

Fluorosubstitution and 7-alkylation as prospective modifications of biologically active 6-aryl derivatives of tricyclic acyclovir and ganciclovir analogues

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Abstract—A series of fluorine containing tricyclic analogues of acyclovir (ACV, **1**) and ganciclovir (GCV, **2**) were synthesized and evaluated for their activity against herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2) and cytostatic activity against HSV-1 thymidine kinase (TK) gene-transduced human osteosarcoma tumour cells. It was found that fluorine substitution reduced the antiviral activity, but most of the new compounds were pronounced cytostatic agents with potency and selectivity similar to those of parental ACV and GCV. Compounds **12**, **13** and **16** seem to be promising as labeled substrates for ¹⁹F NMR studies of the HSV TK–ligand interaction and/or monitoring of their metabolites in cells expressing HSV TK.

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1. Introduction

Our previous studies on modification of the guanine moiety of the antiherpetic drugs acyclovir (ACV, **1**) and ganciclovir (GCV, **2**) in order to modulate their physical and biological properties have shown that introducing of 1,N²-(ethene-1,2-diyl) bridge to transform **1** and **2** into their tricyclic (T) analogues (TACV, **3** and TGCV, **4**), derivatives of 3,9-dihydro-9-oxo-5H-imidazo[1,2-a]purine, together with an appropriate substituent at positions 6 and/or 7 of the appended ring may lead to this aim.^{1,2} At the early stage of this search fluorescent 6-phenyl-TACV (**5**) and 6-phenyl-TGCV (**6**) appeared to be the most promising. The activity of compound **6** against herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2) was very similar to that of the parent ganciclovir, compound **5** being approximately one order of magnitude less active than the parent acyclovir.²

Of the next series of over 20 new tricyclic analogues, which have been evaluated for activity against HSV-1

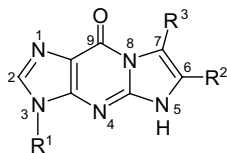
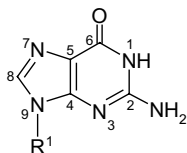
and HSV-2 and subjected to a structure–antiviral activity analysis, 6-(4-MeOPh)-TACV (**7**) and 7-Me-6-Ph-TACV (**8**) and their ganciclovir congeners (**9** and **10**, respectively) showed similar potency and selectivity as the parent compounds. Additionally, they are fluorescent and more lipophilic.³ Further studies using 6-(4-MeOPh)-TACV and 6-(4-MeOPh)-TGCV as lead compounds, recently yielded 6-(4-acyloxyphenyl)-TACV and TGCV analogues, which exhibit stronger fluorescence and antiviral activity similar to that of parent ACV and GCV.⁴ Moreover, they have been identified as potent and highly selective cytostatic agents against HSV-1-thymidine kinase (TK) gene-transduced human osteosarcoma and murine mammary carcinoma tumour cells.⁵

In the present work we prepared a further series of TACV and TGCV derivatives using the aforementioned lead compounds **7**–**10**-derivatives bearing fluorine (compounds **11**–**14**) or trifluoromethyl group (**15**–**17**). We added to this series two nonfluorinated congeners: **18**, an analogue of compound **14**, and **19**, thus combining the structural features of both lead compounds **7** and **8** (Chart 1).

Selective fluorination has been a very effective tool for modifying the reactivity of diverse organic compounds. It has been known that it can have profound and

Keywords: Cytostatic activity; HSV-1 TK gene therapy; Tricyclic acyclovir analogues; Fluorosubstitution; Anti-HSV-1, HSV-2 activity.

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	R ¹		R ¹	R ²	R ³
1	A	3	A	H	H
2	B	4	B	H	H
		5	A	Ph	H
		6	B	Ph	H
		7	A	4-CH ₃ OPh	H
		8	A	Ph	CH ₃
		9	B	4-CH ₃ OPh	H
		10	B	Ph	CH ₃
		11	A	4-FPh	H
		12	B	4-FPh	H
		13	A	4-FPh	CH ₃
		14	A	Ph	4-FPhCH ₂
		15	A	4-CF ₃ Ph	H
		16	B	4-CF ₃ Ph	H
		17	A	4-CF ₃ Ph	CH ₃
		18	A	Ph	PhCH ₂
		19	A	4-CH ₃ OPh	CH ₃

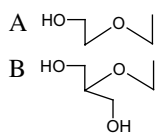


Chart 1.

unexpected results. The literature on this topic is extremely abundant. In the field of nucleosides there are hundreds of analogues described that contain a fluorinated glycone moiety, or are fluorinated at the nucleobase.^{6,7} As to the antiherpetic activity of acyclic analogues examples have been reported in the literature of antivirally efficient nucleobase-fluorinated derivatives⁶ and of inefficient pseudosugar-fluorinated ones.^{8–10}

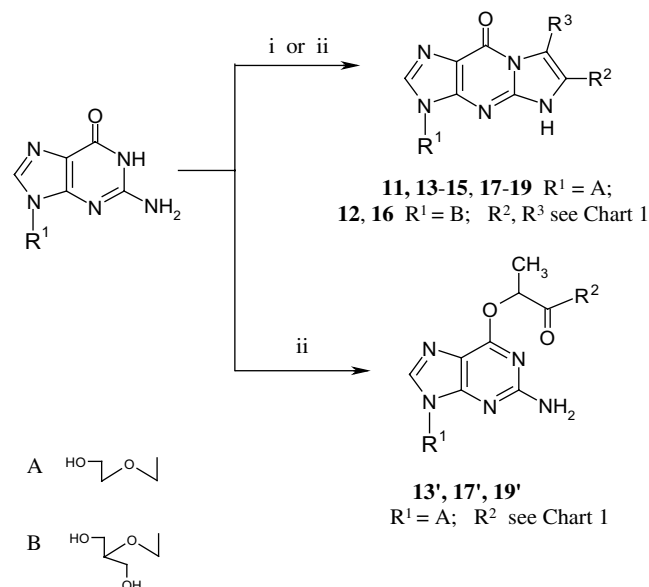
¹⁹F NMR spectroscopy is a convenient tool for in vitro and noninvasively in vivo identifying and monitoring of fluorine-containing metabolites of drugs, for example, fluoropyrimidine drugs.¹¹ This technique also enables to investigate the mode of binding of fluorinated substrates to enzymes as well as conformational properties of complexes formed.¹² The main aim of our present synthetic search was the exploration of new fluorine-substituted tricyclic analogues of ACV and GCV as selective cytostatic agents under conditions of HSV-1 TK gene therapy. Since tricyclic analogues are so far the only compounds for which the interaction with the HSV-1 thymidine kinase in solution has been measured by the ¹H NMR spectrum,¹³ ¹⁹F NMR can be used for studying of the interaction between fluorinated nucleoside analogues and HSV thymidine kinase. The advantage of novel tricyclic acyclovir/ganciclovir analogues as tools for ¹H NMR enzyme–ligand studies over the existing ones is expected upon the following earlier results.¹³ ¹H NMR spectra of the bound structure of the tricyclic ligand with the HSV-1 thymidine kinase in solution have been determined by transferred NOE technique. This technique has not been successful neither in the case of thymidine itself nor of acyclovir. Moreover, in view of the progress on HSV thymidine kinase-mediated anti-

cancer gene therapy, ¹⁹F NMR can also be considered for imaging of the level and distribution of fluorinated tricyclic nucleoside prodrug metabolites.

Introducing the benzyl or 4-fluorobenzyl group into the 7 position of the tricyclic system seemed of interest in view of the potent in vitro antiviral activity found earlier by others^{14,15} and by us¹⁶ for benzyl substituted purines and their analogues.

2. Chemistry

All 6-mono and the majority of the 6,7-disubstituted tricyclic analogues being presently studied were synthesized by an alkylation–condensation reaction of ACV or GCV with the appropriate brominated acetophenones or propiophenones (Scheme 1), according to a previously described method for other tricyclic derivatives.^{2–4} In the case of the reaction of **1** with 2-bromo-4'-substituted propiophenones the yields of 6,7-disubstituted derivatives **13**, **17** and **19** (28%, 19% and 25%, respectively) were much lower in comparison with that of corresponding 6-monosubstituted derivatives **11**, **15** and **7**³ (88%, 71% and 81%, respectively). This was due to the formation of the side products, the most important of which being O⁶-alkylation products **13'** and **17'**. This site of alkylation was unambiguously ascribed on the basis of diagnostic ¹H and ¹³C NMR spectra acquired from our recent work on dimers carrying simultaneously O- and N-substituted guanine moieties.¹⁷ For one of the above dimeric structures, for example, the most conspicuous differences in NMR chemical shifts (δ in ppm) for O versus N regioisomers were, respectively: 2-NH₂, 6.45/7.08; C-5, 113.46/115.62; C-4, 154.28/149.41; C-2, C-6, 159.97, 160.43/153.73, 156.41. The NMR data obtained now for compound **17'** differ from the above O-substitution only within the range of



Scheme 1. Reagents: (i) (1) NaH, DMF, R²COCH₂Br, (2) NH₄OH; (ii) (1) NaH, DMF, R²COCH(Br)CH₃, (2) NH₄OH.

0.2–1.1 ppm. The aforementioned diagnostic data allowed to correct the N-1 alkylated structure we assigned formerly to the products of the reaction of **1** and **2** with 2-bromopropiophenone.³

6,7-Disubstituted compounds **13**, **17** and **19** were prepared analytically pure in spite of their relative instability under standard conditions of chromatography and crystallization. We also tried to obtain their GCV analogues but these products underwent decomposition in the course of purification.

7-Arylalkyl-6-phenyl derivatives **14** and **18** were obtained by arylalkylation of 6-phenyl-TACV (**5**). We have reported previously that aryl substituent in the 6 position of TACV partly directs arylalkylation reactions into unusual positions.¹⁸ Benzylation of **5** with benzyl bromide/K₂CO₃/DMF led to N-5 substituted dominant product (**18a**) in 41% yield, whereas its N-4 isomer (**18b**, 6%) and C-7,N-5 disubstituted derivative (**18c**, 9%), but not C-7 one, were minor products. Literature on arylalkylation of protected or unprotected guanosine or 2'-deoxyguanosine with benzyl bromide and related bromides under alkaline conditions reports on using a variety of bases and solvents. Distribution of products has been shown to depend on solvent and on 4-substituent on the benzylating reagent.¹⁹ In our case, replacement of DMF with methanol in benzylation of **5** resulted in the formation of C-7 benzyl (**18**, 12% yield) and C-7,N-5 dibenzyl (**18c**, 15%) products accompanied by smaller amounts of their N-5 (**18a**, 7%) and N-4 (**18b**, 5%) congeners. Using 4-fluorobenzyl bromide as a reagent, we obtained the products in similar proportions. Benzylation of **5** in 2,2,2-trifluoroethanol (TFE) was regioselectively directed towards the C-7 position (25% yield).

3. Biological results

3.1. Antiviral activity

The newly synthesized compounds **11**–**19** were examined for their inhibitory effects on the replication of a wide

variety of viruses including HSV-1 (strains KOS, F and McIntyre), HSV-2 (strains G, 196 and Lyons), vaccinia virus (VV), vesicular stomatitis virus (VSV), HSV-1 TK[−] ACV^r (TK-deficient acyclovir-resistant HSV-1) strain, varicella-zoster virus (VZV) (strain Oka), TK[−] VZV mutants (strain 07-01) and cytomegalovirus (CMV) (strains AD-169 and Davis) in human embryonic lung (HEL) cell cultures.

In general, introduction of a fluorine (**11**) or trifluoromethyl (**15**) group in the 4 position of the 6-phenyl substituent of **5** decreased or abrogated antiherpetic activity. The compounds were found inactive against VZV, CMV, VV and VSV. Their activity against human herpes simplex virus types 1 and 2 and cytotoxicity are shown in Table 1. For 6-(4-fluorophenyl)-TACV, **11**, there was a substantial loss of activity against HSV-1 and -2 in comparison with the activity of 4-unsubstituted **5** (EC₅₀: 0.2–0.7 µg/mL for HSV-1 and 0.2–1.3 µg/mL for HSV-2). Similarly, compound **12** (EC₅₀ for HSV-1 and HSV-2: 0.384–1.92 µg/mL) and compound **16** (EC₅₀: 0.38–9.6 µg/mL) were less potent than **6** (EC₅₀: 0.005–0.3 µg/mL) by two orders of magnitude. Both TACV analogues (**11** and **15**) and TGCV analogues (**12** and **16**) were relatively cytotoxic.

In the case of the 6,7-disubstituted derivatives **13** and **17**, the decrease of activity was similar to that of the 6-monosubstituted ones. 6-(4-Fluorophenyl)-7-methyl-TACV, **13**, seemed to be the best of the present series, since the decrease of potency noted for this compound was only 3–30-fold compared with that for **8** (EC₅₀ HSV-1: 0.04–0.11 µg/mL; EC₅₀ HSV-2: 0.07–0.16 µg/mL), without loss of selectivity. 7-Methyl-6-(4-trifluoromethylphenyl)-TACV, **17**, was 5–40-fold less active and 5-fold less selective than **13**. 6-(4-Methoxyphenyl)-7-methyl-TACV (**19**) turned out to be effective against particular strains at the same concentrations as **13**, but it was more toxic. 7-Arylalkylated derivatives **14** and **18**, unlike their 7-methyl analogues, were devoid of anti-herpetic activity.

Compounds **11**–**19** were shown to be inactive against a HSV-1 TK[−] ACV^r strain. This observation seems to

Table 1. Activity against human herpes simplex virus type 1 (HSV-1) and 2 (HSV-2) and cytotoxicity of fluoro substituted tricyclic analogues of acyclovir and ganciclovir

Compd	EC ₅₀ (µg/mL) ^a							MCC ^b (µg/mL)
	HSV-1 (KOS)	HSV-1 (F)	HSV-1 (McIntyre)	HSV-2 (G)	HSV-2 (196)	HSV-2 (Lyons)	HSV-1/TK [−]	
11	>16	>16	>16	>16	>16	>16	>16	≥80
12	0.64	0.384	0.384	1.92	1.92	1.92	>80	400
13	0.384	0.384	0.384	1.92	1.92	1.92	>80	400
14	>16	>16	>16	>16	>16	>16	>16	≥80
15	48	16	>16	>16	>16	>16	>16	≥80
16	1.92	0.384	1.92	9.6	9.6	9.6	>80	400
17	9.6	1.92	16	9.6	9.6	9.6	>16	80
18	>16	9.6	>16	>16	>16	>16	>16	≥80
19	0.384	0.384	1.92	1.92	1.92	1.92	>16	≥80
ACV	0.128	0.077	0.384	0.384	0.077	0.077	48	>400
GCV	0.006	0.001	0.019	0.019	0.019	0.006	0.48	>400

^a 50% Effective concentration or compound concentration required to reduce virus-induced cytopathogenicity by 50%.

^b Minimal cytotoxic concentration or compound concentration required to cause a microscopically detectable alteration of normal cell morphology.

Table 2. Inhibitory effects of fluoro substituted tricyclic analogues of acyclovir and ganciclovir on the proliferation of osteosarcoma cells [thymidine kinase-deficient OST cells (OST TK[−]) and OST TK[−] cells transfected with the HSV-1 thymidine kinase gene (OST TK[−]/HSV-1 TK⁺)]

Compd	IC ₅₀ (μM ^a)		SI ^b
	OST TK [−]	OST TK [−] /HSV-1 TK ⁺	
11	92 ± 10	0.27 ± 0.08	341
12	278 ± 64	0.0032 ± 0.0011	86,875
13	122 ± 12	0.043 ± 0.030	2837
14	240 ± 118	0.73 ± 0.34	329
15	368 ± 161	0.20 ± 0.01	1840
16	>500	0.0086 ± 0.0030	58,140
17	≥500	0.35 ± 0.22	1429
18	252 ± 42	0.29 ± 0.08	869
19	62 ± 16	0.012 ± 0.003	5167
ACV	62 ± 29	0.095 ± 0.049	653
GCV	109 ± 71	0.00037 ± 0.00004	294,595

^a 50% Inhibitory concentration or compound concentration required to inhibit tumour cell proliferation by 50%.

^b Selectivity index or ratio of IC₅₀OST TK[−] to IC₅₀OST TK[−]/HSV-1 TK⁺.

suggest that they require phosphorylation by the HSV-1 encoded TK in order to exert their anti-HSV activity in cell culture.

3.2. Cytostatic activity

The substitution of the tricyclic analogues of ACV and GCV with fluorine had a much smaller influence on their cytostatic activity against human osteosarcoma OST TK[−] cells transduced with the HSV-1 TK gene (OST TK[−]/HSV-1 TK⁺) than on the aforementioned antiviral activity against HSV-1 and HSV-2 (Table 2). The selectivity indices of all the new compounds **11–19** (including the 7-arylalkyl substituted compounds) differed from that of parental acyclovir and ganciclovir within less than one order of magnitude. The ganciclovir analogues were 4- or 5-fold lower but those of acyclovir analogues ranged from 2-fold lower (**11**, **14**) to 8-fold higher (**19**). It should be the subject of further research to define the most suitable fluorinated tricyclic nucleoside analogue for the purpose of imaging. In this respect, it is of crucial importance that the ¹⁸F-labeling of the hydroxy-precursor can be performed in a high yield and in a short time period.

4. Conclusions

Compounds **12**, **13** and **16** appear to be the best candidates of the present series for the ¹⁹F NMR studies of the HSV TK–ligand interactions and/or monitoring of their metabolites in cells expressing HSV TK. 6,7-Disubstitution pattern leads to highly potent antiherpetic agents only when the 7-position is substituted by methyl (Table 1, compounds **13**, **17**, **19**). Replacement of the 7-methyl group with the 7-arylalkyl one results in a total loss of antiherpetic activity (Table 1, compounds **14** and **18**). The most interesting compounds should be co-crystallized with HSV-1 TK to gain more insights

in potential interaction sites of the compound pharmacophors with the enzyme.

5. Experimental

5.1. General methods

Melting points were determined on MEL-TEMP II capillary melting point apparatus and are uncorrected. Mass spectra (LR) were measured on a AMD-604 mass spectrometer by LSIMS method with *m*-nitrobenzyl alcohol as a matrix. Elemental analyses were performed by Microanalytical Laboratories of the Institute of Organic Chemistry Polish Academy of Sciences in Warsaw. ¹H, ¹³C and ¹⁹F NMR spectra were recorded on a Unity 300 Varian spectrometer operating at 299.95 MHz, 75.43 MHz and 282.25 MHz, respectively. Tetramethylsilane was used as the internal standard in ¹H and ¹³C NMR, while 10% trifluoroacetic acid in D₂O was used as the external standard in ¹⁹F NMR; the chemical shifts are reported in ppm (δ scale), coupling constants (*J*) in Hz. Thin-layer chromatography (TLC) was performed on Merck precoated 60 F₂₅₄ gel plates. Short column chromatography was carried out on Merck silica gel 60H (40–63 μm).

5.2. Bromoketones

2-Bromo-4'-fluoroacetophenone was purchased from Aldrich. All other bromoketones were obtained previously by diverse methods.^{20a–20d} We found, as described earlier,⁴ that the approach using bromine in tetrahydrofuran solution in the presence of aluminium chloride, was most convenient.²¹

5.2.1. 2-Bromo-4'-(trifluoromethyl)acetophenone^{20a}. Isolated by column chromatography using silica gel and hexane–EtOAc (95:5) for elution to give pure product, which was crystallized on evaporation, 82% yield. MS (LR) 267 (M⁺), 269 (M⁺+2). ¹H NMR (CDCl₃) δ 8.11, 7.78 (2d, 4H, C₆H₄), 4.45 (s, 2H, CH₂).

5.2.2. 2-Bromo-4'-fluoropropiophenone^{20b}. Chromatographed on silica gel column using hexane–EtOAc (95:5) for elution to obtain colorless oil in 78% yield. MS (LR) 230 (M⁺−1), 232 (M⁺+1). ¹H NMR (CDCl₃) δ 8.03–8.10, 7.12–7.20 (2m, 4H, C₆H₄), 5.24 (q, 1H, CH), 1.90 (d, 3H, CH₃).

5.2.3. 2-Bromo-4'-(trifluoromethyl)propiophenone^{20c}. Chromatographed on silica gel column using hexane–EtOAc (95:5) as eluent to obtain colorless oil in 90% yield. MS (LR) 280 (M⁺−1), 282 (M⁺+1). ¹H NMR (CDCl₃) δ 8.15, 7.78 (2d, 4H, C₆H₄), 5.28 (q, 1H, CH), 1.94 (d, 3H, CH₃).

5.2.4. 2-Bromo-4'-methoxypropiophenone^{20d}. Purified by silica gel column using hexane–EtOAc (98:2 → 95:5) to quantitatively afford product as an oil. MS (LR) 242 (M⁺−1), 243 (M⁺), 244 (M⁺+1), 245 (M⁺+2). ¹H NMR (CDCl₃) δ 8.02, 6.96 (2d, 4H, C₆H₄), 5.27 (q, 1H, CH), 3.89 (s, 3H, OCH₃), 1.89 (d, 3H, CH₃).

5.3. General procedure for the alkylation–condensation reactions

To an anhydrous suspension of acyclovir or ganciclovir (1 mmol) in DMF (12 mL or 24 mL, respectively) was added sodium hydride as 60% suspension in oil (1.3 mmol). After being stirred with exclusion of moisture for 1–2 h at room temperature, the resulting solution was treated with bromoketone (1.1 mmol; a solution in 2 mL of DMF). The reaction mixture was stirred at room temperature for the next 2–5 h (for acyclovir) or 5–20 h (for ganciclovir), made alkaline by addition of concentrated aqueous ammonia and left overnight. Volatile materials were evaporated, the residual oil was dissolved in CH_2Cl_2 –MeOH (7.5:1) (in the case of reactions with bromoacetophenones) or (9:1) (in the case of reactions with bromopropiophenones), applied onto a silica gel column and chromatographed in CH_2Cl_2 –MeOH gradient (7.5:1 $\rightarrow$ 6:1) or using CH_2Cl_2 –MeOH (9:1), respectively. Fractions containing the main product were evaporated to dryness and recrystallized.

5.3.1. 3,9-Dihydro-3-[(2-hydroxyethoxy)methyl]-6-(4-fluorophenyl)-9-oxo-5H-imidazo[1,2-a]purine (11). Crude product (302 mg, 88% yield) was subjected to crystallization from MeOH to give yellowish spangles: mp 270–272 °C (dec; soft at ca 250 °C). ^1H NMR ($\text{DMSO}-d_6$) δ 13.14 (br s, 1H, NH), 8.24 (s, 1H, 7-H), 8.08 (s, 1H, 2-H), 7.95–8.00, 7.32–7.38 (2m, 4H, C_6H_4), 5.53 (s, 2H, NCH_2O), 4.70 (t, 1H, OH), 3.48–3.57 (m, 4H, CH_2CH_2). ^{13}C NMR ($\text{DMSO}-d_6$) δ 162.12 (C-4'; $^1J = 246.2$ Hz), 151.17 (C-9), 150.33 (C-3a), 146.45 (C-4a), 139.29 (C-2), 128.17 (C-6), 127.20 (C-2', C-6'; $^3J = 8.6$ Hz), 124.49 (C-1'), 115.99 (C-3', C-5'; $^2J = 22.2$ Hz), 115.38 (C-9a), 103.22 (C-7), 72.31 (NCH_2O), 70.49 (HOCH_2CH_2), 59.85 (HOCH_2). ^{19}F NMR ($\text{DMSO}-d_6$) δ –36.27. Anal. Calcd for $\text{C}_{16}\text{H}_{14}\text{FN}_5\text{O}_3$: C, 55.98; H, 4.11; N, 20.40. Found: C, 55.84; H, 3.96; N, 20.33.

5.3.2. 3,9-Dihydro-3-[(1,3-dihydroxy-2-propoxy)methyl]-6-(4-fluorophenyl)-9-oxo-5H-imidazo[1,2-a]purine (12). Crude isolated product (188 mg, 50% yield) was crystallized from 50% aq EtOH to give white needles: mp > 300 °C (dec at ca 230 °C). ^1H NMR ($\text{DMSO}-d_6$) δ 13.14 (br s, 1H, NH), 8.23 (s, 1H, 7-H), 8.08 (s, 1H, 2-H), 7.95–8.01, 7.32–7.40 (2m, 4H, C_6H_4), 5.62 (s, 2H, NCH_2O), 4.65 (t, 2H, OH, OH), 3.61–3.68 (p, 1H, CH), 3.28–3.49 (m, 4H, CH_2 , CH_2). ^{13}C NMR ($\text{DMSO}-d_6$) δ 161.67 (C-4'; $^1J = 246.2$ Hz), 150.77 (C-9), 149.80 (C-3a), 146.02 (C-4a), 138.85 (C-2), 127.86 (C-6), 126.81 (C-2', C-6'; $^3J = 8.6$ Hz), 124.12 (C-1'), 115.56 (C-3', C-5'; $^2J = 21.6$ Hz), 114.87 (C-9a), 102.67 (C-7), 79.65 (CH), 71.31 (NCH_2O), 60.37 (HOCH_2 , HOCH_2). ^{19}F NMR ($\text{DMSO}-d_6$) δ –36.26. Anal. Calcd for $\text{C}_{17}\text{H}_{16}\text{FN}_5\text{O}_4$: C, 54.69; H, 4.32; N, 18.76. Found: C, 54.49; H, 4.32; N, 18.67.

5.3.3. 3,9-Dihydro-3-[(2-hydroxyethoxy)methyl]-9-oxo-6-(4-trifluoromethylphenyl)-5H-imidazo[1,2-a]purine (15). Product after chromatography: 279 mg (71% yield). Analytical sample was prepared by crystallization from

MeOH to afford colorless crystals: mp > 300 °C (dec at ca 250 °C). ^1H NMR ($\text{DMSO}-d_6$) δ 13.31 (br s, 1H, NH), 8.46 (s, 1H, 7-H), 8.08 (s, 1H, 2-H), 8.15, 7.86 (2d, 4H, C_6H_4), 5.53 (s, 2H, NCH_2O), 4.69 (t, 1H, OH), 3.46–3.57 (m, 4H, CH_2CH_2). ^{13}C NMR ($\text{DMSO}-d_6$) δ 151.21 (C-9), 150.55 (C-3a), 146.72 (C-4a), 139.44 (C-2), 132.01 (C-1'), 128.60 (C-4'; $^2J = 32.4$ Hz), 127.69 (C-6), 125.91 (C-3', C-5'; $^3J = 3.5$ Hz), 125.58 (C-2', C-6'), 124.04 (CF_3 ; $^1J = 272.3$ Hz), 115.44 (C-9a), 105.33 (C-7), 72.39 (NCH_2O), 70.59 (HOCH_2CH_2), 59.92 (HOCH_2). ^{19}F NMR ($\text{DMSO}-d_6$) δ –46.31. Anal. Calcd for $\text{C}_{17}\text{H}_{14}\text{F}_3\text{N}_5\text{O}_3$: C, 51.91; H, 3.59; N, 17.81. Found: C, 51.72; H, 3.52; N, 17.69.

5.3.4. 3,9-Dihydro-3-[(1,3-dihydroxy-2-propoxy)methyl]-9-oxo-6-(4-trifluoromethyl-phenyl)-5H-imidazo[1,2-a]purine (16). Crude isolated material (194 mg, 46% yield) was stirred in H_2O (25 mL) at room temperature for 3 h and filtered. The solid was dried by co-evaporation with toluene (twice), then dissolved in CH_2Cl_2 –MeOH (3:1), concentrated and kept at +5 °C to afford white crystalline precipitate: mp > 300 °C (soft. at 184–186 °C, dec at ca 230 °C). ^1H NMR ($\text{DMSO}-d_6$) δ 13.31 (br s, 1H, NH), 8.46 (s, 1H, 7-H), 8.06 (s, 1H, 2-H), 8.16, 7.86 (2d, 4H, C_6H_4), 5.62 (s, 2H, NCH_2O), 4.63 (t, 2H, OH, OH), 3.59–3.67 (p, 1H, CH), 3.27–3.48 (m, 4H, CH_2 , CH_2). ^{13}C NMR ($\text{DMSO}-d_6$) δ 151.35 (C-9), 150.54 (C-3a), 146.99 (C-4a), 139.40 (C-2), 132.46 (C-1'), 129.38 (C-6), 128.49 (C-4'; $^2J = 31.7$ Hz), 125.90 (C-3', C-5'; $^3J = 3.5$ Hz), 125.65 (C-2', C-6'), 124.10 (CF_3 ; $^1J = 272.0$ Hz), 115.26 (C-9a), 105.15 (C-7), 80.41 (CH), 71.81 (NCH_2O), 60.88 (HOCH_2 , HOCH_2). ^{19}F NMR ($\text{DMSO}-d_6$) δ –46.29. Anal. Calcd for $\text{C}_{18}\text{H}_{16}\text{F}_3\text{N}_5\text{O}_4 \cdot 0.5\text{H}_2\text{O}$: C, 50.00; H, 3.96; N, 16.20. Found: C, 50.05; H, 3.90; N, 15.74.

5.3.5. O^6 -[1-(4-Fluorobenzoyl)ethyl]-9-[(2-hydroxyethoxy)methyl]guanine (13'). Crude solid (21 mg, 6%) was dissolved in *i*PrOH and concentrated to give white powder: mp 194–195 °C (soft. at ca 180 °C). ^1H NMR ($\text{DMSO}-d_6$) δ 8.15, 7.41 (q and t, 4H, C_6H_4), 8.05 (s, 1H, 8-H), 6.45 (q, 1H, CH), 6.28 (br s, 2H, NH_2), 5.43 (s, 2H, NCH_2O), 4.69 (t, 1H, OH), 3.44–3.50 (m, 4H, CH_2CH_2), 1.57 (d, 3H, CH_3).

5.3.6. 3,9-Dihydro-3-[(2-hydroxyethoxy)methyl]-6-(4-fluorophenyl)-7-methyl-9-oxo-5H-imidazo[1,2-a]purine (13). Chromatographically purified product (101 mg, 28% yield) was dissolved in *i*PrOH and the resulting solution was concentrated to obtain white needles: mp 241–243 °C (dec). ^1H NMR ($\text{DMSO}-d_6$) δ 12.50 (br s, 1H, NH), 7.99 (s, 1H, 2-H), 7.60–7.67, 7.35–7.44 (2m, 4H, C_6H_4), 5.47 (s, 2H, NCH_2O), 4.69 (br s, 1H, OH), 3.42–3.54 (m, 4H, CH_2CH_2), 2.78 (s, 3H, 7- CH_3). ^{13}C NMR ($\text{DMSO}-d_6$) δ 161.92 (C-4'; $^1J = 246.3$ Hz), 154.32 (C-9), 150.06 (C-3a), 146.61 (C-4a), 138.89 (C-2), 130.35 (C-2', C-6'; $^3J = 8.5$ Hz), 124.77 (C-1'), 123.93 (C-6), 116.22 (C-7), 115.80 (C-3', C-5'; $^2J = 21.7$ Hz), 115.81 (C-9a), 72.13 (NCH_2O), 70.40 (HOCH_2CH_2), 59.82 (HOCH_2), 11.74 (7- CH_3). ^{19}F NMR ($\text{DMSO}-d_6$) δ –36.54. Anal. Calcd for $\text{C}_{17}\text{H}_{16}\text{FN}_5\text{O}_3$: C, 57.14; H, 4.51; N, 19.60. Found: C, 57.02; H, 4.48; N, 19.39.

5.3.7. O⁶-[1-(4-Trifluoromethylbenzoyl)ethyl]-9-[(2-hydroxyethoxy)methyl]guanine (17'). Amorphous solid after chromatography (102 mg, 24%) was purified by precipitation from *i*PrOH: mp 166–169 °C (soft. at ca 150 °C). ¹H NMR (DMSO-*d*₆) δ 8.24, 7.94 (2d, 4H, C₆H₄), 8.04 (s, 1H, 8-*H*), 6.43 (q, 1H, *CH*), 6.29 (br s, 2H, NH₂), 5.41 (s, 2H, NCH₂O), 4.67 (t, 1H, OH), 3.44–3.48 (m, 4H, CH₂H₂), 1.57 (d, 3H, CH₃). ¹³C NMR (DMSO-*d*₆) δ 197.32 (C=O), 159.45, 158.86 (C-2, C-6), 154.82 (C-4), 140.44 (C-8), 137.88 (C-1'), 132.67 (C-4'; ²*J* = 32.2 Hz), 129.18 (C-2', C-6'), 125.89 (C-3', C-5'; ³*J* = 4.0 Hz), 123.73 (CH₃; ¹*J* = 273.1 Hz), 113.26 (C-5), 72.84 (CH-CH₃), 72.06 (NCH₂O), 70.48 (HOCH₂CH₂), 59.88 (HOCH₂), 17.10 (CH₃).

5.3.8. 3,9-Dihydro-3-[(2-hydroxyethoxy)methyl]-7-methyl-9-oxo-6-(4-trifluoromethyl-phenyl)-5*H*-imidazo[1,2-*a*]-purine (17). Solid material after column chromatography: 77 mg (19%). Crystalline white material precipitated on evaporation of the fractions: mp > 300 °C (dec at 250–253 °C). ¹H NMR (DMSO-*d*₆) δ 12.87 (br s, 1H, NH), 8.01 (s, 1H, 2-*H*), 7.91, 7.81 (2d, 4H, C₆H₄), 5.48 (s, 2H, NCH₂O), 4.69 (t, 1H, OH), 3.47–3.53 (m, 4H, CH₂CH₂), 2.84 (s, 3H, 7-CH₃). ¹³C NMR (DMSO-*d*₆) δ 154.46 (C-9), 150.26 (C-3a), 146.98 (C-4a), 139.10 (C-2), 132.63 (C-1'), 128.77 (C-2', C-6'), 128.52 (C-4'; ²*J* = 32.2 Hz), 125.74 (C-3', C-5'; ³*J* = 3.5 Hz), 123.68 (C-6), 124.10 (CF₃; ¹*J* = 272.0 Hz), 118.01 (C-7), 115.91 (C-9a), 72.26 (NCH₂O), 70.53 (HOCH₂CH₂), 59.94 (HOCH₂), 11.95 (7-CH₃). ¹⁹F NMR (DMSO-*d*₆) δ 15.05. Anal. Calcd for C₁₈H₁₆F₃N₅O₃: C, 53.07; H, 3.96; N, 17.19. Found: C, 52.96; H, 3.87; N, 16.92.

5.3.9. 3,9-Dihydro-3-[(2-hydroxyethoxy)methyl]-6-(4-methoxyphenyl)-7-methyl-9-oxo-5*H*-imidazo[1,2-*a*]-purine (19). Evaporation of appropriate fractions after chromatography gave 94 mg of the compound (25% yield). Analytical sample was obtained by crystallization from MeOH: mp 211–214 °C (dec). ¹H NMR (DMSO-*d*₆) δ 12.59 (br s, 1H, NH), 7.98 (s, 1H, 2-*H*), 7.51, 7.09 (2d, 4H, C₆H₄), 5.47 (s, 2H, NCH₂O), 4.70 (t, 1H, OH), 3.82 (s, 3H, OCH₃), 3.42–3.55 (m, 4H, CH₂CH₂), 2.77 (s, 3H, 7-CH₃). ¹³C NMR (DMSO-*d*₆) δ 159.36 (C-4'), 154.37 (C-9), 150.03 (C-3a), 146.53 (C-4a), 138.87 (C-2), 129.52 (C-2', C-6'), 124.65 (C-1'), 120.50 (C-7), 115.87 (C-9a), 114.31 (C-3', C-5'), 72.19 (NCH₂O), 70.44 (HOCH₂CH₂), 59.89 (HOCH₂), 55.26 (OCH₃), 11.82 (7-CH₃). Anal. Calcd for C₁₈H₁₉N₅O₄·0.75H₂O: C, 56.46; H, 5.40; N, 18.29. Found: C, 56.44; H, 5.08; N, 17.92.

5.4. Alkylation reactions

5.4.1. Benzylolation of **5** with benzyl bromide, K₂CO₃ and DMF as solvent was described in an earlier report.¹⁸

5.4.2. Benzylolation of 5 in MeOH. To a suspension of **5** (540 mg, 1.66 mmol) in anhydrous MeOH (200 mL) was added powdered K₂CO₃ (436 mg, 3.15 mmol). After stirring at room temperature for 1 h, to the resulting solution benzyl bromide (483 mg, 2.82 mmol) was added in one portion and stirring was continued for 4 days.

The mixture was evaporated at 35 °C and the residue was subjected to column chromatography, which was performed in CH₂Cl₂–MeOH (97:3 → 93:7). Evaporation of fractions gave in the order of elution: the mixture of **18c** and **18b** (162 mg; 15% and 5% yield, respectively, on the basis of ¹H NMR spectrum), homogeneous **18a** (49 mg, 7%) and homogeneous **18** (84 mg, 12%).

Compounds **18a–c** were found to be identical by ¹H NMR as described previously.¹⁸

5.4.2.1. 7-Benzyl-3,9-dihydro-3-[(2-hydroxyethoxy)-methyl]-9-oxo-6-phenyl-5*H*-imidazo-[1,2-*a*]-purine (18). White crystals of **18** precipitated from concentrated EtOH solution: mp 224–226 °C (dec). ¹H NMR (DMSO-*d*₆) δ 12.93 (br s, 1H, NH), 7.99 (s, 1H, 2-*H*), 7.47–7.56, 7.10–7.29 (2m, 4H, C₆H₄), 5.49 (s, 2H, NCH₂O), 4.69 (t, 1H, OH), 4.68 (s, 2H, 7-CH₂), 3.47–3.55 (m, 4H, CH₂CH₂). ¹³C NMR (DMSO-*d*₆) δ 153.67 (C-9), 149.92 (C-3a), 146.77 (C-4a), 138.99 (C-2), 140.12, 128.92, 128.81, 128.30, 128.06, 127.74, 127.50, 126.61, 125.79 (Ph, C-6), 117.85 (C-7), 115.88 (C-9a), 72.17 (NCH₂O), 70.40 (HOCH₂CH₂), 59.83 (HOCH₂), 29.88 (7-CH₂). Anal. Calcd for C₂₃H₂₁N₅O₃: C, 66.49; H, 5.10; N, 16.86. Found: C, 66.39; H, 5.15; N, 16.75.

5.4.3. Benzylolation of 5 in 2,2,2-trifluoroethanol (TFE). The same procedure as for reaction in MeOH; reagents: **7** (80 mg, 0.246 mmol), K₂CO₃ (64 mg, 0.463 mmol), benzyl bromide (72 mg, 0.421 mmol), TFE (40 mL). After 1 day of stirring, TLC in CH₂Cl₂–MeOH (9:1) indicated the presence of **18**, product of decomposition and traces of **18a–c** and no substrate in the reaction mixture. Compound **18** was isolated by chromatography as above to yield 26 mg (25%).

5.4.4. 4-Fluorobenzylolation of 5 in MeOH at room temperature. To a suspension of **5** (325 mg, 1.0 mmol) in anhydrous MeOH (100 mL) was added powdered K₂CO₃ (262 mg, 1.9 mmol). After stirring at room temperature for 1 h, to the resulting solution 4-fluorobenzyl bromide (322 mg, 1.7 mmol) was added and the mixture was stirred at room temperature for 4 days. The volatiles were evaporated at 35 °C and the residue was applied as a suspension in CH₂Cl₂–MeOH (97:3) onto a silica gel column. Separation was carried out using CH₂Cl₂–MeOH (97:3 → 95:5) as the eluent and resulted in isolation of compounds **14b** (15 mg, 3% yield), **14a** (18 mg, 4%), **14** (55 mg, 13%) and **5** (94 mg, 29%), in the order of elution.

5.4.5. 4-Fluorobenzylolation of 5 in MeOH at 50 °C. Reagents were added in the same manner as described above; **5** (325 mg, 1.0 mmol), K₂CO₃ (552 mg, 4.0 mmol), 4-fluorobenzyl bromide (643 mg, 3.4 mmol), anhydrous MeOH (50 mL). The solution was stirred at 50 °C for 3 days, and then evaporated. Components of the mixture were separated by column chromatography in CH₂Cl₂–MeOH (97:3 → 9:1) to afford products **14b** (133 mg, 25% yield), **14a** (43 mg, 10%) and **14** (48 mg, 11%), in order of elution.

5.4.5.1. 5,7-Di-(4-fluorobenzyl)-3,9-dihydro-3-[(2-hydroxyethoxy)methyl]-9-oxo-6-phenylimidazo[1,2-a]purine (14b).

¹H NMR (DMSO-*d*₆) δ 8.03 (s, 1H, 2-*H*), 7.48–7.52, 7.40–7.43, 7.00–7.11 (3m, 13H, 2 $\times$ C₆H₄, C₆H₅), 5.50 (s, 2H, NCH₂O), 5.21 (s, 2H, N-5-CH₂), 4.67 (t, 1H, OH), 4.40 (s, 2H, 7-CH₂), 3.41–3.51 (m, 4H, CH₂CH₂). ¹⁹F NMR (DMSO-*d*₆) δ –38.65, –41.27.

5.4.5.2. 3,9-Dihydro-5-(4-fluorobenzyl)-3-[(2-hydroxyethoxy)methyl]-9-oxo-6-phenyl-imidazo[1,2-a]purine (14a).

¹H NMR (DMSO-*d*₆) δ 8.11 (s, 1H, 2-*H*), 7.91 (s, 1H, 7-*H*), 7.49–7.55, 7.02–7.10 (2m, 9H, C₆H₄, C₆H₅), 5.53 (s, 2H, NCH₂O), 5.38 (s, 2H, N-5-CH₂), 4.66 (t, 1H, OH), 3.40–3.52 (m, 4H, CH₂CH₂). ¹⁹F NMR (DMSO-*d*₆) δ –38.67.

5.4.5.3. 3,9-Dihydro-7-(4-fluorobenzyl)-3-[(2-hydroxyethoxy)methyl]-9-oxo-6-phenyl-5*H*-imidazo[1,2-a]purine (14).

Keeping at +5 °C of the concentrated MeOH solution of **14** gave a white crystalline precipitate: mp 243–245 °C (dec). ¹H NMR (DMSO-*d*₆) δ 12.93 (br s, 1H, NH), 7.99 (s, 1H, 2-*H*), 7.45–7.56, 7.04–7.17 (2m, 9H, C₆H₄C₆H₅), 5.48 (s, 2H, NCH₂O), 4.69 (t, 1H, OH), 4.65 (s, 2H, 7-CH₂), 3.45–3.55 (m, 4H, CH₂CH₂). ¹³C NMR (DMSO-*d*₆) δ 160.50 (C-4'; ¹*J* = 242.5 Hz), 153.67 (C-9), 149.94 (C-3a), 146.77 (C-4a), 139.05 (C-2), 136.23 (Ph), 129.28 (C-2', C-6'; ³*J* = 8.1 Hz), 128.95, 127.98, 127.80 126.77 (C-6, Ph), 117.72 (C-7), 115.84 (C-9a), 114.97 (C-3', C-5'; ²*J* = 21.1 Hz), 72.17 (NCH₂O), 70.38 (HOCH₂CH₂), 59.82 (HOCH₂), 29.17 (7-CH₂). ¹⁹F NMR (DMSO-*d*₆) δ –41.38. Anal. Calcd for C₂₃H₂₀FN₅O₃·0.5H₂O: C, 62.44; H, 4.78; N, 15.83. Found: C, 62.40; H, 4.44; N, 15.59.

5.5. Cells

Human embryonic lung (HEL) fibroblasts were obtained from the ATCC (Rockville, MD). Establishment of human osteosarcoma 143B OST TK[–] cells deficient in cytosolic TK and transduced by the HSV-1 TK gene has been described previously.²² The HSV-1 TK gene-transduced cells and their parental cell lines were cultivated in RPMI-1640 medium, supplemented with 10% foetal calf serum, 2 mM L-glutamine and 0.075% NaHCO₃ in a humidified CO₂-controlled incubator at 37 °C.

5.6. Anti-herpes simplex virus (HSV) assays

The anti-HSV assays were based on inhibition of virus-induced cytopathicity in human embryonic lung (HEL) fibroblasts as follows. Confluent cell cultures in 96-well microtiter plates were inoculated with 100 CCID₅₀ of virus, 1 CCID₅₀ being the virus dose required to infect 50% of virus-inoculated cell cultures. After 1 h virus adsorption period, residual virus was removed, and the cell cultures were incubated in the presence of two to fivefold dilutions of the test compounds. Viral cytopathicity was recorded as soon as it reached completion in the control (drug free) virus-infected cell cultures (at day 3 after infection). The cytotoxicity of the test compounds against the cell cultures was read microscopi-

cally on mock-infected HEL cell cultures at 3 days after addition of the compound dilutions.

5.7. Cytostatic activity assays

To evaluate the cytostatic activity of the test compounds against OST TK[–] and OST TK[–]/HSV-1 TK⁺, 10⁴ OST cells/well were seeded in 96-well microtiter plates (Falcon) in 200 μ L cell culture medium at 37 °C, in the presence of five-fold dilutions (in normal growth medium) of the test compounds. After 3 days the OST cells (after being detached with trypsin solution (Gibco)) were counted in a Coulter counter (Coulter Electronics, Harpenden, UK). The IC₅₀ was defined as the drug concentration required to inhibit OST cell proliferation by 50%.

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References and notes

- Boryski, J.; Golankiewicz, B.; De Clercq, E. *J. Med. Chem.* **1991**, *34*, 2380.
- Golankiewicz, B.; Ostrowski, T.; Andrei, G.; Snoeck, R.; De Clercq, E. *J. Med. Chem.* **1994**, *37*, 3187.
- Golankiewicz, B.; Ostrowski, T.; Goslinski, T.; Januszczuk, P.; Zeidler, J.; Baranowski, D.; De Clercq, E. *J. Med. Chem.* **2001**, *44*, 4284.
- Goslinski, T.; Golankiewicz, B.; De Clercq, E.; Balzarini, J. *J. Med. Chem.* **2002**, *45*, 5052.
- Balzarini, J.; Ostrowski, T.; Goslinski, T.; De Clercq, E.; Golankiewicz, B. *Gene Ther.* **2002**, *9*, 1173.
- Barrio, J. R.; Namavari, M.; Phelps, M. E.; Satyamurthy, N. *J. Am. Chem. Soc.* **1996**, *118*, 10408, and references cited therein.
- Examples of reviews: (a) Welch, J. T. *Tetrahedron* **1987**, *43*, 3123; (b) Resnati, G. *Il Farmaco* **1990**, *45*, part I 1043, part II 1137; (c) Pankiewicz, K. W. *Carbohydr. Res.* **2000**, *327*, 87.
- Martin, J. C.; McGee, D. P.; Jeffrey, G. A.; Hobbs, D. W.; Smee, D. F.; Matthews, T. R.; Verheyden, J. P. *J. Med. Chem.* **1986**, *29*, 1384.
- Vemishetti, P.; Saibaba, R.; Panzica, R. P.; Abushanab, E. *J. Med. Chem.* **1990**, *33*, 681.
- Sznajdman, M. L.; Beauchamp, L. M. *Nucleosides Nucleotides* **1996**, *15*, 1315.
- Reviews: (a) Martino, R.; Malet-Martino, M.; Gilard, V. *Curr. Drug Metab.* **2000**, *1*, 271; (b) Wolf, W.; Presant, C. A.; Waluch, V. *Adv. Drug Deliver. Rev.* **2000**, *41*, 55.
- For example: (a) Jenkins, B. G. *Life Sci.* **1991**, *48*, 1227; (b) Roy, B.; Decout, J. L.; Beguin, C.; Fontecave, M.; Allard, P.; Kuprin, S.; Ehrenberg, A. *Biochim. Biophys. Acta* **1995**, *1247*, 284.
- Czaplicki, J.; Bohner, T.; Habermann, A.-K.; Folkers, G.; Milon, A. *J. Biomol. NMR* **1996**, *8*, 261.
- Kelley, J. L.; Linn, J. A.; Krochmal, M. P.; Selway, J. W. *J. Med. Chem.* **1988**, *31*, 2001, and references cited therein.
- Kelley, J. L.; Linn, J. A.; Selway, J. W. *J. Med. Chem.* **1991**, *34*, 157, and references cited therein.

16. Golankiewicz, B.; Januszczyk, P.; Ikeda, S.; Balzarini, J.; De Clercq, E. *J. Med. Chem.* **1995**, *38*, 3558.
17. (a) Goslinski, T.; Golankiewicz, B. *Collect. Czech. Chem. Commun. Symp. Ser. 5* **2002**, *76*; (b) Golankiewicz, B., Goslinski, T. Unpublished results.
18. Ostrowski, T.; Zeidler, J.; Goslinski, T.; Golankiewicz, B. *Nucleos. Nucleot. Nucl.* **2000**, *19*, 1911.
19. (a) Moschel, R. C.; Hudgins, W. R.; Dipple, A. *J. Org. Chem.* **1984**, *49*, 363; (b) Moon, K.-Y.; Moschel, R. C. *Chem. Res. Toxicol.* **1998**, *11*, 696; (c) Vincent, S. P.; Mioskowski, C.; Lebeau, L. *Nucleosides Nucleotides* **1999**, *18*, 2127; (d) Huang, Y.; Johnson, F. *Nucleos. Nucleot. Nucl.* **2002**, *21*, 435.
20. For example: (a) Schröder, J.; Henke, A.; Wenzel, H.; Brandstetter, H.; Stämmler, H. G.; Stämmler, A.; Pfeiffer, W. D.; Tschesche, H. *J. Med. Chem.* **2001**, *44*, 3231; (b) Chenard, B. L. et al. *J. Med. Chem.* **1995**, *38*, 3138; (c) Bartroli, J.; Turmo, E.; Algueró, M.; Boncompte, E.; Vericat, M. L.; García-Rafanell, J.; Forn, J. *J. Med. Chem.* **1995**, *38*, 3918; (d) Zhu, Y.-F.; Struthers, R. S.; Connors, P. J.; Gao, Y.; Gross, T. D.; Saunders, J.; Wilcoxon, K.; Reinhart, G. J.; Ling, N.; Chen, C. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 399.
21. Park, C.-H.; Givens, R. S. *J. Am. Chem. Soc.* **1997**, *119*, 2453.
22. Degreve, B.; Johansson, M.; De Clercq, E.; Karlsson, A.; Balzarini, J. *J. Virol.* **1998**, *72*, 9535.