Available online at www.sciencedirect.com
ScienceDirect
Tetrahedron
Tetrahedron 63 (2007) 11391–11398

Synthesis of phosphonomethoxyethyl or 1,3-bis(phosphonomethoxy)propan-2-yl lipophilic esters of acyclic nucleoside phosphonates

Silvie Vrbková,* Martin Dračínský and Antonín Holý

Centre for Novel Antivirals and Antineoplastics, Gilead Sciences and IOCB Research Center, Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Flemingovo nám. 2, 166 10 Prague 6, Czech Republic

Received 7 October 2006; revised 8 August 2007; accepted 23 August 2007
Available online 30 August 2007

Abstract—A new alternative synthetic pathway towards mono and diesters of acyclic nucleoside phosphonates (PMEA, PMEC and PMEG) or [1,3-bis(phosphonomethoxy)propan-2-yl]adenine bearing one or two hexadecyloxypropyl ester groups $(CH_2)_3O$-*n*-$C_{16}H_{33}$ is reported.

1. Introduction

Considerable effort in medicinal chemistry is directed towards the search for compounds with antiviral activity. We are continually investigating and developing acyclic nucleoside phosphonates (ANPs). These compounds deserve special attention owing to their significant biological activity.[1,2] However, the oral delivery of many acyclic nucleoside phosphonates, e.g., PMEA or (*R*)-PMPA (Fig. 1), is hindered by their poor resorption in the intestine caused by the negative charges in the ANP structure.[3–6] The introduction of a suitable masking moiety to the polar phosphonate group, thus decreasing the polarity of the molecule, can solve this problem. For instance, the application of the dipivoxil group to adefovir (PMEA) or disoproxil group to tenofovir ((*R*)-PMPA) can serve as good examples (Fig. 1).[6–10]

Several other phosphonate-masking groups have been applied to acyclic nucleoside phosphonates, e.g., phosphonamidates, cycloSal esters and lipophilic alkyl esters.[11–13a] The latter having 14–20 carbons in the moiety are most frequently selected.[13a,14] They provide bioavailable prodrugs of ANPs with potency comparable to that of the corresponding non-derivatized drugs.[15–17]

2. Results and discussion

Our previous paper[18] describes the synthesis of a large variety of symmetrical 1,3-bis(phosphonoethoxy)propan-2-yl derivatives of purines and pyrimidines. The present paper discusses the synthetic pathways leading to decreasing the polarity of such prepared bisphosphonic acid derivatives. The hindrance to oral delivery of ANPs is due to the two negative charges. The permeability through the cell membrane, which is decisive for biological activity of bisphosphonates possessing four negative charges in the molecule, must be expected to be even lower. Therefore, we have examined the effect of masking the 1,3-bisphosphonates. For this purpose we used the hexadecyloxypropyl group described earlier in the literature.[13a–16] In principle, it is possible to prepare such lipophilic phosphonates by either activating the corresponding free phosphonic acids or building appropriate lipophilic phosphonate chain by the stepwise procedure.

2.1. Preparation of lipophilic phosphonate derivatives by free phosphonic acid activation

The conversion of free phosphonic acids (PMEA, PMEC, PMEG) to bis[3-{(hexadecyloxy)propyl}ethoxy]methylphosphonate by *N*,*N*′-carbonyldiimidazole, dimethylformamide dineopentyl or 1,3-propylene acetal was not successful.

The free acids of the PME structure derived from adenine, cytosine or guanine (**1a–c**), as depicted in Scheme 1, were first converted by treatment with oxalyl chloride and dimethylformamide into the respective chlorides whose formation was monitored by HPLC. Lipophilic derivatives of

Keywords: Acyclic nucleoside phosphonates (ANPs); PMEA; PMEC; PMEG; Alkoxyalkyl phosphonates; Hexadecyloxypropyl ester groups; Bisphosphonates; Synthesis.
* Corresponding author. E-mail addresses: vrbkova@uochb.cas.cz; dracinsky@uochb.cas.cz; holy@uochb.cas.cz

0040–4020/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2007.08.081

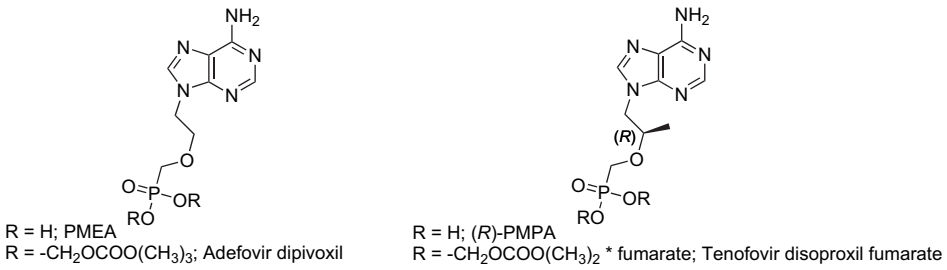


Figure 1. Adefovir and tenofovir.

PME chain **2a–c** were prepared by the subsequent reaction with 3-(hexadecyloxy)propanol in the presence of pyridine and triethylamine at 0 °C under an argon atmosphere. In the reaction, the NH_2 groups of PMEA, PMEG and PMEC are simultaneously transformed into the corresponding amidine(dimethylaminomethylene) derivatives. They are easily released under acidic (PMEC, PMEG) or basic conditions (PMEA). Finally, one of the lipophilic chains was removed exclusively, using the earlier described reaction—by treatment with an excess of LiN_3 in DMF at 100 °C, to give the monoesters **3a**,**c** as the sole products.[19]

This approach can be applied in those cases where the starting compound is easily available. However, a different synthetic strategy has to be used in sensitive systems or compounds that are accessible by multistep syntheses only.

2.2. Stepwise construction of the lipophilic phosphonate

For the synthesis of the lipophilic 1,3-bis(phosphonoethoxy)propan-2-yl derivative, we applied the stepwise building of propan-1,3-diol-2-yl derivative on the adenine ring followed by etherification with the lipophilic phosphorylethyl derivative with a good leaving group (e.g., tosylate **6** or **9**).

Compound **6** was prepared by treatment of 3-(hexadecyloxy)propanol with freshly distilled PCl_3 in pyridine/ether mixture at 0 °C, followed by a standard method for hydroxymethylation and tosylation[20] (Scheme 2). This building block **6** can be directly used as an etherifying/alkylating agent for diol **12** or one lipophilic chain of the intermediate can be easily removed on heating with LiN_3 in DMF to provide compound **9a** (see Scheme 3).

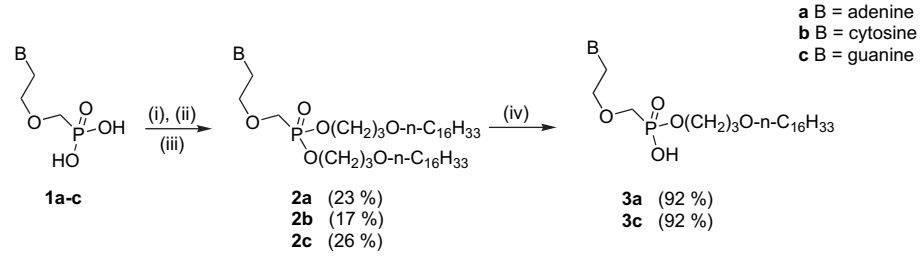
Although compound **6** could be obtained by this procedure, its yield was low. Therefore, we decided to use a different approach. The attempts to convert triethyl phosphite and/or diphenyl phosphite by transesterification to their hexadecyloxypropyl esters failed. We have, therefore, applied the standard method for deprotection of ester groups using the strategy reported in the literature[13a,13b] and depicted in Scheme 3.

The stepwise building of compounds **13** and **14** started with the alkylation of 2-phenyl-1,3-dioxan-5-yl tosylate (**10**). Only N^9 substituted adenine **11** was found in this reaction step, the formation of other possible (N^7) isomer was not observed. NMR analysis was used to identify the position of substitution of the purine ring. All signals of hydrogen and carbon atoms were assigned using 2D-^{1}H,^{13}C HSQC and 2D-^{1}H,^{13}C HMBC experiments. In the case of N^9-isomers, protons on carbon atom next to the purine nitrogen have strong crosspeaks in HMBC spectra with carbons C-4 and C-8 of the purine ring.

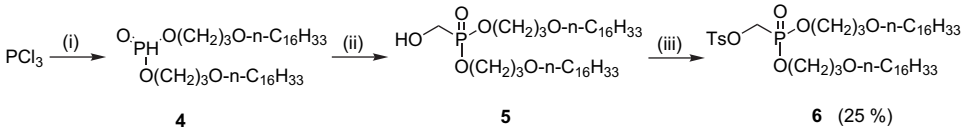
Interestingly, no reaction occurred when the corresponding mesylate or the reaction under Mitsunobu conditions was used for the alkylation. After deprotection of the benzylidene-protecting group, compound **12** was obtained. It was treated with sodium 3-(hexadecyloxy)propyl tosyloxymethylphosphonate (**9b**) in the presence of NaH, in DMF at 50 °C to give the final products **13** and **14** (Scheme 4).

Antiviral activity in vitro against DNA, RNA and retroviruses was determined under the standard conditions.[22,23] Compounds **3a**, **13** and **14** were examined for their inhibitory effect on the replication of varicella-zoster virus (VZV), human cytomegalovirus (HCMV) and herpes simplex virus (HSV-1 and HSV-2) in human embryonic lung (HEL) cells. Further, they were examined for their inhibitory effect on the replication of human immunodeficiency virus (HIV) in human T-lymphocyte (CEM) cells.

The parent compound, PMEA **1a**, is highly active against HIV-1 with the EC_{50} of 6.22±0.73 μmol/l.[24] Against VZV and HIV-2, it exhibited inhibitory activity with the EC_{50}s



Scheme 1. Preparation of lipophilic phosphonate derivatives by the free phosphonic acid activation. (i) Oxalyl chloride, CH_2Cl_2, DMF, reflux; (ii) 3-(hexadecyloxy)propanol, pyridine, Et_3N, CH_2Cl_2, 0 °C; (iii) methanolic ammonia, rt or 80% CH_3COOH, reflux; (iv) LiN_3, DMF, 100 °C.



4 **5** **6** (25 %)

Scheme 2. Synthesis of the lipophilic building block I. (i) 3-(Hexadecyloxy)propanol, pyridine, ether; (ii) $(CH_2O)_n$, Et_3N, 100 °C; (iii) TsCl, DMAP, Et_3N, CH_3CN.

of 7.32 and 6.59, respectively. Although these results are very promising themselves, it is worth mentioning that the introduction of suitable masking moiety to the parent compound has a considerable effect on the antiviral activity. While the lipophilic compound **2a** exhibits a lower activity compared to the corresponding parent compound, the antiviral activity of its mono-lipophilic congener **3a** is increased severalfold in all tested assays (see Table 1).

Also the so-called 'double-PME' lipophilic analogues **13** and **14** proved to be active (see Table 1). While the free bisphosphonic acids showed only negligible activity,[18] their lipophilic congeners **13** and **14** exhibit significant inhibitory activity against herpesviruses, namely varicella-zoster virus and human cytomegalovirus. Also the compounds **13** and **14** showed anti-HSV activity comparable or even better to that observed for the reference compound PMEA. Furthermore, the antiviral, other than anti-HIV, activities of compounds **13** and **14** are higher than in case of parent compound PMEA.

Surprisingly, all lipophilic compounds **3a**, **13** and **14** showed also activity against the Coxsackie virus B4 (HeLa cell culture). In general, in this series of ANPs the activity against RNA viruses is very rare. It remains to be seen whether this finding could result in a new lead.

Furthermore, some of the prepared lipophilic compounds were subject to cytotoxicity measurements. In this case, PMEG **1c** was chosen as the parent and reference compound for the lipophilic analogues. PMEG is an extremely active cytostatic, exhibiting significant activity in vivo in rat and mouse carcinomas and sarcomas.[25–27]

The cytostatic activities of compounds **3c** and PMEG were obtained by classic technique, estimating the cell count in a haematological analyzer. In parallel the cell viability was determined by XTT (Cell Proliferation Kit II, Roche) test. Compounds were tested for cytostatic effect on the cultures of murine leukaemia L1210 cells, human cervix carcinoma HeLa S3 cells, human promyelocytic leukaemia HL60 cells and in human T-lymphoblastoid CCRF-CEM cell line. The presented data (Table 2, CCRF-CEM and HL60 cells) show that the growth inhibitory effect of compound **3c** is two orders of magnitude more efficient than that of parent compound PMEG. The data on viability in case of compound **3c** show significantly low values of IC_{50} compared to the cell count IC_{50}. This difference is proportional to a high extent of apoptosis found in **3c** treated cells.

Also, the PMEA analogues **3a** and **14** showed the cytostatic activity, however, it was necessary to prolong the time to evoke the apoptosis.

In conclusion, this study further confirmed that the introduction of suitable masking moiety to the polar phosphonate group helps with the transport of free phosphonic acids through the cell membrane and may provide bioavailable prodrugs of ANPs with potency comparable or even better to that of the corresponding non-derivatized drugs.

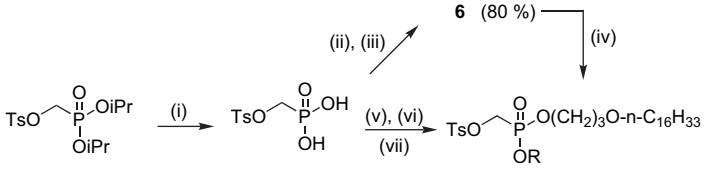
3. Conclusions

A new series of acyclic nucleoside phosphonates with lipophilic ester groups as a part of the phosphonomethoxyethyl or 1,3-bis(phosphonomethoxy)propan-2-yl side chain were prepared. The best results were obtained with activation of acids (PMEA, PMEC, PMEG) with oxalyl chloride and subsequent treatment with 3-(hexadecyloxy)propanol. In the case of the sequential building of 1,3-bis(phosphonomethoxy)propan-2-yl building block on the nucleobase, Beadle's procedure was the strategy of choice.

4. Experimental

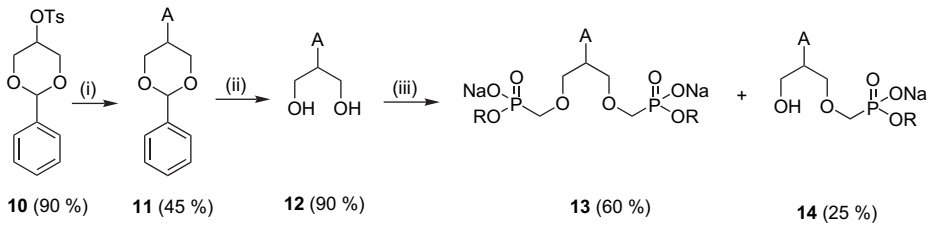
4.1. General

Unless otherwise stated, solvents were evaporated at 40 °C/2 kPa, and compounds were dried at 2 kPa over P_2O_5. Melting points were determined on a Büchi melting point apparatus. NMR spectra were measured on FT NMR spectrometer Varian Unity 500 (^1H at 500 MHz and ^{13}C at 125.7 MHz frequency). Chemical shifts are given in parts



7 **8** **9a** R = H (93 %)
9b R = Na (67 %)

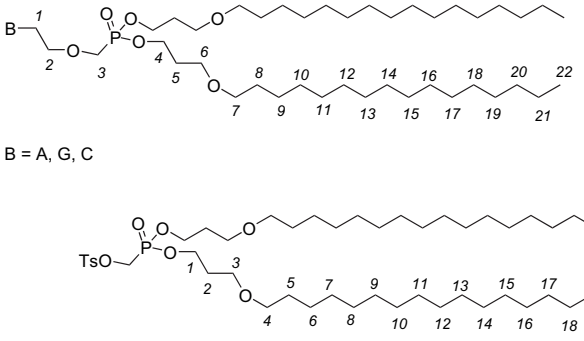
Scheme 3. Synthesis of the lipophilic building block II. (i) TMSBr, rt, CH_3CN; for compound **9a**: (ii) oxalyl chloride, DMF, CH_2Cl_2, reflux; (iii) 3-(hexadecyloxy)propanol, pyridine, Et_3N, CH_2Cl_2, 0 °C; (iv) LiN_3, DMF, 100 °C; for compound **9b**: (v) oxalyl chloride, DMF, CH_2Cl_2, 0 °C, then rt; (vi) 3-(hexadecyloxy)propanol, pyridine, Et_2O, rt; (vii) $NaHCO_3$, 0 °C.



Scheme 4. The stepwise buildup of the lipophilic bisphosphonate derivatives. (i) Cs_2CO_3, DMF, 90 °C; (ii) 80% CH_3COOH, 75 °C; (iii) **9b**, DMF, NaH (60% in mineral oil), 50 °C, R = $-(CH_2)_3-O-n-C_{16}H_{33}$.

per million (δ-scale), coupling constants (J) in hertz. Mass spectra were measured on a ZAB-EQ (Micromass, Manchester, U.K.) spectrometer using FAB (ionization by Xe, accelerating voltage 8 kV, glycerol matrix) or using EI (electron energy 70 eV). UV spectra (λ in nm) were taken on a Beckman Coulter™, DU® 800 spectrophotometer. Elemental analyses were carried out on a Perkin–Elmer CHN Analyser 2400, Series II Sys (Perkin–Elmer, Norwolk, CT, U.S.A.). IR spectra were recorded on a FTIR spectrometer Bruker Equinox 55. Chemicals were purchased from Sigma–Aldrich (Prague, Czech Republic). Dimethylformamide, acetonitrile and dichloromethane were distilled from P_2O_5 and stored over molecular sieves (4 Å). Paraformaldehyde was dried over H_2SO_4.

Numbering of the chain for NMR analysis



4.2. General procedure for the conversion of phosphonic acids to lipophilic phosphonates

Oxalyl chloride (6–7 equiv) was slowly added to the slurry of free phosphonic acid (0.8 mmol of **1a–c**; 1.5 mmol of **8**) and DMF (3 drops) in dry CH_2Cl_2 (25 mL). The resulting mixture was stirred under reflux with $CaCl_2$ protecting tube. The reaction was monitored by analysis of a small aliquot of the reaction mixture with anhydrous MeOH containing 1 drop of triethylamine. The resulting intermediate was analyzed by HPLC (mobile phase: solvent A=water; solvent B=MeOH/water=50:50; solvent C=MeOH; inj. vol=10 µL; UV detection at 254 nm). After completion, the reaction mixture was cooled to room temperature and evaporated in vacuo. The residue in CH_2Cl_2 (15 mL) was treated slowly with pyridine (0.25 mL) at 0 °C and then added to a solution of 3-(hexadecyloxy)propanol (2 equiv) and triethylamine (1.3 mL) in CH_2Cl_2 (15 mL) at −25 °C. The reaction mixture was stirred under argon atmosphere for 5 h and then at the room temperature overnight. The crude mixture was concentrated in vacuo, codistilled with toluene and worked up as described for compounds **2a–c**.

4.2.1. Bis[3-(hexadecyloxy)propyl] [2-(adenin-9-yl)-ethoxy]methylphosphonate (2a). The evaporated and dried crude product was dissolved in methanolic ammonia and stirred at room temperature overnight. The resulting dark viscous residue was evaporated and purified by silica gel chromatography (0–10% MeOH/$CHCl_3$) to yield 0.16 g (23%) of **2a** as yellowish solid (mp=96–98 °C), R_f=0.43 (10%

Table 1. Antiviral activity of studied compounds in vitro

Type of virus	EC_{50}[a] (µM)			
	14	**13**	**3a**	PMEA
Retroviruses				
HIV-1	>32.91	>19.88	0.072	6.22±0.73
HIV-2	>32.91	>19.88	0.14	6.59
CC_{50}[b] (µM)	54.63±8.86	38.86±5.66	3.56±0.18	129.58±5.12
Herpesviruses				
HSV-1	>32.91	>19.88	1.08±0.54	>100
HSV-2	>32.91	>19.88	0.54±0.36	73.21
HCMV				
AD-169 strain	>6.58	>19.88	0.13	>100
Davis strain	>6.58	10.93	<0.058	>100
VZV				
TK^+ OKA strain	>6.58	>3.98	<0.058	30.38
TK^- 07/1 strain	>6.58	3.98	<0.058	7.32
Picornaviruses				
Coxsackie virus B4	>6.58	3.98	>1.44	>100

[a] EC_{50}=50% effective concentration.
[b] CC_{50}=50% cytostatic concentration.

Table 2. Cytostatic activity of studied compounds in vitro

Compound	% of control (IC_{50}, μmol/l) [XTT]			
	L1210	HL60	HeLa S3	CCRF-CEM
3c	15 (0.18±0.02)	(3.6±0.2) [0.059±0.004]	41 (2±0.15)	38 (2±0.12) [0.033±0.004]
PMEG	(3.1±0.17) N.D.	(4.5±0.31) [3.68±0.12]	N.D.	(2.66±0.16) [2.44±0.122]
14	79	92	104	55
3a	62	86	98	45

N.D.—not determined.

MeOH/$CHCl_3$). For $C_{46}H_{88}N_5O_6P$ (838.19) calcd: C, 65.91; H, 10.58; N, 8.36; P, 3.70. Found: C, 65.93; H, 10.59; N, 8.29; P, 3.65. FABMS: 839.62 (MH^+) (80). δ_H (500 MHz, $CDCl_3$) 8.13 and 8.05 (2×s, 2×1H, Pu-8 and Pu-2), 7.08 (br s, 2H, NH_2), 4.32 (t, 2H, J_{1-2} 5.2 Hz, H-1), 3.96 (m, 2×2H, H-4), 3.89 (t, 2H, J_{2-1} 5.2 Hz, H-2), 3.85 (d, 2H, $J_{3\text{-}P}$ 8.0 Hz, H-3), 3.35 and 3.31 (4×t, 2×4H, J_{6-7} 6.5, 6.0 Hz, H-6 and H-7), 1.73 (2×q, 2×2H, J_{5-4} 6.3 Hz, J_{5-6} 6.3 Hz, H-5), 1.47 (2×m, 2×2H, H-8), 1.31–1.25 (br s, 52H, CH_2), 0.86 (2×t, 2×3H, J_{22-21} 7.2 Hz, H-22); δ_C (125.7 MHz, $CDCl_3$) 155.9 (Pu-6), 152.3 (Pu-2), 149.6 (Pu-4), 140.9 (Pu-8), 118.8 (Pu-5), 70.3 (d, $J_{2\text{-}P}$ 11.3 Hz, C-2), 70.1 (C-7), 65.9 (C-6), 63.8 (d, $J_{3\text{-}P}$ 162.7 Hz, C-3), 63.0 (d, $J_{4\text{-}P}$ 6.4 Hz, C-4), 42.4 (C-1), 31.3 (2C), 30.4 (d, $J_{5\text{-}P}$ 5.4 Hz, C-5), 29.0 (2C), 28.9 (2C), 28.8 (2C), 28.6 (2C), 25.6 (2C), 22.0 (2C), 21.2 (2C), 13.9 (2C, C-22). ν_{max} (KBr) 2955, 2919, 2851, 1640, 1600, 1579, 1468, 1417, 1378, 1327, 799, 646, 1250, 1034, 1010, 995, 1126, 1058, 722 cm^{-1}.

4.2.2. Bis[3-(hexadecyloxy)propyl] [2-(cytosin-1-yl)-ethoxy]methylphosphonate (2b). The evaporated mixture was dissolved in 80% CH_3COOH (20 mL) and dioxane (10 mL) and refluxed for 5 h. The crude residue was evaporated and purified by silica gel column chromatography (0–8% MeOH/$CHCl_3$) to yield 0.11 g (17%) of **2b** as brownish solid (mp=98–100 °C), R_f=0.56 (10% MeOH/$CHCl_3$). For $C_{45}H_{88}N_3O_7P$ (814.17) calcd: C, 66.38; H, 10.89; N, 5.16; P, 3.80. Found: C, 66.35; H, 10.82; N, 5.05; P, 3.72. FABMS: 815.0 (MH^+) (60). δ_H (500 MHz, $CDCl_3$) 7.41 (d, 1H, $J_{Py\text{-}6,Py\text{-}5}$ 7.0 Hz, Py-6), 5.72 (d, 1H, $J_{Py\text{-}5,Py\text{-}6}$ 6.3, Py-5), 4.15 (2×q, 2×2H, J_{4-5} 6.8 Hz, $J_{4\text{-}P}$ 6.8 Hz, H-4), 3.97 (br s, 2H, H-1), 3.82 (br s, 2H, H-2), 3.77 (d, 2H, $J_{3\text{-}P}$ 8.4 Hz, H-3), 3.47 (2×t, 2×2H, J_{6-5} 6.1 Hz, H-6), 3.39 (2×t, 2×2H, J_{7-8} 6.7 Hz, H-7), 1.91 (2×p, 2×2H, J_{5-4} 6.2 Hz, J_{5-6} 6.2 Hz, H-5), 1.54 (2×m, 2×2H, H-8), 1.32–1.25 (br s, 52H, CH_2), 0.88 (2×t, 2×3H, J_{22-21} 6.9 Hz, H-22); δ_C (125.7 MHz, $CDCl_3$) 164.8 (Py-4), 155.4 (Py-2), 146.9 (Py-6), 93.3 (Py-5), 71.2 (d, $J_{2\text{-}P}$ 12.0 Hz, C-2), 71.2 (C-7), 66.5 (C-6), 65.1 (d, $J_{3\text{-}P}$ 167.2 Hz, C-3), 63.8 (d, $J_{4\text{-}P}$ 6.5 Hz, C-4), 49.7 (C-1), 31.9 (2C), 30.9 (d, $J_{5\text{-}P}$ 5.9 Hz, C-5), 29.7 (2C), 29.7 (2C), 29.7 (2C), 29.5 (2C), 29.3 (2C), 26.2 (2C), 22.7 (2C), 14.1 (2C, C-22). ν_{max} (KBr) 2957, 2919, 2851, 1654, 1611, 1526, 1491, 1469, 1388, 1380, 1272, 1246, 1122, 1057, 1021, 791, 721 cm^{-1}.

4.2.3. Bis[3-(hexadecyloxy)propyl] [2-(guanin-9-yl)-ethoxy]methylphosphonate (2c). The residue after evaporation was dissolved in 80% CH_3COOH (20 mL) and refluxed for 5 h. The crude residue was evaporated and purified by silica gel column chromatography (0–12% MeOH/$CHCl_3$) to yield 0.18 g (26%) of **2c** as white solid (mp=100–102 °C), R_f=0.4 (20% MeOH/$CHCl_3$). For $C_{46}H_{88}N_5O_7P$ (854.19) calcd: C, 64.68; H, 10.38; N, 8.20; P, 3.63. Found: C, 64.62; H, 10.35; N, 8.19; P, 3.68. FABMS: 855.58 (MH^+) (80). δ_H (500 MHz, $CDCl_3$) 11.92 (br s, 1H, NH), 7.66 (s, 1H, Pu-8), 6.34 (br s, 2H, NH_2), 4.22 (t, 2H, J_{1-2} 6.7 Hz, H-1), 4.15 (2×m, 2×2H, J_{4-5}~$J_{4\text{-}P}$ 6.5 Hz, H-4), 3.92 (t, 2H, J_{2-1} 5.3 Hz, H-2), 3.82 (d, 2H, $J_{3\text{-}P}$ 8.2 Hz, H-3), 3.48 (2×t, 2×2H, J_{6-5} 6.2 Hz, H-6), 3.39 (2×t, 2×2H, J_{7-8} 6.8 Hz, H-7), 1.92 (2×p, 2×2H, J_{5-4}~J_{5-6} 6.2 Hz, H-5), 1.58 (2×m, 2×2H, H-8), 1.32–1.25 (m, 52H, CH_2), 0.88 (2×t, 2×3H, J_{22-21} 7.1 Hz, H-22); δ_C (125.7 MHz, $CDCl_3$) 158.9 (Pu-6), 153.7 (Pu-2), 151.6 (Pu-4), 138.2 (Pu-8), 116.9 (Pu-5), 71.4 (d, $J_{2\text{-}P}$ 10.9 Hz, C-2), 71.2 (C-7), 66.5 (C-6), 65.1 (d, $J_{3\text{-}P}$ 166.5 Hz, C-3), 64.0 (d, $J_{4\text{-}P}$ 6.4 Hz, C-4), 43.1 (C-1), 31.9 (2C, C-8), 30.9 (d, $J_{5\text{-}P}$ 5.9 Hz, C-5), 29.7 (2C), 29.7 (2C), 29.5 (2C), 29.4 (2C), 26.2 (2C), 22.7 (2C), 21.2 (2C), 14.1 (C-22). ν_{max} (KBr) 2923, 2851, 1689, 1654, 1625, 1540, 1481, 1476, 1445, 1398, 1384, 1365, 1260, 1236, 1121, 1072, 1037, 781, 721 cm^{-1}.

4.3. General procedure for the selective removal of 3-(hexadecyloxy)propyl ester group from bis[3-(hexadecyloxy)propyl]phosphonates[20]

LiN_3 (1.1 mmol) was added to a stirred solution of compound **2a** or **2c** (0.16 mmol) or **6** (1.5 mmol) in dry DMF (15 mL). The reaction mixture was stirred with $CaCl_2$ protecting tube at 100 °C for 12 h. The solvent was evaporated in vacuo and the crude residue was purified by silica gel column chromatography (solvent H1—EtOAc/EtOH/acetone/H_2O=4:1:1:1; solvent H3—EtOAc/EtOH/acetone/H_2O=6:1:1:0.5).

4.3.1. 3-(Hexadecyloxy)propyl hydrogen [2-(adenin-9-yl)ethoxy]methylphosphonate (3a). Silica gel column chromatography in system H3 (600 mL) followed by H1 (400 mL) gave compound **3a** (81 mg, 92%) as yellowish solid (mp=78–79 °C), R_f=0.24 (H1). For $C_{27}H_{50}N_5O_5P$ (555.69) calcd: C, 58.36; H, 9.07; N, 12.60; P, 5.57. Found: C, 58.48; H, 8.92; N, 12.45; P, 5.49. FABMS: 556.53 (MH^+) (70). δ_H (500 MHz, $CDCl_3$) 8.21 and 8.19 (2×s, 2×1H, Pu-8 and Pu-2), 4.36 (t, 2H, J_{1-2} 4.4 Hz, H-1), 3.84 (q, 2H, J_{4-5} 6.5 Hz, H-4), 3.80 (br s, 2H, H-2), 3.61 (d, 2H, $J_{3\text{-}P}$ 9.2 Hz, H-3), 3.32 (t, 2H, J_{6-5} 6.2 Hz, H-6), 3.26 (t, 2H, J_{7-8} 6.9 Hz, H-7), 1.71 (p, 2H, J_{5-4}~J_{5-6} 6.4 Hz, H-5), 1.46 (m, 2H, H-8), 1.32–1.21 (m, 26H, CH_2), 0.88 (t, 3H, J_{22-21} 7.0 Hz, H-22); δ_C (125.7 MHz, $CDCl_3$) 155.2 (Pu-6), 151.4 (Pu-2), 149.3 (Pu-4), 142.6 (Pu-8), 118.0 (Pu-5), 71.1 (C-7), 70.9 (d, $J_{2\text{-}P}$ 12.5 Hz, C-2), 66.9 (C-6), 66.3 (d, $J_{3\text{-}P}$ 161.9 Hz, C-3), 62.4 (d, $J_{4\text{-}P}$ 5.6 Hz, C-4), 43.4 (C-1), 31.9 (C-8), 30.9 (d, $J_{5\text{-}P}$ 6.4 Hz, C-5), 29.8, 29.7, 29.7, 29.6, 29.4, 26.1, 22.6, 14.1 (2C, C-22). ν_{max} (KBr) 3427, 3199, 2923, 2853, 1647, 1600, 1578, 1490, 1468, 1419, 1377, 1328, 1238, 1211, 1113, 1077, 995, 799, 720, 649 cm^{-1}.

4.3.2. 3-(Hexadecyloxy)propyl hydrogen [2-(guanin-9-yl)ethoxy]methylphosphonate (3c). Silica gel column chromatography in system H3 (400 mL) followed by H1 (600 mL) gave compound **3c** (84 mg, 92%) as white amorphous solid. R_f=0.19 (H1). For $C_{27}H_{50}N_5O_6P$ (571.69) calcd: C, 56.72; H, 8.82; N, 12.25; P, 5.42. Found: C,

56.68; H, 8.69; N, 12.36; P, 5.43. FABMS: 572.40 (MH^+) (80). NMR spectra of the free acid form of the monoester are unavailable due to poor solubility of compound **3c** in common deuterated solvents and their mixtures. ν_{max} (KBr) 2922, 2852, 2123, 1669, 1649, 1625, 1572, 1541, 1485, 1468, 1412, 1370, 1224, 1187, 1125, 1072, 1030, 781, 720, 704 cm^{-1}.

4.3.3. {[3-(Hexadecyloxy)propoxy](hydroxy)phosphoryl}methyl tosylate (9a). The reaction mixture was obtained according to the above described procedure. Evaporated and purified on silica gel column chromatography (elution solvent 15–20% MeOH/$CHCl_3$) to give 0.76 g (93%) of compound **9a** as white foam. R_f=0.40 (30% MeOH/$CHCl_3$). δ_H (500 MHz, $CDCl_3$) 7.76 (d, 2H, J_{2-3} 7.8 Hz, Ts-2), 7.30 (d, 2H, J_{3-2} 7.8 Hz, Ts-3), 4.04 and 3.88 (br s, 2×2H, OCH_2P and H-1), 3.37 and 3.32 (br s and t, 2×2H, H-3 and H-4), 2.38 (s, 3H, Ts-CH_3), 1.72 (br s, 2H, H-2), 1.50 (br s, 2H, H-5), 1.33–1.20 (br s, 26H, CH_2), 0.88 (t, 3H, J_{19-18} 7.0 Hz, H-19); δ_C (125.7 MHz, $CDCl_3$) 144.8 (Ts-4), 132.4 (Ts-1), 129.9 and 128.2 (Ts-2,3), 71.1 (C-4), 67.2 (C-3), 64.3 and 63.4 (br s, OCH_2P and C-1), 31.9, 30.8, 29.8, 29.7, 29.4, 26.2, 22.7, 21.6 (Ts-CH_3), 14.1 (C-19). ν_{max} (KBr) 2918, 2815, 1598, 1487, 1468, 1400, 1374, 1308, 1295, 1262, 1191, 1179, 1122, 1097, 1061, 1033, 1021, 1010, 819, 770, 721, 702, 664, 579, 554 cm^{-1}.

4.4. [Bis(3-(hexadecyloxy)propoxy)phosphoryl]methyl tosylate (6)

4.4.1. Method A. 3-(Hexadecyloxy)propanol (5.8 mmol, 2.5 equiv) was added to a mixture of freshly distilled PCl_3 (2.3 mmol) and pyridine (3 equiv) in dry diethylether (20 mL) for 15 min keeping the temperature below 10 °C. The mixture was then stirred at room temperature for 3 h, the solid was filtered off and the solution was evaporated and codistilled with toluene. The crude product **4** was used without further purification for the next reaction step. The solution of the crude compound **4** in toluene (30 mL) was treated with Et_3N (0.4 mL). Paraformaldehyde (12 equiv) was added to this mixture and refluxed with the $CaCl_2$ protecting tube for 3 h. The mixture was cooled to room temperature, evaporated and codistilled with CH_2Cl_2. The solution of crude **5** in CH_2Cl_2 (40 mL) was treated with DMAP (0.1 g), Et_3N (0.4 mL) and TsCl (0.5 g), stirred at 0 °C for 2 h and then at room temperature overnight. The reaction mixture was washed with ice-cold water and purified by silica gel column chromatography (HE/EA=2:1, then 3:2) to give 0.45 g (25%) of **6** as white foam. R_f=0.36 (HE/EA=1:1).

4.4.2. Method B. (Diisopropoxyphosphoryl)methyl tosylate **7** (0.5 g, codistilled with acetonitrile) in acetonitrile (20 mL) and $BrSiMe_3$ (2.5 mL) was stirred at room temperature overnight. After evaporation and co-distillation with acetonitrile, the residue was codistilled with water (3×), toluene and finally with CH_2Cl_2. The crude mixture of **8** was evaporated to dryness, and the residue was dissolved in CH_2Cl_2. Treatment with oxalyl chloride and further work up followed the general procedure for the conversion of phosphonic acids to lipophilic bisphosphonates (see above). The crude residue was purified on silica gel column chromatography (HE/EA=2:1, then 3:2) to give 0.95 g (80%) of **6** as white foam. R_f=0.36 (HE/EA=1:1). FABMS: 832.12 (MH^+) (100). δ_H (500 MHz, $CDCl_3$) 7.76 (d, 2H, J_{2-3} 7.8 Hz, Ts-2), 7.30 (d, 2H, J_{3-2} 7.8 Hz, Ts-3), 4.18 (d, 2H, J_{P-CH} 9.9 Hz, OCH_2P), 3.88 (2×m, 2×2H, H-1), 3.42 (2×t, 2×2H, J 6.2 Hz, H-3), 3.38 (2×t, 2×2H, J 6.7 Hz, H-4), 2.40 (s, 3H, Ts-CH_3), 1.80 (2×t, 2×2H, J 6.5 Hz, H-2), 1.50 (2×m, 2×2H, H-5), 1.33–1.25 (br s, 52H, CH_2), 0.88 (2×t, 2×3H, J_{19-18} 7.1 Hz, H-19); δ_C (125.7 MHz, $CDCl_3$) 145.5 (Ts-4), 131.7 (Ts-1), 130.0 and 128.2 (Ts-2,3), 71.1 (2C, C-4), 67.2 (2C, C-3), 64.3 and 63.5 (br s, 3C, OCH_2P and C-1), 31.9 (2C), 29.7 (2C), 29.7 (6C), 29.6 (2C), 29.4 (2C), 26.2 (2C), 22.7 (2C), 21.7 (Ts-CH_3), 14.1 (2C, C-19).

4.5. 2-Phenyl-1,3-dioxan-5-yl tosylate (10)

Tosyl chloride (1.2 equiv) was added to a stirred solution of 2-phenyl-1,3-dioxan-5-ol (5.47 g, 30.35 mmol), DMAP (0.1 equiv) and Et_3N (1.2 equiv) in dry CH_2Cl_2 (80 mL) at 0 °C with the $CaCl_2$ protecting tube. The mixture was stirred at 0 °C for 3 h and at room temperature overnight. The reaction mixture was quenched with ice-cold water (20 mL) and vigorously stirred for 0.5 h. The layers were separated and organic layer was washed with ice water and evaporated. The crystallization from ethanol yielded 9.12 g (90%) of **10** as white solid (mp=127–129 °C), R_f=0.74 (HE/EA=7:3, OCH_{ax}), R_f=0.56 (HE/EA=7:3, OCH_{eq}). For $C_{17}H_{18}O_5S$ (334.39) calcd: C, 61.06; H, 5.43; S, 9.59. Found: C, 60.99; H, 5.38; S, 9.71. FABMS: 335.12 (MH^+) (100). δ_H (500 MHz, DMSO-d_6) 7.89 and 7.52 (2×d, 4H, $H_{arom.}$), 7.36 (m, 5H, $H_{arom.}$), 5.55 (s, 1H, O–C*H*–O), 4.50 (tt, 1H, J 5.3, 5.3, 10.1, 10.1 Hz, O–C*H*), 4.07 (dd, 2H, J 11.3, 5.3 Hz, OCH_2), 3.81 (dd, 2H, J 11.3, 10.3 Hz, OCH_2), 2.44 (s, 3H, CH_3); δ_C (125.7 MHz, DMSO-d_6) 145.8, 137.2, 132.5, 130.6 (2C), 129.2, 128.3 (2C), 127.9 (2C), 126.3 (2C), 100.3 (O–C*H*–O), 68.8 (C*H*), 67.5 (CH_2), 21.3 (CH_3). IR (KBr) 3065, 3034, 1595, 1499, 1493, 1453, 1392, 1355, 1317, 1309, 1296, 1283, 1247, 1220, 1192, 1175, 1143, 1122, 1082, 1041, 1029, 1020, 991, 985, 952, 870, 848, 811, 746, 742, 705, 696, 658, 618, 568, 555, 475, 420 cm^{-1}.

4.6. 9-(2-Phenyl-1,3-dioxan-5-yl)adenine (11)

A suspension of adenine (17 mmol) in dry DMF (50 mL) was treated with Cs_2CO_3 (0.5 equiv) at room temperature with the $CaCl_2$ protecting tube for 0.5 h. The reaction mixture was then heated at 60 °C and **10** (17 mmol, 1.0 equiv) was added. The mixture was stirred at 100 °C for 24 h. The solvent was evaporated and the residue was co-evaporated with toluene. The residue in chloroform was filtered through a Celite pad, evaporated and purified on silica gel column in chloroform/methanol (3–4%). Crystallization from ethanol yielded 1.2 g (45%) of **11** as white amorphous powder. R_f=0.40 (10% MeOH/$CHCl_3$). FABMS: 298.56 (MH^+) (100). δ_H (500 MHz, DMSO-d_6) 8.26 and 8.17 (2×s, 2H, H-2 and H-8), 7.53–7.38 (m, 5H, $H_{arom.}$), 7.30 (br s, 2H, NH_2), 5.74 (s, 1H, O–C*H*–O), 4.86 (m, 1H, N–C*H*), 4.52 (dd, 2H, J_{gem} 10.9 Hz, $J_{CH_2,CH}$ 10.9 Hz, CH_2), 4.39 (dd, 2H, J_{gem} 10.9 Hz, $J_{CH_2,CH}$ 5.0 Hz, CH_2); δ_C (125.7 MHz, DMSO-d_6) 156.3 (C-6), 152.7 (C-2), 149.6 (C-4), 139.8 (C-8), 137.9, 129.2, 128.4, 126.4, 119.0 (C-5), 100.7 (O–CH–O), 68.1 (CH_2), 47.7 (N–CH).

4.7. 9-(1,3-Dihydroxyprop-2-yl)adenine (12)[21]

A solution of **11** (0.3 g, 1.00 mmol) in 80% CH_3COOH (20 mL) was heated at 75 °C for 7 h. The solvent was evaporated. The potentially formed N^6-acetyl group was removed by the methanolysis of the crude product. After neutralization with HCl, methanol was evaporated and the residue was purified by silica gel column chromatography using chloroform/methanol (elution solvent: 30% MeOH/$CHCl_3$) yielded 0.18 g (90%) of **12** as white solid (mp=192–194 °C; lit.=193–195 °C), R_f=0.10 (HE/$CHCl_3$/MeOH=3:2:1). For $C_8H_{11}N_5O_2$ (209.21) calcd: C, 45.93; H, 5.30; N, 33.48. Found: C, 45.83; H, 5.25; N, 33.40. FABMS: 210.13 (MH^+) (100). δ_H (500 MHz, DMSO-d_6) 8.11 and 8.10 (2×s, 2H, H-2 and H-8), 7.17 (br s, 2H, NH_2), 5.04 (t, 2H, OH), 4.51 (m, 1H, N–C*H*), 3.83 (2×m, 2×2H, CH_2); δ_C (125.7 MHz, DMSO-d_6) 156.1 (C-6), 152.1 (C-2), 149.9 (C-4), 140.5 (C-8), 119.0 (C-5), 60.1 (CH_2), 59.2 (N–CH). IR (KBr) 3424, 3350, 3240, 1637, 1613, 1570, 1480, 1335, 1212, 1095, 1087, 1069, 794, 652 cm^{-1}.

4.8. Sodium bis[3-(hexadecyloxy)propyl] [2-(adenin-9-yl)propane-1,3-diyl]bis(oxy)bis(methylene)diphosphonate (13) and sodium 3-(hexadecyloxy)propyl [2-(adenin-9-yl)-3-hydroxypropoxy]methylphosphonate (14)

Sodium hydride (2.5 equiv, 60% in mineral oil) was added to a stirred solution of compound **12** (0.14 g, 0.7 mmol) in dry DMF at 0 °C. After 15 min, compound **9b**[13a] (0.8 g, 1.4 mmol) was added in one portion at room temperature, and the reaction mixture was stirred at 50 °C for 24 h. The mixture was then evaporated and purified by column chromatography, compound **13** was eluted with 30% MeOH/$CHCl_3$ (60% yield, white solid, mp=232–234 °C) followed by compound **14** with 50% MeOH/$CHCl_3$ (25% yield, yellowish solid, mp=91–92 °C), R_f of compound **13**=0.14 (20% MeOH/$CHCl_3$), R_f of compound **14**=0.36 (20% MeOH/$CHCl_3$).

Compound **13**: UV spectrum: ($CHCl_3$) λ_{max}=259 nm (ε_{max}=10,362). Exact mass (FAB HRMS) found: 1006.6118; calculated for $C_{48}H_{91}N_5Na_2O_{10}P$: 1006.6115. FABMS: 1006.9 (MH^+) (80). δ_H (500 MHz, $CDCl_3$) 8.46 and 8.22 (2×br s, 2×1H, Pu-8 and Pu-2), 4.97 (br s, 1H, H-1), 4.02 (m, 8H, H-2 and H-4), 3.76 (br s, 4H, H-3), 3.41 (br s, 4H, H-6), 3.32 (t, 4H, $J_{7,8}$ 6.9 Hz, H-7), 1.80 (br s, 4H, H-5), 1.49 (m, 4H, H-8), 1.32–1.20 (m, 52H, CH_2), 0.88 (t, 6H, J_{CH_3,CH_2} 7.1 Hz, CH_3); δ_C (125.7 MHz, $CDCl_3$) 154.8 (Pu-6), 150.7 (Pu-2), 148.9 (Pu-4), 141.9 (Pu-8), 117.2 (Pu-5), 71.1 (C-7), 70.6 (C-2), 66.9 (C-6), 66.3 (d, $J_{3,P}$ 159.9 Hz, C-3), 62.4 (d, $J_{4,P}$ 6.4 Hz, C-4), 54.1 (C-1), 31.9 (d, $J_{5,P}$ 5.6 Hz, C-5), 30.9 (2C), 30.8 (2C), 29.7 (2C), 29.6 (2C), 29.6 (2C), 29.5 (2C), 29.3 (2C), 26.1 (2C), 22.6 (2C), 14.0 (2C, CH_3). IR (KBr) 2923, 2853, 1634, 1468, 1416, 1379, 1330, 1236, 1216, 1203, 1193, 1128, 1051, 1041, 1014, 799, 720 cm^{-1}.

Compound **14**: UV spectrum: (0.01 M HCl) λ_{max}=259 nm (ε_{max}=5242); (H_2O) λ_{max}=258 nm (ε_{max}=10,254); (0.01 M NaOH) λ_{max}=259 nm (ε_{max}=10,944). Exact mass (FAB HRMS) found: 608.3557; calculated for $C_{28}H_{51}N_5NaO_6P$: 608.3553. FABMS: 608.5 (MH^+) (60). δ_H (500 MHz, $CDCl_3$+CD_3COOD) 8.30 and 8.17 (2×br s, 2×1H, Pu-8 and Pu-2), 4.81 (m, 1H, H-1), 4.05 (m, 3H, CH_2OH and H-2a), 3.94 (dd, 1H, J_{gem} 9.9 Hz, $J_{2,1}$ 4.5 Hz, H-2b), 3.86 (m, 2H, H-4), 3.67 (d, 2H, $J_{3,P}$ 8.6 Hz, H-3), 3.36 (t, 2H, $J_{6,5}$ 6.4 Hz, H-6), 3.29 (t, 2H, $J_{7,8}$ 7.0 Hz, H-7), 1.74 (p, 2H, $J_{5,4}$~$J_{5,6}$ 6.3 Hz, H-5), 1.47 (m, 2H, H-8), 1.31–1.21 (m, 26H, CH_2), 0.88 (t, 3H, J_{CH_3,CH_2} 7.0 Hz, CH_3); δ_C (125.7 MHz, $CDCl_3$+CD_3COOD) 155.5 (Pu-6), 151.4 (Pu-2), 149.0 (Pu-4), 141.8 (Pu-8), 118.0 (Pu-5), 71.6 (C-2), 71.1 (C-7), 66.8 (C-6), 66.4 (d, $J_{3,P}$ 160.0 Hz, C-3), 62.5 (d, $J_{4,P}$ 6.4 Hz, C-4), 61.1 (CH_2OH), 56.5 (C-1), 31.9 (d, $J_{5,P}$ 5.7 Hz, C-5), 30.8, 29.7, 29.6, 29.6, 29.5, 29.3, 26.1, 22.6, 14.1 (CH_3). IR (KBr) 3428, 3267, 3210, 2924, 2853, 1643, 1602, 1577, 1482, 1469, 1417, 1370, 1332, 1245, 1209, 1115, 1073, 994, 799, 720, 651 cm^{-1}.

Acknowledgements

This work is a part of the research project Z4 055 0506. It was supported by the 'Centre for New Antivirals and Antineoplastics' (1M0508), by the Programme of Targeted Projects of Academy of Sciences of the Czech Republic (1QS400550501) and by Gilead Sciences, Inc. (Foster City, CA, USA). Descartes Prize of the European Union HPAW-2002-10096 also supported this project. We would like to thank Ivan Votruba and Eva Tloušťová for cytostatic activity screening. We also would like to thank Jan Balzarini, Armando De Palma, Johan Neyts, Graciela Andrei and Robert Snoeck for antiviral activity screening.

References and notes

1. Holý, A. *Curr. Pharm. Des.* **2003**, *9*, 2567–2592.
2. De Clercq, E.; Holý, A. *Nat. Rev. Drug. Discov.* **2005**, *4*, 928–940.
3. Cundy, K. C.; Shaw, J. P.; Lee, W. A. *Antimicrob. Agents Chemother.* **1994**, *38*, 365–368.
4. Cundy, K. C.; Hitchcock, M. J.; Lee, W. A. *Drug Metab. Dispos.* **1996**, *24*, 738–744.
5. Cundy, K. C. *Clin. Pharmacokinet.* **1999**, *36*, 127–143.
6. Shaw, J. P.; Sueoka, C. M.; Oliyai, R.; Lee, W. A.; Arimilli, M. N.; Kim, C. U.; Cundy, K. C. *Pharm. Res.* **1997**, *14*, 1824–1829.
7. Starrett, J. E., Jr.; Tortolani, D. R.; Hitchcock, M. J.; Martin, J. C.; Mansuri, M. M. *Antiviral Res.* **1992**, *19*, 267–273.
8. Cundy, K. C.; Fishback, J. A.; Shaw, J. P.; Lee, M. L.; Soike, K. F.; Visor, G. C.; Lee, W. A. *Pharm. Res.* **1994**, *11*, 839–843.
9. Cundy, K. C.; Sue, I. L.; Visor, G. C.; Marshburn, J.; Nakamura, C.; Lee, W. A.; Shaw, J. P. *J. Pharm. Sci.* **1997**, *86*, 1334–1338.
10. Shaw, J. P.; Louie, M. S.; Krishnamurthy, V. V.; Arimilli, M. N.; Jones, R. J.; Bidgood, A. M.; Lee, W. A.; Cundy, K. C. *Drug Metab. Dispos.* **1997**, *25*, 362–366.
11. Ballatore, C.; McGuigan, C.; De Clercq, E.; Balzarini, J. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1053–1056.
12. Gërbig, U.; Balzarini, J.; Meier, C. *Antiviral Res.* **2005**, *65*, A94.

13. (a) Beadle, J. R.; Wan, W. B.; Ciesla, S. L.; Keith, K. A.; Hartline, C.; Kern, E. R.; Hostetler, K. Y. *J. Med. Chem.* **2006**, *49*, 2010–2015; (b) Jasko, M. V.; Novicov, N. A.; Tarussova, N. B. *Bioorg. Khim.* **1992**, *20*, 50–54.
14. Wan, W. B.; Beadle, J. R.; Hartline, C.; Kern, E. R.; Ciesla, S. L.; Valiaeva, N.; Hostetler, K. Y. *Antimicrob. Agents Chemother.* **2005**, *49*, 656–662.
15. Hostetler, K. Y.; Rought, S.; Aldern, K. A.; Trahan, J.; Beadle, J. R.; Corbeil, C. *Mol. Cancer Ther.* **2006**, *5*, 156–159.
16. Randhawa, P.; Farasati, N. A.; Shapiro, R.; Hostetler, K. Y. *Antimicrob. Agents Chemother.* **2006**, *50*, 1564–1566.
17. Williams-Aziz, S. L.; Hartline, C. B.; Harden, E. A.; Daily, S. L.; Prichard, M. N.; Beadle, J. R.; Hostetler, K. Y.; Kern, E. R. *Antimicrob. Agents Chemother.* **2005**, *49*, 3724–3733.
18. Vrbovská, S.; Holý, A.; Pohl, R.; Masojídková, M. *Collect. Czech. Chem. Commun.* **2006**, *71*, 543–566.
19. Holý, A. *Synthesis* **1998**, 381–385.
20. Holý, A. *Collect. Czech. Chem. Commun.* **1993**, 649–674.
21. Rosenberg, I.; Holý, A.; Masojídková, M. *Collect. Czech. Chem. Commun.* **1988**, *53*, 2753–2777.
22. Balzarini, J.; Naesens, L.; Slachmuylders, J.; Niphuis, H.; Rosenberg, I.; Holý, A.; Schellekens, H.; De Clercq, E. *AIDS* **1991**, *5*, 21–28.
23. Balzarini, J.; Naesens, L.; Herdewijn, P.; Rosenberg, I.; Holý, A.; Pauwels, R.; Baba, M.; Johns, D.; De Clercq, E. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 332–336.
24. Holý, A.; Günter, J.; Dvořáková, H.; Masojídková, M.; Andrei, G.; Snoeck, R.; Balzarini, J.; De Clercq, E. *J. Med. Chem.* **1999**, *42*, 2064–2086.
25. Rose, W. C.; Crosswell, A. R.; Bronson, J. J.; Martin, J. C. *J. Natl. Cancer Inst.* **1990**, *82*, 510–512.
26. Veselý, J.; Merta, A.; Votruba, I.; Holý, A.; Rosenberg, I. *Neoplasma* **1990**, *37*, 105–110.
27. Krejčová, R.; Horská, K.; Votruba, I.; Holý, A. *Biochem. Pharmacol.* **2000**, *60*, 1907–1913.