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## STUDIES ON THE SYNTHESIS AND METABOLISM OF 14-*epi*-2 $\alpha$ -(3-HYDROXYPROPYL)-19-NORVITAMIN D<sub>3</sub> AND ITS 2 $\beta$ -ISOMER

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This paper is dedicated to Professor Dr. Albert Eschenmoser on the occasion of his 85th birthday.

**Abstract** – Two derivatives of 14-*epi*-1 $\alpha$ ,25-Dihydroxy-19-norvitamin D<sub>3</sub>, 14-*epi*-2 $\alpha$ -(3-hydroxypropyl)-1 $\alpha$ ,25-dihydroxy-19-norvitamin D<sub>3</sub> (14-*epi*-MART-10) and its 2-epimeric analog (14-*epi*-MART-11), were synthesized using Julia coupling reaction to connect between the C5 position (steroidal numbering) of an A-ring precursor ketone derived from (–)-quinic acid and the C6 position of a protected 14-*epi*-CD-ring benzothiazole sulfone. The coupling and deprotection reactions generated a mixture of 14-*epi*-MART-10 and 14-*epi*-MART-11 in a moderate yield. The C2-isomers were then separated as their pivalate forms. The C2-stereochemistry of 2 $\alpha$ - and 2 $\beta$ -isomers was

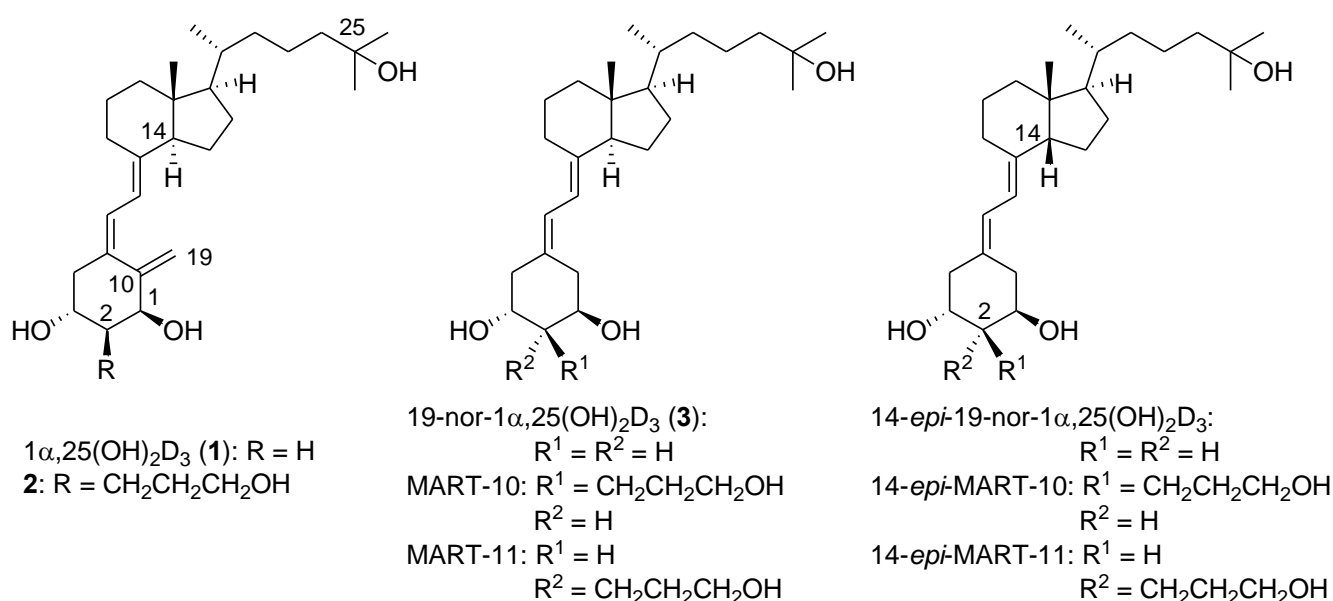
determined by  $^1\text{H}$  NMR studies including NOE experiments. The pivaloyl group was removed under basic conditions to obtain the target molecules of 14-*epi*-MART-10 and 14-*epi*-MART-11. The metabolism of these two new analogs was further studied in a reconstituted cell-free human CYP24A1 system to elucidate the potential mechanism of their super agonistic action on vitamin D receptor. Our results indicate that epimerization at C14 makes the analogs less susceptible to CYP24A1 degradation and therefore more bio-available, leading to enhanced biological activities.

## INTRODUCTION

The biological active form of vitamin D<sub>3</sub>, 1 $\alpha$ ,25-dihydroxyvitamin D<sub>3</sub> (1 $\alpha$ ,25(OH)<sub>2</sub>D<sub>3</sub>, **1**), regulates calcium and phosphorus homeostasis and promotes cell differentiation and apoptosis, and also suppresses autoimmune diseases.<sup>1</sup> Most of the biological actions of 1 $\alpha$ ,25(OH)<sub>2</sub>D<sub>3</sub> are mediated through binding to vitamin D receptor (VDR), which belongs to the nuclear receptor superfamily.<sup>2,3</sup> The potent calcemic activity of 1 $\alpha$ ,25(OH)<sub>2</sub>D<sub>3</sub> prevents its application in pharmacological doses for cancer chemotherapy, even though 1 $\alpha$ ,25(OH)<sub>2</sub>D<sub>3</sub> is able to inhibit cancer cell growth.<sup>4,5</sup> We synthesized a series of active vitamin D<sub>3</sub> analogs with C2-modifications and found some of them showing greater VDR agonistic activity than 1 $\alpha$ ,25(OH)<sub>2</sub>D<sub>3</sub>.<sup>6-10</sup> For example, 2 $\alpha$ -(3-hydroxypropyl)-1 $\alpha$ ,25(OH)<sub>2</sub>D<sub>3</sub> (**2**, Figure 1) showed three-fold higher binding activity for VDR, and the mechanism of the effects of the 2 $\alpha$ -(3-hydroxypropyl) group on enhanced VDR binding has been explained by X-ray crystallographic analysis of the VDR-**2** complex, i.e., the terminal hydroxy group of **2** forms a hydrogen bond with Arg274 and replaces one of the water molecules in the ligand binding domain (LBD) of the VDR to stabilize the complex.<sup>11</sup> On the other hand, we synthesized several highly potent VDR-antagonists (the highest IC<sub>50</sub> = 7.4 pM) with C2 $\alpha$ -functionalization as well as 24-alkyl modification on the lactone ring of Teijin analog, TEI-9647.<sup>12-14</sup>

1 $\alpha$ ,25-Dihydroxy-19-norvitamin D<sub>3</sub> (**3**) is known to have a selective activity profile, combining high potency in inducing differentiation of malignant cells with very low or no calcemic activity.<sup>15-18</sup> The C2-modified active 19-norvitamin D<sub>3</sub> analog, MART-10 (2 $\alpha$ -(3-hydroxypropyl)-1 $\alpha$ ,25-dihydroxy-19-norvitamin D<sub>3</sub>), is 36 times more potent than the natural hormone 1 $\alpha$ ,25(OH)<sub>2</sub>D<sub>3</sub> in inducing HL-60 human promyelocytic leukemia cell differentiation.<sup>19,20</sup> MART-10 is also about 500- to 1000-fold more active than 1 $\alpha$ ,25(OH)<sub>2</sub>D<sub>3</sub> in inhibiting the proliferation of immortalized PZ-HPV-7 normal prostate cells and LNCaP prostate cancer cells, respectively.<sup>21,22</sup>

Moreover, MART-10 was 10-fold more active than  $1\alpha,25(\text{OH})_2\text{D}_3$  in inhibiting the invasion of PC-3 prostate cancer cells<sup>21</sup> and trans-activation of VDRE in LNCaP cells.<sup>22</sup> It has also been reported that the removal of 10(19)-methylene group from  $1\alpha,25(\text{OH})_2\text{D}_3$  eliminates the hydrophobic interactions with the ligand binding domain of VDR, and therefore reduces the VDR binding affinity relative to  $1\alpha,25(\text{OH})_2\text{D}_3$ , as demonstrated by a 70% and 83% drop of VDR binding affinity for the porcine VDR<sup>23</sup> and the calf thymus VDR,<sup>19</sup> respectively. However, the addition of the  $2\alpha$ -(3-hydroxypropyl) group to 19-nor- $1\alpha,25(\text{OH})_2\text{D}_3$  molecule, that results in the formation of MART-10 analog, restores the VDR binding affinity to the level of  $1\alpha,25(\text{OH})_2\text{D}_3$ .<sup>19</sup>

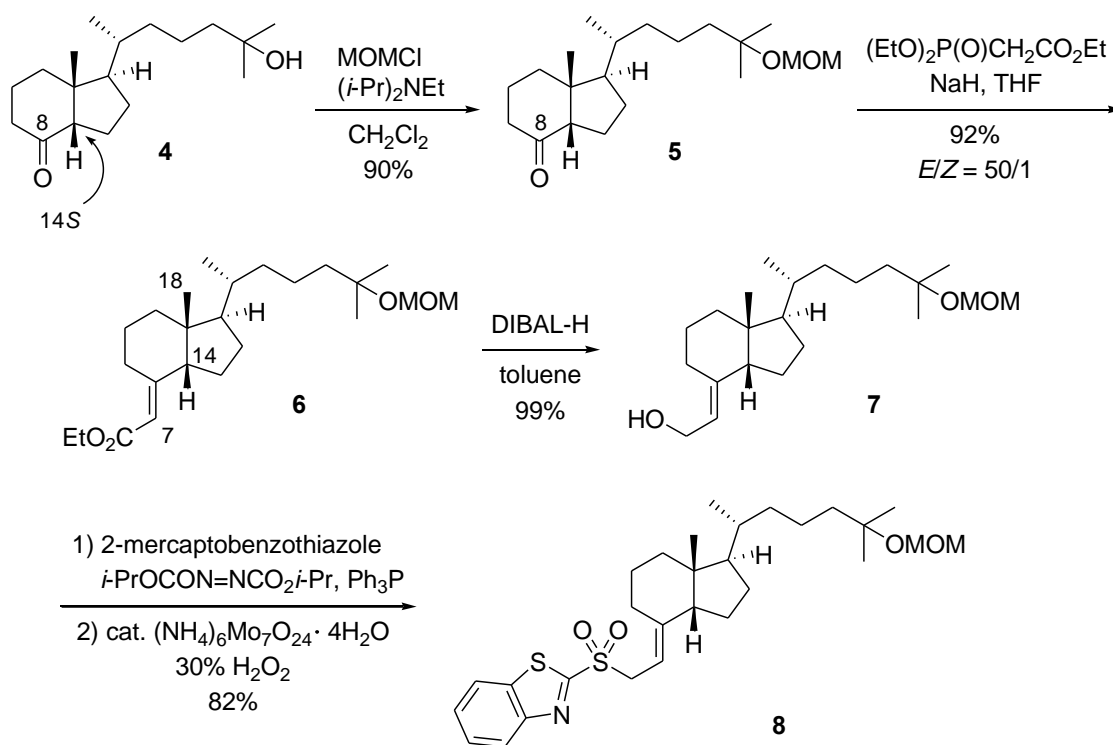


**Figure 1.** Structures of active vitamin D<sub>3</sub> ( $1\alpha,25(\text{OH})_2\text{D}_3$ , **1**),  $2\alpha$ -(3-hydroxypropyl) analog (**2**), 19-nor- $1\alpha,25(\text{OH})_2\text{D}_3$  (**3**), MART-10, MART-11, 14-*epi*-19-nor- $1\alpha,25(\text{OH})_2\text{D}_3$ ,<sup>31</sup> 14-*epi*-MART-10, and 14-*epi*-MART-11

Work from Bouillon's group reported that 14-*epi*-19-norvitamin D analogs, TX522 and TX527, possess a much enhanced antiproliferative action on breast cancer cells *in vitro* and *in vivo*, with much lower calcemic activity compared to  $1\alpha,25(\text{OH})_2\text{D}_3$ .<sup>24</sup> The X-ray crystallographic analysis of the VDR-TX522 complex revealed that TX522 binds to the hVDR with modified contacts of C12-Val300 and C22-Ile268 compared with  $1\alpha,25(\text{OH})_2\text{D}_3$ , which may increase interactions between the VDR and coactivators.<sup>25</sup> Therefore, we synthesized two C2-substituted 14-*epi*-19-norvitamin D<sub>3</sub> analogs, 14-*epi*-MART-10 and 14-*epi*-MART-11, and studied their metabolism by human CYP24A1 in an attempt to explain their potent biological activities *in vitro* and *in vivo*.<sup>26</sup>

## RESULTS AND DISCUSSION

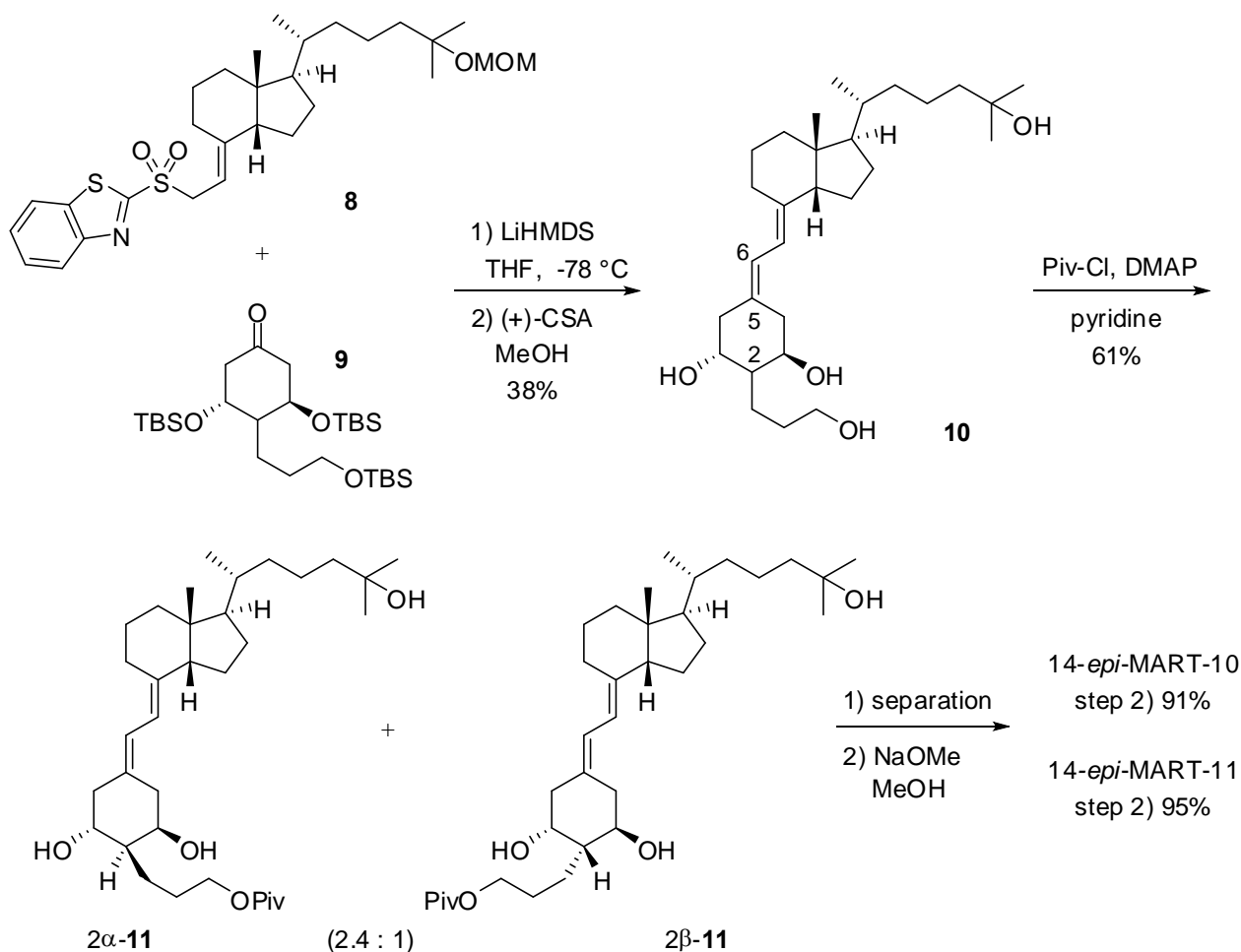
The known 8-oxo-14-*epi*-CD-ring **4**<sup>27</sup> with the 14*S* configuration was converted to sulfone **8**<sup>26</sup> as shown in Scheme 1. The tertiary alcohol **4** was treated with MOM-Cl to give **5** in 90% yield, and the subsequent two-carbon unit elongation at the C8 position was accomplished through Horner-Wadsworth-Emmons reaction, yielding  $\alpha,\beta$ -unsaturated ester **6** in 92%. The *E/Z* ratio of the olefination was ca. 50/1 and the stereochemistry of the major isomer **6** was determined by NOE experiments, in which NOEs were observed between vinylic H7 and H14 and also between H14 and H18. The ester **6** was reduced by DIBAL-H in toluene to yield allylic alcohol **7** in 99% yield. The subsequent sulfonylation with 2-mercaptobenzothiazole under Mitsunobu conditions followed by molybdenum(VI)-catalyzed oxidation afforded sulfone **8** in 82% yield, which was the substrate for the Julia coupling reaction.<sup>28-30</sup>



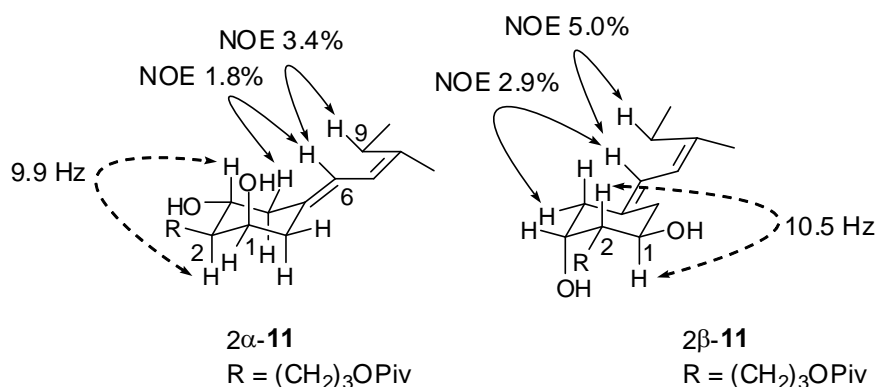
**Scheme 1.** Synthesis of 14-*epi*-CD-ring sulfone **8**

The A-ring part ketone **9**<sup>19</sup> and sulfone **8** were coupled using LiHMDS in THF, and then the protecting groups of the coupling products were removed under acidic conditions to obtain 14-*epi*-2-(3-hydroxypropyl)-19-norvitamin D<sub>3</sub> (**10**) as a diastereoisomeric mixture due to the C2 stereochemistry in 38% yield in two steps (Scheme 2). The products were converted to pivalates **2 $\alpha$ -11** and **2 $\beta$ -11**, which could be separated from each other ( $2\alpha:2\beta = 2.4:1$ ) by HPLC. The stereochemistries of the C2 position of **2 $\alpha$ -11** and **2 $\beta$ -11** were determined by <sup>1</sup>H NMR experiments including NOE

observation as shown in Figure 2.<sup>19</sup> Finally, the pivaloyl group was removed from each isomer under basic conditions to obtain the target molecules, 14-*epi*-MART-10 or 14-*epi*-MART-11.



**Scheme 2.** Julia coupling reaction between A-ring ketone **9** and CD-ring sulfone **8**, and subsequent separation of C2-isomers as pivalates **2α-11** and **2β-11**. Deprotection of each pivaloyl group gave 14-*epi*-MART-10 or 14-*epi*-MART-11



**Figure 2.** Structure determination of **2α-11** and **2β-11** by <sup>1</sup>H NMR experiments<sup>19</sup>

In the previous report, we described biological activities of 14-*epi*-MART-10 and 14-*epi*-MART-11 such as VDR binding affinity (27% and 3% of **1**, respectively), inducing activity of HL-60 cell differentiation (794% and 87% of **1**), potent antiproliferative activity in PZ-HPV-7 cells (14-*epi*-MART-10 showed nearly 10-50 times more active than **1**), transactivation activity of the osteocalcin promoter in HOS (human osteoblast cell line) cells (388% and 54% of **1**), and effects on bone mineral density (BMD) as well as serum and urine calcium concentrations of ovariectomized (OVX) rats.<sup>26,31</sup> 14-*epi*-MART-10 at 0.1  $\mu\text{g}/\text{kg}/\text{day}$  induced a marked increase in BMD in the OVX rats without significant calcemic and calciurinic side-effects, although at the higher dose, such as 1.0  $\mu\text{g}/\text{kg}/\text{day}$ , a hypercalcemia became evident.

CYP24A1-dependent 24-hydroxylation is the first step in the degradation and the termination of various biological activities of  $1\alpha,25(\text{OH})_2\text{D}_3$  and its analogs. Thus, we examined the metabolism of 14-*epi*-MART-10 and 14-*epi*-MART-11 by human CYP24A1 in a reconstituted cell free system. A single metabolite, a putative 24-hydroxy product based on LC/MS analysis, was observed in the metabolism of 14-*epi*-MART-10, 14-*epi*-MART-11, and MART-10 (data not shown). Our previous study revealed that MART-10 is a poor substrate of CYP24A1 based on the fact that the  $k_{\text{cat}}/K_{\text{m}}$  value of MART-10 is only 0.2% of that of  $1\alpha,25(\text{OH})_2\text{D}_3$ .<sup>22</sup> The 3-hydroxypropyl group at C2 and the deletion of the methylene group at C19 in the MART-10 molecule may hinder the binding of MART-10 to the catalytic pocket of CYP24A1. Table 1 shows kinetic parameters of CYP24A1-dependent hydroxylation toward MART-10, 14-*epi*-MART-10 and 14-*epi*-MART-11. Although  $k_{\text{cat}}$  values of three substrates are similar,  $K_{\text{m}}$  values of 14-*epi*-MART-10 and 14-*epi*-MART-11 are significantly higher than that of MART-10, suggesting that both 14-*epi* compounds have lower affinity for CYP24A1 than MART-10. Conformational difference of 14-*epi*-MART-10 from MART-10 might explain the higher  $K_{\text{m}}$  value of 14-*epi*-MART-10. It is noted that  $k_{\text{cat}}/K_{\text{m}}$  value of 14-*epi*-MART-10 is less than 0.1% of  $1\alpha,25(\text{OH})_2\text{D}_3$ . These results indicate much less degradation of 14-*epi*-MART-10 and 14-*epi*-MART-11 by human CYP24A1. This could explain their greater biological activity than  $1\alpha,25(\text{OH})_2\text{D}_3$  *in vitro* and *in vivo* in spite of its lower VDR binding affinity.

**Table 1.** Kinetic parameters of human CYP24A1 for MART-10, 14-*epi*-MART-10, and 14-*epi*-MART-11

Substrate	$k_{\text{cat}}$ ( $\text{min}^{-1}$ )	$K_{\text{m}}$ ( $\mu\text{M}$ )	$k_{\text{cat}}/K_{\text{m}}$
<b>MART-10</b>	<b>2.14 <math>\pm</math> 0.57</b>	<b>10.2 <math>\pm</math> 2.1</b>	<b>0.21 <math>\pm</math> 0.08</b>
<b>14-<i>epi</i>-MART-10</b>	<b>2.60 <math>\pm</math> 0.43</b>	<b>30.0 <math>\pm</math> 3.4</b>	<b>0.087 <math>\pm</math> 0.022</b>
<b>14-<i>epi</i>-MART-11</b>	<b>2.37 <math>\pm</math> 0.37</b>	<b>20.0 <math>\pm</math> 4.8</b>	<b>0.12 <math>\pm</math> 0.04</b>

## CONCLUSION

Two new C2 substituted 14-*epi*-19-norvitamin D<sub>3</sub> analogs, 14-*epi*-MART-10 and 14-*epi*-MART-11, were synthesized using Julia coupling reaction between C5 of A-ring and C6 of 14-*epi*-CD-ring (steroid numbering). The metabolic studies using human CYP24A1 in a reconstituted cell-free system indicate that both analogs have lower binding affinity for human CYP24A1 than MART-10. The results suggest that the epimerization at C-14 position further increases their resistance to the metabolic degradation of the side chain by CYP24A1,<sup>22</sup> and may provide a mechanism for their super agonistic actions *in vitro* and *in vivo*.

## EXPERIMENTAL

### (1*S*,6*R*,7*R*)-7-[(*R*)-6-Methoxymethoxy-6-methylheptan-2-yl]-6-methylbicyclo[4.3.0]nonan-2-one (**5**).

The tertiary alcohol **4** (380 mg, 1.34 mmol) was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (8 mL), and *i*-Pr<sub>2</sub>NEt (1.2 mL, 6.8 mmol) and MOMCl (0.41 mL, 5.4 mmol) were added at 0 °C. After stirring at rt for 18 h, the mixture was partitioned between H<sub>2</sub>O and CH<sub>2</sub>Cl<sub>2</sub>, and the organic layer was washed with 1 M aqueous HCl, H<sub>2</sub>O, and brine, successively, dried over MgSO<sub>4</sub>, filtered, and evaporated. The residue was purified by silica gel column chromatography (hexane/EtOAc = 6:1) to give 390 mg of **5** as a colorless oil in 90% yield.  $[\alpha]_D^{21} +45.6$  (*c* 1.19, CHCl<sub>3</sub>); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ 0.91 (3H, d, *J* = 6.4 Hz), 1.08 (3H, s), 1.24 (6H, s), 0.91-1.59 (8H, m), 1.73-1.93 (6H, m), 2.15-2.17 (1H, m), 2.27-2.34 (3H, m), 3.36 (3H, s), 4.70 (2H, s); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): δ 19.2, 20.2, 21.4, 23.3, 26.5, 27.9, 29.9, 34.6, 36.3, 40.4, 42.5, 49.0, 50.8, 55.4, 55.4, 61.7, 61.8, 76.7, 91.4, 214.6; IR (neat): 2948, 2876, 1709, 1146, 1094, 1044, 918, 756, 723, 710 cm<sup>-1</sup>; EIMS: 324 (M<sup>+</sup>); HREIMS: calcd for C<sub>20</sub>H<sub>36</sub>O<sub>3</sub> 324.2664, found 324.2658.

### Ethyl {(1*S*,2*E*,6*R*,7*R*)-7-[(*R*)-6-Methoxymethoxy-6-methylheptan-2-yl]-6-methylbicyclo[4.3.0]nonan-2-ylidene}acetate (**6**).

To a suspension of NaH (60% in oil, 370 mg, ca. 9.2 mmol) in THF (10 mL) was added (EtO)<sub>2</sub>P(O)CH<sub>2</sub>CO<sub>2</sub>Et (2.4 g, 11 mmol) at 0 °C, and the mixture was stirred at rt overnight. The solution of ketone **5** (500 mg, 1.54 mmol) in THF (5 mL) was added at 0 °C, and the mixture was stirred at rt for 4 days. The reaction mixture was poured into saturated aqueous NH<sub>4</sub>Cl, and extracted with EtOAc. The combined extracts were washed with saturated aqueous NH<sub>4</sub>Cl and brine, dried over MgSO<sub>4</sub>, filtered, and evaporated. The residue was purified by silica gel column chromatography (hexane/EtOAc = 9:1) to give 558 mg of **6** as a colorless oil in 92% yield.  $[\alpha]_D^{21} +34.8$  (*c* 1.32, CHCl<sub>3</sub>); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ 0.89 (3H, d, *J* = 6.4 Hz), 0.95 (3H, s), 0.83-1.65 (24H, m), 1.66-1.73 (3H, m), 1.74-1.91 (1H, m), 2.18 (1H, t, *J* = 8.8 Hz), 2.35-2.47 (1H, m), 3.28 (1H, ddd, *J* = 4.8, 4.8, 16.8 Hz), 3.36 (3H, s), 4.09-4.38 (2H, m), 4.76 (2H, s), 5.65 (1H, s); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): δ 14.4, 19.7, 21.5, 22.0, 22.2, 26.0, 26.4, 27.5, 30.0, 34.0, 34.9, 37.4, 42.3, 46.3, 54.4, 55.1, 58.0, 59.4, 76.3, 90.9, 114.5, 164.9, 166.6;

IR (neat): 2953, 1715, 1640, 1466, 1171, 1146, 1095, 1040, 918, 864, 729, 637  $\text{cm}^{-1}$ ; EIMS: 394 ( $\text{M}^+$ ); HREIMS: calcd for  $\text{C}_{24}\text{H}_{42}\text{O}_4$  394.3083, found 394.3085.

**2-[(1*S*,2*E*,6*R*,7*R*)-7-[(*R*)-6-Methoxymethoxy-6-methylheptan-2-yl]-6-methylbicyclo[4.3.0]nonan-2-ylidene]ethanol (7).**

To the toluene solution (7 mL) of ester **6** (552 mg, 1.40 mmol) was added DIBAL-H (0.99 M solution in toluene, 3.5 mL, 3.5 mmol) at  $-78\text{ }^\circ\text{C}$ . After 1 h stirring at the same temperature, the reaction was quenched by adding saturated aqueous  $\text{Na}_2\text{SO}_4$ . The mixture was extracted with EtOAc, and the combined extracts were washed with brine, dried over  $\text{MgSO}_4$ , filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane/EtOAc = 4:1) to yield 488 mg of **8** as a colorless oil in 99% yield.  $[\alpha]_{\text{D}}^{21} +26.5$  ( $c$  1.08,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR(400 MHz,  $\text{CDCl}_3$ ):  $\delta$  0.77-0.82 (6H, m), 0.87 (3H, s), 1.14 (6H, s), 1.17-1.23 (6H, m), 1.26-1.62 (12H, m), 1.74-1.82 (1H, m), 1.97-2.03 (1H, m), 2.18-2.25 (1H, m), 3.30 (3H, s), 4.02-4.14 (2H, m), 4.64 (2H, s), 5.33 (1H, t,  $J = 6.4$  Hz);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ):  $\delta$  14.2, 19.8, 21.1, 21.6, 22.5, 24.7, 26.3, 26.4, 27.0, 29.2, 34.1, 34.7, 42.2, 45.0, 54.2, 55.1, 57.0, 58.8, 60.4, 76.3, 90.9, 122.3, 143.8; IR (neat): 2948, 2876, 1709, 1146, 1094, 1044, 918, 756, 723, 710  $\text{cm}^{-1}$ ; EIMS: 352 ( $\text{M}^+$ ); HREIMS: calcd for  $\text{C}_{22}\text{H}_{40}\text{O}_3$  352.2977, found 352.2978.

**(1*S*,2*E*,6*R*,7*R*)-2-[2-(Benzothiazole-2-sulfonyl)ethyliden]-7-[(*R*)-6-methoxymethoxy-6-methylheptan-2-yl]-6-methylbicyclo[4.3.0]nonane (8).**

To a solution of 2-mercaptobenzothiazole (209 mg, 1.25 mmol) and  $\text{PPh}_3$  (328 mg, 1.25 mmol) in  $\text{CH}_2\text{Cl}_2$  (2.7 mL) were added a solution of **7** (293 mg, 0.832 mmol) in  $\text{CH}_2\text{Cl}_2$  (2.7 mL) and DIAD (0.36 mL, 1.25 mmol) at  $0\text{ }^\circ\text{C}$ . After 2 h stirring, the mixture was concentrated, and the residue was dissolved in EtOH (5 mL), and to the solution were added 30%  $\text{H}_2\text{O}_2$  (2.7 mL) and  $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}\cdot 4\text{H}_2\text{O}$  (265 mg, 0.215 mmol) at  $0\text{ }^\circ\text{C}$  with stirring. After stirring at rt for 2 h, the mixture was poured into saturated aqueous  $\text{Na}_2\text{SO}_4$  and was extracted with EtOAc. The extracts were combined and washed with brine, dried over  $\text{MgSO}_4$ , filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane/EtOAc = from 20:1 to 3:1) to give 362 mg of **8** as a colorless oil in 82% yield.  $[\alpha]_{\text{D}}^{21} +24.2$  ( $c$  1.02,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  0.73 (3H, s), 0.78 (3H, d,  $J = 6.6$  Hz), 0.85-1.60 (15H, m), 1.21 (6H, s), 1.70-1.78 (1H, m), 1.86 (1H, ddd,  $J = 4.9, 9.8, 14.6$  Hz), 2.07 (1H, dd,  $J = 8.5, 9.0$  Hz), 2.15 (1H, ddd,  $J = 4.9, 4.9, 14.6$  Hz), 3.37 (3H, s), 4.23 (1H, dd,  $J = 7.8, 14.4$  Hz), 4.31 (1H, dd,  $J = 8.3, 14.4$  Hz), 4.71 (2H, s), 5.23 (1H, dd,  $J = 7.8, 8.3$  Hz), 7.59 (1H, ddd,  $J = 1.2, 7.8, 8.1$  Hz), 7.64 (1H, ddd,  $J = 1.2, 8.1, 8.3$  Hz), 8.00 (1H, ddd,  $J = 0.7, 1.2, 7.8$  Hz), 8.22 (1H, ddd,  $J = 0.7, 1.2, 8.3$  Hz);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ):  $\delta$  19.6, 21.3, 21.6, 21.6, 21.9, 25.0, 26.3, 26.4, 26.4, 26.8, 29.1, 34.1, 34.6, 37.2, 42.3, 45.5, 54.1, 54.3, 55.2, 57.3, 60.6, 76.5, 91.2, 107.5, 122.7, 125.7, 128.0, 128.3, 137.4, 153.0, 153.3, 166.4; IR (neat): 3065, 2936, 2361, 2330, 1740, 1470, 1425, 1331, 1150, 1084, 1035, 916,

853, 758, 729, 710, 689, 654, 635  $\text{cm}^{-1}$ ; EIMS: 533 ( $\text{M}^+$ ); HREIMS: calcd for  $\text{C}_{29}\text{H}_{43}\text{O}_4\text{NS}_2$  533.2634, found 533.2636.

**14-Epi-2-(3-hydroxypropyl)-1 $\alpha$ ,25-dihydroxy-19-norvitamin D<sub>3</sub> (a mixture of 14-epi-MART-10 and 14-epi-MART-11).**

To a solution of **8** (49.3 mg, 0.092 mmol) in dry THF (800  $\mu\text{L}$ ) was added LiHMDS (1 M solution in THF, 90  $\mu\text{L}$ , 90  $\mu\text{mol}$ ) at  $-78^\circ\text{C}$  under argon. After stirring at the same temperature for 60 min, a solution of **9** (35.0 mg, 0.066 mmol) in dry THF (800  $\mu\text{L}$ ) was added dropwise to the mixture. After stirring for 2 h, the reaction mixture was quenched by addition of saturated aqueous  $\text{NH}_4\text{Cl}$ , and the mixture was extracted with ether. The organic layer was washed with brine, dried over  $\text{MgSO}_4$ , and filtered. After evaporation of the solvent, the crude mixture was applied on silica gel column chromatography (hexane/EtOAc = from 50:1 to 10:1) to give the crude protected vitamin D mixture. To the solution of the mixture in dry MeOH (0.5 mL) was added (+)-CSA (31 mg, 0.13 mmol) at  $0^\circ\text{C}$  under argon. After stirring at rt for 17 h, the reaction mixture was diluted with EtOAc. The resulting mixture was washed with saturated aqueous  $\text{NaHCO}_3$ , brine, dried over  $\text{MgSO}_4$ , filtered, and evaporated. Purification by silica gel column chromatography (EtOAc/EtOH = 10:1) gave 11.5 mg of diastereomixture **10** as white powder in 38% yield in two steps.

**14-Epi-2 $\alpha$ -(3-pivaloyloxypropyl)-1 $\alpha$ ,25-dihydroxy-19-norvitamin D<sub>3</sub> (2 $\alpha$ -11) and 14-epi-2 $\beta$ -(3-pivaloyloxypropyl)-1 $\alpha$ ,25-dihydroxy-19-norvitamin D<sub>3</sub> (2 $\beta$ -11).**

To the solution of **10** (49.2 mg, 0.106 mmol) in pyridine (0.6 mL) were added DMAP (1.3 mg, 0.01 mmol) and pivaloyl chloride (20  $\mu\text{L}$ , 0.15 mmol), and the mixture was stirred at rt for 14 h. The mixture was diluted with EtOAc, and washed with  $\text{H}_2\text{O}$ , 0.5 M aqueous  $\text{CuSO}_4$ ,  $\text{H}_2\text{O}$ , and brine, successively, and dried over  $\text{MgSO}_4$ , filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane/EtOAc = 1:1) to give 35.3 mg of the mixture of 2 $\alpha$ -**11** and 2 $\beta$ -**11** in 61% yield. The mixture was separated by preparative HPLC (Shim-pack PREP-SIL (H)•Kit, 20 x 250 mm, 9.9 mL/min, hexane/EtOH = 9:1) to afford 23.0 mg of 2 $\alpha$ -**11** and 9.6 mg of 2 $\beta$ -**11**. 2 $\alpha$ -**11**:  $[\alpha]_{\text{D}}^{23} +24.1$  ( $c$  0.26,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR (600 MHz,  $\text{CDCl}_3$ ):  $\delta$  0.88 (3H, d,  $J = 6.6$  Hz), 0.92 (3H, s), 1.00-1.07 (1H, m), 1.20 (9H, s), 1.23 (6H, s), 1.22-1.93 (23H, m), 2.08-2.18 (4H, m), 2.47 (1H, ddd,  $J = 4.9, 4.9, 14.6$  Hz), 2.60 (1H, dd,  $J = 4.7, 12.4$  Hz), 2.90 (1H, dd,  $J = 3.6, 14.0$  Hz), 3.62 (1H, ddd,  $J = 4.7, 9.9, 9.9$  Hz), 4.09-4.12 (1H, m), 4.11 (2H, t,  $J = 6.6$  Hz), 6.00 (1H, d,  $J = 11.3$  Hz), 6.33 (1H, d,  $J = 11.3$  Hz);  $^{13}\text{C}$  NMR (150 MHz,  $\text{CDCl}_3$ ):  $\delta$  19.9, 21.7, 21.9, 22.6, 23.9, 25.0, 26.4, 27.1, 27.4, 29.3, 29.6, 29.9, 29.9, 34.2, 34.8, 34.9, 39.0, 44.3, 44.7, 49.1, 54.9, 58.3, 64.9, 68.5, 71.4, 71.5, 119.3, 124.5, 132.0, 144.3, 179.9; IR (neat): 3431, 2957, 2918, 2847, 2361, 1730, 1718, 1458, 1366, 1289, 1165, 1049, 849  $\text{cm}^{-1}$ ; EIMS: 546 ( $\text{M}^+$ ); HREIMS: calcd for  $\text{C}_{34}\text{H}_{58}\text{O}_5$  546.4284, found 546.4270. 2 $\beta$ -**11**:  $[\alpha]_{\text{D}}^{23} +13.9$  ( $c$  0.22,  $\text{CHCl}_3$ );  $^1\text{H}$

NMR (600 MHz, CDCl<sub>3</sub>):  $\delta$  0.89 (3H, d,  $J = 6.6$  Hz), 0.93 (3H, s), 1.03-1.10 (1H, m), 1.20 (9H, s), 1.22 (3H, s), 1.23 (3H, s), 1.22-1.93 (24H, m), 2.06-2.12 (1H, m), 2.14 (1H, dd,  $J = 8.0, 10.5$  Hz), 2.34 (1H, ddd,  $J = 1.4, 3.9, 13.5$  Hz), 2.41 (1H, d,  $J = 13.5$  Hz), 2.47 (1H, ddd,  $J = 4.7, 4.9, 14.6$  Hz), 3.11 (1H, dd,  $J = 3.6, 12.9$  Hz), 3.54 (1H, ddd,  $J = 4.7, 10.5, 10.5$  Hz), 4.05 (1H, ddd,  $J = 2.8, 2.8, 3.0$  Hz), 4.10 (2H, t,  $J = 6.6$  Hz), 6.05 (1H, d,  $J = 11.3$  Hz), 6.19 (1H, d,  $J = 11.3$  Hz); <sup>13</sup>C NMR (150 MHz, CDCl<sub>3</sub>):  $\delta$  19.8, 21.8, 22.1, 22.4, 25.0, 26.5, 27.1, 27.4, 29.4, 29.5, 29.9, 30.1, 34.3, 34.7, 35.9, 38.1, 39.0, 44.7, 45.6, 45.9, 49.1, 54.9, 58.2, 64.9, 68.7, 71.5, 71.9, 119.2, 125.1, 131.9, 144.5, 197.9; IR (neat): 3447, 2957, 2920, 2361, 2341, 1717, 1701, 1163, 910 cm<sup>-1</sup>; EIMS: 546 (M<sup>+</sup>); HREIMS: calcd for C<sub>34</sub>H<sub>58</sub>O<sub>5</sub> 546.4284, found 546.4270.

#### **14-Epi-2 $\alpha$ -(3-hydroxypropyl)-1 $\alpha$ ,25-dihydroxy-19-norvitamin D<sub>3</sub> (14-epi-MART-10).**

Pivalate 2 $\alpha$ -**11** (3.0 mg, 0.005 mmol) was dissolved in the 10% NaOMe solution in MeOH at 0 °C. After 1.5 h stirring at rt, Amberlyst<sup>®</sup> (H<sup>+</sup> form) was added at 0 °C for neutralization, followed by filtration, and the filtrate was concentrated in vacuo. The residue was purified by preparative thin layer chromatography on silica gel (EtOAc/EtOH = 1:1) to obtain 2.1 mg of 14-epi-MART-10 as a white powder in 91% yield.  $[\alpha]_D^{23} +5.9$  ( $c$  0.17, CHCl<sub>3</sub>); UV(EtOH):  $\lambda_{\max}$  244.5, 252.5, 262.0 nm;  $\lambda_{\min}$  247.5, 259.0 nm. <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>):  $\delta$  0.88 (3H, d,  $J = 6.6$  Hz), 0.92 (3H, s), 1.00-1.08 (1H, m), 1.23 (6H, s), 1.22-1.88 (24H, m), 2.06-2.12 (3H, m), 2.16 (1H, d,  $J = 11.5$  Hz), 2.47 (1H, ddd,  $J = 4.4, 4.4, 14.9$  Hz), 2.60 (1H, dd,  $J = 4.7, 12.4$  Hz), 2.90 (1H, dd,  $J = 4.1, 14.0$  Hz), 3.65 (1H, ddd,  $J = 4.7, 9.9, 9.9$  Hz), 3.71 (2H, t,  $J = 5.8$  Hz), 4.11 (1H, ddd,  $J = 2.8, 2.8, 4.1$  Hz), 6.01 (1H, d,  $J = 11.3$  Hz), 6.32 (1H, d,  $J = 11.3$  Hz); <sup>13</sup>C NMR (150 MHz, CDCl<sub>3</sub>):  $\delta$  19.8, 21.7, 21.9, 22.3, 23.5, 24.8, 26.9, 29.2, 29.4, 30.0, 30.1, 34.1, 34.6, 35.6, 37.9, 44.4, 45.3, 45.5, 48.8, 54.6, 57.9, 63.0, 68.6, 71.1, 71.7, 118.5, 124.3, 131.3, 143.6; IR (neat): 3340, 2920, 1203, 1147, 1051, 801 cm<sup>-1</sup>; EIMS: 462 (M<sup>+</sup>); HREIMS: calcd for C<sub>29</sub>H<sub>50</sub>O<sub>4</sub> 462.3709, found 462.3712.

#### **14-Epi-2 $\beta$ -(3-hydroxypropyl)-1 $\alpha$ ,25-dihydroxy-19-norvitamin D<sub>3</sub> (14-epi-MART-11).**

This compound was obtained by the same procedure as described above from pivalate 2 $\beta$ -**11** (3.0 mg, 0.005 mmol). 14-epi-MART-11 (2.2 mg) was obtained as a white powder in 95% yield.  $[\alpha]_D^{23} +4.3$  ( $c$  0.12, CHCl<sub>3</sub>); UV(EtOH):  $\lambda_{\max}$  244.5, 252.5, 262.0 nm;  $\lambda_{\min}$  247.5, 259.0 nm. <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>):  $\delta$  0.89 (3H, d,  $J = 6.4$  Hz), 0.93 (3H, s), 1.03-1.10 (1H, m), 1.22 (6H, s), 1.22-1.93 (25H, m), 2.06-2.12 (1H, m), 2.14 (1H, dd,  $J = 8.0, 10.0$  Hz), 2.35 (1H, dd,  $J = 3.5, 13.7$  Hz), 2.41 (1H, d,  $J = 13.7$  Hz), 2.46 (1H, ddd,  $J = 4.7, 4.9, 14.6$  Hz), 3.11 (1H, dd,  $J = 4.2, 12.7$  Hz), 3.57 (1H, ddd,  $J = 4.7, 10.0, 10.0$  Hz), 3.70 (2H, t,  $J = 5.6$  Hz), 4.05 (1H, ddd,  $J = 2.9, 2.9, 3.2$  Hz), 6.05 (1H, d,  $J = 11.3$  Hz), 6.19 (1H, d,  $J = 11.3$  Hz); <sup>13</sup>C NMR (150 MHz, CDCl<sub>3</sub>):  $\delta$  19.8, 21.7, 21.8, 22.5, 23.8, 24.9, 27.0, 29.2, 29.5, 29.8, 30.0, 34.1, 34.6, 37.9(2C), 44.0, 44.5, 45.4, 49.0, 54.6, 58.0, 63.0, 68.5, 71.1(2C), 118.5, 123.6,

131.2, 143.2; IR (neat): 3335, 2926, 1456, 1375, 1201, 1147, 1049, 800  $\text{cm}^{-1}$ ; EIMS: 462 ( $\text{M}^+$ ); HREIMS: calcd for  $\text{C}_{29}\text{H}_{50}\text{O}_4$  462.3709, found 462.3715.

### Metabolism of 14-*epi*-MART-10 and 14-*epi*-MART-11 by human CYP24A1 (24R-OHase) in a reconstituted cell-free system

CYP24A1-dependent metabolism of 14-*epi*-MART-10 and 14-*epi*-MART-11 were measured in a reconstituted system containing the membrane fraction.<sup>32-34</sup> The metabolism of MART-10 was also measured to compare it with those of the 14-*epi* compounds. The activities were measured under the conditions as follows: 0.05  $\mu\text{M}$  human CYP24A1, 2.0  $\mu\text{M}$  ADX, 0.2  $\mu\text{M}$  ADR, 0 to 20  $\mu\text{M}$  14-*epi*-MART-10 or 14-*epi*-MART-11 or MART-10 in 100 mM Tris-HCl (pH 7.4) and 1 mM EDTA at 37 °C. The reaction was initiated by the addition of NADPH at a final concentration of 1 mM. Aliquots of the reaction mixture were collected at various time-intervals and extracted with 4 volumes of chloroform-methanol (3:1). The organic phase was recovered and dried under reduced pressure. The resultant residue was dissolved in MeCN and applied to HPLC under the following conditions: column, YMC-Pack ODS-AM (5  $\mu\text{m}$ ) (4.6 mm x 300 mm) (YMC Co., Kyoto, Japan); UV detection, 254 nm; flow-rate, 1.0  $\text{mL min}^{-1}$ ; column temperature, 40 °C; mobile phase, a linear gradient of 20-100% MeCN aqueous solution for the first 25 min followed by 100% MeCN for 10 min for the isolation and identification of 14-*epi*-MART-10 and 14-*epi*-MART-11, MART-10, and their metabolites.

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