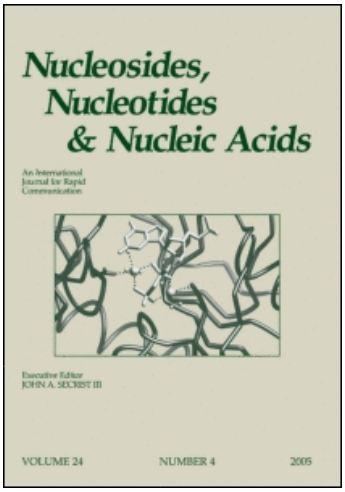

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The Chemical Syntheses of (2'-5')-P-Thioadenylate Dimers, Trimers and Tetramers

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THE CHEMICAL SYNTHESES OF (2'-5')-P-THIOADENYLATE DIMERS, TRIMERS AND TETRAMERS

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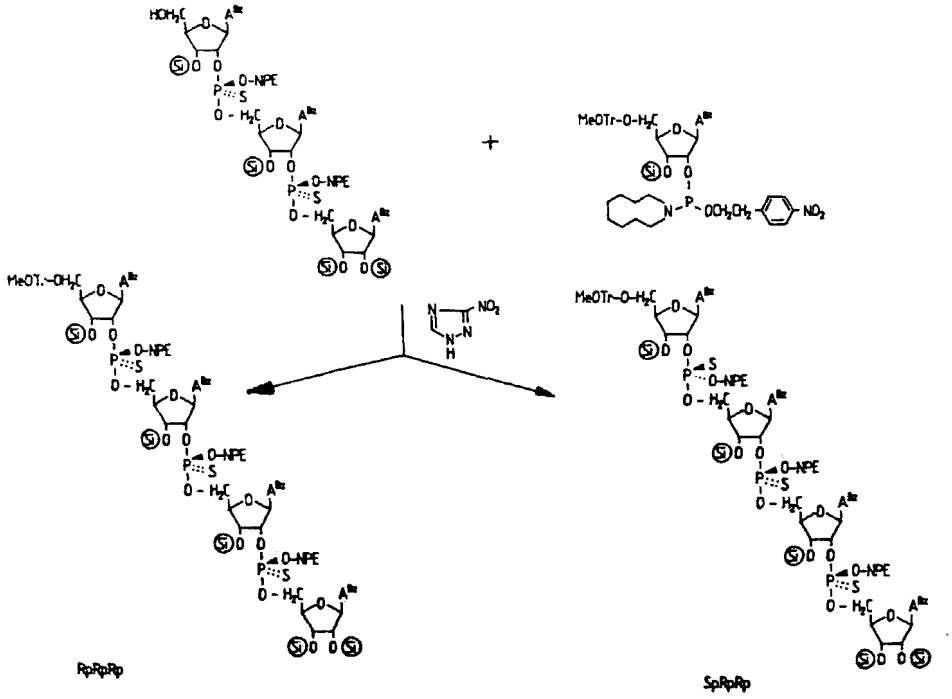
Abstract. The dimeric, trimeric and tetrameric 2'-5'-adenylate phosphothioates were synthesized via the phosphoramidite method using the p-nitrophenylethyl (NPE) group for phosphate protection and followed by sulfur oxidation. The various diastereoisomers were separated by chromatographical means and characterizes in their structures.

The 2'-5'-oligoadenylate (pppA2'p5'A2'p5'A) system is widely accepted to be involved in the antiviral mechanism of interferon[1] and plays an important role in the regulation of cell growth and differentiation. Due to the presence of phosphodiesterases, the phosphodiester linkages of 2-5 oligo As are rapidly degraded[2] and leads to loss of activity. To suppress the digestion several synthetic analogues having modifications in the base[3], the sugar[4] and the phosphate moiety[5] have been prepared. Interesting aspects are expected by the introduction of phosphorothioate functions, which will lead in the case of trimeric 2-5 A to four stereoisomers, which prove beneficial as mechanistic probes to investigate the action of 2'-5'-oligo As.

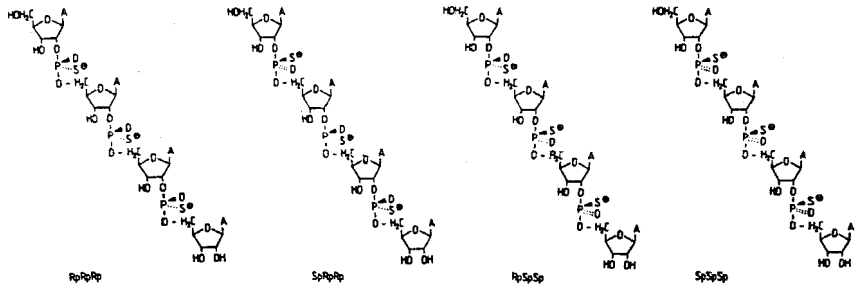
P. Nelson et al.[6] have published the syntheses of dimeric and trimeric phosphorothioates using the phosphite triester approach. Van Boom et al.[7] have recently reported the syntheses of individual diastereomers of 2-5 A core analogues containing one phosphorothioate linkage between the middle and 2'-terminal adenosine unit, whereas Eppstein et



al.[8] have investigated the metabolic stabilities and antiviral activities of RpRp/SpRp and RpSp/SpSp racemic mixtures.

We successfully achieved the syntheses and separation of the two isomeric dimers Rp and Sp, the four isomers of the trimers RpRp, SpRp, RpSp, and SpSp[9] and have now succeeded to prepare four of the eight tetrameric isomers RpRpRp, SpRpRp, SpSpSp and RpSpSp and characterized them by physical means. The syntheses were based upon the two fully protected diastereoisomers RpRp and SpSp thiophospho-trimers, which have first been detritylated and then reacted with N^6-benzoyl-3'-O-tert.butyldimethylsilyl-5'-O-monomethoxytrityl-adenosine-3'-(p-nitrophenylethyl, N-octahydro-azonino)-phosphoramidite[10] by 3-nitro-1,2,4-triazole activation and subsequent oxidation by sulfur to give the diastereoisomeric mixtures RpRpRp + SpRpRp and SpSpSp + RpSpSp respectively.

The separations of the mixtures can be achieved chromatographically on preparative silica gel plates to give yields of 30-50 % each. The final deblocking of the various protec-



ting groups in the four fully protected tetramers was performed by subsequent treatment first with DBU to remove the NPE groups, then with fluoride ion to achieve desilylation and thereafter with ammonia to cleave the benzoyl groups. In the final step the monomethoxytrityl group is removed by acid treatment and then the crude product purified by DEAE Sephadex chromatography yielding 70-80 % of each of the four tetranucleoside-tri-phosphothioates RpRpRp, SpRpRp, SpSpSp and RpSpSp.

The structural and absolute configurational assignments of the tetramers were accomplished analogously to the dimers and trimers[11-13] by HPLC, charge separation, ^{31}P-NMR spectra and enzymatic hydrolyses in comparison to enzymatically synthesized compounds.

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