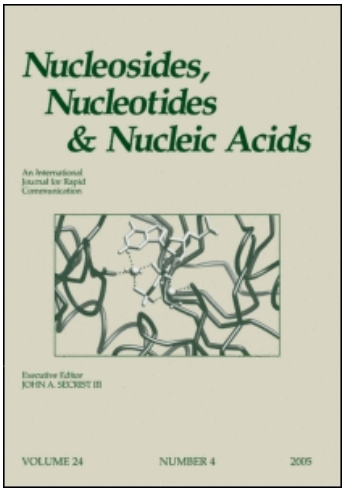

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Stability, Vigor, and Inherent Versatility of Novel Amino Linker and Spacer Phosphoramidites

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Stability, Vigor, and Inherent Versatility of Novel Amino Linker and Spacer Phosphoramidites

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ABSTRACT

Novel amino linker and spacer phosphoramidites were synthesized from methoxyoxalamido (MOX) percursors possessing a secondary hydroxyl, which when phosphitylated endowed stability to the corresponding phosphoramidites. The synthetic strategy is robust, and the chemistry is reactive towards a variety of primary aliphatic diamines and amino alcohols to produce distinctly unique phosphoramidites. The selection of building blocks determines the length and physico-chemical properties of the phosphoramidite tethering arms, and the synthesis can be specifically tailored to suit individual requirement.

Key Words: 5′-Amino modifier phosphoramidites; 5′-Spacer phosphoramidites; Methoxyoxalamido precursor-based synthetic methodology.

Amino modified oligonucleotides are used in DNA microarray production and as intermediates to functionally labeled oligonucleotides.[1,2] Spacers are commonly used to substitute for unknown bases in a sequence, to incorporate hairpin loops, and to position labels at a desired distance from the oligonucleotide.[3,4] The majority of commercially available linkers and spacers are not suitable for reliable large-scale oligonucleotide production because they are not very stable in solution, and the need

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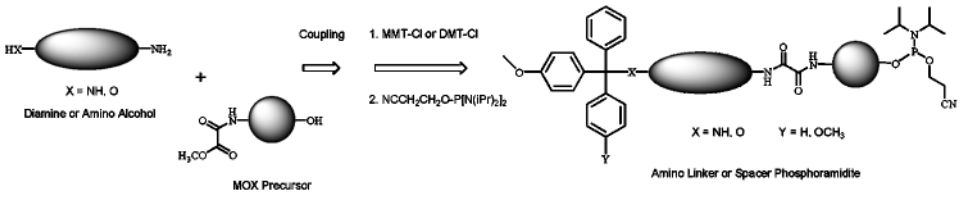

Scheme 1. Synthetic pathway.

to put a freshly-made reagent bottle on the synthesizer substantially increases the production costs for modified oligonucleotides. In addition, the variety of available amino linker and spacer phosphoramidites is limited, and its chemistry is not amenable to ''customization''. Thus, we have designed and synthesized a number of novel amino linker and spacer phosphoramidites based on our proprietary methoxyoxalamido (MOX) precursor strategy that makes up for these shortcomings.

Amino linkers and spacers synthesized from MOX precursors containing a scaffold with a secondary hydroxyl are highly stable in solution. The chemistry[5,6] allows us to synthesize amino linker and spacer phosphoramidites by reaction of the parent precursor with any commercially available primary aliphatic diamine or amino alcohol (Sch. 1).

RESULTS AND DISCUSSION

Based on ^{31}P NMR time course experiments, our phosphoramidites routinely have 50–60% greater stability compared to commercially available ones having a phosphoramidite moiety attached through a primary hydroxyl (data not shown). The coupling yield of our amino linkers and spacers remains high such that modified oligonucleotides can be efficiently made 3–4 weeks after phosphoramidite installation on the synthesizer. This translates to an overall cost savings to the end-user who sporadically incorporates these linkers and spacers into oligonucleotides, since a fresh phosphoramidite preparation per use is no longer necessary. PAGE analysis of amino modified oligonucleotides using our AL10A phosphoramidite showed high yield coupling even after a 30-day sitting on the synthesizer (data not shown).

The robust nature of MOX chemistry allows for the syntheses of diverse phosphoramidites in high yields. The exceptional reactivity of the MOX group toward primary aliphatic amines is responsible for the high stepwise synthetic yields of phosphoramidite intermediates and for the ease by which they are made. In most cases, ether precipitation during workup effectively prepares the intermediates for further synthetic steps and eliminates the need for column chromatography until the final step.

MOX precursor chemistry is extremely versatile and allows us to make phosphoramidites with a variety of spacer lengths, hydrophobicities, arm flexibilities, and elements, such as cleavable diols and disulfide bridges (Fig. 1). Thus, customizing or tailoring the synthesis of an amino linker or spacer phosphoramidite preparation can easily be achieved using this synthetic strategy. Furthermore, it is possible to

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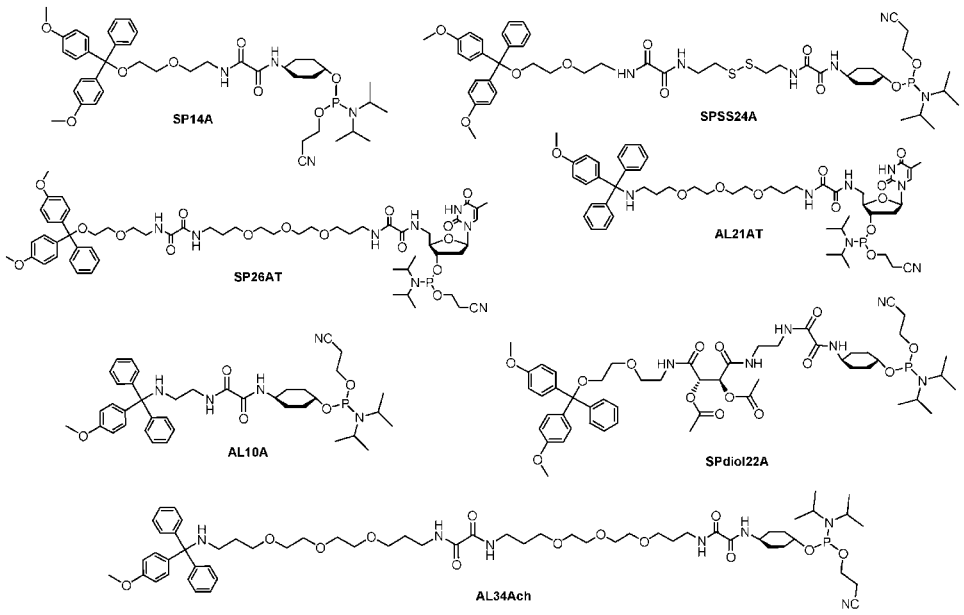

Figure 1. Amino linker and spacer phosphoramidites.

synthesize extremely long tethers, as in the case of AL34Ach, which may serve useful when designing microarrays for optimal oligonucleotide surface density and hybridization properties, and where no charge or low charge density for the linkers is preferable.[7]

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