Labelled Compounds of Interest as Antitumour Agents. Part 4[1]. Deuteration and Tritiation of a Nitroimidazole-Carborane Designed for BNCT.

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Summary

Quenching the anion generated from a 2-(ω-carboranylalkyl)dithiane with ^2H_2O at -78°C and at 0°C introduced deuterium exclusively at C-2 of the carborane. Extension of this model reaction to a bioreductively-targetted carborane allowed the synthesis of 2-[^{2}H]- and 2-[^{3}H]-isotopomers of a nitroimidazole-carborane which is of interest in boron neutron capture therapy (BNCT) of cancer.

Keywords: Nitroimidazole, carborane, boron neutron capture therapy, hypoxia, deuterium oxide, tritiated water.

Introduction

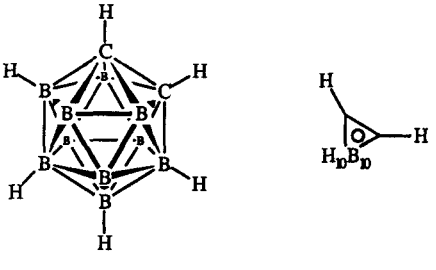

Figure 1. Representations of the structure of carborane. Each B atom has one H atom attached.

Boron neutron capture therapy (BNCT) is of increasing interest in the treatment of various cancers[2-4]. Failures in the early clinical studies were attributed[5-7] to inadequate concentration of the ^{10}B isotope in the tumour tissue or to lack of selectivity of disposition of ^{10}B, leading to damage to normal tissue. To exploit the selective retention[8-12] of 1-substituted 2-nitroimidazoles in hypoxic tumour tissue, we have synthesised[1,13,14] a series of carboranes linked to nitroimidazole. Figure 1 shows the icosahedral structure of carborane. We required radiolabelled material for studies on the biodistribution and selective localisation of the lead compound, the isoxazole-linked nitroimidazole-carborane **3** (Scheme 2). Owing to the complexity of the structure of **3** and the possible consequent complexity of its metabolism, we chose to radiolabel the carborane moiety, since investigation of the biodistribution of the boron was the objective of our study.

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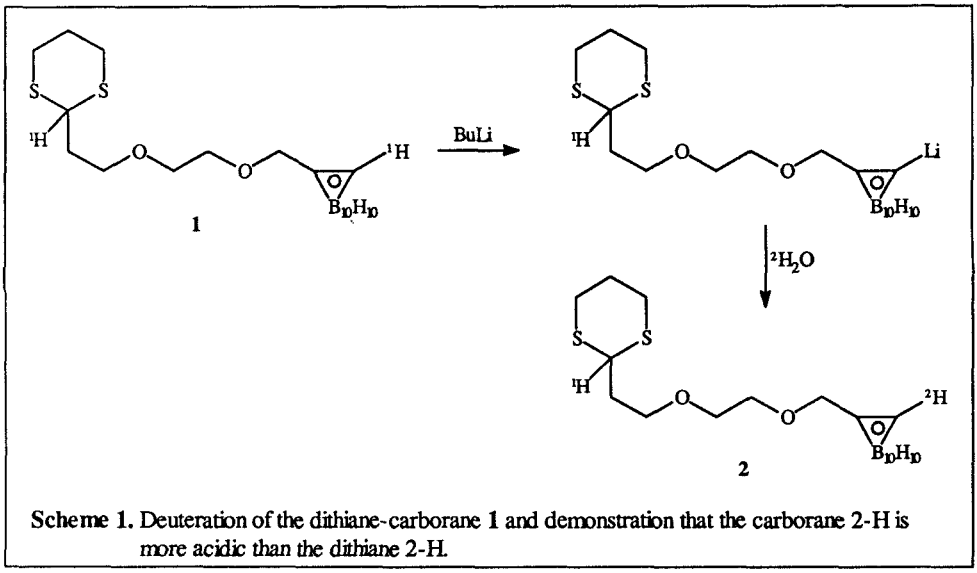
Mizusawa *et al.*[15] have reported tritiation of 1-phenylcarborane by formation of the anion at position 2 of the carborane with butyl lithium at elevated temperature and quenching with tritiated water.

Results and Discussion

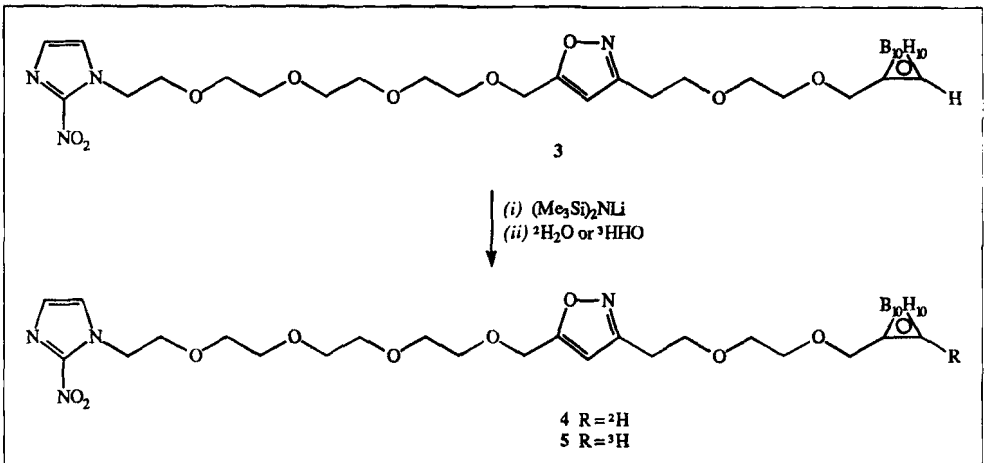
Before embarking on a deprotonation / quenching sequence on the complex molecule **3**, a model study was performed. The dithiane-carborane **1** was synthesised in three steps (alkylation of dithiane anion with 1,2-bis(2-chloroethoxy)ethane, substitution of the chlorine by propyn-3-ol and treatment of the dithiane-alkyne with decaborane(14)) as previously described[16]. Treatment of **1** with one equivalent of butyl lithium at -78°C and quench with deuterium oxide gave exclusively an isotopomer containing only one deuterium (Scheme 1). Deprotonation and reaction at 0°C gave the same material. The vigorous conditions of Mizusawa *et al.*[15] were found to be unnecessary for deprotonation. Since both dithianes[16] and carboranes[15] are known to be deprotonated by butyl lithium, it was important to locate the deuterium by NMR spectroscopy. In the ^{1}H NMR spectrum, the dithiane 2-H was evident as a triplet at δ 4.18 but the broad singlet normally observed at δ 4.1 for the carborane 2-H in deuteriochloroform solvent was absent. The ^{13}C spectrum confirmed the location of the deuterium. The dithiane 2-C appeared as a singlet at δ 43.77 in the broadband ^{1}H-decoupled spectrum, whereas the carborane 2-C gave a 1:1:1 triplet (J = 29 Hz) centred at δ 57.36.



Scheme 1. Deuteration of the dithiane-carborane **1** and demonstration that the carborane 2-H is more acidic than the dithiane 2-H.

Since 2-nitroimidazole has electrophilic reactivity, lithium hexamethyldisilazide was used as a hindered non-nucleophilic base for the deprotonation of the nitroimidazole-carborane **3**. Deprotonation at low temperature, followed by quench with deuterium oxide gave a monodeuterio isotopomer. NMR spectroscopy was again used to locate the deuterium, as deprotonation of the isoxazole at the 4-position is conceivable. The ^{1}H spectrum showed the absence of the carborane 2-H signal at δ 4.1 and the presence of the isoxazole 4-H signal at δ 6.18. The ^{13}C spectrum showed the carborane C-2 signal as the expected 1:1:1 triplet (J = 30 Hz) centred at δ 57.54.

A similar deprotonation of **3**, followed by quench with tritiated water (specific activity 1.8 mCi mmol^{-1}) gave the tritiated isotopomer **5**, although in disappointing chemical yield. Since the deprotonation is shown by the deuterium incorporation model experiment to be quantitative, the specific activity of **5** (248 μCi mmol^{-1}) indicates a significant kinetic tritium isotope effect on the reaction of the carborane anion with the water.



Scheme 2. Deuteration and tritiation of the nitroimidazole-carborane **3**.

This synthesis of the radiolabelled nitroimidazole-carborane **5** provides material for quantitative biodistribution studies to confirm the preliminary biodistribution studies by ^{11}B magnetic resonance spectroscopy *in vivo* of tumour-bearing mice which had received the carborane **3**. These results will be reported elsewhere.

Experimental Section

Tritiated water (1.8 mCi mmol^{-1}, 67 MBq mmol^{-1}) was obtained from ICN; deuterium oxide (99 atom %) was obtained from Aldrich Chemical Co. Ltd. Solutions in dichloromethane were dried with anhydrous magnesium sulphate. Solvents were evaporated under reduced pressure. THF refers to tetrahydrofuran; brine refers to saturated aqueous sodium chloride. NMR spectra were recorded of solutions in $CDCl_3$ with $SiMe_4$ as internal standard using Jeol GX270 and EX400 instruments. IR spectra were recorded on samples as liquid films.

2-[^{2}H]-1-(2-(2-(Dihydro-1,3-dithian-2-yl)ethoxy)ethoxymethyl)-1,2-dicarba*closo*dodecaborane(12) (2). Butyl lithium (2 M in hexanes, 0.25 mL, 0.5 mmol) was added to 1-(2-(2-(dihydro-1,3-dithian-2-yl)ethoxymethyl)ethoxymethyl)-1,2-dicarba*closo*dodecaborane(12) (**1**)[16] (180 mg, 0.5 mmol) in dry THF (5 mL) at -78°C under nitrogen and the mixture was stirred at -78°C for 45 min. Deuterium oxide (0.25 mL) was added and the mixture was warmed to ambient temperature during 30 min. Brine (5 mL) was added and the mixture was extracted with dichloromethane. The

extract was dried and the solvent was evaporated to give 2-[^{2}H]-1-(2-(2-(dihydro-1,3-dithian-2-yl)ethoxy)ethoxymethyl)-1,2-dicarba*closo*dodecaborane(12) (**2**) (180 mg, quant.) as a pale yellow gum: IR ν 2600 (B-H), 2260 (C-D) cm^{-1}; NMR δ_H 1.87 (1 H, m, dithiane 5_{ax}-H), 2.00 (2 H, q, J = 7.0 Hz, dithiane-CH_2), 2.14 (1 H, m, dithiane 5_{eq}-H) 2.3 (10 H, br q J_{B-H} = 160 Hz, $B_{10}H_{10}$), 2.85 (4 H, m, dithiane 4,6-H_4), 3.60 (6 H, m, dithiane-$CH_2CH_2OCH_2CH_2$), 3.96 (2 H, s, carborane-CH_2), 4.18 (1 H, t, J = 7.0 Hz, dithiane 2-H; NMR δ_C 25.74 (CH_2), 30.00 (CH_2), 35.31 (CH_2), 43.77 (dithiane 2-C), 57.36 (t, J_{C-D} = 29 Hz, carborane 2-CD), 67.24 (CH_2), 69.90 (CH_2), 71.18 (CH_2), 72.01 (CH_2), 72.72 (carborane 1-C); mass spectrum (CI) *m/z* ^{10}B/^{11}B isotope cluster centred at 366 (*M* + H).

2-[^{2}H]-1-(2-(2-(5-(2-(2-(2-(2-(2-Nitroimidazol-1-yl)ethoxy)ethoxy)ethoxy)ethoxymethyl)-isoxazol-3-yl)ethyl)ethoxymethyl)-1,2-dicarba*closo*dodecaborane(12) (4). Butyl lithium (2 M in hexanes, 0.05 mL, 0.1 mmol) was added to hexamethyldisilazane (16 mg, 0.1 mmol) in dry THF at -78°C under nitrogen and the mixture was stirred at this temperature for 30 min. The unlabelled nitroimidazole-carborane **3**[16] (60 mg, 0.1 mmol) in dry THF (2 mL) was added and stirring continued at -78°C for 30 min. Deuterium oxide (0.2 mL) was added and the mixture was warmed to ambient temperature during 30 min. Brine (5 mL) was added and the mixture was extracted with dichloromethane. The extract was dried and the solvent was evaporated to give 2-[^{2}H]-1-(2-(2-(5-(2-(2-(2-(2-(2-nitroimidazol-1-yl)ethoxy)ethoxy)ethoxy)ethoxymethyl)isoxazol-3-yl)ethyl)ethoxymethyl)-1,2-dicarba*closo*dodecaborane(12) (**4**) (50 mg, 83%) as a pale yellow gum: IR ν 2590 (B-H), 2270 (C-D) cm^{-1}; NMR δ_H 2.3 (10 H, br q, J_{B-H} = 160 Hz, $B_{10}H_{10}$), 2.92 (2 H, t, J = 6.4 Hz, isoxazole-3-CH_2), 3.60-3.75 (18 H, m, carborane-$CH_2OCH_2CH_2OCH_2$ + $OCH_2CH_2OCH_2CH_2OCH_2CH_2O$), 3.85 (2 H, t, J = 5.0 Hz, imidazole-CH_2CH_2), 3.92 (2 H, s, carborane-CH_2), 4.62 (4 H, m, imidazole-CH_2 + isoxazole-5-CH_2O) 6.18 (1 H, s, isoxazole 4-H), 7.11 (1 H, d, J = 0.8 Hz) and 7.28 (1 H, d, J = 0.8 Hz) (imidazole 4,5-H_2); δ_C 26.75 (CH_2), 29.62 (CH_2), 49.82 (CH_2), 57.54 (t, J_{C-D} = 30 Hz, carborane 2-CD), 61.66 (CH_2), 63.98 (CH_2), 69.95 (CH_2), 69.32 (CH_2), 70.05 (CH_2), 70.31 (CH_2), 70.40 (CH_2), 70.47 (CH_2), 70.58 (CH_2), 71.26 (CH_2), 72.26 (CH_2), 72.52 (carborane 1-C), 103.08 (CH), 127.27 (CH), 127.93 (CH), 161.22 (C_q), 169.03 (C_q); mass spectrum (FAB) *m/z* ^{10}B/^{11}B isotope cluster centred at 616 (*M* + H).

2-[^{3}H]-1-(2-(2-(5-(2-(2-(2-(2-(2-Nitroimidazol-1-yl)ethoxy)ethoxy)ethoxy)ethoxymethyl)-isoxazol-3-yl)ethyl)ethoxymethyl)-1,2-dicarba*closo*dodecaborane(12) (5). The unlabelled nitroimidazole-carborane **3**[16] (60 mg, 0.1 mmol) was deprotonated with lithium hexamethyldisilazide, as for the synthesis of the deuterium isotopomer **4**, Tritiated water (0.025 mL, 25 mCi, 925 MBq) was added and the mixture warmed to ambient temperature during 1 h. Brine (10 mL) was added and the mixture was extracted with dichloromethane. The extract was dried and the solvent was evaporated. Column chromatography (silica gel, ethyl acetate / methanol 10:1) gave 2-[^{3}H]-1-(2-(2-(5-(2-(2-(2-(2-(2-nitroimidazol-1-yl)ethoxy)ethoxy)ethoxy)ethoxymethyl)isoxazol-3-yl)ethyl)ethoxymethyl)-1,2-dicarba*closo*dodecaborane(12) (**5**) (7.8 mg, 13% chemical yield, 3.15 μCi, 248 μCi mmol^{-1}, 9.18 MBq mmol^{-1}) as a colourless gum, identical by t.l.c. with **3** and **4**.

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