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CONCISE ARTICLE

Development of androgen receptor ligands by application of ten-vertex *para*-carborane as a novel hydrophobic core structure†

Shinya Fujii,[a] Kiminori Ohta,[b] Tokuhito Goto,[b] Akifumi Oda,[b] Hiroyuki Masuno,[a] Yasuyuki Endo[b] and Hiroyuki Kagechika*[a]

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We report here the design, synthesis, androgenic activity and anti-androgenic activity of novel derivatives of hexadecahedral 1,10-dicarba-*closo*-decaborane, which is generally called ten-vertex *para*-carborane. The synthesized compounds exhibited very high binding affinity for the androgen receptor and showed anti-androgenic activity toward the androgen-dependent SC-3 cell line. Two compounds also exhibited partial agonistic activity in SC-3 assay. The docking studies of carborane derivatives to AR demonstrate that ten-vertex carborane is useful for development of novel bioactive compounds as well as twelve-vertex carboranes that are well known to be versatile pharmacophores. Ten-vertex carborane, which has not previously been applied in the field of medicinal chemistry, appears to have potential as a hydrophobic pharmacophore with characteristic properties.

Introduction

Closo boranes ($[B_nH_n]^{2-}$,where *n* is the number of vertices)[1] have characteristic properties, such as closed three-dimensional structures and unusual stability, unlike other classes of boranes. Dicarba-*closo*-boranes ($C_2B_{n-2}H_n$),[1,2] a class of *closo* boranes bearing two carbon atoms in place of boron atoms, are among the most stable boron clusters because of their electrically neutral nature and symmetrical, closed structure. Although there has been extensive investigation of dicarba-*closo*-boranes as components of functional molecules and materials, almost all studies have focused on the twelve-vertex dicarba-*closo*-dodecaboranes ($n = 12$, $C_2B_{10}H_{12}$; **1a–c**) (Fig. 1).[3] Twelve-vertex dicarba-*closo*-dodecaboranes, which are usually called twelve-vertex carboranes or more simply carboranes, have a highly symmetrical icosahedral geometry, and remarkable thermal and chemical stability. Focusing on the hydrophobic character of twelve-vertex carboranes, we have investigated their application as hydrophobic building blocks of biological active molecules. We have demonstrated that they are versatile hydrophobic core structures of ligands for nuclear receptors, such as androgen receptor,[4,5] estrogen receptor,[6] retinoid receptors,[7] and vitamin D receptor.[8] Since hydrophobicity of the molecular surface is essential for nuclear receptor ligand function, these results demonstrated that the hydrophobic twelve-vertex carboranes can interact effectively with the hydrophobic surface of the receptor proteins.

Hundreds of twelve-vertex carborane derivatives have been synthesized and investigated. However, there have been only a few investigations of smaller dicarba-*closo*-boranes[9] for liquid crystal applications[10] and electronic materials,[11] and they have not been utilized in the field of medicinal chemistry. We considered that smaller dicarba-*closo*-boranes might have different characteristics as hydrophobic pharmacophores from twelve-vertex carboranes, and the present study was designed to test this idea.

Here, we focused on 1,10-dicarba-*closo*-decaborane ($n = 10$, $C_2B_8H_{10}$; **2**), also called ten-vertex *para*-carborane. Like

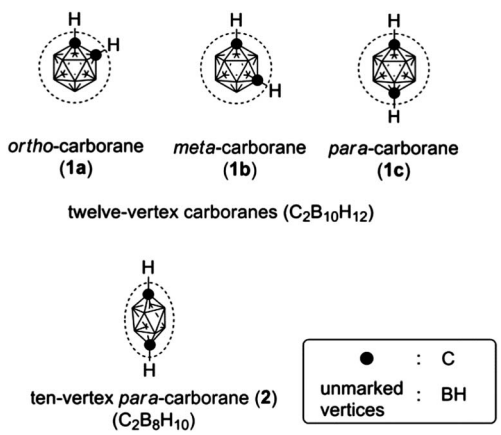

Fig. 1 Structures of carboranes. Hashed circles indicate the overall geometry of the boron clusters.

[a]*Graduate School of Biomedical Science, Institute of Biomaterials and Bioengineering, Tokyo Medical and Dental University, 2-3-10, Kanda-Surugadai, Chiyoda-ku, Tokyo, 101-0062 Japan. E-mail: kage.omc@tmd.ac.jp; Fax: +81-3-5280-8127; Tel: +81-3-5280-8032*
[b]*Faculty of Pharmaceutical Sciences, Tohoku Pharmaceutical University, 4-4-1, Komatsushima, Aoba-ku, Sendai 981-8558, Japan*
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twelve-vertex carboranes, ten-vertex *para*-carborane has a hydrophobic surface, and so is expected to have potential to serve as the hydrophobic core of nuclear receptor ligands. On the other hand, ten-vertex *para*-carborane has an overall ellipsoidal geometry that is quite different from that of the highly symmetrical twelve-vertex carboranes. Therefore, derivatives of ten-vertex *para*-carborane may have activities distinct from those of the corresponding twelve-vertex carborane derivatives. On the basis of these considerations, we designed and synthesized a series of ten-vertex *para*-carborane derivatives as candidate nuclear receptor ligands.

As the biological target, we selected the androgen receptor (AR).[12] AR is a member of the nuclear receptor superfamily of ligand-regulated transcriptional factors[13] specific for testosterone (**3**) or dihydrotestosterone (DHT; **4**), and regulates numerous physiological functions, such as maintenance of the male reproductive system, and bone and muscle homeostasis. AR is also closely related to prostate cancer, and therefore AR antagonists such as flutamide (**5**) and bicalutamide (**7**) are used clinically for treatment of prostate cancer (Fig. 2).[14] Previously we reported the development of two types of potent AR antagonists bearing twelve-vertex carborane as a hydrophobic pharmacophore, *i.e.*, the phenylcarborane derivatives **8–11** (ref. 4) and the carboranylcyclohexenone **12**.[5] In these compounds, the spatial volume of the twelve-vertex carborane cage is essential for antagonistic activity.[15] Therefore, the difference in shape and bulkiness of **2**, compared to **1**, may influence the biological activities. To see whether this is indeed the case, we synthesized ten-vertex *para*-carborane derivatives **13–17** (Fig. 3).

Results and discussion

The crystal structure of AR bound to testosterone shows that the 3-carbonyl group of steroidal ligands interacts with Gln711 and Arg752 of AR.[16] The cyclohexenone ring of twelve-vertex carborane (**12**) corresponds to the hydrophilic pharmacophore of testosterone (**3**). Further, a docking study revealed that the nitro- and cyano groups of **8–11** interact effectively with the same residues through hydrogen bonding.[15] Thus, we designed ten-vertex *para*-carborane derivatives bearing a nitro- or cyanophenyl group (**13–16**) and a cyclohexenone group (**17**) as candidate AR ligands based on our previously developed twelve-vertex carborane-based AR ligands.

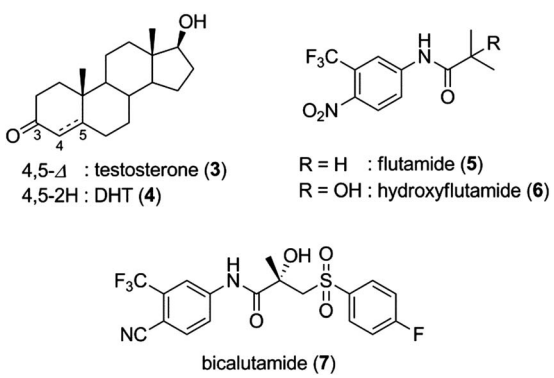


Fig. 2 Structures of endogenous and synthetic AR ligands.

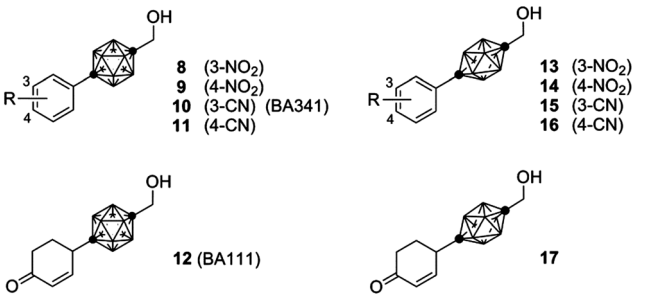
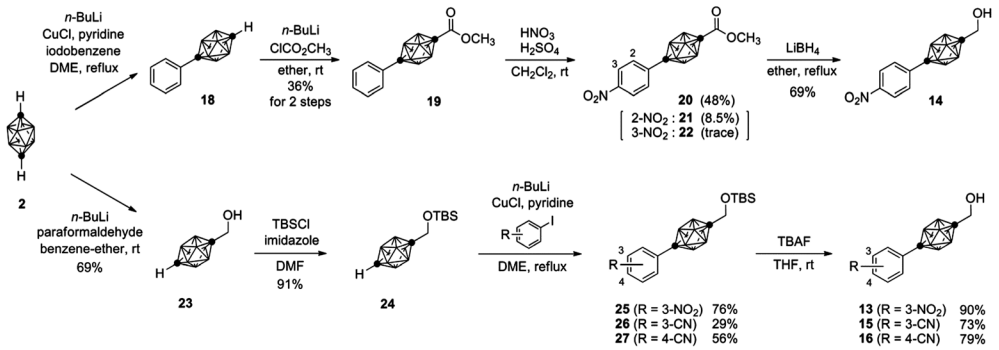


Fig. 3 Structures of the designed ten-vertex carborane derivatives (right) and the corresponding twelve-vertex carborane derivatives (left).

We first examined synthetic methods similar to those used for the twelve-vertex carborane derivatives. The synthetic routes to the phenyl-ten-vertex *para*-carborane derivatives **13–16** are illustrated in Scheme 1. The coupling reaction between *C*-lithiated **2** and iodobenzene using copper(I) chloride[17] gave 1-phenyl-ten-vertex *para*-carborane **18**, and introduction of a methoxycarbonyl group afforded ester **19**. Nitration of **19** yielded the 4-nitrophenyl compound **20** as the main product. However, 3-nitrated derivative **22** was not obtained, and 2-nitrated derivative **21** was obtained as a minor product. In the case of twelve-vertex carborane, nitration of phenylcarborane yielded the 4-nitrophenylcarborane derivative as the major product (*ca.* 60%) and the 3-nitrated derivative as a minor product (*ca.* 20%), while the 2-nitrated compound was not obtained. The difference in orientation of nitration could be attributed to the difference of bulkiness between ten-vertex carborane and twelve-vertex carborane. Reduction of ester **20** with lithium borohydride gave **14**. The 3-nitrophenyl and cyanophenyl derivatives were synthesized by another method. Reaction of **2** and paraformaldehyde gave alcohol **23**, and the hydroxyl group of **23** was protected with TBS to afford **24**. A copper(I)-mediated coupling reaction between *C*-lithiated **24** and iodobenzenes afforded the corresponding phenylcarborane derivatives **25–27**. Finally, removal of the TBS group afforded **13**, **15** and **16** (Scheme 1).

Scheme 2 illustrates the synthesis of cyclohexenone **17**. Reaction of **2** with 1,4-cyclohexanedione-mono-ethyleneketal gave tertiary alcohol **28**. Deprotection of the ketal, followed by dehydration and isomerization of the olefinic double bond with concentrated sulfuric acid, afforded the α,β-unsaturated ketone **29** in one pot. The unsaturated ketone **29** was converted to allyl alcohol **30** by reduction, and protection of the alcohol gave **31**. Reaction of **31** with *n*-butyl lithium and paraformaldehyde afforded alcohol **32**, and deprotection of the MOM group with hydrochloric acid gave diol **33**. Finally, selective oxidation of allyl alcohol using manganese oxide afforded the desired enone **17** (Scheme 2).

The androgenic and anti-androgenic activities of the synthesized molecules were assessed by assay of growth promotion/inhibition potency toward androgen-dependent SC-3 cells (Fig. 4).[18] Fig. 4A shows the cell growth-promoting activity of the carborane derivatives. As we had previously reported, none of the twelve-vertex carborane derivatives **8–12** showed growth-promoting activity, which means these twelve-vertex carborane derivatives do not have AR agonistic activity.[4,5] On the other hand, the cyclohexanone derivative of ten-vertex *para*-carborane



Scheme 1 Synthesis of phenyl-ten-vertex *para*-carborane derivatives.

17 showed significant SC-3 cell growth-promoting activity, and the 3-cyanophenyl derivative **15** also promoted SC-3 cell growth. The other compounds did not exhibit agonistic activity. Fig. 4B shows the antagonistic activity toward SC-3 cell growth promoted by 1 nM testosterone (**3**). All the synthesized molecules exhibited cell growth-inhibitory activity toward SC-3 cells, that is, these compounds have AR antagonistic activity. The nitrophenyl and cyanophenyl derivatives exhibited significant antagonistic potency, and the 3-nitro derivative **13** and the 3-cyano derivative **15** showed the greatest activity, being more potent than hydroxyflutamide **6**, the metabolically activated form of flutamide **5**. The cyclohexenone derivative **17** also exhibited androgen antagonistic activity. There was no significant difference in antagonistic potency between ten-vertex *para*-carborane derivatives and twelve-vertex carborane derivatives, except for the agonistic activity of **15** and **17** at the concentration of 1×10^{-5} M. These results suggest that ten-vertex *para*-carborane derivatives **15** and **17** are partial agonists for AR.

Next, the AR-binding affinity of the ten-vertex *para*-carborane derivatives was examined by a competitive binding assay using ^{3}H-labeled dihydrotestosterone ([^{3}H]DHT) and human AR ligand-binding domain (hAR-LBD),[19] and compared with that of the twelve-vertex derivatives. Fig. 5 shows the dose–response relationship of cyanophenylcarborane derivatives and cyclohexanone derivatives in competitive binding over the concentration range 1×10^{-7} to 1×10^{-4} M. The binding potencies of these ten-vertex derivatives were comparable to those of the twelve-vertex derivatives, and the most potent ten-vertex derivative **15** with the 3-cyano group exhibited higher affinity ($IC_{50} = 7.6 \times 10^{-7}$ M) than the corresponding twelve-vertex derivative **10** ($IC_{50} = 1.9 \times 10^{-6}$ M). With regard to cyclohexanone derivatives, the ten-vertex derivative **17** also exhibited higher affinity for hAR ($IC_{50} = 6.1 \times 10^{-6}$ M) than the twelve-vertex derivative **12** ($IC_{50} = 9.7 \times 10^{-6}$ M). On the other hand, the ten-vertex derivative **16** with the 4-cyano group exhibited lower affinity ($IC_{50} = 1.6 \times 10^{-5}$ M) than the corresponding twelve-vertex derivative **11** ($IC_{50} = 1.0 \times 10^{-5}$ M). It is interesting that

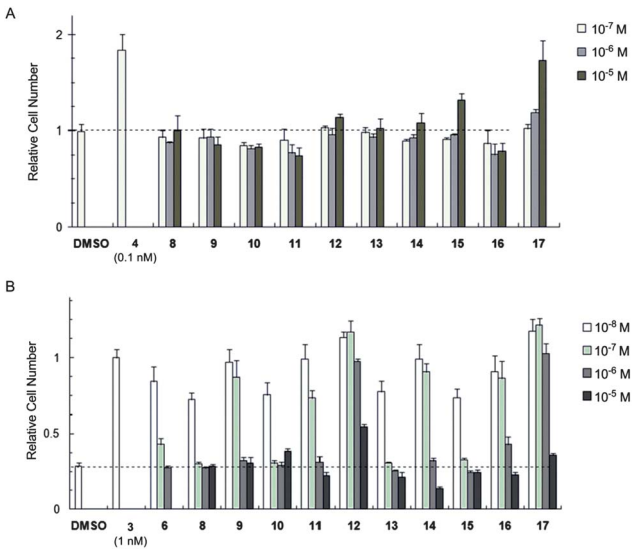
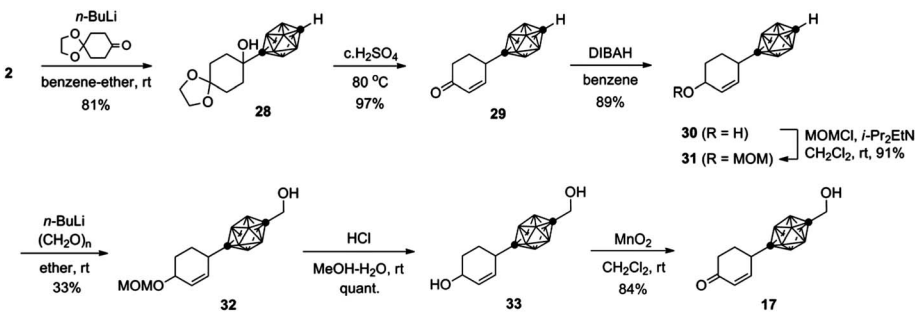


Fig. 4 SC-3 cell proliferation promotion/inhibition assay. (A) SC-3 cell proliferation-promoting activity of test compound only. (B) Inhibitory activity towards testosterone-induced SC-3 cell proliferation. The concentration of testosterone was 1 nM. Cell number was normalized to that in the absence of test compounds, taken as 1.



Scheme 2 Synthesis of cyclohexenone derivative **17**.

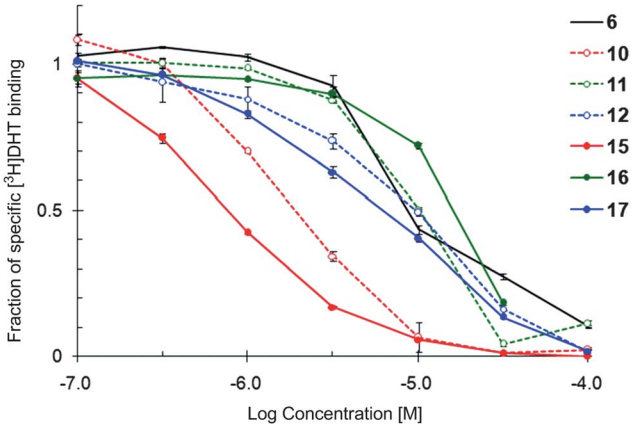


Fig. 5 Competitive binding assay of synthesized ten-vertex carborane derivatives and twelve-vertex carborane derivatives. Solid lines indicate ten-vertex carborane derivatives, and hashed lines indicate twelve-vertex carborane derivatives. The concentration of [^{3}H]DHT was 4 nM.

compounds **15** and **17**, which exhibited partial agonistic activity, showed higher binding affinity than the corresponding twelve-vertex carborane derivatives (Fig. 5). These results suggest that the ten-vertex cage is more favorable as a hydrophobic core of AR ligands than twelve-vertex carborane for exerting androgen-dependent transcriptional activity.

We conducted docking studies of carborane derivatives to AR in order to investigate the difference between the ten-vertex carborane derivative **15** and the corresponding twelve-vertex carborane derivative **10**. Fig. 6 shows the docking simulation of these compounds with AR-LBD by using the co-crystal structure of hAR-LBD with metribolone (PDB:1E3G), by a docking program GOLD.[20] In the calculated binding mode, the ten-vertex carborane derivative **15** is embedded in the ligand-binding pocket of AR in a manner similar to that of **10**. However, the locations of cyano groups, that form key hydrogen-bonds with Gln711 and Arg752, are considerably different from each other. The differences can be attributed to the shape of the clusters. X-ray crystallographic studies reported that ten-vertex *para*-carborane has not only narrower structure than twelve-vertex *para*-carborane but also longer C–C distance (3.39 Å) than twelve-vertex (3.13 Å).[21] We also calculated the molecular volume difference between compounds **10** and **15**; and compound **15** occupies a smaller volume (239 Å^3) than compound **10** (263 Å^3). These results are consistent with our prediction that the spatial volume of the twelve-vertex carborane cage and direction of the cyano

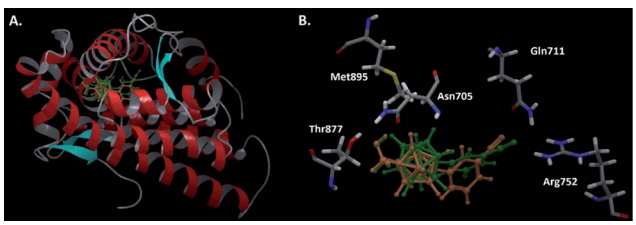


Fig. 6 Docking models of **10** and **15** to hAR-LBD. Compounds **10** and **15** are colored in green and brown, respectively. (A) Overall structure of AR-LBD bound to ligands. (B) Amino acid residue of hAR-LBD around the ligands.

group are essential for full-antagonistic activity of **10**.[15] The difference of cluster geometry can cause the different binding modes of these two compounds, and also induce partial agonistic activity of compound **15**.

Conclusions

We have designed and synthesized derivatives of ten-vertex carborane, which has never previously been used in medicinal chemical applications, and studied their activity as novel AR ligands in comparison with the corresponding twelve-vertex carborane derivatives. The synthesized molecules exhibited potent androgen antagonistic activity, as did the corresponding twelve-vertex carborane derivatives, but the ten-vertex carboranes showed higher AR binding affinity. Among the synthesized molecules, compounds **15** and **17** exhibited partial agonistic activity, in marked contrast to the corresponding twelve-vertex carborane derivatives. Introduction of the ten-vertex cage in place of the twelve-vertex cage may reduce steric interference with the ligand-binding pocket of AR, resulting in partial agonistic activity. Docking simulation suggested that ten-vertex carborane functions as a hydrophobic anchor, and that the characteristic ellipsoidal structure of ten-vertex carborane induces binding modes of the derivatives different from the corresponding twelve-vertex carborane derivatives. Thus, we have shown for the first time that ten-vertex *para*-carborane can function as a hydrophobic core structure of biologically active molecules as well as twelve-vertex carboranes, and may generate a unique profile of biological activities. Structural development studies with ten-vertex and twelve-vertex carboranes may be fruitful approaches to uncover key hydrophobic interactions of other ligand–receptor combinations.

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