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Design and synthesis of iodocarborane-containing ligands with high affinity and selectivity toward ERβ

Kiminori Ohta *, Takumi Ogawa, Yasuyuki Endo

Faculty of Pharmaceutical Sciences, Tohoku Medical and Pharmaceutical University, 4-4-1 Komatsushima, Aoba-ku, Sendai 981-8558, Japan

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ABSTRACT

The selectivity and the binding affinity of previously reported carborane-containing ligands **2** and **3** toward ERβ remains to be optimized. To improve their biological profiles, a series of iodinated carboranyl phenol derivatives (**4**–**6**) were designed and synthesized as prospective ERβ-selective ligands with high affinity. Several iodinated carboranyl phenols showed high relative binding affinity (RBA) values for both ERs, and especially for ERβ, due to suitable hydrophobic interactions of the iodine atoms with the hydrophobic amino acid residues of the ERβ ligand-binding domains. Among these derivatives, 9,10-diiodo-m-carborane **5f** exhibited a more than 100% increase of the RBA values toward ERβ, a 14-fold increased selectivity for ERβ over ERα, and ER-agonistic activity in MCF-7 cell proliferation assays.

Estrogen receptor β (ERβ)[1] was initially cloned from a rat ventral prostate cDNA library as a second form of ERα,[2] and has since shown quite different biological functions compared to ERα.[3] Endogenous estrogen 17β-estradiol (E2, **1**) modulates various physiological processes, including the development and function of the female reproductive system, as well as the maintenance of bone mineral density.[4] However, the therapeutic use of **1** is limited by an increased risk of breast cancer, which has been linked to the activation of ERα (Fig. 1).[5] Nevertheless, the activation of ERβ induces an anti-proliferative effect on breast cancer.[6] ERβ-selective agonists down-regulate the expression of the androgen receptor (AR),[7] inhibit the proliferation and migration of prostate cancer cell lines,[8] and delay the progression of Alzheimer's disease by including the degradation of Aβ aggregates.[9] Thus, ERβ-selective ligands are of interest as potential therapeutic agents for Alzheimer's disease and several other types of cancer, and as probes for ERβ-related molecular biology.[10]

The development of ER-subtype-selective ligands remains a major challenge, as there are only two different amino acid residues in the hydrophobic pocket of the ER-ligand-binding domains (LBDs),[11] i.e., Leu384 and Met421 in ERα are substituted in ERβ by Met336 and Ile373, respectively.[11] Most ERβ-selective ligands obtain their subtype selectivity through electronic, hydrophobic, or steric interactions with these key amino acid residues.[12]

Based on the above theory, we have recently developed carborane-containing ERβ-selective ligands **2**[13] and **3**,[14] which showed high RBA values and high selectivity toward ERβ, respectively (Fig. 1). Although **3** showed much better ERβ selectivity than **2**, it is still not sufficient for the development of drug candidates or biological tools on account of the low binding affinity toward ERβ. An SAR study on the ERβ selectivity of **2** revealed that the iodine atom on the carbon atom of the *m*-carborane cage in **2** increases the binding affinity toward ERβ. Thus, we designed novel iodinated carborane-containing ERβ-selective ligands with high affinity toward ERβ, using **2** as a lead compound. In this paper, we describe the syntheses of several iodinated carboranyl phenols (**4**–**6**) and the evaluation of their ER-binding affinity and selectivity.

The synthesis of iodinated-*o*-carborane **4** is summarized in Scheme 1. 3-Iodo-*o*-carborane **7**, which was prepared from the *o*-carborane-reconstructing reaction of *nido*-7,8-$C_2B_9H_{12}^-$ with BI_3,[15] was treated with 4-methoxyphenylmagnesium bromide in the presence of CuI under Pd-catalyzed coupling conditions to afford 3-(4-methoxyphenyl)-*o*-carborane **8** in 98% yield.[16] Two iodine atoms were introduced on two carbon atoms of the *o*-carborane cage of **8** by consecutive exposure to *n*-BuLi and I_2, which afforded **9** in 91% yield. Subsequently, **9** was treated with BBr_3 to furnish 1,2-diiodo-*o*-carboranylphenol **4** in 96% yield.

Iodinated *m*-carboranes **5a**–**5e** were synthesized from **11** (Scheme 2), which was obtained in 28% yield from the iodination of *m*-carborane **10** with ICl in the presence of $AlCl_3$,[17] followed by a transformation into a copper salt and an Ullman coupling.[18] In this coupling reaction, 7,9-diiodo derivative **12** was also obtained unexpectedly in 23% yield, which suggests that the copper salt of **11** reacts with the iodine liberated during the reaction. The iodinated *m*-carboranes **11** and **12** were treated with BBr_3 to

* Corresponding author.
E-mail address: k-ohta@tohoku-mpu.ac.jp (K. Ohta).

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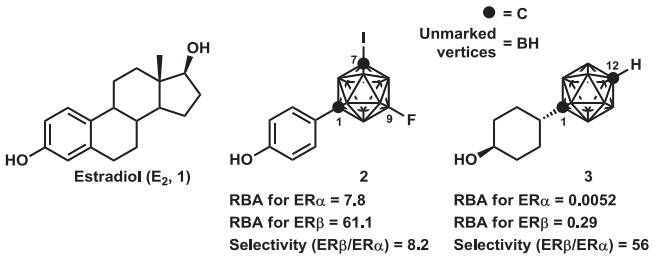
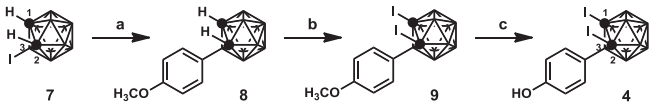
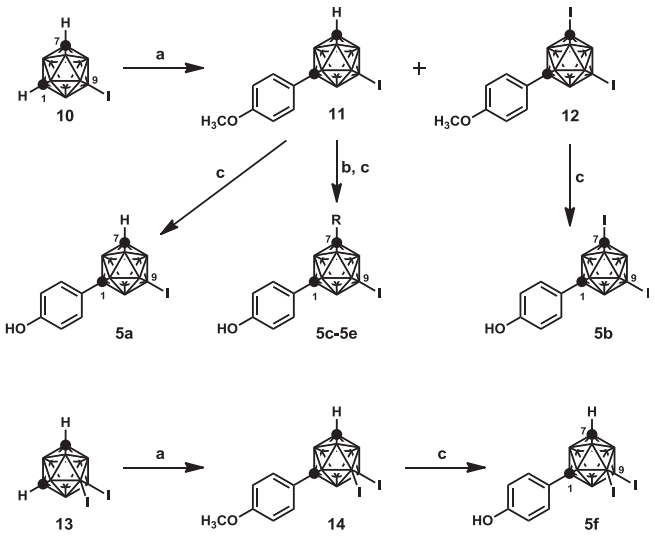


Fig. 1. Structures of 17β-estradiol and carborane-containing ERβ-selective ligands.



Scheme 1. Synthesis of iodinated *o*-carborane **4**. *Reagents and conditions*: (a) 4-methoxyphenylmagnesium bromide, $PdCl_2(PPh_3)_2$, CuI, THF, reflux; (b) (i) *n*-BuLi, Et_2O, 0 °C; (ii) I_2, rt; (c) BBr_3, CH_2Cl_2, 0 °C.

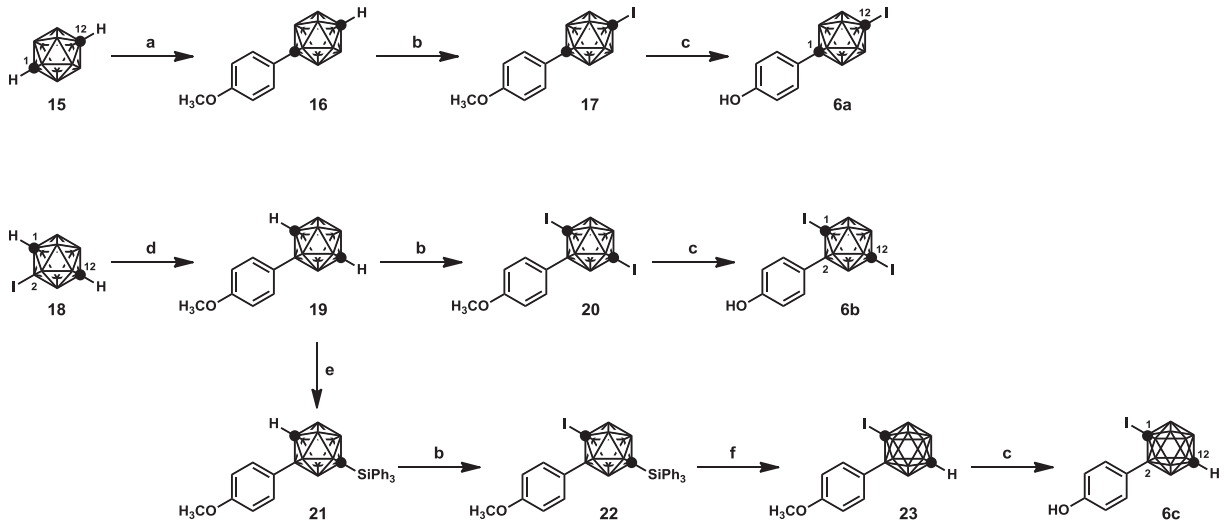


Scheme 2. Synthesis of iodinated *m*-carborane derivatives **5a**–**f**. *Reagents and conditions*: (a) (i) *n*-BuLi, DME, 0 °C; (ii) CuCl, rt; (iii) 4-iodoanisole, pyridine, 100 °C; (b) (i) *n*-BuLi, Et_2O, 0 °C; (ii) R-X, rt; (c) BBr_3, CH_2Cl_2, 0 °C.

afford 9-iodo-*m*-carboranylphenol **5a** and 7,9-diiodo-*m*-carboranylphenol **5b** in 90% and quantitative yield, respectively. Allyl, *n*-butyl, and benzyl groups were introduced into the C–H moiety of the *m*-carborane cage of **11** using the corresponding halide reagents in 68–83% yield, which were treated with BBr_3 to afford the corresponding 9-iodophenol derivatives **5c**, **5d**, and **5e** in 68%, 75%, and 93% yield, respectively. Scheme 2 also includes the synthesis of 9,10-diiodo-*m*-carboranylphenol **5f**. 9,10-Diiodo-*m*-carborane **13**,[19] prepared from the iodination of the corresponding *m*-carborane, was transformed into **14** (65%) under Cu-mediated coupling conditions, followed by a deprotection of the methyl group with BBr_3 to afford **5f** in 61% yield.

Scheme 3 summarizes the synthesis of iodinated *p*-carboranyl phenols **6a**–**6c**. Compound **16**, which was prepared by the Cu-mediated coupling reaction of *p*-carborane **15** with 4-iodoanisole, was consecutively treated with *n*-BuLi and I_2, before a demethylation with BBr_3 afforded 12-iodo-*p*-carboranylphenol **6a** in 33% overall yield. A Pd-catalyzed *B*-arylation of 2-iodo-*p*-carborane **18**, which was obtained from the iodination of *p*-carborane with 4-methoxymagnesium bromide, afforded **19** in 90% yield.[16] Compound **19** was subsequently treated with 2 equivalents of *n*-BuLi, quenched with I_2, and demethylated to afford **6b** in 73% overall yield. One of the less hindered carbon atoms of the *p*-carborane cage of **19** was protected with a triphenylsilyl group,[20] while another carbon atom was iodinated to afford **22** in 20% overall yield. The two protecting groups, i.e., the triphenylsilyl and methyl groups, were separately removed with TBAF and BBr_3, respectively, to afford **6c** in 90% overall yield.

The relative binding affinity (RBA) values toward ERα, ERβ, and the ER-subtype selectivity of the compounds synthesized in this study are summarized in Table 1.[21] Interestingly, several derivatives showed RBA values of more than 100% toward ERβ. Although *o*-carborane **4** showed a high ERβ-binding affinity, the ERβ selectivity was moderate due to the potent binding affinity toward ERα. We found that iodinated *m*-carborane **5a** is an ERβ-selective ligand with high binding affinity toward ERβ. The introduction of an iodine atom onto the carbon atom of the *m*-carborane cage reduced the RBA values toward ERα and ERβ, but the ERβ selectivity of **5b** was almost similar to that of **5a**. On the other hand, the introduction of an alkyl substituent, such as an allyl (**5c**), *n*-butyl (**5d**), or benzyl (**5e**) group, enhanced the ERα-binding affinity under concomitant decrease of the ERβ selectivity. Benzyl derivative **5e** exhibited a remarkable decrease of the binding affinity



Scheme 3. Synthesis of iodinated *p*-carboranes **6a**–**c**. *Reagents and conditions*: (a) (i) *n*-BuLi, DME, 0 °C; (ii) CuCl, rt; (iii) 4-iodoanisole, pyridine, 100 °C; (b) (i) *n*-BuLi, Et_2O, 0 °C; (ii) I_2, rt; (c) BBr_3, CH_2Cl_2, 0 °C; (d) 4-methoxyphenylmagnesium bromide, $PdCl_2(PPh_3)_2$, CuI, THF, reflux; (e) (i) *n*-BuLi, Et_2O, 0 °C; (ii) Ph_3SiCl, rt; (f) TBAF, THF, rt.

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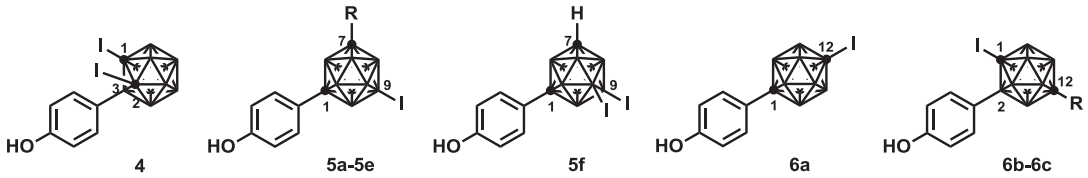
Table 1
RBA values toward ERα and ERβ, and ER-subtype selectivity of the iodinated carboranyl phenols **7**–**9**.[a]



Compound	Carborane	R	RBA[b]		Selectivity[c]	
			ERα	ERβ	ERα/ERβ	ERβ/ERα
4	*o*-Carborane	–	42	242	0.17	5.8
5a	*m*-Carborane	H	13	129	0.10	9.9
5b		I	8.4	81	0.10	9.6
5c		Allyl	17	62	0.27	3.6
5d		*n*-Butyl	29	157	0.18	5.4
5e		Benzyl	28	19	1.50	0.68
5f		–	8.0	111	0.07	14
6a	*p*-Carborane	–	574	888	0.65	1.5
6b		I	18	107	0.17	5.9
6c		H	10	38	0.26	3.8

[a] In all binding assays, which were performed in triplicate ($n = 3$), the test compounds (0.4 nM–4 μM) were examined in the presence of [2,4,6,7-^{3}H]17β-estradiol (4 nM).
[b] RBA values were calculated from the IC_{50} values of E2 and the test compounds, whereby that of E2 was set to 100%.
[c] The ER-subtype selectivity was estimated from the RBA values toward ERα and ERβ.

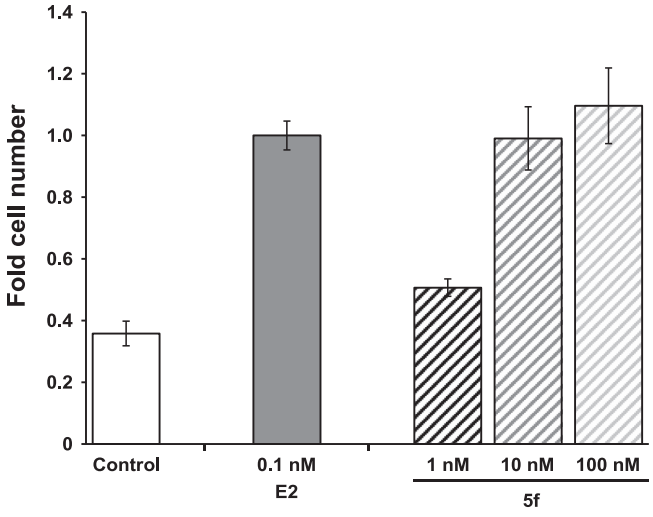


Fig. 2. Concentration-dependent MCF-7 cell proliferation induced by **5f**. MCF-7 cells were incubated with **5f** (100 nM–1 nM) for 5 days; the results are shown as the fold cell number, whereby the value for 0.1 nM E2 was set to 100%; assays were performed in triplicate ($n = 3$).

toward ERβ, and the RBA value of **5e** toward ERα surpassed that of ERβ. 9,10-Diiodo-*m*-carborane **5f** showed a 14-fold higher selectivity toward ERβ than toward ERα with an RBA value of more than 100% for ERβ. Surprisingly, the RBA value of *C*-iodo-*p*-carboranyl phenol **6a** toward ERβ was more than 800%. Unfortunately, the RBA value toward ERα was also more than 500%, resulting in an overall poor ERβ selectivity. These results suggest that the rectilinear *C*-iodo-*p*-carboranyl phenol structure is particularly suitable for the hydrophobic pocket of both ERs. *p*-Carboranes **6b** and **6c** exhibit a bent core structure comparable to that of **4** and **5**, and showed 5.9- and 3.8-fold selectivity toward ERβ, respectively.

The biological profiles, as well as the agonistic and antagonistic activity of **5f** were evaluated by means of a cell proliferation assay using the MCF-7 cell line, which shows estrogen-dependent growth (Fig. 2).[21] **5f** promoted the MCF-7 cell proliferation in a concentration-dependent manner and acted as an ER agonist.

In conclusion, we designed and synthesized various iodinated carboranyl phenols containing *o*-, *m*-, and *p*-carborane cages as prospective ligands with high selectivity and affinity toward ERβ. A competitive binding assay revealed that the introduction of iodine atoms enhanced their ERβ selectivity, which might be caused by a suitable hydrophobic interaction of the iodine atom with the amino acid residues of ERβ LBD. **5f** showed the highest ERβ selectivity, potent ERβ binding affinity, and ER agonistic activity in an MCF-7 cell proliferation assay.

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