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o-Carboranyl derivatives of 1,3,5-*s*-triazines: structures, properties and *in vitro* activities†

Chai-Ho Lee[1], Hong-Gyu Lim[2], Jong-Dae Lee[2], Young-Joo Lee[2], Jaejung Ko[2]***, Hiroyuki Nakamura[3] and Sang Ook Kang[2]***

[1]Department of Chemistry, Wonkwang University, Iksan, Jeonbuk 570-749, South Korea
[2]Department of Chemistry, Korea University, Chochiwon, Chung-nam 339-700, South Korea
[3]Department of Chemistry, Graduate School of Science, Tohoku University, Sendai 980-8578, Japan

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Our objective was to design and synthesize substituted *o*-carboranes carrying the 1,3,5-*s*-triazine units as potential boron neutron capture therapy agents. The preliminary results indicated that selective substitution was successful, and the structural AB_2 (mono-*o*-carboranyl-1,3,5-*s*-triazine) or A_2B (bis-*o*-carboranyl-1,3,5-*s*-triazine) type was used as the starting point for the generation of biologically active species. In the first series, the influence of the structural changes in the central core unit was investigated. Thus, a procedure for the sequential, selective derivatization of cyanuric chloride that allows for the incorporation of *o*-carborane was elucidated. As a result, a variety of mono-, di-, and tri-substituted triazines were produced in good yield. In the second series, the effect of additional amine substituents on the 1,3,5-*s*-triazine was studied. Copyright © 2003 John Wiley & Sons, Ltd.

KEYWORDS: *o*-carboranes; BNCT agents; 1,3,5-*s*-triazines; crystal structure

INTRODUCTION

Triazines are a class of nitrogen-containing cyclic compounds having remarkable thermal and chemical stabilities.[1] Their unusual properties make them uniquely suitable for several specialized applications in the field of materials science and biomedical science. The delocalization of electrons in the ring has been utilized in the preparation of special polymers,[2–6] herbicides,[7,8] and antiviral and anticancer agents.[9] In particular, numerous 1,3,5-*s*-triazine derivatives possess various biological activities, but many of their derivatives still have unexplored pharmacological properties. In this context, and in connection with a research program on 1,3,5-*s*-triazine chemistry and the biological activities undertaken in our laboratories, we have considered that novel, suitably substituted, *o*-carboranyl-1,3,5-*s*-triazines may act as potential boron neutron capture therapy (BNCT) agents.[5]

Our approach was, sequentially and selectively, to displace chlorine atoms from cyanuric chloride with molecules capable of targeting for BNCT, and then, further substituting with various biological functions such as *N*,*N*′-dimethylamine or *N*,*N*′-bis(2-chloroethyl)amine. By simply controlling the stoichiometry, sequential, selective derivatization could be accomplished using lithio-*o*-carboranes as nucleophiles. We now report the synthesis of A_3-, A_2B-, and AB_2-type 1,3,5-*s*-triazine molecules that have an *o*-carborane substituent as A and an amine substituent as B, as shown in Scheme 1. Therefore, the first synthetic methodology for the synthesis of the *o*-carborane-substituted 1,3,5-*s*-triazines was developed and the unique carborane products analyzed by NMR spectroscopy and X-ray crystallography where appropriate.

*Correspondence to: Sang Ook Kang, Department of Chemistry, Korea University, Chochiwon, Chung-nam 339-700, South Korea.
E-mail: sangok@korea.ac.kr
**Correspondence to: Chai-Ho Lee, Department of Chemistry, Wonkwang University, Iksan, Jeonbuk 570-749, South Korea.
***Correspondence to: Jaejung Ko, Department of Chemistry, Korea University, Chochiwon, Chung-nam 339-700, South Korea.
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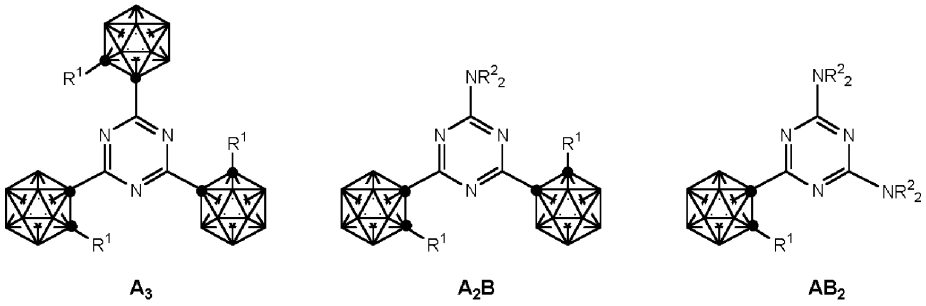
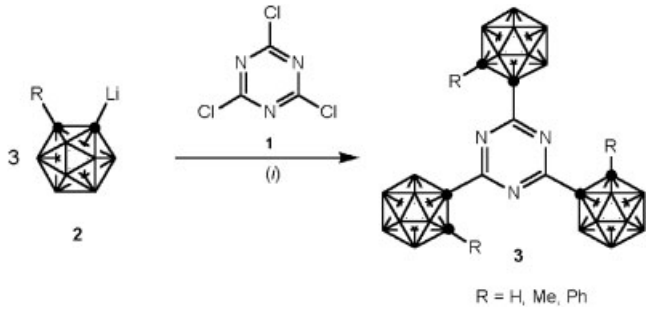
RESULTS AND DISCUSSION

Synthesis of A_3-type 1,3,5-*s*-triazine

As part of our continuing program towards extending the versatility of our lithium *o*-carboranyl addition methodology,[12] the trisubstituted A_3-type 1,3,5-*s*-triazine (**3**) has been prepared by the direct reaction of **2** with **1** in a 3 : 1 stoichiometry.



Scheme 1. R^1 = H, Me, Ph; R^2 = Me, CH_2CH_2Cl.



Scheme 2. Synthesis of **3**. Legend: (*i*) toluene, 70 °C.

As outlined in Scheme 2, cyanuric chloride was reacted with three equivalent amounts of lithio-*o*-carborane (**2**) in toluene for 6 h at 70 °C to yield 2,4,6-tris(*o*-carboranyl)-1,3,5-*s*-triazine (**3**), which was purified by recrystallization from toluene.

Under these conditions, three *o*-carboranyl units lead to complete displacement of the chlorides to yield the trisubstituted *o*-carboranyl-*s*-triazine product in a symmetrical A_3 array. In this case, no color change was observed and the disappearance of the starting material was observed by thin-layer chromatography after 6 h of vigorous stirring at 70 °C. Compounds **3** are moderately stable in air and purified by low-temperature recrystallization in toluene. Since a simple step in this synthesis is high yielding (77–81%), multigram quantities of the A_3 framework complex can be produced with little synthetic effort. All the complexes have been characterized by spectroscopy and by elemental analysis. They have symmetrical C_3 structures, as shown by the presence of a single resonance in the ^1H and ^{13}C spectra of the R-*o*-carboranyl groups, as well as by the ring carbon atoms. In particular, the resonance at *ca* δ 133–168 due to C-(*o*-carborane) in the ^{13}C spectra confirms the presence of the triazine ring.

The X-ray crystallographic analysis of the new A_3 complex of **3c** revealed the expected structure shown in Fig. 1. The asymmetric unit contained two independent, chemically identical molecules of the *o*-carboranyl-substituted 1,3,5-*s*-triazine. Two independent asymmetric moieties of **3c** are shown in Fig. 2. The molecule possesses a three fold proper rotation axis. Each molecule consists of a six-membered triazine ring, which is capped on each carbon

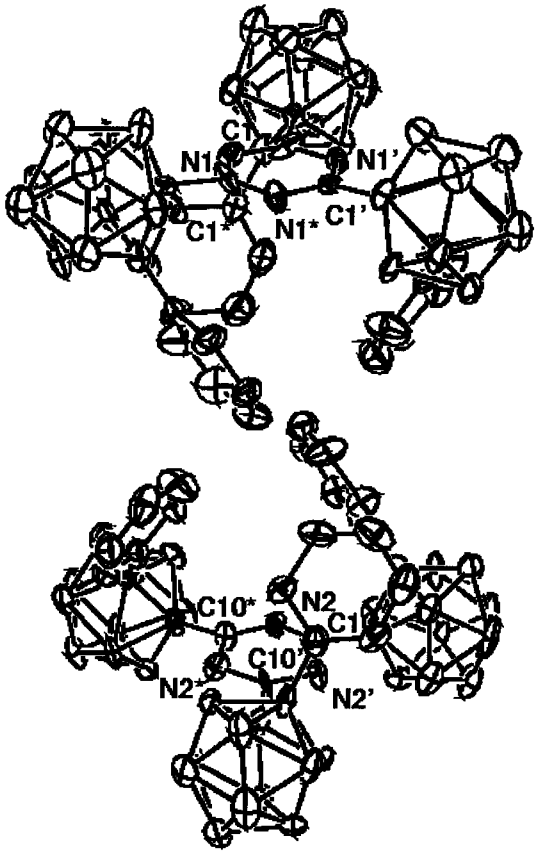


Figure 1. Molecular structure of 2,4,6-(2-phenyl-*o*-carboranyl)-1,3,5-triazine (**3c**). The thermal ellipsoids are drawn at the 30% probability level.

atom with a phenyl-*o*-carboranyl unit. The compound also features three alternating *o*-carboranyl phenyl rings, all of which are located over the triazine ring. The C_3N_3 ring C–N distances (Table 1) are in the double-bond range (average 1.33 Å). This value is similar to the mean bond distances reported in other triazine derivatives, e.g. the averages of 1.34 Å for $(R_3SnS)_3C_3N_3$,[13] 1.36 Å for $Hg_2(CF_3CO_2)_4(TPT)$,[14] 1.35 Å for $[Ni(H_2O)_3(TPT{\cdot}HBr)]^{2+}$,[15] 1.33 Å for $[\mu\text{-}C_3N_3(OMe)(py)_2(pyH)[Ru(CO)_2Cl_2]_2$,[16] and 1.32 Å for $[\mu\text{-}C_3N_3(OH)(py)_2(pyH)][Os(bpy)_2]_2^{3+}$.[17]

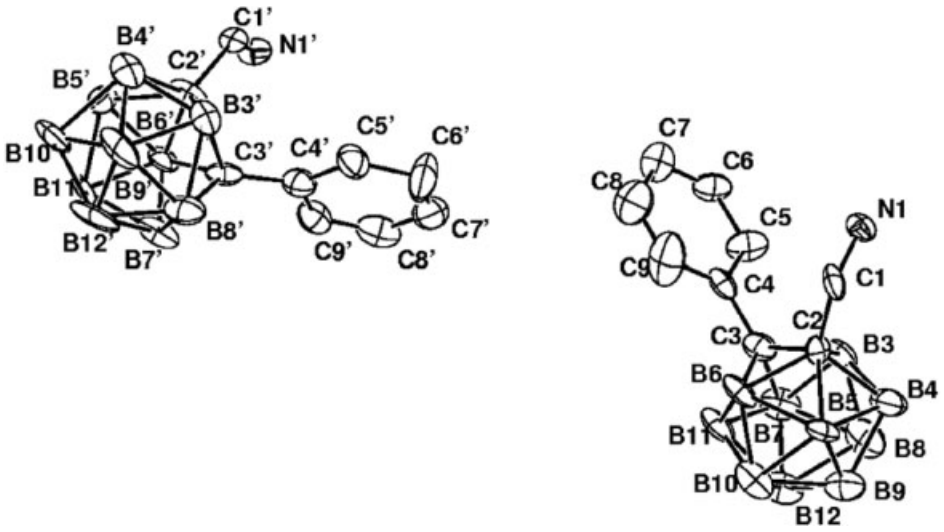


Figure 2. Two independent asymmetric moieties of **3c**. The thermal ellipsoids are drawn at the 30% probability level.

Table 1. Selected interatomic distances (Å) and angles (deg) in **3c**, **5b** and **6a**

3c		5b		6a	
N(1)–C(1)#1	1.31(2)	N(4)–C(6)	1.333(5)	N(1)–C(3)	1.33(2)
N(1)–C(1)	1.35(2)	C(1)–C(4)	1.513(5)	C(1)–C(3)	1.54(2)
N(2)–C(10)	1.32(2)			N(1)–C(5)	1.36(2)
N(2)–C(10)#1	1.36(2)			N(5)–C(5)	1.31(2)
C(1)–N(1)#2	1.31(2)			N(5)–C(8)	1.41(2)
C(1)–C(2)	1.50(2)			N(5)–C(9)	1.53(2)
C(10)–N(2)#2	1.36(2)				
C(10)–C(11)	1.50(2)				
C(11)–C(12)	1.71(2)				
C(1)#1–N(1)–C(1)	114.0(15)	C(6)–N(4)–C(7)	122.2(4)	C(3)–N(1)–C(5)	111.5(14)
C(10)–N(2)–C(10)#1	114.5(14)	C(6)–N(4)–C(8)	120.5(4)	C(3)–N(2)–C(4)	111.6(16)
N(1)#2–C(1)–N(1)	125.9(15)	N(2)–C(4)–C(1)	117.0(3)	C(6)–N(4)–C(7)	123.3(14)
N(1)#2–C(1)–C(2)	115.4(15)	N(3)–C(4)–C(1)	115.4(3)	C(8)–N(5)–C(9)	118.2(15)
N(1)–C(1)–C(2)	118.7(14)	N(4)–C(6)–N(2)	118.1(4)	C(4)–N(4)–C(7)	116.0(16)
C(1)–C(2)–C(3)	118.4(12)	N(4)–C(6)–N(1)	118.3(4)	N(1)–C(3)–C(1)	115.5(12)
N(2)–C(10)–N(2)#2	125.4(13)			N(2)–C(3)–C(1)	113.0(13)
N(2)–C(10)–C(11)	117.5(12)			C(5)–N(5)–C(8)	122.8(18)
N(2)#2–C(10)–C(11)	116.5(10)				

Synthesis of AB_2-type 1,3,5-*s*-triazines

Having shown that the trisubstituted A_3 array could be constructed using the procedure as depicted in Scheme 2, the incorporation of more biologically relevant units was investigated. The readily available lithio-*o*-carboranes[18] provided a way to incorporate *o*-carboranes in the first step of the substitution scheme. Thus, the stoichiometric reaction of lithio-*o*-carborane with cyanuric chloride replaces only one of the chlorine atoms and the remaining chlorines can be subjected to further nucleophilic substitution.

N,*N*′-Dimethylamine units were incorporated into the triazine derivatives because they are known reactive fragments of hexamethylmelamine (HMM).[19] HMM, a 1,3,5-*s*-triazine derivative, has previously been recognized as a clinically effective antitumor agent.[20] Furthermore, a 2,4-diamino-1,3,5-*s*-triazine derivative has received much attention because of its important biological activities.[21] This property makes the AB_2-type molecules promising candidates for delivering boron atoms for the treatment of brain tumors. Our synthetic strategy was to use 2,4-diamino-1,3,5-*s*-triazine as a carrier for ^{10}B, the target molecules being

the AB_2-type array in which the boron functionality was present as a carborane.

Thus, our first objective was the construction of the substituted amines **4** that were needed for attachment of the *N*,*N*′-dimethylamines through an *N*-amine linkage to produce the HMM form. The chlorine groups were sequentially displaced by the *N*,*N*′-dimethylamines to afford the substituted bis-diamines **6** in 60–64% yield. Methyl protons of the *N*,*N*′-dimethylamine moieties in compound **6** gave resonance peaks at around δ 3.2. The ^{1}H NMR data for **6** conform to the structure determined by the X-ray structural study.

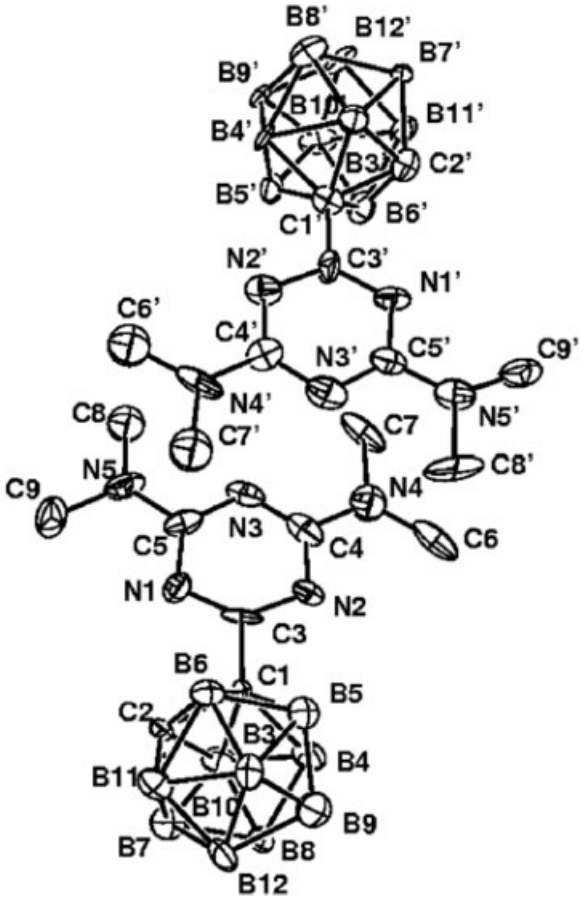
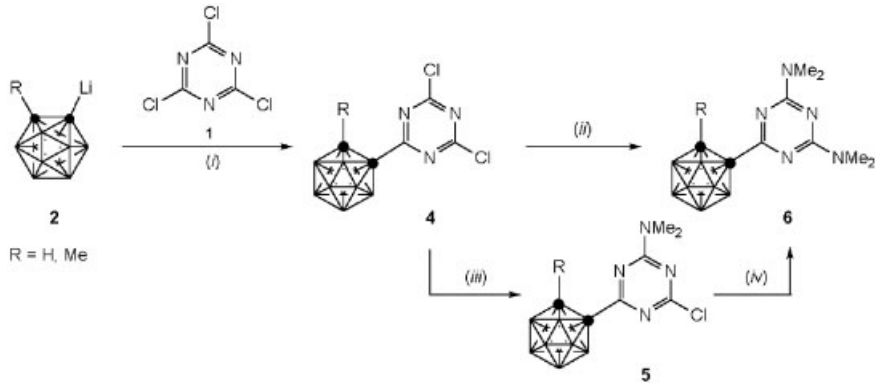


Figure 3. Molecular structure of 4,6-(dimethylamino)-2-(*o*-carboranyl)-1,3,5-triazine (**6a**). The thermal ellipsoids are drawn at the 30% probability level.

The ORTEP diagram in Fig. 3 shows the molecular structure of **6a** and confirms the mono-substituted *o*-carboranyl-*s*-triazine. The asymmetric unit contained two independent, chemically identical molecules of the *o*-carboranyl-substituted 1,3,5-*s*-triazine. The dimethyl amine nitrogen atoms, N(4) and N(5)/N(4′) and N(5′), are coplanar, with deviations of 0.058(24), −0.096(24) Å/0.024(22), −0.036(23) Å, with the triazine ring atoms C(4), N(3), C(5), N(1), C(3), N(2)/C(4′), N(3′), C(5′), N(1′), C(3′), N(2′).

Alternatively, compounds **4** were treated with one equivalent amount of *N*,*N*′-dimethylamine to generate the mono-aminated intermediates **5** in 82–85% yields. The ^{1}H NMR spectra of compounds **5** in Scheme 3 showed resonances of the methyl protons of the *N*,*N*′-dimethylamine unit at around δ 3.3–3.4. In some cases, it was preferable to treat intermediate **5** with additional *N*,*N*′-dimethylamine to provide compounds **6** (the overall yields range from 60 to 64%). Compounds **5** have been isolated as white, transparent crystals. The structural identity of **5** was confirmed by a single crystal X-ray structural study of **5b**, as shown in Fig. 4.

The design of our second framework is a nitrogen mustard that is used extensively in cancer chemotherapy.[22] While nitrogen mustards have chloroalkyl units directly linked to the amine backbone, we were also interested in attaching the triazine units via an additional ethylene spacer.[23,24] The synthesis of the homologous 2-(*o*-carboranyl)-4,6-bis(chloroethyl)amino-1,3,5-*s*-triazine (**7**) started with *N*,*N*′-bis(2-chloroethyl)amine, as shown in Scheme 4. Thus, compound **4** was aminated with chloroethylamine in the presence of potassium carbonate in THF to provide the desired AB_2-type molecule in 44–45% yield.



Scheme 3. Synthesis of **4**, **5**, and **6**. Legend: (*i*) tetrahydrofuran (THF), −78 °C; (*ii*) $2HN(CH_3)_2/K_2CO_3$, THF, 0 to 25 °C; (*iii*) $HN(CH_3)_2/K_2CO_3$, THF, 0 to 25 °C; (*iv*) $HN(CH_3)_2/K_2CO_3$, THF, 0 to 25 °C.

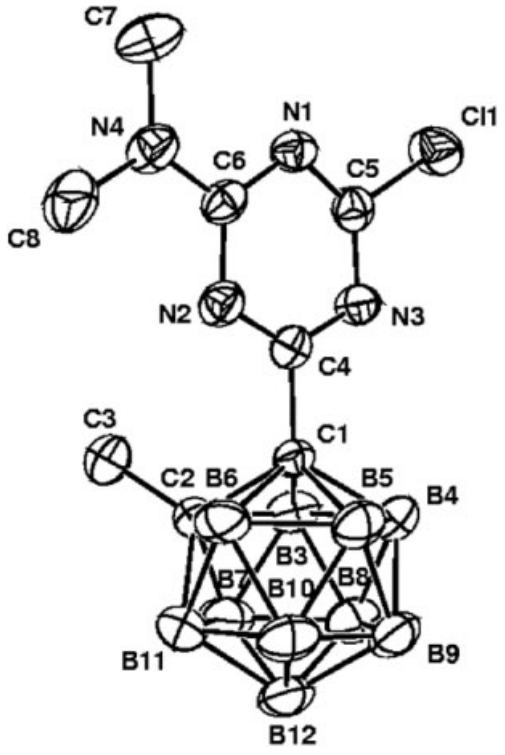


Figure 4. Molecular structure of 6-(chloro)-4-(dimethylamino)-2-(2-methyl-*o*-carboranyl)-1,3,5-triazine (**5b**). The thermal ellipsoids are drawn at the 30% probability level.

The structural characterization was carried out using ^{1}H and ^{13}C NMR and IR spectroscopies. For compound **7**, the peaks at δ 3.5–3.8 and 4.0 are due to the methylene protons of the chloroethyl units, which are at the end of the branch.

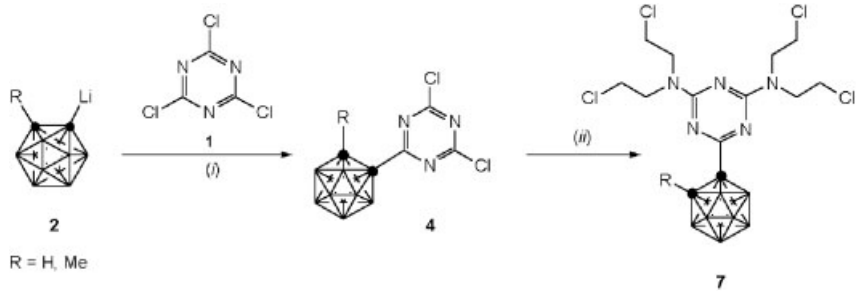
Synthesis of A_2B-type 1,3,5-*s*-triazines

In the final array to be discussed, we wanted to see if it was possible to incorporate two *o*-carboranyl units as well as biologically active amines in the same array. This could be useful for a number of reasons. The presence of high contents of boron atoms could serve as a more efficacious recognition factor in BNCT. In this array, the two *o*-carboranes performed the first substitution. The amines were brought in during the second substitution. The synthetic pathway began with the selective nucleophilic substitution of two chlorine atoms of cyanuric chloride with two equivalent amounts of **2** to yield the 2,4-bis(*o*-carboranyl)-6-chloro-1,3,5-*s*-triazines (**8**), as shown in Scheme 5. The remaining one chlorine atom was replaced with an amine unit to yield either the 2,4-bis(*o*-carboranyl)-6-*N*,*N*′-dimethylamino-1,3,5-*s*-triazines (**9**) or the 2,4-bis(*o*-carboranyl)-6-*N*,*N*′-bis(chloroethyl)amino-1,3,5-*s*-triazines (**10**).

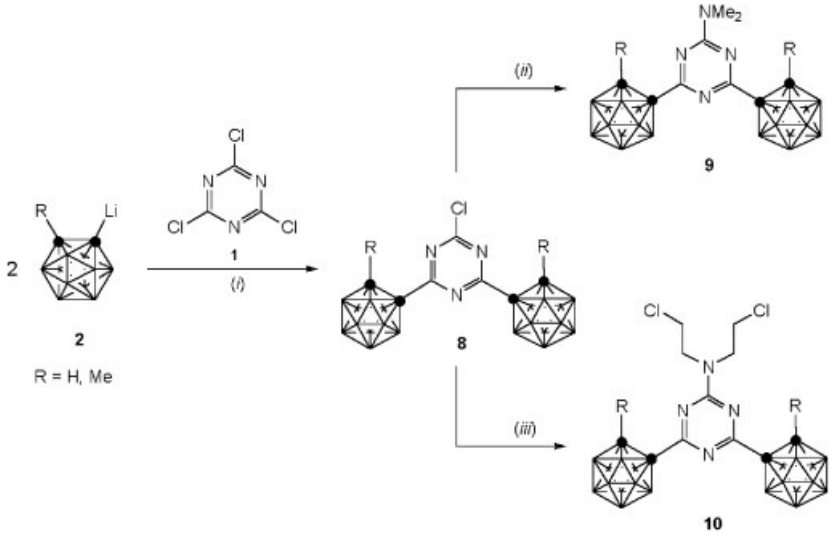
Thus, with two equivalent amounts of **2**, a clean disubstitution of **1** occurred to afford **8** in 65–69% yield after recrystallization, as shown in Scheme 5. The subsequent treatment of **8** with the appropriate amines resulted in mono-amination to afford exclusively the corresponding amines **9** and **10**, based on the ^{1}H NMR spectroscopic analysis of the crude reaction mixture. All complexes have been characterized by spectroscopy and by elemental analysis. Therefore, as the ^{1}H NMR spectra show, the formation of the A_2B-type array reduced the integration of the *N*,*N*′-dialkylamino proton peaks at δ 3.23–3.26 for **9** and δ 2.82–3.49 and 3.69–4.04 for **10** compared with the carboranyl alkyl peaks at around δ 4.4 (R = H) and 2.0 (R = Me). The compounds are both air- and moisture-stable in the solid state. Furthermore, it was proved by an *in vitro* study that compound **9b** highly accumulates into B-16 melanoma cells, although it has a low cytotoxicity (Table 2). The construction of alternative, more polar substituents on the triazines is now under active investigation.

CONCLUSIONS

In summary, the sequential replacement of three chlorine atoms on cyanuric chloride with *o*-carborane nucleophiles provides an efficient route for the systematic synthesis of a variety of A_3-, A_2B-, and AB_2- type 1,3,5-*s*-triazine molecules, which can easily be further one-pot substituted to produce highly active biological molecules for BNCT. Thus, we have developed a general and versatile method for the preparation of triazines flanked with an *o*-carborane. Therefore, the selective nucleophilic substitution is demonstrated to be a mild process, which has great potential in medicinal



Scheme 4. Synthesis of **4** and **7**. Legend: (*i*) THF, −78 °C; (*ii*) $HN(CH_2CH_2Cl)_2/K_2CO_3$, THF, 0 to 25 °C.



Scheme 5. Synthesis of **8**, **9**, and **10**. Legend: (*i*) THF, −78 °C; (*ii*) $HN(CH_3)_2/K_2CO_3$, THF, 0 to 25 °C; (*iii*) $HN(CH_2CH_2Cl)_2/K_2CO_3$, THF, 0 to 25 °C.

Table 2. Cytotoxicity toward B-16 melanoma cells and boron incorporation

Compound	Cytotoxicity IC_{50} (g B/ml)	Boron incorporation (g B/10^6 cells)[a]
6b	13	0.11 ± 0.070
9a	1.7	0.22 ± 0.021
9b	>100	0.14 ± 0.018
10b	31	0.31 ± 0.020
BPA·HCl	10.8	0.14 ± 0.021[b]

[a] B-16 cells were incubated for 3 h at 37 °C with the medium containing the boron compound (1.0×10^{-4} M; 10.8 ppm B).
[b] The concentration for administration was 1.0×10^{-3} M (10.8 ppm B).

chemistry for joining chemically sensitive targeting moieties to pharmacophores for BNCT.

EXPERIMENTAL

General procedures

All manipulations were performed under a dry, oxygen-free nitrogen or argon atmosphere using standard Schlenk techniques. Benzene and toluene were dried and distilled from benzophenone. THF was freshly distilled over potassium benzophenone. ^1H and ^{13}C NMR spectra were recorded on a Varian Mercury 300 spectrometer operating at 300.1 MH_3 and 75.4 MHz. All proton and carbon chemical shifts were measured relative to internal residual chloroform from the lock solvent (99.5% $CDCl_3$) and then referenced to Me_4Si (0.00 ppm). IR spectra were recorded on a Biorad FTS-165 spectrophotometer. Elemental analyses were performed with a Carlo Erba Instruments CHNS-O EA 1108 analyzer. All melting points were uncorrected. Carboranes, dimethyl amine (2 M solution in THF), cyanuric chloride (all Katchem Chemical Co.) and bis(2-chloroethyl)amine hydrochloride (Aldrich) were used without purification.

Synthesis of 2,4,6-(*o*-carboranyl)-1,3,5-*s*-triazine (3a)

To a stirred solution of cyanuric chloride (**1**; 3.69 g, 20 mmol) in toluene (400 ml), which was cooled to 0 °C, 9.01 g (60 mmol) of LiCab^H (**2a**) was added via a side arm. The reaction mixture was stirred at room temperature for 1 h and then stirred for an additional 6 h at 70 °C. After cooling, the reaction mixture was filtered. The volume of the filtrate was reduced; after this had beed allowed to stand at −10 °C for several days, a pale yellow powder of **3a** (7.82 g, 77%) was formed. Anal. Found: C, 21.32; H, 6.61; N, 8.32. Calc. for $C_9H_{33}B_{30}N_3$: C, 21.29; H, 6.55; N, 8.28%. IR spectrum (KBr pellet, cm^{-1}): ν(Cab C–H) 3071(w), ν(B–H) 2599 (s), ν(C=N) 1539, 1376. ^1H NMR (ppm, $CDCl_3$): 3.55 (s, 3H, Cab-*H*). $^{13}C\{^1H\}$ NMR (ppm, $CDCl_3$): 145.6 (s, C_3N_3).

Synthesis of 2,4,6-(2′-methyl-*o*-carboranyl)-1,3,5-*s*-triazine (3b)

A similar procedure was employed as described for **3a**, using **2b**, to give an orange crystalline powder of **3b** (8.91 g, 81%). Anal. Found: C, 26.28; H, 7.22; N, 7.68. Calc. for $C_{12}H_{39}B_{30}N_3$: C, 26.22; H, 7.15; N, 7.64%. IR spectrum (KBr pellet, cm^{-1}): ν(C–H) 2937 (m), ν(B–H) 2583 (s), ν(C=N) 1510, 1367. ^1H NMR (ppm, $CDCl_3$): 2.04 (s, 9H, Cab-*Me*). $^{13}C\{^1H\}$ NMR (ppm, $CDCl_3$): 168.3 (s, C_3N_3), 24.3 (s, Cab-*Me*).

Synthesis of 2,4,6-(2′-phenyl-*o*-carboranyl)-1,3,5-*s*-triazine (3c)

A similar procedure was employed as described for **3a**, using **2c**, to give an orange crystalline powder of **3c** (11.48 g, 78%). Anal. Found: C, 44.18; H, 6.22; N, 5.80. Calc. for $C_{27}H_{45}B_{30}N_3$: C, 44.06; H, 6.16; N, 5.71%. IR spectrum (KBr pellet, cm^{-1}): ν(C–H) 2960 (m), ν(B–H) 2595 (s), ν(C=N) 1523, 1364. ^{1}H NMR (ppm, $CDCl_3$): 7.54–7.23 (m, 15H, Cab-*Ph*). $^{13}C\{^1H\}$ NMR (ppm, $CDCl_3$): 133.32 (s, C_3N_3), 130.43–125.85 (m, Cab-*Ph*).

Synthesis of 4,6-(dichloro)-2-(*o*-carboranyl)-1,3,5-*s*-triazine (4a)

To a solution of cyanuric chloride (**1**; 3.69 g, 20 mmol) in 400 ml of toluene, which was cooled to −78 °C, 3.00 g (20 mmol) of $LiCab^H$ (**2a**) was added via a side arm. The reaction mixture was stirred for 1 h at −78 °C, following which the reaction mixture was warmed slowly to room temperature. The mixture was the maintained at room temperature for 1 h, after which the suspended solid was collected by filtration. The volume of the filtrate was reduced; when this was allowed to stand at −10 °C for several days, a white crystalline powder of **4a** (3.45 g, 59%) was formed.

Synthesis of 4,6-(dichloro)-2-(2′-methyl-*o*-carboranyl)-1,3,5-*s*-triazine (4b)

A similar procedure was employed as described for **4a**, using **2b**, to give a white crystalline powder of **4b** (3.80 g, 62%).

Synthesis of 6-(chloro)-4-(dimethylamino)-2-(*o*-carboranyl)-1,3,5-*s*-triazine (5a)

Dimethylamine (5.0 ml, 10 mmol) was slowly added to a solution of **4a** (2.92 g, 10 mmol) with K_2CO_3 (2.07 g, 1.5 equivalents) in THF (100 ml) at 0 °C. The solution turned yellow upon warming the mixture to room temperature. The reaction mixture was maintained at room temperature for 8 h and the reaction mixture was filtered. The solvent was removed under vacuum, leaving a yellow powder **5a**, which was purified by recrystallization from toluene at −10 °C (2.47 g, 82%). Anal. Found: C, 28.11; H, 5.78; N, 18.80. Calc. for $C_7H_{17}B_{10}Cl\,N_4$: C, 27.95; H, 5.70; N, 18.63%. IR spectrum (KBr pellet, cm^{-1}): ν(CabC–H) 3070 (w), ν(B–H) 2589 (s), ν(C=N) 1560, 1522, 1467, 1359, 1299. ^{1}H NMR (ppm, $CDCl_3$): 4.50 (s, 1H, Cab-*H*), 3.40 (s, 6H, NMe_2). $^{13}C\{^1H\}$ NMR (ppm, $CDCl_3$): 180.1 (s, C_3N_3), 177.6 (s, C_3N_3), 160.6 (s, C_3N_3), 34.9 (s, N Me_2).

Synthesis of 6-(chloro)-4-(dimethylamino)-2-(2′-methyl-*o*-carboranyl)-1,3,5-*s*-triazine (5b)

A similar procedure was employed as described for **5a**, using **4b**, to give an orange crystalline powder of **5b** (2.68 g, 85%). Anal. Found: C, 30.88; H, 5.56; N, 18.00. Calc. for $C_8H_{19}B_{10}Cl\,N_4$: C, 30.72; H, 5.48; N, 17.80%. IR spectrum (KBr pellet, cm^{-1}): ν(C–H) 2960 (m), ν(B–H) 2585 (s), ν(C=N) 1545, 1510, 1430, 1376, 1290. ^{1}H NMR (ppm, $CDCl_3$): 3.32 (s, 6H, NMe_2), 1.79 (s, 3H, Cab-*Me*). $^{13}C\{^1H\}$ NMR (ppm, $CDCl_3$): 179.9 (s, C_3N_3), 174.5 (s, C_3N_3), 168.8 (s, C_3N_3), 35.8 (s, N Me_2), 26.3 (s, Cab-*Me*).

Synthesis of 4,6-(dimethylamino)-2-(*o*-carboranyl)-1,3,5-*s*-triazine (6a)

Dimethylamine (10.0 ml, 20 mmol) was slowly added to a solution of **4a** (2.92 g, 10 mmol) with K_2CO_3 (4.15 g, 3 equivalents) in 100 ml of THF at 0 °C. The solution turned yellow upon warming the mixture to room temperature. The reaction mixture was maintained at room temperature for 24 h and filtered. The solvent was removed under vacuum, leaving a yellow powder of **6a**, which was purified by recrystallization from toluene at −10 °C (1.86 g, 60%). Anal. Found: C, 34.99; H, 7.53; N, 22.67. Calc. for $C_9H_{23}B_{10}N_5$: C, 34.94; H, 7.49; N, 22.63%. IR spectrum (KBr pellet, cm^{-1}): ν(Cab C–H) 3070 (w), ν(B–H) 2610 (s), ν(C=N) 1605, 1497, 1416, 1370. ^{1}H NMR (ppm, $CDCl_3$): 4.44 (s, 1H, Cab-*H*), 3.22 (s, 12H, N Me_2). $^{13}C\{^1H\}$ NMR (ppm, $CDCl_3$): 167.6 (s, C_3N_3), 163.6 (s, C_3N_3), 36.9 (s, N Me_2).

Synthesis of 4,6-(dimethylamino)-2-(2′-methyl-*o*-carboranyl)-1,3,5-*s*-triazine (6b)

A similar procedure was employed as described for **6a**, using **4b**, to give a pale yellow powder of **6b** (2.07 g, 64%). Anal. Found: C, 37.19; H, 7.82; N, 21.69. Calc. for $C_{10}H_{25}B_{10}N_5$. C, 37.13; H, 7.79; N, 21.65%. IR spectrum (KBr pellet, cm^{-1}): ν(C–H) 2934 (m), ν(B–H) 2590 (s), ν(C=N) 1603, 1493, 1416, 1389. ^{1}H NMR (ppm, $CDCl_3$): 3.23 (s, 12H, N Me_2), 1.96 (s, 3H, Cab-*Me*). $^{13}C\{^1H\}$ NMR (ppm, $CDCl_3$): 166.8 (s, C_3N_3), 164.7 (s, C_3N_3), 37.2 (s, N Me_2), 24.2 (s, Cab-*Me*).

Synthesis of 4,6-[bis(chloroethyl)amino]-2-(*o*-carboranyl)-1,3,5-*s*-triazine (7a)

Bis(2-chloroethyl)amine hydrochloride (3.57 g, 20 mmol) was slowly added to a solution of **4a** (2.92 g, 10 mmol) with K_2CO_3 (11.1 g, 8 equivalents) in THF (100 ml) at 0 °C. The solution turned yellow upon warming the mixture to room temperature. The reaction mixture was maintained at room temperature for 24 h and filtered. The solvent was removed under vacuum, leaving a yellow powder of **7a**, which was purified by recrystallization from toluene at −10 °C (2.26 g, 45%). Anal. Found: C, 31.08; H, 5.45; N, 13.97. Calc. for $C_{13}H_{27}B_{10}Cl_4N_5$: C, 31.02; H, 5.41; N, 13.91%. IR spectrum (KBr pellet, cm^{-1}): ν(Cab C–H) 3069 (w), ν(B–H) 2579 (s), ν(C=N) 1568, 1510, 1434, 1367, 1279. ^{1}H NMR (ppm, $CDCl_3$): 4.40 (s, 1H, Cab-*H*), 4.03 (t, 8H, $J_{H-H} = 6$ Hz), 3.79 (t, 8H, $J_{H-H} = 6$ Hz). $^{13}C\{^1H\}$ NMR (ppm, $CDCl_3$): 167.6 (s, C_3N_3), 166.5 (s, C_3N_3), 49.4 (s, $N(CH_2CH_2Cl)_2$), 38.8 (s, $NCH_2CH_2Cl)_2$).

Synthesis of 4,6-[bis(chloroethyl)amino]-2-(2′-methyl-*o*-carborane)-1,3,5-*s*-triazine (7b)

A similar procedure was employed as described for **7a**, using **4b**, to give a pale yellow powder of **7b** (2.28 g, 44%). Anal. Found: C, 32.55; H, 5.69; N, 13.58. Calc. for $C_{14}H_{29}B_{10}Cl_4N_5$: C, 32.50; H, 5.65; N, 13.54%. Mp: 151–153 °C. IR spectrum (KBr pellet, cm^{-1}): ν(C–H) 2940 (m), ν(B–H)

2583 (s), ν(C═N) 1568, 1510, 1433, 1367, 1267. ^{1}H NMR (ppm, $CDCl_3$): 4.01 (t, 8H, N(CH_2CH_2Cl)$_2$, J_{H-H} = 6 Hz), 3.80 (t, 8H, N(CH_2CH_2Cl)$_2$, J_{H-H} = 6 Hz), 2.02 (s, 3H, Cab-*Me*). ^{13}C{^{1}H} NMR (ppm, $CDCl_3$): 167.4 (s, C_3N_3), 164.9 (s, C_3N_3), 51.7 (s, N(CH_2CH_2Cl)$_2$), 40.9 (s, N(CH_2CH_2Cl)$_2$), 24.2 (s, Cab-*Me*).

Synthesis of 6-chloro-2,4-(*o*-carboranyl)-1,3,5-*s*-triazine (8a)

To a stirred solution of cyanuric chloride (**1**; 3.69 g, 20 mmol) in toluene (400 ml), which was cooled to −78 °C, 6.01 g (40 mmol) of LiCab^H (**2a**) was added via a side arm. The reaction mixture was stirred at −78 °C for 1 h, following which the reaction mixture was warmed slowly to room temperature. After being stirred for an additional 3 h, the suspended solid was collected by filtration. The volume of the filtrate was reduced; after allowing this to stand at −10 °C for several days, a white crystalline powder of **8a** was formed (5.20 g, 65%).

Synthesis of 6-chloro-2,4-(2-methyl-*o*-carboranyl)-1,3,5-*s*-triazine (8b)

A similar procedure was employed as described for **8a**, using **2b**, to give a white crystalline powder of **8b** (5.91 g, 69%).

Synthesis of 6-(dimethylamino)-2,4-(*o*-carboranyl)-1,3,5-*s*-triazine (9a)

Dimethylamine (5.0 ml, 10 mmol) was slowly added to a solution of **8a** (4.00 g, 10.0 mmol) with K_2CO_3 (2.07 g, 1.5 equivalents) in THF (100 ml) at 0 °C. The solution turned yellow upon warming the mixture to room temperature. The reaction temperature was maintained at room temperature for 12 h. The solution was decanted and the remaining solids were washed with THF. The THF was then removed in a rotary evaporator, leaving a yellow powder, which was purified by recrystallization from toluene at −10 °C (3.04 g, 74%). Anal. Found: C, 26.49; H, 6.95; N, 13.77. Calc. for $C_9H_{28}B_{20}N_4$: C, 26.46; H, 6.91; N, 13.71%. IR spectrum (KBr pellet, cm^{-1}): ν(Cab C–H) 3072 (w), ν(B–H) 2575 (s), ν(C═N) 1603, 1496, 1415, 1369. ^{1}H NMR (ppm, $CDCl_3$): 4.46 (s, 2H, Cab-*H*), 3.23 (s, 6H, NMe_2). ^{13}C{^{1}H} NMR (ppm, $CDCl_3$): 167.5 (s, C_3N_3), 163.5 (s, C_3N_3), 36.9 (s, N Me_2).

Synthesis of 6-(dimethylamino)-2,4-(2′-methyl-*o*-carboranyl)-1,3,5-*s*-triazine (9b)

A similar procedure was employed as described for **9a**, using **8b**, to give an orange crystalline powder of **9b** (3.23 g, 74%). Anal. Found: C, 30.30; H, 7.43; N, 12.86. Calc. for $C_{11}H_{32}B_{20}N_4$: C, 30.26; H, 7.39; N, 12.83%. IR spectrum (KBr pellet, cm^{-1}): ν(C–H) 2934 (m), ν(B–H) 2594 (s), ν(C═N) 1600, 1495, 1414, 1360. ^{1}H NMR (ppm, $CDCl_3$): 3.26 (s, 6H, NMe_2), 1.97 (s, 6H, Cab-*Me*). ^{13}C{^{1}H} NMR (ppm, $CDCl_3$): 166.5 (s, C_3N_3), 164.5 (s, C_3N_3), 36.8 (s, N Me_2), 24.2 (s, Cab-*Me*).

Synthesis of 4-[bis(chloroethyl)amino]-2,6-(*o*-carboranyl)-1,3,5-*s*-triazine (10a)

Bis(2-chloroethyl)amine hydrochloride (1.78 g, 10.0 mmol) was slowly added to a solution of **8a** (4.00 g, 10.0 mmol) with K_2CO_3 (4.15 g, 3 equivalents) in THF (100 ml) at 0 °C. The solution turned yellow. The reaction mixture was stirred for 30 min at 0 °C and then warmed to room temperature. After being stirred for an additional 12 h, the reaction mixture was filtered. The solvent was removed under vacuum, and the resulting residue was taken up in a minimum of toluene and then recrystallized from this solution by cooling it to −10 °C (3.08 g, 61%). Anal. Found: C, 26.20; H, 6.04; N, 11.12. Calc. for $C_{11}H_{30}B_{20}Cl_2N_4$: C, 26.14; H, 5.98; N, 11.08%. IR spectrum (KBr pellet, cm^{-1}): ν(Cab C–H) 3072 (w), ν(B–H) 2579 (s), ν(C═N) 1566, 1512, 1435, 1364, 1285. ^{1}H NMR (ppm, $CDCl_3$): 4.39 (s, 2H, Cab-*H*), 4.04 (t, 4H, N(CH_2CH_2Cl)$_2$, J_{H-H} = 6 Hz), 3.49 (t, 4H, N(CH_2CH_2Cl)$_2$, J_{H-H} = 6 Hz). ^{13}C{^{1}H} NMR (ppm, $CDCl_3$): 165.5 (s, C_3N_3), 162.4 (s, C_3N_3), 49.4 (s, N(CH_2CH_2Cl)$_2$), 38.8 (s, N(CH_2CH_2Cl)$_2$).

Synthesis of 4-[bis(chloroethyl)amino]-2,6-(2′-methyl-*o*-carboranyl)1,3,5-*s*-triazine (10b)

A similar procedure was employed as described for **10a**, using **8b**, to give a pale yellow powder of **10b** (3.63 g, 68%). Anal. Found: C, 29.30; H, 6.47; N, 10.55. Calc. for $C_{13}H_{34}B_{20}Cl_2N_4$: C, 29.26; H, 6.42; N, 10.50%. IR spectrum (KBr pellet, cm^{-1}): ν(C–H) 2949 (m), ν(B–H) 2592 (s), ν(C═N) 1572, 1506, 1438, 1360, 1309. ^{1}H NMR (ppm, $CDCl_3$): 4.08 (t, 4H, N(CH_2CH_2Cl)$_2$, J_{H-H} = 6 Hz), 3.81 (t, 4H, N(CH_2CH_2Cl)$_2$, J_{H-H} = 6 Hz), 1.98 (s, 6H, Cab-*Me*). ^{13}C{^{1}H} NMR (ppm, $CDCl_3$): 167.1 (s, C_3N_3), 164.8 (s, C_3N_3), 51.9 (s, N(CH_2CH_2Cl)$_2$), 40.9 (s, N(CH_2CH_2Cl)$_2$), 23.7 (s, Cab-*Me*).

X-ray crystallography

Details of the crystal data and a summary of intensity data collection parameters for **3c**, **5b**, and **6a** are given Table 3. Crystals of **3c**, **5b**, and **6a** were grown from ethanol solutions stored at −10 °C. Crystals of **3c**, **5b**, and **6a** were mounted in thin-walled glass capillaries and sealed under argon. The data sets of **3c**, **5b**, and **6a** were collected on an Enraf CAD4 automated diffractometer was used. Molybdenum Kα radiation (λ = 0.7107 Å) was used for all structures. Each structure was solved by the application of direct methods using the SHELXS-86 program[26] and least-squares refinement using SHELXL-97.[27] All non-hydrogen atoms in compounds **3c**, **5b**, and **6a** were refined anisotropically and hydrogen atoms were included in their calculated positions.

Determination of IC_{50}

The boron compounds (20 mg) was dissolved in 1.0 ml of dimethylsulfoxide (DMSO), and the resulting solution was diluted with modified Eagle's medium (10% fetal calf serum (FCS)), or BPA was directly dissolved in the same medium. Using a Falcon 3072 96-well culture plate, the cells (1×10^4 cells/well) were cultured on five wells with the medium containing boron compounds at various concentrations (1–100 ppm), and incubated for 3 days at 37 °C in a CO_2 incubator. It is known that DMSO is non-toxic at a concentration lower than 0.5%. We also confirmed by the control experiment that DMSO was non-toxic at the

Table 3. X-ray crystallographic data and processing parameters for compounds **3c**, **5b** and **6a**

	3c	5b	6a
Molecular formula	$C_{54}H_{90}B_{60}N_6$	$C_8H_{19}B_{10}N_4Cl$	$C_{18}H_{46}B_{20}N_{10}$
Empirical formula	$C_{18}H_{30}B_{20}N_2$		
Formula weight	1472.01	314.82	309.42
Crystal class	Rhombohedral	Monoclinic	Monoclinic
Space group	$R3$	$P2_1/n$	$P2_1$
Z	3	4	2
Cell constants			
a (Å)	12.966(4)	7.7783(6)	7.165(1)
b (Å)		27.077(1)	21.860(4)
c (Å)		8.0781(4)	11.322(2)
V (Å^3)	2073.8(3)	1701.3(2)	1771.5(6)
α or β (deg)	99.69(7)	90.31(5)	92.48(2)
μ (cm^{-1})	0.57	2.18	0.64
Crystal size (mm^3)	0.45 × 0.45 × 0.4	0.4 × 0.4 × 0.5	0.35 × 0.35 × 0.4
D_{calcd} (g cm^{-3})	1.179	1.229	1.160
F(000)	756	648	648
Radiation		Mo Kα ($\lambda = 0.7107$ Å), $T = 293$ K	
θ range (deg)	1.63–25.96	1.50–25.98	1.80–25.97
h, k, l collected	+16, ±16, ±16	+9, +33, ±9	+8, +26, ±13
Reflections collected/unique	8465/5015	3571/3331	3847/3561
Data/restraints/parameters	5012/1/391	3331/0/221	3560/1/433
Goodness-of-fit on F^2	0.93	1.02	1.00
Absolute structure parameter[25]	0.00[a]		0.00[a]
Final R indices [$I > 2\sigma(I)$]	$R_1 = 0.069$	$R_1 = 0.064$	$R_1 = 0.069$
	$wR_2 = 0.146$	$wR_2 = 0.163$	$wR_2 = 0.154$
R indices (all data)	$R_1 = 0.313$	$R_1 = 0.179$	$R_1 = 0.253$
	$wR_2 = 0.258$	$wR_2 = 0.217$	$wR_2 = 0.242$

[a] Flack parameter not reliably determined.
[b] $R_1 = \Sigma||F_o| - |F_c|$ (based on reflections with $F_o^2 > 2\sigma F^2$).
[c] $wR_2 = \{\Sigma[w(F_o^2 - F_c^2)^2]/\Sigma[w(F_o^2)^2]\}^{1/2}$; $w = 1/[\sigma^2(F_o^2) + (0.095P)^2]$; $P = [\max(F_o^2, 0) + 2F_c^2]/3$ (also with $F_o^2 > 2\sigma F^2$).

concentrations shown above. The medium was removed, and the cells were washed three times with phosphate-buffered saline (PBS (−)) and then dyed by crystal violet (0.4% in MeOH) for counting cells on a microplate reader. The results are presented as the concentration of agents that resulted in 50% of the cell number of untreated cultures (IC_{50}).

In vitro boron incorporation into B-16 melanoma cells

B-16 melanoma cells were cultured in Falcon 3025 dishes (150 mm diameter). When the cells had grown to fill up the dish (8.8×10^6 cells/dish), the boron compounds (1.0×10^{-4} M, 10.8 ppm boron) and BPA (1.0×10^{-3} M, 10.8 ppm boron) were added to the dishes. The cells were incubated for 3 h at 37 °C in 20 ml of the medium (MEM, 10% FCS). The cells were washed three times with Ca–Mg-free PBS (−), collected by rubber policeman, digested with 2 ml of 60% $HClO_4$–30% H_2O_2 (1 : 2) solution and then decomposed for 1 h at 75 °C. After filtration with a membrane filter (Millipore, 0.22 μm), the boron concentration was determined by inductively coupled plasma atomic emission spectrometry (Shimadzu, ICPS-1000-III). Three replicate of each experiment were carried out. The average boron concentration of each fraction is indicated in Table 2.

Supporting information available

Crystallographic data have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication nos CCDC-199640 (**3c**), CCDC-199641 (**5b**), and CCDC-199642 (**6a**). Copies of the data can be obtained free of charge on application to the CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).

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REFERENCES

1. Mamalis P, Werbel LM. In *Handbook of Experimental Pharmacology*, Vol. 68, *Antimalarial Drugs II*, Peters W, Michaels WHG (eds). Springer-Verlag: Berlin, 1984; 387.
2. Fink R, Heischkel Y, Thelakkat M, Schmidt H-W, Jonda C, Hüppauff M. *Chem. Mater.* 1998; **10**: 3620.
3. Fink R, Frenz C, Thelakkat M, Schmidt H-W. *Macromolecules* 1997; **30**: 8177.
4. Bauer M. *Acta Polym.* 1992; **43**: 299.
5. Shah PP. *Eur. Polym. J.* 1984; **20**: 519.
6. Quirke JME. In *Comprehensive Heterocyclic Chemistry*, vol. 3, Katritzky CW, Rees CW (eds). Pergamon Press: New York, 1984; 457.
7. Adams C, Thurman E. *J. Environ. Qual.* 1991; **3**: 540.
8. EPA, National Pesticide Survey, Outreach Materials Series, Office of Pesticides and Toxic Substances (OPTS), Washington, DC, 1990.
9. Blaney JM, Hansch C, Silipo C, Vittoria A. *Chem. Rev.* 1984; **84**: 333.
10. Soloway AH, Tjarks W, Barnum BA, Rong FG, Barth RF, Codogni IM, Wilson JG. *Chem. Rev.* 1998; **98**: 1515.
11. Hawthorne MF. *Angew. Chem. Int. Ed. Engl.* 1993; **32**: 950.
12. Ko J, Kang SO. *Adv. Organometal. Chem.* 2001; **47**: 61.
13. Haiduc I, Mahon MF, Molloy KC, Venter MM. *J. Organometal. Chem.* 2001; **627**: 6.
14. Halfpenny J, Small RWH. *Acta Crystallogr. Sect. B* 1982; **38**: 939.
15. Barclay GA, Vagg RS, Watton EC. *Acta Crystallogr Sect. B* 1977; **33**: 3487.
16. Thomas NC, Foley BL, Rheingold AL. *Inorg. Chem.* 1988; **27**: 3426.
17. Paul P, Tyagi B, Bilakhiya AK, Dastidar P, Suresh E. *Inorg. Chem.* 2000; **39**: 14.
18. Viñas C, Benakki R, Teixidor F, Casabó J. *Inorg. Chem.* 1995; **34**: 3844.
19. Rutty CJ, Connors TA. *Biochem. Pharmacol.* 1977; **26**: 2385.
20. Cumber AJ, Ross WCJ. *Chem. Biol. Interact.* 1977; **17**: 349.
21. Kaminsky R, Brun R. *Antimicrob. Agents Chemother.* 1998; **42**: 2858.
22. Haskel CM. In *Cancer Treatment*, 2nd edn. Saunders: Philadelphia, 1990.
23. Haslinger F, Soloway AH. *J. Med. Chem.* 1966; **9**: 792.
24. Soloway AH, Butler DN. *J. Med. Chem.* 1966; **9**: 411.
25. Flack HD. *Acta Crystallogr. Sect. A* 1983; **39**: 876.
26. Sheldrick GM. *Acta Crystallogr. Sect. A* 1990; **46**: 467.
27. Sheldrick GM. *SHELXL97, program for crystal structure refinement, University of Göttingen, Germany, 1997.*