

Supporting Information

for manuscript entitled

Segmented Hybridization Probes: Modulating Target Affinity and Base Pairing Selectivity

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1. Analytical data for modified oligonucleotides.

Compound 7: Yield: 16%; C₁₈ cartridge, CH₃CN in TEAA (0.1 M, pH = 7) gradient: 0% to 50%, elutes with 15% CH₃CN, MALDI-TOF MS for C₁₃₀H₁₆₇N₄₇O₇₈P₁₃ [M-H]⁻: calcd 4036, found 4040.

Compound 8: Yield: 21%; C₁₈ cartridge, CH₃CN in TEAA (0.1 M, pH = 7) gradient: 0% to 50%, elutes with 15% CH₃CN, MALDI-TOF MS for C₁₃₃H₁₇₃N₄₇O₈₀P₁₃ [M-H]⁻: calcd 4110, found 4114.

Compound 9: Yield: 12%; C₁₈ cartridge, CH₃CN in TEAA (0.1 M, pH = 7) gradient: 0% to 50%, elutes with 15% CH₃CN, MALDI-TOF MS for C₁₃₉H₁₈₅N₄₇O₈₃P₁₃ [M-H]⁻: calcd 4242, found 4242.

Compound 11: Yield: 15%; C₁₈ cartridge, CH₃CN in TEAA (0.1 M, pH = 7) gradient: 0% to 50%, elutes with 15% CH₃CN, MALDI-TOF MS for C₁₉₁H₂₄₇N₇₀O₁₁₆P₂₀ [M-H]⁻: calcd 5995, found 5995.

Compound 12: Yield: 7%; C₁₈ cartridge, CH₃CN in TEAA (0.1 M, pH = 7) gradient: 0% to 50%, elutes with 15% CH₃CN, MALDI-TOF MS for C₂₀₉H₂₄₆N₆₈O₁₁₇P₂₀ [M-H]⁻: calcd 5970, found 5971.

Compound 13: Yield: 28%; C₁₈ cartridge, CH₃CN in TEAA (0.1 M, pH = 7) gradient: 0% to 50%, elutes with 15% CH₃CN, MALDI-TOF MS for C₁₉₉H₂₅₈N₇₁O₁₂₃P₂₁ [M-H]⁻: calcd 6259, found 6261.

Compound 14: Yield: 11%; C₁₈ cartridge, CH₃CN in TEAA (0.1 M, pH = 7) gradient: 0% to 50%, elutes with 15% CH₃CN, MALDI-TOF MS for C₁₉₄H₂₅₆N₆₇O₁₂₃P₂₁ [M-H]⁻: calcd 6244, found 6248.

Compound 15: Yield: 27%; C₁₈ cartridge, CH₃CN in TEAA (0.1 M, pH = 7) gradient: 0% to 50%, elutes with 15% CH₃CN, MALDI-TOF MS for C₁₉₄H₂₅₆N₆₇O₁₂₃P₂₁ [M-H]⁻: calcd 6244, found 6248.

Compound 16: Yield: 30%; C₁₈ cartridge, CH₃CN in TEAA (0.1 M, pH = 7) gradient: 0% to 50%, elutes with 15% CH₃CN, MALDI-TOF MS for C₁₉₉H₂₅₇N₇₄O₁₂₁P₂₁ [M-H]⁻: calcd 6268, found 6268.

Compound 19: Yield: 11%, HPLC, CH₃CN gradient: 0% for 5 min to 35% in 45 min, *t_R* = 27 min, MALDI-TOF MS for C₂₁₈H₂₇₃N₇₀O₁₃₃P₂₂ [M-H]⁻: calcd 6782, found 6786.

Compound 20: Yield: 10%, HPLC, CH₃CN gradient: 0% for 5 min to 35% in 45 min, *t_R* = 27 min, MALDI-TOF MS for C₂₁₈H₂₇₃N₇₀O₁₃₃P₂₂ [M-H]⁻: calcd 6782, found 6789.

Compound 21: Yield: 11%, HPLC, CH₃CN gradient: 0% for 5 min to 45% in 45 min, *t_R* = 33 min, MALDI-TOF MS for C₂₂₃H₂₇₄N₇₇O₁₃₁P₂₂ [M-H]⁻: calcd 6806, found 6811.

Compound 22: Yield: 4%, HPLC, CH₃CN gradient: 0% for 5 min to 35% in 45 min, *t_R* = 32 min, MALDI-TOF MS for C₁₅₀H₁₈₈N₅₀O₈₆P₁₄ [M-H]⁻: calcd 4498, found 4499.

Compound 23: Yield: 1%, HPLC, CH₃CN gradient: 0% for 5 min to 35% in 45 min, t_R = 34 min, MALDI-TOF MS for C₁₅₃H₁₉₄N₅₀O₈₈P₁₄ [M-H]⁻: calcd 4572, found 4574.

Compound 24: Yield: 4%, HPLC, CH₃CN gradient: 0% for 5 min to 35% in 28 min, t_R = 26 min, MALDI-TOF MS for C₁₅₉H₂₀₇N₅₀O₉₁P₁₄ [M-H]⁻: calcd 4705, found 4705.

2. Representative Melting curves

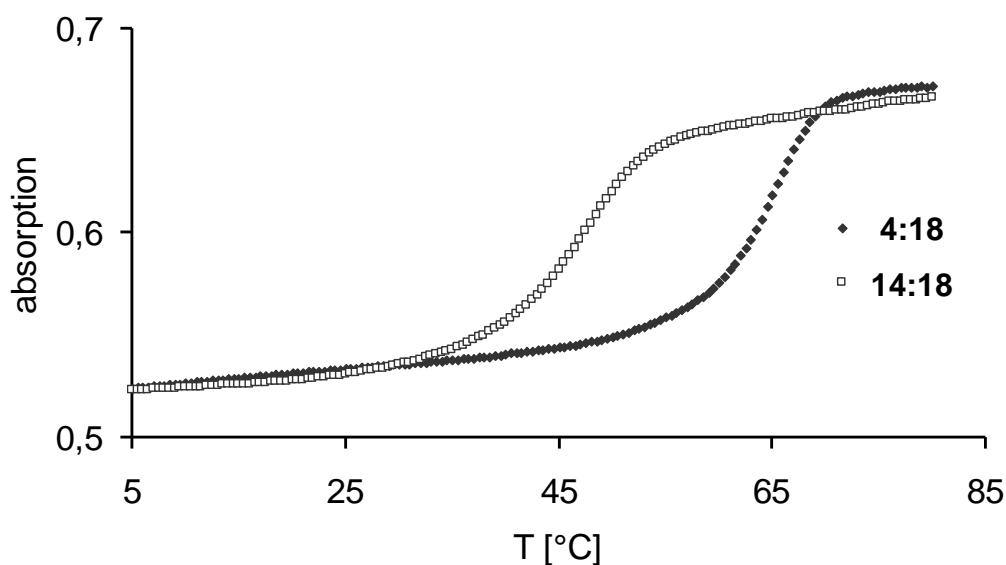


Figure S1: Melting curves of duplexes with segmented probe strand **14** and RNA target **18** (let-7d) and control duplex with unsegmented probe **4** and target **18**. Conditions: probe strands (1.5 μM), RNA target strands (1.5 μM), sodium phosphate buffer (10 mM), pH 7, NaCl (1 M).

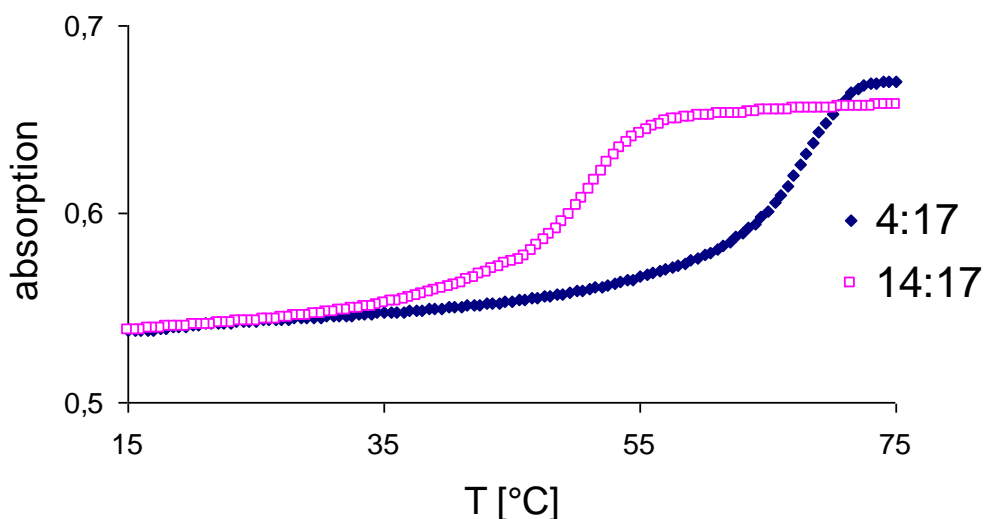


Figure S2: Melting curves of duplexes of segmented probe strand **14** and RNA target **17** (let-7e) and control duplex of unsegmented probe **4** and target **17**. Conditions: probe strands (1.5 μM), RNA target strands (1.5 μM), sodium phosphate buffer (10 mM), pH 7, NaCl (1 M).

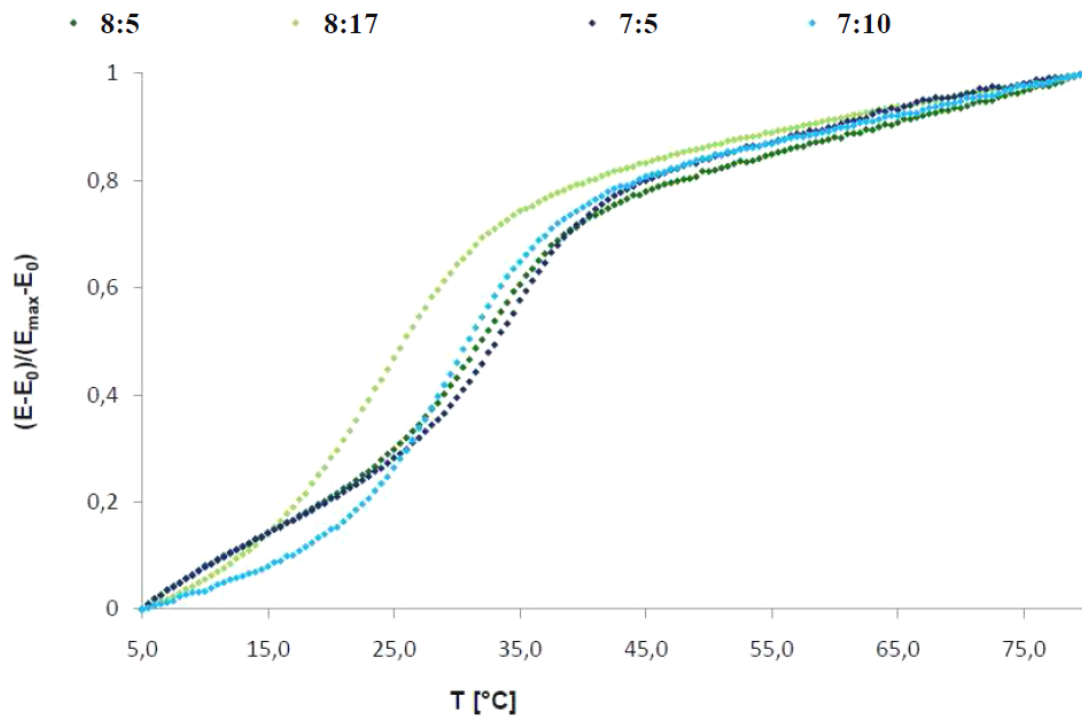
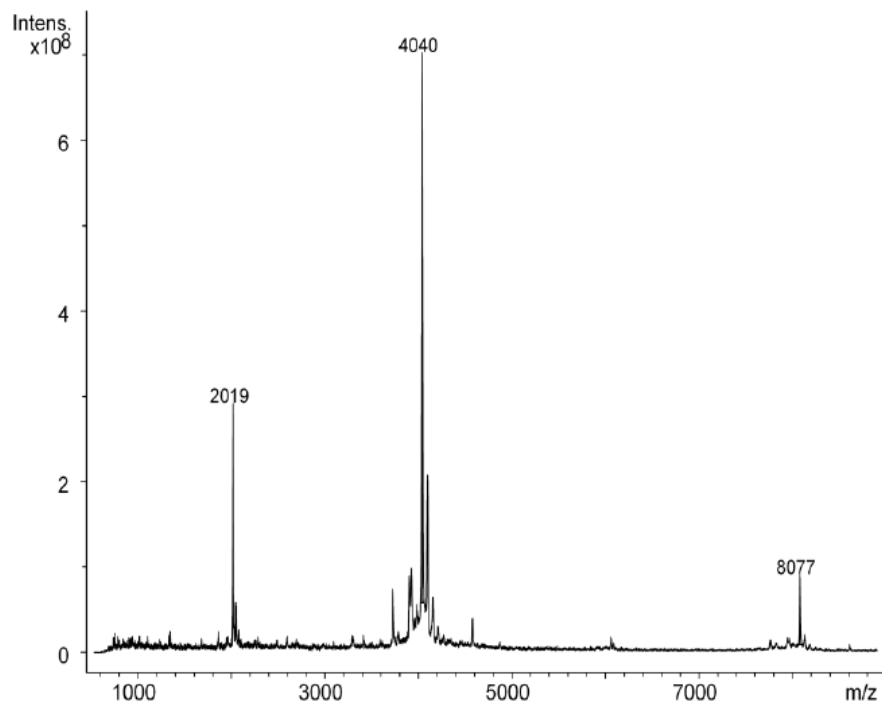
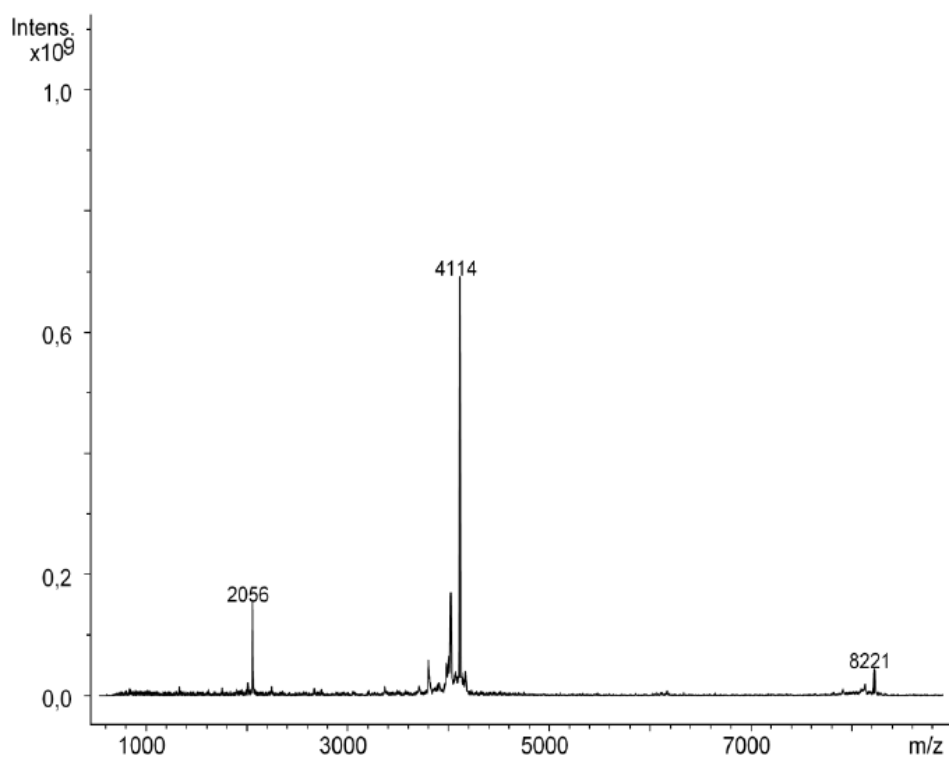


Figure S3: Melting curves of duplexes of segmented probe strand **8** and RNA target **5** (let-7a) or target **17** (let-7e), segmented probe strand **7** and RNA target **5** (let-7a) or target **10** (let-7c). Conditions: probe strands (1.5 μ M), RNA target strands (1.5 μ M), sodium phosphate buffer (10 mM), pH 7, NaCl (1 M).

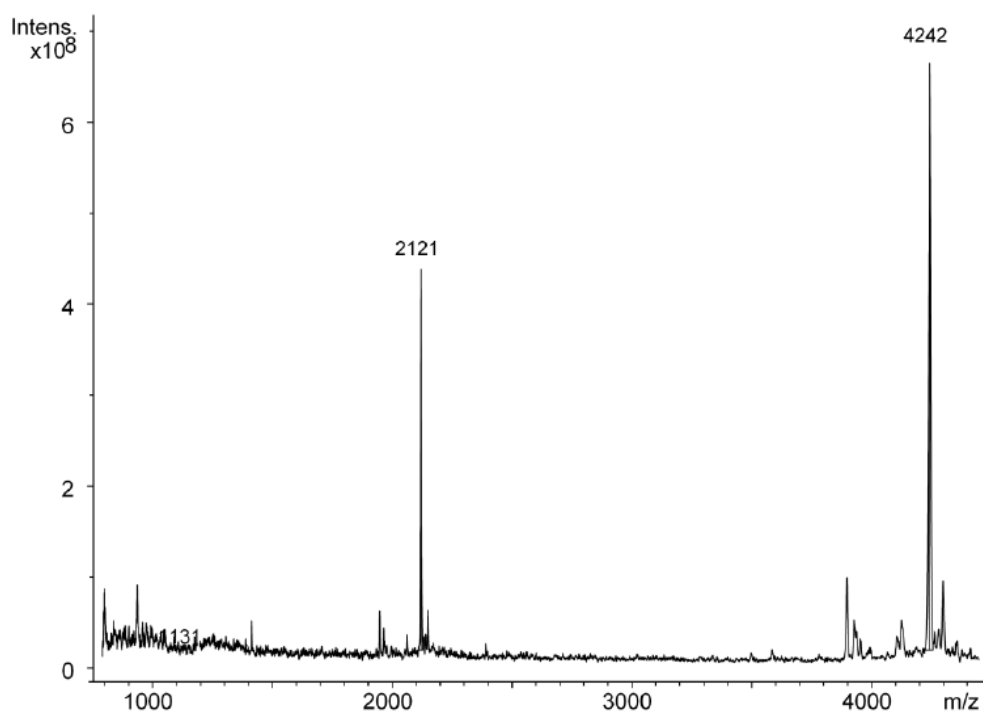
MALDI-TOF mass spectra of oligonucleotides



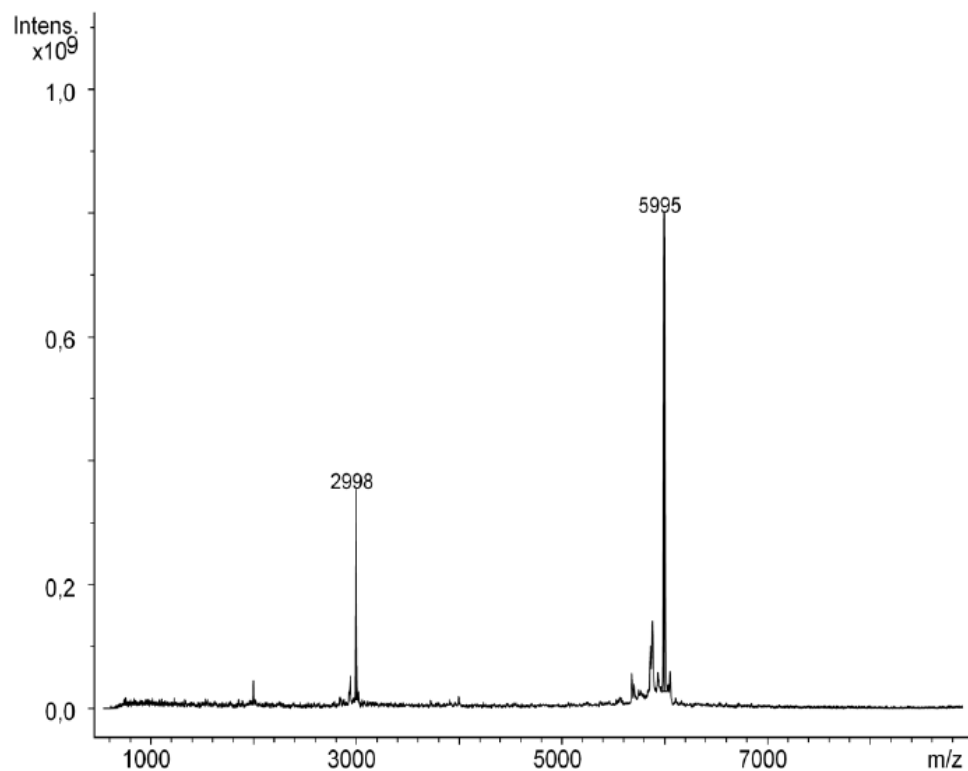
MALDI-TOF mass spectrum of compound **7** *d*(TATACAAC 4-spacer TACTA), calculated mass for [M-H]⁻ 4036.



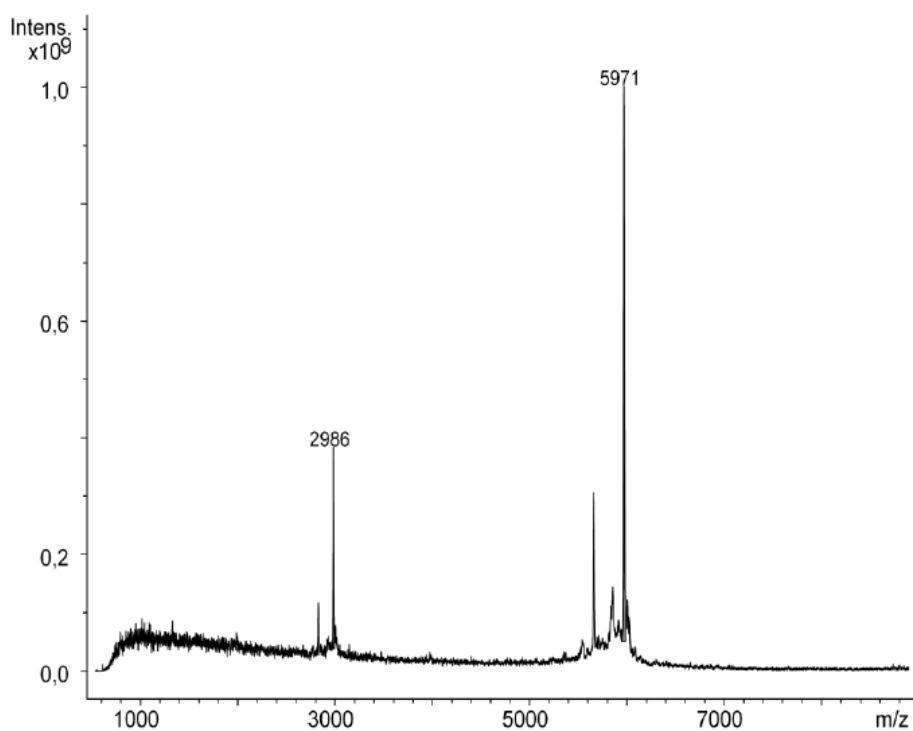
MALDI-TOF mass spectrum of compound **8** *d*(TATACAAC 9-spacer TACTA), calculated mass for [M-H]⁻ 4110.



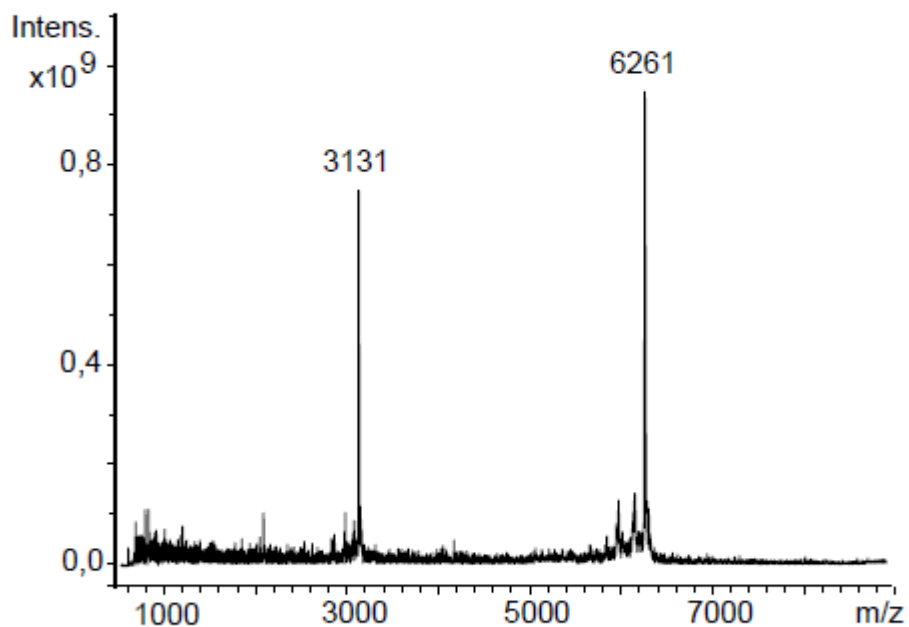
MALDI-TOF mass spectrum of compound **9** *d*(TATACAAC 18-spacer TACTA), calculated mass for [M-H]⁻ 4242.



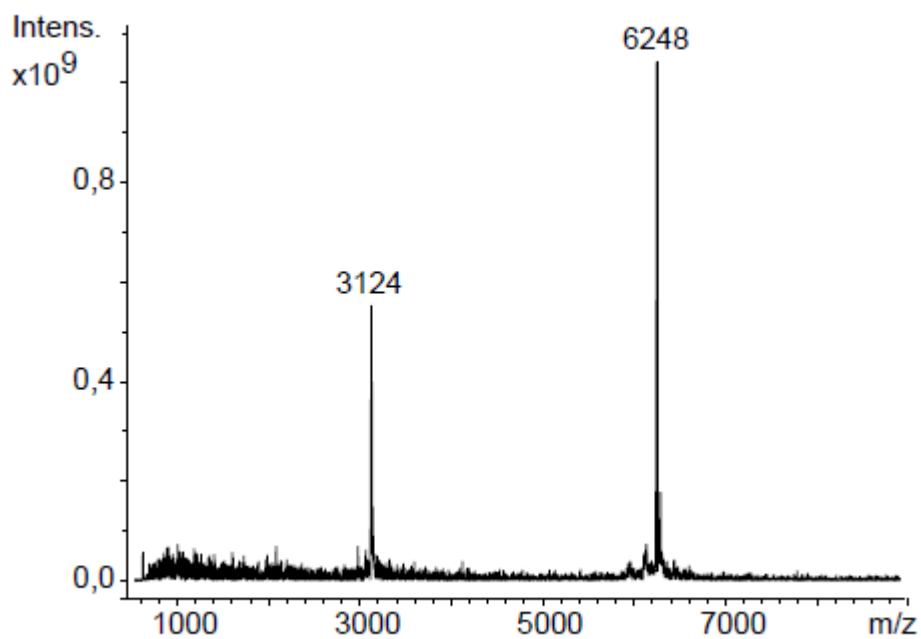
MALDI-TOF mass spectrum of compound **11** *d*(TATACAAC 4-spacer TACTA 4-spacer CTCAA), calculated mass for [M-H]⁻ 5995.



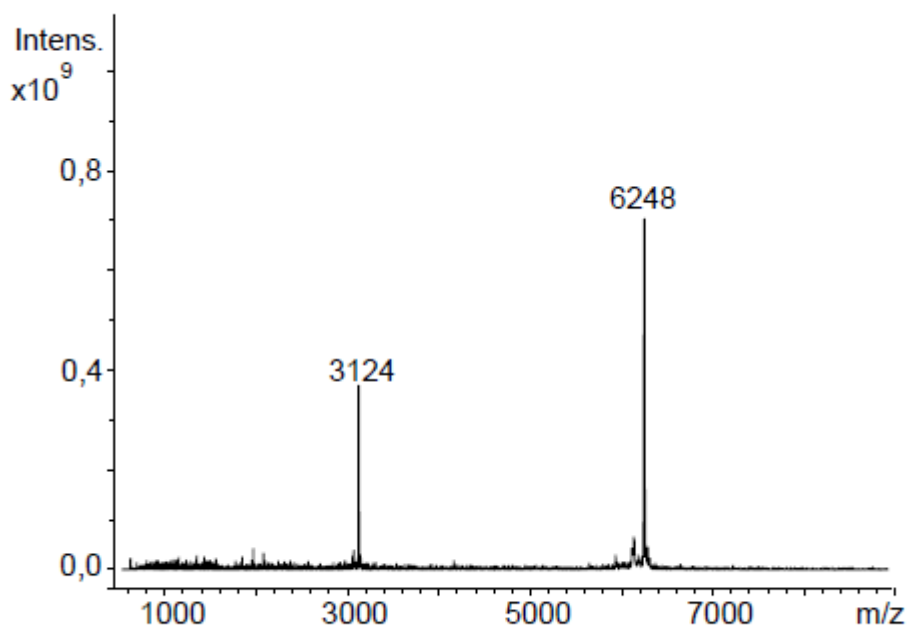
MALDI-TOF mass spectrum of compound **12** *d*(TATACAAC 4-spacer TACT 4-spacer CCTCAAA), calculated mass for $[M-H]^-$ 5970.



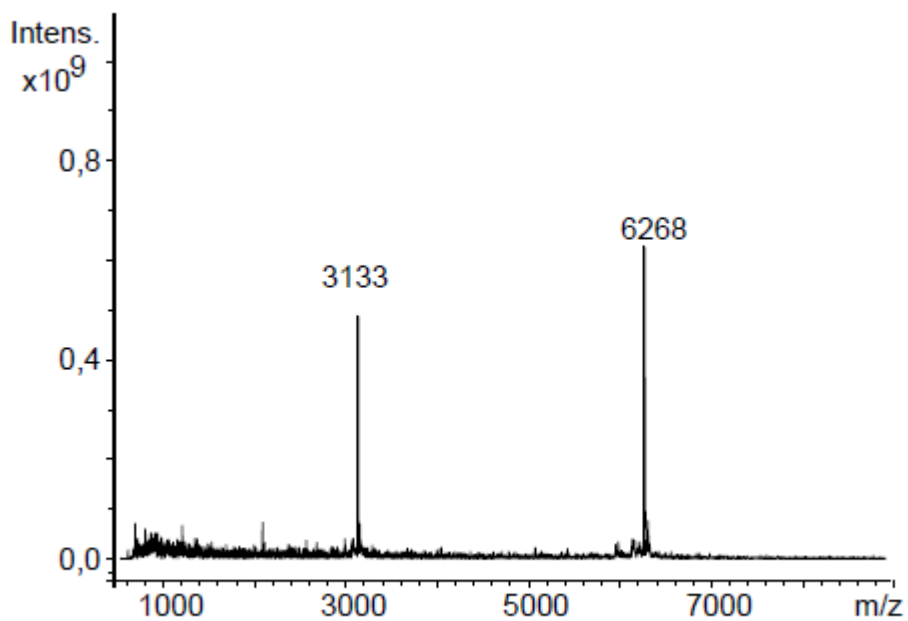
MALDI-TOF mass spectrum of compound **13** *d*(AACTATA 4-spacer AACCTACT 4-spacer CCTCA), calculated mass for $[M-H]^-$ 6259.



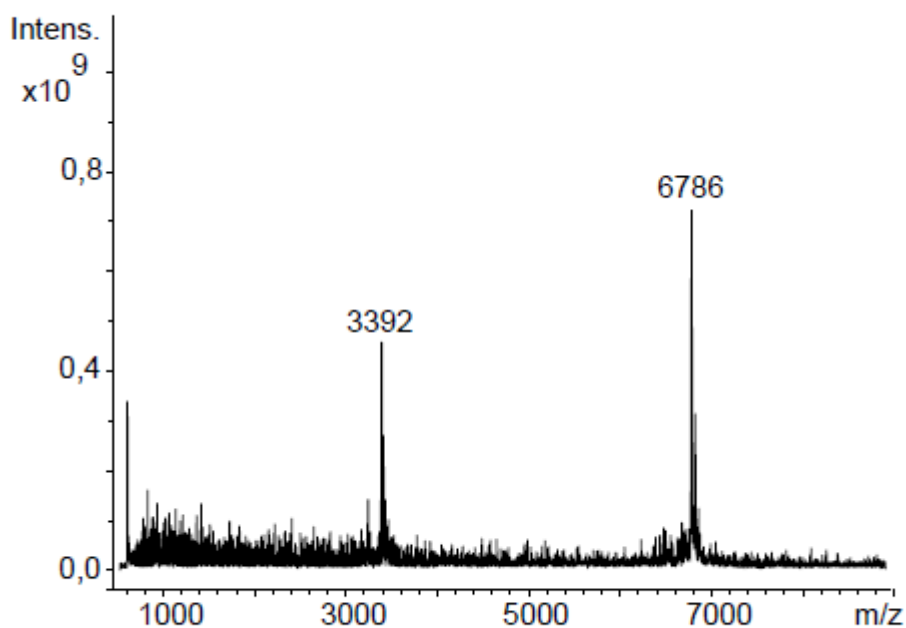
MALDI-TOF mass spectrum of compound **14** *d*(AACTATAC 4-spacer ACCTAC 4-spacer ACCTCA), calculated mass for [M-H]⁻ 6244.



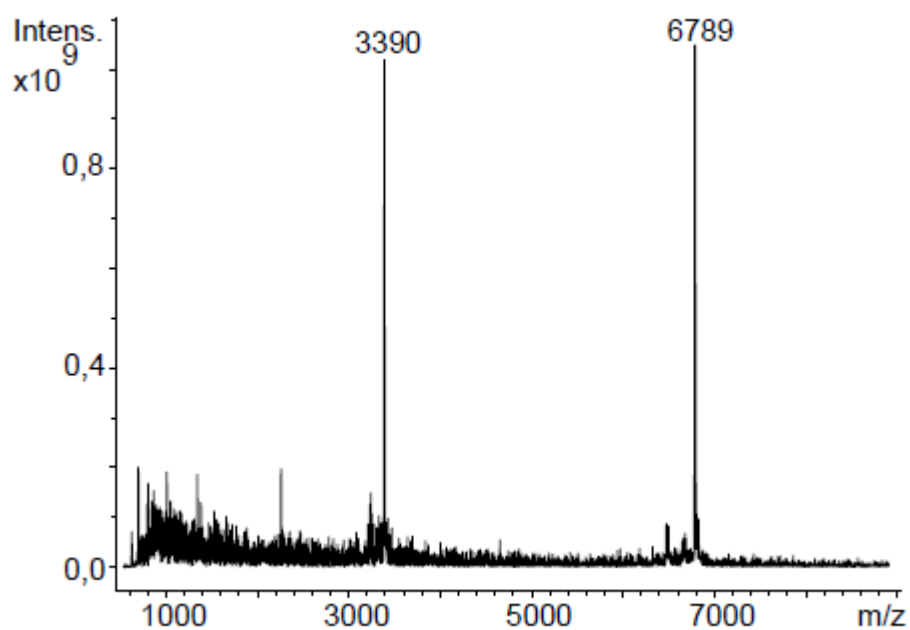
MALDI-TOF mass spectrum of compound **15** *d*(AACTATACA 4-spacer CCTAC 4-spacer ACCTCA), calculated mass for [M-H]⁻ 6244.



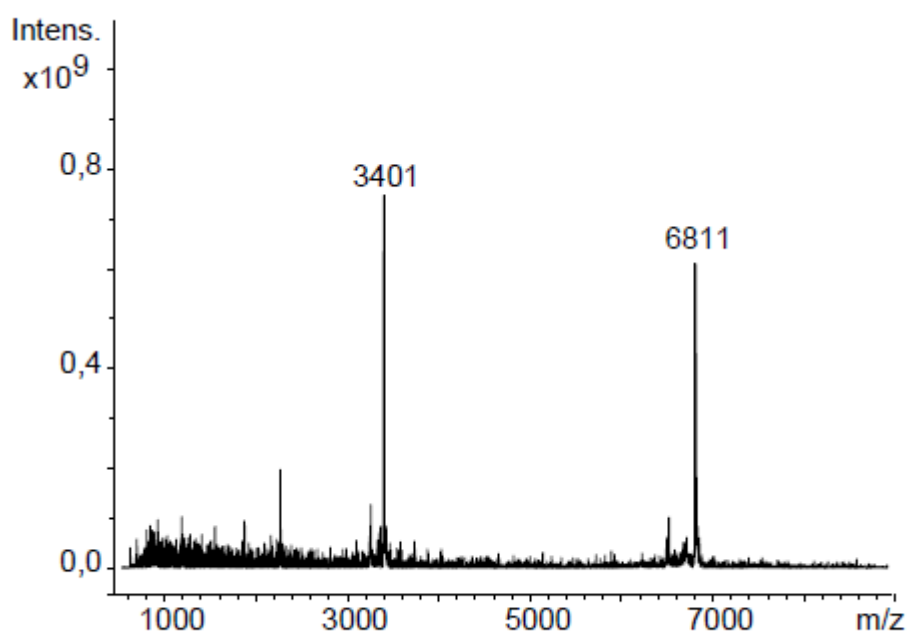
MALDI-TOF mass spectrum of compound **16** *d*(AACTATACAA 4-spacer CTAC 4-spacer ACCTCA), calculated mass for [M-H]⁻ 6268.



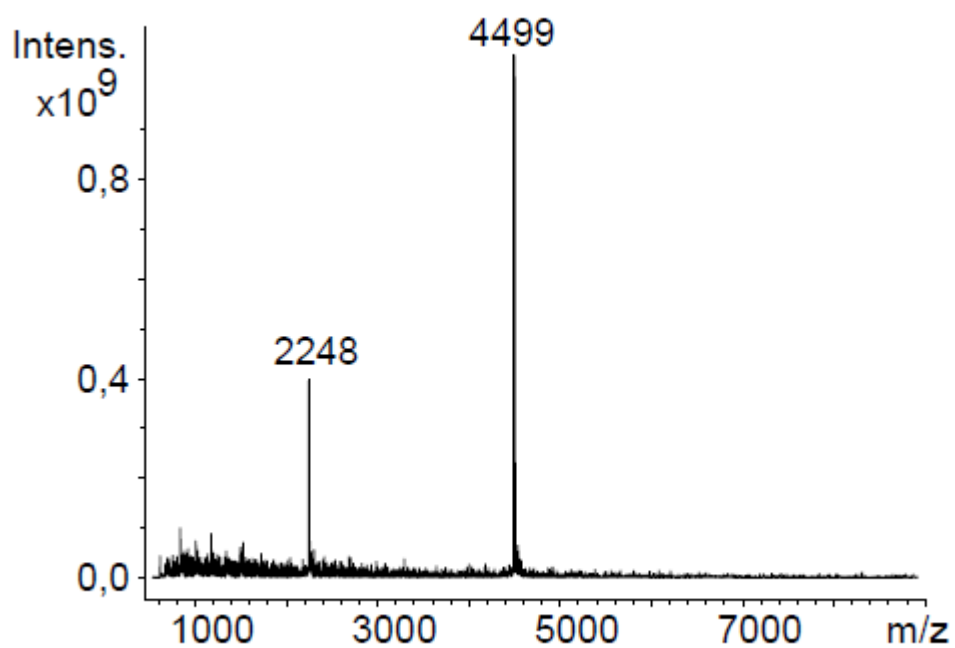
MALDI-TOF mass spectrum of compound **19** *d*(AACTATAC 4-spacer ACCTAC 4-spacer ACCTCA)-*U*_{AQ}, calculated mass for [M-H]⁻ 6782.



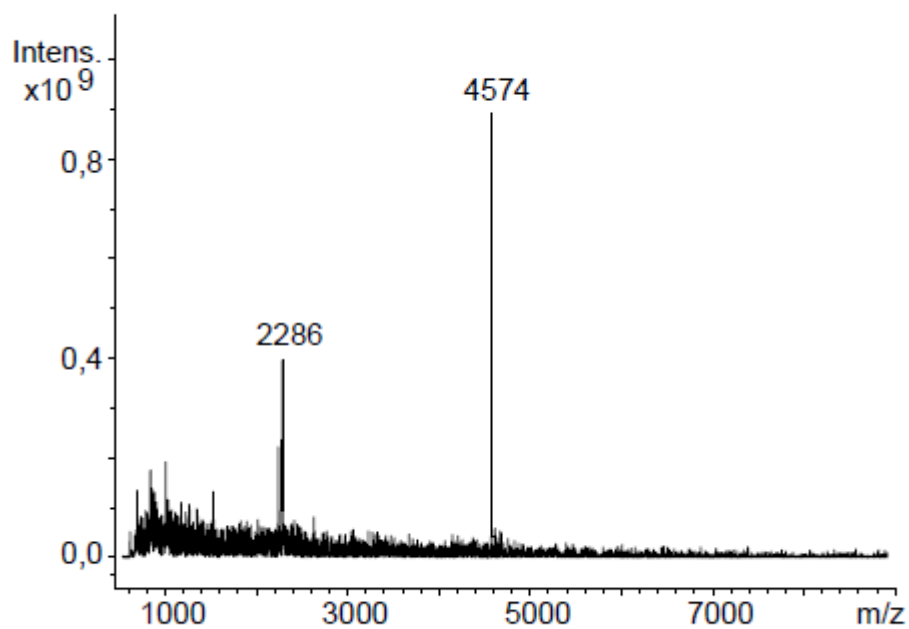
MALDI-TOF mass spectrum of compound **20** *d*(AACTATACA 4-spacer CCTAC 4-spacer ACCTCA)-*U*_{AQ}, calculated mass for [M-H]⁻ 6782.



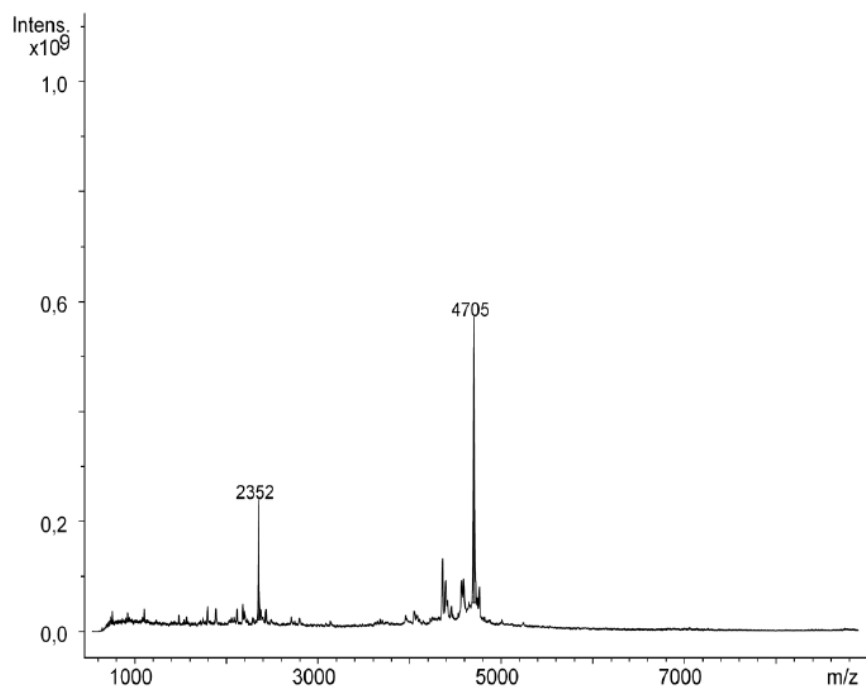
MALDI-TOF mass spectrum of compound **21** *d*(AACTATACAA 4-spacer CTAC 4-spacer ACCTCA)-*U*_{AQ}, calculated mass for [M-H]⁻ 6806.



MALDI-TOF mass spectrum of compound **22** *ogOA-d*(TATACAAC 4-spacer TACTA), calculated mass for [M-H]⁻ 4498.



MALDI-TOF mass spectrum of compound **23** *ogOA-d*(TATACAAC 9-spacer TACTA), calculated mass for [M-H]⁻ 4572.



MALDI-TOF mass spectrum of compound **24** *ogOA-d*(TATACAAC 18-spacer TACTA), calculated mass for [M-H]⁻ 4705.