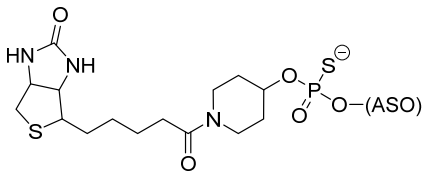
Supplemental Fig. 1

Synthesis of bASO (Ionis AA7141)

5’- (Biotin) – (piperidine linker) - Ges mCes Tes Tes mCes Ads Gds Tds mCds Ads Tds Gds Ads mCds Tds Tes mCes mCes Tes Te - 3’

e = 2’-MOE, s = phosphorothioate (PS), d = deoxy, m = 5’-methyl



Experimental Procedure:

An antisense oligonucleotide (ASO) with a 5’-terminal piperidine group was synthesized using standard phosphoramidite chemistry. 5 mg of ASO was dissolved in sodium tetraborate buffer (0.1 M, pH = 8.5, 100 μl). Biotin NHS was added (Glen Research, 0.5 mg, 3 equiv) and the reaction was stirred vigorously. Acetonitrile was added to achieve a homogenous solution (50 μl). The reaction was heated at 55 °C for 60 seconds then stirred at 35 °C for 1 hour. The reaction mixture was concentrated under reduced pressure. The residue was dissolved in water and purified by High Performance Liquid Chromatography (HPLC) on a strong anion exchange column (Mono Q, GE Healthcare, 16/10, 20 ml, 10 mm, ionic capacity 0.27–0.37 mmol/ml, A=100 mM ammonium acetate, 30% aqueous acetonitrile, B=1.5 M NaBr in A, gradient of 5–60% B over 80 min, Flow 2.0 ml min^{-1}, λ=260 nm). The biotin-conjugated oligonucleotide was desalted using a C18 reverse-phase cartridge (Waters). Oligonucleotides were characterized by ion-pair-HPLC coupled mass spectrometry (MS) analysis with Agilent 1100 MSD system. Yield: 3.4 mg MW (calc) = 7569.11; Found: 7596.40. Purity: 92.3 % (UV), Extinction coefficient: 181.22 mM^{-1} x cm^{-1} @ 260 nm