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SYNTHESIS OF BRANCHED DNA WITH A COMB STRUCTURE

Introduction

By far the most common approach to DNA diagnostics is amplification of the target sequence to produce enough copies for a signal to be observed using conventional detection systems. However, an alternative approach does exist - direct analysis of the target DNA by signal amplification. This latter technique requires that the synthetic oligonucleotide should contain the primary sequence attached to many identical copies of the secondary sequence. It is the detection of the many copies of the secondary sequence that serves to amplify the signal. The resulting branched oligonucleotide has been aptly described as comb-like, with the primary sequence in the handle and the secondary sequences being the teeth of the comb.[1] Using this strategy, as few as 50 molecules of the HIV-1 genome have been quantified in human serum.[2]

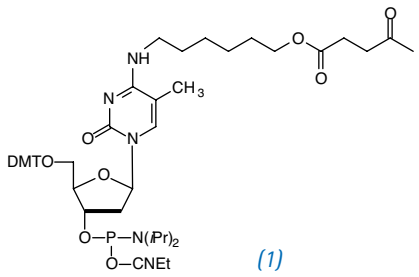
A branching monomer is clearly required to construct comb-like oligonucleotides. For a monomer to generate a branch point in an oligonucleotide sequence, it requires an intermediate with 3 hydroxyl groups. The first hydroxyl is used to generate the phosphoramidite that is necessary for coupling. The second is protected with a DMT group for normal deblocking with acid prior to chain elongation. The third should be protected with a group that is stable during the synthesis of the primary sequence, but is easily removed after completion of the primary sequence, allowing the secondary sequences to be prepared by further synthesis cycles on the original synthesis column. The developers of the comb system from Chiron Corporation evaluated[3] several protecting groups for the third hydroxyl and chose levulinyl (LEV), which is specifically removed using a reagent containing hydrazine hydrate, acetic acid and pyridine. Although non-nucleosidic structures were evaluated, the authors[3] chose to use the dC derivative (1) as the branching monomer.

Synthesis of Simple Branch Structures

This product is very straightforward to use in the synthesis of branched oligos with only a few branch points, as shown in Table 1.

If a non-branching control is desired, simply deprotect in ammonium hydroxide as required by the protecting groups on the nucleobases.

FIGURE 1: **dC BRANCHER PHOSPHORAMIDITE**



(1)

Synthesis of Complex Comb Structures

The synthesis of complex structures is much more involved but the authors[4] offer some suggestions to streamline the process, as shown in Table 2. Using this general procedure, the authors were able to prepare comb oligonucleotides containing as many as 50 branch sites.

References:

(1) T. Horn and M.S. Urdea, *Nucleic Acids Res*, 1989, **17**, 6959-67.
(2) M.L. Collins, et al., *Nucleic Acids Res*, 1997, **25**, 2979-84.
(3) T. Horn, C.A. Chang, and M.S. Urdea, *Nucleic Acids Res*, 1997, **25**, 4842-4849.
(4) T. Horn, C.A. Chang, and M.S. Urdea, *Nucleic Acids Res*, 1997, **25**, 4835-4841.

TABLE 1: SYNTHESIS OF SIMPLE BRANCHED OLIGONUCLEOTIDES

- Carry out the synthesis of the primary sequence with no changes from the regular synthesis cycle.
- Remove the synthesis column from the synthesizer.
- Remove the levulinyl protecting group (Lev) selectively without cleavage of the oligonucleotide from the CPG by treatment with 0.5 M hydrazine hydrate* in 1:1 pyridine/acetic acid.
- Fit the column with syringes and push the solution back and forth across the column.
- Leave for 15 minutes at room temperature.
- Rinse the column with 1.5 mL of 1:1 pyridine/acetic acid (3x) and then 1.5 mL of ACN (3x).
- Dry the CPG support with an argon stream and proceed with the synthesis of the branching sequence.

TABLE 2: SYNTHESIS OF COMPLEX COMB STRUCTURES

Primary Sequence

- Synthesize the primary target detecting sequence on 2000 Å CPG using the standard synthesis cycle.
- Add an additional T20 to the 5' of the target sequence to distance the comb structure from the CPG surface.
- Add dC Brancher Phosphoramidite (B) using the general formula (BTT)n.
- Remove the synthesis column from the synthesizer and using a pair of disposable syringes, remove the Lev protection using 10 mL of 0.5 M hydrazine hydrate* in pyridine:acetic acid (1:1). Pass the solution back and forth through the column for a minimum of 15 minutes. Sequences with many branches may require a longer treatment of 90 minutes.
- Rinse the column with 1.5 mL pyridine:acetic acid (1:1) (3X) and then with 1.5 mL acetonitrile (3X).
- Dry the CPG support with an argon stream and proceed with the synthesis of the secondary "comb" sequence.

Comb Sequence

- Change the synthesis cycle to increase the amidite delivery to correspond to the increase in synthesis scale, (e.g., a 1-µmole synthesis with a 15 branch comb now becomes a 15-µmole synthesis). This can be achieved by increasing the number and time of amidite: activator deliveries.
- Increase the coupling wait time to 3 minutes to maximize coupling efficiency.

***NOTE: HYDRAZINE HYDRATE IS A VIOLENT POISON THAT IS BOTH VOLATILE AND READILY ABSORBED THROUGH SKIN. USE APPROPRIATE SAFETY PRECAUTIONS.**