# Synthesis, Biological Activity, and Conformational Analysis of Four *seco*-D-15,19-*bisnor*-1α,25-Dihydroxyvitamin D Analogues, Diastereomeric at C17 and C20

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The synthesis of four CD-ring-modified 19-*nor*-1α,25-dihydroxyvitamin $D_3$ derivatives lacking C15, referred to as 6C analogues, and diastereomeric at C17 and C20 is described. The synthesis involves an Ireland−Claisen rearrangement of a 3-methyl-substituted ester of (1*R*)-3-methyl-2-cyclohexen-1-ol as the key step, followed by elaboration of the side chain, transformation into a C8 cyclohexanone derivative, and final Wittig−Horner coupling with the 19-*nor* A-ring phosphine oxide. Despite possessing a more flexible side chain than the parent hormone, biological evaluation showed an unexpected superagonistic antiproliferative and prodifferentiating activity (10−50 times higher as compared to that of 1α,25$(OH)_2D_3$) for the diastereomer with the "natural" configuration at C17 and C20. The other diastereomers exhibit a 25−90% decrease in activity. All four analogues show a decreased binding affinity (45% or less), and their calcemic activity is 4−400 times less than that of 1α,25$(OH)_2D_3$. The conformational behavior of their side chain was studied using molecular mechanics calculations, and the result is presented as volume maps. A relative activity volume was determined by subtraction of the volume map of the least active analogue from the volume map of the most active one. This shows three regions corresponding to preferred orientations in space of the side chain of the active analogue. One of these regions was found to overlap with the region that is preferentially occupied by the most active of the four diastereomeric 22-methyl-substituted 1α,25$(OH)_2D_3$ analogues.

## Introduction

The discovery that 1α,25-dihydroxyvitamin $D_3$ (**1**; further abbreviated as 1,25$(OH)_2D_3$), the hormonally active form of vitamin D, possesses, next to its classical calciotropic activity,[1] immunosuppressive activity[2] and is also effective in the inhibition of cellular proliferation and in the induction of cellular differentiation[3] has resulted in a very active search for analogues in which the calcemic activity and the other activities are dissociated.[4] Indeed, the therapeutic utility of the natural hormone in the treatment of certain cancers and skin diseases is severely limited because effective doses provoke hypercalcemia. Notable successes have already been recorded in the discovery of analogues that have such dissociated activity. A few illustrative examples are 20-*epi*-1,25$(OH)_2D_3$ (**2**),[5a] 19-*nor*-1,25$(OH)_2D_3$ (**3**),[6] a 16-ene-23-yne derivative developed by Hoffmann-La Roche (**4**),[7] and 22-oxa-1,25$(OH)_2D_3$ (**5**).[8] The latter compound was the first analogue in which the cell-differentiating and calcemic activities of vitamin D were separated. It has a substantially higher cell-differentiating activity than the natural hormone **1**, but it has low calcemic activity. The comparison of some relevant biological activities for compounds **1**−**5** shown in Table 1 illustrates the possibility of discrimination of the various actions of vitamin D.

**Table 1.** Biological Activities of **1**−**5**[a]

| | binding | | differentiation | calcium |
|---|---|---|---|---|
| entry | VDR (pig) | DBP (human) | HL-60 | Ca serum (mice) |
| **1** | 100 | 100 | 100 | 100 |
| **2** | 88 | 0.2 | 3000 | 800 |
| **3** | 30 | 48 | 90 | 0.3 |
| **4** | 85 | 0 | 950 | 8 |
| **5** | 18 | 0 | 197 | 0.3 |

[a] The activities are presented as relative values, the reference value of 1,25$(OH)_2D_3$ (**1**) being defined as 100. Further details about the methodology are given in the Experimental Section. All data resulted from evaluation in this laboratory (Leuven).

The activity of vitamin D originates from a genomic pathway wherein the hormone binds to the intracellular vitamin D receptor (VDR) which regulates gene transcription and the synthesis of new proteins that are more directly responsible for the biological response.[9] Hence, the VDR−1,25$(OH)_2D_3$ complex, and in particular its conformation, plays a crucial role in vitamin D activity. Structure−function studies have demonstrated that the combined presence of a polar group in the terminal portion of the side chain, such as a (25*R*)- or (24*R*)-hydroxyl group, and a 1α-hydroxyl group is essential for activity. In this respect the structure of 1,25$(OH)_2D_3$ can be regarded as one consisting of a central rigid hydrophobic part, the CD-bicyclic ring system, to

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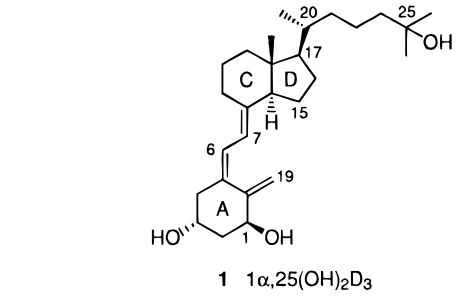
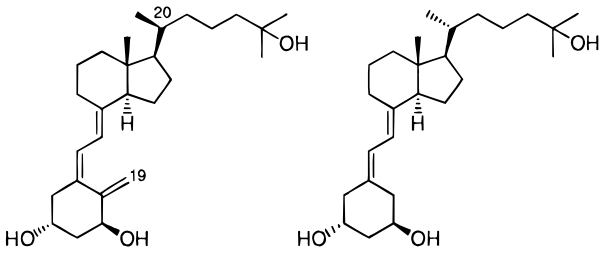
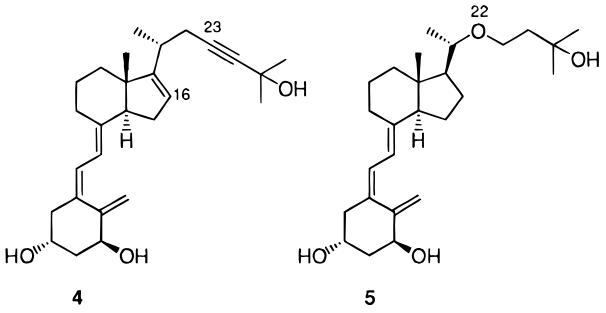


**1** 1α,25(OH)$_2$D$_3$



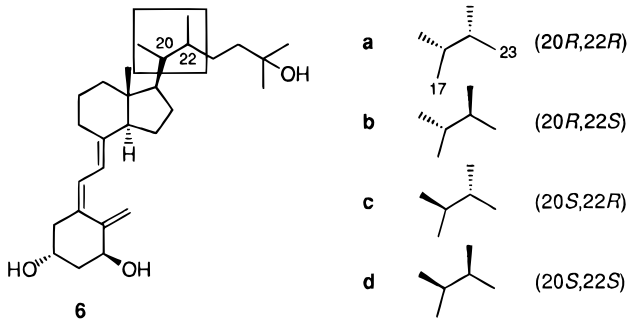
**2** **3**



**4** **5**

which are connected two flexible moieties, i.e., the side chain at C17 and the *seco*-B,A-ring part that is attached to the C-ring via a diene in which the 6,7-bond is freely rotatable. Up to 1995 the search for new analogues has mostly concentrated on derivatives possessing structural changes in the flexible parts of the molecule with a strong preference for the side chain; i.e., more than 278 were recorded in the nonpatent literature in the period 1985−1995.[4] Among these, several possess the inverted stereochemistry at C20, such as the 20-*epi* derivative **2**. Analogue **2** has been reported to be several orders of magnitude more effective than 1,25(OH)$_2$D$_3$ in the inhibition of cellular proliferation and induction of cell differentiation using the human histiocytic lymphoma cell line U937.[5] These observations have led to the claim that no vitamin D analogue study would be complete without an evaluation of both C20-epimeric series.[5b] As a logical corollary, theoretical studies have been directed at establishing a link between biological activity and the conformational profile of the side chain (vide infra).[10] In this context an interesting study of the conformation−function relationship of vitamin D has been recently reported based on the conformational profile of the side chain of the four 22-methyl-substituted diastereomers **6a**−**d** (Chart 1).[11] A few relevant biological activities are shown in Table 2, which indicate a pronounced differential behavior among these four analogues. Conformational studies further showed each of the four derivatives to exhibit preferred domains in space that are occupied by the side chain. Through further comparison with **1** and **2**, preferred side-chain

**Chart 1**



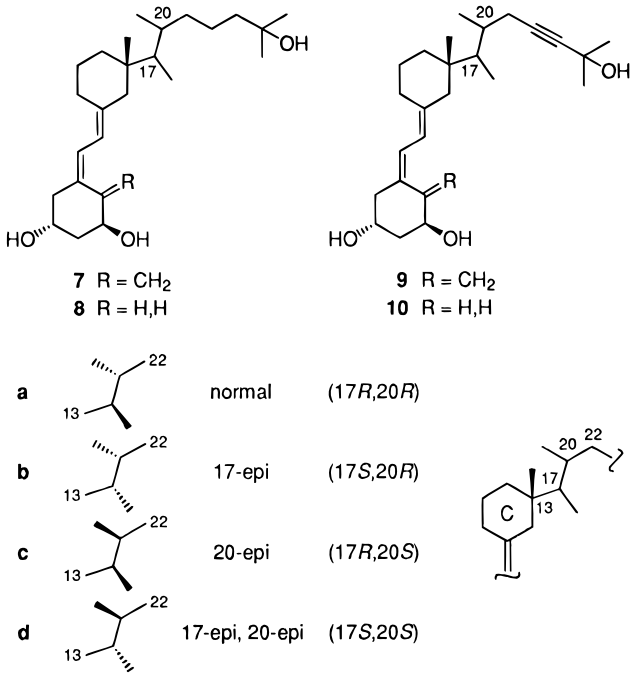
**6**

**Table 2.** Biological Activities of **6a**−**d**[a]

| | binding | | differentiation | calcium |
|---|---|---|---|---|
| entry | VDR (pig) | DBP (rat) | HL-60 | ICA (chick) |
| **1** | 100 | 100 | 100 | 100 |
| **6a** | 1.7 | 0.45 | 1.3 | 25 |
| **6b** | 33 | 67 | 100 | 50 |
| **6c** | 2000 (100) | 0.25 | 11900 (2000) | 50 (100) |
| **6d** | 1 (3) | 0.25 | (8) | (0.1) |

[a] The activities are presented as relative values, the reference value of 1,25(OH)$_2$D$_3$ (**1**) being defined as 100. Data taken from reference 11b. Values in parentheses have been obtained in this laboratory (Leuven).

**Chart 2**



**7** R = $CH_2$ **9** R = $CH_2$
**8** R = H,H **10** R = H,H

**a** normal (17*R*,20*R*)
**b** 17-epi (17*S*,20*R*)
**c** 20-epi (17*R*,20*S*)
**d** 17-epi, 20-epi (17*S*,20*S*)

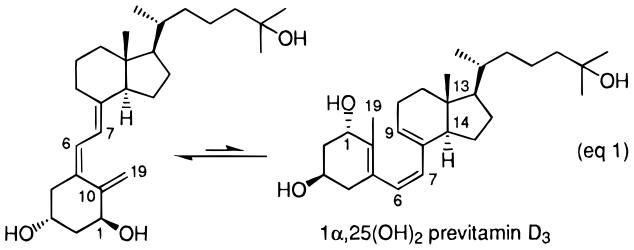
conformations of 1,25(OH)$_2$D$_3$ and its 20-epimer for binding to the VDR were proposed.

In the present paper we report on the synthesis, the biological activity, and the conformational study of the four vitamin D analogues **8a**−**d** (Chart 2), which are structurally characterized by the absence of the five-membered D-ring. Whereas such analogues can be formally identified as 14,16-*seco*-15-*nor* analogues, we rather prefer to focus on the remaining six-membered C-ring of the central part and will further use "6C analogues" as the general term. In a preliminary communication we have already reported on the synthesis of 6C analogues **7a**, **8a**, **9a**, and **10a**,[12] which show variations in the structure of the side chain, i.e., the

natural (**7**, **8**) and the 23-yne (**9**, **10**) side chains, and of the A-ring, i.e., the natural (**7**, **9**) and the corresponding 19-*nor* (**8**, **10**) A-rings. Rather than the *R*,*S*-stereonomenclature, which would vary following the priority convention, for the sake of clarity and consistency, the **a**−**d** designation as shown in Chart 2 will be used throughout the paper for identification of the stereochemical relationship. Also, carbon atoms of the vitamin D skeleton will be referred to according to the conventional steroid numbering.

This comprehensive study has led to two rather remarkable results: (1) in one diastereomeric series, i.e., the **a** series, analogues possess a superagonistic activity as compared to **1**, which is not only unexpected but also highly unusual in view of the greater flexibility of the D-*seco* derivative; (2) the important differences in activities observed in the four diastereomeric series **a**−**d** can be related to relative differences in the conformational profile of the corresponding side chain.

The reason 19-*nor* derivatives (cf. **8**) were developed has a fundamental origin that is related to the well-known thermal equilibrium between vitamin D and previtamin D (eq 1). The latter process constitutes one




of the two pericyclic reactions that intervene in the biosynthesis of vitamin D. The first process is the photochemically induced B-ring opening of 7,8-didehydrocholesterol (known as DHC or provitamin) which leads to previtamin D.[13] A subsequent 1,7-sigmatropic shift involving the 19-methyl hydrogens and position 9 leads to vitamin D in a folded conformation (not shown) characterized by the *s-cis* geometry at the 6,7-bond. Fast rotation at that bond eventually generates vitamin D in the preferred *s-trans* conformation, which also corresponds to the one that is usually depicted. The thermally induced equilibrium mixture of vitamin D and previtamin D contains the vitamin form as the major isomer (88:12 at 80 °C).[14] This is the result of subtle conformational preferences related to the unfavorable position of the previtamin's $\Delta^8$ bond in the *trans*-fused hydrindane system; hence, the vitamin form is preferred.[15] In the 6C analogues, however, one may expect the previtamin form, possessing a more substituted trienic moiety, to become more stable (eq 2). As will be shown later, this is indeed the case. Obviously, the

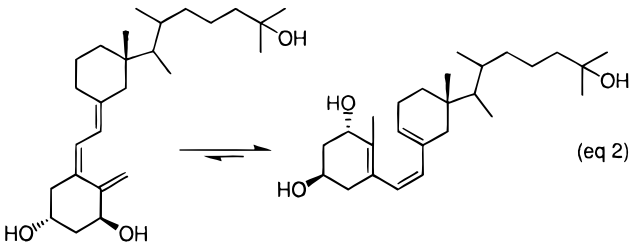
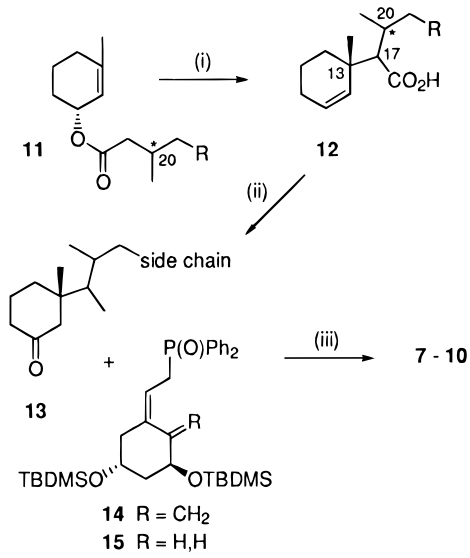



**Chart 3**



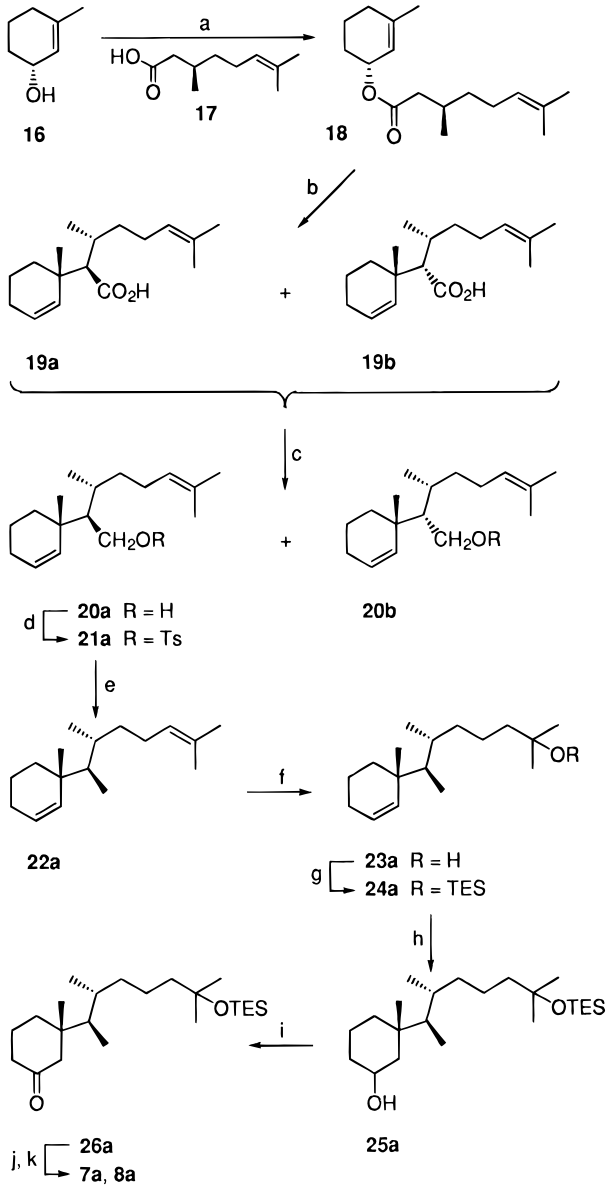

absence of the $\Delta^{10,19}$ exocyclic double bond as in the 19-*nor* derivatives **3**, **8**, and **10** prevents the further rearrangement to the corresponding previtamin. The 19-*nor*-1,25(OH)$_2$D$_3$ previtamin derivative has been shown to be devoid of vitamin D activity.[16]

## Synthesis

The synthesis of the analogues follows a modular strategy based on the following three stages (Chart 3). (i) Establishment of an adequately functionalized six-membered C-ring **12** via the Ireland−Claisen rearrangement[17] of the (*Z*)-enolate silyl ether derived from cyclohexenyl ester **11**: The creation of stereocenter C13 of **12** in the required configuration depends on the stereogenicity of stereocenter C8 in precursor **11**, while the configuration at C17 in **12** is established during the reaction. (ii) The obtained 3-substituted cyclohexene derivative **12** is further transformed into the C8 cyclohexanone derivative **13** in which the desired side chain has been constructed. (iii) The attachment of the A-ring, in its normal or 19-*nor* form, is performed using Lythgoe's strategy[18] involving Wittig−Horner reaction of phosphine oxide **14**[19] or **15**.[6,20] Final removal of the protecting groups then leads to the analogues **7**−**10**.

The synthesis of the 6C analogue **8a** is shown in Scheme 1. It follows the above modular strategy and has been the subject of a preliminary communication.[12] The route centers on the Ireland−Claisen rearrangement of ester **18**.[21] The latter is obtained via esterification of commercially available (*R*)-(+)-citronellic acid (**17**; 98% ee) with (*R*)-3-methyl-2-cyclohexen-1-ol (**16**). The synthesis of the latter proceeds via a sequence that has been previously developed for (*R*)-2-cyclohexenol and involves the enzymatic kinetic resolution of racemic 2-iodo-3-methyl-2-cyclohexen-1-ol as the key step (lipase PS, vinyl acetate, toluene, rt).[21] The Ireland−Claisen rearrangement is performed on the (*Z*)-silyl ketene acetal obtained from **18** through the use of HMPA as cosolvent (LDA, THF−HMPA). Thermal rearrangement of the corresponding TBDMS ether (18 h) leads, after acid workup, to a mixture of diastereomeric acids **19a** and **19b** (ratio >10:1; 58% yield), that could not be separated at this stage. After reduction with lithium

**Scheme 1**[a]

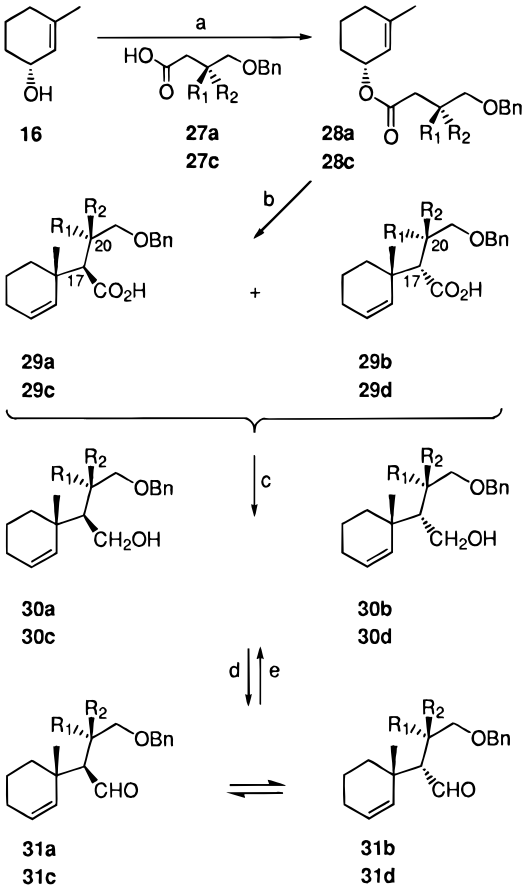



[a] (a) DCC, DMAP, $CH_2Cl_2$ (96%); (b) LDA, THF−HMPA, TBDMSCl, reflux, 16 h (58%); (c) $LiAlH_4$, THF (86%); (d) TsCl, pyridine (95%); (e) $LiAlH_4$, THF (95%); (f) $Hg(OAc)_2$, NaOH; $NaBH_4$ (67%); (g) $Et_3SiCl$, DMAP, imidazole, DMF (92%); (h) 9-BBN, $H_2O_2$ (95%); (i) PDC (80%); (j) **14** or **15**, *n*-BuLi, THF, −78 °C (with **14**: 88%; with **15**: 85%); (k) $Bu_4NF$, THF, rt (**7a**, **8a**: 93%).

aluminum hydride (LAH), the alcohol **20a** was obtained in pure form after HPLC separation. The stereochemical assignment is in line with a boatlike transition state that has been shown to prevail when the (*Z*)-enolate silyl ether is involved in the rearrangement of a six-membered cyclohexene derivative.[22] The same method has been used in the other series and will be discussed in detail later, including the establishment of the assignment via iodolactone formation.

The further conversion of alcohol **20a** into cyclohexanone **26a** involves three stages. (1) After conversion of the primary alcohol into the corresponding tosylate **21a**, the latter is reduced with LAH to yield **22a** (90% overall). (2) The introduction of the 25-hydroxyl group is performed via selective reaction of the trisubstituted side-chain double bond using mercuric acetate, sodium

**Scheme 2**[a]



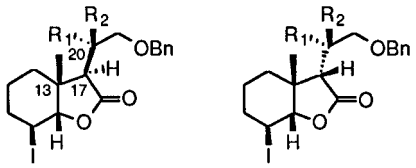

| | $R_1$ | $R_2$ |
|---|---|---|
| **a** | Me | H |
| **b** | Me | H |
| **c** | H | Me |
| **d** | H | Me |

[a] (a) DCC, DMAP, $CH_2Cl_2$ (**a**: 98%; **c**: 92%); (b) LDA, THF−HMPA, TBDMSCl, (**a**, **b**: 78%; **c**, **d**: 64%); (c) $LiAlH_4$, THF (**a**, **b**: 90%; **c**, **d**: 94%); (d) (i) Py·$SO_3$, $Et_3N$, DMSO, $CH_2Cl_2$ (**a**: 81%; **c**: 97%), (ii) DBU, $CH_2Cl_2$, rt; (e) $NaBH_4$, MeOH (**b**: 95%; **d**: 88%).

hydroxide, and sodium borohydride to give **23a** (67% yield), followed by protection as the triethylsilyl (TES) ether (92% yield). (3) Oxygenation of the cyclohexene ring of **24a** is effected by reaction with 9-BBN, followed by hydrogen peroxide (95% yield). This resulted in a diastereomeric mixture (46:54) of alcohols **25a** that was not further separated. Subsequent oxidation with PDC gave ketone **26a** in 80% yield. The introduction of the 19-*nor* A-ring was performed via Wittig−Horner reaction and led, after removal of the protecting groups by treatment with tetrabutylammonium fluoride (TBAF), to analogue **8a** in good yield.

The synthesis of the other analogues **8b**−**d** proceeded via a functionalized truncated side chain. The therefore required homochiral esters **28a** and **28c** (Scheme 2) were obtained via esterification of **16** with acids **27a** and **27c**, respectively. (*S*)-4-Benzyloxy-3-methylbutanoic acid (**27a**) was obtained from methyl (*R*)-3-hydroxy-2-methylpropionate[23] in 70% yield (99% ee), via tosylate displacement with sodium cyanide in DMSO, followed by nitrile hydrolysis with potassium hydroxide. The same reaction sequence starting from methyl (*S*)-3-

**Table 3.** $^1$H NMR Data (500 MHz, $CDCl_3$) of **32a**−**d**: Chemical Shift of H17 and Coupling Constant $^3J_{H17,H20}$



**32a** $R_1$ = Me; $R_2$ = H **32b**
**32c** $R_1$ = H; $R_2$ = Me **32d**

| | **a** | **b** | **c** | **d** |
|---|---|---|---|---|
| $\delta$ (ppm) | 2.55 | 2.85 | 2.82 | 2.47 |
| $J$ (Hz) | 6.8 | 2.9 | 2.9 | 7.5 |

hydroxy-2-methylpropionate led to the enantiomeric acid **27c**. As reported in a previous communication,[12] **27a** can also be obtained via treatment of the expensive (*S*)-3-methyl-$\gamma$-butyrolactone with benzyl alcohol under basic conditions.[24]

After esterification of **16** with homochiral acid **27a** (92% yield), ester **28a** was subjected to the Ireland−Claisen rearrangement sequence (LDA, THF−HMPA; TBDMSCl). This led to a 78% yield of acids **29a** and **29b** (ratio 14:1, respectively). The further sequence involved LAH reduction and HPLC separation of the resulting alcohols **30a** and **30b**.

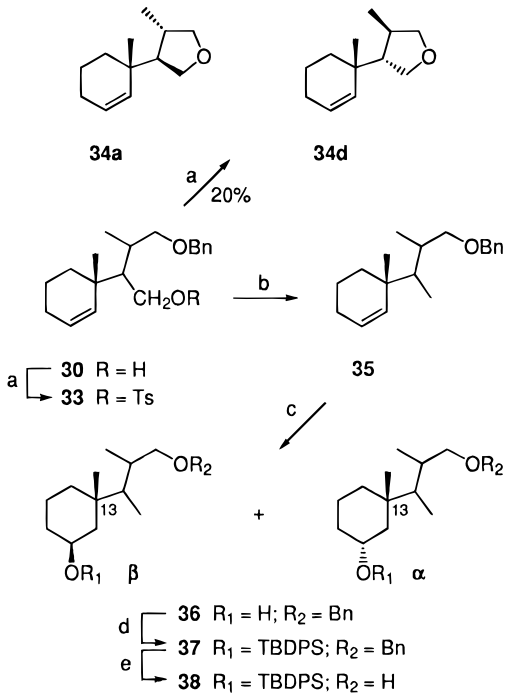
Since the available amount of **30b** was too small for further synthetic work and since variation of the conditions of the Ireland−Claisen rearrangement did not lead to substantial differences in the diastereomeric ratio, we had to recourse to the following sequence for obtaining larger amounts of **30b**. After oxidation of **30a** with sulfur trioxide pyridine complex in DMSO and dichloromethane, the obtained aldehyde **31a** was isomerized in base (DBU, $CH_2Cl_2$, rt, 62 h) to yield an equilibrium mixture of **31a** and **31b** (ratio 55:45, respectively). After reduction of the crude mixture (sodium borohydride, methanol, −10 °C) to the corresponding alcohols, pure **30b** was obtained through HPLC separation.

Using the same reaction conditions Ireland−Claisen rearrangement of diastereomeric ester **28c** led to **29c** and **29d** in a 29:1 ratio, respectively (64% yield). After LAH reduction and HPLC purification, the alcohol **30c** was obtained in 94% yield. In the same way as described above the diastereomeric alcohol **30d** was obtained after reduction of the mixture of aldehydes **31c** and **31d** (ratio 63:37) that resulted from the basic equilibration of **31c**.

The stereochemical assignment of alcohols **30a**−**d** rests on the $^1$H NMR analysis of the lactones **32a**−**d** that were obtained upon treatment of the acids **29** with iodine in acetonitrile. In the case of reaction of the alcohols **29a** and **29b**, the major isomer (**32a**) shows no NOE between Me-18 and H17, whereas a significant NOE is present in the other isomer (**32b**). In the case of **29c** and **29d** structural assignment rests on similar NOE experiments (Table 3) of the two iodo lactones **32c** and **32d**. Whereas **32c** was directly obtained from **29c**, **32d** resulted from iodolactonization of **29d** that was obtained through Jones oxidation of **30d**.

The further transformation of cyclohexenes **30** into the corresponding alcohols **38** is shown in Scheme 3. The reductive removal of the primary hydroxyl group proceeds via the corresponding tosylate **33** (LAH, THF). It is worth mentioning that in the case of **30a** and **30d**

**Scheme 3**[a]



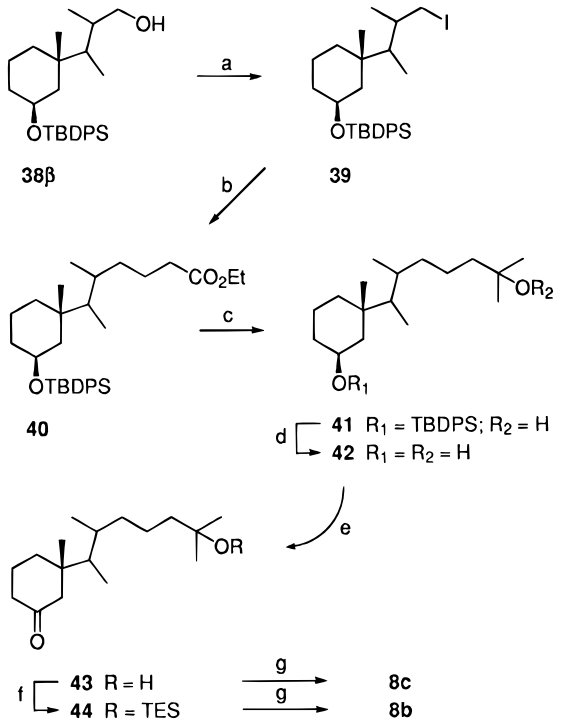
[a] (a) TsCl, pyridine (**a**: 80%; **b**: 100%; **d**: 78%); (b) $LiAlH_4$, THF (**a**: 95%; **b**: 80%; **c**: 89% from **30**; **d**: 80%); (c) 9-BBN, $H_2O_2$, NaOH, EtOH (**a**: 95%; **b**: 84%; **c**: 95%; **d**: 94%); (d) *t*-$BuPh_2SiCl$, DMAP, imidazole, DMF (**a**: 95%; **b**: 93%; **c**: 90%; **d**: 79%); (e) $Pd(OH)_2$, cyclohexene, EtOH (**a**: 85%; **b**: 88%; **c**: 90%; **d**: 85%).

tosylation (tosyl chloride, pyridine, 0 °C) also led to a minor fraction of the *trans*-disubstituted tetrahydrofuran cyclization products **34a** and **34d**, respectively (ca. 20%).[25]

The cyclohexenes **35a**−**d** are further transformed into the corresponding alcohols **38a**−**d** via a sequence involving: (i) treatment with 9-BBN and basic hydrogen peroxide to a mixture of diastereomeric alcohols **36**$\alpha$ and **36**$\beta$, which were not separated at this stage; (ii) protection of the secondary alcohol in **36** as *tert*-butyldiphenylsilyl (TBDPS) ether; (iii) debenzylation of **37** using palladium hydroxide on carbon (Pearlman's catalyst) in cyclohexene−ethanol. The separation of alcohols **38**$\alpha$ and **38**$\beta$ was successful in the case of the **a**−**c** series (HPLC). Although the stereogenicity at C8 is of no further importance since eventually the secondary alcohol will be oxidized, the $\alpha$,$\beta$-structural assignment was possible via difference NOE NMR. Upon irradiation of the methyl group at C13 a strong NOE is observed for the proton at C8 in the case of **38**$\alpha$ (**a**−**c**) while no NOE signal is observed in the case of **38**$\beta$.

As shown in Scheme 4, in the case of the **b** and **c** series the $\beta$-alcohols **38**$\beta$**b** and **38**$\beta$**c** were transformed into the corresponding iodides **39b** and **39c** (triphenylphosphine, iodine). In a following step, part of the side chain was introduced via a nickel-mediated conjugate addition.[26] The required Ni(0) complex was conveniently prepared in situ by simple heating to 60 °C of 1 equiv of nickel(II) chloride·$6H_2O$ with 5 equiv of zinc powder in the presence of ethyl acrylate in pyridine.[27] Addition of iodides **39b** and **39c** to the complex led to esters **40b** and **40c** in high yield. Subsequent Grignard reaction with excess methylmagnesium bromide led to **41** and after silyl deprotection to **42** in high yield.

**Scheme 4**[a]



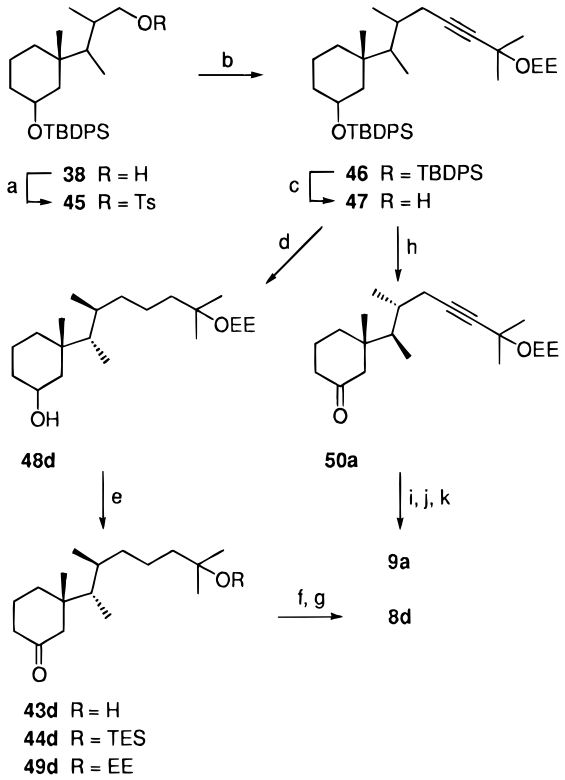

[a] (a) $I_2$, $PPh_3$, imidazole (**b**, **c**: 94%); (b) $NiCl_2$, Zn, $H_2C{=}CHCO_2Et$, pyridine (**b**: 88%; **c**: 91%); (c) MeMgBr, THF (**b**: 86%; **c**: 95%); (d) $Bu_4NF$, THF (**b**: 100%; **c**: 90%); (e) PDC, $CH_2Cl_2$ (**b**: 94%; **c**: 86%); (f) $Et_3SiCl$, DMAP, imidazole, DMF (**b**: 82%); (g) (i) **15**, *n*-BuLi, THF, −78 °C, (ii) $Bu_4NF$, THF (**b**: 100%; **c**: 85%).

Further oxidation gave hydroxy ketones **43b** and **43c**. In the **b** series, the hydroxyl group was protected as TES ether **44b** in view of the subsequent Wittig−Horner reaction with phosphine oxide **15**. Full deprotection using TBAF eventually led to **8b** and the corresponding *Z*-isomer (vide infra). In the **c** series Wittig−Horner condensation was directly performed on the free alcohol **43c** and led after desilylation to **8c**.

The analogue **8d** was obtained employing a different strategy (Scheme 5). The following steps were involved starting from the mixture of **38**α**d** and **38**β**d**: (i) substitution of the corresponding tosylate **45** with the sodium salt derived from the ethoxyethyl ether of 2-methyl-3-butyn-2-ol (NaH, DMSO);[28] (ii) desilylation (TBAF, THF) of **46d**; (iii) hydrogenation of **47d** using 5% $Rh/Al_2O_3$ in ethyl acetate (90%) to alcohol **48d**. Subsequent oxidation with PDC led to a mixture of **49d** and deprotected **43d**. The latter was silylated in situ using triethylsilyl triflate to **44d** and further condensed with the anion derived from phosphine oxide **15**. After full deprotection analogue **8d** was obtained in high yield (95%).

It is appropriate here to comment upon the stereoselectivity of the Wittig−Horner reaction, which is strongly (or even exclusively) in favor of the formation of the *E*-isomer. Only in the case of **44b** a substantial amount of the *Z*-isomer was isolated (ratio *E*/*Z* 7:3). Assignment of the *E*-configuration is based on the analysis of the $^1H$ NMR spectral data. There is an almost perfect correspondence between the data (shift and coupling patterns) that are related to the A,*seco*-B,C-ring part of the molecule in the case of **8a**, **8c**, and **8d** (see Experimental Section). Although spin decoupling ex-

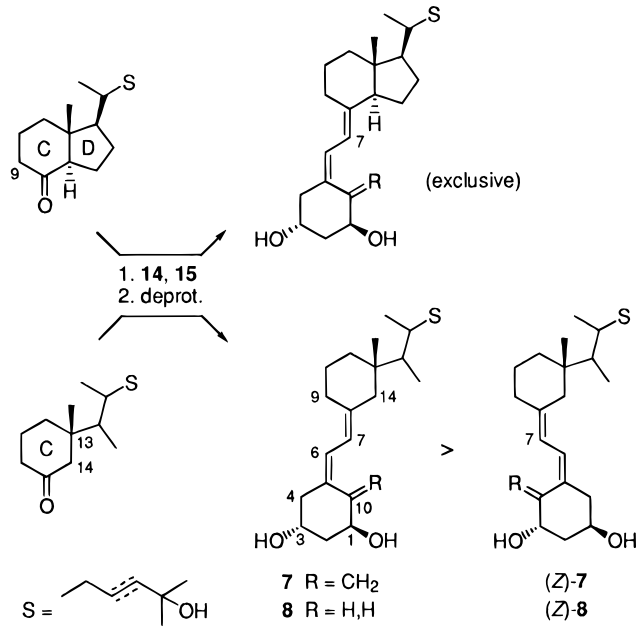
**Scheme 5**[a]



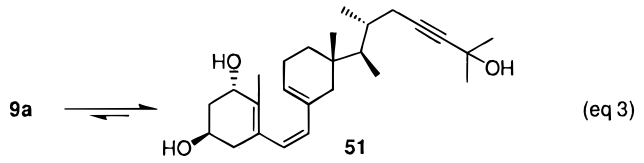

[a] (a) TsCl, pyridine (**d**: 93%); (b) $HC{\equiv}CC(CH_3)_2OEE$, NaH, DMSO (**d**: 57%); (c) $Bu_4NF$, THF (**d**: 94%); (d) $H_2$, 5% $Rh/Al_2O_3$, EtOAc (**d**: 90%); (e) PDC, $CH_2Cl_2$ (**d**: 90%); (f) **15**, *n*-BuLi, THF, −78 °C; (g) $Bu_4NF$, THF (**8d**: 92% from **44**); (h) PDC, $CH_2Cl_2$ (**a**: 85%); (i) **14**, *n*-BuLi, THF, −78 °C (**a**: 89%); (j) PPTS, $CH_2Cl_2$, rt; (k) $Bu_4NF$, THF (**9a**: 92% over 2 steps).

periments did not allow for the unambiguous assignment of the protons at C1 and/or C3 and at C4 and/or C10, the assignments that are given in the Experimental Section are based on the comparison with the corresponding spectral data that were obtained for 1α,-25-$(OH)_2$-2-methylene-19-norvitamin $D_3$ and that have been discussed in detail.[29] Quite surprisingly, NOE difference spectroscopy involving the vinylic proton at C7 did not allow for the distinction between the *E*- and *Z*-configuration of the 7,8-double bond. However, the downfield resonance of the equatorial (β) proton at C9 relative to the resonance of the proton at C14 is in accord with the *E*-configuration. This observation, together with the proven structure of the previtamin derivative **51** (vide infra), leaves no doubt about the preferred formation of the *E*-derivative in the coupling reaction. In the case of **8b** the structural identification rests on the comparison of the shift values observed for protons at C9 and C14 in both isomers (see Experimental Section). The preferred formation of the 7,8*E*-double bond is in line with the stereoselectivity that is observed in the Wittig−Horner reaction of the anion derived from phosphine oxide **14** with the intact *trans*-fused perhydrindanones (Chart 4).[19] Although the different steric environment of the carbonyl (cf. C9 and C14) is probably responsible for the latter observed selectivity, the above results suggest that the quaternary substitution at the farther removed center C13 could also be important. The reason this center is determining in the preferred formation of the *E*-derivatives (and less so in the case of the **b** series) certainly deserves further study.

**Chart 4**




The vitamin/previtamin $D_3$-like equilibrium (see eq 2) was followed for derivative **9a**. The synthesis of the latter is summarized in Scheme 5. It involves, starting from **38**$\beta$**a**, tosylation to **45**$\beta$**a**, introduction of the 23-yne side chain (**46**$\beta$**a**), desilylation to **47**$\beta$**a**, and PDC oxidation to yield cyclohexanone **50a**. The latter is the substrate for the classical Wittig−Horner reaction, which gave, after deprotection, the desired derivative **9a**. The 1,7-sigmatropic shift which generates **51** from **9a** (eq 3) was followed by $^1$H NMR (acetone-$d_6$, 24 °C). Starting from pure **9a** a 1:1 ratio was observed after




325 h; after 2 months the observed ratio amounted to 23:77 in favor of the previtamin derivative. Following the decrease in concentration over a period of 7 half-life values reveals a linear relation from which $k = 3.5 \times 10^{-7}$ s$^{-1}$ is computed.[30] We further note that the pattern observed for the olefinic proton at C9 in **51** ($\delta$ 5.67, m, $W_{1/2} = 8-9$ Hz) provides an indirect indication of the *E*-geometry of the 7,8-double bond in **9a** (cf. stereoselectivity of the Wittig−Horner reaction).

## Biological Evaluation

The biological evaluation of analogues **8a**−**d** has included: (1) determination of binding affinity for the porcine intestinal VDR and (2) for the human vitamin D binding protein (hDBP), (3) antiproliferative activity in vitro on MCF-7 and keratinocyte cell lines and cell-differentiating activity in vitro on HL-60 cell lines, and (4) calcemic activity in vivo in vitamin D-replete normal NMRI mice. Results are given in Table 4. The 6C analogues **8a**−**d** demonstrated low (3% as compared to $1,25(OH)_2D_3$) or no binding affinity for hDBP. Analogue **8a** showed 45% of the binding activity for pig VDR, whereas the affinities of the diastereomeric derivatives

**Table 4.** Biological Activities of **8a**−**d**[a]

| | binding | | cell differentiation and proliferation | | | calcium |
|---|---|---|---|---|---|---|
| entry | VDR (pig) | DBP (human) | HL-60 | MCF-7 | keratinocytes | Ca serum (mice) |
| **1** | 100 | 100 | 100 | 100 | 100 | 100 |
| **8a** | 45 | 3 | 1000 | 2000 | 5000 | 13 |
| **8b** | 8 | 0 | 75 | 75 | 60 | 0.25 |
| **8c** | 10 | 0 | 10 | 40 | 80 | 0.25 |
| **8d** | 9 | 0 | 70 | 90 | 85 | 25 |

[a] The activities are presented as relative values, the reference value of $1,25(OH)_2D_3$ (**1**) being defined as 100.

**8b**−**d** were ≤10% compared to that of $1,25(OH)_2D_3$ (=100% activity). Although **8a** possesses a lower binding affinity for the VDR and exhibits a greater flexibility than the natural hormone, this analogue demonstrates superagonistic antiproliferative and prodifferentiating effects (10−50-fold higher than that of $1,25(OH)_2D_3$). Derivatives **8b**−**d** were less potent than the parent compound in inhibiting cell proliferation or stimulating cell differentiation. Interestingly derivative **8c**, the C20 epimer of the "natural" analogue **8a**, showed decreased biological activity (50−100 times). This finding is in contrast with what has been observed in the case of 20-*epi*-$1,25(OH)_2D_3$ (**2**) that shows a 30-fold[5] increase in cell-differentiating activity. The calcemic activity of all these analogues was decreased compared to that of $1,25(OH)_2D_3$, in particular **8b** and **8c** demonstrated the lowest calcemic effects (400 times less calcemic than $1,25(OH)_2D_3$).

## Conformational Analysis

As mentioned in the Introduction, the *seco*-steroid **1** possesses three different parts:[31] a central rigid *trans*-fused CD-ring system to which are connected two flexible entities, the side chain and the six-membered A-ring attached to C8 via the *s-trans*-diene portion of the *seco*-B-ring. So, in a rough approximation the role of the central hydrophobic part of the molecule may consist in isolating the two flexible parts, each carrying one of the hydroxyl groups, i.e., the 1α-OH and 25- or 24-OH groups, that are essential for recognition by the receptor protein. Presumably the relative orientation of these two polar moieties relative to the central part—which itself probably fits in a hydrophobic pocket of the receptor protein—is essential in determining the pharmacophoric pattern of the molecule.

In view of its flexibility, uncovering the active shape of the natural hormone is a very difficult task. Indeed, it has been well-established that flexible ligands can undergo substantial geometrical distortions away from their calculated or X-ray structure in order to achieve a suitable binding conformation.[32] Since a greater preorganization of the active geometry is expected to result in enhanced binding, a logical step in the search for new active analogues is the development of molecules with reduced mobility in either one (or both) of the flexible parts. With regard to the side-chain portion of the molecule, this basically consists of constraining one or several of the five essentially free rotatable carbon−carbon bonds along the C17−C25 chain. Following this concept 23-yne (e.g. **9**, **10**) and aryl-substituted derivatives have been conceived.[33] In the same context, the synthesis, the biological activity, and the conformational analysis of the four possible 22-

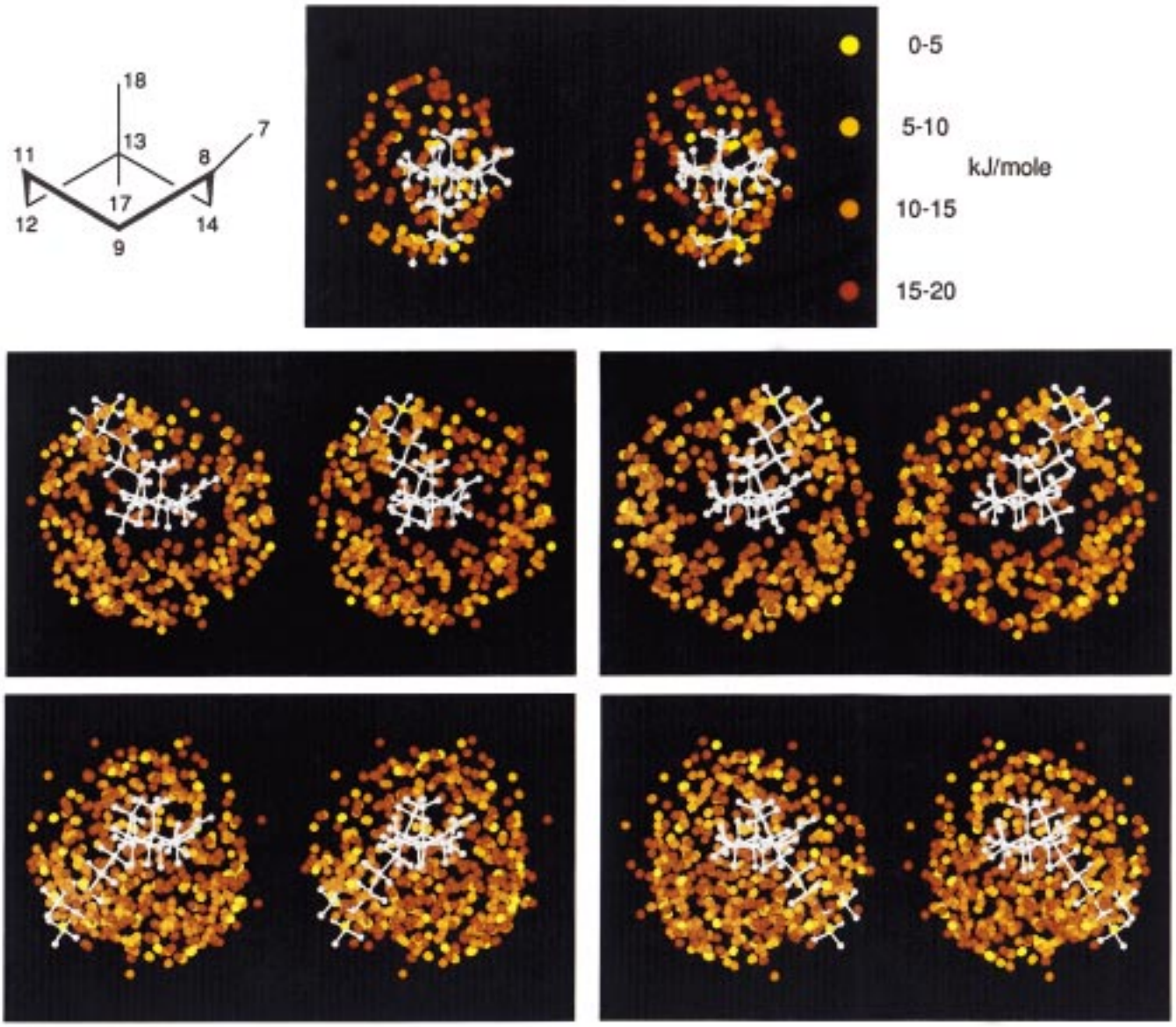



**Figure 1.** Stereoviews of the volume maps representing the conformational behavior of the side chain of $1,25(OH)_2D_3$ (**1**) and 6C analogues **8a**−**d** (truncated model); colored balls indicate positions of O25; energy window: 20 kJ/mol. (Top) Viewing direction and $1,25(OH)_2D_3$ (**1**) with color code. (Middle and bottom) Four diastereomeric 6C analogues **8a**−**d**: middle left, 17*R*,20*R* analogue **8a**; bottom left, 17*R*,20*S* analogue **8c**; middle right, 17*S*,20*S* analogue **8d**; bottom right, 17*S*,20*R* analogue **8b**.

methyl-substituted analogues **6a**−**d**, diastereomeric at C20 and C22, have been recently reported.[11] Among these four, analogue **6c** was found to possess the highest binding affinity for the VDR and a substantially higher potency than $1,25(OH)_2D_3$ (**1**) to induce differentiation of HL-60 cells.

In contrast to the case of **6**, where the introduction of an extraneous 22-methyl group reduces the mobility of the side chain, which is reflected by a rather well-defined and restricted occupation volume for the side chain,[11a] the present case deals with 6C analogues **8** that, by virtue of the absence of the *trans*-fused five-membered D-ring, exhibit a *greater* flexibility than the natural hormone. In this respect, the superagonistic activity of **8a** compared to **1** is remarkable. The development of analogues exhibiting profound structural changes in the CD-portion of the molecule was inspired by the search for derivatives that would exhibit a dissociation in the calcemic and other activities.[34] However, the discovery of analogues possessing superagonistic activity was unexpected. Interestingly, the result of the biological evaluation (Table 4) suggests that analogue **8a** may undergo the necessary geometrical changes to achieve a suitable binding conformation and that this particular geometry is not available for the diastereomeric derivatives **8b**−**d** (or at least substantially less). This hypothesis led us to examine in detail the conformational profile of the side chain in **8a**−**d** using molecular mechanics calculations. In this context, the Riverside group has used the *dot map* concept for defining the region in space that is available for occupation by the flexible side chain.[10] This has revealed in the case of the couples **1** and **2** and the 20-epimers of **5** substantial differences in conformational behavior, which might be determining in the rationalization of the observed differences in biological activity. In the same context, we have recently reported on the use of *volume maps* in the conformational analysis of vitamin D

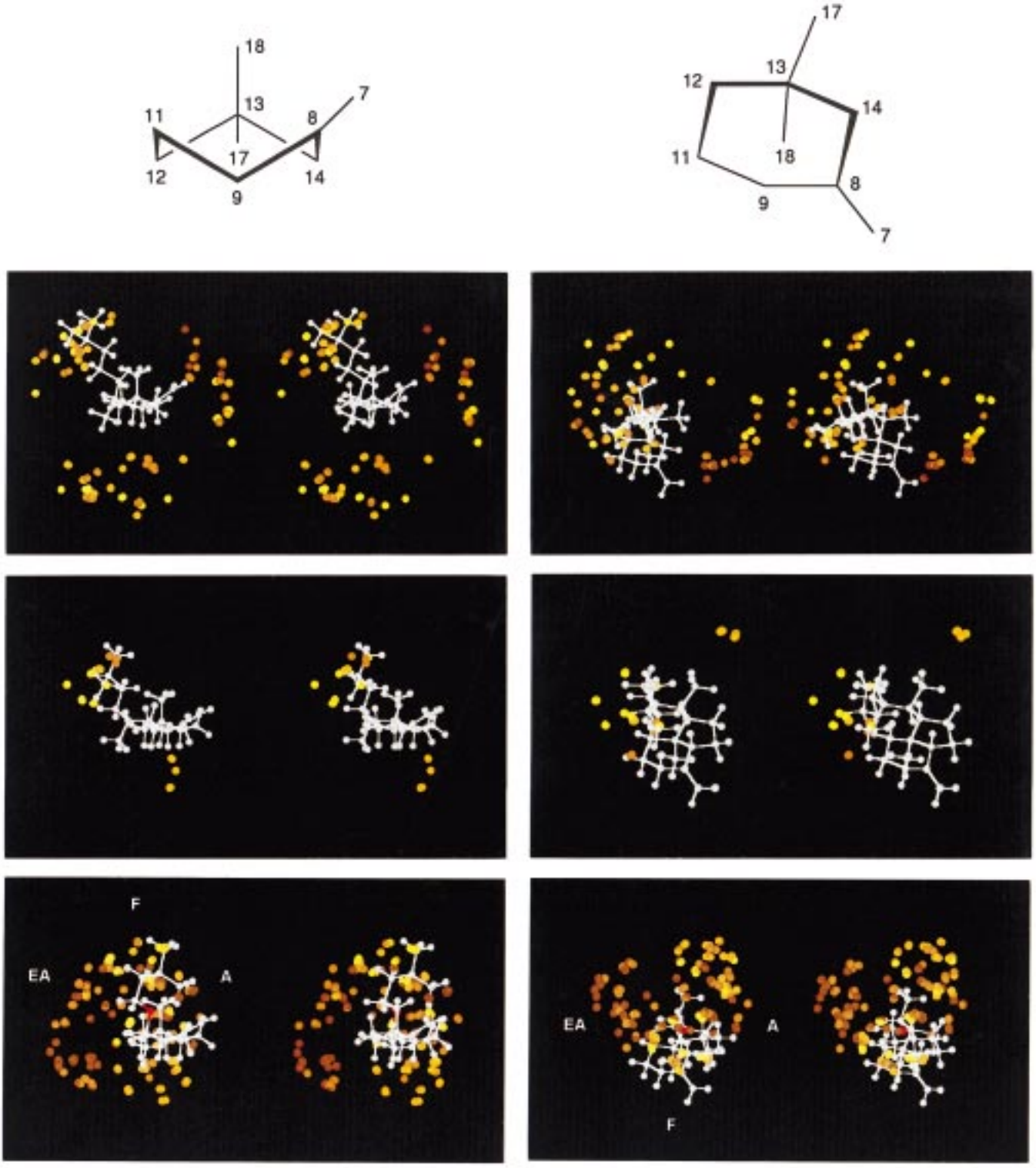



**Figure 2.** Stereoviews of the relative activity volumes determined for **8a** and **6c** and of the volume map of 22-oxa-1,25$(OH)_2D_3$ (**5**): left, view as used in Figure 1; right, view similar to the one used in refs 11a and 38. (Top) Relative activity volume resulting from subtraction of the 6C analogue **8c** from **8a** (volume maps of **8a** and **8c** shown in Figure 1). (Middle) Relative activity volume resulting from subtraction of the 22-methyl-substituted derivative **6d** from **6c** (volume maps of **6c** and **6d** not shown). (Bottom) 22-Oxa-1,25$(OH)_2D_3$ (**5**) with indication of the A-, EA-, and F-regions (see refs 11a and 38).

analogues.[35] In particular, volume maps constitute an extension of the dot map procedure with enhanced possibility for visualization of the conformational behavior of the side chain. Figure 1 shows the volume maps resulting from the analysis of the side chain of 1,25$(OH)_2D_3$ (**1**) and of derivatives **8a**−**d** taking into account all local minimum energy conformations found within 20 kJ/mol of the global minimum. In these maps the molecule is represented using a gray ball-and-stick model corresponding to the global minimum energy form viewed along an axis perpendicular to the C13−C18 bond in line with C9 in front (Figure 1, top left). Typical of the procedure is (1) the replacement of the dots used to indicate the positions of O25 by balls to create an

occupation volume for the side chain and (2) the use of colors to identify particular energy windows. Within the used color palette (yellow-brown) the four different nuances correspond to energy windows of 0−5, 5−10, 10−15, and 15−20 kJ/mol, respectively, the brightest color indicating the most stable forms (Figure 1, top right). It is immediately apparent that the flexibility of analogues **8a**−**d** (1013, 1375, 1408, and 1037 conformations, respectively) compared to, for example, the parent hormone **1** (366 conformations; Figure 1, top) is much greater and that their side chain can occupy regions in space not accessible to **1** (at least within the given energy window). Further inspection of the four maps reveals the pseudo-enantiomeric relationship that exists between **8a** and **8d** and between **8c** and **8b**, respectively.

We have also proposed a volume subtraction procedure for the determination of a *relative activity volume*, a region in space corresponding to the preferred occupation of the side chain of the more active analogue of a pair as compared to the inactive one.[36] To determine a relative activity volume for **8a**, the volume map of the least active **8c** was subtracted from the active **8a** (see Experimental Section for details). This resulted in the reduced volume map presented in Figure 2 in two stereoscopic views (top)[37] and which consists of 177 conformations of **8a** (17% of the original 1013 conformations (0−20 kJ/mol), 72 mol % according to a Boltzmann distribution at 298 K). It shows that within the given energy window the side chain of **8a** can occupy three regions in space which are not or less accessible to the side chain of **8c**. Subtraction of the volume maps of respectively **8b** and **8d** from the one of **8a** led to similar reduced volumes showing the same three regions, although less well-defined (not shown).

In applying the same procedure to the case of the 22-methyl-substituted derivatives **6**, we found that subtraction of the volume in space available to the inactive analogue **6d** from the space available for occupation by the side chain of the active analogue **6c** led to a rather reduced volume that could correspond to the orientation of the side chain in the active conformation of the compound. Stereoscopic images of the so-obtained relative activity volume of **6c**, formed by 38 conformations (18% of the original 210 conformations (0−20 kJ/mol), 88 mol % according to a Boltzmann distribution at 298 K) are shown in Figure 2 (middle).

In a recent study of side-chain analogues, Yamada and co-workers[38] found a strong correlation between the occupation of a region, denoted by the authors as the EA-region (see Figure 2, bottom), and the cell-differentiating potency of the analogue, the side chains of almost all potent analogues being distributed around this EA-region. They also defined a so-called F-region (more or less in front of the EA-region when viewed along the direction shown in Figure 2 on the right) that is typically occupied by the 25-oxygen of analogues possessing moderately high cell-differentiating activity and low calcemic activity, such as 22-oxa-1,25$(OH)_2D_3$ (**5**). The conformational profile of the latter analogue is also shown in Figure 2 (bottom). Comparison of the reduced volume map of **8a** with the reduced volume map of **6c** and with the F-region shown for **5** suggests that, of the three populations of side-chain conformations found in the reduced volume map of **8a**, the F-population could be responsible for the observed biological activity and could thus correspond to the orientation of the side chain in the active conformation of the compound.[37]

In conclusion, we have synthesized four conformationally flexible derivatives (**8a**−**d**) of 1,25$(OH)_2D_3$ (**1**), lacking C15 and C19 and diastereomeric at C17 and C20. Biological evaluation showed an unexpected superagonistic antiproliferative and prodifferentiating activity for diastereomer **8a** with the "natural" configuration at C17 and C20. The conformational behavior of the side chain of **8a**−**d** was studied using molecular mechanics calculations, and the result is presented as volume maps. From the analysis of the relative activity volume obtained by subtraction of the volume map of **8c** from **8a**, we suggest an orientation of the side chain of **8a** which might correspond to the active conformation of the compound.

## Experimental Section

**Synthesis.** All air-sensitive reactions were run under Ar or $N_2$ atmosphere, and reagents were added through septa using oven-dried syringes. $Et_2O$ and THF were distilled from benzophenone ketyl prior to use. Pyridine, $Et_3N$, *i*-$Pr_2NH$, $CH_3CN$, DMF, HMPA, and DMSO were distilled from $CaH_2$. Hexane was distilled from Na, and $CH_2Cl_2$ was distilled from $P_2O_5$. TLC were run on glass plates precoated with silica gel (Merck UV 254 Å). Column and flash chromatography was performed on silica gel (Merck, 70−325 or 230−400 mesh), and HPLC separations were performed on Bio-Sil D 90−10, 10-$\mu$m columns (Bio-Rad) of 1 × 25 cm and 2.2 × 25 cm. IR spectra were obtained on a Perkin-Elmer 1600 series FTIR spectrophotometer using KBr plates with product film or solution in $CH_2Cl_2$. NMR spectra were recorded in $CDCl_3$ (unless otherwise stated) on a Bruker AM-360 or AM-500 spectrometer and are reported relative to $CDCl_3$ ($\delta$ 7.26) as an internal standard. Mass spectra were recorded on a Finnigan 4000 or HP-5988 spectrometer at 70 eV. Optical rotations were measured on a Perkin-Elmer 241 polarimeter.

**(2*R*,3*R*)-3,7-Dimethyl-2-((1*S*)-1-methyl-2-cyclohexenyl)-6-octenoic Acid (19a) and (2*S*,3*R*)-3,7-Dimethyl-2-((1*S*)-1-methyl-2-cyclohexenyl)-6-octenoic Acid (19b).** To a stirred solution of (*R*)-(+)-citronellic acid (**17**; 0.98 g, 5.76 mmol) in $CH_2Cl_2$ (50 mL) were added (*R*)-3-methyl-2-cyclohexen-1-ol (**16**, 84% ee; 0.64 g, 5.76 mmol), DCC (2.96 g, 14.4 mmol), and DMAP (0.73 g, 6 mmol) at 0 °C under $N_2$ atmosphere. After 5 min the reaction mixture was allowed to warm to room temperature and was stirred for 18 h. EtOH (4 mL) and AcOH (4 mL) were added at 0 °C. The mixture was stirred at 0 °C for 20 min and at room temperature for 1 h. $Et_2O$ was added, and the formed white solid was removed by filtration. The filtrate was evaporated under reduced pressure, and the residual liquid was dissolved in $Et_2O$. The solution was washed with $H_2O$, dried over anhydrous $MgSO_4$, and evaporated under reduced pressure. The crude material was chromatographed on silica gel (hexanes−EtOAc 40:1) to give **18** (1.521 g, 96%).

To a stirred solution of *i*-$Pr_2NH$ (456 $\mu$L, 3.27 mmol) in THF (10 mL) was added *n*-BuLi (2.45 M solution in hexane, 1.33 mL, 3.27 mmol) at −15 °C under $N_2$ atmosphere. The reaction mixture was stirred for 20 min, HMPA (3 mL) was added, and the mixture was cooled to −78 °C. A solution of ester **18** (0.77 g, 2.92 mmol) in THF (2 mL) was slowly added, and after 10 min the reaction mixture was allowed to warm to −50 °C and stirred for 20 min. Solid TBDMSCl (491 mg, 3.27 mmol) was added, and stirring was continued for 20 min. The reaction mixture was allowed to warm to room temperature, stirred for 3 h, and then refluxed for 16 h. After cooling an aqueous 5% HCl solution (15 mL) was added; the mixture was stirred at room temperature for 1 h and extracted twice with $Et_2O$. The combined extracts were washed with $H_2O$, dried over

anhydrous $MgSO_4$, and evaporated under reduced pressure. The residual oil was chromatographed on silica gel (hexanes−EtOAc, 25:1 to 5:1) to give an unseparable mixture of **19a** and **19b** (>10:1; 448 mg, 58%): $R_f$ (hexanes−EtOAc, 5:1) 0.35; IR (film) 2930, 1704, 1462, 1381, 1285, 1253, 1202 $cm^{-1}$; $^1H$ NMR (360 MHz) $\delta$ 5.62 (1 H, m), 5.41 (1 H, d, $J$ = 11.0 Hz), 5.09 (1 H, t, $J$ = 6.9 Hz), 2.22 (1 H, d, $J$ = 5.9 Hz), 2.08 (1 H, m), 1.90 (3 H, m), 1.68 (3 H, s), 1.58 (3 H, s), 1.62 (7 H, m), 1.10 (3 H, s), 1.02 (3 H, d, $J$ = 6.8 Hz) ppm; MS $m/z$ 264 ($M^+$, 5), 249 (1), 221 (1), 208 (5), 154 (15), 109 (15), 96 (100). Anal. Calcd for $C_{17}H_{28}O_2$: C, 77.27; H, 10.61. Found: C, 77.37; H, 10.43.

**(2*R*,3*R*)-3,7-Dimethyl-2-((1*S*)-1-methyl-2-cyclohexenyl)-6-octen-1-ol (20a).** To a suspension of $LiAlH_4$ (302 mg, 7.95 mmol) in THF (10 mL) was added a solution of the above acids **19a** and **19b** (420 mg, 1.59 mmol) in THF (5 mL), and the reaction mixture was refluxed for 48 h. Excess $LiAlH_4$ was destroyed by addition of an aqueous 5% HCl solution, and the mixture was repeatedly extracted with $Et_2O$. The combined extracts were washed with $H_2O$, dried over anhydrous $MgSO_4$, and evaporated under reduced pressure. The residue was chromatographed on silica gel (hexanes−EtOAc, 20:1 to 10:1) to afford a mixture of **20a** and **20b** (>10:1; 344 mg, 86%) which was further separated by HPLC (hexanes−EtOAc, 6:1) to give pure **20a**: $R_f$ (hexanes−EtOAc, 5:1) 0.35; IR (film) 3339, 2928, 2871, 1454, 1376, 1028 $cm^{-1}$; $^1H$ NMR (500 MHz) $\delta$ 5.67 (1 H, dt, $J$ = 10.1, 3.5 Hz), 5.53 (1 H, dt, $J$ = 10.1, 1.8 Hz), 5.12 (1 H, tt, $J$ = 6.8, 1.6 Hz), 3.78 (1 H, m), 3.72 (1 H, m), 2.06 (1 H, m), 1.93 (2 H, m), 1.88 (1 H, tt, $J$ = 7.8, 7.8 Hz), 1.70 (2 H. m), 1.68 (3 H, s), 1.61 (2 H, m), 1.59 (3 H, s), 1.50 (1 H, m), 1.40 (1 H, ddd, $J$ = 10.5, 4.5, 1.2 Hz), 1.33 (1 H, m), 1.28 (2 H, m), 1.13 (1 H, m), 1.13 (3 H, d, $J$ = 7.0 Hz), 1.01 (3 H, s) ppm; MS $m/z$ 250 ($M^+$, 5), 232 (3), 219 (10), 137 (40), 95 (100).

**(2*R*,3*R*)-3,7-Dimethyl-2-((1*S*)-1-methyl-2-cyclohexenyl)-6-octene (22a).** To a solution of alcohol **20a** (250 mg, 1 mmol) in pyridine (15 mL) was added TsCl (572 mg, 3 mmol). The light yellow solution was stirred at room temperature for 17 h and then poured into ice water. The mixture was repeatedly extracted with $Et_2O$, and the combined extracts were washed with an aqueous 5% HCl solution and $H_2O$, dried over anhydrous $MgSO_4$, and evaporated under reduced pressure. The residue was chromatographed on silica gel (hexanes−EtOAc, 50:1 to 36:1) to give tosylate **21a** (>95% yield) contaminated with TsCl.

To a suspension of $LiAlH_4$ (342 mg, 9 mmol) in THF (30 mL) was added a solution of the above tosylate **21a** in THF (5 mL), and the reaction mixture was refluxed for 2 h. Excess $LiAlH_4$ was destroyed by addition of EtOH, and the mixture was repeatedly extracted with $Et_2O$. The combined extracts were washed with an aqueous 5% HCl solution and $H_2O$, dried over anhydrous $MgSO_4$, and evaporated under reduced pressure. The residue was chromatographed on silica gel (hexane) to give **22a** (222 mg, 95%): IR (film) 2925, 2865, 1647, 1453, 1378 $cm^{-1}$; $^1H$ NMR (500 MHz) $\delta$ 5.58 (1 H, dt, $J$ = 10.2, 3.5 Hz), 5.45 (1 H, dm, $J$ = 10.2 Hz), 5.12 (1 H, br t, $J$ = 7.0 Hz), 2.02 (1 H, m), 1.92 (2 H, m), 1.85 (1 H, tt, $J$ = 15.4, 7.9 Hz), 1.69 (1 H, m), 1.68 (3 H, s), 1.59 (3 H, s), 1.56 (2 H, m), 1.46 (1 H, m), 1.35 (1 H, m), 0.93 (3 H, s), 0.88 (3 H, d, $J$ = 6.8 Hz), 0.78 (3 H, d, $J$ = 7.1 Hz) ppm; MS $m/z$ 234 ($M^+$, 1), 57 (100).

**(6*R*,7*R*)-2,6,7-Trimethyl-7-((1*S*)-1-methyl-2-cyclohexenyl)heptan-2-ol (23a).** To a suspension of $Hg(OAc)_2$ (346 mg, 1.09 mmol) in $H_2O$ (1.25 mL) and THF (1.25 mL) was added a solution of alkene **22a** (212 mg, 0.906 mmol) in THF (2.5 mL), and the reaction mixture was stirred at room temperature for 2 h. A 3 M NaOH solution (1.09 mL) followed by a 1 M $NaBH_4$ solution in 3 M NaOH (1.09 mL) were added; the mixture was stirred for 10 min and then repeatedly extracted with $Et_2O$. The combined extracts were washed with brine, dried over anhydrous $MgSO_4$, and evaporated under reduced pressure. The residue was chromatographed on silica gel (hexanes−EtOAc, 20:1 to 5:1) to give alcohol **23a** (154 mg, 67%): IR (film) 3364, 2963, 2867, 1462, 1379, 1153 $cm^{-1}$; $^1H$ NMR (500 MHz) $\delta$ 5.58 (1 H, dt, $J$ = 10.3, 3.5 Hz), 5.46 (1 H, dm, $J$ = 10.3 Hz), 1.93 (2 H, m), 1.70 (1 H, m), 1.58 (4 H, m), 1.50−1.34 (5 H, m), 1.26 (2 H, m), 1.21 (6 H, s), 0.94 (3 H, s), 0.88 (3 H, d, $J$ = 7.0 Hz), 0.80 (3 H, d, $J$ = 7.1 Hz) ppm; MS $m/z$ 252 ($M^+$, 1), 95 (100). Anal. Calcd for $C_{17}H_{32}O$: C, 80.95; H, 12.70. Found: C, 81.10; H, 12.95.

**(3*S*)-3-Methyl-3-((1*R*,2*R*)-6-triethylsilyloxy-1,2,6-trimethylheptyl)cyclohexanone (26a).** To a solution of alcohol **23a** (50 mg, 0.2 mmol) in dry DMF (3 mL) were successively added $Et_3SiCl$ (90 mg, 0.6 mmol), imidazole (54 mg, 0.8 mmol), and DMAP (10 mg). The reaction mixture was stirred at room temperature for 20 h and then extracted with $Et_2O$. The combined extracts were washed with an aqueous 5% HCl solution, dried over anhydrous $MgSO_4$, and evaporated under reduced pressure. The residue was chromatographed on silica gel (hexanes−EtOAc, 98:2) to give **24a** (67 mg, 92%).

To a solution of cyclohexene **24a** (121 mg, 0.33 mmol) in THF (8 mL) was added 9-BBN (0.5 M solution in THF, 6.6 mL, 3.3 mmol), and the reaction mixture was refluxed for 30 h. EtOH (1 mL), a 6 M NaOH solution (0.8 mL), and a 30% $H_2O_2$ solution (1.6 mL) were added; the mixture was heated at 50 °C for 1 h and then extracted with $Et_2O$. The combined extracts were washed with an aqueous 5% HCl solution, dried over anhydrous $MgSO_4$, and evaporated under reduced pressure. The residue was chromatographed on silica gel (hexanes−EtOAc, 98:2) to give **25a** (121 mg, 95%).

A mixture of cyclohexanol **25a** (121 mg, 0.34 mmol) and PDC (480 mg, 1.2 mmol) in $CH_2Cl_2$ (9 mL) was stirred at room temperature for 20 h and then filtered through a short pad of silica gel. The crude product was purified by HPLC (hexanes−EtOAc, 20:1) to give **26a** (97 mg, 80%): IR (film) 2958, 1722, 1461, 1381, 1282, 1234, 1042 $cm^{-1}$; $^1H$ NMR (500 MHz) $\delta$ 2.28 (1 H, d, $J$ = 13.2 Hz), 2.26 (2 H, m), 2.06 (1 H, dt, $J$ = 13.2, 1.6 Hz), 1.90 (1 H, m), 1.83 (1 H, m), 1.67 (3 H, m), 1.18 (6 H, s), 0.93 (9 H, t, $J$ = 8.0 Hz), 0.90 (3 H, d, $J$ = 6.9 Hz), 0.86 (3 H, s), 0.79 (3 H, d, $J$ = 7.2 Hz), 0.56 (6 H, q, $J$ = 8.0 Hz) ppm; MS $m/z$ 354 ($M^+$, 10), 353 (5), 173 (30), 111 (60), 55 (100).

**Coupling Reaction of Cyclohexanones 26a, 43c, 44b, 44d, and 50a with Phosphine Oxides 14 and 15—General Procedure.** To a solution of A-ring phosphine oxide **14** (**15**) (0.134 mmol) in dry THF (1.5 mL) was dropwise added *n*-BuLi (2.5 M solution in hexane, 48 $\mu$L, 0.120 mmol) at −78 °C under $N_2$ atmosphere. The formed dark red solution was stirred at −78 °C for 1 h after which a solution of the C8 cyclohexanone (0.034 mmol) in dry THF (0.50 mL) was added. The still red solution was stirred at −78 °C for 2 h and was then allowed to warm to room temperature. The reaction mixture was directly loaded onto a silica gel column, and the reaction product was further purified by HPLC to give the A-ring coupled product.

**(1*R*,3*R*)-5-((*Z*,2*E*)-2-((3*S*)-3-((1*R*,2*R*)-6-Hydroxy-1,2,6-trimethylheptyl)-3-methylcyclohexylidene)ethylidene)-cyclohexane-1,3-diol (8a).** Cyclohexanone **26a** was coupled with phosphine oxide **15** according to the above general procedure. The crude product was purified by HPLC (hexanes−EtOAc, 200:1) to give protected **8a** (85%).

To a solution of protected **8a** (52 mg, 0.07 mmol) in THF (2 mL) was added TBAF (1 M solution in THF, 1.12 mL, 1.12 mmol); the mixture was stirred at room temperature for 14 h and then directly loaded onto a silica gel column ($CH_2Cl_2$−MeOH, 20:1). The product was further purified by HPLC ($CH_2Cl_2$−MeOH, 20:1) to give **8a** (26 mg, 93%): UV (MeOH) $\lambda_{max}$ 249.5 nm; IR (film) 3382, 2935, 1615, 1454, 1380, 1048 $cm^{-1}$; $^1H$ NMR (500 MHz) $\delta$ 6.26 (1 H, d, $J$ = 11.2 Hz, H-6), 5.94 (1 H, d, $J$ = 11.2 Hz, H-7), ~4.09 (2 H, m, H-1 and H-3), 2.67 (1 H, dd, $J$ = 13.3, 3.8 Hz, H-10$\beta$), 2.49 (1 H, dd, $J$ = 13.3, 3.7 Hz, H-4$\alpha$), 2.43 (1 H, m, H-9$\beta$), 2.29 (1 H, dd, $J$ = 13.3, 7.6 Hz, H-10$\alpha$), 2.19 (1 H, dd, $J$ = 13.3, 6.6 Hz, H-4$\beta$), 2.04 (1 H, d, $J$ = 13.0 Hz, H-14$\beta$), 2.00 (1 H, m), 1.90 (1 H, m), 1.86 (1 H, m), 1.84 (1 H, d, $J$ = 13.0 Hz, H-14$\alpha$), 1.70 (1 H, m), 1.56 (3 H, m), 1.21 (6 H, s), 0.89 (3 H, d, $J$ = 6.9 Hz), 0.77 (3 H, d, $J$ = 7.3 Hz), 0.76 (3 H, s) ppm; MS $m/z$ 392 ($M^+$, 1).

**(1*R*)-3-Methyl-2-cyclohexenyl (3*S*)-4-Benzyloxy-3-methylbutanoate (28a) and (1*R*)-3-Methyl-2-cyclohexenyl (3*R*)-4-Benzyloxy-3-methylbutanoate (28c).** Esterification of (*R*)-3-methyl-2-cyclohexen-1-ol (**16**, 80% ee) with (*S*)-(−)-4-benzyloxy-3-methylbutanoic acid (**27a**, 99% ee) was carried out

as described for the preparation of **18**. Column chromatography on silica gel (hexanes−EtOAc, 75:1 to 25:1) followed by HPLC (hexanes−EtOAc, 25:1) gave **28a** (98%) as a colorless oil. In an analogous way esterification of **16** with (*R*)-(−)-4-benzyloxy-3-methylbutanoic acid (**27c**, 99% ee) afforded **28c** (92%). Data of **28a**: $R_f$ (hexanes−EtOAc, 5:1) 0.50; $[\alpha]_D$ = +86.7 (*c* 0.96, $CHCl_3$); IR (film) 3067, 3030, 2924, 2864, 1726, 1672, 1496, 1453, 1377, 1249, 1181, 1097, 1026 $cm^{-1}$; $^1H$ NMR (500 MHz) $\delta$ 7.33 (5 H, m), 5.44 (1 H, m), 5.25 (1 H, m), 4.50 (2 H, s), 3.36 (1 H, dd, *J* = 9.2, 5.9 Hz), 3.31 (1 H, dd, *J* = 9.2, 7.2 Hz), 2.48 (1 H, dd, *J* = 15.0, 5.9 Hz), 2.32 (1 H, m), 2.14 (1 H, dd, *J* = 15.0, 8.0 Hz), 1.95 (2 H, m), 1.74 (2 H, m), 1.70 (3 H, s), 1.65 (2 H, m), 0.99 (3 H, d, *J* = 6.8 Hz) ppm; MS *m*/*z* 302 ($M^+$, 2), 273 (2), 247 (1), 207 (12), 192 (3), 181 (2), 149 (3), 111 (88), 95 (50), 91 (100). Data of **28c**: $R_f$ (hexanes−EtOAc, 5:1) 0.50; $[\alpha]_D$ = +100.4 (*c* 1.01, $CHCl_3$); IR (film) 3056, 3029, 2934, 2865, 1727, 1671, 1496, 1454, 1378, 1248, 1111, 1096 $cm^{-1}$; $^1H$ NMR (500 MHz) $\delta$ 7.33 (5 H, m), 5.43 (1 H, m), 5.24 (1 H, m), 4.51 (1 H, *A*Bd, *J* = 12.4 Hz), 4.49 (1 H, A*B*d, *J* = 12.4 Hz), 3.35 (1 H, dd, *J* = 9.1, 5.9 Hz), 3.30 (1 H, dd, *J* = 9.1, 6.8 Hz), 2.47 (1 H, dd, *J* = 12.0, 6.1 Hz), 2.31 (1 H, m), 2.15 (1 H, dd, *J* = 14.9, 7.9 Hz), 1.94 (2 H, m), 1.74 (2 H, m), 1.70 (3 H, s), 1.65 (2 H, m), 0.99 (3 H, d, *J* = 6.8 Hz) ppm; MS *m*/*z* 302 ($M^+$, 2), 273 (2), 247 (1), 207 (12), 192 (3), 181 (2), 149 (3), 111 (88), 95 (50), 91 (100). Anal. Calcd for $C_{19}H_{26}O_3$: C, 75.46; H, 8.67. Found: C, 75.43; H, 8.80.

**(2*R*,3*S*)-4-Benzyloxy-3-methyl-2-((1*S*)-1-methyl-2-cyclohexenyl)butanoic Acid (29a) and (2*R*,3*R*)-4-Benzyloxy-3-methyl-2-((1*S*)-1-methyl-2-cyclohexenyl)butanoic Acid (29c).** The Ireland−Claisen rearrangement of ester **28a** was carried out according to the procedure described for **18**. The crude material was purified by HPLC (hexanes−EtOAc, 5:1) to give a mixture of **29a** and **29b** (ratio 14:1, 78%) as a colorless oil. In an analogous way **28c** gave a mixture of **29c** and **29d** (ratio 29:1, 64%). Data of **29a**: $R_f$ (hexanes−EtOAc, 5:1) 0.23; IR (film) 3500−3200, 3056, 3015, 2931, 2862, 1700, 1646, 1615, 1451, 1421, 1369, 1256, 1200, 1154, 1097, 1026 $cm^{-1}$; $^1H$ NMR (500 MHz) $\delta$ 7.33 (5 H, m), 5.63 (1 H, ddd, *J* = 9.0, 4.8, 2.7 Hz), 5.40 (1 H, m), 4.50 (2 H, s), 3.52 (1 H, dd, *J* = 9.4, 3.8 Hz), 3.42 (1 H, dd, *J* = 9.4, 6.1 Hz), 2.39 (1 H, d, *J* = 4.3 Hz), 2.18 (1 H, m), 1.94 (1 H, m), 1.90 (1 H, ddd, *J* = 8.7, 5.6, 2.7 Hz), 1.14 (3 H, d, *J* = 7.2 Hz), 1.11 (3 H, s) ppm; MS *m*/*z* 302 ($M^+$, 1), 253 (1), 221 (3), 206 (4), 167 (2), 149 (8), 111 (12), 95 (95), 91 (100). Data of **29c**: $R_f$ (hexanes−EtOAc, 5:1) 0.23; IR (film) 3500−2500, 3056, 3027, 2935, 2873, 1704, 1496, 1454, 1416, 1366, 1256, 1206, 1102 $cm^{-1}$; $^1H$ NMR (500 MHz) $\delta$ 7.33 (5 H, m), 5.64 (1 H, ddd, *J* = 10.1, 7.7, 3.2 Hz), 5.43 (1 H, br d, *J* = 10.3 Hz), 4.55 (1 H, *A*Bd, *J* = 13.0 Hz), 4.51 (1 H, A*B*d, *J* = 13.0 Hz), 3.46 (1 H, dd, *J* = 9.1, 5.7 Hz), 3.40 (1 H, dd, *J* = 9.1, 5.6 Hz), 2.53 (1 H, d, *J* = 3.2 Hz), 2.19 (1 H, m), 1.93 (2 H, m), 1.77 (1 H, m), 1.64 (3 H, m), 1.13 (3 H, s), 1.05 (3 H, d, *J* = 7.0 Hz) ppm; MS *m*/*z* 302 ($M^+$, 1), 253 (1), 221 (3), 206 (4), 167 (2), 149 (8), 111 (12), 95 (95), 91 (100).

**(2*R*,3*S*)-4-Benzyloxy-3-methyl-2-((1*S*)-1-methyl-2-cyclohexenyl)butan-1-ol (30a) and (2*R*,3*R*)-4-Benzyloxy-3-methyl-2-((1*S*)-1-methyl-2-cyclohexenyl)butan-1-ol (30c).** To a suspension of $LiAlH_4$ (628 mg, 16.56 mmol) in THF (40 mL) was added a solution of the mixture of acids **29a** and **29b** (14:1; 1.0 g, 3.31 mmol) in THF (20 mL), and the reaction mixture was refluxed for 43 h. Excess $LiAlH_4$ was destroyed by addition of an aqueous 5% HCl solution, and the mixture was repeatedly extracted with $Et_2O$. The combined extracts were washed with $H_2O$ and brine, dried over anhydrous $MgSO_4$, and evaporated under reduced pressure. The residue was chromatographed on silica gel to afford a mixture of **30a** and **30b** (0.91 g, 95%) which was further separated by HPLC (hexanes−EtOAc, 5:1) to give pure **30a**. In an analogous way the mixture of acids **29c** and **29d** led to **30c** and **30d** (96%), and further HPLC separation gave pure **30c**. Data of **30a**: $R_f$ (hexanes−EtOAc, 2:1) 0.42; $[\alpha]_D$ = +25.2, $[\alpha]_{365}$ = +78.6 (*c* 3.98, $CHCl_3$); IR (film) 3440, 3088, 3064, 3011, 2929, 2865, 1489, 1463, 1448, 1363, 1205, 1097, 1069, 1027 $cm^{-1}$; $^1H$ NMR (500 MHz) $\delta$ 7.33 (5 H), m), 5.62 (1 H, ddd, *J* = 9.0, 4.4, 3.0 Hz), 5.43 (1 H, d, *J* = 10.2 Hz), 4.56 (1 H, *A*Bd, *J* = 11.9 Hz), 4.51 (1 H, A*B*d, *J* = 11.9 Hz), 3.80 (1 H, ddd, *J* = 12.0, 8.2, 3.7 Hz), 3.73 (1 H, ddd, *J* = 12.0, 8.1, 5.6 Hz), 3.70 (1 H, ddd, *J* = 11.4, 5.8, 3.2 Hz), 3.54 (1 H, ddd, *J* = 11.4, 8.0, 5.4 Hz), 2.08 (1 H, m), 1.90 (2 H, m), 1.49 (1 H, ddd, *J* = 5.7, 3.6, 2.1 Hz), 1.13 (3 H, d, *J* = 7.4 Hz), 1.03 (3 H, s) ppm; MS *m*/*z* 288 ($M^+$, 1), 257 (1), 191 (1), 176 (5), 161 (5), 135 (4), 121 (7), 107 (8), 95 (100). Data of **30c**: $R_f$ (hexanes−EtOAc, 2:1) 0.42; $[\alpha]_D$ = −2.53, $[\alpha]_{365}$ = −8.59 (*c* 0.40, $CHCl_3$); IR (film) 3420, 3056, 3016, 2956, 2938, 2865, 1453, 1363, 1273, 1092, 1045, 1027 $cm^{-1}$; $^1H$ NMR (500 MHz) $\delta$ 7.33 (5 H, m), 5.62 (1 H, ddd, *J* = 10.2, 3.5, 3.5 Hz), 5.39 (1 H, d, *J* = 10.2 Hz), 4.57 (1 H, *A*Bd, *J* = 12.4 Hz), 4.52 (1 H, A*B*d, *J* = 12.4 Hz), 3.74 (1 H, dd, *J* = 10.8, 3.1 Hz), 3.56 (1 H, dd, *J* = 10.1, 9.2 Hz), 3.45 (1 H, dd, *J* = 8.7, 4.0 Hz), 3.35 (1 H, dd, *J* = 8.7, 3.6 Hz), 2.15 (1 H, m), 1.92 (2 H, m), 1.60 (4 H, m), 1.09 (3 H, d, *J* = 7.3 Hz), 1.03 (3 H, s) ppm; MS *m*/*z* 288 ($M^+$, 1), 257 (1), 191 (6), 176 (5), 161 (5), 135 (4), 121 (7), 107 (8), 95 (100).

**Isomerization Sequence of Alcohol 30a to 30b and 30c to 30d.** To a solution of **30a** (300 mg, 1.04 mmol) in DMSO (8 mL) and $CH_2Cl_2$ (4 mL) were added $Et_3N$ (527 $\mu$L) and sulfur trioxide pyridine complex (497 mg, 3.13 mmol) at −15 °C. After stirring for 1.5 h at −10 to −4 °C, the reaction mixture was poured into $Et_2O$ and brine. The organic phase was dried over anhydrous $MgSO_4$ and evaporated under reduced pressure. The residue was purified by HPLC (hexanes−EtOAc, 10:1) to afford aldehyde **31a** (242 mg, 81%) as a colorless oil. In an analogous way **30c** gave aldehyde **31c** (97%).

To a solution of **31a** (230 mg, 0.8 mmol) in $CH_2Cl_2$ (20 mL) was added DBU (915 $\mu$L, 5.9 mmol). The reaction mixture was stirred at room temperature for 62 h and concentrated under reduced pressure, and the residue was dissolved in $Et_2O$. The solution was washed with brine, dried over anhydrous $MgSO_4$, and evaporated under reduced pressure. The residue was chromatographed on silica gel to give a mixture of **31a** and the epimeric aldehyde **31b** (ratio 55:45, 225 mg, 98%). In an analogous way **31c** led to a mixture of aldehydes **31c** and **31d** (ratio 63:37, 100%).

To a solution of the aldehyde mixture **31a** and **31b** (240 mg, 0.9 mmol) in MeOH (4.5 mL) was portionwise added $NaBH_4$ (136 mg, 3.6 mmol) at −10 °C, and stirring was continued for 15 min. Brine was added, and the mixture was extracted with $Et_2O$. The combined organic extracts were dried over anhydrous $MgSO_4$ and evaporated under reduced pressure. The residual material was purified by HPLC (hexanes−EtOAc, 5:1) to give **30a** and **30b** (227 mg, 95%) which were further separated to give pure **30b**. In an analogous way **31c** and **31d** led to a mixture of **30c** and **30d** (88%) and further separation to pure **30d**. Data of **30b**: $R_f$ (hexanes−EtOAc, 2:1) 0.42; $[\alpha]_D$ = +24.7, $[\alpha]_{365}$ = +79.0 (*c* 0.33, $CHCl_3$); IR (film) 3410, 3062, 3011, 2926, 2857, 1649, 1598, 1456, 1362, 1090, 1044 $cm^{-1}$; $^1H$ NMR (500 MHz) $\delta$ 7.33 (5 H, m), 5.62 (1 H, ddd, *J* = 9.0, 3.0, 4.5 Hz), 5.46 (1 H, br d, *J* = 10.2 Hz), 4.57 (1 H, *A*Bd, *J* = 12.0 Hz), 4.52 (1 H, A*B*d, *J* = 12.0 Hz), 3.70 (1 H, m), 3.59 (1 H, m), 3.49 (1 H, dd, *J* = 8.8, 4.1 Hz), 3.40 (1 H, dd, *J* = 9.1, 3.0 Hz), 2.19 (1 H, dd, *J* = 7.8, 3.7 Hz), 1.90 (2 H, m), 1.60 (2 H, m), 1.50 (2 H, m), 1.39 (1 H, m), 1.06 (3 H, d, *J* = 7.3 Hz), 1.00 (3H, s) ppm; MS *m*/*z* 288 ($M^+$, 1), 257 (1), 191 (6), 176 (5), 161 (5), 135 (4), 121 (7), 107 (8), 95 (100). Data of **30d**: $R_f$ (hexanes−EtOAc, 2:1) 0.42; $[\alpha]_D$ = +15.88, $[\alpha]_{365}$ = +54.90 (*c* 0.59, $CHCl_3$); IR (film) 3400, 3062, 3011, 2929, 2857, 1593, 1455, 1362, 1095, 1045 $cm^{-1}$; $^1H$ NMR (500 MHz) $\delta$ 7.33 (5 H, m), 5.60 (1 H, ddd, *J* = 10.4, 4.5, 2.8 Hz), 5.00 (1 H, br d, *J* = 10.4 Hz), 4.55 (1 H, *A*Bd, *J* = 11.9 Hz), 4.50 (1 H, A*B*d, *J* = 11.9 Hz), 3.74 (1 H, m), 3.72 (1 H, dd, *J* = 6.3, 3.1 Hz), 3.70 (1 H, dd, *J* = 6.5, 3.2 Hz), 3.53 (1 H, ddd, *J* = 7.0, 4.3, 1.1 Hz), 2.10 (1 H, s), 1.90 (2 H, m), 1.58 (4 H, m), 1.17 (3 H, d, *J* = 7.4 Hz), 1.00 (3 H, s) ppm; MS *m*/*z* 288 ($M^+$, 1), 257 (1), 191 (6), 176 (5), 161 (5), 135 (4), 121 (7), 107 (8), 95 (100).

**(2*S*,3*R*)-4-Benzyloxy-3-methyl-2-((1*S*)-1-methyl-2-cyclohexenyl)butanoic Acid (29d).** To a stirred, ice-cooled solution of alcohol **30d** (40 mg, 0.14 mmol) in acetone (1 mL) was added dropwise Jones reagent (162 $\mu$L) until the mixture turned red-brown. Excess reagent was destroyed by the addition of an aqueous 10% $NaHSO_3$ solution, and the mixture

was extracted with $Et_2O$. The combined extracts were dried over anhydrous $MgSO_4$ and evaporated under reduced pressure. The residue was purified by HPLC (hexanes−EtOAc, 5:1) to give **29d** (25 mg, 60%) as a colorless oil. Alcohol **30b** gave in a similar way acid **29b**. Data of **29d**: $R_f$ (hexanes−EtOAc, 5:1) 0.23; IR (film) 3500−2500, 3056, 3027, 2935, 2873, 1704, 1496, 1454, 1416, 1366, 1256, 1206, 1102 $cm^{-1}$; $^1H$ NMR (500 MHz) $\delta$ 7.33 (5 H, m), 5.74 (1 H, br d, $J$ = 10.3 Hz), 5.63 (1 H, dt, $J$ = 10.3, 3.5 Hz), 4.49 (1 H, *A*Bd, $J$ = 12.0 Hz), 4.45 (1 H, A*B*d, $J$ = 12.0 Hz), 3.64 (1 H, *A*Bd, $J$ = 9.3, 3.4 Hz), 3.38 (1 H, A*B*d, $J$ = 9.3, 6.9 Hz), 2.36 (1 H, d, $J$ = 5.2 Hz), 2.20 (1 H, m), 1.95 (1 H, m), 1.93 (1 H, m), 1.68 (1 H, m), 1.64 (1 H, m), 1.63 (1 H, m), 1.19 (3 H, d, $J$ = 7.0 Hz) 1.12 (3 H, s) ppm; MS *m*/*z* 302 ($M^+$, 1), 253 (1), 221 (3), 206 (4), 167 (2), 149 (8), 111 (12), 95 (95), 91 (100).

**(3*R*,3a*R*,7*S*,7a*S*)-3-((1*S*)-2-Benzyloxy-1-methylethyl)-7-iodo-3a-methylperhydrobenzo[*b*]furan-2-one (32a).** A solution of acid **29a** (45.3 mg, 0.15 mmol) and $I_2$ (76.2 mg, 0.30 mmol) in $CH_3CN$ (0.5 mL) was stirred in the dark at room temperature for 24 h. $Et_2O$ was added, and the organic layer was washed with a saturated aqueous $Na_2SO_3$ solution (2×) and a saturated aqueous $NaHCO_3$ solution (2×), dried over anhydrous $MgSO_4$, and evaporated under reduced pressure. Column chromatography on silica gel afforded iodo lactone **32a** (55.4 mg, 86%) as a light yellow oil. The iodo lactones **32b**−**d** were obtained in a similar way. Data of **32a**: $R_f$ (hexanes−EtOAc, 5:1) 0.36; IR (film) 3060, 2964, 2933, 2863, 1770, 1454, 1358, 1094, 1032 $cm^{-1}$; $^1H$ NMR (500 MHz) $\delta$ 7.30 (5 H, m,), 4.52 (1 H, *A*Bd, $J$ = 11.9 Hz), 4.49 (1 H, A*B*d, $J$ = 11.9 Hz), 4.30 (1 H, d, $J$ = 7.8 Hz), 4.08 (1 H, m), 3.74 (1 H, dd, $J$ = 9.2, 5.3 Hz), 3.57 (1 H, dd, $J$ = 9.2, 5.3 Hz), 2.55 (1 H, d, $J$ = 6.8 Hz), 2.25 (1 H, m), 2.19 (1 H, m), 1.94 (2 H, m), 1.60 (1 H, m), 1.43 (1 H, m), 1.24 (3 H, s), 1.13 (3 H, d, $J$ = 7.0 Hz) ppm; MS *m*/*z* 428 ($M^+$, 1), 386 (2), 337 (6), 280 (8), 221 (2), 195 (22), 153 (20), 135, (4), 91 (100).

**Benzyl (2*S*,3*R*)-2-Methyl-3-((1*S*)-1-methyl-2-cyclohexenyl)butyl Ether (35a), Benzyl (2*S*,3*S*)-2-Methyl-3-((1*S*)-1-methyl-2-cyclohexenyl)butyl Ether (35b), Benzyl (2*R*,3*R*)-2-Methyl-3-((1*S*)-1-methyl-2-cyclohexenyl)butyl Ether (35c), and Benzyl (2*R*,3*S*)-2-Methyl-3-((1*S*)-1-methyl-2-cyclohexenyl)butyl Ether (35d).** To a solution of alcohol **30a** (0.63 g, 2.20 mmol) in pyridine (25 mL) was added TsCl (1.25 g, 0.66 mmol) at 0 °C under $N_2$ atmosphere The mixture was stored in the refrigerator for 24 h, poured into ice water, and extracted with $Et_2O$. The combined organic extracts were washed with an aqueous 5% HCl solution, $H_2O$, and brine, dried over anhydrous $MgSO_4$, and evaporated under reduced pressure. Column chromatography on silica gel (hexanes−EtOAc, 5:1) gave tosylate **33a** (0.77 g, 80%) and the tetrahydrofuran derivative **34a** (∼20%). In an analogous way **30b** gave **33b** (98%) and **30c** gave **33c** (97%), while **30d** gave **33d** (78%) and the tetrahydrofuran derivative **34d** (∼20%).

To a suspension of $LiAlH_4$ (0.464 g, 12.2 mmol) in THF (20 mL) was added a solution of **33a** (0.6 g, 1.36 mmol) in THF (4 mL), and the reaction mixture was refluxed for 4 h. Excess $LiAlH_4$ was destroyed by the addition of EtOH, and the mixture was extracted with $Et_2O$. The combined organic extracts were washed with an aqueous 2.5% HCl solution, $H_2O$, and brine, dried over anhydrous $MgSO_4$, and evaporated under reduced pressure. The residual material was purified by HPLC (hexanes−EtOAc, 8:1) to afford **35a** (0.351 g, 95%) as a colorless oil. In an analogous way **33b** gave **35b** (80% over the two steps), **33c** gave **35c** (94%), and **33d** gave **35d** (80%). Data of **35a**: $R_f$ (hexanes−EtOAc, 5:1) 0.66; $[\alpha]_D$ = +48.5, $[\alpha]_{365}$ = +152.0 (*c* 0.46, $CHCl_3$); IR (film) 3088, 3063, 3012, 2958, 2929, 2869, 1496, 1454, 1366, 1099, 1028 $cm^{-1}$; $^1H$ NMR (500 MHz) $\delta$ 7.33 (5 H, m), 5.59 (1 H, dt, $J$ = 10.2, 4.4 Hz), 5.44 (1 H, br d, $J$ = 10.2 Hz), 4.50 (1 H, *A*Bd, $J$ = 12.0 Hz), 4.46 (1 H, A*B*d, $J$ = 12.0 Hz), 3.58 (1 H, dd, $J$ = 9.1, 3.7 Hz), 3.16 (1 H, dd, $J$ = 9.1, 8.5 Hz), 2.07 (1 H, m), 1.91 (2 H, m), 1.58 (2 H, m), 1.35 (1 H, ddd, $J$ = 9.3, 7.4, 1.9 Hz), 1.02 (3 H, d, $J$ = 6.9 Hz), 0.94 (3 H, s), 0.80 (3 H, d, $J$ = 7.4 Hz) ppm; MS *m*/*z* 272 ($M^+$, 1), 257 (1), 215 (1), 191 (1), 181 (6), 163 (3), 107 (5), 95 (100). Data of **35b**: $R_f$ (hexanes−EtOAc, 5:1) 0.66; $[\alpha]_D$ = +23.1 (*c* 0.32, $CHCl_3$); IR (film) 3086, 3056, 3013, 2956, 2928, 2859, 1496, 1454, 1364, 1100, 1028 $cm^{-1}$; $^1H$ NMR (500 MHz) $\delta$ 7.33 (5 H, m), 5.60 (1 H, dt, $J$ = 10.2, 3.6 Hz), 5.46 (1 H, ddd, $J$ = 9.0, 3.3, 2.0 Hz), 4.52 (1 H, *A*Bd, $J$ = 12.0 Hz), 4.48 (1 H, A*B*d, $J$ = 12.0 Hz), 3.28 (1 H, dd, $J$ = 9.2, 7.3 Hz), 3.21 (1 H, dd, $J$ = 9.2, 7.0 Hz), 2.20 (1 H, ddd, $J$ = 11.0, 7.0, 1.5 Hz), 1.91 (2 H, m), 1.63 (1 H, m), 1.61 (2 H, m), 1.31 (1 H, m), 0.99 (3 H, s), 0.84 (3 H, d, $J$ = 6.9 Hz), 0.75 (3 H, d, $J$ = 7.3 Hz) ppm; MS *m*/*z* 272 ($M^+$, 1), 181 (7), 163 (4), 148 (2), 107 (5), 95 (100). Data of **35c**: $R_f$ (hexanes−EtOAc, 5:1) 0.66; $[\alpha]_D$ = +14.5, $[\alpha]_{365}$ = +38.5 (*c* 0.90, $CHCl_3$); IR (film) 3056, 3016, 2956, 2930, 2862, 1489, 1453, 1378, 1363, 1098, 1028 $cm^{-1}$; $^1H$ NMR (500 MHz) $\delta$ 7.33 (5 H, m), 5.61 (1 H, dt, $J$ = 10.0, 3.6 Hz), 5.46 (1 H, dd, $J$ = 10.0, 1.0 Hz), 4.53 (1 H, *A*Bd, $J$ = 12.1 Hz), 4.49 (1 H, A*B*d, $J$ = 12.1 Hz), 3.26 (1 H, dd, $J$ = 9.2, 7.3 Hz), 3.19 (1 H, dd, $J$ = 9.2, 6.9 Hz), 2.15 (1 H, ddd, $J$ = 11.0, 7.0, 1.7 Hz), 1.93 (2 H, m), 1.62 (2 H, m), 1.56 (2 H, m), 1.37 (1 H, m), 0.96 (3 H, s), 0.86 (3 H, d, $J$ = 6.9 Hz), 0.78 (3 H, d, $J$ = 7.3 Hz) ppm; MS *m*/*z* 272 ($M^+$, 1), 257 (1), 215 (1), 191 (1), 181 (6), 163 (3), 107 (5), 95 (100). Data of **35d**: $R_f$ (hexanes−EtOAc, 5:1) 0.66; $[\alpha]_D$ = −10.57, $[\alpha]_{365}$ = −41.76 (*c* 0.56, $CHCl_3$); IR (film) 3056, 3015, 2954, 2930, 2862, 1646, 1600, 1454, 1374, 1364, 1096, 1026 $cm^{-1}$; $^1H$ NMR (500 MHz) $\delta$ 7.33 (5 H, m), 5.57 (1 H, dt, $J$ = 10.1, 4.0 Hz), 5.43 (1 H, dd, $J$ = 10.1, 1.2 Hz), 4.51 (1 H, *A*Bd, $J$ = 12.1 Hz), 4.47 (1 H, A*B*d, $J$ = 12.1 Hz), 3.61 (1 H, dd, $J$ = 9.1, 3.5 Hz), 3.13 (1 H, dd, $J$ = 9.1, 8.5 Hz), 2.11 (1 H, m), 1.90 (2 H, m), 1.59 (2 H, m), 1.57 (2 H, m), 1.30 (1 H, m), 1.05 (3 H, d, $J$ = 6.9 Hz), 0.96 (3 H, s), 0.77 (3 H, d, $J$ = 7.5 Hz) ppm; MS *m*/*z* 272 ($M^+$, 1), 219 (1), 181 (6), 163 (4), 148 (2), 123 (4), 107 (4), 95 (100), 91 (86).

**(2*S*,3*R*)-3-((1*S*,3*S*)-3-*tert*-Butyldiphenylsilyloxy-1-methylcyclohexyl)-2-methylbutan-1-ol (38a), (2*S*,3*S*)-3-((1*S*,3*S*)-3-*tert*-Butyldiphenylsilyloxy-1-methylcyclohexyl)-2-methylbutan-1-ol (38b), (2*R*,3*R*)-3-((1*S*,3*S*)-3-*tert*-Butyldiphenylsilyloxy-1-methylcyclohexyl)-2-methylbutan-1-ol (38c), and (2*R*,3*S*)-3-((1*S*,3*S*)-3-*tert*-Butyldiphenylsilyloxy-1-methylcyclohexyl)-2-methylbutan-1-ol (38d).** To a solution of **35a** (0.150 g, 0.55 mmol) in THF (14 mL) was added 9-BBN (0.5 M solution in THF, 11 mL, 5.5 mmol), and the mixture was refluxed for 48 h. The organoboranes were oxidized by successively adding EtOH (2.65 mL), 6 N NaOH (1.36 mL), and 30% $H_2O_2$ (2.66 mL). The reaction mixture was heated at 55 °C for 1.5 h and was then extracted with $Et_2O$. The combined organic extracts were washed with an aqueous 5% HCl solution, $H_2O$, and brine, dried over anhydrous $MgSO_4$, and evaporated under reduced pressure. The residual material was purified by HPLC (hexanes−EtOAc, 5:1) to afford the mixture of alcohols **36a** ($\beta$/$\alpha$ diastereomeric ratio 1.5:1, 0.152 g, 95%) as a colorless oil. In an analogous way **35b** gave **36b** ($\beta$/$\alpha$ diastereomeric ratio 1.3:1, 84%), **35c** gave **36c** ($\beta$/$\alpha$ diastereomeric ratio 2:1, 95%), and **35d** gave **36d** ($\beta$/$\alpha$ diastereomeric ratio 2:1, 94%).

To a solution of **36a** (0.145 g, 0.50 mmol) in DMF (3.0 mL) were successively added TBDPSCl (0.21 mL, 0.80 mmol), imidazole (0.053 g, 0.77 mmol), and DMAP (5 mg); the mixture was stirred at room temperature for 16 h and was then diluted with $Et_2O$. The organic phase was washed with 5% HCl, $H_2O$, and brine, dried over anhydrous $MgSO_4$, and evaporated under reduced pressure. The residual material was separated by HPLC (hexanes−EtOAc, 25:1) to give **37a** (0.245 g, 93%). In an analogous way **36b** gave **37b** (93%), **36c** gave **37c** (90%), and **36d** gave **37d** (76%).

Benzyl ether **37a** (250 mg, 0.57 mmol) was dissolved in EtOH (4.0 mL) and cyclohexene (2.0 mL). 20% $Pd(OH)_2$ on carbon (63 mg, 2.5:10 catalyst/substrate by weight) was added, and the suspension was stirred under reflux for 6 h. The catalyst was removed by filtration, and the filtrate was concentrated under reduced pressure. The residual material was separated by HPLC (hexanes−EtOAc, 5:1) to afford alcohols **38**$\beta$**a** and **38**$\alpha$**a** (166 mg, 80%) as colorless oils. In an analogous way **37b** gave **38**$\beta$**b** and **38**$\alpha$**b** (88%), **37c** gave **38**$\beta$**c** and **38**$\alpha$**c** (90%), and **37d** gave **38d** as an unseparable mixture of diastereomers (ratio 2:1; 85%). Data of **38**$\beta$**a**: $R_f$ (hexanes−EtOAc, 5:1) 0.27; IR (film) 3362, 3071, 3049, 2932, 2858, 1472,

1463, 1428, 1377, 1362, 1111, 1070, 1030, 1008 $cm^{-1}$; $^1H$ NMR (500 MHz) $\delta$ 7.66 (4 H, m), 7.42−7.34 (6 H, m), 3.75 (1 H, m), 3.63 (1 H, dd, $J$ = 10.3, 3.1 Hz), 3.20 (1 H, dd, $J$ = 9.8, 9.0 Hz), 1.82 (2 H, m), 1.52 (1 H, m), 1.43 (2 H, m), 1.31 (1 H, m), 1.20 (2 H, m), 1.04 (9 H, s), 0.98 (3 H, d, $J$ = 6.7 Hz), 0.67 (3 H, d, $J$ = 7.4 Hz), 0.56 (3 H, s) ppm; MS $m/z$ 438 ($M^+$, 1), 437 ($M^+$-1, 1), 407 (1), 381 (2), 293 (2), 217 (3), 199 (40), 165 (15), 123 (21), 109 (34), 95 (100). Data of **38**$\beta$**b**: $R_f$ (hexanes−EtOAc, 5:1) 0.27; IR (film) 3367, 3066, 2932, 2855, 1458, 1428, 1383, 1111, 1072, 1037 $cm^{-1}$; $^1H$ NMR (500 MHz) $\delta$ 7.70 (4 H, m), 7.45−7.38 (6 H, m), 3.77 (1 H, ddd, $J$ = 12.0, 9.9, 4.3 Hz), 2.92 (2 H, m), 1.85 (1 H, br d, $J$ = 13.4 Hz), 1.84 (1 H, m), 1.57 (1 H, d, $J$ = 3.3 Hz), 1.54 (1 H, br d, $J$ = 4.4 Hz), 1.48 (1 H, m), 1.29 (1 H, m), 1.14 (1 H, dd, $J$ = 10.5, 13.2 Hz), 1.04 (9 H, s), 0.79 (3 H, s), 0.62 (3 H, d, $J$ = 6.8 Hz), 0.56 (3 H, d, $J$ = 7.2 Hz) ppm; MS $m/z$ 438 ($M^+$, 1), 381 (6), 351 (1), 293 (2), 199 (32), 165 (8), 135 (10), 95 (100). Data of **38**$\beta$**c**: $R_f$ (hexanes−EtOAc, 5:1) 0.27; IR (film) 3351, 3071, 3047, 2932, 2858, 1470, 1428, 1389, 1373, 1111, 1075, 1037 $cm^{-1}$; $^1H$ NMR (500 MHz) $\delta$ 7.67 (4 H, m), 7.42−7.34 (6 H, m), 3.74 (1 H, ddd, $J$ = 15.0, 8.8, 4.4 Hz), 3.33 (2 H, d, $J$ = 7.0 Hz), 1.86 (2 H, m), 1.50 (2 H, m), 1.25 (1 H, m), 1.21 (3 H, m), 1.05 (9 H, s), 0.73 (3 H, d, $J$ = 6.8 Hz), 0.65 (3 H, d, $J$ = 7.2 Hz), 0.55 (3 H, s) ppm; MS $m/z$ 438 ($M^+$, 1), 437 ($M^+$-1, 1), 407 (1), 381 (2), 293 (2), 217 (3), 199 (40), 165 (15), 123 (21), 109 (34), 95 (100).

**Ethyl (5*R*,6*S*)-6-((1*S*,3*S*)-3-*tert*-Butyldiphenylsilyloxy-1-methylcyclohexyl)-5-methylheptanoate (40b) and Ethyl (5*S*,6*R*)-6-((1*S*,3*S*)-3-*tert*-Butyldiphenylsilyloxy-1-methylcyclohexyl)-5-methylheptanoate (40c).** To a solution of alcohol **38**$\beta$**b** (60 mg, 0.137 mmol) in THF (0.75 mL) were successively added $PPh_3$ (54 mg, 0.21 mmol), imidazole (29 mg, 0.42 mmol), and $I_2$ (50 mg, 0.20 mmol) at −10 °C. The mixture was allowed to warm to room temperature and stirred in the dark for 29 h. The reaction mixture was cooled to 0 °C, a saturated aqueous $NaHCO_3$ solution was added, and the mixture was extracted with $Et_2O$. The combined organic extracts were washed with a saturated aqueous $Na_2S_2O_3$ solution and $H_2O$, dried over anhydrous $MgSO_4$, and concentrated under reduced pressure. Column chromatography on silica gel (hexanes−EtOAc, 50:1) afforded **39b** (75 mg, 94%) as a light yellow oil. In an analogous way **38**$\beta$**c** afforded **39c** (94%).

$NiCl_2 \cdot 6H_2O$ (93 mg, 0.39 mmol) in pyridine (2.5 mL) was treated under Ar atmosphere with Zn powder (126 mg, 1.93 mmol) and ethyl acrylate (0.20 mL, 1.93 mol) and then heated at 65 °C for 30 min. The resulting brick-red mixture was cooled to 20 °C, treated with iodide **39b** (70 mg, 0.13 mmol) in pyridine (1.0 mL), and stirred for 2 h. The mixture was poured into EtOAc and filtered through Celite, and the pad was washed with EtOAc. The combined filtrates were washed with an aqueous 1 N HCl solution, $H_2O$, and brine, dried over anhydrous $MgSO_4$, and concentrated under reduced pressure. Column chromatography on silica gel and HPLC purification (hexanes−EtOAc, 8:1) gave ester **40b** (59 mg, 88%) as a colorless oil. In an analogous way **39c** gave **40c** (91%). Data of **40b**: $R_f$ (hexanes−EtOAc, 5:1) 0.61; $[\alpha]_D$ = −10.6 ($c$ 0.15, $CHCl_3$); IR (film) 3056, 2932, 2862, 1737, 1590, 1462, 1428, 1369, 1159, 1111, 1072 $cm^{-1}$; $^1H$ NMR (500 MHz) $\delta$ 7.67 (4 H, m), 7.42−7.34 (6 H, m), 4.12 (2 H, q, $J$ = 7.1 Hz), 3.77 (1 H, ddd, $J$ = 13.5, 9.2, 4.2 Hz), 2.08 (2 H, m), 1.84 (1 H, d, $J$ = 13.4 Hz), 1.67 (1 H, m), 1.48 (2 H, m), 1.31 (1 H, m), 1.25 (3 H, t, $J$ = 7.1 Hz), 1.18 (2 H, m), 1.05 (9 H, s), 0.82 (3 H, s), 0.67 (3 H, d, $J$ = 6.8 Hz), 0.56 (3 H, d, $J$ = 7.1 Hz) ppm; MS $m/z$ 522 ($M^+$, 1), 507 (1), 477 (2), 465 (60), 419 (3), 399 (3), 279 (3), 251 (3), 199 (100), 183 (14), 181 (18), 135 (30), 95 (48). Data of **40c**: $R_f$ (hexanes−EtOAc, 5:1) 0.61; $[\alpha]_D$ = +43.3 ($c$ 0.25, $CHCl_3$); IR (film) 3071, 2932, 2857, 1738, 1463, 1427, 1376, 1246, 1178, 1111, 1072 $cm^{-1}$; $^1H$ NMR (500 MHz) $\delta$ 7.67 (4 H, m), 7.42−7.34 (6 H, m), 4.13 (2 H, q, $J$ = 7.3 Hz), 3.73 (1 H, m), 2.25 (2 H, t, $J$ = 7.5 Hz), 1.84 (1 H, m), 1.66 (1 H, dd, $J$ = 13.6, 6.8 Hz), 1.58 (1 H, m), 1.48 (1 H, m), 1.25 (3 H, t, $J$ = 7.2 Hz), 1.18 (3 H, m), 1.05 (9 H, s), 0.69 (3 H, d, $J$ = 6.7 Hz), 0.62 (3 H, d, $J$ = 7.2 Hz), 0.52 (3 H, s) ppm; MS $m/z$ 522 ($M^+$, 1), 477 (1), 465 (50), 399 (2), 387 (2), 251 (2), 227 (4), 199 (100), 183 (14), 135 (36), 95 (76).

**(3*S*)-3-((1*S*,2*R*)-6-Hydroxy-1,2,6-trimethylheptyl)-3-methylcyclohexan-1-one (43b) and (3*S*)-3-((1*R*,2*S*)-6-Hydroxy-1,2,6-trimethylheptyl)-3-methylcyclohexan-1-one (43c).** To a stirred solution of ester **40b** (55 mg, 0.105 mmol) in dry THF (1.5 mL) at 0 °C under $N_2$ atmosphere was added MeMgBr (3.0 M solution in $Et_2O$, 0.175 mL, 0.525 mmol) over a period of 5 min. Stirring was continued for 2 h, and the reaction mixture was quenched with a saturated aqueous $NH_4Cl$ solution. After extraction with $Et_2O$, the combined extracts were washed with brine, dried over anhydrous $MgSO_4$, and concentrated under reduced pressure. The residual material was purified by HPLC (hexanes−EtOAc, 5:1) to afford **41b** (46 mg, 86%). In an analogous way **40c** gave **41c** (95%).

To a solution of **41b** (32 mg, 0.063 mmol) in THF (1.0 mL) was added TBAF (1 M solution in THF, 0.110 mL, 0.110 mmol) at room temperature under $N_2$ atmosphere. The reaction mixture was stirred for 16 h and then directly loaded onto a silica gel column (hexanes−EtOAc, 2:1). Further purification by HPLC (isooctane−acetone, 1:1) afforded **42b** (17 mg, 100%) as a colorless oil. In an analogous way **41c** gave **42c** (90%).

A mixture of alcohol **42b** (14.5 mg, 0.054 mmol) and PDC (60 mg, 0.160 mmol) in $CH_2Cl_2$ (1.5 mL) was stirred at room temperature for 22 h, and the solution was filtered through a pad of silica gel. HPLC purification (hexanes−EtOAc, 5:1) gave **43b** (13.5 mg, 94%) as a colorless oil. In an analogous way **42c** gave **43c** (86%). Data of **43b**: $R_f$ (hexanes−EtOAc, 1:1) 0.38; $[\alpha]_D$ = −67.7 ($c$ 0.20, $CHCl_3$); IR (film) 3410, 2963, 2923, 1705, 1456, 1421, 1380, 1354, 1154, 1072 $cm^{-1}$; $^1H$ NMR (500 MHz) $\delta$ 2.35 (1 H, d, $J$ = 13.4 Hz), 2.28 (2 H, m), 2.11 (1 H, d, $J$ = 13.4 Hz), 1.88 (2 H, m), 1.74 (1 H, m), 1.69 (1 H, m), 1.53 (1 H, m), 1.45 (2 H, m), 1.32 (2 H, m), 1.27 (1 H, m), 1.22 (3 H, s), 1.21 (3 H, s), 0.87 (3 H, s), 0.80 (3 H, d, $J$ = 6.8 Hz), 0.76 (3 H, d, $J$ = 7.2 Hz) ppm; MS $m/z$ 268 ($M^+$, 1), 253 (4), 235 (1), 201 (1), 169 (4), 140 (4), 123 (5), 111 (74), 83 (34), 69 (38), 55 (100). Data of **43c**: $R_f$ (hexanes−EtOAc, 1:1) 0.38; $[\alpha]_D$ = −1.72, $[\alpha]_{365}$ = −75.0 ($c$ 0.41, $CHCl_3$); IR (film) 3434, 2965, 2933, 1705, 1460, 1378, 1234, 1159, 1087, 1081 $cm^{-1}$; $^1H$ NMR (500 MHz) $\delta$ 2.28 (2 H, m), 2.08 (1 H, m), 1.92 (1 H, m), 1.83 (1 H, ddd, $J$ = 11.5, 9.4, 5.1 Hz), 1.77 (1 H, dd, $J$ = 13.0, 6.1 Hz), 1.66 (2 H, dd, $J$ = 9.3, 4.3 Hz), 1.44 (2 H, m), 1.33 (3 H, m), 1.21 (6 H, s), 0.86 (3 H, s), 0.80 (3 H, d, $J$ = 6.8 Hz), 0.78 (3 H, d, $J$ = 7.2 Hz) ppm; MS $m/z$ 268 ($M^+$, 1), 253 (2), 217 (1), 191 (1), 169 (3), 140 (40), 123 (6), 111 (100), 83 (38), 69 (40), 55 (100).

**(1*R*,3*R*)-5-((*Z*,2*E*)-2-((3*S*)-3-((1*R*,2*S*)-6-Hydroxy-1,2,6-trimethylheptyl)-3-methylcyclohexylidene)ethylidene)-cyclohexane-1,3-diol (8c).** Cyclohexanone **43c** was coupled with phosphine oxide **15** according to the above general procedure. The crude product was purified by HPLC (hexanes−EtOAc, 200:1) to give protected **8c** (89%). Deprotection using TBAF was carried out as described for **8a**, and the product was isolated by loading the crude reaction mixture onto silica gel (hexane−acetone, 2:1) and further purification by HPLC (hexane−acetone, 3:1) to afford analogue **8c** (3.7 mg, 95%) as a white solid: $R_f$ (hexanes−EtOAc, 1:1) 0.08; IR (film) 3386, 2954, 2923, 2720, 1595, 1462, 1426, 1380, 1359, 1154, 1067, 1051 $cm^{-1}$; $^1H$ NMR (500 MHz) $\delta$ 6.23 (1 H, d, $J$ = 11.2 Hz, H-6), 5.95 (1 H, d, $J$ = 11.2 Hz, H-7), 4.11 (1 H, m), 4.07 (1 H, m), 2.68 (1 H, dd, $J$ = 13.4, 3.8 Hz, H-10$\beta$), 2.49 (1 H, dd, $J$ = 13.5, 3.6 Hz, H-4$\alpha$), 2.46 (1 H, m, H-9$\beta$), 2.27 (1 H, dd, $J$ = 13.4, 7.9 Hz, H-10$\alpha$), 2.20 (1 H, dd, $J$ = 13.5, 6.6 Hz), 2.06 (1 H, d, $J$ = 13.4 Hz, H-14$\beta$), 2.00 (1 H, m), 1.91 (1 H, m), 1.84 (1 H, d, $J$ = 13.4 Hz, H-10$\alpha$), 1.76 (1 H, dd, $J$ = 13.8, 7.0 Hz), 1.59 (1 H, m), 1.52 (1 H, m), 1.45 (4 H, m), 1.30 (2 H, m), 1.21 (6 H, s), 0.80 (3 H, d, $J$ = 6.8 Hz), 0.77 (3 H, s), 0.74 (3 H, d, $J$ = 7.1 Hz) ppm; MS $m/z$ 392 ($M^+$, 1), 374 (8), 356 (7), 341 (1), 303 (1), 235 (5), 217 (8), 181 (6), 163 (18), 109 (30), 91 (36), 43 (100).

**(1*R*,3*R*)-5-((*Z*,2*E*)-2-((3*S*)-3-((1*S*,2*R*)-6-Hydroxy-1,2,6-trimethylheptyl)-3-methylcyclohexylidene)ethylidene)-cyclohexane-1,3-diol (8b).** To a solution of **43b** (13 mg, 0.049 mmol) in DMF (750 $\mu$L) were successively added $Et_3SiCl$ (22

mg, 0.147 mmol), imidazole (13 mg, 0.240 mmol), and DMAP (3 mg). The mixture was stirred at room temperature for 16 h and then diluted with $Et_2O$. The organic phase was washed with an aqueous 5% HCl solution and $H_2O$, dried over anhydrous $MgSO_4$, and concentrated under reduced pressure. Column chromatography on silica gel gave silyl ether **44b** (7.6 mg, 82% based on recovered **43b**).

Cyclohexanone **44b** was coupled with phosphine oxide **15** according to the above general procedure to give a mixture of protected **8b** and (*Z*)-**8b** (ratio 7:3, 100%). The mixture was deprotected using TBAF as described for **8c**, and the products were isolated by loading the crude reaction mixture onto silica gel (hexane−acetone, 2:1) and further separation by HPLC (hexane−acetone, 3:1) to afford **8b** and (*Z*)-**8b** (100%) as white solids. Data of **8b**: $R_f$ (hexanes−EtOAc, 1:1) 0.08; IR (film) 3386, 2964, 2923, 2872, 1595, 1431, 1380, 1354, 1149, 1113, 1068, 1051 $cm^{-1}$; $^1H$ NMR (500 MHz) $\delta$ 6.22 (1 H, d, $J$ = 11.3 Hz, H-6), 5.96 (1 H, d, $J$ = 11.3 Hz, H-7), 4.13 (1 H, m), 3.96 (1 H, m), 2.86 (1 H, dd, $J$ = 12.9, 3.6 Hz, H-10$\beta$), 2.43 (1 H, d, $J$ = 13.1 Hz, H-4$\alpha$), 2.37 (1 H, m, H-9$\beta$), 2.25 (1 H, d, $J$ = 13.0 Hz, H-14$\beta$), 2.21 (1 H, dd, $J$ = 13.1, 4.6 Hz, H-4$\beta$), 2.08 (2 H, m), 1.87 (1 H, d, $J$ = 13.0 Hz, H-14$\alpha$), 1.76 (1 H, dd, $J$ = 14.2, 6.3 Hz), 1.70 (1 H, m), 1.60 (1 H, m), 1.50 (1 H, dd, $J$ = 12.7, 4.9 Hz), 1.47 (1 H, m), 1.41 (1 H, m), 1.40 (1 H, d, $J$ = 6.8 Hz), 1.35 (1 H, m), 1.23 (3 H, s), 1.22 (3 H, s), 1.15 (2 H, m), 0.79 (3 H, d, $J$ = 6.8 Hz), 0.78 (3 H, s), 0.69 (3 H, d, $J$ = 7.2 Hz) ppm; MS $m/z$ 392 ($M^+$, 1), 374 (6), 356 (8), 342 (2), 235 (4), 217 (4), 199 (6), 163 (16), 105 (20), 91 (34), 43 (100). Data of (*Z*)-**8b**: $R_f$ (hexanes−EtOAc, 1:1) 0.08; IR (film) 3386, 2964, 2923, 2872, 1595, 1380, 1149, 1113, 1068, 1051 $cm^{-1}$; $^1H$ NMR (500 MHz) $\delta$ 6.21 (1 H, d, $J$ = 11.3 Hz), 6.08 (1 H, d, $J$ = 11.3 Hz), 4.13 (1 H, m), 4.09 (1 H, m), 2.52 (1 H, dd, $J$ = 12.8, 3.4 Hz), 2.40 (1 H, d, $J$ = 13.5 Hz), 2.15 (1 H, dd, $J$ = 12.8, 8.5 Hz), 2.10 (1 H, m), 1.98 (1 H, m), 1.92 (1 H, d, $J$ = 13.5 Hz), 1.76 (1 H, dd, $J$ = 12.2, 8.3 Hz), 1.60 (1 H, m), 1.50 (1 H, dd, $J$ = 14.0, 5.7 Hz), 1.45 (1 H, dd, $J$ = 9.5, 5.1 Hz), 1.42 (1 H, d, $J$ = 9.1 Hz), 1.37 (1 H, m), 1.23 (3 H, s), 1.22 (3 H, s), 1.18 (1 H, m), 0.81 (3 H, d, $J$ = 6.6 Hz), 0.80 (3 H, s), 0.70 (3 H, d, $J$ = 7.2 Hz) ppm; MS $m/z$ 392 ($M^+$, 1), 374 (6), 356 (8), 235 (4), 199 (6), 91 (34), 43 (100).

**(3*S*)-3-((1*R*,2*R*)-6-(1-Ethoxyethoxy)-1,2,6-trimethyl-4-heptynyl)-3-methylcyclohexan-1-ol (47a) and (3*S*)-3-((1*S*,2*S*)-6-(1-Ethoxyethoxy)-1,2,6-trimethyl-4-heptynyl)-3-methylcyclohexan-1-ol (47d).** A solution of **38**$\beta$**a** (98 mg, 0.223 mmol) and TsCl (107 mg, 0.558 mmol) in pyridine (1.5 mL) was stirred at room temperature for 19 h and then poured into ice water. The mixture was extracted with $Et_2O$, and the combined organic extracts were washed with an aqueous 2% HCl solution, dried over anhydrous $MgSO_4$, and concentrated under reduced pressure. The residual material was purified by HPLC (hexanes−EtOAc, 25:1) to afford tosylate **45**$\beta$**a** (120 mg, 91%). In an analogous way **38d** ($\beta$/$\alpha$ diastereomeric mixture) gave **45d** (93%).

NaH (60%, 38 mg, 0.95 mmol) in dry DMSO (1.5 mL) was stirred at 65 °C under $N_2$ atmosphere for 2 h. To the formed light-green solution was dropwise added the ethoxyethyl ether of 2-methyl-3-butyn-2-ol (225 mg, 1.44 mmol) at room temperature. The mixture was stirred for 45 min, and a solution of tosylate **45**$\beta$**a** (68 mg, 0.115 mmol) in DMSO (0.5 mL) was added. Stirring was continued for 45 min, and the reaction mixture was diluted with $Et_2O$. The organic phase was washed with $H_2O$, dried over anhydrous $MgSO_4$, and concentrated under reduced pressure. Column chromatography on silica gel (hexanes−EtOAc, 50:1) gave **46**$\beta$**a** (45 mg, 68%) as a colorless oil. In an analogous way **45d** gave **46d** (9%) and desilylated product **47d** (48%).

To a solution of silyl ether **46**$\beta$**a** (50 mg, 0.087 mmol) in THF (1.5 mL) was added TBAF (1 M solution in THF, 174 $\mu$L, 0.174 mmol). The reaction mixture was stirred at room temperature for 10 h, directly loaded onto a silica gel column, and further purified by HPLC (hexanes−EtOAc, 5:1) to give alcohol **47**$\beta$**a** (28.7 mg, 98%) as a colorless oil. In an analogous way **46d** gave **47d** (94%). Data of **47**$\beta$**a**: $R_f$ (hexanes−EtOAc, 1:1) 0.48; IR (film) 3420, 2977, 2933, 2867, 2233, 1462, 1378, 1253, 1158, 1121, 1082, 1054 $cm^{-1}$; $^1H$ NMR (500 MHz) $\delta$ 5.10 (1H, q, $J$ = 5.44 Hz), 3.76 (1 H, m), 3.67 (1 H, m), 3.50 (1 H, m), 2.01 (2 H, m), 1.89 (1 H, dd, $J$ = 16.3, 10.5 Hz), 1.75 (1 H, m), 1.65 (1 H, m), 1.49 (3 H, s), 1.43 (3 H, s), 1.32 (3 H, d, $J$ = 5.2 Hz), 1.19 (3 H, t, $J$ = 7.0 Hz), 1.03 (3 H, d, $J$ = 6.8 Hz), 0.87 (3 H, s), 0.78 (3 H, d, $J$ = 7.3 Hz) ppm; MS $m/z$ 338 ($M^+$, 1), 323 (1), 294 (1), 249 (2), 233 (2), 199 (7), 151 (8), 123 (2), 109 (24), 73 (100).

**(3*S*)-3-((1*S*,2*S*)-6-(1-Ethoxyethoxy)-1,2,6-trimethylheptyl)-3-methylcyclohexan-1-one (49d).** Alkyne **47d** (20 mg, 0.059 mmol) and 5% Rh on $Al_2O_3$ (20 mg) in EtOAc (1.5 mL) were stirred under $H_2$ atmosphere (1 bar) at room temperature for 4 h. The reaction mixture was filtered through silica gel, and the product was purified by HPLC (hexanes−EtOAc, 5:1) to afford **48d** (18 mg, 90%).

A mixture of **48d** (16 mg, 0.0467 mmol) and PDC (53 mg, 0.140 mmol) in $CH_2Cl_2$ (1.5 mL) was stirred at room temperature for 24 h. The reaction mixture was directly loaded onto a silica gel column, and the product was further purified by HPLC (hexanes−EtOAc, 5:1) to give cyclohexanone **49d** (8.0 mg, 50%) and the deprotected product **43d** (5.0 mg, 40%). Data of **43d**: $R_f$ (hexanes−EtOAc, 1:1) 0.26; IR (film) 3404, 2969, 2944, 2882, 1707, 1459, 1383, 1237, 1159, 1086, 1043 $cm^{-1}$; $^1H$ NMR (500 MHz) $\delta$ 2.28 (2 H, m), 2.15 (1 H, m), 1.88 (1 H, m), 1.82 (1 H, m), 1.70 (1 H, m), 1.68 (1 H, m), 1.47−1.31 (6 H, m), 1.25 (2 H, m), 1.21 (6 H, s), 0.90 (3 H, d, $J$ = 6.8 Hz), 0.87 (3 H, s), 0.79 (3 H, d, $J$ = 7.2 Hz) ppm; MS $m/z$ 268 ($M^+$, 1), 253 (1), 250 (4), 249 (24), 199 (30), 140 (12), 111 (56), 55 (100).

**(1*R*,3*R*)-5-((*Z*,2*E*)-2-((3*S*)-3-((1*S*,2*S*)-6-Hydroxy-1,2,6-trimethylheptyl)-3-methylcyclohexylidene)ethylidene)-cyclohexane-1,3-diol (8d).** A mixture of **43d** (5.0 mg, 0.019 mmol), $CF_3SO_3SiEt_3$ (8.6 mL, 0.038 mmol), and $Et_3N$ (7.92 mL, 0.057 mmol) in $CH_2Cl_2$ (0.5 mL) was stirred at room temperature for 4 h. The reaction mixture was directly loaded onto a silica gel column, and the product was further purified by HPLC (hexanes−EtOAc, 10:1) to give **44d** (5.0 mg, 70%) as a colorless oil.

Cyclohexanone **44d** was coupled with phosphine oxide **15** according to the above general procedure to give protected **8d** (100%). The compound was deprotected using TBAF as described for **8a**, and the product was isolated by loading the crude reaction mixture onto silica gel (hexane−acetone, 2:1) and further separation by HPLC (hexane−acetone, 3:1) to afford **8d** (92% from **44d**) as a white solid: $R_f$ (hexanes−EtOAc, 1:1) 0.08; IR (film) (hexane−acetone, 2.5:1) 0.30; IR (film) 3358, 3036, 2964, 2929, 2872, 1600, 1456, 1431, 1380, 1154, 1087, 1050 $cm^{-1}$; $^1H$ NMR (500 MHz) $\delta$ 6.26 (1 H, d, $J$ = 11.3 Hz, H-6), 5.95 (1 H, d, $J$ = 11.3 Hz, H-7), 4.09 (2 H, m, H-1 and H-3), 2.65 (1 H, dd, $J$ = 13.3, 2.4 Hz, H-10$\beta$), 2.49 (1 H, dd, $J$ = 13.3, 3.8 Hz, H-4$\alpha$), 2.31 (1 H, dd, $J$ = 13.3, 7.6 Hz, H-10$\alpha$), 2.19 (1 H, dd, $J$ = 13.3, 6.9 Hz, H-4$\beta$), 2.15 (1 H, d, $J$ = 13.1 Hz), 1.90 (1 H, d, $J$ = 13.1 Hz), 1.86 (1 H, m), 1.70 (1 H, m), 1.50 (2 H, m), 1.45 (1 H, m), 1.40 (1 H, m), 1.38 (2 H, m), 1.22 (6 H, s), 0.89 (3 H, d, $J$ = 6.8 Hz), 0.76 (3 H, s), 0.72 (3 H, d, $J$ = 7.3 Hz) ppm; MS $m/z$ 392 ($M^+$, 2), 374 (3), 356 (4), 235 (4), 217 (8), 163 (14), 91 (30), 43 (100).

**(3*S*)-3-((1*R*,2*R*)-6-(1-Ethoxyethoxy)-1,2,6-trimethyl-4-heptynyl)-3-methylcyclohexan-1-one (50a).** Alcohol **47a** was oxidized using PDC as described for **42c** to give **50a** (85%) as a colorless oil: $R_f$ (hexanes−EtOAc, 6:1) 0.62; $[\alpha]_D$ = +0.95, $[\alpha]_{365}$ = −24.05 (*c* 0.42, $CHCl_3$); IR (film) 2974, 2935, 2877, 2233, 1713, 1456, 1379, 1313, 1252, 1160, 1122, 1081, 1053 $cm^{-1}$; $^1H$ NMR (500 MHz) $\delta$ 5.10 (1 H, dq, $J$ = 4.9, 1.2 Hz), 3.68 (1 H, m), 3.50 (1 H, m), 2.26 (3 H, m), 2.09 (1 H, m), 1.90 (4 H, m), 1.68 (2 H, m), 1.50 (3 H, s), 1.43 (3 H, s), 1.33 (3 H, d, $J$ = 5.2 Hz), 1.19 (3 H, t, $J$ = 7.0 Hz), 1.06 (3 H, d, $J$ = 6.7 Hz), 0.88 (3 H, s), 0.79 (3 H, d, $J$ = 7.2 Hz) ppm; MS $m/z$ 336 ($M^+$, 1), 321 (1), 292 (1), 263 (5), 247 (9), 221 (2), 191 (3), 177 (4), 149 (5), 135 (13), 111 (33), 73 (100).

**(1*S*,3*R*)-5-((*Z*,2*E*)-2-((3*S*)-3-((1*R*,2*R*)-6-Hydroxy-1,2,6-trimethyl-4-heptynyl)-3-methylcyclohexylidene)ethylidene)-4-methylenecyclohexane-1,3-diol (9a).** Cyclohexanone **50a**

was coupled with phosphine oxide **14** according to the above general procedure to give protected **9a** (100%).

A solution of protected **9a** (16.0 mg, 0.029 mmol) and PPTS (18.5 mg) in $CH_2Cl_2$ (3.5 mL) was stirred at room temperature for 1 h, then directly loaded onto silica gel, and purified by HPLC (hexanes−EtOAc, 5:1) to give ethoxyethyl-deprotected **9a** (∼14.5 mg, 100%).

Ethoxyethyl-deprotected **9a** was desilylated using TBAF as described for **8a**, and the products were isolated by loading the crude reaction mixture onto silica gel (hexane−acetone, 2:1) and further separation by HPLC (hexane−acetone, 3:1) to afford analogue **9a** (100%) as a white solid: $R_f$ (hexanes−EtOAc, 1:1) 0.08; IR (film) 3384, 2955, 2923, 2869, 2232, 1664, 1641, 1619, 1448, 1432, 1371, 1233, 1156, 1051 $cm^{-1}$; $^1H$ NMR (500 MHz) $\delta$ 6.33 (1 H, d, $J = 11.2$ Hz), 6.05 (1 H, d, $J = 11.2$ Hz), 5.33 (1 H, s), 4.99 (1 H, br s), 4.43 (1 H, m), 4.22 (1 H, m), 2.61 (1 H, dd, $J = 13.1$, 4.9 Hz), 2.44 (1 H, m), 2.30 (1 H, dd, $J = 12.8$, 7.2 Hz), 2.26 (1 H, dd, $J = 14.0$, 3.0 Hz), 2.06 (2 H, m), 1.98 (4 H, m), 1.85 (2 H, m), 1.49 (6 H, s), 1.33 (2 H, m), 1.26 (3 H, m), 1.03 (3 H, d, $J = 6.8$ Hz), 0.77 (3 H, s), 0.74 (3 H, d, $J = 7.2$ Hz) ppm; MS *m/z* 400 ($M^+$, 1), 382 (1), 342 (2), 311 (1), 255 (1), 227 (5), 209 (4), 151 (12), 133 (15), 91 (22), 43 (100).

**Preparation of Previtamin 51 from Analogue 9a.** A solution of **9a** (5.2 mg) in acetone-$d_6$ (1.5 mL) in a NMR tube was kept in the dark under $N_2$ atmosphere at 24 °C and the 1,7-sigmatropic hydrogen shift rearrangement was followed by $^1H$ NMR. After 58 days a 23:77 equilibrium mixture of **9a** and **51**, respectively, was obtained. The solvent was removed, and the products were separated by HPLC (RSil CN; hexane−2-propanol−$CH_3CN$, 91:8:1) to give pure previtamin D analogue **51**: $R_f$ (hexanes−EtOAc, 1:1) 0.08; IR (film) 3384, 2955, 2923, 2869, 2232, 1664, 1641, 1619, 1448, 1432, 1371, 1233, 1156, 1051 $cm^{-1}$; $^1H$ NMR (500 MHz) $\delta$ 5.94 (1 H, br d, $J = 12.2$ Hz), 5.67 (1 H, br d, $J = 6.4$ Hz), 5.64 (1 H, br d, $J = 12.2$ Hz), 4.20 (1 H, m), 4.17 (1 H, m), 2.44 (1 H, dd, $J = 16.0$, 4.4 Hz), 2.28 (1 H, dd, $J = 16.0$, 2.8 Hz), 2.11 (2 H, m), 2.08 (1 H, m), 2.02 (1 H, m), 1.98 (1 H, m), 1.83 (1 H, m), 1.72 (3 H, s), 1.50 (3 H, s), 1.49 (3 H, s), 1.04 (3 H, d, $J = 6.9$ Hz), 0.82 (3 H, s), 0.73 (3 H, d, $J = 7.3$ Hz) ppm; MS *m/z* 400 ($M^+$, 1), 382 (1), 342 (2), 311 (1), 255 (1), 227 (5), 209 (4), 151 (12), 133 (15), 91 (22), 43 (100).

**Biological Evaluation.** All analogues published in the literature (compounds **2**−**5**, **6c,** and **6d**) together with the 6C analogues **8a**−**d** were evaluated in our own laboratory (Leuven) as described below.

**Materials.** $1,25(OH)_2D_3$ (**1**) was a gift of M. Uskokovic (Hoffmann-La Roche, Nutley, NJ) and J. P. van de Velde (Duphar, Weesp, The Netherlands). [$^3H$]$1,25(OH)_2D_3$ (specific activity 180 $\mu$Ci/mmol) and [*methyl*-$^3H$]thymidine (specific activity 2 Ci/mmol) were purchased from Amersham (Buckinghamshire, U.K.). Culture media and gentamycin were obtained from Gibco (Roskilde, Denmark). Penicillin and streptomycin were from Boehringer Mannheim (Mannheim, Germany). 4-Nitro blue tetrazolium (NBT) and phorbol 12-myristate 13-acetate were purchased from Sigma (St. Louis, MO). Fetal calf serum (FCS) was obtained from JRH Biosciences (Sera-Lab, W. Sussex, U.K.). Human serum vitamin D binding protein (hDBP) was prepared by affinity chromatography as described previously.[39]

**Cells and Animals.** The human promyelocytic leukemia (HL-60) and breast carcinoma (MCF-7) cell lines were obtained from the American Tissue Culture Co. (Rockville, MD). Vitamin D-replete mice were obtained from the Proefdierencentrum of Leuven (Belgium).

**Affinity for VDR.** The affinity of the analogues of $1,25(OH)_2D_3$ to the VDR was evaluated by their ability to compete with [$^3H$]$1,25(OH)_2D_3$ for binding to high-speed supernatant from intestinal mucosa homogenates obtained from normal pigs. The incubation was performed at 4 °C for 20 h, and phase separation was obtained by addition of dextran-coated charcoal. The relative affinity of the analogues was calculated from their concentration needed to displace 50% of [$^3H$]$1,25(OH)_2D_3$ from its receptor compared with the activity of $1,25(OH)_2D_3$ (assigned a 100% value).

**Affinity for hDBP.** Binding of vitamin D metabolites and analogues to hDBP was performed at 4 °C essentially as described previously.[40] [$^3H$]$1,25(OH)_2D_3$ and $1,25(OH)_2D_3$ or its analogues were added in 5 $\mu$L of ethanol into glass tubes and incubated with hDBP (0.18 $\mu$M) in a final volume of 1 mL (0.01 M Tris-HCl buffer and 0.154 M NaCl, pH 7.4) for 3 h at 4 °C. Phase separation was then obtained by the addition of 0.5 mL of cold dextran-coated charcoal.

**Promyelocytic Leukemia Cells HL-60.** HL-60 cells were seeded at $4 \times 10^4$ cells/mL in 25-$cm^2$ Falcon tissue chambers using RPMI 1640 medium supplemented with 20% FCS and gentamycin (50 $\mu$g/mL). Final volume was 5 mL. Cultures were maintained at 37 °C in a humidified atmosphere of 5% $CO_2$ in air. One day later, $1,25(OH)_2D_3$ or analogues were added to the cell culture in ethanol (final concentration < 0.2%). After 4 days of culture, the dishes were shaken to loosen adherent cells. Cells were washed twice in RPMI, counted, and assayed for differentiation markers (NBT reduction assay). Superoxide production was measured as NBT reducing activity as described previously.[41] HL-60 cells at $1 \times 10^6$/mL were mixed with an equal volume of freshly prepared solution of phorbol 12-myristate 13-acetate (200 ng/mL) and NBT (2 $\mu$g/mL) and incubated for 30 min at 37 °C. The percentage of cells containing black formazan deposits was determined using a hemacytometer.

**Breast Carcinoma Cells MCF-7.** MCF-7 cells were cultured in DMEM/nutrient mixture F12 (HAM) medium supplemented with 10% heat-inactivated FCS, glutamine (2 mM), penicillin (100 units/mL), and streptomycin (0.1 mg/mL). Cultures were maintained at 37 °C in humidified atmosphere of 5% $CO_2$ in air. MCF-7 cells were seeded at $5 \times 10^3$ cells/well in the above-described medium in a 96-well microtiter plate in a final volume of 0.2 mL/well. Triplicate cultures were performed. After 24 h, $1,25(OH)_2D_3$ or analogues were added in the appropriate concentrations for an incubation period of 72 h. Then, 1 $\mu$Ci of [$^3H$]thymidine was added to each well, cells were harvested after 4-h incubation with a Packard harvester, and radioactivity was measured by a Packard Topcount system (Packard, Meriden).

**Keratinocytes.** Human skin keratinocytes were isolated and cultured using a modification of the method of Kitano and Okada[42] and was described previously.[43] Briefly, foreskin was soaked overnight at 4 °C in Dispase (20 units/mL). The epidermis was peeled from the dermis and washed with calcium- and magnesium-free solution for 20 min at 37 °C. The cells were then suspended in keratinocyte medium (Gibco Keratinocytes-SFM with insulin (5 $\mu$g/mL)) supplemented with bovine pituitary extract (50 $\mu$g/mL; Gibco, Paisley, U.K.) and epidermal growth factor (5 ng/mL; Gibco). The cells were plated into culture flasks at $3 \times 10^6$ cells/175-$cm^2$ flask in a final volume of 25 mL and cultured until 75% confluence. Keratinocytes were then seeded in 96-well plates at a concentration of $5 \times 10^3$ cells/0.1 mL of medium/well. The same medium was used as described above but without insulin. After 1 day, serial dilutions of $1,25(OH)_2D_3$ or analogues, in ethanol solution (<0.2% final concentration), were added to the cells in a working volume of 0.1 mL. After an incubation period of 72 h, 1 $\mu$Ci of [$^3H$]thymidine (specific activity 35 Ci/mmol) was added to each well for 2 h. Thereafter, cells were harvested with the Packard Topcount system.

**In Vivo Activity of the $1,25(OH)_2D_3$ Analogues.** The hypercalcemic effect of the analogues was tested in vitamin D-replete normal NMRI mice by daily subcutaneous injection of $1,25(OH)_2D_3$, its analogues, or the solvent during 7 consecutive days, using serum calcium concentration as parameters.

**Conformational Analysis and Molecular Modeling: Volume Maps.** Conformational analysis of the side chain of compounds **1**, **5**, **6c**, **6d**, and **8a**−**d** was carried out using the MacroModel molecular modeling program of Still[44] run on a Digital VAXstation 4000-90A or SiliconGraphics Octane. Molecular mechanics calculations were carried out on model compounds in which the A-ring and diene system up to C6

were replaced by an H atom. Only the most stable chair conformation of the C-ring was taken into account. Rotations with 60° increments were applied to the rotatable C−C bonds of the side chain, while the 25-OH was rotated with increments of 120°. The so-generated starting conformations were minimized using the MM2* force field implementation of MacroModel, and the conformations within 20 kJ/mol of the minimum energy form were retained. Using a PC computer program all conformations of each compound were then overlaid using C13 as a common origin ($x, y, z = 0$), C14 was positioned in the *yz*-plane ($x = 0$), and C18 was made to coincide with the positive *y*-axis ($x, z = 0$). A line drawing was generated of the minimum energy conformation, and the position of O25 in each of the local energy minima within the given energy window was represented by a dot to obtain dot maps. The volume maps shown in Figures 1 and 2 were generated by importing the above dot maps into the CS Chem3D molecular modeling program,[45] creating ball-and-stick models using van der Waals radii of 0.7 and 0.8 Å for C and O, respectively, and applying the appropriate color to O according to the energy of the particular conformation.

**Determination of a Relative Activity Volume by Subtraction.** For subtraction of the dot maps of respectively **6d** from **6c** and **8c** from **8a**, a PC computer program was used which allowed for (1) the generation of a sphere centered around each O25 position in the dot map of **6c** (**8a**) and (2) the determination of the presence of a O25 position of **6d** (**8c**) in each of these spheres. For the procedure both dot maps were oriented and overlaid using C13, C14, and C18 as above. In case no O25 position of **6d** (**8c**) was present in the sphere, the particular O25 position of **6c** (**8a**) was retained. In case a O25 position of **6d** (**8c**) was found in the sphere, the particular O25 position of **6c** (**8a**) was either (a) retained if the energy category of the conformation of **6c** (**8a**) was lower (more stable) than the energy category of the conformation of **6d** (**8c**) or (b) rejected if the energy category of the conformation of **6c** (**8a**) was equal or higher (less stable). For this, four energy categories corresponding to 0−5, 5−10, 10−15, and 15−20 kJ/mol, respectively, were used. The radius of the sphere was varied so as to end up with 15−20% (arbitrarily chosen, but found to give a workable reduced volume area) of the original number of conformations (0−20 kJ/mol) of **6c** and **8a**, respectively. The relative activity volumes shown in Figure 2 (top and middle) were obtained by setting the radius of the sphere to 4.1 Å in the case of **6c** and to 1.65 Å in the case of **8a**.

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## References

(1) Christakos, S.; Raval-Pandya M.; Wernyj, R. P.; Yang, W. Genomic Mechanisms Involved in the Pleiotropic Actions of 1,-25-Dihydroxyvitamin $D_3$. *Biochem. J.* **1996**, *316*, 361−371 and references therein.

(2) Casteels, K.; Bouillon, R.; Waer, M.; Mathieu, C. Immunomodulatory Effects of 1,25-Dihydroxyvitamin $D_3$. *Curr. Opin. Nephrol. Hypertens.* **1995**, *4*, 313−318.

(3) Van Leeuwen, J. P. T. M.; Pols, H. A. P. Vitamin D: Anticancer and Differentiation. In *Vitamin D*; Feldman, D., Glorieux, F. H., Pike, J. W., Eds.; Academic Press: San Diego, CA, 1997; pp 1089−1105.

(4) Bouillon, R.; Okamura, W. H.; Norman, A. W. Structure−Function Relationships in the Vitamin D Endocrine System. *Endocrinol Rev.* **1995**, *16*, 200−257.

(5) (a) Binderup, L.; Latini, S.; Binderup, E.; Bretting, C.; Calverley, M.; Hansen, K. 20-Epi-vitamin $D_3$ Analogues: A Novel Class of Potent Regulators of Cell Growth and Immune Responses. *Biochem. Pharmacol.* **1991**, *42*, 1569−1575. (b) Calverley, M. J.; Binderup, E.; Binderup, L. In *Vitamin D: Gene Regulation. Structure−Function Analysis and Clinical Application*; Norman, A. W., Bouillon, R., Thomasset, M., Eds.; Walter De Gruyter and Co.: Berlin, 1991; pp 163−164.

(6) Perlman, K. L.; Sicinski, R. R.; Schnoes, H. K.; DeLuca, H. F. 1α,25-Dihydroxy-19-nor-vitamin $D_3$, A Novel Vitamin D-Related Compound with Potential Therapeutic Activity. *Tetrahedron Lett.* **1990**, *31*, 1823−1824.

(7) Jung, S. J.; Lee, Y. Y.; De Vos, S.; Elstner, E.; Norman, A. W.; Green, J.; Uskokovic, M.; Koeffler, H. P. 1,25$(OH)_2$-16-Ene-vitamin $D_3$ is a Potent Antileukemic Agent with Low Potential to Cause Hypercalcemia. *Leuk. Res.* **1994**, *101*, 713−718.

(8) Abe, J.; Nakano, T.; Nishii, Y.; Matsumoto, T.; Ogata, E.; Ikeda, K. A Novel Vitamin $D_3$ Analogue, 22-Oxa-1,25-dihydroxyvitamin $D_3$, Inhibits the Growth of Human Breast Cancer in Vitro and in Vivo without Causing Hypercalcemia. *Endocrinology* **1991**, *129*, 832−837.

(9) Haussler, M. R.; Whitfield, G. K.; Haussler, C. A.; Hsieh, J.; Thompson, P. D.; Selznick, S. H.; Dominguez, C. E.; Jurutka, P. W. The Nuclear Vitamin D Receptor: Biological and Molecular Regulatory Properties Revealed. *J. Bone Miner. Res.* **1998**, *13*, 325−349.

(10) Midland, M. M.; Plumet, J.; Okamura, W. H. Effects of C20 Stereochemistry on the Conformational Profile of the Side Chains of Vitamin D Analogues. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 1799−1804.

(11) (a) Yamamoto, K.; Sun, W. Y.; Ohta, M.; Hamada, K.; DeLuca, H. F.; Yamada, S. Conformationally Restricted Analogues of 1α,-25-Dihydroxyvitamin $D_3$ and its 20-Epimer: Compounds for Study of the Three-Dimensional Structure of Vitamin D Responsible for Binding to the Receptor. *J. Med. Chem.* **1996**, *39*, 2727−2737. (b) Yamamoto, K.; Ohta, M.; Umesono, K.; DeLuca, H. F.; Yamada, S. Side-chain conformation and activity of vitamin D: design, synthesis and biological activities of conformationally restricted analogues. In *Vitamin D − Chemistry, Biology and Clinical Applications of the Steroid Hormone*; Norman, A. W., Bouillon, R., Thomasset, M., Eds.; University of California: Riverside; Proceedings of the Tenth Workshop on Vitamin D, Strasbourg, France, May 24−29, 1997; pp 83−84.

(12) Zhu, G.-D.; Chen, Y.; Zhou, X.; Vandewalle, M.; De Clercq, P. J.; Bouillon, R.; Verstuyf, A. Synthesis of CD-ring Modified 1α, 25-Dihydroxy Vitamin D Analogues: C-ring Analogues. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1703−1708.

(13) A leading reference on the photochemistry of 7-DHC in skin: Holick, M. F. McCollum Award Lecture, 1994: Vitamin D − New Horizons for the 21st Century. *Am. J. Clin. Nutr.* **1994**, *60*, 619−630.

(14) Curtin, M. L.; Okamura, W. H. 1α,25-Dihydroxyprevitamin $D_3$: Synthesis of the 9,14,19,19,19-Pentadeuterio Derivative and a Kinetic Study of Its [1,7]-Sigmatropic Shift to 1α,25-Dihydroxyvitamin $D_3$. *J. Am. Chem. Soc.* **1991**, *113*, 6958−6966.

(15) Havinga, E. Vitamin D, Example and Challenge. *Experientia* **1973**, *29*, 1181 and references therein.

(16) Bouillon, R.; Sarandeses, L. A.; Allewaert, K.; Zhao, J.; Mascarenas, J. L.; Mouriño, A.; Vrielynck, S.; De Clercq, P.; Vandewalle, M. Biologic Activity of Dihydroxylated 19-Nor-(Pre) Vitamin $D_3$. *J. Bone Miner. Res.* **1993**, *8*, 1009−1015.

(17) Ireland, R. E.; Mueller, R. H. The Claisen Rearrangement of Allyl Esters. *J. Am. Chem. Soc.* **1972**, *94*, 5897−5898.

(18) (a) Lythgoe, B.; Moran, T. A.; Nambudiry, M. E. N.; Tideswell, J.; Wright, P. W. Calciferol and Its Relatives. Part 22. A Direct Total Synthesis of Vitamin $D_2$ and Vitamin $D_3$. *J. Chem. Soc., Perkin Trans. I* **1978**, 590−595. (b) Kocienski, P. J.; Lythgoe, B.; Ruston, S. Calciferol and Its Relatives. Part 24. A Synthesis of Vitamin $D_4$. *J. Chem. Soc., Perkin Trans. I* **1978**, 1290−1293.

(19) Baggiolini, E. G.; Iacobelli, J. A.; Hennessy, B. M.; Batcho, A. D.; Sereno, J. F.; Uskokovic, M. R. Stereocontrolled Total Synthesis of 1α,25-Dihydroxycholecalciferol and 1α,25-Dihydroxyergocalciferol. *J. Org. Chem.* **1986**, *51*, 3098−3108.

(20) Perlman, K. L.; Swenson, R. E.; Paaren, H. E.; Schnoes, H. K.; DeLuca H. F. Novel Synthesis of 19-nor-Vitamin D Compounds. *Tetrahedron Lett.* **1991**, *32*, 7663−7666.

(21) (a) Takano, S.; Yamada, O.; Iida, H.; Ogasawara, K. Enantiocomplementary Resolution of 2-Phenylthio-2-cyclopentenol and 2-Phenylthio-2-cyclohexenol using the Same Lipase. *Synthesis* **1994**, 592−596. (b) Fukazawa, T.; Hashimoto, T. Synthesis of Enantiomerically Pure (R)−2-Cycloalken-1-ols using Highly Enantioselective Enzymatic Transesterification. *Tetrahedron: Asymmetry* **1993**, *4*, 2323−2326. See also (c) Kawasaki, M.; Suzuki, Y.; Terashima, S. Lithium Aluminum Hydride Partially Decomposed with (−)-*N*-Methylephedrine and 2-Alkylaminopyridine: An Improved Chiral Hydride Useful for the Practical Asymmetric Reduction of Achiral Cyclic Ketones. *Chem. Pharm. Bull.* **1985**, *33*, 52−60. (d) Wu, K.-M.; Okamura, W. H. Hydroxyl-Directing Effects on [1,7]-Sigmatropic Hydrogen Migrations. *J. Org. Chem.* **1990**, *55*, 4025−4033.

(22) Ireland, R. E.; Wipf, P.; Xiang, J.-N. Stereochemical Control in the Ester Enolate Claisen Rearrangement. 2. Chairlike vs Boatlike Transition-State Selection. *J. Org. Chem.* **1991**, *56*, 3572−3582.

(23) White, J. D.; Kawasaki, M. Total Synthesis of (+)−Latrunculin A, an Ichthyotoxic Metabolite of the Sponge *Latrunculia magnifica*, and Its C-15 Epimer. *J. Org. Chem.* **1992**, *57*, 5292−5300.

(24) Chapleo, C. B.; Hallett, P.; Lythgoe, B.; Waterhouse, I.; Wright, P. W. Calciferol and Its Relatives. Part 19. Synthetic Applications of Cyclic Ortho esters: Stereospecific Synthesis of a Bicyclic Alcohol Related to the Vitamins D. *J. Chem. Soc., Perkin Trans. I* **1977**, 1211−1218.
(25) Dehmlow, H.; Mulzer, J.; Seilz, C.; Strecker, A. R.; Kohlmann, A. Stereocontrolled Formation of Polysubstituted Tetrahydrofurans by Debenzylating Cycloetherification. *Tetrahedron Lett.* **1992**, *33*, 3607−3610.
(26) Mascareñas, J. L.; Pérez-Sestelo, J.; Castedo, L.; Mouriño, A. A Short, Flexible Route to Vitamin D Metabolites and Their Side Chain Analogues. *Tetrahedron Lett.* **1991**, *32*, 2813−2816.
(27) Manchand, P. S.; Yiannikouros, G. P.; Belica, P. S.; Madan, P. Nickel-Mediated Conjugate Addition. Elaboration of Calcitriol from Ergocalciferol. *J. Org. Chem.* **1995**, *60*, 6574−6581.
(28) Partridge, J. J.; Faber, S.; Uskokovic, M. R. Vitamin $D_3$ Metabolites I. Synthesis of 25-Hydroxycholesterol. *Helv. Chim. Acta* **1974**, *57*, 764−771.
(29) Sicinski, R. R.; Prahl, J. M.; Smith, C. M.; DeLuca, H. F. New 1α,25-Dihydroxy-19-norvitamin $D_3$ Compounds with High Biological Activity: Synthesis and Biological Evaluation of 2-Hydroxymethyl, 2-Methyl, and 2-Methylene Analogues. *J. Med. Chem.* **1998**, *41*, 4662−4674.
(30) For kinetic data on the natural vitamin−previtamin equilibrium, see ref 14.
(31) Okamura, W. H.; Midland, M. M.; Hammond, M. W.; Rahman, N. A.; Dormanen, M. C.; Nemere, I.; Norman, A. W. Chemistry and Conformation of Vitamin D Molecules. *J. Steroid Biochem. Mol. Biol.* **1995**, *53*, 603−613.
(32) Jorgensen, W. L. Rusting of the Lock and Key Model for Protein-Ligand Binding. *Science* **1991**, *254*, 954−955.
(33) Okamura, W. H.; Palenzuela, J. A.; Plumet, J.; Midland, M. M. Vitamin D: Structure−Function Analyses and the Design of Analogues. *J. Cell. Biochem.* **1992**, *49*, 10−18.
(34) (a) Bouillon, R.; De Clercq, P.; Pirson, P.; Vandewalle, M. Novel Structural Analogues of Vitamin D. Patent PCT/EP 93.202037.3; priority date 09−07−1993. (b) Chen, Y.-J.; De Clercq, P.; Vandewalle, M. Synthesis of New Vitamin $D_3$ Analogues with a Decalin-type CD-Ring. *Tetrahedron Lett.* **1996**, *37*, 9361−9364. (c) Wu, Y.; D'Halleweyn, C.; Van Haver, D.; De Clercq, P.; Vandewalle, M. Synthesis of CD-ring Modified 1α,25-Dihydroxy Vitamin D Analogues: Five-Membered D-ring Analogues. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 923−928. (d) Linclau, B.; De Clercq, P.; Vandewalle, M. Synthesis of CD-ring Modified 1α,25-Dihydroxy Vitamin D Analogues: Six-Membered D-ring Analogues. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 1461−1464. (e) Sabbe, K.; D'Halleweyn, C.; De Clercq, P.; Vandewalle, M.; Bouillon, R.; Verstuyf, A. Synthesis of CD-ring Modified 1α,25-Dihydroxy Vitamin D Analogues: E-ring Analogues. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1697−1702.
(35) Van Haver, D.; De Clercq, P. J. On the Use of Volume Maps in the Conformational Analysis of Vitamin D Analogues. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 1029−1034.
(36) The way a relative activity volume is defined here is reminiscent of the procedure described by Marshall in the active analogue approach; see: Marshall, G. R.; Barry, C. D.; Bosshard, H. E.; Dammkoehler, R. A.; Dunn, D. A. The Conformational Parameter in Drug Design: The Active Analogue Approach. *Computer-Assisted Drug Des.* **1979**, *112*, 205−226.
(37) The views are presented in Figure 2 following a suggestion of one of the reviewers. The second view (right) allows for a better comparison with the standard view used by Yamada (refs 11a and 38).
(38) Yamada, S.; Yamamoto, K.; Masuno, H.; Ohta, M. Conformation-Function Relationship of Vitamin D: Conformational Analysis Predicts Potential Side-Chain Structure. *J. Med. Chem.* **1998**, *41*, 1467−1475.
(39) Van Baelen, H.; Bouillon, R. Purification of the Serum Vitamin D-Binding Protein (DBP) from Different Species. In *Binding Proteins of Steroid Hormones*; Forest, M. G., Pugeat, M., Eds.; John Libbey Eurotext Ltd.: London, 1986; Vol. 149, pp 69−83.
(40) Bouillon, R.; Van Baelen, H.; De Moor, P. Comparative Study of the Affinity of the Serum Vitamin D-Binding Protein. *J. Steroid Biochem.* **1980**, *113*, 1029−1034.
(41) Ostrem, V. K.; Tanaka, Y.; Prahl, J.; DeLuca, H. F.; Ikekawa, N. 24- and 25-Homo-1,25-Dihydroxyvitamin $D_3$: Preferential Activity in Inducing Differentiation of Human Leukemia Cells HL-60 In Vitro. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 2610−2614.
(42) Kitano, Y.; Okada, N. Separation of Epidermal Sheet by Dispase. *Br. J. Dermatol.* **1983**, *108*, 555−560.
(43) Verstuyf, A.; Verlinden, L.; Ling, S.; Wu, Y.; D'Halleweyn, C.; Van Haver, D.; Zhu, G.-D.; Zhu, D.; Chen, Y.; Zhou, X.; Van Baelen, H.; De Clercq, P.; Vandewalle, M.; Bouillon, R. The Biological Activity of Nonsteroidal Vitamin D Hormone Analogues Lacking the C- and D-Rings. *J. Bone Miner. Res.* **1998**, *13*, 549−558.
(44) Mohamadi, F.; Richards, N. G. J.; Guida, W. C.; Liskamp, R.; Lipton, M.; Caufield, C.; Chang, G.; Hendrickson, T.; Still, W. C. MacroModel − An Integrated Software System for Modeling Organic and Bioorganic Molecules using Molecular Mechanics. *J. Comput. Chem.* **1990**, *11*, 440−467.
(45) CS Chem3D, CambridgeSoft Corp., 875 Massachusetts Ave., MA 02139-9586.

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