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Full Paper

Discovery of *ortho*-Carborane-Conjugated Triazines as Selective Topoisomerase I/II Inhibitors

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The cell growth inhibition profile of 2,4-(2-methyl-*ortho*-carboranyl)-4-(dimethylamino)-1,3,5-triazine (TAZ-6) was found to be similar to that of ICRF-193, a topoisomerase II inhibitor, as revealed by COMPARE analysis (correlation coefficient (r) = 0.724). Various mono- and di-*ortho*-carborane-substituted 1,3,5-triazines were synthesized based on the structure of TAZ-6 and tested for their ability to inhibit cell growth and the activities of topoisomerases I and II. Among the compounds synthesized, **3c**, **4c**, and **4f** completely inhibited topoisomerase I activity without affecting topoisomerase II activity, whereas **3a** and **3d** completely inhibited topoisomerase II activity without affecting topoisomerase I activity, at 100 μM.

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Introduction

The proper regulation of DNA topology is vital for cell viability. Topological problems are always associated with DNA replication, transcription, recombination, and chromatin remodelling. Topoisomerases I and II solve those problems by introducing temporary single- (topoisomerase I) or double- (topoisomerase II) strand breaks in DNA.[1–5] Inhibition of the topoisomerases can cause permanent DNA damage, triggering significant cellular events, including apoptosis, and finally cell death. Therefore, they are considered to be an attractive target for the design of chemotherapeutic agents.[6–8] Camptothecin, which was isolated from *Camptotheca acuminate*,[9,10] and its derivatives topotecan and irinotecan are known as topoisomerase I inhibitors,[10–12] whereas doxorubicin and etoposide are known as topoisomerase II inhibitors. These compounds are considered to intercalate DNA.

1,3,5-Triazines are a class of nitrogen-containing cyclic compounds with remarkable thermal and chemical stabilities,[13] and have become one of the important scaffolds for the design of pharmacologically active molecules. In particular, hexamethylmelamine (HMM) was found to be an antitumour agent and is clinically utilized for lung, ovarian, and breast cancers (Figure 1).[14,15] Trimelamol, 2,4,6-tris(*N*-hydroxymethylamino)-1,3,5-triazine, was developed as a water-soluble analogue of HMM. Although phase I and phase II clinical trials have shown that trimelamol demonstrates promising activity toward platinum-refractory ovarian cancer, further clinical development was discontinued due to formulation difficulties.[16] Benzimidazolyl-1,3,5-triazine (ZSTK474) was also developed as a potent antitumour agent[17] and phosphatidylinositol 3-kinase (PI3K) was identified as a molecular target for ZSTK474.[18] Not only antitumour activity,[19,20] but also antiprotozoal,[21] antimalarial,[22] antiviral,[23] antimicrobial,[24,25] and anti-immune activities[26] of 1,3,5-triazines have been reported.

In the meanwhile, much attention has been focussed on carboranes as potential pharmacophores in drug design.[27,28] Carboranes consist of the carbon and boron atoms of the C2B10 core and these atoms are hexacoordinate to compensate for the low electron density, forming 20 triangular faces. Due to the geometric and electronic features of carboranes, their unique interaction with target biomolecules has been designed and studied including estrogen,[29,30] dihydrofolate reductases,[31] and heat shock protein (Hsp) 60.[32]

The 1,3,5-triazine ring has three distinct electrophilic centres that form 2,4,6-mono, di- or tri-substituted, symmetrical and nonsymmetrical triazines bearing different substituents. Cyanuric chloride is the key reagent for the synthesis of these compounds owing to the reactivity of each of the cyanyl chloride units.[13] We sought to utilize the triazine core as a template for the production of potential boron neutron capture therapy (BNCT)[33–35] agents, and recently reported the synthesis of

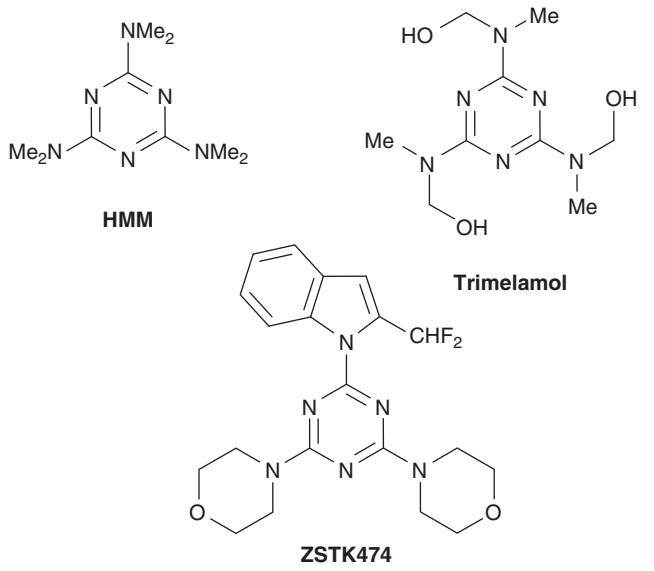


Fig. 1. Structures of 1,3,5-triazines with antitumour activity.

ortho-carborane-containing 1,3,5-triazines.[36,37] We tested these compounds for in vitro antiproliferative activity towards a panel of 39 human cancer cell lines (termed JFCR39), which is comparable to the panel developed by the National Cancer Institute.[38,39] We compared the cell growth inhibitory activities towards JFCR39 of more than 200 conventional compounds, including anticancer drugs and various types of inhibitors, by using COMPARE analysis.[40] This system can be used to predict the molecular target or the mechanism of action of a test compound by determining the correlation coefficient (r) between the cell growth inhibition profiles of the test compound and various reference compounds with known mechanisms of action. Among the compounds synthesized, the cell growth inhibition profile of 2,4-(2-methyl-*ortho*-carboranyl)-4-(dimethylamino)-1,3,5-triazine (TAZ-6) was found to be similar to that of ICRF-193,[41] a topoisomerase II inhibitor, as indicated by COMPARE analysis. In this case, $r = 0.724$. In this study, we synthesized various mono- and di-*ortho*-carborane-substituted 1,3,5-triazines based on the structure of TAZ-6 and examined the structure-activity relationships of these compounds with topoisomerases I and II (Figure 2).

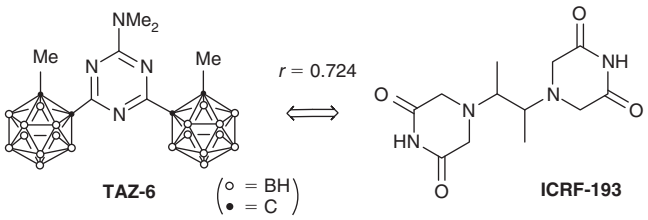
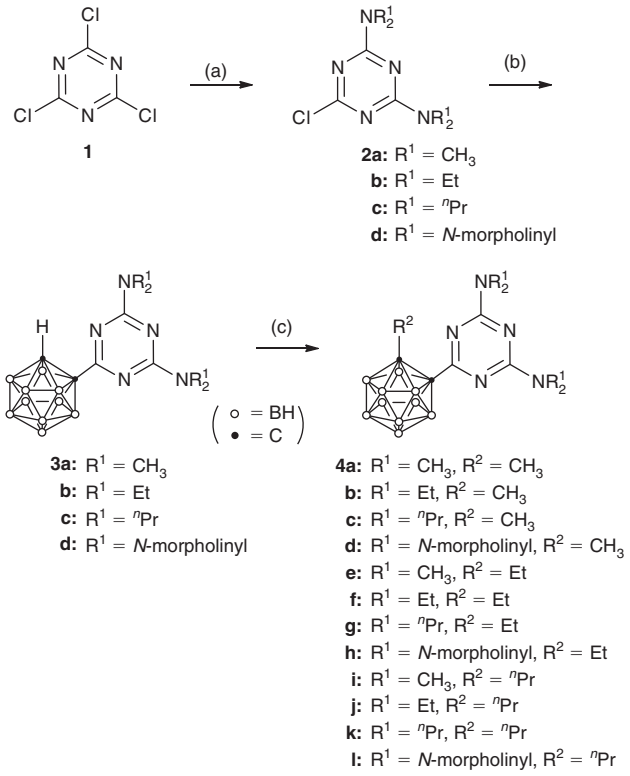


Fig. 2. Structures of di-*ortho*-carboranyl triazine (**TAZ-6**) and topoisomerase II inhibitor (**ICRF-193**).



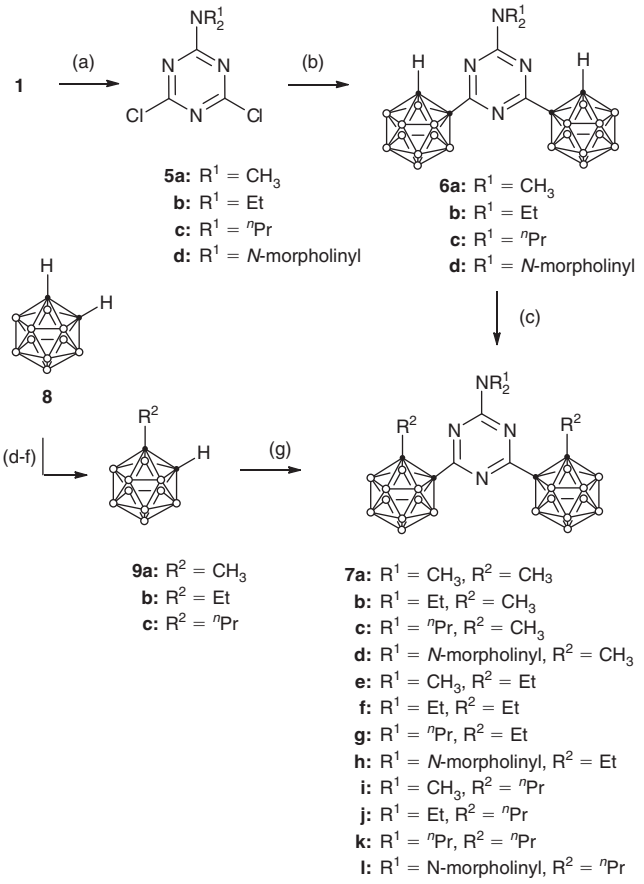
Scheme 1. *Reagents and conditions*: (a) HNR^1_2 (2 equiv), THF, 0°C; (b) lithiated *ortho*-carborane, THF, −10°C; (c) ^{n}BuLi, R^2 I, THF, −10°C.

Results and Discussion

Chemistry

The synthesis of mono-*ortho*-carboranyl triazines **3** and **4** is shown in Scheme 1. Cyanuric chloride **1** was chosen as the starting material and treated with two equivalents of various secondary amines (R^1 = methyl, ethyl, and *n*-propyl) in THF at 0°C to give 2,4-diamino-1,3,5-triazine derivatives **2** in 90–99 % yields.[42] Furthermore, the reaction of **1** with morpholine gave a mixture of mono- and di-substituted triazines in 60 and 29 % yields, respectively. The lithiated *ortho*-carborane prepared from the reaction of *ortho*-carborane with *n*-BuLi in THF was reacted with **2** at −10°C under argon atmosphere to afford the corresponding mono-*ortho*-carborane-substituted 1,3,5-triazines **3** in moderate yields (29–51 %). Deprotonation at the carborane carbon with *n*-BuLi followed by treatment with various iodoalkanes (R^2 = methyl, ethyl, and *n*-propyl) gave alkyl-group-substituted *ortho*-carborane-containing 1,3,5-triazine derivatives **4** in 11–89 % yields. Interestingly, these alkyl-group-substituted compounds have non-equivalent NMR resonances as indicated in the experimental section. For instance, compound **4a** ($R^2 = CH_3$) shows two ^{1}H *N*-methyl singlets at 3.12 and 3.15 ppm and the corresponding ^{13}C signals resonated at 35.9 and 36.1 ppm. These non-equivalent NMR resonances are considered to be caused by their hindered rotation of the triazine-carborane single bond and these phenomena are not observed in compound **3a** (R^2 = H), which has no substituent on the *ortho*-carborane (Scheme 1).

The synthesis of di-*ortho*-carboranyl triazines **6** and **7** is shown in Scheme 2. Cyanuric chloride **1** was treated with one equivalent of various secondary amines in THF at 0°C to give 2-amino-4,6-dichloro-1,3,5-triazine derivatives **5** in good to high yields (65–95 %). The reaction of **5** with the lithiated *ortho*-carborane at −10°C gave the corresponding di-*ortho*-carborane-substituted 1,3,5-triazines **6** in moderate yields (29–34 %). Similarly, the alkyl groups were introduced into the carborane carbon to afford **7** in 39–90 % yields. Alternatively, **9a** ($R^2 = CH_3$), **9b** (R^2 = Et), and **9b** ($R^2 = {}^n$Pr), which were prepared from *ortho*-carborane **8** according to the literature procedure with modification,[43] were treated with ^{n}BuLi and



Scheme 2. *Reagents and conditions*: (a) HNR^1_2 (1 equiv), THF, 0°C; (b) lithiated *ortho*-carborane, THF, −10°C; (c) i) ^{n}BuLi (2.2 equiv); ii) R^2I (2.1 equiv), THF, −10°C; (d) i) ^{n}BuLi (1 equiv), −10°C; ii) TMSCl (1.1 equiv), THF, −10°C to r.t.; (e) i) ^{n}BuLi (1.1 equiv), 0°C; ii) R^2I (1.1 equiv), THF, 0°C to r.t.; (f) TBAF, THF; (g) i) ^{n}BuLi (1.1 equiv), 0°C; ii) **5** (1.1 equiv), THF, 0°C to r.t.

the resulting lithiated *ortho*-carboranes were reacted 5 in THF at 0°C to give the corresponding di-*ortho*-carborane-substituted 1,3,5-triazines **7** in moderate to good yields (Scheme 2).

Biological Evaluation

We first investigated the cell growth inhibitory activities of the 1,3,5-triazine compounds, camptothecin, and etoposide toward HeLa cells using the MTT assay. The results are shown in Table 1. Among the compounds synthesized, compounds **3a** and **3b**, which have no substituent at the carborane moiety, showed the most significant cell growth inhibitory activities and their GI_{50} (the concentration that inhibits 50 % of cell growth) values were 1.8 and 1.5 μM, respectively. Compounds **4a-l**, which have long alkyl chains (R^2) on the carborane moiety, and di-*ortho*-carborane-substituted 1,3,5-triazine compounds (**6a-d**) showed decreased cell growth inhibitory activities. In the cases of compounds **4k** and **6a-d**, their GI_{50} values could not be obtained due to their low solubility in water. Other synthesized di-*ortho*-carborane-substituted 1,3,5-triazines **7b-f** also showed a similar tendency and their GI_{50} values were not detectable (>100 μM: data not shown).

We next investigated the in vitro inhibition of topoisomerases I and II by the compounds. Figure 3 shows supercoiled DNA breaks caused by topoisomerases I and II to form relaxed DNA.[44] Camptothecin, a topoisomerase I inhibitor, inhibited

Table 1. Inhibitory activity of the *ortho*-carborane-conjugated 1,3,5-triazines 3, 4, 6 and 7a towards cell growth and topoisomerases I/II

Compound	Growth inhibition (GI_{50}/μM)[a]	Topo I[b] inhibition	Topo II[c] inhibition
3a	9.2 ± 1.8	–	++
3b	1.8 ± 2.1	+	+
3c	1.5 ± 0.5	++	–
3d	37.1 ± 12.8	–	++
4a	14.2 ± 2.1	–	–
4b	14.1 ± 3.7	+	–
4c	9.6 ± 0.5	++	–
4d	14.7 ± 2.4	–	–
4e	40.9 ± 1.5	–	–
4f	51.0 ± 7.4	++	–
4 g	35.1 ± 5.5	–	–
4h	16.7 ± 0.4	–	–
4i	17.5 ± 0.4	–	–
4j	40.1 ± 2.2	+	–
4k	>100	nd	nd
4l	15.2 ± 0.2	–	–
6a	16.1 ± 5.8	++	++
6b	16.8 ± 2.8	++	++
6c	30.2 ± 15.8	++	++
6d	32.8 ± 5.5	++	++
7a (TAZ-6)	>100	++	++
Camptothecin	<0.4	++	–
Etoposide	2.8 ± 0.2	–	++

[a]The drug concentrations required to inhibit the HeLa cell growth by 50 % (GI_{50}) were determined from semilogarithmic dose-response plots, and results represent mean ± sd of triplicate samples.
[b]Enzymatic activities inhibited partially (+) and completely (++) by compounds at 100 μM. '–' indicates that no inhibition was observed at 100 μM. Camptothecin and etoposide were used as positive controls for topoisomerases I and II, respectively.

supercoiled DNA breaks caused by topoisomerase I partially at 30 μM and completely at 100 μM. Etoposide, a topoisomerase II inhibitor, inhibited supercoiled DNA breaks caused by topoisomerase II completely at 100 μM. Compound **3c** exhibited topoisomerase I inhibition in a concentration-dependent manner without affecting topoisomerase II activity at 30 and 100 μM. We systematically evaluated the inhibitory activities of monocarborane-conjugated 1,3,5-triazines **3a-d** and **4a-l**, and dicarborane-conjugated 1,3,5-triazines **6a-d** and **7a** toward topoisomerases I and II. The results are summarized in Table 1. Selective inhibitions of topoisomerases I and II were observed in several 1,3,5-triazine derivatives. Compounds **3c**, **4b**, **4c**, **4f**, and **4j** selectively inhibited supercoiled DNA breaks caused by topoisomerase I. In particular, compounds **3c**, **4c**, and **4f** exhibited complete inhibition of topoisomerase I activity at 100 μM and the inhibitory activities of these compounds are similar to that of camptothecin. Meanwhile, compounds **3a** and **3d** selectively inhibited supercoiled DNA breaks caused by topoisomerase II and the inhibitory activities of these compounds are similar to that of etoposide (complete inhibition at 100 μM, see Figure 3b). Interestingly, the inhibition of both topoisomerases I and II was observed in di-*ortho*-carborane-substituted 1,3,5-triazines (**6a-d** and **7a**). It is considered that the inhibitory activities of di-*ortho*-carborane-substituted 1,3,5-triazines towards both enzymes would be owing to the high hydrophobic character of *ortho*-carborane, which would aggregate with the enzymes via hydrophobic interactions to render the enzymes inert, although the detailed binding modes of these

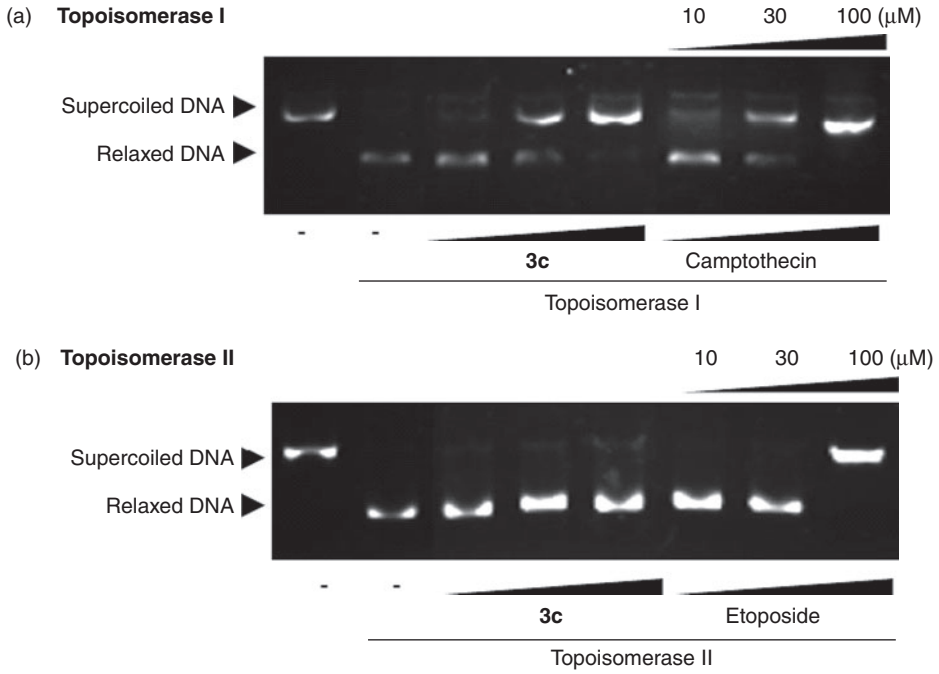


Fig. 3. In vitro inhibition of topoisomerases I and II by compound **3c**. Camptothecin and etoposide were used as positive controls for topoisomerases I and II, respectively. Compound **3c** partially inhibited (+) and completely inhibited (++) topoisomerase I activity at 30 and 100 μM, respectively, without affecting topoisomerase II activity.

compounds to topoisomerases I and II are not clear. Synthesized di-*ortho*-carborane-substituted 1,3,5-triazines **7b-f** also exhibited complete inhibition of both topoisomerases I and II (data not shown).

Conclusion

We have developed *ortho*-carborane-substituted 1,3,5-triazines as selective inhibitors of topoisomerases I and II. The substituents on the *ortho*-carborane are essential for the selective inhibition of topoisomerases I and II. The current findings show the potential of carborane as an alternative pharmacophore in drug design.[27,28] The detailed binding modes of these compounds to topoisomerases I and II are under investigation in our laboratory.

Experimental

General

^{1}H NMR, ^{13}C NMR, and ^{11}B NMR spectra were measured on JEOL JNM-AL 300 (300 MHz) and VARIAN UNITY-INOVA 400 (400 MHz) spectrometers. Chemical shifts are expressed in parts per million (ppm, δ units) relative to internal tetramethylsilane (^{1}H NMR and ^{13}C NMR) or external $BF_3 \cdot Et_2O$ (^{11}B NMR), and the coupling constants are expressed in units of hertz (Hz). IR spectra were measured on a Shimadzu FTIR-8200A spectrometer. High-resolution mass spectra (ESI) were recorded on a Bruker Daltonics micro TOF-15 focus. Elemental analyses were performed by a CE instrument EA1110 CHNS-O automatic elemental analyzer. Analytical thin-layer chromatography (TLC) was performed on glass plates (Merck Kieselgel 60 F254). Column chromatography was performed on silica gel (Merck Kieselgel 70-230 mesh). All reactions were carried out under an argon atmosphere using standard Schlenk techniques. Most chemicals were of analytical grade and used without further purification.

*2,4-Bis(*N,N-*dimethylamino)-6-(*o-*carboran-1-yl)-1,3,5-triazine* ***3a***

To a stirred solution of *o*-carborane (0.29 g, 2.0 mmol) in 10 mL THF at −10°C was added 1.3 mL of *n*-BuLi (1.6 M, 2.0 mmol) via a syringe. Compound **2a** (0.40 g, 2.0 mmol) dissolved in THF (10 mL) was slowly added to the reaction mixture at −10°C, and the reaction temperature was maintained at −10°C for 1 h. The reaction mixture was then warmed slowly to room temperature, stirred for an additional 3 h, and quenched with saturated aqueous NH_4Cl solution. The organic layer was washed with brine, dried with anhydrous $MgSO_4$, and concentrated under reduced pressure. Purification by column chromatography on silica gel with EtOAc/hexane (1:50) gave **3a** in 32 % yield (0.20 g, 0.63 mmol) as a white solid. M*P* = 167–168°C. ν_{max} (KBr)/cm^{-1} 2914, 2573, 1589, 1508, 1379. δ_H (400 MHz, $CDCl_3$) 4.57 (s, 1H), 3.11 (s, 12H). δ_C (100 MHz, $CDCl_3$) 165.8, 164.5, 74.6, 56.1, 36.1, 36.0. δ_B −2.42 to −13.07 (br m, 10B). *m/z* (HR-ESI, positive) Calc. for $C_9H_{23}B_{10}N_5$ $[M\text{-}H]^+$: 312.2962, found: 312.2958.

*2,4-Bis(*N,N-*diethylamino)-6-(*o-*carboran-1-yl)-1,3,5-triazine* ***3b*** was synthesized from **2b** (0.27 g, 1.0 mmol) using the procedure described for **3a** to give **3b** in 43 % yield (0.16 g, 0.43 mmol) as a white solid, mp 110-111°C. ν_{max} (KBr)/cm^{-1} 2571, 1566, 1504, 1435, 1364, 1310. δ_H (400 MHz, $CDCl_3$) 4.52 (s, 1H), 3.52 (q, *J* 7.2, 8H, CH_2 CH_3), 1.12-1.18 (m, 12H, CH_2CH_3). δ_C (100 MHz, $CDCl_3$) 158.6, 156.5, 67.7, 48.9, 34.8, 34.7, 6.3, 5.9. δ_B (96.3MHz, $CDCl_3$) −2.40 to −13.08 (br m, 10B). Anal. Calc. for $C_{13}H_{31}N_5$: C 42.72, H 8.55, N 19.16. Found: C 43.01, H 8.64, N 19.01 %.

*2,4-Bis(*N,N-*di*-n-*propylamino)-6-(*o-*carboran-1-yl)-1,3,5-triazine* ***3c*** was synthesized from **2c** (0.32 g, 1.0 mmol) using the procedure described for **3a** to give **3c** in 62 % yield (0.27 g, 0.64 mmol) as a white solid, mp 95-96°C. ν_{max} (KBr)/cm^{-1} 2932, 2872, 2561, 1578, 1502, 1429. δ_H (400 MHz, $CDCl_3$) 4.48 (s, 1H), 3.44-3.37 (m, 8H), 1.65-1.53 (m, 8H), 0.918-0.871

(m, 12H). δ_C (100 MHz, $CDCl_3$) 165.4, 164.0, 74.8, 55.9, 49.4, 49.3, 21.4, 20.9, 11.5, 11.4. δ_B (96.3MHz, $CDCl_3$) −2.40 to −13.0 (br m, 10B). Anal. Calc. for $C_{17}H_{39}N_5$: C 48.43, H 9.32, N 16.61. Found: C 48.51, H 9.45, N 16.51 %.

*2,4-Bis-(*N-*morpholinyl)-6-(*o-*carboran-1-yl)-1,3,5-triazine* ***3d*** was synthesized from **2d** (0.29 g, 1.0 mmol) using the procedure described for **3a** to give **3d** in 87 % yield (0.34 g, 0.87 mmol) as a white solid, mp 288°C. ν_{max} (KBr)/cm^{-1} 2856, 2584, 1578, 1504, 1445, 1236. δ_H (400 MHz, $CDCl_3$) 4.45 (s, 1H), 3.84-3.66 (m, 16H). δ_C (100 MHz, $CDCl_3$) 166.6, 164.0, 74.2, 66.5, 56.0, 43.7, 43.4. δ_B (96.3MHz, $CDCl_3$) −2.42 to −13.04 (br m, 10B). Anal. Calc. for $C_{13}H_{27}N_5$: C 39.68, H 6.92, N 17.80. Found: C 39.71, H 6.71, N 17.67 %.

*2,4-Bis(*N,N-*dimethyamino)-6-(2-methyl*-o-*carboran-1-yl)-1,3,5-triazine* ***4a***

To a stirred solution of ***3a*** (0.15 g, 0.49 mmol) in 3 mL THF at 0°C was added 0.4 mL of *n*-BuLi (1.6 M, 0.64 mmol) via a syringe. Iodomethane (50 µl, 0.80 mmol) was slowly added to the reaction mixture at 0°C, and the reaction temperature was then warmed slowly to room temperature. After the mixture was quenched with saturated aqueous NH_4Cl solution, the organic layer was washed with brine, dried over anhydrous $MgSO_4$, and concentrated under reduced pressure. Purification by column chromatography on silica gel with EtOAc/hexane (1:50) gave **4a** in 14 % yield (23 mg, 0.070 mmol) as a white solid, mp 170–171°C. ν_{max} (KBr)/cm^{-1} 2935, 2584, 2548, 1593, 1506, 1373. δ_H (400 MHz, $CDCl_3$) 3.15 (s, 6H), 3.12 (s, 6H), 1.97 (s, 3H). δ_C (100 MHz, $CDCl_3$) 164.9, 164.4, 80.0, 75.7, 36.1, 35.9, 24.0. δ_B (96.3MHz, $CDCl_3$) −0.61 to −8.98 (br m, 10B). *m/z* (HR-ESI, positive) Calc. for $C_{10}H_{25}B_{10}N_5$ $[M\text{-}H]^+$: 326.3119, found: 326.3116.

*2,4-Bis(*N,N-*diethyamino)-6-(2-methyl*-o-*carboran-1-yl)-1,3,5-triazine* ***4b*** was synthesized from **3b** (0.18 g, 0.48 mmol) using the procedure described for **4a** to give **4b** in 56 % yield (0.10 g, 0.27 mmol) as a white solid, mp 115-116°C. ν_{max} (KBr)/cm^{-1} 2932, 2584, 1501, 1435. δ_H (400 MHz, $CDCl_3$) 3.58-3.51 (m, 8H), 1.98 (s, 3H), 1.19-1.14 (m, 12H). δ_C (100 MHz, $CDCl_3$) 164.4, 163.9, 80.3, 75.7, 41.9, 41.6, 24.0, 13.4, 12.9. δ_B (96.3MHz, $CDCl_3$) −1.93 to −9.48 (br m, 10B). Anal. Calc. for $C_{14}H_{33}N$: C 44.30, H 8.76, N 18.45. Found: C 44.45, H 8.83, N 18.53 %.

*2,4-Bis(*N,N-n-*propylamino)-6-(2-methyl*-o-*carboran-1-yl)-1,3,5-triazine* ***4c*** was synthesized from **3c** (0.20 g, 0.48 mmol) using the procedure described for **4a** to give **4c** in 90 % yield (0.19 g, 0.43 mmol) as a white solid, mp 80–81°C. ν_{max} (KBr)/cm^{-1} 2966, 2934, 2577, 1562, 1501, 1429. δ_H (400 MHz, $CDCl_3$) 3.46 (t, *J* 7.6, 4H), 3.40 (t, *J* 7.6, 4H), 1.97 (s, 3H), 1.60 (sext, *J* 7.2, 8H), 0.91 (t, *J* 7.2, 12H). δ_C (100 MHz, $CDCl_3$) 164.3, 164.1, 80.4, 75.7, 49.4, 49.3, 24.0, 21.4, 20.9, 11.5, 11.4. δ_B (96.3MHz, $CDCl_3$) −2.06 to −9.45 (br m, 10B). Anal. Calc. for $C_{18}H_{41}N_5$: C 49.62, H 9.49, N 16.08. Found: 49.58, H 9.63, N 15.78 %.

*2,4-Bis-(*N-*morpholinyl)-6-(2-methyl*-o-*carboran-1-yl)-1,3,5-triazine* ***4d*** was synthesized from **3d** (0.20 g, 0.50 mmol) using the procedure described for **4a** to give **4d** in 20 % yield (42 mg, 0.10 mmol) as a white solid, mp 266–267°C. ν_{max} (KBr)/cm^{-1} 2856, 2577, 1574, 1504, 1445, 1234. δ_H (400 MHz, $CDCl_3$) 3.86–3.68 (m, 16H), 1.96 (s, 3H). δ_C (100 MHz, $CDCl_3$) 165.2, 164.4, 79.6, 75.8, 66.7, 66.5, 43.8, 43.5, 24.0. δ_B (96.3MHz, $CDCl_3$) −1.71 to −9.30 (br m, 10B). Anal. Calc. for $C_{14}H_{29}N_5$: C 41.26, H 7.17, N 17.19. Found: C 41.03, H 7.30, N 17.17 %.

*2,4-Bis(*N,N-*dimethyamino)-6-(2-ethyl*-o-*carboran-1-yl)-1,3,5-triazine* ***4e*** was synthesized from **3a** (0.10 g, 0.34 mmol) and iodoethane (35 µL, 0.44 mmol) using the procedure described for **4a** to give **4e** in 11 % yield (13 mg, 0.036 mmol) as a white solid, mp 141–142°C. ν_{max} (KBr)/cm^{-1} 2947, 2554, 1589, 1504, 1406, 1375. δ_H (400 MHz, $CDCl_3$) 3.14 (s, 6H), 3.11(s, 6H), 2.18 (q, *J* 7.6, 2H), 1.05 (t, *J* 7.6, 3H). δ_C (100 MHz, $CDCl_3$) 164.9, 164.2, 81.8, 81.7, 36.1, 35.9, 29.1, 13.9. δ_B (96.3 MHz, $CDCl_3$) −2.34 to −9.68 (br m, 10B). Anal. Calc. for $C_{11}H_{27}N_5$: C 39.15, H 8.06, N 20.75. Found: C 39.36, H 8.16, N 20.56 %.

*2,4-Bis(*N,N-*diethyamino)-6-(2-ethyl*-o-*carboran-1-yl)-1,3,5-triazine* ***4f*** was synthesized from **3b** (0.25 g, 0.68 mmol) and iodoethane (70 µL, 0.88 mmol) using the procedure described for **4a** to give **4f** in 68 % yield (0.18 g, 0.46 mmol) as a white solid, mp 89–90°C. ν_{max} (KBr)/cm^{-1} 2961, 2561, 1570, 1501, 1435. δ_H (400 MHz, $CDCl_3$) 3.58-3,50 (m, 8H), 2,19 (q, *J* 7.6, 2H), 1.19-1.13 (m, 12H), 1.06 (t, *J* 7.6, 3H). δ_C (100 MHz, $CDCl_3$) 164.2, 163.9, 82.1, 81.9, 41.8, 41.6, 29.0, 13.9, 13.4, 12.9. δ_B (96.3MHz, $CDCl_3$) −2.53 to −9.66 (br m, 10B). Anal. Calc. for $C_{15}H_{35}N_5$: C 45.77, H 9.11, N 17.79. Found: C 45.80, H 8.96, N 17.62 %.

*2,4-Bis(*N,N-*di*-n-*propylamino)-6-(2-ethyl*-o-*carboran-1-yl)-1,3,5-triazine* ***4 g*** was synthesized from **3c** (0.18 g, 0.42 mmol) and iodoethane (55 µL, 0.69 mmol) using the procedure described for **4a** to give **4 g** in 83 % yield (0.16 g, 0.35 mmol) as a white solid, mp 52–53°C. ν_{max} (KBr)/cm^{-1} 2966, 2874, 2577, 1578, 1501, 1429. δ_H (400 MHz, $CDCl_3$) 3.46 (t, *J* 7.6, 4H), 3.40 (t, *J* 7.6, 4H), 2.18 (q, *J* 7.6, 2H), 1.67-1.53 (m, 8H), 1.05 (t, *J* 7.6, 3H), 0.906 (t, *J* 7.6, 12H). δ_C (100 MHz, $CDCl_3$) 164.3, 164.0, 82.1, 81.9, 49.4, 49.3, 29.0, 21.5, 20.9, 13.9, 11.5, 11.4. δ_B (96.3MHz, $CDCl_3$) −2.68 to −9.68 (br m, 10B). Anal. Calc. for $C_{19}H_{43}N_5$: C 50.75, H 9.64, N 15.57. Found: C 50.56, H 9.78, N 15.43 %.

*2,4-Bis-(*N-*morpholinyl)-6-(2-ethyl*-o-*carboran-1-yl)-1,3,5-triazine* ***4 h*** was synthesized from **3d** (0.20 g, 0.50 mmol) and iodoethane (50 µL, 0.63 mmol) using the procedure described for **4a** to give **4 h** in 52 % yield (0.11 g, 0.26 mmol) as a white solid, mp 173°C. ν_{max} (KBr)/cm^{-1} 2856, 2579, 1566, 1504, 1232. δ_H (400 MHz, $CDCl_3$) 3.86-3.68 (m, 16H), 2.15 (q, *J* 7.6, 2H), 1.05 (t, *J* 7.6, 3H). δ_C (100 MHz, $CDCl_3$) 165.0, 164.4, 81.9, 81.2, 66.7, 66.5, 43.8, 43.5, 29.2, 13.9. δ_B (96.3MHz, $CDCl_3$) −1.76 to −9.60 (br m, 10B). Anal. Calc. for $C_{15}H_{31}N_5$: C 42.74, H 7.41, N 16.61. Found: C 42.94, H 7.40, N 16.39 %.

*2,4-Bis(*N,N-*dimethyamino)-6-(2*-n-*propyl*-o-*carboran-1-yl)-1,3,5-triazine* ***4i*** was synthesized from **3a** (0.11 g, 0.36 mmol) and iodopropane (39 µL, 0.40 mmol) using the procedure described for **4a** to give **4i** in 23 % yield (29 mg, 0.081 mmol) as a white solid, mp 150-151°C. ν_{max} (KBr)/cm^{-1} 2563, 1589, 1502, 1404. δ_H (400 MHz, $CDCl_3$) 3.14 (s, 6H), 3.12 (s, 6H), 2.10-2.06 (m, 2H), 1.53–1.47 (m, 2H), 0.80 (t, *J* 7.6, 3H). δ_C (100 MHz, $CDCl_3$) 164.9, 164.3, 81.5, 81.0, 37.6, 36.1, 35.9, 22.8, 13.7. δ_B (96.3MHz, $CDCl_3$) −2.22 to −9.47 (br m, 10B). *m/z* (HR-ESI, positive) Calc. for $C_{12}H_{29}B_{10}N_5$ $[M\text{-}H]^+$: 354.3432, found: 354.3433.

*2,4-Bis(*N,N-*diethyamino)-6-(2*-n-*propyl*-o-*carboran-1-yl)-1,3,5-triazine* ***4j*** was synthesized from **3b** (0.18 g, 0.49 mmol) and iodopropane (49 µL, 0.50 mmol) using the procedure described for **4a** to give **4j** in 76 % yield (0.15 g, 0.38 mmol) as a white solid, mp 78°C. ν_{max} (KBr)/cm^{-1} 2972, 2557, 1570, 1506, 1435. δ_H (400 MHz, $CDCl_3$) 3.58-3.51 (m, 8H), 2.11–2.07 (m, 2H), 1.56-1.46 (m, 2H), 1.20-1.13 (m, 12H), 0.814 (t, *J* 7.2, 3H). δ_C (100 MHz, $CDCl_3$) 164.2, 163.9, 81.9, 81.0, 41.8, 41.6,

37.5, 22.9, 13.8, 13.4, 12.9. δ_B (96.3 MHz, $CDCl_3$) −2.56 to −9.715 (br m, 10B). Anal. Calc. for $C_{16}H_{37}N_5$: C 47.15, H 9.15, N 17.18. Found: C 47.12, H 9.16, N 16.91 %.

*2,4-Bis(*N,N-*n-propylamino)-6-(2-*n-*propyl-*o-*carboran-1-yl)-1,3,5-triazine* ***4k*** was synthesized from **3c** (0.25 g, 0.58 mmol) and iodopropane (60 µL, 0.62 mmol) using the procedure described for **4a** to give **4k** in 90 % yield (0.24 g, 0.52 mmol) as a yellow liquid. ν_{max} (KBr)/cm^{-1} 2964, 2359, 1570, 1491, 1327. δ_H (400 MHz, $CDCl_3$) 3.46 (t, *J* 7.6, 4H), 3.41 (t, *J* 7.6, 4H), 2.07 (t, *J* 8.4, 2H), 1.67-1.46 (m, 10H), 0.908 (t, *J* 7.6, 12H), 0.804 (t, *J* 7.6, 3H). δ_C (100 MHz, $CDCl_3$) 164.3, 164.0, 82.0, 81.0, 49.3, 49.3, 37.4, 22.9, 21.5, 20.9, 13.7, 11.5, 11.4. δ_B (96.3 MHz, $CDCl_3$) −2.64 to −9.64 (br m, 10B). *m/z* (HR-ESI, positive) Calc. for $C_{20}H_{45}B_{10}N_5$ $[M-H]^+$: 466.4684, found: 466.4684.

*2,4-Bis-(*N-*morpholinyl)-6-(2-*n-*propyl-*o-*carboran-1-yl)-1,3,5-triazine* ***4l*** was synthesized from **3d** (0.17 g, 0.44 mmol) and iodopropane (43 µL, 0.44 mmol) using the procedure described for **4a** to give **4 L** in 46 % yield (89 mg, 0.20 mmol) as a white solid, mp 210°C. ν_{max} (KBr)/cm^{-1} 2575, 1570, 1504, 1232. δ_H (400 MHz, $CDCl_3$) 3.84-3.67 (m, 16H), 2.04 (t, *J* 8.4, 2H), 1.53-1.43 (m, 2H), 0.795 (t, *J* 7.6, 3H). δ_C (100 MHz, $CDCl_3$) 165.1, 164.4, 81.0, 81.0, 66.7, 66.5, 43.8, 43.5, 37.6, 22.9, 13.8. δ_B (96.3 MHz, $CDCl_3$) −2.30 to −9.61 (br m, 10B). Anal. Calc. for $C_{16}H_{33}N_5$: C 44.12, H 7.64, N 16.08. Found: C 44.07, H 7.73 N 15.91 %.

*2,4-Bis(*o-*carboran-1-yl)-6-(*N,N-*dimethylamino)-1,3,5-triazine* ***6a***

To a stirred solution of *o*-carborane (0.15 g, 1.1 mmol) in 5 mL THF at −10°C was added 0.6 mL of *n*-BuLi hexane solution (1.6 M, 1.1 mmol) via a syringe. Compound **5a** (0.11 g, 0.59 mmol) dissolved in THF (5 mL) was slowly added to the reaction mixture at −10°C, and the reaction temperature was maintained at −10°C for 1 h. The reaction mixture was then warmed slowly to room temperature, stirred for an additional 3 h, and quenched with saturated aqueous NH_4Cl solution. The organic layer was washed with brine, dried over anhydrous $MgSO_4$, and concentrated under reduced pressure. Purification by column chromatography on silica gel with EtOAc/hexane (1:10) gave **6a** in 51 % yield (0.12 g, 0.30 mmol) as a white solid, mp 215°C. ν_{max} (KBr)/cm^{-1} 3072, 2581, 1605, 1497. δ_H (400 MHz, $CDCl_3$) 4.44 (s, 2H), 3.22 (s, 6H). δ_C (100 MHz, $CDCl_3$) 167.4, 163.4, 72.8, 56.0, 36.8. δ_B (96.3MHz, $CDCl_3$) −1.75 to −12.71 (br m, 10B). *m/z* (HR-ESI, negative) Calc. for $C_9H_{28}B_{20}N_4$ $[M-H]^-$: 411.4097, found: 411.4093.

*2,4-Bis(*o-*carboran-1-yl)-6-(*N,N-*diethylamino)-1,3,5-triazine* ***6b*** was synthesized from **5b** (0.14 g, 0.63 mmol) using the procedure described for **6a** to give **6b** in 30 % yield (83 mg, 0.19 mmol) as a white solid, mp 230°C. ν_{max} (KBr)/cm^{-1} 3072, 2581, 1601, 1489. δ_H (400 MHz, $CDCl_3$) 4.41 (s, 2H), 3.60 (q, *J* 7.2, 4H, CH_2CH_3), 1.22 (t, *J* 6.8, 6H, CH_2CH_3). δ_C (100 MHz, $CDCl_3$) 167.4, 162.4, 72.8, 55.9, 43.0, 12.5. δ_B (96.3 MHz, $CDCl_3$) −1.79 to −12.62 (br m, 10B). Anal. Calc. for $C_{11}H_{32}N_4$: C 30.26, H 7.39, N 12.83. Found: C 30.04, H 7.35, N 12.87 %.

*2,4-Bis(*o-*carboran-1-yl)-6-(*N,N-*di-*n-*propylamino)-1,3,5-triazine* ***6c*** was synthesized from **5c** (0.21 g, 0.84 mmol) using the procedure described for **6a** to give **6c** in 62 % yield (0.24 g, 0.52 mmol) as a white solid, mp 157°C. ν_{max} (KBr)/cm^{-1} 2586, 1589, 1499. δ_H (400 MHz, $CDCl_3$) 4.39 (s, 2H), 3.50 (t, *J* 7.6, 4H), 1.63 (sext, *J* 7.6, 4H), 0.942 (t, *J* 7.6, 6H). δ_C (100 MHz, $CDCl_3$) 167.3, 163.0, 72.8, 55.9, 50.1, 20.7, 11.4. δ_B (96.3 MHz, $CDCl_3$) −1.84 to −12.54 (br m, 10B). Anal. Calc. for $C_{13}H_{36}N_4$: C 33.66, H 7.81, N 12.06. Found: C 33.45, H 7.85, N 12.12 %.

*2,4-Bis(*o-*carboran-1-yl)-6-(*N-*morpholinyl)-1,3,5-triazine* ***6d*** was synthesized from **5d** (0.13 g, 0.54 mmol) using the procedure described for **6a** to give **6d** in 38 % yield (92 mg, 0.20 mmol) as a white solid, mp 285°C. ν_{max} (KBr)/cm^{-1} 2590, 1576, 1497, 1283. δ_H (400 MHz, $CDCl_3$) 4.40 (s, 2H), 3.87 (t, *J* 4.8, 4H), 3.78 (t, *J* 4.8, 4H). δ_C (100 MHz, $CDCl_3$) 167.9, 162.9, 72.6, 66.2, 56.0, 44.1. δ_B (96.3MHz, $CDCl_3$) −1.78 to −12.75 (br m, 10B). Anal. Calc. for $C_{11}H_{30}N_4$: C 29.32, H 6.71, N 12.43. Found: C 29.43, H 6.64, N 12.18 %.

*2,4-Bis(2-methyl-*o-*carboran-1-yl)-6-(*N,N-*dimethylamino)-1,3,5-triazine* ***7a***

To a stirred solution of ***9a*** (87 mg, 0.55 mmol) in 5 mL THF at −10°C was added 0.3 mL of *n*-BuLi (1.6 M, 0.50 mmol) via a syringe. Compound **5a** (48 mg, 0.25 mmol) dissolved in THF (5 mL) was slowly added to the reaction mixture at −10°C, and the reaction temperature was maintained at −10°C for 1 h. The reaction mixture was then warmed slowly to room temperature, and stirred for an additional 3 h. The reaction was quenched with saturated aqueous NH_4Cl solution, and the organic layer was washed with brine, dried with anhydrous $MgSO_4$, and concentrated under reduced pressure. Purification by column chromatography on silica gel with EtOAc/hexane (1:100) gave **7a** in 71 % yield (77 mg, 0.18 mmol) as a white solid, mp 177°C. ν_{max} (KBr)/cm^{-1} 2934, 2561, 1605, 1493, 1414. δ_H (400 MHz, $CDCl_3$) 3.27 (s, 6H), 1.98 (s, 6H). δ_C (100 MHz, $CDCl_3$) 166.2, 164.2, 77.8, 76.1, 36.7, 24.0. δ_B (96.3MHz, $CDCl_3$) −0.86 to −8.99 (br m, 10B). Anal. Calc. for $C_{11}H_{32}N_4$: C 36.56, H 8.18, N 11.37. Found: C 36.70, H 8.23, N 11.08 %.

*2,4-Bis(2-methyl-*o-*carboran-1-yl)-6-(*N,N-*diethylamino)-1,3,5-triazine* ***7b*** was synthesized from **5b** (0.11 g, 0.51 mmol) and **9a** (0.15 g, 0.94 mmol) using the procedure described for **7a** to give **7b** in 49 % yield (0.12 g, 0.25 mmol) as a white solid, mp 145°C. ν_{max} (KBr)/cm^{-1} 2968, 2581, 1593, 1489. δ_H (400 MHz, $CDCl_3$) 3.64 (q, *J* 6.8, 4H), 1.97 (s, 6H), 1.25 (t, *J* 6.8, 6H). δ_C (100 MHz, $CDCl_3$) 166.3, 163.2, 77.9, 76.1, 43.0, 24.0, 12.6. δ_B (96.3MHz, $CDCl_3$) −0.89 to −9.01 (br m, 10B). *m/z* (HR-ESI, negative) Calc. for $C_{13}H_{36}B_{20}N_4$ $[M-H]^-$: 467.4723, found: 467.4725.

*2,4-Bis(2-methyl-*o-*carboran-1-yl)-6-(*N,N-*di-*n-*propylamino)-1,3,5-triazine* ***7c***

To a stirred solution of ***6c*** (0.16 g, 0.34 mmol) in 5 mL THF at 0°C was added 0.4 mL of *n*-BuLi hexane solution (1.6 M, 0.68 mmol) via a syringe. Iodomethane (42 µl, 0.68 mmol) was slowly added to the reaction mixture at 0°C, and the reaction temperature was then warmed slowly to room temperature. After the reaction was quenched with saturated aqueous NH_4Cl solution, the organic layer was washed with brine, dried over anhydrous $MgSO_4$, and concentrated under reduced pressure. Purification by column chromatography on silica gel with EtOAc/hexane (1:100) gave **7c** in 90 % yield (0.15 g, 0.31 mmol) as a white solid, mp 167-168°C. ν_{max} (KBr)/cm^{-1} 2968, 2561, 1589, 1497. δ_H (400 MHz, $CDCl_3$) 3.54 (t, *J* 7.6, 4H), 1.97 (s, 6H), 1.66 (sext, *J* 7.6, 4H), 0.97 (t, *J* 7.6, 6H). δ_C (100 MHz, $CDCl_3$) 166.2, 163.7, 77.9, 76.1, 50.1, 24.0, 20.7, 11.3. δ_B (96.3 MHz, $CDCl_3$) −0.77 to −9.02 (br m, 10B). Anal. Calc. for $C_{15}H_{40}N_4$: C 36.56, H 8.18, N 11.37. Found: C 36.39, H 8.32, N 11.25 %.

2,4-Bis(2-methyl-o-*carboran-1-yl)-6-(*N-*morpholinyl)-1,3,5-triazine* ***7d*** was synthesized from **6d** (0.13 g, 0.28 mmol) using the procedure described for **7c** to give **7d** in 67 % yield (90 mg, 0.19 mmol) as a white solid, mp 234°C. ν_{max} (KBr)/cm^{-1} 2590, 1578, 1497, 1281. δ_H (400 MHz, $CDCl_3$) 3.90 (t, *J* 4.8, 4H), 3.80 (t, *J* 4.8, 4H), 1.97 (s, 6H). δ_C (100 MHz, $CDCl_3$) 166.6, 163.6, 77.6, 76.2, 66.1, 44.1, 24.0. δ_B (96.3MHz, $CDCl_3$) −0.76 to −8.97 (br m, 10B). Anal. Calc. for $C_{13}H_{34}N_4$: C 32.62 H 7.16, N 11.71. Found: C 32.55, H 7.19, N 11.81 %.

2,4-Bis(2-ethyl-o-*carboran-1-yl)-6-(*N,N-*dimethylamino)-1,3,5-triazine* ***7e*** was synthesized from **6a** (0.21 g, 0.51 mmol) and iodoethane (80 μL, 1.0 mmol) using the procedure described for **7c** to give **7e** in 62 % yield (0.15 g, 0.32 mmol) as a white solid, mp 210°C. ν_{max} (KBr)/cm^{-1} 2584, 1609, 1491. δ_H (400 MHz, $CDCl_3$) 3.26 (s, 6H), 2.13 (q, *J* 7.6, 4H), 1.05 (t, *J* 7.6, 6H). δ_C (100 MHz, $CDCl_3$) 166.1, 164.3, 82.3, 79.4, 36.7, 29.3, 13.9. δ_B (96.3MHz, $CDCl_3$) −1.34 to −9.32 (br m, 10B). Anal. Calc. for $C_{13}H_{36}N_4$: C 33.60, H 7.81, N 12.06. Found: C 33.63, H 7.79, N 11.76 %.

2,4-Bis(2-ethyl-o-*carboran-1-yl)-6-(*N,N-*diethylamino)-1,3,5-triazine* ***7f*** was synthesized from **5b** (0.27 g, 1.2 mmol) and **9b** (0.38 g, 2.2 mmol) using the procedure described for **7a** to give **7f** in 38 % yield (0.23 g, 0.47 mmol) as a white solid, mp 164°C. ν_{max} (KBr)/cm^{-1} 2984, 2581, 1585, 1489. δ_H (400 MHz, $CDCl_3$) 3.64 (q, *J* 7.2, 4H), 2.13 (q, *J* 7.6, 6H), 1.25 (t, *J* 7.6, 6H), 1.06 (t, *J* 7.2, 6H). δ_C (100 MHz, $CDCl_3$) 166.1, 163.2, 82.3, 79.6, 43.0, 29.3, 13.9, 12.6. δ_B (96.3MHz, $CDCl_3$) −1.35 to −9.20 (br m, 10B). Anal. Calc. for $C_{15}H_{40}N_4$: C 36.56, H 8.18, N 11.37. Found: C 36.70, H 8.23, N 11.08 %.

2,4-Bis(2-ethyl-o-*carboran-1-yl)-6-(*N,N-*di*-n-*propylamino)-1,3,5-triazine* ***7g*** was synthesized from **5c** (75 mg, 0.30 mmol) and **9b** (0.10 g, 0.58 mmol) using the procedure described for **7a** to give **7 g** in 89 % yield (0.14 g, 0.27 mmol) as a white solid, mp 156°C. ν_{max} (KBr)/cm^{-1} 2934, 2573, 1591, 1493. δ_H (400 MHz, $CDCl_3$) 3.53 (t, *J* 7.6, 4H, $CH_2CH_2CH_3$), 2.13 (q, *J* 7.2, 4H, CH_2CH_3), 1.65 (sext, *J* 7.6, 4H), 1.05 (t, *J* 7.6, 6H), 0.965 (t, *J* 7.6, 6H). δ_C (100 MHz, $CDCl_3$) 166.1, 163.8, 82.3, 79.5, 50.1, 29.3, 20.8, 13.9, 11.3. δ_B (96.3MHz, $CDCl_3$) −1.59 to −9.327 (br m, 10B). Anal. Calc. for $C_{17}H_{44}N_4$: C 39.21, H 8.52, N 10.76. Found: C 39.40, H 8.58, N 10.58 %.

2,4-Bis(2-ethyl-o-*carboran-1-yl)-6-(*N-*morpholinyl)-1,3,5-triazine* ***7h*** was synthesized from **6d** (0.13 g, 0.28 mmol) and iodoethane (50 μL, 0.63 mmol) using the procedure described for **7c** to give **7 h** in 66 % yield (95 mg, 0.19 mmol) as a white solid, mp 216°C. ν_{max} (KBr)/cm^{-1} 2575, 1576, 1497. δ_H (400 MHz, $CDCl_3$) 3.91 (t, *J* 4.8, 4H), 3.80 (t, *J* 4.8, 4H), 2.12 (q, *J* 7.6, 4H), 1.06 (t, *J* 7.6, 6H). δ_C (100 MHz, $CDCl_3$) 166.5, 163.7, 82.3, 79.2, 66.1, 44.1, 29.3, 13.9. δ_B (96.3MHz, $CDCl_3$) −1.146 to −9.297 (br m, 10B). *m/z* (HR-ESI, negative) Calc. for $C_{15}H_{38}B_{20}N_4O$ $[M\text{-}H]^-$: 509.4828, found: 509.4827.

2,4-Bis(2-n-*propyl*-o-*carboran-1-yl)-6-(*N,N-*dimethylamino)-1,3,5-triazine* ***7i*** was synthesized from **6a** (0.23 g, 0.57 mmol) and iodpropane (0.11 mL, 1.1 mmol) using the procedure described for **7c** to give **7i** in 37 % yield (0.10 g, 0.21 mmol) as a white solid, mp 183°C. ν_{max} (KBr)/cm^{-1} 2968, 2579, 1605, 1491. δ_H (400 MHz, $CDCl_3$) 3.27 (s, 6H), 2.03 (t, J 8.4, 4H), 1.53–1.44 (m, 4H), 0.823 (t, *J* 7.6, 6H). δ_C (100 MHz, $CDCl_3$) 166.0, 164.2, 81.4, 79.3, 37.7, 36.7, 22.9, 13.7. δ_B (96.3MHz, $CDCl_3$) −1.35 to −9.30 (br m, 10B). Anal. Calc. for $C_{15}H_{40}N_4$: C 36.56, H 8.18, N 11.37. Found: C 36.50, H 8.19, N 11.07 %.

2,4-Bis(2-n-*propyl*-o-*carboran-1-yl)-6-(*N,N-*diethylamino)-1,3,5-triazine* ***7j*** was synthesized from **5b** (65 mg, 0.29 mmol) and **9c** (92 mg, 0.49 mmol) using the procedure described for **7a** to give **7j** in 75 % yield (0.12 g, 0.22 mmol) as a white solid, mp 185°C. ν_{max} (KBr)/cm^{-1} 2968, 2574, 1593, 1491. δ_H (400 MHz, $CDCl_3$) 3.65 (q, *J* 7.2, 4H), 2.03 (t, *J* 8.4, 4H), 1.54–1.43 (m, 4H), 1.25 (t, *J* 7.2, 6H), 0.825 (t, *J* 7.2, 6H). δ_C (100 MHz, $CDCl_3$) 166.1, 163.3, 81.4, 79.4, 42.9, 37.7, 22.9, 13.7, 12.6. δ_B (96.3MHz, $CDCl_3$) −1.28 to −9.30 (br m, 10B). Anal. Calc. for $C_{17}H_{44}N_4$: C 39.21, H 8.52, N 10.76. Found: C 39.27, H 8.56, N 10.52 %.

2,4-Bis(2-n-*propyl*-o-*carboran-1-yl)-6-(*N,N-*di*-n-*propylamino)-1,3,5-triazine* ***7k*** was synthesized from **5c** (63 mg, 0.25 mmol) and **9c** (70 mg, 0.37 mmol) using the procedure described for **7a** to give **7k** in 51 % yield (71 mg, 0.13 mmol) as a white solid, mp 182°C. ν_{max} (KBr)/cm^{-1} 2934, 2565, 1591, 1491. δ_H (400 MHz, $CDCl_3$) 3.55 (t, *J* 7.6, 4H), 2.02 (t, *J* 8.4, 4H), 1.66 (sext, *J* 7.6, 4H), 1.52-1.42 (m, 4H), 0.966 (t, *J* 7.2, 6H), 0.815 (t, *J* 7.2, 6H). δ_C (100 MHz, $CDCl_3$) 166.1, 163.9, 81.4, 79.4, 50.0, 37.7, 22.9, 20.8, 13.7, 11.3. δ_B (96.3MHz, $CDCl_3$) −1.25 to −9.27 (br m, 10B). Anal. Calc. for $C_{19}H_{48}N_4$: C 41.58, H 8.82, N 10.21. Found: C 41.49, H 8.98, N 10.21 %.

2,4-Bis(2-n-*propyl*-o-*carboran-1-yl)-6-(*N,N-*di*-n-*propylamino)-1,3,5-triazine* **7l** was synthesized from **5d** (63 mg, 0.27 mmol) and **9c** (95 mg, 0.51 mmol) using the procedure described for **7a** to give **7l** in 75 % yield (0.11 g, 0.20 mmol) as a white solid, mp 208°C. ν_{max} (KBr)/cm^{-1} 2968, 2567, 1574, 1493. δ_H (400 MHz, $CDCl_3$) 3.92 (t, *J* 4.8, 4H), 3.81 (t, *J* 4.8, 4H), 2.03 (t, *J* 8.4, 4H), 1.54–1.45 (m, 4H), 0.833 (t, *J* 7.2, 6H). δ_C (100 MHz, $CDCl_3$) 166.6, 163.8, 81.5, 79.1, 66.2, 44.1, 37.7, 23.0, 13.7. δ_B (96.3MHz, $CDCl_3$) −1.17 to −9.22 (br m, 10B). Anal. Calc. for $C_{17}H_{42}N_4$: C 38.18, H 7.92, N 10.48. Found: C 38.30, H 7.95, N 10.19 %.

Human Cancer Cell Line Panel Screening: To evaluate drugs for the cell growth inhibition profile, we established a human cancer cell line panel combined with a database. The system as a whole was developed according to the method of the National Cancer Institute,[38,39] with modification. The cell line panel consisted of 39 human cancer cell lines. With this system, we have examined the antiproliferative effect of more than 200 standard compounds, including various anticancer drugs, and established a new database, as described below.

Measurements of Cell Growth Inhibition as Data Analysis. The details of measuring cell growth inhibition are described elsewhere.[39,45] Briefly, the cells were plated at proper density in 96-well plates in RPMI 1640 with 5 % foetal bovine serum and allowed to attach overnight. The cells were exposed to compounds for 48 h. Then, the cell growth was determined according to the sulforhodamine B assay, described by Skehan et al.[46] Data calculations were made according to the method described previously.[40] Absorbance for the control well (C) and the tests well (T) were measured at 525 nm. Moreover, at time 0 (addition of compounds), absorbance for the test well (T_0) was also measured. Using these measurements, cell growth inhibition (percentage of growth) by each concentration of compounds was calculated as: % growth = $100 \times [(T - T_0)/(C - T_0)]$, when $T > T_0$ and % growth = $100 \times [(T - T_0)/T]$, when $T < T_0$. By using the computer to process % growth values, the 50 % growth inhibition parameter (GI_{50}) was determined. The GI_{50} was calculated as $100 \times [(T - T_0)/(C - T_0)] = 50$. The mean graph, which shows the differential growth inhibition of the drug in the cell line panel, was drawn based on a calculation using a set of GI_{50}.[38,45] To analyze the correlation between the mean graphs of compounds A and B, COMPARE computer algorithm was developed to the method described by Paull et al.[39] Correlation coefficients were calculated using the following formula: $r = (\Sigma(x_i - x_m)(y_i - y_m))/(\Sigma(x_i - x_m)^2\Sigma(y_i - y_m)^2)^{1/2}$, where x_i and y_i are log GI_{50} of

compounds A and B, respectively, against each cell line, and x_m and y_m are the mean values of x_i and y_i, respectively.

Topoisomerase I and II assays: Topoisomerase assays were performed according to the manufacturer's instructions of TopoGen. Briefly, 0.5 Ag supercoiled DNA, one unit topoisomerase I, and the compounds were incubated for 30 min at 37°C in 35 mM Tris-HCl (pH 8.0), 72 mM KCl, 5 mM $MgCl_2$, and 5 mM DTT. The reactions were ceased by the addition of 6 loading dye solution and then subjected to electrophoresis on 1 % agarose gels, with or without ethidium bromide. Topoisomerase II assays were prepared as above, except that 125 ng supercoiled DNA and four units topoisomerase II, together with the chemical compounds were incubated for 30 min at 37°C in 10 mM Tris-HCl (pH 7.9), 50 mM NaCl, 50 mM KCl, 5 mM $MgCl_2$, 0.1 mM EDTA, and 1 mM ATP.

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