

Supporting information for publication

“A highly selective phenothiazine-based fluorescence ‘turn-on’ indicator based on cyanide-promoted novel protection/deprotection mechanism”

Bhaskar Garg* and Yong-Chien Ling*

Department of Chemistry, National Tsing Hua University, 101, Section 2, Kuang-Fu Road, Hsinchu, 30013, Taiwan

ycling@mx.nthu.edu.tw; bhaskargarg111@gmail.com

Table of Contents:

General	S2
Synthesis and characterization data	S2-S3
¹ H NMR spectrum of PTZ-AL	S4
¹³ C NMR spectrum of PTZ-AL	S5
¹³ C/ DEPT NMR spectrum of PTZ-AL	S6
Proton-carbon 2D NMR spectrum of PTZ-AL	S7
HRMS (ESI-MS) spectrum of PTZ-AL	S8
¹ H NMR titration profile of PTZ-AL with TBAF	S9
¹ H NMR spectra of PTZ-AL under different conditions	S10
Fluorescence titration (emission) spectra of PTZ-AL with TBACN	S11
Fluorescence titration (emission) spectra of PTZ-AL with thiols	S12-S14
Fluorescence titration (emission) spectra of PTZ-AL with Na ₂ S	S14
Fluorescence emission spectra of PTZ-AL with different species	S15-S16
Crystal data of PTZ-AL	S17-S24

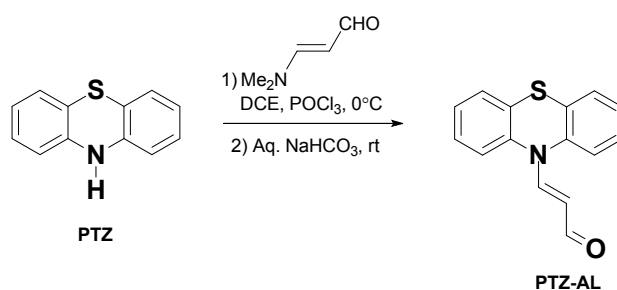
Supporting information for publication

General

Phenothiazine was purchased from Alfa Aesar, UK. 3-(dimethylamino)propenal and 1,2-dichloroethane (99.8%) were purchased from Acros Organics. All the chemicals and reagents received were of highest purity and used without further purification. The Fourier transform infrared spectroscopy (FTIR) was carried out on a Perkin-Elmer system 2000. The ^1H NMR and ^{13}C NMR were recorded on a nuclear magnetic resonance spectrometer (Bruker Cryomagnet, Oxford) operated under 600 MHz (^1H) and 150 MHz (^{13}C), respectively at room temperature. The chemical shifts (δ ppm) are referenced to the respective solvents and splitting patterns are designed as d (doublet), m (multiplet), dd (doublet of doublets), and td (triplet of doublets). The high resolution electrospray ionization mass spectrometry was performed on a Finnigan MAT 95 XP spectrometer. The electronic absorption spectrum was obtained on a SHIMADZU UV-2450 spectrometer and the absorption maxima are expressed in nanometers (nm). The photoluminescent spectra were recorded with a HITACHI F4500 fluorescence spectrophotometer with the excitation and emission slit widths at 5.0 and 10.0 nm, respectively. The column chromatography was carried out using silica gel (100-200 mesh). The TLC analysis was carried out on double coated silica Merk plates. The solvents received were of analytical grade and used without further purification.

Synthesis and Characterization:

Synthesis of (E)-10-(10H-phenothiazin-3'-yl)propenal (**PTZ-AL**)

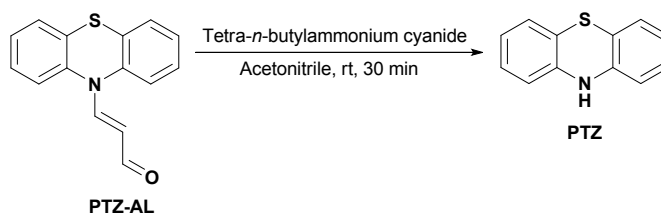


The **PTZ-AL** was synthesized by minor modifications of known procedure.¹ Specifically, 3-(dimethylamino)propenal (0.43 mL, 4.3 mmol) and phosphoryl chloride (0.67 mL, 7.2 mmol) were added dropwise to a stirred solution of phenothiazine (**PTZ**) (1.15 g, 5.8 mmol) in 1,2-dichloroethane (200 mL) at 0°C under nitrogen atmosphere. After stirring 1 h at 0°C , the ice bath was removed and reaction mixture was stirred at room temperature for 2 h. A saturated aqueous

Supporting information for publication

solution of sodium bicarbonate (400 mL) was added and the resulting biphasic mixture was stirred overnight at room temperature. After completion of the reaction, the aqueous phase was separated and organic phase containing the desired compound was dried over anhydrous magnesium sulfate. The solvent was evaporated under reduced pressure and the residue was purified by column chromatography using methanol: chloroform (2: 98, v/v) as eluent. Yield: 70%; ^1H NMR (600 MHz, 25°C , $(\text{CD}_3)_2\text{SO}$): δ = 9.37 (d, J = 8.0 Hz, 1H, -CHO), 8.01 (d, J = 13.6 Hz, 1H, H-3'), 7.61 (d, J = 9.0 Hz, 2H, Ar), 7.51 (d, J = 7.8 Hz, 2H, Ar), 7.44 (t, J = 7.5 Hz, 2H, Ar), 7.31 (t, J = 7.0 Hz, 2H, Ar) and 5.72 (dd, $J_{1,2} = J_{3,4} = 8.0$ Hz, $J_{1,3} = 13.6$ Hz, 1H, H-2'); ^{13}C NMR (150 MHz, 25°C , $(\text{CD}_3)_2\text{SO}$): δ = 191.2 (-CHO), 155.4 CH, 139.5 C, 129.4 C, 128.1 CH, 127.9 CH, 126.9 CH, 123.0 CH and 105.9 CH.

*Synthesis of PTZ by chemical reaction between PTZ-AL and *n*-tetrabutylammonium cyanide (TBACN)*



PTZ-AL (40 mg, 0.16 mmol) was dissolved in acetonitrile (8 mL) in a 10 mL round bottomed flask. To this stirred solution, TBACN (51.5 mg, 1.2 equiv.) was added and the reaction was continued at room temperature. The addition of TBACN generated a light green color in the solution which turned into light pink after 10 min. The progress of the reaction was monitored by TLC. After 30 min, the TLC indicated the complete consumption of PTZ-AL while a new spot appeared on TLC. The reaction was stopped at this point. The R_f of newly formed spot was found similar to that of commercial sample of PTZ. The solvent was evaporated under reduced pressure and column chromatography of the crude, using dichloromethane: hexane (1:1, v/v) as eluent, afforded PTZ as a white solid. Yield: 90%. The structure of as-obtained PTZ was confirmed by ^1H and ^{13}C NMR spectroscopy.

Supporting information for publication

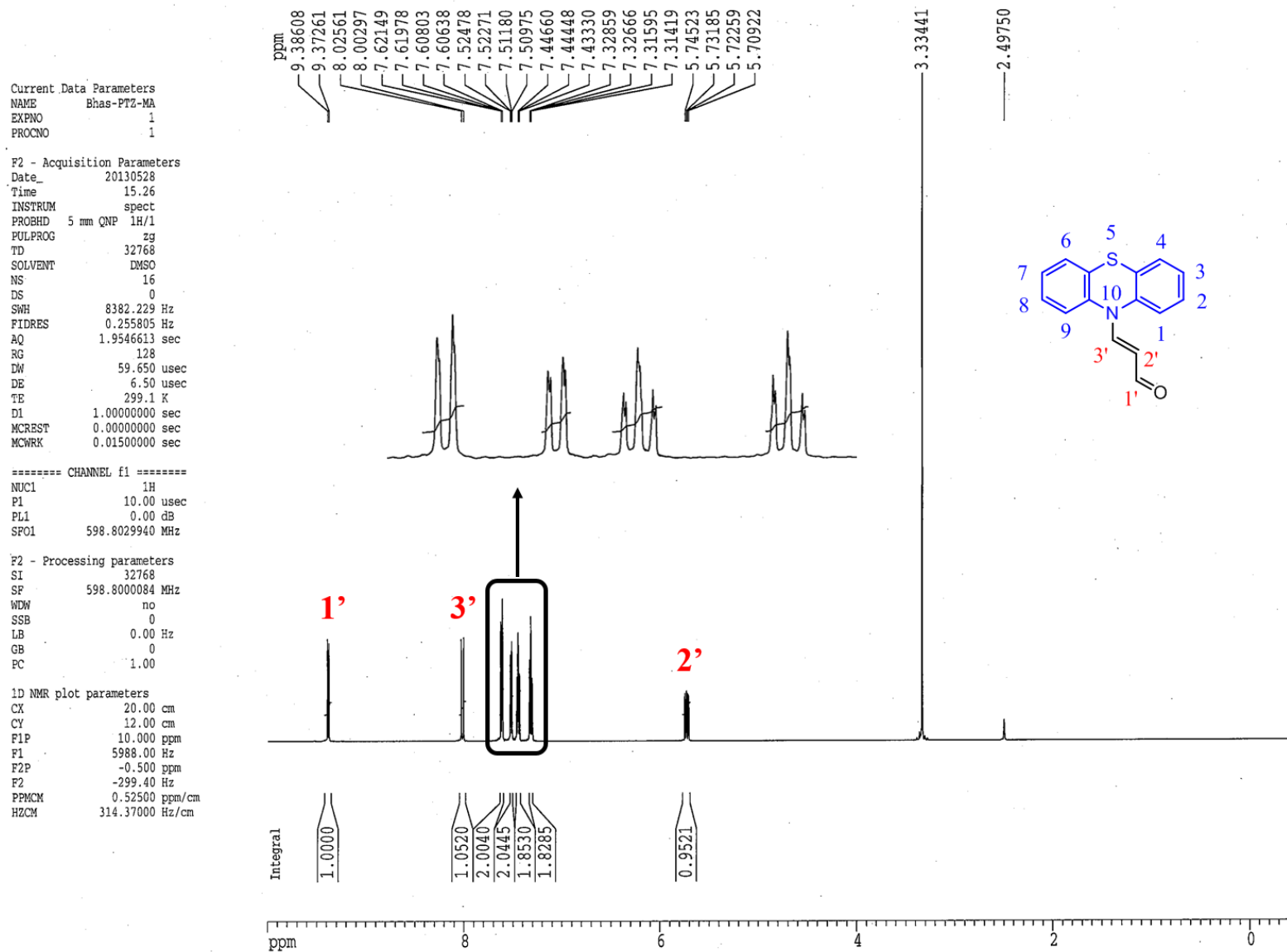


Figure S1 ¹H NMR spectrum of PTZ-AL in (CD₃)₂SO

Supporting information for publication

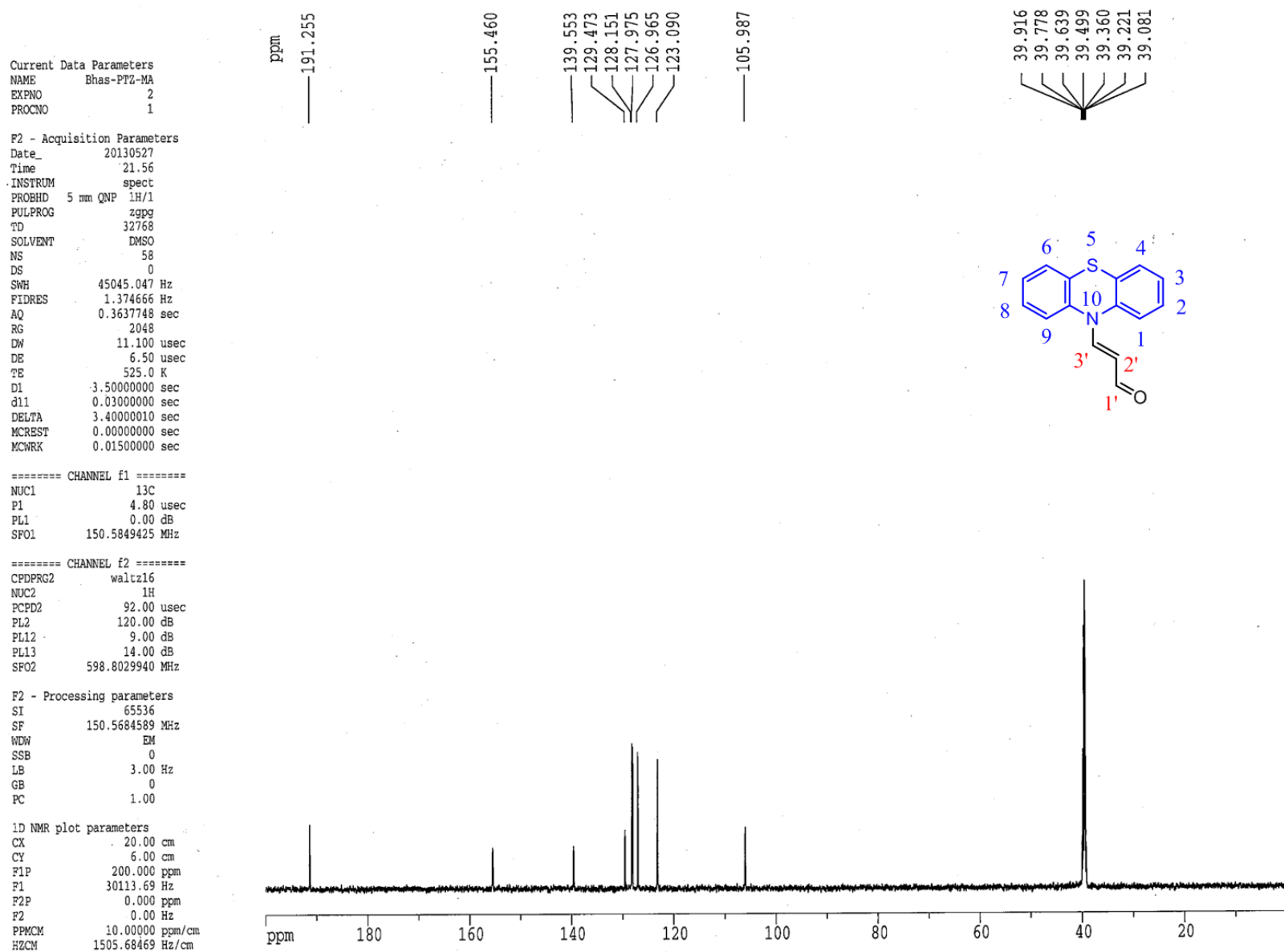


Figure S2 ¹³C NMR spectrum of PTZ-AL in (CD₃)₂SO

Supporting information for publication

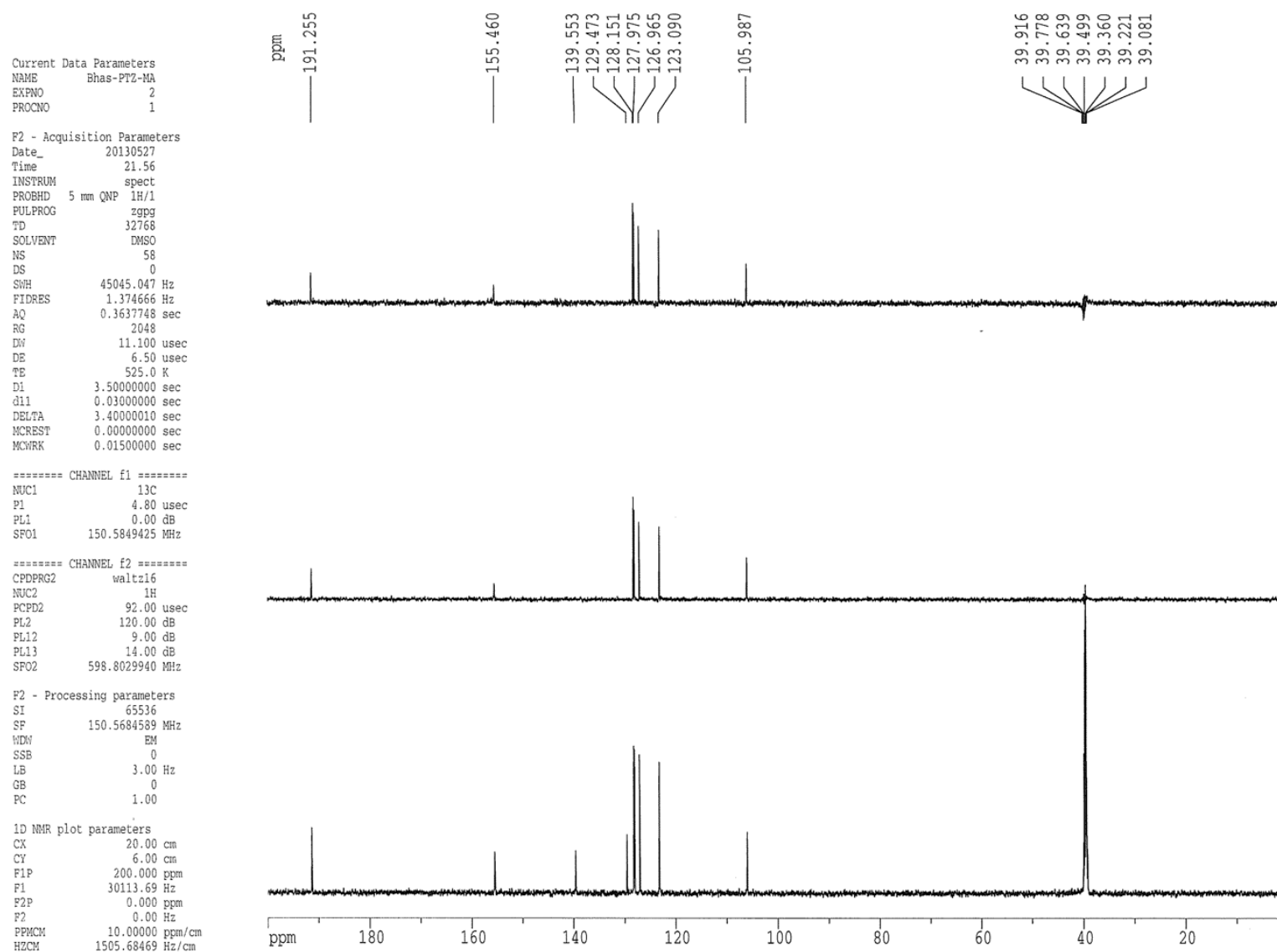


Figure S3 ¹³C/ DEPT NMR spectrum of PTZ-AL in (CD₃)₂SO

Supporting information for publication

```

Current Data Parameters
NAME      Bbas-PTZ-AL
EXPNO    11
PROCNO   1

F2 - Acquisition Parameters
Date_    2019032
Time     14.26
INSTRUM  spect
PROBHD   5 mm QNP 1H/1
PULPROG  zgpgcgpsi
TD        32768
SOLVENT  CDCl3
NS        8
DS        8
SFO1     600.135 MHz
FIDRES   2.944382 Hz
AQ        0.1704436 sec
RG         4096
DM        83.200 usec
DE        6.50 usec
TE        299.7 K
CHST2    145.0000000
d0        0.00000000 sec
d1        1.50000000 sec
d4        0.00172414 sec
d11       0.03000000 sec
d13       0.00000000 sec
d16       0.00000000 sec
d24       0.00089000 sec
DELTA    0.00137480 sec
DELTA1   0.00150800 sec
IMD      0.00001845 sec
MCHSET   0.00000000 sec
MCWPRK  0.25000051 sec
STICHT   128

***** CHANNEL f1 *****
MUC1     1H
P1        9.40 usec
PC        18.00 usec
P2B       1000.00 usec
P4B       3.00 dB
SFO1     500.803472 MHz

***** CHANNEL f2 *****
CPROG2   gmpg
MUC2     13C
P1        10.00 usec
PC        20.00 usec
PCPD2    70.00 usec
P4B       0.00 dB
P4C       19.00 dB
SFO2     150.5854954 MHz

***** GRADIENT CHANNEL *****
GPRAM1   SINE.100
GPRAM2   SINE.100
GPR1     0.00 %
GPR2     0.00 %
GPR3     0.00 %
GPR4     0.00 %
GPR5     80.00 %
GPR6     20.10 %
P16      1000.00 usec

F1 - Acquisition parameters
NUC1     13C
TD        200
SFO1     150.5857 MHz
FIDRES   135.501350 Hz
DM        179.966 ppm
FAMODE   Echo-Antiecho

F2 - Processing parameters
SI        4096
SF        500.8003150 MHz
WDW       Q5SINE
SSB       2
LB        0.00 Hz
GB        0
PC        1.00

F1 - Processing parameters
SI        512
MUC1     echo-antiecho
SF        150.5845554 MHz
WDW       Q5SINE
SSB       4
LB        0.00 Hz
GB        0

2D NMR plot parameters
CK1      14.50 cm
CK2      20.00 cm
F2F1D0   30.331 ppm
F2F1D1   6186.03 Hz
F2F1E1   1.588 ppm
F2F1E2   851.00 Hz
F1F1D0   301.329 ppm
F1F1D1   30133.76 Hz
F1F1E1   37.470 ppm
F1F1E2   4166.21 Hz
F2F1PCN  0.60292 ppm/cm
  
```

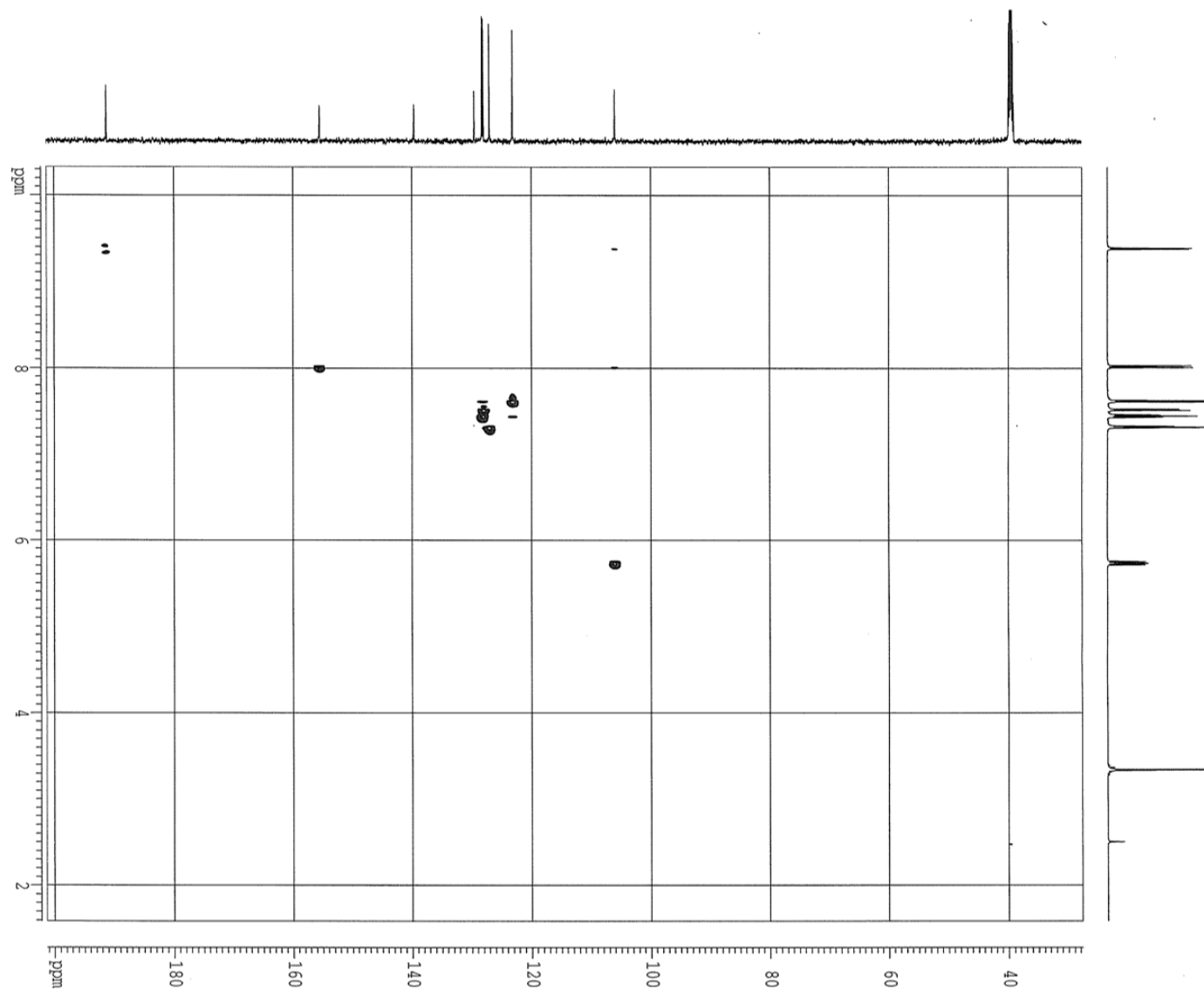


Figure S4 Proton-carbon 2D NMR spectrum of PTZ-AL in $(CD_3)_2SO$

Supporting information for publication

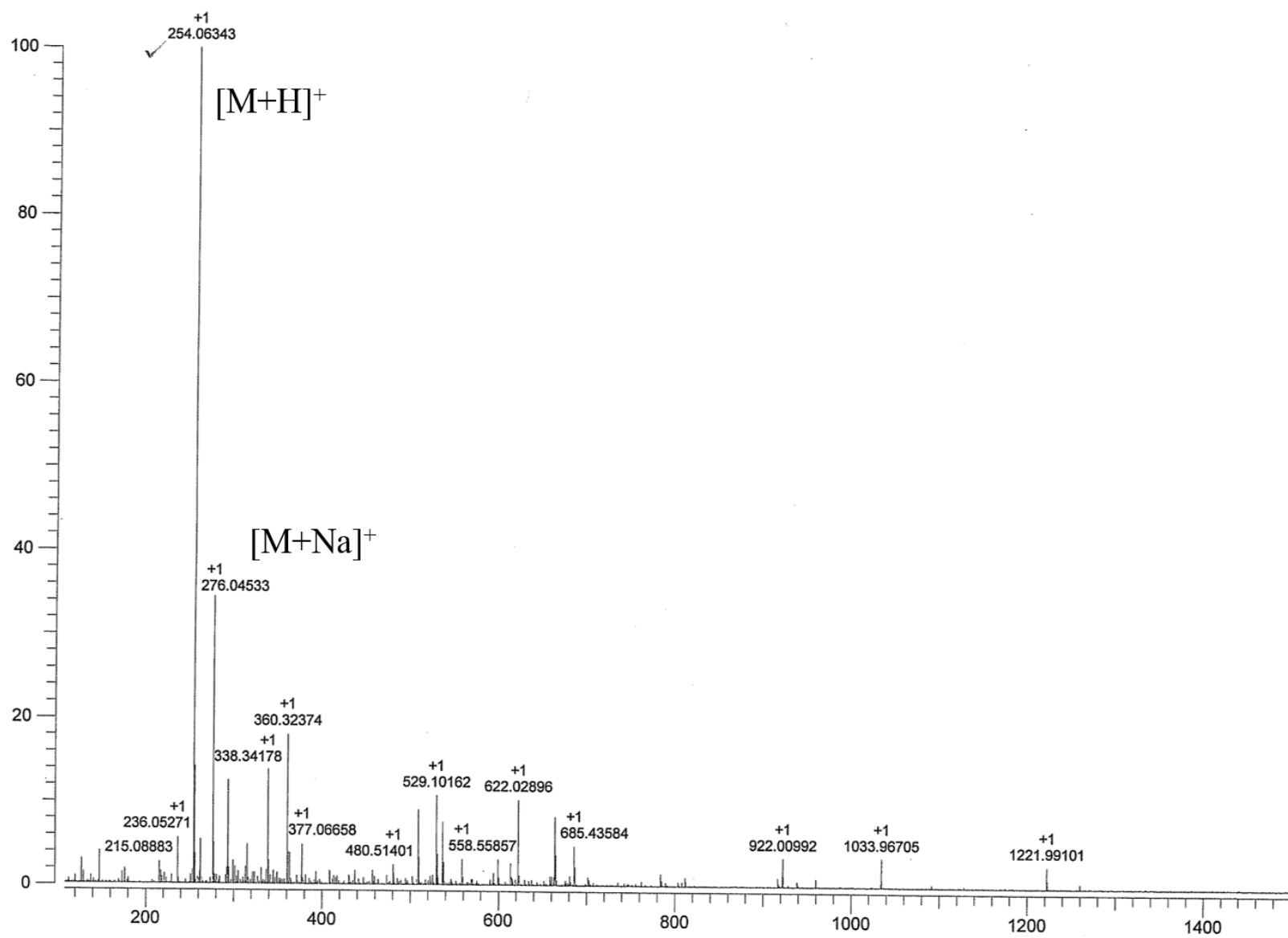


Figure S5 High resolution mass spectrum (ESI-MS) of PTZ-MA in acetonitrile

Supporting information for publication

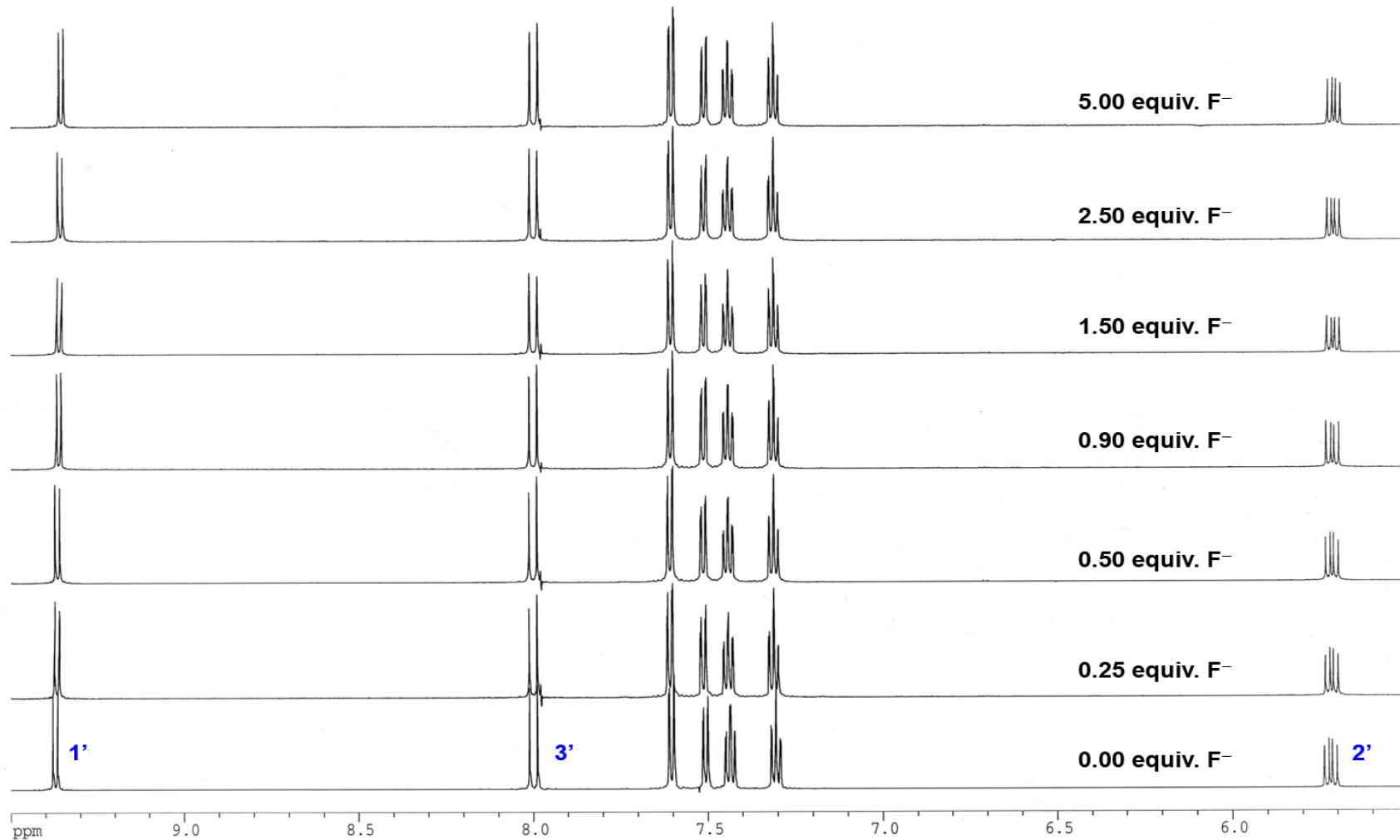


Figure S6 Partial ¹H NMR (600 MHz, (CD₃)₂SO) spectral changes seen upon the addition of TBAF to PTZ-AL (10.0 mM).

Supporting information for publication

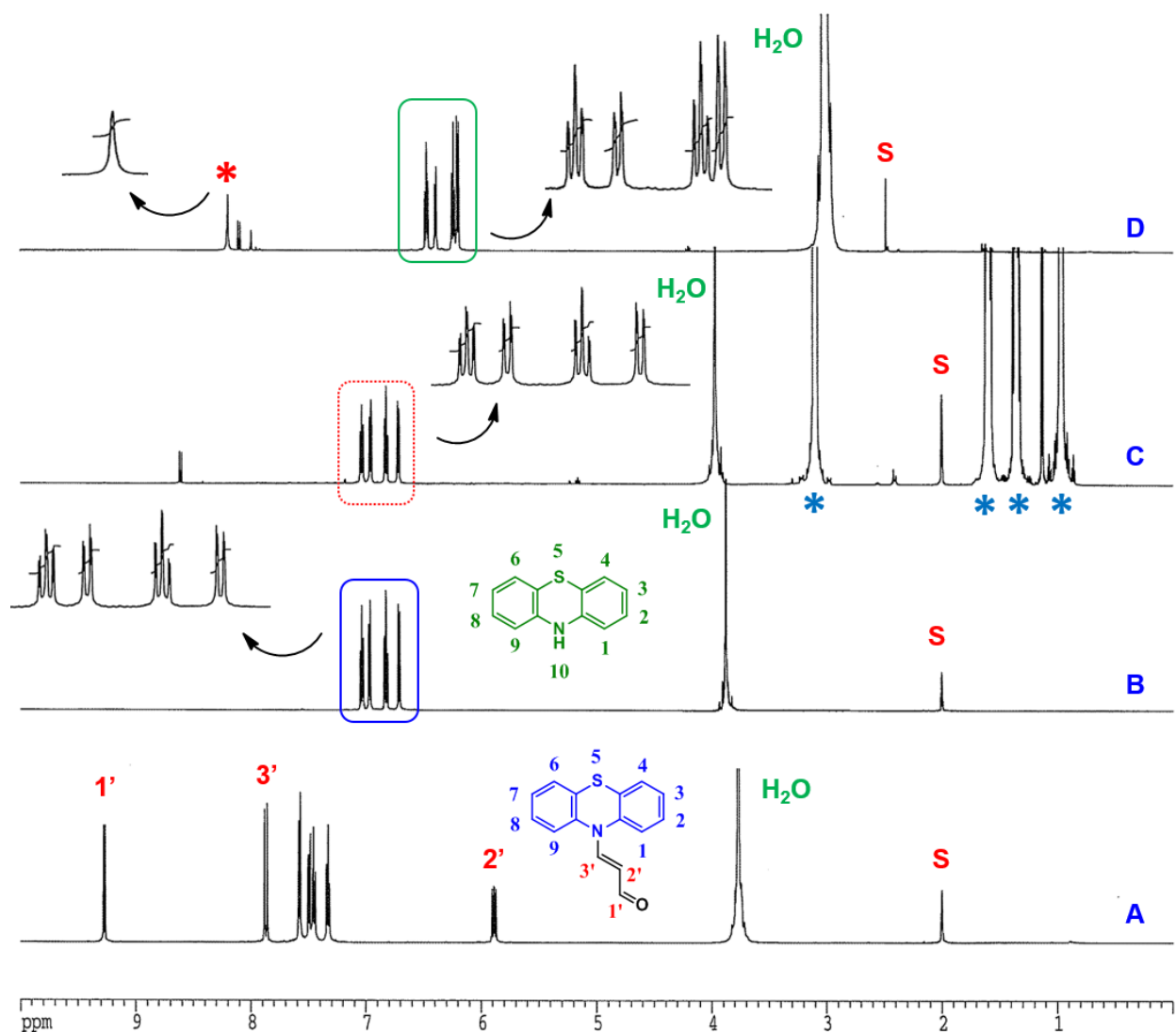


Figure S7 ^1H NMR (600 MHz) spectral changes under different conditions: (A) ^1H NMR spectrum of **PTZ-AL** in a mixture of $\text{CD}_3\text{CN}-\text{D}_2\text{O}$ (2:1, v/v); (B) ^1H NMR spectrum of commercially available **PTZ** in a mixture of $\text{CD}_3\text{CN}-\text{D}_2\text{O}$ (3:1, v/v); (C) ^1H NMR spectral changes seen upon the addition of TBACN ($\text{CD}_3\text{CN}-\text{D}_2\text{O}$ (1:1, v/v), 2 equiv.) to the solution of A (10.0 mM), and (D) ^1H NMR spectral changes seen upon the addition of NaCN ($(\text{CD}_3)_2\text{SO}-\text{D}_2\text{O}$ (1:1, v/v), 2 equiv.) to the solution of **PTZ-AL** ($(\text{CD}_3)_2\text{SO}-\text{D}_2\text{O}$ (2:1, v/v), 10.0 mM). S denotes solvent. The cyan blue stars represent the resonances due to tetrabutylammonium functions. The red star represents the resonance (broad) due to NH group of **PTZ**.

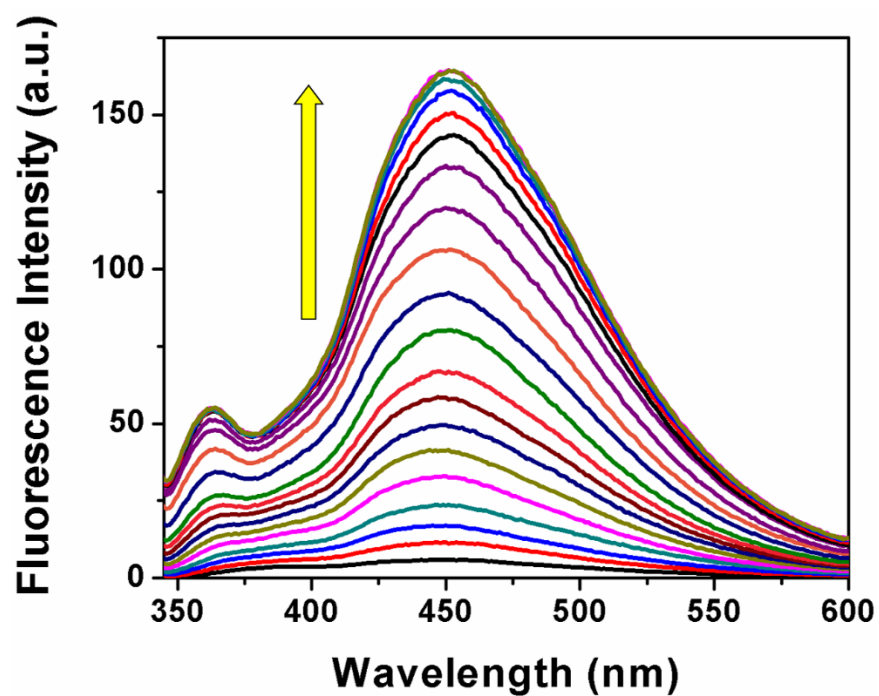


Figure S8 Fluorescence titration (emission) spectra of PTZ-AL (5×10^{-5} M) in acetonitrile-water solution (2:3, v/v) upon incremental addition of TBACN (5×10^{-4} M).

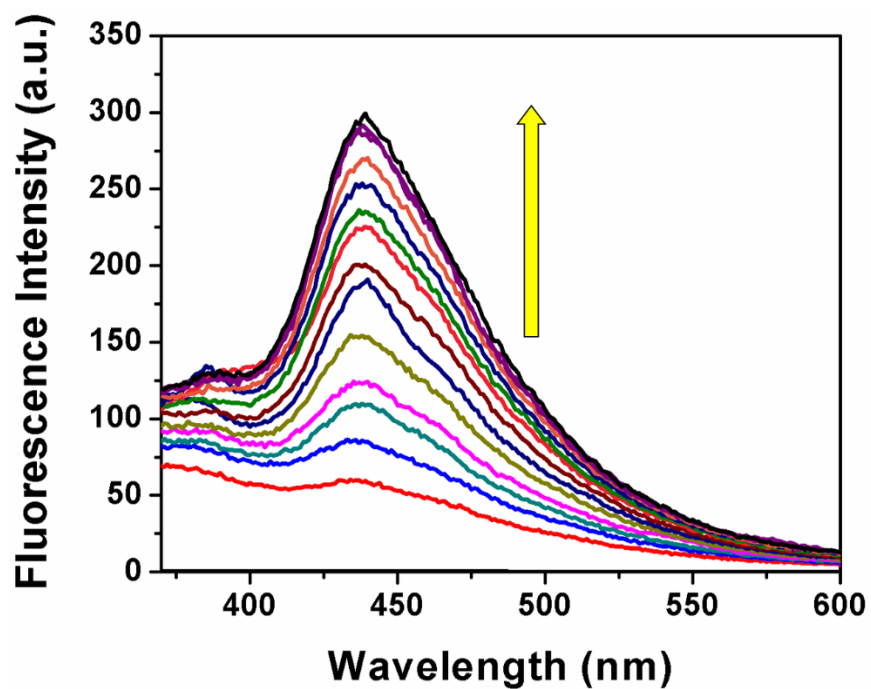


Figure S9 Fluorescence titration (emission) spectra of PTZ-AL (5×10^{-5} M) in acetonitrile-PBS buffer solution (7:3, v/v, pH 7.4) upon incremental addition of TBACN (5×10^{-4} M).

Supporting information for publication

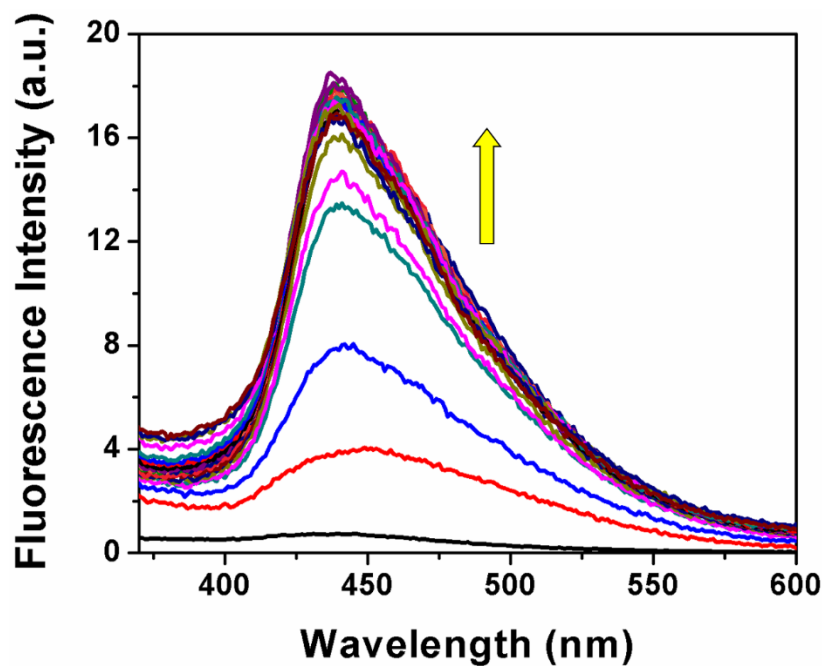


Figure S10 Fluorescence titration (emission) spectra of PTZ-AL (5×10^{-5} M) in acetonitrile-PBS buffer solution (7:3, v/v, pH 7.4) upon incremental addition of cysteine (Cys) (5×10^{-4} M).

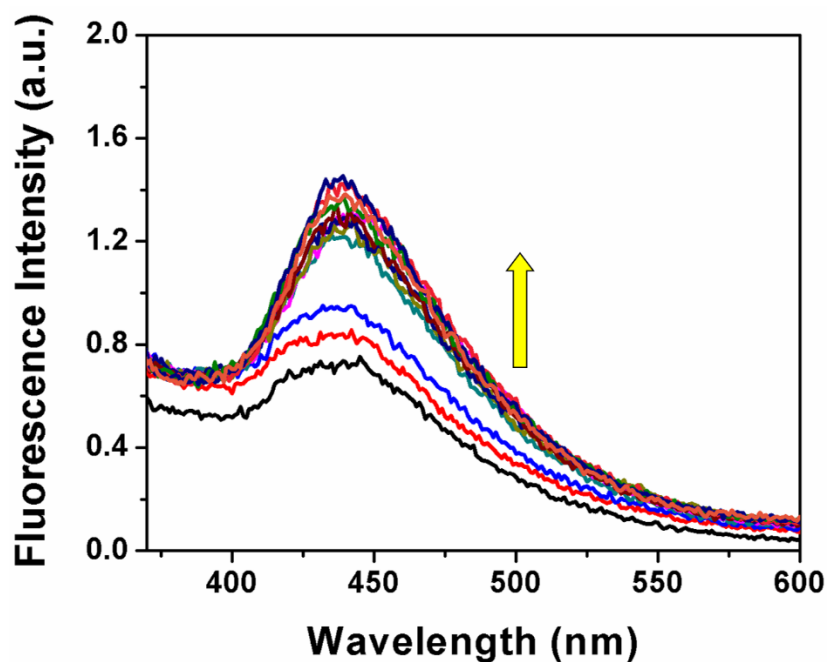


Figure S11 Fluorescence titration (emission) spectra of PTZ-AL (5×10^{-5} M) in acetonitrile-PBS buffer solution (7:3, v/v, pH 7.4) upon incremental addition of homocysteine (Hcy) (5×10^{-4} M).

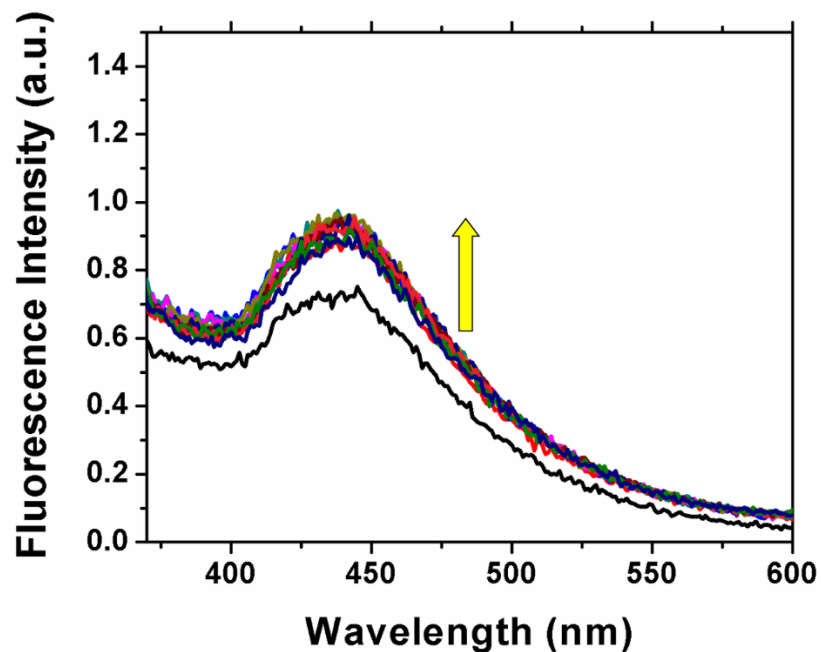


Figure S12 Fluorescence titration (emission) spectra of PTZ-AL (5×10^{-5} M) in acetonitrile-PBS buffer solution (7:3, v/v, pH 7.4) upon incremental addition of glutathione (GSH) (5×10^{-4} M).

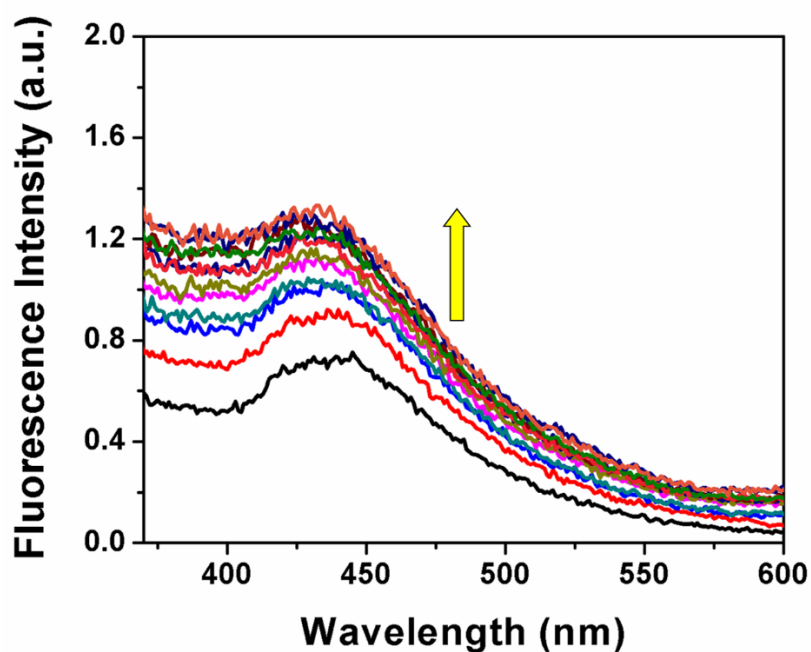


Figure S13 Fluorescence titration (emission) spectra of PTZ-AL (5×10^{-5} M) in acetonitrile-PBS buffer solution (7:3, v/v, pH 7.4) upon incremental addition of 2-mercaptoethanol (2-ME) (5×10^{-4} M).

Supporting information for publication

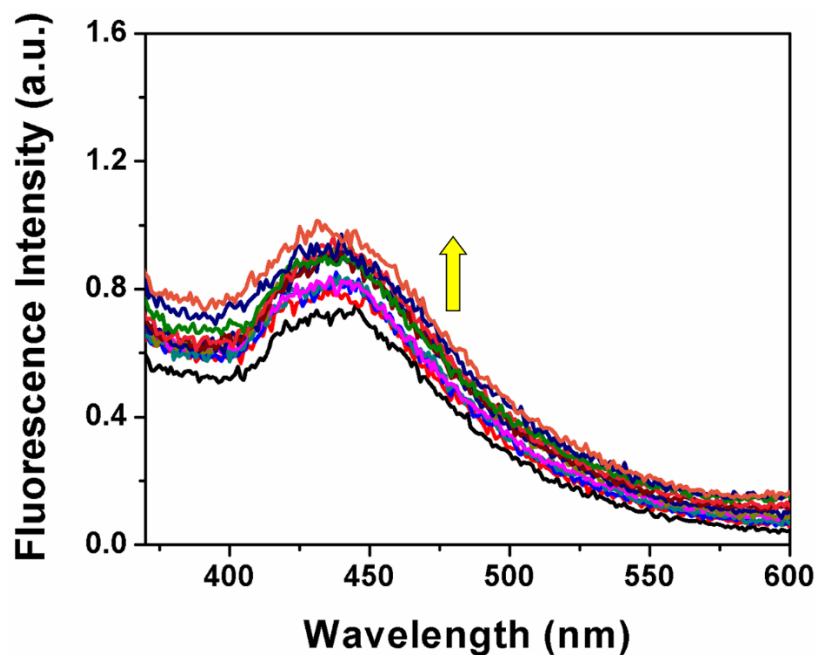


Figure S14 Fluorescence titration (emission) spectra of PTZ-AL (5×10^{-5} M) in acetonitrile-PBS buffer solution (7:3, v/v, pH 7.4) upon incremental addition of mercaptopropionic acid (MPA) (5×10^{-4} M).

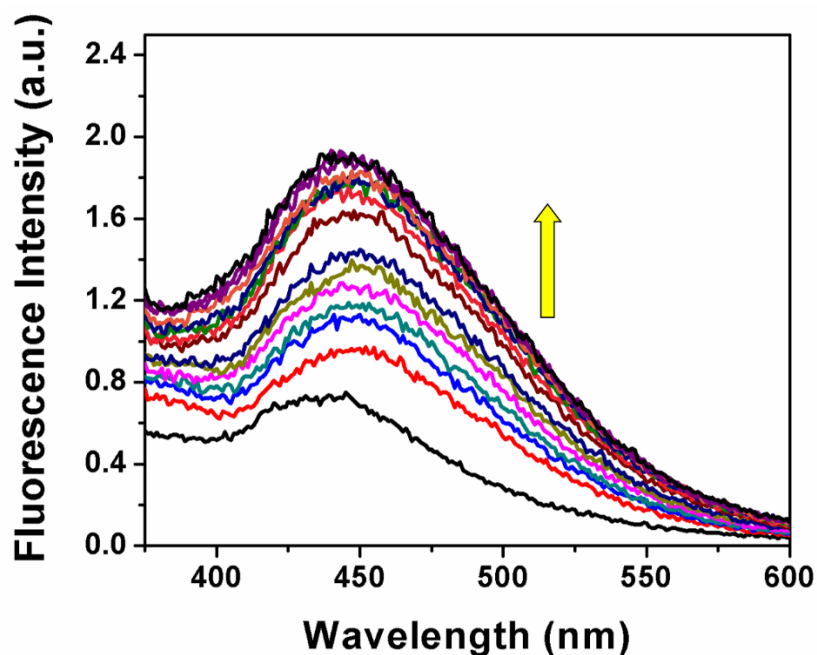


Figure S15 Fluorescence titration (emission) spectra of PTZ-AL (5×10^{-5} M) in acetonitrile-PBS buffer solution (7:3, v/v, pH 7.4) upon incremental addition of sodium sulfide (Na₂S) (5×10^{-4} M).

Supporting information for publication

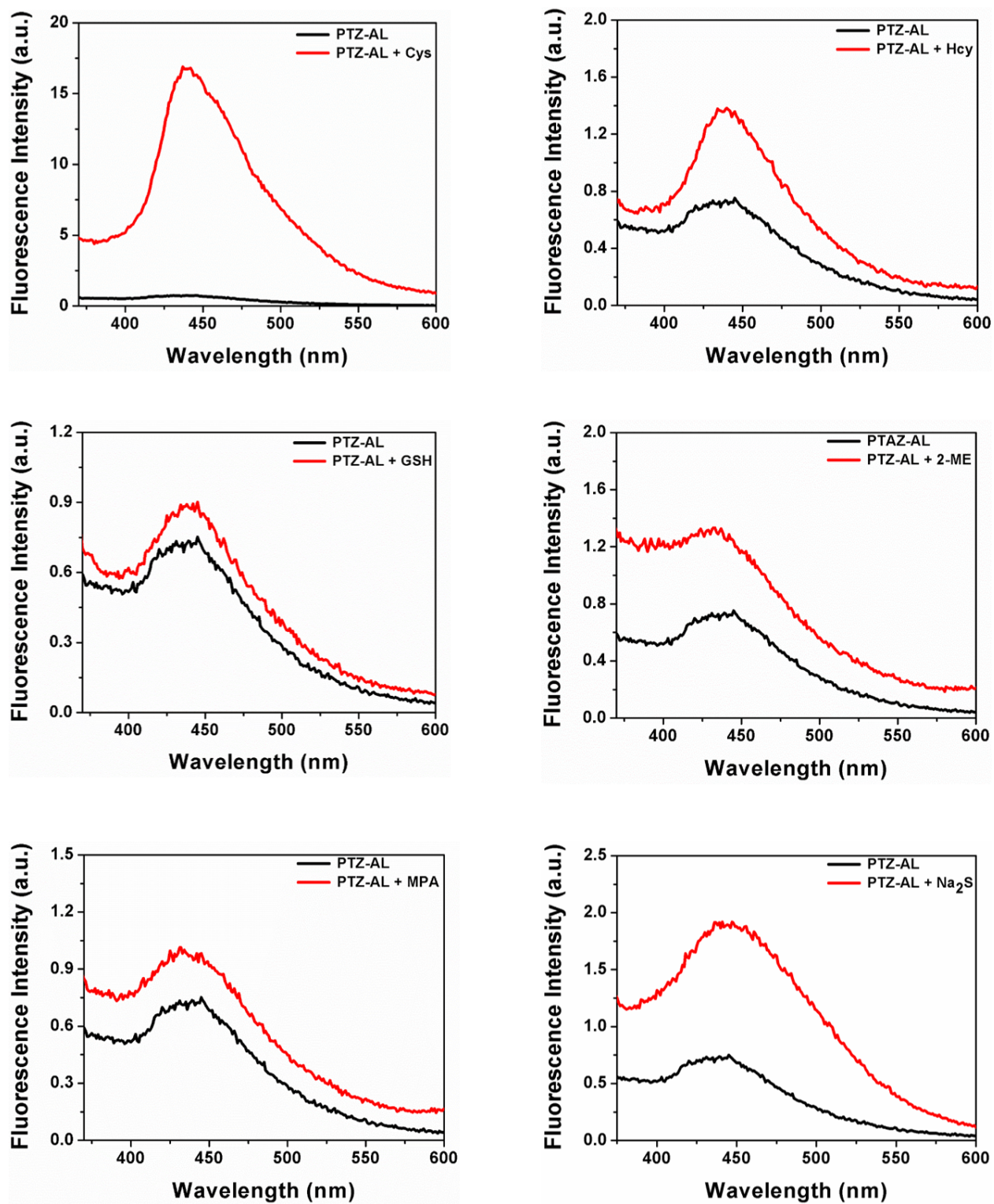


Figure S16 Fluorescence emission spectra of PTZ-AL (5×10^{-5} M) in acetonitrile-PBS buffer solution (7:3, v/v, pH 7.4) with different amino acids and sodium sulfide (5 equiv.).

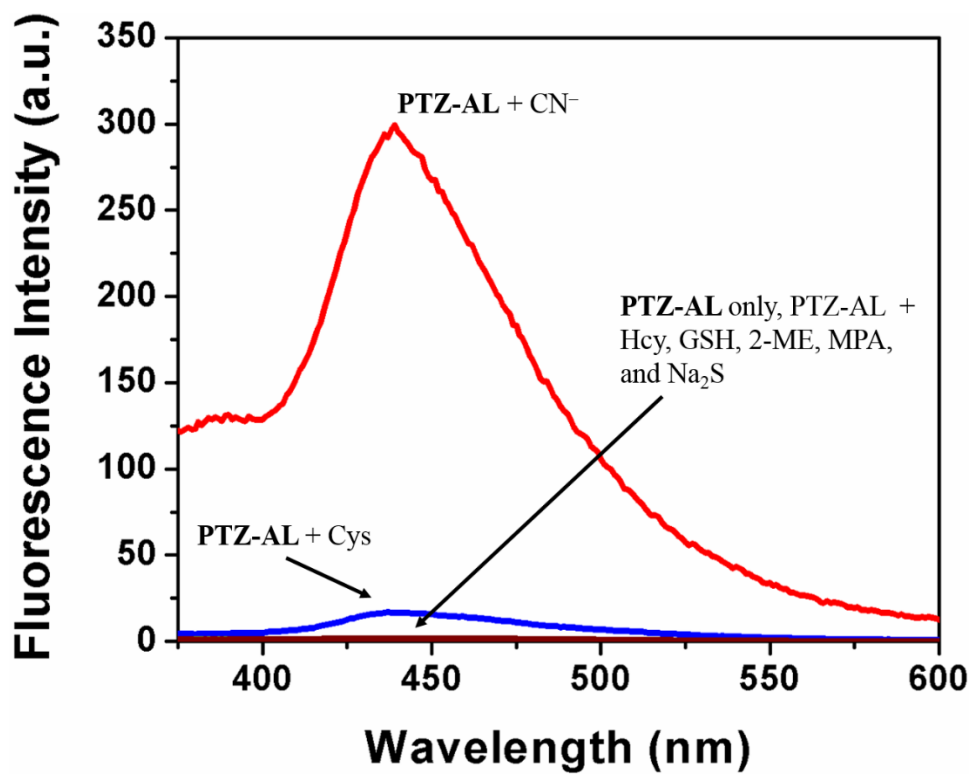


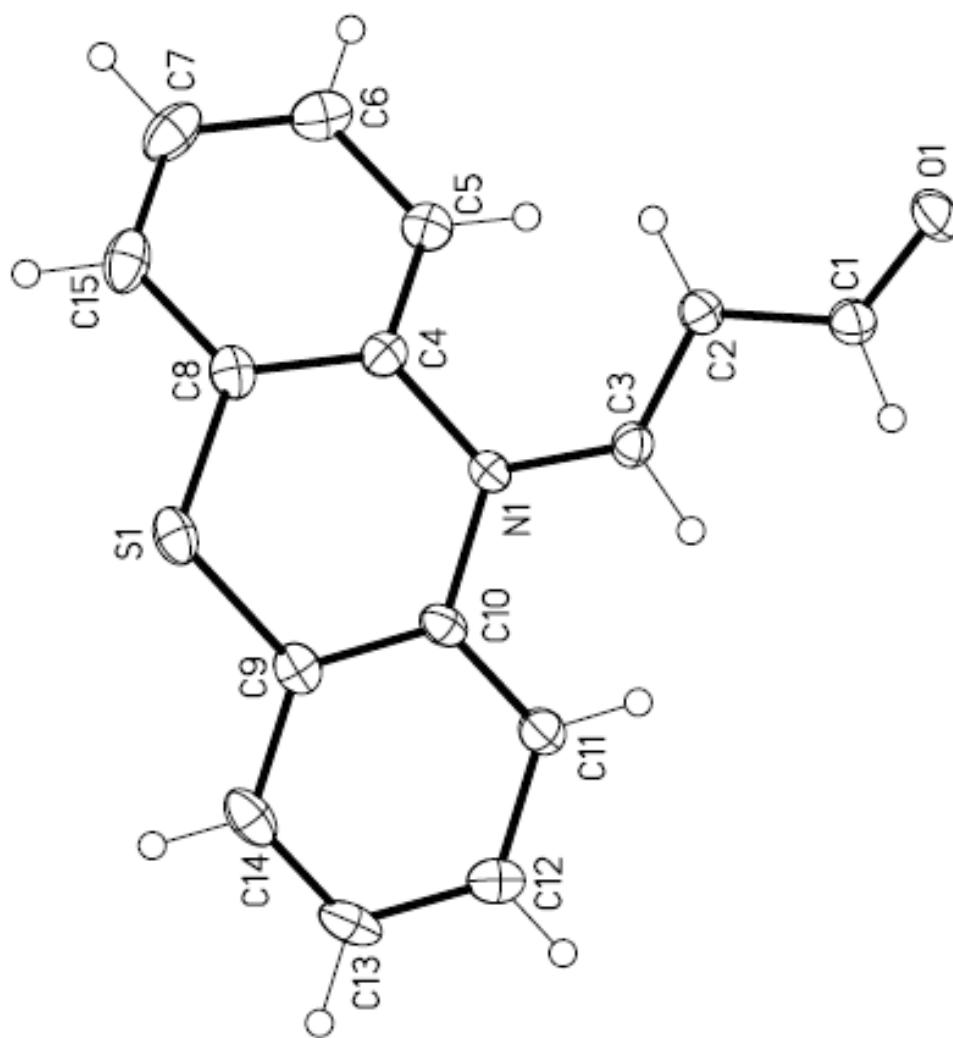
Figure S17 Fluorescence emission spectra of **PTZ-AL** (5×10^{-5} M) in acetonitrile-PBS buffer solution (7:3, v/v, pH 7.4) in the presence of different amino acids, sodium sulfide and TBACN (5 equiv. each).

Supporting information for publication

Crystal data of PTZ-AL

Table 1. Crystal data and structure refinement for 130639LT.

Identification code	130639lt	
Empirical formula	C ₁₅ H ₁₁ N O S	
Formula weight	253.31	
Temperature	100(2) K	
Wavelength	0.71073 Å	
Crystal system	Monoclinic	
Space group	P 1 21/c 1	
Unit cell dimensions	a = 16.1120(13) Å	α = 90°.
	b = 7.8274(7) Å	β = 101.081(2)°.
	c = 9.5930(8) Å	γ = 90°.
Volume	1187.27(17) Å ³	
Z	4	
Density (calculated)	1.417 Mg/m ³	
Absorption coefficient	0.257 mm ⁻¹	
F(000)	528	
Crystal size	0.18 x 0.15 x 0.05 mm ³	
Theta range for data collection	2.90 to 26.46°.	
Index ranges	-17 ≤ h ≤ 20, -9 ≤ k ≤ 5, -11 ≤ l ≤ 11	
Reflections collected	8829	
Independent reflections	2409 [R(int) = 0.0218]	
Completeness to theta = 26.46°	98.7 %	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	0.9486 and 0.8710	
Refinement method	Full-matrix least-squares on F ²	
Data / restraints / parameters	2409 / 0 / 163	
Goodness-of-fit on F ²	1.035	
Final R indices [I > 2σ(I)]	R1 = 0.0302, wR2 = 0.0759	
R indices (all data)	R1 = 0.0371, wR2 = 0.0798	
Largest diff. peak and hole	0.279 and -0.252 e.Å ⁻³	



Ortep view of **PTZ-AL**

Supporting information for publication

Table 2. Atomic coordinates ($\times 10^4$) and equivalent isotropic displacement parameters ($\text{\AA}^2 \times 10^3$) for 130639LT. $U(\text{eq})$ is defined as one third of the trace of the orthogonalized U^{ij} tensor.

	x	y	z	$U(\text{eq})$
S(1)	8675(1)	8387(1)	9617(1)	21(1)
O(1)	5317(1)	7979(1)	3296(1)	24(1)
N(1)	7192(1)	9738(1)	7564(1)	15(1)
C(1)	5550(1)	8310(2)	4560(1)	19(1)
C(2)	6377(1)	8862(2)	5239(1)	17(1)
C(3)	6485(1)	9220(2)	6640(1)	16(1)
C(4)	7987(1)	10071(2)	7156(1)	16(1)
C(5)	8038(1)	11002(2)	5939(1)	18(1)
C(6)	8820(1)	11293(2)	5575(2)	22(1)
C(7)	9553(1)	10704(2)	6440(2)	25(1)
C(8)	8730(1)	9516(2)	8043(1)	18(1)
C(9)	7802(1)	9463(2)	10082(1)	18(1)
C(10)	7140(1)	10017(2)	9024(1)	16(1)
C(11)	6435(1)	10795(2)	9390(1)	18(1)
C(12)	6385(1)	10963(2)	10813(1)	21(1)
C(13)	7051(1)	10423(2)	11864(1)	23(1)
C(14)	7762(1)	9695(2)	11506(1)	22(1)
C(15)	9512(1)	9844(2)	7682(2)	23(1)

Supporting information for publication

Table 3. Bond lengths [\AA] and angles [$^\circ$] for 130639LT.

S(1)-C(8)	1.7659(14)
S(1)-C(9)	1.7683(14)
O(1)-C(1)	1.2265(16)
N(1)-C(3)	1.3642(16)
N(1)-C(4)	1.4329(16)
N(1)-C(10)	1.4355(16)
C(1)-C(2)	1.4317(18)
C(1)-H(1)	0.9500
C(2)-C(3)	1.3509(18)
C(2)-H(2)	0.9500
C(3)-H(3)	0.9500
C(4)-C(5)	1.3921(18)
C(4)-C(8)	1.3981(18)
C(5)-C(6)	1.3892(19)
C(5)-H(5)	0.9500
C(6)-C(7)	1.385(2)
C(6)-H(6)	0.9500
C(7)-C(15)	1.381(2)
C(7)-H(7)	0.9500
C(8)-C(15)	1.394(2)
C(9)-C(14)	1.3915(19)
C(9)-C(10)	1.3930(18)
C(10)-C(11)	1.3930(19)
C(11)-C(12)	1.3895(18)
C(11)-H(11)	0.9500
C(12)-C(13)	1.390(2)
C(12)-H(12)	0.9500
C(13)-C(14)	1.381(2)
C(13)-H(13)	0.9500
C(14)-H(14)	0.9500
C(15)-H(15)	0.9500
C(8)-S(1)-C(9)	98.67(6)
C(3)-N(1)-C(4)	123.71(11)

Supporting information for publication

C(3)-N(1)-C(10)	118.75(11)
C(4)-N(1)-C(10)	117.51(10)
O(1)-C(1)-C(2)	126.50(13)
O(1)-C(1)-H(1)	116.8
C(2)-C(1)-H(1)	116.8
C(3)-C(2)-C(1)	116.49(12)
C(3)-C(2)-H(2)	121.8
C(1)-C(2)-H(2)	121.8
C(2)-C(3)-N(1)	129.80(12)
C(2)-C(3)-H(3)	115.1
N(1)-C(3)-H(3)	115.1
C(5)-C(4)-C(8)	119.44(12)
C(5)-C(4)-N(1)	121.68(12)
C(8)-C(4)-N(1)	118.81(12)
C(6)-C(5)-C(4)	119.96(13)
C(6)-C(5)-H(5)	120.0
C(4)-C(5)-H(5)	120.0
C(7)-C(6)-C(5)	120.34(14)
C(7)-C(6)-H(6)	119.8
C(5)-C(6)-H(6)	119.8
C(15)-C(7)-C(6)	120.13(13)
C(15)-C(7)-H(7)	119.9
C(6)-C(7)-H(7)	119.9
C(15)-C(8)-C(4)	120.05(13)
C(15)-C(8)-S(1)	120.04(11)
C(4)-C(8)-S(1)	119.91(11)
C(14)-C(9)-C(10)	120.05(13)
C(14)-C(9)-S(1)	119.98(10)
C(10)-C(9)-S(1)	119.92(10)
C(9)-C(10)-C(11)	120.02(12)
C(9)-C(10)-N(1)	118.90(12)
C(11)-C(10)-N(1)	121.06(11)
C(12)-C(11)-C(10)	119.53(13)
C(12)-C(11)-H(11)	120.2
C(10)-C(11)-H(11)	120.2
C(11)-C(12)-C(13)	120.13(13)

Supporting information for publication

C(11)-C(12)-H(12)	119.9
C(13)-C(12)-H(12)	119.9
C(14)-C(13)-C(12)	120.45(13)
C(14)-C(13)-H(13)	119.8
C(12)-C(13)-H(13)	119.8
C(13)-C(14)-C(9)	119.73(13)
C(13)-C(14)-H(14)	120.1
C(9)-C(14)-H(14)	120.1
C(7)-C(15)-C(8)	119.98(13)
C(7)-C(15)-H(15)	120.0
C(8)-C(15)-H(15)	120.0

Symmetry transformations used to generate equivalent atoms:

Supporting information for publication

Table 4. Anisotropic displacement parameters ($\text{\AA}^2 \times 10^3$) for 130639LT. The anisotropic displacement factor exponent takes the form: $-2\pi^2 [h^2 a^{*2} U^{11} + \dots + 2 h k a^* b^* U^{12}]$

	U^{11}	U^{22}	U^{33}	U^{23}	U^{13}	U^{12}
S(1)	21(1)	18(1)	20(1)	1(1)	-3(1)	4(1)
O(1)	23(1)	32(1)	15(1)	-5(1)	-1(1)	2(1)
N(1)	15(1)	18(1)	12(1)	0(1)	2(1)	0(1)
C(1)	19(1)	21(1)	17(1)	-2(1)	3(1)	3(1)
C(2)	17(1)	18(1)	15(1)	2(1)	3(1)	1(1)
C(3)	14(1)	16(1)	16(1)	2(1)	2(1)	1(1)
C(4)	16(1)	14(1)	18(1)	-3(1)	4(1)	-1(1)
C(5)	21(1)	15(1)	19(1)	-1(1)	4(1)	0(1)
C(6)	27(1)	17(1)	26(1)	-3(1)	11(1)	-4(1)
C(7)	20(1)	21(1)	38(1)	-7(1)	12(1)	-5(1)
C(8)	19(1)	14(1)	20(1)	-2(1)	0(1)	0(1)
C(9)	20(1)	14(1)	18(1)	1(1)	1(1)	-2(1)
C(10)	20(1)	15(1)	12(1)	1(1)	2(1)	-4(1)
C(11)	19(1)	19(1)	16(1)	0(1)	2(1)	-2(1)
C(12)	26(1)	21(1)	19(1)	-1(1)	8(1)	-2(1)
C(13)	36(1)	22(1)	12(1)	0(1)	5(1)	-6(1)
C(14)	29(1)	17(1)	16(1)	3(1)	-3(1)	-4(1)
C(15)	16(1)	18(1)	33(1)	-6(1)	1(1)	-1(1)

Supporting information for publication

Table 5. Hydrogen coordinates ($\times 10^4$) and isotropic displacement parameters ($\text{\AA}^2 \times 10^{-3}$) for 130639LT.

	x	y	z	U(eq)
H(1)	5142	8188	5148	23
H(2)	6825	8970	4731	20
H(3)	5994	9092	7043	19
H(5)	7539	11439	5357	22
H(6)	8853	11899	4729	27
H(7)	10085	10892	6178	30
H(11)	5990	11209	8671	22
H(12)	5894	11448	11068	26
H(13)	7017	10555	12836	28
H(14)	8222	9355	12228	26
H(15)	10017	9476	8290	28

References

1. A. S.F. Farinha, A. C. Tomé and J. A. S. Cavaleiro, *Tetrahedron Lett.*, 2010, **51**, 2184.