Supplementary Methods

Protocol for preparation and purification of the 11-mer Diels-Alder product

Synthesis of the stereochemically correct 11-mer with the HEG-linked Diels-Alder product was achieved by reaction between N-pentyl maleimide and anthracene-linked 11-mer annealed with 38-mer RNA, and subsequent HPLC purification.

A solution of 0.33 mM 38-mer and 0.2 mM 11-mer anthracene-conjugate in buffer (30 mM Tris-HCl pH 7.4, 300 mM NaCl) was heated to 65 °C for 4 min and allowed to cool to room temperature for 15 min. 80 mM $MgCl_2$ was added and the reaction was initiated by addition of N-pentylmaleimide (20 mM in ethanol) to a final concentration 10%. After incubation for 6 min at room temperature, the reaction was stopped by addition of β-mercaptoethanol. The product-oligomer was recovered by reversed phase HPLC on a semipreparative C18-column (Luna, Phenomenex, 10 x 250 mm) at 45 °C. Flow rate: 3 ml/min; eluent A: 0.1 M triethylammonium acetate (TEAAc) buffer (pH 7.0); eluent B: 0.1 M TEAAC in 80% aq. MeCN (pH 7.0); detection at 260 nm; 1-50% B in A within 15 min followed by 50-60% B in A within 5 min and then 60-74% B in A within 2 min. Fractions containing the purified RNA were desalted by NAP-5 gel filtration (Amersham) and subsequent ethanol precipitation.

Protocol for preparation and purification of Se-labeled RNA oligomers

The 5′-anthracene-HEG-linked 11-mer with U_{Se} at position 2 and C_{Se} at position 10 was converted into the product as described above. The 38-mer was prepared with C_{Se} at positions 15 and 20 and U_{Se} at positions 30 and 33.

Deprotection of the selenium-labeled RNAs and cleavage from the solid support (40 mg; 11-mers, 20 mg; 38-mers) was achieved in the mixture of $MeNH_2$ in ethanol (8 M, 0.65 ml) and $MeNH_2$ in water (40%, 0.65 ml) containing 100 mM DTT for 5 h at 25 °C (11-mers) and for 6.5 h at 30 °C (38-mers), respectively; the solution was then evaporated to dryness. Removal of the 2′-*O*-TOM groups was achieved by treatment with tetrabutylammonium fluoride in tetrahydrofuran (1 M, 0.95 ml) at 42 °C until a clear solution was obtained. The solution was then kept at room temperature for 12 h (11-mers) and for 15 h (38-mers), respectively. The reaction was quenched by the addition of TEAAc buffer (1 M, pH 7.0, 0.95 ml) and the volume of the solution was reduced to 1 ml by evaporation and directly applied on a Sephadex G10 column (30 x 1.5 cm) controlled by UV-detection at 270 nm. The product was eluted with water

(1 ml/min) and the RNA containing fraction was evaporated to dryness. All RNAs were purified by anion-exchange chromatography on a semipreparative Dionex DNAPac column (9 x 250 mm) at 80 °C. Flow rate: 2 ml/min; eluent A: 25 mM Tris-HCl (pH 8.0), 6 M urea; eluent B: 25 mM Tris-HCl (pH 8.0), 0.5 M $NaClO_4$, 6 M urea; detection at 265 nm; 3-10% B in A within 20 min. Fractions containing the purified RNA were desalted by loading onto a C18 SepPak cartridge (Waters/Millipore), followed by elution with 0.1 - 0.2 M $(Et_3NH)HCO_3$, water, and then H_2O/CH_3CN (6/4). Combined fractions containing the RNA were lyophilized to dryness.