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Supporting Information

Phosphate-perylene modified G-quadruplex probes for the detection of Pb^{2+} by fluorescence anisotropy

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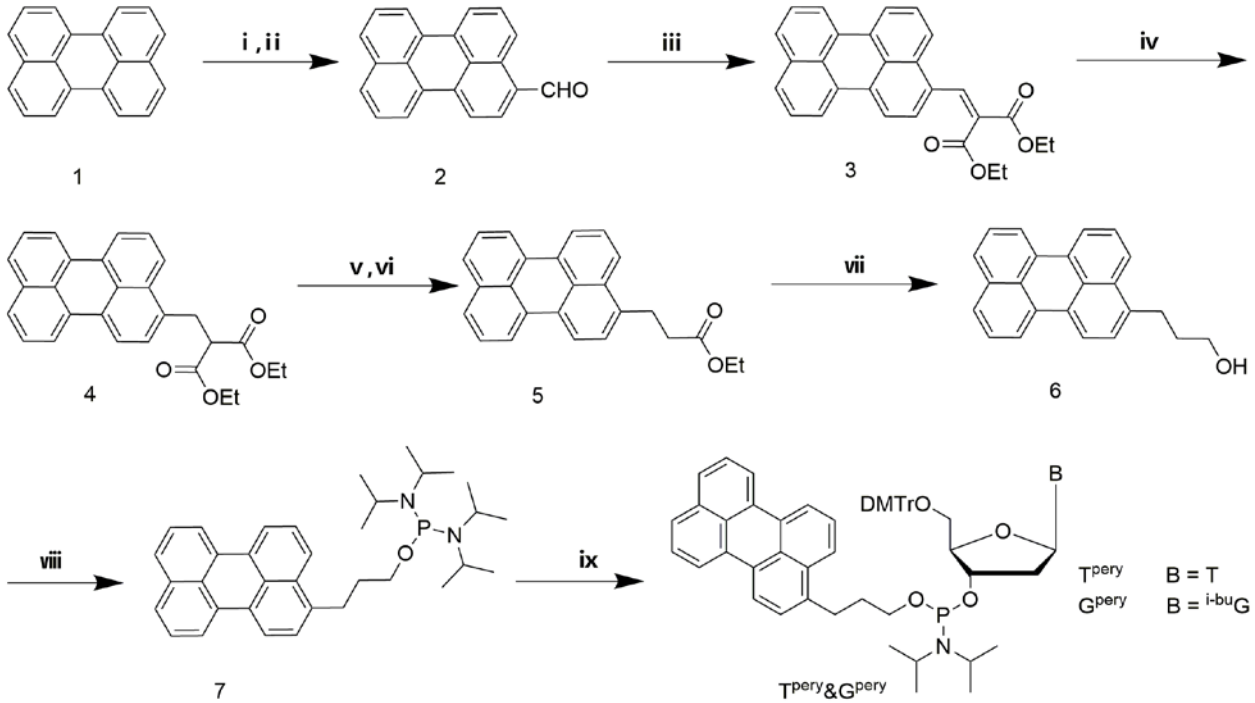
Materials and Methods



Scheme S1. Synthesis of the phosphate-perylene modified phosphoramidites. (i) $SnCl_4$, 1,1-dichlorooxymethylether, 1,2-dichloroethane, 0 °C for 2 h, reflux for 17 h; (ii)H_2O, r.t., 3 h, 60.3%; (iii) diethyl malonate, piperidine, dry methylbenzene, reflux, 48 h, 67.5%; (iv) zinc dust, acetic acid, reflux, 3 h, 93.5%; (v) aqueous KOH (3%), methylbenzene, ethanol, r.t., 3 h; (vi) o-dichlorobenzene, reflux, 1 h, 81.3%; (vii) sodium borohydride, 50% ethanol, THF, 0 °C, overnight, 77.7%; (viii) bis(diisopropyamino) chlorophosphine, triethylamine, anhydrous THF and CH_2Cl_2, N_2, 0 °C to r.t., overnight; (ix) 5'-O-(dimethoxytrityl)deoxy-thymidine for T^{pery} and 5'-O-(dimethoxytrityl)-N-isobutyryldeoxyguanosine for G^{pery}, tetrazole, anhydrous CH_2Cl_2, N_2, r.t., 2h. Combined yield of viii and ix: 37.2% for T^{pery} and 32.0% for G^{pery}, respectively.

General methods and instruments

All chemical reagents used in organic synthesis were analytic pure, while solvents used for reaction and purification were dried and distilled over CaH_2. Merck silica gel C-300 was used in silica gel column chromatography. Lead dichloride was purchased from Aladdin (Purity: 99.999%). All unmodified oligonucleotides were purchased from Sangon Biotechnology Co., Ltd. (Shanghai, China) and all perylene-modified single-stranded oligonucleotides were custom-synthesized on an ABI 394 DNA/RNA synthesizer (Applied Biosystems). Deprotected oligonucleotides were purified with a Waters HPLC system using Waters reverse phase C18 column. The concentrations of oligonucleotides were quantified by measuring absorbance at 260 nm using a Nanodrop 2000 spectrometer (Thermo Scientific). Mass spectra of perylene derivatives with low solubility were performed by EI-MS and mass spectra of all the oligonucleotides were performed by ESI-MS under negative mode. The melting temperature was measured on a Beckman DU800 spectrophotometer equipped with a programmed temperature controller.

Synthesis

3-Perylenecarboxaldehyde (2).

A mixture of perylene (3.61 g, 14.3 mmol) and $SnCl_4$ (3.43 mL, 28.1 mmol) in 1,2-dichloroethane (100 mL) was cooled to 0 °C with an ice-water bath. Then, 1,1-dichlorooxymethylether (1.63 mL, 17.3 mmol) was added dropwise to the mixture over 1 h and the temperature was kept at 0°C for 1 h. After that, the mixture was heated to reflux for 2 h and continued stirring for another 15 h. After cooling to -10 °C, the reaction was quenched by the addition of cold water (100 mL). After 3 h at room temperature, the reaction mixture was extracted with dichloromethane (150 mL). The organic phase was washed with water (3×50 mL), dried over anhydrous magnesium sulfate. After removal of solvents, the residue was purified by silica gel chromatography using PE-EA (8:1) as eluent to give the aldehyde derivative **2** (2.42 g, yield 60.3%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 10.34 (s, 1H), 9.19 (d, 1H), 8.37-8.28 (m, 4H), 7.97 (d, 1H), 7.83 (d, 1H), 7.77 (d, 1H), 7.72 (t, 1H), 7.56 (td, 2H). ^{13}C-NMR (101 MHz, $CDCl_3$) δ= 192.68, 137.51, 137.06, 134.30, 132.19, 131.15, 130.59, 130.00, 129.84, 129.76, 129.22, 128.84, 128.43, 128.06, 126.90, 126.59, 124.53, 122.72, 121.46, 121.00, 119.00.

Diethyl (3-Perylenylmethylene)propanedioate (3).

A mixture of compound **2** (2.42 g, 8.62 mmol), diethyl malonate (2.11 g, 11.2 mmol), and piperidine (2 mL) in dry methylbenzene (100 mL) was heated to reflux for 48 h. The solvent was evaporated in vacuo, and the residue was purified by silica gel chromatography using dichloromethane as eluent to give **3** (2.46 g, 67.5%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 8.40 (s, 1H), 8.26 (d, 1H), 8.22 (t, 2H), 8.13 (d, 1H), 7.85 (d, 1H), 7.72 (t, 2H), 7.62-7.54 (m, 2H), 7.51 (td, 2H), 4.38 (q, 2H), 4.23 (q, 2H), 1.39 (t, 3H), 1.14 (t, 3H). ^{13}C-NMR (101 MHz, $CDCl_3$) δ = 166.30, 164.08, 140.79, 134.53, 133.52, 132.86, 131.72, 130.85, 130.50, 130.07, 128.76, 128.70, 128.60, 128.35, 128.26, 127.38, 127.32, 126.73, 126.62, 123.80, 121.12, 120.87, 120.72, 119.50, 61.76, 61.58, 14.24, 13.90.

Diethyl (3-Perylenylmethyl)propanedioate (4).

A mixture of **3** (2.46 g, 5 mmol), zinc dust (6 g), and acetic acid (150 mL) was refluxed for 3 h. After cooling, the reaction mixture was poured into a mixture of water and benzene. After shaking, the organic layer was separated and dried over anhydrous magnesium sulfate. The solvent was evaporated and the residue was purified by silica gel chromatography using dichloromethane as eluent to give **4** as yellow powder (2.31 g, 93.5%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 8.16 (d, 1H), 8.14 (d, 1H), 8.10 (d, 1H), 8.03 (d, 1H), 7.83 (d, 1H), 7.64 (dd, 2H), 7.51 (t, 1H), 7.44 (td, 2H), 7.35 (d, 1H), 4.19 (m, 4H), 3.85 (t, 1H), 3.64 (d, 2H), 1.22 (t, 6H). ^{13}C-NMR (101 MHz, $CDCl_3$) δ = 169.04, 134.58, 133.52, 132.78, 131.95, 131.20, 131.11, 130.53, 129.10, 128.41, 127.97, 127.87, 127.68, 126.78, 126.54,

123.01, 120.31, 120.20, 120.04, 119.80, 61.60, 52.58, 32.13, 14.07.

Ethyl 3-(3-perylenyl)propanoate (5).

A mixture of **4** (2.31 g, 5.44 mmol), aqueous potassium hydroxide (3%, 65 mL), methylbenzene (50 mL) and ethanol (200 mL) were stirred for 3 h at room temperature. The reaction mixture was washed with saturated aqueous ammonium chloride (100 mL) and was then extracted with dichloromethane (100 mL). The organic layer was evaporated and the residue, without purification, was refluxed in o-dichlorobenzene (75 mL) for 1 h to induce decarboxylation. After the reaction solution was cooled and concentrated, the residue was purified by silica gel chromatography using dichloromethane-methanol (8:1) as eluent to give **5** (1.56 g, 81.3%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 8.21 (d, 1H), 8.18 (d, 1H), 8.15 (d, 1H), 8.10 (d, 1H), 7.86 (d, 1H), 7.67 (dd, 2H), 7.53 (t, 1H), 7.47 (td, 2H), 7.36 (d, 1H), 4.18 (q, 2H), 3.36 (t, 2H), 2.77 (t, 2H), 1.26 (t, 3H). ^{13}C-NMR (101 MHz, $CDCl_3$) δ = 173.02, 136.38, 134.63, 132.83, 131.90, 131.32, 131.27, 130.06, 129.09, 128.47, 127.83, 127.55, 126.79, 126.61, 126.58, 126.55, 123.29, 120.26, 120.20, 120.02, 119.90, 60.58, 34.96, 28.42, 14.27.

3-Perylenepropanol (6).

5 (1.56 g, 4.87 mmol) was dissolved in THF (50 mL) and cooled to 0 ℃ with an ice-water bath. Sodium borohydride (0.738 g, 19.5 mmol) dissolved in 50% ethanol (20 mL) was added to above solution dropwise. The mixture was stirred overnight. Then water was added to quench the reaction. The mixture was concentrated and extracted with dichloromethane. The organic phase was dried over anhydrous sodium sulfate and then evaporated. The residue was purified by silica gel chromatography using dichloromethane as eluent to give compound **6** (1.16 g, 77.7%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 8.22 (d, 1H), 8.19 (d, 1H), 8.16 (d, 1H), 8.13 (d, 1H), 7.91 (d, 1H), 7.67 (m, 2H), 7.52 (t, 1H), 7.47 (td, 2H), 7.37 (d, 1H), 3.79 (t, 2H), 3.14 (t, 2H), 2.06 (m, 2H). ^{13}C-NMR (101 MHz, $CDCl_3$) δ = 137.89, 134.67, 133.04, 131.83, 131.42, 129.70, 129.41, 129.14, 128.52, 127.76, 127.42, 126.93, 126.60, 126.55, 126.42, 123.71, 120.19, 120.16, 120.06, 119.77, 62.50, 33.28, 29.49. MS (EI-TOF^{+}), calculated 310.14, $C_{23}H_{18}O$; measured M^{+} 310.3.

Bis(diisopropyamino)(3-perylenepropoxy) phosphine (7).

6 (310 mg, 1.0 mmoL) was dissolved in anhydrous THF (1mL) containing triethylamine (0.1 mL) under N_2, and the mixture was cooled to 0° C. Bis(diisopropyamino)chlorophosphine (293 mg, 1.1 mmoL) dissolved in anhydrous dichloromethane (1 mL) under N_2 was then added to above solution dropwise. The reaction continued overnight at room temperature and the precipitate was then filtered off. The residue solution was concentrated to give compound **7** as yellow solid. Compound **7** with no need for further purification was directly used in next step.

5'-O-(Dimethoxytrityl)-2'-deoxythymidine-3'-(3-perylene propyl-N,N-diisopropyl) phosphoramidite (T^{pery}).

To the solution of 5'-O-(dimethoxytrityl)deoxythymidine (354 mg, 0.65 mmoL) and tetrazole (80 mg, 1.1 mmoL) in anhydrous dichloromethane (1.5 mL), **7** in anhydrous dichloromethane (1.5 mL) was added under N_2. After about 2 hour reaction at room temperature, the mixture was purified by silica gel column chromatography (PE/EA/TEA, 1:1:0.03) to afford the compound $\mathbf{T^{pery}}$ (336 mg, 0.37 mmoL) as foamy solid with a yield 37.2% for two steps. ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 8.26-8.05 (m, 5H), 7.96-7.73 (m, 1H), 7.72-7.35 (m, 10H), 7.32-7.17 (m, 5H), 6.84-6.76 (m, 3H), 6.48-6.40 (dd, 1H), 4.71 (m, 1H), 4.26-4.08 (m, 2H), 3.80-3.70 (m, 6H), 3.59 (t, 1H), 3.52-3.32 (dd, 2H), 3.17-3.00 (m, 2H), 2.61-2.48 (m, 1H), 2.38-2.28 (m, 1H), 2.19-1.93 (m, 4H), 1.44-1.38 (m, 3H), 1.38-1.24 (m, 12H).^{31}P-NMR (162 MHz, $CDCl_3$) δ = 146.9, 147.6. MS (ESI-TOF$^+$), calculated 983.43, $C_{60}H_{62}N_3O_8P$; measured $[M+Na]^+$ 1006.66.

5'-O-(dimethoxytrityl)-N-isobutyryl-2'-deoxyguanosine-3'-(3-perylenepropyl-N,N-diisopropyl)phosphoramidite(G^{pery}).

To the solution of 5'-O-(dimethoxytrityl)-N-isobutyryl deoxyguanosine (416 mg, 0.65 mmoL) and tetrazole (80 mg, 1.1 mmoL) in anhydrous dichloromethane (1.5 mL), **7** in anhydrous dichloromethane solution (1.5 mL) was added under N_2. After about 2 hour reaction at room temperature, the mixture was purified by silica gel column chromatography (PE/EA/TEA, 1:1:0.03) to afford the compound $\mathbf{G^{pery}}$ (346 mg, 0.32 mmoL) as foamy solid with a yield 32.0% for two steps. ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 8.22-8.07 (m, 4H), 7.88 (dd, 1H), 7.78 (d, 1H), 7.72-7.65 (m, 2H), 7.56-7.44 (m, 5H), 7.40-7.30 (m, 5H), 7.27-7.16 (m, 3H), 6.84-6.68 (m, 4H), 6.24-6.10 (m, 1H), 4.83-4.74 (m, 1H), 4.31 (d, 1H), 3.81-3.58 (m, 9H), 3.37-3.26 (m, 2H), 3.20-2.90 (m, 4H), 2.53-2.45 (m, 1H), 2.13-2.08 (m, 1H), 1.89-1.62 (m, 2H), 1.35-1.23 (m, 12H) , 1.17-1.08 (m, 6H).^{31}P-NMR (162 MHz, $CDCl_3$) δ = 147.3, 147.8. MS (ESI-TOF$^+$), calculated 1078.48, $C_{64}H_{67}N_6O_8P$; measured $[M+H]^+$1080.08, $[M+Na]^+$ 1102.11.

Synthesis, deprotection and purification of oligonucleotides

Phosphate-perylene modified oligonucleotides were synthesized on Applied Biosystems Incorporated 394 synthesizer on 1 μM scale. The coupling yield of $\mathbf{T^{pery}}$ and $\mathbf{G^{pery}}$ was similar to the incorporation efficiency of natural DNA phosphoramidite monomers with extended coupling time (5 min). After oligonucleotide synthesis, fresh concentrated ammonium hydroxide was added to CPGs and the mixture was shaken for 24 hours at room temperature. After concentration, the residue mixture was dissolved in about 400 μL 0.05 M TEAA buffer and was then purified by a Waters HPLC system using Waters reverse phase C18 column (5 μm bead, 9.6 mm×150 mm). Conditions: solvent A, 0.05 M TEAA buffer; solvent B, acetonitrile. 0 - 20 min, B: 0 - 40%; 20 - 30 min, B: 40 - 80%; 30 - 35

min, B: 80 - 100 %; 35 - 38 min, B: 100 - 100 %; 38 - 45 min, B: 100 - 0% B; flow rate: 1 mL/min. The collected fractions were then dried in spin vacuum. The obtained residues were mixed with 80% aqueous acetic acid (500 μL) to remove DMTr group for 30 min at room temperature. After concentration in vacuum, the residue was further purified by HPLC under the same HPLC conditions as above.

Circular dichroism (CD) analysis

CD spectra were obtained on a JASCO J-810 spectropolarimeter at room temperature. Oligonucleotide solutions (3.5 μM) were prepared in 10 mM Tris-HAc buffer (pH 7.5). 100 μM Pb^{2+} or 100 mM K^{+} was added (final volume 200 μL), and then the mixture was allowed to incubate at room temperature for 30 min. CD spectra were recorded between 220 and 450 nm with a 1 mm path length quartz cuvette. The scan rate was set at 200 nm/min with a response time of 1 s and a bandwidth of 1 nm. The spectra were averaged over three scans. The background signal of the 10 mM Tris-HAc (pH 7.5) buffer was subtracted from all CD data.

Fluorescence emission and fluorescence anisotropy analysis.

Oligonucleotides (0.2 μM) were dissolved in the solution of 10 mM Tris-HAc buffer (pH = 7.5). Different concentrations of Pb^{2+} or K^{+} were added to the solution (final volume 350 μL), and the mixture was incubated at room temperature for 30 min before the measurements. The fluorescence spectra of the oligonucleotide solutions were recorded on Cary Eclipse at room temperature. Recording conditions: λ_{ex}= 425 nm; excitation and emission slit 10 nm; PMT 600 V; scan rate 240 nm/min. Fluorescence anisotropy was measured using an F-7000 fluorescence spectrophotometer (Hitachi). All measurements were carried out with a 700 μL quartz cuvette. Recording conditions: λ_{ex}= 420 nm; excitation and emission slit 10 nm; PMT 700 V; scan rate 240 nm/min; the temperature was set at 25 °C. Fluorescence anisotropy (r) was defined as:

$$r = (I_{VV}I_{HH}-I_{VH}I_{HV}) / (I_{VV}I_{HH}+2I_{VH}I_{HV}) \quad (1)$$

where I is the measured fluorescence intensity. The subscripts V and H were defined as the orientation (vertical and horizontal) of the polarizer. The first subscript refers to the orientation of the excitation polarizer and the second one refers the orientation of the emission polarizer.

Table S1. Melting temperatures (*T*m) of all the oligonucleotides at the presence of Pb^{2+} or K^{+}.

sequence	Ion	*T*m (ºC)	Δ*T*m (ºC)
G4-s	**K^{+}**	**72.1**	**-**
	Pb^{2+}	**60.2**	**-**
G4-1	**K^{+}**	**82.4**	**10.3**
	Pb^{2+}	**89.0**	**28.8**
G4-2	**K^{+}**	**69.8**	**-2.3**
	Pb^{2+}	**89.6**	**29.4**
G4-3	**K^{+}**	**62.2**	**-9.9**
	Pb^{2+}	**72.1**	**11.9**
G4-4	**K^{+}**	**75.1**	**3.0**
	Pb^{2+}	**82.2**	**22.0**
G4-6	**K^{+}**	**84.4**	**12.3**
	Pb^{2+}	**89.0**	**28.8**
G4-9	**K^{+}**	**75.6**	**3.5**
	Pb^{2+}	**86.8**	**26.6**
G4-11	**K^{+}**	**82.4**	**10.3**
	Pb^{2+}	**91.5**	**31.3**
G4-14	**K^{+}**	**73.3**	**1.2**
	Pb^{2+}	**83.7**	**23.5**
G4-16	**K^{+}**	**84.9**	**12.8**
	Pb^{2+}	**88.9**	**28.7**
G4-19	**K^{+}**	**88.3**	**16.1**
	Pb^{2+}	**85.6**	**25.4**
TBA-s	**K^{+}**	**49.9**	**-**
	Pb^{2+}	**57.3**	**-**
TBA-3	**K^{+}**	**63.1**	**13.2**
	Pb^{2+}	**86.2**	**28.9**
TBA-4	**K^{+}**	**77.2**	**27.3**
	Pb^{2+}	**89.5**	**32.2**
TBA-7	**K^{+}**	**54.8**	**4.9**
	Pb^{2+}	**72.5**	**15.2**
TBA-9	**K^{+}**	**50.0**	**0.1**
	Pb^{2+}	**76.0**	**18.7**

Table S2. The changing ratios of fluorescence intensity or fluorescence anisotropy between Pb^{2+} and K^{+} using different G-quadruplex probes.

probe	$(F_0/F_{Pb})/(F_0/F_K)$	$\Delta r_{Pb}/\Delta r_K$
G4-1	1.8	7.7
G4-2	1.2	2.0
G4-3	0.7	0.6
G4-4	2.2	1.9
G4-6	0.5	0.2
G4-9	2.9	25.7
G4-11	0.7	0.7
G4-14	1.4	1.7
G4-16	0.9	0.5
G4-19	3.3	2.6
TBA-3	0.9	0.3
TBA-4	0.7	0.1
TBA-7	4.1	3.8
TBA-9	5.6	19.8

Table S3. Recovery experiments of Pb^{2+} in tap water.

$[Pb^{2+}]_{added}$/nM	$[Pb^{2+}]_{measured}$/ nM	Recovery[a]
50	50.77[b]	101.5%
100	101.4[b]	101.4%
500	494.8[b]	99.0%

[a] The data of recovery were calculated by $[Pb^{2+}]_{measured}/[Pb^{2+}]_{added}$ *100%
[b] Mean of three measurements

Table S4. G-quadruplex based methods for the detection of Pb^{2+}

Method	LOD	Real sample	Ref.
Fluorescence (turn-on, ↑)	5 nM	Urine	1
Fluorescence (turn-on, ↑)	8 nM	Not reported	2
Fluorescence(turn-off, ↑)	5 nM	Tap water	3
Electrochemical (signal increase, ↓)	0.5 nM	Not reported	4
Chemiluminescence (turn-off, ↓)	1 nM	Not reported	5
Colorimetric (signal decrease, ↓)	32 nM	Lake water	5
Colorimetric (signal increase, ↑)	1 nM	Tap water	6
Fluorescence anisotropy (signal decrease, ↓)	1 nM	Lake water	7
Fluorescence anisotropy (signal increase, ↑)	24.5 nM	Tap water	This study

Figure S1. Thermal denaturation curves of G-quadruplexes formed by 3.5 μM oligonucleotides at the presence of 100 mM K^+ or 100 μM Pb^{2+} in 10 mM Tris-HAc (pH 7.5) buffer. The absorption was monitored at 295 nm with the increase of temperature.

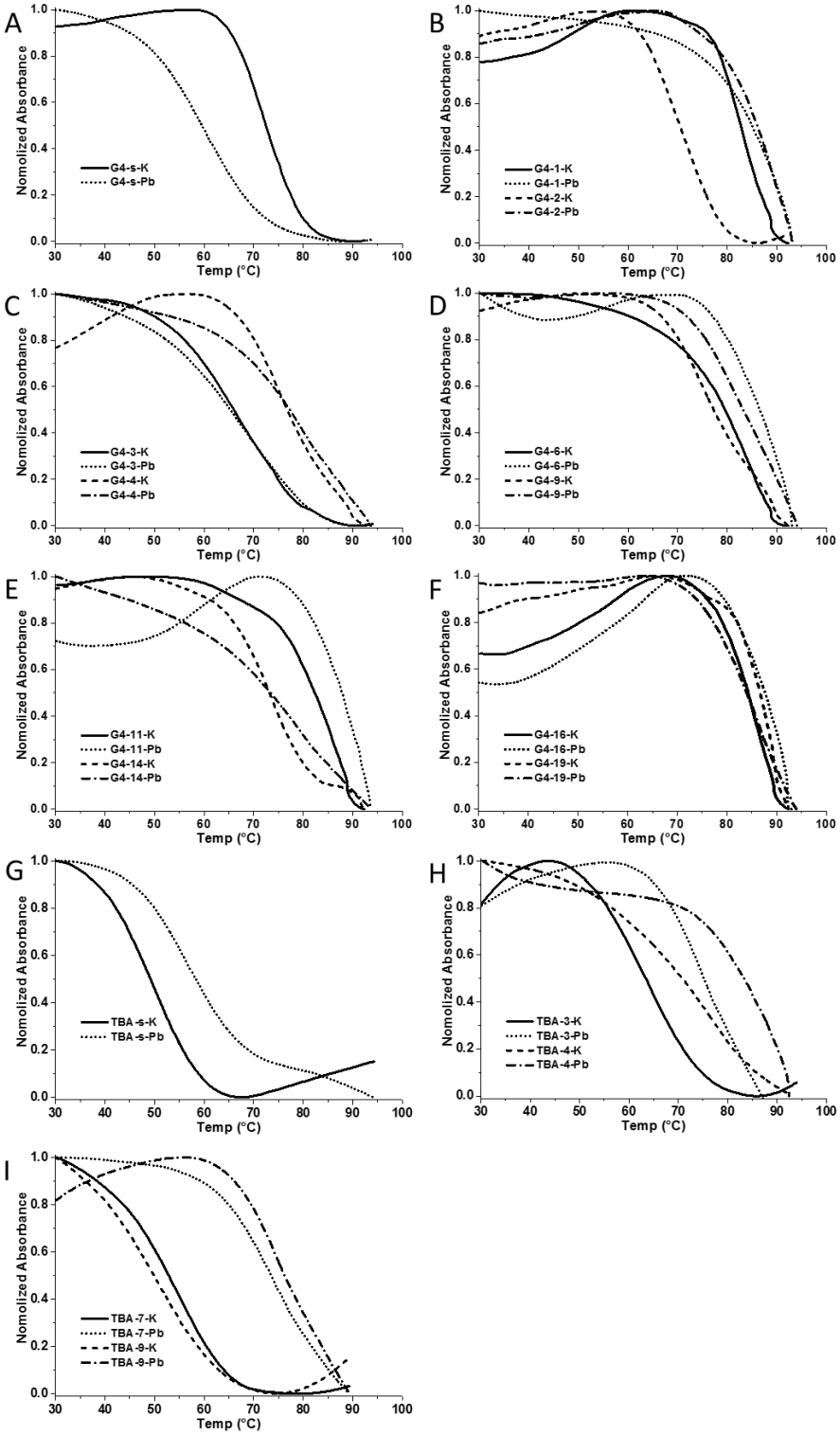


Figure S2. UV-vis absorption spectra for each oligonucleotides (3.5 μM) in the absence of metal ions (black solid line) and at the presence of 100 mM K^+ (red dash line) or 100 μM Pb^{2+} (blue dot line). A 10 mM Tris-HAc buffer (pH 7.5) was used.

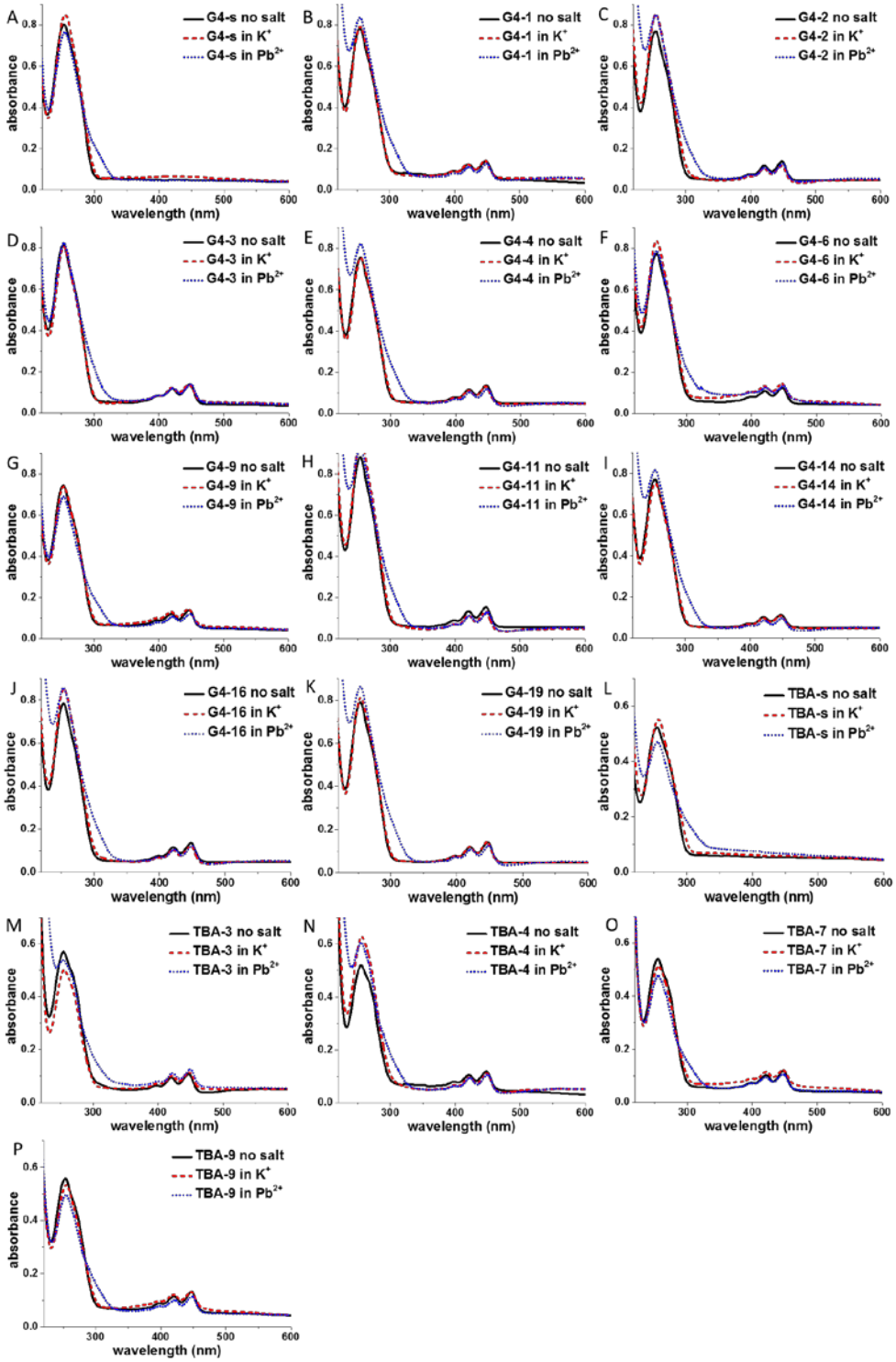


Figure S3. Fluorescence spectra for each oligonucleotides (3.5 μM) in the absence of metal ions (black solid line) and at the presence of 100 mM K^+ (red dash line) or 100 μM Pb^{2+} (blue dot line). A 10 mM Tris-HAc buffer (pH 7.5) was used.

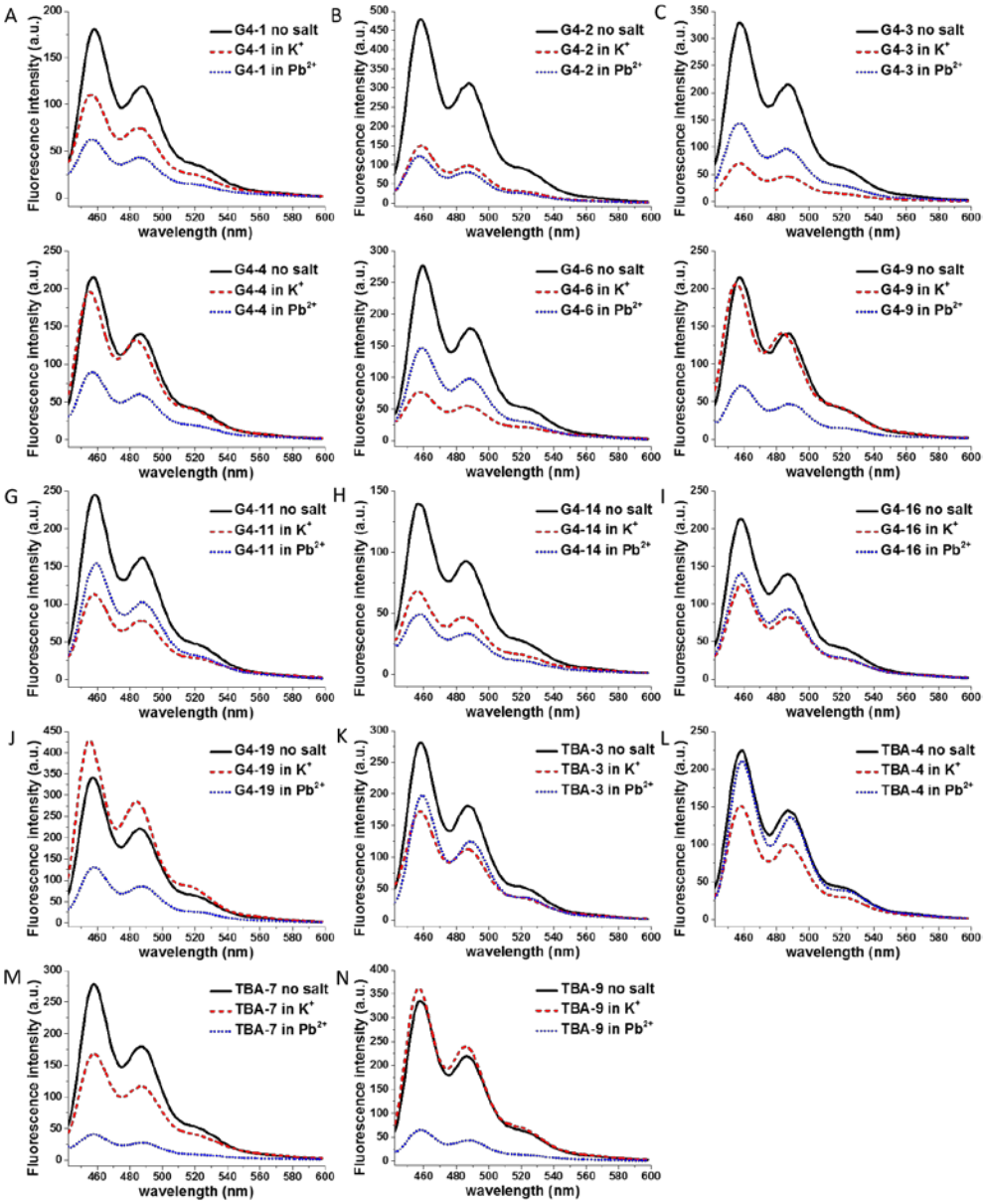


Figure S4. The values of fluorescence anisotropy (*r*) for all the G-quadruplex probes in the absence of metal salts (gray square) and at the presence of 10 μM Pb^{2+} (light gray diamond) or 100 mM K^+ (black circle). λ_{ex}=420 nm; excitation and emission slit 10 nm. Tris-HAc (pH 7.5) buffer:10 mM; oligonucleotides:0.2 μM.

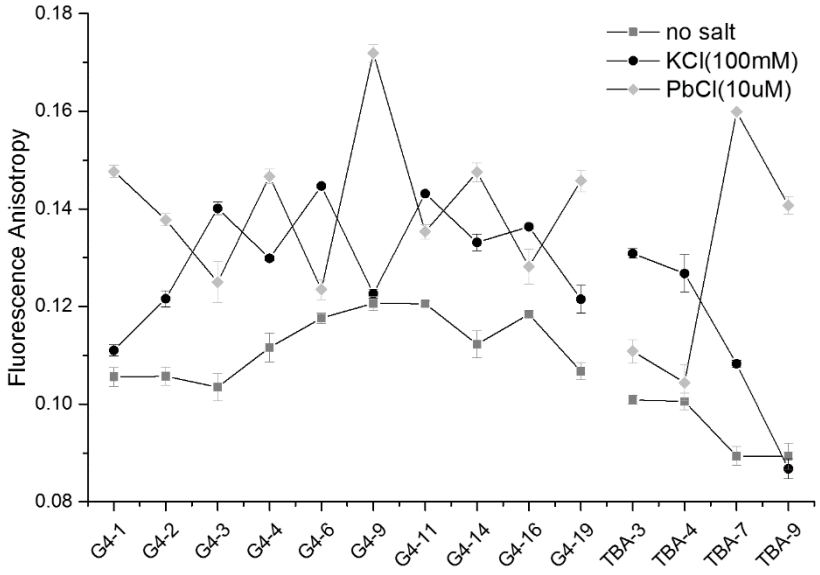


Figure S5. CD titration of 5 μM TBA-s (A), TBA-9 (B), G4-s (C) and G4-9 (D) by the addition of $PbCl_2$. A 10 mM Tris-HAc (pH 7.5) buffer was used. The result showed that TBA-s and TBA-9 tended to bind 1 eq. Pb^{2+} while G4-s and G4-9 tended to bind about 2 eq. Pb^{2+}.

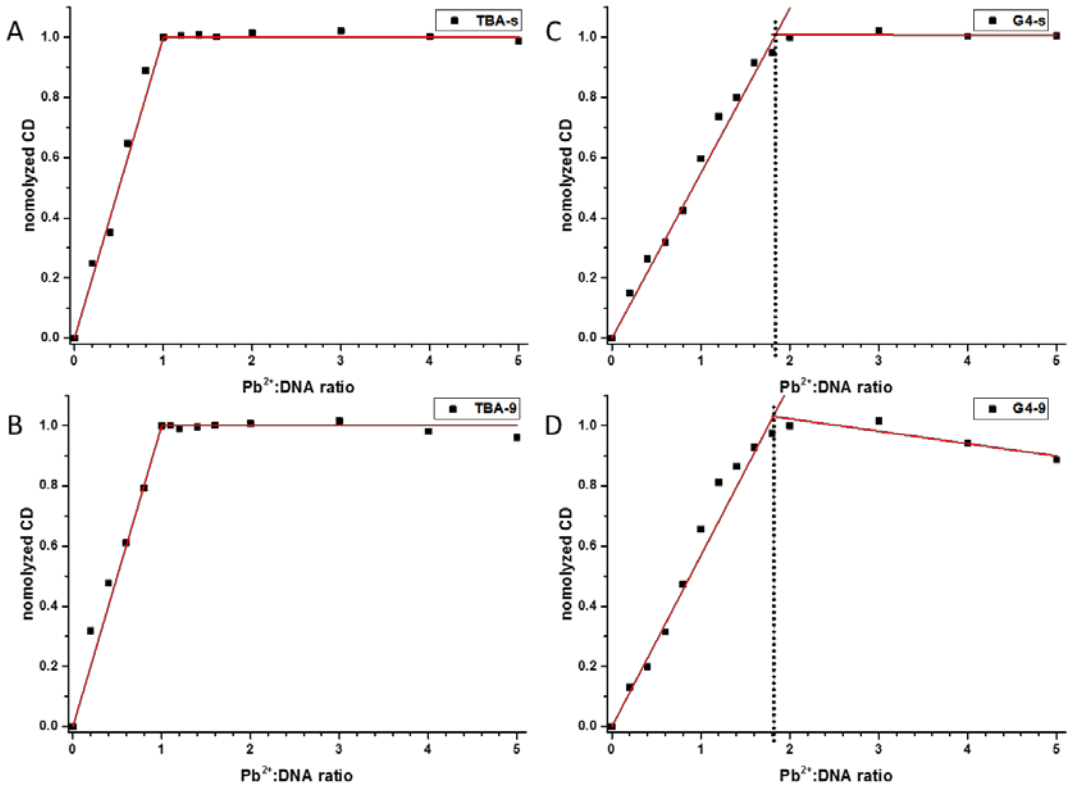


Figure S6. The change of fluorescence intensity of 0.2 μM TBA-9 (A) and G4-9 (B) titrated with increasing concentrations of Pb^{2+} in 10 mM Tris-HAc (pH 7.5) buffer. The insert shows the linear relationship between the change of fluorescence intensity and the logarithm of Pb^{2+} concentrations. The results represent the mean of three measurements, and the error bars represent the standard deviation.

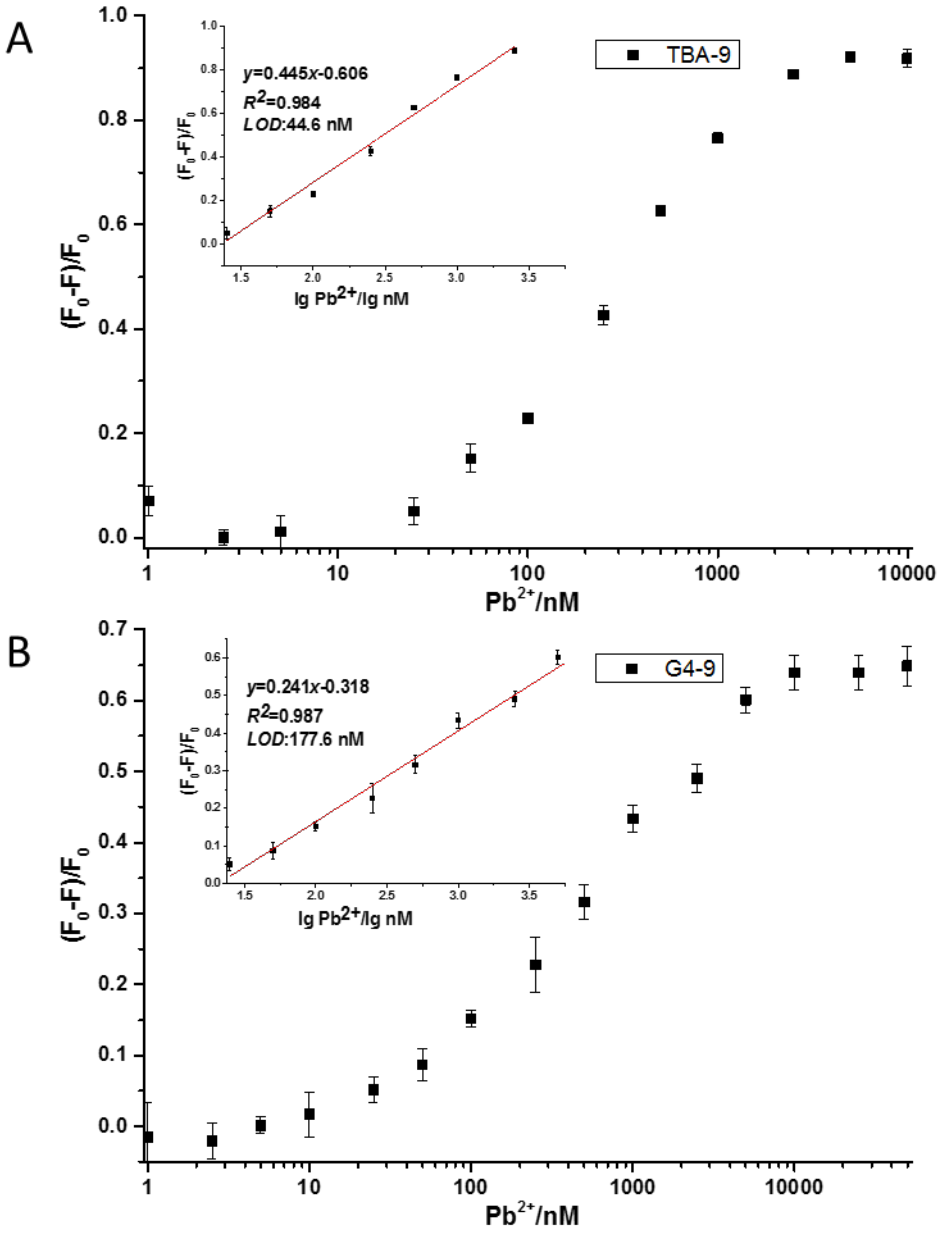


Figure S7. Binding curves showing the bound G-quadruplex probe as a function of Pb^{2+} concentrations. The solid squares show experimental data collected from fluorescence anisotropy (A) and fluorescence intensity (B).

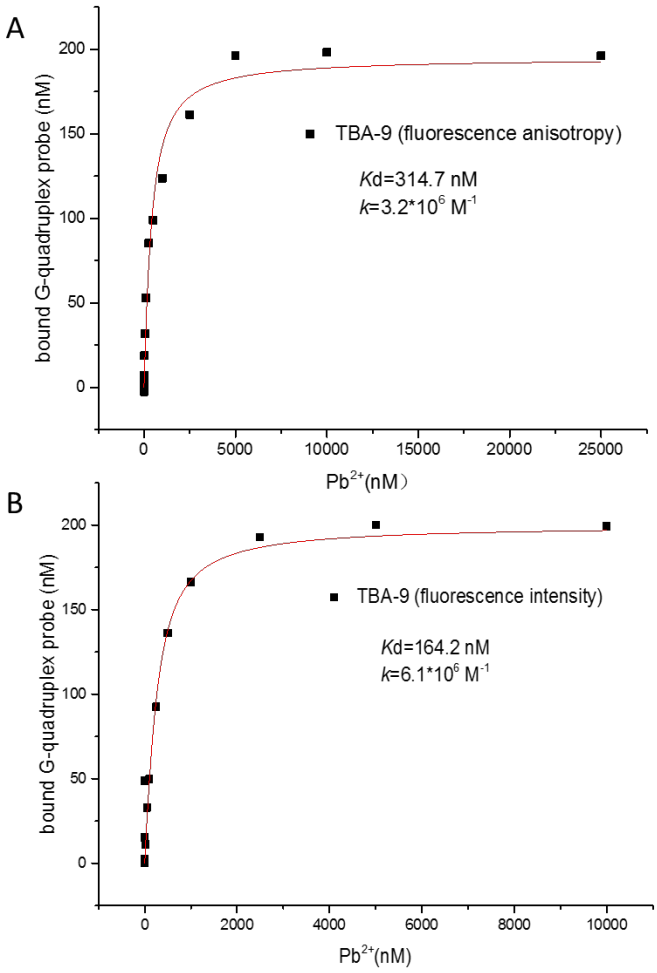


Figure S8. The effect of pH on fluorescence anisotropy of 0.2 μM TBA-9 (solid line) and G4-9 (dash line) at the presence of 10 μM Pb^{2+}. Different pH values of Tris-HAc buffers (10 mM) were used. The results represent the mean of three measurements, and the error bars represent the standard deviation.

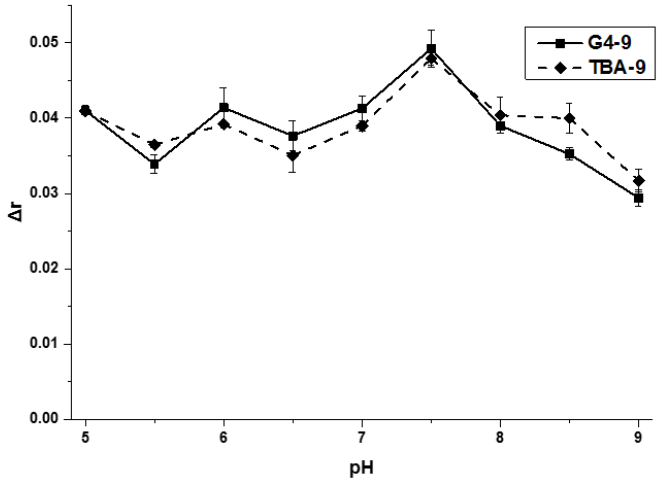


Figure S9. The change of fluorescence anisotropy (Δr) of 0.2 μM TBA-9 and G4-9. The concentration of metal ions: Pb^{2+}, Hg^{2+}, Ag^{+}: 5μM. Both probes showed notable response to Hg^{2+}, which might attribute to the T-Hg^{2+}-T interaction, while Ag^{+} did not trigger the obvious fluorescence anisotropy variation of G4-9 and TBA-9.

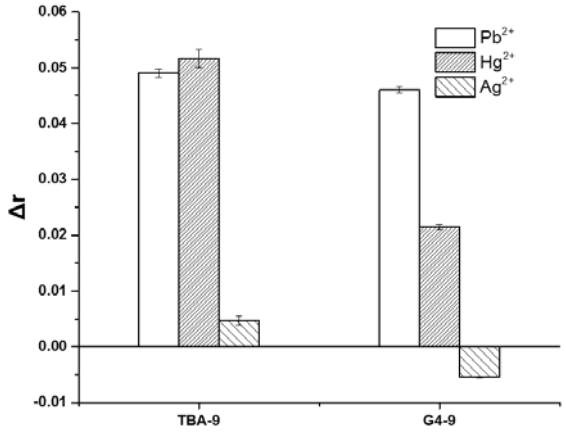


Figure S10. The change of fluorescence anisotropy (Δ*r*) of 0.2 μM TBA-9 titrated with increasing concentrations of Pb^{2+} at the presence of 10 mM K^{+}(A) or 100 mM Na^{+}(B) in 10 mM Tris-HAc (pH 7.5) buffer. The inserts show the linear relationship between the changes of fluorescence anisotropy (Δ*r*) and the logarithm of Pb^{2+} concentrations. The results represent the mean of three measurements, and the error bars represent the standard deviation.

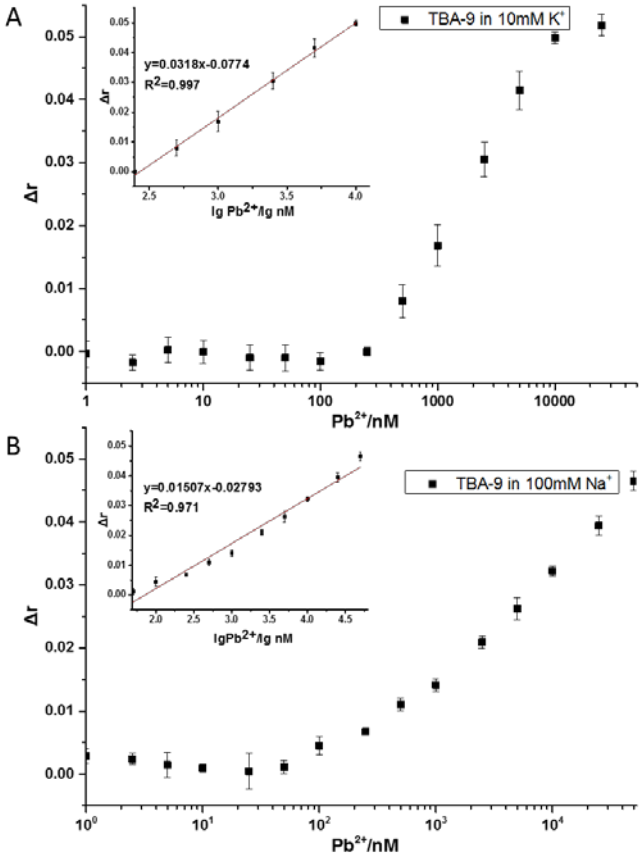
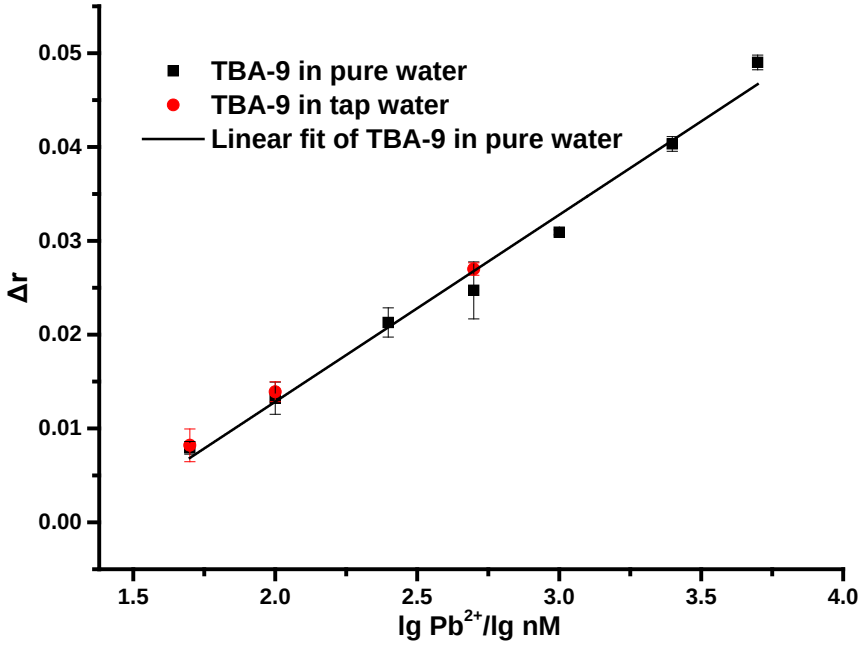
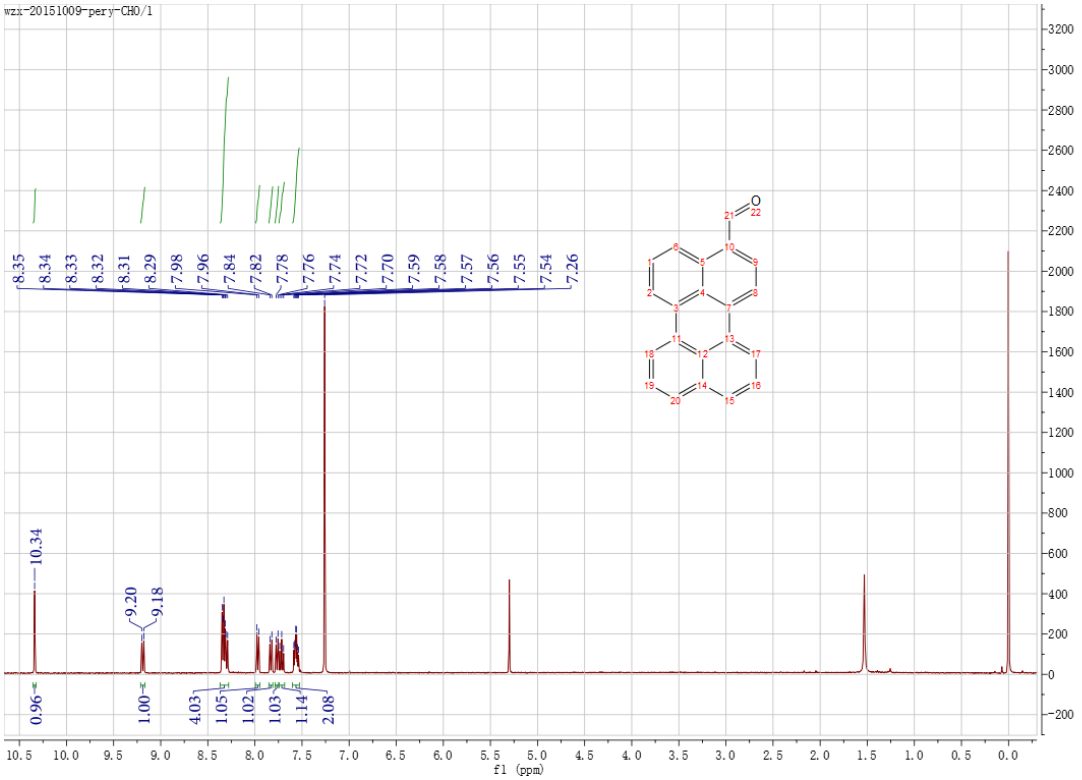
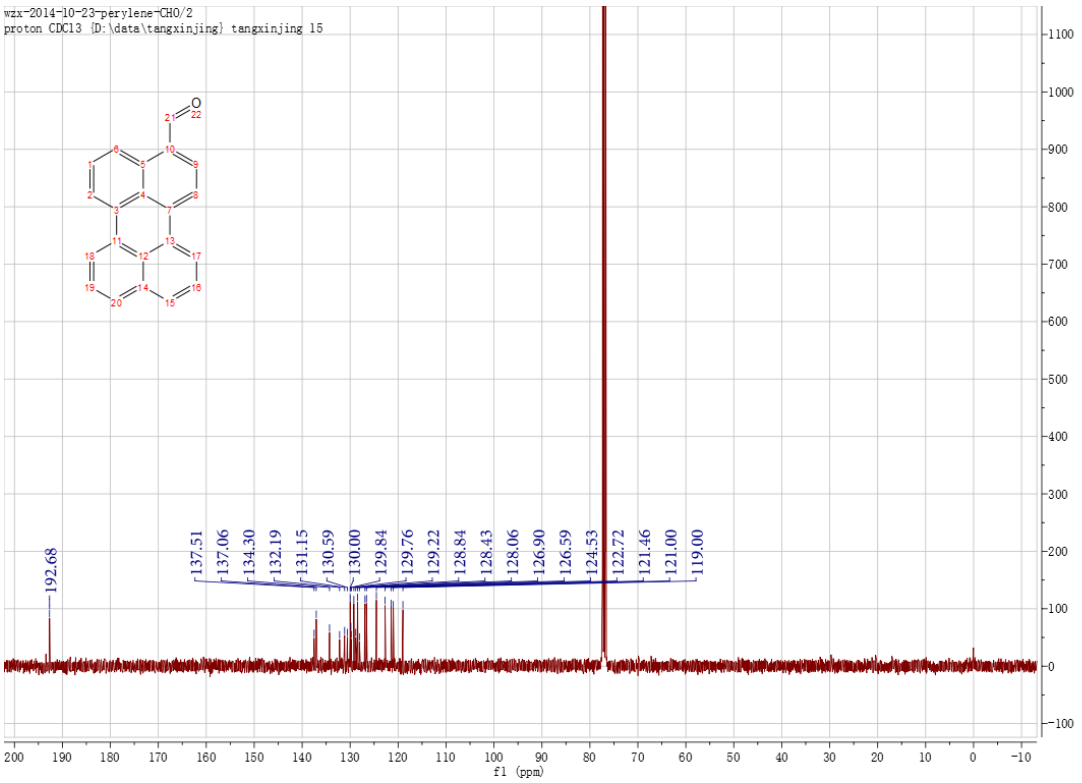


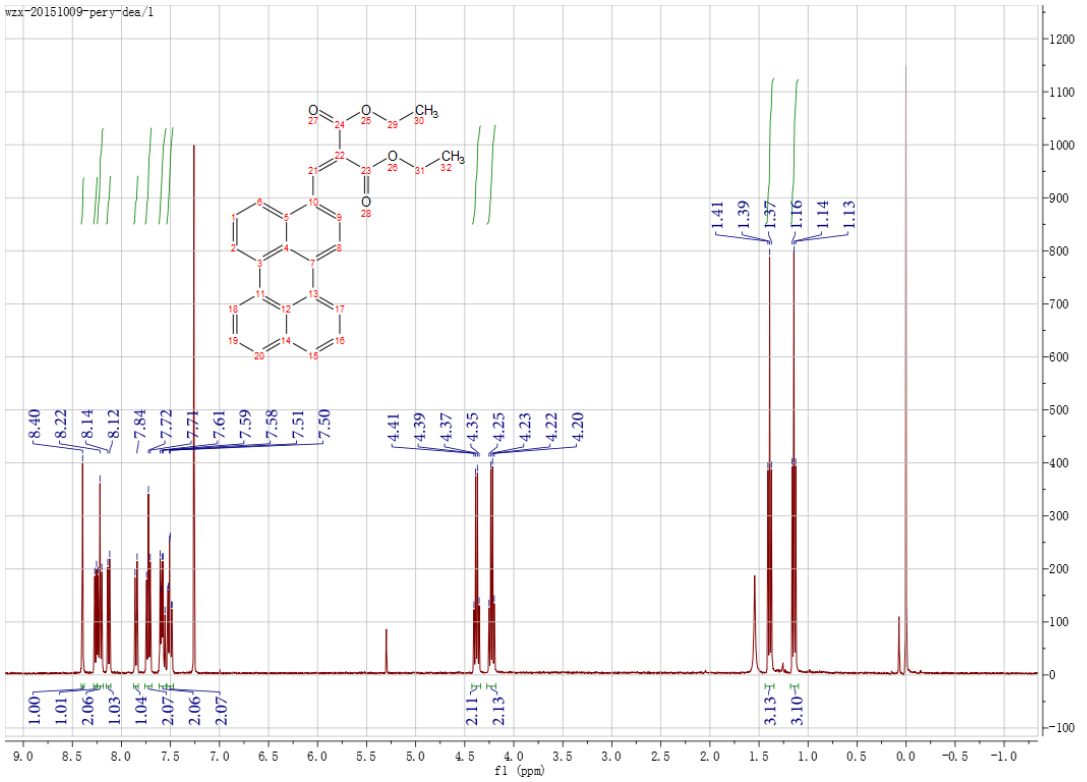
Figure S11. TBA-9 was used for recovery experiments in tap water samples. Tris-HAc (pH 7.5) buffer: 10 mM; oligonucleotides: 0.2 μM. Three different concentrations (50nM, 100nM, 500nM) of Pb^{2+} were added respectively.



The ^{1}H-NMR, ^{13}C-NMR, ^{31}P-NMR and MS spectra in these studies



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8.22
8.14
8.12
7.84
7.72
7.71
7.61
7.59
7.58
7.51
7.50
4.41
4.39
4.37
4.35
4.25
4.23
4.22
4.20
1.41
1.39
1.37
1.16
1.14
1.13
1.00
1.01
2.06
1.03
1.04
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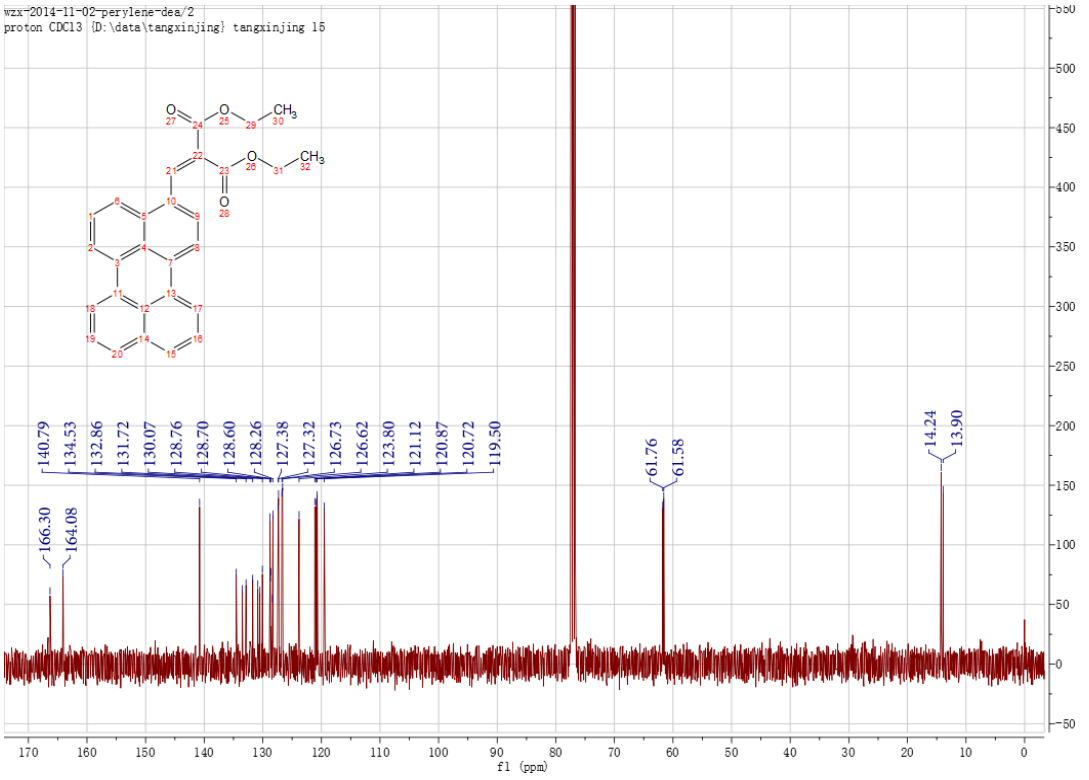
2.11
2.13
3.13
3.10
f1 (ppm)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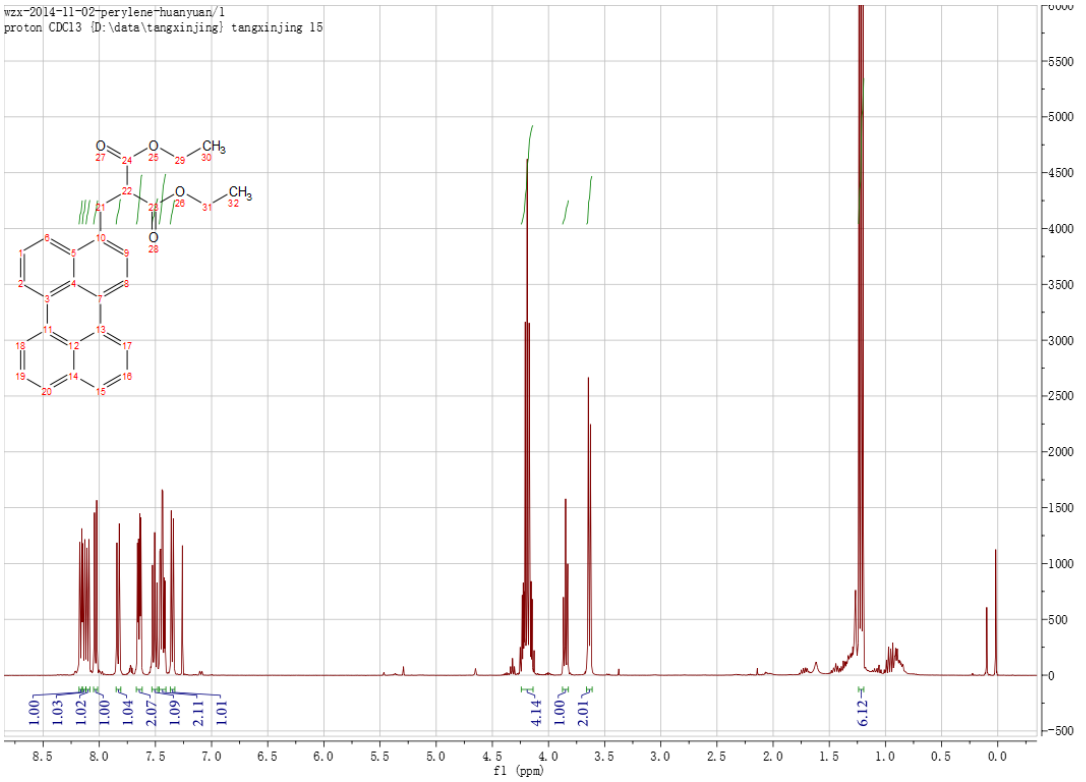
134.53
132.86
131.72
130.07
128.76
128.70
128.60
128.26
127.38
127.32
126.73
126.62
123.80
121.12
120.87
120.72
119.50
166.30
164.08
61.76
61.58
14.24
13.90
f1 (ppm)


wzx-2014-11-02-perylene-huanyuan/1
proton CDCl3 {D:\data\tangxinjing} tangxinjing 15
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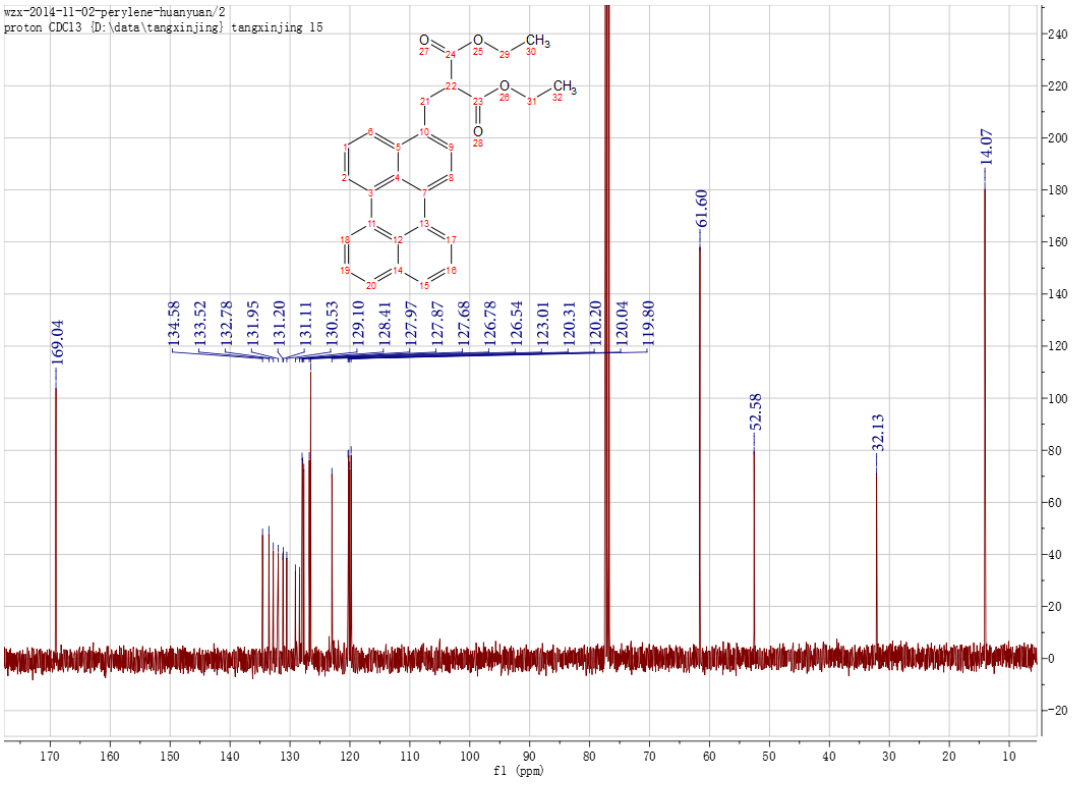
1.03
1.02
1.00
1.04
2.07
1.09
2.11
1.01
4.14
1.00
2.01
6.12
f1 (ppm)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134.58
133.52
132.78
131.95
131.20
131.11
130.53
129.10
128.41
127.97
127.87
127.68
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126.54
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120.31
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120.04
119.80
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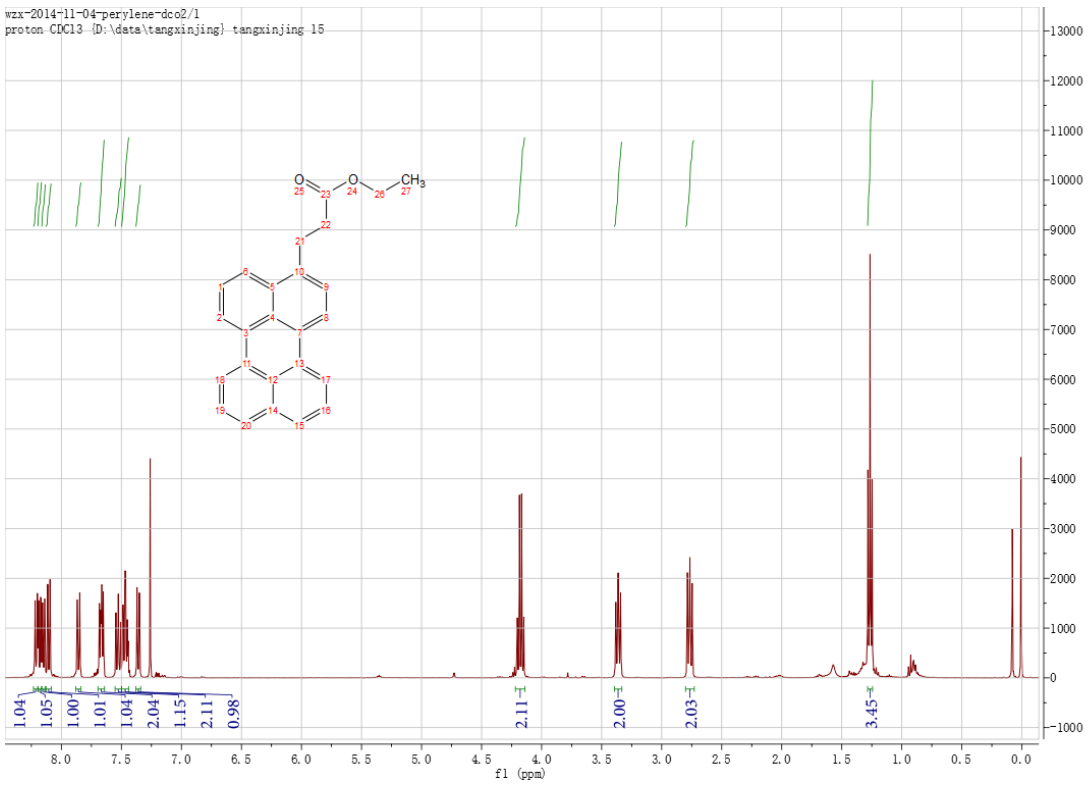
52.58
32.13
14.07
f1 (ppm)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proton CDCl3 {D:\data\tangxinjing} tangxinjing 15
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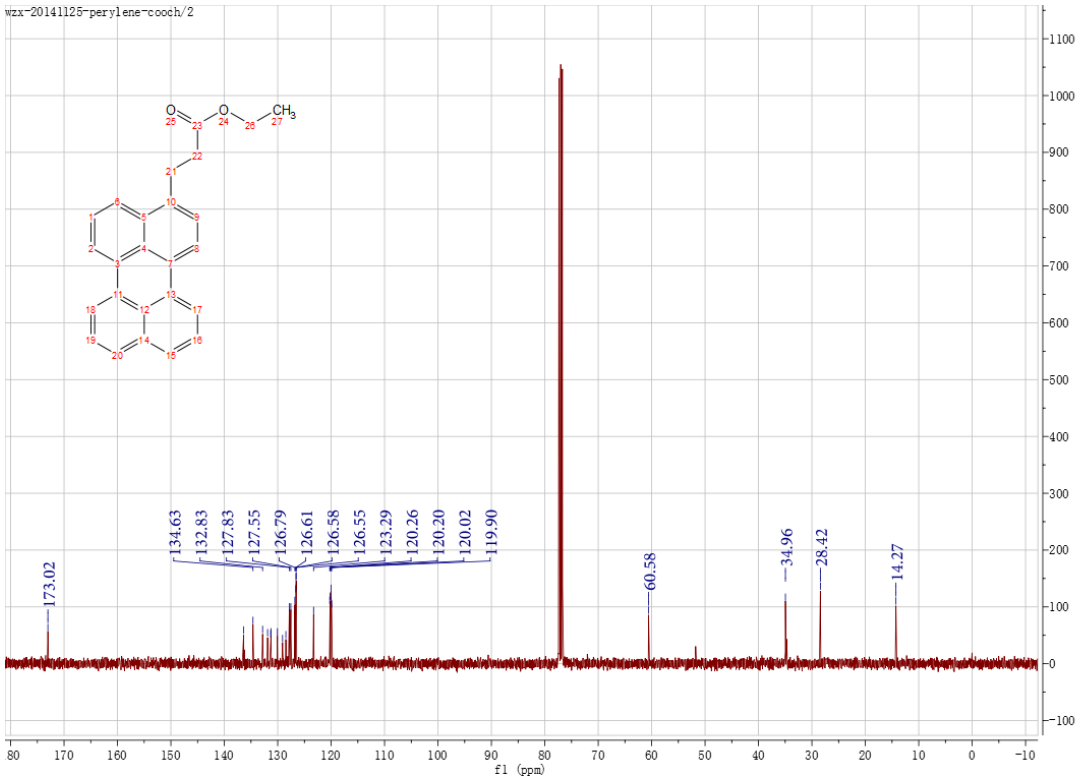
1.01
1.04
2.04
1.15
2.11
0.98
2.11
2.00
2.03
3.45
f1 (ppm)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127.83
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120.02
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34.96
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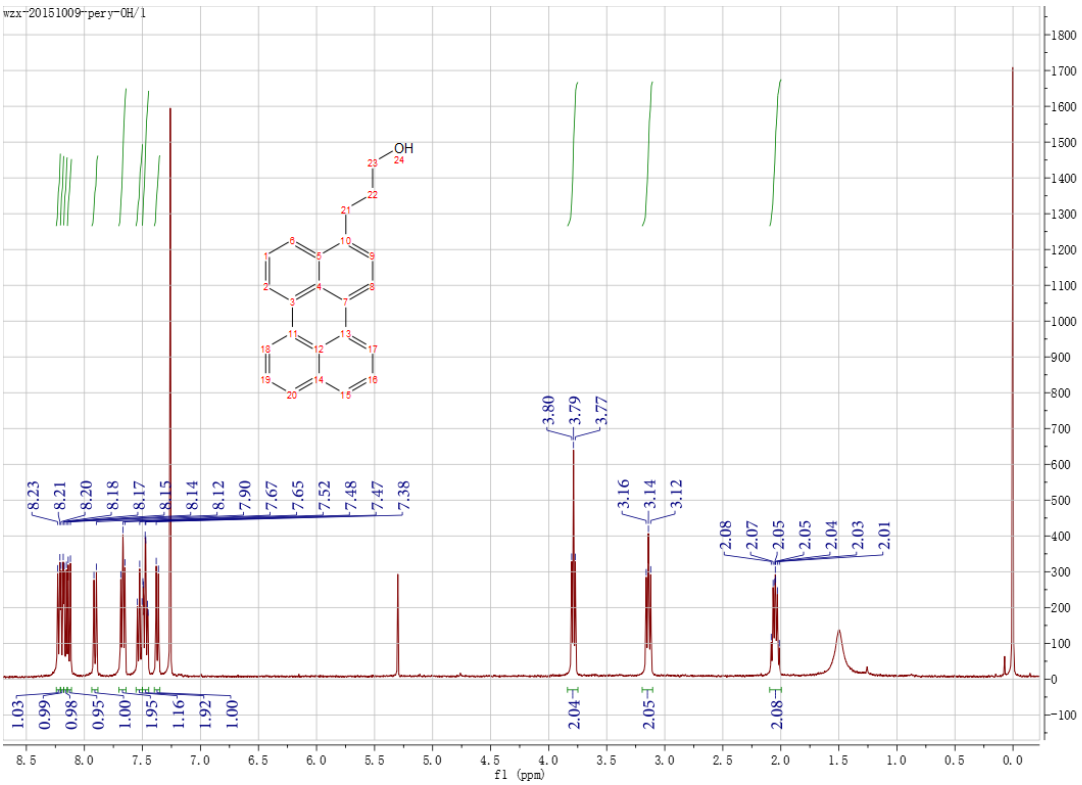
14.27
f1 (ppm)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OH
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8.14
8.12
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7.48
7.47
7.38
3.80
3.79
3.77
3.16
3.14
3.12
2.08
2.07
2.05
2.05
2.04
2.03
2.01
1.03
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0.95
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1.92
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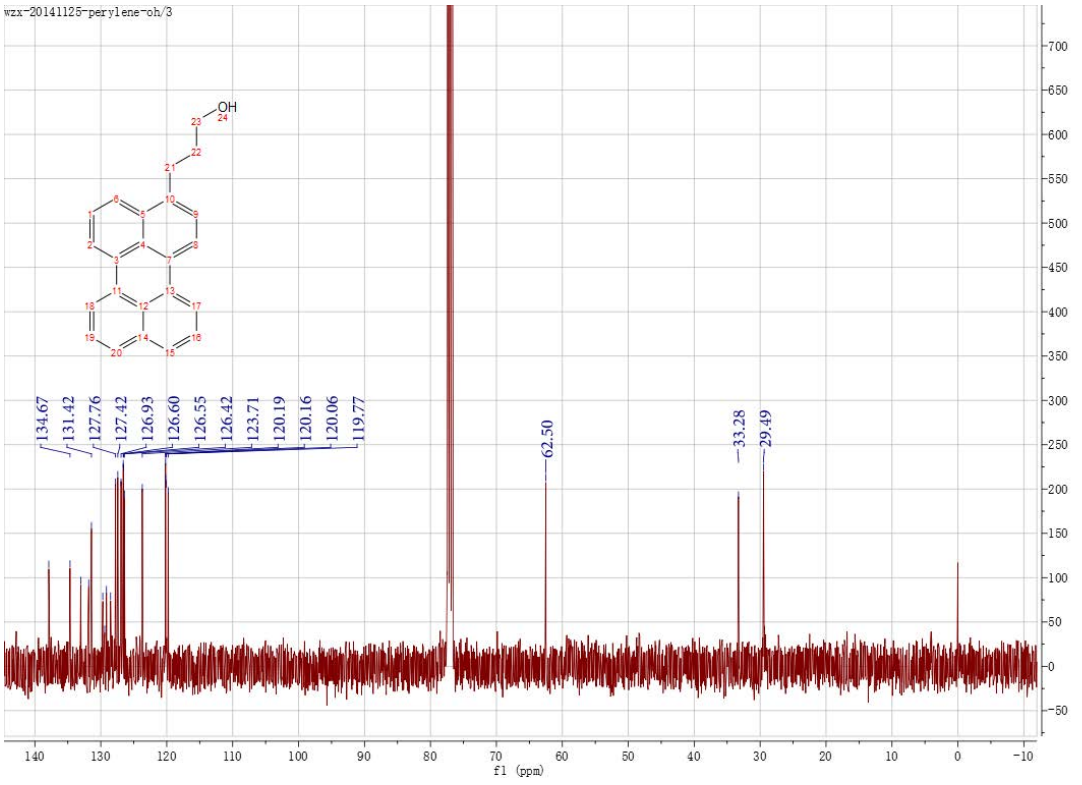
2.05
2.08
f1 (ppm)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OH
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131.42
127.76
127.42
126.93
126.60
126.55
126.42
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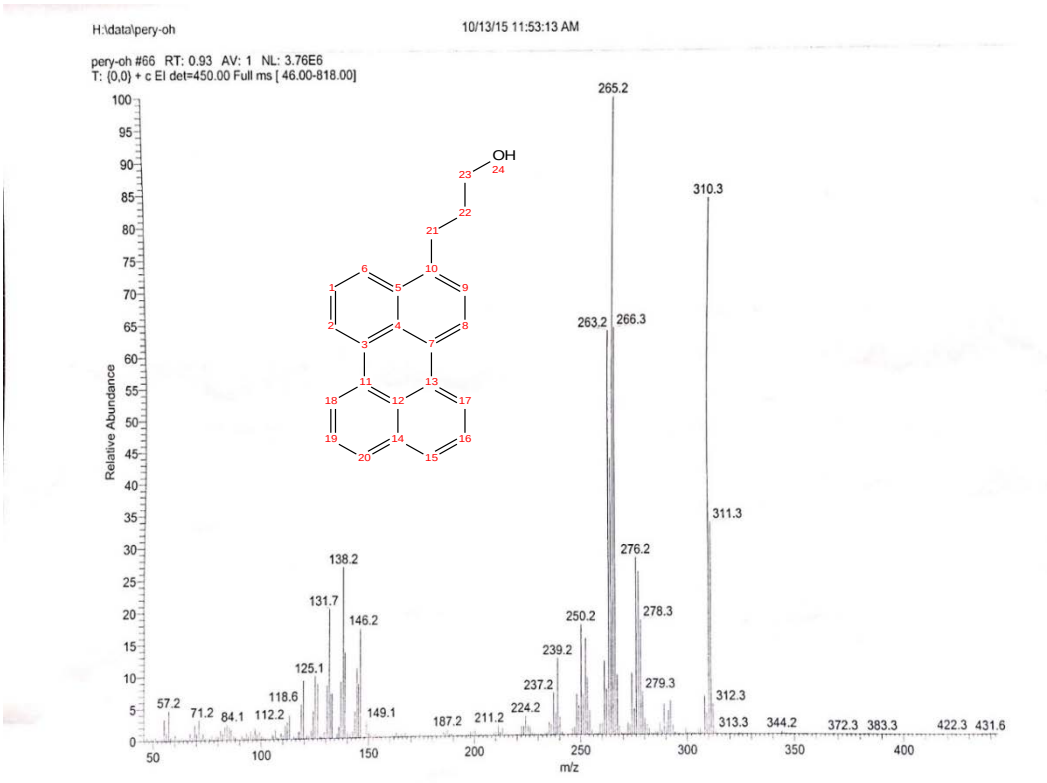
119.77
62.50
33.28
29.49
f1 (ppm)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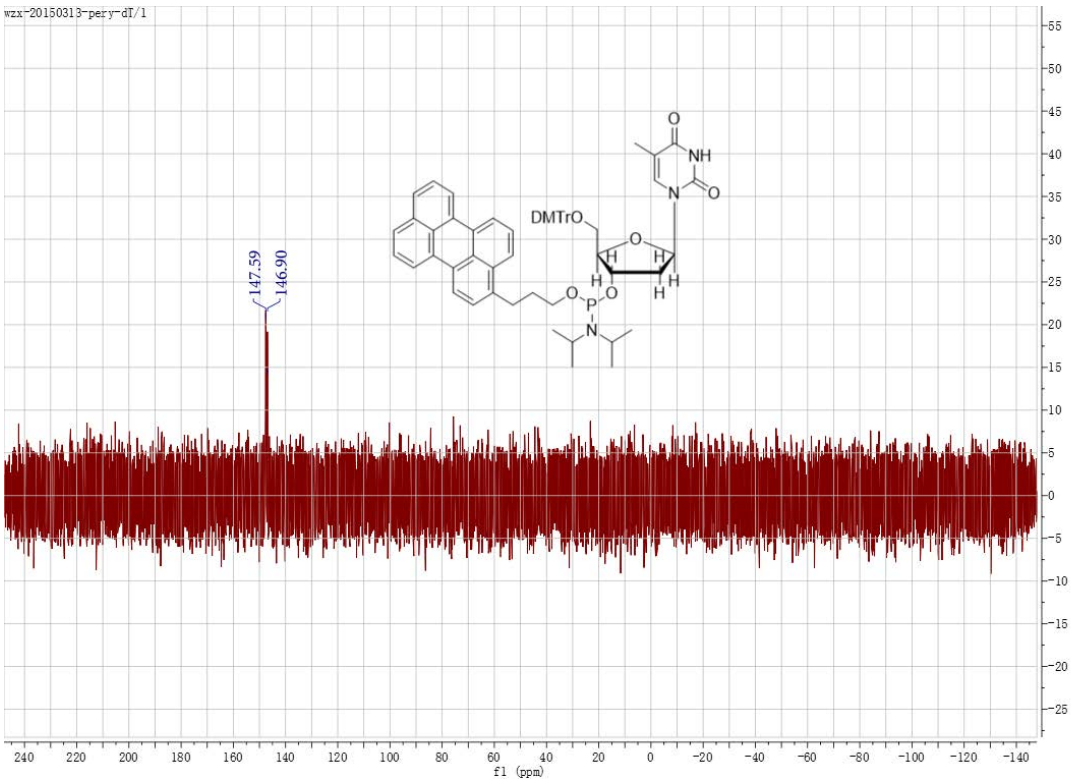
pery-oh #66 RT: 0.93 AV: 1 NL: 3.76E6
T: {0,0} + c EI det=450.00 Full ms [46.00-818.00]
Relative Abundance
m/z
265.2
310.3
263.2
266.3
311.3
276.2
138.2
131.7
146.2
278.3
250.2
239.2
125.1
237.2
279.3
118.6
312.3
57.2
71.2
84.1
112.2
149.1
187.2
211.2
224.2
313.3
344.2
372.3
383.3
422.3
431.6
OH

T^pery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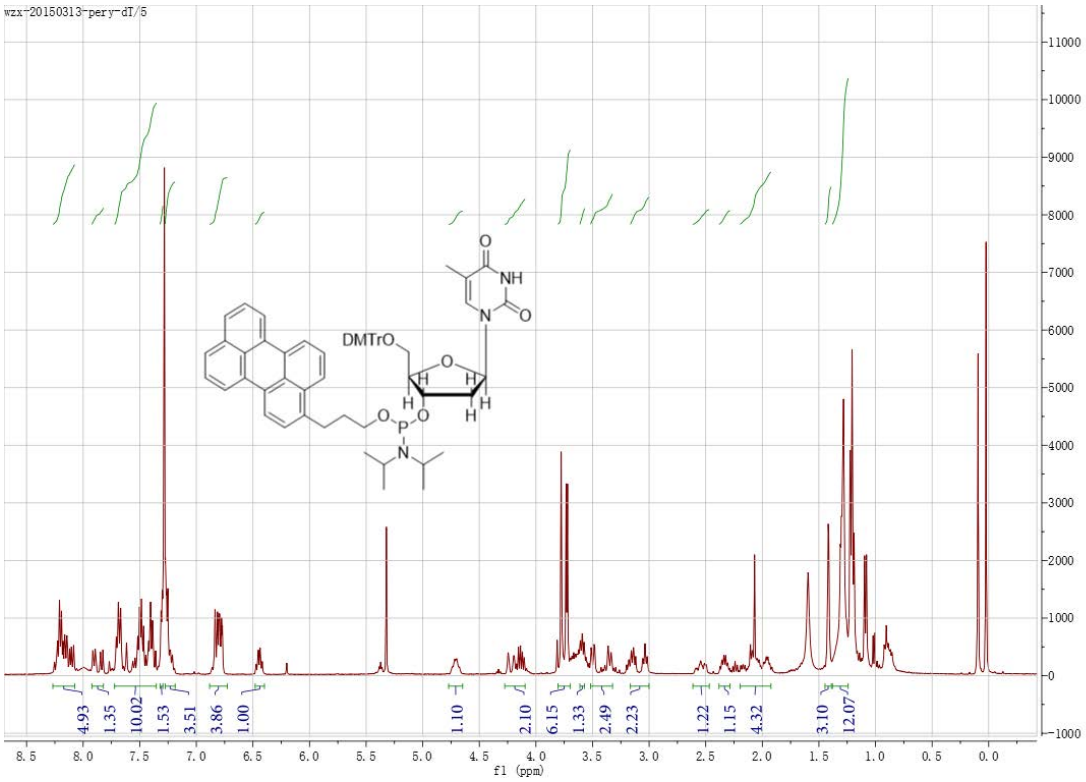
146.90
DMTrO
NH
f1 (ppm)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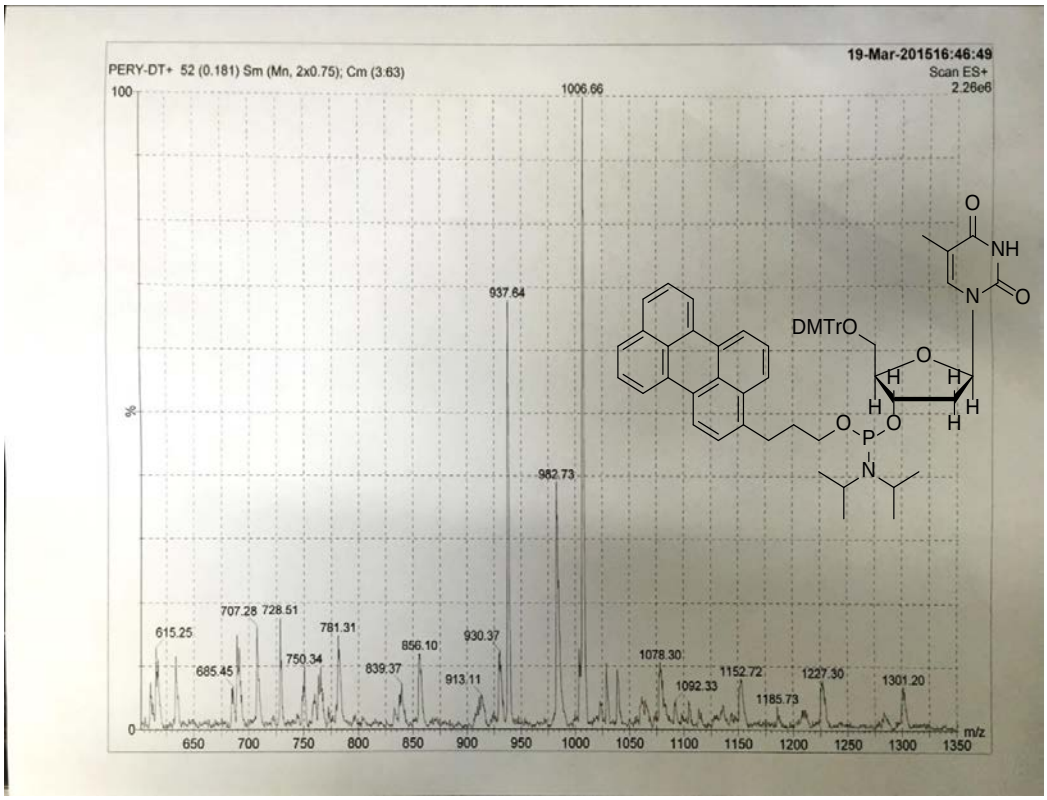
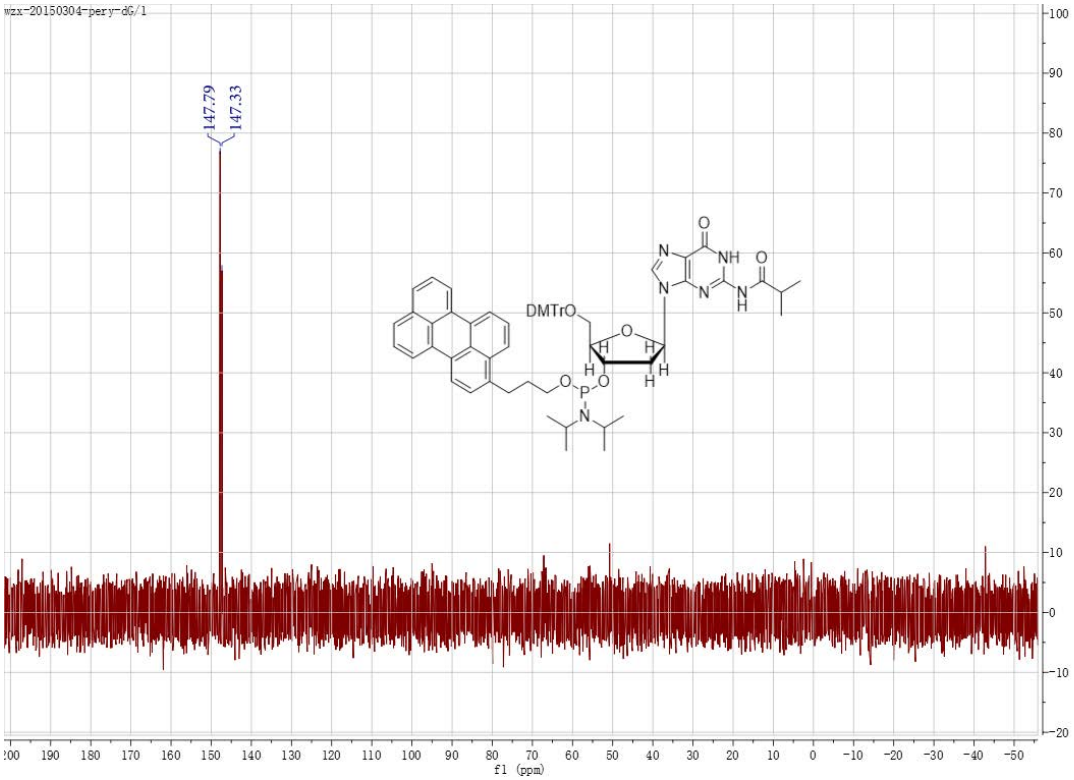
DMTrO
NH
O
N
P
H
4.93
1.35
10.02
1.53
3.51
3.86
1.00
1.10
2.10
6.15
1.33
2.49
2.23
1.22
1.15
4.32
3.10
12.07
f1 (ppm)


PERY-DT+ 52 (0.181) Sm (Mn, 2x0.75); Cm (3:63)
19-Mar-201516:46:49
Scan ES+
2.26e6
1006.66
937.64
982.73
DMTrO
NH
O
N
P
H
615.25
685.45
707.28
728.51
750.34
781.31
839.37
856.10
913.11
930.37
1078.30
1092.33
1152.72
1185.73
1227.30
1301.20
%
m/z

G^{pery}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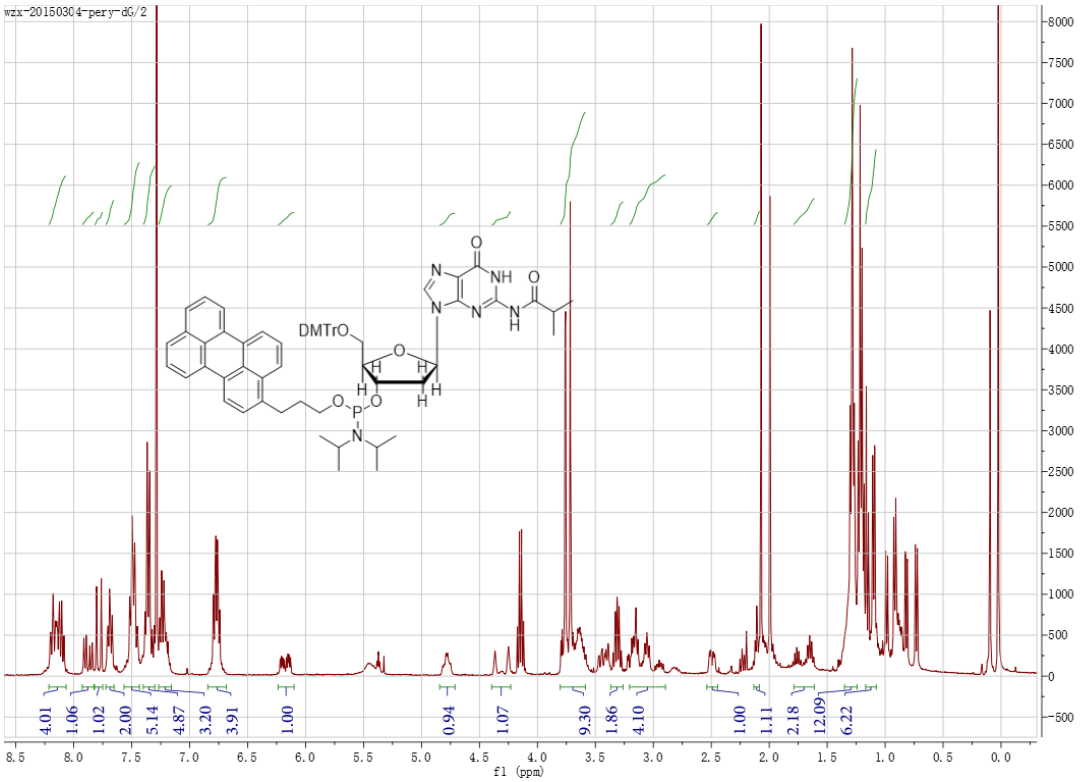
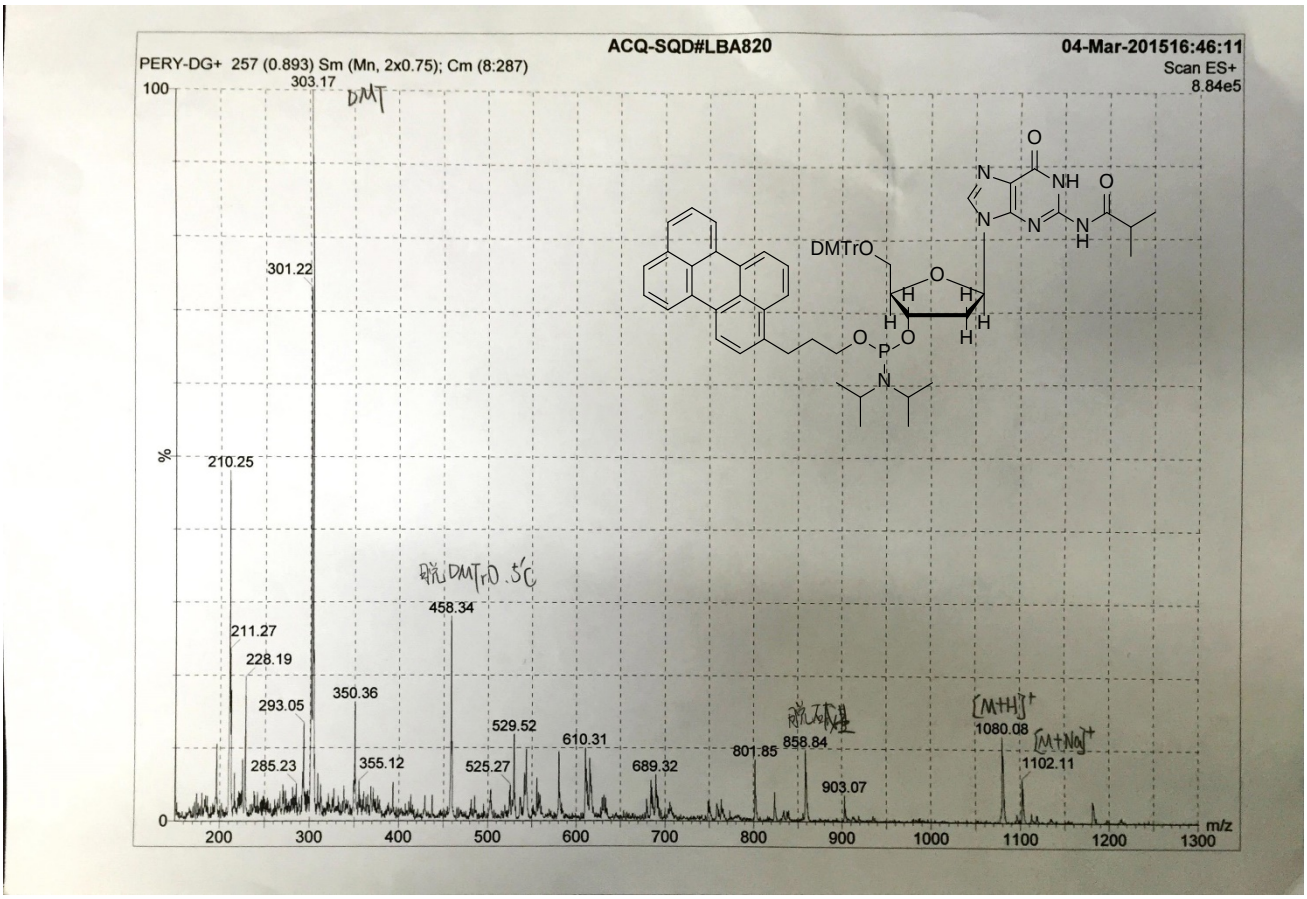
147.33
DMTrO
f1 (ppm)


wzx-20150304-pery-dG/2
DMTrO
4.01
1.06
1.02
2.00
5.14
4.87
3.20
3.91
1.00
0.94
1.07
9.30
1.86
4.10
1.00
1.11
2.18
12.09
6.22
f1 (ppm)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Scan ES+
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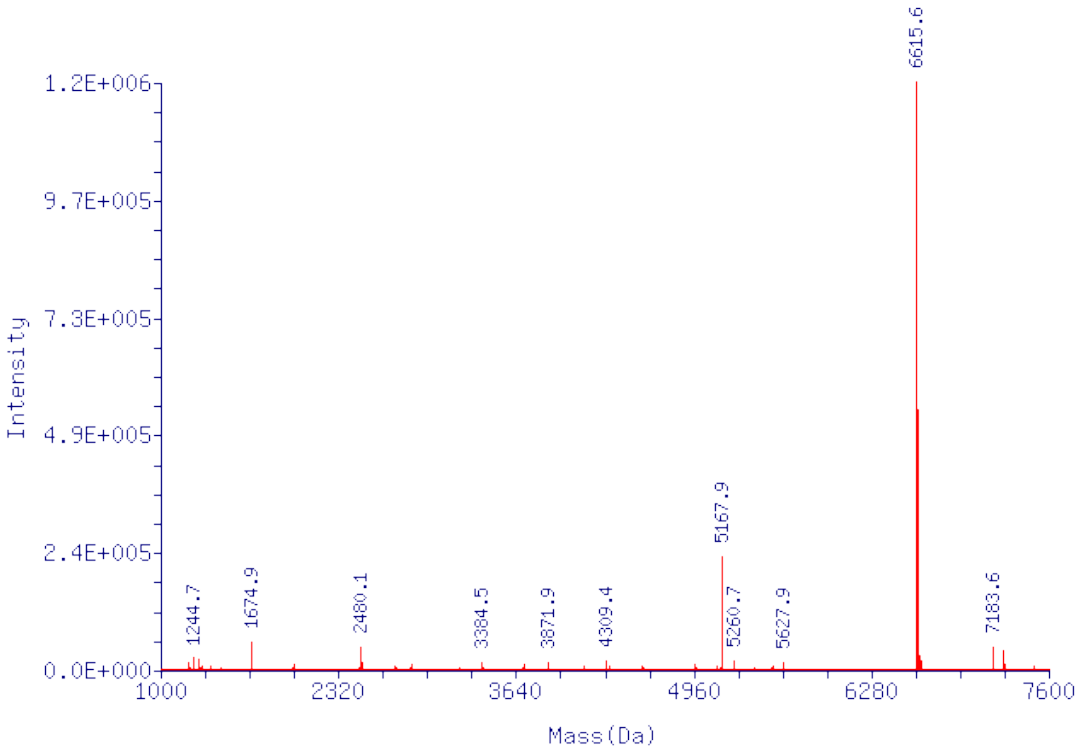
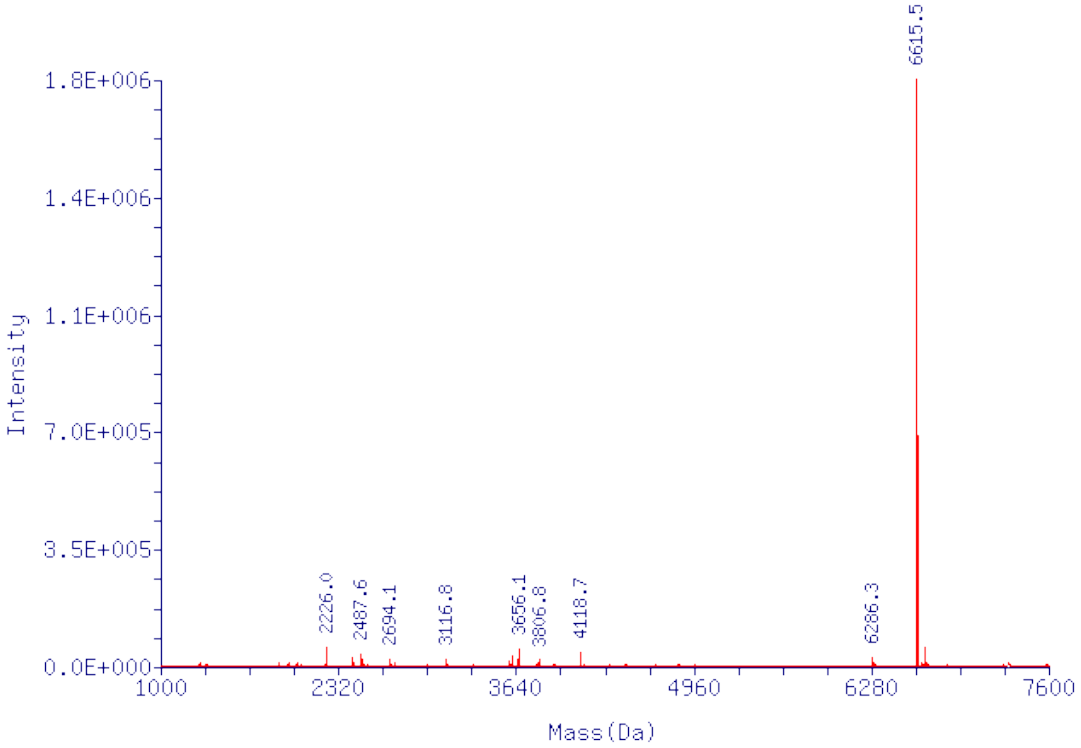
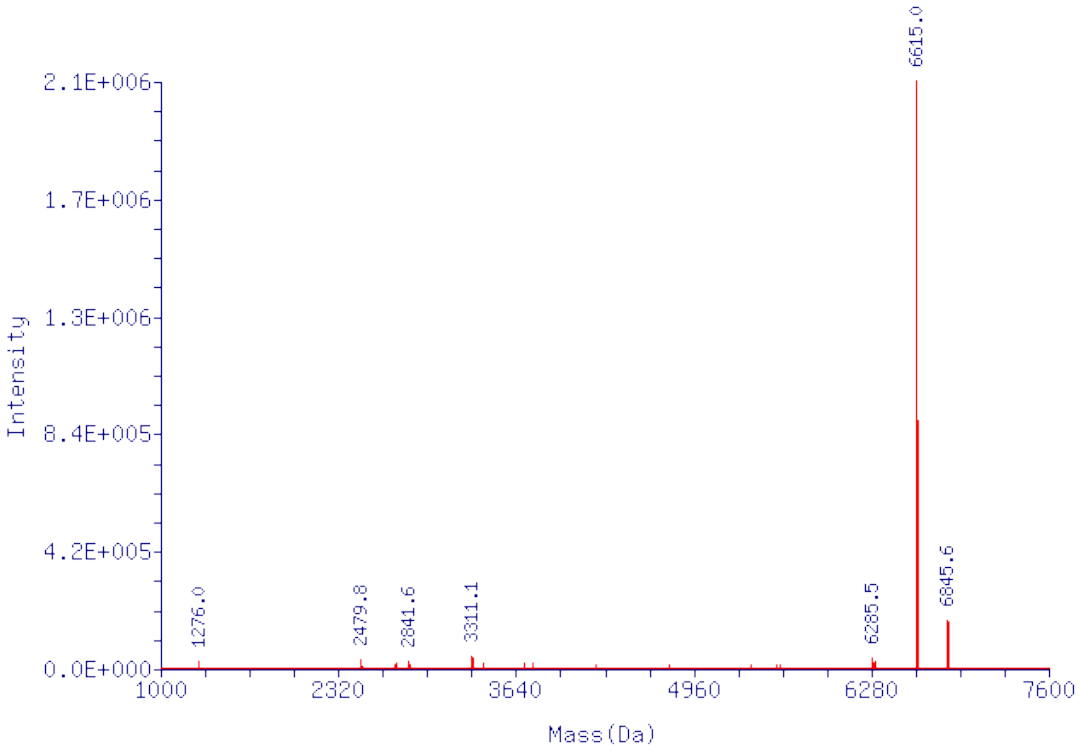
303.17
DMT
301.22
210.25
458.34
211.27
228.19
293.05
350.36
529.52
610.31
285.23
355.12
525.27
689.32
801.85
858.84
903.07
1080.08
1102.11
[M+H]+
[M+Na]+
DMTrO
%
m/z


6615.6
5167.9
1244.7
1674.9
2480.1
3384.5
3871.9
4309.4
5260.7
5627.9
7183.6
Intensity
Mass(Da)

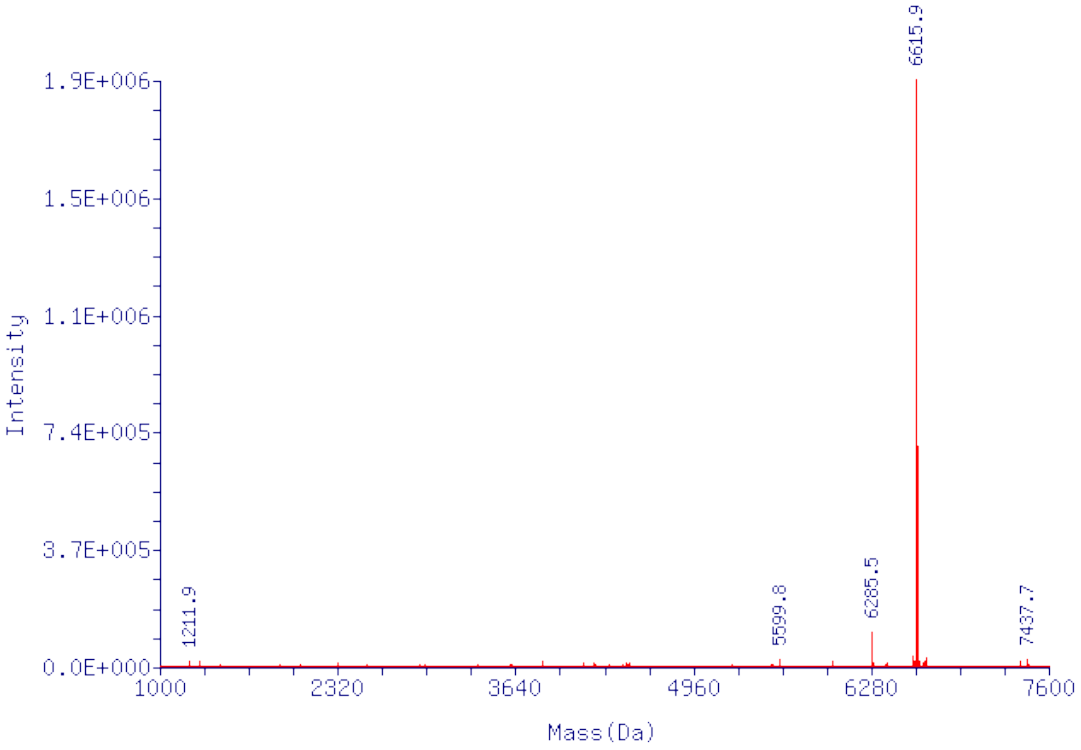
ESI-Mass spectrum of **G4-1**. Calculated: 6614.2; Measured: 6615.6.



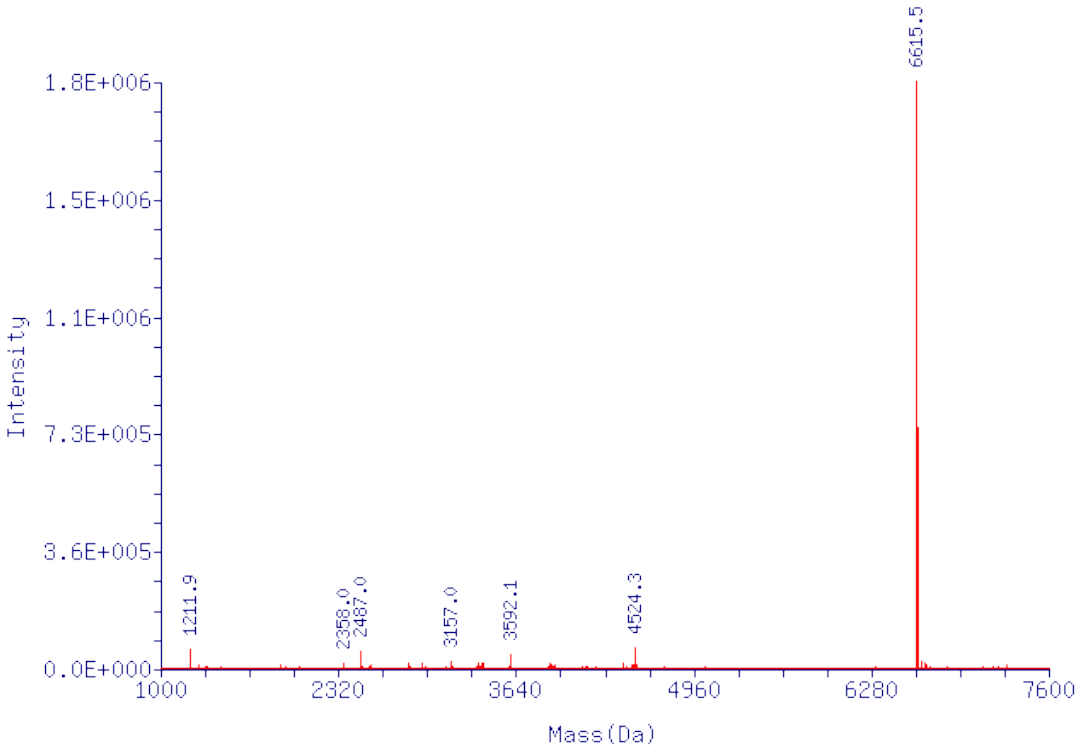
ESI-Mass spectrum of **G4-2**. Calculated: 6614.2; Measured: 6615.5.



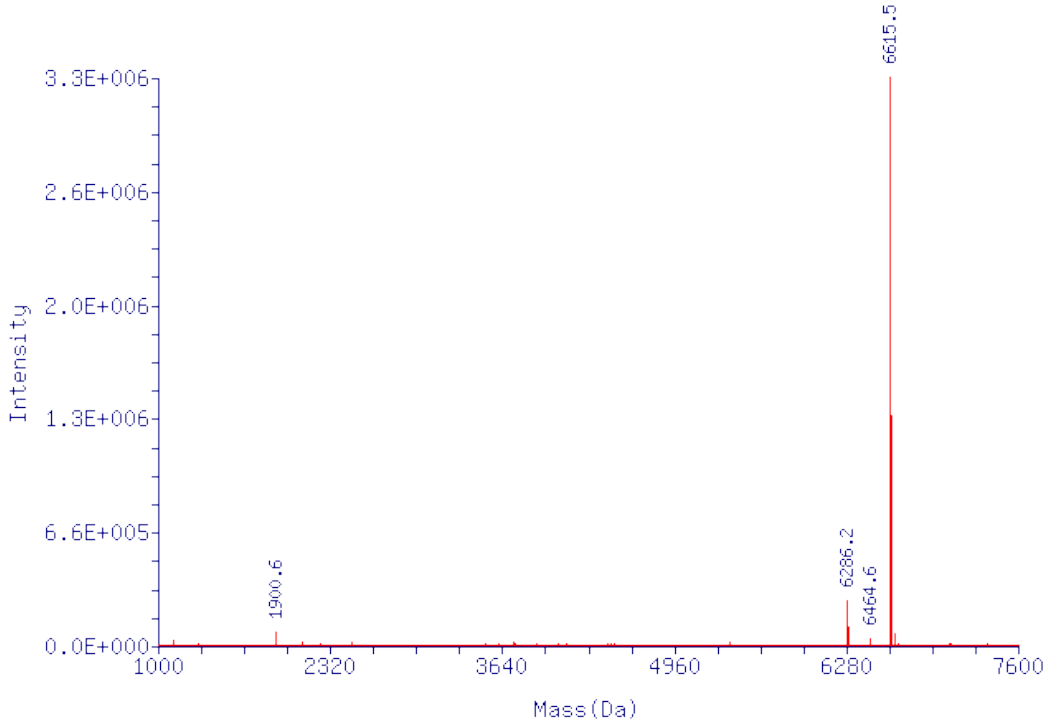
ESI-Mass spectrum of **G4-3**. Calculated: 6614.2; Measured: 6615.0.



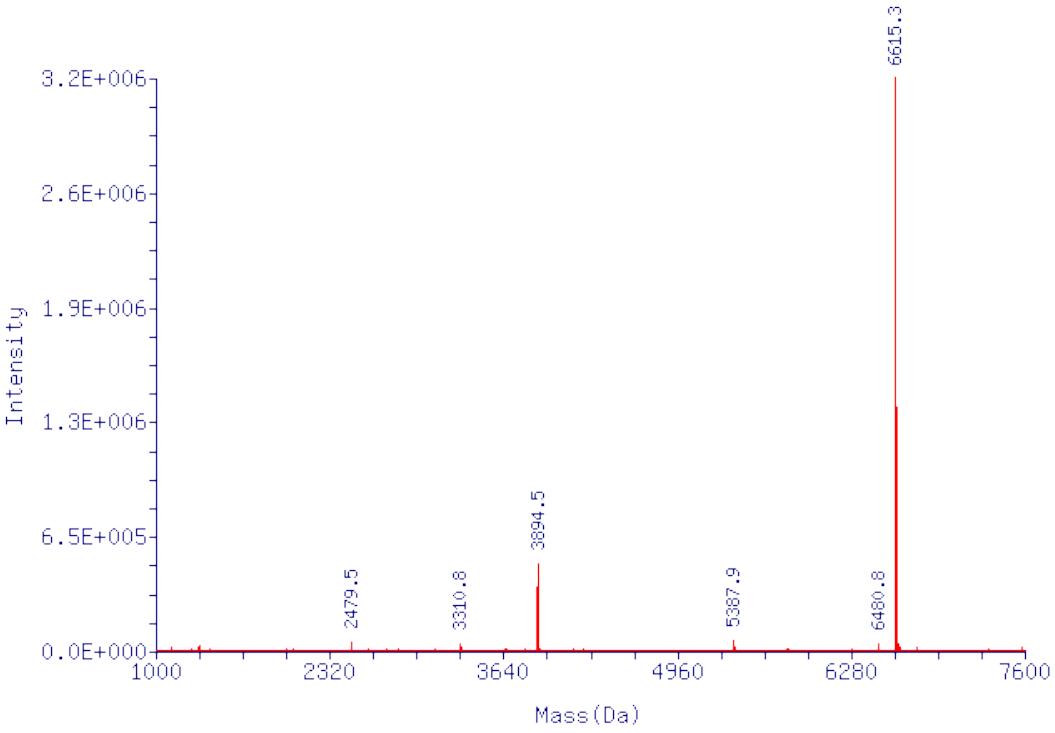
ESI-Mass spectrum of **G4-4**. Calculated: 6614.2; Measured: 6615.9.



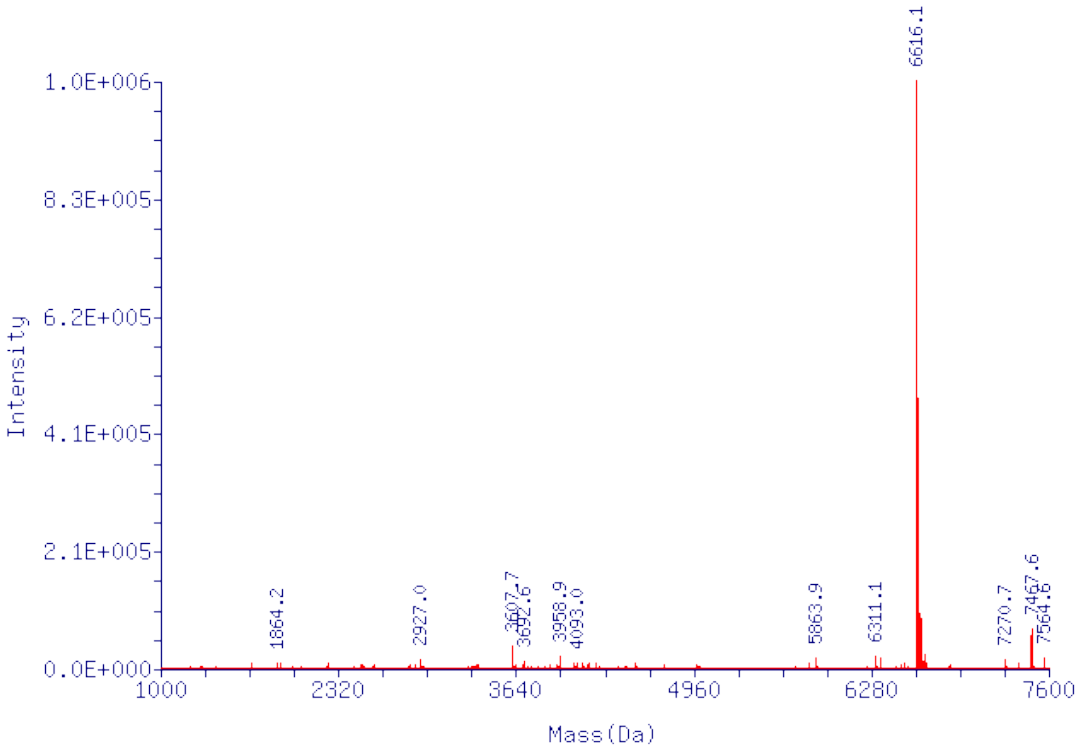
ESI-Mass spectrum of **G4-6**. Calculated: 6614.2; Measured: 6615.5.



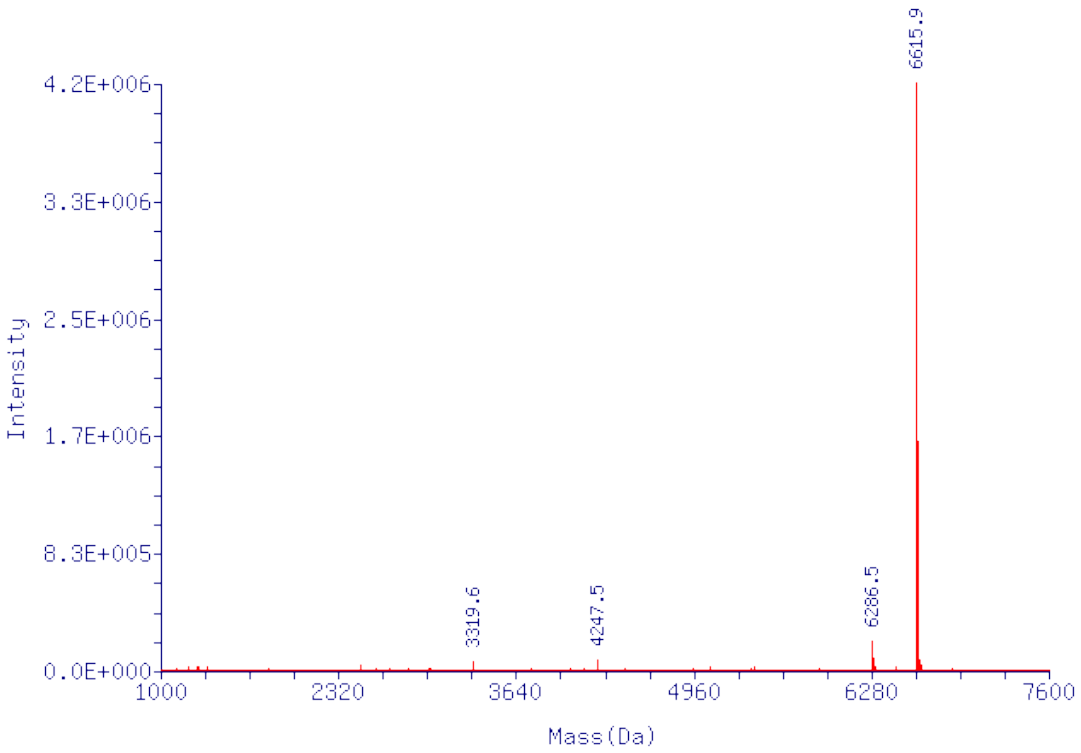
ESI-Mass spectrum of **G4-9**. Calculated: 6614.2; Measured: 6615.5.



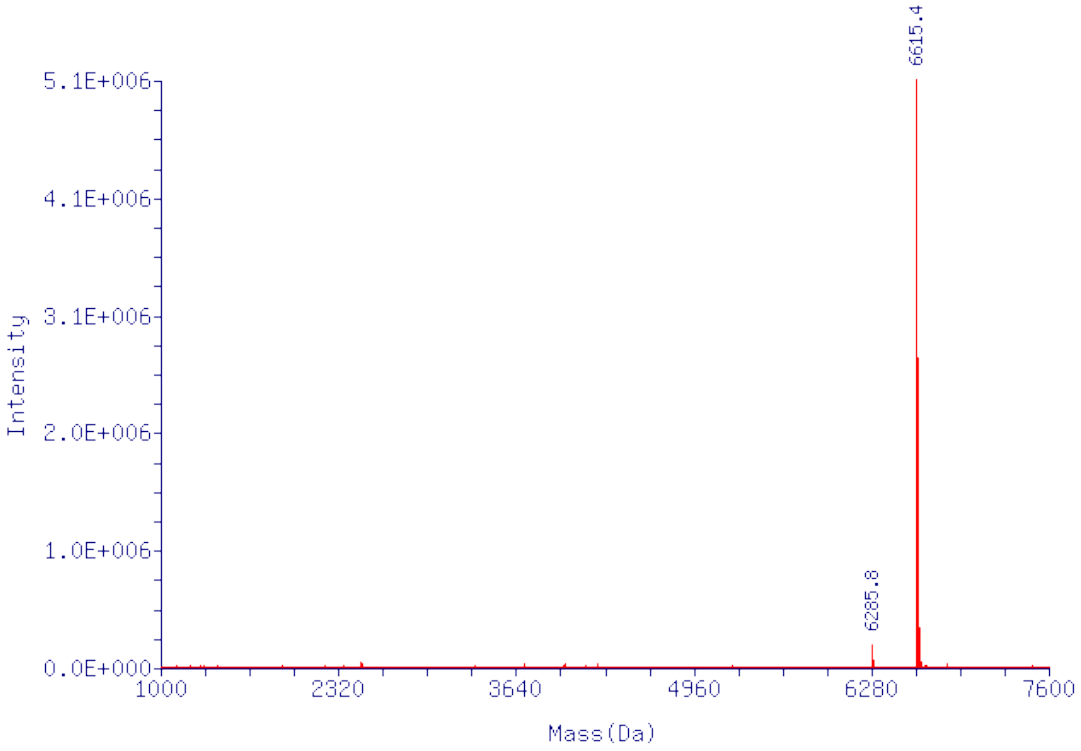
ESI-Mass spectrum of **G4-11**. Calculated: 6614.2; Measured: 6615.3.



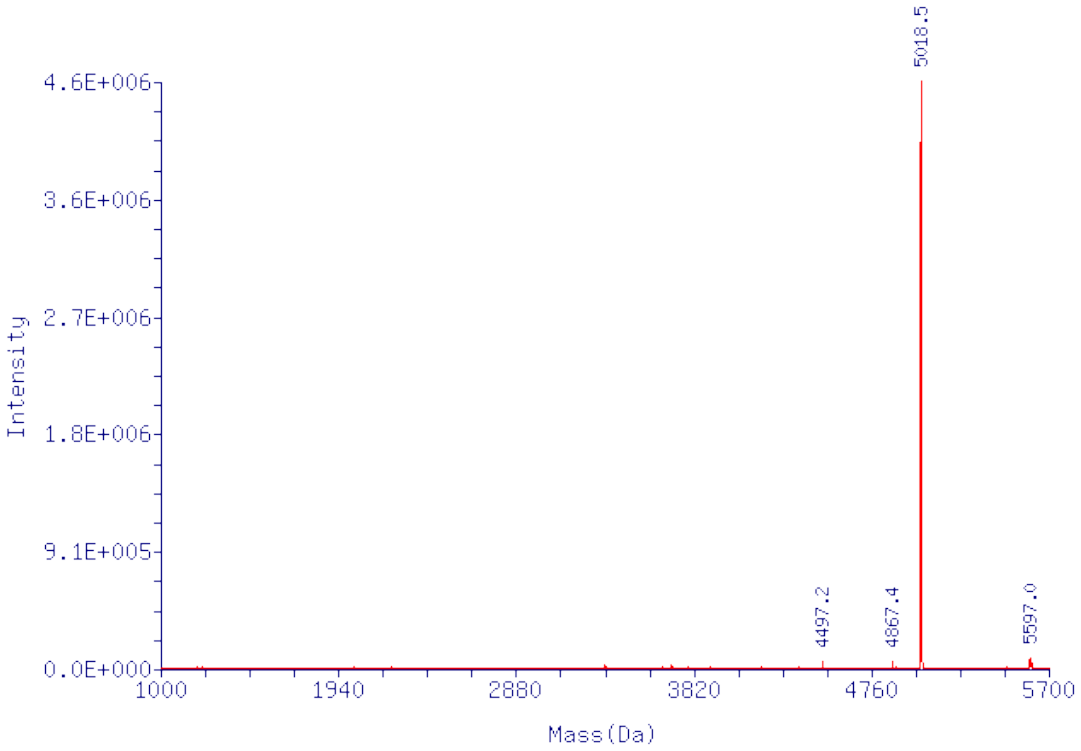
ESI-Mass spectrum of **G4-14**. Calculated: 6614.2; Measured: 6615.1.



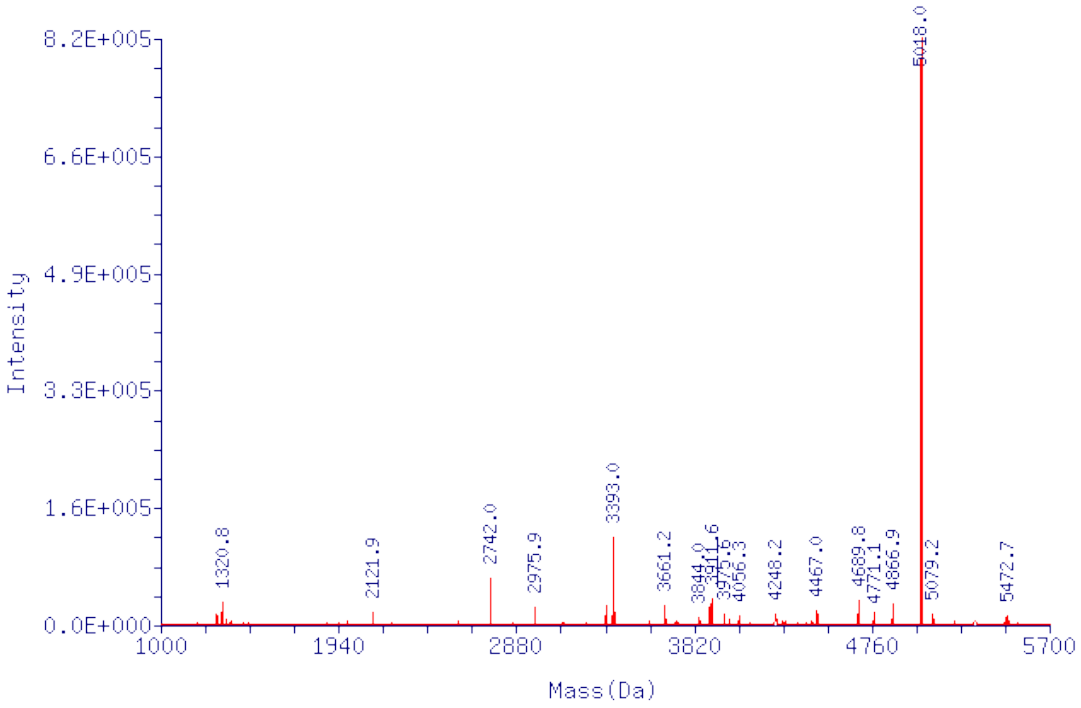
ESI-Mass spectrum of **G4-16**. Calculated: 6614.2; Measured: 6615.9.



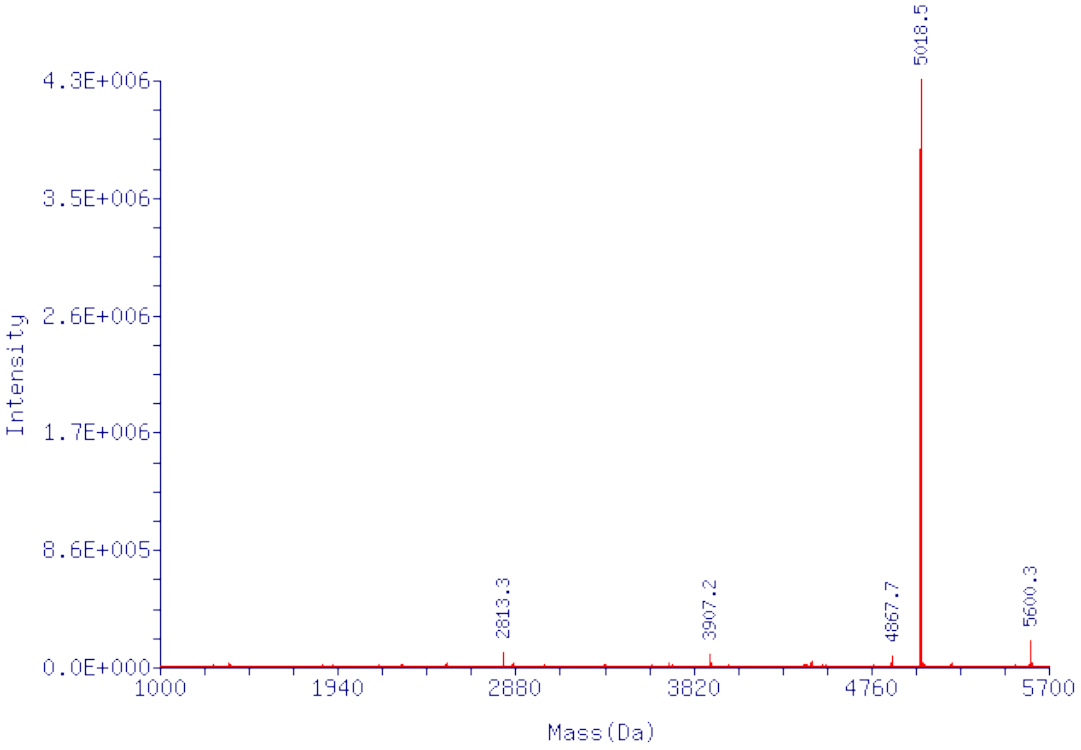
ESI-Mass spectrum of **G4-19**. Calculated: 6614.2; Measured: 6615.4.



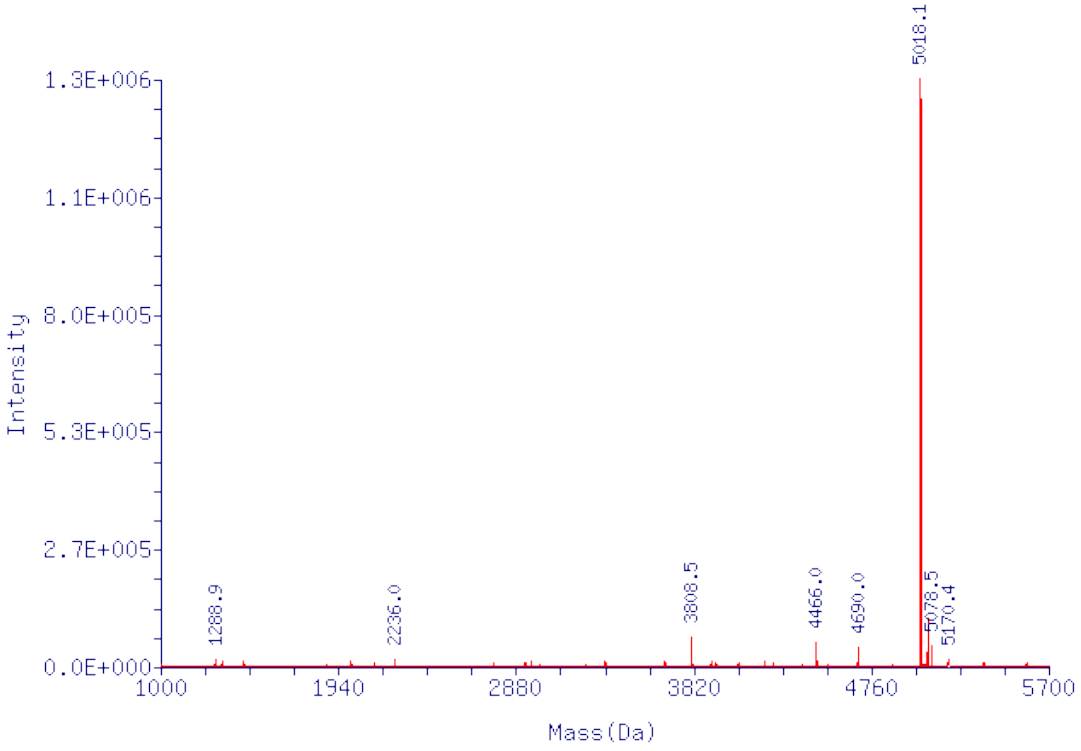
ESI-Mass spectrum of **TBA-3**. Calculated: 5018.3; Measured: 5018.5.



ESI-Mass spectrum of **TBA-4**. Calculated: 5018.3; Measured: 5018.0.



ESI-Mass spectrum of **TBA-7**. Calculated: 5018.3; Measured: 5018.5.



ESI-Mass spectrum of **TBA-9**. Calculated: 5018.3; Measured: 5018.1.

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