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Synthesis of 1α,25-dihydroxyvitamin D_3-26,23-lactams (DLAMs), a novel series of 1α,25-dihydroxyvitamin D_3 antagonist

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Abstract—Novel vitamin D_3 analogs having a lactam structure in their side chains, 1α,25-dihydroxyvitamin D_3-26,23-lactams (DLAMs), were designed based on the principle of regulation of the folding of helix-12 in the vitamin D nuclear receptor (VDR). The new analogs were synthesized via 1,3-dipolar cycloaddition reaction and subsequent reduction of the isoxazolidine as key steps. Among the analogs, (23*S*,25*S*)-DLAM-01 (**4a**) and (23*S*,25*S*)-DLAM-1P (**5a**) bind strongly to VDR. Moreover, these analogs were found to inhibit the differentiation of HL-60 cells induced by 1α,25-dihydroxyvitamin D_3.
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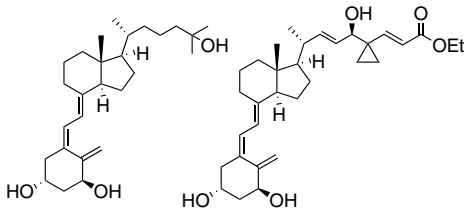
1. Introduction

1α,25-Dihydroxyvitamin D_3 (1,25-D_3) (**1**), the hormonally active form of vitamin D_3, is an endogenous ligand for the nuclear vitamin D receptor (VDR), which is a member of the steroid/thyroid/androgen nuclear receptor super-family, and plays critical roles in a variety of biological activities, including regulation of calcium homeostasis, bone mineralization and control of cellular growth, differentiation, and apoptosis.[1] The VDR contains a DNA-binding domain (DBD), which is formed by two zinc-finger motifs that are characteristic of the nuclear receptor super-family, and a ligand-binding domain (LBD), which consists of 11 α-helical structures, containing a short trans-activation function 2 (AF-2) domain.[2] Among these helixes, helix-12, the last one, plays an important role in the trans-activation activity.[3] The agonistic signaling of **1** is induced through a conformational change of the LBD, that is, folding of helix-12 to form a lid over the LBD pocket, which contains **1**.[4] Approximately 3000 analogs of **1** have been synthesized as candidate ligands for VDR so far, and many of them were reported to show agonistic activity.[1b]

When VDR ligands inhibit the folding or induce the mis-folding of helix-12, these compounds should be antagonists, which are expected to be effective for treatment of Paget's disease.[5] However, only two types of compounds, ZK168281 (**2**)[6] and its analogs from Schering and TEI-9647 (**3**)[7] from Teijin, have so far been reported as 1,25-D_3 antagonists (Chart 1).



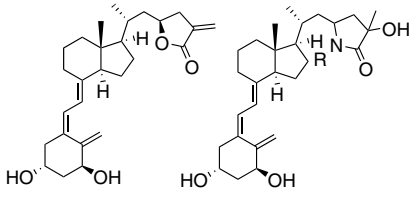


Chart 1.

Keywords: Vitamin D; Antagonist; Lactam.
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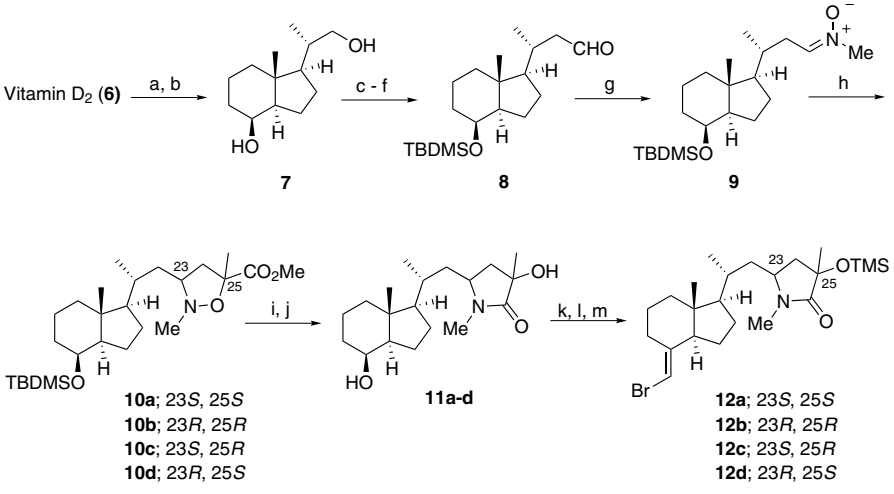
We have recently succeeded in developing the nonanilide type androgen antagonist on the basis of the principle of inhibiting folding of the helix-12 of the nuclear androgen receptor, a member of the nuclear receptor superfamily.[8] Based upon this strategy, we decided to look for a new type of VDR antagonist. In this paper, we describe the design, synthesis and biological evaluations of DLAMs **4** and **5** as novel antagonists having a lactam moiety in their side chain. The former was designed as a mis-folding inducer type antagonist, which was expected to show partial antagonistic/agonistic activity, and the latter was designed as a folding inhibitor type of antagonist, which was expected to show full antagonistic activity.

2. Design and synthesis

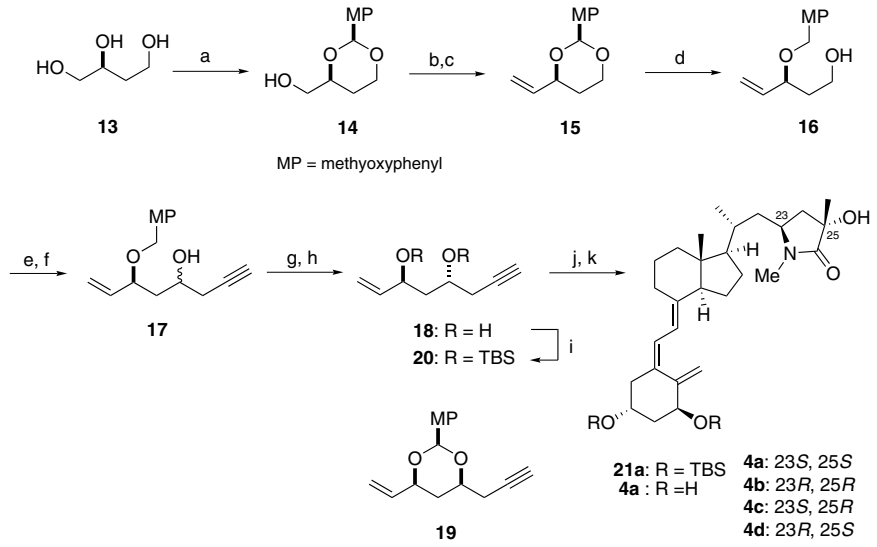
At the outset of the design of the new 1,25-D_3 analogs, the lactam skeleton was chosen as a key component to install in the side chain, since related analogs having a lactone skeleton on their side chain are considered to be promising structures for binding to the VDR.[9] We thus newly designed the DLAM series, in which a variety of substituents can be installed on the nitrogen atom of the lactam. These substituents were expected to sterically influence the position of helix-12 so as to inhibit the folding (e.g., having bulky group like benzyl group **5**) or the induce mis-folding (e.g., having less bulky group like methyl group **4**), which would result in VDR antagonistic activity. It had also aroused our interest that few nitrogen-containing 1,25-D_3 analogs with biological activities have yet been reported.[10]

The syntheses of DLAM-01 (**4**) and DLAM-1P (**5**) are depicted in Schemes 1–3. The CD-ring synthon **12** was synthesized from vitamin D_2 (**6**) via the Inhoffen–Lythgoe diol (**7**) and the aldehyde **8** (Scheme 1). The aldehyde **8** was reacted with *N*-methylhydroxylamine to give the nitrone **9**, which was subsequently reacted with methyl methacrylate to give the isoxazolidine **10** with four possible diastereomers at C23 and C25.[11a] These four diastereomers were separated with HPLC to give **10a–d** in 26%, 28%, 10%, and 10% yields, respectively. Their stereochemistries were determined by comparison with the reported data of ^{1}H NMR spectra and $[\alpha]_D$ values by Uskokovic and co-workers.[11] Each of the diastereomers **10a–d** was then carried on to the corresponding CD-ring synthon **12a–d**. Thus, the deprotection of the TBDMS ether of **10** with PTS, and reduction of the N–O bond with hydrogen in the presence of palladium on carbon gave the lactams **11** in 80% yield. The secondary alcohol of **11** was oxidized with TPAP–NMO,[12] and the tertiary alcohol was protected as the TMS ether with TMS-imidazole in 80% yield. Finally, Wittig reaction was conducted with bromomethyl triphenyl phosphonium bromide and LHMDS[13] to afford the vinyl bromides **12** in 25–71% yield.

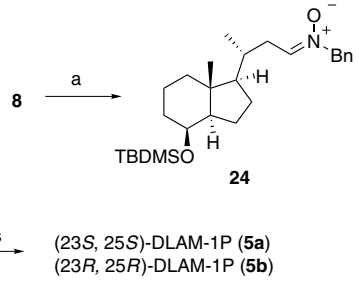
In contrast, the A-ring synthon **20**[14] was prepared from the triol **13** derived from (*S*)-malic acid (Scheme 2). Reaction of the triol **13** with *p*-anisaldehyde dimethylacetal in the presence of a catalytic amount of camphorsulfonic acid gave the six-membered benzylidene alcohol **14** in 59% yield. After oxidation of the primary alcohol of **14** under Swern conditions, the aldehyde generated was reacted with the Wittig reagent to give **15** in 73% yield. The *p*-methoxybenzylidene acetal of **15** was selectively reduced with DIBAH at 0 °C to afford the primary alcohol **16** in 70% yield. The oxidation of the primary alcohol of **16** with Dess–Martin reagent[15] followed by reaction with propargylmagnesium bromide gave **17** in 67% yield as a 1:1 diastereomeric mixture. The reaction of **17** with DDQ and subsequent purification by silica gel column chromatography provided the diol **18** and acetal **19** in 25% and 26% yields, respec-



Scheme 1. Reagents and conditions: (a) O_3, CH_2Cl_2, −78 °C; (b) $NaBH_4$, MeOH, 0 °C, 90% (two steps); (c) TsCl, DMAP, CH_2Cl_2, 0 °C; (d) TBDMSOTf, 2,6-lutidine, CH_2Cl_2, 0 °C; (e) NaCN, DMSO, 90 °C; (f) DIBAL-H, CH_2Cl_2, 0 °C, 41% (four steps); (g) MeNHOH·HCl, Et_3N, CH_2Cl_2; (h) methyl methacrylate, toluene, 110 °C, then HPLC separation, **10a** (26%), **10b** (28%), **10c** (10%), **10d** (10%); (i) *p*-TsOH, MeOH; (j) Pd/C, H_2, 80% (two steps); (k) TPAP, NMO, MS4A, CH_2Cl_2; (l) TMS-Im, CH_2Cl_2; (m) $BrCH_2PPh_3$·Br, LiHMDS, toluene, −78 °C, **12a** (25%), **12b** (42%), **12c** (49%), **12d** (71%) (three steps).



Scheme 2. Reagents and conditions: (a) *p*-anisaldehyde dimethylacetal, CSA, CH_2Cl_2, 59%; (b) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, −78 °C; (c) PPh_3CH_3Br, *n*-BuLi, THF, 0 °C, 73% (two steps); (d) DIBAH, CH_2Cl_2, 0 °C, 70%; (e) Dess–Martin reagent, CH_2Cl_2, 89%; (f) propargylmagnesium bromide, Et_2O, 0 °C, 67%; (g) DDQ, CH_2Cl_2; (h) silica gel, **18** (25%), **19** (26%); (i) TBSOTf, 2,6-lutidine, CH_2Cl_2, 0 °C, 99%; (j) **12a**–**d**, $Pd_2(dba)_3CHCl_3$, Et_3N, 100 °C, **21a** (62%), **21b** (90%), **21c** (43%), **21d** (53%); (k) HF-Py, THF, **4a** (53%), **4b** (73%), **4c** (67%), **4d** (98%).



Scheme 3. Reagents and conditions: (a) BnNHOH·HCl, Et_3N, CH_2Cl_2.

tively. The diol **18** was reacted with TBSOTf to give **20** in 99% yield. The coupling reaction of **20** with **12a**–**d** was performed under palladium-catalyzed reaction conditions[14] to give **21a**–**d** in 43–90% yield. The silyl ethers were deprotected with HF-pyridine to give DLAM-01 (**4a**–**d**) in 53–98% yield. DLAM-1P (**5**) was also synthesized similarly using *N*-benzylhydroxylamine (Scheme 3). In this case, only (23*S*,25*S*)-**5a** and (23*R*,25*R*)-**5b** were isolated after HPLC purification at the final stage. These stereochemistries were determined by comparison with the ^1H NMR spectra of **4a**–**d**.[16]

3. Results and discussion

The relative binding affinities of the DLAM **4a**–**d** and **5a**–**b** to the chick intestinal VDR were examined. A competitive receptor binding assay for the synthesized compounds was performed using VDR from chick intestine as described previously.[17] Chick intestinal 1,25-D_3 receptor was obtained from Yamasa Biochemical and dissolved in 0.05 M phosphate buffer (pH 7.4) containing 0.3 M KCl and 5 mM dithiothreitol just before use. The receptor solution (500 μL, 0.35 mg protein) was pre-incubated with 5 μL of ethanol solution of **1** or an analog at various concentrations for 60 min at 25 °C. Then, the receptor mixture was left to stand for 24 h with 0.1 nM [^{3}H]-1,25-D_3 (Amersham) at 4 °C. The bound and free [^{3}H]-1,25-D_3 were separated by treatment with dextran-coated charcoal for 30 min at 4 °C and centrifuged at 3000 rpm for 10 min. The radioactivity of the supernatant (500 μL) with Atomlight (PerkinElmer) was then counted. The relative binding affinities of DLAM derivatives to VDR are summarized in Table 1. The (23*S*,25*S*)-DLAM-01 (**4a**) and (23*S*,25*S*)-DLAM-1P (**5a**) bound strongly to VDR, while (23*S*,25*R*)-DLAM-01 (**4c**) and (23*R*,25*S*)-DLAM-01 (**4d**) did not bind to VDR in the concentration range examined.

Next we investigated the ability of DLAMs to induce HL-60 cell differentiation, a typical agonistic activity of 1,25-D_3 (**1**), by way of the NBT reducing activity method.[18] Among the DLAM series, (23*R*,25*R*)-DLAM-1P (**5b**) induced cell differentiation apparently at a high concentration of 10^{-5} M. On the other hand, **4a** and **5a**, which have strong affinity for the VDR, scarcely

Table 1. Relative binding affinities to VDR

Analog	IC_{50} (nM)
1	0.5
4a	10.2
4b	50
4c	>100
4d	>100
5a	1.9
5b	30

IC_{50} values determined by in vitro competition binding to chick intestinal VDR versus [^{3}H]-**1**.

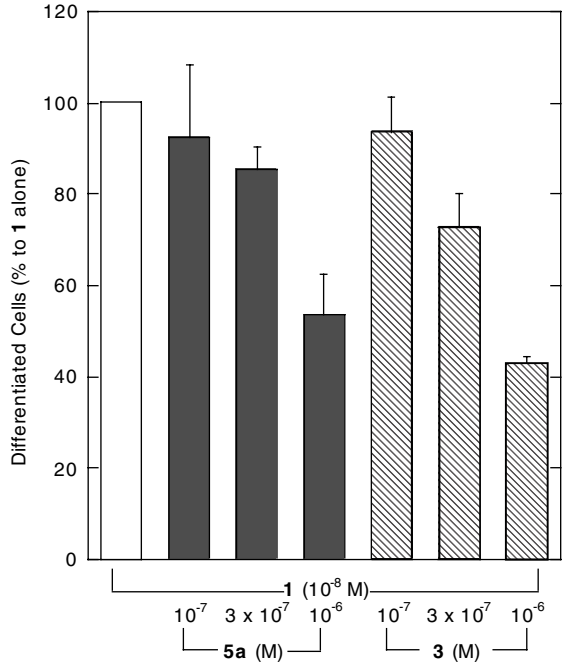


Figure 1. Effects of **5a** on **1**-induced HL-60 cell differentiation as examined with NBT-reducing activity assay. HL-60 cells were treated with **5a** or TEI-9647 (**3**) in the presence of 10^{-8} M **1** for 96 h, and NBT-reducing activity was examined. The activity of each analog was normalized to the result obtained for **1**, which was set to 100%.

induced cell differentiation even at the high concentration (data not shown).

These results prompted us to explore the possibilities of these analogs, especially **4a** and **5a**, as antagonists to VDR. Thus, we next examined the inhibitory activity of the compounds on HL-60 cell differentiation induced by 10^{-8} M 1,25-D_3 (**1**), using the NBT reducing activity method.[18] The analogs **4a** and **5a** inhibited the cell differentiation induced by **1** at 10^{-6} M with the potency of 12% and 47%, respectively. Especially, **5a** inhibited the cell differentiation with almost comparable efficacy to that of TEI-9647 (**3**) (Fig. 1). Those biological data, though preliminary, show that the newly developed analogs DLAM **4a** and **5a** are new antagonists of 1,25-D_3 (**1**). To elicit the antagonistic activity, the '*S*' configurations at C23 and C25 of DLAM seem to be crucial, and the bulkiness of the substituent on nitrogen also seems to be an important factor (**4a** vs **5a**). These facts strongly suggest that the substituent on nitrogen interacts with helix-12 and control its folding as antagonistic positions, as expected.

4. Conclusions

We have developed the DLAMs as a novel series of 1,25-D_3 analogs. Some members of this series, **4a** and **5a**, show antagonistic activity against **1**, thereby providing the third example of 1,25-D_3 antagonist analogs. The configuration of the lactam structure and the bulkiness of the substituents on nitrogen of DLAM are suggested to be important for antagonistic activity, and further investigations of SAR studies are in progress.

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16. Spectra data for **5a**: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.40–7.14 (m, 5H), 6.37 (d, $J = 11.1$ Hz, 1H), 6.01 (d, $J = 11.5$ Hz, 1H), 5.33 (s, 1H), 5.02 (s, 1H), 4.44 (m, 1H), 4.23 (m, 1H), 4.08–3.97 (m, 2H), 3.49 (m, 1H), 2.82 (dd, $J = 4.7$, 12.8 Hz, 1H), 2.60 (dd, $J = 2.1$, 12.8 Hz, 1H), 2.31 (m, 1H), 2.27 (dd, $J = 7.7$, 13.3 Hz, 1H), 2.10–0.87 (m, 23H), 0.77 (d, $J = 6.4$ Hz, 3H), 0.53 (s, 3H); m/z 534 ($M+H^+$); HRMS calcd for $C_{34}H_{48}NO_4$ 534.3583, found 534.3501. Data for **5b**: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.36–7.18 (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), 4.43 (m, 1H), 4.23 (m, 1H), 4.06 (d, $J = 15.0$ Hz, 1H), 3.50 (m, 1H), 2.81 (dd, $J = 3.4$, 12.4 Hz, 1H), 2.60 (dd, $J = 2.6$, 13.3 Hz, 1H), 2.37 (dd, $J = 7.7$, 13.3 Hz, 1H), 2.32 (m, 1H), 2.04–0.99 (m, 23H), 0.86 (d, $J = 5.6$ Hz, 3H), 0.48 (s, 3H); m/z 534 ($M+H^+$); HRMS calcd for $C_{34}H_{48}NO_4$ 534.3583, found 534.3492.
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