

Photoregulating RNA digestion using azobenzene linked dumbbell antisense oligodeoxynucleotides

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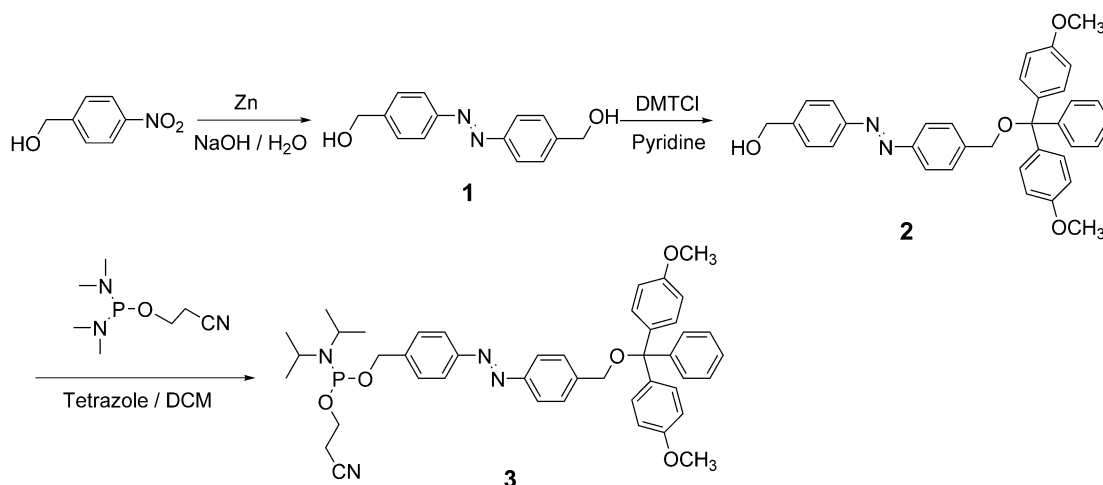
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1. Synthetic procedure of 2-cyanoethyl-4-O-{[4-(4,4'-dimethoxytrityl)-O-methyl-diazenyl]benzyl}-N,N'-diisopropylaminophosphoramidite

Figure S1. Synthetic procedure of azobenzene phosphoramidite.



Compound 1. 4,4'-bis(hydroxyethyl)-azobenzene: 6.0 g (26 mmol)

4-nitrobenzyl alcohol was added to 70 mL NaOH (5.7 M) aqueous solution, and 7.0 g (100 mmol) Zn power was added slowly. After addition of the materials, the solution was refluxed with stirring vigorously. After 24 h, the reaction mixture was filtered, and the filtered solid was suspended in hot methanol until the azo components were dissolved completely. Air was bubbled to the azobenzene methanol solution and refluxed for 10 h. Upon concentration on vacuum evaporator, the resulting methanol solution was slowly cooled to give orange solid 3.2 g, with yield 50 %. ¹H NMR (DMSO, 400 M): δ 7.87 (d, 4H), 7.54 (d, 4H), 5.39 (t, 2H), 4.61 (d, 4H). ¹³C NMR (DMSO, 101 M): δ 105.84, 146.24, 127.09, 122.34, 62.43.

Compound 2. 4-hydroxymethyl-4'-O-(4,4'-dimethoxytrityl)-azobenzene: 1.5 g (6.5 mmol) of 4,4'-bis(hydroxyethyl)-azobenzene was dissolved in 30 mL dry

pyridine. Part of pyridine was distilled to remove water residue in the solution. After cooling the solution, 2.1 g (6.5 mmol) 4,4'-dimethoxytrityl chloride was added while stirring vigorously. The reaction was monitored by TLC. The reaction mixture was concentrated and the residue was purified by silica gel chromatography with CH₂Cl₂/MeOH (volume ratio: 100/2) to give orange solids (2.0 g), yield 34%. ¹H NMR (CDCl₃, 400 M): δ 7.90 (m, 4H), 7.52 (m, 6H), 7.42 (m, 4H), 7.24 (m, 3H), 6.85 (m, 4H), 4.73 (s, 2H), 4.26 (s, 2H) 3.78 (s, 6H). ¹³C NMR (CDCl₃, 101 M): δ 158.56, 152.05, 151.84, 144.99, 144.44, 142.58, 136.19, 130.1, 128.2, 127.93, 127.46, 127.34, 126.87, 122.98, 122.82, 113.22, 86.63, 65.31, 64.40, 55.24.

Compound 3. 2-Cyanoethyl-4-O-[[4-(4,4'-dimethoxytrityl)-O-methyl-diazenyl]]

benzyl}-N,N'-diisopropylaminophosphoramidite: To the solution of 4-hydroxymethyl-4'-O-(4,4'-dimethoxytrityl)-azobenzene (0.160 g, 0.29 mmol) dissolved in 2 mL dry acetonitrile, 0.120 g 2-cyanoethyl-N,N,N',N'-tetraisopropylphosphora-diamidite and 21 mg (0.30 mmol) 1H-tetrazole were added. The mixture was stirred under N₂ and the reaction was monitored by TLC (yield was estimated above 90%). After an hour, the solution was filtered using hydrophobic membrane filter and moved to a vial for application to DNA synthesis without purification, and completion of the reaction was identified by ³¹PNMR. ³¹PNMR (CDCl₃, 162 M): δ 148.92, 148.77.

2. Structural characteristics of all azobenzene linked dumbbell asODNs before and after UV irradiation.

Figure S2. UV-Vis spectral changes of 4,4'-bis(hydroxyethyl)-azobenzene and **Az18-4** through illumination with UV or visible light. Photoirradiation experiments of 4,4'-bis(hydroxyethyl)-azobenzene were performed in 3:7 MeOH/H₂O at room temperature with UV irradiation (365 nm, 7 mW/cm², *trans*-to-*cis*) and visible irradiation (>400 nm, 11 W, *cis*-to-*trans*), and **Az18-4** was dissolved in 1×PBS and irradiated. Inset: plots of absorbance at maximum absorption of azobenzene as a function of UV irradiation time and visible irradiation time.

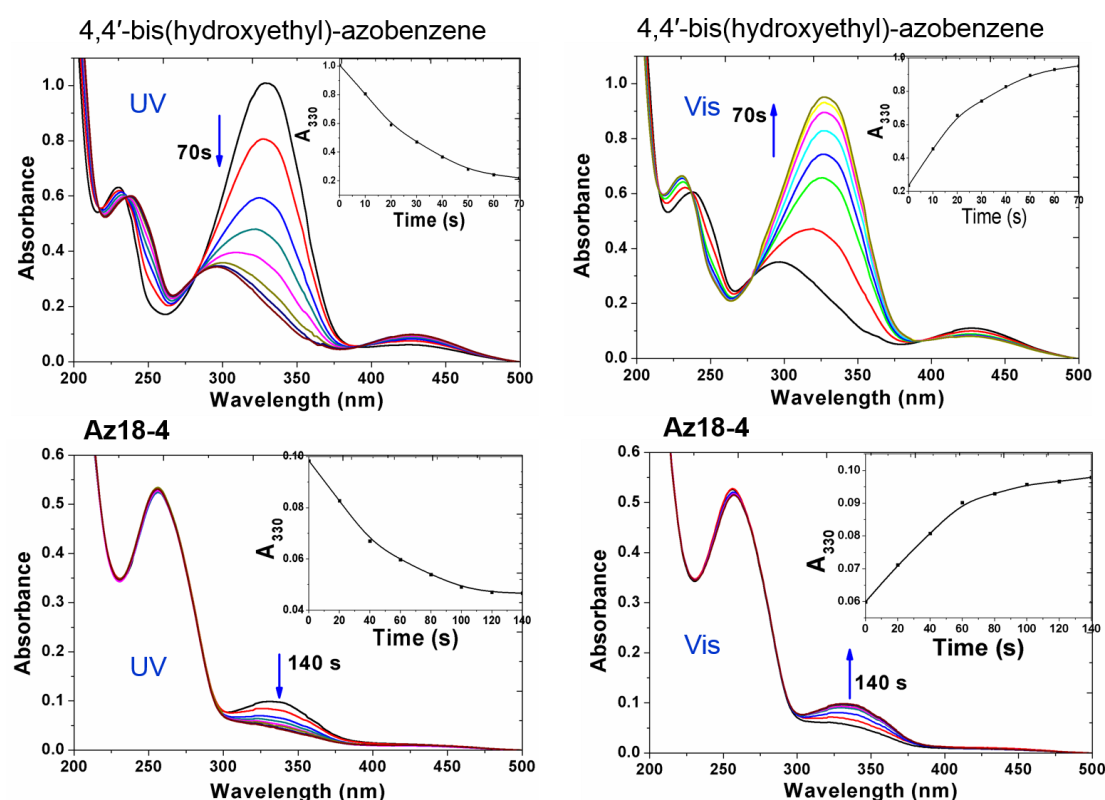
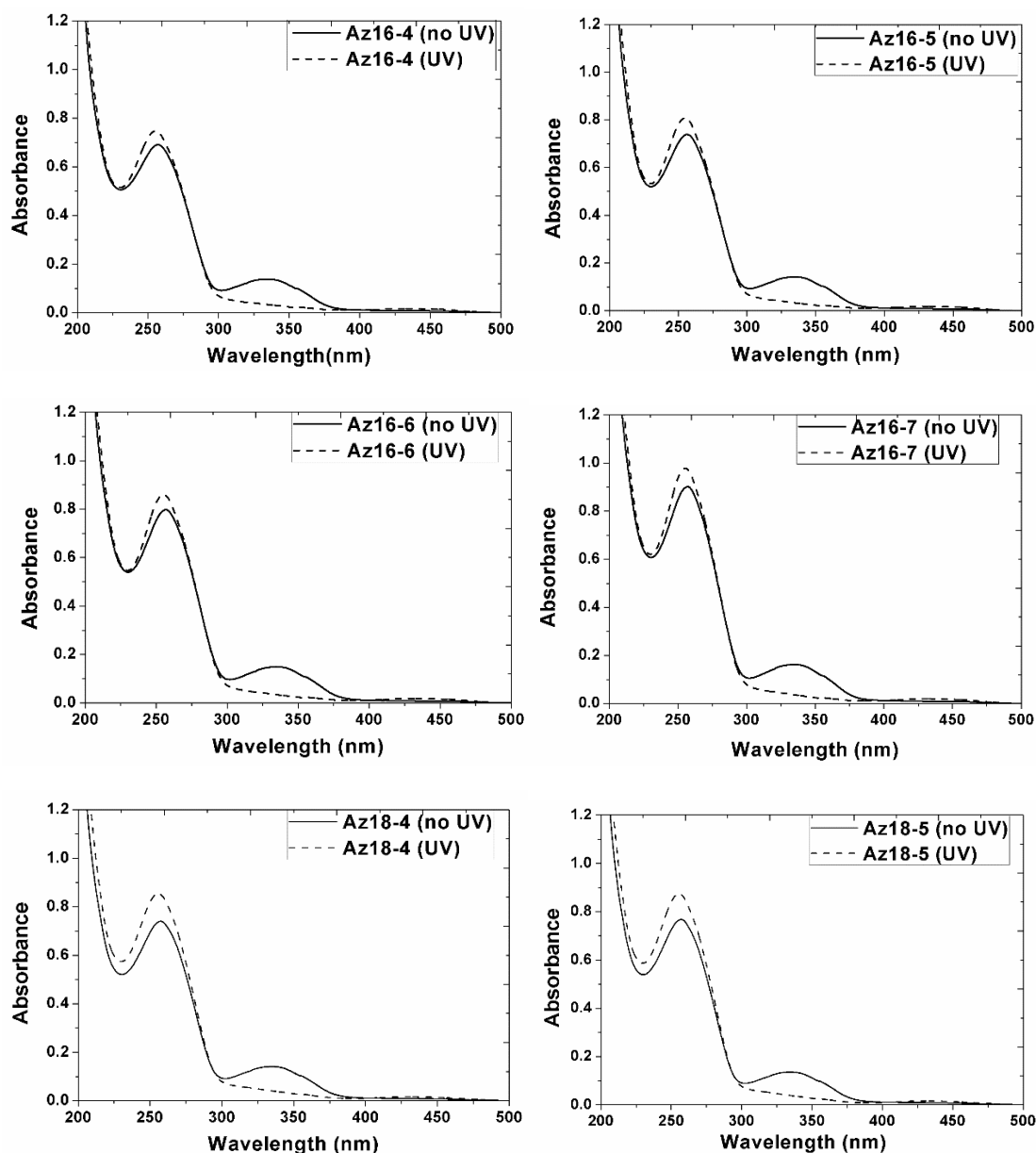


Figure S3. UV/vis absorbance spectra of the respective isomeric forms of all azobenzene linked dumbbell ODNs before and after light illumination. The samples (2 μM) were irradiated with UV (365 nm, $7\text{mW}/\text{cm}^2$, *trans*-to-*cis*) in 1 \times PBS until absorbance changes were no longer observable.



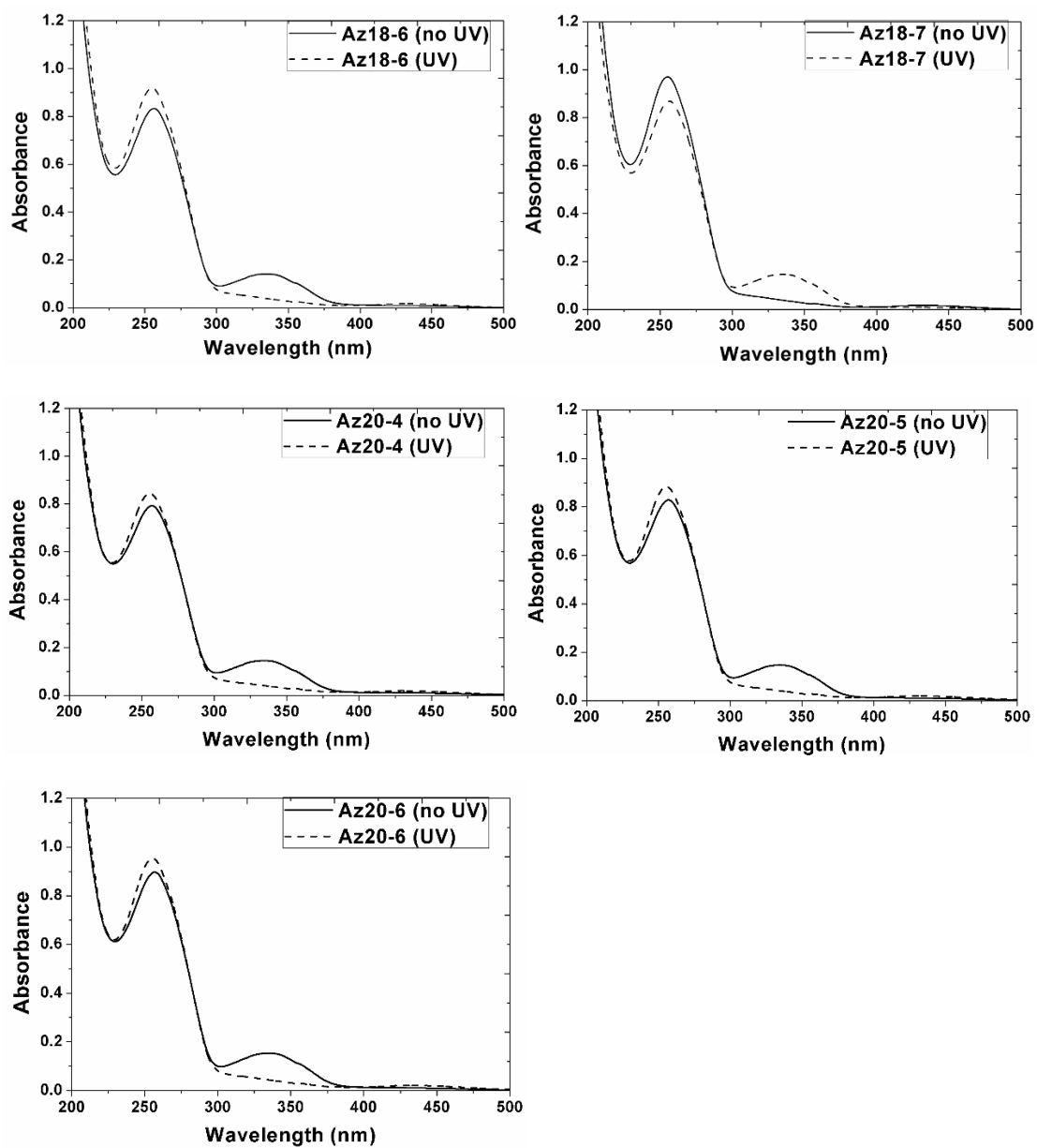


Figure S4. Native polyacrylamide gel electrophoresis of all azobenzene linked dumbbell asODNs before and after light illumination (365 nm, 7 mW/cm²). The samples from left to right were corresponding to *trans*-Az18-4, *cis*-Az18-4, *trans*-Az18-5, *cis*-Az18-5, *trans*-Az18-6, *cis*-Az18-6, *trans*-Az18-7, *cis*-Az18-7.

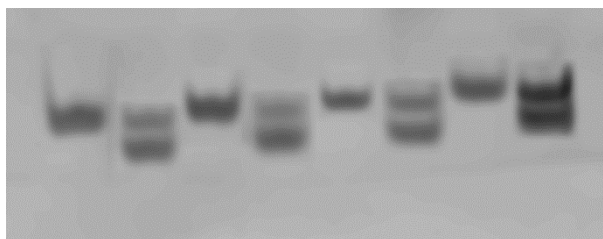
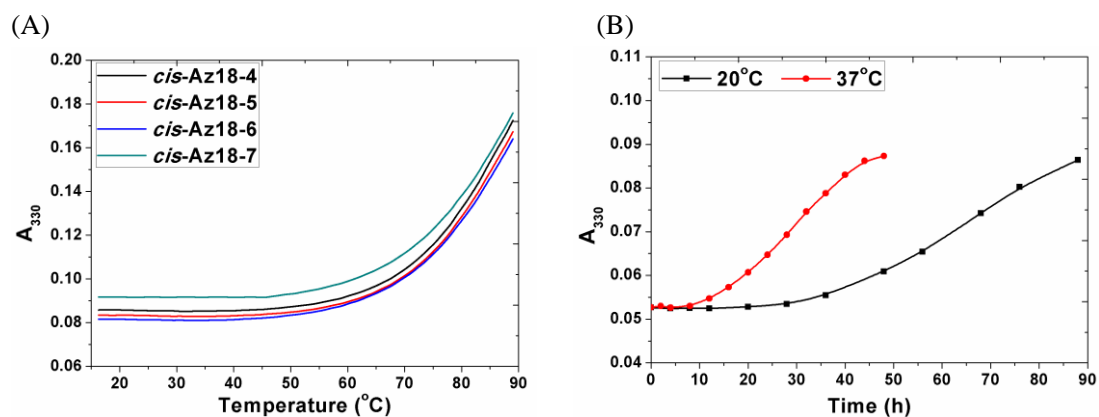


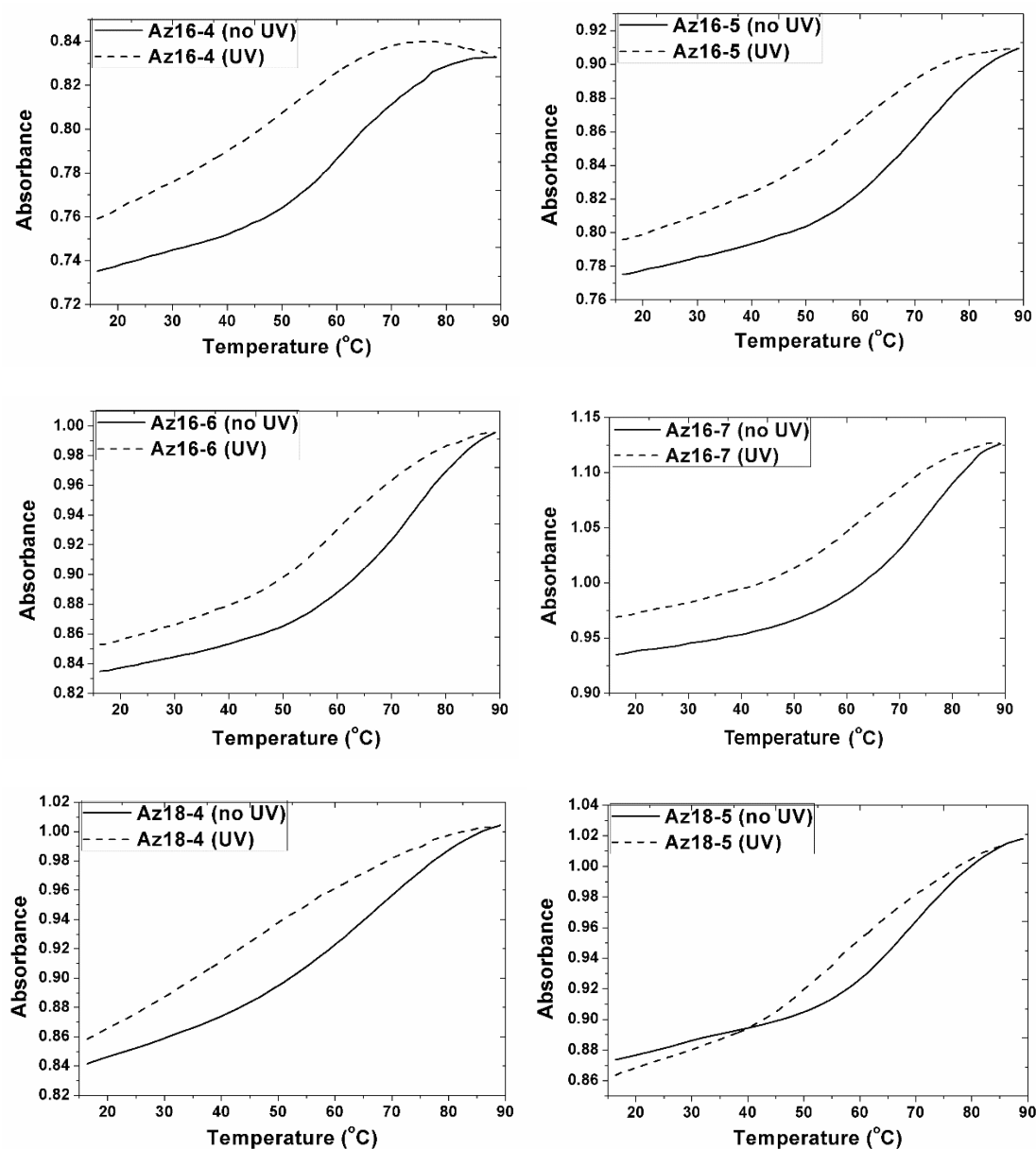
Figure S5. A) Plots of absorbance at maximum absorption of azobenzene for *cis*-Az18-4, *cis*-Az18-5, *cis*-Az18-6 and *cis*-Az18-7 in 1×PBS as a function of temperature; B) Plots of absorbance at maximum absorption of azobenzene for *cis*-Az18-4 with increasing time at 20°C or 37°C.

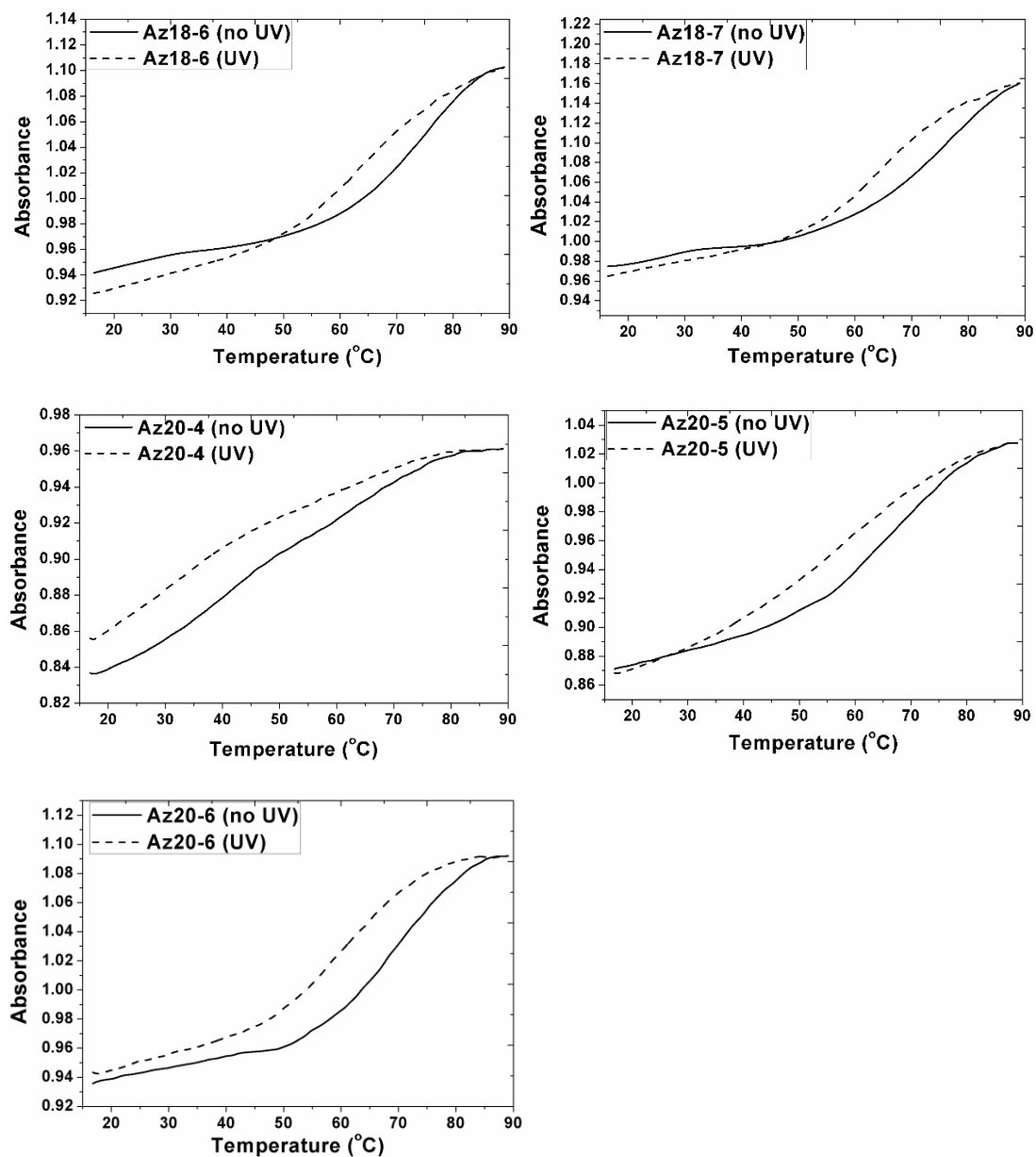


3. Typical melting curves of all azobenzene linked dumbbell asODNs before and after UV irradiation

Figure S6. A) Typical melting curves of all azobenzene linked dumbbell asODNs themselves. The black and dashed lines present the melting curves before and after irradiation with UV light (365 nm, 7 mW/cm²), respectively. UV melting experiments were performed in 1×PBS and ODN concentrations were adjusted to 2 μM. B) Typical T_m derivative (dA/dT) plot of UV melting curve for Az18-4.

A)





B)

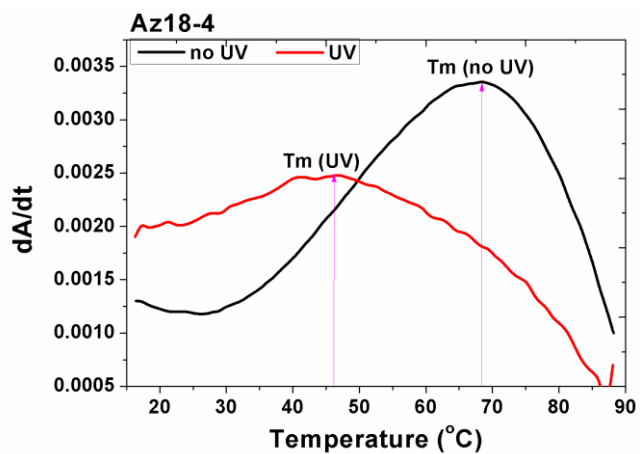
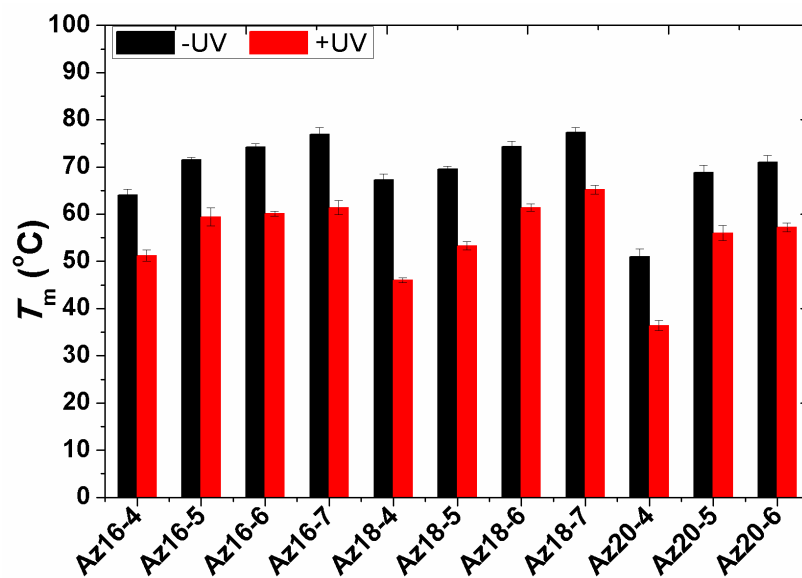


Figure S7. T_m comparison of azobenzene linked dumbbell asODNs before and after UV irradiation. Red column represents T_m s of *cis*-azobenzene linked ODNs, and black column represents T_m s of *trans*-azobenzene linked ODNs.



4. Photoregulation of binding target RNA with azobenzene linked dumbbell asODNs

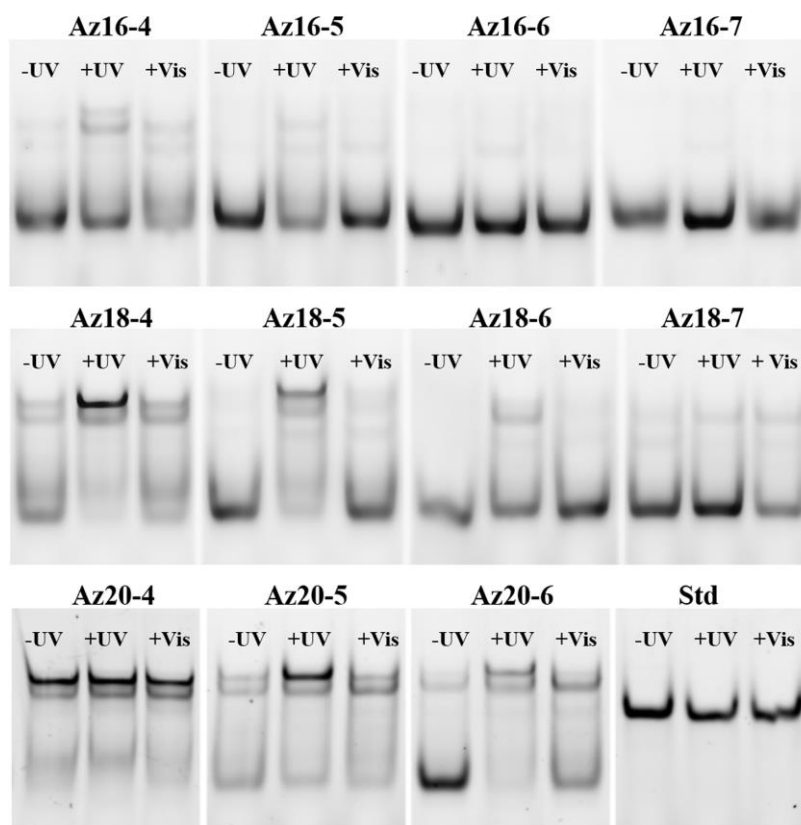
Table S1. The ratios of the binding of azobenzene linked dumbbell asODNs (2 μ M, 10 μ M and 20 μ M) with the complementary FAM-labeled RNA (1 μ M) in 5 μ L 1 \times PBS buffer after 30 min incubation at 37°C. UV irradiation was performed for the mixture of dumbbell asODNs and FAM-RNA within 2 min.

Az16-4	Lane1	Lane2	Lane3	Lane4	Lane5	Lane6	Lane7
	0 μ M	2 μ M	10 μ M	20 μ M	2 μ M	10 μ M	20 μ M
UV	–	–	–	–	+	+	+
ODN/RNA(%)	0	4.8	8.1	8.3	53.6	53.7	57.0
		4.9	4.3	8.1	14.1	18.5	18.6
RNA (%)	100	90.3	87.6	83.6	32.3	27.8	24.4
Az16-5	Lane1	Lane2	Lane3	Lane4	Lane5	Lane6	Lane7
	0 μ M	2 μ M	10 μ M	20 μ M	2 μ M	10 μ M	20 μ M
UV	–	–	–	–	+	+	+
ODN/RNA(%)	0				17.5	8.3	11.3
		3.6	7.2	17.7		18.1	21.6
RNA (%)	100	96.4	92.8	82.3	82.5	73.6	67.1
Az16-6	Lane1	Lane2	Lane3	Lane4	Lane5	Lane6	Lane7
	0 μ M	2 μ M	10 μ M	20 μ M	2 μ M	10 μ M	20 μ M
UV	–	–	–	–	+	+	+
ODN/RNA(%)	0	0	2.6	3.3	0	3.3	4.4
RNA (%)	100	100	97.4	96.7	100	96.7	95.6
Az16-7	Lane1	Lane2	Lane3	Lane4	Lane5	Lane6	Lane7
	0 μ M	2 μ M	10 μ M	20 μ M	2 μ M	10 μ M	20 μ M
UV	–	–	–	–	+	+	+
ODN/RNA(%)	0	0	1.3	2.3	0	2.3	2.2
RNA (%)	100	100	98.7	97.7	100	97.7	97.8

Az18-4	Lane1	Lane2	Lane3	Lane4	Lane5	Lane6	Lane7
	0 μ M	2 μ M	10 μ M	20 μ M	2 μ M	10 μ M	20 μ M
UV	–	–	–	–	+	+	+
ODN/RNA(%)	0	4.6	4.9	4.8	48.4	47.5	50.5
		3.2	3.2	8.3	10.0	8.8	20.8
		2.6	3.8	7.4	13.6	19.1	18.4
RNA (%)	100	89.6	87.9	79.6	27.7	24.6	20.3
Az18-5	Lane1	Lane2	Lane3	Lane4	Lane5	Lane6	Lane7
	0 μ M	2 μ M	10 μ M	20 μ M	2 μ M	10 μ M	20 μ M
UV	–	–	–	–	+	+	+
ODN/RNA(%)	0				15.5	14.6	14.9
		2.6	3.3	2.0	12.5	16.2	21.2
		3.3	4.3	7.1	5.1	7.5	11.0
RNA (%)	100	94.1	92.4	90.9	66.9	61.7	52.9
Az18-6	Lane1	Lane2	Lane3	Lane4	Lane5	Lane6	Lane7
	0 μ M	2 μ M	10 μ M	20 μ M	2 μ M	10 μ M	20 μ M
UV	–	–	–	–	+	+	+
ODN/RNA(%)	0					4.0	5.9
		0	4.2	7.8	0	2.1	5.0
RNA (%)	100	100	95.8	92.8	100	93.9	89.1
Az18-7	Lane1	Lane2	Lane3	Lane4	Lane5	Lane6	Lane7
	0 μ M	2 μ M	10 μ M	20 μ M	2 μ M	10 μ M	20 μ M
UV	–	–	–	–	+	+	+
ODN/RNA(%)	0					3.2	3.2
		0	2.6	4.3	0	5.1	4.2
RNA (%)	100	100	97.4	95.7	100	91.7	92.6
Az20-4	Lane1	Lane2	Lane3	Lane4	Lane5	Lane6	Lane7
	0 μ M	2 μ M	10 μ M	20 μ M	2 μ M	10 μ M	20 μ M
UV	–	–	–	–	+	+	+
ODN/RNA(%)	0	50.0	51.4	50.8	65.9	69.8	67.3

		27.5	28.2	30.3	23.5	21.9	25.1
RNA (%)	100	22.5	20.4	18.9	10.6	8.3	7.6
Az20-5	Lane1	Lane2	Lane3	Lane4	Lane5	Lane6	Lane7
	0 μ M	2 μ M	10 μ M	20 μ M	2 μ M	10 μ M	20 μ M
UV	–	–	–	–	+	+	+
ODN/RNA(%)	0	21.8	17.0	21.1	48.8	45.9	46.6
		34.4	44.1	42.7	26.8	31.3	34.1
RNA (%)	100	43.8	38.9	36.2	24.4	22.8	19.3
Az20-6	Lane1	Lane2	Lane3	Lane4	Lane5	Lane6	Lane7
	0 μ M	2 μ M	10 μ M	20 μ M	2 μ M	10 μ M	20 μ M
UV	–	–	–	–	+	+	+
ODN/RNA(%)	0	18.1	20.4	25.2	38.6	30.4	34.7
		11.6	14.1	21.5	18.3	30.7	34.2
RNA (%)	100	70.3	65.5	53.3	43.1	38.9	31.1

Figure S8. Native PAGE gels (20%) of the binding of azobenzene linked dumbbell asODNs (10 μ M) with the complementary FAM-labeled RNA (1 μ M) in 5 μ L 1 \times PBS buffer after 30 min incubation at 37°C. 2 min UV irradiation and 2 min Vis irradiation after 2 min UV irradiation were performed for the mixture of dumbbell asODNs and FAM-RNA.



5. Photoregulation of RNA digestion with azobenzene linked dumbbell asODNs

Table S2. The fractions of RNA degradation at 37 °C for all azobenzene linked dumbbell asODNs before and after 2 min UV irradiation with 70 min incubation.

ODNs	Cleaved RNA (%)		
	-UV	+UV	+UV/-UV
Az16-4	12.1±1.5	39.3±3.4	3.2
Az16-5	5.2±0.9	19.3±2.1	3.7
Az16-6	1.8±1.2	10.9±2.3	6.1
Az16-7	0±0	0±0	--
Az18-4	19.9±1.9	83.5±3.7	4.2
Az18-5	4.9±1.4	36.7±2.6	7.5
Az18-6	1.6±1.0	11.2±2.2	7.0
Az18-7	1.4±0.8	1.9±0.7	1.4
Az20-4	54.8±2.6	87.2±4.1	1.6
Az20-5	41.2±4.9	61.5±4.1	1.5
Az20-6	16.3±1.4	38.4±3.1	2.4
Std	100	100	1

Figure S9. Denaturing PAGE (20%) of photomodulation of the RNA digestion by RNase H in presence of 1 μ M FAM-labeled RNA, 0.05 μ M azobenzene linked asODNs (**Az16-4**, **Az16-5**, **Az16-6**, **Az16-7**, **Az18-6**, **Az18-7**, **Az20-4**, **Az20-5**, **Az20-6** and **Std**) and 0.25 U RNase H in 25 μ L RNase H buffer without or with UV irradiation (365 nm, 7 mW/cm²) at 37 $^{\circ}$ C with increasing incubation time. Aliquots of each incubated sample were removed at 0 min, 10 min, 25 min, 45 min and 70 min.

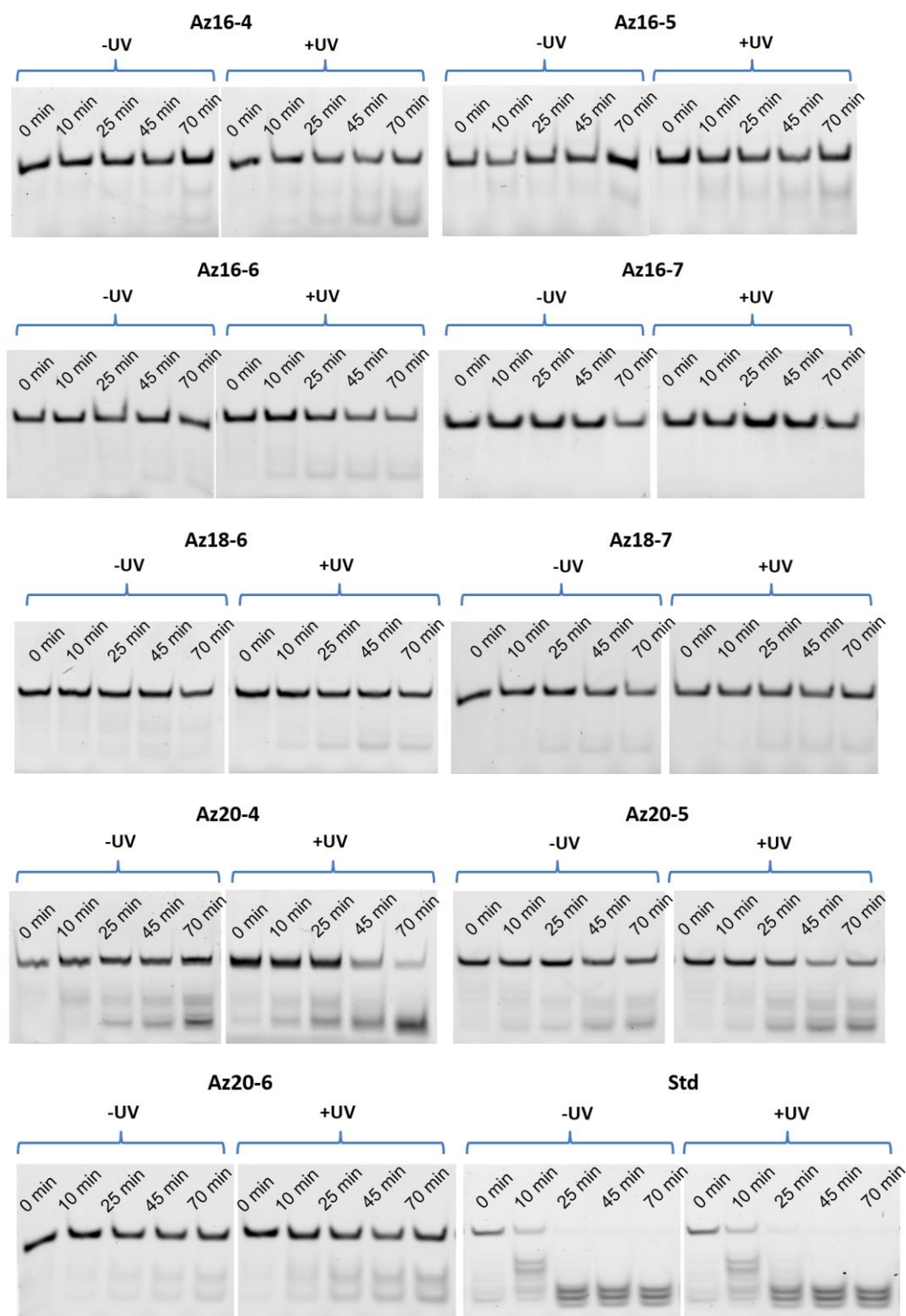


Table S3. The fractions of RNA degradation at 37 °C for all azobenzene linked dumbbell asODNs before and after 2 min UV irradiation. Quantitative analysis of RNA digestion was performed with each incubated sample at 0 min, 10 min, 25 min, 45 min and 70 min.

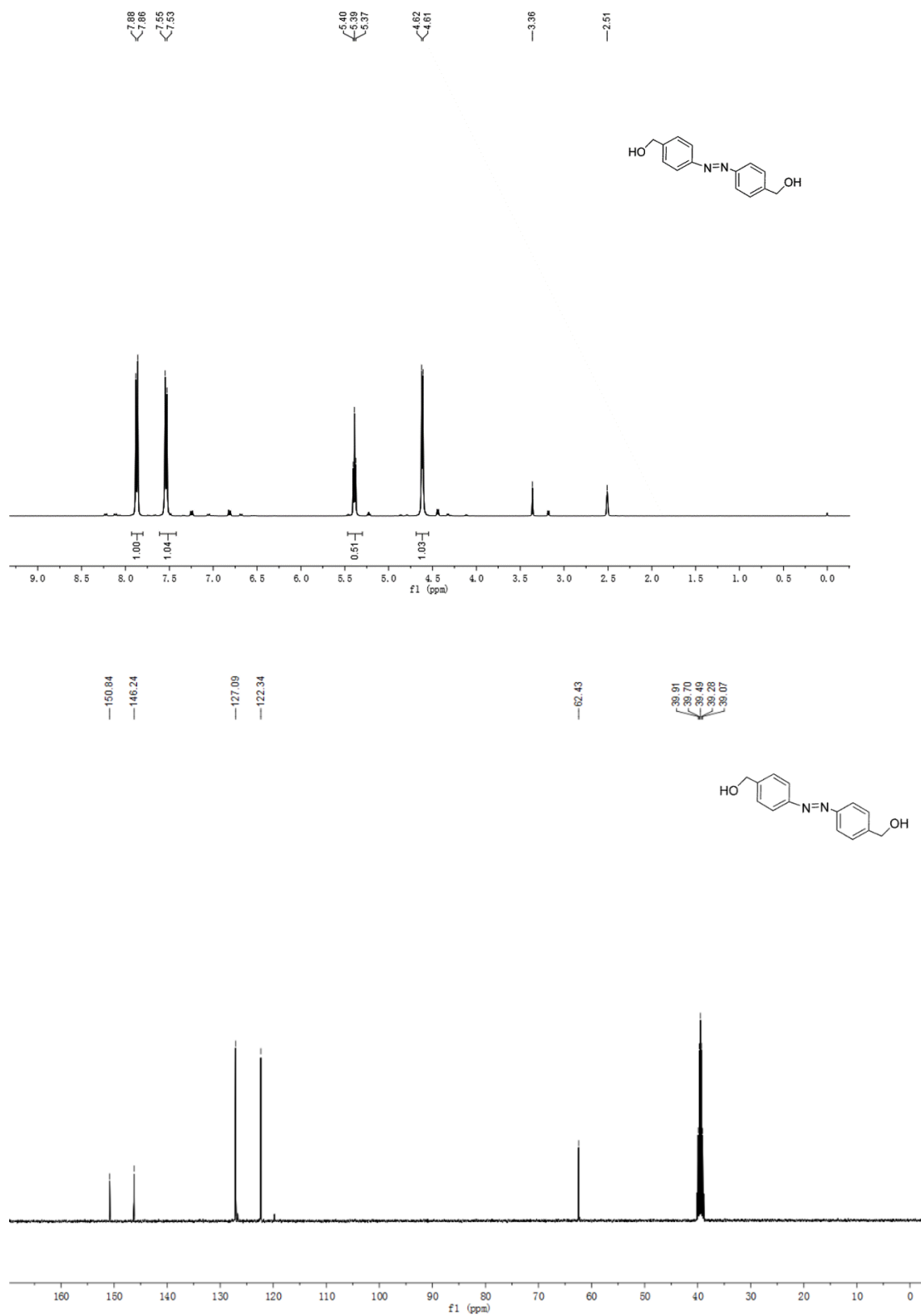
Az16-4	Lane1	Lane2	Lane3	Lane4	Lane5	Lane6	Lane7	Lane8	Lane9	Lane10
UV	–	–	–	–	–	+	+	+	+	+
Uncleaved RNA (%)	100	99.2	98.8	97.3	86.2	100	89.2	84.6	84.6	62.3
Cleaved RNA (%)	0	0.8	1.2	0.9	5.1	0	1.1	1.1	1.4	9.4
				1.8	8.7		9.7	14.3	14.0	28.3
Az16-5	Lane1	Lane2	Lane3	Lane4	Lane5	Lane6	Lane7	Lane8	Lane9	Lane10
UV	–	–	–	–	–	+	+	+	+	+
Uncleaved RNA (%)	100	97.8	99.0	98.8	95.2	100	86.5	87.6	81.4	78.6
Cleaved RNA (%)	0	2.2	1.0	1.2	4.8	0	13.3	11.9	18.3	20.5
							0.2	0.5	0.3	0.9
Az16-6	Lane1	Lane2	Lane3	Lane4	Lane5	Lane6	Lane7	Lane8	Lane9	Lane10
UV	–	–	–	–	–	+	+	+	+	+
Uncleaved RNA (%)	100	100	99.5	98.5	98.7	100	99.2	92.0	89.1	87.2
Cleaved RNA (%)	0	0	0.5	0.6	0.8	0	0.8	8.0	10.9	12.8
				0.9	0.5					
Az16-7	Lane1	Lane2	Lane3	Lane4	Lane5	Lane6	Lane7	Lane8	Lane9	Lane10
UV	–	–	–	–	–	+	+	+	+	+
Uncleaved RNA (%)	100	100	100	100	100	100	100	100	100	100
Cleaved RNA (%)	0	0	0	0	0	0	0	0	0	0
Az18-4	Lane1	Lane2	Lane3	Lane4	Lane5	Lane6	Lane7	Lane8	Lane9	Lane10

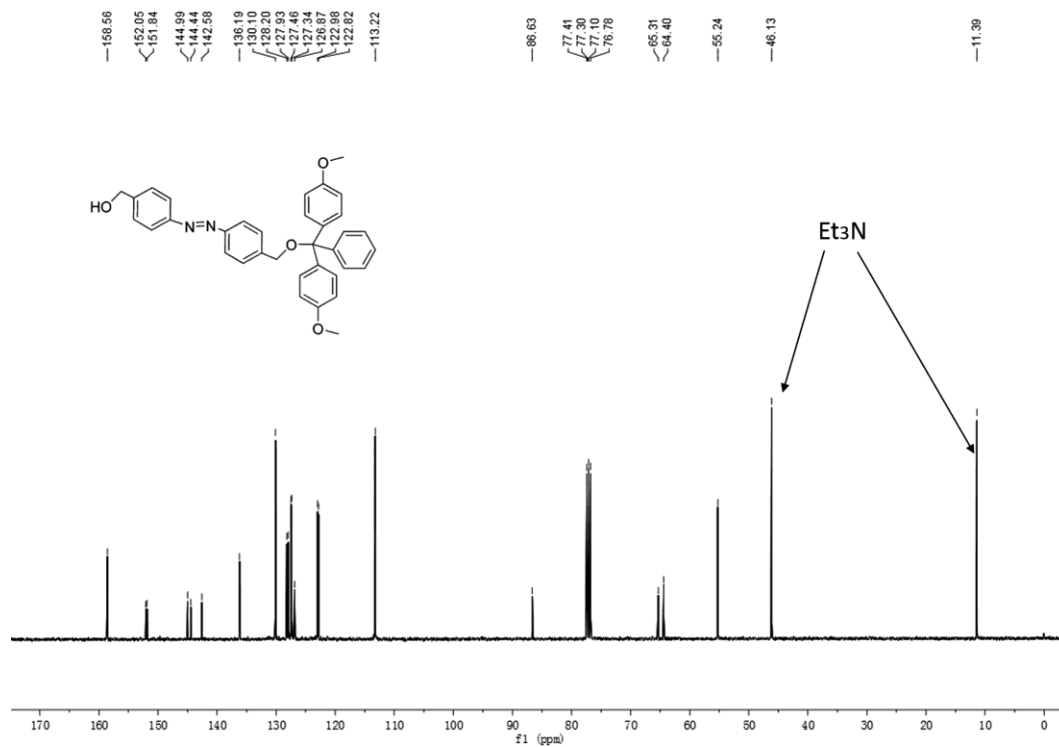
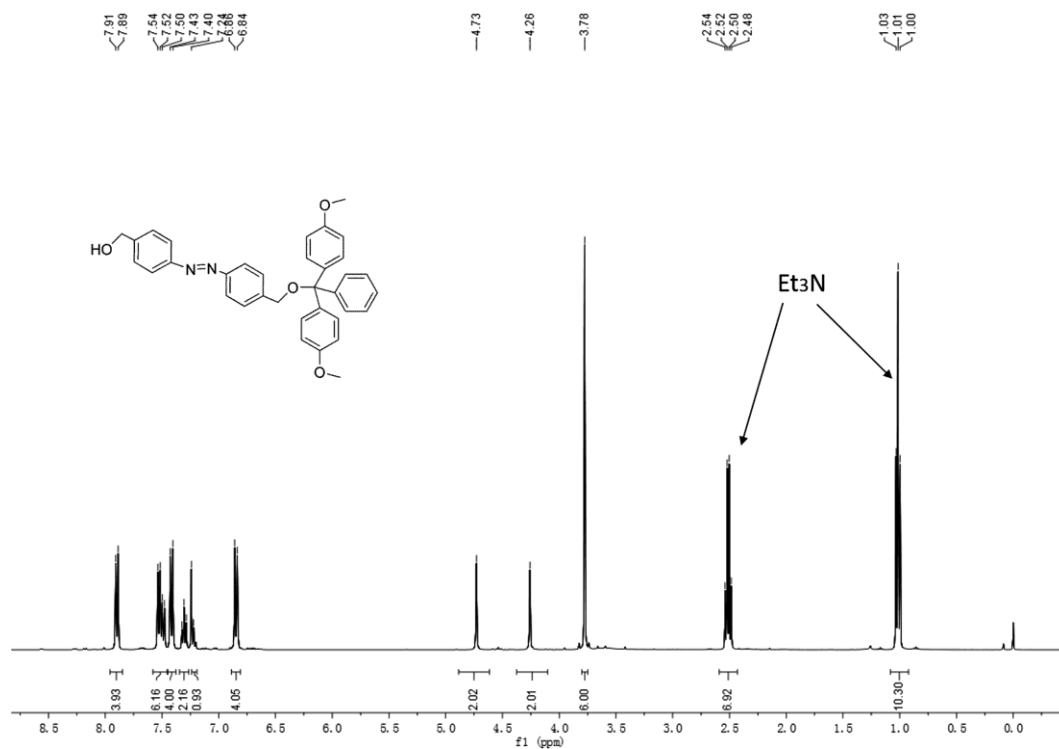
UV	–	–	–	–	–	+	+	+	+	+
Uncleaved	100	98.7	91.5	87.1	79.7	100	64.5	37.4	23.6	16.8
RNA (%)										
Cleaved	0	1.3	8.5	12.9	20.3	0	6.8	10.5	10.2	7.2
RNA (%)							28.7	52.1	66.2	76.0
Az18-5	Lane1	Lane2	Lane3	Lane4	Lane5	Lane6	Lane7	Lane8	Lane9	Lane10
UV	–	–	–	–	–	+	+	+	+	+
Uncleaved	100	99.7	99.0	97.3	95.8	100	94.4	84.8	71.4	63.3
RNA (%)										
Cleaved	0	0.3	1.0	2.7	4.2	0	5.6	15.2	8.1	10.8
RNA (%)									20.5	25.9
Az18-6	Lane1	Lane2	Lane3	Lane4	Lane5	Lane6	Lane7	Lane8	Lane9	Lane10
UV	–	–	–	–	–	+	+	+	+	+
Uncleaved	100	100	99.1	98.7	98.6	100	99.2	94.2	90.9	90.6
RNA (%)										
Cleaved	0	0	0.5	0.7	0.9	0	0.8	5.8	9.1	9.4
RNA (%)			0.4	0.6	0.5					
Az18-7	Lane1	Lane2	Lane3	Lane4	Lane5	Lane6	Lane7	Lane8	Lane9	Lane10
UV	–	–	–	–	–	+	+	+	+	+
Uncleaved	100	100	98.8	97.9	97.9	100	100	98.7	98.2	98.2
RNA (%)										
Cleaved	0	0	1.2	2.1	2.1	0	0	1.3	1.8	1.8
RNA (%)										
Az20-4	Lane1	Lane2	Lane3	Lane4	Lane5	Lane6	Lane7	Lane8	Lane9	Lane10
UV	–	–	–	–	–	+	+	+	+	+
Uncleaved	100	96.3	84.0	66.6	46.0	97.8	81.7	66.8	40.8	12.4
RNA (%)										
Cleaved	0	3.7	16.0	8.8	14.3	1.2	7.3	10.8	59.2	87.6
RNA (%)				24.6	39.7	1.0	11.0	22.4		

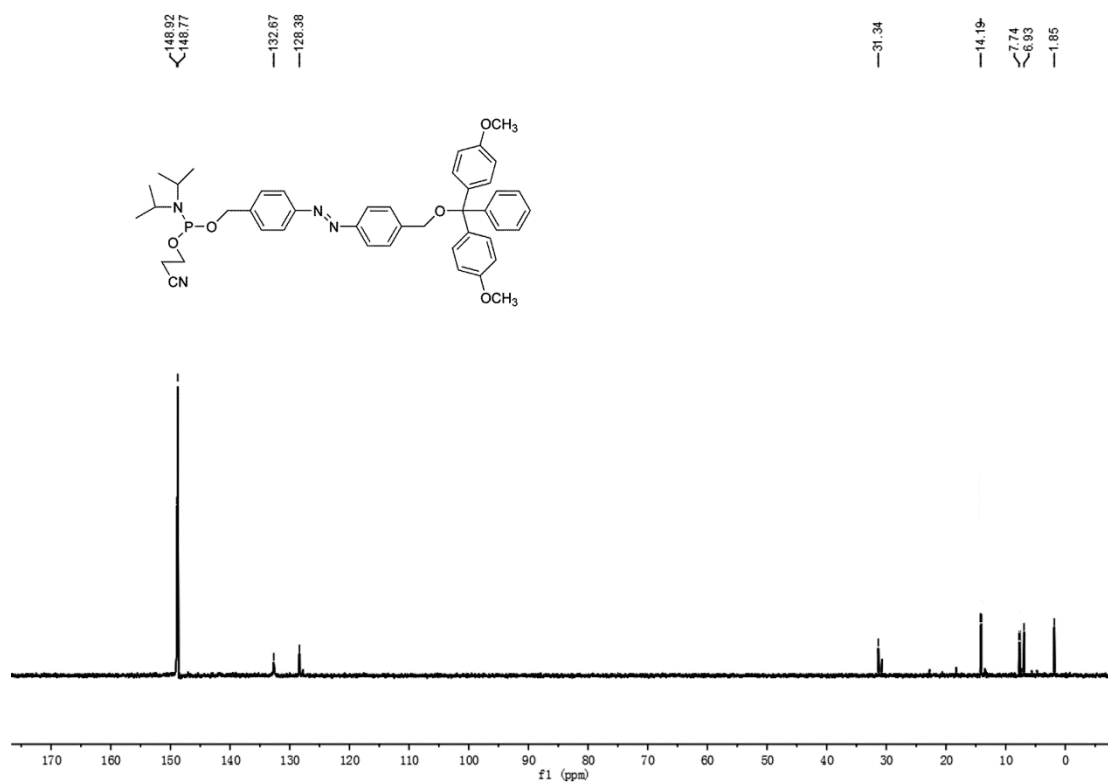
Az20-5	Lane1	Lane2	Lane3	Lane4	Lane5	Lane6	Lane7	Lane8	Lane9	Lane10
UV	–	–	–	–	–	+	+	+	+	+
Uncleaved	100	96.2	94.2	65.8	55.2	100	98.1	65.9	31.2	34.4
RNA (%)										
Cleaved	0	1.7	2.6	12.9	13.3	0	1.9	8.8	18.8	16.8
RNA (%)		2.1	3.2	21.3	31.5			25.3	50.0	48.8
Az20-6	Lane1	Lane2	Lane3	Lane4	Lane5	Lane6	Lane7	Lane8	Lane9	Lane10
UV	–	–	–	–	–	+	+	+	+	+
Uncleaved	100	98.8	96.6	95.1	82.6	100	96.0	75.9	64.0	62.8
RNA (%)										
Cleaved	0	0.8	1.9	2.4	8.9	0	2.3	12.7	19.8	20.3
RNA (%)		0.4	1.5	2.5	8.5		1.7	11.4	16.2	16.9

6. Characterization of all azobenzene derivatives

Figure S10. ^1H NMR, ^{13}C NMR and ^{31}P NMR of azobenzene derivatives







³¹P spectra of 2-Cyanoethyl-4-O-[[4-(4,4'-dimethoxytrityl)-O-methyl-diazenyl]benzyl]-N,N'-diisopropylaminophosphoramidite

7. Characterization of all azobenzene linked dumbbell asODNs

Table S4. Values for ESI-MS of azobenzene linked dumbbell asODNs.

Name	Sequence	Calculated	Found
Az16-4	5'-CGTTazoAACGTTTCGGACCGTAazoTACG-3'	7952.31	7954.2
Az16-5	5'-ACGTTazoAACGTTTCGGACCGTAazoTACGG-3'	8594.73	8596.5
Az16-6	5'-AACGTTazoAACGTTTCGGACCGTAazoTACGGT-3'	9212.14	9213.0
Az16-7	5'-AAACGTTazoAACGTTTCGGACCGTAazoTACGGTC-3'	9814.53	9819.9
Az18-4	5'-GTTGazoCAACGTTTCGGACCGTATazoATAC-3'	8569.72	8571.4
Az18-5	5'-CGTTGazoCAACGTTTCGGACCGTATazoATACG-3'	9188.11	9190.1
Az18-6	5'-ACGTTGazoCAACGTTTCGGACCGTATazoATACGG-3'	9830.53	9832.1
Az18-7	5'-AACGTTGazoCAACGTTTCGGACCGTATazoATACGGT-3'	10447.94	10450.2
Az20-4	5'-TTGGazoCCAACGTTTCGGACCGTATTazoAATA-3'	9187.13	9188.2
Az20-5	5'-GTTGGazoCCAACGTTTCGGACCGTATTazoAATAC-3'	9805.52	9807.1
Az20-6	5'-CGTTGGazoCCAACGTTTCGGACCGTATTazoAATACG-3'	10423.91	10426.1

Figure S11. ESI-MS spectra of azobenzene linked dumbbell asODNs.

