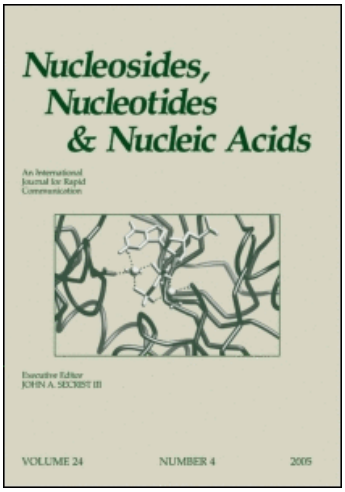

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DEOXYOLIGONUCLEOTIDES WITH INVERTED POLARITY: SYNTHESIS AND USE IN TRIPLE-HELIX FORMATION

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Abstract: Deoxyoligonucleotides with inverted polarity (containing a 3'-3' junction) are described. The ability of these oligonucleotides to bind to duplex DNA utilizing various linker elements is discussed.

Triple-helix formation by deoxyoligonucleotides has become an area of intense research [1]. At the present time only polypurine tracts are recognized by the third strand, limiting the number of target sites in duplex DNA. The third strand of a triple-helix lies in the major groove and allows for the possibility to bind to alternate strands of the duplex DNA by crossing the major groove. In an effort to increase the number of biologically relevant targets for triple-helix formation we have utilized DNA with inverted polarity (at least one 3'-3' linkage) to allow for binding to opposite strands of duplex DNA. Recently, Horne and Dervan reported alternate strand triple-helix formation utilizing 1,2-dideoxyribose as the linker element[2]. Reported herein is the results of our work on alternate strand recognition. An oligonucleotide with inverted polarity, and the proper linker element, is able to switch back from one purine tract to another on opposite strands of the target DNA. We have termed this type of deoxyoligonucleotide a 'switchback'.

The target sequence and binding scheme chosen for this work is shown in Figure 1, the corresponding switchback oligonucleotide has a 3'-3' internucleotide junction and two 5' ends. At the junction point the duplex DNA contains base pairs that do not form hydrogen-bonds with the third strand and are referred to as null base pairs. Figure 1 shows that there is two separate binding domains, one on each strand of the duplex. The length of the oligonucleotides for this study was 18 bases, 9 bases recognizing each binding domain separated by the linker element.

The synthesis of deoxyoligonucleotides with inverted polarity has been described and requires the use of 3'-DMT protected nucleosides [3]. The deoxyoligonucleotides were prepared by standard H-phosphonate chemistry [4] with the first nucleoside attached to the CPG via the 5' hydroxyl.

1 Null Base Pair

```
          5' C T T T T T C T T 3'
5' A G A A A G A A A A A G A A A T T T C T T C T T T T T C T 3'
3' T C T T T C T T T T T C T T T A A A G A A G A A A A A G A 5'
                              3' T T T C T T C T T T 5'
```

2 Null Base Pairs

```
          5' C T T T T T C T T 3'
5' A G A A A G A A A A A G A A A A T T C T T C T T T T T C T 3'
3' T C T T T C T T T T T C T T T T A A G A A G A A A A A G A 5'
                              3' T T C T T C T T T T 5'
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FIGURE 1: Triple-helix binding scheme for 3'-3' switchback oligonucleotides

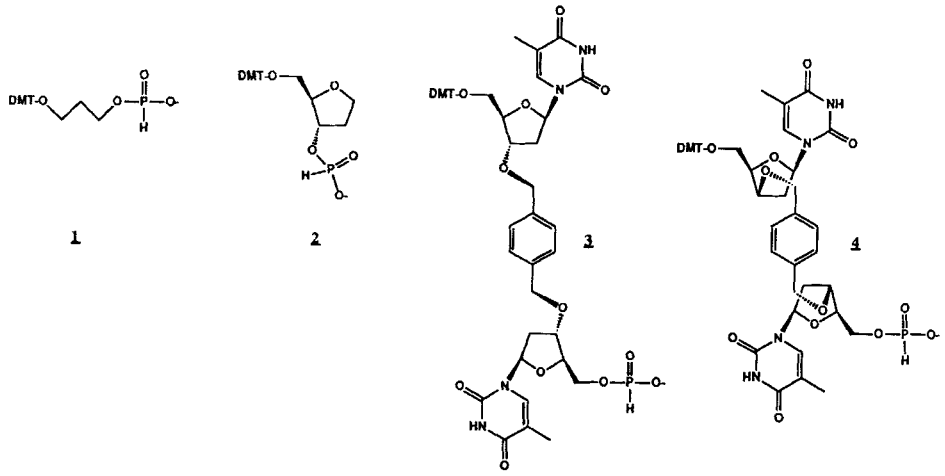

FIGURE 2: 3'-3' Linkers; 1) Propylene glycol (PG)
2) 1,2-dideoxyribose (ddR) 3) p-dRibose dimer
4) p-dXylose dimer

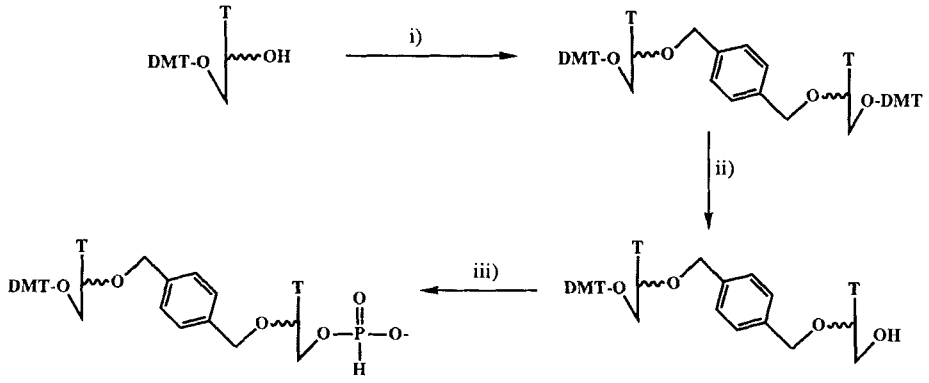

FIGURE 3: i) NaH (xs) / NaI (1 eq) / α,α' dibromo-p-xylene (0.5 eq) / THF / 45° C / 24 hrs; ii) 5% DCA CH_2Cl_2/MeOH (1/1) / 15 min / 25° C; iii) phosphitylation [5]

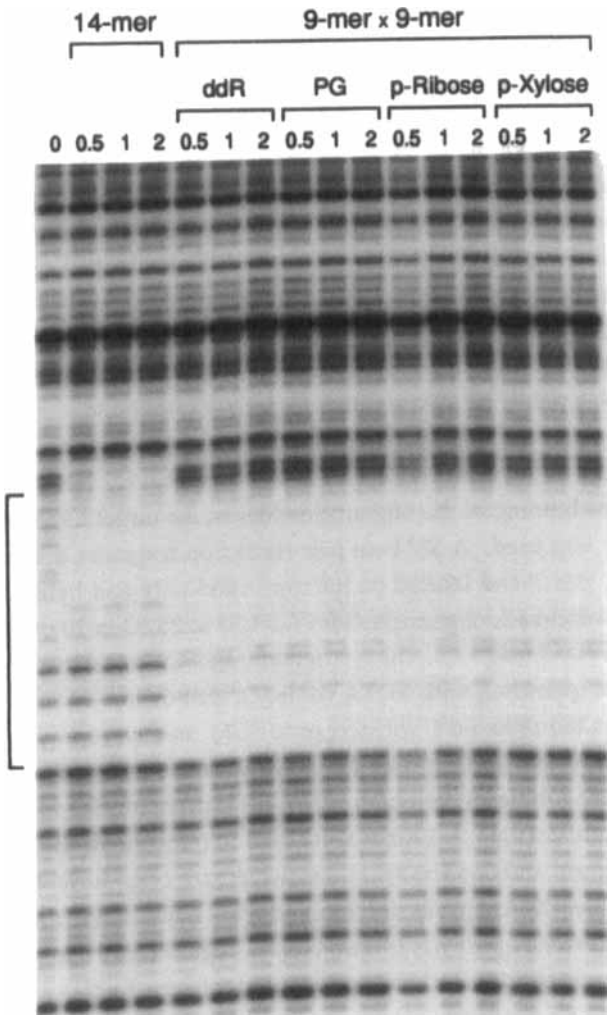

FIGURE 4: Audioradiogram derived from DNAse footprint analysis of 9x9 switchback deoxyoligonucleotides. 6% polyacrylamide/7 M urea gel. Concentration of third strand is 0.5, 1.0 and 2.0 μM
14-mer: Control for one binding domain;
ddR: 1,2-dideoxyribose linker (2), 2 null base pairs;
PG: propylene glycol linker (1), 2 null base pairs;
p-Ribose: 2'-deoxyribofuranosyl thimine dimer (3), 2 null base pairs;
p-Xylose: 2'-deoxyxylofuranosyl thimine dimer (4), 1 null base pair.

Two approaches to link the two binding domains were investigated, namely; 1) diol derived linkers and 2) ether linked dinucleosides. The diol derived linkers were prepared from propylene glycol(1, PG) and 1,2-dideoxyribose (2, ddR) (Figure 2). Two types of ether linked dimers were prepared, linkers derived from 2'-deoxyribofuranosyl thymine (3) and 2'-deoxyxylofuranosyl thymine (4) (Figure 2). The ether linked dimers were synthesized with a DMT protecting group at one of the 5'- hydroxyls and a H-phosphonate at the other 5'- hydroxyl as shown if Figure 3. Incorporation of the dinucleoside into the deoxyoligonucleotide provides the inverted junction with the dinucleoside becoming the first bases in each binding domain. Because of the difference in configuration of the two sugars it would be expected that the two ether linked switchbacks would bind to target sites with different null base pairs, the xylose dimer (4) would target one less null base pair than the ribose dimer (3). Because of the central null base pairs in the target these two oligonucleotides will target a 19-mer and 20-mer respectively (see Figure 1).

To assess binding of the oligonucleotides to the target DNA duplex a DNase protection assay was used. A 327 base pair restriction fragment, containing the target sequence insert, was 3'-end labeled on the top strand only and hybridized with the corresponding switchback oligonucleotides for 1 hr at 25 °C in 20 mM MES/10 mM $MgCl_2$/100 mM NaCl (pH = 6). The restriction fragment was digested for 1 minute with DNAse and quenched with EDTA (20 mM). Following ethanol precipitation the samples were loaded onto a 6% polyacryamide/7M urea gel, run until bromophenol blue reached the bottom and exposed for 24 hrs give the audioradiogram in Figure 4. The footprint data compares the oligonucleotides containing the four linkers at a conc. of 0.5, 1.0 and 2.0 μM. A 14-mer that binds to only one of the binding domains is included as a control and confirms that the switchback oligonucleotide binds at the correct binding site. DNAse protection of the duplex DNA by the switchback oligonucleotides extends past the 14-mer control region confirming that the switchback oligonucleotides are binding to both domains. By this assay the 18-mer switchback binds at a similar concentration as the 14-mer control.

Switchback oligonucleotides can extend the number of target sequences for triple-helix formation by allowing binding to alternate strands of the duplex. We have described the synthesis and binding of a number of oligonucleotides with 3'-3' junctions. In principle this could be expanded to include 5'-5' switchbacks as well.

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