7 Summary

A novel approach for interference mapping with nucleotide analogs has been established. The use of 3‘-S-phosphorthiolates as a tag for nucleotide analogs was presented. The advantage of this 3‘-S-phosphorothiolate tag is derived from the only moderate consequences it has on the conformation and the reactive phosphorous. Moreover, the 3‘-S-phosphorothiolate linkage could be cleaved selectively and quantitatively in the presence of silver ions without damaging the native phosphordiester bond. This enables a quantitative determination of band intensities in the interference approach.

The utility of the novel approach was confirmed by investigating the importance of 2‘-hydroxy groups of uridine within the Hammerhead-Ribozyme. 3‘-S-phosphorothiolate tagged nucleoside analogs were incorporated into oligonucleotides by means of solid phase synthesis. The investigation of the partial incorporation of 3‘-phosphorothiolate modified amidites into oligonucleotides and the silver cleavage reactions were carried out with a model-oligonucleotide.

First, the 3‘-S-phosphorothiolate modifications at uridines in the catalytic core of the Hammerhead-Ribozyme were introduced to test whether the tag interferes with catalytic activity. The experiments did not reveal any effect. The combined introduction of the 2’-deoxy modification with the 3’-S-phosphorothiolate tag revealed no effect at position U16.1, a weak interference at position U7 and a strong interference at position U4. The result of the interference analysis correlate well with the results from single substitution experiments. While the results for positions U16.1 and U7 are consistent with previous observations, the strong interference at position U4 was unexpected in comparison with earlier reports. The prominent effect at position U4 may be due to the specific conformation of the particular ribozyme sequence used in this study. The interference effect, however, fits well into the proposed cleavage mechanism based on crystallographic data.

The new method may have general applications to any reaction involving RNA or DNA in which precursor and products can be separated. The 3’-S-phosphorthiolate tag may be combined with different base and sugar modifications to simultaneously allocate the importance of individual functional groups. A cluster of different tagged modifications could be used to rapidly identify the active centers of ribozymes or the binding domains of aptamers. Moreover, it is conceivable to combine the tag with modifications, such as 2’-O-methyl, 2’-amino, 2’-fluoro that render the RNA more stable against nuclease degradation.