

Electronic Supplement

EXPERIMENTAL

Materials

HPLC grade acetonitrile was from Tedia Company Inc. (Fairfield, OH, USA). HPLC grade methanol, reagent grade ammonium acetate and glacial acetic acid were from Fisher Scientific (Springfield, NJ, USA). Phosphorothioate oligonucleotides (21-mer: 5'-d(TCGTGCTTTTGTTGTTTCGC)-3'; and 23-mer: 5'-d(TCGTGCTTTTGTTGTTTCGCGT)-3' with phosphorothioate internucleotide linkages at every nucleotide residue) were obtained from Biosearch Technologies (Novato, CA, USA). Phosphorothioates were used as received after being reconstituted in deionized, distilled and autoclaved water to a concentration of 100 μM . Re

HILIC

Chromatographic analysis was performed on an Agilent Technologies 1200 HPLC system (Santa Clara, CA, USA) equipped with a binary pump, vacuum chambered microdegasser and a thermostatically controlled well plate sampler and column compartment. A TSKgel Amide 80 column from TOSOH Bioscience (Montgomeryville, PA, USA), 150 mm x 2 mm i.d. with 3 mm particle size, was used for the separation of the phosphorothioate oligonucleotides. Mobile phase A was 5 mM ammonium acetate, pH 5.8 and Mobile phase B was 100% acetonitrile. After optimization, gradient elution was performed starting at 15% A, a ramp to 30% A at 6% min^{-1} followed by another ramp from 30–40% A at 0.75% min^{-1} . A rapid re-equilibration step back to 15% A was then applied for 150 s. A 10-min column regeneration period was required before subsequent injections. HPLC was conducted at a flow rate of 100 $\mu\text{L min}^{-1}$ with the column thermostatted to 35 $^{\circ}\text{C}$. Oligonucleotides in water were mixed in equimolar amounts and diluted with 75%/25% acetonitrile: methanol with 1 μL injections used throughout.

LTQ-MS

For on-line LC-MS, the eluent from the HILIC separation was directed into a Thermo Scientific (Waltham, MA) LTQ™ linear ion trap mass spectrometer. Operating parameters were capillary temperature of 300 $^{\circ}\text{C}$, spray voltage of 4 kV, sheath gas at 50 arbitrary units, auxiliary gas at 15 arbitrary units and sweep gas to 15 arbitrary units with a fused silica capillary voltage of -34 V. ESI-MS data was obtained in negative polarity, with a divert valve to control HILIC eluent into the mass spectrometer only during relevant portions of the HPLC gradient. Mass spectra were collected in both full scan (m/z 200-2000) and selected ion monitoring (SIM) modes. The mass spectrometer was autotuned using direct infusion ESI-MS analysis of a 2.5 μM solution of the 23-mer phosphorothioate oligonucleotide. Masses and expected charge state m/z values for the phosphorothioate oligonucleotides were calculated using the Mongo Oligo mass

calculator v2.06 (<http://library.med.utah.edu/masspec/mongo.htm>) with appropriate sulfur for oxygen substitutions