Bipartite Cysteine Display Probes for the Detection of RNA Splice Variants

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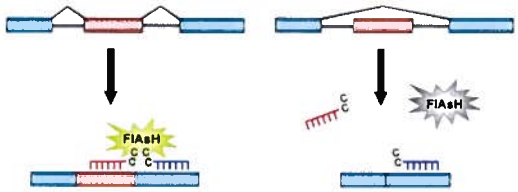
We are developing a method for detection of RNA and applying this method to detect RNA splicing. Although there are numerous methods developed for detection of oligonucleotides there are a limited number of strategies for detection of RNA splicing. Simple oligonucleotide detection methods can be problematic because the unspliced form has half of the correct sequence of the spliced product.

Using bipartite tetracysteine display, two oligonucleotides complementary to two different exons were functionalized with dicysteine peptides. Upon proper splicing the dicysteine motifs binds FlAsH molecule and induce fluorescence. The FlAsH molecule shows a two to three fold increase in fluorescence signal in the presence of properly spliced RNA sequences compared to samples that don't have the correct splice variant at micromolar concentrations. We have successfully detected nanomolar concentrations of the target splice variant. Because this method is simple and allows rapid detection of RNA it can be used for high throughput screening of small molecules that activate or inhibit splicing.



An Efficient One Step Preparation Of Alkyl Mida Boronates Through a Hydroboration-Direct Trapping Process

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Alkyl boranes and many boronic acids are notoriously unstable. This sensitive nature often impedes the use of these versatile functional groups and the installation of alkyl boranes and boronic acids often must be performed immediately prior to their usage. N-Methyliminodiacetic acid (MIDA) boronates which are readily converted to boronic acids with mild aqueous base have shown remarkable stability in being carried through multistep synthesis. In this report, we describe our discovery of a novel direct MIDA-alkyl exchange process on alkyl boranes, which led to the development of a new efficient way to prepare alkyl MIDA boronates through a hydroboration-trapping reaction. In addition, we demonstrate that MIDA boronates are tolerated in a multistep synthesis towards a building block in the total synthesis of amphotericin B. The successful Suzuki coupling of this building block with a model coupling partner is also described which illustrates a rare example of C_{sp3} alkyl boronic acid coupling in a highly complex system.