J. Med. Chem. **2000**, *43*, 4247–4265

Synthesis, Biological Evaluation, and Conformational Analysis of A-Ring Diastereomers of 2-Methyl-1,25-dihydroxyvitamin D_3 and Their 20-Epimers: Unique Activity Profiles Depending on the Stereochemistry of the A-Ring and at C-20

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Received June 19, 2000

All eight possible A-ring diastereomers of 2-methyl-1,25-dihydroxyvitamin D_3 (**2**) and 2-methyl-20-*epi*-1,25-dihydroxyvitamin D_3 (**3**) were convergently synthesized. The A-ring enyne synthons **19** were synthesized starting with methyl (*S*)-(+)- or (*R*)-(−)-3-hydroxy-2-methylpropionate (**8**). This was converted to the alcohol **14** as a 1:1 epimeric mixture in several steps. After having been separated by column chromatography, each isomer led to the requisite A-ring enyne synthons **19** again as 1:1 mixtures at C-1. Coupling of the resulting A-ring enynes **20a**–**h** with the CD-ring portions **5a**,**b** in the presence of a Pd catalyst afforded the 2-methyl analogues **2a**–**h** and **3a**–**h** in good yield. In this way, all possible A-ring diastereomers were synthesized. The synthesized analogues were biologically evaluated both in vitro and in vivo. The potency was highly dependent on the stereochemistry of each isomer. In particular, the $\alpha\alpha\beta$-isomer **2g** exhibited 4-fold higher potency than 1α,25-dihydroxyvitamin D_3 (**1**) both in bovine thymus VDR binding and in elevation of rat serum calcium concentration and was twice as potent as the parent compound in HL-60 cell differentiation. Furthermore, its 20-epimer, that is, 20-*epi*-$\alpha\alpha\beta$ **3g**, exhibited exceptionally high activities: 12-fold higher in VDR binding affinity, 7-fold higher in calcium mobilization, and 590-fold higher in HL-60 cell differentiation, as compared to 1α,25-dihydroxyvitamin D_3 (**1**). Accordingly, the double modification of 2-methyl substitution and 20-epimerization resulted in unique activity profiles. Conformational analysis of the A-ring by ^{1}H NMR and an X-ray crystallographic analysis of the $\alpha\alpha\beta$-isomer **2g** are also described.

Introduction

1α,25-Dihydroxyvitamin D_3 (**1**) is a hormonally active metabolite of vitamin D_3 and functions as a regulator of calcium and phosphorus homeostasis.[1] The discovery by Suda et al. that **1** exhibits a variety of biological activities such as cell differentiation and proliferation, besides the classical functions, stimulated renewed interest in both the chemistry and biology of **1**.[2] Over the past two decades, extensive studies have focused on the physiological roles of **1**, and a large number of analogues have been synthesized and biologically evaluated to investigate the structure–activity relationships (SARs) and to develop potential therapeutic agents.[3]

Many of the analogues synthesized so far are altered in the side chain, providing many useful analogues with high potency or selective activity.[4] For example, 24,24-difluoro-1α,25-dihydroxyvitamin D_3 is the first analogue that exhibits higher potency than **1**.[5] 22-Oxa-1α,25-dihydroxyvitamin D_3 (OCT),[6] 26,27-dihomo-1α,25-dihydroxyvitamin D_3,[7] and 20-*epi*-1α,25-dihydroxyvitamin D_3[8] show more potent activity in cell differentiation than in calcemic effects. Notably, the combination of these three side chain modifications together with 24-homologation led to a highly potent analogue, 20-*epi*-22-oxa-1α,25-dihydroxy-24,26,27-trihomovitamin D_3 (KH-1060).[8] In recent years, modification of the A-ring has attracted much attention because it can afford useful analogues exhibiting unique activity profiles as well: for example, 2β-(3-hydroxypropoxy)-1α,25-dihydroxyvitamin D_3 (ED-71),[9] 1α,25-dihydroxy-19-norvitamin D_3,[10] and 1-hydroxymethyl-25-hydroxyvitamin D_3.[11] Modification of the CD-ring has also been investigated, giving a highly active analogue.[12] It is noteworthy that nonsteroidal vitamin D mimics, i.e., compounds showing vitamin D

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10.1021/jm000261j CCC: $19.00 © 2000 American Chemical Society
Published on Web 10/03/2000

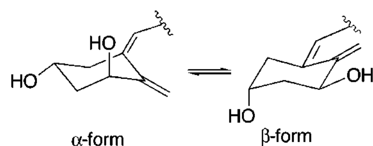
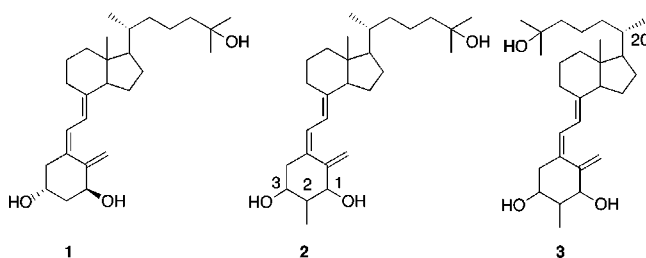


Figure 1. Conformational equilibrium of 1α,25-dihydroxyvitamin D_3.



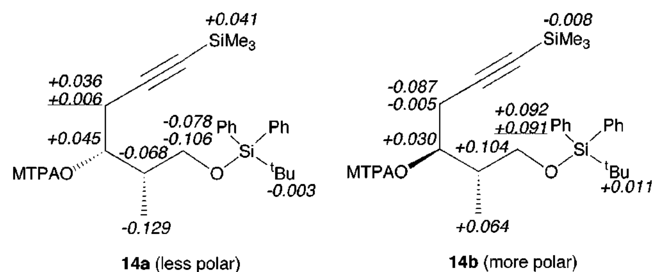


Figure 2. Determination of absolute stereochemistry at the C-3 position.

activity without having *seco*-steroidal vitamin D structure, have been found quite recently.[13]

1α,25-Dihydroxyvitamin D_3 (**1**) exerts its functions through interaction with its specific receptor, the vitamin D receptor (VDR),[14] belonging to the nuclear receptor superfamily.[15] The binding of ligand **1** to VDR triggers the whole sequence of biological responses: conformational change, heterodimerization with the retinoid X receptor (RXR), binding to DNA, and transcriptional activities. Therefore, in recent years, this specific interaction has been the focus of attention with respect to structure−function relationships.[16] In particular, interaction of the side chain with VDR is well-documented,[17] and Yamada et al. showed that potential side chain structures can be predicted by conformational analysis using molecular mechanics.[18] Furthermore, differential interaction of compounds having the natural side chain and their 20-epimers has been demonstrated.[19] In contrast, however, little is known about the conformation−activity relationships of the A-ring. Okamura et al. reported that the A-ring of **1** equilibrates between two chair conformers, the α- and β-form (Figure 1), a in 1:1 ratio as determined by ^{1}H NMR analysis,[20] and they proposed that the β-form, in which the 1α-hydroxyl occupies the equatorial position, may be responsible for biological activity.[21] This conformational equilibrium of the A-ring was confirmed by ^{13}C NMR analysis.[22] Recent studies on the 1-alkyl-1,25-dihydroxyvitamin D_3 analogues by Yamada et al. seemed to support this proposal,[23] but DeLuca et al. concluded that, on the contrary, the axial orientation of the 1α-hydroxy group is essential for biological activity from studies with 1α,25-dihydroxy-19-norvitamin D_3 analogues.[24] Quite recently, Norman et al. proposed a three-dimensional model of the ligand binding domain of the human VDR based on the X-ray crystallographic atomic coordinates of that of the rat α1-thyroid receptor and suggested that the optimal shape of a ligand is one where the plane of the A-ring in relation to the CD-ring is at some intermediate angle (0−90°).[25]

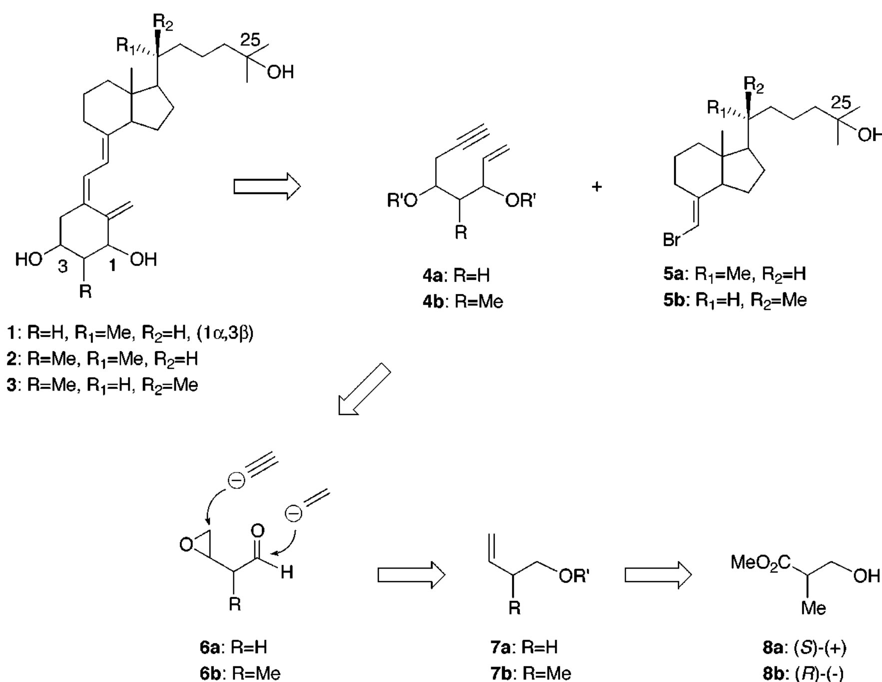
In view of the results of A-ring modification, as well as the conformation−activity relationships in the A-ring as stated above, we have synthesized all eight possible diastereomers of 2-methyl-1,25-dihydroxyvitamin D_3 (**2**), demonstrating that the potency of the analogues varies depending on the configuration not only of the C-1 and C-3 hydroxy groups but also of the 2-methyl group. Consequently, 2α-methyl-1α,25-dihydroxyvitamin D_3 (ααβ-isomer) showed higher potency than **1**.[26a] This remarkable effect of the 2-methyl substitution and the results of the 20-epimerization previously reported prompted us to further synthesize all possible diastereomers of 2-methyl-20-*epi*-1,25-dihydroxyvitamin D_3 (**3**), modified both in the A-ring and in the side chain. Biological evaluation of these double-modified analogues gave rise to an exceptionally highly active analogue.[26b] Herein, we report in detail the synthesis of all eight possible diastereomers of 2-methyl-1,25-dihydroxyvitamin D_3 (**2**) and its 20-epimers (**3**). The results of biological evaluation and conformational analysis, including an X-ray crystallographic analysis, of these analogues are also described.

Results and Discussion

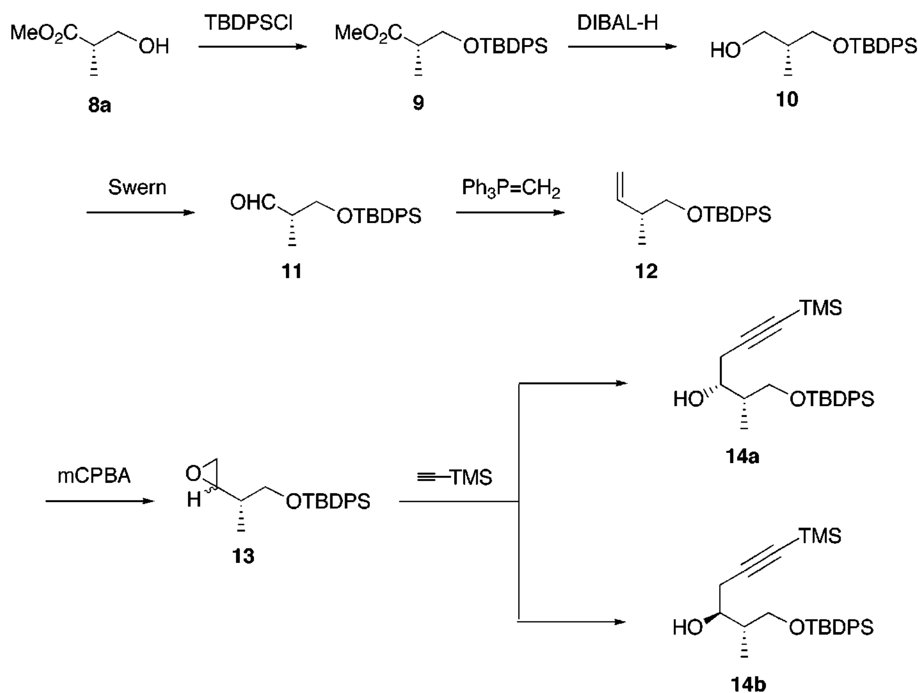
Chemistry. For the synthesis of analogues modified both in the A-ring and in the side chain, the convergent method is undoubtedly advantageous over the classical steroidal approach, because it can be more effective and flexible. Separate preparation of the requisite two units, followed by coupling, would produce a wide variety of analogues. Several convergent methods have been reported since 1981,[27] and of these, the procedure reported by Trost et al. using palladium-catalyzed coupling of the A-ring enyne synthon **4** with the CD-ring portion (**5**) seems to be the most useful (Scheme 1).[28] In this method, cyclization of the A-ring and coupling with the CD-ring proceeds in a single step, facilitating the synthesis of the A-ring moiety and its modification.

Preparations of both the 20-natural- and 20-*epi*-CD-ring portions, **5a**[28] and **5b**,[26b,c] respectively, have been reported. The A-ring enyne synthon **4a** can be synthesized in several different ways, but they do not seem to be applicable to analogue synthesis.[29] Therefore, we have developed a practical method for the synthesis of **4a**, which is versatile and applicable to a wide variety of A-ring analogues including diastereomers. It is based on introduction of an acetylene unit and a vinyl group into the epoxy-aldehyde (or its equivalent) precursor **6a** derived from 3-buten-1-ol (**7a**). The epimeric mixture obtained at the introduction of each unit is separable by chromatography, and thus all the A-ring diastereomers are available. Indeed, we have synthesized all the A-ring diastereomers of **1** and its 20-epimers.[26c] Utilizing this method, the 2-methyl-substituted A-ring enyne **4b** may be synthesized starting from methyl 3-hydroxy-2-methylpropionate (**8**) through the corresponding 3-buten-1-ol derivative **7b** and the epoxy-aldehyde precursor **6b**. Preparation of **7b** from **8** should be straightforward. Since both enantiomers of **8** are com-

Scheme 1



Scheme 2

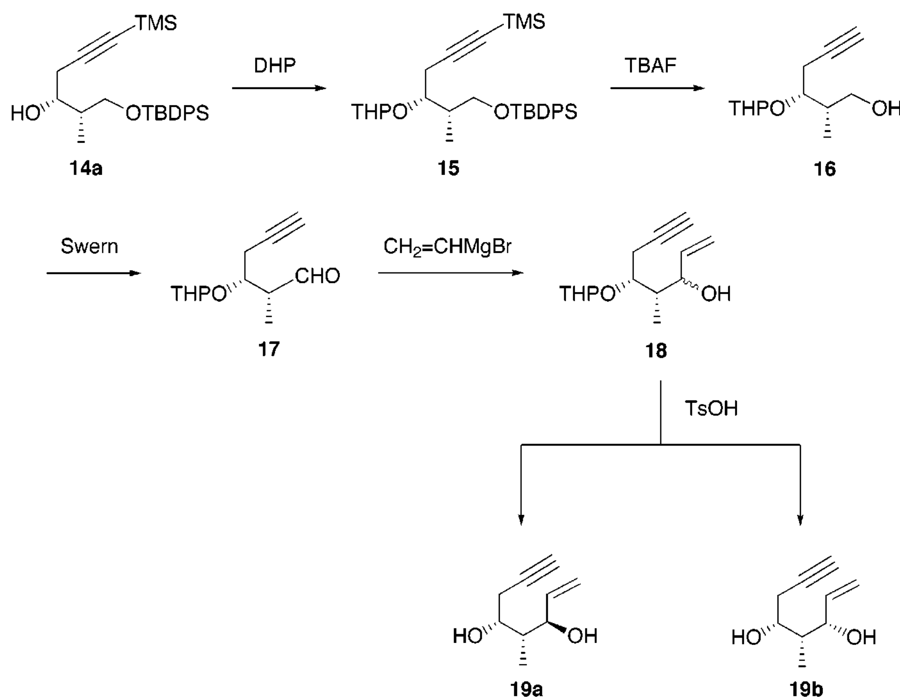


mercially available, all the enantiomers would be easily accessible in the same way.

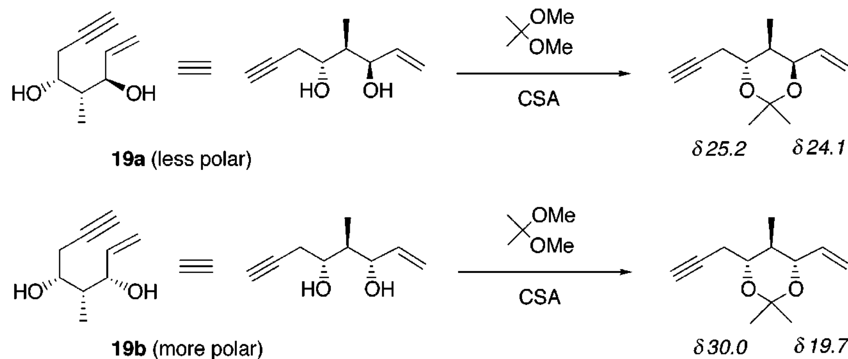
Conversion of methyl (*S*)-(+)-3-hydroxy-2-methylpropionate (**8a**) (Scheme 2) to the requisite 3-buten-1-ol derivative **12** was accomplished in excellent yield in a conventional manner: protection of the primary alcohol by TBDPSCl, then DIBAL-H reduction of the ester **9** to give the alcohol **10**, followed by Swern oxidation, and finally the Wittig reaction of the resultant aldehyde **11**. Racemization of the aldehyde **11** occurred to the extent of 5−10%, and the optical purity of all intermediates after this stage is 80−90%. Treatment of the olefin **12** thus obtained with mCPBA afforded the epoxide **13** as a 1:1 mixture in 97% yield. The acetylene unit was introduced by the reaction of **13** with ethynyltrimethylsilane/BuLi−BF_3 etherate, giving a 1:1 mixture of the alcohols **14a** (less polar) and **14b** (more polar) in 85% yield. These isomers were readily separable by silica gel column chromatography, and the absolute configuration at C-3 (the vitamin D numbering) of each isomer was determined by the ^{1}H NMR analysis of its MTPA esters.[30] The results are shown in Figure 2. Thus, the less polar isomer **14a** has the (3*R*)-configuration (3*β*-isomer), whereas the more polar isomer **14b** has the (3*S*)-configuration (3α-isomer). The optical purity of each isomer was also determined by this analysis.

Protection of the 3-OH in the 3*β*-isomer **14a** (Scheme 3) with THP to give **15**, followed by treatment with

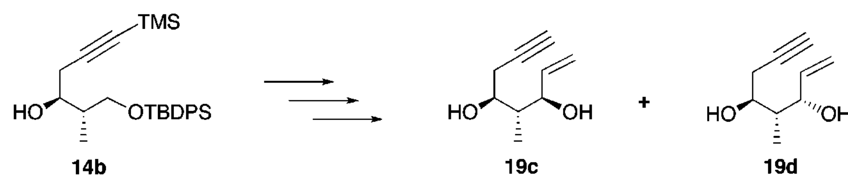
Scheme 3



Scheme 4



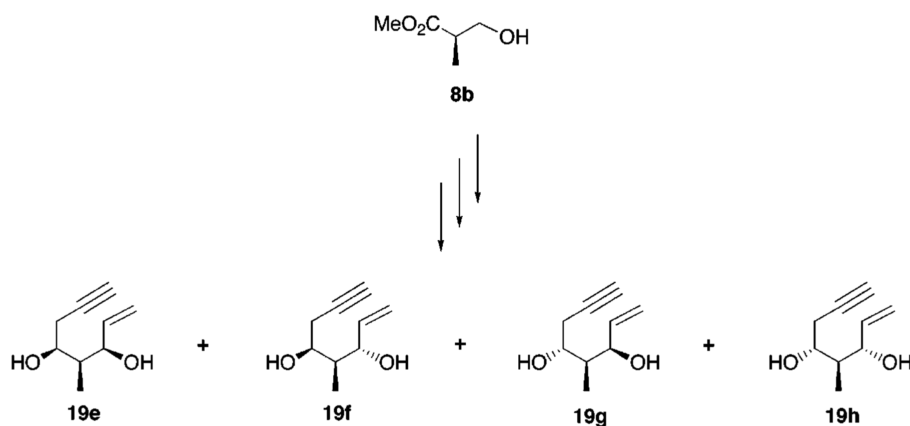
Scheme 5



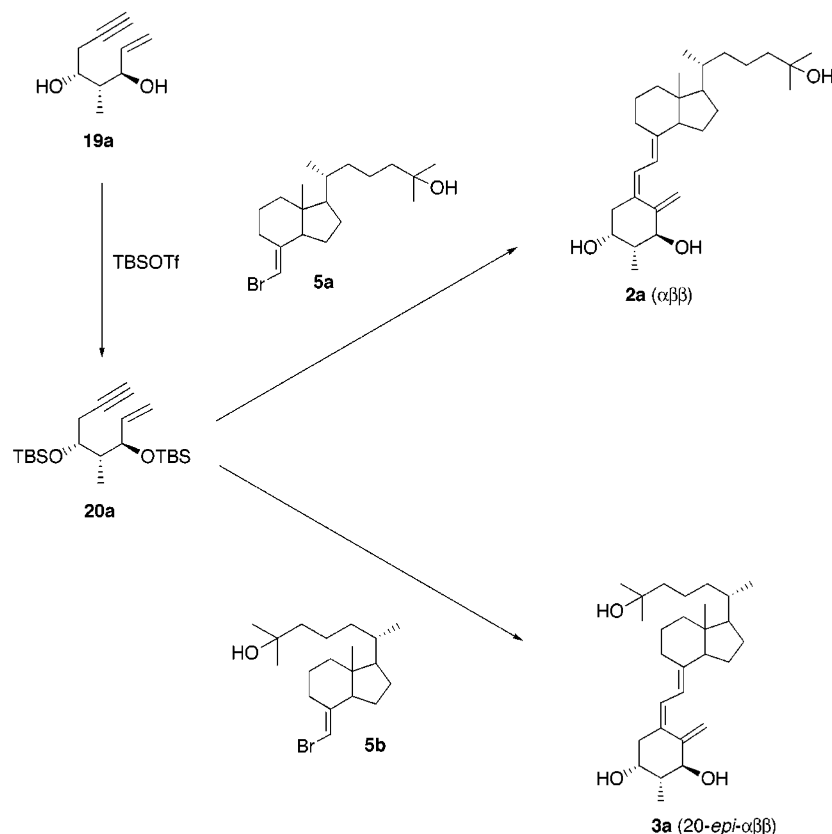
TBAF, furnished the primary alcohol **16**, which was then oxidized by the Swern procedure to give the aldehyde **17** in excellent yield. When another protective group (MEM, MOM) was used instead of THP, epimerization occurred at C-2 of the aldehyde **17**, i.e., only the THP protective group suppressed the epimerization. The reaction of **17** with vinylmagnesium bromide in the presence of $CeCl_3$ afforded a 1:1 mixture of the diasteromeric allyl alcohols **18** in 92% yield. After removal of the THP protecting group, the resulting mixture of the requisite A-ring enyne synthons **19a**,**b** was readily separated by silica gel column chromatography. The relative stereochemistry of the 1,3-diol in each isomer was determined by ^{13}C NMR analysis of its acetonides.[31] The results are shown in Scheme 4. The less polar isomer **19a** was shown to be 1,3-*anti*, in other words, the $\alpha\beta\beta$-isomer (the Greek letters denote the configurations of C-1, -2, and -3, respectively, in the vitamin D numbering), and the more polar isomer **19b** was 1,3-*syn*, the $\beta\beta\beta$-isomer. In the same way, the other 2β-Me isomers **19c**,**d** were obtained from the 3α-isomer **14b** (Scheme 5). Furthermore, starting from the enantiomeric **8b** and following exactly the same sequence of reactions from **8a** to **19a**−**d**, the corresponding enantiomers **19e**−**h** were synthesized (Scheme 6). Thus, we have accomplished the synthesis of all eight possible diastereomers of 2-Me-substituted A-ring enyne synthons.

Coupling of the above A-ring enynes with the CD-ring portions proceeded smoothly, as described in the literature.[28] Scheme 7 shows the results for the case of the $\alpha\beta\beta$-isomer **19a**. The diol **19a** was protected by TBS to afford **20a**, which was coupled with the natural-CD-ring portion **5a** by using palladium catalysis, followed by

Scheme 6



Scheme 7



deprotection with camphorsulfonic acid (CSA) in MeOH, and the product was finally purified by recycling HPLC to give the 2β-Me analogue **2a** ($\alpha\beta\beta$-isomer) in good yield. On the other hand, coupling of **20a** with the 20-*epi*-CD-ring portion **5b** afforded the corresponding 20-*epi* analogue **3a** ($\alpha\beta\beta$-isomer). In the same manner, the rest of the analogues (**2b**–**h** and **3b**–**h**) were synthesized. Thus, we have synthesized a total of sixteen 2-Me-substitued analogues.

Posner and co-workers found that, in diastereomeric pairs differing only by inversions of stereochemistry at positions 1–3, but not in the CD-ring or in the side chain, the ^{1}H NMR signals of C-18 and C-19 showed a characteristic pattern of chemical shifts that is related to the sign of optical rotation of the compound. By means of these criteria, they tentatively assigned the stereochemistry of the diastereomers compared with that of closely related vitamin D analogues.[32] The 2-Me analogues **2a**–**h**, however, showed a similar characteristic ^{1}H NMR pattern and sign of optical rotation, but they are not correlated to the stereochemistry of the diastereomers (Table 1). The stereochemistry of these analogues was unambiguously assigned by means of well-established procedures as indicated above.

The analogue **2g** ($\alpha\alpha\beta$-isomer) gave a crystal suitable for X-ray crystallographic analysis. The ORTEP drawing and the molecular packing along the *c* axis are shown in Figures 3 and 4, respectively. These results confirmed the A-ring stereochemistry determined by spectroscopic methods as described above. Both the molecular structure and the conformation are quite similar to those of 1α,25-dihydroxyvitamin D$_3$ (**1**).[33] The A-ring exists exclusively in the β-form, in which the C-1 and C-3 hydroxyl groups occupy equatorial and axial positions, respectively. Stabilization of this conformer in the solid state may be due to the formation of a hydrogen bond

Table 1. Characteristic Physical Constants (selected ^{1}H NMR data and optical rotation values) of the Synthesized Analogues **2a**−**h**

compd	stereochemistry C-1	C-2	C-3	$[\alpha]_D$	^{1}H NMR (δ) C-18	C-19
2f	β	α	α	+27.3	0.54	5.35, 5.01
2a	α	β	β	−35.2	0.55	5.37, 5.02
2e	α	α	α	+87.0	0.54	5.23, 4.98
2b	β	β	β	−44.8	0.56	5.25, 5.01
2c	α	β	α	+58.0	0.54	5.31, 5.05
2h	β	α	β	−47.7	0.55	5.30, 5.07
2g	α	α	β	+41.1	0.53	5.28, 5.01
2d	β	β	α	−11.2	0.55	5.28, 5.02

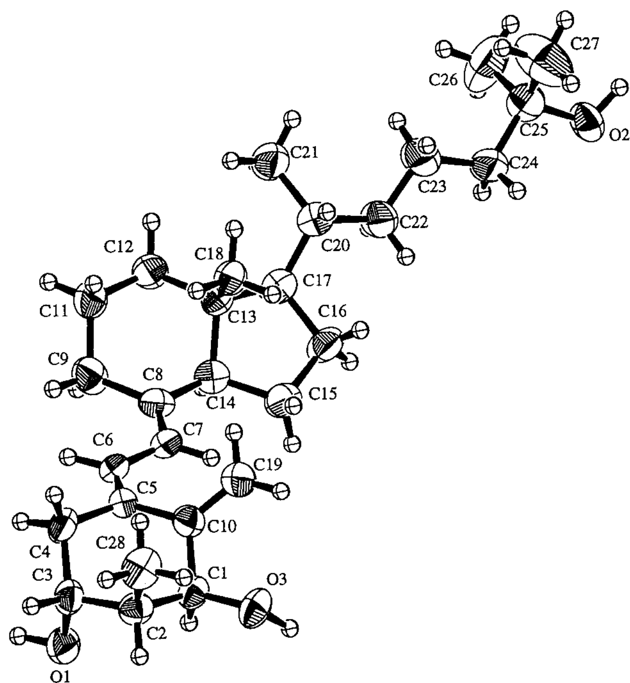


Figure 3. ORTEP drawing of the crystal structure of 2α-methyl-1α,25-dihydroxyvitamin D_3 (**2g**).

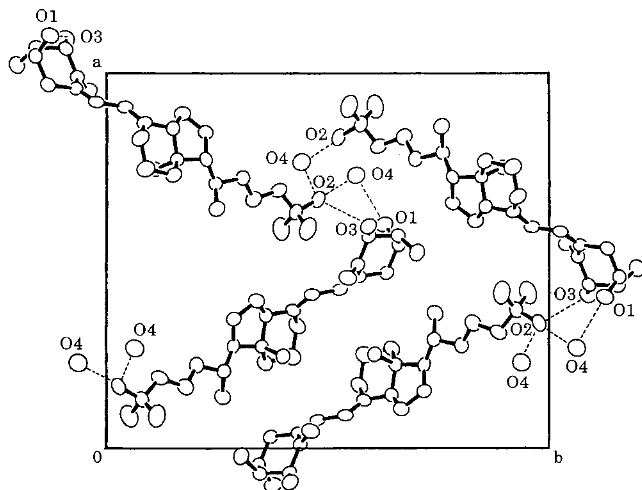


Figure 4. Molecular packing in the unit cell of the crystal of 2α-methyl-1α,25-dihydroxyvitamin D_3 (**2g**).

network involving the C-1, C-3, and C-25 hydroxyl groups and water molecules included in the crystal.

To gain information on the A-ring conformation−activity relationships, the solution conformations of the A-ring of the synthesized analogues were examined by means of ^{1}H NMR analyses together with molecular mechanics calculation. First, the conformations of **2a**,**g**, both of which have natural OH configurations, were analyzed by ^{1}H NMR (Figure 5). As a solvent, $CDCl_3$ was used because virtually no solvent dependency was observed among $CDCl_3$, CD_3OD, and DMSO-d_6. COSY and TOCSY experiments allowed assignment of all A-ring signals. The conformational equilibrium was deduced from the vicinal coupling constant between H(3α)−H(4β) by the method based on the data reported for cyclohexanol protons ($J_{ax,ax}$ = 11.1 Hz, $J_{eq,eq}$ = 2.7 Hz),[34] which is widely accepted for analysis of the A-ring of vitamin D compounds.[20,23] In the case of **2g**, the signals of H(4α) and H(4β) both appeared as doublets of doublets (dd), and the $J_{3\alpha-4\beta}$ value of 7.7 Hz indicated a ratio of 60:40 in favor of the α-form. This was supported by the NOESY experiment. NOEs of H(2β)−H(4β) and 2-Me−H(4α) were observed, which suggested that both chair conformers, α- and β-form, respectively, were involved. When the ^{1}H NMR spectrum of **2g** was measured at −50 °C in $CDCl_3$, broadening of the A-ring signals, in particular the 2-Me signal, was apparent, again supporting the above conclusion. In the case of **2a**, however, the signal of H(4β) was a doublet of doublets (dd), whereas that of H(4α) was a broad doublet owing to allylic coupling with H(6). The conformational equilibrium of **2a** deduced from the $J_{3\alpha-4\beta}$ value of 4.8 Hz showed a ratio of 25:75 in favor of the β-form. In this case, the NOESY experiment did not give clear results, while the allylic coupling between H(4α) and H(6) suggested a major contribution of the β-form in the conformational equilibrium of **2a**.

On the basis of the preliminary results with **2a**,**g**, the ^{1}H NMR spectra of all the synthesized analogues **2a**−**h** and **3a**−**h** were analyzed. The ^{1}H NMR spectra were taken in $CDCl_3$−D_2O to exclude the coupling of H(1) and H(3) with OH protons in these cases. Analyses by COSY and decoupling experiments allowed the assignment of all A-ring signals and the coupling constants. There was no marked difference in chemical shifts between each pair of the 20-natural and 20-*epi*-isomers, except for the chemical shifts due to the 20-methyl groups. The ^{1}H NMR spectral data of the A-ring protons of **2a**−**h** are summarized in Table 2. In addition to the above analysis using vicinal coupling constants between H(3)−H(4), those of H(1)−H(2) and H(2)−H(3) were utilized. In every case, the A-ring is in equilibrium between two chair conformations, the α- and β-forms (Figure 6), as indicated by the analysis of the coupling patterns of H(2) and H(3). In this situation, all H(3) signals should appear as doublets of triplets (dt) because the coupling constant due to H(2)−H(3) is the same as one of those from H(3)−H(4α) and H(3)−H(4β). Likewise, the H(2) signals should appear as doublets of doublets of quartets (ddq) for 1,3-*trans* compounds, and triplets of quartets (tq) for 1,3-*cis* compounds. The conformational equilibria were deduced from the vicinal coupling constants based on the data reported for cyclohexanol protons ($J_{ax,ax}$ = 11.1 Hz, $J_{eq,eq}$ = 2.7 Hz)[34] as stated above. In every case, one was favored over the other, but the extent varied depending on the stereochemistry. Consequently, in the cases of 1,3-*cis* compounds (**b**, **c**, **e**, and **h**), the form in which both hydroxyl groups occupied axial positions was predominant because of hydrogen bond formation between these groups. On the other hand, the form in

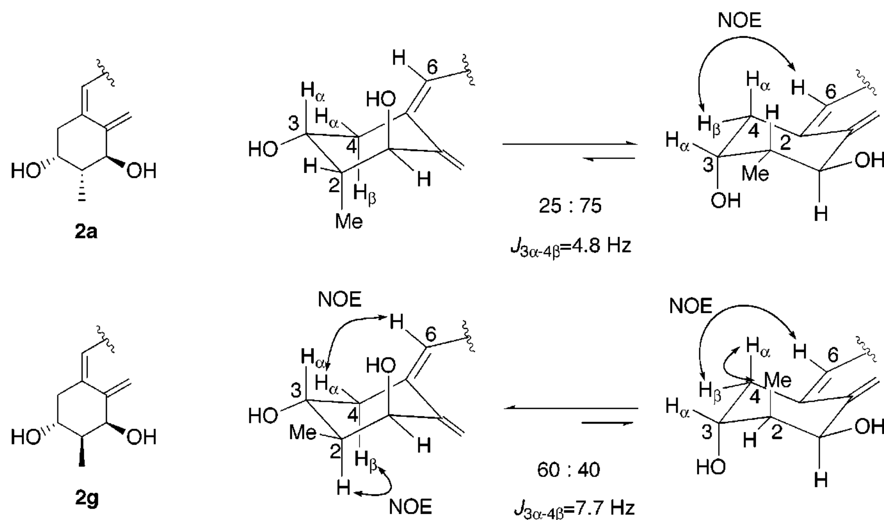


Figure 5. Conformational equilibria and observed NOEs of 2a,g.

Table 2. ¹H NMR Data of A-Ring Protons of the Synthesized Analogues **2a**–**h**[a]

compd	H(1)	H(2)	H(3)	H(4α)	H(4β)
2a	4.01	1.78	4.03	2.52	2.42
	d	ddq	dt	dd[b]	dd
	(9.2)	(9.2, 2.6, 7.0)	(4.8, 2.6)	(13.6, 2.7)	(13.6, 4.8)
2b	4.17	1.90	3.90	2.50	2.59
	d	tq	dt	dd[b]	dd
	(2.4)	(2.4, 7.0)	(3.4, 2.4)	(14.0, 2.4)	(14.0, 3.4)
2c	3.90	2.00	3.65	2.34	2.64
	d	tq	dt	dd	dd
	(5.9)	(5.9, 7.0)	(3.3, 6.0)	(13.7, 6.0)	(13.7, 3.3)
2d	4.26	1.87	3.81	2.24	2.66
	d	ddq	dt	dd	dd
	(3.3)	(8.4, 3.3, 7.0)	(4.3, 8.4)	(13.2, 8.4)	(13.2, 4.3)
2e	4.17	1.92	3.90	2.58	2.50
	d	tq	dt	dd	dd[b]
	(2.7)	(2.7, 7.0)	(4.0, 2.7)	(14.0, 4.0)	(14.0, 2.7)
2f	4.04	1.86	4.06	2.42	2.51
	d	ddq	dt	dd	dd
	(8.4)	(8.4, 2.9, 7.0)	(5.9, 2.9)	(13.6, 5.9)	(13.6, 2.9)
2g	4.30	1.92	3.84	2.67	2.23
	d	ddq	dt	dd	dd
	(3.3)	(7.7, 3.3, 7.2)	(4.0, 7.7)	(13.2, 4.0)	(13.2, 7.7)
2h	3.96	2.12	3.72	2.65	2.36
	d	tq	dt	dd	dd
	(4.4)	(5.1, 7.0)	(3.0, 5.1)	(13.9, 3.0)	(13.9, 5.1)

[a] The spectra were recorded at 400 MHz in $CDCl_3$–D_2O. [b] Each signal was observed as a broad doublet because of long-range coupling with H(6). The vicinal coupling constants between H(3) and H(4) were recorded in decoupling experiments designed to exclude the coupling with H(6).

which the 2-Me group occupied the equatorial position was favored in 1,3-*trans* compounds (**a**, **d**, **f**, and **g**) because of 1,3-diaxial interaction between 2-Me and H(4). However, the equilibrium ratio varies even within the 1,3-*cis* and the 1,3-*trans* groups: for example, the ratio in the isomer **e** is 85:15, but it is only 60:40 in the isomer **c**. This should be due to the disposition of the 2-methyl group; in the favored α-conformer, it is equatorial in **e** but axial in **c**. In these cases, calculation of energy difference and estimation of the ratio would be valuable. Therefore, molecular mechanics calculation using MM2* was carried out with model compounds lacking the side chain.[35] As expected, the two chair conformers are the most stable conformers in each isomer, and the calculated values of energy difference and equilibrium ratio at 25 °C are shown in parentheses in Figure 6. These results were in good agreement with the experimental data, which well-supported the ¹H NMR analyses. Thus, the orientations of the two hydroxyl groups and the 2-methyl group affect the equilibrium ratio, and as a result, the equilibrium is biased to various extents in a direction that depends on the A-ring stereochemistry.

Biological Evaluation. The biological activities of the synthesized analogues were evaluated in comparison with those of 1α,25-dihydroxyvitamin D_3 (**1**). The results are summarized in Tables 3 and 4. It is well-recognized that the 1α,3β-configuration is crucial for potent vitamin D activity and epimerization of each hydroxyl group dramatically decreases the activity by about 100 times for 1β and 10 times for 3α.[36] In addition to that, the configuration of the 2-methyl group was found considerably to alter the activity. Consequently, the potency was highly dependent on the stereochemistry of the A-ring substituents. Thus, the ααβ-isomer **2g** showed much higher activity than the parent compound **1** among the 20-natural analogues. Most interestingly, the combination of A-ring modification and 20-epimerization, double modification, brought about a remarkable alteration of the potency, and the 20-*epi*-ααβ-isomer **3g** exhibited exceptionally high potency.

Binding affinity to the VDR was examined using bovine thymus.[37] Among the 20-natural analogues, most of the 1α-hydroxy compounds (**2a**,**e**,**g**) exhibited significant to high VDR affinity, whereas the 1β-hydroxy analogues (**2b**,**d**,**f**,**h**) had virtually no affinity, as expected, in accordance with previous findings. Furthermore, a comparison of the 2-epimeric pairs of these 1α-compounds revealed that the 2α-methyl isomers **2e**,**g** showed much higher potency than the corresponding 2β-methyl isomers **2c**,**a**, respectively. Accordingly, the 2α-methyl group enhances the VDR affinity, whereas the 2β-methyl group decreases the affinity. Thus, the ααβ-isomer **2g** showed 4-fold higher affinity than 1α,25-dihydroxyvitamin D, while the αβα-isomer **2c** had virtually no affinity, despite having a 1α-configuration. Compared to the 20-natural counterparts, each 20-*epi*-analogue showed much higher VDR affinity, ranging from 3- to 10-fold higher. In particular, the 20-*epi*-ααβ-isomer **3g** showed 12-fold higher affinity, and the 2β-

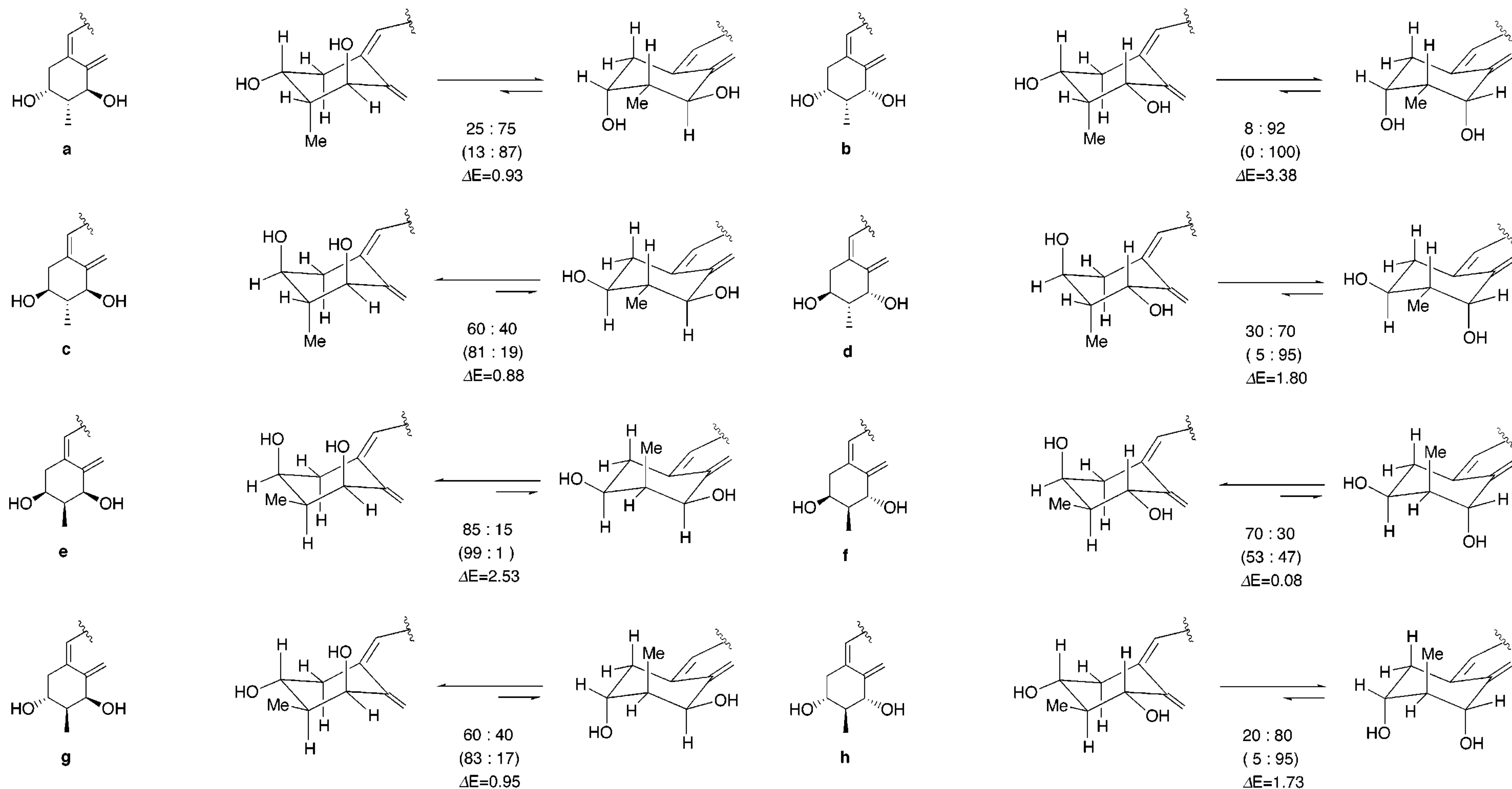


Figure 6. A-Ring conformational equilibria of the synthesized analogues. The numbers show the equilibrium ratio deduced from the ^{1}H NMR analysis. The calculated ratio at 25 °C and energy difference values (kcal/mol) are shown in parentheses.

Table 3. Relative Potency of the Synthesized Analogues with Respect to 1α,25-Dihydroxyvitamin D_3 (**1**)[a]

compd[b]	VDR[c]	Ca[d]	HL-60[e]	DBP[f]
1α,25-$(OH)_2VD_3$ (**1**)	100	100	100	100
2a (αββ)	13	2	10	79
2b (βββ)	<0.1	NT[g]	1.5	1000
2c (αβα)	0.3	NT	1.5	21
2d (ββα)	0.8	NT	3	1300
2e (ααα)	4	NT	13	45
2f (βαα)	<0.1	NT	0.5	1200
2g (ααβ)	400	400	200	68
2h (βαβ)	<0.1	NT	1	200
3a (20-*epi*-αββ)	160	115	2600	<0.3
3b (20-*epi*-βββ)	<0.1	NT	1	<0.3
3c (20-*epi*-αβα)	<0.1	NT	6	<0.3
3d (20-*epi*-ββα)	7	19	190	<0.3
3e (20-*epi*-ααα)	17	144	730	<0.3
3f (20-*epi*-βαα)	<0.1	NT	1	<0.3
3g (20-*epi*-ααβ)	1200	655	59000	<0.3
3h (20-*epi*-βαβ)	<0.1	NT	3	<0.3

[a] The potency of **1** is normalized to 100. [b] The Greek letters denote the configurations at C-1, C-2, and C-3, respectively. [c] Binding affinity to bovine thymus vitamin D receptor. [d] Increasing effect on serum calcium level in normal rats. [e] Differentiation-inducing effect on HL-60 cells. [f] Binding affinity to calf serum vitamin D binding protein. [g] NT, not tested.

methyl-20-*epi* analogue **3a** (20-*epi*-αββ) showed comparable potency to **1**. The affinity of **3g** is as strong as that of (22*R*)-22-methyl-20-*epi*-1α,25-dihydroxyvitamin D_3, which has the highest VDR binding affinity so far known.[18b] The VDR binding affinity of 20-*epi*-1α,25-dihydroxyvitamin D_3 relative to that of 1α,25-dihydroxyvitamin D_3 (normalized to 100) is reported to be 120 for chick intestinal VDR and 500 for bovine thymus VDR.[8] Therefore, the double modification of 2-methyl substitution and 20-epimerization seemed to have additive effects on VDR binding.

The rank order of potency for HL-60 cell differentiation[38] among each series of compounds **2a**−**h** and **3a**−**h** was essentially parallel to that of VDR binding. Thus, among the 20-natural analogues, **2g** was twice as potent as 1α,25-dihydroxyvitamin D_3 (**1**), **2a**,**e** showed significant activity, and the others had only weak activity. However, 20-epimerization enhanced the potency far more than expected from the change of VDR binding affinity. Most notably, the potency of the 20-*epi*-ααβ-isomer **3g** is 590 times higher than that of **1**, which is comparable in activity to KH-1060, the most potent analogue reported to date.[8] Compared to the 36-fold relative enhancement of 20-*epi*-1α,25-dihydroxyvitamin D_3 vs 1α,25-dihydroxyvitamin D_3,[26c] that of 20-*epi*-ααβ **3g** vs ααβ **2g** is much greater, approximately 295 times. Therefore, this effect should be due to the combination of 2α-methyl introduction and 20-epimerization, the double modification. Again, 2β-methyl substitution decreased the activity, as seen in the analogue **3a**, the 2-epimer of **3g**, but the compound still showed 26 times higher potency than **1**.

A similar trend to those of VDR binding and HL-60 cell differentiation was found in calcemic activity. In the elevation of serum calcium concentration using normal SD male rats,[39] the ααβ-isomer **2g** also exhibited 4-fold higher potency than **1**, whereas its 2-methyl epimer **2a** had quite low activity. Again, 20-epimerization enhanced the potency, but in this case, the extent was not as much as for HL-60 differentiation: the 20-*epi*-ααβ-isomer **3g** showed 6.5-fold higher potency than **1** and the potency of the 20-*epi*-αββ-isomer **3a** was comparable to that of **1**. Intestinal calcium transport and bone calcium mobilization were also examined by using vitamin D-deficient rats.[40] The results, shown in Table 4, were in good agreement with those for elevation of rat serum calcium concentration. Among the 20-natural analogues, only the ααβ-isomer **2g** showed activity of 4−5 times higher than that of **1**, and the other isomers had virtually no activity. In these cases, 20-epimerization had no enhancing effect on the activity; only the

Table 4. Intestinal Calcium Transport (ICT) and Bone Calcium-Mobilizing (BCM) Activities of the 2-Methyl Analogues in Vitamin D-Deficient Rats on a Low-Calcium Diet[a]

compd	amount (pmol/day/ 7 days)	ICT serosal/ mucosal ratio (mean ± SEM)	BCM serum Ca in mg/100 mL (mean ± SEM)
none (control)	0	3.8 ± 0.28	4.1 ± 0.26
1	260	5.0 ± 0.20**	5.5 ± 0.12***
2a (αββ)	260	4.3 ± 0.27	4.1 ± 0.20
	500	4.3 ± 0.16	5.0 ± 0.09‡
2b (βββ)	260	4.2 ± 0.32	5.0 ± 0.10‡
	500	3.9 ± 0.17	4.9 ± 0.17‡
2c (αβα)	260	4.0 ± 0.32	5.2 ± 0.19***
	500	3.3 ± 0.21	5.4 ± 0.08***
none (control)	0	2.8 ± 0.21	3.8 ± 0.11
1	260	4.9 ± 0.25***	6.8 ± 0.25***
2d (ββα)	260	3.0 ± 0.44	4.1 ± 0.13
	500	3.4 ± 0.21	3.8 ± 0.12
2e (ααα)	260	3.8 ± 0.05	4.2 ± 0.31
	500	5.0 ± 0.73***	4.1 ± 0.18
2f (βαα)	260	3.1 ± 0.29	3.9 ± 0.14
	500	3.0 ± 0.22	3.9 ± 0.09
none (control)	0	4.4 ± 0.31	4.3 ± 0.12
1	260	8.8 ± 0.32***	5.5 ± 0.15**
2g (ααβ)	65	9.3 ± 0.52***	6.0 ± 0.08***
	260	7.3 ± 0.60***	7.8 ± 0.25***
2h (βαβ)	500	7.0 ± 1.10***	4.4 ± 0.06
	1000	7.2 ± 0.99***	4.3 ± 0.10
none (control)	0	2.3 ± 0.49	4.4 ± 0.15
1	130	3.6 ± 0.13†	5.4 ± 0.19‡
	260	4.9 ± 0.33***	5.5 ± 0.26‡
3a (20-*epi*-αββ)	130	3.8 ± 0.32†	4.6 ± 0.10
	260	4.6 ± 0.57***	4.4 ± 0.16
3b (20-*epi*-βββ)	260	4.2 ± 0.57*	4.9 ± 0.18
	500	3.9 ± 0.38*	5.0 ± 0.17
none (control)	0	4.3 ± 0.48	4.7 ± 0.12
1	260	6.3 ± 0.39***	6.0 ± 0.17***
3c (20-*epi*-αβα)	260	5.6 ± 0.69**	4.4 ± 0.09
	500	5.4 ± 0.99**	4.3 ± 0.04
3d (20-*epi*-ββα)	130	5.5 ± 0.70**	4.4 ± 0.05
	260	5.5 ± 0.43**	4.4 ± 0.08
3f (20-*epi*-βαα)	260	5.0 ± 0.56	4.3 ± 0.13
	500	4.9 ± 0.35	4.4 ± 0.08
none (control)	0	2.7 ± 0.34	4.6 ± 0.16
1	260	5.1 ± 0.24***	6.1 ± 0.25***
3e (20-*epi*-ααα)	260	4.4 ± 0.19‡	4.6 ± 0.19
	500	4.0 ± 0.17	4.6 ± 0.04
3g (20-*epi*-ααβ)	50	4.3 ± 0.70‡	5.8 ± 0.21***
	100	5.2 ± 0.60***	7.8 ± 0.23***
3h (20-*epi*-βαβ)	500	4.4 ± 0.25‡	4.8 ± 0.15
	1000	3.4 ± 0.34	4.7 ± 0.13

[a] Weanling male rats were maintained on a vitamin D-deficient and 0.47% Ca diet for 1 week and then switched to a vitamin D-deficient and low-calcium diet containing 0.02% Ca for an additional 3 weeks. During the last week, they were dosed daily with a vitamin D analogue for 7 consecutive days. All doses were administered intraperitoneally in 0.1 mL of propylene glycol/ethanol (95:5). The control group received the vehicle. Determinations were made 24 h after the last dose. There were 5−6 rats/group. Statistical analysis was done by the use of Student's *t*-test. Differences from control in each panel: $^{***}p < 0.001$, $^{**}p < 0.005$, $^{‡}p = 0.005$, $^{*}p = 0.01$, $^{†}p < 0.025$.

20-*epi*-$\alpha\alpha\beta$-isomer **3g** was as active as **2g** and all other isomers showed very little activity.

In binding to the vitamin D binding protein (DBP) using fetal calf serum DBP,[41] the 1β-isomers showed high affinity, while the 1α-isomers had only poor affinity, which was consistent with previous findings (Table 3).[42] Moreover, all the 20-*epi* analogues were very poor ligands for DBP, showing approximately 300 times less affinity than **1**. These results imply that 20-epimerization remarkably decreases the DBP binding activity irrespective of the A-ring stereochemistry. This may be related in part to the high potency of the 20-*epi* analogues in HL-60 cell differentiation.

It is noteworthy that 20-*epi*-$\beta\beta\alpha$ **3d** was significantly active in HL-60 cell differentiation despite having a 1β-hydroxy configuration, which should impart low or no activity. Compared to 1α,25-dihydroxyvitamin D_3, **3d** showed 7% VDR binding affinity, 2-fold greater HL-60 differentiation activity, and about one-fifth of the calcium-mobilizing activity. Since all the compounds epimeric at either one of the 1, 2, 3, and 20 positions, 20-*epi*-$\alpha\beta\alpha$ **3c**, 20-*epi*-$\beta\alpha\alpha$ **3f**, 20-*epi*-$\beta\beta\beta$ **3b**, and $\beta\beta\alpha$ **2d**, respectively, exhibited virtually no activity, the combination of all these configurations is essential for activity. It is plausible that these effects arise from rotation or turnover of the molecule due to having opposite configurations at all those positions, so that it can mimic the 20-*epi*-$\alpha\alpha\beta$ **3g** or $\alpha\alpha\beta$ **2g**.

We also examined transactivation of target genes in transfected cells and modulation of cell-surface CD11b antigen expression in HL-60 cells in order to gain more insight into the biological action of these 2-methyl analogues.[43] As already reported, the rank orders of the transcriptional potencies were essentially parallel to those of the VDR binding affinity and HL-60 cell differentiation: that is, 20-*epi*-$\alpha\alpha\beta$ **3g** was the most potent and exhibited exceptionally high activity, and $\alpha\alpha\beta$ **2g** and 20-*epi*-$\alpha\beta\beta$ **3a** showed activity comparable to or greater than that of **1**.[43a] In further evaluation of biological activities, we surprisingly found that the structural requirement for inducing apoptosis of HL-60 cells was clearly different from that for inducing differentiation of these cells. In contrast to the requirement of the 1α- and 3β-hydroxyl groups for differentiation activity, compounds bearing the 1β-hydroxyl group together with the 3α- or 3β-hydroxyl group were potent stimulators of apoptosis of these cells.[43b] Therefore, these findings provided useful information not only for structure–function studies of 1α,25-dihydroxyvitamin D_3 analogues but also for the development of therapeutic agents for the treatment of leukemia and other cancers.

One of the objectives of this study was to gain information about the A-ring conformation–activity relationship, as mentioned in the Introduction. Modification by the 2-methyl group was intended to alter the equilibrium between the α- and β-conformers so that we could examine which conformer contributes more to the activity. Unfortunately, however, this study does not permit any decisive conclusion. The most potent isomer **g** favored the α-conformer, whereas the second most potent **a** favored the β-conformer, and the energy difference between the two conformers was not large: a 60:40 ratio for **g** and a 75:25 ratio for **a**. In this situation, the A-ring may adopt either conformation when it binds to the receptor. The configuration of the 2-methyl group seems to influence the potency because the isomers bearing the 2α-methyl group are much more potent than their counterparts, as seen in $\alpha\alpha\beta$ **g** vs $\alpha\beta\beta$ **a** and in $\alpha\alpha\alpha$ **e** vs $\alpha\beta\alpha$ **c**. Therefore, the 2α-methyl group may have a specific favorable interaction with the receptor, for example, a hydrophobic interaction. A steric effect is also plausible: that is, there may be a hydrophobic pocket in the receptor into which only the 2α-methyl group can fit well. Quite recently, a crystal structure of a mutant VDR in complex with 1α,25-dihydroxyvitamin D_3[44] and three-dimensional modeling of the VDR ligand binding domain[45] have been reported. Interestingly, the experimental data show that there is an additional space near position 2 of the A-ring, which could accommodate the 2-methyl group. Further studies are required to elucidate the A-ring conformation–activity relationship and the significance of the 2α-methyl substitution.[46]

Conclusions

In this study, we have synthesized all possible A-ring diastereomers of 1α,25-dihydroxyvitamin D_3 and its 20-epimers utilizing our newly developed procedure for synthesis of A-ring enyne synthon. The advantage of this procedure is that all possible A-ring diastereomers are readily accessible in the same way, which is in marked contrast to the known procedures. Biological evaluation of all these analogues demonstrated that the activities are highly dependent on the configuration not only of the C-1 and C-3 hydroxyl groups but also of the 2-methyl group, providing unique activity profiles. 2α-Methyl substitution with 1α,3β-hydroxyl groups increased the VDR binding, calcium mobilization, and HL-60 cell differentiation activities, and further modification by 20-epimerization enhanced the potencies. Thus, we found the exceptionally potent analogue **3g**, which is as potent as KH-1060, the most potent analogue reported to date. Moreover, the analogue **3d** exhibited significant activities, despite having a 1β-configuration. Further evaluation of the biological activities of all the 2-methyl analogues led to the finding that the structural requirement for inducing apoptosis of HL-60 cells was clearly different from that for inducing differentiation of these cells. These results show that the synthesis of all possible diastereomers is an effective approach to fully understanding SARs. The information provided by this study should cast light on the physiological roles of vitamin D and its SAR for various biological activities.

Experimental Section

General. Melting points were determined by using a Yanagimoto hot-stage apparatus and are uncorrected. NMR spectra were recorded on a JEOL GSX-400 or a Bruker DMX-500 spectrometer. Chemical shifts are expressed in ppm relative to tetramethylsilane. Mass spectra (MS) and high-resolution mass spectra (HRMS) were recorded on a JEOL JMS D-300 mass spectrometer. Infrared spectra were recorded on a Jasco FT/IR-8000 spectrometer and are expressed in cm^{-1}. Ultraviolet spectra were recorded with a Hitachi 200-10 spectrophotometer. Optical rotations were determined by using a Jasco DIP-370 digital polarimeter. Elemental analyses were carried out in the Microanalytical Laboratory, Faculty of Pharmaceutical Sciences, University of Tokyo, and were within 0.3% of the theoretical values. Recycling preparative HPLC was performed on a Waters LC equipped with a 510 HPLC

pump and 484 tunable absorbance detector. Analytical HPLC was performed on a Waters M600E LC system equipped with a 490E tunable absorbance detector. Analyses were conducted by using reversed-phase HPLC (Lichrosorb RP-18 column, 4 × 250 mm, 1.0 mL/min, acetonitrile:water = 60:40) and retention times (t_R) are expressed in minutes. Crystallographic data were collected on a Rigaku AFC7S diffractometer with graphite-monochromated Cu Kα radiation.

Methyl (*S*)-3-[(*tert*-Butyldiphenylsilyl)oxy]-2-methylpropionate (9). To a solution of methyl (*S*)-3-hydroxy-2-methylpropionate (10.4 g, 88 mmol) and imidazole (13.2 g, 194 mmol) in dry CH_2Cl_2 (20 mL) was added TBDPSCl (25 mL, 97 mmol) at 0 °C with stirring. After the ice bath was removed, the reaction mixture was stirred at room temperature for 1 h. The reaction mixture was diluted with water and the whole was extracted with CH_2Cl_2. The organic layer was washed with brine, dried over magnesium sulfate and filtered. Evaporation of the filtrate gave a crude mixture, from which **9** (31.4 g) was separated by silica gel column chromatography (2% ethyl acetate−*n*-hexane) as a colorless oil in quantitative yield: $[\alpha]_D$ +13.8 (c = 1.52, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 1.04 (9 H, s), 1.15 (3 H, d, J = 7.0 Hz), 2.72 (1 H, d, quint, J = 7.0, 5.8 Hz), 3.67 (3 H, s), 3.73 (1 H, dd, J = 9.8, 6.4 Hz), 7.35−7.44 (6 H, m), 7.64−7.68 (4 H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.5 (q), 19.2 (s), 26.7 (q), 42.2 (d), 51.5 (q), 65.9 (t), 127.7 (d), 129.7 (d), 133.5 (s), 135.6 (d), 175.3 (s); IR (neat) 1741 cm^{-1}; MS m/z 356 $(M)^+$, 325 $(M - OMe)^+$; HRMS m/z $(M)^+$ calcd 356.1808 for $C_{21}H_{28}O_3Si$, found 356.1796.

(*R*)-3-[(*tert*-Butyldiphenylsilyl)oxy]-2-methylpropan-1-ol (10). To a solution of **9** (3.54 g, 9.93 mmol) dissolved in dry toluene was added DIBAL-H (1.0 M in toluene, 19.8 mL) at −78 °C under argon. Stirring was continued for 1 h at −78 °C, then ethyl acetate was added to the reaction mixture to destroy the excess reagent. The mixture was washed with 0.5 N HCl (aq) and then with brine, dried over magnesium sulfate and filtered. The solvent was removed under reduced pressure to give a residue, from which **10** (2.90 g) was separated by silica gel column chromatography (4−10% ethyl acetate−*n*-hexane) as a colorless oil in 90% yield: $[\alpha]_D$ +1.5 (c = 1.34, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 0.83 (3 H, d, J = 7.0 Hz), 1.06 (9 H, s), 1.99 (1 H, m), 2.58 (1 H, m), 3.60 (1 H, dd, J = 10.1, 7.6 Hz), 3.68 (2 H, m), 3.72 (1 H, dd, J = 10.1, 4.6 Hz), 7.37−7.46 (6 H, m), 7.67−7.69 (4 H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.2 (q), 19.2 (s), 26.7 (q), 37.6 (d), 67.5 (t), 68.6 (t), 127.8 (d), 129.8 (d), 133.20 (s), 133.21 (s), 135.57 (d), 135.58 (d); IR (neat) 3383, 1472, 1428 cm^{-1}; MS m/z 271 $(M - {}^tBu)^+$; HRMS m/z $(M - {}^tBu)^+$ calcd for $C_{16}H_{19}O_2Si$ 271.1155, found 271.1159.

(*S*)-3-[(*tert*-Butyldiphenylsilyl)oxy]-2-methylpropanal (11). A solution of oxalyl chloride (1.8 mL, 21 mmol) in CH_2Cl_2 was added to a solution of DMSO (3.0 mL, 44 mmol) in CH_2Cl_2, and the mixture was stirred at −78 °C for 1 h under an argon atmosphere. The resulting mixture was transferred to a solution of **10** (3.40 g, 10 mmol) in CH_2Cl_2 at −78 °C and the mixture was stirred for 30 min. Subsequently, Et_3N (11.5 mL) was added to the mixture followed by stirring for 1 h, while the temperature was elevated from −78 to 0 °C. The reaction mixture was extracted with ethyl acetate after the addition of water. The organic phase was washed with brine, dried over magnesium sulfate and filtered. The solvent was removed under reduced pressure to give a residue, from which **11** (3.37 g) was separated by silica gel column chromatography (4% ethyl acetate−*n*-hexane) as a colorless oil in 99% yield: $[\alpha]_D$ +4.0 (c = 1.28, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 1.04 (9 H, s), 1.10 (3 H, d, J = 7.0 Hz), 2.56 (1 H, m), 3.87 (1 H, ddd, J = 10.0, 6.1, 4.8 Hz), 7.37−7.46 (6 H, m), 7.63−7.67 (4 H, m), 9.77 (1 H, d, J = 1.5 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.2 (q), 19.2 (s), 26.8 (q), 41.9 (d), 67.6 (t), 127.8 (d), 129.8 (d), 133.07 (s), 133.12 (s), 135.9 (d), 204.4 (d); IR (neat) 1732, 1472, 1428 cm^{-1}; MS m/z 326 $(M)^+$, 269 $(M - {}^tBu)^+$; HRMS m/z $(M)^+$ calcd for $C_{20}H_{26}O_2Si$ 326.1708, found 326.1705.

(*R*)-4-[(*tert*-Butyldiphenylsilyl)oxy]-3-methylbut-1-ene (12). To a suspension of methyltriphenylphosphonium bromide (2.2 g, 7.4 mmol) in THF (15 mL) was added with stirring *n*-BuLi (1.6 M solution in *n*-hexane, 5.2 mL, 9.3 mmol) under argon at 0 °C. The resulting mixture was stirred for 20 min, then a solution of **11** (1.2 g, 3.7 mmol) in THF (15 mL) was added to it. The reaction mixture was further stirred for 15 min and then for 45 min at room temperature. Saturated NH_4Cl (aq) was added to the mixture, and the whole was extracted with ethyl acetate. The organic layer was washed with brine, dried over magnesium sulfate, filtered and concentrated. The crude product was purified by silica gel column chromatography (2% ethyl acetate−*n*-hexane) to give **12** (1.1 g) as a colorless oil in 92% yield: $[\alpha]_D$ +12.1 (c = 0.90, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 1.03 (3 H, d, J = 7.0 Hz), 1.05 (9 H, s), 2.39 (1 H, m), 3.49 (1 H, dd, J = 9.7, 6.7 Hz), 3.57 (1 H, dd, J = 9.7, 6.1 Hz), 5.01 (3 H, m), 7.35−7.44 (6 H, m), 7.65−7.68 (4 H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 16.2 (q), 19.3 (s), 26.9 (q), 40.2 (d), 68.5 (t), 114.1 (t), 127.6 (d), 129.5 (d), 134.0 (s), 135.7 (d), 141.4 (d); IR (neat) 1472, 1428 cm^{-1}; MS m/z 267 $(M - {}^tBu)^+$; HRMS m/z $(M - {}^tBu)^+$ calcd for $C_{17}H_{19}OSi$ 267.1205, found 267.1202.

(2*S*,3*RS*)-1-[(*tert*-Butyldiphenylsilyl)oxy]-2-methyl-3,4-epoxybutane (13). To a solution of **12** (1.0 g, 3.1 mmol) in dry CH_2Cl_2 (25 mL) was added mCPBA (1.4 g, 7.4 mmol) under argon. The resultant solution was stirred for 15 min at 0 °C, then further stirred overnight after removal of the ice bath. The whole mixture was extracted with ethyl acetate after the addition of water. The organic layer was washed with brine and dried over magnesium sulfate. Evaporation of the solvent afforded a residue, from which **13** (1.1 g) was separated by silica gel column chromatography (2% Et_2O−*n*-hexane) as a colorless oil in quantitative yield: ^1H NMR (400 MHz, $CDCl_3$) δ 0.99 (3 H, d, J = 6.8 Hz), 1.05 (9 H × 3/5, s), 1.07 (9 H × 2/5, s), 1.58 (1 H, m), 2.54 (1 H × 2/5, dd, J = 5.0, 2.7 Hz), 2.60 (1 H × 3/5, dd, J = 5.0, 2.7 Hz), 2.73 (1 H × 2/5, dd, J = 5.0, 4.3 Hz), 2.76 (1 H × 3/5, dd, J = 5.0, 4.3 Hz), 2.85 (1 H × 3/5, ddd, J = 7.0, 4.3, 2.7 Hz), 2.97 (1 H × 2/5, ddd, J = 7.0, 4.3, 2.7 Hz), 3.62 (1 H, dd, J = 6.7, 3.4 Hz), 3.70 (1 H, dd, J = 7.0, 5.0 Hz), 7.36−7.45 (6 H, m), 7.64−7.70 (4 H, m); IR (neat) 1472, 1427, 1294 cm^{-1}; MS m/z 283 $(M - {}^tBu)^+$; HRMS m/z $(M - {}^tBu)^+$; calcd for $C_{17}H_{19}O_2Si$ 283.1154, found 283.1167.

(2*S*,3*R*)-1-[(*tert*-Butyldiphenylsilyl)oxy]-2-methyl-6-(trimethylsilyl)hex-5-yn-3-ol (14a) and (2*S*,3*S*)-1-[(*tert*-Butyldiphenylsilyl)oxy]-2-methyl-6-(trimethylsilyl)hex-5-yn-3-ol (14b). Under an argon atmosphere, ethynyltrimethylsilane (9.4 mL, 67 mmol) was dissolved in THF, and *n*-BuLi (1.6 M in *n*-hexane solution, 36 mL, 57 mmol) was added to the mixture at 0 °C. The whole was stirred for 20 min then cooled to −78 °C. The resulting mixture was added to a stirred solution of **13** (6.47 g, 19 mmol) in THF at −78 °C. The reaction mixture was stirred at −78 °C for 50 min after addition of BF_3·Et_2O (4.7 mL, 38 mmol). The reaction was quenched by the addition of satd NH_4Cl (aq), and the whole was extracted with ethyl acetate. The organic layer was washed with brine, dried over magnesium sulfate, filtered and concentrated. This crude residue was purified by silica gel column chromatography (5% ethyl acetate−*n*-hexane) to give **14a** (3.42 g, 41%, less polar) and **14b** (4.33 g, 52%, more polar), both as colorless oils.

14a: $[\alpha]_D$ +5.0 (c = 0.54, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 0.15 (9 H, s), 0.91 (3 H, d, J = 7.1 Hz), 1.07 (9 H, s), 1.96 (1 H, m), 2.46 (1 H, dd, J = 10.6, 6.4 Hz), 2.54 (1 H, dd, J = 10.6, 4.9 Hz), 2.84 (1 H, d, J = 3.1 Hz), 3.67 (1 H, dd, J = 10.4, 6.4 Hz), 3.75 (1 H, m), 3.79 (1 H, dd, J = 10.4, 4.3 Hz), 7.37−7.46 (6 H, m), 7.65−7.68 (4 H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 0.1 (q), 13.5 (q), 19.1 (s), 26.7 (t), 26.8 (q), 39.2 (d), 67.6 (t), 73.6 (d), 86.9 (s), 103.6 (s), 127.7 (d), 129.8 (d), 133.0 (s), 133.6 (d), 135.6 (d); IR (neat) 3491, 2179 cm^{-1}; MS m/z 423 $(M - Me)^+$, 365 $(M - TMS)^+$, 308 $(M - TMS - {}^tBu)^+$; HRMS m/z $(M - Me)^+$ calcd for $C_{25}H_{35}O_2Si_2$ 423.2243, found 423.2209.

14b: $[\alpha]_D$ −10.4 (c = 0.58, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 0.14 (9 H, s), 1.00 (3 H, d, J = 7.0 Hz), 1.06 (9 H, s), 1.92 (1 H, m), 2.42 (1 H, dd, J = 10.1, 7.0 Hz), 2.50 (1 H, dd, J = 10.1, 4.3 Hz), 2.84 (1 H, d, J = 3.1 Hz), 3.67 (1 H, dd, J = 10.2, 6.4 Hz), 3.75 (1 H, dd, J = 10.2, 4.2 Hz), 3.97 (1 H, dd, J = 10.4, 4.3 Hz), 7.37−7.46 (6 H, m), 7.65−7.68 (4 H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 0.1 (q), 10.1 (q), 19.2 (s), 26.1 (t),

26.9 (q), 38.3 (d), 68.1 (t), 72.2 (d), 86.8 (s), 103.8 (s), 127.8 (d), 129.7 (d), 129.8 (d), 133.0 (s), 133.2 (s), 135.6 (d), 135.6 (d); IR (neat) 3484, 2176 cm^{-1}; MS *m/z* 381 (M − ^{t}Bu)$^+$; HRMS *m/z* (M − ^{t}Bu)$^+$ calcd for $C_{22}H_{29}O_2Si_2$ 381.1706, found 381.1714.

General Procedure for Syntheses of MTPA Esters. A solution of each of the above alcohols dissolved in dry CH_2Cl_2 was treated with DMAP (2 equiv) and (*R*)- or (*S*)-MTPACl (2 equiv) at room temperature under an argon atmosphere. The reaction solution was purified by preparative TLC (10% ethyl acetate−*n*-hexane) without pretreatment to afford the corresponding MTPA ester.

(*S*)-MTPA ester of 14a: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.116 (9 H, s), 0.820 (3 H, d, *J* = 7.0 Hz), 1.048 (9 H, s), 2.186 (1 H, q, *J* = 6.1 Hz), 2.588 (1 H, dd, *J* = 11.0, 6.7 Hz), 2.751 (1 H, dd, *J* = 11.0, 6.7 Hz), 3.486 (1 H, dd, *J* = 10.3, 5.4 Hz), 3.537 (1 H, dd, *J* = 10.3, 5.8 Hz), 3.57 (3 H, s), 5.317 (1 H, dd, *J* = 10.3, 6.7 Hz), 7.28−7.45 (9 H, m), 7.49−7.54 (2 H, m), 7.59−7.65 (4 H, m).

(*R*)-MTPA ester of 14a: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.075 (9 H, s), 0.949 (3 H, d, *J* = 7.0 Hz), 1.051 (9 H, s), 2.254 (1 H, q, *J* = 6.7 Hz), 2.552 (1 H, dd, *J* = 11.6, 6.1 Hz), 2.745 (1 H, dd, *J* = 11.6, 5.2 Hz), 3.42 (3 H, s), 3.564 (1 H, dd, *J* = 10.7, 5.8 Hz), 3.643 (1 H, dd, *J* = 10.7, 6.5 Hz), 5.272 (1 H, dd, *J* = 10.6, 5.8 Hz), 7.28−7.45 (9 H, m), 7.50−7.56 (2 H, m), 7.59−7.65 (4 H, m).

(*S*)-MTPA ester of 14b: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.116 (9 H, s), 0.861 (3 H, d, *J* = 7.0 Hz), 1.067 (9 H, s), 2.276 (1 H, q, *J* = 6.1 Hz), 2.565 (1 H, dd, *J* = 10.6, 5.8 Hz), 2.703 (1 H, dd, *J* = 10.6, 6.1 Hz), 3.46 (3 H, s), 3.491 (1 H, m), 3.532 (1 H, m), 5.487 (1 H, dd, *J* = 9.8, 5.8 Hz), 7.28−7.46 (9 H, m), 7.49−7.56 (2 H, m), 7.60−7.69 (4 H, m).

(*R*)-MTPA ester of 14b: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.124 (9 H, s), 0.797 (3 H, d, *J* = 6.7 Hz), 1.056 (9 H, s), 2.172 (1 H, q, *J* = 6.7 Hz), 2.652 (1 H, t, *J* = 6.7 Hz), 2.708 (1 H, m), 3.339 (1 H, m), 3.441 (1 H, m), 3.58 (3 H, s), 5.457 (1 H, dd, *J* = 10.3, 6.1 Hz), 7.28−7.46 (9 H, m), 7.49−7.55 (2 H, m), 7.61−7.65 (4 H, m).

(4*R*,5*S*)-6-[(*tert*-Butyldiphenylsilyl)oxy]-5-methyl-4-[(2-tetrahydropyranyl)oxy]-1-(trimethylsilyl)hex-1-yne (15). To a solution of **14a** (2.15 g, 4.9 mmol) and DHP (0.76 mL, 8.3 mmol) in dry CH_2Cl_2 (10 mL) was added PPTS (160 mg, 0.64 mmol) at room temperature, and the resulting mixture was allowed to stand overnight. The mixture was diluted with ether, then washed with brine and dried over magnesium sulfate. Evaporation of the solvent gave a residue, from which **15** (2.51 g) was separated by silica gel column chromatography (1% ethyl acetate−*n*-hexane) as a colorless oil in 98% yield: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.127 (9 H × 1/2, s), 0.135 (9 H × 1/2, s), 0.95 (3 H, d, *J* = 7.0 Hz), 1.058 (9 H × 1/2, s), 1.061 (9 H × 1/2, s), 1.52 (4 H, m), 1.74 (2 H, m), 2.13 (1 H, m), 2.38 (1 H × 1/2, dd, *J* = 17.1, 7.3 Hz), 2.46 (1 H × 1/2, dd, *J* = 17.1, 4.6 Hz), 2.54 (1 H × 1/2, dd, *J* = 17.1, 5.5 Hz), 2.66 (1 H × 1/2, dd, *J* = 17.1, 5.8 Hz), 3.45 (1 H, m), 3.64 (2 H, m), 3.77 (1 H, m), 3.90 (1 H × 1/2, dt, *J* = 7.3, 4.9 Hz), 4.02 (1 H × 1/2, m), 4.66 (1 H × 1/2, t, *J* = 3.1 Hz), 4.86 (1 H, dd, *J* = 4.3, 2.7 Hz), 7.35−7.44 (6 H, m), 7.65−7.70 (4 H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ −0.05 (q), 0.003 (q), 12.8 (q), 13.2 (q), 19.2 (s), 19.3 (t), 19.7 (t), 22.3 (t), 24.1 (t), 25.4 (t), 25.5 (t), 26.8 (q), 26.9 (q), 30.7 (t), 30.8 (t), 38.2 (d), 40.0 (d), 62.1 (t), 62.6 (t), 65.4 (t), 65.8 (t), 76.3 (d), 77.7 (d), 85.8 (s), 98.3 (d), 99.3 (d), 104.9 (s), 127.7 (d), 129.7 (d), 133.9 (s), 134.0 (s), 135.7 (d); IR (neat) 2176, 1362, 1250, 1113, 1078, 1028 cm^{-1}; MS *m/z* 522 (M)$^+$, 507 (M − Me)$^+$; HRMS *m/z* (M)$^+$ calcd for $C_{31}H_{46}O_3Si_2$ 522.2986, found 522.2986.

(2*S*,3*R*)-2-Methyl-3-[(2-tetrahydropyranyl)oxy]hex-5-yn-1-ol (16). To a stirred solution of **15** (1.13 g, 2.2 mmol) in THF (20 mL) was added TBAF (1.0 M THF solution, 8.8 mL, 8.8 mmol), and the resulting mixture was stirred at room temperature for 4 h. Water was added, and the mixture was extracted with ethyl acetate. The organic phase was washed with brine, dried over magnesium sulfate and filtered. Evaporation of the filtrate gave a residue, from which **16** (450 mg) was separated by silica gel column chromatography (20% ethyl acetate−*n*-hexane) as a colorless oil in 96% yield: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.99 (3 H × 1/2, d, *J* = 6.7 Hz), 1.01 (3 H × 1/2, d, *J* = 7.0 Hz), 1.52 (4 H, m), 1.74 (2 H, m), 1.99 (1 H × 1/2, t, *J* = 2.7 Hz), 2.00 (1 H × 1/2, t, *J* = 2.7 Hz), 2.16 (1 H, m), 2.33 (1 H × 1/2, m), 2.38 (1 H × 1/2, ddd, *J* = 17.1, 6.1, 2.8 Hz), 2.56 (1 H × 1/2, ddd, *J* = 17.1, 4.0, 2.8 Hz), 2.63 (1 H × 1/2, ddd, *J* = 17.1, 4.0, 2.8 Hz), 2.72 (1 H × 1/2, ddd, *J* = 17.1, 7.0, 2.8 Hz), 3.48 (3 H × 1/2, m), 3.70 (2 H, m), 3.98 (3 H × 1/2, m), 4.70 (1 H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.9 (q), 14.3 (q), 19.6 (t), 21.2 (t), 22.5 (t), 23.5 (t), 25.0 (t), 25.2 (t), 30.9 (t), 31.1 (t), 37.5 (d), 39.0 (d), 62.8 (t), 64.6 (t), 65.2 (t), 70.0 (d), 70.1 (d), 77.1 (d), 80.8 (s), 80.9 (s), 81.8 (d), 100.0 (d), 101.2 (d); IR (neat) 3310, 3021, 2176, 1375, 1248, 1046 cm^{-1}.

(2*R*,3*R*)-2-Methyl-3-[(2-tetrahydropyranyl)oxy]hex-5-ynal (17). A solution of oxalyl chloride (0.8 mL, 9.3 mmol) in CH_2Cl_2 (5 mL) was added to a solution of DMSO (1.3 mL, 19 mmol) in CH_2Cl_2 (5 mL), and the mixture was stirred at −78 °C for 1 h under an argon atmosphere. The resulting mixture was transferred to a solution of **16** (1.04 g, 4.9 mmol) in CH_2Cl_2 (20 mL) at −78 °C and the mixture was stirred for 30 min. Subsequently, Et_3N (5.0 mL) was added to the mixture followed by stirring for 1 h, while the temperature was allowed to rise from −78 to 0 °C. The reaction mixture was extracted with ethyl acetate after the addition of water. The organic phase was washed with brine, dried over magnesium sulfate and filtered. The solvent was removed under reduced pressure to give a residue, from which **17** (989 mg) was separated by silica gel column chromatography (20% ethyl acetate−*n*-hexane) as a colorless oil in 96% yield: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.12 (3 H × 1/2, d, *J* = 7.2 Hz), 1.15 (3 H × 1/2, d, *J* = 7.2 Hz), 1.57 (4 H, m), 1.76 (2 H, m), 2.02 (1 H × 1/2, t, *J* = 2.8 Hz), 2.04 (1 H × 1/2, t, *J* = 2.8 Hz), 2.44 (1 H × 1/2, ddd, *J* = 17.4, 5.1, 2.8 Hz), 2.57 (1 H × 1/2, ddd, *J* = 17.1, 6.1, 2.8 Hz), 2.38 (1 H × 1/2, ddd, *J* = 17.1, 6.1, 2.8 Hz), 2.56 (1 H × 1/2, ddd, *J* = 17.4, 5.5, 2.8 Hz), 2.73 (1 H × 1/2, ddd, *J* = 17.4, 6.7, 2.6 Hz), 2.82 (1 H × 1/2, dquint, *J* = 2.1, 7.0 Hz), 2.90 (1 H × 1/2, dquint, *J* = 2.1, 7.0 Hz), 3.53 (1 H, m), 3.81 (1 H × 1/2, m), 3.96 (1 H, m), 4.10 (1 H × 1/2, dd, *J* = 11.8, 5.6 Hz), 4.70 (1 H × 1/2, m), 4.82 (1 H × 1/2, m), 9.82 (1 H × 1/2, d, *J* = 1.5 Hz), 9.85 (1 H × 1/2, d, *J* = 2.1 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 10.3 (q), 10.5 (q), 19.4 (t), 19.6 (t), 21.0 (t), 23.3 (t), 25.2 (t), 25.4 (t), 30.6 (t), 30.7 (t), 49.0 (d), 49.7 (d), 62.7 (t), 62.9 (t), 70.5 (d), 71.3 (d), 74.2 (d), 79.7 (s), 80.1 (s), 97.0 (d), 100.6 (d), 203.7 (d), 204.1 (d); IR (neat) 2942, 2876, 2174, 1724 cm^{-1}.

(3*RS*,4*S*,5*R*)-4-Methyl-5-[(2-tetrahydropyranyl)oxy]oct-1-en-7-yn-3-ol (18). A suspension of $CeCl_3$ (595 mg, 2.4 mmol) in dry THF (7 mL) was stirred at 0 °C for 2 h under argon atmosphere. To the mixture was added at −78 °C a solution of vinylmagnesium bromide (1.0 M in THF, 2.25 mL, 6.0 mmol), and the mixture was further stirred for 2 h at −78 °C. A solution of **17** (190 mg, 0.90 mmol) in THF (5 mL) was introduced into the thus prepared mixture. Stirring was continued for 2 h, then the reaction was quenched by the addition of satd NH_4Cl (aq). Extraction with ethyl acetate followed by evaporation of the solvent afforded a residue, from which **18** (197 mg) was separated by silica gel column chromatography (20% ethyl acetate−*n*-hexane) as a colorless oil in 92% yield: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.85 (3 H × 1/4, d, *J* = 7.0 Hz), 0.88 (3 H × 1/4, d, *J* = 7.3 Hz), 0.90 (3 H × 1/4, d, *J* = 7.0 Hz), 0.93 (3 H × 1/4, d, *J* = 7.0 Hz), 1.52 (4 H, m), 1.73 (2 H, m), 2.01 (1 H, m), 2.17 (1 H, m), 2.40 (1 H × 1/2, m), 2.59 (1 H, m), 2.68 (1 H × 1/4, d, *J* = 3.7 Hz), 2.80 (1 H × 1/2, m), 2.88 (1 H × 1/4, d, *J* = 4.6 Hz), 3.37 (1 H × 1/4, d, *J* = 4.3 Hz), 3.52 (1 H, m), 3.73 (1 H × 3/4, m), 3.80 (1 H × 1/4, d, *J* = 4.3 Hz), 3.97 (1 H × 5/4, m), 4.13 (1 H × 1/4, m), 4.19 (1 H × 1/4, m), 4.51 (1 H × 1/4, m), 4.70 (1 H, m), 4.81 (1 H × 1/4, m), 5.24 (2 H, m), 5.88 (1 H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 9.8 (q), 10.7 (q), 12.4 (q), 13.4 (q), 19.4 (t), 19.5 (t), 20.2 (t), 21.5 (t), 22.8 (t), 23.1 (t), 23.5 (t), 24.9 (t), 25.1 (t), 25.2 (t), 25.3 (t), 30.8 (t), 31.0 (t), 31.1 (t), 39.8 (d), 41.1 (d), 41.8 (d), 42.2 (d), 62.5 (t), 62.8 (t), 63.8 (t), 65.6 (t), 69.7 (d), 70.0 (d), 70.0 (d), 70.1 (d), 70.3 (d), 72.1 (d), 75.5 (d), 76.4 (d), 76.9 (d), 77.2 (d), 80.5 (d), 80.6 (s), 80.8 (s), 81.5 (s), 81.5 (d), 98.1 (d), 100.3 (d), 100.5 (d), 101.5 (d), 114.0 (t), 114.5 (t), 115.6 (t), 116.1 (t), 139.1

(d), 139.5 (d), 139.9 (d); IR (neat) 3451, 3309, 2120, 1642, 1217, 1182, 1074 cm^{-1}.

(3*R*,4*R*,5*R*)-4-Methyloct-1-en-7-yne-3,5-diol (19a) and (3*S*,4*R*,5*R*)-4-Methyloct-1-en-7-yne-3,5-diol (19b). To a solution of **18** (315 mg, 1.3 mmol) in methanol (10 mL) was added TsOH·H_2O (25 mg, 0.13 mmol), and the mixture was allowed to stand at room temperature for 1 h. After evaporation of methanol, brine was added to the mixture and the whole was extracted with ether. The organic layer was dried over magnesium sulfate, filtered and concentrated. The crude mixture was purified by silica gel column chromatography (10% ethyl acetate−*n*-hexane) to give **19a** (79 mg, 39%, less polar) and **19b** (75 mg, 37%, more polar) each as a colorless oil.

19a: $[\alpha]_D$ −20.2 (*c* = 0.58, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 0.90 (3H, d, *J* = 7.0 Hz), 1.95 (1H, dquint, *J* = 2.8, 7.0 Hz), 2.08 (1H, t, *J* = 2.8 Hz), 2.43 (1H, ddd, *J* = 17.1, 7.0, 2.8 Hz), 2.54 (1H, ddd, *J* = 17.1, 4.6, 2.8 Hz), 2.72 (1H, d, *J* = 5.5 Hz), 2.96 (1H, d, *J* = 4.6 Hz), 3.79 (1H, dd, *J* = 7.0, 4.6 Hz), 4.44 (1H, dtt, *J* = 7.0, 5.5, 1.5 Hz), 5.23 (1H, dt, *J* = 10.7, 1.5 Hz), 5.32 (1H, dt, *J* = 17.1, 1.5 Hz), 5.94 (1H, ddd, *J* = 17.1, 10.7, 5.5 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 11.6 (q), 25.5 (t), 41.3 (d), 70.9 (q), 72.6 (d), 74.3 (d), 80.6 (s), 115.6 (t), 138.2 (d); IR (neat) 3384, 3308, 3015, 2120, 1644 cm^{-1}.

19b: $[\alpha]_D$ +9.1 (*c* = 0.68, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 0.83 (3 H, d, *J* = 7.0 Hz), 1.83 (1 H, m), 2.07 (1 H, t, *J* = 2.8 Hz), 2.41 (1 H, ddd, *J* = 16.8, 6.7, 2.8 Hz), 2.58 (1 H, ddd, *J* = 16.8, 4.0, 2.8 Hz), 2.88 (1 H, brs), 3.41 (1 H, brs), 3.74 (1 H, m), 4.14 (1 H, t, *J* = 7.0 Hz), 5.19 (1 H, dq, *J* = 10.4, 0.9 Hz), 5.27 (1 H, dq, *J* = 17.1, 1.2 Hz), 5.88 (1 H, ddd, *J* = 17.1, 10.4, 7.3 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.7 (q), 25.4 (t), 42.2 (d), 70.8 (q), 73.9 (d), 78.0 (d), 80.6 (s), 117.0 (t), 139.3 (d); IR (neat) 3387, 3308, 3083, 2120, 1643 cm^{-1}.

General Procedure for Syntheses of Acetonides. Each of the above enyne compounds (5 mg) dissolved in acetone (0.4 mL) was treated with dimethoxypropane (0.1 mL) and CSA (1.5 mg, 0.2 equiv) at room temperature. Evaporation of the solvent afforded a residue, from which the corresponding acetonide was purified by silica gel column chromatography (5% ethyl acetate−*n*-hexane).

Acetonide of 19a: ^1H NMR (400 MHz, $CDCl_3$) δ 0.90 (3 H, d, *J* = 7.0 Hz), 1.39 (3 H, s), 1.40 (3 H, s), 1.86−1.92 (1 H, m), 2.01 (1 H, t, *J* = 2.8 Hz), 2.44 (1 H, ddd, *J* = 17.4, 6.1, 2.8 Hz), 2.48 (1 H, ddd, *J* = 17.4, 5.5, 2.8 Hz), 3.49 (1 H, dt, *J* = 7.6, 5.8 Hz), 4.43 (1 H, ddt, *J* = 6.1, 5.2, 1.5 Hz), 5.17 (1 H, dt, *J* = 10.7, 1.2 Hz), 5.26 (1 H, dt, *J* = 17.4, 1.2 Hz), 5.79 (1 H, ddd, *J* = 17.4, 10.7, 6.1 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.9 (q), 24.1 (q), 25.2 (q), 29.7 (t), 39.8 (d), 69.7 (s), 70.6 (d), 73.0 (d), 81.0 (d), 100.9 (s), 115.8 (t), 139.6 (t).

Acetonide of 19b: ^1H NMR (400 MHz, $CDCl_3$) δ 0.82 (3 H, d, *J* = 6.7 Hz), 1.45 (3 H, s), 1.49 (3 H, s), 1.51−1.61 (1 H, m), 2.01 (1 H, t, *J* = 2.7 Hz), 2.42 (1 H, ddd, *J* = 17.4, 5.5, 2.7 Hz), 2.52 (1 H, ddd, *J* = 17.4, 4.0, 2.7 Hz), 3.68 (1 H, ddd, *J* = 10.1, 5.8, 4.0 Hz), 3.91 (1 H, ddt, *J* = 10.1, 7.3, 1.5 Hz), 5.24 (1 H, dt, *J* = 7.3, 1.5 Hz), 5.29 (1 H, dd, *J* = 17.4, 1.5 Hz), 5.76 (1 H, ddd, *J* = 17.4, 10.1, 7.3 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.2 (q), 19.7 (q), 29.7 (t), 30.0 (q), 39.8 (d), 69.7 (s), 70.6 (d), 73.0 (d), 81.0 (d), 100.9 (s), 115.8 (t), 135.6 (t).

(3*R*,4*R*,5*S*)-4-Methyloct-1-en-7-yne-3,5-diol (19c) and (3*S*,4*R*,5*S*)-4-Methyloct-1-en-7-yne-3,5-diol (19d). These compounds were obtained by the same procedures as described above for **19a**,**b**, starting from **14b** instead of **14a**.

19c: $[\alpha]_D$ +20.1 (*c* = 0.74, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 0.93 (3 H, d, *J* = 7.0 Hz), 1.85 (1 H, qt, *J* = 7.0, 2.4 Hz), 2.04 (1 H, t, *J* = 2.8 Hz), 2.35 (1 H, ddd, *J* = 16.8, 6.7, 2.4 Hz), 2.37 (1 H, t, *J* = 2.8 Hz), 2.50 (1 H, ddd, *J* = 16.8, 7.0, 2.4 Hz), 2.94 (1 H, t, *J* = 2.4 Hz), 4.11 (1 H, tt, *J* = 7.0, 2.4 Hz), 4.44 (1 H, m), 5.20 (1 H, dt, *J* = 10.7, 1.5 Hz), 5.28 (1 H, dq, *J* = 17.4, 1.5 Hz), 5.90 (1 H, ddd, *J* = 17.4, 10.7, 4.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 4.8 (q), 24.9 (t), 40.4 (d), 70.5 (q), 74.0 (d), 76.8 (d), 81.0 (s), 114.9 (t), 139.4 (d); IR (neat) 3385, 3300, 2978, 2912, 2120, 1649 cm^{-1}.

19d: $[\alpha]_D$ −18.1 (*c* = 0.73, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 1.01 (3 H, d, *J* = 7.3 Hz), 1.86 (1 H, m), 2.03 (1 H, t, *J* = 2.8 Hz), 2.33 (1 H, ddd, *J* = 16.8, 6.1, 2.8 Hz), 2.45 (1 H, d, *J* = 4.6 Hz), 2.48 (1 H, ddd, *J* = 16.8, 5.2, 2.8 Hz), 2.77 (1 H, d, *J* = 3.7 Hz), 4.19 (2 H, m), 5.23 (1 H, dt, *J* = 10.1, 1.5 Hz), 5.23 (1 H, dt, *J* = 10.7, 1.5 Hz), 5.34 (1 H, dt, *J* = 17.1, 1.5 Hz), 5.92 (1 H, ddd, *J* = 17.1, 10.4, 5.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 10.4 (q), 24.3 (t), 40.6 (d), 70.4 (d), 76.7 (d), 81.2 (s), 115.9 (t), 139.8 (d); IR (neat) 3567, 3303, 2977, 2917, 2120, 1649 cm^{-1}.

The following four compounds (**19e**−**h**) were prepared by the same procedures as described above, starting from **8b** instead of **8a**.

(3*R*,4*S*,5*S*)-4-Methyloct-1-en-7-yne-3,5-diol (19e): $[\alpha]_D$ −12.7 (*c* = 0.70, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 0.83 (3 H, d, *J* = 7.3 Hz), 1.83 (1 H, m), 2.08 (1 H, t, *J* = 2.8 Hz), 2.41 (1 H, ddd, *J* = 16.8, 6.7, 2.8 Hz), 2.58 (1 H, ddd, *J* = 16.8, 3.7, 2.8 Hz), 2.78 (1 H, brs), 3.35 (1 H, brs), 3.75 (1 H, ddd, *J* = 8.5, 6.7, 3.7 Hz), 4.12 (1 H, m), 5.20 (1 H, dq, *J* = 10.2, 1.5 Hz), 5.27 (1 H, dq, *J* = 17.4, 2.7 Hz), 5.87 (1 H, ddd, *J* = 17.4, 10.1, 7.3 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.7 (q), 25.4 (t), 42.5 (d), 70.9 (d), 73.9 (d), 78.0 (d), 80.6 (s), 117.0 (t), 138.3 (d); IR (neat) 3387, 3308, 3083, 2120, 1644 cm^{-1}.

(3*S*,4*S*,5*S*)-4-Methyloct-1-en-7-yne-3,5-diol (19f): $[\alpha]_D$ −30.3 (*c* = 0.70, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 0.90 (3 H, d, *J* = 7.3 Hz), 1.96 (1 H, dquint, *J* = 7.3, 2.8 Hz), 2.07 (1 H, t, *J* = 2.8 Hz), 2.42 (1 H, ddd, *J* = 16.8, 7.0, 2.8 Hz), 2.54 (1 H, ddd, *J* = 16.8, 4.6, 2.8 Hz), 2.73 (1 H, brs), 2.96 (1 H, brs), 3.79 (1 H, q, *J* = 6.7 Hz), 4.43 (1 H, m), 5.23 (1 H, dt, *J* = 10.7, 1.5 Hz), 5.32 (1 H, dt, *J* = 17.1, 1.5 Hz), 5.93 (1 H, ddd, *J* = 17.1, 10.7, 5.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 11.6 (q), 25.6 (t), 41.4 (d), 71.0 (d), 72.7 (d), 74.5 (d), 80.5 (s), 115.6 (t), 138.3 (d); IR (neat) 3384, 3308, 3086, 3015, 2120, 1644 cm^{-1}.

(3*R*,4*S*,5*R*)-4-Methyloct-1-en-7-yne-3,5-diol (19g): $[\alpha]_D$ +26.9 (*c* = 0.68, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 1.01 (3 H, d, *J* = 7.3 Hz), 1.86 (1 H, m), 2.03 (1 H, t, *J* = 2.8 Hz), 2.33 (1 H, ddd, *J* = 16.8, 6.1, 2.8 Hz), 2.48 (1 H, ddd, *J* = 16.8, 7.9, 2.4 Hz), 2.49 (1 H, d, *J* = 2.4 Hz), 2.79 (1 H, d, *J* = 3.4 Hz), 4.17 (2 H, m), 5.23 (1 H, dt, *J* = 10.4, 1.5 Hz), 5.33 (1 H, dt, *J* = 17.1, 1.5 Hz), 5.92 (1 H, ddd, *J* = 17.1, 10.4, 5.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 10.4 (q), 24.3 (t), 40.5 (d), 70.3 (d), 76.7 (d × 2), 81.2 (s), 115.8 (t), 138.8 (d); IR (neat) 3385, 3308, 3085, 2120, 1644 cm^{-1}.

(3*S*,4*S*,5*R*)-4-Methyloct-1-en-7-yne-3,5-diol (19h): $[\alpha]_D$ −24.3 (*c* = 0.84, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 0.93 (3 H, d, *J* = 7.0 Hz), 1.85 (1 H, ddq, *J* = 2.8, 2.4, 7.0 Hz), 2.04 (1 H, t, *J* = 2.8 Hz), 2.37 (1 H, ddd, *J* = 16.8, 6.7, 2.8 Hz), 2.41 (1 H, d, *J* = 3.4 Hz), 2.50 (1 H, ddd, *J* = 16.8, 7.0, 2.4 Hz), 2.97 (1 H, d, *J* = 2.4 Hz), 4.10 (1 H, ddt, *J* = 10.1, 7.0, 2.4 Hz), 4.44 (1 H, m), 5.20 (1 H, dt, *J* = 10.7, 1.5 Hz), 5.29 (1 H, dt, *J* = 17.4, 1.5 Hz), 5.90 (1 H, ddd, *J* = 17.4, 10.7, 6.7 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 4.7 (q), 24.8 (t), 40.3 (d), 70.4 (d), 74.0 (d), 76.8 (d), 81.0 (s), 114.9 (t), 139.4 (d); IR (neat) 3424, 3308, 3018, 2120, 1640 cm^{-1}.

(3*R*,4*R*,5*R*)-3,5-Bis[(*tert*-butyldimethylsilyl)oxy]-4-methyloct-1-en-7-yne (20a). To a solution of **19a** (58 mg, 0.38 mmol) in CH_2Cl_2 (5 mL) was added 2,6-lutidine (0.18 mL, 1.5 mmol) and subsequently TBSOTf (0.34 mL, 1.5 mmol) at 0 °C. The mixture was stirred for 1 h, water was added to the solution, and the whole was extracted with ethyl acetate. The organic phase was washed with brine, dried over magnesium sulfate and filtered. The solvent was removed under reduced pressure to give a residue, from which **20a** (141 mg) was separated by silica gel column chromatography (2% ethyl acetate−*n*-hexane) as a colorless oil in 98% yield: $[\alpha]_D$ +4.6 (*c* = 1.30, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 0.01 (3 H, s), 0.05 (3 H, s), 0.07 (3 H, s), 0.11 (3 H, s), 0.88 (3 H, d, *J* = 7.0 Hz), 0.89 (9 H, s), 0.90 (9 H, s), 1.78 (1 H, dquint, *J* = 4.9, 7.0 Hz), 1.93 (1 H, t, *J* = 2.8 Hz), 2.26 (1 H, ddd, *J* = 16.8, 7.0, 2.8 Hz), 2.40 (1 H, ddd, *J* = 16.8, 4.3, 2.8 Hz), 3.86 (1 H, dt, *J* = 7.0, 4.3 Hz), 4.11 (1 H, ddt, *J* = 7.3, 5.8, 1.8 Hz), 5.09 (1 H, dt, *J* = 7.0, 4.3 Hz), 5.14 (1 H, dt, *J* = 17.4, 1.8 Hz), 5.84 (1 H, ddd, *J* = 17.4, 10.1, 7.3 Hz), 5.94 (1 H, ddd, *J* = 17.1, 10.7, 5.5 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ −4.7 (q), −4.6 (q), −4.2 (q), −3.8 (q), 9.7 (q), 18.12 (s), 18.23 (s), 23.7 (t), 25.87 (q), 25.94

(q), 46.1 (d), 69.6 (d), 72.0 (d), 75.5 (d), 82.6 (s), 115.2 (t), 140.9 (d); IR (neat) 3310, 3018, 2957, 2932, 2120, 1642, 1520, 1472, 1215, 837, 752, 669 cm^{-1}; MS *m/z* 382 $(M)^+$, 367 $(M - Me)^+$, 325 $(M - {}^tBu)^+$; HRMS *m/z* $(M)^+$ calcd for $C_{21}H_{42}O_2Si_2$ 382.2724, found 382.2725.

The following seven compounds (**20b**−**h**) were prepared by the same procedure as described for **20a**.

(3*S*,4*R*,5*R*)-3,5-Bis[(*tert*-butyldimethylsilyl)oxy]-4-methyloct-1-en-7-yne (20b): $[\alpha]_D$ −10.4 (*c* = 0.96, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 0.02 (3 H, s), 0.057 (3 H, s), 0.063 (3 H, s), 0.11 (3 H, s), 0.78 (3 H, d, *J* = 7.0 Hz), 0.86 (6 H, s), 0.90 (12 H, s), 1.89 (1 H, dquint, *J* = 5.5, 7.0 Hz), 1.93 (1 H, t, *J* = 2.8 Hz), 2.26 (1 H, ddd, *J* = 16.8, 7.0, 2.8 Hz), 2.39 (1 H, ddd, *J* = 16.8, 4.0, 2.8 Hz), 3.97 (1 H, ddd, *J* = 6.7, 5.2, 4.0 Hz), 4.12 (1 H, ddt, *J* = 6.7, 6.4, 1.2 Hz), 5.09 (1 H, dq, *J* = 10.4, 1.2 Hz), 5.16 (1 H, dq, *J* = 17.1, 1.5 Hz), 5.75 (1 H, ddd, *J* = 17.1, 10.4, 6.1 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ −4.9 (q), −4.7 (q), −4.2 (q), −3.0 (q), 9.8 (q), 18.11 (s), 18.13 (s), 23.6 (t), 25.87 (q), 25.89 (q), 45.0 (d), 69.5 (d), 71.4 (d), 74.9 (d), 82.5 (s), 115.2 (t), 139.6 (d); IR (neat) 3310, 3018, 2957, 2932, 2120, 1520, 1256, 1215 cm^{-1}; MS *m/z* 382 $(M)^+$, 367 $(M - Me)^+$, 325 $(M - {}^tBu)^+$; HRMS *m/z* $(M)^+$ calcd for $C_{21}H_{42}O_2Si_2$ 382.2724, found 382.2719.

(3*R*,4*R*,5*S*)-3,5-Bis[(*tert*-butyldimethylsilyl)oxy]-4-methyloct-1-en-7-yne (20c): $[\alpha]_D$ +3.5 (*c* = 2.06, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 0.01 (3 H, s), 0.049 (3 H, s), 0.051 (3 H, s), 0.08 (3 H, s), 0.89 (18 H, s), 0.92 (3 H, d, *J* = 7.0 Hz), 1.89 (1 H, dquint, *J* = 4.0, 6.7 Hz), 1.95 (1 H, t, *J* = 2.8 Hz), 2.38 (2 H, dd, *J* = 5.8, 2.8 Hz), 3.88 (1 H, ddd, *J* = 6.4, 6.1, 4.0 Hz), 5.10 (1 H, dq, *J* = 10.4, 0.9 Hz), 5.14 (1 H, dq, *J* = 17.4, 0.9 Hz), 5.81 (1 H, ddd, *J* = 17.4, 10.4, 7.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ −4.8 (q), −4.6 (q), −4.0 (q), −3.9 (q), 9.7 (q), 9.4 (q), 18.14 (s), 18.23 (s), 25.5 (t), 25.90 (q), 25.93 (q), 46.1 (d), 70.1 (d), 71.0 (d), 75,2 (d), 81.8 (s), 115.3 (t), 140.8 (d); IR (neat) 3081, 2957, 2124, 1646 cm^{-1}; MS *m/z* 382 $(M)^+$, 367 $(M - Me)^+$, 325 $(M - {}^tBu)^+$; HRMS *m/z* $(M)^+$ calcd for $C_{21}H_{42}O_2Si_2$ 382.2724, found 382.2723.

(3*S*,4*R*,5*S*)-3,5-Bis[(*tert*-butyldimethylsilyl)oxy]-4-methyloct-1-en-7-yne (20d): $[\alpha]_D$ −5.8 (*c* = 0.79, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 0.03 (3 H, s), 0.06 (3 H, s), 0.07 (3 H, s), 0.08 (3 H, s), 0.76 (3 H, d, *J* = 7.0 Hz), 0.889 (9 H, s), 0.892 (9 H, s), 1.91 (1 H, dquint, *J* = 3.7, 7.0 Hz), 1.97 (1 H, t, *J* = 2.8 Hz), 2.38 (2 H, m), 4.02 (2 H, m), 5.09 (1 H, dt, *J* = 10.4, 0.9 Hz), 5.13 (1 H, dt, *J* = 17.1, 0.9 Hz), 5.73 (1 H, ddd, *J* = 17.1, 10.1, 7.6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ −4.6 (q), −4.5 (q), −4.0 (q), −3.4 (q), 9.1 (q), 18.1 (s), 18.2 (s), 23.8 (t), 25.86 (q), 25.95 (q), 43.7 (d), 70.1 (d), 70.6 (d), 76.0 (d), 81.5 (s), 115.9 (t), 140.0 (d); IR (neat) 3316, 3079, 2957, 2932, 2887, 2859, 2122, 1645, 1472, 1462, 1254, 1075, 837, 775 cm^{-1}; MS *m/z* 382 $(M)^+$, 367 $(M - Me)^+$, 325 $(M - {}^tBu)^+$; HRMS *m/z* $(M)^+$ calcd for $C_{21}H_{42}O_2Si_2$ 382.2724, found 382.2724.

(3*R*,4*S*,5*S*)-3,5-Bis[(*tert*-butyldimethylsilyl)oxy]-4-methyloct-1-en-7-yne (20e): $[\alpha]_D$ +11.3 (*c* = 1.05, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 0.01 (3 H, s), 0.06 (3 H, s), 0.06 (3 H, s), 0.11 (3 H, s), 0.78 (3 H, d, *J* = 7.0 Hz), 0.86 (9 H, s), 0.90 (9 H, s), 1.88 (1 H, dquint, *J* = 5.5, 6.7 Hz), 1.93 (1 H, t, *J* = 2.8 Hz), 2.26 (1 H, ddd, *J* = 16.8, 7.0, 2.4 Hz), 4.12 (1 H, ddt, *J* = 6.7, 5.2, 1.8 Hz), 5.09 (1 H, dq, *J* = 10.4, 1.2 Hz), 5.15 (1 H, dq, *J* = 17.1, 1.2 Hz), 5.75 (1 H, ddd, *J* = 17.1, 10.4, 6.7 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ −4.9 (q), −4.7 (q), −4.2 (q), −4.1 (q), 9.9 (q), 18.13 (s), 18.15 (s), 23.5 (t), 25.88 (q), 25.91 (q), 45.0 (d), 69.5 (d), 71.4 (d), 75.0 (d), 82.5 (s), 115.2 (t), 139.6 (d); IR (neat) 3310, 3020, 2957, 2932, 2121, 1520, 1256, 1215 cm^{-1}; MS *m/z* 382 $(M)^+$, 367 $(M - Me)^+$, 325 $(M - {}^tBu)^+$; HRMS *m/z* $(M)^+$ calcd for $C_{21}H_{42}O_2Si_2$ 382.2724, found 382.2719.

(3*S*,4*S*,5*S*)-3,5-Bis[(*tert*-butyldimethylsilyl)oxy]-4-methyloct-1-en-7-yne (20f): $[\alpha]_D$ −4.9 (*c* = 1.30, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 0.01 (3 H, s), 0.05 (3 H, s), 0.07 (3 H, s), 0.10 (3 H, s), 0.88 (3 H, d, *J* = 7.0 Hz), 0.89 (9 H, s), 0.90 (9 H, s), 1.78 (1 H, m), 1.93 (1 H, t, *J* = 2.8 Hz), 2.26 (1 H, ddd, *J* = 16.8, 7.0, 2.8 Hz), 2.40 (1 H, ddd, *J* = 16.8, 4.3, 2.8 Hz), 3.85 (1 H, dt, *J* = 7.0, 4.3 Hz), 4.11 (1 H, ddt, *J* = 7.3, 5.8, 1.5 Hz), 5.10 (1 H, dq, *J* = 10.1, 0.6 Hz), 5.14 (1 H, dq, *J* = 17.4, 0.9 Hz), 5.84 (1 H, ddd, *J* = 17.4, 10.1, 7.3 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ −4.64 (q), −4.56 (q), −4.2 (q), −3.8 (q), 9.7 (q), 18.14 (s), 18.25 (s), 23.8 (t), 25.89 (q), 25.96 (q), 46.1 (d), 69.6 (d), 72.0 (d), 75.5 (d), 82.6 (s), 115.2 (t), 140.9 (d); IR (neat) 3310, 3019, 2957, 2932, 2120, 1644, 1520, 1472, 1256, 1215, 837, 752, 669 cm^{-1}; MS *m/z* 382 $(M)^+$, 367 $(M - Me)^+$, 325 $(M - {}^tBu)^+$; HRMS *m/z* $(M)^+$ calcd for $C_{21}H_{42}O_2Si_2$ 382.2724, found 382.2727.

(3*R*,4*S*,5*R*)-3,5-Bis[(*tert*-butyldimethylsilyl)oxy]-4-methyloct-1-en-7-yne (20g): $[\alpha]_D$ +10.3 (*c* = 1.15, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 0.03 (3 H, s), 0.06 (3 H, s), 0.07 (3 H, s), 0.08 (3 H, s), 0.76 (3 H, d, *J* = 7.0 Hz), 0.889 (9 H, s), 0.891 (9 H, s), 1.90 (1 H, m), 1.97 (1 H, t, *J* = 2.8 Hz), 2.38 (2 H, m), 4.01 (2 H, m), 5.10 (1 H, dq, *J* = 10.1, 0.9 Hz), 5.13 (1 H, dq, *J* = 17.1, 0.9 Hz), 5.74 (1 H, ddd, *J* = 17.1, 10.1, 7.6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ −4.6 (q), −4.5 (q), −4.0 (q), −3.4 (q), 9.5 (q), 18.15 (s), 18.22 (s), 25.5 (t), 25.9 (q), 26.0 (q), 43.8 (d), 70.1 (d), 70.6 (d), 76.0 (d), 81.6 (s), 115.9 (t), 140.0 (d); IR (neat) 3310, 3021, 2958, 2930, 2121, 1524, 1256, 1215 cm^{-1}; MS *m/z* 382 $(M)^+$, 367 $(M - Me)^+$, 325 $(M - {}^tBu)^+$; HRMS *m/z* $(M)^+$ calcd for $C_{21}H_{42}O_2Si_2$ 382.2724, found 382.2724.

(3*S*,4*S*,5*R*)-3,5-Bis[(*tert*-butyldimethylsilyl)oxy]-4-methyloct-1-en-7-yne (20h): $[\alpha]_D$ −0.3 (*c* = 1.85, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 0.01 (3 H, s), 0.049 (3 H, s), 0.051 (3 H, s), 0.08 (3 H, s), 0.89 (18 H, s), 0.92 (3 H, d, *J* = 7.0 Hz), 1.85 (1 H, m), 1.96 (1 H, t, *J* = 2.8 Hz), 2.39 (2 H, dd, *J* = 6.7, 2.8 Hz), 3.88 (1 H, ddd, *J* = 6.6, 6.1, 4.0 Hz), 4.08 (1 H, m), 5.10 (1 H, dq, *J* = 10.4, 0.9 Hz), 5.14 (1 H, dq, *J* = 17.4, 0.9 Hz), 5.81 (1 H, ddd, *J* = 17.4, 10.4, 7.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ −4.8 (q), −4.6 (q), −4.0 (q), −3.8 (q), 9.4 (q), 18.15 (s), 18.24 (s), 25.5 (t), 25.91 (q), 25.94 (q), 43.9 (d), 70.0 (d), 70.9 (d), 75.3 (d), 81.8 (s), 115.3 (t), 140.8 (d); IR (neat) 3310, 3020, 2957, 2932, 2121, 1520, 1256, 1215 cm^{-1}; MS *m/z* 367 $(M - Me)^+$, 325 $(M - {}^tBu)^+$; HRMS *m/z* $(M - Me)^+$ calcd for $C_{20}H_{39}O_2Si_2$ 367.2488, found 367.2487.

(5*Z*,7*E*)-(1*S*,2*R*,3*R*)-2-Methyl-9,10-*seco*-5,7,10(19)-cholestatriene-1,3,25-triol (2a). To a mixture of $Pd_2(dba)_3 \cdot CHCl_3$ (13 mg, 0.012 mmol), PPh_3 (30 mg, 0.12 mmol) and Et_3N (1.5 mL) was added a solution of **5a** (44 mg, 0.12 mmol) and **20a** (61 mg, 0.16 mmol) in toluene (3 mL). The resulting mixture was stirred at room temperature for 10 min and subsequently heated to reflux for 6 h. Water was added to the cooled reaction mixture, and the whole was extracted with ether. The organic layer was washed with brine, dried over magnesium sulfate and filtered. Evaporation of the filtrate afforded a residue, from which the silylated vitamin was separated by silica gel column chromatography (10% ethyl acetate−*n*-hexane) as a colorless oil. The product thus obtained was dissolved in methanol (3 mL) and treated with CSA (28 mg, 0.12 mmol). The resulting mixture was stirred for 16 h at room temperature. The reaction mixture was poured into satd $NaHCO_3$ (aq) and the whole was extracted with ether. The extract was washed with brine, dried over magnesium sulfate and filtered. The solvent was evaporated under reduced pressure to give a residue, from which **2a** (20 mg) was separated by silica gel column chromatography (50% ethyl acetate−*n*-hexane) as a white solid in 41% yield. Further purification for biological evaluation was conducted by using reversed-phase recycle HPLC (YMC-Pack ODS column, 20 × 150 mm, 9.0 mL/min, acetonitrile:water = 70:30). Analytical HPLC was conducted by using reversed-phase HPLC (Lichrosorb RP-18 column, 4 × 250 mm, 1.0 mL/min, acetonitrile: water = 60:40) to give **2a** (t_R = 18.28 min) as a single homogeneous peak: $[\alpha]_D$ −35.2 (*c* = 0.46, $CHCl_3$); UV (EtOH) λ_{max} 263 nm, λ_{min} 227 nm; ^1H NMR (400 MHz, $CDCl_3-D_2O$) δ 0.55 (3 H, s), 0.94 (3 H, d, *J* = 6.3 Hz), 1.15 (3 H, d, *J* = 7.0 Hz), 1.22 (6 H, s), 1.78 (1 H, ddq, *J* = 9.2, 2.6, 7.0 Hz), 2.42 (1 H, dd, *J* = 13.6, 4.8 Hz), 2.52 (1 H, br. d, *J* = 13.6 Hz), 2.82 (1 H, dd, *J* = 11.9, 3.7 Hz), 4.01 (1 H, d, *J* = 9.2 Hz), 4.03 (1 H, dt, *J* = 4.8, 2.6 Hz), 5.02 (1 H, t, *J* = 1.8 Hz), 5.37 (1 H, t, *J* = 1.8 Hz), 6.03 (1 H, d, *J* = 11.2 Hz), 6.35 (1 H, d, *J* = 11.2 Hz); ^1H NMR (500 MHz, $CDCl_3$) δ 0.55 (3 H, s), 0.94 (3 H, d, *J* = 6.5 Hz), 1.15 (3 H, d, *J* = 6.9 Hz), 1.22 (6 H, s), 1.78 (1 H, ddq, *J* = 9.4, 2.6, 6.9 Hz), 2.42 (1 H, dd, *J* = 13.9, 4.8 Hz), 2.53 (1 H, br. d, *J* = 13.7 Hz), 2.82 (1 H, dd, *J* = 11.9, 3.9 Hz), 4.02 (2

H, m), 5.02 (1 H, t, *J* = 1.8 Hz), 5.37 (1 H, t, *J* = 1.8 Hz), 6.03 (1 H, d, *J* = 11.3 Hz), 6.35 (1 H, d, *J* = 11.7 Hz); ^{1}H NMR (500 MHz, CD_3OD) δ 0.57 (3 H, s), 0.96 (3 H, d, *J* = 6.5 Hz), 1.07 (3 H, d, *J* = 6.9 Hz), 1.17 (6 H, s), 1.76 (1 H, ddq, *J* = 9.5, 2.6, 6.9 Hz), 2.37 (1 H, dd, *J* = 14.0, 5.6 Hz), 2.42 (1 H, br. d, *J* = 14.0 Hz), 2.85 (1 H, dd, *J* = 12.5, 4.5 Hz), 3.95 (1 H, d, *J* = 9.0 Hz), 3.98 (1 H, dt, *J* = 5.6, 2.6 Hz), 4.97 (1 H, t, *J* = 2.2 Hz), 5.38 (1 H, t, *J* = 1.9 Hz), 6.08 (1 H, d, *J* = 11.9 Hz), 6.27 (1 H, d, *J* = 11.0 Hz); ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.50 (3 H, s), 0.90 (3 H, d, *J* = 6.5 Hz), 0.94 (3 H, d, *J* = 6.8 Hz), 1.04 (6 H, s), 1.58 (1 H, ddq, *J* = 9.8, 2.7, 6.8 Hz), 2.24 (2 H, m), 2.77 (1 H, dd, *J* = 13.3, 5.2 Hz), 3.76 (1 H, d, *J* = 9.0 Hz), 3.81 (1 H, dt, *J* = 7.0, 3.3 Hz), 4.76 (1 H, dd, *J* = 2.7, 1.2 Hz), 5.26 (1 H, dd, *J* = 2.7, 1.8 Hz), 5.97 (1 H, d, *J* = 11.6 Hz), 6.14 (1 H, d, *J* = 11.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 11.9 (q), 13.8 (q), 18.7 (q), 20.7 (t), 22.2 (t), 23.6 (t), 27.6 (t), 29.0 (t), 29.2 (q), 29.3 (q), 36.0 (d), 36.3 (t), 40.4 (t), 43.6 (t), 44.4 (t), 44.6 (d), 45.9 (s), 56.3 (d), 56.5 (d), 70.9 (d), 71.1 (s), 74.7 (d), 111.0 (t), 117.0 (d), 124.9 (d), 133.4 (s), 143.1 (s), 147.5 (s); MS *m/z* 430 $(M)^+$ 412 $(M - H_2O)^+$, 394 $(M - 2H_2O)^+$, 376 $(M - 3H_2O)^+$; HRMS *m/z* $(M)^+$ calcd for $C_{28}H_{46}O_3$ 430.3447, found 430.3450.

The following seven compounds (**2b**−**h**) were prepared by the same procedure as described for **2a**.

(5*Z*,7*E*)-(1*R*,2*R*,3*R*)-2-Methyl-9,10-*seco*-5,7,10(19)-cholestatriene-1,3,25-triol (2b): t_R = 28.05 min; $[\alpha]_D$ −44.8 (*c* = 0.30, $CHCl_3$); UV (EtOH) λ_{max} 264 nm, λ_{min} 226 nm; ^{1}H NMR (400 MHz, $CDCl_3-D_2O$) δ 0.56 (3 H, s), 0.94 (3 H, d, *J* = 6.2 Hz), 1.22 (6 H, s), 1.23 (3 H, d, *J* = 7.0 Hz), 1.90 (1 H, tq, *J* = 2.4, 7.0 Hz), 2.50 (1 H, br. d, *J* = 14.0 Hz), 2.59 (1 H, dd, *J* = 14.0, 3.4 Hz), 2.85 (1 H, dd, *J* = 12.1, 3.4 Hz), 3.90 (1 H, dd, *J* = 3.4, 2.4 Hz), 4.17 (1 H, d, *J* = 2.4 Hz), 5.01 (1 H, d, *J* = 1.8 Hz), 5.25 (1 H, d, *J* = 2.1 Hz), 6.09 (1 H, d, *J* = 11.4 Hz), 6.48 (1 H, d, *J* = 11.4 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 11.9 (q), 13.9 (q), 18.8 (q), 20.7 (t), 22.3 (t), 23.6 (t), 27.6 (t), 29.1 (t), 29.2 (q), 29.3 (q), 36.1 (d), 36.4 (t), 40.1 (t), 40.4 (t), 44.4 (t), 45.1 (t), 45.9 (s), 56.3 (d), 56.5 (d), 71.1 (s), 72.6 (d), 78.9 (d), 114.3 (t), 117.0 (d), 126.3 (d), 131.1 (s), 143.2 (s), 147.4 (s); MS *m/z* 430 $(M)^+$, 412 $(M - H_2O)^+$, 394 $(M - 2H_2O)^+$, 376 $(M - 3H_2O)^+$; HRMS *m/z* $(M)^+$ calcd for $C_{28}H_{46}O_3$ 430.3447, found 430.3446.

(5*Z*,7*E*)-(1*S*,2*R*,3*S*)-2-Methyl-9,10-*seco*-5,7,10(19)-cholestatriene-1,3,25-triol (2c): t_R = 18.98 min; $[\alpha]_D$ +58.0 (*c* = 0.83, $CHCl_3$); UV (EtOH) λ_{max} 261 nm, λ_{min} 226 nm; ^{1}H NMR (400 MHz, $CDCl_3-D_2O$) δ 0.54 (3 H, s), 0.94 (3 H, d, *J* = 6.4 Hz), 1.05 (3 H, d, *J* = 7.0 Hz), 1.22 (6 H, s), 2.00 (1 H, tq, *J* = 5.9, 7.0 Hz), 2.34 (1 H, dd, *J* = 13.7, 6.0 Hz), 2.64 (1 H, dd, *J* = 13.7, 3.3 Hz), 2.83 (1 H, dd, *J* = 12.2, 4.0 Hz), 3.65 (1 H, dt, *J* = 3.3, 6.0 Hz), 3.90 (1 H, d, *J* = 5.9 Hz), 5.05 (1 H, d, *J* = 1.5 Hz), 5.31 (1 H, d, *J* = 1.5 Hz), 6.02 (1 H, d, *J* = 11.4 Hz), 6.41 (1 H, d, *J* = 11.4 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 11.9 (q), 14.9 (q), 18.7 (q), 20.7 (t), 22.2 (t), 23.5 (t), 27.6 (t), 29.0 (t), 29.2 (q), 29.3 (q), 36.0 (d), 36.4 (t), 40.5 (t), 42.7 (t), 44.2 (d), 44.4 (t), 45.9 (s), 56.3 (d), 56.5 (d), 71.1 (s), 73.3 (d), 77.9 (d), 113.5 (t), 117.0 (d), 125.0 (d), 131.9 (s), 143.2 (s), 146.1 (s); MS *m/z* 430 $(M)^+$, 412 $(M - H_2O)^+$, 394 $(M - 2H_2O)^+$, 376 $(M - 3H_2O)^+$; HRMS *m/z* $(M)^+$ calcd for $C_{28}H_{46}O_3$ 430.3447, found 430.3447.

(5*Z*,7*E*)-(1*R*,2*R*,3*S*)-2-Methyl-9,10-*seco*-5,7,10(19)-cholestatriene-1,3,25-triol (2d): t_R = 18.39 min; $[\alpha]_D$ −11.2 (*c* = 0.43, $CHCl_3$); UV (EtOH) λ_{max} 265 nm, λ_{min} 226 nm; ^{1}H NMR (400 MHz, $CDCl_3-D_2O$) δ 0.55 (3 H, s), 0.94 (3 H, d, *J* = 6.4 Hz), 1.11 (3 H, d, *J* = 7.0 Hz), 1.22 (6 H, s), 1.87 (1 H, ddq, *J* = 8.4, 3.3, 7.0), 2.24 (1 H, dd, *J* = 13.2, 8.4 Hz), 2.66 (1 H, dd, *J* = 13.2, 4.3 Hz), 2.82 (1 H, dd, *J* = 11.8, 3.6 Hz), 3.81 (1 H, dt, *J* = 4.3, 8.4 Hz), 4.26 (1 H, d, *J* = 3.3 Hz), 5.02 (1 H, d, *J* = 1.7 Hz), 5.28 (1 H, d, *J* = 1.7 Hz), 6.02 (1 H, d, *J* = 11.4 Hz), 6.40 (1 H, d, *J* = 11.4 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 11.9 (q), 12.8 (q), 18.8 (q), 20.8 (t), 22.3 (t), 23.6 (t), 27.6 (t), 29.0 (t), 29.2 (q), 29.3 (q), 36.0 (d), 36.4 (t), 40.5 (t), 44.1 (t), 44.4 (t), 44.5 (d), 45.9 (s), 56.3 (d), 56.5 (d), 71.1 (s), 71.6 (d), 76.1 (d), 113.7 (t), 117.0 (d), 124.9 (d), 133.0 (s), 143.3 (s), 146.8 (s); MS *m/z* 430 $(M)^+$, 412 $(M - H_2O)^+$, 394 $(M - 2H_2O)^+$, 376 $(M - 3H_2O)^+$; HRMS *m/z* $(M)^+$ calcd for $C_{28}H_{46}O_3$ 430.3447, found 430.3445.

(5*Z*,7*E*)-(1*S*,2*S*,3*S*)-2-Methyl-9,10-*seco*-5,7,10(19)-cholestatriene-1,3,25-triol (2e): t_R = 27.11 min; $[\alpha]_D$ +87.0 (*c* = 0.30, $CHCl_3$); UV (EtOH) λ_{max} 265 nm, λ_{min} 227 nm; ^{1}H NMR (400 MHz, $CDCl_3-D_2O$) δ 0.54 (3 H, s), 0.93 (3 H, d, *J* = 6.4 Hz), 1.22 (6 H, s), 1.22 (3 H, d, *J* = 7.0 Hz), 1.92 (1 H, tq, *J* = 2.7, 7.0 Hz), 2.50 (1 H, br. d, *J* = 14.0 Hz), 2.58 (1 H, dd, *J* = 14.0, 4.0 Hz), 2.85 (1 H, dd, *J* = 12.2, 3.4 Hz), 3.90 (1 H, dt, *J* = 4.0, 2.7 Hz), 4.17 (1 H, d, *J* = 2.7 Hz), 4.98 (1 H, d, *J* = 1.8 Hz), 5.23 (1 H, d, *J* = 1.8 Hz), 6.03 (1 H, d, *J* = 11.0 Hz), 6.48 (1 H, d, *J* = 11.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.0 (q), 13.5 (q), 18.7 (q), 20.8 (t), 22.2 (t), 23.4 (t), 27.6 (t), 29.0 (t), 29.1 (q), 29.3 (q), 36.1 (d), 36.4 (t), 40.2 (d), 40.5 (t), 44.4 (t), 44.9 (t), 45.9 (s), 56.3 (d), 56.5 (d), 71.1 (s), 72.5 (d), 78.7 (d), 114.1 (t), 126.3 (d), 130.9 (d), 143.2 (s), 147.2 (s); MS *m/z* 430 $(M)^+$, 412 $(M - H_2O)^+$, 394 $(M - 2H_2O)^+$, 376 $(M - 3H_2O)^+$; HRMS *m/z* $(M)^+$ calcd for $C_{28}H_{46}O_3$ 430.3447, found 430.3446.

(5*Z*,7*E*)-(1*R*,2*S*,3*S*)-2-Methyl-9,10-*seco*-5,7,10(19)-cholestatriene-1,3,25-triol (2f): t_R = 18.61 min; $[\alpha]_D$ +27.3 (*c* = 0.10, $CHCl_3$); UV (EtOH) λ_{max} 264 nm, λ_{min} 228 nm; ^{1}H NMR (400 MHz, $CDCl_3-D_2O$) δ 0.54 (3 H, s), 0.93 (3 H, d, *J* = 6.4 Hz), 1.12 (3 H, d, *J* = 7.0 Hz), 1.22 (6 H, s), 1.86 (1 H, ddq, *J* = 8.4, 2.9, 7.0 Hz), 2.42 (1 H, dd, *J* = 13.6, 5.9 Hz), 2.51 (1 H, dd, *J* = 13.6, 2.9 Hz), 2.82 (1 H, dd, *J* = 12.2, 2.4 Hz), 4.04 (1 H, d, *J* = 8.4 Hz), 4.06 (1 H, dt, *J* = 5.9, 2.9 Hz), 5.01 (1 H, t, *J* = 1.8 Hz), 5.35 (1 H, t, *J* = 1.8 Hz), 6.00 (1 H, d, *J* = 11.2 Hz), 6.36 (1 H, d, *J* = 11.2 Hz); MS *m/z* 430 $(M)^+$, 412 $(M - H_2O)^+$, 394 $(M - 2H_2O)^+$, 376 $(M - 3H_2O)^+$; HRMS *m/z* $(M)^+$ calcd for $C_{28}H_{46}O_3$ 430.3447, found 430.3447.

(5*Z*,7*E*)-(1*S*,2*S*,3*R*)-2-Methyl-9,10-*seco*-5,7,10(19)-cholestatriene-1,3,25-triol (2g): t_R = 19.60 min; mp 136 °C (recrystallization from ethyl acetate); $[\alpha]_D$ +41.1 (*c* = 0.20, $CHCl_3$); UV (EtOH) λ_{max} 265 nm, λ_{min} 226 nm; ^{1}H NMR (400 MHz, $CDCl_3-D_2O$) δ 0.53 (3 H, s), 0.93 (3 H, d, *J* = 6.4 Hz), 1.08 (3 H, d, *J* = 7.0 Hz), 1.22 (6 H, s), 1.92 (1 H, ddq, *J* = 7.7, 3.3, 7.2 Hz), 2.23 (1 H, dd, *J* = 13.2, 7.7 Hz), 2.67 (1 H, dd, *J* = 13.2, 4.0 Hz), 2.82 (1 H, dd, *J* = 12.5, 4.0 Hz), 3.84 (1 H, dt, *J* = 4.0, 7.4 Hz), 4.30 (1 H, d, *J* = 3.3 Hz), 5.01 (1 H, d, *J* = 1.8 Hz), 5.28 (1 H, dd, *J* = 1.8, 1.1 Hz), 6.01 (1 H, d, *J* = 11.4 Hz), 6.39 (1 H, d, *J* = 11.4 Hz); ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.53 (3 H, s), 0.93 (3 H, d, *J* = 6.5 Hz), 1.08 (3 H, d, *J* = 6.9 Hz), 1.22 (6 H, s), 1.92 (1 H, ddq, *J* = 7.8, 3.5, 6.9 Hz), 2.24 (1 H, dd, *J* = 13.3, 7.7 Hz), 2.67 (1 H, dd, *J* = 13.5, 4.0 Hz), 2.82 (1 H, dd, *J* = 12.8, 4.4 Hz), 3.84 (1 H, m), 4.31 (1 H, dd, *J* = 3.5, 4.2 Hz), 5.01 (1 H, d, *J* = 1.6 Hz), 5.28 (1 H, br. s), 6.01 (1 H, d, *J* = 11.3 Hz), 6.39 (1 H, d, *J* = 11.2 Hz); ^{1}H NMR (500 MHz, CD_3OD) δ 0.56 (3 H, s), 0.96 (3 H, d, *J* = 6.5 Hz), 1.03 (3 H, d, *J* = 6.9 Hz), 1.16 (6 H, s), 1.79 (1 H, ddq, *J* = 8.1, 3.5, 6.9 Hz), 2.17 (1 H, dd, *J* = 13.2, 8.2 Hz), 2.59 (1 H, dd, *J* = 13.3, 4.1 Hz), 2.86 (1 H, dd, *J* = 12.4, 3.9 Hz), 3.72 (1 H, dt, *J* = 4.2, 8.1 Hz), 4.22 (1 H, d, *J* = 3.5 Hz), 4.89 (1 H, d, *J* = 2.5 Hz), 5.22 (1 H, d, *J* = 1.8 Hz), 6.08 (1 H, d, *J* = 11.2 Hz), 6.32 (1 H, d, *J* = 11.0 Hz); ^{1}H NMR (500 MHz, DMSO-d_6) δ 0.49 (3 H, s), 0.84 (3 H, d, *J* = 6.9 Hz), 0.90 (3 H, d, *J* = 6.4 Hz), 1.04 (6 H, s), 1.73 (1 H, ddq, *J* = 6.8, 3.7, 6.4 Hz), 2.05 (1 H, dd, *J* = 13.3, 7.1 Hz), 2.45 (1 H, dd, *J* = 13.3, 3.4 Hz), 2.78 (1 H, dd, *J* = 13.3, 4.6 Hz), 3.60 (1 H, dt, *J* = 4.2, 7.1 Hz), 4.13 (1 H, d, *J* = 3.4 Hz), 4.76 (1 H, d, *J* = 2.6 Hz), 5.16 (1 H, d, *J* = 2.0 Hz), 5.98 (1 H, d, *J* = 11.3 Hz), 6.18 (1 H, d, *J* = 11.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 11.9 (q), 12.4 (q), 18.7 (q), 20.7 (t), 20.7 (t), 22.2 (t), 23.5 (t), 27.6 (t), 29.0 (q), 29.2 (q), 29.3 (q), 36.0 (d), 36.4 (t), 40.5 (t), 43.5 (t), 44.2 (t), 44.4 (d), 45.9 (s), 56.3 (d), 56.5 (d), 71.1 (s), 71.7 (d), 76.1 (d), 75.5 (d), 113.2 (t), 117.0 (d), 124.9 (d), 133.0 (s), 143.3 (s), 146.7 (s); MS *m/z* 430 $(M)^+$, 412 $(M - H_2O)^+$, 394 $(M - 2H_2O)^+$, 376 $(M - 3H_2O)^+$; HRMS *m/z* $(M)^+$ calcd for $C_{28}H_{46}O_3$ 430.3447, found 430.3449. Anal. ($C_{28}H_{46}O_3 \cdot H_2O$) C, H.

(5*Z*,7*E*)-(1*R*,2*S*,3*R*)-2-Methyl-9,10-*seco*-5,7,10(19)-cholestatriene-1,3,25-triol (2h): t_R = 20.06 min; $[\alpha]_D$ −47.7 (*c* = 0.84, $CHCl_3$); UV (EtOH) λ_{max} 262 nm, λ_{min} 226 nm; ^{1}H NMR (400 MHz, $CDCl_3-D_2O$) δ 0.55 (3 H, s), 0.94 (3 H, d, *J* = 6.4 Hz), 1.02 (3 H, d, *J* = 7.0 Hz), 1.22 (6 H, s), 2.12 (1 H, tq, *J* = 5.1, 7.0 Hz), 2.36 (1 H, dd, *J* = 13.9, 5.1 Hz), 2.65 (1 H, dd, *J* = 13.9, 3.0 Hz), 2.83 (1 H, dd, *J* = 12.5, 4.3 Hz), 3.72 (1

H, dt, $J = 3.0, 5.1$ Hz), 3.96 (1 H, d, $J = 4.4$ Hz), 5.07 (1 H, d, $J = 1.7$ Hz), 5.30 (1 H, d, $J = 1.7$ Hz), 6.05 (1 H, d, $J = 11.3$ Hz), 6.42 (1 H, d, $J = 11.3$ Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 11.9 (q), 14.9 (q), 18.7 (q), 20.7 (t), 22.2 (t), 23.6 (t), 27.6 (t), 29.0 (t), 29.2 (q), 36.1 (d), 36.3 (t), 40.4 (t), 42.0 (t), 43.2 (d), 44.4 (t), 45.9 (s), 56.3 (d), 56.5 (d), 71.1 (s), 73.3 (d), 78.5 (d), 114.4 (t), 116.9 (d), 125.2 (d), 131.8 (s), 143.2 (s), 143.2 (s), 145.7 (s); MS m/z 430 (M)$^+$, 412 (M − H_2O)$^+$, 394 (M − 2H_2O)$^+$, 376 (M − 3H_2O)$^+$; HRMS m/z (M)$^+$ calcd for $C_{28}H_{46}O_3$ 430.3447, found 430.3447.

The following eight compounds (**3a**−**h**) were prepared by the same procedure as described for **2a** using **5b** instead of **5a**.

(5*Z*,7*E*)-(1*S*,2*R*,3*R*,20*S*)-2-Methyl-9,10-*seco*-5,7,10(19)-cholestatriene-1,3,25-triol (3a): $t_R = 16.16$ min; UV (EtOH) λ_{max} 263 nm, λ_{min} 228 nm; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.55 (3 H, s), 0.85 (3 H, d, $J = 6.4$ Hz), 1.15 (3 H, d, $J = 6.7$ Hz), 1.21 (6 H, s), 2.42 (1 H, dd, $J = 13.9, 4.9$ Hz), 2.52 (1 H, br. d, $J = 13.9$ Hz), 2.82 (1 H, dd, $J = 11.9, 4.0$ Hz), 4.02 (2 H, m), 5.02 (1 H, t, $J = 1.8$ Hz), 5.37 (1 H, t, $J = 1.8$ Hz), 6.03 (1 H, d, $J = 11.3$ Hz), 6.35 (1 H, d, $J = 11.3$ Hz); MS m/z 430 (M)$^+$, 412 (M − H_2O)$^+$, 394 (M − 2H_2O)$^+$, 376 (M − 3H_2O)$^+$; HRMS m/z (M)$^+$ calcd for $C_{28}H_{46}O_3$ 430.3447, found 430.3441.

(5*Z*,7*E*)-(1*R*,2*R*,3*R*,20*S*)-2-Methyl-9,10-*seco*-5,7,10(19)-cholestatriene-1,3,25-triol (3b): $t_R = 16.81$ min; UV (EtOH) λ_{max} 265 nm, λ_{min} 226 nm; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.55 (3 H, s), 0.85 (3 H, d, $J = 6.7$ Hz), 1.22 (6 H, s), 1.23 (3 H, d, $J = 7.3$ Hz), 2.17 (1 H, d, $J = 4.3$ Hz), 2.50 (1 H, br.d, $J = 14.0$ Hz), 2.59 (1 H, dd, $J = 14.0, 3.7$ Hz), 2.79 (1 H, d, $J = 7.6$ Hz), 2.85 (1 H, dd, $J = 12.5, 4.9$ Hz), 3.91 (1 H, m), 4.17 (1 H, m), 5.01 (1 H, d, $J = 2.1$ Hz), 5.25 (1 H, d, $J = 1.8$ Hz), 6.09 (1 H, d, $J = 11.3$ Hz), 6.48 (1 H, d, $J = 11.3$ Hz); MS m/z 430 (M)$^+$, 412 (M − H_2O)$^+$, 394 (M − 2H_2O)$^+$, 376 (M − 3H_2O)$^+$; HRMS m/z (M)$^+$ calcd for $C_{28}H_{46}O_3$ 430.3447, found 430.3446.

(5*Z*,7*E*)-(1*S*,2*R*,3*S*,20*S*)-2-Methyl-9,10-*seco*-5,7,10(19)-cholestatriene-1,3,25-triol (3c): $t_R = 16.88$ min; UV (EtOH) λ_{max} 262 nm, λ_{min} 228 nm; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.54 (3 H, s), 0.85 (3 H, d, $J = 6.4$ Hz), 1.06 (3 H, d, $J = 7.0$ Hz), 1.22 (6 H, s), 2.12 (1 H, d, $J = 2.8$ Hz), 2.34 (1 H, dd, $J = 14.7, 7.0$ Hz), 2.60 (1 H, br.s), 2.64 (1 H, dd, $J = 13.4, 2.8$ Hz), 2.84 (1 H, dd, $J = 11.6, 3.1$ Hz), 3.65 (1 H, m), 3.90 (1 H, m), 5.05 (1 H, d, $J = 1.8$ Hz), 5.30 (1 H, s), 6.02 (1 H, d, $J = 11.3$ Hz), 6.41 (1 H, d, $J = 11.3$ Hz); MS m/z 430 (M)$^+$, 412 (M − H_2O)$^+$, 394 (M − 2H_2O)$^+$, 376 (M − 3H_2O)$^+$; HRMS m/z (M)$^+$ calcd for $C_{28}H_{46}O_3$ 430.3447, found 430.3447.

(5*Z*,7*E*)-(1*R*,2*R*,3*S*,20*S*)-2-Methyl-9,10-*seco*-5,7,10(19)-cholestatriene-1,3,25-triol (3d): $t_R = 16.29$ min; UV (EtOH) λ_{max} 266 nm, λ_{min} 228 nm; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.54 (3 H, s), 0.85 (3 H, d, $J = 6.4$ Hz), 1.10 (3 H, d, $J = 6.7$ Hz), 1.22 (6 H, s), 1.68 (2 H, m), 1.85 (2 H, m), 1.98 (2 H, m), 2.24 (1 H, dd, $J = 13.4, 8.5$ Hz), 2.65 (1 H, dd, $J = 13.4, 4.3$ Hz), 2.82 (1 H, dd, $J = 12.2, 4.3$ Hz), 3.81 (1 H, m), 4.27 (1 H, m), 5.02 (1 H, d, $J = 2.1$ Hz), 5.28 (1 H, d, $J = 1.8$ Hz), 6.02 (1 H, d, $J = 11.3$ Hz), 6.40 (1 H, d, $J = 11.3$ Hz); MS m/z 430 (M)$^+$, 412 (M − H_2O)$^+$, 394 (M − 2H_2O)$^+$, 376 (M − 3H_2O)$^+$; HRMS m/z (M)$^+$ calcd for $C_{28}H_{46}O_3$ 430.3447, found 430.3446.

(5*Z*,7*E*)-(1*S*,2*S*,3*S*,20*S*)-2-Methyl-9,10-*seco*-5,7,10(19)-cholestatriene-1,3,25-triol (3e): $t_R = 24.60$ min; UV (EtOH) λ_{max} 264 nm, λ_{min} 226 nm; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.53 (3 H, s), 0.85 (3 H, d, $J = 6.4$ Hz), 1.21 (6 H, s), 1.22 (3 H, d, $J = 7.0$ Hz), 2.09 (1 H, d, $J = 4.6$ Hz), 2.49 (1 H, br. d, $J = 14.7$ Hz), 2.58 (1 H, dd, $J = 14.0, 3.7$ Hz), 2.80 (1 H, d, $J = 7.9$ Hz), 2.85 (1 H, m), 3.91 (1 H, m), 4.17 (1 H, m), 4.98 (1 H, d, $J = 2.1$ Hz), 5.23 (1 H, d, $J = 1.8$ Hz), 6.03 (1 H, d, $J = 11.3$ Hz), 6.48 (1 H, d, $J = 11.3$ Hz); MS m/z 430 (M)$^+$, 412 (M − H_2O)$^+$, 394 (M − 2H_2O)$^+$, 376 (M − 3H_2O)$^+$; HRMS m/z (M)$^+$ calcd for $C_{28}H_{46}O_3$ 430.3447, found 430.3447.

(5*Z*,7*E*)-(1*R*,2*S*,3*S*,20*S*)-2-Methyl-9,10-*seco*-5,7,10(19)-cholestatriene-1,3,25-triol (3f): $t_R = 16.93$ min; UV (EtOH) λ_{max} 263 nm, λ_{min} 228 nm; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.53 (3 H, s), 0.85 (3 H, d, $J = 6.4$ Hz), 1.13 (3 H, d, $J = 6.7$ Hz), 1.21 (6 H, s), 1.69 (2 H, m), 1.84 (2 H, m), 1.98 (2 H, m), 2.41 (1 H, dd, $J = 13.7, 5.5$ Hz), 2.51 (1 H, dd, $J = 13.4, 2.4$ Hz), 2.82 (1 H, m), 4.05 (1 H, m), 5.01 (1 H, t, $J = 1.8$ Hz), 5.35 (1 H, t, $J = 1.8$ Hz), 6.01 (1 H, d, $J = 11.6$ Hz), 6.36 (1 H, d, $J = 11.6$ Hz); MS m/z 430 (M)$^+$, 412 (M − H_2O)$^+$, 394 (M − 2H_2O)$^+$, 376 (M − 3H_2O)$^+$; HRMS m/z (M)$^+$ calcd for $C_{28}H_{46}O_3$ 430.3447, found 430.3445.

(5*Z*,7*E*)-(1*S*,2*S*,3*R*,20*S*)-2-Methyl-9,10-*seco*-5,7,10(19)-cholestatriene-1,3,25-triol (3g): $t_R = 17.62$ min; UV (EtOH) λ_{max} 266 nm, λ_{min} 226 nm; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.53 (3 H, s), 0.85 (3 H, d, $J = 6.7$ Hz), 1.08 (3 H, d, $J = 6.8$ Hz), 1.21 (6 H, s), 2.23 (1 H, dd, $J = 13.4, 7.9$ Hz), 2.67 (1 H, dd, $J = 13.4, 4.0$ Hz), 2.83 (1 H, m), 3.83 (1 H, ddd, $J = 7.9, 4.4, 4.0$ Hz), 4.29 (1 H, d, $J = 3.3$ Hz), 5.01 (1 H, d, $J = 1.8$ Hz), 5.28 (1 H, m), 6.01 (1 H, d, $J = 11.3$ Hz), 6.39 (1 H, d, $J = 11.3$ Hz); MS m/z 430 (M)$^+$, 412 (M − H_2O)$^+$, 394 (M − 2H_2O)$^+$, 376 (M − 3H_2O)$^+$; HRMS m/z (M)$^+$ calcd for $C_{28}H_{46}O_3$ 430.3447, found 430.3443.

(5*Z*,7*E*)-(1*R*,2*S*,3*R*,20*S*)-2-Methyl-9,10-*seco*-5,7,10(19)-cholestatriene-1,3,25-triol (3h): $t_R = 17.68$ min; UV (EtOH) λ_{max} 261 nm, λ_{min} 226 nm; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.55 (3 H, s), 0.85 (3 H, d, $J = 6.4$ Hz), 1.02 (3 H, d, $J = 7.0$ Hz), 1.22 (6 H, s), 1.83 (2 H, m), 2.00 (2 H, m), 2.11 (1 H, m), 2.27 (1 H, d, $J = 5.2$ Hz), 2.36 (1 H, dd, $J = 14.0, 5.5$ Hz), 2.65 (1 H, dd, $J = 13.7, 2.8$ Hz), 2.77 (1 H, d, $J = 7.0$ Hz), 2.84 (1 H, dd, $J = 12.2, 4.3$ Hz), 3.72 (1 H, m), 3.97 (1 H, t, $J = 4.9$ Hz), 5.07 (1 H, d, $J = 2.1$ Hz), 5.30 (1 H, d, $J = 2.1$ Hz), 6.04 (1 H, d, $J = 11.3$ Hz), 6.43 (1 H, d, $J = 11.3$ Hz); MS m/z 430 (M)$^+$, 412 (M − H_2O)$^+$, 394 (M − 2H_2O)$^+$, 376 (M − 3H_2O)$^+$; HRMS m/z (M)$^+$ calcd for $C_{28}H_{46}O_3$ 430.3447, found 430.3445.

X-ray Crystallographic Analysis of 2g. A colorless prismatic crystal with dimensions of 0.20 × 0.12 × 0.32 mm^3 was obtained by recrystallization from ethyl acetate. The observed cell parameters are as follows: $C_{28}H_{48}O_4$, M_r = 448.68, orthorhombic, $P2_12_12_1$; $a = 18.432(1)$, $b = 21.680(1)$, $c = 6.770(1)$ Å; $V = 2705.2(4)$ Å^3, $Z = 4$, $Dx = 1.102$ g/cm^3; λ(Cu Kα) = 1.54178 Å, μ(Cu Kα) = 5.58 cm^{-1}; $F(000) = 992.00$, room temperature. The structure was solved by direct methods and expanded using Fourier techniques. The non-hydrogen atoms were refined anisotropically by full matrix least-squares calculations. Hydrogen atoms were included but not refined. $R = 0.054$, $R_w = 0.056$ for 1861 reflections ($I > 1.50\sigma(I)$). Further X-ray crystallographic data including bond lengths and angles, H-atom coordinates, anisotropic thermal parameters, and structure factors ($F_o - F_c$ tables) for this compound are available as Supporting Information.

Binding to Vitamin D Receptor (VDR). Bovine thymus 1α,25-dihydroxyvitamin D_3 receptor was obtained from Yamasa Biochemical (Chiba, Japan) 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, 0.23 mg protein) was pre-incubated with 50 μL of an ethanol solution of 1α,25-dihydroxyvitamin D_3 or an analogue at various concentrations for 60 min at 25 °C. Then, the receptor mixture was left to stand overnight with 0.1 nM [^{3}H]-1α,25-dihydroxyvitamin D_3 at 4 °C. The bound and free [^{3}H]-1α,25-dihydroxyvitamin D_3 were separated by treatment with dextran-coated charcoal for 30 min at 4 °C, followed by centrifugation at 3000 rpm for 10 min. The supernatant (500 μL) was mixed with ACS-II (9.5 mL) (Amersham, England) and the radioactivity was counted. The relative potency of the analogues was calculated from their concentration needed to displace 50% of [^{3}H]-1α,25-dihydroxyvitamin D_3 from its receptor compared with the activity of 1α,25-dihydroxyvitamin D_3 (assigned a 100% value).

Binding to Vitamin D Binding Protein (DBP). Fetal calf serum (FCS) was diluted 2500 times with 50 mM phosphate-buffered saline (pH 7.0) and used as a source of DBP. The incubation mixture used contained diluted FCS (200 μL), [^{3}H]-25-hydroxyvitamin D_3 (480 pg, 50000 dpm/tube) dissolved in 100 μL of 50 mM phosphate-buffered saline containing 0.01% Triton X-100 (pH 7.0), and various amounts of vitamin D_3 analogues (0−1 μg in 10 μL ethanol/tube) to be tested. After overnight incubation at 4 °C, 500 μL of dextran-coated charcoal suspension was added to each tube, and bound and free vitamins were separated by vigorous mixing, followed by

centrifugation at 2000*g* for 10 min. Aliquots (500 μL) of the supernatant were counted using a liquid scintillation counter.

Assay for HL-60 Cell Differentiation. Nitro blue tetrazolium (NBT) reducing activity was used as a cell differentiation marker. HL-60 cells were cultured in RPMI-1640 medium supplemented with 10% heat-inactivated FCS. Exponentially proliferating cells were collected, suspended in fresh medium and seeded in culture plates (Falcon, Becton Dickinson and Co., Franklin Lakes, NJ). Cell concentration at seeding was adjusted to 2×10^4 cells/mL and the seeding volume was 1 mL/well. 1α,25-Dihydroxyvitamin D_3 or an analogue dissolved in ethanol was added to the culture medium at 0.1% volume and culture was continued for 96 h at 37 °C in a humidified atmosphere of 5% CO_2/air without medium change. The same amount of vehicle was added to the control culture. NBT reducing assay was performed according to the method of Collins et al.[38] Briefly, cells were collected, washed with PBS, and suspended in serum-free medium. NBT/TPA solution (dissolved in PBS) was added. Final concentrations of NBT and TPA were 0.1% and 100 ng/mL, respectively. Then, the cell suspensions were incubated at 37 °C for 25 min. After incubation, cells were collected by centrifugation and resuspended in PBS. Cytospin smears were prepared, and the counter-staining of nuclei was done with Kemechrot solution. At least 500 cells/preparation were observed.

In Vivo Calcium-Regulating Assay. Six-week-old normal SD male rats were divided into several groups of five rats, each receiving oral administration of 1.0, 10.0 or 100.0 μg/kg of 1α,25-dihydroxyvitamin D_3 or an analogue in 2 mL/kg of 0.1% Triton X-100 solution. The resulting increases in serum calcium were measured at 8, 24, 48 and 72 h after administration by the *o*-cresolphthalein complexone method. The relative activity of each analogue with respect to 1α,25-dihydroxyvitamin D_3 was evaluated as follows: the dose−response effect of each analogue was calculated at the time when the effect was maximum and expressed as the dosage required to elevate the serum calcium level by 1 mg/dL.

Measurement of Intestinal Calcium Transport and Bone Calcium Mobilization. Male weanling SD rats were maintained on a vitamin D-deficient diet containing normal level of calcium and phosphorus (0.47% Ca, 0.3% P) for 7 days, then were placed on a vitamin D-deficient and reduced calcium diet (0.02% Ca, normal P) for the duration of the experiment. After 21 days on the deficient diet, the animals were dosed daily with the indicated analogues for 7 days. Each analogue was suspended in 5% ethanol and 95% propylene glycol (0.1 mL) and injected intraperitoneally. The control groups received the vehicle. There were 5 or 6 animals/group. The determinations were made 24 h after the last dose. The rats were sacrificed under ether anesthesia by decapitation; their blood and intestines were collected and used immediately to determine calcium transport activity and serum calcium concentration. Calcium was measured in the presence of 0.1% lanthanum chloride by means of a Perkin-Elmer atomic absorption spectrometer model 3110. Intestinal calcium transport was determined by the everted intestinal sac method using the proximal 10 cm of intestine. Statistical analysis was done by the use of Student's *t*-test. Intestinal calcium transport is expressed as serosal:mucosal ratio of calcium in the sac to the calcium in the final incubation medium, or S/M. Bone calcium mobilization represents the rise in serum calcium of the rats maintained on a very-low-calcium diet. In that measurement, the rise in serum calcium must arise from bone and hence is a determination of bone calcium mobilization.

Acknowledgment. This work was supported by a grant-in-aid from the Ministry of Education, Science and Culture of Japan and a grant from the Hayashi Memorial Foundation for Female Natural Scientists.

Supporting Information Available: X-ray crystallographic data and variable temperature ^{1}H NMR spectra for **2g**. This material is available free of charge via the Internet at http://pubs.acs.org.

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