SYNTHESIS AND BIOLOGICAL ACTIVITY OF A TUBERCIDIN ANALOG of 2-5A

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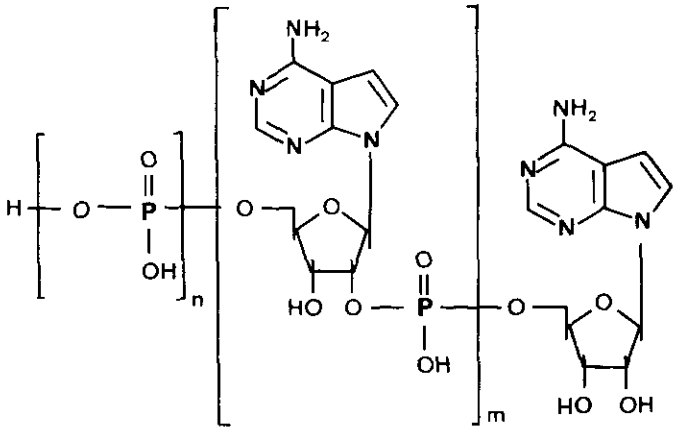
Previous studies from this laboratory have demonstrated that oligonucleotide binding to the 2-5A-dependent endonuclease (RNase L) of mouse L cells decreases dramatically upon substitution of the adenine rings of 2-5A by uracil, cytosine, or hypoxanthine. To explore the structural requirements for base binding more closely, we prepared the tubercidin analog of 2-5A [ppp5'-(c^7A)2'p5'(c^7A)2'p5'(c^7A)] by lead ion-catalyzed polymerization of 5'-phosphoroimidazolide of c^7A to give 2',5'-($pc^7A)_3$ which was converted to the triphosphate via pyrophosphate displacement on the phosphoroimidazolide. This material and intermediates were thoroughly characterized by enzymatic digestions and NMR. The 5'-monophosphate, 2',5'($pc^7A)_3$, as well as the 5'-triphosphate, 2',5'-pp($pc^7A)_3$, were bound to RNase L of mouse L cells approximately as well as 2-5A itself as judged by displacement of radiolabeled ppp5'A2'p5'A2'p5'A2'p5'A3'p5'Cp from the nuclease. Nonetheless, the 5'-triphosphate, 2',5'-pp($pc^7A)_3$ did not activate RNase L as judged by its ability to inhibit protein synthesis in encephalomyocarditis viral RNA-programmed extracts of L cells under conditions where 2-5A caused a 50% inhibition of translation at 10^{-9}M. These results suggest that while the N-7 atoms of the adenine rings of 2-5A may not be involved in binding to RNase L, they are critical for the activation of the enzyme.



m = 1, 2, 3

n = 0, 1, 2, 3