, dt, $J=4.7$, 9.0 Hz), 4.44 (1H, d, $J=3.3$ Hz), 5.10 (1H, d, $J=1.7$ Hz), 5.39 (1H, bs), 6.01 (1H, d, $J=11.3$ Hz), 6.42 (1H, d, $J=11.3$ Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.2, 19.9, 20.2, 22.1, 22.8, 23.6, 27.1, 29.1, 29.2, 29.3, 29.4, 29.7, 30.3, 31.3, 39.4, 40.2, 44.3, 44.4, 44.5, 46.0, 49.1, 53.0, 56.3, 62.8, 68.0, 68.5, 70.5, 71.1, 73.6, 113.6, 117.0, 124.8, 132.8, 143.2, 146.6; HREIMS calcd for $C_{35}H_{60}O_5$ (M^+) 560.4441, found 560.4450.

20. Enyne **15** (20.0 mg, 36.9 μmol) was converted to the 2α-hydroxypropoxy analogue (**2c**, 5.2 mg, 24% yield in two steps) as a white solid according to the procedure described above for **2a**. Data for **2c**: $[\alpha]_D^{20}$ −33.6 (*c*, 0.13, $CHCl_3$); UV (EtOH) λ_{max} 267 nm, λ_{min} 228 nm; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.53 (3H, s), 1.21 (12H, s), 2.25 (1H, dd, $J=9.3$, 12.9 Hz), 2.68 (1H, dd, $J=4.5$, 12.9 Hz), 2.83 (1H, dd, $J=3.5$, 9.3 Hz), 3.88 (1H, dt, $J=3.5$, 9.3 Hz), 4.37 (1H, d, $J=3.5$ Hz), 5.00 (1H, d, $J=1.7$ Hz), 5.28 (1H, bs), 6.00 (1H, d, $J=11.2$ Hz), 6.39 (1H, d, $J=11.2$ Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.2, 19.9, 20.2, 22.1, 23.5, 27.1, 29.1, 29.2, 29.3, 29.4, 31.3, 31.9, 39.4, 40.2, 41.0, 44.4, 44.5, 46.0, 53.0, 56.3, 61.3, 68.4, 68.5, 71.1, 71.9, 84.5, 116.2, 117.2, 125.5, 131.6, 143.5, 144.3; HREIMS calcd for $C_{35}H_{60}O_6$ (M^+) 576.4390, found 576.4396.

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23. Potencies in inducing HL-60 cell differentiation of compounds **1a** and **1b** were 200 and 240, respectively; see refs 5 and 7. It appears that the activity of the double side chain analogues is characterized with regard to 20-*epi* analogues of **1**; see ref 11. Further details in this context and the activity of **1c** will be published elsewhere.