MATERIALS AND EXPERIMENTS

Materials. Phosphoramidite monomers (A, G, C, T), CPG columns, reagents for DNA synthesis, and Poly-Pak cartridges were obtained from Glen Research. Other reagents necessary for the syntheses of base surrogates were purchased from Tokyo Chemical Industry, Wako, and Aldrich. Unmodified DNA was purchased from Integrated DNA Technologies.

Syntheses of probes. All the fluorescent probes were synthesized on an ABI 3400 DNA/RNA Synthesizer using phosphoramidite monomers carrying natural nucleobases or **F**, **L**, **Q**, or **gQ**. Syntheses of phosphoramidite monomers **F**, **L**, and **Q** were reported previously.[1][2] After work-up, probes were purified by reversed-phase high-performance liquid chromatography (HPLC) and characterized using MALDI-TOF mass spectroscopy (Autoflex II, Bruker Daltonics). Mass spectrometry data are shown in the appendix.

Spectroscopic measurements. Fluorescence spectra were measured in microcells on a JASCO model FP-8500 equipped with a programmable temperature controller. Excitation wavelengths were 440 nm for fluorophore **F** and 455 nm for fluorophore **L**. In order to monitor emission at equilibrium, the fluorescence spectrum was measured after heating to 80 ^{o}C followed by a gradual decrease (4 ^{o}C min^{-1}) of temperature to 20 ^{o}C in TBM (Tris-Borate Magnesium) buffer, pH 7.0 with 10, 100, or 1000 mM $MgCl_2$). UV/Vis spectra were obtained with a Shimadzu UV-1800 by measuring absorbance spectra at 20 ^{o}C.

Measurement of melting temperature (T_m). Melting curves were obtained with a Shimadzu UV-1800 by measuring the change of absorbance at 260 nm versus temperature. The T_m was determined from the maximum in the second derivative of the melting curve for Hoogsteen base pairs and in first derivative for the Watson-Crick base pairs. Both the heating and cooling curves were measured, and the reported T_m value is the average. The temperature ramp was 0.5 ^{o}C/min. Solution conditions were 1.0 µM TFO linear probe, 1.0 µM DNA-A or AR-as, 1.1 µM DNA-T or AR-s, 90 mM TBM buffer pH 7.0 with 10, 100, or 1000 mM $MgCl_2$. It should be noted that addition of excess DNA-T ensures all DNA-A strands form duplex, which avoids the unintended hybridization to single stranded DNA-A (linear probe/DNA-A) while the detection.

Plasmid construction. The multiple cloning site in pcDNA 4/TO/myc-His C[3] was cut with restriction enzyme Xho I. The self-complementary insert (5'-TCGAAAAGAAGAAAAGAGAGAA-3'), which includes the target binding region and which is complementary to the ends resulting from Xho I cleavage was ligated in the plasmid using T4 DNA ligase. This yielded plasmids with various numbers of the repeat sequence. The newly constructed plasmids were transformed into competent *E. coli*, colonies were selected, amplified, and sequenced. Sequences of relevant regions of each plasmid are shown in Table S2 and the appendix.

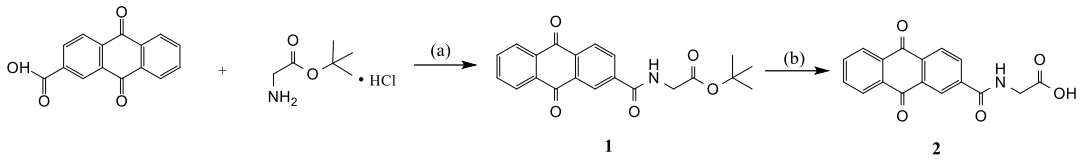
Primer design and PCR. Primers were designed to hybridize near the target region. The predicted melting temperature was 69 °C as determined using the NEB T_m Calculator. The forward primer sequence was 5'-GCTCCCAGTAGGAATTCTGCAGATATCC-3', and the reverse primer sequence was 5'-CATACCGGTCATCATCACCATCACCAT-3'. For amplification, the protocol for Phusion® High-Fidelity DNA Polymerase (M0530) from NEW ENGLAND BioLabs was used. The reaction volume was 50 µL. The expected length of products for plasmids 0, 2, and 5 were 124, 168, and 232 base pairs, respectively. The general PCR program was as follows: Initial Denaturation (98 °C, 30 s)→[Denaturation (98 °C, 10 s)→Renaturation (69 °C, 30 s)→Extension (72 °C, product 0: 4 s, product 2: 5 s, product 5: 6 s)]*n(Cycles)→Final extension (72 °C, 600 s)→Cooling (4 °C, hold).For the real-time PCR reaction the program was as follows: Initial Denaturation (98 °C, 30 s)→[Denaturation (98 °C, 30 s)→Renaturation (37 °C, 60 s, fluorescence measurement point)→Extension (72 °C, product 2: 5 s, product 5: 6 s)]*n(Cycles)→Final extension (72 °C, 600 s)→Cooling (37 °C, hold). The amplification curves of qPCR were obtained with Roche LightCycler® 96 Instrument by measuring fluorescence emission at 514 nm. Excitation wavelength was 470 nm. Same PCR sample within probe solution (42.6 µM, 1.17 µl) and 5.0 µl glycerol was prepared before measurement.

Native PAGE. Probe **3L1gQ** (38.2 µM, 1.0 µl) and crude PCR product (8 µl) in 19 µl sterile water was mixed with 3.0 µl 10X loading buffer containing bromophenol blue (0.03%) and glycerol (60 vol%). Gels were stained with SYBR™ Gold and analyzed using a Typhoon FLA 9500 (GE Healthcare) by exciting with a 473-nm laser. On non-denaturing gels, we could detect perylene emission from the high-order complex (duplex or triplex).

[1] H. Kashida, N. Kondo, K. Sekiguchi, H. Asanuma, *Chem. Commun.* **2011**, *47*, 6404–6406.
[2] H. Asanuma, H. Hayashi, J. Zhao, X. Liang, A. Yamazawa, T. Kuramochi, D. Matsunaga, Y. Aiba, H. Kashida, M. Komiyama, *Chem. Commun.* **2006**, 5062-5064
[3] H. Asanuma, M. Akahane, R. Niwa, H. Kashida, Y. Kamiya, *Angew. Chemie Int. Ed.* **2015**, *54*, 4315–4319.

Supporting Figures and Tables (Scheme S1-S2, Fig. S1-S12, Tables S1-S3)

Synthesis of anthraquinone derivative: gQ phosphoramidite monomer



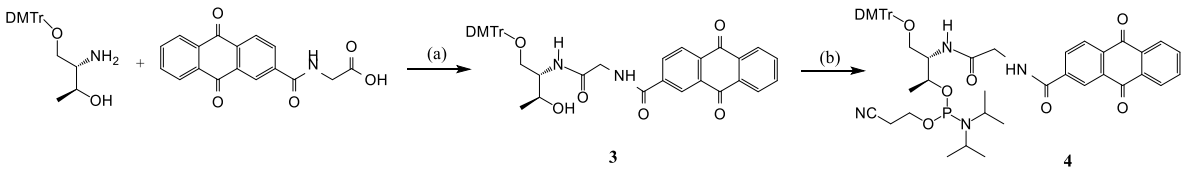
Scheme S1. Synthesis of anthraquinone with glycine linker, **gQ**. Reagent and conditions: a) EDC, HOBt, in DMF and Et_3N, room temperature 3 h, 70.6%; b) triethylsilane, TFA, in CH_2Cl_2, room temperature, 3 h, 89.0%.

a) Synthesis of compound **1**

Glycine tert-butyl ester hydrochloride (0.84 g, 5.0 mmol, 1.0 eq) dissolved in 20 mL DMF was added dropwise to Et_3N (2.4 ml, 17.5 mmol, 3.5 eq) and stirred. Next, 1-hydroxybenzotriazole (1.22 g, 8.0 mmol, 1.6 eq), anthraquinone-2-carboxylic acid (1.26 g, 5.0 mmol, 1.0 eq), and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (1.53 g, 8.0 mmol, 1.6 eq) in 15 ml DMF were added, and the solution was stirred for 3-4 hours. The product was extracted with AcOEt and washed twice with aqueous $NaHCO_3$. The solvent was removed by evaporation to afford compound **1** (1.3 g, 3.5 mmol, yield: 70%). ^{1}H-NMR [$CDCl_3$, 500 MHz] δ= 8.63 (d, *J*= 1.5 Hz, 1H), 8.35 (d, *J*= 8.0 Hz, 1H), 8.28 (m, 3H), 7.81 (m, 2H), 7.03 (t, *J*= 4.5 Hz,1H), 4.20 (d, *J*= 5.0 Hz, 2H), 1.52 (s, 9H). ^{13}C-NMR [CDCl3, 125 MHz] δ = 182.4, 182.3, 168.9, 165.5, 138.8, 135.3, 134.42, 134.41, 133.5, 133.4, 133.0, 127.9, 127.42, 127.38, 125.3, 82.8, 42.7, 28.1. HRMS (FAB) Calcd for $C_{21}H_{19}NO_5$ (M) 365.1297; found 366.1270.

b) Synthesis of compound **2**

To compound **1** (1.29 g, 3.5 mmol, 1.0 eq) dissolved in 10 ml CH_2Cl_2 was added Et_3N (1.40 ml, 8.8 mmol, 2.5 eq), the solution was stirred, and 20 mL TFA was added. After 4 hours with stirring, the solvent was removed by evaporation, and the product was dissolved in diethyl ether and isolated by filtration. Removal of the solvent by vacuum evaporation, yielded compound **2** (0.95 g, 3.1 mmol, yield: 89%). ^{1}H-NMR [DMSO, 500 MHz] δ = 9.35 (t, *J*= 6.0 Hz, 1H), 8.67 (s, 1H), 8.35 (dd, J_1= 1.5 Hz, J_2= 8.0 Hz, 1H), 8.30(d, *J*= 8.0 Hz, 1H), 8.25 (m, 2H), 7.95 (m, 2H), 4.00 (d, *J*= 6.0 Hz, 2H). ^{13}C-NMR [DMSO, 125 MHz] δ = 182.6, 182.5, 171.5, 165.4, 139.0, 135.1, 133.6, 133.5, 133.4, 133.3, 129.3, 128.7, 127.3, 127.0, 126.0, 125.8, 41.8. HRMS (FAB) Calcd for $C_{17}H_{11}NO_5$ ($M+H^+$) 310.0715; found 310.0662.



Scheme S2. Synthesis of **gQ** on D-threoninol scaffold. Reagent and conditions: a) DCC, HOBt, in DMF, room temperature overnight, 99%; b) $(iPr)_2NP(Cl)(OCH_2CH_2CN)$, Et_3N, CH_2Cl_2, N_2 0 °C → room temperature, 1 h, 56%.

c) Synthesis of compound **3**

Anthraquinone-2-carboxylic acid (0.37 g, 1.2 mmol, 1.2 eq) was coupled with DMT-D-threoninol (1.00 mmol) in the presence of dicyclohexylcarbodiimide (0.25 g, 1.2 mmol) and 1-hydroxybenzotriazole (0.18 g, 1.2 mmol) in 50 mL DMF. The reaction was stirred at room temperature overnight, and the product was extracted with 200 mL AcOEt and isolated by filtration. The product was washed twice with saturated aqueous $NaHCO_3$ and twice with saturated aqueous NaCl to remove unreacted reagents, and dried over $MgSO_4$. The solvent was removed by evaporation, and the product was purified by silica gel column chromatography ($CHCl_3$: Et_3N = 100:3 -> $CHCl_3$: CH_3OH : Et_3N = 95:5:3) to afford **3** (0.99 mmol, yield 99%). ^{1}H-NMR [$CDCl_3$, 500 MHz] δ= 8.65 (d, *J*= 1.0 Hz, 1H), 8.27-8.19(m, 4H), 8.00(t, *J*= 5.0 Hz,1H), 7.77 (tdd, J_1= 2.0 Hz, J_2= 7.5 Hz, J_3= 10.5 Hz, 2H), 7.37 (m, 2H), 7.25-7.20(m, 6H), 7.14-7.09 (m, 2H), 6.76 (dd, 4H), 4.35 (dd, J_1= 5.0 Hz, J_2= 16.5 Hz, 1H), 4.10-4.13(m, 2H), 4.06 (m, 1H), 3.71 (d, *J*= 0.5 Hz, 6H), 3.39 (dd, J_1= 5.0 Hz, J_2= 9.5 Hz, 1H), 3.28 (dd, J_1= 4.0 Hz, J_2= 9.5 Hz, 1H), 1.15 (d, *J*= 6.0 Hz, 3H). ^{13}C-NMR [$CDCl_3$, 125 MHz]δ=182.25, 182.24, 169.5, 165.8, 158.5, 144.4, 138.4, 135.6, 135.5, 135.1, 134.4, 134.3, 133.24, 133.22, 133.18, 133.05, 129.9, 128.0, 127.9, 127.6, 127.4, 127.2, 126.9, 125.8, 113.2, 86.5, 67.8, 64.1, 55.2, 54.6, 46.0, 44.0, 20.1, 9.91. HRMS (FAB) Calcd for $C_{42}H_{38}N_2O_8$(M) 698.2628. Found 697.4104

d) Synthesis of compound **4**

Et_3N (0.48 ml, 3.6 mmol, 5 eq) and 2-cyanoethyldiisopropylchlorophosphoramidite (0.32 ml, 1.4 mmol, 2 eq) were added to a solution of compound **3** (0.50 g, 0.72 mmol) in CH_2Cl_2 (3.0 ml) at 0 °C. The mixture was stirred 20 min at 0 °C and then for 40 min at room temperature under nitrogen. $CHCl_3$ was added to the reaction mixture, and the solvent was removed by evaporation. Silica gel column chromatography (AcOEt : Hexane: Et_3N = 33: 66: 3 ->50: 50: 3) afforded **4** (0.37 g, 0.40 mmol, yield 56%). ^{1}H-NMR [$CDCl_3$, 500 MHz] δ = 8.67 (m, 1H), 8.22-8.19 (m, 4H), 8.00-7.87 (m, 1H), 7.77 (m, 2H), 7.42 (m, 2H), 7.31-7.15 (m, 7H), 6.80-6.76 (m, 4H), 6.60-6.46 (m, 1H), 4.37 (m, 1H), 4.28-4.12 (m, 3H), 3.71-3.62 (m, 6H) , 3.57-3.41 (m, 4H), 3.34-3.16 (m, 2H) , 2.59-2.41 (m, 2H), 1.22 (m, 3H), 1.12-0.92 (m, 12H). ^{13}C-NMR [$CDCl_3$, 125 MHz] δ= 180.5, 180.3, 167.1, 167.0, 163.8, 156.6, 142.9, 142.8, 136.70, 136.65, 134.13, 134.05, 134.03, 133.3, 132.5, 131.6, 131.5, 131.4, 131.1, 131.0, 128.3, 128.22, 128.15, 126.4, 126.3, 126.0, 125.8, 125.47, 125.45, 125.0, 124.0, 123.9, 116.5, 116.2,

111.22, 111.20, 84.4, 84.3, 67.6, 67.5, 66.7, 66.6, 61.0, 60.6, 56.4, 56.3, 56.1, 56.0, 53.3, 52.8, 52.7, 51.7, 42.1, 42.0, 41.3, 41.2, 22.83, 22.77, 22.5, 22.4, 18.53, 18.48, 17.82, 17.80. ^{31}P-NMR [$CDCl_3$, 121 MHz] δ = 148.0, 147.6. HRMS (FAB) Calcd for $C_{51}H_{55}N_4O_9(M+H^+)$ 899.3785; found 899.3034.

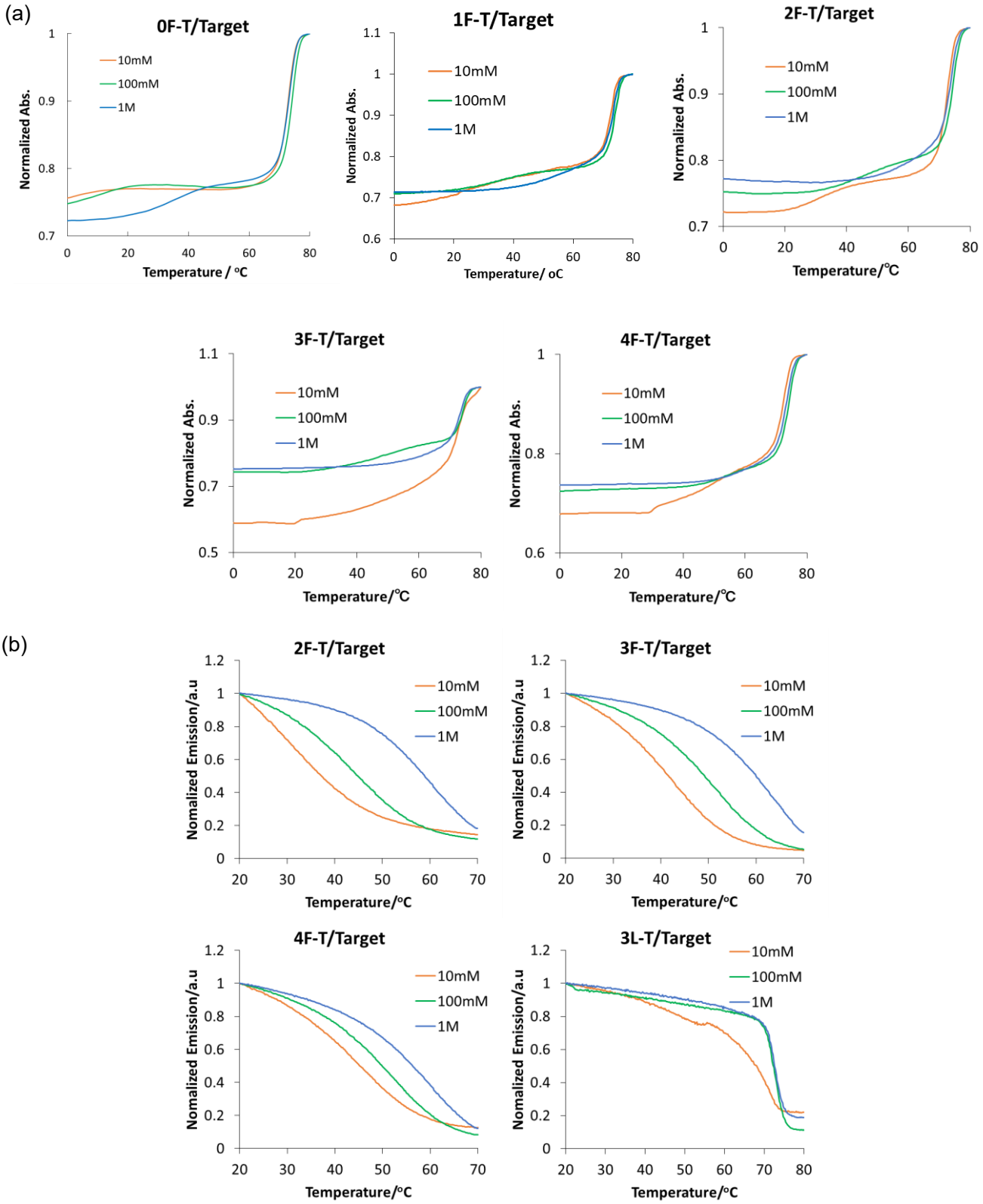


Figure S1. (a) UV-melting curves and (b) fluorescence-melting curves. Conditions: 1.0 μM indicated TFO linear probe and DNA-A, 1.1 μM DNA-T in 90 mM TBM buffer, pH 7.0 with 10 mM, 100 mM, or 1 M $MgCl_2$, 80 °C -indicated temperature °C, 0.5 °C / min. For **F**-type perylene, excitation wavelength: 440 nm. For **L**-type perylene, excitation wavelength: 455 nm.

Table S1. Calculated T_m values (°C) of the triplex formations

Linear probe	10mM	100mM	1M
0F-T	**n.d**	**n.d**	**35.8**
1F-T	**17.4**	**32.7**	**55.3**
2F-T	**32.5**	**44.5**	**59.6**
3F-T	**42.6**	**53.1**	**63.9**
4F-T	**44.4**	**52.1**	**60.1**
3L-T	**65[a]**	**72.7**	**72.7**
0L-X	**40.2**	**40.0**	**n.d**
3L-X	**n.d**	**62.7**	**60.7**
3L1gQ	**65.3**	**61.9**	**60.6**

a. The T_m value was estimated from intensity, because the melting curve did not show a clear transition.

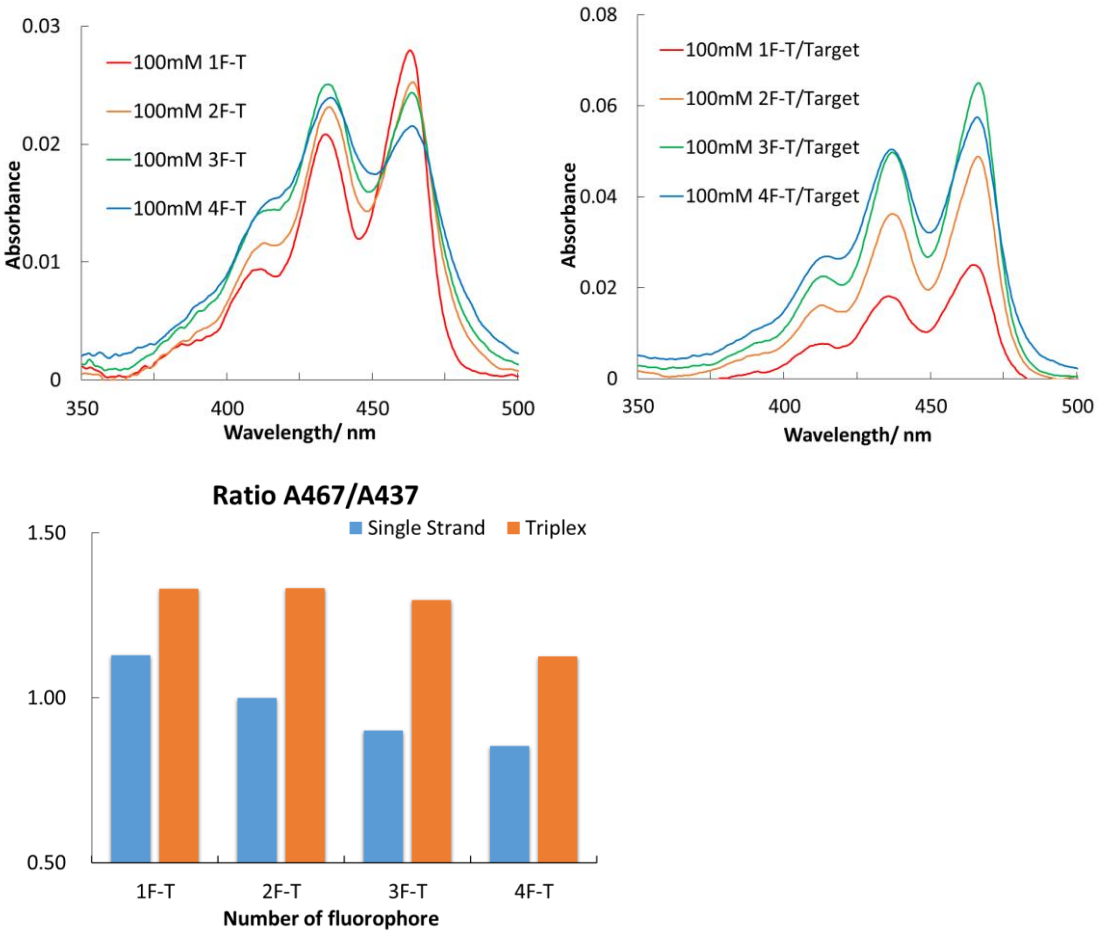


Figure S2. UV-Vis spectra of TFO linear probes. Conditions: 1.0 µM TFO linear probe, 1.0 µM DNA-A and 1.1 µM DNA-T in 90 mM TBM buffer, pH 7, 100 mM $MgCl_2$, 20 °C. In the absorption spectra, the A467/A437 ratio of the triplex was the same for triplexes with **1F-T**, **2F-T**, and **3F-T**, in which perylenes are separated by nucleobases via intercalation. The lower A467/A437 ratio in the triplex of **4F-T** suggests that interaction between perylenes in the triplex state suppressed emission intensity.

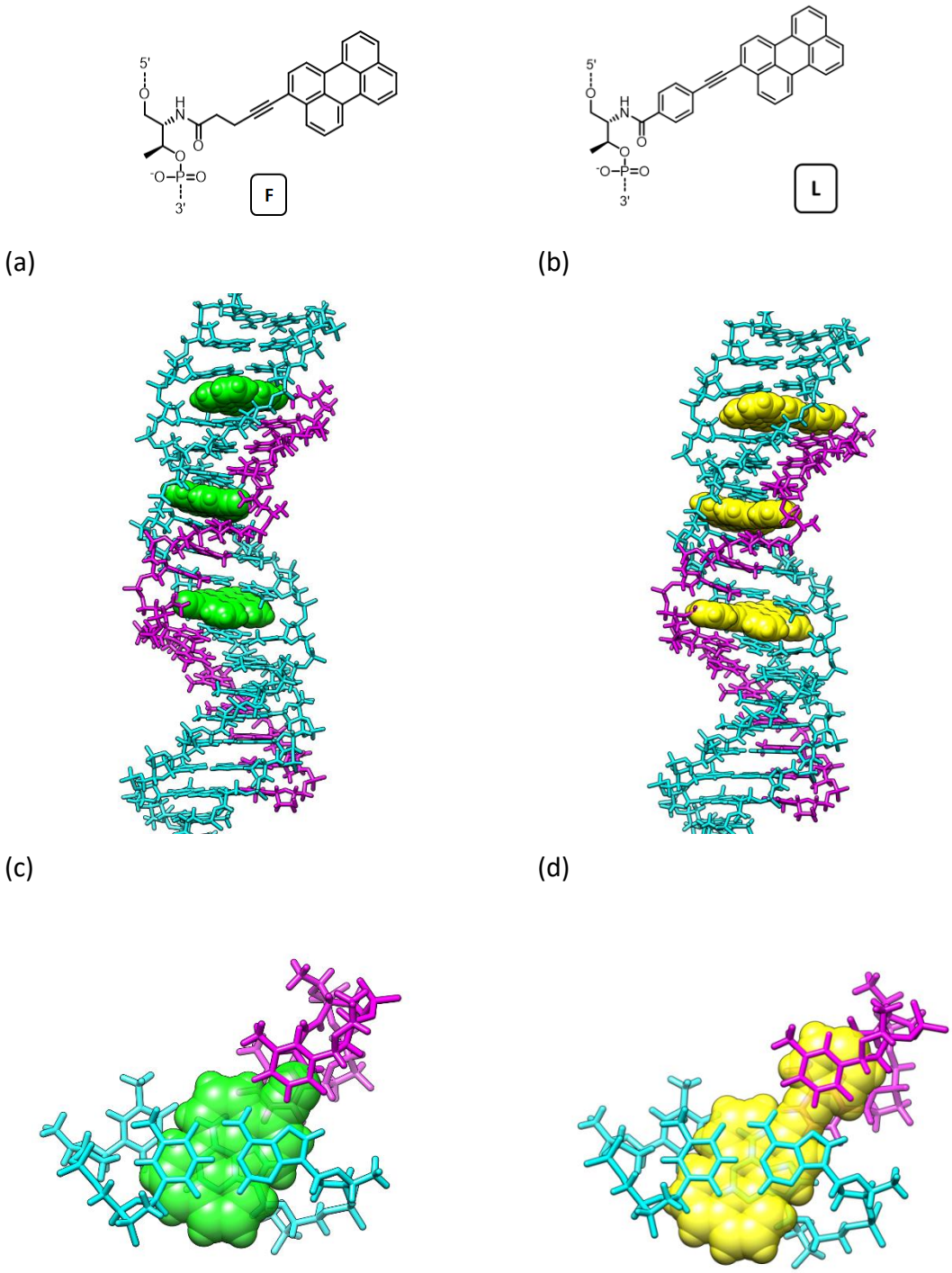
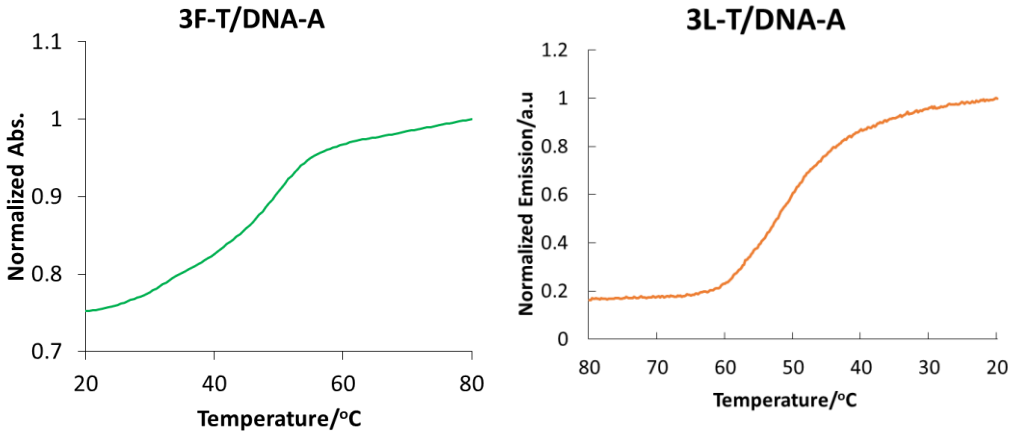
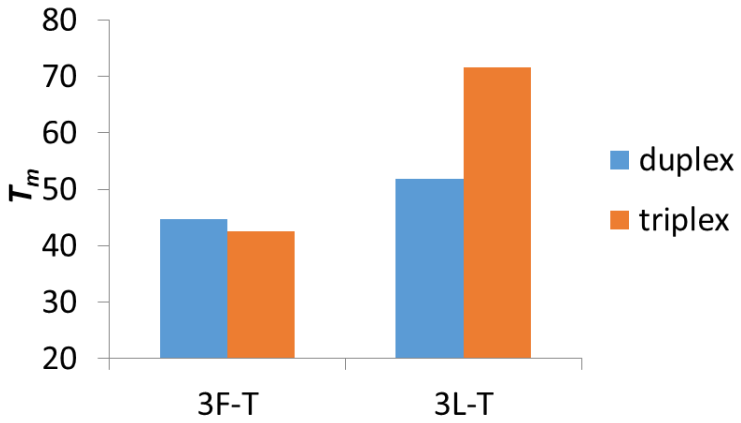


Figure S3. Energy minimized structures of triplexes of (a) **3F-T**/dsDNA and (b) **3L-T**/dsDNA. (c and d) Enlarged views of the structure in a and b. The dsDNA, probe strand, and perylenes are presented as cyan, magenta, and green/yellow respectively.



(a)



(b)

Figure S4. (a) UV-melting curve of **3F-T**/DNA-A and fluorescence-melting curve **3L-T**/DNA-A. (b) T_m comparison between triplex and linear probe/DNA-A duplex. Conditions: 1.0 μM TFO linear probe, 1.0 μM DNA-A, and 1.1 μM DNA-T in 90 mM TBM buffer, pH 7.0, 10 mM $MgCl_2$. T_m values: **3F-T**/DNA-A, 44.8 °C; **3F-T**/Target duplex, 42.6 °C; **3L-T**/DNA-A, 51.8 °C; **3L-T**/Target duplex, 72.7 °C. Excitation wavelengths were 440 nm for **F**-type perylene and 455 nm for **L**-type perylene.

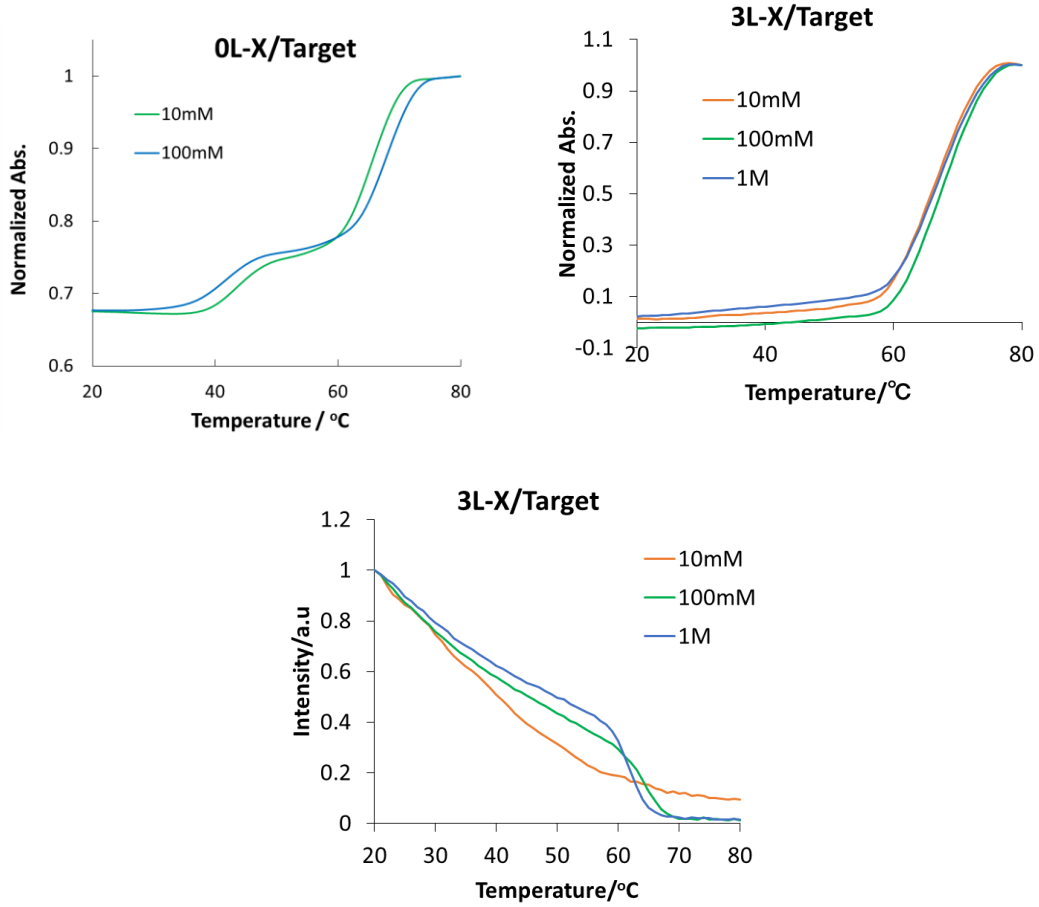


Figure S5. UV-melting and Fluorescence-melting curves. Conditions: 1.0 µM TFO linear probes, 1.0 µM AR-as and 1.1 µM AR-s in 90 mM TBM buffer, pH 7.0 with indicated concentration of $MgCl_2$, 80-20 °C, 0.5 °C / min. Excitation wavelength: 455 nm.

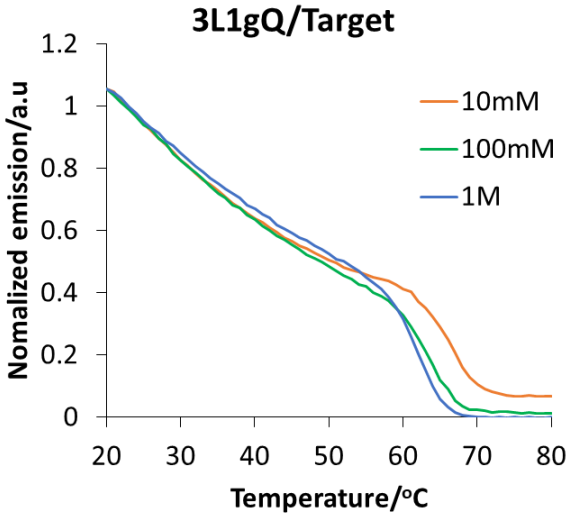


Figure S6. Fluorescence-melting curves of **3L1gQ**/Target duplex. Conditions: 1.0 µM TFO linear probe and AR-as, 1.1 µM AR-s in 90 mM TBM buffer, pH 7.0 with 10 mM, 100 mM, or 1 M $MgCl_2$, 80 °C -20 °C, 0.5 °C / min. Excitation wavelength: 455 nm

Table S2. Sequence design incorporating **Q**

Target DNA duplex		
Target	**DNA-A**	**5’-CGTCGGTTT-AAAAAAAAAAAAA-TTTCGTGGC-3’**
DNA duplex	**DNA-T**	**3’-GCAGCCAAA-TTTTTTTTTTTTT-AAAGCACCG-5’**
Probes	**3F-T**	5’-FTTTFTTTFTTTTTTT-3’
	1Q3F	5’-QTTTFTTTFTTTFTTTT-3’
	2Q3F	5’-QTTTFTTTFTTTFTTTTQ-3’
	2Q3F-2	5’-QTTTFTTTFTTTFTTTQT-3’

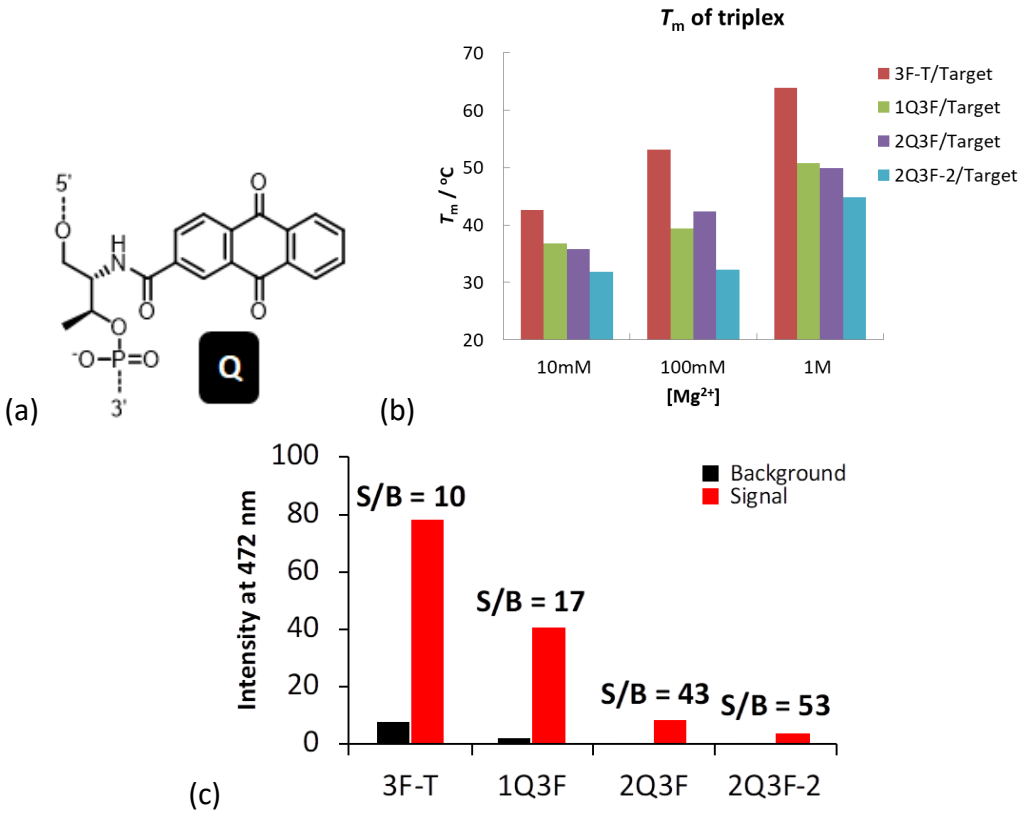


Figure S7 (a) Chemical structure of anthraquione **Q**. (b) *T_ms* of triplexes of TFO linear probes with target dsDNA. Conditions: 1.0 μM TFO linear probe, 1.0 μM DNA-A and 1.1 μM DNA-T in 90 mM TBM buffer, pH 7.0, and 10 mM, 100 mM, and 1 M $MgCl_2$. (c) Emission intensities at 472 nm and S/B ratios. Conditions: 1.0 μM TFO linear probe, 1.0 μM DNA-A and 1.1 μM DNA-T in 90 mM TBM buffer, pH 7.0, 100 mM $MgCl_2$, 20 °C. Excitation wavelength: 440 nm.

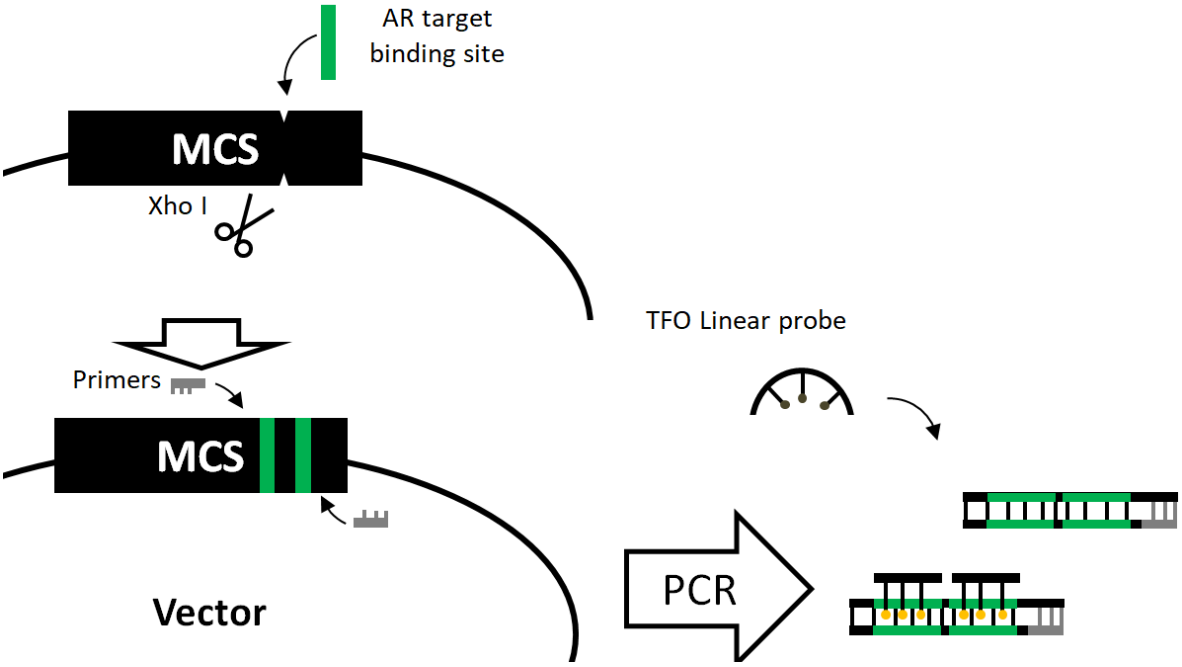


Figure S8. Scheme of plasmid construction.

Table S3. Sequence of PCR products containing *AR* gene sequence. Underlining indicates Xho I restriction site; sequence in red font is *AR* gene sequence.

Product	Sequence
Product 0	…5’-CAGTGGCGGCCGCTCGAGGTCACCCA-3’…
Product 1	…5’-CAGTGGCGGCCGCTCGATTCTCTCTTTTCTTCTTTTCGAGGTCACCCA-3’…
Product 2	…5’-CAGTGGCGGCCGCTCGATTCTCTCTTTTCTTCTTTTCGATTCTCTCTTTTCTTCTTTTCGAGGTCACCCA-3’…
Product 5	…5’-CAGTGGCGGCCGCTCTTCTCTCTTTTCTTCTTTTCGAAAAGAAGAAAAGAGAGAATCGATTCTCTCTTTTCTTCTTTTCGAAAAGAAGAAAAGAGAGAATCGAAAAGAAGAAAAGAGAGAATCGAGGTCACCCA-3’…

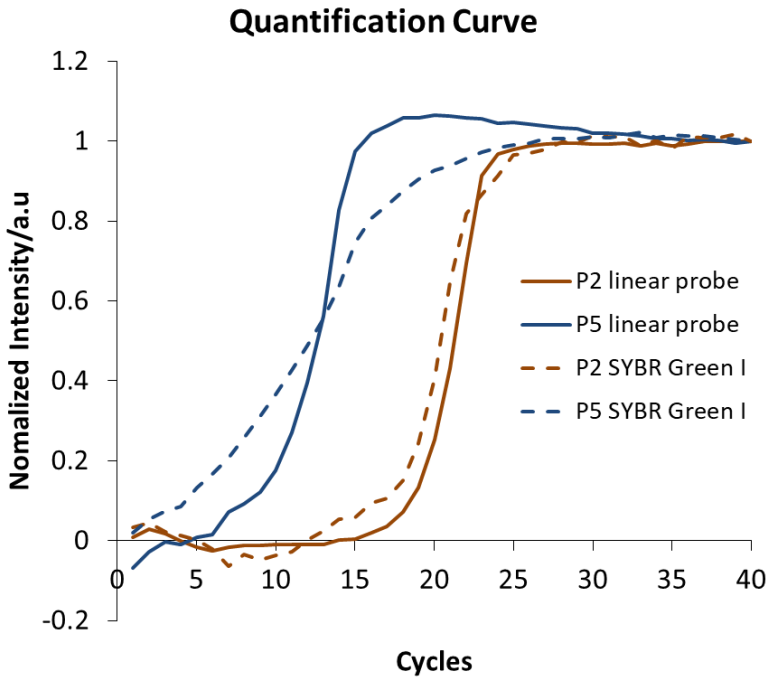


Figure S9. Amplification curves of real-time PCR. Conditions: 1 µM **3L1gQ** or SYBR green I, 5 µL glycerol in 50 µl PCR sample. Excitation wavelength: 470 nm, emission wavelength: 514 nm.

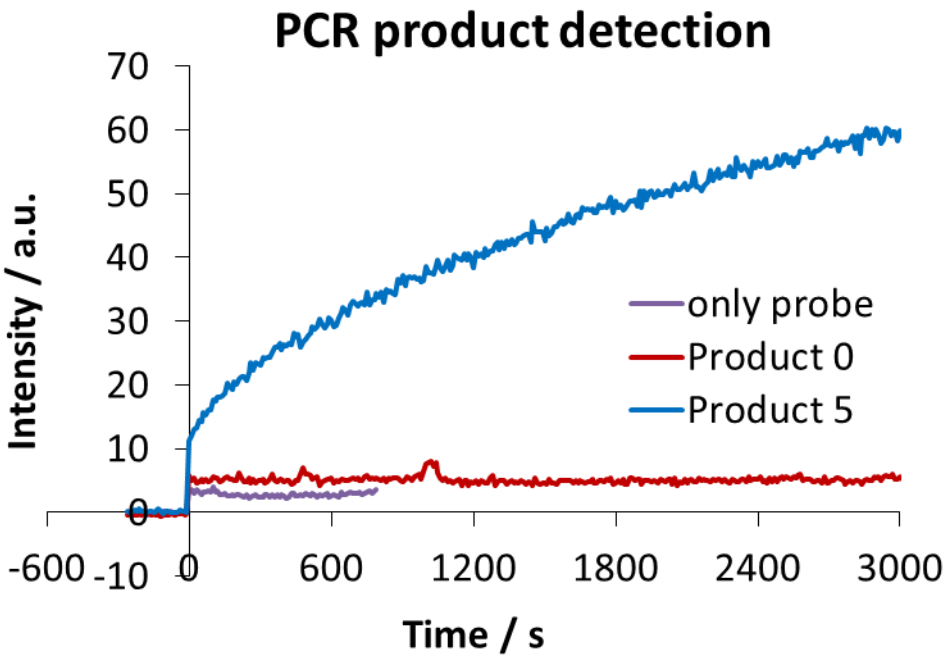


Figure S10. Fluorescence emission at 514 nm after **3L1gQ** was added to PCR product. Conditions: 100 nM **3L1gQ**, 20 nM purified product from amplification of plasmid 5 (100 nM target sites) or 20 nM purified product from amplification of plasmid 0. Conditions: 200 µL 90 mM TBM buffer, pH 7.0, 100 mM $MgCl_2$, 20 ^{o}C. Excitation wavelength: 455 nm, emission wavelength: 514 nm.

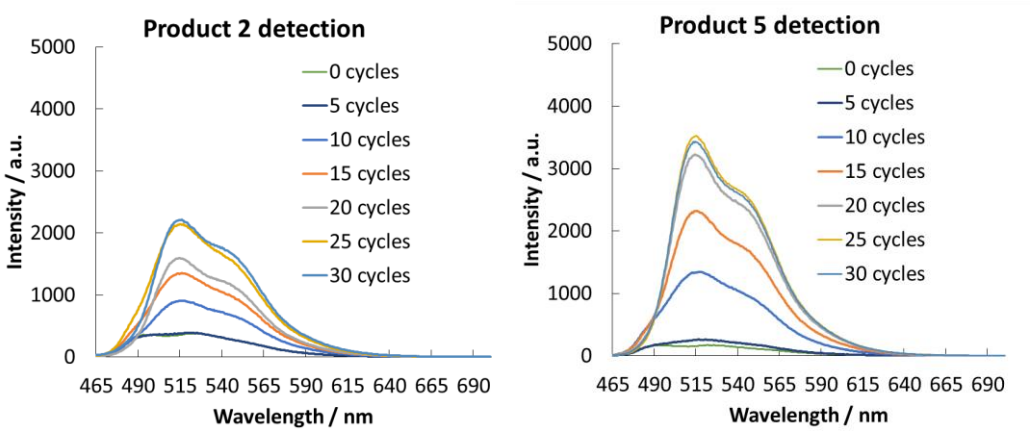


Figure S11. Fluorescence spectra of PCR products from amplification of plasmid 2 (left) and plasmid 5 (right) after indicated numbers of cycles. Conditions: 1.0 µM linear probe, 50 µL PCR product, 9 vol% glycerol in 150 µL 90 mM TBM buffer, pH 7.0 and 100 mM $MgCl_2$, 20 °C. Excitation wavelength: 455 nm.

Lane	1	2	3	4	5	6
Linear probe (3L1gQ)	-	+	-	+	-	+
product 0	+	+	-	-	-	-
product 5	-	-	+	+	-	-
product 2	-	-	-	-	+	+

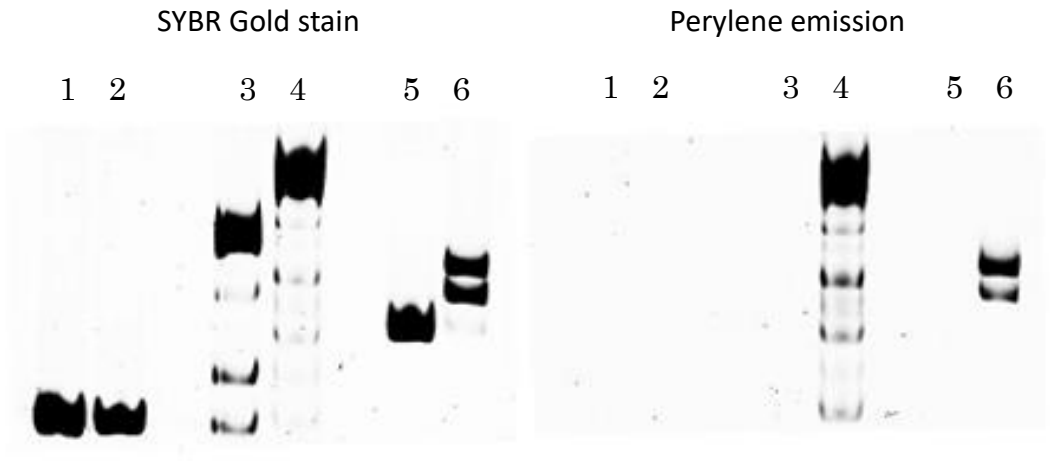


Figure S12. Native PAGE of PCR products. Fluorescence of perylene was recorded before staining by SYBR Gold.

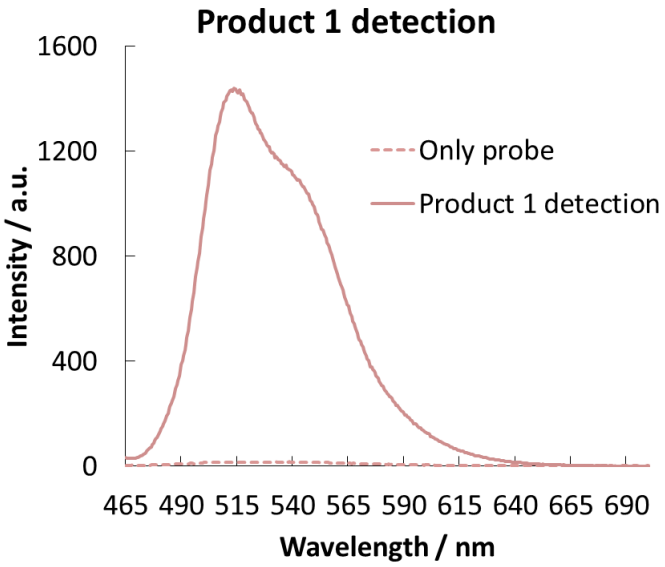


Figure S13. Direct fluorescence detection of dsDNA after PCR. Conditions: 1.0 μM **3L1gQ**, 50 μL crude PCR product, 9 vol% glycerol within 90 mM TB buffer, pH 7.0 with 100 mM $MgCl_2$ in a final volume of 200 μL, 20 °C. ex. = 455 nm, sensitivity: high.

Table S4. Sequence of mismatch *AR gene sequence*. (Red font indicates the MM site)

		Sequence
Probe	**3L1gQ**	5'-**L**TTT**L**CTT**L**CTTTTC**gQ**TCTCTT-3'
1MM	**1MM-ARas**	5'-CTACT-AAAGAAGAAAGGAGAGAA-GAATC-3'
	1MM-ARs	3'-GATGA-TTTCTTCTTTCCTCTCTT-CTTAG-5'
2MM	**2MM-ARas**	5'-CTACT-AAAGAAGAAGGGAGAGAA-GAATC-3'
	2MM-ARs	3'-GATGA-TTTCTTCTTCCCTCTCTT-CTTAG-5'

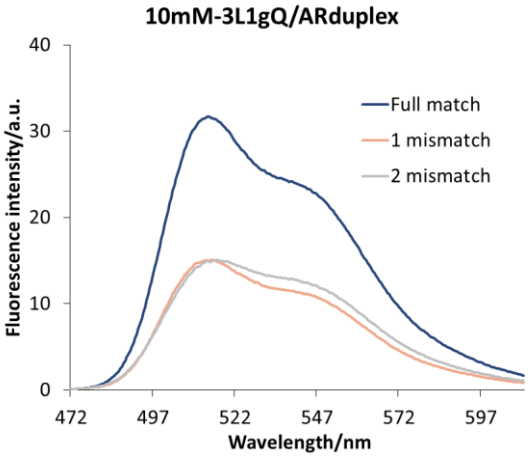
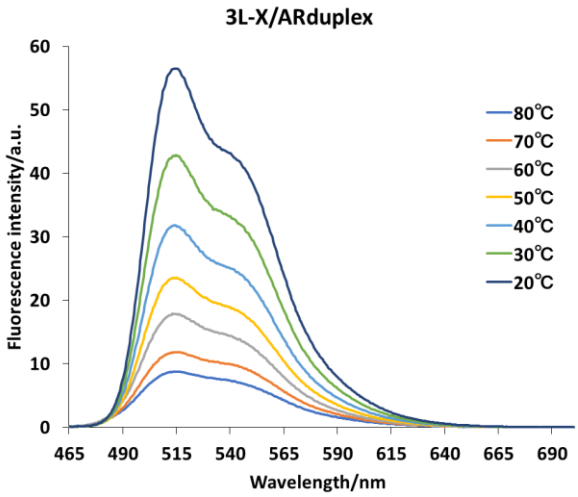


Figure S14. Fluorescence spectra while **3L1gQ** detecting full-match AR gene, 1MM-AR gene and 2MM-AR gene, respectively. Condition: 1.0 μM **3L1gQ** and 1μM indicated AR-as, 1.1μM indicated AR-s in 90 mM TBM buffer ($MgCl_2$= 10 mM), pH 7.0, ex.=455 nm, sensitivity: low.

(a)



(b)

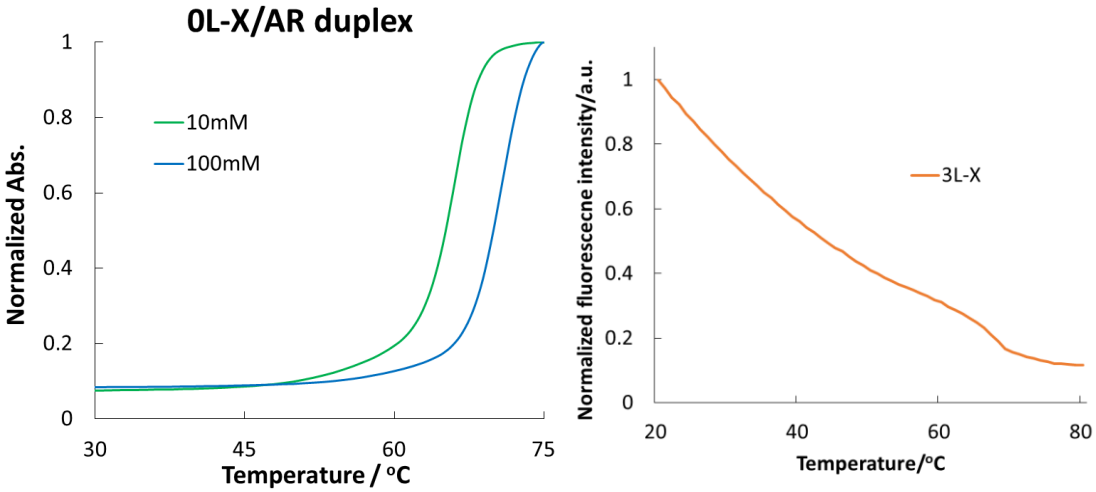


Figure S15. (a) Fluorescence spectra of **3L-X** under pH 5.5. (b)UV-melting curves of 0L-X/AR duplex (left) and fluorescence-melting curve (right) of **3L-X**/AR duplex in the MES buffer. Condition: 1.0 μM indicated linear probe and AR-as, 1.1 μM AR-s in 90 mM MES buffer, pH 5.5 with 100mM $MgCl_2$, 80-20 °C, 0.5 °C / min, sensitivity: low.

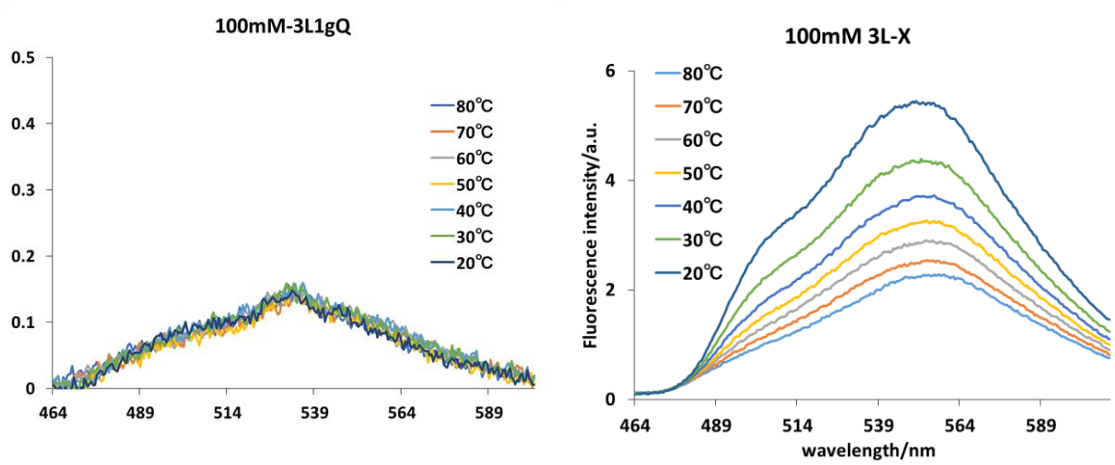


Figure S16. Fluorescence spectra of sing-stranded linear probe **3L1gQ** (left) and **3L-X** (right). Condition: 1.0 μM indicated linear probe in 90 mM TB buffer, pH 7.0 with 100 mM $MgCl_2$, ex.=455 nm, sensitivity: low.

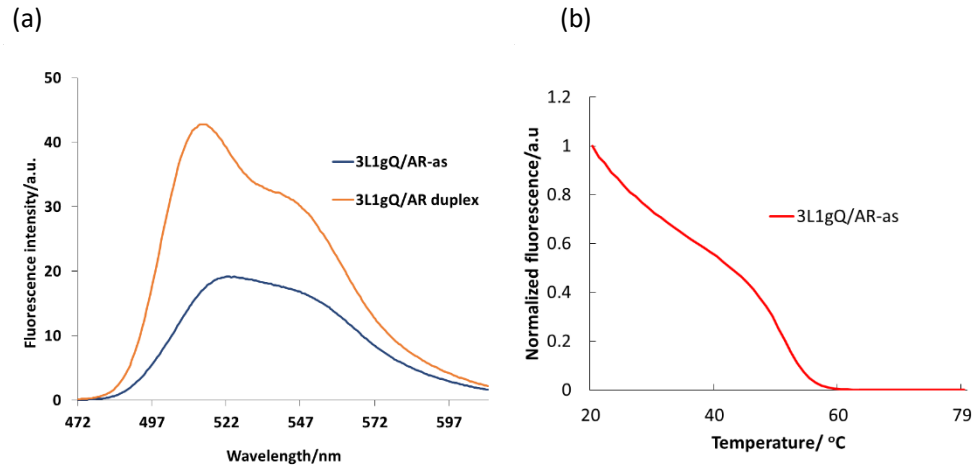
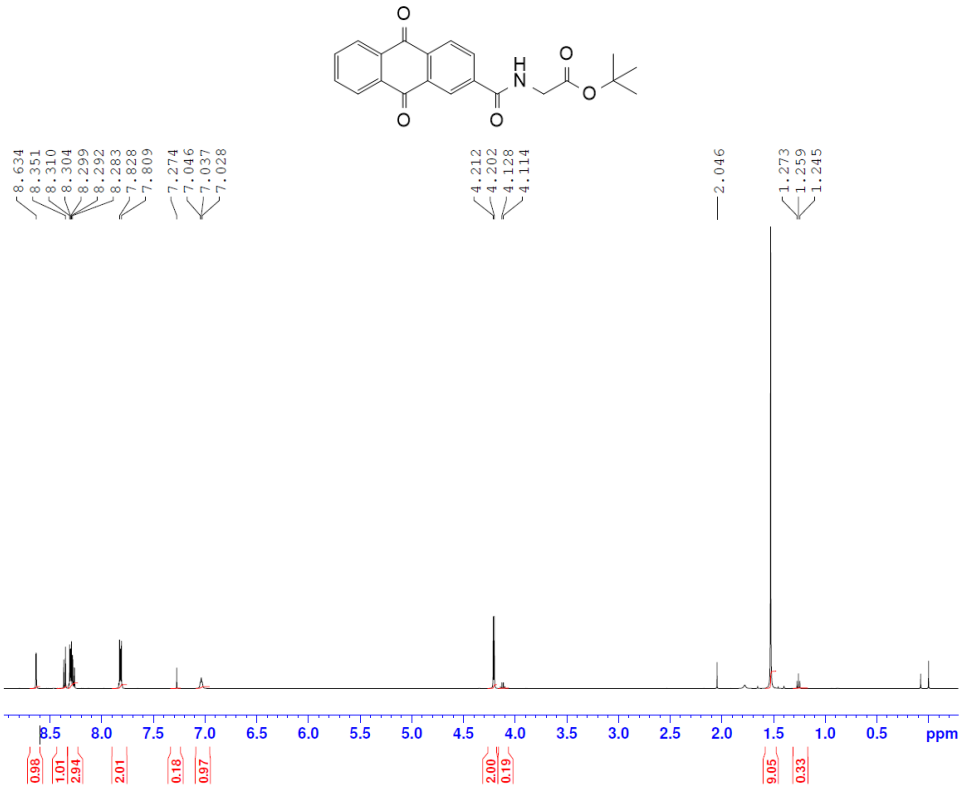


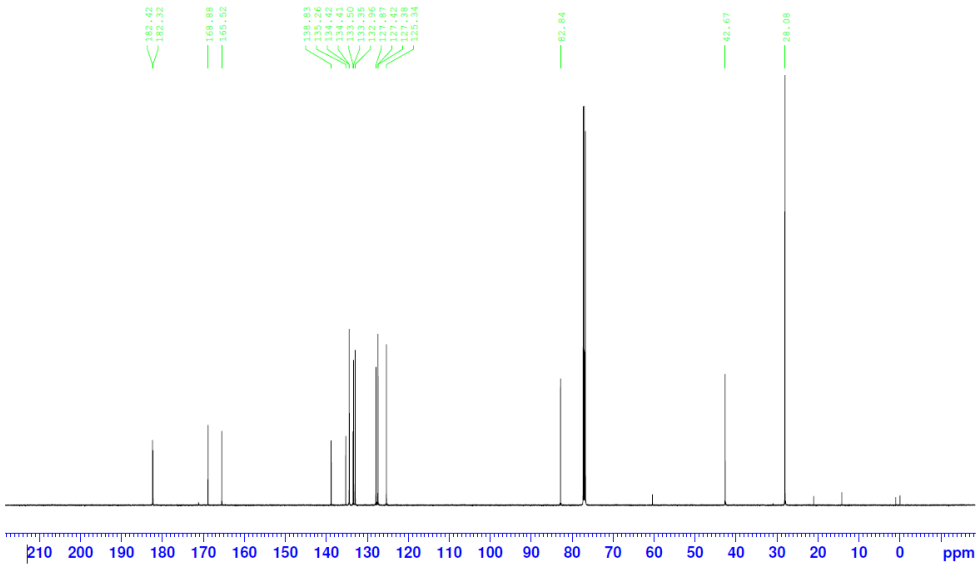
Figure S17. (a) Fluorescence spectra of indicated complex: 3L1gQ/AR-as (blue) and triplex: 3L1gQ/AR-as/AR-s (orange). (b) Fluorescence-melting curve of 3L1gQ/AR-as duplex. Condition: 1.0 μM **3L1gQ** and 1μM indicated AR-as, 1.1μM indicated AR-s in 90 mM TBM buffer ($MgCl_2$=100 mM), pH 7.0 with, ex.=455 nm, sensitivity: low.

3. Appendix

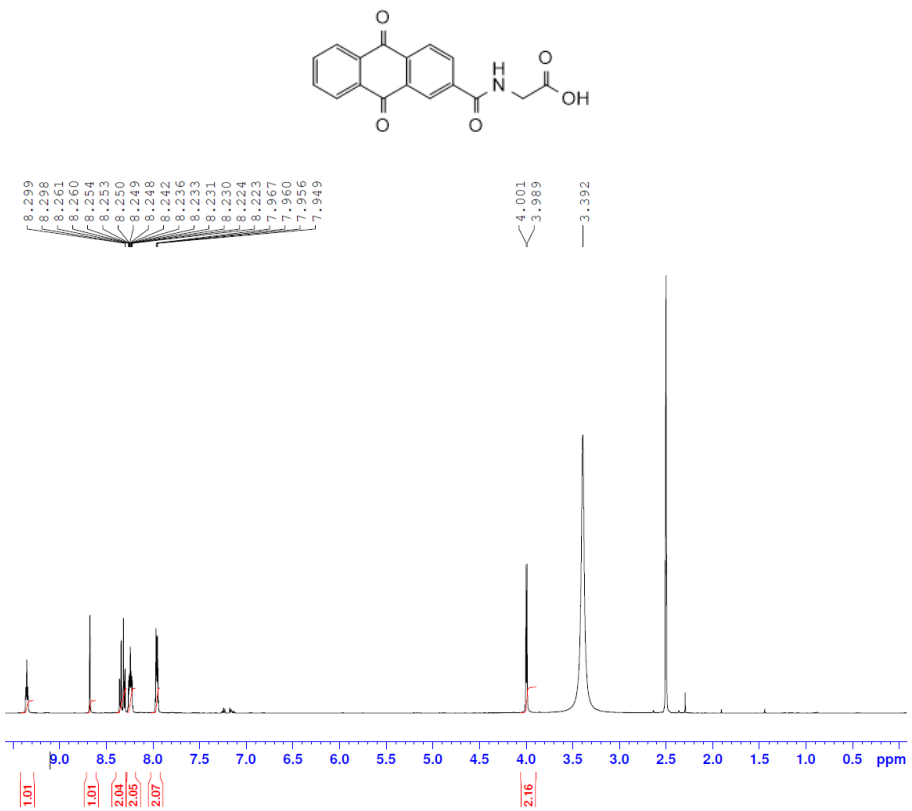
3-1 NMR spectra



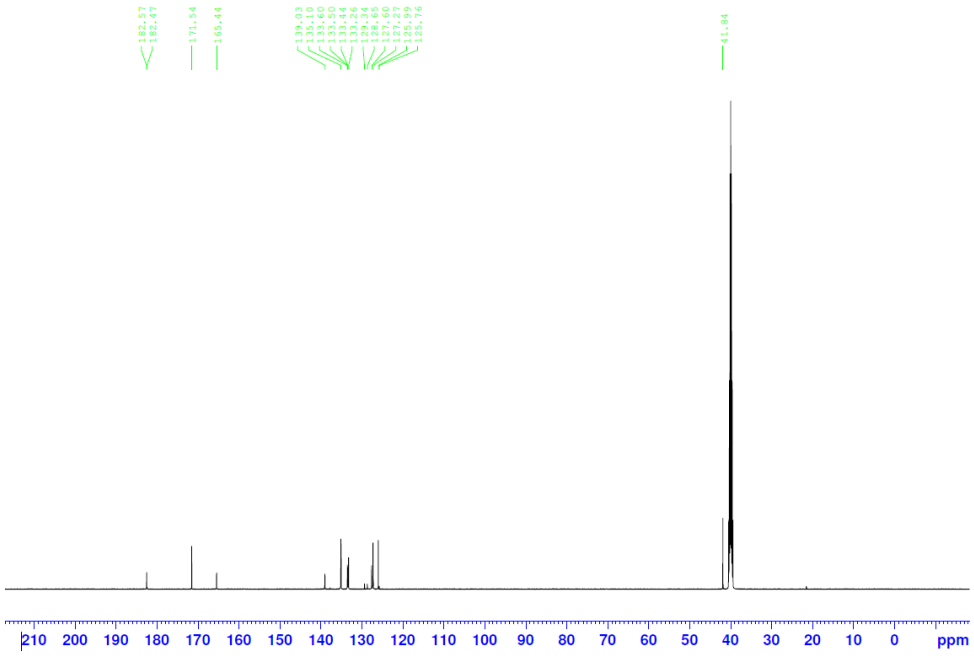
^{1}H-NMR spectrum of **1**



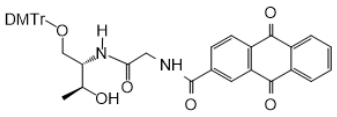
^{13}C-NMR spectrum of **1**

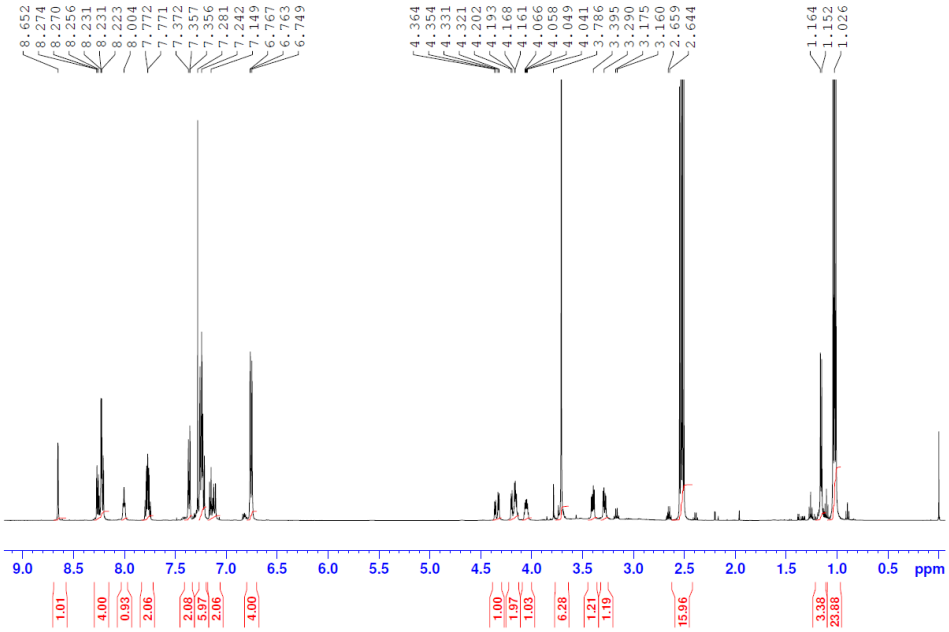


^{1}H-NMR spectrum of **2**

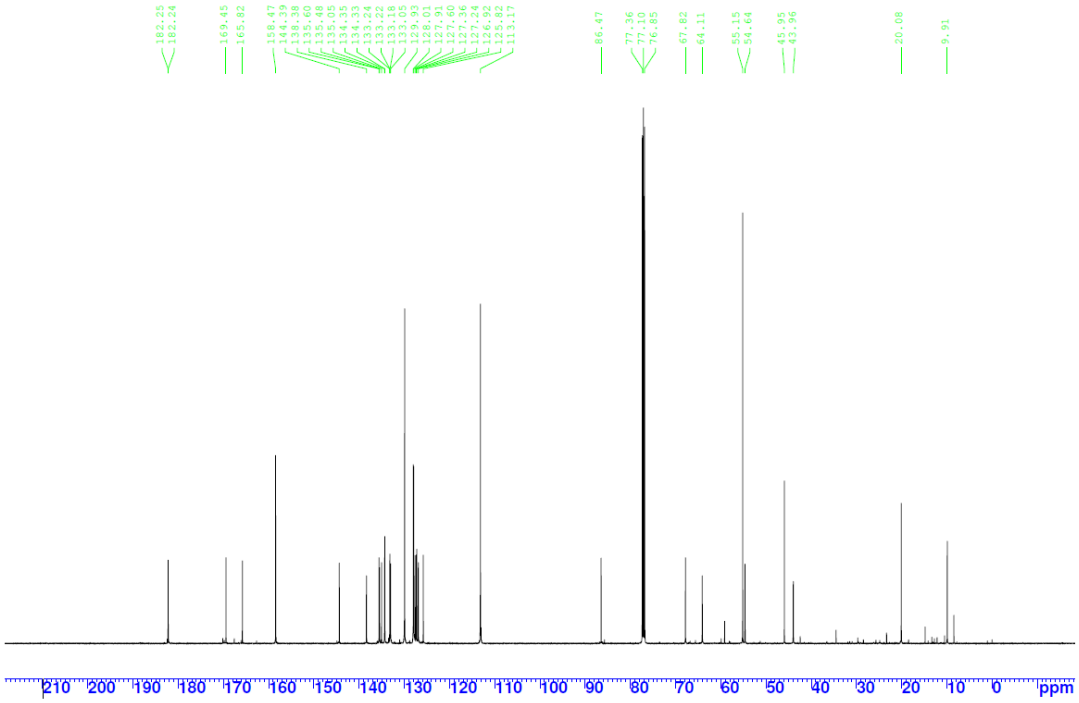


^{13}C-NMR spectrum of **2**

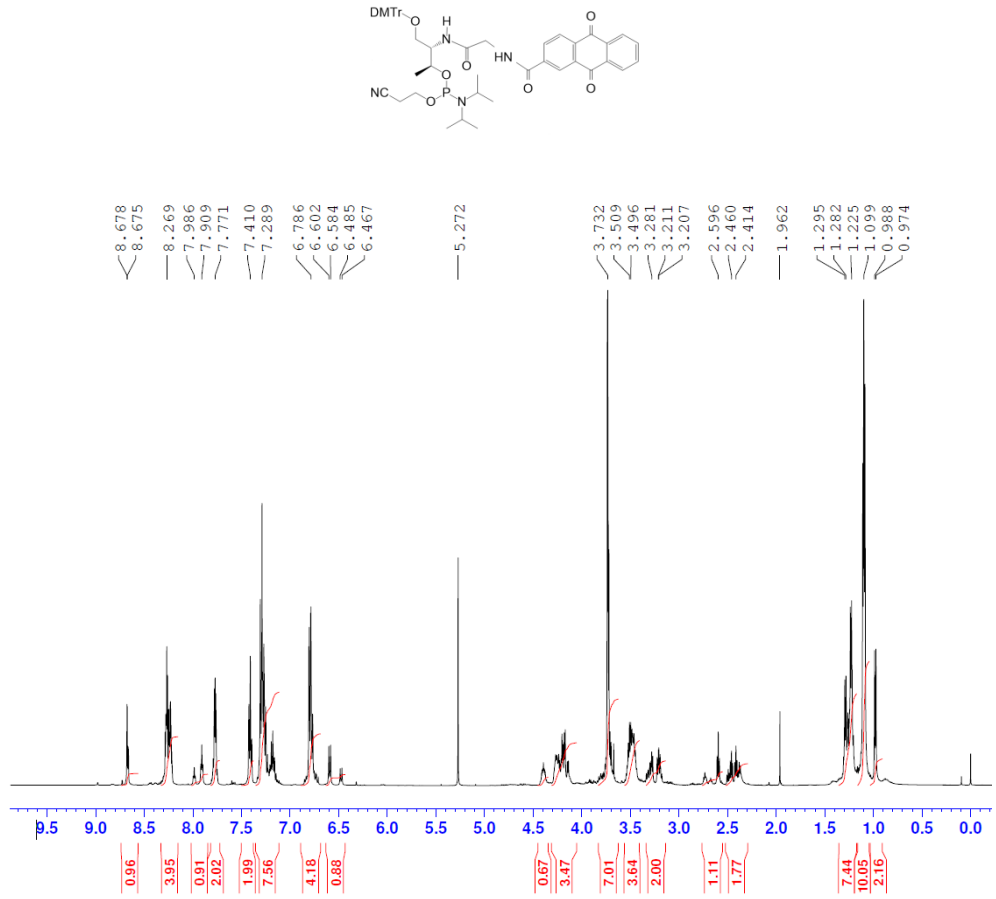




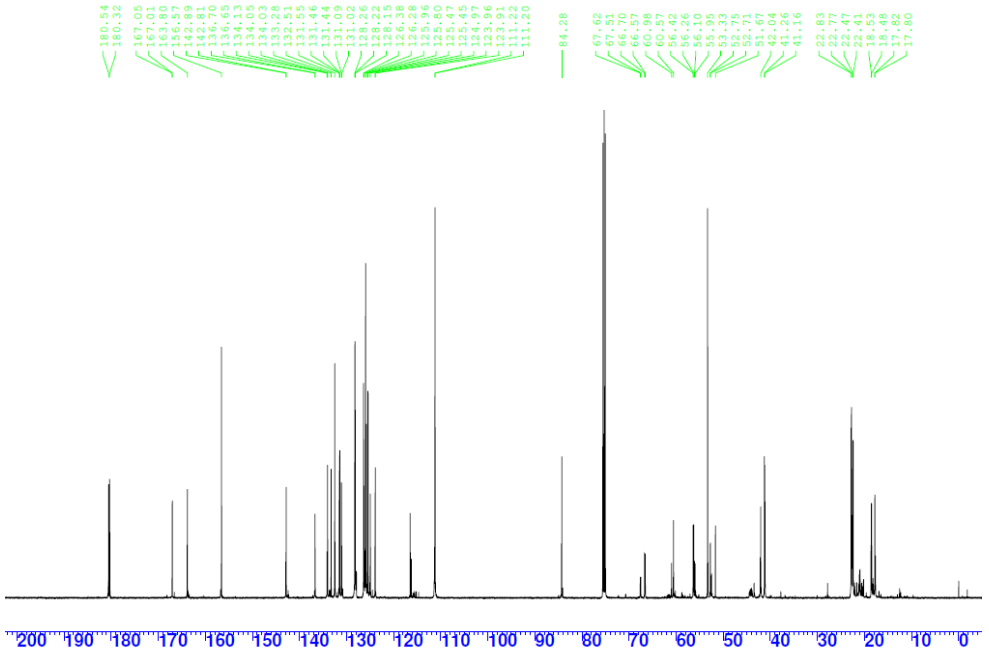
^{1}H-NMR spectrum of **3**



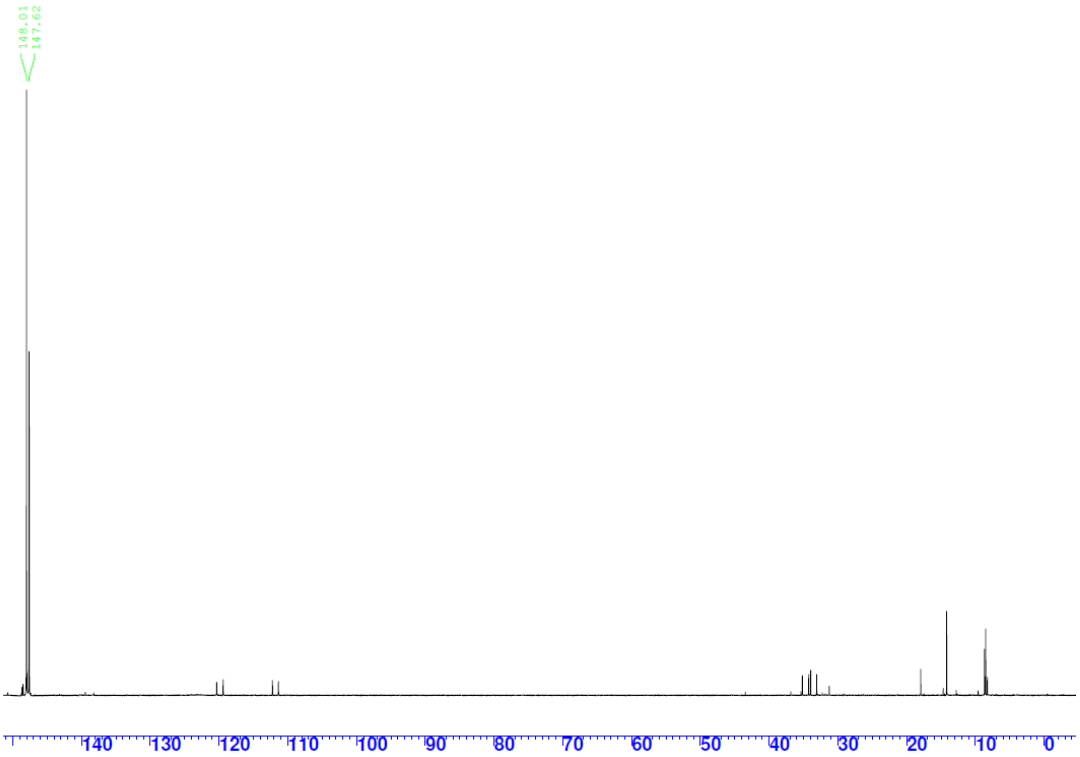
^{13}C-NMR spectrum of **3**



^{1}H-NMR spectrum of **4**



^{13}C-NMR spectrum of **4**

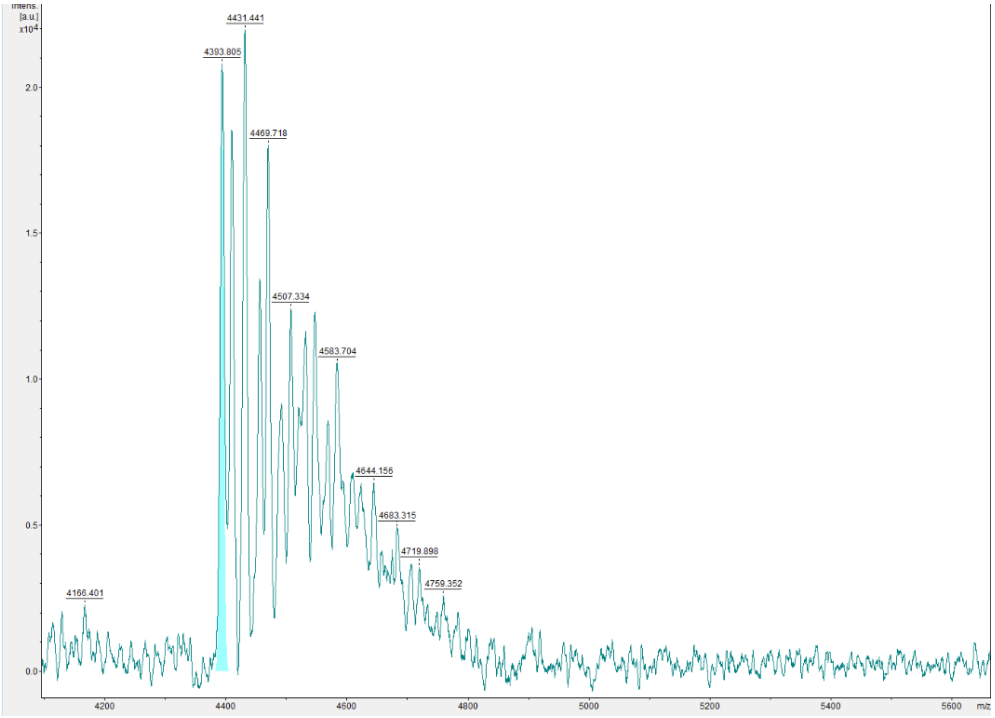


^{31}P-NMR spectrum of **4**

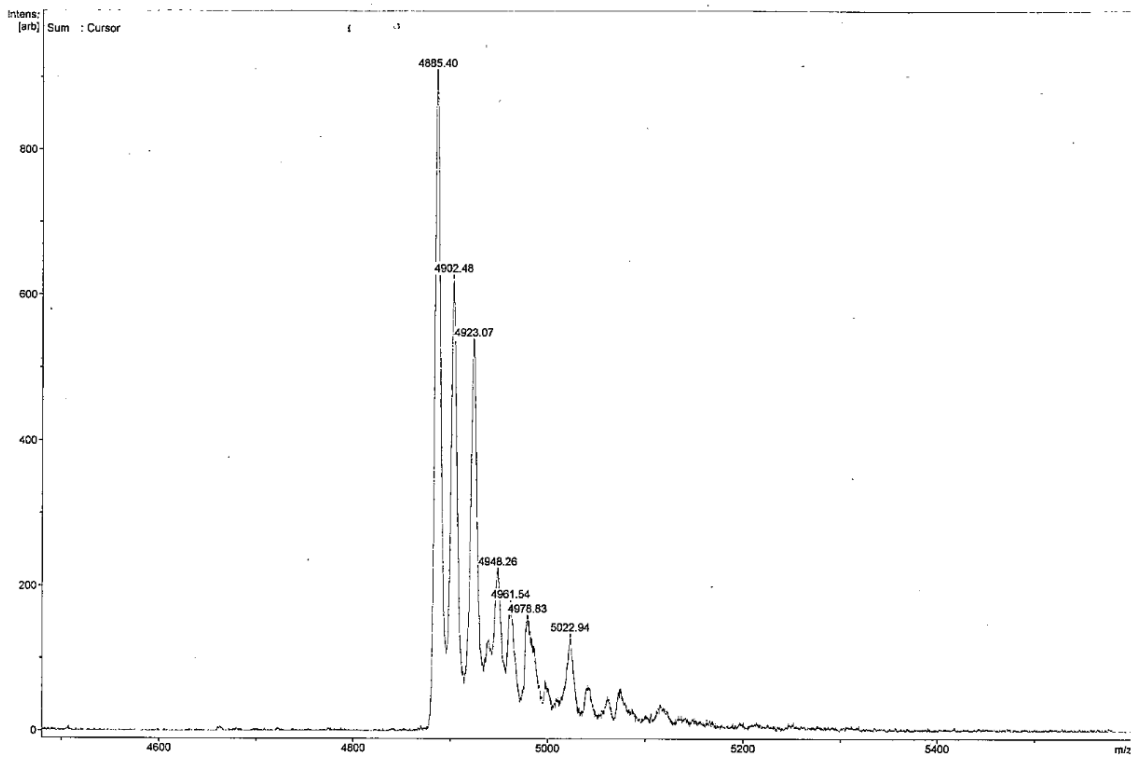
3-2. Results of MALDI-TOF MS

TFO linear probe	Theoretical	Observed
1F-T	4387.83	4393.81
2F-T	4884.97	4885.40
3F-T	5382.11	5383.68
4F-T	5879.25	5880.65
3L-T	5532.17	5528.68
3L-X	6977.42	6976.45
3L1gQ	7436.98	7434.67

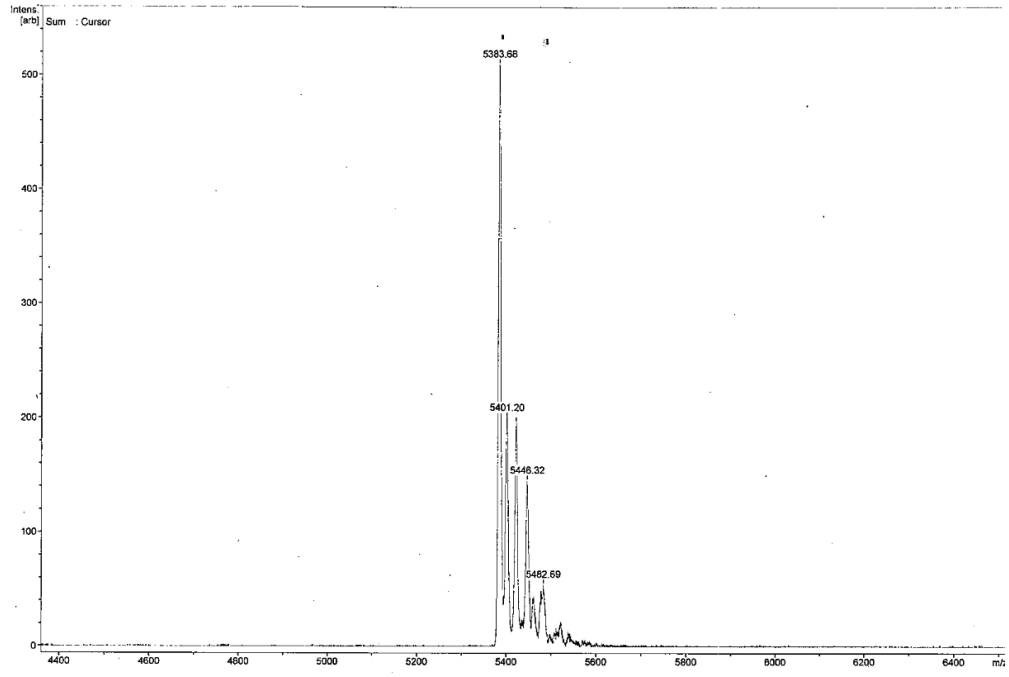
1F-T



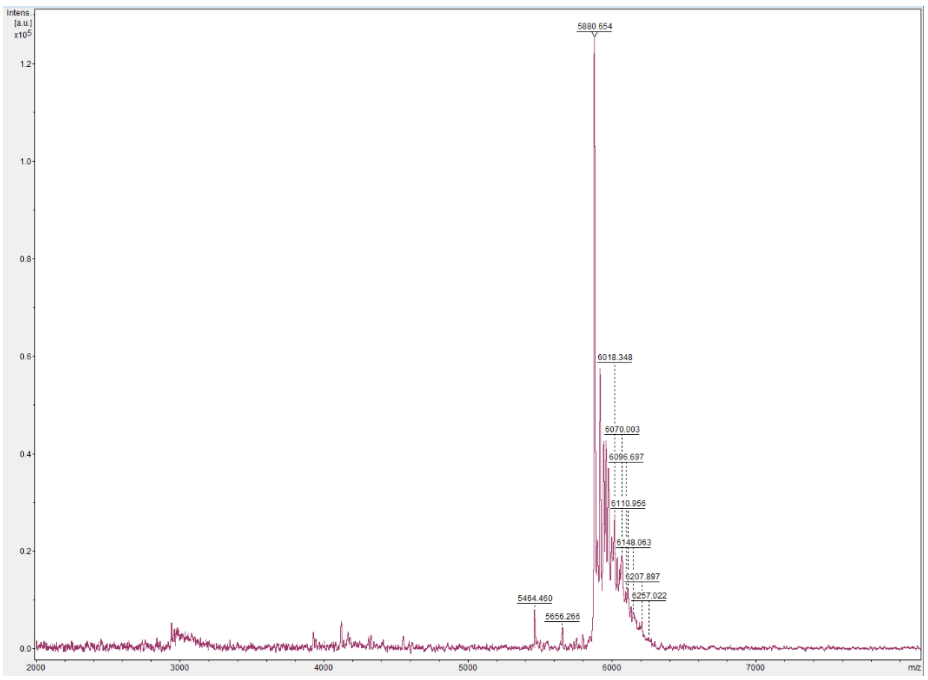
2F-T


Intens.
[arb]
Sum : Cursor
4885.40
4902.48
4923.07
4948.26
4961.54
4978.83
5022.94
800
600
400
200
0
4600
4800
5000
5200
5400
m/z

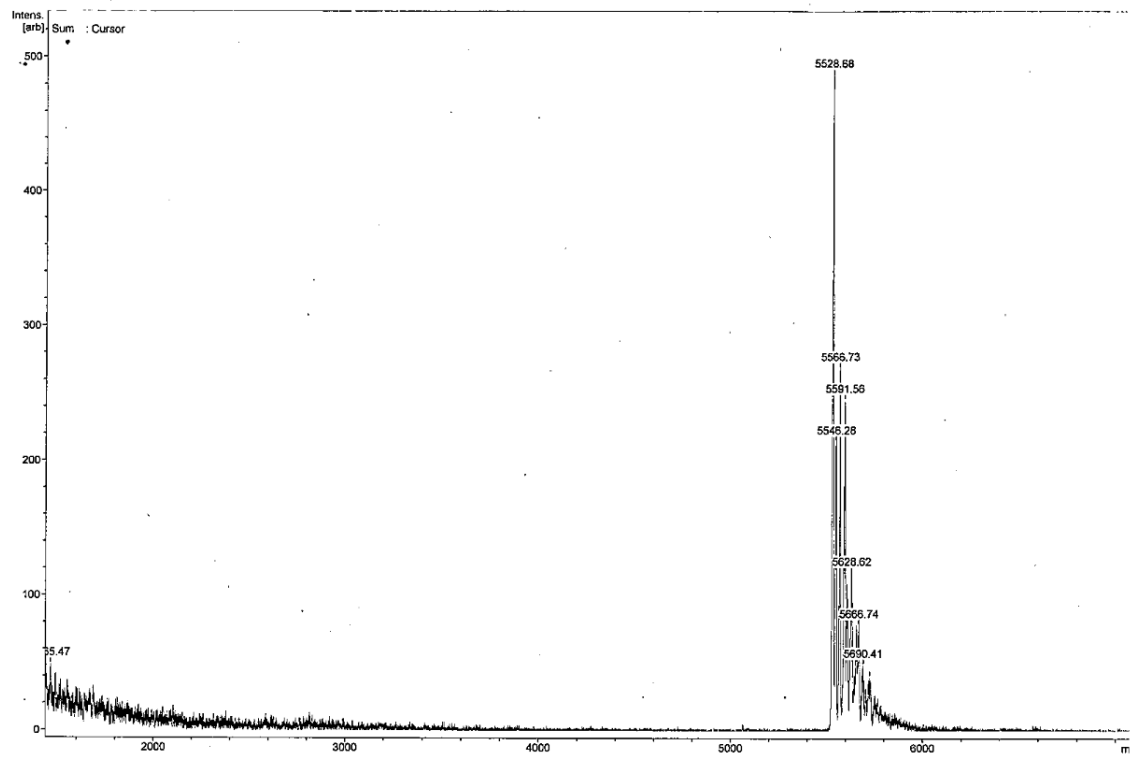
3F-T


Intens.
[arb]
Sum : Cursor
5383.68
5401.20
5446.32
5482.69
500
400
300
200
100
0
4400
4600
4800
5000
5200
5400
5600
5800
6000
6200
6400
m/z

4F-T


Intens.
[a.u.]
x10^5
5880.654
6018.348
6070.003
6096.697
6110.956
6148.063
6207.897
6257.022
5464.460
5656.266
m/z

3L-T


Intens.
[arb]
Sum : Cursor
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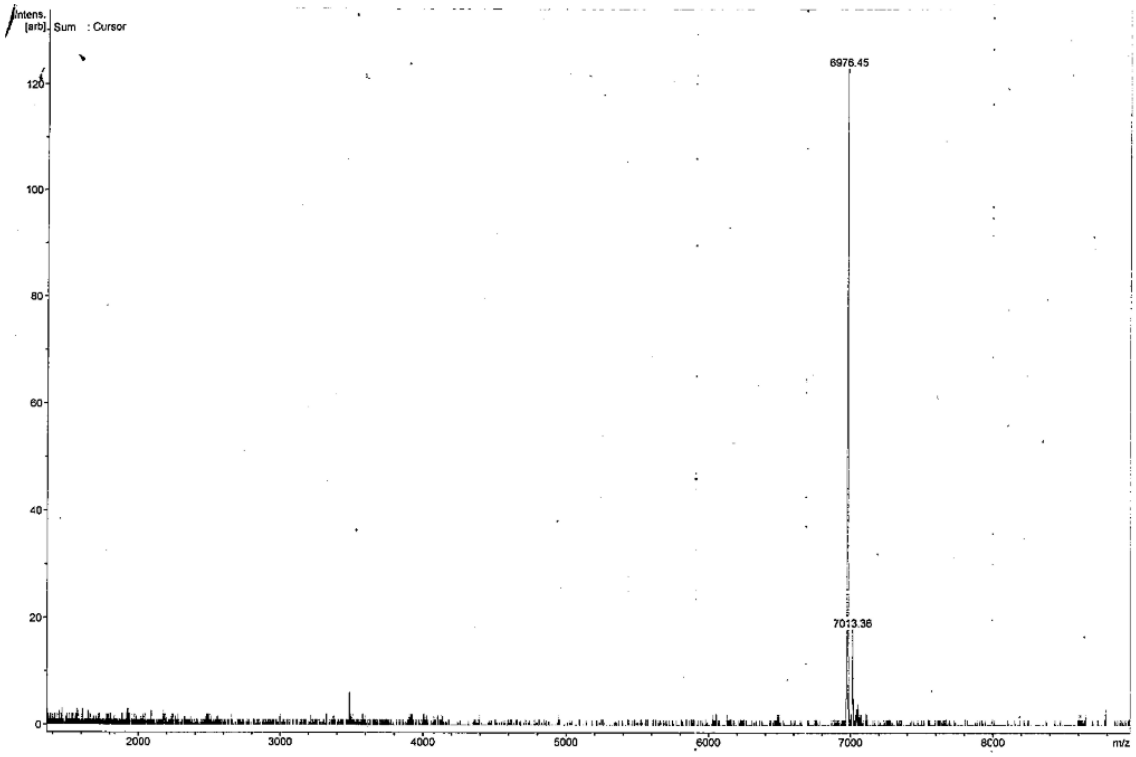
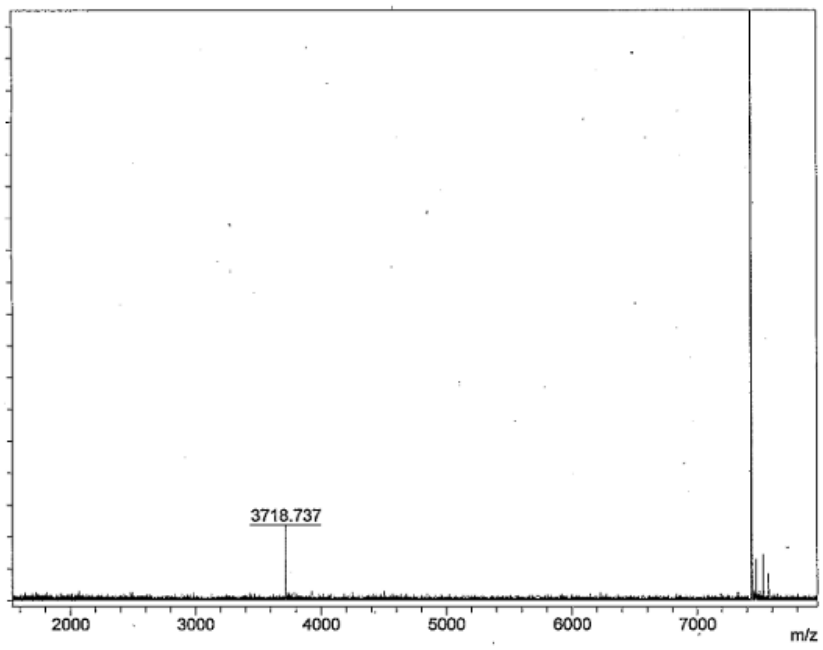
5566.73
5591.56
5548.28
5628.62
5666.74
5690.41
35.47
m

3L-X



3L1gQ

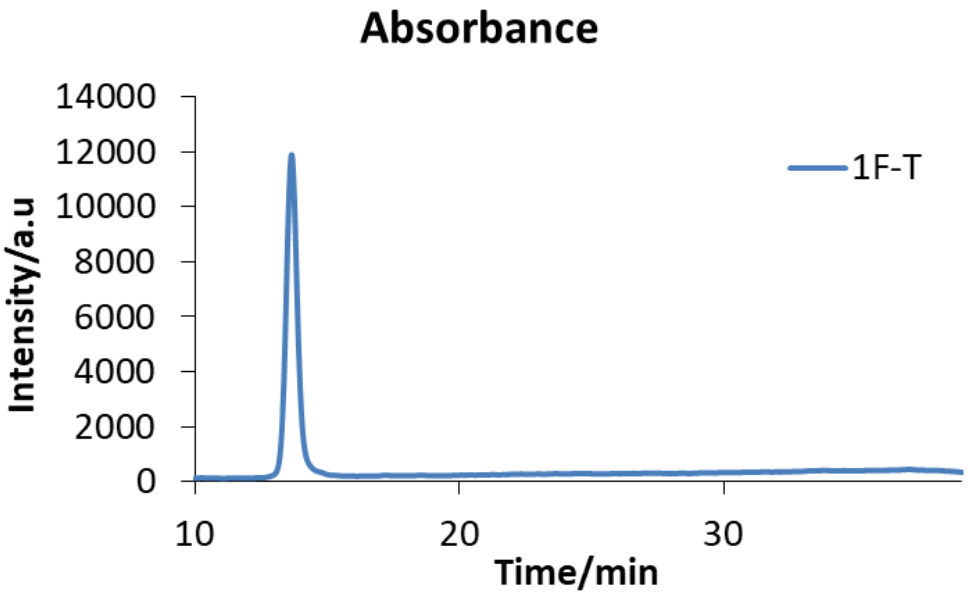


m/z	SN	Quality Fac.	Res.	Intens.	Area
3718.737	14.4		46945	46.00	6
7434.674	201.4		15755	657.00	437

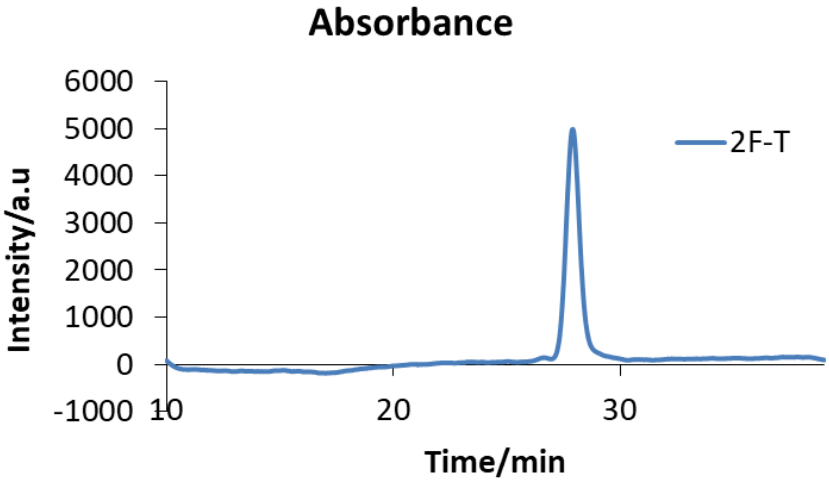
3-3. Results of HPLC:

Buffer A: 50 mM ammonium formate

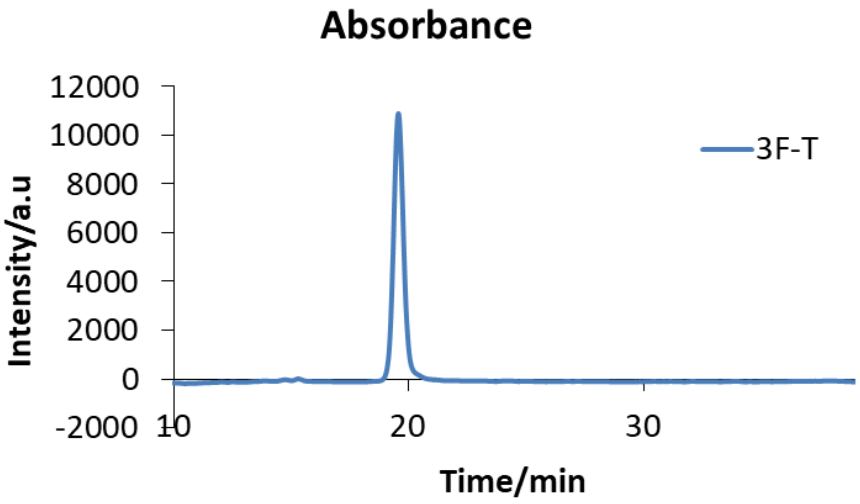
Buffer B: mixture of 50 mm ammonium formate and acetonitrile (50:50, v/v)



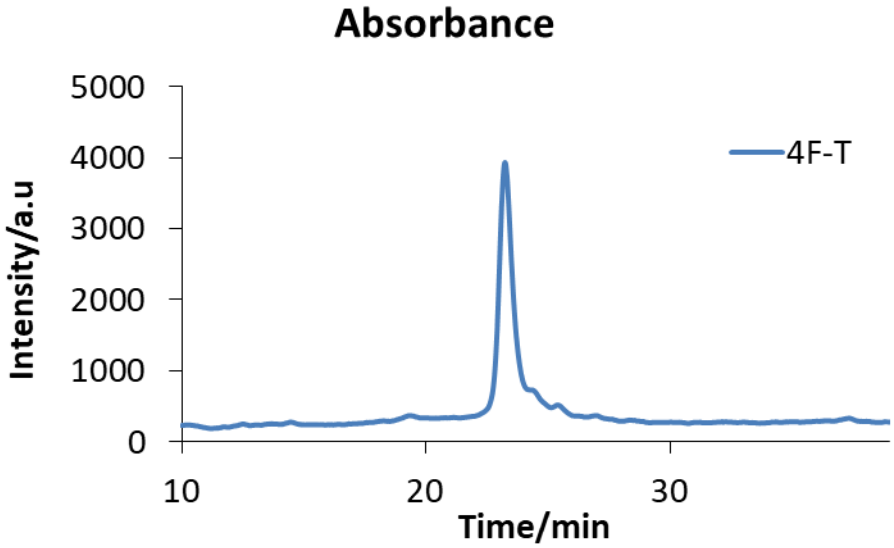
From 52% buffer B to 62% buffer B.



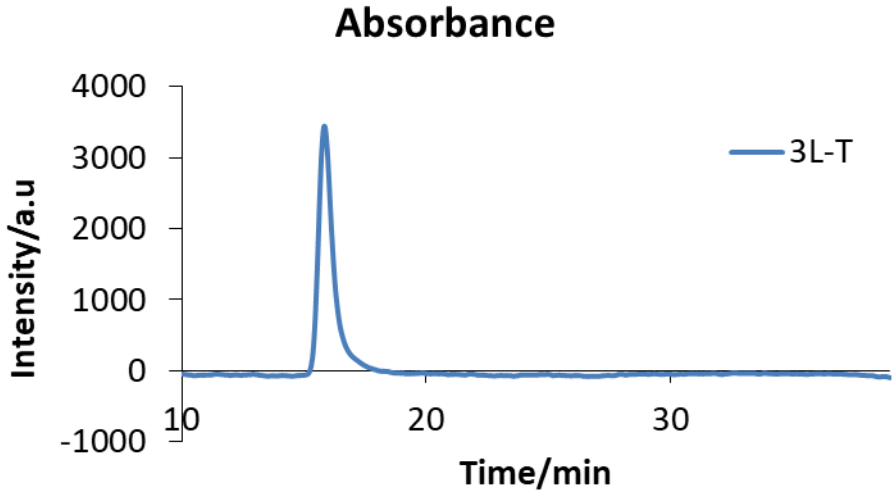
From 45% buffer B to 55% buffer B.



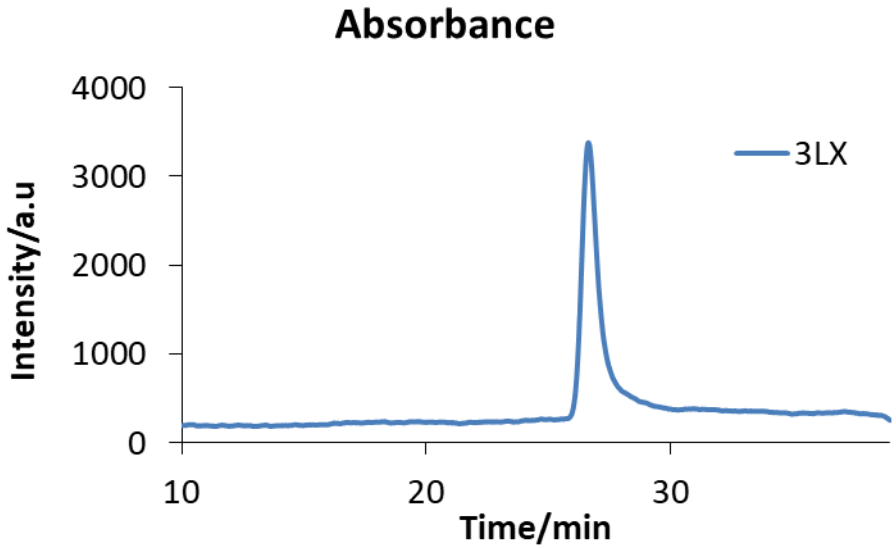
From 52% buffer B to 62% buffer B.



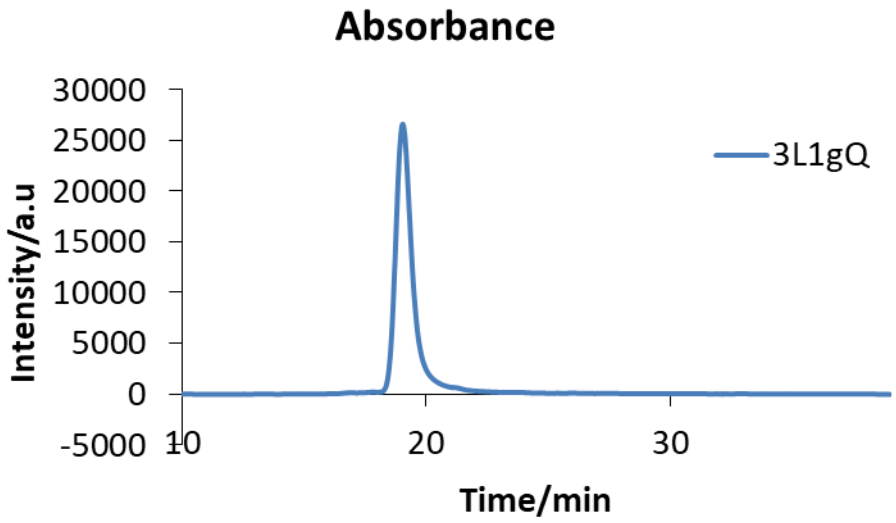
From 52% buffer B to 62% buffer B.



From 70% buffer B to 80% buffer B.



From 70% buffer B to 80% buffer B.



From 65% buffer B to 75% buffer B.

3-4 Sequence of plasmid

Orange font designates Xho I restriction site and gray indicates sites complementary to forward and reverse primers used for PCR.

NNNNNNNNNNNNGCTTATGGACACACCGAGGANGTCATCAAGGAGTTCATGCAGTTCAAGGTGCGCATGGAGGGCTCCGTG
AACGGCCACTACTTCGAGATCGAGGGCGAGGGCGAGGGCAAGCCCTACGAGGGCACCCAGACCGCCAAGCTGCAGGTGACC
AAGGGCGGCCCCCTGCCCTTCGCCTGGGACATCCTGTCCCCCCAGTTCCAGTACGGCTCCAAGGCCTACGTGAAGCACCCCGCC
GACATCCCCGACTACATGAAGCTGTCCTTCCCCGAGGGCTTCACCTGGGAGCGCTCCATGAACTTCGAGGACGGCGGCGTGGT
GGAGGTGCAGCAGGACTCCTCCCTGCAGGACGGCACCTTCATCTACAAGGTGAAGTTCAAGGGCGTGAACTTCCCCGCCGACG
GCCCCGTAATGCAGAAGAAGACTGCCGGCTGGGAGCCCTCCACCGAGAAGCTGTACCCCCAGGACGGCGTGCTGAAGGGCGA
GATCTCCCACGCCCTGAAGCTGAAGGACGGCGGCCACTACACCTGCGACTTCAAGACCGTGTACAAGGCCAAGAAGCCCGTGC
AGCTGCCCGGCAACCACTACGTGGACTCCAAGCTGGACATCACCAACCACAACGAGGACTACACCGTGGTGGAGCAGTACGAG
CACGCCGAGGCCCGCCACTCCGGCTCCCAGTAGGAATTCTGCAGATATCCAGCACAGTGGCGGCCGCTCGAGGTCACCCATTCG
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TCGACTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGCCCCTCCCCCGTGCCTTCCTTGACCCTGGAAGGTGCCACTCCCACTGT
CCTTTCCTAATAAAATGAGGAAATTGCATCGCATTGTCTGAGTNGTGTCATTCTATTCTGGGGGGTGGGGGTTGGGGCNNNNCA
GCAANGGNNNNNATTGGGAAGACAATANCNNNNTGCTGGGGANGCNGTGGGNCTCTANNGNNTTCNGNAGNCGAANGA
ANCANNCTGGGGGNNNNANGGGNGNNNNNNNNNNNNNNNGCNNTGNTNNNNCNGN