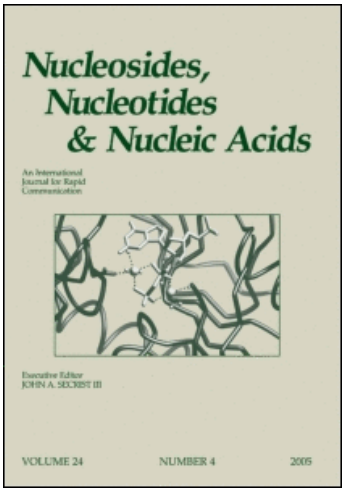

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SYNTHESIS AND BIOLOGICAL EVALUATION OF BORONATED NUCLEOSIDES FOR BORON NEUTRON CAPTURE THERAPY (BNCT) OF CANCER

W. Tjarks,[1,*] J. Wang,[2] S. Chandra,[3] W. Ji,[1] J. Zhuo,[1] A. J. Lunato,[1] C. Boyer,[4] Q. Li,[4] E. V. Usova,[2] S. Eriksson,[2] G. H. Morrison,[3] and G. Y. Cosquer[1]

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ABSTRACT

Several N-3 substituted carboranyl Thd analogs were synthesized. These agents as well as some non-boronated nucleosides were evaluated in phosphoryl transfer assays with recombinant human TK1 and TK2. For some carboranyl thymidine analogs, TK1 phosphorylation rates approached 38% that of thymidine. Their *in vitro* cytotoxicty appeared to correlate with the TK1 levels in the tested cells. In some cases increased uptake in tumor cell nuclei compared with the surrounding cytoplasm was detected *in vitro*.

INTRODUCTION

The development of ^{10}B-containing compounds for the treatment of cancer by boron neutron capture therapy (BNCT) requires the synthesis and evaluation of

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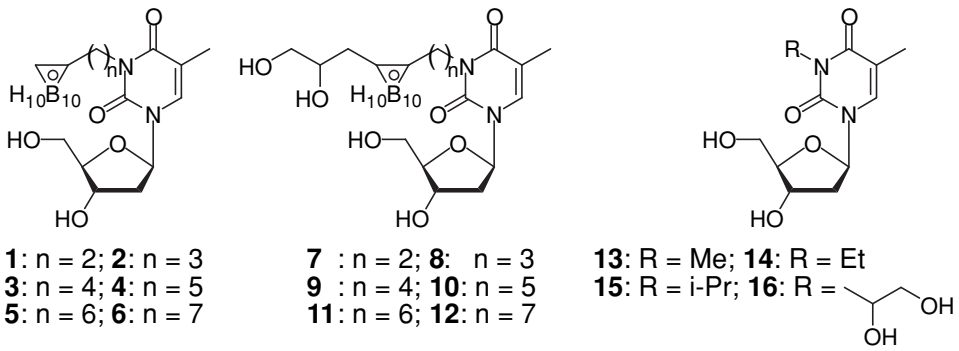

Figure 1. N-3 substituted Thd analogs.

non-toxic agents that selectively target malignant cells in contrast with adjacent normal tissue and are retained intracellulary. The boron content localized within the tumor cells is activated by an external flux of thermal neutrons at the time of treatment generating highly destructive $^{4}He^{2+}$ and $^{7}Li^{3+}$ ions by a neutron capture reaction [$^{10}B(n,\alpha)^{7}Li$]. These ions have path lengths of <10 μm in biological tissue. Because of this limited range, the caused lethal damage is largely restricted to the tumor (1).

Boronated nucleosides have significant potential as BNCT agents. They may be converted to the corresponding 5′-monophosphates by phosphorylating enzymes and consequently entrapped in malignant cells due to the acquired charged phosphate group. Minimum requirement for selective accumulation and persistence of a boronated nucleoside in malignant versus benign cells is that phosphorylating enzymes with substrate specificity for such a nucleoside have significantly elevated activity levels in malignant cells. This is in particular the case for cytosolic Thd kinase (TK1). Therefore, this enzyme appears to be a primary target of boronated nucleosides for BNCT (2).

Previously, we have evaluated several C-5 substituted carboranyl 2′-deoxyuridine analogs and compounds **3–6** (Fig. 1) in phosphoryl transfer assays with human TK1 and human mitochondrial Thd kinase (TK2) (3). The latter kinase has moderate activity levels both in proliferating and non-proliferating cells and its substrate specificity is significantly different from that of TK1 (2). The obtained results indicated that only the N-3 substituted 2′-deoxyuridines (**3–6**) were good substrates for TK1, presumably the therapeutically relevant Thd kinase isoform. We have now prepared an extended series of N-3 substituted Thds (Fig. 1, **1–16**) for further evaluation in phosphoryl transfer assays as well as *in vitro* uptake-, subcellular distribution-, and toxicity studies.

RESULTS AND DISCUSSION

Compounds **1–6** (3), **15** (4), and **16** (5) were synthesized as described previously. The synthesis of compounds **7–12** is described at the example of

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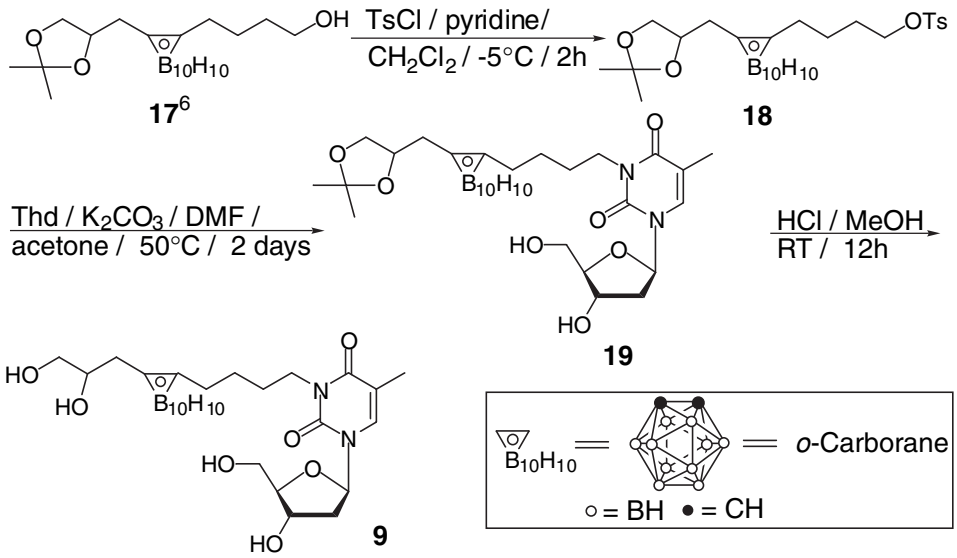

Scheme 1.

compound **9** (Scheme 1). All new compounds (**1**, **2**, **7**–**12**) were analyzed by ^{1}H-NMR, ^{13}C-NMR, and high resolution MS.

Phosphoryl transfer assay were carried out as described previously (3). N-3 substituted Thd derivatives **1**–**15** were good substrates for TK1 while they were, if at all, poor substrates for TK2 (Table 1). Only a small methyl group at N-3 was also tolerated by the latter kinase (**13**). A polar dihydroxypropyl group directly attached to N-3 (**16**) was not accepted by TK1. Compounds **7**–**12**, containing a dihydroxypropyl group at the second carbon atom of the carborane cluster, appeared to have better TK1 substrate characteristics than their counterparts **1**–**6** lacking this group. No obvious influence of the tether length on the TK1 substrate characteristics of **7**–**12** could be observed. In case of **1**–**6**, TK1 activity levels

Table 1. Phosphorylation of Compounds **1**–**16** by Recombinant TK1 and TK2 Relative to Thd

Comp.	TK1	TK2	Comp.	TK1	TK2	Comp.	TK1	TK2
Thd	1	1	Thd	1	1	Thd	1	1
1	0.20	–	**7**	0.38	<0.01	**13**	0.43	0.05
2	0.17	–	**8**	0.33	<0.01	**14**	0.43	<0.01
3	0.21	–	**9**	0.38	<0.01	**15**	0.17	–
4	0.05	–	**10**	0.28	<0.01	**16**	–	–
5	0.08	–	**11**	0.35	<0.01			
6	0.09	–	**12**	0.13	<0.01			

(–): monophosphate product not detectable; ATP and substrate concentrations were 100 μM; the DMSO concentration in all assays was 0.0625%

deteriorated from **3** to **4** under the applied experimental conditions. Activity levels observed for **13** and **14** indicate that smaller boron moieties than the carborane cage may be considered as N-3 substituents. In conclusion, more detailed studies are necessary to define the criteria, (e.g. hydrophilicity, tether length, and structural features) for optimal TK1 substrate characteristics of N-3 substituted Thds.

In vitro ion microscopy (7) studies of compound **3** using T98G glioblastoma cells resulted in intracellular boron concentrations ranging from 300 to 590 ppm with a cell/medium concentration ratio of ~75 to 1. Most cells showed a homogeneous boron distribution throughout all cellular compartments but for some cells slightly higher boron concentrations could be observed in chromosomal regions. Compound **8** appeared to be about 4.8 times more toxic in HT1080/TK^+ human fibrosarcoma cells transfected with human TK1 than in ccd18co normal colon epithelium cells in the crystal violet cytotoxicity assay (8). Since the TK1 activity is approximately 5 times higher in the former cell line, the cytotoxicity observed for compounds **8** may correlate with its TK1 substrate characteristics.

ACKNOWLEDGMENTS

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