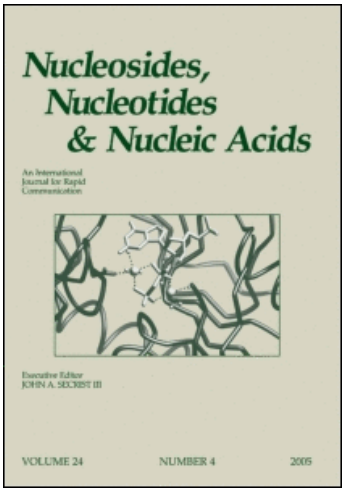

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Solid Supported Chemical 5'-Phosphorylation of Oligodeoxy-Ribonucleotides that can be Monitored by Trityl Cation Releasi Application to Gene Synthesis

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SOLID SUPPORTED CHEMICAL 5'-PHOSPHORYLATION OF OLIGODEOXYRIBONUCLEOTIDES THAT CAN BE MONITORED BY TRITYL CATION RELEASE APPLICATION TO GENE SYNTHESIS.

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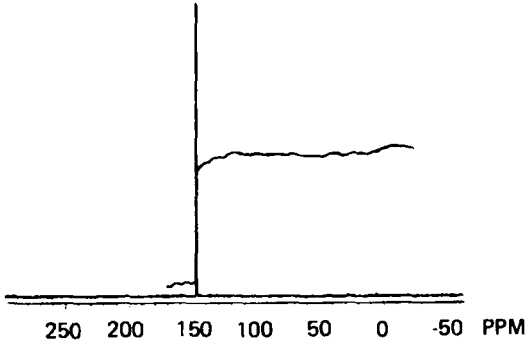
ABSTRACT: The gene for human lysozyme was assembled from oligonucleotides that were chemically phosphorylated with a novel phosphorylation reagent.

In order to circumvent the use of T4 polynucleotide kinase and ATP for the 5'-phosphorylation of synthetic oligodeoxyribonucleotides after deprotection and purification, we and others (1,2) have developed chemical phosphorylation reagents that can be used on automatic DNA synthesis instruments. However there is no convenient way to determine the coupling efficiency of these compounds.

We now report a phosphorylation procedure that can be monitored by the release of the orange dimethoxytrityl cation after acid treatment (3). The phosphite-derived compound (2-cyanoethoxy)-2-(2'-O-4,4'-dimethoxytrityloxyethyl sulfonyl)ethoxy-N,N-diisopropylaminophosphine was designed

(a) t = 0

LINE #	HEIGHT	FREQ (HZ)	PPM
1	1283.88	10676.56	146.435
2	29.57	9897.53	135.750
3	21.66	430.02	5.898



(b) t = 14 days at 20°C (50 mM)

LINE #	HEIGHT	FREQ (HZ)	PPM
1	26.10	10718.33	147.008
2	28.81	10705.76	146.835
3	1599.02	10676.63	146.436
4	33.33	10649.60	146.065
5	31.10	854.14	135.750
6	29.20	854.14	11.715
7	32.28	428.33	5.874
8	50.03	382.11	5.240

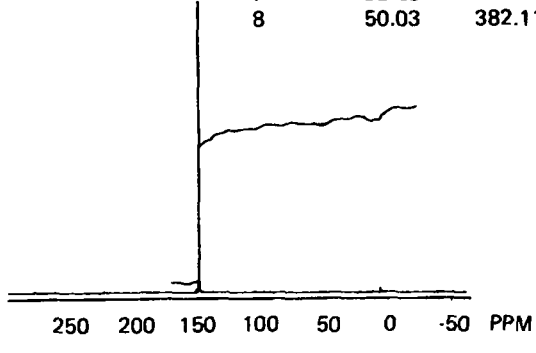
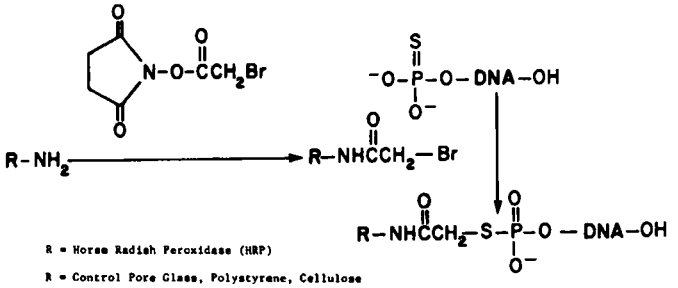


FIGURE 1. 31-P-NMR of phosphitylating compound.



SCHEME I.

and synthesized. The phosphitylating compound is very stable as determined by 31-P-NMR of a 50 mM solution in dry acetonitrile after a period of 14 days at 20 C (Figure 1). The reagent is coupled to solid-supported fragments and oxidized under the same conditions as nucleoside phosphoramidites. The resultant 5'-phosphate oligomers are then fully deprotected in standard ammonium hydroxide solution at elevated temperature.

Reverse-phase HPLC, silica gel TLC and 31-P-NMR analyses of chemically phosphorylated thymidine were compared with a variety of phosphorus derivatives of thymidine. All evidence suggested that the nucleoside was converted to the 5'-phosphate form quantitatively with the reagent. By substituting sulfur for iodine in the oxidation step (4) the 5'-phosphorothioate was obtained in high yield.

The 5'-phosphorothioate oligonucleotides can be used with maleimido- and bromoacetyl-containing reagents to

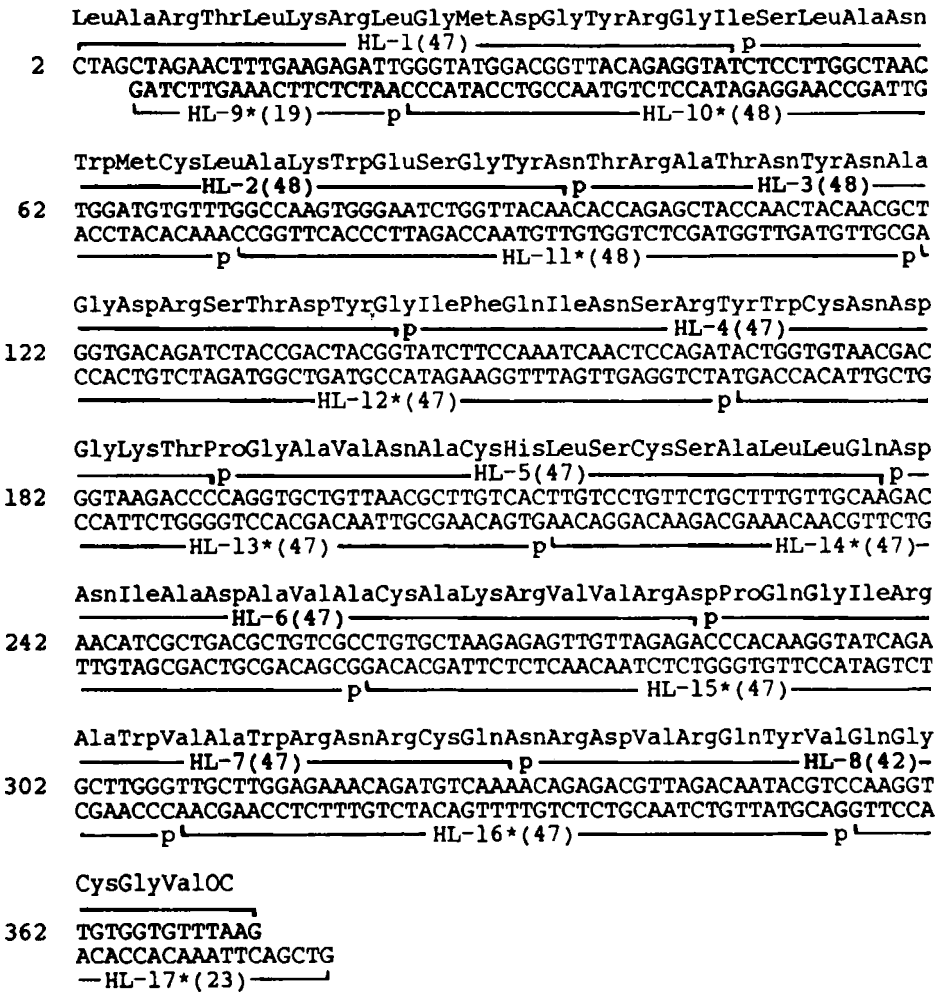


FIGURE 2. Human lysozyme amino acid- and synthetic gene sequences.

introduce non-radioactive labels (such as horse radish peroxidase) and to immobilize DNA to solid supports (5) (See Scheme 1).

A complete gene for human lysozyme (373 bp) was assembled from oligonucleotides that were chemically phospho-

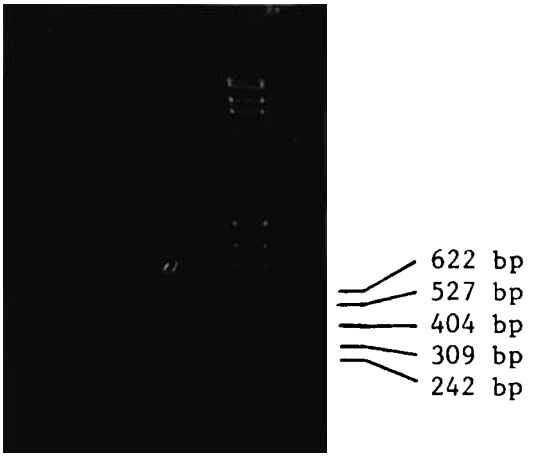


FIGURE 3. Agarose gel analysis of synthetic human lysozyme ligation (lane 1). Lane 2 is pBR322 cut with Hpa2; the sizes are shown on the right. Lane 3 is ØX174 DNA cut with Hae3.

rylated with the new reagent. The gene was designed from the amino acid sequence using yeast prefered codons (6). The sequences of the 17 oligomers employed (17 to 48 nucleotides long) are shown in Figure 2, where the chemically phosphorylated 5' ends are indicated with "p". Analysis in a 1.5% agarose gel of the "one-shot" ligation of the 17 fragments shows one band of the appropriate size (Figure 3). Through restriction map analysis, 10 out of 12 clones were shown to contain the correct size insert. Subsequent DNA sequence analysis of two of these clones was in complete accord with the designed oligonucleotides.

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