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Synthesis, susceptibility to enzymatic phosphorylation, cytotoxicity and *in vitro* antiviral activity of lipophilic pyrimidine nucleoside/carborane conjugates

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This paper is dedicated to Prof. Dr. Narayan S. Hosmane on his 70th birthday and in recognition of his outstanding contributions to boron cluster chemistry.

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ABSTRACT

We synthesized a series of new uridine and 2′-deoxyuridine conjugates of the *o*-carborane cluster attached at C-5 through a linker comprising the ethynyl group and/or triazole ring separated by alkane chains. The obtained conjugates have low or medium toxicity and are phosphorylated moderately by nucleoside kinases TK1 and TK2 and efficiently by dCK. Low toxicity and susceptibility to phosphorylation makes them candidates for application as boron carriers for boron neutron capture therapy (BNCT), with compound **15** phosphorylated efficiently by all three enzymes as the best hit.

1. Introduction

During the past decades, a great number of nucleoside derivatives and analogues have been synthesized and tested in the search for therapeutically useful agents [1]. Since that time, more than 90 drugs have been approved for the treatment of viral infections [2] and cancer therapies [3], and roughly half of them are nucleoside-derived compounds. Three antiviral drugs (idoxuridine, trifluridine, and brivudine) have been approved in the 5-substituted 2′-deoxyuridine analogue drug group. Uridines modified at C-5 with unsaturated hydrocarbon substituents have been found to be especially interesting [2,4,5], with brivudine [5-(2-bromovinyl)-2′-deoxyuridine], a potent inhibitor of virus replication, as the best-known example [5]. Several other 5-alkynyl modified pyrimidine nucleosides were also found to be efficient antiviral agents, especially against the members of the *Herpesviridae* family, such as herpes simplex virus 1 and 2 (HSV-1, HSV-2), varicella-zoster virus (VZV), at low μM concentrations [6–8]. Interestingly, some of these derivatives were also active as inhibitors of *Mycobacterium* species [9].

Recently uridine and 2′-deoxyuridine derivatives bearing a *p*-carborane modification attached through an ethynyl linker at the C-5 of uracil have been found to demonstrate potent and specific activity against human cytomegalovirus (HCMV) [10]. Interestingly, in contrast to carborane nucleoside conjugates, described recently cobalt bis(1,2-dicarbollide) (−1) conjugates of uridine or 2′-deoxyuridine demonstrated a lack of antiviral activity within the nontoxic concentration range [11]. Still another field of boron cluster nucleoside conjugate research that has received extensive attention in recent years is their application as boron carriers for boron neutron capture therapy (BNCT) in cancer.

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Herein, we describe an extension of our previous work [10] towards the synthesis and biological evaluation of uridine derivatives modified with the *o*-carborane cluster attached at C-5 *via* a longer linker containing triple bond and/or a triazole ring obtained using Sonogashira coupling and a Husigen-Meldal-Sharpless "click reaction" [12].

2. Results and discussion

2.1. Synthesis of uridine and 2′-deoxyuridine o-carborane cluster acceptors bearing terminal triple bond

The synthesis of 5-ethynyl-uridine (**2**) and 5-ethynyl-2′-deoxyuridine (**3**) (Scheme 2) was performed according to the literature [10,13]. A series of new 5-alkynyl-uridines and 2′-deoxyuridines (**9**–**11**, **12**–**14**) were synthesized *via* Sonogashira coupling as depicted in Scheme 1. 5-Iodonucleoside (**4**,**5**) (1 eq.) and diverse alkane-1,n-diynes (**6**–**8**) containing linear alkane chains (**6**, n = 3; **7**, n = 4; **8**, n = 5) were coupled at $Pd(PPh_3)_4$ (0.1 eq.), CuI (0.2 eq.) and triethylamine (TEA) (2 eq.) in dimethylformamide (DMF) at 55 °C (24 h).

In the of case reactions of compound **4** or **5** with diyne **7** (n = 4), the expected formation of products **10** and **13** was followed by subsequent cyclization leading to **10a** and **13a** and the formation of a mixture of **10** and **10a**, and **13** and **13a** products. The cyclic and acyclic products were separated by silica gel column chromatography and characterized by TLC and ^1H and ^{13}C NMR. A diagnostic signal in the ^1H NMR spectrum corresponding to the proton in the dihydrofuran ring can be observed for compounds **10a** and **13a** at 6.43 ppm and 6.44 ppm, respectively. Interestingly, the ratio of the acyclic (**10**, **13**) and cyclic (**10a**, **13a**) products is affected by the type of a palladium catalyst used. Thus, the use of $Pd(PPh_3)_4$ provides **10** and **10a**, and **13** and **13a** in a ratio ca. 2:1. For $Pd(PPh_3)_2Cl_2$, only the products of cyclization **10a** and **13a** were obtained, with no detectable amount of **10** and **13**.

2.2. Synthesis of o-carborane and uridine or 2′-deoxyuridine conjugates

The target *o*-carborane and uridine or 2′-deoxyuridine conjugates were obtained in a convenient, one-step procedure based on the copper(I)-catalysed Husigen-Meldal-Sharpless 1,3-dipolar cycloaddition of azides and alkynes to give triazoles ("click chemistry") (Scheme 2) [14–17]. The "click chemistry" approach was used previously for the attachment of ionic *nido*-*o*-carborane clusters or metallacarboranes to the N-3 position of thymidine functionalized with an alkyl substituent bearing a terminal ethynyl group [18].

More recently, a "click chemistry" methodology was used to synthesize all four canonical nucleosides: thymidine (T), 2′-deoxycytidine (dC), 2′-deoxyadenosine (dA) and 2′-deoxyguanosine (dG) modified with an *o*-carborane cluster at various locations within the nucleobase. Their phosphonamidites are suitable for automated synthesis of modified DNA with the preparation of the *o*-carborane cluster [19].

In an approach described herein, a suitable uridine or 2′-deoxyuridine boron cluster acceptor bearing a terminal ethynyl group (**2**, **3**, and **9**–**14**) and *o*-carborane cluster donor (**1**) equipped with a terminal azide group (Scheme 2) was dissolved in a mixture of *t*BuOH:H_2O (1:1 v/v) in the presence of a catalytic amount of $CuSO_4 \times 5H_2O$ and sodium ascorbate. The reaction was performed at room temperature for 24 h and then for the next 3 h at 50 °C. The yields of products **15**–**22** after isolation and purification by silica gel column chromatography was 42%–72%. Higher yields were obtained for 2′-deoxyuridine then compared to the uridine derivatives used, such as *o*-carborane cluster acceptors (compounds **16**, **20**–**22**). The lack of terminal hydrogen which is the case of internal alkynes prevents the Cu catalyzed reaction resulting in inertness of the alkyne groups in compounds **9**–**14** during the "click chemistry" cycloaddition.

All the obtained conjugates bearing boron clusters attached at the C-5 of the nucleoside through a linker containing a triple bond and a triazole ring prepared *via* "click chemistry", as well as uridine or 2′-deoxyuridine boron cluster acceptors bearing a terminal ethynyl group (Scheme 2), were tested *in vitro* for cytotoxicity in five cell lines and for antiviral activity against selected RNA and DNA viruses.

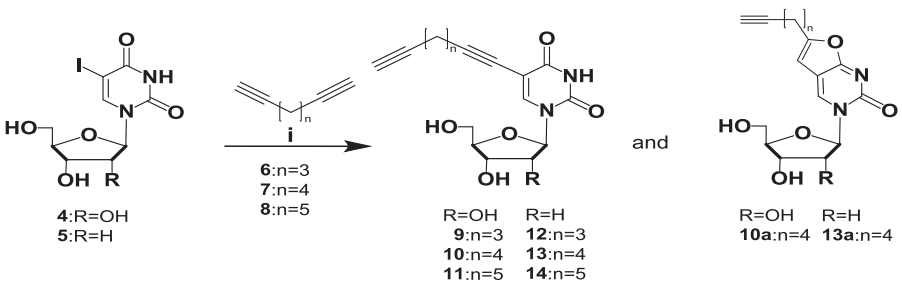
2.3. Biological investigations

2.3.1. In vitro cytotoxicity and antiviral activity assays

Cytotoxicity was compared in five cell lines: MRC-5 (human foetal lung fibroblasts), Vero (African Green Monkey kidney epithelial cells), A549 (human lung adenocarcinoma epithelial cells), LLC-MK2 (rhesus monkey kidney epithelial cells), and L929 (mouse fibroblasts). The cytotoxicity of compounds **9**–**21** was established by measurement of the 50% cytotoxic concentration (CC_{50}) using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) staining as described previously [20,21] and compared with the unmodified uridine and 2′-deoxyuridine.

In general, low toxicity or a lack of toxicity in the cell lines tested was observed for all 5-alkynyl uridine and 2′-deoxyuridne derivatives **9**–**14**, though toxicity varies from line to line (Table 1). The *o*-carborane and uridine or 2′-deoxyuridine conjugates **15**–**21** clearly demonstrate higher toxicity, though the cytotoxic effect is still moderate (CC_{50} from 20 μM to 340 μM).

Compounds **9**–**21** have been screened for antiviral activity against human cytomegalovirus (HCMV), herpes simplex virus type 1 (HSV-1), encephalomyocarditis virus (EMCV), human parainfluenza virus type 3 (HPIV-3), and vesicular stomatitis virus



Scheme 1. Uridine (**9**–**11**) and 2′-deoxyuridine (**12**–**14**) *o*-carborane cluster acceptors with a triple bond.

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