# Protein-Binding Polyhedral Boranes II: DL-*S*-(10-Dimethylsulfidooctahydrodecaborane)methionine

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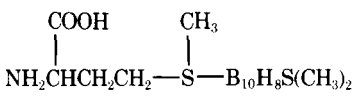
**Abstract** □ An analog of methionine, DL-*S*-(10-dimethylsulfidooctahydrodecaborane)methionine, was synthesized from fully protected homoserine. A protected dipeptide with glycine is described.

**Keyphrases** □ Boranes—synthesis of a decaboranemethionine derivative, a neutral polyhedral borane with protein-incorporating functions, potential tumor antibody-binding agent □ Protein binding—polyhedral borane synthesized, potential tumor antibody-binding agent □ Methionine analog—synthesis of a neutral polyhedral borane with protein-incorporating functions, potential tumor antibody-binding agent

The development of neutral polyhedral boranes with protein-incorporating functions offers the opportunity of binding such structures to tumor antibodies. Treatment of a tumor by neutron-capture therapy then could be quite specific (1–3). As part of continuing efforts in this area (4), DL-*S*-(10-dimethylsulfidooctahydrodecaborane)methionine (I), an analog of the amino acid methionine, substituted at the side-chain sulfur with a polyhedral borane, was synthesized.



I

This work is providing the basis for several lines of research designed to synthesize a water-soluble, boron-containing polypeptide. This amino acid will be used to synthesize low and medium weight polymers to attach to tumor antibodies. In this way, large numbers of boron atoms can be localized in and at the level of the cell. Variable solubility characteristics of the products can be obtained by copolymerization with other amino acids

## DISCUSSION

Compound I can by synthesized from DL-homoserine (II) or DL-homoserine lactone (5) (III) by the sequence outlined in Schemes I and II. Compound II or III is subjected to carbobenzyloxylation; then treatment of the sodium salt (IV) with *p*-nitrobenzyl toluenesulfonate (6) yields the *p*-nitrobenzyl ester (V). The toluenesulfonate (VI) is then formed by treatment of (V) with *p*-toluenesulfonyl chloride and pyridine. Reaction of VI with tetramethylammonium 1-methylsulfido-10-dimethylsulfidooctahydrodecaborane (7) results in the fully protected amino acid (VII).

This derivative can be partially or totally deprotected using routine methods of peptide chemistry, except that catalytic hydrogenation must be avoided since this procedure is known to degrade the borane cage (4). Some transformations are shown in Scheme II. Treatment of VII with sodium or potassium hydroxide in methanol or acetone at room temperature gives the free acid (VIII), which can be isolated through the dicyclohexylammonium salt. Reaction of VIII with hydrogen bromide in acetic acid yields the hydrobromide salt from which I can be isolated. This free amino acid is more soluble in methanol than in cold water, presumably due to the effect of the unusual side chain.

Compound VIII was also used to form a dipeptide (IX) by reaction with glycine ethyl ester and dicyclohexylcarbodiimide. Other coupling methods were not tested, but no difficulty is anticipated with the mixed anhydride or active ester methods, since this amino acid is stable to the reagents and conditions used.

## EXPERIMENTAL[1]

***N*-Carbobenzoxy-DL-homoserine *p*-Nitrobenzyl Ester (V)**—*Method A*—The method was identical to that used previously for the preparation of the L-isomer (8). The yield was 22%, mp 108–110°; IR: 3400 (OH, NH), 1720 ($CH_2OC{=}ONH$), 1600, 1520, and 1350 ($NO_2$) $cm^{-1}$; NMR (deuterochloroform): δ 8.2 (2, d, aromatic), 7.4 (7, m, aromatic), 5.8 (1, d, NH), 5.25 (2, s, $NO_2C_6H_4CH_2$), 5.15 (2, s, $C_6H_5CH_2$), 4.5 (1, m, $NHCHCO$), 3.7 (2, q, $CH_2OH$), 2.8 (1, OH), and 2.0 (2, m, $CHCH_2CH_2OH$).

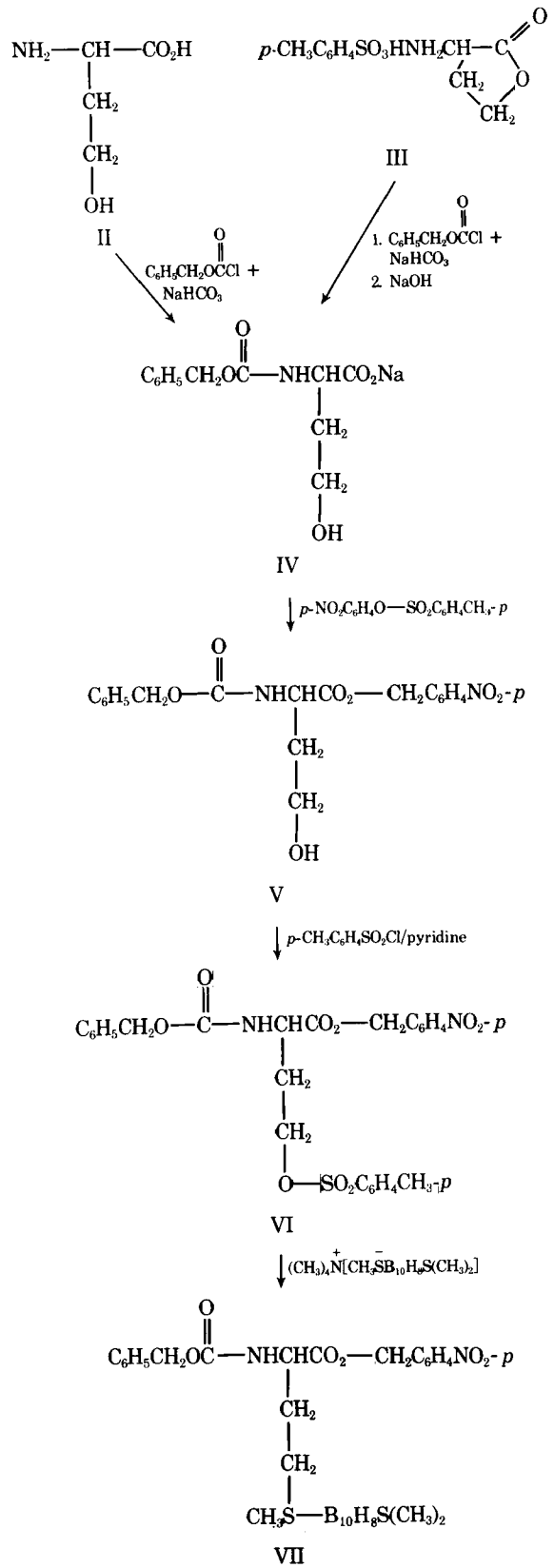
*Anal.*—Calc. for $C_{19}H_{20}N_2O_7$: C, 58.76; H, 5.19; N, 7.21. Found: C, 59.10; H, 4.94; N, 7.23.

*Method B*—Homoserine lactone *p*-toluenesulfonate (6) (40 g, 0.146 mole) was added to about 250 ml of water containing 25 g of sodium bicarbonate (0.31 mole). Benzyl chloroformate (23 ml, 0.16 mole) was added in four portions over 1 hr, and the mixture was allowed to stir for 3 hr more. Then the solution was extracted twice with 200 ml of ethyl acetate. The organic solution was washed with water, dried over sodium sulfate, and evaporated. Crystallization of the residue from ethyl acetate–hexane gave 20.5 g (60%), mp 108–110° [lit. (9) mp 110–112°].

The lactone (8 g, 34 mmoles) was dissolved in about 20 ml of methanol, and 4 *N* NaOH was added until the pH remained at 11 for 15 min (8 ml, 32 mmoles). Then the solution was evaporated to dryness several times with benzene. The residue was dissolved in 40 ml of dimethylformamide and 80 ml of acetone, *p*-nitrobenzyl toluenesulfonate (10.75 g, 35 mmoles) was added, and the mixture was refluxed for 1 hr. After evaporation, the residue was partitioned between ethyl acetate and water. The organic layer was dried over sodium sulfate and evaporated to give an oil. The oil crystallized from ether to give 11 g (89%) of white crystals, mp 88–93°. Recrystallization from ethyl acetate–hexane gave 7.94 g of an ester (64%), mp 108–110°, identical with that obtained by Method A.

***N*-Carbobenzoxy-*O*-*p*-toluenesulfonate-DL-homoserine *p*-Nitrobenzyl Ester (VI)**—Compound V (6.94 g, 17.9 mmoles) was dissolved in 40 ml of pyridine at 0°, and 4.78 g (25 mmoles) of *p*-toluenesulfonyl chloride was added. The reaction mixture was kept at 0° overnight. Ethyl acetate and iced 7 *N* $H_2SO_4$ were then added, and the layers separated. The organic layer was then washed with 2 *N* $H_2SO_4$, sodium bicarbonate solution, and saline, dried over sodium sulfate, and evaporated. The residue was crystallized from ethyl acetate–hexane to give 7.2 g (75%) of white crystals, mp 98–101°, and recrystallized to give 6.1 g, mp 102–104°; IR (potassium bromide): 3350 (NH), 1760 (ester), 1715 ($CH_2O$-C=O), 1600, 1520, 1370 ($NO_2$), 1350, and 1200 ($OSO_2$) $cm^{-1}$; NMR (deuterochloroform): δ 8.2 (2, d, $C_6H_4NO_2$), 7.7 (2, d, $C_6H_4NO_2$), 7.3 (9, m, aromatic), 5.4 (1, d, NH), 5.2 (2, s, $NO_2C_6H_4CH_2$), 5.1 (2, s, $C_6H_5CH_2$), 4.5 (1, m, —CH—), 4.1 (2, t, $—CH_2O—$), 2.5 (3, s, $CH_3C_6H_5$), and 2.2 (2, m, —CH—).

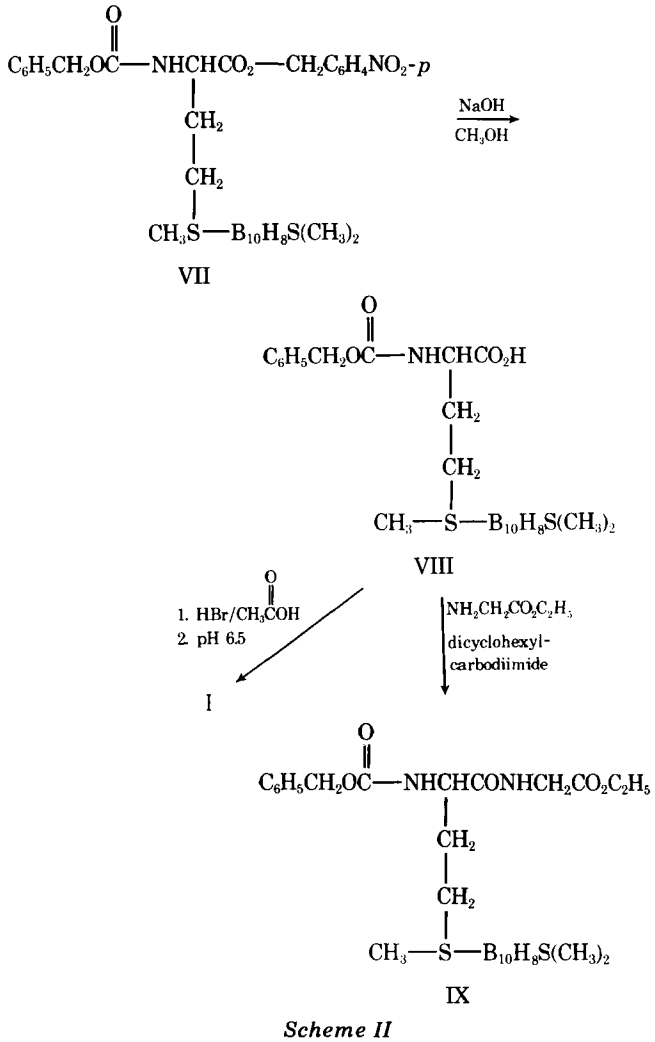
[1] Melting points were determined using a Mel-Temp melting-point apparatus and are corrected. Elemental analyses were performed by Midwest Microlab, Inc., Indianapolis, Ind. IR spectra were determined on a Perkin-Elmer 127 Infracord, and NMR spectra were determined on a Varian T-60. Silica gel hard-layer TLC plates were purchased from Analtech, Inc. Boron-containing compounds were visualized on TLC with an acidic 1% $PdCl_2$ spray.



*Scheme I*

*Anal.*—Calc. for $C_{26}H_{26}N_2O_9S$: C, 57.56; H, 4.83; N, 5.16. Found: C, 57.46; H, 4.61; N, 5.23.

**N-Carbobenzoxy-DL-S-(10-dimethylsulfidooctahydrodecaborane)methionine p-Nitrobenzyl Ester (VII)**—Compound VI (1.2 g, 2.34 mmoles) was dissolved in acetone and added to a solution of tetramethylammonium 1-methylsulfido-10-dimethylsulfidooctahydrodecaborane (0.7 g, 2.34 mmoles) in 7 ml of dimethylformamide. Although a precipitate occurred within 1–2 min, TLC indicated only about 50% reaction after 12 hr at room temperature. After 45 min at 50°, all of the starting boron salt was reacted. Ethyl acetate was added and washed several times with water, dried over magnesium sulfate, and evaporated.



*Scheme II*

Chromatography of the resulting oil on silica gel–chloroform gave 1.2 g of an oil, which could be obtained as a noncrystalline foam by vacuum evaporation of a methanol or acetone solution, mp 59–65°; IR: 3150 (NH), 2500 (BH), 1750 (ester), 1720 ($CH_2O$-C=O), 1600, 1520, and 1360 ($NO_2$) $cm^{-1}$; NMR (deuterochloroform): δ 8.2 (2, d, $NO_2C_6H_4$), 7.6 (2, d, $NO_2C_6H_4$), 7.3 (5, s, $C_6H_5$), 7.0 (1, m, NH), 5.3 (2, s, $NO_2C_6H_4CH_2$), 5.1 (2, s, $C_6H_5CH_2$), 4.7 (1, m, —CH—), 3.6 (2, m, $CH_2S$), 3.1 (9, s, $SCH_3$), and 2.6 (2, m, —CH—).

*Anal.*—Calc. for $C_{22}H_{36}B_{10}N_2O_6S_2$: C, 44.26; H, 6.07; N, 4.69; S, 10.74. Found: C, 43.92; H, 6.08; N, 4.43; S, 10.52.

**N-Carbobenzoxy-DL-S-(10-dimethylsulfidooctahydrodecaborane)methionine (VIII)**—Compound VII (1.2 g, 2 mmoles) was stirred with 0.6 ml (2.4 mmoles) of 4 *N* NaOH in an acetone–water solution (15 ml) for 1 hr at room temperature. Water was added, and the mixture was extracted with ethyl acetate. The aqueous layer was acidified and extracted three times with ethyl acetate. The organic solution was dried over magnesium sulfate and evaporated to an oil. A solution of 0.6 ml of dicyclohexylamine in ethyl acetate was added, and 0.6 g (48%) of white crystals were obtained, mp 183–186°.

Recrystallization from methanol–acetone–ether–hexane gave 0.4 g, mp 187.5–190°; IR (potassium bromide): 3400 (NH), 2500 (BH), and 1720 ($CH_2OC$=O) $cm^{-1}$; NMR (dimethyl sulfoxide-$d_6$–deuterium oxide): δ 7.3 (5H, s, $C_6H_5$), 5.1 (2H, s, $C_6H_5CH_2$), 3.4 (1, m, —CH—), 3.0 (6H, s, $SCH_3$), 2.9 (3H, s, $SCH_3$), 2.5 (4H, m, $—CH_2CH_2—$), and 1.0–2.0 (22H, m, $2C_6H_{11}$).

*Anal.*—Calc. for $C_{27}H_{54}B_{10}N_2O_4S_2$: C, 50.45; H, 8.47. Found: C, 50.58; H, 8.33.

**DL -S-(10 - Dimethylsulfidooctahydrodecaborane)methionine (I)**—The oily free acid, VIII (1.4 g, 3 mmoles), was treated with 20 ml of 15% HBr–acetic acid for 1 hr. Ether was added, and the mixture was cooled. A white solid was obtained, which was recrystallized from methanol–ether, yielding 1 g, mp 152° dec. The hydrobromide salt (0.21 g) was suspended in water, and the pH was adjusted to 6 with dilute sodium hydroxide solution. The resulting white solid was insoluble in cold water but soluble in hot water and in methanol. It was ninhydrin positive and palladium chloride positive, and it had an $R_f$ of 0.41 (methanol) and a melting point of 185°; IR (potassium bromide): 3550, 3350, broad ($NH_2$), 2980, 2900 ($CH_2$), 2500 (BH), 1600 ($CO_2^-$), 1500 ($CH_2$), 1400, 1320, 1040, and 1000 $cm^{-1}$.

A satisfactory microanalysis could not be obtained due to incomplete combustion and the formation of boron carbide.

***N*-Carbobenzoxy-DL-*S*-(10-dimethylsulfidooctahydrodecaborane)methionylglycine Ethyl Ester (IX)**—The dicyclohexylammonium salt of VIII (330 mg, 0.51 mmole) was converted to the acid by treatment with 2 *N* $H_2SO_4$ and extraction into ethyl acetate. After drying over magnesium sulfate and evaporation, the residue was dissolved in 10 ml of methylene chloride plus 1 ml of dimethylformamide and cooled to 0°. Glycine ethyl ester hydrochloride (140 mg, 1 mmole) was added along with 0.14 ml (1 mmole) of triethylamine. Dicyclohexylcarbodiimide (123 mg, 0.6 mmole) was then introduced. After the mixture was stirred overnight, it was filtered, evaporated, and partitioned between ethyl acetate and water.

The organic layer was washed sequentially with 2 *N* $H_2SO_4$, sodium bicarbonate solution, and saline. This layer was then dried over magnesium sulfate and evaporated. The residue was purified by preparatory TLC to give 50.2 mg of recovered acid VIII ($R_f$ 0.57, methanol) and 89.9 mg of the dipeptide ($R_f$ 0.25, 20% ethyl acetate–chloroform) as a noncrystalline foam, mp 81–83° (begins to change structure at 65°); IR (chloroform): 3400 (NH), 3030, 2930 (CH), 2500 (BH), 1720 ($CH_2OC$=ONH), 1705 (ester), 1670 (amide), and 1200 (ester) $cm^{-1}$; NMR (deuterochloroform): δ 7.3 (5H, s, $C_6H_5$), 5.5–6.0 (2H, m, 2NH), 3.9–4.3 (7H, m, $NHCH(CH_2CH_2$—$CONHCH_2COCH_2CH_3)$, and 3.0 (9H, d, 3SCH), and 1.0–1.5 (5H, m, $CH_2CH_3$ and $CHCH_2CH_2$).

*Anal.*—Calc. for $C_{19}H_{38}B_{10}N_2O_5S_2$: C, 41.75; H, 7.01; S, 11.71. Found: C, 42.03; H, 7.24; S, 11.91.

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# High-Speed Liquid Chromatographic Determination of Procaine in Pharmaceuticals


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**Abstract** □ The operating conditions for a quantitative method of determining procaine in pharmaceutical preparations by high-speed liquid chromatography are described. The presence of decomposition products and the possible interference of other ingredients usually present in pharmaceutical preparations were found to have no effect. The method, because of its simplicity, is highly suited for routine analysis of pharmaceutical preparations containing procaine.



**Keyphrases** □ Procaine—high-speed liquid chromatographic analysis, pharmaceutical preparations □ High-speed liquid chromatography—analysis, procaine, pharmaceutical preparations


Several methods for the quantitative determination of procaine and its hydrochloride in pharmaceutical preparations are available. The USP XVIII (1) monographs for procaine hydrochloride and sterile procaine hydrochloride incorporate a method based on titration with standard sodium nitrite solution. This analytical scheme is satisfactory for the drug substance or its solution, but other substances containing a primary amine would interfere with the analysis. The assay for procaine hydrochloride in procaine hydrochloride injection requires an extraction procedure, followed by spectrophotometric determination (2). The USP does not recommend any method of assay for the procaine portion in penicillin G procaine, although a method is recommended for the penicillin moiety (3).

Several colorimetric (4–6), spectrophotometric (7), and acidimetric (8) methods have been developed for the determination of procaine. These methods show high sensitivity but do not have the specificity and simplicity that would be anticipated with a chromatographic procedure. In addition, since these procedures often require separation of the procaine from the interfering substances, they are quite time consuming.