is also more intense and narrower than MLCT I in the parent species (Table V). Band Ib, MLCT I in the diadduct $BF_3$ species, is apparently very strong and broad. Its high oscillator strength may be attributed to even greater mixing of metal and bpz orbitals caused by the strongly accepting nature of the two $BF_3$ groups. The broadness may reflect significant splitting of the d ($t_{2g}$ in $O_h$) manifold since one of the three d orbitals ($b_2$) is strongly favored for mixing and hence stabilization relative to the other two.

In conclusion, the protonation and $BF_3$ data provide evidence for significant mixing between metal and ligand orbitals and the stabilization of a complex whose back-donating character may be very significant.

**Acknowledgment.** The authors are indebted to Prof. I. M. Walker for useful discussions. We also thank the Natural Sciences and Engineering Research Council of Canada (Ottawa) and the Office of Naval Research (Washington, DC) for financial support. This work is part of a joint ONR Contract with Prof. A. J. Bard.

**Registry No.** $Mo(CO)_4bpz$, 80907-60-4; $Mo(CO)_4(bpzH)^+$, 95891-49-9; $Mo(CO)_4(bpzBF_3)$, 95891-50-2; $Mo(CO)_4(bpz(BF_3)_2)$, 95891-51-3; $W(CO)_4bpz$, 80925-51-5; $W(CO)_4(bpzH)^+$, 95891-52-4; $W(CO)_4(bpzBF_3)$, 95891-53-5; $W(CO)_4(bpz(BF_3)_2)$, 95891-54-6.

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Contribution from the Department of Chemistry and Biochemistry, University of California, Los Angeles, Los Angeles, California 90024

# Synthesis and Antibody-Labeling Studies with the *p*-Isothiocyanatobenzene Derivatives of 1,2-Dicarba-*closo*-dodecaborane(12) and the Dodecahydro-7,8-dicarba-*nido*-undecaborate(1−) Ion for Neutron-Capture Therapy of Human Cancer. Crystal and Molecular Structure of $Cs^+[$*nido*-7-(*p*-$C_6H_4NCS$)-9-I-7,8-$C_2B_9H_{11}]^-$

EUGENE A. MIZUSAWA, MICHAEL R. THOMPSON, and M. FREDERICK HAWTHORNE*

*Received January 3, 1985*

To investigate the boron-10 labeling of tumor-localizing antibodies, new boron-containing compounds that can be used to conjugate antibody protein have been synthesized. These include the *p*-isothiocyanatophenyl derivatives of 1,2-dicarba-*closo*-dodecaborane(12) and the dodecahydro-7,8-dicarba-*nido*-undecaborate(1−) ion. Antibody-labeling studies using some of these compounds are described. In addition, the cesium salt of the 7-(*p*-isothiocyanatophenyl)-9-iodododecahydro-7,8-dicarba-*nido*-undecaborate(1−) ion, synthesized using formal $I^+$ that had been generated by the action of *N*-chloro-*p*-toluenesulfonamide on sodium iodide in phosphate buffer, has been characterized by single-crystal, X-ray diffraction methods. The compound crystallized in the monoclinic space group $P2_1/c$, with $a$ = 13.809 (6) Å, $b$ = 12.509 (5) Å, $c$ = 10.601 (3) Å, $\beta$ = 95.79 (3)°, and $Z$ = 4. Diffraction data to $2\theta$ = 54° (Mo K$\alpha$ radiation) were collected on a Syntex P$\bar{1}$ diffractometer, and the structure was solved by conventional heavy-atom methods. The final discrepancy indices were $R$ = 0.051 and $R_w$ = 0.061, for 2664 independent reflections. The structure was found to be disordered, with the title compound and its enantiomer occupying the same positions in the unit cell.

## Introduction

The potential use of boron-containing compounds in cancer therapy is based on the nuclear property of the boron-10 isotope to absorb thermal neutrons (0.025 eV), releasing an $\alpha$-particle and recoiling lithium-7 ion with an average total kinetic energy of 2.4 MeV:

$$^{10}_{5}B + {}^{1}_{0}n \rightarrow [^{11}_{5}B] \rightarrow {}^{7}_{3}Li + {}^{4}_{2}He + 2.4\ MeV$$

This type of therapy consists of localizing boron-10 in the tumor mass, followed by external thermal neutron irradiation of the area. The resulting fission fragments, having a range in tissue of less than 10 $\mu$m,[1] would deposit the 2.4-MeV energy released in an area confined only to the tumor and to those normal cells immediately surrounding the tumor area.

In 1936,[2] G. L. Locher first proposed the therapeutic possibilities of neutron capture using boron and other elements possessing high-thermal neutron-capture cross sections. In 1940, in vitro studies by Kruger demonstrated the feasibility of boron-10, thermal neutron-capture therapy.[3] In this study, tumor tissues that had been mixed with boric acid and irradiated with thermal neutrons exhibited reduced capacities to be transplanted into mice. Later that same year, Zahl, Cooper, and Dunning, using tumor-bearing mice, claimed a 45% cure or regression due to the boron-10, thermal neutron process.[4] In 1949 Conger and Giles examined the effect of thermal neutrons on plant rootlets.[5] They reported that when boron-10 was introduced, the extent of chromosome damage that was observed was proportional to the amount of boron-10 present.

For normal brain tissue, the entry of foreign chemicals from the bloodstream is prevented by a physiological phenomenon called the blood brain barrier. In the case of most brain tumors, the blood brain barrier is diminished, and on this basis, L. E. Farr in 1951–1952 at the Brookhaven National Laboratory[6-8] and W. H. Sweet 10 years later at the Massachusetts General Hospital[9,10] attempted to treat patients with brain tumors using intravenous boron-10-enriched borax injections followed by thermal neutron irradiation. In both studies, the results were not encouraging, as serious injury to the scalp and blood vessels of the normal brain area was observed.[11]

In Japan, H. Hatanaka has been treating patients with brain tumors since 1972 using boron-10-enriched $Na_2B_{12}H_{11}SH$,[12] and he reports a 33.3% (10/30) 5-year survival with a mean survival time of 36.9 ± 14.8 months.[13] The longest surviving patient is

(1) Tractenberg, M. C.; Kornblith, P. L., Hauptli, J. *Brain Res.* **1972**, *38*, 279.
(2) Locher, G. L. *Am. J. Roentgenol. Radium Ther.* **1936**, *36*, 1.
(3) Kruger, P. G. *Proc. Natl. Acad. Sci. U.S.A.* **1940**, *26*, 181.
(4) Zahl, P. A.; Cooper, F. S.; Dunning, J. A. *Proc. Natl. Acad. Sci. U.S.A.* **1940**, *26*, 591.
(5) Conger, A. D.; Giles, N. H., Jr. *Genetics* **1950**, *35*, 397.
(6) Farr, L. E. *Acta Unio Int. Cancrum* **1955**, *11*, 5.
(7) Farr, L. E.; Sweet, W. H.; Locksley, H. B.; Robertson, J. S. *Trans. Am. Neurol. Assoc.* **1954**, *79*, 110.
(8) Godwin, J. T.; Farr, L. E.; Sweet, W. H.; Robertson, J. S. *Cancer* **1955**, *8*, 601.
(9) Sweet, W. H.; Soloway, A. H.; Brownell, G. L. *Acta Unio Int. Cancrum* **1966**, *16*, 1216.
(10) Sweet, W. H.; Soloway, A. H.; Wright, R. L. *J. Pharmacol. Exp. Ther.* **1962**, *137*, 263.
(11) Brownell, G. L., Murray, B. W.; Sweet, W. H.; Wellum, G. R.; Soloway, A. H. *Proc. Natl. Cancer Conf.* **1973**, *7*, 827.
(12) Hatanaka, H. In "Synthesis and Applications of Isotopically Labeled Compounds"; Duncan, W. P., Susan, A. B., Eds.; Elsevier: Amsterdam, 1983; pp 167–174.
(13) Hatanaka, H. *Proc. First Int. Symp. Neutron Capture Ther.* **1984**, *1*, 384.

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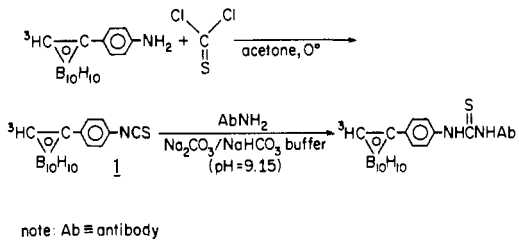


**Figure 1.** Synthesis and antibody binding of 2-(*p*-isothiocyanatophenyl)-1,2-dicarba-*closo*-[1-$^3$H]dodecaborane(12) (**1**).

now 62 years old, 11.5 years after boron-10, thermal neutron-capture therapy. These encouraging results have prompted other investigators to seek new methods of localizing boron-10 in brain tumors and other tumor types.[14] This includes a concept that was first suggested by Bale in 1952,[15] the use of boron-10 labeled, tumor-localizing antibodies for neutron-capture therapy.

The major goal of this study is to utilize the thermal neutron-capturing reaction of boron-10 for selective tumor cell destruction using boron-10-labeled, tumor-localizing antibodies. Research in the interdependent chemical and immunological areas of this multidisciplinary project is presented.

## Results and Discussion

The boron-10 labeling of goat anticarcinoembryonic antigen IgG (anti-CEA) using the *p*-[1,2-dicarba-*closo*-[1-$^3$H]dodecaboran(12)-2-yl]benzenediazonium ion ([$^3$H]DBD) has been investigated,[16] and in vivo studies with hamsters bearing GW-39 tumors demonstrated that anti-CEA labeled with three carborane cages, i.e., 30 boron atoms, retained selective localization in tumors.[17] With this reagent, up to 13 carborane cages could be conjugated to each antibody molecule, but increased precipitation of antibody protein was observed as the number of carborane cages that were conjugated to each antibody molecule was increased. Whether the antibody protein loss was due to denaturation by the boron-10-labeling conditions or due to a decrease in the solubility of the carborane–antibody conjugate was unknown. To further investigate the boron-10 labeling of anti-CEA, the hydrophobic 1-(*p*-isothiocyanatophenyl)-1,2-dicarba-*closo*-dodecaborane(12) (**1**) and the water-soluble sodium salt of the 1-(*p*-isothiocyanatophenyl)dodecahydro-7,8-dicarbaundecaborate(1−) ion were synthesized and used in antibody protein conjugation studies.

The utility of the isothiocyanate group for antibody protein labeling has been extensively studied with respect to fluorescent antibody techniques.[18] Riggs et al.[19] were first to synthesize fluorescein isothiocyanate, by treating aminofluorescein with thiophosgene. Reacting with an alkaline solution of antibody, fluorescein isothiocyanate forms a thiocarbamide bond with free antibody amino groups, with a maximum combining number of about 15 for intact antibody protein. The synthesis and protein labeling using 1-[S(CH$_3$)(CH$_2$C$_6$H$_4$NCS)]-10-S(CH$_3$)$_2$B$_{10}$H$_8$ for boron-10 thermal neutron-capture therapy has been reported,[20] and more recently, a water-soluble deca-*B*-chloro-1,2-dicarba-*closo*-dodecaborane(12) isothiocyanate derivative has been synthesized and incorporated into poly-L-lysine.[21]

By the procedure illustrated in Figure 1, 2-(*p*-isothiocyanatophenyl)-1,2-dicarba-*closo*-[1-$^3$H]dodecaborane(12) (**1**($^3$**H**)), a water-insoluble antibody conjugation reagent, was synthesized in high yield. The water-soluble antibody conjugation reagent, sodium 7-(*p*-isothiocyanatophenyl)dodecahydro-7,8-dicarba-*nido*-undecaborate(1−) (**4**), was synthesized following the reaction scheme illustrated in Figure 2. The previously synthesized[16] 1-(*p*-aminophenyl)-1,2-dicarba-*closo*-dodecaborane(12) was first degraded in refluxing ethanolic potassium hydroxide, followed by precipitation with aqueous tetramethylammonium chloride to yield (CH$_3$)$_4$N$^+$[*nido*-7-(*p*-C$_6$H$_4$NH$_2$)-7,8-C$_2$B$_9$H$_{11}$]$^-$ (**2**). This aminophenyl monoanion was then treated with thiophosgene in anhydrous acetone at 0 °C to give the tetramethylammonium salt **3**. The sodium salt **4** was obtained with sodium cation-exchange resin eluted with 1:1 acetonitrile/water. From the same procedures, the 93% $^{10}$B-enriched and tritium-radiolabeled forms of **1**–**4** were synthesized. The specific activities of the tritium-radiolabeled species **3**($^3$**H**) and **4**($^3$**H**) were approximately 1% of the specific activity of the 2-(*p*-aminophenyl)-1,2-dicarba-*closo*-[1-$^3$H]dodecaborane(12) precursor. This loss of radioactivity is the result of base-catalyzed hydrogen exchange of the carborane C-$^3$H during the carborane cage degradation step using ethanolic KOH. Fortunately, the specific activities of **3**($^3$**H**) and **4**($^3$**H**) were sufficient for this study, without resorting to the use of C$_2$H$_5$O$^3$H and KO$^3$H in base-degradation reaction. The unlabeled products **1**–**3** were characterized by elemental analyses and standard spectroscopic techniques, which included IR, $^1$H NMR, and $^{11}$B NMR.

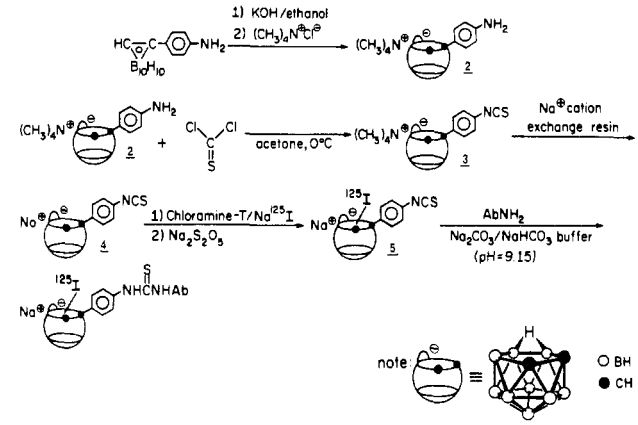


**Figure 2.** Syntheses of (CH$_3$)$_4$N$^+$[*nido*-7-(*p*-C$_6$H$_4$NH$_2$)-7,8-C$_2$B$_9$H$_{11}$]$^-$ (**2**), (CH$_3$)$_4$N$^+$[*nido*-7-(*p*-C$_6$H$_4$NCS)-7,8-C$_2$B$_9$H$_{11}$]$^-$ (**3**), Na$^+$[*nido*-7-(*p*-C$_6$H$_4$NCS)-7,8-C$_2$B$_9$H$_{11}$]$^-$ (**4**), and Na$^+$[*nido*-7-(*p*-C$_6$H$_4$NCS)-9-$^{125}$I-7,8-C$_2$B$_9$H$_{10}$]$^-$ (**5**).

In an earlier animal study,[17] the in vivo tumor localization of boron-10 by anti-CEA was indirectly measured. Anti-CEA was first boron-10 labeled, and the resulting carborane–antibody conjugate was $^{125}$I radiolabeled by the iodination of tyrosine phenoxide groups present in the antibody molecule. From the amount of $^{125}$I radioactivity counted in weighed tissue samples, calculation of the percent injected antibody dose per gram of tissue for each sample was made, from which boron-10 concentrations were calculated from the previously determined number of carborane cages bound per antibody molecule. In the search for a more direct method of determining the tumor localization of boron-10, the 7-(*p*-isothiocyanatophenyl)-9-iodododecahydro-7,8-dicarba-*nido*-undecaborate(1−) ion (**5**) was synthesized and characterized for later use in antibody protein conjugation as illustrated in Figure 2. Unlike the earlier animal study, the carborane and not the antibody will carry the $^{125}$I radiolabel.

Monohalogenated derivatives of the dodecahydro-7,8-dicarba-*nido*-undecaborate(1−) ion were first reported by Olsen and Hawthorne.[22] In this work, the products obtained from the reaction of elemental iodine with the *nido*-7,8-C$_2$B$_9$H$_{12}^-$ ion in absolute ethanol were determined by $^{11}$B NMR to be *nido*-9-I-7,8-C$_2$B$_9$H$_{11}^-$ and its enantiomer, *nido*-11-I-7,8-C$_2$B$_9$H$_{11}^-$. This reaction was viewed as iodination of the carborane cage monoanion by electrophilic substitution.

(14) For representative work, see: *Proc. First Int. Symp. Neutron Capture Ther.* **1984**, *1*.
(15) Bale, W. F. *Proc. Natl. Cancer Conf.* **1952**, *2*, 967.
(16) Mizusawa, E.; Dahlman, H. L.; Bennett, S. J.; Goldenberg, D. M.; Hawthorne, M. F. *Proc. Natl. Acad. Sci. U.S.A.* **1982**, *79*, 3011.
(17) Goldenberg, D. M.; Sharkey, R. M.; Primus, F. J.; Mizusawa, E.; Hawthorne, M. F. *Proc. Natl. Acad. Sci. U.S.A.* **1984**, *81*, 560.
(18) Kawamura, A., Ed. "Fluorescent Antibody Techniques and Their Applications"; University of Tokyo Press: Tokyo, 1969; Chapter 3.
(19) Riggs, J. L.; Seiwald, R. J.; Burckhalter, J.; Downs, C. M.; Metcalf, T. G. *Am. J. Path.* **1958**, *34*, 1081.
(20) Sneath, R. L., Jr.; Soloway, A. H.; Dey, A. S. *J. Med. Chem.* **1974**, *17*, 796.
(21) Gabel, D.; Walczyna, R. *Z. Naturforsch., C: Biosci.* **1982**, *37C*, 1038.
(22) Olsen, F. P.; Hawthorne, M. F. *Inorg. Chem.* **1965**, *4*, 1839.

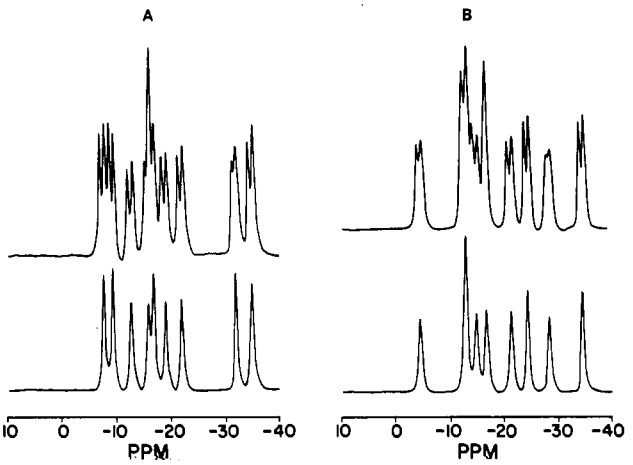


**Figure 3.** $^{11}B$ NMR spectra: A, $(CH_3)_4N^+[nido\text{-}7\text{-}(p\text{-}C_6H_4NCS)\text{-}7,8\text{-}C_2B_9H_{11}]^-$ (**3**); B, $(CH_3)_4N^+[nido\text{-}7\text{-}(p\text{-}C_6H_4NCS)\text{-}9\text{-}I\text{-}7,8\text{-}C_2B_9H_{10}]^-$ (**5**).

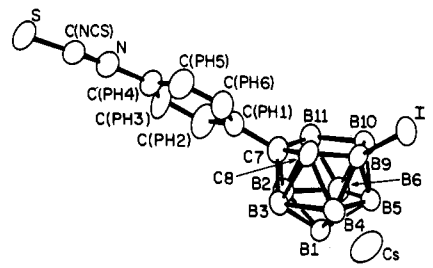


**Figure 4.** Molecular X-ray structure of one enantiomer of **5**: $Cs^+[nido\text{-}7\text{-}(p\text{-}C_6H_4NCS)\text{-}9\text{-}I\text{-}7,8\text{-}C_2B_9H_{10}]^-$.

The iodinated species **5** could be synthesized from the reaction of elemental iodine with **3** in absolute ethanol. In a more relevant preparation of **5**, the sodium salt **4**, dissolved in 0.05 M phosphate buffer, pH 7.4 (PB), was reacted with incipient $I^+$ that had been generated by the action of *N*-chloro-*p*-toluenesulfonamide (chloramine-T) on sodium iodide in PB. This procedure was patterned after the antibody radioiodination procedure of Greenwood and Hunter.[23] Generation of incipient $I^+$ by the action of "iodogen" on sodium iodide in PB could also be used.[24] The iodinated product was converted to the tetramethylammonium salt (**5**), recrystallized from acetone/water, and characterized by elemental analyses, IR, $^1H$ NMR, and $^{11}B$ NMR. The rapid separation of the sodium salt **4** and the sodium salt of the iodinated product was achieved by HPLC using a C-18 reverse-phase column eluted with 50:50 acetonitrile/water and may be of use in later radioiodination experiments. The proton-decoupled and -coupled $^{11}B$ NMR spectra at 160.4 MHz for **3** (A) and **5** (B) are shown in Figure 3 for comparison. In the decoupled spectrum of **3** (A, lower), each boron atom gives rise to a separate resonance peak. In the decoupled spectrum of **5** (B, lower), there are eight resonance peaks with two coincident resonances at $\delta$ 12.9. Inspection of the proton-coupled spectra (upper) reveals that 16 of the 17 peaks exhibit splitting. The exceptional peak in spectrum B at $\delta$ 16.77 remains a singlet, indicating iodine substitution at one of the boron atoms in **5**. In order to determine which boron atom was bonded to iodine, a single-crystal X-ray structural analysis was performed. The result of this analysis shows the structure of the iodinated product to be that of the 7-(*p*-isothiocyanatophenyl)-9-iododecahydro-7,8-dicarba-*nido*-undecaborate(1−) ion and its enantiomer.

Single crystals of the racemic cesium salt, $Cs^+[nido\text{-}7(8)\text{-}(p\text{-}C_6H_4NCS)\text{-}9(11)\text{-}I\text{-}7,8\text{-}C_2B_9H_{10}]^-$ suitable for X-ray diffraction were obtained from **5**. The unit cell parameters are given in Table I as part of a summary of data for this study. The final positional parameters are listed in Table II and the final thermal parameters in Table III. During the course of the X-ray crystal structure determination, it was revealed that the two enantiomers coincidently crystallize in the unit cell. The molecular structure of one

**Table I.** Summary of Crystal Data for $Cs^+[nido\text{-}7\text{-}(p\text{-}C_6H_4NCS)\text{-}9\text{-}I\text{-}7,8\text{-}C_2B_9H_{10}]^-$

| | |
|---|---|
| $M_r$ | 526.43 |
| $D_{measd}$ (21 °C) | 1.9 g cm$^{-3}$ |
| $D_{calcd}$ | 1.915 g cm$^{-3}$ |
| $Z$ | 4 |
| cryst size | 0.42 × 0.20 × 0.18 mm$^3$ |
| space gp | $P2_1/c$ |
| $a$ | 13.809 (6) Å |
| $b$ | 12.509 (5) Å |
| $c$ | 10.601 (3) Å |
| $\beta$ | 95.79 (3)° |
| $V$ | 1822 (1) Å$^3$ |
| temp of data collcn | 21 °C |
| λ(Mo Kα) | 0.7107 Å |
| scan mode | $\theta/2\theta$ |
| $2\theta$ max | 54.0° |
| scan rate | 0–45°; 6.0° min$^{-1}$<br>45–54°; 4.0° min$^{-1}$ |
| scan range | 1.25° below $K\alpha_1$<br>1.25° above $K\alpha_2$ |
| no. of reflcns measd | 3998 |
| no. of unique reflcns with $I > (3\sigma(I)$ | 2664 |
| std and decay | 008, 713, 055, 90$\bar{2}$, 56$\bar{4}$, 22$\bar{7}$; no observable decay |
| $R$ | 0.051 |
| $R_w$ | 0.061 |
| goodness of fit | 1.76 |
| data/parameters | 12.8 |

**Table II.** Positional Parameters for $Cs^+[nido\text{-}7\text{-}(p\text{-}C_6H_4NCS)\text{-}9\text{-}I\text{-}7,8\text{-}C_2B_9H_{10}]^-$ [a]

| atom | $x$ | $y$ | $z$ |
|---|---|---|---|
| Cs | 0.4480 (1) | 0.3914 (1) | 0.1831 (1) |
| I | 0.39074 (4) | 0.06792 (5) | 0.07552 (5) |
| S | −0.4361 (2) | 0.3063 (2) | −0.1115 (3) |
| N | −0.2762 (6) | 0.2079 (7) | 0.0093 (8) |
| C(NCS) | −0.3417 (6) | 0.2497 (8) | −0.0421 (9) |
| B1 | 0.2270 (6) | 0.1322 (8) | 0.4535 (9) |
| B2 | 0.1107 (6) | 0.0778 (8) | 0.4119 (8) |
| B3 | 0.1487 (6) | 0.1956 (8) | 0.3339 (9) |
| B4 | 0.2772 (7) | 0.1807 (8) | 0.3170 (10) |
| B5 | 0.3124 (6) | 0.0523 (8) | 0.3736 (9) |
| B6 | 0.2089 (7) | −0.0101 (8) | 0.4390 (8) |
| B9 | 0.2812 (6) | 0.0699 (8) | 0.2143 (8) |
| B10 | 0.2465 (7) | −0.0514 (8) | 0.2868 (9) |
| B11 | 0.1200 (6) | −0.0302 (8) | 0.3061 (9) |
| C7 | 0.0951 (5) | 0.0942 (6) | 0.2524 (7) |
| C7′ [b] | 0.1708 (9) | 0.0367 (11) | 0.1836 (12) |
| C8 | 0.1849 (10) | 0.1330 (14) | 0.1936 (15) |
| C(Ph1) | −0.0036 (5) | 0.1155 (6) | 0.1841 (7) |
| C(Ph2) | −0.0884 (6) | 0.0877 (10) | 0.2362 (10) |
| C(Ph3) | −0.1796 (6) | 0.1186 (10) | 0.1748 (10) |
| C(Ph4) | −0.1846 (6) | 0.1738 (7) | 0.0662 (9) |
| C(Ph5) | −0.1025 (7) | 0.1990 (8) | 0.0102 (9) |
| C(Ph6) | −0.0131 (6) | 0.1701 (8) | 0.0710 (8) |
| H(C7′) [b] | 0.1527 | 0.0119 | 0.0985 |
| H(B1) | 0.2464 | 0.1698 | 0.5510 |
| H(B10) | 0.2771 | −0.1349 | 0.2687 |
| H(B11) | 0.0670 | −0.1025 | 0.3018 |
| H(B2) | 0.0509 | 0.0773 | 0.4793 |
| H(B3) | 0.1152 | 0.2772 | 0.3489 |
| H(B4) | 0.3300 | 0.2510 | 0.3183 |
| H(B5) | 0.3911 | 0.0357 | 0.4152 |
| H(B6) | 0.2153 | −0.0684 | 0.5233 |
| H(C8) | 0.1775 | 0.1706 | 0.1137 |
| H(Ph2) | −0.0856 | 0.0490 | 0.3132 |
| H(Ph3) | −0.2379 | 0.1011 | 0.2127 |
| H(Ph5) | −0.1073 | 0.2383 | −0.0690 |
| H(Ph6) | 0.0453 | 0.1891 | 0.0322 |

[a] Standard deviations in parentheses. [b] Refined atoms that are present in the $Cs^+[nido\text{-}8\text{-}(p\text{-}C_6H_4NCS)\text{-}11\text{-}I\text{-}7,8\text{-}C_2B_9H_{10}]^-$ enantiomer.

(23) Greenwood, F. C.; Hunter, W. M. *Biochem. J.* **1963**, *89*, 114.
(24) Fraker, P. J.; Speck, J. C. *Biochem. Biophys. Res. Commun.* **1978**, *80*, 849.

**Table III.** Thermal Parameters for $Cs^+[nido\text{-}7\text{-}(p\text{-}C_6H_4NCS)\text{-}9\text{-}I\text{-}7,8\text{-}C_2B_9H_{10}]^-$ [a,b]

| atom | $U_{11}$ | $U_{12}$ | $U_{33}$ | $U_{12}$ | $U_{13}$ | $U_{23}$ |
|---|---|---|---|---|---|---|
| Cs | 773 (5) | 871 (5) | 718 (4) | -311 (4) | -163 (3) | 251 (4) |
| I | 423 (3) | 724 (4) | 538 (3) | 99 (3) | 204 (2) | 49 (3) |
| S | 528 (14) | 640 (17) | 1329 (27) | 142 (13) | -360 (16) | -159 (18) |
| N | 519 (44) | 610 (50) | 861 (58) | 140 (39) | -183 (41) | -160 (45) |
| C(NCS) | 442 (45) | 528 (53) | 761 (60) | 9 (42) | -64 (43) | -177 (49) |
| B1 | 370 (44) | 505 (58) | 468 (50) | -9 (40) | 41 (38) | -60 (43) |
| B2 | 392 (42) | 474 (52) | 432 (46) | 64 (41) | 77 (36) | 57 (42) |
| B3 | 344 (43) | 519 (56) | 513 (53) | 56 (40) | 137 (39) | -48 (44) |
| B4 | 408 (48) | 489 (59) | 596 (59) | -54 (41) | 109 (43) | -47 (47) |
| B5 | 362 (43) | 496 (57) | 439 (47) | 34 (38) | 62 (36) | -75 (41) |
| B6 | 417 (46) | 566 (60) | 407 (47) | 18 (43) | 144 (38) | 37 (43) |
| B9 | 302 (39) | 572 (57) | 442 (46) | -9 (40) | 73 (34) | 54 (44) |
| B10 | 441 (48) | 489 (59) | 453 (48) | 41 (41) | 123 (39) | -25 (42) |
| B11 | 388 (45) | 442 (52) | 522 (53) | 0 (39) | 116 (40) | 76 (44) |
| C7 | 333 (36) | 492 (49) | 445 (41) | 26 (33) | 75 (31) | 126 (36) |
| C7′ | 311 (66) | 254 (70) | 328 (68) | -7 (54) | 64 (53) | 11 (55) |
| C8 | 296 (73) | 689 (125) | 419 (85) | -22 (74) | 69 (63) | 105 (80) |
| C(Ph1) | 410 (41) | 424 (45) | 477 (45) | 41 (35) | 57 (34) | 24 (37) |
| C(Ph2) | 350 (43) | 1142 (90) | 745 (65) | -29 (51) | 124 (42) | 256 (63) |
| C(Ph3) | 302 (43) | 1132 (90) | 812 (70) | 45 (50) | 102 (44) | 240 (66) |
| C(Ph4) | 426 (45) | 443 (51) | 694 (59) | 68 (37) | -138 (41) | -49 (44) |
| C(Ph5) | 549 (54) | 701 (64) | 616 (57) | 39 (48) | -62 (44) | 142 (50) |
| C(Ph6) | 423 (44) | 703 (64) | 584 (53) | 34 (42) | 86 (39) | 103 (47) |

[a] Hydrogen atoms were each assigned an isotropic $B$ value of 7 where $B = U8\pi^2$. The anisotropic temperature factor $T$ is defined as $T = \exp[-2\pi^2(a^{*2}U_{11}h^2 + b^{*2}U_{22}k^2 + c^{*2}U_{33}l^2 + 2a^*b^*U_{12}hk + 2a^*c^*U_{13}hl + 2b^*c^*U_{23}kl)]$. [b] Standard deviations in parentheses.

of these enantiomers, $Cs^+[nido\text{-}7\text{-}(p\text{-}C_6H_4NCS)\text{-}9\text{-}I\text{-}7,8\text{-}C_2B_9H_{10}]^-$, is shown in Figure 4, and the important interatomic distances and angles are listed in Table IV.

From the molecular structure, the electrophilic substitution by iodine can be seen to have taken place on B9, the boron atom in the top belt of the *nido*-7,8-$C_2B_9$ cage that is adjacent to the unsubstituted carbon atom. The B9–I bond distance is 2.214 (8) Å, and there is nothing unusual about the *nido*-7,8-$C_2B_9$ cage geometry. The *p*-isothiocyanatophenyl group is bonded to C7, with C7–C(Ph1) bond distance of 1.501 (10) Å. The isothiocyanate group, which remains intact during the aqueous chloramine-T/sodium iodide iodination of **4**, has an N–C(NCS) bond distance of 1.135 (10) Å, a C(NCS)–S bond distance of 1.596 (9) Å, a C(NCS)–N–C(Ph4) bond angle of 168.7 (10)°, and an N–C(NCS)–S bond angle of 178.1 (10)°.

Comparison of the isothiocyanate group in the cesium salt with other covalently bound isothiocyanate groups that were determined by X-ray crystallography shows great similarities: $[(CH_3)_3CN\text{-}BNCS]_4$,[25] N–C = 1.172 (10) Å, C–S = 1.560 (8) Å, X–N–C = 176.4 (8)°, N–C–S = 173.3 (7)°; 6-$B_{10}H_{13}NCS$,[26] N–C = 1.149 (5) Å, C–S = 1.581 (4) Å, X–N–C = 171.0 (6)°, N–C–S = 178.1 (6)°; $NH_3\text{-}BH_2NCS$,[27] N–C = 1.137 (8) Å, C–S = 1.627 (6) Å, X–N–C = 177.5 (6)°, N–C–S = 179.2 (5)°. The bridging hydrogen could not be located because of the enantiomeric molecular disorder, but it is presumed to be located over the B10–B11 bond.

The boron-10, antibody-labeling procedure using 2-(*p*-isothiocyanatophenyl)-1,2-dicarba-*closo*-[1-$^3$H]dodecaborane(12) (**1**($^3$**H**)) and the tritium-radiolabeled sodium salt of the 7-(*p*-isothiocyanatophenyl)dodecahydro-7,8-dicarba-*nido*-undecaborate(1−) ion (**4**($^3$**H**)) followed the general method described by Marshall et al.[28] The water-insoluble **1**($^3$**H**) required dissolution in *N*,*N*-dimethylformamide and the sodium salt **4**($^3$**H**) in water, before their addition to antibody solutions in 0.5 M sodium carbonate/bicarbonate buffer, pH 9.5. For both reagents, antibody protein conjugation took place overnight at room temperature.

In the case of **1**($^3$**H**) labeling of normal goat IgG (NG IgG), the maximum number of conjugated carborane cages attained per antibody molecule was 1. In a 20% *N*,*N*-dimethylformamide/80% water solution, most of the carborane precipitated before it could react with the antibody protein. If the proportion of *N*,*N*-dimethylformamide was increased to keep more of the **1**($^3$**H**) dissolved, increased precipitation of antibody protein by the organic solvent was observed. Similar results were obtained with tetrahydrofuran to dissolve **1**($^3$**H**).

The boron-10, NG IgG labeling results using the water-soluble **4**($^3$**H**) are listed in Table V. A maximum combining number of 20 was achieved, but as in the case of the *p*-[1,2-dicarba-*closo*-[1-$^3$H]dodecaboran(12)-2-yl]benzenediazonium ion,[16] increased loss of antibody protein was observed as the reagent to antibody

**Table IV.** Selected Interatomic Distances (Å) and Bond Angles (deg) for $Cs^+[nido\text{-}7\text{-}(p\text{-}C_6H_4NCS)\text{-}9\text{-}I\text{-}7,8\text{-}C_2B_9H_{10}]^-$ [a]

| Interatomic Distances (Å) | | | |
|---|---|---|---|
| I–B9 | 2.214 (8) | B4–B9 | 1.767 (14) |
| S–C(NCS) | 1.596 (9) | B5–B6 | 1.826 (12) |
| N–C(NCS) | 1.135 (10) | B5–B9 | 1.714 (13) |
| N–C(Ph4) | 1.411 (10) | B5–B10 | 1.786 (13) |
| B1–B2 | 1.759 (13) | B6–B10 | 1.819 (13) |
| B1–B3 | 1.769 (13) | B6–B11 | 1.791 (13) |
| B1–B4 | 1.773 (13) | C7–C8 | 1.523 (16) |
| B1–B5 | 1.819 (12) | C7–B11 | 1.681 (12) |
| B1–B6 | 1.802 (14) | C7–C(Ph1) | 1.501 (10) |
| B2–B3 | 1.794 (13) | C8–B9 | 1.543 (17) |
| B2–B6 | 1.747 (13) | B9–B10 | 1.788 (14) |
| B2–C7 | 1.695 (11) | B10–B11 | 1.799 (12) |
| B2–B11 | 1.769 (14) | C(Ph1)–C(Ph2) | 1.388 (11) |
| B3–B4 | 1.811 (12) | C(Ph1)–C(Ph6) | 1.375 (11) |
| B3–C7 | 1.666 (12) | C(Ph2)–C(Ph3) | 1.412 (13) |
| B3–C8 | 1.796 (18) | C(Ph3)–C(Ph4) | 1.338 (13) |
| B4–B5 | 1.766 (14) | C(Ph4)–C(Ph5) | 1.369 (12) |
| B4–C8 | 1.832 (18) | C(Ph5)–C(Ph6) | 1.382 (12) |

| Bond Angles (deg) | | | |
|---|---|---|---|
| N–C(NCS)–S | 178.1 (10) | C(Ph1)–C7–B3 | 116.7 (6) |
| C(NCS)–N–C(Ph4) | 168.7 (10) | C(Ph1)–C7–C8 | 118.9 (8) |
| N–C(Ph4)–C(Ph3) | 119.3 (9) | C(Ph1)–C7–B11 | 118.2 (6) |
| N–C(Ph4)–C(Ph5) | 119.4 (9) | I–B9–B4 | 119.0 (6) |
| C(Ph2)–C(Ph1)–C(Ph6) | 117.5 (8) | I–B9–B5 | 122.2 (5) |
| C(Ph2)–C(Ph1)–C7 | 121.7 (7) | I–B9–C8 | 123.2 (7) |
| C(Ph6)–C(Ph1)–C7 | 120.7 (7) | I–B9–B10 | 120.4 (6) |
| C(Ph1)–C(Ph2)–C(Ph3) | 119.8 (9) | C7–C8–B9 | 120.3 (12) |
| C(Ph2)–C(Ph3)–C(Ph4) | 120.3 (8) | C8–B9–B10 | 103.3 (8) |
| C(Ph3)–C(Ph4)–C(Ph5) | 121.3 (8) | B9–B10–B11 | 103.2 (6) |
| C(Ph4)–C(Ph5)–C(Ph6) | 118.5 (8) | B10–B11–C7 | 105.4 (6) |
| C(Ph5)–C(Ph6)–C(Ph1) | 122.6 (8) | B11–C7–C8 | 106.6 (8) |
| C(Ph1)–C7–B2 | 121.4 (6) | | |

[a] Standard deviations in parentheses.

(25) Clarke, P. T.; Powell, H. M. *J. Chem. Soc. B* **1966**, 1172.
(26) Kendall, D. S.; Lipscomb, W. N. *Inorg. Chem.* **1973**, *12*, 2915.
(27) Kendall, D. S.; Lipscomb, W. N. *Inorg. Chem.* **1973**, *12*, 2920.
(28) Marshall, J. D., Jr.; Eveland, W. C.; Smith, C. W. *Proc. Soc. Exp. Biol. Med.* **1958**, *98*, 898.

**Table V.** Carborane Coupling of NG IgG Using $Na^+[nido\text{-}7\text{-}(p\text{-}C_6H_4NCS)\text{-}7,8\text{-}C_2B_9H_{11}]^-$ (Tritiated)

| molar ratio | carborane cage/antibody molecule[a] | recovery of antibody protein, % |
|---|---|---|
| 5:1 | 0.8 | 91 |
| 10:1 | 2.1 | 89 |
| 25:1 | 9.3 | 49 |
| 50:1 | 17.6 | 12 |
| 100:1 | 20.0 | 6 |

[a] mM carborane/mM NG IgG based on $1.84 \times 10^{10}$ cpm/mol of carborane.

**Table VI.** Affinity Chromatography of Boron-10-Labeled Anti-CEA and NG IgG Using $Na^+[nido\text{-}7\text{-}(p\text{-}C_6H_4NCS)\text{-}7,8\text{-}C_2B_9H_{11}]^-$ (Tritiated)

| | IgG protein | |
|---|---|---|
| sample (carborane/IgG)[a] | recovery, % | immunoreactivity, % |
| $^{131}$I-labeled anti-CEA IgG control (0) | | 62.5[b] |
| [$^3$H] anti-CEA IgG (0.5) | 97 | 59.2[b] |
| [$^3$H] anti-CEA IgG (2.0) | 100 | 60.0[b] |
| [$^3$H] anti-CEA IgG (5.4) | 95 | 55.0[b] |
| [$^3$H] anti-CEA IgG (8.0) | 96 | 59.3[b] |
| [$^3$H] anti-CEA IgG (12.3) | 93 | 57.7[b] |
| [$^3$H] NGIgG (9.3) | 93 | 10.1 |

[a] mM carborane/mM IgG based on $1.84 \times 10^{10}$ cpm/mol of carborane. [b] Corrected for nonspecific binding to Sepharose 4B.

molar reaction ratio was steadily increased. Nevertheless, at a 25:1 **4**($^3$**H**):antibody molar ratio, up to nine carborane cages were bound per antibody molecule with a 49% recovery of antibody protein. More recently, 12 carborane cages were bound to anti-CEA with 55% recovery of antibody protein. Affinity chromatography results listed in Table VI show that anti-CEA immunoreactivity is retained by antibody samples that had been labeled with up to 12 carborane cages with **4**($^3$**H**).

The usefulness of the water-soluble sodium salt **4** for conjugation to anti-CEA IgG and other antibodies has been demonstrated above. The novelty of this reagent lies in its propensity to react with radioiodine under well-established conditions and further conjugate to antibody in a subsequent step. This sequence provides a radioiodine tracer attached directly to the boron-10 reagent rather than adjacent antibody protein and more nearly ensures the quantification of boron-10 concentration in tissues of interest using radiometric methods. Furthermore, the reagent **5** may prove to be a uniquely effective carrier of radioiodine for in vivo as well as in vitro experiments that require a radiolabel indirectly attached to protein.

## Experimental Section

**Physical Measurements and Materials.** Proton FT nuclear magnetic resonance spectra ($^1$H FT NMR, 200.133 MHz) were obtained on a Bruker WP-200 FTNMR spectrometer. Chemical shifts are reported in ppm ($\delta$) and are referenced to internal $Me_4Si$. For compounds in which B–H protons are present, all terminal B–H proton signals appear as an envelope of broadened resonances between $\delta$ 0 and $\delta$ 4, and all bridging B–H–B protons give rise to broadened resonances that are centered around $\delta$ −2.5. Boron-11 FT nuclear magnetic resonance spectra ($^{11}$B FT NMR, 126.7 MHz) were obtained with a spectrometer constructed by Professor F. A. L. Anet, Department of Chemistry and Biochemistry, University of California, Los Angeles, CA. Additional $^{11}$B FT NMR spectra (160.4 MHz) were obtained on a Bruker WM-500 spectrometer at the Southern California Regional NMR Facility, California Institute of Technology, Pasadena, CA. Chemical shifts are reported in ppm ($\delta$) and are referenced to an external sample of $BF_3{\cdot}OEt_2$ (0.00 ppm). The following abbreviations have been used when reporting $^1$H and $^{11}$B NMR spectra: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; prefix br, a broadened signal. Infrared spectra (IR) were recorded as Nujol mineral oil mulls on Perkin-Elmer 137 and 710B spectrophotometers and are referenced to polystyrene (1601 cm$^{-1}$). Ultraviolet spectra (UV) were taken on a Cary Model 219 UV/vis spectrophotometer, and melting points were determined on a Thomas-Hoover capillary apparatus and are uncorrected. Elemental analyses were performed by Schwarzkopf Microanalytical Laboratory, Woodside, NY, and Galbraith Laboratories, Knoxville, TN. Analytical results that are indicated by element symbols were within ±0.4% o the calculated values.

Ion-exchange chromatography was run, using cation-exchange resin (Bio-Rad AGSOW-X8, 20–50 mesh, Bio-Rad Laboratories, Richmond, CA), eluted with 50:50 acetonitrile/water. High-performance liquid chromatography (HPLC) was run, using a 4.6 mm × 150 mm Ultrasphere ODS reverse-phase column (C-18, 5 $\mu$m) and Altex 110A pumps, interfaced with an Altex 420 programmer. A Hitachi Model 100-40 variable-wavelength UV/vis detector equipped with an Altex 155-00 flow cell was used for peak detection. A drybox-enclosed Cahn Model RTL electrobalance was used to weigh samples for specific activity and molar extinction coefficient determinations. Tritium radioactivity was measured in Budget Solve scintillation cocktail (Research Products International, Elk Grove Village, IL), using a Packard TRI-CARB Model 3255 liquid scintillation spectrometer. X-ray crystallographic data were collected at 21 °C on a Syntex P$\bar{1}$ diffractometer.

All reactions were run under positive nitrogen atmosphere. Unless otherwise indicated, all solvents were purified by standard procedures and distilled under nitrogen before use. Boron-10-enriched decaborane(14) (93% enrichment) was purchased from Callery Chemical Co., Callery, PA. Normal goat IgG (NG IgG) was purchased from Miles Laboratories, Elkhart, IN, and goat anticarcinoembryonic antigen IgG (anti-CEA IgG) was provided by D. M. Goldenberg, Center for Molecular Medicine and Immunology at the School of Medicine and Dentistry of New Jersey, Newark, NJ. The Bio-Rad protein assay was used to determine the concentrations of all boron-10-labeled antibody samples.

**1-(*p*-Isothiocyanatophenyl)-1,2-dicarba-*closo*-dodecaborane(12) (1).** Under nitrogen, 1.0 g (4.2 mmol) of 1-($p$-aminophenyl)-1,2-dicarba-*closo*-dodecaborane(12)[16] was dissolved in 25 mL of anhyrous acetone and cooled to 0 °C. From a disposable syringe, 1.5 mL (19.7 mmol) of thiophosgene was quickly added to the reaction flask and the resultant mixture stirred for 1 h at 0 °C. Afterward, 10 mL of water was added, the reaction was stirred for an additional 1 h at room temperature, and with the addition of 25 mL of ethyl acetate, a separation between the aqueous and organic phases was observed. The organic phase was isolated, filtered, and dried over anhydrous sodium sulfate. Following filtration, the solvent was removed under reduced pressure on a rotary evaporator and the crude product was placed under high vacuum. Recrystallization from hot hexanes gave 0.94 g (3.4 mmol, 81%) of the desired product (mp 119–121 °C).

Anal. ($C_9H_{15}B_{10}NS$) C, H, B, N, S. Infrared spectrum: 3080 (m), 2600 (str), 2075 (str), 1600 (m), 1500 (m), 925 (m), 830 (m) cm$^{-1}$. NMR spectra ($\delta$): $^1$H (acetone-$d_6$, room temperature) 5.24 (br s, 1 H, carborane C–H), 7.58 (m, 4 H, para-substituted phenyl); $^{11}$B (160.4 MHz, acetone, room temperature) 2.85, 1.03, −3.50, −5.38, −7.18.

By the above procedure, 93% $^{10}$B-enriched 1-($p$-isothiocyanatophenyl)-1,2-dicarba-*closo*-dodecaborane(12) and the radiolabeled 2-($p$-isothiocyanatophenyl-1,2-dicarba-*closo*-[1-$^3$H]dodecaborane(12) (1.115 Ci/mol) were synthesized.

**Tetramethylammonium 7-(*p*-Aminophenyl)dodecahydro-7,8-dicarba-*nido*-undecaborate(1−), $(CH_3)_4N^+[nido\text{-}7\text{-}(p\text{-}C_6H_4NH_2)\text{-}7,8\text{-}C_2B_9H_{11}]^-$ (2).** Under nitrogen, 1.13 g (16.8 mmol) of potassium hydroxide (85%) was dissolved in 175 mL of absolute ethanol with stirring and slight warming. The flask was cooled, 2.00 g (8.5 mmol) of 1-($p$-aminophenyl)-1,2-dicarba-*closo*-dodecaborane(12) was added, and the reaction was stirred overnight at reflux temperature. Upon cooling, excess carbon dioxide was passed through the solution, the reaction was filtered, and the solvent was removed under reduced pressure on a rotary evaporator. The remaining solid was dissolved in water and filtered, and the product was precipitated with excess aqueous tetramethylammonium chloride. The product was collected in a sintered-glass funnel, washed with water, and recrystallized from ethanol/water, giving 2.01 g (6.7 mmol, 79% yield) of $((CH_3)_4N^+[nido\text{-}7\text{-}(p\text{-}C_6H_4NH_2)\text{-}7,8\text{-}C_2B_9H_{11}]^-$ (mp 155–156 °C).

Anal. ($C_{12}H_{29}B_9N_2$) C, H, B, N. Infrared spectrum: 3290 (w), 2500 (str), 1615 (w), 1510 (m), 950 (w) cm$^{-1}$. NMR spectra ($\delta$): $^1$H (acetone-$d_6$, room temperature) 3.28 (br s, 1 H, carborane C–H), 3.43 (s, 12 H, tetramethylammonium), 4.22 (br s, 2 H, $NH_2$), 6.68 (m, 4 H, para-substituted phenyl): $^{11}$B 160.4 MHz, acetone, room temperature) −8.54, −9.60, −13.14, −15.69, −17.70, −18.39, −22.11, −32.06, −35.21.

The sodium salt of the 7-($p$-aminophenyl)dodecahydro-7,8-dicarba-*nido*-undecaborate(1−) ion was obtained by the sodium cation exchange of the tetramethylammonium salt **2** using cation-exchange resin eluted with 50:50 acetonitrile/water.

By the above procedure, the 93% $^{10}$B-enriched and tritium-radiolabeled (8 mCi/mol) forms of the 7-($p$-aminophenyl)dodecahydro-7,8-dicarba-*nido*-undecaborate(1−) ion were synthesized.

**Tetramethylammonium 7-(*p*-Isothiocyanatophenyl)dodecahydro-7,8-dicarba-*nido*-undecaborate(1−), $(CH_3)_4N^+[nido\text{-}7\text{-}(p\text{-}C_6H_4NCS)\text{-}7,8\text{-}$**

$C_2B_9H_{11}]^-$ (**3**). Under nitrogen, 0.85 g (2.8 mmol) of **2** was dissolved in 8.5 mL of anhydrous acetone and cooled to 0 °C. From a microliter pipet, 0.240 mL (3.2 mmol) of thiophosgene was quickly added to the reaction flask and stirred for 1 h at 0 °C. After 1 h, the ice bath was removed, 17 mL of water was added, and the reaction was kept stirring while open to the air until a precipitate was observed. An additional 20 mL of water was added, and the reaction was stirred for another hour. The precipitate was collected in a sintered-glass funnel, washed with water, and placed under high vacuum. Recrystallization from acetone/water gave 0.83 g (2.4 mmol, 87% yield) of the straw-colored product, which was observed to decompose above 175 °C.

Anal. ($C_{13}H_{27}B_9N_2S$) C, H, B, N, S. Infrared spectrum: 2470 (str), 2065 (str), 1500 (m), 950 (m), 845 (m) $cm^{-1}$. NMR spectra ($\delta$): $^1H$ (acetone-$d_6$, room temperature) 2.21 (br s, 1 H, carborane C–H), 3.45 (s, 12 H, tetramethylammonium), 7.18 (m, para-substituted phenyl); $^{11}B$ (160.4 MHz, acetone, room temperature) −7.53, −9.20, −12.57, −15.69, −16.62, −18.82, −21.77, −31.65, −34.62. Molar extinction coefficient at 295 nm (70:30 acetonitrile/water) $\epsilon = 2.3 \times 10^4$ $M^{-1}$ $cm^{-1}$.

The sodium salt of the 7-(*p*-isothiocyanatophenyl)dodecahydro-7,8-dicarba-*nido*-undecaborate(1−) ion (**4**), obtained by the sodium cation exchange of the tetramethylammonium salt **3**, has a molar extinction coefficient at 295 nm in 70:30 acetonitrile/water of $2.2 \times 10^4$ $M^{-1}$ $cm^{-1}$. At 288 nm in water the molar extinction coefficient was determined to be $2.3 \times 10^4$ $M^{-1}$ $cm^{-1}$.

By the above procedure, the 93% $^{10}B$-enriched and tritium-radiolabeled (8 mCi/mol) forms of the 7-(*p*-isothiocyanatophenyl)dodecahydro-7,8-dicarba-*nido*-undecaborate(1−) ion were synthesized.

**Tetramethylammonium 7-(*p*-Isothiocyanatophenyl)-9-iododecahydro-7,8-dicarba-*nido*-undecaborate(1−), $(CH_3)_4N^+[$*nido*-7-(*p*-$C_6H_4NCS)$-9-I-7,8-$C_2B_9H_{10}]^-$ (5).** In a 50-mL, round-bottom flask was dissolved 1.30 g (4.5 mmol) of **4** in 20 mL of 0.05 M phosphate buffer, pH 7.4 (PB), with stirring. In another flask, 2.5 mL of a 2.0 M solution of sodium iodide in PB and 2.5 mL of a freshly prepared 2.0 M solution of sodium *N*-chloro-*p*-toluenesulfonamide in PB were mixed and added to the flask containing **4**, with stirring. After 5 min, the crude product was precipitated with excess aqueous tetramethylammonium chloride, collected in a sintered-glass funnel, and washed with water. Repeated acetone/water recrystallizations gave 1.16 g (2.5 mmol, 55% yield) of the iodinated product, which was observed to decompose above 230 °C.

In another preparation of **5**, 0.150 g (0.44 mmol) of **3** was dissolved in 30 mL of absolute ethanol under nitrogen, with stirring and gentle heating. Dissolved in 25 mL of absolute ethanol, 0.127 g (0.50 mmol) of elemental iodine was slowly added to the reaction flask, which was allowed to stir overnight at room temperature. The colorless solution was filtered, and the solvent was removed under reduced pressure on a rotary evaporator. The remaining residue was dissolved in water, and the crude product, which was precipitated by the addition of excess aqueous tetramethylammonium chloride, was collected in a sintered-glass funnel and washed with water. Repeated acetone/water recrystallizations gave 0.120 g (0.26 mmol, 52% yield) of product.

Anal. ($C_{13}H_{26}B_9N_2SI$) H, B, N, S, I; C: calcd, 33.47; found, 32.97. Infrared spectrum: 2500 (str), 2075 (tr), 1500 (m), 950 (m), 850 (m), 820 (m) $cm^{-1}$. NMR spectra ($\delta$): $^1H$ (acetone-$d_6$, room temperature) 2.84 (br s, 1 H, carborane C–H), 3.46 (s, 12 H, tetramethylammonium), 7.24 (m, 4 H, para-substituted phenyl); $^{11}B$ (160.4 MHz, acetone, room temperature) −4.68, −12.99, −14.94, −16.77, −21.24, −24.30, −28.25, −34.40.

The sodium salt of **5** was obtained by the sodium cation exchange of the tetramethylammonium salt. Dissolving the sodium salt in water and adding excess aqueous cesium chloride gave the cesium salt as a precipitate, which was collected in a sintered-glass funnel, washed with water, and dried under high vacuum.

**Crystal and Molecular Structure of $Cs^+[$*nido*-7-(*p*-$C_6H_4NCS)$-9-I-7,8-$C_2B_9H_{10}]^-$.** Single crystals that were suitable for the X-ray diffraction study of $Cs^+[$*nido*-7-(*p*-$C_6H_4NCS)$-9-I-7,8-$C_2B_9H_{10}]^-$ were obtained from the slow evaporation of a benzene/heptane solution at 20 °C. The density of the straw-colored crystals, measured by flotation in a mixture of bromoform and benzene at 20 °C, was 1.9 g $cm^{-3}$. A transparent, irregularly shaped crystal was mounted on the end of a glass fiber with epoxy cement, placed on an *x*, *y*, *z* goniometer, and optically centered on a Syntex P$\bar{1}$ diffractometer equipped with a molybdenum tube [$\lambda(K\alpha)$ = 0.7107 Å] and a graphite monochromator.

The compound was found to crystallize in the monoclinic space group $P2_1/c$-$C_{2h}$ (No. 14)[29] with $a$ = 13.809 (6) Å, $b$ = 12.509 (5) Å, $c$ = 10.601 (3) Å, $\beta$ = 95.79 (3)°, and V = 1822 (1) Å$^3$. For $Z$ = 4, the calculated density was 1.915 g $cm^{-3}$.

A total of 3998 independent reflections were measured using the $\theta/2\theta$ scanning technique over a $2\theta$ range of 54°. Background radiation was calculated with a locally edited version of CARESS.[30] Of the 3988 independent reflections measured, 2664 had $I > 3\sigma(I)$. To monitor experimental consistency, six standard reflections were measured every 300 reflections and decay of these standard reflections during data collection was not observed.

The *x*, *y*, *z* coordinates for the cesium and iodine atoms were deduced from a three-dimensional Patterson map. Phases calculated from these atoms allowed successive location of the positions of 19 of the 20 remaining non-hydrogen atoms in the asymmetric unit by iterative least-squares and Fourier calculations. Two peaks of nearly equal intensity were located by a difference Fourier calculation and appeared to be disordered sites for the remaining non-hydrogen atom. Inspection of positional and thermal parameters for all non-hydrogen atoms in the asymmetric unit revealed that the two enantiomers, $Cs^+[$*nido*-7-(*p*-$C_6H_4NCS)$-9-I-7,8-$C_2B_9H_{10}]^-$ and $Cs^+[$*nido*-8-(*p*-$C_6H_4NCS)$-11-I-7,8-$C_2B_9H_{10}]^-$, coincidently crystallize in the unit cell. The C8 atom of $Cs^+[$*nido*-7-(*p*-$C_6H_4NCS)$-9-I-7,8-$C_2B_9H_{10}]^-$ and the C7′ atom of $Cs^+[$*nido*-8-(*p*-$C_6H_4NCS)$-11-I-7,8-$C_2B_9H_{10}]^-$ were included in subsequent cycles of least-squares refinement with each atom assigned a 0.50 site occupancy factor. In the final cycles of least-squares refinement, all non-hydrogen thermal motion parameters were refined anisotropically, and for hydrogen atoms, the positional parameters were fixed with idealized geometry and C–H bond distances of 0.95 Å and B–H bond distances of 1.15 Å. All hydrogen atoms were each assigned a fixed isotropic $B$ value of 7. The bridging hydride atom of the molecule was not included in the least-squares refinement. The final unweighted value of $R = \sum||F_o| - |F_c||/\sum|F_o|$ was 0.051 and $R_w = \sum[w(|F_o| - |F_c|)^2/\sum w|F_o|^2]^{1/2}$ had a value of 0.061, with weights being taken as $w = [1/\sigma(F_o)]^2$. Structure factor calculations were made with atomic form factors taken from Cromer and Mann,[31] and anomalous dispersion corrections were applied to the scattering factors of the cesium, iodine, and sulfur atoms.[32] Structure factor tables are included as supplementary material.

**Boron-10 Labeling of Antibody Protein Using 2-(*p*-Isothiocyanatophenyl)-1,2-dicarba-*closo*-[1-$^3H$]dodecaborane(12) (1($^3$H)) and the Tritium-Radiolabeled Sodium Salt of the 7-(*p*-Isothiocyanatophenyl)-dodecahydro-7,8-dicarba-*nido*-undecaborate(1−) Ion (4($^3$H)).** Equal volumes of 1 mg/mL of antibody protein solution in normal saline and 0.5 M sodium carbonate/bicarbonate buffer pH 9.5 were mixed, and the desired amount of **1($^3$H)** dissolved in *N*,*N*-dimethylformamide or **4($^3$H)** dissolved in water was added with stirring at room temperature. The crystalline **1($^3$H)** was easily handled and could be accurately weighed. The hygroscopic **4($^3$H)** was first dissolved in water and its concentration determined from the molar extinction coefficient at 288 nm, $\epsilon_{288} = 2.3 \times 10^4$ $M^{-1}$ $cm^{-1}$. After stirring overnight at room temperature, the sample was extensively dialyzed against 0.05 M PB saline, pH 7.4, and passed through a Millex-GV 0.22-$\mu$m filter unit (Millipore Corp., Bedford, MA). The protein concentration of the boron-10-labeled antibody sample was determined from the Bio-Rad protein assay, and tritium counting (1.0 mL of sample in 10.0 mL scintillation cocktail) allowed the calculation of the extent of boron-10 labeling from the 1.115 Ci/mol for **1($^3$H)** or 8 mCi/mol for **4($^3$H)** specific activity values.

The method used to measure carborane–antibody conjugate immunoreactivity by affinity chromatography has been described.[16]

**Acknowledgment.** This research was supported by U.S. Public Health Service Grant CA31753-02S1.

**Registry No.** **1**, 96055-92-4; **2**·$N(CH_3)_4^+$, 91997-72-7; **2**·$Na^+$, 96095-19-1; **3**, 96095-21-5; **4**, 96095-22-6; **5**·$N(CH_3)_4^+$, 96055-94-6; **5**·$Na^+$, 96055-95-7; **5**·$Cs^+$, 96055-96-8; 1-(*p*-aminophenyl)-1,2-dicarba-*closo*-dodecaborane(12), 23738-81-0.

**Supplementary Material Available:** Table of the observed and calculated structure factors (12 pages). Ordering information is given on any current masthead page.

(29) "International Tables for X-Ray Crystallography", Kynoch Press: Birmingham, England, 1969; Vol. I, p 99.

(30) All calculations were performed on the UCLA, Chemistry and Biochemistry Departmental DEC VAX 11/780 using the Departmental Crystallographic Program Package (locally written data reduction and absorption programs, and locally edited versions of CARESS, PROFILE, ORFLS, ORFFE, and ORTEP).

(31) Cromer, D. T.; Mann, J. L. *Acta Crystallogr., Sect. A: Cryst. Phys., Diffr., Theor. Gen. Crystallogr.* **1968**, *A24*, 321.

(32) Cromer, D. T. *Acta Crystallogr.* **1965**, *18*, 17.