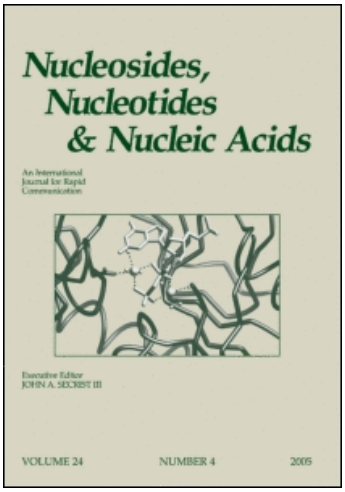

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Phosphorothioate Analogs OF 2-5A: Activation / Inhibition of Rnase L and Inhibition of HIV-1 Reverse Transcriftase

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To cite this Article Suhadolnik, Robert J. , Lebleu, Bernard , Pfleiderer, Wolfgang , Charubala, Ramamurthy , Montefiori, David C. , Mitchell, William M. , Sobol Jr., Robert W. , Li, Shi Wu , Kariko, Katalin and Reichenbach, Nancy L.(1989) 'Phosphorothioate Analogs OF 2-5A: Activation / Inhibition of Rnase L and Inhibition of HIV-1 Reverse Transcriftase', Nucleosides, Nucleotides and Nucleic Acids, 8: 5, 987 — 990
To link to this Article: DOI: 10.1080/07328318908054260
URL: http://dx.doi.org/10.1080/07328318908054260

NUCLEOSIDES & NUCLEOTIDES, 8(5&6), 987-990 (1989)

PHOSPHOROTHIOATE ANALOGS OF 2-5A: ACTIVATION / INHIBITION OF RNASE L AND INHIBITION OF HIV-1 REVERSE TRANSCRIPTASE

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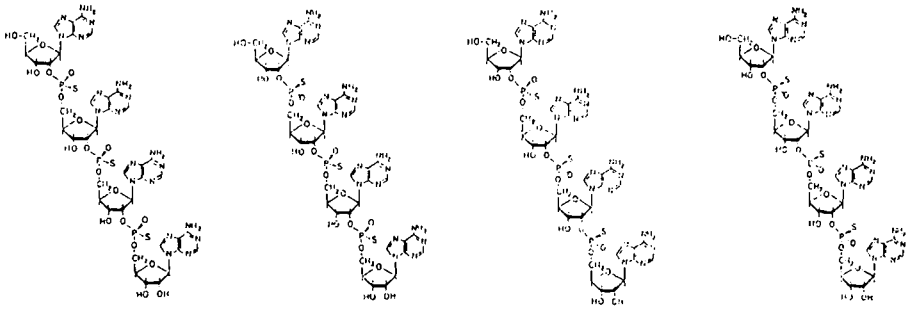
Chemically and enzymatically synthesized diastereomeric 2',5'-phosphorothioate dimer, trimer and tetramer cores and their 5'-mono- and triphosphates demonstrate marked differences in their ability to bind to and activate RNase L from L929 cell extracts in radiobinding, core-cellulose and rRNA cleavage assays[1,2] (Fig. 1). These are the first 2-5A cores that are able to bind to and activate RNase L. The enzymatically synthesized 2',5'-phosphorothioate dimer and trimer 5'-triphosphates can also bind to and activate RNase L[1].



RpRpRp SpRpRp RpSpSp SpSpSp

Figure 1. Structures of four diastereomers of the 2',5'-phosphorothioate tetramer cores.

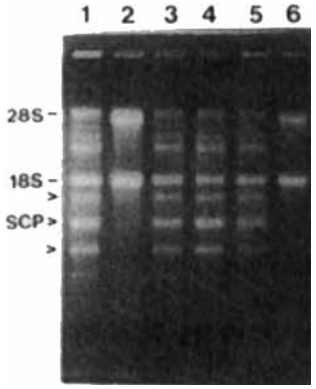


Figure 2. Ribosomal RNA cleavage assay with 2',5'-phosphorothioate tetramer 5'-monophosphates. L929 cell extracts were incubated in the absence (lane 2) or the presence of p_3A_3 at 10^{-8} M (lane 1), pRpSpSp at 10^{-8} M (lane 3), pSpRpRp at 10^{-8} M (lane 4), pRpRpRp at 10^{-8} M (lane 5), or pSpSpSp at 10^{-3} M (lane 6). The positions of 28S and 18S rRNA are shown. The arrows indicate the positions of the well-characterized specific cleavage products (SCP) of RNase L.

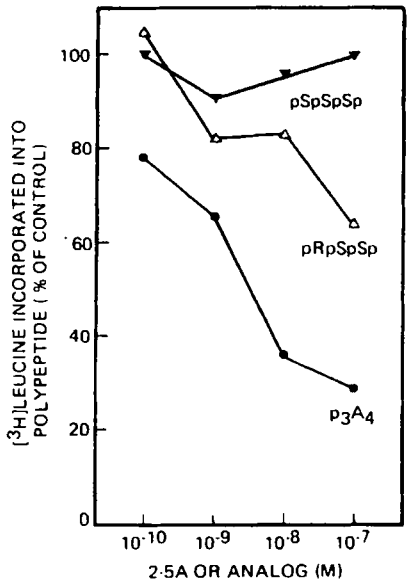


Figure 3. Inhibition of cellular protein synthesis by the 2',5'-phosphorothioate tetramer 5'-monophosphates in intact L929 cells. The procedure for the calcium phosphate coprecipitation technique was as described[4]. Control cultures were treated with calcium phosphate but no oligonucleotides. The incorporation of [^{3}H]leucine in control cultures was taken as 100% (1500 dpm). p_3A_4 (●); pRpSpSp (△); pSpSpSp (▼).

Table 1. Antiviral Effects of 2-5$A_{4\ oxred}$ and pSpSp in Intact HeLa Cells

First injection	Second injection	VSV Yield (pfu/100 cells)	log (pfu/100 cells)
H_2O	H_2O	2.5×10^4	4.39
H_2O	1 μM 2-5$A_{4\ oxred}$	1.3×10^2	2.11
H_2O	100 nM 2-5$A_{4\ oxred}$	2.2×10^3	3.30
1 μM pSpSp	H_2O	7×10^3	3.80
1 μM pSpSp	1 μM 2-5$A_{4\ oxred}$	8.4×10^3	3.90
1 μM pSpSp	100 nM 2-5$A_{4\ oxred}$	2.2×10^4	4.30
1 μM pSpSp + 1 μM 2-5$A_{4\ oxred}$		8.4×10^3	3.90

One hundred HeLa cells were microinjected[5] with 0.5 pL each of 2-5$A_{4\ oxred}$ [3' modified 2-5A_4 which is more stable than authentic 2-5A_4] or pSpSp at the indicated concentrations. One hour later, cells were challenged with VSV at a m.o.i. of 10 and the virus titers were determined 18 hours later by an end-point on L929 cells.

Binding and Activation of RNase L. The chemically synthesized 2',5'-phosphorothioate tetramer cores (i.e., RpRpRp, SpRpRp, RpSpSp and SpSpSp) and corresponding 5'-monophosphates bind to RNase L in L929 cell extracts as determined by radiobinding assay[3]. Activation of RNase L by the 2',5'-phosphorothioate tetramer cores and 5'-monophosphates was measured in rRNA cleavage assays with L929 cell extracts[2]. The RpRpRp, SpRpRp and RpSpSp tetramer cores (1×10^{-7} M) activate RNase L to cleave 28S and 18S rRNA to specific cleavage products (SCP). The SpSpSp tetramer core (1×10^{-3} M) does not activate RNase L. The pRpRpRp, pSpRpSp and pRpSpSp (1×10^{-8} M) activate RNase L to cleave 28S and 18S rRNA (Fig. 2, lanes 2-4). However, the pSpSpSp tetramer (1×10^{-5} M) does not activate RNase L (Fig. 2, lane 5).

Inhibition of Cellular Protein Synthesis. Studies were done with intact cells to determine whether the in vitro data could be extrapolated to the inhibition of protein synthesis in the intact cell. This was accomplished by the calcium phosphate coprecipitation technique[4]. The pRpSpSp but not the pSpSpSp tetramer 5'-monophosphate inhibited cellular protein synthesis in a dose-dependent manner (Fig. 3). These data indicate that the inhibition of protein synthesis proceeds via the activation of RNase L.

Antiviral Effects in Intact Cells. A 2-5A molecule capable of selectively inhibiting RNase L activation has been an elusive goal as a means to precisely assess the involvement of RNase L in the interferon-induced antiviral and antiproliferative cascade. The demonstrated ability of the pSpSp analog to bind to but not activate RNase L, plus its significantly increased stability[2], makes it most attractive for in vivo studies. Microinjection of the pSpSp analog into the cytoplasm of HeLa cells did not inhibit multiplication of VSV at concentrations as high as 10^{-6} M, whereas 100 nM 2-5$A_{4\ oxred}$ exhibited a substantial antiviral effect (Table 1). When injected prior to 2-5$A_{4\ oxred}$, the pSpSp analog inhibits the antiviral effect of 2-5$A_{4\ oxred}$.

Inhibition of HIV-1 Reverse Transcriptase and Anti-HIV Activity. The phosphorothioate analogs of 2-5A inhibit human immunodeficiency virus type 1 reverse transcriptase (HIV-1 RT) in viral lysates and inhibit replication of HIV-1 in MT-2 cells[6]. HIV-1 RT was not inhibited by authentic 2',5'-adenylate trimer core (A_3) or 5'-triphosphate (p_3A_3) at 0.25 - 256 μM. In contrast, a concentration-dependent inhibition of HIV-1 RT was observed with the 2',5'-phosphorothioate tetramer 5'-monophosphates (30 - 58% inhibition at 2.5 μM). The enzymatically synthesized RpRp trimer 5'-triphosphate was the most potent inhibitor of HIV-1 RT (50% inhibition at 0.5 μM). The pRpSpSp analog was shown to have antiviral activity against HIV-1 in an in vitro MT-2 cell microtiter infection assay[6]. This inhibition of RT and HIV-1 replication may represent a new and important role of 2',5'-oligoadenylate analogs in the control of retrovirus replication.

Supported in part by NIH research grant P01 CA29545.

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