

## MODIFICATION OF SELECTED ANTI-HCMV DRUGS WITH LIPOPHILIC BORON CLUSTER MODULATOR

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**Abstract:** Methods for the modification of ganciclovir (GCV), acyclovir (ACV), cidofovir (CDV) and valganciclovir (VCDV) with boron cluster have been developed. Toxicity of the new derivatives was evaluated in adherent cells; no cytotoxicity was observed in five different cell lines up to 1000  $\mu\text{M}$  with the exception of modified valganciclovir which was cytotoxic above 300  $\mu\text{M}$ . The compounds were active against HCMV or HSV-1 by cytopathic effect or plaque reduction assays. None of the tested compounds had activity against HPIV-3 or VSV.

**Keywords:** nucleosides, boron clusters, anti-HCMV drugs.

Human cytomegalovirus (HCMV), a *betaherpesviridae*, infects about 60% of adults in developed world and more than 90% of third world populations (1). In the immunocompetent host initial infection and reactivation of latent infection are usually asymptomatic. However, in hosts with impaired cellular immune functions, such as transplant recipients, persons infected with human immunodeficiency virus (HIV) or undergoing anticancer chemotherapy and/or radiotherapy, the full pathogenic potential of the virus may be realized (2, 3).

Other kinds of problem prevail in perinatal cases of HCMV infection. HCMV is the most common cause of viral intrauterine infection affecting from 0.4 to 2.3% of live-born infants. In pregnant, immunocompetent women infections with HCMV are usually asymptomatic or resembling mononucleosis, and often give rise to undetected latent infections and reinfections. However, severe infections may occur among congenitally infected fetuses and infants due to immaturity of immune system in neonates (4).

Investigations over many years indicate that HCMV load is the critical factor in pathogenesis: symptomatic patients exhibit elevated levels of HCMV replication, compared with those in person who remain asymptomatic (5). In addition, the classic view of HCMV as slowly replicating virus is

challenged by recent *in vivo* finding suggesting that active replication occurs dynamically in the human host, with doubling time of approximately one day (6). It should be therefore pointed out that with currently available anti-HCMV drugs the virus load can be often successfully controlled. Though the existing armamentarium of anti-HCMV drugs is limited, antiviral therapies for HCMV have been developed and employed as either prophylactic or pre-emptive therapies to prevent HCMV disease or as direct treatment for the disease if HCMV DNAemia in plasma, serum, and whole blood is detected (7).

Although antiviral chemotherapy does not completely eradicate the virus, HCMV treatments have improved dramatically over the past decade: in 1995 a ganciclovir (Cytovene®) pill was approved to prevent HCMV, in 1996 cidofovir (Vistide®) was approved for intravenous use, in 1998 formivirsen (Vitracene®) was approved for injection into the eye to treat retinitis, and in 2001 a valganciclovir (Valcyte®) was certified. Recently high-dose of anti-herpes simplex virus (HSV) drug acyclovir (Zovirax®) has also been used as prophylactic against HCMV with some successes. The list is completed by the foscarnet (Foscavir®), a structural mimic of the pyrophosphate inhibiting viral DNA polymerase, known from many years.

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However, the currently available anti-HCMV drugs have several drawbacks that limit their clinical utility (8). Some of the compounds have limited oral bioavailability and thus must be administered intravenously. In addition, most of the anti-HCMV drugs exhibit significant toxicity. The emergence of drug-resistant viral strains also poses an increasing problem for disease management. Since most of the approved anti-HCMV compounds share a similar mechanism of action, targeting the viral DNA polymerase, mutant viruses resistant to one drug are commonly resistant to others, although this is not a rule. Finally, the safety and efficacy of the currently available drugs in the treatment of congenital HCMV infection is the subject of some debate despite the availability of results from several trials (9-11). Thus, there is still a strong need to identify new targets for anti-HCMV chemotherapy and to develop novel antiviral compounds and treatment strategies.

Anti-infectious disease drugs bearing essential boron component forms an area of medicinal chemistry still awaiting exploration. Herein, we propose use of carboranes - members of the vast boron cluster family for modification of selected anti-HCMV drugs. The use of carboranes as pharmacophores was made popular by the pioneering works of Endo and colleagues (12). However, prior to the introduction of this terminology, the hydrophobicity of carboranes was already used to trigger desired biological actions (13). Due to their spherical shape and pronounced lipophilicity, in most cases carboranes are integrated as substitutes for organic ring systems. The literature comprises numerous examples in which carboranes are used as surrogates for heterocycles, annulated carbon rings, or most popularly, due to the benzene analogy, for substituted or unsubstituted phenyl rings (14).

Demonstrated by  $\beta$ -D-2',3'-didehydro-2',3'-dideoxythymidine (D4T),  $\beta$ -D-2',3'-didehydro-2',3'-dideoxy-5-fluorocytidine (D-D4FC), and certain others 2',3'-didehydro-2',3'-dideoxy-nucleosides (d4N) potent antiviral activity against HIV and HBV encouraged synthesis of the carborane-containing d4-nucleosides (15). In this direction number of modified sugar residues was introduced to the carborane-containing nucleosides and the obtained derivatives were tested for antiviral and anticancer activity (16).

In this communication, an approach to the synthesis and preliminary evaluation of new type of potential therapeutically active compounds, derivatives of known anti-HCMV preparations, bearing phosphoric or phosphonate acid residue and highly

lipophilic *para*-carborane cluster (1,12-dicarba-closo-dodecaborane,  $C_{2}B_{10}H_{12}$ ), is described.

## EXPERIMENTAL

### Materials

Most of the chemicals were obtained from Aldrich Chemical Company and used without further purification unless stated. GCV, ACV, CDV and VCDV were purchased from TCS Industry LTD (P.R. China). Flash chromatography was performed using silica gel 60 (230-400 mesh, ASTM, Aldrich Chemical Company).  $R_f$  values refer to 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 by use of UV light (254 nm) or 0.5% acidic solution of  $PdCl_2$  in HCl/methanol for boron containing derivatives. The yields were not optimized.

(2-Hydroxypropyl)- $\beta$ -cyclodextrin (HPBCD), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium (MTT), and fetal bovine serum (FBS) were bought from Sigma-Aldrich, Co. (USA). Minimum essential medium Eagle (MEM), Dulbecco's modified Eagle's medium (DMEM), L-glutamine, penicillin G - streptomycin, dimethyl sulfoxide (DMSO) and methyl blue as well as GCV and ACV used as reference in cytotoxicity and antiviral activity tests, were from Sigma-Aldrich (Germany, UK, USA). Dimethylformamide (DMF) was from POCH (Poland) and sodium dodecyl sulfate (SDS) was from Bioshop (Canada). The 96-well culture plates were bought from Nunc (Denmark).

The human diploid embryonic lung MRC-5 cells (ATCC CCL-171), human lung A549 cells (ATCC CCL-185), monkey kidney cells LLC-MK2 (ATCC CCL-7.1) and Vero (ATCC CCL-81), were bought from American Type Culture Collection (USA). Human cytomegalovirus Towne strain (HCMV, ATCC VR-977), human parainfluenza virus type 3 - C243 strain (HPIV-3, ATCC VR-93), human herpes simplex type 1 - McIntyre strain (HSV-1, ATCC VR-539) were bought from ATCC. The mouse fibroblast L-929 cell line and vesicular stomatitis virus (VSV) were provided by Prof. Z. Blach-Olszewska (Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Poland).

The purity of compounds: **11**, **14**, **17**, **20** and **22** was established by HPLC and was more than 95%. **11**,  $t_R = 16.49$  min; **14**,  $t_R = 21.51$  min; **17**,  $t_R = 22.98$  and  $23.21$  min (two separable signals for two diastereomers); **20**,  $t_R = 22.35$  min; **22**,  $t_R = 21.33$  min (for HPLC conditions see below).

## Chemistry

The structures of all the compounds were confirmed by NMR. For modified ganciclovir **11**  $^1\text{H}$ -,  $^{13}\text{C}$ -,  $^{31}\text{P}$ - and  $^{11}\text{B}$ -NMR spectra were recorded on a Bruker Avance DPX 250 MHz spectrometer equipped with BB inverse probe-head, the spectra for  $^1\text{H}$ ,  $^{13}\text{C}$ ,  $^{31}\text{P}$ , and  $^{11}\text{B}$  nuclei were recorded at 250.13 MHz, 62.90 MHz, 101.26 MHz and 80.25 MHz, respectively. Tetramethylsilane was used as standard for  $^1\text{H}$ -NMR and  $^{13}\text{C}$ -NMR, 75% aqueous solution of  $\text{H}_3\text{PO}_4$  was used as standard for  $^{31}\text{P}$ -NMR,  $\text{BF}_3/(\text{C}_2\text{H}_5)_2\text{O}$  was used as standard for  $^{11}\text{B}$ -NMR.

For modified acyclovir **14**, cidofovir **17**, **20** and valganciclovir **22**  $^1\text{H}$ -,  $^{13}\text{C}$ -,  $^{31}\text{P}$ - and  $^{11}\text{B}$ -NMR spectra were recorded on a Bruker Avance III 600 MHz spectrometer equipped with direct ATM probe, the spectra for  $^1\text{H}$ ,  $^{13}\text{C}$ ,  $^{31}\text{P}$  and  $^{11}\text{B}$  nuclei were recorded at 600.26 MHz, 150.94 MHz, 242.99 MHz and 192.59 MHz, respectively. Deuterated solvents were used as standards for  $^1\text{H}$ -NMR and  $^{13}\text{C}$ -NMR., 75% aqueous solution of  $\text{H}_3\text{PO}_4$  was used as standard for  $^{31}\text{P}$ -NMR.  $\text{BF}_3/(\text{C}_2\text{H}_5)_2\text{O}$  was used as standard for  $^{11}\text{B}$ -NMR. All chemical shifts are reported in ppm ( $\delta$ ) relative to external standards. The following abbreviations were used to explain the multiplicities: s = singlet, d = doublet, dd = doublet of doublets, t = triplet, dt = doublet of triplets, q = quartet, quin = quintet, bs = broad singlet, m = multiplet. Coupling constants are reported in hertz.

Fast atom bombardment (FAB, Gly) mass spectra were recorded on a Finnigan MAT (Bremen, Germany). The MS  $m/z$  were measured in a positive and negative mode. Calculation of the theoretical molecular mass was performed using "Show Analysis Window" option in ChemDraw 8.0 program. The calculated  $m/z$  corresponds to calculated values based on the average mass of the elements consisting natural isotopes. In practice, usually the most intense signal in multiple peaks corresponds closely to the molecular weight.

UV measurements were performed on GBC Cintra10 UV-VIS spectrometer (Dandenong, Australia). Samples for UV experiments, ca. 0.5  $A_{260}$  ODU (optical unit) of each compound were dissolved in 95%  $\text{C}_2\text{H}_5\text{OH}$ . The measurement was performed at ambient temperature.

HPLC analysis of compounds **11**, **14**, **17**, **20** and **22** was performed on the Hewlett-Packard 1050 system, using Econosil RP C18 5  $\mu\text{m}$ ,  $4.6 \times 250$  mm column. All analyses were run at ambient temperature. HPLC conditions were as follows: 20 min from 0% D to 100% D, 5 min 100% D, 5 min to 0% D. Buffer A contained 0.1 M TEAB (pH 7.0) in a mix-

ture of acetonitrile (for HPLC, J.T. Baker, Deventer, The Netherlands) and water (MiliQ) (1 : 49, v/v), buffer D contained 0.1 M TEAB (pH 7.0) in a mixture of acetonitrile and water (3 : 2, v/v). Flow rate 1.0 mL/min, the UV detection was performed at  $\lambda = 268$  nm.

## Stability in buffers with different pH and Eagle's minimal essential medium

The stock solution of compounds **11**, **14**, **17**, **20** and **22** was prepared by dissolving a sample of the compound (ca. 0.3 mg) in DMSO (Sigma Aldrich) (ca. 1  $\mu\text{L}$ ) followed by water solution containing 1% of hydroxypropyl- $\beta$ -cyclodextrin (Sigma Aldrich) (49  $\mu\text{L}$ ). Final concentration of the resultant homogeneous solution of the test compounds was  $1 \times 10^{-2}$  M. The working solutions were prepared by mixing suitable aliquots of the stock and buffer with desired pH (4, 7, 9) or Eagle's minimal essential medium (Sigma Aldrich) (with Eagle's salts, L-glutamine,  $\text{NaHCO}_3$ , and 2% serum) (50  $\mu\text{L}$ ). The final concentration of the compounds was  $0.5 \times 10^{-2}$  M, the final concentration of the DMSO and HPBCD did not exceed 1% and 0.5%, respectively.

## Stability in buffers with different pH

All the tests were conducted at 50°C in Wealtec Block Heater, protecting solutions of the compounds against light to avoid potential photolytic effect and against oxygen. Aliquots (0.01 mg, ca. 5-8  $\mu\text{L}$ ) were withdrawn from the incubation mixture after 1, 2, 4, 6, 8, 12 and 24 h, placed into Eppendorf tube and stored at -80°C before HPLC analysis.

## Stability in Eagle's minimal essential medium

All the tests were carried out at 37°C in Wealtec Block Heater. Aliquots (0.01 mg, ca. 5-8  $\mu\text{L}$ ) were withdrawn from the incubation mixture after every 1 h during first 12 h then after 24 and 48 h. Next, the procedure was as described above.

## Determination of lipophilicity using RP-TLC analysis of compounds **11**, **14**, **17**, **20**, **22** and unmodified counterparts: ganciclovir, acyclovir, cidofovir and valganciclovir (37)

Sample of the compounds (0.1 mg) was dissolved in methanol (POCH, Poland) (40  $\mu\text{L}$ ) and DMSO (Aldrich) (2  $\mu\text{L}$ ) then aliquot of the solution (1  $\mu\text{L}$ ) was spotted on the RP-TLC plate, 1.5 cm from the bottom edge. TLC chromatography was performed on  $5 \times 10$  cm pre-coated  $\text{RP}_{18}$   $\text{F}_{254\text{S}}$  plates from Merck. The plates were developed in glass chamber previously saturated with mobile phase

vapor for 30 min. Mobile phases: mixture of acetonitrile (POCH, Poland) and water (MiliQ) with acetonitrile content between 45 and 80% (v/v), for **14**, **17**, **20** and **22**, 10 and 40% (v/v), for **11**, and between 10 and 25% (v/v) for the unmodified counterparts with 5% increments. All measurements were performed at ambient temperature. After development and drying of the plates, the spots were visualized under UV light at  $\lambda = 254$  nm.  $R_f$  values were the means from three independent experiments.  $R_M$  values were calculated from experimental  $R_f$  by use of the equation  $R_M = \log(1/R_f - 1)$ .  $R_M$  values were then extrapolated to zero acetonitrile concentration by use of equation  $R_M = R_{M0} + aC$ , where  $C$  is the concentration of acetonitrile in the mobile phase (% v/v).

#### Synthesis of 2*N*-isobutyryl-9-[[1,3-dihydroxypropan-2-yl]oxy]methyl]guanine (**4**)

Compound **1** (0.5 g, 1.96 mmol) was dried by co-evaporation with anhydrous pyridine (3 × 4 mL) and then suspended in anhydrous pyridine (20 mL). To the resultant suspension trimethylchlorosilane (3.06 mL, 24.11 mmol) was added. After stirring for 15 min at room temperature, to formed 9-[[1,3-*O*,*O*-di(*tert*-butyldimethylsilyl)propan-2-yl]oxy]methyl]guanine (**2**) isobutyric anhydride (3.25 mL, 19.59 mmol) was added *in situ* and the solution was maintained at room temperature for 3 h. The reaction mixture was then cooled in an ice bath and next, water (5 mL) was added. After 5 min, resultant 2*N*-isobutyryl-9-[[1,3-*O*,*O*-di(*tert*-butyldimethylsilyl)propan-2-yl]oxy]methyl]guanine (**3**) was treated without isolation, with 25% aqueous ammonia (5 mL), then the reaction was stirred for additional 15 min. The solution was then evaporated to near dryness and to the residue water (15 mL) was added. The water solution was washed with a mixture of ethyl acetate and ethyl ether (5 mL, 1:1, v/v), then the water and organic layers were separated. The organic layer was extracted with water (15 mL), then the water fractions were combined and evaporated to dryness under vacuum yielding crude product **4**. The crude product was purified by silica gel column chromatography (230-400 mesh, 20 g) using gradient elution: 0-40% CH<sub>3</sub>OH/CH<sub>2</sub>Cl<sub>2</sub>.

Yield: 76.8%;  $R_f$ : 0.54 (CH<sub>2</sub>Cl<sub>2</sub> : CH<sub>3</sub>OH, 8 : 2, v/v); UV (95% C<sub>2</sub>H<sub>5</sub>OH,  $\lambda$ , nm):  $\lambda_{\min} = 225, 272, \lambda_{\max} = 255, 260, 281, \lambda_{\text{sh}} = 257$ ; <sup>1</sup>H NMR (250 MHz, CD<sub>2</sub>Cl<sub>2</sub>,  $\delta$ , ppm): 1.24 (d, 6H, 2 × CH<sub>3</sub> of isobutyryl group), 2.50-2.55 (m, 1H, CH of isobutyryl group), 3.50-3.90 (m, 5H, 1H-2, 2H-1, 2H-3), 5.59 (s, 2H, H-4), 7.76 (s, 1H, H-8 from guanine); MS ( $m/z$ ): 326.2 [M+1]<sup>+</sup>, 324.2 [M - 1]<sup>-</sup>.

#### Synthesis of 2*N*-isobutyryl-9-[[1-(dimethoxytrityl-3-hydroxypropan-2-yl)oxy]methyl]guanine and 2*N*-isobutyryl-9-[[1-(1-hydroxy-3-dimethoxytritylpropan-2-yl)oxy]methyl]guanine (**5**), racemic mixture

Compound **4** (0.49 g, 1.51 mmol) was dried by co-evaporation with anhydrous pyridine (3 × 3 mL), then was suspended in anhydrous pyridine (6 mL). Next, triethylamine (271  $\mu$ L, 1.94 mmol) and 4,4'-dimethoxytrityl chloride (0.56 g, 1.66 mmol) were added and the reaction mixture was stirred at room temperature. After 4 h, to the reaction mixture methanol (9.5 mL) was added and the whole was stirred for 10 min, then solvents were evaporated to near dryness and the residue was dissolved in dichloromethane containing 1% of triethylamine (50 mL). The obtained solution was washed once with saturated solution of sodium hydrogen carbonate (20 mL). The organic layer was dried over anhydrous magnesium sulfate, filtered and then the solvents were evaporated. Crude product was purified by column chromatography on silica gel column (230-400 mesh, 30 g) using gradient elution: 0-10% CH<sub>3</sub>OH/CH<sub>2</sub>Cl<sub>2</sub>.

Yield: 53%;  $R_f$ : 0.29 (CH<sub>2</sub>Cl<sub>2</sub> : CH<sub>3</sub>OH, 95 : 5, v/v); UV (95% C<sub>2</sub>H<sub>5</sub>OH,  $\lambda$ , nm):  $\lambda_{\min} = 223, 269, \lambda_{\max} = 236, 277, \lambda_{\text{sh}} = 258$ ; <sup>1</sup>H NMR (250 MHz, CD<sub>2</sub>Cl<sub>2</sub>,  $\delta$ , ppm): 1.21 (d, 6H, 2 × CH<sub>3</sub> of isobutyryl group), 2.53-2.59 (m, 2H, 1H-2, CH of isobutyryl group), 3.16-3.62 (2 × m, 4H, 2H-1, 2H-3), 3.82 (s, 6H, 2 × OCH<sub>3</sub> of dimethoxytrityl group), 5.63 (d, 2H, H-4), 6.83-6.85 (m, 4H, 2 × CH<sub>3</sub>Oph of dimethoxytrityl group), 7.26-7.44 (m, 9H, dimethoxytrityl group), 7.78 (s, 1H, H-8 from guanine), 11.89 (s, 1H, NH-3 from guanine); <sup>13</sup>C NMR (62.90 MHz, CD<sub>2</sub>Cl<sub>2</sub>,  $\delta$ , ppm): 19.06 (2 × CH<sub>3</sub> of isobutyryl group), 36.67 (CH of isobutyryl group), 55.60 (2 × OCH<sub>3</sub> of dimethoxytrityl group), 63.09 (C-1), 64.18 (C-3), 73.28 (C-4), 79.49 (C-2), 86.69 (C-methylidene of dimethoxytrityl group), 113.44 (C of dimethoxytrityl group), 121.44 (C-5 from guanine), 127.20, 128.18, 128.38, 130.34, 136.15 (C of dimethoxytrityl group), 139.47 (C-8 from guanine), 145.24 (C of dimethoxytrityl group), 148.59 (C-2 from guanine), 149.20 (C-6 from guanine), 156.11 (C-4 from guanine), 159.05 (C of dimethoxytrityl group), 179.48 (CO from guanine); HPLC  $t_R$ : 23.06 min.

#### Synthesis of 2*N*-isobutyryl-9-[[1-(*O*-dimethoxytrityl-3-propoxy-2-yl)oxy]methyl]guanine 3-*O*-phosphonate and 2*N*-isobutyryl-9-[[1-(propoxy-3-*O*-methoxytrityl-2-yl)oxy]methyl]guanine 1-*O*-phosphonate (**6**), triethylammonium salt, racemic mixture

Imidazole (0.5 g, 7.36 mmol) was dissolved in tetrahydrofuran (25.7 mL) and the solution was cooled to  $-10^{\circ}\text{C}$  in dry ice/acetone bath. Then, phosphorous trichloride (209  $\mu\text{L}$ , 2.4 mmol) was added with vigorous stirring followed by triethylamine (1.06 mL, 7.71 mmol) mixed with tetrahydrofuran (856  $\mu\text{L}$ ). The reaction mixture was stirred for 30 min at  $-10^{\circ}\text{C}$  and then was cooled to  $-78^{\circ}\text{C}$  in dry ice/acetone bath. To the solution of resultant phosphorimidazolidite intermediate a protected ganciclovir **5** (0.43 g, 0.69 mmol) in tetrahydrofuran (17.7 mL) was added *in situ*, dropwise, during 30 min, and next, the reaction mixture was stirred at  $-78^{\circ}\text{C}$ . After 1 h 2 M TEAB (25.5 mL) was added followed by dichloromethane (50 mL). The organic layer was separated, washed once with 2 M TEAB (25.5 mL), dried over anhydrous magnesium sulfate, filtered and evaporated under reduced pressure to dryness. Crude product **6** was purified by silica gel column chromatography (230-400 mesh, 16 g) using gradient elution: 0-50%  $\text{CH}_3\text{OH}/\text{CH}_2\text{Cl}_2$  (with 1% of triethylamine added).

Yield: 79.7%;  $R_f$ : 0.31 ( $\text{CH}_2\text{Cl}_2$  :  $\text{CH}_3\text{OH}$ , 8 : 2, v/v); UV (95%  $\text{C}_2\text{H}_5\text{OH}$ ,  $\lambda$ , nm):  $\lambda_{\text{min}} = 223$ , 271,  $\lambda_{\text{max}} = 236$ , 282,  $\lambda_{\text{sh}} = 252$ , 256, 275;  $^1\text{H}$  NMR (250 MHz,  $\text{CH}_3\text{OD}$ ,  $\delta$ , ppm): 1.13 (d, 6H, 2  $\times$   $\text{CH}_3$  of isobutyryl group), 1.27 (m, 9H, 3  $\times$   $\text{CH}_3$  of triethylamine), 2.72 (m, 1H, CH of isobutyryl group), 2.97-3.03 (m, 2H, H-3), 3.15 (q, 6H, 3  $\times$   $\text{CH}_2$  of triethylamine), 3.80-4.25 (m, 3H, 2H-1, 1H-2), 3.73 (s, 6H, 2  $\times$   $\text{OCH}_3$  of dimethoxytrityl group), 6.68 (d, 1H, P-H,  $^1J_{\text{P-H}} = 662.25$  Hz), 5.69 (d, 2H, H-4), 6.74-6.77 (m, 4H, 2  $\times$   $\text{CH}_3\text{O}$  of dimethoxytrityl group), 7.05-7.20 (m, 9H, dimethoxytrityl group), 8.15 (s, 1H, H-8 from guanine);  $^{13}\text{C}$  NMR (62.90 MHz,  $\text{CH}_3\text{OD}$ ,  $\delta$ , ppm): 9.22 (C of triethylamine), 19.49 (2  $\times$   $\text{CH}_3$  of isobutyryl group), 36.96 (CH of isobutyryl group), 55.71 (2  $\times$   $\text{OCH}_3$  of dimethoxytrityl group), 64.86 (C-1, C-3), 73.99 (C-4), 79.76 (C-2), 87.25 (C-methylidene of dimethoxytrityl group), 113.99, 127.71, 128.68, 129.18, 131.09, 137.01, 137.17 (C of dimethoxytrityl group), 141.76 (C-8 from guanine), 146.17, 159.99 (C of dimethoxytrityl group), 215.32 (C of CO from guanine);  $^{31}\text{P}$  NMR (101.26 MHz,  $\text{CH}_3\text{OD}$ ,  $\delta$ , ppm): 6.11 (s, 1P, PH of phosphonate group); MS (m/z): 690.4 [ $\text{M} - 1$ ]; HPLC  $t_R$ : 18.81 min.

**Synthesis of 2*N*-isobutyryl-9-[(1-*O*-dimethoxytrityl-3-propoxy-2-yl)oxy]methyl]guanine-(3-propoxy-1-yl)-*para*-carborane 3-*O*-phosphonate and 2*N*-isobutyryl-9-[(1-propoxy-3-*O*-dimethoxytrityl-2-yl)oxy]methyl]guanine-(3-propoxy-1-yl)-*para*-carborane 1-*O*-phosphonate (**7**), mixture of diastereomers**

Compound **6** (0.15 mg, 0.22 mmol) and 1-(3-hydroxypropyl)-*para*-carborane (40.3 mg, 0.2 mmol) were mixed together and dried by co-evaporation with anhydrous pyridine (3  $\times$  2 mL) (21). The gummy residue was dissolved in pyridine (2 mL) and while stirring pivaloyl chloride (73.5  $\mu\text{L}$ , 0.6 mmol) was added in one portion. After reaction completion (ca. 0.5 h, TLC control) 2 M TEAB (200  $\mu\text{L}$ ) was added into reaction mixture which was then concentrated by evaporation under vacuum, then, the residue was portioned between dichloromethane (6 mL) and 0.5 M TEAB (200  $\mu\text{L}$ ). The organic layer was collected, dried over anhydrous magnesium sulfate, then the solvent was evaporated. The crude product containing modified *H*-phosphonate **7** was purified on a silica gel column (230-400 mesh, 9 g) using gradient elution: 0-3%  $\text{CH}_3\text{OH}/\text{CH}_2\text{Cl}_2$  (with 1% of triethylamine added).

Yield: 58%;  $R_f$ : 0.65 ( $\text{CH}_2\text{Cl}_2$  :  $\text{CH}_3\text{OH}$ , 9 : 1, v/v); UV (95%  $\text{C}_2\text{H}_5\text{OH}$ ,  $\lambda$ , nm):  $\lambda_{\text{min}} = 223$ , 270,  $\lambda_{\text{max}} = 236$ , 282,  $\lambda_{\text{sh}} = 253$ , 260, 276;  $^1\text{H}$  NMR (250 MHz,  $\text{CD}_2\text{Cl}_2$ ,  $\delta$ , ppm): 1.00-2.50 (m, 10H, carborane), 1.25 (d, 6H, 2  $\times$   $\text{CH}_3$  of isobutyryl group), 1.40 (m, 2H,  $\text{CH}_2$ -2 from linker), 1.70 (m, 2H,  $\text{CH}_2$ -1 from linker), 2.58 (m, 1H, CH of isobutyryl group), 2.75 (bs, 1H, CH of carborane), 3.20 (m, 2H,  $\text{CH}_2\text{O}$  from linker), 3.82-4.50 (m, 5H, 2H-1, 2H-3, 1H-2), 3.81 (s, 6H, 2  $\times$   $\text{OCH}_3$  of dimethoxytrityl group), 5.54 (2d, 2H, H-4), 6.83-6.88 (m, 4H, 2  $\times$   $\text{CH}_3\text{O}$  of dimethoxytrityl group), 7.27-7.41 (m, 9H, dimethoxytrityl group), 7.75 and 7.77 (2s, 1H, H-8 from guanine), 6.74 and 6.83 (2d, 1H, P-H,  $^1J_{\text{P-H}} = 711.65$  Hz and  $^1J_{\text{P-H}} = 710.64$  Hz), 12.05 (bs, 1H, NH from guanine);  $^{13}\text{C}$  NMR (62.90 MHz,  $\text{CD}_2\text{Cl}_2$ ,  $\delta$ , ppm): 14.28 ( $\text{CH}_3$  of isobutyryl group), 22.43 ( $\text{CH}_3$  of isobutyryl group), 25.42 ( $\text{CH}_2$ -2 from linker), 30.34 ( $\text{CH}_2$ -1 from linker), 31.72 (CH of isobutyryl group), 50.80 (2  $\times$   $\text{OCH}_3$  of dimethoxytrityl group), 54.17 (CH of carborane), 58.41 ( $\text{CH}_2\text{O}$  from linker), 60.10 (C-1), 60.53 (C-3), 67.80 and 68.22 (C-4), 71.86 (C-2), 81.96 (C-methylidene of dimethoxytrityl group), 108.14 and 108.68 (C of dimethoxytrityl group), 116.53 (C-5 from guanine), 122.48, 123.43, 123.52, 125.48, 131.17 (C of dimethoxytrityl group), 134.16 (C-8 from guanine), 140.26 (C of dimethoxytrityl group), 144.23 (C-2 from guanine), 144.67 (C-6 from guanine), 151.02 (C-4 from guanine), 154.29 (C of dimethoxytrityl group), 175.15 (C of CO from guanine);  $^{11}\text{B}$  NMR (80.25 MHz,  $\text{CD}_2\text{Cl}_2$ ,  $\delta$ , ppm) decoupled: -15.54, -13.21 (s, 10B, B of carborane); coupled: -14.52 (t, 10B, BH of carborane);  $^{31}\text{P}$  NMR (101.26 MHz,  $\text{CD}_2\text{Cl}_2$ ,  $\delta$ , ppm): 7.82, 9.46 (2s, 1P, PH of phosphonate group); MS (m/z): 877.4 [ $\text{M} + 2$ ] $^+$ ; HPLC  $t_R$ : 23.08 min.

**Synthesis of 2*N*-isobutyryl-9-[[*(1-O*-dimethoxytrityl-3-propoxy-2-yl)oxy]methyl]guanine-(3-propoxy-1-yl)-*para*-carborane 3-*O*-phosphate and 2*N*-isobutyryl-9-[[*(1-propoxy-3-O*-dimethoxytrityl-2-yl)oxy]methyl]guanine-(3-propoxy-1-yl)-*para*-carborane 1-*O*-phosphate (8), triethylammonium salt, racemic mixture**

Compound 7 (0.11 g, 0.13 mmol) was dissolved in solution of carbon tetrachloride : triethylamine : *N*-methylimidazole (15.6 mL, 9 : 0.5 : 0.5, v/v/v) and then water (1.74 mL) was added. The reaction mixture was stirred at room temperature for 1 h, then dichloromethane (87 mL) was added and the whole was extracted with 0.1 M TEAB (3 × 43 mL). The organic layer was dried over magnesium sulfate, filtered and solvent evaporated. Crude product was purified by silica gel column chromatography (230-400 mesh, 6 g) using gradient elution: 0-20% CH<sub>3</sub>OH/CH<sub>2</sub>Cl<sub>2</sub> (with 1% of triethylamine added).

Yield: 95%; R<sub>f</sub>: 0.46 (CH<sub>2</sub>Cl<sub>2</sub> : CH<sub>3</sub>OH, 8 : 2, v/v); UV (95% C<sub>2</sub>H<sub>5</sub>OH, λ, nm): λ<sub>min</sub> = 223, 271, λ<sub>max</sub> = 236, 281, λ<sub>sh</sub> = 252, 259, 275; <sup>1</sup>H NMR (250 MHz, CD<sub>2</sub>Cl<sub>2</sub>, δ, ppm): 1.17-1.28 (m, 15H, 2 × CH<sub>3</sub> of isobutyryl group, 3 × CH<sub>3</sub> of triethylamine), 1.25-4.00 (m, 10H, carborane), 1.31-1.61 (m, 4H, CH<sub>2</sub>-2 from linker, CH<sub>2</sub>-1 from linker), 2.68 (m, 1H, CH of isobutyryl group), 2.89 (q, 6H, 3 × CH<sub>2</sub> of triethylamine), 3.00 (s, 1H, CH of carborane), 3.40-4.00 (m, 7H, 2H-1, 1H-2, 2H-3, CH<sub>2</sub>O from linker), 3.76 (s, 6H, 2 × OCH<sub>3</sub> of dimethoxytrityl), 5.50-5.75 (m, 2H, H-4), 6.77-6.80 (m, 4H, 2 × CH<sub>3</sub>Oph of dimethoxytrityl group), 7.00-7.40 (m, 9H, dimethoxytrityl group), 7.80 (s, 1H, H-8 from guanine); <sup>13</sup>C NMR (62.90 MHz, CD<sub>2</sub>Cl<sub>2</sub>, δ, ppm): 6.63 (3 × CH<sub>3</sub> of triethylamine), 17.08 (2 × CH<sub>3</sub> of isobutyryl group), 28.64 (CH<sub>2</sub>-2 from linker), 33.65 (CH of isobutyryl group), 35.50 (CH<sub>2</sub>-1 from linker), 43.83 (3 × CH<sub>2</sub> of triethylamine), 53.36 (2 × OCH<sub>3</sub> of dimethoxytrityl group), 56.50 (CH of carborane), 62.35 (C-1, C-3), 76.29 (C-2), 84.12 (C-methylidene of dimethoxytrityl group), 111.18 (C of dimethoxytrityl group), 113.82 (CH 5 from guanine), 124.93, 125.93, 126.26, 128.11 (C of dimethoxytrityl group), 134.05 (C-8 from guanine), 156.74 (C of dimethoxytrityl group); <sup>11</sup>B NMR (80.25 MHz, CD<sub>2</sub>Cl<sub>2</sub>, δ, ppm) decoupled: -15.13, -12.63 (2s, 10B, B of carborane); coupled: -14.44 (10B, t, BH of carborane); <sup>31</sup>P NMR (101.26 MHz, CD<sub>2</sub>Cl<sub>2</sub>, δ, ppm): -1.43 (s, 1P, PH of phosphate group); MS (m/z): 891.4 [M - 1]; HPLC t<sub>R</sub>: 25.83 min.

**Synthesis of 9-[[*(1-hydroxy-3-propoxy-2-yl)oxy*]methyl]guanine-(3-propoxy-1-yl)-*para*-carborane 3-*O*-phosphate and 9-[[*(1-propoxy-3-hydroxy-2-yl)oxy*]methyl]guanine-(3-propoxy-1-yl)-*para*-carborane 1-*O*-phosphate (11), sodium salt, racemic mixture**

**yl)oxy]methyl]guanine-(3-propoxy-1-yl)-*para*-carborane 1-*O*-phosphate (10), triethylammonium salt, racemic mixture**

Compound 8 (73 mg, 0.08 mmol) was dissolved in 25% aqueous ammonia (20 mL), then the solution was kept at 55°C for 19 h. The reaction mixture was next degassed under stream of nitrogen and water was evaporated under vacuum yielding a crude racemic mixture of 9-[[*(1-O*-dimethoxytrityl-3-propoxy-2-yl)oxy]methyl]guanine-(3-propoxy-1-yl)-*para*-carborane 3-*O*-phosphate and 9-[[*(1-propoxy-3-O*-dimethoxytrityl-2-yl)oxy]methyl]guanine-(3-propoxy-1-yl)-*para*-carborane 1-*O*-phosphate (9). Crude compound 9, without purification, was dissolved in a mixture of glacial acetic acid and dichloromethane (25 mL, 8 : 2, v/v). The solution was stirred at room temperature for 1 h. Then, to the reaction mixture methanol was added (10 mL) and stirring was continued for next 10 min. The mixture was next concentrated under vacuum and product was isolated by silica gel column chromatography (230-400 mesh, 4 g) using gradient elution: 10-35% CH<sub>3</sub>OH/CH<sub>2</sub>Cl<sub>2</sub>.

Yield: 61.5%; R<sub>f</sub>: 0.24 (CH<sub>2</sub>Cl<sub>2</sub> : CH<sub>3</sub>OH, 7 : 3, v/v, developed threefold); UV (95% C<sub>2</sub>H<sub>5</sub>OH, λ, nm): λ<sub>min</sub> = 225, λ<sub>max</sub> = 254, λ<sub>sh</sub> = 274; <sup>1</sup>H NMR (600 MHz, DMSO, δ, ppm): 1.27 (m, 9H, 3 × CH<sub>3</sub> of triethylamine), 1.26-1.33 (m, 2H, CH<sub>2</sub>-2 from linker), 1.50-2.20 (m, 10H, carborane), 1.67-1.70 (m, 2H, CH<sub>2</sub>-1 from linker), 2.52 (q, 6H, 3 × CH<sub>2</sub> of triethylamine), 3.27-3.39 (m, 5H, 2H-1, 2H-3, CH of carborane), 3.46-3.49 (m, 2H, CH<sub>2</sub>O from linker), 3.61-3.69 (m, 1H, H-2), 5.42 (s, 2H, H-4), 6.61 (bs, 2H, NH<sub>2</sub>), 7.80 (s, 1H, H-8 from guanine), 10.72 (s, 1H, NH); <sup>13</sup>C NMR (150.95 MHz, DMSO, δ, ppm): 30.50 (CH<sub>2</sub>-2 from linker), 35.13 (CH<sub>2</sub>-1 from linker), 54.04 (CH of carborane), 59.99 (C-1, C-3), 63.09 (CH<sub>2</sub>O from linker), 71.25 (C-4), 78.42 (C-2), 116.56 (C-5 from guanine), 137.66 (C-8 from guanine), 151.36 (C-4 from guanine), 154.10 (C-2 from guanine), 156.92 (C-6 from guanine); <sup>11</sup>B NMR (192.59 MHz, DMSO, δ, ppm) decoupled: -15.10, -12.62 (s, 10B, B of carborane); coupled: -15.08, -12.56 (2d, 10B, BH of carborane); <sup>31</sup>P NMR (242.99 MHz, DMSO, δ, ppm): -0.42 (s, 1P, PH of phosphate group); MS (m/z): 519.4 [M], 581.2 [M + Cu<sup>2+</sup>]; HPLC t<sub>R</sub>: 16.46 min.

**Synthesis of 9-[[*(1-hydroxy-3-propoxy-2-yl)oxy*]methyl]guanine-(3-propoxy-1-yl)-*para*-carborane 3-*O*-phosphate and 9-[[*(1-propoxy-3-hydroxy-2-yl)oxy*]methyl]guanine-(3-propoxy-1-yl)-*para*-carborane 1-*O*-phosphate (11), sodium salt, racemic mixture**

Compound **10** (25.7 mg, 0.05 mmol) was dissolved in 60% acetonitrile in 0.1 M TEAB (2 mL), then the solution was added to the stirred solution of 1 M sodium iodide in acetone (19.8 mL). The mixture was stirred at room temperature for 10 min, the resultant precipitate of sodium salt **11** was separated by centrifugation, washed with acetone (8 × 10 mL, HPLC grade) then dried under vacuum.

Yield: 91%;  $R_f$ : 0.20 (CH<sub>2</sub>Cl<sub>2</sub> : CH<sub>3</sub>OH, 6 : 4, v/v); UV (95% C<sub>2</sub>H<sub>5</sub>OH,  $\lambda$ , nm):  $\lambda_{\min}$  = 243, 266,  $\lambda_{\max}$  = 253, 274; <sup>1</sup>H NMR (400 MHz, D<sub>2</sub>O,  $\delta$ , ppm): 1.20-2.90 (m, 10H, carborane), 1.34 (bs, 2H, CH<sub>2</sub>-2 from linker), 1.61 (bs, 2H, CH<sub>2</sub>-1 from linker), 2.99 (bs, 1H, CH of carborane), 3.58-3.87 (m, 7H, CH<sub>2</sub>O from linker, 2H-1 2H-3, 1H-2), 5.58-5.61 (m, 2H, H-4), 7.91-7.94 (m, 1H, H-8 from guanine); <sup>11</sup>B NMR (128.33 MHz, D<sub>2</sub>O,  $\delta$ , ppm) decoupled: -16.25, -13.88 (s, 10B, B of carborane); coupled: -15.02, -14.45 (2d, 10B, BH of carborane); <sup>31</sup>P NMR (161.92 MHz, D<sub>2</sub>O,  $\delta$ , ppm): 1.49 (s, 1P, PH of phosphate group); MS (m/z): 540.3 [M - 2]<sup>-</sup>; HPLC:  $t_R$ : 16.49 min.

#### Synthesis of 9-[(1-oxoethoxy)methyl]guanine-(3-propoxy-1-yl)-*para*-carborane 1-*O*-phosphonate (**14**), racemic mixture

Compound **13** (47.8 mg, 0.165 mmol) and 1-(3-hydroxypropyl)-*para*-carborane (48 mg, 0.24 mmol) were dissolved in a mixture of acetonitrile and pyridine (3 : 1, v/v), then to the stirred solution pivaloyl chloride (55.5  $\mu$ L, 0.45 mmol) was added in one portion (22). The reaction mixture was stirred for 2 h at room temperature, then 2 M TEAB (150  $\mu$ L) was added, next, the solvents were evaporated under reduced pressure. Crude product **14** was purified by silica gel column chromatography (230-400 mesh, 5 g) using gradient elution: 0-12% CH<sub>3</sub>OH/CH<sub>2</sub>Cl<sub>2</sub>.

Yield: 58%;  $R_f$ : 0.55 (CH<sub>2</sub>Cl<sub>2</sub> : CH<sub>3</sub>OH, 8 : 2, v/v); UV (95% C<sub>2</sub>H<sub>5</sub>OH,  $\lambda$ , nm):  $\lambda_{\min}$  = 222, 264,  $\lambda_{\max}$  = 255, 275; <sup>1</sup>H NMR (600 MHz, DMSO,  $\delta$ , ppm): 1.0-3.00 (m, 10H, carborane), 1.39-1.44 (m, 2H, CH<sub>2</sub>-2 from linker), 1.68-1.71 (m, 2H, CH<sub>2</sub>-1 from linker), 3.65-3.67 (m, 2H, H-2), 3.78-3.81 (m, 2H, CH<sub>2</sub>O from linker), 4.01-4.09 (m, 2H, H-1), 5.37 (s, 2H, H-4), 6.75 (d, 1H, P-H, <sup>1</sup>J<sub>P-H</sub> = 706.44), 7.81 (s, 1H, H-8 from guanine); <sup>13</sup>C NMR (150.94 MHz, DMSO,  $\delta$ , ppm): 29.92 (CH<sub>2</sub>-2 from linker), 34.36 (CH<sub>2</sub>-1 from linker), 59.33 (CH of carborane), 64.00 (CH<sub>2</sub>O from linker), 64.41 (C-1), 67.88 (C-2), 72.03 (C-4), 116.68 (C-5 from guanine), 137.88 (C-8 from guanine), 151.50 (C-4 from guanine), 154.30 (C-2 from guanine), 157.10 (C-6 from guanine); <sup>11</sup>B-NMR (192.59 MHz, DMSO,  $\delta$ , ppm) decoupled:

-15.04, -12.63 (s, 10B, B of carborane); coupled: -15.04, -12.63 (2d, 10B, BH of carborane); <sup>31</sup>P NMR (242.99 MHz, DMSO,  $\delta$ , ppm): 9.35 (s, 1P, PH of phosphonate group); MS (m/z): 474.3 [M + 1]<sup>+</sup>, 537.2 [M+1+Gly]<sup>+</sup>; RP HPLC  $t_R$ : 21.51 min.

#### Synthesis of (S)-1-[(2-oxo-1,4,2-dioxaphosphorinan-5-yl)methyl]cytosine 2-*O*-(3-propoxy-1-yl)-*para*-carborane (**17**), mixture of diastereomers

A cyclic cidofovir (**16**) (48 mg, 0.09 mmol) and 1-(3-bromopropyl)-*para*-carborane (120.4 mg, 0.45 mmol) were mixed together, dissolved in dimethylformamide (6.4 mL) then heated at 80°C (23). After 1 h the reaction mixture was cooled to room temperature and solvent evaporated under vacuum. The crude product **17** was purified by silica gel column chromatography (230-400 mesh, 5 g) using gradient elution: 0-20% CH<sub>3</sub>OH/CH<sub>2</sub>Cl<sub>2</sub>.

Yield: 58%;  $R_f$ : 0.45 (CH<sub>2</sub>Cl<sub>2</sub> : CH<sub>3</sub>OH : NH<sub>3</sub> aq. : H<sub>2</sub>O, 80 : 20 : 1 : 1, v/v/v/v); UV (95% C<sub>2</sub>H<sub>5</sub>OH,  $\lambda$ , nm):  $\lambda_{\min}$  = 253,  $\lambda_{\max}$  = 272; <sup>1</sup>H NMR (600 MHz, CD<sub>2</sub>Cl<sub>2</sub>,  $\delta$ , ppm): 1.50-1.70 (m, 10H, carborane), 1.49-1.75 (m, 4H, CH<sub>2</sub>-2 from linker, CH<sub>2</sub>-1 from linker), 2.66 (bs, 1H, CH of carborane), 3.37-3.44 (m, 1H, H-7), 3.53-3.57 (m, 1H, H-7), 3.82-3.87 (m, 2H, CH<sub>2</sub>O from linker), 3.91-4.05 (m, 5H, 2H-3, 1H-5, 1H-7, 1H-7), 4.21-4.27 (m, 2H, H-6), 5.69 (t, 1H, H-5 from cytidine), 7.18 (d, 0.5H, H-6 from cytidine, <sup>1</sup>J<sub>H-H</sub> = 7.2 Hz), 7.21 (d, 0.5H, H-6 from cytidine, <sup>1</sup>J<sub>H-H</sub> = 7.2 Hz); <sup>13</sup>C NMR (150.94 MHz, CD<sub>2</sub>Cl<sub>2</sub>,  $\delta$ , ppm): 31.05 and 31.09 (CH<sub>2</sub>-2 from linker), 35.65 and 35.77 (CH<sub>2</sub>-1 from linker), 49.78 and 50.02 (C-7), 59.38 (CH of carborane), 63.93 and 64.89 (C-3), 65.37 and 66.49 (CH<sub>2</sub>O from linker), 71.68 and 73.17 (C-6), 74.73 and 75.34 (C-5), 94.66 (C-5 from cytidine), 147.69 (C-6 from cytidine), 156.99 (C-2 from cytidine), 166.62 (C-4 from cytidine); <sup>11</sup>B-NMR (192.59 MHz, CD<sub>2</sub>Cl<sub>2</sub>,  $\delta$ , ppm) decoupled: -15.02, -12.61 (s, 10B, B of carborane); coupled: -15.03, -12.61 (2d, 10B, BH of carborane); <sup>31</sup>P NMR (242.99 MHz, CD<sub>2</sub>Cl<sub>2</sub>,  $\delta$ , ppm): 10.16, 11.82 (2s, 1P, PH); MS (m/z): 446.3 [M + 1]<sup>+</sup>, 631.6 [M + 1 + Gly + Gly]<sup>+</sup>; HPLC  $t_R$ : 22.98 and 23.21 min (two diastereomers).

#### Ethylene glycol-(3-*para*-carboranyl)propionic acid ester (**19**)

The procedure was performed under anhydrous conditions, with a positive pressure of argon. 3-(*para*-Carboranyl)propionic acid (**18**) (60 mg, 0.28 mmol) was dissolved in dry dichloromethane (1.73 mL), then DMAP (5.1 mg, 0.042 mmol) and ethylene glycol (61.92  $\mu$ L, 1.11 mmol) was added (21). Next, the solution was cooled to 0-4°C in an ice bath

and DCC (74.52 mg, 0.36 mmol) was added. The reaction mixture was stirred at room temperature for 16 h and after reaction completion the solvent was evaporated. Crude product was purified by silica gel column chromatography (230–400 mesh, 6 g) using gradient elution: 25–60% diethyl ether in hexane.

Yield: 81%;  $^1\text{H}$  NMR (600 MHz,  $\text{CD}_3\text{OD}$ ,  $\delta$ , ppm): 1.00–2.80 (m, 10H, carborane), 2.04 (t, 2H,  $\text{CH}_2$ -2), 2.29 (t, 2H,  $\text{CH}_2$ -1), 3.19 (bs, 1H, CH of carborane), 3.73 (t, 2H,  $\text{CH}_2$ -6), 4.14 (t, 2H,  $\text{CH}_2$ -5); MS (m/z): 261.3  $[\text{M} + 1]^+$ .

**(S)-1-[(2-Oxo-1,4,2-dioxaphosphorinan-5-yl)methyl]cytosine 2-O-[ethylene glycol-(3-*para*-carboranyl)propionic acid ester] (20), mixture of diastereomers**

A cyclic cidofovir (**16**) (11.37 mg, 0.02 mmol) and ethylene glycol-(3-*para*-carboranyl)propionic acid ester (**19**) (8 mg, 0.03 mmol) were dissolved in dimethylformamide (172.3  $\mu\text{L}$ ), next, *N,N*-diisopropylethylamine (9.69  $\mu\text{L}$ ) and benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate (26.67 mg, 0.05 mmol) were added (**23**). The reaction mixture was stirred under argon at 40°C for 1.5 h, then solvent was removed under vacuum and the crude product was purified by silica gel column chromatography (230–400 mesh, 1 g) using gradient elution: 0–30%  $\text{CH}_3\text{OH}/\text{CH}_2\text{Cl}_2$ .

Yield: 48%;  $R_f$ : 0.45 ( $\text{CH}_2\text{Cl}_2$  :  $\text{CH}_3\text{OH}$  :  $\text{NH}_3$  aq. :  $\text{H}_2\text{O}$ , 80 : 20 : 1 : 1 v/v/v/v); UV (95%  $\text{C}_2\text{H}_5\text{OH}$ ,  $\lambda$ , nm):  $\lambda_{\text{min}} = 252$ ,  $\lambda_{\text{max}} = 276$ ;  $^1\text{H}$  NMR (600 MHz,  $\text{CD}_2\text{Cl}_2$ ,  $\delta$ , ppm): 1.00–3.00 (m, 10H, carborane), 2.00–2.08 (m, 2H,  $\text{CH}_2$ -2 from linker), 2.24–2.29 (m, 2H,  $\text{CH}_2$ -1 from linker), 2.77 (bs, 1H, CH of carborane), 3.57–3.62 (m, 2H,  $\text{CH}_2$ -6 from linker), 3.68–3.74 (m, 2H,  $\text{CH}_2$ -5 from linker), 4.07–4.22 (m, 5H, 2H-7, 2H-3, 1H-5), 4.35–4.38 (m, 2H, 2H-6), 5.84 (t, 1H, H-5 from cytidine), 7.32 (d, 0.5H, H-6 from cytidine), 7.36 (d, 0.5H, H-6 from cytidine);  $^{13}\text{C}$  NMR (150.94 MHz,  $\text{CD}_2\text{Cl}_2$ ,  $\delta$ , ppm): 34.03 and 34.07 ( $\text{CH}_2$ -2 from linker), 34.14 and 34.18 ( $\text{CH}_2$ -1 from linker), 49.91 and 50.04 (C-7), 59.51 (CH of carborane), 62.51 and 62.55 ( $\text{CH}_2$ -6 from linker), 64.00 and 64.10 (C-3), 71.17 and 71.82 (C-6), 73.16 and 73.20 ( $\text{CH}_2$ -5 from linker), 75.22 and 75.61 (C-5), 94.82 and 94.91 (C-5 from cytidine), 147.78 and 147.84 (C-6 from cytidine), 156.79 and 156.81 (C-2 from cytidine), 166.66 and 166.68 (C-4 from cytidine), 171.95 and 172.01 (CO from cytidine);  $^{11}\text{B}$  NMR (192.59 MHz,  $\text{CD}_2\text{Cl}_2$ ,  $\delta$ , ppm) decoupled: –14.98, –12.66 (s, 10B, B of carborane); coupled: –14.98, –12.67 (2d, 10B, BH of carborane);  $^{31}\text{P}$  NMR (242.99 MHz,  $\text{CD}_2\text{Cl}_2$ ,  $\delta$ , ppm): 10.69, 12.54 (2s, 1P, PH); MS (m/z): 504.3  $[\text{M} + 1]^+$ ; HPLC  $t_R$ : 22.35 min.

**9-[(1,3-dihydroxypropan-2-yl)oxy]methyl]guanine (2S)-2-amino-*N*-[1-(3-*para*-carboranyl)propionyl]-3-methylbutanoate (22)**

1-(3-*para*-Carboranyl)propionic acid (**18**) (12.2 mg, 0.06 mmol) and *N,N'*-dicyclohexylcarbodiimide were dissolved in dimethylformamide (385  $\mu\text{L}$ ) (**21**). The solution was cooled to 0°C in dry ice/acetone bath and next *N*-methylmorpholine (6.22  $\mu\text{L}$ ) was added. The reaction mixture was stirred for 2 h at 0°C and then 9-[(1,3-dihydroxypropan-2-yl)oxy]methyl]guanine (2S)-2-amino-3-methylbutanoate (**21**) (40 mg, 0.11 mmol) was added. The reaction was continued for 19 h at room temperature, then the solvents were evaporated to dryness. The crude product was purified by silica gel column chromatography (230–400 mesh, 3 g) using gradient elution: 0–10%  $\text{CH}_3\text{OH}/\text{CH}_2\text{Cl}_2$ .

Yield: 60%;  $R_f$ : 0.30 ( $\text{CH}_2\text{Cl}_2$  :  $\text{CH}_3\text{OH}$ , 8 : 2, v/v); UV (95%  $\text{C}_2\text{H}_5\text{OH}$ ,  $\lambda$ , nm):  $\lambda_{\text{min}} = 222$ ,  $\lambda_{\text{max}} = 255$ ,  $\lambda_{\text{sh}} = 274$ ;  $^1\text{H}$  NMR (600 MHz, DMSO,  $\delta$ , ppm): 0.78 (d, 6H, 2  $\times$   $\text{CH}_3$  from valine,  $^1J_{\text{H-H}} = 6.72$  Hz), 1.0–3.00 (m, 10H, carborane), 1.83–1.90 (m, 3H,  $\text{CH}_2$ -2 from linker,  $\text{CH}(\text{CH}_3)_2$  from valine), 2.02–2.09 (m, 2H,  $\text{CH}_2$ -1 from linker), 3.29–3.41 (m, 2H, 2H-1), 3.65 (bs, 1H, CH of carborane), 3.74–3.76 (m, 0.5H, H-2), 3.81–3.83 (m, 0.5H, H-2), 4.04–4.07 (m, 1H, CH-5 from valine), 4.09–4.10 (m, 0.5H, H-3), 4.10–4.11 (m, 0.5H, H-3), 4.20–4.21 (2d, 0.5H, H-3), 4.22–4.23 (2d, 0.5H, H-3), 4.79 (q, 1H, OH from ganciclovir), 5.39 (s, 0.5H,  $\text{NCH}_2\text{O}$ ), 5.40 (s, 0.5H,  $\text{NCH}_2\text{O}$ ), 6.50 (bs, 2H,  $\text{NH}_2$  from guanine), 7.77 (s, 0.5H, H-8 from guanine), 7.78 (s, 0.5H, H-8 from guanine), 8.05–8.08 (m, 1H, NH from valine), 10.63 (bs, 1H, NH from guanine);  $^{13}\text{C}$  NMR (150.94 MHz, DMSO,  $\delta$ , ppm): 18.04 and 18.98 ( $\text{CH}_3$  from valine), 29.75 ( $\text{CH}(\text{CH}_3)_2$  from valine), 33.80 ( $\text{CH}_2$ -2 from linker), 34.38 ( $\text{CH}_2$ -1 from linker), 57.34 and 57.42 (C-5), 59.19 (CH-carborane), 60.29 and 60.43 (C-1), 71.09 and 71.34 (C-3), 71.09 and 71.34 (C of  $\text{NCH}_2\text{O}$ ), 76.58 and 76.86 (C-2), 116.60 (C-5 from guanine), 137.60 (C-8 from guanine), 151.40 (C-4 from guanine), 154.01 (C-2 from guanine), 156.83 (C-6 from guanine), 170.48 (C-3 from linker), 171.43 (C-6 from valine);  $^{11}\text{B}$  NMR (192.59 MHz,  $\text{CD}_2\text{Cl}_2$ ,  $\delta$ , ppm) decoupled: –15.05, –12.61 (s, 10B, B of carborane); MS (m/z): 552.4  $[\text{M} - 1]^+$ ; RP HPLC  $t_R$ : 21.33 min.

**Biological investigations**

**Cells and viruses**

The MRC-5, LLC-MK2, Vero and L929 cells were propagated in MEM and A549 cells were grown in DMEM. Both media were supplemented with 10% inactivated FBS, 2 mM L-glutamine and

100 units/mL penicillin G + 100 µg/mL streptomycin. The cells were incubated in growth medium at 37°C in a humidified 5% CO<sub>2</sub>. The HCMV strain Towne was used in the plaque reduction assay. The laboratory strains of viruses: HPIV-3 (C243), HSV-1 (McIntyre) and VSV were used in the cytopathic effect (CPE) reduction assays.

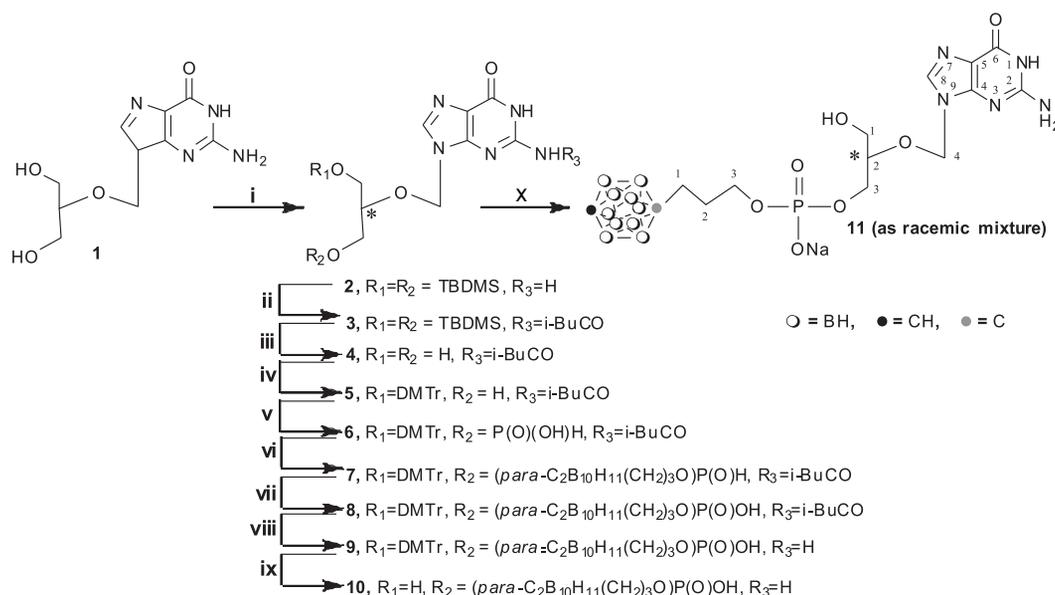
### Cytotoxicity assay

Cytotoxicity of the compounds for MRC-5, A549, LLC-MK2, Vero and L-929 was monitored by inhibition of cell growth. The cells were seeded at  $2 \times 10^4$  cells/well in 96-well microtiter plates and allowed to proliferate at 37°C for 24 h in growth medium (38). Confluent monolayers of cells were treated with different concentrations of the compounds (three wells for each concentration). The compounds were suspended in distilled water (compound **11**) or DMSO (compounds **14**, **17** and **20**) and then in MEM supplemented with 2% of FBS, 2 mM L-glutamine and antibiotic as a test medium. Additionally, to the compounds suspended in DMSO, HPBCD was added. The final concentrations of DMSO and HPBCD in the medium were 0.1% and 0.5%, respectively. After a 2-day incubation at 37°C in 5% CO<sub>2</sub>, the number of viable cells was determined by the formazan method based on

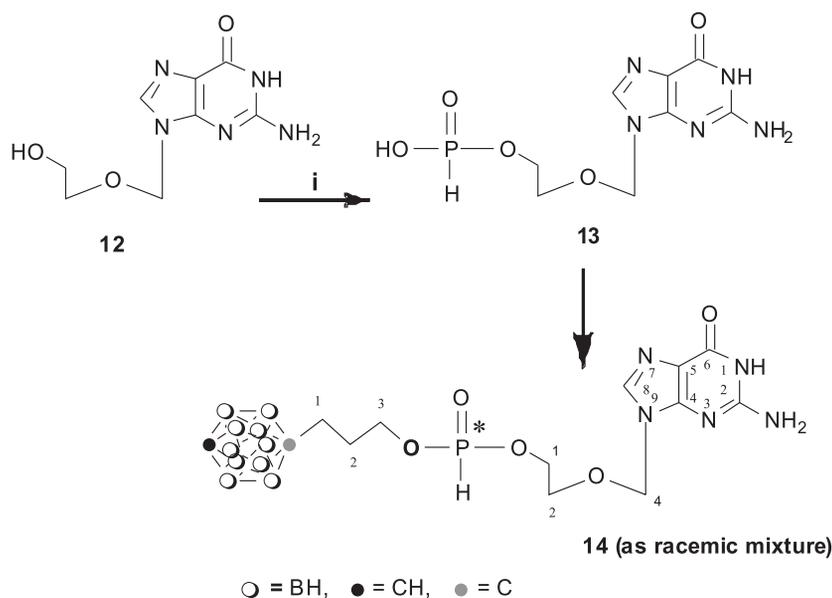
conversion of tetrazolium salt MTT to formazan by living cells (38, 39). Cytotoxicity of the compounds is expressed as the 50% cytotoxic concentration (CC<sub>50</sub>), which is the concentration required to reduce cell growth by 50% of the (untreated) control. The cell variability was evaluated as the mean value density resulting from six mock-treated cell controls. Selectivity index (SI) of the compound was determined as the ratio of CC<sub>50</sub> for cell growth to IC<sub>50</sub> for viral plaque formation or virus-induced cytopathicity.

### Antiviral assay

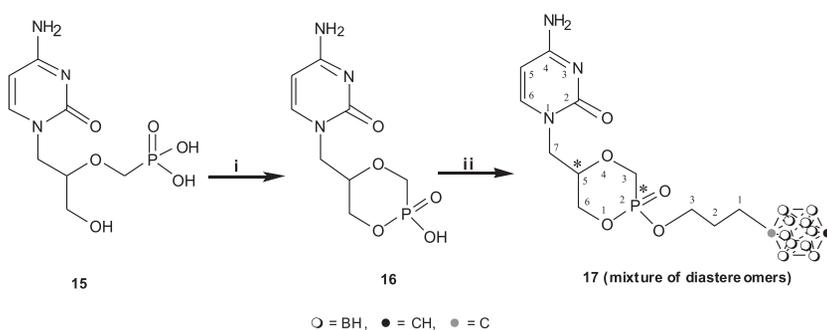
The compounds **11**, **14**, **17** and **20** were evaluated for their ability to inhibit the replication of HCMV, HPIV-3, HSV-1 and VSV *in vitro*. Unmodified GCV, ACV, CDV were used as controls. For plaque reduction assay, confluent MRC-5 cells grown in 96-well microtiter plates were inoculated with HCMV at an input of 20 PFU (plaque forming units) per well. After 2 h adsorption period, residual virus was removed, and the infected cells were further incubated with a maintenance MEM containing varying concentrations of the compounds (0.01 µM - 1 mM). After 7 days of incubation at 37°C (5% CO<sub>2</sub>), the cells were fixed with methanol for 15 min and stained with 0.05% methylene blue



Scheme 1. Synthesis of ganciclovir phosphate modified with *para*-carborane cluster, 9-[(1-hydroxy-3-propoxy-2-yl)oxy]methyl] guanine-(3-propoxy-1-yl)-*para*-carborane 3-*O*-phosphate and 9-[[[1-(3-propoxy-3-hydroxy-2-yl)oxy]methyl]guanine-(3-propoxy-1-yl)-*para*-carborane 1-*O*-phosphate (**11**), racemic mixture; center of chirality marked with a star (\*): i. TMSCl, pyridine, 25°C, 15 min.; ii. (i-BuCO)<sub>2</sub>O, 25°C, 3 h; iii. 25% NH<sub>3</sub>aq, 0°C, 15 min; iv. DMTrCl, Et<sub>3</sub>N, pyridine, 25°C, 4 h; v. imidazole, PCl<sub>3</sub>, Et<sub>3</sub>N, THF, -78°C, 1 h, TEAB; vi. PvCl, *para*-C<sub>2</sub>B<sub>10</sub>H<sub>11</sub>(CH<sub>2</sub>)<sub>3</sub>OH, pyridine, 25°C, 35 min; vii. CCl<sub>4</sub>:Et<sub>3</sub>N:*N*-methylimidazole (9:0.5:0.5, v/v/v), H<sub>2</sub>O, 25°C, 1 h; viii. 25% NH<sub>3</sub>aq, 55°C, 19 h; ix. 80% CH<sub>3</sub>COOH, rt, 1 h; x. precipitation with NaI as sodium salt



Scheme 2. Synthesis of acyclovir *H*-phosphonate modified with *para*-carborane cluster, 9-[(1-oxoethoxy)methyl]guanine-(3-propoxy-1-yl)-*para*-carborane 1-*O*-phosphonate (**14**), racemic mixture; center of chirality marked with a star (\*): i.  $\text{PCl}_3$ ,  $(\text{MeO})_3\text{PO}$ ,  $5^\circ\text{C}$ , 6 h, next  $20^\circ\text{C}$ , 2 h; ii.  $\text{PvCl}$ , *para*- $\text{C}_2\text{B}_{10}\text{H}_{11}(\text{CH}_2)_3\text{OH}$ ,  $\text{CH}_3\text{CN}$ :pyridine (3:1, v/v),  $25^\circ\text{C}$ , 2 h



Scheme 3. Synthesis of cidofovir cyclic phosphate modified with *para*-carborane cluster, (*S*)-1-[(2-oxo-1,4,2-dioxaphosphorinan-5-yl)methyl]cytosine 2-*O*-(3-propoxy-1-yl)-*para*-carborane (**17**), mixture of diastereomers; center of chirality marked with a star (\*): i. DCC *N,N'*-dicyclohexyl-4-morpholinecarboxamide, DMF,  $85^\circ\text{C}$ , 5 h; ii. *para*- $\text{C}_2\text{B}_{10}\text{H}_{11}(\text{CH}_2)_3\text{Br}$ , DMF,  $80^\circ\text{C}$ , 1 h

for 15 min (40). The number of HCMV plaques was counted under microscope. Antiviral activity was expressed as compound concentration required to reduce the number of viral plaques to 50% of control (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 culture were inoculated with 100  $\mu\text{l}$  of the respective virus suspension in test medium (HPIV-3 on LLC-MK2, HSV-1 on Vero and VSV on L-929

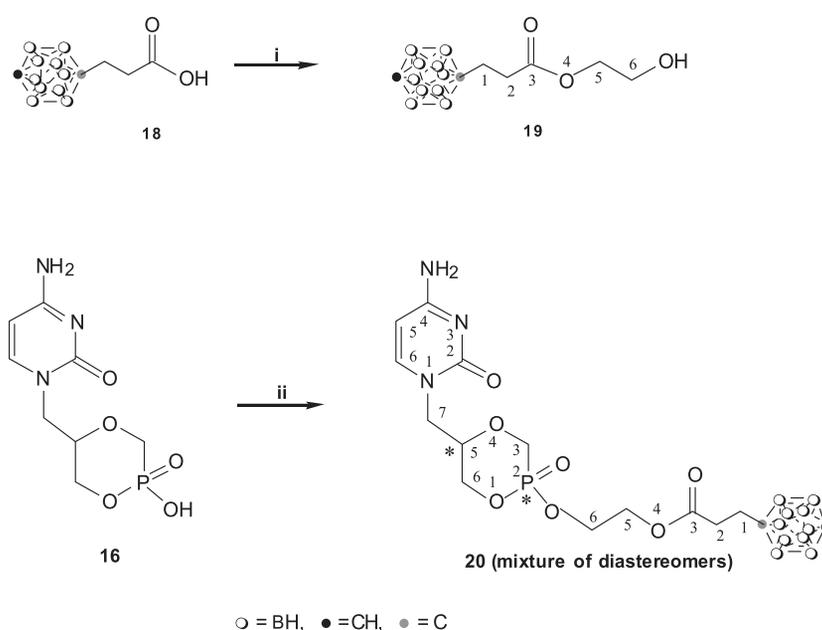
containing approximately 100  $\text{CCID}_{50}$  (50% cell culture infective doses)/well. After adsorption at  $37^\circ\text{C}$  for 1 h, virus inoculum was removed and medium containing varying concentrations of the test compounds was added. The cells monolayers were treated with the compounds for 48-72 h, until typical CPE was visible. Viral infection was evaluated by CPE and MTT assays as described above. Antiviral activity was expressed as  $\text{IC}_{50}$  (50% inhibitory concentration) virus-induced cytopathicity by 50% compared to the untreated control.

## RESULTS

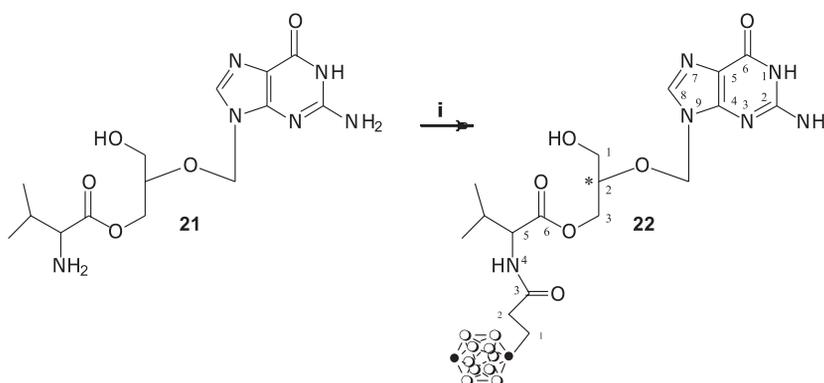
## Chemistry

The target ganciclovir phosphate modified with *para*-carborane cluster **11** was obtained in the procedure involving: 1) protection of 6*N* amino and hydroxyl functions of GCV (**1**), 2) phosphorylation/phosphorylation, 3) boron cluster addition and 4) removal of the protecting groups (Scheme 1). Thus first, 6*N* amino function of compound **1** was

protected in the reaction with isobutyric anhydride ((*i*-BuCO)<sub>2</sub>O) in pyridine solution using transient protection of hydroxyl functions with trimethylsilyloxane groups (TBDMS) (**18**). Next, 1- or 3-hydroxyl function was protected with dimethoxytrityl group (DMTr) under standard conditions yielding compound **5** (**19**). Then, 6*N* and 1- or 3-hydroxyl-protected GCV **5** has been transformed into corresponding fully protected 1-*O*/3-*O*-(*H*-phosphonate) monoester **6** according to PCl<sub>3</sub>/imida-



Scheme 4. Synthesis of cidofovir cyclic phosphate modified with *para*-carborane cluster, (S)-1-[(2-oxo-1,4,2-dioxaphosphorinan-5-yl)methyl]cytosine 2-*O*-[ethylene glycol-(3-*para*-carboranyl)propionic acid ester] (**20**), mixture of diastereomers; center of chirality marked with a star (\*): i. ethylene glycol, DMAP, DCC, CH<sub>2</sub>Cl<sub>2</sub>, rt, 16 h; ii. DIPEA, PyBOP, DMF, 40°C, 1.5 h



Scheme 5. Synthesis of valganciclovir modified with *para*-carborane cluster, 9-[[1-(1,3-dihydroxypropan-2-yl)oxy]methyl]guanine (2*S*)-2-amino-*N*-[1-(3-*para*-carboranyl)-propionyl]-3-methylbutanoate (**22**); center of chirality marked with a star (\*): i. DMF, 3-(*para*-carboranyl)propionic acid, *N*-methylmorpholine, DCC

zole method (20). Thus, to cooled to  $-78^{\circ}\text{C}$  and stirred mixture of imidazole, triethylamine ( $\text{Et}_3\text{N}$ ) and phosphorus trichloride ( $\text{PCl}_3$ ) in tetrahydrofuran (THF), yielding phosphorimidazolidite intermediate, a solution of **5** in the same solvent was added. After reaction completion solution of triethylammonium bicarbonate buffer (TEAB) was added and the whole was left to warm to room temperature spontaneously. After standard work-up the product was isolated by silica gel column chromatography. Boron cluster attachment providing diester **7** has been achieved using *H*-phosphonate chemistry to couple **6** with 1-(3-hydroxypropyl)-*para*-carborane (21). Resultant fully protected intermediate **7** was

next oxidized to transform *H*-phosphonate group into natural phosphate one yielding compound **8**. Subsequently, 6*N* isobutyryl group and 1-*O*/3-*O*-DMTr group has been removed with 25% aqueous ammonia ( $\text{NH}_3$  aq.) and 80% acetic acid ( $\text{CH}_3\text{COOH}$ ), respectively, then final product was precipitated as sodium salt.

Acyclovir phosphonate modified with *para*-carborane cluster **14** was obtained in a simple, two-step procedure (Scheme 2). Thus first, acyclovir **12**, without protection of 6*N* amino function, has been transformed into corresponding monoester according to *H*-phosphonate method using  $\text{PCl}_3$ /imidazole system as described above for GCV, then the result-

Table 1. Lipophilicity, stability in buffers with different pH and in culture medium of GCV, ACV, CDV and VGCV compounds.

Compound	Lipophilicity		Stability in buffers			Stability in cell culture medium <sup>k</sup>
	RP-TLC $R_{M0}$	RP-HPLC $t_r$ [min]	$t_{1/2}$ [h]			
			pH: 4	7	9	$t_{1/2}$ [h]
<b>11</b>	1.85 <sup>a</sup>	16.49 <sup>c</sup>	0.47	stable <sup>l</sup>	stable <sup>l</sup>	stable <sup>m</sup>
<b>14</b>	1.87 <sup>b</sup>	21.51 <sup>f</sup>	0.26	6.61	1.20	5.7
<b>17</b>	2.34 <sup>c</sup>	22.98, 23.21 <sup>g,i</sup>	j	j	3.76	j
<b>20</b>	2.14	22.35 <sup>e</sup>	0.21	3.43	0.72	3.64
<b>22</b>	2.61 <sup>d</sup>	21.33 <sup>h</sup>	0.21	stable <sup>j</sup>	34.83	n

<sup>a</sup> $R_{M0}$  for unmodified GCV =  $-0.22$ , <sup>b</sup> $R_{M0}$  for unmodified ACV =  $-0.15$ , <sup>c</sup> $R_{M0}$  for unmodified CDV =  $-0.71$ , <sup>d</sup> $R_{M0}$  for unmodified VGCV =  $0.63$ , <sup>e</sup> $t_r$  for unmodified GCV =  $5.62$  min, <sup>f</sup> $t_r$  for unmodified ACV =  $5.91$  min, <sup>g</sup> $t_r$  for unmodified CDV =  $3.89$  min, <sup>h</sup> $t_r$  for unmodified VGCV =  $8.56$  min, two diastereomers separable by RP-HPLC, <sup>i</sup>compound **17** is transformed into a stable monohydrate, according to the FAB-MS ( $-Ve$ ) analysis, <sup>j</sup>Eagle's minimal essential medium; <sup>k</sup>no signs of degradation observed during 24 h; <sup>m</sup>no signs of degradation observed during 48 h, <sup>n</sup>10% degradation during 48 h.

Table 2. Antiviral activities against different virus strains of GCV, ACV, CDV and VGCV compounds<sup>a</sup>.

Compound	Antiviral activity, $\text{IC}_{50}$ [mM]	
	HCMV (MRC5)	HSV-1 (Vero)
<b>11</b>	0.41	75
GCV	0.33	20
<b>14</b>	80	0.008
ACV	15	0.004
<b>17</b>	540	nd
<b>20</b>	2.8	nd
CDV	0.4	nd
<b>22</b>	15	nd
VGCV	0.06	nd

<sup>a</sup>The  $\text{CC}_{50}$  of the all compounds studied was higher than  $1000 \mu\text{M}$  in all cell lines used: MRC5, L-929, LLC-MK2 and Vero with the exception of compound **22**: MRC5:  $550$ , L-929:  $366$ , LLC-MK2:  $612$  and Vero:  $318 \mu\text{M}$ ; <sup>b</sup>nd = not determined. None of the compounds was active against VSV in L-929 cell line and HPIV-3 in LLC-MK2 cell line up to  $1000 \mu\text{M}$  (for compound **22** up to  $366$  and  $612 \mu\text{M}$ , respectively).

ant compound **13** was esterified with 1-(3-hydroxypropyl)-*para*-carborane, an alcohol bearing boron cluster using *H*-phosphonate method (20, 22).

Cidofovir **17** modified with *para*-carborane cluster attached through an alkyl linker was obtained in a convenient and simple, two-step procedure (Scheme 3) involving transformation of cidofovir **15** in acyclic form into cyclic derivative **16** (cCDV) which was next converted into cyclic triester **17** in the reaction with 1-(3-bromopropyl)-*para*-carborane used as boron cluster donor (23). The literature method described for esterification of cCDV with alkyl bromides was adapted (23).

Derivative **20** containing *para*-carborane cluster attached through a linker equipped with carboxylic acid ester function is another example of cidofovir modification with boron cluster proposed herein. The synthetic strategy involving P-O conjugation between cCDV and an ethylene glycol-ester linked modification (e.g., amino acids), masking the negative charge on the cCDV was already exploited successfully to increase the cidofovir bioavailability (24). Compound **20** was obtained in a simple esterification reaction between cCDV **16** and ethylene glycol-(3-*para*-carboranyl)propionic acid ester (**19**) using benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate (PyBOP) as condensing agent (Scheme 4) (24).

Finally, the 3-(*para*-carborane)propionoamide of valganciclovir (**22**), has been synthesized analogously as dipeptide conjugates reported previously (25, 26). Condensation between an amino function of valine residue in valganciclovir (VGCV) and 1-(3-*para*-carboranyl)propionic acid (**18**) at the presence of DCC (*N,N'*-dicyclohexylcarbodiimide) as condensing agent provided expected product with high yield (Scheme 5) (27).

All the final products have been characterized using UV, IR, <sup>1</sup>H-, <sup>13</sup>C-, <sup>31</sup>P- and <sup>11</sup>B-NMR, FAB-MS and chromatographic methods. Boron cluster modified ganciclovir, acyclovir and cidofovir phosphates or phosphonates: **11**, **14**, **17**, **20** and compound **22** were tested for their stability in buffers with different pH and cell culture medium (Table 1). The lipophilicity ( $R_{M0}$ ) was estimated using RP-TLC method. Then, the phosphorus containing derivatives **11**, **14**, **17**, **20** and compound **22** have been tested for cytotoxicity and antiviral activity (Table 2). The cytotoxicity assays was performed on confluent monolayers of MRC-5, Vero, L929, LLC-MK2 and A549 cells. Examined compounds did not affect the growth of cells at concentration up to 1000  $\mu$ M, with the exception of compound **22** which revealed cytotoxicity above 300  $\mu$ M. We did not observe major

differences between cytotoxicity for compounds **11**, **14**, **17** and **20** and unmodified GCV, ACV and CDV.

### Biological investigations

The derivatives **11**, **14**, **17**, **20** and **22** were tested first for anti-HCMV activity using plaque reduction assay, in comparison with GCV, ACV, CDV and VGCV, respectively. The obtained IC<sub>50</sub> values are shown in Table 2. Ganciclovir phosphate **11** exhibited similar degree of anti-HCMV activity to unmodified GCV, at 0.41  $\mu$ M compound **11** achieved 50% reduction in plaque formation. The compounds **14**, **17** and **22** showed lower antiviral activity against HCMV compared with ACV, CDV and VGCV, respectively. As showed in Table 2, the compounds **11** and **14** were also effective in inhibiting the HSV-1 replication. Acyclovir phosphonate **14** showed remarkable activity at the low IC<sub>50</sub> range (8 nM), *versus* unmodified ACV (4 nM). In comparison to unmodified GCV, almost 4-fold lower inhibition of HSV-1 with ganciclovir phosphate **11** was observed. GCV, ACV and CDV phosphate modified with *para*-carborane cluster and analogue of valganciclovir **22** were not inhibitors of RNA viruses' replication. These compounds did not inhibit the HPIV-3 and VSV replication even at a concentration of 1000  $\mu$ M.

### DISCUSSION AND CONCLUSIONS

The DNA polymerase of herpes viruses provides a major target for antiviral drugs. It can be inhibited by compounds which require conversion to the active triphosphates by phosphokinases and can act as terminators blocking extension of the replicating DNA molecule. It was shown that GCV, ACV, CDV effectively inhibit HCMV and HSV-1 replication by targeting the viral DNA polymerase (17). Because of the problems associated with currently available antiviral compounds there is a continuous need for new compounds with antiviral activity, high bioavailability and low toxicity.

The major characteristic of the GCV, ACV and CDV derivatives **11**, **14** and **17** is the presence of phosphoric or phosphonate acid residue and lipophilic modification in the form of boron cluster. This fulfills two major requirements for the potential nucleoside pro-drugs: 1) the presence of phosphate residue helping to bypass the limiting first phosphorylation step prerequisite for nucleoside antivirals activity, and 2) the presence of lipophilic structural element facilitating permeation through biological membranes of the otherwise lipophobic nucleoside

phosphates and phosphonates, and potentially increasing the molecule's bioavailability (28). In addition, methods for the attachment of boron cluster through a linker bearing carboxylic acid ester or amide function have been also developed using cyclic CDV and VGCV as substrates for the synthesis of modification **20** and **22**.

Strategies to improve the antiviral activity and oral absorption of GCV, ACV and CDV phosphates or phosphonates by covalent attachment of lipophilic group has been already tested and proved beneficial in many cases. Often increased antiviral activity over the activity of underivatized counterparts and increased oral bioavailability has been observed (29-33).

The concept of increasing the lipophilicity of nucleoside phosphates with boron cluster modification was tested by us previously. A series of biologically important adenosine phosphates such as AMP, cAMP and ATP modified with *para*-carborane group have been synthesized and their logP measured. It was found that the lipophilicity of adenosine phosphates modified with boron cluster was three orders of magnitude higher than that of unmodified counterparts. Moreover, the resistance towards phosphatases in human blood serum of the modified adenosine phosphates increased substantially (34).

Similar effect was observed for modification of GCV, ACV and CDV. Indeed, substantial increase of the lipophilicity measured by RP-TLC method was observed for all boron cluster modifications in comparison with unmodified counterpart (Table 1). It is worthy to point out that an increase of lipophilicity was observed even for compounds **11** and **14** bearing lipophobic phosphate or *H*-phosphonate functions. The lipophilicity measured as affinity to RP-HPLC column ( $t_R$ ) increased also dramatically as shown by more than threefold increase of  $t_R$  for all modified derivatives, **11**: 16.49 min (GCV: 5.62 min), **14**: 21.51 min (ACV: 5.91 min), **17**: 22.98 min and 23.21 min, **20**: 22.35 min (CDV: 3.89 min) and **22**: 21.33 min (VGCV: 8.57 min). Desirable increase of lipophilicity of GCV, ACV and CDV pro-drugs causes lower water solubility which, apart of other aspects, complicates the cytotoxicity and antiviral activity assays and hampers the reproducibility of the obtained results. The cyclodextrin derivatives are classical surfactants used to improve lipophilic drugs solubility in water and are used as components of drug delivery systems (35, 36). We have found that addition of (2-hydroxypropyl)- $\beta$ -cyclodextrin (HPBCD) improves solubility of compounds **11**, **14**, **17**, **20** and **22** in

buffers and cell culture media satisfactorily and facilitates biological evaluation.

The stability of compounds **11**, **14**, **17**, **20** and **22** in buffered aqueous solutions at 50°C was evaluated by incubation of each compound at pH 4, 7 and 9. The rate of disappearance of the tested material was estimated by HPLC and the half-life was determined from the percentage of undegraded compound – time curve. The half-life is expressed as the time required to reduce the original concentration of the tested compound by 50%. An effect of pH on the compounds' stability was observed with the lowest stability at acidic pH and the highest at pH 7. The stability in the Eagle's minimal essential medium used in cytotoxicity and antiviral activity assays followed in general the similar pattern as in buffered solutions at pH 7. The stability in cell culture medium increased in order **14** < **20** << **22** <<< **11** (Table 1). In the case of modified cidofovir **17**, most probably formation of stable hydrate occurs. The formation of the hydrate was confirmed by its isolation and FAB-MS analysis. The emerging after longer time products of **11**, **14**, **17**, **20** and **22** degradation are in the process of isolation and analysis.

In this study, we have demonstrated that ganciclovir phosphate, acyclovir *H*-phosphonate and cidofovir and valganciclovir derivatives modified with lipophilic boron cluster module are inhibitory to the replication of HCMV and HSV-1 at their non-toxic concentration to the host cells. These compounds exhibited low cytotoxicity and in the case of compound **11** high selectivity index, more than 2400, which is similar to GCV.

The stereochemistry of the boron cluster modified GCV, ACV and CDV derivatives **11**, **14**, **17** and **20** should be noticed as a possible factor affecting their biological activity. Due to the presence of the centers of chirality at the carbon atom and/or phosphorus they are formed as a mixture of enantiomers (compound **11** and **14**) or diastereomers (compound **17** and **20**). Biological properties of the chiral components of the racemic or diastereomeric pairs may be different. More in-depth work is necessary to define these effects. The obtained results suggest that compound **11** could be considered as promising candidate for the future development of the new type of derivatives with anti-HCMV activity and for *in vivo* analysis.

In summary, we have synthesized novel GCV, ACV, CDV and VGCV conjugates bearing boron cluster as lipophilic modification. Activity against selected DNA and RNA virus strains of these modifications was tested. High activity of compound **11** against HCMV and of compound **14** against HSV-1

was observed. Although the new molecules did not prove more active than original drug, based on previous study of lipophilic derivatives of many antiviral compounds, boron cluster may increase bioavailability and its conjugates can be considered as a new type of pro-drugs for further structural optimization and biological evaluation.

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