Recl. Trav. Chim. Pays-Bas **114**, 43–50 (1995)
SSDI 0165-0513(94)00084-2

0165–0513/95/02043-08$09.50

Full Papers

Role of the stereochemistry of 3′-fluoro-3′-deoxy analogues of 2–5A in binding to and activation of mouse RNase L

Elena N. Kalinichenko [#], Tatjana L. Podkopaeva [#], Nicolai E. Poopeiko [#], Merike Kelve [§], Mart Saarma [§], Igor A. Mikhailopulo [§,*], Jacquelien E. van den Boogaart [†] and Cornelis Altona [†]

[#] *Institute of Bioorganic Chemistry, Byelorussian Academy of Sciences, 220141 Minsk, Byelorussia (C.I.S.),*
[§] *Institute of Chemical Physics and Biophysics, Estonian Academy of Sciences, 200026 Tallinn, Estonia,*
[†] *Leiden Institute of Chemistry, Gorlaeus Laboratories, Leiden University, P.O. Box 9502, 2300 RA Leiden, The Netherlands*
(Received August 3, 1994)

Abstract. The synthesis of two sets of analogues of 2–5A trimer containing 9-(3-fluoro-3-deoxy-β-D-xylo-furanosyl)adenine (A^F) or 3′-fluoro-3′-deoxyadenosine (A_F) at different positions of the chain is described, along with the preparation of the corresponding 5′-monophosphates and 5′-diphosphorylated (core) trimers. The ability of each *ribo* and *xylo* isomeric pair of fluorodeoxy analogues of 2–5A (*i*) to compete with $p_3(A2'p)_3A3'[^{32}P]pC3'p$ for binding to RNase L in L_{929} cell extracts, and (*ii*) to activate the partially purified RNase L from L_{929} cell extracts to hydrolyze poly(U)[^{3}H], was compared to that of the related 3′-deoxy analogue [*Torrence* et al., J. Biol. Chem. **263**, 1131 (1988)] and the parent trimer, p_3A_3, using radiobinding and RNase L-(2′,5′)pentaadenylate(core)-agarose assays, respectively. Evidence is presented to show that the stereochemistry of the trimers plays an important role, specifically in the second process. The most striking observation is that, compared to 2–5A, $p_3A(A^F)A$ was found to be nine times more effective an activator of RNase L, whereas isomeric $p_3A(A_F)A$ is 30 times less effective.

Introduction [a]

The important role of 5′-triphosphorylated (2′,5′)oligoadenylates [mainly pppA2′-5′A2′-5′A; p_3A_3; (2–5A)] in the antiviral action of interferon is now well recognized (for a review, see Ref. 1). These oligomers bind to and subsequently activate 2–5A-dependent endoribonuclease (RNase L) resulting in the cleavage of virus mRNA and, hence, in inhibition of protein synthesis. The synthesis of a broad spectrum of 2–5A analogues allowed us to define some structural and conformational factors that are important for both processes. One of the aims of these investigations is the search for new antiviral and/or antitumor agents which could be eventually used instead of interferon in some chemotherapeutic applications. Thus, it was demonstrated that phosphorothioate and 3′-deoxyadenosine (cordycepin) analogues of 2–5A inhibit human immunodeficiency virus (HIV) infection via inhibition of reverse transcriptase[2–4]. It was also reported that (2′,5′)oligoadenylate core (5′-dephosphorylated) trimers modified at the 2′(3′)-terminus effectively prevent the rejection of transplanted kidneys in rabbits and monkeys, ensure the normal functioning of the transplanted organ, and slow the growth of the T-helper and T-killer cells in experimental animals during the post-operative period[5].

A series of reports demonstrated that diverse functionalities of each individual nucleoside fragment of 2–5A contribute highly specifically in binding to and activation of RNase L. For instance, using sequence-specific 2–5A analogues, in which the 3′-hydroxyl group of each adenosine residue was sequentially replaced by hydrogen, it was demonstrated that (*i*) the replacement of the 5′-terminal adenosine by cordycepin results in a three-fold decrease in activation ability; (*ii*) by contrast, the same modification of the central adenosine fragment causes decreases in binding (nine-fold *vs.* the parent 2–5A) and activation (500–1000-fold); (*iii*) a *ca.* ten-fold increase in both binding and activation ability occurs upon substitution of a cordycepin residue at the 2′(3′)-terminus[6].

[a] **Abbreviations**: 2–5A = 5′-triphosphoadenylyl(2′-5′)adenylyl(2′-5′)adenosine, p_3A_3; pA_3 = phosphoadenylyl(2′-5′)adenylyl(2′-5′)adenosine; A_3 = 5′-dephosphorylated p_3A_3, 2–5A core; A^F = 9-(3-fluoro-3-deoxy-β-D-xylofuranosyl)adenine; A_F = 3′-fluoro-3′-deoxyadenosine; RNase L = 2–5A-dependent endo-ribonuclease; BAP = bacterial alkaline phosphatase; DBU = 1,8-diazabicyclo[5.4.0]undec-7-ene; DMAP = 4-(dimethylamino)pyridine; DMSO = dimethyl sulfoxide; HEPES = 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; HPLC = high-performance liquid chromatography; NPE = 2-(4-nitrophenyl)ethyl; MTr = monomethoxytrityl = (4-methoxyphenyl)diphenylmethyl; poly(U) = polyuridylic acid; TLC = thin-layer chromatography; TEAB = triethylammonium hydrogen carbonate.

Table I ^1H *NMR data of the fluorodeoxy derivatives of adenosine.* [a]

No.	H2 H8	H1′	H2′	H3′	H4′	H5′ H5″	Others
8	8.54 s 8.21 s	5.98 d	4.72 m	5.06 m	4.26 m	3.12 m	7.94–7.22 m (5H, arom.) 6.22 d (1H, 2′-OH) 4.96 t (1H, 5′-OH)
10	8.62 s 8.07 s	6.27 s	4.75 br.d	5.11 dd	4.66 ddt	3.60 dd 3.48 br.dd	9.27 br.s (1H, NH) 7.96–6.8 m (19H, arom.) 3.76 s (3H, OCH_3)
12	8.63 s 8.20 s	6.53 br.s	5.80 br.d	5.29 dd	4.52 ddt	3.66 dd 3.56 dd	8.09–6.82 m (24H, arom.) 3.77 s (3H, OCH_3)
14	8.63 s 8.36 s	6.46 d	5.92 dd	5.35 dd	4.52 m	4.02 d	8.05–7.33 m (15H, arom.)
18	8.74 s 8.28 s	6.17 d	5.17 m	5.38 dd	4.54 m	3.96 br.d	11.26 br.s (1H, NHBz) 8.12–6.85 m (23H, arom.) ≈ 4.45 m (2H, P-O-CH_2-CH_2) 3.74 s (3H, OCH_3) 2.92 m (2H, P-O-CH_2-CH_2)
9	8.80 s 8.76 s	6.14 d	5.04 m	5.18 dd	4.35 dt	3.70 m	11.26 s (1H, NHBz) 8.08–7.53 m (5H, arom.) 6.06 d (1H, 2′-OH) 5.39 t (1H, 5′-OH)
11	8.72 s 8.22 s	6.12 d	5.15 ddd	5.17 dd	4.50 dt	3.50 dd 3.36 dd	9.12 s (1H, NHBz) 8.05–6.75 m (19H, arom.) 3.75 s (3H, OCH_3)
13	8.52 s 8.26 s	6.55 d	6.29 ddd	5.59 dd	4.58 dt	3.59 dd 3.47 dd	8.06–6.8 m (24H, arom.) 3.79 s (3H, OCH_3)
15	8.66 s 8.20 s	6.34 d	6.08 ddd	5.58 dd	4.64 br.d	4.02 br.d 3.86 br.d	8.02–7.27 m (15H, arom.)
19	8.62 s 8.58 s	6.19 d	5.64 m	5.36 dd	4.42 m	[b]	11.22 br.s (1H, NHBz) 8.09–6.84 m (23H, arom.) ≈ 4.40 m (2H, P-O-CH_2-CH_2) 3.73 s (3H, O-CH_3) 2.65 m (2H, P-O-CH_2-CH_2)

[a] Chemical shifts (δ_{TMS}, ppm). The spectra of compounds **8**, **18**, **9** and **19** were measured in DMSO-d_6, compounds **10**, **12**, **14**, **11** and **13** in $CDCl_3$ and **15** in a mixture of $CDCl_3$ and CD_3OD. [b] Resonance signals are overlapped by an intense signal of HOD.

Recently, we reported on the detailed conformational analysis of 2–5A trimer cores containing 9-(3-fluoro-3-deoxy-β-D-xylofuranosyl)adenine (A^F) or 3′-fluoro-3′-deoxyadenosine (A_F) at different positions of the chain[7]. It was found that the fluoro substituent governs the conformation of the sugar ring as follows: the A^F residues display mainly N-type and the A_F residues exhibit almost pure S-type conformation compared to the adenosine fragments in the trimers which appear rather flexible, as is also the case with the parent trimer, A_3. Moreover, for all fluoro-substituted ribonucleosides an *anti* base orientation is found as opposed to $(A^F)A_2$ and $A(A^F)A$. In the latter two trimers indications for the presence of a *syn* conformation of the base in A^F residues were found. Some of these conformational peculiarities correlate rather well with the data[7,8] on the binding of 5′-monophosphates of these fluoro-substituted trimers to RNase L and the susceptibility to (2′-5′)phosphodiesterase degradation.

It was also reported that the phagocytic activity of macrophages[9] and the lytic activity of human natural killer cells[10] are strongly dependent on both the configuration of the fluorine atom and on the position of fluorodeoxy adenine nucleoside in the sequence of the 2–5A analogue. Again, these data revealed a relationship between oligonucleotide conformation and biological properties of fluorodeoxy analogues of 2–5A.

More recently, we studied the reactivity of *anti*-(2′,5′)oligoadenylate mice antisera with the same set of fluorodeoxy analogues of 2–5A trimer core. It was found that the conformational features of the analogues are an important factor in polyclonal antibody recognition[11].

In the present paper, this investigation has been further extended to the evaluation of the role of conformational factors in the interaction between fluoro-substituted analogues of 2–5A and RNase L.

Results and discussion

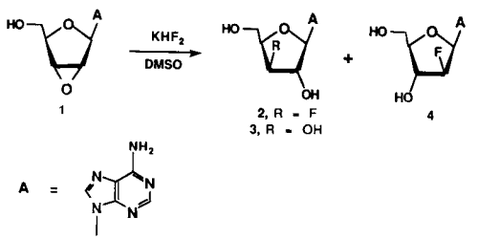
Chemistry

The starting material for the preparation of A^F was readily available 9-(2,3-anhydro-β-D-ribofuranosyl)adenine

Table II Coupling constants (Hz) for fluorodeoxy derivatives of adenosine [a].

No.	J(1′2′)	J(2′3′)	J(3′4′)	J(F2′)	J(F3′)	J(F4′)	J(4′5′) = J(4′5″)	Others
8	2.8			13.7	48.6	28.6		4.3 (OH, 2′)
10	< 1.0	< 1.0	2.0	14.0	51.0	30.0	5.4	9.6 (5′,5″)
12	< 1.0	< 1.0	2.2	12.6	49.5	24.7	6.6	9.6 (5′,5″)
14	2.4	< 1.0	3.4	13.8	51.0	27.6	5.4	
18	1.8	< 1.0	2.0	15.6	51.6	28.0	6.0	
9	8.0	3.8	< 1.0	22.0	54.0	27.9	3.9	6.6 (OH, 2′) 6.0 (OH, 5′)
11	7.5	4.5	< 1.0	22.5	54.0	27.0	3.9	10.8 (5′,5″)
13	8.1	4.5	< 1.0	20.4	54.0	25.8	4.5	10.8 (5′,5″)
15	8.2	4.5	< 1.0	22.8	54.0	27.5	–	12.6 (5′,5″)
19	7.2	3.0	< 1.0	22.8	54.0	25.8	[b]	

[a] See footnote a of Table I. [b] See footnote b of Table I.



Scheme 1. Synthesis of xylo-fluoride **2** *in the presence of dibenzo-18-crown-6.*

(**1**)[12]. Its treatment with KHF_2 in the presence of crown ether led to *xylo*-fluoride **2** in a yield of 42% after column chromatography on silica gel. The 9-(β-D-xylofuranosyl)adenine (**3**) and 9-(2-fluoro-2-deoxy-β-D-arabinofuranosyl)adenine (**4**) were also isolated from the reaction mixture (*cf.* Ref. 13) (Scheme 1). The synthesis of ribofluoride **5** is described in separate publications[14,15]. The appropriately protected mononucleosides **14, 15** and **25** and phosphodiester **18, 19** and **24** building blocks (Schemes 2 and 3) were prepared essentially by literature procedures[16-18].

^{1}HNMR data for fluorodeoxy derivatives of adenosine are listed in Tables I and II.

A careful study of the benzoylation of compound **20** with benzoyl chloride showed that in the presence of a catalytic amount of 4-(dimethylamino)pyridine the reaction leads to the predominant formation of 3′-*O*-benzoate **23**. In this way, benzoates **21, 22** and **23** were separated by column chromatography on silica gel in 10, 15 and 64% yields, respectively. This method is an advantageous alternative to the one previously published which used benzoyl cyanide treatment[16,19-21]. Detritylation of **21** gave N^6,3′-*O*,2′-*O*-tribenzoyladenosine which was also employed as the 2′(3′)-terminal fragment along with **25** in some syntheses.

A phosphotriester methodology was applied to synthesize the trimers (Scheme 4). The assembly of the trimers was performed in good overall yield by condensation in succession and combination of (*i*) the monomeric building blocks to synthesize the 5′-detritylated dimers **26–31**, and (*ii*) the dimers obtained with phosphodiesters to give the 5′-detritylated trimers **32–38** (Scheme 4; Tables III and IV). The condensations were performed in chloroform solution using a mixture of 2,4,6-triisopropylbenzenesulfonyl chloride and *N*-methylimidazole (in a molar ratio of 1:3)[22-24]. Deprotection of trimers **32–38** was performed by sequential deblocking of phosphotriester groups with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) and subsequent treatment with saturated methanolic ammonia followed by DEAE-Sephadex A-25 (HCO_3^- form) chromatography in a linear gradient of TEAB buffer. The deblocked core trimers were obtained in the form of Na^+ salts as amorphous powders according to *Moffatt*[25] and their structures were unequivocally established by NMR analysis[7]. The 5′-mono- and -triphosphates of fluoro-substituted trimers were prepared according to literature procedures[26,27]. Digestion of both 5′-mono- and -triphosphates with bacterial alkaline phosphatase gave the corresponding core trimers.

no.	B	R	R_1	no.
2	A	H	H	**5**
6	A^{Bz_2}	Bz	Bz	**7**
8	A^{Bz}	H	H	**9**
10	A^{Bz}	MTr	H	**11**
12	A^{Bz_2}	MTr	Bz	**13**
14	A^{Bz_2}	H	Bz	**15**
16	A^{Bz}	MTr	-P(O)(OoClPh)ONPE	**17**
18	A^{Bz}	MTr	-P(O)O$^-$ONPE	**19**

Scheme 2. Summary of protected A^F *and* A_F *building blocks.*

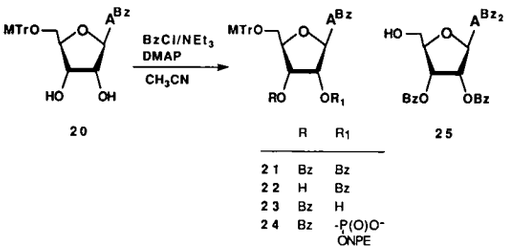
Table III Data on the synthesis and physical properties of the protected dimers **26-31** and trimers **32-38**.

First component		Second component		Product									
				Yield		R_f values		UV spectra (MeOH)					
No.	Amt.	No.	Amt.	No.	%	D	C	λ_{max}, nm			log ε		
25	0.15	**24**	0.16	**26**	80	0.44		233		278	4.96		4.81
25	0.15	**18**	0.16	**27**	83		0.67	233		278	4.83		4.76
14	0.10	**24**	0.11	**28**	91		0.56			278		4.83	
25	0.30	**19**	0.33	**29**	86		0.35	233		278	4.93		4.74
15	0.17	**24**	0.19	**30**	82		0.34	236		279	4.82		4.78
15	0.17	**18**	0.19	**31**	85		0.38	235	257sh	277	4.82	4.73	4.76
26	0.11	**18**	0.12	**32**	92		0.25	233		279	5.11		5.02
27	0.055	**24**	0.06	**33**	91		0.23	233		279	5.07		5.00
28	0.047	**24**	0.057	**34**	83		0.26	234		279	5.11		5.05
26	0.18	**19**	0.20	**35**	86	0.51		233	259sh	274	4.96	4.83	4.83
29	0.21	**24**	0.23	**36**	81	0.45		232	260sh	275	5.26	5.11	5.13
30	0.07	**24**	0.08	**37**	87	0.43		233		278	5.25		5.09
31	0.016	**24**	0.018	**38**	82	0.53		235	257sh	277	4.97	4.81	4.84

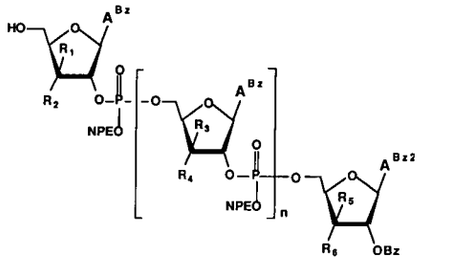
Table IV Isolated yields and HPLC data for the fluorodeoxy analogues of 2–5A core, 5′-mono- and 5′-triphosphate.

Structure of core trimer	Isolated yield (%)			HPLC retention time (min)		
	Core	5′-MP	5′-TP	Core	5′-MP [a]	5′-TP [c]
$(A^F)A_2$	72	85	32	18.72 [a]	10.46	28.60
$A(A^F)A$	70	68	33	22.82 [a]	13.10	30.00
$A_2(A^F)$	71	73	36	23.91 [a]	13.12	17.90
$(A_F)A_2$	71	63	32	23.26 [b]	15.26	27.10
$A(A_F)A$	76	60	52	18.34 [b]	14.18	31.50
$A_2(A_F)$	74	63	27	20.68 [b]	14.49	24.30
$A(A^F)A_F$	65	68	25	24.40 [a]	16.10	36.20

[a] The data were obtained on a Zorbax ODS C18 column. [b] Ultrasphere ODS column. [c] Separon SG×NH_2 (see experimental).



Scheme 3. Benzoylation of **20**.



no.	n	R_1	R_2	R_3	R_4	R_5	R_6
26	0	H	OBz	-	-	H	OBz
27	0	F	H	-	-	H	OBz
28	0	H	OBz	-	-	F	H
29	0	H	F	-	-	H	OBz
30	0	H	OBz	-	-	H	F
31	0	F	H	-	-	H	F
32	1	F	H	H	OBz	H	OBz
33	1	H	OBz	F	H	H	OBz
34	1	H	OBz	H	OBz	F	OBz
35	1	H	F	H	OBz	H	OBz
36	1	H	OBz	H	F	H	OBz
37	1	H	OBz	H	OBz	H	F
38	1	H	OBz	F	H	H	F

Scheme 4. Summary of protected dimers (n = 0) and trimers (n = 1).

Biochemical studies

In previous accounts of our investigations, we have shown that (*i*) sequence-specific fluoro-substituted analogues of 2–5A trimer core are stereochemically modified oligomers[7], and (*ii*) the differences, sometimes drastic, in biological properties reflect their conformational peculiarities[7–11]. Although our conformational analyses have been performed on the core trimers, we assume that the presence of a 5′-triphosphate group at the 5′-terminus does not lead to essential conformational changes of fluorinated residues owing to their conformational rigidity[7]. It should be stressed that the fluorine atom is able to form hydrogen bonds and that substitution of the fluorine atom for a hydroxyl group does not create any steric hindrances.

Our aim in the present investigation was to define the role of stereochemical factors implicated in the binding and activation processes of RNase L using fluoro-substituted analogues of 2–5A. It is noteworthy that RNase L is a functionally stereoselective enzyme as shown by in-depth studies by *Suhadolnik* et al.[28–30] using the unique spectrum of phosphorothioate analogues of 2–5A. One of the most interesting characteristics of RNase L is that the binding process is independent of the activation process[29]. Furthermore, each pair of *xylo* and *ribo* fluoro-substituted analogues of 2–5A compares favourably with the corresponding 3′-deoxy analogue extensively studied by *Torrence* et al.[6,31]. Thus, on the one hand, a pair of A^F and A_F substituted analogues is structurally closely related to a corresponding cordycepin analogue, while on the other hand, the furanose rings of both *xylo* and *ribo* fluorodeoxy nucleoside fragments are conformationally biased toward N- and S-type[7], respectively, affecting the base orientation specifically in A^F 5′-terminal and central residues (*vide supra*). Thus, the fluorodeoxy analogues under consideration are valuable tools for the study of stereochemical aspects of binding and activation processes of RNase L relating to pentofuranose ring conformation and *syn/anti* adenine base orientation.

The ability of fluoro-substituted analogues of 2–5A to bind to RNase L was examined in radiobinding assays according to Ref. 22; the data are summarized in Table V and some displacement curves are illustrated in Figure 1. These data display the changes within an order of magnitude, implying that the stereochemical modifications of the type considered are not of crucial importance in this process. The 5′-triphosphates were bound to RNase L with higher efficiency than the corresponding 5′-monophosphates[8] except for $p_3A(A_F)A$ and $p_3A_2(A_F)$ (*cf.* Ref. 6). Note at this point that $p_3A(A^F)A$ displays a relatively weak binding, in contrast to its outstanding activation property, see below.

Several interesting stereochemical aspects of RNase L activation are revealed by the use of fluoro-substituted analogues of 2–5A in RNase L-(2′,5′)pentaadenylate-(core)-agarose assays by monitoring the degradation of poly(U)[^{3}H] (Table V; Figure 2). The analogues in which the 5′-terminal adenosine of 2–5A was replaced by *xylo*

Table V Biological activities of 3′-fluoro-3′-deoxy analogues of 2–5A

Compound	Poly(U)[^{3}H]degradation		Radiobinding	
	(IC_{50}, M·10^{-9}) [a]	Rel. act. [b]	(IC_{50}, M·10^{-9}) [a]	Rel. act. [b]
p_3A_3	2.8 (±1) (n = 6)	1.0	2.2 (±1) (n = 3)	1.0
$p_3(A^F)A_2$	4.2 (±2) (n = 4)	0.66	20 (±10) (n = 4)	0.1
$p_3A(A^F)A$	0.32 (±0.15) (n = 3)	8.75	7 (±2) (n = 4)	0.3
$p_3A_2(A^F)$	6 (±3) (n = 5)	0.5	3.6 (±2) (n = 7)	0.6
$p_3(A_F)A_2$	9 (±3.5) (*n* = 3)	0.3	5 (±2) (*n* = 3)	0.45
$p_3A(A_F)A$	90 (±20) (n = 4)	0.03	22 (±10) (n = 3)	0.1
$p_3A_2(A_F)$	28 (±15) (n = 4)	0.1	6 (±2) (n = 3)	0.4
$p_3A(A^F)A_F$	32 (±20) (n = 4)	0.09	10 (n = 2)	0.2

[a] See experimental; standard errors of mean are shown in parentheses; the results given represent the mean of n experiments. [b] Relative activity was calculated by dividing the IC_{50} of p_3A_3 by the IC_{50} of an analogue; the lower the value, the less active the analogue.

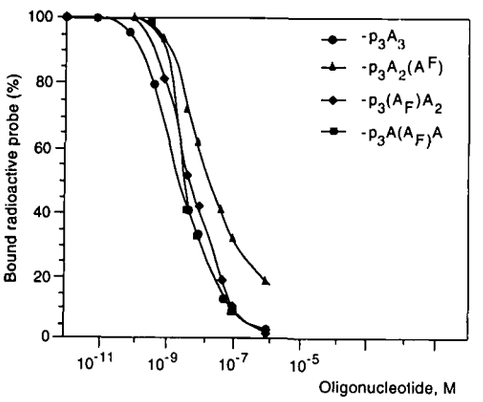


Figure 1. Ability of 2-5A and its fluorodeoxy analogues to inhibit the binding of the radioactive probe to RNase L (see experimental).

or *ribo* fluorodeoxy nucleosides, $p_3(A^F)A_2$ or $p_3(A_F)A_2$, retained 66 and 30% of the activity of the parent trimer, respectively. This observation is in good accordance with the data[6] for the related cordycepin analogue.

We suggest that a *syn* orientation of the base in combination with predominant N-type conformation in A^F at the 5'-terminus[7] account for the more effective activation of RNase L by $p_3(A^F)A_2$ *vs.* $p_3(A_F)A_2$. This suggestion is consistent with an earlier report on the conformation of the cordycepin (2',5')-trimer core for which an overwhelming preference for the N-conformation has been found in all furanose residues[33], unfortunately, no data were available regarding the base orientation. One can also speculate that a significant decrease in activation of RNase L following replacement of 5'-terminal adenosine of 2-5A with 8-bromoadenosine[34-36] or 8-methyladenosine[37] is related to the introduction of bulky groups which promote a *syn* orientation of the base[34], thus adversely affecting this process.

Replacement of the central adenosine fragment of 2-5A with A^F or A_F brought about profound differences in activation of RNase L. The $p_3A(A_F)A$ was approximately 30 times less effective in this process than the parent trimer, similar to the behaviour of the related cordycepin analogue[6]. It is noteworthy that at a concentration of 1.10^{-6} M (oligonucleotide equivalents) of $p_3A(A_F)A$, breakdown of poly(U) into acid-soluble fragments reached a maximum value of *ca.* 67(±6)% compared to above 85(±5)% in the presence of the parent 2-5A at the same concentration (Figure 2). This result is reminiscent of the finding that an analogous compound, (2'5')p_3A(3'dA)A,

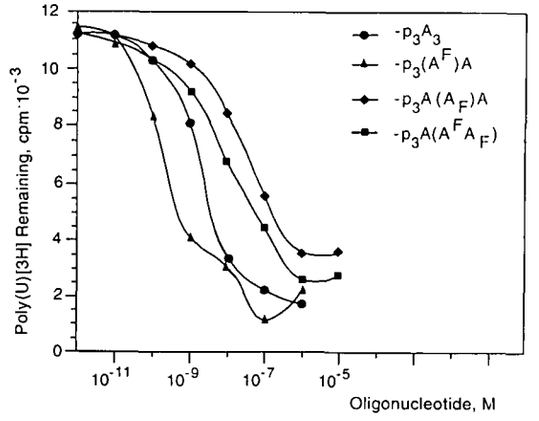


Figure 2. RNase L-(2',5')pentaadenylate(core)-agarose assay for the activation of RNase L by 2-5A and its fluorodeoxy analogues (see experimental).

failed to cause degradation of all the substrate and reached a maximum value of 65% at 1.10^{-6} M[31]. In contrast to this, there was a *ca.* nine-fold increase in the activation of RNase L by $p_3A(A^F)A$ compared to the parent 2-5A. In other words, the *xylo* fluoro-substituted analogue, $p_3A(A^F)A$, displayed a *ca.* 280-fold higher activating ability of RNase L than its *ribo* counterpart, $p_3A(A_F)A$. The importance of stereochemical factors seems understandable in terms of the observed patterns of RNase L activation. These findings are not consistent with an earlier conclusion that the 3'-hydroxyl group of the central adenosine fragment of 2-5A is needed for effective activation of RNase L[6,31]. It seems probable that the intrinsic spatial arrangement of $p_3A(A^F)A$ facilitates the formation of a productive complex between RNase L, poly(U), and the modifier, in which the presence of the 3'-hydroxyl group of the central adenosine residue is not implicated. When the 3'-hydroxyl group of the 2'(3')-terminal residue of 2-5A is replaced by a fluorine atom in the *xylo* configuration, activation ability decreases slightly; the *ribo* fluorodeoxy analogue, $p_3A_2(A_F)$, was 10-fold less active than 2-5A. Note that the corresponding cordycepin analogue was approximately 5-10 times more active than 2-5A[6,31]. The decreased activation ability of $p_3A_2(A_F)$ may be correlated to the almost pure S-type conformation of the fluorodeoxy pentofuranose ring of A_F, whereas the A^F residue in $p_3A_2(A^F)$ (and in its cordycepin counterpart[33]) displays mainly N-type sugar[7]. Another explanation invokes an unfavourable *anti* base orientation at the 2'(3')-terminus in both $p_3A_2(A_F)$ and $p_3A_2(A^F)$[7], albeit that the *anti* range is most likely populated in the case of the related cordycepin analogue.

The study on pA_3 analogues in which one or more of the adenosine fragments were replaced by 8-bromoadenosine has revealed the possible correlation between biological activities of these compounds and their conformation[34]. It was suggested that the binding of pA_3 to RNase L required (besides an *anti* orientation of the 5'-terminal base; *vide supra*) (*i*) a *syn*/N-type conformational combination of the central nucleotide, and (*ii*) S-type sugar and/or a *syn* orientation of the 2'(3')-terminal base[34]. Similar observations were made for 8-methyladenosine-substituted analogues of 2-5A[37]. The 2-5A analogues with 8-bromoadenosine and 8-methyladenosine at the 2'(3')-terminus displayed *ca.* 10-fold increase of RNase L activating ability compared with 2-5A[35,37]. It is noteworthy that the substitution of H-8 of adenosine residue(s) by a bulky bromine atom results in an *anti* → *syn* change in the conformation about the N-glycosidic bond[34] and the population of *syn* conformation in the case of 8-methyladenosine is markedly increased[38]. At the same time this type of modification leads to the introduction of an additional steric factor, the contribution of which to the binding to and the activation of RNase L cannot be definitively ascertained[34-37].

On the basis of the observations reported here, the *anti*/*syn* stereochemical differences between the fluoro-substituted analogues $p_3A_2(A_F)$ and $p_3A_2(A^F)$ on the one hand, and related 8-bromo and 8-methyl analogues on the other hand, are responsible for the distinct patterns of RNase L activation. Our findings point to the *syn* base orientation as a major contribution to this process. The low ability of $p_3A(A^F)A_F$ to activate RNase L supports this suggestion. It is also apparent that activating ability of the bis-substituted analogue of 2-5A is hard to predict on the basis of the biological properties of related monosubstituted analogues (*cf.* Ref. 37).

In summary, the use of fluoro-substituted analogues of 2-5A has contributed essential information to the analysis of the stereochemical course of RNase L activation. The *syn* orientation of the bases at the 5'-terminus and the

central position, together with a favoured N-type conformation of pentofuranose residues, seem to be required for the formation of a productive complex between RNase L, poly(U), and 2–5A or its analogues. In addition, *syn* orientation of the base at the 2′(3′)-terminus appears to influence the RNase L activation in a positive manner. These conclusions seem to be rather paradoxical and we may raise the question whether or not one or more of the adenine bases of 2–5A adopt a *syn* orientation at a transition stage of RNase L activation as a special property of this modifier. This question cannot be answered at the present time. Whatever the case may be, our findings support and extend earlier conclusions[29] that RNase L has functional stereoselectivity and that binding is independent of activation.

Experimental

General

UV spectra were recorded on a Specord UV-VIS spectrophotometer (Carl Zeiss, Germany). ^{1}H-NMR spectra were recorded with a Bruker WM-360 (Bruker, Germany) spectrometer with tetramethylsilane as an internal standard (s, singlet; d, doublet; t, triplet; m, multiplet; br.s, broad signal).
Bacterial alkaline phosphatase (BAP) was purchased from Boehringer (Germany).
Thin-layer chromatography (TLC) was carried out on F 1500 LS 254 silica gel plates (Schleicher & Schuell, Germany). As solvent systems were used: ethyl-acetate/hexane, 5:7 (A); ethyl-acetate/hexane, 7:5 (B); chloroform/methanol, 24:1 (C); chloroform/methanol, 19:1 (D); chloroform/methanol, 4:1 (E); 2-propanol/25% aq. ammonia/water, 7:1:2 (F); 1-butanol/acetic acid/water, 5:3:2 (G).
Column chromatography was performed on silica gel L 40/100 μ or 100/400 μ (Chemapol, Czechoslovakia).
High-performance liquid chromatography (HPLC) was carried out with (*i*) a Bruker LC 31 (Bruker, Germany) apparatus with a Zorbax ODS C18 column (150×4.6 mm) using a linear gradient (0 → 50%) of buffer B (MeOH/H_2O, 1:1, v/v) in buffer A [50 mM $(NH_4)H_2PO_4$, pH 7.0] at a flow rate of 1 ml/min (time of analysis was 25 min), or a Separon SG×NH_2 column (150×3.3 mm) (Czechoslovakia) using a linear gradient (0 → 50%) of buffer B (1.0 M $LiClO_4$/0.16 mM NH_4OAc/20% CH_3CN) in buffer A (0.4 M $LiClO_4$/0.16 mM NH_4OAc/20% CH_3CN) (1 ml/min; 40 min), and (*ii*) an Altex (Altex, USA) apparatus with an Ultrasphere ODS column (250×4.6 mm) using a linear gradient (0 → 50%) of buffer B (MeOH/H_2O, 1:1, v/v) in buffer A [50 mM $(NH_4)H_2PO_4$, pH 7.0] (1 ml/min; 70 min).
The solutions of compounds in organic solvents were dried with anhydrous sodium sulfate for 4 h. The reactions were carried out at room temperature, unless stated otherwise.
Melting points were determined with a Boethius (Germany) apparatus and are uncorrected.

Interaction of 9-(2,3-anhydro-β-D-ribofuranosyl)adenine (1) *with* KHF_2

A mixture of **1** (0.42 g, 1.72 mmol), KHF_2 (0.47 g, 6.03 mmol) and dibenzo-18-crown-6 (1.96 g, 5.44 mmol) in 8 ml of anhydrous DMSO was heated at 130–135 °C for 16 h. After cooling the reaction mixture was diluted with 150 ml water and washed with chloroform (5×40 ml). The water phase was separated, evaporated, and the products were chromatographed on a silica gel column (100 ml) eluting with chloroform (500 ml) and then with a linear methanol gradient (0 → 25%, v/v; 2×500 ml) in chloroform. The fractions containing individual products were collected, evaporated and crystallized from appropriate solvents.

9-(3-Fluoro-3-deoxy-β-D-xylofuranosyl)adenine (**2**). 198 mg (41.5%), m.p. 215–217 °C (from ethanol) [reported[13]: m.p. 212–214 °C (from ethanol)] R_f 0.36 (E). UV (H_2O) λ_{max} nm (lg ϵ): 260 (4.15).

9-(2-Fluoro-2-deoxy-β-D-arabinofuranosyl)adenine (**4**). 16 mg (3.5%), m.p. 231–233 °C (from ethanol) [reported[39]: m.p. 231–234 °C (from ethanol)]; R_f 0.34 (E). UV (H_2O) λ_{max} nm (lg ϵ): 260 (4.14). CD (H_2O) λ_{max} ([θ].10^{-3}): 280 (0), 255 (−2.7), 234 (0), 214 (−2.3), 210 (0). ^{1}H-NMR (DMSO-d_6): 8.30 (d, 1H, $J_{8,2'F}$ 1.6, 8-H), 8.20 (s, 1H, 2-H), 7.28 (br.s, 2H, NH_2), 6.43 (dd, 1H, $J_{1',2'}$ 4.45, $J_{1',2'F}$ 13.6, 1′-H), 6.05 (br.s, 1H, 3′-OH), 5.22 (dt, 1H, $J_{2',3'}$ 4.45, $J_{2',2'F}$ 56, 2′-H), 4.31 (m, 1H, $J_{3',4'}$ 4.45, $J_{3',2'F}$ 4.0, 3′-H), 3.89 (m, 1H, 4′-H), ~3.70 (m, 2H, 5′-H) (*cf.* the data reported in Ref. 40).

9-(β-D-Xylofuranosyl)adenine (**3**). 55 mg (12%), identical with the authentic sample[41] in all respects.

9-(3-Fluoro-3-deoxy-β-D-xylofuranosyl)-N^6*-benzoyladenine* (**8**)

Method A. Benzoyl chloride (0.54 g, 0.44 ml, 3.8 mmol) was added to a stirred solution of xyloside **2** (0.2 g, 0.74 mmol) in pyridine (5.5 ml) at 0 °C; the mixture was then allowed to warm to room temperature and stirred for 12 h. The reaction mixture was poured into a mixture of ice and water (300 ml); the ice was allowed to melt and the resulting precipitate was collected by filtration, washed with water and dried *in vacuo* to give 0.49 g (96%) of tetrabenzoate **6** as an amorphous powder, R_f 0.84 (B). Without purification, this material (0.49 g, 0.715 mmol) was dissolved in an ethanol/pyridine mixture (12 ml, 1:1; v/v) and treated with 2 M NaOH. After stirring for 5 min, the mixture was neutralized with Dowex 50×8 (PyH^+ form) and then filtered. The filtrate was evaporated and the residue was dissolved in water (30 ml) and washed with ether (3×30 ml). The water phase was evaporated and the residue was crystallized from water to give 0.22 g (83%) of **8** with m.p. 117–118 °C; R_f 0.28 (D). UV (MeOH) λ_{max} nm (lg ϵ): 235 (4.11), 281 (4.30).

Method B. Trimethylsilyl chloride (1.54 g, 1.8 ml, 14 mmol) was added to a solution of xyloside **2** (0.71 g, 2.63 mmol) in pyridine (4 ml) and the reaction mixture was stirred for 15 min. Benzoyl chloride (2.19 g, 1.7 ml, 14 mmol) was then added and stirring was continued for 2 h. The reaction mixture was cooled to 0 °C and water (2.8 ml, 0 °C) was added while stirring. After 5 min, 25% aqueous ammonia (6 ml) was added and stirring was continued for 30 min. The reaction mixture was evaporated and the residue was partitioned between water (70 ml) and ethyl acetate (50 ml). The organic phase was dried, evaporated, and the residue was crystallized to give 0.87 g (89%) of **8**.

N^6*-Benzoyl-3′-fluoro-3′-deoxyadenosine* (**9**). Compound **9** was obtained, as described above (Method A) for compound **8**, starting from 0.1 g (0.37 mmol) of **5** to give 0.09 g (72% combined yield) of **9** with m.p. 220–222 °C (from water); R_f 0.26 (D). UV (MeOH) λ_{max} nm (lg ϵ): 236 (4.11), 282 (4.30).

9-[5-O-(Monomethoxytrityl)-3-fluoro-3-deoxy-β-D-xylofuranosyl]-N^6*-benzoyladenine* (**10**)

A mixture of **8** (0.2 g, 0.54 mmol) and monomethoxytrityl chloride (0.35 g, 1.13 mmol) in dry pyridine (3 ml) was stirred for 12 h and then poured into a mixture of ice and water (300 ml) under vigorous stirring. The resulting precipitate was collected by filtration, washed with water and dried *in vacuo*. The product was purified on a column of silica gel (100 ml) eluting with a linear methanol gradient (0 → 10%, v/v; 2×500 ml) in chloroform to give 0.3 g (87%) of **10** as an amorphous powder; R_f 0.39 (D). UV (MeOH) λ_{max} nm (lg ϵ): 232 (4.36), 280 (4.30).

N^6*-Benzoyl-3′-fluoro-3′-deoxy-5′-O-(monomethoxytrityl)adenosine* (**11**). Compound **11** was obtained as described above, starting from 0.5 g (1.34 mmol) of **9** to give 0.82 g (95%) of **11** with m.p. 114–116 °C (from ethanol); R_f 0.39 (D).

9-[2-O-Benzoyl-3-fluoro-3-deoxy-5-O-(monomethoxytrityl)-β-D-xylofuranosyl]-N^6,N^6*-dibenzoyl-adenine* (**12**) *and* N^6,N^6,2′*-O-tribenzoyl-3′-fluoro-3′-deoxy-5′-O-(monomethoxytrityl)-adenosine* (**13**). Benzoylation (*vide supra* for **6**) of 0.23 g (0.36 mmol) of **10** afforded 0.3 g (96%) of **12**: m.p. 111–113 °C (from ethanol); R_f 0.45 (A). Similarly, starting from 0.73 g (1.13 mmol) of **11**, 0.95 g (98%) of **13** was obtained: m.p. 121–122 °C (from ethanol); R_f 0.43 (A).

9-(2-O-Benzoyl-3-fluoro-3-deoxy-β-D-xylofuranosyl)-N^6,N^6*-dibenzoyladenine* (**14**) *and* N^6,N^6,2′*-O-tribenzoyl-3′-fluoro-3′-deoxyadenosine* (**15**). Detritylation[16] of 0.3 g (0.35 mmol) of **12** afforded 0.19 g (93%) of **14**: m.p. 168–169 °C; R_f 0.15 (A). Similarly, starting from 0.95 g (1.11 mmol) of **13**, 0.54 g (83%) of **15** was obtained: m.p. 193–195 °C; R_f 0.12 (A).

9-[3-Fluoro-3-deoxy-5-O(-monomethoxytrityl)-β-D-xylofuranosyl]-N^6*-benzoyladenine 2′-[2-chlorophenyl 2-(4-nitrophenyl)ethyl phosphate]* (**16**) *and* N^6*-benzoyl-3′-fluoro-3′-deoxy-5′-O-(monomethoxytrityl)adenosine-2′-[2-chlorophenyl 2-(4-nitrophenyl)ethyl phosphate]* (**17**). Phosphorylation of 0.18 g (0.28 mmol) of **10** with 2-chlorophenyl

phosphoditriazolidate followed by treatment with 2-(4-nitrophenyl)ethanol as described[16], afforded 0.25 g (93%) of triester **16** as an amorphous powder; R_f 0.54 (C). Similarly, starting from 0.05 g (0.08 mmol) of **11**, 0.07 g (92%) of triester **17** was obtained as an amorphous powder; R_f 0.53 (C).

Triethylammonium 9-[3-fluoro-3-deoxy-5-O-(monomethoxytrityl)-β-D-xylofuranosyl]-N^6-benzoyladenine-2'-[2-(4-nitrophenyl)ethyl phosphate] (**18**) *and triethylammonium* N^6*-benzoyl-3'-fluoro-3'-deoxy-5'-O-(monomethoxytrityl)adenosine-2'-[2-(4-nitrophenyl)ethyl phosphate]* (**19**). Treatment of 0.25 g (0.25 mmol) of triester **16** with 4-nitrobenzaldoxime, followed by work-up as described[16], afforded 0.19 g (79%) of diester **18** as an amorphous powder; R_f 0.84 (G). Similarly, starting from 0.07 g (0.071 mmol) of triester **17**, 0.05 g (83%) of diester **19** was obtained as an amorphous powder; R_f 0.84 (G).

Benzoylation of N^6*-benzoyl-5'-O-(monomethoxytrityl)adenosine* (**20**) *with benzoyl chloride*

To a stirred solution of **20** (0.91 g, 1.41 mmol)[17] in a mixture of anhydrous acetonitrile (18 ml), triethylamine (2.5 ml) and 4-(dimethylamino)pyridine (DMAP) (0.013 g, 0.11 mmol), freshly distilled benzoyl chloride (0.24 g, 0.197 ml, 1.69 mmol) was added. After stirring for 30 min, the reaction mixture was poured into a mixture of ice and water (600 ml) under vigorous stirring. After melting of the ice, the resulting precipitate was collected by filtration, washed with water and dried *in vacuo*. The products were chromatographed on a silica gel column (70 ml) eluting with a linear ethyl-acetate gradient (25 → 100%, v/v; 2×400 ml) in hexane. The fractions containing individual products were collected, evaporated, and crystallized to give *N*^{6},2'-*O*,3'-*O*-tribenzoyl-5'-*O*-(monomethoxytrityl)adenosine (**21**) [0.1 g (10%); R_f 0.38 (B)], *N*^{6},2'-*O*-dibenzoyl-5'-*O*-(monomethoxytrityl)adenosine (**22**) [0.16 g (15%); R_f 0.21 (B)] and *N*^{6},3'-*O*-dibenzoyl-5'-*O*-(monomethoxytrityl)adenosine (**23**) [0.67 g (64%); R_f 0.11 (B)]. The compounds **21–23** were identical to the authentic samples[16] in all respects.

Triethylammonium N^6,3'-O-*dibenzoyl-5'-O-(monomethoxytrityl)-adenosine-2'-[2-(4-nitrophenyl)ethyl phosphate]* (**24**). Compound **24** was obtained as described above for compounds **18** and **19**.

Synthesis of the core (2',5') trimers (**32–38**)

Synthesis of dimers **26–31** and then trimers **32–38** was performed according to previously described methodology[16] consisting of (i) condensation of phosphodiesters **18, 19**, or **24** with 2'-terminal building blocks **14, 15**, or **25**[17] and subsequent detritylation, and (ii) condensation of each individual dimer obtained with phosphodiesters **18, 19**, or **24** and subsequent detritylation.

To a solution of phosphodiester (0.11 mmol) and 2'-terminal building block or dimer (0.1 mmol) in alcohol-free anhydrous chloroform (0.5 ml), 2,4,6-triisopropylbenzenesulfonyl chloride (0.3 mmol) and *N*-methylimidazole (0.9 mmol) were added and the reaction mixture was stirred for 30 min. The reaction mixture was poured into hexane (200 ml), the resulting precipitate was collected by filtration, dried *in vacuo* and then dissolved in 2% solution of *p*-toluenesulfonic acid in dichloromethane/methanol (7:3, v/v; 15 ml). After stirring for 5 min, the solution was diluted with chloroform (15 ml) and washed with 0.05 M phosphate buffer, pH 7.0 (2×30 ml). The organic phase was separated, dried, evaporated and purified by silica gel column chromatography (60 ml). The product was eluted with a linear methanol gradient (0 → 5%, v/v; 2×300 ml) in chloroform. The product-containing fractions were collected, evaporated to a volume of 2 ml, and precipitated in hexane (200 ml). The compounds were obtained as amorphous solids in 80–92% yields.

Deprotection of trimers **32–38** was performed by the sequential deblocking of phosphate groups and subsequent treatment with methanol, saturated with ammonia at 0 °C. A trimer (0.1 mmol) was dissolved in 0.5 M solution of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in pyridine (30 ml) and stirred for 2 h. After the addition of 15 ml of a 1 M solution of acetic acid in pyridine, the mixture was evaporated and then co-evaporated with pyridine (2×10 ml). The residue was dissolved in saturated methanolic ammonia (20 ml), kept for 20 h and evaporated. The residue was chromatographed on a DEAE-Sephadex A-25 (HCO_3^- form; 100 ml) column using a linear gradient (0.001 → 0.6 M; pH 7.6; 2×500 ml) of TEAB buffer. The product-containing fractions were collected and evaporated. Deblocked core (2',5') trimers were obtained in the form of Na^+ salts as amorphous powders according to Moffatt[18]. The physicochemical data are summarized in Tables III and IV.

The (2',5')pentaadenylate (core), A_5, was obtained in a similar way; yield 49%; R_f 0.22 (F), 0.03 (G); UV (H_2O), λ_{max}: 261 nm, hypochromicity 27.3%; HPLC (Zorbax ODS C18) retention time (R_t) 18.27 min, under these HPLC conditions, the following R_t values were found for A_2, A_3, and A_4: 21.93, 21.34, and 19.38 min, respectively.

Synthesis of the (2',5')trimer 5'-monophosphates

The synthesis of the (2',5')trimer 5'-monophosphates was accomplished from 5'-deprotected trimers **32–38** by reaction with pyrophosphoryl chloride[26]. To a suspension of (2',5')trimer (0.1 mmol) in 2 ml of anhydrous ethyl acetate, pyrophosphoryl chloride (0.07 ml, 0.5 mmol) was added and the reaction mixture was stirred for 3.5 h at 0 °C. The reaction was stopped by the addition of ice; the mixture was neutralized with cold saturated $NaHCO_3$ and evaporated. The residue was dissolved in 0.5 M solution of DBU in pyridine (20 ml) and stirred for 20 h. After the addition of 12 ml of a 1M solution of acetic acid in pyridine, the mixture was evaporated and then co-evaporated with pyridine (2×5 ml). The residue was dissolved in 25 ml saturated methanolic ammonia, kept for 20 h, evaporated, and purified by column chromatography as described above for core trimers using a linear gradient (0.001 → 0.8 M; pH 7.6; 2×500 ml) of TEAB buffer. Deblocked 5'-monophosphates were obtained in the form of Na^+ salts as amorphous powders; the yields and HPLC data are summarized in Table IV.

pA_3 was obtained analogously starting from the corresponding 5'-deblocked trimer[16]; HPLC (Zorbax ODS C18), R_t 11.05 min; TLC [Kieselgel 60 F_{254} glass plates (Merck, Germany); solvents system: 2-propanol/25% aq. ammonia/water, 11:7:2], R_f 0.62. Quantitative digestion of 5'-monophosphates with BAP[6] using HPLC gave the corresponding core trimers.

(2',5')Trimer 5'-triphosphates

Preparation of the (2',5')trimer 5'-triphosphates was performed from the deblocked core trimers using phosphorus oxychloride in trimethyl phosphate for selective monophosphorylation of the 5'-hydroxyl function followed by a condensation step with bis(tributylammonium)-pyrophosphate[27]. Phosphorus oxychloride (0.328 mmol) was added to a suspension of (2',5')oligomer (0.0041 mmol) in 0.2 ml of trimethyl phosphate at 0 °C and the reaction mixture was stirred for 3.5 h. A mixture of 0.5 M bis(tributylammonium) pyrophosphate in anhydrous *N,N*-dimethylformamide (1 ml) and tributylamine (0.1 ml) was then added and stirring was continued for 30 min. The reaction mixture was poured into 0.5 M TEAB (3 ml), stirred at 20 °C for 3 h, and evaporated. The residue was chromatographed on a DEAE Sephadex A-25 (HCO_3^- form, 40 ml) column using a linear gradient (0.001 → 1.0 M, pH 7.6; 2×500 ml) of TEAB buffer. The product-containing fractions were collected and evaporated. Triphosphates were obtained in the form of Na^+ salts as amorphous powders.

The purity of all synthesized 5'-triphosphates was checked by HPLC (Table IV) and TLC and further confirmed by degradation leading to the corresponding core trimers under the action of BAP[6].

The p_3A_3 was obtained analogously starting from A_3[16] and was found to be identical (HPLC, TLC) to the authentic sample (Pharmacia, Sweden); HPLC (Zorbax ODS C18), R_t 9.18 min; TLC [Kieselgel 60 F_{254} glass plates (Merck, Germany); solvents system: 2-propanol/25% aq. ammonia/water, 11:7:2], R_f 0.25.

Radiobinding assay

The preparation of mouse L_{929} cell extracts was performed according to *Torrence* and *Friedman*[42]. The probe used for radiobinding assays, $p_3(A2'p)_3A3'[^{32}P]pC3'p$, was synthesized and purified by HPLC as described by *Knight* et al[32]. Radiobinding assays were performed as described by *Knight* et al.[32]; the data are expressed as a concentration (calculated for each trimer 5'-triphosphate, taking into account the hypochromicity data for the corresponding core trimer[11]) for 50% displacement of the radioactive probe from RNase L (IC_{50}) and summarized in Table V; representative results are depicted in Figure 1.

Functional assay for 2-5A and its fluorodeoxy analogues

This was performed as described by *Silverman*[43] with the following modification: (*i*) poly(U)[^{3}H] (20–72 Ci/mmol, Amersham) was used instead of poly(U)3'[^{32}P]pC3'p; (*ii*) (2',5')pentaadenylate (core) was linked to hydrazide agarose (Chematex, Estonia).

Linkage of (2',5')pentaadenylate to agarose was conducted as described by *Silverman*[43]. The concentration of (2',5')oligoadenylate

bound to agarose was determined by treating an aliquot of agarose with 0.3 M KOH at 37 °C for 18 h and measuring the absorbance of the supernatant at 260 nm. (2',5')Oligoadenylate was covalently attached to the agarose in a yield greater than 95%. (2',5')Pentaadenylate (core)-agarose (32 μm of A_5 in the assay) was washed by resuspending and centrifuging at 1000×g for 5 min at 2 °C in 2×10 vol of freshly made buffer A [11.5 mM HEPES, pH 7.6; 104 mM KCl; 5.8 mM $Mg(OAc)_2$; 8.8 mM 2-mercaptoethanol; 1.2 mM ATP, pH 7; and 100 μg/ml leupeptin]. Washed agarose was resuspended in a mixture of 1 vol. of mouse L cell extract (60 μg/protein/assay) and 1.6×buffer A. This mixture was incubated with occasional shaking for 1 h at 0 °C, then RNase L-(2',5')pentaadenylate(core)-agarose complex was washed in the same way as mentioned above with 3×10 vol of ice-cold buffer A. After the final centrifugation, the complex was resuspended in 1.25×buffer A. The mixture of 15 μL of the complex in buffer A and 5 μL of H_2O or solution of varying amounts of trimer 5'-triphosphate was preincubated for 20 min on ice and 5 μL poly(U)[^{3}H] (*ca.* 12000 cpm in the assay) was then added. The reaction mixture was incubated at 30 °C for 2 h. Poly(U)[^{3}H] breakdown into acid-soluble fragments was monitored by mixing the assay mixture (25 μL) with 1.0 ml of 5% trichloroacetic acid, 0.2% pyrophosphate, plus 100 μL of 5 mg/ml of yeast RNA on ice. The samples were filtered on presoaked 30 glass-fiber circles, 25 mm diameter (Schleicher & Schuell), and washed with 2×10 ml of 5% trichloroacetic acid, 0.2% pyrophosphate and once with 5 ml of 95% ethanol. The radioactivity on the dried filters was determined by scintillation counting.

The p_3A_3 was used as a control for each series of assays; the data are expressed as a concentration (calculated for trimer 5'-triphosphate, taking into account the hypochromicity data for the corresponding core trimer) needed to effect a 50% degradation of poly(U)[^{3}H] (IC_{50}) and summarized in Table V; representative results are depicted in Figure 2.

After submission of this paper we found that data on radiobinding and activation of mouse RNase L by two of the ribo-congeners reported here, $p_3(A_F)AA$ and $p_3A(A_F)A$, have been published recently by *Torrence* et al[44]. Their data are in good agreement with ours.

Acknowledgements

I.A.M. is deeply grateful to the *Alexander von Humboldt-Stiftung* (Bonn-Bad-Godesberg, Germany) for the partial financial support of this work.

References

1 *P.F. Torrence*, In *P.F. Torrence*, Ed., "Biological response modifiers. New approaches to disease intervention", Academic Press, Orlando, FL, 1985, pp. 77–105.

2 *D.C. Montefiori, R.W. Sobol, S.W. Li, N.L. Reichenbach, R.J. Suhadolnik, R. Charubala, W. Pfleiderer, A. Modliszewski, W.E. Robinson, Jr.* and *W.M. Mitchell*, Proc. Natl. Acad. Sci. USA **86**, 7191 (1989).

3 *W.E.G. Mueller, B.E. Weiler, R. Charubala, W. Pfleiderer, L. Leserman, R.W. Sobol, R.J. Suhadolnik* and *H.C. Schroeder*, Biochemistry **30**, 2027 (1991).

4 *H.C. Schroeder, R.J. Suhadolnik, W. Pfleiderer, R. Charubala* and *W.E.G. Mueller*, Int. J. Biochem. **24**, 55 (1992).

5 *I.A. Mikhailopulo, Z.Yu. Tkachuk, E.A. Baran, L.V. Tkachuk, A.V. Koslov, I.I. Slukvin, E.A. Kvasyuk, T.I. Kulak* and *G.Kh. Matsuka*, In 4th International Symposium on Molecular Aspects of Chemotherapy, 23–25 June, 1993, Gdansk, Poland. Abstract 61.

6 *P.F. Torrence, D. Brozda, D. Alster, R. Charubala* and *W. Pfleiderer*, J. Biol. Chem. **263**, 1131 (1988).

7a *J.E. van den Boogaart, E.N. Kalinichenko, T.L. Podkopaeva, I.A. Mikhailopulo* and *C. Altona*, Eur. J. Biochem. **221**, 759 (1994);

b Two different families of conformers of the D-furanose ring in common nucleosides and nucleotides are commonly distinguished: N-type (North), roughly C3'-*endo* (C3' up, on the same side as C5', ^{3}E); S-type (South), roughly C2'-*endo* (^{2}E), see *C. Altona*, Recl. Trav. Chim. Pays-Bas **101**, 413 (1982).

8 *E.N. Kalinichenko, T.L. Podkopaeva, M. Kelve, M. Saarma* and *I.A. Mikhailopulo*, Biochem. Biophys. Res. Commun. **167**, 20 (1990).

9 *M. Kelve, A. Kuuskalu* and *E.N. Kalinichenko*, In 5th International Congress on Cell Biology, 26–31 July, 1992, Madrid, Spain. Abstracts Section 13.4, No. 269.

10 *M. Kelve, E.N. Kalinichenko, T.L. Podkopaeva, M. Saarma* and *I.A. Mikhailopulo*, J. Interferon Res. **12** (Suppl. 1), S.120 (1992).

11 *E.N. Kalinichenko, T.L. Podkopaeva, I.A. Mikhailopulo, M. Kelve, M. Saarma, J.E. van den Boogaart* and *C. Altona*, unpublished observations.

12 *A.A. Akhrem, G.V. Zaitseva, E.N. Kalinichenko* and *I.A. Mikhailopulo*, Bioorgan. Khim. (Moscow) **2**, 1325 (1976).

13 *M.J. Robins, Y. Fouron* and *R. Mengel*, J. Org. Chem. **39**, 1564 (1974).

14 *I.A. Mikhailopulo, T.I. Pricota, N.E. Poopeiko, G.G. Sivets, E.I. Kvasyuk, T.V. Sviryaeva, L.P. Savochkina* and *R.S. Beabealashvilli*, FEBS Lett. **250**, 139 (1989).

15 *I.A. Mikhailopulo, N.E. Poopeiko, T.I. Pricota, G.G. Sivets, E.I. Kvasyuk, J. Balzarini* and *E. De Clercq*, J. Med. Chem. **34**, 2195 (1991).

16 *E.I. Kvasyuk, T.I. Kulak, N.B. Khripach, I.A. Mikhailopulo, E. Uhlmann, R. Charubala* and *W. Pfleiderer*, Synthesis **6**, 535 (1987).

17 *R. Lohrmann* and *H.G. Khorana*, J. Am. Chem. Soc. **86**, 4188 (1964).

18 *G.S. Ti, B.L. Gaffney* and *R.A. Jones*, J. Am. Chem. Soc. **104**, 1316 (1982).

19 *E.I. Kvasyuk, T.I. Kulak, G.V. Zaitseva, I.A. Mikhailopulo, R. Charubala* and *W. Pfleiderer*, Tetrahedron Lett. **25**, 3683 (1984).

20 *E.I. Kvasyuk, T.I. Kulak, G.V. Zaitseva, I.A. Mikhailopulo* and *W. Pfleiderer*, Bioorgan. Khim. (Moscow). **10**, 506 (1984).

21 *E.I. Kvasyuk, T.I. Kulak, E.N. Kalinichenko, T.L. Podkopaeva, I.A. Mikhailopulo* and *W. Pfleiderer*, Bioorgan. Khim. (Moscow) **11**, 1227 (1985).

22 *V.A. Efimov, S.V. Reverdatto* and *O.G. Chakhmakcheva*, Bioorgan. Khim. (Moscow) **8**, 231 (1982).

23 *V.F. Zarytova, E.M. Ivanova* and *V.P. Romanenko*, Dokl. Akad. Nauk USSR (Moscow) **265**, 878 (1982).

24 *V.F. Zarytova, E.M. Ivanova* and *V.P. Romanenko*, Bioorgan. Khim. (Moscow) **9**, 516 (1983).

25 *J.G. Moffatt*, Can. J. Chem. **42**, 599 (1964).

26 *D.A. Eppstein, Y.V. Marsh, B.B. Schryver, M.A. Larsen, J.W. Barnett, J.P.H. Verheyden* and *E.J. Prisbe*, J. Biol. Chem. **257**, 13390 (1982).

27 *A. Nyilas, L. Vrang, A. Drake, B. Oberg* and *J. Chattopadhyaya*, Acta Chem. Scand. **B 40**, 678 (1986).

28 *K. Kariko, R.W. Sobol Jr., L. Suhadolnik, S.W. Li, N.L. Reichenbach, R.J. Suhadolnik, R. Charubala* and *W. Pfleiderer*, Biochemistry **26**, 7127 (1987).

29 *K. Kariko, S.W. Li, R.W. Sobol Jr., R.J. Suhadolnik, R. Charubala* and *W. Pfleiderer*, Biochemistry **26**, 7136 (1987).

30 *R.J. Suhadolnik, C. Lee, K. Kariko* and *S.W. Li*, Biochemistry **26**, 7143 (1987).

31 *R. Charubala, W. Pfleiderer, D. Alster, D. Brozda* and *P.F. Torrence*, Nucleosides & Nucleotides **8**, 273 (1989).

32 *M. Knight, D.H. Wreschner, R.H. Silverman* and *I.M. Kerr*, Methods Enzymol. **79**, 216 (1981).

33 *J. Doornbos, R. Charubala, W. Pfleiderer* and *C. Altona*, Nucleic Acids Res. **11**, 4569 (1983).

34 *Y.Th. van den Hoogen, C.M.A. Hilgersom, D. Brozda, K. Lesiak, P.F. Torrence* and *C. Altona*, Eur. J. Biochem. **182**, 629 (1989).

35 *K. Lesiak* and *P.F. Torrence*, J. Biol. Chem. **262**, 1961 (1987).

36 *K. Lesiak* and *P.F. Torrence*, J. Med. Chem. **29**, 1015 (1986).

37 *Y. Kitade, Y. Nakata, K. Hirota, Y. Maki, A. Pabuccuoglu* and *P.F. Torrence*, Nucleic Acids Res. **19**, 4103 (1991).

38 *R. Stolarski, A. Pohorille, L. Dudycz* and *D. Shugar*, Biochim. Biophys. Acta **610**, 1 (1980).

39 *J.A. Wright, N.F. Taylor* and *J.J. Fox*, J. Org. Chem. **84**, 2632 (1969).

40 *C.K. Chu, J. Matulic-Adamic, J.-T. Huang, T.-Ch. Chou, J.H. Burchenal, J.J. Fox* and *K.A. Watanabe*, Chem. Pharm. Bull. **37**, 336 (1989).

41 *N.E. Poopeiko, E.I. Kvasyuk, I.A. Mikhailopulo* and *M.J. Lidaks*, Synthesis **6/7**, 605 (1985).

42 *P.F. Torrence* and *R.M. Friedman*, J. Biol. Chem. **254**, 1259 (1979).

43 *R.H. Silverman*, Anal. Biochem. **144**, 450 (1985).

44 *T. Kovacs, A. Pabuccuoglu, K. Lesiak* and *P.F. Torrence*, Bioorganic Chem. **21**, 192 (1993).