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Highly Potent Cell Differentiation-Inducing Analogues of 1α,25-Dihydroxyvitamin D_3: Synthesis and Biological Activity of 2-Methyl-1,25-dihydroxyvitamin D_3 with Side-Chain Modifications

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Abstract—Eight 2-methyl substituted analogues of 20-*epi*-22*R*-methyl-1α,25-dihydroxyvitamin D_3 (**5**) and 20-*epi*-24,26,27-trihomo-22-oxa-1α,25-dihydroxyvitamin D_3 (**6**: KH-1060) were convergently synthesized. Preparation of the CD-ring portions with modified side chains of **5** and **6**, followed by palladium-catalyzed cross-coupling with the A-ring enyne synthons (**20a–d**), (3*S*,4*S*,5*R*)-, (3*S*,4*R*,5*R*)-, (3*S*,4*S*,5*S*)- and (3*R*,4*R*,5*S*)-3,5-bis[(*tert*-butyldimethylsilyl)oxy]-4-methyloct-1-en-7-yne, afforded two sets of four A-ring stereoisomers of 20-*epi*-2,22-dimethyl-1,25-dihydroxyvitamin D_3 (**7a–d**) and 20-*epi*-24,26,27-trihomo-2-methyl-22-oxa-1,25-dihydroxyvitamin D_3 (**8a–d**). The biological profiles of the hybrid analogues were assessed in terms of affinity for vitamin D receptor (VDR) and HL-60 cell differentiation-inducing activity in comparison with the natural hormone. The combined modifications of the A-ring at the 2-position and the side chain yielded analogues with high potency.

Introduction

The hormonally active metabolite of vitamin D, 1α,25-dihydroxyvitamin D_3 (**1**), is now recognized as an important cell-cycle regulator, which influences cell proliferation, differentiation and apoptosis, in addition to its classical role in calcium–phosphorus homeostasis.[1] Therefore, analogues of 1α,25-dihydroxyvitamin D_3 should be promising candidates to treat cancer, psoriasis and numerous other diseases caused by cell-cycle disorders. Most biological responses to 1α,25-dihydroxyvitamin D_3 are mediated by a ligand-inducible transcription factor, vitamin D receptor (VDR), which is a member of the nuclear receptor superfamily.[2] Therefore, analogues with high VDR affinity are of great interest since the whole sequence of actions is considered to be triggered by the formation of the ligand–VDR complex.

In order to investigate the structure–function relationship of the natural hormone, we synthesized all eight possible A-ring diastereomers of 2-methyl-1,25-dihydroxyvitamin D_3 (**3**) and found that the potencies of the analogues depend on the configuration not only of the C-1 and C-3 hydroxyl groups, but also of the 2-methyl group.[3] In particular, 2α-methyl-1α,25-dihydroxyvitamin D_3 (**3a**: ααβ-isomer; the Greek letters denote the configurations of C-1, C-2 and C-3, respectively, in the vitamin D numbering system) exhibited 4-fold higher VDR binding potency than **1**, while its 2-epimer, 2β-methyl-1α,25-dihydroxyvitamin D_3 (**3b**: αββ-isomer), showed one-eighth of the activity of **1**. This simple A-ring modification yielded for the first time an analogue (**3a**) with significantly higher VDR binding activity than the parent hormone.

20-*epi*-1α,25-Dihydroxyvitamin D_3 (**2**) is noteworthy due to its high activity in cell differentiation with relatively low calcemic effect; its biological profile is clearly distinct from that of the natural hormone.[4] The VDR binding potency of **2** relative to **1** (normalized to 100) was estimated to be 400[5] by us and 500[4a] by others using bovine thymus VDR. We synthesized the above 2-methyl analogues with 20-epimerization, obtaining all eight possible A-ring stereoisomers (**4**), and found that 2-methyl substitution and 20-epimerization had additive

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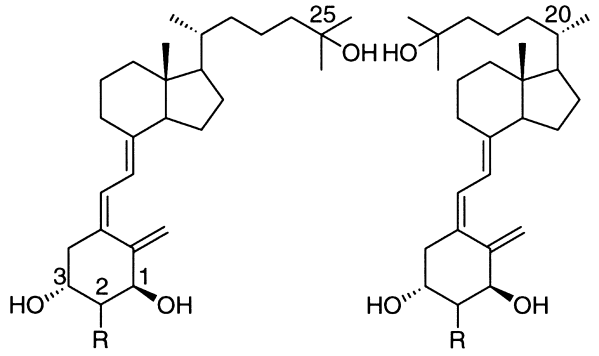
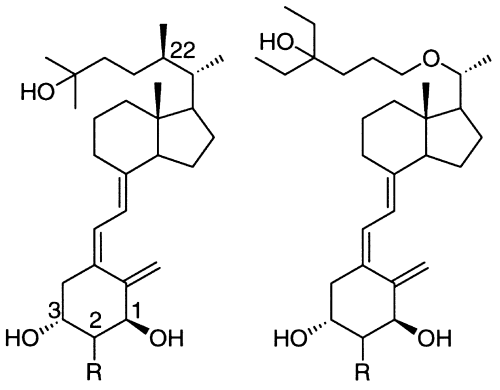


1: R = H
3: R = CH_3

2: R = H
4: R = CH_3

Chart 1.



5: R = H
7: R = CH_3

6: R = H (KH-1060)
8: R = CH_3

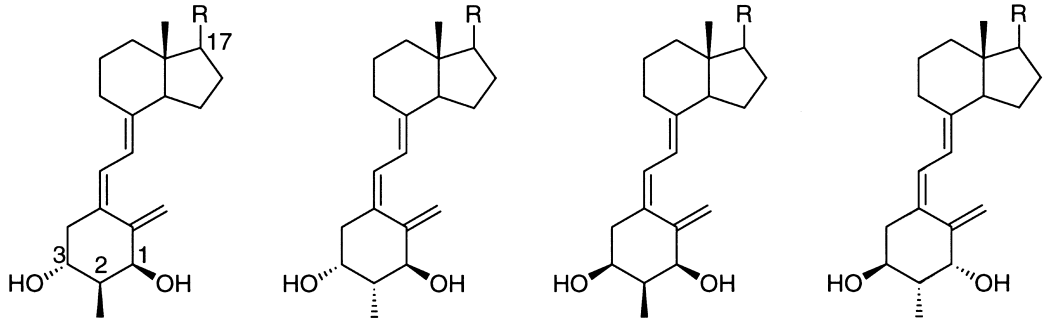
Chart 2.

effects on the VDR binding and cell differentiation-inducing activities.[6,7] Thus, 20-*epi*-2α-methyl-1α,25-dihydroxyvitamin D_3 (**4a**: ααβ-isomer) exhibited 12-fold higher affinity to VDR and its 2-epimer, 20-*epi*-2β-methyl-1α,25-dihydroxyvitamin D_3 (**4b**: αββ-isomer), showed 1.6-fold higher affinity than **1**. On the other hand, four of the eight 20-*epi* analogues possessed higher cell differentiation-inducing activity than **1**, i.e., the ααβ-, αββ-, ααα- and ββα-isomers, which correspond to **4a**, **4b**, **4c** and **4d**, respectively, as illustrated in Chart 3.

Those remarkable effects of double modification prompted us, in the present work, to design 2-methyl analogues modified in the side chain, in order to study further the structure–activity relationships of the hybrid analogues. Four potent A-ring parts, ααβ-, αββ-, ααα- and ββα-isomers, and two characteristic side chains were selected. The first side chain was that of 20-*epi*-22*R*-methyl-1α,25-dihydroxyvitamin D_3 (**5**), which has 11-fold higher affinity to bovine thymus VDR and 20-fold higher affinity to porcine intestinal VDR than **1**, and is the most active side-chain analogue found to date in terms of VDR binding affinity.[8] The second side chain was that of 20-*epi*-24,26,27-trihomo-22-oxa-1α,25-dihydroxyvitamin D_3 (**6**: KH-1060), which is the most potent known analogue in terms of cell differentiation and immunosuppressive effects.[4b] In this paper, syntheses and biological profiles of the 2-methyl analogues of 20-*epi*-22*R*-methyl-1,25-dihydroxyvitamin D_3 (**7a–d**) and 20-*epi*-24,26,27-trihomo-22-oxa-1,25-dihydroxyvitamin D_3 (**8a–d**) are disclosed.

Synthesis

Convergent synthesis can be more effective and flexible for the synthesis of a variety of analogues than the



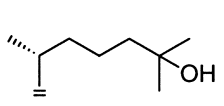
ααβ-isomer
3a
4a
7a
8a

αββ-isomer
3b
4b
7b
8b

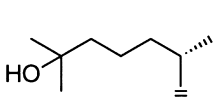
ααα-isomer
3c
4c
7c
8c

ββα-isomer
3d
4d
7d
8d

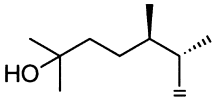
R: **3** series



R: **4** series



R: **7** series



R: **8** series

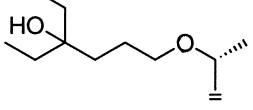
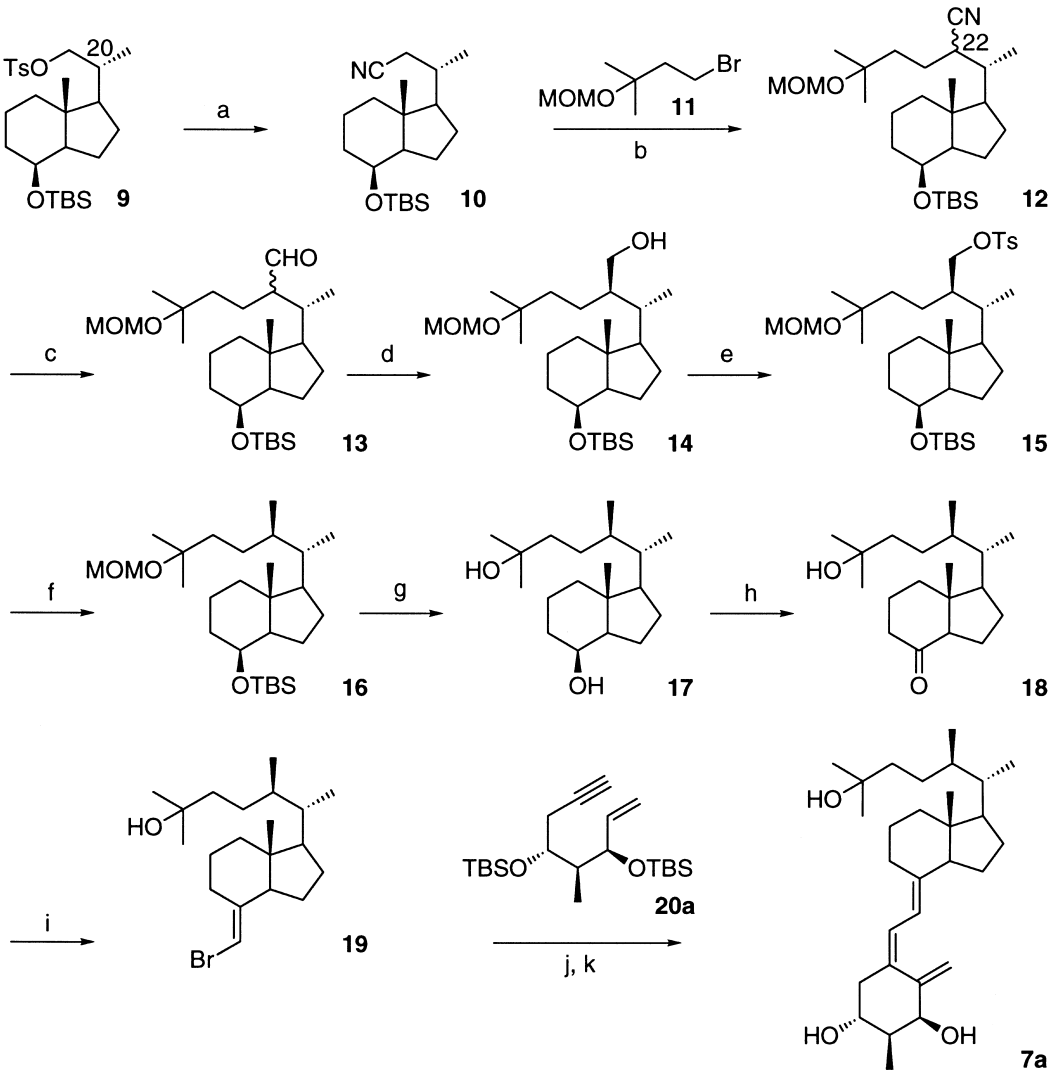


Chart 3.

classical steroidal approach.[9] In particular, Trost's convergent method[10] using palladium-catalyzed coupling of the A-ring enyne synthon with the CD-ring portion, applied by us in the synthesis of A-ring analogues,[3,5,6,11] seems most useful. The 2-methyl-A-ring synthons can be easily obtained according to our procedure, a part of which was reported in ref 3. Therefore, we focused on the synthesis of CD-ring portions with modified side chains.

Scheme 1 outlines the synthesis of the 20-*epi*-22*R*-methyl-CD-ring portion **19**[12] and the subsequent coupling with an A-ring enyne **20a**,[3] as exemplified by the ααβ-isomer, to produce the 2α-methyl analogue **7a**. The tosylate **9**[5] of unnatural 20(*S*)-configuration was converted to the nitrile **10** in 99% yield using sodium cyanide. Condensation of **10** with the side-chain moiety **11**[13] using LDA as a base furnished **12** as an approximately 5:1 epimeric mixture at C-22 in 77% yield. Subsequent reduction of this nitrile mixture **12** with DIBAL-H, followed by $NaBH_4$, afforded the corresponding C-22 epimeric alcohols in 79% yield (two steps), from which the alcohol **14** of the desired 22(*S*)-configuration was separated by chromatography as a major product. The absolute configuration at C-22 was determined by X-ray crystallography of the diol **17** (Chart 4).[14] Reductive elimination of a hydroxyl group in **14** via the tosylate **15** produced the desired CD-ring portion **16**. Removal of both protecting groups in **16** with CSA afforded the corresponding diol **17** in 95% yield. The resulting secondary alcohol was oxidized with PDC to give the ketone **18** in 99% yield. Finally, bromomethylenation of **18** furnished the requisite 20-*epi*-22*R*-methyl-CD-ring synthon **19**. Each of the four selected A-ring enyne synthons (**20a**, **20b**, **20c** and **20d**),[15] prepared separately as we previously reported,[3] was coupled with the 20-*epi*-22*R*-methyl-CD-ring **19** using the palladium catalyst, followed by deprotection to give the 2-methyl analogue (**7a**, **7b**, **7c** and **7d**). In this way, a set of four stereoisomers of 20-*epi*-2,22-dimethyl-1,25-dihydroxyvitamin D_3 was synthesized.

Scheme 2 shows the synthesis of the CD-ring portion of KH-1060 (**26**), and the subsequent coupling with an A-ring enyne **20a** to afford the 2α-methyl substituted



Scheme 1. (a) NaCN/DMSO, 90 °C, 99%; (b) LDA/THF, −78 to 0 °C, 77%; (c) DIBAL-H/CH_2Cl_2, −10 °C, 90%; (d) $NaBH_4$/MeOH, rt, 74%; (e) TsCl/pyridine, rt, 99%; (f) $LiAlH_4$/ether, rt, 89%; (g) CSA/MeOH, rt, 95%; (h) PDC/CH_2Cl_2, rt, 99%; (i) $Ph_3P^+CH_2Br\cdot Br^-$, NaHMDS/THF, 50%; (j) $Pd_2(dba)_3\cdot CHCl_3$–PPh_3–Et_3N/toluene, reflux; (k) CSA/MeOH, rt.

KH-1060 analogue (**8a**). The synthetic approach to the CD-ring ketone **25** was previously described in a communication.[16] Similar procedures and coupling conditions were employed here. The alcohol **21**[17] was coupled with the side-chain moiety **22**[18] using potassium hydride as a base in the presence of 18-crown-6 ether to give the protected diol **23** in 86% yield. Removal of both TBS protecting groups in **23** by a quick treatment with TFA furnished the diol **24** in 81% yield. Oxidation of **24** with PDC, followed by bromomethylenation, afforded the requisite CD-ring portion **26**. Finally, in the same manner as described above, each of the four A-ring enyne synthons (**20a**, **20b**, **20c** and **20d**)[15] was coupled with the CD-ring **26** using the palladium catalyst, followed by deprotection to give the 2-methyl substituted KH-1060 analogue (**8a**, **8b**, **8c** and **8d**). Thus, syntheses of two sets of four 2-methyl analogues of 20-*epi*-22*R*-methyl-1,25-dihydroxyvitamin D_3 (**7a–d**) and 20-*epi*-24,26,27-trihomo-22-oxa-1,25-dihydroxyvitamin D_3 (**8a–d**) were accomplished.

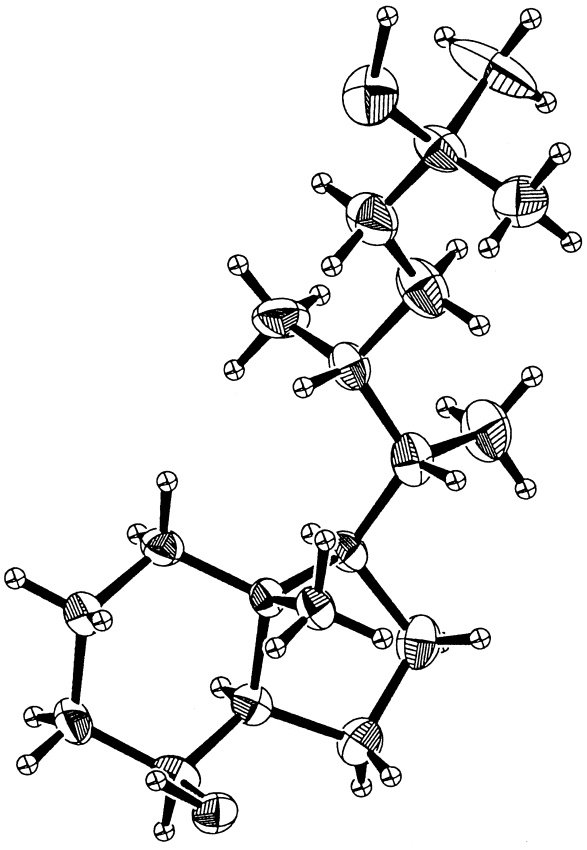
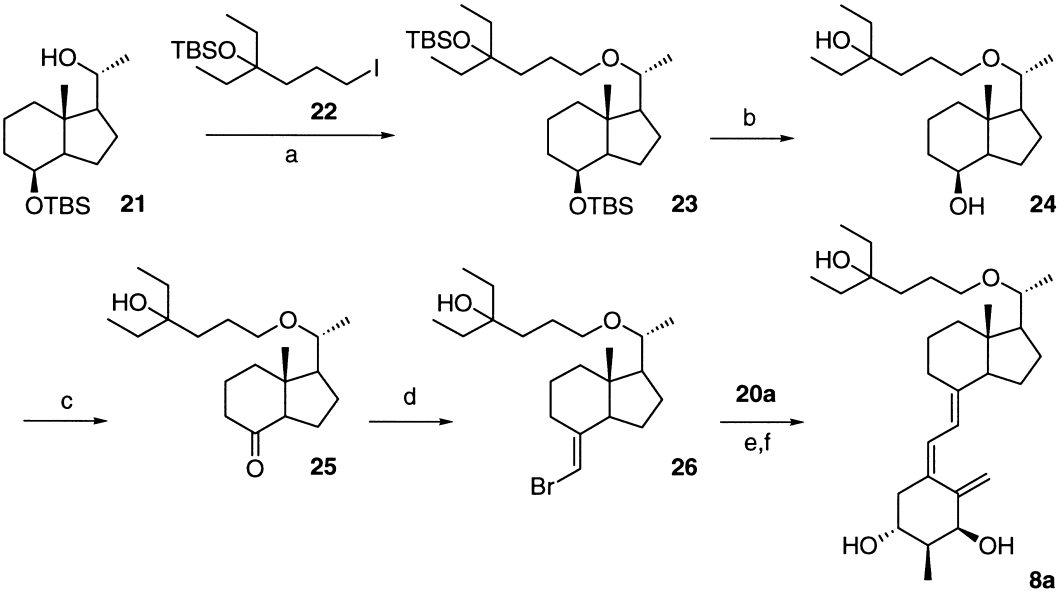


Chart 4.

Biological Evaluation

The biological activities of the synthesized analogues (**7a–d**, **8a–d**) are summarized in Table 1 in comparison with those of the natural hormone **1**, together with the 2-methyl (**3a–d**)[3,7] and 20-*epi*-2-methyl analogues (**4a–d**).[6,7] The activities were highly dependent not only on the stereochemistry in the A-ring, but also on the side-chain character. Thus, the double modification yielded analogues with unique activity profiles.

In the vitamin D receptor binding assay using bovine thymus VDR,[19] **7a** and **7b**, both of which have natural hydroxyl configurations, exhibited 2-fold higher affinity than 1α,25-dihydroxyvitamin D_3 irrespective of the orientation of the 2-methyl group. The analogue **7c**, the 3-epimer of **7a**, showed rather high affinity to VDR, with half the potency of the natural hormone **1**, whereas **7d** exhibited low affinity. The introduction of a 22*R*-methyl group into 20-*epi*-1α,25-dihydroxyvitamin D_3 (**2**) produced 20-*epi*-22*R*-methyl-1α,25-dihydroxyvitamin D_3 (**5**) with approximately 2.8-fold elevated VDR binding affinity.[8] Activation by 22*R*-methyl introduction was also observed in the cases of **7b** (versus **4b**) and **7c** (versus



Scheme 2. (a) KH, 18-crown-6/THF, rt, 86%; (b) TFA/CH_2Cl_2, 0 °C, 81%; (c) PDC/CH_2Cl_2, rt, 80%; (d) $Ph_3P^+CH_2Br \cdot Br^-$, NaHMDS/THF, 33%; (e) $Pd_2(dba)_3 \cdot CHCl_3$–PPh_3–Et_3N/toluene, reflux; (f) CSA/MeOH, rt.

Table 1. Biological activites of the 2-methyl analogues of 1α,25-dihydroxyvitamin D_3[a]

	VDR[b]	HL-60[c]			VDR	HL-60	
1	100	100		**2**	400[f]	3571[f]	
3a	400[d]	258[e]	(200)[d]	**4a**	1200[g]	9688[e]	(59,000)[g]
3b	13[d]	54[e]	(10)[d]	**4b**	160[g]	1722[e]	(2600)[g]
3c	4[d]	22[e]	(13)[d]	**4c**	17[g]	705[e]	(730)[g]
3d	0.8[d]	0[e]	(3)[d]	**4d**	7[g]	135[e]	(190)[g]
5	1100[h]	20,000[h]		**6**[i]	29	6430	
7a	200	6190		**8a**	70	7500	
7b	200	5420		**8b**	2.5	2610	
7c	50	4330		**8c**	0.5	820	
7d	3	58		**8d**	< 0.1	6	

[a]The potencies of 1α,25-dihydroxyvitamin D_3 (**1**) are normalized to 100.
[b]Bovine thymus.
[c]Cell differentiation was assessed in terms of expression of CD11b. Values in parentheses are results using NBT reductivity instead of expression of CD11b.
[d]Ref 3.
[e]Ref 7.
[f]Ref 5.
[g]Ref 6.
[h]Ref 8.
[i]For comparison, **6** was synthesized and biologically evaluated in our laboratory.

4c). As regards HL-60 cell differentiation-inducing activities,[20] **7a** and **7b** showed 62- and 54-fold higher potency than **1**, respectively. Interestingly, **7c** exhibited 43-fold higher activity than **1**, having almost comparable activity to **7a** and **7b** in potency. 3-Epimerization generally results in a marked reduction of potency and has recently been reported to be involved in the metabolic pathway not only of the natural hormone **1**, but also of several side-chain analogues.[21] In the case of **7a**, however, 3-epimerization would afford **7c** with high cell-differentiating activity, which might be advantageous for in vivo administration. The cell-differentiation-inducing activity of **7d** was low but significant, in spite of the 1β-hydroxyl configuration.

In VDR binding assay using bovine thymus, **8a** showed 70% affinity while **8b** showed 2.5% affinity, compared with **1**. The VDR binding affinity of KH-1060 (**6**) relative to **1**, normalized to 100, was reported to be 120 for chick intestinal VDR.[4b] Our estimate using bovine thymus VDR was 29, and the lower affinity of **6** may be explained by the difference of the VDR source. Introduction of a 2α-methyl group into **6** to produce the analogue **8a** increased the VDR binding activity. The analogue **8c**, the 3-epimer of **8a**, showed low affinity to VDR and **8d** exhibited virtually no affinity. The rank order of potency to induce HL-60 cell differentiation among **8a**–**d** was almost parallel to that of VDR binding affinity. Although the KH-1060 analogues (**8a**–**c**) appeared to be relatively poor ligands for VDR, their cell differentiation-inducing activities were high. In the case of **8a**, which exhibited 70% affinity to VDR relative to **1**, the potency in cell differentiation was 75-fold higher than that of **1**. In contrast, the corresponding 20-*epi*-22*R*-methyl analogue **7a** exhibited 62-fold higher activity in cell differentiation, although its VDR affinity was 2-fold higher than that of **1**. Thus, the double modification resulted in synergistic effects on HL-60 cell differentiation-inducing activity in the case of the KH-1060 analogues. The 2-epimerization of **8a** reduced the activity in cell differentiation by approximately 1/3, while the 3-epimerization did so by 1/9. These results revealed that the potency of the 2-methyl analogues with KH-1060 side chains was affected by the A-ring stereochemistry.

High potency of **5** in VDR binding was explained by anchoring of the 25-hydroxyl group to the right position in the receptor due to the introduction of the 22*R*-methyl group.[8] The effect of the 22*R*-methyl substitution to restrict the side-chain conformation seems to be independent of the A-ring or the CD-ring structure. However, introduction of a 2α-methyl group into each parent (**1**, **2**, **5** and **6**) afforded analogues (**3a**, **4a** and **8a**) with elevated VDR affinity by 2- to 4-fold, except for the case of the 20-*epi*-22*R*-methyl analogue (**7a**). The combined modification of 2α-methyl and 20-*epi*-22*R*-methyl introduction resulted in an analogue with lower affinity than each parent, instead of a 'super ligand' to VDR. In addition, cell differentiation-inducing activities of **7a**–**c** suggested that the potency of the 20-*epi*-22*R*-methyl analogues was barely affected by the orientation of the 2-methyl or the 3-hydroxyl group, implying that the double-modified analogues would be dominated by the side-chain character. These unique effects in hybrid analogues might need to be explained by an alternative concept. Further studies are required to elucidate the structure–activity relationships of these compounds. On the other hand, the cell differentiation-inducing activity of KH-1060 (**6**) was elevated by the 2α-methyl substitution to yield **8a** with 75-fold higher activity than **1**. The immunosuppresive effect of **8a**, one of the characteristic activities of KH-1060,[4] would be of great interest.

In summary, we have synthesized a total of eight 2-methyl analogues with side-chain modifications (**7a**–**d**, **8a**–**d**) by employing the convergent method using palladium catalyst. The above procedure should be versatile for the synthesis of a variety of analogues, in particular those with A-ring and/or side-chain modifications. Biological evaluation revealed that further modification in the side chain differentially affected the potency of the 2-methyl analogues depending on the side-chain character. Thus, the 2-methyl analogues with 20-*epi*-22*R*-methyl side chains exhibited a reduced dependency on the A-ring stereochemistry. In contrast, the potency of the corresponding KH-1060 analogues was affected by the A-ring stereochemistry and the double modification resulted in synergistic effects on HL-60 cell differentiation-inducing activity. These novel analogues with high potency should be useful tools in research on the biology of vitamin D. The results of broad-spectrum biological screening of the analogues will be reported in due course.

Experimental

Melting points were determined by using a Yanagimoto hot-stage melting point apparatus and are uncorrected. NMR spectra were recorded on a JEOL GSX-400 spectrometer. Chemical shifts are expressed in ppm

relative to tetramethylsilane. Mass spectra (EI) and high-resolution mass spectra (HRMS) were recorded on a JMS-SX 102A. 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. Crystallographic data were collected on a Rigaku AFC7S diffractometer with graphite-monochromated Cu-K_α radiation.

(20*S*)-De-A,B-8β-[(*tert*-butyldimethylsilyl)oxy]-20-(cyanomethyl)pregnane (10). To a solution of **9** (1.70 g, 3.54 mmol) in DMSO (30 mL) was added NaCN (370 mg, 7.08 mmol). The resulting mixture was stirred at 90 °C for 4 h. The reaction mixture was cooled, then water was added. After extraction of the mixture with ethyl acetate, the organic layer was washed with brine, dried over magnesium sulfate and filtered. Evaporation of the filtrate afforded a crude mixture, from which **10** (1.12 g) was separated by silica gel column chromatography (ethyl acetate:*n*-hexane = 5:95) in 99% yield as a colorless oil.

$[\alpha]_D = +21.1$ ($c = 3.0$, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.00 (3H, s), 0.01 (3H, s), 0.89 (9H, s), 0.92 (3H, s), 1.06 (3H, d, $J = 6.7$ Hz), 2.38 (1H, dd, $J = 16.8$, 6.7 Hz), 2.45 (1H, dd, $J = 16.7$, 4.0 Hz), 4.00 (1H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ −5.3 (q), −4.9 (q), 13.9 (q), 17.4 (t), 17.9 (s), 19.5 (q), 22.6 (t), 23.9 (t), 25.7 (q), 26.9 (t), 31.7 (d), 34.1 (t), 40.1 (t), 41.9 (s), 52.7 (d), 55.0 (d), 69.2 (d), 118.9 (s); FTIR (neat) 2932, 2858, 2251, 1462, 1253, 1167, 1084, 1022, 837, 810, 775 cm^{-1}; MS 335 $[M]^+$, 320 $[M-Me]^+$, 278 $[M-t\text{-}Bu]^+$; HRMS calcd for $[C_{20}H_{37}NOSi]$ 335.2644, found 335.2652.

(20*S*,22*RS*)-De-A,B-8β-[(*tert*-butyldimethylsilyl)oxy]-22-cyano-25-[(methoxymethyl)oxy]cholestane (12). To a solution of diisopropylamine (0.92 mL, 6.56 mmol) in THF (5 mL) was added *n*-BuLi solution (1.54 M in *n*-hexane, 4.26 mL, 6.56 mmol) with stirring at 0 °C under an argon atmosphere. The resulting mixture was stirred for 30 min at 0 °C. The LDA solution was cooled to −78 °C, then a solution of **10** (1.10 g, 3.28 mmol) in THF (7 mL) was added. After stirring for 30 min, a solution of **11** (1.38 g, 6.56 mmol) in THF (10 mL) was added, and the reaction mixture was stirred for 1 h at −78 °C. After an additional one hour at 0 °C, the reaction was quenched by adding saturated aqueous NH_4Cl solution. The whole was extracted with ethyl acetate. The organic solution was washed with brine, dried over magnesium sulfate, filtered and concentrated. The crude product was separated by silica gel column chromatography (ethyl acetate:*n*-hexane = 5:95) to give **12** (1.17 g) in 77% yield as a 22-epimeric mixture.

^{1}H NMR (400 MHz, $CDCl_3$) δ 0.00 (3H, s), 0.02 (3H, s), 0.89 (9H, s), 0.91 (3H×1/6, s), 0.93 (3H×5/6, s), 0.98 (3H×5/6, d, $J = 6.4$ Hz), 1.03 (3H×1/6, d, $J = 7.0$ Hz), 2.72 (1H×1/6, m), 2.89 (1H×5/6, ddd, $J = 8.9$, 5.5, 3.4 Hz), 3.36 (3H, s), 4.01 (1H, m), 4.69 (2H, s); ^{13}C NMR (100 MHz, $CDCl_3$) (major) δ −5.3 (q), −4.9 (q), 13.9 (q), 14.3 (q), 17.5 (t), 17.9 (s), 22.6 (t), 25.6 (t), 25.7 (q), 25.9 (q), 26.5 (q), 27.2 (t), 34.1 (t), 36.4 (d), 36.6 (d), 40.0 (t), 40.3 (t), 41.9 (s), 52.8 (d), 54.3 (d), 55.2 (q), 69.3 (d), 75.6 (s), 91.1 (t), 121.4 (s), (minor) δ −5.3 (q), −4.9 (q), 14.0 (q), 14.5 (q), 17.0 (t), 17.9 (s), 20.7 (t), 22.9 (t), 25.6 (t), 26.0 (q), 26.1 (q), 26.3 (q), 26.5 (t), 34.2 (t), 36.1 (d), 36.2 (d), 39.7 (t), 40.0 (t), 42.2 (s), 52.2 (d), 54.4 (d), 55.1 (d), 55.0 (q), 69.1 (d), 75.6 (s), 91.0 (t), 122.7 (s); FTIR (neat) 3021, 2934, 2251, 1472, 1387, 1215, 926, 837, 760 cm^{-1}; MS 465 $[M]^+$, 450 $[M-Me]^+$, 408 $[M-t\text{-}Bu]^+$; HRMS calcd for $[C_{27}H_{51}NO_3Si]$ 465.3638, found 465.3637.

(20*S*,22*RS*)-De-A,B-8β-[(*tert*-butyldimethylsilyl)oxy]-22-formyl-25-[(methoxymethyl)oxy]cholestane (13). To a solution of **12** (940 mg, 2.02 mmol) in CH_2Cl_2 (15 mL) was added DIBAL-H solution (0.95 M in *n*-hexane, 2.34 mL, 2.22 mmol) with stirring at −10 °C under an argon atmosphere. After stirring for 1 h, the reaction was quenched by adding 4% aqueous HCl solution. The whole was extracted with ethyl acetate. The organic solution was washed with brine, dried over magnesium sulfate, filtered and concentrated. The crude product was separated by silica gel column chromatography (ethyl acetate:*n*-hexane = 8:92) to give **13** (846 mg) in 90% yield as a colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 0.00 (3H, s), 0.01 (3H, s), 0.72 (3H×1/6, d, $J = 7.0$ Hz), 0.89 (9H, s), 0.90 (3H×5/6, d, $J = 6.7$ Hz), 0.96 (3H×5/6, s), 1.00 (3H×1/6, s), 2.46 (1H×1/6, m), 2.59 (1H×5/6, m), 3.36 (3H, s), 4.00 (1H, m), 4.69 (2H, s), 9.26 (1H×1/6, s), 9.81 (1H×5/6, s); ^{13}C NMR (100 MHz, $CDCl_3$) (major) δ −5.3 (q), −4.9 (q), 14.2 (q), 14.4 (q), 17.6 (t), 18.0 (s), 21.7 (t), 22.8 (t), 25.7 (q), 26.1 (q), 26.3 (q), 27.2 (t), 34.3 (t), 36.3 (d), 39.9 (t), 40.6 (t), 42.5 (s), 52.9 (d), 53.9 (d), 54.4 (d), 55.1 (q), 69.3 (d), 76.0 (s), 91.0 (t), 206.6 (d), (minor) δ −5.3 (q), −4.9 (q), 14.3 (q), 14.7 (q), 17.0 (t), 18.0 (s), 21.1 (t), 22.9 (t), 26.0 (q), 26.2 (q), 26.5 (q), 27.0 (t), 34.4 (t), 36.0 (d), 40.0 (t), 40.2 (t), 42.3 (s), 52.7 (d), 52.8 (d), 53.0 (d), 55.0 (q), 69.4 (d), 76.1 (s), 90.9 (t), 205.3 (d); FTIR (neat) 3157, 2934, 1794, 1717, 1472, 1253, 1036, 909, 733 cm^{-1}; MS 468 $[M]^+$, 453 $[M-Me]^+$; HRMS calcd for $[C_{27}H_{52}O_4Si]$ 468.3634, found 468.3626.

(20*S*,22*S*)-De-A,B-8β-[(*tert*-butyldimethylsilyl)oxy]-22-hydroxymethyl-25-[(methoxymethyl)oxy]cholestane (14). A solution of **13** (810 mg, 1.73 mmol) in methanol (10 mL) was treated with $NaBH_4$ (134 mg, 3.46 mmol) with stirring at 0 °C. Stirring was continued for 2 h at room temperature, then the reaction was quenched by adding water. The whole was extracted with ethyl acetate. The organic solution was washed with brine, dried over magnesium sulfate, filtered and concentrated. The crude product was separated by silica gel column chromatography (ethyl acetate:*n*-hexane = 10:90) to give **14** (603 mg, 74%, more polar) and its 22-epimer (110 mg, 14%, less polar), both as colorless oils.

$[\alpha]_D = +17.5$ ($c = 0.92$, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ −0.01 (3H, s), 0.00 (3H, s), 0.73 (3H, d,

$J = 7.0$ Hz), 0.88 (9H, s), 0.92 (3H, s), 1.22 (6H, s), 3.37 (3H, s), 3.41 (1H, dd, $J = 10.7$, 8.2 Hz), 3.76 (1H, dd, $J = 10.7$, 3.7 Hz), 3.99 (1H, m), 4.71 (2H, q, $J = 7.3$ Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ −5.3 (q), −4.9 (q), 13.6 (q), 13.8 (q), 17.6 (t), 18.0 (s), 22.9 (t), 24.4 (t), 25.8 (q), 26.2 (q), 26.5 (q), 27.5 (t), 34.4 (t), 35.8 (t), 39.8 (t), 40.3 (t), 42.4 (s), 43.2 (d), 53.1 (d), 53.6 (d), 54.1 (q), 63.6 (t), 69.4 (d), 76.5 (s), 91.0 (t); FTIR (neat) 3574, 3538, 3406, 3158, 2934, 1381, 1253, 1093, 909, 733 cm^{-1}; MS 470 $[M]^+$; HRMS calcd for $[C_{27}H_{54}O_4Si]$ 470.3791, found 470.3798.

(22*R*)-Isomer: $[\alpha]_D = +20.2$ ($c = 1.11$, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.00 (3H, s), 0.01 (3H, s), 0.75 (3H, d, $J = 7.0$ Hz), 0.89 (9H, s), 0.94 (3H, s), 1.16 (6H, s), 3.37 (3H, s), 3.51 (1H, dd, $J = 10.7$, 7.9 Hz), 3.62 (1H, dd, $J = 10.7$, 4.9 Hz), 4.00 (1H, m), 4.72 (2H, q, $J = 7.3$ Hz); FTIR (neat) 3634, 3453, 2932, 2859, 1468, 1381, 1038, 922, 872, 837 cm^{-1}.

(20*S*,22*S*)-De-A,B-8β-[(*tert*-butyldimethylsilyl)oxy]-25-(methoxymethyl)oxy-22-[(*p*-tolylsulfonyl)oxymethyl]cholestane (15). To a solution of **14** (780 mg, 1.66 mmol) in pyridine (10 mL) was added *p*-tosyl chloride (475 mg, 2.49 mmol) with stirring at 0 °C under an argon atmosphere. The resulting mixture was stirred overnight at room temperature. The reaction mixture was poured into 4% aqueous HCl solution. After extraction with ethyl acetate, the organic layer was washed with brine, dried over magnesium sulfate and filtered. Evaporation of the filtrate afforded a crude mixture, from which **15** (1.02 g) was separated by silica gel column chromatography (ethyl acetate:*n*-hexane = 10:90) in 99% yield as a colorless oil.

$[\alpha]_D = +3.53$ ($c = 0.85$, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ −0.02 (3H, s), 0.00 (3H, s), 0.67 (3H, d, $J = 7.0$ Hz), 0.86 (3H, s), 0.87 (9H, s), 1.18 (6H, s), 1.91 (1H, m), 2.44 (3H, s), 3.35 (3H, s), 3.82 (1H, t, $J = 8.9$ Hz), 3.95 (1H, m), 4.07 (1H, dd, $J = 9.2$, 3.7 Hz), 4.67 (2H, m), 7.34 (2H, d, $J = 7.9$ Hz), 7.79 (2H, d, $J = 7.9$ Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ −5.3 (q), −4.9 (q), 13.2 (q), 13.6 (q), 17.5 (t), 17.9 (s), 21.5 (q), 22.7 (t), 24.0 (t), 25.7 (q), 26.2 (q), 26.5 (q), 27.6 (t), 34.3 (t), 35.1 (d), 39.8 (t), 39.4 (t), 39.7 (d), 39.9 (t), 42.2 (s), 52.9 (d), 53.4 (d), 55.1 (d), 69.3 (d), 71.8 (t), 76.0 (s), 91.0 (t), 128.1 (d), 129.8 (d), 133.2 (s), 144.6 (s); FTIR (neat) 2932, 1531, 1468, 1176, 1096, 1038, 910, 733 cm^{-1}.

(20*S*,22*R*)-De-A,B-8β-[*tert*-butyldimethylsilyl)oxy]-25-[(methoxymethyl)oxy]-22-methylcholestane (16). A solution of **15** (420 mg, 0.67 mmol) in dry ether (10 mL) was treated with $LiAlH_4$ (250 mg, 6.7 mmol) with stirring at 0 °C. The resulting suspension was allowed to stand overnight with stirring at room temperature. The mixture was diluted with ether and the whole was filtered through a small pad of silica gel. Evaporation of the filtrate afforded a residue, from which **16** (271 mg, 89%) and **14** (30 mg, 9.5%) were separated by silica gel column chromatography (ethyl acetate:*n*-hexane = 5:95), both as colorless oils.

$[\alpha]_D = +17.9$ ($c = 1.54$, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.00 (3H, s), 0.01 (3H, s), 0.68 (3H, d, $J = 6.7$ Hz), 0.74 (3H, d, $J = 7.0$ Hz), 0.89 (9H, s), 0.92 (3H, s), 1.21 (6H, s), 3.37 (3H, s), 4.00 (1H, m), 4.71 (2H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ −5.3 (q), −4.9 (q), 12.0 (q), 13.7 (q), 13.8 (q), 17.7 (t), 18.0 (s), 22.9 (t), 25.8 (q), 26.3 (q), 26.4 (q), 27.6 (t), 29.9 (t), 34.5 (t), 34.7 (d), 38.1 (d), 40.0 (t), 40.3 (t), 42.2 (s), 52.2 (d), 53.6 (d), 55.1 (q), 69.5 (d), 76.4 (s), 91.0 (t); FTIR (neat) 2934, 1381, 1253, 1165, 1096, 1038, 911, 733 cm^{-1}; MS 439 $[M-Me]^+$, 393 $[M-OCH_2OCH_3]^+$; HRMS calcd for $[C_{26}H_{51}O_3Si]$ 439.3608, found 439.3611.

(20*S*,22*R*)-De-A,B-22-methylcholestane-8β,25-diol (17). A solution of **16** (300 mg, 0.66 mmol) in MeOH (5 mL) was treated with CSA (306 mg, 1.32 mmol) at room temperature. The reaction mixture was stirred at room temperature for 2 days. After the reaction was completed, saturated aqueous $NaHCO_3$ solution was added to the mixture. The whole was extracted with ethyl acetate. The organic layer was washed with brine, dried over magnesium sulfate and filtered. Purification by silica gel column chromatography (ethyl acetate:*n*-hexane = 20:80) afforded **17** (185 mg) in 95% yield as a solid, which was recrystallized from ethyl acetate to give analytically pure **17** as colorless prisms.

Mp 139–141 °C (recryst. from ethyl acetate); $[\alpha]_D = +36.7$ ($c = 0.91$, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.69 (3H, d, $J = 6.7$ Hz), 0.76 (3H, d, $J = 6.7$ Hz), 0.93 (3H, s), 1.21 (6H, s), 1.96 (1H, m), 4.08 (1H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.0 (q), 13.5 (q), 13.7 (q), 17.4 (t), 22.3 (t), 27.3 (t), 29.1 (q), 29.3 (q), 30.2 (t), 33.6 (t), 34.7 (d), 38.0 (d), 40.0 (t), 41.9 (s), 42.1 (t), 52.7 (d), 53.4 (d), 69.4 (d), 71.1 (s); FTIR (neat) 3339, 2923, 1456, 1375, 1266, 1161, 1113, 1069, 1034, 987, 906, 729 cm^{-1}; MS 278 $[M-H_2O]^+$, 260 $[M-2H_2O]^+$; HRMS calcd for $[C_{19}H_{34}O]$ 278.2610, found 278.2611. Anal. calcd for $C_{19}H_{36}O_2$: C, 76.97; H, 12.24; found: C, 77.06; H, 12.01.

(20*S*,22*R*)-De-A,B-25-hydroxy-22-methylcholestan-8-one (18). Pyridinium dichromate (PDC, 1.04 g, 2.76 mmol) was added to a stirred mixture of **17** (205 mg, 0.69 mmol) in dry CH_2Cl_2 (10 mL) at room temperature under argon. The mixture was stirred at room temperature for 4 h, and separated by silica gel column chromatography (ethyl acetate:*n*-hexane = 5:95) to give the corresponding ketone **18** (200 mg) as a colorless oil in 99% yield.

$[\alpha]_D = -7.9$ ($c = 1.77$, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.63 (3H, s), 0.73 (3H, d, $J = 6.7$ Hz), 0.81 (3H, d, $J = 7.0$ Hz), 1.22 (6H, s), 2.23 (2H, m), 2.46 (1H, dd, $J = 11.3$, 7.6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.0 (q), 12.3 (q), 13.8 (q), 18.8 (t), 23.9 (t), 27.6 (t), 29.1 (q), 29.3 (q), 30.1 (t), 35.0 (d), 38.2 (d), 38.6 (t), 40.9 (t), 42.0 (t), 49.9 (s), 53.6 (d), 62.0 (d), 71.0 (s), 212.2 (s); FTIR (neat) 3158, 2967, 2878, 1705, 1469, 1385, 1219, 1098, 908, 740 cm^{-1}; MS 294 $[M]^+$, 276 $[M-H_2O]^+$, 261 $[M-H_2O-Me]^+$; HRMS calcd for $[C_{19}H_{34}O_2]$ 294.2559, found 294.2555.

(*E*)-(20*S*,22*R*)-De-A,B-8-bromomethylene-22-methylcholestan-25-ol (19). Sodium hexamethyldisilazide (NaHMDS) (1.0 M in THF, 3.40 mL, 3.40 mmol) was

added to (bromomethyl)triphenylphosphonium bromide (1.55 g, 3.55 mmol) in THF (1.5 mL) at −60 °C under argon. After 1 h, a solution of **18** (210 mg, 0.71 mmol) in THF (5 mL) was added. After an additional one hour at room temperature, the reaction mixture was diluted with *n*-hexane and the whole was filtered over Celite®. The filtrate was concentrated, then purified by silica gel column chromatography (ethyl acetate:*n*-hexane = 10:90) to give **19** (132 mg) as a colorless oil in 50% yield.

$[\alpha]_D = +87.4$ ($c = 0.54$, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 0.55 (3H, s), 0.71 (3H, d, $J = 6.4$ Hz), 0.78 (3H, d, $J = 6.7$ Hz), 1.21 (6H, s), 2.87 (1H, m), 5.65 (1H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 11.7 (q), 12.1 (q), 13.8 (q), 21.8 (t), 22.5 (t), 27.7 (t), 29.1 (q), 29.3 (q), 30.2 (t), 31.0 (t), 35.0 (d), 38.7 (d), 39.5 (t), 42.1 (t), 45.5 (s), 52.7 (d), 55.9 (d), 71.1 (s), 97.5 (d), 145.2 (s); FTIR (neat) 3381, 2964, 2872, 1632, 1466, 1377, 1088 cm^{-1}; MS 370 $[M]^+$; HRMS calcd for $[C_{20}H_{35}O^{79}Br]$ 370.1870, found 370.1871.

(5*Z*,7*E*)-(1*S*,2*S*,3*R*,20*S*,22*R*)-2,22-Dimethyl-9,10-seco-5,7,10(19)-cholestatriene-1,3,25-triol (7a). A mixture of $(dba)_3Pd_2{\cdot}CHCl_3$ (4.0 mg, 0.0039 mmol), Ph_3P (9.0 mg, 0.034 mmol) and triethylamine (1 mL) in toluene (1 mL) was stirred for 10 min at room temperature, then a solution of the A-ring moiety **20a**[3] (15 mg, 0.039 mmol) and the CD-ring moiety **19** (22 mg, 0.059 mmol) in toluene (0.5 mL) was added. After having been heated at reflux for 6 h and diluted with pentane, the reaction mixture was filtered through a pad of silica gel with ether. After evaporation of the solvent, the crude mixture was dissolved in methanol (1.5 mL) and treated with CSA (9.0 mg, 0.039 mmol) at room temperature for 2 days. After removal of the solvent, the residue was subjected to silica gel column chromatography (ethyl acetate:*n*-hexane = 50:50) to give **7a** (5.4 mg) as a colorless oil in 30% yield. Further purification for biological evaluation was conducted by using reversed-phase recycle HPLC (YMC-Pack ODS column, 20 mm × 150 mm, 9.0 ml/min, acetonitrile: water = 85:15).

$[\alpha]_D = +10.0$ ($c = 0.11$, $CHCl_3$); UV (EtOH) λ_{max} 265 nm, λ_{min} 228 nm; ^1H NMR (400 MHz, $CDCl_3$) δ 0.53 (3H, s), 0.71 (3H, d, $J = 5.8$ Hz), 0.78 (3H, d, $J = 6.7$ Hz), 1.08 (3H, d, $J = 7.0$ Hz), 1.21 (6H, s), 2.23 (1H, dd, $J = 13.1$, 7.9 Hz), 2.67 (1H, dd, $J = 13.1$, 4.0 Hz), 2.83 (1H, m), 3.85 (1H, m), 4.31 (1H, m), 5.01 (1H, d, $J = 1.5$ Hz), 5.28 (1H, d, $J = 1.5$ Hz), 6.02 (1H, d, $J = 11.3$ Hz), 6.39 (1H, d, $J = 11.3$ Hz); MS 444 $[M]^+$, 426 $[M-H_2O]^+$, 408 $[M-2H_2O]^+$, 393 $[M-2H_2O-Me]^+$, 375 $[M-3H_2O-Me]^+$; HRMS calcd for $[C_{29}H_{48}O_3]$ 444.3603, found 444.3603.

(5*Z*,7*E*)-(1*S*,2*R*,3*R*,20*S*,22*R*)-2,22-Dimethyl-9,10-seco-5,7,10(19)-cholestatriene-1,3,25-triol (7b). This compound was obtained by the same procedure as described for **7a** using **20b** instead of **20a**, in 23% yield.

$[\alpha]_D = -79.1$ (c = 0.12, $CHCl_3$); UV (EtOH) λ_{max} 263 nm, λ_{min} 227 nm; ^1H NMR (400 MHz, $CDCl_3$) δ 0.54 (3H, s), 0.71 (3H, d, $J = 6.1$ Hz), 0.78 (3H, d, $J = 6.7$ Hz), 1.15 (3H, d, $J = 6.7$ Hz), 1.21 (6H, s), 2.42 (1H, dd, $J = 13.7$, 4.9 Hz), 2.52 (1H, d, $J = 13.7$ Hz), 2.83 (1H, m), 4.03 (2H, m), 5.02 (1H, s), 5.37 (1H, s), 6.04 (1H, d, $J = 11.0$ Hz), 6.35 (1H, d, $J = 11.0$ Hz); MS 444 $[M]^+$, 426 $[M-H_2O]^+$, 408 $[M-2H_2O]^+$, 393 $[M-2H_2O-Me]^+$, 375 $[M-3H_2O-Me]^+$; HRMS calcd for $[C_{29}H_{48}O_3]$ 444.3603, found 444.3606.

(5*Z*,7*E*)-(1*S*,2*S*,3*S*,20*S*,22*R*)-2,22-Dimethyl-9,10-seco-5,7,10(19)-cholestatriene-1,3,25-triol (7c). This compound was obtained by the same procedure as described for **7a** using **20c** instead of **20a**, in 31% yield.

$[\alpha]_D = +86.8$ (c = 0.04, $CHCl_3$); UV (EtOH) λ_{max} 264 nm, λ_{min} 228 nm; ^1H NMR (400 MHz, $CDCl_3$) δ 0.53 (3H, s), 0.71 (3H, d, $J = 5.8$ Hz), 0.78 (3H, d, $J = 6.7$ Hz), 1.21 (6H, s), 2.49 (1H, d, $J = 13.7$ Hz), 2.58 (1H, dd, $J = 13.7$, 4.0 Hz), 2.83 (1H, m), 3.84 (1H, m), 4.18 (1H, m), 4.98 (1H, d, $J = 1.8$ Hz), 5.23 (1H, d, $J = 1.8$ Hz), 6.04 (1H, d, $J = 11.0$ Hz), 6.48 (1H, d, $J = 11.0$ Hz); MS 444 $[M]^+$, 426 $[M-H_2O]^+$, 408 $[M-2H_2O]^+$, 393 $[M-2H_2O-Me]^+$; HRMS calcd for $[C_{29}H_{48}O_3]$ 444.3603, found 444.3603.

(5*Z*,7*E*)-(1*R*,2*R*,3*S*,20*S*,22*R*)-2,22-Dimethyl-9,10-seco-5,7,10(19)-cholestatriene-1,3,25-triol (7d). This compound was obtained by the same procedure as described for **7a** using **20d** instead of **20a**, in 46% yield.

$[\alpha]_D = -56.5$ ($c = 0.23$, $CHCl_3$); UV (EtOH) λ_{max} 265 nm, λ_{min} 226 nm; ^1H NMR (400 MHz, $CDCl_3$) δ 0.54 (3H, s), 0.71 (3H, d, $J = 5.8$ Hz), 0.78 (3H, d, $J = 6.7$ Hz), 1.11 (3H, d, $J = 6.7$ Hz), 1.21 (6H, s), 2.24 (1H, m), 2.66 (1H, dd, $J = 13.1$, 4.0 Hz), 2.82 (1H, dd, $J = 12.5$, 4.0 Hz), 3.82 (1H, m), 4.27 (1H, m), 5.02 (1H, d, $J = 1.5$ Hz), 5.28 (1H, d, $J = 1.5$ Hz), 6.02 (1H, d, $J = 11.3$ Hz), 6.39 (1H, d, $J = 11.3$ Hz); MS 444 $[M]^+$, 426 $[M-H_2O]^+$, 408 $[M-2H_2O]^+$, 393 $[M-2H_2O-Me]^+$, 375 $[M-3H_2O-Me]^+$; HRMS calcd for $[C_{29}H_{48}O_3]$ 444.3603, found 444.3606.

(20*R*)-De-A,B-8β-[(*tert*-butyldimethylsilyl)oxy]pregnan-20-ol (21).[17] ^1H NMR (400 MHz, $CDCl_3$) δ −0.01 (3H, s), 0.01 (3H, s), 0.89 (9H, s), 1.00 (3H, s), 1.12 (3H, d, $J = 6.1$ Hz), 3.74 (1H, m), 4.01 (1H, d, $J = 2.4$ Hz); MS 312 $[M]^+$, 297 $[M-Me]^+$; HRMS calcd for $[C_{18}H_{36}O_2Si]$ 312.2485, found 312.2465.

(20*S*)-Isomer. ^1H NMR (400 MHz, $CDCl_3$) δ 0.01 (3H, s), 0.02 (3H, s), 0.88 (9H, s), 0.97 (3H, s), 1.20 (3H, d, $J = 6.1$ Hz), 3.68 (1H, m), 3.99 (1H, d, $J = 2.7$ Hz); MS 312 $[M]^+$, 297 $[M-Me]^+$; HRMS calcd for $[C_{18}H_{36}O_2Si]$ 312.2485, found 312.2483.

(20*R*)-De-A,B-8β,25-bis[(*tert*-butyldimethylsilyl)oxy]-24,26,27-trihomo-22-oxacholestane (23). To a stirred suspension of potassium hydride (30%, 200 mg, 3.30 mmol) and 18-crown-6 ether (698 mg, 2.64 mmol) in THF (5 mL) was added **21** (688 mg, 2.20 mmol) under an argon atmosphere. The mixture was stirred for 15 min at room temperature, then a solution of side-chain moiety **22** (1.30 g, 3.52 mmol) in THF (5 mL) was

added dropwise to it. The resulting mixture was stirred overnight at room temperature, then cooled and water was added to destroy the excess reagent. After extraction 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 **23** (1.03 g) was separated by silica gel column chromatography (ethyl acetate:*n*-hexane = 1:4) in 86% yield as a colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ −0.01 (3H, s), 0.01 (3H, s), 0.06 (6H, s), 0.815 (3H, t, J = 7.6 Hz), 0.823 (3H, t, J = 7.6 Hz), 0.86 (9H, s), 0.89 (9H, s), 0.94 (3H, s), 1.04 (3H, d, J = 5.8 Hz), 3.12 (1H, m), 3.25 (1H, dq, J = 9.8, 6.1 Hz), 3.56 (1H, m), 4.00 (1H, m); MS 525 $[M-Et]^+$; HRMS calcd for $[C_{30}H_{61}O_3Si_2]$ 525.4159, found 525.4155.

(20*R*)-De-A,B-24,26,27-trihomo-22-oxacholestane-8β, 25-diol (24). A solution of **23** (240 mg, 0.44 mmol) in CH_2Cl_2 (4 mL) was treated with TFA (1.5 mL) at 0 °C with stirring. The mixture was stirred for 45 min, then diluted with water and extracted with ethyl acetate. The organic layer was washed with brine, and dried over magnesium sulfate. Evaporation of the filtrate afforded a residue, from which **24** (116 mg) was separated by silica gel column chromatography (ethyl acetate:hexane = 1:4) in 81% as a colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 0.871 (3H, t, J = 7.6 Hz), 0.874 (3H, t, J = 7.6 Hz), 0.95 (3H, s), 1.06 (3H, d, J = 5.8 Hz), 1.92 (2H, q, J = 7.6 Hz), 1.94 (2H, q, J = 7.6 Hz), 2.10 (1H, m), 3.16 (1H, dt, J = 8.9, 6.4 Hz), 3.28 (1H, dq, J = 9.8, 5.8 Hz), 3.57 (1H, dt, J = 8.9, 6.4 Hz), 4.10 (1H, m); MS 308 $[M-H_2O]^+$; HRMS calcd for $[C_{20}H_{36}O_2]$ 308.2715, found 308.2716.

(20*R*)-De-A,B-24,26,27-trihomo-25-hydroxy-22-oxacholestan-8-one (25). A stirred mixture of **24** (164 mg, 0.50 mmol) and powdered 4 Å MS (60 mg) in CH_2Cl_2 (4 mL) was treated with PDC (472 mg, 1.25 mmol) at room temperature. The mixture was stirred at room temperature for 1 h under argon, and separated by silica gel column chromatography (ethyl acetate:hexane = 1:6) to give the corresponding ketone **25** (130 mg, 80%) as a colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 0.55 (3H, s), 0.777 (3H, t, J = 7.6 Hz), 0.781 (3H, t, J = 7.6 Hz), 0.90 (3H, d, J = 5.8 Hz), 1.83 (4H, q, J = 7.6 Hz), 2.69 (1H, dd, J = 11.0, 7.3 Hz), 3.07 (1H, dt, J = 9.2, 6.2 Hz), 3.17 (1H, m), 3.49 (1H, dt, J = 9.2, 6.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 7.4 (q), 7.5 (q), 12.9 (q), 18.2 (q), 19.4 (t), 24.0 (t), 24.1 (t), 25.1 (t), 27.2 (t), 31.4 (t), 39.0 (t), 41.2 (t), 49.9 (s), 56.8 (d), 61.5 (d), 67.8 (t), 77.6 (d), 94.8 (s), 212.0 (s); FTIR (neat) 3750, 2968, 2880, 1776, 1714, 1458, 1373, 1336, 1217, 1169 cm^{-1}; MS 306 $[M-H_2O]^+$; HRMS calcd for $[C_{20}H_{34}O_2]$ 306.2559, found 306.2559.

(20*R*)-De-A,B-8-bromomethylene-24,26,27-trihomo-22-oxacholestan-25-ol (26). A stirred suspension of (bromomethyl)triphenylphosphonium bromide (719 mg, 1.65 mmol) in THF (4 mL) was treated with NaHMDS (1.0 M in THF, 1.6 mL, 1.6 mmol) at −60 °C under argon. The mixture was stirred at −60 °C for 1 h, then a solution of **25** (107 mg, 0.33 mmol) in THF (2 mL) was added to it. The resulting solution was stirred at −60 °C for 70 min, then at room temperature for 2 h. The reaction was quenched by addition of a small amount of water. After extraction with ethyl acetate, the organic layer was washed with brine, dried over magnesium sulfate and concentrated. The residue was purified by silica gel column chromatography (ethyl acetate:hexane = 1:5) to afford the desired olefin **26** (44 mg) as a pale yellow oil in 33% yield.

^{1}H NMR (400 MHz, $CDCl_3$) δ 0.57 (3H, s), 0.84 (3H, t, J = 7.6 Hz), 0.85 (3H, t, J = 7.3 Hz), 1.09 (3H, d, J = 5.8 Hz), 1.46 (4H, m), 2.00 (1H, ddd, J = 12.2, 6.7, 1.5 Hz), 2.16 (1H, m), 2.88 (1H, m), 3.22 (1H, dt, J = 8.9, 6.1 Hz), 3.27 (1H, dq, J = 10.1, 5.8 Hz), 3.56 (1H, dt, J = 8.9, 6.1 Hz), 5.63 (1H, t, J = 1.5 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 7.8 (q), 7.9 (q), 12.5 (q), 18.2 (q), 22.2 (t), 22.5 (t), 24.2 (t), 25.0 (t), 30.9 (t), 31.1 (t), 35.6 (t), 39.7 (t), 45.5 (s), 55.4 (d), 56.1 (d), 68.8 (t), 74.1 (s), 78.1 (d), 97.2 (d), 145.2 (s); FTIR (neat) 3447, 2964, 2876, 2363, 1456, 1371, 1336, 1130, 1105, 949 cm^{-1}; MS 371 $[M-Et]^+$; HRMS calcd for $[C_{19}H_{32}O_2{}^{79}Br]$ 371.1586, found 371.1585.

(5*Z*,7*E*)-(1*S*,2*S*,3*R*,20*R*)-24,26,27-Trihomo-2-methyl-22-oxa-9,10-seco-5,7,10(19)-cholestatriene-1,3,25-triol (8a). A mixture of $(dba)_3Pd_2{\cdot}CHCl_3$ (6.0 mg, 0.0062 mmol), Ph_3P (15 mg, 0.056 mmol) and triethylamine (1 mL) in toluene (0.5 mL) was stirred for 10 min at rt, then a solution of the A-ring moiety **20a** (30 mg, 0.076 mmol) and the CD-ring moiety **26** (25 mg, 0.062 mmol) in toluene (0.5 mL) was added. After having been heated at reflux for 6 h and diluted with pentane, the reaction mixture was filtered through a pad of silica gel with ether. After evaporation of the solvent, the crude mixture was dissolved in methanol (2 mL) and treated with CSA (6.0 mg, 0.026 mmol) at room temperature overnight. After removal of the solvent, the residue was subjected to silica gel column chromatography (ethyl acetate:*n*-hexane = 2:3) to give **8a** (1.7 mg) as a colorless oil in 6% yield. Further purification for biological evaluation was conducted by using reversed-phase recycle HPLC (YMC-Pack ODS column, 20 mm × 150 mm, 9.0 mL/min, acetonitrile:water = 80:20).

$[\alpha]_D^{22} = +253$ (c = 0.00316, EtOH); UV (EtOH) λ_{max} 265 nm, λ_{min} 226 nm; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.55 (3H, s), 0.848 (3H, t, J = 7.6 Hz), 0.853 (3H, t, J = 7.3 Hz), 1.08 (6H, d, J = 6.4 Hz), 1.46 (4H, m), 1.91 (1H, ddq, J = 3.4, 7.3, 6.4 Hz), 2.01 (1H, t, J = 9.8 Hz), 2.14 (1H, d, J = 12.2 Hz), 2.23 (1H, dd, J = 13.4, 7.9 Hz), 2.67 (1H, dd, J = 13.4, 4.6 Hz), 2.83 (1H, dd, J = 12.5, 4.0 Hz), 3.22 (1H, dt, J = 8.9, 5.8 Hz), 3.26 (1H, dq, J = 9.8, 6.4 Hz), 3.57 (1H, dt, J = 8.9, 6.1 Hz), 3.84 (1 H, m), 4.29 (1H, m), 5.01 (1H, d, J = 1.8 Hz), 5.27 (1H, d, J = 0.9 Hz), 5.99 (1H, d, J = 11.3 Hz), 6.40 (1H, d, J = 11.3 Hz); FTIR (neat) 3387, 2964, 2876, 1454, 1373, 1332, 1103, 949 cm^{-1}; MS 474 $[M]^+$, 456 $[M-H_2O]^+$, 438 $[M-2H_2O]^+$, 420 $[M-3H_2O]^+$; HRMS calcd for $[C_{30}H_{50}O_4]$ 474.3709, found 474.3718.

(5*Z*,7*E*)-(1*S*,2*R*,3*R*,20*R*)-24,26,27-Trihomo-2-methyl-22-oxa-9,10-seco-5,7,10(19)-cholestatriene-1,3,25-triol (8b). This compound was obtained by the same procedure as described for **8a** using **20b** instead of **20a**, in 38% yield.

$[\alpha]_D^{24} = +434$ ($c = 0.00115$, EtOH); UV (EtOH) λ_{max} 263 nm, λ_{min} 227 nm; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.56 (3H, s), 0.848 (3H, t, $J = 7.6$ Hz), 0.853 (3H, t, $J = 7.6$ Hz), 1.09 (3H, d, $J = 5.8$ Hz), 1.15 (3H, d, $J = 7.0$ Hz), 1.46 (4H, m), 1.79 (1H, ddq, $J = 2.6$, 9.2, 7.0 Hz), 2.00 (1H, t, $J = 9.9$ Hz), 2.16 (1H, d, $J = 12.5$ Hz), 2.42 (1H, dd, $J = 13.4$, 5.2 Hz), 2.52 (1H, dd, $J = 13.4$ Hz), 2.83 (1H, dd, $J = 12.2$, 4.0 Hz), 3.22 (1H, dt, $J = 8.9$, 5.6 Hz), 3.27 (1H, dq, $J = 9.8$, 6.1 Hz), 3.56 (1H, dt, $J = 8.9$, 6.1 Hz), 4.00 (1H, m), 4.02 (1H, m), 5.02 (1H, t, $J = 1.8$ Hz), 5.37 (1H, t, $J = 1.8$ Hz), 6.01 (1H, d, $J = 11.3$ Hz), 6.35 (1H, d, $J = 11.3$ Hz); FTIR (neat) 3406, 2963, 2930, 2876, 2235, 1639, 1456, 1373, 1332, 1217, 1105, 993, 949 cm^{-1}; MS 474 $[M]^+$, 456 $[M-H_2O]^+$, 438 $[M-2H_2O]^+$, 420 $[M-3H_2O]^+$; HRMS calcd for $[C_{30}H_{50}O_4]$ 474.3709, found 474.3710.

(5*Z*,7*E*)-(1*S*,2*S*,3*S*,20*R*)-24,26,27-Trihomo-2-methyl-22-oxa-9,10-seco-5,7,10(19)-cholestatriene-1,3,25-triol (8c). This compound was obtained by the same procedure as described for **8a** using **20c**, instead of **20a** in 40% yield.

$[\alpha]_D^{22} = +534$ ($c = 0.00187$, EtOH); UV (EtOH) λ_{max} 265 nm, λ_{min} 226 nm; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.55 (3H, s), 0.849 (3H, t, $J = 7.6$ Hz), 0.855 (3H, t, $J = 7.3$ Hz), 1.09 (3H, d, $J = 5.8$ Hz), 1.22 (3H, d, $J = 7.3$ Hz), 1.47 (4H, m), 1.92 (1H, tq, $J = 2.7$, 7.3 Hz), 2.01 (1H, t, $J = 9.5$ Hz), 2.16 (1H, d, $J = 8.9$ Hz), 2.50 (1H, d, $J = 13.7$ Hz), 2.58 (1H, dd, $J = 13.7$, 4.0 Hz), 2.86 (1H, dd, $J = 12.5$, 4.0 Hz), 3.22 (1H, dt, $J = 8.9$, 5.8 Hz), 3.27 (1H, dq, $J = 9.8$, 5.8 Hz), 3.56 (1H, dt, $J = 8.9$, 6.4 Hz), 3.91 (1H, m), 4.17 (1H, m), 4.97 (1H, d, $J = 2.1$ Hz), 5.23 (1H, d $J = 1.8$ Hz), 6.01 (1H, d, $J = 11.3$ Hz), 6.48 (1H, d, $J = 11.3$ Hz); FTIR (neat) 3383, 2964, 2876, 2365, 2239, 1736, 1649, 1456, 1373, 1334, 1271, 1217, 1062, 1030, 970, 949 cm^{-1}; MS 474 $[M]^+$, 456 $[M-H_2O]^+$, 438 $[M-2H_2O]^+$, 420 $[M-3H_2O]^+$; HRMS calcd for $[C_{30}H_{50}O_4]$ 474.3709, found 474.3711.

(5*Z*,7*E*)-(1*R*,2*R*,3*S*,20*R*)-24,26,27-Trihomo-2-methyl-22-oxa-9,10-seco-5,7,10(19)-cholestatriene-1,3,25-triol (8d). This compound was obtained by the same procedure as described for **8a** using **20d** instead of **20a**, in 10% yield.

$[\alpha]_D^{21} = +114$ ($c = 0.00262$, EtOH); UV (EtOH) λ_{max} 264 nm, λ_{min} 226 nm; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.56 (3H, s), 0.848 (3H, t, $J = 7.6$ Hz), 0.853 (3H, t, $J = 7.6$ Hz), 1.09 (3H, d, $J = 6.1$ Hz), 1.10 (3H, d, $J = 7.3$ Hz), 1.46 (4H, m), 1.86 (1H, ddq, $J = 3.4$, 8.2, 7.3 Hz), 2.00 (1H, t, $J = 9.1$ Hz), 2.15 (1H, d, $J = 12.4$ Hz), 2.24 (1H, dd, $J = 13.4$, 8.2 Hz), 2.67 (1H, dd, $J = 13.4$, 4.3 Hz), 2.83 (1H, dd, $J = 12.2$, 4.0 Hz), 3.22 (1H, dt, $J = 8.5$, 6.1 Hz), 3.27 (1H, dq, $J = 9.8$, 5.8 Hz), 3.56 (1H, dt, $J = 8.5$, 6.1 Hz), 3.82 (1H, dt, $J = 8.2$, 4.3 Hz), 4.27 (1H, m), 5.01 (1H, d, $J = 2.1$ Hz), 5.27 (1H, d $J = 1.8$ Hz), 6.00 (1H, d, $J = 11.3$ Hz), 6.40 (1H, d, $J = 11.3$ Hz); FTIR (neat) 3406, 2964, 2876, 1649, 1454, 1371, 1332, 1103, 972, 949 cm^{-1}; MS 474 $[M]^+$, 456 $[M-H_2O]^+$, 438 $[M-2H_2O]^+$; HRMS calcd for $[C_{30}H_{50}O_4]$ 474.3709, found 474.3711.

(5*Z*,7*E*)-(1*S*,3*R*,20*R*)-24,26,27-Trihomo-22-oxa-9,10-seco-5,7,10(19)-cholestatriene-1,3,25-triol (6).[4] For comparison, KH-1060 (**6**) was synthesized by the same procedure as described for **8a** using (3*S*,5*R*)-3,5-bis[(*tert*-butyldimethylsilyl)oxy]oct-1-en-7-yne[5] instead of **20a**, in 30% yield.

UV (EtOH) λ_{max} 262 nm, λ_{min} 226 nm; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.56 (3H, s), 0.848 (3H, t, $J = 7.3$ Hz), 0.852 (3H, t, $J = 7.6$ Hz), 1.08 (6H, d, $J = 6.1$ Hz), 1.46 (4H, m), 2.31 (1H, dd, $J = 13.1$, 6.4 Hz), 2.60 (1H, dd, $J = 13.1$, 3.7 Hz), 2.83 (1H, dd, $J = 11.9$, 4.0 Hz), 3.22 (1H, dt, $J = 8.9$, 6.1 Hz), 3.28 (1H, dq, $J = 9.8$, 5.8 Hz), 3.60 (1H, dt, $J = 8.9$, 6.1 Hz), 4.23 (1H, m), 4.43 (1H, m), 5.00 (1H, m), 5.32 (1H, t, $J = 1.5$ Hz), 5.99 (1H, d, $J = 11.0$ Hz), 6.39 (1H, d, $J = 11.0$ Hz).

X-ray crystallographic analysis of 17. A colorless prismatic crystal with dimensions of 0.30 × 0.10 × 0.40 mm was obtained by recrystallization from ethyl acetate. The observed cell parameters were as follows: $C_{19}H_{36}O_2$, $Mr = 296.49$, orthorhombic, $P2_12_12$, $a = 9.911(14)$ Å, $b = 19.205(10)$ Å, $c = 9.825(15)$ Å, $V = 1870(6)$ Å^3, $Z = 4$, $Dx = 1.053$ g/cm^3, λ(Cu*K*α) = 1.54178 Å, μ(Cu*K*α) = 5.01 cm^{-1}, $F(000) = 664.00$, 23 °C. 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. $R = 0.079$, $Rw = 0.097$ for 1136 reflections ($I > 2.00\ \sigma(I)$).

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 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 and centrifuged at 3000 rpm for 10 min. The radioactivity of the supernatant (500 μL) with ACS-II (9.5 mL) (Amersham, England) was then counted.

Cell surface antigen expression analysis. HL-60 cells were seeded at 10^5 cells/well in 24-well plates, and incubated for 72 h with between 10^{-12} and 10^{-7} M 1α,25-dihydroxyvitamin D_3 or an analogue at 37 °C in a humidified atmosphere of 5% carbon dioxide in air. The cells were then washed with PBS and adjusted to 2×10^6 cells/100 μL of Diluent solution [phosphate-buffered saline (minus Mg^{2+}, minus Ca^{2+}) containing 1% BSA and 1% NaN_3]. Aliquots of cell suspension (100 μL) were incubated with 10 μL of the human

monoclonal fluorescein isothiocyanate-conjugated CD11b antibody for 30 min at room temperature in the dark. The cells were washed once with Diluent solution and then fixed in 500 μL of PBS containing 2% paraformaldehyde. Fluorescence was read on a Becton Dickinson FACSan^{TM} at an excitation wavelength of 490 nm and emission wavelength of 520 nm. Results were recorded as the mean fluorescence index, which is the product of the % fluorescence and the mean fluorescence intensity, with 10^4 cells being counted per treatment.

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13. The side-chain moiety **11**, 4-bromo-2-methylbutan-2-ol MOM-ether, was synthesized via two-step conversion [(a) MeMgBr/THF, 0 °C, 1 h, 66%. (b) MOMCl/ $^{i}Pr_2NEt$, rt, 13.5 h, 77%] of commercially available ethyl 3-bromopropionate. **11**: ^1H NMR (400 MHz, $CDCl_3$) δ 1.24 (6H, s), 2.11 (2H, m), 3.36 (3H, s), 3.46 (2H, m), 4.69 (2H, s); MS 195 [M−Me]$^+$.
14. Introduction of a 22*R*-methyl group into 20-*epi*-1α,25-dihydroxyvitamin D_3 was predicted by computational methods to restrict the dihedral angle at C(17-20-22-23) to *anti* in ref 8a. Our experimental data show that the dihedral angle at C(17-20-22-23) in **17** is 169.2(7)°, suggesting that the *anti* conformer also dominates in the case of **17** in the solid state.
15. The A-ring synthons, **20a**, **20b**, **20c** and **20d**, correspond to (3*S*,4*S*,5*R*)-, (3*S*,4*R*,5*R*)-, (3*S*,4*S*,5*S*)- and (3*R*,4*R*,5*S*)-3,5-bis[(*tert*-butyldimethylsilyl)oxy]-4-methyloct-1-en-7-yne.
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18. The side-chain moiety **22**, 3-ethyl-6-iodohexan-3-ol TBS ether, was synthesized via four-step conversion [(a) EtMgBr/THF, −78 °C, 3 h, 94%. (b) TsCl/pyridine, 0 °C, 1 h, 96%. (c) TBSOTf/2,6-lutidine, 0 °C, 1 h, quant. (d) NaI/DMF, rt, 6 h, 92%] of commercially available γ-butylolactone. **22**: ^1H NMR (400 MHz, $CDCl_3$) δ 0.77 (6H, s), 0.84 (6H, t, J = 7.3 Hz), 0.87 (9H, s), 1.46 (2H, q, J = 7.3 Hz), 1.48 (2H, q, J = 7.3 Hz), 1.51 (2H, m), 1.83 (2H, m), 3.18 (2H, t, J = 7.0 Hz); MS 355 [M−Me]$^+$; HRMS calcd for [$C_{13}H_{28}IOSi$] 355.0954, found 355.0953.
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