

Supporting information

Bridged Nucleic Acid Conjugates at 6'-Thiol: Synthesis, Hybridization Properties and Nuclease Resistances

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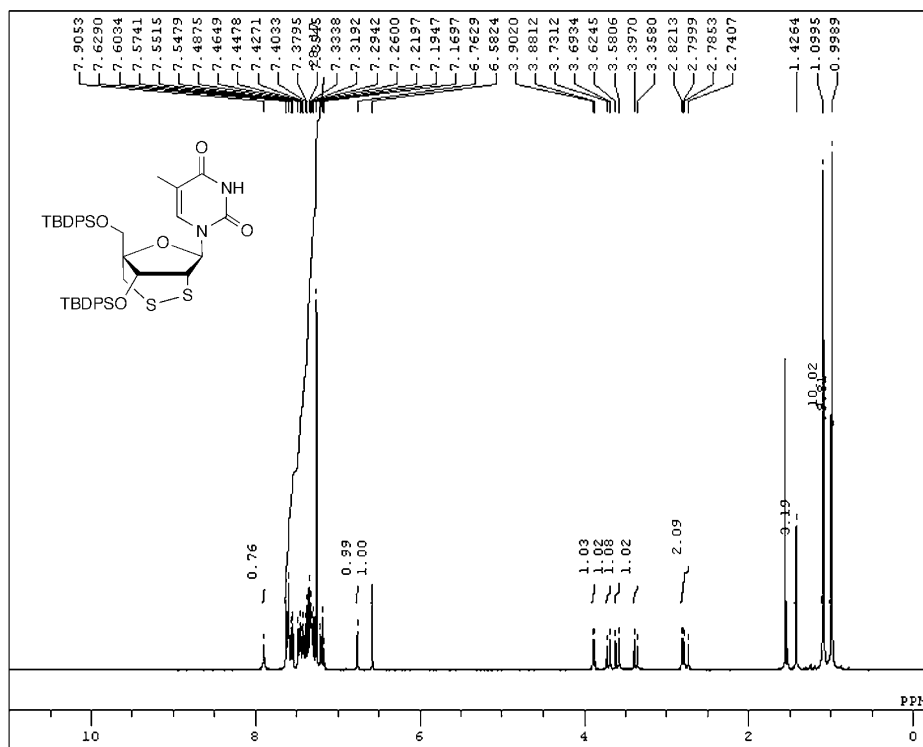
E-mail: kodama@phs.osaka-u.ac.jp; obika@phs.osaka-u.ac.jp

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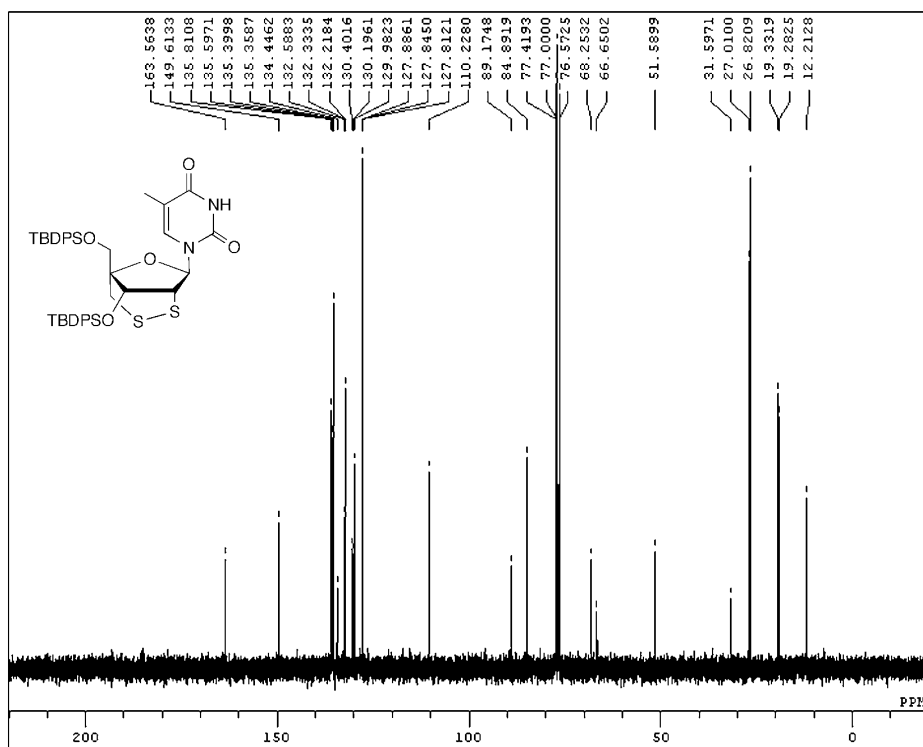
1. ^1H -, ^{13}C - and ^{31}P -NMR spectra of new compounds
2. HPLC, MALDI-MS and ESI-MS data for oligonucleotide conjugates
3. UV melting profiles

1. ^1H -, ^{13}C - and ^{31}P -NMR spectra of new compounds

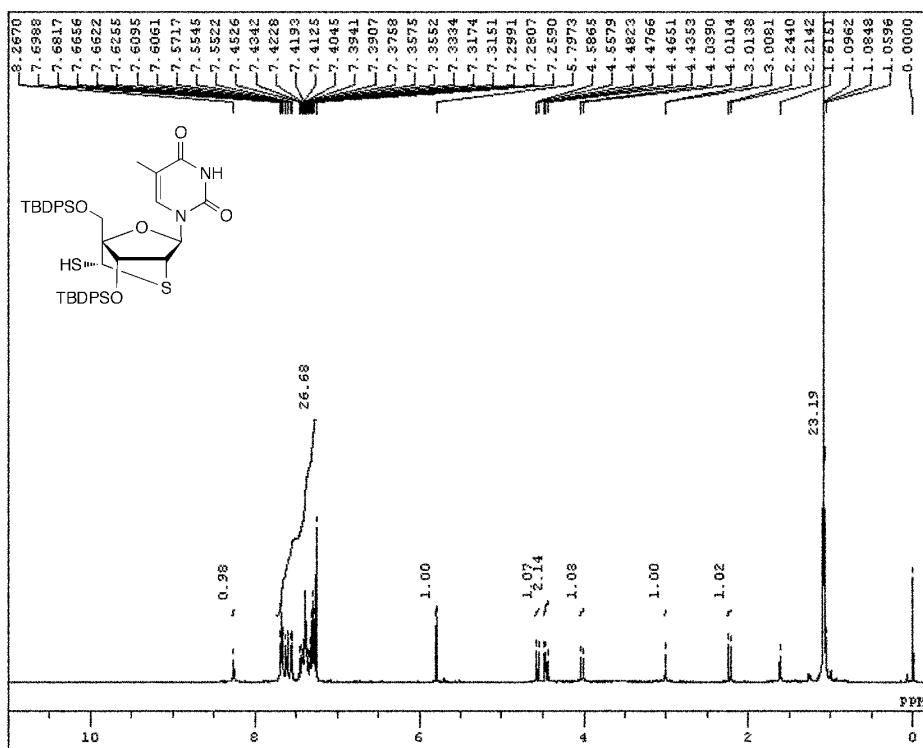
Compound 2 (^1H -NMR)



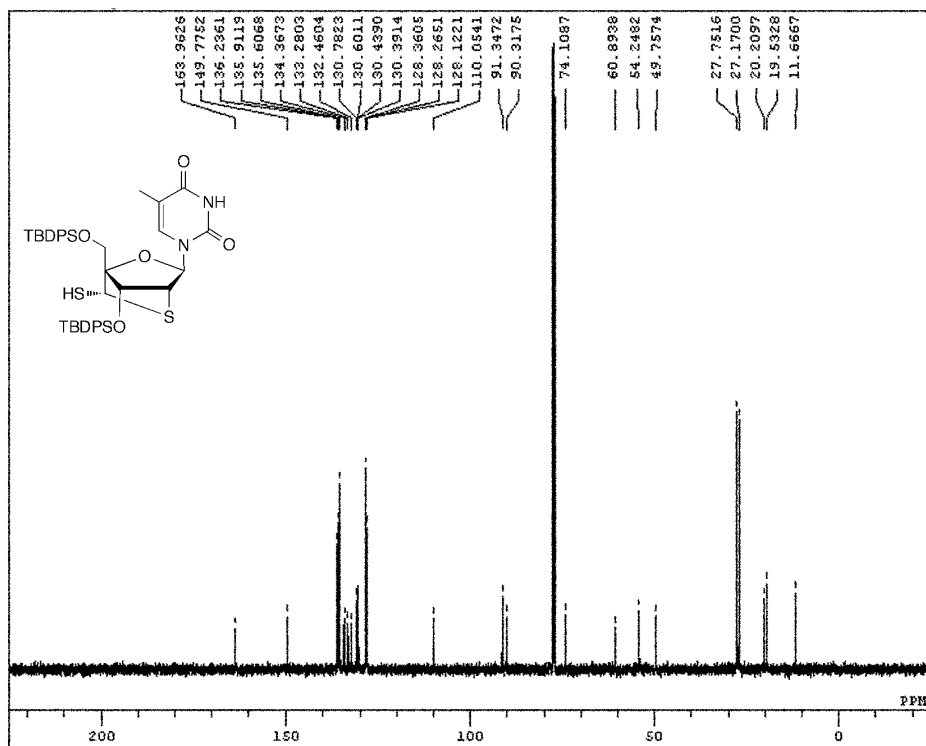
Compound 2 (^{13}C -NMR)



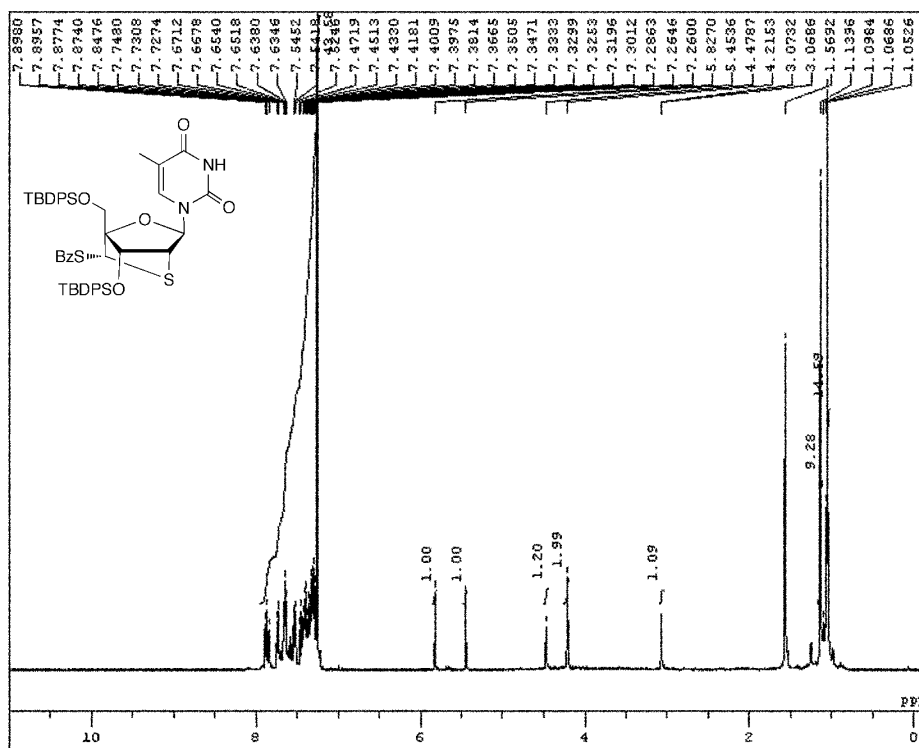
Compound **3** ($^1\text{H-NMR}$)



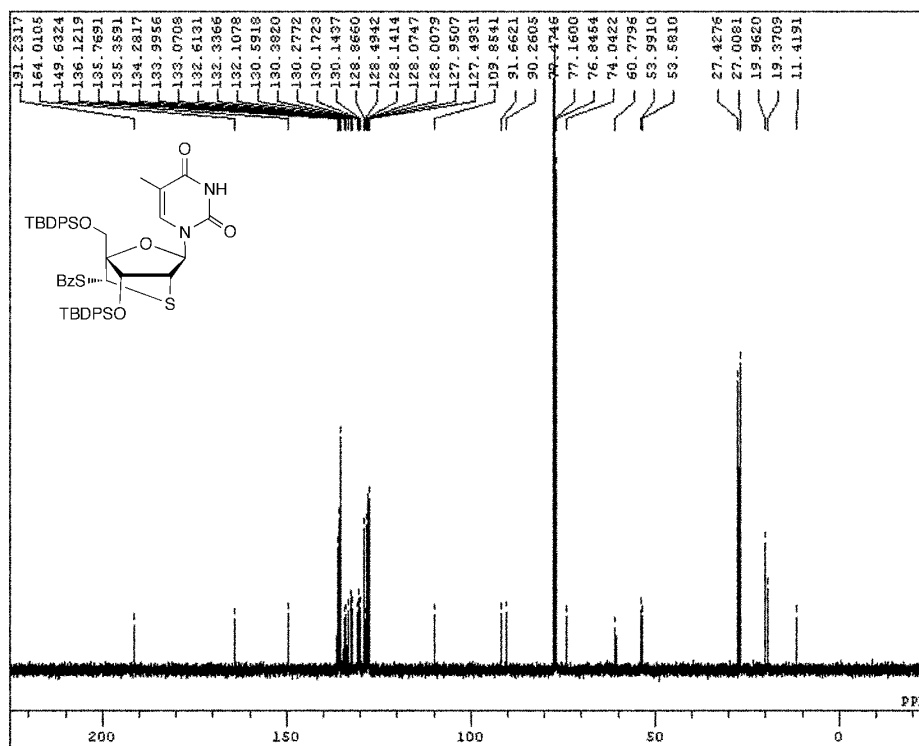
Compound **3** ($^{13}\text{C-NMR}$)



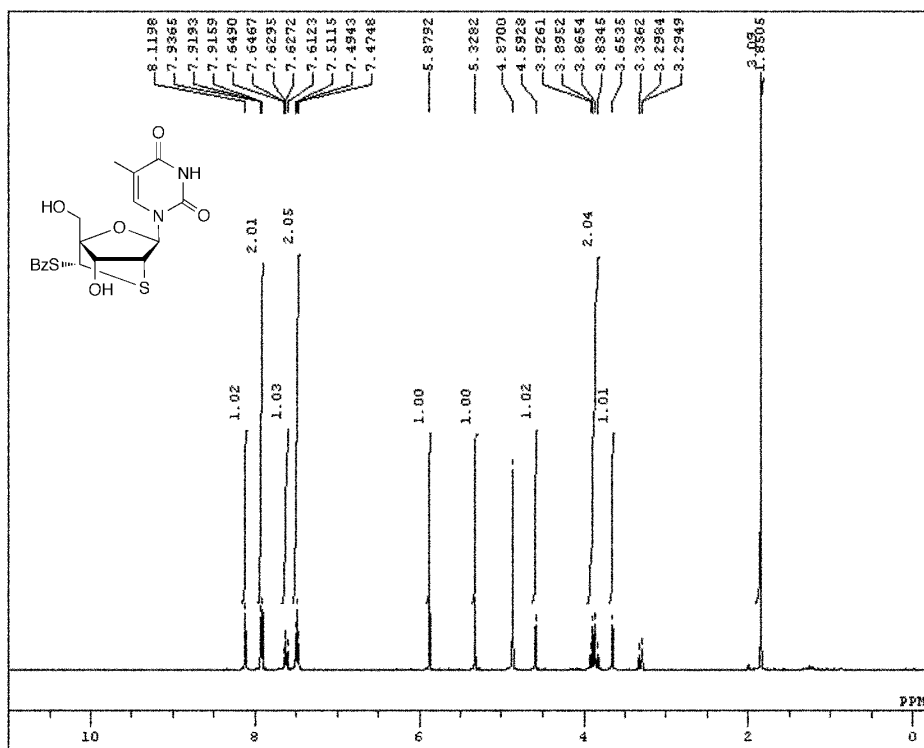
Compound 4 ($^1\text{H-NMR}$)



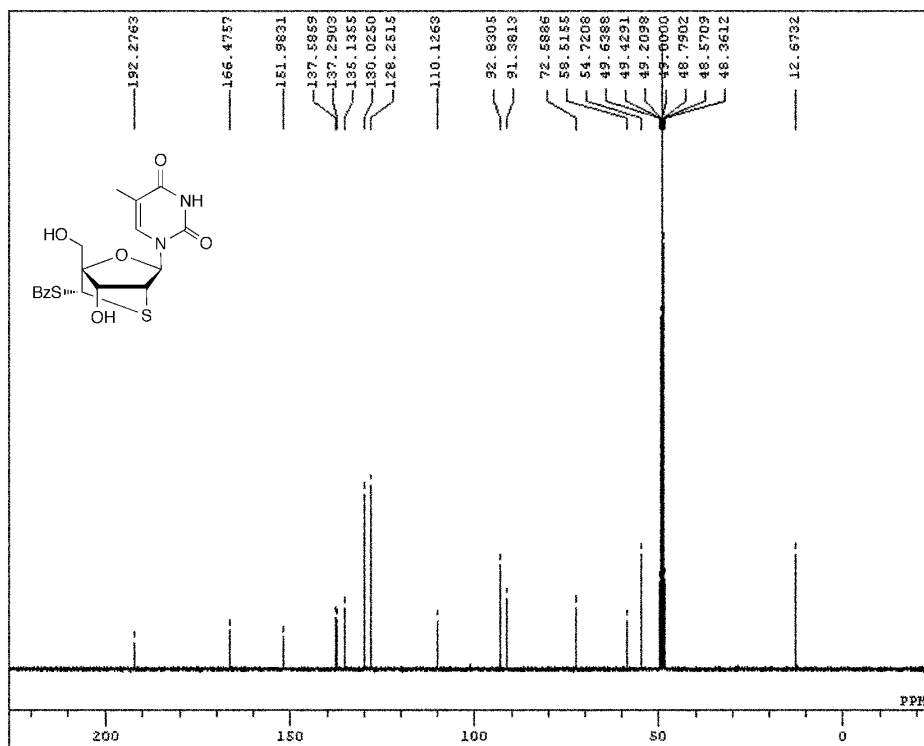
Compound 4 ($^{13}\text{C-NMR}$)



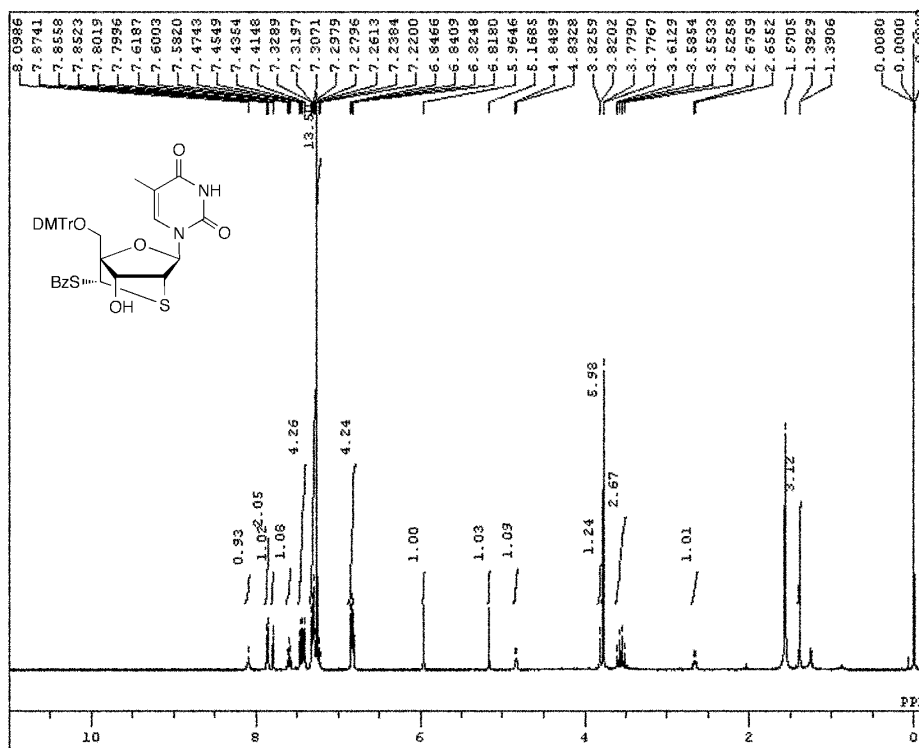
Compound **5** ($^1\text{H-NMR}$)



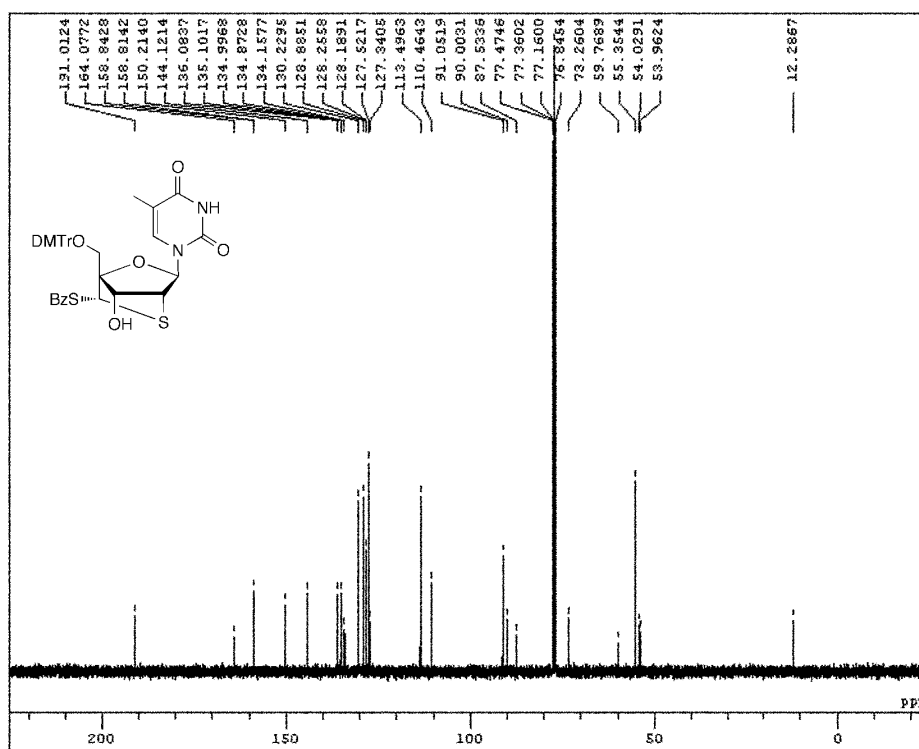
Compound **5** ($^{13}\text{C-NMR}$)



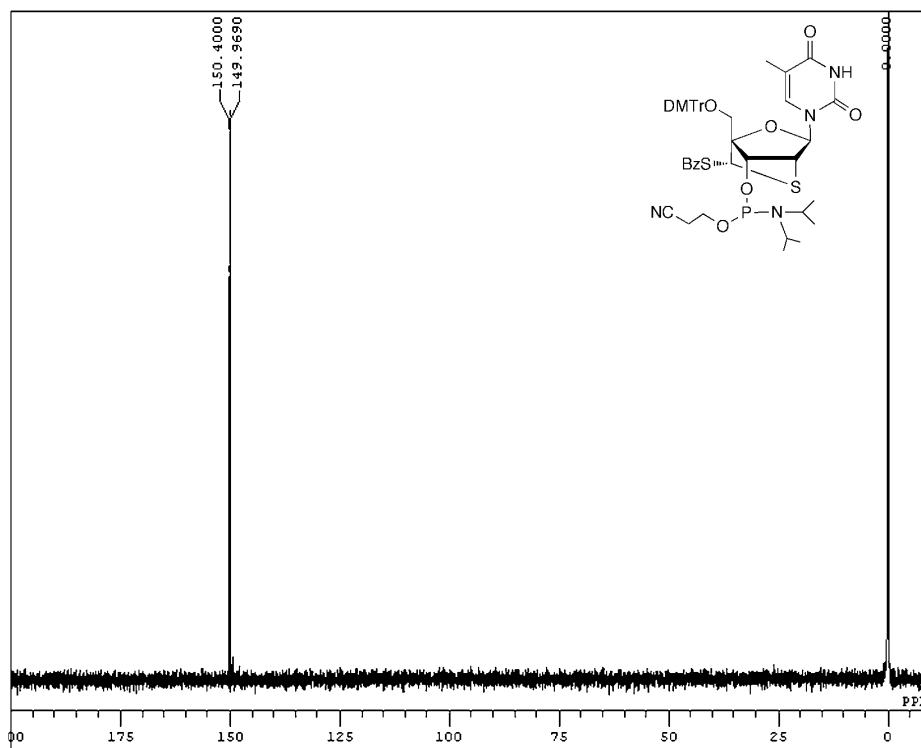
Compound 6 (¹H-NMR)



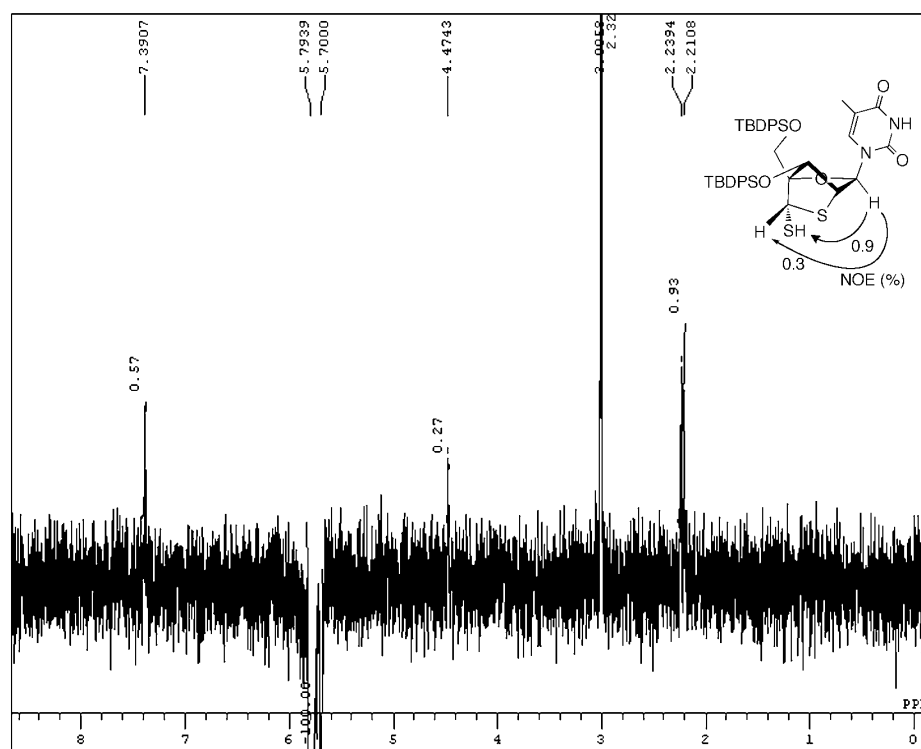
Compound 6 (¹³C-NMR)



Compound 7 (^{31}P -NMR)



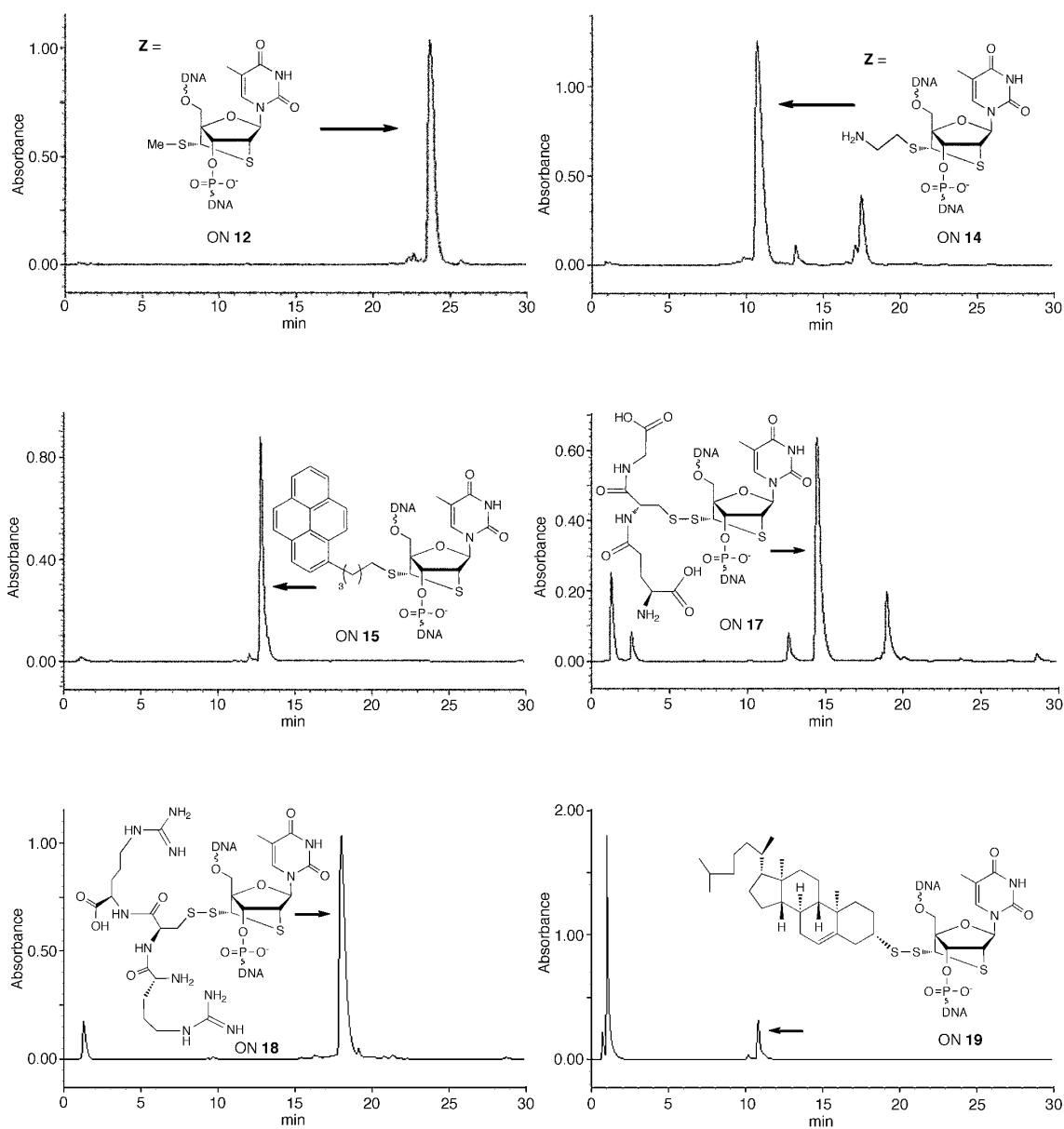
Compound 3 (NOE experiment)



2. HPLC- and MALDI MS-data for oligonucleotide conjugates

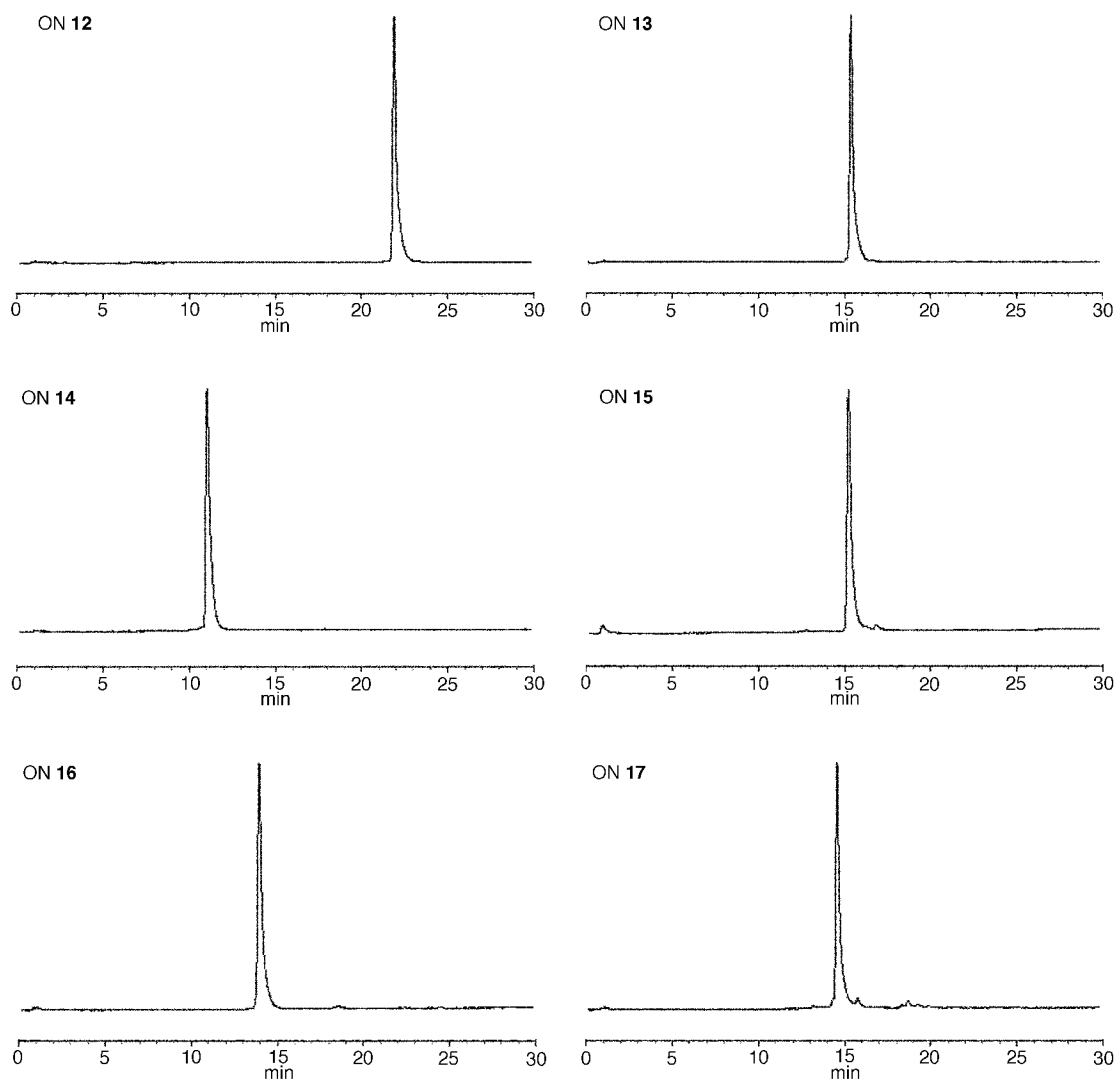
2-1. RP-HPLC analyses of solution-phase conjugations

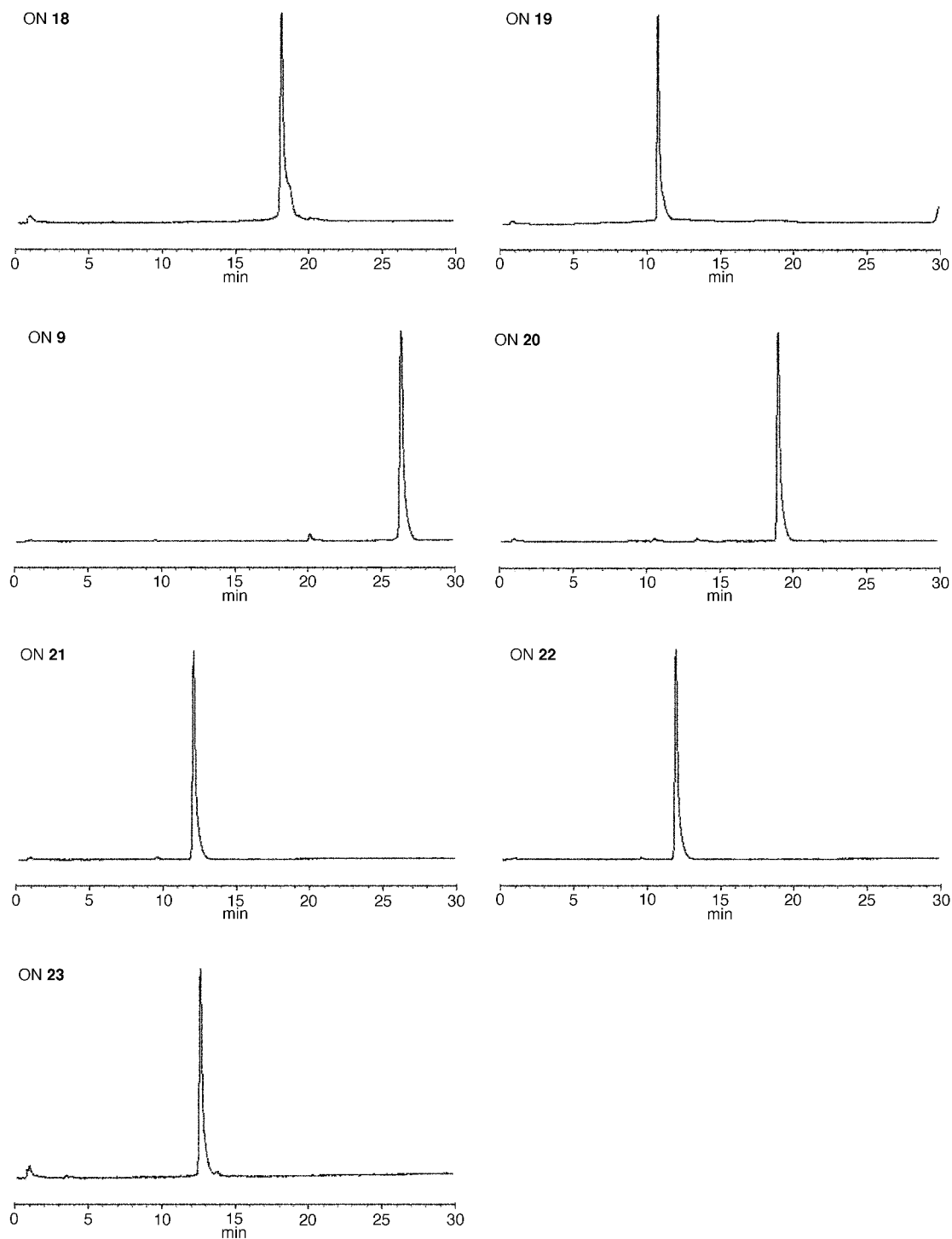
Each reaction mixture of conjugation was analyzed by RP-HPLC using XBridge™ Shield RP 18 2.5 μm (4.6 x 50 mm) with a linear gradient of MeCN (6 to 12 % for ON 12, 14, 17 and 18, 10 to 50 % for ON 15, or 30 to 80% for ON 19 over 30 min) in 0.1 M triethylammonium acetate (pH = 7.0).



2-2. RP-HPLC analyses of purified ONs

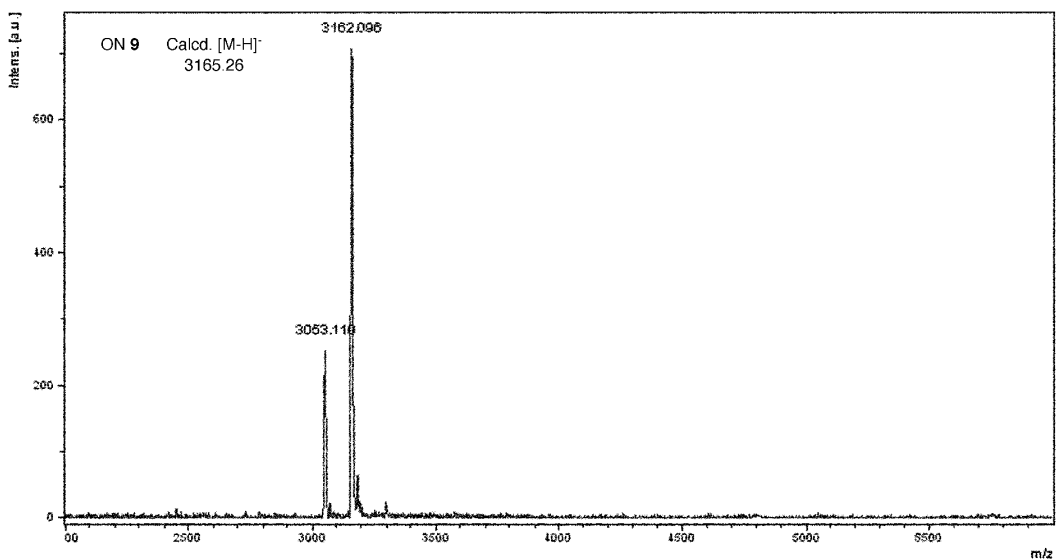
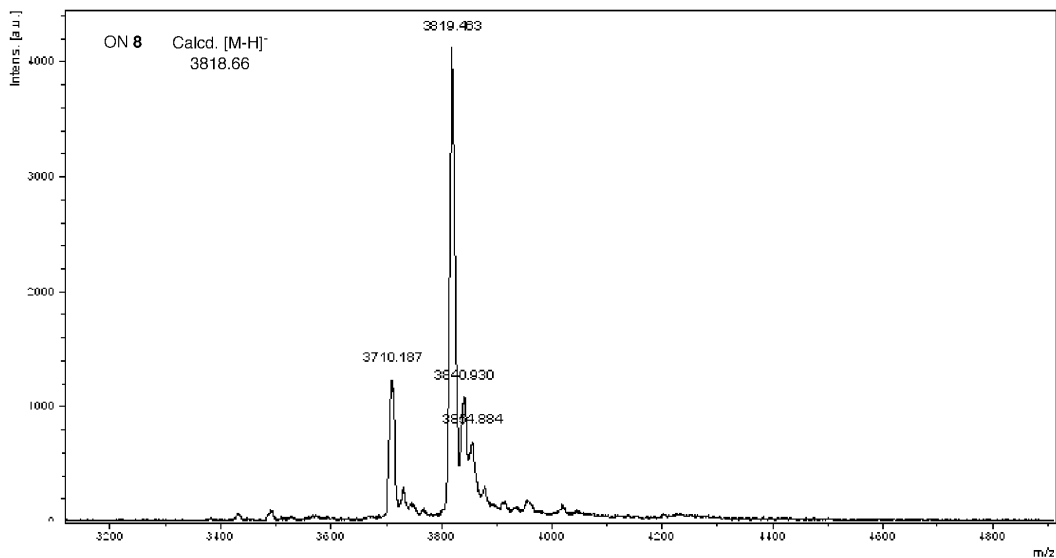
Each purified ON was analyzed by RP-HPLC using XBridge™ Shield RP 18 2.5 μm (4.6 x 50 mm) with a linear gradient of MeCN (6 to 12 % for ON **12-14** and **16-18**, 15 to 25 % for ON **15**, 30 to 80% for ON **19**, or 8 to 14 % for ON **9** and **20-23** over 30 min) in 0.1 M triethylammonium acetate (pH = 7.0).



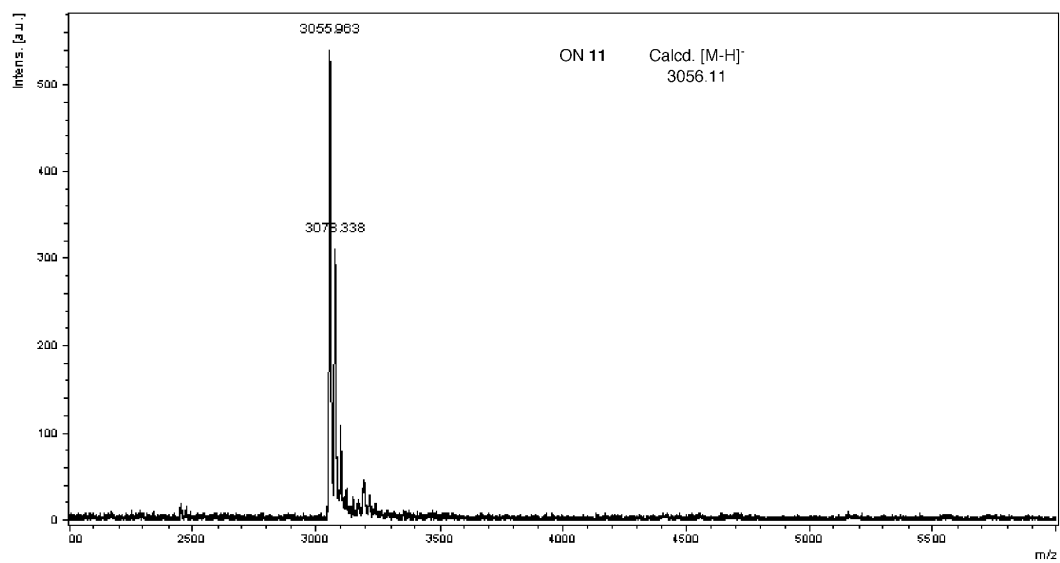
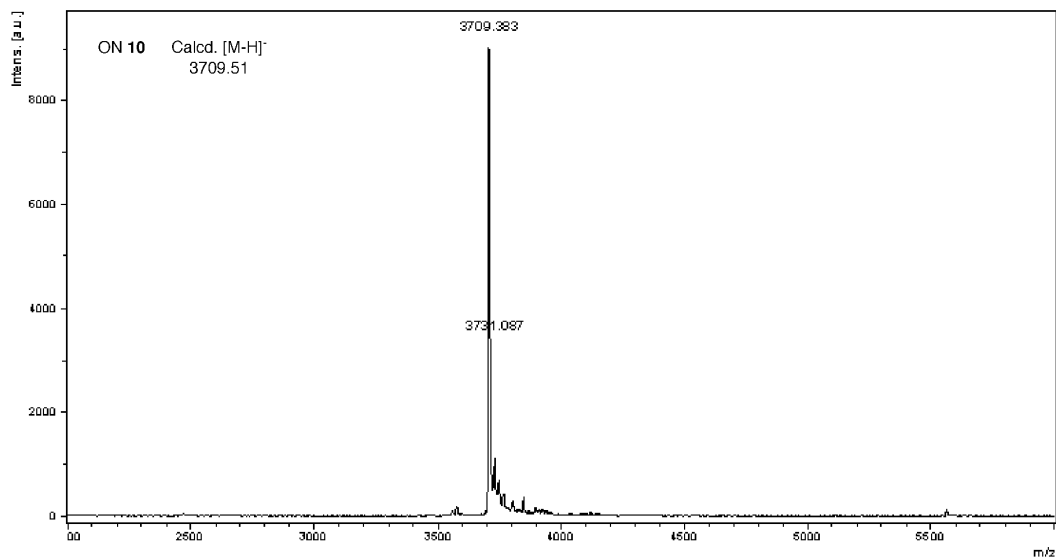


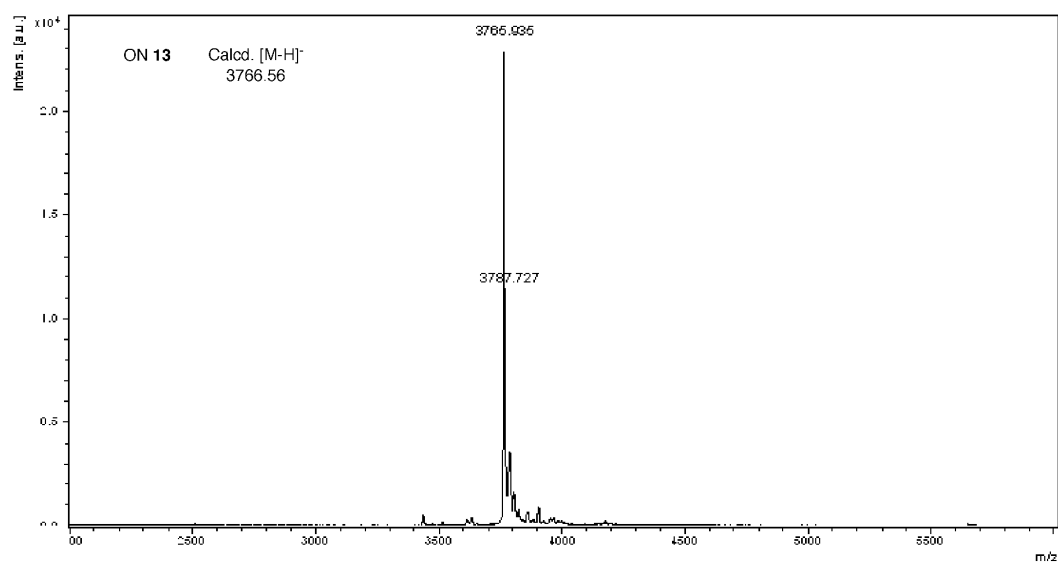
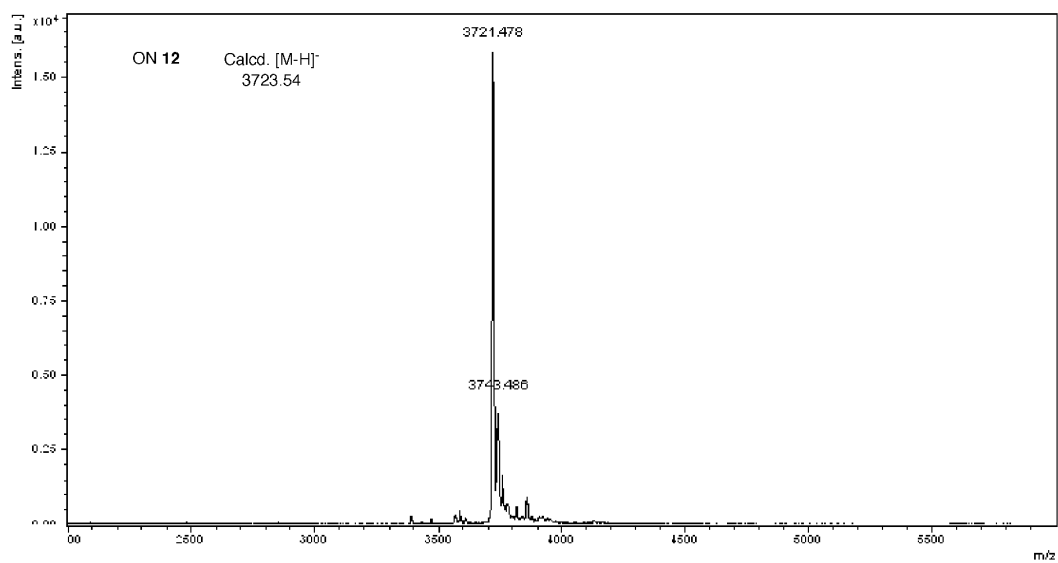
The percent purities of ONs calculated from UV absorption at 260 nm were over 90%.

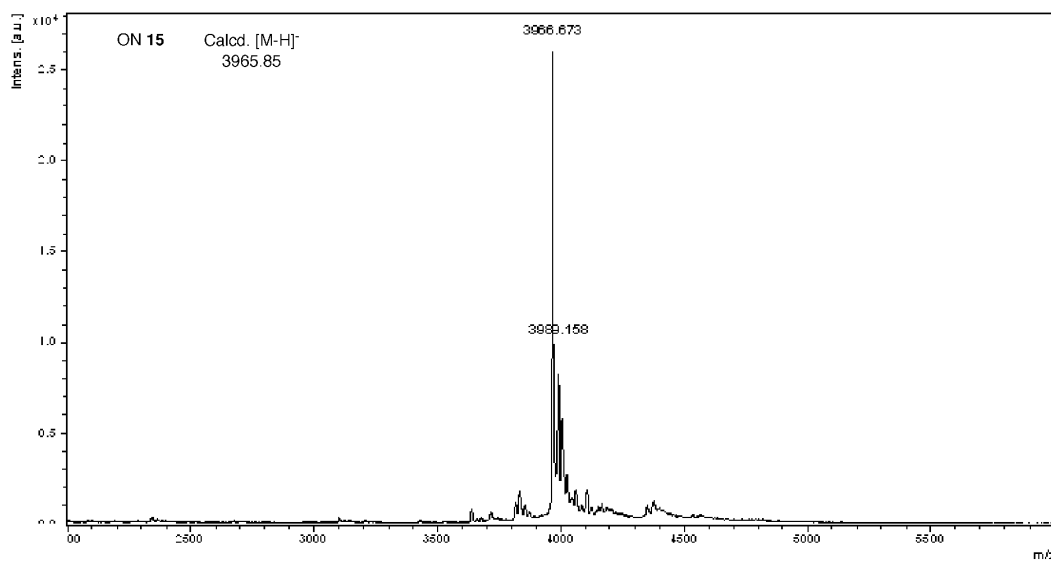
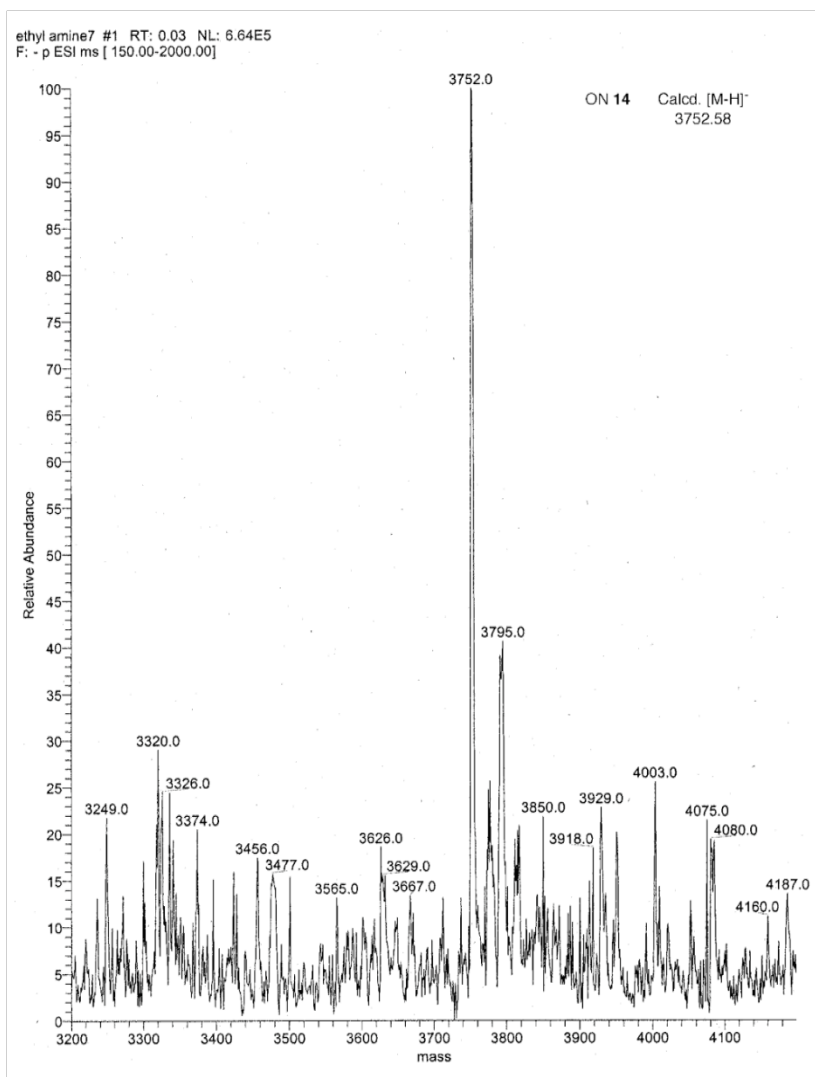
2-3. MALDI-TOF-MS data for ON 8-13, 15-23 and ESI-MS datum for ON 14

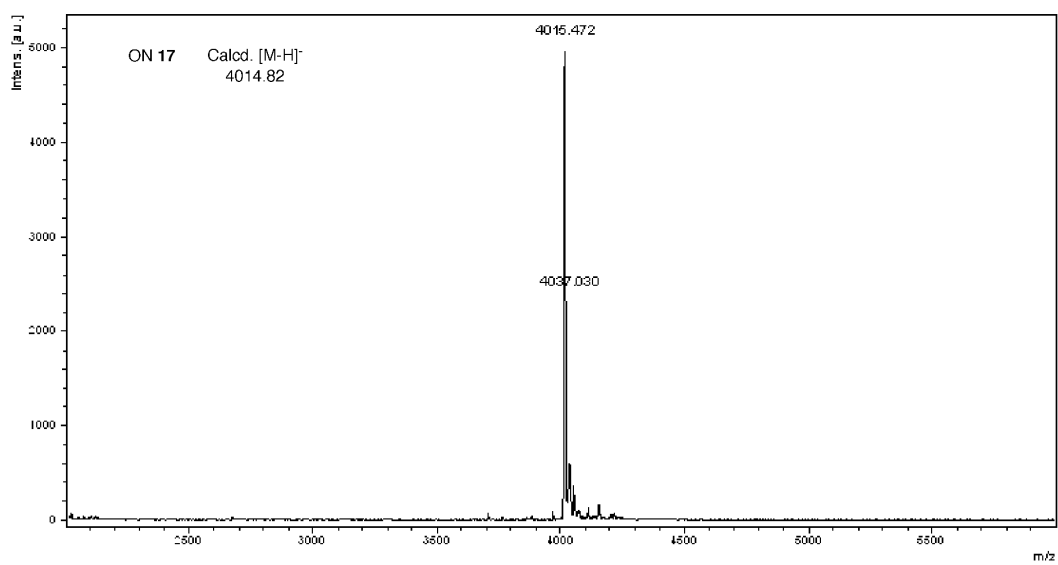
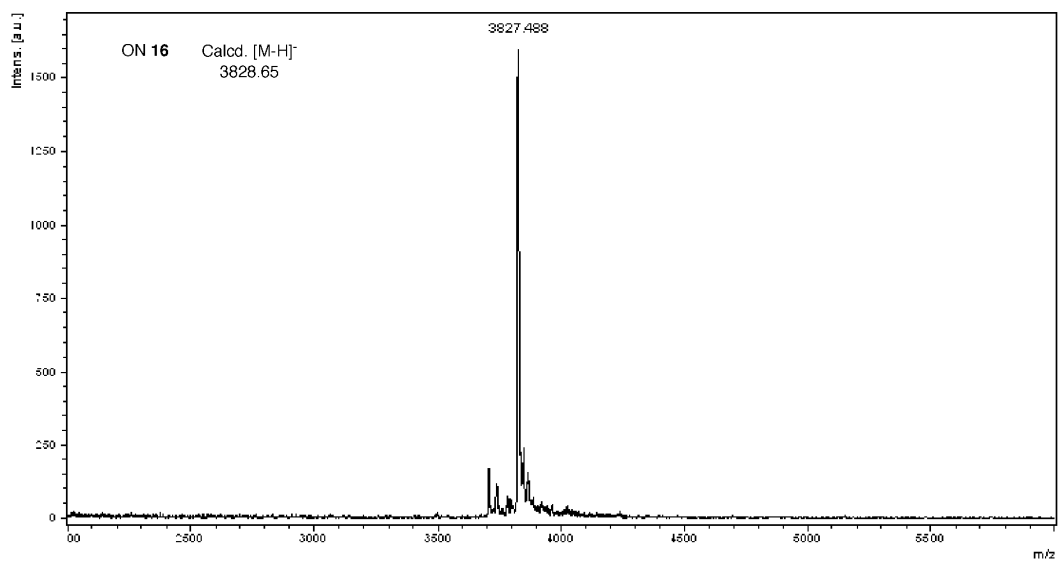


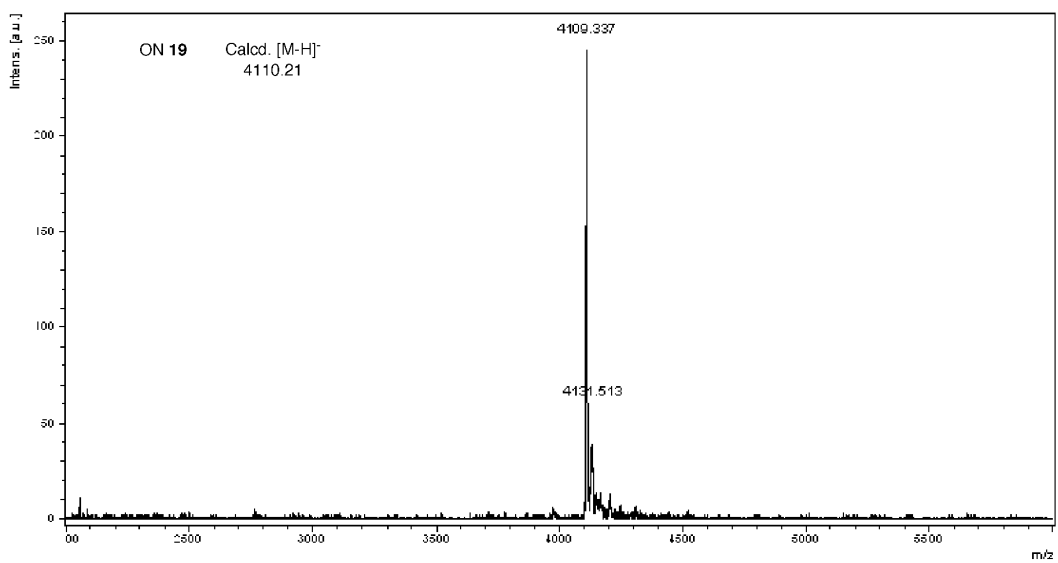
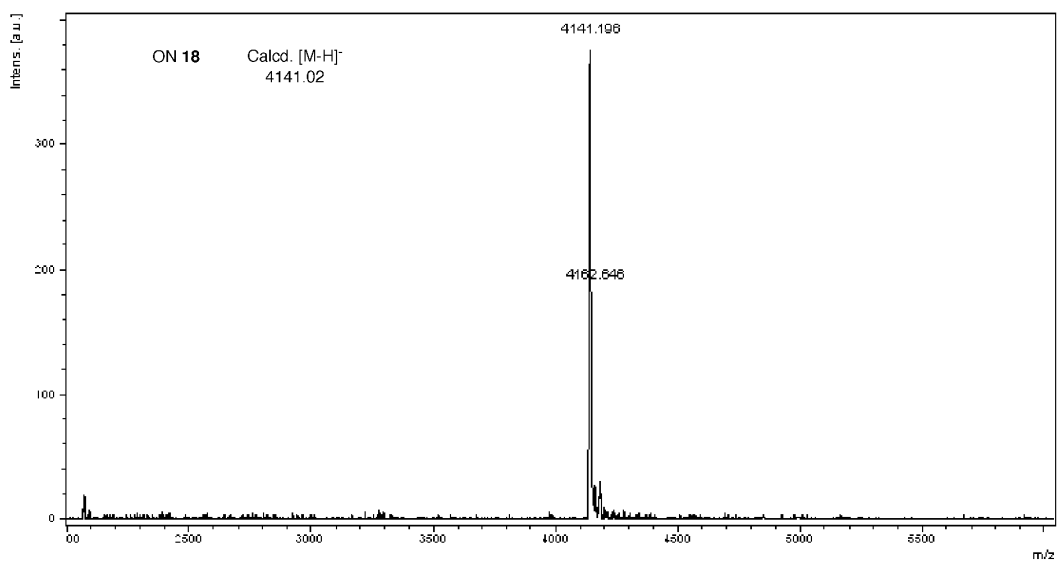
The peaks corresponding to 110 lower molecular weights than ON 8 and 9 were found in the MS data probably because the disulfide linkages were cleaved on MALDI-MS plate.

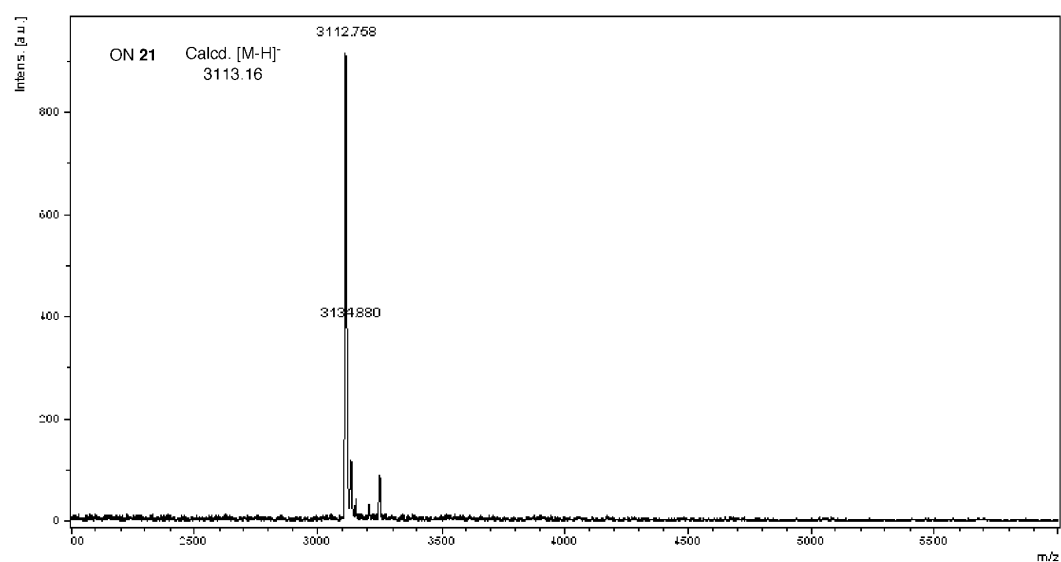
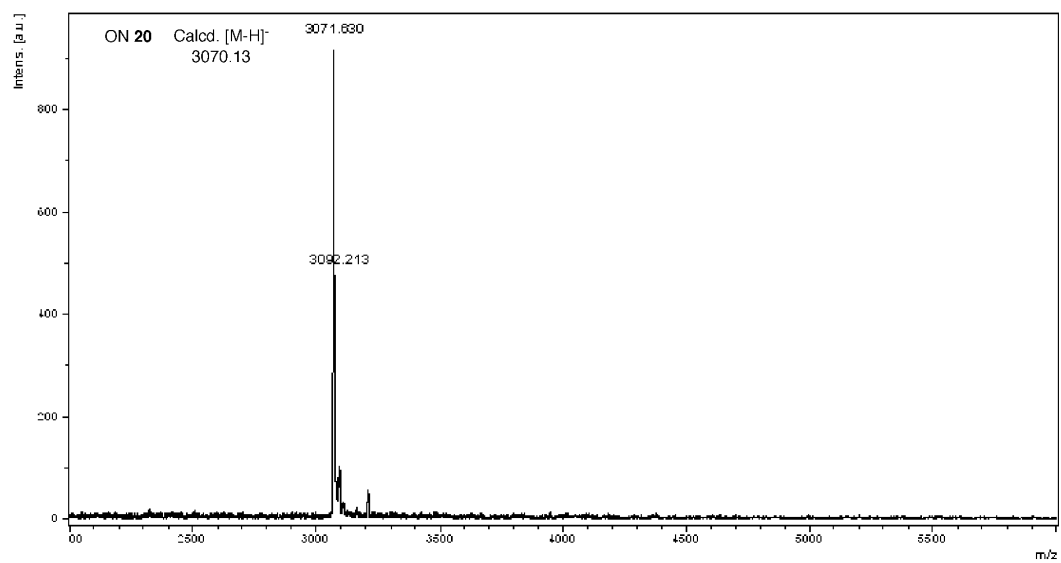


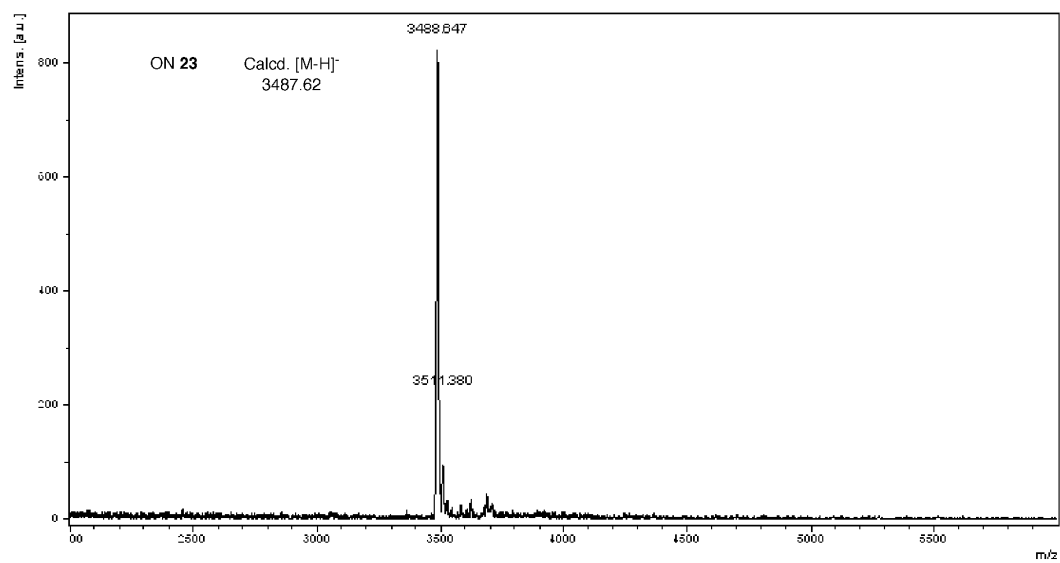
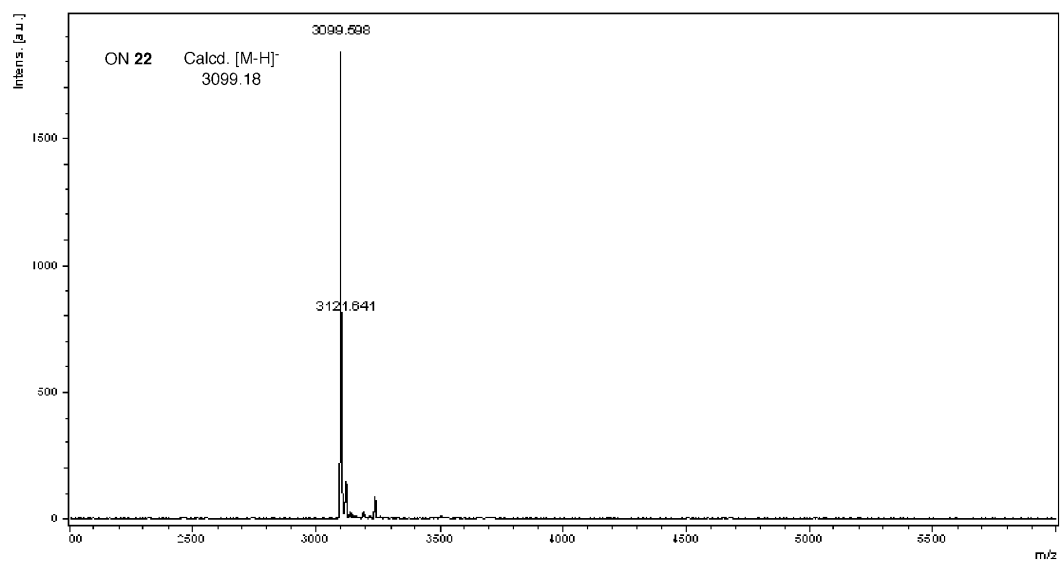












3. UV melting profiles (Figure S1 and S2)

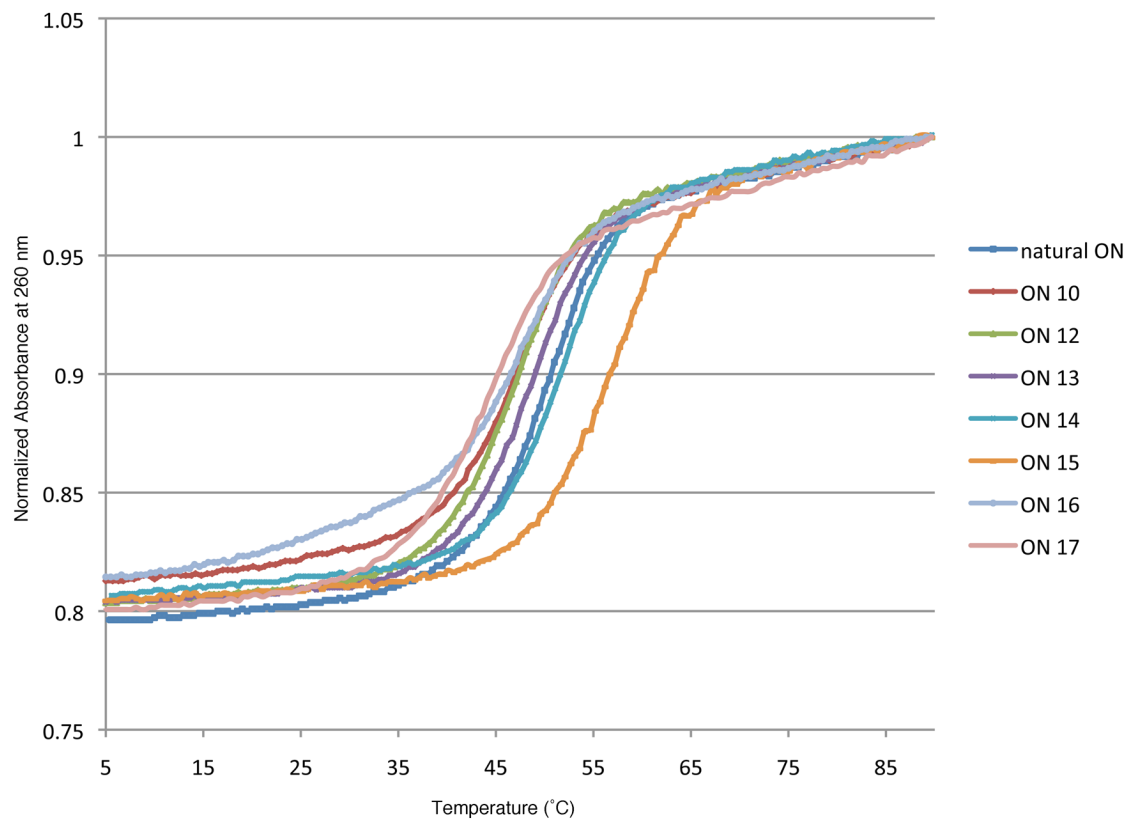


Figure S1 UV melting curves for the duplexes formed by conjugated ONs and the target DNA strand, 5'-d(AGCAAAAACGC)-3'. Conditions for natural ON and ON **12-17**: 10 mM sodium phosphate buffer (pH = 7.2), 100 mM NaCl solution, and 4 μ M each oligonucleotide, for ON **10**: 10 mM sodium phosphate buffer (pH= 7.4), 100 mM NaCl solution, 400 μ M TCEP, and 4 μ M each oligonucleotide. The melting profiles were recorded at 260 nm from 5 to 90 $^{\circ}$ C at a scan rate of 0.5 $^{\circ}$ C/min.

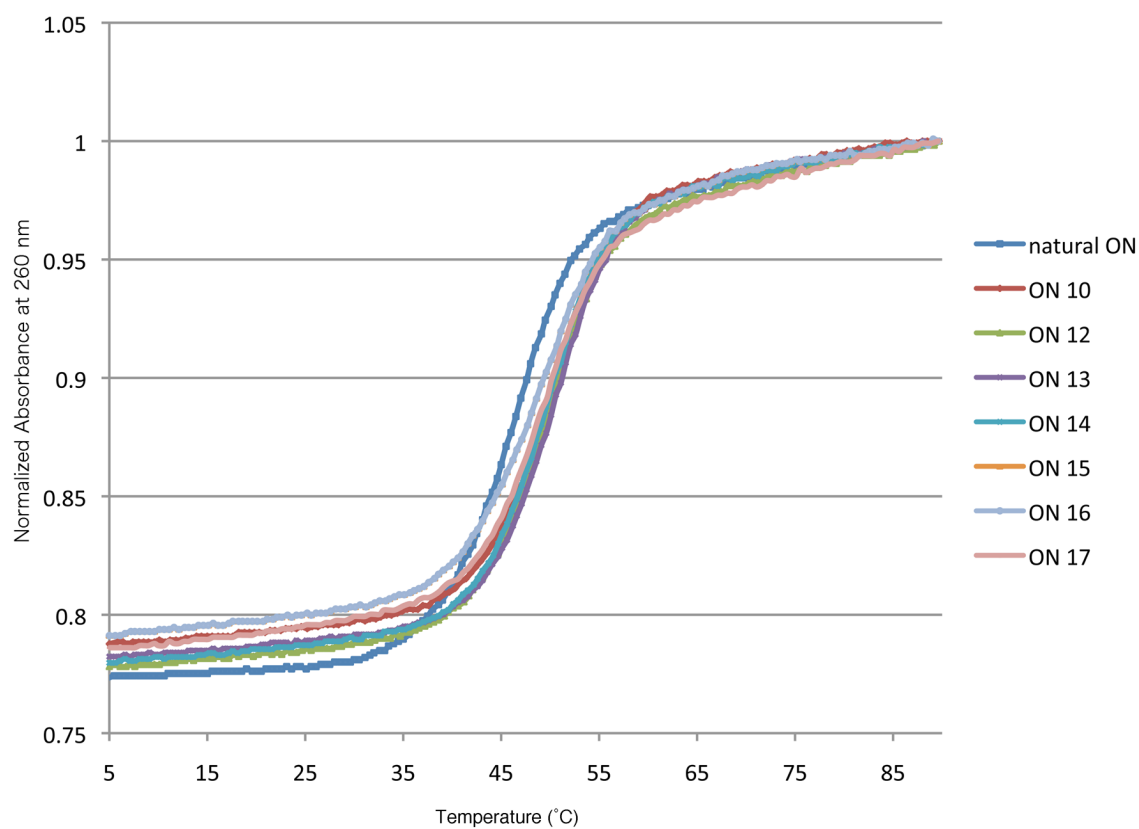


Figure S2 UV melting curves for the duplexes formed by conjugated ONs and the target RNA strand, 5'-r(AGCAAAAACGC)-3'. Conditions for natural ON and ON **12-17**: 10 mM sodium phosphate buffer (pH = 7.2), 100 mM NaCl solution, and 4 μ M each oligonucleotide, for ON **10**: 10 mM sodium phosphate buffer (pH= 7.4), 100 mM NaCl solution, 400 μ M TCEP, and 4 μ M each oligonucleotide. The melting profiles were recorded at 260 nm from 5 to 90 $^{\circ}$ C at a scan rate of 0.5 $^{\circ}$ C/min.