CN 108863961 B
2021.05.25

201810860615.X
2018.08.01

CN 108863961 A
2018.11.23

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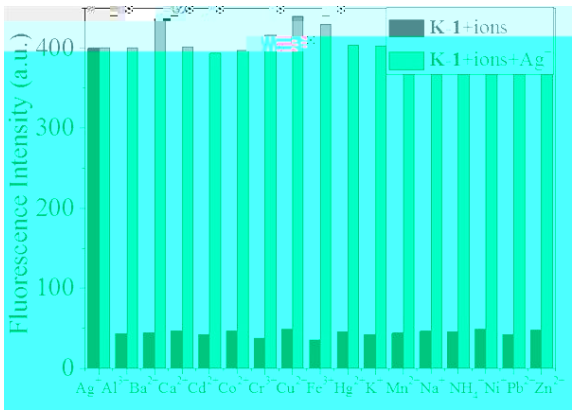
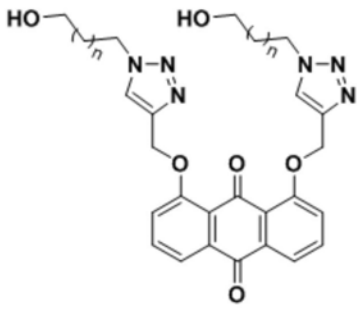
CN 103694259 A,2014.04.02
CN 104111244 A,2014.10.22
CN 107973785 A,2018.05.01
CN 102952155 A,2013.03.06
CN 102875623 A,2013.01.16
CN 106928295 A,2017.07.07
CN 107674036 A,2018.02.09
KR 20110081697 A,2011.07.14
US 8927733 B2,2015.01.06

权利要求书2页 说明书6页 附图8页

HepG2





n 1 4

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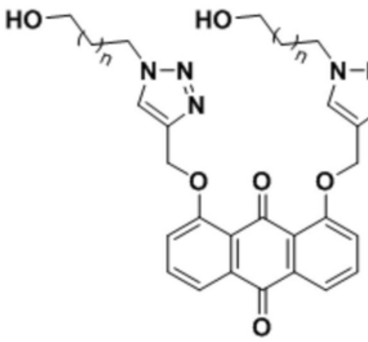
CN 108863961 B

1. ᥶ ῲ ἰ ضي G ῲ



ἰ n 1 4

,

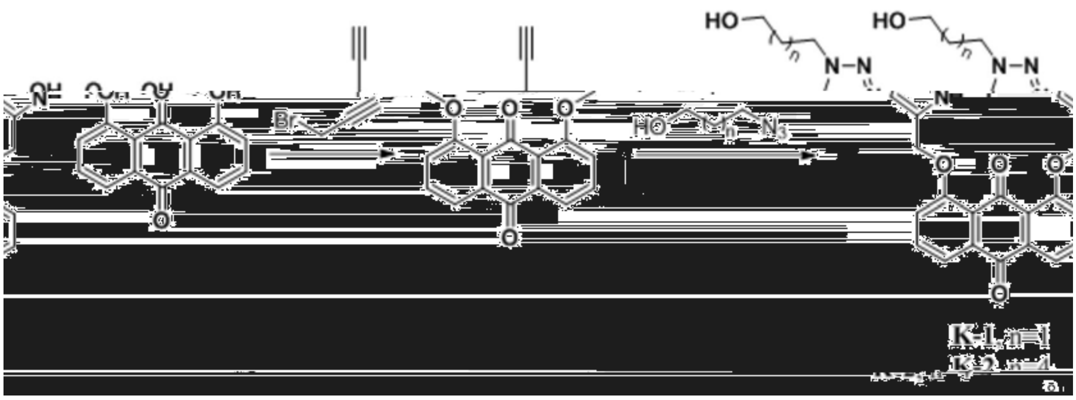
2. ≠ 1 ᥶ ῲ L ἰ ضي G ἴ r

S1 1,8 Ꮡ N,N Ꮡ Ṗ ᵭ ˜ . ⷵ غ 3 1 Ụ G ἴ ἰ . ⷵ ἴ TLC Ʌ ἴ ῃ Ụ Ꮖ غ Ꮡ . ἴ́ ἴ r Ụ № ⌠1,8 Ꮡ

S2 N,N Ꮡ Ṗ ᵭ ˜ ŋ . G 80 100 ἴ ἴ TLC Ʌ ἴ ῃ Ụ № ⌠ŋ ŋ

S3 S2 ⌠ ŋ ŋ غ S1 ⌠ 1,8 Ꮡ H_2O THF ⷵ ꓶ Ṗ ᵭ ˜ غ L شخ ضي ῲ Ð G 50 60 ἴ ἰ . ἴ TLC Ʌ ἴ ῃ Ụ Ꮖ غ Ꮡ ἴ́ ἴ r Ụ № ⌠ ᵒᵒ ᥶ ῲ K 1 K 2

ⷵ ἴ



3. ≠ 2 ᥶ ῲ L ἰ ضي G S1 1,8 Ꮡ . ⷵ 3 1 8.38:42:33.5

4. ≠ 2 ᥶ ῲ L ἰ ضي G S2 ŋ . 3:1

5. ≠ 2 ᥶ ῲ L ἰ ضي G S3 1,8 Ꮡ L شخ ŋ ŋ

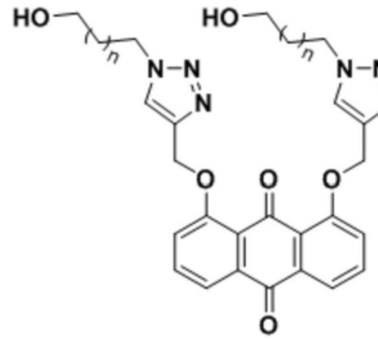
1.58:0.46:0.91:6.08 H_2O THF ﾞ ╕ H_2O THF r 1:1 1:3

6. ≠ 1 սե ῼ ضي №
r

7. ≠ 6 ὶ ضي G ւե ῼ G ج ί r γ ꞕ ˘ ╕ └ 0.05mmol/L ῼ ‰ γ HEPES ‖ ╕ └ ▯ Ữ 30μL ῼ ‰ №≢ 3mL ▯ Ữ ﾞ 5minＵ ﾞ ضي 466nm ῼ ΰ Ῥ γ شم ῼ ΰ شم └ ‰ ⌠ ῼ ΰ ‰ 30μL ῼ ‰ 3mL ج ﾞ 5minＵ ﾞ ضي 466nm ῼ ΰ Ῥ ῼ ΰ ╕ ˜ ῼ ΰ ‰ ⌠ ج HEPES ‖ pH 6 8 ῼ ῼ Đ ΰ Ex 10.0nm ΰ Em 20.0nm ΰ 340nm ڗ 1s

8. ≠ 7 ὶ ضي G ւե ῼ ضي 0.05 0.5mmol/L مخ ῤ ₮ ῼ ڗ مخ ῤ Շ

9. ≠ 7 ὶ ضي G HEPES ‖ pH 7.2



ὢ ἳ ضي G ὢ ἳ n 1 ,

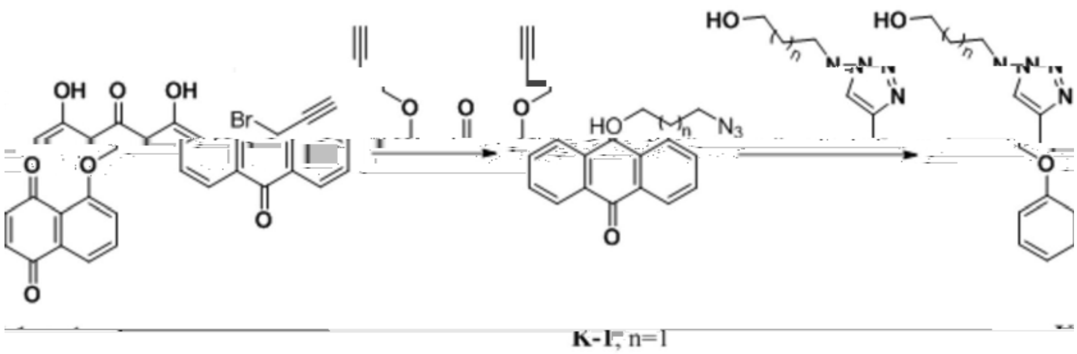
4

ΰ ㎓ ὢ L ἳ ضي G ΐ r

S1 1,8 ҍ N,N ҍ ΈP ⱴ ˜ . غ3 1 U G ἳ ἳ . ἳ TLC /l ἳ ŋ U Ԛ غҍ . ΰ ἳ r U № ⌠1,8 ҍ

S2 N,N ҍ ΈP ⱴ ˜ ŋ . G 80 100 ἳ ἳ TLC /l ἳ ŋ U № ⌠ŋ ŋ

S3 S2 ⌠ ŋ ŋ غ S1 ⌠ 1,8 ҍ H_2O THF ╕ ΈP ⱴ ˜ غL شخ ضي ὢ Ð G 50 60 ἳ ἳ . ἳ TLC /l ἳ ŋ U Ԛ غҍ ΰ ἳ r U № ⌠ ㎓ ὢ K-1 K-2 ἳ



K-1, n=1
K-2, n=4

。

п S1 1,8 ҍ . 3 1 8.38:42:33.5

п S2 ŋ . 3:1

п S3 1,8 ҍ L شخ ŋ ŋ 1.58:0.46:0.91:6.08 H_2O THF ╕ H_2O THF r 1:1 1:3

ΰ ㎓ ὢ ضي № r

r

п ሡ ῷ G ج

ἷ ἵ r ɣ ҥ ˀ ╕ └ 0.05mmol/L ῷ

‰ ɣ HEPES ǁ ╕ └ Ữ 30μL ῷ

‰ №≢ 3mL Ữ ﲾ 5minU ﲾ ضي 466nm

ῷ ΰ ῬP ɣ شم ῷ ΰ شم └ ‰ ⌠

ῷ ΰ ‰ 30μL ῷ ‰ 3mL ج

ﲾ 5minU ﲾ ضي 466nm ῷ ΰ ῬP ῷ ΰ

ꞁ ˜ ῷ ΰ ‰ ⌠ ج

HEPES ǁ pH 6 8 ῷ ῷ Đ ΰ Ex 10.0nm ΰ Em 20.0nm ΰ 340nm ڗ 1s

п ሡ ῷ ضي 0.05 0.5mmol/L مخ ṗ ₮ ῷ ڗ مخ ṗ

ꝺ

п HEPES ǁ pH 7.2

п ሡ ῷ G ṗ غ

῎E ἷ ἵ r ꞔ r HepG2 PBS ǁ U 1mmol/L ሡ ῷ G 37 Ī 30min ῬP ꞔ r HepG2 1mmol/L $AgNO_3$ PBS ǁ G 37 Ī 30min ῬP PBS ǁ 3 U Olympus FV 1000 ῷ ῍ ῍ ῷ ῎E ⌠ ᶉ ˜ Uꞔ r HepG2 ῷ ῎E

ΰ ἵ ɣ

1 ΰ └ ሡ ῷ ἵ ɣ ἳ ضيꞔ r HepG2 ṗ ῎E ج ἵ ضي E Ṿ

2 ΰ └ ሡ ῷ ضي 0.05 0.5mmol/L مخ ṗ ₮ ῷ ڗ مخ ṗ

ꝺ

3 ΰ └ ሡ ῷ ῀ ≢ ῍ ἷ

4 ΰ └ ሡ ῷ ﲾ ۷ ἵ ڃ . E Ṿ

يحـ1 ῷ K 1 ᶉ ˜ (1.0mmol/L) ῷ ἵ يحـ

يحـ2 ضي ῷ K 1(0.05mmol/L) ῷ ≢ ƀ يحـ

يحـ3 ῷ K 1 ῷ ῷ

يحـ4 λ 466nm ῷ K 1 ƀ يحـ

يحـ5 Job (λem 466nm)

يځ6 ῳ̈ K 1 ῳ̈ Benesi Hildebrandيځ

يځ7 pH ṿ ῳ̈ ڗ يځ

"V` j–7 Âيځ8 ῳ̈ K 1(0.05mmol/L) HepG2 ῳ̈ ˝Eيځ

05]Я5] ı 9 ĥ l' شخD Я0

1 .92(m ,4H) .^{13}C NMR(150MHz ,DMSO)δ183 .2 ,181 .1 ,157 .5 ,142 .3 ,134 .2 ,125 .0 ,123 .9 , 120 .9 ,118 .8 ,62 .6 ,57 .4 ,46 .8 ,33 .0 .ESI(+) HRMS(m/z) : [M+Na]$^{+}$calcd .for $C_{26}H_{26}N_6O_6Na$ 541 .1806 found 541 .1802)

ώ K 2 ^{1}H NMR(400MHz ,DMSO)δ8 .25(s ,2H) ,7 .75 7 .69(m ,6H) ,5 .34(s , 4H) ,4 .70(s ,2H) ,4 .41 4 .33(m ,4H) ,3 .35(t ,J 12 .6 ,6 .4Hz ,4H) ,1 .85 1 .75(m ,4H) ,1 .43 1 .33(m ,4H) ,1 .32 1 .25(m ,4H) ,1 .25 1 .15(m ,4H) .^{13}C NMR(150MHz ,DMSO)δ183 .2 ,181 .1 , 157 .5 ,135 .9 ,133 .1 ,128 .6 ,120 .4 ,119 .1 ,117 .7 ,62 .8 ,57 .4 ,46 .8 ,30 .1 ,29 .2 ,26 .1 , 25 .4 .ESI(+) HRMS(m/z) : [M+Na]+calcd .for $C_{32}H_{38}N_6O_6Na$ 625 .2745 found 625 .2745)

ъ 2

ώ ώ Ð

ΰ 10 .0nm ΰ 20 .0nm ΰ 340nm ζ 1s

ج 1cmώ ΐ ضمغ

ץ ח ˚ ╕ G 5mL ‰ L 1 .0 × 10 ^{2}mol/L ώ

K 1 K 2 ΰ 25μL G 5mL ώ

50μM 'P HEPES Ⅱ L 1 .0 × 10 ^{3}mol/L HEPES Ⅱ

È

≠ Job's Plot L ↓ K 1 Ag^+ 0.25mmol/L
K 1 Ag^+ λem 466nm 5
K 1 Ag^+ 1:1 K 1 1:1
K 1 K 1λ 466nm
≠ Benesi Hildebrand 1/[[Ag+](Fmax Fmin)]
1/(F Fmin) y 7.31×10^{4}/Ka+0.003(R2 0.978) 6
Ka 1368M

$$\frac{1}{F-F_{min}} = \frac{1}{K_a(F_{max}-F_{min})[Ag^+]} + \frac{1}{F_{max}-F_{min}}$$

6
pH K 1 K 1+Ag^+
pH K 1 K 1+Ag^+ λem 466nm
5 pH 5.0 11.0 K 1 pH
6.0 8.0 K 1+Ag^+ pH 6.0 8.0 K 1 Ag^+ pH
Ag^+ HEPES
7
K 1 Ag^+
K 1 Ag^+ HepG2
(PBS) 1mM K 1 37 30min PBS 3
1mM $AgNO_3$ PBS 37 30min PBS
3 Olympus FV 1000 8
Ag^+ HepG2
2

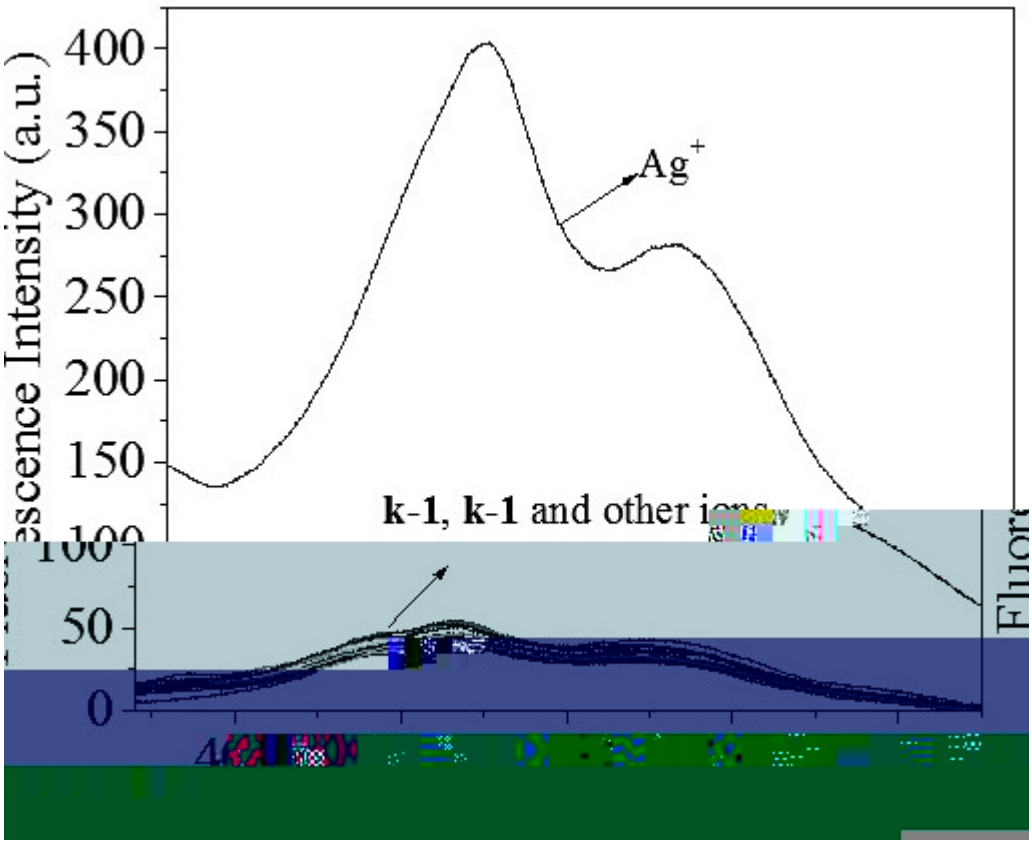


图1

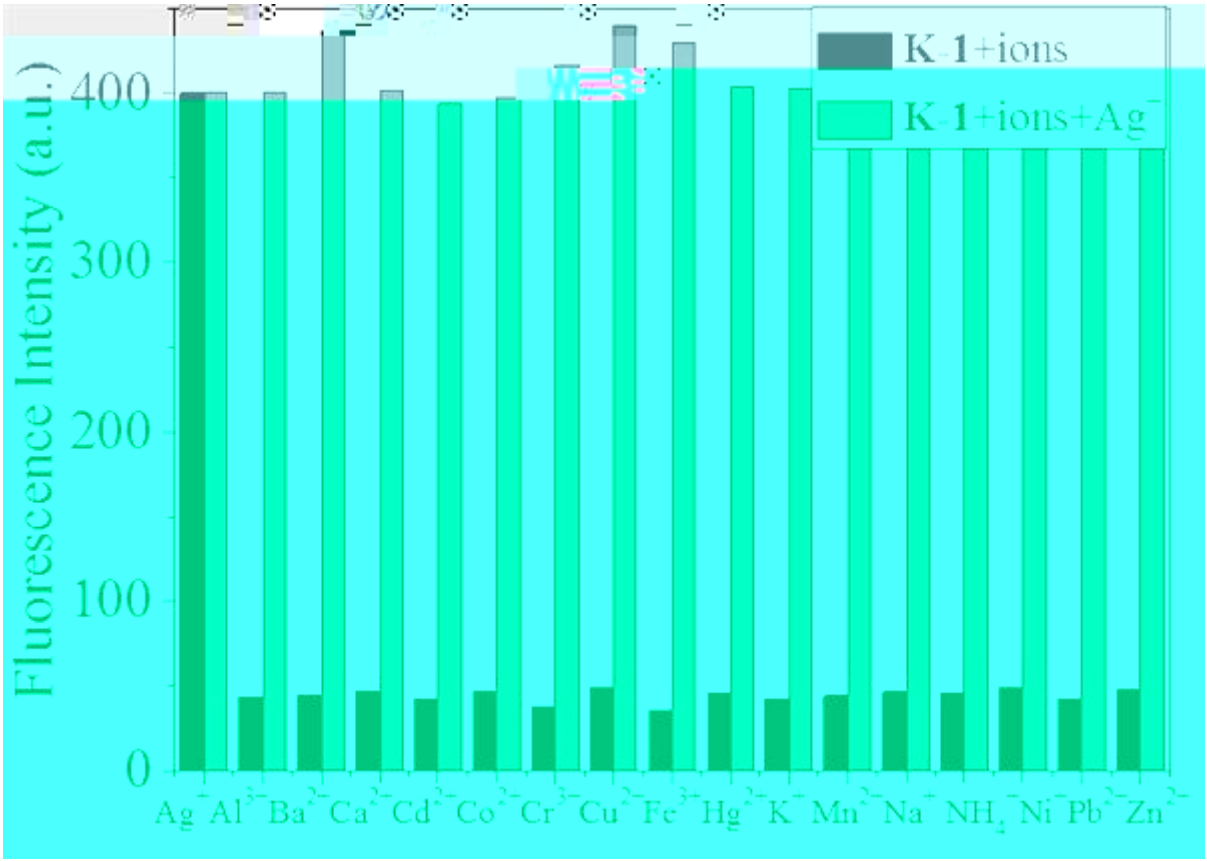

K-1+ions
K-1+ions+Ag+
Fluorescence Intensity (a.u.)
0
100
200
300
400
Ag+ Al3+ Ba2+ Ca2+ Cd2+ Co2+ Cr3+ Cu2+ Fe3+ Hg2+ K+ Mn2+ Na+ NH4+ Ni2+ Pb2+ Zn2+

图 2

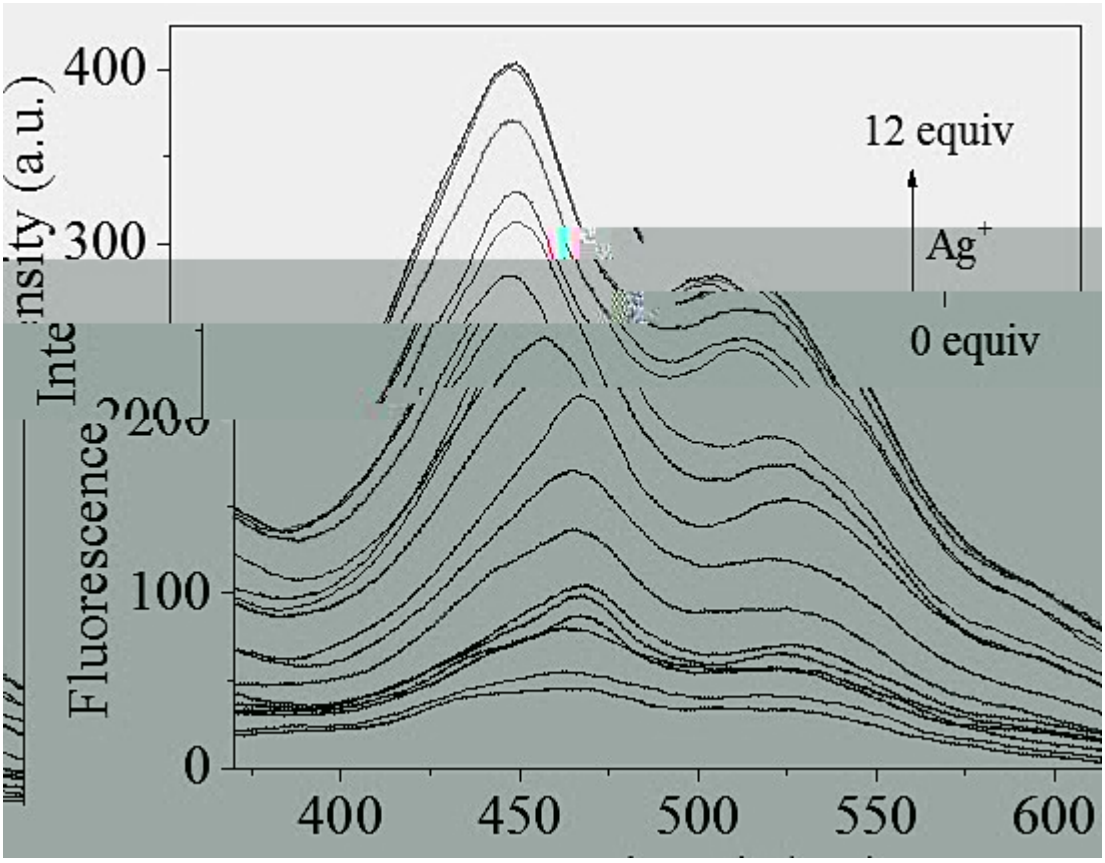

400
300
200
100
0
Fluorescence Intensity (a.u.)
400
450
500
550
600
12 equiv
Ag+
0 equiv

图 3

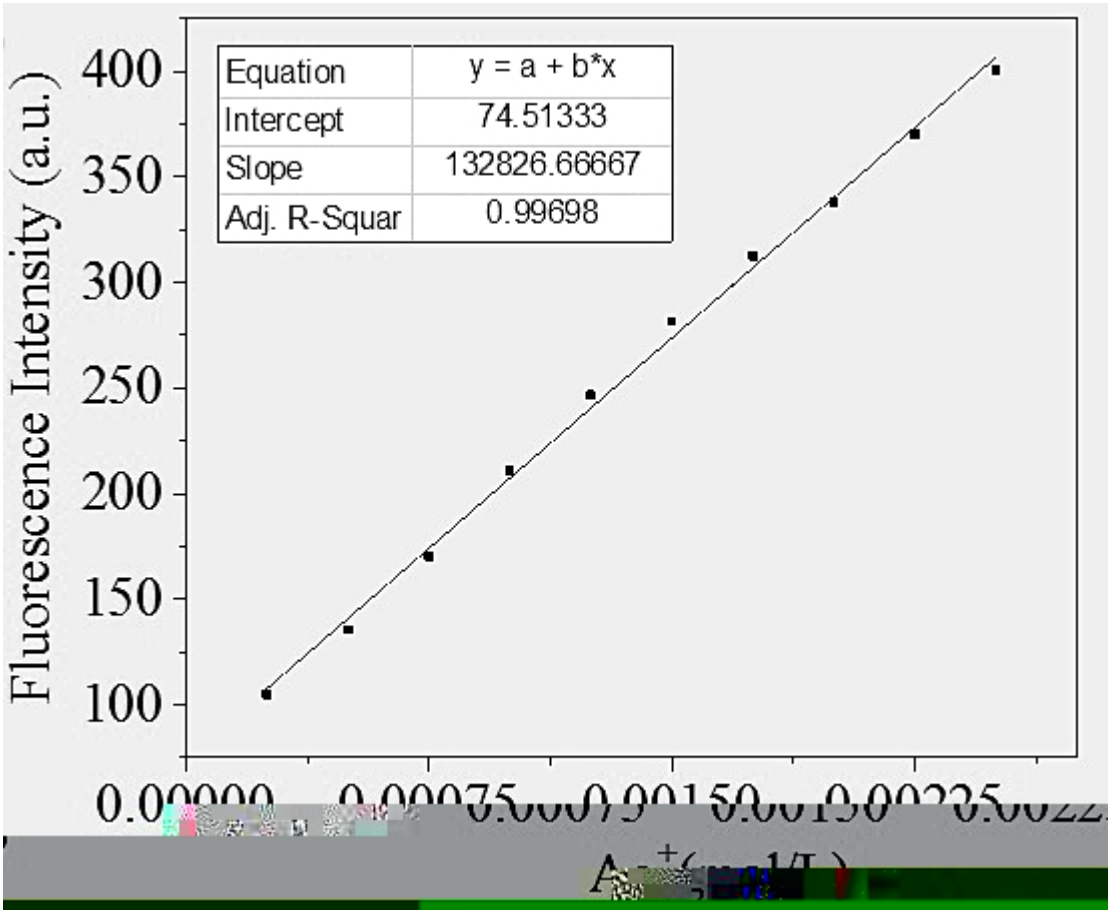

Equation
y = a + b*x
Intercept
74.51333
Slope
132826.66667
Adj. R-Squar
0.99698
Fluorescence Intensity (a.u.)
400
350
300
250
200
150
100
0.00000

图4

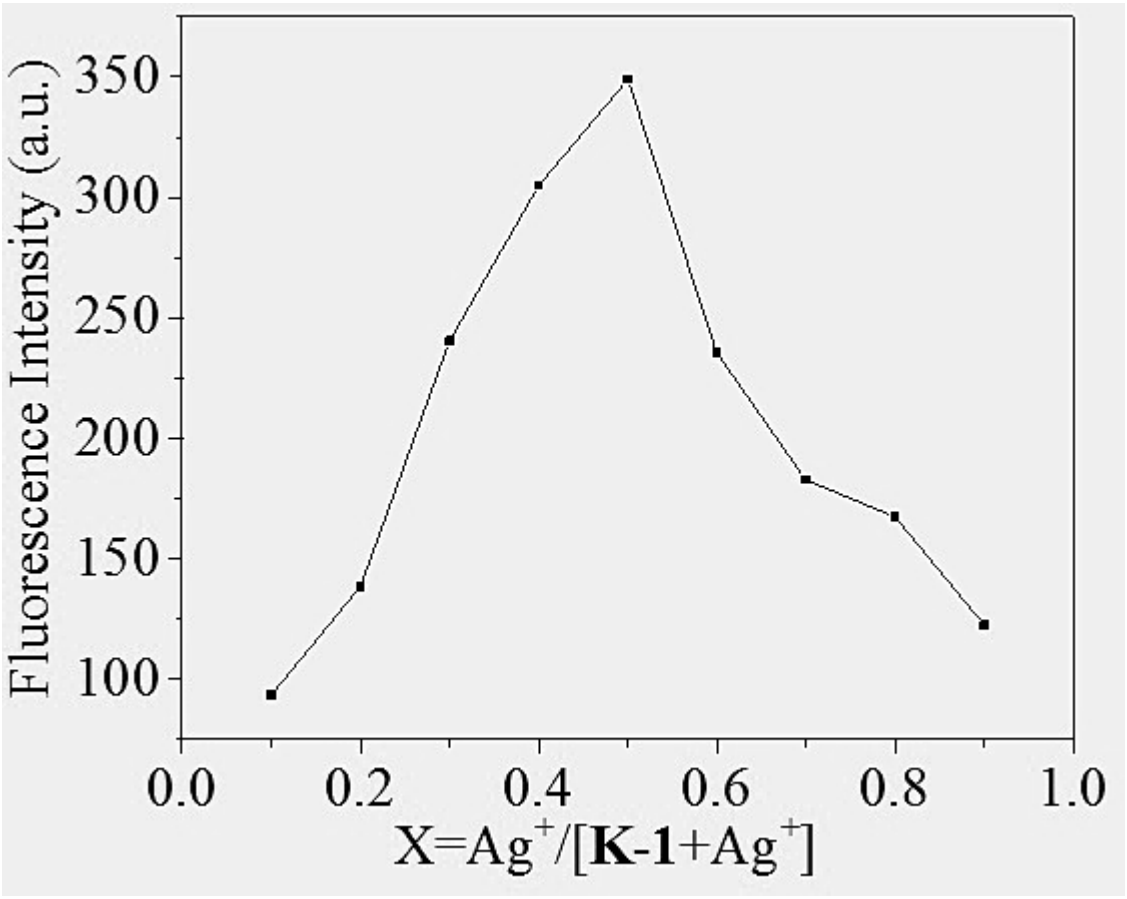

Fluorescence Intensity (a.u.)
350
300
250
200
150
100
0.0
0.2
0.4
0.6
0.8
1.0
X=Ag+/[K-1+Ag+]

图5

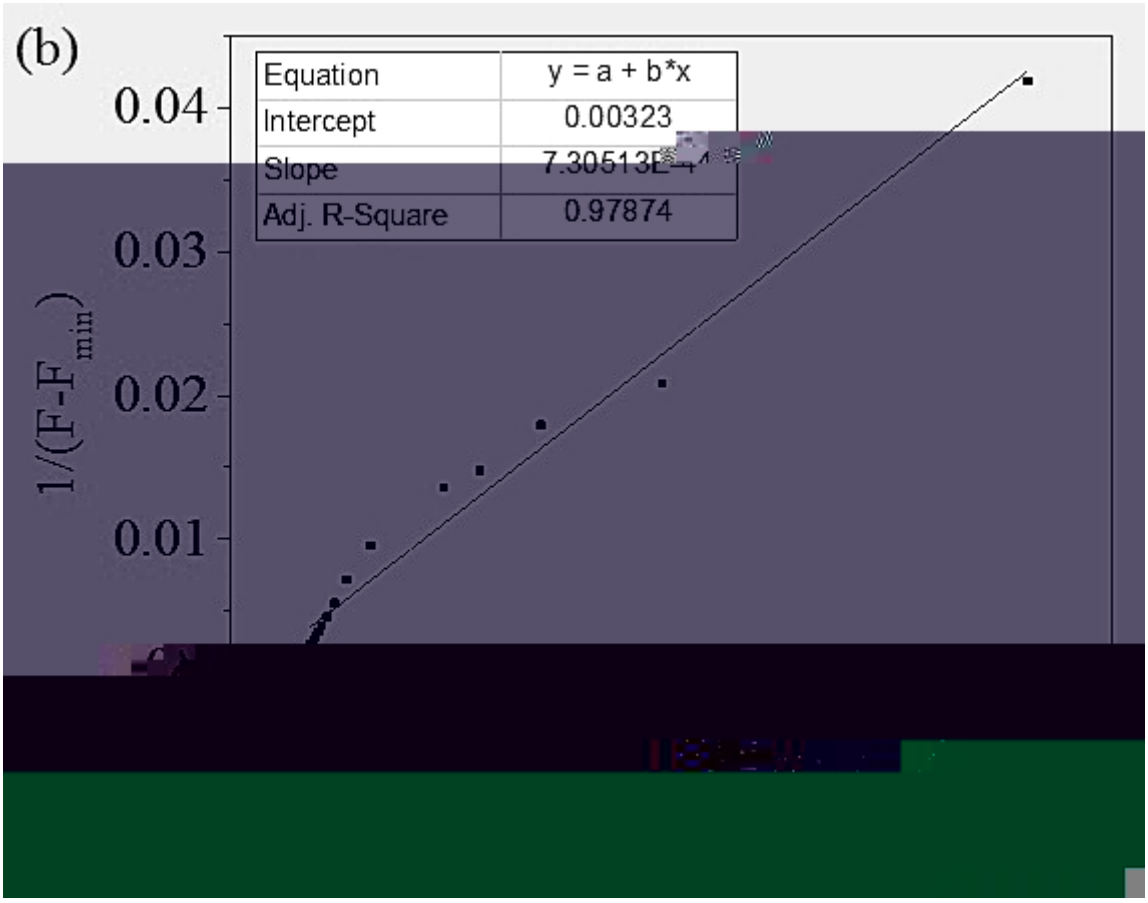

(b)
Equation
y = a + b*x
Intercept
0.00323
Slope
Adj. R-Square
0.97874
0.04
0.03
0.02
0.01
1/(F-F min)

图6

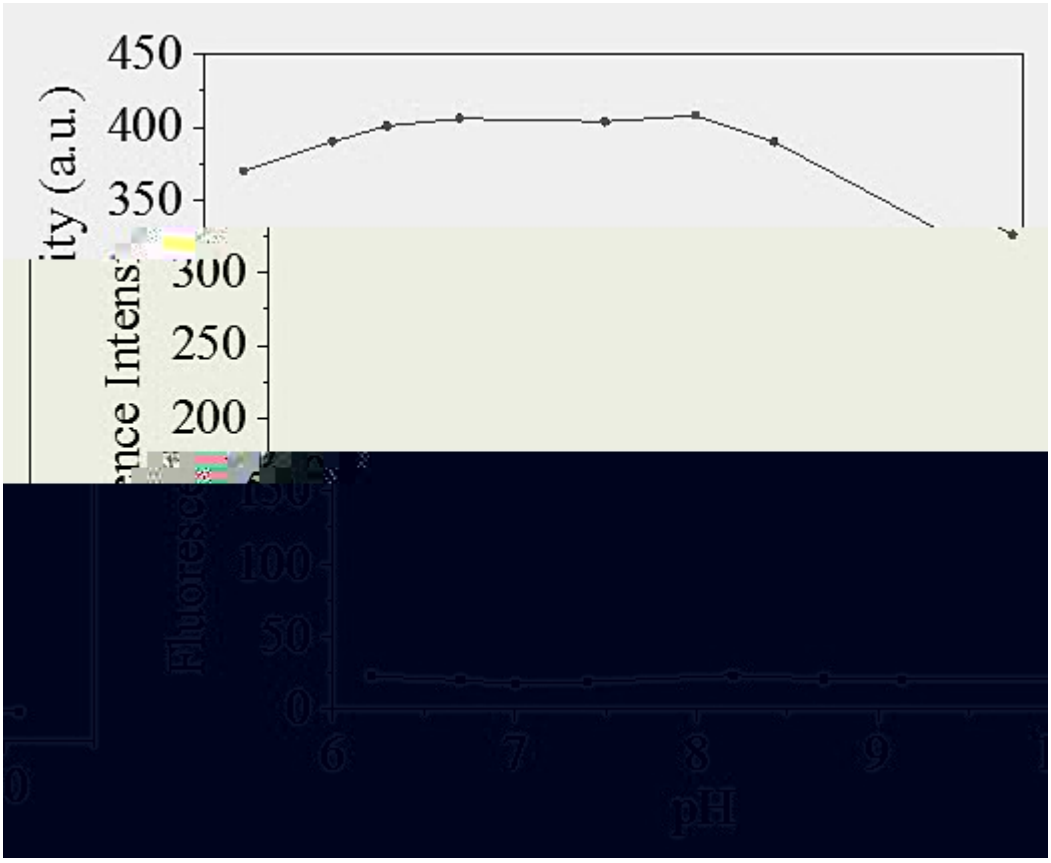


图7

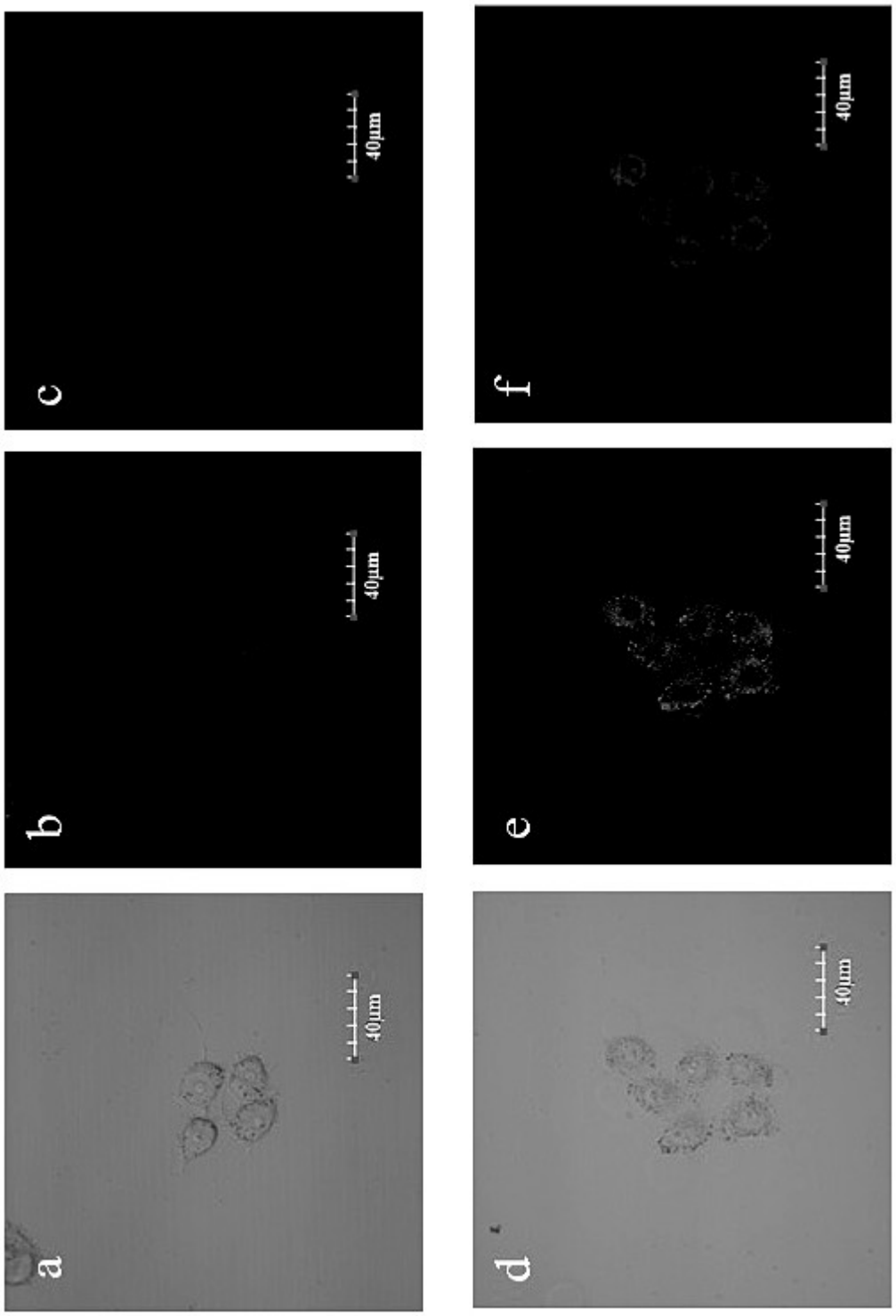


图8