Efficient Fluorescent Recognition of ATP/GTP by a Water-Soluble Bisquinolinium Pyridine-2,6-Dicarboxamide Compound. Crystal Structures, Spectroscopic Studies and Interaction Mode with DNA

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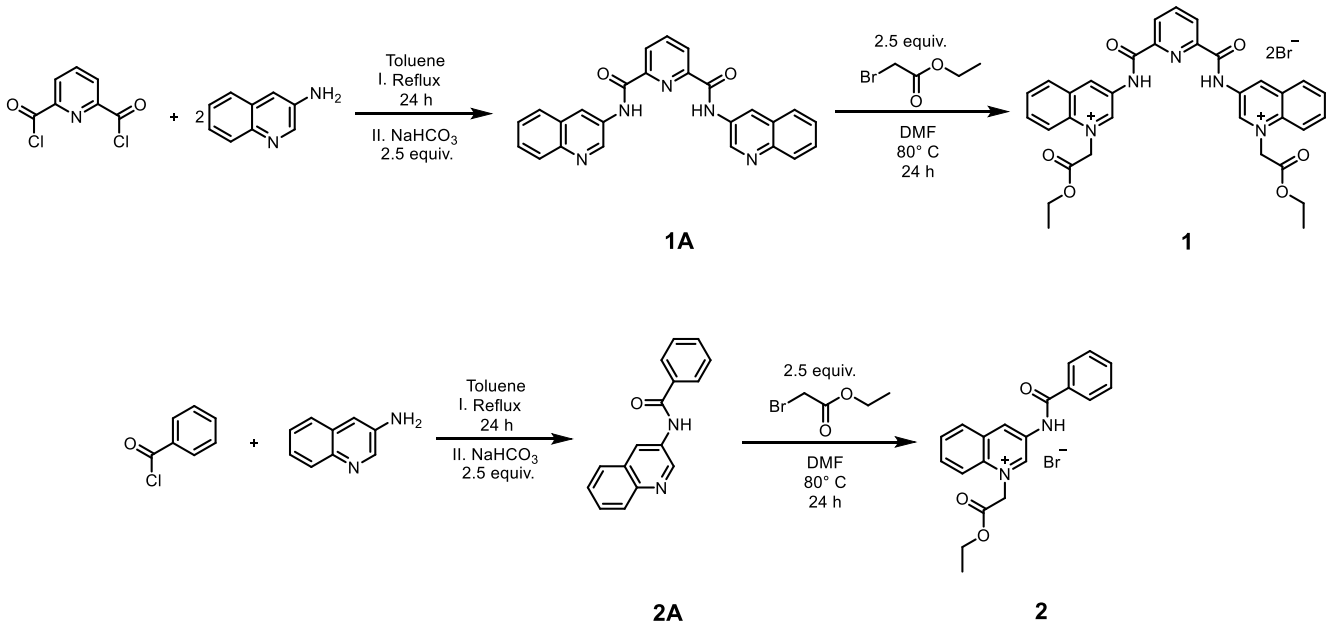
General Considerations: All reagents for synthesis and analysis were of analytical grade and used without further purification: 3-aminoquinoline (Aldrich, 98%); 2,6-pyridinedicarbonyl dichloride (Aldrich, 97 %); ethyl bromoacetate (Aldrich, 98 %); silver trifluoromethanesulfonate (Aldrich, 98%); sodium chloride extra pure (Acros Organics, 99.99 %), sodium acetate (Sigma-Aldrich, 99.5%), sodium phosphate monobasic dihydrate (Aldrich 99.0%), sodium sulfate (Aldrich 99.9 % A.C.S reagent), and sodium pyrophosphate decahydrate (Sigma-Aldrich 99.0 %) were used as received. 5′-nucleotides & nucleosides: guanosine and disodium salts of ATP, ADP, AMP, CTP, GTP, UTP (Aldrich, 85-95%), sodium pyrophosphate tetrabasic (PPi) (Aldrich, 95%). Deoxyribonucleic acid sodium salt from salmon testes (Aldrich), ethidium bromide (Aldrich, 95%). Solvents were purified and dried using standard procedures. Deuterated solvents were purchased from Aldrich. MOPS buffer solutions were prepared by adjusting pH with NaOH to desired values with bidistilled water. MOPS buffer solution (40 mM, pH 7.0) was prepared with double distilled water. Fluorescence spectra were recorded on an Agilent Cary Eclipse spectrophotometer equipped with a thermostated cell holder. UV-Vis spectra were recorded on an Agilent Cary 100 UV-VIS spectrophotometer. Electrospray ionization mass spectra were obtained with a Waters CapLC-coupled Micromass Q-ToF Ultima ESI-instrument. DART ionization mass spectrum of **1** was obtained with a JEOL model JMS-T100LC. EI ionization mass spectrum of **2** was obtained with MStation JMS-700. ^{1}H, ^{13}C, and ^{31}P NMR spectra were recorded on a Bruker Advance DPX 300 spectrometer at 300 MHz. Combustion analysis was performed with a Thermo Scientific Flash 2000 Organic Elemental Analyzer.



Scheme S1. Synthesis of **1** and **2**.

Synthesis of N, N'-bis(3-quinoline) pyridine-2,6-dicarboxiamide, ***1A***.

2,6-pyridinedicarbonyl dichloride (126.9 mg, 0.62 mmol) was dissolved in 50 mL of dry toluene with 3-aminoquinoline (185.9 mg, 1.29 mmol) the solution was stirred at room temperature. The solution was heated to reflux for 4 h. Thus, a brief period of cooling a yellow precipitate was separated from the reaction mixture by vacuum filtration and washed with 10 mL of cold acetone with a cold solution of sodium bicarbonate 3 % m/v (20 mL) a white powder was obtained (236.2 mg, 0.563 mmol, yield: 94 %). **^{1}H-NMR** (300MHz, DMSO-d_6) δ= 11.45 (s, 2H), 9.37 (d, J = 2.40 Hz, 2H), 9.00 (d, J = 2.30 Hz, 2H), 8.49 (m Hz, 2H), 8.49 (*dd*, $^{3}J_{HH}$ =8.62 Hz, $^{3}J_{HH}$ =6.70 Hz, 1H), 8.08 – 8.04 (m, 4H), 7.76 – 7.70 (m, 2H), 7.67 – 7.62 (m, 2H). **^{13}C-NMR** (75 MHz, DMSO) δ 162.40, 148.44, 146.07, 144.79, 140.41, 131.80, 128.71, 128.52, 127.98, 127.74, 127.28, 125.76, 124.52. **MS** (DART+) m/z= 420.1, Calcd. [$C_{25}H_{17}0_2N_5$+ H^+= 420.1]. **IR** (ATR) 3249.79*w* (N-H), 3098.01*w* (C-H, arom.), 1682.79*m* (C=O), 1529.82*m* (N-H).

Synthesis of 3,3'-((pyridine-2,6-dicarbonyl)bis(azanediyl))bis(1-(2-ethoxy-2-oxoethyl)quinolin-1-ium) bromide, ***1***.

Compound **1A** (64.8 mg, 0.154 mmol) was dissolved in dry DMF (2 mL) and added to ethyl bromoacetate (262 µL, 0.943 mmol) to be stirred at room temperature. Acetone (30 mL) was added, and the reaction mixture was heated to reflux for 48 h.

The yellow precipitate was separated from the reaction mixture by vacuum filtration and washed with 10 mL of cold methanol to finally obtain a yellow powder (77.4 mg, 0.103 mmol yield: 66 %).

^{1}H-NMR (400 MHz, DMSO-d_6) δ= 12.12 (s, 2H), *10.39 (d, J = 2.40 Hz, 2H),* 9.89 (d, *J* = 1.36 Hz, 2H), 8.62 – 8.57 (m, 4H), 8.50 (*dd*, $^{3}J_{HH}$ =8.52 Hz, $^{3}J_{HH}$ =6.96 Hz, 1H, 8.45 (d, *J* = 9.16 Hz, 2H), 8.23 (td, *J* = 8.42, 1.00 Hz, 2H), 8.08 (t, *J* = 7.59 Hz, 2H), 6.36 (s, 4H), 4.28 (q, *J* = 7.10 Hz, 4H), 1.28 (t, *J* = 7.08 Hz, 6H). **^{13}C-NMR** (125 MHz, DMSO-d_6) δ 166.10, 162.60, 147.59, 145.92, 141.03, 137.58, 135.75, 134.90, 132.25, 130.50, 130.34, 129.21, 126.72, 118.86, 62.58, 57.99, 14.01. **MS** (ESI^+) m/z= 673.7, Calcd. [$C_{33}H_{31}O_6N_5Br^+$= 673.14]. **IR** (ATR) 3304.18*w* (N-H), 2975.58*w* (C-H, arom.), 1742.64*m* (C=O), 1675.32*m* (N-H), 1545.42*m* (N-H), 1371.41*m* (-CH_3), 1203.64*m* (C-O). **Anal. Calcd**. For $C_{33}H_{31}Br_2N_5O_6$: C, 52.61; H, 4.15; N, 9.30. Found: C, 52.53; H, 4.27; N, 9.14.

*Synthesis of N-(quinolin-3-yl)benzamide, **2A**.*

3-aminoquinoline (88.1 mg, 0.611 mmol) was dissolved in 23 mL dry toluene with benzoyl chloride (70 µL, 0.588 mmol),, thus, the solution was stirred at room temperature. The reaction mixture was heated under reflux for 48 h. A yellow precipitate was separated from the reaction mixture by vacuum filtration, after that, the precipitate was washed with cold acetone (10 mL) and with a cold solution of sodium bicarbonate 3 %m/v (13 mL) a beige powder was obtained (79.4 mg, 0.320 mmol, yield: 53 %). **^{1}H-NMR** (300MHz, DMSO-d_6) δ= 10.77 (s, 1H), 9.18 (d, J = 2.33 Hz, 1H), 8.87 (d, J =2.16 Hz, 1H), 8.08 – 7.94 (m, 4H), 7.73 – 7.52 (m, 5H). **^{13}C-NMR** (75 MHz, DMSO) δ 166.37, 145.36, 144.01, 134.31, 133.04, 132.15, 128.67, 128.45, 128.27, 127.95, 127.91, 127.88, 127.35, 124.10. **MS** (EI) m/z= 248.08, Calcd. [$C_{16}H_{12}N_2O^{\bullet+}$= 248.09441]. **IR** (ATR) 3470.48*w* (N-H), 3060.49 *w* (C-H, arom.), 1650.75*m* (C=O), 1556.20*m* (N-H).

*Synthesis of 3-benzamido-1-(2-ethoxy-2-oxoethyl)quinolin-1-ium bromide, **2**.*

A solution of **2A** (50.2 mg, 0.202 mmol) in dry acetone (25 mL), ethyl bromoacetate (224 µL, 2.025 mmol) was added at room temperature. The solution was heated to reflux for 74 h. After that time diethyl ether (32 mL) was added and the solution was cooled by 0.5 h to obtain a beige solid. The solution was filtered and washed with cold diethyl ether (15 mL) a beige powder was obtained (29.1 mg, 0.070 mmol, yield: 35 %). Anion exchange for triflate salt of **2** was carried out with silver triflate in CH_3OH/H_2O (9/1, v/v).

^{1}H-NMR (300MHz, DMSO-d_6) = δ 11.43 (s, 1H), 10.04 (d, J = 2.22 Hz, 1H), 9.45 (d, J = 2.3 Hz, 1H), 8.53 (dd, $^{3}J_{HH}$ = 8.30, $^{4}J_{HH}$ = 0.88 Hz, 1H), 8.40 (d, J =8.96 Hz, 1H), 8.18 (ddd, $^{3}J_{HH}$ = 8.76, $^{3}J_{HH}$ = 7.15, $^{4}J_{HH}$ = 1.47 Hz, 1H), 8.11 – 8.00 (m, 3H), 7.77 – 7.58 (m, 3H), 6.25 (s, 2H), 4.26 (q, J = 7.10 Hz, 2H), 1.26 (t, J = 7.10 Hz, 3H). **^{13}C-NMR** (75 MHz, DMSO-d_6) δ 166.35, 166.18, 145.56, 136.63, 135.54, 134.68, 133.54, 132.97, 130.44, 130.31, 129.38, 128.95, 128.01, 118.75, 62.63, 58.10, 14.00. **MS** (ESI^{+}) m/z= 334.7, Calcd. [$C_{20}H_{19}N_2O_3^{+}$= 335.1]. **IR** (ATR) 3411.51*w* (N-H), 3029.37*w* (C-H, arom.), 2951.74*w* (C-H), 1748.19*m* (C=O), 1673.64*m* (N-H), 1570.51*m* (N-H), 1374.45*m* (-CH_3), 1289.56*m* (C-N), 1208.84*m* (C-O). **Anal. Calcd**. For $C_{20}H_{19}BrN_2O_3$: C, 57.84; H, 4.61; N, 6.75. Found: C, 57.53; H, 4.77; N, 6.54.

Table S1. Crystallographic data for bromide of **1** and triflate of **2**.

	1	**2**
Empirical formula	$C_{70}H_{84}Br_4N_{10}O_{19}$	$C_{21}H_{19}F_3N_2O_6S$
Formula weight	1689.11	484.44
Temperature	100(2) K	100(2) K
Wavelength	0.71073 Å	1.54178 Å
Crystal system	Triclinic	Monoclinic
Space group	P-1	P 21/c
Unit cell dimensions	a = 10.06590(10) Å α = 91.9737(5)° b = 11.5585(2) Å β = 100.2535(5)° c = 16.0001(2) Å γ = 92.6788(5)°	a = 9.3162(2) Å α = 90° b = 15.1106(4) Å β = 93.2292(18)° c = 15.1042(4) Å γ = 90°
Volume	1828.10(4) $Å^3$	2122.89(9) $Å^3$
Z	1	4
Density (calculated)	1.534 Mg/m^3	1.516 Mg/m^3
Absorption coefficient	2.279 mm^{-1}	1.985 mm^{-1}
F(000)	866	1000
Crystal size	0.250 x 0.219 x 0.120 mm^3	0.289 x 0.175 x 0.040 mm^3
Theta range for data collection	1.765 to 27.445°	4.142 to 68.296°
Index ranges	-13<=h<=13, -14<=k<=14, -20<=l<=20	-10<=h<=11, -17<=k<=18, -17<=l<=11
Reflections collected	46156	16120
Independent reflections	8342 [R(int) = 0.0208]	3834 [R(int) = 0.0284]
Completeness to theta = 25.242°	99.9 %	98.8 %
Absorption correction	None	None
Refinement method	Full-matrix least-squares on F^2	Full-matrix least-squares on F^2
Data / restraints / parameters	8342 / 375 / 608	3834 / 0 / 302
Goodness-of-fit on F^2	1.052	1.023
Final R indices [I>2sigma(I)]	R1 = 0.0261, wR2 = 0.0641	R1 = 0.0368, wR2 = 0.0979
R indices (all data)	R1 = 0.0292, wR2 = 0.0654	R1 = 0.0429, wR2 = 0.1022
Extinction coefficient	n/a	n/a
Largest diff. peak and hole	0.548 and -0.462 $e.Å^{-3}$	0.681 and -0.339 $e.Å^{-3}$

Table S2. Hydrogen bonds for bromide of **1** [Å and °].

D-H ··· A	d(D-H)	d(H ··· A)	d(D ··· A)	<(DHA)
O(7)-H(7A)...Br(2)	0.841(13)	2.394(14)	3.2176(15)	166(2)
O(7)-H(7B)...Br(1)	0.80(2)	2.42(2)	3.2168(14)	177(2)
O(8)-H(8A)...Br(1)	0.842(7)	2.79(3)	3.577(6)	155(7)
O(8)-H(8B)...Br(2)	0.83(4)	2.76(4)	3.552(5)	161(6)
O(8A)-H(8C)...Br(1)	0.842(10)	2.78(5)	3.58(2)	160(13)
O(8A)-H(8D)...Br(2)	0.842(10)	2.76(5)	3.56(2)	158(12)
O(9)-H(9A)...Br(2)#1	0.860(17)	2.385(18)	3.2443(16)	178(3)
O(10)-H(10A)...O(9)	0.892(18)	1.907(19)	2.781(2)	166(3)
N(2)-H(2N)...O(7)	0.828(15)	2.149(17)	2.9300(19)	157(2)
N(4)-H(4N)...O(7)	0.838(15)	2.082(16)	2.8912(19)	162(2)

Symmetry transformations used to generate equivalent atoms:

#1 -x+1,-y,-z+1

Table S3. Hydrogen bonds for triflate of **2** [Å and °].

D-H ··· A	d(D-H)	d(H ··· A)	d(D ··· A)	<(DHA)
N(1)-H(1)...O(6)#1	0.83(2)	2.12(2)	2.903(2)	159(2)
C(8)-H(8)...O(1)	0.95	2.26	2.737(2)	110.3
C(8)-H(8)...O(5)	0.95	2.41	3.179(2)	137.9
C(15)-H(15)...O(6)#1	0.95	2.51	3.217(2)	130.8
C(17)-H(17A)...O(4)#2	0.99	2.38	3.220(2)	142.7
C(17)-H(17B)...O(5)	0.99	2.31	3.242(2)	157.3
C(19)-H(19A)...O(1)#3	0.99	2.43	3.165(2)	130.9

Symmetry transformations used to generate equivalent atoms:
#1 x-1,y,z #2 -x+1,y-1/2,-z+1/2 #3 -x+1,-y+1,-z

Table S4. Interaction energies and Gibbs free interaction energies in kcal/mol for **1**, obtained at the PBEh-3c method

Nucleotide	E_{int}	G_{int}
ATP	-40.30	-21.68
GTP	-37.91	-20.95
CTP	-35.27	-18.41
UTP	-22.58	-8.41

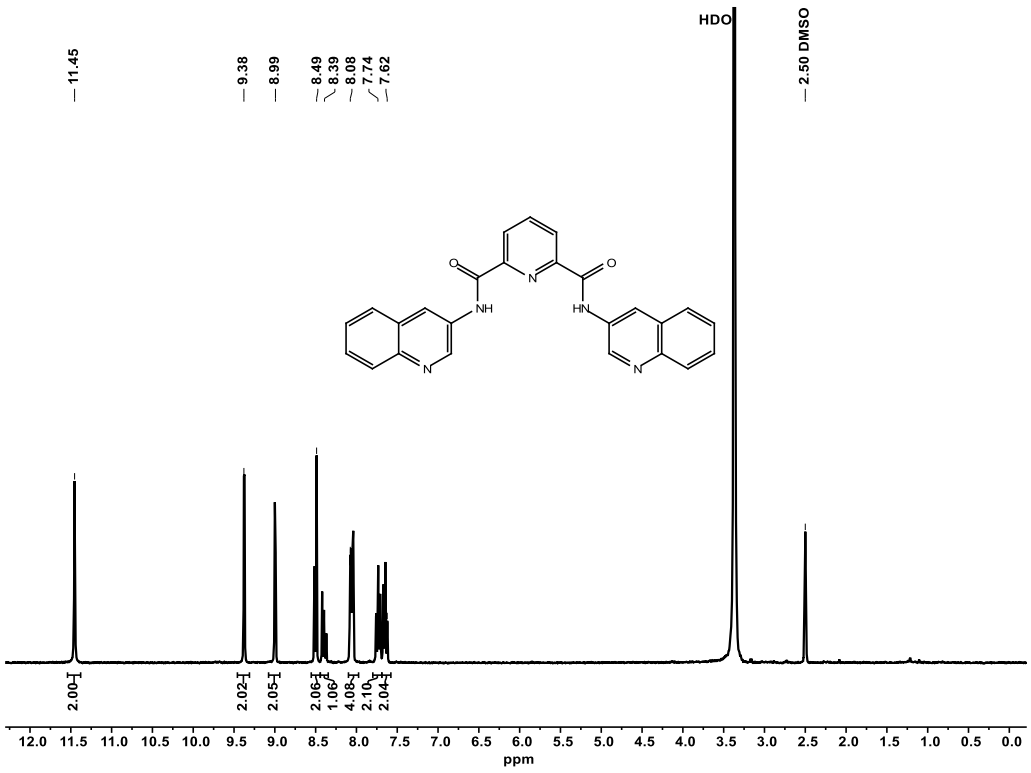


Fig. S1 ^{1}H NMR spectrum of **1A** in DMSO-d_6.

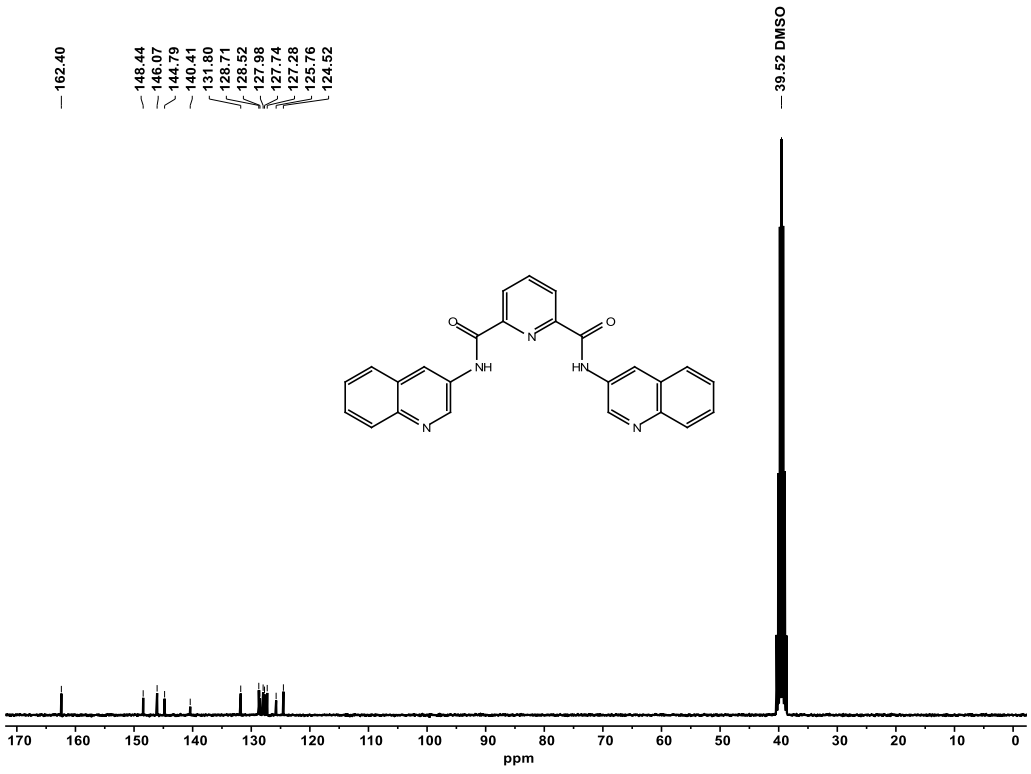


Fig. S2 ^{13}C NMR spectrum of **1A** in DMSO-d_6.

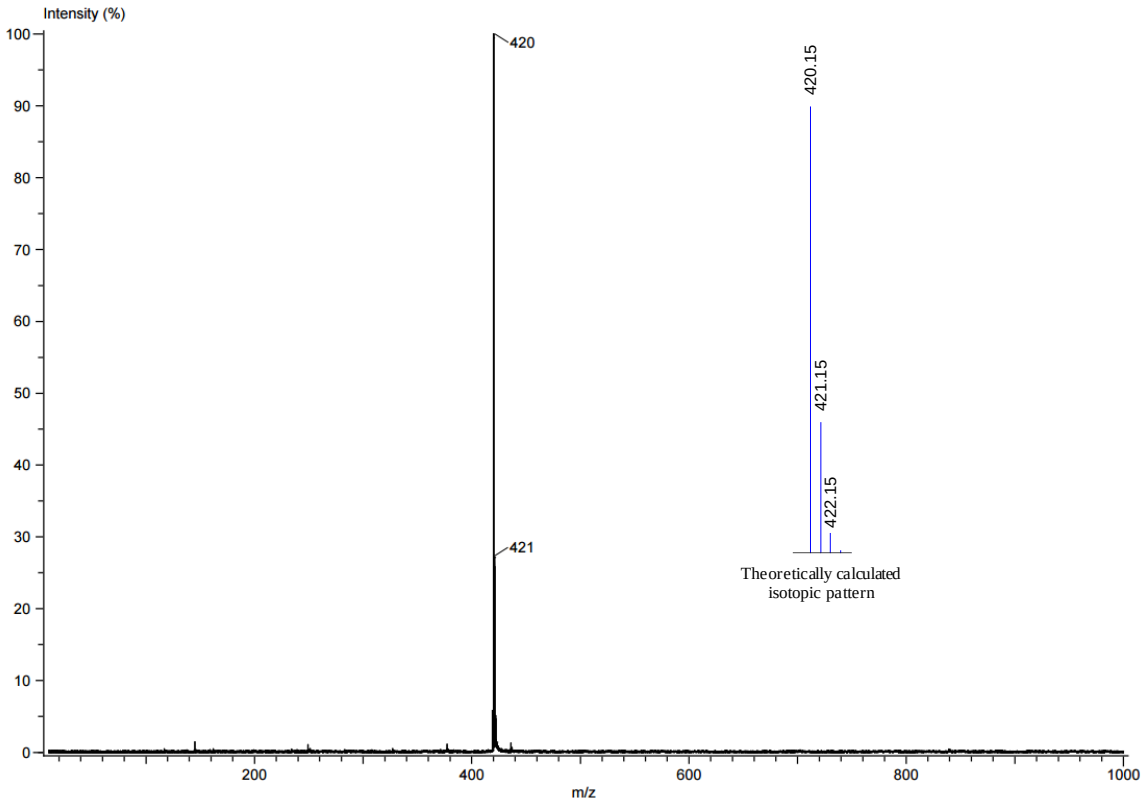


Fig. S3 Positive scan MS-DART **1A** + H^+ [$C_{25}H_{18}N_5O_2^+$= 420.14550].

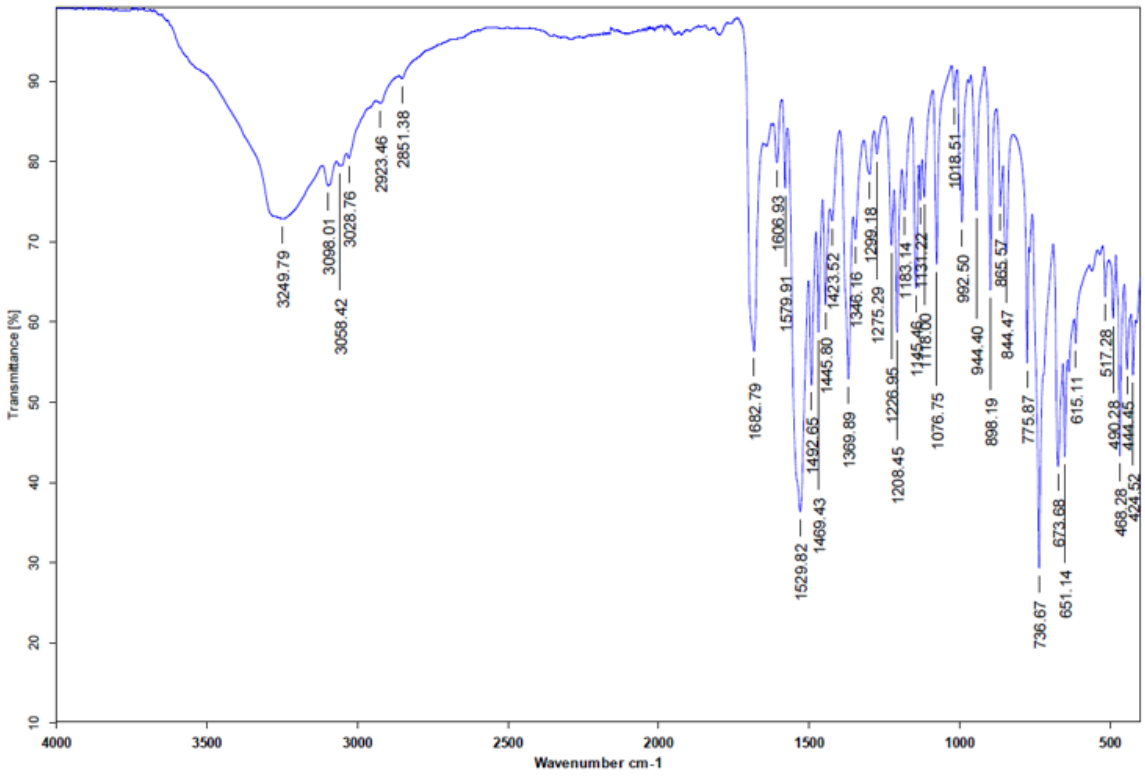


Fig. S4 IR-ATR spectrum of **1A**.

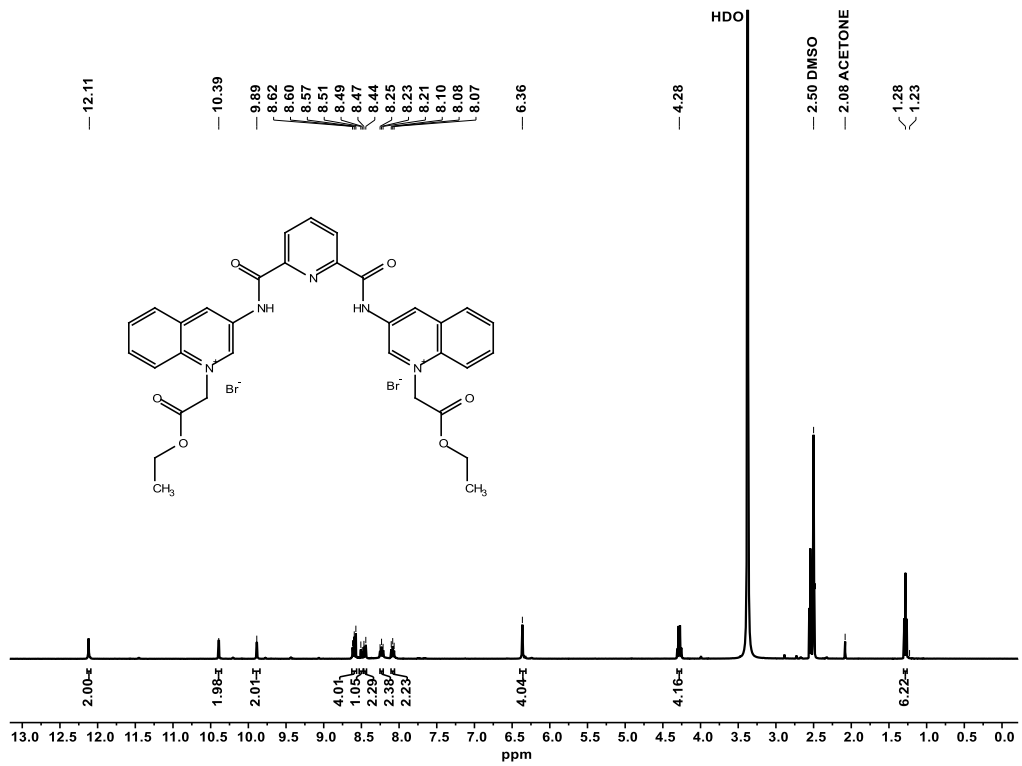


Fig. S5 ^{1}H NMR spectrum of **1** in DMSO-d_6.

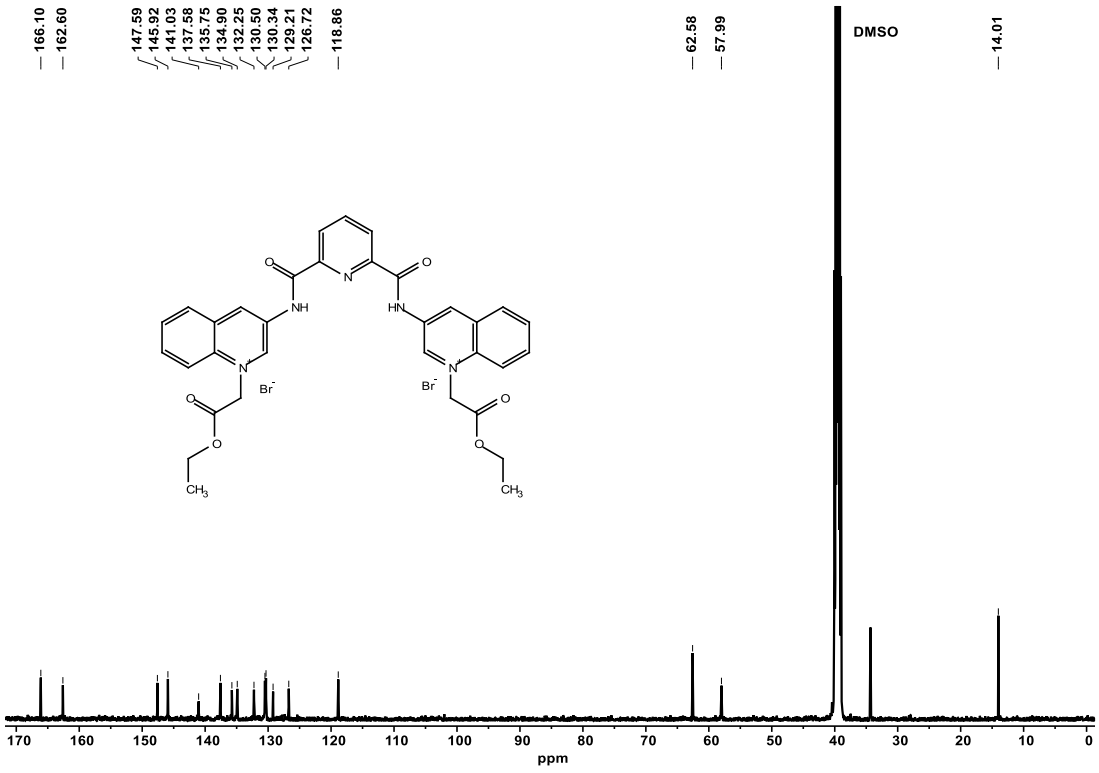


Fig. S6 ^{13}C NMR spectrum of **1** in DMSO-d_6.

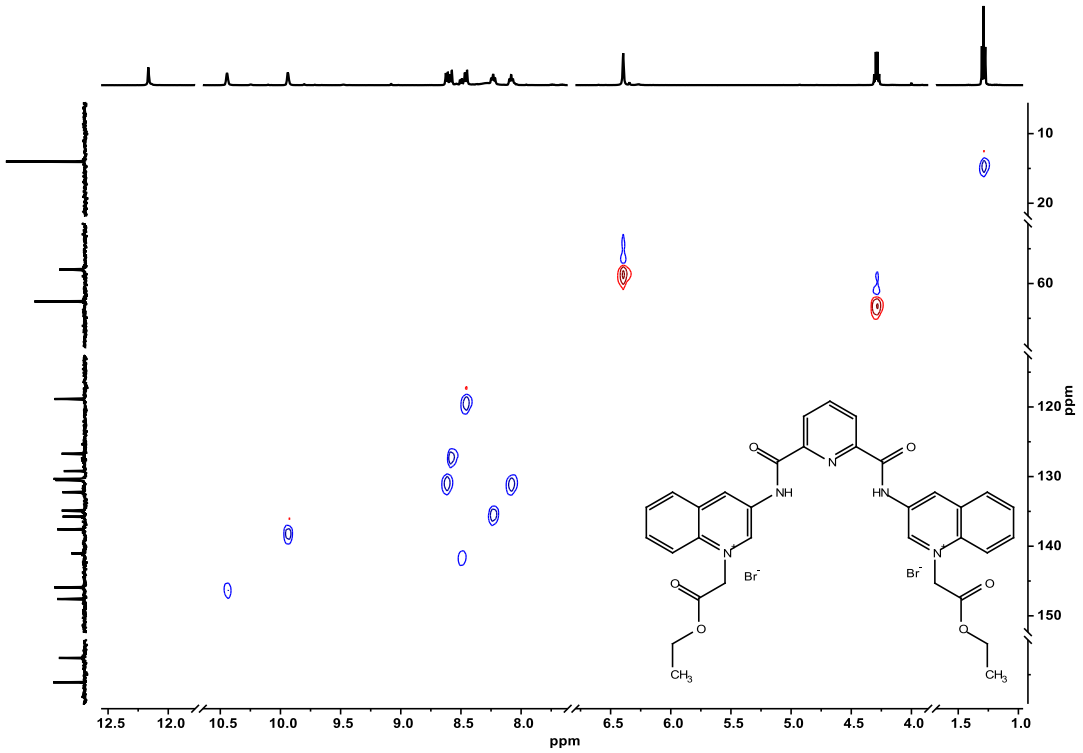


Fig. S7 HSQC spectrum of **1**in DMSO-d_6.

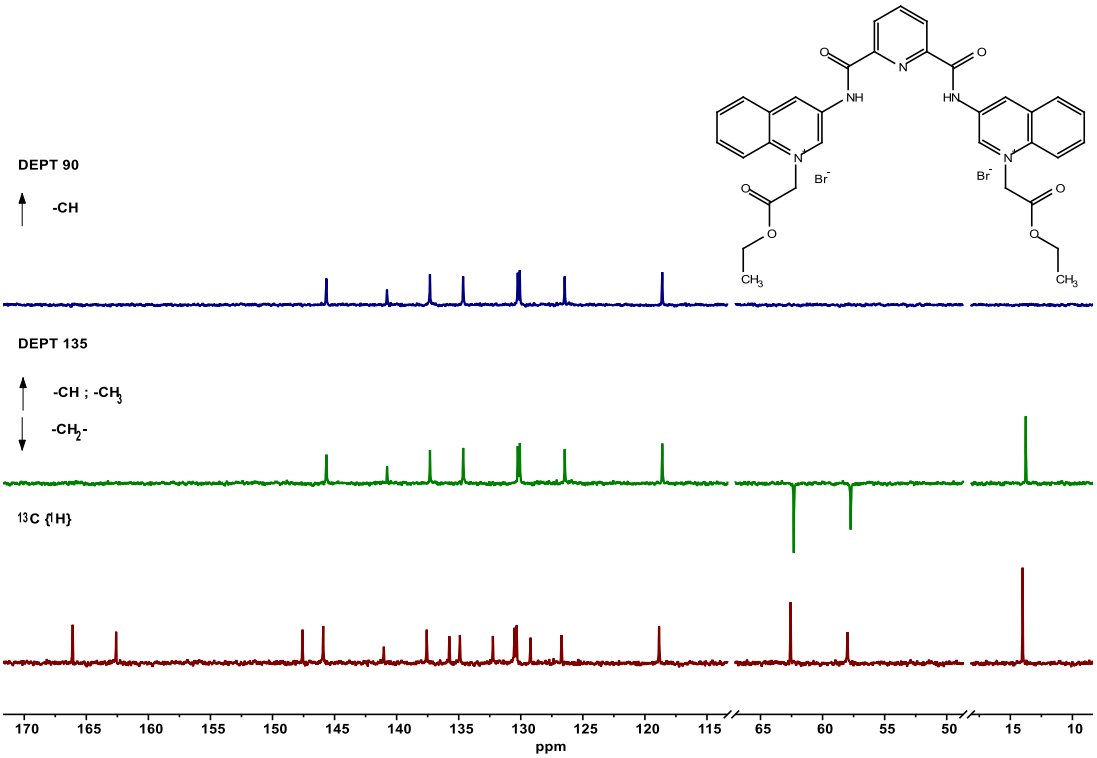


Fig. S8 DEPT spectrum of **1** in DMSO-d_6.

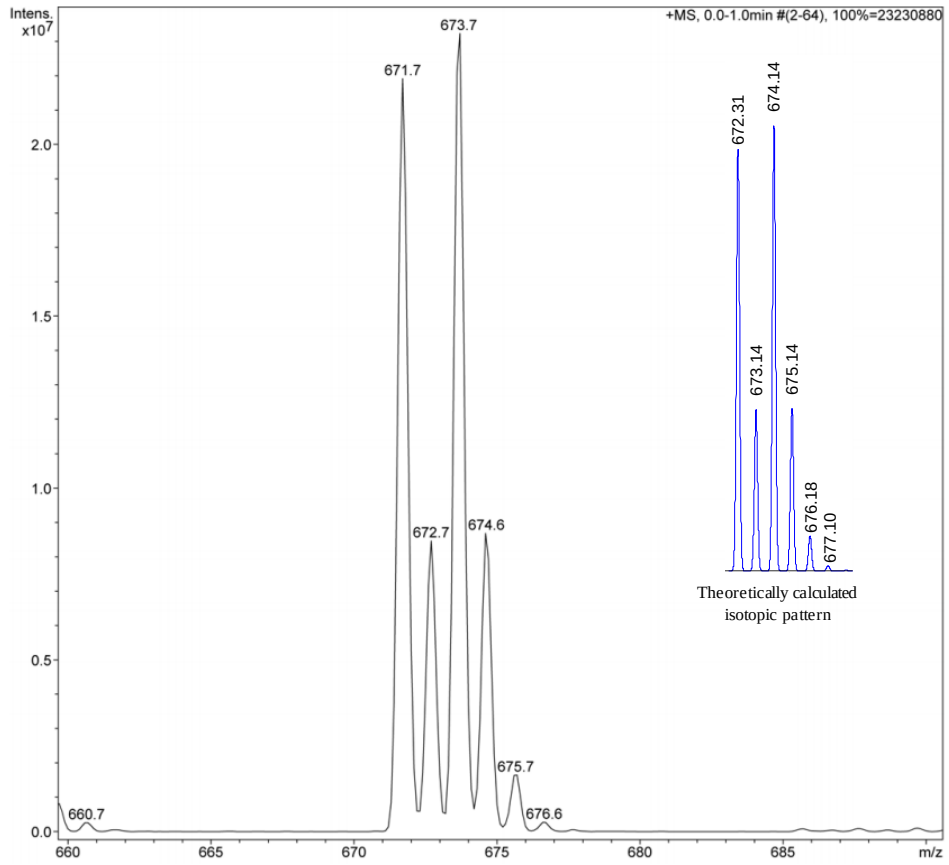


Fig. S9 Positive scan MS-ESI spectrum of **1** + Br^- [$C_{33}H_{31}BrN_5O_6^+$= 672.14522].

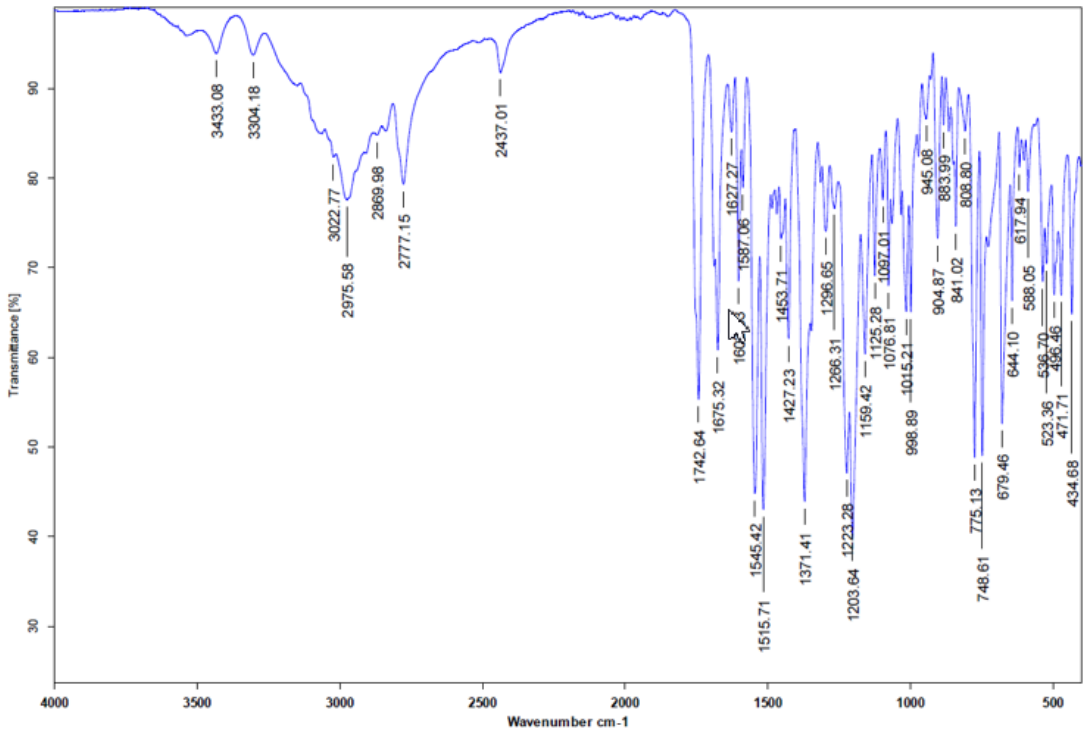


Fig. S10 IR-ATR spectrum of **1**.

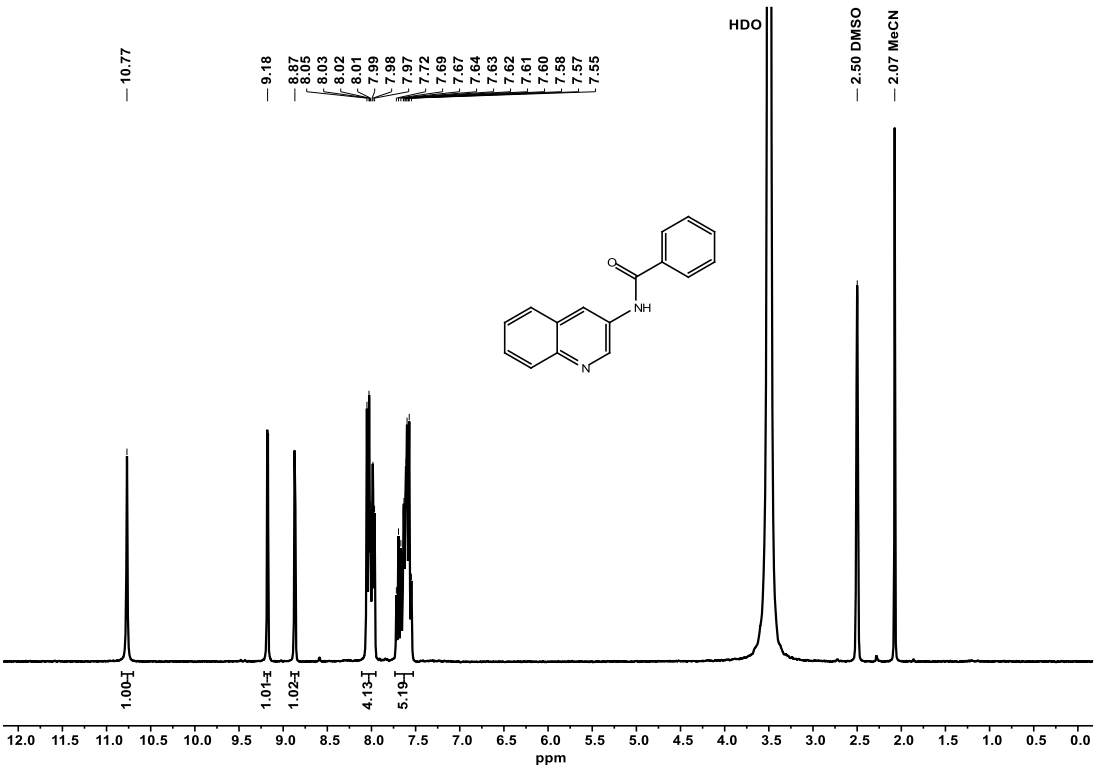


Fig. S11 ^{1}H NMR spectrum of **2A** in DMSO-d_6.

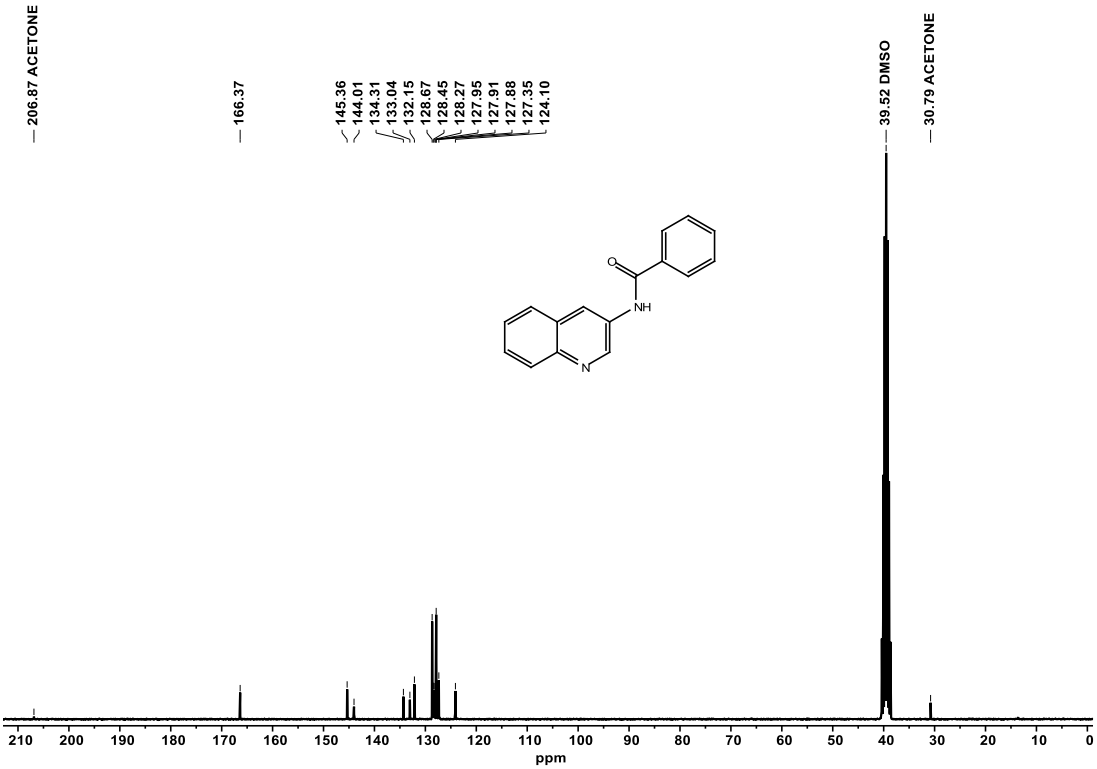


Fig. S12 ^{13}C NMR spectrum of **2A** in DMSO-d_6.

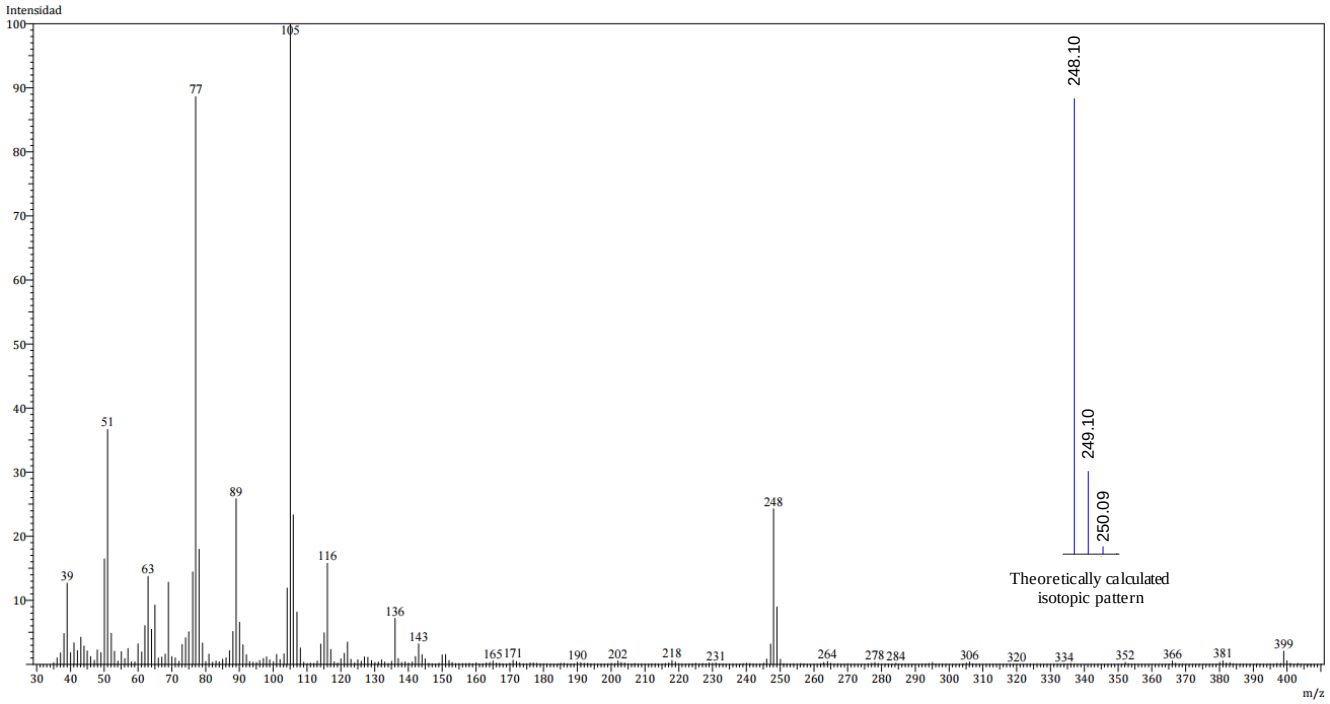


Fig. S13 Positive scan MS-EI **2A** [$C_{16}H_{12}N_2O^{\bullet+}$= 248.09441].

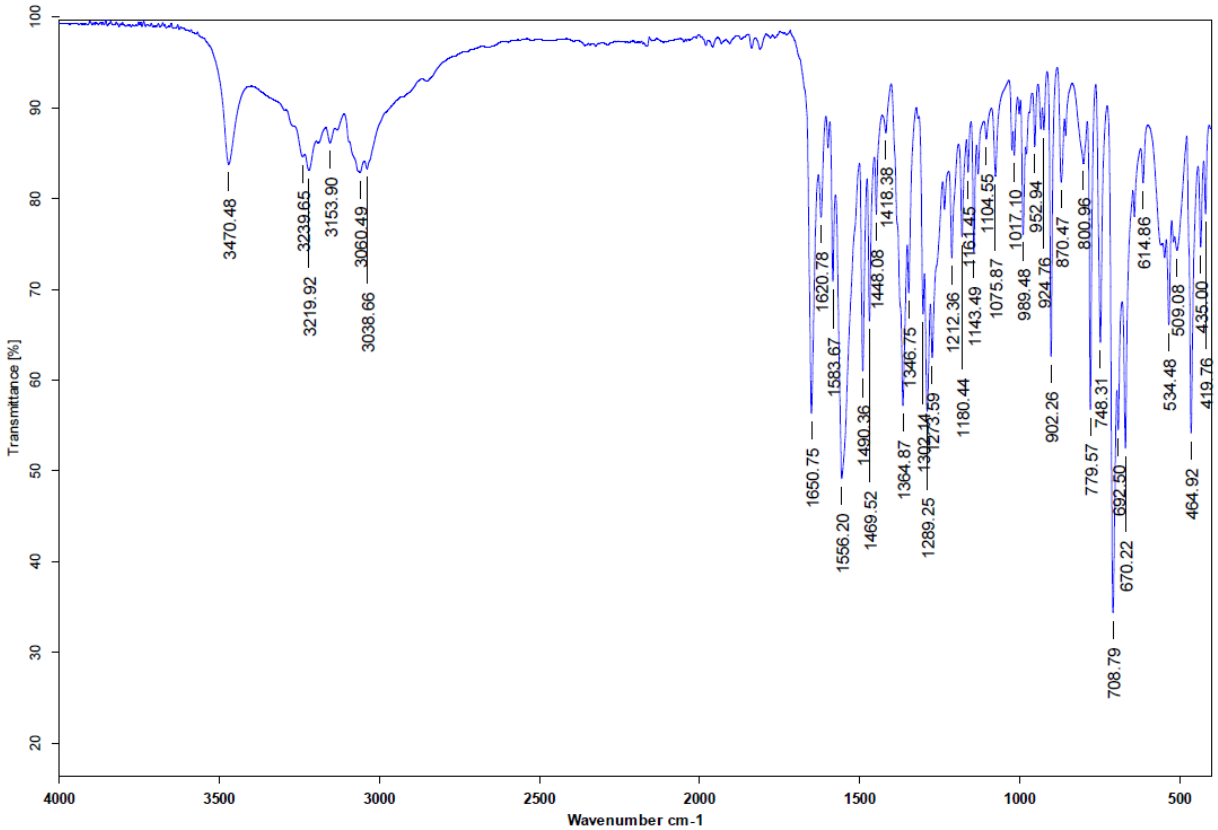


Fig S14. IR-ATR spectrum of **2A**.

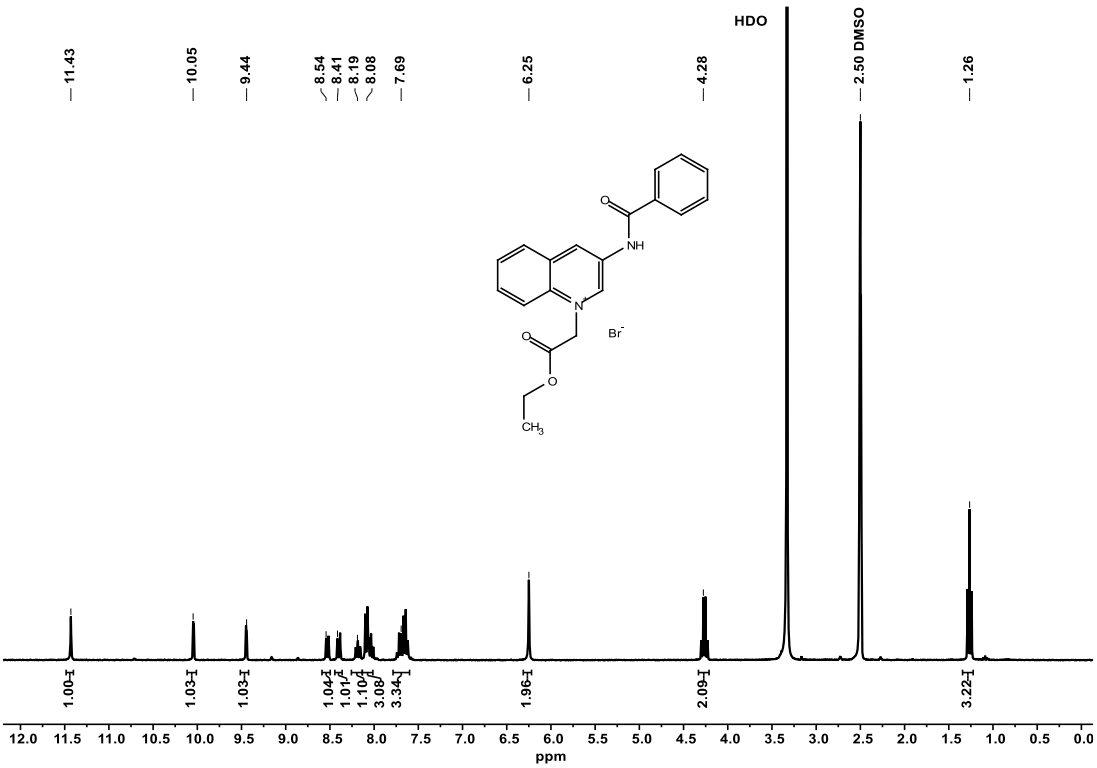


Fig. S15 ^{1}H NMR spectrum of **2** in DMSO-d_6.

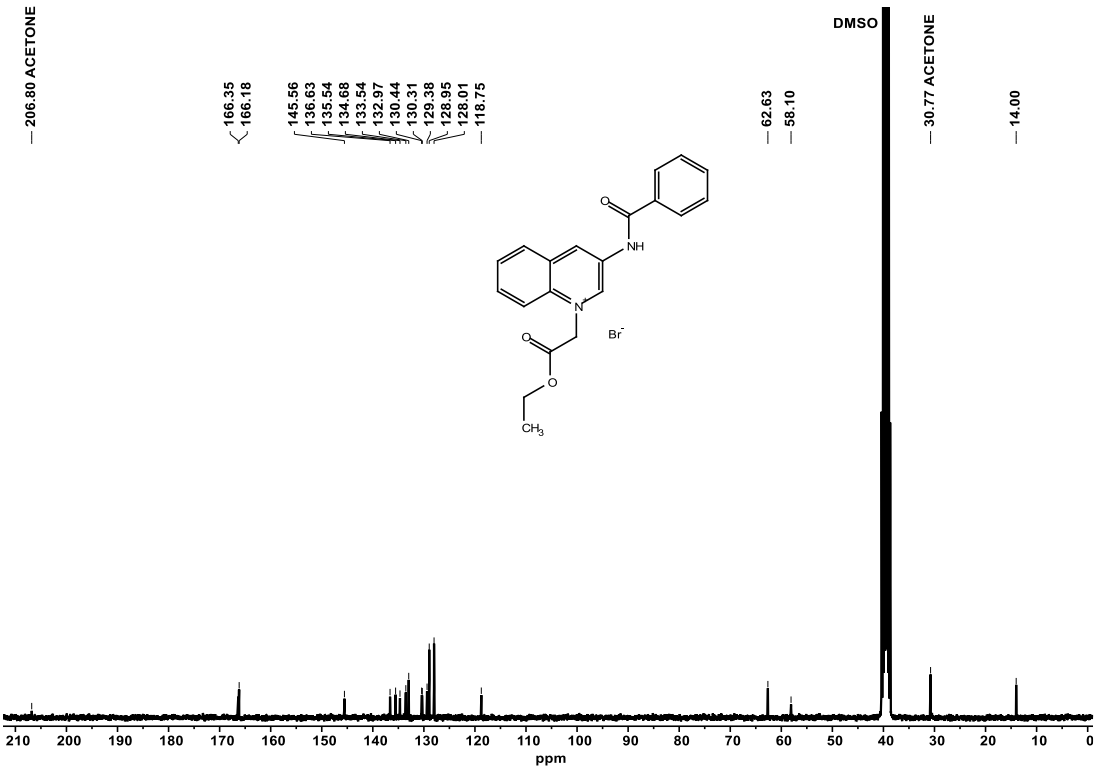


Fig. S16 ^{13}C NMR spectrum of **2** in DMSO-d_6.

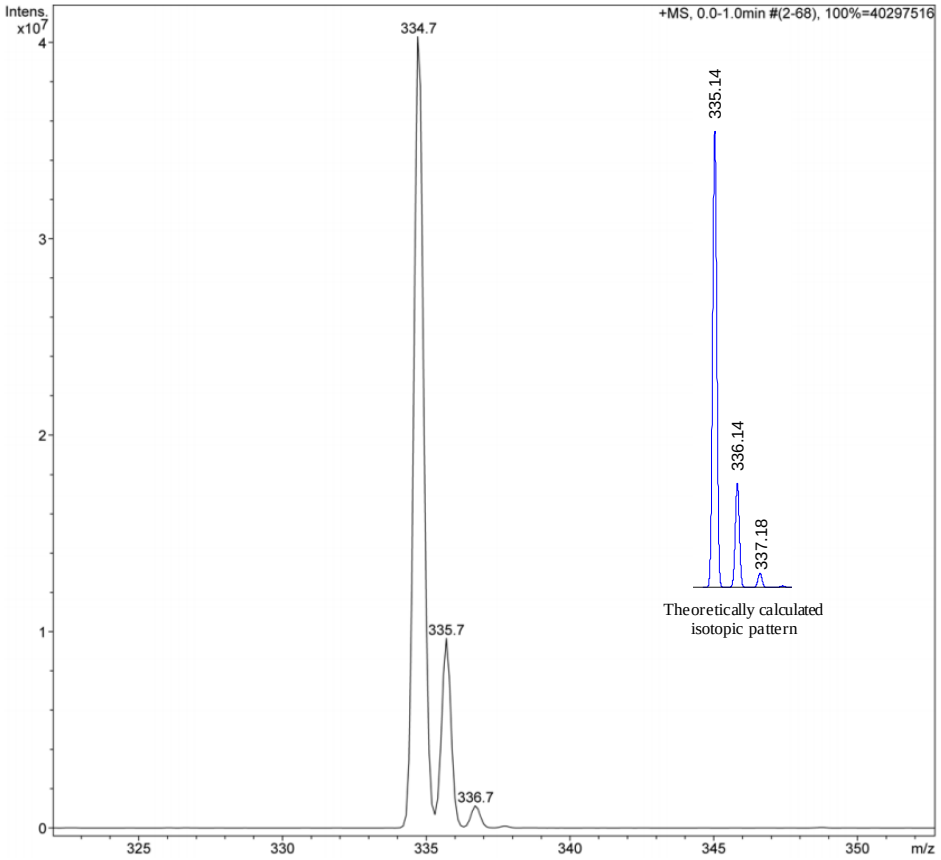


Fig. S17 Positive scan MS-ESI spectrum of **2** [$C_{20}H_{19}N_2O_3^+$= 335.13902].

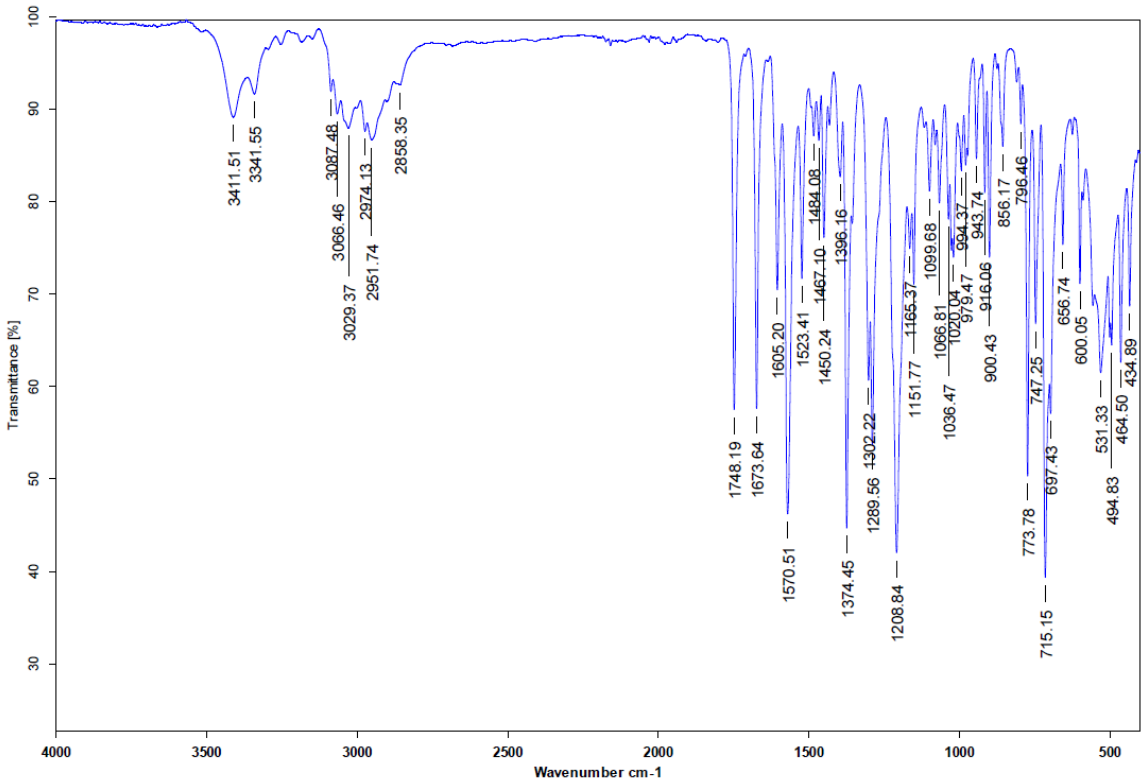


Fig. S18 IR-ATR spectrum of **2**.

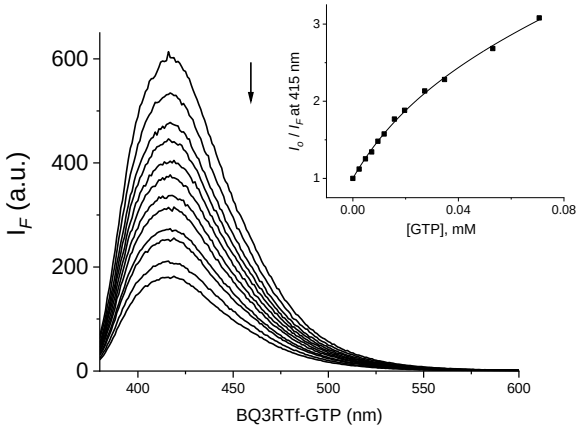


Fig. S19. Changes of emission spectra (λ_{ex}= 350 nm) of buffered aqueous solutions at pH= 7.0 of **1** (15 μM) upon addition of increasing amounts of GTP. The inset shows the S–V plot at 415 nm.

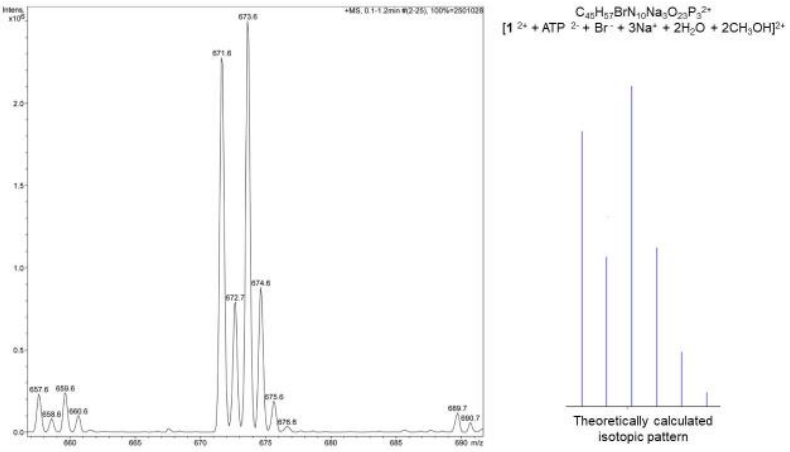


Fig. S20. Positive scan MS-ESI spectrum of **1** + 1.0 equiv. of sodium salt of ATP.

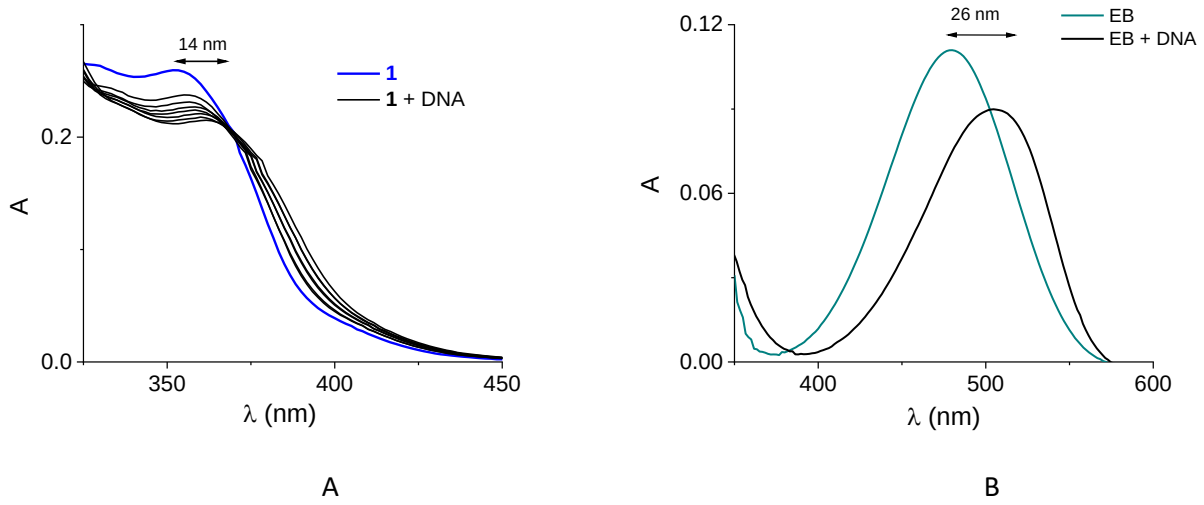


Fig. S21. The effect of DNA on the absorption spectra of (A) **1** (20 μM) and (B) EB (10 μM) in buffered aqueous solutions at pH= 7.0 (5.0 mM Tris.HCl, 25 °C). Final DNA concentration (120 μM). The ionic strength was adjusted to 50.0 mM of NaCl.

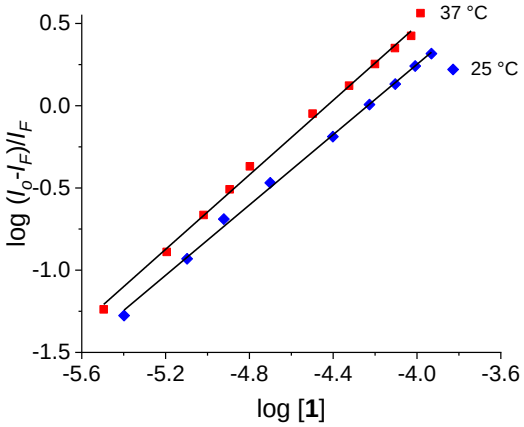


Fig. S22. The double-log plot of quenching (λ_{ex}= 520 nm) effect of **1** on ds-DNA-EB complex fluorescence at pH= 7.0 (10 m MOPS) and different temperatures.

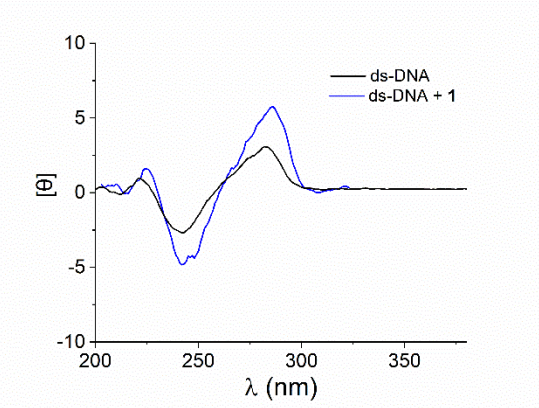


Fig. S23. CD-spectra of free ds-DNA (40 μM) and ds-DNA in the presence of **1** (40 μM) at pH= 7.0 (10 mM MOPS, 25°C). The ionic strength was adjusted to 50.0 mM of NaCl.