The structural characterization of RNA molecules remains challenging due to the high conformational flexibility of nucleotide residues, their propensity to adopt multiple secondary and tertiary structures, and their susceptibility to structural rearrangements induced, for example, by experimental conditions. Although X-ray crystallography is an effective method for determining high-resolution RNA structures, crystallizing RNA molecules is often difficult and becomes the limiting step in the structural analysis process. For instance, RNA hairpins tend to unwind and form alternative duplex structures at high RNA and salt concentrations typically used for crystallization. Consequently, many biologically important native RNA motifs cannot be captured in a homogeneous crystalline state without additional stabilization strategies.

The aim of this dissertation was to develop new strategies for stabilizing RNA structures. Two approaches were applied: covalent stabilization using non-nucleosidic linkers and stabilization using a small-molecule ligand.

In the first strategy, a series of aromatic linkers were designed, synthesized, and incorporated into the RNA chain to covalently connect the 3’ and 5’ ends while preserving the native RNA geometry. In the second approach, the ability of a naphthyridine carbamate dimer (NCD) to stabilize RNA was investigated. It was demonstrated that the binding of a small-molecule ligand can stabilize RNA structures through mechanisms fundamentally different from covalent methods. NCD acts as a clamp linking nucleotide residues localized either within the same RNA molecule or between two adjacent RNA molecules, serving as a “molecular glue.”

The final part of this thesis focuses on the biophysical and biochemical evaluation of the modified RNA constructs, including thermal denaturation studies monitored by UV spectroscopy, circular dichroism measurements, and in vitro cleavage assays using Dicer nuclease. These methods were used to assess how different stabilization strategies influence the RNA structural integrity, folding behavior, and biological compatibility.

In summary, this dissertation presents two strategies for stabilizing RNA molecules for structural studies. The results obtained expand our understanding of how both covalent non-nucleosidic linkers and small-molecule ligands can be used to overcome the challenges associated with RNA crystallization.