Scientific paper

Labeled Undecahydro-*closo*-dodecaborates Based on Azo Dyes for Boron Neutron Capture Therapy: Synthesis, Characterization, and Visualization in Cells

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Received: 02-06-2011

Abstract

A general approach to the synthesis of novel boronated azo dyes derived from undecahydro-*closo*-dodecaborates as a convenient preparation of dye labeled for boron neutron capture therapy (BNCT) is described. The method focused on the synthesis of two classes of dye-labeled dodecaborate anions. One is the reaction of $(CH_3)_4NB_{12}H_{11}NH_3^-$ with $NaNO_2$ in acetonitrile/water to give its diazonium salt which consequently reacted with substituted phenols to produce boronated azo-dyes ($B_{12}H_{11}$–N=N–Ar$^-$, Ar = 4-HOC_6H_5, 1-naphthol, 2-naphthol, 2,3-$(HO)_2C_6H_4$, 3-MeO-4-HOC_6H_4, 2-HO-5-$MeOC_6H_4$, and 4-Me_2N-C_6H_4). The second is the reaction of aryldiazonium salt as couplers with disodium salt of dodecaborates ($B_{12}H_{11}X^{2-}$, X = SH or OH) to yield substituted dodecaborate azo-dyes ($HXB_{12}H_{10}$–N=N–Ar$^-$, Ar = *para*-bromo, *para*-nitro, *para*-carboxy, *meta*-carboxy, *para*-sulfonamide, and *para*-sulfonic acid). The results show the expected effect of the various substituents on the efficiency of the coupling reactions. Extension of similar strategies to tyrosine and 5-(*para*-aminophenyl)-10,15,20-triphenylporphyrin diazonium salt, we succeeded to get dodecaborate anion containing amino acid or porphyrin as candidates for BNCT, respectively. Dye-labeled dodecaborates were obtained in acceptable yields. The proposed methodology provides not only a convenient way to synthesize libraries of boron cluster modified azo dyes for various applications but also for the visualization of boron clusters in cells.

Keywords: Azo dyes, BNCT, porphyrin, amino acid, boron clusters, dodecaborate

1. Introduction

The principle of boron neutron capture therapy (BNCT) for cancer treatment is dependent upon the irradiation of ^{10}B with a beam of low energy neutrons to produce high energy α-particles and lithium-7 nuclei.[1,2] These particles dissipate their kinetic energies before traveling a distance equivalent to one cell diameter (~10 μm), enabling them to precisely kill tumor cells. To afford selective treatment by BNCT it is essential that targeted delivery of boron is achieved; this has been realized using strategies that involve synthetic chemical, biochemical and biophysical approaches.[3–5] Successful BNCT highly depends on the sufficient and selective boron delivery to the tumor cells. Therefore, the development of boron compounds that accumulate in the tumor cells in the appropriate concentrations is essential for BNCT.

The advantages of $B_{12}H_{12}^{2-}$ icosahedron are its hydrophilic properties and simple methods of the parent anion synthesis from ^{10}B-enriched raw material; additionally it is known to be harmless to man and is therefore an interesting compound for BNCT. Whereas the main problems of the $B_{12}H_{12}^{2-}$ synthetic chemistry compared to that of carborane are the absence of a distinguished reaction center due to its high, nearly spherical, symmetry and high reactivity with respect to electrophiles often giving mixtures of products with various substitution degrees. To avoid this complication, the primary introduction of a reaction center (–SH, $–NH_3^+$, –OH, or –I) is necessary.[6–13]

Azo dyes are among the most useful synthons in the chemist's toolbox, and new, high-yield methods for their

synthesis are always welcome. A review of the literature demonstrates that boronated azo dyes have been versatile building blocks for biomedicine,[14–16] material science[17] and basic organic chemistry of boranes.[18,19] In recent years, dye-labeling with azobenzene derivatives has become important for many biologically relevant products such as DNA probes,[20] biochemical analogs,[21] lipids,[22] cytokines,[23] cells,[24] polymers[25,26] and monolayers.[27,28] Such dye-labeling facilitates the isolation and detection of biologically active molecules.[29–32] Boronic acid azo dyes have been known for over 40 years; they were used for investigations in the treatment of cancer by BNCT.[33,34] Some of azo compounds containing boron atoms were found to be effective in the medical treatment of brain tumors.[14,15] It had been known that a number of azo compounds containing negative atoms or groups such as halogens, hydroxyl, and nitro groups are also effective, if irradiated, even without boron atoms. However, the azo compounds containing boron atoms are more effective with respect to the accumulation in tumors than the compounds without boron atoms. A number of boronated azo dyes were obtained by coupling *ortho*-hydroxybenzeneboronic acids anhydride with the diazonium chlorides of benzene, *para*-bromobenzene, sulfonamides and nitrobenzene.[18] It was expected that if boron atoms were introduced into sulfonamides by coupling them with boronic acids, the antibiotic effect would be even more remarkable. A fairly recent development has been the study of the effect of saccharides on the color of dyes containing the boronic acid functionality.[35,36] Coupling reaction of carborane diazonium salts with 2-naphthol resulted in the formation of azo dyes as boron labeled antibodies to carcinoembryonic antigen.[16] Azo dyes of $B_{10}H_{10}^{2-}$ salts were also prepared by coupling reaction with aryldiazonium ions in acetonitrile to give apically substituted $B_{10}H_{10}^{2-}$ derivatives related to the azobenzenes.[19,37] In contrast to the extremely rapid reactions of $B_{10}H_{10}^{2-}$, $B_{12}H_{12}^{2-}$ did not react with aryldiazonium ions in acetonitrile.[37] Reaction of $B_{10}H_9NH_3^-$ with benzenediazonium ion led to coupling and the formation of an azo dye containing the $-NH_3^+$ group as a substituent on the B_{10} polyhedron.

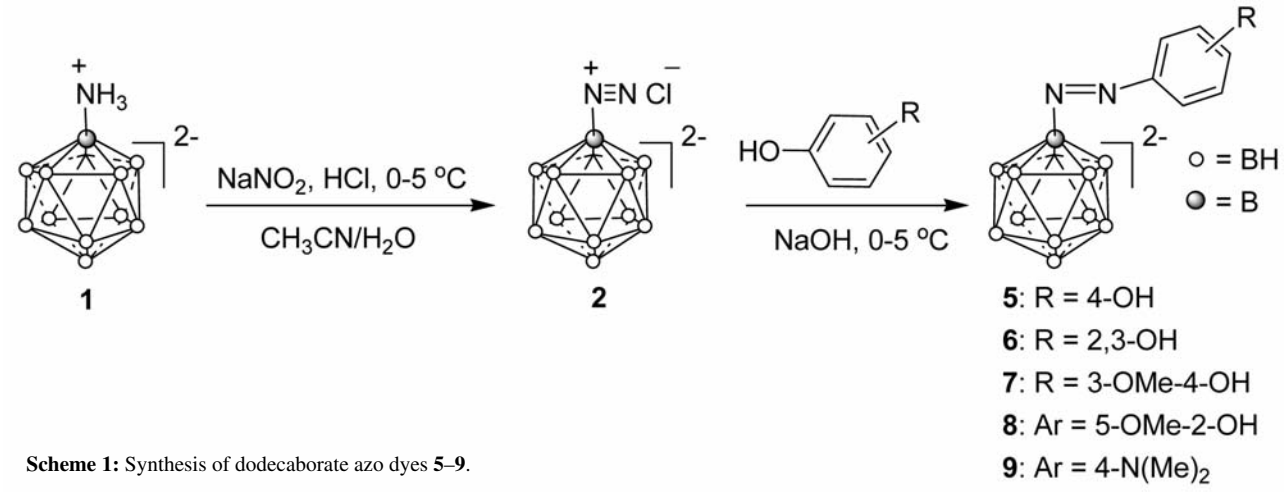
We wish to report herein the extremely facial azo coupling reaction which occurs between a variety of hydroxyl arenes and the sodium salt of amine dodecaborate ($NaB_{12}H_{11}NH_3^-$, **1**) after conversion to its diazonium salt **2**. The resulting products are highly colored dyes and as such are monosubstituted dodecaborate anions. We also report the azo coupling of aryldiazonium salts with disodium salts of dodecaborates ($Na_2B_{12}H_{11}SH^{2-}$, **3** or $Na_2B_{12}H_{11}OH^{2-}$, **4**) to give disubstituted dodecaborate anions.

2. Results and Discussion

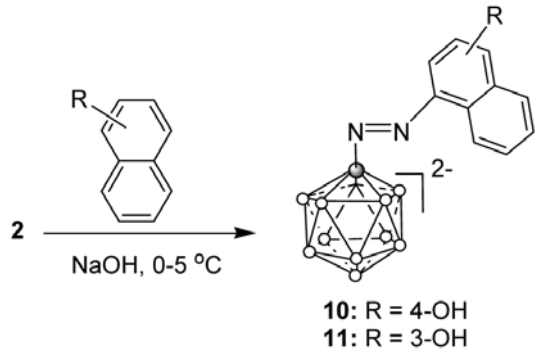
2. 1. Synthesis of Undecahydro-*closo*-dodecaborates Based Azo Dyes

The formation of stable undecahydro-*closo*-dodecaborate anions containing azo group seems to be one of the characteristic features of the chemistry of polyhedral boron hydrides. Polyhedron *closo*-borane dianions such as ($B_{12}H_{12}^{2-}$) are regarded as three-dimensional aromatic species.[38] Consequently, their reactivity and bonding properties have received attention because of the analogy to arenes. Three distinct features of azo dye labeled dodecaborates have called to our attention the possibility of applying this approach to the synthesis of BNCT agents: *(1) in photoresponsive reporters to monitor, regulate or control the activity of boronated prodrugs; (2) the synthetic operation can be accomplished in a benign solvent, usually water; and (3) azo groups are structural motifs with considerable medicinal and agrochemical potential.*[39]

In our synthetic strategy, two types of undecahydro-*closo*-dodecaborate azo dye anions **5**–**23** building blocks were constructed as illustrated in Schemes 1–6. First, the procedure prior to the synthesis of the dyes obviously requires the conversion of **1** to the diazonium salt **2**, and then coupling with phenols (e.g. phenol, 1-naphthol, 2-naphthol, catechol, 2-methoxyphenol, 4-methoxyphenol and *N*,*N*-dimethylaminobenzene) for 2 h in acetonitri-



Scheme 1: Synthesis of dodecaborate azo dyes **5**–**9**.

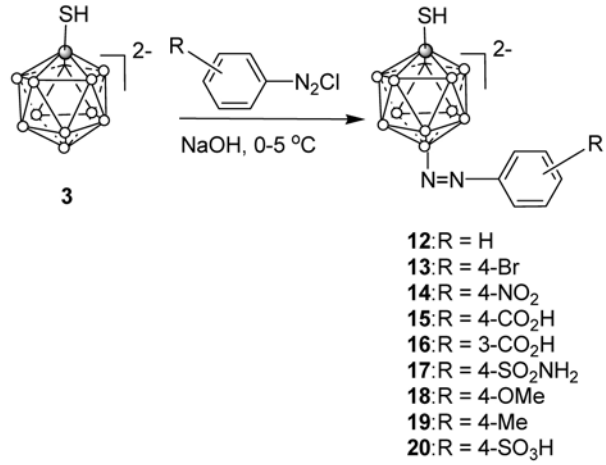


Scheme 2: Synthesis of dodecaborate azo dyes **10** and **11**.

le/water (1:1) at 0 °C using NaOH as a base (Schemes 1 and 2). The isolated yields **5**–**11** were ranging from 35–86%. Regardless of the substituent on the phenols, the yield of catechol diazo compound **8** was low and substantial amounts of impurities were produced. The low yields are not surprising when it is taken into account that dodecaboratediazonium salts undergo hemolytic dediazotization reactions under certain reducing conditions.

It has been reported that *para*-hydroquinone and ascorbic acid can reduce diazonium salts.[40] EPR studies have shown that even under acidic conditions catechol can reduce 4-methoxyphenyldiazonium ion through one electron reduction to form the aryl radical intermediate.[41,42] Azo dyes of catechol are of interest due to their chromophoric nature and the bidentate character of their *ortho* phenolic hydroxyl groups. These properties have made them useful for metal complexation studies[43] and for spectroscopic measurement of cation concentrations.[44,45] Their wider utility is limited by a lack of generally applicable, yet efficient, methods for their synthesis. The limitations of existing methods became obvious in our attempts to prepare catechol azo compounds for use as chromophoric substrates for redox enzymes. There are at least two factors affecting the dediazotization reaction. One factor concerns the structure of the reducing agent, which determines the transition state of the redox reaction. The primary factor, however, is the reducing ability of the reducing agent. It has been shown that molecules with oxidation potentials higher than 1 V versus normal hydrogen electrode (NHE) are poor reducing agents for diazonium salts.[46] Taking the dediazotization reaction into account, it was not unexpected that phenol gave the highest yield from diazo coupling compared to guaiacol (2-methoxyphenol) and catechol. Guaiacol, which has a peak current potential (0.52 V) lower than that of phenol (0.60 V) but higher than that of catechol (0.42 V), gave yields in diazo coupling between that obtained for catechol and phenol. The peak current potential for catechol is comparable to that of *para*-hydroquinone. Presumably, guaiacol can reduce diazonium salts, albeit to a lesser extent than catechol. An important aspect to consider for catechol in a diazo coupling reaction is its tendency to oxidize to unstable *ortho*-quinone. Oxidation of catechol becomes increasingly rapid with increasing pH (more alkaline). However, a high pH also increases the reactivity of catechol to diazo coupling. It is the balancing of these two characteristics of catechol which leads to compromises in the yield of reaction. In the case of *ortho*-nitro-, *para*-nitro- and *ortho*-cyanophenol, we did not observe any thin-layer chromatographic evidence for the presence of diazocoupling species in these reactions.

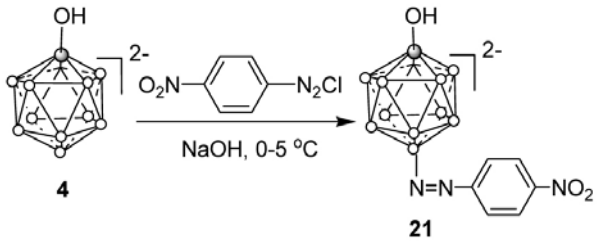
We next turned our attention to prepare second types of azo dyes containing undecahydro-*closo*-dodecaborate azo dye anions. Scheme 3 demonstrates our successful approach to the synthesis of 12-(arylazo)mecaptoundecahydro-*closo*-dodecaborate anions **12**–**20**. In this case the diazonium ions of aniline or its derivatives (*para*-bromo, *para*-nitro, *para*-carboxy, *meta*-carboxy, *para*-sulfonamide, *para*-methoxy, *para*-tolyl, and *para*-sulfonic acid) were then coupled by nucleophilic substitution with the mercaptododecaborate anion as a substrate.



Scheme 3: Synthesis of dodecaborate azo dyes **12**–**20**.

The resulting colored compounds **5**–**20** were converted to their tetramethylammonium salts and then purified by flash column chromatography using MeOH/CH_2Cl_2 (1:4) as a mobile phase. These coupling reactions are accompanied by side reactions that have not been thoroughly studied. Although a high pH increases the reactivity of diazo coupling reactions, when the direct coupling method was used for the synthesis of compounds **5**–**20**, it was found that apart from the consideration of pH, efficiency of diazo coupling reactions depended on the nature of substituents on the aryl diazonium salts as well. Aryldiazonium salts with electron-withdrawing substituents, such as nitro and sulfonic acid, gave higher yields of diazo product than phenyldiazonium, whereas aryldiazonium salts with electron-donating substituents, such as methyl or methoxy, gave lower yields. The dependency of the yield of diazo product on the nature of the diazonium salt

(see experimental section) is in agreement with the premise that electron-withdrawing groups increase the reactivity of diazonium salts. However, these groups also promote the ability of the diazonium salt to accept an electron in homolytic dediazotization reactions.[47] The results show the expected effect of the various substituents on the efficiency of the coupling reaction between **3** with aryl diazonium salt. Conversely to **3**, treatment of phenyldiazonium salt with **4** failed to produce the desired boronated azo dyes. However, diazocoupling reaction of **4** with 4-nitrophenyldiazonium salt gave azo compound **21** in good yield (Scheme 4).

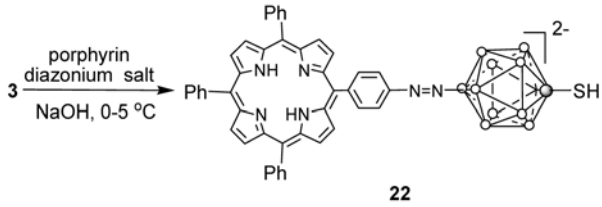


Scheme 4: Synthesis of dodecaborate azo dye **21.**

Furthermore, the reaction of aryldiazonium salts with **4** was substantially slower than their reaction with BSH. Compared to **3** or $B_{12}H_{12}^{2-}$, the reactivity of B_{12} cluster is strongly affected by substitution on B1 atom. The expected effect of the various substituents is an important factor on the efficiency of the coupling reaction between **4** with aryl diazonium salt. Another factor which limits the reaction of **4** for diazo coupling reactions is the presence of the OH group, which increases the deshielding due to the *–I* effect of the oxygen atom compared with compound **3**.[48] Furthermore, the reactivity of the SH group in **3** does not resemble that of an SH group bonded to a carbon; rather, its reactivity resembles more closely that of an organic hydroxyl group.[8]

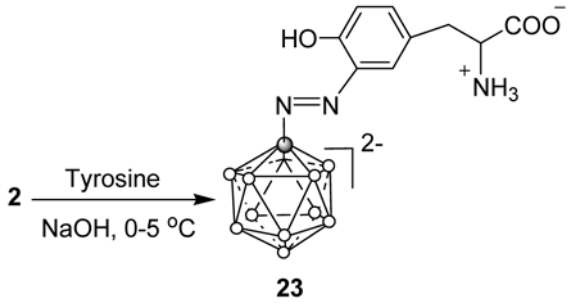
2. 2. Candidates for Boron Carriers for BNCT

An efficient BNCT agent should be able to deliver a therapeutic amount of ^{10}B to tumors (> 20 μg/g) with high selectivity and low systemic toxicity.[1] The advantage of boron cluster containing compounds is that they can deliver high concentrations of boron to tumor cells with tolerable toxicity per molecule of BNCT agent administered. Polyhedral borane anions of *closo*-$B_{12}H_{12}^{2-}$ type have been utilized for this purpose because of their known chemistry, hydrophilic properties, high boron content, and chemical stability. The compounds currently used for clinical trials of BNCT applied to malignant brain tumors are the sulfhydryl boron hydride $Na_2B_{12}H_{11}SH$ (BSH) and the amino acid *para*-boronophenylalanine (BPA), which yield tumor to blood boron concentration ratios of 1:1 and 3:1, respectively.[49,50] It would be useful if combinations of these and/or new boron carriers could achieve higher tumor-selective ratios without undesirable chemotoxicity. On the other hand, the family of dyes most extensively studied with respect to photodynamic therapy (PDT) and BNCT are the porphyrins and related macrocycles (e.g., chlorines, phthalocyanines, and porphyrazines).[1,51] In many aspects, these drugs appear to act similarly. For a porphyrin to be useful in either of these therapies, it should be preferentially accumulated or retained in the tumor tissue with respect to the surrounding healthy tissue. There are several uptake mechanisms for porphyrins into tumor cells,[52] but there exists no general rule for the nature of the structure-uptake correlation. Using the BNCT and PDT approaches as references, we synthesized boronated porphyrin **22** from **3** using the diazocoupling reaction. Compound **3** was treated with 1.0 equiv of diazonium salt of 5-(*para*-aminophenyl)-10,15,20-triphenylporphyrin at 0 °C to afford **22** in 87% yield (Scheme 5). This new strategy in the synthesis of boronated compounds may be used to optimize BNCT and PDT therapies. Moreover, some of the boron-containing porphyrins could be used in other fields of medicine.[53]



Scheme 5: Synthesis of dodecaborate azo dye **22.**

In addition to the boronated porphyrins approach, the amino acid boron delivery system has been attracting attention because it can deliver high therapeutic amounts of boron to the tumor tissue.[54] In recent years, encouraging clinical results have been obtained using 4-dihydroxyborylphenylalanine (BPA) as the tumor-specific boronated agent.[55] It is believed that the amino acids are preferentially taken up by growing tumor cells. Amino acids might therefore be useful vehicles for transporting boron to tumor tissue. To date, a variety of amino acids have



Scheme 6: Synthesis of dodecaborate azo dye **23.**

been used to deliver boron to tumor cells.[54] The studies have been focused on the synthesis of amino acids containing only one boron atom. In the present study, we demonstrated a new route for the preparation of the first *closo*-dodecaborate amino acid *via* azo coupling reaction (Scheme 6).

For tyrosine coupling, it was necessary to prepare the requisite diazonium salt of **1**. The reaction of diazonium salt **2** with 1.0 equiv of tyrosine at 0 °C gave **23** in 67% yield, after purification by column chromatography.

2. 3. Spectral Properties of Dyes

To gain information about the structures of the boronated azo-dyes NMR spectroscopy, IR spectroscopy, UV-Vis, mass spectrometry, and elemental analysis were conducted. Peak assignments for ^{11}B, ^{1}H, and ^{13}C NMR spectra of all compounds **5**–**23** in CD_3CN are listed in the Experimental Section. ^{11}B NMR spectra of boronated azo dyes were consistent with the proposed structure and proved the site of attachment of the azo linkage to be at an apical position of the B1 or B_{12} polyhedron. ^{1}H NMR signals for aromatic protons of the azo substituted derivatives of $B_{12}H_{11}NH_3^{2-}$ (**5**–**11** and **23**) appeared in the range of 8.8–6.8 ppm, and the BH protons at ca. 0.5–2.2 ppm. The disappearance of NH_3 signals of $B_{12}H_{11}NH_3^{2-}$ indicated the formation of a diazonium salt that is available for further coupling, as expected from the diazocoupling reaction of **1** with phenols (Figure 1).

Whereas ^{13}C NMR signals for aromatic carbons (C and CH) appeared at ca. 159, 145, 130, 128 and 113 ppm, respectively, the new signal appeared at low field region (ca. 145 ppm) in ^{13}C NMR spectra corresponding to C–N=N of the boronated azo dyes. Relative integrations of the signals at ca. 8.8–6.8 ppm (CH-aromatic), at ca. 6.0–5.4 ppm (OH), and 1.8–0.45 ppm ($B_{12}H_{11}^{2-}$) demonstrated the nearly quantitative incorporation of phenol(s) into the dodecaborate diazonium salt of **1**. The ^{1}H NMR spectra of the boronated compounds **12**–**22** revealed signals characteristic of both $B_{12}H_{11}X^{2-}$ (X = SH or OH) derivatives and aniline derivatives, with disappearance of NH_2 signal of the aniline derivatives. Similarly, the ^{13}C NMR spectra of **12**–**22** showed a new peak at ca. 145 ppm that corresponded to C–N=N which is located at a slightly

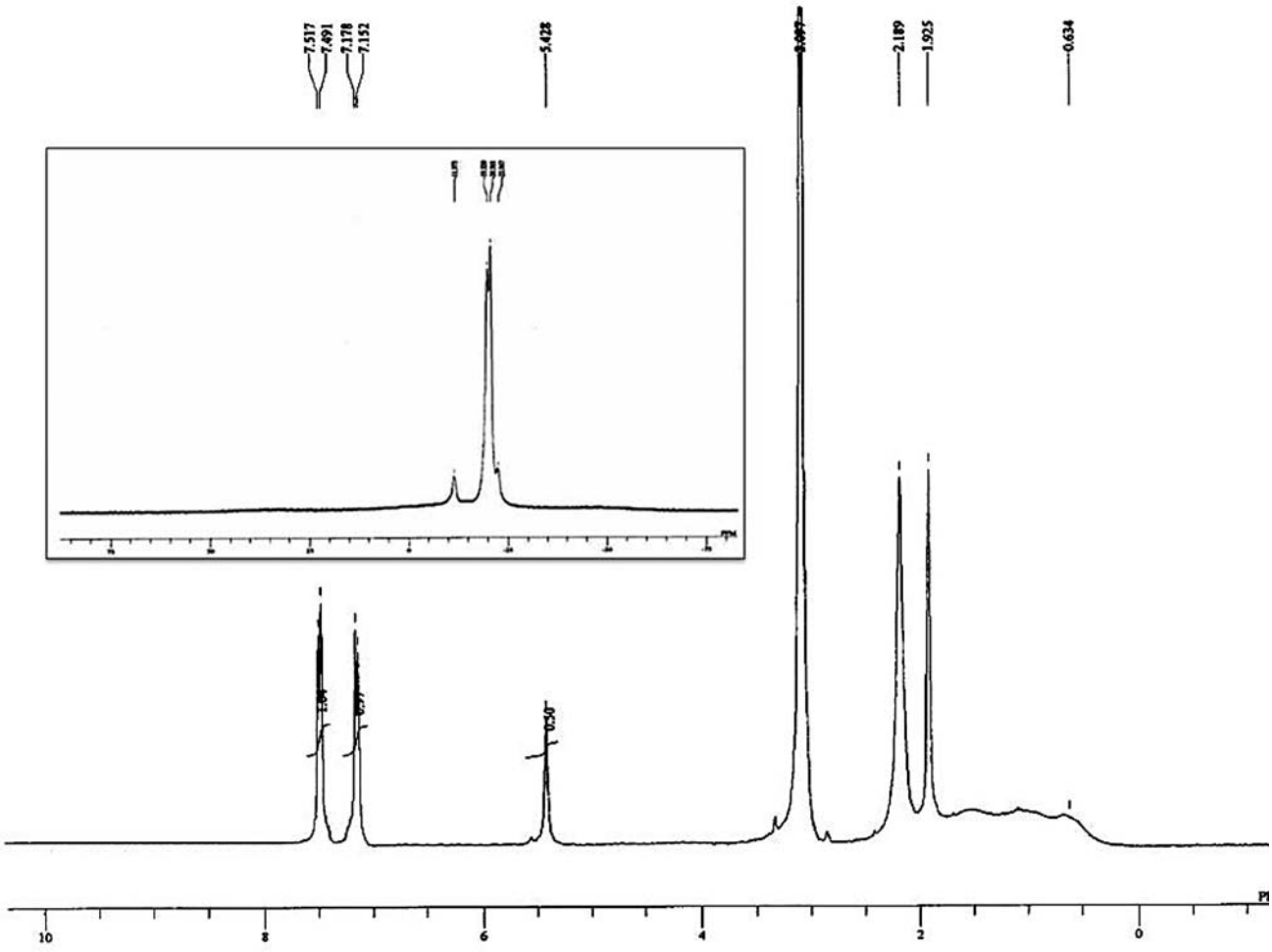


Figure 1: 300 MHz ^{1}H NMR spectrum of compound **5** (inset: 96.3 MHz ^{11}B NMR spectrum) in CD_3CN at 20 °C.

lower field than the signal of the methylene group of C–NH_2 of the starting aniline derivatives at 139 ppm.

The ^{11}B NMR spectra presented a characteristic shielding pattern over a quite remarkable range of ca. –22 to –11.5 ppm for the azo dyes of BNH (**5–11** and **23**), showing only minor differences in the overall ^{11}B NMR cluster shielding patterns. These spectra showed the 1:5:5:1 pattern typical of monosubstituted B_{12} derivatives (Figure 1). In contrast, the spectra of **12–22** showed the 1:1:5:5 pattern typical of disubstituted B_{12} derivatives. The ^{11}B NMR spectra of compounds **12–22** consisted of singlets at –9.25 and –10.25 ppm and a multiplet in the range of –21.08 to –22.5 ppm. The low field singlets were assigned to two equatorial boron atoms. The singlets represented the points of attachment of the SH or OH and azo groups to the B_1 and B_{12} cages which corresponded to apical boron atoms, respectively.

All synthesized boronated compounds were characterized by electrospray ionization mass spectrometry (ESI-MS). Generally, ESI-MS is considered to be the softest of all known MS ionization methods. Consequently, the fragmentation of sample ions is usually not observed unless it is deliberately induced by collision-induced dissociation in the nozzle-skimmer region (sCID) or MS/MS. Hence, the negative-ion ESI mass spectra of compounds **5–11** and **23** showed only the signal of a singly charged ion whose mass and typical isotopic pattern of boron isotopes (^{10}B and ^{11}B) suggest the molecular formula at $m/z = M^-$. The ESI mass spectra of **12–22** showed only the signal of the doubly charged molecular anion that was attributed to $m/z = M^-/2$.

Azo dyes have characteristic stretching modes that are suitable for study by IR spectroscopy. Free $B_{12}H_{11}NH_3^-$ (**1**) exhibited a strong absorption bands at 3285 and 1625 cm^{-1} due to the asymmetric stretching and the bending vibration of the NH group, respectively. These absorption bands of the NH group completely disappeared in the IR spectra of compounds **5–11**. This was replaced with new medium absorption band within regions 1596–1585 cm^{-1}, which are characteristic of N=N groups. The IR spectra of **14** and **21** contained absorption bands located at ca. 1515 and 1332 cm^{-1}, which could be attributed to the vibrational mode of the NO_2 group, while the SO_2 group of compounds **18** and **21** has two vibrational frequencies at ca. 1355 and 1152 cm^{-1}. Furthermore, the IR spectra of the compounds **15**, **16** and **23** confirmed the presence of the C=O group which resonates at 1716–1725 cm^{-1}. The vibrational frequencies of the B–H bond, ν(B–H), or the B–B bond, ν(B–B), were not sensitive to the click reactions. For compounds **5–23** ν(B–H) lay in the 2499–2585 cm^{-1} region, whereas ν(B–B) varied from 1049 to 1045 cm^{-1}. Among the frequencies of the $B_{12}H_{12}^{2-}$ moiety: ν(B–H) 2486 to 2462 cm^{-1}; ν(B–B) 1073 to 1057 cm^{-1};[13] only slight differences were found among the compounds, indicating that intracluster bonding was not perturbed by the substitution of the icosahedron.

Figure 2 represents the electronic spectra of diazonium salt **2** and azododecaborates in acetonitrile. The band of shortest wavelength appearing in the range 215–245 nm was best ascribed to π–π^* transition of the benzenoid system and dodecaborate cluster of the compounds.

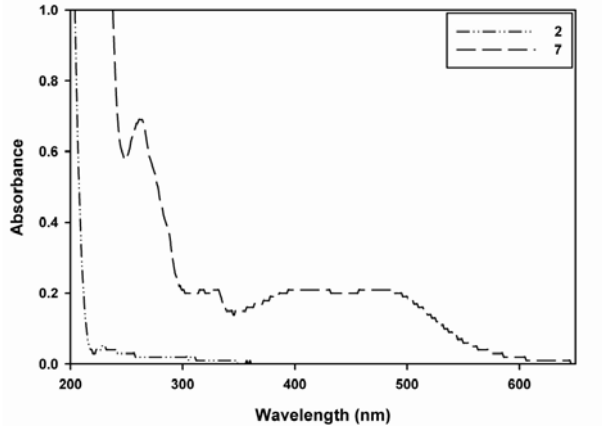


Figure 2: Electronic absorption spectra of compounds **2** and **7** in CH_3CN.

The band observed in the UV region at 285–290 nm was assigned to n–π^* electronic transition of OH or N=N groups. The long wavelength band ranging from 385 to 525 nm corresponds to the azo form. This band was capable of being assigned to π–π^* transition involving the whole electronic system of the compounds with a considerable charge-transfer (CT) character. Such a CT originated mainly from the azododecaborate to the benzene moiety, i.e. this band was due to intramolecular CT transition. In the case of porphyrin azo dye **22**, it was difficult to draw an unambiguous conclusion as to whether the π system of the azo dye interacts with the π system of the porphyrin ring. The Soret band of tetraphenyporphyrin (λ_{max} ~410 nm) is found alongside with the broad absorption band of the azo dye residue (λ_{max} ~585 nm), which does not permit a confident judgment to be made on whether transfer of π electron density from the azo dye residue to the porphyrin ring has taken place. However, the sharp reduction in intensity of the Soret band and the growth in intensity of the electronic transition and also their bathochromic shift indicate the existence of such interactions.

2. 4. Visualization of Compound 3 by Azo Dye Reaction with Benzene Diazonium Salt in HeLa Cells.

The development of technology for the chemical modification of compounds in, or on, living cells under physiological conditions has become an important issue for the dynamic imaging of drugs in medicinal chemistry.

We examined the azo reaction of compound **3** with benzene diazonium salt in HeLa cells. The cells were plated on dishes and incubated at 37 °C for 24 h. Then, they were treated with compound **3** (1 mM) for 3 h. After fixing the cells with 4% paraformaldehyde in PBS for 10 min, the azo reaction was performed with benzene diazonium salt. UV-Vis spectra are shown in Figure 3.

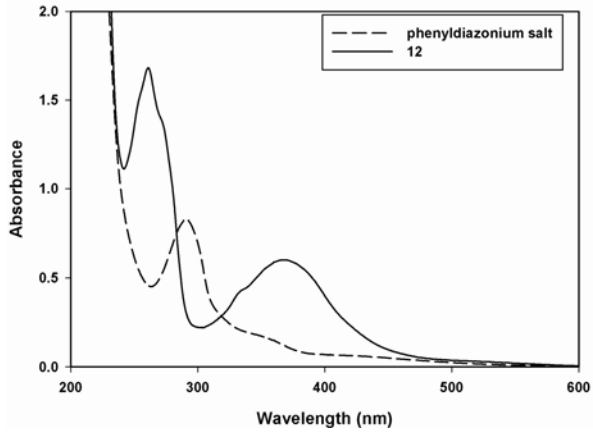


Figure 3: Absorption spectra of compound **12** and phenyldiazonium salt in HeLa cells.

HeLa cells treated with DMSO and benzene diazonium salt did not show any visible bands. In contrast, cells treated with compound **3** and benzene diazonium salt showed yellow color.

3. Conclusions

A novel and rapid spectrophotometric visualization method for the determination of dodecaborate anions is proposed in this paper. This convenient and efficient method for the preparation under mild and simple reaction conditions of azo dye labeled dodecaborate anions has been developed by reacting amino-, hydroxyl- or mercaptoundecahydro-*closo*-dodecaborates with phenols or benzene diazonium salts at low temperature. The synthesis of boronated amino acid and porphyrin was also achieved by the azo coupling reaction, which may be useful in the boron delivery system for neutron capture therapy. All the azo dye labeled products were isolated in good yields. It was possible to obtain suitable colored dodecaborate anions with maximum absorption peaks ranging from 385 to 475 nm by choosing the appropriate azo chromophore. All azo compounds under investigation displayed two or three bands in the UV region in acetonitrile as the solvent. The first and second bands attributed to π–π* transition in benzenoid system and dodecaborate moiety, respectively, whereas the third band in UV region was assigned to n–π* electronic transition. In the visible region, all the compounds displayed main broad visible band, which were attributed to intramolecular CT transition. The current reactions require only benign reaction conditions and simple workup and purification procedures. Finally, we demonstrated the azo coupling reaction of compound **3** with benzene diazonium salt in HeLa cells to give a yellow color solution as a marker of compound **3**. We believe that this study not only provides synthetic applications but also clarifies the biological mechanism of dodecaborate derivatives for neutron capture therapy.

4. Experimental Section

4. 1. General Remarks

^{1}H NMR and ^{13}C NMR spectra were measured on a JEOL JNM-AL 300 (300 MHz) and VARIAN UNITY-INOVA 400 (400 MHz) spectrometers. Chemical shifts of ^{1}H NMR and ^{13}C NMR were expressed in parts per million (ppm, δ units), and coupling constant (*J*) values were in hertz (Hz). ^{11}B NMR spectra were recorded on a JEOL JNM-AL 300 spectrometer (96.3 MHz) and the chemical shifts were reported in δ units relative to external BF_3 Et_2O in $CDCl_3$. IR (cm^{-1}) spectra were determined as KBr disc on a Shimadzu FTIR-8600PC spectrometer. Electron spray ionization (ESI) mass spectra were recorded on a Shimadzu LCMS-2010 eV spectrometer. UV-Vis spectra were measured on a Shimadzu 2450 PC spectrophotometer within the wavelength range 200–700 nm. Elemental analyses were performed by a CE instrument EA1110 CHNS-O automatic elemental analyzer. All compounds gave elemental analysis within ±0.4 of the theoretical values. Analytical thin-layer chromatography (TLC) was performed on a glass plates of silica gel 60 GF_{254} (Merck). Visualization was achieved by UV light (254 nm), I_2, $KMnO_4$, or $PdCl_2$. Preparative TLC was carried out using 0.75 mm layers of silica gel 60 GF_{254} (Merck) made from water slurries on glass plates of dimensions 20 × 20 cm^2, followed by drying in air at 100 °C. Plate chromatography was conducted on Sigma-Aldrich TLC plates silica gel matrix, H × W 20 cm × 20 cm. All chemicals and solvents used in this study were of analytical grade. Melting points were recorded on Gallenkamp apparatus. Most chemicals were of analytical grade and used without further purification. 5-(*para*-Aminophenyl)-10,15,20-triphenylporphyrin, $Na_2B_{12}H_{11}SH$, $[(CH_3)_4N]B_{12}H_{11}NH_3$ and $Na_2B_{12}H_{11}OH$ were prepared as described in literature and stored at –20 °C.[6–8,56]

4-Hydroxyphenyl-*azo*-undecahydro-*closo*-dodecaborate(–1) Tetramethylammonium Salt (5).

To a stirring solution of **1** (232 mg, 1.0 mmol) in acetonitrile/water (10 mL, 1:1) at 0 °C was added 2 M HCl (2 mL). A 0 °C solution of $NaNO_2$ (210 mg, 3.0 mmol) in acetonitrile/water (5 mL, 1:1) was added dropwise to the reaction mixture, and stirred for 20 min. To

this solution, phenol (95 mg, 1.0 mmol) in 5 mL NaOH (100 mg, 2.5 mmol) was slowly added and the reaction mixture was stirred for 30 min at the same temperature. The reaction was allowed to warm to room temperature and stirred for an additional 3 h. The resulting solution was acidified with 1 M HCl. The solvents were evaported in vacuo. The product was purified by TLC using MeOH/CH_2Cl_2 (1:4) as a mobile phase to give **5** (220 mg, 65%) as a yellow solid. R_f = 0.29, mp 195–197 °C. IR (KBr, cm^{-1}) ν 3606 (OH), 3259, 3207, 3097 (CH), 2495 (BH), 1596 (N=N), 1525 (C=C),1469, 1410, 1384 (CH), 1249 (C–O), 1161 (CN), 1101, 1055 (B–B), 970, 883, 723 (CH). ^{1}H NMR (300 MHz, CD_3CN): δ 7.51 (d, 2H, J_{CH} = 7.8 Hz, CH-aromatic), 7.17 (d, 2H, J_{CH} = 7.8 Hz, CH-aromatic), 5.42 (s, 1H, OH), 3.09 (s, 12H, $N(CH_3)_4$), 1.75–0.45 (m, 11H, $B_{12}H_{11}$). ^{13}C NMR (75 MHz, CD_3CN): δ 159.62 (1C, C–O), 146.98 (1C, C–N=N), 125.07, 115.99 (4C, C-aromatic), 56.25 (4C, $N(CH_3)_4$). ^{11}B NMR (96.3 MHz; CD_3CN): δ –12.52 (bs, 1B, B1), –20.34 (d, J_{BH} = 41.69 Hz, 10B, B2–11), –22.78 (bs, 1B, B12). MS (ESI–): *m/z* 261.5 (100, M^-). Anal. Calcd. for $C_{10}H_{28}B_{12}N_3O$: C, 35.74; H,8.4; N, 12.5%. Found: C, 35.56; H, 8.27; N, 12.39%.

(1,2-Dihydroxy-4-azo-benzene)undecahydro-*closo*-dodecaborate(–1) Tetramethylammonium Salt (6).

A deoxygenated solution of **1** (232 mg, 1.0 mmol) in acetonitrile/water (10 mL, 1:1) was injected dropwise to a deoxygenated solution of catechol (110 mg, 1.0 mmol) at 0 °C under an N_2 atmosphere while the pH of the reaction mixture was kept between 6 and 7 by addition of the appropriate amount of deoxygenated K_2CO_3 (1 M) solution. The reaction mixture was kept stirring under N_2 at 0 °C for 30 min by which time the product precipitated. Precipitation was driven to completion by the addition of a few drops of dilute HCl (10%). The precipitate was filtered and washed with heptane to give **6** (123 mg, 35%) as a yellow solid. R_f = 0.21, mp 235–237 °C. IR (KBr, cm^{-1}) ν 3605, 3585 (OH), 3259, 3205, 3095 (CH), 2495 (BH), 1595 (N=N), 1575 (C=C), 1485, 1410, 1385 (CH), 1245 (C–O), 1161 (CN), 1105, 1045 (B–B), 975, 882, 721 (CH). ^{1}H NMR (300 MHz, CD_3CN): δ 8.85 (s, 1H, OH), 8.32 (s, 1H, OH), 7.82 (d, 1H, J_{CH} = 7.72 Hz, CH-aromatic), 7.44 (dd, 1H, J_{CH} = 8.41 Hz, J_{CH} = 2.32 Hz, CH-aromatic), 6.99 (d, 1H, J_{CH} = 8.35 Hz, CH-aromatic), 3.05 (s, 12H, $N(CH_3)_4$), 1.79–0.52 (m, 11H, $B_{12}H_{11}$). ^{13}C NMR (75 MHz, CD_3CN): δ 162.34, 160.32 (2C, C-O), 145.78 (1C, C–N=N), 128.05, 126.32, 124.64 (3C, C-aromatic), 56.25 (4C, $N(CH_3)_4$). ^{11}B NMR (96.3 MHz; CD_3CN): δ –12.39 (bs, 1B, B1), –21.42 (d, J_{BH} = 45.23 Hz, 10B, B2–11), –22.53 (bs, 1B, B12). MS (ESI–): *m/z* 277.6 (100, M^-). Anal. Calcd. for $C_{10}H_{29}B_{12}N_3O_2$: C, 34.02; H,8.28; N, 11.90%. Found: C, 33.82; H, 7.84; N, 11.69%.

(4-Hydroxy-3-methoxy-1-azo-benzene)undecahydro-*closo*-dodecaborate(–1) Tetramethylammonium Salt (7).

This compound was prepared from **1** (232 mg, 1.0 mmol) and 2-methoxyphenol (124 mg, 1.0 mmol) as a coupler, using the procedure described for **5** to give **7** (264 mg, 72%) as a red solid. R_f = 0.27, mp 187–189 °C. IR (KBr, cm^{-1}) ν 3595 (OH), 3262, 3207, 3098 (CH), 2489 (BH), 1585 (N=N), 1582 (C=C), 1487, 1412, 1385 (CH), 1247 (C–O), 1162 (CN), 1105, 1045 (B–B), 972, 885, 723 (CH). ^{1}H NMR (300 MHz, CD_3CN): δ 7.02–6.69 (m, 3H, CH-aromatic), 5.95 (s, 1H, OH), 3.74 (s, 3H, OMe), 3.05 (s, 12H, $N(CH_3)_4$), 1.74–0.45 (m, 11H, $B_{12}H_{11}$). ^{13}C NMR (75 MHz, CD_3CN): δ 159.21, 158.56 (2C, C–O), 143.97 (1C, C–N=N), 128.74, 126.82, 125.03 (3C, C-aromatic), 56.25 (4C, $N(CH_3)_4$), 55.16 (1C, O–CH_3). ^{11}B NMR (96.3 MHz; CD_3CN): δ –12.37 (bs, 1B, B1), –21.39 (d, J_{BH} = 43.29 Hz, 10B, B2–11), –22.47 (bs, 1B, B12). MS (ESI–): *m/z* 292.5 (100, M^-). Anal. Calcd. for $C_{11}H_{31}B_{12}N_3O_2$: C, 35.99; H,8.51; N, 11.45%. Found: C, 35.78; H, 8.21; N, 11.39%.

(2-Hydroxy-3-methoxy-1-azo-benzene)undecahydro-*closo*-dodecaborate(–1) Tetramethylammonium Salt (8).

This compound was prepared from **1** (232 mg, 1.0 mmol) and 4-methoxyphenol (124 mg, 1.0 mmol) as a coupler, using the procedure described for **5** to give **8** (275 mg, 75%) as a red solid. R_f = 0.25, mp 219–221 °C. IR (KBr, cm^{-1}) ν 3601 (OH), 3265, 3205, 3092 (CH), 2492 (BH), 1591 (N=N), 1665 (C=C), 1485, 1415, 1385 (CH), 1242 (C–O), 1165 (CN), 1105, 1045 (B–B), 971, 885, 722 (CH). ^{1}H NMR (300 MHz, CD_3CN): δ 7.25, 6.89, 6.85 (m, 3H, CH-aromatic), 5.99 (s, 1H, OH), 3.76 (s, 3H, OMe), 3.07 (s, 12H, $N(CH_3)_4$), 1.78–0.51 (m, 11H, $B_{12}H_{11}$). ^{13}C NMR (75 MHz, CD_3CN): δ 159.67, 159.05 (2C, C–O), 145.32 (1C, C–N=N), 128.75, 126.88, 125.12 (3C, C-aromatic), 56.26 (4C, $N(CH_3)_4$), 55.19 (1C, O–CH_3). ^{11}B NMR (96.3 MHz; CD_3CN): δ –12.77 (bs, 1B, B1), –21.42 (d, J_{BH} = 45.25 Hz, 10B, B2–11), –22.49 (bs, 1B, B12). MS (ESI–): *m/z* 292.6 (100, M^-). Anal. Calcd. for $C_{11}H_{31}B_{12}N_3O_2$: C, 35.99; H,8.51; N, 11.45%. Found: C, 35.84; H, 8.17; N, 11.21%.

[4-(*N,N*-Dimethylamino)phenylazo]undecahydro-*closo*-dodecaborate(–1) Tetramethylammonium Salt (9).

This compound was prepared from **1** (232 mg, 1.0 mmol) and *ortho*-methoxyphenol (121 mg, 1.0 mmol) as a coupler, using the procedure described for **5** to give **9** (312 mg, 86%) as a red solid. R_f = 0.22, mp 267–269 °C. IR (KBr, cm^{-1}) ν 3255, 3203, 3095 (CH), 2495 (BH), 1587 (N=N), 1557 (C=C), 1485, 1415, 1385 (CH), 1165 (CN), 1105, 1045 (B–B), 975, 885, 725 (CH). ^{1}H NMR (300 MHz, CD_3CN): δ 7.77 (d, 2H, J_{CH} = 9.55 Hz, CH-aromatic), 6.72 (d, 2H, J_{CH} = 13.68 Hz, CH-aromatic), 3.15 (s, 6H, $N(Me)_2$), 3.05 (s, 12H, $N(CH_3)_4$), 1.78–0.51 (m, 11H, $B_{12}H_{11}$). ^{13}C NMR (75 MHz, CD_3CN): δ 152.11 (1C, C–N), 143.79 (1C, C–N=N), 126.75, 115.53 (4C, C-aromatic), 56.25 (4C, $N(CH_3)_4$), 42.21 (1C, $N(CH_3)_2$). ^{11}B NMR (96.3 MHz; CD_3CN): δ –12.75 (bs, 1B, B1), –21.46

(d, J_{BH} = 49.21 Hz, 10B, B2–11), –22.52 (bs, 1B, B12). MS (ESI–): *m/z* 289.4 (100, M^-). Anal. Calcd. for $C_{12}H_{34}B_{12}N_4$: C, 39.58; H, 9.41; N, 15.39%. Found: C, 39.41; H, 9.28; N, 15.17%.

(1-Naphthol-4-azo)undecahydro-*closo*-dodecaborate(–1) Tetramethylammonium Salt (10).

This compound was prepared from **1** (232 mg, 1.0 mmol) and 1-naphthol (144 mg, 1.0 mmol) as a coupler, using the procedure described for **5** to give **10** (278 mg, 72%) as a yellow solid. R_f = 0.24, mp 186–188 °C. IR (KBr, cm^{-1}) ν 3589 (OH), 3262, 3205, 3095 (CH), 2497 (BH), 1591 (N=N), 1579 (C=C), 1458, 1412, 1379 (CH), 1250 (C–O), 1168 (CN), 1104, 1047 (B–B), 964, 886, 719 (CH). ^{1}H NMR (300 MHz, CD_3CN): δ 7.03–7.91 (m, 7H, CH-aromatic), 6.55 (bs, 1H, OH), 3.11 (s, 12H, $N(CH_3)_4$), 1.81–0.52 (m, 11H, $B_{12}H_{11}$). ^{13}C NMR (75 MHz, CD_3CN): δ 160.03 (1C, C–O), 145.15 (1C, C–N=N), 129.85, 128.05, 126.47, 125.97, 123.99, 122.11, 115.76, 113.45 (8C, C-aromatic), 56.26 (4C, $N(CH_3)_4$). ^{11}B NMR (96.3 MHz; CD_3CN): δ –12.79 (bs, 1B, B1), –21.55 (bs, 10B, B2–11), –23.12 (bs, 1B, B12). MS (ESI–): *m/z* 312.8 (100, M^-). Anal. Calcd. for $C_{14}H_{31}B_{12}N_3O$: C, 43.43; H,8.07; N, 10.85%. Found: C, 43.29; H, 7.76; N, 10.64%.

(2-Naphthol-1-azo)undecahydro-*closo*-dodecaborate(–1) Tetramethylammonium Salt (11).

This compound was prepared from **1** (232 mg, 1.0 mmol) and 2-naphthol (144 mg, 1.0 mmol) as a coupler, using the procedure described for **5** to give **11** (305 mg, 79%) as a reddish brown solid. R_f = 0.28, mp 216–218 °C. IR (KBr, cm^{-1}) ν 3602 (OH), 3265, 3202, 3092 (CH), 2489 (BH), 1595 (N=N), 1581 (C=C), 1457, 1415, 1381 (CH), 1238 (C–O), 1165 (CN), 1105, 1045 (B–B), 965, 885, 723 (CH). ^{1}H NMR (300 MHz, CD_3CN): δ 8.18–7.28 (m, 7H, CH-aromatic), 6.89 (bs, 1H, OH), 3.05 (s, 12H, $N(CH_3)_4$), 1.79–0.47 (m, 11H, $B_{12}H_{11}$). ^{13}C NMR (75 MHz, CD_3CN): δ 158.12 (1C, C–O), 143.55 (1C, C–N=N), 129.77, 127.66, 126.47, 125.97, 123.99, 118.22 (8C, C-aromatic), 56.22 (4C, $N(CH_3)_4$). ^{11}B NMR (96.3 MHz; CD_3CN): δ –12.49 (bs, 1B, B1), –21.52 (d, J_{BH} = 42.73 Hz, 10B, B2–11), –22.85 (bs, 1B, B12). MS (ESI–): *m/z* 312.6 (100, M^-). Anal. Calcd. for $C_{14}H_{31}B_{12}N_3O$: C, 43.43; H,8.07; N, 10.85%. Found: C, 43.22; H, 7.82; N, 10.69%.

12-(Phenylazo)mercaptoundecahydro-*closo*-dodecaborate(–2) Ditetrabutylammonium Salt (12). A 0 °C solution of aniline hydrochloride (130 mg, 1.0 mmol) and 1 M HCl (1 mL) in deionized (DI) water (5 mL) was treated with a 0 °C solution of $NaNO_2$ (210 mg, 3.0 mmol) in DI water (5 mL), and the mixture was stirred at 0 °C for 30 min. To the stirred solution of diazonium salt was slowly added disodium salt of **3** (322 mg, 1.0 mmol) dissolved in 10 mL DI water at the same temperature. The mixture was stirred at 0 °C for 30 min and at room temperature for 3 h. The aqueous solution was filtered and tetrabutylammonium chloride (278 mg, 1.0 mmol) was added, resulting in a precipitate that was filtered off. The product was purified by TLC using MeOH/CH_2Cl_2 (1:4) as eluent to yield **12** (572 mg, 75%) as a yellow solid. R_f = 0.33, mp 178–180 °C. IR (KBr, cm^{-1}) ν 3030, 2965 (CH), 2495 (BH), 1591 (N=N), 1525 (C=C), 1485, 1415, 1385 (CH), 1165 (CN), 1105, 1045 (B–B), 971, 725, 675 (CH). ^{1}H NMR (300 MHz, CD_3CN): δ 7.49–7.35 (m, 5H, CH-aromatic), 3.11 (m, 16H, $N(CH_2–)_4$), 1.57 (m, 16H, $N(CH_2CH_{2-})_4$), 1.38 (m, 16H, $N(CH_2CH_2CH_{2-})_4$), 0.95 (t, J = 14.41 Hz, 24H, $N(CH_2CH_2CH_2CH_3)_4$), 1.81–0.55 (m, 10H, $B_{12}H_{11}$). ^{13}C NMR (75 MHz, CD_3CN): δ 145.51 (1C, C–N=N), 130.21, 129.56, 124.67 (5C, C-aromatic), 59.25 (8C, $N(CH_{2-})_4$), 24.34 (8C, $N(CH_2CH_{2-})_4$), 20.25 (8C, $N(CH_2CH_2CH_{2-})_4$), 13.75 (8C, $N(CH_2CH_2CH_2CH_3)_4$). ^{11}B NMR (96.3 MHz; CD_3CN): δ –9.51 (bs, 1B, B1), –10.52 (bs, 1B, B12), –21.46 (d, J_{BH} = 53.21 Hz, 10B, B2–11). MS (ESI–): *m/z* 138.6 (100, M^-/2). Anal. Calcd. for $C_{38}H_{88}B_{12}N_4S$: C, 59.82; H, 11.63; N, 7.34%. Found: C, 59.63; H, 11.39; N, 7.12%.

12-(4-Bromophenylazo)mercaptoundecahydro-*closo*-dodecaborate(–2) Ditetrabutylammonium Salt (13).

This compound was prepared from **3** (322 mg, 1.0 mmol) and *para*-bromoaniline (172 mg, 1.0 mmol), using the procedure described for **12** to give **13** (581 mg, 69%) as an orange solid. R_f = 0.32, mp 196–198 °C. IR (KBr, cm^{-1}) ν 3025, 2985 (CH), 2492 (BH), 1585 (N=N), 1534 (C=C), 1485, 1415, 1385 (CH), 1165 (CN), 1105, 1045 (B–B), 975, 723, 672 (CH). ^{1}H NMR (300 MHz, CD_3CN): δ 7.69 (d, 2H, J_{CH} = 13.44 Hz, CH-aromatic), 7.15 (d, 2H, J_{CH} = 13.32 Hz, CH-aromatic), 3.08 (m, 16H, $N(CH_2–)_4$), 1.56 (m, 16H, $N(CH_2CH_{2-})_4$), 1.38 (m, 16H, $N(CH_2CH_2CH_{2-})_4$), 0.95 (t, J = 13.55 Hz, 24H, $N(CH_2CH_2CH_2CH_3)_4$), 1.72–0.47 (m, 10H, $B_{12}H_{11}$). ^{13}C NMR (75 MHz, CD_3CN): δ 143.98 (1C, C–N=N), 132.78, 130.87, 122.73 (5C, C-aromatic), 59.25 (8C, $N(CH_{2-})_4$), 24.35 (8C, $N(CH_2CH_{2-})_4$), 20.28 (8C, $N(CH_2CH_2CH_{2-})_4$), 13.76 (8C, $N(CH_2CH_2CH_2CH_3)_4$). ^{11}B NMR (96.3 MHz; CD_3CN): δ –9.65 (bs, 1B, B1), –10.61 (bs, 1B, B12), –21.51 (d, J_{BH} = 48.41 Hz, 10B, B2–11). MS (ESI–): *m/z* 178.3 (100, M^-/2). Anal. Calcd. for $C_{38}H_{87}B_{12}BrN_4S$: C, 54.22; H, 10.42; N, 6.66%. Found: C, 53.96; H, 10.18; N, 6.39%.

12-(4-Nitrophenylazo)mercaptoundecahydro-*closo*-dodecaborate(–2) Ditetrabutylammonium Salt (14).

para-Nitroaniline (138 mg, 1.0 mmol) was dissolved in a mixture of methanol (20 mL) and concentrated HCl (5 mL). Isoamyl nitrite (1.34 mL, 0.01 mol) was added to this solution at 0 °C. A yellow precipitate gradually appeared. The reaction mixture was kept stirring at 0 °C for 45 min. Using the direct coupling procedure described above, compound **3** (322 mg, 1.0 mmol) was added to give **14** (695 mg, 86%) as a red solid. R_f = 0.34, mp

244–246 °C. IR (KBr, cm^{-1}) ν 3030, 2985 (CH), 2495 (BH), 1585 (N=N), 1535 (C=C), 1515, 1335 (NO_2), 1485, 1415, 1385 (CH), 1165 (CN), 1105, 1045 (B–B), 972, 725, 671 (CH). ^{1}H NMR (300 MHz, CD_3CN): δ 8.31 (d, 2H, J_{CH} = 7.82 Hz, CH-aromatic), 7.96 (d, 2H, J_{CH} = 9.53 Hz, CH-aromatic), 3.08 (m, 16H, $N(CH_2-)_4$), 1.58 (m, 16H, $N(CH_2CH_2-)_4$), 1.36 (m, 16H, $N(CH_2CH_2CH_2-)_4$), 0.96 (t, J = 14.02 Hz, 24H, $N(CH_2CH_2CH_2CH_3)_4$), 1.76–0.52 (m, 10H, $B_{12}H_{11}$). ^{13}C NMR (75 MHz, CD_3CN): δ 149.52 (1C, C–NO_2), 145.02 (1C, C–N=N), 130.29, 125.13 (4C, C-aromatic), 59.26 (8C, $N(CH_2-)_4$), 24.28 (8C, $N(CH_2CH_2-)_4$), 20.25 (8C, $N(CH_2CH_2CH_2-)_4$), 13.75 (8C, $N(CH_2CH_2CH_2CH_3)_4$). ^{11}B NMR (96.3 MHz; CD_3CN): δ –9.75 (bs, 1B, B1), –10.87 (bs, 1B, B12), –21.74 (d, J_{BH} = 59.12 Hz, 10B, B2–11). MS (ESI–): *m/z* 161.5 (100, $M^-/2$). Anal. Calcd. for $C_{38}H_{87}B_{12}N_5O_2S$: C, 56.49; H, 10.85; N, 8.67%. Found: C, 56.23; H, 10.69; N, 8.43%.

12-(4-Carboxyphenylazo)mercaptoundecahydro-*closo*-dodecaborate(–2) Ditetrabutylammonium Salt (15).

This compound was prepared from **3** (322 mg, 1.0 mmol) and *para*-carboxyaniline (137 mg, 1.0 mmol), using the procedure described for **12** to give **15** (629 mg, 78%) as a red solid. R_f = 0.24, mp 232–234 °C. IR (KBr, cm^{-1}) ν 3496 (OH), 3026, 2989 (CH), 2487 (BH), 1725 (C=O), 1585 (N=N), 1527 (C=C), 1485, 1410, 1386 (CH), 1162 (CN), 1105, 1047 (B–B), 972, 722, 673 (CH). ^{1}H NMR (300 MHz, CD_3CN): δ 7.45 (d, 2H, J_{CH} = 9.12 Hz, CH-aromatic), 6.89 (d, 2H, J_{CH} = 7.25 Hz, CH-aromatic), 3.11 (m, 16H, $N(CH_2-)_4$), 1.57 (m, 16H, $N(CH_2CH_2-)_4$), 1.37 (m, 16H, $N(CH_2CH_2CH_2-)_4$), 0.96 (t, J = 12.63 Hz, 24H, $N(CH_2CH_2CH_2CH_3)_4$), 1.75–0.51 (m, 10H, $B_{12}H_{11}$). ^{13}C NMR (75 MHz, CD_3CN): δ 172.43 (1C, C=O), 145.41 (1C, C–N=N), 131.86, 129.75, 127.54 (5C, C-aromatic), 59.26 (8C, $N(CH_2-)_4$), 24.45 (8C, $N(CH_2CH_2-)_4$), 20.25 (8C, $N(CH_2CH_2CH_2-)_4$), 13.79 (8C, $N(CH_2CH_2CH_2CH_3)_4$). ^{11}B NMR (96.3 MHz; CD_3CN): δ –9.51 (bs, 1B, B1), –10.21 (bs, 1B, B12), –21.69 (d, J_{BH} = 57.22 Hz, 10B, B2–11). MS (ESI–): *m/z* 160.8 (100, $M^-/2$). Anal. Calcd. for $C_{39}H_{88}B_{12}N_4O_2S$: C, 58.05; H, 10.99; N, 6.94%. Found: C, 57.79; H, 10.73; N, 6.68%.

12-(3-Carboxyphenylazo)mercaptoundecahydro-*closo*-dodecaborate(–2) Ditetrabutylammonium Salt (16).

This compound was prepared from **3** (322 mg, 1.0 mmol) and *para*-carboxyaniline (137 mg, 1.0 mmol), using the procedure described for **12** to give **16** (629 mg, 78%) as a red solid. R_f = 0.29, mp 210–212 °C. IR (KBr, cm^{-1}) ν 3502 (OH), 3028, 2985 (CH), 2492 (BH), 1716 (C=O), 1591 (N=N), 1525 (C=C), 1485, 1411, 1387 (CH), 1160 (CN), 1105, 1045 (B–B), 975, 725, 675 (CH). ^{1}H NMR (300 MHz, CD_3CN): δ 7.81–7.45 (m, 4H, CH-aromatic), 3.11 (m, 16H, $N(CH_2-)_4$), 1.56 (m, 16H, $N(CH_2CH_2-)_4$), 1.35 (m, 16H, $N(CH_2CH_2CH_2-)_4$), 0.95 (t, J = 13.55 Hz, 24H, $N(CH_2CH_2CH_2CH_3)_4$), 1.76–0.50 (m, 10H, $B_{12}H_{10}$). ^{13}C NMR (75 MHz, CD_3CN): δ 172.67 (1C, C=O), 145.32 (1C, C–N=N), 132.03, 130.87, 129.96, 128.54, 127.61 (5C, C-aromatic), 59.26 (8C, $N(CH_2-)_4$), 24.39 (8C, $N(CH_2CH_2-)_4$), 20.26 (8C, $N(CH_2CH_2CH_2-)_4$), 13.75 (8C, $N(CH_2CH_2CH_2CH_3)_4$). ^{11}B NMR (96.3 MHz; CD_3CN): δ –9.24 (bs, 1B, B1), –10.72 (bs, 1B, B12), –21.71 (d, J_{BH} = 59.12 Hz, 10B, B2–11). MS (ESI–): *m/z* 160.6 (100, $M^-/2$). Anal. Calcd. for $C_{39}H_{88}B_{12}N_4O_2S$: C, 58.05; H, 10.99; N, 6.94%. Found: C, 57.86; H, 10.79; N, 6.72%.

12-(4-Sulfonamidophenylazo)mercaptoundecahydro-*closo*-dodecaborate(–2) Ditetrabutylammonium Salt (17).

This compound was prepared from **3** (322 mg, 1.0 mmol) and sulfanilamide (172 mg, 1.0 mmol), using the procedure described for **12** to give **17** (665 mg, 79%) as an orange solid. R_f = 0.27, mp 196–198 °C. IR (KBr, cm^{-1}) ν 3365 (NH_2), 3025, 2985 (CH), 2495 (BH), 1592 (N=N), 1535 (C=C), 1485, 1414, 1384 (CH), 1353, 1150 (SO_2), 1165 (CN), 1105, 1045 (B–B), 975, 723, 672 (CH). ^{1}H NMR (300 MHz, CD_3CN): δ 7.52 (d, 2H, J_{CH} = 8.22 Hz, CH-aromatic), 6.97 (d, 2H, J_{CH} = 8.22 Hz, CH-aromatic), 3.08 (m, 16H, $N(CH_2-)_4$), 1.56 (m, 16H, $N(CH_2CH_2-)_4$), 1.38 (m, 16H, $N(CH_2CH_2CH_2-)_4$), 0.95 (t, J = 13.55 Hz, 24H, $N(CH_2CH_2CH_2CH_3)_4$), 1.75-0.51 (m, 10H, $B_{12}H_{10}$). ^{13}C NMR (75 MHz, CD_3CN): δ 145.07 (1C, C–N=N), 132.62, 130.43, 125.99 (5C, C-aromatic), 59.25 (8C, $N(CH_2-)_4$), 24.35 (8C, $N(CH_2CH_2-)_4$), 20.28 (8C, $N(CH_2CH_2CH_2-)_4$), 13.76 (8C, $N(CH_2CH_2CH_2CH_3)_4$). ^{11}B NMR (96.3 MHz; CD_3CN): δ –9.55 (bs, 1B, B1), –10.87 (bs, 1B, B12), –21.74 (d, J_{BH} = 59.46 Hz, 10B, B2–11). MS (ESI–): *m/z* 178.5 (100, $M^-/2$). Anal. Calcd. for $C_{38}H_{89}B_{12}N_5O_2S_2$: C, 54.20; H, 10.65; N, 8.32%. Found: C, 53.88; H, 10.33; N, 8.09%.

12-(4-Methoxyphenylazo)mercaptoundecahydro-*closo*-dodecaborate(–2) Ditetrabutylammonium Salt (18).

This compound was prepared from **3** (322 mg, 1.0 mmol) and *para*-anisidine (123 mg, 1.0 mmol), using the procedure described for **12** to give **18** (412 mg, 52%) as a red solid. R_f = 0.35, mp 215–217 °C. IR (KBr, cm^{-1}) ν 3030, 2985 (CH), 2485 (BH), 1589 (N=N), 1526 (C=C), 1485, 1412, 1387 (CH), 1165 (CN), 1105, 1047 (B–B), 972, 725, 676 (CH). ^{1}H NMR (300 MHz, CD_3CN): δ 7.87 (d, 2H, J_{CH} = 8.25 Hz, CH-aromatic), 6.93 (d, 2H, J_{CH} = 7.62 Hz, CH-aromatic), 3.78 (s, 3H, OMe), 3.08 (m, 16H, $N(CH_2-)_4$), 1.55 (m, 16H, $N(CH_2CH_2-)_4$), 1.36 (m, 16H, $N(CH_2CH_2CH_2-)_4$), 0.95 (t, J = 13.72 Hz, 24H, $N(CH_2CH_2CH_2CH_3)_4$), 1.79-0.53 (m, 10H, $B_{12}H_{10}$). ^{13}C NMR (75 MHz, CD_3CN): δ 159.21 (1C, C–O), 144.98 (1C, C–N=N), 130.21, 125.75 (4C, C-aromatic), 59.24 (8C, $N(CH_2-)_4$), 55.32 (1C, OCH_3), 24.45 (8C, $N(CH_2CH_2-)_4$), 20.25 (8C, $N(CH_2CH_2CH_2-)_4$), 13.77 (8C, $N(CH_2CH_2CH_2CH_3)_4$). ^{11}B NMR (96.3 MHz; CD_3CN): δ –9.19 (bs, 1B, B1), –10.53 (bs, 1B, B12), –21.71 (d, J_{BH} =

55.35 Hz, 10B, B2–11). MS (ESI–): *m/z* 153.8 (100, $M^-/2$). Anal. Calcd. for $C_{39}H_{90}B_{12}N_4OS$: C, 59.07; H, 11.44; N, 7.07%. Found: C, 58.89; H, 11.29; N, 6.91%.

12-(4-Methylphenylazo)mercaptoundecahydro-*closo*-dodecaborate(–2) Ditetrabutylammonium Salt (19).

This compound was prepared from **3** (322 mg, 1.0 mmol) and *para*-toluidine (107 mg, 1.0 mmol), using the procedure described for **12** to give **19** (427 mg, 55%) as a red solid. R_f = 0.42, mp 228–230 °C. IR (KBr, cm^{-1}) ν 3030, 2985 (CH), 2491 (BH), 1586 (N=N), 1525 (C=C), 1485, 1411, 1385 (CH), 1165 (CN), 1105, 1045 (B–B), 975, 722, 675 (CH). ^{1}H NMR (300 MHz, CD_3CN): δ 7.75 (d, 2H, J_{CH} = 8.52 Hz, CH-aromatic), 7.15 (d, 2H, J_{CH} = 8.52 Hz, CH-aromatic), 3.08 (m, 16H, $N(CH_2)_4$), 2.35 (s, 1H, CH_3), 1.55 (m, 16H, $N(CH_2CH_2)_4$), 1.35 (m, 16H, $N(CH_2CH_2CH_2)_4$), 0.97 (t, J = 14.21 Hz, 24H, $N(CH_2CH_2CH_2CH_3)_4$), 1.82–0.57 (m, 10H, $B_{12}H_{10}$). ^{13}C NMR (75 MHz, CD_3CN): δ 145.13 (1C, C–N=N), 129.52, 125.75 (4C, C-aromatic), 59.24 (8C, $N(CH_2)_4$), 55.35, 24.46 (8C, $N(CH_2CH_2)_4$), 21.05 (1C, CH_3), 20.22 (8C, $N(CH_2CH_2CH_2)_4$), 13.75 (8C, $N(CH_2CH_2CH_2CH_3)_4$. ^{11}B NMR (96.3 MHz; CD_3CN): δ –8.81 (bs, 1B, B1), –10.65 (bs, 1B, B12), –21.89 (d, J_{BH} = 52.95 Hz, 10B, B2–11). MS (ESI–): *m/z* 145.8 (100, $M^-/2$). Anal. Calcd. for $C_{39}H_{90}B_{12}N_4S$: C, 60.29; H, 11.68; N, 7.21%. Found: C, 59.91; H, 11.49; N, 6.92%.

12-(4-Sulfonylphenylazo)mercaptoundecahydro-*closo*-dodecaborate(–2) Ditetrabutylammonium Salt (20).

This compound was prepared from **3** (322 mg, 1.0 mmol) and sulfanilic acid (173 mg, 1.0 mmol), using the procedure described for **12** to give **20** (750 mg, 89%) as a red solid. R_f = 0.37, mp 239–241 °C. IR (KBr, cm^{-1}) ν 3392 (OH), 3030, 2985 (CH), 2494 (BH), 1591 (N=N), 1525 (C=C), 1485, 1411, 1385 (CH), 1355, 1152 (SO_2), 1165 (CN), 1105, 1045 (B–B), 973, 722, 673 (CH). ^{1}H NMR (300 MHz, CD_3CN): δ 7.75 (d, 2H, J_{CH} = 8.42 Hz, CH-aromatic), 7.05 (d, 2H, J_{CH} = 8.42 Hz, CH-aromatic), 3.11 (m, 16H, $N(CH_2)_4$), 1.57 (m, 16H, $N(CH_2CH_2)_4$), 1.38 (m, 16H, $N(CH_2CH_2CH_2)_4$), 0.97 (t, J = 13.72 Hz, 24H, $N(CH_2CH_2CH_2CH_3)_4$), 1.79-0.51 (m, 10H, $B_{12}H_{10}$). ^{13}C NMR (75 MHz, CD_3CN): δ 145.43 (1C, C–N=N), 132.86, 130.92, 126.12 (5C, C-aromatic), 59.25 (8C, $N(CH_2)_4$), 24.46 (8C, $N(CH_2CH_2)_4$), 20.25 (8C, $N(CH_2CH_2CH_2)_4$), 13.77 (8C, $N(CH_2CH_2CH_2CH_3)_4$). ^{11}B NMR (96.3 MHz; CD_3CN): δ –10.71 (bs, 1B, B1), –19.85 (bs, 1B, B12), –21.83 (d, J_{BH} = 56.19 Hz, 10B, B2–11). MS (ESI–): *m/z* 178.6 (100, $M^-/2$). Anal. Calcd. for $C_{38}H_{88}B_{12}N_4O_3S_2$: C, 54.14; H, 10.52; N, 6.65%. Found: C, 53.86; H, 10.29; N, 6.32%.

12-(4-Nitrophenylazo)oxyundecahydro-*closo*-dodecaborate(–2) Ditetrabutylammonium Salt (21). This compound was prepared from sodium salt of **4** (204 mg, 1.0 mmol) and 4-nitroaniline (138 mg, 1.0 mmol), using the procedure described for **12** to give **21** (704 mg, 89%) as a reddish brown solid. R_f = 0.33, mp 177–179 °C. IR (KBr, cm^{-1}) ν 3035, 2987 (CH), 2491 (BH), 1589 (N=N), 1530 (C=C), 1519, 1332 (NO_2), 1485, 1415, 1385 (CH), 1165 (CN), 1105, 1045 (B–B), 975, 723, 674 (CH). ^{1}H NMR (300 MHz, CD_3CN): δ 8.36 (d, 2H, J_{CH} = 8.24 Hz, CH-aromatic), 7.89 (d, 2H, J_{CH} = 7.92 Hz, CH-aromatic), 3.08 (m, 16H, $N(CH_2)_4$), 1.57 (m, 16H, $N(CH_2CH_2)_4$), 1.35 (m, 16H, $N(CH_2CH_2CH_2)_4$), 0.97 (t, J = 14.21 Hz, 24H, $N(CH_2CH_2CH_2CH_3)_4$), 1.76–0.52 (m, 10H, $B_{12}H_{11}$). ^{13}C NMR (75 MHz, CD_3CN): δ 150.17 (1C, C–NO_2), 145.34 (1C, C–N=N), 130.55, 125.29 (4C, C–aromatic), 59.36 (8C, $N(CH_2)_4$), 24.12 (8C, $N(CH_2CH_2)_4$), 20.28 (8C, $N(CH_2CH_2CH_2)_4$), 13.75 (8C, $N(CH_2CH_2CH_2CH_3)_4$). ^{11}B NMR (96.3 MHz; CD_3CN): δ –9.59 (bs, 1B, B1), –11.21 (bs, 1B, B12), –21.99 (d, J_{BH} = 54.72 Hz, 10B, B2–11). MS (ESI–): *m/z* 152.5 (100, $M^-/2$). Anal. Calcd. for $C_{38}H_{86}B_{12}N_5O_3$: C, 57.71; H, 10.96; N, 8.86%. Found: C, 57.58; H, 10.73; N, 8.61%.

12-(5-Phenylazo-10,15,20-triphenylporphyrin)mercaptoundecahydro-*closo*-dodecaborate(–2) Ditetrabutylammonium Salt (22).

This compound was prepared from **3** (322 mg, 1.0 mmol) and the diazonium salt of 5-(*para*-aminophenyl)-10,15,20-triphenylporphyrin[56] (785 mg, 1.25 mmol), using the procedure described for **12** to give **22** (688 mg, 53%) as a violet solid. R_f = 0.25, mp 255–257 °C. IR (KBr, cm^{-1}) ν 3392 (OH), 3030, 2985 (CH), 2495 (BH), 1592 (N=N), 1527 (C=C), 1485, 1410, 1385 (CH), 1165 (CN), 1105, 1045 (B–B), 975, 725, 675 (CH). ^{1}H NMR (300 MHz, CD_3CN): δ 8.92–8.59 (m, 8H, β-pyrrole), 8.21–7.03 (m, 19H, CH-aromatic), 3.08 (m, 16H, $N(CH_2)_4$), 1.57 (m, 16H, $N(CH_2CH_2)_4$), 1.37 (m, 16H, $N(CH_2CH_2CH_2)_4$), 0.96 (t, J = 13.72 Hz, 24H, $N(CH_2CH_2CH_2CH_3)_4$), 1.76–0.54 (m, 10H, $B_{12}H_{10}$), –3.25 (s, 2H, NH). ^{13}C NMR (75 MHz, CD_3CN): δ 145.43 (1C, C–N=N), 162.32, 153.43, 139.05, 138.32, 135.92, 131.19, 130.5, 128.45, 126.77, 125.22, 122.53, 120.16, 105.12, 79.52, 78.26, 77.98, 76.89 (44C, C-aromatic), 59.25 (8C, $N(CH_2)_4$), 24.46 (8C, $N(CH_2CH_2)_4$), 20.25 (8C, $N(CH_2CH_2CH_2)_4$), 13.77 (8C, $N(CH_2CH_2CH_2CH_3)_4$). ^{11}B NMR (96.3 MHz; CD_3CN): δ –9.11 (bs, 1B, B1), –10.88 (bs, 1B, B12), –21.91 (d, J_{BH} = 56.32 Hz, 10B, B2–11). MS (ESI–): *m/z* 178.6 (407.3, $M^-/2$). Anal. Calcd. for $C_{76}H_{112}B_{12}N_8S$: C, 70.24; H, 8.69; N, 8.62%. Found: C, 69.89; H, 8.41; N, 8.45%.

2-Ammonio-3-[4-hydroxy-3-(undecahydro-*closo*-dodecaboratediazenyl)phenyl]propanoate(–1) Tetramethylammonium Salt (23).

This compound was prepared from **1** (232 mg, 1.0 mmol) and L-tyrosine (181 mg, 1.0 mmol) as a coupler, using the procedure described for **5** to give **23** (284 mg, 67%) as a yellow solid. R_f = 0.23, mp 191–193 °C. IR (KBr, cm^{-1}) ν 3605, 3597 (NH_2 and OH), 3203, 3095

(CH), 2492 (BH), 1719 (C=O), 1589 (N=N), 1557 (C=C), 1485, 1415, 1385 (CH), 1165 (CN), 1105, 1045 (B–B), 975, 885, 725 (CH). ^{1}H NMR (300 MHz, CD_3CN): δ 7.26 (s, 1H, CH-aromatic), 6.99 (d, 2H, J_{CH} = 12.52 Hz, CH-aromatic), 5.47 (m, 1H, OH), 4.71 (bs, 2H, NH_2), 4.07 (s, 2H, CH_2), 3.15 (t, 1H, J_{CH} = 21.2 Hz, CH), 3.05 (s, 12H, $N(CH_3)_4$), 1.78–0.51 (m, 11H, $B_{12}H_{11}$). ^{13}C NMR (75 MHz, CD_3CN): δ 170.98 (1C, C=O), 155.41 (1C, C–O), 145.85 (1C, C–N=N), 130.85, 128.49, 120.9 (4C, C-aromatic), 56.25 (4C, $N(CH_3)_4$), 54.67 (1C, NH_2–CH), 37.27 (1C, CH_2). ^{11}B NMR (96.3 MHz; CD_3CN): δ –12.54 (bs, 1B, B1), –22.85 (d, J_{BH} = 46.22 Hz, 10B, B2–11), –23.52 (bs, 1B, B12). MS (ESI–): *m/z* 349.5 (100, M^-). Anal. Calcd. for $C_{13}H_{34}B_{12}N_4O_3$: C, 36.81; H, 8.08; N, 13.21%. Found: C, 36.61; H, 7.89; N, 12.94%.

4. 2. Azo Dye Reaction of Mercaptododecaborate (3) in Cells

The human cervical carcinoma cell line HeLa cells were plated on p35 dishes (1×10^4 cells) and incubated at 37 °C for 24 h. After compound **3** (1 mM) treatment for 3 h, the cells were washed with PBS and fixed in 4% paraformaldehyde in PBS for 10 min. After washing with PBS, the cells were permeabilized with 0.1% Triton X-100 in PBS for 10 min, and blocked with 1% bovine serum albumin in PBS for 10 min. The azo reactions with compound **3** and diazobenzene were established at 4 °C to give a yellow color immediately. UV-Vis spectrum of this solution was measured on a Shimadzu 2450 PC spectrophotometer within the wavelength range 200–700 nm.

5. References

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Povzetek

Opisan je splošni pristop k sintezi novih borovih azo barvil, izhajajoč iz undekahidro-*closo*-dodekaboratov. Predstavljen postopek predstavlja priročno metodo priprave označenih barvil, primernih za terapijo z borovim zajetjem nevtronov (BNCT). Metoda je primerna za sintezo dveh serij barvil, označenih z dodekaboratnimi anioni. Prvo serijo pripravimo z reakcijo med $(CH_3)_4NB_{12}H_{11}NH_3^-$ in $NaNO_2$ v zmesi acetonitrila in vode, pri čemer nastane diazonijeva sol, ki v naslednji stopnji reagira s substituiranimi fenoli. Tako nastanejo boronatna azo barvila ($B_{12}H_{11}$–N=N–Ar^-, Ar = 4-HOC_6H_5, 1-naftol, 2-naftol, 2,3-$(HO)_2C_6H_4$, 3-MeO-4-HOC_6H_4, 2-HO-5-$MeOC_6H_4$ in 4-Me_2N-C_6H_4). Drugo serijo pripravimo z reakcijo med arildiazonijevo soljo kot partnerico pri pripajanju in dinatrijevo soljo dodekaboratnega aniona ($B_{12}H_{11}X^{2-}$, X = SH ali OH), pri čemer nastanejo substituirana dodekaboratna azo barvila ($HXB_{12}H_{10}$–N=N–Ar^-, Ar = *para*-bromo, *para*-nitro, *para*-karboksi, *meta*-karboksi, *para*-sulfonamide in *para*-sulfonska kislina). Rezultati kažejo pričakovane vplive različnih substituentov na učinkovitost reakcij pripajanja. Razširitev opisane strategije na tirozin in diazonijevo sol 5-(*para*-aminofenil)-10,15,20-trifenilporfirina je omogočila pripravo dodekaboratnega aniona, ki je vseboval bodisi aminsko skupino ali pa porfirinski sistem. Obe pripravljeni spojini sta dobra kandidata za BNCT. Dodekaboratna barvila so bila pripravljena s sprejemljivimi izkoristki. Opisana metodologija omogoča enostavno sintezo knjižnic azo barvil, modificiranih z različnimi borovimi klastri. Tovrstna barvila so primerna za različne uporabe, omogočajo pa celo vizualizacijo borovih klastrov znotraj celic.