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Synthesis and Biological Activity of 2,22-Dimethylene Analogues of 19-Norcalcitriol and Related Compounds

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ABSTRACT: Continuing our search for vitamin D analogues we explored modification of the steroidal side chain and inserted a methylene moiety in position C-22 together with either lengthening the side chain or introducing a ring at the terminal end. Our conformational studies confirmed that the presence of a methylene group attached to C-22 restricts conformational flexibility of the side chain which can result in changes in biological characteristics of a molecule. All synthesized 1α,25-dihydroxy-2,22-dimethylene-19-norvitamin D_3 analogues proved equal to calcitriol in their ability to bind to VDR and most of them exert significantly higher differentiation and transcriptional activity than calcitriol. The most active compounds were characterized by the presence of an elongated side chain or 26,27-dimethylene bridge. The synthetic strategy was based on the Wittig-Horner coupling of the known A-ring phosphine oxide with the corresponding Grundmann ketones prepared from a 20-epi-Inhoffen-Lythgoe diol derived from vitamin D_2.

INTRODUCTION

Physiological actions of 1α,25-dihydroxyvitamin D_3 [calcitriol, 1α,25-$(OH)_2D_3$, **1**, Figure 1) are exerted through the vitamin D receptor (VDR),[1-3] a member of the steroid receptor gene family of transcription factors.[4]

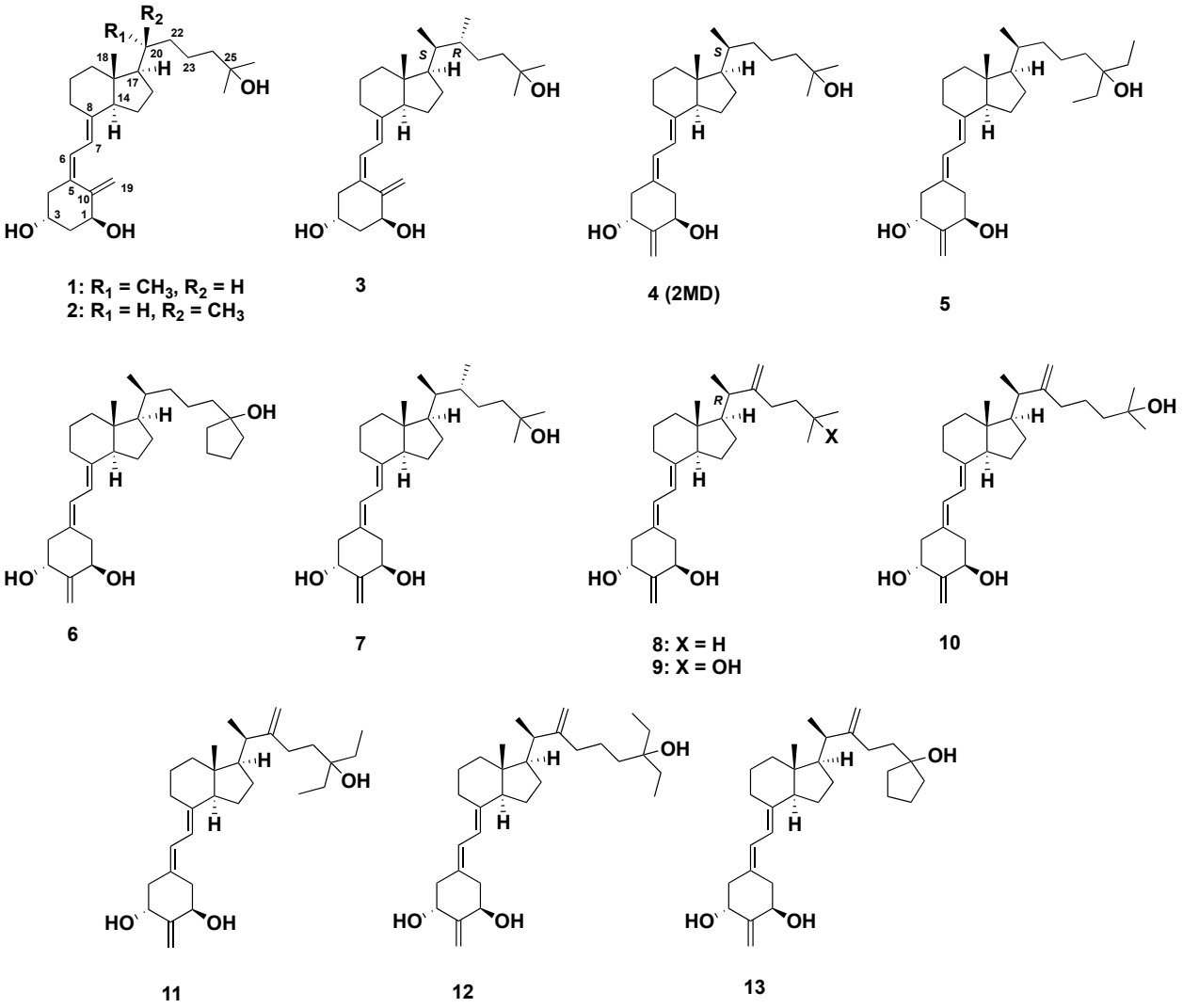


Figure 1. Chemical structure of 1α,25-dihydroxyvitamin D_3 (calcitriol, **1**), (20*S*)-1α,25-dihydroxyvitamin D_3 (**2**), the previously synthesized compounds **3**-**7**, and 2,22-dimethylene analogues described in this work **8**-**13**.

Superagonistic analogs of calcitriol have been attractive targets of synthetic efforts because of their potential use as drugs for osteoporosis and other bone disorders.[5-8] Almost three decades

ago Binderup and collaborators established that epimerization at C-20 in the calcitriol molecule enhances VDR affinity.[9] Moreover, 20-epi calcitriol (**2**) showed a 2–5 fold increased calcemic activity in comparison with calcitriol **1**.[10] It was also proved that the presence of an additional (22*R*)-methyl group (compound **3**) further elevates biological potency.[11] An even more dramatic increase in activity was observed for 2MD (**4**),[12-16] the 2-methylene-19-nor analogue of calcitriol discovered by us in 1998,[17] and characterized by an inverted configuration at C-20. Next, preparation of 2MD homologues **5** and **6**,[18] which both proved to be very calcemic, confirmed an observation that elongation of the steroidal side chain at C-26 and C-27 causes an enhancement of calcemic activity of vitamin D.[19,20] Further modification of the 2MD side chain achieved in our laboratory[21] was based on the earlier studies[11] and involved introduction of an additional 22-methyl substituent. All possible diastereomers at C-20 and C-22 were synthesized and according to the expectations (see below), (20*S*,22*R*)-22-methyl analog **7** had the highest biological activity, both *in vitro* and *in vivo*. Taking these facts into account, we considered modification of the 2MD molecule at C-22 by substituting it with a methylene group (analog **9**) and coupled this structural change with side chain elongation (analogs **10**-**13**). In addition, the importance of the 25-hydroxyl group was readdressed by elimination of it (analog **8**).

The concept of vitamin D side-chain modification was developed by many research groups, both in industry and academia. These combined efforts resulted in the preparation of many calcitriol analogs of promising biological properties.[22-24] In 2000 Moras group solved a crystalline structure of the VDR(LBD)-**1** complex and proved that 25-hydroxyl group of the natural hormone creates hydrogen bonds with His-305 and His-397.[25] This valuable findings allowed scientists to perform docking experiments of the structurally modified calcitriol analogs to the VDR. However, crystal structures of the VDR complexes with 20-epi-calcitriol analogs possessing different side chains, published one year later,[26] indicated - on the contrary to the general belief that different receptor

conformations would be induced - conserved ligand-binding pocket with the same histidine residues anchoring terminal side-cahain hydroxyl present in the examined steroidal ligands. Thus, these crystallographic structures provided surprising information that the aliphatic side chain of the vitamin D compounds can sometimes be forced to adopt energetically unfavorable conformations. Since steroidal ligand must change its side-chain conformation to fit into the ligand binding pocket and anchor to the respective amino acids, energy expenditure associated with this process may be relevant to its biological activity.

As a consequence, these studies led to the realization that conformation of flexible steroidal side chain and the spatial position of its terminal hydroxyl group, play an important role in the binding of the modified vitamin D molecule to VDR and subsequent physiological actions. First "dot maps" presenting conformational flexibility of the vitamin D side chain by showing positions of the terminal 25-oxygen in the energetically favorable conformers was reported by Okamura and his collaborators.[27,28] Later, an interesting structure-function hypothesis was formulated by Yamada and collaborators in a series of publications.[11,29-32] In their concept of „active space region", Japanese scientists postulated that VDR affinity and other biological functions (cell differentiation, transactivation potency) of calcitriol analogs can be predicted by conformational analysis of their side chains. Thus, the most stable conformers are identified and location of their 25-oxygen can be assigned to the one of the spatial regions (EG, G, EA and A). The authors analyzed the conformation of over 50 potent calcitriol analogs and they proved that this method can be especially useful for compounds possessing additional side chain substituents (for instance at C-22) causing severe restriction of the side chain mobility due to hampered rotation around the 20,22-bond. Therefore, in the following years, several research groups, especially these led by Yamada[33-34] and Mourino,[35-39] developed syntheses of various conformationally restricted vitamin D analogs.

Applied to the analogs described in this paper, the "active space group concept" predicted the proposed analogs would exert significant biological activity and thus, the syntheses pursued.

RESULTS AND DISCUSSION

Conformational analysis. Since the presence of a sp^2 carbon atom 22 in our planned structures of 22-methylene 2MD analogs obviously confines their side chain rotation, we performed systematic conformational searches[21,40] of the corresponding model 8-methylene *des*-A,B-compounds possessing side chains analogous to those present in vitamins **9-13**. Then, according to a protocol described by us previously in detail,[40] three-dimensional „dot maps" were created indicating the spatial regions potentially occupied by the 25-oxygen present in the energetically preferred conformations of the examined compounds (Figure 1S, Supporting Information). Since the results of structural analysis in all cases gave similar results, indicating that the planned 22-methylene analogs would exert high biological activity, comparable to the parent 2MD, their synthesis was undertaken and described in this work.

Chemistry. Synthesis of the target vitamins **8-13** was achieved by applying Lythgoe's strategy[41-43] based on the Wittig-Horner reaction. A-Ring phosphine oxide **14** (Figure 2), described by us previously[17], was coupled with corresponding Grundmann ketones **15a-f** which in turn were obtained from protected hydroxy aldehyde **16** (Scheme 1S, Supporting Information).

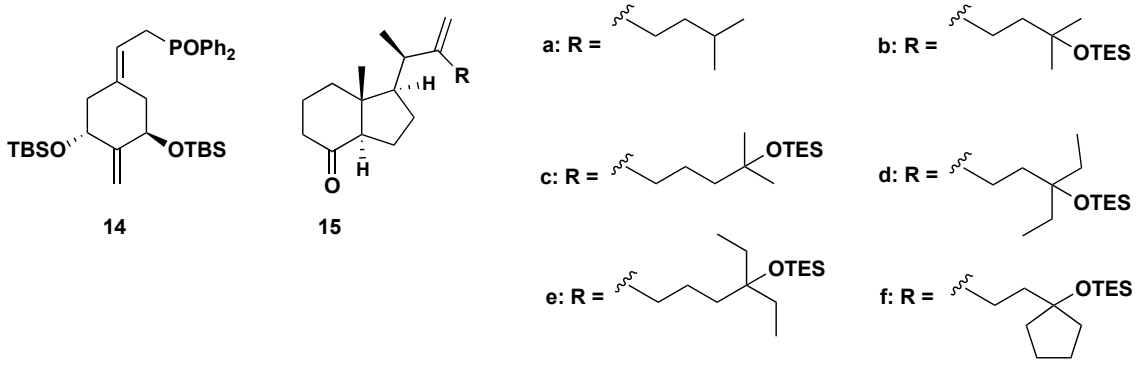
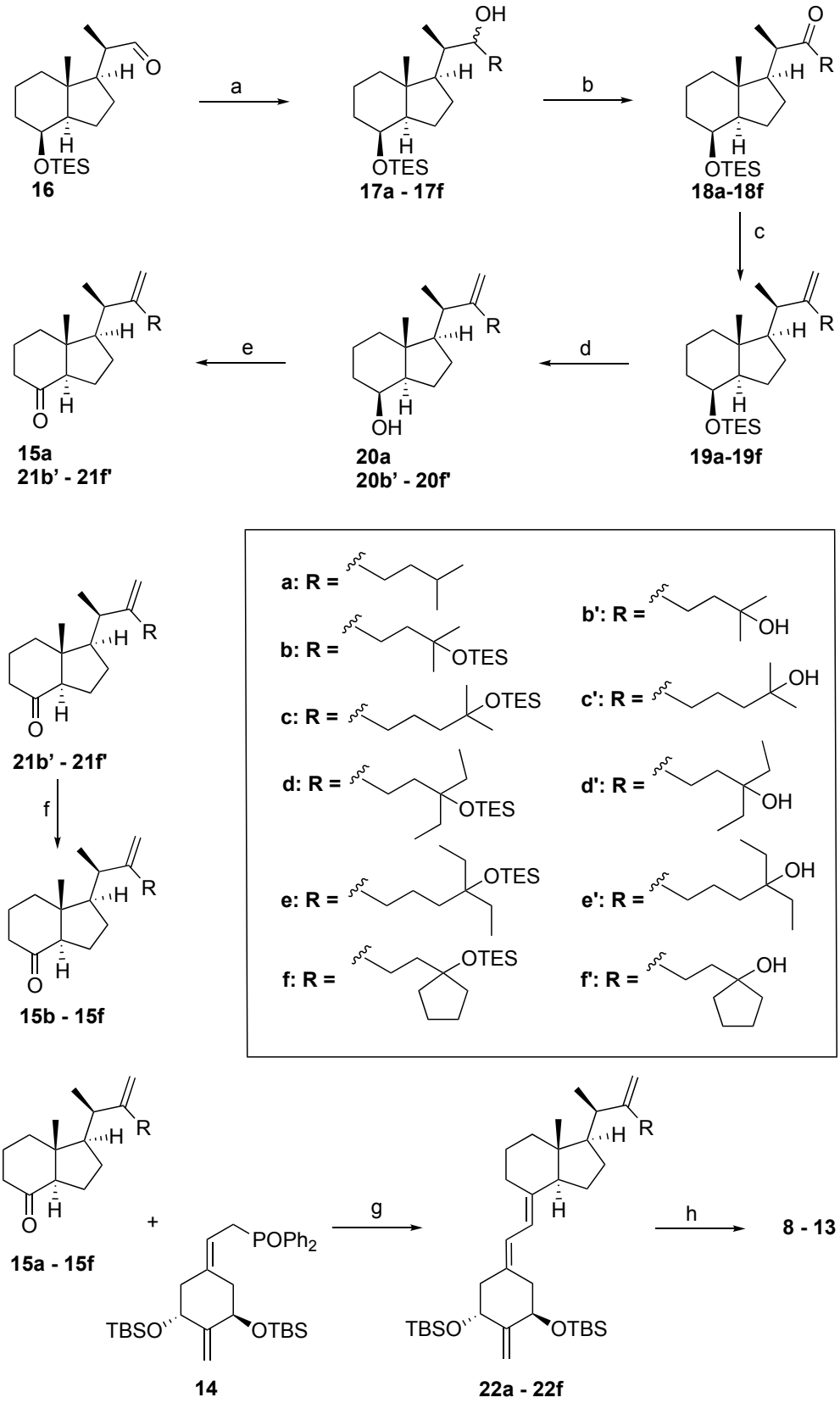


Figure 2. The building blocks used for the synthesis of target compounds.

For the preparation of **16** we used slightly modified literature procedures; the synthesis is described in the Supporting Information. The availability of this compound allowed us to carry out its reactions with the different Grignard reagents RMgBr (Scheme 1), generated from the corresponding bromides **A-F**; their preparation is described in Supporting Information. The formed 22-alcohols (steroidal numbering) **17a-f** were obtained in a very good yield as mixtures of 22-epimers (with 20*R*,22*R*-isomers prevailing). Their oxidation with Dess-Martin periodinane provided single 22-keto compounds **18a-18f**. The obtained ketones were then subjected to Wittig methylenation and in the formed products **19a-19f** hydroxyl groups were deprotected by treatment with tetrabutylammonium fluoride. The resulting hydrindanol **20a** and the diols **20b'-20f'** were subjected to oxidation with PDC to give the corresponding Grundmann ketone analog **15a** and **21b'-21f'**. The latter series of compounds, containing a 25-hydroxyl group, were silylated and the 25-OTES derivatives **15b-15f** were efficiently obtained. Synthesis of the desired C/D-building blocks **15a-15f** allowed their coupling with the A-ring phosphine oxide **14**. Removal of the silyl protecting groups in the formed 19-norvitamin D compounds **22a-22f** furnished the target 2,22-dimethylene-19-nor-1α,25-$(OH)_2D_3$ compounds **8-13**.

Biological Evaluation. All of the newly synthesized 22-methylenated vitamins (**8-13**) were examined for biological activity, using both *in vitro* and *in vivo* testing, and the results were compared to 1α,25-$(OH)_2D_3$ (**1**) and 2MD (**4**). The data presented here (Table 1) show that introduction of a 22-methylene moiety did not dramatically affect the binding affinity of the ligands to the full-length recombinant rat receptor unless it was coupled with removal of the 25-hydroxyl group.

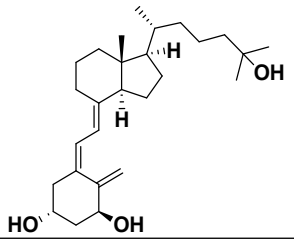
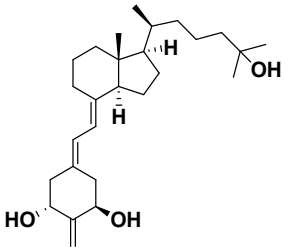
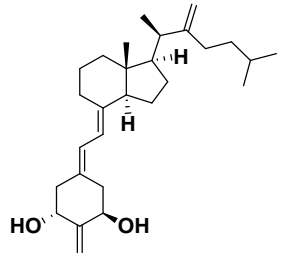
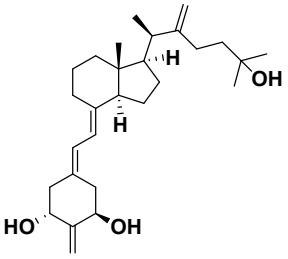
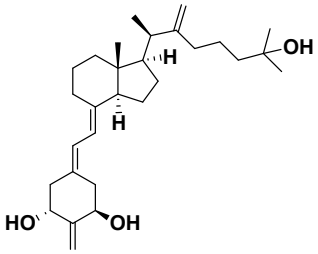

a
b
c
d
e
f
g
h
16
17a - 17f
18a-18f
19a-19f
20a
20b' - 20f'
15a
21b' - 21f'
15b - 15f
15a - 15f
14
22a - 22f
8 - 13
a: R =
b: R =
c: R =
d: R =
e: R =
f: R =
b': R =
c': R =
d': R =
e': R =
f': R =
OTES
OH
POPh2
TBSO
OTBS

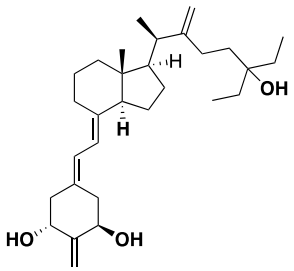
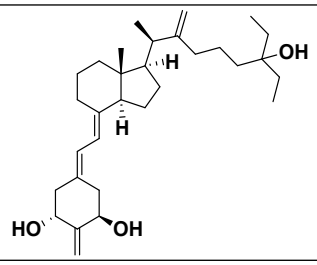
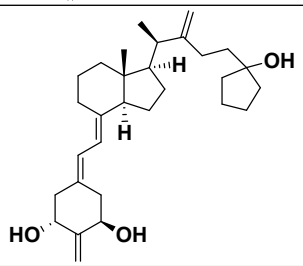
Scheme 1. (a) Mg, bromide **A-F**, THF (**17a**: 92%; **17b**: 98%; **17c**: 99%; **17d**: 98%; **17e**: 95%; **17f**: 94%); (b) Dess-Martin periodinane, CH_2Cl_2 (**18a**: 91%; **18b**: 96%; **18c**: 92%; **198**: 97%; **18e**: 89%; **18f**: 87%**);** (c) $Ph_3P^+CH_3Br^-$, *t*-BuOK, THF (**19a**: 92%; **19b**: 97%; **19c**: 97%; **19d**: 75%; **19e**: 80%; **19f**: 68%); (d) TBAF, THF (**20a**: 97%; **20b'**: 96%; **20c'**: 96%; **20d'**: 86%; **20e'**: 95%; **20f'**: 91%); (e) PDC, CH_2Cl_2 (**15a**: 87%; **21b'**: 90%; **21c'**: 88%; **21d'**: 89%; **21e'**: 91%; **21f'**: 90%); (f) TESOTf, 2,6-lutidine, CH_2Cl_2 (**15b**: 98%; **15c**: 95%; **15d**: 99%; **15e**: 84%; **15f**: 91%); (g) *n*-BuLi, THF (**22a**: 96%; **22b**: 75%; **22c**: 92%; **22d**: 85%; **22e**: 94%; **22f**: 88%); (h) TBAF, THF (**8**: 71%; **9**: 77%; **10**: 50%; **11**: 53%; **12**: 43%; **13**: 50%).

Elimination of the 25-hydroxyl group resulted in nearly two logs less binding affinity. Testing in cultured cells provided more distinction amongst the compounds. All 22-methylene analogues, with the exception of **8**, proved to be more potent than the natural hormone **1** in the cell-based assays. Compounds **10** and **12** showed the highest ability to elicit cellular differentiation of human promyelocytic HL-60 cells into monocytes, exceeding that of 2MD twice, and 1α,25-$(OH)_2D_3$ 50-fold. The transcriptional activity of the synthesized compounds was examined in ROS cells harboring a luciferase gene driven by the 24-hydroxylase (*CYP24A1*) promoter. The 24-homo analog **10** as well as compound **13** were the most potent at inducing transcription being 20 times as active as the native hormone **1**. The remaining analogues with the exception of 25-desoxy-compound **8**, induced activation of the *CYP24A1* gene similar or slightly more than 1α,25-$(OH)_2D_3$ but less than 2MD (**4**). Overall, the *in vitro* findings were as predicted with elongation of the side chain resulting in more potent analogues and removal of the 25-hydroxyl group diminishing VDR binding and cellular activities

In contrast to the *in vitro* testing, all 22-methylene analogues, showed increased efficacy compared to the native hormone in intestinal calcium transport (Figure 3-8). While intestinal activity saturated with 780 pmol of 1 α,25$(OH)_2D_3$, it continued to increase in animals administered the 22-methylene analogues. The explanation for these differences in efficacy is the topic of a subsequent investigation

Table 1. Relative VDR Binding Activities,[a] HL-60 Differentiating Activities,[b] and Transcriptional Activities[c] of the Vitamin D Hormone (**1**), 2MD (**4**) and the Vitamin D Analogues **8-13**.

Compd Structure	Comp. No.	VDR Binding[a]	HL-60[b] differentiation	24OHase[c] transcription
		K_i ratio	ED_{50} ratio	ED_{50} ratio
	**1**	1	1	1
	**4**	1	25	29
	**8**	0.01	0.1	0.05
	**9**	0.5	4	2.5
	**10**	1	50	20

	**11**	1	10	6.7
	**12**	0.5	50	6.7
	**13**	1	29	20

[a]Competitive binding of 1α,25-$(OH)_2D_3$ (**1**) and the synthesized vitamin D analogues to the full-length recombinant rat vitamin D receptor. The experiments were carried out in duplicate on two different occasions. The K_i values are derived from the dose-response curves and represent the inhibition constant when radiolabeled 1α,25-$(OH)_2D_3$ is present at 1 nM and a K_d of 0.2 nM is used. The numbers shown in the Table are expressed as the average ratio of the 1α,25-$(OH)_2D_3$ K_i to the K_i for the analogue. [b]Induction of differentiation of HL-60 promyelocytes to monocytes by 1α,25-$(OH)_2D_3$ (**1**) and the synthesized vitamin D analogues. Differentiation state was determined by measuring the percentage of cells reducing nitro blue tetrazolium (NBT). The experiment was repeated in duplicate two times. The ED_{50} values are derived from the dose-response curves and represent the analogue concentration capable of inducing 50% maturation. The numbers shown in the Table are expressed as the average ratio of the 1α,25-$(OH)_2D_3$ ED_{50} to the ED_{50} for the analogue. [c]Transcriptional assay in rat osteosarcoma cells stably transfected with a 24-hydroxylase gene reporter plasmid. The ED_{50} values are derived from dose-response curves and represent the analogue concentration capable of increasing the luciferase activity by 50%. The numbers shown in the Table are expressed as the average ratio of the 1α,25-$(OH)_2D_3$ ED_{50} to the ED_{50} for the analogue.

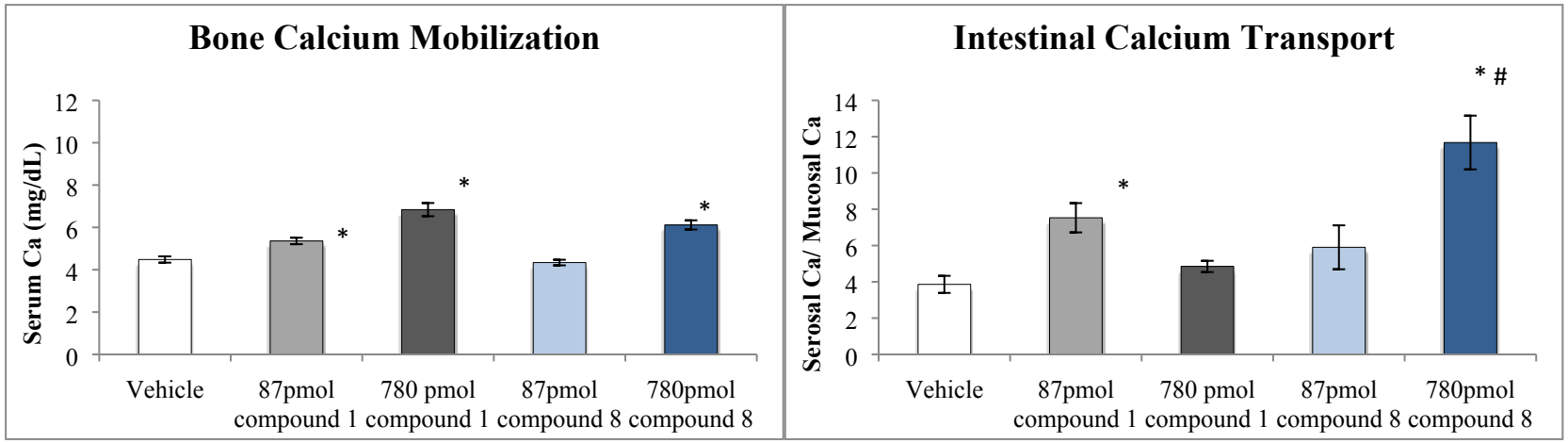


Figure 3. Bone calcium mobilization and intestinal calcium transport of 1α,25-$(OH)_2D_3$ (**1**) and the synthesized 22-methylene compound **8**. Each group contains 5 to 7 rats. Statistical significance ($p < 0.05$) is denoted by an asterisk (*) (comparison to vehicle) or a pound sign (#) (comparison to 1α,25-$(OH)_2D_3$).

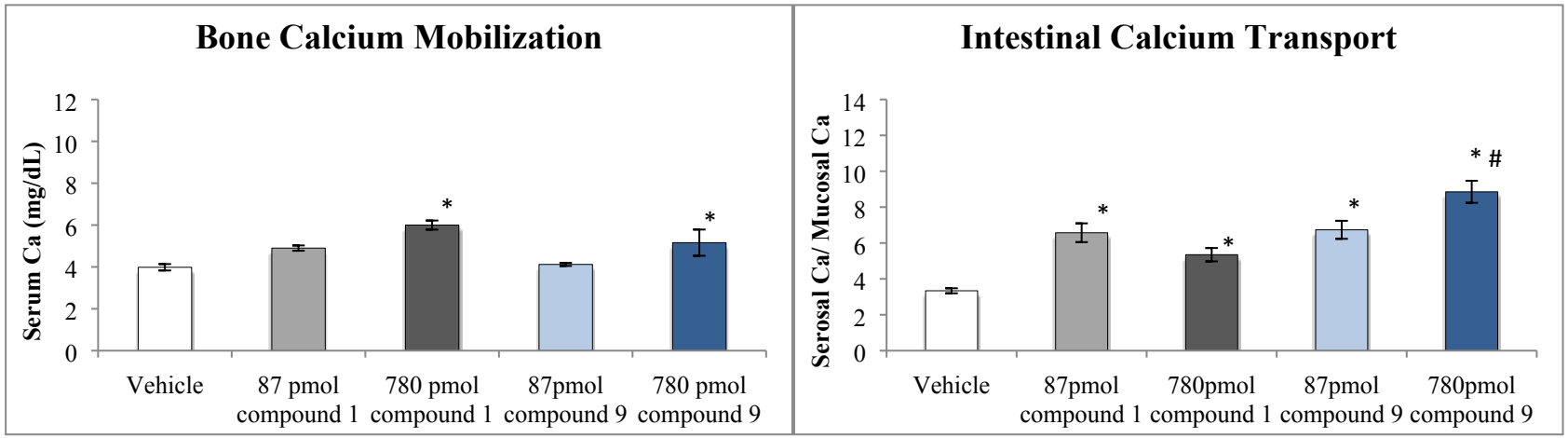


Figure 4. Bone calcium mobilization and intestinal calcium transport of 1α,25-$(OH)_2D_3$ (**1**) and the synthesized 22-methylene compound **9**. Each group contains 5 to 7 rats. Statistical significance ($p < 0.05$) is denoted by an asterisk (*) (comparison to vehicle) or a pound sign (#) (comparison to 1α,25-$(OH)_2D_3$).

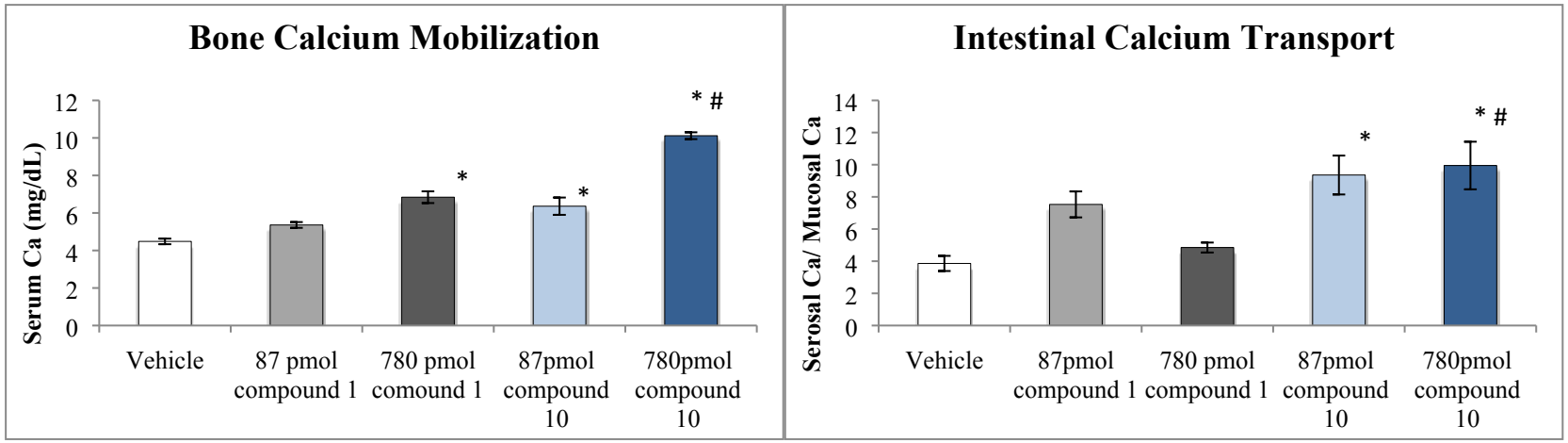


Figure 5. Bone calcium mobilization and intestinal calcium transport of 1α,25-$(OH)_2D_3$ (**1**) and the synthesized 22-methylene compound **10**. Each group contains 5 to 7 rats. Statistical significance ($p < 0.05$) is denoted by an asterisk (*) (comparison to vehicle) or a pound sign (#) (comparison to 1α,25-$(OH)_2D_3$).

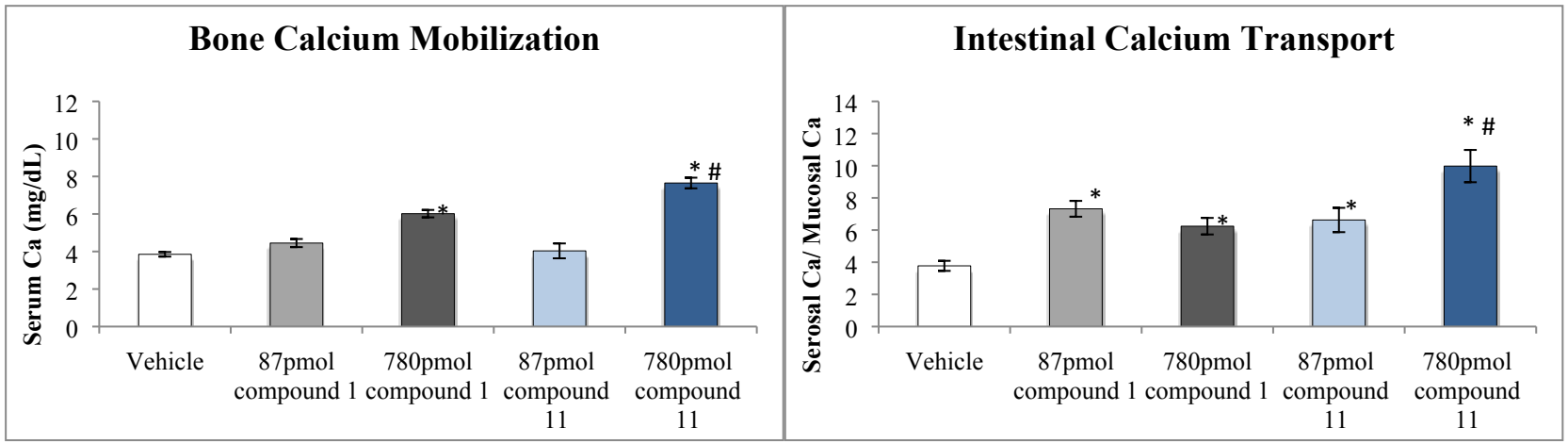


Figure 6. Bone calcium mobilization and intestinal calcium transport of 1α,25-$(OH)_2D_3$ (**1**) and the synthesized 22-methylene compound **11**. Each group contains 5 to 7 rats. Statistical significance ($p < 0.05$) is denoted by an asterisk (*) (comparison to vehicle) or a pound sign (#) (comparison to 1α,25-$(OH)_2D_3$).

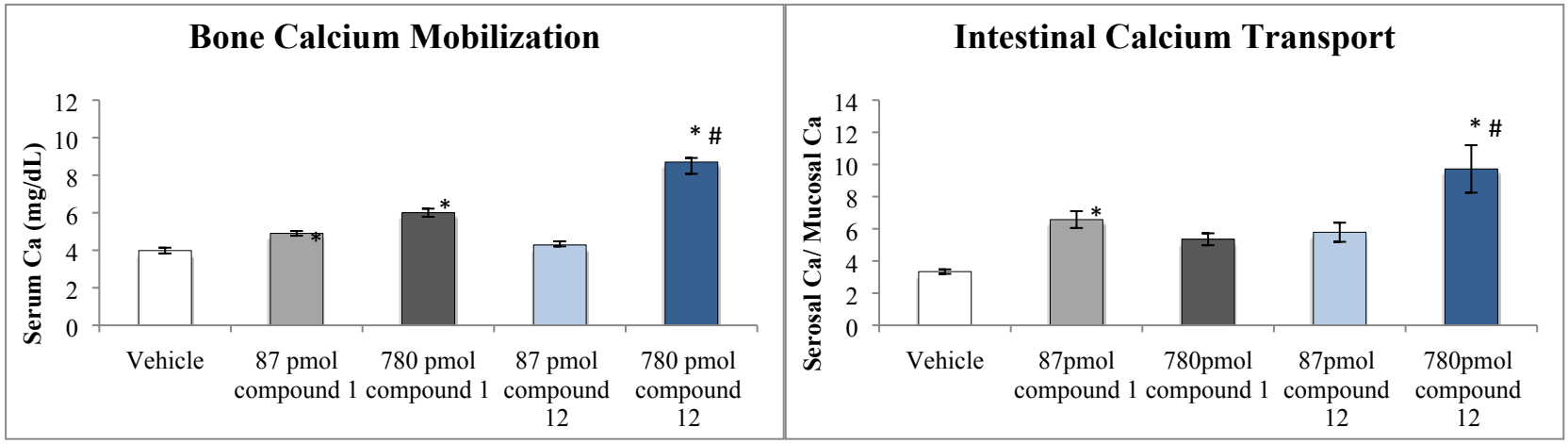


Figure 7. Bone calcium mobilization and intestinal calcium transport of 1α,25-$(OH)_2D_3$ (**1**) and the synthesized 22-methylene compound **12**. Each group contains 5 to 7 rats. Statistical significance ($p < 0.05$) is denoted by an asterisk.

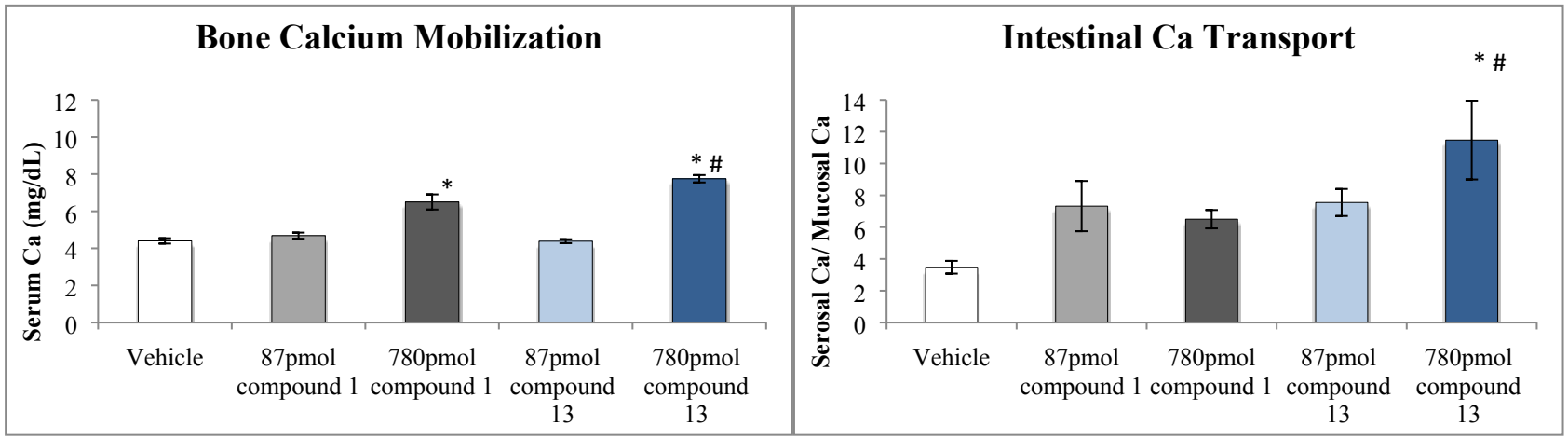


Figure 8. Bone calcium mobilization and intestinal calcium transport of 1α,25-$(OH)_2D_3$ (**1**) and the synthesized 22-methylene compound **13**. Each group contains 5 to 7 rats. Statistical significance ($p < 0.05$) is denoted by an asterisk (*) (comparison to vehicle) or a pound sign (#) (comparison to 1α,25-$(OH)_2D_3$).

Bone calcium mobilization activity of the analogues provided more separation amongst the 22-methylene analogues. Compounds **10** and **12** with elongated side chains in the 20*R*-configuration were the most potent which is consistent with the results obtained *in vitro*. The analog with the five-membered ring, compound **13**, as well as compound **11**, also show increased bone calcium mobilization activity compared to the native hormone. The remainder of the 22-methylene analogues were in the same potency range as the native hormone.

Overall, two new analogs, compounds **10** and **12**, were generated that possess increased biological potency compared to both 1α,25-$(OH)_2D_3$ and 2MD. Both of these compounds have elongated sides chains which has previously been shown to increase biological activity. The reason for this heightened activity is unknown.

CONCLUSIONS

As predicted by the "active space region" model, the addition of a 22-methylene group coupled with side chain elongation resulted in analogues with more potent cellular differentiation, cellular transactivation, intestinal calcium transport and bone calcium mobilization activities. Interestingly, the addition of a five-membered ring also increased biological potency, but not quite to the same extent as aliphatic extension (**13** vs **10** and **12**). This difference is most noticeable in the cell differentiaton assay, which might suggest that flexibility of the side chain is important for eliciting cellular differentiation of human promyelocytic HL-60 cells into monocytes.

The results of the *in vivo* testing in bone indicate only analogues **10-13** are more potent than the native hormone. These results indicate that a simple 22-methylene addition (compound **9**) without chain elongation or ring addition is not sufficient to cause differential bone calcium mobilization activity compared to 1α,25-$(OH)_2D_3$. Both a 22-methylene moiety as well as chain elongation are necessary for increasing *in vivo* potency in the bone. In the intestine on the other

hand, all analogues were more potent than 1α,25-$(OH)_2D_3$. Thus, all biological activity, with the exception of the bone activity, is predicted by the active space region concept.

EXPERIMENTAL SECTION

Chemistry. Ultraviolet (UV) absorption spectra were obtained on a Shimadzu UV-1800 UV spectrophotometer in 100% EtOH. All nuclear magnetic resonance spectra were recorded in deuterochloroform using Bruker DMX-400 (400 MHz) and Bruker DMX-500 (500 MHz). Chemical shifts (δ) are reported in parts per million relative to $(CH_3)_4$ Si (δ 0.00) as an internal standard. Abbreviations used are singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m). High resolution mass spectra were registered on LCT (TOF) or Mass Quattro LC spectrometers. High-performance liquid chromatography (HPLC) was performed on a Waters Associates liquid chromatograph equipped with a model 6000A solvent delivery system, model U6K Universal injector, and model 486 tunable absorbance detectors. Solvents were dried and distilled following standard procedures.

The purity of final compounds was determined by HPLC, and they were judged at least 99% pure. Two HPLC columns (9.4 mm × 25 cm Zorbax-Sil and 9.4 mm × 25 cm Zorbax Eclipse XDB-C18) were used as indicated in Table 2 (Supporting Information). The purity and identity of the synthesized vitamins were additionally confirmed by inspection of their ^{1}H NMR and high-resolution mass spectra.

Preparation of the aldehyde **16** and the alkyl bromides **A**-**F** has been described in Supporting Information.

(20*R*,22*R*)- and (20*R*,22*S*)-8β-[(Triethylsilyl)oxy]-*des*-A,B-cholestan-22-ols (17a). A solution of isopentyl magnesium bromide, prepared from 1-bromo-3-methylbutane (**A**, 393 μL, 3.28 mmol) and magnesium powder (78 mg, 3.21 mmol) in anhydrous THF (6 mL) as described in Supporting Information, was added to the solution of the aldehyde **16** (212 mg, 653 μmol) in anhydrous THF (3 mL) at -78 °C under argon. After 30 min a cooling bath was removed, and the mixture was allowed to reach room temperature. Reaction was quenched by addition of an aqueous NH_4Cl solution, extracted with ethyl acetate, dried (Na_2SO_4) and concentrated. The resulting product was applied on a silica Sep-Pak cartridge and eluted with hexane/ethyl acetate (95:5) to give product **17a** (226 mg, 92%) as a colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$; signals of the main 20*R*,22*R*-isomer are listed) δ 0.555 (6H, q, J = 7.8 Hz, 3 × $SiCH_2$), 0.801 (3H, d, J = 6.8 Hz, 21-H_3), 0.889 (6H, d, J = 6.7 Hz, 26- and 27-H_3), 0.922 (3H, s, 18-H_3), 0.948 (9H, t, J = 7.8 Hz, 3 × $SiCH_2C\underline{H}_3$), 3.38 (1H, m, 22-H), 4.04 (1H, narr m, 8α-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 4.9, 6.9, 11.1, 13.9, 17.7, 22.6, 22.8, 27.2, 28.1, 32.8, 34.6, 35.6, 38.9, 40.4, 41.9, 52.6, 53.1, 69.3, 72.8; HRMS (ESI) exact mass calculated for $C_{24}H_{50}NO_2Si$ ($M^+ + NH_4$) 412.3610, found 412.3611.

(20*R*,22*R*)- and (20*R*,22*S*)-8β,25-Bis[(triethylsilyl)oxy]-*des*-A,B-cholestan-22-ols (17b). A solution of Grignard reagent, prepared from 1-bromo-3-methyl-3-[(triethylsilyl)oxy]butane (**B**; 1.19 mL, 4.48 mmol) and magnesium powder (114 mg, 4.69 mmol) in anhydrous THF, was added to the solution of the aldehyde **16** (308 mg, 950 μmol) in anhydrous THF (3.3 mL) at -78 °C under argon. After analogous procedure as described above for **17a**, product **17b** (489 mg, 98%) was obtained as a colorless oil.

^{1}H NMR (500 MHz, $CDCl_3$; signals of the main 20*R*,22*R*-isomer are listed) δ 0.554 and 0.577 (6H and 6H, each q, J = 8.0 Hz, 6 × $SiCH_2$), 0.813 (3H, d, J = 6.8 Hz, 21-H_3), 0.914 (3H, s, 18-H_3),

0.944 and 0.947 (9H and 9H, each t, J = 8.0 Hz, 6 × $SiCH_2C\underline{H}_3$), 1.215 (6H, s, 26- and 27-H_3), 3.79 (1H, m, 22-H), 4.04 (1H, narr m, 8α-H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 4.9, 6.7, 6.9, 7.1, 11.2, 13.9, 17.7, 22.8, 27.1, 29.8, 29.8, 29.9, 34.6, 35.6, 39.1, 40.3, 41.9, 41.9, 52.5, 53.0, 69.3, 73.2, 73.3; HRMS (ESI) exact mass calculated for $C_{30}H_{62}O_3Si_2$ ($M^+ + Na^+$) 549.4135, found 549.4130.

(20*R*,22*R*)- and (20*R*,22*S*)-8β,25-Bis[(triethylsilyl)oxy]-*des*-A,B-24-homocholestan-22-ols (17c). A solution of Grignard reagent, prepared from 1-bromo-4-methyl-4-[(triethylsilyl)oxy]pentane (**C**; 1.02 mL, 3.25 mmol) and magnesium powder (78 mg, 3.21 mmol) in anhydrous THF (5.3 mL), was added to the solution of the aldehyde **16** (210 mg, 647 μmol) in anhydrous THF (3 mL) at -78 °C under argon. After analogous procedure as described above for **17a**, product **17c** (340 mg, 99%) was obtained as a colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$; signals of the main 20*R*,22*R*-isomer are listed) δ 0.554 and 0.566 (6H and 6H, each q, J = 7.8 Hz, 6 × $SiCH_2$), 0.800 (3H, d, J = 6.8 Hz, 21-H_3), 0.922 (3H, s, 18-H_3), 0.942 and 0.962 (9H and 9H, each t, J = 7.8 Hz, 6 × $SiCH_2C\underline{H}_3$), 1.193 and 1.197 (3H and 3H, each s, 26- and 27-H_3), 3.86 (1H, m, 22-H), 4.04 (1H, narr m, 8α-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 4.9, 6.8, 6.9, 7.1, 11.1, 13.9, 17.7, 21.3, 22.8, 27.2, 29.7, 29.9, 34.6, 35.6, 39.0, 40.4, 41.9, 45.2, 52.7, 53.1, 69.3, 72.6, 73.3; HRMS (ESI) exact mass calculated for $C_{31}H_{64}O_3Si_2Na$ (M^+ + Na) 563.4292, found 563.4293.

(20*R*,22*R*)- and (20*R*,22*S*)-8β,25-Bis[(triethylsilyl)oxy]-*des*-A,B-26,27-dihomocholestan-22-ols (17d). A solution of Grignard reagent, prepared from 1-bromo-3-ethyl-3-[(triethylsilyl)oxy]pentane (**D**; 1.32 mL, 4.28 mmol) and magnesium powder (103 mg, 4.24 mmol) in anhydrous THF (6.3 mL), was added to the solution of the aldehyde **16** (278 mg, 858 μmol) in anhydrous THF (3 mL) at -78 °C under argon After analogous procedure as described above for **17a**, product **17d** (459 mg, 98%) was obtained as a colorless oil.

^{1}H NMR (500 MHz, $CDCl_3$; signals of the main 20*R*,22*R*-isomer are listed) δ 0.554 and 0.578 (6H and 6H, each q, *J* = 7.9 Hz, 6 × $SiCH_2$), 0.809 (3H, d, *J* = 6.6 Hz, 21-H_3), 0.830 [6H, t, *J* = 7.4 Hz, 2 × $C(CH_2C\underline{H}_3)$], 0.917 (3H, s, 18-H_3), 0.947 (18H, t, *J* = 7.9 Hz, 6 × $SiCH_2C\underline{H}_3$), 3.78 (1H, m, 22-H), 4.04 (1H, narr m, 8α-H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 4.9, 6.9, 7.0, 7.3, 8.3, 8.4, 11.2, 13.8, 17.7, 22.8, 27.1, 29.0, 31.6, 31.8, 34.6, 35.4, 39.1, 40.3, 41.9, 52.6, 53.0, 69.3, 73.5, 78.1; HRMS (ESI) exact mass calculated for $C_{32}H_{67}O_3Si_2$ ($M^+ + H^+$) 555.4628, found 549.4625.

(20*R*,22*R*)- and (20*R*,22*S*)-8β,25-Bis[(triethylsilyl)oxy]-*des*-A,B-24,26,27-trihomocholestan-22-ols (17e). A solution of Grignard reagent, prepared from 1-bromo-4-ethyl-4-[(triethylsilyl)oxy]hexane (**E**; 0.94 mg, 2.9 mmol) and magnesium powder (63 mg, 2.59 mmol) in anhydrous THF (4.2 mL) was added to the solution of the aldehyde **16** (171 mg, 528 μmol) in anhydrous THF (2 mL) at -78 °C under argon. After analogous procedure as described above for **17a**, the resulting product was applied on a silica Sep-Pak cartridge and eluted with hexane/ethyl acetate (93:7) to give **17e** (285 mg, 95%) as a colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$; signals of the main 20*R*,22*R*-isomer are listed) δ 0.553 and 0.573 (6H and 6H, each q, *J* = 7.8 Hz, 6 × $SiCH_2$), 0.800 (3H, d, *J* = 6.5 Hz, 21-H_3), 0.824 [(6H, t, *J* = 7.4 Hz, 2 × $C(CH_2C\underline{H}_3)$], 0.924 (3H, s, 18-H_3), 0.945 and 0.949 (9H and 9H, each t, *J* = 7.8 Hz, 6 × $SiCH_2C\underline{H}_3$), 3.86 (1H, narr m, 22-H), 4.04 (1H, narr m, 8α-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 4.9, 6.8, 6.9, 7.1, 7.3, 8.3, 11.1, 13.9, 17.7, 20.6, 22.9, 27.2, 31.4, 31.7, 34.6, 35.8, 39.0, 40.4, 41.9, 52.7, 53.1, 69.3, 72.5, 78.1; HRMS (ESI) exact mass calculated for $C_{33}H_{68}O_3Si_2Na$ (M^+ + Na) 591.4600, found 591.4598.

(20*R*,22*R*)- and (20*R*,20*S*)-8β-[(Triethylsilyl)oxy]-24-{1'-[(triethylsilyl)oxy]cyclopentyl}-*des*-A,B-25,26,27-trinorcholestan-22-ols (17f). A solution of Grignard reagent, prepared from 1-(2'-bromoethyl)-1-[(triethylsilyl)oxy]cyclopentane (**F**; 1.514 g, 4.58 mmol) and magnesium turnings

(119 mg, 4.89 mmol) in anhydrous THF (7 mL) was added to the solution of the aldehyde **16** (401 mg, 1.24 mmol) in anhydrous THF (4 mL) at -78 °C under argon. After analogous procedure as described above for **17a**, product **17f** (643 mg, 94%) was obtained as a colorless oil.

^{1}H NMR (500 MHz, $CDCl_3$; signals of the main 20*R*,22*R*-isomer are listed) δ 0.556 and 0.587 (6H and 6H, each q, *J* = 7.9 Hz, 6 × $SiCH_2$), 0.817 (3H, d, *J* = 6.8 Hz, 21-H_3), 0.917 (3H, s, 18-H_3), 0.948 (18H, t, *J* = 7.9 Hz, 6 × $SiCH_2C\underline{H}_3$), 3.80 (1H, m, 22-H), 4.04 (1H, narr m, 8α-H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 4.9, 6.6, 6.9, 7.2, 11.2, 13.8, 17.7, 22.8, 23.5, 23.6, 27.1, 30.2, 34.6, 38.8, 39.3, 39.6, 39.9, 40.4, 41.9, 52.6, 53.0, 69.3, 73.4, 84.6; HRMS (ESI) exact mass calculated for $C_{32}H_{65}O_3Si_2$ ($M^+ + H^+$) 553.4472, found 553.4467

(20*R*)-8β-[(Triethylsilyl)oxy]-*des*-A,B-cholestan-22-one (18a). The mixture of the epimeric alcohols **17a** (226 mg, 570 μmol) and Dess-Martin periodinane (295 mg, 696 μmol) in anhydrous methylene chloride (15 mL) was vigorously stirred at room temperature for 2 h. The reaction was quenched with aqueous $NaHCO_3$ solution, extracted with methylene chloride, dried (Na_2SO_4) and concentrated. The crude product was applied on a silica Sep-Pak cartridge and eluted with hexane/ethyl acetate (98:2) to afford the ketone **18a** (204 mg, 91%) as a colorless oil.

^{1}H NMR (500 MHz, $CDCl_3$) δ 0.544 (6H, q, *J* = 7.8 Hz, 3 × $SiCH_2$), 0.894 (6H, d, *J* = 6.4 Hz, 26- and 27-H_3), 0.900 (3H, s, 18-H_3), 0.938 (9H, t, *J* = 7.8 Hz, 3 × $SiCH_2C\underline{H}_3$), 0.990 (3H, d, *J* = 7.0 Hz, 21-H_3), 4.02 (1H, narr m, 8α-H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 4.9, 6.9, 14.3, 17.0, 17.5, 22.4, 22.4, 25.6, 27.6, 32.2, 39.4, 39.7, 41.5, 47.1, 52.4, 53.2, 69.0, 215.8; HRMS (ESI) exact mass calculated for $C_{24}H_{50}NO_2Si$ ($M^+ + NH_4$) 412.3611, found 412.3615.

(20*R*)-8β,25-Bis[(triethylsilyl)oxy]-*des*-A,B-cholestan-22-one (18b). The oxidation of the alcohols **17b** (489 mg, 929 μmol) with Dess-Martin periodinane (475 mg, 1.12 mmol) in anhydrous

methylene chloride (35 mL), performed as described above for **17a**, afforded the ketone **18b** (466 mg, 96%) as a colorless oil.

^{1}H NMR (500 MHz, $CDCl_3$) δ 0.545 (6H, q, J = 8.0 Hz, 3 × $SiCH_2$), 0.565 (6H, q, J = 7.9 Hz, 3 × $SiCH_2$), 0.904 (3H, s, 18-H_3), 0.938 (9H, t, J = 8.0 Hz, 3 × $SiCH_2C\underline{H}_3$), 0.942 (9H, t, J = 7.9 Hz, 3 × $SiCH_2C\underline{H}_3$), 0.998 (3H, d, J = 6.9 Hz, 21-H_3), 1.204 (6H, s, 26- and 27-H_3), 4.02 (1H, narr m, 8α-H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 4.9, 6.6, 6.9, 7.1, 14.2, 17.1, 17.5, 22.3, 25.6, 29.8, 30.0, 34.5, 36.7, 37.9, 39.3, 41.7, 47.3, 52.4, 53.4, 69.1, 72.4, 216.1; HRMS (ESI) exact mass calculated for $C_{30}H_{61}O_3Si_2$ ($M^+ + H^+$) 525.4159, found 525.4149.

(20*R*)-8β,25-Bis[(triethylsilyl)oxy]-*des*-A,B-24-homocholestan-22-one (18c). The oxidation of the alcohols **17c** (340 mg, 629.6 μmol) with Dess-Martin periodinane (330 mg, 778 μmol) in anhydrous methylene chloride (20 mL), performed as described above for **17a**, afforded the ketone **18c** (313 mg, 92%) as a colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 0.549 and 0.556 (6H and 6H, each q, J = 7.8 Hz, 6 × $SiCH_2$), 0.899 (3H, s, 18-H_3), 0.938 (18H, t, J = 7.8 Hz, 6 × $SiCH_2C\underline{H}_3$), 0.982 (3H, d, J = 6.9 Hz, 21-H_3), 1.202 (6H, s, 26- and 27-H_3), 4.02 (1H, narr m, 8α-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 4.9, 6.7, 6.9, 7.1, 14.4, 16.9, 17.5, 18.5, 22.3, 29.7, 34.5, 39.5, 42.3, 44.6, 46.9, 52.5, 53.2, 69.1, 73.3, 215.4; HRMS (ESI) exact mass calculated for $C_{31}H_{62}O_3Si_2Na$ (M^+ + Na) 561.4135, found 561.4138.

(20*R*)-8β,25-Bis[(triethylsilyl)oxy]-*des*-A,B-26,27-dihomocholestan-22-one (18d). The oxidation of the alcohols **17d** (447 mg, 807 μmol) with Dess-Martin periodinane (412 mg, 972 μmol) in anhydrous methylene chloride (30 mL), performed as described above for **17a**, afforded the crude product which was applied on a silica Sep-Pak cartridge and eluted with hexane/ethyl acetate (96:4) to yield the ketone **18d** (434 mg, 97%) as a colorless oil.

^{1}H NMR (500 MHz, $CDCl_3$) δ 0.545 and 0.575 (6H and 6H, each q, J = 7.9 Hz, 6 × $SiCH_2$), 0.827 and 0.832 [3H and 3H, each t, J = 7.5 Hz, 2 × $C(CH_2C\underline{H}_3)$], 0.904 (3H, s, 18-H_3), 0.938 and 0.943 (9H and 9H, each t, J = 7.9 Hz, 6 × $SiCH_2C\underline{H}_3$), 0.995 (3H, d, J = 6.9 Hz, 21-H_3), 4.02 (1H, narr m, 8α-H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 4.9, 6.9, 7.0, 7.3, 8.40, 8.44, 14.3, 17.1, 17.5, 22.3, 25.6, 31.5, 31.8, 34.5, 36.2, 39.5, 41.6, 47.3, 52.4, 53.4, 69.1, 78.4, 216.0; HRMS (ESI) exact mass calculated for $C_{32}H_{65}O_3Si_2$ ($M^+ + H^+$) 553.4462, found 553.4465.

(20*R*)-8β,25-Bis[(triethylsilyl)oxy]-*des*-A,B-24,26,27-trihomocholestan-22-one (18e). The oxidation of the alcohols **17e** (275 mg, 484 μmol) with Dess-Martin periodinane (250 mg, 589 μmol) in anhydrous methylene chloride (15 mL), performed as described above for **17a**, afforded the crude product which was applied on a silica Sep-Pak cartridge and eluted with hexane/ethyl acetate (98:1) to yield the ketone **18e** (245 mg, 89%) as a colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 0.545 and 0.564 (6H and 6H, each q, J = 7.9 Hz, 6 × $SiCH_2$), 0.826 (6H, t, J = 7.4 Hz, 26- and 27-H_3), 0.901 (3H, s, 18-H_3), 0.938 (18H, t, J = 7.9 Hz, 6 × $SiCH_2C\underline{H}_3$), 0.985 (3H, d, J = 6.9 Hz, 21-H_3), 4.02 (1H, narr m, 8α-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 4.9, 6.9, 7.0, 7.2, 8.2, 14.4, 16.9, 17.5, 17.8, 22.3, 25.6, 31.7, 34.5, 38.4, 39.8, 41.6, 42.3, 47.0, 52.5, 53.2, 69.1, 78.1, 215.4; HRMS (ESI) exact mass calculated for $C_{33}H_{67}O_3Si_2$ ($M^+ + H^+$) 567.4624, found 567.4631.

(20*R*)-8β-[(Triethylsilyl)oxy]-24-{1'-[(triethylsilyl)oxy]cyclopentyl}-*des*-A,B-25,26,27-trinorcholestan-22-one (18f). The oxidation of the alcohols **17f** (643 mg, 1.232 mmol) with Dess-Martin periodinane (628 mg, 1.48 mmol) in anhydrous methylene chloride (40 mL), performed as described above for **17a**, afforded the crude product which was applied on a silica Sep-Pak cartridge and eluted with hexane/ethyl acetate (93:7) to yield the ketone **18f** (558 mg, 87%) as a colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 0.549 and 0.581 (6H and 6H, each q, J = 7.9 Hz, 6 × $SiCH_2CH_3$), 0.913 (3H, s, 18-H_3), 0.943 and 0.946 (9H and 9H, each t, J = 7.9 Hz, 3 × $SiCH_2CH_3$), 0.999 (3H, d, J = 6.9 Hz, 21-H_3), 2.52 (1H, m), 2.63 (2H, m), 4.03 (1H, narr m, 8α-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 4.9, 6.5, 6.8, 7.1, 14.2, 16.9, 17.5, 22.2, 23.4, 25.6, 34.9, 37.3, 39.7, 39.9, 41.6, 47.2, 52.4, 5.3, 69.0, 83.8, 215.7; HRMS (ESI) exact mass calculated for $C_{32}H_{62}O_3Si_2Na$ (M^+ + Na) 573.4135, found 573.4147.

(20*R*)-22-Methylene-8β-[(triethylsilyl)oxy]-*des*-A,B-cholestane (19a). Potassium *tert*-butoxide (347 mg, 3.1 mmol) was added to a stirred suspension of methyl triphenylphosphonium bromide (1.1 g, 3.1 mmol) in anhydrous THF (16 mL) at 0 °C. The mixture was warmed up to room temperature and stirred for 30 min. A solution of the ketone **18a** (204 mg, 517 μmol) in THF (3 mL) was added *via* cannula at 0 °C under argon. Cooling bath was removed and stirring was continued at room temperature for 28 h. Water was added and the mixture was extracted with ethyl acetate, dried (Na_2SO_4) and concentrated. The residue was applied on a silica Sep-Pak cartridge and eluted with hexane/diethyl ether (98:2) to afford compound **19a** (187 mg, 92%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 0.544 (6H, q, J = 7.9 Hz, 3 × $SiCH_2$), 0.897 (3H, s, 18-H_3), ca. 0.91 (3H, 21-H_3), ca. 0.92 (6H, d, J = 6.3 Hz, 26- and 27-H_3), 0.941 (9H, t, J = 7.9 Hz, 3 × $SiCH_2CH_3$), 1.94 (2H, br t, J = 6.8 Hz), 2.17 (1H, m), 4.02 (1H, narr m, 8α-H), 4.63 and 4.76 (1H and 1H, each s, =CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ 4.9, 6.9, 13.1, 17.7, 20.6, 22.5, 22.7, 22.8, 27.0, 28.3, 29.3, 34.7, 37.2, 39.5, 42.3, 43.5, 53.1, 54.0, 69.4, 107.9, 155.3; HRMS (ESI) exact mass calculated for $C_{25}H_{52}NOSi$ (M^+ + NH_4) 410.3818, found 410.3820.

(20*R*)-22-Methylene-8β,25-bis[(triethylsilyl)oxy]-*des*-A,B-cholestane (19b). Solution of the ketone **18b** (451 mg, 860 μmol) in THF (5 mL) was treated with the ylide generated from methyl triphenylphosphonium bromide (1.831 g, 5.13 mmol) and potassium *tert*-butoxide (580 mg,

5.16 mmol) in anhydrous THF (25 mL) at 45 °C for 20 h. Heating bath was removed, mixture was allowed to reach room temperature and water was added. Materials were extracted with ethyl acetate, dried (Na_2SO_4) and concentrated. The residue was applied on a silica Sep-Pak cartridge and eluted with hexane/diethyl ether (95:5) to afford compound **19b** (435 mg, 97%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 0.544 and 0.571 (6H and 6H, each q, each J = 8.0 Hz, 6 × SiC$\underline{H}_2$CH$_3$), 0.904 (3H, s, 18-H$_3$), ca. 0.93 (3H, 21-H$_3$), 0.942 and 0.949 (9H and 9H, each t, each J = 8.0 Hz, 6 × SiCH$_2$C$\underline{H}_3$), 1.224 (6H, s, 26- and 27-H$_3$), 2.04 (2H, m), 2.19 (1H, m), 4.02 (1H, narr m, 8α-H), 4.60 and 4.76 (1H and 1H, each br s, =CH$_2$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 4.9, 6.8, 6.9, 7.1, 13.0, 17.6, 20.4, 22.5, 25.5, 27.0, 29.9, 30.1, 34.7, 39.3, 42.2, 42.8, 43.9, 53.1, 53.9, 69.4, 73.2, 107.6, 155.3; HRMS (ESI) exact mass calculated for $C_{31}H_{63}O_2Si_2$ ($M^+ + H^+$) 523.4366, found 523.4355.

(20*R*)-22-Methylene-8β,25-bis[(triethylsilyl)oxy]-*des*-A,B-24-homocholestane (19c). Solution of the ketone **18c** (313 mg, 582 μmol) in THF (4 mL) was treated with the ylide generated from methyl triphenylphosphonium bromide (1.24 g, 3.49 mmol) and potassium *tert*-butoxide (392 mg, 3.50 mmol) in anhydrous THF (20 mL) at room temperature for 48 h. Water was added and the mixture was extracted with ethyl acetate, dried (Na_2SO_4) and concentrated. The residue was applied on a silica Sep-Pak cartridge and eluted with hexane/diethyl ether (99:1) to afford compound **19c** (303 mg, 97%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 0.545 and 0.583 (6H and 6H, each q, J = 7.7 Hz, 6 × SiC$\underline{H}_2$CH$_3$), 0.904 - 0.963 (24H, 6 × SiCH$_2$C$\underline{H}_3$, 18- and 21-H$_3$), 1.202 (6H, s, 26- and 27-H$_3$), 1.92 (2H, m), 2.17 (1H, m), 4.02 (1H, narr m, 8α-H), 4.66 and 4.78 (1H and 1H, each s, =CH$_2$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 4.9, 6.8, 6.9, 7.1, 13.1, 17.6, 20.5, 22.5, 22. 6, 26.9, 29.8, 30.0, 32.1, 34.7, 39.4, 42.2, 43.3, 45.2, 53.1, 53.9, 69.4, 73.4, 108.1, 154.9; HRMS (ESI) exact mass calculated for $C_{32}H_{64}O_2Si_2Na$ (M^+ + Na) 559.4343, found 559.4343.

(20*R*)-22-Methylene-8β,25-bis[(triethylsilyl)oxy]-*des*-A,B-26,27-dihomocholestane (19d). Solution of the ketone **18d** (419 mg, 759 μmol) in THF (4 mL) was treated with the ylide generated from methyl triphenylphosphonium bromide (1.742 g, 4.88 mmol) and potassium *tert*-butoxide (481 mg, 4.28 mmol) in anhydrous THF (20 mL) at 45 °C for 23 h. Heating bath was removed, mixture was allowed to reach room temperature and water was added. Materials were extracted with ethyl acetate, dried (Na_2SO_4) and concentrated. The residue was applied on a silica Sep-Pak cartridge and eluted with hexane/diethyl ether (97:3) to afford compound **19d** (314 mg, 75%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 0.545 and 0.579 (6H and 6H, each q, J = 8.0 Hz, 6 × SiC$\underline{H}_2$CH$_3$), 0.837 and 0.851 [3H and 3H, each t, J = 7.5 Hz, 2 × C(CH$_2$C$\underline{H}_3$)], 0.905 (3H, s, 18-H$_3$), ca. 0.93 (3H, 21-H$_3$), 0.942 and 0.950 (9H and 9H, each t, J = 8.0 Hz, 6 × SiCH$_2$C$\underline{H}_3$), 1.94 (2H, m), 2.17 (1H, m), 4.02 (1H, narr m, 8α-H), 4.62 and 4.77 (1H and 1H, each br s, =CH$_2$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 4.9, 6.9, 7.3, 8.3, 8.4, 13.0, 17.6, 20.5, 22.5, 25.1, 27.0, 31.6, 31.9, 34.7, 36.5, 39.6, 42.2, 43.8, 531, 53.9, 69.4, 78.1, 107.7, 155.3; HRMS (ESI) exact mass calculated for $C_{33}H_{67}O_2Si_2$ ($M^+ + H^+$) 551.4679, found 551.4655.

(20*R*)-22-Methylene-8β,25-bis[(triethylsilyl)oxy]-*des*-A,B-24,26,27-trihomocholestane (19e). Solution of the ketone **18e** (245 mg, 432 μmol) in THF (2 mL) was treated with the ylide generated from methyl triphenylphosphonium bromide (1.02 g, 2.86 mmol) and potassium *tert*-butoxide (323 mg, 2.87 mmol) n anhydrous THF (15 mL) at room temperature for 10 days. Water was added and the mixture was extracted with ethyl acetate, dried (Na_2SO_4) and concentrated. The residue was applied on a silica Sep-Pak cartridge and eluted with hexane/diethyl ether (98:2) to afford compound **19e** (195 mg, 80%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 0.544 and 0.569 (6H and 6H, each q, J = 7.8 Hz, 6 × SiC$\underline{H}_2$CH$_3$), 0.823 [6H, t, J = 7.4 Hz, 2 × C(CH$_2$C$\underline{H}_3$)], 0.904 (3H, s, 18-H$_3$), 0.929 (3H, d, J = 6.1 Hz, 21-H$_3$),

0.941 and 0.944 (9H and 9H, each t, J = 7.8 Hz, 6 × $SiCH_2C\underline{H}_3$), 1.92 (2H, m), 2.17 (1H, m), 4.02 (1H, narr m, 8α-H), 4.65 and 4.78 (1H and 1H, each s, $=CH_2$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 4.9, 6.9, 7.1, 7.3, 8.3, 8.4, 13.1, 17.6, 20.4, 21.9, 22.5, 27.0, 31.5, 31.8, 32.1, 34.7, 39.1, 39.4, 42.2, 43.5, 53.1, 53.9, 69.6, 78.2, 108.1, 155.0; HRMS (ESI) exact mass calculated for $C_{34}H_{69}O_2Si_2$ ($M^+ + H^+$) 565.4836, found 565.4828.

(20*R*)-22-Methylene-8β-[(triethylsilyl)oxy]-24-{1'-[(triethylsilyl)oxy]cyclopentyl}-*des*-A,B-25,26,27-trinorcholestane (19f). Solution of the ketone **18f** (491 mg, 892 μmol) in THF (4 mL) was treated with the ylide generated from methyl triphenylphosphonium bromide (869 mg, 2.43 mmol) and potassium *tert*-butoxide (347 mg, 3.09 mmol) in anhydrous THF (25 mL), as it was described for **18e**, providing after the analogous work-up compound **19f** (332 mg, 68%).

^1H NMR (400 MHz, $CDCl_3$) δ 0.546 and 0.584 (6H and 6H, each q, J = 7.9 Hz, 6 × $SiC\underline{H}_2CH_3$), 0.909 (3H, s, 18-H_3), 0.93 (3H, d, J = 6.15 Hz, 21-H_3), 0.943 and 0.949 (9H and 9H, each t, each J = 7.9 Hz, 6 × $SiCH_2C\underline{H}_3$), 2.08 (2H, m), 2.19 (1H, m), 4.02 (1H, narr m, 8α-H), 4.62 and 4.76 (1H and 1H, each s, $=CH_2$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 4.9, 6.9, 7.1, 7.3, 8.3, 8.4, 13.1, 17.6, 20.4, 21.9, 22.5, 27.0, 31.5, 31.8, 32.1, 34.7, 39.1, 39.4, 42.2, 43.5, 53.1, 53.9, 69.4, 78.2, 108.1, 155.0; HRMS (ESI) exact mass calculated for $C_{33}H_{64}O_2Si_2$ ($M^+ + H^+$) 549.4440, found 549.4449.

(20*R*)-22-Methylene-*des*-A,B-cholestan-8β-ol (20a). To a solution of protected alcohol **19a** (61.3 mg, 157.1 μmol) in THF (9 mL) was added tetrabutylammonium fluoride (1.0 M in THF; 760 μL, 760 μmol) at room temperature under argon. The stirring was continued for 20 h, brine was added and the mixture was extracted with ethyl acetate. The organic extracts were dried (Na_2SO_4) and evaporated. The residue was purified on a silica Sep-Pak cartridge using hexane/ethyl acetate (8:2) to give alcohol **20a** (42 mg, 97%) as colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 0.909 (6H, d, J = 6.7 Hz, 26- and 27-H_3), 0.925 (3H, s, 18-H_3), 0.944 (3H, d, J = 6.9 Hz, 21-H_3), 1.95 (2H, m), 2.18 (1H, m), 4.02 (1H, narr m, 8α-H), 4.64 (1H, d, J = 1.2 Hz, one of =CH_2), 4.78 (1H, s, one of =CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.1, 17.4, 20.5, 22.0, 22.6, 22.8, 26.8, 28.2, 29.1, 3.6, 37.1, 39.0, 41.9, 43.5, 52.6, 53.7, 69.3, 108.1, 155.0; HRMS (ESI) exact mass calculated for $C_{19}H_{38}NO$ ($M^+ + NH_4$) 296.2954, found 296.2959.

(20*R*)-22-Methylene-*des*-A,B-cholestane-8β,25-diol (20b'). Solution of protected diol **19b** (71.5 mg, 129.4 μmol) in THF (1 mL) was treated with tetrabutylammonium fluoride (1.0 M in THF; 742 μL, 742 μmol) as described for **19a**. The crude product was purified on a silica Sep-Pak cartridge using hexane/ethyl acetate (9:1) to give the diol **20b'** (39 mg, 96%) as colorless oil.

^{1}H NMR (500 MHz, $CDCl_3$) δ 0.928 (3H, s, 18-H_3), 0.959 (3H, d, J = 6.9 Hz, 21-H_3), 1.24 (6H, s, 26- and 27-H_3), 2.04 (2H, m), 2.21 (1H, m), 4.07 (1H, narr m, 8α-H), 4.65 (1H, d, J = 1.2 Hz, one of =CH_2), 4.81 (1H, s, one of =CH_2); ^{13}C NMR (125 MHz, $CDCl_3$) δ 13.0, 17.3, 20.3, 21.9, 25.4, 26.8, 29.2, 29.3, 33.5, 38.9, 41.8, 41.9, 43.7, 52.5, 53.5, 69.2, 70.9, 108.4, 154.3; HRMS (ESI) exact mass calculated for $C_{19}H_{35}O_2$ ($M^+ + H^+$) 295.2637, found 295.2636.

(20*R*)-22-Methylene-*des*-A,B-24-homocholestane-8β,25-diol (20c'). Solution of protected diol **19c** (109 mg, 203.3 μmol) in THF (1 mL) was treated with tetrabutylammonium fluoride (1.0 M in THF; 1.16 mL, 1.16 mmol) as described for **19a**. The crude product was purified on a silica Sep-Pak cartridge using hexane/ethyl acetate (8:2) to give the diol **20c'** (60 mg, 96%) as colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 0.926 (3H, s, 18-H_3), 0.943 (3H, d, J = 6.9 Hz, 21-H_3), 1.233 (6H, s, 26- and 27-H_3), 1.96 (2H, m), 2.18 (1H, m), 4.02 (1H, narr m, 8α-H), 4.68 and 4.82 (1H and 1H, each s, =CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.1, 17.2, 20.5, 22.9, 23.2, 26.8, 29.7, 29.8, 32.2, 33.9, 39.7, 42.6, 43.2, 45.2, 53.4, 54.1, 69.5, 71.3, 108.2, 154.8; HRMS (ESI) exact mass calculated for $C_{20}H_{36}O_2Na$ (M^+ + Na) 331.2613, found 331.2613.

(20*R*)-22-Methylene-*des*-A,B-26,27-dihomocholestan-8β,25-diol (20d'). Solution of protected diol **19d** (73 mg, 132 μmol) in THF (1 mL) was treated with tetrabutylammonium fluoride (1.0 M in THF; 756 μL, 756 μmol) as described for **19a** providing the diol **20d'** (37 mg, 86%) as colorless oil.

^{1}H NMR (500 MHz, $CDCl_3$) δ 0.873 and 0.878 [3H and 3H, each t, J = 7.5 Hz, 2 × C($CH_2C\underline{H}_3$)], 0.928 (3H, s, 18-H_3), 0.958 (3H, d, J = 6.9 Hz, 21-H_3), 1.97 (2H, m), 2.21 (1H, m), 4.07 (1H, narr m, 8α-H), 4.67 (1H, narr m, one of =CH_2), 4.82 (1H, s, one of =CH_2); ^{13}C NMR (125 MHz, $CDCl_3$) δ 7.7, 7.8, 13.0, 17.3, 20.4, 21.9, 24.6, 26.8, 30.9, 33.6, 36.3, 39.0, 41.9, 43.7, 52.5, 53.6, 69.3, 74.8, 108.4, 154.5; HRMS (ESI) exact mass calculated for $C_{21}H_{39}O_2$ ($M^+ + H^+$) 323.2950, found 323.2949.

(20*R*)-22-Methylene-*des*-A,B-24,26,27-trihomocholestane-8β,25-diol (20e'). Solution of protected diol **19e** (165 mg, 292.5 μmol) in THF (1.5 mL) was treated with tetrabutylammonium fluoride (1.0 M in THF; 1.68 mL, 1.68 mmol) as described for **19a** providing the diol **20e'** (92 mg, 95%) as colorless oil.

^{1}H NMR (500 MHz, $CDCl_3$) δ 0.865 [6H, t, J = 7.5 Hz, 2 × C($CH_2C\underline{H}_3$)], 0.923 (3H, s, 18-H_3), 0.942 (3H, d, J = 6.8 Hz, 21-H_3), 1.94 (2H, m), 2.18 (1H, m), 4.07 (1H, narr m, 8α-H), 4.67 and 4.81 (1H and 1H, each s, =CH_2); ^{13}C NMR (125 MHz, $CDCl_3$) δ 7.7, 7.8, 13.0, 17.3, 20.4, 21.5, 21.9, 26.8, 30.97, 31.0, 31.8, 33.6, 38.3, 38.9, 41.9, 43.4, 52.5, 53.6, 69.3, 74.7, 108.4, 154.4; HRMS (ESI) exact mass calculated for $C_{22}H_{44}NO_2$ ($M^+ + NH_4$) 354.3367, found 354.3369.

(20*R*)-22-Methylene-24-(1'-hydroxycyclopentyl)-*des*-A,B-25,26,27-trinorcholestan-8β-ol (20f'). Solution of protected diol **19f** (216 mg, 394.4 μmol) in THF (2 mL) was treated with tetrabutylammonium fluoride (1.0 M in THF; 2.25 μL, 2.25 mmol) as described for **19a** providing the diol **20f'** (114 mg, 91%) as colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 0.928 (3H, s, 18-H_3), 0.959 (3H, d, J = 6.7 Hz, 21-H_3), 2.04 (2H, m), 2.11 (1H, m), 4.06 (1H, narr m, 8α-H), 4.65 and 4.81 (1H and 1H, each s, =CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.9, 17.3, 20.3, 21.9, 25.9, 26.7, 33.5, 39.6, 39.7, 39.7, 41.9, 43.7, 52.5, 53.5, 69.2, 82.4, 102.3, 108.2, 154.6; HRMS (ESI) exact mass calculated for $C_{21}H_{37}O_2$ ($M^+ + H^+$) 321.2789, found 321.2785.

(20*R*)-22-Methylene-*des*-A,B-cholestan-8-one (15a). The alcohol **20a** (81 mg, 291 μmol) and pyridinium dichromate (468 mg, 2.17 mmol) in anhydrous methylene chloride (5 mL) was stirred vigorously at room temperature overnight. The reaction mixture was then filtered through a pad of Celite (washed with methylene chloride) and the solvents were removed under reduced pressure. The crude product was applied on a silica Sep-Pak cartridge and eluted with hexane/diethyl ether (98:2) to yield the ketone **15a** (70 mg, 87%) as a colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 0.615 (3H, s, 18-H_3), 0.927 (6H, d, J = 6.6 Hz, 26- and 27-H_3), 0.977 (3H, d, J = 6.9 Hz, 21-H_3), 2.43 (1H, dd, J = 11.5, 7.7 Hz, 14α-H), 4.68 (1H, narr m, one of =CH_2), 4.80 (1H, d, J = 1.5 Hz, one of =CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.0, 18.6, 20.4, 22.6, 22.8, 24.1, 27.1, 28.2, 29.0, 37.0, 37.6, 41.9, 49.9, 53.7, 61.9, 108.6, 154.4, 212.0; HRMS (ESI) exact mass calculated for $C_{19}H_{36}NO$ ($M^+ + NH_4$) 294.2797, found 294.2798.

(20*R*)-25-Hydroxy-22-methylene-*des*-A,B-cholestan-8-one (21b'). The solution of diol **20b'** (38 mg, 129 μmol) in anhydrous methylene chloride (2.5 mL) was treated with pyridinium dichromate (194 mg, 516 μmol) as described for **20a**. The crude product was applied on a silica Sep-Pak cartridge and eluted with hexane/diethyl ether (75:25) to yield the ketone **21b'** (35 mg, 90%) as a colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 0.619 (3H, s, 18-H_3), 0.995 (3H, d, J = 6.9 Hz, 21-H_3), 1.273 (6H, s, 26- and 27-H_3), 2.43 (1H, dd, J = 11.5, 7.8 Hz, 14α-H), 4.70 (1H, narr m, one of =CH_2), 4.84

(1H, s, one of $=CH_2$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.0, 18.6, 20.3, 24.1, 25.5, 27.1, 29.3, 29.4, 37.6, 40.99, 419, 44.2, 49.9, 53.7, 61.92, 70.9, 108.9, 153.9, 212.0; HRMS (ESI) exact mass calculated for $C_{19}H_{36}NO_2$ ($M^+ + NH_4$) 310.2746, found 310.2742.

(20*R*)-25-Hydroxy-22-methylene-*des*-A,B-24-homocholestan-8-one (21c'). The solution of diol **20c'** (132 mg, 428,6 μmol) in anhydrous methylene chloride (8 mL) was treated with pyridinium dichromate (690 mg, 3.19 mmol) as described for **20a**. The crude product was applied on a silica Sep-Pak cartridge and eluted with hexane/ethyl acetate (7:3) to yield the ketone **21c'** (116 mg, 88%) as a colorless oil.

^1H NMR (400 MHz, $CDCl_3$) δ 0.612 (3H, s, 18-H_3), 0.976 (3H, d, J = 6.8 Hz, 21-H_3), 1.243 (6H, s, 26- and 27-H_3), 2.17 (2H, m), 2.25 (1H, dd, J = 13.7, 4.9 Hz), 2.43 (1H, dd, J = 11.2, 7.8 Hz, 14α-H), 4.72 and 4.84 (1H and 1H, each s, $=CH_2$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.0, 18.5, 20.3, 22.4, 24.0, 27.0, 29.2, 29.3, 31.9, 37.6, 40.9, 43.7, 43.9, 49.97, 53.7, 61.9, 70.8, 108.9, 153.8, 212.0; HRMS (ESI) exact mass calculated for $C_{20}H_{38}NO_2$ ($M^+ + NH_4$) 324.2903, found 329.2899.

(20*R*)-25-Hydroxy-22-methylene-*des*-A,B-26,27-dihomocholestan-8-one (21d'). The solution of diol **20d'** (27 mg, 84 μmol) in anhydrous methylene chloride (1.5 mL) was treated with pyridinium dichromate (135 mg, 359 μmol) as described for **20a**. The crude product was applied on a silica Sep-Pak cartridge and eluted with hexane/diethyl ether (8:2) to yield the ketone **21d'** (24 mg, 89%) as a colorless oil.

^1H NMR (400 MHz, $CDCl_3$) δ 0.618 (3H, s, 18-H_3), 0.889 [6H, t, J = 7.4 Hz, 2 × $C(CH_2C\underline{H}_3)$], 0.993 (3H, d, J = 6.9 Hz, 21-H_3), 2.42 (1H, dd, J = 11.2, 7.9 Hz, 14α-H), 4.71 (1H, narr m, one of $=CH_2$), 4.83 (1H, s, one of $=CH_2$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 7.8, 12.0, 18.6, 20.3, 24.0, 24.6, 27.0, 30.9, 31.0, 36.25, 37.7, 40.9, 44.1, 49.9, 53.7, 61.9, 74.5, 108.9, 154.1, 212.0; HRMS (ESI) exact mass calculated for $C_{21}H_{40}NO_2$ ($M^+ + NH_4$) 338.3058, found 338.3041.

(20*R*)-25-Hydroxy-22-methylene-*des*-A,B-24,26,27-trihomocholestan-8-one (21e'). The solution of diol **20e'** (92 mg, 274 μmol) in anhydrous methylene chloride (5 mL) was treated with pyridinium dichromate (440 mg, 1.17 mmol) as described for **20a**. The crude product was applied on a silica Sep-Pak cartridge and eluted with hexane/ethyl acetate (9:1) to yield the ketone **21e'** (75 mg, 91%) as a colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 0.614 (3H, s, 18-H_3), 0.875 [6H, t, J = 7.4 Hz, 2 × $CH_2C\underline{H}_3$)], 0.976 (3H, d, J = 6.9 Hz, 21-H_3), 2.17 (2H, m), 2.26 (1H, dd, J = 13.7, 5.4 Hz), 2.41 (1H, dd, J = 11.2, 7.8 Hz, 14α-H), 4.72 and 4.83 (1H and 1H, each s, =CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ 7.8, 12.0, 18.6, 20.3, 24.1, 30.9, 31.1, 31.9, 37.6, 38.4, 41.0, 43.8, 49.9, 53.7, 61.9, 74.6, 108.9, 153.9, 212.1; HRMS (ESI) exact mass calculated for $C_{22}H_{38}O_2Na$ (M^+ + Na) 357.2770, found 357.2273.

(20*R*)-24-(1'-Hydroxycyclopentyl)-22-methylene-*des*-A,B-25,26,27-trinorcholestan-8-one (21f'). The solution of diol **20f'** (39 mg, 122 μmol) in anhydrous methylene chloride (2.2 mL) was treated with pyridinium dichromate (197 mg, 525 μmol) as described for **20a**. The crude product was applied on a silica Sep-Pak cartridge and eluted with hexane/ethyl acetate (9:1) to provide the ketone **21f'** (35 mg, 90%) as a colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 0.618 (3H, s, 18-H_3), 0.995 (3H, d, J = 6.8 Hz, 21-H_3), 2.42 (1H, dd, J = 11.3, 7.8 Hz, 14α-H), 4.70 and 4.83 (1H and 1H, each s, =C$\underline{H}_2$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.0, 18.5, 20.3, 23.7, 24.1, 25.9, 27.1, 37.6, 39.8, 40.9, 44.2, 49.9, 53.6, 61.9, 108.7, 154.2, 212.0; HRMS (ESI) exact mass calculated for $C_{21}H_{35}O_2$ (M^+ + H^+) 319.2632, found 319.2619.

(20*R*)-22-Methylene-25-[(triethylsilyl)oxy]-*des*-A,B-cholestan-8-one (15b). To the solution of alcohol **21b'** (35 mg, 120 μmol) in anhydrous methylene chloride (2 mL) and 2,6-lutidine (37 μL, 320 μmol) at -40 °C, triethylsilyl trifluoromethanesulfonate (67 μL, 296 μmol) was added dropwise.

The reaction mixture was stirred for 30 min and saturated $NaHCO_3$ was added. Cooling bath was removed, and the mixture was allowed to warm up to room temperature. Then it was extracted with methylene chloride, dried (Na_2SO_4) and concentrated. The residue was applied on a silica Sep-Pak cartridge and eluted with hexane/ethyl acetate (98:2) to give compound **15b** (48 mg, 98%) as a colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 0.584 (6H, q, J = 7.9 Hz, 3 × SiC$\underline{H}_2$CH$_3$), 0.618 (3H, s, 18-H$_3$), 0.961 (9H, t, J = 7.9 Hz, 3 × SiCH$_2$C$\underline{H}_3$), 0.987 (3H, d, J = 6.7 Hz, 21-H$_3$), 1.247 (6H, s, 26- and 27-H$_3$), 2.26 (1H, dd, J = 13.5, 5.3 Hz), 2.42 (1H, dd, J = 11.3, 7.8 Hz, 14α-H), 4.66 (1H, narr m, one of =CH$_2$), 4.80 (1H, s, one of =CH$_2$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 6.8, 7.1, 11.9, 18.6, 20.3, 24.1, 25.5, 27.1, 29.8, 30.1, 37.6, 41.2, 42.9, 44.2, 49.9, 53.8, 61.9, 73.1, 108.4, 154.5, 212.1; HRMS (ESI) exact mass calculated for $C_{25}H_{50}NO_2Si$ ($M^+ + NH_4$) 424.3611, found 424.3611.

(20*R*)-22-Methylene-25-[(triethylsilyl)oxy]-*des*-A,B-24-homocholestan-8-one (15c). The solution of alcohol **21c'** (114 mg, 372.5 μmol) in anhydrous methylene chloride (7 mL) and 2,6-lutidine (113 μL, 1.01 mmol) was treated with *tert*-butyldimethylsilyl trifluoromethanesulfonate (220 μL, 949 μmol) as described above for **21b'**. The crude product was applied on a silica Sep-Pak cartridge and eluted with hexane/ethyl acetate (9:1) to give compound **15c** (149 mg, 95%) as a colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 0.571 (6H, q, J = 7.9 Hz, 3 × Si C$\underline{H}_2$CH$_3$), 0.617 (3H, s, 18-H$_3$), 0.949 (9H, t, J = 7.9 Hz, 3 × SiCH$_2$C$\underline{H}_3$), 0.974 (3H, d, J = 6.5 Hz, 21-H$_3$), 1.217 (6H, s, 26- and 27-H$_3$), 2.16 (2H, m), 2.26 (1H, dd, J = 13.5, 5.3 Hz), 2.42 (1H, dd, J = 11.3, 7.8 Hz, 14α-H), 4.71 and 4.82 (1H and 1H, each s, =CH$_2$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 6.7, 7.1, 12.0, 18.6, 20.3, 22.5, 24.06, 27.1, 29.8, 30.0, 31.9, 37.6, 41.0, 43.7, 45.2, 49.9, 53.7, 61.9, 73.3, 108.8, 154.2, 212.0; HRMS (ESI) exact mass calculated for $C_{26}H_{52}NO_2Si$ ($M^+ + NH_4$) 438.3767, found 438.3759.

(20*R*)-22-Methylene-25-[(triethylsilyl)oxy]-*des*-A,B-26,27-dihomocholestan-8-one (15d). The solution of alcohol **21d'** (24 mg, 75 μmol) in anhydrous methylene chloride (1.3 mL) and 2,6-lutidine (23 μL, 198 μmol) was treated with *tert*-butyldimethylsilyl trifluoromethanesulfonate (42 μL, 186 μmol) as described above for **21b'**. The crude product was applied on a silica Sep-Pak cartridge and eluted with hexane/ethyl acetate (9:1) to give compound **15d** (33 mg, 99%) as a colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 0.591 (6H, q, J = 7.9 Hz, 3 × SiC$\underline{H}_2$CH$_3$), 0.619 (3H, s, 18-H$_3$), 0.853 and 0.858 [3H and 3H, each t, J = 7.5 Hz, 2 × C(CH$_2$C$\underline{H}_3$)], 0.961 (9H, t, J = 7.9 Hz, 3 × SiCH$_2$C$\underline{H}_3$), 0.984 (3H, d, J = 6.6 Hz, 21-H$_3$), 2.42 (1H, dd, J = 11.3, 7.8 Hz, 14α-H), 4.68 (1H, narr m, one of =CH$_2$), 4.81 (1H, s, one of =CH$_2$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 7.09, 7.30, 8.33, 8.38, 12.00, 18.59, 20.41, 24.07, 25.2, 27.1, 31.6, 31.9, 36.4, 41.0, 44.2, 49.9, 53.8, 61.9, 78.0, 108.5, 154.6, 212.1; HRMS (ESI) exact mass calculated for $C_{27}H_{54}NO_2Si$ ($M^+ + NH_4$) 452.3924, found 452.3913.

(20*R*)-22-Methylene-25-[(triethylsilyl)oxy]-*des*-A,B-24,26,27-trihomocholestan-8-one (15e). The solution of alcohol **21e'** (75 mg, 224 μmol) in anhydrous methylene chloride (4 mL) and 2,6-lutidine (69 μL, 595 μmol) was treated with *tert*-butyldimethylsilyl trifluoromethanesulfonate (125 μL, 553 μmol) as described above for **21b'**. The crude product was applied on a silica Sep-Pak cartridge and eluted with hexane/ethyl acetate (99:1) to give compound **15e** (85 mg, 84%) as a colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 0.577 (6H, q, J = 7.8 Hz, 3 × SiC$\underline{H}_2$CH$_3$), 0.618 (3H, s, 18-H$_3$), 0.835 [6H, t, J = 7.4 Hz, 2 × C(CH$_2$C$\underline{H}_3$)], 0.952 (9H, t, J = 7.8 Hz, 3 × SiCH$_2$C$\underline{H}_3$), 0.979 (3H, d, J = 6.0 Hz, 21-H$_3$), 2.18 (2H, m), 2.26 (1H, dd, J = 13.6, 5.3 Hz), 2.42 (1H, dd, J = 11.3, 7.9 Hz,

14α-H), 4.71 and 4.83 (1H and 1H, each s, $=CH_2$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 7.0, 7.2, 8.3, 12.0, 18.6, 20.31, 21.9, 24.1, 27.1, 31.6, 31.8, 32.0, 37.6, 39.0, 41.0, 43.9, 50.0, 53.7, 61.9, 78.1, 108.8, 154.2, 212.2; HRMS (ESI) exact mass calculated for $C_{28}H_{53}O_2Si$ ($M^+ + H^+$) 449.3810, found 449.3813.

(20*R*)-22-Methylene-24-{1'-[(triethylsilyl)oxy]cyclopentyl}-*des*-A,B-25,26,27-trinorcholestan-8-one (15f). The solution of alcohol **21f'** (34 mg, 107 μmol) in anhydrous methylene chloride (1.8 mL) and 2,6-lutidine (32 μL, 279 μmol) was treated with *tert*-butyldimethylsilyl trifluoromethanesulfonate (61 μL, 271 μmol) as described above for **21b'**. The crude product was applied on a silica Sep-Pak cartridge and eluted with hexane/ethyl acetate (97:3) to give compound **15f** (42 mg, 91%) as a colorless oil.

^1H NMR (400 MHz, $CDCl_3$) δ 0.594 (6H, q, J = 7.9 Hz, 3 × $SiC\underline{H}_2$), 0.618 (3H, s, 18-H_3), 0.835 [6H, t, J = 7.4 Hz, 2 × $C(CH_2C\underline{H}_3)$], 0.952 (9H, t, J = 7.9 Hz, 3 × $SiCH_2C\underline{H}_3$), 0.979 (3H, d, J = 6.0 Hz, 21-H_3), 2.18 (2H, m), 2.26 (1H, dd, J = 13.6, 5.3 Hz), 2.42 (1H, dd, J = 11.3, 7.9 Hz, 14α-H), 4.71 and 4.83 (1H and 1H, each s, $=CH_2$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 7.0, 7.2, 8.3, 12.0, 18.6, 20.3, 21.9, 24.1, 27.1, 31.5, 31.8, 32.0, 37.6, 39.0, 41.0, 43.9, 50.0, 53.7, 61.9, 78.1, 108.8, 154.2, 212.1; HRMS (ESI) exact mass calculated for $C_{27}H_{49}O_2Si$ ($M^+ + H^+$) 433.3497, found 433.3442.

(20*R*)-1α-[(*tert*-Butyldimethylsilyl)oxy]-2,22-dimethylene-19-norvitamin D_3 *tert*-butyldimethylsilyl ether (22a). To a solution of phosphine oxide **14** (55.3 mg, 95 μmol) in anhydrous THF (0.5 mL) at 0 °C was slowly added *n*-BuLi (1.6 M in hexanes; 60 μL, 96 μmol) under argon with stirring. The solution turned red. The mixture was cooled to -78 °C, and precooled (-78 °C) solution of the ketone **15a** (17.5 mg, 63.4 μmol) in anhydrous THF (200 μL + 200 μL) was slowly added. The mixture was stirred under argon at -78 °C for 4 h and at 4 °C for 19 h. Brine

was added, the mixture was extracted with ethyl acetate, dried (Na_2SO_4) and evaporated. The residue was purified on a silica Sep-Pak cartridge (0→2% ethyl ether/hexane) to give protected 19-norvitamin D derivative **22a** (39 mg, 96%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 0.025, 0.047, 0.066, and 0.077 (each 3H, each s, 4 × $SiCH_3$), 0.519 (3H, s, 18-H_3), 0.865 and 0.894 (9H and 9H, each s, 2 × Si-*t*-Bu), 0.916 (6H, d, *J* = 6.7 Hz, 26- and 27-H_3), 0.966 (3H, d, *J* = 6.6 Hz, 21-H_3), 2.19 (2H, m), 2.32 (1H, dd, *J* = 13.2, 2.7 Hz, 10β-H), 2.45 (1H, dd, *J* = 12.9, 4.5 Hz, 4α-H), 2.51 (1H, dd, *J* = 13.2, 5.9 Hz, 10α-H), 2.81 (1H, dd, *J* = 12.9, 3.0 Hz, 9β-H), 4.42 (2H, m, 1β- and 3α-H), 4.64, 4.79, 4.92, and 4.96 (each 1H, each s, 2 × =CH_2), 5.83 and 6.21 (1H and 1H, each d, *J* = 11.1 Hz, 7- and 6-H); ^{13}C NMR (125 MHz, $CDCl_3$) δ -5.1, -4.9, 11.6, 18.2, 18.3, 20.5, 21.7, 22.6, 22.8, 23.3, 25.7, 25.8, 27.2, 28.2, 28.7, 29.0, 37.1, 38.5, 39.2, 44.4, 45.7, 47.7, 53.8, 56.2, 71.6, 72.5, 106.2, 108.1, 116.1, 122.4, 132.7, 141.3, 152.9, 155.2; HRMS (ESI) exact mass calculated for $C_{40}H_{72}O_2Si_2Na$ (M^+ + Na) 663.4969, found 663.4974.

(20*R*)-1α-[(*tert*-Butyldimethylsilyl)oxy]-2,22-dimethylene-25-[(triethylsilyl)oxy]-19-norvitamin D_3 *tert*-butyldimethylsilyl ether (22b). The solution of ketone **15b** (11 mg, 25 μmol) in anhydrous THF (200 μL + 100 μL) was slowly added to the THF solution (0.5 mL) of the anion, generated from the phosphine oxide **14** (29 mg, 50 μmol) and *n*-BuLi (1.6 M in hexanes; 32 μL, 51 μmol), under argon at -78 °C with stirring. After analogous procedure as described above for **22a**, protected 19-norvitamin D derivative **22b** (20 mg, 75%) was obtained as a colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 0.017, 0.038, 0.059, and 0.069 (each 3H, each s, 4 × $SiCH_3$), 0.514 (3H, s, 18-H_3), 0.568 (6H, q, *J* = 8.0 Hz, 3 × $SiCH_2$), 0.857 and 0.886 (9H and 9H, each s, 2 × Si-*t*-Bu), 0.947 (9H, t, *J* = 8.0 Hz, 3 × $SiCH_2C\underline{H}_3$), 0.961 (3H, d, *J* = 6.8 Hz, 21-H_3), 1.227 (6H, s, 26- and 27-H_3), 2.18 (2H, m), 2.32 (1H, dd, *J* = 13.2, 2.4 Hz, 10β-H), 2.45 (1H, dd, *J* = 12.9, 4.5 Hz, 4α-H), 2.50 (1H, dd, *J* = 13.2, 5.9 Hz, 10α-H), 2.81 (1H, dd, *J* = 12.9, 3.0 Hz, 9β-H), 4.41 (2H, narr

m, 1β- and 3α-H), 4.61, 4.77, 4.92, and 4.96 (each 1H, each s, 2 × =CH_2), 5.82 and 6.20 (1H and 1H, each d, J = 11.1 Hz, 7- and 6-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ -5.1, -4.9, 6.8, 7.1, 11.5, 18.1, 18.2, 20.4, 21.7, 23.3, 25.6, 25.7, 25.8, 27.2, 28.7, 29.6, 29.9, 30.0, 38.5, 39.1, 42.9, 44.7, 45.7, 47.6, 53.8, 56.2, 71.6, 72.5, 73.2, 106.2, 108.8, 116.05, 122.4, 132.7, 141.3, 152.9, 155.2; HRMS (ESI) exact mass calculated for $C_{40}H_{71}O_2Si_2$ (M^+ -TBSO + H^+) 639.4992, found 639.4991.

(20*R*)-1α-[(*tert*-Butyldimethylsilyl)oxy]-2,22-dimethylene-25-[(triethylsilyl)oxy]-24-homo-19-norvitamin D_3 *tert*-butyldimethylsilyl ether (22c). The solution of ketone **15c** (28.7 mg, 68.3 μmol) in anhydrous THF (300 μL + 200 μL) was slowly added to the THF solution (0.5 mL) of the anion, generated from the phosphine oxide **14** (61.5 mg, 106 μmol) and *n*-BuLi (1.6 M in hexanes; 67 μL, 107 μmol), under argon at -78 °C with stirring. After analogous procedure as described above for **22a**, protected 19-norvitamin D derivative **22c** (50 mg, 92%) was obtained as a colorless oil.

^{1}H NMR (500 MHz, $CDCl_3$) δ 0.025, 0.047, 0.067, and 0.078 (each 3H, each s, 4 × $SiCH_3$), 0.521 (3H, s, 18-H_3), 0.567 (6H, q, J = 7.9 Hz, 3 × $SiCH_2$), 0.864 and 0.895 (9H and 9H, each s, 2 × Si-*t*-Bu), 0.946 (9H, t, J = 7.9 Hz, 3 × $SiCH_2C\underline{H}_3$), 0.961 (3H, d, J = 6.8 Hz, 21-H_3), 1.210 (6H, s, 26- and 27-H_3), 2.17 (2H, m), 2.32 (1H, dd, J = 13.3, 3.0 Hz, 10β-H), 2.45 (1H, dd, J = 12.8, 4.5 Hz, 4α-H), 2.51 (1H, dd, J = 13.3, 5.9 Hz, 10α-H), 2.80 (1H, dd, J = 13.5, 3.7 Hz, 9β-H), 4.42 (2H, m, 1β- and 3α-H), 4.67, 4.81, 4.92, and 4.97 (each 1H, each s, 2 × =CH_2), 5.83 and 6.21 (1H and 1H, each d, J = 11.1 Hz, 7- and 6-H); ^{13}C NMR (125 MHz, $CDCl_3$) δ -5.1, -4.9, -4.7, 6.8, 7.1, 11.6, 15.3, 18.2, 20.4, 21.7, 22.5, 23.3, 25.7, 27.2, 28.7, 29.8, 29.9, 31.9, 38.5, 39.1, 44.2, 45.2, 45.8, 47.6, 53.7, 56.2, 65.7, 71.6, 72.5, 73.4, 106.3, 108.2, 116.1, 122.4, 132.7, 141.3, 152.9, 154.8; HRMS (ESI) exact mass calculated for $C_{47}H_{92}NO_3Si_3$ (M^+ + NH_4) 802.6385, found 802.6391.

(20*R*)-1α-[(*tert*-Butyldimethylsilyl)oxy]-2,22-dimethylene-25-[(triethylsilyl)oxy]-26,27-dihomo-19-norvitamin D_3 *tert*-butyldimethylsilyl ether (22d). The solution of ketone **15d** (11 mg, 25 μmol) in anhydrous THF (200 μL + 100 μL) was slowly added to the THF solution (0.3 mL) of the anion, generated from the phosphine oxide **14** (22 mg, 38 μmol) and *n*-BuLi (1.6 M in hexanes; 24 μL, 38 μmol), under argon at -78 °C with stirring. After analogous procedure as described above for **22a**, protected 19-norvitamin D derivative **22d** (17 mg, 85%) was obtained as a colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 0.025, 0.048, 0.067, and 0.078 (each 3H, each s, 4 × $SiCH_3$), 0.525 (3H, s, 18-H_3), 0.585 (6H, q, *J* = 7.9 Hz, 3 × $SiCH_2$), 0.846 and 0.851 [3H and 3H, each t, *J* = 7.5 Hz, 2 × $C(CH_2C\underline{H}_3)$], 0.864 and 0.895 (9H and 9H, each s, 2 × Si-*t*-Bu), 0.957 (9H, t, *J* = 7.9 Hz, 3 × $SiCH_2C\underline{H}_3$), 0.961 (3H, d, *J* = 6.8 Hz, 21-H_3), 2.18 (2H, m), 2.33 (1H, dd, *J* = 13.1, 2.4 Hz, 10β-H), 2.45 (1H, dd, *J* = 12.9, 4.5 Hz, 4α-H), 2.50 (1H, dd, *J* = 13.1, 5.9 Hz, 10α-H), 2.83 (1H, dd, *J* = 12.9, 3.0 Hz, 9β-H), 4.42 (2H, m, 1β- and 3α-H), 4.63, 4.79, 4.92, and 4.97 (each 1H, each s, 2 × =CH_2), 5.83 and 6.22 (1H and 1H, each d, *J* = 11.0 Hz, 7- and 6-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ -5.1, -4.9, -4.7, 6.8, 7.1, 8.3, 11.6, 18.1, 18.3, 20.5, 21.7, 23.3, 25.1, 25.7, 25.8, 27.2, 28.7, 29.6, 31.7, 31.9, 37.0, 38.5, 39.3, 44.6, 45.7, 47.6, 53.8, 56.2, 71.6, 72.5, 78.1, 106.3, 107.9, 116.1, 122.4, 132.7, 141.3, 152.9, 155.2; HRMS (ESI) exact mass calculated for $C_{42}H_{75}O_2Si_2$ (M^+ -TBSO + H^+) 667.5305, found 667.5316.

(20*R*)-1α-[(*tert*-Butyldimethylsilyl)oxy]-2,22-dimethylene-25-[(triethylsilyl)oxy]-24,26,27-trihomo-19-norvitamin D_3 *tert*-butyldimethylsilyl ether (22e). The solution of ketone **15e** (25 mg, 56 μmol) in anhydrous THF (300 μL + 200 μL) was slowly added to the THF solution (0.5 mL) of the anion, generated from the phosphine oxide **14** (50 mg, 86 μmol) and *n*-BuLi (1.6 M in hexanes; 32 μL, 51 μmol), under argon at -78 °C with stirring. After analogous procedure as

described above for **22a**, protected 19-norvitamin D derivative **22e** (43 mg, 94%) was obtained as a colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 0.026, 0.047, 0.068, and 0.078 (each 3H, each s, 4 × $SiCH_3$), 0.524 (3H, s, 18-H_3), 0.567 (6H, q, *J* = 7.8 Hz, 3 × $SiCH_2$), 0.830 [6H, t, *J* = 7.4 Hz, 2 × $C(CH_2C\underline{H}_3)$], 0.866 and 0.895 (9H and 9H,each s, 2 × Si-*t*-Bu), 0.950 (9H, t, *J* = 7.8 Hz, 3 × $SiCH_2C\underline{H}_3$), 0.966 (3H, d, *J* = 6.8 Hz, 21-H_3), 2.18 (2H, m), 2.33 (1H, dd, *J* = 13.2, 2.6 Hz, 10β-H), 2.45 (1H, dd, *J* = 12.9, 4.5 Hz, 4α-H), 2.51 (1Hdd, *J* = 13.2, 5.9 Hz, 10α-H), 2.80 (1H, br d, *J* ~ 14 Hz, 9β-H), 4.42 (2H, m, 1β- and 3α-H), 4.67, 4.81, 4.92, and 4.97 (each 1H, each s, 2 × =CH_2), 5.83 and 6.20 (1H and 1H, each d, *J* = 11.1 Hz, 7- and 6-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ -5.1, -4.9, -4.7, 7.1, 7.3, 8.3, 11.6, 14.1, 18.2, 18.2, 20.4, 21.7, 21.9, 23.3, 25.8, 25.8, 27.2, 28.7, 31.6, 31.8, 32.1, 38.6, 39.1, 39.2, 44., 45.8, 47.6, 53.8, 56.2, 71.6, 72.4, 78.2, 106.3, 108.3, 116.1, 122.4, 132.7, 141.3, 152.9, 154.9; HRMS (ESI) exact mass calculated for $C_{49}H_{92}O_3Si_3Na$ (M^+ + Na) 835.6252, found 835.6253.

(20*R*)-1α-[(*tert*-Butyldimethylsilyl)oxy]-2,22-dimethylene-24-{1'-[(triethylsilyl)oxy] cyclopentyl}-19,25,26,27-tetranorvitamin D_3 *tert*-butyldimethylsilyl ether (23f). The solution of ketone **15f** (25 mg, 56 μmol) in anhydrous THF (300 μL + 200 μL) was slowly added to the THF solution (0.5 mL) of the anion, generated from the phosphine oxide **14** (24 mg, 56 μmol) and *n*-BuLi (1.6 M in hexanes; 40 μL, 65 μmol), under argon at -78 °C with stirring. After analogous procedure as described above for **22a**, protected 19-norvitamin D derivative **22f** (39 mg, 88%) was obtained as a colorless oil.

^{1}H NMR (500 MHz, $CDCl_3$) δ 0.025, 0.047, 0.067, and 0.078 (each 3H, each s, 4 × $SiCH_3$), 0.524 (3H, s, 18-H_3), 0.588 (6H, q, *J* = 7.9 Hz, 3 × $SiC\underline{H}_2CH_3$), 0.864 and 0.894 (9H and 9H, each s, 2 × Si-*t*-Bu), 0.953 (9H, t, *J* = 7.9 Hz, 3 × $SiCH_2C\underline{H}_3$), 0.967 (3H, d, *J* = 6.8 Hz, 21-H_3), 2.32 (1H, dd, *J* = 13.0, 2.8 Hz, 10β-H), 2.45 (1H, dd, *J* = 12.7, 4.5 Hz, 4α-H), 2.51 (1H, dd, *J* = 13.3, 5.9 Hz,

10α-H), 2.82 (1H, br d, J = 12.8 Hz, 9β-H), 4.42 (2H, m, 1β- and 3α-H), 4.62, 4.79, 4.92, and 4.97 (each 1H, each s, 2 × =CH_2), 5.83 and 6.21 (1H and 1H, each d, J = 11.1 Hz, 7- and 6-H); ^{13}C NMR (125 MHz, $CDCl_3$) δ -5.1, -4.9, -4.8, 6.6, 7.2, 11.6, 14.1, 18.2, 18.2, 20.6, 21.6, 22.7, 23.6, 25.7, 25.8, 27.3, 28.7, 31.6, 38.5, 39.2, 39.6, 39.8, 39.9, 44.7, 45.7, 47.6, 53.7, 56.2, 71.6, 72.5, 84.6, 106.3, 107.7, 116.1, 122.4, 132.7, 141.3, 152.9, 155.2; HRMS (ESI) exact mass calculated for $C_{42}H_{73}O_2Si_2$ (M^+ - $C_6H_{15}OSi$) 665.5144, found 665.5135.

(20*R*)-1α-Hydroxy-2,22-dimethylene-19-norvitamin D_3 (8). To a solution of protected vitamin **22a** (39 mg, 60.8 μmol) in THF (3 mL) was added tetrabutylammonium fluoride (1.0 M in THF; 2.9 mL, 2.9 mmol) at room temperature under argon. The stirring was continued for 20 h, brine was added and the mixture was extracted with ethyl acetate. The organic extracts were dried (Na_2SO_4) and evaporated. The residue was purified by HPLC (9.4 mm × 25 cm Zorbax-Sil column, 4 mL/min) using hexane/2-propanol (95:5) solvent system; 19-norvitamin **8** (17.91 mg, 71%) was collected at R_V 35 mL. Analytical sample of the vitamin was obtained after reversed-phase HPLC (9.4 mm × 25 cm Zorbax Eclipse XDB-C18 column, 4 mL/min) using methanol/water (96:4) solvent system (R_V 37 mL).

UV (EtOH) λ_{max} 244, 252 (ε 42 200), 261 nm; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.544 (3H, s, 18-H_3), 0.935 (6H, br d, J = 7.0 Hz, 26- and 27-H_3), 0.985 (3H, d, J = 7.0 Hz, 21-H_3), 2.29 (1H, dd, J = 13.0, 9.0 Hz, 10α-H), 2.34 (1H, dd, J = 13.0, 6.0 Hz, 4β-H), 2.58 (1H, dd, J = 13.0, 3.6 Hz, 4α-H), 2.82 (1H, dd, J = 14.0, 4.5 Hz, 9β-H), 2.87 (1H, dd, J = 13.0, 4.5 Hz, 10β-H), 4.48 (1H, dd, J = 9.0, 4.5 Hz, 1β-H), 4.51 (1H, narr m, 3α-H), 4.66, 4.80, 5.10, and 5.13 (each 1H, each s, 2 × =CH_2), 5.89 and 6.37 (1H and 1H, each d, J = 11.5 Hz, 7- and 6-H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 11.5, 14.2, 20.5, 21.8, 22.8, 22.8, 23.4, 27.2, 28.2, 28.9, 37.2, 38.1, 39.0, 44.5, 45.8, 45.9, 53.8, 56.3,

70.7, 71.8, 107.8, 108.2, 115.3, 124.3, 130.4, 143.6, 151.9, 155.1; HRMS (ESI) exact mass calculated for $C_{28}H_{45}O_2$ ($M^+ + H^+$) 413.3419, found 413.3412.

(20*R*)-1α,25-Dihydroxy-2,22-dimethylene-19-norvitamin D_3 (9). The solution of protected vitamin **22b** (19 mg, 24.7 μmol) in THF (1 mL) was treated with tetrabutylammonium fluoride (1.0 M in THF; 1.1 mL, 1.1 mmol) at room temperature under argon. After analogous procedure as described above for **8**, the crude product was purified by HPLC (9.4 mm × 25 cm Zorbax-Sil column, 4 mL/min) using hexane/2-propanol (95:5) solvent system; vitamin **9** (8.13 mg, 77%) was collected at R_V 36 mL. Analytical sample of the vitamin was obtained after reversed-phase HPLC (9.4 mm × 25 cm Zorbax Eclipse XDB-C18 column, 4 mL/min) using methanol/water (87:13) solvent system (R_V 29 mL).

UV (EtOH) λ_{max} 244, 252 (ε 42 500), 261 nm; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.530 (3H, s, 18-H_3), 0.983 (3H, d, *J* = 7.0 Hz, 21-H_3), 1.262 (6H, s, 26- and 27-H_3), 2.21 (1H, m), 2.29 (1H, dd, *J* = 13.1, 8.4 Hz, 10α-H), 2.33 (1H, dd, *J* = 13.4, 6.1 Hz, 4β-H), 2.56 (1H, dd, *J* = 13.4, 3.8 Hz, 4α-H), 2.80 (1H, dd, *J* = 14.0, 4.4 Hz, 9β-H), 2.87 (1H, dd, *J* = 13.1, 4.6 Hz, 10β-H), 4.47 (2H, narr m, 1β- and 3α-H), 4.65, 4.82, 5.09, and 5.11 (each 1H, each s, 2 × =CH_2), 5.89 and 6.35 (1H and 1H, each d, *J* = 11.3 Hz, 7- and 6-H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 11.6, 20.3, 21.7, 23.3, 25.4, 27.2, 28.3, 28.9, 29.3, 29.3, 38.1, 38.9, 41.8, 44.6, 45.7, 45.8, 53.6, 56.2, 70.6, 71.0, 71.8, 107.7, 108.5, 115.3, 124.2, 130.4, 143.5, 151.9, 154.4; HRMS (ESI) exact mass calculated for $C_{28}H_{48}NO_3$ ($M^+ + NH_4$) 446.3634, found 446.3624.

(20*R*)-1α,25-Dihydroxy-2,22-dimethylene-24-homo-19-norvitamin D_3 (10). The solution of protected vitamin **22c** (50 mg, 64 μmol) in THF (3.5 mL) was treated with tetrabutylammonium fluoride (1.0 M in THF; 3.7 mL, 3.7 mmol) at room temperature under argon. After analogous procedure as described above for **8**, the crude product was purified by HPLC (9.4 mm × 25 cm

Zorbax-Sil column, 4 mL/min) using hexane/2-propanol (92:8) solvent system; vitamin **10** (13.91 mg, 50%) was collected at R_V 45 mL. Analytical sample of the vitamin was obtained after HPLC (9.4 mm × 25 cm Zorbax Eclipse XDB-C18 column, 4 mL/min) using methanol/water (85:15) solvent system (R_V 52 mL).

UV (EtOH) λ_{max} 244, 252 (ε 42 100), 261 nm; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.525 (3H, s, 18-H_3), 0.964 (3H, d, *J* = 7.0 Hz, 21-H_3), 1.227 (6H, s, 26- and 27-H_3), 2.29 (1H, dd, *J* = 13.0, 8.5 Hz, 10α-H), 2.34 (1H, dd, *J* = 13.2, 6.0 Hz, 4β-H), 2.58 (1H, dd, *J* = 13.2, 3.6 Hz, 4α-H), 2.82 (1H, dd, *J* = 14.0, 4.5 Hz, 9β-H), 2.87 (1H, dd, *J* = 13.0, 4.5 Hz, 10β-H), 4.48 (1H, dd, *J* = 8.5, 4.5 Hz, 1β-H), 4.51 (1H, m, 3α-H), 4.66, 4.80, 5.10, and 5.13 (each 1H, each s, 2 × =CH_2), 5.89 and 6.37 (1H and 1H, each d, *J* = 11.5 Hz, 7- and 6-H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 11.7, 18.4, 20.4, 21.7, 22.4, 23.2, 27.1, 28.9, 29.2, 29.3, 31.8, 38.1, 38.9, 43.9, 44.2, 45.7, 45.8, 53.7, 56.2, 58.4, 70.6, 71.1, 71.6, 107.7, 108.4, 115.3, 124.1, 130.4, 143.4, 151.9, 154.4; HRMS (ESI) exact mass calculated for $C_{29}H_{50}NO_3$ ($M^+ + NH_4$) 460.3791, found 465.3794.

(20*R*)-1α,25-Dihydroxy-26,27-dihomo-2,22-dimethylene-19-norvitamin D_3 (11). The solution of protected vitamin **22d** (17 mg, 21.3 μmol) in THF (1.5 mL) was treated with tetrabutylammonium fluoride (1.0 M in THF; 1.34 mL, 1.34 mmol) at room temperature under argon. After analogous procedure as described above for **8**, the crude product was purified by HPLC (9.4 mm × 25 cm Zorbax-Sil column, 4 mL/min) using hexane/2-propanol (95:5) solvent system; vitamin **11** (5.118 mg, 53%) was collected at R_V 27 mL. Analytical sample of the vitamin was obtained after reversed-phase HPLC (9.4 mm × 25 cm Zorbax Eclipse XDB-C18 column, 4 mL/min) using methanol/water (9:1) solvent system (R_V 29 mL).

UV (EtOH) λ_{max} 244.5, 252 (ε 42 000), 261.5 nm;^{1}H NMR (500 MHz, $CDCl_3$) δ 0.529 (3H, s, 18-H_3), 0.878 and 0.884 [3H and 3H, each t, *J* = 7.5 Hz, 2 × C(CH_2C$\underline{H}_3$)], 0.981 (3H, d, *J* = 6.9 Hz,

21-H_3), 2.21 (1H, m), 2.29 (1H, dd, *J* = 13.0, 8.3 Hz, 10α-H), 2.33 (1H, dd, *J* = 13.3, 6.1 Hz, 4β-H), 2.57 (1H, dd, *J* = 13.3, 3.8 Hz, 4α-H), 2.79 (1H, dd, *J* = 13.7, 4.3 Hz, 9β-H), 2.85 (1H, dd, *J* = 13.1, 4.5 Hz, 10β-H), 4.47 (2H, m, 1β-H and 3α-H), 4.67, 4.82, 5.09, and 5.11 (each 1H, each s, 2 × =CH_2), 5.89 and 6.35 (1H and 1H, each d, *J* = 11.3 Hz, 7- and 6-H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 7.73, 7.79, 11.6, 20.4, 21.7, 23.3, 24.5, 27.2, 28.9, 30.9, 31.0, 36.2, 38.1, 39.1, 44.6, 45.7, 45.8, 53.6, 56.2, 70.6, 71.8, 74.6, 107.7, 108.5, 115.3, 124.2, 130.3, 143.5, 151.9, 154.6; HRMS (ESI) exact mass calculated for $C_{30}H_{52}NO_3$ ($M^+ + NH_4$) 474.3947, found 474.3960.

(20*R*)-1α,25-Dihydroxy-2,22-dimethylene-24,26,27-trihomo-19-norvitamin D_3 (12). The solution of protected vitamin **22e** (43 mg, 52.9 μmol) in THF (3 mL) was treated with tetrabutylammonium fluoride (1.0 M in THF; 3.2 mL, 3.2 mmol) at room temperature under argon. After analogous procedure as described above for **8**, the crude product was purified by HPLC (9.4 mm × 25 cm Zorbax-Sil column, 4 mL/min) using hexane/2-propanol (92:8) solvent system; vitamin **12** (10.5 mg, 43%) was collected at R_V 25 mL. Analytical sample of the vitamin was obtained after HPLC (9.4 mm × 25 cm Zorbax Eclipse XDB-C18 column, 4 mL/min) using methanol/water (9:1) solvent system (R_V 38 mL).

UV (EtOH) λ_{max} 244, 252 (ε 41 900), 261 nm; ^{1}H NMR (750 MHz, $CDCl_3$) δ 0.526 (3H, s, 18-H_3), 0.868 [6H, d, *J* = 7.5 Hz, 2 × C($CH_2C\underline{H}_3$)], 0.965 (3H, d, *J* = 6.8 Hz, 21-H_3), 2.17 (1H, m), 2.28 (1H, dd, *J* = 12.8, 8.3 Hz, 10α-H), 2.33 (1H, dd, *J* = 13.5, 6.0 Hz, 4β-H), 2.56 (1H, dd, J = 13.5, 3.8 Hz, 4α-H), 2.80 (1H, br d, *J* = 12.0 Hz, 9β-H), 2.85 (1H, dd, *J* = 12.8, 4.5 Hz, 10β-H), 4.45 (1H, dd, *J* = 8.3, 4.5 Hz, 1β-H), 4.48 (1H, m, 3α-H), 4.68, 4.81, 5.01, and 5.11 (each 1H, each s, 2 × =CH_2), 5.88 and 6.35 (1H and 1H, each d, *J* = 11.3 Hz, 7- and 6-H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 7.77, 7.79, 11.6, 14.2, 20.3, 21.5, 21.7, 23.3, 24.1, 27.1, 28.0, 30., 31.0, 38.1, 38.4, 38.9, 44.3,

45.7, 45.8, 53.7, 56.2, 60.4, 70.6, 71.6, 74.6, 107.7, 108.4, 115.3, 124.1, 130.5, 143.4, 151.9, 154.5; HRMS (ESI) exact mass calculated for $C_{31}H_{50}O_3Na$ (M^+ + Na) 493.3658, found 493.3658.

(20*R*)-1α-Hydroxy-2,22-dimethylene-24-(1'-hydroxycyclopentyl)-19,25,26,27-tetranorvitamin D_3 (13). The solution of protected vitamin **22f** (37.5 mg, 47.05 μmol) in THF (2.3 mL) was treated with tetrabutylammonium fluoride (1.0 M in THF; 2.6 mL, 2.6 mmol) at room temperature under argon. After analogous procedure as described above for **8**, the crude product was purified by HPLC (9.4 mm × 25 cm Zorbax-Sil column, 4 mL/min) using hexane/2-propanol (90:10) solvent system; vitamin **13** (10.7 mg, 50%) was collected at R_V 40 mL. Analytical sample of the vitamin was obtained after HPLC (9.4 mm × 25 cm Zorbax Eclipse XDB-C18 column, 4 mL/min) using methanol/water (85:15) solvent system (R_V 60 mL).

UV (EtOH) λ_{max} 244.5, 252.5 (ε 42 300), 261.5 nm; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.528 (3H, s, 18-H_3), 0.983 (3H, d, *J* = 6.9 Hz, 21-H_3), 1.99 (1H, m), 2.12 (1H, m), 2.21 (1H, m), 2.28 (1H, dd, *J* = 12.8, 8.2 Hz, 10α-H), 2.33 (1H, dd, *J* = 13.5, 6.0 Hz, 4β-H), 2.56 (1H, dd, *J* = 13.5, 3.7 Hz, 4α-H), 2.79 (1H, br d, *J* = 12.0 Hz, 9β-H), 2.86 (1H, dd, *J* = 12.8, 4.5 Hz, 10β-H), 4.45 (1H, dd, *J* = 8.2, 4.5 Hz, 1β-H), 4.48 (1H, m, 3α-H), 4.65, 4.81, 5.08, and 5.10 (each 1H, each s, 2 × =CH_2), 5.88 and 6.35 (1H and 1H, each d, *J* = 11.2 Hz, 7- and 6-H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 11.6, 20.3, 21.7, 23.3, 23.7, 25.8, 27.2, 28.9, 38.1, 38.9, 39.5, 39.7, 39.8, 44.6, 45.7, 45.8, 53.6, 56.2, 70.6, 71.8, 82.5, 107.7, 108.3, 115.3, 124.2, 130.4, 143.4, 151.9, 154.7; HRMS (ESI) exact mass calculated for $C_{30}H_{45}O_2$ (M^+ - OH) 437.3415, found 437.3424.

Biological Studies. ***1. In Vitro Studies.*** VDR binding, HL-60 differentiation, and 24-hydroxylase transcription assays were performed as previously described.[44,45]

2. In Vivo Studies. 2.1. Bone Calcium Mobilization and Intestinal Calcium Transport. Male, weanling Sprague-Dawley rats were purchased from Harlan (Indianapolis, IN). The animals were group housed and placed on Diet 11 (0.47% Ca) + AEK oil for one week followed by Diet 11 (0.02% Ca) + AEK oil for 3 weeks. The rats were then switched to a diet containing 0.47% Ca[46] for one week followed by two weeks on a diet containing 0.02% Ca. Dose administration began during the last week on 0.02% Ca diet. Four consecutive intraperitoneal doses were given approximately 24 hours apart. Twenty four hours after the last dose, blood was collected from the severed neck and the concentration of serum calcium determined as a measure of bone calcium mobilization. The first 10 cm of the intestine was also collected for the intestinal calcium transport analysis using the everted gut sac method.[45] Please note, the intestinal calcium transport assay saturates when doses of 780 pmol 1α,25-$(OH)_2D_3$ are administered. Thus, the reason values will often be lower than those attained with a smaller dose.

All animals were managed in accordance with University of Wisconsin standards and protocols for animal care and use. Our experiments were approved by the College of Agricultural and Life Sciences Institutional Animal Care and Use Committee.

2.2. Statistical Analysis. All statistical analyses were done using the SAS mixed model procedures and Dunnett's pairwise comparisons (SAS Institute, Inc. Cary, N.C., USA).

Molecular Modeling. The molecular mechanics studies were used to establish the energy-minimized structures of the model 8-methylene *des*-A,B-compounds (Figure 1S, Supporting Information). The calculation of optimized geometries and steric energies was initially carried out using the algorithm from the MM+ HyperChem (release 8.0) software package (Autodesk, Inc.). MM+ is an all-atom force field based on the MM2 functional form. The procedure used for

generation of the respective side-chain conformers and finding the global minimum structures was analogous to that described by us previously[40] and involved the Conformational Search module.

ASSOCIATED CONTENT

Supporting Information 1. Purity criteria of the vitamin D analogues **8-13**, their ^{1}H and ^{13}C NMR spectra, spectral data of the synthesized compounds, conformational analysis of model 8-methylene *des*-A,B-compounds, preparation of the aldehyde **16** and the alkyl bromides **A-F**, ^{1}H and ^{13}C NMR spectra of the synthesized intermediate compounds. **Molecular Formula Strings** together with the associated biochemical and biological data (smiles.csv). This material is available free of charge via the Internet at http://pubs.acs.org.

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ABBREVIATIONS USED

1α,25-$(OH)_2D_3$, 1α,25-dihydroxyvitamin D_3; 2MD, (20*S*)-1α,25-dihydroxy-2-methylene-19-norvitamin D_3; VDR, vitamin D receptor**.**

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Table of Contents Graphic

Synthesis and Biological Activity of 2,22-Dimethylene Analogues of 19-Norcalcitriol and Related Compounds.

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