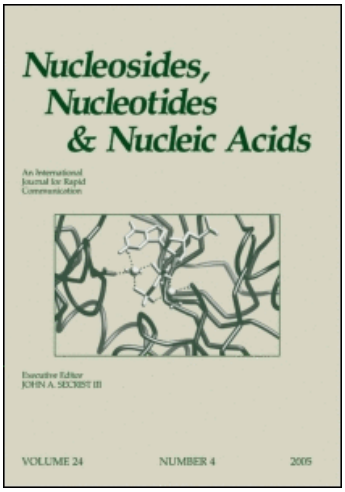

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Novel Biotin Phosphoramidites with Super-long Tethering Arms

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ABSTRACT

A number of novel biotin phosphoramidites, possessing exceptionally long and uncharged tethering arms, were synthesized from methoxyoxalamido (MOX) and succinimido (SUC) precursors. Included among these monomers is a uridine derivative with the biotin moiety attached through the 2′-position. Some of these phosphoramidites were used to make 5′-biotinylated primers, which were applied in direct sequencing of genomic DNA and capture of Sanger fragment pools.

Key Words: Biotin phosphoramidites; Biotin labeled oligonucleotides; Long tethering.

Biotin is widely used for DNA/RNA detection/isolation due to the extremely high affinity of the biotin-streptavidin interaction (association constant 10^{15}/M). There are numerous methods for the attachment of biotin moieties to oligonucleotides, but the easiest and most efficient is through the use of biotin phosphoramidites on the DNA/RNA synthesizer. We synthesized a number of novel biotin phosphoramidites with tethering arms of up to 39 atoms in length (Fig. 1). Among these is a

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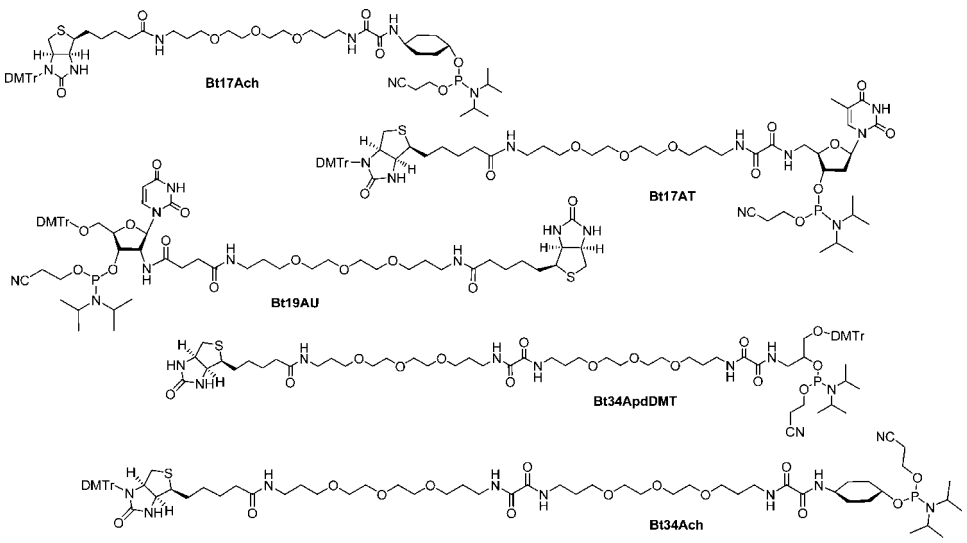

Figure 1. Biotin amidites with long tethers.

uridine derivative, Bt19AU, with a biotin moiety attached through the 2′-position. Our biotin phosphoramidites were synthesized based on proprietary methoxyoxalamido (MOX) and succinimido (SUC) precursor strategies from scaffolds having a phosphoramidite moiety attached through a secondary hydroxyl. To ensure efficient coupling of these amidites we used ETT as the catalyst. For the sterically more hindered Bt19AU the coupling time was set to 10 min. All other amidites were coupled within 3–5 min. They are highly stable in solution, since the coupling efficiency remains equally high for at least 2 weeks after phosphoramidite installation on the synthesizer, and are ideal for the streamlined production of biotinylated oligonucleotides.

Studies of oligonucleotide immobilization on a solid surface have shown that factors, such as charge density, rigidity, hydrophobicity, sterics, and tethering arm length, are crucial for optimal hybridization and nucleic acid processing events staged at the surface.[1–3] These are the factors that we kept in mind in the preparation of our ''super-long'' biotin amidites.

Our synthetic strategy enables us to synthesize not only tethering arms of extraordinary lengths, but also of diverse hydrophobicities. For this study we chose a relatively polar spacer diamine, 4,7,10-trioxa-1,13-tridecanediamine, since we believed it was the most suitable in terms of flexibility and biological applicability. MOX chemistry[4,5] is amenable to subsequent serial additions of MOX synthons on precursors to extend the length of our tethers to any desired length during monomer synthesis. The high reactivity of the MOX group toward primary aliphatic amines guarantees excellent step-wise yields throughout the synthetic pathway.

The oxalamido and amide bonds in our tethers are responsible for partial rigidity, and it is possible to increase this rigidity through the use of shorter spacer diamines coupled sequentially in greater numbers during monomer synthesis. Some rigidity in the linkers may be necessary to assure that the biotin moiety is sufficiently exposed and extended for steptavidin capture.

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All of the linker arms of our biotin amidites are uncharged, and this should be beneficial for applications involving immobilized oligonucleotides on a solid support, since electrostatic repulsion by the arms to target DNA is non-existent.[2] Our longest amidite, Bt34Ach, has a 39-atom tethering arm and lies within the optimal length for maximum hybridization yields.[2]

A number of 5′-biotinylated primers were synthesized using biotin phosphoramidites with both super-long tethering arms and with shorter ones to do a comparative analysis on their usage for direct sequencing of genomic DNA. These primers were used in sequencing reactions, and the Sanger fragment pools were subsequently captured with paramagnetic streptavidin beads in a 15 min incubation. The use of commercial BiotinTEG with a 16-atom linker gave a capturing efficiency of 35–40%. When the length of the linker was increased to 22 atoms in the case of Bt17Ach, the capturing efficiency accordingly increased to 40–45%. By utilizing the longest arm of Bt34Ach, it was possible to capture 90–95% of Sanger fragments. These results lead us to conclude that, by using exceptionally long uncharged tethers to attach biotin to oligonucleotides, we may be able to alleviate the problem of low binding capacity of long DNA fragments to surface-bound streptavidin.[6]

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