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Structural diversity of nucleoside phosphonic acids as a key factor in the discovery of potent inhibitors of rat T-cell lymphoma thymidine phosphorylase

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ABSTRACT

Structurally diverse, sugar-modified, thymine-containing nucleoside phosphonic acids were evaluated for their ability to inhibit thymidine phosphorylase (TP, EC 2.4.2.4) purified from spontaneous T-cell lymphomas of an inbred Sprague-Dawley rat strain. From a large set of tested compounds, among them a number of pyrrolidine-based derivatives, 10 nucleotide analogues with IC_{50} values below 1 μM were selected. Out of them, four compounds strongly inhibited the enzyme with IC_{50} values lying in a range of 11–45 nM. These most potent compounds might be bi-substrate analogues.

Thymidine phosphorylase (TP), the catabolic enzyme cleaving thymidine by a phosphorolytic mechanism (Fig. 1), has attracted the attention of many research groups.[1–8]

The enzyme, identical to the platelet-derived endothelial cell growth factor (PDECGF),[9–12] is involved in angiogenesis and chemotaxis in human tumors,[10,11,13,14] and thus can be considered as a target in cancer treatment. Elevated levels of TP found in colorectal, ovarian, pancreatic, and breast tumors,[4,15] and at other hyperproliferative disease states such as rheumatoid arthritis[16] and psoriasis,[17] correlate with increased hypoxia. The inhibition of TP may result in reduction of tumor growth and metastasis,[18–25] and it also potentiates[26] the antiproliferative effect of nucleoside drugs such as 5-(*E*)-(bromovinyl)-2′-deoxyuridine, 2′-deoxy-5-trifluoromethyluridine, 2′-deoxy-5-iodouridine, and 5-fluoro-2′-deoxyuridine which are substrates of TP.[27] As follows from the survey of literature, the inhibition of TP represents a promising target in cancer chemotherapy.[28–32]

One of the most potent inhibitors of human TP is 5-chloro-6-[1-(2-iminopyrrolidinyl)methyl]uracil hydrochloride **1**[21] (TPI, Fig. 2) which inhibits the enzyme competitively with $K_i = 17$ nM.[29] In vivo, TPI increases the proportion of apoptotic cancer cells in TP-positive tumors and suppresses the growth of the tumors in mouse model.[21]

A recently published study on the inhibition of TP, purified from rat T-cell lymphomas of Sprague-Dawley rat strain, by phosphonomethoxyalkyl derivatives of thymine (Fig. 2) **2a** (FPMPT), **2b** (HPMPT), **2c** (PMPT), and **2d** (PMET) showed that these compounds were competitive inhibitors of both thymidine and inorganic phosphate.[6] It suggests that these phosphonic acids are bi-substrate

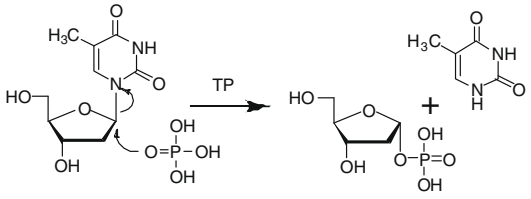

Figure 1. TP-catalysed thymidine cleavage.

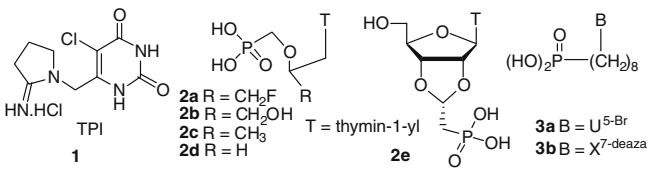

Figure 2. Examples of TP inhibitors.

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analogues. They possess considerable inhibitory potency towards T-cell lymphoma TP but not to rat liver, *Escherichia coli*, and human TPs. The competitive type of inhibition of compounds **2a–c** towards thymidine and inorganic phosphate resembles the character of inhibition of 8-phosphonooctyl derivatives **3a** and **3b** towards *E. coli* TP published by Perez-Perez and co-workers [33] In contrast to compounds **2a–d** and **3a, b**, the nucleoside phosphonic acid **2e** (Fig. 2) was reported to be a potent inhibitor of human recombinant TP with K_i = 236 nM.[34,35]

These findings prompted us to undertake a structure–activity study on the inhibition of TP, purified from spontaneous T-cell lymphomas of an inbred Sprague-Dawley rat strain,[6] with structurally diverse thymine-containing nucleoside phosphonic acids.

About 200 compounds that were prepared during our studies on various types of phosphonate analogues of nucleotides over last years were tested as potential inhibitors of rat T-cell lymphoma TP.[6] Out of this large pool of compounds, we selected those that reduced TP activity to at least 40% at the 1:10 ratio of the inhibitor versus thymidine as substrate (Table 1). In order to select most potent inhibitors, we measured the IC_{50} values of eleven promising nucleoside phosphonates and uncovered 10 submicromolar-level inhibitors. Among them, four very potent ones exhibited IC_{50} values within 11–45 nM range at 100 μM concentration of thymidine

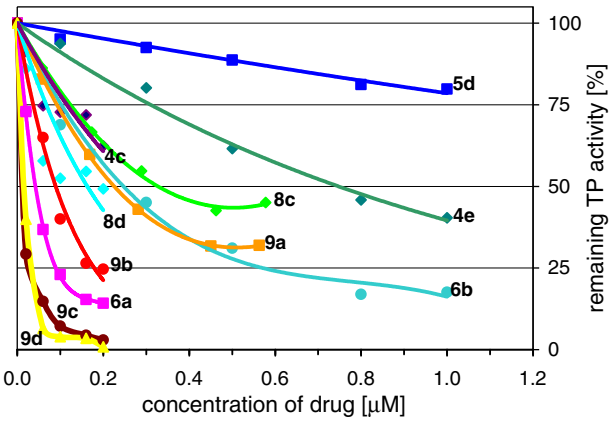
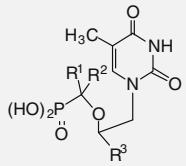
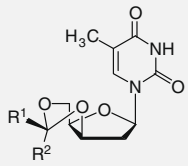
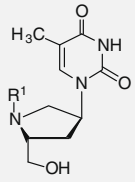
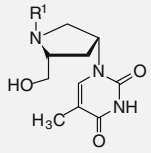
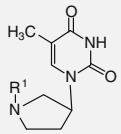
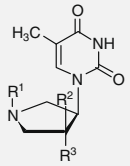


Figure 3. T-lymphoma TP inhibition by selected phosphonates: determination of IC_{50} values (for experimental conditions, see Table 1).

Table 1
Nucleoside phosphonic acids-based inhibitors of T-cell lymphoma thymidine phosphorylase

Inhibitor					Residual activity of TP[a] (%)	IC_{50}[a,b] (nM)
No.	Structure	R^1	R^2	R^3		
4a		$-(CH_2)_5-$		H–	21	–
4b		$-(CH_2)_4-$		H–	14	–
4c		H–	H–	$CH_2{=}CH_2CH_2O-$	10	~300[c]
4d		Ph–	H–	H–	9	–
4e		H–	H–	PhO–	1	750
5a		$(HO)_2(O)P-$	Ph–	–	47	–
5b		$(HO)_2(O)P-$	$BrCH_2-$	–	26	–
5c		$BrCH_2$	$(HO)_2(O)P-$	–	20	–
5d		Ph	$(HO)_2(O)P-$	–	4	–
6a		$(HO)_2(O)PC(O)-$	–	–	2	45
6b		$(HO)_2(O)PCH_2-$	–	–	0	250
7		$(HO)_2(O)PCH_2-$	–	–	17	–
8a		$(HO)_2(O)PCH_2C(O)-$	–	–	59	–
8b		$(HO)_2(O)PCH_2-$	–	–	29	–
8c		$(HO)_2(O)PC(O)-$	–	–	3	350
8d		$(HO)_2(O)PC(S)-$	–	–	9	190
9a		$(HO)_2(O)PC(O)-$	H–	HO–	4	220
9b		$(HO)_2(O)PCH_2-$	HO–	H–	1	45
9c		$(HO)_2(O)PC(O)-$	HO–	H–	0	11
9d		$(HO)_2(O)PC(S)-$	HO–	H–	0	15

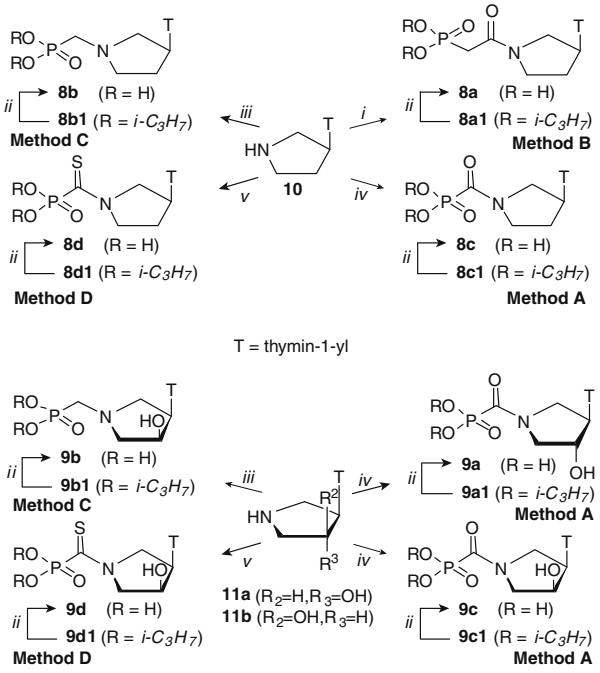
[a] TP assay conditions: 20 mM bis-Tris–HCl, pH 6.7, 1 mM EDTA, 2 mM DTT, 100 μM [^{3}H-methyl]thymidine, 200 μM PO_4^{3-}, 25.5 pU enzyme, 10 μM inhibitor; K_m = 83.2 ± 8.69 μM.
[b] Inhibitor concentration 0–1 μM.
[c] Estimated value (see Fig. 3).

(Table 1, Fig. 3). These potent inhibitors contain prolinol[36] and pyrrolidine nucleoside skeletons.[37,38]

Chemistry: Compounds **4a–e** were prepared according to Ref. 39, **5a–d** according to Ref. 40, and **6a**, **6b** and **7** according to Ref. 37. The synthesis of compounds **8a–d** and **9a–d** is shown in the Scheme 1. Both these groups of compounds were synthesized by a two-step procedure. Diisopropyl esters **8a1–d1** and **9a1–d1** obtained in the first step were treated, in the second step, with bromotrimethylsilane to remove the alkyl groups from the phosphonate.

Thus, condensation of diisopropylphosphonoacetic acid[41] with pyrrolidine nucleoside **10**[38] by EDC in the presence of DMAP, yielded the *N*-diisopropylphosphonoacetyl derivatives **8a1**. Mannich reaction[42] of diisopropyl phosphite and aqueous formaldehyde with pyrrolidine nucleosides **10** and **11b** resulted in the *N*-diisopropylphosphonomethyl derivatives **8b1** and **9b1**, respectively. The diisopropylphosphonocarbonyl derivatives **8c1**, **9a1**, and **9c1** were prepared by the reaction of the respective pyrrolidine nucleosides **10**, **11a**, and **11b** with phenyl diisopropylphosphonoformate.[43] Finally, methyl diisopropylphosphonodithioformate[44] afforded, by the reaction with pyrrolidine nucleosides **10** and **11b**, the *N*-diisopropylphosphonothiocarbonyl derivatives **8d1** and **9d1**. All diisopropyl esters were treated with bromotrimethylsilane to afford free phosphonic acids **8a–d** and **9a–d**. Compounds **6a**, **6b**, **7** and **9a–d** were prepared as single diastereomers whereas compounds **8a–d** were racemates.

Inhibition: Two compounds from the series **4a–e** and **5a–d** exhibited promising inhibition effect in the selection assay (see residual activity, Table 1) but IC_{50} determination revealed that only **4e** was a submicromolar-level inhibitor with IC_{50} = 750 nM (Fig. 3). The profile of TP inhibition by the second compound, the phosphonate **5d**, suggested that its IC_{50} value would probably be within micromolar range. Interestingly, the common feature of **4e** and **5d**, and also of the inactive **4d**, is the presence of phenyl ring close to the phosphoryl moiety which suggests that suitable mutual position of these groups could influence the inhibitory properties of the mentioned compounds.



Scheme 1. Synthesis of pyrrolidine nucleoside phosphonic acids. Reagents and conditions: (i) $(i\text{PrO})_2(\text{O})\text{PCH}_2\text{COOH}$, EDC, DMF; (ii) Me_3SiBr, DMF; (iii) $(i\text{PrO})_2(\text{O})\text{PH}$, aq CH_2O, 60 °C; (iv) $(i\text{PrO})_2(\text{O})\text{PC(O)OPh}$, DMF; (v) $(i\text{PrO})_2(\text{O})\text{PC(S)SMe}$, DMF.

The transition state for thymidine phosphorolysis catalysed by TP likely involves formation of a partial positive charge on both O4′ and C1′ atoms of the 2′-deoxyribosyl moiety.[5] Supposing that the similar mechanism takes place also in case of the rat lymphoma TP, we examined two types of nucleoside phosphonates containing either prolinol (**6a**, **6b**, **7**) or pyrrolidine (**8a–d**, **9a–d**) rings, instead of the deoxyribose, bearing a phosphonate moiety attached to the nitrogen atom of pyrrolidine ring (Table 1). The common feature of compounds **6–9** (except for **8a**) was isostericity, that is, the identical distance between N1 atom of the thymine moiety and phosphorus atom, given by the number of bridging atoms connecting these two positions. This arrangement allowed us to correlate the inhibitory activities of compounds bearing structurally diverse but isosteric phosphonate moieties with their structure, and thus draw certain conclusions concerning the structural requirements of TP for inhibitor(s).

Prolinol- and pyrrolidine-based nucleotides with the *N*-phosphonomethyl moiety **6b**, **7** and **8b**, **9b**, respectively, bearing a positive charge located on the pyrrolidine nitrogen atom, exhibited an interesting inhibitory profile. Both the active compounds **9b** (IC_{50} = 45 nM) and **6b** (IC_{50} = 250 nM) contain, in contrast to the inactive **8b**, a hydroxy group which is very important for further interaction in a TP binding site. On the other hand, the compound **7** (related to **6b**) was found to be inactive, probably due to a different orientation of the thymine nucleobase.

An interesting comparison can be made between the inhibitory activity of compounds **9a–d** and **6a,b**. The replacement of the *N*-phosphonomethyl moiety in **9b** for *N*-phosphonocarbonyl (compound **9c**) and *N*-phosphonothiocarbonyl (compound **9d**) has led to further decrease in IC_{50} values (11 nM and 15 nM for **9c** and **9d**, respectively). Similarly, the *N*-phosphonocarbonyl compound **6a** is also a more potent inhibitor than the *N*-phosphonomethyl derivative **6b**. All three compounds **9b–d** have the hydroxy group *cis*-oriented to the thymine nucleobase, which is of key importance for the inhibitory activity: the compound **9a**, a *trans* congener of **9c**, exhibited a more than one order of magnitude higher IC_{50} value than that of **9c**. In addition, **9c,d** are not positively charged like **9b**, due to the presence of amide or thioamide linkages. Both these linkages can be viewed as hydrogen bond acceptors. Another difference between **9b** and **9c,d** is in the conformation of the phosphonate moieties attached to the pyrrolidine nitrogen, as found by NMR spectroscopy. In contrast to the *N*-phosphonomethyl linkage in **9b** which is, surprisingly, predominantly *trans*-oriented to the thymine nucleobase, the carbon atom of the carbonyl and thiocarbonyl groups lies, together with pyrrolidine nitrogen and both the neighboring carbon atoms, in one plane due to a conjugation of nitrogen free electron pair and the electron-rich carbonyl and thiocarbonyl groups. The importance of the *cis*-orientation of thymine and the vicinal hydroxyl in compounds **9b–d** for the inhibitory activity is obvious when the activities of parent compounds **8b–d** lacking the hydroxyl are compared. For **8c,d**, we found that the IC_{50} values were more than one order of magnitude greater than those for **9c,d**. The replacement of the *N*-phosphonocarbonyl group in **8c** for the *N*-phosphonoacetyl (compound **8a**), which is one methylene group longer, led to the loss of activity. The inhibitory activity was also tested with the positively charged prolinol and pyrrolidine nucleosides **6**, **7** (R^1 = H) and **8**, **9** (R^1 = H) but no inhibitory effect was observed. We can conclude that the presence of a positive charge does not seem to play any crucial role in the inhibition of the SD-lymphoma TP by nucleoside phosphonic acids. Since no X-ray structure is known as yet of a complex of TP with nucleoside phosphonic acid-based inhibitor, we can hardly make more detailed conclusions on the structural requirements of TP for inhibitor. The most active compounds **9c** and **9d** were also tested for inhibition of TP from *E. coli*, and human and rat liver. However, no inhibitory activity was found.

In conclusion, a study on the inhibition of thymidine phosphorylase, purified from SD-lymphoma, by a large number of structurally diverse nucleoside phosphonic acids revealed submicromolar-level inhibitors with IC_{50} values in a concentration range of 11–750 nM (measured at 100 μM thymidine and 200 μM phosphate). The most potent pyrrolidine nucleoside phosphonate-based inhibitors **6a**, **9b**, **9c**, and **9d** exhibited IC_{50} values of 45, 45, 11, and 15 nM, respectively, and they could be bi-substrate inhibitors. A new class of potent SD-lymphoma TP inhibitors based on pyrrolidine nucleoside phosphonic acids was thus discovered. The structures of these most active pyrrolidine compounds **9b–d** differ in the type of phosphonate residue, that is, either *N*-phosphonomethyl, *N*-phosphonocarbonyl, or *N*-phosphonothiocarbonyl moieties. The inhibitor **6a**, on the other hand, is a prolinol nucleotide with *N*-phosphonocarbonyl group. The structure–activity study revealed that both the presence of the phosphonate moiety in the N–C–P arrangement and a *cis* orientation of the vicinal hydroxyl and thymine nucleobase in pyrrolidine nucleoside phosphonic acids are necessary for a strong inhibition of SD-lymphoma TP. Concerning **9c** and **9d** as lead structures, a further improvement of their inhibitory activity might be achieved by replacement of the pyrrolidine ring by either azetidine or piperidine heterocycles. Also, the replacement of the thymine nucleobase by a uracil moiety substituted in the 5- or 6-position by an electronegative substituent, which increases the acidity of N1–H bond[45] and thus the binding to the TP catalytic site (known in the case of human TP), could be beneficial.

We found that the most active compounds **9c** and **9d**, however, did not inhibit the *E. coli*, human, and rat liver TPs. The explanation for the observed inactivity of both compounds toward the TP isolated from the liver of healthy rats may be important for understanding the processes connected with TP functions in tumors. Experiments attempting to resolve whether this difference arises due to spontaneous and unique mutation of the TP gene expressed in tumor tissue are currently being considered.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.12.081.

References and notes

1. Cole, C.; Foster, A. J.; Freeman, S.; Jaffar, M.; Murray, P. E.; Stratford, I. J. *Anti-Cancer Drug Des.* **1999**, *14*, 383.
2. Griffiths, L.; Stratford, I. J. *Br. J. Cancer* **1997**, *76*, 689.
3. Folkman, J. *J. Natl. Cancer Inst.* **1996**, *88*, 1091.
4. Pérez-Pérez, M. J.; Priego, E. M.; Hernández, A. I.; Camarasa, M. J.; Balzarini, J.; Liekens, S. *Mini-Rev. Med. Chem.* **2005**, *5*, 1113.
5. Birck, M. R.; Schramm, V. L. *J. Am. Chem. Soc.* **2004**, *126*, 2447.
6. Votruba, I.; Pomeisl, K.; Tloušťová, E.; Holý, A.; Otová, B. *Biochem. Pharmacol.* **2005**, *69*, 1517.
7. Nencka, R.; Votruba, I.; Hřebabecký, H.; Tloušťová, E.; Horská, K.; Masojídková, M.; Holý, A. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 1335.
8. Nencka, R.; Votruba, I.; Hřebabecký, H.; Jansa, P.; Tloušťová, E.; Horská, K.; Masojídková, M.; Holý, A. *J. Med. Chem.* **2007**, *50*, 6016.
9. Moghaddam, A.; Bicknell, R. *Biochemistry* **1992**, *31*, 12141.
10. Furukawa, T.; Yoshimura, A.; Sumizawa, T.; Haraguchi, M.; Akiyama, S.-I.; Fukui, K.; Ishizawa, M.; Yamada, Y. *Nature* **1992**, *356*, 668.
11. Brown, N. S.; Jones, A.; Fujiyama, C.; Harris, A. L.; Bicknell, R. *Cancer Res.* **2000**, *60*, 6298.
12. Usuki, K.; Saras, J.; Waltenberger, J.; Miyazono, K.; Pierce, G.; Thomason, A.; Heldin, C. H. *Biochem. Biophys. Res. Commun.* **1992**, *184*, 1311.
13. Ishikawa, F.; Miyazono, K.; Hellman, U.; Drexler, H.; Wernstedt, C.; Hagiwara, K.; Usuki, K.; Takaku, F.; Risau, W.; Heldin, C.-H. *Nature* **1989**, *338*, 557.
14. Haraguchi, M.; Miyadera, K.; Uemura, K.; Sumizawa, T.; Furukawa, T.; Yamada, K.; Akiyama, S.-i.; Yamada, Y. *Nature* **1994**, *368*, 198.
15. Moghaddam, A.; Zhang, H. T.; Fan, T. P.; Hu, D. E.; Lees, V. C.; Turley, H.; Fox, S. B.; Gatter, K. C.; Harris, A. L.; Bicknell, R. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 998.
16. Takeuchi, M.; Otsuka, T.; Matsui, N.; Asai, K.; Hirano, T.; Moriyama, A.; Isobe, I.; Eksioglu, Y. Z.; Matsukawa, K.; Kato, T.; Tada, T. *Arthritis Rheum.* **1994**, *37*, 662.
17. Creamer, D.; Jaggar, R.; Allen, M.; Bicknell, R.; Barker, J. *Br. J. Dermatol.* **1997**, *137*, 851.
18. Folkman, J. *New Engl. J. Med.* **1971**, *285*, 1182.
19. Folkman, J. Tumor Angiogenesis, fourth ed.. In *Cancer Medicine*; Holland, J. F., Bast, R. C., Morton, D. L., Frei, E., Kufe, D. W., Weichselbaum, R. R., Eds.; Williams & Wilkens: Baltimore, MD, 1996; Vol. 1, p 181.
20. Folkman, J. *J. Natl. Cancer Inst.* **1990**, *82*, 4.
21. Matsushita, S.; Nitanda, T.; Furukawa, T.; Sumizawa, T.; Tani, A.; Nishimoto, K.; Akiba, S.; Miyadera, K.; Fukushima, M.; Yamada, Y.; Yoshida, H.; Kanzaki, T.; Akiyama, S. *Cancer Res.* **1999**, *59*, 1911.
22. Pocher, F.; Spadari, S. *Curr. Cancer Drug Targets* **2001**, *1*, 141.
23. Matter, A. *Drug Discovery Today* **2001**, *6*, 1005.
24. Cristofanelli, M.; Charnsangavej, C.; Hortobagyi, G. N. *Nat. Rev. Drug Disc.* **2002**, *1*, 415.
25. Davis, D. W.; McConkey, D. J.; Zhang, W.; Herbst, R. S. *Bio Techniques.* **2003**, *34*, 1048.
26. Bronckaers, A.; Gago, F.; Balzarini, J.; Liekens, S. *Med. Res. Rev.* **2009**, *29*, 903.
27. Desgranges, C.; Razaka, G.; Rabaud, M.; Bricaud, H.; Balzarini, J.; De Clercq, E. *Biochem. Pharmacol.* **1983**, *32*, 3583.
28. Fukushima, M.; Suzuki, N.; Emura, T.; Yano, S.; Kazuno, H.; Tada, Y. *Biochem. Pharmacol.* **2000**, *59*, 1227.
29. Liekens, S.; De Clercq, E.; Neyts, J. *Biochem. Pharmacol.* **2001**, *61*, 253.
30. Klein, R. S.; Lenzi, M.; Lim, T. H.; Hotchkiss, K. A.; Wilson, P.; Schwartz, E. L. *Biochem. Pharmacol.* **2001**, *62*, 1257.
31. Murray, P. E.; McNally, V. A.; Lockyer, S. D.; Williams, K. J.; Stratford, I. J.; Jaffar, M. *Bioorg. Med. Chem.* **2002**, *10*, 525.
32. Liekens, S.; Bilsen, F.; De Clercq, E.; Priego, E. M.; Camarasa, M. J.; Pérez-Pérez, M. J. *FEBS Lett.* **2002**, *510*, 83.
33. Esteban-Gamboa, A.; Balzarini, J.; Esnouf, R.; De Clercq, E.; Camarasa, M. J.; Perez-Perez, M. J. *J. Med. Chem.* **2000**, *43*, 971.
34. Li, Y. Studies in Nucleoside Chemistry. Synthesis of Purine and Pyrimidine Nucleoside Phosphorylase Inhibitors. Total Synthesis of the Sulfated Nucleoside Glycoside HF-7, 2000, Ph.D. Dissertation, Cornell University, Ithaca, NY.
35. Allan, A. L.; Gladstone, P. L.; Price, M. L. P.; Hopkins, S. A.; Juarez, J. C.; Doñate, F.; Ternansky, R. J.; Shaw, D. E.; Ganem, B.; Li, Y.; Wang, W.; Ealick, S. *J. Med. Chem.* **2006**, *49*, 7807.
36. Vaněk, V.; Budějínský, M.; Rinnová, M.; Rosenberg, I. *Tetrahedron* **2009**, *65*, 862.
37. Kočalka, P.; Pohl, R.; Rejman, D.; Rosenberg, I. *Tetrahedron* **2006**, *62*, 5763.
38. Rejman, D.; Kočalka, P.; Budějínský, M.; Pohl, R.; Rosenberg, I. *Tetrahedron* **2007**, *63*, 1243.
39. Rosenberg, I.; Králíková, Š. *Collect. Czech. Chem. Commun., Special Issue* **1996**, *61*, S81.
40. Endová, M.; Masojídková, M.; Budějínský, M.; Rosenberg, I. *Tetrahedron* **1998**, *54*, 11187.
41. Magerlein, B. J.; Kagan, F. *J. Am. Chem. Soc.* **1960**, *82*, 593.
42. Rejman, D.; Pohl, R.; Kočalka, P.; Masojídková, M.; Rosenberg, I. *Tetrahedron* **2009**, *65*, 3673.
43. Noren, J. O.; Helgstrand, E.; Johansson, N. G.; Misiorny, A.; Stening, G. *J. Med. Chem.* **1983**, *26*, 264.
44. Grisley, D. W. *J. Org. Chem.* **1961**, *26*, 2544.
45. Reigan, P.; Edwards, P. N.; Gbaj, A.; Cole, C.; Barry, S. T.; Page, K. M.; Ashton, S. E.; Luke, R. W. A.; Douglas, K. T.; Stratford, I. J.; Jaffar, M.; Bryce, R. A.; Freeman, S. *J. Med. Chem.* **2005**, *48*, 392.