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# Systematic SAR study of the side chain of nonsecosteroidal vitamin $D_3$ analogs

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ABSTRACT

A series of nonsecosteroidal vitamin $D_3$ analogs with carboxylic acid were explored. Through our systematic SAR studies on the side chain moiety, compound **6b** was identified as the optimal compound showing excellent vitamin D receptor (VDR) agonistic activity. Compound **6b** had the diethyl group in the terminal which was bound by (*E*)-olefin linker to the bisphenyl core. Calculating the volume of the side chain showed that the diethyl group in **6b** filled the hydrophobic region of VDR with the ideal packing coefficient based on the 55% rule, and that this resulted in the most potent in vitro activity.

## 1. Introduction

Since the first cloning of steroid receptors in the mid-1980s, 48 nuclear receptors have been reported in human and classified into seven subfamilies.[1–5] Nuclear receptors are ligand-dependent transcription factors which are located in cytosol. They need specific ligands, such as retinoids, thyroid hormone, or vitamin $D_3$, to move into the nucleus and bind to DNA, and then regulate the expression of genes. Despite ligand variation, the structural components of nuclear receptors are similar to each other with few exceptions. They consist of two major parts which bind to a hinge region; one is the N-terminus region which contains a DNA-binding domain and an activation function 1 (AF-1) domain, the other one is the C-terminus region which contains a ligand-binding domain (LBD) including an AF-2 domain. The DNA-binding domain is the site at which hormone responsive element (HRE) binds. The AF-1 domain is capable of ligand-independent transcription activity but, in contrast, transcription in the AF-2 domain is activated ligand-dependently.[1,6]

Because many genes associated to various diseases are regulated by nuclear receptors, the ligands binding to nuclear receptors are very attractive drug targets.[7] Extended research is now ongoing on various nuclear receptors, such as androgen receptor, estrogen receptor, glucocorticoid receptor, PPARs, RXR, and VDR, using classic steroidal compounds and unique nonsteroidal compounds.[8] In VDR, many groups reported new ligands and their various types of activity, as well as the SAR around 25-hydroxyl moiety of the secosteroidal skeleton, such as side chain linker length and hydrophobic volume.[9] The 25-hydroxy group in secosteroidal vitamin $D_3$ has a very important role in the vitamin D activation pathway and also in binding to VDR. Furthermore, modifying the hydrophobic volume around 26 and 27 methyl groups in $1,25(OH)_2D_3$ (**1**) is an effective way to modulate VDR agonistic activity.[9–11]

In 1999, Boehm et al. reported the first nonsecosteroidal vitamin $D_3$, LG190178 (**2**).[12] Since then, some groups reported derivatives of LG190178; however, the information on the side chain part is very limited and a detailed SAR is still not available.[13–18]

We previously reported compound **3** as a novel nonsecosteroidal vitamin $D_3$ agonist which has a VDR agonistic effect both in vitro and in vivo.[19] Compound **3** has very unique carboxylic acid and hydroxy groups in the A-part (Fig. 1). From X-ray crystallographic analysis of **4**/VDR complex, these functional groups made a salt bridge interaction and formed a tight hydrogen bonding network in VDR.

To the bisphenyl skeleton with gamma hydroxyl carboxylic acid moiety in the A-part, in this study we attempted to introduce multiple-bond linkers ((*E*) and (*Z*)-olefins, and acetylene) to minimize the flexibility and to fix the direction of the side chain, and then modified the terminal hydrophobic volume of the side chain systematically. As a result, we found potent VDR agonists and obtained detailed SAR information of the side chain.

In VDR, the LBD, especially the region occupied by the side chain of the ligand is composed of a lot of hydrophobic amino acids, such as valine, leucine, isoleucine, and phenylalanine. The other interesting feature of the VDR/ligand complex is that the ligand is completely surrounded by VDR. In the area of host–guest chemistry, Mecozzi and Rebek proposed that when a guest molecule is completely surrounded by the host, the recognition of the

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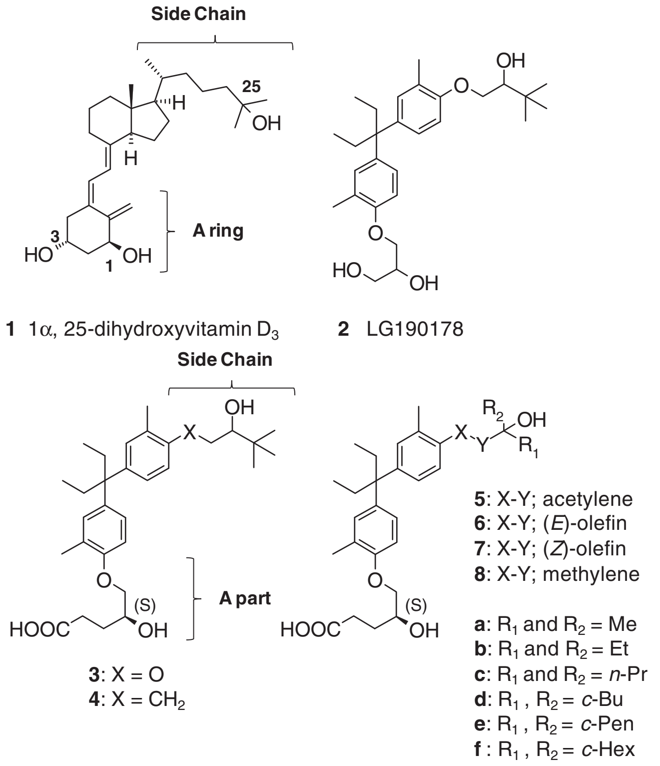


**Figure 1.** Structures of secosteroids 1α, 25-dihydroxyvitamin $D_3$ (**1**), nonsecosteroidal analog LG190178 (**2**) and a series of nonsecosteroidal carboxylic acid derivatives (**3**, **4**, **5a–f**, **6a–f**, **7b**, and **8b**). Part labeled A of **3** and **4** (lower left) shows the structural correspondence to the A ring of secosteroids (upper left).

guest by the host is firstly governed by the volume of the guest, and they then showed that the ideal volume occupancy of an inclusion guest compound in hydrophobic and confined spaces of a host receptor was 55 ± 9%.[20] In order to examine whether the concept of host–guest chemistry could be applied to the VDR/ligand system or not, we performed volume calculations similar to those done by Mecozzi et al. to our compounds and VDR. After calculating the packing coefficient (PC) between the side chain and the corresponding hydrophobic pocket of VDR, we were interested to find that the optimal PC for the hydrophobic side chain moiety of our compounds and the SAR of the side chains of VDR ligand follow the rule for host–guest recognition.

## 2. Chemistry

The synthesis of compounds **5a–f**, **6a–f**, **7b**, and **8b** is shown in Scheme 1. Compound **9** was prepared by a previously reported method.[19] Sonogashira coupling with trimethylsilylacetylene in the presence of palladium catalyst afforded acetylene **10** and the consequent removal of trimethylsilyl group using tetrabutylammonium fluoride (TBAF) gave acetylene **11** in very good yield. Alkylation of **11** with *n*-butyl lithium and various ketones gave corresponding alcohols **12a–f** in moderate yields. These acetylene alcohols were selectively reduced to (*E*)-olefins **13a–f** by Red-Al®. Acetylene **12b** was converted to (*Z*)-olefin **14b** and saturated methylene **15b** in the hydrogenation condition of Lindlar catalyst and palladium hydroxide on carbon, respectively. All phenol intermediate was alkylated by tosylate **16** in the presence of potassium carbonate to afford lactones **17a–f**, **18a–f**, **19b**, and **20b** in good yield. These lactones were hydrolyzed to gamma hydroxyl carboxylic acids **5a–f**, **6a–f**, **7b**, and **8b** in good yield. All compounds (**5a–f**, **6a–f**, **7b**, and **8b**) were stored in a freezer as an ethanol solution after adding an equivalent amount of 1 N NaOH to avoid retro lactone formation.

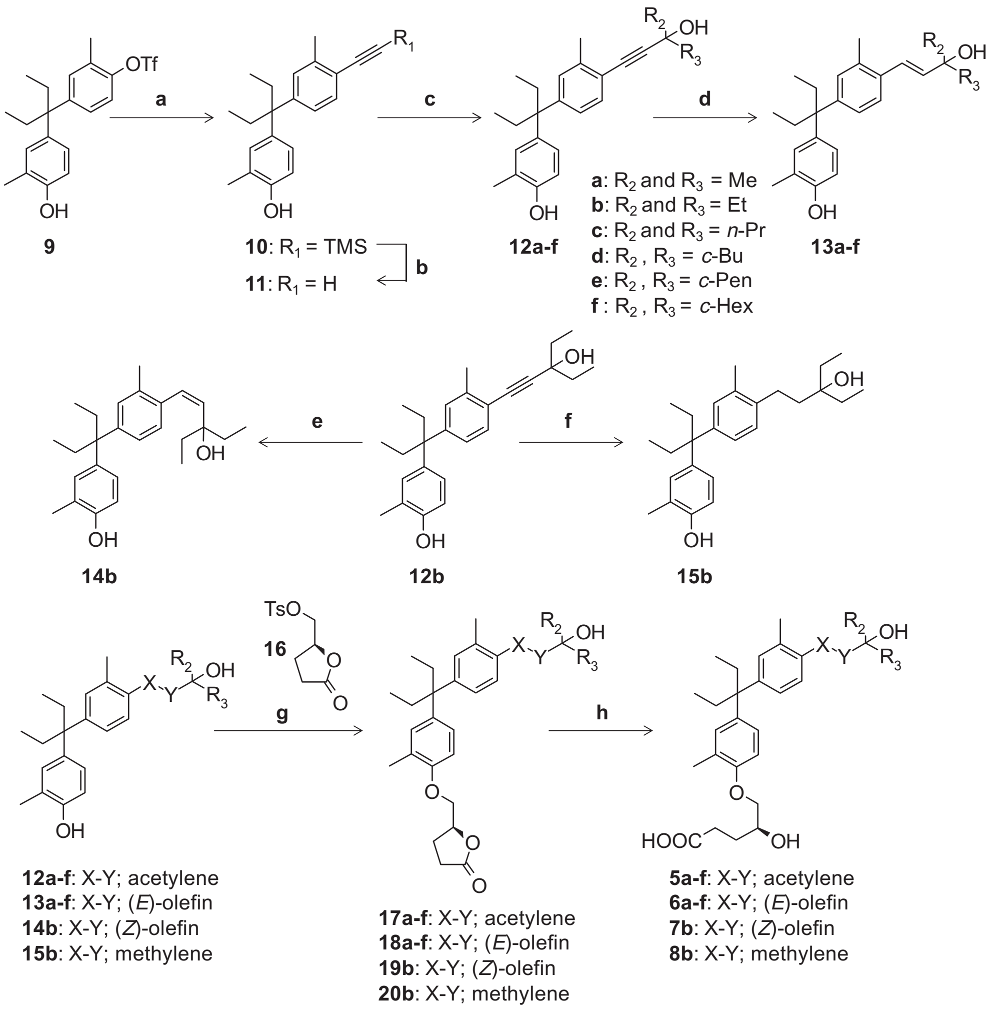
## 3. Results and discussion

All synthesized compounds were evaluated by reporter gene assay (RGA) in MG-63 cells which contains vitamin D responsive element (VDRE) sequence derived from mouse osteopontine promoter as a vitamin D agonistic activity.[19] VDRE RGA activity is represented by a relative $EC_{50}$ value based on $1,25(OH)_2D_3$ being assigned as 100% (a higher figure means stronger activity).

At first, we examined the impact of the linker between the bisphenyl core and the terminal hydroxy group of the side chain moiety, X–Y in Table 1. We considered that an ether linker, as in LG190178, is flexible and the position of the hydroxy group, which has to make hydrogen bond with VDR, on the side chain with ether linker is not fixed and could be distributed to the widespread areas of conformational space. To reduce the flexibility of the side chain and to find a more potent VDR agonist by restricting the positions occupied by the terminal hydroxy group on the side chain, we replaced the flexible ether linker with acetylene (**5b**) and olefins (**6b** and **7b**). These multiple-bond linkers were expected to rigidify the conformation of the side chain and define the position of the terminal hydrophobic moiety and hydroxy group in a restricted space. Methylene linker (**8b**) was used as a standard of the flexible carbon linker (Table 1). Compared to **3**, which has an identical side chain to LG190178, **5b** with acetylene linker and **8b** with methylene linker exhibited the same level of activity as **3** in VDRE RGA. In olefin analogs, (*E*)-olefin **6b** showed the activity five time higher than that of **3** and being equal to $1,25(OH)_2D_3$ in VDRE RGA, but the activity of (*Z*)-olefin **7b** was reduced dramatically when compared to **3**. It was speculated that the difference in activity of (*E*) and (*Z*)-olefins is because the terminal diethyl group of (*Z*)-olefin could collide with VDR and cause significant steric repulsion, while the hydroxy group of the side chain of (*E*)-olefin could make the hydrogen bond interactions with VDR properly so that the diethyl group of (*E*)-olefin could occupy the hydrophobic pocket of VDR appropriately.

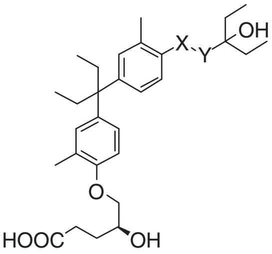
In order to estimate the difference in activity between **5b** with acetylene linker and **6b** with (*E*)-olefin linker, the docking models of **5b** and **6b** were constructed based on crystallographic structure of compound **4** in complex with VDR (PDB code: 3AZ3) (Fig. 2).[19] The compounds **5b** and **6b** were manually docked into the crystal structure of VDR using software MOLOC.[21] Compounds **5b** and **6b** were optimized using the MAB force field with fixed VDR structure. In the models, the carboxylic acid of A-part is formed a salt bridge with Arg274 and hydrogen-bonded to the crystallographic water. The hydroxy group of A-part makes hydrogen bond to the crystal water and makes a hydrogen bonding network with Ser278, Tyr143 and Arg274 via crystal water, in the same manner as compound **4**. Hydrophobic diethyl moieties of the side chain of **5b** and **6b** are superposed well to each other as shown in Figure 2; thus, we could not explain the difference in activity between **5b** and **6b** from the position and shape of the hydrophobic moiety.

The difference in the activity of (*E*)-olefin and acetylene analogs could be explained by the loss of rotational entropy in the bonds of the side chain. Database searches of crystallographic structures of small molecules by Cambridge structural database (CSD) indicated that the bond rotation of CC triple bond is free (Fig. 3a).[22] This result accords with the description that the barrier of the rotation of CC triple bond is low.[23] In contrast, the bond rotation along (*E*)-olefin is very restricted (Fig. 3b), although the number of hits in a CSD search for the query was three. Thus, no significant loss of bond rotational entropy along (*E*)-olefin bonds occurs when (*E*)-olefin linker analogs bind to VDR. But, in the case of acetylene linker



**Scheme 1.** Reagents and conditions: (a) TMS acetylene, CuI, Pd($PPh_3$)$_4$, 110 °C, acetonitrile, 74%; (b) TBAF, room temp, THF, 97%; (c) *n*-BuLi, ketones, −78 °C, THF, 54–90%; (d) Red-Al®, 0 °C, THF, 74–94%; (e) Lindlar catalyst, room temp, MeOH, 62%; (f) $Pd(OH)_2$/C, room temp, MeOH, 67%; (g) $K_2CO_3$, **16**, 100 °C, DMF, 54–90%; (h) 1 N KOH, room temp, MeOH, 71–98%.

**Table 1**
VDRE reporter gene activity of various side chain analogs

| | Compound | X–Y | Ratio of VDRE reporter activity[a] (%) |
|---|---|---|---|
|  | **5b** | C≡C | 21 |
| | **6b** | (*E*)-CH=CH | 105 |
| | **7b** | (*Z*)-CH=CH | 0.62 |
| | **8b** | $CH_2CH_2$ | 26 |
| | **3** | | 20 |
| | LG190178 | | 6.7 |
| | $1,25(OH)_2D_3$ | | 100 |

[a] $1,25(OH)_2D_3$ ratio of $EC_{50}$. $1,25(OH)_2D_3$ was assigned as 100% (higher figure means stronger activity).

analogs, the loss of rotational entropy along CC triple bond was plain to see because the hydroxy group at the terminal hydrophobic moiety has to interact with two His residues of VDR making the bond rotation of CC triple bond fixed when the acetylene linker analog binds to VDR.

The other possible explanation for the difference in activity between **5b** and **6b** is the hydrophobicity of the side chain. The *C*Log*P*

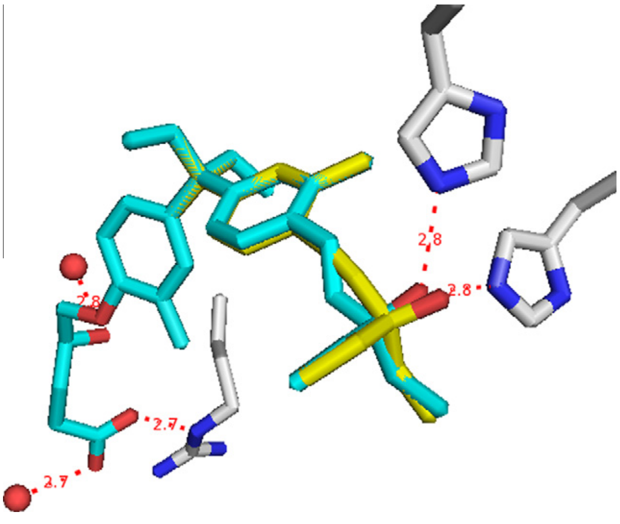


**Figure 2.** Superposition of **5b** and **6b**. Compound 6b, which has a (*E*)-olefin linker, is depicted in cyan. Compound **5b**, which has an acetylene linker, is depicted in yellow. The side chains of the residue of VDR, which make hydrogen bond with **6b**, are depicted in white. Crystallographic waters, which make hydrogen bonds with **6b**, are depicted as red spheres. Hydrogen bonds are depicted by red dotted line and their distances are annotated in red characters.

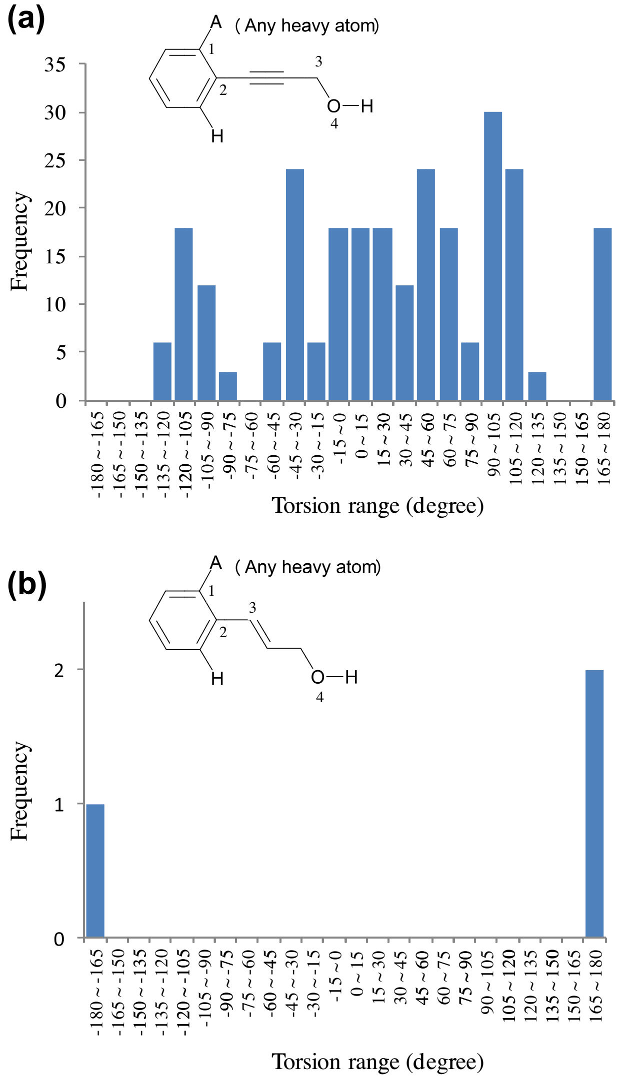
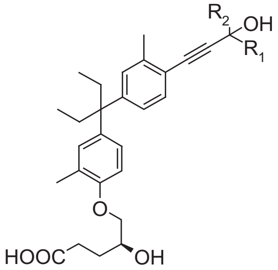
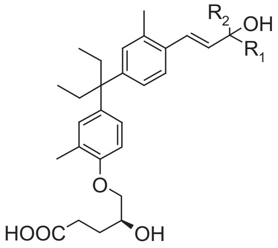


**Figure 3.** Histograms of the torsion distribution along acetylene and (*E*)-olefin. Substructure queries for CSD search are depicted on the histogram. Torsion is defined for atoms 1-2-3-4. (a) Histogram for torsion along acetylene. (b) Histogram for torsion along (*E*)-olefin.

**Table 2**
VDRE RGA activity of acetylene and (*E*)-olefin side chain analogs

| | Compound | $R_1$ | $R_2$ | Ratio of VDRE reporter activity[a] (%) |
|---|---|---|---|---|
|  | **5a** | Me | Me | 5.2 |
| | **5b** | Et | Et | 21 |
| | **5c** | *n*-Pr | *n*-Pr | 0.25 |
| | **5d** | *c*-Bu | | 3.0 |
| | **5e** | *c*-Pen | | 20 |
| | **5f** | *c*-Hex | | 7.8 |
|  | **6a** | Me | Me | 10 |
| | **6b** | Et | Et | 105 |
| | **6c** | *n*-Pr | *n*-Pr | 1.6 |
| | **6d** | *c*-Bu | | 9.0 |
| | **6e** | *c*-Pen | | 31 |
| | **6f** | *c*-Hex | | 55 |
| | LG190178 | | | 6.7 |
| | $1,25(OH)_2D_3$ | | | 100 |

[a] $1,25(OH)_2D_3$ ratio of $EC_{50}$. $1,25(OH)_2D_3$ was assigned as 100% (higher figure means stronger activity).

value for acetylene **5b** is 6.65 and that for (*E*)-olefin **6b** is 7.30.[24] Hansch–Fujita hydrophobic π constant of the acetylene group is 0.40 and of the olefin group is 0.82. These values indicated that (*E*)-olefin analogs are more hydrophobic than acetylenes, and this difference in hydrophobicity could affect the VDR activity.

Regarding the in vitro rat metabolic stability, acetylene (**5b**) and (*E*)-olefin (**6b**) showed good stability. On the other hand, methylene analog (**8b**) showed significantly poor stability, possibly due to the benzylic oxidation. From both the activity and the metabolic stability viewpoints, we selected acetylene (**5b**) and (*E*)-olefin (**6b**) as linkers for further modification to investigate the SAR for the side chain, especially for the terminal hydrophobic moiety.

We systematically modified the terminal hydrophobic moiety of the side chain using acetylene and (*E*)-olefin linkers (Table 2). The length of the terminal alkyl chain was changed from one to three for branched alkyl analogs **5a**–**c** and **6a**–**c**. The ring size was changed from four to six for cyclic alkyl analogs **5d**–**f** and **6d**–**f**. With an acetylene linker, the substitution of dimethyl (**5a**) for diethyl group (**5b**) showed four times reduction in VDRE RGA activity. The replacement of diethyl group (**5b**) with di-*n*-propyl (**5c**) exhibited more than 80 times reduction in activity. When compared to the activity of **5b**, the cyclic alkyl analogs of the terminal hydrophobic group (**5d–f**) exhibited the same activity in **5e** with a cyclopentyl group, seven times reduction in activity in **5d** with a cyclobutyl group, and 2.7 times reduction in **5f** with a cyclohexyl group. In the case of (*E*)-olefin analogs, diethyl group (**6b**) showed the most potent activity (105%) and the smaller dimethyl group (**6a**) exhibited ten times reduction in activity and the larger di-*n*-propyl group (**6c**) exhibited more than 65 times reduction, respectively when compared to **6b**. In the case of cyclic alkyl analogs of (*E*)-olefin, VDRE RGA activity rose as ring size became larger, 9% for cyclobutyl (**6d**), 31% for cyclopentyl (**6e**) and 55% for cyclohexyl (**6f**), respectively.

From the results of the optimization of hydrophobic moiety using both acetylene and (*E*)-olefin linkers, (*E*)-olefin analogs exhibited more potent in vitro activity than the corresponding acetylene analogs. The reason for the stronger activity of (*E*)-olefin analogs is thought to be a small loss of entropy of bond rotation in the side chain and/or higher hydrophobicity as described above for **5b** and **6b**.

The trend of the SARs for acetylene and (*E*)-olefin analogs was the same for the acyclic alkyl series; diethyl group was the optimal for both linkers. But, the SARs were slightly different for the cyclic alkyl series; cyclopentyl was most active for acetylene linker and cyclohexyl was most potent for (*E*)-olefin linker.

The docking model of **6b** suggested, as shown in Figure 4, that two ethyl groups of **6b** are located in the hydrophobic pocket, which is formed by Leu230, Val234, Ile268, Tyr401, Leu414, Val418, and Phe422 of VDR. The SAR of the side chain indicated that the size and shape of the hydrophobic moiety in the side chain could have a strong influence on the VDR agonistic activity. To evaluate the effect of the hydrophobic moiety in the side chain of the ligand on VDR agonistic activity, we modeled the docking pose for each compounds and calculated the volume of the side chain and its packing coefficient (PC) as a ratio of the volume of VDR pocket accommodating the side chain of the ligand.

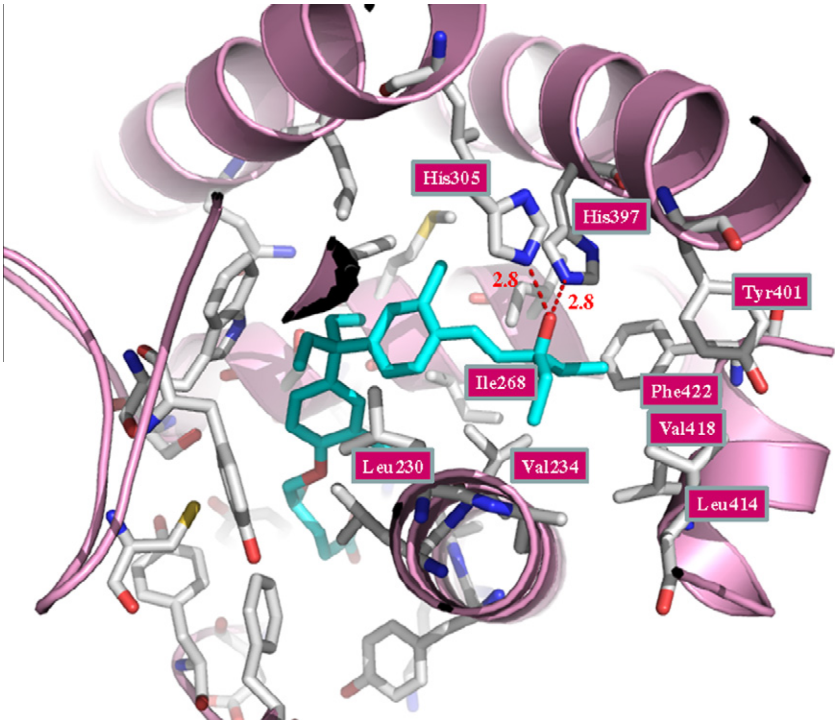


**Figure 4.** Hydrophobic pocket around the side chain of compound **6b**. Amino acid residues of VDR within 6 Å from compound **6b** are depicted by white sticks. Key hydrogen bonding interactions are shown by red dotted lines and each distance is indicated in angstrom (Å).

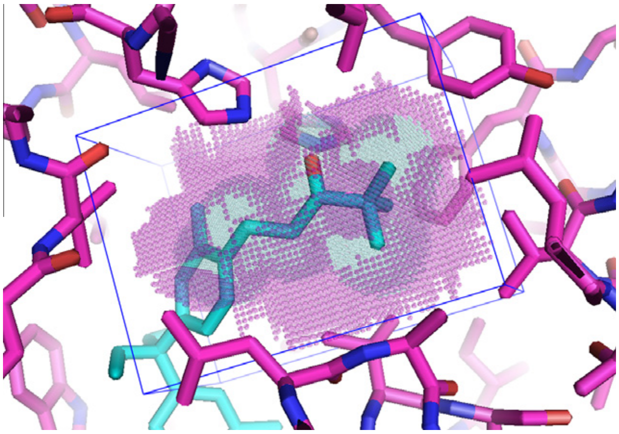


**Figure 5.** Side chain volume ($V_{\text{side chain}}$, 118 Å$^3$, cyan), $V_{\text{void space}}$ (90 Å$^3$, magenta) and the corresponding LBD volume ($V_{\text{LBD}}$, 208 Å$^3$, magenta plus cyan) within the defined rectangular box (blue) in the co-crystal structure of **4** and VDR (PDB 3AZ3). PC of **4** was calculated as 57% by $V_{\text{side chain}}/V_{\text{LBD}} \times 100$ (%).

The compounds **5a–f** and **6a–f** were manually docked into the crystallographic structure of VDR and the structure of the ligand was optimized in the same manner as the docking of **6b** to VDR. The volume occupied by the side chain of the compound ($V_{\text{side chain}}$), the volume not occupied by either the ligand or VDR ($V_{\text{void space}}$) in the VDR pocket, and the volume of the VDR pocket ($V_{\text{LBD}}$) were calculated by in-house programs (see Fig. 5 for the graphical image of the values $V_{\text{side chain}}$, $V_{\text{void space}}$ and $V_{\text{LBD}}$ and refer to Section 5.3 for details of the calculations). $V_{\text{LBD}}$ was calculated as 208 Å$^3$.

The packing coefficient (PC) values were calculated as follows:

$$\mathrm{PC}(\%) = (V_{\text{side chain}}/V_{\text{LBD}}) \times 100$$

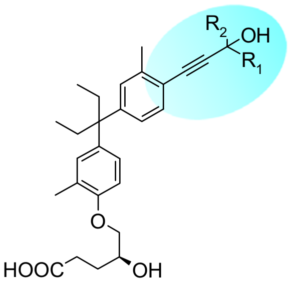
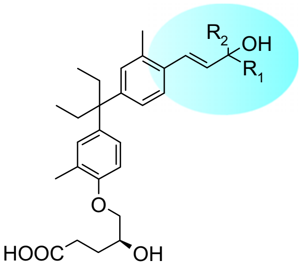
where $V_{\text{LBD}} = V_{\text{side chain}} + V_{\text{void space}}$.

Table 3 shows the calculated volume $V_{\text{side chain}}$ and PC for compounds **5a–f** and **6a–f**. (*E*)-Olefin analogs showed slightly larger volume (1–2 Å$^3$) than that of corresponding acetylene analogs. There was a difference of 51 Å$^3$ between $V_{\text{side chain}}$ of the smallest dimethyl group analogs (**5a** and **6a**) and that of the largest di-*n*-propyl group analogs (**5c** and **6c**). The increasing volume order was diMe < *c*-Bu < *c*-Pen < diEt < *c*-Hex < di-*n*-Pr, which was the same tendency in both acetylenes and (*E*)-olefins.

Figure 6 shows the correlations between PC and VDRE RGA activity. In acetylene analogs, cyclopentyl group (**5e**, PC = 52%) and diethyl group (**5b**, PC = 55%) showed higher RGA activity than the other four analogs. Meanwhile, in (*E*)-olefin analogs, diethyl group in **6b** showed optimal PC (56%) and either decreasing the volume to have dimethyl group (**6a**, PC = 44%) or increasing the volume to have di-*n*-propyl group (**6c**, PC = 68%) reduced the activity. Cyclopentyl group (**6e**) and cyclohexyl group (**6f**) both had moderate RGA activity of 31% and 55%, respectively, and the PC values of these analogs were in the range of 56 ± 3%. Almost the same tendency was seen in acetylene analogs in spite of the maximum VDRE reporter activity being less potent than in (*E*)-olefins.

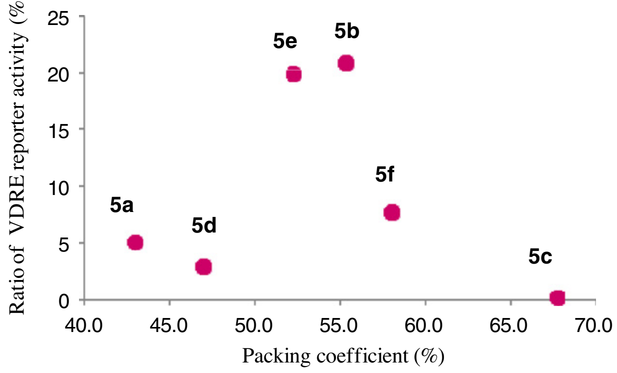
These results suggest that the lipophilic VDR region occupied by the side chain of a ligand has an optimal PC value for exhibiting high activity. It was thought that, when the PC value is lower than optimal, the number of van der Waals interactions between the side chain atoms and VDR becomes lower and the enthalpic gain from various non-bonded interactions is reduced. On the other hand, when the PC value is higher than the optimal, two reasons could be considered. One of the reasons might be that a large number of van der Waals interactions are formed resulting in a large enthalpic gain. However, at the same time, the dynamic mobility of both protein and ligand becomes reduced, which leads to unfavorable complexation entropy that compensates for the enthalpic gain.[25] So the optimal PC value is achieved by the most moderate balance between enthalpic gain from hydrophobic interaction and entropy loss from limited mobility. The other reason might be a ligand with a large volume makes van der Waals collisions

**Table 3**
Calculated volume results of the side chain moieties of **4**, **5a–f**, and **6a–f** and corresponding PCs

| | Compound | $R_1$ | $R_2$ | Volume $V_{\text{side chain}}$[a] (Å$^3$) | PC[b] (%) |
|---|---|---|---|---|---|
| | **4** | | | 118 | 57 |
|  | **5a** | Me | Me | 89 | 43 |
| | **5b** | Et | Et | 115 | 55 |
| | **5c** | *n*-Pr | *n*-Pr | 141 | 68 |
| | **5d** | *c*-Bu | | 98 | 47 |
| | **5e** | *c*-Pen | | 109 | 52 |
| | **5f** | *c*-Hex | | 121 | 58 |
|  | **6a** | Me | Me | 91 | 44 |
| | **6b** | Et | Et | 117 | 56 |
| | **6c** | *n*-Pr | *n*-Pr | 142 | 68 |
| | **6d** | *c*-Bu | | 99 | 48 |
| | **6e** | *c*-Pen | | 110 | 53 |
| | **6f** | *c*-Hex | | 122 | 59 |

[a] Calculated volume of extracted side chain moiety from whole molecule (highlighted in blue in the structure).
[b] PC was calculated as follows; $(V_{\text{side chain}}/208\ \text{Å}^3) \times 100$ (%).

(a): acetylene



(b): (*E*)-olefin

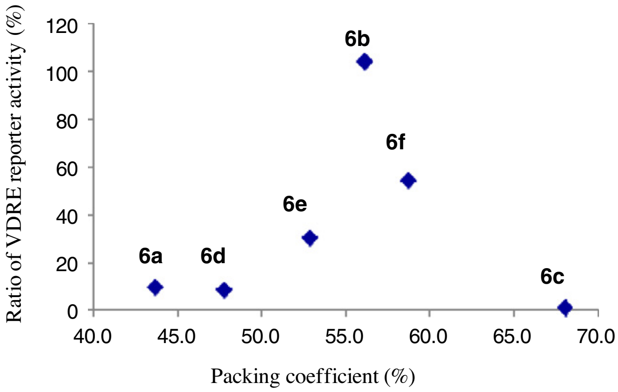


**Figure 6.** The correlation between activity and PC; (a) **5a–f** (acetylene linker series) and (b) **6a–f** ((*E*)-olefin linker series).

with VDR and the collisions induce the conformational change of VDR resulting in high energy state VDR.

Mecozzi and Rebek showed that the ideal volume occupancy of guest compounds included in hydrophobic confined spaces of host capsular synthetic receptors was 55 ± 9%.[20] Diederich et al. applied this 55% rule in the enzyme pocket and showed optimal packing coefficiency of hydrophobic moiety of inhibitors in the apolar pocket to be around 55%.[25,26] These ratios are consistent with our results, thus, our case is a second example that the 55% rule is validated for protein–ligand recognition.

From these results, compound **6b** was the ligand with an optimum size of side chain moiety in VDR. When one is trying to fill the vacant hydrophobic region of a protein by modifying the ligand, it might be difficult to design a molecule that will exhibit optimal activity just by checking its shape and its fit to the receptor via modeling. In such a case, optimizing the ligand size by considering PC with the protein could be a useful tool for analog design and optimization.

## 4. Conclusion

A series of nonsecosteroidal vitamin $D_3$ analogs with carboxylic acid were explored. Through our systematic SAR studies on the side chain moiety, compound **6b** was identified as the optimal compound, showing excellent in vitro potency and metabolic stability. Compound **6b** had the diethyl group connected by (*E*)-olefin linker to the bisphenyl core. (*E*)-Olefin linker of **6b** has a conformational rigidity and thus has the advantage that the entropy loss of bond rotation is less than that of acetylene analog **5b** when binding to VDR. The diethyl group at the terminal of the side chain of **6b** has an ideal PC (56%) for the hydrophobic pocket of VDR. When filling a hydrophobic region of a protein by a ligand, the 55% rule could be a useful tool for analog design and optimization.

## 5. Experimental

### 5.1. Chemistry: general

Purchased reagents and solvents were used without further purification unless otherwise noted. $^1$H and $^{13}$C NMR spectra were carried out on VARIAN 400-MR spectrophotometers; chemical shifts are reported in parts per million (ppm) downfield from that of internal tetramethylsilane (TMS). Mass spectrophotometry was measured with Waters ACQUITY SQD electrospray ionization (ESI) system. High-resolution mass spectra (HRMS) were recorded on Thermo Fisher Scientific LTQ Orbitrap XL (ESI) instruments. Optical rotations were measured on a HORIBA SEPA-200 polarimeter (sodium D line at 25 °C). Chromatographic purification was carried out using Merck silica gel 60 (column) or Merck silica gel 60 $PF_{254}$ (preparative TLC).

#### 5.1.1. 4-[1-Ethyl-1-(3-methyl-4-trimethylsilanylethynylphenyl)propyl]-2-methylphenol (10)

To a solution of **9** (25 g, 60.0 mmol) in MeCN (300 mL) were added triethylamine (25.1 mL, 180.1 mmol), (trimethylsilyl)acetylene (25.5 mL, 180.1 mmol), CuI (1.14 g, 6.00 mmol), and $Pd(PPh_3)_4$ (6.93 g, 6.00 mmol). The mixture was stirred at 110 °C for 18 h under nitrogen atmosphere and concentrated. The mixture was poured into $H_2O$ and products were extracted with AcOEt. The extracts were dried over anhydrous $MgSO_4$ and concentrated. The obtained residue was chromatographed on silica gel (*n*-hexane/AcOEt = 15:1) to afford **10** (16 g, 74%) as colorless oil. $^1$H NMR ($CDCl_3$) $\delta$: 0.24 (9H, s), 0.58 (6H, t, $J = 7.3$ Hz), 2.02 (4H, q, $J = 7.3$ Hz), 2.17 (3H, s), 2.38 (3H, s), 4.58 (1H, br s), 6.64 (1H, d, $J = 9.0$ Hz), 6.81–6.86 (2H, m), 6.92 (1H, dd, $J = 8.1$, 1.7 Hz), 6.99

(1H, s), 7.30 (1H, d, *J* = 8.0 Hz). $^{13}$C NMR (CDCl$_3$) $\delta$: 151.5, 149.9, 140.4, 139.7, 131.3, 130.6, 129.0, 126.5, 125.4, 122.8, 119.6, 114.0, 104.4, 97.4, 49.0, 28.9, 20.9, 16.0, 8.3, 0.0. MS (ESI negative): 363 (M−H)$^-$.

### 5.1.2. 4-[1-Ethyl-1-(4-ethynyl-3-methylphenyl)-propyl]-2-methyl phenol (11)

To a solution of **10** (18.02 g, 49.4 mmol) in THF (247 mL) was added tetrabutylammonium fluoride (1 M in THF, 74.1 mL, 74.1 mmol). The mixture was stirred for 30 min and concentrated. The residue was diluted with AcOEt and the mixture was washed with H$_2$O and brine, dried over anhydrous MgSO$_4$, and concentrated. The obtained residue was chromatographed on silica gel (*n*-hexane/AcOEt = 10:1) to afford **11** (14.08 g, 97%) as pale yellow oil. $^{1}$H NMR (CDCl$_3$) $\delta$: 0.59 (6H, t, *J* = 7.3 Hz), 2.03 (4H, q, *J* = 7.4 Hz), 2.19 (3H, s), 2.40 (3H, s), 3.22 (1H, s), 4.53 (1H, br s), 6.65 (1H, d, *J* = 8.0 Hz), 6.83–6.87 (2H, m), 6.94 (1H, dd, *J* = 8.0, 1.8 Hz), 7.01 (1H, s), 7.33 (1H, d, *J* = 8.0 Hz). $^{13}$C NMR (CDCl$_3$) $\delta$: 151.5, 150.1, 140.4, 139.8, 131.8, 130.6, 129.1, 126.6, 125.5, 122.8, 118.7, 114.1, 82.9, 80.3, 49.1, 29.0, 20.9, 16.1, 8.3. MS (ESI negative): 291 (M−H)$^-$.

### 5.1.3. General procedure for alkylation of acetylene group of 11

To a solution of **11** (4.28 g, 14.69 mmol) in THF (92 mL) was added *n*-butyllithium (2.5 M in hexane, 14.7 mL, 36.73 mmol) at −78 °C and the mixture was stirred for 30 min. To the mixture was added ketone (44.07 mmol) at −78 °C and the mixture was stirred for 3 h. Then the mixture was poured into satd NH$_4$Cl aq solution and products were extracted with AcOEt. The extracts were dried over anhydrous Na$_2$SO$_4$ and concentrated. The obtained residue was chromatographed on silica gel (*n*-hexane/AcOEt = 6:1) to afford alcohol.

### 5.1.4. 4-{1-Ethyl-1-[4-(3-hydroxy-3-methyl-1-butynyl)-3-methyl phenyl]-propyl}-2-methylphenol (12a)

The yield was 56%. Colorless oil. $^{1}$H NMR (CDCl$_3$) $\delta$: 0.58 (6H, t, *J* = 7.2 Hz), 1.62 (6H, s), 2.02 (4H, q, *J* = 7.3 Hz), 2.17 (3H, s), 2.35 (3H, s), 5.79 (1H, br s), 6.65 (1H, d, *J* = 8.2 Hz), 6.82–6.85 (2H, m), 6.93 (1H, dd, *J* = 8.0, 1.6 Hz), 7.00 (1H, s), 7.25 (1H, d, *J* = 8.0 Hz). $^{13}$C NMR (CDCl$_3$) $\delta$: 151.7, 149.7, 140.1, 139.2, 131.0, 130.6, 129.1, 126.4, 125.5, 122.9, 119.1, 114.1, 97.1, 81.3, 65.9, 49.0, 31.6, 29.0, 20.9, 16.2, 8.3. MS (ESI negative): 349 (M−H)$^-$.

### 5.1.5. 4-{1-Ethyl-1-[4-(3-ethyl-3-hydroxy-1-pentynyl)-3-methyl phenyl]-propyl}-2-methylphenol (12b)

The yield was 62%. Colorless oil. $^{1}$H NMR (CDCl$_3$) $\delta$: 0.59 (6H, t, *J* = 7.2 Hz), 1.11 (6H, t, *J* = 7.3 Hz), 1.72–1.78 (4H, m), 2.02 (4H, q, *J* = 7.2 Hz), 2.18 (3H, s), 2.37 (3H, s), 4.83 (1H, br s), 6.65 (1H, d, *J* = 8.2 Hz), 6.82–6.86 (2H, m), 6.93 (1H, dd, *J* = 8.1, 1.9 Hz), 7.00 (1H, s), 7.27 (1H, d, *J* = 8.2 Hz). $^{13}$C NMR (CDCl$_3$) $\delta$: 151.4, 149.6, 140.4, 139.1, 131.3, 130.6, 129.1, 126.6, 125.4, 122.6, 119.4, 114.0, 94.9, 83.5, 72.8, 49.0, 34.6, 29.0, 21.0, 16.0, 8.7, 8.3. MS (ESI negative): 377 (M−H)$^-$.

### 5.1.6. 4-{1-Ethyl-1-[4-(3-hydroxy-3-propyl-1-hexynyl)-3-methyl phenyl]-propyl}-2-methylphenol (12c)

The yield was 54%. Colorless oil. $^{1}$H NMR (CDCl$_3$) $\delta$: 0.59 (6H, t, *J* = 7.3 Hz), 0.98 (6H, t, *J* = 7.2 Hz), 1.59–1.65 (4H, m), 1.68–1.73 (4H, m), 2.02 (4H, q, *J* = 7.3 Hz), 2.18 (3H, s), 2.36 (3H, s), 4.57 (1H, br s), 6.65 (1H, d, *J* = 8.6 Hz), 6.82–6.86 (2H, m), 6.93 (1H, dd, *J* = 8.1, 1.9 Hz), 7.00 (1H, d, *J* = 1.8 Hz), 7.26 (1H, d, *J* = 8.0 Hz). $^{13}$C NMR (CDCl$_3$) $\delta$: 151.8, 149.6, 140.0, 139.1, 131.2, 130.6, 129.1, 126.4, 125.4, 123.0, 119.3, 114.0, 95.4, 83.4, 72.0, 49.0, 44.5, 29.0, 21.0, 17.7, 16.1, 14.3, 8.3. MS (ESI negative): 405 (M−H)$^-$.

### 5.1.7. 4-{1-Ethyl-1-[4-(1-hydroxycyclobutylethynyl)-3-methyl phenyl]-propyl}-2-methylphenol (12d)

The yield was 69%. Colorless oil. $^{1}$H NMR (CDCl$_3$) $\delta$: 0.59 (6H, t, *J* = 7.3 Hz), 1.82–1.90 (2H, m), 2.03 (4H, q, *J* = 7.3 Hz), 2.18 (3H, s), 2.33–2.37 (2H, m), 2.38 (3H, s), 2.49–2.55 (2H, m), 4.69 (1H, br s), 6.65 (1H, d, *J* = 8.0 Hz), 6.82–6.86 (2H, m), 6.94 (1H, dd, *J* = 8.1, 1.9 Hz), 7.01 (1H, s), 7.28 (1H, d, *J* = 8.2 Hz). $^{13}$C NMR (CDCl$_3$) $\delta$: 151.7, 149.7, 140.0, 139.2, 131.0, 130.5, 129.0, 126.3, 125.4, 123.0, 119.1, 114.0, 95.8, 82.5, 68.4, 48.9, 38.8, 28.9, 20.8, 16.1, 12.9, 8.3. MS (ESI negative): 361 (M−H)$^-$.

### 5.1.8. 4-{1-Ethyl-1-[4-(1-hydroxycyclopentylethynyl)-3-methyl phenyl]-propyl}-2-methylphenol (12e)

The yield was 70%. Colorless oil. $^{1}$H NMR (CDCl$_3$) $\delta$: 0.59 (6H, t, *J* = 7.3 Hz), 1.70–1.88 (4H, m), 1.99–2.06 (8H, m), 2.18 (3H, s), 2.36 (3H, s), 4.69 (1H, br s), 6.65 (1H, d, *J* = 8.8 Hz), 6.82–6.86 (2H, m), 6.93 (1H, dd, *J* = 8.0, 1.6 Hz), 7.00 (1H, d, *J* = 1.4 Hz), 7.26 (1H, d, *J* = 8.0 Hz). $^{13}$C NMR (CDCl$_3$) $\delta$: 152.0, 149.4, 139.3, 138.8, 130.7, 130.2, 128.8, 126.0, 125.2, 123.0, 119.2, 113.8, 96.4, 82.5, 74.6, 48.7, 42.3, 28.7, 23.6, 20.6, 16.1, 8.1. MS (ESI negative): 375 (M−H)$^-$.

### 5.1.9. 4-{1-Ethyl-1-[4-(1-hydroxycyclohexylethynyl)-3-methyl phenyl]-propyl}-2-methylphenol (12f)

The yield was 90%. Colorless oil. $^{1}$H NMR (CDCl$_3$) $\delta$: 0.59 (6H, t, *J* = 7.3 Hz), 1.57–1.78 (8H, m), 1.99–2.06 (6H, m), 2.18 (3H, s), 2.38 (3H, s), 4.71 (1H, br s), 6.65 (1H, d, *J* = 8.0 Hz), 6.82–6.86 (2H, m), 6.94 (1H, dd, *J* = 8.1, 1.9 Hz), 7.00 (1H, d, *J* = 1.8 Hz), 7.28 (1H, d, *J* = 8.2 Hz). $^{13}$C NMR (CDCl$_3$) $\delta$: 151.8, 149.6, 139.8, 139.0, 131.0, 130.4, 129.0, 126.3, 125.3, 123.0, 119.2, 114.0, 95.8, 83.5, 69.5, 48.9, 40.0, 28.9, 25.1, 23.5, 21.0, 16.2, 8.3. MS (ESI negative): 389 (M−H)$^-$.

### 5.1.10. General procedure for reduction of acetylene group of 12a–f

To a solution of acetylene (0.327 mmol) in THF (3.0 mL) was added Red-Al® (65% in toluene, 0.294 mL, 0.980 mmol) at 0 °C and the mixture was stirred for 2 h. The mixture was quenched with satd NaHCO$_3$ aq solution, diluted with AcOEt, and Celite was added. After stirring overnight, the mixture was filtered through Celite pad and concentrated. The obtained residue was purified by preparative TLC (*n*-hexane/AcOEt = 1:1) to afford olefin.

### 5.1.11. 4-{1-Ethyl-1-[4-((*E*)-3-hydroxy-3-methyl-1-butenyl)-3-methylphenyl]-propyl}-2-methylphenol (13a)

The yield was 82%. Colorless oil. $^{1}$H NMR (CDCl$_3$) $\delta$: 0.60 (6H, t, *J* = 7.2 Hz), 1.42 (6H, s), 2.03 (4H, q, *J* = 7.3 Hz), 2.18 (3H, s), 2.29 (3H, s), 5.25 (1H, br s), 6.22 (1H, d, *J* = 15.8 Hz), 6.65 (1H, d, *J* = 8.4 Hz), 6.77 (1H, d, *J* = 16.0 Hz), 6.84 (1H, dd, *J* = 8.1, 2.2 Hz), 6.89 (1H, d, *J* = 2.0 Hz), 6.93–6.97 (2H, m), 7.31 (1H, d, *J* = 8.4 Hz). $^{13}$C NMR (CDCl$_3$) $\delta$: 151.5, 148.3, 140.6, 137.9, 134.5, 132.6, 130.6, 129.9, 126.6, 126.0, 124.6, 123.9, 122.7, 114.0, 71.4, 48.8, 29.9, 29.1, 20.1, 16.1, 8.4. MS (ESI negative): 351 (M−H)$^-$.

### 5.1.12. 4-{1-Ethyl-1-[4-((*E*)-3-ethyl-3-hydroxy-1-pentenyl)-3-methylphenyl]-propyl}-2-methylphenol (13b)

The yield was 85%. Colorless oil. $^{1}$H NMR (CDCl$_3$) $\delta$: 0.61 (6H, t, *J* = 7.3 Hz), 0.92 (6H, t, *J* = 7.4 Hz), 1.64 (4H, q, *J* = 7.5 Hz), 2.04 (4H, q, *J* = 7.4 Hz), 2.19 (3H, s), 2.30 (3H, s), 4.73 (1H, br s), 6.01 (1H, d, *J* = 16.0 Hz), 6.65 (1H, d, *J* = 8.2 Hz), 6.75 (1H, d, *J* = 16.0 Hz), 6.87 (1H, dd, *J* = 8.4, 2.3 Hz), 6.91 (1H, d, *J* = 2.0 Hz), 6.93–6.97 (2H, m), 7.30 (1H, d, *J* = 8.8 Hz). $^{13}$C NMR (CDCl$_3$) $\delta$: 151.4, 148.0, 140.7, 135.8, 134.4, 133.2, 130.6, 129.9, 126.7, 125.9, 124.7, 122.5, 114.0, 76.1, 48.8, 33.3, 29.1, 20.2, 16.1, 8.4, 7.9. MS (ESI negative): 379 (M−H)$^-$.

### 5.1.13. 4-{1-Ethyl-1-[4-((*E*)-3-hydroxy-3-propyl-1-hexenyl)-3-methylphenyl]-propyl}-2-methylphenol (13c)

The yield was 89%. Colorless oil. $^{1}$H NMR ($CDCl_3$) $\delta$: 0.61 (6H, t, $J$ = 7.3 Hz), 0.92 (6H, t, $J$ = 7.3 Hz), 1.33–1.43 (4H, m), 1.55–1.62 (4H, m), 2.04 (4H, q, $J$ = 7.2 Hz), 2.19 (3H, s), 2.30 (3H, s), 5.04 (1H, br s), 6.05 (1H, d, $J$ = 15.8 Hz), 6.65 (1H, d, $J$ = 8.2 Hz), 6.73 (1H, d, $J$ = 15.8 Hz), 6.86 (1H, dd, $J$ = 8.4, 2.2 Hz), 6.91 (1H, d, $J$ = 2.0 Hz), 6.94–6.97 (2H, m), 7.29 (1H, d, $J$ = 8.6 Hz). $^{13}$C NMR ($CDCl_3$) $\delta$: 151.4, 148.0, 140.6, 136.3, 134.4, 133.1, 130.6, 129.9, 126.6, 125.9, 125.2, 124.7, 122.6, 114.0, 75.9, 48.8, 43.7, 29.1, 20.2, 16.9, 16.1, 14.5, 8.4. MS (ESI negative): 407 (M−H)$^-$.

### 5.1.14. 4-(1-Ethyl-1-{4-[(*E*)-2-(1-hydroxycyclobutyl)-vinyl]-3-methylphenyl}-propyl)-2-methylphenol (13d)

The yield was 91%. Colorless oil. $^{1}$H NMR ($CDCl_3$) $\delta$: 0.60 (6H, t, $J$ = 7.2 Hz), 1.61–1.70 (1H, m), 1.79–1.89 (1H, m), 1.96–2.07 (2H, m), 2.04 (4H, q, $J$ = 7.3 Hz), 2.18 (3H, s), 2.23–2.30 (2H, m), 2.30 (3H, s), 5.63 (1H, br s), 6.34 (1H, d, $J$ = 15.8 Hz), 6.65 (1H, d, $J$ = 8.4 Hz), 6.82 (1H, d, $J$ = 15.8 Hz), 6.84 (1H, dd, $J$ = 8.1, 2.1 Hz), 6.89 (1H, d, $J$ = 1.8 Hz), 6.94–6.98 (2H, m), 7.35 (1H, d, $J$ = 8.0 Hz). $^{13}$C NMR ($CDCl_3$) $\delta$: 151.6, 148.4, 140.3, 134.5, 134.2, 132.5, 130.5, 129.9, 126.5, 125.9, 124.7, 124.4, 122.8, 114.0, 75.4, 48.8, 36.3, 29.1, 20.1, 16.1, 12.2, 8.4. MS (ESI negative): 363 (M−H)$^-$.

### 5.1.15. 4-(1-Ethyl-1-{4-[(*E*)-2-(1-hydroxycyclopentyl)-vinyl]-3-methylphenyl}-propyl)-2-methylphenol (13e)

The yield was 74%. Colorless oil. $^{1}$H NMR ($CDCl_3$) $\delta$: 0.60 (6H, t, $J$ = 7.1 Hz), 1.70–1.83 (6H, m), 1.87–1.96 (2H, m), 2.03 (4H, q, $J$ = 7.2 Hz), 2.17 (3H, s), 2.28 (3H, s), 6.12 (1H, br s), 6.25 (1H, d, $J$ = 15.8 Hz), 6.65 (1H, d, $J$ = 8.2 Hz), 6.80–6.88 (3H, m), 6.93–6.97 (2H, m), 7.31 (1H, d, $J$ = 8.0 Hz). $^{13}$C NMR ($CDCl_3$) $\delta$: 151.7, 148.2, 140.2, 136.1, 134.3, 132.7, 130.5, 129.9, 126.4, 125.9, 124.5, 124.2, 122.9, 113.9, 75.9, 48.7, 40.6, 29.1, 23.6, 20.1, 16.1, 8.4. MS (ESI negative): 377 (M−H)$^-$.

### 5.1.16. 4-(1-Ethyl-1-{4-[(*E*)-2-(1-hydroxycyclohexyl)-vinyl]-3-methylphenyl}-propyl)-2-methylphenol (13f)

The yield was 94%. Colorless oil. $^{1}$H NMR ($CDCl_3$) $\delta$: 0.60 (6H, t, $J$ = 7.2 Hz), 1.50–1.75 (10H, m), 2.03 (4H, q, $J$ = 7.0 Hz), 2.17 (3H, s), 2.28 (3H, s), 5.65 (1H, br s), 6.20 (1H, d, $J$ = 16.0 Hz), 6.65 (1H, d, $J$ = 8.2 Hz), 6.80–6.84 (2H, m), 6.88 (1H, d, $J$ = 2.0 Hz), 6.93–6.97 (2H, m), 7.31 (1H, d, $J$ = 7.8 Hz). $^{13}$C NMR ($CDCl_3$) $\delta$: 151.6, 148.3, 140.4, 137.5, 134.5, 132.8, 130.5, 129.9, 126.5, 125.9, 124.8, 124.6, 122.8, 114.0, 72.3, 48.8, 38.1, 29.1, 25.4, 22.1, 20.1, 16.1, 8.4. MS (ESI negative): 391 (M−H)$^-$.

### 5.1.17. 4-{1-Ethyl-1-[4-((*Z*)-3-ethyl-3-hydroxy-1-pentenyl)-3-methylphenyl]-propyl}-2-methylphenol (14b)

To a solution of **12b** (100 mg, 0.264 mmol) in MeOH (4 mL) was added Lindlar catalyst (5% palladium on calcium carbonate; poisoned with lead, 5 mg), the mixture was stirred at room temperature under hydrogen overnight. Then the mixture was filtered through Celite pad and concentrated. The obtained residue was purified by preparative TLC (*n*-hexane/AcOEt = 2:1) to afford **14b** (62 mg, 62%) as a white powder. $^{1}$H NMR ($CDCl_3$) $\delta$: 0.58 (6H, t, $J$ = 7.2 Hz), 0.92 (6H, t, $J$ = 7.4 Hz), 1.54 (4H, q, $J$ = 7.4 Hz), 2.02 (4H, q, $J$ = 7.2 Hz), 2.19 (3H, s), 2.22 (3H, s), 4.70 (1H, br s), 5.55 (1H, d, $J$ = 12.7 Hz), 6.49 (1H, d, $J$ = 12.5 Hz), 6.63 (1H, d, $J$ = 8.4 Hz), 6.84 (1H, dd, $J$ = 8.2, 2.2 Hz), 6.90 (1H, d, $J$ = 1.8 Hz), 6.94–6.97 (2H, m), 7.11 (1H, d, $J$ = 8.2 Hz). $^{13}$C NMR ($CDCl_3$) $\delta$: 151.4, 148.4, 140.7, 136.7, 135.5, 133.5, 130.5, 129.6, 127.8, 127.6, 126.7, 125.3, 122.5, 114.1, 78.0, 48.8, 34.2, 29.1, 20.6, 16.1, 8.4, 8.3. MS (ESI positive): 403 (M+Na)$^+$.

### 5.1.18. 4-{1-Ethyl-1-[4-(3-ethyl-3-hydroxypentyl)-3-methyl phenyl]-propyl}-2-methylphenol (15b)

To a solution of **12b** (33.0 mg, 0.087 mmol) in MeOH (2 mL) was added 10% palladium hydroxide (20 mg), the mixture was stirred at room temperature under hydrogen overnight. The mixture was filtered through Celite pad and concentrated. The obtained residue was purified by preparative TLC (*n*-hexane/AcOEt = 2:1) to afford **15b** (22.4 mg, 67%) as colorless oil. $^{1}$H NMR ($CDCl_3$) $\delta$: 0.59 (6H, t, $J$ = 7.3 Hz), 0.91 (6H, t, $J$ = 7.5 Hz), 1.56 (4H, q, $J$ = 7.5 Hz), 1.64–1.69 (2H, m), 2.03 (4H, q, $J$ = 7.4 Hz), 2.19 (3H, s), 2.25 (3H, s), 2.54–2.59 (2H, m), 4.96 (1H, br s), 6.64 (1H, d, $J$ = 8.4 Hz), 6.85 (1H, dd, $J$ = 8.4, 2.2 Hz), 6.90–6.93 (3H, m), 7.00 (1H, d, $J$ = 8.6 Hz). $^{13}$C NMR ($CDCl_3$) $\delta$: 151.4, 146.3, 140.8, 137.4, 134.6, 130.6, 129.8, 127.7, 126.7, 125.7, 122.5, 114.0, 74.9, 48.7, 38.8, 30.9, 29.2, 26.7, 19.6, 16.1, 8.4, 7.8. MS (ESI positive): 405 (M+Na)$^+$.

### 5.1.19. General procedure for alkylation of phenol group of12a–f, 13a–f, 14b, and 15b

To a solution of phenol (0.394 mmol) in DMF (4 ml) was added $K_2CO_3$ (0.985 mmol) and the mixture was stirred at room temperature for 30 min. Then, toluene-4-sulfonic acid (*S*)-5-oxo-tetrahydro-furan-2-ylmethyl ester (0.591 mmol) was added and the mixture was stirred at 100 °C for 15 h. The mixture was poured into satd $NH_4Cl$ aq solution and products were extracted with AcOEt. The extracts were washed with $H_2O$ and brine, dried over anhydrous $Na_2SO_4$, and concentrated. The obtained residue was chromatographed on silica gel (*n*-hexane/AcOEt = 5:1–3:1) to afford lactone.

### 5.1.20. (*S*)-5-(4-{1-Ethyl-1-[4-(3-hydroxy-3-methyl-1-butynyl)-3-methylphenyl]-propyl}-2-methylphenoxymethyl)-dihydro-furan-2-one (17a)

The yield was 75%. Colorless oil. $[\alpha]_{D}^{25}$ +17.7 ($c$ 1.17, $CHCl_3$). $^{1}$H NMR ($CDCl_3$) $\delta$: 0.58 (6H, t, $J$ = 7.3 Hz), 1.62 (6H, s), 2.03 (4H, q, $J$ = 7.3 Hz), 2.14 (3H, s), 2.29–2.31 (1H, m), 2.36 (3H, s), 2.42–2.45 (1H, m), 2.52–2.61 (1H, m), 2.72–2.81 (1H, m), 4.06 (1H, dd, $J$ = 10.4, 3.3 Hz), 4.16 (1H, dd, $J$ = 10.3, 3.2 Hz), 4.87–4.89 (1H, m), 6.66 (1H, d, $J$ = 8.6 Hz), 6.86 (1H, d, $J$ = 2.0 Hz), 6.91–6.94 (2H, m), 6.98 (1H, s), 7.26 (1H, d, $J$ = 7.2 Hz). $^{13}$C NMR ($CDCl_3$) $\delta$: 177.2, 154.0, 149.4, 140.9, 139.2, 131.0, 130.7, 129.0, 126.1, 125.6, 125.4, 119.2, 109.8, 97.3, 81.1, 77.8, 69.3, 65.7, 49.0, 31.6, 28.9, 28.3, 24.0, 20.9, 16.6, 8.3. MS (ESI positive): 471 (M+Na)$^+$.

### 5.1.21. (*S*)-5-(4-{1-Ethyl-1-[4-(3-ethyl-3-hydroxy-1-pentynyl)-3-methylphenyl]-propyl}-2-methylphenoxymethyl)-dihydrofuran-2-one (17b)

The yield was 73%. Colorless oil. $[\alpha]_{D}^{25}$ +13.6 ($c$ 1.10, $CHCl_3$). $^{1}$H NMR ($CDCl_3$) $\delta$: 0.59 (6H, t, $J$ = 7.3 Hz), 1.11 (6H, t, $J$ = 7.4 Hz), 1.70–1.83 (4H, m), 2.03 (4H, q, $J$ = 7.2 Hz), 2.14 (3H, s), 2.27–2.35 (1H, m), 2.37 (3H, s), 2.40–2.48 (1H, m), 2.52–2.61 (1H, m), 2.73–2.81 (1H, m), 4.06 (1H, dd, $J$ = 10.3, 3.4 Hz), 4.16 (1H, dd, $J$ = 10.4, 3.3 Hz), 4.86–4.92 (1H, m), 6.66 (1H, d, $J$ = 8.6 Hz), 6.87 (1H, d, $J$ = 1.8 Hz), 6.92–6.94 (2H, m), 6.99 (1H, s), 7.27 (1H, d, $J$ = 8.2 Hz). $^{13}$C NMR ($CDCl_3$) $\delta$: 177.2, 154.0, 149.4, 140.9, 139.2, 131.3, 130.7, 129.1, 126.1, 125.7, 125.4, 119.5, 109.9, 95.0, 83.4, 77.8, 72.7, 69.3, 49.0, 34.6, 28.9, 28.3, 24.0, 21.0, 16.6, 8.7, 8.3. MS (ESI positive): 499 (M+Na)$^+$.

### 5.1.22. (*S*)-5-(4-{1-Ethyl-1-[4-(3-hydroxy-3-propyl-1-hexynyl)-3-methylphenyl]-propyl}-2-methylphenoxymethyl)-dihydro-furan-2-one (17c)

The yield was 78%. Colorless oil. $[\alpha]_{D}^{25}$ +12.1 ($c$ 0.73, $CHCl_3$). $^{1}$H NMR ($CDCl_3$) $\delta$: 0.58 (6H, t, $J$ = 7.3 Hz), 0.98 (6H, t, $J$ = 7.1 Hz), 1.55–1.60 (4H, m), 1.68–1.73 (4H, m), 2.03 (4H, q, $J$ = 7.3 Hz), 2.14 (3H, s), 2.27–2.35 (1H, m), 2.36 (3H, s), 2.39–2.49 (1H, m), 2.53–2.61 (1H, m), 2.73–2.82 (1H, m), 4.06 (1H, dd, $J$ = 10.3, 3.4 Hz), 4.17 (1H, dd, *J* = 10.2, 3.3 Hz), 4.86–4.92 (1H, m), 6.66 (1H, d, *J* = 8.4 Hz), 6.87 (1H, d, *J* = 2.0 Hz), 6.90–6.94 (2H, m), 6.98 (1H, s), 7.26 (1H, d, *J* = 8.0 Hz). $^{13}$C NMR (CDCl$_3$) $\delta$: 177.2, 154.0, 149.4, 141.0, 139.2, 131.3, 130.7, 129.1, 126.1, 125.7, 125.4, 119.5, 109.8, 95.6, 83.3, 77.9, 71.8, 69.3, 49.1, 44.5, 29.0, 28.3, 24.1, 21.1, 17.7, 16.6, 14.3, 8.3. MS (ESI positive): 527 (M+Na)$^+$.

### 5.1.23. (*S*)-5-(4-{1-Ethyl-1-[4-(1-hydroxycyclobutylethynyl)-3-methylphenyl]-propyl}-2-methylphenoxymethyl)-dihydrofuran-2-one (17d)

The yield was 83%. Colorless oil. $[\alpha]_{\mathrm{D}}^{25}$ +18.4 (*c* 1.43, CHCl$_3$). $^{1}$H NMR (CDCl$_3$) $\delta$: 0.59 (6H, t, *J* = 7.3 Hz), 1.82–1.88 (2H, m), 2.04 (4H, q, *J* = 7.3 Hz), 2.14 (3H, s), 2.30–2.35 (2H, m), 2.38 (3H, s), 2.41–2.61 (5H, m), 2.73–2.81 (1H, m), 4.06 (1H, dd, *J* = 10.4, 3.5 Hz), 4.17 (1H, dd, *J* = 10.7, 3.0 Hz), 4.86–4.91 (1H, m), 6.66 (1H, d, *J* = 8.4 Hz), 6.87 (1H, d, *J* = 1.8 Hz), 6.93 (2H, dd, *J* = 8.2, 1.8 Hz), 7.00 (1H, s), 7.28 (1H, d, *J* = 8.2 Hz). $^{13}$C NMR (CDCl$_3$) $\delta$: 177.2, 154.0, 149.5, 140.9, 139.3, 131.1, 130.7, 129.0, 126.1, 125.7, 125.4, 119.2, 109.8, 96.0, 82.4, 77.9, 69.3, 68.4, 49.0, 38.8, 28.9, 28.3, 24.0, 20.9, 16.6, 12.9, 8.3. MS (ESI positive): 483 (M+Na)$^+$.

### 5.1.24. (*S*)-5-(4-{1-Ethyl-1-[4-(1-hydroxycyclopentylethynyl)-3-methylphenyl]-propyl}-2-methylphenoxymethyl)-dihydrofuran-2-one (17e)

The yield was 58%. Colorless oil. $[\alpha]_{\mathrm{D}}^{25}$ +13.7 (*c* 0.83, CHCl$_3$). $^{1}$H NMR (CDCl$_3$) $\delta$: 0.58 (6H, t, *J* = 7.3 Hz), 1.75–1.90 (4H, m), 1.99–2.07 (8H, m), 2.14 (3H, s), 2.27–2.34 (1H, m), 2.36 (3H, s), 2.41–2.48 (1H, m), 2.52–2.61 (1H, m), 2.72–2.81 (1H, m), 4.06 (1H, dd, *J* = 10.4, 3.5 Hz), 4.16 (1H, dd, *J* = 10.4, 3.3 Hz), 4.85–4.91 (1H, m), 6.66 (1H, d, *J* = 8.6 Hz), 6.86 (1H, d, *J* = 2.0 Hz), 6.90–6.94 (2H, m), 6.98 (1H, s), 7.26 (1H, d, *J* = 8.0 Hz). $^{13}$C NMR (CDCl$_3$) $\delta$: 177.2, 154.0, 149.4, 141.0, 139.2, 131.0, 130.7, 129.0, 126.1, 125.7, 125.4, 119.4, 109.8, 96.3, 82.1, 77.9, 75.1, 69.3, 49.0, 42.6, 28.9, 28.3, 24.1, 23.4, 20.9, 16.6, 8.3. MS (ESI positive): 497 (M+Na)$^+$.

### 5.1.25. (*S*)-5-(4-{1-Ethyl-1-[4-(1-hydroxycyclohexylethynyl)-3-methylphenyl]-propyl}-2-methylphenoxymethyl)-dihydrofuran-2-one (17f)

The yield was 72%. Colorless oil. $[\alpha]_{\mathrm{D}}^{25}$ +13.4 (*c* 1.17, CHCl$_3$). $^{1}$H NMR (CDCl$_3$) $\delta$: 0.59 (6H, t, *J* = 7.2 Hz), 1.56–1.77 (8H, m), 1.99–2.06 (6H, m), 2.14 (3H, s), 2.26–2.35 (1H, m), 2.38 (3H, s), 2.40–2.48 (1H, m), 2.52–2.60 (1H, m), 2.72–2.81 (1H, m), 4.06 (1H, dd, *J* = 10.3, 3.4 Hz), 4.16 (1H, dd, *J* = 10.3, 3.2 Hz), 4.85–4.90 (1H, m), 6.66 (1H, d, *J* = 8.6 Hz), 6.87 (1H, d, *J* = 2.0 Hz), 6.91–6.95 (2H, m), 6.99 (1H, s), 7.28 (1H, d, *J* = 8.2 Hz). $^{13}$C NMR (CDCl$_3$) $\delta$: 177.2, 154.0, 149.4, 140.9, 139.2, 131.1, 130.7, 129.0, 126.1, 125.6, 125.4, 119.4, 109.8, 96.2, 83.3, 77.8, 77.2, 69.3, 49.0, 40.1, 28.9, 28.3, 25.2, 24.0, 23.5, 21.1, 16.6, 8.3. MS (ESI positive): 506 (M+NH$_4$)$^+$.

### 5.1.26. (*S*)-5-(4-{1-Ethyl-1-[4-((*E*)-3-hydroxy-3-methyl-1-butenyl)-3-methylphenyl]-propyl}-2-methylphenoxymethyl)-dihydrofuran-2-one (18a)

The yield was 84%. Colorless oil. $[\alpha]_{\mathrm{D}}^{25}$ +20.8 (*c* 1.25, CHCl$_3$). $^{1}$H NMR (CDCl$_3$) $\delta$: 0.60 (6H, t, *J* = 7.2 Hz), 1.42 (6H, s), 2.04 (4H, q, *J* = 7.2 Hz), 2.14 (3H, s), 2.28–2.36 (1H, m), 2.30 (3H, s), 2.40–2.48 (1H, m), 2.50–2.62 (1H, m), 2.72–2.81 (1H, m), 4.06 (1H, dd, *J* = 10.4, 3.3 Hz), 4.16 (1H, dd, *J* = 10.4, 3.1 Hz), 4.86–4.91 (1H, m), 6.23 (1H, d, *J* = 15.8 Hz), 6.66 (1H, d, *J* = 8.6 Hz), 6.77 (1H, d, *J* = 16.0 Hz), 6.90–6.97 (4H, m), 7.32 (1H, d, *J* = 8.8 Hz). $^{13}$C NMR (CDCl$_3$) $\delta$: 177.2, 153.9, 148.0, 141.2, 138.0, 134.5, 130.7, 130.0, 129.9, 127.9, 126.2, 125.9, 124.6, 123.7, 109.8, 77.9, 71.2, 69.3, 48.8, 29.9, 29.0, 28.3, 24.0, 20.1, 16.6, 8.4. MS (ESI positive): 473 (M+Na)$^+$.

### 5.1.27. (*S*)-5-(4-{1-Ethyl-1-[4-((*E*)-3-ethyl-3-hydroxy-1-pentenyl)-3-methylphenyl]-propyl}-2-methylphenoxymethyl)-dihydrofuran-2-one (18b)

The yield was 90%. Colorless oil. $[\alpha]_{\mathrm{D}}^{25}$ +11.9 (*c* 0.24, CHCl$_3$). $^{1}$H NMR (CDCl$_3$) $\delta$: 0.60 (6H, t, *J* = 7.3 Hz), 0.92 (6H, t, *J* = 7.4 Hz), 1.60–1.67 (4H, m), 2.05 (4H, q, *J* = 7.3 Hz), 2.15 (3H, s), 2.28–2.38 (1H, m), 2.31 (3H, s), 2.39–2.49 (1H, m), 2.52–2.61 (1H, m), 2.73–2.82 (1H, m), 4.06 (1H, dd, *J* = 10.3, 3.4 Hz), 4.17 (1H, dd, *J* = 10.3, 3.4 Hz), 4.86–4.91 (1H, m), 6.01 (1H, d, *J* = 16.0 Hz), 6.66 (1H, d, *J* = 8.4 Hz), 6.74 (1H, d, *J* = 16.0 Hz), 6.93–6.97 (4H, m), 7.30 (1H, d, *J* = 8.8 Hz). $^{13}$C NMR (CDCl$_3$) $\delta$: 177.2, 153.9, 147.9, 141.3, 135.9, 134.4, 133.3, 130.7, 129.9, 126.2, 125.8, 125.6, 124.8, 109.9, 77.9, 76.0, 69.3, 48.8, 33.3, 29.1, 28.3, 24.1, 20.2, 16.6, 8.4, 7.9. MS (ESI positive): 496 (M+NH$_4$)$^+$.

### 5.1.28. (*S*)-5-(4-{1-Ethyl-1-[4-((*E*)-3-hydroxy-3-propyl-1-hexenyl)-3-methylphenyl]-propyl}-2-methylphenoxymethyl)-dihydrofuran-2-one (18c)

The yield was 72%. Colorless oil. $[\alpha]_{\mathrm{D}}^{25}$ +12.5 (*c* 1.19, CHCl$_3$). $^{1}$H NMR (CDCl$_3$) $\delta$: 0.60 (6H, t, *J* = 7.3 Hz), 0.92 (6H, t, *J* = 7.3 Hz), 1.33–1.43 (4H, m), 1.55–1.61 (4H, m), 2.04 (4H, q, *J* = 7.2 Hz), 2.15 (3H, s), 2.27–2.37 (1H, m), 2.30 (3H, s), 2.39–2.48 (1H, m), 2.52–2.60 (1H, m), 2.73–2.82 (1H, m), 4.06 (1H, dd, *J* = 10.4, 3.3 Hz), 4.16 (1H, dd, *J* = 10.4, 3.3 Hz), 4.85–4.91 (1H, m), 6.05 (1H, d, *J* = 15.8 Hz), 6.66 (1H, d, *J* = 8.4 Hz), 6.73 (1H, d, *J* = 16.0 Hz), 6.91–6.98 (4H, m), 7.29 (1H, d, *J* = 8.8 Hz). $^{13}$C NMR (CDCl$_3$) $\delta$: 177.2, 153.9, 147.8, 141.2, 136.5, 134.4, 133.2, 130.7, 129.8, 126.2, 125.8, 125.6, 125.1, 124.7, 109.8, 77.9, 75.7, 69.3, 48.8, 43.7, 29.1, 28.3, 24.1, 20.2, 16.9, 16.6, 14.5, 8.4. MS (ESI positive): 529 (M+Na)$^+$.

### 5.1.29. (*S*)-5-[4-(1-Ethyl-1-{4-[(*E*)-2-(1-hydroxycyclobutyl)-vinyl]-3-methylphenyl}-propyl)-2-methylphenoxymethyl]-dihydrofuran-2-one (18d)

The yield was 90%. Colorless oil. $[\alpha]_{\mathrm{D}}^{25}$ +21.4 (*c* 0.95, CHCl$_3$). $^{1}$H NMR (CDCl$_3$) $\delta$: 0.60 (6H, t, *J* = 7.1 Hz), 1.61–1.71 (1H, m), 1.79–1.89 (1H, m), 2.05 (4H, q, *J* = 7.2 Hz), 2.15 (3H, s), 2.20–2.35 (5H, m), 2.31 (3H, s), 2.37–2.48 (1H, m), 2.49–2.59 (1H, m), 2.71–2.80 (1H, m), 4.05 (1H, dd, *J* = 10.5, 3.4 Hz), 4.16 (1H, dd, *J* = 9.9, 3.0 Hz), 4.84–4.90 (1H, m), 6.35 (1H, d, *J* = 15.8 Hz), 6.66 (1H, d, *J* = 8.4 Hz), 6.82 (1H, d, *J* = 15.8 Hz), 6.91–6.98 (4H, m), 7.36 (1H, d, *J* = 8.4 Hz). $^{13}$C NMR (CDCl$_3$) $\delta$: 177.1, 153.9, 148.1, 141.2, 134.5, 130.7, 130.0, 129.9, 127.9, 126.2, 125.9, 125.6, 124.7, 124.3, 110.0, 77.9, 75.2, 69.3, 48.8, 36.4, 29.1, 28.2, 24.0, 20.1, 16.5, 12.2, 8.3. MS (ESI positive): 485 (M+Na)$^+$.

### 5.1.30. (*S*)-5-[4-(1-Ethyl-1-{4-[(*E*)-2-(1-hydroxycyclopentyl)-vinyl] -3-methylphenyl}-propyl)-2-methylphenoxymethyl]-dihydrofuran-2-one (18e)

The yield was 54%. Colorless oil. $[\alpha]_{\mathrm{D}}^{25}$ +13.2 (*c* 1.20, CHCl$_3$). $^{1}$H NMR (CDCl$_3$) $\delta$: 0.60 (6H, t, *J* = 7.3 Hz), 1.72–1.95 (8H, m), 2.04 (4H, q, *J* = 7.2 Hz), 2.14 (3H, s), 2.28–2.36 (1H, m), 2.31 (3H, s), 2.38–2.48 (1H, m), 2.52–2.60 (1H, m), 2.72–2.81 (1H, m), 4.06 (1H, dd, *J* = 10.3, 3.4 Hz), 4.16 (1H, dd, *J* = 10.1, 3.2 Hz), 4.85–4.90 (1H, m), 6.26 (1H, d, *J* = 16.0 Hz), 6.66 (1H, d, *J* = 8.6 Hz), 6.83 (1H, d, *J* = 16.0 Hz), 6.91–6.97 (4H, m), 7.33 (1H, d, *J* = 8.6 Hz). $^{13}$C NMR (CDCl$_3$) $\delta$: 177.2, 153.9, 147.9, 141.3, 136.4, 134.4, 132.8, 130.7, 129.9, 126.2, 125.9, 125.5, 124.6, 124.1, 109.8, 82.3, 77.9, 69.3, 48.8, 40.7, 29.0, 28.3, 24.0, 23.7, 20.2, 16.6, 8.4. MS (ESI positive): 499 (M+Na)$^+$.

### 5.1.31. (*S*)-5-[4-(1-Ethyl-1-{4-[(*E*)-2-(1-hydroxycyclohexyl)-vinyl]-3-methylphenyl}-propyl)-2-methylphenoxymethyl]-dihydrofuran-2-one (18f)

The yield was 71%. Colorless oil. $[\alpha]_{\mathrm{D}}^{25}$ +18.1 (*c* 1.29, CHCl$_3$). $^{1}$H NMR (CDCl$_3$) $\delta$: 0.60 (6H, t, *J* = 7.2 Hz), 1.52–1.74 (10H, m), 2.04

(4H, q, $J$ = 7.2 Hz), 2.15 (3H, s), 2.27–2.36 (1H, m), 2.30 (3H, s), 2.38–2.48 (1H, m), 2.51–2.61 (1H, m), 2.73–2.81 (1H, m), 4.06 (1H, dd, $J$ = 10.3, 3.4 Hz), 4.16 (1H, dd, $J$ = 10.2, 3.3 Hz), 4.85–4.90 (1H, m), 6.21 (1H, d, $J$ = 16.0 Hz), 6.66 (1H, d, $J$ = 8.4 Hz), 6.81 (1H, d, $J$ = 16.0 Hz), 6.91–6.97 (4H, m), 7.32 (1H, d, $J$ = 8.8 Hz). $^{13}$C NMR (CDCl$_3$) $\delta$: 177.2, 153.9, 148.0, 141.2, 138.0, 134.5, 132.9, 130.7, 129.9, 126.2, 125.9, 125.5, 124.6, 124.5, 109.8, 77.9, 71.9, 69.3, 48.8, 38.1, 29.0, 28.3, 25.5, 24.0, 22.1, 20.1, 16.6, 8.4. MS (ESI positive): 513 (M+Na)$^+$.

### 5.1.32. (*S*)-5-(4-{1-Ethyl-1-[4-((*Z*)-3-ethyl-3-hydroxy-1-pentenyl)-3-methylphenyl]-propyl}-2-methylphenoxymethyl)-dihydrofuran-2-one (19b)

The yield was 85%. Colorless oil. $[\alpha]_D^{25}$ +31.7 ($c$ 0.36, CHCl$_3$). $^1$H NMR (CDCl$_3$) $\delta$: 0.58 (6H, t, $J$ = 7.2 Hz), 0.92 (6H, t, $J$ = 7.4 Hz), 1.54 (4H, q, $J$ = 7.5 Hz), 2.03 (4H, q, $J$ = 7.4 Hz), 2.16 (3H, s), 2.22 (3H, s), 2.27–2.37 (1H, m), 2.40–2.49 (1H, m), 2.53–2.61 (1H, m), 2.74–2.82 (1H, m), 4.06 (1H, dd, $J$ = 10.3, 3.4 Hz), 4.17 (1H, dd, $J$ = 10.4, 3.3 Hz), 4.86–4.91 (1H, m), 5.55 (1H, d, $J$ = 12.5 Hz), 6.49 (1H, d, $J$ = 12.7 Hz), 6.66 (1H, d, $J$ = 9.2 Hz), 6.91–6.96 (4H, m), 7.11 (1H, d, $J$ = 8.2 Hz). $^{13}$C NMR (CDCl$_3$) $\delta$: 177.2, 153.9, 148.2, 141.2, 136.7, 135.5, 133.6, 130.6, 129.6, 127.7, 127.6, 126.3, 125.6, 125.2, 109.9, 78.0, 77.9, 69.3, 48.8, 34.2, 29.1, 28.3, 24.1, 20.6, 16.6, 8.4, 8.3. MS (ESI positive): 501 (M+Na)$^+$.

### 5.1.33. (*S*)-5-(4-{1-Ethyl-1-[4-(3-ethyl-3-hydroxypentyl)-3-methyl phenyl]-propyl}-2-methylphenoxymethyl)-dihydrofuran-2-one (20b)

The yield was 78%. Colorless oil. $[\alpha]_D^{25}$ +12.9 ($c$ 1.09, CHCl$_3$). $^1$H NMR (CDCl$_3$) $\delta$: 0.59 (6H, t, $J$ = 7.2 Hz), 0.90 (6H, t, $J$ = 7.4 Hz), 1.55 (4H, q, $J$ = 7.6 Hz), 1.63–1.68 (2H, m), 2.03 (4H, q, $J$ = 14.9 Hz), 2.15 (3H, s), 2.25 (3H, s), 2.28–2.36 (1H, m), 2.38–2.49 (1H, m), 2.52–2.61 (3H, m), 2.73–2.81 (1H, m), 4.06 (1H, dd, $J$ = 10.4, 3.5 Hz), 4.16 (1H, dd, $J$ = 10.3, 3.2 Hz), 4.85–4.91 (1H, m), 6.66 (1H, d, $J$ = 8.4 Hz), 6.89–6.97 (4H, m), 7.00 (1H, d, $J$ = 8.6 Hz). $^{13}$C NMR (CDCl$_3$) $\delta$: 177.2, 153.8, 146.2, 141.5, 137.5, 134.7, 130.7, 129.8, 127.8, 126.2, 125.7, 125.5, 109.8, 77.9, 74.6, 69.3, 48.7, 38.8, 30.9, 29.1, 28.3, 26.7, 24.1, 19.6, 16.6, 8.4, 7.8. MS (ESI positive): 503 (M+Na)$^+$.

### 5.1.34. General procedure for hydrolysis of lactone group of 17a–f, 18a–f, 19b, and 20b

To a solution of lactone (0.355 mmol) in methanol (7.5 ml), 1 N KOH solution (0.7 ml, 0.700 mmol) was added. The mixture was stirred at room temperature for 2 h. The mixture was poured into satd NH$_4$Cl aq solution and products were extracted with AcOEt. The extracts were washed with brine, dried over MgSO$_4$, and concentrated. The obtained residue was chromatographed on silica gel (5% MeOH/CH$_2$Cl$_2$) to afford gamma hydroxyl carboxylic acid. All compounds were stocked at −30 °C as a 1 mg/mL EtOH solution with 1.0 equiv of 1 N NaOH solution to avoid retro lactone formation.

### 5.1.35. (*S*)-5-(4-{1-Ethyl-1-[4-(3-hydroxy-3-methyl-1-butynyl)-3-methylphenyl]-propyl}-2-methylphenoxy)-4-hydroxypentanoic acid (5a)

The yield was 71%. Colorless amorphous. $[\alpha]_D^{25}$ +2.04 ($c$ 0.49, CHCl$_3$). $^1$H NMR (CD$_3$OD) $\delta$: 0.59 (6H, t, $J$ = 7.2 Hz), 1.56 (6H, s), 1.78–1.88 (1H, m), 1.93–2.01 (1H, m), 2.06 (4H, q, $J$ = 7.3 Hz), 2.15 (3H, s), 2.33 (3H, s), 2.34–2.39 (2H, m), 3.90 (2H, d, $J$ = 5.1 Hz), 3.93–3.99 (1H, m), 6.77 (1H, d, $J$ = 8.6 Hz), 6.83 (1H, d, $J$ = 1.8 Hz), 6.94 (2H, dd, $J$ = 8.3, 1.9 Hz), 7.00 (1H, s), 7.21 (1H, d, $J$ = 8.2 Hz). $^{13}$C NMR (CD$_3$OD) $\delta$: 183.5, 157.2, 151.8, 142.1, 141.1, 132.8, 132.3, 131.1, 128.1, 127.9, 127.4, 121.9, 112.2, 99.6, 82.5, 74.1, 72.3, 66.9, 49.8, 36.4, 32.8, 32.4, 30.8, 21.9, 17.6, 9.6. HRMS (ESI negative): Calcd for C$_{29}$H$_{37}$O$_5$ 465.2646. Found: 465.2642 (M−H)$^-$.

### 5.1.36. (*S*)-5-(4-{1-Ethyl-1-[4-(3-ethyl-3-hydroxy-1-pentynyl)-3-methylphenyl]-propyl}-2-methylphenoxy)-4-hydroxypentanoic acid (5b)

The yield was 76%. Colorless amorphous. $[\alpha]_D^{25}$ +1.68 ($c$ 0.60, CHCl$_3$). $^1$H NMR (CD$_3$OD) $\delta$: 0.59 (6H, t, $J$ = 7.2 Hz), 1.09 (6H, t, $J$ = 7.4 Hz), 1.66–1.85 (5H, m), 1.95–2.03 (1H, m), 2.07 (4H, q, $J$ = 7.3 Hz), 2.15 (3H, s), 2.34 (3H, s), 2.41–2.57 (2H, m), 3.90 (2H, d, $J$ = 5.1 Hz), 3.93–3.99 (1H, m), 6.76 (1H, d, $J$ = 8.6 Hz), 6.85 (1H, d, $J$ = 2.0 Hz), 6.94 (2H, dd, $J$ = 8.2, 1.8 Hz), 7.00 (1H, s), 7.23 (1H, d, $J$ = 8.0 Hz). $^{13}$C NMR (CD$_3$OD) $\delta$: 178.4, 157.1, 151.8, 142.3, 141.1, 133.1, 132.4, 131.2, 128.1, 127.9, 127.4, 122.0, 112.3, 97.3, 84.9, 74.2, 74.0, 71.2, 50.0, 36.5, 32.0, 31.0, 30.8, 22.1, 17.6, 10.1, 9.6. HRMS (ESI negative): Calcd for C$_{31}$H$_{41}$O$_5$ 493.2959. Found: 493.2958 (M−H)$^-$.

### 5.1.37. (*S*)-5-(4-{1-Ethyl-1-[4-(3-hydroxy-3-propyl-1-hexynyl)-3-methylphenyl]-propyl}-2-methylphenoxy)-4-hydroxypentanoic acid (5c)

The yield was 95%. Colorless amorphous. $[\alpha]_D^{25}$ +2.00 ($c$ 0.50, CHCl$_3$). $^1$H NMR (CD$_3$OD) $\delta$: 0.59 (6H, t, $J$ = 7.3 Hz), 0.98 (6H, t, $J$ = 7.1 Hz), 1.57–1.72 (8H, m), 1.78–1.88 (1H, m), 1.93–2.02 (1H, m), 2.06 (4H, q, $J$ = 7.3 Hz), 2.15 (3H, s), 2.33 (3H, s), 2.37–2.42 (2H, m), 3.90 (2H, d, $J$ = 5.3 Hz), 3.93–3.99 (1H, m), 6.77 (1H, d, $J$ = 8.6 Hz), 6.83 (1H, d, $J$ = 2.0 Hz), 6.92–6.96 (2H, m), 7.00 (1H, s), 7.21 (1H, d, $J$ = 8.0 Hz). $^{13}$C NMR (CD$_3$OD) $\delta$: 182.5, 157.2, 151.8, 142.2, 141.0, 133.0, 132.3, 131.2, 128.1, 127.9, 127.4, 122.0, 112.2, 97.8, 84.7, 74.1, 73.2, 72.1, 49.8, 46.7, 35.5, 32.1, 30.8, 22.1, 19.8, 17.6, 15.7, 9.6. HRMS (ESI negative): Calcd for C$_{33}$H$_{45}$O$_5$ 521.3272. Found: 521.3279 (M−H)$^-$.

### 5.1.38. (*S*)-5-(4-{1-Ethyl-1-[4-(1-hydroxycyclobutylethynyl)-3-methylphenyl]-propyl}-2-methylphenoxy)-4-hydroxypentanoic acid (5d)

The yield was 95%. Colorless amorphous. $[\alpha]_D^{25}$ +2.52 ($c$ 0.95, CHCl$_3$). $^1$H NMR (CD$_3$OD) $\delta$: 0.59 (6H, t, $J$ = 7.2 Hz), 1.80–1.90 (3H, m), 1.92–2.01 (1H, m), 2.07 (4H, q, $J$ = 7.3 Hz), 2.15 (3H, s), 2.27–2.39 (4H, m), 2.36 (3H, s), 2.43–2.49 (2H, m), 3.90 (2H, d, $J$ = 4.9 Hz), 3.93–3.99 (1H, m), 6.78 (1H, d, $J$ = 8.6 Hz), 6.84 (1H, d, $J$ = 1.8 Hz), 6.93–6.97 (2H, m), 7.01 (1H, d, $J$ = 1.4 Hz), 7.24 (1H, d, $J$ = 8.0 Hz). $^{13}$C NMR (CD$_3$OD) $\delta$: 183.5, 157.2, 151.9, 142.1, 141.1, 132.9, 132.3, 131.2, 128.1, 127.9, 127.4, 121.9, 112.2, 98.4, 83.5, 74.1, 72.3, 69.8, 49.8, 40.8, 36.4, 32.4, 30.8, 21.9, 17.6, 14.7, 9.6. HRMS (ESI negative): Calcd for C$_{30}$H$_{37}$O$_5$ 477.2646. Found: 477.2639 (M−H)$^-$.

### 5.1.39. (*S*)-5-(4-{1-Ethyl-1-[4-(1-hydroxycyclopentylethynyl)-3-methylphenyl]-propyl}-2-methylphenoxy)-4-hydroxypentanoic acid (5e)

The yield was 83%. Colorless amorphous. $[\alpha]_D^{25}$ +2.22 ($c$ 0.63, CHCl$_3$). $^1$H NMR (CD$_3$OD) $\delta$: 0.59 (6H, t, $J$ = 7.3 Hz), 1.75–1.88 (5H, m), 1.93–2.03 (5H, m), 2.06 (4H, q, $J$ = 7.4 Hz), 2.15 (3H, s), 2.33 (3H, s), 2.35–2.40 (2H, m), 3.90 (2H, d, $J$ = 5.1 Hz), 3.93–3.99 (1H, m), 6.77 (1H, d, $J$ = 8.6 Hz), 6.83 (1H, d, $J$ = 1.8 Hz), 6.94 (2H, dd, $J$ = 8.2, 2.0 Hz), 6.99 (1H, s), 7.21 (1H, d, $J$ = 8.0 Hz). $^{13}$C NMR (CD$_3$OD) $\delta$: 183.2, 157.2, 151.8, 142.2, 141.1, 132.8, 132.3, 131.1, 128.1, 127.9, 127.4, 122.1, 112.2, 98.7, 83.4, 76.4, 74.1, 72.3, 50.0, 44.2, 36.1, 32.3, 30.8, 25.2, 21.9, 17.6, 9.6. HRMS (ESI negative): Calcd for C$_{31}$H$_{39}$O$_5$ 491.2803. Found: 491.2813 (M−H)$^-$.

### 5.1.40. (*S*)-5-(4-{1-Ethyl-1-[4-(1-hydroxycyclohexylethynyl)-3-methylphenyl]-propyl}-2-methylphenoxy)-4-hydroxypentanoic acid (5f)

The yield was 92%. Colorless amorphous. $[\alpha]_D^{25}$ +2.36 ($c$ 0.76, CHCl$_3$). $^1$H NMR (CD$_3$OD) $\delta$: 0.59 (6H, t, $J$ = 7.2 Hz), 1.57–1.77 (8H, m), 1.80–1.88 (1H, m), 1.93–2.02 (3H, m), 2.07 (4H, q, $J$ = 7.3 Hz), 2.15 (3H, s), 2.34–2.39 (2H, m), 2.35 (3H, s), 3.90 (2H, d, *J* = 4.9 Hz), 3.93–3.99 (1H, m), 6.77 (1H, d, *J* = 8.6 Hz), 6.83 (1H, d, *J* = 2.0 Hz), 6.93–6.97 (2H, m), 7.00 (1H, s), 7.23 (1H, d, *J* = 8.0 Hz). $^{13}$C NMR ($CD_3OD$) $\delta$: 183.5, 157.2, 151.8, 142.2, 141.1, 132.9, 132.3, 131.2, 128.1, 127.9, 127.4, 122.0, 112.2, 98.4, 84.9, 74.1, 72.3, 70.6, 50.0, 42.0, 36.4, 32.4, 30.8, 27.4, 25.5, 22.1, 17.6, 9.6. HRMS (ESI negative): Calcd for $C_{32}H_{41}O_5$ 505.2959. Found: 505.2953 (M−H)$^-$.

### 5.1.41. (*S*)-5-(4-{1-Ethyl-1-[4-((*E*)-3-hydroxy-3-methyl-1-butenyl)-3-methylphenyl]-propyl}-2-methylphenoxy)-4-hydroxypentanoic acid (6a)

The yield was 90%. Colorless amorphous. $[\alpha]_D^{25}$ +2.73 (*c* 0.73, $CHCl_3$). $^1$H NMR ($CD_3OD$) $\delta$: 0.59 (6H, t, *J* = 7.2 Hz), 1.37 (6H, s), 1.78–1.88 (1H, m), 1.93–2.01 (1H, m), 2.06 (4H, q, *J* = 7.3 Hz), 2.14 (3H, s), 2.27 (3H, s), 2.34–2.40 (2H, m), 3.90 (2H, d, *J* = 5.3 Hz), 3.93–3.98 (1H, m), 6.19 (1H, d, *J* = 15.8 Hz), 6.76 (1H, d, *J* = 8.6 Hz), 6.77 (1H, d, *J* = 16.0 Hz), 6.84 (1H, d, *J* = 2.0 Hz), 6.91–6.97 (3H, m), 7.30 (1H, d, *J* = 8.0 Hz). $^{13}$C NMR ($CD_3OD$) $\delta$: 183.5, 157.1, 150.3, 142.6, 140.3, 136.5, 135.5, 132.4, 131.8, 128.1, 127.9, 127.8, 126.7, 125.8, 112.2, 74.1, 72.6, 72.3, 50.0, 36.4, 32.4, 31.0, 30.9, 21.1, 17.6, 9.6. HRMS (ESI negative): Calcd for $C_{29}H_{39}O_5$ 467.2803. Found: 467.2795 (M−H)$^-$.

### 5.1.42. (*S*)-5-(4-{1-Ethyl-1-[4-((*E*)-3-ethyl-3-hydroxy-1-penteny1)-3-methylphenyl]-propyl}-2-methylphenoxy)-4-hydroxypentanoic acid (6b)

The yield was 85%. Colorless amorphous. $[\alpha]_D^{25}$ +4.10 (*c* 0.20, $CHCl_3$). $^1$H NMR ($CD_3OD$) $\delta$: 0.60 (6H, t, *J* = 7.2 Hz), 0.92 (6H, t, *J* = 7.4 Hz), 1.63 (4H, q, *J* = 7.4 Hz), 1.78–1.88 (1H, m), 1.93–2.00 (1H, m), 2.06 (4H, q, *J* = 7.3 Hz), 2.15 (3H, s), 2.27 (3H, s), 2.34–2.40 (2H, m), 3.90 (2H, d, *J* = 5.1 Hz), 3.93–3.99 (1H, m), 5.99 (1H, d, *J* = 16.0 Hz), 6.75 (1H, d, *J* = 16.2 Hz), 6.76 (1H, d, *J* = 8.4 Hz), 6.85 (1H, d, *J* = 2.0 Hz), 6.92 (1H, s), 6.96 (2H, dd, *J* = 8.5, 2.2 Hz), 7.28 (1H, d, *J* = 8.0 Hz). $^{13}$C NMR ($CD_3OD$) $\delta$: 183.5, 157.1, 150.2, 142.6, 137.8, 136.3, 135.8, 132.4, 131.8, 128.1, 127.9, 127.8, 127.8, 126.7, 112.1, 77.7, 74.1, 72.3, 49.4, 36.4, 35.0, 32.4, 30.9, 21.2, 17.6, 9.7, 9.3. HRMS (ESI negative): Calcd for $C_{31}H_{43}O_5$ 495.3116. Found: 495.3124 (M−H)$^-$.

### 5.1.43. (*S*)-5-(4-{1-Ethyl-1-[4-((*E*)-3-hydroxy-3-propyl-1-hexenyl)-3-methylphenyl]-propyl}-2-methylphenoxy)-4-hydroxypentanoic acid (6c)

The yield was 93%. Colorless amorphous. $[\alpha]_D^{25}$ +2.58 (*c* 1.01, $CHCl_3$). $^1$H NMR ($CD_3OD$) $\delta$: 0.59 (6H, t, *J* = 7.2 Hz), 0.92 (6H, t, *J* = 7.3 Hz), 1.32–1.46 (4H, m), 1.54–1.59 (4H, m), 1.78–1.88 (1H, m), 1.92–2.01 (1H, m), 2.05 (4H, q, *J* = 7.3 Hz), 2.14 (3H, s), 2.26 (3H, s), 2.33–2.39 (2H, m), 3.89 (2H, d, *J* = 5.3 Hz), 3.92–3.98 (1H, m), 6.01 (1H, d, *J* = 16.0 Hz), 6.72 (1H, d, *J* = 16.2 Hz), 6.76 (1H, d, *J* = 8.6 Hz), 6.84 (1H, d, *J* = 2.0 Hz), 6.91 (1H, s), 6.95 (2H, dd, *J* = 8.4, 2.2 Hz), 7.26 (1H, d, *J* = 8.0 Hz). $^{13}$C NMR ($CD_3OD$) $\delta$: 183.5, 157.1, 150.2, 142.6, 138.5, 136.3, 135.8, 132.4, 131.8, 128.1, 127.8, 127.8, 127.3, 126.6, 112.2, 77.3, 74.1, 72.3, 49.5, 45.6, 36.4, 32.4, 30.9, 21.2, 18.9, 17.6, 15.9, 9.7. HRMS (ESI negative): Calcd for $C_{33}H_{47}O_5$ 523.3429. Found: 523.3432 (M−H)$^-$.

### 5.1.44. (*S*)-5-[4-(1-Ethyl-1-{4-[(*E*)-2-(1-hydroxycyclobutyl)-vinyl]-3-methylphenyl}-propyl)-2-methylphenoxy]-4-hydroxypentanoic acid (6d)

The yield was 98%. Colorless amorphous. $[\alpha]_D^{25}$ +2.11 (*c* 0.87, $CHCl_3$). $^1$H NMR ($CD_3OD$) $\delta$: 0.60 (6H, t, *J* = 7.2 Hz), 1.64–1.76 (1H, m), 1.79–1.89 (2H, m), 1.93–2.01 (1H, m), 2.07 (4H, q, *J* = 7.3 Hz), 2.15 (3H, s), 2.18–2.30 (4H, m), 2.28 (3H, s), 2.34–2.40 (2H, m), 3.90 (2H, d, *J* = 5.1 Hz), 3.93–3.98 (1H, m), 6.33 (1H, d, *J* = 15.8 Hz), 6.77 (1H, d, *J* = 8.6 Hz), 6.81–6.85 (2H, m), 6.92–6.98 (3H, m), 7.35 (1H, d, *J* = 8.0 Hz). $^{13}$C NMR ($CD_3OD$) $\delta$: 183.5, 157.1, 150.4, 142.6, 136.8, 136.5, 135.4, 132.4, 131.9, 128.1, 127.9, 127.8, 126.7, 126.1, 112.2, 76.8, 74.1, 72.3, 49.4, 38.1, 36.4, 32.4, 30.9, 21.1, 17.6, 14.0, 9.6. HRMS (ESI negative): Calcd for $C_{30}H_{39}O_5$ 479.2803. Found: 479.2795 (M−H)$^-$.

### 5.1.45. (*S*)-5-[4-(1-Ethyl-1-{4-[(*E*)-2-(1-hydroxycyclopentyl)-vinyl] -3-methylphenyl}-propyl)-2-methylphenoxy]-4-hydroxypentanoic acid (6e)

The yield was 97%. Colorless amorphous. $[\alpha]_D^{25}$ +2.77 (*c* 0.87, $CHCl_3$). $^1$H NMR ($CDCl_3$) $\delta$: 0.60 (6H, t, *J* = 7.2 Hz), 1.71–1.78 (8H, m), 1.79–1.88 (1H, m), 1.93–2.01 (1H, m), 2.07 (4H, q, *J* = 7.3 Hz), 2.15 (3H, s), 2.28 (3H, s), 2.36–2.41 (2H, m), 3.91 (2H, d, *J* = 5.1 Hz), 3.94–3.99 (1H, m), 6.22 (1H, d, *J* = 15.8 Hz), 6.77 (1H, d, *J* = 8.6 Hz), 6.84 (1H, d, *J* = 16.0 Hz), 6.85 (1H, d, *J* = 2.0 Hz), 6.91–6.98 (3H, m), 7.32 (1H, d, *J* = 8.0 Hz). $^{13}$C NMR ($CD_3OD$) $\delta$: 183.2, 157.1, 150.2, 142.6, 138.7, 136.3, 135.6, 132.4, 131.8, 128.1, 127.8, 127.8, 126.7, 126.2, 112.2, 83.9, 74.1, 72.3, 50.0, 42.2, 36.1, 32.3, 30.9, 25.6, 21.1, 17.6, 9.6. HRMS (ESI negative): Calcd for $C_{31}H_{41}O_5$ 493.2959. Found: 493.2955 (M−H)$^-$.

### 5.1.46. (*S*)-5-[4-(1-Ethyl-1-{4-[(*E*)-2-(1-hydroxycyclohexyl)-vinyl]-3-methylphenyl}-propyl)-2-methylphenoxy]-4-hydroxypentanoic acid (6f)

The yield was 98%. Colorless amorphous. $[\alpha]_D^{25}$ +2.26 (*c* 1.06, $CHCl_3$). $^1$H NMR ($CD_3OD$) $\delta$: 0.59 (6H, t, *J* = 7.3 Hz), 1.30–1.41 (2H, m), 1.51–1.77 (8H, m), 1.79–1.89 (1H, m), 1.93–2.02 (1H, m), 2.06 (4H, q, *J* = 7.3 Hz), 2.14 (3H, s), 2.27 (3H, s), 2.34–2.40 (2H, m), 3.90 (2H, d, *J* = 5.1 Hz), 3.93–3.99 (1H, m), 6.16 (1H, d, *J* = 15.8 Hz), 6.76 (1H, d, *J* = 8.6 Hz), 6.81 (1H, d, *J* = 16.0 Hz), 6.84 (1H, d, *J* = 2.0 Hz), 6.91–6.97 (3H, m), 7.30 (1H, d, *J* = 8.2 Hz). $^{13}$C NMR ($CD_3OD$) $\delta$: 183.5, 157.1, 150.3, 142.6, 140.4, 136.4, 135.7, 132.4, 131.8, 128.1, 127.8, 127.8, 126.6, 126.5, 112.2, 74.1, 73.5, 72.3, 49.8, 39.8, 36.4, 32.4, 30.9, 27.7, 24.1, 21.1, 17.6, 9.6. HRMS (ESI negative): Calcd for $C_{32}H_{43}O_5$ 507.3116. Found: 507.3108 (M−H)$^-$.

### 5.1.47. (*S*)-5-(4-{1-Ethyl-1-[4-((*Z*)-3-ethyl-3-hydroxy-1-pentenyl)-3-methylphenyl]-propyl}-2-methylphenoxy)-4-hydroxypentanoic acid (7b)

The yield was 71%. Colorless amorphous. $[\alpha]_D^{25}$ +4.84 (*c* 0.33, $CHCl_3$). $^1$H NMR ($CD_3OD$) $\delta$: 0.59 (6H, t, *J* = 7.3 Hz), 0.86 (6H, t, *J* = 7.4 Hz), 1.43–1.55 (4H, m), 1.77–1.87 (1H, m), 1.94–2.01 (1H, m), 2.07 (4H, q, *J* = 7.3 Hz), 2.14 (3H, s), 2.19 (3H, s), 2.38–2.46 (2H, m), 3.90 (2H, d, *J* = 5.1 Hz), 3.95–3.98 (1H, m), 5.60 (1H, d, *J* = 12.9 Hz), 6.47 (1H, d, *J* = 12.7 Hz), 6.76 (1H, d, *J* = 8.6 Hz), 6.84 (1H, d, *J* = 2.0 Hz), 6.92–6.98 (3H, m), 7.11 (1H, d, *J* = 8.6 Hz). $^{13}$C NMR ($CD_3OD$) $\delta$: 181.5, 157.1, 150.3, 142.6, 139.2, 137.1, 136.4, 132.5, 131.3, 130.3, 130.1, 128.1, 127.8, 126.8, 112.2, 79.0, 74.1, 71.9, 50.0, 35.4, 34.6, 31.8, 30.9, 21.5, 17.6, 9.6, 9.4. HRMS (ESI negative): Calcd. for $C_{31}H_{43}O_5$ 495.3116. Found: 495.3126 (M−H)$^-$.

### 5.1.48. (*S*)-5-(4-{1-Ethyl-1-[4-(3-ethyl-3-hydroxypentyl)-3-methyl phenyl]-propyl}-2-methylphenoxy)-4-hydroxypentanoic acid (8b)

The yield was 91%. Colorless amorphous. $[\alpha]_D^{25}$ +1.88 (*c* 0.64, $CHCl_3$). $^1$H NMR ($CD_3OD$) $\delta$: 0.59 (6H, t, *J* = 7.2 Hz), 0.92 (6H, t, *J* = 7.5 Hz), 1.55 (4H, q, *J* = 7.5 Hz), 1.60–1.64 (2H, m), 1.79–1.89 (1H, m), 1.94–2.01 (1H, m), 2.05 (4H, q, *J* = 7.3 Hz), 2.15 (3H, s), 2.25 (3H, s), 2.35–2.41 (2H, m), 2.55–2.59 (2H, m), 3.91 (2H, d, *J* = 5.1 Hz), 3.94–4.00 (1H, m), 6.76 (1H, d, *J* = 8.4 Hz), 6.85 (1H, d, *J* = 2.0 Hz), 6.88–6.93 (2H, m), 6.95 (1H, dd, *J* = 8.5, 2.2 Hz), 6.99 (1H, d, *J* = 8.0 Hz). $^{13}$C NMR ($CD_3OD$) $\delta$: 183.5, 157.1, 148.6, 142.8, 140.0, 136.6, 132.4, 131.9, 129.9, 128.1, 127.7, 112.2, 76.4, 74.1, 72.3, 50.0, 41.0, 36.4, 32.4, 32.4, 31.0, 28.7, 20.6, 17.6, 9.7, 9.1. HRMS (ESI negative): Calcd. for $C_{31}H_{45}O_5$ 497.3272. Found: 497.3270 (M−H)$^-$.

### 5.2. VDRE reporter gene assay

MG-63 cells were plated at $2 \times 10^3$ cells/200 μL/well in a 96-well white cell-culture plate and were incubated at 37 °C in 5% $CO_2$ incubator for 24 h. Then, MG-63 cells were cotransfected with 0.05 μg of the pGV2-basic/VDRE-luciferase vector which contained three repeats of the VDRE sequence from mouse osteopontin promoter and 0.001 μg of pRL-SV40 vector (Promega Corporation, WI, USA) using Lipofectamine (Invitrogen). The cells were added to minimum essential medium (MEM) containing 5% fetal bovine serum treated with dextran-coated charcoal (DCC-FBS) and were incubated for 8 h. The cells were treated with the serial diluted compounds (final concentrations were $10^{-7}$–$10^{-11}$ mol/L with 0.1% DMSO) and were incubated for an additional 3 days. After removing the supernatants, the cells were lysed in cell-lysis buffer and luciferase activity was measured by DLR™ Assay System (Promega Corporation, WI, USA) and the luminescence was detected by Wallac ALVO SX 1420 multi-label counter (PerkinElmer, Inc., MA, USA). The half maximal effective concentrations ($EC_{50}$) were determined and the inductive activity was calculated as the ratio of the $EC_{50}$ value of the compounds to that of $1,25(OH)_2D_3$ (Solvay Pharmaceuticals, Weesp, The Netherlands) which was used as a positive control.

### 5.3. Modeling and volume calculations

The compounds **5a–f** and **6a–f** were manually docked in the crystal structure of VDR (PDB code 3AZ3). The conformations of compounds were optimized using the MAB force field as implemented in a program MOLOC[21] by fixing the VDR structure.

In order to calculate the PC values, the volume occupied by the side chain of the compound ($V_{\text{side chain}}$) was calculated first.

To calculate $V_{\text{side chain}}$, the space around the volumes occupied by all of the side chains of **5a–f** and **6a–f** should be defined. The rectangular box (see the rectangular box depicted by blue lines in Fig. 5) was defined so as to include all of the side chain volume of the compounds **5a**–**f** and **6a**–**f**. To determine the size of the rectangular box, the minimum and maximum limits for *x*, *y* and *z* directions of all side chain atoms for each compound were calculated by comparing x coordinate of the atom ± van der Waals (vdW) radius, *y* ± vdW and *z* ± vdW for each atom of the side chain. The calculation was performed only for the side chain moiety, $C\text{-}C{\equiv}C\text{-}C(OH)\text{-}R_1R_2$ and $C\text{–}CH{=}CH\text{-}C(OH)R_1R_2$, of the compound. 3D coordinates of the side chain moiety were extracted from the modeled structure. The radii for atomic spheres were 1.70 Å for carbon atoms, 1.55 Å for nitrogen atoms, 1.52 Å for oxygen atoms, and 1.80 Å for sulfur atoms as defined by Bondi.[27] Hydrogen atoms were ignored in the calculation. Minimum and maximum limits for *x*, *y*, *z* directions for all of the compounds were determined by comparing the minimum and maximum limits of **5a**–**f** and **6a**–**f**. The rectangular box for the side chain was defined as minimum and maximum limits for each *x*, *y* and *z* direction with ±0.75 Å margins. The calculations of minimum and maximum limits for *x*, *y*, *z* directions plus ±0.75 Å margins resulted in a rectangular box of 8.3 Å × 11.3 Å × 7.4 Å. The grid points were generated with 0.1 Å grid spacing within the rectangular box.

$V_{\text{side chain}}$ for each compound (see the grid points colored by cyan in Fig. 5) was calculated as follows; all the grid points within the rectangular box were examined to see whether they were within the vdW sphere of each atom of the side chain of the compound. The number n is defined as the number of the grid points within the vdW radius for each atom of the side chain. $V_{\text{side chain}}$ was calculated as $n \times (0.1\ \text{Å})^3$.

$V_{\text{void space}}$, which is the volume in the VDR pocket not occupied by either ligand atoms or VDR atoms, (see the grid points colored by magenta in Fig. 5) was calculated for each compound as follows; all the grid points within the rectangular box were examined to see whether they lay outside the vdW sphere of each atom of VDR and outside the vdW sphere of each atom of the compound. The number of grid point m was defined as the number of grid points that are not within the vdW radius for each atom of either the compound or VDR. $V_{\text{void space}}$ was calculated as $m \times (0.1\ \text{Å})^3$.

$V_{\text{LBD}}$, which represents a volume of the pocket of VDR, is defined as a summation of $V_{\text{side chain}}$ and $V_{\text{viod space}}$. The value of $V_{\text{LBD}}$ was 208 Å$^3$.

The packing coefficient (PC) values were calculated as follows;

$$PC(\%) = V_{\text{sidechain}}/V_{\text{LBD}} \times 100$$

where $V_{\text{side chain}} = n \times (0.1\ \text{Å})^3$, $V_{\text{void space}} = m \times (0.1\ \text{Å})^3$, $V_{\text{LBD}} = V_{\text{side chain}} + V_{\text{void space}}$.

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