Synthesis of Carboranes Containing Nucleoside Bases. Unexpectedly High Cytostatic and Cytocidal Toxicity towards Cancer Cells

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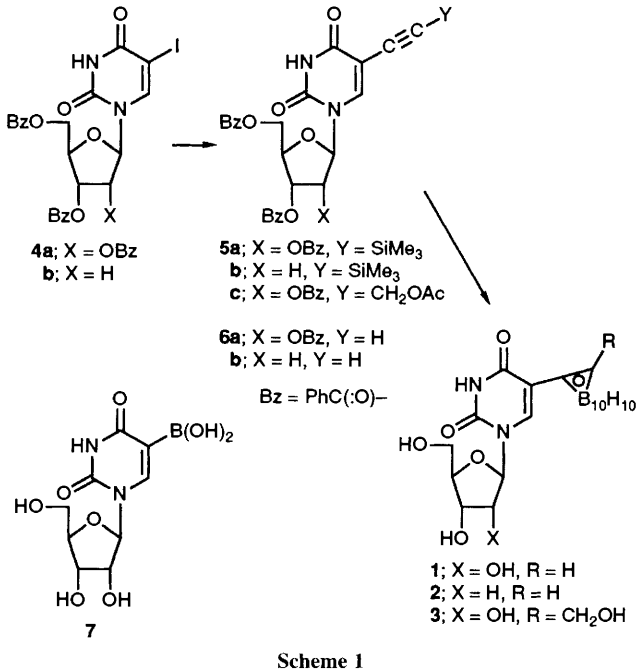
5-Carboranyluridine **1**, 5-carboranyldeoxyuridine **2** and 5-hydroxymethylcarboranyluridine **3** have been prepared; they show unexpectedly high cytotoxicity towards cancer cells, with IC_{50} values being *ca.* 10^{-5} mol dm^{-3}.

As ^{10}B carriers for boron neutron capture therapy, carboranes attached to nucleoside bases are promising.[1] Primarily, most attention has been paid to developing new ^{10}B carriers which exhibit high tumour/normal cell ratios.[1] We now report that certain carboranes attached to uridine exhibit unexpectedly high cytotoxity towards cancer cells and may be potentially useful not only as anticancer drugs but also as ^{10}B carriers, since a synergistic effect may enhance the killing activity towards cancer cells.

5-Carboranyluridine **1** (5-B_{10}U), 5-carboranyldeoxyuridine **2** (5-B_{10}UD) and 5-hydroxymethylcarboranyluridine **3** (5-H-B_{10}U) were prepared from the corresponding acetylenes as shown in Scheme 1. The iodouridines[2] **4** were converted to the corresponding acetylenic derivatives **5** by treatment with the acetylenes in the presence of 10 mol % $PdCl_2$–$2PPh_3$ and CuI in tetrahydrofuran (THF)–Et_3N. The preparation of **5a** is representative. To a THF solution (30 ml) of **4a** (3 mmol), $PdCl_2$ (0.3 mmol), PPh_3 (0.6 mmol) and CuI (0.6 mmol) were added triethylamine (1.2 ml) and trimethylsilylacetylene (6 mmol), and the resulting mixture was stirred at 40 °C for 2 h under an Ar atmosphere. The solvent was removed under vacuum. The crude product was purified by silica gel column chromatography using benzene–AcOEt (5 : 1) as eluent, to give **5a** in 75% yield (2.25 mmol): ^1H NMR ($CDCl_3$) δ 8.24 (s, 1H), 7.74 (s, 1H), 7.32–8.14 (m, 15H), 6.30 (d, *J* 5.9 Hz, 1H), 5.86 (dd, *J* 5.9, 3.7 Hz, 1H), 5.74 (dd, *J* 5.9, 5.9 Hz, 1H), 4.76 (m, 3H) and 0.18 m (m, 9H). A similar procedure gave **5b** in 64% yield, and **5c** in 62% yield.



Scheme 1

Treatment of **5a** with Bu_4NF in THF at room temperature gave **6a** in nearly quantitative yield. However, the desilylation of **5b** needed much stronger conditions; heating of **5b** with 2 equiv. of Et_4NBr–KF in MeCN for 4 h gave **6b**. To a toluene solution (10 ml) of **6a** (0.25 mmol) and decaborane (0.3 mmol) was added propionitrile (5 mmol) under Ar, and the mixture was refluxed for 18 h. The solvents were removed at room temperature under vacuum. The product was purified by silica gel column chromatography using CH_2Cl_2–EtOH (100 : 1) as eluent, to give 2′,3′,5′-tri-*O*-benzoyl-5-carboranyluridine (the benzoyl protected form of **1**) (117 mg) in 67% yield; ^1H NMR ($CDCl_3$) δ 8.50 (br s, 1H), 7.76 (s, 1H), 7.34–8.14 (m, 15H), 6.19 (d, *J* 5.9, Hz, 1H), 5.93 (dd, *J* 5.5, 4.0 Hz, 1H), 5.76 (dd, *J* 5.5, 5.5 Hz, 1H), 5.61 (br s, 1H), 4.81 (dd, *J* 11.4, 3.7 Hz, 1H), 4.77 (m, 2H), 4.68 (dd, *J* 11.4, 3.7 Hz, 1H) and 1.0–3.0 (br). The benzoyl groups were removed with NaOMe–MeOH, giving **1** in 67% yield from **5a**. A similar procedure produced **2** in 55% yield from **5b**. The decaboration of **5c** followed by debenzoylation afforded **3** in 40% yield. The choice of Lewis base in the decaboration step was important to obtain high chemical yields;[3] the use of propionitrile gave the best result for the preparation of **1** and **2**, whereas the use of Et_2S produced a higher yield in the case of **3**.

The growth inhibition of several mouse tumour cell lines with the boron compounds **1–3** and **7** is summarized in Table 1, compound **7** (5BU) having been prepared by the known

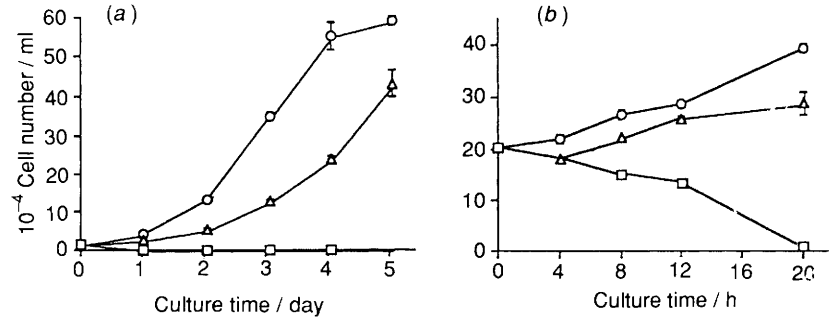


Fig. 1 Kinetics of the growth inhibition of P388 cells by 5-B_{10}U **1** (*a*) in a 5 day-culture and (*b*) in a 20 h culture. For general details see Table 1. P388 cells (2×10^4 ml^{-1}) were cultured with two doses of 5-B_{10}U for 5 days and were enumerated by a Coulter counter; (*b*) P388 cells (20×10^4 ml^{-1}) were cultured with two doses of 5-B_{10}U for 20 h and were enumerated by the Trypan Blue exclusion method. Values are represented as mean ± S.E. ($n = 4$). (○) Control; (△) 5-B_{10}U (3×10^{-5} mol dm^{-3}); (□) 5-B_{10}U (1×10^{-4} mol dm^{-3}).

Table 1 Growth inhibition of several mouse tumour cell lines with the boron compounds **1–3** and **7**[a]

	10^5 IC_{50}/mol dm^{-3}			
Cell line	5-B_{10}U **1**	5-B_{10}UD **2**	5-B_{10}U **3**	5-B_1U **7**
P-388	2.20	1.99	5.60	>30
L1210	3.98	2.98	>30	>30
B-16	3.16	2.82	10	>30
MBL-2	3.09	2.23	11.9	>30
MethA	3.98	3.16	>30	>30

[a] The boron compounds were dissolved in 75% ethanol in phosphate-buffered saline as a stock solution (9×10^{-2} mol dm^{-3}) and stored at −20 °C for at most two months. Before use, the stock solution was diluted in RPMI1640 medium. The final concentration of the ethanol diluent was 0.085% and this ethanol concentration had no effect on the growth of any of the tumour cell lines tested. Precipitates of the compounds were not observed in culture under microscopy. Murine leukaemia cells P388, L1210, MBL-2, melanoma cells B16, and sarcoma cells MethA were suspended in RPMI1640 medium supplemented with 5% foetal calf serum. Tumour cells at 2×10^4 ml^{-1} were cultured with different doses of each compound in 24-well tissue culture plates in a CO_2-incubator at 37 °C for 3 days. Cells were enumerated using a Coulter counter and results are presented as the concentration of agent that resulted in 50% of the cell number of untreated cultures.

procedure for comparison.[1b] 5-B_{10}U and 5-B_{10}UD exhibited high growth inhibition towards all the cell lines tested, with IC_{50} values of the order of 10^{-5} mol dm^{-3}. Time curves of growth inhibition of P388 cells by 5-B_{10}U are shown in Fig. 1. There was no growth of P388 cells in the presence of 1×10^{-4} mol dm^{-3} 5-B_{10}U during the 5 days observed [Fig. 1(*a*)] and the short time assay with a large number of cells showed that 5-B_{10}U at a dose of 3×10^{-5} mol dm^{-3} apparently inhibited cell proliferation and at 1×10^{-4} mol dm^{-3} completely killed P388 cells after 20 h (Fig. 1(*b*)]. It is interesting that 5-B_{10}U, a uracil derivative, exhibited a potent killing effect on tumour cells, whereas 5-fluorouracil[4] (5-FU) is a cytostatic agent, inhibiting DNA/RNA synthesis. These findings indicate that novel boron derivatives such as 5-B_{10}U could serve as neutron capture agents having direct cytotoxicity towards tumour cells, if the cells selectively take up the ^{10}B carriers.

We acknowledge financial support from Nagase Science and Technology Foundation.

Received, 3rd July 1991; Com. 1/03355G

References

1 (*a*) A. K. M. Anisuzzaman, F. Alam and A. H. Soloway, *Polyhedron*, 1990, **9**, 891. For nucleoside bases containing boronic acid; (*b*) R. F. Schinazi and W. H. Prusoff, *J. Org. Chem.*, 1985, **50**, 841; (*c*) Y. Yamamoto, T. Seko, F. G. Rong and H. Nemoto, *Tetrahedron Lett.*, 1989, **30**, 7191; Y. Yamamoto, *Pure Appl. Chem.*, 1991, **63**, 423.

2 M. J. Robins, P. J. Barr and J. Giziewiciz, *Can. J. Chem.*, 1982, **60**, 554.

3 It is well known that the reaction of alkynes with decaborane gives 1,2-dicarba-*closo*-dodecaborane: T. L. Heying, J. W. Ager, Jr., S. L. Clark, D. J. Mangold, H. L. Goldstein, M. Hillman, R. J. Polak and J. W. Szymanski, *Inorg. Chem.*, 1963, **2**, 1089; M. M. Flein, J. Bobinski, N. Maves, N. Schwartz and M. Cohen, *Inorg. Chem.*, 1963, **2**, 1111. However, this procedure has not been used for the preparation of carboranes bonded to nucleoside bases. For another synthesis see ref. 1(*a*).

4 C. Heidelberger, N. K. Chaudhuri, P. Danneberg, D. Mooren, L. Griesbach, R. Duschinsky, R. J. Schinitzer, E. Pleven and J. Scheiner, *Nature*, 1957, **179**, 663.