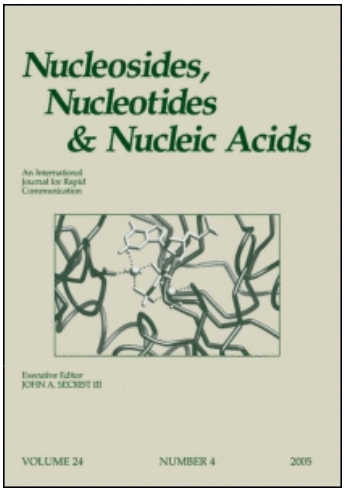

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THE SYNTHESIS OF BRANCHED OLIGONUCLEOTIDES AS SIGNAL AMPLIFICATION MULTIMERS FOR USE IN NUCLEIC ACID ASSAYS

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Abstract: Branched oligonucleotides have been synthesized using phosphoramidite derivatives with two protected hydroxyl functions. These molecules are employed for a label amplification strategy used in DNA probe diagnostics.

Organisms, such as human immunodeficiency virus and non-A, non-B hepatitis virus, occur in low numbers in infected human blood. Direct detection by immunoassay methods has proven difficult. The sensitivity necessary (probably 10-1000 organisms) may be possible with nucleic acid assay methods.

Our approach to the sensitivity problem has been to develop signal amplification techniques that can permit the specific attachment of many labels per hybridization probe-target nucleic acid complex. We have previously reported the synthesis of large oligonucleotide complexes (amplification multimers) for this purpose (1). The branched nucleic acids, which contain multiple sites for the hybridization of small enzyme labeled probes (2) were synthesized in solution by chemical cross-linking of oligonucleotides with three evenly spaced alkylamine cytidine residues. Amplification multimers have proven very useful in nucleic acid analysis since many labels can be incorporated specifically.

We have developed new methods for the controlled automated synthesis of amplification multimers containing hundreds of

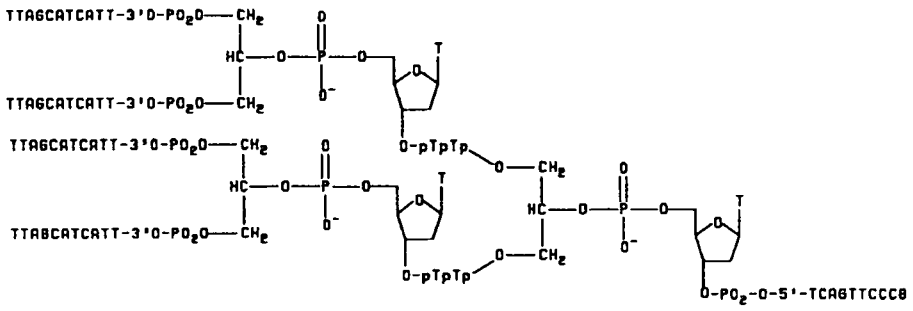

FIG. 1 "Fork" type of branched DNA.

nucleotides. These nucleic acid networks have been designed to maximize the number of labels that can be bound to the probe-target complex and to minimize non-specific binding. The approach involves the use of a new class of phosphoramidite reagent that, upon coupling to a solid phase synthetic oligonucleotide, introduces two hydroxyl functions. After limited deprotection, these two hydroxyl functions act as branch points from a primary fragment for the synthesis of multiple copies of secondary oligonucleotides. Multiple incorporations of branch points allow for the synthesis of as many as 32 copies of a secondary sequence on a primary fragment.

Two types of branched DNA have been synthesized: "fork" and "comb" structures. Fork structures are synthesized by protecting both hydroxyl groups of the branching phosphoramidite reagent with the same function. Branching occurs from the 5'-terminus. The compound 2-[1,3-bis-(4,4'-dimethoxytrityl)glyceryloxy]-methoxy-N,N-diisopropyl-amino-phosphine has been employed for the synthesis of forked oligonucleotides (Figure 1). Comb structures, on the other hand, require two different hydroxyl protection functions that can be removed under different conditions. For instance, a dimethoxytrityl and either FMOC or levulinyl protection can

be used. After the incorporation of several comb branching derivatives in the primary sequence, the FMOC or levulinyl protection is removed with t-butylamine/ pyridine or hydrazine hydrate/pyridine/acetic acid, respectively. Subsequent nucleoside phosphoramidite coupling yields secondary fragments from internal points within the primary sequence.

It has been necessary to modify blocking group strategies and deprotection reagents for efficient synthesis of these molecules. The position and spacing of the branches has proven important for maximization of enzyme labeled probe binding.

The new amplification multimers have been successfully applied in a rapid and non-radioisotopic assay format to the analysis of several target nucleic acids, including the hepatitis B virus, a plasmid of Chlamydia trachomatis, two genomic sequences of Neisseria gonorrhoeae, and the ß-lactamase gene, TEM-1, and the tetracycline resistance determinant, tetM, in several bacteria. As few as 1000 organisms have been reliably detected.

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