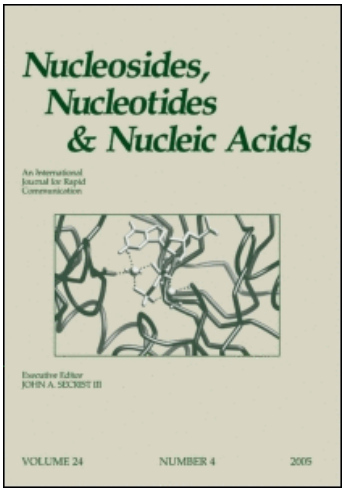

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

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To cite this Article Charubala, R. , Pfleiderer, W. , Suhadolnik, R. J. and Sobol, R. W.(1991) 'Chemical Synthesis and Biological Activity of 2'-5'-Phosphorothioate Tetramer Cores', Nucleosides, Nucleotides and Nucleic Acids, 10: 1, 383 — 388
To link to this Article: DOI: 10.1080/07328319108046484
URL: http://dx.doi.org/10.1080/07328319108046484

NUCLEOSIDES & NUCLEOTIDES, 10(1-3), 383-388 (1991)

CHEMICAL SYNTHESIS AND BIOLOGICAL ACTIVITY OF 2'-5'-PHOSPHOROTHIOATE TETRAMER CORES

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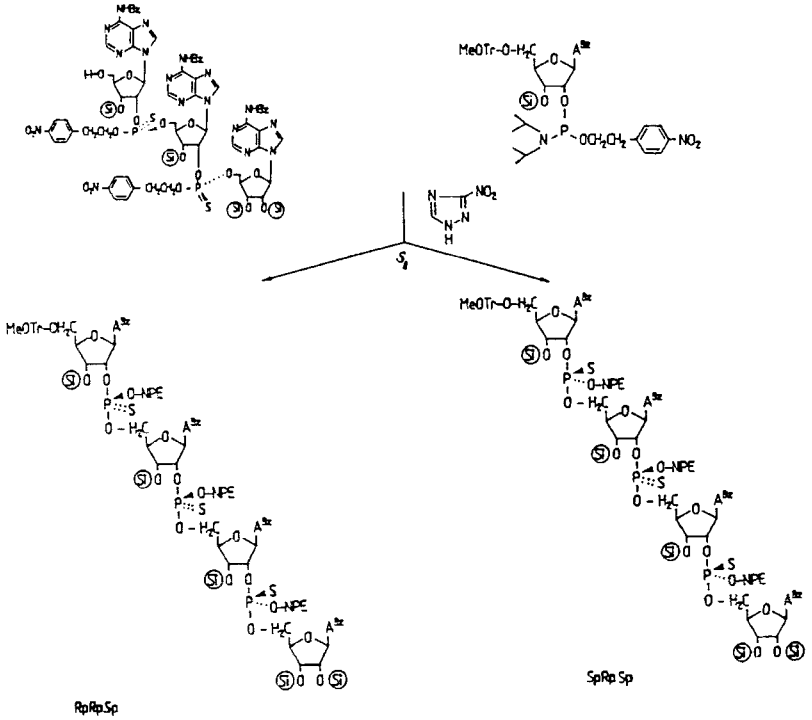
Abstract. The chemical synthesis of P-thioadenylyl-(2'-5')-P-thioadenylyl-(2'-5')-P-thioadenylyl-(2'-5')-adenosines was performed and the eight diastereomers were separated and characterized. All diastereomers bind to RNase L, seven of which also activate this enzyme, whereas the SpSpSp isomer turned out to be an antagonist of RNase L.

The 2-5A synthetase/RNase L pathway is an integral part of the antiviral mechanism of interferon and may also be involved in the regulation of cell growth[1]. 2-5A (ppp5'A-(2'p5'A)$_n$) is synthesized from ATP by the dsRNA-dependent 2'-5'-oligoadenylate synthetase, and exerts its biological effect by binding to and activating its only known target enzyme, the unique endoribonuclease RNase L at subnanomolar concentrations[1]. We have reported on studies directed towards the elucidation of the structural requirements for binding to and activation of RNase L, utilising enzymatically[2] and chemically synthesized 2'-5'-phosphorothioate trimers and tetramers[3,4]. We present now the chemical syntheses, physical characterization, and biological activity of the four missing additional diastereomers of 2'-5'-phosphorothioate tetramer cores.

We successfully achieved the syntheses and separation of the two isomeric dimers Rp and Sp, the four trimers RpRp, SpRp, RpSp, and SpSp[5] and four of the eight possible tetra-

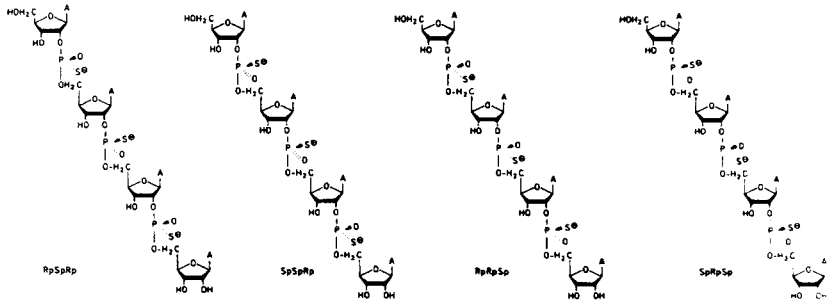
mers RpRpRp, SpRpRp, RpSpSp, and SpSpSp[6] and characterized them by chemical means.



In order to complete the series of the eight possible diastereomeric 2'-5'-phosphorothioate tetramers we have recently synthesized the remaining four tetramers, namely the RpRpSp, SpRpSp, RpSpRp, and SpSpRp isomers starting from the fully protected RpSp and SpRp phosphorothioate trimers, which have been first detritylated and then condensed with N^6-benzoyl-3'-O-[(tert-butyldimethyl)-silyl]-5'-O-(monomethoxytrityl)-adenosine-3'-(p-nitrophenylethyl, N,N-diisopropyl)-phosphoramidite by 3-nitro-1,2,4-triazole activation and subsequent oxidation by sulfur to give the diastereomeric mixtures RpRpSp+SpRpSp and RpSpRp+SpSpRp respectively.

The separations of the mixtures can be achieved by chromatographical means to give yields of 30-50 % each. The final deblocking of the various protecting groups in the fully protected tetramers was performed by subsequent treatment

with p-TsOH to remove the monomethoxytrityl group, followed by DBU to cleave off the NPE groups, then with ammonia to deblock the benzoyl groups and finally with fluoride ion for desilylation. The crude products were purified by DEAE Sephadex chromatography yielding 70-80 % of each of the four tetramers.



The structural assignments were accomplished by HPLC, charge separation and ^{31}P-NMR spectra.

The ability of these 2'-5'-phosphorothioate tetramer cores to compete with $p_3A_4(^{32}P)pCp$ for binding to RNase L in L929 cell extracts was compared to that of 2'-5'-p_3A_4 and 2'5'-A_4, in radiobinding assays[7]. As shown in FIG. 1 there is no significant difference in the affinity of the phosphorothioate analogs to RNase L from L929 cell extracts (IC_{50} = 2 x 10^{-7}M). This is in support with our earlier report that RNase L cannot distinguish between the Rp or Sp stereoconfiguration with respect to binding to RNase L from L929 cells[3] or from extracts of interferon treated Hela cells[4].

The activation of RNase L by these four tetramer cores was determined by two independent methods, 1) by core cellulose assay (FIG. 2)[8] in which RNase L from L929 cell extracts is immobilised and partially purified on 2-5 A_4-core cellulose and utilized to hydrolyse poly (U)(^{32}P)pCp, and 2) by rRNA cleavage assay which is based on the 2-5A-dependent activation of RNase L and subsequent hydrolysis of rRNA to characteristic specific cleavage products (SCP)[9]. The RpRpSp

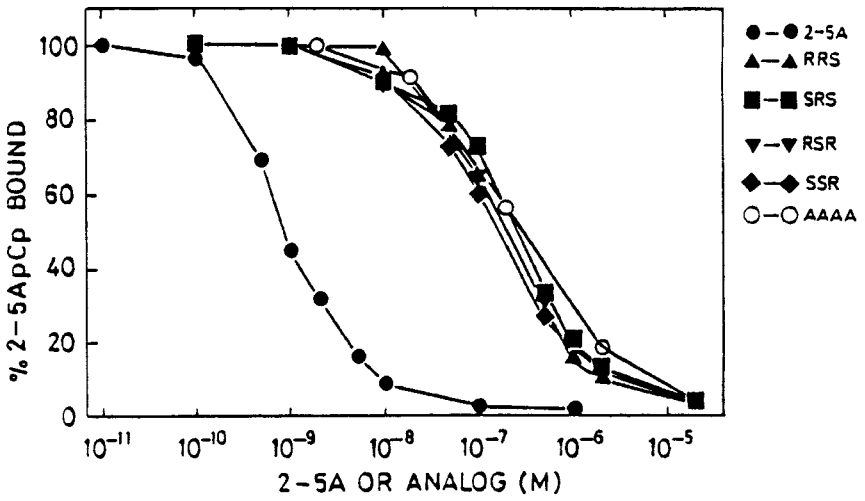


FIG. 1 - Binding of p_3A_4, A_4 core, and 2',5'-phosphorothiate tetramer core analogs to RNase L. 2',5'-p_3A_4 (●), 2',5'-A_4 core (○), RpRpSp (▲), SpRpSp (■), RpSpRp (▼), and SpSpRp (◆) were compared for their ability to compete with the $p_3A_4[^{32}P]pCp$ probe for binding to RNase L in L929 cell extracts in the radiobinding assay[1].

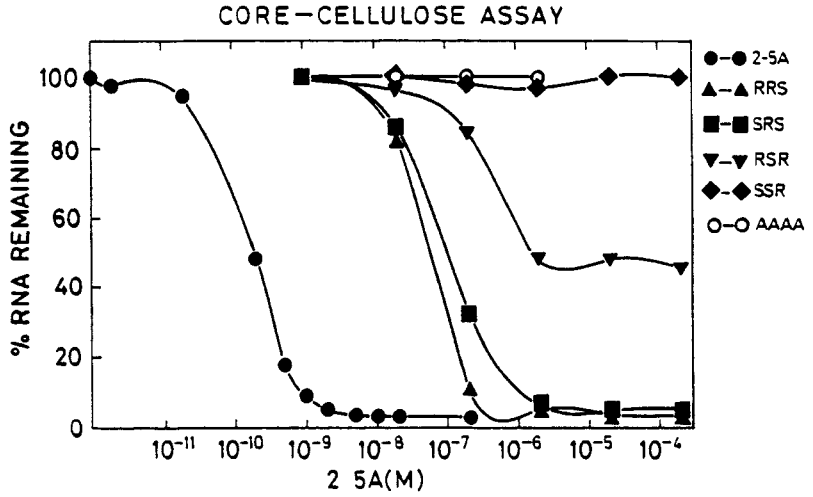


FIG. 2 - Ability of 2',5'-phosphorothioate tetramer cores to activate the partially purified RNase L from L929 cell extracts as determined by the hydrolysis of poly(U) $[^{32}P]pcp$ in the core-cellulose assay[3]. Activation of RNase L was determined by the conversion of poly(U) $[^{32}P]pCp$ to acid-soluble fragments after incubation. One hundred percent represents 21,000 dpm of labeled poly(U) $[^{32}P]pCp$ bound to glass fiber filters. 2',5'-p_3A_4 (●), 2',5'-A_4 core (○), RpRpSp (▲), SpRpSp (■), RpSpRp (▼), and SpSpRp (◆).

and SpRpSp tetramer cores will activate RNase L in both methods at a concentration of 10^{-7} M. Subsequently the RpSpRp and SpSpRp tetramer cores are less active and require a concentration of 10^{-6} M to activate RNase L. However, the 2'-5'-phosphorothioate tetramer SpSpSp as well as authentic 2-5 A_4 core are completely inactive at concentrations as high as 10^{-3} M.

These results show that the phosphorothioates with only Sp chirality in each internucleotide linkage are completely inactive 2-5 A analogs and will be useful as antagonists of 2-5 A action. Recently the antagonistic properties of pSpSpSp were exemplified in VSV infected HeLa cells[4]. The comicroinjection of 2-5 A and pSpSpSp analog into HeLa cells, followed by infection with VSV, completely antagonised the antiviral activity of 2-5 A.

Additionally, the highly stable and phosphodiesterase resistant 2'-5'-phosphorothioates have also been shown to act as effective inhibitors of HIV-1 reverse transcriptase and HIV-1 infection[10].

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