Article

1,2-Diphenyl-*o*-carborane and its Chromium Derivatives: Synthesis, Characterization, X-ray Structural Studies, and Biological Evaluations

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Abstract: The objective of this study is to design and synthesize substituted η^6-chromium tricarbonyl metal complexes carrying *o*-carborane units as potential boron neutron capture therapy (BNCT) agents. In this study, 1,2-Diphenyl-*o*-carborane units were used as starting materials to generate biologically active species. We investigated how the structural changes of 1,2-diphenyl-*o*-carborane substituted with chromium(0) tricarbonyl affect the biological properties; $[(CO)_3Cr]Ph_2C_2$ (**2**) and $[(CO)_3Cr]_2Ph_2C_2$ (**3**) species were produced in moderate yields. The molecular structures of compounds **1–3** were identified and established by infrared (IR), ^1H, ^{11}B, and ^{13}C nuclear magnetic resonance (NMR), and X-ray crystallography analyses. Crystal structures of *o*-carboranyl chromium complexes **1** [a = 10.859(1) Å, b = 24.953(3) Å, c = 13.938(2) Å, β = 111.854(2)º], **2** [a = 10.621(3) Å, b = 17.056(5) Å, c = 12.174(4) Å, β = 106.622(5)º], and **3** [a = 17.540(2) Å, b = 18.060(2) Å, c = 19.484(4) Å, α = 105.746(2)º, β = 110.226(2)º, γ = 91.256(2)º] were obtained. In vitro study using B16 and CT26 cancer cells containing the triphenyl-*o*-carboranyl chromium (0) complexes **Ph3C2BCr2** and **Ph3C2BCr3**, which we have previously reported, the compounds **2** and **3** accumulated at higher levels than compounds **Ph3C2BCr2** and **Ph3C2BCr3**. However, the phenylated *o*-carboranyl chromium(0) complexes have been found to be more cytotoxic than *p*-boronophenylalanine (BPA).

Keywords: 1,2-Diphenyl-*o*-carborane; Chromium Metal Complexes; Boron Neutron Capture Therapy; Biological Evaluation; Cytotoxicity

1. Introduction

Boron neutron capture therapy (BNCT) is a promising treatment for a variety of central nervous system (CNS) disorders, especially brain tumors. For example, BNCT is currently used as an adjunct to surgery for the treatment of glioblastoma multiforme [1]. The effect of BNCT is to suppress brain micrometastases that cannot be treated surgically or for which other treatment methods are not available [2]. BNCT destroys cancer cells with energy from targeted radiation bursts by reacting boron isotopes delivered to malignant cells with neutrons. Upon uptake into the cells, ^{10}B is externally irradiated with neutrons and becomes unstable $[^{11}B]^*$, decaying to lithium ($^7Li^{3+}$) and releasing high-energy particles ($^4He^{2+}$). Another advantage of boron is that many ^{10}B atoms form a polyhedral cluster, such as $B_{10}H_{10}^{2-}$ and $B_{12}H_{12}^{2-}$, enabling a high concentration of boron in the molecule, so that a satisfactory therapeutic effect can be expected. The high selectivity of neutron capture by the boron atoms and the effectiveness of treatment by it makes an acceptable alternative to other chemotherapy methods that destroy cancer cells by high toxicity.

o-Carborane, namely 1,2-dicarba-*closo*-dodecaborane, $C_2B_{10}H_{12}$, is a boron-rich compound with an icosahedral structure and a diameter similar to that of a rotating benzene ring, nearly 1 nm, with high symmetry and remarkable stability under various conditions [3]. This cluster contains ten boron and two carbon atoms, making it well suited as a BNCT

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agent [4,5], and also has potential in other fields of drug discovery, molecular imaging, and targeted radionuclide therapy [6]. However, despite of these advantages, the high toxicity and low water solubility of boron agents remains a significant problem impairing the further clinical treatment [7].

Recently, as shown in Chart 1, we reported the successful synthesis and structural characterization of 1,2,3-triphenyl-*o*-carboranes and the corresponding chromium complexes (**Ph3C2B**, **Ph3C2BCr2**, and **Ph3C2BCr3**) [8,9]. Among transition-metal π-complexes, η⁶-arene chromium(0) tricarbonyl complexes have been the subject of extensive development due to their significant applications in organic synthesis [10,11]. Moreover, the use of metal carbonyls in bioorganometallic chemistry is being increasingly developed to enhance the potential of chromium complexes [12–15].

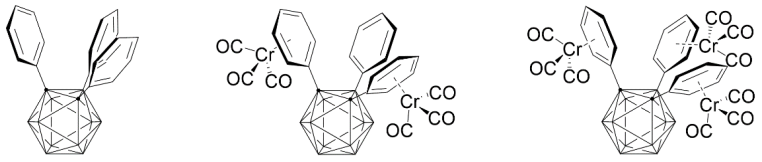


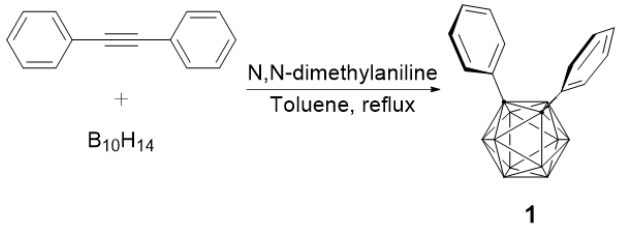
Chart 1. Molecular structure of 1,2,3-triphenyl-*o*-carborane and its chromium(0) tricarbonyl complexes **Ph3C2B**, **Ph3C2BCr2**, and **Ph3C2BCr3**.

To the best of our knowledge, this is the first example of the application of η⁶-arene chromium(0) tricarbonyl-substituted *o*-carborane compounds in BNCT. Many studies have reported the introduction of various aryl groups to carbons and/or borons in *o*-carborane. However, no biological characteristics have yet been reported for them. This study is significant because it demonstrates the potential of phenylated *o*-carboranes with η⁶-chromium(0) tricarbonyl groups as potential BNCT agents.

2. Results and Discussion

2.1. Synthesis of 1,2-Diphenyl-o-carborane and Corresponding Chromium Metal Complexes

We previously reported the detailed synthesis of compounds **Ph3C2B**, **Ph3C2BCr2**, and **Ph3C2BCr3** [8,9]. Compound **1** was synthesized by a modified procedure than previous results [18,19] as shown in Schemes 1. 1,2-Diphenyl-*o*-carborane (**1**) was prepared from decaborane ($B_{10}H_{14}$) and diphenylacetylene in moderate yield in toluene solvent using *N*,*N*-dimethylaniline as a base. As a result, we first synthesized icosahedral *o*-carborane with phenyl substituents on the two carbons (Scheme 1).



Scheme 1. Preparation of 1, 2-diphenyl-*o*-carborane (**1**).

To compare with previous results, we tried to confirm the electron-withdrawing ability of *o*-carborane by reacting chromium(0) hexacarbonyl [$Cr(CO)_6$] with two phenyl groups introduced into *o*-carborane [20]. The reaction of **1** with $Cr(CO)_6$ produced η⁶-phenyl-coordinated mono- and bis-chromium complexes (**2** and **3**) in moderate yields through stoichiometric reactions, as shown in Schemes 2. Interestingly, these results showed that 1,2-diphenyl-*o*-carborane prefers stoichiometric reactions to 1,2,3-triphenyl-*o*-carborane.

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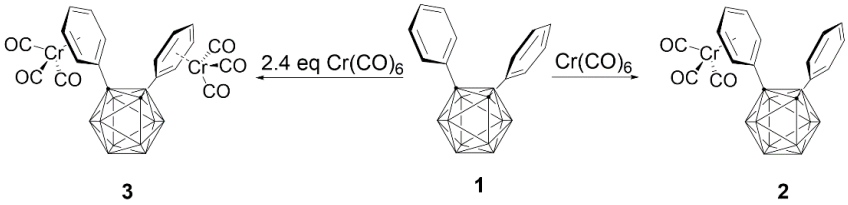

Scheme 2. Preparation of mono- and bis- chromium complexes (**2** and **3**).

2.2. IR and NMR spectroscopy

The structure of 1,2-diphenyl-*o*-carborane (**1**) was proposed based on the assignments the ^{1}H, ^{11}B, and ^{13}C NMR resonances before confirmation by X-ray diffraction (XRD). The ^{1}H NMR spectrum of compound **1** showed resonances at around 7.35–7.31 ppm for the two phenyl rings. The ^{11}B NMR spectrum showed resonances at around δ –11.18 and –2.52. In the ^{11}B NMR, it can be seen that only two peaks appear due to high intramolecular symmetry. This pattern was confirmed to be consistent with the previously result [19]. The ^{13}C NMR spectrum showed resonances at around δ 132.3–126.7. The infrared (IR) spectra of compounds **2** and **3** showed characteristic absorption bands of C≡O units at 1965,1890 (**2**), 1965, and 1892 cm^{-1} (**3**). These values are lower than those of $(C_6H_6)Cr(CO)_3$ [21–25]. The IR and NMR spectra clearly indicate Cr coordination to the phenyl groups of **2** and **3**, with chemical shifts to the downfield region of δ 7.70–7.33 and 141.5–128.7 in the ^{1}H and ^{13}C NMR spectra, respectively.

2.3. X-ray Structural Studies of 1,2-Diphenyl-o-carborane and Corresponding Chromium Complexes

The selected crystallographic data and a summary of the intensity data collection parameters for **1**, **2**, and **3** are presented in Tables 1 and 2, respectively. Detailed information on the structural determinations and structural features of compounds **1**, **2**, and **3** are provided in the Supplementary Materials and Appendix A. Additionally, the molecular structures and structural characteristics of **Ph3C2B**, **Ph3C2BCr2**, and **Ph3C2BCr3** are also included (Figures S1–S3 and Tables S10 and S11). Single-crystal X-ray structure determination revealed the structural authenticity of each compound and suggested alteration of the electronic structure based on the changes in the C–C distance of carborane cage [26]. The C1–C2 bond distance of **1** is 1.726(2) Å, which is significantly longer than the C1–C2 bond distance of the unsubstituted *o*-carborane [1.629(6) and 1.630(6) Å]. Moreover, comparison of the C–C distances in compounds **1** and **Ph3C2B** (Table S11) showed that the bonds in **Ph3C2B** are slightly longer than those in compound **1**. Compound **Ph3C2B** contains further phenyl decorations at the boron atom while maintaining the active compound **1** platform [27,28].

Table 1. Crystal data and structure refinement of **1** – **3**.

	1	**2**	**3**
Identification code	K120504	K130805	K131105
Empirical formula	$C_{14}H_{20}B_{10}$	$C_{17}H_{20}B_{10}Cr_1O_3$	$C_{20}H_{20}B_{10}Cr_2O_6$
Formula weight	296.40	432.43	568.46
Temperature	293(2) K	293(2) K	293(2) K
Wavelength	0.71073 Å	0.71073 Å	0.71073 Å
Crystal system, space group	Monoclinic, $P2_1/n$	Monoclinic, $P2_1/n$	Triclinic, $P\bar{1}$
Unit cell dimensions	a = 10.859(1) Å b = 24.953(3) Å, β = 111.854(2)º c = 13.938(2) Å	a = 10.621(3) Å b = 17.056(5) Å, β = 106.622(5)º c = 12.174(4) Å	a = 17.540(2) Å, α = 105.746(2)º b = 18.060(2) Å, β = 110.226(2)º c = 19.484(3) Å, γ = 91.256(2)º
Volume	3505.3(8)	2113.2(1)	
Z, D_{calc}	8, 1.123	4, 1.359	

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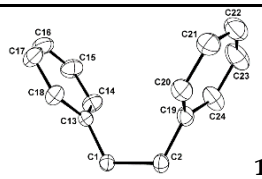
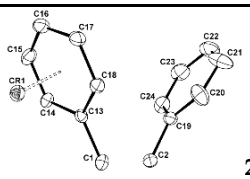
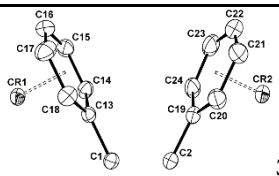
$F(000)$	1232.0	880.0	5528.0(1)
Crystal size	0.15, 0.13, 0.12	0.17, 0.15, 0.13	2, 0.342
θ range for data collection	1.63 to 28.37	2.12 to 28.46	572
Limiting indices	$-14 \le h \le 14$, $-33 \le k \le 32$, $-18 \le l \le 18$	$-14 \le h \le 14$, $-22 \le k \le 22$, $-16 \le l \le 16$	0.20, 0.20, 0.15
Reflections collected/unique	35826/8726 [R(int) = 0.0421]	28490/5305 [R(int) = 0.0238]	1.17 to 28.38
Completeness to θ = 28.38	99.3%	99.2%	$-23 \le h \le 23$, $-24 \le k \le 24$, $-26 \le l \le 25$
Refinement method	Full-matrix least-squares on F^2	Full-matrix least-squares on F^2	44259/18014 [R(int) = 0.0398]
Data/restraints/parameters	8726/0/581	5305/0/360	65.0%
Goodness-of-fit on F^2	1.008	1.053	Full-matrix least-squares on F^2
Final R indices [$I > 2\theta(I)$]	$^aR_1$ = 0.0616, $^bwR_2$ = 0.1545	$^aR_1$ = 0.0332, $^bwR_2$ = 0.0958	18014/0/1409
R indices (all data)	$^aR_1$ = 0.1037, $^bwR_2$ = 0.1897	$^aR_1$ = 0.0388, $^bwR_2$ = 0.1018	0.902
Largest diff. peak and hole	0.220 and –0.355 e.Å^{-3}	0.364 and –0.242 e.Å^{-3}	$^aR_1$ = 0.0563, $^bwR_2$ = 0.1505
			$^aR_1$ = 0.0877, $^bwR_2$ = 0.1612
			0.687 and –0.375 e.Å^{-3}

$^aR_1 = \sum||F_o|-|F_c|| / \sum|F_o|$ (based on reflections with $F_o^2 > 2\sigma F^2$), $^bwR_2 = [\sum[w(F_o^2-F_c^2)^2]/\sum[w(F_o^2)^2]]^{1/2}$; $w = 1/[\sigma^2(F_o^2)+(0.095P)^2]$; , $P = [\max(F_o^2, 0)+2F_c^2]/3$(also with $F_o^2 > 2\sigma F^2$).

Table 2. Comparison of selected bond lengths (Å), angles (º), and torsion angles (º) for **1** – **3**.

	 **1**	 **2**	 **3**
Phc-c (av)	1.376	1.400	1.406
Phc-c (av)−Cr		2.210	2.212
Cabc-c	1.726(2)	1.740(2)	1.724(4)
Cent−Cr		1.702	1.696(av)
Cr–CO		1.856(av)	1.851(av)
C1–C13	1.507(2)	1.499(2)	1.502(4)
C2–C19	1.501(2)	1.500(2)	1.510(4)
C13–C1–C2	118.3(1)	116.6(1)	116.4(2)
C19–C2–C1	119.0(1)	119.6(1)	116.1(2)
C1-C2-C19-C20	84.1(2)	86.1(2)	105.8(3)
C2-C1-C13-C14	81.7(2)	102.3(1)	112.4(3)

Single crystals of **1** and its chromium(0) tricarbonyl complexes, **2** and **3**, suitable for X-ray crystallography, were obtained from dichloromethane solutions by slow evaporation. The general structural characteristics of compound **1** and its chromium complex are shown in Table 2. The molecular structures of compounds **1**, **2**, and **3** are shown in Figs 1, 2, and 3. Compound **1** was already reported in 1993 by Lewis and Welch [29]. Comparing the data, it can be seen that the bond lengths, angles and dihedral angles are nearly identical (Fig. 1).

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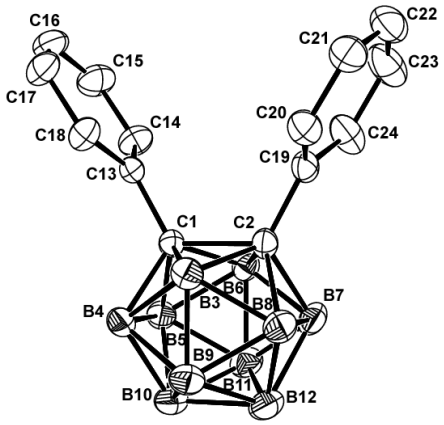

Figure 1. ORTEP drawing (30% probability for thermal ellipsoids) of **1** (the hydrogen atoms are omitted for clarity).

As shown in Fig. 2, the chromium atom of compound **2** is coordinated to the phenyl ring of the carbon atom of *o*-carborane via a π-bond, similar to the previous results [8,9]. The chromium metal adopts the typical three-legged 'piano stool' geometry, with the expected geometrical parameters. The chromium metals are centered approximately over the phenyl rings, giving rise to Cr1–C_6H_5 face (centroid) distances of 1.702 Å. The average C–C bond length in the coordinated phenyl ring is 1.410 Å, which is 0.028 Å longer than the average bond length of 1.382 Å for C–C bonds within the non-coordinated phenyl ring. The Cr–C_{Ph} and Cr–CO bonds had average values of 2.210 and 1.856 Å, respectively, which were within the normal range [30]. The average C–O bond length in the chromium-coordinated carbonyl ligands is 1.143 Å, which is slightly shorter than that reported η^6-arene chromium(0) tricarbonyl complexes [31–35]. As expected, the C1–C2 bond distance is 1.740(2) Å, a slightly increase over compound **1**, and is essentially in the range of non-bonding character [36–39]. Furthermore, as shown in Table 2 and S6, the torsion angles of the two phenyl rings of **1** and **2** changed upon coordination with the chromium atom.

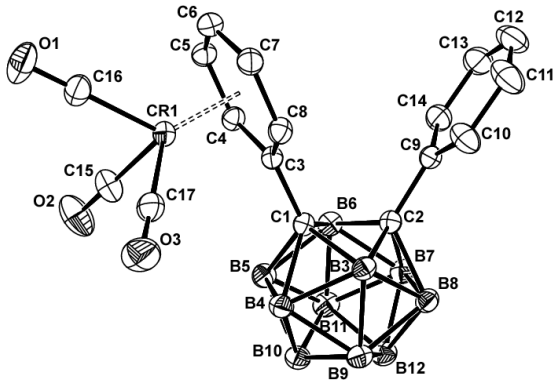

Figure 2. ORTEP drawing (30% probability for thermal ellipsoids) of **2** (the hydrogen atoms are omitted for clarity).

The X-ray crystal structure of **3** (Fig. 3) reveals that the dichromium atoms adopt an η^6-coordination with the two phenyl rings. The single crystal X-ray diffraction study of **3** revealed crystallization in the triclinic space group *P*–1. Interestingly, even though it is a triclinic space group, the APEX3 program identified the Z value as 8, which made it difficult to solve. In the end, this was considered to be recognized by four molecules as one molecule, and the structure was interpreted by adjusting the Z value to 2. The geometrical parameters of complex **3** were within the expected ranges (Table 2). The average C–C bond length in the chromium coordinated phenyl rings is 1.406 Å, which is 0.03 Å longer than the average bond length of 1.376 Å for the C–C bonds in **1**. The Cr–C_{Ph} and Cr–CO bond

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lengths were within the normal range [30], with average values of 2.212 and 1.851 Å, respectively. The average C–O bond length in the chromium-coordinated carbonyl ligands is 1.138 Å, which is significantly shorter than those of reported η⁶-arene chromium(0) tricarbonyl complexes [31–35]. As shown in Table 2, the chromium metals are centered approximately over the phenyl rings, giving rise to Cr1/Cr2–C_6H_5 face (centroid) distances of 1.687 and 1.705 Å, respectively. Interestingly, the C1–C2 bond length of **3** is shorter than that of **1** and **2**, it was shown that contraction of this bond is due to favorable phenyl ring π* and carboranyl σ* orbital interactions do not occur in **3**, as previous results [8,9]. As shown in Table 2 and S9, the torsion angles of the phenyl rings of **1** and **3** or **2** and **3** changed upon coordination with the chromium atoms.

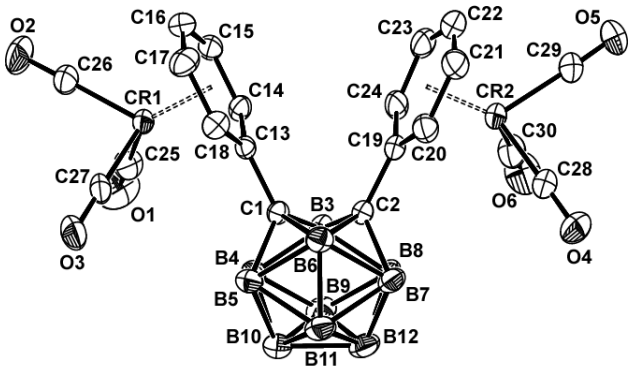

Figure 3. ORTEP drawing (30% probability for thermal ellipsoids) of **3** (the hydrogen atoms are omitted for clarity).

2.4. Determination of IC_{50} and Incorporation of Boron into B16 and CT26 Cells

B16 mouse melanoma and CT26 colon carcinoma cells were treated with compounds **2**, **3**, **Ph3C2BCr2**, and **Ph3C2BCr3** for 3 d, after which the cell viability was determined using the MTT [30-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay. Compounds **2**, **3**, **Ph3C2BCr2**, and **Ph3C2BCr3** showed higher cytotoxicity than BPA (Table 3), with IC_{50} (the half maximal inhibitory concentration) values in the range of 0.091–0.736 μM. Interestingly, the 1,2,3-triphenyl-*o*-carboranyl chromium tricarbonyl complexes (**Ph3C2BCr2** and **Ph3C2BCr3**) showed higher cytotoxicity to the B16 and CT26 cells than complexes **2** and **3**. The higher cytotoxicity of compounds **Ph3C2BCr2** and **Ph3C2BCr3** to B16 cells may be a result of the difference in the mechanism by which the phenyl groups and chromium(0) tricarbonyl moieties induce cell toxicity. Compounds **2**, **3**, **Ph3C2BCr2**, and **Ph3C2BCr3** exhibited similar activities in the CT26 and B16 cells, with IC_{50} values in the range of 0.089–0.833 μM.

We next examined the level of intracellular accumulation of compounds **2**, **3**, **Ph3C2BCr2**, and **Ph3C2BCr3** by determining their boron concentrations using ICP-OES. The intracellular boron uptake of compounds **2**, **3**, **Ph3C2BCr2**, and **Ph3C2BCr3** in B16 and CT26 cells was higher than that of BPA (Table 3). Boron uptake from both bis- and tris-chromium tricarbonyl-substituted compounds, which included the 1,2,3-triphenyl-o-carboranyl chromium tricarbonyl complexes (i.e., **Ph3C2BCr2** and **Ph3C2BCr3**), was lower. These results show that the introduction of phenyl groups or chromium metals into the carborane backbone increases cytotoxicity rather than an increase in boron accumulation in cancer cells.

Table 3. Cytotoxicity (IC_{50}) and Boron accumulation of B16 melanoma and CT26 colon carcinoma cells.

Compds	B16		CT26	
	Cytotoxicity IC_{50} (M)[a]	**Boron Accumulation (ppm)[b]**	**Cytotoxicity IC_{50} (M)[a]**	**Boron Accumulation (ppm)[b]**

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2	0.736×10^{-6} (± 0.01)	0.825 ± 0.003	0.833×10^{-6} (± 0.03)	0.755 ± 0.009
3	0.681×10^{-6} (± 0.04)	0.620 ± 0.002	0.314×10^{-6} (± 0.07)	0.694 ± 0.002
5	0.411×10^{-6} (± 0.06)	0.384 ± 0.006	0.164×10^{-6} (± 0.05)	0.402 ± 0.002
6	0.091×10^{-6} (± 0.03)	0.221 ± 0.001	0.089×10^{-6} (± 0.08)	0.247 ± 0.001
BPA	4.871×10^{-5} (± 0.03)	0.103 ± 0.002	3.862×10^{-3} (± 0.04)	0.514 ± 0.001

[a]B16 melanoma and CT26 colon carcinoma cancer cells (5×10^3 cells) were incubated for 72 h in the presence of compounds **2**, **3**, **4**, and **6**, and then the percentages of viable cells were determined by MTT assay. The drug concentrations required to inhibit cell viability by 50% (IC_{50}) were determined from semi-logarithmic concentration-response plots, and the results represent the means ± s.d. of triplicate samples. [b]B16 and CT26 cells (5×10^5 cells) were incubated for 3 h in the presence of compounds **2**, **3**, **4**, and **6** or BPA (10.8 ppm). After three times washes, the accumulated boron concentrations were determined by inductively coupled plasma optical emission spectroscopy (ICP-OES). The values are the means ± s.d. from three samples.

3. Materials and Methods

3.1. General Procedure

All manipulations were performed under dry nitrogen or argon atmosphere using standard Schlenk techniques. Tetrahydrofuran (THF) was distilled from sodium and benzophenone under nitrogen atmosphere. Elemental analyses were performed using a Carlo Erba Instruments CHNS-O EA 1108 analyzer. High-resolution tandem mass spectrometry (JMS-HX 110/110A, Jeol Ltd.) data were acquired at the Korean Basic Science Institute. ^{1}H, ^{11}B, and ^{13}C NMR spectra were recorded using a Bruker 600 spectrometer operating at 600.1, 150.9, and 192.6 MHz, respectively. All ^{11}B chemical shifts were referenced to $BF_3{\cdot}O(C_2H_5)_2$ (0.0 ppm), with a negative sign indicating an upfield shift. All proton and carbon chemical shifts were measured relative to the internal residual $CHCl_3$ in the lock solvent (99.9% $CDCl_3$). Decaborane was purchased from Katchem. *N*,*N*-dimethylaniline, 1,2-diphenylacetylene, n-BuLi (2.5 M in hexane), and chromium hexacarbonyl [$Cr(CO)_6$] were purchased from Aldrich Chemicals.

3.2. Crystal Structure Determination

Crystals of **1**, **2**, and **3** were obtained from toluene or CH_2Cl_2, sealed in glass capillaries under argon atmosphere, and mounted on a diffractometer. Preliminary examination and data collection were performed using a Bruker SMART CCD detector system single crystal X-ray diffractometer equipped with a sealed-tube X-ray source (40 kV × 50 mA), using graphite-monochromated Mo-Kα radiation (λ = 0.71073 Å). The preliminary unit cell constants were determined using a set of forty-five narrow-frame (0.3° in ω) scans. Double-pass scanning was used to exclude noise. The collected frames were integrated using an orientation matrix determined from the narrow-frame scans. The SMART software package was used for data collection and SAINT was used for frame integration [16]. The final cell constants were determined by global refinement of the *xyz* centroids of the reflections harvested from the entire dataset. Structure solution and refinement were performed using the SHELXTL-PLUS software package [17].

3.3. Cell Viability Assay (MTT Assay)

The boron compounds were dissolved in dimethylsulfoxide (DMSO), and the resulting solution was diluted with Dulbecco's modified Eagle's medium (DMEM) (10% FCS), or *p*-boronophenylalanine (BPA) was directly dissolved in the same medium. In a 96-well culture plate (Falcon 3072), B16 mouse melanoma and CT26 colon carcinoma cells (5×10^3 cells/well) were cultured in five wells with medium containing the boron compounds at various concentrations, followed by incubation at for 72 h at 37 °C in a CO_2 incubator. DMSO is non-toxic at concentrations less than 0.5%, and control experiments confirmed the non-toxicity of DMSO at the concentrations used in the present experiments. After incubation, the medium was removed, the cells were washed three times with phosphate-buffered saline [PBS (–)], and the CellTiter 96® Aqueous Non-Radioactive Cell

 doi:10.20944/preprints202306.0167.v1

Proliferation Assay (MTT) was used to count the cells on a microplate reader. The concentration that resulted in a cell culture with 50% of the number of cells in the corresponding untreated group (IC_{50}) is summarized in Table 3.

3.4. In Vitro Boron Incorporation into B16 and CT26 Cancer Cells

B16 and CT26 cancer cells were cultured in Falcon 3025 dishes (150 mm diameter). When the cell population increased to fill the dish (5×10^5 cells/dish), the boron compounds and BPA (10 μM) were added to the dishes. The cells were then incubated for 3 h at 37 °C in DMEM (20 mL of 10% FBS). The cells were washed three times with Ca/Mg-free PBS (–), collected with a rubber policeman, digested with a mixture of 60% $HClO_4$–30% H_2O_2 (1:2) solution (2 mL), and finally decomposed for 1 h at 75 °C. After filtration through a membrane filter (Millipore, 0.22 mm), the boron concentration was determined using an inductively coupled plasma optical emission spectroscopy (ICP-OES) instrument [Avio 220 Max, PerkinElmer, Massachusetts, U.S.A)]. Each experiment was performed in triplicate.

3.5. Synthesis of 1,2-Diphenyl-o-carborane (1)

1,2-Diphenylethyne (2.2 g, 12.0 mmol) and $B_{10}H_{14}$ (decaborane) (1.22 g, 10.0 mmol) were dissolved in dry toluene (100 mL) at room temperature under argon atmosphere. *N,N*-dimethylaniline (2.78 mL, 24.0 mmol) was added to the reaction mixture, and the mixture was stirred at 110 °C for 24 h. After cooling, the solid residue was filtered off and the solvent was evaporated to dryness. The crude mixture was purified using silica gel column chromatography (hexane as the eluent). Recrystallization from CH_2Cl_2 provided **1** as colorless crystals (2.31 g, 78%). HRMS: Calcd. for $[^{12}C_{14}{}^{1}H_{20}{}^{11}B_{10}]^+$ 296.2568. Found: 296.2571. IR spectrum (KBr pellet, cm^{-1}): ν(C_{Ar}–H) 3024, ν(B–H) 2589, ν(C=C_{Ar}) 1601, 1500. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.35 (m, 5H, Ph-*H*), 7.31 (m, 5H, Ph-*H*). ^{11}B NMR ($CDCl_3$, 192.6 MHz) δ –2.52, –11.18. ^{13}C NMR ($CDCl_3$, 150.9 MHz) δ 132.3, 130.1, 129.8, 129.2, 126.7 (*Ph*), 84.0 (C_{cab}).

3.6. Synthesis of 1-(Phenyl-η^6-chromium(0) tricarbonyl)-2-phenyl-o-carborane (2)

Compound **1** (0.3 g, 1.0 mmol) and 1.2 equiv of $[Cr(CO)_6]$ (0.26 g, 1.2 mmol) were dissolved in a mixture of THF (5 mL) and di-n-butyl ether (50 mL). The mixture was refluxed for 72 h. The resulting dark reddish solution was cooled to room temperature and filtered through Celite. The solvent was evaporated under reduced pressure. After evaporation, the crude reaction mixture was purified by column chromatography [CH_2Cl_2:hexane (1:1) as the eluent] to give the chromium complex **2**, which was then recrystallized from toluene to obtain red crystals. Yield: 88% (0.38 g, 0.88 mmol). HRMS: Calcd. for $[^{12}C_{17}{}^{1}H_{20}{}^{11}B_{10}{}^{52}Cr_{1}{}^{16}O_3]^+$ 432.1821. Found: 432.1824. IR spectrum (KBr pellet, cm^{-1}): ν(C_{Ar}–H) 3023, 3020, ν(B–H) 2588, ν(C≡O) 1965,1890. ^{1}H NMR ($CDCl_3$, 600.1 MHz) δ 7.62 (m, 5H, Ph-*H*), 7.33 (m, 5H, Ph-*H*). ^{11}B NMR ($CDCl_3$, 192.6 MHz) δ –2.28, –4.11, –9.51, –11.45. ^{13}C NMR ($CDCl_3$, 150.9 MHz) δ 231.4 (Cr–*C*O), 141.5, 137.9, 134.8, 132.7,131.3,130.8,128.7 (Ph), 85.6 (Ph–*C*cab).

3.7. Synthesis of 1,2-bis(phenyl-η^6-chromium(0) tricarbonyl)-o-carborane (3)

A procedure analogous to the preparation of **2** was used to obtain red crystals. Yield: 57% (0.32 g, 0.57 mmol). HRMS: Calcd. for $[^{12}C_{20}{}^{1}H_{20}{}^{11}B_{10}{}^{52}Cr_{2}{}^{16}O_6]^+$ 568.1073. Found: 568.1077. IR spectrum (KBr pellet, cm^{-1}): ν(C_{Ar}–H) 3021, ν(B–H) 2583, 2589; ν(C≡O) 1965, 1892. ^{1}H NMR ($CDCl_3$, 600.1 MHz) δ 7.70 (m, 4H, Ph-*H*), 7.48 (m, 3H, Ph-*H*), 7.39 (m, 3H, Ph-*H*). ^{11}B NMR ($CDCl_3$, 192.6 MHz) δ –2.65, –11.48. ^{13}C NMR ($CDCl_3$, 150.9 MHz) δ 233.1 (Cr-*C*O), 141.5, 140.3, 138.5, 135.1, 133.8, 131.1 (*Ph*), 86.7 (Ph-Ccab).

4. Conclusions

 doi:10.20944/preprints202306.0167.v1

In conclusion, we described the synthesis, X-ray structures, and biological activities of a series of 1,2-diphenyl- and 1,2,3-triphenyl-*o*-carborane and the corresponding chromium(0) tricarbonyl transition metal complexes, which can be easily stoichiometrically substituted with transition metals to produce highly active biological molecules for BNCT. We presented a general and versatile method for the sequential introduction of phenyl groups into *o*-carborane and stoichiometrically-coordinated transition metal complexes using chromium(0) hexacarbonyl. Diphenyl- or triphenyl-*o*-carborane and its chromium complexes show higher cytotoxicity than *p*-boronophenylalanine in B16 and CT26 cancer cell lines, however, the boron accumulation is higher than that of *p*-boronophenylalanine.

Supplementary Materials: The following supporting information can be downloaded at: www.mdpi.com/xxx/s1, Figure S1–S3: Molecular structures of **Ph3C2B**, **Ph3C2BCr2**, and **Ph3C2BCr3**; Table S1–S9: Bond lengths (Å), angles (º), and torsion angles (º) of compounds **1**, **2**, and **3**; Table S10 and S11: Crystal data and structure refinement and comparison of selected bond lengths (Å), angles (º), and torsion angles (º) for **1**–**3** and **Ph3C2B**, **Ph3C2BCr2**, and **Ph3C2BCr3**.

Author Contributions: Conceptualization, J.D.L.; Chemical experiments, T.J.H., D.K.I. and S.M.K.; Designed and performed biological tests, D.K.I. and J.D.L..; validation, J.D.L.; formal analysis, T.J.H., D.K.I., and S.M.K.; investigation, T.J.H., D.K.I. and S.M.K.; data curation, T.J.H. and J.D.L.; writing—original draft preparation, T.J.H. and S.M.K.; writing—review and editing, J.D.L.; supervision, J.D.L.; project administration, J.D.L.; funding acquisition, J.D.L.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Supplementary Materials are available online. Figures S1–S6: X-ray structures of compounds **1**, **2**, **3**, **Ph3C2B**, **Ph3C2BCr2** and **Ph3C2BCr3**, Tables S1–S6. Detailed information on the structural determinations and structural features of compounds **1**, **2**, **3**, **Ph3C2B**, **Ph3C2BCr2** and **Ph3C2BCr3** are provided in the Supplementary Materials.

Acknowledgments: This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (2022R1F1A1074095). We thank Prof. Kun-Ho Lee (Department of Biomedical Science, Chosun University) for assistance with the biological evaluation and discussion.

Conflicts of Interest: The authors declare no conflict of interest.

Appendix A

CCDC 2262119, 2262120, and 2262121 contain supplementary crystallographic data for compounds **1**, **2**, and **3**, respectively. These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB2 1EZ, UK; Fax: +44 1223 336033; or deposit@ccdc.cam.ac.uk). Additional Supporting Information can be found online in the Supporting Information tab.

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