Electronic Supplementary Information

Phosphate-sensing with (di-(2-picolyl)amino)quinazolines based on fluorescence on-off system

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Fig. S1: pH profile

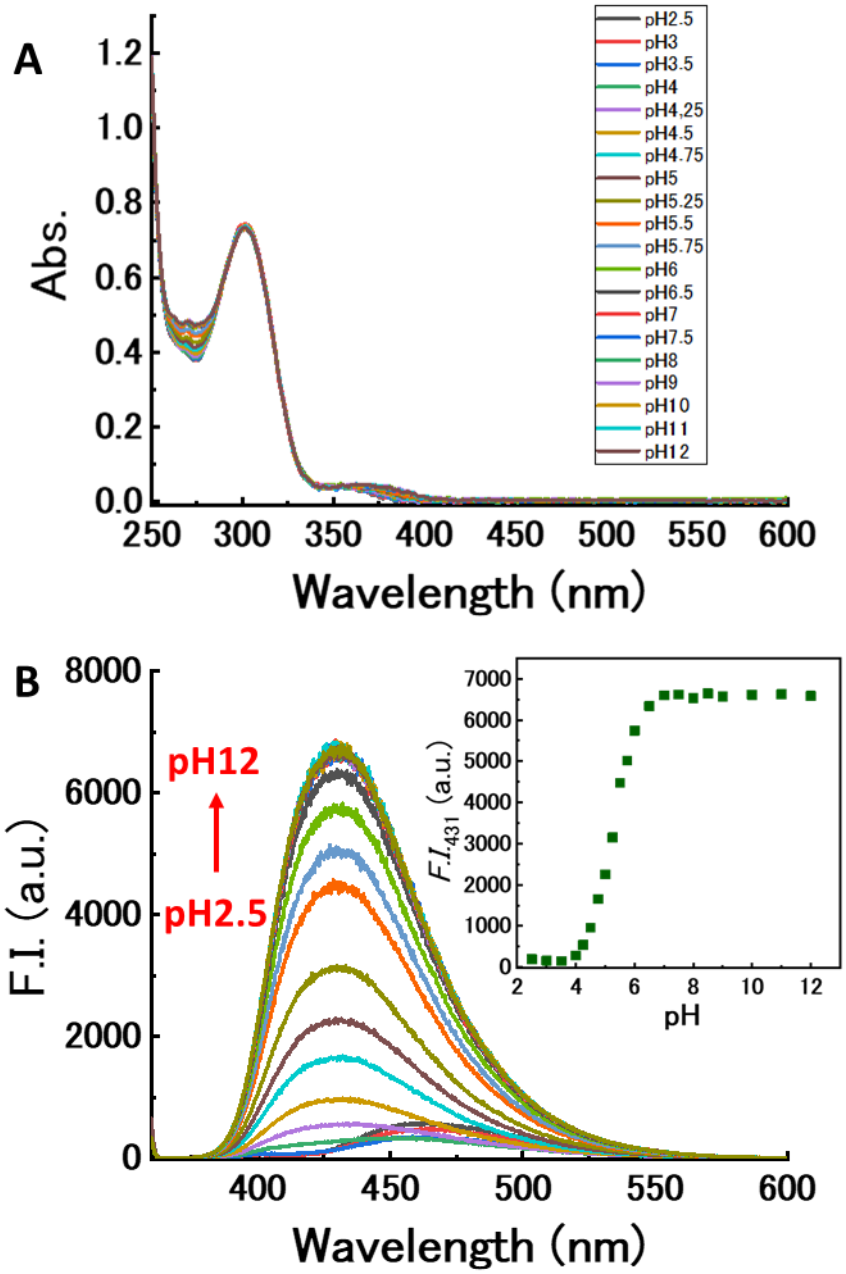


Fig. S1 (A) Absorption and (B) Fluorescence spectra of **dpa-QZ1** with various pH in 1% DMSO-99% water (v/v), adjusted by HEPES/NaOH buffer, at 25 °C (λ_{ex} = 355 nm). [**dpa-QZ1**] = 10 μM, [HEPES buffer] = 5.0 mM, [$NaNO_3$] = 0.10 M

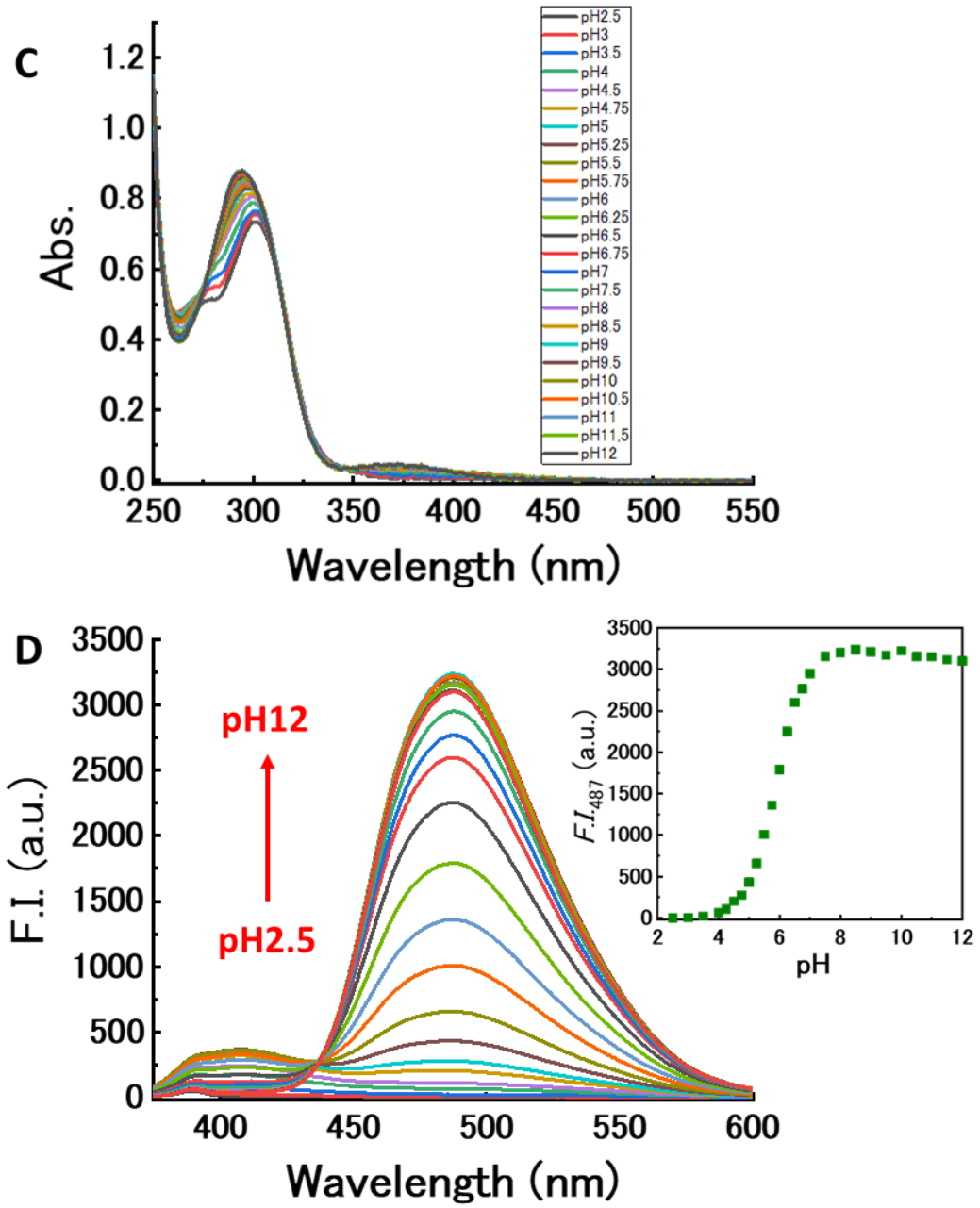
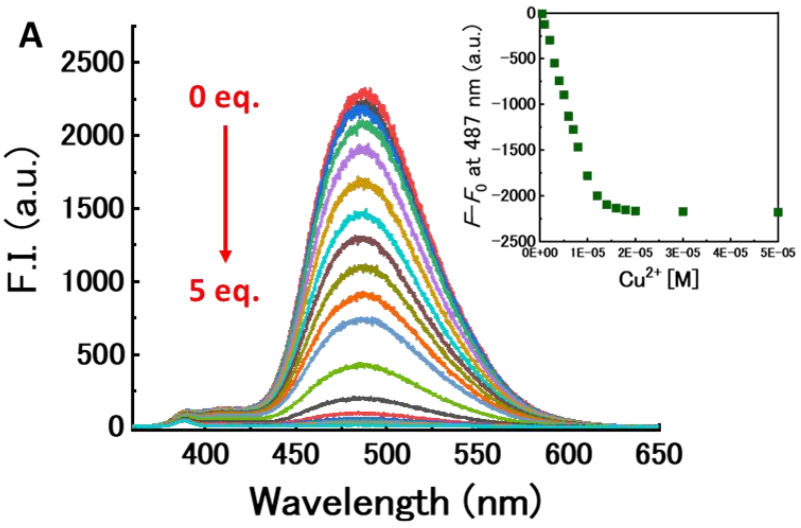
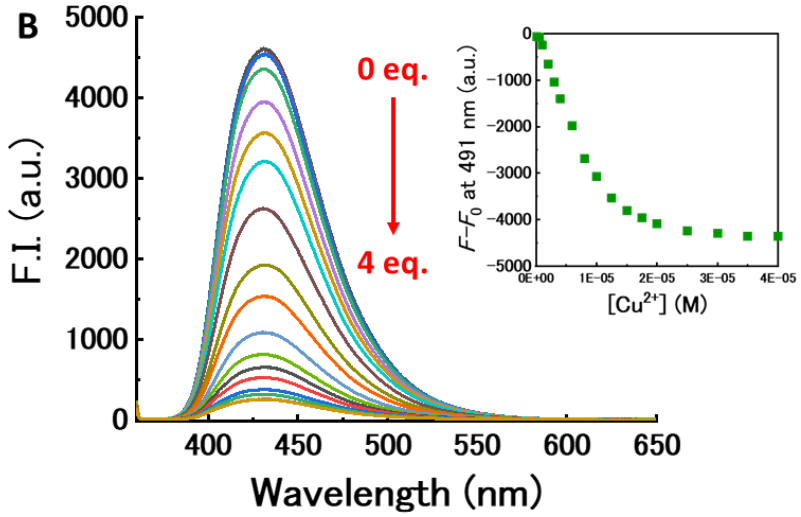


Fig. S1 (C) Absorption and (D) Fluorescence spectra of **dpa-QZ2** with various pH in 1% DMSO-99% water (v/v), adjusted by HEPES/NaOH buffer, at 25 °C (λ_{ex} = 345 nm). [**dpa-QZ2**] = 10 μM, [HEPES buffer] = 5.0 mM, [$NaNO_3$] = 0.10 M

Fig. S2: Cu Titration Curve



Binding Constant: 5.7 x 10^5 [M^{-1}]



Binding Constant: 1.1 x 10^7 [M^{-1}]

Fig. S2 Fluorescence spectra and Fluorescence change at fluorescence maximum wavelength of **dpa-QZ1** and **dpa-QZ2** with Cu^{2+} in 1% DMSO-99% water (v/v), pH7.4 adjusted by HEPES/NaOH buffer, at 25 °C ($\lambda_{ex}^{\textbf{dpa-QZ1}}$ = 355 nm, $\lambda_{ex}^{\textbf{dpa-QZ2}}$ = 345 nm). [**probe**] = 10 μM, [$Cu(NO_3)_2$] = 0 – 50 μM, [$NaNO_3$] = 0.10 M.

Fig. S3: Binding Constants of Cu-dpa-QZ1 to Phosphoric Acids

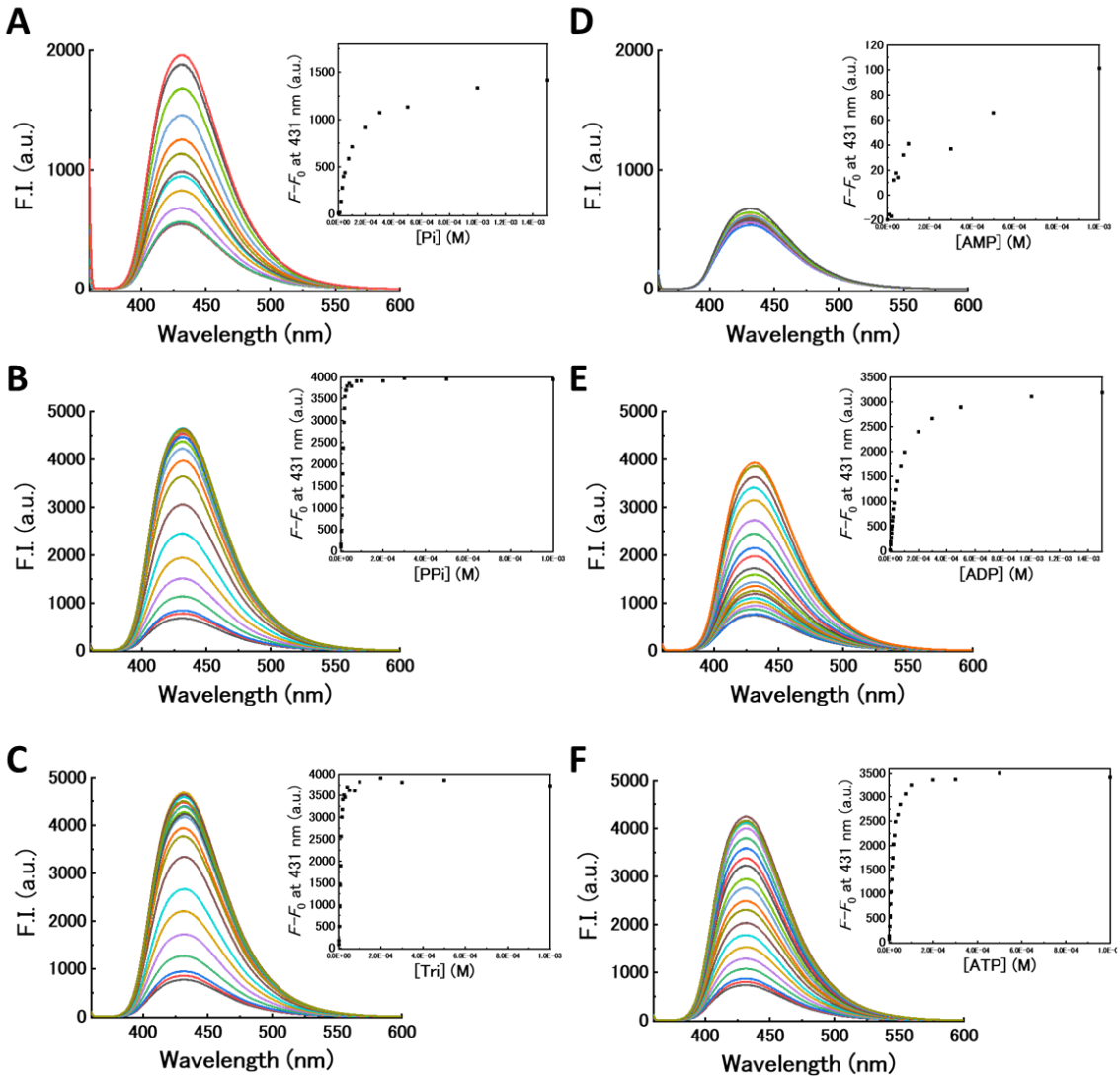


Fig. S3 Fluorescence spectra and Fluorescence change at 431 nm of **Cu-dpa-QZ1** against (A) Pi, (B) PPi, (C) Tri, (D) AMP, (E) ADP and (F) ATP in 1% DMSO-99% water (v/v), pH7.4 adjusted by HEPES/NaOH buffer, at 25 °C (λ_{ex} = 355 nm). [**dpa-QZ1**] = 0.01 mM, [$Cu(NO_3)_2$] = 0.02 mM, [phosphoric acid] = 0 - 4 mM, [HEPES buffer] = 5.0 mM, [$NaNO_3$] = 0.10 M.

Fig. S4: Binding Constants of Cu-dpa-QZ2 to Phosphoric Acids

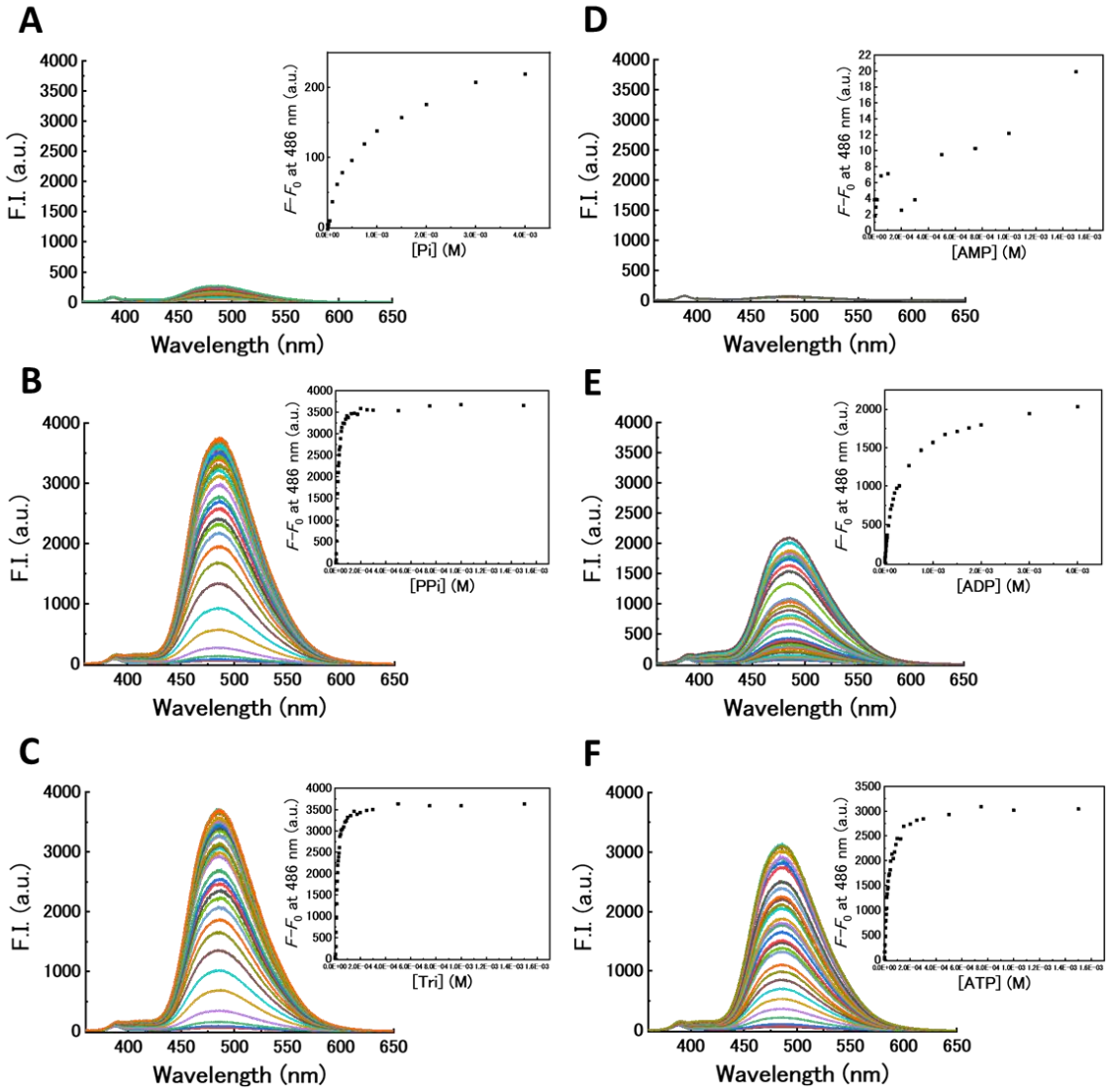


Fig. S4 Fluorescence spectra and Fluorescence change at 486 nm of **Cu-dpa-QZ2** against (A) Pi, (B) PPi, (C) Tri, (D) AMP, (E) ADP and (F) ATP in 5% DMSO-95% water (v/v), pH7.4 adjusted by HEPES/NaOH buffer, at 25 °C (λ_{ex} = 345 nm). [**dpa-QZ2**] = 0.01 mM, [$Cu(NO_3)_2$] = 0.02 mM, [phosphoric acid] = 0 - 4 mM, [HEPES buffer] = 5.0 mM, [$NaNO_3$] = 0.10 M.

Fig. S5: Comparison Studies of Blue Shift under Hydrophobic Condition

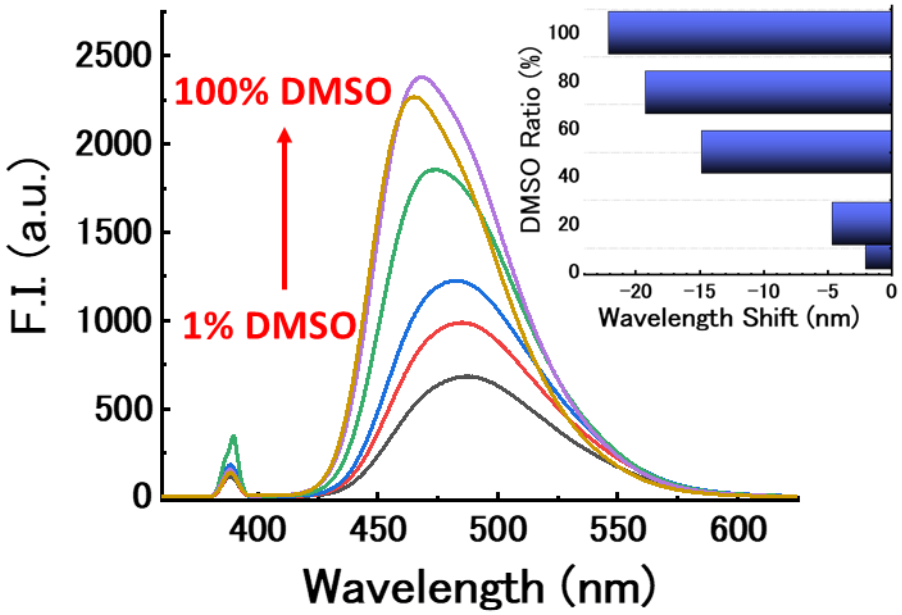


Fig. S5 Fluorescence spectra of **dpa-QZ2** with DMSO concentration changes in 1% - 100% DMSO in 1% DMSO - 99% water (v/v), and their wavelength shifts, pH7.4 adjusted by HEPES/NaOH buffer, at 25 °C (λ_{ex} = 345 nm). [**dpa-QZ2**] = 10 μM, [HEPES buffer] = 5.0 mM, [$NaNO_3$] = 0.10 M.

Fig. S6: Metal Ion Selectivity of dpa-QZ1 in the presence of CyDs

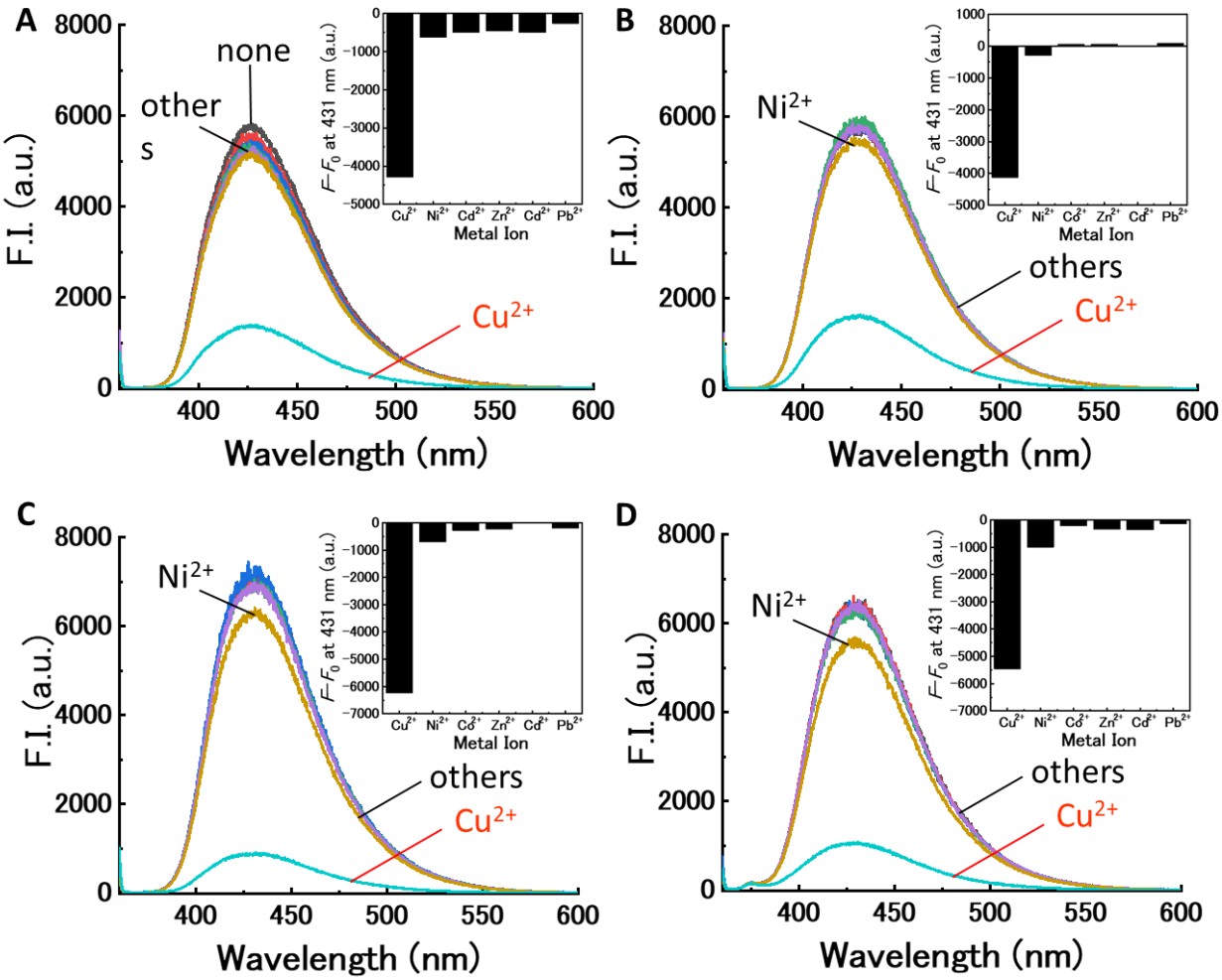


Fig. S6 Fluorescence spectra of **dpa-QZ1** (λ_{ex} = 355 nm) with various metals in the presence of (A) β-CyD, (B) γ-CyD, (C) FPB-β-CyD and (D) FPB-γ-CyD in 1% DMSO – 99% water (v/v), pH7.4 adjusted by HEPES/NaOH buffer, at 25 °C. F-F_0 is the fluorescence intensity difference at fluorescence maximum wavelength. [**dpa-QZ1**] = 10 μM, [metal$(NO_3)_2$] = 20 μM, [CyD] = 5.0 mM, [FPB-CyD] = 0.50 mM, [HEPES buffer] = 5.0 mM, [$NaNO_3$] = 0.10 M.

Fig. S7: Metal Ion Selectivity of dpa-QZ2 in the presence of CyDs

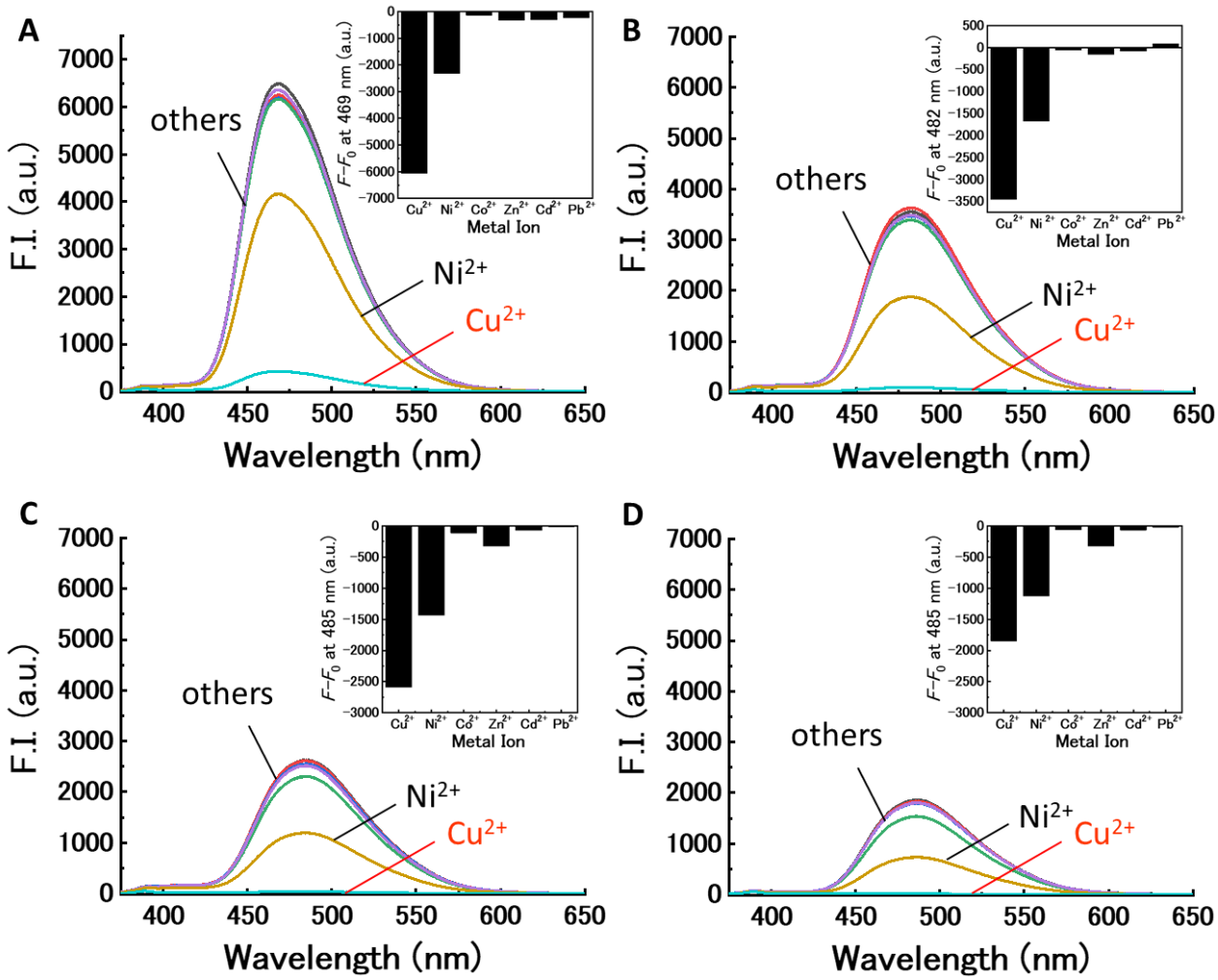


Fig. S7 Fluorescence spectra of **dpa-QZ2** (λ_{ex} = 345 nm) with various metals in the presence of (A) β-CyD, (B) γ-CyD, (C) FPB-β-CyD and (D) FPB-γ-CyD in 1% DMSO – 99% water (v/v), pH7.4 adjusted by HEPES/NaOH buffer, at 25 °C. F-F_0 is the fluorescence intensity difference at fluorescence maximum wavelength. [**dpa-QZ2**] = 10 μM, [metal$(NO_3)_2$] = 20 μM, [CyD] = 5.0 mM, [FPB-CyD] = 0.50 mM, [HEPES buffer] = 5.0 mM, [$NaNO_3$] = 0.10 M.

Fig. S8: Phosphoric Acids Recognition of Cu-dpa-QZ1 in the presence of CyDs

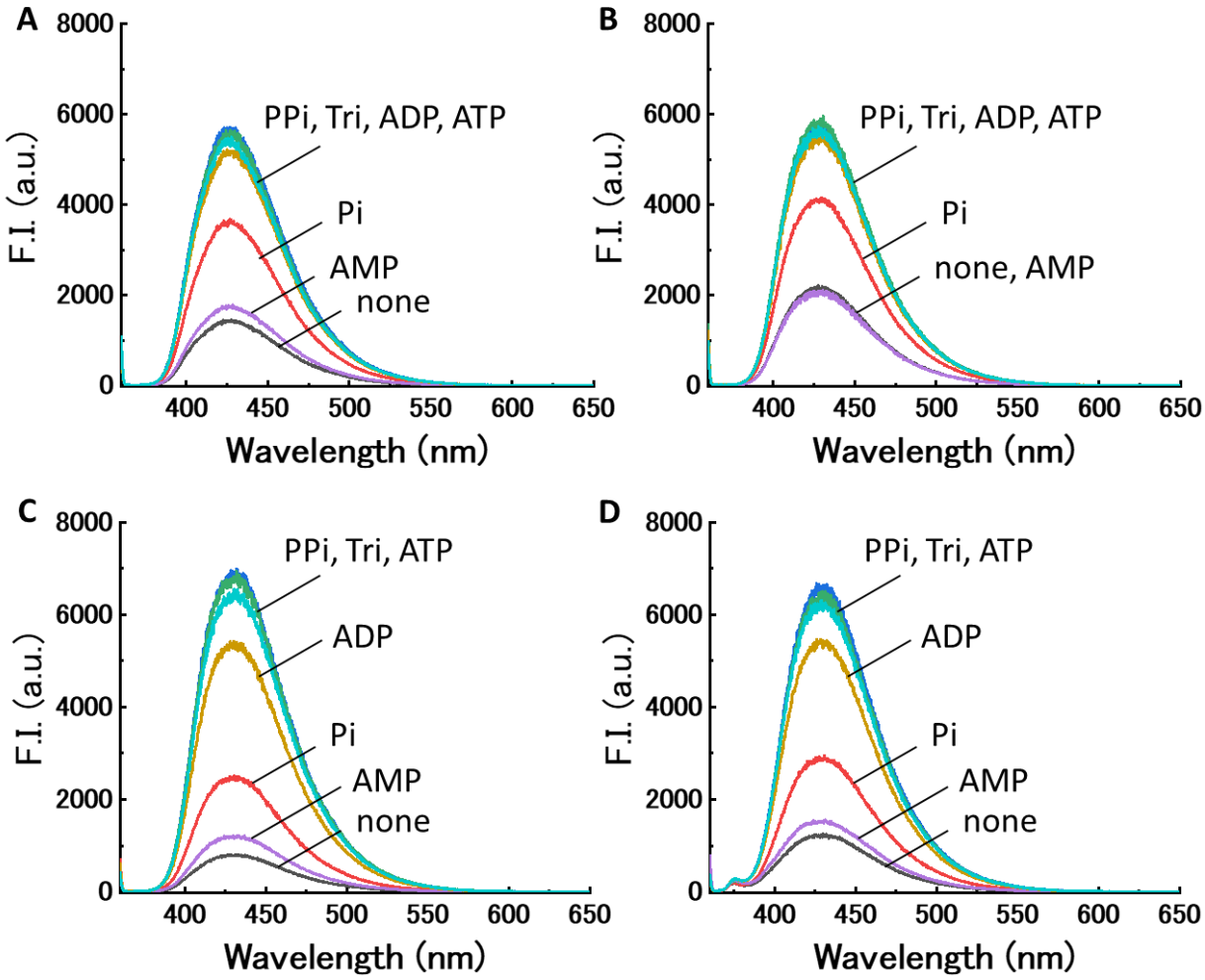


Fig. S8 Fluorescence spectra of **Cu-dpa-QZ1** (λ_{ex} = 355 nm) with phosphoric acid derivatives (Pi, PPi, Tri, AMP, ADP and ATP) in the presence of (A) β-CyD, (B) γ-CyD, (C) FPB-β-CyD, and (D) FPB-γ-CyD in 1% DMSO – 99% water (v/v), pH7.4 adjusted by HEPES/NaOH buffer, at 25 °C. F-F_0 is the fluorescence intensity difference at fluorescence maximum wavelength. [**dpa-QZ1**] = 10 μM, [$Cu(NO_3)_2$] = 20 μM, [CyD] = 5.0 mM, [FPB-CyD] = 0.50 mM, [phosphoric acid] = 1.0 mM, [HEPES buffer] = 5.0 mM, [$NaNO_3$] = 0.10 M.

Fig. S9: Phosphoric Acids Recognition of Cu-dpa-QZ2 in the presence of CyDs

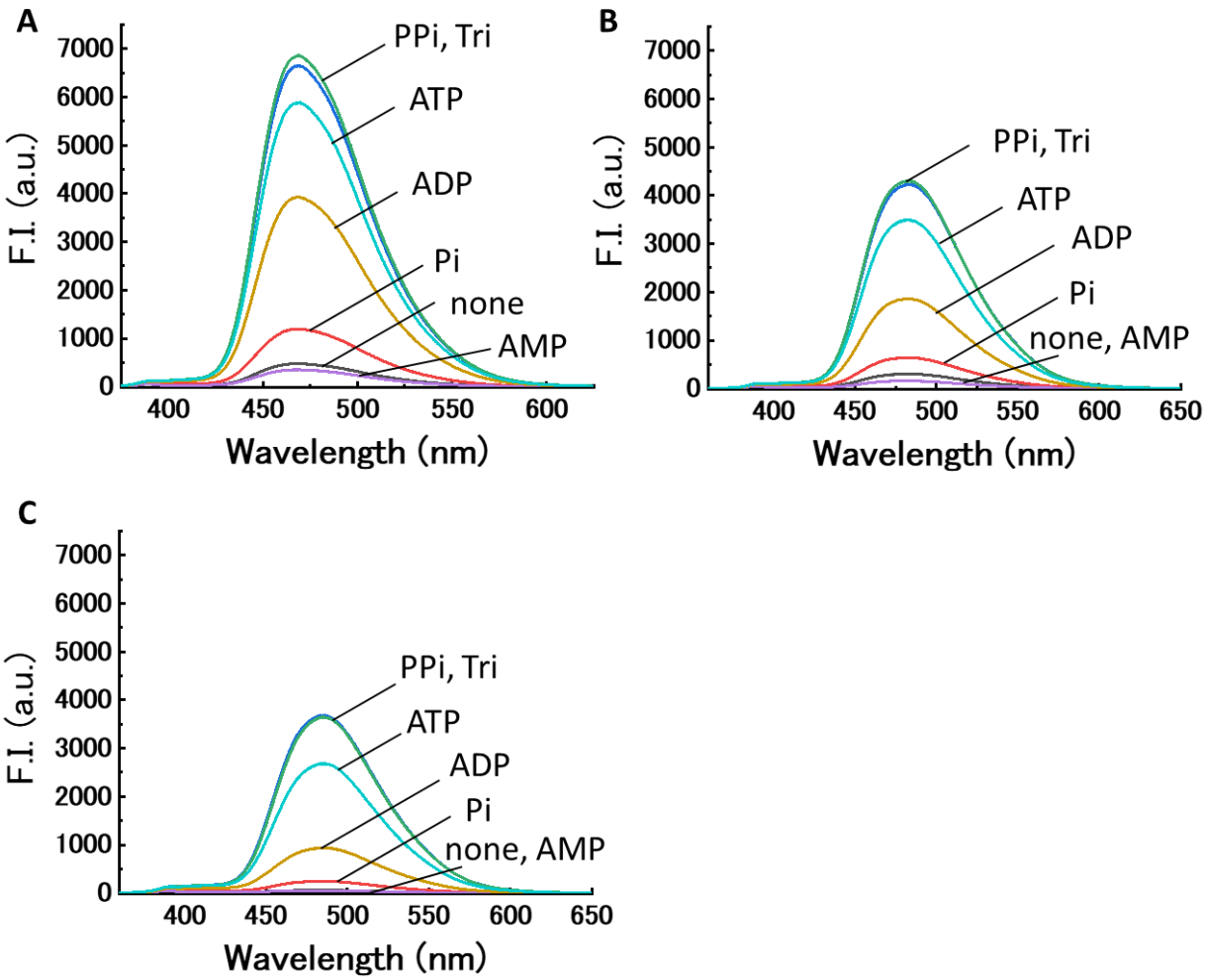


Fig. S9 Fluorescence spectra of **Cu-dpa-QZ2** (λ_{ex} = 345 nm) with phosphoric acid derivatives (Pi, PPi, Tri, AMP, ADP and ATP) in the presence of (A) β-CyD, (B) γ-CyD, and (C) FPB-β-CyD in 1% DMSO – 99% water (v/v), pH7.4 adjusted by HEPES/NaOH buffer, at 25 °C. F-F_0 is the fluorescence intensity difference at fluorescence maximum wavelength. [**dpa-QZ2**] = 10 μM, [$Cu(NO_3)_2$] = 20 μM, [CyD] = 5.0 mM, [FPB-CyD] = 0.50 mM, [phosphoric acid] = 1.0 mM, [HEPES buffer] = 5.0 mM, [$NaNO_3$] = 0.10 M.

Fig. S10: Phosphoric Acids Recognition of Cu-dpa-QZ2 in the presence of CyDs

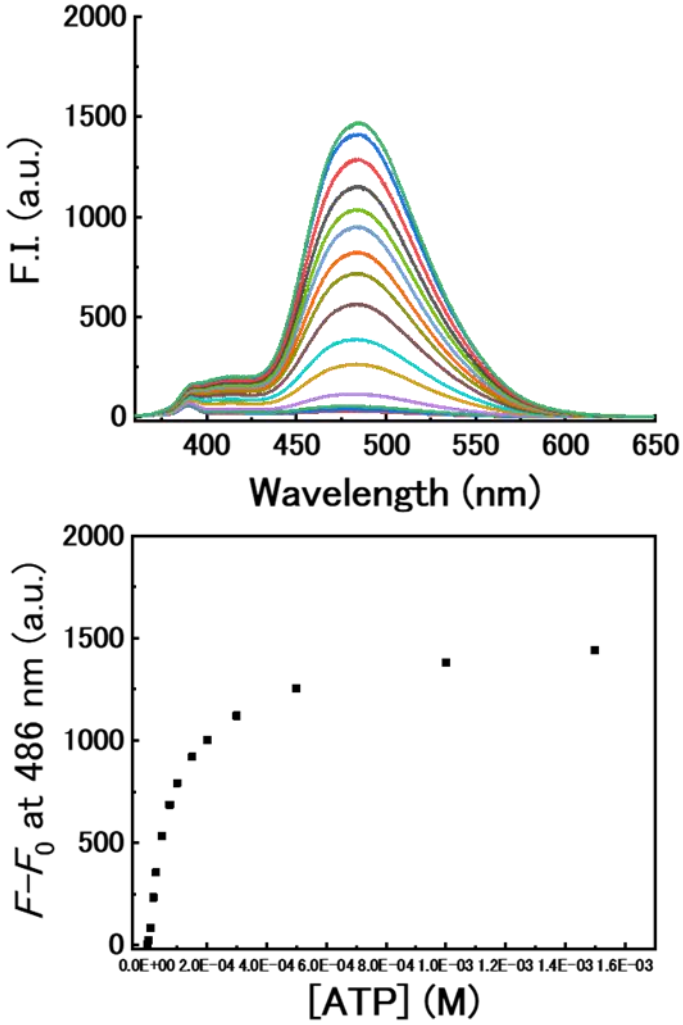


Fig. S10 Fluorescence spectra and Fluorescence change at 486 nm of **Cu-dpa-QZ2/FPB-γ-CyD** against ATP in 1% DMSO-99% water (v/v), pH7.4 adjusted by HEPES/NaOH buffer, at 25 °C (λ_{ex} = 345 nm). [**dpa-QZ2**] = 0.01 mM, [FPB-γ-CyD] = 0.50 mM, [$Cu(NO_3)_2$] = 0.02 mM, [phosphoric acid] = 0 – 1.5 mM, [HEPES buffer] = 5.0 mM, [$NaNO_3$] = 0.10 M.

Fig. S11: Fluorescence response of dpa-QZ2 to FPB-γ-CyD

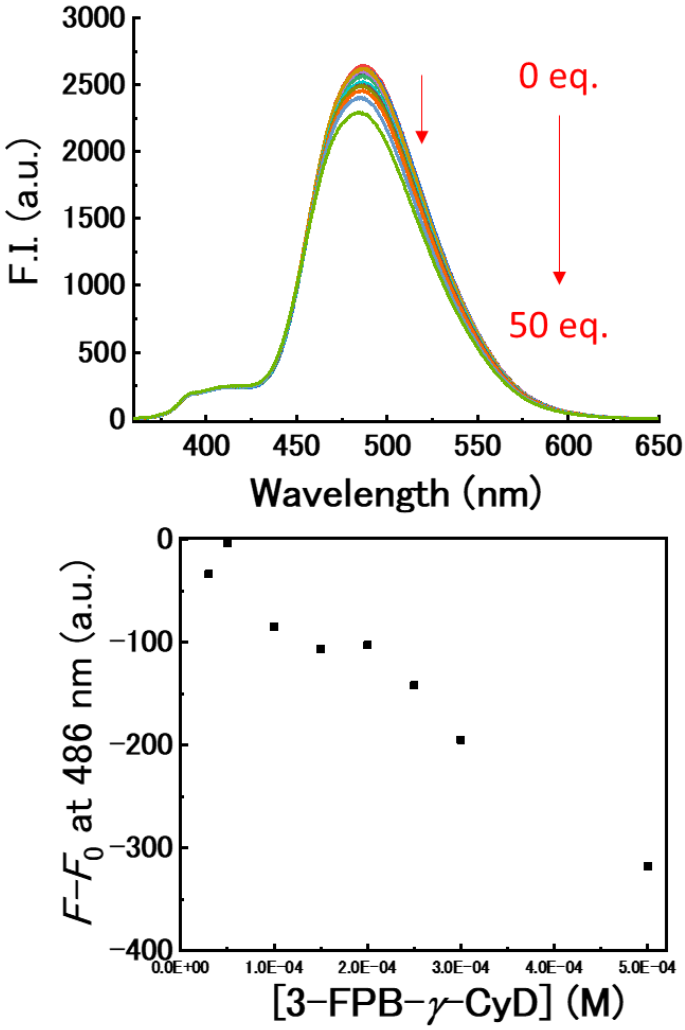


Fig. S11 Fluoroscence response of **dpa-QZ2** to FPB-γ-CyD (0 – 0.5 mM) in 1% DMSO - 99% water (v/v), and their wavelength shifts, pH7.4 adjusted by HEPES/NaOH buffer, at 25 °C (λ_{ex} = 345 nm). [**dpa-QZ2**] = 10 μM, [HEPES buffer] = 5.0 mM, [$NaNO_3$] = 0.10 M.

Calculation of the Binding Constants

The apparent 1:1 binding constants (K_{app}) of host molecules with guest molecules (i.e. **dpa-QZ** with metal ions and **Cu-dpa-QZ** with phosphoric acids) were determined from the changes in the fluorescence spectra. With an increase in concentration of guest molecules, the fluorescence intensity was also changed (Fig. S3-S5). On the assumption that the fluorescence change is only induced by the formation of a 1:1 complex (HG) between host molecules (H) and guest molecules (G), the binding constant K_{app} can be defined as follows:

<Symbols used>

$[H]_t$: total concentration of host molecules

$[G]_t$: total concentration of guest molecules

F: fluorescent intensity ϕ: fluorescence quantum yield β: device constant

<Theoretical formulas for curve fitting method>

$$H + G \rightleftharpoons^{K_{app}} HG$$

$$K_{app} = \frac{[HG]}{[H][G]} \quad \ldots(1)$$

$$[H]_t = [H] + [HG] \quad \ldots(2)$$

$$[G]_t = [G] + [HG] \quad \ldots(3)$$

From equations (1), (2) and (3)

$$[G] = \frac{-(1 + K_{app}([H]_t - [G]_t) + \sqrt{(1 + K_{app}([H]_t - [G]_t)^2 + 4K_{app}[G]_t}}{2K_{app}} \quad \ldots(4)$$

In the case that the concentration of a fluorescence chemical species A is very low, the fluorescence intensity F can be expressed as follows,

$$F = \beta\phi[A] \quad \ldots(5)$$

Therefore, the fluorescence intensity difference can be expressed as:

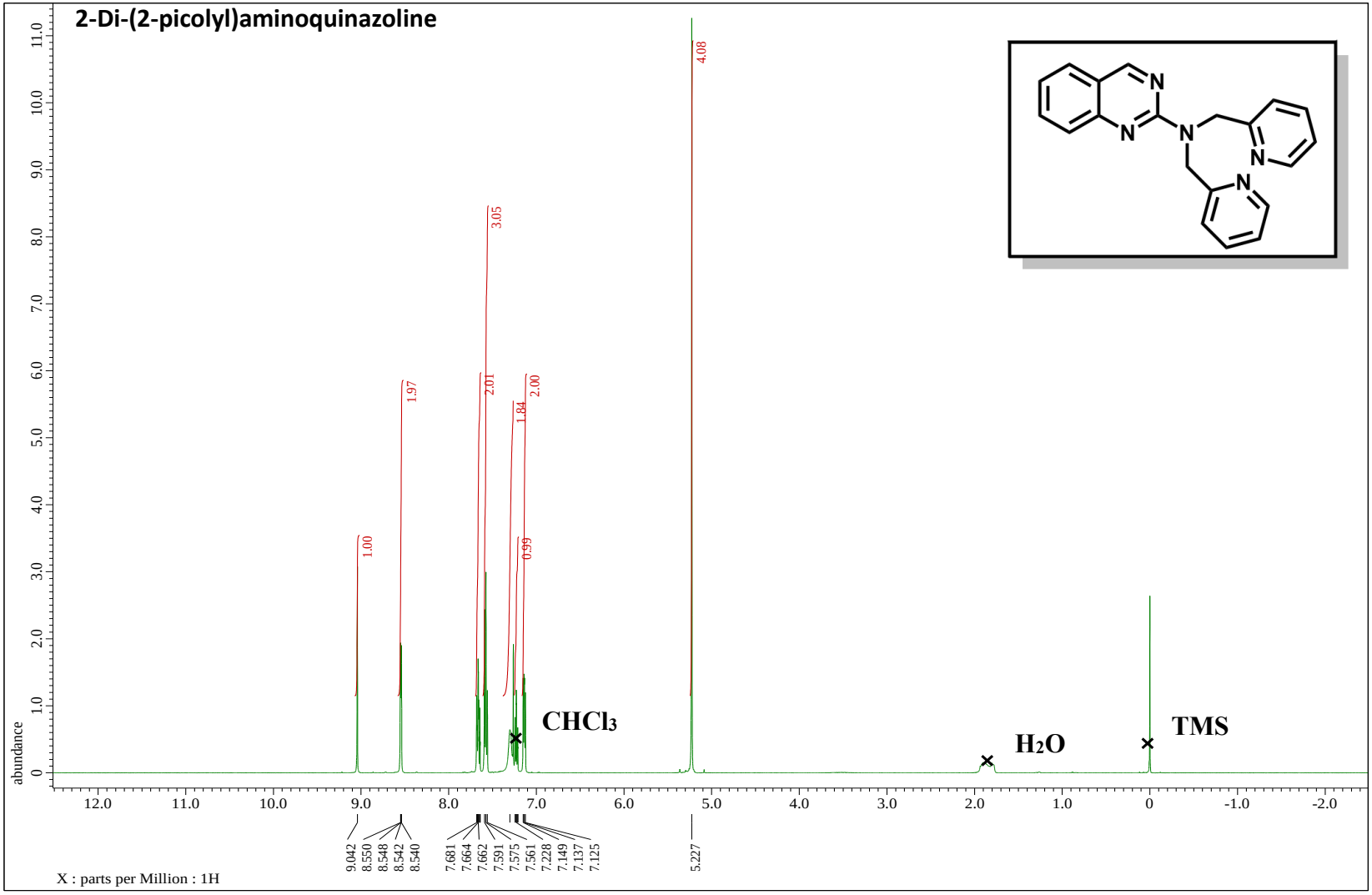
$$F_{HG} - F_0 = (\beta\phi_H[H] + \beta\phi_{HG}[HG]) - \beta\phi_H[H] \quad \ldots(6)$$

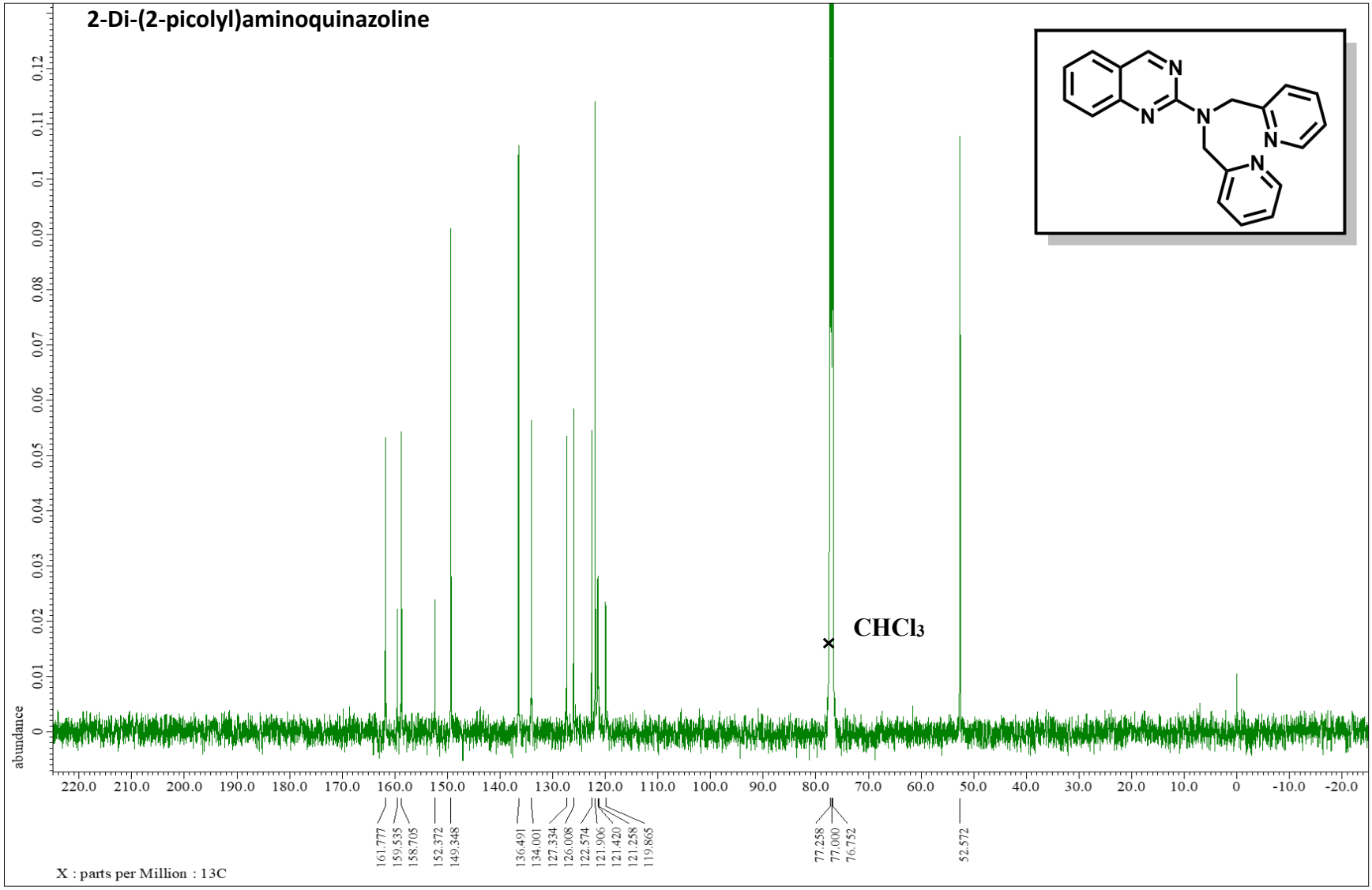
From equations (6), the fluorescence intensity difference can be expressed as a function of [G] and $[H]_t$, using equations (1), (3) and (4),

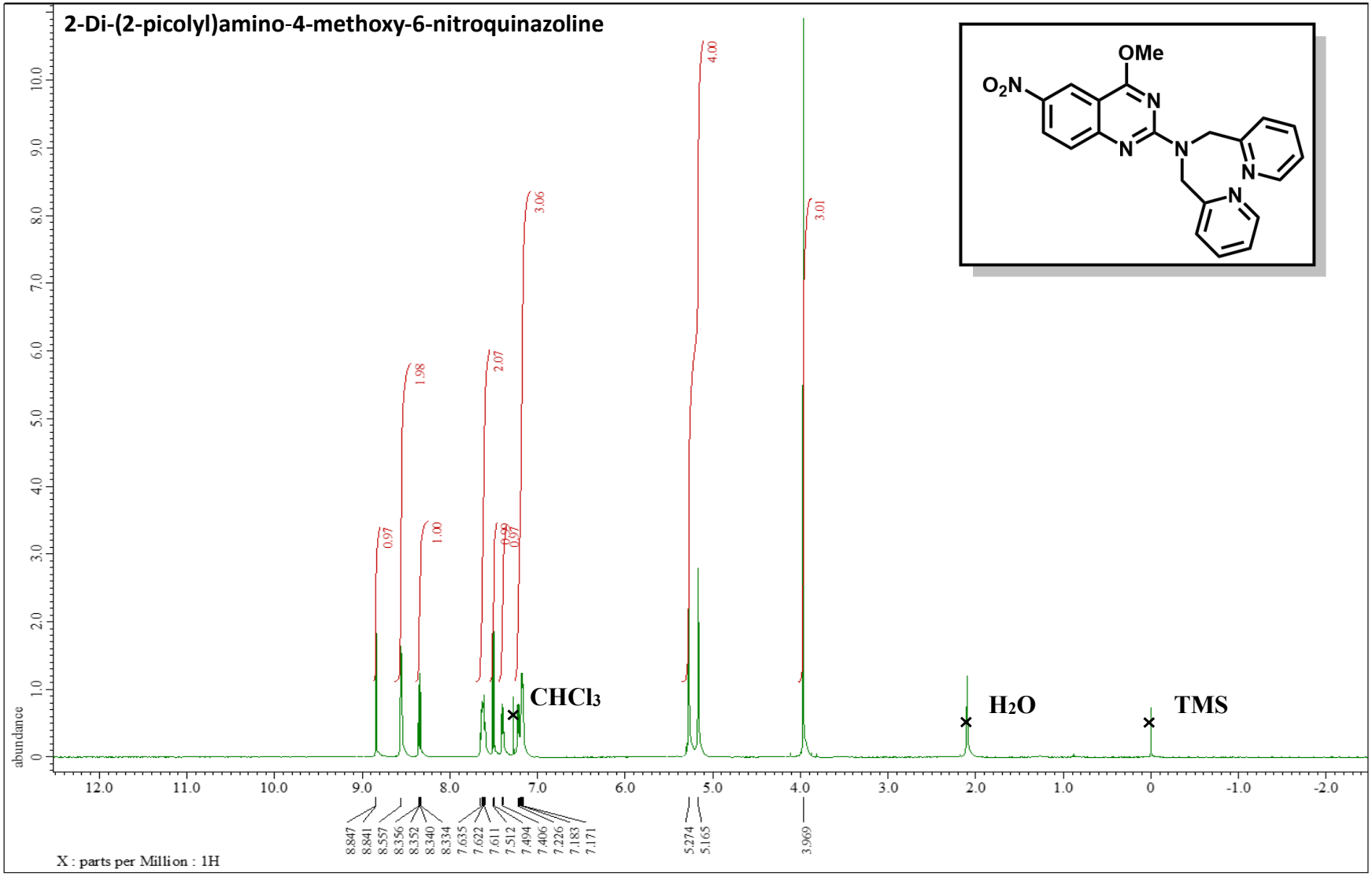
$$F_{HG} - F_0 = \frac{[G_t]\left(-1-K_{app}([G_t]-[H_t])+\sqrt{\left(1+K_{app}([G_t]-[H_t])\right)^2+4K_{app}[H_t]}\right)\left(\frac{F_{HG}}{[HG]}-\frac{F_0}{[G_t]}\right)}{1-K_{app}([G_t]-[H_t])+\sqrt{\left(1+K_{app}([G_t]-[H_t])\right)^2+4K_{app}[H_t]}} \quad \ldots(7)$$

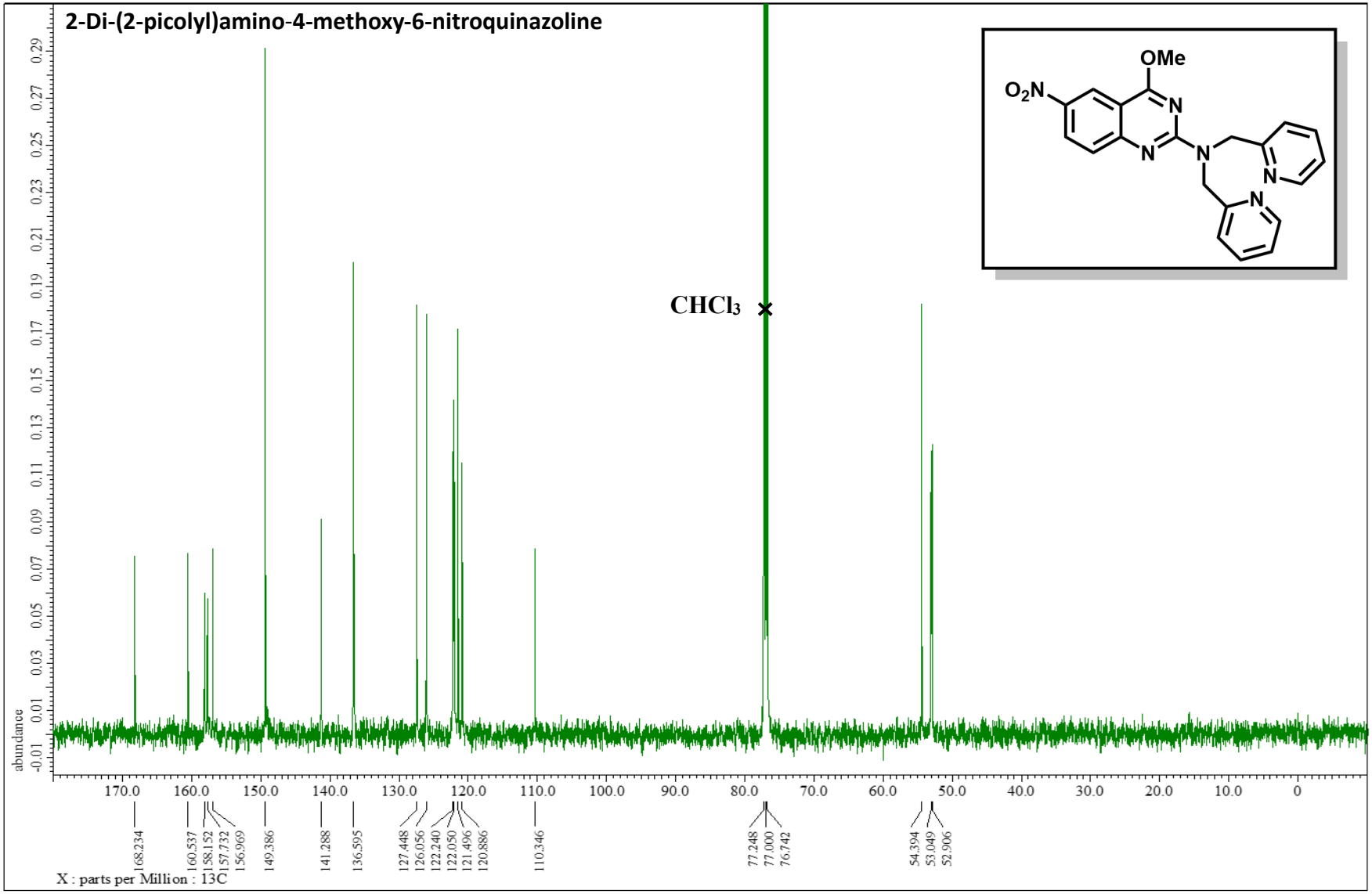
Therefore, from the equation (7), the apparent binding constant for the 1:1 inclusion complex was calculated by the least-square curve fitting analysis (KaleidaGraph^R 4.0 software).

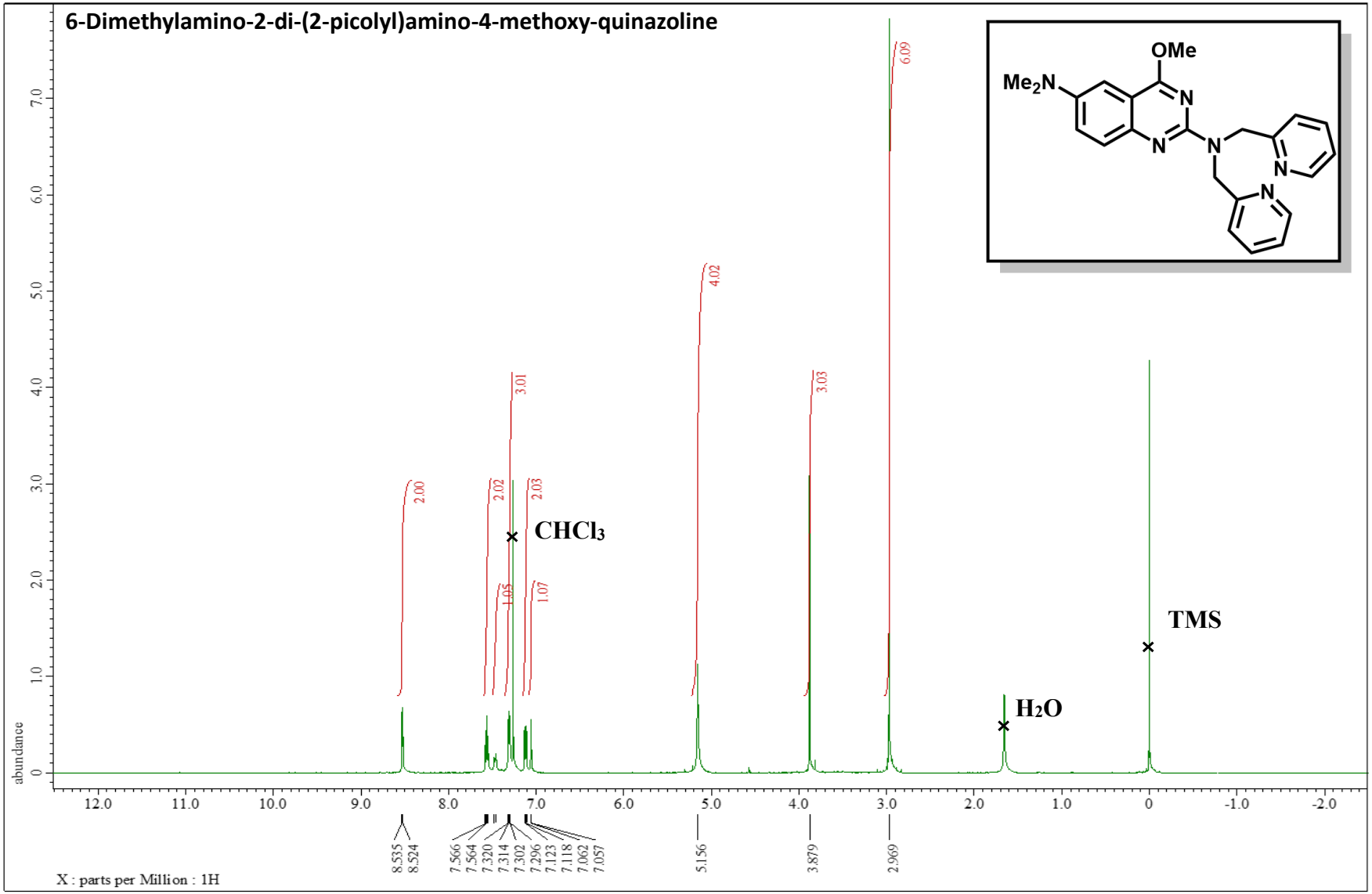
NMR Spectra

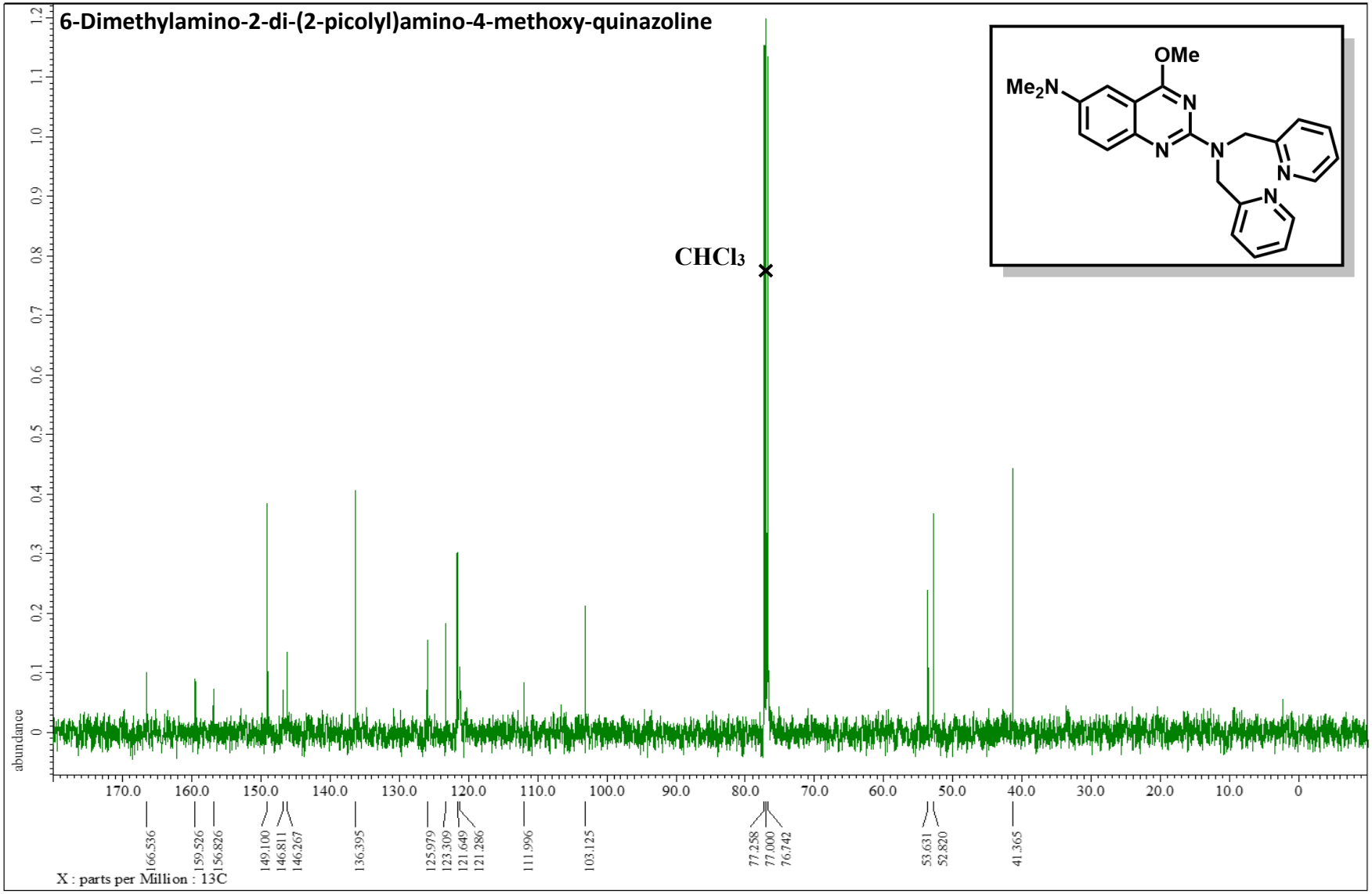

2-Di-(2-picolyl)aminoquinazoline
abundance
X : parts per Million : 1H
CHCl3
H2O
TMS
4.08
3.05
2.01
2.00
1.97
1.84
1.00
0.99
9.042
8.550
8.548
8.542
8.540
7.681
7.664
7.662
7.591
7.575
7.561
7.228
7.149
7.137
7.125
5.227


2-Di-(2-picolyl)aminoquinazoline
CHCl3
abundance
X : parts per Million : 13C
161.777
159.535
158.705
152.372
149.348
136.491
134.001
127.334
126.008
122.574
121.906
121.420
121.258
119.865
77.258
77.000
76.752
52.572

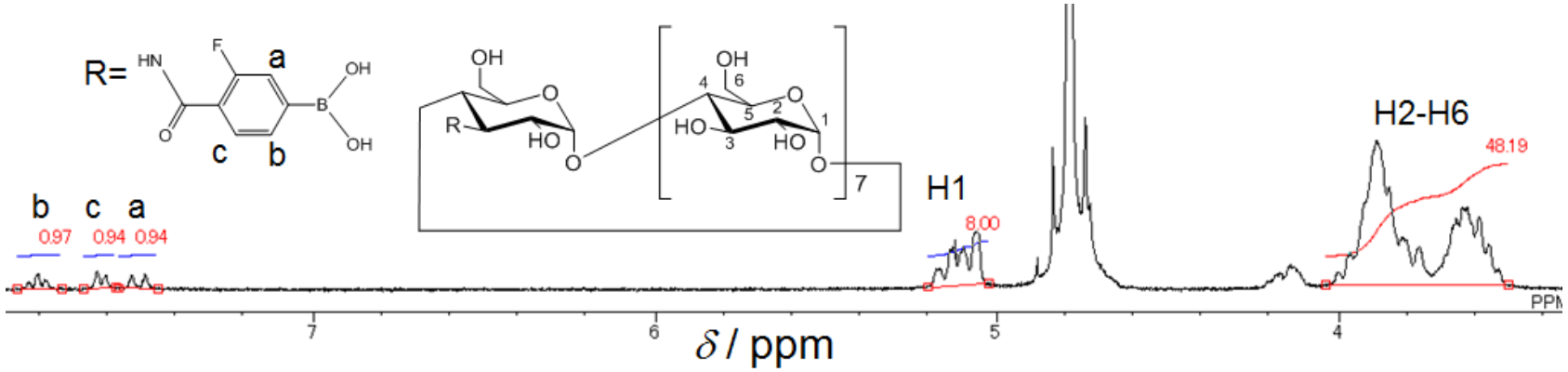

2-Di-(2-picolyl)amino-4-methoxy-6-nitroquinazoline
OMe
O2N
N
N
N
N
N
4.00
3.06
3.01
2.07
1.98
1.00
0.97
0.99
0.97
CHCl3
H2O
TMS
abundance
12.0
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0
-1.0
-2.0
8.847
8.841
8.557
8.356
8.352
8.340
8.334
7.635
7.622
7.611
7.512
7.494
7.406
7.226
7.183
7.171
5.274
5.165
3.969
X : parts per Million : 1H


2-Di-(2-picolyl)amino-4-methoxy-6-nitroquinazoline
OMe
O2N
N
N
N
N
N
CHCl3
abundance
-0.01
0.01
0.03
0.05
0.07
0.09
0.11
0.13
0.15
0.17
0.19
0.21
0.23
0.25
0.27
0.29
170.0
160.0
150.0
140.0
130.0
120.0
110.0
100.0
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0
168.234
160.537
158.152
157.732
156.969
149.386
141.288
136.595
127.448
126.056
122.240
122.050
121.496
120.886
110.346
77.248
77.000
76.742
54.394
53.049
52.906
X : parts per Million : 13C


6-Dimethylamino-2-di-(2-picolyl)amino-4-methoxy-quinazoline
OMe
Me2N
N
N
N
N
N
CHCl3
H2O
TMS
abundance
X : parts per Million : 1H
6.09
4.02
3.03
3.01
2.00
2.02
2.03
1.05
1.07
8.535
8.524
7.566
7.564
7.320
7.314
7.302
7.296
7.123
7.118
7.062
7.057
5.156
3.879
2.969


6-Dimethylamino-2-di-(2-picolyl)amino-4-methoxy-quinazoline
CHCl3
OMe
Me2N
N
N
N
N
N
abundance
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
1.1
1.2
170.0
160.0
150.0
140.0
130.0
120.0
110.0
100.0
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0
166.536
159.526
156.826
149.100
146.811
146.267
136.395
125.979
123.309
121.649
121.286
111.996
103.125
77.258
77.000
76.742
53.631
52.820
41.365
X : parts per Million : 13C

FPB-γ-CyD



FPB-β-CyD