Template-Mediated Synthesis of Lariat-RNA & DNA

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Figure S1: The predicted favorable secondary structure of the lariat DNA precursor **4** under MES buffer ligation conditions (4°C). Predicted structures were obtained from the DNA MFOLD server available at: <http://www.bioinfo.rpi.edu/applications/mfold/old/dna/form1.cgi>. The 3',5'-branchpoint T₂₀ extension was omitted for calculation purposes. The bases in the loop are numbered with respect to their position from the 5'-terminus of the molecule. Blue dots represent standard Watson-Crick (WC) base-pairing while the red dot represents a G-T wobble base pair.

Figure S2: Thermal denaturation analysis of nicked and ligated 4 and comparison to the nicked DNA complementary region (1 + 2) and full DNA complementary region (3). All compounds were hybridized to their DNA splint sequence, 5 (1:1). T_m 's were obtained in 10 mM Tris-HCl, pH 7.5, 10 mM NaCl.

Figure S3: Circular dichroic signatures of the intermolecular complexes used in the CNBr-mediated ligation of 4. CD's were obtained in 10 mM Tris-HCl pH 7.5, 10 mM NaCl buffer. (A): Comparison of the associated and unassociated nicked forms of 4. (B): Comparison of the nicked and ligated forms of 4 complexed to 5 (4 °C). Under the low ionic strength conditions used (10 mM NaCl), the single stranded DNA splint 5 is not highly structured in solution, however it demonstrates a weak B-type helical signature (**Figure S3-A**). On the other hand, the spectral profile of the nicked lariat precursor 4 indicates that the Y-structure, in its unbound state is B-form as well, but is more structured than the single-stranded splint, given the amplified magnitude of the positive and negative cotton effects. Complexation of the splint 5 to nicked precursor 4 results in a CD profile that is strictly B-form in character, and appears to be a simple summation of their individual spectral signatures. No perturbation of the helical structure is observed upon binding of the splint to the precursor lariat (**Figure S3-A**). When the template is bound to the closed, circular form of 4 (lariat DNA) a decrease in the amplitudes of both the positive and negative CD signals occurs (**Figure S3-B**). Furthermore, the major CD band centered at 280 nm in the nicked complex is red-shifted by approximately 5 nm in the ligated complex. This suggests that although the template has threaded through the loop and is bound to its complementary region, points of local disruption, likely at the terminal regions of the complex are found along the helix axis.

Figure S4: Analysis of the CNBr-mediated ligation of 8 in the presence of 1:1 complementary DNA splint 5 or RNA splint 9 by 12% denaturing PAGE (8.3 M urea). Lane 1: negative control 8 (no complementary splint); Lane 2: CNBr ligation of 8 in the presence of DNA splint 5; Lane 3: CNBr ligation of 8 in the presence of RNA splint 9. All ligation reactions contained 10^{-4} M precursor and complementary splint dissolved in 0.25 M MES pH 7.6, 20 mM MgCl_2 buffer.

Figure S5: Characterization of ligated 8 by T_m analysis and comparison to the nicked (6) and full (7) template regions of 8 by hybridization to the complementary RNA splint 9. Analyses were conducted in 10 mM Tris-HCl pH 7.5, 10 mM NaCl buffer.