Bioorganic & Medicinal Chemistry Letters 22 (2012) 181–185

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Activation of human RNase L by 2′- and 5′-*O*-methylphosphonate-modified oligoadenylates

Ondřej Páv, Natalya Panova, Jan Snášel, Eva Zborníková, Ivan Rosenberg *

Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Flemingovo n. 2, 166 10 Prague 6, Czech Republic

ARTICLE INFO

Article history:
Received 22 September 2011
Revised 8 November 2011
Accepted 10 November 2011
Available online 23 November 2011

Keywords:
Human recombinant RNase L
Phosphonate
Oligoadenylate
FRET

ABSTRACT

To determine the influence of internucleotide linkage and sugar ring conformation, and the role of 5′-terminal phosphate, on the activation of human RNase L, a series of 2′- and 5′-*O*-methylphosphonate-modified tetramers were synthesized from appropriate monomeric units and evaluated for their ability to activate human RNase L. Tetramers pAAAp_cX modified by *ribo*, *arabino* or *xylo* 5′-phosphonate unit p_cX activated RNase L with efficiency comparable to that of natural activator. Moreover, incorporation of phosphonate linkages ensured the stability against cleavage by nucleases. The substitution of 5′-terminal phosphate for 5′-terminal phosphonate in tetramer p_cXAAA afforded tetramers with excellent activation efficiency and with complete stability against cleavage by phosphomonoesterases.

The short 2′,5′-oligoadenylates (2-5A) play a significant role in the interferon-induced antiviral defence mechanism of cells.[1,2] In the presence of double-stranded RNA, interferon induces expression of the 2′,5′-oligoadenylate synthetase utilizing ATP as a substrate for the synthesis of 5′-phosphorylated 2-5As, (pp)p5′Ap(Ap)$_n$A2′ (where *n* is mainly 1 or 2).[3] These oligonucleotides bind to a latent endoribonuclease RNase L and subsequently activate it. The activated enzyme is capable of cleaving single-stranded RNA and thus of preventing expression of viral proteins. Activity of RNase L is regulated by a specific 2′,5′-exonuclease (2′,5′-phosphodiesterase) that cleaves 2-5A to AMP and ATP.[4] It has been shown that 2-5A also plays an important role in the regulation of cell growth and differentiation, antiproliferative functions of interferons, and in apoptosis.[2,5]

Successful use of RNase L pathway in targeting several viral strains (HIV[6]; RSV[7]; EMCV[8]; VSV, VV, HPV[9]; Mengovirus[10]) has been reported, making RNase L agonists candidates for antiviral therapy. Moreover, a role of RNase L in suppression of prostate cancer and leukemia has been reported.[4,11] Abnormalities in the RNase L pathway, its hyperactivity, and increased levels in blood samples have been reported in patients with chronic fatigue syndrome (CFS). This also makes the RNase L antagonists of potential interest.[12]

Any successful biological application of oligoadenylates would depend on the stability of these compounds in cells, particularly on the resistance of the 2',5' internucleotide linkages against a specific nuclease cleavage. Protection of the 5′-phosphate group, a key structural feature of 2–5A's, against cleavage by phosphomonoes terases is also a significant issue. In connection with these requirements, modified oligonucleotides bearing phosphonate intern-ucleotide linkages have been shown to offer remarkable protection against nucleases.[13] These compounds containing structurally diverse types of phosphonate linkages have attracted our attention for a long time.[13]

In our previous work,[14] we presented a study on the influence of the 2′-*O*-methylphosphonate internucleotide linkage in 2-5A analoges on the process of binding to, and activating of, murine RNase L. We synthesized a library of 2-5A tetramers in which one or more adenylate residues were replaced by either *ribo* $\mathbf{X_a}$, *arabino* $\mathbf{X_b}$, or *xylo* $\mathbf{X_c}$ adenosine-2′-phosphonate unit Xp_c (Fig. 1). We found that tetramers pAAA modified by *ribo* $\mathbf{X_a}$ and *xylo* $\mathbf{X_c}$ 2′-phosphonate units activated RNase L with efficiency comparable to that of natural pA_4. Surprisingly, the tetramer pAAXp_cA modified by *arabino* $\mathbf{X_b}$ 2′-phosphonate unit was unable to activate RNase L, and seems to be a potent inhibitor of the enzyme. Moreover, the phosphonate 2-5A's exhibited increased stability against cleavage by nucleases compared to the natural 2-5A.

Here we report an extended study on the synthesis and investigation of biological properties of 2′- and 5′-*O*-methylphosphonate-modified oligoadenylates as potential agonists and antagonists of human RNase L (Figs. 1 and 2). We focused on the influence of internucleotide linkage on the activation of human RNase L, and the role of 5′-phosphate and sugar ring conformation in these processes.

First we prepared the appropriate monomers. Synthesis of 2′-phosphonate monomers was already published in Pav et al.[14]

* Corresponding author. Tel.: +420 220 183 381; fax: +420 220 183 531.
E-mail address: ivan@uochb.cas.cz (I. Rosenberg).

0960-894X/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2011.11.040

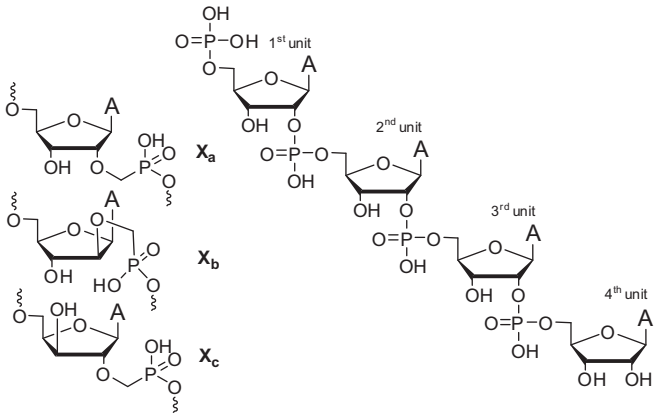


Figure 1. Oligoadenylate tetramers modified by *ribo* $\mathbf{X_a}$, *arabino* $\mathbf{X_b}$, and *xylo* $\mathbf{X_c}$ adenosine-2'-phosphonate units.

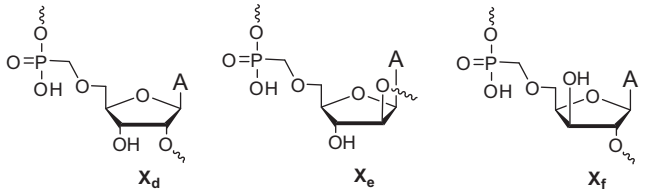


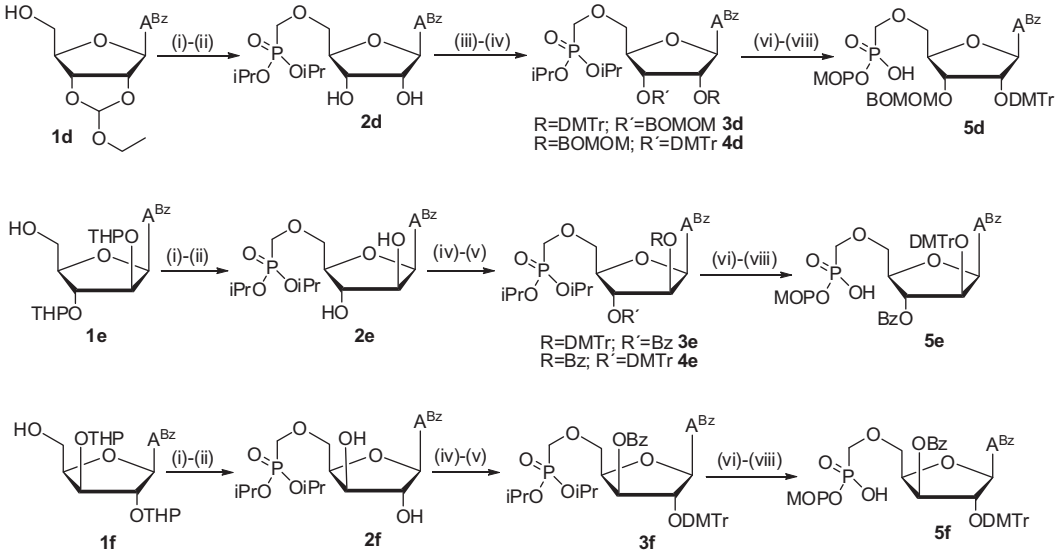
Figure 2. *Ribo* $\mathbf{X_d}$, *arabino* $\mathbf{X_e}$, and *xylo* $\mathbf{X_f}$ adenosine-5'-phosphonate units.

5′-*O*-Methylphosphonate monomers were prepared according to Scheme 1. Suitably protected nucleosides **1** were phosphonylated with diisopropyl tosyloxymethylphosphonate in the presence of sodium hydride to afford deprotected 5′-phosphonates **2** upon acidic cleavage of ethoxymethylidene (**1d**) and tetrahydropyranyl (**1e**, **1f**) groups. A mixture of fully protected nucleotides **3d** and **4d** was prepared by the reaction of *ribo* 5′-phosphonate **2d** and benzoyloxymethoxymethyl chloride (BOMOM–Cl) in the presence of dibutyltin dichloride, followed by dimethoxytritylation with dimethoxytrityl chloride in the presence of silver triflate. The reason for the introduction of benzoyloxymethoxymethyl group (BOMOM) emerges from the synthesis of 5′-phosphonate-modified oligoadenylates in the 5′→2′ direction. The BOMOM group was recently introduced for the synthesis of RNA in reverse direction by our group.[15] Similarly, *arabino* nucleoside-5′-phosphonate (**2e**) was first dimethoxytritylated with dimethoxytritylchloride in the presence of silver triflate and then protected with benzoyl group to afford a mixture of regiomers **3e** and **4e**. Dimethoxytritylation of *xylo* nucleoside-5′-phosphonate **2f** followed by reaction with benzoyl cyanide afforded 2′-dimethoxytrityl derivative **3f** as the only product. Due to the *trans*-2′,3′-diol configuration in *arabino* and *xylo* monomers, there is no need to use any special protecting group such as BOMOM. Thus, the benzoyl group which, in this particular case, is fully compatible with the solid phase synthesis and deprotection cycle, was used.

Since the mixtures of 2′,3′-regioisomers **3d**, **4d** and **3e**, **4e** were not separable by silica gel chromatography, the separation was accomplished by preparative HPLC to afford the desired phosphonates **3d** and **3e**. The fully protected phosphonates **3** were treated with bromotrimethylsilane and subsequently esterified with 4-methoxy-1-oxido-2-pyridylmethanol (MOP-OH) in the presence of 2-chloro-5,5-dimethyl-1,3,2-dioxaphosphorinane-2-oxide (CDDO) and methoxypyridine-*N*-oxide (MPNO) to afford the appropriate phosphonomethyl monomers **5**.

Once all monomeric building blocks were prepared, various oligoadenylate tetramers were synthesised in both the 2′→5′ and the 5′→2′ directions (Table 1). Solid-phase synthesis was performed on a 1 μmol scale in the 2′→5′ direction (2′-phosphonate series) using [2(3)-*O*-benzoyl-5-*O*-dimethoxytrityl-1-(6-*N*-benzoyladenin-9-yl)-β-D-ribofuranos-3(2)-*O*-succinoyl]LCAA-CPG and in the 5′→2′ direction (5′-phosphonate series) using [2-[2-(4,4'-dimethoxytrityloxy)ethylsulfonyl]ethyl-succinoyl]LCAA-CPG as solid supports. In case of the synthesis in 2′→5′ direction, the 5′-terminal phosphate was introduced by 2-[2-(4,4′-dimethoxytrityloxy)-ethylsulfonyl]ethyl-(2-cyanoethyl)-(*N*,*N*-diisopropyl)-phosphoramidite. Synthesis, deprotection and purification of the modified tetramers were performed using the protocols published earlier in Pav et al.[15]

As was mentioned above, one of the aims of the presented study was to define the role of stereochemical factors influencing the process of activation of human RNase L, especially those associated with internucleotide linkage. Thus, we introduced the *ribo*, *arabino*, and *xylo*-configured 2′- and 5′-phosphonate units $\mathbf{X_{a-c}}$ and $\mathbf{X_{d-f}}$, respectively, (Figs. 1 and 2) into individual positions of oligoadeny-



Scheme 1. Synthesis of 5′-*O*-methylphosphonate derivatives of adenine nucleosides. Reagents and conditions: (i) $TsOCH_2PO(OiPr)_2$, NaH, DMF, rt; (ii) 80% AcOH, rt; (iii) BOMOM–Cl, Bu_2SnCl_2, DIPEA, DCE, 80 °C; (iv) DMTr–Cl, CF_3SO_3Ag, py, rt; (v) BzCN, TEA, ACN, rt; (vi) Me_3SiBr, 2,6-lutidine, ACN, rt; (vii) 4-methoxy-1-oxido-2-pyridylmethanol, CDDO, MPNO, py, rt; (viii) PhSH, Et_3N, DMF, rt.

Table 1
Evaluated 2′- and 5′-Phosphonate-modified oligoadenylates

Natural activator							
6	pAAAA						
2'-Phosphonate-modification (Xp$_c$) *ribo* **X$_a$**, *arabino* **X$_b$**, and *xylo* **X$_c$**							
7	pXp$_c$AAA	**10**	pXp$_c$Xp$_c$AA	**13**	pXp$_c$Xp$_c$Xp$_c$A		
8	pAXp$_c$AA	**11**	pXp$_c$AXp$_c$A				
9	pAAXp$_c$A	**12**	pAXp$_c$Xp$_c$A				
5'-Phosphonate-modification (p$_c$X) *ribo* **X$_d$**, *arabino* **X$_e$**, and *xylo* **X$_f$**							
14	p$_c$XAAA	**18**	p$_c$Xp$_c$XAA	**24**	p$_c$Xp$_c$Xp$_c$XA	**28**	p$_c$Xp$_c$Xp$_c$Xp$_c$X
15	pAp$_c$XAA	**19**	p$_c$XAp$_c$XA	**25**	p$_c$Xp$_c$XAp$_c$X		
16	pAAp$_c$XA	**20**	p$_c$XAAp$_c$X	**26**	p$_c$XAp$_c$Xp$_c$X		
17	pAAAp$_c$X	**21**	pAp$_c$Xp$_c$XA	**27**	pAp$_c$Xp$_c$Xp$_c$X		
		22	pAp$_c$XAp$_c$X				
		23	pAAp$_c$Xp$_c$X				

lates (Table 1). The introduction of these units into oligoadenylates variously changed the length of the internucleotide linkage, the conformation of the sugar–phosphate backbone, the conformation of the sugar part of the respective phosphonate unit, and distribution of a negative charge along the oligoadenylate strand in the place of modified internucleotide linkage.

The ability of modified oligoadenylates to activate human RNase L was monitored by the cleavage of FRET substrate [CY5]-r(C_{11}-UU-C_7)-[BHQ2] using fluorescence reader. For more details see Supplementary data. The natural activators pA_4 (**6**) and pA_3 exhibited identical EC_{50} values of 17 nM. The EC_{50} values of two sets of phosphonate-modified tetramers summarized in Table 2 were calculated from regression curves (see Supplementary data). The remaining tetramers containing two, or more phosphonate units exhibited EC_{50} values above 10 μM.

In general, there was a strong relationship between the position of modified linkage and the ability of modified 2-5A to activate the enzyme. Comparing the influence of sugar part configuration, the *ribo* modification was readily tolerated whereas the *arabino* and *xylo* modifications afforded tetramers with lower activation ability. In contrast to murine RNase L where 2′-phosphonate linkage was very well tolerated,[14] the 5′-phosphonate linkage was better tolerated by human RNase L than the 2′-phosphonate one. The introduction of more than one phosphonate unit resulted in a significant decrease in activation ability.

Unlike murine RNase L which requires pA_4 tetramer for the activation, pA_3 trimer is able to activate human RNase L.[2] Therefore, the fourth unit is known for not being crucial for the activation process. Nonetheless, modification of this unit may still afford a tetramer with improved properties such as enhanced binding to the active site or higher stability against cleavage by nucleases. The incorporation of 5′-phosphonate linkage between the third and the fourth unit afforded tetramers (**17d**, **17e**, **17f**) with activation efficiency comparable or better to that of natural activator. The 2′-phosphonate tetramers **9a** and **9b** modified at the same site retained a moderate activation ability. Importantly, both phospho-

Table 2
EC_{50} values (nM) of the selected activators of human RNase L

		EC_{50} [nM][a]		
Natural activator				
6	pAAAA	**17**	-	-
2'-Phosphonate-modification (Xp$_c$)		*ribo* **X$_a$**	*arabino* **X$_b$**	*xylo* **X$_c$**
7	pXp$_c$AAA	>1000	>1000	300
8	pAXp$_c$AA [b]	>1000	>1000	>1000
9	pAAXp$_c$A [c]	200	1000	>1000
5'-Phosphonate-modification (p$_c$X)		*ribo* **X$_d$**	*arabino* **X$_e$**	*xylo* **X$_f$**
14	p$_c$XAAA	53	1000	300
15	pAp$_c$XAA	>1000	>1000	>1000
16	pAAp$_c$XA [b]	300	>1000	1000
17	pAAAp$_c$X [c]	15	6.5	4.2

[a] Average value from three independent measurements ±(5–10)%.
[b] Exonuclease cleaves the tetramers **8** and **16** to trimers pAXp$_c$A and pAAp$_c$X, respectively.
[c] Completely stable against exonucleases.

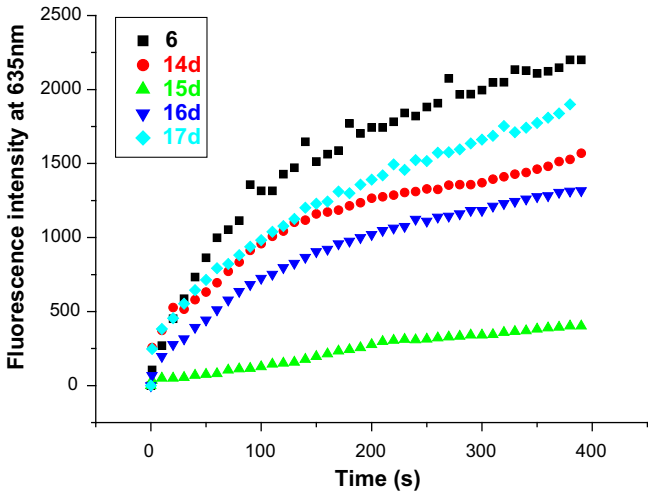


Figure 3. Cleavage of FRET substrate in the presence of natural activator **6** and tetramers modified by *ribo* 5′-phosphonate unit $\mathbf{X_d}$ (**14d**, **15d**, **16d**, **17d**).

nate linkages at this position ensure the stability against cleavage by exonucleases.[14,15] Interestingly, the position of the methylene group in the phosphonate 2',5' internucleotide linkages of regioisomeric pairs of *ribo* tetramers (**7a** and **15d**, **8a** and **16d**, and **9a** and **17d**) substantially influences their EC_{50} values as obvious from the Table 2.

Tetramers pAXp$_c$AA **8** and pAp$_c$XAA **15** bearing *ribo*, *arabino*, and *xylo* modifications at the second position exhibited a considerably reduced activation ability. The second unit is essential for the binding of a tetramer to RNase L[2,16] and it seems that any modification of the internucleotide linkage at this position has a detrimental effect. This finding is in agreement with the data we obtained in our study dealing with murine RNase L.[14]

Interestingly, the tetramers p$_c$XAAA **14** bearing 5′-terminal phosphonate instead of 5′-terminal phosphate retained the activation ability. Especially the *ribo* tetramer **14d** was a very potent activator of human RNase L with EC_{50} value of 53 nM. The 5′-terminal phosphate is a key structural feature of 2-5As and its cleavage by phosphomonoesterases leads to an immediate loss of the activation ability of the tetramer. Thus, the presence of 5′-terminal phosphonate affords an activator with complete stability against 5'-dephosphorylation by phosphomonoesterases.[17] However, this tetramer **14** is not resistant against exonucleases because of the presence of natural units at second, third, and fourth positions. The incorporation of the *ribo* 5'-phosphonate unit ($\mathbf{X_d}$) at the fourth position of tetramer instead of the natural unit provided both phosphomonoesterase and exonuclease stable *ribo* tetramer p$_c$XAAp$_c$X **20** but with the EC_{50} value of 2 μM.

Except for tetramers **17d**, **17e**, and **17f** which were potent activators of RNase L, there was only a moderate activation ability of *arabino*- and *xylo*-configured phosphonates (**7c**, **9b**, **14e**, **14f**, **16f**).

The tetramers modified by *ribo* 5′-phosphonate unit $\mathbf{X_d}$ (**14d**, **15d**, **16d**, **17d**) represent an example of considerable differences encountered in the activation efficiency depending on the position of the modification (Fig. 3, Table 2). Tetramer **17d** modified at the fourth position exhibited the activity comparable to that of natural activator. Modification at the third position (**16d**) led to a moderate loss in the activation ability, whereas modification of the second unit (**15d**) resulted in a complete loss of the activation ability. Incorporation of 5′-phosphonate unit at the first position (**14d**) afforded a very potent activator of the enzyme.

The antagonist activity of modified tetramers was examined by the cleavage reaction in the presence of natural activator and phosphonate tetramer at a 1:1 ratio. None of the prepared oligoadenylates exhibited antagonist activity under these conditions.

In conclusion, we prepared two sets of oligoadenylates modified by regioisomeric 2′- and 5′-phosphonate units. We found that tetramers pAAAp$_c$X modified by *ribo* (**17d**), *arabino* (**17e**), and *xylo* (**17f**) 5′-phosphonate units activated RNase L with efficiency comparable to that of natural activator. Moreover, the modification at this position provided tetramers possesing stability of the 2'-*O*-*P*-*CH$_2$*-*O*-5' internucleotide linkage against cleavage by exonucleases forming the 5'-nucleotides. Our earlier study on the modified oligonucleotides containing the regioisomeric 3'-*O*-*P*-*CH$_2$*-*O*-5' linkage showed their complete stability against nucleases of L1210 cell free extract and phosphodiesterase I.[15,17,18] The substitution of 5′-terminal phosphate for 5′-terminal phosphonate in tetramer p$_c$XAAA **14d** afforded tetramer with excellent activation efficiency and with complete stability against cleavage by phosphomonoesterases but not against exonucleases, however, there is no problem to modify the 2'(3')-end of p$_c$XAAA **14d** to prevent the exonuclease cleavage. The improved stability observed with compounds **14d** and **17d–f** is anticipated to facilitate in vivo evaluation of these oligoadenylates.

Acknowledgements

The support by the grant 202/09/0193 (Czech Science Foundation) and Research centers KAN200520801 (Acad. Sci. CR) and LC060061 (Ministry of Education, CR) under the Institute research project Z40550506 is gratefully acknowledged.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.11.040.

References and notes

1. Williams, B. R. G.; Kerr, I. M. *Nature* **1978**, *276*, 88.
2. Player, M. R.; Torrence, P. F. *Pharmacol. Ther.* **1998**, *78*, 55.
3. Torrence, P. F.; Imai, J.; Lesiak, K.; Jamoulle, J. C.; Sawai, H. *J. Med. Chem.* **1984**, *27*, 726.
4. Adah, S. A.; Bayly, S. F.; Cramer, H.; Silverman, R. H.; Torrence, P. F. *Curr. Med. Chem.* **2001**, *8*, 1189.
5. Castelli, J. C.; Hassel, B. A.; Maran, A.; Paranjape, J.; Hewitt, J. A.; Li, X.; Hsu, Y.; Silverman, R. H.; Youle, R. J. *Cell Death Differ.* **1998**, *5*, 313.
6. Sobol, R. W.; Henderson, E. E.; Kon, N.; Shao, J.; Hitzges, P.; Mordechai, E.; Reichenbach, N. L.; Charubala, R.; Schirmeister, H.; Pfleiderer, W.; Suhadolnik, R. J. *J. Biol. Chem.* **1995**, *270*, 5963; Műller, W. E. G.; Weiler, B. E.; Charubala, R.; Pfleiderer, W.; Leserman, L.; Sobol, R. W.; Suhadolnik, R. J.; Schröder, H. C. *Biochemistry* **1991**, *30*, 2027; Torrence, P. F.; Maitra, R. K.; Lesiak, K.; Khamnei, S.; Zhou, A.; Silverman, R. H. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 1300; Player, M. R.; Maitra, R. K.; Silverman, R. H.; Torrence, P. F. *Antivir. Chem. Chemother.* **1998**, *9*, 213.
7. Wyde, P. R. *Antivir. Res.* **1998**, *39*, 63; Barnard, D. L.; Sidwell, R. W.; Xiao, W.; Player, M. R.; Adah, S. A.; Torrence, P. F. *Antivir. Res.* **1999**, *41*, 119; Verheijen, J. C.; Chen, L.; Bayly, S. F.; Torrence, P. F.; van der Marel, G. A.; van Boom, J. H. *Nucleosides Nucleotides Nucleic Acids* **2000**, *19*, 1821; Leaman, D. W.; Longano, F. J.; Okicki, J. R.; Soike, K. F.; Torrence, P. F.; Silverman, R. H.; Cramer, H. *Virology* **2002**, *292*, 70; Xu, Z.; Kuang, M.; Okicki, J. R.; Cramer, H.; Chaudhary, N. *Antivir. Res.* **2004**, *61*, 195.
8. Chebath, J.; Benech, P.; Revel, M.; Vigneron, M. *Nature* **1987**, *330*, 587.
9. Zhou, A.; Paranjape, J.; Brown, T. L.; Nie, H.; Nai, Z. S.; Dong, B.; Chang, A.; Trapp, B.; Fairchild, R.; Colmenares, C.; Silverman, R. H. *EMBO J.* **1997**, *16*, 6355; Maitra, R. K.; Silverman, R. H. *J. Virol.* **1998**, *72*, 1146.
10. Williams, B. R.; Golgher, R. R.; Brown, R. E.; Gilbert, C. S.; Kerr, I. M. *Nature* **1979**, *282*, 582; Liang, S.; Quirk, D.; Zhou, A. *IUBMB Life* **2006**, *58*, 508.
11. Silverman, R. H. *Cytokine Growth Factor Rev.* **2007**, *18*, 381.
12. Suhadolnik, R. J.; Reichenbach, N. L.; Hitzges, P.; Sobol, R. W.; Peterson, D. L.; Henry, B. *Clin. Infect. Dis.* **1994**, *18*, S96–104; Lombardi, V. C.; Ruscetti, F. W.; Das Gupta, J.; Pfost, M. A.; Hagen, K. S.; Peterson, D. L. *Science* **2009**, *326*, 585; Van Kuppeveld, F. J. M.; de Jong, A. S.; Lanke, K. H.; Verhaegh, G. W.; Melchers, W. J. G.; Swanink, C. M. A.; Bleijenberg, G.; Netea, M. G.; Galama, J. M. D.; van der Meer, J. W. M. BMJ 2010; 340: c1018. doi: 10.1136/bmj.c1018.
13. Endová, M.; Masojídková, M.; Buděšínský, M.; Rosenberg, I. *Tetrahedron* **1998**, *54*, 11151; Endová, M.; Masojídková, M.; Buděšínský, M.; Rosenberg, I. *Tetrahedron* **1998**, *54*, 11187; Rosenberg, I. In *Frontiers in Nucleosides and*

In Schinazi, R. F., Liotta, D. C., Eds.; IHL Press: Tucker, GA, 2004; pp 519–548; Rejman, D.; Masojídková, M.; De Clercq, E.; Rosenberg, I. *Nucleosides Nucleotides Nucleic Acids* **2001**, *20*, 1497; Králíková, Š.; Masojídková, M.; Buděšínský, M.; Rosenberg, I. *Nucleosides Nucleotides Nucleic Acids* **2003**, *22*, 329–347; Páv, O.; Barvík, I.; Buděšínský, M.; Masojídková, M.; Rosenberg, I. *Org. Lett.* **2007**, *9*, 5469.

14. Páv, O.; Protivínská, E.; Pressová, M.; Collinsová, M.; Jiráček, J.; Snášel, J.; Masojídková, M.; Buděšínský, M.; Rosenberg, I. *J. Med. Chem.* **2006**, *49*, 3955.
15. Páv, O.; Košiová, I.; Barvík, I.; Pohl, R.; Buděšínský, M.; Rosenberg, I. *Org. Biomol. Chem.* **2011**, *9*, 6120.
16. Tanaka, N.; Nakanishi, M.; Kusakabe, Y.; Goto, Y.; Kitade, Y.; Nakamura, K. T. *EMBO J.* **2004**, *23*, 3929.
17. Holý, A.; Rosenberg, I. *Collect. Czech. Chem. Commun.* **1982**, *47*, 3447.
18. Rejman, D.; Snášel, J.; Liboska, R.; Točík, Z.; Pačes, O.; Králíková, Š.; Rinnová, M.; Koiš, P.; Rosenberg, I. *Nucleosides Nucleotides Nucleic Acids* **2001**, *20*, 819.