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SYNTHESIS AND *IN VITRO* EVALUATION OF SUGAR-MODIFIED CARBORANYLURIDINES

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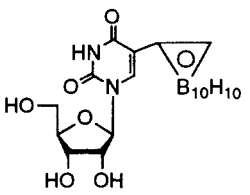
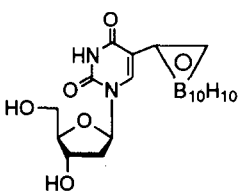
Abstract : Sugar-part modified carboranyluridines **5** and **6** were synthesized. Although cytotoxicities of **5** and **6** were similar to those of **1** and **2** having ordinary sugar parts, selective uptake of **6** into TIG-1-20 cells was accomplished by changing the sugar part to a sulfur containing one. Copyright © 1996 Elsevier Science Ltd

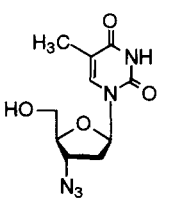
Boron neutron capture therapy (BNCT) of cancer is based on the nuclear reaction that occurs when boron-10 is irradiated with thermal neutrons to yield high-energy alpha particles and recoiling lithium-7 nuclei. A key requirement of BNCT is the selective delivery of an adequate concentration of boron-10 to tumors (30 μg/g tumor tissue).[1] Caluculations have shown[2] that the therapeutic effectiveness will be 2-5 times greater if ^{10}B-containing compounds are localized within the cell nucleus in contrast with the same ^{10}B concentration uniformly distributed throughout the cell. This fact has been the rationale for the attempts to synthesize boron containing nucleic acid precursors. Such structures may achieve higher concentration differentials in rapidly proliferating cancer cells compared to the mitotically less active normal cells. Our group was the first to synthesize 5-carboranyluridine (**1**, CU) and 5-carboranyldeoxyuridine (**2**, CDU).[3] Actually, cellular uptake of CU and CDU into malignant cells was very high[4] in comparison with the previously known ^{10}B carriers, such as *p*-boronophenylalanine (BPA) and sodium mercaptoundecahydrododecaborate ($Na_2B_{12}H_{11}SH$, so-called BSH) which have been used clinically for treatment of skin cancers and brain tumors, respectively. Unfortunately, however, those carboranyl-uridine **1** and -deoxyuridine **2** exhibited relatively high toxicity toward normal tissue.



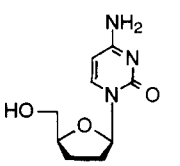
1 (CU)



2 (CDU)

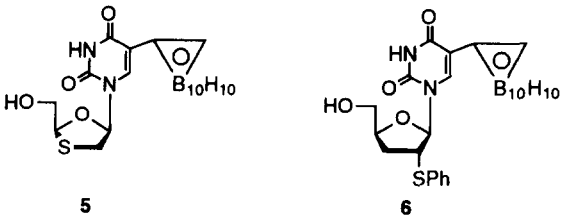


3 (AZT)



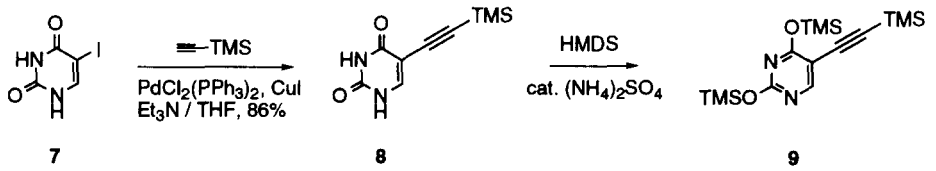
4 (ddC)

It has been reported that the toxicity of antiretroviral agents, such as AZT (**3**) and ddC (**4**), can be suppressed by changing the structure of sugar moieties.[5] It occured to us that sugar modified carboranyluridines might exhibit lower toxicity with high cellular uptake. We wish to report the synthesis of sugar modified 5-orthocarboranyluridines **5** and **6**, and their biological properties.



5 6

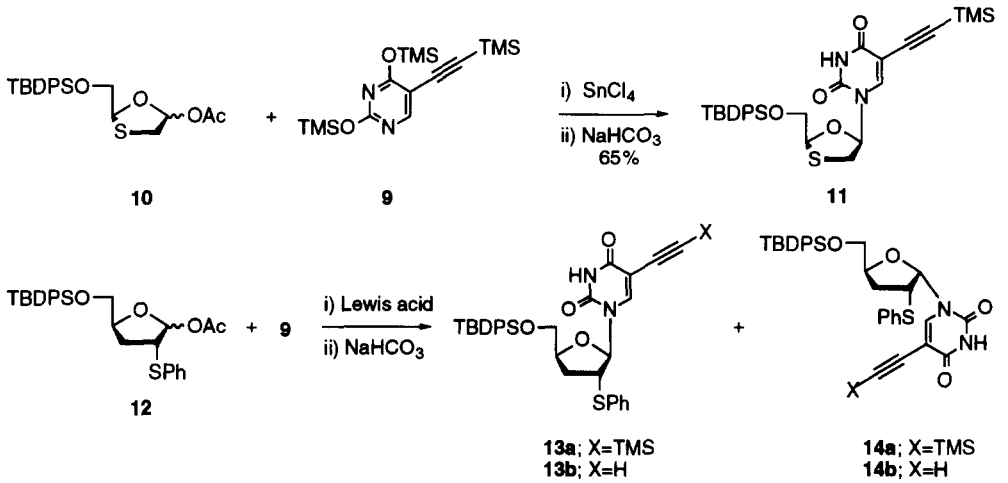
Synthesis of the base part of **5** and **6** was carried out by the literature procedure[6] (Scheme 1).



7 8 9

Scheme 1. Synthesis of 5-alkynylated uracil

Carbon-carbon triple bond necessary for carborane formation was introduced to 5 position of uracil by coupling reaction of 5-iodouracil and trimethylsilylacetylene under Pd catalyst. Treatment of **8** with hexamethyldisilazane produced persilylated compound **9**, which was used in the next condensation step (Scheme 2).



10 9 11

12

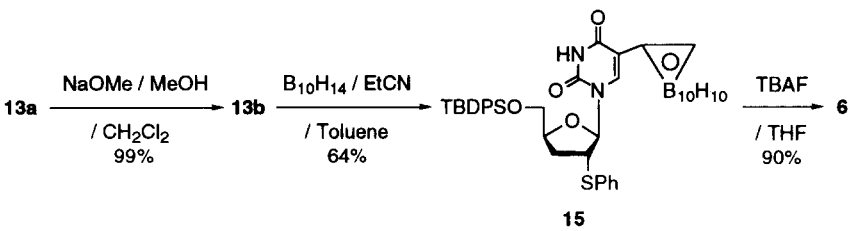
13a; X=TMS
13b; X=H

14a; X=TMS
14b; X=H

Scheme 2. Coupling of base and sugar parts

For initial trial, we chose **10**[7] and **12**[9,10] as sugar parts, because it is known that they may decrease toxicity[8] and exhibit high β selectivity in the Lewis acid mediated nucleoside synthesis.[8,10,11] The structure unit **10** can be seen in the sugar moiety of antiretroviral agent 3TC, and **12** is involved as a sugar moiety of intermediate of antiretroviral agent d4T synthesis.[10,11] The condensation reaction of **9** and **10** using $SnCl_4$ gave the β anomer **11** as a sole product. The $SnCl_4$ mediated reaction of **9** with **12** afforded the β anomer **13a** with very high selectivity along with small amounts of **14a** (**13a**/**14a** = 93/7), but the yield of the condensation

products was ca. 50%. The use of TMSOTf instead of $SnCl_4$ led to higher yield of β products with lower stereoselectivity; a mixture of **13a** and **13b** was isolated in 66% yield and the ratio of **13** to **14** was 84:16. Partial removal of TMS group took place by using TMSOTf as a Lewis acid. Treatment of **13a** with NaOMe/MeOH gave **13b** in essentially quantitative yield. The reaction of **13b** with $B_{10}H_{12}(EtCN)_2$, formed in situ from $B_{10}H_{14}$ and EtCN, in toluene gave carboranyluridine **15** in which a hydroxy group was protected by TBDPS group. Removal of TBDPS group from **15** using TBAF afforded the desired compound **6** ($[\alpha]^{23}_D$ -74.7° (*c* 1.00, CH_3OH)) in 57% overall yield from **13**.



Scheme 3. Synthesis of **6**

In the case of **11**, the silyl groups (TMS and TBDPS) were removed once and the 5'OH group was protected by benzoyl group. The resulting acetylene derivative with a free acetylenic C-H bond was treated with $B_{10}H_{12}(EtCN)_2$ to give the corresponding carborane compound. Removal of benzoyl group afforded the desired uridine **5** ($[\alpha]^{25}_D$ +0.82° (*c* 0.50, CH_3OH))[12] in 40% overall yield from **11**.

Cytotoxicities of these sugar-modified carboranyluridines toward B-16 melanoma cells and TIG-1-20 fibroblast cells were evaluated in terms of IC_{50} value. B-16 melanoma cells are a representative cancer cell and TIG-1-20 fibroblast cells have been used as a model of normal cells.[13] Results are shown in Table 1.

Table 1. Cytotoxicities toward cancer and normal cells

	IC_{50} ($M/10^{-5}$, M=mol/dm^3)	
	B-16	TIG-1-20
1	[a] 3.8	[a] 2.5
5	2.0±0.4	1.0±0.1
6	0.67±0.11	0.47±0.06

[a] See Reference (14).

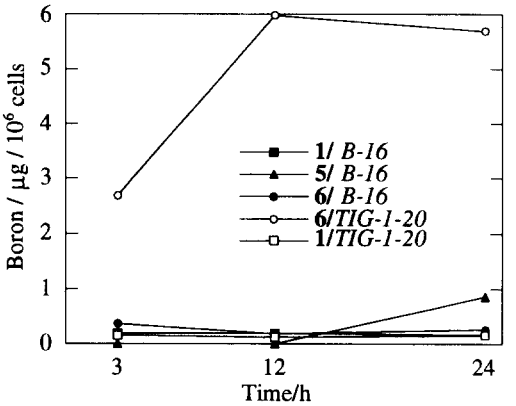


Figure 1. Cellular uptake of carboranyluridines.
6/TIG-1-20 ; Compounds **6** was incubated into TIG-1-20 cells at the IC_{50} concentration.

Obviously, decrease of toxicity was not observed by the sugar modification. This is presumably due to high lipophilic character of carborane moiety. Although the initial aim to decrease toxicity was not successful, we found very interesting difference between B-16 and TIG-1-20 on cellular uptake of boron (Fig 1). Compounds **1**[14], **5**, and **6** were incubated to each cells at the concentration of IC_{50} value and boron incorporation was monitored by the method described previously.[13] **6** was incorporated into B-16 cells as much as **1**, and boron uptake of **1** into B-16 was same as that into TIG-1-20. However, **6** was highly accumulated in TIG-1-20; that is to say, selective uptake was observed. These observations suggest that selective delivery of B-10 carriers to cell lines may be achieved by modification of sugar parts of uridines. Researches based upon this idea are in progress.

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