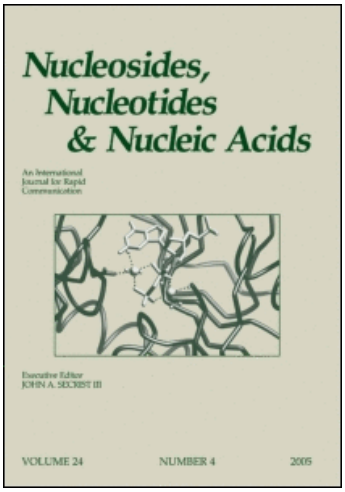

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Boronated Thymidine Analogues for Boron Neutron Capture Therapy

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BORONATED THYMIDINE ANALOGUES FOR BORON NEUTRON CAPTURE THERAPY

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□ *Concise synthetic methods for synthesizing 3-carboranyl thymidine analogues (3CTAs) modified with cyclic and acyclic alcohols have been developed. The synthesis of these potential boron neutron capture therapy (BNCT) agents and their preliminary biological evaluation is described.*

Keywords Boron Neutron Capture Therapy (BNCT); 3-Carboranyl Thymidine Analogues (3CTAs)

INTRODUCTION

Boron neutron capture therapy (BNCT) is a binary system for the treatment of cancer.[1] In order for this therapy to be effective, the targeted cancer cells must attain a sufficient concentration of ^{10}B and, thus, delivery systems for this non-radioactive boron isotope have to be developed that selectively target tumor cells. Boronated nucleosides have been considered as very attractive BNCT agents because of their potential metabolic properties, which could result in their selective accumulation and retention in tumor cells.[1] Previously, we have synthesized several types of

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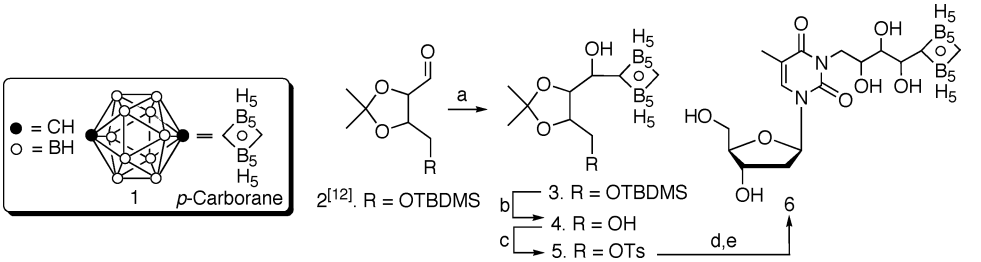


SCHEME 1 (a) *p-Carborane* (**1**)/n-BuLi/THF, 12 h, RT, yield: 63%; (b) TBAF/THF, 1 h, −78°C, yield: 92%; (c) TsCl/pyridine: CH_2Cl_2 (1:6), 12 h, RT, yield: 54%; (d) Thd/K_2CO_3/DMF:acetone (1:1), 24–48 h, 50°C; (e) 17% HCl in MeOH, 14 h, RT, yield: 49% from **5**.

3-carboranyl substituted thymidine analogues (3CTAs).[2–5] These boronated nucleoside prodrugs presumably accumulate selectively in tumor cells due to 5′-monophosphorylation by human thymidine kinase 1 (hTK1), which is highly active in rapidly proliferating tumor cells but not in non-dividing normal cells.[6] Most of these earlier 3CTAs contain a 1,2-disubstituted *o*-carborane cluster linked with thymidine through an alkyl spacer to the 1-position and a dihydroxypropyl group attached to the 2-position of the carborane cluster to partially compensate for the highly hydrophobic properties of this cage structure.[2] Here, we describe the synthesis of novel *p*-carboranyl 3CTAs, hydrophilically enhanced with cyclic and acyclic alcohol functions with more than two hydroxyl groups, and their preliminary biological evaluation. The design and synthesis of these novel 3CTAs was based on the following considerations. First, the *o*-carborane cluster covalently linked to the 5-position of 2′-deoxyuridine was found to be unstable and was partially degraded to the corresponding *nido*-carborane under in vitro conditions.[7] In contrast, the *p*-carborane cluster is presumably stable under in vitro and in vivo conditions.[8] Secondly, the current lead compound of our 3CTA library, N5-2OH, was found to be insoluble in water and a 50% aqueous DMSO was necessary to solubilize N5-2OH for in vivo studies.[9] Hydrophilic attachments with more than 3 hydroxyl groups may improve the water solubility of 3CTAs. Thirdly, a dihydroxypropyl group attached to the 2-position of the *o*-caborane cage may fold back onto the thymidine moiety, thereby interfering with binding to the active site of hTK1. On the other hand, a linear 1,12-disubstitution in a *p*-carboranyl 3CTA would project a hydrophilic attachment away from the nucleoside scaffold and may thereby enhance delivery properties such as water-solubility while decreasing interference with the binding to hTK1. Finally, attachment of hydroxyl groups in the spacer unit between *p*-carborane cage and the thymidine scaffold may also be suitable to achieve some of the objectives outlined above.

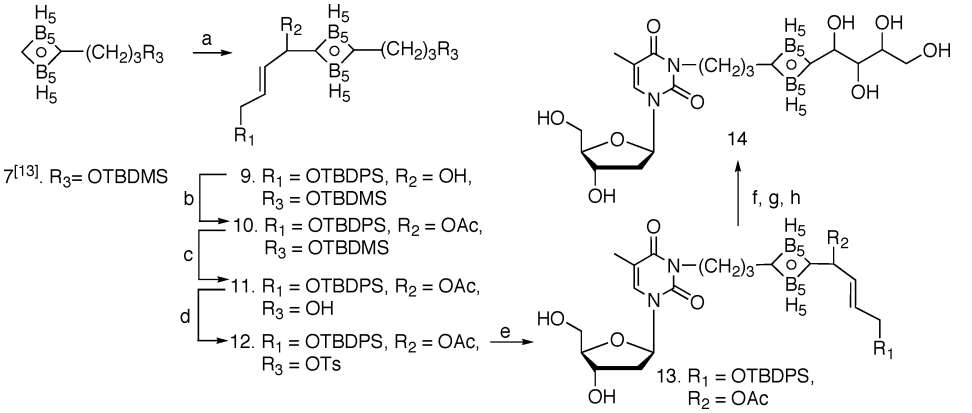
METHODS

Proton and carbon-13 NMR spectra were obtained on Bruker 250 MHz and 400 MHz instruments. High-resolution electron spray mass spectra (HR-ESI) were produced at the Ohio State University Campus Chemical Instrumentation Center (OSU-CCIC) and the Ohio State University Department of Chemistry. The structures of all synthesized compounds were confirmed by NMR and/or MS spectroscopy (see ^{1}H-NMR, ^{13}C-NMR, and HR-MS data of target compounds **6**[1], **14**[2], and **17**[3] in the footnotes). Recombinant hTK1 was expressed and purified as described previously.[5] The activity of recombinant hTK1 (3CTA phosphorylation rates) was followed by ADP production measured by the change in absorbance at 340 nm, caused by NADH oxidation in a coupled enzyme system with pyruvate kinase and lactate dehydrogenase. The standard reaction mixture contained 40 μM 3CTA, 20 mM Tris-HCl pH 7.6, 50 mM KCl, 2 mM $MgCl_2$, 0.5 mM ATP, 5 mM DTT, 1 mM phosphoenolpyruvate, 0.5 units/mL pyruvate kinase, 0.5 units/mL lactate dehydrogenase, 0.1 mM NADH, and 600 ng hTK1 in a total volume of 0.25 mL. The reaction was performed at 24°C with a Cary 3 spectrophotometer (Varian Techtron, Mulgrave, Australia) and started by the addition of thymidine or the 3CTAs. The enzyme activity values were calculated from the slope of the absorbance graph. The activity of hTK1 with 20 μM Thd was 640 nmol dTMP formed per min and mg hTK1 protein. In vitro cytotoxicity assays with CEM cells were carried out adapting experimental procedures previously described by us for 3CTAs with similar structural features as those described here.[9,10] In vitro boron uptake and retention studies in L929 wild-type [TK1(+)] cells, L929 TK1(−) cells,

[1]**6:** ^{1}H NMR (CD_3OD) δ: 1.84 (d, 3H, CH_3), 2.09–2.12 (m, 2H, H-2′), 3.18 (s, 1H, H-$C_{carborane}$), 3.34–3.36 (m, 1H, CH-OH), 3.51–3.52 (m, 1H, CH-OH), 3.68–3.76 (m, 3H, H-5′ and H-4′), 3.81–3.85 (m, 2H, CH_2-N), 4.08–4.17 (m, 1H, CH-OH), 4.30–4.35 (m, 1H, H-3′), 6.22 (t, 1H, H-1′), 7.79 (d, 1H, H-6); ^{13}C NMR (CD_3OD) δ: 165.86 (C-4), 152.79 (C-2), 136.55 (C-6), 110.72 (C-5), 88.91 (C-4′), 87.27 (C-1′), 73.11 (C-O), 72.92 (C-O), 72.17 (C-O), 72.04 (C-3′), 62.73 (C-5′), 44.87 (CH_2-N), 41.46 (C-2′), 13.21 (CH_3); MS (HR-ESI) $C_{16}H_{32}B_{10}N_2O_8Na$ $(M+Na)^+$ calcd: 513.2987, found 513.3016.

[2]**14:** ^{1}H NMR (CD_3OD) δ: 1.44–1.47 (m, 2H, CH_2), 1.66–1.70 (m, 2H, CH_2-$C_{carborane}$), 1.88 (d, 3H, CH_3), 2.14–2.28 (m, 1H, H-2′), 3.40–3.58 (m, 5H, CH-OH and CH_2-OH), 3.68–3.92 (m, 5H, H-4′, H-5′, and CH_2-N), 4.36–4.38 (m, 1H, H-3′), 6.27 (t, 1H, H-1′), 7.81 (d, 1H, H-6); ^{13}C NMR (CD_3OD) δ: 165.28 (C-4), 152.20 (C-2), 136.58 (C-6), 110.65 (C-5), 89.11 (C-1′), 87.10 (C-4′), 75.17 (C-O), 72.66 (C-O), 72.41 (C-3′), 72.05 (C-O), 63.74 (CH_2-O), 62.73 (C-5′), 41.56 (CH_2-N), 41.29 (C-2′), 36.29 (C-$C_{carborane}$), 28.70 (CH_2), 13.14 (CH_3); MS (HR-ESI) $C_{19}H_{38}B_{10}N_2O_9Na$ $(M+Na)^+$ calcd: 569.3489, found: 569.3480.

[3]**17:** ^{1}H NMR ($CDCl_3$) δ: 1.47–1.50 (m, 4H, CH_2), 1.56–1.58 (m, 2H, CH_2), 1.89 (d, 3H, CH_3), 1.93–1.98 (m, 2H, CH_2-$C_{carborane}$), 2.29–2.44 (m, 4H, CH_2-$C_{carborane}$ and H-2′), 3.78–3.98 (m, 9H, CH-OH, H-5′, H-4′, and CH_2-N), 4.58 (m, 1H, H-3′), 4.80 (d, 1H, CH(OH)-O), 6.12 (t, 1H, H-1′), 7.28 (d, 1H, H-6); ^{13}C NMR ($CDCl_3$) δ: 162.35 (C-4), 150.78 (C-2), 135.21 (C-6), 110.78 (C-5), 109.71 (C(OH)-O), 90.17(C-O), 88.06 (C-4′), 87.18 (C-1′), 78.97 (C-O), 71.99 (C-3′), 70.72 (C-O), 62.83 (C-5′), 55.30 (C-O), 41.55 (CH_2-N), 40.88 (C-2′), 40.44 (C-$C_{carborane}$), 37.98 (C-$C_{carborane}$), 29.55 (CH_2), 27.53 (CH_2), 26.78 (CH_2), 13.71 (CH_3); MS (HR-ESI) $C_{23}H_{44}B_{10}N_2O_{10}Na$ $(M+Na)^+$ calcd: 639.3909, found: 639.3904.

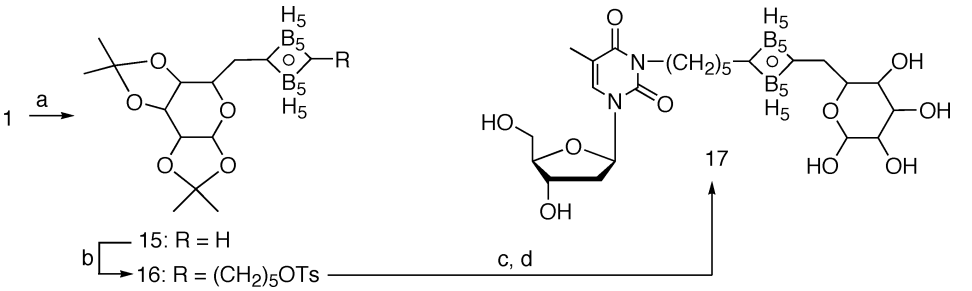


SCHEME 2 (a) BuLi/THF, *trans*-4-(*tert*-butyldiphenylsiloxy)but-2-en-1-al (**8**),[14] 12 h, RT, yield: 57%; (b) AcCl/pyridine/CH_2Cl_2, 3 h, RT, yield: 74%; (c) CCl_4/MeOH (1:1), ultrasonic bath, 10 h, yield: 67%; (d) TsCl/pyridine: CH_2Cl_2 (1:6), 3 h, RT, 80%; (e) Thd/K_2CO_3/DMF:acetone (1:1), 24–48 h, 50°C, yield: 60%; (f) TBAF/THF, 2 h, −78°C; (g) K_2CO_3/MeOH, 12 h, RT, yield: 53% from **13**; (h) OsO_4/acetone:H_2O (20:1), 6 h, RT, yield: 53%.

CEM wild-type [TK1(+)], and CEM TK1(−) were carried following exactly experimental procedures described previously by us for other 3CTAs[9] and cellular boron concentrations were determined by direct current plasma-atomic emission spectroscopy (DCP-AES).[11]

SYNTHESIS

The synthesis of target compounds **6**, **14**, and **17** are described in detail in Schemes 1–3. Various starting materials for the synthesis of compounds **6**, **14**, and **17** were prepared according to previously described methods[12–15] as indicated in Schemes 1–3. It is noteworthy that the TBDMS protective group in compound **10** could be removed selectively in the presence of the TBDPS group using ultrasonification.



SCHEME 3 (a) n-BuLi/1,2:3,4-*bis*-*o*-(methylethylidene)-4-methylbenzenesulfonate-α-D-galactopyranose,[15] 4 h, RT, yield: 20%; (b) 1,5-pentanediol-di-*p*-tosylate/n-BuLi, 4 h, RT, yield: 50%; (c) Thd/K_2CO_3/DMF:acetone(1:), 24–48 h, 50°C, yield: 65%; (d) 17% HCl in MeOH, 15 h, RT, yield: 85%.

PRELIMINARY BIOLOGICAL STUDIES

No significant phosphorylation by hTK1 was observed for compound **17**. However, the hTK1 phosphorylation rates of **6** and **14** were 64 and 47%, respectively, relative to that of thymidine. Compound **14** was not a substrate for deoxycytidine kinase (dCK), and, if at all, only a poor substrate for thymidine kinase 2 (TK2). The in vitro IC_{50} values of **14** in both CEM wild-type [TK1(+)] and CEM TK1(−) cells were >250 μM. Twenty-four-hour incubation of L929 wild-type cells with **14** (75 μM) resulted in the accumulation of 46 μg B/10^9 (∼1 g) cells. Boron retention following additional 12 h propagation of the cells in compound-free medium was 22%. Identical experimental conditions in L929 TK1(−) cells resulted in the accumulation of 10 μg B/10^9 cells and 0% retention. The results obtained in uptake/retention studies with CEM wild-type [TK1(+)] and CEM TK1(−) were comparable to those with L929 cells (data not shown).

CONCLUSION

Simple and efficient methods for synthesizing 3CTAs modified with cyclic and acyclic alcohols were developed. Compound **17** was not efficiently phosphorylated by hTK1. This could be due to the bulky galactopyranose group attached to the 12-postion of the *p*-carborane cage, which may interfere with the binding of this compound to the active site of hTK1. However, both compound **6** and compound **14** had higher relative phosphorylation rates than N5-2OH.[2] The cellular uptake profile of compound **14** in L929 wild-type [TK1(+)] cells and L929 TK1 (−) cells was comparable to that of N5-2OH while the compound seemed to be significantly less toxic than N5-2OH.[10] The physicochemical properties of compound **6** and **14** were improved compared with those of N5-2OH as demonstrated by the ratio of apolar surface to polar surface areas of these compounds (calculated with VEGA ZZ 2.0.4 software, Milano, Italy), which are 2.42, 2.06, and 2.90, respectively. Overall, the obtained preliminary results indicate that compounds **6** and **14** might be superior to N5-2OH, the current lead compound of our 3CTA library, as BNCT agents. More detailed studies are currently underway in our laboratories to substantiate the findings presented here.

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