Practical Synthesis and Evaluation of the Biological Activities of 1α,25-dihydroxyvitamin D_3 Antagonists, 1α,25-dihydroxyvitamin D_3-26,23-lactams. Designed on the Basis of the Helix 12-Folding Inhibition Hypothesis

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A practical synthetic route to novel vitamin D antagonists of DLAM (1α,25-dihydroxyvitamin D_3-26,23-lactam) was developed from vitamin D_2 via the 1,3-dipolar cycloaddition reaction as a key step. Six DLAM derivatives (24 compounds) with a variety of nitrogen substituents and stereochemistries at C23 and C25 were synthesized. Among these new derivatives, (23*S*,25*S*)-DLAM isomers bound effectively to VDRs and showed antagonistic activity in the HL-60 cell differentiation inhibition assay. The importance of the substituent on the nitrogen of DLAMs for antagonistic activity was also suggested by computational docking studies.

Introduction

1α,25-Dihydroxyvitamin D_3 (**1**) (1,25-D_3) (Figure 1), which is a hormonally active form of vitamin D_3, exhibits various physiological actions, including the 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 vitamin D receptor (VDR), which is a member of the nuclear receptor (NR) superfamily.[2] Like all NRs, the VDR has a DNA-binding domain and a ligand-binding domain (LBD), which is formed by 12 α-helices, containing an activation function 2 (AF-2) domain.[3] Among these helices, the carboxyl-terminal α-helix (helix 12) plays an important role in the regulation of the transcriptional activity of the receptor.[4] The binding of the ligands causes a conformational change within the LBD, that is, the closure of helix 12 similar to that of a mouse trap.

Moreover, the LBD is involved in a variety of reversible interactions with nuclear proteins, such as other NRs, coactivators (CoAs), and corepressors (CoRs).[5] The VDR favors binding with a CoR in the absence of its ligands (apo form) and acts as a transcriptional suppressor of the responsive genes. In the apo form, helix 12 takes an open conformation. In response to 1,25-D_3, helix 12 is stabilized in the active closed conformation (holo form), and CoA is recruited and binds to a specific site located in helix 12, which results in the transcriptional enhancement of the responsive genes.[4b,6] These 1,25-D_3-modulated protein−protein interactions/dissociations are the central molecular events of nuclear 1,25-D_3 signaling. Therefore, synthetic VDR ligands that inhibit helix 12 folding or the optimal positioning of helix 12 in its agonistic conformation should have the potential to act as antagonist.

So far, more than 3000 analogues of 1,25-D_3 have been synthesized as candidate ligands for VDRs, and most of them have been reported to show agonistic activity. However, the 1,25-D_3 antagonists are expected to be effective for the treatment of metabolic bone disease, represented by Paget's disease,[7] and/or become tools for the elucidation of VDR function as well as the mode of action of 1,25-D_3. Only two types of ligands (except our compounds, vide infra), the 25-carboxylic ester **2** (ZK168281)[8] and 26,23-lactone **3** (TEI-9647)[7g,9] have been reported as antagonists so far (Figure 1). Compound **3** is an analogue of

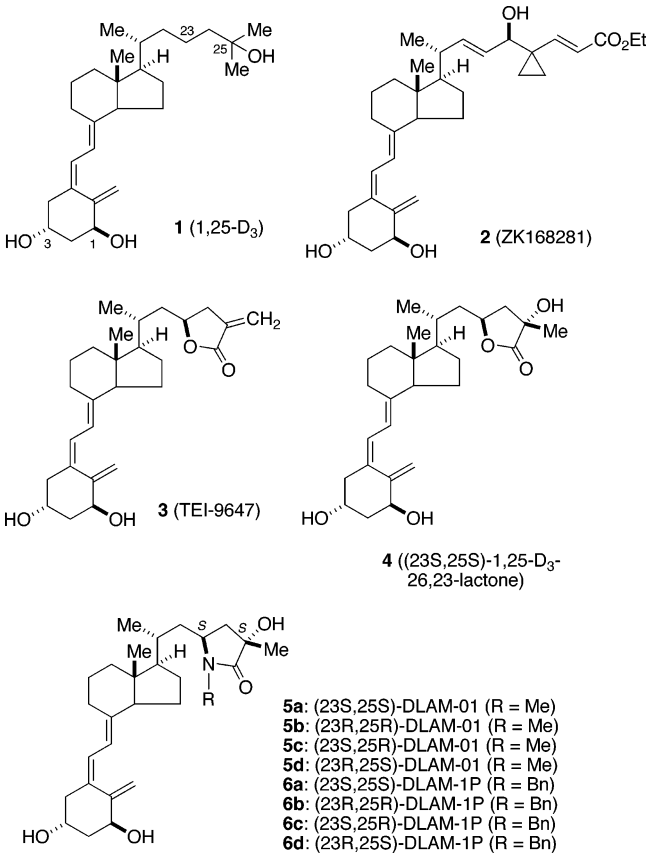

Figure 1. Structures of vitamin D_3 and its derivatives.

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the natural metabolite compound **4**, whereas compound **2** is a derivative of the cyclopropyl-containing anti-psoriasis drug calcipotriol (MC903).[10] Recently, we have reported novel types of vitamin D antagonist DLAMs **5** (DLAM-01) and **6** (DLAM-1P),[11] which were designed on the basis of the principle of inhibition of folding of helix 12 in the NR.[12] Among the new derivatives, **6a** ((23*S*,25*S*)-DLAM-1P) was found to competitively bind to VDRs with 1,25-D_3 and to inhibit the differentiation of HL-60 cells induced by 1,25-D_3. In this article, we describe computer-assisted docking studies of **6a** with VDRs and report the results of the structure−activity relationship studies of DLAM derivatives **7**−**11** and their novel and practical synthetic method.

Results and Discussion

Docking Studies of 6a with VDRs. We have recently introduced a novel type of 1,25-D_3 antagonists, DLAMs, which have a lactam structure in the side chain.[11] However, the antagonistic activities of DLAMs is not potent, that is, even the most active derivative among them, **6a** ((23*S*,25*S*)-DLAM-1P), possesses a 70-fold weaker antagonistic activity than that of the known antagonist **3**. As a precursor to further SAR studies of DLAMs, we conducted a docking study of **6a** with VDRs.

So far, six crystal structures of complexes consisting of recombinant VDR−LBDs (Δ165−215) and vitamin D_3 derivatives, that is, **1** (1,25-D_3),[13] 20-epi-1,25-D_3 (MC1288),[14] 26,-27-dimethyl-24-homo-20-epi-22-oxa-1,25-D_3 (KH1060),[14] calcipotriol,[15] seocalcitol,[15] and 19-nor-14-epi-23-yne-1,25-D_3 (TX522),[16] have been reported. They show practically the same VDR−LBD and, therefore, validate the assumption that the new DLAMs would also bind in the same way. We used the VDR−LBD X-ray structure of the VDR−LBD/**1** complex for the docking studies with **6a**.

First, computer-assisted reconstruction studies of the VDR−LBD/**1** complex were performed to ascertain the reliability of the docking calculation. Namely, 1,25-D_3 was deleted from the reported X-ray structure of the VDR−LBD/**1** complex (pdb 1DB1), and the remaining VDR−LBD structure was used as a rigid receptor model. Energy minimization of the complex structure with VDR−LBD and **1** was performed with the CHARMm force field[17] until a gradient convergence of less than 0.01 kcal/mol Å was reached. The obtained docking structure was consistent with the X-ray structure, supporting the reliability of our calculation method. Compound **4** ((23*S*,-26*S*)-1α,25-dihydroxyvitamin D_3-26,23-lactone), which is structurally related to **6a**, was docked under the same conditions and gave a docking structure similar to that of 1,25-D_3 (**1**). Next, we conducted docking studies of **6a** with VDR−LBD on the basis of the above results. The strain energy of **6a** in the energy-minimized structure of the complex with VDR−LBD was calculated to be 29.4 kcal/mol, which is clearly higher than those of 1,25-D_3 (3.9 kcal/mol) and lactone **4** (11.6 kcal/mol). This calculated value, which suggests the unfavorable/weak binding of **6a** to VDR compared to that of **1** and **4**, is not consistent with the moderate binding activity of **6a** to VDR, that is, **6a** binds to VDR with an efficacy similar to that with **4**.[11] The result suggests that the coformation of the VDR−LBD moiety in the VDR−LBD/**6a** complex is different from that of the VDR−LBD/**1** and VDR−LBD/**4** complexes. In fact, as mentioned above, compound **6a** was initially designed as a ligand that inhibits helix 12-folding.[12] Hence, we next performed docking to an artificial VDR−LBD template lacking the helix 12 moiety. The helix 12 moiety as well as the ligand molecule was deleted from the X-ray structure of the VDR−LBD/**1** complex, and the resulting substructure was used as a rigid receptor model for docking with **6a.** In this case, the strain energy of **6a** in the energy-minimized structure was decreased to 10.0 kcal/mol, suggesting that there is indeed an unfavorable interaction between **6a** and helix 12. To examine this interaction, helix 12 was brought back to the original position within VDR−LBD (Figure 2), resulting in a conflict between the benzyl group on the nitrogen atom of **6a** and the Phe422 residue in helix 12. This interaction could well be the cause of the antagonistic activity of **6a**, that is, the regulation/inhibition of the folding of helix 12 and, therefore, the nature of the substituent on the nitrogen was expected to affect the antagonistic activity of DLAM. We therefore performed SAR studies focusing on the substituents in the lactam moiety of DLAM.

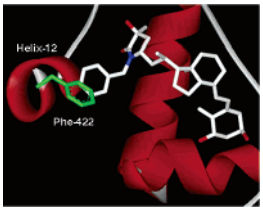
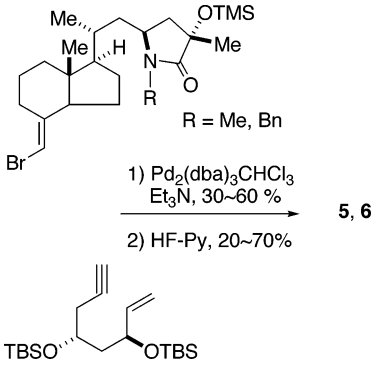


Figure 2. Docking structure of **6a** with VDR−LBD in the presence of helix-12.

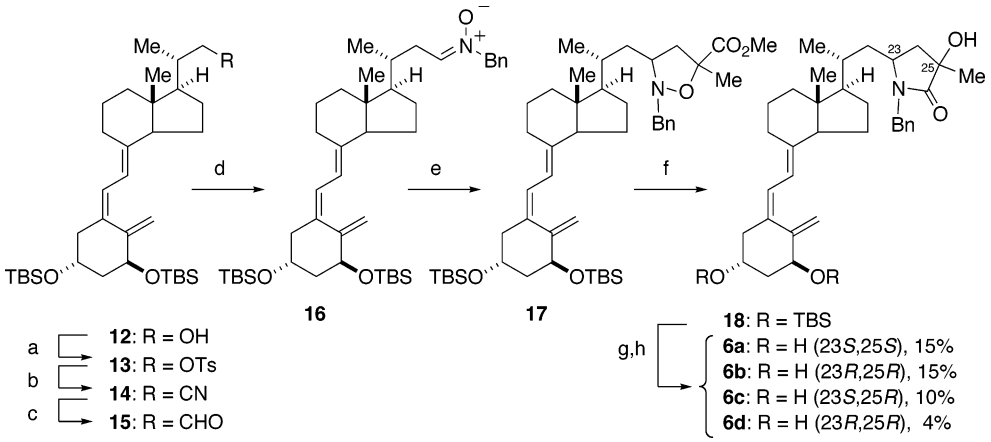
Scheme 1. Synthesis of DLAM-01 (**5**) and 1P (**6**) by a Convergent Route



Structure−Activity Relationship Studies on DLAM Derivatives. Synthesis of DLAM. We have previously reported the synthesis of **5** (DLAM-01) and **6** (DLAM-1P) by means of a convergent strategy as illustrated in Scheme 1.[11] Though this synthetic route was flexible enough to install various types of A-ring moieties, the yields in the coupling reaction of the A-ring and CD-ring synthons using the palladium catalyst[18] and the subsequent deprotection reaction were not consistent, and this approach seemed unsuitable for preparing substantial amounts of a range of DLAM derivatives for biological testing. Thus, we decided to develop an alternative synthesis of DLAM for the preparation of derivatives with various lactam substituents.

Compound **6** was synthesized directly from vitamin D_2 (Scheme 2). Alcohol **12** was synthesized from vitamin D_2 using the Calverley method[10] via the selective oxidation of the 1α position[19] and the oxidative degradation of the side chain. The primary alcohol of **12** was reacted with *p*-toluenesulfonyl chloride followed by sodium cyanide to give nitrile **14** in 95% yield (2 steps). The nitrile group was reduced with DIBAL-H to give aldehyde **15** in 96% yield. Reaction of aldehyde **15** with *N*-benzylhydroxylamine gave nitrone **16**, which was further reacted with methyl methacrylate to give isoxazolidine **17** in 77% yield with four diastereomers at C23 and C25.[20] The reduction of the N−O bond of the isoxazolidine in the presence of $Mo(CO)_6$[21] gave lactam **18** in 55% yield. The TBS group

Scheme 2. Practical Synthesis of DLAM-1P (**6**) from VD_2[a]



[a] Reagents and Conditions: (a) TsCl, DMAP, CH_2Cl_2, 95%; (b) NaCN, DMSO 90 °C, 96%; (c) DIBAL-H, CH_2Cl_2, 0 °C, 96%; (d) BnNHOH−HCl, Et_3N, CH_2Cl_2; (e) methyl methacrylate, toluene, 90 °C, 77% (2 steps); (f) $Mo(CO)_6$, $NaBH_4$, CH_3CN-H_2O, 90 °C, 55%; (g) HF·Py, THF, 0 °C; (h) HPLC separation.

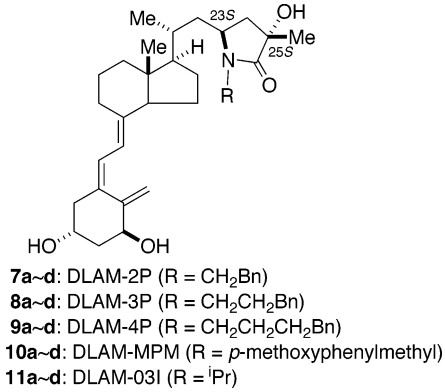


Figure 3. Structures of DLAMs **7**−**11**. The suffixes **a**−**d** refer to the stereoisomers at C23 and C25. **a**: (23*S*,25*S*), **b**: (23*R*,25*R*), **c**: (23*S*,25*R*), and **d**: (23*R*,25*S*).

was deprotected with HF-pyridine, and HPLC separation gave (23*S*,25*S*)-**6a**, (23*R*,25*R*)-**6b**, (23*S*,25*R*)-**6c**, and (23*R*,25*S*)-**6d** in 15, 15, 10, and 4% yields, respectively. The stereochemistries of these compounds were determined by comparison with the spectral data of authentic compounds prepared by the convergent method.[11,20] **7** (DLAM-2P), **8** (DLAM-3P), **9** (DLAM-4P), **10** (DLAM-MPM), and **11** (DLAM-03I) were similarly synthesized by changing the corresponding hydroxylamine (Figure 3).

Evaluation of the Biological Activities of DLAMs. The relative binding affinity of DLAMs **6**−**11** to the VDR was examined. The experiments were repeated at least three times. The values differed from experiment to experiment, but the results were basically reproducible (especially the order of potency of the compounds). A competitive receptor binding assay for the six types of compounds (24 compounds in total) was performed using chick intestinal VDR,[22] and the results are summarized in Table 1. The DLAM derivatives with (23*S*,25*S*) stereochemistries showed higher VDR affinity than the other corresponding diastereoisomers. Among them, **7a** ((23*S*,25*S*)-DLAM-2P) exhibited the strongest binding affinity, which was about 8% of that of **1** (for comparison, **3** and **4** show binding affinities of about 12% and 8% of that of **1**, respectively, under the same conditions).[23]

Next we examined the antagonistic activity of DLAMs **6**−**11** by the use of an HL-60 cell differentiation-inducing assay system.[24] First, the ability of DLAMs to induce HL-60 cell differentiation, a typical agonistic activity of **1**, was examined by the NBT reducing-activity method.[25] DLAMs **6**−**11**, which have aromatic groups on the lactam, scarcely induced HL-60 cell differentiation even at the high concentrations ($>10^{-6}$ M) (data not shown). However, **11a** ((23*S*,25*S*)-DLAM-03I) induced cell differentiation at a high concentration ($>10^{-6}$ M) (data not shown). Next, we investigated the antagonistic activity of DLAMs, that is, the inhibitory activity of DLAMs on HL-60 cell differentiation induced by 10^{-8} M concentration of **1** was tested using the NBT reducing-activity method (Table 1). Among the six kinds of DLAM derivatives (24 compounds), all of the isomers with (23*S*,25*S*) stereochemistries showed antagonistic activity, and most of the other isomers did not. Among the non-(*23S*,25*S*) stereoisomers, only **7c** ((23*S*,25*R*)-DLAM-2P) showed weak antagonistic activity. Compound **7a** ((23*S*,25*S*)-DLAM-2P) showed the most potent antagonistic activity, with an efficacy 3 times higher than that of **6a** ((23*S*,25*S*)-DLAM-1P).[26] The order of the antagonistic activity of

Table 1. VDR Binding Affinity and Antagonistic Activity of DLAM Derivatives **6**−**11**

DLAM	VDR binding affinity[a]	antagonistic activity[b] (IC_{50}, nM)
6a	2.74	700
6b	0.25	NA[c]
6c	0.18	NA
6d	0.25	NA
7a	8	207
7b	0.51	NA
7c	0.34	>2000
7d	0.19	NA
8a	0.68	2200
8b	0.19	NA
8c	0.09	NA
8d	0.03	NA
9a	5.24	390
9b	0.3	NA
9c	0.09	NA
9d	0.1	NA
10a	2.2	660
10b	0.3	NA
10c	0.09	NA
10d	0.1	NA
11a	1.52	>1000
11b	0.14	NA
11c	0.14	NA
11d	0.11	NA

[a] The potency of **1** 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**. [c] NA = not an antagonist.

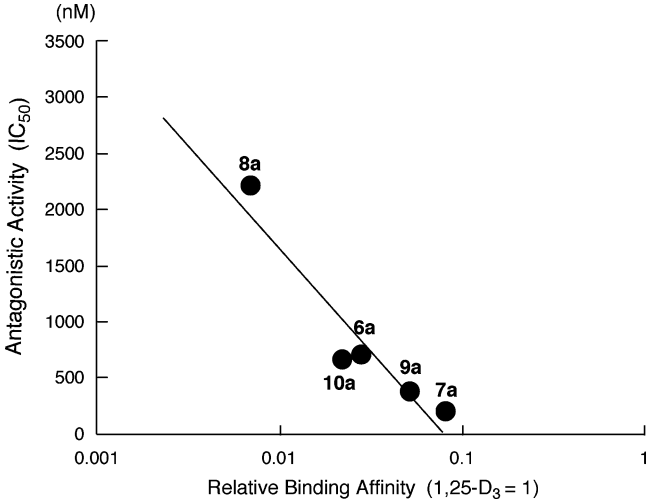


Figure 4. Relationships of VDR binding affinity and antagonistic activity of (23*S*,25*S*)-DLAM derivatives **6a**−**10a**.

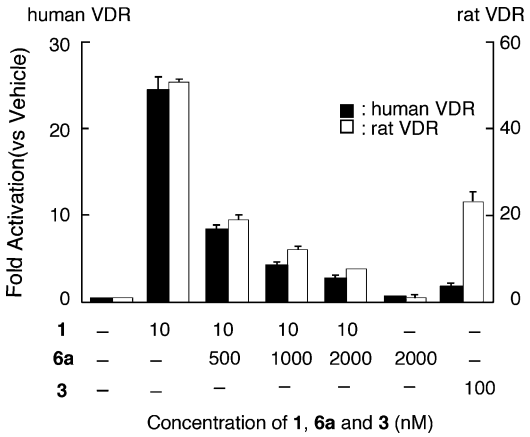


Figure 5. Transcriptional assay of **6a** with human and rat VDRs.

DLAMs was correlated with that of their binding affinity to the VDR (Figure 4).

The effect of **6a** on the transcriptional activation activity of VDRs was examined using a luciferase reporter gene assay system.[9a] The assay was conducted using COS-7 cells transfected with human or rat VDR genes as well as 25(OH)-D_3-24-hydroxylase genes containing VDRE (vitamin D responsive element) as a reporter gene.[27] As shown in Figure 5, **6a** ((23*S*,25*S*)-DLAM-1P) did not induce 25(OH)-D_3-24-hydroxylase gene expression in the human or rat VDR transfected system even at a high concentration ($>2 \times 10^{-6}$ M), which is consistent with the finding that **6a** is ineffective as a VDR agonist. As expected, **6a** showed a dose-dependent inhibitory activity on 25(OH)-D_3-24-hydroxylase gene expression induced by 10^{-8} M of **1** in both human and rat VDR transfected systems. These results suggest that the (23*S*,25*S*)-DLAM derivatives inhibit the activation of VDR at the transcriptional level irrespective of the species difference of human and rat VDRs. It is noteworthy that **3** (TEI-9647) shows agonistic activity toward rat VDRs at the transcriptional level.[28] These results suggest that the molecular mechanisms of VDR antagonistic activity elicited by DLAMs and **3** are different. Compound **3** (TEI-9647) may elicit its VDR antagonistic activity through alkylation of the cysteine residue in the LBD of the human VDR.[9f,28] The ineffectiveness of **3** as an antagonist toward rat VDRs can be interpreted in terms of the lack of corresponding cysteine residues in the LBDs of rat VDRs. In contrast, DLAMs are expected to elicit their VDR antagonistic activity through the inhibition of helix 12-folding, which is a general feature of VDR activation; therefore, these compounds can be expected to be species-nonspecific VDR antagonists such as the ZK analogues (Figure 1, compound **2**).

Conclusions

We have developed a practical synthetic route for DLAM derivatives from vitamin D_2 and used it to obtain new DLAM derivatives with various substituents on the nitrogen atom of the lactam moiety. Among these new derivatives, (23*S*,25*S*)-DLAM isomers effectively bind to VDRs, and they also showed antagonistic activity in the HL-60 cell differentiation inhibition assay. The role of the substituent on the nitrogen of DLAMs for antagonistic activity was investigated by computational docking studies. Further SAR studies of DLAMs and investigations of the biological activities are in progress.

Experimental Section

Synthesis of DLAMs. General Procedures. Flash chromatography was performed on Silica gel 60 (spherical, particle size 0.040−0.100 mm; Kanto Kagaku). Optical rotations were measured on a JASCO DIP polarimeter 370, using the sodium D line. IR spectra were measured with a JASCO VALOR−III FT-IR spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded on JEOL JNM-ECP500 instrument. Mass spectra were recorded on JEOL JMS-HX110 spectrometer with *m*-nitrobenzyl alcohol as the matrix.

1α,3β-Bis-(*tert*-Butyldimethylsilyoxy)-9,10-secopregna-5(*Z*),7(*E*),10(19)-triene-20(*R*)-methyl *p*-toluenesulfonate (13). To a solution of alcohol **12** (575.8 mg, 1.00 mmol) in dichloromethane (6 mL) was added DMAP (245 mg, 2.0 mmol) and *p*-toluenesulfonyl chloride (248 mg, 1.3 mmol), and the mixture was stirred for 3 h at room temperature. To the reaction mixture was added H_2O, and the organic layer was extracted with dichloromethane. The extracts were dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by silica gel chromatography (hexane/ethyl acetate = 10:1) to give **13** (697 mg, 0.96 mmol, 95%). $[\alpha]^{24}_D$ +44.06 (*c* 1.81, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.78 (d, J = 8.3 Hz, 2H), 7.34 (d, J = 8.3 Hz, 2H), 6.22 (d, J = 11.0 Hz, 1H), 6.00 (d, J = 11.2 Hz, 1H), 5.17 (d, J = 1.5 Hz, 1H), 4.84 (d, J = 2.2 Hz, 1H), 4.37 (dd, J = 3.4, 6.3 Hz, 1H), 4.19 (m, 1H), 3.98 (dd, J = 3.2, 9.3 Hz, 1H), 3.80 (dd, J = 6.3, 9.3 Hz, 1H), 2.82 (d, J = 12.4 Hz, 1H), 2.44 (m, 4H), 2.21 (dd, J = 7.4, 13.1 Hz, 1H), 1.97−1.15 (m, 14H), 0.99 (d, J = 6.6 Hz, 3H), 0.88 (s, 18H) 0.50 (s, 3H), 0.06 (m, 12H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 148.3, 144.5, 140.3, 135.3, 133.1, 129.7, 127.9, 123.0, 118.1, 111.2, 75.6, 72.0, 67.5, 55.9, 52.2, 46.0, 45.7, 40.2, 36.5, 28.7, 26.9, 25.8, 23.3, 22.1, 21.6, 17.0, 11.9, −4.7, −4.8, −5.1; *m*/*z* 729 (M + H$^+$); HRMS: calcd for $C_{41}H_{69}O_5SSi_2$, 729.4404; found, 729.4384.

1α,3β-Bis-(*tert*-butyldimethylsilyoxy)-20(*R*)-cyanomethyl-9,10-secopregna-5(*Z*),7(*E*),10(19)-triene (14). A mixture of **13** (618 mg, 0.85 mmol) and sodium cyanide (250 mg, 5.10 mmol) in DMSO (10 mL) was stirred at 90 °C for 2 h. To the reaction mixture was added H_2O, and the organic layer was extracted with ethyl acetate. The extracts were dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by silica gel chromatography (hexane/ethyl acetate = 50:1) to give **14** (496 mg, 0.85 mmol, 96%). $[\alpha]^{24}_D$ +43.59 (*c* 3.37, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.23 (d, J = 11.2 Hz, 1H), 6.02 (d, J = 11.0 Hz, 1H), 5.18 (d, J = 1.5 Hz, 1H), 4.86 (d, J = 2.2 Hz, 1H), 4.38 (dd, J = 3.7, 6.6 Hz, 1H), 4.19 (m, 1H), 2.84 (d, J = 12.0 Hz, 1H), 2.45 (d, J = 10.0 Hz, 1H), 2.39−2.20 (m, 3H), 2.06−1.26 (m, 14H), 1.18 (d, J = 6.6 Hz, 3H), 0.88 (s, 18H) 0.56 (s, 3H), 0.07 (m, 12H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 148.3, 140.1, 135.5, 123.0, 119.0, 118.3, 111.2, 72.0, 67.5, 56.0, 55.1, 46.0, 45.7, 44.8, 40.2, 33.9, 29.7, 28.7, 27.5, 25.8, 24.8, 23.3, 22.0, 19.4, 12.0, −4.7, −4.8, −5.1; *m*/*z* 584 (M + H$^+$); HRMS: calcd for $C_{35}H_{62}NO_2Si_2$, 584.4319; found, 584.4306.

1α,3β-Bis-(*tert*-butyldimethylsilyoxy)-20(*R*)-formylmethyl-9,10-secopregna-5(*Z*),7(*E*),10(19)-triene (15). To a solution of **14** (563 mg, 0.96 mmol) in dichloromethane (10 mL) DIBAL-H (0.94 M in *n*-hexane, 1.23 mL, 1.16 mmol) was added at 0 °C and stirred for 2 h. To the reaction mixture was added saturated aqueous NH_4Cl (0.5 mL), and the resulting mixture was diluted with dichloromethane. The mixture was dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by silica gel chromatography (hexane/ethyl acetate = 100:1) to give **15** (540 mg, 0.92 mmol, 96%). $[\alpha]^{24}_D$ +19.66 (*c* 1.79, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.75 (m, 1H), 6.24 (d, J = 11.2 Hz, 1H), 6.03 (d, J = 11.0 Hz, 1H), 5.19 (d, J = 2.0 Hz, 1H), 4.87 (d, J = 2.4 Hz, 1H), 4.38 (dd, J = 3.4, 6.3 Hz, 1H), 4.19 (m, 1H), 2.84 (d, J = 12.2 Hz, 1H), 2.49−2.45 (m, 2H), 2.25−1.27 (m, 16H), 1.03 (d, J = 6.6 Hz, 3H), 0.89 (s, 18H) 0.59 (s, 3H), 0.07 (m, 12H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 203.3, 148.3, 140.4, 135.3, 123.0, 118.1, 111.2, 72.0, 67.5, 56.2, 50.8, 46.0, 45.8, 44.8, 40.4, 31.9, 28.8, 27.9, 25.8, 23.4, 22.1, 20.1, 12.0, −4.7, −4.8, −5.1; *m*/*z* 587 (M + H$^+$); HRMS: calcd for $C_{35}H_{63}O_3Si_2$, 587.4316; found, 587.4295.

1α,3β-Bis-(*tert*-butyldimethylsilyoxy)-20(*R*)-benzyliminomethyl-9,10-secopregna-5(*Z*),7(*E*),10(19)-triene *N*-oxide (16). To a solution of aldehyde **15** (63 mg, 0.11 mmol) in dichloromethane (2 mL) was added *N*-benzylhydroxylamine hydrochloride (35 mg, 0.22 mmol) and triethylamine (60 μL, 0.43 mmol) at room temperature, and the mixture was stirred for 2 h. The reaction mixture was cooled to 0 °C and saturated aqueous NH_4Cl was added. The organic layer was extracted with dichloromethane, and the extracts were dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by silica gel chromatography (chloroform/methanol = 20:1) to give **16** (78 mg, 0.11 mmol, 100%). $[\alpha]^{24}_D$ +39.84 (*c* 2.42, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.39−7.32 (m, 5H), 6.78 (brs, 1H), 6.22 (d, J = 11.0 Hz, 1H), 6.00 (d, J = 11.0 Hz, 1H), 5.17 (s, 1H), 4.94 (brs, 2H), 4.85 (m, 1H), 4.36 (m, 1H), 4.18 (m, 1H), 2.81 (d, J = 11.2 Hz, 1H), 2.43 (d, J = 12.8 Hz, 1H), 2.21 (m, 1H), 1.96−1.19 (m, 16H), 0.94−0.87 (m, 21H) 0.51 (s, 3H), 0.05 (s, 12H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 148.1, 140.5, 135.1, 132.8, 129.2, 128.9, 123.0, 118.0, 111.2, 72.0, 69.1, 67.4, 56.5, 56.1, 46.0, 45.7, 44.7, 40.4, 34.3, 33.5, 28.7, 27.7, 25.8, 23.3, 22.0, 19.9, 11.9, −4.7, −4.9, −5.1; *m*/*z* 692 (M + H$^+$); HRMS: calcd for $C_{42}H_{70}NO_3Si_2$, 692.4894; found, 692.4920.

1α,3β-Bis-(*tert*-butyldimethylsilyoxy)-20(*R*)-(2-benzyl-5-methoxycarbonyl-5-methyl-isoxazolidine-3-yl)methyl-9,10-secopregna-5(*Z*),7(*E*),10(19)-triene (17). A mixture of nitrone **16** (78 mg, 0.11 mmol) and methyl methacrylate (60 μL, 0.56 mmol) in toluene (4 mL) was heated at 90 °C for 2 h. The reaction mixture was concentrated in vacuo. The residue was purified by silica gel chromatography (hexane/ethyl acetate = 10:1) to give **17** as four diastereomer mixtures (68 mg, 0.086 mmol, 77%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.40−7.31 (m), 6.23 (d, J = 10.0 Hz), 6.01 (d, J = 10.8 Hz), 5.18 (m), 4.86 (m), 4.38 (m), 4.19 (m), 4.13−3.88 (m), 3.76 (s), 3.13−1.26 (m), 0.89 (s), 0.69 (d, J = 6.1 Hz), 0.54−0.50 (m), 0.06 (s); *m*/*z* 793 (M + H$^+$); HRMS: calcd for $C_{47}H_{78}NO_5Si_2$, 792.5419; found, 792.5408.

1α,3β-Bis-(*tert*-butyldimethylsilyoxy)-20(*R*)-(1-benzyl-3-hydroxy-3-methyl-pyrrolidin-2-one-5-yl)methyl-9,10-secopregna-5(*Z*),7(*E*),10(19)-triene (18). To a solution of isoxsazolidine **17** (41 mg, 0.052 mmol) in CH_3CN-H_2O (7:1, 4 mL) was added $Mo(CO)_6$ (74 mg, 0.28 mmol) and $NaBH_4$ (1 mg, 0.026 mmol), and the resulting mixture was stirred at 90 °C for 4 h. The reaction mixture was filtered through a pad of Celite, and the filtrates were concentrated in vacuo. The residue was purified by silica gel chromatography (ethyl acetate) to give **18** as four diastereomer mixtures (22 mg, 0.028 mmol, 55%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.38−7.20 (m), 6.23−6.21 (m), 6.02−5.99(m), 5.18 (s), 4.86 (m), 4.37 (m), 4.19 (m), 4.09 (d, J = 15.0 Hz), 3.98 (d, J = 15.0 Hz), 3.52 (m), 3.30 (m), 2.81 (m), 2.43 (m), 2.27−1.25 (m), 1.10−0.87 (m) 0.54−0.46 (m), 0.10−0.06 (m); *m*/*z* 763 (M + H$^+$); HRMS: calcd for $C_{46}H_{76}O_4Si_2$, 762.5313; found, 762.5348.

20(*R*)-(1-Benzyl-3-hydroxy-3-methyl-pyrrolidin-2-one-5-yl)-methyl-9,10-secopregna-5(*Z*),7(*E*),10(19)-triene-1α,3β-diol (6). To a solution of **18** (22 mg, 0.029 mmol) in THF (2 mL) was added HF·Py (0.9 mL) at 0 °C, and the mixture was stirred for 5 h. The reaction mixture was diluted with ethyl acetate, and $NaHCO_3$ (solid) was added. The mixture was washed with H_2O and brine, and the organic layer was dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by silica gel chromatography (chloroform/methanol = 5:1) to give **6** as four diastereomer mixtures. These mixtures were further purified by HPLC (PEGASIL Silica 60−5 column, φ 20 × 250 mm, Senshu Pack, hexane/ethyl acetate/IPA = 51:45:4) to give **6a**−**d** in 15% (2.3 mg, 4.3 μmol), 15% (2.3 mg, 4.3 μmol), 10% (1.5 mg, 2.8 μmol), and 4% (0.58 mg, 1.1 μmol, 3.8%) yields, respectively.

Spectral Data for (23*S*, 25*S*)-DLAM-1P (6a). $[\alpha]^{24}_D$ +3.54 (*c* 0.20, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.33−7.22 (m, 5H), 6.37 (d, J = 11.5 Hz, 1H), 6.01 (d, J = 11.1 Hz, 1H), 5.32 (s, 1H), 4.99 (s, 1H), 4.97 (d, J = 11.1 Hz, 1H), 4.43 (m, 1H), 4.23 (m, 1H), 3.97 (d, J = 12.0 Hz, 1H), 3.51 (m, 1H), 2.82 (d, J = 12.4 Hz, 1H), 2.59 (d, J = 10.3 Hz, 1H), 2.32 (dd, J = 6.4, 13.7 Hz, 1H), 2.27 (dd, J = 7.7, 13.7 Hz, 1H), 2.05−1.84 (m, 5 H), 1.66 (dd, J = 5.1, 13.7 Hz, 1H), 1.68−1.17 (m, 12 H), 1.49 (s, 3H), 0.88 (m, 2H), 0.77 (d, J = 6.0 Hz, 3H), 0.53 (s, 3H).

Spectral Data for (23*R*, 25*R*)-DLAM-1P (6b). $[\alpha]^{24}_D$ +13.73 (*c* 0.32, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.33−7.18 (m, 5H), 6.36 (d, J = 11.1 Hz, 1H), 6.00 (d, J = 11.1 Hz, 1H), 5.34 (s, 1H), 5.00 (s, 1H), 4.98 (d, J = 15.0 Hz, 1H), 4.44 (m, 1H), 4.23 (m, 1H), 4.06 (d, J = 15.4 Hz, 1H), 3.50 (m, 1H), 2.81 (d, J = 12.8 Hz, 1H), 2.60 (d, J = 9.8 Hz, 1H), 2.48 (brs, 1H), 2.36 (dd, J = 7.7, 13.3 Hz, 1H), 2.31 (dd, J = 6.4, 13.3 Hz, 1H), 2.04 (m, 1H), 1.92 (m, 3H), 1.77 (dd, J = 5.1, 13.3 Hz, 1H), 1.66−1.20 (m, 11H), 1.50 (s, 3H), 1.03 (m, 2H), 0.88 (m, 1H), 0.86 (d, J = 6.4 Hz, 3H), 0.48 (s, 3H).

Spectral Data for (23*S*, 25*R*)-DLAM-1P (6c). $[\alpha]^{24}_D$ +13.75 (*c* 0.155, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.34−7.18 (m, 5H), 6.36 (d, J = 11.1 Hz, 1H), 6.00 (d, J = 11.5 Hz, 1H), 5.32 (s, 1H), 5.00 (d, J = 14.5 Hz, 1H), 4.99 (s, 1H), 4.43 (m, 1H), 4.23 (m, 1H), 4.06 (d, J = 15.2 Hz, 1H), 3.27 (m, 1H), 2.82 (d, J = 12.4 Hz, 1H), 2.76 (brs, 1H), 2.60 (d, J = 13.5 Hz, 1H), 2.33 (m, 1H), 2.21 (dd, J = 6.4, 12.8 Hz, 1H), 2.19−1.95 (m, 5H), 1.73 (dd, J = 8.1, 12.8 Hz, 1H), 1.68−1.20 (m, 12H), 1.35 (s, 3H), 0.88 (m, 4H), 0.76 (d, J = 6.4 Hz, 3H), 0.51 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 176.8, 147.6, 142.6, 136.1, 133.2, 128.7, 127.9, 127.7, 124.8, 117.3, 111.9, 73.9, 70.8, 66.9, 56.9, 56.3, 51.3, 45.9, 45.2, 44.1, 42.8, 40.6, 40.5, 39.7, 32.8, 29.0, 27.9, 24.7, 23.5, 22.2, 18.6, 12.0; *m*/*z* 556 (M+Na$^+$); HRMS: calcd for $C_{34}H_{47}NO_4Na$, 556.3403; found, 556.3366.

Spectral Data for (23*R*, 25*S*)-DLAM-1P (6d). $[\alpha]^{24}_D$ −11.38 (*c* 0.058, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.33−7.14 (m, 5H), 6.36 (d, J = 11.1 Hz, 1H), 6.00 (d, J = 11.5 Hz, 1H), 5.33 (s, 1H), 5.01 (d, J = 15.0 Hz, 1H), 4.99 (s, 1H), 4.43 (m, 1H), 4.23 (m, 1H), 4.06 (d, J = 15.2 Hz, 1H), 3.28 (m, 1H), 2.81 (d, J = 12.8 Hz, 1H), 2.68 (brs, 1H), 2.60 (d, J = 16.7 Hz, 1H), 2.32 (m, 1H), 2.27 (dd, J = 6.4, 12.8 Hz, 1H), 2.17−1.92 (m, 5H), 1.87 (dd, J = 8.1, 12.8 Hz, 1H), 1.75−1.22 (m, 11H), 1.39 (s, 3H), 1.06 (m, 2H), 0.87 (d, J = 6.4 Hz, 3H), 0.48 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 177.0, 147.6, 142.6, 136.1, 133.1, 128.7, 127.7, 127.6, 124.9, 117.2, 111.8, 74.0, 70.8, 66.9, 57.3, 56.1, 52.9, 45.9, 45.2, 44.5, 42.9, 42.2, 40.5, 40.3, 34.5, 29.0, 27.7, 24.7, 23.4, 22.2, 20.2, 11.9; *m*/*z* 556 (M+Na$^+$); HRMS: calcd for $C_{34}H_{47}NO_4Na$, 556.3403; found, 556.3433.

20(*R*)-(1-Phenylethyl-3-hydroxy-3-methyl-pyrrolidin-2-one-5-yl)methyl-9,10-secopregna-5(*Z*),7(*E*),10(19)-triene-1α,3β-diol (7). DLAM-2P (**7**) was obtained from the corresponding nitrone, which was prepared using aldehyde **15** and 2-phenylethylhydroxylamine (**19**), as four diastereomer mixtures. These mixtures were purified by HPLC (PEGASIL Silica 60−5 column, φ 20 × 250 mm, Senshu Pack, hexane/ethyl acetate/IPA = 14:81:5) to give **7a**−**d** in 26, 28, 8, and 11% yields, respectively.

Spectral Data for (23*S*, 25*S*)-DLAM-2P (7a). $[\alpha]^{24}_D$ +11.78 (*c* 0.52, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.31−7.21 (m, 5H), 6.37 (d, J = 11.1 Hz, 1H), 6.01 (d, J = 11.1 Hz, 1H), 5.32 (s, 1H), 4.99 (s, 1H), 4.43 (dd, J = 4.3, 7.7 Hz, 1H), 4.23 (m, 1H), 3.79 (m, 1H), 3.47 (m, 1H), 3.19 (m, 1H), 2.90 (m, 1H), 2.85−

2.77 (m, 2H), 2.60 (d, J = 9.8 Hz, 1H), 2.32 (dd, J = 6.8, 13.3 Hz, 1H), 2.23 (dd, J = 7.3, 13.3 Hz, 1H), 2.01−1.21 (m, 20H), 1.41 (s, 3H), 0.87 (d, J = 5.6 Hz, 3H), 0.55 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 176.0, 147.7, 142.5, 138.8, 133.2, 128.8, 128.6, 126.6, 124.8, 117.3, 111.7, 74.0, 70.7, 66.9, 56.9, 56.3, 52.7, 45.9, 45.2, 42.9, 42.2, 40.5, 39.8, 39.5, 33.7, 33.1, 29.0, 28.0, 25.9, 23.5, 22.2, 18.6, 12.0; m/z 548 (M + H$^+$); HRMS: calcd for $C_{35}H_{50}NO_4$, 548.3740; found, 548.3758.

Spectral Data for (23*R*, 25*R*)-DLAM-2P (7b). $[\alpha]^{24}_D$ +32.12 (c 0.57, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.31−7.20 (m, 5H), 6.37 (d, J = 11.1 Hz, 1H), 6.02 (d, J = 11.1 Hz, 1H), 5.34 (s, 1H), 5.00 (s, 1H), 4.44 (dd, J = 4.3, 7.7 Hz, 1H), 4.24 (m, 1H), 3.83 (m, 1H), 3.48 (m, 1H), 3.18 (m, 1H), 2.91 (m, 1H), 2.85−2.76 (m, 2H), 2.60 (dd, J = 3.0, 13.3 Hz, 1H), 2.53 (brs, 1H), 2.32 (dd, J = 7.7, 13.3 Hz, 1H), 2.02−1.20 (m, 19H), 1.69 (dd, J = 5.1, 13.3 Hz, 1H), 1.49 (s, 3H), 0.96 (d, J = 6.8 Hz, 3H), 0.56 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 176.0, 147.7, 142.5, 138.7, 133.3, 128.8, 128.6, 126.6, 124.8, 117.3, 111.8, 73.9, 70.8, 66.9, 57.4, 56.2, 53.9, 46.0, 45.2, 42.9, 42.8, 41.8, 41.1, 40.4, 35.1, 33.6, 29.0, 27.9, 25.9, 23.5, 22.3, 20.3, 12.0; m/z 548 (M + H$^+$); HRMS: calcd for $C_{35}H_{50}NO_4$, 548.3740; found, 548.3704.

Spectral Data for (23*S*, 25*R*)-DLAM-2P (7c). $[\alpha]^{24}_D$ +56.13 (c 0.16, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.30−7.19 (m, 5H), 6.37 (d, J = 11.5 Hz, 1H), 6.02 (d, J = 11.1 Hz, 1H), 5.33 (s, 1H), 4.99 (s, 1H), 4.44 (dd, J = 4.3, 7.7 Hz, 1H), 4.23 (m, 1H), 3.97 (m, 1H), 3.26 (m, 1H), 3.19 (m, 1H), 2.90 (m, 1H), 2.83 (d, J = 13.3 Hz, 1H), 2.76 (m, 1H), 2.61 (d, J = 13.3 Hz, 1H), 2.31 (dd, J = 6.4, 13.3 Hz, 1H), 2.14 (dd, J = 6.4, 12.8 Hz, 1H), 2.01−1.13 (m, 19H), 1.62 (dd, J = 8.1, 12.8 Hz, 1H), 1.25 (s, 3H), 0.89 (d, J = 6.0 Hz, 3H), 0.54 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 176.6, 147.6, 142.5, 138.4, 133.2, 128.7, 128.6, 126.6, 124.8, 117.3, 111.8, 73.6, 70.8, 66.8, 56.9, 56.3, 51.8, 45.9, 45.2, 42.8, 40.9, 40.5, 39.9, 39.8, 33.7, 32.7, 29.0, 27.9, 24.7, 23.5, 22.2, 18.7, 12.0; m/z 548 (M + H$^+$); HRMS: calcd for $C_{35}H_{50}NO_4$, 548.3740; found, 548.3763.

Spectral Data for (23*R*, 25*S*)-DLAM-2P (7d). $[\alpha]^{24}_D$ +1.81 (c 0.22, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.28−7.16 (m, 5H), 6.36 (d, J = 11.5 Hz, 1H), 6.01 (d, J = 11.5 Hz, 1H), 5.32 (s, 1H), 4.99 (s, 1H), 4.43 (m, 1H), 4.22 (m, 1H), 3.96 (m, 1H), 3.26 (m, 1H), 3.18 (m, 1H), 2.89 (m, 1H), 2.81 (m, 1H), 2.72 (m, 1H), 2.58 (m, 1H), 2.57−2.27 (m, 2H), 2.17 (dd, J = 6.4, 12.4 Hz, 1H), 2.02−1.13 (m, 18H), 1.75 (dd, J = 8.1, 12.4 Hz, 1H), 1.23 (s, 3H), 0.96 (d, J = 6.4 Hz, 3H), 0.55 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 176.6, 147.7, 142.6, 138.2, 133.2, 128.8, 128.6, 126.7, 124.9, 117.3, 111.8, 73.7, 70.8, 66.9, 57.4, 56.2, 53.4, 46.0, 45.2, 42.9, 42.4, 41.5, 40.8, 40.4, 34.8, 33.6, 29.0, 28.0, 24.6, 23.5, 22.3, 20.4, 12.0; m/z 548 (M + H$^+$); HRMS: calcd for $C_{35}H_{50}NO_4$, 548.3740; found, 548.3693.

20(*R*)-(1-Phenylpropyl-3-hydroxy-3-methyl-pyrrolidin-2-one-5-yl)methyl-9,10-secopregna-5(*Z*),7(*E*),10(19)-triene-1α,3β-diol (8). DLAM-3P (**8**) was obtained from the corresponding nitrone, which was prepared by aldehyde **15** and 3-phenylpropylhydroxylamine (**20**), as four diastereomer mixtures. These mixtures were purified by HPLC (PEGASIL Silica 60−5 column, ϕ 20 × 250 mm, Senshu Pack, hexane/ethyl acetate/IPA = 19:78:3) to give **8a**−**d** in 21, 21, 14, and 11% yields, respectively.

Spectral Data for (23*S*, 25*S*)-DLAM-3P (8a). $[\alpha]^{24}_D$ +10.47 (c 0.45, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.28 (m, 2H), 7.19 (m, 3H), 6.37 (d, J = 11.1 Hz, 1H), 6.02 (d, J = 11.1 Hz, 1H), 5.33 (s, 1H), 4.99 (s, 1H), 4.43 (dd, J = 4.3, 7.7, Hz, 1H), 4.23 (m, 1H), 3.68 (m, 1H), 3.60 (m, 1H), 3.01 (m, 1H), 2.83 (d, J = 12.8 Hz, 1H), 2.63 (m, 3H), 2.32 (m, 1H), 2.29 (dd, J = 7.3, 13.3 Hz, 1H), 2.07−1.25 (m, 21H), 1.60 (dd, J = 5.6, 13.3 Hz, 1H), 1.43 (s, 3H), 0.95 (d, J = 5.6 Hz, 3H), 0.57 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 176.1, 147.7, 142.4, 141.3, 133.3, 128.4, 128.3, 126.0, 124.8, 117.3, 111.7, 74.1, 70.7, 66.8, 56.9, 56.5, 52.2, 45.9, 45.2, 42.9, 40.5, 40.1, 39.9, 39.6, 33.1, 28.8, 28.0, 25.8, 23.5, 22.2, 18.7, 12.1; m/z 562 (M + H$^+$); HRMS: calcd for $C_{36}H_{52}NO_4$, 562.3896; found, 562.3869.

Spectral Data for (23*R*, 25*R*)-DLAM-3P (8b). $[\alpha]^{24}_D$ +26.19 (c 0.57, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.27 (m, 2H), 7.18 (m, 3H), 6.36 (d, J = 11.1 Hz, 1H), 6.02 (d, J = 11.5 Hz, 1H), 5.35 (s, 1H), 5.00 (s, 1H), 4.44 (m, 1H), 4.24 (m, 1H), 3.61 (m, 2H), 3.00 (m, 1H), 2.83 (m, 1H), 2.61 (m, 3H), 2.37 (dd, J = 7.7, 13.3 Hz, 1H), 2.32 (dd, J = 6.4, 13.3 Hz, 1H), 2.04−1.25 (m, 21H), 1.71 (dd, J = 5.1, 13.3 Hz, 1H), 1.43 (s, 3H), 0.97 (d, J = 6.8 Hz, 3H), 0.53 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 176.3, 147.6, 142.5, 141.2, 133.3, 128.4, 128.3, 126.0, 124.8, 117.3, 111.7, 74.1, 70.7, 66.9, 57.3, 56.2, 53.8, 45.9, 45.2, 42.8, 41.9, 41.0, 40.6, 40.4, 35.1, 33.1, 28.7, 25.8, 23.5, 22.3, 20.3, 12.0; m/z 562 (M + H$^+$); HRMS: calcd for $C_{36}H_{52}NO_4$, 562.3896; found, 562.3926.

Spectral Data for (23*S*, 25*R*)-DLAM-3P (8c). $[\alpha]^{24}_D$ +56.21 (c 0.65, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.29 (m, 2H), 7.19 (m, 3H), 6.37 (d, J = 11.1 Hz, 1H), 6.01 (d, J = 11.1 Hz, 1H), 5.33 (s, 1H), 4.99 (s, 1H), 4.43 (dd, J = 4.3, 7.7 Hz, 1H), 4.23 (m, 1H), 3.63 (m, 1H), 3.48 (m, 1H), 3.01 (m, 1H), 2.83 (d, J = 12.8 Hz, 1H), 2.60 (m, 3H), 2.31 (dd, J = 6.4, 13.3 Hz, 1H), 2.23 (m, 1H), 2.04−1.24 (m, 22H), 1.33 (s, 3H), 0.95 (d, J = 6.0 Hz, 3H), 0.55 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 176.6, 147.6, 142.5 141.2, 133.3, 128.5, 128.3, 126.1, 124.8, 117.3, 111.8, 73.8, 70.8, 66.9, 56.9, 56.3, 51.8, 45.9, 45.2, 42.8, 40.8, 40.5, 39.9, 39.8, 33.2, 32.8, 29.0, 27.9, 24.9, 23.5, 22.2, 18.7, 12.0; m/z 562 (M + H$^+$); HRMS: calcd for $C_{36}H_{52}NO_4$, 562.3896; found, 562.3881.

Spectral Data for (23*R*, 25*S*)-DLAM-3P (8d). $[\alpha]^{24}_D$ +3.58 (c 0.24, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.29 (m, 2H), 7.18 (m, 3H), 6.38 (d, J = 11.1 Hz, 1H), 6.03 (d, J = 11.1 Hz, 1H), 5.35 (s, 1H), 5.01 (s, 1H), 4.44 (m, 1H), 4.24 (m, 1H), 3.66 (m, 1H), 3.43 (m, 1H), 3.02(m, 1H), 2.83 (d, J = 12.8 Hz, 1H), 2.60 (m, 3H), 2.32 (dd, J = 6.4, 13.3 Hz, 1H), 2.27 (dd, J = 6.4, 12.8 Hz, 1H), 2.08−1.06 (m, 21H), 1.81 (dd, J = 7.3, 12.8 Hz, 1H), 1.33 (s, 3H), 1.00 (d, J = 6.4 Hz, 3H), 0.54 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 176.6, 147.7, 142.5, 141.3, 133.3, 128.5, 128.3, 126.1, 124.8, 117.3, 111.6, 73.9, 70.7, 66.9, 57.4, 56.2, 53.4, 45.9, 45.2, 42.9, 42.4, 40.8, 40.4, 40.3, 34.8, 33.3, 29.0, 27.9, 24.7, 23.5, 22.3, 20.4, 12.0; m/z 562 (M + H$^+$); HRMS: calcd for $C_{36}H_{52}NO_4$, 562.3896; found, 562.3892.

20(*R*)-(1-Phenylbutyl-3-hydroxy-3-methyl-pyrrolidin-2-one-5-yl)methyl-9,10-secopregna-5(*Z*),7(*E*),10(19)-triene-1α,3β-diol (9). DLAM-4P (**9**) was obtained from the corresponding nitrone, which was prepared by using aldehyde **15** and 4-phenylbutylhydroxylamine (**21**), as four diastereomer mixtures. These mixtures were purified by HPLC (PEGASIL Silica 60−5 column, ϕ 20 × 250 mm, Senshu Pack, hexane/ethyl acetate/IPA = 15:82:3) to give **9a**−**d** in 17, 17, 12, and 10% yields, respectively.

Spectral Data for (23*S*, 25*S*)-DLAM-4P (9a). $[\alpha]^{24}_D$ +12.89 (c 0.94, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.27 (m, 2H), 7.17 (m, 3H), 6.37 (d, J = 11.1 Hz, 1H), 6.02 (d, J = 11.5 Hz, 1H), 5.33 (s, 1H), 4.99 (s, 1H), 4.43 (m, 1H), 4.23 (m, 1H), 3.64−3.56 (m, 2H), 2.96 (m, 1H),2.84 (d, J = 12.8 Hz, 1H), 2.69−2.59 (m, 3H), 2.32 (dd, J = 6.8, 14.5 Hz, 1H), 2.29 (m, 1H), 2.01−1.25 (m, 23H), 1.60 (m, 1H), 1.42 (s, 3H), 0.92 (brs, 3H), 0.57 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 175.9, 147.7, 142.5, 141.9, 133.2, 128.4, 128.3, 125.8, 124.8, 117.3, 111.7, 74.2, 70.7, 66.9, 56.9, 56.3, 52.0, 45.9, 45.2, 42.9, 40.5, 40.1, 39.8, 39.6, 35.3, 33.1, 29.0, 28.2, 28.0, 26.5, 25.9, 23.5, 22.2, 18.6, 12.0; m/z 576 (M + H$^+$); HRMS: calcd for $C_{37}H_{54}NO_4$, 576.4053; found, 576.4082.

Spectral Data for (23*R*, 25*R*)-DLAM-4P (9b). $[\alpha]^{24}_D$ +24.27 (c 1.05, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.26 (m, 2H), 7.16 (m, 3H), 6.37 (d, J = 11.5 Hz, 1H), 6.03 (d, J = 11.5 Hz, 1H), 5.34 (s, 1H), 5.00 (s, 1H), 4.44 (m, 1H), 4.23 (m, 1H), 3.63 (m, 1H), 3.58 (m, 1H), 3.00 (m, 1H), 2.96 (m, 1H), 2.83 (d, J = 12.4 Hz, 1H), 2.62 (m, 3H), 2.36 (dd, J = 7.7, 13.3 Hz, 1H), 2.32 (dd, J = 6.4, 13.3 Hz, 1H), 2.05−1.17 (m, 23H), 1.70 (dd, J = 5.6, 13.3 Hz, 1H), 1.42 (s, 3H), 0.97 (d, J = 6.4 Hz, 3H), 0.54 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 176.0, 147.7, 142.4, 141.9, 133.3, 128.4, 128.3, 125.8, 124.7, 117.3, 111.6, 74.1, 70.7, 66.8, 57.3, 56.1, 53.3, 45.9, 45.2, 42.9, 41.8, 40.8, 40.5, 40.4, 35.3, 35.0, 29.0, 28.3, 27.8, 26.3, 25.8, 23.4, 22.3, 20.3, 11.9; m/z 576 (M + H$^+$); HRMS: calcd for $C_{37}H_{54}NO_4$, 576.4053; found, 576.4033.

Spectral Data for (23*S*, 25*R*)-DLAM-4P (9c). $[\alpha]^{24}_D$ +44.34 (c 0.61, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.27 (m, 2H), 7.17 (m, 3H), 6.37 (d, J = 11.1 Hz, 1H), 6.02 (d, J = 11.1 Hz, 1H),

5.33 (s, 1H), 4.99 (s, 1H), 4.44 (m, 1H), 4.23 (m, 1H), 3.62 (m, 1H), 3.41 (m, 1H), 2.97 (m, 1H), 2.83 (d, J = 12.5 Hz, 1H), 2.70−2.57 (m, 3H), 2.31 (dd, J = 6.4, 13.3 Hz, 1H), 2.22 (dd, J = 6.4, 12.4 Hz, 1H), 2.01−1.20 (m, 25H), 1.68 (dd, J = 7.7, 12.4 Hz, 1H), 1.33 (s, 3H), 0.91 (d, J = 6.0 Hz, 3H), 0.55 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 176.7, 147.6, 142.5, 141.9, 133.3, 128.8, 128.4, 125.9, 124.8, 117.4, 111.9, 73.9, 70.8, 66.8, 56.9, 56.4, 51.6, 45.9, 45.2, 42.8, 40.8, 40.5, 39.9, 39.7, 35.2, 32.8, 29.0, 28.3, 27.9, 26.5, 24.9, 23.5, 22.2, 18.6, 12.0; m/z 576 (M + H$^+$); HRMS: calcd for $C_{37}H_{54}NO_4$, 576.4053; found, 576.4015.

Spectral Data for (23*R*, 25*S*)-DLAM-4P (9d). $[\alpha]^{24}_D$ −5.87 (*c* 0.41, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.28 (m, 2H), 7.17 (m, 3H), 6.38 (d, J = 11.5 Hz, 1H), 6.03 (d, J = 11.1 Hz, 1H), 5.34 (s, 1H), 5.01 (s, 1H), 4.44 (m, 1H), 4.23 (m, 1H), 3.68 (m, 1H), 3.37 (m, 1H), 2.98 (m, 1H), 2.83 (d, J = 12.8 Hz, 1H), 2.68−2.59 (m, 3H), 2.33 (m, 1H), 2.27 (dd, J = 6.4, 12.4 Hz, 1H), 2.04−1.25 (m, 23H), 1.81 (dd, J = 7.7, 12.4 Hz, 1H), 1.33 (s, 3H), 0.98 (d, J = 6.4 Hz, 3H), 0.55 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 176.6, 147.7, 142.6, 141.9, 133.2, 128.8, 128.4, 125.9, 124.8, 117.3, 111.7, 73.9, 70.8, 66.9, 57.4, 56.2, 53.1, 46.0, 45.2, 42.9, 42.4, 40.7, 40.4, 40.1, 35.3, 34.7, 29.0, 28.5, 27.9, 26.5, 24.8, 23.5, 22.3, 20.4, 11.9; m/z 576 (M + H$^+$); HRMS: calcd for $C_{37}H_{54}NO_4$, 576.4053; found, 576.4066.

20(*R*)-(1-(4-Methoxybenzyl)-3-hydroxy-3-methyl-pyrrolidin-2-one-5-yl)methyl-9,10-secopregna-5(*Z*),7(*E*),10(19)-triene-1α,3β-diol (10). DLAM-MPM (**10**) was obtained from the corresponding nitrone, which was prepared by using aldehyde **15** and 4-methoxyphenylmethylhydroxylamine (**22**), as four diastereomer mixtures. These mixtures were purified by HPLC (PEGASIL Silica 60−5 column, ϕ 20 × 250 mm, Senshu Pack, hexane:ethyl acetate:IPA = 15:82:3) to give **10a**−**d** in 17, 25, 14, and 14% yields, respectively.

Spectral Data for (23*S*, 25*S*)-DLAM-MPM (10a). $[\alpha]^{24}_D$ −11.68 (*c* 0.68, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.15 (d, J = 8.6 Hz, 2H), 6.84 (d, J = 8.6 Hz, 2H), 6.36 (d, J = 11.1 Hz, 1H), 6.01 (d, J = 11.1 Hz, 1H), 5.32 (s, 1H), 4.99 (s, 1H), 4.92 (d, J = 15.0 Hz, 1H), 4.43 (m 1H), 4.23 (m, 1H), 3.90 (d, J = 15.0 Hz, 1H), 3.79 (s, 3H), 3.48 (m, 1H), 2.82 (d, J = 12.4 Hz, 1H), 2.59 (d, J = 13.7 Hz, 1H), 2.32 (m, 1H), 2.25 (dd, J = 7.7, 13.3 Hz, 1H), 2.08−1.25(m, 14H), 1.65 (dd, J = 5.1, 13.3 Hz, 1H), 1.48 (s, 3H), 0.89−0.84 (m, 5H), 0.79 (d, J = 6.4 Hz, 3H), 0.53 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 176.1, 159.1, 147.7, 142.6, 133.2, 129.3, 127.9, 124.8, 117.3, 114.1, 111.7, 74.2, 70.7, 66.9, 56.8, 56.3, 55.3, 51.4, 45.9, 45.2, 43.7, 42.9, 40.4, 39.7, 39.3, 33.2, 29.0, 28.0, 26.1, 23.5, 22.2, 18.7, 12.0; m/z 564 (M + H$^+$); HRMS: calcd for $C_{35}H_{50}NO_5$ 564.3689; found, 564.3729.

Spectral Data for (23*R*, 25*R*)-DLAM-MPM (10b). $[\alpha]^{24}_D$ +17.00 (*c* 1.10, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.12 (d, J = 8.6 Hz, 2H), 6.85 (d, J = 8.6 Hz, 2H), 6.36 (d, J = 11.1 Hz, 1H), 6.01 (d, J = 11.1 Hz, 1H), 5.33 (s, 1H), 5.00 (s, 1H), 4.93 (d, J = 15.0 Hz, 1H), 4.44 (dd, J = 4.3,7,7 Hz 1H), 4.23 (m, 1H), 3.97 (d, J = 15.4 Hz, 1H), 3.79 (s, 3H), 3.47 (m, 1H), 2.82 (d, J = 12.4 Hz, 1H), 2.59 (d, J = 13.3 Hz, 1H), 2.34 (dd, J = 7.7, 13.3 Hz, 1H), 2.32 (dd, J = 6.4, 12.8 Hz, 1H), 2.08−1.89 (m, 5H), 1.76 (dd, J = 5.1, 13.3 Hz, 1H), 1.69−0.99 (m, 14H), 1.49 (s, 3H), 0.87 (d, J = 6.9 Hz, 3H), 0.50 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 176.4, 159.0, 147.7, 142.6, 133.2, 129.0, 127.9, 124.8, 117.3, 114.1, 111.7, 74.1, 70.8, 66.9, 57.3, 56.2, 55.3, 53.0 45.9, 45.2, 44.2, 42.9, 41.7, 40.7, 40.4, 35.0, 29.0, 27.8, 26.0, 23.5, 22.3, 20.1, 11.9; m/z 576 (M + H$^+$); HRMS: calcd for $C_{35}H_{50}NO_5$ 564.3689; found, 564.3694.

Spectral Data for (23*S*, 25*R*)-DLAM-MPM (10c). $[\alpha]^{24}_D$ +15.58 (*c* 0.46, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.11 (d, J = 8.6 Hz, 2H), 6.84 (d, J = 8.6 Hz, 2H), 6.36 (d, J = 11.1 Hz, 1H), 6.01 (d, J = 11.1 Hz, 1H), 5.33 (s, 1H), 4.99 (s, 1H), 4.94 (d, J = 14.5 Hz, 1H), 4.43 (dd J = 4.3, 7.7 Hz, 1H), 4.23 (m, 1H), 3.90 (d, J = 15.0 Hz, 1H), 3.79 (s, 3H), 3.26 (m, 1H), 2.82 (d, J = 12.4 Hz, 1H), 2.59 (dd, J = 3.0, 13.7 Hz, 1H), 2.31 (m, 1H), 2.19 (dd, J = 6.4, 12.8 Hz, 1H), 2.02−1.14 (m, 20H), 1.71 (dd, J = 8.1, 12.8 Hz, 1H), 1.33 (s, 3H), 0.88 (m, 1H), 0.78 (d, J = 6.4 Hz, 3H), 0.52 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 176.7, 159.1, 147.6, 142.6, 133.2, 129.2, 128.2, 124.8, 117.3, 114.1, 111.9, 74.0, 70.9, 66.9, 56.9, 56.3, 55.3, 51.0, 45.9, 45.3, 43.5, 42.8, 40.6, 40.5, 39.8, 32.9, 29.0, 27.9, 24.7, 23.5, 22.2, 18.7, 12.0; m/z 576 (M + H$^+$); HRMS: calcd for $C_{35}H_{50}NO_5$ 564.3689; found, 564.3718.

Spectral Data for (23*R*, 25*S*)-DLAM-MPM (10d). $[\alpha]^{24}_D$ +3.49 (*c* 0.53, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.08 (d, J = 8.6 Hz, 2H), 6.85 (d, J = 8.6 Hz, 2H), 6.36 (d, J = 11.1 Hz, 1H), 6.01 (d, J = 11.1 Hz, 1H), 5.33 (s, 1H), 4.99 (s, 1H), 4.95 (d, J = 15.0 Hz, 1H), 4.44 (m 1H), 4.22 (m, 1H), 3.98 (d, J = 15.0 Hz, 1H), 3.80 (s, 3H), 3.25 (m, 1H), 2.82 (d, J = 12.4 Hz, 1H), 2.59 (d, J = 14.1 Hz, 1H), 2.32 (m, 1H), 2.24 (dd, J = 6.4, 12.4 Hz, 1H), 2.13−1.22 (m, 21H), 1.86 (dd, J = 7.7, 12.4 Hz, 1H), 1.36 (s, 3H), 1.09 (m, 2H), 0.88 (d, J = 6.5 Hz, 3H), 0.50 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 176.9, 159.1, 147.7, 142.6, 133.2, 129.0, 128.2, 124.9, 117.3, 114.1, 111.7, 74.0, 70.8, 66.9, 57.4, 56.2, 55.3, 52.9, 45.9, 45.3, 43.9, 42.9, 42.2, 40.5, 40.4, 38.8, 34.5, 29.0, 27.8, 24.7, 23.5, 22.3, 20.3, 11.9; m/z 576 (M + H$^+$); HRMS: calcd for $C_{35}H_{50}NO_5$ 564.3689; found, 564.3706.

20(*R*)-(1-(4-Isopropyl-3-hydroxy-3-methyl-pyrrolidin-2-one-5-yl)methyl-9,10-secopregna-5(*Z*),7(*E*),10(19)-triene-1α,3β-diol (11). DLAM-03I (**11**) was obtained from the corresponding nitrone, which was prepared by using aldehyde **15** and commercially available isoprorylhydroxylamine hydrochloride (**23**), as four diastereomer mixtures. These mixtures were purified by HPLC (ODS-AM, ϕ 30 × 250 mm, YMC−Pack, A = 95% H_2O/CH_3CN, B = 0.5% H_2O/40% MeOH/CH_3CN; A:B = 2:3) to give **11a**−**d** in 4, 5, 5, and 6% yields, respectively.

Spectral Data for (23*S*, 25*S*)-DLAM-03I (11a). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.38 (1H, d, J = 11.5 Hz), 6.03 (1H, d, J = 11.2 Hz), 5.33 (1H, s), 5.00 (1H, s), 4.44 (1H, br s), 4.24 (1H, br s), 4.02−3.92 (1H, m), 3.73−3.63 (1H, m), 2.87−2.80 (1H, m), 2.64−2.21 (4H, m), 2.07−1.22 (25H, m), 1.00 (3H, d, J = 5.9 Hz), 0.59 (3H, s); m/z 486 (M + H$^+$); HRMS: calcd for $C_{30}H_{48}NO_4$, 486.3583; found, 486.3531.

Spectral Data for (23*R*, 25*R*)-DLAM-03I (11b). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.38 (1H, d, J = 11.2 Hz), 6.03 (1H, d, J = 10.7 Hz), 5.33 (1H, s), 5.00 (1H, s), 4.44 (1H, br s), 4.24 (1H, br s), 3.95−3.84 (1H, m), 3.66−3.55 (1H, m), 2.88−2.80 (1H, m), 2.65−2.26 (4H, m), 2.08−1.08 (25H, m), 1.02 (3H, d, J = 6.3 Hz), 0.65 (3H, s); m/z 486 (M + H$^+$); HRMS: calcd for $C_{30}H_{48}NO_4$, 486.3583; found, 486.3541.

Spectral Data for (23*S*, 25*R*)-DLAM-03I (11c). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.38 (1H, d, J = 11.2 Hz), 6.02 (1H, d, J = 11.5 Hz), 5.34 (1H, s), 5.00 (1H, s), 4.44 (1H, br s), 4.24 (1H, br s), 3.97−3.88 (1H, m), 3.60−3.49 (1H, m), 2.87−2.80 (1H, m), 2.63−2.53 (2H, m), 2.32 (1H, dd, J = 12.6, 6.7 Hz), 2.20 (1H, dd, J = 12.6, 6.7 Hz), 2.05−1.20 (25H, m), 0.99 (3H, d, J = 6.3 Hz), 0.57 (3H, s); m/z 486 (M + H$^+$); HRMS: calcd for $C_{30}H_{48}NO_4$, 486.3583; found, 486.3572.

Spectral Data for (23*R*, 25*S*)-DLAM-03I (11d). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.38 (1H, d, J = 11.2 Hz), 6.03 (1H, d, J = 11.2 Hz), 5.33 (1H, s), 5.00 (1H, s), 4.43 (1H, br s), 4.23 (1H, br s), 3.89−3.80 (1H, m), 3.48−3.40 (1H, m), 2.87−2.80 (1H, m), 2.63−2.50 (2H, m), 2.32 (1H, dd, J = 12.7, 6.5 Hz), 2.25 (1H, dd, J = 12.7, 6.8 Hz), 2.10−1.15 (25H, m), 1.03 (3H, d, J = 6.6 Hz), 0.57 (3H, s); m/z 486 (M + H$^+$); HRMS: calcd for $C_{30}H_{48}NO_4$, 486.3583; found, 486.3564.

Binding to the Vitamin D Receptor (VDR). Nuclear 1,25-D_3 receptor protein was prepared from chick intestine at Teijin Pharma. The VDR (0.2 mg) was dissolved in 1 mL of 25 mM phosphate buffer (pH 7.4) containing 0.1 M KCl, 1 mM dithiothreitol, and gelatin (1 mg). This solution was mixed with (26,27-methyl-^{3}H)-1,25-D_3 (Amersham Biosciences Corp., 15 000 dpm, 180 Ci/mmol, dissolved in 10 ml of ethanol) and various concentrations of a test compound (dissolved in 40 mL ethanol) in a polypropylene tube (Walter Sarstedt, 12 × 75 mm) and incubated for 60 min at 25 °C. Then the mixture was cooled to 4 °C, and 40% poly(ethylene glycol) 6000 solution (1 mL) was added to each tube, and the tubes were mixed vigorously and centrifuged at 2260*g* for 60 min at 4 °C. The resulting pellet was dissolved in a scintillation cocktail (DuPont, 10 mL), and the radio activity was counted with a liquid scintillation

counter (Beckman, model LS6500). The relative binding affinity of the test compounds for VDR was calculated from the concentration necessary to displace 50% of (26,27-methyl-^{3}H)-1,25-D_3 from VDR. The relative affinity thus measured for 1,25-D_3 was defined as 100.

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 RPMI-1640 (Life Technologies) medium supplemented with 10% heat-inactivated fetal bovine serum (FBS). The cell concentration at seeding was adjusted to 3×10^3 cells/mL, and the seeding volume was 1 mL/well. To assess the vitamin D_3-agonistic activity of test compounds, the HL-60 cells were incubated in the presence or absence of 1,25-D_3 (a positive control) or a test compound (added to the culture in 1 mL of ethanol solution) and incubated for 96 h at 37 °C in a humidified atmosphere of 5% CO_2/air without a medium change. To assess the vitamin D_3-antagonistic activity of test compounds, the HL-60 cells were incubated with various concentrations of a test compound (added to the culture in 1 mL of ethanol solution) in the presence of 1×10^{-5} M 1,25-D_3 (added to the culture in 1 mL of ethanol solution). After incubation, the nitroblue tetrazolium (NBT)-reducing activity of the HL-60 cells was measured. The HL-60 cells were collected by centrifugation, washed with phosphate-buffered saline (PBS), and re-suspended in the medium. To the cell suspension was added NBT (Tokyo Kasei Kogyo) and 12-*O*-tetradecanoylphorbol-13-acetate (TPA, Wako). The final concentrations of NBT and TPA were 0.1% and 100 ng/mL, respectively. Then, the mixture was incubated at 37 °C for 25 min, and the cells were collected by centrifugation and re-suspended in PBS. Cytospin smears were prepared, the counter-staining of the nuclei was done with a Kemechrot solution, and the ratio of NBT-positive cells was counted under a microscope.

Transactivation Assay. The COS-7 cells were maintained in DMEM (Dulbecco's modified Eagle's medium), supplemented with DCC-treated 10% FBS (JRH Bioscience). A VDR expression vector was prepared by inserting a full-length human VDR gene into the multicloning sites of the pTracer expression plasmid (Stratagene). A reporter gene plasmid was prepared by inserting the promoter region of the human 24-hydroxylase (Cyp24) gene containing two sets of VDRE, which was obtained by polymerase chain reaction (PCR), into pGL3-basic (Promega). The COS-7 cells (4×10^4 cells/well) were cotransfected with the VDR expression vector (0.025 mg) and the reporter gene plasmid (0.25 mg) by using the FuGENE6 (Roche) method. To standardize the transfection efficiency, pRL-TK (0.025 mg, Promega) was also cotransfected at the same time. Transfection was continued for 4 h, and then the medium was exchanged with a fresh a-MEM medium containing 10% FBS and various concentrations of a test compound. The mixture was incubated for 24 h, and then the cells were washed with Dulbecco's phosphate-buffered saline (D-PBS). The luciferase activity of the treated cells was measured by using the dual-luciferase reporter assay system (Promega) and the fluoroskan ascent FL (Thermo Labsystems) according to the protocol recommended by the supplier.

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Supporting Information Available: Synthesis of hydroxylamines **19**−**22** and ^{1}H NMR spectral data for **6**−**11**. This material is available free of charge via the Internet at http://pubs.acs.org.

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