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Synthesis and Biological Evaluation of 3′-Carboranyl Thymidine Analogues

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Abstract—Boron neutron capture therapy (BNCT) is a chemoradio-therapeutic method for the treatment of cancer. It depends on the selective targeting of tumor cells by boron-containing compounds. One category of BNCT agents with potential to selectively target tumor cells may be thymidine derivatives substituted at the 3′-position with appropriate boron moieties. Thus, several thymidine analogues were synthesized with a carborane cluster bound to the 3′-position either through an ether or a carbon linkage. The latter are the first reported carborane-containing nucleosides in which the carboranyl entity is directly linked to the carbohydrate portion of the nucleoside by a carbon–carbon bond. Low but significant phosphorylation rates in the range of 0.18% that of thymidine were observed for the carbon-linked 3′-carboranyl thymidine analogues in phosphoryl transfer assays using recombinant preparations of thymidine kinases 1 (TK1) and thymidine kinases 2 (TK2). Some of the ether-linked 3′-carboranyl thymidine analogues appeared to be slightly unstable under acidic as well as phosphoryl transfer assay conditions and were, if at all, poor substrates for TK1.

Boron neutron capture therapy (BNCT) is a chemoradio-therapeutic method for the treatment of cancer that is based on the capability of ^{10}B to undergo fission after the capture of external low energy neutrons generating highly cell toxic $^{4}He^{2+}$ and $^{7}Li^{3+}$ ions. Cells that are exposed to these high linear energy transfer (LET) fission products will be killed with high probability. However, both ions can travel only approximately one cell-diameter thus damaging only cells in which they are generated. The prerequisite for successful BNCT is therefore the selective targeting of tumor cells by boron-containing compounds.[1,2]

Boronated nucleosides may be promising candidates for the selective targeting of tumor cells for BNCT because of their potential metabolic fate. For example, the key step for the intracellular entrapment of a boronated thymidine derivative may be the phosphorylation by cytosolic thymidine kinase (TK1) to the corresponding 5′-monophosphate. This would occur primarily in proliferating tumor cells since the expression of TK1 is tightly regulated during the cell cycle and the active enzyme is found only in S-phase cells.[3] TK1 is therefore widely distributed and expressed in all proliferating neoplastic cells but it is virtually absent in all non-proliferating normal tissue.[3]

For many years, TK1 was considered to have the most stringent substrate specificity among all nucleoside kinases allowing only phosphorylation of native thymidine/2′-deoxyuridine and, to a limited extent, analogues with minor modifications at either the 5-position or the 3-position.[3] Recently, however, we have evaluated several thymidine and 2′-deoxyuridine analogues substituted with bulky carboranylalkyl groups at N-3 and C-5, respectively, in phosphoryl transfer assays with recombinant human TK1. The obtained results indicated that 3-substituted carboranyl thymidines but not 5-substituted carboranyl 2′-deoxyuridines were good substrates for TK1.[4]

In order to determine to which degree the TK1 substrate characteristic of thymidine is preserved when its 3′-position is substituted with a bulky carborane cluster,

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we have synthesized several 3′-substituted carboranyl thymidine analogues with varying physicochemical properties and dimensions and evaluated their potential as substrates for TK1 in phosphoryl transfer assays using recombinant enzyme preparations. The substrate specificities of TK1 and mitochondrial thymidine kinase (TK2) differ significantly.[3] Therefore, we have also determined the phosphorylation rates of the synthesized boronated nucleoside analogues with recombinant preparations of the latter enzyme. A thymidine derivative with a cyanoborane substituent at the 3′-position (Fig. 1) has been described previously by Burnham et al.[5] and its phosphorylation rates with TK1 and TK2 were also determined.

Selective *O*-alkylation of the 3′-position of compound **2**[6] using propargyl bromide and sodium hydride in refluxing THF under N_2 overnight gave compound **3** in 89% yield. Compound **4** was obtained by refluxing **3** in toluene for 6 h with a decaborane–diethyl sulfide complex[1] that was formed in situ. Toluene was evaporated and the remaining residue was stirred in methanol containing 80% aqueous acetic acid for 30 min at room temperature to remove the monomethoxytrityl (mMTr) group. Compound **4** was obtained in 36% overall yield. Base promoted degradation[7] of the *closo*-carborane cage of compound **4** using pyrrolidine in dichloromethane at 0 °C for 1 h yielded compound **5** which was precipitated from aqueous solution as the tetraphenylphosphonium salt in 76% yield. For phosphoryl transfer assays, compound **5** was converted to the sodium salt using Dowex 50X8–100 (Na^+ form) as described previously.[7] Target compound **6** was obtained in 76% yield by selective 3′-*O*-alkylation of **2** using iodopropyl-*p*-carborane[8] followed by acidic hydrolysis of the 5′-mMTr-protective group. Reactions conditions applied for the synthesis of **6** were identical to those applied for the synthesis of **3** and **4** (Scheme 1).

The 3′-ether linkage in **4** and **5**, but not **6**, appeared to be slightly unstable during reactions, workup, and phosphoryl transfer assays since the appearance of small quantities of thymidine/thymidine-5′-monophosphate could be detected during these processes. Consequently the syntheses of compounds **12**, **13**, and **14** was undertaken to eliminate the risk of hydrolysis by linking the carboranyl entity directly to the ribose moiety through a carbon–carbon bond (Scheme 2). Compounds **7** and **10** were synthesized via multistep syntheses as described by Sanghvi et al.[10] and Fandor et al.,[11] respectively. Compound **7** was added to 6-fold excess of the Wittig reagent ($Ph_3P{=}CBr_2$) in dichloromethane and reacted for 24 h in accordance to a procedure described by Lebreton et al.[12] to give the dibromo-olefin **8** in 74% yield. Compound **8** was refluxed for 12 h in the presence of potassium hydroxide in isopropyl alcohol/water (4:1) to give 3′-ethynylthymidine (**9**) in 39% yield. Under these reaction conditions, the 5′-TBDPS-protective group and both bromine atoms of **8** were removed and/or eliminated. The analytical data[13] of **9** were identical to those of the 3′-ethynylthymidine prepared previously by Sahlberg[14] using a different synthetic route. The 5′-hydroxyl group of compounds **9** and **10** were then protected by an acetyl group using acetic anhydride in pyridine/dichloromethane (1/10) for 12 h to provide compounds **11** and **12** in 85 and 79% yield, respectively. Compound **11** and **12** were refluxed with a decaborane-acetonitrile complex[7] in benzene for 4 h. Benzene was evaporated and the residues stirred at room temperature in methanol/H_2O in the presence of K_2CO_3 for 4 h to yield both compounds **13** and **14** in ~10% overall. It was difficult to purify compound **14** by column chromatography because the reaction mixture contained a minor amount of compound **10** and the R_f values of both compounds were similar in various solvent systems [e.g., **10** = 0.22; **14** = 0.28, **9** = 0.20; **13** = 0.40 (acetonitrile:toluene = 1:1)]. When compound **12** was reacted with a decaborane–acetonitrile complex and the subsequent the removal of the acetyl group was carried for 12 h under reflux condition, the *nido*-carboranyl thymidine derivative **15** was obtained in 44% overall yield.

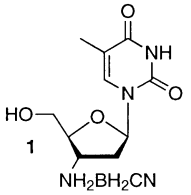
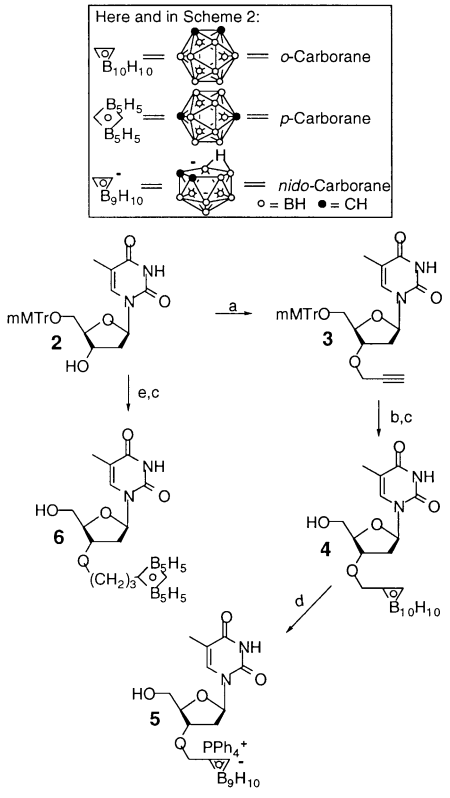
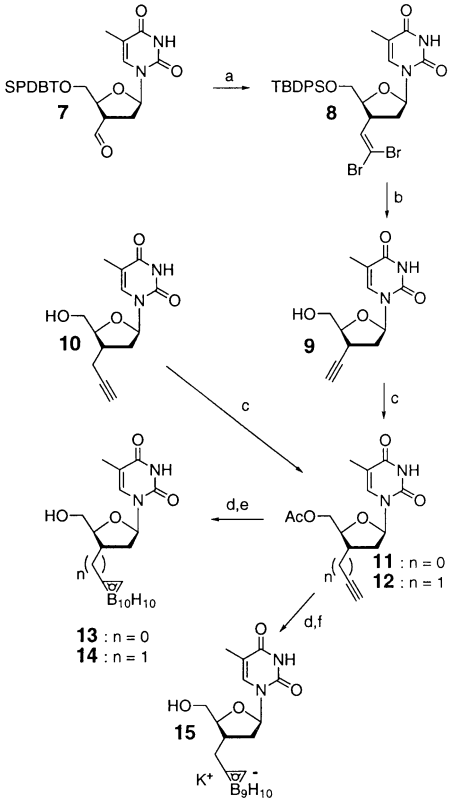


Figure 1.



Scheme 1. (a) NaH, 80% propargylbromide, THF, reflux, 12 h, 89%; (b) decaborane (CAUTION),[9] diethyl sulfide, toluene, reflux, 6 h; (c) 80% AcOH, MeOH, 40 °C, 4 h; (d) pyrrolidine, CH_2Cl_2, 0 °C, 1 h, 76%; (e) NaH, iodopropyl-*p*-carborane, reflux, 12 h.

Phosphoryl transfer assays using recombinant human TK1 and TK2 were carried out as reported previously[4] with **1**, **4**–**6**, and **13**–**15** (Table 1). Significant phosphorylation rates with TK1 could be observed for compounds **1** and **13** indicating that the 3′-position of thymidine is a preferred site for structural modifications that are tolerated by this important kinase. Significant phosphorylation was also observed for compound **4**. Unfortunately, the already indicated instability of the ether linkage in **4** prevented an exact quantification of its TK1 phosphorylation rate. Compound **5**, the negatively charged *nido*-counterpart of **4**, and compound **6**, containing a slightly larger and more lipophilic *para*-carboranylpropylether at C-3′ instead of the *ortho*-carboranylmethylether in **4**, were apparently not substrates for TK1. Compound **13** was clearly the best substrate and the activity decreased drastically with one carbon extension (**14**) or with the negatively charged *nido* form (**15**) of the carboranyl substitution. This clearly demonstrates the narrow window for any type of modification that TK1 can accept. The TK2 phosphorylation rates for **13** and **14** were slightly higher than those observed previously for 3-carboranyl thymidine analogues.[4]



Scheme 2. (a) $Ph_3P{=}CBr_2$, CH_2Cl_2, 25 °C, 12 h, 74%; (b) KOH, *i*-PrOH/H_2O (4/1), reflux, 12 h, 39%; (c) Ac_2O, pyridine/CH_2Cl_2 (1/10), 25 °C,12 h; (d) decaborane (CAUTION),[9] CH_3CN, benzene, reflux, 4 h; (e) K_2CO_3, methanol/H_2O, 25 °C, 1 h, ~10%; (f) (only for **12**) K_2CO_3, methanol/H_2O, reflux, 12 h.

Table 1. Phosphorylation of 3′-carboranyl thymidine analogues

Compd	TK1	TK2
Thd	100	100
1	5.6 (±0.3)[a]	<0.1
4	+	nd
5	<0.1	nd
6	<0.1	nd
13	8.1 (±0.3)	1.2 (±0.3)
14	0.8 (±0.3)	0.5 (±0.2)
15	0.1 (±0.02)	<0.1

Detailed experimental conditions for the phosphoryl transfer assays have been described previously.[4] Obtained values for thymidine (Thd) were set to 100. The final DMSO assay concentration was 1%, substrate and ATP concentrations were 100 μM.

[a]Values are means of three experiments, standard deviation is given in parentheses. In the cases of **4**, **5**, and **6** only one measurement was carried out. nd, Not determined; +, a phosphorylation product could be detected but it could not be quantified accurately because **4** was unstable under the assay conditions.

In conclusion, we have synthesized six new carboranyl thymidine derivatives. Compounds **4** and **5** appeared to be hydrolytically cleaved to some extend at the 3′-ether linkage during reactions, work-up, and phosphoryl transfer assays. This prompted the synthesis of compounds **13**, **14**, and **15** in which the carboranyl entities are linked to the ribose moiety through carbon–carbon bonds. These compounds are the first reported carborane-containing nucleosides with a carbon linkage between the carborane cluster and the carbohydrate portion of the nucleosides. The results of TK1 phosphoryl transfer assays with compounds **1**, **4**–**6**, and **13**–**15** indicated that this enzyme, presumably the therapeutically relevant thymidine kinase isoform, can tolerate bulky and highly lipophilic carboranyl substituents at the 3′-position of thymidine. Our results suggest that the synthesis and evaluation of 3′-substituted boronated thymidines with further improved steric and physico-chemical properties as potential delivery agents for clinical BNCT is warranted.

Acknowledgements

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13. **4**: ^{1}H NMR (300 MHz, CD_3OD) 1.87 (s, 3H), 2.24–2.26 (m, 1H), 2.49–2.51 (m, 1H), 3.73 (m, 2H), 3.96–4.08 (m, 3H), 4.21 (m, 1H), 4.62 (s, br, 1H), 6.19 (dd, 1H), 7.75 (s, 1H); ^{13}C NMR (75 MHz, CD_3OD) δ 12.5, 38.0, 61.1, 63.0, 71.5, 74.7, 82.2, 86.2, 111.8, 138.0, 152.4, 166.3. *m/z* (HR-FAB$^-$) 397.2753, calcd for $C_{13}H_{26}B_{10}N_2O_5(M-H)^-$ 397.2774. **5**: ^{1}H NMR (300 MHz, CD_3OD) δ −2.80 (s, br, 1H), 1.87 (s, 3H), 2.14–2.35 (m, 2H), 3.75 (m, 2H), 3.96–4.02 (m, 3H), 4.19 (m, 1H), 6.21 (dd, 1H), 7.48–7.95 (m, 21H); ^{13}C NMR (75 MHz, CD_3OD) δ 12.5, 38.5, 63.5, 78.1, 79.2, 86.3, 86.7, 111.6, 118.5, 120.3, 131.6, 136.0, 136.7, 138.3, 152.4, 166.4. *m/z* (HR-FAB$^-$) 388.2736, calcd for $C_{13}H_{26}B_9N_2O_5$ $(M)^-$ 388.2726. **6**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.40–1.48 (m, 2H), 1.62–1.71 (m, 2H), 1.91 (s, 3H), 2.25–2.31 (m, 2H), 2.64 (s, br, 1H), 3.21–3.29 (m, 2H), 3.69–3.76 (m, 1H), 3.88–3.95 (m, 1H), 3.99 (q, 1H), 4.06 (m, 1H), 6.03 (dd, 1H), 7.32 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 12.51, 29.42, 35.54, 36.92, 58.09, 62.84, 68.46, 79.05, 84.98, 87.46, 111.09, 137.04, 150.16, 163.40; *m/z* (HR-FAB$^-$) 425.3100, calcd for $C_{15}H_{30}B_{10}N_2O_5$ $(M-H)^-$ 425.3088; **8**: ^{1}H NMR (250 MHz, $CDCl_3$) 1.08 (s, br, 9H), 1.60 (d, 3H), 2.26–2.33 (m, 2H), 3.35–3.41 (m, 1H), 3.72–3.86 (m, 2H), 4.00–4.07 (m, 1H), 6.15–6.20 (t, 1H), 6.32–6.35 (d, 1H), 7.34–7.69 (m, 11H), 9.21 (s, 1H); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 12.19, 19.40, 27.02, 37.70, 42.18, 63.28, 84.32, 84.53, 92.28, 111.18, 127.93, 128.70, 130.02, 130.08, 132.50, 132.91, 135.06, 135.40, 135.56, 136.76, 150.38, 163.61; *m/z* (HRESI) 671.0345, calcd for $C_{28}H_{32}N_2O_4Br_2Si$ $(M+Na)^+$ 671.0378 **11**: ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.90–1.91 (d, 3H), 2.11 (s, 3H), 2.19 (s, 1H), 2.41–2.54 (m, 2H), 2.86–3.12 (m, 1H), 4.11–4.16 (m, 1H), 4.30–4.44 (m, 2H), 6.08–6.12 (t, 1H), 7.28 (s, 1H); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 12.48, 20.83, 29.80, 39.17, 62.19, 72.02, 80.33, 82.78, 85.49, 110.80, 135.21, 150.18, 163.79, 170.30; *m/z* (HREI) 292.1053, calcd for $C_{14}H_{16}N_2O_5$ $(M)^+$ 292.1059. **12**: ^{1}H NMR (250 MHz, CD_3OD) δ 1.84–1.85 (d, 3H), 2.04 (s, 3H), 2.09 (s, 1H), 2.25–2.37 (m, 5H), 3.95–4.10 (m, 1H), 4.26–4.39 (m, 2H), 5.99–6.04 (t, 1H), 7.51–7.56 (q, 1H); ^{13}C NMR (62.5 MHz, CD_3OD) δ 12.57, 20.77, 20.98, 30.67, 38.38, 65.17, 71.87, 81.76, 83.65, 86.53, 111.29, 137.73, 152.27, 166.42, 172.36; *m/z* (HRESI) 307.1272, calcd for $C_{15}H_{18}N_2O_5$ $(M+H)^+$ 307.1294. **13**: ^{1}H NMR (250 MHz, CD_3OD) δ 1.78 (s, br, 3H), 2.34–2.46 (m, 2H), 3.16–3.25 (m, 1H), 3.72–3.85 (m, 2H), 3.98–4.04 (m, 1H), 4.62 (s, 1H), 5.96 (t, 1H), 7.84 (q, 1H); ^{13}C NMR (62.5 MHz, CD_3OD) δ 12.4, 41.60, 43.38, 61.95, 64.16, 77.28, 83.48, 86.42, 111.02, 138.19, 152.27, 166.47; *m/z* (HRESI) 391.2–656, calcd for $C_{12}H_{24}N_2O_4B_{10}$ $(M+Na)^+$ 391.2643. **14**: ^{1}H NMR (250 MHz, CD_3OD) δ 1.83 (s, br, 3H), 2.08–2.18 (m, 1H), 2.29–2.50 (m, 5H), 3.54–3.82 (m, 3H), 4.53 (s, 1H), 5.98 (t, 1H), 7.81 (q, 1H); ^{13}C NMR (62.5 MHz, CD_3OD) δ 12.48, 38.63, 40.26, 41.52, 61.45, 64.29, 75.90, 86.50, 111.19, 138.27, 152.31, 166.49; *m/z* (HRESI) 405.2837, calcd for $C_{13}H_{26}N_2O_4B_{10}$ $(M+Na)^+$ 405.2801. **15**: ^{1}H NMR (250 MHz, CD_3OD) 1.59–1.62 (m, 2H), 1.83 (q, br, 1H), 1.83–1.94 (m, 1H), 1.96–2.56 (m, 5H), 3.56–3.82 (m, 3H), 5.95–5.99 (t, 1H), 7.90–7.93 (q, 1H); ^{13}C NMR (62.5 MHz, CD_3OD) δ 12.53, 40.27, 41.17, 43.29, 61.54, 62.68, 86.49, 110.86, 138.51, 152.47, 166.60; *m/z* (HRESI) 372.2805, calcd for $C_{13}H_{26}N_2O_4B_9$ $(M)^-$ 372.2777.
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