

Supporting information for:

Development of a wash-free homogeneous immunoassay method for the detection of
tetracycline in environmental samples

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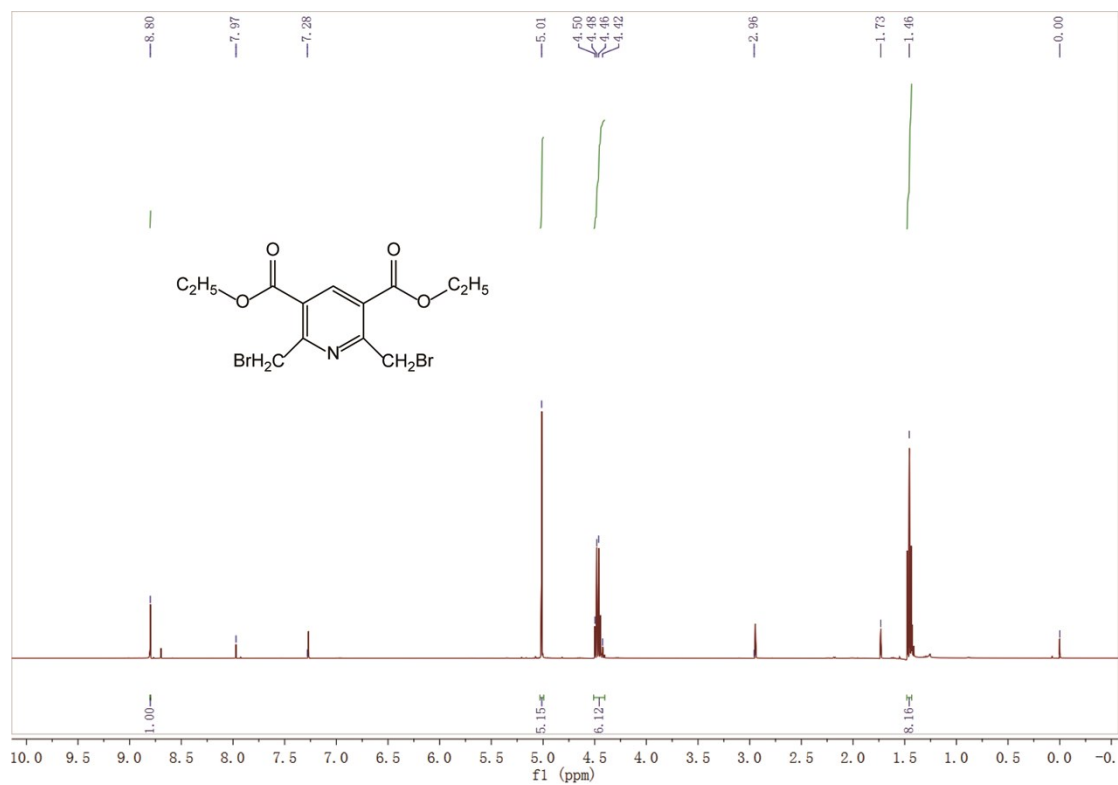
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A1-10-20190724_190723112138#128 RT: 0.81 AV: 1 NL: 4.21E5
T: ITMS + c ESI Full ms [100.00-1000.00]

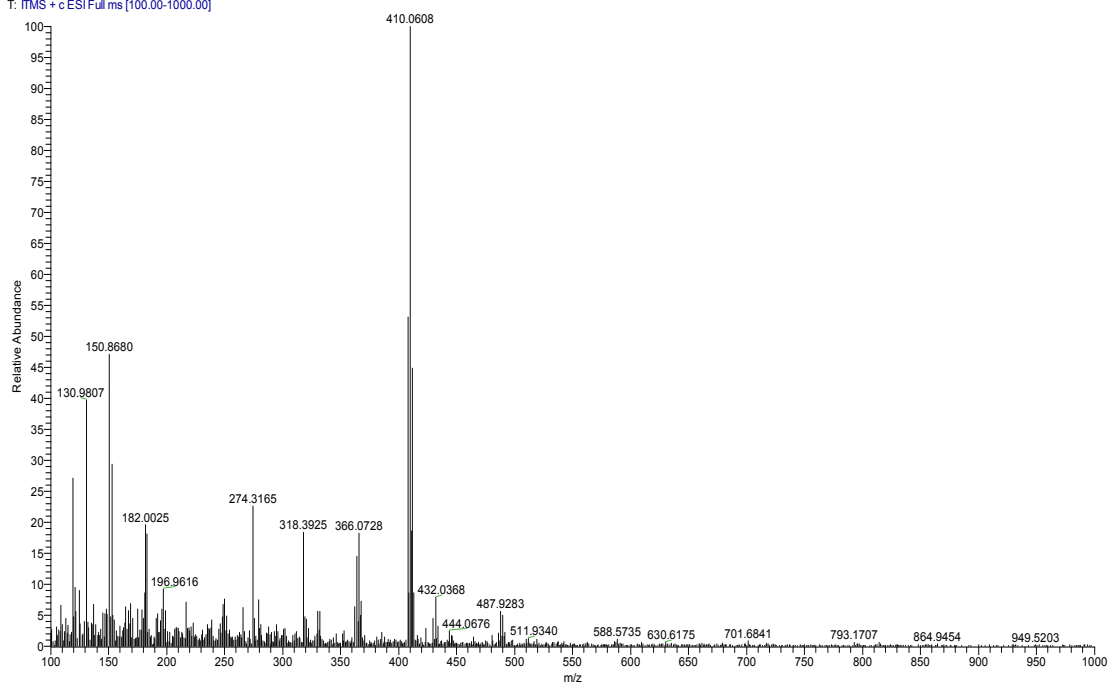
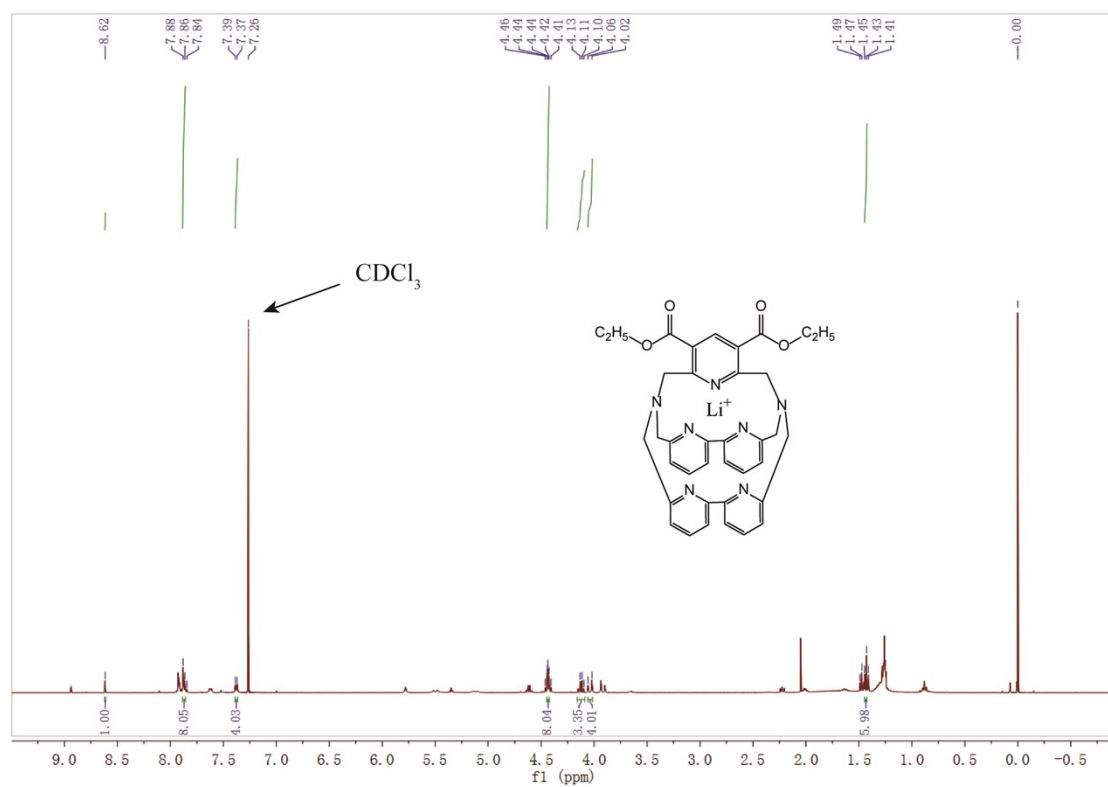


Fig. S1. The ¹H-NMR and mass spectrum of 2 (CDCl₃, 400MHz) δ: 1.45 (t, 2CH₃); 4.46 (dd, 2CH₂); 5.01 (s, 4H); 8.79 (s, 1H). MS: C₁₃H₁₅O₄NBr₂=409.09, [C₁₃H₁₅O₄NBr₂+H⁺]⁺=410.06.



A: 20190924_190925201653 #72 RT: 0.13 AV: 1 NL: 5.46E7
T: ITMS + c ESI Full ms [150.00-750.00]

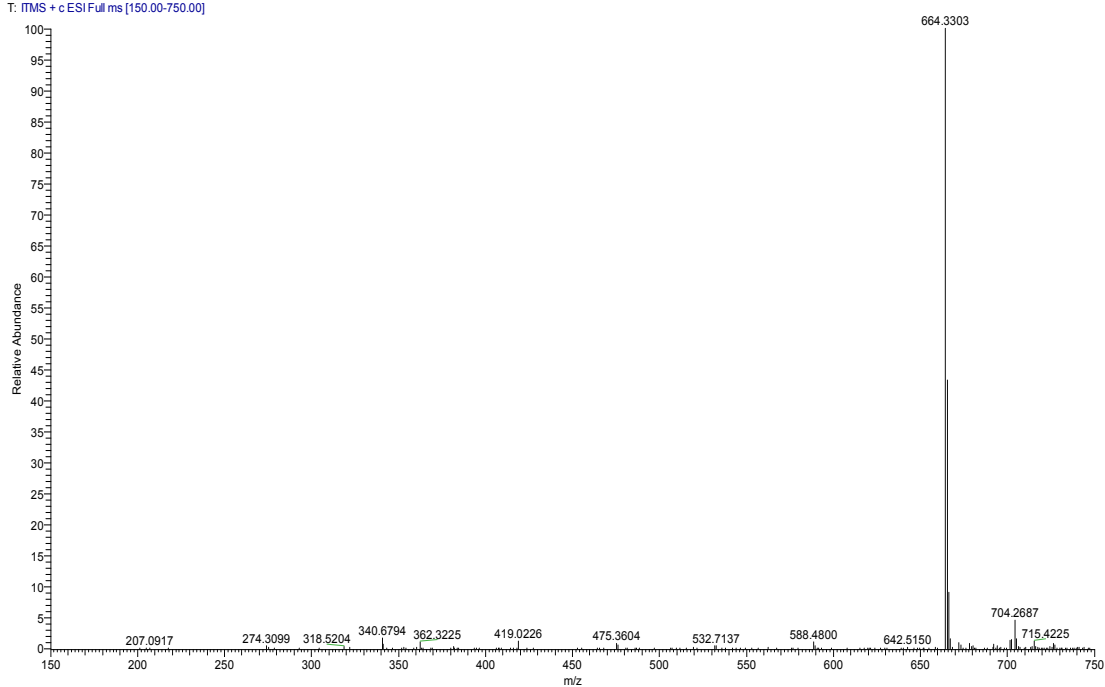


Fig. S2. The ^1H -NMR and mass spectrum of 7 ^1H -NMR (CDCl_3 , 400HMz) δ : 1.45 (t, 2CH_3); 3.93 (dd, 2CH_2); 4.05 (dd, 2CH_2); 4.44 (dd, 2CH_2); 7.39 (d, 4H); 7.88 (d, 8H); 8.62 (s, 1H). MS: $\text{C}_{37}\text{H}_{35}\text{O}_4\text{N}_7=641.73$, $[\text{C}_{37}\text{H}_{35}\text{O}_4\text{N}_7+\text{Na}^+]^+=664.33$.

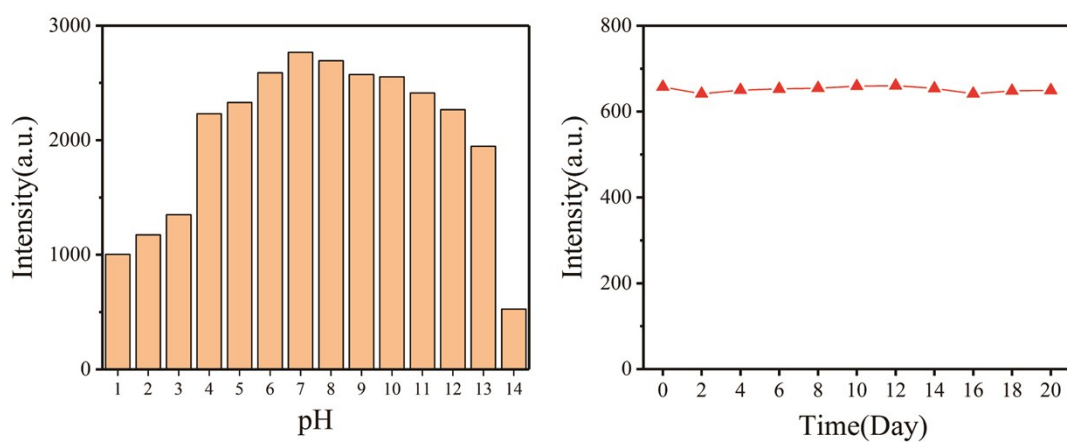


Fig. S3. (a) Fluorescence intensities of Eu³⁺ cryptate at different pH values. (b) Effect of storage time on fluorescence intensity of Eu³⁺ cryptate. (Average of triplicate measurements)

Table S1. The parameters of UPLC-MS for detecting TC

Time (min)	Column Temperature (°C)	Flow Rate (mL/min)	0.1% Formic acid (%)	100% methyl alcohol (%)	Column	Injection volume (μL)
0	40	0.3	95	5	C18 column (2.1mm ×50mm ;1.5um)	10
1	40	0.3	95	5		
3.5	40	0.3	50	50		
4.0	40	0.3	10	90		
5.0	40	0.3	10	90		
5.1	40	0.3	90	10		
6.0	40	0.3	90	10		

Table S2. The parameters of MS for detecting TC

Parameters	Values
Polarity	Positive
Microscans	1
Maximum target	1e6
Maximum inject time	50
Sheath gas flow rate	5
Spray voltage (KV)	3.5
Capillary temperature (°C)	320
Aux gas heater temperature (°C)	30