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Polymethylcarborane as a Novel Bioactive Moiety: Derivatives with Potent Retinoid Antagonistic Activity

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Abstract—4-[(Deca-*B*-methyl-1,12-dicarba-*closo*-dodecaboran-1-yl)carbamoyl]benzoic acid and its congeners showed potent antagonistic activity at concentrations of 10^{-7}–10^{-8} M on the differentiation-inducing action of retinoids towards human promyelocytic leukemia HL-60 cells. This is the first example of derivatives of polymethylcarborane, which resembles C_{60} in size, with biological activity.

Interest in topologically spherical molecules (e.g., dodecahedrane[1]) began in the 1980s, and following the discovery of fullerenes in the subsequent decade, has led to applications in supramolecular chemistry[2] and medicinal chemistry.[3] Recently, another family of topologically spherical molecules, including dodecamethyl-1,12-di-carba-*closo*-dodecaborane (12) (**1**)[4] and dodecamethyl-*closo*-dodecaborate (2-) anion,[5] has been reported by Hawthorne et al. The polymethylcarborane **1** provides a unique hydrophobic surface due to its methyl substituents, and is termed a 'camouflaged carborane'. It is noteworthy that the size of **1** resembles that of C60. However, the difference between the electron-rich molecular surface of C60 and the fully hydrophobic surface of **1** should lead to marked differences in molecular interactions. We are interested in new medicinal applications of boron cluster compounds, dicarba-*closo*-dodecaboranes (carboranes), which are smaller than **1**, as hydrophobic pharmacophores. We have reported examples of the design, synthesis and biological evaluation of potent nuclear receptor ligands, such as retinoids,[6] estrogens,[7] and other biologically active molecules containing carborane moiety.[8] Polymethylcarboranes may also have potential as a new bulky and hydrophobic unit for biologically active molecules. Several functionalization of polymethylcarboranes have been reported by Hawthorne et al.[9] We have reported the preparation[10] and unique reactivity[11] of deca-*B*-methyl-1,12-dicarba-*closo*-dodecaboran(12)-1-carboxylic acid (**2**) and their derivatives. In this paper, we describe the synthesis and biological evaluation of novel retinoidal antagonists bearing polymethylcarborane. This is the first example of a potential medicinal application of this unique molecule (Fig. 1).

Retinoic acid (all-*trans*, **3**) has a broad spectrum of biological activities related to cellular differentiation and proliferation and is essential for normal embryonic development in vertebrates.[12] These biological responses are mediated by binding to and activation of the specific retinoic acid receptors (RARs)[13] followed by modulation of target gene transcription by the complex. The retinoidal actions are also modulated by retinoid X receptors (RXRs), which bind 9-*cis*-retinoic acid.[14] Recent work on the design of synthetic retinoids[12,15] and the availability of 3-D structural information[16] have revealed the structural requirements for the appearance of retinoidal activity. High binding affinity for RAR requires a carboxylic acid moiety and an appropriate hydrophobic group, such as in retinobenzoic acid, Am80 (**4**). Recently, we have reported potent retinoid agonists (**5**) bearing a carborane cage as a hydrophobic pharmacophore.[6] We have also found that a retinobenzoic acid with a bulkier hydrophobic group, such as the pentacyclotetradecane (diamantyl) group (TD550, **6**)[17] or with heterocyclic ring structure (LE540, **7**),[18] exhibited anti-retinoidal activity toward human promyelocytic leukemia cells (HL-60) Fig. 2. These results led us to synthesize and investigate compounds (**8**–**16**) having carboranes or polymethylcarboranes as a hydrophobic moiety, as shown in Fig. 3. In the icosahedral cage structures throughout this paper, closed circles (●) represent carbon atoms and other vertices represent boron atoms or BH units (Fig. 2).

The syntheses of the designed molecules are summarized in Scheme 1. 1,2-Dicarba-*closo*-dodecaborane

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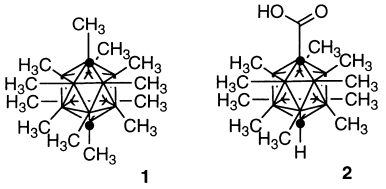


Figure 1. Structures of globular polymethylcarboranes.

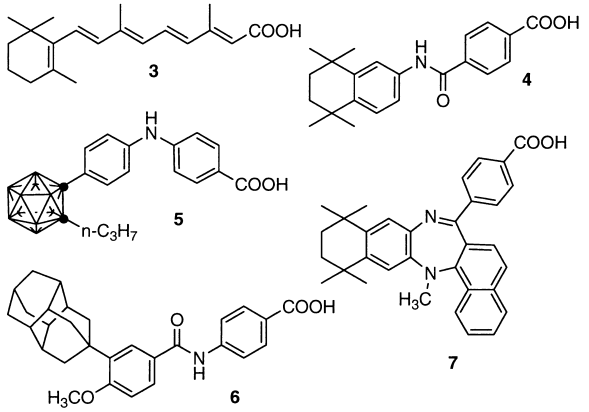


Figure 2. Structures of potent retinoid agonists and antagonists.

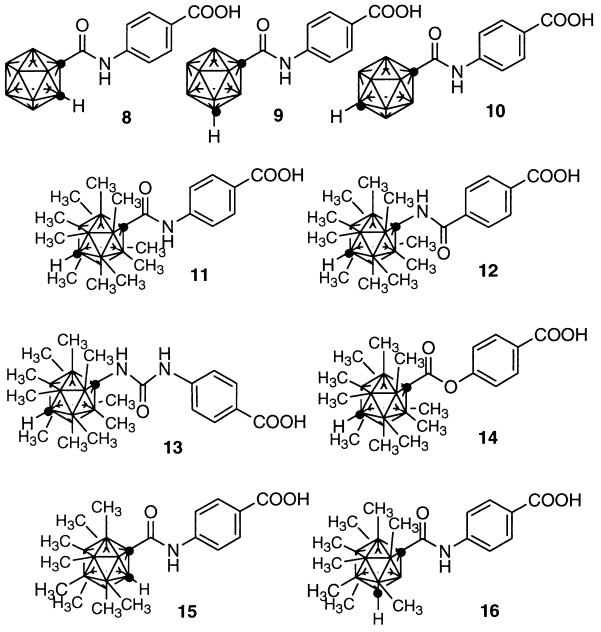
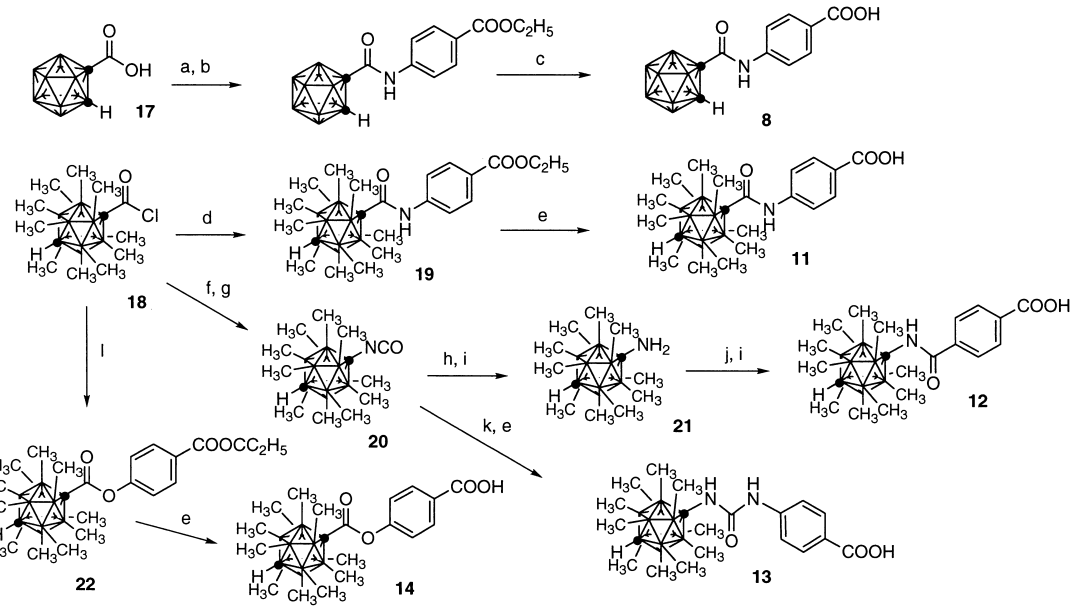


Figure 3. The designed compounds with a carborane or polymethylcarborane moiety (**8**–**16**).

(*o*-carborane)-1-carboxylic acid (**17**) was readily transformed to acid chloride by thionyl chloride. The reaction of the acid chloride with methyl 4-aminobenzoate (in pyridine, room temperature, 1 h) followed by alkaline hydrolysis of the methyl ester (KOH, EtOH, 80 °C, 1 h) afforded compound **8** in an usual manner. Compounds **9** and **10** were also prepared from *m*- or *p*-carborane-1-carboxylic acid. However, the reactivity of the carboxyl group of polymethylcarborane-1-carboxylic acid **2** was greatly reduced by the steric effect of the surrounding five *B*-methyl groups. For example, esterification of **2** did not proceed by acid-catalyzed condensation with alcohol and the methyl ester of **2** was not hydrolyzed under acidic or basic conditions. However, the acid chloride (**18**) was prepared as a stable crystalline form.[10] Condensation of **18** with ethyl 4-aminobenzoate under drastic conditions (in *o*-dichlorobenzene, 180 °C, 24 h) gave the amide **19**, which was hydrolyzed under basic conditions (KOH, EtOH, 80 °C, 1 h) to afford compound **11** in quantitative yield (2 steps). The acid chloride **18** was transformed to the stable crystalline isocyanate **20** by reaction with sodium azide in DMF, followed by thermolysis (toluene, reflux) in a yield of 96%. The isocyanate **20** was further transformed to the amine **21** by methanolysis followed by alkaline hydrolysis, in 79% yield. Condensation of the amine **21** with terephthalic acid monomethyl ester chloride (in *o*-dichlorobenzene, 180 °C, 18 h, 53%) followed by alkaline hydrolysis afforded compound **12**. The isocyanate **20** was allowed to react with ethyl 4-aminobenzoate (neat, 180 °C, 24 h, 51%) followed by alkaline hydrolysis to afford compound **13**. The acid chloride **18** was allowed to react with ethyl 4-hydroxybenzoate (DMAP, pyridine 70 °C, 1 h, 81%) to give the ester **22**, which was hydrolyzed (KOH, EtOH, 80 °C, 1 h, y: quantitative) to give compound **14**. 4,5,7,8,9,10,11,12-Octamethyl-1,2-dicarba-*closo*-dodecaborane[19] and 4,5,6,8,9,10,11,12-octamethyl-1,7-dicarba-*closo*-dodecaborane[19] were transformed to their 1-acids in a similar manner to that used for preparation of **2**.[10] The acids are more reactive than the acid **2** owing to the lack of some methyl groups around the carbon atom. The acids were converted to compounds **15** and **16** in a similar manner to that used for the preparation of **8**. The structures of the compounds **8**–**16** were confirmed by a broad array of spectroscopic and analytic data including ^{1}H NMR, HRMS and elemental analysis.[20]

The biological activities of compounds **8**–**16** were evaluated in terms of the activity to induce differentiation of HL-60 cells to mature granulocytes.[21] The differentiated cells were identified by nitro blue tetrazolium (NBT) reduction assay.[21] All the synthetic compounds by themselves were completely inactive as differentiation inducers below 1×10^{-6} M. Next, we evaluated the antagonistic activity of the synthetic compounds functionally in terms of the inhibitory potency on the differentiation-inducing activity of Am80 (**4**) in HL-60 cells. Although the compounds bearing carborane **8**–**10** were completely inactive in the assay, the compounds bearing polymethylcarborane **11**–**16** clearly inhibited the activity of Am80. The effects of compounds **11**, **14** and the typical retinoid antagonist LE540 (**7**) on differentiation induced by 1×10^{-9} M Am80 are shown in Figure 4. The IC_{50} values of the all compounds on 1×10^{-9} M Am80-induced HL-60 cell differentiation are shown in Table 1. As reported previously,[18] LE540 (**7**) antagonized the differentiation-inducing activity of Am80, with IC_{50} value of 1.7×10^{-8} M. Compounds **11**, **12** and **14** showed almost the same activity as **7**. Replacement of the linking group (between the benzoic acid residue and hydrophobic group) of **11** with a reversed amide group or ester group did not affect the potent antagonistic activity. However, elongation of the linking group of **11** with a urea group



Scheme 1. Synthesis of the designed compounds bearing a carborane or polymethylcarborane moiety. key: (a) $SOCl_2$, benzene, heat; (b) ethyl 4-aminobenzoate, DMAP/CH_2Cl_2; (c) KOH/H_2O-THF; (d) ethyl 4-aminobenzoate/*o*-dichlorobenzene, 180 °C; (e) KOH/H_2O-EtOH; (f) NaN_3/DMF; (g) toluene, heat; (h) MeOH, heat; (i) KOH/H_2O-MeOH; (j) terephthalic acid monomethylester chloride/*o*-dichlorobenzene, 180 °C; (k) ethyl 4-aminobenzoate, 180 °C; (l) ethyl 4-hydroxybenzoate, DMAP/ pyridine.

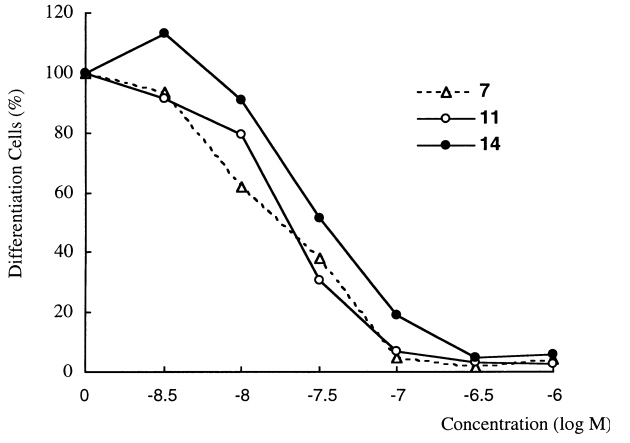


Figure 4. Antagonistic activities of **11**, **14** and **7** on 1×10^{-9} M Am80-induced HL-60 cell differentiation. The vertical scale is the percentage of differentiated cells evaluated from NBT reduction assay, and the horizontal scale is the molar concentration of test compounds.

Table 1. Antagonistic activities of the compounds bearing a carborane or polymethylcarborane moiety (**8**–**16**)

Compound	IC_{50} M[a]
LE540 (**7**)	1.7×10^{-8}
8	Inactive[b]
9	Inactive
10	Inactive
11	2.9×10^{-8}
12	2.8×10^{-8}
13	6.1×10^{-7}
14	3.5×10^{-8}
15	1.1×10^{-7}
16	1.6×10^{-7}

[a]IC_{50} values were determined as the concentration of a test compound which reduced by half the percentage of differentiated HL-60 cells induced by 1×10^{-9} M Am80.
[b]'Inactive' means there was no detectable activity below 1×10^{-6} M test compound.

diminished the activity. Change of the overall shape of the decamethyl-*p*-carborane cage of **11** by introducing a smaller octamethylcarborane cage (**15**, **16**) somewhat diminished the activity. In general, introduction of a bulky hydrophobic group at an appropriate position of the agonist molecule results in a conformational change of the receptor–ligand complex to afford antagonistic activity. The polymethylcarboranyl group seems to play the role of a bulky and hydrophobic region for the appearance of antagonistic activity.

In conclusion, we have developed novel polymethyl-carborane-containing molecules with potent retinoidal antagonistic 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 results of this study demonstrate that polymethylcarboranes, like carboranes, can be employed as the hydrophobic moiety of biologically active molecules.

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20. Spectral data of polymethylcarborane-containing compounds **11**–**16** were as follows: **11**: colorless needles (CH_2Cl_2-*n*-hexane) mp 285–286 °C; ^{1}H NMR ($CDCl_3$) 0.12, 0.27 (each 15H, s, B-CH_3), 2.27 (1H, s, CH), 7.13 (1H, bs, NH), 7.45 (2H, d, J=8.8 Hz), 8.01 (2H, d, J=8.8 Hz). Anal. calcd For $C_{20}B_{10}H_{37}NO_3$: C, 53.67; H, 8.33; N, 3.13. Found: C, 53.68; H, 8.22; N, 3.01. **12**: colorless leaflets (ethyl acetate-*n*-hexane) mp 259 °C; ^{1}H NMR ($CDCl_3$) 0.11, 0.18 (each 15H, s, B-CH_3), 2.04 (1H, s, CH), 5.60 (1H, s, NH), 7.66 (2H, d, J=8.8 Hz), 8.12 (2H, d, J=8.8 Hz). Anal. calcd for $C_{20}B_{10}H_{37}NO_3$: C, 53.67; H, 8.33; N, 3.13. Found: C, 53.38; H, 8.33; N, 3.13. **13**: colorless leaflets (methanol) mp >300 °C; ^{1}H NMR (DMSO-d_6) 0.14, 0.20 (each 15H, s, B-CH_3), 2.77 (1H, s, CH), 5.57 (1H, s, NH), 7.48 (2H, d, J=8.8 Hz), 7.92 (2H, d, J=8.8 Hz), 9.49 (1H, s, NH). Anal. calcd for $C_{20}B_{10}H_{38}N_2O_3$: C, 51.92; H, 8.28; N, 6.06. Found: C, 51.63; H, 8.09; N, 5.77. **14**: colorless needles (ethyl acetate-*n*-hexane) mp 257–258 °C; ^{1}H NMR ($CDCl_3$) 0.12, 0.24 (each 15H, s, B-CH_3), 2.27 (1H, s, CH), 7.03 (2H, d, J=9.0 Hz), 8.08 (2H, d, J=9.0 Hz). Anal. calcd for $C_{20}B_{10}H_{36}O_4$: C, 53.55; H, 8.09. Found: C, 53.44; H, 8.01. **15**: colorless needles (CH_2Cl_2-*n*-hexane) mp 216–217 °C; ^{1}H NMR ($CDCl_3$) −0.19 (3H, s, B-CH_3), −0.17 (3H, s, B-CH_3), 0.06 (6H, s, B-CH_3), 0.20 (6H, s, B-CH_3), 0.38 (6H, s, B-CH_3), 3.99 (1H, bs, CH), 7.43 (1H, s, NH), 7.56 (2H, d, J=8.5 Hz), 8.10 (2H, d, J=8.8 Hz); HRMS: calcd for $C_{18}H_{33}^{10}B_2^{11}B_8NO_3$, 419.3469; found, 419.3466. **16**: colorless prisms (CH_2Cl_2-*n*-hexane) mp 236–237 °C; ^{1}H NMR ($CDCl_3$) −0.07 (3H, s, B-CH_3), 0.02 (3H, s, B-CH_3), 0.23 (6H, s, B-CH_3), 0.24 (6H, s, B-CH_3), 0.28 (6H, s, B-CH_3), 2.43 (1H, bs, CH), 7.54 (1H, s, NH), 7.56 (2H, d, J=8.5 Hz), 8.07 (2H, d, J=8.8 Hz). Anal. calcd for $C_{18}B_{10}H_{33}NO_3$: C, 51.53; H, 7.93; N, 3.34. Found: C, 51.37; H, 7.69; N, 3.31.
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