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Synthesis and Characterization of 7-$(CH_3)_3$N-4-{2,4-$(NO_2)_2C_6H_3S$}-*nido*-7-$CB_{10}H_{11}$ and its Biodistribution in C57B16 Mice Bearing B16 Melanoma

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7-$(CH_3)_3$N-4-{2,4-$(NO_2)_2C_6H_3S$}-nido-7-$CB_{10}H_{11}$ has been synthesized through a Friedel–Crafts substitution reaction on 7-$(CH_3)_3$N-*nido*-7-$CB_{10}H_{12}$. A biodistribution study in mice with implanted B16 melanoma indicates that the compound locates in neoplastic tissue at concentrations which suggest that its use in ^{10}B neutron capture therapy may be feasible

Keywords: carbaborane; carborane; neutron capture therapy; melanoma; biodistribution; boron

INTRODUCTION

There is considerable resurgence of interest in ^{10}B neutron capture therapy (^{10}BNCT) for the treatment of cancer.[1] The technique involves the delivery of a compound which is preferentially accumulated in tumour tissue, and its irradiation with neutrons to induce short-range high-LET radiation within the malignancy by the capture reaction represented in Scheme 1.

$$^{10}_{5}B + {}^{1}_{0}n \rightarrow {}^{7}_{3}Li^* + {}^{4}_{2}He + 2.31\ MeV$$

$$\downarrow {}^{94\%}$$

$${}^{7}_{3}Li + \gamma + 0.48\ MeV$$

$$\overset{6\%}{\rightarrow} {}^{7}_{3}Li + {}^{4}_{2}He + 2.79\ MeV$$

Scheme 1

For an adequate radiation dose to the malignancy, the compound should have a high boron content, and this is usually achieved by a cluster system.

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The boron cluster compounds that have been studied for application in ^{10}BNCT have normally been derivatives of the anion $[B_{12}H_{12}]^{2-}$ and the neutral carborane $C_2B_{10}H_{12}$. We have studied routes to substitute monocarbon carborane cluster compounds to extend the range and versatility of the compounds appropriate to BNCT.[2] Sulphur-substituted derivatives of 7-trimethylamine-7-carba-*nido*-undecaborane(12), **1**, have been reported[2] to be formed by a Friedel–Crafts substitution reaction with disulphur dichloride. $[B_{12}H_{12}]^{2-}$ reacted with 2,4-$(NO_2)_2C_6H_3SCl$ in acetonitrile to produce the monosubstituted derivative.[3]

Hypoxic cells have been targeted by bioreducible systems such as nitroimidazoles,[4] and nitroacetophenone has been used as a radiosensitizer.[5] We have therefore combined a nitroaromatic system with a boron cluster to attempt to induce selective incorportion of boron into a tumour system.

In this work, 7-$(CH_3)_3$N-*nido*-7-$CB_{10}H_{12}$, **1**, was treated under Friedel–Crafts conditions with 2,4-dinitrophenylsulphenyl chloride to produce 7-$(CH_3)_3$N-4-{2,4-$(NO_2)_2C_6H_3S$}-7-$CB_{10}H_{11}$, **2**, which was characterized by ^{11}B and ^{1}H NMR spectroscopy. The biodistribution of **2** was also investigated in C57B16 male mice, inoculated with B16 melanoma cells.

EXPERIMENTAL

Solvents and reagents

Dichloromethane was dried by distillation from calcium hydride. 2,4-$(NO_2)_2C_6H_3SCl$ was handled in a glove box and used as supplied.

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7-$(CH_3)_3$N-7-$CB_{10}H_{12}$ was prepared by the published method[6] and recrystallized from acetonitrile and water before use.

NMR spectra

These were recorded on a Bruker AMX400 spectrometer (^{1}H, 400 MHz; ^{11}B, 128 MHz) on solutions in CD_3CN. Chemical shifts, in ppm, are quoted as positive to high frequency of the reference standards, $Si(CH_3)_4$ and $BF_3(Et_2O)$.

Synthesis of 7-$(CH_3)_3$N-4-{2,4-$(NO_2)_2C_6H_3S$}-7-$CB_{10}H_{11}$

Compound **1** (0.66 g, 3.4 mmol) and $AlCl_3$ (0.53 g, 4 mmol) were suspended in 20 ml of dry CH_2Cl_2 and cooled to −10 °C. 2,4-$(NO_2)_2C_6H_3SCl$ (0.8 g, 3.6 mmol) in 10 ml of CH_2Cl_2 was added dropwise to the suspension under nitrogen. The dark red solution was refluxed for 6 h until it was a clear yellow colour. Once cool, the solution was concentrated on a rotary evaporator and passed through a flash silica-gel column eluted with CH_2Cl_2. The first compound off the column was unreacted **1** and the second compound was 4-{2,4-$(NO_2)_2C_6H_3S$}-7-$(CH_3)_3$N-7-$CB_{10}H_{11}$ (0.17 g, 0.44 mmol; 13%). Found. C, 31.01; H, 5.15; N, 8.78%. $C_{10}H_{23}B_{10}O_4S$ requires: C, 30.84; H, 5.95; N, 10.79%.

Biodistribution study

Compound **2** was dissolved in 45% ethanol because it was insoluble in water. This ethanolic concentration was the minimum possible which would allow sufficient solubility of compound **2** and was tolerated by the strain of mice used (our unpublished data). The solution (0.2 ml containing 50 μg of boron per g body weight) was injected intraocularly into C57B16 male mice (20–23 g), 11 days after the hosts were inoculated s.c. with B16 melanoma cells. The animals were sacrificed by decapitation 1, 6 and 24 h after injection (six animals for every time point).

The successful treatment of neoplasms by the BNCT method requires a boron concentration gradient between tumour and tissue adjacent to it (B in tumour/B in adjacent tissue $\gg 1$) after sufficient time has elapsed to allow clearance of the blood. Therefore the tumour, tumour bed, muscle and skin were analysed. Additionally the blood, urine and tissues of excretory organs (kidneys, liver, lung) were also analysed. The organs were excised, rinsed in physiological saline, dried with filter paper and weighed. The boron-10 content was determined by prompt gamma spectroscopy in the IR-8 reactor at Kurchatov Research Centre, Russia.

Table 1 ^{1}H and ^{11}B NMR data for **2**

Chemical shift, δ(ppm)	Peak multiplicity	Relative intensity	Assignment position
2.5	doublet	1 BH	5
−9.4	doublet	2 BH	2, 3
−12.9	doublet	1 BH	8
−14.3	doublet	1 BH	11
−21.6	singlet +	1 BS	4
−21.6	doublet	2 BH	9, 10
−25.2	doublet	1 BH	1
−32.4	doublet	1 BH	6
8.47	doublet	1 CH	3 (aromatic
8.37	doublet of	1 CH	5 (aromatic)
8.21	doublets		
8.08	doublet	1 CH	6 (aromatic)
3.13	singlet	9 CH_3	$N(CH_3)_3$

RESULTS AND DISCUSSION

Characterization of 7-$(CH_3)_3$N-4-{2,4-$(NO_2)_2C_6H_3S$}-7-$B_{10}H_{11}$, 2

The ^{11}B and ^{1}H NMR data on **2** are presented in Table 1. The ^{11}B data clearly indicate the absence of a plane of symmetry in the molecule, and the singlet assigned to the B4 atom is in the same chemical-shift region as reported for other position-4 sulphur-substituted compounds of **1**.[1b] The ^{1}H data confirm the nature of the substituents, and thus establish the compound as 7-$(CH_3)_3$N-4-{2,4-$(NO_2)_2C_6H_3S$}-7-$B_{10}H_{11}$ as shown in Fig. 1.

Initial attempts to cleave the ring carbon–sulphur bond by hydrolysis in aqueous base or by sodium in liquid ammonia resulted in isolation of unchanged **2**.

Biological distribution of 2 in mice

After administration of **2**, the level of boron-10 in the tumour (Table 2) was observed to be only moderate and this level rapidly decreased with time: the highest tumour boron content was found after 1 h (15.7 μg g^{-1}) and after 6 and 24 h was 8.5 and 4.2 μg g^{-1} respectively. There was a

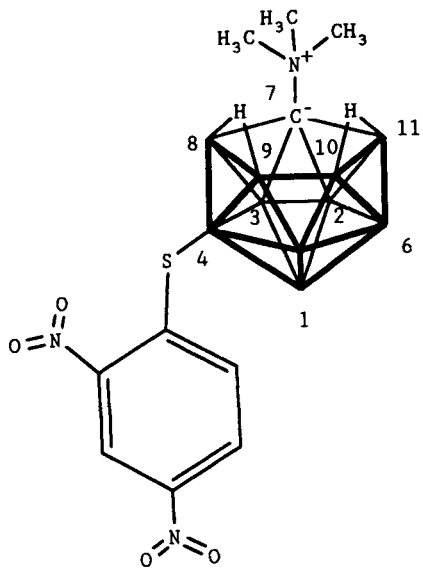

Figure 1 Compound **1**.

slight concentration of boron in the tumour bed, and with time this content increased from 2.4 μg g^{-1} after 1 h to 7.1 μg g^{-1} in 24 h. Simultaneously, the blood boron content was 3, 2 and 1.5 times larger than in the tumour and was 5.7, 3.9 and 3.2 μg (g blood)$^{-1}$ after 1, 6 and 24 h respectively. The clearance of the blood was adequate for the goals of BNCT. The muscles adjacent to the tumour collected small amounts of boron (2.5, 1.1 and 1.3 μg g^{-1} respectively. Like many compounds of boron which had been studied previously, the boron content in the skin was higher than that in the tumour and the boron was slowly eliminated from the skin. After 1 h there was 20.5 μg g^{-1}, and after 6 and 24 h 16 and 7.1 μg g^{-1} respectively.

Table 2 Boron-10 content of **2** in Tissue (μg g^{-1})

	Time after administration of compound		
	1 h	6 h	24 h
Blood	5.7 ± 0.04[a]	3.9 ± 0.11	3.21 ± 0.11
Urine	562 ± 146.3	160 ± 78.9	258.5 ± 126.8
Skin	20.5 ± 4.4	16 ± 3.6	7.1 ± 2.8
Tumour	15.7 ± 0.12	8.5 ± 1.9	4.2 ± 1.5
Tumour bed	2.4 ± 0.2	3.2 ± 0.5	7.1 ± 0.13
Muscle	2.5 ± 0.8	1.1 ± 0.3	1.3 ± 0.2
Liver	6.7 ± 0.4	3.4 ± 0.6	5.9 ± 1.6
Kidneys	26.7 ± 13.8	12.8 ± 5.5	16.5 ± 0.15
Lung	28.4 ± 0.8	29.4 ± 0.6	44.3 ± 0.8

CONCLUSIONS

Compound **2** is easily prepared by the Friedel–Crafts substitution of **1** by 2,4-dinitrophenylsulphenyl chloride. The biodistribution study indicates that **2** locates in neoplastic tissue at concentrations that are appropriate for BNCT. The tumour/blood and tumour/muscle ratios were both greater than 1 at all times, although the tumour/skin ratio was consistently less than 1.

Further evaluation would be required to assess the feasibility of using **2** in BNCT, and it would also be desirable to synthesize water-soluble derivatives of the compound.

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