

## SYNTHESIS OF A DODECADEOXYRIBOOLIGONUCLEOTIDE CONTAINING A 3'-THIO ANALOGUE OF THYMIDINE PHOTODIMER

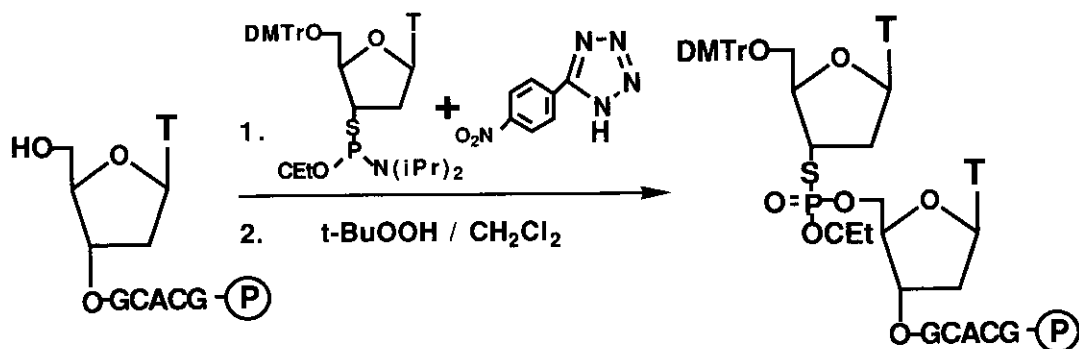
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**Abstract**---3'-Thio analogue of thymidine 3'-phosphate (Tsp) was incorporated into a dodecadeoxyribonucleotide at the 5'-side of dithymidylate to yield dGCACGTspTGCACG. The oligonucleotide was synthesized by the phosphoramidite method and converted to the *cis-syn* thymine dimer derivative by irradiation with uv, then isolated by high performance liquid chromatography.

DNA lesions have been investigated as a biologically important problem and several DNA repair enzymes are known to play essential roles in fidelity of DNA replication.<sup>1</sup> The *cis-syn* thymine dimer (T[c,s]T) is the major DNA lesion caused by uv lights and repair enzymes for this lesion as well as other pyrimidine photodimers such as (6-4) and Dewar photoproducts have been found to be related to genetic diseases such as xeroderma pigmentosum.<sup>2</sup> We have been studying recognition of the thymidine dimer by T4 endonuclease 4V, which hydrolyzes the glycoside linkage of the 3'-side thymine of the dimer then cleaves the 3'-phosphate by  $\beta$ -elimination.<sup>3</sup> Although site-directed mutagenesis<sup>4</sup> and the three dimensional structure obtained by X-ray crystallography<sup>5</sup> of the enzyme suggest several important amino acid residues are involved in its activities, it is necessary to investigate interactions between the enzyme and a substrate in detail. In this paper we report a synthesis of a substrate analogue that contains a thymine dimer bearing the 3'-deoxy-3'-thiothymidine on the 5'-side. 5'-O-Dimethoxytrityl-3'-deoxy-3'-thiothymidine was synthesized from 5'-O-dimethoxytritylthymidine<sup>6</sup> via the 2, 3'-O-cyclothymidine derivative. The cyclonucleoside was further treated with alkali to yield the 3'-xylo-3'-thio analogue, which was mesylated then treated with sodium thiobenzoate.<sup>7</sup> The resulting thio derivative was deacylated and the 3'-sulfhydryl groups phosphitylated with 2-cyanoethyl *N,N*-diisopropyl chlorophosphoramidite<sup>8</sup> to give the key intermediate for the synthesis of a dodecadeoxyribonucleotide (dGCACGTspTGCACG). The oligonucleotide was synthesized using a DNA synthesizer with a stronger activating reagent (p-nitrophenyltetrazole),<sup>7,9</sup> and a milder reagent (*tert*-butylhydroperoxide) for oxidation<sup>10</sup> for the thio derivative. The coupling reaction was repeated three times. (Scheme 1)

The dodecamer was purified by reverse-phase high performance liquid chromatography (hplc) and characterized by the susceptibility to iodine of the P-S bond. The purified dodecamer (Figure 1a) was treated with a mixture of iodine-water-pyridine to yield two hexamers as shown in Figure 1b. The uv-temperature profile of the analogue and its complementary strand was observed and the  $T_m$  found to be 59 °C in 0.01 M sodium cacodyl (pH 7.0) and 0.1 M sodium chloride. The  $T_m$  of dGCACGTspTGCACG with the complementary strand was 59 °C and that for the *cis-syn* thymine dimer duplex was 54 °C. These results indicate that the substitution of the 3'-O with 3'-S does not affect the thermal stability of the duplex.



d G C A C G T s p T G C A C G

(P) = controlled pore glass

CeT = 2-cyanoethyl

Scheme 1

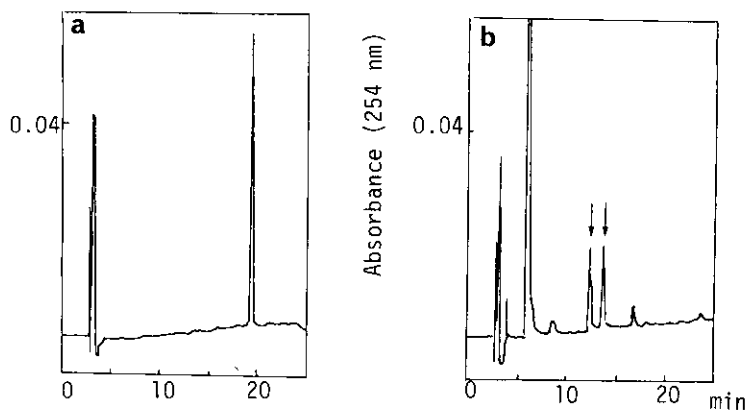


Figure 1. Hplc of oligomers.  
 (a) The dodecamer.  
 (b) Cleaved hexamers.  
 column: TSKgel DEAE-2SW  
 solv.A: 20%  $\text{CH}_3\text{CN}$   
 solv.B: 20%  $\text{CH}_3\text{CN}$  plus  
 2 M  $\text{HCOONH}_4$   
 gradient(B): 15 to 35%  
 20 min  
 flow rate: 1 ml/min

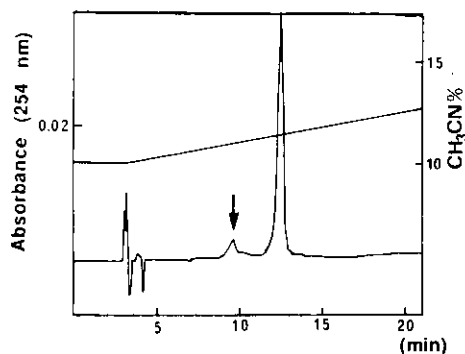


Figure 2. Isolation of the photo-product by hplc. The product indicated by an arrow was separated from the starting material (main peak).

column: CHEMCOSORB 5-ODS-H  
 solv.A: 0.1 M triethylammonium acetate plus  
 5%  $\text{CH}_3\text{CN}$   
 solv.B: 0.1 M triethylammonium acetate plus  
 25%  $\text{CH}_3\text{CN}$   
 flow rate: 1 ml/min

The thio analogue was converted to the thymine dimer derivative by irradiation with uv light around 280 nm using conditions described previously.<sup>3</sup> The product was isolated by reverse-phase hplc (Figure 2) and its photoreversal property confirmed by reirradiation. The major photoproduct was assumed to be the cis-syn isomer, since formation of the *trans-syn* isomer was proved to be difficult in an oligonucleotide. The photoproduct from the thio analogue was mixed with the complementary 15-mer to form a duplex and tested for its ability to bind to the endonuclease. The dissociation constant was found to be  $2.7 \times 10^{-7}$ , which is one order larger than the natural thymine dimer substrate. Cleavage of the glycosidic linkage and the phosphodiester linkage in the analogue duplex were also slower than those for the thymine dimer duplex with the same chain length. These results indicated involvement of the 3'-oxygen of thymidine at the cleavage site in the reaction with the enzyme. Detailed kinetics for the 3'-thio analogue will be reported elsewhere.

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