Nucleobase-modified antisense oligonucleotides containing 5-(phenyltriazol)-2'-deoxyuridines nucleotides induce exon-skipping *in vitro*.

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1. Synthesis of the oligonucleotides

The synthesis of the 5-(phenyltriazol)-2′-deoxyuridine building block is described in ChemBioChem, 2007, 8, p. 2106 (original synthesis) and in J. Org. Chem., 2015, 80, p. 9592 (improved synthesis).[1,2] The oligonucleotides were synthesized on an AKTA Oligopilot synthesizer (GE Healthcare Life Sciences) in 1.0 µmol scale loaded on 500 Å controlled-pore glass (CPG) supports using the phosphoramidite approach and following the manufacturer's protocol. Double coupling (2 × 5 min) cycles were used for commercial phosphoramidites and prolonged coupling times (20 minutes) were used for the 5-(phenyltriazol)-2′-deoxyuridine phosphoramidite. The phosphoramidites were activated using 1H-tetrazole and incorporated into oligonucleotides via manual couplings: 10 µmol of the modified phosphoramidite was dissolved in anhydrous CH_3CN (2 mL), treated with a solution of the activator, and infused into the reaction compartment. Xanthane hydride was used as oxidizing agent for preparing the phosphorothioate oligonucleotides. The stepwise coupling efficiencies were monitored by measuring the absorbance of the trityl cation at 495 nm, which in in general were between 95—100% for the commercial phosphoramidites, and 90—100% for the modified phosphoramidites. The final 5′-terminal DMT group in the oligonucleotides was kept on for purification purposes. The final crude oligonucleotides on solid support were treated with aqueous NH_3 (28%, 1 mL) overnight at 55 °C.

2. Purification of the oligonucleotides

After cleavage from the support, the oligonucleotides were filtered, and the filtrates were evaporated to dryness at 45 °C by a steady flow of N_2, and dissolved in an aqueous triethylammonium acetate buffer (500 µL, 0.05 M, pH 7.4). The oligonucleotides were purified by a reversed-phase HPLC on a Waters 600 system using Xterra MS C18 10 µm (7.8 × 50 mm) columns and Xterra MS C18 10 µm (7.8 × 10 mm) precolumns. Elution was performed with 100% eluent A over 2 min, followed by a linear gradient down to 30% eluent A over 38 min, then a wash with 100% eluent B over 10 min, 100% eluent A over 10 min. (Eluent A = triethylammonium acetate (0.05 M, pH 7.4). Eluent B = 75% CH_3CN/H_2O (3:1, v/v)). The pure fractions were pooled and evaporated at 45 °C. The 5′-terminal DMT group was removed by treatment with acetic acid (80% in H_2O, 100 µL) for 30 min, upon which an aqueous solution of NaOAc (15 µL, 3.0 M), an aqueous solution of $NaClO_4$ (15 µL, 5.0 M), and pure acetone (1 mL) were added. The oligonucleotides precipitated overnight at -20 °C. The supernatant was removed from the sedimented solid (centrifugation, 12,000 rpm, 10 min at 2 °C), and the remaining pellet was washed with cold acetone (3 × 1 mL) and dissolved in 500 µL Milli-Q® H_2O. Mass spectra of the oligonucleotides were recorded on a MALDI-TOF mass spectrometry instrument in ES+ mode using an MSP AnchorChip 600/96

microScout Target (Bruker Daltonics) with a matrix consisting of 3-hydroxypicolinic acid (3HPA) and diammonium hydrogen citrate. All oligonucleotides were obtained in >85% purity and the determined m/z values were within ±4 amu (i.e. ~1‰) of the calculated masses. The concentrations of the purified oligonucleotides were determined by the optical density at 260 nm, assuming the molar absorptivities of the oligonucleotides equal the sum of each constituent nucleotide monomers.

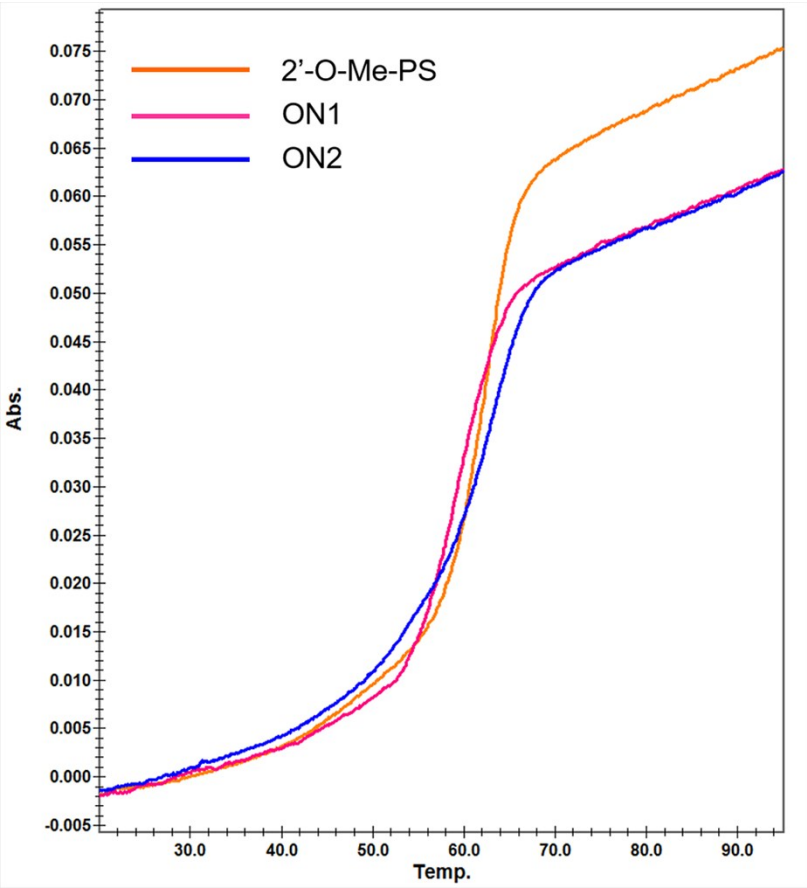

Fig S1. Melting temperature curves of the 2'-OMePS AO, ON1 and ON2.

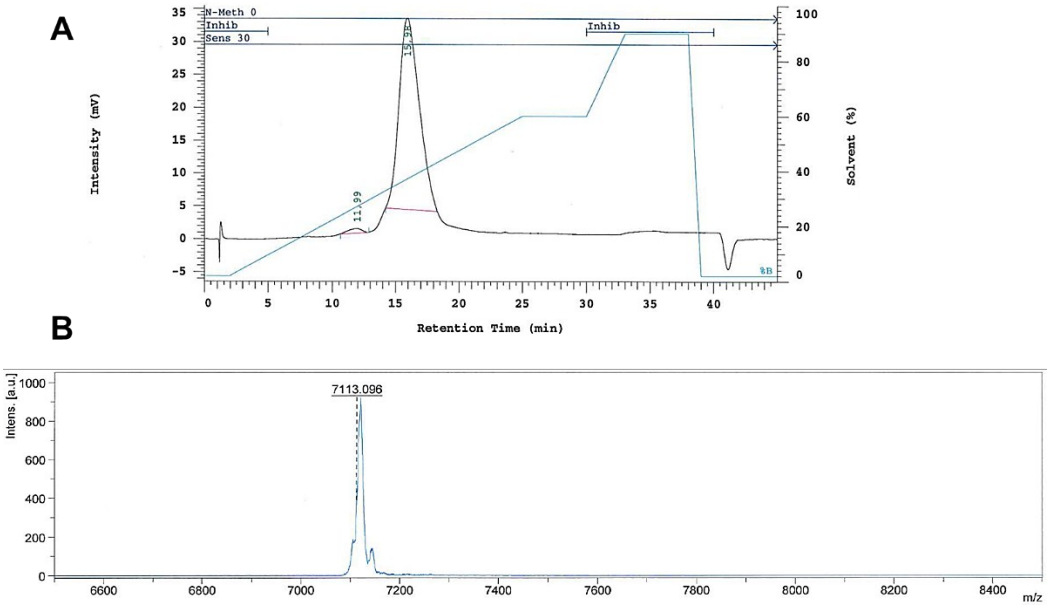

Fig S2. A. HPLC trace of ON1; B. MALDI-TOF analysis of ON1.

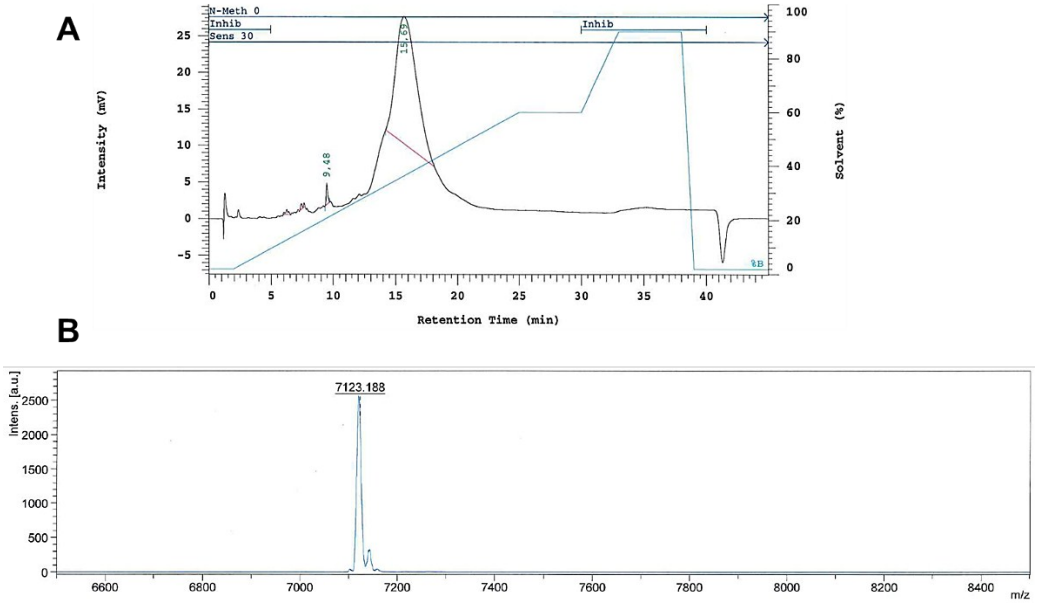

Fig S3. A. HPLC trace of ON2; B. MALDI-TOF analysis of ON2.

References

1. P. Kocalka, N. K. Andersen, F. Jensen, P. Nielsen, *Chembiochem.,* 2007, **8**, 2106-2116.
2. M. Hornum, P. Kumar, P. Podsiadly, P. Nielsen, *J. Org. Chem.* 2015, **80**, 9592−9602.