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2-Methylene 19-nor-25-dehydro-1α-hydroxyvitamin D_3 26,23-lactones: Synthesis, biological activities and molecular basis of passive antagonism

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Abstract—To investigate the molecular mechanism of vitamin D receptor (VDR) antagonists having no structurally bulky group interfering with helix 12 of the ligand-binding domain of the VDR, we have synthesized four diastereomers at C(20) and C(23) of 19-nor-1α-hydroxyvitamin D_3 25-methylene-26,23-lactone bearing a 2MD-type A-ring. All four analogs showed significant VDR affinity. Transactivation was tested by using Cos7 cells and HEK293 cells. In both types of cells, **LAC67a** showed little transactivation potency and inhibited the activation induced by the natural hormone concentration-dependently, indicating that **LAC67a** works as an antagonist for the VDR in these cells. **LAC67b**, **LAC82a** and **LAC82b** similarly acted as VDR antagonists in Cos7 cells, but in HEK293 cells they behaved as potent VDR agonists. Docking of four lactones into the VDR–LBD, followed by structural analysis, demonstrated that each lactone lacks the hydrophobic interaction with helix12 necessary for maintaining the active conformation of the VDR, indicating that these lactones are passive-type antagonists. Furthermore, each docking structure explained the characteristic transactivation profiles of the four lactones. On the basis of our present findings, we suggest that the ligand acts as an agonist if there are appropriate coactivators in the cells to bind to the looser VDR–ligand complex, and as an antagonist if there are no such appropriate coactivators. The molecular basis of the passive antagonism is discussed in detail.

1α,25-Dihydroxyvitamin D_3[1,25-$(OH)_2D_3$, **1**] plays an important role in regulation of calcium and bone metabolism, cellular differentiation and proliferation, and immune responses.[1] The majority of these actions are mediated by the vitamin D receptor (VDR), which is a member of the nuclear receptor superfamily and functions as a ligand-dependent transcriptional factor.[1,2] Upon ligand binding, the VDR undergoes conformational change to form the AF2 surface to allow binding of a coactivator.[3] In the absence of the ligand, a corepressor binds to the AF2 surface.[3,4]

Two types of nuclear receptor antagonists have been reported. The selective estrogen receptor modulators (SERM), 4-hydroxytamoxifen and raloxifene, constitute the first group of antagonists, which have a bulky substituent that creates spatial restriction with residues on helix 12 (H12) of estrogen receptor α in the active conformation and prevents the H12 from occupying the correct position on the AF2 surface.[5,6] Ligands of this type are called active antagonists. The other group of antagonists has no such bulky substituent. For example, progesterone works as an antagonist of the mineralocorticoid receptor (MR), although this compound is easily accommodated in the ligand-binding pocket (LBP).[7] Recently, Bledsoe et al. have solved and reported the X-ray crystal structure of the MR ligand-binding domain (LBD) complexed with progesterone, in which progesterone interacts poorly with helix 3 (H3) and helix 10/11 (H10/11) of MR–LBD.[8] This inferior interaction results in insufficient interactions among

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H3, helix 5 (H5), H10/11, and H12 mediated by the ligands, so that the correct AF2 surface cannot form, and thus progesterone behaves as an antagonist.[7,8] Ligands of this type are known as passive antagonists.

Thousands of vitamin D analogues have been synthesized to date. Most of them act as VDR agonists, whereas only two known types act as VDR antagonists. As active antagonists, ZK compounds such as ZK168281 **2** have been developed by the Schering group (Chart 1).[9] These compounds have a bulky ester group at the side chain terminal that causes spatial restriction with H12 of the active conformation of the VDR. The other antagonists are TEI compounds, such as TEI9647 **3b** and TET9648 **3a**, developed by the Teijin group.[10,11] They have a methylene lactone on the side chain, and no bulky structure directed toward H12. In the course of our studies to develop VDR antagonists for treatment of metabolic bone disease such as Paget disease[11b] and to investigate the molecular basis of the VDR antagonism, we designed and synthesized four diastereomers of 2-methylene 19-nor-25-dehydro-1α-hydroxyvitamin D_3 26,23-lactone (**LAC67a**, **LAC67b**, **LAC82a**, and **LAC82b**) as the candidates of passive antagonist. These are analogs of TEI compounds having methylene lactone on the side chain. The 2-methylene 19-nor structure of the A-ring was selected because the 19-nor structure is known to be more stable than the conjugated triene structure of the hormone and is easily synthesized. Insertion of a methylene group into C(2) was expected to increase the VDR affinity and the biological activity, as in the case of 2MD compound.[12] Since no VDR antagonist with a 20-*epi*-configuration had yet been reported, **LAC82a** and **LAC82b** were also designed to evaluate the effects of the stereochemistry at C(20). In this report, we describe the synthesis and biological activities of four diastereomers of vitamin D_3 derivatives having methylene lactone on the side chain (**LAC67a**, **LAC67b**, **LAC82a**, and **LAC82b**), and their interaction with the receptor. In addition, the molecular basis of these lactones acting as passive antagonists was investigated by docking analysis.

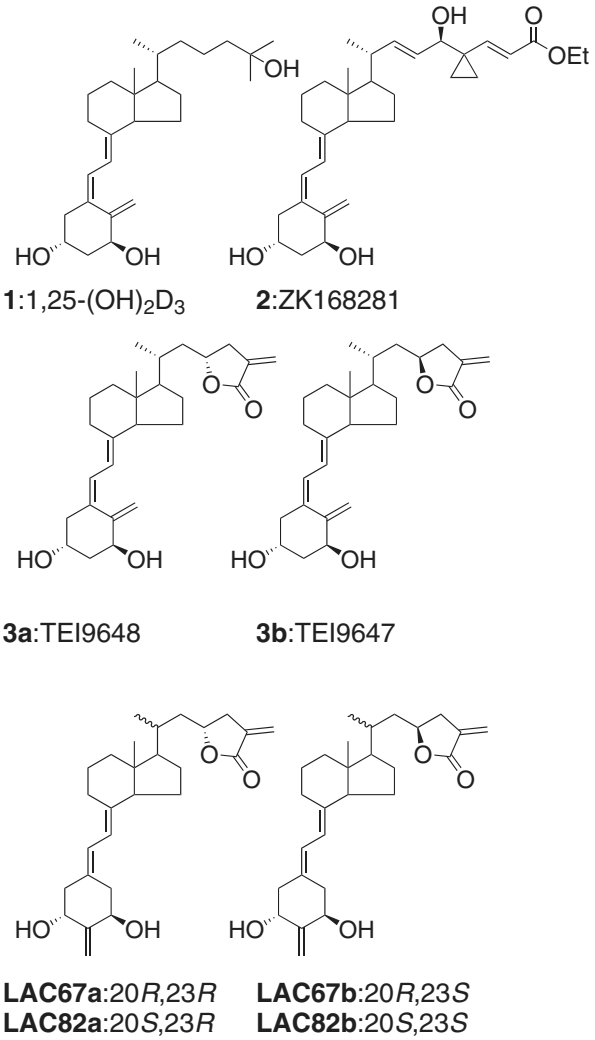


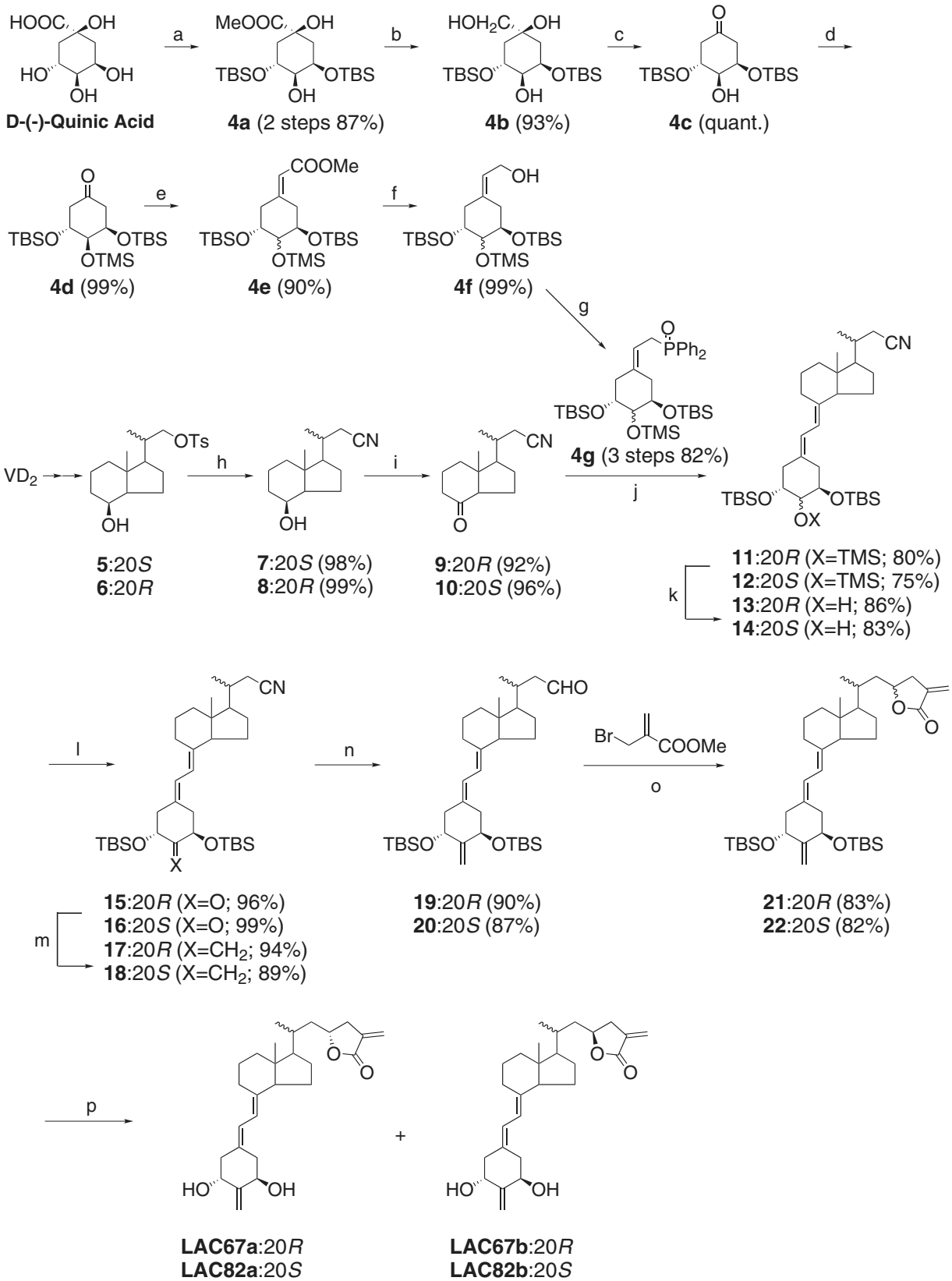
Chart 1. Structures of $1,25\text{-}(OH)_2D_3$ and its analogs.

1. Synthesis

Four methylene lactone compounds **(LAC67a, LAC67b**, **LAC82a**, and **LAC82b**) were synthesized using the Wittig–Horner reaction of Grundmann's ketone derivative **9/10** with A-ring phosphine oxide **4g** derived from (−)-quinic acid, followed by 2-methylenation and subsequent methylene lactonization (Scheme 1).

Phosphine oxide **4g** was derived from (−)-quinic acid in 59% overall yield by a modification of DeLuca's method. Conversion of (−)-quinic acid to methyl ester **4a** and conversion of keto compound **4c** to phosphine oxide **4g** have been reported by DeLuca's group[13a] and Shimizu's group,[13b] respectively. We reduced methyl ester **4a** with $NaBH_4$ instead of DIBAL[14] to give **4b**, which was oxidatively cleaved to give keto compound **4c**. Phosphine oxide **4g** was combined with Grundmann's ketone derivative **9**, derived from vitamin D_2, to give the 23-cyanide **11** at 80% yield. Removal of the protecting group followed by oxidation of the 2-hydroxyl group of **13** afforded the corresponding 2-keto compound **15**, which was then treated with Wittig reagent to give the 2-methylene compound **17**. The cyano group of **17** was reduced by DIBAL to give the aldehyde **19**. This aldehyde was reacted with an organo-chromium complex, prepared from allylic bromide and low-valent chromium (II) derived from $CrCl_3$ by reduction with $LiAlH_4$, to give the lactone derivative **21**.[15] This method is a one-step allylation–lactonization reaction that was developed by Kittaka's group.[16] Methylene lactone **21** was obtained as a 2:3 mixture in terms of the stereochemistry at C23. Since treatment of lactone **21** with *n*-Bu_4NF gave a complex mixture of various compounds, TBDMS groups protecting the two hydroxyl groups were removed by acid-hydrolysis to give a mixture of **LAC67a** and **LAC67b**, which were then separated by HPLC. Stereochemistry at C(23) of **LAC67a** and **LAC67b** was determined by the Kusumi–Mosher method[17] described below.

LAC82a and **LAC82b** were synthesized by the same procedure as **LAC67a** and **LAC67b** (Scheme 1).



Scheme 1. Synthetic scheme of lactones. Reagents: (a) 1—MeOH, *p*-TsOH; 2—TBSCl, Et_3N; (b) $NaBH_4$; (c) $NaIO_4$; (d) TMSCl, imidazole; (e) $Me_3SiCH_2CO_2Me$, LDA; (f) DIBAL-H; (g) 1—*p*-TsCl, *n*-BuLi, then Ph_2PH, *n*-BuLi; 2—10% H_2O_2; (h) KCN, DMSO; (i) TPAP, NMO, Molecular Sieves 4A; (j) *n*-BuLi; (k) AcOH; (l) $(COCl)_2$, DMSO, Et_3N; (m) $MeP^+Ph_3Br^-$, *n*-BuLi; (n) DIBAL-H; (o) $CrCl_3$, $LiAlH_4$; (p) CSA, MeOH.

2. Determination of stereochemistry at C(23)

Stereochemistry at C(23) of each lactone was determined by the Kusumi–Mosher method (Scheme 2). Methylene lactone **21** was reduced by DIBAL to give a mixture of 23,26-diols **23a** and **23b**, which were separable by silica gel column chromatography. The primary alcohol of each compound was selectively protected as the pivalate. Then, the pivalate esters (**25a** and **25b**) were converted to the corresponding *S*- and *R*-MTPA esters of the 23-hydroxyl group (**27a** and **28a**; **27b** and **28b**). Chemical shift differences between the *S*-MTPA ester and the corresponding *R*-MTPA ester are shown in Figure 1a and b. This analysis clearly indicated that the 23,26-diol **23a** has a 23*R*-configuration. This diol **23a** was converted to the corresponding lactone **LAC67a** by oxidation with MnO_2, followed by removal of the TBDMS group. HPLC analysis showed that the methylene lactone derived from **23a** co-migrated with **LAC67a**. Based on these analyses we determined that **LAC67a** and **LAC67b** have a 23*R*- and 23*S*-configuration, respectively. In 20-*epi*-compounds, each stereochemistry at C(23) of **LAC82a** and **LAC82b** was determined to be *R* and *S*, respectively, according to the same method (Fig. 1c and d).

3. Biological activities

Biological activities in vitro are summarized in Table 1. Binding affinity for the VDR was evaluated by competitive-binding assay using rat recombinant VDR–LBD prepared in our laboratory (Fig. 2).[18] The binding affinity of **LAC67b** was 1/7 as potent as the natural hormone **1**, while that of the 23*R*-isomer **LAC67a** was 1/200 as potent as the hormone **1**. Interestingly, both 20-epimers **LAC82a** and **LAC82b** showed VDR-binding potency intermediate (1/50) between **LAC67a** and **LAC67b**. These results indicated that all four lactones are VDR ligands having significant affinity. The finding that **LAC67b** with a 23*S*-configuration showed stronger VDR affinity than its 23*R*-isomer **LAC67a** is in agreement with the properties of the TEI compounds, TEI9647 **3b** with a 23*S*-configuration having stronger affinity than its 23*R*-epimer TEI9648 **3a**.[10,19] However, this was not the case for synthetic 20-*epi* compounds because **LAC82a** and **LAC82b** had almost the same VDR affinity.

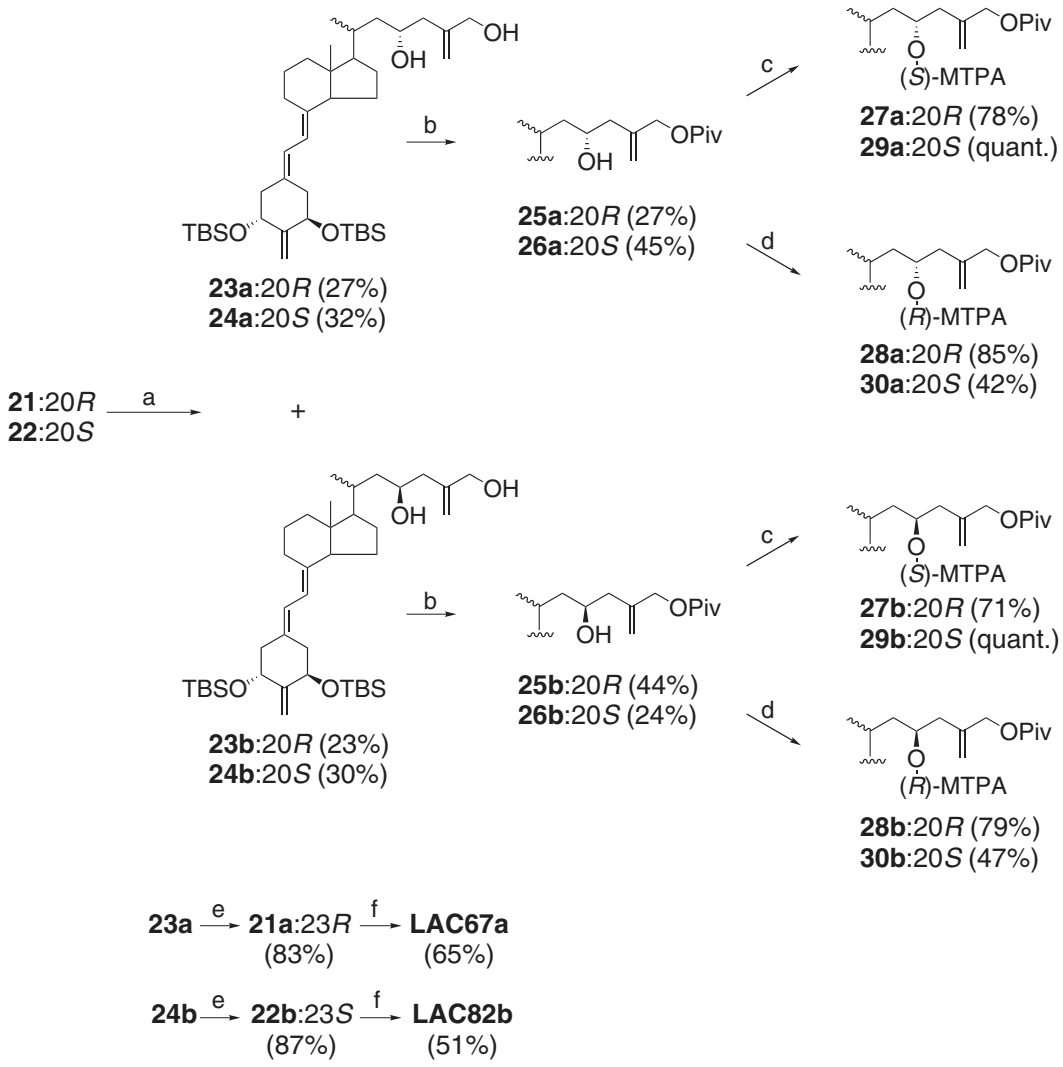
The ability of methylene lactones to induce transcription of a vitamin D-responsive gene was tested using a rat osteopontin luciferase reporter gene assay system in Cos7 cells.[20] It is well known that, in this assay, 1,25-$(OH)_2D_3$ (**1**) increases luciferase activity in a concentration-dependent manner. As shown in Figure 3a, **LAC67a** and **LAC67b** activated the VDR in a concentration-dependent manner, but their efficacy of maximal activation was only 14% and 11% of that of the hormone (**1**), respectively, indicating that both lactones are extremely weak partial VDR agonists. Antagonistic activity was evaluated by inhibition of VDR stimulation by 10 nM 1,25-$(OH)_2D_3$ (**1**). As shown in Figure 3c, both compounds inhibited the transactivation induced by 1,25-$(OH)_2D_3$ (**1**) concentration-dependently, indicating they can work as VDR antagonists. Interestingly, both the EC_{50} and IC_{50} of **LAC67b** in the transactivation assay were lower than those of **LAC67a**, in agreement with their VDR affinity.

Interestingly, **LAC82a** and **LAC82b** showed significant agonistic activity that is 34% and 43% efficacy compared with the hormone (**1**), respectively (Fig. 3b), and their EC_{50} values were 6 and 10 nM. These results clearly indicate that **LAC82a** and **LAC82b** are also partial agonists. Inversion of stereochemistry from 20*R* to 20*S* enhanced transactivation in terms of both efficacy and potency (EC_{50}). As expected, both compounds reduced the transactivation induced by hormone (**1**) to their own efficacy of maximal activation (Fig. 3d). **LAC82b** with the 23*S*-configuration showed stronger antagonistic activity than **LAC82a** with the 23*R*-configuration, in agreement with **LAC67b** and TEI9647 **3b** with the 23*S*-configuration.[10,21] We found that 20-epimerization (from **LAC67a** to **LAC82a**; from **LAC67b** to **LAC82b**) enhanced both agonistic and antagonistic activities.

To evaluate the effects of cell type, we performed the same transactivation assay using HEK293 cells. As also observed in Cos7 cells, **LAC67a** had little transactivation potency, whereas **LAC67b** showed significant agonistic activity (Fig. 4a). **LAC67a**, but not **LAC67b**, significantly reduced the transactivation stimulated by 1,25-$(OH)_2D_3$ (Fig. 4c). These results indicate that **LAC67a** acts almost as an antagonist in HEK293 cells, whereas **LAC67b** acts as a partial agonist with potent activity. The 20-epimer lactones **LAC82a** and **LAC82b** showed strong transactivation potency (Fig. 4b). As expected, they showed little antagonistic activity (Fig. 4d), indicating that both behave almost as full agonists in HEK293 cells.

4. Receptor interaction

Docking analysis was performed by the procedure described in the Experimental section. Figure 5a shows the X-ray crystal structure of VDR–LBD complexed with 1,25-$(OH)_2D_3$.[22] Figure 5c–f show the docking models of **LAC67a**, **LAC67b**, **LAC82a**, and **LAC82b** with the VDR–LBD, and their superposition is shown in Figure 5b. As shown in Figure 5, all of these four analogs, **LAC67a**, **LAC67b**, **LAC82a**, and **LAC82b**, were accommodated in the VDR–LBP where their hydroxyl groups at the 1α- and 3β-positions form pincer-type hydrogen bonds with Ser237 and Arg274, and Tyr143 and Ser278, respectively. Furthermore, the carbonyl group at C(26) forms a pincer-type hydrogen bond with His305 and His397, as in the case of 1,25-$(OH)_2D_3$ **1**. Differences from 1,25-$(OH)_2D_3$ **1** were found in hydrophobic interactions between amino acid residues on H12, including the tail of H11 and the ligand. While the terminal C(26)-methyl group of 1,25-$(OH)_2D_3$ **1** directly interacted with residues Val418, Phe422 on H12 and Tyr401 on H11 (Fig. 5a), the terminal methylene group of **LAC67a** is rather too distant from these three residues to interact directly (Fig. 5c). This docking mode



Scheme 2. Synthesis of MTPA esters. Reagents: (a) DIBAL-H; (b) PivCl, pyridine; (c) *R*-MTPACl, Et_3N, DMAP; (d) *S*-MTPACl, Et_3N, DMAP; (e) MnO_2; (f) CSA, MeOH.

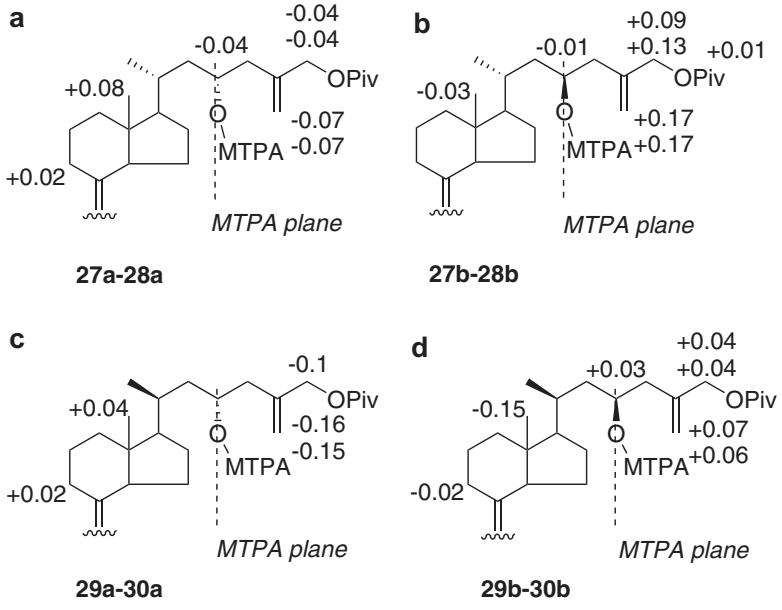


Figure 1. Determination of stereochemistry at C(23). Chemical shift differences.

Table 1. Biological activities of synthetic lactone compounds

	VDR binding[a] EC_{50} (M)	Cos7[b]		HEK293[c]	
		Transact.[d] EC_{50} (M)	Inhibit.[e] IC_{50} (M)	Transact.[d] EC_{50} (M)	Inhibit.[e] IC_{50} (M)
1,25-$(OH)_2D_3$ (**1**)	1×10^{-10}	5×10^{-10}	—	1×10^{-9}	—
LAC67a	2×10^{-8}	1.5×10^{-7}	1×10^{-6}	2×10^{-7}	3×10^{-7}
LAC67b	7×10^{-10}	2.5×10^{-8}	3×10^{-7}	1×10^{-9}	ND
LAC82a	5×10^{-9}	6×10^{-9}	3×10^{-7}	2×10^{-8}	ND
LAC82b	5×10^{-9}	1×10^{-8}	1×10^{-7}	1.5×10^{-8}	ND

[a] Competitive binding of 1,25-$(OH)_2D_3$ (**1**) and synthetic lactones to the rat vitamin D receptor. The EC_{50} values are derived from dose–response curves and represent the analog concentration required for 50% displacement of the radio-labeled 1,25-$(OH)_2D_3$ from the receptor protein. The experiments were carried out in duplicate.

[b] Transactivation was evaluated by dual luciferase assay using a full-length human VDR expression plasmid (pCMX-hVDR), a reporter plasmid containing three copies of the mouse osteopontin VDRE (SPPx3-TK-Luc), and the internal control plasmid containing sea pansy luciferase expression constructs (pRL-CMV) in Cos7 cells as described previously.[20]

[c] Transactivation potencies were evaluated using a full-length hVDR expression plasmid (pCMX-hVDR) and a reporter plasmid containing three copies of the mouse osteopontin VDRE (SPPx3-TK-Luc) in HEK293 cells as described previously.[31]

[d] Agonistic activity. The EC_{50} values are derived from dose–response curves and represent the analog concentration capable of inducing 50% maximal transactivation response. All experiments were carried out in triplicate.

[e] Antagonistic activity. The IC_{50} values are derived from dose–response curves and represent the analog concentration capable of reducing 50% maximal transactivation response.

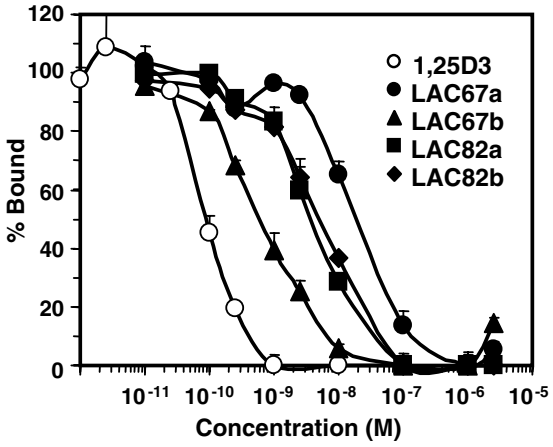


Figure 2. Competitive-binding assay of synthetic lactones.

suggests that **LAC67a** can bind to VDR but cannot form a transcriptionally active conformation in which H12 folds back ligand-dependently to form the AF2-surface. In the complex of **LAC67b**, Tyr401 and Val418 occupy the appropriate positions, but Phe422 is located far from the ligand (Fig. 5d).

20-*epi*-Compounds, **LAC82a** and **LAC82b**, also showed moderate contacts with Tyr401 and Val418 and weak contact with Phe422 (Fig. 5e and f). Compared with **LAC67a** and **LAC67b**, $C(22)H_2$ of the 20-*epi*-lactones **LAC82a** and **LAC82b** interacts more intimately with Val300. This close contact, similar to that observed in the complex of 20-*epi*-1,25-$(OH)_2D_3$, might be the reason why 20-*epi*-compounds have more potent activity than 20-normal compounds.[23–25]

5. Discussion

Almost all of the X-ray crystal structures of the VDR–LBD complexed with a ligand revealed that the distance between the terminal alkyl group of the vitamin D side chain and Val418, Phe422, and Tyr401 at the C-terminal of the VDR is within 4.7 Å.[22,26–30] These results indicate that intimate interaction is needed in order to form a transcriptionally active conformation of the VDR. Docking of synthetic lactones into the VDR–LBD and structural analysis yielded insights into the molecular basis of VDR passive antagonism.

As shown in Figure 5, the hydroxyl groups at the 1α- and 3β-positions of the four lactones form pincer-type hydrogen bonds with Ser237 and Arg274, and Tyr143 and Ser278, respectively, and the carbonyl group at C(26) forms a pincer-type hydrogen bond with His305 and His397, as is the case for 1,25-$(OH)_2$ D_3 **1**. Construction of these complete six hydrogen bonds is one of the most important reasons why lactones can bind to the VDR. However, docking models of the four lactones demonstrated that hydrophobic interaction with the C-terminal of the VDR is not enough for full agonist action. In the case of **LAC67a**, all of the three residues Tyr401, Val418, and Phe422 are far from the side chain of this ligand, as shown in Figure 5c, indicating the looser packing of H12 including the tail of H11. We concluded that the lack of these hydrophobic interactions is the reason why **LAC67a** works as an antagonist both in Cos7 as well as HEK293 cells. The docking model of **LAC67b** indicates that **LAC67b** has, in addition to six complete hydrogen bonds, appropriate hydrophobic interactions with Tyr401 and Val418, but not Phe422. This proper interaction with Tyr401 and Val418 would be one reason why **LAC67b** works as a potent agonist in HEK293 cells. HEK293 cells, but not Cos7 cells, might have an appropriate coactivator that can bind to the AF2 surface of the VDR docked with **LAC67b**.

This implies that **LAC67b** might work as a tissue- or cell-selective agonist for the VDR. Recently, we have clarified the level of CYP24A1 mRNA in several cell types treated with **LAC67a** and **LAC67b** by real time PCR, and suggested that **LAC67b** might act as a selective VDR modulator (SVDRM).[31] SVDRMs would

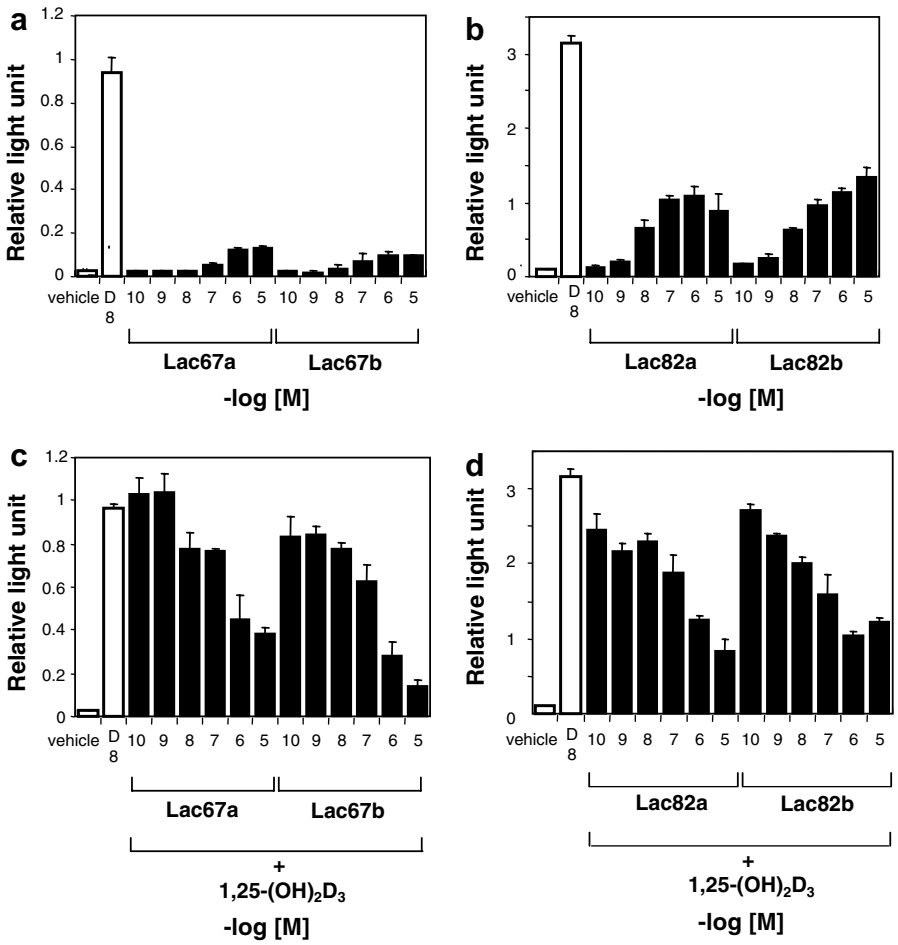


Figure 3. Transactivation of synthetic lactones in Cos7 cells. The activities were evaluated by dual luciferase assay using a full-length human VDR expression plasmid (pCMX-hVDR), a reporter plasmid containing three copies of the mouse osteopontin VDRE (SPPx3-TK-Luc), and the internal control plasmid containing sea pansy luciferase expression constructs (pRL-CMV) in Cos7 cells as described previously.[20,25] (a) agonistic activity of **LAC67a** and **LAC67b**, (b) agonistic activity of **LAC82a** and **LAC82b**, (c) antagonistic activity of **LAC67a** and **LAC67b** in the presence of 10 nM 1,25-$(OH)_2D_3$, (d) antagonistic activity of **LAC82a** and **LAC82b** in the presence of 10 nM 1,25-$(OH)_2D_3$.

act like as SERMs. Certain constituents in each cell are important for determining cell specificity. For example, the antagonistic function of ZK159222 has been shown to depend on the cell-specific ratio between VDR and RXR proteins.[32] Kato's group have reported that depletion of serum from the culture medium converted TEI9647 **3b** from an antagonist to an agonist of VDR-mediated transactivation, whereas it retained antagonistic activity in the presence of serum.[33] Taken together with our previous report, these findings indicate that **LAC67a** is a VDR antagonist while **LAC67b** might be a SVDRM.[31]

Docking models of **LAC82a** and **LAC82b** show six complete hydrogen bonds and moderate interactions with Tyr401 and Val418, but not Phe422. Since these hydrophobic interactions are insufficient, the activity of these compounds might depend on the cellular environment. Therefore, the ligand would act as an agonist if there are appropriate coactivators in that cell, whereas it would act as an antagonist if proper proteins are lacking. These observations demonstrate that the four synthetic lactones are passive antagonists.

The mechanism of the antagonistic activity of the methylene lactone compound TEI9647 **3b** has been reported by the Ishizuka and Norman group. They reported that the importance of Cys403 and Cys410 of human VDR for expression of the antagonistic activity of TEI9647 **3b** suggests a mechanism involving Michael-type addition of these cysteines to the methylene lactone.[34,35] On the other hand, the same group, using a ligand exchange assay, demonstrated that TEI9647 **3b** bound to VDR is freely exchanged with 1,25-$(OH)_2D_3$ **1** in vitro.[36] Since **LAC67b** has completely the same structure at the side chain as TEI9647 **3b**, the mechanism of its antagonistic action is thought to be quite similar to that of the latter. **LAC67b** acts as a VDR antagonist in Cos7 cells transfected with human VDR, whereas this compound functions as a potent VDR agonist in HEK293 cells transfected with human VDR. The latter fact that **LAC67b** can function as a potent VDR agonist suggests that this compound does not form a covalent bond with the human VDR. Therefore, we suggest that methylene lactones such as **LAC67b** and TEI9647 **3b** inhibit the transactivation as passive antagonists, and not via covalent bond formation.

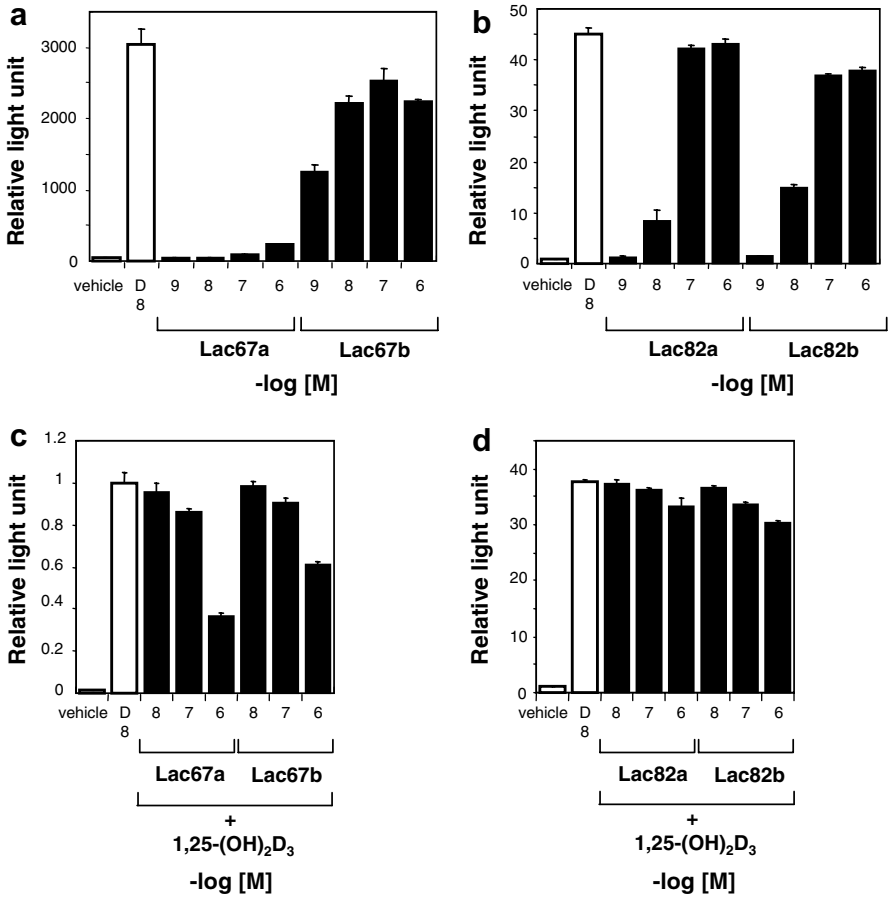


Figure 4. Transactivation of four methylene lactones in HEK293 cells. Transactivation potencies were evaluated using a full-length hVDR expression plasmid (pCMX-hVDR) and a reporter plasmid containing three copies of the mouse osteopontin VDRE (SPPx3-TK-Luc) in HEK293 cells as described previously.[31] (a) agonistic activity of **LAC67a** and **LAC67b**, (b) agonistic activity of **LAC82a** and **LAC82b**, (c) antagonistic activity of **LAC67a** and **LAC67b** in the presence of 10 nM 1,25-$(OH)_2D_3$, (d) antagonistic activity of **LAC82a** and **LAC82b** in the presence of 10 nM 1,25-$(OH)_2D_3$.

6. Conclusions

We synthesized four methylene lactone analogs and evaluated their affinity for the VDR as well as their agonistic and antagonistic activities. Based on data for their biological activities and a docking study, we concluded that these methylene lactones are passive antagonists lacking hydrophobic interactions with H12 of the VDR. We have already synthesized and reported another type of antagonist having a bulky substituent of the adamantine ring at the side chain. Further experiments are now in progress to study the molecular mechanism of VDR antagonism using these two types of synthetic antagonist.

7. Experimental

^{1}H NMR spectra were recorded in $CDCl_3$ at 400 MHz and chemical shifts are reported as δ units relative to tetramethylsilane or solvent signal as an internal standard. ^{13}C NMR spectra were recorded at 100 MHz. High and low resolution mass spectra were obtained on a JEOL JMS-AX505HA spectrometer at 70 eV. Relative intensities are given in parentheses in low mass. IR spectra were recorded on a JASCO FT/IR-300E spectrometer. UV spectra were recorded on a BECKMAN DU7500 spectrophotometer. All air and moisture sensitive reactions were carried out under argon atmosphere.

7.1. (3*R*,5*R*)-3,5-Bis{*tert*-butyl(dimethyl)silyl]oxy}-1-(hydroxymethyl)-1,4-cyclohexanediol (4b)

To a solution of methyl ester **4a** (10.4 g, 24 mmol) in EtOH (60 mL) was added $NaBH_4$ (2.8 g, 75 mmol), and the mixture was stirred at 0 °C for 5 h. The reaction was quenched with H_2O and brine at 0 °C and was extracted with AcOEt. The organic layer was washed with brine, dried over $MgSO_4$, and evaporated. The residue was chromatographed on silica gel (40 g, hexane/AcOEt = 17:3) to give alcohol **4b** (9.1 g, 93.3%) as a colorless solid.

4b ^{1}H NMR δ 0.11 and 0.12 and 0.15 and 0.16 (each 3H, s, SiMe), 0.90 and 0.91 (each 9H, s, *t*-Bu), 1.34 (1H, dd, J = 12.9, 10.9 Hz), 1.50 (1H, dd, J = 14.5, 2.4 Hz), 2.00 (2H, m), 2.12 (1H, dd, J = 8.2, 4.7 Hz), 2.28 (1H, d, J = 2.9 Hz), 3.33 (2H, m, CH_2OH), 3.41 (1H, dd, J = 11.0, 4.7 Hz), 4.11 (1H, ddd, J = 10.7, 9.0, 4.7 Hz), 4.32 (1H, m), 4.53 (1H, br s, O*H*).

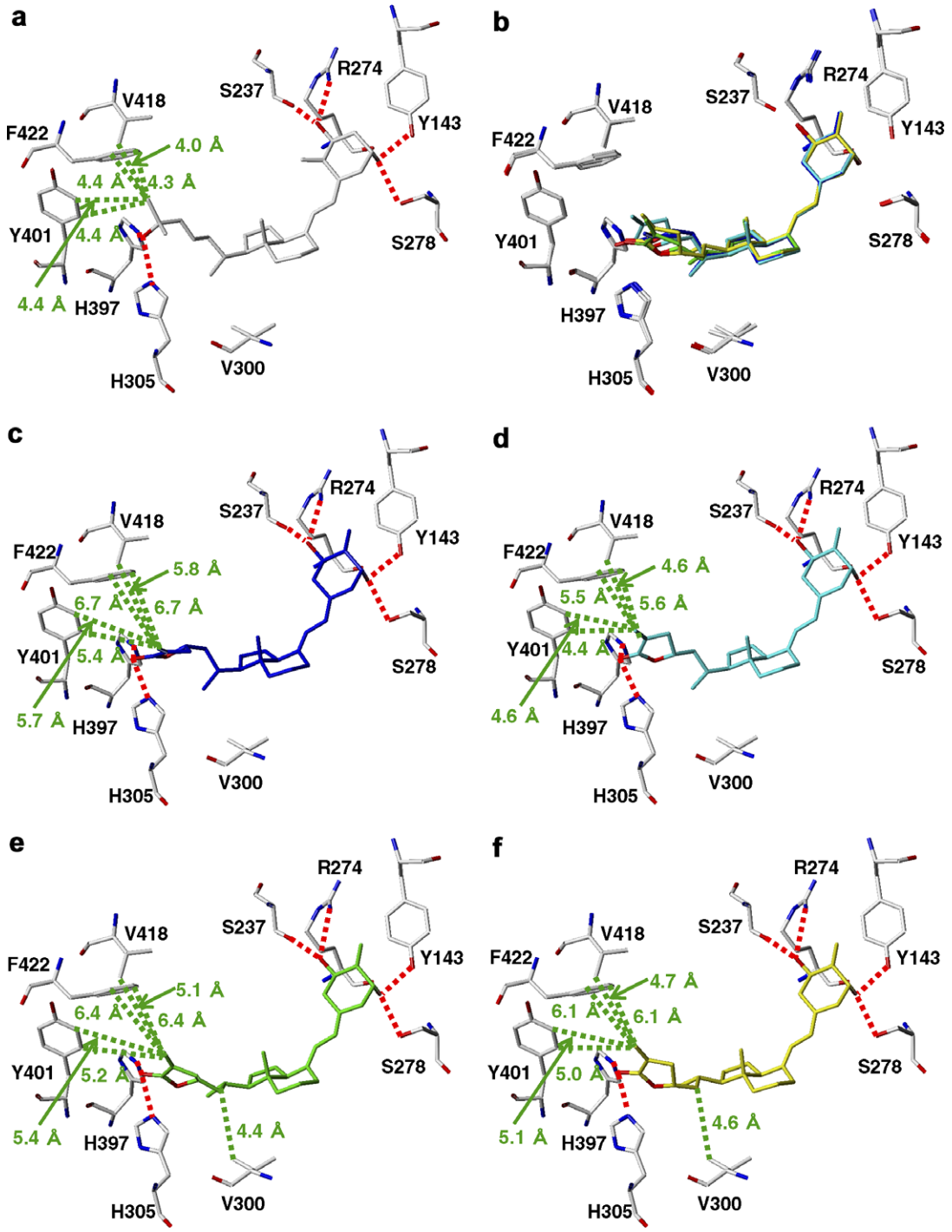


Figure 5. Docking models of **LAC67a**, **LAC67b**, **LAC82a** and **LAC82b** into the VDR–LBD. Hydrogen bonds and hydrophobic interactions are depicted as dotted red and green lines, respectively. (a) 1,25-$(OH)_2D_3$ and VDR–LBD complex. 26-Methyl group of 1,25-$(OH)_2D_3$ makes intimate van der Waals contacts with Tyr401 (Y401), Val418 (V418), and Phe422 (F422). (b) Superposition of four docking models. **LAC67a**, **LAC67b**, **LAC82a** and **LAC82b** in the VDR–LBP are drawn in blue, cyan, green, and yellow color, respectively. (c) **LAC67a** and VDR–LBD complex. (d) **LAC67b** and VDR–LBD complex. (e) **LAC82a** and VDR–LBD complex. f. **LAC82b** and VDR–LBD complex.

7.2. (3*R*,5*R*)-3,5-Bis{*tert*-butyl(dimethyl)silyl]oxy}-4-hydroxycyclohexanone (4c)

To a solution of alcohol **4b** (951.6 mg, 2.34 mmol) in MeOH (30 mL) was added sodium periodate-saturated water (8 mL), and the mixture was stirred at 0 °C for 3 h. The reaction was poured into brine and was extracted with AcOEt. The organic layer was washed with brine, dried over $MgSO_4$, and evaporated. The residue was chromatographed on silica gel (30 g, hexane/AcOEt = 23:2) to give ketone **4c** (900.0 mg, quant.) as a colorless solid.

4c ^{1}H NMR δ 0.06 (6H, s, SiMe × 2), 0.08 and 0.09 (each 3H, s, SiMe), 0.85 and 0.90 (each 9H, s, *t*-Bu), 2.25 (1H, m), 2.45 (1H, m), 2.60 (1H, m), 2.77 (1H, dd, J = 14.4,

3.3 Hz), 3.80 (1H, m, H-4), 4.27 (2H, m, H-3, 5). ^{13}C NMR δ −5.1, −4.94, −4.88, −4.7, 17.9, 18.0, 25.6 (3 carbons), 25.7 (3 carbons), 44.3, 46.0, 69.4, 70.0, 72.6, 207.3.

7.3. (3*R*)-3-[(4*S*,7a*R*)-4-Hydroxy-7a-methyloctahydro-1*H*-inden-1-yl]butanenitrile (7)

To a solution of tosylate **5** (130.1 mg, 0.36 mmol) in dry DMSO (500 μL) was added KCN (46.5 mg, 0.71 mmol), and the mixture was stirred at 70 °C for 1.5 h. The reaction was quenched with H_2O at 0 °C and was extracted with AcOEt. The organic layer was washed with brine, dried over $MgSO_4$, and evaporated. The residue was chromatographed on silica gel (5 g, hexane/AcOEt = 1:1) to give cyanide **7** (78.1 mg, 98.2%) as a colorless solid.

7 ^{1}H NMR δ 0.96 (3H, s, H-18), 1.15 (3H, d, J = 6.6 Hz, H-21), 2.25 (1H, dd, J = 16.7, 6.9 Hz, H-22), 2.35 (1H, dd, J = 16.7, 3.8 Hz, H-22), 4.09 (1H, m, H-8). ^{13}C NMR δ 13.8, 17.5, 19.3, 22.6, 24.8, 27.2, 33.2, 33.7, 40.2, 42.1, 52.5, 55.3, 69.1, 119.1. MS *m*/*z* (%): 221 (M^+, 15), 206 (45), 188 (15), 163 (15), 135 (20), 125 (20), 111 (100).

7.4. (3*S*)-3-[(4*S*,7a*R*)-4-Hydroxy-7a-methyloctahydro-1*H*-inden-1-yl]butanenitrile (8)

Compound **8** was obtained from **6** by the same procedure as described for **7** (yield 98.5%) as a colorless solid.

8 ^{1}H NMR δ 0.94 (3H, s, H-18), 1.07 (3H, d, J = 6.7 Hz, H-21), 2.43 (2H, m, H-22), 4.09 (1H, m, H-8). ^{13}C NMR δ 14.0, 17.5, 19.7, 22.4, 24.2, 27.1, 31.9, 33.6, 40.1, 41.8, 52.4, 55.0, 69.1, 118.9. IR (neat) 3493, 2930, 2871, 2248, 1454, 1167, 991, 954 cm^{-1}. MS *m*/*z* (%): 221 (M^+, 20), 206 (60), 188 (15), 163 (15), 135 (35), 125 (25), 111 (100), 93 (30). HRMS calcd for $C_{14}H_{23}ON$ 221.1780, found 221.1781.

7.5. (3*R*)-3-[(7a*R*)-7a-Methyl-4-oxooctahydro-1*H*-inden-1-yl]butanenitrile (9)

To a solution of cyanide **7** (286.6 mg, 1.30 mmol) in dry CH_2Cl_2 (3 mL) were added Molecular Sieves 4A (150 mg) and *N*-methylmorpholine *N*-oxide (NMO, 1.26 g, 10.8 mmol) at room temperature. After 10 min, tetrapropylammonium perruthenate (TPAP, 24.6 mg, 0.070 mmol) was added to the mixture, and the reaction mixture was stirred for 2 h at same temperature. The mixture was chromatographed on silica gel (20 g, hexane/AcOEt = 1:1) to give ketone **9** (260.5 mg, 91.5%) as a colorless solid.

9 ^{1}H NMR δ 0.67 (3H, s, H-18), 1.21 (3H, d, J = 6.6 Hz, H-21), 2.52 (1H, dd, J = 11.7, 7.6 Hz, H-14). ^{13}C NMR δ 12.6, 19.1, 19.4, 23.9, 24.8, 27.4, 33.2, 38.6, 40.8, 49.7, 55.1, 61.6, 118.6, 211.2. IR (neat) 2957, 2875, 2244, 1710, 1463, 1383, 1233 cm^{-1}. MS *m*/*z* (%): 219 (M^+, 70), 204 (80), 179 (25), 176 (90), 163 (60), 124 (40), 96 (45).

7.6. (3*S*)-3-[(7a*R*)-7a-Methyl-4-oxooctahydro-1*H*-inden-1-yl]butanenitrile (10)

Compound **10** was obtained from **8** by the same procedure as described for **9** (yield 95.8%) as a colorless solid.

10 ^{1}H NMR δ 0.62 (3H, s, H-18), 1.08 (3H, d, J = 6.6 Hz, H-21), 2.49 (1H, dd, J = 11.6, 7.7 Hz, H-14). ^{13}C NMR δ 12.9, 19.0, 19.7, 24.0, 24.4, 27.1, 32.0, 38.6, 40.8, 49.5, 54.8, 61.6, 118.5, 211.2. MS *m*/*z* (%): 219 (M^+, 65), 204 (100), 191 (28), 176 (80), 163 (45), 124 (58), 96 (65).

7.7. (3*R*)-3-{(1*R*,3*R*,7*E*)-1,3-Bis{*tert*-butyl(dimethyl)silyl]-oxy}-2-[(trimethylsilyl)oxy]-9,10-secoestra-5,7-dien-17-yl}butanenitrile (11)

To a solution of phosphine oxide **4** (1.18 g, 1.80 mmol, a 2:3 mixture of diastereomers at C(4)) in dry THF (10 mL) at −78 °C was added slowly *n*-BuLi (1.59 M hexane solution, 1.1 mL, 1.79 mmol), and the resulting dark orange solution was stirred for 15 min. To this colored solution was added a solution of ketone **9** (258.7 mg, 1.18 mmol) in dry THF (5 mL), the reaction mixture was stirred for 1.5 h at same temperature, and then the mixture was allowed to warm to 0 °C for 2 h. The reaction was quenched with saturated NH_4Cl aq solution at 0 °C and was extracted with AcOEt. The organic layer was washed with brine, dried over $MgSO_4$, and evaporated. The residue was chromatographed on silica gel (20 g, hexane/AcOEt = 19:1) to give compound **11** (683.7 mg, 79.8%, a 2:3 mixture of C(2) epimers) as colorless oil.

11 (a 2:3 mixture of C(2) epimers) ^{1}H NMR δ 0.0–0.1 (12H, s, SiMe × 4), 0.120, 0.125 (2:3) (9H, s, TMS), 0.56, 0.57 (2:3) (3H, s, H-18), 0.854, 0.867 (2:3) and 0.873, 0.891 (3:2) (each 9H, s, *t*-Bu), 1.17 (3H, d, J = 6.6 Hz, H-21), 2.80 (1H, m, H-9), 3.54, 3.59 (3:2) (1H, m, H-2), 3.80 (1H, m, H-3), 3.88, 3.92 (3:2) (1H, m, H-1), 5.79, 5.82 (2:3) (1H, d, J = 11.0 Hz, H-7), 6.09, 6.12 (3:2) (1H, d, J = 11.0 Hz, H-6). IR (neat) 2953, 2856, 2245, 1620, 1471, 1251, 1095, 837, 775 cm^{-1}. MS *m*/*z* (%): 659 (M^+, 20), 528 (30), 527 (70), 470 (90), 424 (80), 380 (50), 306 (30), 147 (30), 73 (60). HRMS calcd for $C_{37}H_{69}O_3NSi_3$ 659.4585, found 659.4597.

7.8. (3*S*)-3-{(1*R*,3*R*,7*E*)-1,3-Bis{[*tert*-butyl(dimethyl)silyl]-oxy}-2-[(trimethylsilyl)oxy]-9,10-secoestra-5,7-dien-17-yl} butanenitrile (12)

Compound **12** was obtained from **10** by the same procedure as described for **11** (yield 74.7%, a 2:3 mixture of C(2) epimers) as colorless oil.

12 (a 2:3 mixture of C(2) epimers) ^{1}H NMR δ −0.1–0.1 (12H, s, SiMe × l4), 0.119, 0.123 (2:3) (9H, s, TMS), 0.54, 0.55 (2:3) (3H, s, H-18), 0.85, 0.86 (2:3) and 0.87, 0.89 (3:2) (each 9H, s, *t*-Bu), 1.09 (3H, d, J = 6.6 Hz, H-21), 2.80 (1H, m, H-9), 3.54, 3.59 (3:2) (1H, m, H-2), 3.80 (1H, m, H-3), 3.88, 3.92 (3:2) (1H, m, H-1), 5.79, 5.82 (2:3) (1H, d, J = 11.1 Hz, H-7), 6.08, 6.11

(3:2) (1H, d, J = 11.1 Hz, H-6). IR (neat) 2954, 2928, 2856, 2247, 1729, 1471, 1251, 1095, 837 cm^{-1}. MS m/z (%): 659 (M^+, 10), 602 (8), 527 (35), 470 (40), 424 (38), 309 (58), 256 (52), 236 (55), 138 (60), 75 (100). HRMS calcd for $C_{37}H_{69}O_3NSi_3$ 659.4585, found 659.4579.

7.9. (3*R*)-3-((1*R*,3*R*,7*E*)-1,3-Bis{[*tert*-butyl(dimethyl)silyl]-oxy}-2-hydroxy-9,10-secoestra-5,7-dien-17-yl)butanenitrile (13)

A solution of compound **11** (532.6 mg, 0.81 mmol, a 2:3 mixture of C(2) epimers) in AcOH/THF/H_2O (8:8:1, 17 mL) was stirred at room temperature for 22.5 h. The reaction was quenched with 5% $NaHCO_3$ aq solution at 0 °C and was extracted with AcOEt. The organic layer was washed with brine, dried over $MgSO_4$, and evaporated. The residue was chromatographed on silica gel (20 g, hexane/AcOEt = 49:1 then 19:1) to give compound **11** (38.4 mg, 7.2%, a 2:3 mixture of C(2) epimers) and compound **13** (colorless oil, 405.6 mg, 85.5%, a 2:3 mixture of C(2) epimers).

13 (a 2:3 mixture of C(2) epimers) ^{1}H NMR δ 0.0–0.2 (12H, s, SiMe × 4), 0.55, 0.56 (2:3) (3H, s, H-18), 0.84, 0.86 (2:3) and 0.87, 0.89 (3:2) (each 9H, s, *t*-Bu), 1.17 (3H, d, J = 6.6 Hz, H-21), 2.80 (1H, m, H-9), 3.51, 3.58 (3:2) (1H, m, H-2), 3.91, 3.99 (3:2) (1H, m, H-3), 3.99 (1H, m, H-1), 5.79 (1H, d, J = 11.2 Hz, H-7), 6.14, 6.17 (3:2) (1H, d, J = 11.2 Hz, H-6). IR (neat) 3481, 2953, 2930, 2857, 2246, 1717, 1471, 1254, 1090, 837, 779 cm^{-1}. MS m/z (%): 587 (M^+, 5), 530 (7), 438 (5), 398 (100), 306 (15), 165 (10), 129 (10), 75 (40), 73 (18).

7.10. (3*S*)-3-((1*R*,3*R*,7*E*)-1,3-Bis{[*tert*-butyl(dimethyl)silyl]-oxy}-2-hydroxy-9,10-secoestra-5,7-dien-17-yl)butanenitrile (14)

Compound **14** was obtained from **12** by the same procedure as described for **13** (yield 82.6%, a 2:3 mixture of C(2) epimers) as colorless oil.

14 (a 2:3 mixture of C(2) epimers) ^{1}H NMR δ −0.1–0.2 (12H, s, SiMe × 4), 0.53, 0.55 (2:3) (3H, s, H-18), 0.85, 0.86 (2:3) and 0.87, 0.89 (3:2) (each 9H, s, *t*-Bu), 1.09 (3H, d, J = 6.6 Hz, H-21), 2.80 (1H, m, H-9), 3.51, 3.58 (3:2) (1H, m, H-2), 3.91, 3.99 (3:2) (1H, m, H-3), 3.99 (1H, m, H-1), 5.80 (1H, d, J = 11.2 Hz, H-7), 6.13, 6.16 (3:2) (1H, d, J = 11.2 Hz, H-6). IR (neat) 3555, 2952, 2855, 2246, 1462, 1254, 1085, 836 cm^{-1}. MS m/z (%): 587 (M^+, 3), 530 (8), 438 (10), 398 (100), 380 (10), 306 (20), 257 (10), 236 (15), 129 (15), 73 (25). HRMS calcd for $C_{34}H_{61}O_3NSi_2$ 587.4190, found 587.4196.

7.11. (3*R*)-3-((1*R*,3*R*,7*E*)-1,3-Bis{[*tert*-butyl(dimethyl)silyl]oxy}-2-oxo-9,10-secoestra-5,7-dien-17-yl)butanenitrile (15)

To a solution of oxalyl chloride (2.0 M CH_2Cl_2 solution, 315 μL, 0.63 mmol) in dry CH_2Cl_2 (1 mL) was added a solution of DMSO (90 μL, 1.27 mmol) in dry CH_2Cl_2 (500 μL) at −78 °C. After 10 min, a solution of compound **13** (308.1 mg, 0.53 mmol, a 2:3 mixture of C(2) epimers) in dry CH_2Cl_2 (2.5 mL) was added to the mixture, and the reaction mixture was stirred for 15 min at same temperature. Then, to this solution was added Et_3N (363 μL, 2.62 mmol), and mixture was stirred at −78 °C for 30 min and at 0 °C for 1 h. The reaction was quenched with H_2O at 0 °C and was extracted with CH_2Cl_2. The organic layer was washed with brine, dried over $MgSO_4$, and evaporated. The residue was chromatographed on silica gel (10 g, hexane/AcOEt = 9:1) to give ketone **15** (294.8 mg, 96.0%) as a colorless oil.

15 ^{1}H NMR δ 0.05 and 0.08 (each 3H, s, SiMe), 0.06 (6H, s, SiMe × 2), 0.57 (3H, s, H-18), 0.86 and 0.88 (each 9H, s, *t*-Bu), 1.18 (3H, d, J = 6.6 Hz, H-21), 2.83 (1H, m, H-9), 4.35 (1H, dd, J = 6.4, 4.2 Hz, H-3), 4.53 (1H, dd, J = 8.7, 5.5 Hz, H-1), 5.81 (1H, d, J = 11.1 Hz, H-7), 6.33 (1H, d, J = 11.1 Hz, H-6). ^{13}C NMR δ −5.00, −4.95, −4.7, −4.6, 12.3, 18.3, 18.5, 19.6, 22.3, 23.4, 25.0, 25.9 (3 carbons), 26.0 (3 carbons), 27.7, 28.9, 37.7, 40.3, 41.6, 45.9, 46.7, 55.3, 56.2, 74.5, 74.8, 116.4, 119.1, 124.5, 129.5, 142.3, 208.7. IR (neat) 2952, 2929, 2856, 2245, 1738, 1470, 1254, 1098, 836 cm^{-1}. MS m/z (%): 585 (M^+, 2), 570 (3), 528 (100), 396 (62), 230 (40), 75 (62), 73 (32). HRMS calcd for $C_{34}H_{59}O_3NSi_2$ 585.4033, found 585.4055.

7.12. (3*S*)-3-((1*R*,3*R*,7*E*)-1,3-Bis{[*tert*-butyl(dimethyl)silyl]-oxy}-2-oxo-9,10-secoestra-5,7-dien-17-yl)butanenitrile (16)

Compound **16** was obtained from **14** by the same procedure as described for **15** (yield 99.2%) as a colorless oil.

16 ^{1}H NMR δ 0.04 and 0.08 (each 3H, s, SiMe), 0.05 (6H, s, SiMe × 2), 0.55 (3H, s, H-18), 0.86 and 0.88 (each 9H, s, *t*-Bu), 1.09 (3H, d, J = 6.7 Hz, H-21), 2.82 (1H, m, H-9), 4.35 (1H, dd, J = 6.4, 4.2 Hz, H-3), 4.53 (1H, dd, J = 8.6, 5.5 Hz, H-1), 5.81 (1H, d, J = 11.2 Hz, H-7), 6.33 (1H, d, J = 11.2 Hz, H-6). ^{13}C NMR δ −5.1, −5.0, −4.8, −4.7, 12.3, 18.2, 18.4, 19.6, 22.2, 23.4, 24.9, 25.81 (3 carbons), 25.85 (3 carbons), 27.6, 28.8, 34.0, 37.6,40.2, 45.8, 46.6, 55.2, 56.1, 74.4, 74.7, 116.3, 119.0, 124.5, 129.4, 142.2, 208.5. MS m/z (%): 528 ($M^+-C(CH_3)_3$, 100), 510 (4), 396 (42), 378 (12), 325 (15), 259 (10), 147 (12), 73 (45).

7.13. (3*R*)-3-((1*R*,3*R*,7*E*)-1,3-Bis{*tert*-butyl(dimethyl)silyl]oxy}-2-methylene-9,10-secoestra-5,7-dien-17-yl)butanenitrile (17)

To a suspension of methyltriphenylphosphonium bromide (527.3 mg, 1.48 mmol) in dry THF (1.5 mL) at 0 °C was added slowly *n*-BuLi (1.59 M hexane solution, 923 μL, 1.47 mmol), and the resulting yellow solution was stirred for 1 h. To this colored solution was added a solution of ketone **15** (285.9 mg, 0.49 mmol) in dry THF (2 mL), the reaction mixture was stirred at 0 °C for 1 h, and then at room temperature for 1 h. The reaction was quenched with H_2O at 0 °C and was extracted with AcOEt. The organic layer was washed with brine, dried over $MgSO_4$, and evaporated. The residue was chromatographed on silica gel (10 g, hexane/

AcOEt = 19:1) to give 2-methylene **17** (268.4 mg, 94.2%) as a colorless oil.

17 ^{1}H NMR δ 0.02 and 0.05 and 0.06 and 0.08 (each 3H, s, SiMe), 0.57 (3H, s, H-18), 0.86 and 0.89 (each 9H, s, *t*-Bu), 1.18 (3H, d, J = 6.6 Hz, H-21), 2.82 (1H, m, H-9), 4.43 (2H, m, H-3, 1), 4.92 and 4.97 (each 1H, s, –C=CH_2), 5.84 (1H, d, J = 11.2 Hz, H-7), 6.21 (1 H, d, J = 11.2 Hz, H-6). ^{13}C NMR δ −4.9, −4.71, −4.66, −4.64, 12.4, 18.36, 18.44, 19.7, 22.3, 23.4, 25.0, 25.98 (3 carbons), 26.04 (3 carbons), 27.8, 28.8, 34.1, 38.8, 40.4, 45.8, 47.8, 55.4, 56.2, 71.8, 72.7, 106.6, 116.7, 119.2, 122.4, 133.5, 140.4, 153.1. IR (neat) 2952, 2891, 2856, 2246, 1472, 1256 cm^{-1}.

7.14. (3*S*)-3-((1*R*,3*R*,7*E*)-1,3-Bis{*tert*-butyl(dimethyl)silyl] oxy}-2-methylene-9,10-secoestra-5,7-dien-17-yl)butanenitrile (18)

Compound **18** was obtained from **16** by the same procedure as described for **17** (yield 88.5%) as a colorless oil.

18 ^{1}H NMR δ 0.02 and 0.05 and 0.06 and 0.08 (each 3H, s, SiMe), 0.55 (3H, s, H-18), 0.86 and 0.89 (each 9H, s, *t*-Bu), 1.10 (3H, d, J = 6.7 Hz, H-21), 2.83 (1H, m, H-9), 4.42 (2H, m, H-3, 1), 4.92 and 4.97 (each 1H, s, –C=CH_2), 5.85 (1H, d, J = 11.2 Hz, H-7), 6.20 (1H, d, J = 11.2 Hz, H-6). ^{13}C NMR δ −4.9, −4.7, −4.6 (2 carbons), 12.6, 18.37, 18.45, 19.9, 22.2, 23.5, 24.5, 25.99 (3 carbons), 26.04 (3 carbons), 27.5, 28.7, 32.8, 38.8, 40.3, 45.5, 47.8, 55.0, 56.1, 71.8, 72.7, 106.6, 116.8, 119.0, 122.4, 133.5, 140.3, 153.1. IR (neat) 2934, 2885, 2857, 2247, 1472, 1257 cm^{-1}.

7.15. (3*R*)-3-((1*R*,3*R*,7*E*)-1,3-Bis{*tert* -butyl(dimethyl)silyl] oxy}-2-methylene-9,10-secoestra-5,7-dien-17-yl)butanal (19)

To a solution of 2-methylene **17** (113.3 mg, 0.19 mmol) in dry CH_2Cl_2 (1 mL) was added a solution of DIBAL-H (1.01 M solution in toluene, 252 μL, 0.25 mmol) at 0 °C for 1.5 h. The reaction was quenched with 10% potassium sodium tartrate aq solution at 0 °C and was extracted with CH_2Cl_2. The organic layer was washed with brine, dried over $MgSO_4$, and evaporated. The residue was chromatographed on silica gel (5 g, hexane/AcOEt = 49:1) to give aldehyde **19** (102.3 mg, 90.0%) as a colorless oil.

19 ^{1}H NMR δ 0.02 and 0.04 and 0.06 and 0.07 (each 3H, s, SiMe), 0.59 (3H, s, H-18), 0.86 and 0.89 (each 9H, s, *t*-Bu), 1.02 (3H, d, J = 6.5 Hz, H-21), 2.81 (1H, m, H-9), 4.42 (2H, m, H-3, 1), 4.91 and 4.97 (each 1H, s, –C=CH_2), 5.83 (1H, d, J = 11.2 Hz, H-7), 6.20 (1H, d, J = 11.2 Hz, H-6), 9.76 (1H, dd, J = 3.3, 1.2 Hz, H-23). ^{13}C NMR δ −4.9, −4.7, −4.6 (2 carbons), 12.3, 18.37, 18.45, 20.3, 22.4, 23.5, 25.98 (3 carbons), 26.04 (3 carbons), 28.1, 28.8, 32.1, 38.8, 40.6, 45.9, 47.8, 51.0, 56.40, 56.43, 71.8, 72.7, 106.5, 116.6, 122.5, 133.3, 140.8, 153.1, 203.6. MS *m*/*z* (%): 586 (M^+, 12), 529 (10), 454 (100), 366 (34), 322 (12), 234 (12), 75 (32), 73 (30).

7.16. (3*S*)-3-((1*R*,3*R*,7*E*)-1,3-Bis{*tert*-butyl(dimethyl)silyl]oxy}-2-methylene-9,10-secoestra-5,7-dien-17-yl)butanal (20)

Compound **20** was obtained from **18** by the same procedure as described for **19** (yield 87.3%) as a colorless oil.

20 ^{1}H NMR δ 0.02 and 0.04 and 0.06 and 0.07 (each 3H, s, SiMe), 0.58 (3H, s, H-18), 0.86 and 0.89 (each 9H, s, *t*-Bu), 0.95 (3H, d, J = 6.6 Hz, H-21), 2.26 (1H, ddd, J = 15.9, 9.6, 3.4 Hz, H-22), 2.67 (1H, dd, J = 15.9, 3.1 Hz, H-22), 2.82 (1H, m, H-9), 4.42 (2H, m, H-3, 1), 4.92 and 4.97 (each 1H, s, –C=CH_2), 5.85 (1H, d, J = 11.2 Hz, H-7), 6.20 (1H, d, J = 11.2 Hz, H-6), 9.75 (1 H, dd, J = 3.4, 1.0 Hz, H-23). ^{13}C NMR δ −4.9, −4.7, −4.6 (2 carbons), 12.7, 18.4, 18.5, 20.0, 22.2, 23.5, 26.0 (3 carbons), 26.1 (3 carbons), 27.4, 28.8, 31.1, 38.8, 40.7, 45.8, 47.8, 50.3, 56.2, 56.3, 71.8, 72.7, 106.5, 116.7, 122.5, 133.4, 140.6, 153.1, 203.4.

7.17. 5-[(2*R*)-2-((1*R*,3*R*,7*E*)-1,3-Bis{*tert*-butyl(dimethyl)silyl]oxy}-2-methylene-9,10-secoestra-5,7-dien-17-yl)propyl]-3-methylenedihydro-2(3*H*)-furanone (21)

To a suspension of $CrCl_3$ (74.8 mg, 0.47 mmol) in dry THF (1 mL) was added slowly $LiAlH_4$ (1.0 M solution in THF, 235 μL, 0.24 mmol) at 0 °C, and the mixture was stirred at room temperature for 45 min. To the mixture were added a solution of methyl 2-(bromomethyl)acrylate (34 μL, 0.28 mmol) in dry THF (300 μL) and a solution of aldehyde **19** (68.9 mg, 0.12 mmol) in dry THF (750 μL) at room temperature, and the resulting mixture was stirred at the same temperature for 30 min. The reaction was quenched with H_2O and 1N HCl at 0 °C and was extracted with AcOEt. The organic layer was washed with brine, dried over $MgSO_4$, and evaporated. The residue was chromatographed on silica gel (5 g, hexane/AcOEt = 49:1) to give methylene lactone **21** (amorphous solid, 63.6 mg, 82.7%, a 2:3 mixture of diastereomers at C(23)) and 23-alcohol (a colorless oil, 4 mg, 5.8%).

21 (a 2:3 mixture of C(23) epimers) ^{1}H NMR δ −0.0–0.1 (12H, s, SiMe × 4), 0.557, 0.562 (2:3) (3H, s, H-18), 0.86 and 0.890, 0.894 (2:3) (each 9H, s, *t*-Bu), 1.02, 1.03 (3:2) (3H, d, J = 6.3 Hz, H-21), 2.82 (1H, m), 3.06 (1H, m), 4.42 (2H, m, H-3, 1), 4.63 (1H, m, H-23), 4.91 and 4.97 (each 1H, s, –C=CH_2), 5.62 (1H, t, J = 2.4 Hz, H-27), 5.83 (1H, d, J = 11.2 Hz, H-7), 6.21 (1H, d, J = 11.2 Hz, H-6), 6.22 (1H, m, H-27). MS *m*/*z* (%): 654 (M^+, 12), 597 (8), 522 (78), 465 (18), 366 (35), 251 (18), 197 (15), 75 (100), 73 (38).

7.18. 5-[(2*S*)-2-((1*R*,3*R*,7*E*)-1,3-Bis{*tert*-butyl(dimethyl)silyl]oxy}-2-methylene-9,10-secoestra-5,7-dien-17-yl)propyl]-3-methylenedihydro-2(3*H*)-furanone (22)

Compound **22** was obtained from **20** by the same procedure as described for **21** (yield 81.8%, 2:3 mixture of C(23) epimers) as amorphous solid.

22 (a 2:3 mixture of C(23) epimers) ^{1}H NMR δ −0.1–0.1 (12H, s, SiMe × 4), 0.54, 0.55 (2:3) (3H, s, H-18), 0.859,

0.861 (3:2) and 0.89 (each 9H, s, *t*-Bu), 0.92, 0.95 (2:3) (3H, d, J = 6.7 Hz, H-21), 2.81 (1H, m), 3.06 (1 H, m), 4.42 (2H, m, H-3, 1), 4.63 (1H, m, H-23), 4.92 and 4.97 (each 1H, s, –C=CH_2), 5.66 (1H, m, H-27), 5.84 (1H, d, J = 11.2 Hz, H-7), 6.18 (1H, d, J = 11.2 Hz, H-6), 6.27 (1H, m, H-27).

7.19. 5-{(2*R*)-2-[(1*R*,3*R*,7*E*)-1,3-Dihydroxy-2-methylene-9,10-secoestra-5,7-dien-17-yl]propyl}-3-methylenedihydro-2(3*H*)-furanone (LAC67a, b)

To a solution of methylene lactone **21** (26.0 mg, 0.040 mmol, 2:3 mixture of C(23) epimers) in MeOH (1 mL) was added CSA (20.4 mg, 0.088 mmol) at room temperature for 1.3 h. The reaction was quenched with 5% $NaHCO_3$ at 0 °C and was extracted with AcOEt. The organic layer was washed with brine, dried over $MgSO_4$, and evaporated. The residue was chromatographed on silica gel (5 g, AcOEt) to give lactone analogue **LAC67** (5.7 mg, 54.0%, a 2:3 mixture of C(23) epimers) as a colorless solid. The mixture was separated by HPLC (LiChrosorb Si 60, Hibar RT 250-10, 7 μm, hexane/AcOEt = 7:3, 5 mL/min) to give **LAC67a** (2.7 mg) and **LAC67b** (2.1 mg). The purity of **LAC67a** and **LAC67b** was proved to be about 100% by HPLC.

LAC67a (less polar) ^{1}H NMR δ 0.58 (3H, s, H-18), 1.02 (3H, d, J = 6.5 Hz, H-21), 4.51 (2H, m, H-3, 1), 4.65 (1H, m, H-23), 5.09 and 5.12 (each 1H, s, –C=CH_2), 5.63 (1H, t, J = 2.4 Hz, H-27), 5.89 (1H, d, J = 11.2 Hz, H-7), 6.23 (1H, t, J = 2.8 Hz, H-27), 6.35 (1H, d, J = 11.2 Hz, H-6). IR (neat) 3384, 2942, 2823, 1748 cm^{-1}. MS *m*/*z* (%): 426 (M$^+$, 10), 390 (50), 375 (12), 285 (12), 252 (100), 250 (60), 197 (32), 105 (25). HRMS calcd for $C_{27}H_{38}O_4$ 426.2770, found 426.2794. UV (95%EtOH): λ_{max} 245, 254, 263 nm.

LAC67b (more polar) ^{1}H NMR δ 0.57 (3H, s, H-18), 1.04 (3H, d, J = 6.3 Hz, H-21), 4.50 (2H, m, H-3, 1), 4.60 (1H, m, H-23), 5.10 and 5.12 (each 1H, s, –C=CH_2), 5.63 (1H, t, J = 2.4 Hz, H-27), 5.89 (1H, d, J = 11.5 Hz, H-7), 6.23 (1H, t, J = 2.8 Hz, H-27), 6.36 (1H, d, J = 11.5 Hz, H-6). IR (neat) 3452, 2923, 2853, 1759 cm^{-1}. MS *m*/*z* (%): 426 (M$^+$, 10), 390 (80), 375 (20), 285 (20), 251 (100), 197 (42), 105 (40). HRMS calcd for $C_{27}H_{38}O_4$ 426.2770, found 426.2750. UV (95% EtOH): λ_{max} 245, 253, 263 nm.

7.20. 5-{(2*S*)-2-[(1*R*,3*R*,7*E*)-1,3-Dihydroxy-2-methylene-9,10-secoestra-5,7-dien-17-yl]propyl}-3-methylenedihydro-2(3*H*)-furanone (LAC82a, b)

LAC82 was obtained from **22** by the same procedure as described for **LAC67** (yield 99.1%) as a colorless solid. The mixture was separated by HPLC (LiChrosorb Si 60, Hibar RT 250-10, 7 μm, hexane/AcOEt = 7:3, 5 mL/min) to give **LAC82a** and **LAC82b**. The purity of **LAC82a** and **LAC82b** was proved to be about 100% by HPLC.

LAC82a (more polar) ^{1}H NMR δ 0.56 (3H, s, H-18), 0.94 (3H, d, J = 6.4 Hz, H-21), 4.49 (2H, m, H-3, 1), 4.61 (1H, m, H-23), 5.10 and 5.11 (each 1H, s, –C=CH_2), 5.63 (1H, t, J = 2.4 Hz, H-27), 5.89 (1H, d, J = 11.3 Hz, H-7), 6.23 (1H, t, J = 2.7 Hz, H-27), 6.36 (1H, d, J = 11.3 Hz, H-6). IR (neat) 3421, 2929, 2875, 1762 cm^{-1}. MS *m*/*z* (%): 426 (M$^+$, 30), 390 (70), 375 (20), 285 (25), 251 (100), 197 (50), 157 (40), 105 (58). HRMS calcd for $C_{27}H_{38}O_4$ 426.2770, found 426.2787. UV (95% EtOH): λ_{max} 245, 253, 263 nm.

LAC82b (less polar) ^{1}H NMR δ 0.57 (3H, s, H-18), 0.95 (3H, d, J = 6.6 Hz, H-21), 4.49 (2H, m, H-3, 1), 4.64 (1H, m, H-23), 5.09 and 5.12 (each 1H, s, –C=CH_2), 5.62 (1H, t, J = 2.4 Hz, H-27), 5.89 (1H, d, J = 11.2 Hz, H-7), 6.23 (1H, t, J = 2.8 Hz, H-27), 6.34 (1H, d, J = 11.2 Hz, H-6). IR (neat) 3421, 2936, 2875, 1759 cm^{-1}. MS *m*/*z* (%): 426 (M$^+$, 20), 408 (28), 390 (90), 375 (28), 285 (30), 251 (100), 197 (55), 157 (30), 105 (55). HRMS calcd for $C_{27}H_{38}O_4$ 426.2770, found 426.2759. UV (95% EtOH): λ_{max} 245, 253, 263 nm.

7.21. (6*R*)-6-((1*R*,3*R*,7*E*)-1,3-Bis{[*tert*-butyl(dimethyl)silyl]-oxy}-2-methylene-9,10-secoestra-5,7-dien-17-yl)-2-methylene-1,4-heptanediol (23a, b)

To a solution of methylene lactone **21** (21.0 mg, 0.032 mmol) in dry toluene (1 mL) was added a solution of DIBAL-H (1.01 M solution in toluene, 137 μL, 0.14 mmol) at 0 °C for 1 h. The reaction was quenched with 10% potassium sodium tartrate aq solution at 0 °C and was extracted with AcOEt. The organic layer was washed with brine, dried over $MgSO_4$, and evaporated. The residue was chromatographed on silica gel (7 g, hexane/AcOEt = 4:1) to give 23,26-diol **23a** (a colorless oil, 5.7 mg, 27.0%, less polar) and diol **23b** (a colorless oil, 4.9 mg, 23.2%, more polar).

23a (less polar) ^{1}H NMR δ 0.02 and 0.05 and 0.06 and 0.08 (each 3H, s, SiMe), 0.58 (3H, s, H-18), 0.85 and 0.90 (each 9H, s, *t*-Bu), 0.99 (3H, d, J = 6.5 Hz, H-21), 2.83 (1H, m, H-9), 3.87 (1H, m, H-23), 4.12 (2H, s, H-26), 4.42 (2H, m, H-3, 1), 4.92 and 4.97 (each 1H, s, –C=CH_2), 4.98 and 5.15 (each 1H, s, H-27), 5.84 (1H, d, J = 11.1 Hz, H-7), 6.22 (1H, d, J = 11.1 Hz, H-6). MS *m*/*z* (%): 658 (M$^+$, 8), 640 (17), 526 (32), 508 (100), 454 (50), 366 (56), 234 (23), 147 (17), 75 (75), 73 (68).

23b (more polar) ^{1}H NMR δ 0.02 and 0.05 and 0.06 and 0.08 (each 3H, s, SiMe), 0.56 (3H, s, H-18), 0.86 and 0.89 (each 9H, s, *t*-Bu), 1.01 (3H, d, J = 6.4 Hz, H-21), 2.82 (1H, m, H-9), 3.86 (1H, m, H-23), 4.13 (2H, s, H-26), 4.42 (2H, m, H-3, 1), 4.92 and 4.97 (each 1H, s, –C=CH_2), 5.00 and 5.17 (each 1H, s, H-27), 5.84 (1H, d, J = 11.2 Hz, H-7), 6.21 (1H, d, J = 11.2 Hz, H-6). MS *m*/*z* (%): 658 (M$^+$, 10), 640 (18), 526 (45), 508 (100), 454 (63), 366 (65), 236 (32), 234 (30), 147 (22), 138 (21), 75 (92), 73 (90).

7.22. (6*S*)-6-((1*R*,3*R*,7*E*)-1,3-Bis{[*tert*-butyl(dimethyl)silyl]oxy}-2-methylene-9,10-secoestra-5,7-dien-17-yl)-2-methylene-1,4-heptanediol (24a, b)

Compound **24** was obtained from **22** by the same procedure as described for **23a** and **23b** (**24a** 31.8% more polar, **24b** 29.7% less polar) as colorless oil, respectively.

24a (more polar) ^{1}H NMR δ 0.02 and 0.05 and 0.06 and 0.08 (each 3H, s, SiMe), 0.55 (3H, s, H-18), 0.86 and 0.89 (each 9H, s, *t*-Bu), 0.91 (3H, d, J = 6.7 Hz, H-21), 2.82 (1H, m, H-9), 3.86 (1H, m, H-23), 4.12 (2H, s, H-26), 4.42 (2H, m, H-3, 1), 4.92 and 4.97 (each 1H, s, –C=CH_2), 5.00 and 5.16 (each 1H, s, H-27), 5.84 (1H, d, J = 11.1 Hz, H-7), 6.21 (1H, d, J = 11.1 Hz, H-6).

24b (less polar) ^{1}H NMR δ 0.02 and 0.05 and 0.06 and 0.08 (each 3H, s, SiMe), 0.58 (3H, s, H-18), 0.86 and 0.89 (each 9H, s, *t*-Bu), 0.91 (3H, d, J = 6.9 Hz, H-21), 2.82 (1H, m, H-9), 3.86 (1H, m, H-23), 4.11 (2H, s, H-26), 4.42 (2H, m, H-3, 1), 4.92 (1H, s, –C=CH_2), 4.969 and 4.975 (each 1H, s, H-27, –C=CH_2), 5.14 (1H, s, H-27), 5.84 (1H, d, J = 11.1 Hz, H-7), 6.21 (1H, d, J = 11.1 Hz, H-6).

7.23. 2-((2*R*,4*R*)-4-{(4*E*)-4-[2-((3*R*,5*R*)-3,5-Bis{[*tert*-butyl(dimethyl)silyl]oxy}-4-methylenecyclohexylidene) ethylidene]-7a-methyloctahydro-1H-inden-1-yl}-2-hydroxypentyl)-2-propenyl pivalate (25a)

To a solution of 23,26-diol **23a** (18.4 mg, 0.028 mmol) in dry CH_2Cl_2 (1 mL) were added dry pyridine (9.5 μL, 0.12 mmol) and PivCl (4.5 μL, 0.036 mmol), and the mixture was stirred at 0 °C for 22.5 h. The reaction was quenched with H_2O at 0 °C and was extracted with CH_2Cl_2. The organic layer was washed with brine, dried over $MgSO_4$, and evaporated. The residue was chromatographed on silica gel (5 g, hexane/AcOEt = 49:1 then 4:1) to give pivalate ester **25a** (a yellow oil, 5.5 mg, 26.5%) and 23,26-diol **23a** (5.6 mg, 30.4%).

25a ^{1}H NMR δ 0.02 and 0.05 and 0.06 and 0.08 (each 3H, s, SiMe), 0.58 (3H, s, H-18), 0.85 and 0.90 (each 9H, s, *t*-Bu), 0.98 (3H, d, J = 6.4 Hz, H-21), 1.23 (9H, s, –COC(CH_3)$_3$), 2.82 (1H, m, H-9), 3.88 (1H, m, H-23), 4.42 (2H, m, H-3, 1), 4.55 (2H, s, H-26), 4.91 and 4.97 (each 1H, s, –C=CH_2), 5.04 and 5.14 (each 1H, s, H-27), 5.84 (1H, d, J = 11.1 Hz, H-7), 6.22 (1H, d, J = 11.1 Hz, H-6). MS *m*/*z* (%): 742 (M^+, 20), 724 (3), 640 (5), 610 (100), 592 (42), 508 (40), 454 (92), 366 (90), 234 (25), 197 (20), 147 (22), 73 (98).

7.24. 2-((2*S*,4*R*)-4-{(4*E*)-4-[2-((3*R*,5*R*)-3,5-Bis{[*tert*-butyl (dimethyl)silyl]oxy}-4-methylenecyclohexylidene)ethylidene]-7a-methyloctahydro-1H-inden-1-yl}-2-hydroxypentyl)-2-propenyl pivalate (25b)

Compound **25b** was obtained from **23b** by the same procedure as described for **25a** (yield 43.7%) as a yellow oil, with the recovered **23b** (8.2%).

25b ^{1}H NMR δ 0.02 and 0.05 and 0.06 and 0.08 (each 3H, s, SiMe), 0.55 (3H, s, H-18), 0.86 and 0.89 (each 9H, s, *t*-Bu), 1.00 (3H, d, J = 6.1 Hz, H-21), 1.23 (9H, s, –COC(CH_3)$_3$), 2.82 (1H, m, H-9), 3.85 (1H, m, H-23), 4.43 (2H, m, H-3, 1), 4.53 and 4.58 (each 1H, d, J = 13.7 Hz, H-26), 4.92 and 4.97 (each 1H, s, –C=CH_2), 5.06 and 5.17 (each 1H, s, H-27), 5.83 (1H, d, J = 11.1 Hz, H-7), 6.21 (1H, d, J = 11.1 Hz, H-6). MS *m*/*z* (%): 742 (M^+, 20), 724 (5), 640 (3), 610 (92), 592 (45), 508 (42), 454 (92), 366 (95), 234 (30), 197 (20), 147 (22), 73 (100).

7.25. 2-((2*R*,4*S*)-4-{(4*E*)-4-[2-((3*R*,5*R*)-3,5-Bis{[*tert*-butyl (dimethyl)silyl]oxy}-4-methylenecyclohexylidene)ethylidene]-7a-methyloctahydro-1*H*-inden-1-yl}-2-hydroxypentyl)-2-propenyl pivalate (26a)

Compound **26a** was obtained from **24a** by the same procedure as described for **25a** (yield 45.3%) as a yellow oil, with the recovered **24a** (34.7%).

26a ^{1}H NMR δ 0.03 and 0.05 and 0.07 and 0.08 (each 3H, s, SiMe), 0.55 (3H, s, H-18), 0.86 and 0.90 (each 9H, s, *t*-Bu), 1.23 (9H, s, –COC(CH_3)$_3$), 2.82 (1H, m, H-9), 3.85 (1H, m, H-23), 4.43 (2H, m, H-3, 1), 4.53 and 4.58 (each 1H, d, J = 13.6 Hz, H-26), 4.92 and 4.97 (each 1H, s, –C=CH_2), 5.06 and 5.17 (each 1H, s, H-27), 5.84 (1H, d, J = 11.1 Hz, H-7), 6.21 (1H, d, J = 11.1 Hz, H-6).

7.26. 2-((2*S*,4*S*)-4-{(4*E*)-4-[2-((3*R*,5*R*)-3,5-Bis{[*tert*-butyl (dimethyl)silyl]oxy}-4-methylenecyclohexylidene)ethylidene]-7a-methyloctahydro-1*H*-inden-1-yl}-2-hydroxypentyl)-2-propenyl pivalate (26b)

Compound **26b** was obtained from **24b** by the same procedure as described for **25a** (yield 23.8%) as a yellow oil, with the recovered **24b** (45.4%).

26b ^{1}H NMR δ 0.02 and 0.05 and 0.06 and 0.08 (each 3H, s, SiMe), 0.59 (3H, s, H-18), 0.86 and 0.90 (each 9H, s, *t*-Bu), 1.23 (9H, s, –COC(CH_3)$_3$), 2.82 (1H, m, H-9), 3.88 (1H, m, H-23), 4.42 (2H, m, H-3, 1), 4.54 (2H, s, H-26), 4.92 and 4.97 (each 1H, s, –C=CH_2), 5.04 and 5.15 (each 1H, s, H-27), 5.84 (1H, d, J = 11.0 Hz, H-7), 6.21 (1H, d, J = 11.0 Hz, H-6).

7.27. (1*R*)-1-[(2*R*)-2-((1*R*,3*R*,7*E*)-1,3-Bis{[*tert*-butyl(dimethyl)silyl]oxy}-2-methylene-9,10-secoestra-5,7-dien-17-yl) propyl]-3-{[(2,2-dimethylpropanoyl)oxy]methyl}-3-butenyl (2*S*)-3,3,3-trifluoro-2-methoxy-2-phenylpropanoate (27a)

To a solution of pivalate ester **25a** (4.0 mg, 0.0054 mmol) in dry CH_2Cl_2 (100 μL) were added Et_3N (7.5 μL, 0.054 mmol), DMAP (6.2 mg, 0.051 mmol), and a solution of *R*-MTPACl (5.1 μL, 0.027 mmol) in dry CH_2Cl_2 (40 μL), the reaction mixture was stirred for 15 min at room temperature. The reaction was quenched with H_2O at 0 °C and was extracted with CH_2Cl_2. The organic layer was washed with brine, dried over $MgSO_4$, and evaporated. The residue was chromatographed on silica gel (5 g, hexane/AcOEt = 9:1) to give *S*-MTPA ester **27a** (3.8 mg, 78.0%) as a colorless oil.

27a ^{1}H NMR δ 0.03 and 0.05 and 0.07 and 0.08 (each 3H, s, SiMe), 0.48 (3H, s, H-18), 0.86 and 0.89 (each 9H, s, *t*-Bu), 0.98 (3H, d, J = 6.3 Hz, H-21), 1.23 (9H, s, –COC(CH_3)$_3$), 2.82 (1H, m, H-9), 3.51 (3H, s, –OCH_3), 4.41 (2H, m, H-3, 1), 4.48 and 4.53 (each 1H, d, J = 13.7 Hz, H-26), 4.92 and 4.97 (each 1H, s, –C=CH_2), 4.93 and 5.05 (each 1H, s, H-27), 5.38 (1H,

m, H-23), 5.83 (1H, d, J = 11.1 Hz, H-7), 6.21 (1H, d, J = 11.1 Hz, H-6), 7.38 (3H, m, Ph-3, 4, 5), 7.53 (2H, m, Ph-2, 6).

7.28. (1*R*)-1-[(2*R*)-2-((1*R*,3*R*,7*R*)-1,3-Bis{[*tert*-butyl(dimethyl)silyl]oxy}-2-methylene-9,10-secoestra-5,7-dien-17-yl)propyl]-3-{[(2,2-dimethylpropanoyl)oxy]methyl}-3-butenyl (2*R*)-3,3,3-trifluoro-2-methoxy-2-phenylpropanoate (28a)

In a similar manner to that for the synthesis of **27a** from **25a**, a crude product, which was obtained from **25a** (4.5 mg, 0.0061 mmol), Et_3N (8.4 μL, 0.061 mmol), DMAP (6.7 mg, 0.055 mmol), and *S*-MTPACl (5.7 μL, 0.030 mmol) in dry CH_2Cl_2 at room temperature for 15 min, was purified by chromatographed on silica gel (5 g, hexane/AcOEt = 9:1) to give *R*-MTPA ester **28a** (5.0 mg, 84.8%) as a colorless oil.

28a ^{1}H NMR δ 0.03 and 0.05 and 0.07 and 0.08 (each 3H, s, SiMe), 0.40 (3H, s, H-18), 0.87 and 0.89 (each 9H, s, *t*-Bu), 1.23 (9H, s, –COC(CH_3)$_3$), 2.80 (1H, m, H-9), 3.53 (3H, s, –OCH_3), 4.41 (2H, m, H-3, 1), 4.52 and 4.57 (each 1H, d, J = 13.7 Hz, H-26), 4.92 and 4.97 (each 1H, s, –C=CH_2), 5.00 and 5.12 (each 1H, s, H-27), 5.42 (1H, m, H-23), 5.81 (1H, d, J = 11.0 Hz, H-7), 6.20 (1H, d, J = 11.0 Hz, H-6), 7.37 (3H, m, Ph-3, 4, 5), 7.53 (2H, m, Ph-2, 6).

7.29. (1*S*)-1-[(2*R*)-2-((1*R*,3*R*,7*E*)-1,3-Bis{[*tert*-butyl(dimethyl)silyl]oxy}-2-methylene-9,10-secoestra-5,7-dien-17-yl)propyl]-3-{[(2,2-dimethylpropanoyl)oxy]methyl}-3-butenyl (2*S*)-3,3,3-trifluoro-2-methoxy-2-phenylpropanoate (27b)

Compound **27b** was obtained from **25b** by the same procedure as described for **27a** (yield 70.6%) as a colorless oil.

27b ^{1}H NMR δ 0.02 and 0.05 and 0.06 and 0.08 (each 3H, s, SiMe), 0.51 (3H, s, H-18), 0.86 and 0.89 (each 9H, s, *t*-Bu), 1.22 (9H, s, –COC(CH_3)$_3$), 2.82 (1H, m, H-9), 3.52 (3H, s, –OCH_3), 4.43 (2H, m, H-3, 1), 4.52 and 4.56 (each 1H, d, J = 13.7 Hz, H-26), 4.92 and 4.97 (each 1H, s, –C=CH_2), 5.01 and 5.14 (each 1H, s, H-27), 5.32 (1H, m, H-23), 5.82 (1H, d, J = 11.1 Hz, H-7), 6.21 (1H, d, J = 11.1 Hz, H-6), 7.39 (3H, m, Ph-3, 4, 5), 7.53 (2H, m, Ph-2, 6).

7.30. (1*S*)-1-[(2*R*)-2-((1*R*,3*R*,7*E*)-1,3-Bis{[*tert*-butyl(dimethyl)-silyl]oxy}-2-methylene-9,10-secoestra-5,7-dien-17-yl)propyl]-3-{[(2,2-dimethylpropanoyl)oxy]methyl}-3-butenyl (2*R*)-3,3,3-trifluoro-2-methoxy-2-phenylpropanoate (28b)

Compound **28b** was obtained from **25b** by the same procedure as described for **28a** (yield 79.2%) as a colorless oil.

28b ^{1}H NMR δ 0.03 and 0.05 and 0.06 and 0.08 (each 3H, s, SiMe), 0.54 (3H, s, H-18), 0.86 and 0.90 (each 9H, s, *t*-Bu), 1.21 (9H, s, –COC(CH_3)$_3$), 2.82 (1H, m, H-9), 3.53 (3H, s, –OCH_3), 4.42 (2H, m, H-3, 1), 4.43 (2H, s, H-26), 4.84 (1H, s, H-27), 4.92 (1H, s, –C=CH_2), 4.97 (2H, s, H-27, –C=CH_2), 5.33 (1H, m, H-23), 5.83 (1H, d, J = 11.0 Hz, H-7), 6.21 (1H, d, J = 11.0 Hz, H-6), 7.38 (3 H, m, Ph-3, 4, 5), 7.52 (2H, m, Ph-2, 6).

7.31. (1*R*)-1-[(2*S*)-2-((1*R*,3*R*,7*E*)-1,3-Bis{[*tert*-butyl(dimethyl)silyl]oxy}-2-methylene-9,10-secoestra-5,7-dien-17-yl)propyl]-3-{[(2,2-dimethylpropanoyl)oxy]methyl}-3-butenyl (2*S*)-3,3,3-trifluoro-2-methoxy-2-phenylpropanoate (29a)

Compound **29a** was obtained from **26a** by the same procedure as described for **27a** (yield quant.) as a colorless oil.

29a ^{1}H NMR δ 0.03 and 0.05 and 0.06 and 0.08 (each 3H, s, SiMe), 0.53 (3H, s, H-18), 0.86 and 0.90 (each 9H, s, *t*-Bu), 1.21 (9H, s, –COC(CH_3)$_3$), 2.83 (1H, m, H-9), 3.54 (3H, s, –OCH_3), 4.42 (4 H, m, H-26, 3, 1), 4.85 (1H, s, H-27), 4.93 (1H, s, –C=CH_2), 4.98 (2H, s, H-27, –C=CH_2), 5.34 (1H, m, H-23), 5.84 (1H, d, J = 11.0 Hz, H-7), 6.21 (1H, d, J = 11.0 Hz, H-6), 7.38 (3H, m, Ph-3, 4, 5), 7.53 (2H, m, Ph-2, 6).

7.32. (1*R*)-1-[(2*S*)-2-((1*R*,3*R*,7*R*)-1,3-Bis{[*tert*-butyl(dimethyl)silyl]oxy}-2-methylene-9,10-secoestra-5,7-dien-17-yl)propyl]-3-{[(2,2-dimethylpropanoyl)oxy]methyl}-3-butenyl (2*R*)-3,3,3-trifluoro-2-methoxy-2-phenylpropanoate (30a)

Compound **30a** was obtained from **26a** by the same procedure as described for **28a** (yield 41.9%) as a colorless oil.

30a ^{1}H NMR δ 0.02 and 0.05 and 0.06 and 0.07 (each 3H, s, SiMe), 0.49 (3H, s, H-18), 0.86 and 0.89 (each 9H, s, *t*-Bu), 1.21 (9H, s, –COC(CH_3)$_3$), 2.81 (1H, m, H-9), 3.52 (3H, s, –OCH_3), 4.42 (2H, m, H-3, 1), 4.52 (2H, s, H-26), 4.92 and 4.97 (each 1H, s, –C=CH_2), 5.01 and 5.13 (each 1H, s, H-27), 5.34 (1H, m, H-23), 5.82 (1H, d, J = 11.0 Hz, H-7), 6.20 (1H, d, J = 11.0 Hz, H-6), 7.38 (3 H, m, Ph-3, 4, 5), 7.52 (2H, m, Ph-2, 6).

7.33. (1*S*)-1-[(2*S*)-2-((1*R*,3*R*,7*E*)-1,3-Bis{[*tert*-butyl(dimethyl)silyl]oxy}-2-methylene-9,10-secoestra-5,7-dien-17-yl)propyl]-3-{[(2,2-dimethylpropanoyl)oxy]methyl}-3-butenyl (2*S*)-3,3,3-trifluoro-2-methoxy-2-phenylpropanoate (29b)

Compound **29b** was obtained from **26b** by the same procedure as described for **27a** (yield 99.8%) as a colorless oil.

29b ^{1}H NMR δ 0.04 and 0.05 and 0.07 and 0.08 (each 3H, s, SiMe), 0.29 (3H, s, H-18), 0.88 and 0.89 (each 9H, s, *t*-Bu), 1.23 (9H, s, –COC(CH_3)$_3$), 2.79 (1H, m, H-9), 3.54 (3H, s, –OCH_3), 4.42 (2 H, m, H-3, 1), 4.53 and 4.58 (each 1H, d, J = 13.6 Hz, H-26), 4.93 and 4.97 (each 1H, s, –C=CH_2), 5.00 and 5.12 (each 1H, s, H-27), 5.40 (1H, m, H-23), 5.80 (1H, d, J = 11.2 Hz, H-7), 6.19 (1H, d, J = 11.2 Hz, H-6), 7.36 (3H, m, Ph-3, 4, 5), 7.53 (2H, m, Ph-2, 6).

7.34. (1*S*)-1-[(2*S*)-2-((1*R*,3*R*,7*E*)-1,3-Bis{[*tert*-butyl(dimethyl)silyl]oxy}-2-methylene-9,10-secoestra-5,7-dien-17-yl) propyl]-3-{[(2,2-dimethylpropanoyl)oxy]methyl}-3-butenyl (2*R*)-3,3,3-trifluoro-2-methoxy-2-phenylpropanoate (30b)

Compound **30b** was obtained from **26b** by the same procedure as described for **28a** (yield 46.5%) as a colorless oil.

30b ^{1}H NMR δ 0.03 and 0.05 and 0.07 and 0.08 (each 3H, s, SiMe), 0.44 (3H, s, H-18), 0.87 and 0.90 (each 9H, s, *t*-Bu), 1.23 (9H, s, –COC(CH_3)$_3$), 2.81 (1H, m, H-9), 3.48 (3H, s, –OCH_3), 4.42 (2H, m, H-3, 1), 4.49 and 4.54 (each 1H, d, J = 13.5 Hz, H-26), 4.93 (2H, s, H-27, –C=CH_2), 4.97 (1H, s, –C=CH_2), 5.06 (1H, s, H-27), 5.37 (1H, m, H-23), 5.83 (1H, d, J = 11.1 Hz, H-7), 6.20 (1H, d, J = 11.1 Hz, H-6), 7.39 (3H, m, Ph-3, 4, 5), 7.53 (2H, m, Ph-2, 6).

7.35. (5*R*)-5-[(2*R*)-2-((1*R*,3*R*,7*E*)-1,3-Bis{[*tert*-butyl(dimethyl)silyl]oxy}-2-methylene-9,10-secoestra-5,7-dien-17-yl)propyl]-3-methylenedihydro-2(3*H*)-furanone (21a)

To a solution of diol **23a** (11.4 mg, 0.017 mmol) in dry CH_2Cl_2 (2 mL) was added MnO_2 (77.7 mg, 0.89 mmol), and the mixture was stirred at room temperature for 76 h. The mixture was chromatographed on silica gel (5 g, hexane/AcOEt = 4:1) to give methylene lactone **21a** (9.4 mg, 83.1%).

21a ^{1}H NMR δ 0.02 and 0.05 and 0.07 and 0.08 (each 3H, s, SiMe), 0.57 (3H, s, H-18), 0.86 and 0.90 (each 9H, s, *t*-Bu), 1.02 (3H, d, J = 6.5 Hz, H-21), 4.43 (2H, m, H-3, 1), 4.65 (1H, m, H-23), 4.91 and 4.97 (each 1H, s, –C=CH_2), 5.62 (1H, t, J = 2.4 Hz, H-27), 5.84 (1H, d, J = 11.2 Hz, H-7), 6.22 (1H, d, J = 11.2 Hz, H-6), 6.23 (1H, t, J = 2.7 Hz, H-27).

7.36. (5*S*)-5-[(2*S*)-2-((1*R*,3*R*,7*E*)-1,3-Bis{[*tert*-butyl(dimethyl)silyl]oxy}-2-methylene-9,10-secoestra-5,7-dien-17-yl) propyl]-3-methylenedihydro-2(3*H*)-furanone (22b)

Compound **22b** was obtained from **24b** by the same procedure as described for **21a** (yield 86.9%).

22b ^{1}H NMR δ 0.02 and 0.05 and 0.07 and 0.08 (each 3H, s, SiMe), 0.56 (3H, s, H-18), 0.86 and 0.90 (each 9H, s, *t*-Bu), 0.95 (3H, d, J = 6.5 Hz, H-21), 4.42 (2H, m, H-3, 1), 4.64 (1H, m, H-23), 4.92 and 4.97 (each 1H, s, –C=CH_2), 5.62 (1H, t, J = 2.4 Hz, H-27), 5.84 (1H, d, J = 11.2 Hz, H-7), 6.21 (1H, d, J = 11.2 Hz, H-6), 6.23 (1H, t, J = 2.8 Hz, H-27). MS *m*/*z* (%): 654 (M^+, 3), 522 (10), 454 (12), 440 (12), 366 (15), 313 (40), 147 (20), 75 (100), 73 (70).

7.37. (5*R*)-5-{(2*R*)-2-[(1*R*,3*R*,7E)-1,3-Dihydroxy-2-methylene-9,10-secoestra-5,7-dien-17-yl]propyl}-3-methylenedihydro-2(3*H*)-furanone (LAC67a)

LAC67a was obtained from **21a** by the same procedure as described for **LAC67** (yield 64.9%). The compound was identified as **LAC67a** by HPLC analysis; Lichrosorb Si 60, 5 μm, Hexane/AcOEt = 7:3, flow rate 2 mL/min, retention time 34.2 min.

7.38. (5*S*)-5-{(2*S*)-2-[(1*R*,3*R*,7*E*)-1,3-Dihydroxy-2-methylene-9,10-secoestra-5,7-dien-17-yl]propyl}-3-methylenedihydro-2(3*H*)-furanone (LAC82 b)

LAC82b was obtained from **22a** by the same procedure as described for **LAC67** (yield 51.1%). The compound was identified as **LAC82b** by HPLC analysis; Lichrospher Si 60, 5 μm, hexane/AcOEt = 1:1, flow rate 2 mL/min, retention time 8.9 min.

7.38.1. Competitive-binding assay, rat VDR. The rat VDR–LBD (amino acids 115–423) was expressed as an amino-terminal His-tagged protein in *Escherichia coli* BL21 (DE3) pLys S (Novagen).[37] The cells were lysed by sonication and the supernatants were diluted 1000 times in 50 mM Tris buffer (100 mM KCl, 5 mM DTT, 0.5% Chaps, pH 7.5) containing bovine serum albumin (100 μg/mL). This solution of crude rVDR–LBD was pipetted into glass culture tubes. A solution containing an increasing amount of 1α,25-$(OH)_2D_3$ or the synthetic analogs in 15 μL of EtOH was added to the receptor solution in each tube and the mixture was vortexed 2–3 times. The mixture was incubated for 1 h at room temperature. [^{3}H]-1,25-$(OH)_2D_3$ (specific activity, 6.62 TBq/mmol, ca. 5000 dpm) in 15 μL of EtOH was added, vortexed 2–3 times, and the whole mixture was then allowed to stand at 4 °C for 18 h. At the end of the second incubation, 200 μL of dextran-coated charcoal suspension (purchased from Yamasa Shoyu) was added to bind any free ligands (or to remove free ligands) and the sample was vortexed. After 30 min at 4 °C, bound and free [^{3}H]-1,25-$(OH)_2D_3$ were separated by centrifugation at 3000 rpm for 15 min at 4 °C. Aliquots (500 μL) of the supernatant were mixed with 9.5 mL of ACS-II scintillation fluid (Amersham, Buckinghamshire, U.K.) and submitted for radioactivity counting. Each assay was performed at least twice in duplicate.

7.38.2. Transfection and transactivation assay. Cos7 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 5% fetal bovine serum (FBS). Cells were seeded on 24-well plates at a density of 2×10^4 per well. After 24 h, the cells were transfected with a reporter plasmid containing three copies of the mouse osteopontin VDRE (5′-GGTTCAcgaGGTTCA, SPPx3-TK-Luc), a wild-type hVDR expression plasmid (pCMX-hVDR), and the internal control plasmid containing sea pansy luciferase expression constructs (pRL-CMV) by the lipofection method as described previously.[18,23,24] After 4 h incubation, the medium was replaced with fresh DMEM containing 5% charcoal-treated FCS (HyClone, UT, USA). The next day, the cells were treated with either the ligand or ethanol vehicle and cultured for 24 h. Cells in each well were harvested with a cell lysis buffer, and the luciferase activity was measured with a luciferase assay kit (Toyo Ink, Inc., Japan). Transactivation measured by the luciferase activity was normalized with the internal control. All experiments were done in triplicate.

7.38.3. Cell culture and cotransfection assay. Human embryonic kidney (HEK) 293 cells were cultured in

DMEM containing 5% FBS, 100 U/mL penicillin, and 100 μg/mL streptomycin at 37 °C in a humidified atmosphere containing 5% CO_2. Transfections in HEK293 cells were performed by the calcium phosphate coprecipitation assay as described previously.[31,38] Eight hours after transfection, compounds were added. Cells were harvested after 16–24 h and were assayed for luciferase and β-galactosidase activities using a luminometer and a microplate reader (Molecular Devices, Sunnyvale, CA). Luciferase data were normalized to the internal β-galactosidase control and represent the means ± SD of triplicate assays.

7.38.4. Graphical manipulations and ligand docking. Graphical manipulations were performed using SYBYL 7.3 (Tripos, St. Louis). The atomic coordinates of the human VDR–LBD (residues 118–427 Δ166–216) crystal structure were retrieved from Protein Data Bank (code: 2α-methyl-1,25-$(OH)_2D_3$,[29] 2HB8; 20-*epi*-1,25-$(OH)_2D_3$,[26] 1IE9). **LAC67a** and **LAC67b** were docked into the ligand-binding pocket of the VDR–LBD[29] manually by superposition with the 2α-methyl-1,25-$(OH)_2D_3$ at the A- to D-ring. **LAC67a** and **LAC67b** in the LBP of the VDR–LBD were minimized on Tripos force field with 100 times iterations.

LAC82a and **LAC82b** were docked into the ligand-binding pocket of the VDR–LBD[26] manually by superposition with the 20-*epi*-1,25-$(OH)_2D_3$ at the A- to D-ring. **LAC82a** and **LAC82b** in the LBP of the VDR–LBD were minimized on Tripos force field with 100 times iterations.

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