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Boron Cluster-based Development of Potent Nonsecosteroidal Vitamin D Receptor Ligands: Direct Observation of Hydrophobic Interaction between Protein Surface and Carborane

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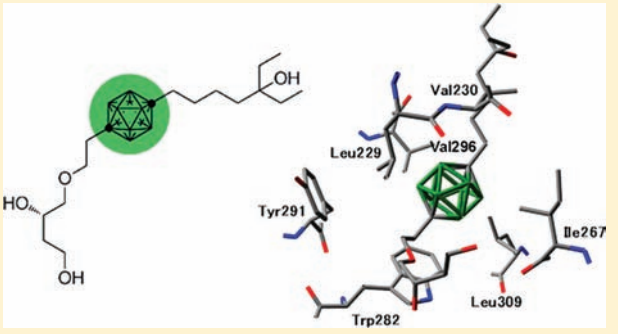
Supporting Information

ABSTRACT: We report here the design and synthesis of a novel vitamin D receptor (VDR) agonist whose hydrophobic core structure is *p*-carborane (1,12-dicarba-*closo*-dodecaborane, an icosahedral carbon-containing boron cluster having remarkable thermal and chemical stability and a characteristically hydrophobic B−H surface). This carborane-based VDR ligand exhibited moderate vitamin D activity, comparable to that of the natural hormone, despite its simple and flexible structure. X-ray structure analysis provided direct evidence that the carborane cage binds to the hydrophobic surface of the ligand-binding pocket of the receptor, promoting transition to the active conformation. These results indicate that the spherical B−H surface of carborane can function efficiently as a hydrophobic anchor in binding to the receptor surface, thereby allowing induced fitting of the three essential hydroxyl groups on the alkyl chains to the appropriate positions for interaction with the VDR binding site, despite the entropic disadvantage of the flexible structure. We suggest that carborane structure is a promising option in the design of novel drug candidates.



INTRODUCTION

Icosahedral carboranes (dicarba-*closo*-dodecaboranes; Figure 1), which are polyhedral carbon-containing boron clusters, have a bulky spherical structure and exhibit high hydrophobicity as well as having high thermal and chemical stability.[1] The remarkable stability of carboranes makes their derivatives particularly suitable for specialized applications, e.g., in polymers for high-temperature use and as neutron-shielding materials. In the field of life sciences, carboranes are considered to be candidates for boron neutron capture therapy (BNCT) of cancer, based on the nuclear reaction between ^{10}B nuclei and thermal neutrons.[2] Various types of compounds have been prepared, including amino acid derivatives, nucleic acid derivatives, and porphyrin derivatives, with the aim of incorporating ^{10}B atoms selectively into cancer cells,[3] but the carborane cage has been regarded as simply a boron carrier. Little attention has been paid to the possibility of using the spherical hydrophobic B−H surface, the most characteristic feature of carboranes, to obtain biologically active molecules.[4] Hydrophobic interaction plays an essential role in interactions between proteins and bioactive compounds, and therefore the use of carboranes as a hydrophobic core could make it possible to develop a novel class of biologically active molecules. In particular, hydrophobic structure is important for nuclear receptor ligands, such as steroid hormones and activated vitamins, to bind effectively to their specific receptors.[5] We have applied the carborane cage as a hydrophobic structural moiety of nuclear receptor ligands and developed novel carborane-based ligands for nuclear receptors, such as androgen receptor (AR),[6] estrogen receptor (ER),[7] and retinoid receptors.[8]

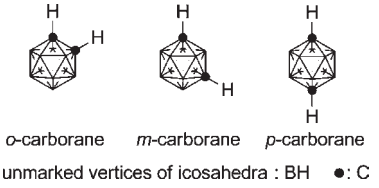


Figure 1. Structures of carboranes.

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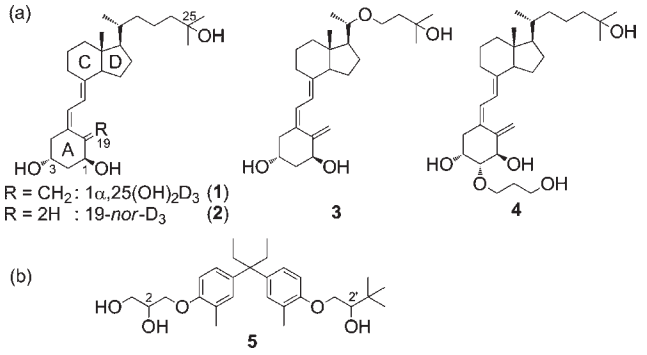


Figure 2. Structures of (a) vitamin D derivatives with secosteroid structure and (b) a nonsecosteroidal VDR ligand.

These carborane-based nuclear receptor ligands bearing an unnatural hydrophobic core structure, i.e., nonsteroidal and nonterpenoidal ligands, are advantageous for separation of pleiotropic receptor functions as well as having excellent chemical stability.

Vitamin D receptor (VDR) is the ligand-inducible nuclear receptor for vitamin D and plays important roles in many physiological processes, including calcium and phosphate homeostasis, bone metabolism, immune regulation, cell proliferation, and differentiation.[9] VDR is activated by binding of the endogenous agonist, 1α,25-dihydroxyvitamin D_3 [1α,25$(OH)_2D_3$; **1**], a metabolically activated form of vitamin D_3, and regulates expression of specific target genes. VDR and its ligands have significant roles in the pathogenesis and therapy of diseases, such as osteoporosis, arthritis, psoriasis, and cancers. Thus, thousands of VDR ligands have been synthesized and biologically evaluated as drug candidates, leading to the clinical application of maxacalcitol (**3**) and eldecalcitol (**4**).[10]

Structurally, almost all of the developed VDR ligands with high potency have the same secosteroidal skeleton as **1** or **2**, consisting of the A-ring bearing two hydroxyl groups, a conjugated diene or triene moiety, the CD-ring, and a side chain (Figure 2). Although secosteroidal derivatives have high potency, their structural complexity, synthetic inconvenience, and chemical instability are disadvantageous for potential clinical application. Further, there are strict structural requirements, such as the substituent configuration on the A ring in the secosteroid structure. Though some work has been done to develop nonsecosteroidal VDR ligands, such as des-C and/or D ring derivatives of **1**[11] and bisphenol derivatives such as **5**,[12] in the past decade, it remains important to discover potent nonsecosteroidal VDR ligands. Nonsecosteroidal compounds would be useful as clinical drug candidates and as tools for investigation of VDR function, by analogy with nonsteroidal ligands of steroid hormone receptors, such as the ER ligands tamoxifen and raloxifene[13] and the AR ligands flutamide and bicalutamide. Herein, we describe the development of nonsecosteroidal VDR ligands, based on the carborane cage, and we also report the direct observation, by means of X-ray crystal structure analysis, of the interaction between a carborane-based VDR ligand and the specific receptor.[14]

RESULTS

Design and Synthesis of Carborane-Based VDR Ligand Candidates. Four structural elements are required for effective binding to VDR: one is a hydrophobic core with appropriate bulkiness, that corresponds to the CD-ring of **1**, and the others are three appropriately positioned hydroxyl groups. We assumed that the CD-ring of secosteroidal structure could be replaced by a carborane cage, from the viewpoints of spatial size, hydrophobicity, and stability. Regarding hydrophilic moieties, structure–activity relationship studies on secosteroidal vitamin D analogs have revealed that the configuration and conformation of hydroxyl groups, especially the hydroxyl groups at the 1- and 3-positions of the A-ring, are critical for vitamin D potency. We assumed, however, that a flexible dihydroxyalkyl group would be able to function as an alternative to the dihydroxylated A-ring, if the hydrophobic carborane cage functions as potent anchor in binding. Generally, a flexible backbone is entropically less effective than cyclic structure to retain active conformation. However, if the carborane cage interacts strongly with the hydrophobic surface of the receptor, the flexible alkyl chains might permit induced fitting of the three hydroxyl groups to the positions required for VDR binding, so that the whole molecule would function as a VDR ligand. A few opened A-ring analogs of **1** were synthesized, and application of the flexible diol as the hydrophilic pharmacophore of VDR ligands had been suggested.[15] Further, the lack of conjugated diene or triene structure would make the compound more stable chemically. On the basis of the above considerations, we designed a series of dialkylcarborane derivatives which have three hydroxyl groups bound to flexible acyclic alkyl or ether chains (Figure 3).

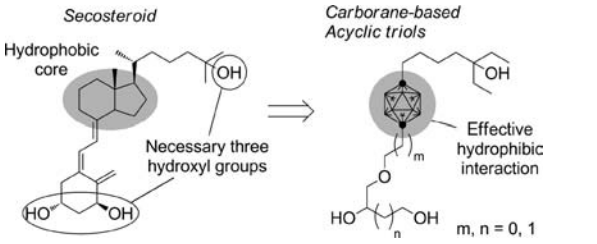
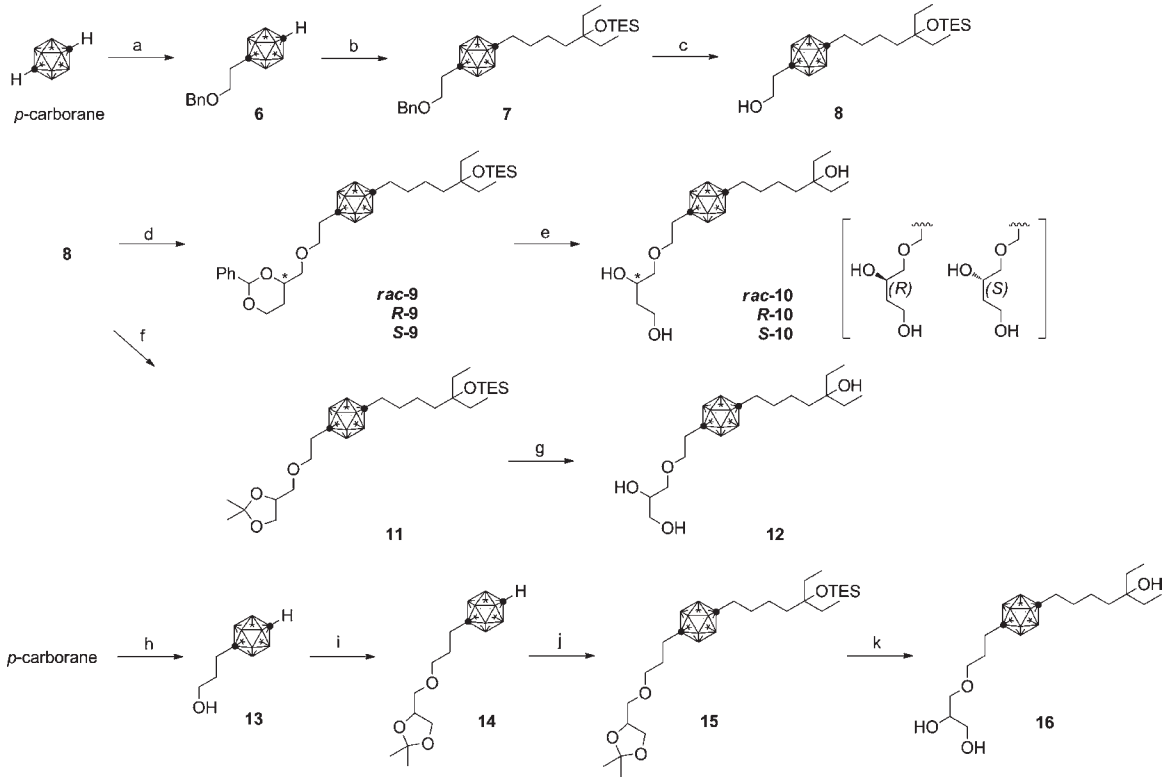


Figure 3. Design strategy for novel nonsecosteroidal VDR ligands bearing a carborane cage.

As a hydrophobic core, we chose *p*-carborane, considering the geometry of the carbon atoms, at which substituents are introduced and the fact that it has the highest hydrophobicity among the three stereoisomers of carborane.[16] Diethylcarbinol was selected as the side chain terminal in place of the dimethylcarbinol of **1** based on previous reports that indicated diehylcarbinol moiety enhanced the vitamin D potency.[17] An ether oxygen atom was introduced into the dihydroxyalkyl chain to provide efficient synthetic approaches. In addition, the less hindered oxa tethering chain may be favorable for avoiding steric repulsion between the chain and the hydrophobic carborane core.[18]

The designed carborane derivatives were synthesized from *p*-carborane (Scheme 1). The hydrogen atom on carbon of the carborane cage is sufficiently acidic to be removed by a strong base, such as butyl lithium, and the resulting C-lithiated form of carborane is a good nucleophilic reagent.[19] Thus, a 2-benzyloxyethyl group was introduced at the carbon atoms of *p*-carborane with *n*-butyl lithium and benzyl 2-bromoethyl ether to afford **6**. Introduction of a 5-ethyl-5-triethylsilyloxyheptyl group, corresponding to the side chain of **1**, gave **7**, and subsequent removal of the benzyl group gave the common intermediate **8**. Ether formation of **8** with appropriate tosylates gave the corresponding triol precursor **9** or **11**. Deprotection of three hydroxyl groups under acidic conditions afforded the target compounds **10** and **12**.

Scheme 1. Synthetic Scheme of Designed Carborane Derivatives[a]



[a] Conditions: (a) *n*-BuLi, benzyl 2-bromoethyl ether, THF, 44%; (b) *n*-BuLi, 7-bromo-3-ethyl-3-triethylsilyloxyheptane, THF, ether, 96%; (c) H_2, Pd/C, EtOH-THF, 91%; (d) NaH, 4-(*p*-toluenesulfonyloxymethyl)-2-phenyl-1,3-dioxane, DMF, 17%; (e) HCl, MeOH-THF-H_2O, 96%; (f) NaH, 4-(*p*-toluenesulfonyloxymethyl)-2,2-dimethyl-1,3-dioxolane, DMF, 45%; (g) HCl, MeOH-THF-H_2O, 92%; (h) *n*-BuLi, trimethylene oxide, ether, 36%; (i) NaH, 4-(*p*-toluenesulfonyloxymethyl)-2,2-dimethyl-1,3-dioxolane, DMF, 26%; (j) *n*-BuLi, 7-bromo-3-ethyl-3-triethylsilyloxyheptane, THF, 94%; and (k) HCl, MeOH-THF-H_2O, 71%. Bn = benzyl, DMF = *N*,*N*-dimethylformamide, TES = triethylsilyl, and THF = tetrahydrofuran.

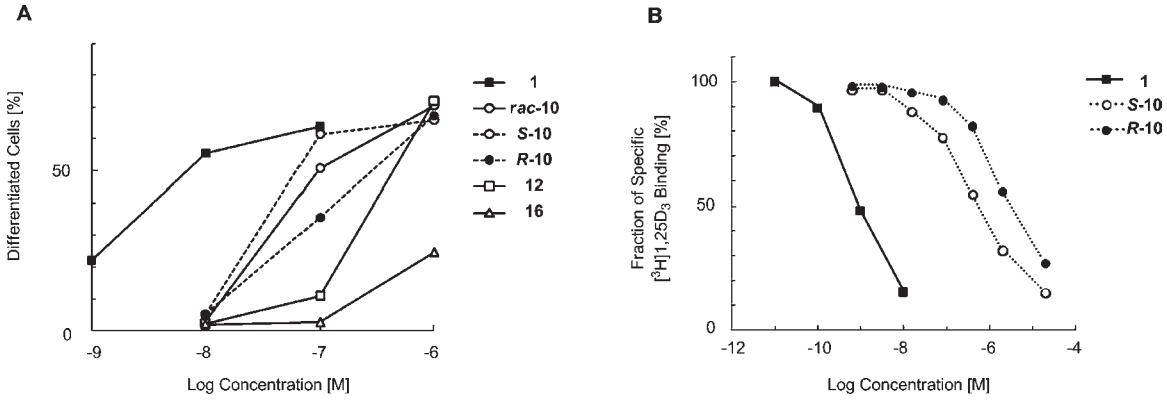


Figure 4. Biological evaluation of carborane derivatives. (A) HL-60 cell differentiation-inducing potency of carborane compounds in the concentration range of 10^{-9} to 10^{-6} M. Cell differentiation was determined as the ratio of NBT-positive cells. (B) Competitive binding assay of carborane derivatives using bovine thymus VDR-LBD and [^{3}H]1α,25(OH)$_2$D$_3$.

Two stereoisomers of **10** were also synthesized by use of the corresponding chiral tosylates.

Compound **16** bearing the other diol structure was also prepared from *p*-carborane in four steps. The lithiated form of *p*-carborane reacted with trimethylene oxide to afford 3-hydroxypropylcarborane **13**, and then ether formation gave **14**. The side chain moiety was introduced into **14** to afford **15**, and removal of protective groups under acidic conditions gave the triol **16**.

HL-60 Cell Differentiation-Inducing Activity. Vitamin D activity of the synthesized molecules **10**, **12**, and **16** was evaluated in terms of cell differentiation-inducing activity toward human acute promyelocytic leukemia cell line HL-60.[20] These carborane derivatives induced HL-60 cell differentiation in a dose-dependent manner (Figure 4A), and racemic **10** exhibited substantial activity; its potency was approximately one-twentieth of that of **1** and nearly equal to the reported potency of 19-*nor*-D$_3$ (**2**).[9,15]

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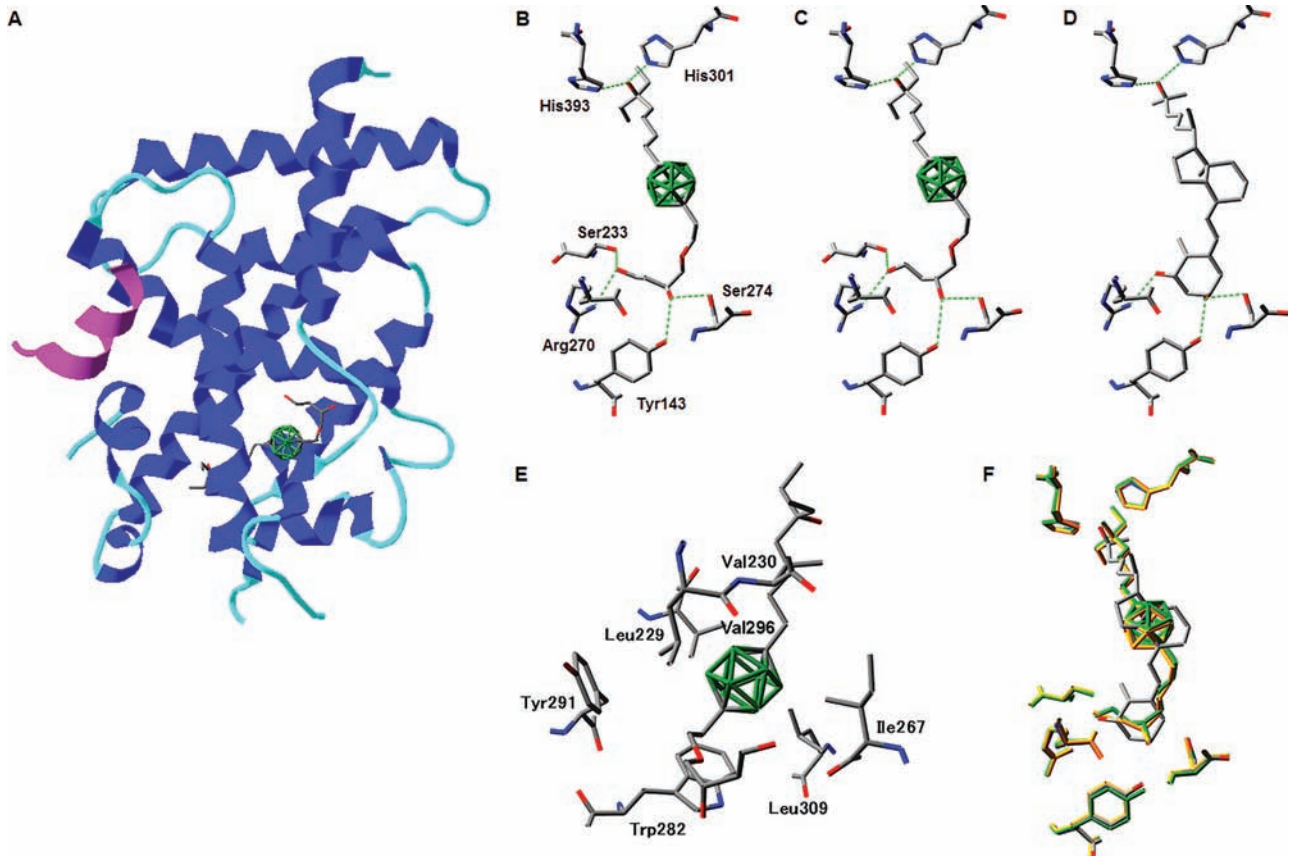

Figure 5. Structures of ligand-bound rat VDR-LBD and coactivator peptide (derived from DRIP205) complexes. (A) Structure of rat VDR-LBD bound to (*S*)-**10**. The coactivator peptide is shown in magenta. (B) Binding mode of (*S*)-**10** to VDR. (C) Binding mode of (*R*)-**10** to VDR. (D) Binding mode of **1** to VDR (PDB ID; 1RK3, ref 21). (E) Hydrophobic residues near the carborane cage of (*S*)-**10**. (F) Superimposition of ligand-binding pockets of the rat VDR-LBD complexed with the ligands: orange for complex of (*S*)-**10**, green for (*R*)-**10** and gray for 1α,25(OH)$_2$D$_3$ (**1**).

Therefore we prepared two enantiomers of **10** and investigated the vitamin D activities. Among the two enantiomers, the *S*-isomer ((*S*)-**10**) exhibited higher potency than the *R*-isomer ((*R*)-**10**). Therefore, *S*-configuration of the hydroxyl group is preferred for vitamin D activity, though the difference of potency between the two isomers was not very large. Compound **12**, bearing 1,2-diol structure, exhibited moderate activity, being less potent than compound **10**. Compound **16** exhibited quite low potency.

VDR-Binding Affinity of Carborane Derivatives. The binding affinity of selected compounds was examined by competitive binding assay using [^{3}H]1α,25(OH)$_2$D$_3$ and bovine thymus VDR ligand-binding domain (LBD) to determine whether the carborane derivatives bind directly to VDR. Figure 4B shows the dose−response curves of receptor binding of the carborane derivatives. Compounds (***S***)-**10** and (*R*)-**10** exhibited dose-dependent binding (IC_{50} = 0.64 and 4.1 μ*M*, respectively). Although their binding potency was lower than that estimated from their functional potency toward HL-60 cells, low binding affinity of nonsecosteroidal VDR ligands was also reported in the case of bisphenol derivatives. Some physicochemical properties of these synthetic ligands may influence the potency, depending on the in vitro assay system. Nevertheless, the binding data were consistent with the results of HL-60 cell differentiation assay: the *S*-isomer of **10** exhibited higher potency than the *R*-isomer.

Structure of Complexes of Novel Carborane Derivatives with VDR. In order to understand the molecular basis of the interaction between the carborane hydrophobic core structure and the specific receptor protein, X-ray crystal structure analyses of VDR-LBD bound to (*S*)-**10** and (*R*)-**10** were conducted using the rat VDR-LBD, whose amino acid sequence in the ligand-binding pocket is identical to that of human VDR-LBD. The structure of rat VDR-LBD complexed with (*S*)-**10** in the presence of the coactivator peptide was determined at 1.93 Å resolution (PDB ID; 3VJS), and that of the complex of the less potent stereoisomer (*R*)-**10** at 2.0 Å resolution (PDB ID; 3VJT).

Figure 5A shows the structure of the complex of VDR-LBD and the most potent carborane derivative (*S*)-**10**. The overall structure of the VDR-LBD bound to (*S*)-**10** is essentially similar to that of VDR-LBD bound to **1** (PDB ID; 1RK3).[21] The coactivator peptide is bound to the AF-2 surface and is located at a typical position for agonist binding. This is a significant result, since the ligand-inducible activation of VDR depended on the three-dimensional structure of holo-VDR. VDR changes its conformation dramatically upon binding to the agonist. Depending on the nature of the induced three-dimensional structural change of VDR, a ligand may act as a partial agonist or antagonist. Carborane derivatives bearing flexible acyclic hydroxyalkyl chains can induce the active conformation of VDR, even though their structure is essentially different from that of secosteroid derivative **1**.

The acyclic triol (*S*)-**10** is located in the same pocket of the LBD as **1**. The structure of (*S*)-**10** in the ligand-binding pocket is shown in detail in Figure 5B. In the complex, the carborane

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Table 1. Comparison of Hydrogen-Bond Distances between Ligand and Amino Acid Residues

	(*S*)-**10**, Å	(*R*)-**10**, Å	1α,25(OH)$_2$D$_3$ (**1**), Å
Ser 233 (Og)···O	2.79	2.70	2.75
Arg 270 (Nh)···O	2.94	2.88	2.83
Tyr 143 (Oh)···O	2.83	3.19	2.67
Ser 274 (Og)···O	2.96	3.24	2.94
His 301 (Ne) ···O	2.68	2.69	2.85
His 393 (Ne) ···O	2.94	3.01	2.70

moiety of (*S*)-**10** binds at the hydrophobic region of the VDR LBD, where the CD ring of **1** would be located (Figure 5E). The carborane is surrounded by hydrophobic amino acids residues (Leu229, Val230, Ile267, Trp282, Tyr291, Val296, and Leu309). The three hydroxyl groups of (*S*)-**10** form hydrogen bonds to residues of the receptor in a similar manner to that in the case of **1** (Figure 5B, D and Table 1). Thus, the terminal primary hydroxyl group of the 1,3-diol part of (*S*)-**10**, corresponding to the 1α-hydroxyl group of **1**, forms hydrogen bonds to Ser233 (OH···O: 2.79 Å) and Arg270 (OH···O: 2.94 Å). The secondary hydroxyl group of (*S*)-**10**, corresponding to the 3-hydroxyl group of **1**, interacts with Tyr143 (OH···O: 2.83 Å) and Ser274 (OH···O: 2.96 Å). The tertiary alcohol of the other alkyl chain interacts with His301 (OH···N: 2.68 Å) and His393 (OH···N: 2.94 Å), which interact with the 25-hydroxyl group of **1**.

The stereoisomer (*R*)-**10** also binds to the ligand-binding pocket of the VDR-LBD, and the overall structure of the complex is essentially similar to the active conformation induced by **1** or (*S*)-**10**. Figure 5C shows the interaction of (*R*)-**10** with the VDR-LBD. The hydroxyl groups of (*S*)-**10** and (*R*)-**10** are placed in similar positions in the two complexes, though the carbon backbones are located in slightly different positions, especially in the region of the terminal diol (Figure 5F). This conformational difference enables the hydroxyl groups with different stereochemistry to interact similarly with the same amino acid residues of the receptor. A comparison of **1** and the carborane derivatives indicates that the hydroxyl groups of these molecules are located at the same positions. On the other hand, the locations of the carbon atoms in the backbone of the carborane derivatives are considerably different from those of **1**, especially at the alkyl chain bearing two hydroxyl groups (corresponding to the A-ring) and the conjugated diene part of **1**. These results imply that the positions of the three hydroxyl groups in the carborane derivatives are adjustable to form appropriate hydrogen-bonds for VDR ligand function due to the flexible nature of the molecules.

DISCUSSION

We designed the unique flexible acyclic triols as a novel type of VDR ligand, focusing on the highly hydrophobic character of the icosahedral carborane cage. These flexible acyclic triols, designed as vitamin D analogs without the A-ring and conjugated diene/triene structures, exhibited potent activity, comparable to that of secosteroid **1**. Generally, an acyclic structure is entropically disadvantageous in interaction with a receptor, in comparison to a rigid cyclic structure. However, in the case of the acyclic VDR ligands developed here, flexibility of the diol moiety seems to be favorable for inducing vitamin D activity. Though (*S*)-**10** exhibited the highest potency toward HL-60 cells among the synthesized molecules, its enantiomer (*R*)-**10** also exhibited considerable activity. In addition, compound **12**, which has a 1,2-diol system, also showed moderate potency. These results contrast with the structure−activity relationship of secosteroidal compounds, in which the position and configuration of the hydroxyl groups are critical for vitamin D potency.[9] These results suggest that a suitable conformation of the two hydroxyl groups corresponding to the hydroxyl groups of **1** is induced as a consequence of the molecular flexibility of **10**.

The X-ray crystal structures revealed the molecular basis of the activity of these novel VDR ligands bearing a carborane cage, providing the first direct evidence that a carborane derivative can bind to a specific receptor protein.[22] Although the acyclic **10** exhibited relatively weak binding affinity, the X-ray structure supports the idea that **10** functions as a VDR agonist by inducing the active conformation of the receptor. In the crystal structure, the primary and secondary hydroxyl groups of the diol moiety of **10** correspond to the 1α- and 3-hydroxyl groups of **1**, respectively. It is reasonable that compound (*S*)-**10** is a more potent VDR ligand than the *R*-isomer, because (*S*)-**10** has the same stereochemistry of the secondary hydroxyl group as the 3-hydroxyl group of **1**. The fact that the difference of activity between the two enantiomers of **10** is rather small indicates that the flexible side chain can allow the two hydroxyl groups to take appropriate positions for hydrogen bonding without conformational instability. The locations of carbon atoms in the acyclic diol moiety and tethering methylene chain are rather different from the locations of the A-ring and the conjugated diene of **1**. The straight-chain backbone adopts a curvilinear conformation, and the hydroxyl groups can take suitable positions to interact with the receptor. Thus, the flexibility of acyclic triols is thought to be important for their vitamin D activity, and this is the reason why the structure−activity relationship is so different from that of secosteroids. These observations should be useful for further ligand development. A comparison with the reported crystal structure of the nonsecosteroidal VDR ligand (2*S*,2′*R*)-**5**,[23] the most potent stereoisomer of LG190178 (**5**),[24] indicates that the carborane-based VDR ligand (*S*)-**10** forms hydrogen bonds more effectively. All the hydroxyl groups of (*S*)-**10** form direct hydrogen bonds to the protein, whereas the terminal hydroxyl group of (2*S*,2′*R*)- **5** does not interact directly with the receptor. The combination of the carborane core and flexible acyclic side chain structure appears to make it easily possible for a polar substituent to take a suitable position for hydrogen-bond formation. These findings will be helpful for further development of carborane-containing bioactive molecules. Further, the finding that linear acyclic triols can function as vitamin D ligands represents a breakthrough in the development of nonsecosteroidal VDR ligands. The molecules synthesized in this study are expected to be versatile lead compounds for development of new-generation VDR ligands.

CONCLUSIONS

In conclusion, we have developed a novel nonsecosteroidal VDR ligand by exploiting the hydrophobicity of *p*-carborane. The novel VDR ligand exhibited potent vitamin D activity, comparable to that of the natural hormone, despite its flexible structure. X-ray crystallographic analysis revealed that the hydrophobic, spherical B−H surface of carborane could function as a

potent hydrophobic anchor in binding to the protein surface. The effective hydrophobic interaction between carborane and the receptor protein thus counteracted the entropic disadvantage of interaction of a flexible molecule. This is the first direct observation of the interaction of a boron cluster with a specific receptor protein. Our results open the way for further applications of carboranes in the field of chemical biology and medicinal chemistry

EXPERIMENTAL SECTION

Chemistry. *General Remarks.* NMR spectra were recorded on Bruker AVANCE 400 or AVANCE 500 spectrometers. Chemical shifts are reported in ppm as δ values from tetramethylsilane. Data are reported as follows: chemical shift, multiplicity (s, singlet; d, doublet; t, triplet; q quartet; br, broad; and m, multiplet), coupling constants (Hz), and integration. Mass spectra were collected on a Bruker Daltonics microTOF-2focus or JEOL AX505H in the positive and negative ion modes. Melting points were obtained on a Yanagimoto micro melting point apparatus without correction.

*1-(2-Benzyloxyethyl)-1,12-dicarba-closo-dodecaborane (**6**).* A 1.6 M solution of *n*-BuLi in *n*-hexane (9.53 mL, 15.3 mmol) was added to a solution of *p*-carborane (2.00 g, 13.9 mmol) in THF (80 mL) at 0 °C, and the mixture was stirred at room temperature for 10 min under Ar. Then, benzyl 2-bromoethyl ether (4.48 g, 20.8 mmol) was added to the mixture at 0 °C, and stirring was continued at room temperature for 16 h. The reaction mixture was poured into water and extracted with ethyl acetate. The organic layer was washed with brine, dried over sodium sulfate, and then concentrated. Purification by silica gel column chromatography (eluent; hexane) gave **6** (1.70 g, 6.11 mmol, 44%) as a white wax. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.30−7.20 (m, 5 H), 4.39 (s, 2 H), 3.26 (t, J = 7.1 Hz, 2 H), 3.0−1.3 (br m, 10 H), 2.64 (br s, 1 H), 1.95 (t, J = 7.1 Hz, 2 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 137.9, 128.4, 127.6, 127.5, 81.5, 72.9, 68.6, 58.5, 38.1; MS (EI+) m/z 279 [$(M - H)^+$: calcd for $C_{11}H_{21}B_{10}O$, 279].

*1-(2-Benzyloxyethyl)-12-(5-triethylsilyloxy-5-ethylheptyl)-1,12-dicarba-closo-dodecaborane (**7**).* A 1.6 M solution of *n*-BuLi in *n*-hexane (4.08 mL, 6.54 mmol) was added to a solution of **6** (1.60 g, 5.75 mmol) in THF (20 mL) at 0 °C, and the mixture was stirred at room temperature for 15 min under Ar. Then, 7-bromo-3-ethyl(3-triethylsilyloxy)-heptane (1.70 g, 5.04 mmol) was added to the mixture at 0 °C, and stirring was continued at room temperature for 40 h. The reaction mixture was poured into water and extracted with ethyl acetate. The organic layer was washed with brine, dried over sodium sulfate, and then concentrated. Purification by silica gel column chromatography (eluent; hexane/ethyl acetate, 100/1 to 20/1) gave **7** (2.60 g, 4.86 mmol, 96%) as a colorless oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.31−7.25 (m, 5 H), 4.39 (s, 2 H), 3.25 (t, J = 7.1 Hz, 2 H), 3.0−1.3 (br m, 10 H), 1.95 (t, J = 7.1 Hz, 2 H), 1.60 (m, 2 H), 1.39 (q, J = 7.5 Hz, 4 H), 1.27 (m, 2 H), 1.08 (m, 4 H), 0.92 (t, J = 7.9 Hz, 9 H), 0.78 (t, J = 7.3 Hz, 6 H), 0.54 (q, J = 7.9 Hz, 6 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 137.9, 128.4, 127.6, 127.5, 79.9, 77.9, 75.8, 72.9, 68.8, 38.3, 37.9, 36.9, 31.6, 30.2, 23.3, 8.3, 7.2, 7.0; HRMS (ESI+) m/z 559.4725 [$(M + Na)^+$: calcd for $C_{26}H_{54}B_{10}NaO_2Si$, 559.4721].

*1-(2-Hydroxyethyl)-12-(5-triethylsilyloxy-5-ethylheptyl)-1,12-dicarba-closo-dodecaborane (**8**).* A mixture of **7** (2.50 g, 4.67 mmol) and 10% palladium on carbon (250 mg) in ethanol (40 mL) and THF (5 mL) was stirred at room temperature for 3 days under atmospheric pressure of hydrogen. Insoluble materials were filtered off through Celite, and the filtrate was concentrated. Purification by silica gel column chromatography (eluent; hexane/ethyl acetate, 10/1) gave **8** (1.90 g, 91%) as a white solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.44 (dt, J = 5.8 Hz, 7.0 Hz, 2 H), 3.3−1.3 (br m, 10 H), 1.89 (t, J = 7.0 Hz, 2 H), 1.61 (m, 2 H), 1.37 (q, J = 7.9 Hz, 4 H), 1.26 (m, 3 H), 1.07 (m, 4 H), 0.92 (t, J = 7.7 Hz, 9 H), 0.78 (t, J = 7.3 Hz, 6 H), 0.52 (q, J = 7.9 Hz, 6 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 80.0, 77.9, 75.6, 61.4, 39.8, 38.3, 37.9, 31.57, 31.54, 30.2, 23.2, 8.3, 7.2, 7.0; HRMS (ESI+) m/z 469.4254 [$(M + Na)^+$: calcd for $C_{19}H_{48}B_{10}NaO_2Si$, 469.4251].

*1-{2-[(2-Phenyl-1,3-dioxan-4-yl)methoxy]ethyl}-12-(5-triethylsilyloxy-5-ethylheptyl)-1,12-dicarba-closo-dodecaborane (rac-**9**).* Compound **8** (500 mg, 1.12 mmol) was added to a suspension of sodium hydride (58 mg, 60% in oil, 1.46 mmol) in DMF (11 mL) at 0 °C, and the mixture was stirred at room temperature for 50 min under Ar. Then, 4-(*p*-toluenesulfonyloxymethyl)-2-phenyl-1,3-dioxane (1.00 g, 2.87 mmol) was added to the mixture at 0 °C, and stirring was continued at room temperature for 40 h. The reaction mixture was poured into water and extracted with ethyl acetate. The organic layer was washed with brine, dried over sodium sulfate, and then concentrated. Purification by silica gel column chromatography (eluent; hexane/ethyl acetate, 20/1 to 5/1) gave *rac*-**9** (120 mg, 0.193 mmol, 17%) as a colorless oil with 50% recovery of starting material. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.48 (m, 2H), 7.34 (m, 3H), 5.51 (s, 1H), 4.27 (m, 1 H), 3.99 (m, 2 H), 3.50 (dd, J = 10.3 Hz, 5.9 Hz, 1 H), 3.38 (dd, J = 10.3 Hz, 4.6 Hz, 1 H), 3.3−1.3 (br m, 10 H), 3.26 (m, 2 H), 1.91 (t, J = 7.1 Hz, 2 H), 1.75−1.90 (m, 2 H), 1.58−1.49 (m, 2 H), 1.39 (q, J = 7.7 Hz, 4 H), 1.27 (m, 2 H), 1.09 (m, 4 H), 0.92 (t, J = 7.9 Hz, 9 H), 0.78 (q, J = 7.5 Hz, 6 H), 0.53 (q, J = 8.1 Hz, 6 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 138.4, 128.8, 128.2, 126.1, 101.2, 79.9, 77.9, 76.2, 75.7, 73.6, 69.9, 66.8, 38.3, 37.9, 36.9, 31.6, 30.2, 28.0, 23.2, 8.3, 7.2, 7.0; HRMS (ESI+) m/z 645.5099 [$(M + Na)^+$: calcd for $C_{30}H_{60}B_{10}NaO_4Si$, 645.5090].

Compounds *R*-**9** and *S*-**9** were also prepared from the corresponding tosylates. All spectra data were the same as those of *rac*-**9**.

*1-[2-(2,4-Dihydroxybutoxy)ethyl]-12-(5-ethyl-5-hydroxyheptyl)-1,12-dicarba-closo-dodecaborane (rac-**10**).* Compound *rac*-**9** (100 mg, 0.161 mmol) was dissolved in methanol (4 mL) and THF (1 mL), and then 2 M hydrochloric acid (0.5 mL) was added to the solution at 0 °C. The mixture was stirred for 16 h at room temperature, poured into water, and extracted with ethyl acetate. The organic layer was washed with brine, dried over sodium sulfate, and then concentrated. Purification by silica gel column chromatography (eluent; hexane/ethyl acetate, 1/1) gave *rac*-**10** (65 mg, 0.155 mmol, 96%) as a white solid. mp 79−80 °C (*n*-hexane); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.97 (m, 1 H), 3.84 (m, 2 H), 3.34 (dd, J = 9.2 Hz, 3.5 Hz, 1 H), 3.3−1.3 (br m, 10 H), 3.24 (m, 3H), 2.60 (d, J = 3.2 Hz, 1 H), 2.32 (t, J = 5.3 Hz, 1 H), 1.90 (t, J = 6.7 Hz, 2 H), 1.70−1.55 (m, 4 H), 1.40 (q, J = 7.5 Hz, 4 H), 1.32−1.27 (m, 2 H), 1.16−1.08 (m, 4 H), 1.00 (s, 1H), 0.82 (t, J = 7.5 Hz, 6 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 79.9, 75.7, 74.7, 74.4, 70.1, 69.5, 61.0, 37.76, 37.73, 36.9, 34.6, 30.9, 30.0, 22.9, 7.7; HRMS (ESI+) m/z 443.3922 [$(M + Na)^+$: calcd for $C_{17}H_{42}B_{10}NaO_4$, 443.3909].

Compounds *R*-**10** and *S*-**10** were also prepared from *R*-**9** and *S*-**9**, respectively. All spectra data were the same as those of *rac*-**10**.

*1-{2-[(2,2-Dimethyl-1,3-dioxolan-4-yl)methoxy]ethyl}-12-(5-triethylsilyloxy-5-ethylheptyl)-1,12-dicarba-closo-dodecaborane (**11**).* Compound **8** (400 mg, 0.899 mmol) was added to a suspension of sodium hydride (47 mg, 60% in oil, 1.17 mmol) in DMF (10 mL) at 0 °C, and the mixture was zstirred at room temperature for 50 min under Ar. Then, 4-(*p*-toluenesulfonyloxymethyl)-2,2-dimethyl-1,3-dioxolane (500 mg, 1.74 mmol) was added to the mixture at 0 °C, and stirring was continued at room temperature for 40 h. The reaction mixture was poured into water and extracted with ethyl acetate. The organic layer was washed with brine, dried over sodium sulfate, and then concentrated. Purification by silica gel column chromatography (eluent; hexane/ethyl acetate, 20/1 to 5/1) gave **11** (230 mg, 0.401 mmol, 45%) as a colorless oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.19 (m, 1 H), 4.02 (dd, J = 8.2 Hz, 6.4 Hz, 1 H), 3.69 (dd, J = 8.2 Hz, 6.4 Hz, 1 H), 3.40 (dd, J = 9.7 Hz, 5.5 Hz, 1 H), 3.33 (dd, J = 9.7 Hz, 5.5 Hz, 1 H), 3.3−1.3 (br m, 10 H), 3.22 (m, 2 H), 1.90 (t, J = 7.5 Hz, 2 H), 1.58 (m, 2 H), 1.40 (s, 3 H), 1.38 (q, J = 7.7 Hz, 4 H), 1.35 (s, 3 H), 1.27 (m, 2 H), 1.08

(m, 4 H), 0.92 (t, J = 7.7 Hz, 9 H), 0.78 (t, J = 7.5 Hz, 6 H), 0.54 (q, J = 7.9 Hz, 6 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 109.4, 79.9, 77.9, 75.6, 74.5, 71.6, 69.9, 66.7, 38.3, 37.9, 36.7, 31.6, 30.2, 26.7, 25.4, 23.2, 8.3, 7.2, 7.0; HRMS (ESI+) m/z 583.4935 [$(M + Na)^+$: calcd for $C_{25}H_{58}B_{10}NaO_4Si$, 583.4933].

*1-[2-(2,3-Dihydroxypropoxy)ethyl]-12-(5-ethyl-5-hydroxyheptyl)-1,12-dicarba-closo-dodecaborane (**12**).* Compound **11** (200 mg, 0.349 mmol) was dissolved in methanol (6 mL) and THF (2 mL), and then 2 M hydrochloric acid (2 mL) was added to the solution at 0 °C. The mixture was stirred for 16 h at room temperature, poured into water, and extracted with ethyl acetate. The organic layer was washed with brine, dried over sodium sulfate, and then concentrated. Purification by silica gel column chromatography (eluent; hexane/ethyl acetate, 1/1) gave **12** (130 mg, 0.321 mmol, 92%) as a white solid. mp 104−105 °C (n-hexane), ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.79 (m, 1 H), 3.65 (m, 1H), 3.62 (m, 1 H), 3.42−3.36 (m, 2 H), 3.3−1.3 (br m, 10 H), 3.27−3.20 (m, 2 H), 2.46 (d, J = 5.5 Hz, 1 H), 1.99 (m, 1 H), 1.90 (t, J = 6.8 Hz, 2 H), 1.60 (m, 2 H), 1.40 (q, J = 7.5 Hz, 4 H), 1.30−1.26 (m, 2 H), 1.14−1.08 (m, 4 H), 1.00 (s, 1 H), 0.82 (t, J = 7.5 Hz, 6 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 79.9, 75.7, 74.5, 72.2, 70.4, 69.7, 63.9, 37.76, 37.72, 36.9, 30.9, 30.0, 22.9, 7.7; HRMS (ESI+) m/z 429.3758 [$(M + Na)^+$: calcd for $C_{16}H_{40}B_{10}NaO_4$, 429.3753].

*1-(3-Hydroxypropyl)-1,12-dicarba-closo-dodecaborane (**13**).* A 1.57 M solution of n-BuLi in n-hexane (24.3 mL, 38.1 mmol) was added to a solution of p-carborane (5.00 g, 34.7 mmol) in ether (120 mL) at 0 °C, and the mixture was stirred at room temperature for 1 h. Then, trimethylene oxide (2.21 g, 38.1 mmol) was added to the mixture, and stirring was continued at room temperature for 16 h. The reaction mixture was poured into water and extracted with ether. The organic layer was washed with brine, dried over magnesium sulfate, and then concentrated. Purification by silica gel column chromatography (eluent; hexane/ethyl acetate, 8:1) gave **13** (36%) as a white solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.49 (t, J = 6.3 Hz, 2 H), 3.0−1.4 (br m, 10 H), 2.66 (br s, 1 H), 1.76−1.70 (m, 2 H), 1.47−1.39 (m, 2 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 84.1, 61.8, 58.1, 35.3, 32.2; MS (EI+) m/z 210 [$(M - H)^+$: calcd for $C_5H_{17}B_{10}O$, 201].

*1-{3-[(2,2-Dimethyl-1,3-dioxolan-4-yl)methoxy]propyl}-1,12-dicarba-closo-dodecaborane (**14**).* Compound **13** (900 mg, 4.45 mmol) was added to a suspension of sodium hydride (214 mg, 60% in oil, 5.34 mmol) in DMF (18 mL) at 0 °C, and the mixture was stirred at room temperature for 1.0 h under Ar. Then 4-(p-toluenesulfonyloxymethyl)-2,2-dimethyl-1,3-dioxolane (1.20 g, 4.19 mmol) was added to the mixture at 0 °C, and stirring was continued at room temperature for 20 h. The reaction mixture was poured into water and extracted with ethyl acetate. The organic layer was washed with brine, dried over sodium sulfate, and then concentrated. Purification by silica gel column chromatography (eluent; hexane/ethyl acetate, 10/1 to 3/1) gave **14** (350 mg, 1.11 mmol, 26%) as a colorless oil. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 4.19 (m, 1 H), 4.02 (dd, J = 8.2 Hz, 6.4 Hz, 1 H), 3.69 (dd, J = 8.2 Hz, 6.4 Hz, 1 H), 3.45 (dd, J = 9.7 Hz, 5.5 Hz, 1 H), 3.38−3.26 (m, 3 H), 3.3−1.3 (br m, 10 H), 2.65 (br s, 1 H), 1.71 (m, 2 H), 1.46 (m, 1H), 1.43 (s, 3 H), 1.38 (s, 3 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 109.4, 84.2, 74.6, 71.7, 70.5, 66.7, 58.0, 35.5, 29.3, 26.7, 25.4; HRMS (ESI+) m/z 341.2868 [$(M + Na)^+$: calcd for $C_{11}H_{28}B_{10}NaO_3$, 341.2864].

*1-{3-[(2,2-Dimethyl-1,3-dioxolan-4-yl)methoxy]propyl}-12-(5-triethylsilyloxy-5-ethylheptyl)-1,12-dicarba-closo-dodecaborane (**15**).* A 1.6 M solution of n-BuLi in n-hexane (0.900 mL, 1.43 mmol) was added to a solution of **14** (350 mg, 1.11 mmol) in THF (5 mL) at 0 °C, and the mixture was stirred at room temperature for 10 min under Ar. Then, 7-bromo-3-ethyl-(3-triethylsilyloxy)heptane (640 mg, 1.90 mmol) was added to the mixture at 0 °C, and stirring was continued at room temperature for 40 h. The reaction mixture was poured into water and extracted with ethyl acetate. The organic layer was washed with brine, dried over sodium sulfate, and then concentrated. Purification by silica gel column chromatography (eluent; hexane/ethyl acetate, 100/1 to 10/1) gave **15** (600 mg, 1.05 mmol, 94%) as a colorless oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.20 (m, 1 H), 4.02 (dd, J = 8.2 Hz, 6.4 Hz, 1 H), 3.66 (dd, J = 8.2 Hz, 6.4 Hz, 1 H), 3.42 (dd, J = 9.9 Hz, 5.7 Hz, 1 H), 3.36−3.24 (m, 3 H), 3.3−1.3 (br m, 10 H), 1.70−1.65 (m, 2 H), 1.62−1.55 (m, 2 H), 1.46−1.34 (m, 6 H), 1.40 (s, 3 H), 1.35 (s, 3 H), 1.30−1.24 (m, 2 H), 1.10−1.05 (m, 4 H), 0.92 (t, J = 8.1 Hz, 9 H), 0.78 (t, J = 7.5 Hz, 6 H), 0.54 (q, J = 7.9 Hz, 6 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 109.4, 79.4, 78.6, 77.9, 74.6, 71.7, 70.5, 66.7, 38.3, 37.9, 34.3, 31.6, 30.2, 29.4, 26.7, 25.4, 23.3, 8.3, 7.2, 7.0; HRMS (ESI+) m/z 597.5097 [$(M + Na)^+$: calcd for $C_{26}H_{60}B_{10}NaO_4Si$, 597.5089].

*1-[3-(2,3-Dihydroxypropoxy)propyl]-12-(5-ethyl-5-hydroxyheptyl)-1,12-dicarba-closo-dodecaborane (**16**).* Compound **15** (600 mg, 1.05 mmol) was dissolved in methanol (15 mL) and THF (5 mL), and then 2 M hydrochloric acid (2 mL) was added to the solution at 0 °C. The mixture was stirred for 20 h at room temperature, poured into water, and extracted with ethyl acetate. The organic layer was washed with brine, dried over sodium sulfate, and then concentrated. Purification by silica gel column chromatography (eluent; hexane/ethyl acetate, 1/1) gave **16** (310 mg, 0.741 mmol, 71%) as a white solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.82 (m, 1 H), 3.69 (m, 1 H), 3.60 (m, 1 H), 3.45−3.38 (m, 2 H), 3.3−1.3 (br m, 10 H), 3.29 (t, J = 6.2 Hz, 2 H), 2.40 (d, J = 4.9 Hz, 1 H), 1.90 (dd, J = 6.8 Hz, 5.4 Hz, 1 H), 1.70−1.57 (m, 4 H), 1.45−1.37 (m, 2 H), 1.40 (q, J = 7.5 Hz, 4 H), 1.32−1.27 (m, 2 H), 1.14−1.08 (m, 4 H), 1.00 (s, 1 H), 0.82 (t, J = 7.5 Hz, 6 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 79.3, 78.4, 74.5, 72.3, 70.48, 70.41, 64.0, 37.7, 34.3, 30.9, 30.1, 29.4, 22.9, 7.7; HRMS (ESI+) m/z 443.3916 [$(M + Na)^+$: calcd for $C_{17}H_{42}B_{10}NaO_4$, 443.3909].

Biology. *Assay of HL-60 Cell Differentiation-Inducing Activity.* HL-60 cells were cultured in RPMI-1640 medium supplemented with 5% FBS and penicillin G and streptomycin at 37 °C under 5% CO2 in air. The cells were diluted to 8.0 × 10^4 cell/mL with RPMI-1640 (5% FBS), and ethanol solution of a test compound was added to give 10^{-9} to 10^{-6} M final concentration. Control cells were treated with the same volume of ethanol alone. 1α,25(OH)$_2$D$_3$ was always assayed at the same time as a positive control. The cells were incubated at 37 °C under 5% CO_2 in air for 4 days. The percentage of differentiated cells was determined by nitro-blue tetrazolium (NBT) reduction assay. Cells were incubated at 37 °C for 20 min in RPMI-1640 (5% FBS) and an equal volume of phosphate-buffered saline (PBS) containing NBT (0.2%) and 12-O-tetradecanoylphorbol 13-acetate (TPA; 200 ng/mL). The percentage of cells containing blue-black formazan was determined in a minimum of 200 cells.

Competitive Binding Assay with Bovine Thymus VDR. Binding to bovine thymus VDR was evaluated according to the reported procedure. Bovine thymus VDR was purchased from Yamasa Biochemical (Choshi, Chiba, Japan) and dissolved in 0.05 M phosphate buffer (pH 7.4) containing 0.3 M KCl and 5 mM dithiothreitol just before use. The receptor solution (500 μL) in an assay tube was incubated with 0.072 nM [^{3}H] 1α,25(OH)$_2$D$_3$, together with graded amounts of each vitamin D analogue or vehicle for 19 h at 4 °C. Bound and free [^{3}H] 1α,25(OH)$_2$D$_3$ were separated on dextran-coated charcoal for 20 min at 4 °C. The assay tubes were centrifuged at 1000 g for 10 min. The radioactivity of the supernatant was counted, and nonspecific binding was subtracted. These experiments were done in duplicate.

X-ray Crystallographic Analysis. In this study, the method reported by Vanhooke et al. was used with some modifications to prepare the crystals of VDR complexes.[21] The rat VDR-LBD (residues 116−423, Δ165−211) was cloned as an N-terminal His$_6$-tagged fusion protein into the pET14b expression vector and overproduced in *Escherichia coli* C41. The cells were grown at 37 °C in LB medium (including ampicillin 100 mg/L) and subsequently induced for 6 h with 15 μM isopropyl-β-D-thiogalactopyranoside (IPTG) at 23 °C. The purification procedure included affinity chromatography on a Ni-NTA column, followed by dialysis and cation-exchange chromatography (SP-Sepharose). After tag removal by thrombin digestion, protease was removed by filtration

through a HiTrap benzamidine column, and the protein was further purified by gel filtration on a Superdex200 column. The purity and homogeneity of the rVDR−LBD were assessed by SDS-PAGE.

Purified rVDR−LBD solution was concentrated to about 0.75 mg/mL by ultrafiltration. To an aliquot (800 μL) of the protein solution was added a ligand (ca. 10 equiv), then the solution was further concentrated to about 1/8, and a solution (25 mM Tris−HCl, pH 8.0; 50 mM NaCl; 10 mM DTT; 0.02% NaN_3) of coactivator peptide (H_2N-KNHPMLMNLLKDN-$CONH_2$) derived from DRIP205 was added. This solution of VDR/ligand/peptide was allowed to crystallize by the vapor diffusion method using a series of precipitant solutions containing 0.1 M MOPS−NaOH (pH 7.0), 0.1−0.4 M sodium formate, 12−22% (w/v) PEG4000, and 5% (v/v) ethylene glycol. Droplets for crystallization were prepared by mixing 2 μL of complex solution and 1 μL precipitant solution, and droplets were equilibrated against 500 μL precipitant solution at 20 °C.

Prior to diffraction data collection, crystals were soaked in a cryoprotectant solution containing 0.1 M MOPS−NaOH (pH 7.0), 0.1−0.4 M sodium formate, 15−20% PEG4000, and 17−20% ethylene glycol. Diffraction data sets were collected at 100 K in a stream of nitrogen gas at beamline BL-6A of KEK-PF (Tsukuba, Japan). Reflections were recorded with an oscillation range per image of 1.0°. Diffraction data were indexed, integrated, and scaled using the program HKL2000 (HKL Research Inc.). The structures were solved by molecular replacement with the program CNS[25] using rat VDR-LBD coordinates (PDB code: 1RK3), and finalized sets of atomic coordinates were obtained after iterative rounds of model modification and refinement with CNS by simulated annealing, positional minimization, water molecule identification, and restrained individual isotropic B-value refinement.

■ ASSOCIATED CONTENT

Supporting Information. ^{1}H NMR spectra and ^{13}C NMR spectra of synthesized compounds and details of preparation of 3-ethyl-3-triethylsilyloxy-7-bromoheptane, 4-(*p*-toluenesulfonyloxymethyl)-2-phenyl-1,3-dioxane, and 4-(*p*-toluenesulfonyloxymethyl)-2,2-dimethyl-1,3-dioxolane. This material is available free of charge via the Internet at http://pubs.acs.org.

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