Synthesis of Carboranylthiouracils for Neutron Capture Therapy of Malignant Melanoma

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Several 1,2-*closo*-dodecaboranes have been synthesized from the corresponding alkynyl-substituted thiouracils to provide carboranes attached to functional group capable of being covalently incorporated into structures of potential use in the treatment of melanoma by boron neutron capture therapy (**BNCT**). The carboranyl functionalities were attached at the 5, 6 and N-1 positions of the thiouracil molecule.

Introduction

In a study of the biosynthesis of melanin, Whittaker[1] showed that thiouracil was incorporated into the melanin polymer, most probably due to its capacity to react with dopaquinone and possibly other intermediates in the melanin biosynthetic pathway.[1] The same author then suggested that thiouracil derivatives would be suitable vehicles for radioisotopic labels or for cytotoxic moieties in the diagnosis and therapy of this type of cancer. This suggestion was soon extended to the study of other simple radiolabeled thiouracils, both *in vitro* and *in vivo*.[2]

The use of thiouracil as a vehicle for stable nuclei such as ^{10}B for neutron capture therapy (NCT) of melanoma was first discussed by Fairchild and co-workers[2a] in 1982, and since then a number of boron-containing thiouracils have been synthesized for this purpose (Chart 1). Thus Roberto and Larsson[3,4] have synthesized the decaborane adduct of 5-(dimethylamino)methylthiouracil (**1**), which accumulates in murine melanotic melanomas, and Tjarks[5] has reported the synthesis of four 5-(dihydroxyboryl)thiouracils (**2–5**) as well as the carborane (**6**) and the corresponding *nido* derivative (**6a**).

Our aim was also to use thiouracil as a vehicle for ^{10}B, target molecules being thiouracils in which the boron functionality was present as a carborane, 1,2-dicarbadodecaborane, a dodecahedron cage of ten boron atoms and two carbon atoms, the large number of boron atoms having a clear advantage for NCT. Our first objective was therefore a number of thiouracils bearing an alkyl group containing a triple bond for elaboration to a carborane. Positions 5 and 6 of thiouracil were selected for attachment of the boron functionality. The synthesis of three thiouracils (**7–9**) bearing acetylenic groups in these positions (Chart 2) has been reported.[6] The present paper describes the continuation of this work with the preparation of the carboranes of this series and its extension to the synthesis of a thiouracil in which a carborane function is attached to one of the nitrogens.

Experimental Section

^1H and ^{13}C NMR spectra were recorded on a JEOL JNM-GX-400 Fourier transform spectrometer. In all cases the solvent was $(CD_3)_2SO$. Infrared spectra were run on a BioRad FTS-60 FTIR spectrometer, samples being prepared as KBr disks. Mass spectra were recorded on a Vacuum Generator 12-12 quadrupole FT mass spectrometer (matrix, PEG 200; collision gas, xenon) using an atom beam at 8 kV and 2 mA. Elemental analyses were performed at the Microanalytical Unit, ANU, Canberra, ACT, Australia.

5-[3-(*o*-Carboran-1-ylpropyl)]-6-methylthiouracil (DBTU-1). 5-(4-Pentynyl)-6-methylthiouracil (**7**) (2.08 g, 10 mmol), hexamethyldisilazane (32 mL), and dry (molecular sieves) dioxane (16 mL) to which trimethylchlorosilane (10 drops) had been added were heated under reflux for 18 h to give a clear, almost colorless solution of the bis(trimethylsilyl) derivative. After removal of all volatile material in vacuo, the residue in dry toluene (50 mL) was stirred and refluxed with the decaborane–acetonitrile adduct (2.10 g) for 4 h. After removal of the solvent the residual gum was left in contact with ethanol (50 mL) overnight; the solution was heated, filtered from a small amount of precipitate, and evaporated leaving a yellow foam which was chromatographed on silica (80 g), the eluting solvent being ethyl acetate–petroleum ether (bp 60–80 °C) (1:1). The carborane was obtained as a chromatographically homogeneous crystalline solid (1.32 g, 44%). For analysis a sample was recrystallized from methanol from which it slowly separated as prisms, mp 260–262 °C. Anal. Calcd for $C_{10}H_{22}N_2OSB_{10}$: C, 36.8; H, 6.8; N, 8.6. Found: C, 36.9; H, 6.7; N, 8.5. IR (max): 1645 (CO); 2576 (BH) cm^{-1}. ^1H NMR: δ 1.48, m, H3; 2.09, s, CH3; 2.33, m, H3, H5; 5.16, bs, H1. ^{13}C NMR: δ 63.60, J_{CH} = 196.3 Hz, C1; 76.89, C2; 36.51, J_{CH} = 131.4 Hz, C3; 23.71, J_{CH} = 129.5 Hz, C4; 28.23, J_{CH} = 129.6 Hz, C5; 16.16, J_{CH} = 129.5 Hz, C6; 161.76, C7; 113.90, C8; 174.33, 174.45, C9; 149.17, 149.31, C10. FAB-MS: m/z 327 (M + H) (+ve mode) (glycerol).

5-(*o*-Carboran-1-ylmethyl)-6-methylthiouracil (DBTU-2). This carborane was prepared by the method described above. Chromatography of the crude product (4.10 g) (obtained from acetylenic thiouracil, 4.50 g, 15 mmol) on SiO_2-60 in ethyl acetate–petroleum ether (1:2) gave the pure product, (1.20 g, 27%), mp 296–298 °C (darkens from 270 °C). Anal. Calcd for $C_8H_{18}N_2OSB_{10}$: C, 32.2; H, 6.1; N, 9.4. Found: C, 32.3; H, 6.2; N, 9.1°. IR (max): 1642 (CO); 2576 (BH) cm^{-1}. ^1H NMR: δ 2.13, s, CH_3; 3.32, bs, H3; 5.01, bs, H1. ^{13}C NMR: δ 63.11, J_{CH} = 197.9 Hz, C1; 75.88, C2; 32.32, J_{CH} = 135.1 Hz, C3; 17.11, J_{CH} = 132.0 Hz, C4; 161.68, C5; 110.99, C6; 175.17, C7; 152.21, C8. FAB-MS: m/z 299 (M + H) (+ve mode) (glycerol).

6-[2-(*o*-Carboran-1-ylethyl)]thiouracil (DBTU-3). Prepared as above (10 mmol scale) this compound crystallized from methanol after purification by chromatography using ethyl acetate–petroleum ether (1:3) (0.41 g, 14%), mp 305–308 °C dec. Anal. Calcd for $C_8H_{18}N_2OSB_{10}$: C, 32.2, H, 6.0; N, 9.4. Found: C, 32.2; H, 6.1; N, 9.4. IR (max): 1668 (CO), 2587 (BH) cm^{-1}. ^1H NMR: δ 2.55, m, H 3&4; 5.12, bs, H1; 5.77, s, H6. ^{13}C NMR: δ 63.50, J_{CH} = 194.0 Hz, C1; 75.48, C2; 34.56, J_{CH} = 135.5 Hz, C3; 31.34, 31.40, J_{CH} = 134.7 Hz, C4; 161.38, C5; 104.16, J_{CH} = 174.0 Hz, C6; 176.37, 176.50, C7; 154.19, C8. FAB-MS: m/z 299 (M + H) (+ve mode); 298 (M) (–ve mode).

Alkylation of *S*-Benzylthiouracil with 3-Prop-1-ynyl Bromide. A solution of *S*-benzylthiouracil (**10**) (10.9 g, 50 mmol) and 3-prop-1-ynyl bromide (12.0 g, 0.10 mol) in dimethyl sulfoxide (100 mL) was stirred with anhydrous potassium carbonate (21.0 g, 150 mmol) for 24 h. After dilution with water (200 mL) the solution was extracted with chloroform (3 × 80 mL); the extract was washed with 10% sodium hydroxide (3 × 50 mL) and then water (3 × 50 mL). Evaporation of the dried ($MgSO_4$) solution left a dark oil (14.0 g), from which most of the color was removed on passage down a column of silica (65 g) in methylene chloride to give

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(1) Whittaker, J. R. *J. Biol. Chem.* **1971**, *246*, 6217.
(2) (a) Fairchild, R. G.; Packer, S.; Greenberg, D.; Som, P.; Brill, A. B.; Fand, I.; McNally, W. P. *Cancer Res.* **1982**, *42*, 5126. (b) Larsson, B.; Olander, K.; Dencker, L.; Holmqvist, L. *Br. J. Cancer* **1982**, *46*, 538. (c) van Langevelde, A.; Bakker, C. N. M.; Broxterman, H. J.; Journee-de Korver, J. G.; Kaspersen, F. M.; Oosterhuis, J. A.; Pauwels, E. K. J. *Eur. J. Nucl. Med.* **1983**, *8*, 45. (d) Coderre, J. A.; Packer, S.; Fairchild, R. G.; Greenberg, D.; Laster, B.; Micca, P.; Fand, I. *J. Nucl. Med.* **1986**, *27*, 1157.
(3) Roberto, A.; Larsson, B. S. *Strahlenther. Onkol.* **1989**, *165*, 165.
(4) Larsson, B.; Dencker, L.; Olander, K.; Roberto, A. *Abstracts*; First International Conference on Skin Melanoma, Venice, Italy, May, 1984; p 41.
(5) Tjarks, W.; Gabel, D. *J. Med. Chem.* **1989**, *34*, 315.
(6) Wilson, J. G. *Pigment Cell Res.* **1989**, *2*, 297.

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Chart 1

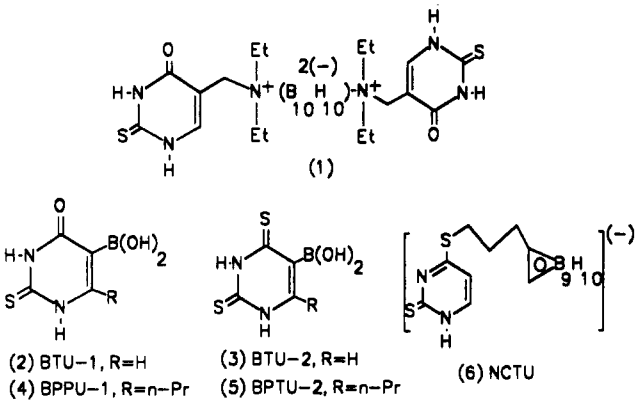
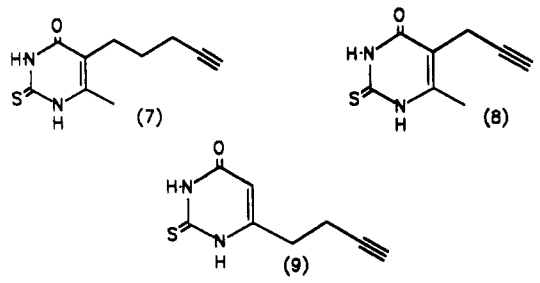


Chart 2



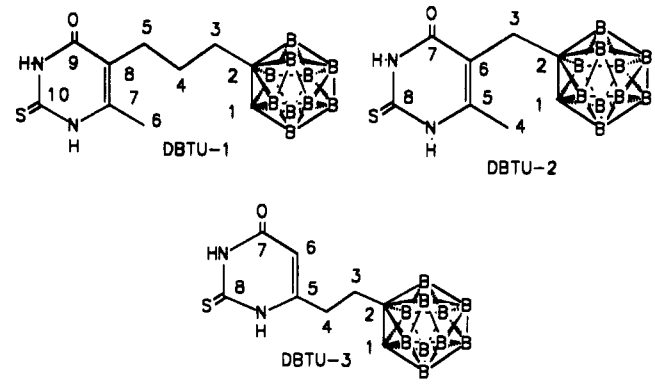
a light brown oil (11.4 g). Flash chromatography on silica (100 g) with 20% ethyl acetate/petroleum ether (bp 60–80 °C) as the eluting solvent afforded two products. **2-Benzylthio-4-(3-prop-1-ynyloxy)pyrimidine (11)** (5.4 g, 49%), which was eluted first, was obtained as a clear, pale yellow-brown oil, bp 200–230 °C (0.5–0.9 mmHg) (Kugelrohr). Anal. Calcd for $C_{14}H_{12}N_2OS$: C, 65.6; H, 4.7; N, 10.9. Found: C, 65.3; H, 5.0; N, 11.1°. IR (max) (liquid film): 1568 (arom) cm^{-1}. ^{1}H NMR: δ 4.42, s, S-CH2; 4.95, d, J = 2.4 Hz, O-CH2; 2.51, t, J = 2.4 Hz, acetylenic H; 6.48, d, J = 5.8 Hz, H6; 8.27, d, J = 5.8 Hz, H5; 7.25, m, 7.43, m, arom.

Elution with 30% ethyl acetate/petroleum ether gave the second product, **2-benzylthio-*N*-3-(3-prop-1-ynyl)-pyrimidin-4-one (12)** (4.4 g, 40%), which was obtained as a clear gum that crystallized on standing, mp 55–57 °C. Recrystallization of a sample from petroleum ether gave flat needles, mp 69–71 °C. Anal. Calcd for $C_{14}H_{12}N_2OS$: C, 65.6; H, 4.7; N, 10.9. Found: C, 65.2; H, 4.9; N, 10.7. IR (max): 1672 (CO) cm^{-1}. ^{1}H NMR: δ 4.47, s, S-CH2; 4.82, d, J = 2.6 Hz, N-CH2; 2.26, t, J = 2.6 Hz, acetylenic H; 6.23, d, J = 6.6 Hz, H5; 7.76, d, J = 6.4 Hz, H6; 7.32, m, 7.40, m, arom.

2-Benzylthio-*N*-3-(*o*-carboran-1-ylmethyl)pyrimidin-4-one (13). The thiouracil (**12**) (4.3 g, 17 mmol) and decaborane–acetonitrile adduct (3.8 g, 1.1 equiv) were refluxed with stirring in toluene (90 mL) for 6 h. After removal of the solvent the residue was extracted twice with boiling methanol (50 and 20 mL). The combined extracts were filtered and concentrated until crystals began to separate. After several hours the carborane was collected and washed with cold solvent (1.94 g, 31%), mp 167 °C. Anal. Calcd for $C_{14}H_{22}N_2OSB_{10}$: C, 44.9; H, 5.9; N, 7.5. Found: C, 45.0; H, 6.0; N, 7.5°. IR (max): 1669 (CO), 2598 (BH) cm^{-1}. ^{1}H NMR: δ 4.52, s, S-CH2; 5.06, 4.41, ABq, J = 16.3 Hz, N-CH2; 5.12, bs, H1; 6.30, d, J = 6.4 Hz, H5; 7.93, d, J = 6.6 Hz, H6; 7.40, m, 7.30, m, arom.; 1.40–2.90, br, BH. FAB-MS: m/z 374 (5%, M); 283 (100%, M – 91).

***N*-3-(*o*-Carboran-1-ylmethyl)thiouracil (14) (DBTU-4).** The thiouracil (**13**) (2.0 g, 5.3 mmol) was added to a solution of aluminum bromide (obtained from Fluka as colorless crystals under heptane)(3.20 g, 2 equiv) in toluene (50 mL). After being stirred at 55–60 °C for 5 h the reaction mixture was cooled and treated cautiously with water (50 mL). The solid that separated over several hours was collected (0.77 g) and combined with the crystals obtained on evaporation of the toluene layer (0.54 g). Chromatography on silica using ethyl acetate–petrol (1:2) gave the pure product (0.61 g, mp 268–270 °C) (43%). Anal. Calcd for $C_7H_{16}N_2OSB_{10}$: C, 29.6; H, 5.6; N, 9.9. Found: C, 29.5; H, 5.5; N, 10.0°. IR (max): 1669 (CO), 2608 (BH) cm^{-1}. ^{1}H NMR: δ 5.10, bs, H1; 5.40, 4.86, ABq, J = 14.8 Hz, H3; 6.02, d, J = 7.5 Hz, H5; 7.52, q, J = 7.5, 5.5 Hz (smaller splitting was removed on addition of D_2O), H6. ^{13}C NMR: δ 63.27, J_{CH} = 198.7 Hz, C1; 73.93, C2; 48.74, J_{CH} = 148.2 Hz, C3; 141.94, J_{CH} = 185.2 Hz, C6; 104.60, J_{CH} = 178.8 Hz, C5; 178.09, C4; 160.67, C7. FAB-MS: m/z 283 (81%, M – H, –ve mode).

Chart 3



Results and Discussion

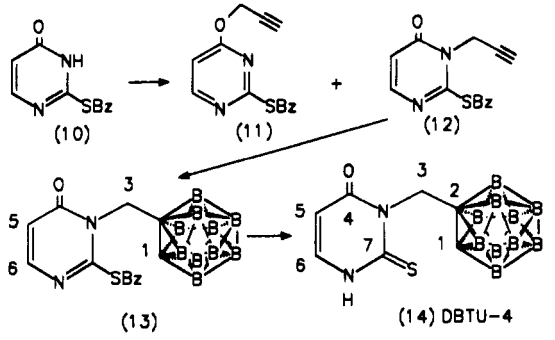
The carboranes **DBTU-1, DBTU-2,** and **DBTU-3** were prepared from the corresponding acetylenic alkylthiouracils in two steps: (1) conversion to their bis(trimethylsilyl) derivatives by reaction with hexamethyldisilazane, which removed the labile protons on the nitrogen and sulfur, and (2) reaction of these derivatives with the decaborane acetonitrile adduct. The three carboranes, which were isolated in consistently low yields, are high melting crystalline compounds (Chart 3). Their spectra were in complete agreement with their structures. In the proton NMR spectra (in DMSO-d_6) the hydrogen on the cage carbon atom of all three compounds occurred as a broad singlet with the expected chemical shift (5.0–5.2ppm). The IR spectra were characterized by strong absorptions due to the B–H stretching frequency at 2570–2600 cm^{-1}. Their carbonyl absorption bands are from 20 to 40 wavenumbers lower than those of the parent thiouracils; the carbonyl adsorption of **DBTU-3**, however, was the same as that of the parent thiouracil. The mass spectra of the three compounds have prominent pseudomolecular ion clusters in the positive FAB mode (glycerol matrix), the distribution of the peaks being in excellent agreement with the calculated values.

Alkylation of thiouracil as a means of preparing *N*-alkylthiouracils is always complicated by preferential S-alkylation.[7] If, however, the sulfur is protected by a group capable of later removal, the preparation of N-substituted thiouracils is possible. Thiouracil was therefore converted into *S*-benzylthiouracil which on further alkylation with propargyl bromide in the presence of sodium ethoxide afforded a mixture of several products. The NMR spectrum of this mixture indicated the presence of mono- and dialkyl derivatives reslting from alkylation at both nitrogen and oxygen. When, however, alkylation was carried out under milder conditions by stirring the reactants in DMSO at ambient temperature in the presence of potassium carbonate, two products (**11**) and (**12**) were formed. These were separated by flash chromatography and have been assigned the structures shown in Scheme 1 on the basis of spectral evidence.

Reaction of *N*-3-(3-prop-1-ynyl)-*S*-benzylthiouracil (**12**) with decaborane–acetonitrile adduct in toluene or benzene afforded carborane **13** in reasonable yield (30%). The IR spectrum of this compound had a carbonyl absorption at 1668 cm^{-1}, very close to that of its precursor, and the characteristic strong B–H stretching band at 2594 cm^{-1}. In the NMR spectrum the resonance of the cage carbon proton was at 5.12 ppm and the methylene protons between the cage and the heterocyclic ring formed an AB quartet, 5.06, 4.41 ppm, J = 16.3 Hz. The benzyl group was smoothly

(7) Brown, D. J. *The Pyrimidines*; Interscience: New York, 1962; p 282.

Scheme 1



removed with anhydrous aluminum bromide in toluene to give 3-(*o*-carboran-1-ylmethyl)thiouracil (**14**) (**DBTU-4**) in 60% yield. This carborane was also high melting, 268–272 °C, and its IR spectrum was characterized by a carbonyl absorption at 1666 cm^{-1}, also close to the corresponding absorptions of its two precursors. In the NMR spectrum the cage carbon proton appeared at 5.10ppm and the methylene protons again formed an AB quartet, 5.40, 4.86 ppm, $J = 14.8$ Hz. The most significant resonance was that of the 6 proton at 7.52 ppm which was a quartet formed by coupling with both the 5 proton ($J = 7.5$ Hz) and the proton on the adjacent nitrogen ($J = 5.5$ Hz). On the addition of D_2O to the sample the quartet collapsed to a doublet, 7.50 ppm ($J = 7.5$ Hz). This confirms the structure shown in Scheme 1.

DBTU-1 was found to accumulate in certain melanoma lines to give a boron concentration 11 times that of the monoboronothiouracil, **BTU-1**.[8] Solubility, however, was a problem. To overcome this obstacle complexation with a liposome, a process known as liposome entrapment, was investigated in the case of **DBTU-1** and **DBTU-2** with the result that increased concentrations of these compounds in several melanoma lines were observed.[9] When tested in nude mice bearing the Harding–Passey melanoma, **DBTU-4** proved to be rather toxic.[10] At a dose which the animals survived, biodistribution studies indicated a level of 12 μg/g of tumor, about half the concentration required for effective BNCT.

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(9) Moore, D. E.; Chandler, A. K.; Corderoy-Buck, S.; Wilson, J. G.; Allen, B. J. *Progress in Neutron Capture Therapy for Cancer*; Allen, B. J., Moore, D. E., Harrington, B. V., Eds.; Plenum Press: New York, 1992; p 451.

(10) Corderoy-Buck, S.; Wilson, J. G.; Gabel, D.; Tjarks, W.; Moore, D. E.; Chandler, A. K. Proceedings of the 4th Australia-Japan Workshop for Malignant Melanoma, Kobe, Japan, Feb. 13–16th, 1990.