Supporting Information

Photoswitching properties of hairpin ODNs with azobenzene derivatives in the loop

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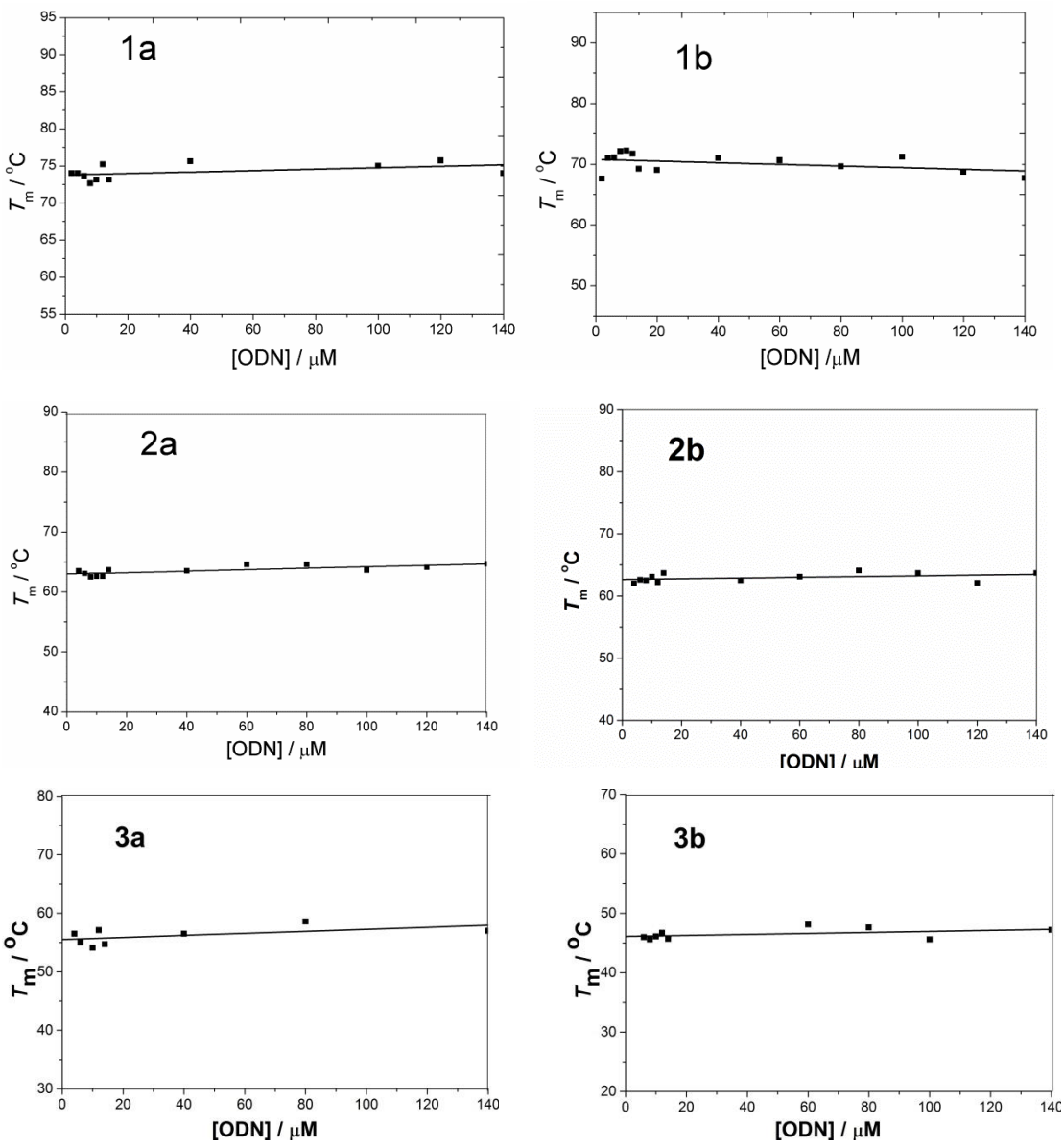
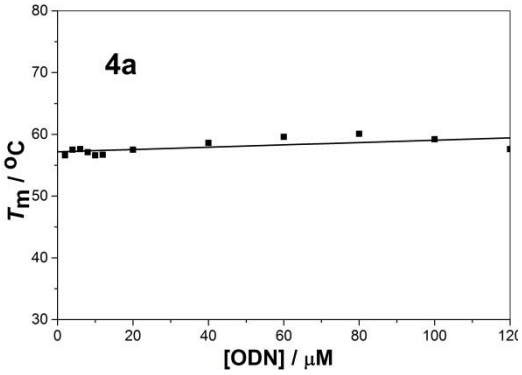
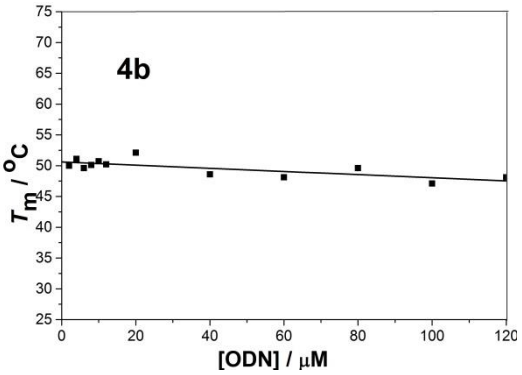
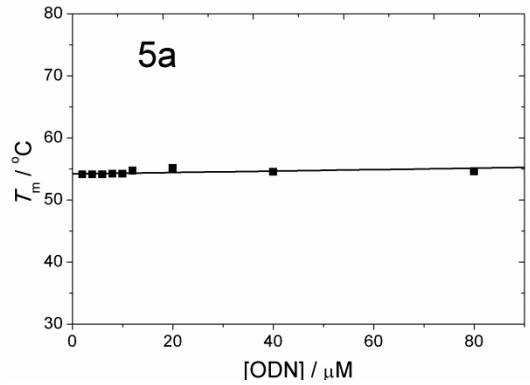
1. Melting temperature of azobenzene modified ODNs in different concentrations

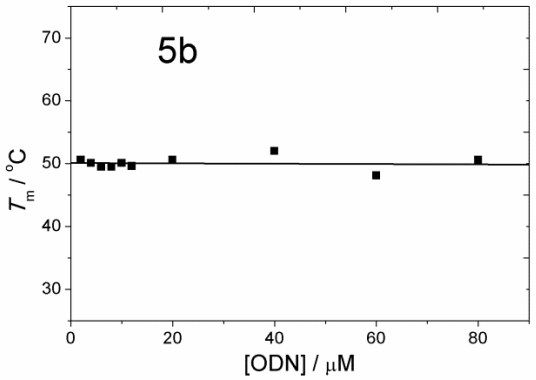
Figure S1. The melting temperatures of azobenzene modified ODNs versus their concentrations from 2 μM to 140 μM in 100 mM NaCl buffer containing 0.1 mM Na_2EDTA / 10 mM Na_2HPO_4-NaH_2PO_4, pH=7.0.

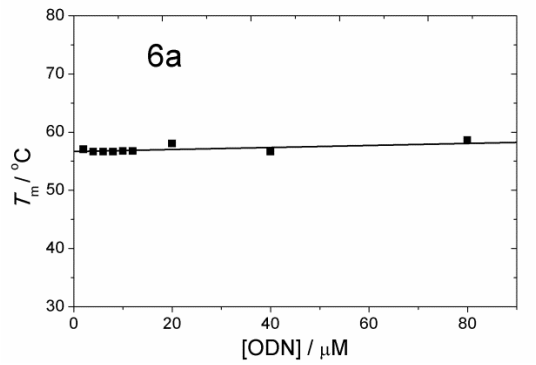


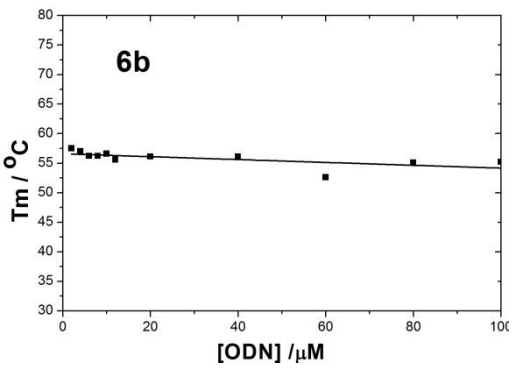

4a
Tm / oC
[ODN] / μM

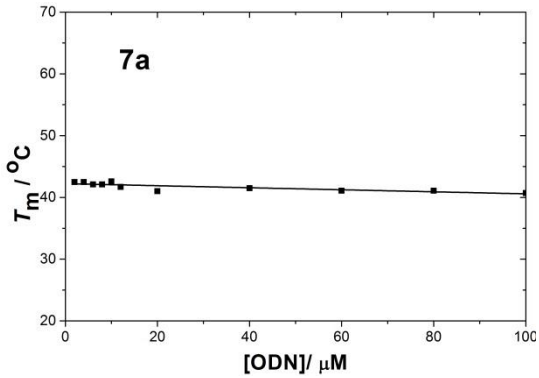

4b
Tm / oC
[ODN] / μM

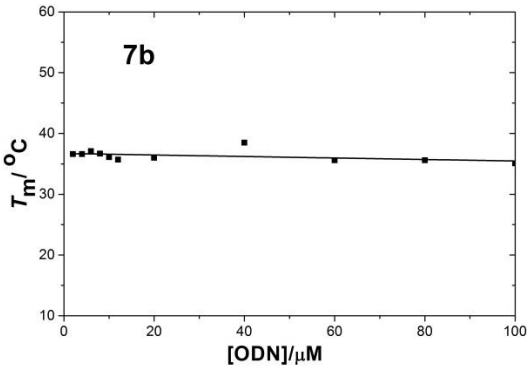

5a
Tm / °C
[ODN] / μM

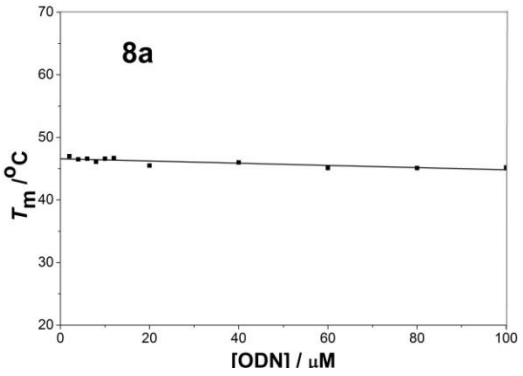

5b
Tm / °C
[ODN] / μM

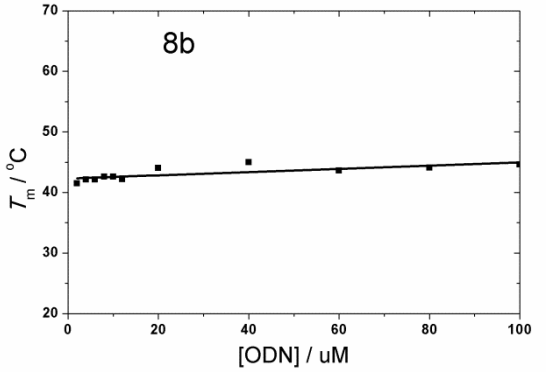

6a
Tm / °C
[ODN] / μM

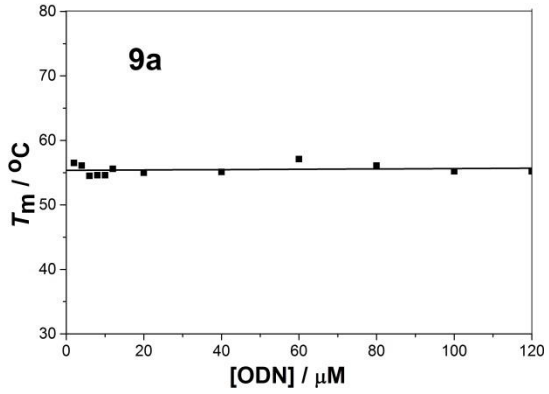

6b
Tm / oC
[ODN] /μM

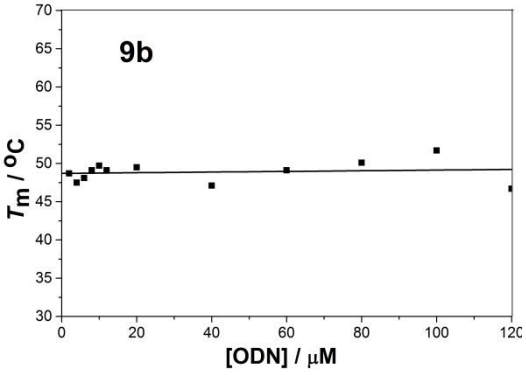

7a
Tm / oC
[ODN]/ μM

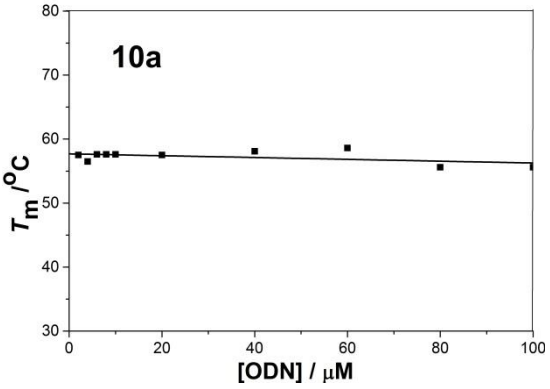

7b
Tm / oC
[ODN]/μM

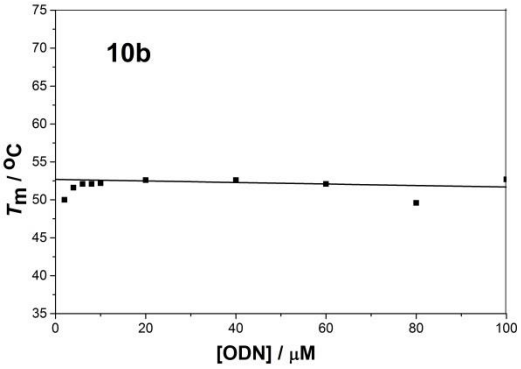

8a
T_m /°C
[ODN] / μM


8b
T_m / °C
[ODN] / uM


9a
Tm / °C
[ODN] / μM


9b
Tm / °C
[ODN] / μM


10a
T_m /°C
[ODN] / μM


10b
Tm / °C
[ODN] / μM

2. Native PAGE of azobenzene modified ODNs

Figure S2. Native polyacrylamide gel electrophoresis pattern for trans-azobenzene linked hairpins. **(a)** Lane1: hairpin marker 5′-GATCTTTTGATC-3′ (**2c**), Lane2: [**2a**] = 10 μM, Lane3: [**2a**] = 100 μM, Lane4: [**2b**] = 10 μM, Lane5: [**2b**] = 100 μM, Lane6: [**3a**] = 10 μM, Lane7: [**3a**] = 100 μM, Lane8: [**3b**] = 10 μM, Lane9: [**3b**] = 100 μM, Lane10: hairpin marker, 5′-AAAGTTTTCTTT-3′ (**3c**). **(b)** Lane1: hairpin marker 5′-AAAAATTTTTTTTT-3′ (**9c**), Lane2: [**9a**] = 10 μM, Lane3: [**9a**] = 100 μM, Lane4: [**9b**] = 10 μM, Lane5: [**9b**] = 100 μM, Lane6: [**10a**] = 10 μM, Lane7: [**10a**] = 100 μM, Lane8: [**10b**] = 10 μM, Lane9: [**10b**] = 100 μM, Lane10: hairpin marker, 5′-AAAAAATTTTTTTTT-3′ (**10c**). **(c)** Lane1: hairpin marker 5′-AATAG**TTTT**CTATT-3′ (**5c**), Lane2: [**5a**] = 10 μM, Lane3: [**5a**] = 100 μM, Lane4: [**5b**] = 10 μM, Lane5: [**5b**] = 100 μM, Lane6: hairpin marker, 5′-AATAC**TTTT**GTATT -3′ (**6c**), Lane7: [**6a**] = 10 μM, Lane8: [**6a**] = 100 μM, Lane9: [**6b**] = 10 μM, Lane10: [**6b**] = 100 μM. **(d)** Lane1: hairpin marker 5′-AATAA**TTTT**TTATT-3′ (**7c**), Lane2: [**7a**] = 10 μM, Lane3: [**7a**] = 100 μM, Lane4: [**7b**] = 10 μM, Lane5: [**7b**] = 100 μM, Lane6: hairpin marker, 5′-AATAT**TTTT**ATATT-3′ (**8c**), Lane7: [**8a**] = 10 μM, Lane8: [**8a**] = 100 μM, Lane9: [**8b**] = 10 μM, Lane10: [**8b**] = 100 μM. The samples were loaded into a 20% native gel and the gel was run for 2h under 150V at room temperature.

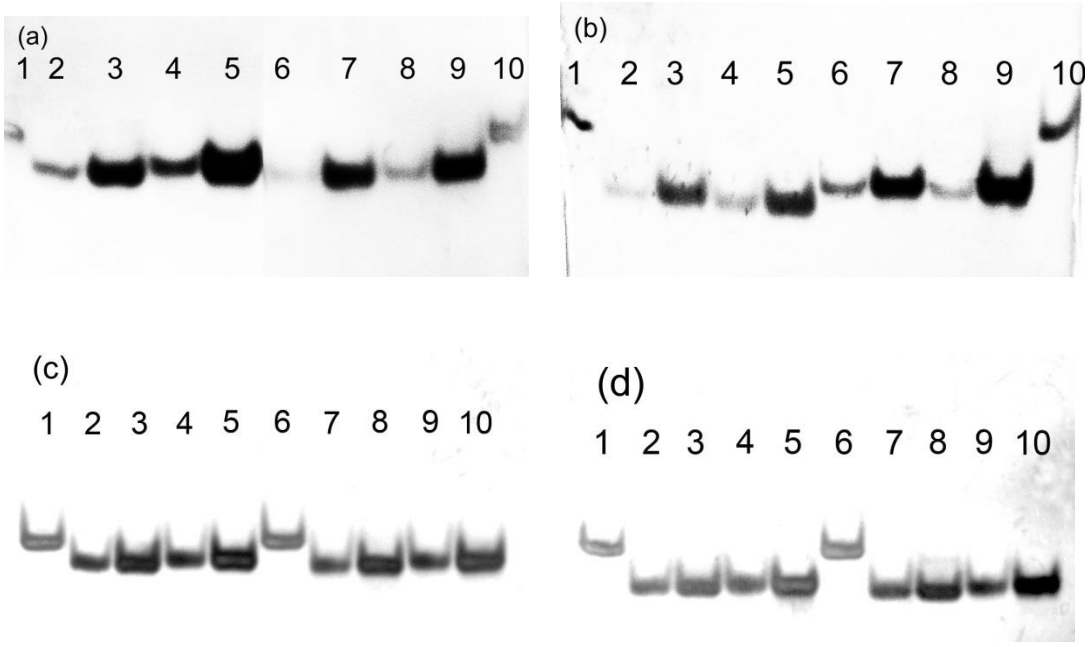
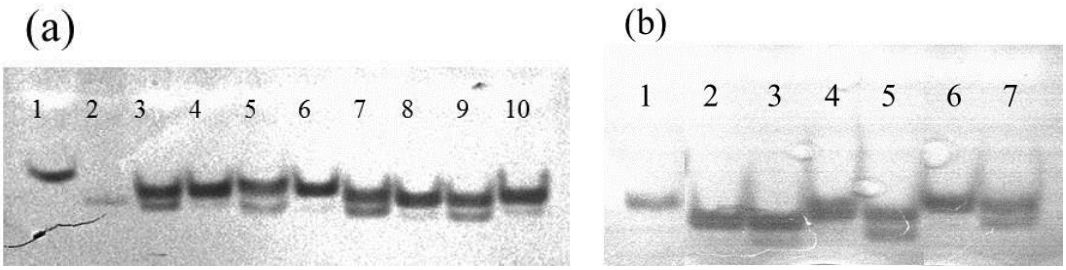


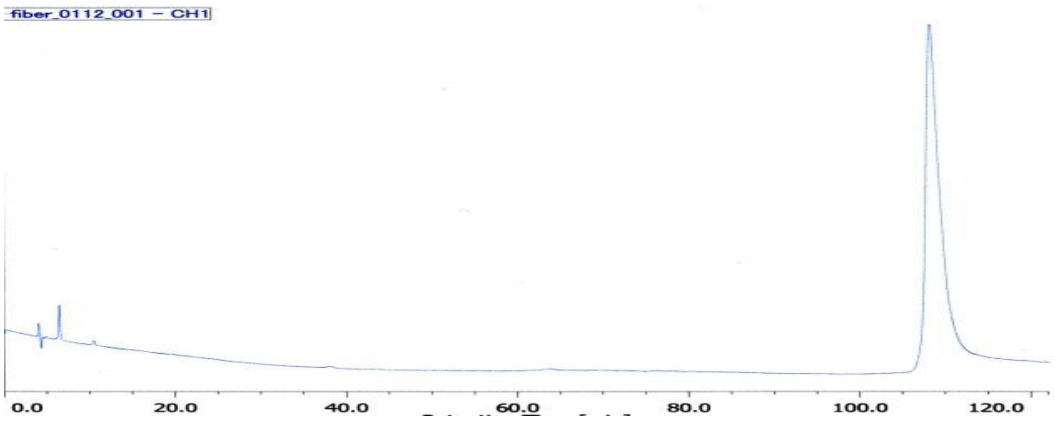
Figure S3. Native polyacrylamide gel electrophoresis pattern for trans-azobenzene and cis-azobenzene linked hairpins. **(a)** Lane1: hairpin marker 5′-GATCTTTTGATC-3′ (**2c**), Lane2: hairpin marker 5′-AATAGTTTTCTATT-3′, Lane3: cis-**1a**, Lane4: trans-**1a**, Lane5: cis-**1b**, Lane6: trans-**1b**, Lane7: cis-**5a**, Lane8: trans-**5a**, Lane9: cis-**5b**, Lane10: trans-**5b**. **(b)** Lane1: hairpin marker 5′-AATAGTTTTCTATT-3′, (**2c**), Lane2: trans-**10a**, Lane3: cis-**10a**, Lane4: trans-**9a**, Lane5: cis-**9a**, Lane6: trans-**8a**, Lane7: cis-**8a**. The samples were loaded into a 20% native gel and the gel was run for 4h under 80V at room temperature.



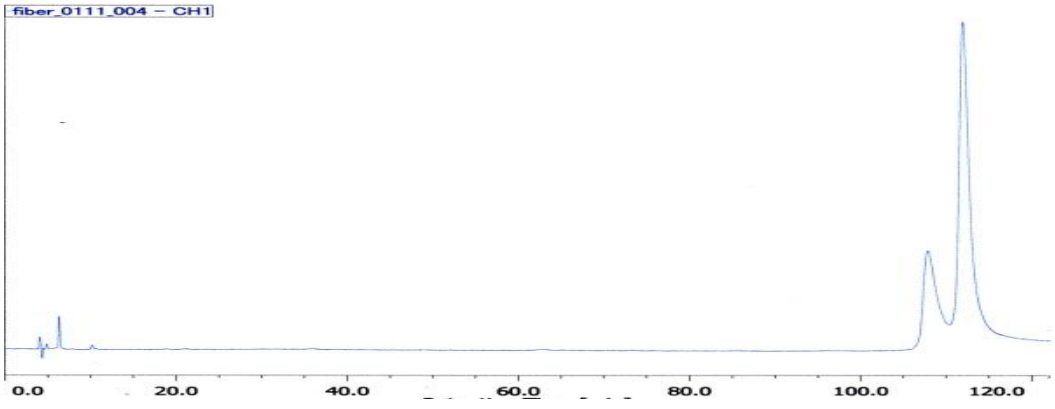
3. Typical HPLC analysis of trans-form and cis-form of azobenzene linked hairpins.

Figure S4 Comparison of HPLC charts of trans-**1a (**before UV light irradiation**)** and cis-**1a (**after UV light irradiation **).** Experiments were performed in a HPLC (JASCO LC-2000 Plus) with TSKgel ODS-80Ts (TOSOH, Japan) column. Set up the wavelength of UV detector at 260 nm and the flow rate at 0.5 mL min^{-1}. The sample was separated at 25 ℃, and 0.01 M TEAA (pH7.0) and 50% MeOH were used as an elute solvent.

Trans-**1a**



Cis-**1a**



4. UV spectral change of azobenzene linker and azobenzene modified ODNs with UV irradiation

Figure S5. UV absorption spectra of **Az1**, **Az2** as well as **Az1** and **Az2** linked conjugates **3a**, **3b** in the trans form in 3:7 MeOH/H_2O.

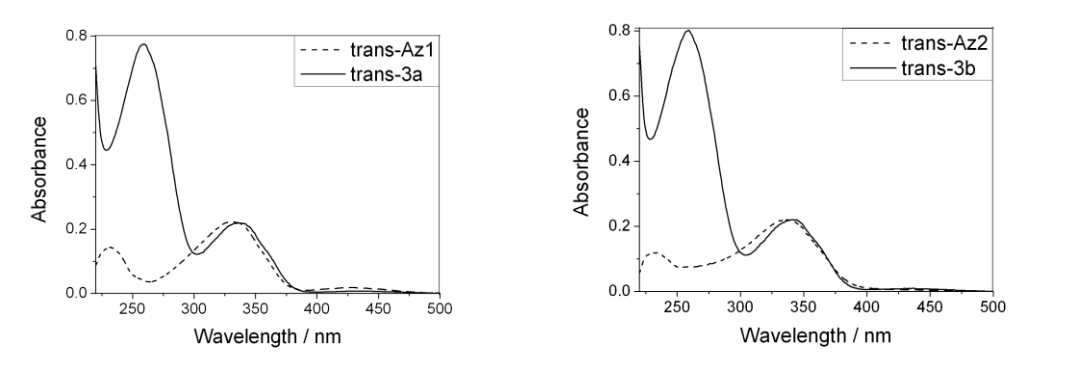
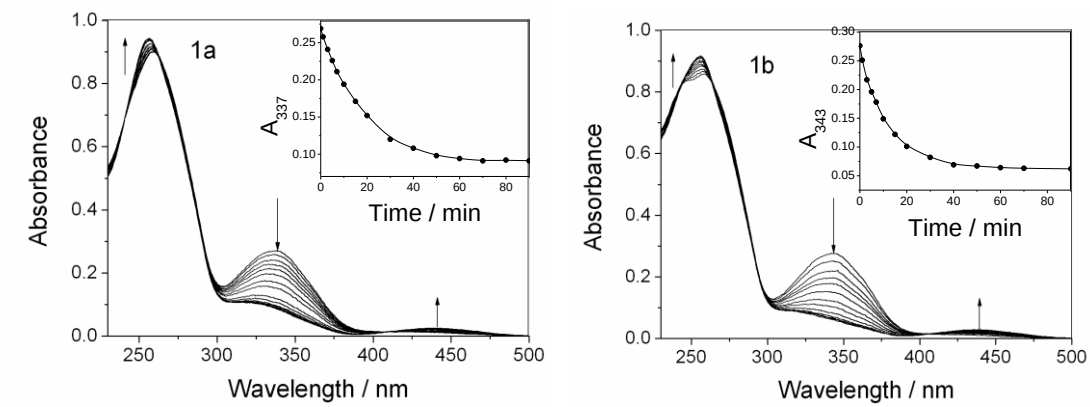
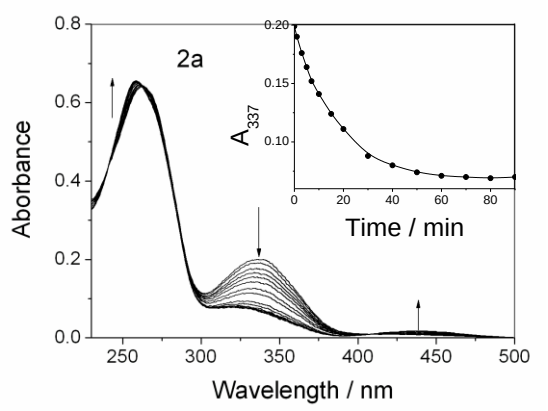
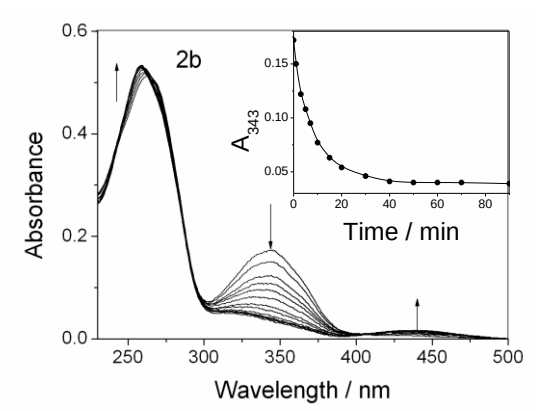
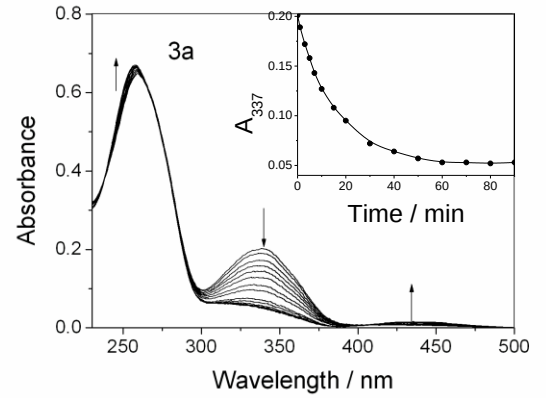


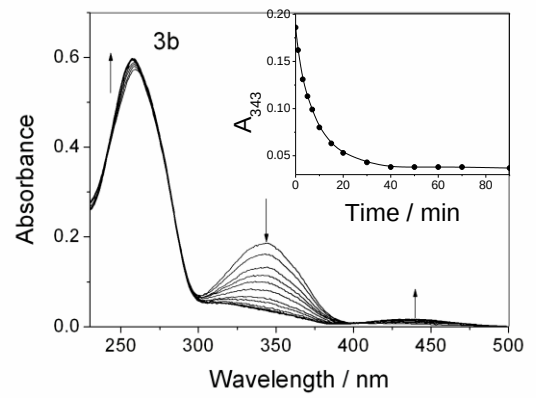
Figure S6. UV spectral changes of **Az1**-ODN **1a**-**10a** and **Az2**-ODN **1b**-**10b** by illuminating UV light. UV spectra were measured in 10 mM phosphate buffer (pH 7.0) containing 100 mM NaCl and 0.1 mM EDTA, the concentrations of ODNs were 10 μM. Inset: plots of absorbance at maximum absorption of azobenzene as a function of UV irradiation time.

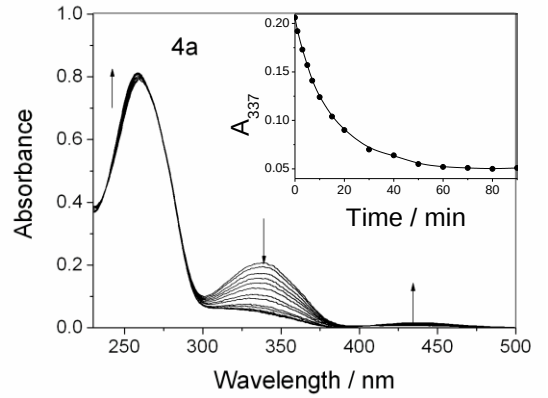


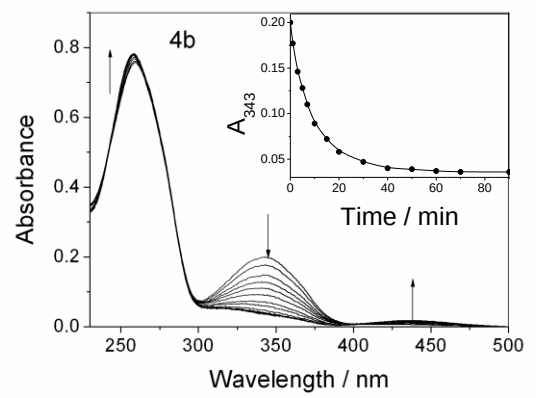

2a
Aborbance
Wavelength / nm
A337
Time / min

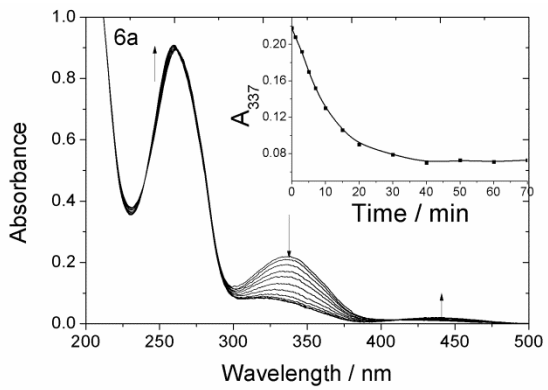

2b
Absorbance
Wavelength / nm
A343
Time / min

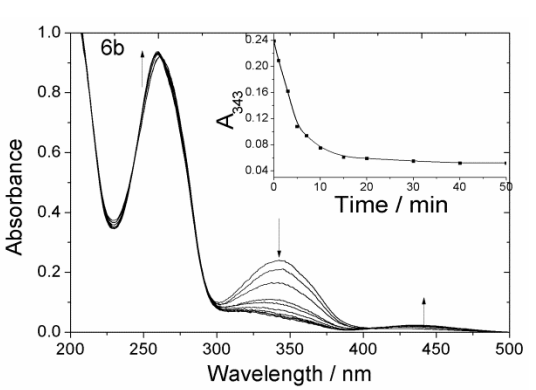

3a
Absorbance
Wavelength / nm
A337
Time / min

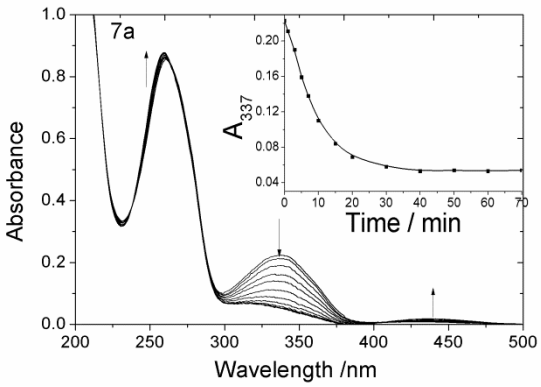

3b
Absorbance
Wavelength / nm
A343
Time / min

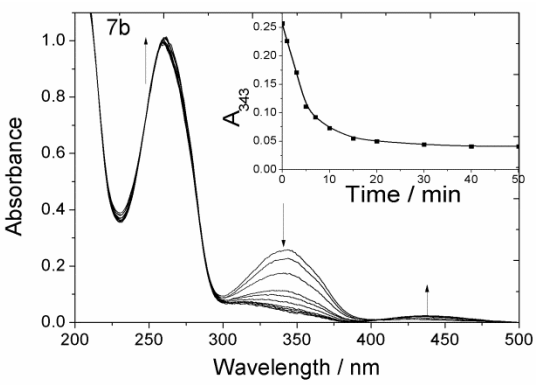

4a
Absorbance
Wavelength / nm
A337
Time / min

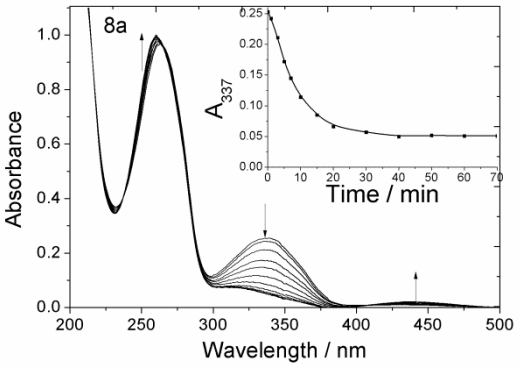

4b
Absorbance
Wavelength / nm
A343
Time / min

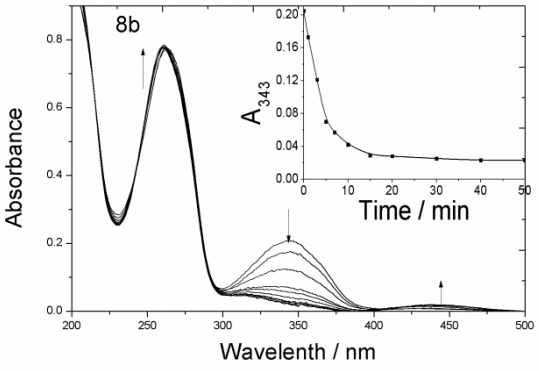

6a
Absorbance
Wavelength / nm
A337
Time / min

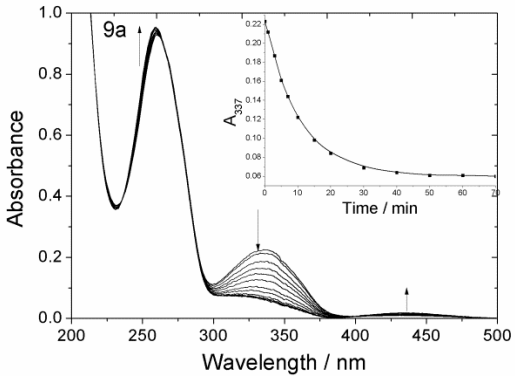

6b
Absorbance
Wavelength / nm
A343
Time / min

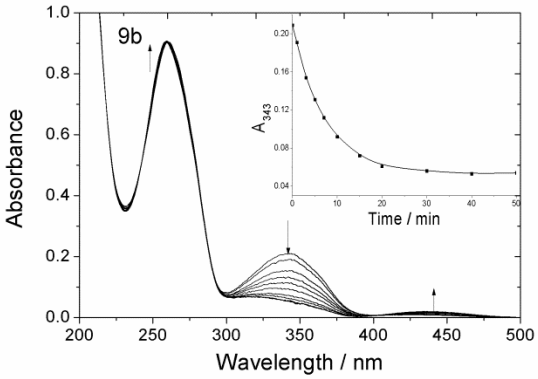

7a
Absorbance
Wavelength /nm
A337
Time / min

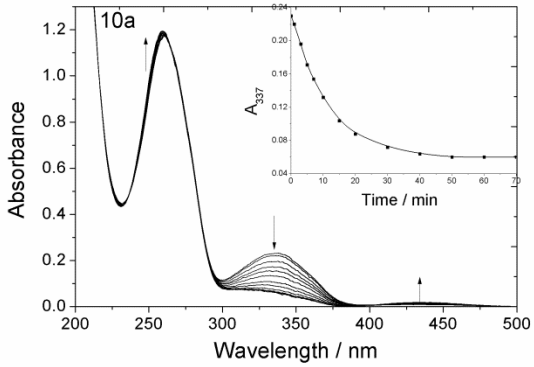

7b
Absorbance
Wavelength / nm
A343
Time / min

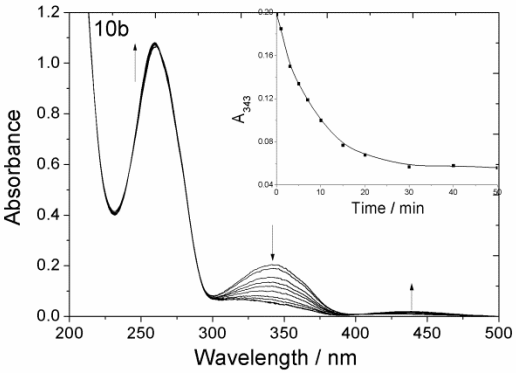

8a
Absorbance
Wavelength / nm
A337
Time / min


8b
Absorbance
Wavelenth / nm
A343
Time / min


9a
Absorbance
Wavelength / nm
A337
Time / min

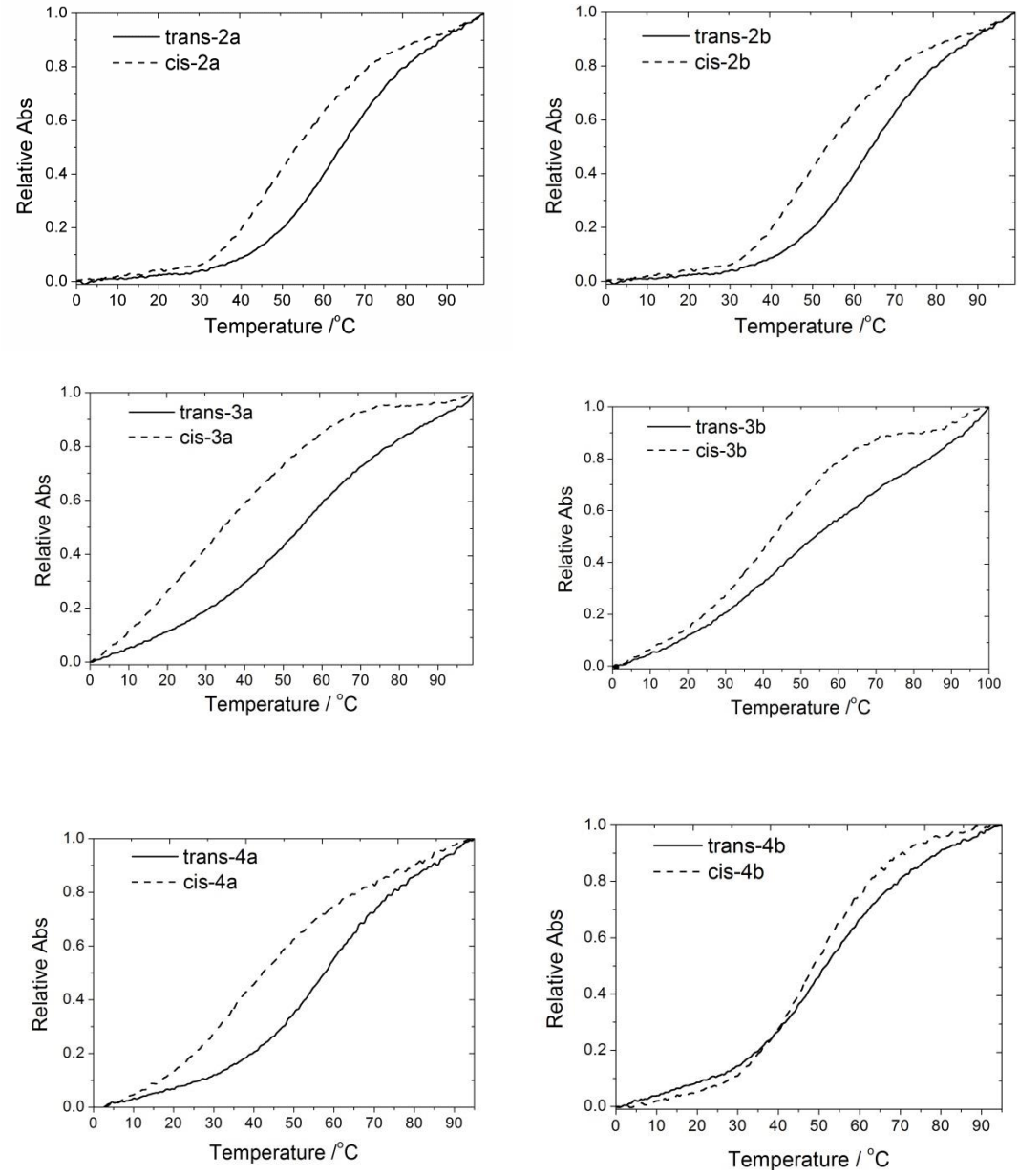

9b
Absorbance
Wavelength / nm
A343
Time / min

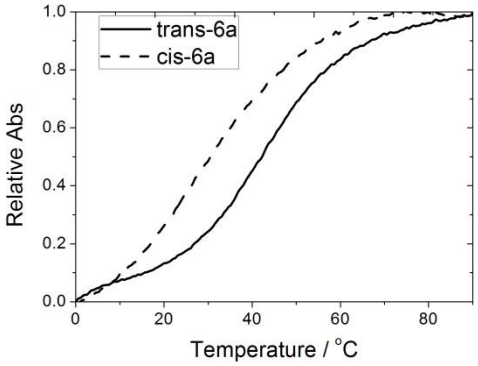

10a
Absorbance
Wavelength / nm
A337
Time / min

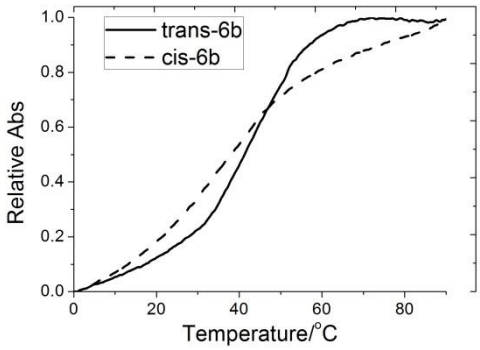

10b
Absorbance
Wavelength / nm
A343
Time / min

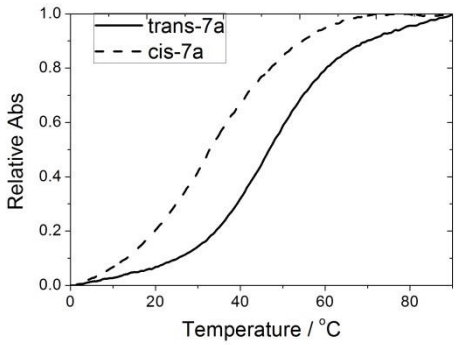
5. Typical UV melting curves for trans-form (solid line) and cis-form (broken line) of azobenzene modified ODNs

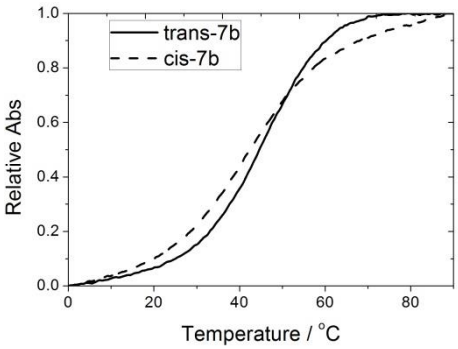
Figure S7. Comparison of UV melting curves for trans-ODNs (solid line) and cis-ODNs (broken line). UV melting experiments were performed in a 10 mM phosphate buffer (pH 7.0) containing 100 mM NaCl and 0.1 mM EDTA. ODN concentrations were adjusted to 6 μM.

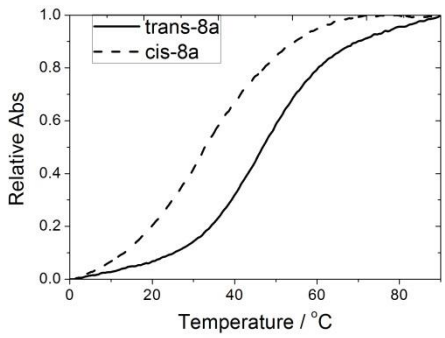


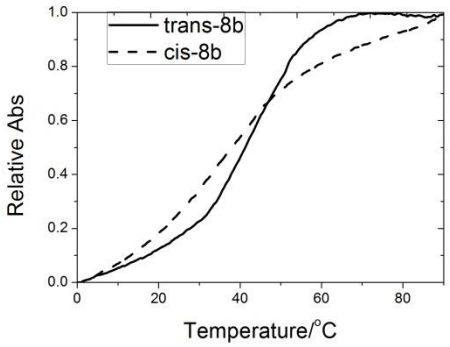

trans-6a
cis-6a
Relative Abs
Temperature / °C

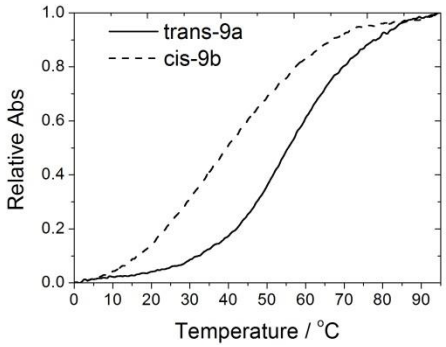

trans-6b
cis-6b
Relative Abs
Temperature/°C

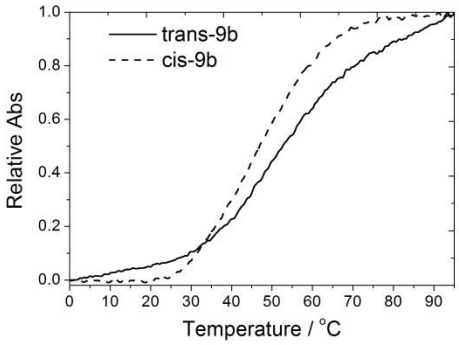

trans-7a
cis-7a
Relative Abs
Temperature / °C

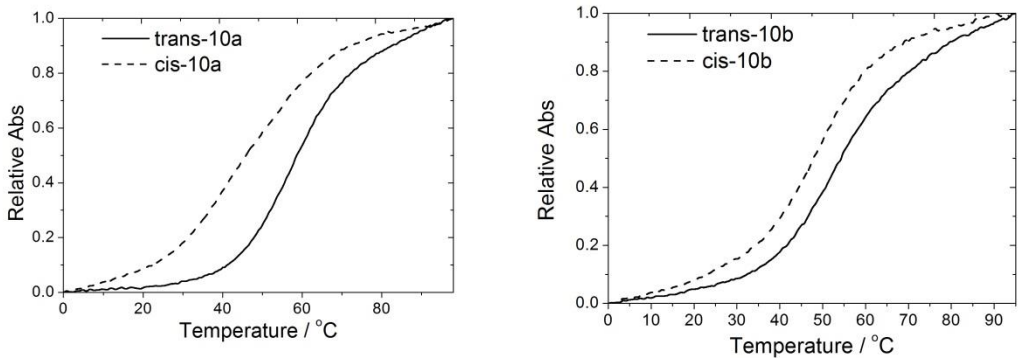

trans-7b
cis-7b
Relative Abs
Temperature / °C


trans-8a
cis-8a
Relative Abs
Temperature / °C


trans-8b
cis-8b
Relative Abs
Temperature/°C


trans-9a
cis-9b
Relative Abs
Temperature / °C


trans-9b
cis-9b
Relative Abs
Temperature / °C



6. CD spectra of Az1 and Az2 as well as azobenzene modified ODNs with or without UV irradiation

Figure S8. Comparison of CD spectra of trans-form (solid line) and cis-form (dash line) of **Az1** and **Az2** at 4 °C. CD spectra were measured in 3:7 MeOH/H_2O, the concentrations of **Az1** and **Az2** were 10 μM.

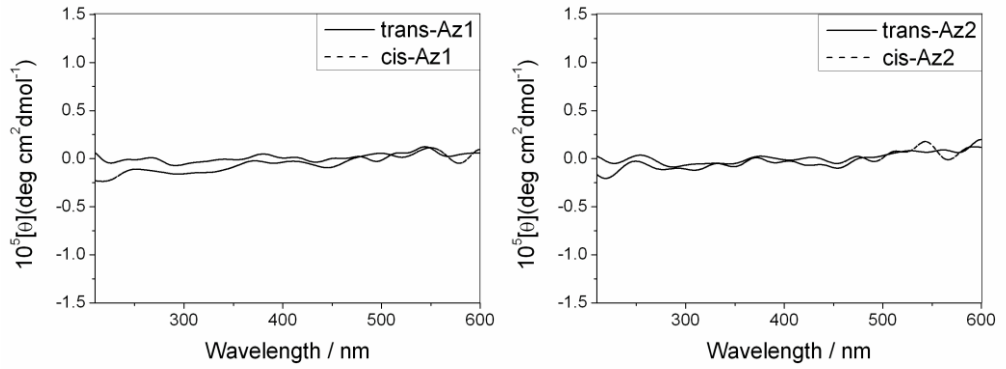
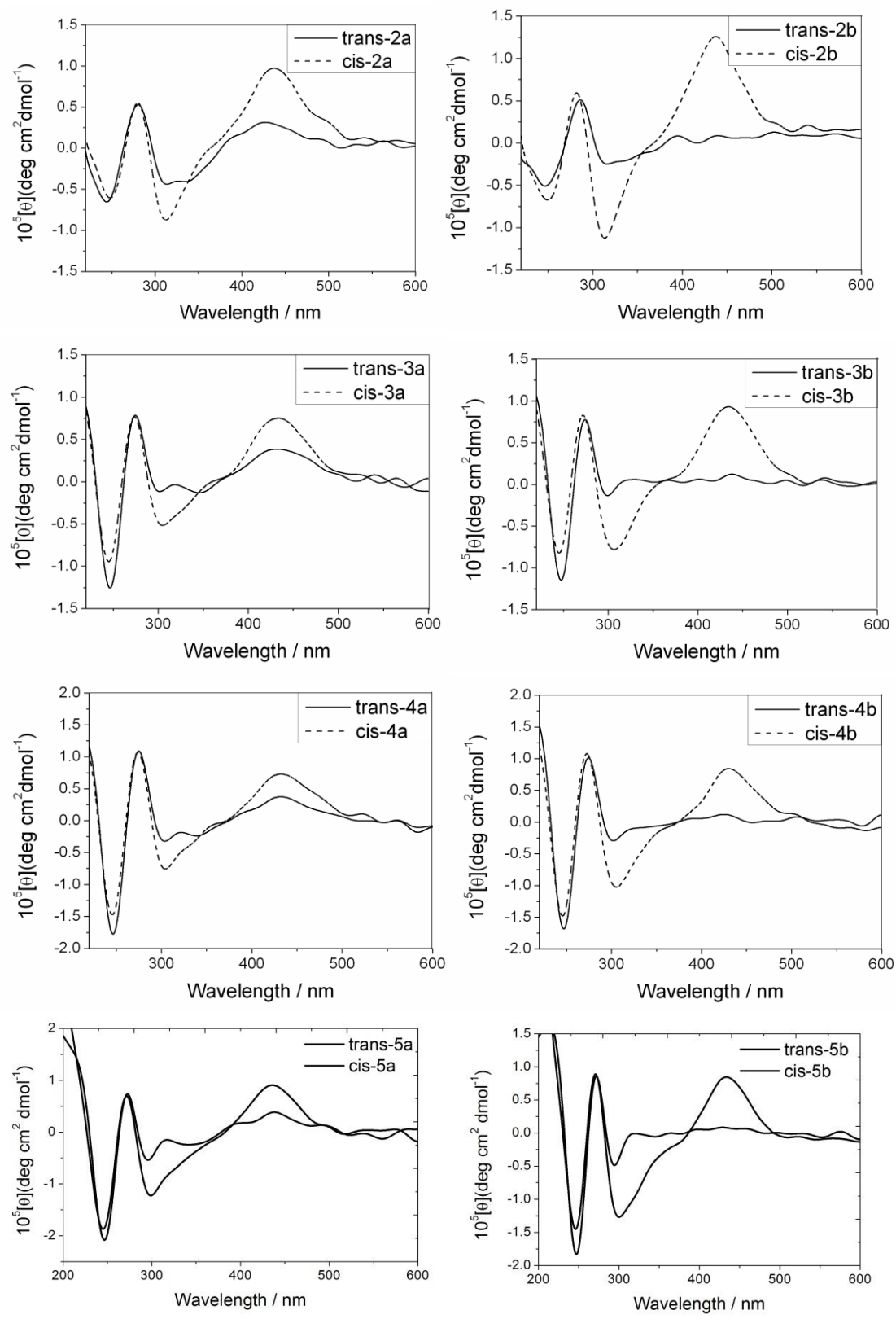
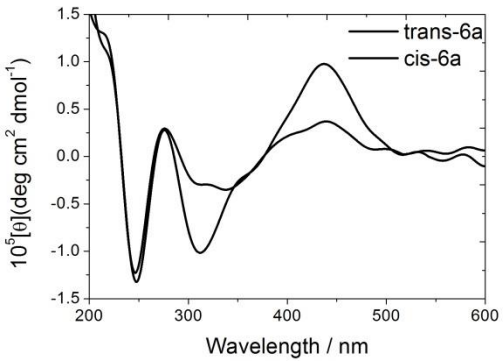
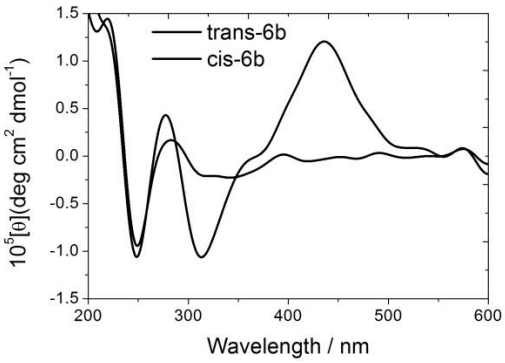
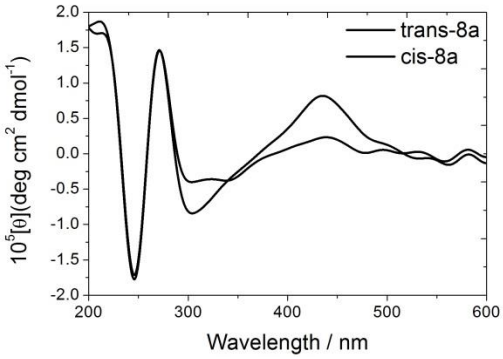


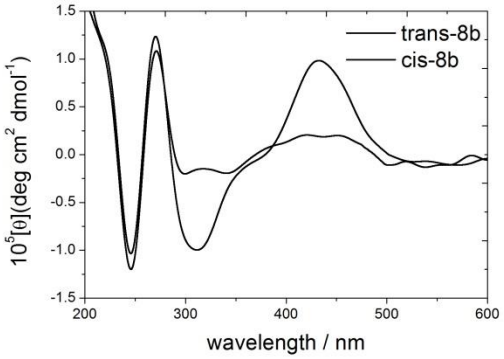
Figure S9. Comparison of CD spectra of trans-DNAs (solid line) and cis-DNAs (dash line) at 4 ^{o}C. CD spectra were measured in a 10 mM phosphate buffer (pH 7.0) containing 100 mM NaCl and 0.1 mM EDTA, the concentrations of ODNs were 10 μM.

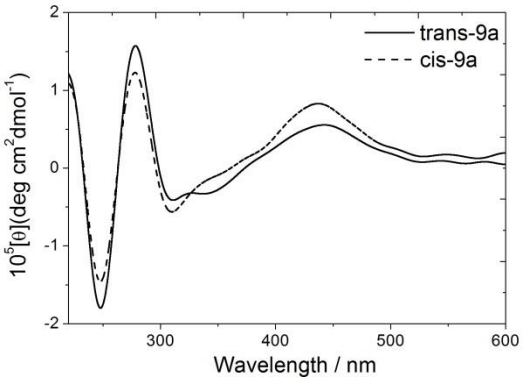


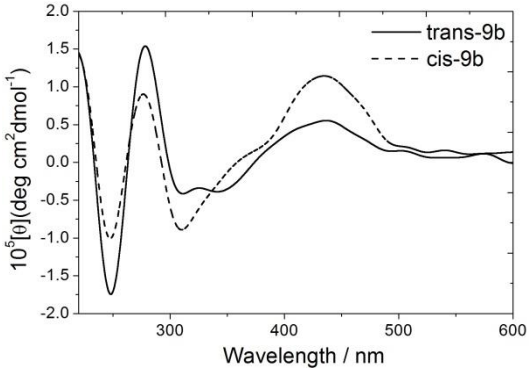

trans-6a
cis-6a
$10^5[\theta](\text{deg cm}^2\text{ dmol}^{-1})$
Wavelength / nm

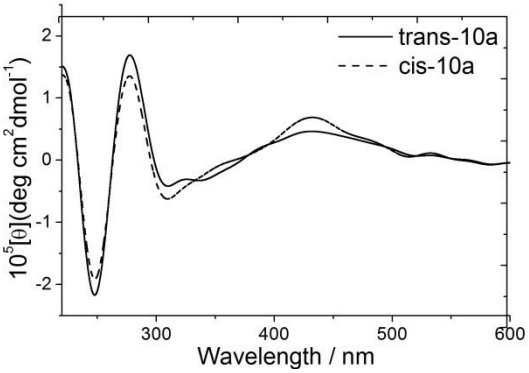

trans-6b
cis-6b
$10^5[\theta](\text{deg cm}^2\text{ dmol}^{-1})$
Wavelength / nm

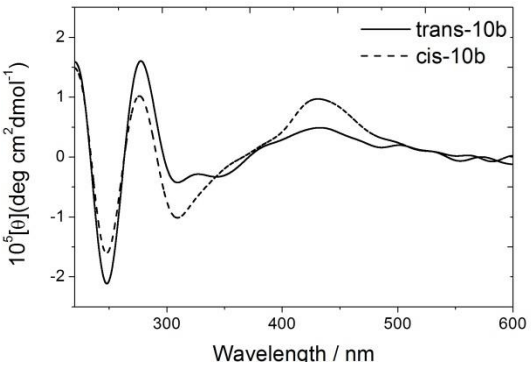

trans-8a
cis-8a
$10^5[\theta](\text{deg cm}^2\text{ dmol}^{-1})$
Wavelength / nm


trans-8b
cis-8b
$10^5[\theta](\text{deg cm}^2\text{ dmol}^{-1})$
wavelength / nm


trans-9a
cis-9a
$10^5[\theta](\text{deg cm}^2\text{dmol}^{-1})$
Wavelength / nm

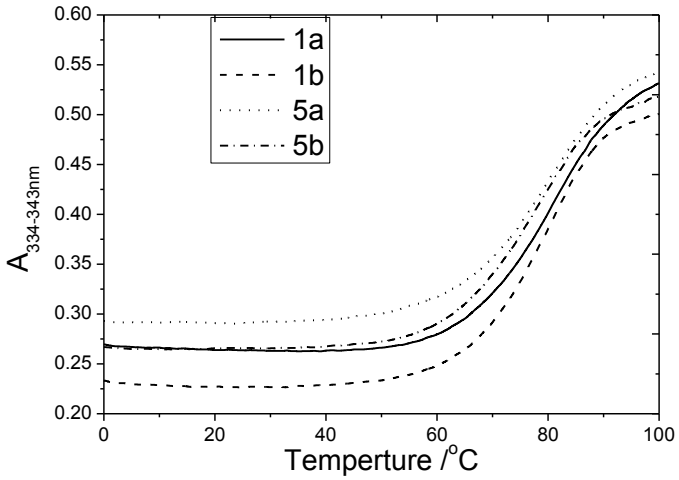

trans-9b
cis-9b
$10^5[\theta](\text{deg cm}^2\text{dmol}^{-1})$
Wavelength / nm


trans-10a
cis-10a
$10^5[\theta](\text{deg cm}^2\text{dmol}^{-1})$
Wavelength / nm


trans-10b
cis-10b
$10^5[\theta](\text{deg cm}^2\text{dmol}^{-1})$
Wavelength / nm

7. Isomerization behavior of cis-form of azobenzene modified ODNs upon heating

Figure S10. Plots of absorbance at maximum absorbance for cis-**1a** (solid line), cis-**1b** (broken line), cis-**5a** (dot line) and cis-**5b** (broken dot line) as a function of temperature (heating rate: 0.5 ℃ mim^{-1}). Experiment was performed in a 10 mM phosphate buffer (pH 7.0) containing 100 mM NaCl and 0.1 mM EDTA. ODN concentrations were adjusted to 10 μM.

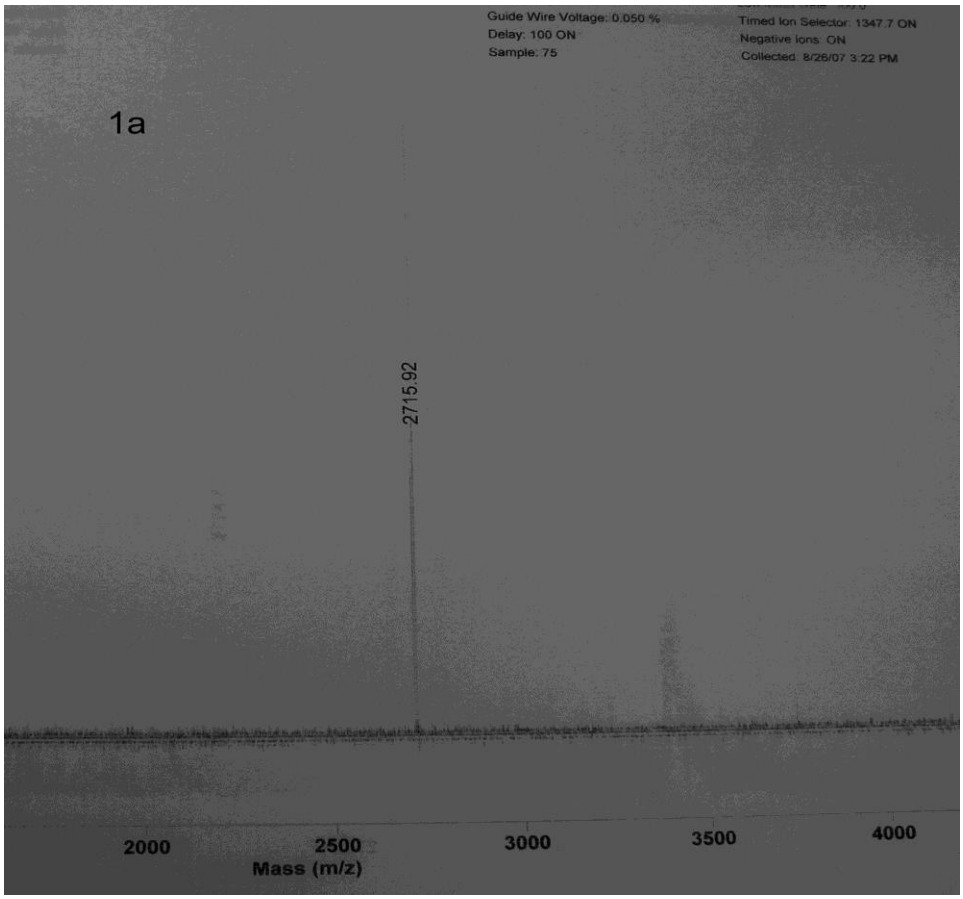


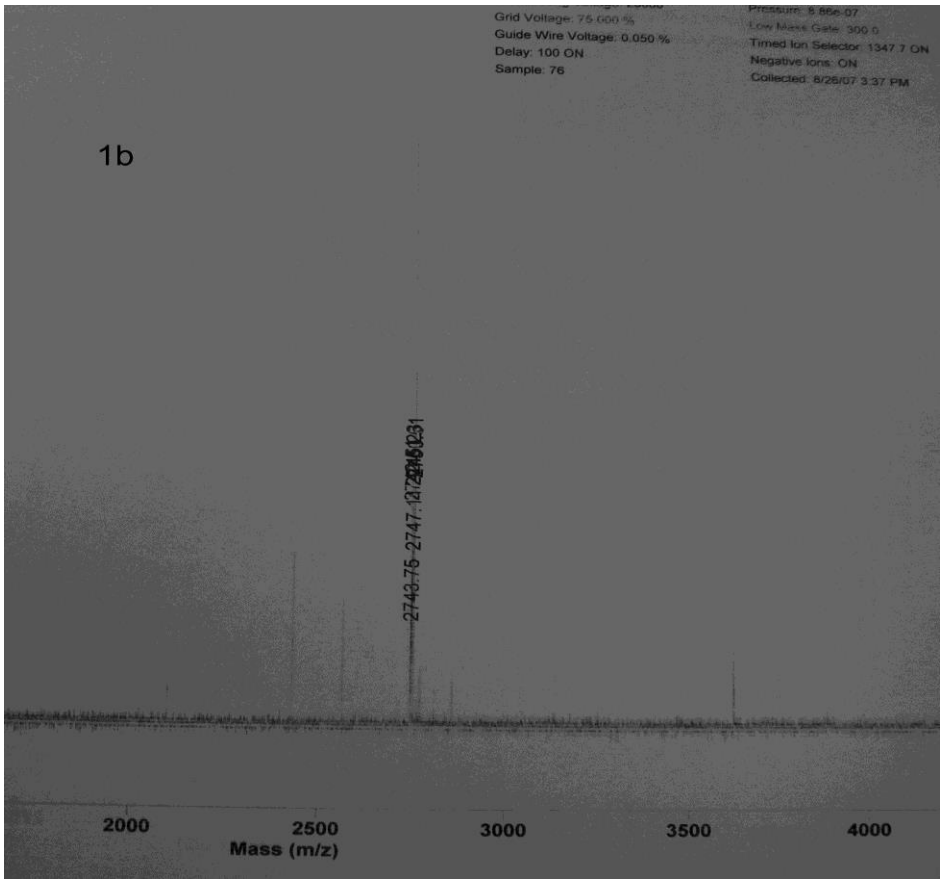
8. MS of azobenzene modified ODNs

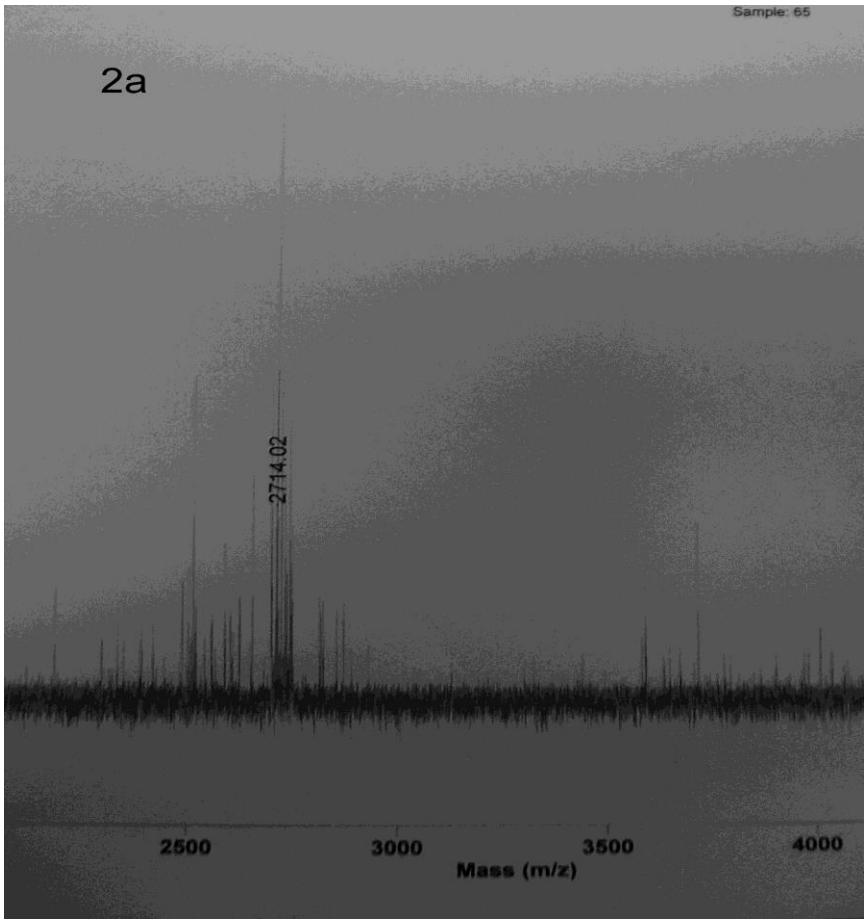
Table S1. The results for MS of the azobenzene modified hairpins.

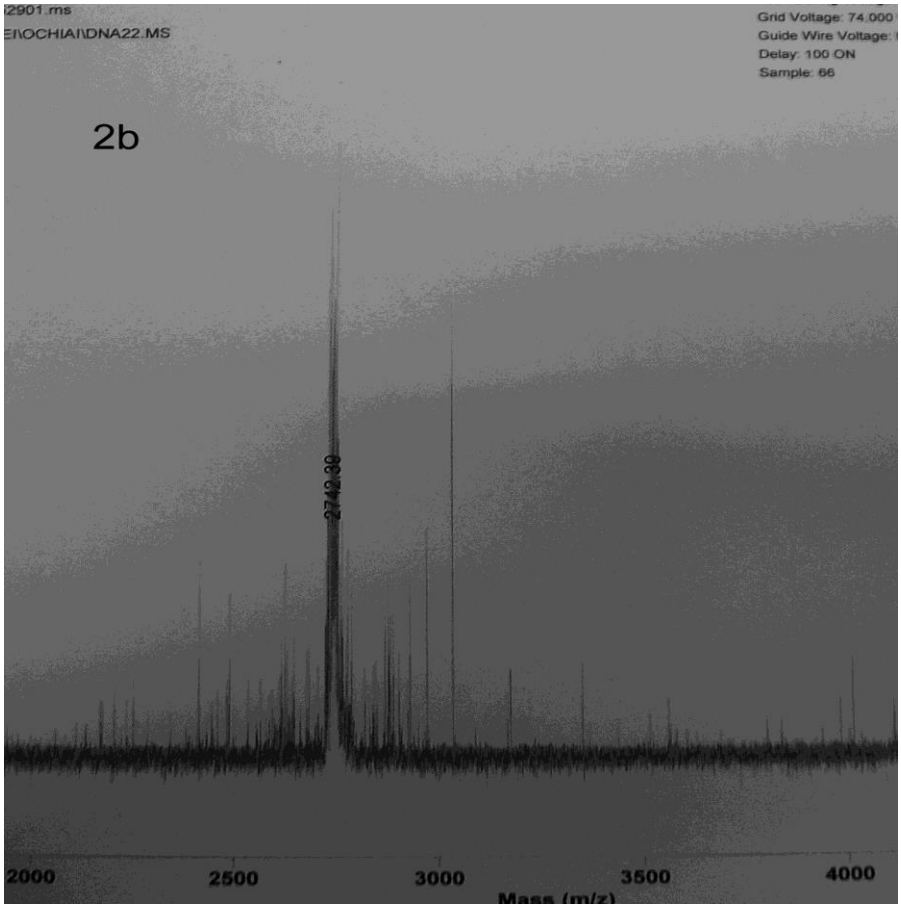
	Found	Calculated		Found	Calculated
1a	2715.9	2714.8	**1b**	2742.5	2742.9
2a	2714.0	2713.8	**2b**	2742.4	2741.9
3a	2713.4	2712.8	**3b**	2741.7	2740.9
4a	3330.3	3330.2	**4b**	3359.4	3358.3
5a	3331.5	3330.2	**5b**	3359.6	3358.3
6a	3332.6	3330.2	**6b**	3360.2	3358.3
7a	3331.9	3329.2	**7b**	3359.6	3357.3
8a	3331.0	3329.2	**8b**	3357.5	3357.3
9a	3329.7	3329.2	**9b**	3358.0	3357.3
10a	3947.2	3946.6	**10b**	3975.6	3974.7

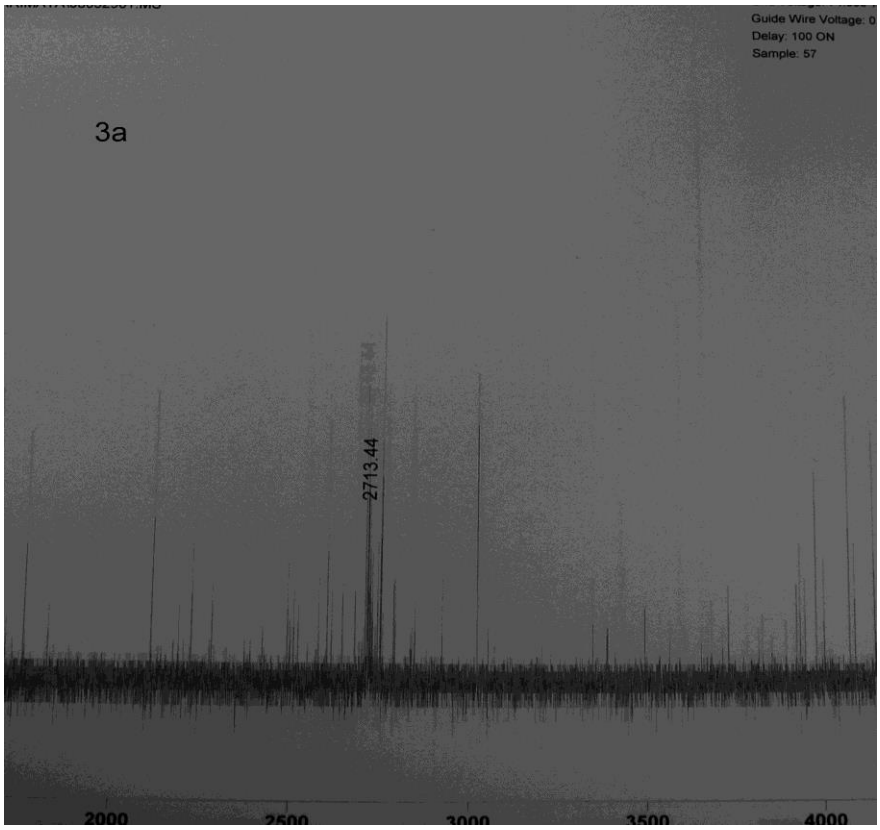
Figure S11. MALDI-TOF-MS of azobenzene modified ODNs.

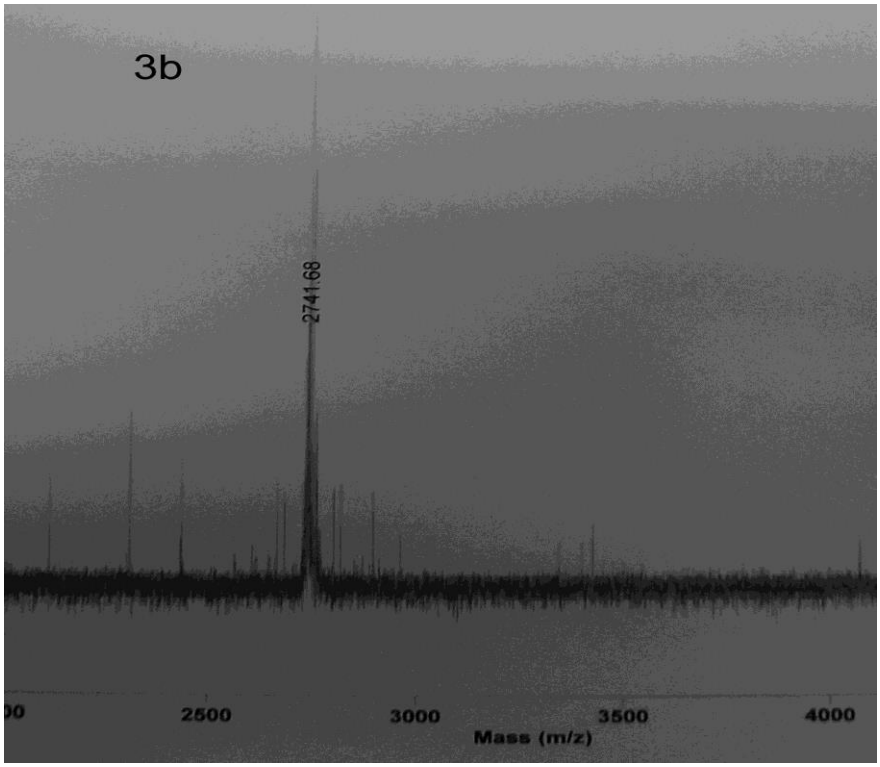


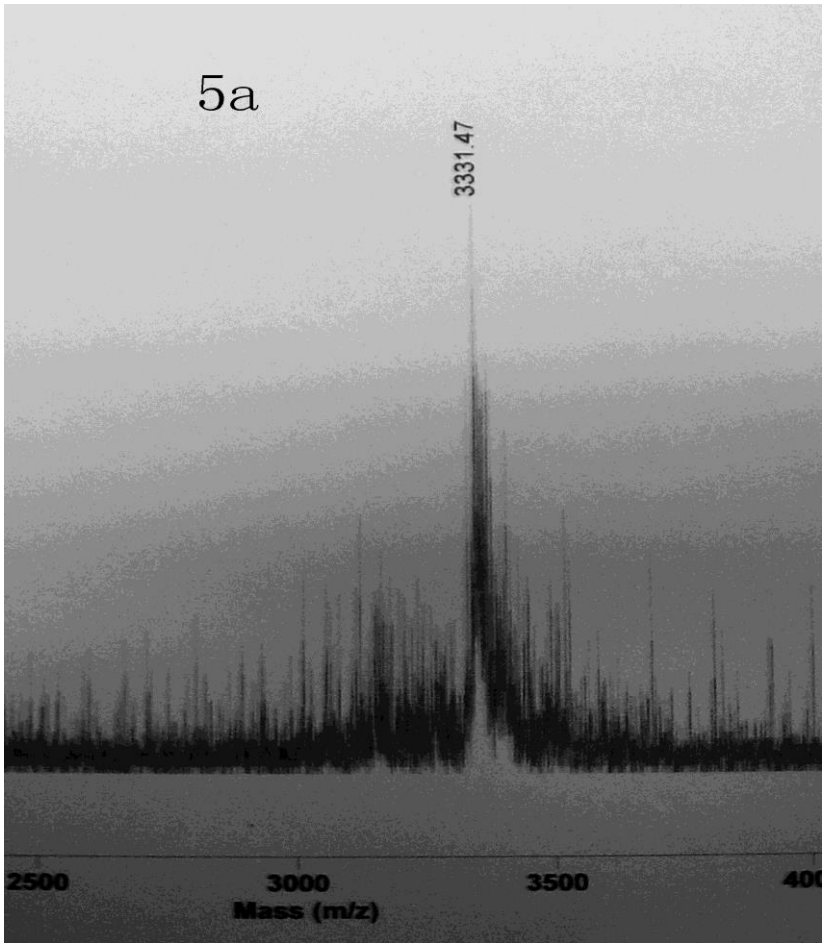


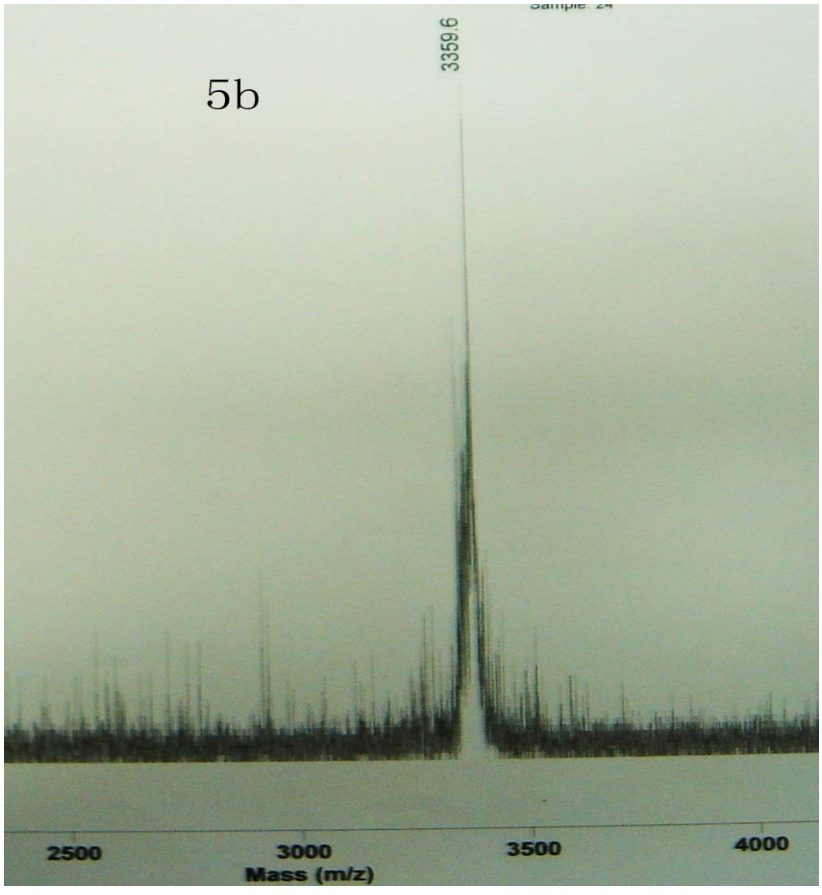

Sample: 65
2a
2714.02
2500
3000
3500
4000
Mass (m/z)

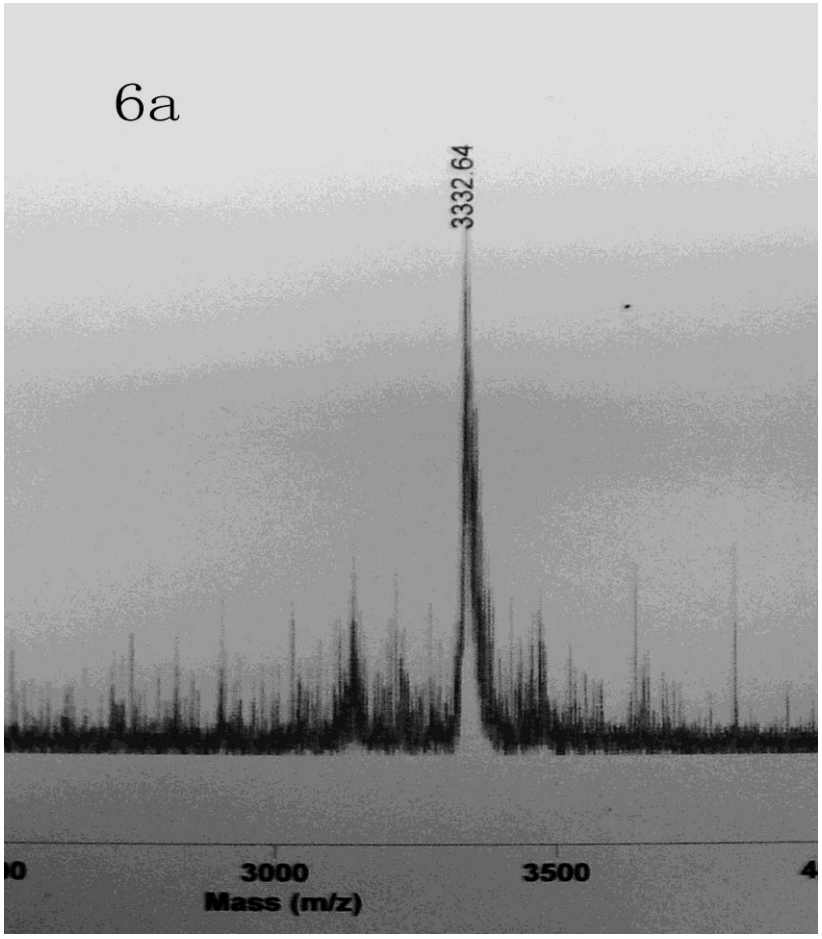

2901.ms
E:\OCHIAI\DNA22.MS
Grid Voltage: 74.000
Guide Wire Voltage:
Delay: 100 ON
Sample: 66
2b
2714.02
2000
2500
3000
3500
4000
Mass (m/z)

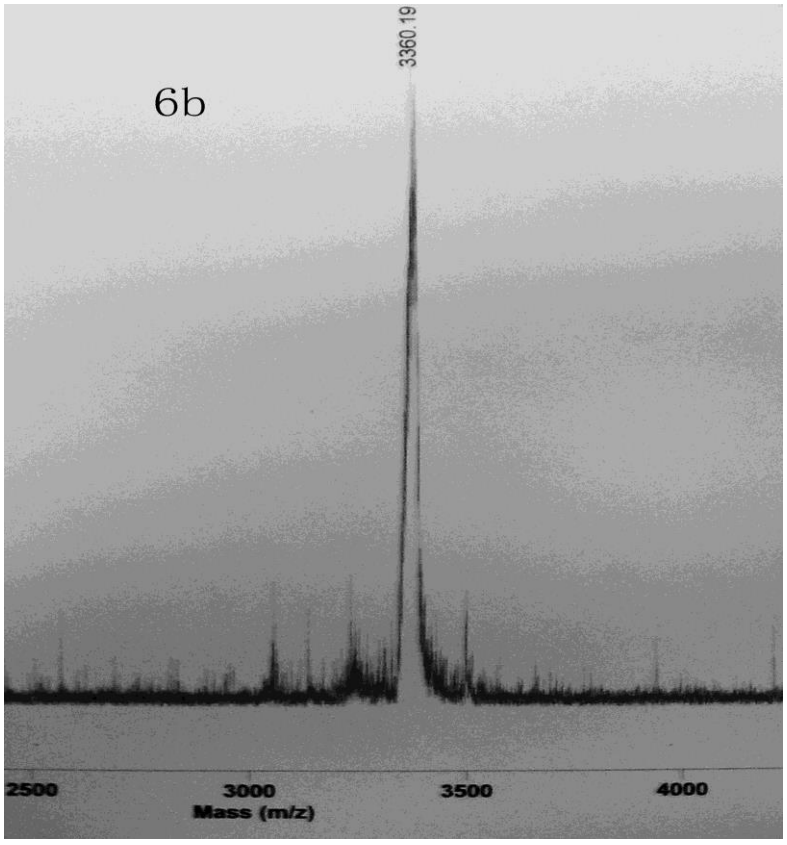

Guide Wire Voltage: 0
Delay: 100 ON
Sample: 57
3a
2713.44
2000
2500
3000
3500
4000

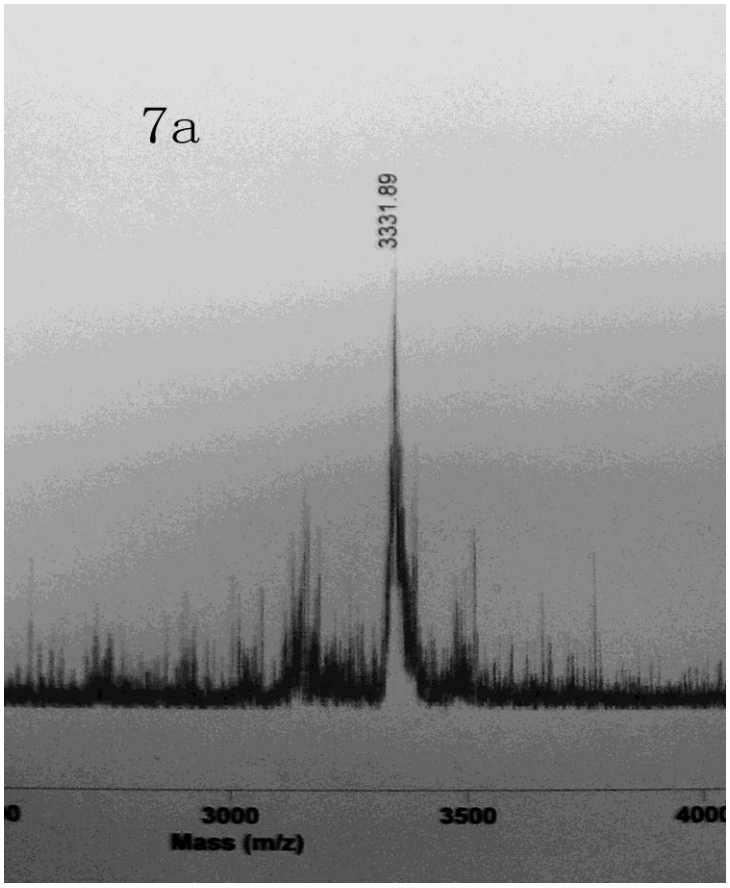

3b
2741.68
2500
3000
3500
4000
Mass (m/z)

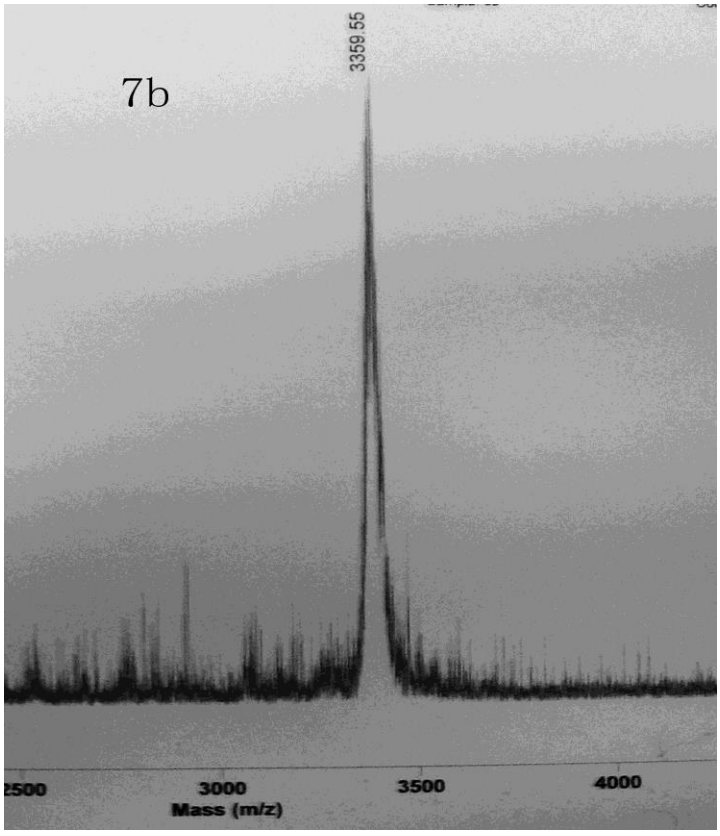

5a
3331.47
2500
3000
3500
Mass (m/z)

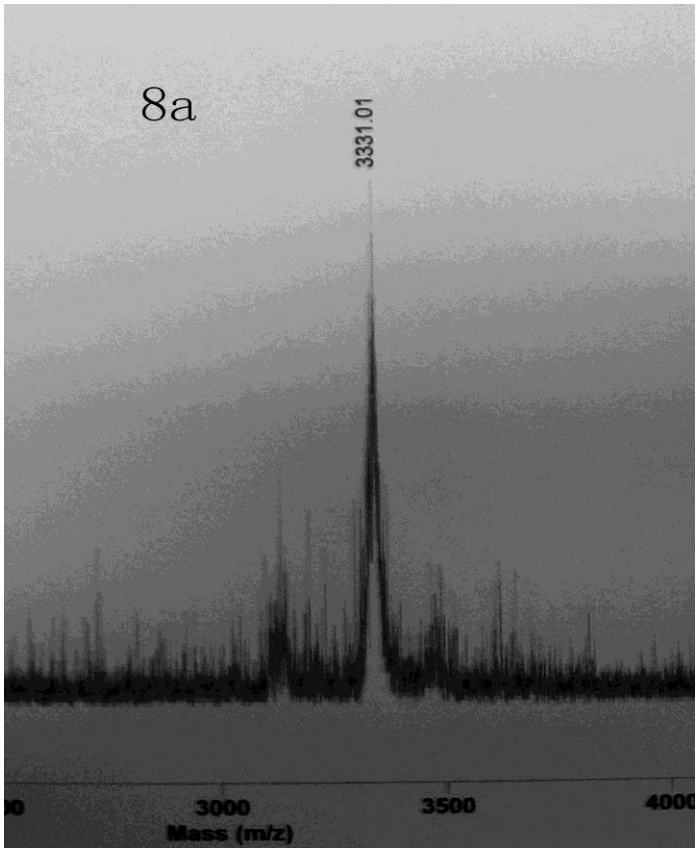

5b
3359.6
2500
3000
3500
4000
Mass (m/z)

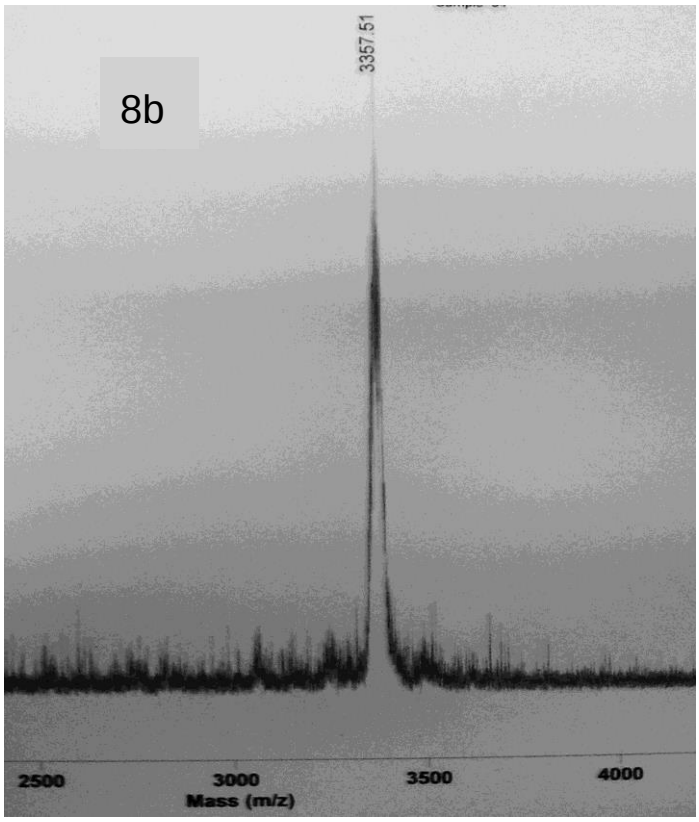

6a
3332.64
3000
3500
Mass (m/z)


6b
3360.19
2500
3000
3500
4000
Mass (m/z)


7a
3331.89
3000
3500
Mass (m/z)


7b
3359.55
3000
3500
4000
Mass (m/z)


8a
3331.01
3000
Mass (m/z)
3500


8b
3357.51
2500
3000
Mass (m/z)
3500
4000