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Increased Hydrophobicity and Estrogenic Activity of Simple Phenols with Silicon and Germanium-Containing Substituents

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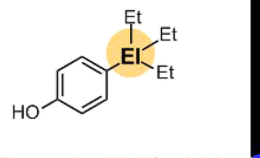
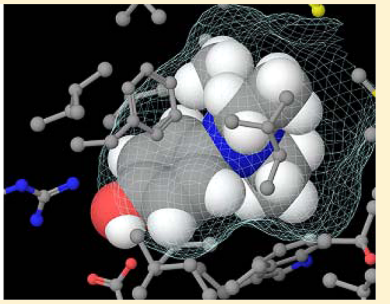
ABSTRACT: Here, we report the systematic synthesis and characterization of simple phenols bearing a trialkyl(aryl)silyl or trialkyl(aryl)germyl functional group as a hydrophobic substituent. These silicon and germanium analogues exhibited higher hydrophobicity than the corresponding carbon analogues, with a difference in log *P* value of approximately 0.6, independent of the alkyl(aryl) species. Trimethylsilylphenol and trimethylgermylphenol exhibited smaller pK_a values than the corresponding carbon analogue or unsubstituted phenol, indicating that trialkylsilyl and trialkylgermyl functional groups have a negative substituent constant (σ). The trialkylsilyl- and trialkylgermylphenols exhibited more potent estrogenic activity as compared with the carbon analogues. The substituent parameters and structure−activity relationship reported here may be helpful for drug discovery utilizing the heavier group 14 elements.



El	logP	ER EC_{50} (nM)
C	5.03	23
Si	5.69	3.4
Ge	5.71	2.1



INTRODUCTION

Hydrophobic interaction, as well as hydrogen bonding and other polar interactions, plays an essential role in the interactions between bioactive compounds and their target proteins.[1] Thus, the application of novel hydrophobic structures is expected to be useful in the development of bioactive compounds. For example, we have demonstrated the utility of carboranes (carbon-containing boron clusters)[2] as the hydrophobic pharmacophore of specific ligands for several nuclear receptors, including retinoid receptors,[3] vitamin D receptor,[4] estrogen receptor (ER),[5] and androgen receptor (AR).[6] In these examples, the boron cluster interacts with hydrophobic amino acid residues of the ligand-binding pocket of the receptors.[4,5] Thus, we considered that the introduction of other hydrophobic moieties might elicit distinctive chemical and pharmacological properties.

One strategy for the development of drug candidates bearing novel hydrophobic substructures is sila-substitution of hydrocarbons.[7] Several silicon-containing analogues of bioactive compounds, such as retinoids Am555S (**1**)[8] and sila-Am80 (**2**)[9] and the topoisomerase inhibitor karenitecin (BNP1350) **3**,[10] have been reported, and these compounds exhibit unique properties distinct from those of the parent carbon analogues (Figure 1). Germanium, one of the heavier group 14 atoms, has also been considered as a possible component of bioactive compounds, but there are only a few reports describing biologically active germanium-containing molecules. The characteristic properties of these silicon- and germanium-containing derivatives result at least partly from the change of their inherent hydrophobicity compared with the corresponding carbon analogues. Although it is known that the hydrophobicity of silicon analogues is higher than that of corresponding carbon analogues,[7,11] there have been few investigations to compare the hydrophobicity of alkylsilicon and alkylgermanium substituents. Because systematic determination of hydrophobicity parameters of these heavier group 14 atom-containing substituents could be useful for the design of novel drug candidates, we set out to synthesize a series of simple phenols bearing a trialkyl(aryl)silyl or trialkyl(aryl)-germyl functional group and to compare their hydrophobicity, substituent constant, and biological activity with those of the carbon analogues.

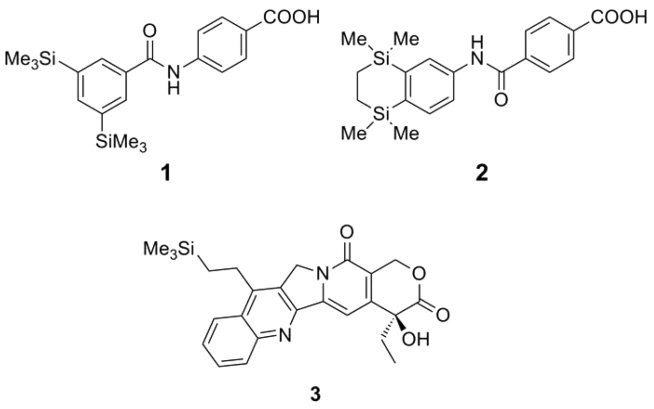


Figure 1. Examples of sila-substituted bioactive substances.

As silicon and germanium-containing compounds, we designed 4-trialkyl(aryl)silylphenols and 4-trialkyl(aryl)-

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gemylphenols (Figure 2). In addition, because 4-substituted phenols are pharmacophores for estrogen receptor (ER)

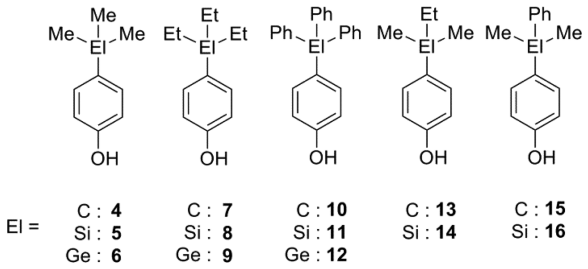


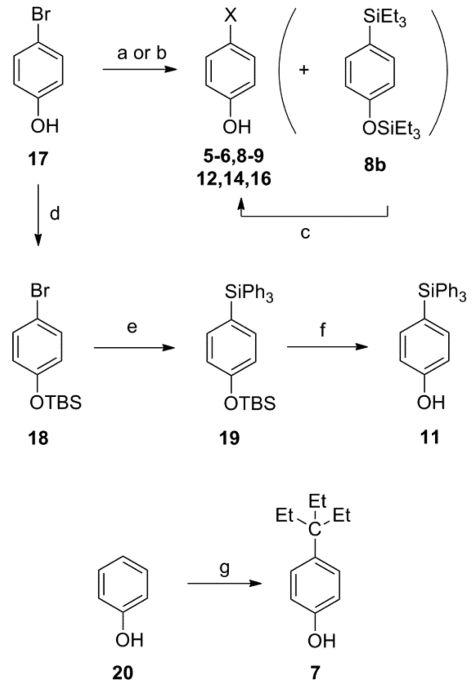
Figure 2. Structures of compounds investigated in this study.

ligands,[12] we aimed to investigate the structure (element)–estrogenic activity relationship of the designed compounds.

RESULTS AND DISCUSSION

Synthesis. Synthesis of the silicon and germanium-containing phenols is summarized in Scheme 1. Lithiation of

Scheme 1. Synthesis of the Designed 4-Substituted Phenols[a]

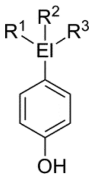


[a]Conditions: (a) *n*-BuLi, trialkylsilyl chloride, or trialkylgermyl chloride, THF, −78 to 0 °C, 31% quant; (b) *t*-BuLi, trialkylsilyl chloride, or trialkylgermyl chloride, THF, −78 to 0 °C, 59% for **6**; (c) TBAF, THF, 0 °C; (d) TBSCl, imidazole, DMF, rt, 78%; (e) *t*-BuLi, triphenylsilyl chloride, THF, −78 °C to rt; (f) TBAF, THF, 0 °C, 50% from **18**; (g) 3-ethyl-3-pentanol, TFA, rt, 86%.

4-bromophenol **17** followed by reaction with trialkylsilyl chloride or trialkylgermyl chloride gave the desired 4-trialkylsilylphenols and 4-trialkylgermylphenols. A silyl or germyl group was also introduced at the phenolic oxygen of **17**, but this was usually removed under aqueous workup conditions. However, in the case of triethylsilylphenol **8**, the triethylsilyl group on the phenolic oxygen remained after workup and had to be removed by hydrolysis. Introduction of the triphenylsilyl group into **17** did not proceed, so first the hydroxyl group was protected with a TBS group and then the triphenylsilyl group was introduced. The carbon analogue **7** corresponding to triethylsilylphenol **8** and triethylgermylphenol **9** was synthesized from phenol by alkylation under acidic conditions. Other carbon analogues **4**, **10**, **13**, and **15** are commercially available and were purchased.

Hydrophobicity. The hydrophobicity of compounds **4**–**16** was determined as the octanol–water partition coefficient (P) using an HPLC method.[13] Table 1 summarizes the log P values

Table 1. Hydrophobicity Parameter of 4-Substituted Phenols



compd	R^1, R^2, R^3	El	log P	π[a]
phenol			1.46	
4	Me_3	C	3.50	+2.04 (+1.98)[b]
5		Si	4.12	+2.66 (+2.59)[b]
6		Ge	4.11	+2.65
7	Et_3	C	5.03	+3.57
8		Si	5.69	+4.23
9		Ge	5.71	+4.25
10	Ph_3	C	*6.25*[c]	*+4.79*
11		Si	*6.83*[c]	*+5.37*
12		Ge	*6.97*[c]	*+5.51*
13	Me_2Et	C	4.04	+2.58
14		Si	4.64	+3.18
15	Me_2Ph	C	4.31	+2.85
16		Si	4.91	+3.45

[a]Hansch–Fujita hydrophobicity parameter of each substituent. [b]Values cited from ref 11. [c]These log P values are tentative because the hydrophobicity was beyond the range of determination of log P by the HPLC method ($0 < \log P < 6$).

of the compounds and the Hansch–Fujita hydrophobicity parameter π of the substituents,[14] which was deduced by subtraction of the log P value of phenol (1.46) from the observed log P value. Among the trimethyl derivatives **4**–**6**, silicon analogue **5** exhibited a log P value of 4.12, which is 0.62 larger than that of carbon analogue **4**. The hydrophobicity parameter π of the trimethylsilyl group was 2.66, which is close to the reported value.[11] Trimethylgermylphenol **6** exhibited a log P value of 4.11, which is almost the same as that of **5** and 0.61 larger than that of **4**. As for the other derivatives, such as triethyl and triphenyl derivatives, the silicon analogues also exhibited larger log P values than the corresponding carbon analogues with differences in the log P value of 0.5–0.7. The differences of log P values between the silicon analogues and corresponding germanium analogues were again small. It is interesting that the differences of log P values between silicon analogues and the corresponding carbon analogues were approximately the same, independent of the substituents on these elements. The difference of hydrophobicity can be mainly attributed to difference of bond length of the central atom, and so it is reasonable that the germanium analogues exhibited log P values similar to those of corresponding silicon analogues (covalent radii: C, 77 pm; Si, 117 pm; Ge, 122 pm).

Acidity. The acidity of the phenolic hydroxyl group was also determined for selected compounds (Table 2). The pK_a values

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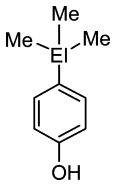
Table 2. pK_a Values of Trimethylsilyl- and Trimethylgermylphenols

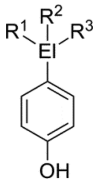


compd	El	pK_a	σ^a
phenol		10.44	
4	C	10.54	+0.10
5	Si	10.06	−0.38
6	Ge	10.20	−0.24

of silicon analogue **5** (10.06) and germanium analogue **6** (10.20) were smaller than that of phenol (10.44), indicating that these substituents behave as electron-withdrawing groups. It is well-known that pK_a values and substituent constants σ are affected by the solvent system used and depend on the structure of compounds.[15] Our results obtained in 20% aqueous methanol were consistent with reported results for benzoic acid derivatives and phenol derivatives in aqueous ethanol.[15,16]

Estrogenic Activity. Estrogen receptor (ER) is a member of the nuclear receptor superfamily, and its ligand is the endogenous estrogen estradiol (E_2).[17] We have previously reported that simple phenols bearing a hydrophobic substituent at the 4-position act as ER ligands,[18] and here we compared the estrogenic activities of the silicon and germanium-containing phenols with those of the parent phenols by assay of their ability to promote estrogen-dependent proliferation of human breast cancer cell line MCF-7 (Table 3).[19] The trialkyl derivatives exhibited marked MCF-7 cell proliferation-promoting activity. Among the tested compounds, triethylsilylphenol **8** and triethylgermylphenol **9** exhibited potent activity with EC_{50} values of 3.4 and 2.1 nM, respectively, being more potent than the corresponding carbon analogue **7** (EC_{50} = 23 nM). Regarding other trialkyl derivatives, the silyl and germyl derivatives also exhibited more potent activity than the corresponding carbon analogues. The triphenyl derivatives **10**, **11**, and **12** all exhibited only moderate MCF-7 cell proliferation-promoting activity. These results suggest that increase of hydrophobicity of the trialkyl substructure (R_3E_l−) increases affinity for ER, but a large increase of hydrophobic volume results in loss of full ER agonistic activity.

Table 3. Proliferation-Promoting Activity of 4-Substituted Phenols toward MCF-7 Cells



compd	R	El	EC_{50} [nM]^a
4	Me_3	C	870
5		Si	63
6		Ge	100
7	Et_3	C	23
8		Si	3.4
9		Ge	2.1
10	Ph_3	C	(16%)^b
11		Si	(29%)^b
12		Ge	(47%)^b
13	Me_2Et	C	190
14		Si	41
15	Me_2Ph	C	38
16		Si	16

^{a}EC_{50} values for MCF-7 cell proliferation-promoting activity were evaluated. Cell proliferation assay was performed in triplicate (n = 3). ^{b}Maximum response in comparison with E_2.

Next, we examined transcriptional activity of compounds **7**, **8**, and **9** with potent activities in MCF-7 cell assay, using T47D-Kbluc cell line that expresses an estrogen-responsive luciferase reporter (Figure 3A).[20] Triethylsilyl derivative **8** exhibited the most potent ER agonistic activity, and triethylgermyl derivative **9** also exhibited higher potency than that of carbon analogue **7**. We also examined the ER-binding affinity of the triethyl derivatives. The binding affinity was examined by competitive binding assay using [^{3}H]E_2 and human ERα (Figure 3B).[21] Triethylsilylphenol **8** and triethylgermylphenol **9** exhibited dose-dependent binding, and the affinities of these compounds exceeded that of the carbon analogue **7** by approximately 1 order of magnitude. These results suggest that the increased MCF-7 cell proliferation-promoting activity of these compounds is induced by stronger binding to and activation of ER.

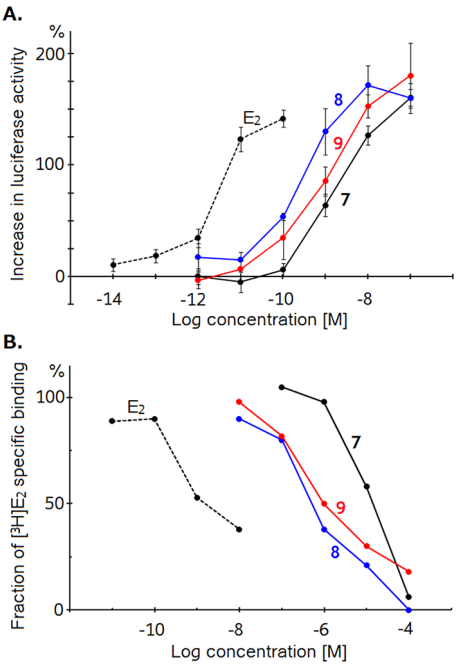


Figure 3. (A) Luciferase assay of compounds **7**−**9** using T47D-Kbluc cell line. (B) Competitive binding assay of compounds **7**−**9** using human ERα and [^{3}H]E_2. The concentration of [^{3}H]-E_2 was 1.0×10^{-8} M.

To understand the interaction between the hydrophobic substructure of these simple phenols and the receptor surface, we conducted docking simulations using the cocrystal structure of hERα-LBD with estradiol (E_2) (PDB ID: 1G50)[22] by a docking program AutoDock.[23] Figure 4 shows the docking models of silyl analogue **8**. In the calculated structure, phenolic hydroxyl group of **8** forms hydrogen bonds to residues of ER (E353 and R394) in a similar manner to that in case of E_2. The

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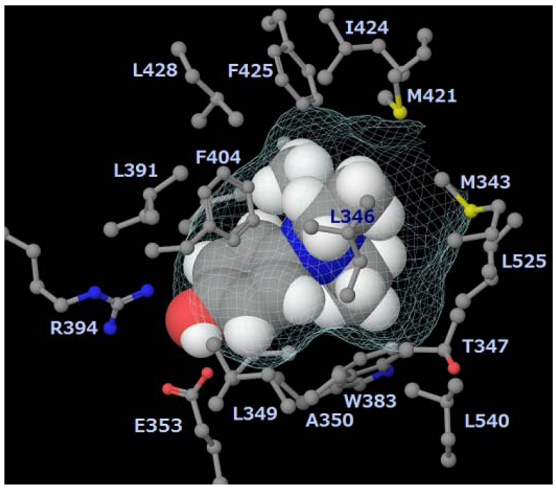

Figure 4. Docking model of triethylsilylphenol **8** and hER-LBD. Side chains of residues near the ligand (<4 Å) are displayed. Protein surface is indicated as a light-blue mesh.

hydrophobic triethylsilyl moiety at 4-position of **8** binds at the hydrophobic region of ER-LBD and is surrounded by many hydrophobic amino acid residues (Figure 4). This calculation suggests that hydrophobicity of 4-position of phenol is essential to ER binding and also supports the idea that increase of hydrophobicity of the substituent at the 4-position increases the affinity for ER. Many structure−activity relationship studies about ER ligands revealed that various factors affect their ligand potency, in particular, location and direction of the second hydroxyl group corresponding to 17-hydroxyl group of E_2 that interacts with H524 of ER.[12] In this study, interestingly, increase of hydrophobicity without significant change of molecular shape significantly increases the affinity to ER and the estrogenic activity. These findings are helpful for development of novel ER ligands. These results further suggest that simple sila-substitution or germa-substitution of bioactive compounds may result in significant enhancement of biological activity.

■ CONCLUSION

We prepared various phenols bearing a trialkylated group 14 element substituent at the 4-position and determined the hydrophobicity of the functional groups. Trialkylsilyl and trialkylgermyl groups exhibited higher hydrophobicity than the corresponding carbon analogues, with a difference in log *P* value of approximately 0.6 in both cases, and the difference in log *P* value versus the carbon analogue was independent of the substituents on these elements. We also determined the substituent constant (σ value) of the trimethyl derivatives and demonstrated that trialkylsilyl and trialkylgermyl substituents function as electron-withdrawing groups in terms of their effect on the phenolic hydroxyl group. Sila-substitution and germa-substitution of the simple phenols examined here resulted in significant enhancement of estrogenic activity. This approach may also be applicable to various ER ligands or other bioactive compounds. Further, the parameters and structure−activity relationship presented here should be helpful in application of the heavier group 14 elements for drug development.

■ EXPERIMENTAL SECTION

Chemistry. All reagents were purchased from Sigma-Aldrich Chemical Co., Tokyo Kasei Kogyo Co., Wako Pure Chemical Industries, and Kanto Chemical Co., Inc. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AVANCE 400 spectrometer or a Bruker AVANCE 500 spectrometer. Chemical shifts for ^{1}H NMR and ^{13}C NMR are reported as parts per million (ppm) relative to chloroform (7.26 ppm for ^{1}H NMR and 77.00 ppm for ^{13}C NMR). 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. Melting points were taken on a Yanagimoto micro melting point apparatus. Mass spectra were obtained with a Daltonics microTOF-2focus and a JEOL AX-505 spectrometer. The purity of compounds was determined by elemental analysis, confirming ≥95% purity. Elemental analyses were carried out by Yanaco MT-6 CHNCORDER spectrometer.

Preparation of Compounds. *General Procedure A for Silyl Compounds **5**, **14**, and **16**.* Under argon atmosphere, a solution of *n*-BuLi in *n*-hexane (3.0 equiv for *p*-bromophenol) was added dropwise to a solution of *p*-bromophenol in THF at −78 °C, and the mixture was stirred at room temperature for 1 h. Trialkyl(aryl)silyl chloride (3.0 equiv for *p*-bromophenol) was added dropwise at −78 °C, and the mixture was stirred at room temperature for several hours. The reaction was quenched with saturated aqueous ammonium chloride, and then the mixture was extracted with ethyl acetate and dried with sodium sulfate and evaporated. The residue was purified by column chromatography (hexane/EtOAc) and gave the desired compound.

*General Procedure B for Germyl Compounds **6**, **9**, and **12**.* Under argon atmosphere, a solution of *t*-BuLi in *n*-pentane (3.0 equiv for *p*-bromophenol) was added dropwise to a solution of *p*-bromophenol in THF at −78 °C. The mixture was stirred at room temperature for 1 h. Trialkyl(aryl)lgermyl chloride (2.0−10 equiv) was added dropwise at −78 °C. The mixture was stirred at room temperature for 4.5 h, and then the reaction was quenched with saturated aqueous ammonium chloride. The solvent was extracted with ethyl acetate and dried with sodium sulfate and evaporated. The residue was purified by column chromatography (hexane/EtOAc) and gave the desired compound.

*4-Trimethylsilylphenol (**5**).* Prepared by the general procedure A (quant). The compound was recrystallized by water to afford colorless crystals. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.25 (s, 9 H), 4.70 (s, 1 H), 6.84 (d, J = 8.5 Hz, 2 H), 7.41 (d, J = 8,5 Hz, 2 H). ^{13}C NMR (125 MHz, $CDCl_3$) δ −0.8, 115.9, 135.7, 135.4, 138.3, 141.0, 159.1. Anal. Calcd for $C_9H_{14}OSi + 1/8H_2O$: C, 64.14; H, 8.52. Found: C, 64.17; H, 8.46.

*4-Trimethylgermylphenol (**6**).* Prepared by the general procedure B (59%). Distillation under reduced pressure gave the title compound as a colorless solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.36 (s, 9 H), 4.63 (s, 1 H), 6.84 (d, J = 8.4 Hz, 2 H), 7.35 (d, J = 8.5 Hz, 2 H). ^{13}C NMR (125 MHz, $CDCl_3$) δ −1.66, 115.5, 133.5, 134.4, 155.7. Anal. Calcd For $C_9H_{14}OGe + 1/8H_2O$: C, 50.73; H, 6.74. Found: C, 50.87; H, 6.78.

*4-(1,1-Diethylpropyl)phenol (**7**).* A solution of phenol (211 mg, 2.24 mmol) in TFA (4.0 mL) was treated with 3-ethyl-3-pentanol (0.34 mL, 2.46 mmol). Stirring was continued at room temperature for 16 h. The solution was concentrated, and the residue was diluted with CH_2Cl_2. The organic layer was washed with water, saturated aqueous sodium bicarbonate, and brine. The combined organic layer was dried with sodium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography (hexane/EtOAc, 10:1) and gave 340 mg (79%) of **7** as colorless solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.65 (t, J = 7.4 Hz, 9 H), 1.63 (q, J = 7.4 Hz, 6 H), 6.77 (d, J = 8.8 Hz, 2 H), 7.16 (d, J = 8,8 Hz, 2 H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 7.9, 28.7, 73.0, 114.6, 128.0, 139.5, 152.8. Anal. Calcd for $C_{10}H_{20}O$: C, 81.20; H, 10.48. Found: C, 81.23; H, 10.47. Melting point 78.9−79.5 °C.

*4-Triethylsilylphenol (**8**).* Under argon atmosphere, a solution of *n*-BuLi in *n*-hexane (5.5 mL, 8.7 mmol) was added dropwise at −78 °C to a solution of *p*-bromophenol (502 mg, 2.9 mmol) in 3.0 mL of THF. The mixture was stirred at room temperature. Triethylchlorosilane (1.5 mL, 8.7 mmol) was added dropwise at −78 °C, and then THF (4 mL) added. The mixture was stirred at room temperature for 2 h, and then the reaction was quenched with saturated aqueous ammonium chloride. The solvent was extracted with ethyl acetate and

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dried with sodium sulfate and evaporated. The residue was purified by column chromatography (hexane) to yield 806 mg (86%) of **8b** as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.76 (q, J = 7.8 Hz, 6 H), 0.76 (q, J = 7.9 Hz, 6 H), 0.96 (t, J = 7.8 Hz, 9 H), 1.01 (t, J = 7.9 Hz, 9 H), 6.84 (d, J = 8.4 Hz, 2 H), 7.34 (d, J = 8,4 Hz, 2 H). A tetrabutylammonium fluoride solution (1.0 M in THF, 1.6 mL, 1.6 mmol) was added dropwise to a solution of **8b** (758 mg, 2.35 mmol) in 7.0 mL of THF at 0 °C, and then the mixture was stirred for 20 min. The mixture was poured into 100 mL of water and extracted with ethyl acetate. The organic layers were dried with sodium sulfate and concentrated. The product was purified by silica gel chromatography (hexane/EtOAc, 8:1) and distillation under reduced pressure gave 130 mg (27%) of **8** as a colorless solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.77 (q, J = 7.8 Hz, 6 H), 0.96 (t, J = 7.8 Hz, 9 H), 4.65 (s, 1 H), 6.84 (d, J = 8.5 Hz, 2 H), 7.38 (d, J = 8,4 Hz, 2 H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 3.5, 7.4, 114.9, 128.5, 135.8, 156.1. Anal. Calcd for $C_{12}H_{20}OSi$: C, 69.17; H, 9.67. Found: C, 69.00; H, 9.49. Melting point 30.8−32.2 °C.

*4-Triethylgermylphenol (**9**).* Prepared by the general procedure B (54%). Distillation under reduced pressure gave the title compound as a colorless solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.93−0.99 (m, 6 H), 1.04−1.08 (m, 9 H), 4.62 (s, 1H), 6.84 (d, J = 8.5 Hz, 2 H), 7.31 (d, J = 8.4 Hz, 2 H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 4.24, 8.91, 115.03, 130.56, 135.3, 155.6. Anal. Calcd for $C_{12}H_{20}OGe$: C, 56.98; H, 7.97. Found: C, 56.75; H, 7.90.

*4-Bromo(t-butyldimethylsilyloxy)benzene (**18**).* Imidazole (469 mg, 6.92 mmol) and TBSCl (781 mg, 5.19 mmol) were added to a solution of *p*-bromophenol (599 mg, 3.46 mmol) in DMF (10 mL) and then stirred for 1 h at 0 °C. The reaction was quenched with addition of water. The mixture was extracted with ethyl acetate and dried with sodium sulfate and then evaporated to yield 779.9 mg (78.2%) of the title compound. ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.2 (s, 6 H), 0.99 (s, 9 H), 6.72 (d, J = 8.9 Hz, 2 H), 7.32 (d, J = 8.8 Hz, 2 H).

*4-Triphenylsilylphenol (**11**).* Under argon atmosphere, a solution of *t*-BuLi in *n*-pentane (2.5 mL, 3.9 mmol) was added dropwise at −78 °C to a solution of **18** (564 mg, 1.95 mmol) in 10 mL of THF. The mixture was stirred at room temperature for 1 h. Chlorotriphenylsilane (1.15 g, 3.9 mmol) in 3 mL of THF was added dropwise at −78 °C. The mixture was stirred at room temperature for 2.5 day, and then the mixture was quenched with saturated aqueous ammonium chloride. The solvent was extracted with ethyl acetate and dried with sodium sulfate and evaporated. The residue was purified by column chromatography (hexane/EtOAc, 8:1) to yield 596 mg of the intermediate **19**. A TBAF solution (1.0 M in THF, 1.6 mL, 1.6 mmol) was added dropwise to the imtermediate **19** (55.6 mg, 0.12 mmol) in 2 mL of THF at 0 °C and stirred for 15 min. The reaction mixture was poured into water and extracted with ethyl acetate. The organic layer was dried with sodium sulfate and concentrated. The product was purified by silica gel chromatography (hexane/EtOAc, 9:1) to give 27 mg (50%, 2 steps) of the title compound as a colorless crystals. Recrystallization from dichloromethane/hexane gave colorless crystals. ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.8 (s, 1 H), 6.86 (d, J = 8.5 Hz, 2 H), 7.36−7.56 (m, 15 H), 7.57 (d, J = 8.0 Hz, 2 H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 115.1, 125.2, 127.8, 129.5, 134.5, 136.3, 138.2, 156.9. Anal. Calcd for $C_{24}H_{20}OSi$: C, 81.77; H, 5.72. Found: C, 81.54; H, 5.99. Melting point 236.7−237.6 °C.

*4-Triphenylgermylphenol (**12**).* Prepared by the general procedure B (54%). Recrystallization from hexane/EtOAc gave a colorless solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.75 (s, 1 H), 6.88 (d, J = 8.5 Hz, 2 H), 7.38−7.54 (m, 15 H), 7.5 (d, J = 7.6 Hz, 2 H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 115.5, 127.0, 128.2, 129.1, 135.3, 136.3, 136.9, 156.4. Anal. Calcd for $C_{24}H_{20}OGe$: C, 72.60; H, 5.08. Found: C, 72.35; H, 5.24. Melting point 228.3−229.4 °C.

*4-Ethyldimethylsilylphenol (**14**).* Prepared by the general procedure A (31%). Distillation under reduced pressure gave colorless solids. ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.22 (s, 6 H), 0.7 (q, J = 7.9 Hz, 2 H), 0.94 (t, J = 7.9 Hz, 3 H), 4.67 (s, 1 H), 6.83 (d, J = 8.5 Hz, 2 H), 7.39 (d, J = 8.5 Hz, 2 H). ^{13}C NMR (125 MHz, $CDCl_3$) δ −3.38, 7.39, 7.59, 114.9, 130.6, 135.2, 156.1. Anal. Calcd for $C_{10}H_{16}OSi$: C, 66.61; H, 8.94. Found: C, 66.65; H, 8.66.

*4-Dimethylphenylsilylphenol (**16**).* Prepared by the general procedure A (39%). Distillation under reduced pressure gave colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.53 (s, 6 H), 4.73 (s, 1 H), 6.84 (d, J = 8.5 Hz, 2 H), 7.33−7.39 (m, 3 H), 7.41 (d, J = 8.4 Hz, 2 H), 7.50−7.55 (m, 2 H). ^{13}C NMR (125 MHz, $CDCl_3$) δ −2.23, 114.9, 127.8, 129.0, 129.3, 134.1, 135.9, 138.6, 156.4. HRMS Calcd for $C_{14}H_{16}OSi$ [M + H]: 228.0970. Found: 228.0976.

Determination of log *P*. The 1-octanol/water partition coefficient, log *P*, was determined by HPLC method based on the OECD Guideline for Testing Chemicals 117.[13] the measurement was performed on a Mightysil RP-18 GP 250−4.6 (5 μm) (Kanto Chemical Co. Inc., Japan) by using an HPLC instrument (UV/vis detector (UV-2077, JASCO), pump (PU-2089, JASCO), and oven (CO-965, JASCO)). The injection volume was 10 μL, and the flow-rate was 1.0 mL/min in all cases. The compounds were detected by measuring UV absorption at 240 and 230 nm. The temperature of the column was kept at 40.0 (±0.1) °C during the measurement. The mobile phase was methanol−aqueous 0.1 M phosphoric acid system, changing the methanol concentration from 80% (v/v) to 60% (v/v) by 5% (v/v) concentration steps. The dead time t_0 was measured with thiourea as the unretained compound. Each measurement was performed in triplicate, and the mean was used for the further calculations. The capacity factor of each compound in 100% aqueous eluent, log k_w, was calculated by extrapolation of the line fitted on the measured log k values of methanol−aqueous mobile phase. For the reference compounds (phenol, *p*-cresol, 4-chlorophenol, 4-phenylphenol, 2,4,6-trichlorophenol, diphenylether, and pentachlorophenol), the calculatd log k_w values were plotted against log P values, and the calibration graph was determined (log P = 1.1369 + 0.1886, R^2 = 0.9966). The log P values of tested phenols were obtained by interpolation of the calculated capacity factors log k_w on the calibration graph.

Determination of pK_a. The pK_a values were determined by measurement of the change of absorbance at λ_{max} in the pH-dependent UV spectra of ionic species in 20:80 (w/w) methanol−phosphate buffer (in the pH range of 7.0−12.0). The values of pH obtained with the pH meter were corrected by using the equation: pH* = pH (recorded) − d (d = 0.01).[24] The pH values at which the concentration of free phenol and ionic phenoxide is equal are determined as pK_a values.

Cell Proliferation Assay Using MCF-7 cell lines. Cells of the human breast adenocarcinoma line MCF-7 were routinely cultivated in DMEM supplemented with 10% FBS, 100 IU/mL penicillin, and 100 mg/mL streptomycin at 37 °C in a 5% CO_2 humidified incubator. On the day before an assay, MCF-7 cells were switched to DMEM (low glucose, phenol red-free supplemented with 5% stripped FBS, 100 IU/mL penicillin, and 100 mg/mL streptomycin) Cells were trypsinized from the maintenance dish with phenol red-free trypsin-EDTA and seeded in a 96-well plate at a density of 1.2×10^3 cells per final volume of 100 μL of DMEM supplemented with 5% stripped FBS, 100 IU/mL penicillin, and 100 mg/mL streptomycin. After 24 h, the medium was replaced with 90 μL of fresh DMEM and 10 μL of drug solution. Final concentration of compounds was 10^{-12} M to $^{-6}$ M. Cells were incubated for 7 days, and medium was replaced once after 4 days. At the end of the incubation time, proliferation was evaluated by using WST-8. Then 10 μL of WST-8 was added to microcultures and cells were incubated for 2 h. The absorbance at 450 nm was measured. This parameter is related to the number of living cells in the culture. The number of living cells was plotted against the logarithm of the concentration, and then the sigmoidal fit was carried out using the KaleidaGraph4.01 to calculate EC_{50} values.

T47D-Kbluc ER Reporter Assay. T47D-Kbluc cells were routinely cultivated in RPMI1640 medium supplemented with 10% FBS at 37 °C in a 5% CO_2 humidified incubator. Cells were trypsinized from the maintenance dish with phenol red-free trypsin and seeded in a 96-well plate at a density of 2.0×10^4 cells per final volume of 90 μL of DMEM (low glucose, phenol red-free supplemented with 8% stripped FBS). After incubation at 37 °C for 24 h, 10 μL of drug solution (1% DMSO in medium) was added. Final concentration of compounds was 10^{-14} M to 10^{-7} M, and final

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concentration of DMSO was 0.1%. After incubation for 24 h, half volume of medium was removed and 50 μL of Steady-Glo (Promega Co.) was added. After 5 min, the chemiluminescence was measured with microplate reader.

hER Binding Assay. The recombinant human ER was purchased from Wako Pure Chemical Industries, Ltd. Bio-Gel HT hydroxylapatite (Bio-Rad, Hercules, CA) was washed five times with the buffer (50 mM Tris-HCl, 1 mM KH_2PO_4, pH 7.2). The hydroxylapatite slurry was adjusted to 50% (by volume) hydroxylapatite in the suspension. The ER was diluted with binding buffer (50 mM Tris, pH 7.5, 10% glycerol, 0.1 mM butylated hydroxyanisole, 10 mM mercaptoethanol, 0.5% yeast extract) to a protein concentration of 2−4 nM. Then 2 μL of 10^{-6} M [^{3}H]estradiol solution in DMSO was added to each tube, followed by 2 μL aliquots of the competitor solutions in DMSO. After addition of 200 μL of ER solution to each tube, the tubes were placed in the refrigerator. The final incubation conditions were the following: 10^{-8} M [^{3}H]-estradiol, 10^{-4}−10^{-8} M competitor. After 14−18 h, the bound ligand was assayed by adsorption on hydroxylapatite for 15 min at 0 °C, followed by three washes with 1 mL of 0.05 M Tris, pH 7.3. After the last wash, the hydroxylapatite pellet was resuspended in 0.3 mL of EtOH and radioactivity was counted in 7 mL of scintillation cocktail (ACS II). All experiments were performed in duplicate.

Molecular Modeling. Structure of LBD of human ER alpha was prepared from the Protein Data Bank accession 1g50. The structure added for polar hydrogens, and partial atomic charges were assigned using AutoDockTools (ADT).[23] Structures of ligand were optimized using MOPAC 2012 (Stewart J. J. P., Stewart Computational Chemistry, Colorado Springs, CO, USA, HTTP://OpenMOPAC.net (2012)) with PM3 parameters and partial atomic charges of them were assigned using ADT. Molecular docking was performed using AutoDock 4.2 with Genetic Algorithm. Autodock parameters for silicon atom Rii = 1.60 and εii = 0.875 were used.

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS USED

ER, estrogen receptor; TBAF, tetra-*n*-butylammonium fluoride; TBS, *t*-butyldimethylsilyl

REFERENCES

(1) *Protein−Ligand Interactions (Methods and Principles in Medicinal Chemistry)*; Gohlke, H., Ed.; Wiley-VCH: Weinheim, Germany, 2012.

(2) (a) *The Borane, Carborane, Carbocation Continuum*; Casanova, J., Ed.; Wiley Interscience: New York, 1998. (b) *Carboranes*, 2nd ed.; Grimes, R. N., Ed.; Academic Press: New York, 2011. For reviews of carboranes: (c) Bregadze, V. I. Dicarba-*closo*-dodecaboranes $C_2B_{10}H_{12}$ and their derivatives. *Chem. Rev.* **1992**, *92*, 209−223. (d) Soloway, A. H.; Tjarks, W.; Barnum, B. A.; Rong, F.-G.; Barth, R. F.; Codogni, I. M.; Wilson, J. G. The chemistry of neutron capture therapy. *Chem. Rev.* **1998**, *98*, 1515−1562.

(3) (a) Iijima, T.; Endo, Y.; Tsuji, M.; Kawachi, E.; Kagechika, H.; Shudo, K. Dicarba-*closo*-dodecaboranes as a pharmacophore. Retinoidal antagonists and potential agonists. *Chem. Pharm. Bull.* **1999**, *47*, 398−404. (b) Endo, Y.; Yaguchi, K.; Kawachi, E.; Kagechika, H. Polymethylcarborane as a novel bioactive moiety: derivatives with potent retinoid antagonistic activity. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1733−1736. (c) Endo, Y.; Iijima, T.; Yaguchi, K.; Kawachi, E.; Inoue, N.; Kagechika, H.; Kubo, K.; Itai, A. Structure−activity study of retinoid agonists bearing substituted dicarba-*closo*-dodecaborane. Relation between retinoidal activity and conformation of two aromatic nuclei. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1307−1311.

(4) (a) Fujii, S.; Masuno, H.; Taoda, Y.; Kano, A.; Wongmayura, A.; Nakabayashi, M.; Ito, N.; Shimizu, M.; Kawachi, E.; Hirano, T.; Endo, Y.; Tanatani, A.; Kagechika, H. Boron cluster-based development of potent nonsecosteroidal vitamin D receptor ligands: direct observation of hydrophobic interaction between protein surface and carborane. *J. Am. Chem. Soc.* **2011**, *133*, 20933−20941. (b) Wongmayura, A.; Fujii, S.; Ito, S.; Kano, A.; Taoda, Y.; Kawachi, E.; Kagechika, H.; Tanatani, A. Novel vitamin D receptor ligands bearing a spherical hydrophobic core structure: comparison of bicyclic hydrocarbon derivatives with boron cluster derivatives. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 1756−1760.

(5) (a) Endo, Y.; Iijima, T.; Yamakoshi, Y.; Yamaguchi, M.; Fukasawa, H.; Shudo, K. Potent estrogenic agonists bearing dicarba-*closo*-dodecaborane as a hydrophobic pharmacophore. *J. Med. Chem.* **1999**, *42*, 1501−1504. (b) Endo, Y.; Iijima, T.; Yamakoshi, Y.; Fukasawa, H.; Miyaura, C.; Inada, M.; Kubo, A.; Itai, A. Potent agonists based on carborane as a hydrophobic skeletal structure: a new medicinal application of boron clusters. *Chem. Biol.* **2001**, *8*, 341−355. (c) Endo, Y.; Yoshimi, T.; Ohta, K.; Suzuki, T.; Ohta, S. Potent estrogen receptor ligands based on bisphenols with a globular hydrophobic core. *J. Med. Chem.* **2005**, *48*, 3941−3944.

(6) (a) Fujii, S.; Goto, T.; Ohta, K.; Hashimoto, Y.; Suzuki, T.; Ohta, S.; Endo, Y. Potent androgen antagonists based on carborane as a hydrophobic core structure. *J. Med. Chem.* **2005**, *48*, 4654−4662. (b) Fujii, S.; Hashimoto, Y.; Suzuki, T.; Ohta, S.; Endo, Y. A new class of androgen receptor antagonists bearing carborane in place of a steroidal skeleton. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 227−230. (c) Fujii, S.; Ohta, K.; Goto, T.; Kagechika, H.; Endo, Y. Acidic heterocycles as novel hydrophilic pharmacophore of androgen receptor ligands with a carborane core structure. *Bioorg. Med. Chem.* **2009**, *17*, 344−350. (d) Fujii, S.; Yamada, A.; Tomita, K.; Nagano, M.; Goto, T.; Ohta, K.; Harayama, T.; Endo, Y.; Kagechika, H. *p*-Carborane-based androgen antagonists active in LNCaP cells with a mutated androgen receptor. *Med. Chem. Commun.* **2011**, *2*, 877−880. (e) Fujii, S.; Ohta, K.; Goto, T.; Oda, A.; Masuno, H.; Endo, Y.; Kagechika, H. Development of androgen receptor ligands by application of ten-vertex *para*-carborane as a novel hydrophobic core structure. *Med. Chem. Commun.* **2012**, *3*, 680−684.

(7) (a) Showell, G. A.; Mills, J. S. Chemistry challenges in lead optimization: silicon isosteres in drug discovery. *Drug Discovery Today* **2003**, *8*, 551−556. (b) Mills, J. S.; Showell, G. A. Exploitation of silicon medicinal chemistry in drug discovery. *Expert Opin. Investig. Drugs* **2004**, *13*, 1149−1157. (c) Gately, S.; West, R. Novel therapeutics with enhanced biological activity generated by the strategic introduction of silicon isosteres into known drug scaffolds. *Drug Dev. Res* **2007**, *68*, 156−163.

(8) (a) Yamakawa, T.; Kagechika, H.; Kawachi, E.; Hashimoto, Y.; Shudo, K. Retinobenzoic acids. 5. Retinoidal activities of compounds having a trimethylsilyl or trimethylgermyl group(s) in human promyelocytic leukemia cells HL-60. *J. Med. Chem.* **1990**, *33*, 1430−1437. (b) Higginbotham, K. B.; Lozano, R.; Brown, T.; Patt, Y. Z.; Arima, T.; Abbruzzese, J. L.; Thomas, M. B. A phase I/II trial of TAC-101, an oral synthetic retinoid, in patients with advanced hepatocellular carcinoma. *J. Cancer Res. Clin. Oncol.* **2008**, *134*, 1325−1335.

(9) Tacke, R.; Müller, V.; Buettner, M. W.; Lippert, W. P.; Bertermann, R.; Daiss, J. O.; Khanwalkar, H.; Furst, A.; Gaudon, C.; Gronemeyer, H. Synthesis and pharmacological characterization of disila-AM80 (disila-tamibarotene) and disila-AM580, silicon analogues of the RARα-selective retinoid agonists AM80 (tamibarotene) and AM580. *ChemMedChem* **2009**, *4*, 1797−1802.

(10) (a) New highly lipophilic camptothecin BNP1350 is an effective drug in experimental human cancer Van Hattum, A. H.;

Pinedo, H. M.; Schluper, H. M. M.; Hausheer, F. H.; Boven, E. *Int. J. Cancer* **2000**, *88*, 260−266. (b) Daud, A.; Valkov, N.; Centeno, B.; Derderian, J.; Sullivan, P.; Munster, P.; Urbas, P.; DeConti, R. C.; Berghorn, E.; Liu, Z.; Hausheer, F.; Sullivan, D. Phase II Trial of Karenitecin in Patients with Malignant Melanoma: Clinical and Translational Study. *Clin. Cancer Res.* **2005**, *11*, 3009−3016. (c) Munster, P. N.; Daud, A. I. Preclinical and clinical activity of the topoisomerase I inhibitor, karenitecin, in melanoma. *Expert Opin. Investig. Drugs* **2011**, *20*, 1565−1574.

(11) Hansch, C.; Leo, A.; Unger, S. H.; Kim, K. H.; Nikaitani, D.; Lien, E. J. "Aromatic" substituent constants for structure−activity correlations. *J. Med. Chem.* **1973**, *16*, 1207−1216.

(12) Katzenellenbogen, J. A. The 2010 Philip S. Portoghese Medicinal Chemistry Lectureship: Addressing the "Core Issue" in the Design of Estrogen Receptor Ligands. *J. Med. Chem.* **2011**, *54*, 5271−5282.

(13) *OECD Guideline for Testing of Chemicals 117, Partition Coefficient (n-octanol/water), High Performance Liquid Chromatography (HPLC) Method*; March 1989.

(14) Fujita, T.; Iwasa, J.; Hansch, C. A new substituent constant, π, derived from partition coefficients. *J. Am. Chem. Soc.* **1964**, *86*, 5175−5180.

(15) Chatt, J.; Williams, A. A. The nature of the co-ordinate link. Part IX. The dissociation constants of the acids p-R_3M-C_6H_4-CO_2H (M = C, Si, Ge, and Sn, and R = Me and Et) and the relative strengths of dπ−pπ-bonding in the M−Car bond. *J. Chem. Soc.* **1954**, 4403−4411.

(16) Benkeser, R. A.; Krysiak, H. R. The conjugate ability of the trimethylsilyl group. *J. Am. Chem. Soc.* **1953**, *75*, 2421−2425.

(17) (a) Evans, R. M. The steroid and thyroid hormone receptor superfamily. *Science* **1988**, *240*, 889−894. (b) McEwan, I. J. Gene regulation through chromatin remodeling by members of the nuclear receptor superfamily. *Biochem. Soc. Trans.* **2000**, *28*, 369−373.

(18) Yamakoshi, Y.; Otani, Y.; Fujii, S.; Endo, Y. Dependence of estrogenic activity on the shape of the 4-alkyl substituent in simple phenols. *Biol. Pharm. Bull.* **2000**, *23*, 259−261.

(19) Ohta, K.; Chiba, Y.; Ogawa, T.; Endo, Y. Promising core structure for nuclear receptor ligands: design and synthesis of novel estrogen receptor ligands based on diphenylamine skeleton. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 5050−5053.

(20) Wilson, V. S.; Bobseine, K.; Gray, L. E., Jr. Development and characterization of a cell line that stably expresses an estrogen-responsive luciferase reporter for the detection of estrogen receptor agonist and antagonists. *Toxicol. Sci.* **2004**, *81*, 69−77.

(21) Carlson, K. E.; Choi, I.; Gee, A.; Katzenellenbogen, B. S.; Katzenellenbogen, J. A. Altered ligand binding properties and enhanced stability of a constitutively active estrogen receptor: evidence that an open pocket conformation is required for ligand interaction. *Biochemistry* **1997**, *36*, 14897−14905.

(22) Eiler, S.; Gangloff, M.; Duclaud, S.; Moras, D.; Ruff, M. Overexpression, purification, and crystal structure of native ER alpha LBD. *Protein Expression Purif.* **2001**, *2*, 165−173.

(23) Morris, G. M.; Huey, R.; Lindstrom, W.; Sanner, M. F.; Belew, R. K.; Goodsell, D. S.; Olson, A. J. Autodock4 and AutoDockTools4: automated docking with selective receptor flexiblity. *J. Comput. Chem.* **2009**, *16*, 2785−2791.

(24) Bates, R. G. *Determination of pH, Theory and Practice*; Wiley: New York, 1973.