An efficient method for the Incorporation of molecular probes at multiple/specific sites in RNA: levulinyl protection for 2′-ACE®, 5′-silyl oligoribonucleotide synthesis

Xiaoqin Cheng, Shawn Begay, Randy Rauen, Kelly Grimsley, Kaizhang He, Michael Delaney
Dharmacon, A Horizon Discovery Group Company, Lafayette, CO, USA

Abstract
Molecular probes have found wide application in the study of biomolecules within living systems. Oligonucleotides that are labeled with molecular probes are an invaluable tool for monitoring DNA and RNA processing for both in vitro and in vivo applications. Solid-phase oligoribonucleotide synthesis facilitates relatively straightforward and efficient incorporation of molecular probes at the 5′-end of DNA or RNA. However, modifying the 3′-end of an oligonucleotide generally requires either post-synthetic strategies or immobilization of the molecular probe to the solid support. The former process is subject to low yields due to potentially inefficient coupling while the latter strategy is restricted by the stability of the modification to repeated exposure to synthesis reagents. Similarly, internal labeling of oligonucleotides with molecular probes is largely limited to post-synthetic processing and subject to coupling efficiencies associated with this process for labeling steps. Finally, the need to differentially label oligonucleotides with distinct moieties in specific terminal and internal positions adds yet another layer of complexity in the generation of these important molecular tools.

In order to improve the labeling efficiency and ease of preparation of internal or 3′-terminal sites of oligoribonucleotides, we have developed a method for labeling these positions while the oligonucleotide remains immobilized on the solid support. We have applied a method to selectively de-block a levulinyl-protected hydrazyl group at a variety of different sites within an oligonucleotide and to selectively label these positions by the use of phosphoramidite-activated molecular probes. Conditions used to remove the levulinyl protecting group are mild and compatible with the 2′-ACE®, 5′-Silyl oligoribonucleotide synthesis platform, resulting in excellent yields of high-quality, full length modified oligoribonucleotides.

Methods
Phosphoramidites containing the Lev Protecting Group were designed in a manner such that minimal disruption in hybridization would occur. Amides were synthesized using standard 5′-Silyl-2′-ACE® RNA phosphoramidite synthesis conditions.

Figure 1. Representative example of labeling oligonucleotides internally using activated ester approaches. Key: A- = unlabeled oligonucleotide, B= Fluorescein-labeled oligonucleotide, C= Dabcyl labeled oligonucleotide, D= Dual-labeled oligonucleotide, E= Phosphorus-labeled oligonucleotide, F= Fluorescein-phosphorus-labeled oligonucleotide.

Results
Conclusions
Incorporation of molecular probes at defined sites within synthetic RNA was accomplished using the 2′-ACE®-5′-Silyl RNA synthesis platform in combination with the Levulinyl protecting group. Selective removal of the Levulinyl group with hydrazine was accomplished while the RNA molecule is retained on solid support allowing for efficient coupling of phosphoramidite activated molecular probes at specific sites within the RNA molecule. This process results in excellent yields of modified full length RNA and superior qualities.

References