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Boron-Based Sensors

^{11}B NMR/MRI Sensing of Copper(II) Ions In Vitro by the Decomposition of a Hybrid Compound of a *nido*-*o*-Carborane and a Metal Chelator

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Abstract: Cu^{2+} is closely correlated with certain types of physiological and pathological events. Therefore, the development of a noninvasive methodology for detecting Cu^{2+} is important for understanding its biological role and relationship with disease. Herein, we report on the development of a Cu^{2+}-specific ^{11}B NMR/MRI probe that contains a chelator unit, TriMEDA. The method is based on the rapid complete deboronation of *nido*-*o*-carborane by Cu^{2+} under physiological conditions.

Introduction

It is known that Cu^{2+} is an abundant element in bio-organisms (an essential element in humans) and functions as a crucial cofactor for numerous enzymes such as tyrosinase, cytochrome c oxidase, and superoxide dismutase.[1] Although the level of Cu^{2+} in the serum of a normal human being ranges from 18.5 to 32 μM,[2] current research indicates that it is altered in many diseases such as the Menkes syndrome,[3] Willson's disease,[4] amyotrophic lateral sclerosis,[5] Alzheimer's disease,[6] and Parkinson's disease.[7] Additionally, high levels of Cu^{2+} in the blood may induce the progress of certain types of cancer.[8] Thus, the development of imaging probes for detecting Cu^{2+} is important to understand its metabolism and role in pathological events.[9]

A number of fluorescence-based probes for Cu^{+} and Cu^{2+} have been reported to date.[10] However, the fluorescence detection of $Cu^{+/2+}$ ions has limitations in terms of the impermeability of biological tissue. On the other hand, magnetic resonance imaging (MRI) is considered to be a powerful technique for the detection of molecules and the diagnosis of various diseases, because it is noninvasive and capable of producing three-dimensional images of opaque tissue with a high degree of spatial and temporal resolution.[11]

In this context, we have focused on the use of ^{11}B NMR probes for the detection of d-block metal ions, because ^{11}B is one of the ultratrace elements found in living systems.[12] We previously reported on the use of phenylboronic acid with pendant cyclen for the detection of d-block metals (cyclen = 1,4,7,10-tetraazacyclododecane).[13,14] The C–B bond is cleaved upon the formation of a metal complex with d-block metals to give $B(OH)_3$ in aqueous solution at neutral pH (Scheme 1), which can then be applied to the in-cell ^{11}B NMR spectroscopic detection of Zn^{2+}, Cu^{2+}, and other d-block metal ions. More recently, we found that *o*-carborane derivatives such as **1**, **2**, and **3** decompose in the presence of Cu^{2+} ions.[15,16] Mechanistic studies strongly suggest that Cu^{2+} oxidizes the *nido*-forms of **1**–**3** (**1′**–**3′**), which are produced upon the depletion of one molecule of $B(OH)_3$ by a nucleophile such as metal-bound H_2O or OH^-, and the further decomposition reaction is promoted by Cu^{2+}, resulting in the release of ten molecules of $B(OH)_3$ in total (Scheme 1a). The $B(OH)_3$ released from **1**–**3** was successfully detected by ^{11}B NMR spectroscopy and MRI on the basis of its differing chemical shift (δ = 20 ppm) and its shorter relaxation time (less than 10 ms) relative to those of *closo*-/*nido*-*o*-carboranes (δ = –10 to –40 ppm and 100 ms). However, further improvement of this decomposition reaction is necessary for achieving the facile and rapid detection of Cu^{2+} ions, because the reaction with **3′** requires a long time (t > 96 h) and a high temperature (50 °C) for completion.

Herein, we report on *nido*-carborane derivatives **4** bearing *N*,*N*,*N′*-trimethylethylenediamine (TriMEDA) as a chelator unit for improving the decomposition reactivity by virtue of a proximity effect (Scheme 1b). It was expected that **4** would form a complex **5** with Cu^{2+} more efficiently than other metal complexes, because the affinity of the TriMEDA unit for Cu^{2+} is higher than those for Mn^{2+}, Fe^{2+}, or Zn^{2+},[17] which results in

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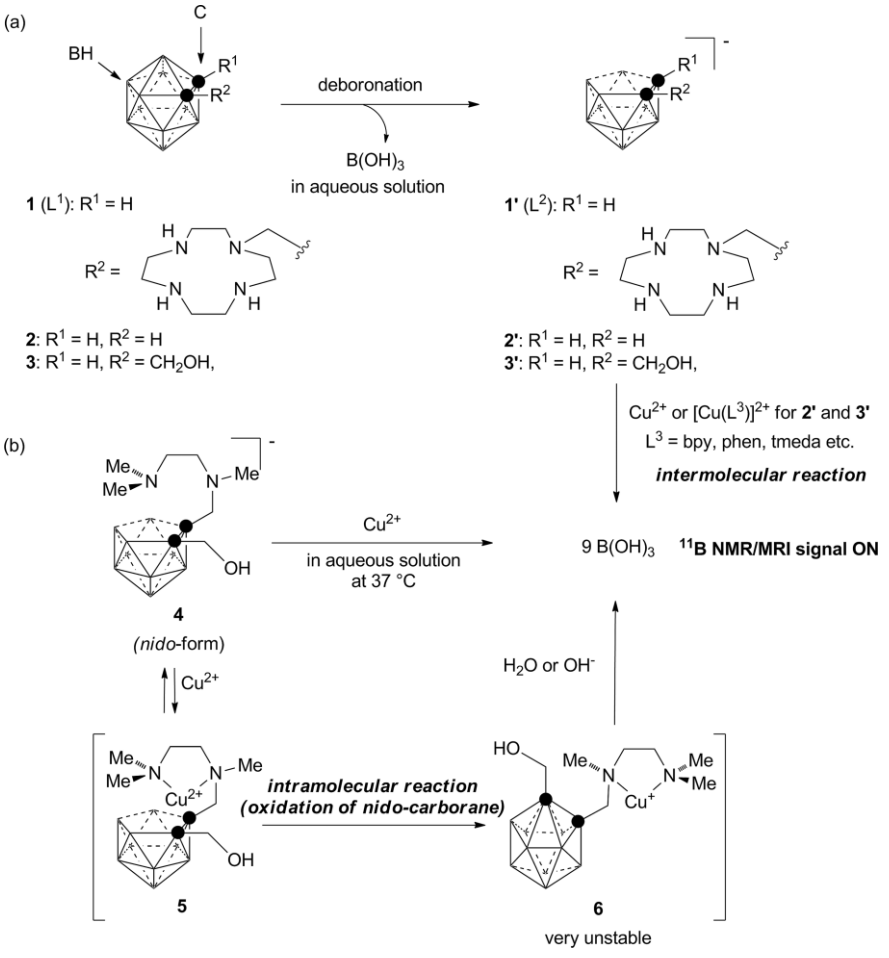


Scheme 1. Detection of Cu^{2+} ions by ^{11}B NMR/MRI on the basis of (a) the decomposition reaction of *o*-carborane derivatives **1**–**3** and (b) the reaction of **4** reported in this work.

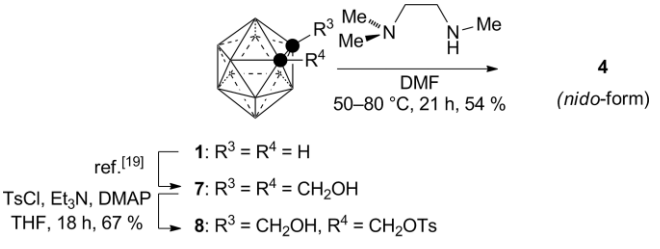
considerable acceleration of the decomposition reaction even at room temperature to release 9 equiv. of $B(OH)_3$.

Results and Discussion

Design and Synthesis of a Hybrid Compound of *nido*-*o*-Carborane and Chelator 4

In previous attempts to assess the effect of the chelator on the Cu^{2+}-induced decomposition of the *o*-carborane derivative, we found out that *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TMEDA) facilitated the Cu^{2+}-promoted decomposition reaction of **3** over a wide pH range (pH 5–10).[15,17,18] Thus, we designed *nido*-*o*-carborane derivative **4** bearing TriMEDA in order to improve the reaction rate by virtue of a proximity effect.

The synthesis of **4** was conducted as shown in Scheme 2. Diol **7** was prepared from *o*-carborane **1** by the reported method.[19] It then reacted with tosyl chloride in the presence of triethylamine and dimethylaminopyridine (DMAP) to afford monotosyl compound **8**. The desired compound **4** was converted from **8** by substitution of the tosylate with *N*,*N*,*N*′-trimethylethylenediamine accompanied by the loss of 1 equiv. of $B(OH)_3$ from the *o*-carborane unit under these reaction conditions.



Scheme 2. Synthesis of *nido*-carborane derivative **4**.

Decomposition Reaction of a Hybrid Compound of *nido*-*o*-Carborane and Chelator 4 in the Presence of Cu^{2+}

Probe **4** was treated with 1 equiv. of Cu^{2+} in DMSO/0.5 M HEPES buffer (pH 7) (1:1) at 37 °C ([**4**] = 1 mM), and the progress of the reaction was monitored by ^{11}B NMR spectroscopy (Figure 1a) and an azomethine-H assay (Figure 1b), as reported in our previous paper.[15] In Figure 1a, broad peaks were observed at –10 to –40 ppm corresponding to the *o*-carborane part of metal-free **4** (the top spectrum in Figure 1a), and the addition of Cu^{2+} to **4** induced considerable broadening of these ^{11}B NMR signals (the second spectrum from the top in Figure 1a), possibly due to the paramagnetic effect of Cu^{2+}, indicating the formation of **5**. After incubation of **5** at 37 °C, the intensity of

its ^{11}B NMR signals at about 20 ppm corresponding to $B(OH)_3$ increased with time and reached a plateau at 8 h (solid circles in Figure 1b), suggesting that **4** had completely decomposed. In comparison, the decomposition of **2′** or **3** (1 mM) in the presence of 1 equiv. of Cu(TMEDA) (1 mM) was slower than that of **4** under the same conditions (empty circles and triangles in Figure 1b). These results indicate that the decomposition of **4** by Cu^{2+} proceeds in an intramolecular manner (in Cu^{2+} complex such as **5**) rather than by an intermolecular mechanism.

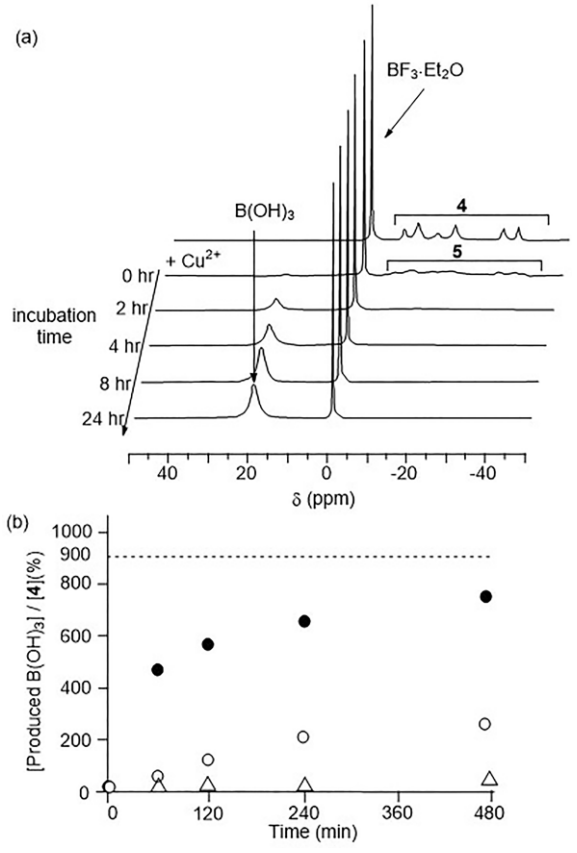


Figure 1. (a) $^{11}B\{^1H\}$ NMR (128 MHz) spectrum of **4** (1.4 mM) in the absence of Cu^{2+} (top) and $^{11}B\{^1H\}$ NMR (128 MHz) spectra of **4** (1 mM) in the presence of 1 equiv. of Cu^{2+} (1 mM) in DMSO/0.5 M HEPES buffer (pH 7)/D_2O (5:4:1, 1 mL in total) at 37 °C after incubation for 0, 2, 4, 8, and 24 h. $BF_3{\cdot}Et_2O$ (2.5 % in $CDCl_3$) was used as an external reference, as shown in Figure S1 in the Supporting Information. (b) Reaction of **2′** (empty circles), **3** (empty triangles), and **4** (solid circles) (1 mM) with 1 equiv. of Cu^{2+} (1 mM) in DMSO/0.5 M HEPES buffer (pH 7)/D_2O (5:4:1, 0.5 mL in total) at 37 °C. The reaction was quenched by the addition of 1 N aq. HCl after incubation for 0, 0.5, 1.0, 2.0, 4.0, and 8.0 h and analyzed by an azomethine-H assay.

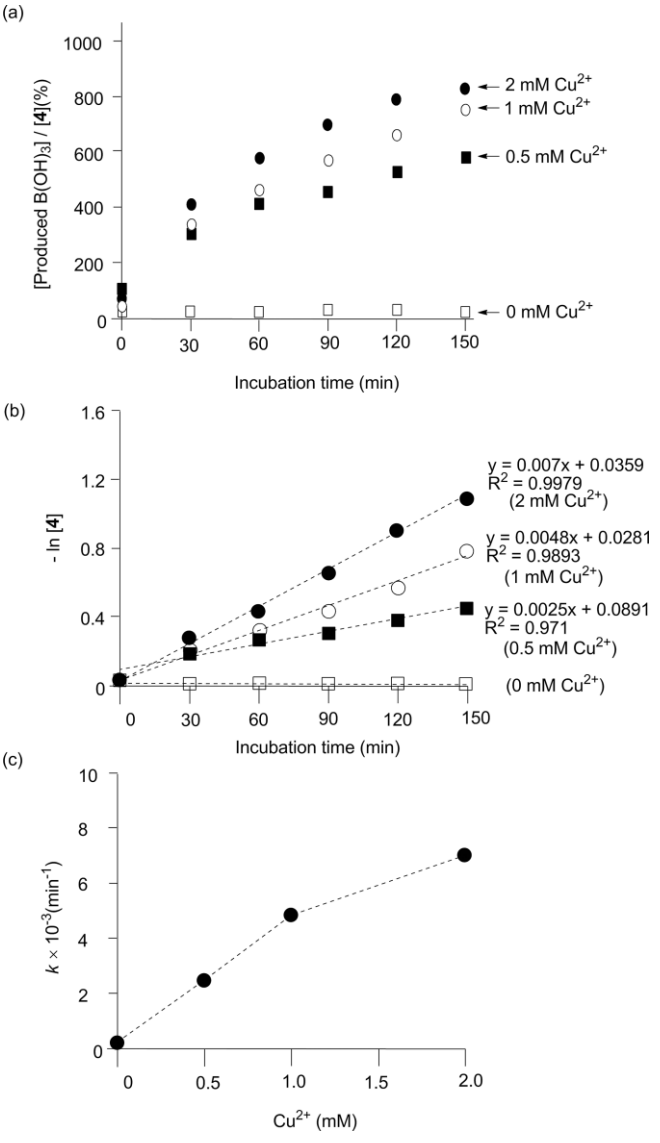


Figure 2. (a) Decomposition reaction of **4** (solid circles) (2 mM) with different numbers of equivalents of Cu^{2+} (0, 0.5, 1.0, and 2.0 mM) in DMSO/0.5 M HEPES buffer (pH 7) (1:1, 0.5 mL in total) at 37 °C. The reaction was quenched by 1 N aq. HCl after incubation for 0, 0.5, 1.0, 1.5, 2.0, and 2.5 h and analyzed by an azomethine-H assay. (b) Kinetic profile of the decomposition reaction of **4**. (c) The effect of Cu^{2+} concentration on the rate of the decomposition reaction.

Effect of the Number of Equivalents of Cu^{2+} on the Decomposition Rate of 4

The decomposition of **4** (2 mM) in the presence of different concentrations of Cu^{2+} (0, 0.25, 0.5, and 1 equiv.) in DMSO/0.5 M HEPES buffer (1:1) at 37 °C was monitored over the range 0–150 min by an azomethine-H assay. As shown in Figure 2, the rate of decomposition of **4** (2 mM) is dependent on the Cu^{2+} concentration ($k = 7 \times 10^{-3}$, 4.8×10^{-3}, and 2.5×10^{-3} min^{-1} at $[Cu^{2+}] = 2.0$, 1.0, and 0.5 mM, respectively).

Decomposition Reaction of 4 in the Presence of Various Metals

The decomposition of **4** in the presence of various d-block metal ions (Mg^{2+}, Ca^{2+}, Mn^{2+}, $Fe^{2+/3+}$, Co^{2+}, Ni^{2+}, $Cu^{+/2+}$, Zn^{2+}, and Cd^{2+}) was monitored by ^{11}B NMR spectroscopy. Figure 3a displays the change in the $^{11}B\{^1H\}$ NMR spectra (128 MHz) of **4** (1.4 mM) in the presence of salts of these metals (2 mM) in DMSO/HEPES buffer (0.5 M, pH 7)/D_2O (5:4:1) at 37 °C [(^1H) depicts H-decoupled measurement]. As shown in Figure 3a, a strong ^{11}B NMR signal corresponding to $B(OH)_3$ was observed

after the incubation of **4** with Cu^{2+} for 4 h, and the spectral change at this point (in the spectra recorded for the range δ = –10 to –40 ppm) was negligible in the presence of other metal ions, suggesting the selectivity of the method for Cu^{2+}. Although ^{11}B NMR signals for $B(OH)_3$ were observed in the presence of Cu^{+}, it is considered that this decomposition is promoted by Cu^{2+}, which is possibly produced by the oxidation of Cu^{+} by air, because the chemical yields of $B(OH)_3$ were decreased considerably in the presence of antioxidants such as sodium ascorbate (NaAsc). The chemical yields of $B(OH)_3$ released from **4** (1.4 mM) in the aforementioned reactions were also determined by azomethine-H assays, as summarized in Figure 3b, in which trace amounts of $B(OH)_3$ were observed in the presence of other d-block metal ions such as $Fe^{2+/3+}$, Mn^{2+}, Co^{2+}, and so on. These results suggest that the selectivity of **4** for Cu^{2+} is better than that of previously reported *o*-carborane derivatives such as **1** and **3**.[15]

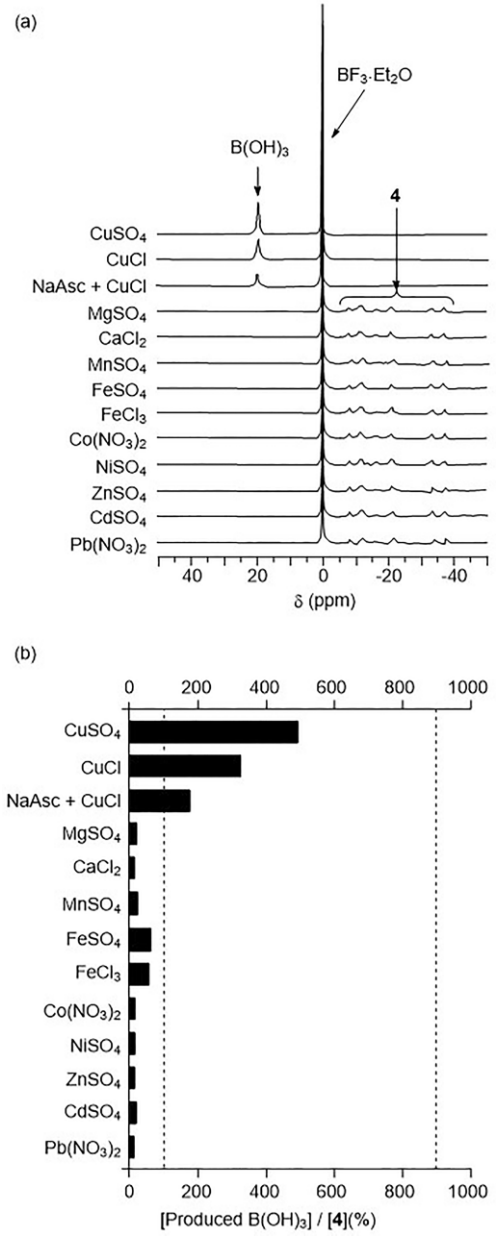


Figure 3. Decomposition reaction of **4** (1.4 mM) in the presence of Cu^{2+}, Cu^{+}, Cu^{+}+NaAsc, Mg^{2+}, Ca^{2+}, Mn^{2+}, Fe^{2+}, Fe^{3+}, Co^{2+}, Ni^{2+}, Zn^{2+}, Cd^{2+}, and Pb^{2+} (2 mM) in DMSO/0.5 M HEPES buffer (pH 7)/D_2O (5:4:1, 0.5 mL in total) at 37 °C after incubation for 4 h. The reaction was quenched by addition of 1 N aq. HCl containing 10 % D_2O (0.5 mL), and concentrations of $B(OH)_3$ were measured by $^{11}B\{^1H\}$ NMR spectroscopy (a) and azomethine-H assay (b). For $^{11}B\{^1H\}$ NMR spectroscopy experiments, 2.5 % BF_3·Et_2O in $CDCl_3$ was used as external reference.

Electrochemical Analysis of 4

To examine the mechanism responsible for the rapid decomposition of **4** in the presence of Cu^{2+}, the electrochemical properties of **4** were evaluated. We had previously found that the reactivity of *nido*-carborane derivatives with Cu^{2+} is strongly correlated with their oxidation potential.[15] The oxidation potential of **4** (1 mM) was measured by cyclic voltammetry and compared with that of **3′**. As shown in Figure 4, **4** exhibits two irreversible oxidation potentials at 0.38 and 0.70 V vs. Ag/AgCl. It is hypothesized that these two irreversible oxidation peaks correspond to the oxidation of *nido*-form **4** to $\mathbf{4_{ox}}$ and the further oxidation of $\mathbf{4_{ox}}$, respectively (Figure 5). The oxidation potentials of **4** (*nido*-form) are less positive than those of **1′** and **3′**, suggesting that **4** is more easily oxidized than **1′** and **3′** by Cu^{2+}.

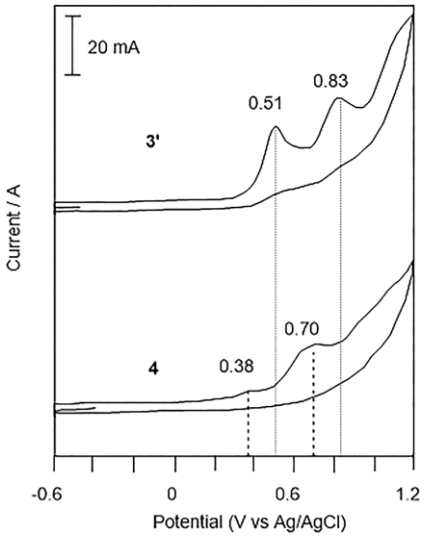


Figure 4. Cyclic voltammograms of **3′** and **4** (1 mM) recorded in DMF solutions of tetrabutylammonium hexafluorophosphate (0.1 M) with a glassy carbon electrode (0.1 mV s^{-1}) starting on reduction (potentials vs. Ag/AgCl).

In addition, intrinsic complexation constants (K_1: the first complexation constants between ligand and metal to form 1:1 complexes) of ethylenediamine (en) with Cu^{2+}, Fe^{2+}, and Mn^{2+} are reported to be $10^{10.5}$, $10^{4.3}$, and $10^{2.7}$ M^{-1},[17] respectively, implying that the Cu^{2+} complex of **4** having a TriMEDA unit is much more stable than its Mn^{2+} and Fe^{2+} complexes. Therefore, it was concluded that rapid and selective decomposition of **4** by Cu^{2+} is attributable to the less positive redox potential of **4**

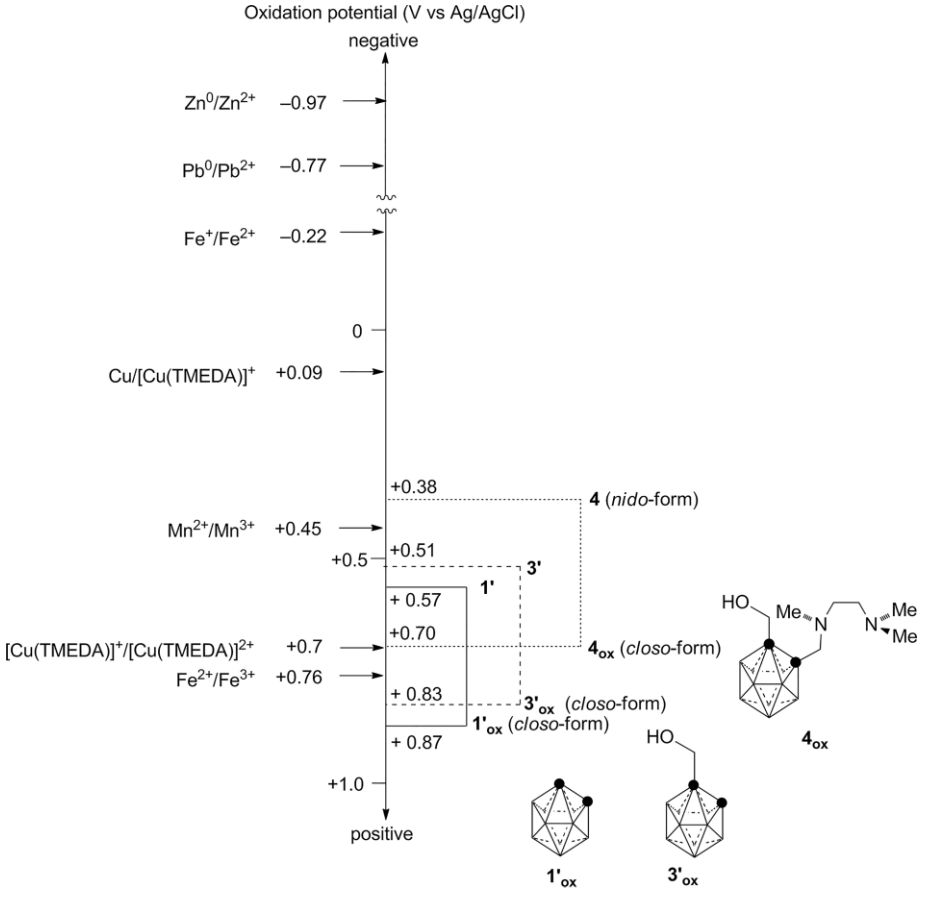


Figure 5. Summary of oxidation potentials of **1′**, **3′**, and **4** (*nido*-form) with redox potentials of Cu, Fe, Pb, Mn, and Zn.

relative to that of complex $[Cu(TMEDA)]^{2+}$ (+ 0.7 V) and its stable complexation with Cu^{2+}. It is considered that $\mathbf{4_{ox}}$, which corresponds to **6** derived by the oxidation of **5** (Scheme 1), is a reactive intermediate that immediately decomposes in aqueous solution, because this species was hardly detected in the ^{11}B NMR spectroscopy experiment.[20]

Cellular Uptake of 3, 4, 7, and 9 in Jurkat T and HeLa S3 Cells

The intracellular uptake of **3**, **4**, **7**, and **9**[13] was determined by ICP-AES (249.733 nm) or ICP-MS, and the results are shown in Figure 6. Cellular uptake of other *closo*-*o*-carborane derivatives **3**, **7**, and **9** was observed to some extents, while the uptake of **4** by Jurkat T cells was quite low (0.022 fmol as molecule [0.20 fmol as boron atom per cell]). These results indicated that **4** would be suitable for the detection of Cu^{2+} in vitro.

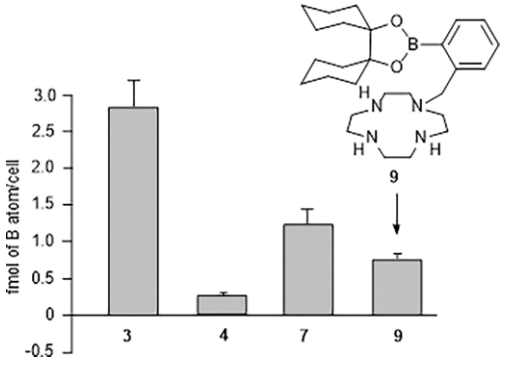


Figure 6. Concentrations of boron atoms of **3**, **4**, **7**, and **9**[13] in Jurkat T (for **4** and **9**) or HeLa-S3 (for **3** and **7**) cells (ca. 4.0×10^6 cells) determined by ICP-MS (for **4**) or ICP-AES (for **3** and **7**) after incubation with **3** (33 μM), **4** (24 μM), **7** (33 μM), and **9** (33 μM) for 1 h at 37 °C and lysis with RIPA buffer. Intracellular concentrations of **3**, **4**, **7**, and **9** were determined on the basis of the results of the control experiments, in which Jurkat T cells were incubated with DMSO alone. The error bars display the standard deviation obtained from three independent experiments.

^{11}B Magnetic Resonance Imaging (MRI) of Cu^{2+} with Probe 4

These results enabled us to conduct ^{11}B MRI experiments for the detection of Cu^{2+} ions by **4** in solutions. Reactions of **4** (1 mM) were conducted in the presence of 1 equiv. of Cu^{2+}, Mn^{2+}, Fe^{3+}, or Zn^{2+} in DMSO/0.5 M HEPES buffer (pH 7) incubated at 37 °C for 8 h. As shown in the upper left part of Figure 7, samples were prepared in four different tubes (spots 1–4), which contained **4** (1 mM) and no metal or Cu^{2+}, Mn^{2+}, Fe^{3+},

or Zn^{2+} (spot 1), $B(OH)_3$ (9 mM) (spot 2), no metal or Cu^{2+}, Mn^{2+}, Fe^{3+}, or Zn^{2+} (1 mM) (spot 3), and blank (spot 4), and the results were monitored by ^{11}B MRI analysis. Data for ^{11}B MRI were acquired by using an ultra-short echo time (UTE) pulse sequence with short TE (echo time) and TR (repetition time) values (199 μs and 30 ms, respectively) because of the short relaxation times of $B(OH)_3$.[15] As shown in Figure 7, a strong ^{11}B MRI signal was observed in spot 1 only in the presence of Cu^{2+} ions, while no signal was detected in samples containing **4** and the other metals. Thus, selective imaging of copper based on the decomposition reaction of **4** can be achieved by ^{11}B MRI. In the ^1H MRI spectrum (Figure S2 in the Supporting Information), the brightness of solutions containing Mn^{2+} or Cu^{2+} ions (spots 1 and 3 in Figure S2) is greater than that of solutions without Cu^{2+} (spots 2 and 4), because the relaxation time of the ^1H signal corresponding to H_2O was reduced by the paramagnetic relaxation enhancement of Cu^{2+} and Mn^{2+}.[21]

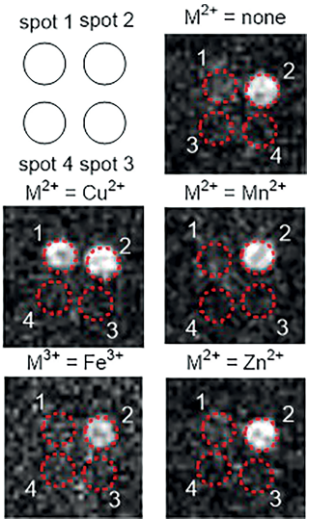


Figure 7. In the upper left is an illustration of the samples used in the MRI experiments: Each sample contains a reaction mixture of 1 mM **4** with no metal or 1 mM of Cu^{2+}, Mn^{2+}, Fe^{2+}, or Zn^{2+} in DMSO/0.5 M HEPES buffer (1:1) after incubation for 8 h at 37 °C (spot 1), a reference solution of 9 mM $B(OH)_3$ in DMSO/0.5 M HEPES buffer (1:1) (spot 2), a solution of no metal or each metal alone in DMSO/0.5 M HEPES buffer (1:1) (spot 3), and a blank consisting of DMSO/0.5 M HEPES buffer (1:1) (spot 4). The other pictures show actual ^{11}B MRI images of samples containing no metal, Cu^{2+}, Mn^{2+}, Fe^{2+}, or Zn^{2+}. ^{11}B NMR images were acquired by a two-dimensional ultra-short echo time sequence (UTE2D) with BF1 values of about 128.392 MHz, TE = 199 μs, and TR = 30 ms.

Determination of Cu^{2+} Concentration with Probe 4 by ^{11}B NMR/MRI

Finally, we observed changes in the ^{11}B NMR spectra and MRI images of **4** (2 mM) with increasing concentrations of Cu^{2+} (0, 0.02, 0.10, 0.20, 1.00, and 2.00 mM) in aqueous solution after an incubation period of 8 h. As shown in Figure 8, ^{11}B NMR/MRI signals of $B(OH)_3$ increased with increasing Cu^{2+} concentration. Although the change in the $^{11}B\{^1H\}$ spectrum of **4** was observed even in the presence of 0.02 mM of Cu^{2+} ion, the ^{11}B MRI signals were not observed at this concentration, possibly because of weaker signal sensitivity of ^{11}B MRI than that of $^{11}B\{^1H\}$ NMR spectroscopy (due to the difference in the instrumental setup).[22]

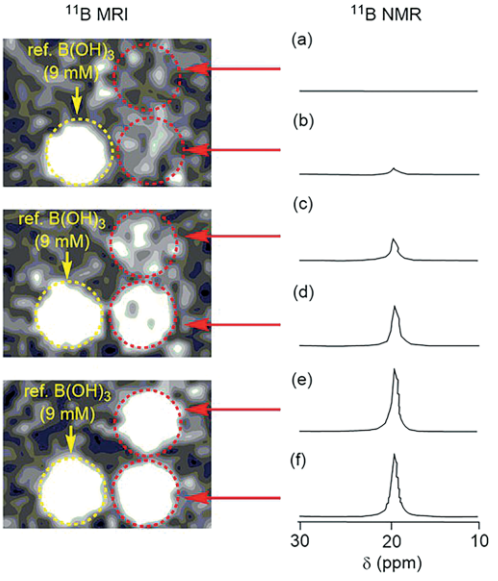


Figure 8. ^{11}B MRI and $^{11}B\{^1H\}$ NMR (128 MHz) spectra of **4** (2 mM) in DMSO/0.5 M HEPES buffer (pH 7)/D_2O (5:4:1, 0.5 mL in total) after incubation with various concentrations [(a) 0, (b) 0.02, (c) 0.10, (d) 0.20, (e) 1.00, and (f) 2.00 mM] of Cu^{2+} at 37 °C. After 8 h, 0.5 mL of the reaction mixtures were mixed with 0.5 mL of 10 % D_2O in 1 N aq. HCl before the NMR spectroscopic measurement. A 2.5 % solution of $BF_3{\cdot}Et_2O$ in $CDCl_3$ was used as the external reference. ^{11}B NMR images were acquired by a two-dimensional ultra-short echo time sequence (UTE2D) with BF1 values of about 128.392 MHz, TE = 199 μs and TR = 30 ms.

Conclusions

We report the decomposition of *nido-o*-carborane compound **4** bearing TriMEDA in the presence of Cu^{2+} under physiological conditions to release 9 equiv. of $B(OH)_3$. It was also found that **4** is decomposed by Cu^{2+} even in the presence of a catalytic amount (0.25 equiv.) of Cu^{2+}. Moreover, the selective detection of Cu^{2+} ions by ^{11}B MRI by using **4** was demonstrated and explained by the relationship of the redox potentials of **4** and Cu^{2+} and by the selective complexation of **4** with Cu^{2+}. We conclude that these results represent useful information for the development of new methods for the detection of transition metal ions and the design and synthesis of ^{11}B MRI (NMR spectroscopy) probes.

Experimental Section

General Information: *o*-Carborane and the organic solvents for spectroscopic analysis were purchased from WAKO CHEMICALS Co., Ltd.; $ZnSO_4{\cdot}7H_2O$ and $NiSO_4{\cdot}6H_2O$ were purchased from Yoneyama Chemical Industry Co. Ltd.; $Zn(NO_3)_2{\cdot}6H_2O$, $Cd(NO_3)_2{\cdot}4H_2O$, $Co(NO_3)_2{\cdot}6H_2O$, and $FeSO_4{\cdot}7H_2O$ were purchased from Kanto Chemical Co. Ltd.; $FeCl_2{\cdot}4H_2O$, $Cu(NO_3)_2{\cdot}3H_2O$, and CuCl were purchased from WAKO CHEMICALS Co., Ltd.; anhydrous $CaCl_2$, $Pb(NO_3)_2$, $CuSO_4{\cdot}5H_2O$, and CuI were obtained from Nacalai Tesque, Inc.; $MnSO_4{\cdot}H_2O$ was purchased from Sigma–Aldrich Co.; azomethine-H reagent was purchased from Dojindo. All aqueous solutions were prepared by using deionized and distilled water. The Good's buffer reagent HEPES [*N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic

acid, pK_a = 7.5] was obtained from Dojindo. All other reagents and solvents were of the highest commercial quality available and were used without further purification, unless otherwise noted. Melting points were measured with a YANACO Micro Melting Point Apparatus. IR spectra were recorded with a JASCO FTIR-410 and a Perkin-Elmer Spectrum100 spectrophotometer at room temperature. ^{1}H (400 MHz), ^{13}C (100 MHz), and ^{11}B (128 MHz) NMR spectra were recorded with a JEOL Lambda 400 spectrometer. ^{1}H (300 MHz) and ^{13}C (75 MHz) NMR spectra were recorded with a JEOL Always 300 spectrometer. Chemical shifts (δ) in $CDCl_3$ were determined relative to an internal reference of tetramethylsilane (TMS) and $CDCl_3$ for ^{1}H and ^{13}C NMR spectroscopy, respectively. ^{11}B NMR spectra were measured in a quartz NMR tube by using boron trifluoride diethyl ether complex in $CDCl_3$ as an external reference (δ = 0 ppm). Elemental analyses were performed with a Perkin–Elmer CHN 2400 analyzer. Electrospray ionization (ESI) mass spectra were recorded with a JEOL JMS-SX102A and a Varian 910-MS instrument. Thin-layer chromatography (TLC) was performed by using Merck Silica 5554 (silica gel) TLC plates. Silica gel column chromatography was performed by using Fuji Silysia Chemical FL-100D.

1,2-Dicarbadecaborate-1-hydroxymethyl-2-ethyl-4-methylbenzensulfonate (8): To a THF solution of **7**[19] (15 mL, 300 mg, 1.47 mmol) were added triethylamine (306 μL, 2.20 mmol), dimethylaminopyridine (18 mg, 0.15 mmol), and TsCl (279.3 mg, 1.47 mmol) at 0 °C, and the reaction mixture was stirred at room temperature for 18 h. The reaction was quenched with HCl (1 N aq.), and the mixture was extracted with AcOEt, washed with brine, and dried with anhydrous Na_2SO_4. After filtration, the solvents were removed, and the residue was purified by silica gel column chromatography (hexane/AcOEt = 4:1) to afford **8** as a white solid (350 mg, 67 % yield). ^{1}H NMR (400 MHz, CD_3OD, 25 °C, TMS): δ = 7.79 (d, J = 8.4 Hz, 2 H), 7.40 (d, J = 7.8 Hz, 2 H), 4.55 (s, 2 H), 4.17 (d, J = 6.9 Hz, 2 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C, TMS): δ = 146.16 (2 C), 131.63 (2 C), 130.28, 128.02, 79.25, 72.98, 67.42, 63.93 ppm. ^{11}B{^{1}H} NMR (128 MHz, CD_3OD, 25 °C, BF_3·Et_2O): δ = –10.4, –11.4, –14.8, –17.3, –18.4, –22.1, –33.0, –37.4 ppm. IR (ATR): $\tilde{\nu}$ = 3528.84, 2580.74, 2560.76, 1595.87, 1372.67, 1189.71, 1171.59, 1080.72, 997.61, 855.66, 661.61 cm^{-1}. HRMS (EI): calcd. for $C_{11}H_{22}B_{10}O_4S^+$ [M + H]$^+$ 360.2169; found 360.2191. $C_{10}H_{18}B_{10}O_2$ (278.35): calcd. C 36.86, H 6.19, N 0.00; found C 36.95, H 6.10, N 0.11.

Hybrid Compound of *nido*-Carborane and TriMEDA (Compound 4): To a solution of **8** (179 mg, 0.5 mmol) in DMF (2.0 mL) was added *N*,*N*,*N*′-trimethylethylenediamine (325 μL, 2.5 mmol), and the mixture was stirred at 50 °C for 21 h. After concentration in vacuo, the residue was purified by silica gel column chromatography ($CHCl_3$/MeOH = 4:1) and then precipitated with H_2O to afford compound **4** as a white solid (30 mg, 20 % yield). ^{1}H NMR (300 MHz, [D_6]DMSO, 25 °C, TMS): δ = 3.75–3.25 (m, 9 H), 2.82–2.78 (m, 3 H), 2.58 (m, 2 H), 2.46 ppm (m, 4 H) ppm. ^{13}C NMR (100 MHz, [D_6]DMSO, 25 °C, TMS): δ = 65.63, 62.38, 53.22, 51.36, 44.28, 43.93, 43.59, 42.99, 40.65 ppm; ^{11}B{^{1}H} NMR (128 MHz, [D_6]DMSO, 25 °C, BF_3·Et_2O): δ = –6.9, –11.1, –16.0, –21.2, –33.4, –36.9 ppm. IR (ATR): $\tilde{\nu}$ = 2524.73, 2494.71, 1659.93, 1504.92, 1465.83, 983.69 cm^{-1}. HRMS (ESI): calcd. for $C_9H_{27}B_9N_2O$ + H$^+$ [M + H]$^+$ 278.2961; found 278.3082.

General Procedure for Detection of d-Block Metal Ions with ^{11}B NMR Spectroscopy (Figures 1a and 3a): Sample solutions of **4** (20 mM) in DMSO (0.04 mL) were prepared and mixed with a solution of a metal salt in DMSO/HEPES buffer/D_2O (9:9:1) ([metal salt] = 1.05 mM), 0.76 mL). These resulting mixtures were incubated in screw-capped vials at a given temperature and for a given time. Then, an aliquot of the reaction mixture (0.66 mL) was placed into a quartz NMR tube and analyzed by ^{11}B NMR spectroscopy (128 MHz) with a sweep width of 38022 Hz, 4096 data points, a 45° pulse width, a 0.16 second recycle time, and 2850 scans. Each spectrum was processed with 4.6 Hz line broadening by using BF_3·Et_2O in $CDCl_3$ as an external reference (δ = 0 ppm).[15]

Azomethine-H Assay for the Decomposition of Carborane Derivatives by Metal Ions (Figures 1b and 3b)

Preparation of Reaction Samples (Solution A): For **3** and **4**, solutions (0.45 mL) of each compound in DMSO/HEPES buffer (5:4 for **3** and **4**) ([**3** and **4**] = 1.25 mM) were prepared, to which a solution (0.05 mL) of the metal salt in D_2O was added ([metal salt] = 20 mM). These mixtures were incubated at 37 °C in a screw-capped vial for the decomposition reaction. Then, an aliquot of the reaction mixture (50 μL of solution A) was taken at given reaction times (after 0, 1, 2, 4, 6, and 8 h), to which HCl (50 μL, 1 N aq.) was added. The concentrations of $B(OH)_3$ produced by the decomposition reaction of carborane derivatives were determined by azomethine-H assay as previously reported (details are described in the Supporting Information).[15]

Cyclic Voltammetry of Carborane Derivatives and Metal Ions (Figure 4): Cyclic voltammetry measurements were performed with a BAS model 660A electrochemical analyzer at room temperature in DMF containing nBu$_4$N(PF_6) (0.1 M) as the supporting electrolyte in a standard one-component cell equipped with a 3 mm outer diameter glassy carbon working electrode, a platinum wire counter electrode, and the Ag/AgCl reference electrode (Ag/AgCl in MeCN containing 0.01 M $AgNO_3$ and 0.1 M nBu$_4$NPF_6). All solutions were deoxygenated by bubbling with argon for at least 10 min immediately before the measurement.

Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES) or Mass Spectrometry (ICP-MS) Experiments (Figure 6): Jurkat T cells were incubated in 100 mm-coating dishes under an atmosphere of 5 % CO_2 at 37 °C for 72 h. The numbers of cells were counted (ca. 4.0 × 10^6 cells), and these cells were incubated in RPMI 1640 or DMEM/DMSO (1000:3.3, v/v) with **3** (33 μM), **4** (24 μM), **7** (33 μM), and **9** (30 μM) under an atmosphere of 5 % CO_2 at 37 °C for 30 min. The culture medium solutions were transferred into 50 mL centrifuge tubes and centrifuged at 4 °C (1400 rpm for 7 min). The medium was removed, and Jurkat cells were transferred to a 15 mL centrifuge tube with RPMI 1640 or DMEM (5 mL × 2). After centrifugation at 1400 rpm and 4 °C for 7 min, the medium solution was removed. After being transferred to a 1.5 mL Eppendorf tube with RPMI (0.45 mL × 2), the Jurkat cells were centrifuged at 2000 rpm and 4 °C for 10 min, and the medium solution was removed. Radio-immunoprecipitation assay (RIPA) buffers (500 μL) were then added, and the sample was kept in the centrifuge tube at 0 °C for 30 min. The solutions in these centrifuge tubes were centrifuged at 15000 rpm and 4 °C for 10 min, and the supernatant liquid (400 μL) (portion A) was taken and diluted with HCl (5 mL, 1 N aq.) and made up to 10 mL with H_2O, and this mixture was then centrifuged at 3000 rpm and 4 °C for 10 min before ICP-AES analysis with a Shimadzu ICPE-9000 or ICP-MS analysis with a PerkinElmer NexION 300 instrument.

Magnetic Resonance Imaging of Probes in the Presence of Cu^{II} (Figures 7 and 8): For the experiment in Figure 7, a solution (1 mL) of **4** (1 mM) and each metal [$CuSO_4$·$5H_2O$, $MnSO_4$, $FeCl_3$, or $ZnSO_4$ (1 mM)] in DMSO/0.5 M HEPES buffer (pH 7) (1:1) was incubated at 37 °C for 8 h in a screw-capped vial. For the experiment in Figure 8, a solution (0.5 mL) of **4** (2 mM) and each metal ($CuSO_4$·$5H_2O$, $MnSO_4$, $FeCl_3$, or $ZnSO_4$) (2 mM) in DMSO/0.5 M HEPES buffer (pH 7) (1:1) was incubated at 37 °C for 8 h in a screw-capped vial, and the reaction was then quenched by addition of HCl (0.5 mL, 1 N in H_2O/

D_2O 9:1). Solutions of $B(OH)_3$ (9 mM) or metals (1 mM) in DMSO/0.5 M HEPES buffer (pH 7) (1:1) were prepared as reference solution. NMR imaging experiments were performed with a 9.4 T Avance I MicroImaging system (Bruker BioSpin, Rheinstetten, Germany) with a Micro2.5 gradient coil and $^1H/^{11}B$ dual tuned RF coil (dia. 25 mm). ^{11}B NMR images were acquired by a two-dimensional ultra-short echo time sequence (UTE2D) with the following parameters: TE = 199 μs; TR = 30 ms; Flip angle: 60 deg; matrix size: 64 × 64; FOV = 60 × 60 mm; slice thickness: 20 mm; number of projections: 202. The scan time was 16 min.

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Keywords: Carboranes · Copper · Chelates · Sensors · Magnetic resonance imaging

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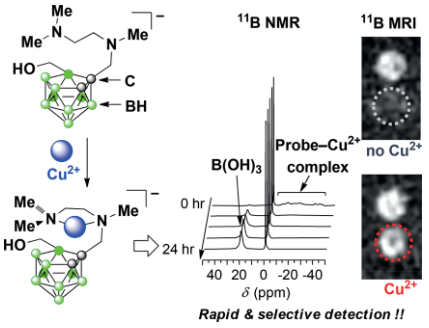

A hybrid molecule made up of a *nido-o*-carborane and a metal chelator decomposes rapidly in the presence of Cu^{2+} under mild conditions. This probe is useful for the ^{11}B NMR/MRI sensing of Cu^{2+} in vitro.

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