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SYNTHESIS AND BIOLOGICAL ACTIVITIES OF VDR ANTAGONISTS; 25-MODIFIED 1α,25-DIHYDROXYVITAMIN D_3-26,23-LACTAM (DLAM) DERIVATIVES[†]

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Abstract – Three series of DLAM (1α,25-dihydroxyvitamin D_3-26,23-lactam) derivatives (9 compounds) with 25-modification were synthesized. The biological evaluation of these derivatives revealed that the bulkiness at the 25-position of the DLAM causes a decrease in both the binding affinity for VDR and antagonistic activity.

1α,25-Dihydroxyvitamin D_3 (**1**, Figure 1) (1,25-D_3), which is a hormonally active form of vitamin D_3, exhibits various physiological actions including regulation of calcium homeostasis, bone mineralization, proliferation and differentiation of various types of cells, and immune modulation.[1] Most of these actions of 1,25-D_3 are mediated by its specific receptor, vitamin D receptor (VDR), which is a member of the nuclear receptor (NR) superfamily and acts as ligand-dependent gene transcriptional factor with coactivators.[2, 3]

More than 3000 analogs of 1,25-D_3 have been synthesized as candidate ligands for VDR, and most of them were reported to show agonistic activities. Only four types of ligands, the 25-carboxylic ester derivative **2** (ZK168281),[4] 26,23-lactone **3** (TEI-9647),[4c,5] AD-47 (**4**)[6] and DLAM (1α,25-dihydroxyvitamin D_3-26,23-lactam),[7] have been reported as antagonists so far (Figure 1). These VDR antagonists are expected to be effective for the treatment of metabolic bone disease represented by Paget's disease[8] as well as biological tools for the elucidation of VDR function and the mode of action of 1,25-D_3.

We have recently synthesized new DLAM derivatives having various aralkyl group on the nitrogen of the lactam ring ((23*S*,25*S*)-DLAM-1P (5)~4P (**8**)) and their stereoisomers. The biological activities of these compounds were evaluated, and the orders of the VDR binding affinity and antagonistic activity of these DLAM derivatives were found to be correlated.[7c] In our continuous structure-activity relationship (SAR) studies on DLAM derivatives, we focused on the substitution at C-25 position on DLAM, since some of the analogs on 1,25-D_3 were reported to increase their binding affinities to VDR and/or activities by modifying the alkyl chain at C-25.[9] Herein, we described the synthesis of C-25 modified new DLAM derivatives and evaluation of their VDR binding affinity and antagonistic activity.

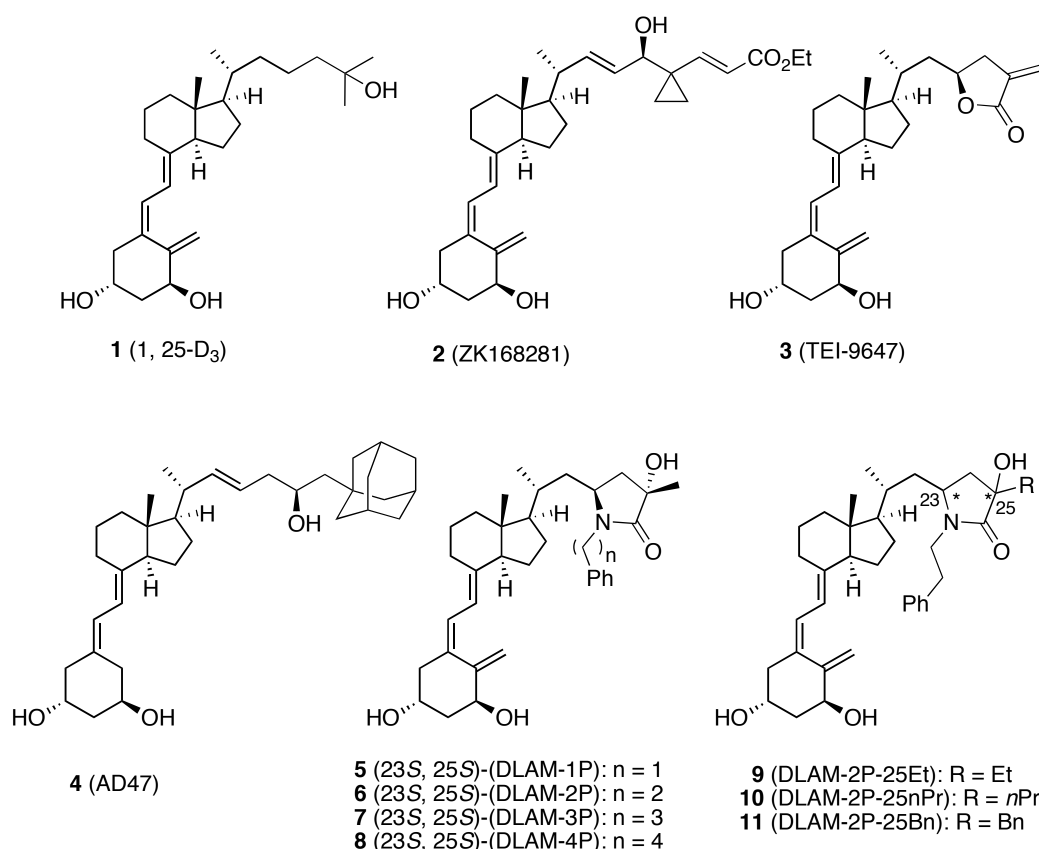
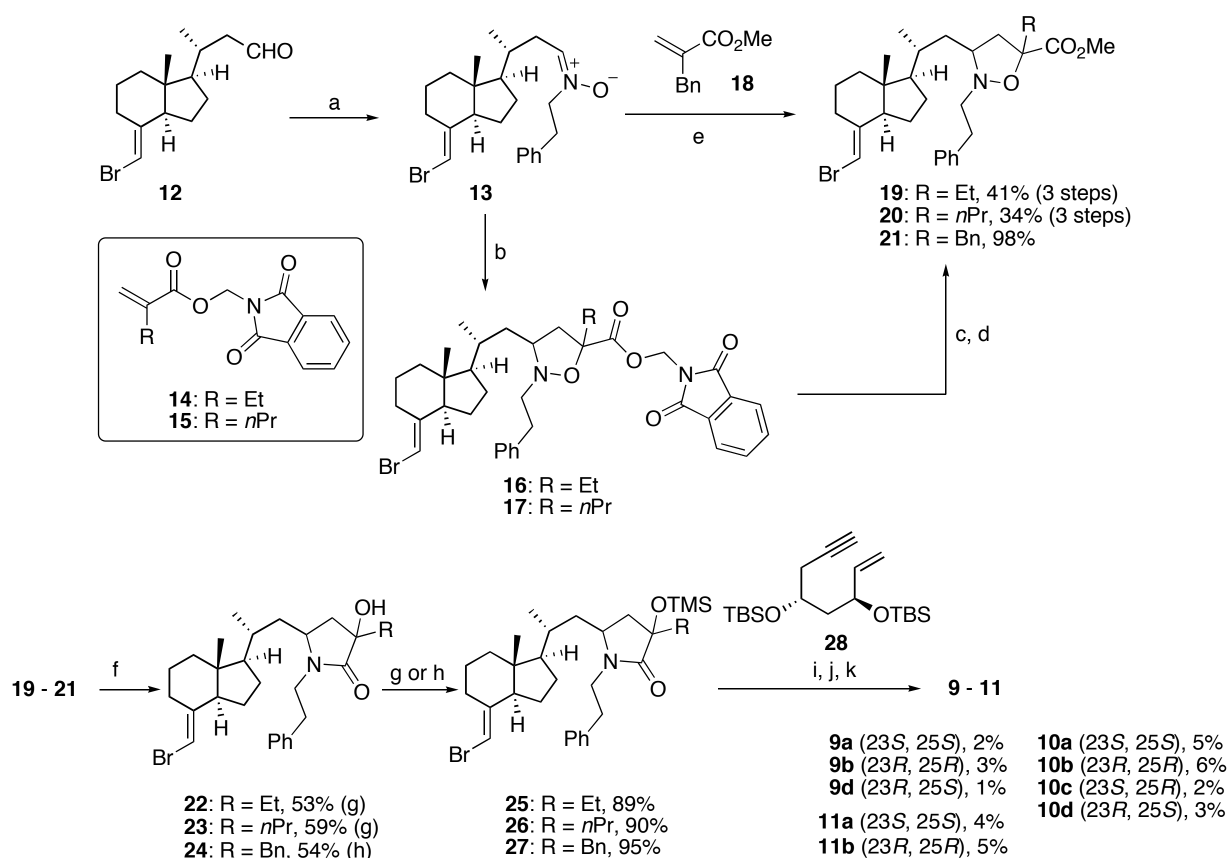


Figure 1. Structures of 1,25-VD_3 and VDR antagonists of ZK168281 (**2**), TEI-9647 (**3**), AD47 (**4**), and DLAMS (**5~8**) including new derivatives **9~11**.

Outset of our SAR studies, DLAM-2P was selected as a core skeleton for the structural development since one of its stereoisomers of (23*S*,25*S*)-DLAM-2P (**6**) showed the highest binding affinity for VDR with strongest antagonistic activity among the DLAMs.[7c] Thus, based upon our 1,3-diploar cycloaddition methodology, C-25 modified new DLAM-2P derivatives (**9~11**) were synthesized (Scheme 1).

The aldehyde **12**[10] was reacted with 2-phenylethylhydroxylamine to give the nitrone **13**, which was subsequently reacted with the acrylic acid derivatives **14**, **15**[11] and **18**[12] to give the isoxazolidines **16**, **17**, and **21**, respectively, with four possible diastereomers at C23 and C25. The phthalimidomethyl ester of **16** and **17** was converted into methyl ester by hydrolysis with hydrazine followed by esterification of the resulting carboxylic acid with trimethylsilyl diazomethane to give **19** and **20**. Reduction of the N-O bond in **19**-**21** with molybdenum hexacarobonyl simultaneous cyclization took place to give lactams **22**, **23** and **24**. The tertiary alcohol of **22**-**24** was protected as trimethylsilyl ether to give the CD-ring synthon **25**-**27**. These CD-ring synthons **25**-**27** were reacted with enyne **28**[14] to construct the vitamin D_3 triene skeleton in the presence of palladium catalyst. Finally, deprotection of the TBS and TMS groups with TBAF in THF at 50 °C gave **9**-**11** as a mixture of diastereomers at C23 and C25. These stereoisomers were separated with HPLC. In case of **9** and **11**, three and two out of four possible diastereomers, i.e., **9a**, **9b** and **9d**, and **11a** and **11b**, were isolated, respectively.[15]



Scheme 1. Synthesis of C-25 modified DLAM derivatives **9**-**11**. (a) 2-phenylethylhydroxylamine, Et_3N, CH_2Cl_2. (b) **14** or **15**, toluene, 90 °C. (c) NH_2NH_2, *n*-butanol. (d) $TMSCHN_2$, benzene/ MeOH (9/1). (e) toluene, 90 °C. (f) $Mo(CO)_6$, $MeCN/H_2O$ (7/1), 90 °C. (g) TMS-imidazole, CH_2Cl_2. (h) TMSOTf, 2,6-lutidine, CH_2Cl_2, 0 °C. (i) $Pd_2(dba)_3CHCl_3$, Ph_3P, Et_3N, toluene, 120 °C. (j) TBAF, THF, 50 °C. (k) HPLC separation.

The C-25 modified new DLAM-2P derivatives (**9-11**) in hand,[16] the biological activities of these compounds were evaluated (Table 1). Firstly, the VDR binding affinity was examined by using the chick intestinal VDR as described previously.[17] The VDR affinities of **9-11** were varied by the stereochemistries at C23 and C25, and substituents at C25. The (23*S*,25*S*)-stereoisomers of **9-11** showed higher binding affinities than other stereoisomers, and their binding affinities of **9a-11a** were revealed to decrease with increasing their bulkiness at C-25-position, and compound **6** had the highest affinity with VDR (entries 1, 2, 5, and 9). Next we examined the antagonistic activities of **9-11** by the NBT-reduction method[18] in terms of IC_{50} for differentiation of HL-60 cells induced by 10 nM of **1**. As a result, only the DLAM derivatives with (23*S*,25*S*) stereochemistries of **9a-11a** showed antagonistic activities. As in the case of the binding affinities for VDR, the antagonistic activities of them were reduced with increasing their bulkiness at C-25 position (entries 2, 5, and 9). With these experiments, structural development of DLAM-2P at C-25 with bulky substituents resulted in decreasing both VDR binding affinity and antagonistic activity. Since the VDR antagonistic activity of DLAM derivatives are elicited through the binding with VDR, sterically bulky group at C-25 was realized to cause only negative factors for binding by preventing the interaction of 25-hydroxyl group with His305 and His379 residues in VDR.[19]

Table 1. Binding affinity for VDR and antagonistic activity of DLAMs **9-11**.

entry	DLAM	VDR binding affinity [a]	antagonistic activity [b] (IC_{50}, nM)
1	**6**	8[7c]	194[7c]
2	**9a**	3.89	560
3	**9b**	0.56	NA [c]
4	**9d**	0.19	NA
5	**10a**	1.09	580
6	**10b**	0.75	NA
7	**10c**	0.10	NA
8	**10d**	0.15	NA
9	**11a**	0.74	>3000
10	**11b**	1.10	NA

[a] The potency of the 1,25-D_3 is normalized to 100.
[b] The antagonistic activity was assessed in terms of IC_{50} for the differentiation of HL-60 cells induced by 10 nM of 1,25-D_3.
[c] NA = not antagonist

In conclusion, structural development of DLAM at C-25 position was examined. The C-25 modified DLAM-2P derivatives **9-11** were synthesized, and evaluated their VDR binding affinity and antagonistic activity. As a result, the biological activities of both VDR binding affinity and antagonistic activity of these derivatives were decreased in relation to increasing the bulkiness at C-25 position. This result suggests that, as in the case of 1,25-D_3 binding for VDR, the interaction between the 25-hydroxyl group of the DLAM and the VDR plays an important role in binding, and the steric hinderence at C-25 position in DLAM would affect negatively to the binding affinity and antagonistic activity in this case. Further SAR studies of DLAM derivatives and investigation to the variety of biological activities are in progress.

EXPREMENTAL

General

Flash column chromatography was performed on KP-SIL (Biotage Silica, 40-63 μm). NMR spectra were measured on JEOL AL-400 magnetic resonance spectrometers. Mass spectra were measured on Agilent LC/ MSD SL (Liquid Chromatograph mass spectrometer) and Shimadzu LCMS-IT-TOF (Liquid Chromatograph mass spectrometer-ion trap-Time of flight).

Preparation of DLAMs 9-11.

2-Ethylacrylic acid phthalimidomethyl ester (14). To a suspension of 2-ethylacrylic acid **29** (250 mg, 2.5 mmol) in DMF (8 mL) was added *N*-bromophthalimide (660 mg, 2.8 mmol) and KF (291 mg, 5.0 mmol), and the mixture was stirred at 80 ˚C for 12 h under argon atmosphere. The reaction mixture was concentrated. To the residue was added H_2O (80 mL) and extracted with CH_2Cl_2. The organic layer was dried over $MgSO_4$ and concentrated. The residue was purified by flash column chromatography on KP-SIL (hexane / AcOEt = 4 / 1) to give **14** (463 mg, 72%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.94 (dd, *J* = 5.4, 3.2 Hz, 2 H), 7.79 (dd, *J* = 2.8, 1.4 Hz, 2 H), 6.16 (d, *J* = 1.0 Hz, 1 H), 5.80 (s, 2 H), 5.57 (d, *J* = 1.5 Hz, 1 H), 2.35-2.25 (m, 2 H), 1.06 (t, *J* = 7.4 Hz, 3 H).

2-Propylacrylic acid phthalimidomethyl ester (15). Similar to the synthesis of **14**, a crude product, which was obtained from 2-propylacrylic acid **30** (300 mg, 2.6 mmol), *N*-bromophthalimide (695 mg, 2.9 mmol) and KF (306 mg, 5.3 mmol) in DMF (8 mL) at 80 ˚C for 12 h, was purified by flash column chromatography on KP-SIL (hexane / AcOEt = 4 / 1) to give **15** as colorless oil (643 mg, 90%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.94 (dd, *J* = 5.4, 2.7 Hz, 2 H), 7.79 (dd, *J* = 5.4, 2.7 Hz, 2 H), 6.16 (d, *J* = 0.7 Hz, 1 H), 5.80 (s, 2 H), 5.57 (d, *J* = 1.5 Hz, 1 H), 2.32-2.23 (m, 2 H), 1.55-1.43 (m, 2 H), 0.96-0.89 (m, 3 H).

***N*-[3-(4-Bromomethylene-7α-methyloctahydroinden-1-yl)-1-butylidene]phenetylamine *N*-oxide (13).** To a suspension of 3-(4-bromomethylene-7α-methyloctahydroinden-1-yl)butyraldehyde **12**[10] (788 mg, 2.64 mmol) in CH_2Cl_2 (25 mL) was added 2-phenyethylhydroxylamine (722 mg, 5.26 mmol) and Et_3N (5.0 mL), and the mixture was stirred at rt for 1 h under argon atmosphere. To the mixture was added saturated NH_4Cl aq. at 0 ˚C, and the aqueous layer was extracted with CH_2Cl_2. The organic layer was dried over $MgSO_4$ and concentrated. The residue was purified by flash column chromatography on KP-SIL (hexane / AcOEt = 2 / 1) to give **13** (1.28 g, 99%) as colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31-7.20 (m, 5 H), 6.38 (t, *J* = 5.7 Hz, 1 H), 5.64 (s, 1 H), 3.97 (t, *J* = 6.7 Hz, 2 H), 3.23-3.19 (m, 2 H), 2.88-2.86 (m, 1 H), 2.46 (dt, *J* = 17.2, 4.4 Hz, 1 H), 2.28-2.19 (m, 1 H), 1.97-1.13 (m, 12 H), 0.79 (d, *J* = 6.6 Hz, 3 H), 0.53 (s, 3 H).

3-[2-(4-Bromomethylene-7a-methyloctahydroinden-1-yl)propyl]-5-ethyl-2-phenethylisoxazolidine-5-carboxylic acid methyl ester (19). To a suspension of **13** (577 mg, 1.43 mmol) in toluene (10 mL) was added the suspension of **14** (463 mg, 1.79 mmol) in toluene (5 mL), and the mixture was stirred at 90 ˚C for 12 h under argon atmosphere. The resulting mixture was concentrated. The residue was roughly purified by flash column chromatography on KP-SIL (hexane / AcOEt = 4 / 1). The resulting crude product was dissolved in *n*-butanol (25 mL). To the mixture was added hydrazine (3.3 mL), and stirred at 90 ˚C for 1 h under argon atmosphere. To the mixture was added water at 0 ˚C, and concentrated. The residue was diluted with AcOEt, and washed with 1N HCl. The organic layer was dried over $MgSO_4$ and concentrated. The residue was roughly purified by flash column chromatography on KP-SIL (hexane / AcOEt = 4 / 1). The resulting crude product was dissolved in benzene / MeOH = 9 / 1 (5 mL). To the mixture was added $TMSCHN_2$ (1.0 mL, 0.59 mmol), and stirred at rt for 15 min. The resulting mixture was concentrated to give **19** (269 mg, 41%) as colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32-7.15 (m, 5 H), 5.64 (brs, 1 H), 3.79-3.75 (m, 3 H), 3.10-0.72 (m, 30 H), 0.57-0.52 (m, 3 H).

3-[2-(4-Bromomethylene-7a-methyloctahydroinden-1-yl)propyl]-5-propyl-2-phenethylisoxazolidine-5-carboxylic acid methyl ester (20). Similar to the synthesis of **19**, to a suspension of **13** (700 mg, 1.74 mmol) in toluene (10 mL) was added the suspension of **15** (643 mg, 2.35 mmol) in toluene (7 mL), and the mixture was stirred at 90 ˚C for 12 h under argon atmosphere. The resulting mixture was concentrated. The residue was roughly purified by flash column chromatography on KP-SIL (hexane / AcOEt = 4 / 1). The resulting crude product was dissolved in *n*-butanol (25 mL). To the mixture was added hydrazine (3.6 mL), and stirred at 90 ˚C for 1 h under argon atmosphere. To the mixture was added water at 0 ˚C, and concentrated. The residue was diluted with AcOEt, and washed with 1N HCl. The organic layer was dried over $MgSO_4$ and concentrated. The residue was roughly purified by flash column chromatography on KP-SIL (hexane / AcOEt = 4 / 1). The resulting crude product was dissolved

in benzene / MeOH = 9 / 1 (5 mL). To the mixture was added $TMSCHN_2$ (1.1 mL, 0.64 mmol), and stirred at rt for 15 min. The resulting mixture was concentrated to give **20** (269 mg, 34%) as colorless oil. ^1H NMR (400 MHz, $CDCl_3$) δ 7.32-7.16 (m, 5 H), 5.64 (brs, 1 H), 3.78-3.74 (m, 3 H), 3.10-0.80 (m, 32 H), 0.57-0.51 (m, 3 H).

5-[2-(4-Bromomethylene-7a-methyloctahydroinden-1-yl)propyl]-3-ethyl-3-hydroxy-1-phenethyl-pyrrolidin-2-one (22). To a suspension of **19** (269 mg, 0.50 mmol) in MeCN / H_2O = 7 / 1 (8 mL) was added $Mo(CO)_6$ (205 mg, 0.78 mmol), and the mixture was stirred at 90 ˚C for 12 h under argon atmosphere. The resulting mixture was filtered over celite and concentrated. The residue was purified by flash column chromatography on KP-SIL (hexane / AcOEt = 2 / 1) to give **22** (132 mg, 53%) as colorless oil. ^1H NMR (400 MHz, $CDCl_3$) δ 7.33-7.16 (m, 5 H), 5.66 (brs, 1 H), 3.85-3.73 (m, 1 H), 3.55-3.46 (m, 1 H), 3.32-3.16 (m, 1 H), 2.94-2.64 (m, 3 H), 2.28-0.82 (m, 24 H), 0.61-0.56 (m, 3 H).

5-[2-(4-Bromomethylene-7a-methyloctahydroinden-1-yl)propyl]-3-propyl-3-hydroxy-1-phenethyl-pyrrolidin-2-one (23). Similar to the synthesis of **22**, a crude product, which was obtained from **20** (307 mg, 0.56 mmol) and $Mo(CO)_6$ (230 mg, 0.87 mmol) in CH_3CN / H_2O = 7 / 1 (8 mL) at 90 ˚C for 12 h, was purified by preparative TLC (silica gel; hexane / AcOEt = 1/ 1) to give **23** as colorless oil (171 mg, 59%). ^1H NMR (400 MHz, $CDCl_3$) δ 7.33-7.17 (m, 5 H), 5.66 (brs, 1 H), 3.84-3.72 (m, 1 H), 3.55-3.47 (m, 1 H), 3.25-3.15 (m, 1 H), 2.95-2.73 (m, 3 H), 2.29-0.83 (m, 26 H), 0.60-0.55 (m, 3 H).

5-[2-(4-Bromomethylene-7a-methyloctahydroinden-1-yl)propyl]-3-ethyl-1-phenethyl-3-trimethyl-silanyloxypyrrolidin-2-one (25). To a suspension of **22** (132 mg, 0.26 mmol) in CH_2Cl_2 (5 mL) was added trimethylsilyl-imidazole (0.2 mL, 1.30 mmol), and the mixture was stirred at rt for 12 h under argon atmosphere. To the resulting mixture was added water at 0 ˚C. The aqueous layer was extracted with CH_2Cl_2. The organic layer was dried over $MgSO_4$ and concentrated. The residue was purified by preparative TLC (silica gel; hexane / AcOEt = 5 / 1) to give **25** as colorless oil (133 mg, 89%). ^1H NMR (400 MHz, $CDCl_3$) δ 7.33-7.16 (m, 5 H), 5.68-5.64 (m, 1 H), 3.77-3.50 (m, 2 H), 3.28-3.10 (m, 1 H), 2.92-2.75 (m, 3 H), 2.23-0.75 (m, 24 H), 0.58 (d, J = 4.6 Hz, 3 H), 0.16-0.10 (m, 9 H).

5-[2-(4-Bromomethylene-7a-methyloctahydroinden-1-yl)propyl]-3-propyl-1-phenethyl-3-trimethyl-silanyloxypyrrolidin-2-one (26). Similar to the synthesis of **25**, a crude product, which was obtained from **23** (171 mg, 0.33 mmol) and trimethylsilylimidazole (0.25 mL, 1.65 mmol) in CH_2Cl_2 (5 mL) at rt for 12 h, was purified by preparative TLC (silica gel; hexane / AcOEt = 5 / 1) to give **26** as colorless oil (174 mg, 90%). ^1H NMR (400 MHz, $CDCl_3$) δ 7.33-7.17 (m, 5 H), 5.68-5.64 (m, 1 H), 3.76-3.52 (m, 2 H), 3.26-3.10 (m, 1 H), 2.92-2.73 (m, 3 H), 2.23-0.86 (m, 26 H), 0.58 (d, J = 5.1 Hz, 3 H), 0.15-0.11 (m, 9 H).

3-Benzyl-5-[2-(4-bromomethylene-7a-methyloctahydroinden-1-yl)propyl]-3-hydroxy-1-phenethyl-pyrrolidin-2-one (24). The mixture of **13** (240 mg, 0.93 mmol) and 2-benzylacrylic acid methyl ester

18[12] (530 mg, 3.00 mmol) in toluene (5 mL) was stirred at 90 °C for 12 h under argon atmosphere. The resulting mixture was concentrated. The residue was roughly purified by flash column chromatography on KP-SIL (hexane / AcOEt = 20 / 1). The crude product was dissolved in MeCN / H_2O = 7 / 1 (12 mL). To the resulting mixture was added $Mo(CO)_6$ (240 mg, 0.91 mmol), and the mixture was stirred at 90 °C for 12 h under argon atmosphere. The resulting mixture was filtered over celite and concentrated. The residue was purified by flash column chromatography on KP-SIL (hexane / AcOEt = 2 / 1) to give **24** (178 mg, 2steps 53%) as an amorphous solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31-7.18 (m, 10 H), 5.65 (s, 1 H), 3.80-3.68 (m, 1 H), 3.44-3.43 (m, 1 H), 3.20-3.16 (m, 1 H), 3.05-2.73 (m, 5 H), 2.17-1.00 (m, 16 H), 0.86-0.82 (m, 3 H), 0.54-0.50 (m, 3 H).

3-Benzyl-5-[2-(4-bromomethylene-7a-methyloctahydroinden-1-yl)propyl]-1-phenethyl-3-trimethyl-silanyloxypyrrolidin-2-one (27). To the solution of **24** (178 mg, 0.32 mmol) in CH_2Cl_2 (3.5 mL) was added TMSOTf (70 μL, 0.39 mmol) and 2,6-lutidine (55 μL, 0.47 mmol). The resulting mixture was stirred at 0 °C for 1 h under argon atmosphere. To the mixture was added saturated $NaHCO_3$ aq. at 0 °C, and the aqueous layer was extracted with CH_2Cl_2. The organic layer was dried over $MgSO_4$ and concentrated. The residue was purified by flash column chromatography on KP-SIL (hexane / AcOEt = 20 / 1) to give **27** (190 mg, 95%) as colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33-7.15 (m, 10 H), 5.65 (brs, 1 H), 3.75-3.44 (m, 2 H), 3.30-3.18 (m, 1 H), 3.14-2.54 (m, 5 H), 2.12-1.02 (m, 16 H), 0.94-0.82 (m, 3 H), 0.55-0.43 (m, 3 H), 0.26-0.15 (m, 9 H).

20(*R*)-(3-Ethyl-3-hydroxy-1-phenethylpyrrolidin-2-one-5-yl)methyl-9,10-secopregna-5(*Z*),7(*E*),10(19)-triene-1α,3β-diol (9). The mixture of $Pd_2(dba)_3CHCl_3$ (23.0 mg, 0.023 mmol) and PPh_3 (47.0 mg, 0.18 mmol) in toluene / Et_3N = 1 / 1 (3.6 mL) was stirred at rt for 10 min under argon atmosphere. To the resulting mixture was added the solution of **25** (87.0 mg, 0.15 mmol) and **28**[14] (66.0 mg, 0.18 mmol) in toluene (1.8 mL), and refluxed at 120 °C for 1 h under argon atmosphere. The resulting mixture was diluted with AcOEt, then added saturated NH_4Cl aq. at rt. The aqueous layer was extracted with AcOEt. The combined organic layer was washed with brine, dried over $MgSO_4$ and concentrated. The residue was roughly purified by preparative TLC (silica gel; hexane / AcOEt = 5 / 1). The crude product was dissolved in THF (3 mL). To the resulting solution was added the 1 M solution of TBAF in THF (0.44 mL, 0.44 mmol), and stirred at 50 °C for 2 h under argon atmosphere. To the resulting mixture was added saturated NH_4Cl aq. at rt. The aqueous layer was extracted with AcOEt. The combined organic layer was washed with brine, dried over $MgSO_4$ and concentrated. The residue was roughly purified by preparative TLC (silica gel; hexane / acetone = 1 / 1) to give **9** as three diastereomers mixtures. These mixtures were further purified by HPLC three times [first; CHIRALPAK AD column, ϕ 20 x 250 mm, DAICEL, hexane / EtOH = 7 / 3, second; ODS-AM column, ϕ 30 x 250 mm, YMC, eluent A / B = 3 / 7 (Eluent A; H_2O / MeCN = 95 / 5, eluent B; MeCN / MeOH = 6 / 4 + 0.5% H_2O), third; CHIRALPAK AD

column, ϕ 20 x 250 mm, DAICEL, hexane / EtOH = 85 / 15] to give **9a** (1.2 mg, 2%), **9b** (2.2 mg, 3%) and **9d** (0.6 mg, 1%), respectively.

Spectral data for (23*S*,25*S*)-DLAM-2P-25Et (**9a**):[16] ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33-7.18 (m, 5 H), 6.38 (d, *J* = 11.5 Hz, 1 H), 6.02 (d, *J* = 11.0 Hz, 1 H), 5.33 (s, 1 H), 5.00 (s, 1 H), 4.43 (brs, 1 H), 4.23 (brs, 1 H), 3.80-3.70 (m, 1 H), 3.55-3.45 (m, 1 H), 3.25-3.15 (m, 1 H), 2.93-2.75 (m, 3 H), 2.63-2.55 (m, 1 H), 2.32 (dd, *J* = 13.7, 6.7 Hz, 1 H), 2.11 (dd, *J* = 13.7, 7.3 Hz, 1 H), 2.04-1.10 (m, 22 H), 0.96 (t, *J* = 7.4 Hz, 3 H), 0.88 (d, *J* = 5.6 Hz, 3 H), 0.56 (s, 3 H); HRMS calcd for $C_{36}H_{51}NO_4$ 562.3891, found 562.3915.

Spectral data for (23*R*,25*R*)-DLAM-2P-25Et (**9b**):[16] ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34-7.19 (m, 5 H), 6.38 (d, *J* = 11.2 Hz, 1 H), 6.03 (d, *J* = 11.2 Hz, 1 H), 5.34 (s, 1 H), 5.01 (s, 1 H), 4.44 (brs, 1 H), 4.25 (brs, 1 H), 3.85-3.77 (m, 1 H), 3.55-3.47 (m, 1 H), 3.25-3.15 (m, 1 H), 2.95-2.74 (m, 3 H), 2.61 (dd, *J* = 13.7, 3.4 Hz, 1 H), 2.32 (dd, *J* = 13.4, 6.7 Hz, 1 H), 2.20 (dd, *J* = 13.5, 7.7 Hz, 1 H), 2.12-1.15 (m, 22 H), 1.05-0.91 (m, 6 H), 0.56 (s, 3 H); HRMS calcd for $C_{36}H_{51}NO_4$ 562.3891, found 562.3905.

Spectral data for (23*R*,25*S*)-DLAM-2P-25Et (**9d**):[16] ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32-7.16 (m, 5 H), 6.38 (d, *J* = 11.0 Hz, 1 H), 6.03 (d, *J* = 11.2 Hz, 1 H), 5.34 (s, 1 H), 5.01 (s, 1 H), 4.44 (brs, 1 H), 4.24 (brs, 1 H), 4.06-3.95 (m, 1 H), 3.35-3.27 (m, 1 H), 3.24-3.14 (m, 1 H), 2.95-2.69 (m, 3 H), 2.64-2.57 (m, 1 H), 2.42 (s, 1 H), 2.36-2.23 (m, 2 H), 2.04-1.06 (m, 19 H), 1.00 (d, *J* = 6.3 Hz, 3 H), 0.85 (t, *J* = 7.4 Hz, 3 H), 0.58 (s, 3 H); HRMS calcd for $C_{36}H_{51}NO_4$ 562.3891, found 562.3921.

20(*R*)-(3-Hydroxy-1-phenethyl-3-propylpyrrolidin-2-one-5-yl)methyl-9,10-secopregna-5(*Z*),7(*E*),10(19)-triene-1α,3β-diol (10). Similar to the synthesis of **9**, a crude product, which was obtained from the solution of $Pd_2(dba)_3CHCl_3$ (23.0 mg, 0.023 mmol) and PPh_3 (47.0 mg, 0.18 mmol) in toluene / Et_3N = 1 / 1 (3.6 mL), and the solution of **26** (86.9 mg, 0.15 mmol) and **28**[14] (66.0 mg, 0.18 mmol) in toluene (1.8 mL) at 120 ˚C for 1 h, was treated with 1 M TBAF in THF (0.56 mL, 0.56 mmol) at 50 ˚C for 2 h. After usual work up, the crude product was roughly purified by preparative TLC (silica gel; hexane / acetone = 1 / 1) to give **10** as four diastereomers mixtures. These mixtures were further purified by HPLC twice [first; ODS-AM column, ϕ 30 x 250 mm, YMC, Eluent A / B = 3 / 7 (eluent A; H_2O / MeCN = 95 / 5, eluent B; MeCN / MeOH = 6 / 4 + 0.5% H_2O), second; CHIRALPAK AD column, ϕ 20 x 250 mm, DAICEL, hexane / EtOH = 7 / 3] to give **10a** (4.5 mg, 5%), **10b** (5.1 mg, 6%), **10c** (1.6 mg, 2%), and **10d** (2.7 mg, 3%) respectively.

Spectral data for (23*S*,25*S*)-DLAM-2P-25nPr (**10a**):[16] ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33-7.18 (m, 5 H), 6.38 (d, *J* = 11.0 Hz, 1 H), 6.02 (d, *J* = 11.2 Hz, 1 H), 5.33 (s, 1 H), 5.00 (s, 1 H), 4.43 (brs, 1 H), 4.23 (brs, 1 H), 3.81-3.70 (m, 1 H), 3.55-3.45 (m, 1 H), 3.25-3.16 (m, 1 H), 2.93-2.75 (m, 3 H), 2.63-2.56 (m, 1 H), 2.32 (dd, *J* = 13.4, 6.6 Hz, 1 H), 2.20-1.10 (m, 25 H), 0.96 (t, *J* = 7.0 Hz, 3 H), 0.88 (d, *J* = 5.6 Hz, 3 H), 0.56 (s, 3 H); HRMS calcd for $C_{37}H_{53}NO_4$ 576.4047, found 576.4054.

Spectral data for (23*R*,25*R*)-DLAM-2P-25nPr (**10b**):[16] ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34-7.19 (m, 5 H), 6.38 (d, *J* = 11.2 Hz, 1 H), 6.03 (d, *J* = 11.2 Hz, 1 H), 5.34 (s, 1 H), 5.01 (s, 1 H), 4.44 (brs, 1 H), 4.24 (brs, 1 H), 3.85-3.75 (m, 1 H), 3.54-3.46 (m, 1 H), 3.25-3.14 (m, 1 H), 2.94-2.72 (m, 3 H), 2.61 (dd, *J* = 13.5, 3.3 Hz, 1 H), 2.32 (dd, *J* = 13.4, 6.7 Hz, 1 H), 2.25-2.15 (m, 2 H), 2.06-1.11 (m, 23 H), 1.00-0.93 (m, 6 H), 0.56 (s, 3 H); HRMS calcd for $C_{37}H_{53}NO_4$ 576.4047, found 576.4069.

Spectral data for (23*S*,25*R*)-DLAM-2P-25nPr (**10c**):[16] ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32-7.17 (m, 5 H), 6.37 (d, *J* = 11.2 Hz, 1 H), 6.01 (d, *J* = 11.5 Hz, 1 H), 5.33 (s, 1 H), 5.00 (s, 1 H), 4.43 (brs, 1 H), 4.23 (brs, 1 H), 4.06-3.96 (m, 1 H), 3.33-3.25 (m, 1 H), 3.22-3.13 (m, 1 H), 2.93-2.70 (m, 3 H), 2.64-2.56 (m, 1 H), 2.49 (s, 1 H), 2.32 (dd, *J* = 13.3, 7.0 Hz, 1 H), 2.21 (dd, *J* = 13.1, 6.4 Hz, 1 H), 2.04-1.10 (m, 23 H), 0.91 (d, *J* = 6.3 Hz, 3 H), 0.86 (t, *J* = 7.0 Hz, 3 H), 0.55 (s, 3 H); HRMS calcd for $C_{37}H_{53}NO_4$ 576.4047, found 576.4072.

Spectral data for (23*R*,25*S*)-DLAM-2P-25nPr (**10d**):[16] ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31-7.15 (m, 5 H), 6.38 (d, *J* = 11.0 Hz, 1 H), 6.03 (d, *J* = 11.2 Hz, 1 H), 5.34 (s, 1 H), 5.01 (s, 1 H), 4.44 (brs, 1 H), 4.24 (brs, 1 H), 4.07-3.97 (m, 1 H), 3.35-3.28 (m, 1 H), 3.23-3.14 (m, 1 H), 2.95-2.69 (m, 3 H), 2.64-2.57 (m, 1 H), 2.49 (s, 1 H), 2.36-2.23 (m, 2 H), 2.06-1.05 (m, 23 H), 1.00 (d, *J* = 6.6 Hz, 3 H), 0.87 (t, *J* = 7.0 Hz, 3 H), 0.58 (s, 3 H); HRMS calcd for $C_{37}H_{53}NO_4$ 576.4047, found 576.4080.

20(*R*)-(3-Benzyl-3-hydroxy-1-phenethylpyrrolidin-2-one-5-yl)methyl-9,10-secopregna-5(*Z*),7(*E*),10(19)-triene-1α,3β-diol (11). Similar to the synthesis of **9**, a crude product, which was obtained from the solution of $Pd_2(dba)_3CHCl_3$ (11.0 mg, 0.011 mmol) and PPh_3 (22.0 mg, 0.084 mmol) in toluene / Et_3N = 1/ 1 (2 mL), and the solution of **27** (45.0 mg, 0.07 mmol) and **28**[14] (31.0 mg, 0.084 mmol) in toluene (1 mL) at 120 ˚C for 2 h, was treated with 1 M TBAF in THF (0.14 mL, 0.14 mmol) at 50 ˚C for 4 h. After usual work up, the crude product was roughly purified by preparative TLC (silica gel; hexane / acetone = 1 / 1) to give **11** as two diastereomers mixtures. These mixtures were further purified by HPLC twice [first; CHIRALPAK AD column, ϕ 20 x 250 mm, DAICEL, hexane / EtOH = 8 / 2, second; ODS-AM column, ϕ 30 x 250 mm, YMC, eluent A / B = 25 / 75 (eluent A; H_2O / MeCN = 95 / 5, eluent B; MeCN / MeOH = 6 / 4 + 0.5% H_2O)] to give **11a** (3.0 mg, 4%) and **11b** (4.4 mg, 5%).

Spectral data for (23*S*,25*S*)-DLAM-2P-25Bn (**11a**):[16] ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32-7.17 (m, 10 H), 6.38 (d, *J* = 11.2 Hz, 1 H), 6.02 (d, *J* = 11.2 Hz, 1 H), 5.35 (s, 1 H), 5.01 (s, 1 H), 4.44 (brs, 1 H), 4.24 (brs, 1 H), 3.76-3.66 (m, 1 H), 3.50-3.40 (m, 1 H), 3.24-3.16 (m, 1 H), 3.05-2.58 (m, 6 H), 2.62-2.59 (m, 1H), 2.37 (s, 1H), 2.32 (dd, *J* = 13.4, 6.6 Hz, 1H), 2.38-1.12 (m, 19 H), 0.80 (d, *J* = 5.1 Hz, 3 H), 0.51 (s, 3 H); HRMS calcd for $C_{41}H_{53}NO_4$ 624.4047, found 624.4072.

Spectral data for (23*R*,25*R*)-DLAM-2P-25Bn (**11b**):[16] ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32-7.18 (m, 10 H), 6.37 (d, *J* = 11.2 Hz, 1 H), 6.01 (d, *J* = 11.0 Hz, 1 H), 5.34 (s, 1 H), 5.00 (s, 1 H), 4.44 (brs, 1 H), 4.23 (brs, 1 H), 3.80-3.69 (m, 1 H), 3.47-3.38 (m, 1 H), 3.23-3.12 (m, 1 H), 3.05-2.70 (m, 6 H), 2.64-2.55 (m,

1 H), 2.45 (s, 1 H), 2.32 (dd, J = 13.4, 6.6 Hz, 1H), 2.15-1.05 (m, 19 H), 0.84 (d, J = 6.6 Hz, 3 H), 0.51 (s, 3 H); HRMS calcd for $C_{41}H_{53}NO_4$ 624.4047, found 624.4077.

Vitamin D receptor (VDR) binding assay[17]

[26,27-*Methyl*-^{3}H]-1α,25-dihydroxyvitamin D_3 (180 Ci/ mmol, 15000 dpm) and various amounts of DLAM analogs to be tested were dissolved in 50 μL of absolute ethanol in 12 x 75 mm polypropylene tubes. The chick intestinal VDR (0.2 mg) and 1 mg of gelatin in 1 mL of phosphate buffer solution (pH 7.4) were added to each tube in an ice bath. The assay tubes were incubated in shaking water bath for 1 h at 25 ˚C and then chilled in an ice bath. Polypropylene glycol 6000 (40%, 1 mL) in distilled water was added to each tube, and the tubes were mixed vigorously and centrifuged ant 2260 x *g* for 60 min at 4 ˚C. The resulting pellet was cut off into a scintillation vial containing 10 mL of dioxane-based scintillation fluid and the radioactivity was counted with Beckman liquid scintillation counter (model LC6500). The relative potency of the analogs were calculated from their concentration needed to displace 50% of [26,27-*Methyl*-^{3}H]-1α,25-dihydroxyvitamin D_3 from the receptor compared with the activity of 1α,25-dihydroxyvitamin D_3 (assigned a 100% value).

Assay for HL-60 cell differentiation: The human promyelocytic leukemia cell line HL-60 was purchased from a cell bank (Japanese Cancer Research Resources Bank, cell#: JCRB0085). The HL-60 cells were cultured in RPMI1640 medium supplemented with 10% FBS. The cell concentration at seeding was adjusted to 2 x 10^4 cells / mL, and the seeding volume was 1 mL / well. To assess the vitamin D_3-antagonistic activities of test compounds, the HL-60 cells were incubated with various concentrations (1 x 10^{-4} M to 3 x 10^{-3} M) of a test compound (added to the culture in 1 μL of ethanol solution) in the presence of 1 x 10^{-5} M 1α,25-dihydroxyvitamin D_3 (added to the culture in 1 μL of ethanol solution) for 96 h at 37 ˚C in a humidified atmosphere of 5% CO_2/air without a medium change. The same amount of vehicle was added to the control culture. NBT-reducing assay was performed according to the method of Collins.[18] Briefly, cells were collected, washed with PBS, and suspended in serum-free medium. NBT / TPA solution (dissolved in PBS) was added. Final concentrations of NBT and TPA were 0.1% and 100 ng / mL, respectively. Then, the cell suspensions were incubated at 37 ˚C for 25 min. After incubation, cells were collected by centrifugation and resuspended in FCS. Cytospin smears were prepared, the counter-staining of nuclei was done with Kemechrot solution, and the ratio of NBT-positive cells was counted under microscope.

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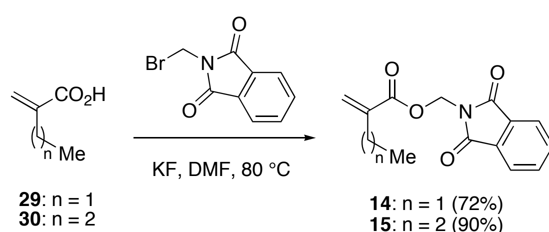
11. We applied phthalimidomethyl ester as the protective group of the carboxyl group of **29** and **30** because the boiling points of the methyl ester of **29** and **30** are relatively low to handle.[20,21] Synthesis of phtalimidoester **14** and **15** were shown in Scheme 2.



Scheme 2. Synthesis of phtalimide ester **14** and **15**.

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15. We couldn't get all four diastereomers of **9** and **11**, though we obtained four diastereomers of **10**. We presumed that the amount of the final product **9** was too small (9.8 mg) so that we lost one diastereomer during the HPLC separation process. In case of **11**, only two diatereomers of **21** were generated during the 1,3-dipolarcycloaddition reaction of **13** and **18** because of the steric hinderance of the benzyl group in **18**. Thus, two major isomers of **11a** and **11b** were obtained at the final separation process.
16. Stereochemistries at C23 and C25 of **9**-**11** were determined by comparison with the ^{1}H NMR spectral data for DLAM-2P (**6**).[7c] The typical chemical shifts of each stereoisomers for **9**-**11** were underlined in their experiment sections.
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