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Research Paper

Potent estrogen agonists based on carborane as a hydrophobic skeletal structure
A new medicinal application of boron clusters

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Abstract

Background: Carboranes (dicarba-*closo*-dodecaboranes) are a class of carbon-containing polyhedral boron-cluster compounds having remarkable thermal stability and exceptional hydrophobicity. Applications of the unique structural and chemical properties offered by icosahedral carboranes in boron neutron capture therapy have received increasing attention over the past 30 years. However, these features of carboranes may allow another application as a hydrophobic pharmacophore in biologically active molecules that interact hydrophobically with receptors.

Results: We have designed candidate estrogen-receptor-binding compounds having carborane as a hydrophobic skeletal structure and synthesized them. The most potent compound bearing a carborane cage exhibited activity at least 10-fold greater than that of 17β-estradiol in the luciferase reporter gene assay. Estrogen receptor-α-binding data for the compound were consistent with the results of the luciferase reporter gene assay. The compound also showed potent in vivo effects on the recovery of uterine weight and bone loss in ovariectomized mice.

Conclusion: Further development of the potent carborane-containing estrogenic agonists described here, having a new skeletal structure and unique characteristics, should yield novel therapeutic agents, especially selective estrogen receptor modulators. Furthermore, the suitability of the spherical carborane cage for binding to the cavity of the estrogen receptor-α ligand-binding domain should provide a basis for a similar approach to developing novel ligands for other steroid receptors.

Keywords: Carborane; Estrogen ligand; Hydrophobic interaction; Nuclear receptor

1. Introduction

The icosahedral carboranes (dicarba-*closo*-dodecaboranes), which were first synthesized in the 1960s, have unusual properties, such as (1) high boron content, (2) remarkable thermal and chemical stability, (3) a hydrophobic surface, and (4) a spherical geometry [1]. The carboranes were utilized in medicinal chemistry in the field of boron neutron capture therapy (BNCT) for incorporation of large numbers of boron atoms into tumor cells. Since the early applications of simple boron-containing compounds for BNCT, various compounds have been synthesized by adding carborane units to amino acids, nucleic acids, porphyrins, DNA binders and carborane-containing antibodies [2,3]. Among the properties of carboranes, the high boron content and chemical stability are useful for BNCT. However, their highly hydrophobic character prevents the incorporation of these molecules into tumor cells. Therefore, derivatization of carborane-containing molecules has been conducted to obtain water solubility [4]. On the other hand, from the standpoint of receptor–ligand complexation, the exceptional hydrophobic character of carboranes [5,6] may allow their use as a hydrophobic pharmacophore in biologically active molecules. This concept has been realized by the synthesis of several biologically active peptides, in which phenylalanine residues were replaced with (*o*-carboranyl)alanine [7–10].

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However, little attention has been paid to the possible use of carborane as a hydrophobic skeletal structure, in the field of drug design. We have been engaged in the design and synthesis of new nuclear receptor ligands [11,12] and signal transduction modulators [13,14], and have recognized the importance of hydrophobic interaction in receptor–ligand complexation. The difference of binding constants between a ligand having a suitable hydrophobic group and a ligand without such a group sometimes reaches 100 ~ 10000 times. To evaluate the utility of icosahedral carborane as a hydrophobic component for drug design [15–17], we focused on the design and synthesis of estrogen agonists bearing a carborane cage as a hydrophobic skeletal structure [18].

The steroid hormone estrogen influences the growth, differentiation, and functioning of many target tissues. Estrogens play an important role in the female and male reproductive systems, and also in bone maintenance, in the central nervous system and in the cardiovascular system. The first step in the appearance of these activities is mediated by the binding of hormonal ligands to the estrogen receptors (ER) α [19] and β [20]. The hormone-bound ER undergoes a conformational change, allowing the receptor to dimerize. The dimer functions as a transcription factor that mediates biological response by binding to specific promoter elements of DNA to initiate gene transcription. Compounds that either induce or inhibit cellular estrogen responses have potential value as biochemical tools and candidates for drug development. In this article, we describe the synthesis and biological evaluation of novel carborane-containing estrogenic agonists, which are more potent than 17β-estradiol.

2. Results

2.1. Ligand design

Since the discovery of the non-steroidal estrogen diethylstilbestrol [21], many stilbenes, triarylethylenes and other aromatic compounds have been synthesized and shown to possess estrogenic activity. Many estrogen agonists and antagonists [22] have been developed as agents for regulating fertility, preventing and controlling hormone-responsive breast cancer, and post-menopausal hormone replacement. Estrogenic activity has been found in a large range of structural prototypes, including non-steroidal compounds isolated from plants, such as flavonoids [23]. On the other hand, the health risks of the estrogenic compounds that are either present in the environment or used as chemicals have become of interest in recent years [24]. One of the reasons for the estrogenic activity of such a wide variety of organic chemicals appears to be that ER binding is primarily the result of interaction between the receptor and a phenolic residue. Furthermore, the estrogenic activity is known to depend on the shape of the alkyl substituent in simple phenols having a bulky alkyl group at the 4-position. Therefore, high binding affinity for ER and the appearance of substantial estrogenic activity require an appropriate hydrophobic group adjacent to the phenolic ring, other than two hydrogen-bonding groups such as a phenolic hydroxyl group and another hydroxyl group located at a suitable position on the molecule. The C, D rings on the natural hormone, 17β-estradiol (**1**), play an important role in stabilizing the ligand–receptor complex by hydrophobic interactions. The size of the carborane cage seems to be appropriate for the hydrophobic skeletal structure in place of the C, D ring structure of **1**. Substitution of the two carbon atoms of the carborane isomers should allow suitable fixation of the direction of the functional groups. On the basis of these considerations, we designed the compound BE120 (**2c**), which seems to contain all the essential molecular recognition components for ER. Fig. 1 shows a structural comparison of 17β-estradiol (**1**) and **2c**. As shown in Fig. 1, we also designed compounds **2a**, **2b** and **2d**–**f** to evaluate the effects of substituents and of the distance of the aliphatic hydroxyl group from the hydrophobic skeleton. In icosahedral

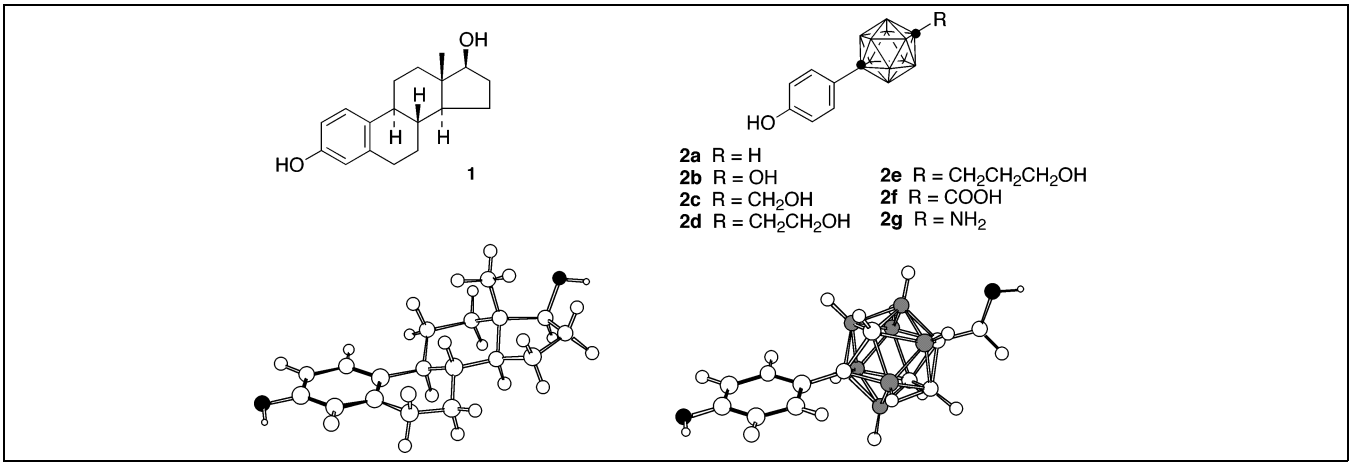

Fig. 1. Structures of 17β-estradiol (**1**) and designed compounds (**2**). (Bottom) Structural comparison of **1** and **2c**.

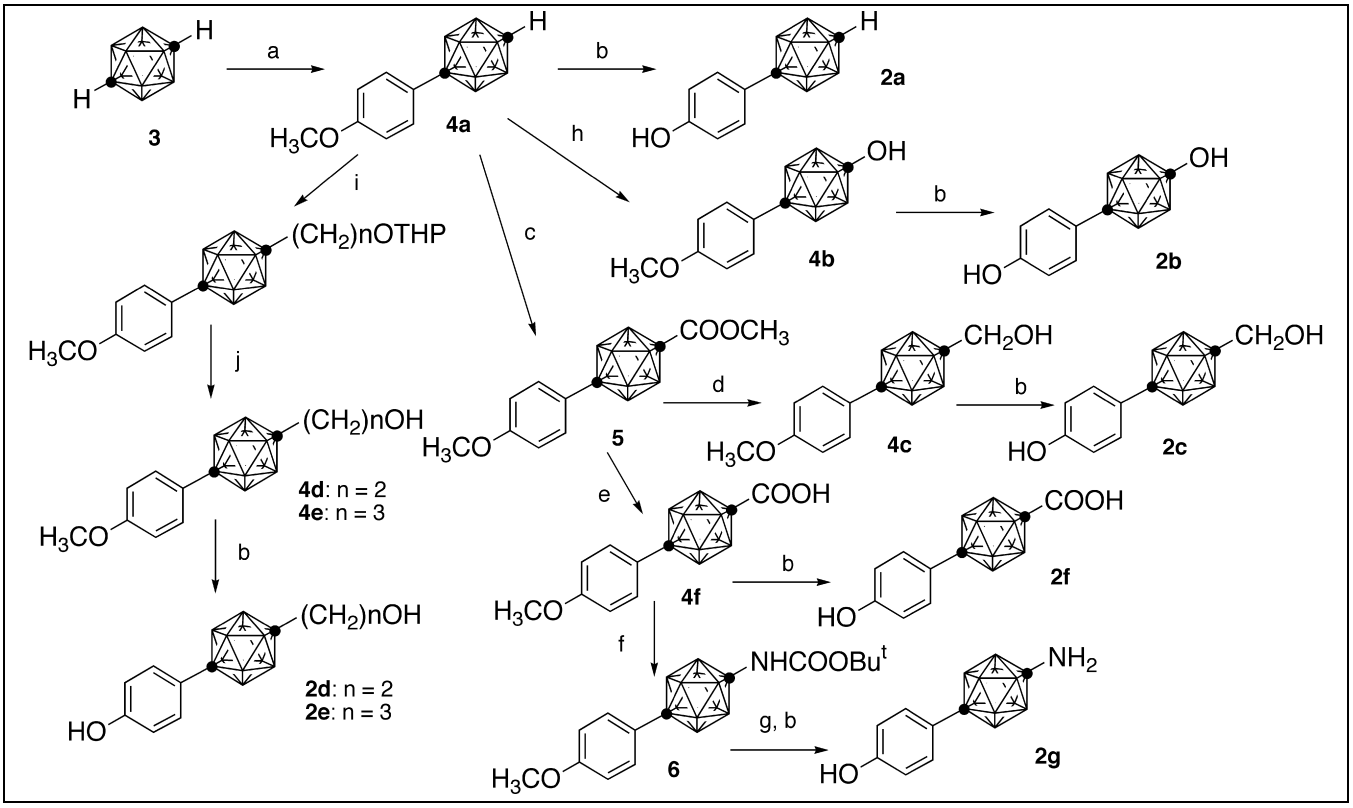

Fig. 2. Synthetic route used for the preparation of the estrogen ligands bearing carborane. Key: (a) (1) *n*-BuLi, CuCl/DME, (2) *p*-iodoanisole/pyridine, reflux; (b) BBr_3/CH_2Cl_2; (c) (1) *n*-BuLi/benzene–Et_2O, (2) $ClCOOCH_3$; (d) $LiAlH_4$/THF; (e) KOH/H_2O–THF; (f) DPPA, Et_3N, DMAP/*t*-BuOH reflux; (g) CF_3COOH/CH_2Cl_2; (h) (1) *n*-BuLi/benzene–Et_2O, (2) $(C_6H_5COO)_2$/benzene–Et_2O; (i) (1) *n*-BuLi/benzene–Et_2O, (2) $Br(CH_2)_nOTHP$; (j) *p*-TsOH·H_2O/MeOH.

cage structures throughout this paper, closed circles (●) represent carbon atoms and other vertices represent BH units.

2.2. Ligand synthesis

The designed molecules **2** were synthesized from 1,12-dicarba-*closo*-dodecaborane (*p*-carborane, **3**) as shown in Fig. 2. 1-(4-Methoxyphenyl)-1,12-dicarba-*closo*-dodecaborane (**4a**), which was prepared by coupling of the C-copper (I) derivative of **3** with 4-iodoanisole in dimethoxyethane in the presence of pyridine in 60% yield [25], was demethylated with boron tribromide to afford compound **2a** in 93% yield. The methoxyphenylcarborane **4a** was converted to the *C*-methoxycarbonyl derivative **5** by reaction of the lithiate of **4a** with methyl chloroformate (91%). After reduction of **5** with $LiAlH_4$, demethylation gave 1-hydroxymethyl-12-(4-hydroxyphenyl)-1,12-dicarba-*closo*-dodecaborane (BE120, **2c**) in 98% yield. 1-Hydroxy-12-(4-hydroxyphenyl)-1,12-dicarba-*closo*-dodecaborane (**2b**) was prepared by oxidation of the lithiate of **4a** with benzoyl peroxide [26] followed by demethylation (75%). Hydrolysis of the ester group of **5** followed by demethylation afforded 12-(4-hydroxyphenyl)-1,12-dicarba-*closo*-dodecaborane-1-carboxylic acid (**2f**) in 98% yield. The acid **4f** was converted to the C-*t*-butoxycarbonylamino derivative (**6**) in 41% yield by means of the modified Curtius rearrangement [27] employing diphenylphosphoryl azide (DPPA), 4-*N,N*-dimethylaminopyridine (DMAP) and *tert*-butanol. Deprotection of the Boc group of **6** followed by demethylation gave 1-amino-12-(4-hydroxyphenyl)-1,12-dicarba-*closo*-dodecaborane (**2g**) in a quantitative yield. Compound **4a** was converted to **4d** by reaction of the lithiate of **4a** with 2-(2-bromoethoxy)tetrahydro-2*H*-pyran followed by deprotection of the THP group with *p*-toluenesulfonic acid (45%). Demethylation of the methoxy group gave 1-hydroxyethyl-12-(4-hydroxyphenyl)-1,12-dicarba-*closo*-dodecaborane (**2d**) in 88% yield. Similarly, introduction of a C3 unit into **4a** afforded 1-hydroxypropyl-12-(4-hydroxyphenyl)-1,12-dicarba-*closo*-dodecaborane (**2e**).

2.3. Transactivational properties and ER-binding affinity of the designed carborane-containing molecules

The estrogenic activities of the synthesized compounds were examined by the luciferase reporter gene assay [28], in which a rat ERα expression plasmid [29] and a reporter plasmid, which contains five copies of estrogen response elements, are transiently transfected into COS-1 cells. 17β-Estradiol at 1×10^{-10}–1×10^{-8} M induced the expression of luciferase in a dose-dependent manner. This activation by 17β-estradiol was dependent upon the expression of ER

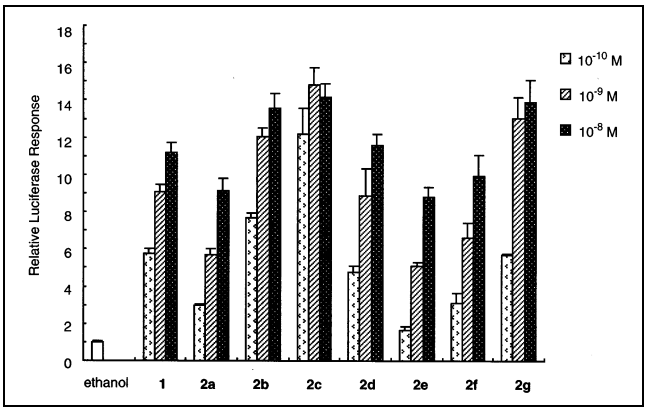

Fig. 3. Transcriptional activation by the estrogen ligands bearing carborane (**2a**–**g**). COS-1 cells were transfected with EREx5-pGL-TK and pCl-rERα and incubated with the compounds at the indicated concentrations (10^{-10}–10^{-8} M). Results are shown as means ± S.D. for triplicate transfections.

and was completely inhibited by estrogen antagonists (tamoxifen and ICI 164,384). The results of the estrogenic activity assay of our carborane-containing molecules (**2a**–**g**) are summarized in Fig. 3. Compound **2a**, which is a simple phenol bearing *p*-carboranyl at the 4-position, exhibited potent transcriptional activity in the concentration range of 1×10^{-10}–1×10^{-8} M; its potency is much higher than that of 4-alkylphenols. 1-Phenyl-1,12-dicarba-*closo*-dodecaborane, which lacks the hydroxyl group, showed no activity. On the other hand, the activity of compound **2b**, which has a second hydrogen-bonding group on carbon of the carborane cage, was significantly increased. The potency of **2b** was greater than that of 17β-estradiol. BE120 (**2c**), which has a hydroxymethyl group on the carborane cage, afforded the most potent activity, being at least 10 times more potent than 17β-estradiol. Further insertion of methylene units in the substituent on the carborane cage (**2d** and **2e**) decreased the activity. The activity of **2g** with an amino group on the carborane cage seemed to be somewhat stronger than that of 17β-estradiol, and **2f** with a carboxylic acid moiety exhibited moderate activity.

ERα-binding assays were performed on the three active

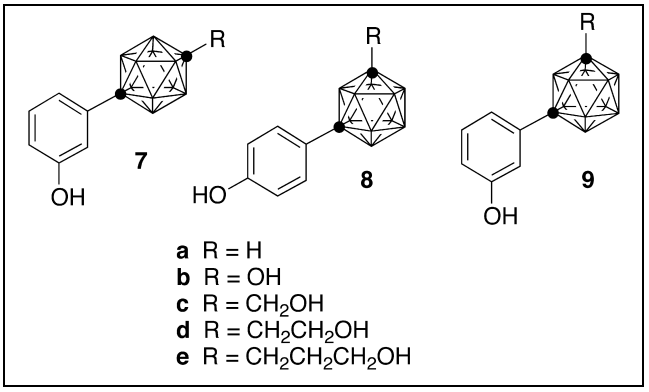

Fig. 4. Designed compounds for the structure–activity study of carborane-containing estrogens.

compounds (**2a**, **2c** and **2g**) and 17β-estradiol to confirm that the gene-regulatory activity correlated with the binding affinity for the ERα. The assays were done by measurement of inhibition of [6,7-^{3}H]17β-estradiol binding (K_d = 0.4 nM) to human recombinant ERα (PanVera), using the nitrocellulose filter binding assay method. The ERα-binding data for these compounds are consistent with the results of the luciferase reporter gene assay. Compounds **2a** and **2g** showed strong affinity for ERα; their potency was almost the same as that of 17β-estradiol. The most active compound in the luciferase reporter gene assay, **2c**, also showed the highest affinity for ERα, and its affinity was higher than that of 17β-estradiol. The K_i values of **2a**, **2c**, and **2g** for ERα were 0.40, 0.10, and 0.65 nM, respectively.

2.4. Structure–activity relations of the carborane-containing estrogens

To investigate the relation between the activity and the geometry and separation distance of the two hydroxyl groups on the phenylcarborane skeleton, we designed and synthesized compounds bearing a *para*-substituent on the carborane cage and a 3-hydroxyl group on the benzene nucleus (**7a**–**e**), compounds bearing a substituted *meta*-carborane cage and a 4-hydroxyl group on the benzene nucleus (**8a**–**e**) and compounds bearing a substituted *meta*-carborane cage and a 3-hydroxyl group on the benzene nucleus (**9a**–**e**), in the same manner as described for **2a**–**2e** (Fig. 4). The transcriptional activity of compounds **7**, **8**, and **9** is summarized in Fig. 5. The activity of the compounds bearing a *para*-substituent on the carborane cage and a 3-hydroxyl group on the benzene nucleus (**3**) was somewhat weaker than that of **2**, and showed the same tendency upon insertion of methylene groups as in the series of **2**. However, the potency of 1-hydroxymethyl-12-(4-hydroxyphenyl)-1,12-dicarba-*closo*-dodecaborane **7c** was still greater than that of 17β-estradiol. The activity

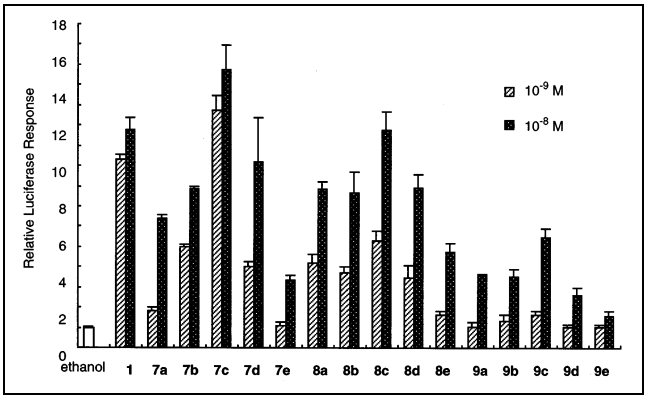

Fig. 5. Transcriptional activation by the estrogen ligands bearing carborane (**7**–**9**). COS-1 cells were transfected with EREx5-pGL-TK and pCl-rERα and incubated with the compounds at the indicated concentrations (10^{-10}–10^{-8} M). Results are shown as means ± S.D. for triplicate transfections.

of the compounds bearing a *meta*-substituent on the carborane cage and a 4-hydroxyl group on the benzene nucleus (**8**) was weaker than that of **7**. Further, the activity of the compounds bearing a *meta*-substituent on the carborane cage and a 3-hydroxyl group on the benzene nucleus (**9**) was greatly decreased, being similar to that of the typical estrogenic alkylphenol 4-*tert*-octylphenol.

2.5. *Effects of the carborane-containing estrogens on uterine atrophy and bone loss in ovariectomized mice*

Estrogen deficiency results in uterine atrophy and a marked bone loss, and these phenomena can be restored by estrogen administration [30]. Using ovariectomized (OVX) mice, we compared the effects of 17β-estradiol and **2c** on the uterus. The uterine weight decreased markedly in OVX mice, indicating that the mice were estrogen-deficient as shown in Fig. 6. As reported previously, 17β-estradiol (100 ng/day) restored the decreased uterine weight in OVX mice to a level higher than that of the sham-operated mice. Compound **2c** (100 ng/day) also restored the uterine weight of OVX mice, and its potency was similar to that of **1** (Fig. 6A). This indicates that **2c** exhibits a potent estrogenic action in the uterus in vivo.

To determine the effects of **2c** on bone mass, OVX mice were treated for 4 weeks with 100 ng/day of **2c** and the femurs were subjected to radiographic analysis. X-ray analysis showed a marked loss of mineralized cancellous bone, especially in the distal metaphysis of the femur, in OVX mice [31]. Treatment with 17β-estradiol or **2c** at 100 ng/day markedly and similarly prevented the bone loss in the distal metaphysis in OVX mice (Fig. 6B). The effects of **2c** on bone mass were determined by measuring bone mineral density (BMD) at a distal region of the femur. BMD was significantly reduced by OVX but recovered completely on the administration of 100 ng/day of **2c** to the sham level. 17β-Estradiol at the same dosage also restored the BMD, as reported previously. These results suggest that **2c** binds to estrogen receptor(s) present in uterus and bone, and exhibits estrogen-like effects in both tissues.

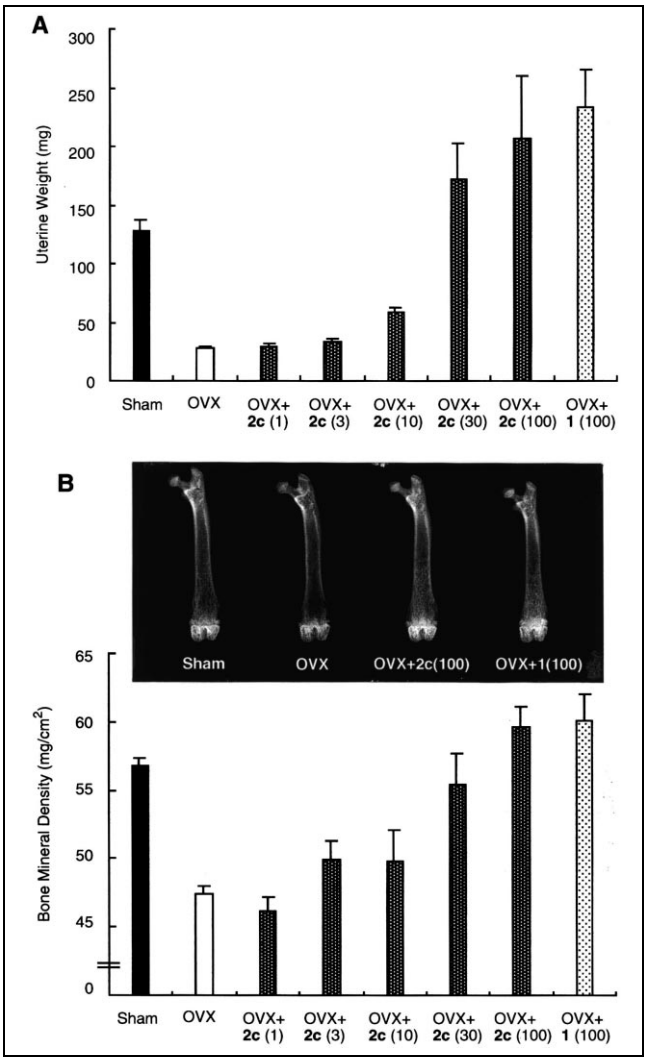

Fig. 6. Effects of **1** and **2c** on the uterine weight and bone mineral density (BMD) of femurs in OVX mice. Mice were sham-operated or OVX, and some of the OVX mice were treated with 1–100 ng/day of **2c** or 100 ng/day of **1** immediately after the operation. Numbers in parentheses are doses of the test compounds per day (ng). At 4 weeks after the operation, uterine weight (A) and BMD of the distal femur (B) were measured. The upper panel in B shows soft X-ray radiograms of the femurs collected from animals of each group. Note that marked bone loss occurred in the femoral cancellous bone in OVX mice, and that the bone loss was completely prevented by the treatment with 100 ng/day of **2c** or **1**. Data are expressed as the means ± S.E.M. of 6–8 mice.

3. Discussion

Recent studies on the three-dimensional structure of the complex formed by 17β-estradiol and the human ERα ligand-binding domain (hERαLBD) have revealed the structural requirements for the appearance of estrogenic activity [32,33]. 17β-Estradiol is oriented in the hERα ligand-binding pocket by two types of contacts: hydrogen bonding at both ends and hydrophobic van der Waals contacts along the body of the skeleton. The phenolic hydroxyl group is hydrogen-bonded to the glutamate (Glu-353) of hERαLBD and the 17-β-hydroxyl group is hydrogen-bonded to the δ-nitrogen of His-524. Docking simulations to this structure (1ERE: Fig. 7A) would be useful to clarify the relation between the three-dimensional structures and high activity of carborane-containing estrogens. These simulations were performed using an automatic docking program (ADAM; not publicly distributed, only available for collaborators on contract basis) [34]. The most potent carborane-containing estrogen **2c** was well-fitted to the cavity of the X-ray structure of hERαLBD. Compound **2c** is oriented in the cavity as shown in Fig. 7B by two types of contacts: hydrogen bonding at both ends and hydrophobic van der Waals contacts along the body of the skeleton, as found for 17β-estradiol (**1**) in the X-ray structure. Compound **2b**,

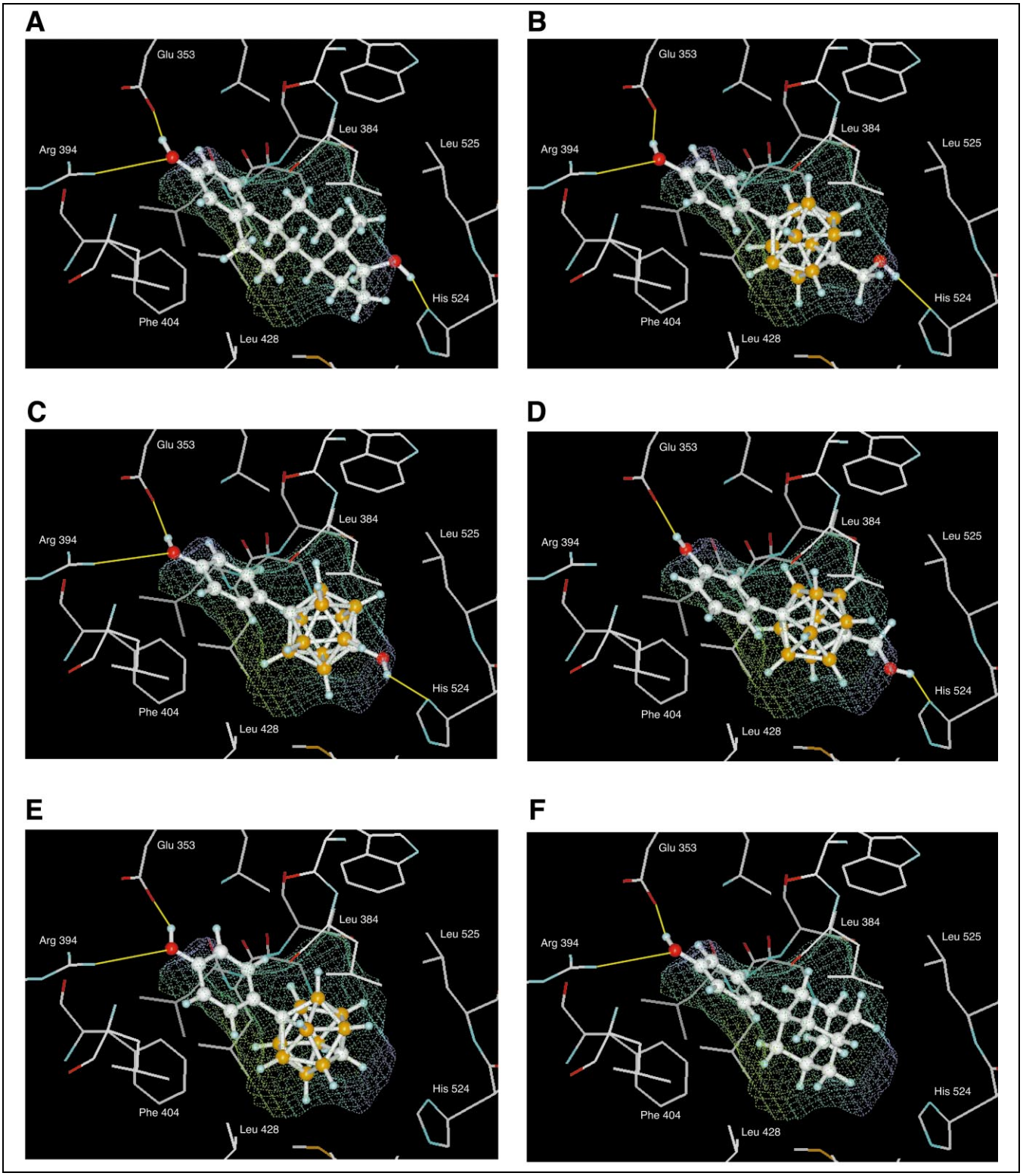

Fig. 7. The structure of the complex formed by 17β-estradiol (**1**) (A) and hERαLBD and the most stable docking models for **2c** (B), **2b** (C), **7c** (D), **2a** (E), and 4-(1-adamantyl)phenol (F) to the hERαLBD. The colored cage represents the allowed region for carbon and boron atoms in ligands, calculated from the protein structure. Intermolecular hydrogen bonds (distance less than 3.2 Å) are shown with yellow lines.

with a hydroxyl group directly substituted on the carborane cage, and the 3-hydroxy isomer **7c** were also well-fitted to the cavity of hERαLBD (Fig. 7C,D). Table 1 shows the heteroatom–heteroatom distances of compounds **2a**, **2b**, **2c**, **3b**, **7c**, **8c**, and **9c** in the most stable docking models, and that in the crystal structure of **1**. The relation between heteroatom–heteroatom distances and biological activity suggested that three distances within 3.2 Å seem to be

Table 1
Heteroatom–heteroatom distances in Å (hydrogen bond distances) in the most stable docking models

Ligand	Phenolic-OH/353Glu	Phenolic-OH/349Arg	Alcoholic-OH/524His	E_{inter} (kcal/mol)[a]
1	2.59	3.36	2.57	−54.67
2a	2.71	3.17	–	−45.88
2b	2.80	3.24	2.91	−47.76
2c	2.64	2.89	2.82	−51.60
7b	3.41	(4.59)	3.04	−45.63
7c	3.14	(4.08)	2.80	−48.00
8c	2.97	3.32	(5.05)	−49.17
9c	3.09	(3.98)	2.93	−45.34

[a] E_{inter}: interaction energy between protein and ligand in the most stable docking models.

effective for hydrogen bondings. However, the interactions of the ligands to the receptor cannot be explained only in terms of hydrogen bonding, but also hydrophobic interaction. The lack of one hydrogen bond might be compensated for by an effective hydrophobic interaction. The hydrophobic interaction along the spherical carborane cage seems to produce a stronger interaction than that in the case of 17β-estradiol. This may be the reason for the high activity of compound **2a**, which lacks a hydroxyl group for hydrogen bonding to the δ-nitrogen of His-524 (Fig. 7E). Compound **2a** has significant potency compared to well-known estrogenic 4-alkylphenols [35], 4-cycloalkylphenols [36], and 4-(1-adamantyl)phenol [36] (Fig. 7F). Therefore, use of this new hydrophobic and spherical component, the carborane cage, for molecular drug design should make it possible to develop a wide variety of biologically active molecules, especially nuclear receptor ligands.

In summary, we have developed novel carborane-containing molecules with potent estrogenic activity. The unique character of biologically active molecules containing a carborane skeleton may give rise to unusual membrane transport characteristics and metabolism, compared with conventional active molecules. The superagonistic properties of the carborane-containing compounds raise the possibility that structure–function studies could lead to the development of more selective estrogen agonists and antagonists, which could be useful as therapeutic agents for a wide variety of conditions.

4. Significance

Carboranes (dicarba-*closo*-dodecaboranes) are a class of carbon-containing polyhedral boron-cluster compounds having remarkable thermal stability and exceptional hydrophobicity. These features of carboranes may allow their use as a hydrophobic pharmacophore in biologically active molecules that interact hydrophobically with receptors. We therefore designed candidate estrogen-receptor-binding compounds having carborane as a hydrophobic moiety (**2**) and synthesized them. Their estrogenic activity was examined by the luciferase reporter gene assay using COS-1 cells transfected with rat ERα expression plasmid and an appropriate reporter plasmid. The most potent compound bearing a carborane cage (BE120, **2c**) exhibited potent activity in the concentration range of 1×10^{-10}–1×10^{-8} M; its potency is at least 10-fold greater than that of 17β-estradiol. In vitro ERα-binding data for these compounds were consistent with the results of the luciferase reporter gene assay. These results confirm that the gene-regulatory activity of the carborane-containing compounds is correlated with their binding affinity for the ER. The most active compound in the luciferase reporter gene assay, BE120, also showed the highest affinity for ERα. The compound also showed potent in vivo effects on the recovery of uterine weight and bone loss in OVX mice. Development of the potent carborane-containing estrogenic agonists described here should yield novel candidate therapeutic agents, especially selective ER modulators. Furthermore, the suitability of the spherical carborane cage for binding to the cavity of ERαLBD should provide a basis for a similar approach to developing novel ligands for other steroid receptors.

5. Materials and methods

5.1. Synthesis

5.1.1. 1-(4-Methoxyphenyl)-1,12-dicarba-closo-dodecaborane (*4a*)

To a solution of 1,12-dicarba-*closo*-dodecaborane (**3**, 3.5 g, 24.3 mmol) in 1,2-dimethoxyethane (95 ml) was added dropwise a 1.54 M solution of *n*-BuLi in hexane (16.6 ml, 25.6 mmol) at 0°C under Ar. The mixture was stirred at room temperature for 30 min, and CuCl (3.13 g, 31.6 mmol) was added in one portion. Stirring was continued at room temperature for 1 h, then pyridine (14.7 ml, 183 mmol) was added, and 4-iodoanisole (5.97 g, 25.5 mmol) was further added in one portion, and the mixture was heated at 100°C for 48 h. After cooling, the reaction mixture was diluted with Et_2O and stirred at room temperature for 3 h. Insoluble materials were filtered off through Celite. The filtrate was washed with 2 N HCl, aq. $Na_2S_2O_3$, H_2O, and brine, dried over Na_2SO_4, then concentrated. Purification by silica gel column chromatography (eluent: hexane to hexane/AcOEt, 10:1) gave **4a** (60%) and 1,12-bis(4-methoxyphenyl)-1,12-dicarba-*closo*-do-

decaborane (13%). **4a**: colorless needles (hexane): mp 102–103°C; ^{1}H NMR ($CDCl_3$) δ 1.50–3.30 (10 H, br m), 2.75 (1 H, br s), 3.74 (3 H, s), 6.68 (2 H, d, J = 9.1 Hz), 7.11 (2 H, d, J = 9.1 Hz); HRMS calc. for $C_9H_{18}B_{10}O$ 250.2361, found 250.2374.

*5.1.2. 1-(4-Hydroxyphenyl)-1,12-dicarba-closo-dodecaborane (**2a**)*

To a solution of **4a** (100 mg, 0.399 mmol) in CH_2Cl_2 (1 ml) was added dropwise a 1 M solution of BBr_3 in CH_2Cl_2 (0.48 ml) at −78°C. The mixture was stirred at room temperature for 2 h, then poured into ice water, and extracted with CH_2Cl_2. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated. Purification by silica gel flash column chromatography (eluent: hexane/AcOEt, 10:1) gave **2a** (93%). **2a**: colorless needles (CH_2Cl_2–hexane); mp 193–194°C; ^{1}H NMR ($CDCl_3$) δ 1.40–3.20 (10 H, br m), 2.75 (1 H, br s), 4.73 (1 H, br s), 6.61 (2 H, d, J = 9.0 Hz), 7.07 (2 H, d, J = 9.0 Hz); HRMS calcd for $C_8H_{16}B_{10}O$ 236.2204, found 236.2227. Anal. calcd for $C_8H_{16}B_{10}O$: C, 40.66; H, 6.82. Found C, 40.67; H, 6.79.

*5.1.3. 1-Hydroxy-12-(4-hydroxyphenyl)-1,12-dicarba-closo-dodecaborane (**2b**)*

To a solution of **4a** (300 mg, 1.20 mmol) in benzene/Et_2O (2:1, 3 ml) was added dropwise a 1.54 M solution of *n*-BuLi in hexane (1.56 ml, 2.40 mmol) at 0°C under Ar. The mixture was stirred at room temperature for 30 min. The solution was cooled at 0°C and benzyl peroxide (145 mg, 0.601 mmol) in benzene/Et_2O (2:1, 3 ml) was added dropwise, then the mixture was stirred at room temperature for 2 h. The reaction was quenched with 10% aqueous HCl and extracted with AcOEt. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated. Purification by silica gel flash column chromatography (eluent: hexane/AcOEt, 10:1) gave **4b** (75%) as a colorless solid: ^{1}H NMR ($CDCl_3$) δ 1.60–3.40 (10 H, br m), 2.87 (1 H, s), 3.73 (3 H, s), 6.67 (2 H, d, J = 8.6 Hz), 7.12 (2 H, d, J = 8.6 Hz); HRMS calcd for $C_9H_{18}B_{10}O_2$ 266.2310, found 266.2304. **2b** was prepared from **4b** by the same method as used for the preparation of **2a**. Purification by recrystallization gave **2b** (95%). **2b**: colorless needles (CH_2Cl_2–hexane); mp 181–183°C; ^{1}H NMR ($CDCl_3$) δ 1.50–3.40 (10 H, br m), 4.70 (1 H, bs), 6.60 (2 H, d, J = 8.8 Hz), 7.07 (2 H, d, J = 8.8 Hz); HRMS calcd for $C_8H_{16}B_{10}O_2$ 252.2153, found 252.2173. Anal. calcd for $C_8H_{16}B_{10}O_2 \cdot 1/2H_2O$: C, 36.77; H, 6.56. Found C, 36.42, H, 6.57.

*5.1.4. 1-Methoxycarbonyl-12-(4-methoxyphenyl)-1,12-dicarba-closo-dodecaborane (**5**)*

To a solution of **4a** (500 mg, 2.00 mmol) in benzene/Et_2O (2:1, 15 ml) was added dropwise a 1.54 M solution of *n*-BuLi in hexane (1.56 ml, 2.40 mmol) at 0°C under Ar. The mixture was stirred at room temperature for 30 min and cooled to 0°C, then methyl chloroformate (227 mg, 2.40 mmol) was added dropwise and stirring was continued at room temperature for 3 h. The reaction was quenched with water and the whole was extracted with AcOEt. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated. Purification by silica gel flash column chromatography (eluent: hexane/AcOEt, 50:1) gave **5** (91%). **5**: colorless prisms (hexane); mp 108–109°C; ^{1}H-NMR ($CDCl_3$) δ 1.60–3.40 (10 H, br m), 3.65 (3 H, s), 3.74 (3 H, s), 6.68 (2 H, d, J = 9.1 Hz), 7.08 (2 H, d, J = 9.1 Hz); Anal. calcd for $C_{11}H_{20}B_{10}O_3$: C, 42.84; H, 6.54. Found C, 42.82; H, 6.29.

*5.1.5. 1-Hydroxymethyl-12-(4-hydroxyphenyl)-1,12-dicarba-closo-dodecaborane (**2c**)*

To a suspension of $LiAlH_4$ (25.8 mg, 0.680 mmol) in THF (3 ml) was added dropwise **5** (150 mg, 0.486 mmol) in THF (2 ml) at 0°C, and the mixture was stirred at room temperature for 2.5 h. The reaction was quenched with 2 N HCl and the whole was extracted with AcOEt. The organic layer was washed with water and brine, dried over Na_2SO_4, then concentrated to give 1-hydroxymethyl-12-(4-methoxyphenyl)-1,12-dicarba-*closo*-dodecaborane (**4c**) (99%). **4c**: colorless prisms (CH_2CH_2–hexane); mp 151–152°C; ^{1}H NMR ($CDCl_3$) δ 1.50–3.30 (10 H, br m), 3.55 (2 H, s), 3.74 (3 H, s), 6.68 (2 H, d, J = 9.2 Hz), 7.11 (2 H, d, J = 9.2 Hz). Anal. calcd for $C_{10}H_{20}B_{10}O_2$: C, 42.84; H, 7.19. Found C, 42.94; H, 6.96. **2c** was prepared from **4c** by the same method as used for the preparation of **2a**. Purification by silica gel flash column chromatography (eluent: hexane/AcOEt, 4:1) gave **2c** (100%). **2c**: colorless needles (CH_2Cl_2–hexane); mp 184–185°C; ^{1}H NMR ($CDCl_3$) δ 1.50–3.30 (10 H, br m), 3.54 (2 H, s), 4.87 (1 H, br), 6.61 (2 H, d, J = 8.9 Hz), 7.06 (2 H, d, J = 8.9 Hz); HRMS calcd for $C_9H_{18}B_{10}O_2$ 266.2310, found 266.2310. Anal. calcd for $C_9H_{18}B_{10}O_2$: C, 40.59; H, 6.81. Found C, 40.30, H, 6.81.

*5.1.6. 1-Hydroxyethyl-12-(4-hydroxyphenyl)-1,12-dicarba-closo-dodecaborane (**2d**)*

To a solution of **4a** (200 mg, 0.80 mmol) in benzene/Et_2O (2:1, 7.5 ml) was added dropwise a 1.54 M solution of *n*-BuLi in hexane (0.623 ml, 0.953 mmol) at 0°C under Ar. The mixture was stirred at room temperature for 30 min and cooled to 0°C, then *O*-tetrahydropyranyl-2-bromoethanol (231 mg, 1.20 mmol) was added dropwise and stirring was continued at room temperature for 15 h. The reaction was quenched with water and the whole was extracted with AcOEt. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated. Purification by silica gel flash column chromatography (eluent: hexane/CH_2Cl_2, 2:1) gave the C-alkylated product (45%). ^{1}H NMR ($CDCl_3$) δ 1.45–1.82 (6 H, m), 1.50–3.20 (10 H, br m), 1.98 (2 H, t, J = 7.0 Hz), 3.17 (1 H, dt, J = 9.9, 7.6 Hz), 3.45–3.51 (1 H, m), 3.55 (1 H, dt, J = 10.1, 7.6 Hz), 3.73 (3 H, s), 3.80 (1 H, m), 4.49 (1 H, m), 6.67 (2 H, d, J = 9.2 Hz), 7.10 (2 H, d, J = 9.2 Hz). The compound (112 mg, 0.296 mmol) was dissolved in MeOH/CH_2Cl_2 (3:1, 4 ml), and TsOH·H_2O (5.6 mg, 0.0294 mmol) was added. The mixture was stirred at room temperature for 15 h. The reaction was quenched with saturated aqueous $NaHCO_3$, then the mixture was extracted with CH_2Cl_2. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated to give **4d** (100%). ^{1}H NMR ($CDCl_3$) δ 1.50–3.30 (10 H, br m), 1.95 (2 H, t, J = 7.0 Hz), 3.48 (1 H, t, J = 7.0 Hz), 3.73 (3 H, s), 6.67 (2 H, d, J = 9.0 Hz), 7.10 (2 H, d, J = 9.0 Hz). **2d** was prepared from **4d** by the same method as used for the preparation of **2a**. Purification by silica gel flash column chromatography (eluent: hexane/AcOEt, 4:1) gave **2d** (88%). **2d**: colorless needles (CH_2Cl_2–hexane); mp 180–181°C; ^{1}H NMR ($CDCl_3$) δ 1.30 (1 H, t, J = 5.1 Hz), 1.50–

3.30 (10 H, br m), 1.95 (2 H, t, $J = 7.0$ Hz), 3.48 (1 H, dt, $J = 7.0$, 5.1 Hz), 4.75 (1 H, s), 6.60 (2 H, d, $J = 9.0$ Hz), 7.06 (2 H, d, $J = 9.0$ Hz). HRMS calcd for $C_{10}H_{20}B_{10}O_2$ 280.2466, found 280.2462. Anal. calcd for $C_{10}H_{20}B_{10}O_2$: C, 42.84; H, 7.19. Found C, 42.79; H, 7.46.

*5.1.7. 1-Hydroxyethyl-12-(4-hydroxyphenyl)-1,12-dicarba-closo-dodecaborane (**2e**)*

4a (750 mg, 3.00 mmol) was converted to **4e** (64%) in the same manner as used for the preparation of **4d**. ^{1}H NMR ($CDCl_3$) δ 1.17 (1 H, bs), 1.40–3.30 (10 H, br m), 1.46 (2 H, m), 1.78 (2 H, m), 3.50 (2 H, m), 3.73 (3 H, s), 6.67 (2 H, d, $J = 9.0$ Hz), 7.10 (2 H, d, $J = 9.0$ Hz). **2e** was prepared from **4e** by the same method as used for the preparation of **2a**. Purification by silica gel flash column chromatography (eluent: hexane/AcOEt, 4:1) gave **2d** (92%). **2d**: colorless needles (CH_2Cl_2–hexane); mp 185–186°C; ^{1}H NMR ($CDCl_3$) δ 1.10–3.30 (10 H, br m), 1.45 (2 H, m), 1.77 (2 H, m), 3.50 (2 H, t, $J = 6.1$ Hz), 4.70 (1 H, bs), 6.60 (2 H, d, $J = 9.0$ Hz), 7.06 (2 H, d, $J = 9.0$ Hz). Anal. calcd for $C_{11}H_{22}B_{10}O_2$: C, 44.88; H, 7.53. Found C, 44.58, H, 7.32.

*5.1.8. 1-Hydroxycarbonyl-12-(4-hydroxyphenyl)-1,12-dicarba-closo-dodecaborane (**2f**)*

To a solution of **5** (260 mg, 0.843 mmol) in THF (3 ml) was added 1 N KOH (4.22 ml), and the mixture was stirred at room temperature for 17 h. The reaction was quenched with 2 N HCl and the whole was extracted with AcOEt. The organic layer was washed with water and brine, dried over Na_2SO_4, then concentrated to give 1-hydroxycarbonyl-12-(4-methoxyphenyl)-1,12-dicarba-*closo*-dodecaborane (**4f**) (quant.). ^{1}H NMR (DMSO-d_6) δ 1.60–3.40 (10 H, br m), 3.69 (3 H, s), 6.78 (2 H, d, $J = 9.1$ Hz), 7.08 (2 H, d, $J = 9.1$ Hz), 14.06 (1 H, br). **2f** was prepared from **4f** by the same method as used for the preparation of **2b** (quant.). **2f**: colorless needles (AcOEt–CH_2Cl_2–hexane); mp 249–252°C; ^{1}H NMR (DMSO-d_6) δ 1.60–3.40 (10 H, br m), 6.57 (2 H, d, $J = 8.8$ Hz), 6.94 (2 H, d, $J = 8.8$ Hz), 9.58 (1 H, s), 14.04 (1 H, br); HRMS calcd for $C_9H_{16}B_{10}O_3$ 280.2102, found 280.2114. Anal. calcd for $C_9H_{16}B_{10}O_3$: C, 38.56; H, 5.75. Found C, 38.39, H, 5.82.

*5.1.9. 1-tert-Butoxycarbonylamino-12-(4-methoxyphenyl)-1,12-dicarba-closo-dodecaborane (**6**)*

A mixture of **4f** (140 mg, 0.476 mmol), Et_3N (144 mg, 1.42 mmol), 4-dimethylaminopyridine (11.6 mg, 0.0949 mmol) and diphenylphosphoryl azide (196 mg, 0.712 mmol) in *t*-BuOH (8 ml) was refluxed for 24 h, then concentrated. The residue was taken up in AcOEt, washed with water and brine, dried over Na_2SO_4, then concentrated. Purification by silica gel flash column chromatography (eluent: hexane/AcOEt, 20:1) gave **6** (41%). **6**: colorless needles (hexane); mp 148–149°C; ^{1}H NMR ($CDCl_3$) δ 1.39 (9 H, s), 1.60–3.40 (10 H, br m), 3.73 (3 H, s), 4.89 (1 H, s), 6.67 (2 H, d, $J = 9.0$ Hz), 7.11 (2 H, d, $J = 9.0$ Hz).

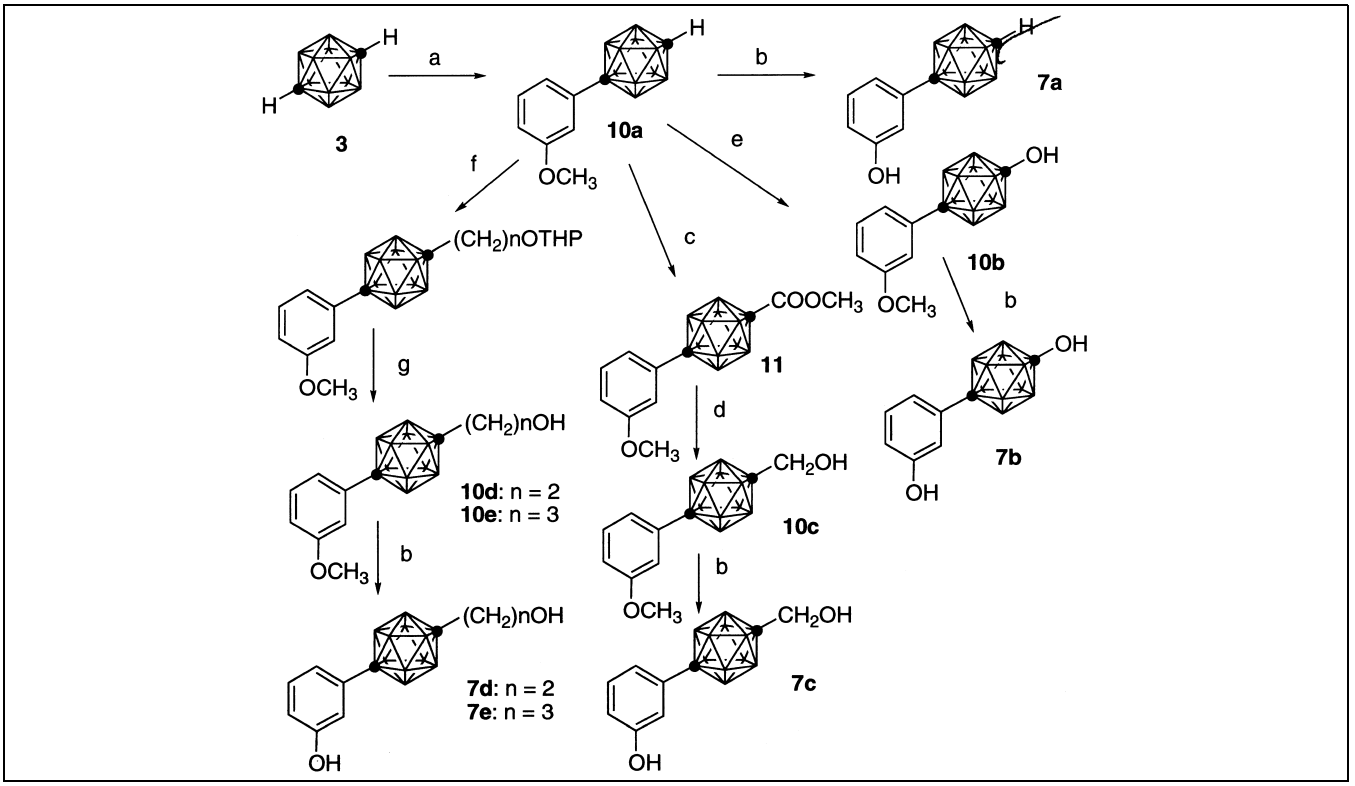

Fig. 8. Synthetic route used for the preparation of the estrogen ligands bearing carborane (**7**). Key: (a) (1) *n*-BuLi, CuCl/DME, (2) *m*-iodoanisole/pyridine, reflux; (b) BBr_3/CH_2Cl_2; (c) (1) *n*-BuLi/benzene–Et_2O, (2) $ClCOOCH_3$; (d) $LiAlH_4$/THF; (e) (1) *n*-BuLi/benzene–Et_2O, (2) $(C_6H_5COO)_2$/benzene–Et_2O; (f) *n*-BuLi/benzene–Et_2O, (2) $Br(CH_2)_nOTHP$; (g) *p*-TsOH·H_2O/MeOH.

*5.1.10. 1-Amino-12-(4-hydroxyphenyl)-1,12-dicarba-closo-dodecaborane (**2g**)*

To a solution of **6** (62 mg, 0.170 mmol) in CH_2Cl_2 (2 ml) was added TFA (0.4 ml), and the mixture was stirred at room temperature for 2.5 h. The reaction was quenched with saturated aqueous $NaHCO_3$ and the whole was extracted with CH_2Cl_2. The organic layer was washed with brine, dried over Na_2SO_4, then concentrated to give 1-amino-12-(4-methoxyphenyl)-1,12-dicarba-*closo*-dodecaborane (100%) as colorless needles (CH_2Cl_2–hexane). mp 155–156°C; ^{1}H NMR ($CDCl_3$) δ 1.50–3.30 (10 H, br m), 3.73 (3 H, s), 6.67 (2 H, d, J = 9.0 Hz), 7.11 (2 H, d, J = 9.0 Hz). **2g** was prepared from the amine by the same method as used for the preparation of **2a**. The reaction mixture was poured into cold saturated aqueous $NaHCO_3$ and extracted with AcOEt. The organic layer was washed with brine, dried over Na_2SO_4 and concentrated. Purification by silica gel flash column chromatography (eluent: hexane/AcOEt, 5:1) gave **2g** (100%). **2g**: colorless needles (CH_2Cl_2–hexane); mp 169–171°C; ^{1}H NMR ($CDCl_3$) δ 1.50–3.30 (10 H, br m), 2.05 (2 H, br s), 4.81 (1 H, s), 6.59 (2 H, d, J = 9.0 Hz), 7.06 (2 H, d, J = 9.0 Hz); HRMS calcd for $C_8H_{17}B_{10}NO$ 251.2313, found 251.2299. Anal. calcd for $C_8H_{17}B_{10}NO$: C, 36.91; H, 6.97; N, 5.38. Found C, 37.20; H, 6.90; N, 5.28.

*5.1.11. 1-(3-Methoxyphenyl)-1,12-dicarba-closo-dodecaborane (**10a**)*

Compound **10a** was prepared from 1,12-dicarba-*closo*-dodecaborane (**3**) and 3-iodoanisole by the same method as used for preparation of **4a** (Fig. 8). Purification by silica gel column chromatography (eluent: hexane/AcOEt, 100:1 to 10:1) gave **10a** (61%) and 1,12-bis(3-methoxyphenyl)-1,12-dicarba-*closo*-dodecaborane (10%). **10a**: colorless solid; ^{1}H NMR ($CDCl_3$) δ 1.50–3.30 (10 H, br m), 2.78 (1 H, br s), 3.75 (3 H, s), 6.73–6.76 (2 H, m), 6.79 (1 H, m), 7.08 (1 H, m).

*5.1.12. 1-(3-Hydroxyphenyl)-1,12-dicarba-closo-dodecaborane (**7a**)*

Compound **7a** was prepared from **10a** by the same method as used for preparation of **2a**. Purification by silica gel flash column chromatography (eluent: hexane/AcOEt, 10:1) gave **7a** (100%). **7a**: colorless needles (CH_2Cl_2–hexane); mp 163–164°C; ^{1}H NMR ($CDCl_3$) δ 1.50–3.30 (10 H, br m), 2.78 (1 H, br s), 4.67 (1 H, s), 6.66–6.69 (2 H, m), 6.78 (1 H, m), 7.03 (1 H, m). Anal. calcd for $C_8H_{16}B_{10}O$: C, 40.66; H, 6.82. Found C, 40.36; H, 6.64.

*5.1.13. 1-Hydroxy-12-(3-hydroxyphenyl)-1,12-dicarba-closo-dodecaborane (**7b**)*

Compound **10b** was prepared from **10a** by the same method as used for preparation of **4b**. Purification by silica gel flash column chromatography (eluent: hexane/AcOEt, 10:1) gave **10b** (65%) as colorless solid: ^{1}H NMR ($CDCl_3$) δ 1.60–3.40 (10 H, br m), 2.97 (1 H, s), 3.74 (3 H, s), 6.72–6.77 (2 H, m), 6.80 (1 H, ddd, J = 1.0, 1.9, 7.9 Hz), 7.07 (1 H, dt, J = 0.9, 7.9 Hz). Compound **7b** was prepared from **10b** by the same method as used for preparation of **2b**. Purification by silica gel flash column chromatography (eluent: hexane/AcOEt, 5:1) gave **7b** (96%). **7b**: colorless needles (CH_2Cl_2–hexane); mp 185–186°C; ^{1}H NMR ($CDCl_3$) δ 1.50–3.40 (10 H, br m), 4.70 (1 H, br), 6.66–6.71 (2 H, m), 6.78 (1 H, br d) 7.02 (1 H, t, J = 8.0 Hz). Anal. calcd for $C_8H_{16}B_{10}O_2$: C, 38.08; H, 6.39. Found C, 38.31; H, 6.43.

*5.1.14. 1-Methoxycarbonyl-12-(3-methoxyphenyl)-1,12-dicarba-closo-dodecaborane (**11**)*

Compound **11** was prepared from **10a** by the same method as used for preparation of **5**. Purification by silica gel flash column chromatography (eluent: hexane/AcOEt, 20:1) gave **11** (87%) as colorless solid: ^{1}H NMR ($CDCl_3$) δ 1.60–3.50 (10 H, br m), 3.65 (3 H, s), 3.75 (3 H, s), 6.71–6.78 (3 H, m).

*5.1.15. 1-Hydroxymethyl-12-(3-hydroxyphenyl)-1,12-dicarba-closo-dodecaborane (**7c**)*

Compound **7c** was prepared from **11** by the same method as used for preparation of **2c**. Purification by silica gel flash column chromatography (eluent: hexane/AcOEt, 4:1) gave **7c** (100%). **7c**: colorless needles (CH_2Cl_2–hexane); mp 134–135°C; ^{1}H NMR ($CDCl_3$) δ 1.50–3.30 (10 H, br m), 1.58 (1 H, t, J = 7.3 Hz), 3.55 (2 H, d, J = 7.3 Hz), 4.70 (1 H, s), 6.67–6.69 (2 H, m), 6.77 (1 H, m), 7.03 (1 H, m). Anal. calcd for $C_9H_{18}B_{10}O_2$: C, 40.59; H, 6.81. Found C, 40.35; H, 6.51.

*5.1.16. 1-Hydroxyethyl-7-(3-hydroxyphenyl)-1,12-dicarba-closo-dodecaborane (**7d**)*

Compound **10d** was prepared from **10a** by the same method as used for preparation of **4d** (66%). **10d**: colorless solid; ^{1}H NMR ($CDCl_3$) δ 1.28 (1 H, t, J = 5.6 Hz), 1.50–3.30 (10 H, br m), 1.95 (2 H, t, J = 7.0 Hz), 3.48 (2 H, dt, J = 5.6, 7.0 Hz), 3.74 (3 H, s), 6.73–6.79 (3 H, m), 7.07 (1 H, m). Compound **7d** was prepared from **10d** by the same method as used for preparation of **2d**. Purification by silica gel flash column chromatography (eluent: hexane/AcOEt, 3:1) gave **7d** (92%). **7d**: colorless needles (CH_2Cl_2); mp 186–187°C; ^{1}H NMR ($CDCl_3$) δ 1.30 (1 H, t, J = 4.9 Hz), 1.50–3.30 (10 H, br m), 1.95 (2 H, t, J = 7.0 Hz) 3.48 (2 H, dt, J = 4.9, 7.0 Hz), 4.74 (1 H, s), 6.65–6.69 (2 H, m), 6.76 (1 H, m), 7.02 (1 H, m); HRMS calcd for $C_{10}H_{20}B_{10}O_2$ 280.2466; found 280.2466. Anal. calcd for $C_{10}H_{20}B_{10}O_2 \cdot 0.1_2O$: C, 42.56; H, 7.21. Found C, 42.39; H, 6.95.

*5.1.17. 1-Hydroxypropyl-12-(3-hydroxyphenyl)-1,12-dicarba-closo-dodecaborane (**7e**)*

Compound **10e** was prepared from **10a** by the same method as used for preparation of **4e** (69%). **10e**: colorless solid; ^{1}H NMR ($CDCl_3$) δ 1.14 (1 H, t, J = 5.2 Hz), 1.40–3.30 (10 H, br m), 1.45 (2 H, m), 1.78 (2 H, m), 3.50 (2 H, dt, J = 5.2, 6.1 Hz), 3.74 (3 H, s), 6.72–6.80 (3 H, m), 7.07 (1 H, m). Compound **7e** was prepared from **10e** by the same method as used for preparation of **2e**. Purification by silica gel flash column chromatography (eluent: hexane/AcOEt, 3:1) gave **2e** (99%). **2e**: colorless needles (CH_2Cl_2); mp 211–212°C; ^{1}H NMR (DMSO-d_6) δ 1.25 (2 H, m), 1.40–3.20 (10 H, br m), 1.73 (2 H, m) 3.23 (2 H, dt, J = 5.1,5.7 Hz), 4.39 (1 H, t, J = 5.1 Hz), 6.57–6.59 (2 H, m), 6.64 (1 H, m), 7.01 (1 H, m), 9.54 (1 H, s). Anal. calcd for $C_{11}H_{22}B_{10}O_2$: C, 44.88; H, 7.53. Found C, 44.64; H, 7.24.

*5.1.18. 1-(4-Methoxyphenyl)-1,7-dicarba-closo-dodecaborane (**13a**)*

To a solution of 1,7-dicarba-*closo*-dodecaborane (**12**, 3.5 g, 24.3 mmol) in 1,2-dimethoxyethane (95 ml) was added dropwise a 1.54 M solution of *n*-BuLi in hexane (16.6 ml, 25.6 mmol) at 0°C under Ar (Fig. 9). The mixture was stirred at room temperature for 30 min, then CuCl (3.13 g, 31.6 mmol) was added in one portion and stirring was continued at room temperature for 1 h. Pyridine (14.7 ml, 183 mmol) was added, then 4-iodoanisole (5.97 g, 25.5 mmol) was further added in one portion, and the mixture was heated at 100°C for 48 h. After cooling, the reaction mixture was diluted with Et_2O and stirred at room temperature for 15 h. Insoluble materials were filtered off through Celite. The filtrate was washed with 2 N HCl, aq. $Na_2S_2O_3$, H_2O and brine, dried over Na_2SO_4, then concentrated. Purification by silica gel column chromatography (eluent: hexane to hexane/AcOEt, 30:1) gave **13a** (42%) and 1,7-bis(4-methoxyphenyl)-1,7-dicarba-*closo*-dodecaborane (17%). **13a**: colorless needles (hexane); mp 100–101°C; ^{1}H NMR ($CDCl_3$) δ 1.50–3.70 (10 H, br m), 3.04 (1 H, br s), 3.77 (3 H, s), 6.76 (2 H, d, J = 9.2 Hz), 7.33 (2 H, d, J = 9.2 Hz); HRMS calcd for $C_9H_{18}B_{10}O$ 250.2361, found 250.2361.

*5.1.19. 1-(4-Hydroxyphenyl)-1,7-dicarba-closo-dodecaborane (**8a**)*

Compound **8a** was synthesized from **13a** by the same method as used for preparation of **2a**. Purification by silica gel flash column chromatography (eluent: hexane/AcOEt, 10:1) gave **8a**. **8a**: colorless needles (CH_2Cl_2–hexane); mp 180–181°C; ^{1}H NMR ($CDCl_3$) δ 1.50–3.70 (10 H, br m), 3.04 (1 H, br s), 4.81 (1 H, s), 6.69 (2 H, d, J = 8.9 Hz), 7.28 (2 H, d, J = 8.9 Hz); HRMS calcd for $C_8H_{16}B_{10}O$ 236.2204, found 236.2206. Anal. calcd for $C_8H_{16}B_{10}O$: C, 40.66; H, 6.82. Found: C, 40.52; H, 6.68.

*5.1.20. 1-Hydroxy-7-(4-hydroxyphenyl)-1,7-dicarba-closo-dodecaborane (**8b**)*

Compound **13b** was prepared from **13a** by the same method as used for preparation of **4b**. Purification by silica gel flash column chromatography (eluent: hexane/CH_2Cl_2, 1:1) gave **13b** (41%) as colorless solid: ^{1}H NMR ($CDCl_3$) δ 1.40–4.10 (10 H, br m), 3.31 (1 H, s), 3.78 (3 H, s), 6.76 (2 H, d, J = 8.9 Hz), 7.32 (2 H, d, J = 8.9 Hz); HRMS calcd for $C_9H_{18}B_{10}O_2$ 266.2310, found 266.2299. Compound **8b** was prepared from **13b** by the same method as used for preparation of **2b**. Purification by silica gel flash column chromatography (eluent: hexane/AcOEt, 3:1) gave **8b** (93%). **8b**: colorless needles (CH_2Cl_2–hexane); mp 168–169°C ^{1}H NMR ($CDCl_3$) δ 1.40–4.00 (10 H, br m), 4.80 (1 H, br), 6.70 (2 H, d, J = 9.0 Hz), 7.28 (2 H, d, J = 9.0 Hz). Anal. calcd for $C_8H_{16}B_{10}O_2$: C, 38.08; H, 6.39. Found C, 37.84; H, 6.30.

*5.1.21. 1-Methoxycarbonyl-7-(4-methoxyphenyl)-1,7-dicarba-closo-dodecaborane (**14**)*

Compound **14** was prepared from **13a** by the same method as used for preparation of **5**. Purification by silica gel flash column

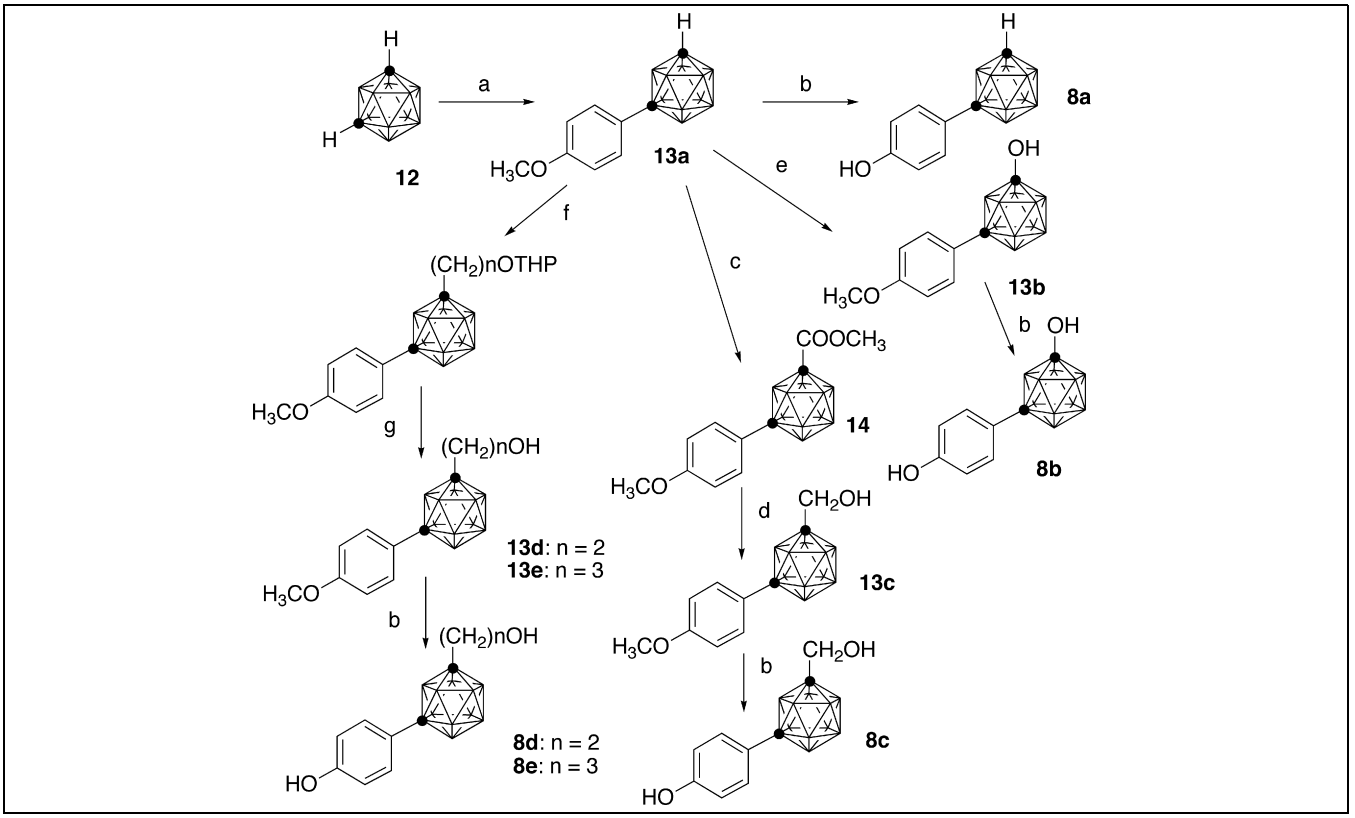

Fig. 9. Synthetic route used for the preparation of the estrogen ligands bearing carborane (**8**). Key: (a) (1) *n*-BuLi, CuCl/DME, (2) *p*-iodoanisole/pyridine, reflux; (b) BBr_3/CH_2Cl_2; (c) (1) *n*-BuLi/benzene–Et_2O, (2) $ClCOOCH_3$; (d) $LiAlH_4$/THF; (e) (1) *n*-BuLi/benzene–Et_2O, (2) $(C_6H_5COO)_2$/benzene–Et_2O; (f) (1) *n*-BuLi/benzene–Et_2O, (2) $Br(CH_2)_nOTHP$; (g) *p*-TsOH·H_2O/MeOH.

chromatography (eluent: hexane/AcOEt, 20:1) gave **14** (82%). **14**: colorless needles (hexane); mp 47–48°C; ^{1}H NMR ($CDCl_3$) δ 1.50–3.70 (10 H, br m), 3.76 (3 H, s), 3.78 (3 H, s), 6.77 (2 H, d, J = 9.2 Hz), 7.33 (2 H, d, J = 9.2 Hz); HRMS calcd for $C_{11}H_{26}B_{10}O_3$ 308.2416, found 308.2428.

5.1.22. *1-Hydroxymethyl-7-(4-hydroxyphenyl)-1,7-dicarba-closo-dodecaborane (**8c**)*

Compound **8c** was prepared from **14** by the same method as used for preparation of **2c**. Purification by silica gel flash column chromatography (eluent: hexane/AcOEt, 3:1) gave **8c** (79%). **8c**: colorless needles (CH_2Cl_2–hexane); mp 173–174°C; ^{1}H NMR ($CDCl_3$) δ 1.50–3.80 (10 H, br m), 1.86 (1 H, br s), 3.87 (2 H, s), 4.83 (1 H, s), 6.69 (2 H, d, J = 8.9 Hz), 7.28 (2 H, d, J = 8.9 Hz); HRMS calcd for $C_9H_{18}B_{10}O_2$ 266.2310, found 266.2303. Anal. calcd for $C_9H_{18}B_{10}O_2$: C, 40.59; H, 6.81. Found C, 40.57; H, 6.88.

5.1.23. *1-Hydroxyethyl-7-(4-hydroxyphenyl)-1,7-dicarba-closo-dodecaborane (**8d**)*

Compound **13d** was prepared from **13a** by the same method as used for preparation of **4d** (63%). **13d**: colorless solid; ^{1}H NMR ($CDCl_3$) δ 1.20–3.30 (10 H, br m), 2.28 (2 H, t, J = 6.9 Hz), 3.67 (2 H, t, J = 6.9 Hz), 3.78 (3 H, s), 6.76 (2 H, d, J = 9.0 Hz), 7.32 (2 H, d, J = 9.0 Hz). Compound **8d** was prepared from **13d** by the same method as used for preparation of **2d**. Purification by silica gel flash column chromatography (eluent: hexane/AcOEt, 2:1) gave **8d** (90%). **8d**: colorless needles (CH_2Cl_2–hexane); mp 156–157°C; ^{1}H NMR ($CDCl_3$) δ 1.50–3.50 (10 H, br m), 2.28 (2 H, t, J = 6.9 Hz), 3.67 (2 H, t, J = 6.9, Hz), 4.79 (1 H, s), 6.69 (2 H, d, J = 8.9 Hz), 7.28 (2 H, d, J = 8.9 Hz); HRMS calcd for $C_{10}H_{20}B_{10}O_2$ 280.2466, found: 280.2470. Anal. calcd for $C_{10}H_{20}B_{10}O_2$: C, 42.84; H, 7.19. Found C, 42.53; H, 6.75.

5.1.24. *1-Hydroxypropyl-7-(4-hydroxyphenyl)-1,7-dicarba-closo-dodecaborane (**8e**)*

Compound **13e** was prepared from **13a** by the same method as used for preparation of **4e** (84%). **13e**: colorless solid; ^{1}H NMR ($CDCl_3$) δ 1.20–3.40 (10 H, br m), 1.25 (1 H, br s), 1.67 (2 H, m), 2.12 (2 H, m), 3.60 (2 H, t, J = 6.1 Hz), 3.78 (3 H, s), 6.75 (2 H, d, J = 9.0 Hz), 7.32 (2 H, d, J = 9.0 Hz). Compound **8e** was prepared from **13e** by the same method as used for preparation of **2e**. Purification by silica gel flash column chromatography (eluent: hexane/AcOEt, 2:1) gave **8e** (82%). **8e**: colorless needles (CH_2Cl_2–hexane); mp 157–158°C; ^{1}H NMR ($CDCl_3$) δ 1.23 (1 H, t, J = 5.1 Hz), 1.40–3.80 (10 H, br m), 1.67 (2 H, m), 2.11 (2 H, m) 3.60 (2 H, dt, J = 5.1, 5.3 Hz), 4.81 (1 H, s), 6.68 (2 H, d, J = 8.9 Hz), 7.28 (2 H, d, J = 8.9 Hz). Anal. calcd for $C_{11}H_{22}B_{10}O_2$: C, 44.88; H, 7.53. Found C, 44.61; H, 7.24.

5.1.25. *1-(3-Methoxyphenyl)-1,7-dicarba-closo-dodecaborane (**15a**)*

Compound **15a** was prepared from 1,7-dicarba-*closo*-dodecaborane (**12**) and 3-iodoanisole by the same method as used for preparation of **13a** (Fig. 10). Purification by silica gel column chromatography (eluent: hexane/AcOEt, 100:1 to 50:1) gave **15a** (39%) and 1,7-bis(3-methoxyphenyl)-1,7-dicarba-*closo*-dodecaborane (14%). **15a**: colorless solid; ^{1}H NMR ($CDCl_3$) δ 1.50–3.70 (10 H, br m), 3.05 (1 H, br s), 3.79 (3 H, s), 6.82 (1 H, dd, J = 2.4, 8.2 Hz), 6.96 (1 H, t, J = 2.4 Hz), 7.01 (1 H, br d, J = 8.2 Hz), 7.16 (1 H, t, J = 8.2 Hz).

5.1.26. *1-(3-Hydroxyphenyl)-1,7-dicarba-closo-dodecaborane (**9a**)*

Compound **9a** was synthesized from **15a** by the same method as used for preparation of **2a**. Purification by silica gel flash column chromatography (eluent: hexane/AcOEt, 5:1) gave **9a** (100%). **9a**: colorless needles (CH_2Cl_2–hexane); mp 140–141°C; ^{1}H NMR ($CDCl_3$) δ 1.40–3.80 (10 H, br m), 3.05 (1 H, br s), 4.70 (1 H, s), 6.75 (1 H, dd, J = 2.4, 8.1 Hz), 6.90 (1 H, t, J = 2.4 Hz), 6.99 (1 H, br d, J = 8.1 Hz), 7.11 (1 H, t, J = 8.1 Hz). Anal. calcd for $C_8H_{16}B_{10}O$: C, 40.66; H, 6.82. Found C, 40.48; H, 6.56.

5.1.27. *1-Hydroxy-7-(3-hydroxyphenyl)-1,12-dicarba-closo-dodecaborane (**9b**)*

Compound **15b** was prepared from **15a** by the same method as used for preparation of **4b**. Purification by silica gel flash column chromatography (eluent: hexane/CH_2Cl_2, 1:1) gave **15b** (44%) as colorless solid: ^{1}H NMR ($CDCl_3$) δ 1.60–4.00 (10 H, br m), 3.30 (1 H, s), 3.79 (3 H, s), 6.83 (1 H, dd, J = 2.2, 8.1 Hz), 6.95 (1 H, t, J = 2.2 Hz), 7.00 (1 H, m), 7.17 (1 H, t, J = 8.1 Hz). Compound **9b** was prepared from **15b** by the same method as used for preparation of **2b**. Purification by silica gel flash column chromatography (eluent: hexane/AcOEt, 3:1) gave **9b** (100%). **9b**: colorless needles (CH_2Cl_2–hexane); mp 135–136°C; ^{1}H NMR ($CDCl_3$) δ 1.40–4.00 (10 H, br m), 4.70 (1 H, br), 6.76 (1 H, ddd, J = 1.0, 2.5, 8.1 Hz), 6.89 (1 H, t, J = 2.5 Hz), 6.99 (1 H, ddd, J = 1.0, 2.5, 8.1 Hz), 7.12 (1 H, t, J = 8.1 Hz). Anal. calcd for $C_8H_{16}B_{10}O_2$: C, 38.08; H, 6.39. Found C, 37.79; H, 6.37.

5.1.28. *1-Methoxycarbonyl-7-(3-methoxyphenyl)-1,7-dicarba-closo-dodecaborane (**16**)*

Compound **16** was prepared from **15a** by the same method as used for preparation of **5**. Purification by silica gel flash column chromatography (eluent: hexane/AcOEt, 20:1) gave **16** (76%) as colorless solid: ^{1}H NMR ($CDCl_3$) δ 1.50–3.70 (10 H, br m), 3.77 (3 H, s), 3.79 (3 H, s), 6.84 (1 H, dd, J = 2.4, 8.2 Hz), 6.96 (1 H, t, J = 2.4 Hz), 7.01 (1 H, br d, J = 8.2 Hz), 7.17 (1 H, t, J = 8.2 Hz).

5.1.29. *1-Hydroxymethyl-7-(3-hydroxyphenyl)-1,7-dicarba-closo-dodecaborane (**9c**)*

Compound **9c** was prepared from **16** by the same method as used for preparation of **2c**. Purification by silica gel flash column chromatography (eluent: hexane/AcOEt, 3:1) gave **9c** (91%). **9c**: colorless needles (CH_2Cl_2–hexane); mp 140–141°C; ^{1}H NMR ($CDCl_3$) δ 1.50–3.80 (10 H, br m), 1.91 (1 H, t, J = 7.2 Hz), 3.87 (2 H, d, J = 7.2 Hz), 4.90 (1 H, s), 6.76 (1 H, ddd, J = 0.9, 2.2, 8.0 Hz), 6.91 (1 H, t, J = 2.2 Hz), 6.99 (1 H, ddd, J = 0.9, 2.2, 8.0 Hz), 7.11 (1 H, t, J = 8.0 Hz). Anal. calcd for $C_9H_{18}B_{10}O_2$: C, 40.59; H, 6.81. Found C, 40.33; H, 6.81.

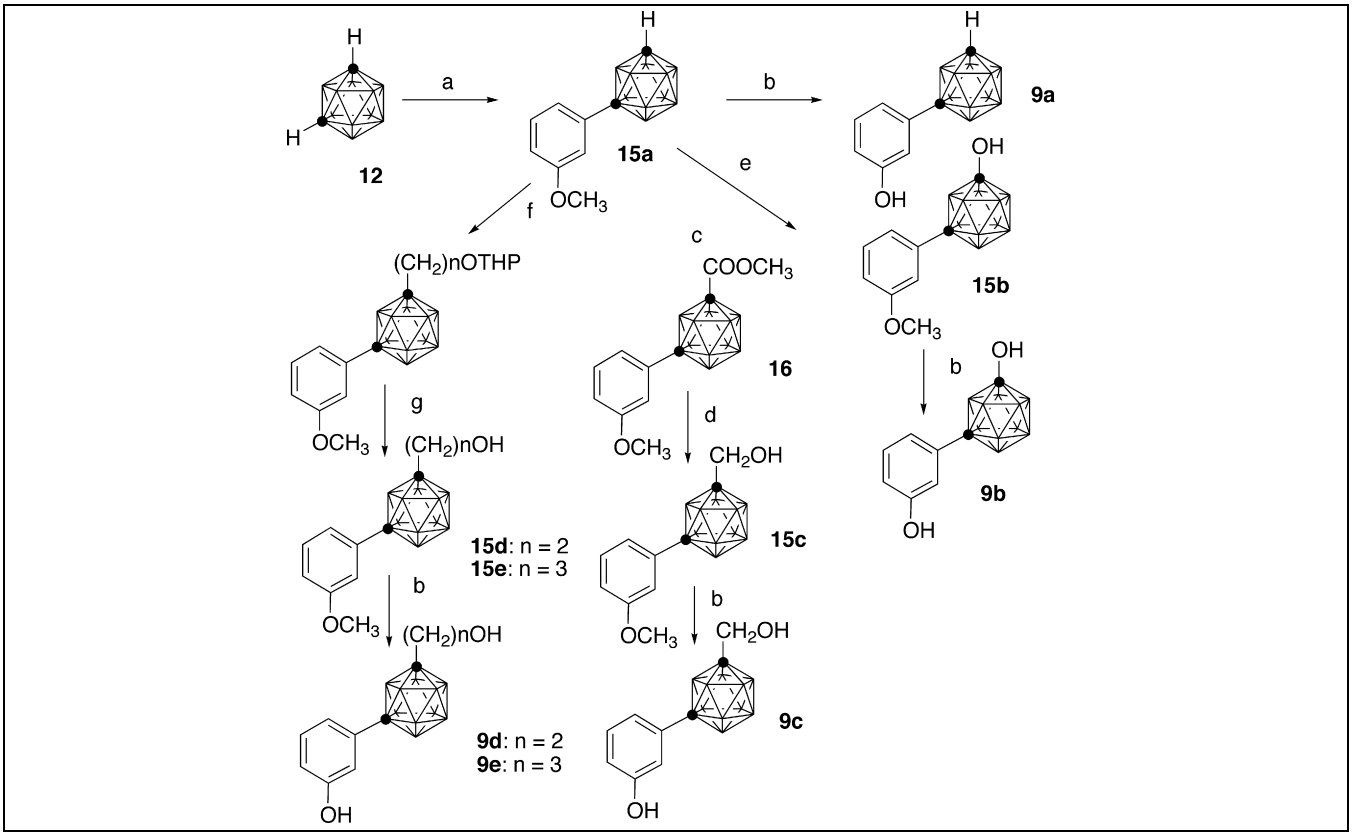

Fig. 10. Synthetic route used for the preparation of the estrogen ligands bearing carborane (**9**). Key: (a) (1) *n*-BuLi, CuCl/DME, (2) *m*-iodoanisole/pyridine, reflux; (b) BBr_3/CH_2Cl_2; (c) (1) *n*-BuLi/benzene–Et_2O, (2) $ClCOOCH_3$; (d) $LiAlH_4$/THF; (e) (1) *n*-BuLi/benzene–Et_2O, (2) $(C_6H_5COO)_2$/benzene–Et_2O; (f) (1) *n*-BuLi/benzene–Et_2O, (2) $Br(CH_2)_nOTHP$; (g) *p*-TsOH·H_2O/MeOH.

*5.1.30. 1-Hydroxyethyl-7-(3-hydroxyphenyl)-1,7-dicarba-closo-dodecaborane (**9d**)*

Compound **15d** was prepared from **15a** by the same method as used for preparation of **4d** (52%). **15d**: colorless solid; ^{1}H NMR ($CDCl_3$) δ 1.40 (1 H, t, J = 5.4 Hz), 1.50–3.60 (10 H, br m), 2.28 (2 H, t, J = 6.9 Hz), 3.67 (2 H, dt, J = 5.4, 6.9 Hz), 3.79 (3 H, s), 6.82 (1 H, dd, J = 2.2, 8.1 Hz), 6.95 (1 H, s), 7.00 (1 H, br d, J = 8.1 Hz), 7.16 (1 H, t, J = 8.1 Hz). Compound **9d** was prepared from **15d** by the same method as used for preparation of **2d**. Purification by silica gel flash column chromatography (eluent: hexane/AcOEt, 3:1) gave **15d** (100%). **15d**: colorless needles (CH_2Cl_2–hexane); mp 126–127°C; ^{1}H NMR ($CDCl_3$) δ 1.42 (1 H, t, J = 5.5 Hz), 1.30–3.60 (10 H, br m), 2.28 (2 H, t, J = 6.5 Hz) 3.68 (2 H, dt, J = 5.5, 6.5 Hz), 4.84 (1 H, s), 6.75 (1 H, ddd, J = 0.9, 2.1, 7.9 Hz), 6.89 (1 H, t, J = 2.1 Hz), 6.98 (1 H, ddd, J = 0.9, 2.1, 7.9 Hz), 7.11 (1 H, t, J = 7.9 Hz). Anal. calcd for $C_{10}H_{20}B_{10}O_2$: C, 42.84; H, 7.19. Found C, 42.65; H, 6.90.

*5.1.31. 1-Hydroxypropyl-7-(3-hydroxyphenyl)-1,7-dicarba-closo-dodecaborane (**9e**)*

Compound **15e** was prepared from **15a** by the same method as used for preparation of **4e** (84%). **15e**: colorless solid; ^{1}H NMR ($CDCl_3$) δ 1.24 (1 H, t, J = 5.0 Hz), 1.50–3.50 (10 H, br m), 1.67 (2 H, m), 2.11 (2 H, m), 3.60 (2 H, dt, J = 5.0, 6.0 Hz), 3.79 (3 H, s), 6.82 (1 H, dd, J = 2.4, 8.2 Hz), 6.96 (1 H, t, J = 2.4 H), 7.00 (1 H, br d, J = 8.2 Hz), 7.16 (1 H, t, J = 8.2 Hz). Compound **9e** was prepared from **15e** by the same method as used for preparation of **2e**. Purification by silica gel flash column chromatography (eluent: hexane/AcOEt, 3:1) gave **9e** (90%). **9b**: colorless needles (CH_2Cl_2–hexane); mp 106–107°C; ^{1}H NMR ($CDCl_3$) δ 1.26 (1 H, t, J = 5.1 Hz), 1.50–3.70 (10 H, br m), 1.67 (2 H, m), 2.11 (2 H, m), 3.60 (2 H, dt, J = 5.1, 5.6 Hz), 4.87 (1 H, s), 6.75 (1 H, ddd, J = 0.7, 2.1, 8.1 Hz), 6.90 (1 H, t, J = 2.1 Hz), 6.98 (1 H, ddd, J = 0.7, 2.1, 8.1 Hz), 7.11 (1 H, t, J = 8.1 Hz). Anal. calcd for $C_{11}H_{22}B_{10}O_2$: C, 44.88; H, 7.53. Found C, 44.58; H, 7.35.

5.2. Transfection and luciferase assays

The ER expression plasmid, pCI-rERα, was constructed by inserting the rat uterus ER cDNA (pUCER6, which was obtained from the Health Science Research Resources Bank, Osaka, Japan) into the expression vector pCI-neo (Promega). The reporter plasmid, EREx5-pGL-TK, was constructed by introducing five copies of the estrogen response element (ERE), caAGGTCAcccT-GACCTcc, and the herpes simplex virus thymidine kinase promoter into the *Nhe*I–*Hin*dIII sites of the pGL3-Basic luciferase reporter vector (Promega). The COS-1 cells were obtained from the Japanese Cancer Research Resources Bank (JCRB) and were maintained in Dulbecco's modified essential medium (DMEM), supplemented with 5% fetal bovine serum (FBS). For the reporter gene assay, COS-1 cells were seeded in 24-well tissue culture plates at 6×10^4 cells per well with 5% charcoal-stripped FBS/

DMEM without phenol red. The cells were cultured at 37°C in 5% CO_2 overnight and allowed to attach to the plates. Then, the medium was removed and the transfection was performed with the cationic lipid transfection reagent Tfx-20 (Promega). Liposomes were formed by incubating 100 ng of pCI-rERα, 50 ng of EREx5-pGL-TK, and 100 ng of the reference plasmid pCMVβ (Clontech) with 0.75 μl of Tfx-20 in phenol red-free DMEM (final 200 μl) for 10–15 min at room temperature. These DNA/Tfx-20 mixtures were added to the cells and the culture plates were returned to the CO_2 incubator. After 2 h, 800 μl of DMEM supplemented with charcoal-stripped FBS (final 0.5%) and 5 μl of ethanol solution of ligands were added. After an additional 18 h of incubation, the cells were harvested, and the luciferase assay was performed with the Luciferase Assay System (Promega). The luciferase activities were normalized to β-galactosidase activities. Each condition was assayed in triplicate.

5.3. ERα ligand-binding assays

The ligand-binding activity of ERα was determined by the nitrocellulose filter binding assay method. ERα (0.5 μg/tube, PanVera Co. Ltd.) was diluted with a binding assay buffer (20 mM Tris–HCl pH 8.0, 0.3 M NaCl, 1 mM EDTA pH 8.0, 10 mM 2-mercaptoethanol, 0.2 mM phenylmethylsulfonyl fluoride) and incubated with 4 nM [6,7-^{3}H]17β-estradiol in the presence or absence of an unlabeled competitor at 4°C for 16 h. The incubation mixture was absorbed by suction onto a nitrocellulose membrane that had been soaked in binding assay buffer. The membrane was washed twice with buffer (20 mM Tris–HCl pH 8.0, 0.3 M NaCl) and then with 25% ethanol in distilled water. Radioactivity that remained on the membrane was measured in Atomlight (NEN) using a liquid scintillation counter.

5.4. Animal studies

Eight-week-old female mice of the ddy strain were obtained from Japan SLC Inc. (Shizuoka, Japan). Mice were either sham-operated or OVX, and some of the OVX mice were subcutaneously administered either 1–100 ng/day of **2c** or 100 ng/day of 17β-estradiol (**1**) dissolved in polyethyleneglycol 300 using a miniosmotic pump (Alza Co.), immediately after the surgery. At 4 weeks after the surgery, the mice were killed and the weight of the uterus was measured, and the right and left femurs were removed to measure BMD.

5.5. Radiographic analysis of the femur

Radiographic photographs of the femurs were taken with soft X-rays (model CMB-2, SOFTEX). The BMD of the femurs was measured by dual X-ray absorptiometry (model DCS-600R, Aloka). The bone mineral content (BMC) of the mouse femur was correlated closely with the ash weight. BMD was calculated by dividing BMC of the measured area by the area. The scanned area was divided into three parts: proximal femur, midshaft, and distal femur.

5.6. Docking simulation

The most stable docking models for all compounds were estimated using the automatic docking program ADAM version 4.11 [34]. Energy minimization of the docking models was done by the SANDER module of the AMBER 5 program package. Energy decomposition analyses of the final docking models were done by the ANAL module of AMBER 5 [37]. The structure of the human ERαLBD was obtained from the crystal structure of the ERαLBD complex (PDB code 1ERE A-chain) by removing the 17β-estradiol molecule. All hydrogen atoms that are missing in the crystal structure but should exist in the protein structure were computationally located at appropriate positions and optimized further by the AMBER program.

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