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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.

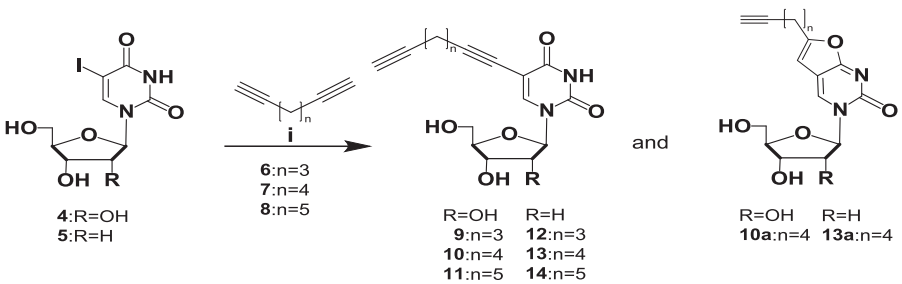
2.3. Biological investigations

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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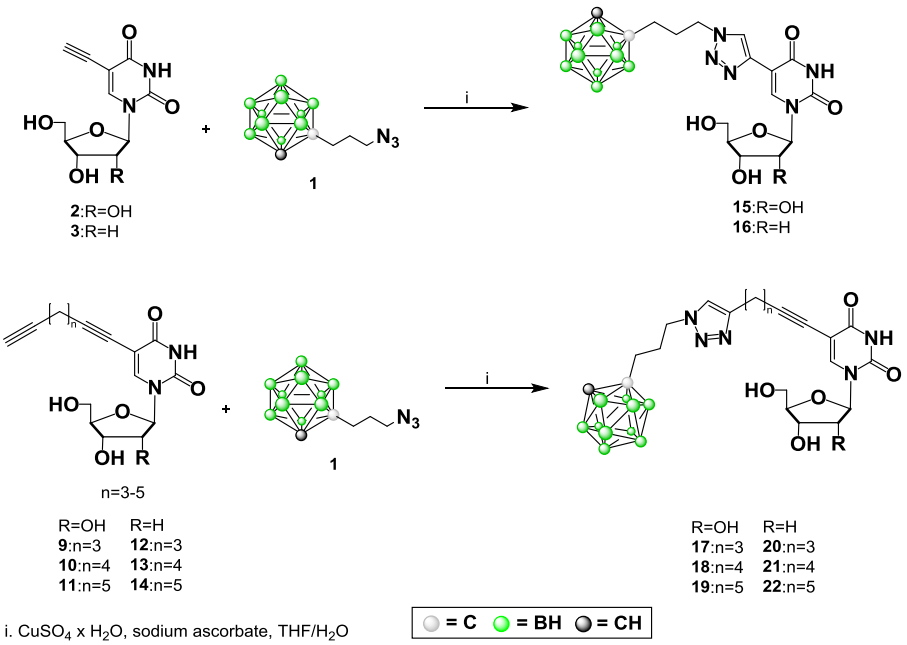

Scheme 2. Synthesis of *o*-carborane and uridine or 2′-deoxyuridine conjugates.

Table 1
Cytotoxicity of compounds **9**–**17**, and **19**–**21**.

Compound	Cell line, CC_{50} (μM)				
	MRC-5	A549	LLC-MK2	L929	Vero
U	960 ± 56.57	>1000	>1000	>1000	>1000
dU	>1000	>1000	>1000	>1000	>1000
9	950 ± 17.3	947.7 ± 3.5	>1000	690 ± 17.3	>1000
10	126.7 ± 11.5	250 ± 8.7	453.3 ± 5.3	263.3 ± 28.4	125.03 ± 17.3
11	749.3 ± 5.8	671.7 ± 5.8	748 ± 6.9	606.7 ± 6.1	423.3 ± 11.5
12	723.3 ± 23.1	850 ± 17.3	>1000	406.7 ± 11.5	773.3 ± 23.1
13	970 ± 30	621.7 ± 22.5	998.3 ± 2.97	>1000	966.7 ± 28.9
14	753.3 ± 11.5	770.3 ± 9	812 ± 6.9	572 ± 6.9	265.3 ± 8.1
15	40.7 ± 1.53	337.3 ± 1.53	205.3 ± 2.52	240.7 ± 1.15	239.3 ± 1
16	88.7 ± 1.15	263.3 ± 2.52	235.7 ± 2.08	56.3 ± 0.58	206 ± 2.52
17	70.9 ± 0.31	338.7 ± 1.15	289 ± 1.73	66.1 ± 0.31	181.3 ± 2.52
19	29 ± 0.2	29.5 ± 0.31	92.3 ± 1.53	26.9 ± 0.31	81.5 ± 0.31
20	68.9 ± 0.5	219 ± 2	314.7 ± 1.53	39.1 ± 0.42	292.7 ± 1.15
21	21.8 ± 0.2	21.3 ± 0.23	64 ± 0.2	23.5 ± 0.31	62.7 ± 0.23

18, **22**- not determined.

(VSV). The testing revealed a lack of antiviral activity of the all the tested compounds against the examined DNA and RNA viruses within the nontoxic concentrations (Table 1 SI).

2.3.2. Enzymatic phosphorylation

Phosphorylation of the nucleosides and their analogues is an important step in nucleoside metabolism and therapeutic nucleoside prodrug activation so that it becomes an antiviral agent and/or a cytotoxic anticancer drug [2,22]. This step is also crucial for nucleoside BNCT applications since it is assumed that boron cluster-modified nucleosides are preferentially accumulated in rapidly multiplying tumour cells as a result of phosphorylation to the corresponding nucleotide, leading to trapping of the charged compound inside the cells [23]. In turn, unwanted phosphorylation of the nucleoside drugs contributes to the side effects of these therapeutics [24].

There are four nucleoside kinases in human cells that are responsible for the phosphorylation of deoxynucleosides. Of these, 2′-deoxycytidine kinase (dCK) and thymidine kinase 1 (TK1) are the cytosolic deoxynucleoside kinases that are involved in the phosphorylation of natural nucleosides and many antiviral and anticancer nucleoside analogues [25]. TK2 and 2′-deoxyguanosine kinase (dGK) are the two mitochondrial deoxynucleoside kinases that have been involved in the activation of certain biologically active nucleoside analogues. The cytosolic enzymes, dCK and TK1, show a special expression pattern so that dCK is expressed at high levels in lymphocytic tissues, such as the spleen and thymus, while TK1 is expressed in all rapidly proliferating cells and tissues, including tumour cells. A series of studies have been performed where boron containing pyrimidine nucleosides have been designed for trapping by TK1-mediated phosphorylation in the tumour cells. This approach has led to promising BNCT activity in brain tumour animal cell models [26]. Additionally, pyrimidine, as well as purine nucleosides modified with metallacarboranes, were tested for their susceptibility to enzymatic phosphorylation [27].

Herein, we compared the susceptibility of boron cluster-containing conjugates **15**–**22** to phosphorylation. It seems that within the series of compounds studied, the efficacy of phosphorylation depends mainly upon two factors: 1) the type of nucleoside kinase used (TK1, TK2 or dCK), and 2) the type and length of the

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linker between C-5 of the nucleobase and the boron cluster. All the nucleosides studied were poorly phosphorylated by TK1 and TK2 with an exception of compound **15** which contains a shorter linker than the other derivatives and a triazole ring instead of triple bond directly attached at carbon 5 of the nucleobase (Fig. 1). Phosphorylation catalysed by dCK occurred with a much higher yield for almost all of compounds **15**–**22**. There was an especially notable increase of the phosphorylation level for compounds **17** and **20**–**22**. Efficient phosphorylation by dCK can be explained by the known promiscuity of this enzyme in comparison to both thymidine kinases. Long and rigid linkers comprising both the ethynyl group and triazole ring separated by alkane chains seems acceptable for dCK, but not for TK1 and TK2. However, the fact that all three enzymes occur together with various other kinases in all types of cells substantiates the expectation that the uridine and 2′-deoxyuridine boron cluster conjugates described herein can be phosphorylated *in vivo*.

3. Conclusions

A convenient method for the synthesis of uridine and 2′-deoxyuridine conjugates of the *o*-carborane cluster attached through a linker at the C-5, based on Sonogashira coupling and Husigen-Meldal-Sharpless “click reaction”, was developed. All the obtained nucleoside boron cluster conjugates have low or medium toxicity in the studied cell lines and do not express antiviral activity below their CC50 values for cytotoxicity. They are phosphorylated by TK1 and TK2 with low efficacy but are good substrates for dCK. Low toxicity and susceptibility to phosphorylation make them candidates for application as boron carriers for BNCT, with compound **15**, which is phosphorylated efficiently by all three enzymes, as the best hit.

4. Experimental section

4.1. Chemistry

4.1.1. Materials and methods

Most of the chemicals and solvents were obtained from Sigma-Aldrich Chemical Company (St. Louis, MO, USA) and used without further purification. *o*-Carborane was purchased from KatChem (Prague, Czech Republic), 5-iodouridine and 5-iodo-2′-deoxyuridine were purchased from Carbosynth (Berkshire, United Kingdom). Flash chromatography was performed on silica gel 60 (230–400 mesh, ASTM, Sigma-Aldrich Chemical Company) (St. Louis, MO, USA). R_f values refer to the analytical TLC performed using pre-coated silica gel 60 F254 plates purchased from Sigma-Aldrich (Steinheim, Germany) and developed in the solvent system indicated. Compounds were visualized using UV light (254 nm) or 0.5% acidic solution of $PdCl_2$ in HCl/methanol for boron-containing derivatives. The yields were not optimized.

4.1.1.1. Nuclear magnetic resonance (NMR). ^{1}H, ^{13}C and ^{11}B NMR spectra were recorded on a Bruker Avance III 600 MHz spectrometer equipped with a BB inverse probe-head, the spectra for ^{1}H, ^{13}C, and ^{11}B nuclei that were recorded at 600.26 MHz, 150.94 MHz and 192.59 MHz, respectively. Tetramethylsilane was used as a standard for ^{1}H and ^{13}C NMR, and $BF_3/(C_2H_5)_2O$ was used as a standard for ^{11}B NMR. All chemical shifts are reported in ppm (δ) relative to the internal standards. The following abbreviations were used to explain the multiplicities: s = singlet, d = doublet, bs = broad singlet, m = multiplet. Coupling constants (J) are given in Hertz.

4.1.1.2. Mass spectrometry. Electrospray ionization (ESI) mass spectra were recorded on a PurIon S (Teledyne ISCO, Lincoln, NY, USA). The ionization was achieved by ESI in positive ion mode (ESI+) and negative ion mode (ESI−). The capillary voltage was set to 2.5 kV. The source temperature was 200 °C, and the desolvation temperature was 350 °C. Nitrogen was used as a desolvation gas (35 L/min, purity >99%, nitrogen generator EURUS35 LCMS, E-DGSi SAS, France). The theoretical molecular masses of the compounds were calculated using the “Show Analysis Window” option in the ChemDraw Ultra 12.0 program. The calculated *m/z* corresponds to the average mass of the compounds consisting of natural isotopes.

4.1.1.3. Ultraviolet spectroscopy measurements (UV). UV measurements were performed on a GBC Cintra10 UV-VIS spectrometer

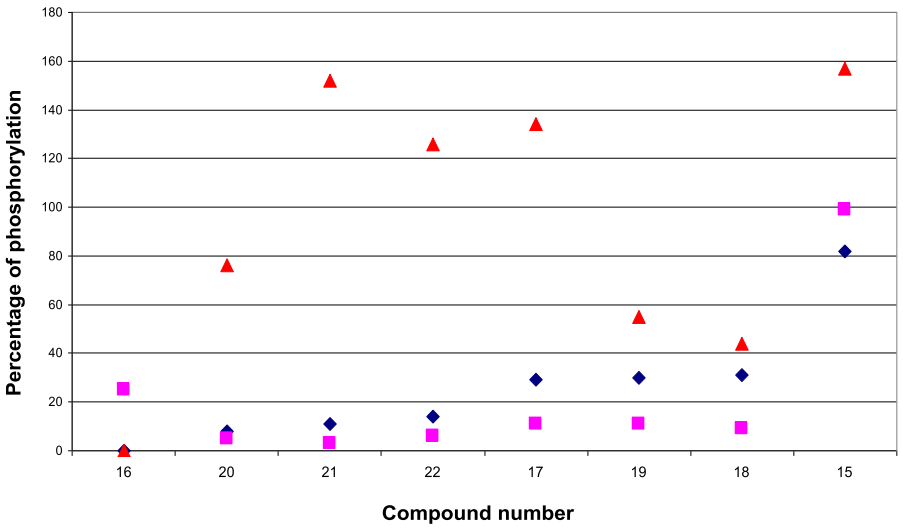

Fig. 1. Comparison of susceptibility to phosphorylation by TK1 (◆), TK2 (■) and dCK (▲) of boron cluster-modified uridine and 2′-deoxyuridine derivatives: 5-{[3-propyl-(1,2-dicarba-*closo*-dodecaborane-2-yl)]-1N-1,2,3-triazole-4-yl}2′-deoxyuridine (**16**), 5-{[3-propyl-(1,2-dicarba-*closo*-dodecaborane-2-yl)]-1N-1,2,3-triazole-4-yl}(pent-1-yl)-2′-deoxyuridine (**20**), 5-{[3-propyl-(1,2-dicarba-*closo*-dodecaborane-2-yl)]-1N-1,2,3-triazole-4-yl}(hex-1-yl)-2′-deoxyuridine (**21**), 5-{[3-propyl-(1,2-dicarba-*closo*-dodecaborane-2-yl)]-1N-1,2,3-triazole-4-yl}(hept-1-yl)-2′-deoxyuridine (**22**), 5-{[3-propyl-(1,2-dicarba-*closo*-dodecaborane-2-yl)]-1N-1,2,3-triazole-4-yl}(pent-1-yl)uridine (**17**), 5-{[3-propyl-(1,2-dicarba-*closo*-dodecaborane-2-yl)]-1N-1,2,3-triazole-4-yl}(hept-1-yl)uridine (**19**), 5-{[3-propyl-(1,2-dicarba-*closo*-dodecaborane-2-yl)]-1N-1,2,3-triazole-4-yl}(hex-1-yl)uridine (**18**), 5-{[3-propyl-(1,2-dicarba-*closo*-dodecaborane-2-yl)]-1N-1,2,3-triazole-4-yl}uridine (**15**). The values for tested compounds are expressed in percentage, relative to the unmodified nucleoside (U or dU) phosphorylation.

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(Dandenong, Australia). Samples for the UV experiments and the ca. 0.5 A_{260} ODU of each compound were dissolved in 96% C_2H_5OH. The measurements were performed at ambient temperature.

Synthesis of 1-(3-azidopropanyl)-1,12-dicarba-*closo*-dodecaborane (**1**) was performed as described previously with minor modifications [28].

Synthesis of 5-ethynyl uridine (**2**) and 5-ethynyl-2′-deoxyuridine (**3**) were performed as described previously with minor modifications [10,13].

4.1.2. General procedure for the synthesis of 5-(alkane-1,n-diynyl] nucleoside (**9**–**14**)

The procedure was performed under anhydrous conditions, with a positive argon pressure. $Pd(PPh_3)_4$ (0.0068–0.0272 mmol, 0.0078–0.0312 g) and CuI (0.0136–0.054 mmol, 0.0026–0.0104 g) dissolved in dry degassed DMF (0.375–1.500 mL) in the round-bottom flask were added to 5-iodonucleoside (**4**, **5**) (0.068–0.272 mmol, 0.024–0.100 g) with alkane-1, n-diyne (**6**–**8**) (0.680–2.720 mmol, 0.0817–0.252 g) and TEA (0.136–0.544 mmol, 0.0138–0.0552 g) and the mixture was stirred for 24 h at 55 °C. Then the solvent was removed *in vacuo* and the solid residue was dissolved in CH_2Cl_2 (0.7–2.0 mL). The resultant solution was washed with water (3 × 0.5–1.0 mL) and 0.5% EDTA (1 × 1 mL). Next the organic phase was dried over $MgSO_4$ and evaporated to dryness yielding 0.082–0.430 g of the crude product. This was purified by column chromatography, eluting with methanol in CH_2Cl_2 (0–10%) to afford 0.0112–0.0451 g of pure product with a yield 29–50%.

9: Yield 50%. ^{1}H NMR (600 MHz, CD_3OD) δ (ppm): 1.776–1.823 (m, 2H, CH_2), 2.269 (t, 1H, alkynyl group-CH, $J = 2.4$), 2.368 (dt, 2H, CH_2-alkynyl group, $J = 3$; 7.2), 2.541 (t, 2H, CH_2-nucleoside, $J = 7.2$), 3.772–3.921 (m, 2H, H-5′, H-5″), 4.043–4.059 (m, 1H, H-4′), 4.195–4.226 (m, 2H, H-3′, H-2′), 5.927 (d, 1H, H-1′, $J = 4.2$ Hz), 8.307 (s, 1H, H-6); ^{13}C NMR (150.94 MHz, $CDCl_3$) δ (ppm): 19.24 (CH_2-alkynyl group), 20.22 (CH_2-nucleoside), 29.82 (CH_2), 63.09 (C-5′), 71.01 (alkynyl group-CH), 72.05 (C-3′), 76.89 (C-2′), 85.21 (C-alkynyl group), 87.38 (C-4′), 91.95 (C-1′), 102.19 (C-5), 145.50 (C-6), 152.50 (C=O), 165.57 (C=O), TLC CH_2Cl_2/MeOH (9:1 v/v) $R_f = 0.36$.

10: Yield 29%. ^{1}H NMR (600 MHz, CD_3OD) δ (ppm): 1.674–1.754 (m, 4H, 2 × CH_2), 2.230–2.273 (m, 3H, alkynyl group-CH, CH_2), 2.449 (t, 2H, CH_2, $J = 7.2$), 3.770–3.918 (m, 2H, H-5′, H-5″), 4.042–4.058 (m, 1H, H-4′), 4.193–4.226 (m, 2H, H-3′, H-2′), 5.927 (d, 1H, H-1′, $J = 4.2$), 8.288 (s, 1H, H-6); ^{13}C NMR (150.94 MHz, $CDCl_3$) δ (ppm): 19.55 (CH_2-alkynyl group), 20.66 (CH_2-nucleoside), 29.62 (CH_2), 29.76 (CH_2), 63.03 (C-5′), 70.66 (alkynyl group-CH), 72.11 (C-3′), 76.90 (C-2′), 85.73 (C-alkynyl group), 87.42 (C-4′), 91.94 (C-1′), 102.32 (C-5), 145.39 (C-6), 152.57 (C=O), 165.66 (C=O), TLC CH_2Cl_2/MeOH (9:1 v/v) $R_f = 0.43$.

10a: Yield 14%. ^{1}H NMR (600 MHz, CD_3OD) δ (ppm): 1.620–1.669 (m, 2H, CH_2), 1.843–1.898 (m, 2H, CH_2), 2.239–2.292 (m, 3H, alkynyl group-CH, CH_2), 2.773 (t, 2H, CH_2, $J = 7.2$), 3.865–4.067 (m, 2H, H-5′, H-5″), 4.164–4.179 (m, 1H, H-4′), 4.198–4.224 (m, 2H, H-3′, H-2′), 6.015 (d, 1H, H-1′, $J = 1.2$), 6.434 (s, 1H, cyclic), 8.979 (s, 1H, H-6); ^{13}C NMR (150.94 MHz, $CDCl_3$) δ (ppm): 19.65 (CH_2), 28.00 (CH_2), 29.45 (CH_2), 29.91 (CH_2), 62.12 (C-5′), 70.76 (C-3′), 77.88 (C-2′), 86.87 (C-4′), 95.13 (C-1′), 101.81 (C-5), 139.64 (C-6), 158.08 (C-O), 162.09 (C=O), TLC CH_2Cl_2/MeOH (9:1 v/v) $R_f = 0.27$.

11: Yield 46%. ^{1}H NMR (600 MHz, CD_3OD) δ (ppm): 1.556–1.640 (m, 6H, 3 × CH_2), 2.208–2.240 (m, 3H, alkynyl group-CH, CH_2), 2.425 (t, 2H, CH_2, $J = 7.2$), 3.771–3.917 (m, 2H, H-5′, H-5″), 4.042–4.059 (m, 1H, H-4′), 4.195–4.229 (m, 2H, H-3′, H-2′), 5.927 (d, 1H, H-1′, $J = 4.2$), 8.272 (s, 1H, H-6); ^{13}C NMR (150.94 MHz, $CDCl_3$) δ (ppm): 19.92 (CH_2-alkynyl group), 21.08 (CH_2-nucleoside), 29.96 (CH_2), 30.08 (CH_2), 30.23 (CH_2), 63.05 (C-5′), 70.46 (alkynyl group-CH), 72.13 (C-3′), 76.87 (C-2′), 86.01 (C-acetyl), 87.44 (C-4′), 91.91 (C-1′), 102.39 (C-5), 145.33 (C-6), 152.53 (C=O), 165.59 (C=O); TLC CH_2Cl_2/MeOH (9:1 v/v) $R_f = 0.38$.

12: Yield 49%. ^{1}H NMR (600 MHz, CD_3OD) δ (ppm): 1.777–1.824 (m, 2H, CH_2), 2.270 (t, 1H, alkynyl group-CH, $J = 3$), 2.318–2.381 (m, 4H, CH_2, H-2′, H-2″), 2.541 (t, 2H, CH_2, $J = 7.2$), 3.760–3.862 (m, 2H, H-5′, H-5″), 3.958–3.974 (m, 1H, H-4′), 4.425–4.447 (m, 1H, H-3′), 6.278 (t, 1H, H-1′, $J = 6.6$), 8.248 (s, 1H, H-6); ^{13}C NMR (150.94 MHz, $CDCl_3$) δ (ppm): 19.25 (CH_2-alkynyl group), 20.22 (CH_2-nucleoside), 29.84 (CH_2), 42.63 (C-2′), 63.61 (C-5′), 71.01 (alkynyl group-CH), 73.03 (C-3′), 85.21 (C-alkynyl group), 87.93 (C-1′), 90.09 (C-4′), 102.09 (C-5), 145.34 (C-6), 152.26 (C=O), 165.65 (C=O); TLC CH_2Cl_2/MeOH (9:1 v/v) $R_f = 0.40$.

13: Yield 34%. ^{1}H NMR (600 MHz, CD_3OD) δ (ppm): 1.676–1.754 (m, 4H, 2 × CH_2), 2.229–2.355 (m, 5H, alkynyl group-CH, CH_2, H-2′, H-2″), 2.450 (t, 2H, CH_2, $J = 7.2$), 3.759–3.858 (m, 2H, H-5′, H-5″), 3.957–3.974 (m, 1H, H-4′), 4.423–4.445 (m, 1H, H-3′), 6.279 (t, 1H, H-1′, $J = 6.6$), 8.230 (s, 1H, H-6); ^{13}C NMR (150.94 MHz, $CDCl_3$) δ (ppm): 19.55 (CH_2-alkynyl group), 20.67 (CH_2-nucleoside), 29.63 (CH_2), 29.78 (CH_2), 42.62 (C-2′), 63.64 (C-5′), 70.66 (alkynyl group-CH), 73.06 (C-3′), 85.72 (C-alkynyl group), 87.92 (C-1′), 90.10 (C-4′), 102.25 (C-5), 145.22 (C-6), 152.30 (C=O), 165.68 (C=O); TLC CH_2Cl_2/MeOH (9:1 v/v) $R_f = 0.36$.

13a: Yield 16%. ^{1}H NMR (600 MHz, CD_3OD) δ (ppm): 1.620–1.669 (m, 2H, CH_2), 1.843–1.898 (m, 2H, CH_2), 2.215–2.654 (m, 5H, alkynyl group-CH, CH_2, 2′, 2″), 2.774 (t, 2H, CH_2, $J = 7.2$), 3.811–3.937 (m, 2H, H-5′, H-5″), 4.085–4.103 (m, 1H, H-4′), 4.412–4.436 (m, 1H, H-3′), 6.337–6.351 (m, 1H, H-1′), 6.444 (s, 1H, cyclic), 8.870 (s, 1H, H-6); ^{13}C NMR (150.94 MHz, $CDCl_3$) δ (ppm): 19.65 (CH_2), 28.02 (CH_2), 29.41 (CH_2), 29.91 (CH_2), 43.85 (C-2′), 63.34 (C-5′), 78.61 (C-3′), 90.68 (C-4′), 90.80 (C-1′), 101.94 (C-5), 139.33 (C-6), 157.83 (C-O), 161.97 (C=O); TLC CH_2Cl_2/MeOH (9:1 v/v) $R_f = 0.26$.

14: Yield 49%. ^{1}H NMR (600 MHz, CD_3OD) δ (ppm): 1.576–1.641 (m, 6H, 3 × CH_2), 2.209–2.355 (m, 5H, alkynyl group-CH, CH_2, H-2′, H-2″), 2.426 (t, 2H, CH_2, $J = 7.2$), 3.760–3.857 (m, 2H, H-5′, H-5″), 3.959–3.976 (m, 1H, H-4′), 4.426–4.447 (m, 1H, H-3′), 6.278 (t, 1H, H-1′, $J = 6.6$), 8.210 (s, 1H, H-6); ^{13}C NMR (150.94 MHz, $CDCl_3$) δ (ppm): 19.92 (CH_2-alkynyl group), 21.08 (CH_2-nucleoside), 30.05 (CH_2), 30.18 (CH_2), 30.25 (CH_2), 42.61 (C-2′), 63.65 (C-5′), 70.45 (alkynyl group-CH), 73.08 (C-3′), 85.99 (C-alkynyl group), 87.91 (C-1′), 90.11 (C-4′), 102.31 (C-5), 145.15 (C-6), 152.27 (C=O), 165.67 (C=O); TLC CH_2Cl_2/MeOH (9:1 v/v) $R_f = 0.31$.

4.1.3. General procedure for the synthesis of (**15**–**22**)

To 5-alkyne nucleoside (**2**, **3**, **9**, **11**–**13**) (0.036–0.047 mmol, 0.0100–0.0164 g) was dissolved in a mixture of THF/H_2O (0.5–0.7 mL, 1:1 v/v) $CuSO_4 \times H_2O$ (0.0044–0.0047 mmol, 0.001–0.0012 g), 1-(3-azidopropanyl)-1,12-dicarba-*closo*-dodecaborane (**1**) (0.0040–0.0051 mmol, 0.010–0.012 g) and sodium ascorbate (0.0080–0.0094 mmol, 0.0016–0.0020 g) were added. The mixture was stirred for 24 h at RT and 3 h at 50 °C. Then, the solvent was removed *in vacuo* and the residue was purified by column chromatography, eluting with methanol in CH_2Cl_2 (0–5%) to afford 0.008–0.0144 g of pure product with a yield 42–57%.

15: Yield 52%. ^{1}H NMR (600 MHz, CD_3OD) δ (ppm): 2.136–2.187 (m, 2H, CH_2), 2.344–2.373 (m, 2H, CH_2), 3.794–3.943 (m, 2H, H-5′, H-5″), 4.079–4.097 (m, 1H, H-4′), 4.239 (t, 1H, H-3′, $J = 4.8$), 4.315 (t, 1H, H-2′, $J = 4.8$), 4.463 (t, 2H, CH_2-triazole, $J = 6.6$), 4.575 (bs, 1H, B-C-H), 6.052 (d, 1H, H-1′, $J = 4.8$), 8.352 (s, 1H, H-6), 8.661 (s, 1H, CH-triazole); ^{13}C NMR (150.94 MHz, CD_3OD) δ (ppm): 31.86 (CH_2), 36.63 (CH_2), 51.20 (CH_2-triazole), 63.54 (C-5′), 64.78 (C-carborane), 72.50 (C-3′), 76.80 (C-2′), 87.66 (C-4′), 91.81 (C-1′), 107.90 (C-5), 125.15 (C-6), 139.30 (CH-triazole), 146.81 (C-triazole), 152.83 (C=O), 164.15 (C=O); ^{11}B NMR (192.59 MHz, CD_3OD) δ (ppm): −15.03-11.71 (m, 4 B), −9.56 (s, 2 B), −5.91 (s, 2 B), −2.74 (s, 2 B); TLC

CH_2Cl_2/MeOH (9:1 v/v) $R_f = 0.40$; MS (ESI, −VE): $m/z = 495$ $[M]^-$, calcd for $C_{16}H_{29}B_{10}N_5O_6 = 495.54$.

16: Yield 42%. ^{1}H NMR (600 MHz, CD_3OD) δ (ppm): 2.304–2.412 (m, 6H, 2 × CH_2, H-2′), 3.793–3.898 (m, 2H, H-5′, H-5″), 4.002–4.020 (m, 1H, H-4′), 4.452–4.488 (m, 3H, CH_2-triazole, H-3′), 4.574 (bs, 1H, B-C-H), 6.381 (t, 1H, H-1′, $J = 6.0$), 8.351 (s, 1H, H-6), 8.653 (s, 1H, CH-triazole); ^{13}C NMR (150.94 MHz, CD_3OD) δ (ppm): 31.86 (CH_2), 36.63 (CH_2), 42.55 (C-2′), 51.20 (CH_2-triazole), 63.96 (C-5′), 64.79 (C-carborane), 73.34 (C-3′), 87.97 (C-1′), 90.16 (C-4′), 107.71 (C-5), 125.08 (C-6), 139.06 (CH-triazole), 141.93 (C-triazole), 152.52 (C═O), 164.20 (C═O); ^{11}B NMR (192.59 MHz, CD_3OD) δ (ppm): −13.02-11.69 (m, 4 B), −9.54 (s, 2 B), −5.91 (s, 2 B), −2.75 (s, 2 B); TLC CH_2Cl_2/MeOH (9:1 v/v) $R_f = 0.45$; MS (ESI, −VE): $m/z = 478$ $[M-1]^-$, calcd for $C_{16}H_{29}B_{10}N_5O_5 = 479.54$.

17: Yield 48%. ^{1}H NMR (600 MHz, CD_3OD) δ (ppm): 1.924–1.969 (m, 2H, CH_2), 2.116–2.155 (m, 2H, CH_2), 2.314–2.334 (m, 2H, CH_2), 2.456 (t, 2H, alkynyl group-CH_2, $J = 6.6$), 2.905 (t, 2H, C(triazole)-CH_2, $J = 7.2$), 3.789–3.940 (m, 2H, H-5′, H-5″), 4.054–4.070 (m, 1H, H-4′), 4.207–4.232 (m, 2H, H-2′, H-3′), 4.463 (t, 2H, CH_2-triazole, $J = 6.6$), 4.558 (bs, 1H, B-C-H), 5.921 (d, 1H, H-1′, $J = 3.6$), 7.859 (s, 1H, CH-triazole), 8.349 (s, 1H, H-6); ^{13}C NMR (150.94 MHz, CD_3OD) δ (ppm): 20.43 ($\underline{CH_2}$-C-alkynyl group), 26.14 ($\underline{CH_2}$-C-triazole), 30.03 (CH_2), 31.84 (CH_2), 36.63 (CH_2), 51.08 (CH_2-triazole), 62.93 (C-5′), 64.75 (C-carborane), 72.03 (C-3′), 76.98 (C-2′), 87.39 (C-4′), 91.96 (C-1′), 102.12 (C-5), 145.57 (C-6), 152.50 (C═O), 165.60 (C═O); ^{11}B NMR (192.59 MHz, CD_3OD) δ (ppm): −13.02-11.71 (m, 4 B), −9.55 (s, 2 B), −5.92 (s, 2 B), −2.74 (s, 2 B); TLC CH_2Cl_2/MeOH (9:1 v/v) $R_f = 0.27$; MS (ESI, −VE): $m/z = 560$ $[M-1]^-$, calcd for $C_{21}H_{35}B_{10}N_5O_6 = 561.64$.

18: Yield 65%. ^{1}H NMR (600 MHz, CD_3OD) δ (ppm): 1.622–1.660 (m, 4H, 2 × CH_2), 1.852–1.878 (m, 2H, CH_2), 2.124–2.140 (m, 2H, CH_2), 2.303–2.331 (m, 2H, CH_2), 2.461 (t, 2H, alkynyl group-CH_2, $J = 7.2$), 2.780 (t, 2H, C(triazole)-CH_2, $J = 7.2$), 3.792–3.944 (m, 2H, H-5′, H-5″), 4.122–4.144 (m, 1H, H-4′), 4.204–4.220 (m, 2H, H-2′, H-3′), 4.397 (t, 2H, CH_2-triazole, $J = 6.6$), 4.558 (bs, 1H, B-C-H), 5.927 (d, 1H, H-1′, $J = 3.0$), 7.832 (s, 1H, CH-triazole), 8.354 (s, 1H, H-6); ^{13}C NMR (150.94 MHz, CD_3OD) δ (ppm): 20.61 ($\underline{CH_2}$-C-alkynyl group), 25.83 ($\underline{CH_2}$-C-triazole), 29.33 (CH_2); 30.39 (CH_2), 31.90 (CH_2), 36.64 (CH_2), 51.04 (CH_2-triazole), 62.88 (C-5′), 64.76 (C-carborane), 72.01 (C-3′), 77.03 (C-2′), 87.36 (C-4′), 91.98 (C-1′), 102.23 (C-5), 124.36 (CH-triazole), 145.50 (C-6), 152.51 (C═O), 165.59 (C═O); TLC CH_2Cl_2/MeOH (9:1 v/v) $R_f = 0.28$; MS (ESI, −VE): $m/z = 574$ $[M-1]^-$, calcd for $C_{22}H_{37}B_{10}N_5O_6 = 575.67$.

19: Yield 44%. ^{1}H NMR (600 MHz, CD_3OD) δ (ppm): 1.519–1.568 (m, 2H, CH_2), 1.618–1.666 (m, 2H, CH_2), 1.716–1.766 (m, 2H, CH_2), 2.092–2.143 (m, 2H, CH_2), 2.331–2.302 (m, 2H, CH_2), 2.427 (t, 2H, alkynyl group-CH_2, $J = 6.6$), 2.760 (t, 2H, C(triazole)-CH_2, $J = 7.2$), 3.780–3.927 (m, 2H, H-5′, H-5″), 4.049–4.065 (m, 1H, H-4′), 4.204–4.234 (m, 2H, H-2′, H-3′), 4.392 (t, 2H, CH_2-triazole, $J = 6.6$), 4.559 (bs, 1H, B-C-H), 5.930 (d, 1H, H-1′, $J = 3.6$), 7.793 (s, 1H, CH-triazole), 8.296 (s, 1H, H-6); ^{13}C NMR (150.94 MHz, CD_3OD) δ (ppm): 21.03 ($\underline{CH_2}$-C-alkynyl group), 27.11 ($\underline{CH_2}$-C-triazole), 30.20 (CH_2), 30.22 (CH_2), 30.88 (CH_2), 31.89 (CH_2), 36.63 (CH_2), 51.04 (CH_2-triazole), 62.99 (C-5′), 64.76 (C-carborane), 72.08 (C-3′), 76.94 (C-2′), 87.42 (C-4′), 91.98 (C-1′), 102.35 (C-5), 124.32 (CH-triazole), 145.39 (C-6), 152.23 (C═O), 165.59 (C═O); ^{11}B NMR (192.59 MHz, CD_3OD) δ (ppm): −13.03-11.71 (m, 4 B), −9.56 (s, 2 B), −5.92 (s, 2 B), −2.76 (s, 2 B); TLC CH_2Cl_2/MeOH (9:1 v/v) $R_f = 0.26$; MS (ESI, −VE): $m/z = 589$ $[M-1]^-$, calcd for $C_{23}H_{39}B_{10}N_5O_6 = 590.39$.

20: Yield 57%. ^{1}H NMR (600 MHz, CD_3OD) δ (ppm): 1.936–1.984 (m, 2H, CH_2), 2.115–2.155 (m, 2H, CH_2), 2.242–2.381 (m, 4H, CH_2, H-2′), 2.461 (t, 2H, alkynyl group-CH_2, $J = 6.6$), 2.898 (t, 2H, C(triazole)-CH_2, $J = 7.2$), 3.760–3.876 (m, 2H, H-5′, H-5″), 3.958–3.984 (m, 1H, H-4′), 4.399 (t, 2H, CH_2-triazole, $J = 6.6$), 4.434–4.456 (m, 1H, H-3′), 4.557 (bs, 1H, B-C-H), 6.285 (t, 1H, H-1′, $J = 6.6$), 7.869 (s, 1H, CH-triazole), 8.289 (s, 1H, H-6); ^{13}C NMR (150.94 MHz, CD_3OD) δ (ppm): 20.46 (CH_2), 26.13 (CH_2), 29.98 (CH_2), 31.66 (CH_2), 36.64 (CH_2), 42.69 (C-2′), 51.10 (CH_2-triazole), 63.61 (C-5′), 64.76 (C-carborane), 73.02 (C-3′), 87.93 (C-1′), 90.12 (C-4′), 102.08 (C-5), 145.40 (C-6), 152.26 (C═O), 163.94 (C═O); ^{11}B NMR (192.59 MHz, CD_3OD) δ (ppm): −13.04-11.72 (m, 4 B), −9.57 (s, 2 B), −5.91 (s, 2 B), −2.76 (s, 2 B); TLC CH_2Cl_2/MeOH (9:1 v/v) $R_f = 0.40$; MS (ESI, −VE): $m/z = 545$ $[M]^-$, calcd for $C_{21}H_{35}B_{10}N_5O_5 = 545.36$.

21: Yield 55%. ^{1}H NMR (600 MHz, CD_3OD) δ (ppm): 1.619–1.667 (m, 2H, CH_2), 1.859–1.900 (m, 2H, CH_2), 2.108–2.194 (m, 2H, CH_2), 2.232–2.356 (m, 4H, CH_2, H-2′), 2.458 (t, 2H, alkynyl group-CH_2, $J = 6.6$), 2.780 (t, 2H, C(triazole)-CH_2, $J = 7.2$), 3.766–3.875 (m, 2H, H-5′, H-5″), 3.959–3.983 (m, 1H, H-4′), 4.396 (t, 2H, CH_2-triazole, $J = 6.6$), 4.431–4.458 (m, 1H, H-3′), 4.552 (bs, 1H, B-C-H), 6.283 (t, 1H, H-1′, $J = 6.6$), 7.838 (s, 1H, CH-triazole), 8.279 (s, 1H, H-6); ^{13}C NMR (150.94 MHz, CD_3OD) δ (ppm): 20.87 (CH_2), 26.75 (CH_2), 29.88 (CH_2), 30.38 (CH_2), 31.88 (CH_2), 36.63 (CH_2), 42.70 (C-2′), 51.04 (CH_2-triazole), 63.63 (C-5′), 64.75 (C-carborane), 73.01 (C-3′), 87.92 (C-1′), 90.10 (C-4′), 102.22 (C-5), 124.40 (CH-triazole), 145.31 (C-6), 152.27 (C═O), 165.64 (C═O); ^{11}B NMR (192.59 MHz, CD_3OD) δ (ppm): −13.02-11.73 (m, 4 B), −9.57 (s, 2 B), −5.93 (s, 2 B), −2.76 (s, 2 B); TLC CH_2Cl_2/MeOH (9:1 v/v) $R_f = 0.50$; MS (ESI, −VE): $m/z = 558$ $[M-2]^-$, calcd for $C_{22}H_{37}B_{10}N_5O_6 = 560.38$.

22: Yield 72%. ^{1}H NMR (600 MHz, CD_3OD) δ (ppm): 1.532–1.571 (m, 2H, CH_2), 1.621–1.657 (m, 2H, CH_2), 1.730–1.755 (m, 2H, CH_2), 2.101–2.129 (m, 2H, CH2), 2.212–2.390 (m, 4H, H-2′, H-2″, CH_2), 2.430 (t, 2H, alkynyl group-CH_2, $J = 7.2$), 2.759 (t, 2H, C(triazole)-CH_2, $J = 7.2$), 3.760–3.864 (m, 2H, H-5′, H-5″), 3.964–3.980 (m, 1H, H-4′), 4.389 (t, 2H, CH_2-triazole, $J = 6.0$), 4.431–4.454 (m, 1H, H-3′), 4.561 (bs, 1H, B-C-H), 6.267–6.295 (m, 1H, H-1′), 7.794 (s, 1H, CH-triazole), 8.240 (s, 1H, H-6); ^{13}C NMR (150.94 MHz, CD_3OD) δ (ppm): 19.93 (CH_2), 21.04 (CH_2), 21.09 (CH_2), 27.10 (CH_2), 30.76 (CH_2), 31.91 (CH_2), 36.63 (CH_2), 42.66 (C-2′), 51.01 (CH_2-triazole), 63.62 (C-5′), 64.76 (C-carborane), 73.06 (C-3′), 87.92 (C-1′), 90.11 (C-4′), 102.29 (C-5), 124.29 (CH-triazole), 145.20 (C-6), 152.26 (C═O), 165.65 (C═O); TLC CH_2Cl_2/MeOH (9:1 v/v) $R_f = 0.50$; MS (ESI, −VE): $m/z = 572$ $[M-1]^-$, calcd for $C_{23}H_{39}B_{10}N_5O_6 = 573.70$.

4.2. Biological investigations

4.2.1. Cytotoxicity assay

The MRC-5 (ATCC CCL-171; American Type Culture Collection, Rockville, MD), A549 (ATCC CCL-185), LLC-MK2 (ATCC-CCL-7.1), Vero (ATCC CCL-81), and L929 (ATCC CCL-1) cells were propagated in Eagle's minimal essential medium (EMEM) supplemented with 10% inactivated foetal bovine serum (FBS), 2 mM L-glutamine, and 100 units/mL penicillin G with 100 mg/mL streptomycin (Sigma-Aldrich Co., Ayrshire, UK). The cells were cultured at 37 °C in a humidified 5% CO_2 atmosphere. The cytotoxic activities of compounds **9**–**17** and **19**–**21** were examined against the cell lines using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma-Aldrich Co.) assay as described previously [21,29]. Briefly, the cells were seeded at 2×10^4 cells per well into 96-well microtiter plates and allowed to proliferate at 37 °C for 24 h. Confluent monolayers of cells were treated with different concentrations of compounds (0.01–1000 μM) in triplicate or replaced with fresh medium (untreated controls). The compounds were first dissolved in DMSO (Sigma-Aldrich Co.) to form drug solutions and then suspended in EMEM supplemented with 2% FBS and antibiotics. The final concentration of DMSO in the medium was 0.1%. After a 2-day incubation at 37 °C in 5% CO_2, the number of viable cells was determined by the formazan method based on the conversion of tetrazolium salt MTT to formazan by the living cells [29,30]. The CC_{50} was defined as the concentration required to reduce the cell number by 50% compared to that for the untreated

controls. The cell viability was evaluated as the mean value density resulting from six mock-treated cell controls. The CC_{50} was calculated by linear regression analysis of the dose response curves obtained from the data.

4.2.2. Antiviral assay

The compounds **9**–**17**, and **19**–**21** were evaluated for their ability to inhibit the replication of selected DNA (HSV-1, HCMV) and RNA (HPIV-3, EMCV, and VSV) viruses *in vitro*. For the plaque reduction assay, confluent MRC-5 cells grown in 96-well microtiter plates were inoculated with 20 PFU (plaque forming units) of HCMV per well. After a 2-h adsorption period, the residual virus was removed, and the infected cells were further incubated with EMEM supplemented with 2% FBS containing serial dilutions of the compounds (0.01 μM-1000 μM). After 5–7 days of incubation at 37 °C (5% CO_2), the cells were fixed with methanol for 15 min and stained with 0.05% methylene blue for 15 min. The number of HCMV plaques was counted under microscope. The IC_{50} (50% inhibitory concentration) was determined as the compound concentration required to reduce the number of viral plaques to 50% of the inoculum (virus infected but untreated).

For cytopathic effect (CPE), inhibitory assays were carried out in one-day-old confluent cell monolayers growing in 96-well microtiter plates. The cell cultures were inoculated with 100 μL of respective virus suspension in the medium (HPIV-3 on LLC-MK2, HSV-1 on Vero, VSV on L929, and EMCV on A549) containing approximately 100 $CCID_{50}$ (50% cell culture infective doses)/well. After adsorption at 37 °C for 1 h, virus inoculum was removed, and the medium containing the various concentrations of the compounds was added. The cell monolayers were treated with the compounds for 48–72 h until typical CPE was visible. The viruses that induced CPE were measured by inverted light microscopy and evaluated by the MTT assay. Untreated virus controls and uninfected untreated cell controls were included in all the assays. Antiviral activity was expressed as the IC_{50} (50% inhibitory concentration) and concentration producing 50% inhibition of virus-induced cytopathic effect compared to the untreated control [29].

4.2.3. Phosphorylation transfer assay (PTA), adenosine kinase (ADK)

The assay was performed as described previously with minor modifications [31]. The reaction mixtures contained 0.25 mM of compounds **15**–**20**, and uridine or 2′-deoxyuridine were used as standards, 50 mM Tris-HCl (pH 7.6), 0.5 mg/mL BSA (bovine serum albumin), 5 mM $MgCl_2$, 125 mM KCl, 10 mM dithiothreitol (DTT), and 125 μM ATP (with 0.03 μM [γ −32 P]-ATP and 0.5 μg of the enzyme (TK1, TK2 or DCK). The reaction mixtures were incubated at 37 °C for 20 min. Following the incubation period, the enzyme was heat inactivated for 5 min at 100 °C. The reaction mixture was centrifuged (10 min, 6000 rpm) and 2-μL sample portions were spotted on silica gel TLC plates. The TLC plates were developed in a solvent system containing 1-propanol, NH_{3aq} (25%) and H_2O (7:1:2 v/v). The radiograms were obtained by exposure to a storage phosphor screen and documented with a Typhoon 8600 scanner (Molecular Dynamics). The radiograms were quantified using the Quantity One (ver. 4.6.6 basic, BIORAD) computer program. Values for the tested compounds were expressed relative to the unmodified nucleoside. The analyses were made in triplicate and are presented as the mean values with standard deviation (±SD) quantified in Excel (Microsoft Office, Professional Edition, 2003).

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Appendix A. Supplementary data

Supplementary data related to this article can be found at doi 10.1016/j.jorganchem.2018.03.026.

References

[1] P. Herdwijn (Ed.), Modified Nucleosides: in Biochemistry, Biotechnology and Medicine, Wiley-VCH, Weinheim, 2008.
[2] E. De Clercq, G. Li, Clin. Microbiol. Rev. 29 (2016) 695–747.
[3] J. Shelton, X. Lu, J.A. Hollenbaugh, J.H. Cho, F. Amblard, R.F. Schinazi, Chem. Rev. 116 (2016) 14379–14455.
[4] O. Sari, V. Roy, J. Balzarini, R. Snoeck, G. Andrei, L.A. Agrofoglio, Eur. J. Med. Chem. 53 (2012) 220–228.
[5] E. De Clercq, Biochem. Pharmacol. 68 (2004) 2301–2315.
[6] W.A. Cristofoli, L.I. Wiebe, E. De Clercq, G. Andrei, R. Snoeck, J. Balzarini, E.E. Knaus, J. Med. Chem. 50 (2007) 2851–2857.
[7] M.V. Skorobogatyi, A.V. Ustinov, I.A. Stepanova, A.A. Pchelintseva, A.L. Petrunina, V.L. Andronova, G.A. Galegov, A.D. Malakhov, V.A. Korshun, Org. Biomol. Chem. 4 (2006) 1091–1096.
[8] E. De Clercq, J. Descamps, J. Balzarini, J. Giziewicz, P.J. Barr, M.J. Robins, J. Med. Chem. 26 (1983) 661–666.
[9] D. Rai, M. Johar, T. Manning, B. Agrawal, D.Y. Kunimoto, R. Kumar, J. Med. Chem. 48 (2005) 7012–7017.
[10] M. Białek-Pietras, A.B. Olejniczak, E. Paradowska, M. Studzińska, P. Suski, A. Jabłońska, Z.J. Leśnikowski, J. Organomet. Chem. 798 (2015) 99–105.
[11] A. Ilinova, A. Semioshkin, I. Lobanova, V.I. Bregadze, A.F. Mironov, E. Paradowska, M. Studzińska, A. Jabłońska, M. Białek-Pietras, Z.J. Leśnikowski, Tetrahedron 70 (2014) 5704–5710.
[12] M.V. Skorobogatyi, A.A. Pchelintseva, A.L. Petrunina, I.A. Stepanova, V.L. Andronova, G.A. Galegov, A.D. Malakhov, V.A. Korshun, Tetrahedron 62 (2006) 1279–1287.
[13] S. Meneni, I. Ott, C.D. Sergeant, A. Sniady, R. Gust, R. Dembinski, Bioorg. Med. Chem. 15 (2007) 3082–3088.
[14] V.V. Rostovtsev, L.G. Green, V.V. Fokin, K.B. Sharpless, Angew. Chem. 114 (2002) 2708–2711.
[15] M. Meldal, C.W. Tornoe, Chem. Rev. 108 (2008) 2952–3015.
[16] F. Amblard, J.H. Cho, R.F. Schinazi, Chem. Rev. 109 (2009) 4207–4220.
[17] A.H. El-Saqheer, T. Brown, Chem. Soc. Rev. 39 (2010) 1388–1405.
[18] B.A. Wojtczak, A.B. Olejniczak, Z.J. Lesnikowski, in: S.L. Beaucage (Ed.), Current Protocols in Nucleic Acid Chemistry, John Wiley & Sons, Inc., 2009, pp. 1–26 (chapter 4), unit 4.37.
[19] M. Matuszewski, A. Kiliszek, W. Rypniewski, Z.J. Lesnikowski, A.B. Olejniczak, New J. Chem. 39 (2015) 1202–1221.
[20] T. Mosmann, J. Immunol. Meth. 65 (1983) 55–63.
[21] Z.J. Lesnikowski, E. Paradowska, A.B. Olejniczak, M. Studzinska, P. Seekamp, U. Schüßler, D. Gabel, R.F. Schinazi, J. Plešek, Bioorg. Med. Chem. 13 (2005) 1168–4175.
[22] A. Varga, C. Lionne, B. Roy, Curr. Drug Metabol. 17 (2016) 237–252.
[23] H.K. Agarwal, A. Khalil, K. Ishita, W. Yang, R.J. Nakkula, L.C. Wu, T. Ali, R. Tiwari, Y. Byun, R.F. Barth, W. Tjarks, Eur. J. Med. Chem. 100 (2015) 197–209.
[24] B. Munch-Petersen, J. Piškur, in: G.J. Peters (Ed.), Cancer Drug Discovery and Development: Deoxynucleoside Analogs in Cancer Therapy, Humana Press Inc., Totowa, NJ, 2007, pp. 53–79.
[25] S. Eriksson, B. Kierdaszuk, B. Munch-Petersen, B. Oberg, N.G. Johansson, Biochem. Biophys. Res. Commun. 176 (1991) 586–592.
[26] R.F. Barth, W. Yang, G. Wu, M. Swindall, Y. Byun, S. Narayanasamy, W. Tjarks, K. Tordoff, M.L. Moeschberger, S. Eriksson, P.J. Binns, K.J. Riley, Proc. Natl. Acad. Sci. U.S.A. 15 (2008) 17493–17497.
[27] B.A. Wojtczak, A.B. Olejniczak, L. Wang, S. Eriksson, Z.J. Leśnikowski, Nucleos Nucleot. Nucleic Acids 32 (2013) 571–588.
[28] G.V.S. Reddy, G.V. Rao, R.V.K. Subramanyam, D.S. Iyengar, Synth. Commun. 30 (2000) 2233–2237.
[29] A.B. Olejniczak, A.M. Adamska, E. Paradowska, M. Studzińska, P. Suski, Z.J. Leśnikowski, Acta Pol. Pharm. 70 (2013) 489–504.
[30] K. Berg, M.B. Hansen, S.E. Nielsen, APMIS 98 (1990) 156–162.
[31] G.T. Lu, Y.Q. Tang, C.Y. Li, R.F. Li, S.Q. An, J.X. Feng, Y.Q. He, B.L. Jiang, D.J. Tang, J.L. Tang, J. Bacteriol. 191 (2009) 3639–3648.