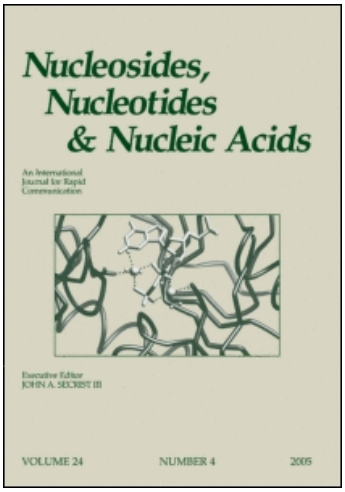

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SYNTHESIS AND BIOLOGICAL ACTIVITY OF NEW 2',5'-OLIGONUCLEOTIDES

Evgeny I. Kvasyuk[a*], Tamara I. Kulak[a], Igor A. Mikhailopulo[a], Robert J. Suhadolnik[b], Earl E. Henderson[c], Susan E. Horvath[b], Ming-Xu Guan[c], and Wolfgang Pfleiderer[d*]

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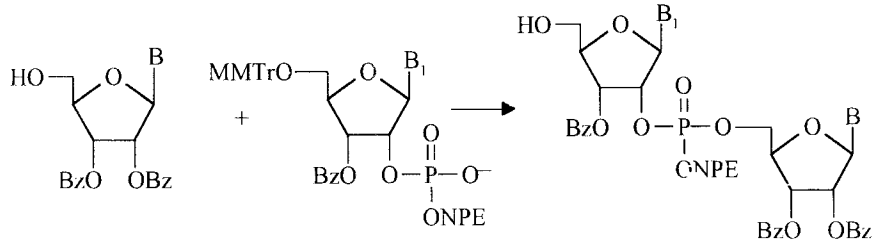
ABSTRACT: Some new 2',5'-oligonucleotide trimers containing the antiviral nucleoside ribavirin and the cytokine 6-benzylaminopurine riboside in different position of the oligos have been synthesized. Some of the trimers showed biological inhibitions of HIV-1 reverse transcriptase, HIV-1 induced syncytia formation and activation of RNase L.

2',5'-Oligoadenylates play an important role in one of the mechanisms of antiviral action of interferon[1]. Furthermore, naturally occurring 2',5'-oligoadenylates (both, not phosphorylated and 5'-phosphorylated) have shown different kinds of biological activity[2,3]. Recently, some of the sugar modified trimers of 2',5'-oligoadenylates were found to be inhibitors of HIV-1 reverse transcriptase (RT)[4-6]. It appears that each individual nucleoside residue of 2',5'-oligoadenylates may assume a fundamentally different role in inhibition of RT or RNase L activation. This supposition prompted us to synthesize a new type of 2',5'-oligoadenylate trimers containing the known antiviral nucleoside 1-(β-D-ribofurano-syl)-3-carboxamido-1,2,4-triazole (ribavirin) and the cytokine 6-benzylaminopurine riboside in different positions of the trimer instead of the adenosine residue, and to investigate their ability to inhibit HIV-1 replication, and RNase L activation.

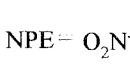
The syntheses of new 2',5'-oligonucleotides have been achieved by the phosphotriester method. Selectively blocked nucleosides **1-3** and 2'-phosphodiester derivatives **4-6** used for the synthesis of oligonucleotides have been obtained from the corresponding unprotected nucleosides according to known procedures[7]. Condensation of the 2'-terminal units **1-3** and the building blocks **4-6** in the presence of TPSCl/tetrazole 1:3, as catalysts and followed by detritylation with 2% TsOH in CH_2Cl_2/MeOH 7:3 afforded the 5'-OH containing dimers **7-12**, respectively. The final condensation of **4-6** and **7-12**, followed by treatment with 0.5 M DBU/Py, TsOH/CH_2Cl_2/MeOH and conc. NH_4OH led to the corresponding trimers **13-20** which were isolated by ion exchange chromatography with overall yields of 30-50% from all steps.

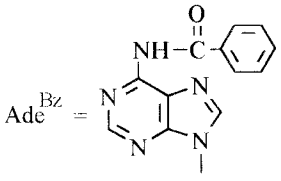


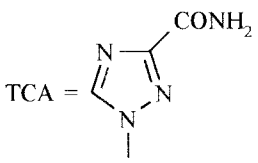
N	B
1	Ade^{Bz}
2	TCA
3	BAP

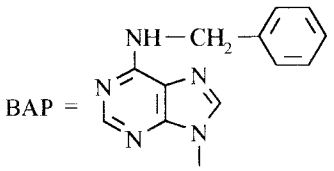
N	B_1
4	Ade^{Bz}
5	TCA
6	BAP

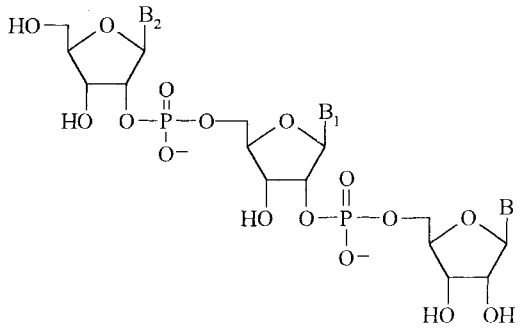
N	B	B_1
7	Ade^{Bz}	Ade^{Bz}
8	TCA	Ade^{Bz}
9	BAP	Ade^{Bz}
10	Ade^{Bz}	TCA
11	Ade^{Bz}	BAP
12	BAP	BAP











N	B	B_1	B_2
13	Ade	Ade	Ade
14	TCA	Ade	Ade
15	Ade	TCA	Ade
16	Ade	Ade	TCA
17	BAP	Ade	Ade
18	Ade	BAP	Ade
19	Ade	Ade	BAP
20	BAP	BAP	BAP

All synthesized trimers were tested in 10 μM concentration for RNase L activation, and inhibition of RT and HIV-1 induced syncytia formation by assays described previously[6]. Summarized data in comparison to the activity of the 2',5'-oligoadenylate trimer core **13**, are presented in the following table.

TABLE. Biological activities of 2',5'-oligonucleotides

Compound N	RNase L[a]	Syn[b]	RT[c]
13	50.0	3.0	33.0
14	87.7	1.6	99.7
15	9.4	7.0	99.7
16	0	1.2	99.5
17	37.4	>1500	33.4
18	34.8	>1500	7.6
19	0	>1500	12.0*
20	13.6	>1500	4.0*

[a] - Activation of RNase L (% activity);

[b] - Inhibition of Syncytia formation (fold reduction);

[c] - Inhibition of HIV-1 RT activity (% inhibition);

*- Instead of inhibition, an increase in HIV-1 RT activity was observed.

Ribavirin derivatives **14-16** inhibited HIV-1 induced syncytia formation to the same extent as trimer **13**. However, compounds **13-16** inhibited RT 33%,99.7%, 99.7%, and 99.5%, respectively. The activation of RNase L by **13-16** was 50%, 87.7%, 9.4%, and 0%, respectively. These data show that the adenyl moiety at the 2',3'-terminus induces a pronounced effect for RNase L activation[8]. With **14** and **15**, the inhibition of HIV-1 replication can be attributed in part to the inhibition of RT activity plus the activation of RNase L. Most important is the observation that independent of the position of the N^6-benzylaminopurine moiety in **17-20**, HIV-1 induced syncytia formation was inhibited >1500-fold. However, **17-20** activated RNase L only by 37.4%, 34.8%, 0%, and 13.6%, respectively which has to be seen in comparison to a 50% activation of RNase L by the trimer **13**. The presented data support the hypothesis that the adenyl moiety at the 2',3'-terminus of the naturally occurring 2',5'-oligoadenylate trimer is stimulating the activation of RNase L.

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