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Activation of RNase L by Phosphorothioate Analogs of 2-5A Trimer and Tetramer Cores. R.J. Suhadolnik, K. Kariko, S.W. Li, R.W. Sobol, Jr., N.L. Reichenbach, W. Pfleiderer, and R. Charubala, Temple University School of Medicine, Philadelphia, PA, USA; Universitat Konstanz, Konstanz, Germany.

The binding and activation processes of the specific antiviral enzyme, RNase L, have been examined using four chemically synthesized diastereomeric 2,5-phosphorothioate trimer cores, four tetramer cores and their 5'-monophosphates. Stereochemical modification of the internucleotide linkages does not adversely affect binding to RNase L. However, marked differences were observed in the activation of RNase L. Three of the four 2,5-phosphorothioate trimer cores and two tetramer cores are able to activate RNase L at 10^{-5} and 10^{-8} M, respectively. RNase L activation by the 5'-monophosphates was at 10^{-7} to 10^{-8} M. Although the phosphorothioates with SpSp, RpSpSp, SpSpSp and pSpSp internucleotide linkages are capable of binding to RNase L, they are unable to activate RNase L at 10^{-3}, 10^{-3}, 10^{-3} and 10^{-5} M and competitively inhibit RNase L activation by p_3A_3. The results presented are consistent with the hypothesis that the binding process is independent of the activation process of RNase L.

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Chemical Synthesis of Phosphorothioate Analogs of 2-5A Trimer and Tetramer. W. Pfleiderer and R. Charubala, Universität Konstanz, Konstanz, Germany.

As part of continuing studies on the antiviral agent pppA2'p5'A2'p5'A [2-5A] and its core, we have introduced the phosphorothioate function into the internucleotide linkages of 2-5A trimer and tetramer cores. We have achieved the syntheses of all four stereoisomeric P-thioadenylyl-(2'-5')-P-thioadenylyl-(2'-5')-adenosines starting from N^6-benzoyl-3'-O-t-butyldimethylsilyl-5'-O-monomethoxytrityladenosine and N^6-benzoyl-2',3'-di-t-butyldimethylsilyladenosine by applying the phosphoramidite approach for build-up of the thiophosphotriester function. Phosphitylation at the 2'-OH group was performed with chloro-p-nitrophenylethoxy-N-octahydroazonino-phosphate to the phosphoramidite which was condensed under catalysis of 3-nitro-1,2,4-triazole and subsequent oxidation by sulfur. After chromatographic separation, each diastereomer was detritylated and converted to the corresponding diastereomeric trimer pair. Resolution and purification of the individual trimers were achieved by silica gel chromatography. Deprotection of the dimer and trimer cores has been achieved follwoed by purification by DEAE-Sephadex. Four of the eight phosphorothioate tetramer cores have been similarly prepared. The phosphorothioate analogs have subsequently been used as mechanistic probes of the binding and activation processes of the specific antiviral enzyme, RNase L (see accompanying abstract).