

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. $\text{Mo}(\text{CO})_4\text{bpz}$, 80907-60-4; $\text{Mo}(\text{CO})_4(\text{bpzH})^+$, 95891-49-9; $\text{Mo}(\text{CO})_4(\text{bpzBF}_3)$, 95891-50-2; $\text{Mo}(\text{CO})_4(\text{bpz}(\text{BF}_3)_2)$, 95891-51-3; $\text{W}(\text{CO})_4\text{bpz}$, 80925-51-5; $\text{W}(\text{CO})_4(\text{bpzH})^+$, 95891-52-4; $\text{W}(\text{CO})_4(\text{bpzBF}_3)$, 95891-53-5; $\text{W}(\text{CO})_4(\text{bpz}(\text{BF}_3)_2)$, 95891-54-6.

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 $\text{Cs}^+[\text{nido-7-(p-C}_6\text{H}_4\text{NCS)-9-I-7,8-C}_2\text{B}_9\text{H}_{11}]^-$

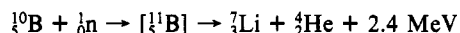
EUGENE A. MIZUSAWA, MICHAEL R. THOMPSON, and M. FREDERICK HAWTHORNE*

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 $2\theta = 54^\circ$ (Mo $K\alpha$ radiation) were collected on a Syntex P1 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 α -particle and recoiling lithium-7 ion with an average total kinetic energy of 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 μm ,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 Laboratory⁶⁻⁸ 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 $\text{Na}_2\text{B}_{12}\text{H}_{11}\text{SH}$,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

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- (13) Hatanaka, H. *Proc. First Int. Symp. Neutron Capture Ther.* **1984**, *1*, 384.

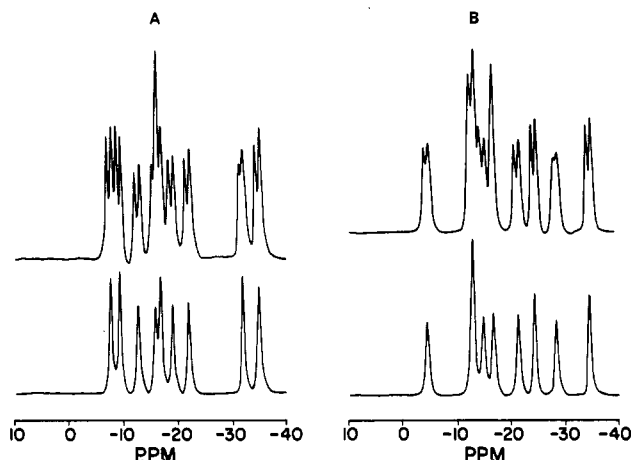


Figure 3. ¹¹B NMR spectra: A, (CH₃)₄N⁺[nido-7-(p-C₆H₄NCS)-7,8-C₂B₉H₁₁]⁻ (3); B, (CH₃)₄N⁺[nido-7-(p-C₆H₄NCS)-9-I-7,8-C₂B₉H₁₀]⁻ (5).

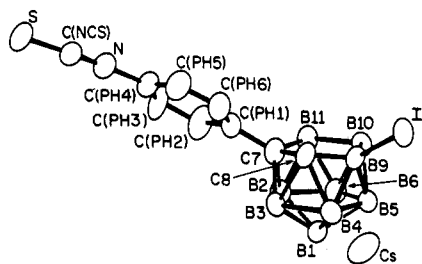


Figure 4. Molecular X-ray structure of one enantiomer of 5: Cs⁺[nido-7-(p-C₆H₄NCS)-9-I-7,8-C₂B₉H₁₀]⁻.

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 ¹¹B 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 ¹¹B 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 δ 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 δ 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*-isothiocyantophenyl)-9-iodododecahydro-7,8-dicarba-*nido*-undecaborate(1-) ion and its enantiomer.

Single crystals of the racemic cesium salt, Cs⁺[nido-7(8)-(p-C₆H₄NCS)-9(11)-I-7,8-C₂B₉H₁₀]⁻ 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

Table I. Summary of Crystal Data for Cs⁺[nido-7-(p-C₆H₄NCS)-9-I-7,8-C₂B₉H₁₀]⁻

<i>M_r</i>	526.43
<i>D</i> _{meas} (21 °C)	1.9 g cm ⁻³
<i>D</i> _{calcd}	1.915 g cm ⁻³
<i>Z</i>	4
cryst size	0.42 × 0.20 × 0.18 mm ³
space gp	<i>P</i> 2 ₁ / <i>c</i>
<i>a</i>	13.809 (6) Å
<i>b</i>	12.509 (5) Å
<i>c</i>	10.601 (3) Å
β	95.79 (3)°
<i>V</i>	1822 (1) Å ³
temp of data collcn	21 °C
λ(Mo Kα)	0.7107 Å
scan mode	θ/2θ
2θ max	54.0°
scan rate	0–45°; 6.0° min ⁻¹ 45–54°; 4.0° min ⁻¹
scan range	1.25° below Kα ₁ 1.25° above Kα ₂
no. of reflns measd	3998
no. of unique reflns with <i>I</i> > (3σ(<i>I</i>))	2664
std and decay	008, 713, 055, 902, 564, 227; no observable decay
<i>R</i>	0.051
<i>R_w</i>	0.061
goodness of fit	1.76
data/parameters	12.8

Table II. Positional Parameters for Cs⁺[nido-7-(p-C₆H₄NCS)-9-I-7,8-C₂B₉H₁₀]⁻^a

atom	<i>x</i>	<i>y</i>	<i>z</i>
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-8-(p-C₆H₄NCS)-11-I-7,8-C₂B₉H₁₀]⁻ enantiomer.

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 coincidentally crystallize in the unit cell. The molecular structure of one

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Table III. Thermal Parameters for $\text{Cs}^+[\text{nido-7-(p-C}_6\text{H}_4\text{NCS)-9-I-7,8-C}_2\text{B}_9\text{H}_{10}]^-$ ^{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)

^aHydrogen atoms were each assigned an isotropic B value of 7 where $B = U/8\pi^2$. The anisotropic temperature factor T is defined as $T = \exp[-2\pi^2(a^2U_{11}h^2 + b^2U_{22}k^2 + c^2U_{33}l^2 + 2a^*b^*U_{12}hk + 2a^*c^*U_{13}hl + 2b^*c^*U_{23}kl)]$. ^bStandard deviations in parentheses.

of these enantiomers, $\text{Cs}^+[\text{nido-7-(p-C}_6\text{H}_4\text{NCS)-9-I-7,8-C}_2\text{B}_9\text{H}_{10}]^-$, 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}_2\text{B}_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}_2\text{B}_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: $[(\text{CH}_3)_3\text{CN-BNCS}]_4$,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)°; $6\text{-B}_{10}\text{H}_{13}\text{NCS}$,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)°; $\text{NH}_3\cdot\text{BH}_2\text{NCS}$,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*-[1-³H]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}) 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

Table IV. Selected Interatomic Distances (Å) and Bond Angles (deg) for $\text{Cs}^+[\text{nido-7-(p-C}_6\text{H}_4\text{NCS)-9-I-7,8-C}_2\text{B}_9\text{H}_{10}]^-$ ^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)		

^aStandard deviations in parentheses.

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*-[1-³H]dodecaboran(12)-2-yl]benzenediazonium ion,¹⁶ increased loss of antibody protein was observed as the reagent to antibody

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Table V. Carborane Coupling of NG IgG Using Na⁺[nido-7-(p-C₆H₄NCS)-7,8-C₂B₉H₁₁]⁻ (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 1.84×10^{10} cpm/mol of carborane.

Table VI. Affinity Chromatography of Boron-10-Labeled Anti-CEA and NG IgG Using Na⁺[nido-7-(p-C₆H₄NCS)-7,8-C₂B₉H₁₁]⁻ (Tritiated)

sample (carborane/IgG) ^a	IgG protein	
	recovery, %	immuno-reactivity, %
¹²⁵ 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 1.84×10^{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(³H):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(³H).

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 (¹H FT NMR, 200.133 MHz) were obtained on a Bruker WP-200 FTNMR spectrometer. Chemical shifts are reported in ppm (δ) and are referenced to internal Me₄Si. For compounds in which B-H protons are present, all terminal B-H proton signals appear as an envelope of broadened resonances between δ 0 and δ 4, and all bridging B-H-B protons give rise to broadened resonances that are centered around δ -2.5. Boron-11 FT nuclear magnetic resonance spectra (¹¹B 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 ¹¹B 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 (δ) and are referenced to an external sample of BF₃·OEt₂ (0.00 ppm). The following abbreviations have been used when reporting ¹H and ¹¹B 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 Mi-

croanalytical Laboratory, Woodside, NY, and Galbraith Laboratories, Knoxville, TN. Analytical results that are indicated by element symbols were within ±0.4% of 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 μm) 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 PI 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 antineuroembryonic 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-Isothiocyantophenyl)-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 anhydrous 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. (C₉H₁₅B₁₀NS) 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 (δ): ¹H (acetone-*d*₆, room temperature) 5.24 (br s, 1 H, carborane C-H), 7.58 (m, 4 H, para-substituted phenyl); ¹¹B (160.4 MHz, acetone, room temperature) 2.85, 1.03, -3.50, -5.38, -7.18.

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

Tetramethylammonium 7-(p-Aminophenyl)dodecahydro-7,8-dicarba-*nido*-undecaborate(1-), (CH₃)₄N⁺[nido-7-(p-C₆H₄NH₂)-7,8-C₂B₉H₁₁]⁻ (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 ((CH₃)₄N⁺[nido-7-(p-C₆H₄NH₂)-7,8-C₂B₉H₁₁]⁻) (mp 155–156 °C).

Anal. (C₁₂H₂₉B₉N₂) C, H, B, N. Infrared spectrum: 3290 (w), 2500 (str), 1615 (w), 1510 (m), 950 (w) cm⁻¹. NMR spectra (δ): ¹H (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, NH₂), 6.68 (m, 4 H, para-substituted phenyl); ¹¹B 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% ¹⁰B-enriched and tritium-radio-labeled (8 mCi/mol) forms of the 7-(p-aminophenyl)dodecahydro-7,8-dicarba-*nido*-undecaborate(1-) ion were synthesized.

Tetramethylammonium 7-(p-Isothiocyantophenyl)dodecahydro-7,8-dicarba-*nido*-undecaborate(1-), (CH₃)₄N⁺[nido-7-(p-C₆H₄NCS)-7,8-

C₂B₉H₁₁]⁺ (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. (C₁₃H₂₇B₉N₂S) C, H, B, N, S. Infrared spectrum: 2470 (str), 2065 (str), 1500 (m), 950 (m), 845 (m) cm⁻¹. NMR spectra (δ): 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); ¹¹B (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) ε = 2.3 × 10⁴ 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 × 10⁴ M⁻¹ cm⁻¹. At 288 nm in water the molar extinction coefficient was determined to be 2.3 × 10⁴ M⁻¹ cm⁻¹.

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

Tetramethylammonium 7-(*p*-isothiocyanatophenyl)-9-iodododecahydro-7,8-dicarba-*nido*-undecaborate(1-), (CH₃)₄N⁺[*nido*-7-(*p*-C₆H₄NCS)-9-I-7,8-C₂B₉H₁₀]⁻ (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. (C₁₃H₂₆B₉N₂SI) 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 (δ): 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); ¹¹B (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*-C₆H₄NCS)-9-I-7,8-C₂B₉H₁₀]⁻. Single crystals that were suitable for the X-ray diffraction study of Cs⁺[*nido*-7-(*p*-C₆H₄NCS)-9-I-7,8-C₂B₉H₁₀]⁻ 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 P1 diffractometer equipped with a molybdenum tube [λ(Kα) = 0.7107 Å] and a graphite monochromator.

The compound was found to crystallize in the monoclinic space group *P*₂₁/*c*-C_{2h} (No. 14)²⁹ with *a* = 13.809 (6) Å, *b* = 12.509 (5) Å, *c* =

10.601 (3) Å, β = 95.79 (3)°, and *V* = 1822 (1) Å³. For *Z* = 4, the calculated density was 1.915 g cm⁻³.

A total of 3998 independent reflections were measured using the θ/2θ scanning technique over a 2θ range of 54°. Background radiation was calculated with a locally edited version of CARESS.³⁰ Of the 3988 independent reflections measured, 2664 had *I* > 3σ(*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*-C₆H₄NCS)-9-I-7,8-C₂B₉H₁₀]⁻ and Cs⁺[*nido*-8-(*p*-C₆H₄NCS)-11-I-7,8-C₂B₉H₁₀]⁻, coincidentally crystallize in the unit cell. The C8 atom of Cs⁺[*nido*-7-(*p*-C₆H₄NCS)-9-I-7,8-C₂B₉H₁₀]⁻ and the C7' atom of Cs⁺[*nido*-8-(*p*-C₆H₄NCS)-11-I-7,8-C₂B₉H₁₀]⁻ 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* = Σ||*F*_o| - |*F*_c||/Σ|*F*_o| was 0.051 and *R*_w = Σ[w(|*F*_o| - |*F*_c||)²/Σw|*F*_o|²]^{1/2} had a value of 0.061, with weights being taken as *w* = [1/σ(*F*_o)²]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(³H)) and the Tritium-Radiolabeled Sodium Salt of the 7-(*p*-isothiocyanatophenyl)-dodecahydro-7,8-dicarba-*nido*-undecaborate(1-) Ion (4(³H)).** 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(³H) dissolved in *N,N*-dimethylformamide or 4(³H) dissolved in water was added with stirring at room temperature. The crystalline 1(³H) was easily handled and could be accurately weighed. The hygroscopic 4(³H) was first dissolved in water and its concentration determined from the molar extinction coefficient at 288 nm, ε₂₈₈ = 2.3 × 10⁴ 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-μm 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(³H) or 8 mCi/mol for 4(³H) 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(CH₃)₄⁺, 91997-72-7; 2-Na⁺, 96095-19-1; 3, 96095-21-5; 4, 96095-22-6; 5-N(CH₃)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.

(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, ORFELS, ORFFE, and ORTEP).

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