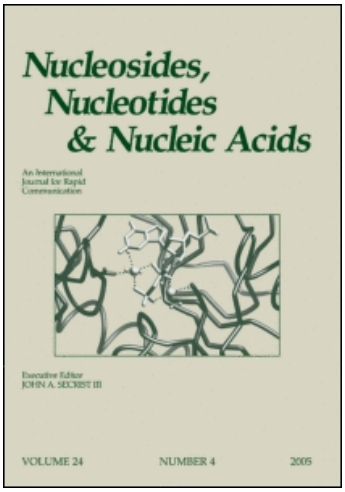

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Synthesis and Biological Activity of New Sugar Modified 2',5'-Oligoadenylate Trimers

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SYNTHESIS AND BIOLOGICAL ACTIVITY OF NEW SUGAR MODIFIED 2',5'-OLIGOADENYLATE TRIMERS

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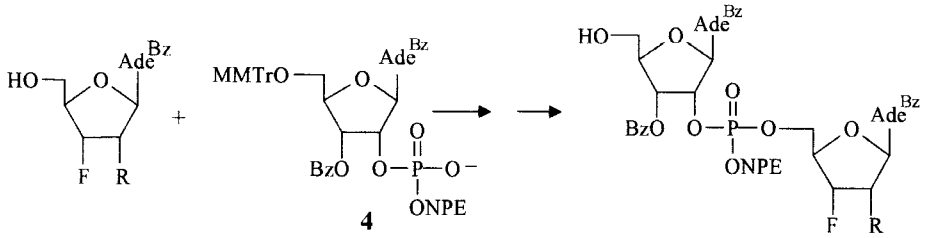
ABSTRACT: New 2',5'-oligonucleotide trimers containing 2',3'-dideoxy-3'-fluoro derivatives of adenosine at the 2'-terminal end of oligomers have been synthesized. Some of the synthesized trimers showed biological inhibitions of HIV-1 reverse transcriptase and HIV-1 induced syncytia formation.

Naturally occuring 2',5'-oligoadenylates (mainly trimers, both 5'-phosphorylated and unphosphorylated) have shown different kinds of biological activity[1-4]. However, these compounds are rapidly inactivated in the cell by some enzymes[5,6]. Many analogues of 2',5'-oligoadenylates have been synthesized to overcome this problem, and to achieve new approaches to antiviral and antitumoral therapy[7-15]. One of this analogues, cordycepin trimer core[16,17], was found to be a biologically active compound with metabolic stability[18]. Later it was found to be also an inhibitor of HIV-1 reverse transcriptase (RT)[19,20]. As far as each individual nucleoside residue of 2',5'-oligoadenylates may assume a different role in inhibition of RT or RNase L activation, we have synthesized some new 3'-fluoro containing trimers with double modification at the 2',3'-terminus of the oligos, as potential metabolic stable 2',5'-oligoadenylates. The ability of synthesized trimers to inhibit HIV-1 replication and to improve RNase L activation have been also investigated.

Selectively blocked nucleosides **1-3**, used for the synthesis of trimers, have been obtained from the corresponding unprotected nucleosides[21-23], respectively, using the transient protection method[24]. The synthesis of the 2'-phosphodiester **4** has been described by us earlier[25]. Condensation of the 2'-terminal units **1-3** and the building block **4** 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 **5-7**, respectively. The final condensation of **5-7** and **4**, followed by treatment with 0.5 M DBU/Py, TsOH/CH_2Cl_2/MeOH and conc. NH_4OH led to the

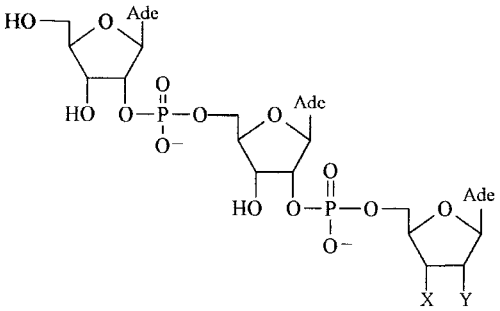
corresponding trimers **8-11** which were isolated by ion exchange chromatography with overall yields of 40-50% from all steps. Catalytic hydrogenolysis of the azido derivative **8** in the presence of 10% Pd/C in EtOH gave 2'-amino-3'-fluoro-2',3'-dideoxy containing trimer **11**.



4

N	R
1	N_3
2	H
3	Cl

N	R
5	N_3
6	H
7	Cl



N	X	Y
8	F	N_3
9	F	H
10	F	Cl
11	F	NH_2
12	OH	OH

The synthesized trimers were tested for recombinant human GST-RNase L activation, and inhibition of RT and HIV-1 induced syncytia formation by assays described previously[26]. Summarized data in comparison to the activity of the 2',5'-oligoadenylate trimer core **12**, are presented in the following table.

Compound **9-11** inhibited induced syncytia formation 7.0, 2.0, and 9.0 fold, respectively, compared to 3.0 fold reduction with unmodified trimer **12**. Trimers **8-10** inhibited HIV-1 RT activity 99.7%, which compares with a 33% inhibition of HIV-1 RT by the compound **12**. The previously obtained data about a 96% inhibition of HIV-1 RT by the cordycepin trimer core[27] and the results presented here, show that hydroxyl group at either the C2' or C3' position of the trimer **12** are not essential for the inhibition of HIV-1 RT activity. When 2',5'-oligoadenylate **12** is modified at the C2' or C3' position either fluorine, chlorine, azido and amino substituent, recombinant human GST-RNase L is not activated to hydrolyze poly(U)-3'-[^{32}P]pCp compared to a 50% activation of GST-RNase L by the trimer **12**. These results, and the data a 12% activation

TABLE. Biological activities of the synthesized trimers.

Comp.	X	Y	RNase L[a]	Syn[b]	RT[c]
8	F	N_3	0	-	99.7
9	F	H	0	7.0	99.7
10	F	Cl	0	2.0	99.7
11	F	NH_2	0	9.0	-
12	OH	OH	50	3.0	33.0

[a]- RNase L is the percent hydrolysis of [^{32}P]poly(U) in the presence of 10 nM p_3A_3 and 10 μM for the remaining trimers.
[b]- Syn is the fold reduction in HIV-1 replication, as determined by Syncytia formation.
[c]-RT is the percent inhibition of HIV-1 RT.
The value given in the mean of duplicate determination with a variance of ±5-10%.

of GST-RNase L by the both cordycepin trimer core and its conjugate with vitamin E at the 2'-terminus of the trimer[27], indicate that the oxygen of the hydroxyl group at the C2'-position of the 2',3'-terminus is essential for the activation of GST-RNase L.

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REFERENCES

1. Kimchi A., Shure H., Revel M. *Nature* (London). **1979**, *282*, 849.
2. Leanderson T., Nordfelth R., Lundgren E. *Biochem. Biophys. Res. Commun.* **1982**, *107*, 511.
3. Devash Y., Biggs S., Sela J. *Science* **1982**, *216*, 1415.
4. DevashY., Gera A., Willis D.H., Reichman M., Pfleiderer W., Charubala R., Sela I., Suhadolnik R.J. *J. Biol. Chem.* **1984**, *259*, 3482.
5. Eppstein D.A., Barnet J.W., Marsh Y.Y., Gosselin G., Imbach J.L. *Nature* (London). **1987**, *302*, 723.
6. Herdewijn P., Charubala R., De Clercq E., Pfleiderer W. *Helv. Chim. Acta.* **1989**, *72*, 1739.
7. Kwiatkowski M., Gioelli C., Chattopadhyaya J.B., Oberg B., Drake A.F., *Chem. Scripta* **1982**, *19*, 49.
8. Mikhailov S.N., Pfleiderer W. *Tetrahedron Lett.* **1985**, *26*, 2059.
9. Herdewijn P., Charubala R., Pauwels R., De Clercq E., Pfleiderer W. *Nucleosides & Nucleotides* **1987**, *6*, 443.
10. Kariko K., Sobol R.W., Suhadolnik L., Li S.W., Reichenbach N.L., Suhadolnik R.J., Pfleiderer W. *Biochemistry* **1987**, *26*, 7127.
11. Herdewijn P., Charubala R., Pfleiderer W. *Helv, Chim. Acta.* **1989**, *72*, 1729.
12. Charubala R., Pfleiderer W., Sobol R.W., Li S.W., Suhadolnik R.J. *Helv, Chim. Acta.* **1989**, *72*, 1354.

13. Kitade Y., Nakata Y., Hirota K., Maki Y., Pabuccuoglu A., Torrence P.F. *Nucl. Acids Res.* **1991**, *19*, 4103.

14. Pfleiderer W., Himmelsbach F., Charubala R. *Bioorgan. Med. Chem. Lett.* **1994**, *8*, 1047.

15. Kvasyuk E.I., Kulak T.I., Mikhailopulo I.A., Charubala R., Pfleiderer W. *Helv, Chim. Acta.* **1995**, *78*, 1777.

16. Charubala R., Pfleiderer W. *Tetrahedron Lett.* **1980**, *21*, 4077.

17. Charubala R., Uhlmann E., Himmelsbach F., Pfleiderer W. *Helv, Chim. Acta.* **1987**, *70*, 2028.

18. Doetsch P.W., Suhadolnik R.J., Sawada Y., Mosca J.D., Flick M.B., Reichenbach N.L., Dang A.Q., Wu J.M., Charubala R., Pfleiderer W., Henderson E.E. *Proc. Natl. Acad. Sci. U.S.A.* **1981**, *78*, 6699.

19. Müller 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.

20. Schröder H.C., Suhadolnik R.J., Pfleiderer W., Charubala R., Müller W.E.G. *Int. J. Biochem.* **1992**, *24*, 55.

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

22. Chiedgeavadze Z.G., Beabealashvilli R.S., Scamrov A.V., Kvasyuk E.I., Zaitseva G.V., Mikhailopulo I.A., Kowollik G., Langen P. *FEBS Lett.* **1985**, *183*, 275.

23. 2',3'-Dideoxy-2'-chloro-3'-fluoroadenosine have been obtained as a gift from Dr. Tamara Pricota (Institute of Bioorganic Chemistry Byelorussian Academy of Sciences, Minsk).

24. Ti G.S., Gaffney B.L., Jones A.A. *J. Am. Chem. Soc.* **1982**, *104*, 1316.

25. Kvasyuk E.I., Kulak T.I., Khripach N.B., Mikhailopulo I.A., Uhlmann E., Charubala R., Pfleiderer W. *Synthesis* **1987**, 535.

26. Wasner M., Suhadolnik R.J., Horvath S.E., Adelson M.E., Kon N., Guang M.-X., Henderson E.E., Pfleiderer W. *Helv, Chim. Acta.* **1996**, *79*, 609.

27. Wasner M., Suhadolnik R.J., Horvath S.E., Adelson M.E., Kon N., Guang M.-X., Henderson E.E., Pfleiderer W. *Helv, Chim. Acta.* **1996**, *79*, 619.