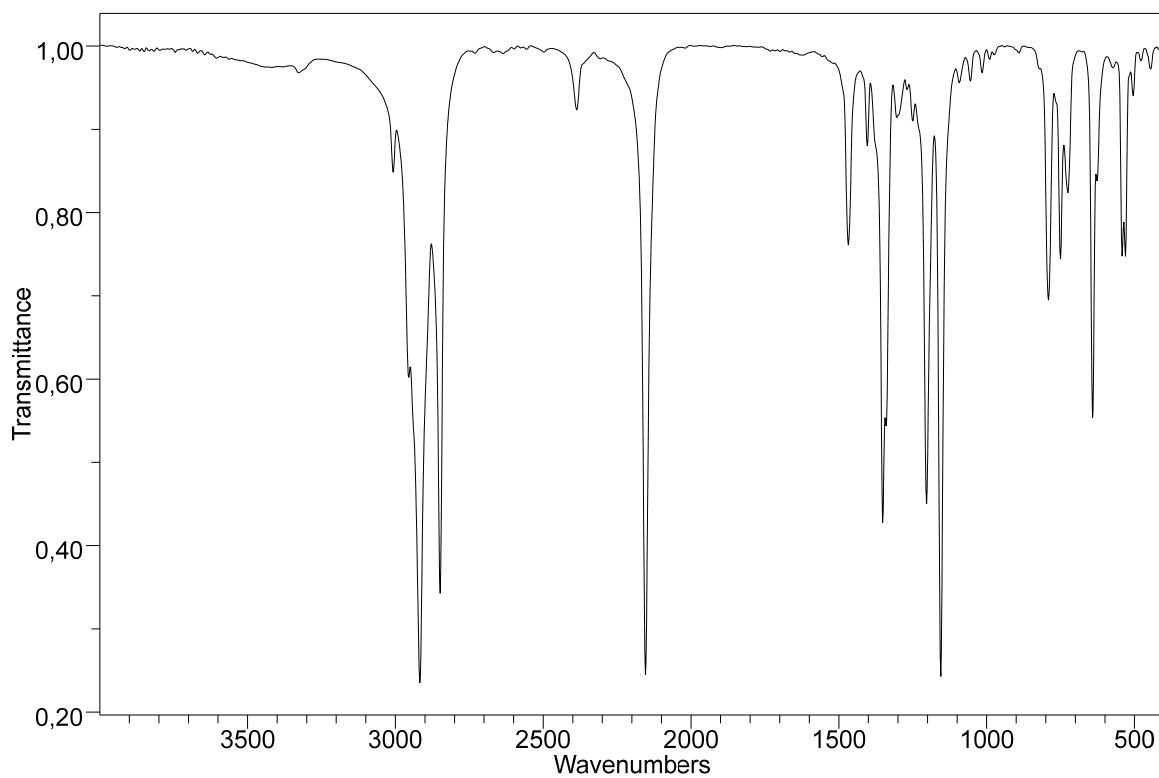


# Supplementary Materials: Novel lipid-oligonucleotide conjugates containing long-chain sulfonyl phosphoramidate groups: synthesis and biological properties

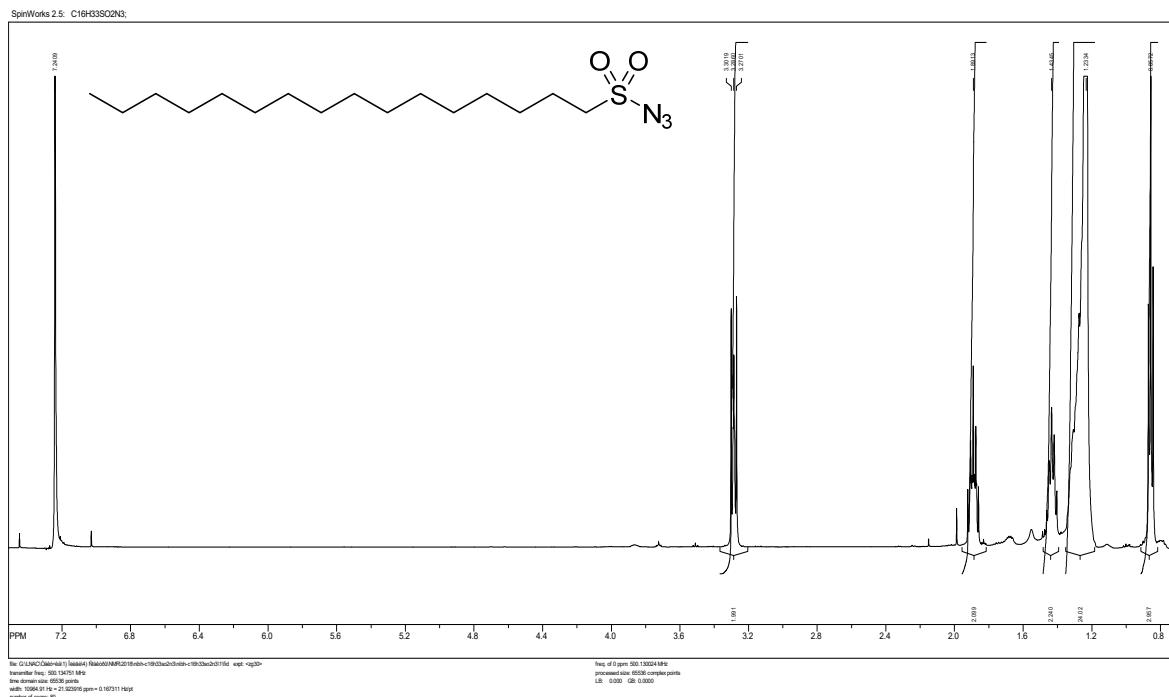
Alina Derzhalova, Oleg Markov, Alesya Fokina, Yasuo Shiohama, Timofei Zatsepin, Masayuki Fujii, Marina Zenkova and Dmitry Stetsenko

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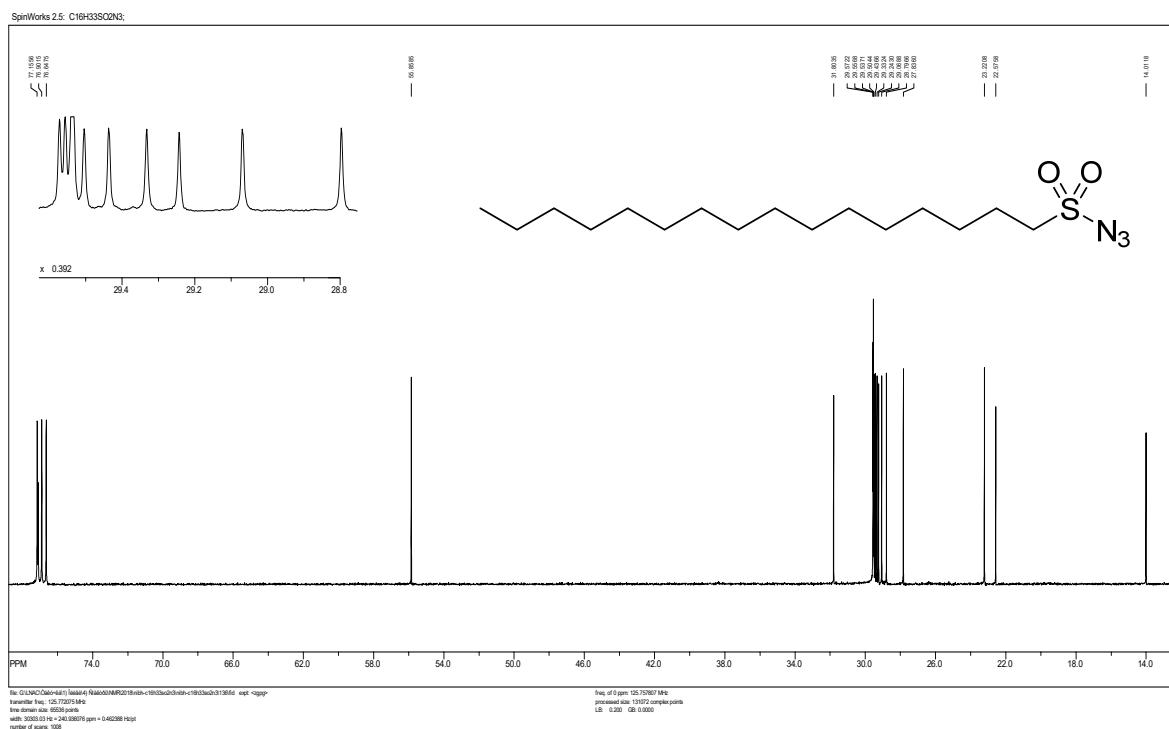
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**Figure S1.** IR spectrum of 1-hexadecanesulfonyl azide.



**Figure S2.** <sup>1</sup>H NMR spectrum of 1-hexadecanesulfonyl azide.



**Figure S3.** <sup>13</sup>C NMR spectrum of 1-hexadecanesulfonyl azide.

## RP-HPLC and ESI-MS data for oligonucleotides

Instrument: Ultimate 3000 chromatography unit with LCQ Fleet mass-spectrometry detector.

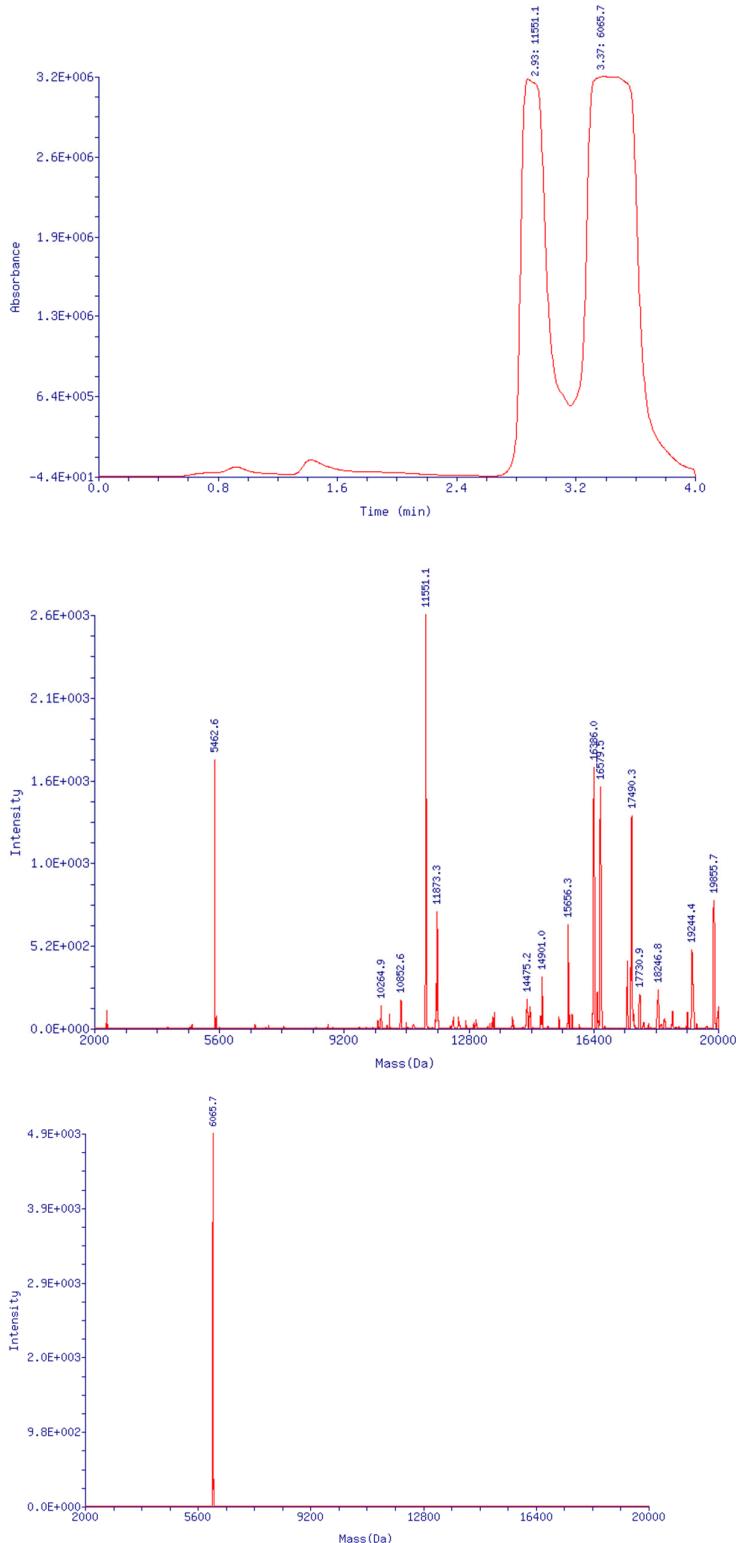
LC conditions: flow 0.3 ml/min, temperature 45°C, buffer A: 10 mM diisopropylamine, 15 mM hexafluoroisopropanol (HFIP) in water; buffer B: 10 mM diisopropylamine, 15 mM HFIP in 80% acetonitrile. Step elution 0–1 min 100% A, 1–3.5 min 100% B. UV detection at 260 nm. MS: ESI, negative mode.

**Table S1.** The list of oligonucleotide samples analyzed.

Code	Oligonucleotide sequence, 5'-3'
<b>1δ-F</b>	a <sup>d</sup> gt ctc gac ttg cta cc-Flu
<b>2δ-F</b>	a <sup>d</sup> g <sup>d</sup> t ctc gac ttg cta cc-Flu
<b>1η-F</b>	a <sup>ng</sup> t ctc gac ttg cta cc-Flu
<b>2η-F</b>	a <sup>ng</sup> t ctc gac ttg cta cc-Flu
<b>1δ</b>	a <sup>d</sup> gt ctc gac ttg cta cc
<b>2δ</b>	a <sup>d</sup> g <sup>d</sup> t ctc gac ttg cta cc

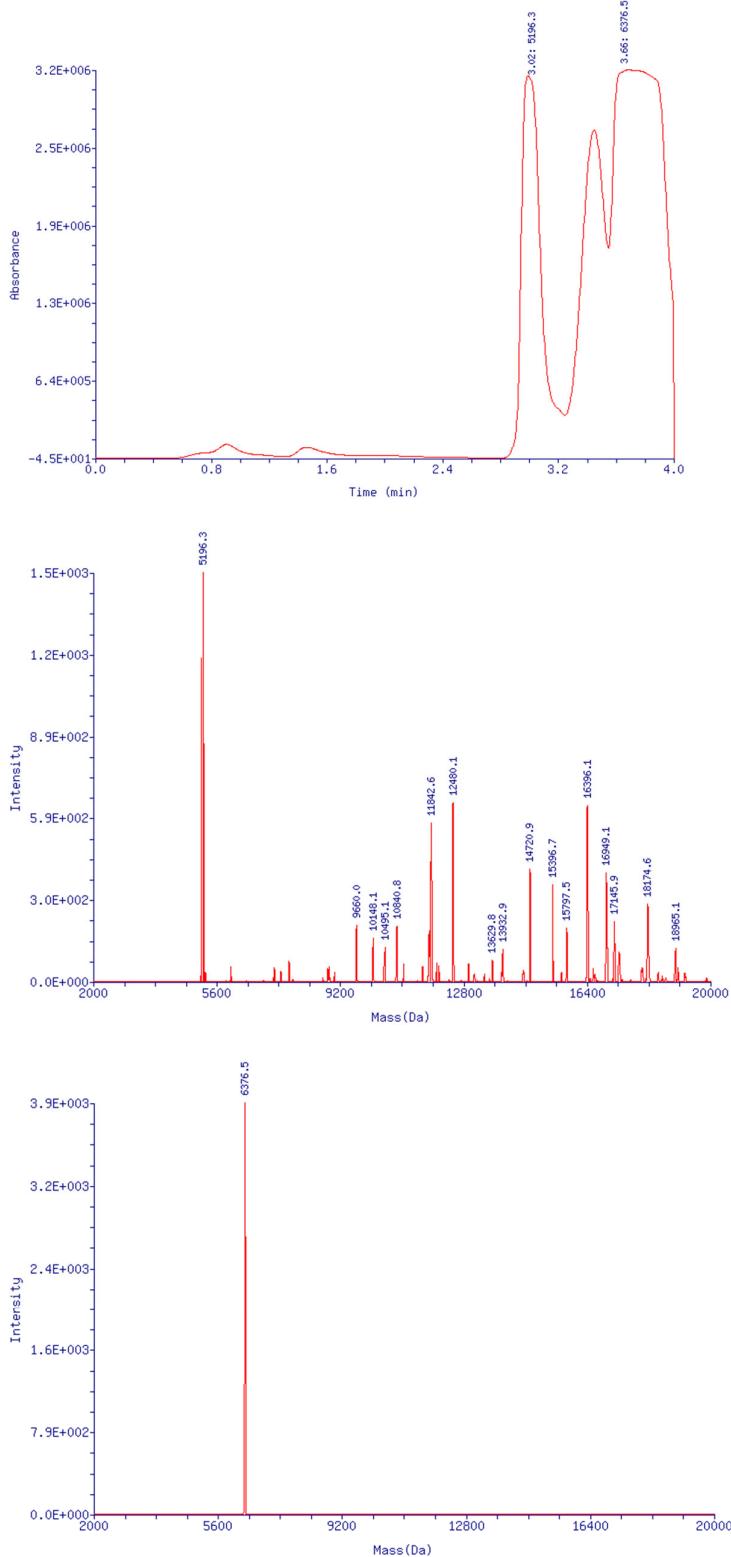
**1δ-F**Sequence: 5'-a<sup>δ</sup>gt ctc gac ttg cta cc-Flu

Calculated Mass: 6060 Da



**2δ-F**Sequence: 5'-a<sup>d</sup>g<sup>d</sup>t ctc gac ttg cta cc-Flu

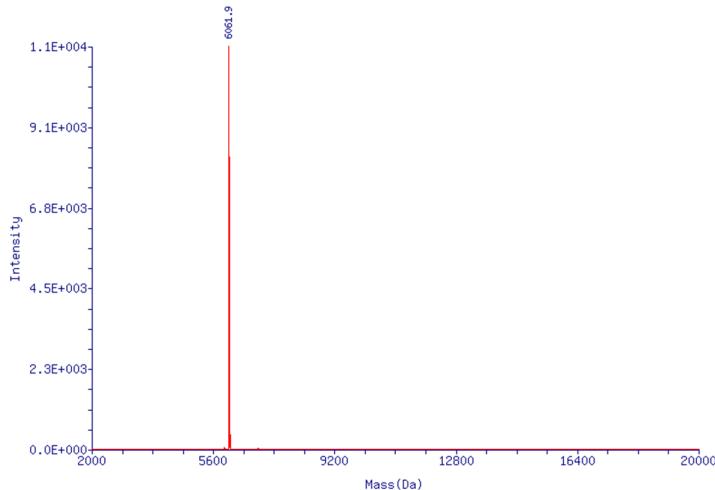
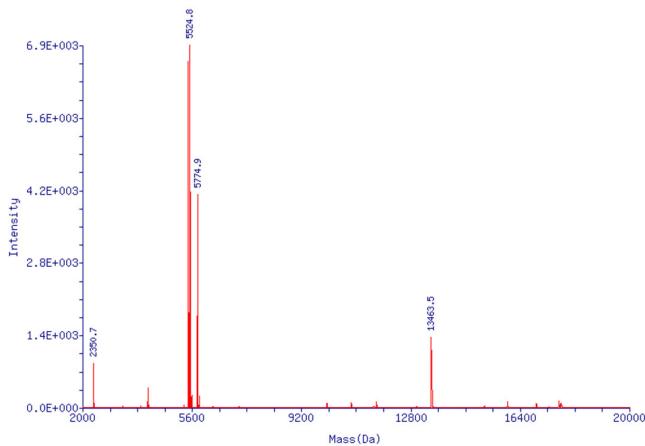
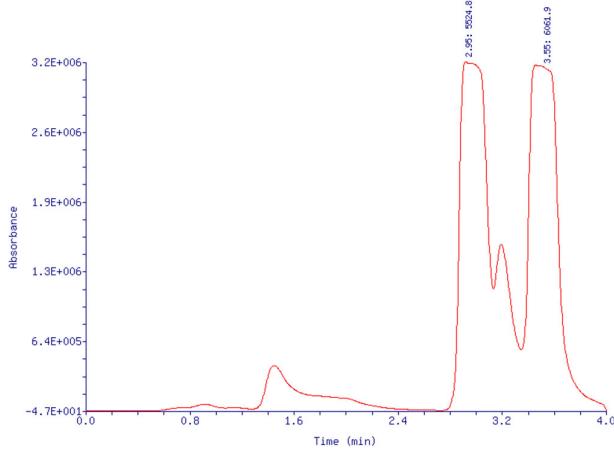
Calculated Mass: 6370 Da



**1η-F**

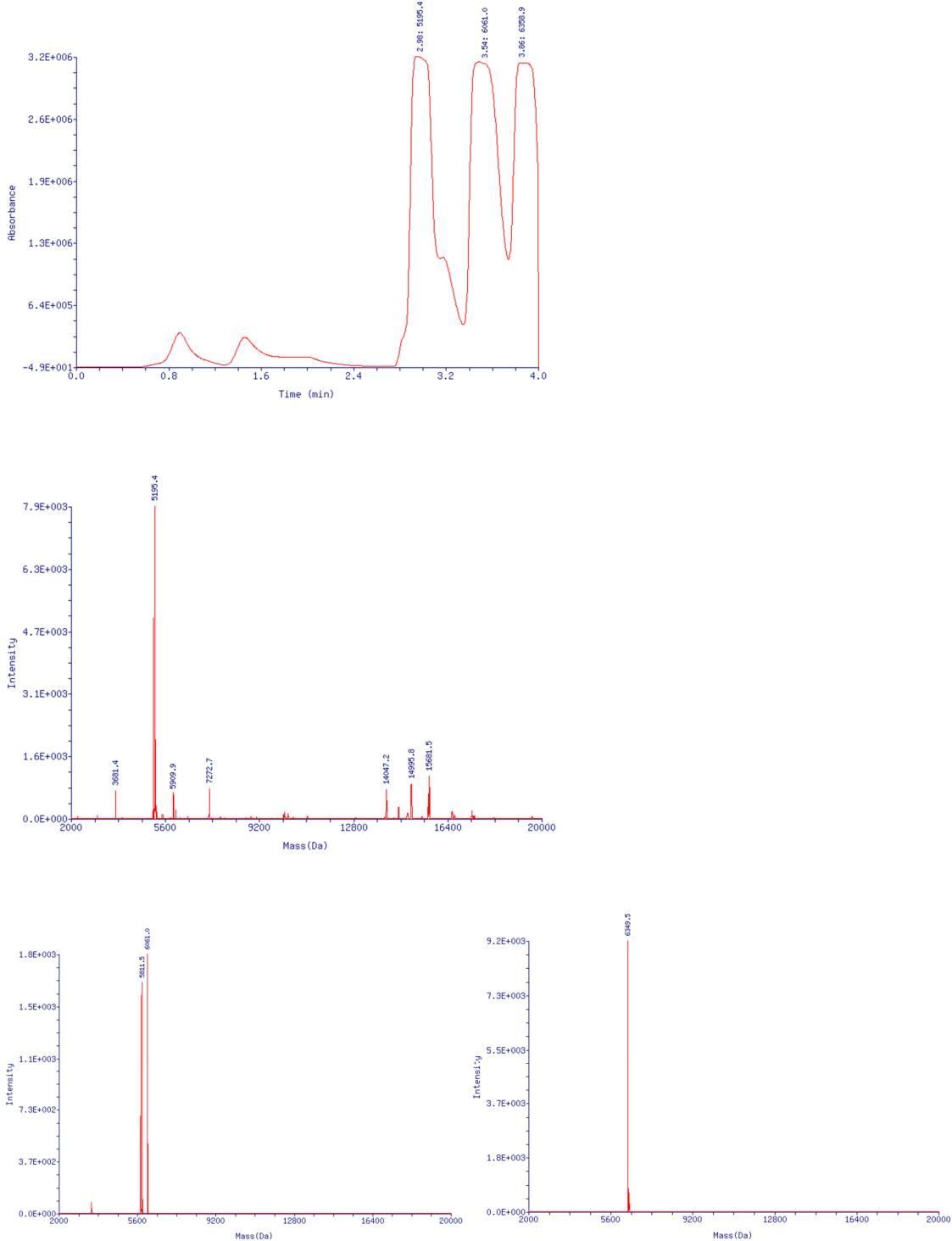
Sequence: 5'-agt ctc gac ttg cta cc-Flu

Calculated Mass: 6056 Da



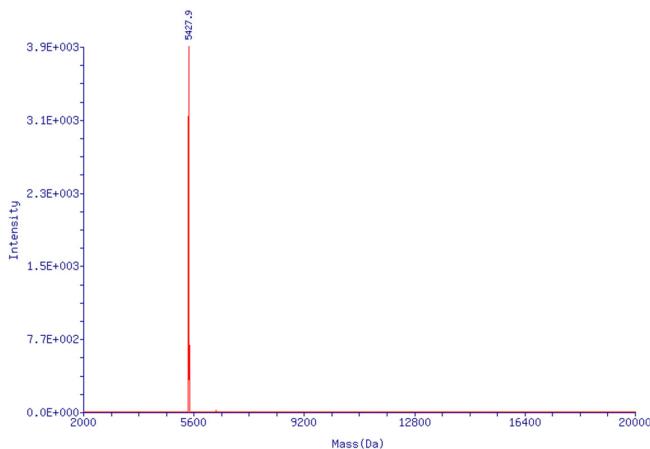
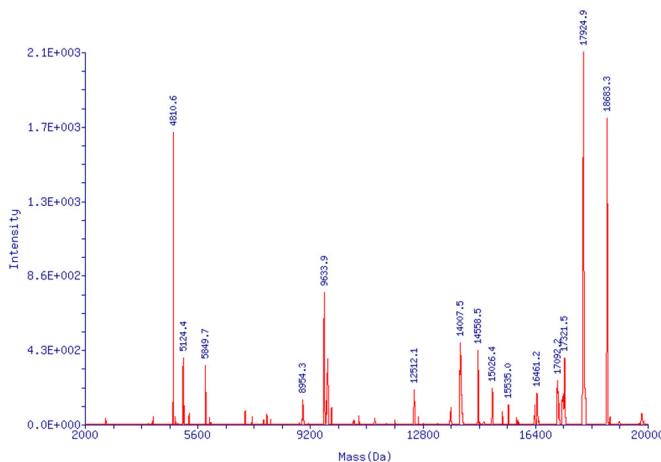
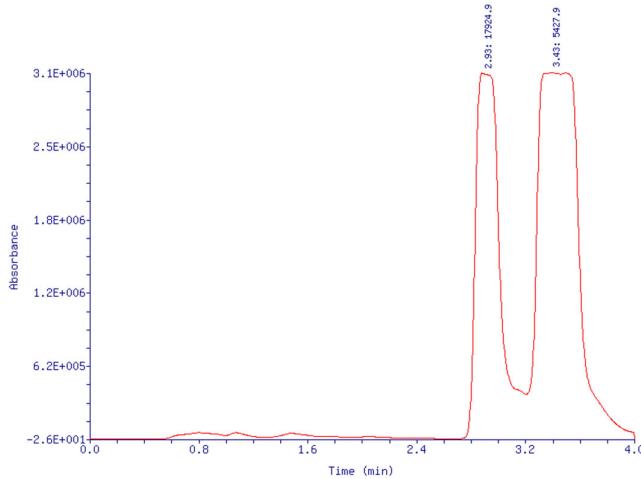
**2η-F**Sequence: 5'-a<sup>n</sup>g<sup>n</sup>t ctc gac ttg cta cc-Flu

Calculated Mass: 6345 Da



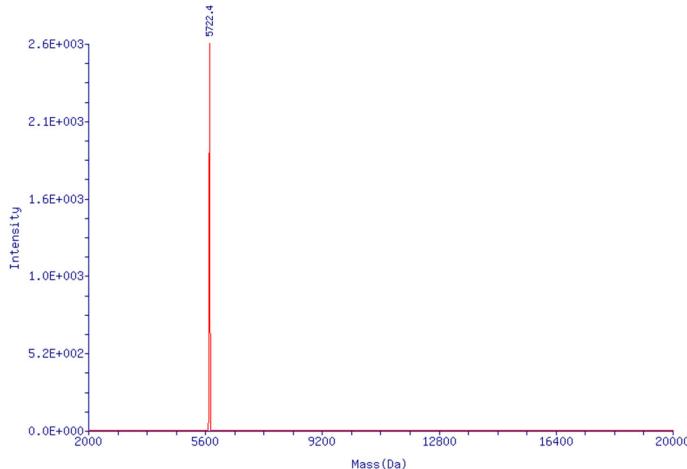
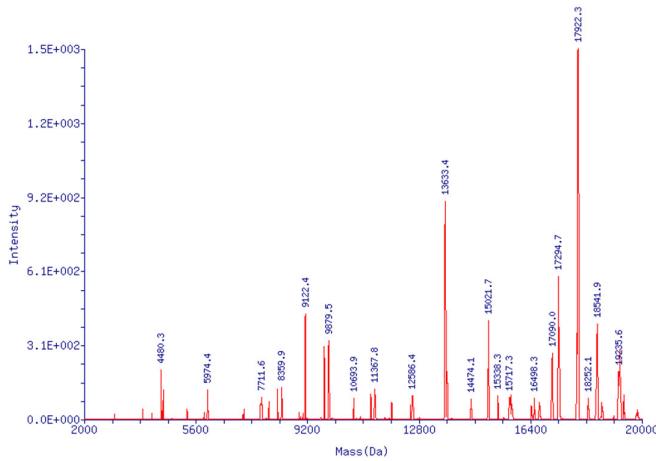
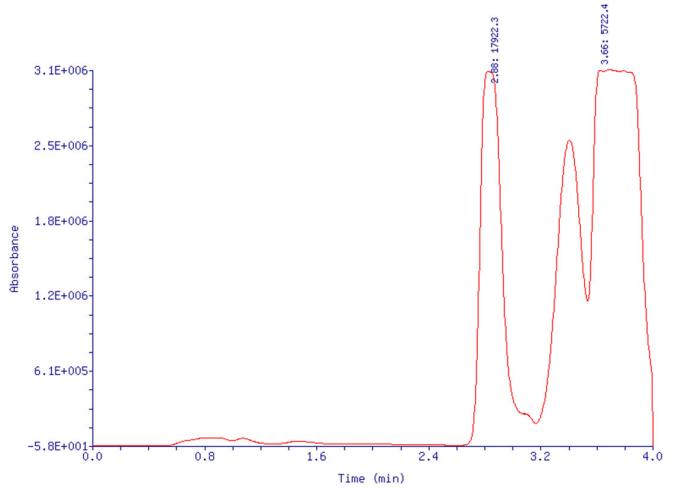
**18**Sequence: 5'-a<sup>5</sup>gt ctc gac ttg cta cc

Calculated Mass: 5430 Da



**2δ**Sequence: 5'-a<sup>d</sup>g<sup>d</sup>t ctc gac ttg cta cc

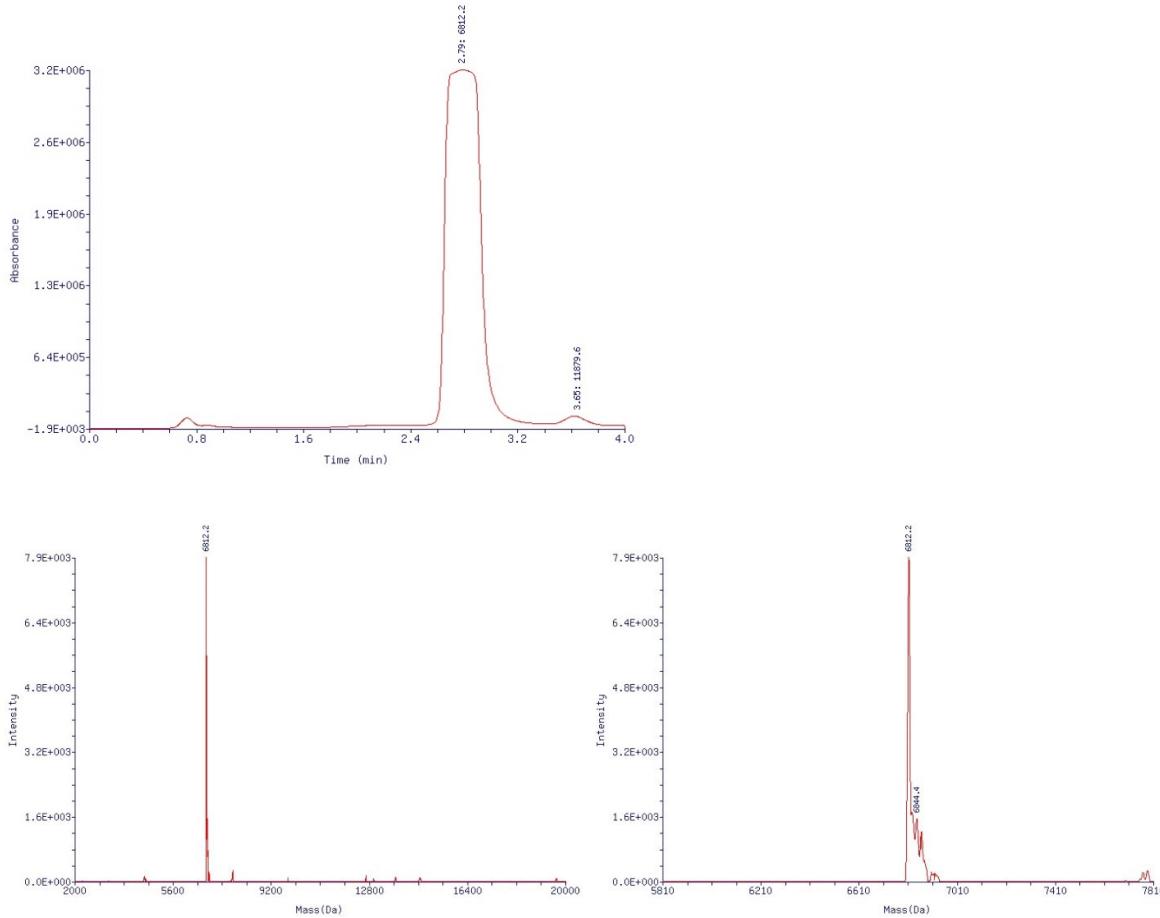
Calculated Mass: 5718 Da



**RGMa antisense**

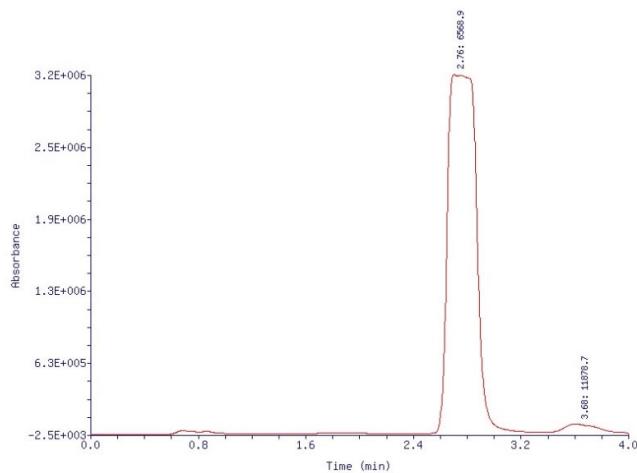
Sequence: 5'-AAGUUUCUUGAAGAUGAUGGUG

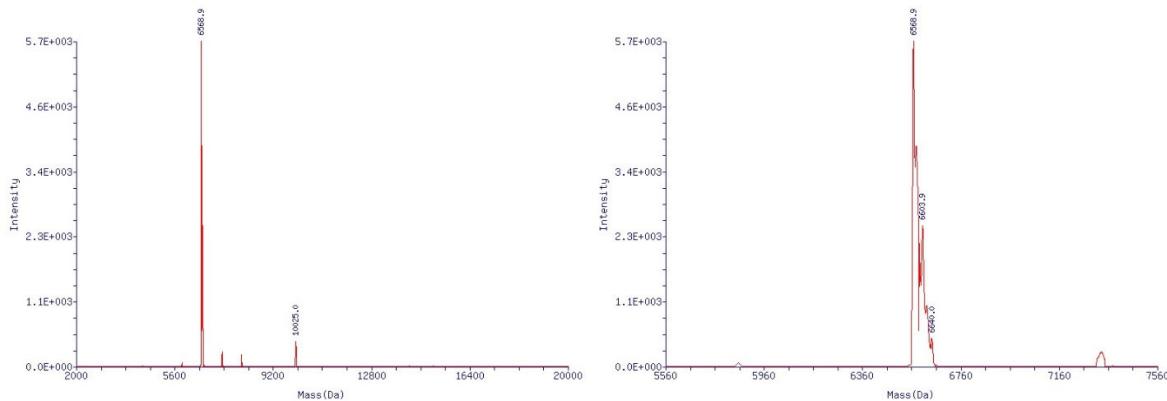
Calculated Mass: 6806.2 Da

**RGMa sense**

Sequence: 5'- CCAUCAUCUUCAAGAACUUCC

Calculated Mass: 6563.99 Da

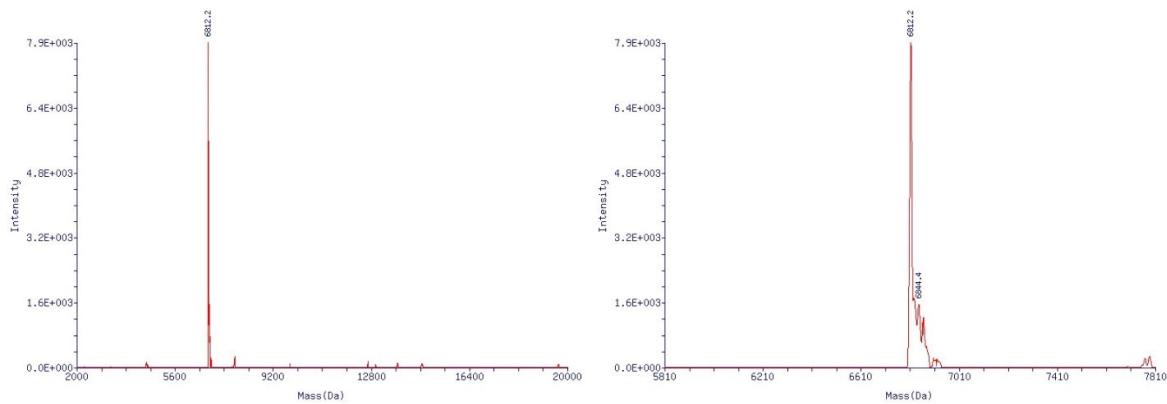
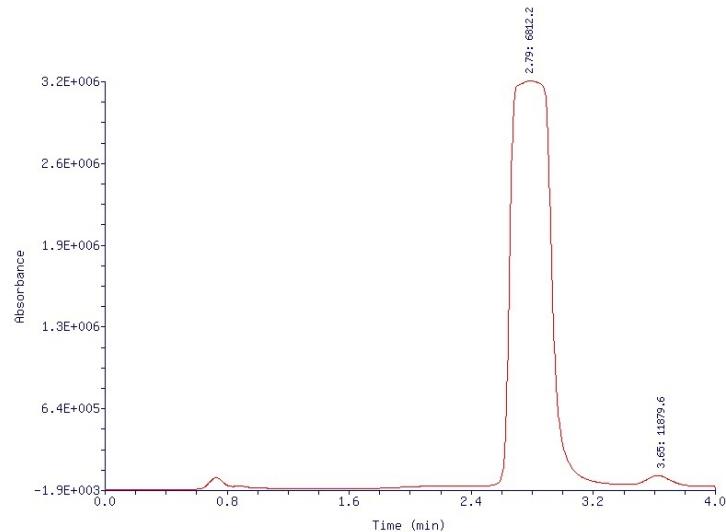




## 2H-RGMa antisense

Sequence: 5'-AAGUUUCUUGAAGAUGAUGGUG

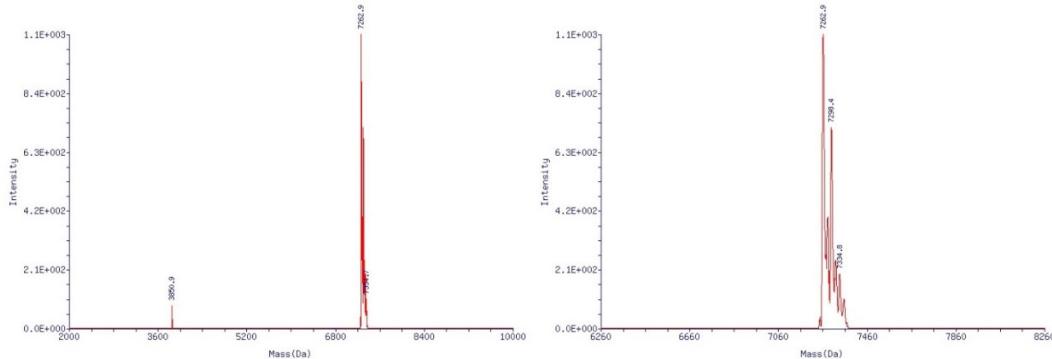
Calculated Mass: 6806.2 Da



## 2H-RGMa sense

Sequence: 5'-CCAUCAUCUCAAGAACUUC<sup>n</sup>C<sup>n</sup>-p

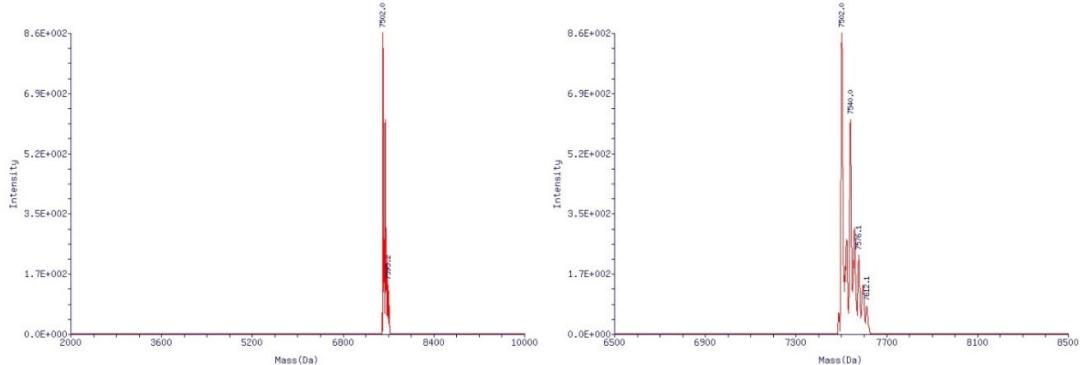
Calculated Mass: 7260.0 Da



## 4H-RGMa antisense

Sequence: 5'-AAGUUUCUUGAAGAUGAUGGC<sup>n</sup>C<sup>n</sup>-p

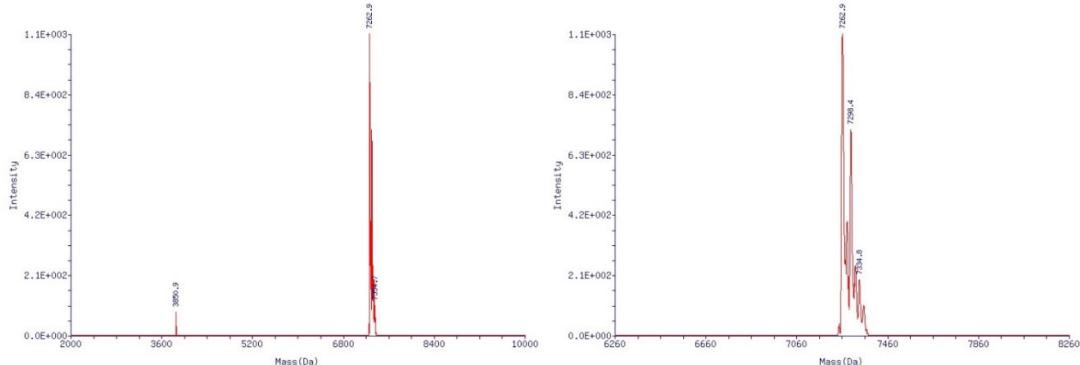
Calculated Mass: 7501.2 Da



## 4H-RGMa sense

Sequence: 5'-CCAUCAUCUCAAGAACUUC<sup>n</sup>C<sup>n</sup>-p

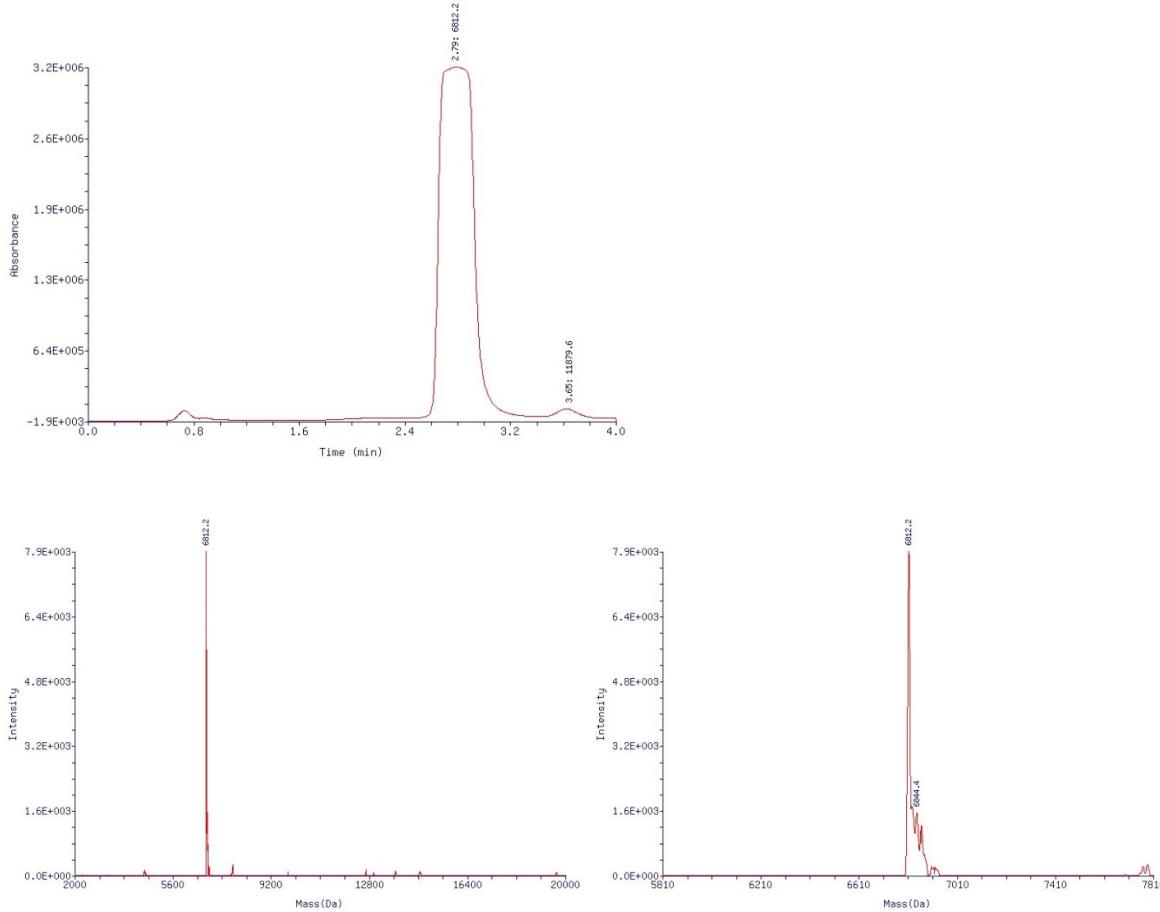
Calculated Mass: 7260.0 Da



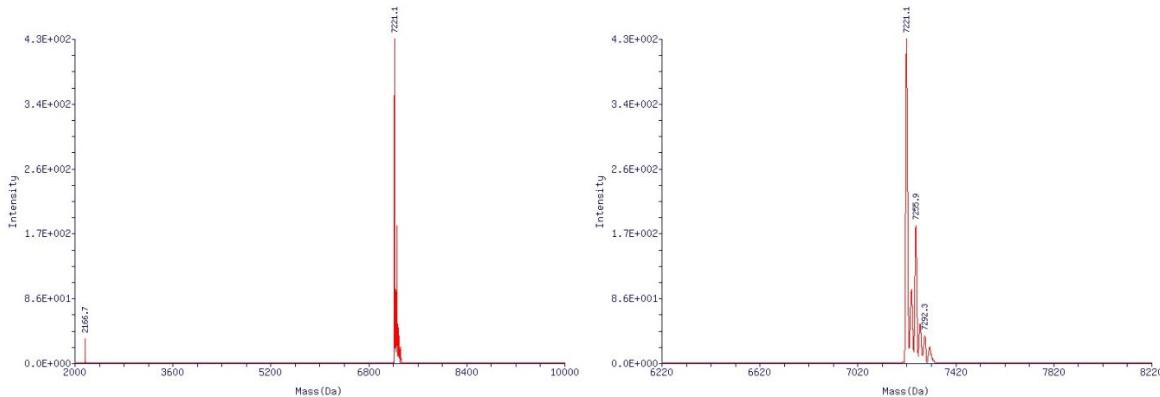
**2Δ-RGMa antisense**

Sequence: 5'-AAGUUUCUUGAAGAUGAUGGUG

Calculated Mass: 6806.2 Da

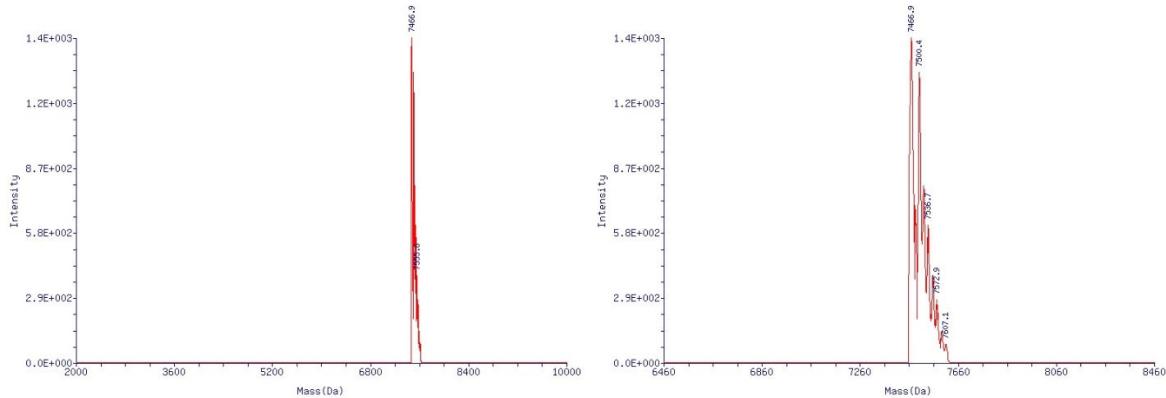
**2Δ-RGMa sense**Sequence: 5'-CCAUCAU<sup>C</sup>UCAAGAACUUC<sup>C</sup>-p

Calculated Mass: 7219.4 Da

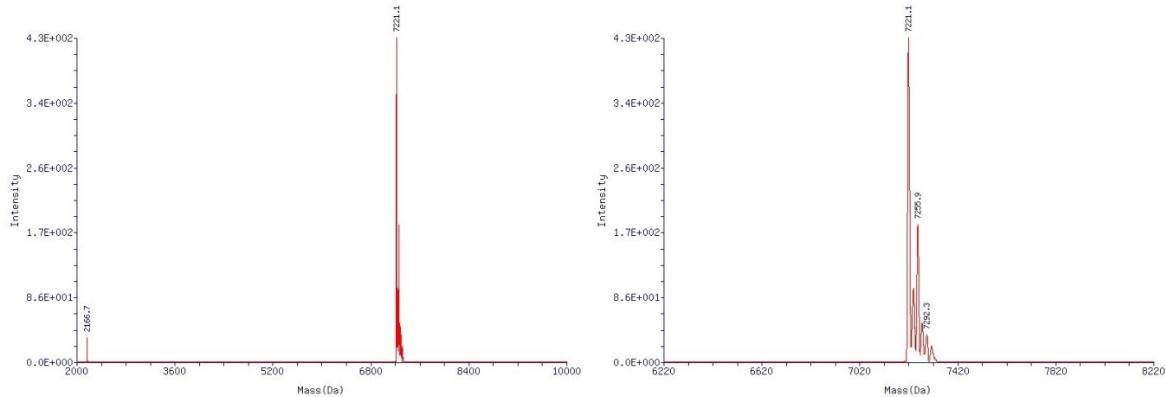


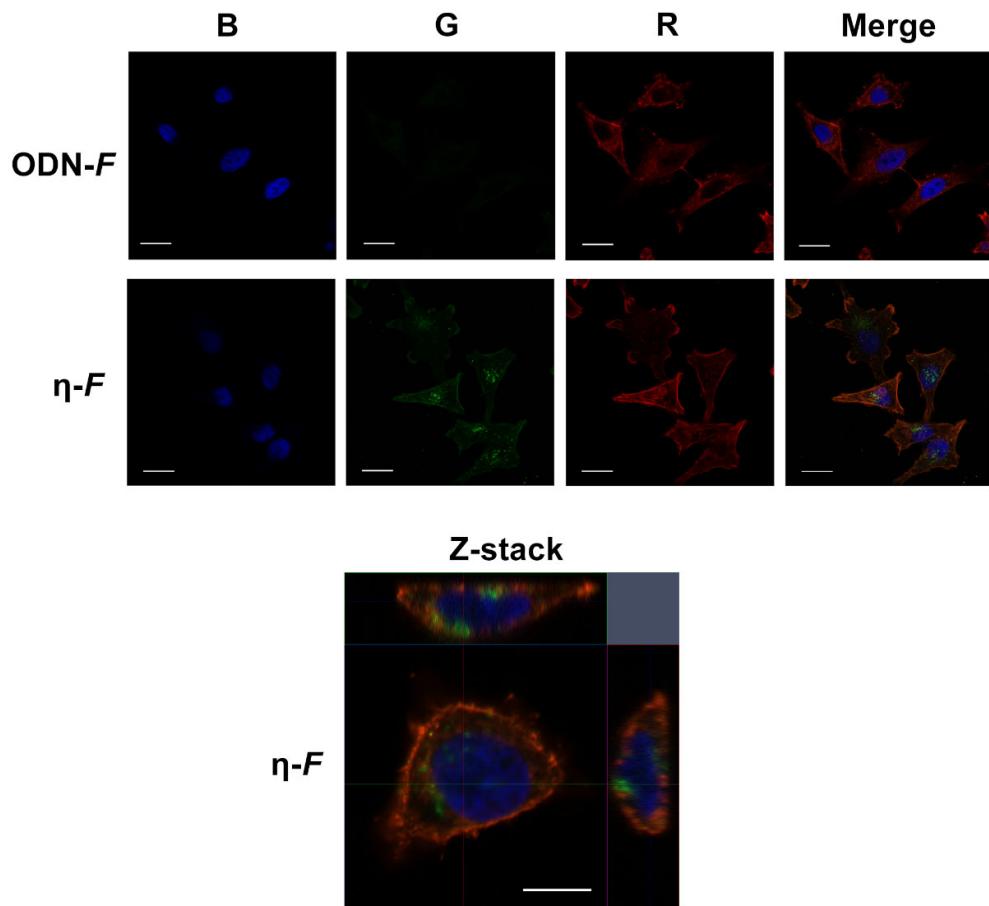
**4Δ-RGMa antisense**Sequence: 5'-AAGUUUCUUGAAGAUGAUGGC<sup>d</sup>C<sup>d</sup>-p

Calculated Mass: 7460.6 Da

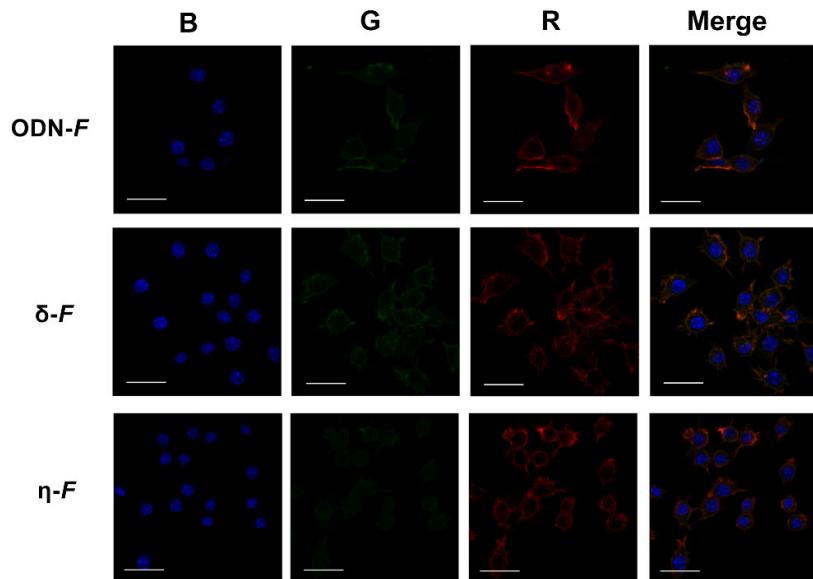
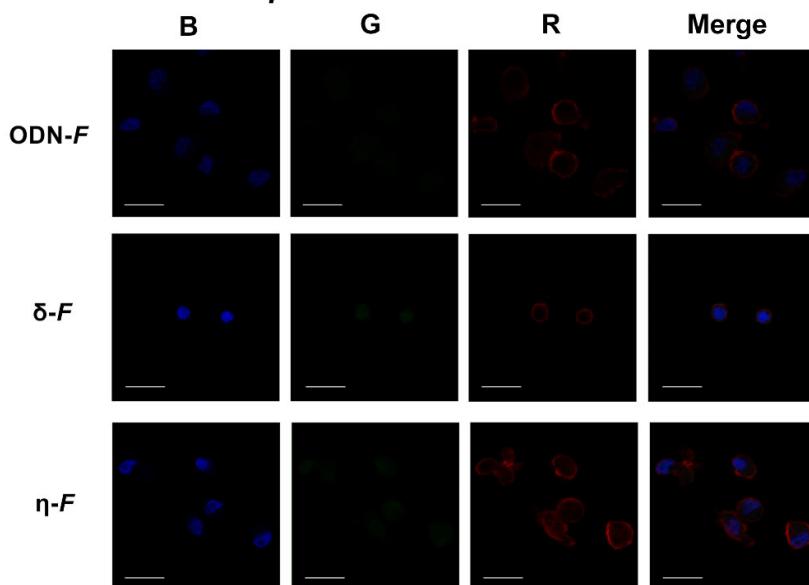
**4Δ-RGMa sense**Sequence: 5'-CCAUCAUCAUCUCAAGAACUUCC<sup>d</sup>C<sup>d</sup>-p

Calculated Mass: 7219.4 Da





**Figure S4.** Intracellular accumulation of  $\eta$ -F conjugate in KB-8-5 cells. Analysis of the samples was performed 24 h after addition of fluorescein-labeled conjugates ( $5 \mu\text{M}$ ) to the cells using  $\alpha$ Plan-Apochromat 100x/1.46 Oil DIC M27 objective. Three-channel (BGR) pictures were obtained using staining by DAPI (nuclei staining) (B); fluorescein-labeled conjugates (G); Phalloidin-iFluor532 (cytoskeleton staining) (R). Scale bars: top panel –  $20 \mu\text{m}$ , Z-stack –  $10 \mu\text{m}$ .

**RAW264.7 cells****THP-1-induced Mφ**

**Figure S5.** Intracellular accumulation of  $\delta$ -F and  $\eta$ -F conjugates in RAW264.7 cells and THP-1-induced macrophages. Analysis of the samples was performed 24 h after addition of fluorescein-labeled conjugates (5  $\mu$ M) to the cells using  $\alpha$ Plan-Apochromat 100x/1.46 Oil DIC M27 objective. Three-channel (BGR) pictures were obtained using staining by DAPI (nuclei staining) (B); fluorescein-labeled conjugates (G); Phalloidin-iFluor532 (cytoskeleton staining) (R). Scale bars: 20  $\mu$ m.