cochemical properties of angiotensin peptides[19b,23,26] and, besides determining the solubility properties responsible for the countercurrent distribution behavior, may also be important for the biological activities. This would explain the great loss of activity on going from angiotensin to [MeAla^7]angiotensin to [Ala^7]angiotensin to [β-Ala^7]-angiotensin. It would also explain why the Pro^2-containing retroenantiomers are inactive, while those containing more flexible residues retain a substantial portion of the activities of the respective parent compounds.

Acknowledgment. This work was done with the technical assistance of Jose M. Pontinha and Jose Roque C. Santos and was supported by grants from Financiadora de Estudos e Projetos (FINEP), the Sao Paulo Research Foundation (FAPESP), and the Brazilian National Council for Scientific and Technological Development (CNPq).

References and Notes

(1) M. C. Khosla, R. R. Smeby, and F. M. Bumpus in "Angiotensin", I. H. Page and F. M. Bumpus, Ed., Springer-Verlag, Berlin, 1974, p 126.
(2) T. B. Paiva, G. Goissis, L. Juliano, M. E. Miyamoto, and A. C. M. Paiva, *J. Med. Chem.*, **17**, 238 (1974).
(3) M. M. Shemyakin, Yu. A. Ovchinnikov, and V. T. Ivanov, *Angew. Chem., Int. Ed. Engl.*, **8**, 492 (1969).
(4) J. Rudinger in "Drug Design", E. J. Ariens, Ed., Academic Press, New York, N.Y., 1971, p 319.
(5) M. M. Shemyakin, Yu. A. Ovchinnikov, V. T. Ivanov, and A. V. Evstratov, *Nature (London)*, **213**, 413 (1967).
(6) T. Wieland, B. Penke, and C. Birr, *Justus Liebigs Ann. Chem.*, **759**, 71 (1972).
(7) J. M. Stewart and D. W. Wooley in "Hypotensive Peptides", E. G. Erdös, N. Back, and F. Sicuteri, Ed., Springer, Berlin, 1966, p 23; K. Vogler, P. Lantz, W. Lergier, and W. Haefely, *Helv. Chim. Acta*, **49**, 390 (1966).
(8) D. Chung and C. H. Li, *Biochim. Biophys. Acta*, **136**, 570 (1967).
(9) C. F. Hayward and J. S. Morley in "Peptides 1974", Y. Wolman, Ed., Wiley, New York, N.Y., 1975, p 287.
(10) K. Hofmann, W. D. Pekhan, and A. Rheiner, *J. Am. Chem. Soc.*, **78**, 238 (1956).
(11) N. Izumia, H. Uchino, and T. Yamashita, *Nippon Kagaku Zasshi*, **79**, 420 (1958).
(12) E. Wunsch, G. Fries, and A. Zwick, *Chem. Ber.*, **91**, 542 (1958).
(13) T. Fujii and S. Sakakibara, *Bull. Chem. Soc. Jpn.*, **47**, 3146 (1974).
(14) E. Schnabel, *Justus Liebigs Ann. Chem.*, **702**, 188 (1967).
(15) (a) R. B. Merrifield, *J. Am. Chem. Soc.*, **85**, 2149 (1963); (b) R. B. Merrifield, *Adv. Enzymol.*, **32**, 221 (1969); (c) J. M. Stewart and J. Young, "Solid Phase Peptide Synthesis", W. H. Freeman, San Francisco, Calif., 1969.
(16) R. B. Merrifield, *J. Am. Chem. Soc.*, **86**, 304 (1964).
(17) F. C. Westall, *J. Org. Chem.*, **37**, 3363 (1972).
(18) E. Kaiser, R. L. Colescott, C. D. Bossinger, and P. I. Cook, *Anal. Biochem.*, **34**, 595 (1970).
(19) (a) T. B. Paiva, L. Juliano, V. L. A. Nouailhetas, and A. C. M. Paiva, *Eur. J. Pharmacol.*, **25**, 191 (1974); (b) L. Juliano and A. C. M. Paiva, *Biochemistry*, **13**, 2445 (1974).
(20) T. B. Paiva and A. C. M. Paiva, *Br. J. Pharmacol. Chemother.*, **15**, 557 (1960).
(21) T. B. Paiva and A. C. M. Paiva, *J. Pharmacol. Exp. Ther.*, **130**, 177 (1960).
(22) R. H. Andreatta and H. A. Scheraga, *J. Med. Chem.*, **14**, 489 (1971).
(23) R. Deslauriers, A. C. M. Paiva, K. Schaumburg, and I. C. P. Smith, *Biochemistry*, **14**, 878 (1975).
(24) G. R. Marshall and H. E. Bosshard, *Circ. Res.*, **30**, II-143 (1972); G. R. Marshall, H. E. Bosshard, N. C. Eilers, and P. Needleman in "Chemistry and Biology of Peptides", J. Meienhofer, Ed., Ann Arbor Science Publishers, Ann Arbor, Mich., 1972, p 571.
(25) D. Gagnon, W. K. Park, and D. Regoli, *Br. J. Pharmacol.*, **43**, 409 (1971).
(26) L. Juliano, P. Boschcov, and A. C. M. Paiva, *Biochemistry*, **13**, 4263 (1974).

Protein-Binding Polyhedral Boranes. 3[1]

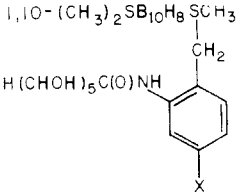
R. L. Sneath, Jr., J. E. Wright, A. H. Soloway,* S. M. O'Keefe, A. S. Dey, and W. D. Smolnycki

Department of Medicinal Chemistry, College of Pharmacy and Allied Health Professions, Northeastern University, Boston, Massachusetts 02115. *Received November 20, 1975*

A series of polyhedral borane derivatives containing protein-binding functional groups has been synthesized. Problems encountered in earlier studies (low incorporation levels, gross precipitation of conjugates) have been overcome by including a water-solubilizing gluconamide group in the structure. This modification has allowed high levels of boron to be covalently bound to HGG, forming a completely water-soluble conjugate.

In anticipation of the expected isolation of tumor-specific antibodies,[2] several research groups have investigated the attachment of boron-containing moieties to proteins.[1,3-5] These investigations aim to demonstrate the feasibility of transporting boron into neoplasms, via antibody carriers, thereby providing the basis for therapeutic neutron-capture irradiation. Toward this end, we have reported the synthesis of a number of polyhedral borane derivatives containing protein-binding functional groups and their incorporation into bovine serum albumin (BSA) and human γ-globulin (HGG).[1] These results have indicated that proteins may be appropriately modified by our method. Unfortunately, the derivatives which were used in those initial experiments reduced the aqueous solubility of the proteins, especially HGG, so severely that only a small number of boron-containing labels could be incorporated prior to denaturation of the conjugate.

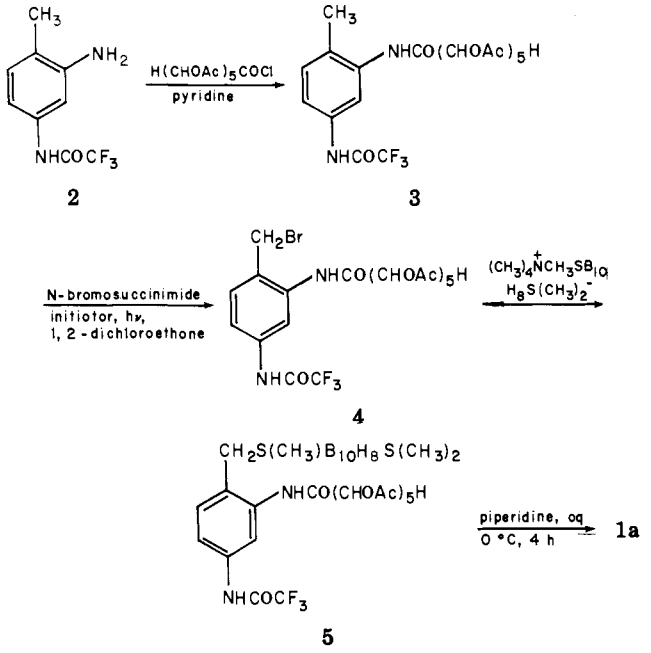
Chart I. Structures of the Protein Binding Polyhedral Borane Derivatives



1a, X = NH_2
b, X = NCS
c, X = CO_2H

In seeking to overcome this problem, we have undertaken the attachment of a water-solubilizing group onto the appropriate polyhedral borane derivatives. For reasons

Scheme I



which are well established, the use of a nonionic label is essential.[2] Consequently we have sought to incorporate a covalent side chain, the gluconamide group, into the protein-binding handle. Syntheses of homologues shown in Chart I were undertaken.

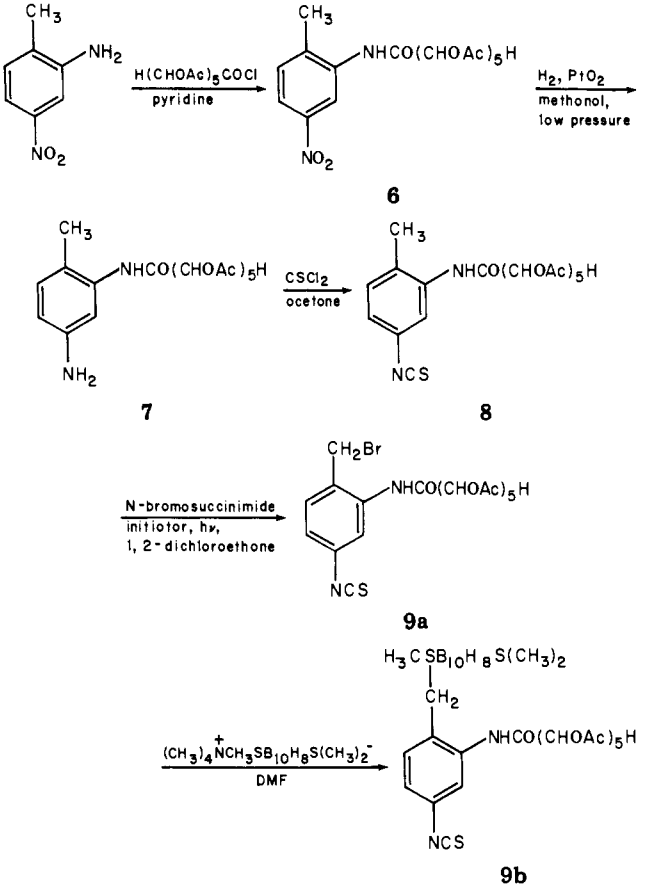
The structures shown in Chart I are numbered conventionally. Retention of the *R* configuration in the carbohydrate group is assumed, while the racemic nature of the asymmetric center at sulfur is apparent, due to nonspecificity of the benzylation reaction.

Throughout the preparation of **1a–c**, the gluconamide function is protected by acetoxy groups. These are removed prior to coupling in order to minimize self-condensation while providing the necessary water solubility in the conjugate. Schemes I–III outline the syntheses.

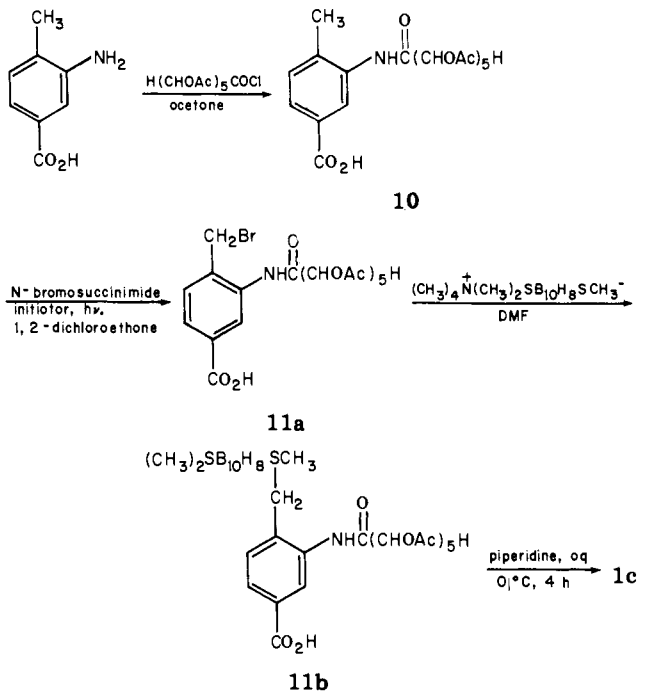
In Scheme I, the starting material **2** was prepared using standard procedures commencing with 3-nitro-4-toluidine.[6,7] Compound **2** was acylated in pyridine, using 2,3,4,5,6-pentaacetyl-(*R*)-gluconyl chloride.[8] Selective free-radical bromination of **3** gave the desired benzyl bromide derivative **4**, which was allowed to react without purification with the appropriate demethylated borane derivative to furnish **5**.[10] This latter substance could be more easily purified with the elimination of the residual impurities remaining from the two previous steps. The final procedure was the hydrolysis of both the acetoxy and trifluoroacetoxy protective groups using cold aqueous piperidine. Under these conditions, the gluconamide linkage is preserved. It was found that the deprotected amine could be isolated and purified for identification purposes or could be coupled to protein in situ without the necessity of isolation.

The synthesis of the isothiocyanate derivative is given in Scheme II. This preparation was accomplished via a sequence similar to that for **1a**. 4-Nitro-2-toluidine was acylated with 2,3,4,5,6-pentaacetyl-(*R*)-gluconyl chloride to give **6**. This was hydrogenated over Adam's catalyst to the corresponding amine **7** which reacted with thiophosgene to yield the desired isothiocyanate **8**.[11] Bromination of this compound formed the benzyl halide **9a**, which reacted readily with the demethylated boron hydride derivative to give **9b**. An advantage of this route is the introduction of boron in the last step prior to deprotection

Scheme II



Scheme III



and coupling. This is an important feature, since the economical preparation of such compounds from ^{10}B enriched precursors will require high-yield steps once boron has been introduced into the structure. Compound **1b** was not isolated per se, since the reactive isothiocyanate group may be capable of intermolecular reactions with the carbohydrate function. Therefore **1b** was generated in situ, prior to protein binding.

The third compound, **11b**, was prepared by a related sequence, shown in Scheme III, beginning with 3-amino-4-methylbenzoic acid. Acylation of this compound with 2,3,4,5,6-pentaacetylgluconyl chloride was carried out in acetone, in order to avoid complications which arise from deprotonation of the carboxylic acid group by more basic solvents. Using this procedure, **10** was isolated, characterized, and brominated to form **11a**. This latter compound was allowed to react without further purification with the demethylated borane derivative. In this way, **11b** was easily formed and separated in high purity. Finally, the protective groups were removed as in Scheme I, and **1c** was isolated and characterized.

The isolation of species **1a** and **1c** was difficult, gave low yields, and offered no advantage over in situ deprotection and coupling to proteins. Table I lists the compounds which we have characterized, along with their physical and analytical data.

Protein-Binding Studies. Compounds **1a–c** differ structurally only in their protein binding functional groups, thus allowing a comparison between amino, isothiocyanate, and carboxylic acid groups with respect to their relative boron to HGG binding efficiencies. The preferred functional group may be assessed on the basis of its ability to incorporate, covalently, the highest levels of boron into protein, without gross denaturation. Precipitation of conjugate has served as our criterion for denaturation throughout this work, although antigen specificity, a more refined index of conformation, will be considered in a later study. Compound **1a** was incorporated into the free carboxylic acid residues of HGG by carbodiimide activation.[13] Compound **1b** was directly coupled to the protein's free amino groups via spontaneous thiourea coupling.[12] **1c** was attached to free amine groups on the protein by means of carbodiimide activation.[13]

Realistic assessment of protein-binding efficiencies was assured by the use of standard solutions of protein, borane, soluble carbodiimide, and diluent, from which aliquots were transferred into the reaction mixtures allowing precise control of the boron to protein mole ratios. At the same time, protein concentration and total reaction volume were maintained at constant values throughout all of these experiments. The covalent nature of the conjugation was confirmed by gel permeation chromatography of the reaction mixtures. The material which was collected for the final boron and protein analyses included only that fraction containing purified conjugate.

Although maximum molar ratios of boron to protein were not attained at the hapten levels employed in these experiments, the values reported herein (Table II) do reach and surpass the minimum estimated therapeutic level.[14] The extent of incorporation varies predictably, in view of the differing target sites on the protein. The results indicate that the availability of a side-chain group for conjugation with the appropriate borane derivative may depend upon its position in the protein and, hence, its exposure to the entering label.

Experimental Section

Apparatus and Reagents. Melting points were determined using a Thomas-Hoover apparatus and are corrected. Elemental analyses were performed by Schwartzkopf Microanalytical Laboratories, Woodside, N.Y., and by Instranal Laboratories, Rensselaer, N.Y. Infrared spectra of the compounds, pressed into KBr wafers, were obtained using a Perkin-Elmer 127 Infracord spectrometer. NMR spectra of samples dissolved in polysol-*d* were obtained on a Varian T-60 instrument. Boron[15] and protein[16] were analyzed using standard procedures.

Synthesis of 3-amino-4-methyltrifluoroacetanilide (**2**) was readily accomplished by acylation of 3-nitro-4-methylaniline with trifluoroacetic anhydride followed by hydrogenation of the 3-nitro-4-methyltrifluoroacetanilide (mp 133–135 °C) over platinum oxide to form the amine **2** (mp 113–115 °C).

The phosphate buffer (pH 8.0) was prepared by the standard method.[17]

The preparation of $(CH_3)_4N^+$-1,10-$B_{10}H_8S(CH_3)_2SCH_3^-$[1,10] and pentaacetylgluconyl chloride has been described previously.

The immunoglobulin G, Human Cohn fraction II, molecular weight 160 000, was obtained from Sigma Chemical Co.

Acylation with Pentaacetylgluconyl Chloride. The following procedure was used for the preparation of compounds **3** and **6**. To 3 g of 3-amino-4-methyltrifluoroacetanilide in 2.5 ml of dry pyridine at 0 °C, 8 g of pentaacetylgluconyl chloride was added over a 2-h period with continuous stirring. The stirring was continued for 5 h at 0 °C; then the solution was poured into 400 ml of water and extracted with four 50-ml portions of ethyl acetate. The combined ethyl acetate extracts were washed with 25 ml of 10% aqueous cupric sulfate solution to remove any residual pyridine, dried over anhydrous sodium sulfate, and filtered. The filtrate was concentrated under reduced pressure, and the solid so formed was recrystallized from methylene chloride in hexane to give 6.3 g (75%) of **3**, mp 209–210 °C.

The same procedure was used to convert 4-nitro-2-toluidine to compound **6**, but the corresponding 2-toluidine-4-carboxylic acid was acylated by the following alternative procedure to form compound **10**.

Into 100 ml of anhydrous acetone were dissolved 2 g of 2-toluidine-4-carboxylic acid and 6 g of pentaacetylgluconyl chloride. The solution refluxed for 4 h, then was cooled to room temperature, and freed of acetone under reduced pressure. The resulting white solids were recrystallized from 100 ml of ethyl acetate, 50 vol % in cyclohexane, giving 4.2 g (58% yield) of **10**, mp 95 °C dec.

3-Pentaacetylgluconamido-4-methylphenyl Isothiocyanate (8). 3-Pentaacetylgluconamido-*p*-toluidine (**7**, 1 g, 1.96 mol) was dissolved in 15 ml of acetone. To the stirred solution maintained at 0 °C was added 2 ml of thiophosgene. After 1 h, 10 ml of H_2O was added and stirring was continued for an additional hour in order to decompose residual thiophosgene. To the mixture, an equal volume of ethyl acetate was added and the nonaqueous layer partitioned and collected. The aqueous layer was extracted a second time and the combined extracts were dried over anhydrous sodium sulfate. Solvent removal under reduced pressure gave a product which could be recrystallized from methylene chloride to yield 840 mg of **8** (77% yield), mp 161–163 °C.

Preparation of the Benzyl Bromides 4, 9a, and 11a. The procedures for preparing **4**, **9a**, and **11a** are similar throughout and exploit the solubilities of **3**, **8**, and **10** in 1,2-dichloroethane. The procedure for **4** is representative.

2-Pentaacetylgluconamido-4-(trifluoroacetamido)benzyl Bromide (4). To a solution of 3.0 g (4.95 mmol) of **3** in 150 ml of 1,2-dichlorethane was added 884 mg of *N*-bromosuccinimide and 100 mg of 2,2′-azobis(2-methylpropionitrile). The solution was refluxed for 4 h under a flood lamp. Then it was cooled and filtered and the solvent removed under reduced pressure. The resulting solids were extracted with three 100-ml portions of ethyl ether and the combined extracts diluted with boiling hexane and then cooled to recrystallize the product, yielding 2.5 g of **4** (75% yield), mp 163–167 °C.

1′-Dimethylsulfido-10′-(2-pentaacetylgluconamido-4-trifluoroacetamidobenzyl)methylsulfidooctahydrodecaborane (5). To 10 ml of dry dimethylformamide was added 1 g of $(CH_3)_4N$-1,10-$B_{10}H_8S(CH_3)_2SCH_3$ and 2.29 g of **4**. The resulting solution was stirred for 24 h and then filtered to remove tetramethylammonium bromide. Slow addition of methylene chloride precipitated 1.7 g of large pure crystals of **5** which were filtered, washed in 5 ml of methylene chloride, and dried in vacuo (70% yield): mp 123–126 °C.

1-Dimethylsulfido-10′-(2-pentaacetylgluconamido-4-thiocyanatobenzyl)methylsulfidooctahydrodecaborane (9b). To a stirred solution of 620 mg of $(CH_3)_4N$-1,10-$B_{10}H_8S(CH_3)_2SCH_3$ (2.07 mmol) in 10 ml of dimethylformamide was added 1.3 g (2.06 mmol) of 2-pentaacetylgluconamido-4-thiocyanatobenzyl bromide (**9**). A precipitate formed almost immediately as the solution was stirred at room temperature. After 12 h, the mixture was poured slowly with rapid stirring into 400 ml of H_2O.

Table I. Chemical and Physical Data for the Second Generation of Protein-Binding Polyhedral Boranes, Their Precursors, and Intermediates

Compd	Formula (analyses)[a]	Mp, °C	Ir, cm^{-1}[b]	NMR[c]	% yield
3	$C_{25}H_{29}N_2O_{12}F_3$ (C, H, N)	209-210	3575 (m), 1720 (s), 1610 (m), 1510 (m), 1370 (m), 1210 (s)	2.1 (s, 12 H), 2.2 (s, 3 H), 2.3 (s, 3 H), 4.2 (t, 2 H), 5.8 (m, 4 H), 7.8 (m, 3 H)	75
4	$C_{25}H_{28}N_2O_{12}F_3Br$	162-166 dec	3275 (m), 3150 (m), 1720 (s), 1610 (m), 1430 (m), 1380 (m), 1210 (s)	2.1 (s, 12 H), 2.3 (s, 3 H), 4.5 (m, 2 H), 5.6 (m, 6 H), 7.8 (m, 3 H)	67
5	$C_{23}H_{45}N_2O_{12}S_2F_3B_{10}$ (C, H, N, S, F, B)	123-126	3300 (m), 2500 (s), 1730 (s), 1600 (m), 1490 (m), 1210 (s), 1050 (m)	2.1 (m, 12 H), 2.2 (s, 3 H), 2.9 (d, 3 H), 3.1 (s, 6 H), 4.2 (t, 2 H), 5.7 (m, 4 H), 7.8 (m, 3 H)	70
8	$C_{24}H_{28}N_2O_{11}S$ (C, H, N, S)	161-163	3200 (m), 2100 (s), 1720 (s), 1360 (m), 1210 (s), 1060 (m)	2.2 (s, 12 H), 2.3 (s, 3 H), 2.4 (s, 3 H), 4.4 (m, 2 H), 5.6 (m, 4 H), 7.4 (m, 3 H)	77
9a	$C_{24}H_{27}N_2O_{11}SBr$	154-156	3350 (m), 2150 (s), 1740 (s), 1700 (m), 1380 (m), 1220 (s), 1060 (m)	2.1 (s, 12 H), 2.4 (s, 3 H), 4.2 (t, 2 H), 4.6 (s, 2 H), 5.6 (m, 4 H), 7.2 (m, 3 H)	76
9b	$C_{27}H_{44}N_2O_{11}S_3B_{10}$ (C, H, N, S)	87-90 dec	2500 (s), 2100 (s), 1730 (s), 1360 (m), 1210 (m), 1020 (m)	2.0 (s, 12 H), 2.2 (s, 3 H), 2.8 (s, 3 H), 3.0 (s, 6 H), 4.2 (m, 2 H), 4.5 (s, 2 H), 5.5 (m, 4 H), 8.0 (m, 3 H)	75
10	$C_{24}H_{29}NO_{13}$ (C, H, N)	95 dec	3300 (m), 1740 (s), 1530 (m), 1380 (m), 1220 (s), 1060 (m)	2.0 (s, 12 H), 2.2 (s, 3 H), 2.3 (s, 3 H), 4.3 (t, 2 H), 5.5 (m, 4 H), 7.8 (m, 3 H)	58
11a	$C_{24}H_{28}NO_{13}Br$ (C, H, N)	125-128 dec	3200 (m), 1740 (s), 1430 (m), 1380 (m), 1220 (s), 1050 (m)	2.0 (s, 12 H), 2.3 (s, 3 H), 4.3 (t, 2 H), 4.5 (s, 2 H), 5.6 (m, 4 H), 7.8 (m, 3 H)	50
11b	$C_{27}H_{45}NB_{10}O_{13}S_2$ (C, H, N, S)	138-141 dec	3300 (m), 2950 (m), 2500 (s), 1735 (s), 1430 (m), 1380 (m), 1220 (s), 1060 (m)	2.0 (s, 12 H), 2.2 (s, 3 H), 3.0 (s, 9 H), 4.2 (t, 2 H), 4.8 (s, 2 H), 5.5 (m, 4 H), 7.9 (m, 3 H)	80
1c	$C_{17}H_{35}O_8NS_2B_{10}$ (C, H, N)	69 dec	3300 (m), 2910 (m), 2490 (s), 1620 (m), 1420 (m), 1400 (m), 1140 (s), 960 (s), 880 (s)	3.1 (s, 6 H), 3.4 (s, 3 H), 3.8 (m, 2 H), 4.2 -4.8 (m, 7 H), 4.9 (s, 6 H), 8.0 (m, 3 H), 9.0 (s, 1 H)	27
1a	$C_{16}H_{36}O_6N_2S_2B_{10}$ (C, H, N)	70 dec	3350 (m), 3000 (m), 2920 (m), 2500 (s), 1670 (m), 1600 (m), 1530 (m), 1480 (m), 1450 (m), 1420 (m), 1400 (m), 1140 (s), 960 (m), 880 (m)	3.1 (s, 6 H), 3.4 (s, 3 H), 3.8 (m, 2 H), 4.2-4.8 (m, 7 H), 4.9 (s, 5 H), 5.9 (s, 3 H), 8.0 (m, 3 H)	23

[a] Microanalyses for the elements within 0.2% of theoretical values. [b] KBr wafer method. [c] Chemical shifts in ppm downfield from Me_4Si (multiplicity, number of protons).

Table II. Molar Concentrations of Boron and Protein and Their Mole Ratios in the Coupling Reaction Mixtures and in the Purified Conjugate

Compd	Reaction mixture [Boron][a] (M × 10³)	Reaction mixture [Protein] (M × 10⁶)	Reaction mixture [Boron/protein] (molar ratio)	Conjugate [boron/protein] (molar ratio)
1a	3.96	6.50	610	26
	5.28	6.50	810	82
	7.92	6.50	1200	99
1b	3.90	6.50	600	410
	5.20	6.50	800	590
	7.80	6.50	1200	1100
1c	3.75	6.70	500	85
	5.00	6.70	750	120
	7.50	6.70	1100	260

[a] Calculated as gram-formula weights of the element per liter of solution.

The resulting solid was filtered, dried, and recrystallized from a 2-propanol–H_2O–1.2 g mixture (75% yield): mp 87–90 °C dec.

1′-Dimethylsulfido-10′-(2-pentaacetylgluconamido-4-carboxybenzyl)methylsulfidooctahydrodecaborane (11b). To 100 mg (0.16 mmol) of **11a** in 10 ml was added 49 mg (0.16 mmol) of $(CH_3)_4N$-1,10-$B_{10}H_8S(CH_3)_2SCH_3$. The mixture was stirred at room temperature for 6 h. A precipitate formed almost immediately. The DMF was removed under reduced pressure and the solid obtained was partitioned between ethyl acetate and water. The organic layer was dried and reduced in volume. The product was precipitated by adding hexane and refrigerating the solution. The yield was 112 mg (80%), mp 138–141 °C dec.

Removal of the Protective Groups. **1a** and **1c** were isolated and purified by the procedure given below. This procedure was also used for deprotection of **9b** but that compound was not isolated for identification prior to its use in the protein-binding experiments.

1′-Dimethylsulfido-10′-(2-gluconamido-4-carboxybenzyl)methylsulfidooctahydrodecaborane (1c). A solution of 150 mg (0.196 mmol) of **11b** in 4 ml of piperidine at 0 °C was stirred for 4 h. During this period, 4 g of ice was added slowly. When the reaction was complete, as determined by TLC (12% NH_4NO_3, Cellulose DEAE; R_f 0.5 for **1c**, <0.4 for precursors), the water and piperidine were removed under reduced pressure. The residue was taken up in 10 ml of tetrahydrofuran and filtered. The filtrate was dried over anhydrous sodium sulfate, filtered, and stripped to dryness. The resulting solids were recrystallized from an ethyl acetate–hexane mixture to give 30 mg of **1c** (28% of yield), mp 69 °C dec.

Application of this same procedure to compound **5** gave **1a**, mp 70 °C dec, in 23% yield. Spectral and analytical studies, summarized in Table I, confirmed the identity of these products and their precursors.

Protein-Binding Procedures. Incorporation of 1a and 1c into Proteins. Stock solution A was prepared by diluting 17.28 mg of **1a** and 6.45 mg of 1-ethyl-3-(3′-dimethylaminopropyl)-carbodiimide hydrochloride to 25 ml with pH 8 phosphate buffer at 0 °C. Stock solution B was prepared by dilution of 69.0 mg of immunoglobulin G to 25 ml with pH 8 phosphate buffer at 0 °C. Three 25-ml Erlenmeyer flasks, precooled to 0 °C, were quickly filled by pipet as shown in Table III.

The solutions were maintained at 0 °C for 4.1 h and then fractionated on a 30-cm Sephadex G 25–80 column, with pH 8 buffer, discarding the initial 38-ml void volume. The following 15-ml dilution volume was collected, as determined by standardization with blue dextran. The collected fractions were analyzed for boron and protein to obtain the data shown in Table II. Protein recoveries were found to exceed 90%.

Table III. Volumes of Solution A, B, and pH 8 Buffer Used to Achieve the Given Boron to Protein Ratios

Sample no.	Boron/protein molar ratios	Volumes, ml: Soln A	Soln B	pH 8 buffer
1	610	3	4	3
2	810	4	4	2
3	1200	6	4	0

The incorporation of **1c** into immunoglobulin was accomplished by the method given above, except that stock solution A was prepared using 20.54 mg of **1c** and 6.56 mg of the water-soluble carbodiimide.

Incorporation of 1b into Protein. **9b** (25.28 mg) was dissolved in 3 ml of *p*-dioxane and cooled to 0 °C on an ice bath. Trimethylamine (1.0 ml) 25% aqueous was added and the mixture allowed to remain at 0–5 °C for 10 min, adjusted to pH 8 with 6 N HCl, and then diluted to 25 ml with pH 8 buffer to make solution A. Immunoglobulin G (70.45 mg) was diluted to 25 ml with pH 8 buffer to prepare solution B. Three reaction mixtures were prepared from the stock solutions as indicated in Table III. The reaction mixtures remained at 0 °C for 4 h and then were purified on Sephadex as described above.

Acknowledgment. This work was supported in part by the National Institute of Health Grant CA12246 and the Ruth M. Israel Fund for Cancer Research. We are especially indebted to Mrs. Janette Messer of the Massachusetts General Hospital for the boron and protein analyses.

References and Notes

(1) For paper 1 of this series, see R. L. Sneath, A. H. Soloway, and A. S. Dey, *J. Med. Chem.*, **17**, 796 (1974); for paper 2, see P. M. Jacobs, R. L. Sneath, A. H. Soloway, and A. S. Dey, *J. Pharm. Sci.*, **65**, 604 (1976).
(2) G. Klein, *Fed. Proc., Fed. Am. Soc. Exp. Biol.*, **28**, 1739 (1969), and ref 3, p 450.
(3) M. F. Hawthorne, R. J. Wiersema, and M. Takasugi, *J. Med. Chem.*, **15**, 449 (1972).
(4) A. G. Mallinger, E. L. Jozwiak, Jr., and J. C. Carter, *Cancer Res.*, **32**, 1947 (1972).
(5) W. N. Lipscomb, *J. Med. Chem.*, **17**, 785, 792 (1974).
(6) M. L. Wolfrom and H. B. Bhat, *Chem. Commun.*, **6**, 146 (1966).
(7) R. Adams and F. L. Cohen, "Organic Syntheses", Collect. Vol. V, Wiley, New York, N.Y., 1973, p 240.
(8) C. E. Braun and C. D. Cook in ref 7, p 887.
(9) A. Kalier in ref 7, p 825.
(10) W. H. Knoth, W. R. Hertler, and E. L. Muetterties, *Inorg. Chem.*, **4**, 280 (1965).
(11) H. Tilles in "Organic Sulfur Compounds", N. Kharasch, Ed., Vol. II, Pergamon, Oxford, 1966, p 311.
(12) P. Edman, *Acta Chem. Scand.*, **4**, 283 (1950).
(13) J. C. Sheehan, J. Preston, and P. A. Cruickshank, *J. Am. Chem. Soc.*, **87**, 2492 (1965); G. R. Stark, *Adv. Protein Chem.*, **24**, 261 (1970).
(14) K. Dohan, personal communication.
(15) A. H. Soloway and J. R. Messer, *Anal. Chem.*, **36**, 433 (1964).
(16) E. Layne, *Methods Enzymol.*, **3**, 448–450 (1957).
(17) R. A. Robinson and R. H. Stokes, "Electrolyte Solutions", Butterworths, London, 1955.