THE SYNTHESIS OF 5' THIOANALOGS OF POLYDEOXYRIBONUCLEOTIDES

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Summary: Oligo 5'-thiodeoxythymidylates and oligo 5'-thiodeoxyadenylates were prepared via displacement of tosylates by thiophosphate mono- and diesters. The 5' ends contain O-tosyl or S-phosphoryl groups, while the 3' ends terminate in deoxythymidine. The types of starting materials used for such polymerization were 5'-O-tosyl nucleoside 3' cyanoethyl phosphorothionates and the corresponding monoesters. Both oligonucleotide analogs possess secondary structure according to spectral evidence.

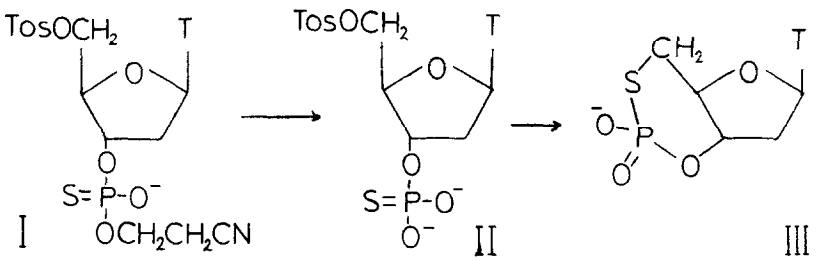
The substitution of the 5' oxygen in polydeoxyribonucleotides with sulfur represents a minimal structural deviation which might pass unrecognized by some important enzymes of nucleic acids metabolism. The interest in such polynucleotide analogs is further evoked by the attractiveness of their synthesis *via* displacement reaction. Since Akerfeldt's early synthesis of S-alkyl phosphorothioates (1), his method has been applied to the synthesis of the nucleotide analog 5'-thio-IMP (2) and two dinucleoside monophosphate analogs (3). While displacement reactions involving 5' tosylates and nucleoside monophosphates require relatively high temperatures (4), monoesters of thiophosphoric acid may react even in the cold. In the course of our studies on the stepwise synthesis of 5'-thiooligothymidylates, we found that sterically unhindered diesters of phosphorothionates, although quite stable in water at 20°, also participate in displacement in suitable solvents like dimethylsulfoxide and N,N-dimethylformamide. Here we report on polymerization experiments involving both mono- and diesters and resulting in the formation of oligo 5'-thiodeoxythymidylates and oligo 5'-thiodeoxyadenylates.

The starting materials 5'-O-tosyldeoxyribonucleoside 3' cyanoethyl phosphorothionates were prepared as follows. 5'-O-tosylthymidine (5) was reacted with an excess of $PSCl_3$ in pyridine solution (5°, 6 hrs); then treated

sucessively with hydracrylonitrile and $Et_3NH \cdot HCO_3$, and the reaction product was purified on a DEAE cellulose column. 5'-O-Tosylthymidine 3' cyanoethyl phosphorothionate(I) thus obtained in 25% yield was homogeneous on paper chromatography, electrophoresis and TLC*. In similar manner, 5'-O-tosyl-N^6-benzoyldeoxyadenosine 3' cyanoethyl phosphorothionate and 5'-O-tosyl-N^6-acetyldeoxycytidine 3' cyanoethyl phosphorothionate were prepared in 49 and 15% yield, respectively.

On treatment with dilute alkali at 20°, I and the corresponding deoxyadenosine and deoxycytidine derivatives hydrolyzed to the monoesters, type II, which underwent cyclization to the nucleoside 5'-S:3'-O-cyclic phosphates (III, Scheme 1, example of thymidine). The full analysis of the crystalline III including NMR, ORD and CD spectra has been completed, and it confirms the proposed structure.

Scheme 1



The polymerization of I was carried out at a scale of 0.1 mmole in concentrated dimethylformamide solution at 45° for one week. This was followed by a few hours exposure to an excess of 5'-O-tosylthymidine and by hydrolysis with ammonia. The separation of the shortest oligomers was performed by gel filtration on a column of Sephadex G-25 in 0.1 M triethylammonium bicarbonate. (Figure 1). The last two peaks comprised almost pure cyclic mono- and dinucleotide which were resistant to purified snake venom phosphodiesterase. The oligomers larger than trimers were further distributed on Sephadex G-50

*The following solvent systems were used: 2-propanol-conc. NH_4OH-H_2O 7:1:2, 1-butanol-ethanol-0.5 M CH_3COONH_4 5:3:2, for paper chromatography; 1-butanol acetone-H_2O 8:1:1, for TLC; and 0.05 M Na-phosphate pH 7.2, for electrophoresis.

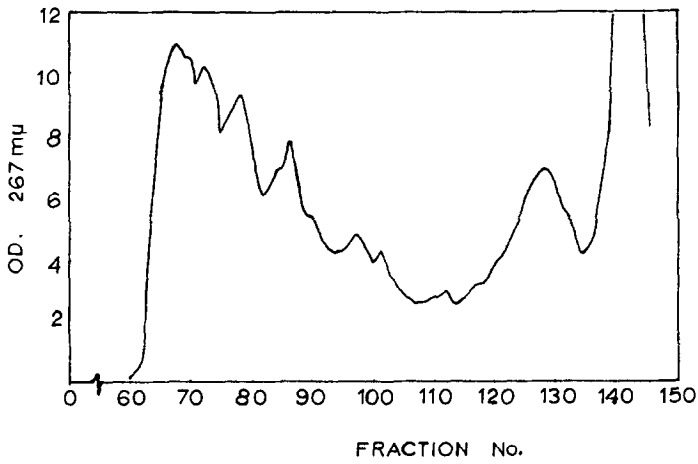


Figure 1. Separation of oligomers on Sephadex G-25 (2.5 x 100 cm) in 0.1 M triethylammonium bicarbonate; fractions (2.4 ml) collected every 10 min.

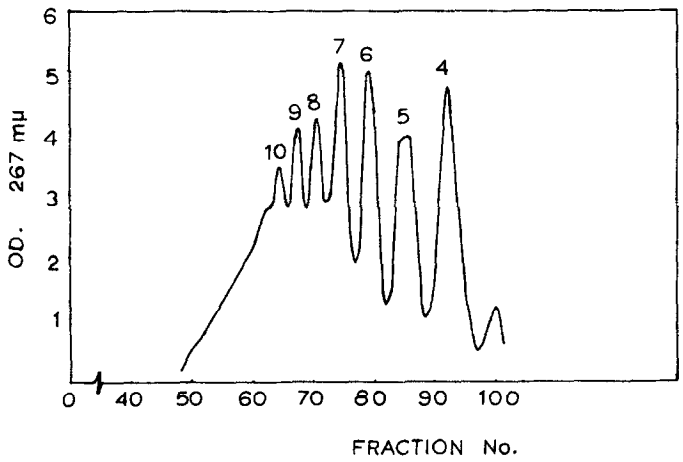
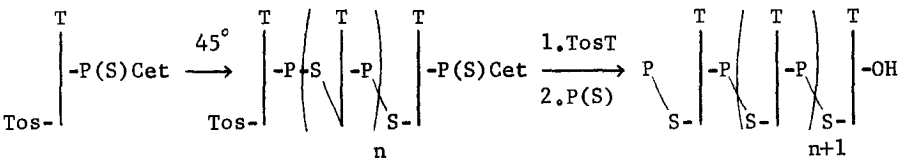


Figure 2. Fractions 60-95 from Figure 1 redistributed on Sephadex G-50 superfine (2.1 x 109 cm) in 0.1 M triethylammonium bicarbonate; fractions (2.4 ml) collected every 10 min. The numbers above the peaks indicate the chainlength.

according to Narang *et al.* (6). In Figure 2, the peak of the decamer is still discernible, but longer polymers are clearly present. While the peaks 4 and 5 still contained some cyclic oligonucleotides, the preceding fractions consisted of linear oligonucleotides which were degraded by venom phosphodiesterase in rather sluggish manner. Thymidine 5'-S-thiophosphate (3) and

5'-O-tosylthymidine were identified as the products of the hydrolysis by paper chromatography and electrophoresis. An ORD and CD study to be published separately reveals the presence of secondary structures and brings further support for the correctness of the general formula shown in scheme 2.

Scheme 2



The more reactive monesters, 5'-O-tosylthymidine 3'-thiophosphate and 5'-O-tosyl-N^6-benzoyldeoxyadenosine 3'-thiophosphate, underwent polymerization in concentrated dimethylformamide or in water solutions at lower temperature. After 2-3 days at 20 to 35°, the polymerization was terminated by treatment with 5'-O-tosylthymidine, and, in some cases, by an additional treatment with inorganic thiophosphate (Scheme 2). The N-benzoyl protecting groups were split off with ammonia, and the oligomers were separated as described above. The chain length distributions were comparable to that obtained *via* diester polymerization. The oligo 5'-thiodeoxyadenylate fraction, n=5-8, exhibited the expected hypochromicity (7) on mixing with poly rU, while the oligo 5'-thiodeoxythymidylate, n=10, was only slightly hypochromic with poly rA at the ionic strength of 0.15 M at 20°.

The uncomplicated nature of these polymerizations provides a new incentive to further work on a variety of poly 5'-S-deoxyribonucleotides which should be investigated with respect to the action of DNA polymerase, RNA polymerase and polynucleotide ligase. The possibility of a purely chemical replication is implicit in this approach, which might have also some prebiotic relevance. Some of these poly 5'-thionucleotides are potential interferon inducers in analogy to other sulfur containing polynucleotides (8).

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