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(54) Title: ANTIVIRAL DRUG DERIVATIVE

(57) Abstract: The subject of the present invention is an antiviral drug derivative, preferably against HCMV, containing a boron cluster connected with the drug via a connecting group and preferentially a phosphoric or H-phosphonic acid.



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Antiviral drug derivative

The subject of the present invention is a derivative of an antiviral drug, preferably against HCMV, containing a boron cluster connected with the drug through a connecting group and preferentially a phosphoric or H-phosphonic acid residue. The present invention is for use in medicine.

Human cytomegalovirus (HCMV), a beta-herpesviridae, infects about 60% of adults in developed world and more than 90% of third world populations. 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 chemo- and/or radiotherapy, the full pathogenic potential of the virus may be realized .

The currently available anti-HCMV drugs have several drawbacks that limit their clinical utility. 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 are the subject of some debate despite the availability of results from one randomised controlled trials. 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 of compounds, for modification of selected anti-HCMV drugs. The use of carbaboranes as pharmacophores was made popular by the pioneering work of Endo and colleagues. However, prior to the introduction of this terminology, the hydrophobicity of carbaboranes was already used to trigger desired biological actions (Schwyzer, R., H.Q. Do, A.N. Eberle, J-L. Fauchère. 1981. Synthesis and Biological Properties of Enkephalin-like Peptides Containing Carboranylalanine in Place of Phenylalanine. *Helv. Chim. Acta*, 64, 2078-2083). Due to their spherical shape in most cases carbaboranes are integrated as substitutes for organic ring systems. The literature comprises different examples in which carbaboranes are used as

surrogates for heterocycles, annulated carbon rings, or most popularly, due to the benzene analogy, for substituted or unsubstituted phenyl rings (Lesnikowski 2007, Boron units as pharmacophors – New applications and opportunities of boron cluster chemistry. Coll. Czech. Chem. Commun., 72: 1646-1658). WO 96/14073 discloses methods and compositions for the treatment of urogenital cancer, particularly prostate, bladder and kidney cancer using boron-neutron therapy. Compounds according to said invention used in the above-mentioned therapy are nucleosides and oligonucleotides containing carborane groups conjugated with an appropriate nucleic base through phosphate, thiophosphate or selenophosphate residues. These compounds are characteristic in that they are more lipophilic. Another patent, US 6,838,574, discloses a drug having as its active substance a dicarba-closo-dodecaborane derivative or its pharmacologically permissible salt. The borane group is conjugated with an aromatic ring either directly or through a connecting group. Compounds according to the invention exhibit an affinity for RAR and RXR retinoic acid receptors, and can be used as agonists or antagonists of these receptors. EP 1364954 describes a compound being a dicarba-closo-dodecaborane derivative or its pharmacologically permissible salt, which is useful as an agent modulating vitamin D activity or as an agent augmenting vitamin D activity. The compound according to the cited invention may be described with the formula R1-X-R2, where X denotes a dicarba-closo-dodecaboranedyl group, whereas R1 and R2 are hydroxyl derivatives of alkyl, aryl, etc. groups. Among the antiviral drugs used, the biggest majority is constituted by preparations that are nucleoside derivatives. It is commonly accepted that they are phosphorylated to monophosphate form by the HCMV kinase encoded by the gene UL97. The conversion into triphosphates occurs with the activity of cellular enzymes. The nucleoside triphosphates derived from the medicinal preparations compete with natural nucleotides. When incorporated into the HCMV DNA strand, they promote the termination of DNA chains and/or inhibition of DNA polymerase activity. Examples of medicinal preparations with such activity include gancyclovir, cidofovir, valcyclovir, valgancyclovir and acyclovir (Talarico C.L. 1999, 43:1941-1946; Leśnikowski Z.J. 2002, 75:242-249, Lischka P. Curr. Opin Pharmac., 2008, 8:541-48). Orally administered valacyclovir and valgancyclovir are initially hydrolysed to their active form by intracellular esterases, which is not necessary in the case of oskarnet or cidofovir. Despite all, there is still a need for antiviral drugs characterised by low toxicity, with strong antiviral activity that would be useful for oral administration and which exhibit strong lipophilic properties, and which preferentially contain a phosphoric or H-phosphonic acid residue.

The subject of the present invention is a derivative of an antiviral drug, preferably against HCMV, containing a boron cluster connected with the drug via a connecting group and preferably a phosphoric acid or H-phosphonic acid residue. Equally preferentially, derivatives of an antiviral drug according to the present invention is characterised in that the antiviral drug is selected from a group comprising gancyclovir, acyclovir, cidofovir, valgancyclovir, valacyclovir and pencyclovir. More preferentially, the antiviral drug derivative according to the present invention contains connecting group defined by the formula: $-(\text{CH}_2)_n\text{W}^m\text{J}^k(\text{CH}_2)_h\text{Tf}$ - where f is 0-3, h is 0 do 5, k is from 0 to 6, m is 0 or 1, n is from 0 to 5 W is O, S, C(O), S(O), S(O)₂, Se, NR (where R = H, alkyl, haloalkyl, alkoxyalkyl or aryl), T is XP(Z)(Y)X₁ (where X = O, S, Se, alkyl, haloalkyl, alkoxyalkyl, aryl) and also CH=CH, CC, N=N, CHOH and CHN₃; where X₁ = O, S, Se, alkyl, haloalkyl, alkoxyalkyl, aryl) and also CH=CH, CC, N=N, CHOH and CHN₃; Z = O, S, Se; Y = OH, SH, SeH, H or alkyl, haloalkyl, alkoxyalkyl, aryl or halogen, in particular fluorine), and also CH=CH, CC, N=N, CHOH and CHN₃. Equally preferably, An antiviral drug derivative is characterised in that the boron cluster is one of the following groups: 1,2-dicarba-closo-dodecarborane (ortho-carboranyl), 1,7-dicarba-closo-dodecarborane (meta-carboranyl), 1,12-dicarba-closo-dodecarborane (para-carboranyl), 7,8-dicarba-nido-undecarborane (nido-carboranyl), closo-dodecarborane and their derivatives substituted on a carbon or boron atom.

The major characteristic of the obtained derivatives of antiviral drugs presented invention 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 by pass the limiting first phosphorylation step prerequisite for nucleoside antivirals activity, and 2) 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

Example embodiments of the present invention are shown in the attached figures which Fig.1 shows synthesis of ganciclovir phosphate modified with para-carborane cluster: 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 (11), racemic mixture; center of chirality marked with a star (*), where i. TMSCl, pyridine, 25°C, 15 min.; ii. (i-BuCO)₂O, 25°C, 3h; iii. 25% NH₃aq, 0°C, 15 min; iv. DMTrCl, Et₃N, pyridine, 25°C, 4h; v. imidazole, PCl₃, Et₃N, THF; ii.

TEAB. -78°C, 1h; vi. PvCl , para-C2B10H11(CH₂)₃OH, pyridine, 25°C, 35 min; vii. CCl₄:Et₃N:N-methylimidazole (9:0.5:0.5, v/v), H₂O, 25°C, 1h; ix. 25%NH₃aq., 55°C, 19h; x. a) 80%CH₃COOH, rt, 1h; b) precipitation with NaI as sodium salt., Fig. 2. shows synthesis of acyclovir H-phosphonate modified with para-carborane cluster: 9-[(1-oxyethoxy)methyl]guanine-(3-propoxy-1-yl)-para-carborane 1-O-phosphonate (14), racemic mixture; center of chirality marked with a star (*), where i. PCl_3 , (MeO)₃PO, 5°C, 6 h, next 20°C, 2h; ii. vi. PvCl , para-C2B10H11(CH₂)₃OH, CH₃CN:pyridine (3:1, v/v), 25°C, 2h, Fig. 3 show 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 (*), where i. DCC, N,N'-dicyclohexyl-4-morpholinecarboxamidine, DMF, 85°C, 5 h; ii. para-C2B10H11(CH₂)₃Br, DMF, 80°C, 1 h and fig. 4 shows 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 (*), where and in stage 18-19 i. ethylene glycol, DMAP, DCC, CH₂Cl₂, rt, 16h and and in stage 16 – 20 i. 19, DIPEA, PyBOP, DMF, 40°C, 1.5h. Fig 5 shows synthesis of ganciclovir modified with para-carborane cluster: 9-[(1,3-dihydroxypropan-2-yl)oxy]methyl}guanine (2S)-2-amino-N-[1-(3-para-carboranyl)-propionyl]-3-methylbutanoate (22) where i. DMF, 3-(para-carboranyl)propionic acid, N-methylmorpholine, DCC.

The target ganciclovir phosphate modified with para-carborane cluster 11 was obtained in multi-step procedure involving 1) protection of 6N amino and hydroxyl functions of ganciclovir (1), 2) phosphorylation, 3) boron cluster addition and 4) removal of the protecting group (fig. 1). Thus first, 6N amino function of compound 1 was protected in the reaction with isobutyric anhydride in pyridine solution using transient protection of hydroxyl functions with trimethylsiloxane groups (Ti et al., 1982 Transient Protection: Efficient One-Flask Syntheses of Protected Deoxynucleosides. J. Am. Chem. Soc., 104: 1316-1319.). Next 1- or 3-hydroxyl function was protected with dimethoxytrityl group under standard conditions (Schaller et al, 1963 Studies on Polynucleotides. XXIV. The Stepwise Synthesis of Specific Deoxyribopolynucleotides (4). Protected Derivatives of Deoxyribonucleosides and New Syntheses of Deoxyribonucleoside-3" Phosphates. J. Am. Chem. Soc., 85: 3821-3827.) yielding compound 5. Next, 6N and 1- or 3-hydroxyl-protected ganciclovir 5 has been transformed into corresponding fully protected 1-O/3-O-(H-phosphonate)monoester 6

according to PCl_3 /imidazole method (Stawinski, 2005 Di- and oligonucleotide synthesis using H-phosphonate chemistry. p. 81-100. in P. Herdewijn (ed.), *Methods in Molecular Biology*, vol. 288: *Oligonucleotide Synthesis: Methods and Applications*, Humana Press INC., Totowa, NJ.). Thus, to cooled to -78° and stirred mixture of imidazole, triethylamine and phosphorus trichloride in tetrahydrofuran yielding phosphorimidazolite intermediate, solution of 5 in the same solvent was added. After reaction completion solution of TEAB buffer was added and whole warmed 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 via H-phosphonate method in condensation reaction of 6 with 1-(3-hydroxypropyl)-para-carborane (Stawinski, 2005). Resultant fully protected intermediate 7 was next oxidized to transform H-phosphonate group into natural phosphate one yielding compound 8. Subsequently, 6N isobutyryl group and 1-O/3-O-dimethoxytrityl group has been removed with 25% aqueous ammonia and 80% acetic acid, respectively, then final product was precipitated as sodium salt.

Contrary to the ganciclovir derivative, acyclovir phosphonate modified with para-carborane cluster 14 was obtained in a simple, two-step procedure (fig 2). Thus first acyclovir 12, without protection of 6N amino function, has been transformed into corresponding monoester 6 according to PCl_3 /imidazole method as described above for ganciclovir (Stawinski, 2005) then the resultant compound 13 was esterified with 1-(3-hydroxypropyl)-para-carborane, an alcohol bearing boron cluster, using H-phosphonate method.

Cidofovir phosphate 17 modified with para-carborane cluster was obtained in a convenient and simple, two-step procedure (fig 3) involving transformation of cidofovir 15 in acyclic form into cyclic counterpart 16 which was next converted into cyclic triester 17 in the reaction with 1-(3-bromopropyl)-para-carborane used as boron cluster donor.

All the isolated intermediates and final products have been fully characterized by UV, IR, ^1H -, ^{13}C -, ^{31}P - and ^{11}B -NMR, FAB-MS and chromatographic methods. The concept of increasing the lipophilicity of nucleoside phosphates with boron cluster modification was tested by Wojtczak et al., 2008 Highly lipophilic adenosine phosphates bearing para-carborane ($\text{C}_2\text{B}_{10}\text{H}_{11}$) modification. *Coll. Czech. Chem. Commun.*, 73: 175-186.). Several biologically important adenosine phosphates such as AMP, cAMP and ATP modified with para-carborane cluster have been synthesized and their logP measured. It was found that the lipophilicity of adenosine phosphates modified with boron cluster was three order of magnitude higher than that of unmodified counterparts. Moreover, the resistance towards

phosphatases in human blood serum of the modified adenosine phosphates increased substantially

Example 1 Cytotoxicity assay

5 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 2×10^4 cells/well in 96-well microtiter plates and allowed to proliferate at 37°C for 24 h in growth medium (Lesnikowski et al. 2005 Towards new boron carriers for boron neutron capture therapy: Metallacarboranes and their nucleoside conjugates. *Bioorg. Med. Chem.*,13: 4168-4175.). Confluent monolayers of cells
10 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 and 17) and then in MEM supplemented with 2% of FBS, 2 mM L-glutamine and antibiotic as a maintenance medium. Additionally, to the compounds suspended in DMSO, HPBCD was added. The final concentrations of DMSO and HPBCD in the medium
15 were 0.1% and 0.5%, respectively. After a 2-day incubation at 37°C in 5% CO₂, the number of viable cells was determined by the formazan method based on conversion of tetrazolium salt MTT to formazan by living cells (Berg et al., 1990; A new sensitive bioassay for precise quantification of interferon activity as measured via the mitochondrial dehydrogenase function in cells (MTT-method. 1990. *APMIS*, 98: 156-162). Cytotoxicity of the compounds
20 is expressed as the 50% cytotoxic concentration (CC₅₀), which is the concentration required to reduce cell growth by 50% of the (untreated) control. Selectivity index (SI) of the compounds were determined as the ratio of CC₅₀ for cell growth to IC₅₀ for viral plaque formation or virus-induced cytopathicity. (see results in Table 1)

25 Example 2 Antiviral assay.

The compounds 11, 14 and 17 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 control. 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
30 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-1mM). After 7 days of incubation at 37°C (5% CO₂), the cells were fixed with methanol for 15 min and stained with 0.05% methylene blue for 15 min (Freitas et 1993 Efficacy of ganciclovir in combination with other antimicrobial agents against cytomegalovirus in vitro

and in vivo. Antivir. Res., 20: 1-12.). 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 CPE reduction assays, confluent LLC-MK2 cells were infected with HPIV-3, confluent Vero and A549 cells were infected with HSV-1 and confluent L-929 cells were infected with VSV at 100 CCID₅₀ (50% cell culture infective doses)/well for 1 h. After that 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 (Yu et al., 2010 Antiviral activity of recombinant Cyanovirin-N against HSV-1. Virol. Sin. 25, 432-439.). Antiviral activity was expressed as IC₅₀ (50% inhibitory concentration) virus-induced cytopathicity by 50% compared to the untreated control. (see results in Table 1)

Table 1.

Cytotoxic activity					
Compound	CC ₅₀ (M) for cell lines				
	MRC-5	L929	LLC-MK2	A549	Vero
11	4 000	> 1 250	> 1 250	> 1 250	> 1 250
GCV	3 050	> 1 250	> 1 250	> 1 250	> 1 250
14	> 1 000	> 1 000	> 1 000	> 1 000	> 1 000
ACV	> 5 000	> 5 000	3 340	3 760	> 5 000
17	> 1 000	> 1 000	> 1 000	> 1 000	> 1 000
CDV	> 5 000	> 5 000	> 5 000	1 000	> 5 000

Antiviral activity			
Compound	IC50 (M) for viruses		
	CMV (MRC-5)	HSV-1 (A549)	HSV-1 (Vero)
11	7	60	75
GCV	0,65	50	50
14	160	0,08	0,08
ACV	30	0,04	0,04
17	540	nd	nd
CDV	0,4	nd	nd

11– gancyclovir (GCV) modified with a para-carboranyl cluster;

sodium salt of 9-(1-hydroxy-2-propoxymethyl)guanyl (3-propyloxy)-para-carboranyl phosphate

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14– acyclovir (ACV) modified with a para-carboranyl cluster;

9-(2-hydroxyethoxymethyl)guanyl (3-propyloxy)-para-carboranyl 3'-H-phosphonate

17 –cidofowoir (CDV) modified with a para-carboranyl cluster: (S)-1-[(2-oxo-1,4,2-

10 dioksafosforinan-5-yl)methyl]cytosine 2-O-(3-propoxy-1-yl)-para-carborane,

Modified GCV, ACV and CDV are characterised by low toxicity and strong antiviral activity against the examined herpes viruses in comparison to their parental drugs.

CMV – cytomegaly virus

15 HSV-1 and HSV-2 – herpes simplex viruses type 1 and 2

Example 3 synthesis of compound 13

Synthesis of 9-[(1-oxyethoxy)methyl]guanine 1-O-phosphonate (13) was performed according to the literature procedure (Galegov et al., 1997 Synthesis and antiherpetic activity of acyclovir phosphoesters. Bioorg. Khim., 23: 906-909.).

Example 4 synthesis of compound 16

Synthesis of (S)-1-[(2-hydroxy-2-oxo-1,4,2-dioxaphosphorinan-5-y)methyl]cytosine (16) was performed according to the literature procedure (Krecemerova et al., 2007 2007. Ester prodrugs of cyclic 1-(S)-[3-hydroxy-2-(phosphonomethoxy)propyl]-5-azacytosine: Synthesis and antiviral activity. J. Med. Chem., 50: 5765-5772.).

Example 5 synthesis of compound 19

Synthesis of 3-(para-carborane)propionic acids (19) was performed according to literature procedure (Malmquist, 1996 Asymmetric synthesis of p-carboranylalanine (p-Car) and 2-methyl-o-carboranylalanine (Me-o-Car). Tetrahedron, 52:9207-9218).

Example 6 synthesis of compound 4

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

9-{[(1,3-dihydroxypropan-2-yl)oxy]methyl} guanine (1) (500 mg, 1.96 mmol) was dried by co-evaporation with anhydrous pyridine (3 x 4 ml) and then suspended in 20 ml of anhydrous pyridine. 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 2N-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 (1:1, v/v, 5 ml) 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 using a stepwise gradient of methanol in

dichloromethane. Yield: 489.45 mg (76.8 %). TLC (CH₂Cl₂:CH₃OH, 8:2, v/v): R_f = 0.54; UV-Vis (95% C₂H₅OH): λ_{min}=225 nm, λ_{min} =272 nm, λ_{max}=255 nm, λ_{max}=260 nm, λ_{max}=281, λ_{sh}=257 nm; ¹H NMR (250 MHz, CD₂Cl₂, 25°C, TMS): δ = 1.24 (d, 6H, 2 x CH₃ 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 (Gly, FAB, +Ve): m/z = 326.2 [M+1]⁺, MS (Gly, FAB, -Ve): m/z = 324.2 [M-1]⁻ (C₁₃H₁₉N₅O₅, calculated 325.14).

Example 7 synthesis of compound 5

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

2N-isobutyryl-9-(1,3-dihydroxy-2-propoxymethyl)guanine (4) (489.45 mg, 1.51 mmol) was dried by co-evaporation with anhydrous pyridine (3 x 3 ml) then was suspended in anhydrous

15 pyridine (6 ml). Next triethylamine (271 μl, 1.94 mmol) and 4,4'-dimethoxytrityl chloride (560.9 mg, 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

20 once with saturated solution of sodium hydrogen carbonate (20 ml). The organic layer was dried over anhydrous magnesium sulphate, filtered and then the solvents were evaporated. Crude product was purified by column chromatography on silica gel column using a stepwise gradient of methanol in dichloromethane to elute the product 5. Yield: 500.3 mg (53 %). TLC (CH₂Cl₂:CH₃OH, 95:5 v/v): R_f = 0.29; RP-HPLC (Hypersil Gold C18 RP, 250 x 4.6 mm, 5

25 μm; flow 1 ml/min; room temperature; 0 min – 100 % A, 0-20 min 0-100 % D, 20-25 min 100 % D, 25-30 min 0-100 % A, A = 2% CH₃CN/0.1M TEAB, D = 60% CH₃CN/0.1M TEAB): R_t = 23.06 min; UV-Vis (95% C₂H₅OH): λ_{min} = 223 nm, λ_{min} = 269 nm, λ_{max} = 236 nm, λ_{max} = 277 nm, λ_{sh} = 258 nm; ¹H NMR (250 MHz, CD₂Cl₂): δ = 1.21 (d, 6H, 2 x CH₃ of isobutyryl group), 2.53-2.59 (m, 2H, 1H-2, 1CH of isobutyryl group), 3.16-3.62 (2 x m, 4H, 2H-1, 2H-3), 3.82 (s, 6H, 2 x OCH₃ of dimethoxytrityl group), 5.63 (d, 2H, H-4),

30 6.83-6.85 (m, 4H, 2 x CH₃OPh 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); ¹³C NMR (62.90 MHz, CD₂Cl₂): δ = 19.06 (CH₃ of isobutyryl group), 36.67 (CH of isobutyryl group), 55.60 (OCH₃ of dimethoxytrityl group), 63.09, 64.18 (C-1, 3), 73.28 (C-4), 79.49 (C-2), 86.69 (C-

methylenedioxy 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 (C of CO from guanine).

Example 8 synthesis of compound 6

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

Imidazole (501.33 mg, 7.36 mmol) was dissolved in tetrahydrofuran (25.7 ml) and the solution was cooled to -10 °C in dry ice/acetone bath. Then phosphorous trichloride (209 µl, 2.4 mmol) was added with vigorous stirring followed by triethylamine (1.06 ml, 7.71 mmol) mixed with tetrahydrofuran (856 µl). The reaction mixture was stirred for 30 min at -10 °C and then was cooled to -78 °C in dry ice/acetone bath. To the solution of resultant phosphorimidazolidite intermediate a protected ganciclovir 5 (430 mg, 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 °C. After 1 h 2M TEAB (25.5 ml) was added followed by dichloromethane (50 ml). The organic layer was separated, washed once with of 2M TEAB (25.5 ml), dried over anhydrous magnesium sulphate, filtrated and evaporated under reduced pressure to the dryness. Crude product 6 was purified by silica-gel column chromatography using a stepwise gradient of methanol in dichloromethane with 1 % of triethylamine added, as eluting solvent system. Yield: 377.5 mg (79.7 %). TLC (CH₂Cl₂:CH₃OH, 8:2, v/v): R_f = 0.31; RP-HPLC (Hypersil Gold C18 RP; 250 x 4.6 mm; 5 µm; flow 1 ml/min; room temperature; 0 min – 100 % A, 0-20 min 0-100 % D, 20-25 min 100 % D, 25-30 min 0-100 % A, A=2% CH₃CN/0.1M TEAB, D=60% CH₃CN/0.1M TEAB): R_t = 18.81 min; UV-Vis (95% C₂H₅OH): λ_{min.} = 223 nm, λ_{min.} = 271 nm λ_{max.} = 236 nm, λ_{max.} = 282 nm, λ_{sh.} = 252 nm, λ_{sh.} = 256 nm, λ_{sh.} = 275 nm; ¹H NMR (250 MHz, CH₃OD): δ = 1.13 (d, 6H, 2 x CH₃ of isobutyryl group), 1.27 (m, 9H, 3 x CH₃ of triethylamine), 2.72 (m, 1H, CH of isobutyryl group), 2.97-3.03 (m, 2H, H-3), 3.15 (q, 6H, CH₂ of triethylamine), 3.80-4.25 (m, 3H, 2H-1, 1H-2), 3.73 (s, 6H, 2 x OCH₃ of dimethoxytrityl group), 6.68 (d, 1JP-H=662.25, 1H, P-H), 5.69 (d, 2H, H-4), 6.74-6.77 (m, 4H, 2 x CH₃OPh of dimethoxytrityl group), 7.05-7.20 (m, 9H, dimethoxytrityl group), 8.15 (s, 1H, H-8 from guanine); ¹³C NMR (62.90 MHz, CH₃OD): δ = 9.22 (C of triethylamine), 19.49 (CH₃ of isobutyryl group), 36.96 (CH of

isobutyryl group), 55.71 (OCH₃ 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); ³¹P NMR (101.26 MHz, CH₃OD): δ = 6.11; MS (FAB, Gly, -Ve): m/z = 690.4 [M-1]⁻ (C₃₄H₃₈N₅O₉P, calculated: 691.24 (100%)).

Example 9 synthesis of compound 7

Synthesis of 2N-isobutyryl-9-[[[(1-O-dimethoxytrityl-3-propoxy-2-yl)oxy]methyl]guanine-(3-propoxy-1-yl)-para-carborane 3-O-phosphonate and 2N-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 (151.5 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 x 2 ml).

The gummy residue was dissolved in pyridine (2 ml) and while stirring pivaloyl chloride (73.5 μ l, 0.6 mmol) was added in one portion. After reaction completion (ca. 0.5 h, TLC control) 2M TEAB (200 μ l) was added into reaction mixture then whole was concentrated by evaporation under vacuum, then the residue was portioned between dichloromethane (6 ml) and 0.5M TEAB (200 μ l). The organic layer was collected, dried over anhydrous magnesium sulphate then the solvent was evaporated. The crude product containing modified H-phosphonate 7 was purified on a silica gel column using a stepwise gradient of methanol in dichloromethane, with 1 % triethylamine added. Yield: 111.7 mg (58%). TLC (CH₂Cl₂:CH₃OH, 9:1, v/v): R_f = 0.65; RP-HPLC (Hypersil Gold C18 RP; 250 x 4.6 mm; 5 μ m; flow 1 ml/min; room temperature; 0 min – 100 % A, 0-20 min 0-100 % D, 20-25 min 100 % D, 25-30 min 0-100 % A, A=2% CH₃CN/0.1M TEAB, D=60% CH₃CN/0.1M TEAB): R_t = 23.08 min; UV-Vis (95% C₂H₅OH): λ_{min} = 223 nm, λ_{min} = 270 nm, λ_{max} = 236 nm, λ_{max} = 282, λ_{sh} = 253 nm, λ_{sh} = 260 nm, λ_{sh} = 276 nm; ¹H NMR (250 MHz, CD₂Cl₂): δ = 1.00-2.50 (m, 10H, carborane), 1.25 (d, 6H, 2 x CH₃ of isobutyryl group), 1.40 (m, 2H, CH₂-2 from linker), 1.70 (m, 2H, CH₂-1 from linker), 2.58 (m, 1H, CH of isobutyryl group), 2.75 (bs, 1H, CH of carborane), 3.20 (m, 2H, CH₂O from linker), 3.82-4.50 (m, 5H, 2H-1, 2H-3, 1H-2), 3.81 (s, 6H, 2 x OCH₃ of dimethoxytrityl group), 5.54 (2d, 2H, H-4), 6.83-6.88 (m, 4H, 2 x CH₃O⁺Ph 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, 1JP-H=711.65 and 1JP-H=710.64), 12.05 (bs, 1H, NH from guanine); ¹³C NMR (62.90 MHz,

CD₂Cl₂): δ = 14.28 (CH₃ of isobutyryl group), 22.43 (CH₃ of isobutyryl group), 25.42 (CH₂-2 from linker), 30.34 (CH₂-1 from linker), 31.72 (CH of isobutyryl group), 50.80 (OCH₃ of dimethoxytrityl group), 54.17 (CH of carborane), 58.41 (CH₂O from linker), 60.10, 60.53 (C-1, C-3), 67.80 and 68.22 (C-4), 71.86 (C-2), 81.96 (C-methylidene of dimethoxytrityl group), 108.14, 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); ¹¹B NMR (80.25 MHz, CD₂Cl₂), decoupled: δ = -15.54, -13.21 (s, 10B, carborane); coupled: δ = -14.52 (t, 10B, carborane); ³¹P NMR (101.26 MHz, CD₂Cl₂): δ = 7.82 and 9.46; MS (FAB, Gly, +Ve): m/z = 877.4 [M+2]⁺ (C₃₉H₅₄B₁₀N₅O₉P calculated: 875.47 (100%)).

Example 10 synthesis of compound 8

Synthesis of 2N-isobutyryl-9-[[[(1-O-dimethoxytrityl-3-propoxy-2-yl)oxy]methyl]guanine-(3-propoxy-1-yl)-para-carborane 3-O-phosphate and 2N-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

H-Phosphonate 7 (111.7 mg, 0.13 mmol) was dissolved in solution of carbon tetrachloride:triethylamine:N-methylimidazole (15,6 ml, 9:0.5:0.5, 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 x 43 ml). The organic layer was dried over magnesium sulphate, filtrated and solvent evaporated. Crude product was purified by silica gel column chromatography using stepwise gradient of methanol in dichloromethane. Yield: 108.1 mg (95 %). TLC (CH₂Cl₂:CH₃OH, 8:2, v/v): R_f = 0.46; RP-HPLC (Hypersil Gold C18 RP; 250 x 4.6 mm; 5 μ m; flow 1 ml/min; room temperature; 0 min – 100 % A, 0-20 min 0-100 % D, 20-25 min 100 % D, 25-30 min 0-100 % A, A = 2% CH₃CN/0.1M TEAB, D = 60% CH₃CN/0.1M TEAB): R_t = 25.83 min; UV-Vis (95% C₂H₅OH): $\lambda_{min.}$ = 223 nm, $\lambda_{min.}$ = 271 nm, $\lambda_{max.}$ = 236 nm, $\lambda_{max.}$ = 281, $\lambda_{sh.}$ = 252 nm, $\lambda_{sh.}$ = 259 nm, $\lambda_{sh.}$ = 275 nm; ¹H NMR (250 MHz, CD₂Cl₂): δ = 1.17-1.28 (m, 15H, 2 x CH₃ of isobutyryl group, 3 x CH₃ of triethylamine), 1.25-4.00 (m, 10H, carborane), 1.31-1.61 (m, 4H, CH₂-2 from linker, CH₂-1 from linker), 2.68 (m, 1H, CH of isobutyryl group), 2.89 (q, 6H, CH₂ of triethylamine), 3.00 (s, 1H, CH of carborane), 3.40-4.00 (m, 7H, 2H-1, 1H-2, 2H-3, CH₂O from linker), 3.76 (s, 6H, 2 x OCH₃ of dimethoxytrityl), 5.50-5.75 (m,

2H, H-4), 6.77-6.80 (m, 4H, 2 x CH₃OPh of dimethoxytrityl group), 7.00-7.40 (m, 9H, dimethoxytrityl group), 7.80 (s, 1H, H-8 from guanine); ¹³C NMR (62.90 MHz, CD₂Cl₂): δ = 6.63 (CH₃ of triethylamine), 17.08 (CH₃ of isobutyryl group), 28.64 (CH₂-2 from linker), 33.65 (CH of isobutyryl group), 35.50 (CH₂-1 from linker), 43.83 (CH₂ of triethylamine), 53.36 (OCH₃ of dimethoxytrityl group), 56.50 (CH of carborane), 62.35 (C-1, 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); ¹¹B NMR (80.25 MHz, CD₂Cl₂): decoupled: δ = -15.13, -12.63 (2s, 10B, carborane); coupled: δ = -14.44 (t, 10B, carborane); ³¹P NMR (101.26 MHz, CD₂Cl₂): δ = -1.43; MS (FAB, Gly, -Ve): m/z = 891.4 [M-1]⁻ (C₃₉H₅₄B₁₀N₅O₁₀P, calculated 892.46 (100%)).

Example 11 synthesis of compound 10

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 (10), triethylammonium salt, racemic mixture. Phosphate 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 (8:2, v/v, 25 ml). The solution was stirred at room temperature for 1 h. Next, to the reaction mixture methanol was added (10 ml) then stirring was continued for next 10 min. The mixture was next concentrated under vacuum and product was isolated by silica gel column chromatography using a stepwise gradient of methanol in dichloromethane yielding 26.1 mg (61.5 % yield) of final product as triethylammonium salt (10). TLC (CH₂Cl₂:CH₃OH, 7:3, v/v): R_f = 0.24 (the TLC plates was developed tree fold); RP-HPLC (Hypersil Gold C18 RP; 250 x 4.6 mm; 5 μm; flow 1 ml/min; room temperature; 0 min – 100 % A, 0-20 min 0-100 % D, 20-25 min 100 % D, 25-30 min 0-100 % A, A = 2% CH₃CN/0.1M TEAB, D = 60% CH₃CN/0.1M TEAB): R_t = 16.46 min; UV-Vis (95% C₂H₅OH): λ_{min} = 225 nm, λ_{max} = 254 nm, λ_{sh} = 274 nm; ¹H NMR (600 MHz, DMSO): δ = 1.27 (m, 9H, 3 x CH₃ of triethylamine), 1.26-1.33 (m, 2H, CH₂-2 from linker), 1.50-2.20

(m, 10H, carborane), 1.67-1.70 (m, 2H, CH₂-1 from linker), 2.52 (q, 6H, CH₂ of triethylamine), 3.27-3.39 (m, 5H, 2H-1, 2H-3, CH-carborane), 3.46-3.49 (m, 2H, CH₂O from linker), 3.61-3.69 (m, 1H, H-2), 5.42 (s, 2H, H-4), 6.61 (bs, 2H, NH₂), 7.80 (s, 1H, H-8 from guanine), 10.72 (s, 1H, NH); ¹³C NMR (150.95 MHz, DMSO): δ = 30.50 (CH₂-2 from linker), 35.13 (CH₂-1 from linker), 54.04 (CH of carborane), 59.99 (C-1, 3), 63.09 (CH₂O 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); ¹¹B NMR (192.59 MHz, DMSO), decoupled: δ = -15.10, -12.62 (s, 10B, carborane); coupled: δ = -15.08, -12.56 (2 x d, 10B, carborane); ³¹P NMR (242.99 MHz, DMSO): δ = -0.42; MS (FAB, Gly, -Ve): m/z = 519.4 [M], 581.2 [M+Cu₂+]⁻ (C₁₄H₃₀B₁₀N₅O₇P, calculated: 519.29 (100%)).

Example 12 synthesis of compound 11

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.

To obtain sodium salt of the final product 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 1M 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 x 10 ml, HPLC grade) then dried under vacuum. Yield: 20.32 mg (91 %). TLC (CH₂Cl₂:CH₃OH, 6:4, v/v) : R_f = 0.20; RP-HPLC (Hypersil Gold C18 RP; 250 x 4.6 mm; 5 μ m; flow 1 ml/min; room temperature; 0 min – 100 % A, 0-20 min 0-100 % D, 20-25 min 100 % D, 25-30 min 0-100 % A, A=2% CH₃CN/0.1M TEAB, D=60% CH₃CN/0.1M TEAB): R_t = 16.49 min; UV-Vis (95% C₂H₅OH): $\lambda_{\min}$. = 243 nm, $\lambda_{\min}$. = 266 nm, $\lambda_{\max}$. = 253 nm, $\lambda_{\max}$. = 274 nm; ¹H NMR (400 MHz, D₂O): δ = 1.20-2.90 (m, 10H, BH-carborane), 1.34 (bs, 2H, CH₂-2 from linker), 1.61 (bs, CH₂-1 from linker), 2.99 (bs, 1H, CH of carborane), 3.58-3.87 (m, 7H, CH₂O from linker, 2H-1 2H-3, 1H-2), 5.58-5.61 (m, 2H, H-4), 7.91-7.94 (m, 1H, H-8); ¹¹B NMR (128.33 MHz, D₂O): decoupled: δ = -16.25, -13.88 (s, 10B, carborane); coupled: δ = -15.02, -14.45 (2 x d, 10B, carborane); ³¹P NMR (161.92 MHz, D₂O): δ = 1.49 (t); MS (FAB, Gly, -Ve): m/z = 540.3 [M-2]⁻ (C₁₄H₂₉B₁₀N₅NaO₇P, calculated 542.27 (100%)).

Example 13 synthesis of compound 14

Synthesis of 9-[(1-oxyethoxy)methyl]guanine-(3-propoxy-1-yl)-para-carborane 1-O-phosphonate (14)

9-[(1-Oxyethoxy)methyl]guanine-(3-propoxy-1-yl)-para-carborane 1-O-phosphonate (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 μ l, 0.45 mmol) was added in one portion. The reaction mixture was stirred for 2h at room temperature then 2M TEAB (150 μ l) was added, next the solvents were evaporated under reduced pressure. Crude product 14 was purified by silica gel column chromatography using a stepwise gradient of methanol in dichloromethane. Yield: 45.5 mg (58 %). TLC (CH₂Cl₂:CH₃OH, 8:2, v/v): R_f = 0.55; RP-HPLC: (Hypersil Gold C18 RP; 250 x 4.6 mm; 5 μ m; flow 1 ml/min; room temperature; 0 min – 100 % A, 0-20 min 0-100 % D, 20-25 min 100 % D, 25-30 min 0-100 % A, A = 2% CH₃CN/0.1M TEAB, D = 60% CH₃CN/0.1M TEAB): R_t = 21.51 min; UV-Vis (95% C₂H₅OH): λ_{min} = 222 nm, λ_{min} = 264 nm, λ_{max} = 255 nm, λ_{max} = 275 nm; ¹H-NMR (600 MHz, DMSO): δ = 1.0-3.00 (m, 10H, BH-carborane), 1.39-1.44 (m, 2H, CH₂-2 from linker), 1.68-1.71 (m, 2H, CH₂-1 from linker), 3.65-3.67 (m, 2H, H-2), 3.78-3.81 (m, 2H, CH₂O from linker), 4.01-4.09 (m, 2H, H-1), 5.37 (s, 2H, H-4), 6.75 (d, 1JP-H=706.44, 1H, P-H), 7.81 (s, 1H, H-8); ¹³C-NMR (150.94 MHz, DMSO): δ = 29.92 (CH₂-2 from linker), 34.36 (CH₂-1 from linker), 59.33 (CH-carborane), 64.00 (CH₂O 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); ¹¹B-NMR (192.59 MHz, DMSO): decoupled: δ = -15.04, -12.63 (s, 10B, carborane); coupled: δ = -15.04, -12.63 (2 x d, 10B, carborane); ³¹P-NMR (242.99 MHz, DMSO): δ = 9.35; MS (FAB, Gly, +Ve): m/z = 474.3 [M+1]⁺ and 537.2 [M+1+Gly]⁺ (C₁₃H₂₈B₁₀N₅O₅P, calculated: 473.28 (100%)).

Example 14 synthesis of compound 17

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. 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 using a stepwise gradient of methanol in dichloromethane. Yield: 22.48 mg (58%). TLC (CH₂Cl₂:

CH₃OH:NH₃aq:H₂O, 80:20:1:1, v/v): R_f = 0.45; RP-HPLC (Hypersil Gold C18 RP; 250 x 4.6 mm; 5 µm; flow 1 ml/min; room temperature; 0 min – 100 % A, 0-20 min 0-100 % D, 20-25 min 100 % D, 25-30 min 0-100 % A, A = 2% CH₃CN/0.1M TEAB, D = 60% CH₃CN/0.1M TEAB): R_t = 22.98 and 23.21 min (two diastereomers); UV-Vis (95% C₂H₅OH): λ_{min}. = 253 nm, λ_{max}. = 272 nm; ¹H-NMR (600 MHz, CD₂Cl₂): δ = 1.50-1.70 (m, 10H, BH-carborane), 1.49-1.75 (m, 4H, CH₂-2 from linker, CH₂-1 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, CH₂O from linker), 3.91-4.05 (m, 5H, 2H-3, 1H-5, 2H-7, 2H-7), 4.21-4.27 (m, 2H, H-6); ¹³C-NMR (150.94 MHz, CD₂Cl₂): δ = 31.05 and 31.09 (CH₂-2 from linker), 35.65 and 35.77 (CH₂-1 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 (CH₂O 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); ¹¹B-NMR (192.59 MHz, CD₂Cl₂): decoupled: δ = -15.02, -12.61 (s, 10B, carborane); coupled: δ = -15.03, -12.61 (2d, 10B, carborane); ³¹P-NMR (242.99 MHz, CD₂Cl₂): δ = 10.16 (s) and 11.82 (s); MS (FAB, Gly, +Ve): m/z = 446.3 [M+1]⁺, 631.6 [M+1+Gly+Gly]⁺ (C₁₃H₂₉B₁₀N₃O₅P, calculated: 445.28 (100%)).

Example 15 synthesis of compound 19

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 µl, 1.11 mmol) was added. 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 16h and after reaction completion the solvent was evaporated. Crude product was purified by silica gel column chromatography using a stepwise gradient of diethyl ether in hexane. Yield: 58.6 mg (81 %). ¹H-NMR (600 MHz, CD₃OD): δ = 1.00-2.80 (m, 10H, BH-carborane), 2.04 (t, 2H, CH₂-2 from linker), 2.29 (t, 2H, CH₂-1 from linker), 3.19 (bs, 1H, CH of carborane), 3.73 (t, 2H, CH-6), 4.14 (t, 2H, CH₂-5); MS (FAB, Gly, + Ve): m/z = 261.3 [M+1]⁺ (C₇H₂₀B₁₀O₃, calculated: 260.24 (100%)).

Example 16 synthesis of compound 20

(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. Cidofovir (16) (11.37 mg, 0.02 mmol) and 3-(para-carborane)propionic acids (19) (8 mg, 0.03 mmol) were dissolved in

DMF (172.3 μ l), next DIPEA (9.69 μ l) and (benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate (PyBOP) (26.67 mg, 0.05 mmol) were added. 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 two silica gel columns chromatography: first, using a stepwise gradient of methanol in dichloromethane and second, using a stepwise gradient of acetone in diethyl ether. Yield: 4.94 mg (48%). TLC (CH₂Cl₂:CH₃OH:NH₃:H₂O, 80:20:1:1, v/v): R_f = 0.45; RP-HPLC (Hypersil Gold C18 RP; 250 x 4.6 mm; 5 μ m; flow 1 ml/min; room temperature; 0 min – 100 % A, 0-20 min 0-100 % D, 20-25 min 100 % D, 25-30 min 0-100 % A, A = 2% CH₃CN/0.1M TEAB, D = 60% CH₃CN/0.1M TEAB): R_t = 22.35 min. UV-Vis (95% C₂H₅OH): λ_{min} = 252 nm, λ_{max} = 276 nm; ¹H-NMR (600 MHz, CD₂Cl₂): δ = 1.00-3.00 (m, 10H, BH-carborane), 2.00-2.08 (m, 2H, CH₂-2 from linker), 2.24-2.29 (m, 2H, CH₂-1 from linker), 2.77 (bs, 1H, CH of carborane), 3.57-3.62 (m, 2H, CH₂-6 from linker), 3.68-3.74 (m, 2H, CH₂-5 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 citidine), 7.32 (d, 1H, H-6 from citidine), 7.36 (d, 1H, H-6 from citidine); ¹³C-NMR (150.94 MHz, CD₂Cl₂): δ = 34.03 and 34.07 (CH₂-2 from linker), 34.14 and 34.18 (CH₂-1 from linker), 49.91 and 50.04 (C-7), 59.51 (CH of carborane), 62.51 and 62.55 (CH₂-6 from linker), 64.00 and 64.10 (C-3), 71.17 and 71.82 (C-6), 73.16 and 73.20 (CH₂-5 from linker), 75.22 and 75.61 (C-5), 94.82 and 94.91 (C-5 from citidine), 147.78 and 147.84 (C-6 from citidine), 156.79 and 156.81 (C-2 from citidine), 166.66 and 166.68 (C-4 from citidine), 171.95 and 172.01 (CO from citidine); ¹¹B-NMR (192.59 MHz, CD₂Cl₂): δ = decoupled: -14.98, -12.66 (s, 10B, carborane); coupled: -14.98, -12.67 (2 d, 10B, carborane); ³¹P-NMR (242.99 MHz, CD₂Cl₂): δ = 10.69 (s) and 12.54 (s); MS (FAB, Gly, +Ve): m/z = 504.3 [M+1]⁺ (C₁₅H₃₀B₁₀N₃O₇P, calculated: 503.28 (100%)).

Example 17 synthesis of compound 22

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 DMF (385 μ l). The solution was cooled to 0 °C in dry ice/acetone bath and next *N*-methylmorpholine (6.22 μ l) was added. The reaction mixture was stirred for 2 h at 0 °C and after then 9-[(1,3-dihydroxypropan-2-yl)oxy]methyl}guanine (2S)-2-amino-3-methylbutanoate (**20**) (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 using a stepwise gradient of methanol in dichloromethane. Yield: 19.5 mg (62 %). TLC

(CH₂Cl₂:CH₃OH, 8:2, v/v): R_f= 0.30; RP-HPLC (Hypersil Gold C18 RP; 250 x 4.6 mm; 5 μm; flow 1 ml/min; room temperature; 0 min – 100 % A, 0-20 min 0-100 % D, 20-25 min 100 % D, 25-30 min 0-100 % A, A=2% CH₃CN/0.1M TEAB, D=60% CH₃CN/0.1M TEAB): R_t = 21.33 min; UV-Vis (95% C₂H₅OH): λ_{min}= 222 nm, λ_{max}= 255 nm, λ_{sh}= 274 nm; MS (FAB, Gly, -Ve): m/z = 552.4 [M-1]⁻ (C₁₉H₃₆B₁₀N₆O₆, calculated: 553.37 (100%)); ¹¹B-NMR (192.59 MHz, CD₂Cl₂): δ = decoupled: -15.05, -12.61 (s, 10B, carborane);

Example 18 Stability

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 sample of the compound (ca. 0.3 mg,) in DMSO (Sigma Aldrich) (ca. 1 μl) followed by water solution containing 1% of (2-hydroxypropyl)-beta-cyclodextrin HPBCD) (Sigma Aldrich) (49 μl) to the concentration of the compound 1 x 10⁻²M. The working solutions of 11, 14, 17, 20 and 22 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, NaHCO₃, and 2% serum) (50 μl). The final concentration of the compounds was 0.5 x 10⁻²M; 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 μl) 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 under conditions as described above.

Stability in Eagle's minimal essential medium: All the test were carried out at 37 °C in Wealtec Block Heater. Aliquots (0.01 mg, ca. 5-8 μl) were withdrawn from the incubation mixture after every 2 h during first 12 h then after 24 and 48 h. Next the procedure was as described above. See results in Table 2

Example 19 lipophilicity

Determination of lipophilicity using RP-TLC analysis (Bajda et al., 2007) of compounds 11, 14, 17, 20, 22 and unmodified counterparts: ganciclovir, aciclovir, cidofovir and valganciclovir. Sample of the compounds (0.1 mg) was dissolved in methanol (POCH, Poland) (40 μl) and DMSO (Aldrich) (2 μl) then aliquate of the solution (1 μl) was spotted on

the RP-TLC plates, 1.5 cm from the bottom edge. TLC chromatography was performed on 5x10 cm precoated RP18 F254S plates from Merck. The plates were developed in glass chamber previously saturated with mobil-phase vapor for 30 min. Mixture of acetonitrile (POCH, Poland) and water (MiliQ) with acetonitrile content between 45 and 80% (v/v), for modified acyclovir 14, cidofovir 17, cidofovir 20 and valganciclovir 22, 10 and 40% (v/v), for modified ganciclovir 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. Rf values were means from three independent experiments. RM values were calculated from experimental Rf by use of the equation $RM=\log(1/Rf-1)$. RM values were then extrapolated to zero acetonitrile concentration by use of equation $RM=RM_0+aC$, where C is the concentration of acetonitrile in the mobile phase (%v/v).(see results in Table2)

Table 2.

Lipophilicity, stability in buffers with different pH and in human serum of GCV, ACV and CDV compounds

Compound	Lipophilicity	Stability in buffers			Stability in culture medium
		pH			
		4	7	9	
		t1/2[h]			t1/2[h]
11	1.85 ^a	0.47	stable	stable	stable
14	1.87 ^b	0.26	6.61	1.20	5.7
17	2.34 ^c	^d	^d	3.76	^d
20	2.14	0.21	3.43	0.72	3.64
22	2.61	0.21	stable	34.83	304

^aRM0 for unmodified GCV = -0.22, ^bRM0 for unmodified ACV = -0.15, ^cRM0 for unmodified CDV = -0.71, ^d hydrate formation

Claims

1. An antiviral drug derivative, preferentially against HCMV, containing a boron cluster connected with the drug through a connecting group and, preferentially, a phosphoric or H-phosphonic acid.

2. An antiviral drug derivative according to claim 1, characterised in that the antiviral drug is selected from a group comprising gancyclovir, acyclovir, cidofovir, valgancyclovir, valacyclovir and pencyclovir.

3. An antiviral drug derivative according to any of claims from 1 to 2 characterised in that the connecting group is defined by the formula $-(\text{CH}_2)_n-(\text{W})_m]_k(\text{CH}_2)_h\text{Tf}$ - where f is 0-3, h is 0 to 5, k is from 0 to 6, m is 0 or 1, n is from 0 to 5 W is O, S, C(O), S(O), S(O)₂, Se, NR (where R = H, alkyl, haloalkyl, alkoxyalkyl or aryl), T is XP(Z)(Y)X₁ (where X = O, S, Se, alkyl, haloalkyl, alkoxyalkyl, aryl) and also CH=CH, CC, N=N, CHOH and CHN₃; where X₁ = O, S, Se, alkyl, haloalkyl, alkoxyalkyl, aryl) and also CH=CH, CC, N=N, CHOH and CHN₃; Z = O, S, Se; Y = OH, SH, SeH, H or alkyl, haloalkyl, alkoxyalkyl, aryl or halogen, in particular fluorine), and also CH=CH, CC, N=N, CHOH and CHN₃

4 An antiviral drug derivative according to claim 1, characterized in that the boron cluster is 1,2-dicarba-closo-dodecarborane (ortho-carboranyl), 1,7-dicarba-closo-dodecarborane (meta-carboranyl), 1,12-dicarba-closo-dodecarborane (para-carboranyl), 7,8-dicarba-nido-undecarborane (nido--carboranyl), closo-dodecarborane or their derivatives substituted on a carbon or boron atom.

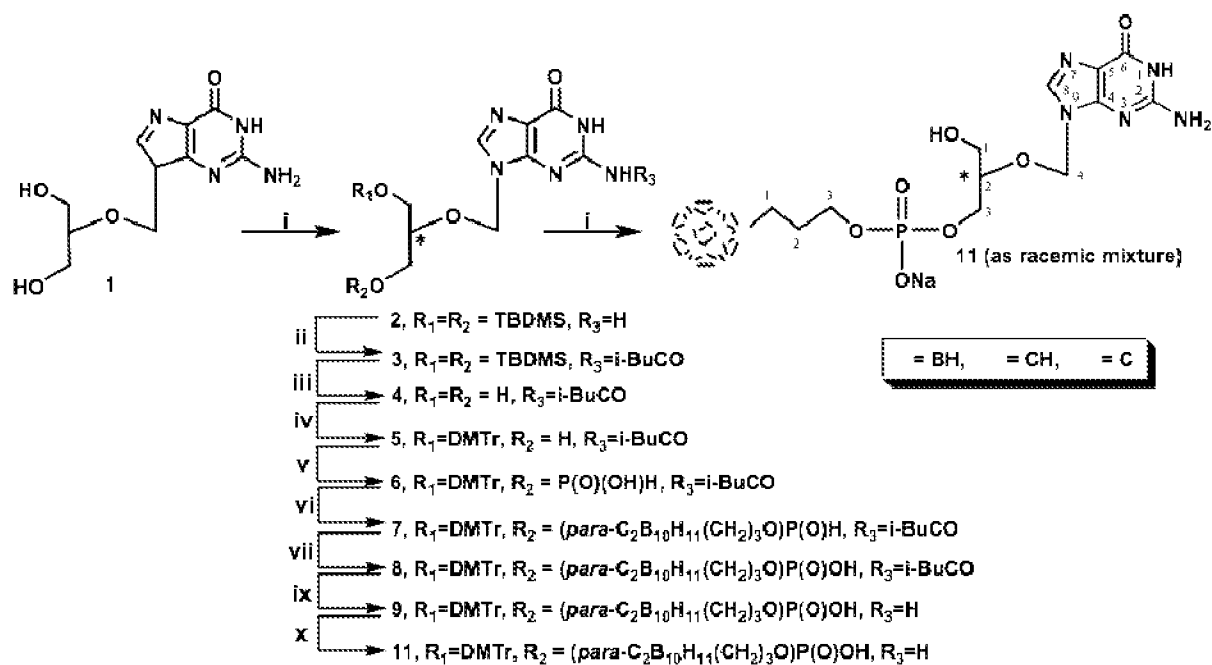


Fig.1

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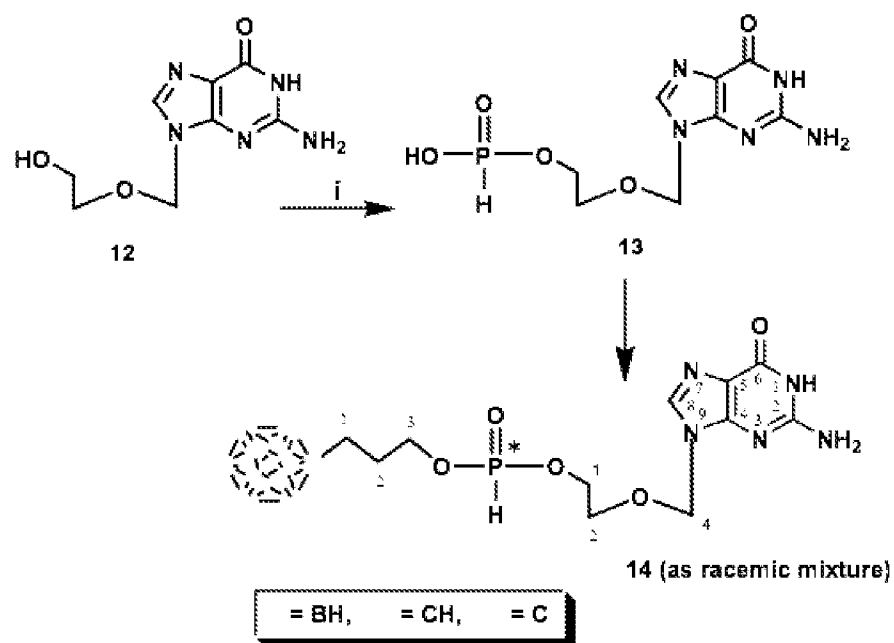


Fig 2.

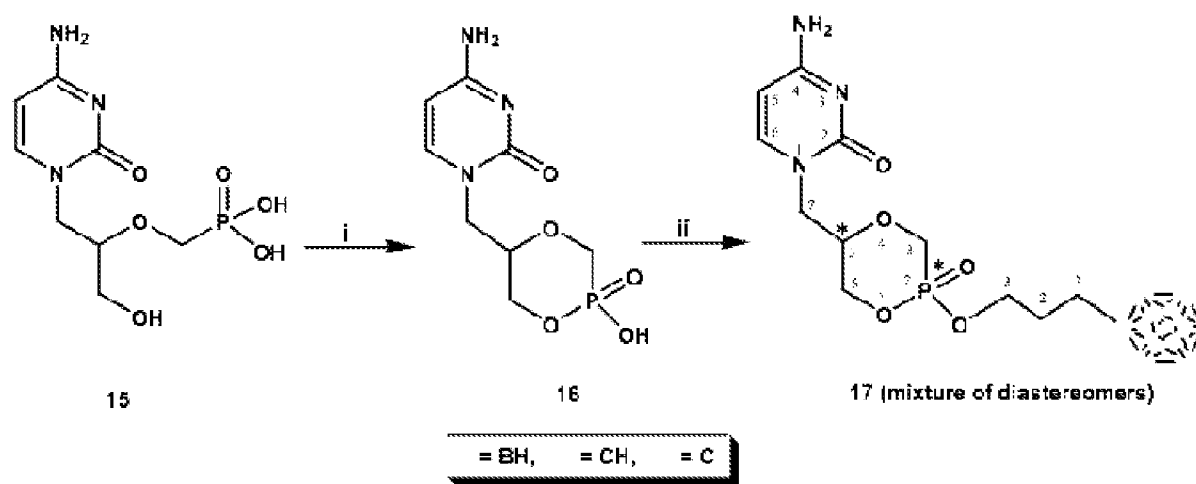
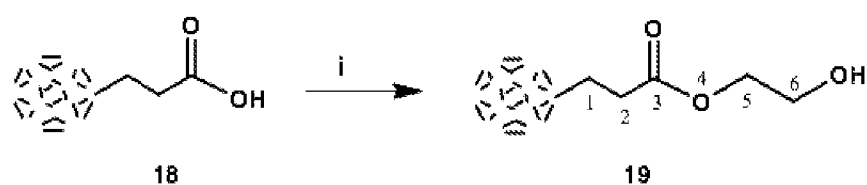


Fig 3.



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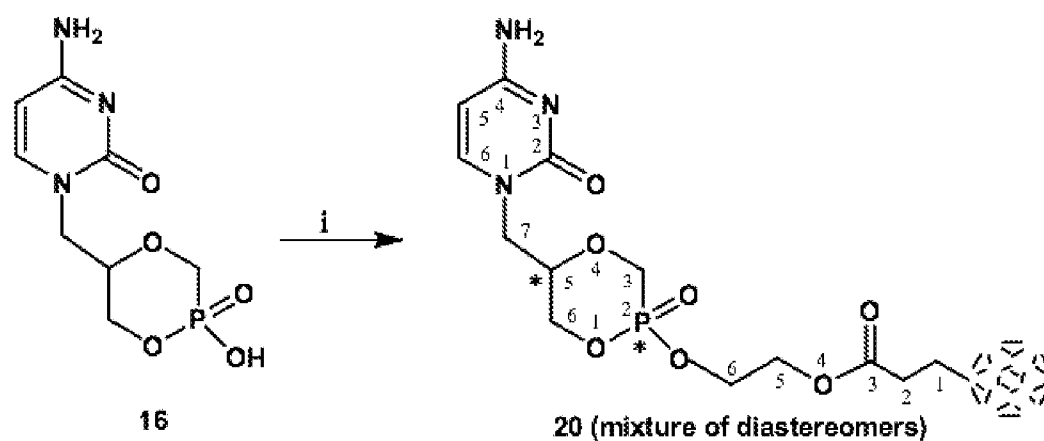
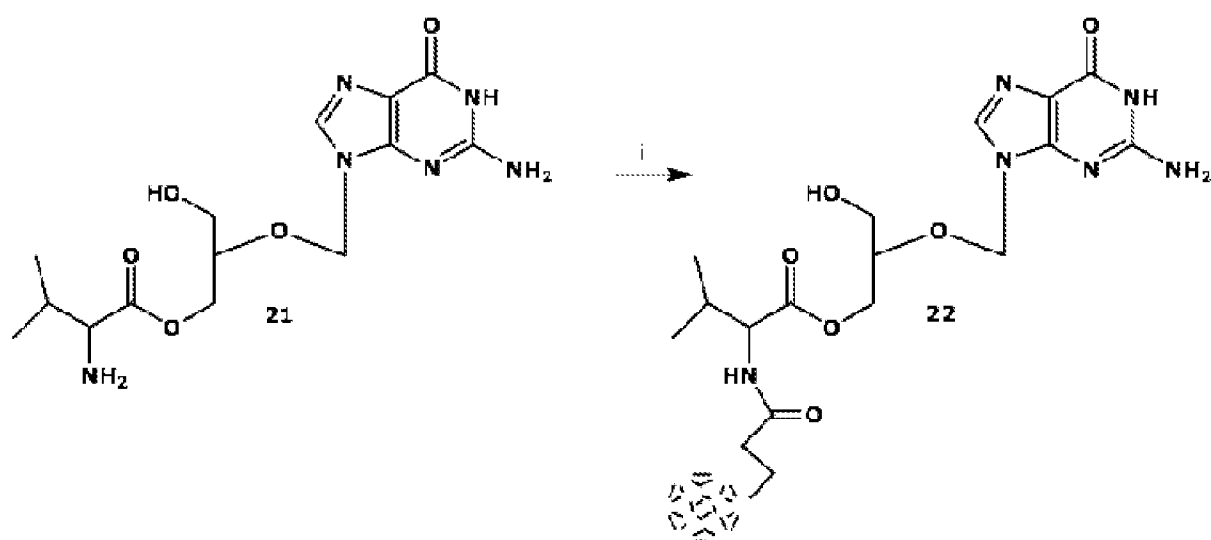


Fig. 4.

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INTERNATIONAL SEARCH REPORT

International application No
PCT/PL2011/050012

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07F5/02 A61K31/69 A61P31/12
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07F A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, CHEM ABS Data, BEILSTEIN Data, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 96/14073 A1 (UNIV EMORY [US]) 17 May 1996 (1996-05-17) cited in the application page 79; claim 1	1-4
A	----- US 6 838 574 B1 (ENDO YASUYUKI [JP]) 4 January 2005 (2005-01-04) cited in the application page 65; claim 1	1-4
A	----- EP 1 364 954 A1 (KAGECHIKA HIROYUKI [JP]) 26 November 2003 (2003-11-26) cited in the application page 12; compound 8 page 14; compound 10 page 16; compound 16 ----- -/-	1-4



Further documents are listed in the continuation of Box C.



See patent family annex.

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Date of the actual completion of the international search

7 July 2011

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INTERNATIONAL SEARCH REPORT

International application No

PCT/PL2011/050012

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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A	US 2005/043246 A1 (MITRA ASHIM K [US]) 24 February 2005 (2005-02-24) page 25; claim 1 -----	1-4
X	US 2009/149482 A1 (MITRA ASHIM K [US] ET AL) 11 June 2009 (2009-06-11) page 25; claims 1, 10 -----	1-4

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International application No

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