Sensing Organic Amines and Quantitative Monitoring Intracellular pH Change Using Fluorescent Self-Assembly System

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Experimental Section

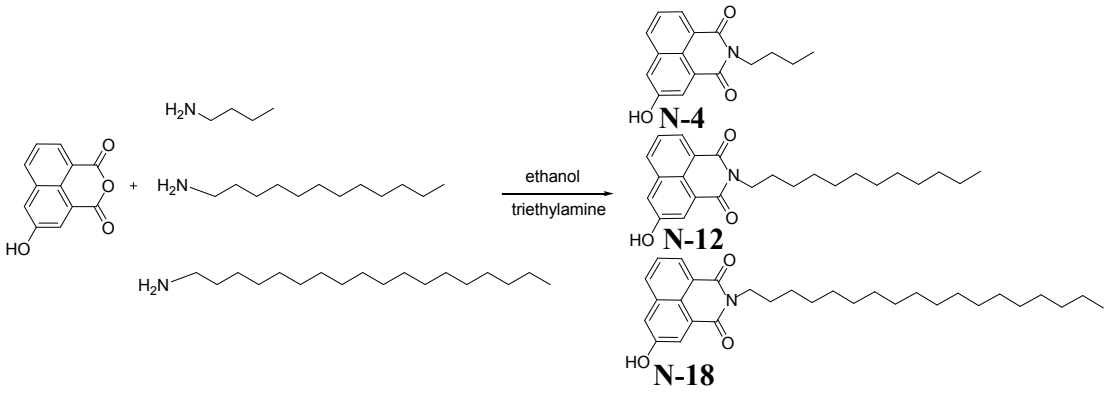
Reagents and materials: 3-Hydroxy-1,8-naphthalic anhydride was purchased from shanghai Titan technology Co., Ltd. Butylamine, dodecanamine and octadecylamine were provided from reagent company. All other reagents were analytically pure.

Gelation test: The detailed gelation process was the same as that of the previous references[17, 30].

Cells culture and imaging: HepG2 cells were cultured in Dulbecco's modified Eagle medium (DMEM) containing 10% fetal bovine serum and 1% antibiotic–antimycotic at 37 ^{0}C in a 5% CO_2/95% air incubator. Fluorescence imaging, cells (4×10^3 per well) were passed on confocal dishes and incubated for 24 h. Before the staining experiments, the cells were washed twice with PBS.

Cell viability: Cytotoxicity was assessed by performing CCK8 assay on the HepG2 cells. Cells were seeded into a 96-well plate at 2×10^3/well and were cultured at 37^{O}C and 5% CO_2 for 24 h. Different concentrations of **N-4** (0, 1, 2.5, 5, 7.5 and 10 μ M) were then added to the wells. After incubation for 5 or 10 h, CCK8 (0.5 mg/mL) was added to each well and the plate was incubated for 1 h. The optical densities at 490 nm were measured.

Instrumentation conditions: the detail instrumentation conditions were the same as that of the previous references[17, 30].



Scheme 1 Synthetic route of compounds N-4, N-12 and N-18.

Compounds N-4, N-12 and **N-18** were synthesized according to literature 1.

Synthesis of N-4: 3-hydroxyl-1,8-Naphthalimide (1.0 g, 4.67 mmol), n-Butylamine (0.41 g, 5.60 mmol) and triethylamine (5 mL) were mixed in ethanol (20 mL). The reaction mixture was heated for refluxing and stirred for 12 h under a nitrogen atmosphere. After the reaction was over, large amount of solvent was removed under reduced pressure, and the residue was filtrated and washed with cold ethanol for three times. The compound **N-4** as a yellow powder was obtained with the yield of 65%; ^{1}HNMR (400 MHz, $CDCl_3$): δ 8.44 (d, J = 7.6 Hz, 1H), 8.31 (d, J = 2.4 Hz, 1H), 8.06 (t, J = 7.6 Hz, 1H), 7.68 (t, J = 7.6 Hz, 1H), 7.58 (d, J = 2.4 Hz, 1H), 6.49 (s, 1H), 4.18 (t, J = 7.6 Hz, 2H), 1.72 (m, 2H), 1.47 (m, 2H), 0.98 (t, J = 7.2 Hz, 3H); ^{13}CNMR (100 MHz, $CDCl_3$): δ 164.4, 155.1, 133.4, 132.5, 128.9, 127.5, 116.3, 40.4, 30.2, 20.4, 13.8. HRMS calculated for $C_{16}H_{16}NO_3$ $[M+H]^+$ 270.1130, found: 270.1133.

Synthesis of N-12: The synthesis procedure was the same as **N-4**. The brown product **N-12** was obtained with the yield of 72 %; ^{1}HNMR (400 MHz, $CDCl_3$): δ 8.42 (d, J = 7.6 Hz, 1H), 8.38 (d, J = 2.4 Hz, 1H), 8.04 (d, J = 7.6 Hz, 1H), 7.66 (t, J = 7.6 Hz, 1H), 7.58 (d, J = 2.4 Hz, 1H), 4.17 (t, J = 7.6 Hz, 2H), 1.73 (m, 2H), 1.42-1.24 (m, 18H), 0.87 (t, J = 7.2 Hz, 3H); ^{13}CNMR (100 MHz, $CDCl_3$): δ 164.5, 164.3, 155.2, 133.4, 132.6, 128.8, 127.4, 123.8, 123.4, 122.4, 116.5, 40.8, 31.9, 29.6, 29.4, 29.3, 28.1, 27.2, 22.7, 14.1; HRMS calculated for $C_{24}H_{32}NO_3$ $[M+H]^+$ 382.2382, Found: 382.2364.

Synthesis of N-18: The synthesis procedure was the same as **N-4**. The brown product **N-18** was obtained with the yield of 83 %; ^{1}HNMR (400 MHz, $CDCl_3$): δ 8.43 (d, J = 7.6 Hz, 1H), 8.34 (d, J = 2.4 Hz, 1H), 8.05 (d, J = 7.6 Hz, 1H), 7.67 (d, J = 7.6 Hz, 1H), 7.58 (d, J = 2.4 Hz, 1H), 4.17 (t, J = 7.6 Hz, 2H), 1.73 (m, 2H), 1.42-1.24 (m, 30H), 0.87 (t, J = 7.2 Hz, 3H); ^{13}CNMR (100 MHz,

$CDCl_3$): δ 164.3, 155.0, 133.4, 132.5, 128.8, 127.4, 124.0, 123.5, 122.5, 122.4, 116.4, 40.7, 31.9, 29.7, 29.6, 29.4, 28.1, 27.2, 22.7, 14.1; HRMS calculated for $C_{30}H_{44}NO_3$ $[M+H]^+$ 466.3321, Found: 466.3330.

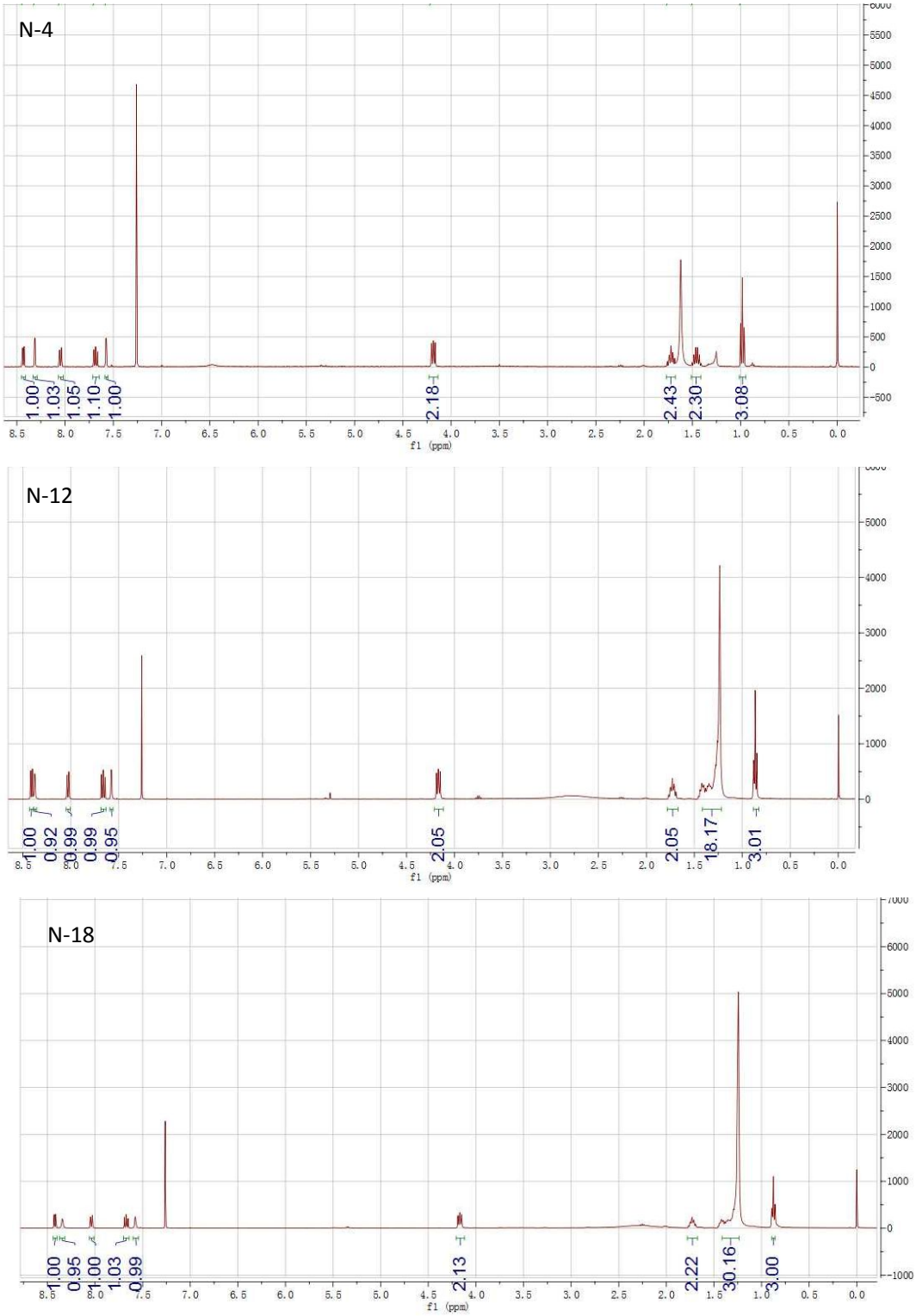
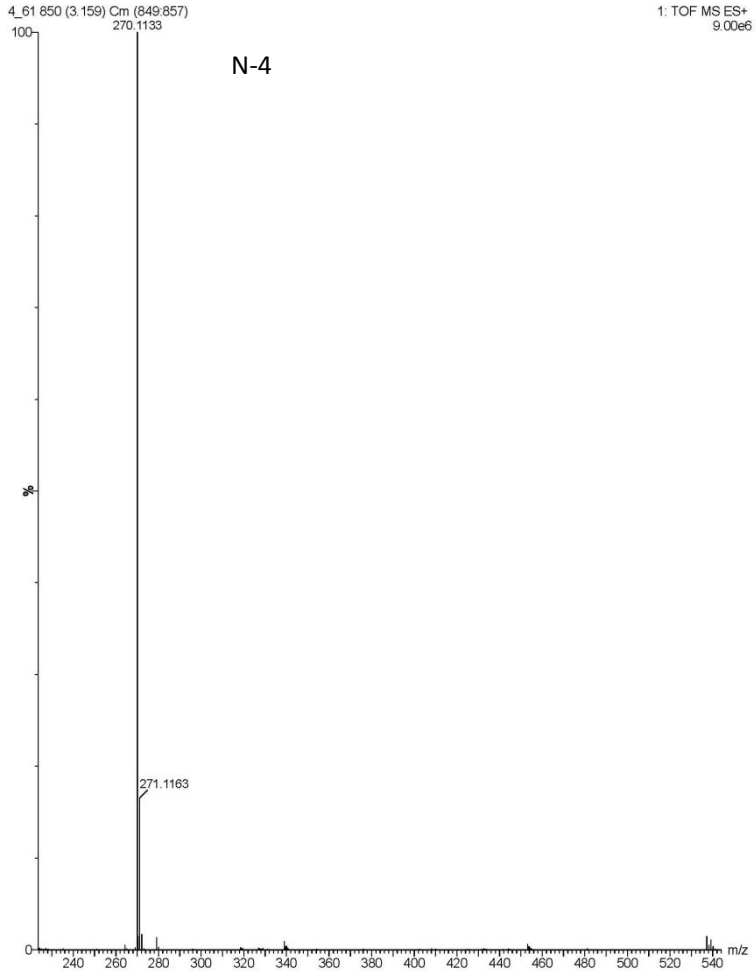


Fig. S1 ^{1}HNMR spectra of **N-4**, **N-12** and **N-18** in $CDCl_3$.


4_61 850 (3.159) Cm (849:857)
270.1133
1: TOF MS ES+
9.00e6
100
%
N-4
271.1163
0
240 260 280 300 320 340 360 380 400 420 440 460 480 500 520 540
m/z

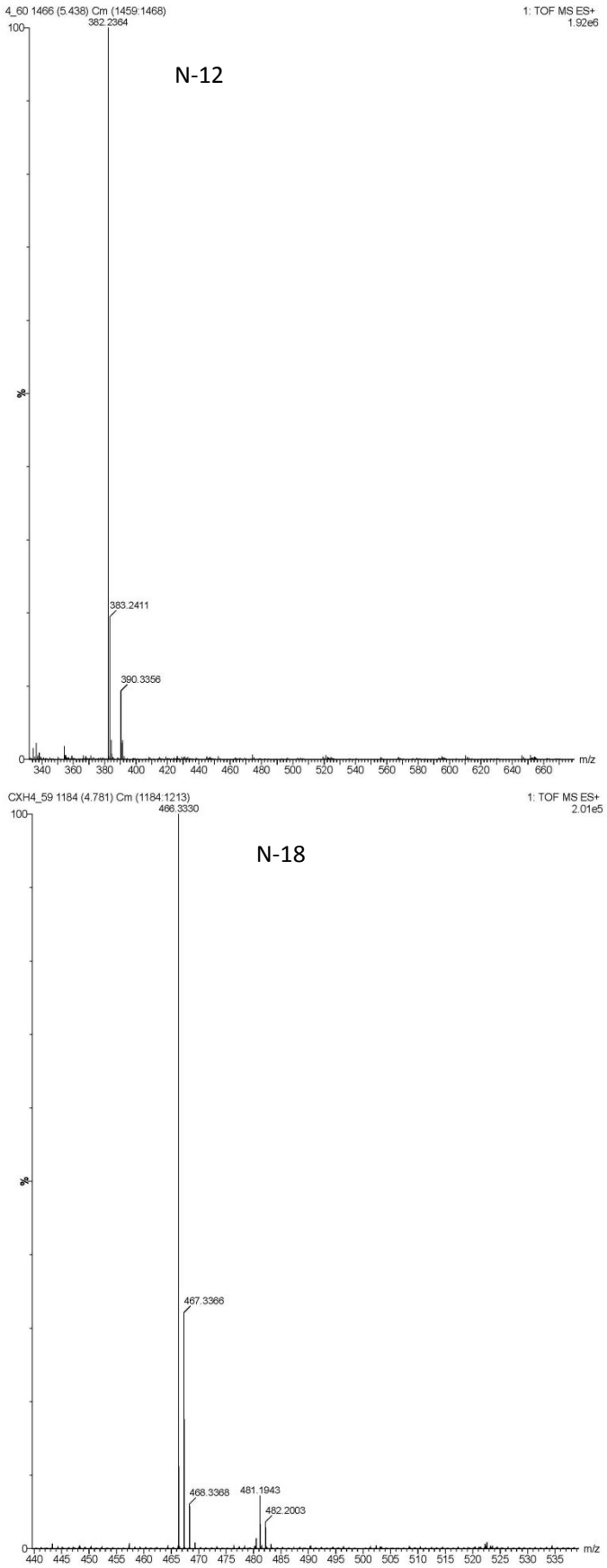


Fig. S2 HRMS spectra of **N-4**, **N-12** and **N-18**.

Table S1 EDX experimental results of xerogels N-18 from different solvents.

sample	element	content	sample	element	content
xerogel in n-hexane	C	78.22 %	xerogel in acetonitrile	C	79.00 %
	N	5.28 %		N	5.75 %
	O	16.49 %		O	15.26 %
xerogel in toluene	C	81.52 %	xerogel in petroleum	C	79.96 %
	N	5.47 %		N	6.82 %
	O	13.01 %		O	13.23 %
xerogel in DMF/H_2O	C	77.46 %	xerogel in DMSO/H_2O	C	78.16 %
	N	6.39 %		N	6.28 %
	O	16.14 %		O	15.56 %

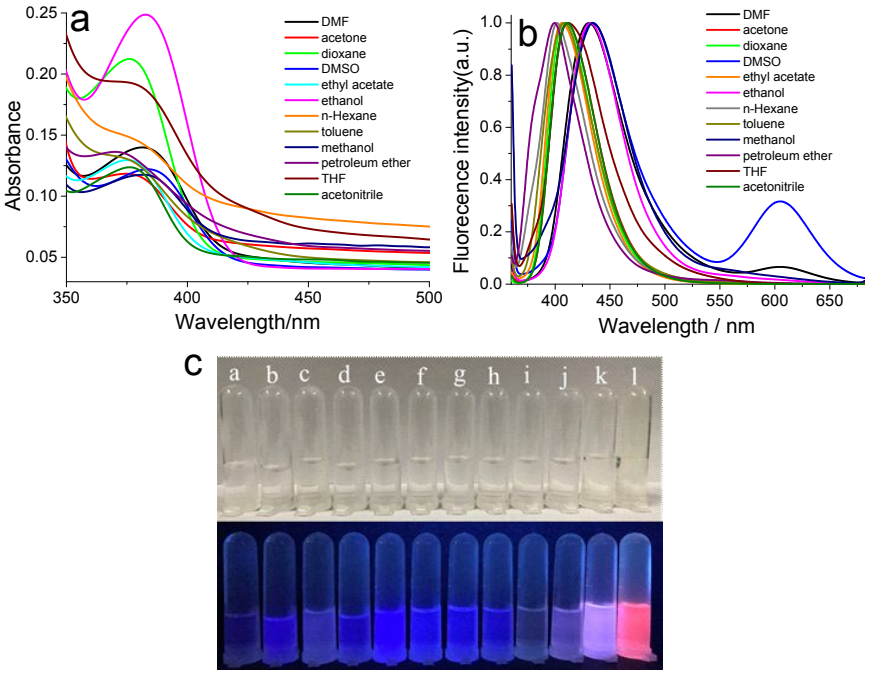


Fig. S3 a) UV-vis absorption spectra of **N-18** in different solvents; b) Fluorescence emission spectra of **N-18** in different solvents; c) The images of **N-18** solutions in different solvents; a) for petroleum ether; b) for n-hexane; c) for acetonitrile; d) ethyl acetate; e) for 1,4-dioxane; f) for THF; g) for toluene; h) for acetone; i) for methanol; j) for ethanol; k) for DMF and l) for DMSO. The concentration of **N-18** solution was 10^{-5} M.

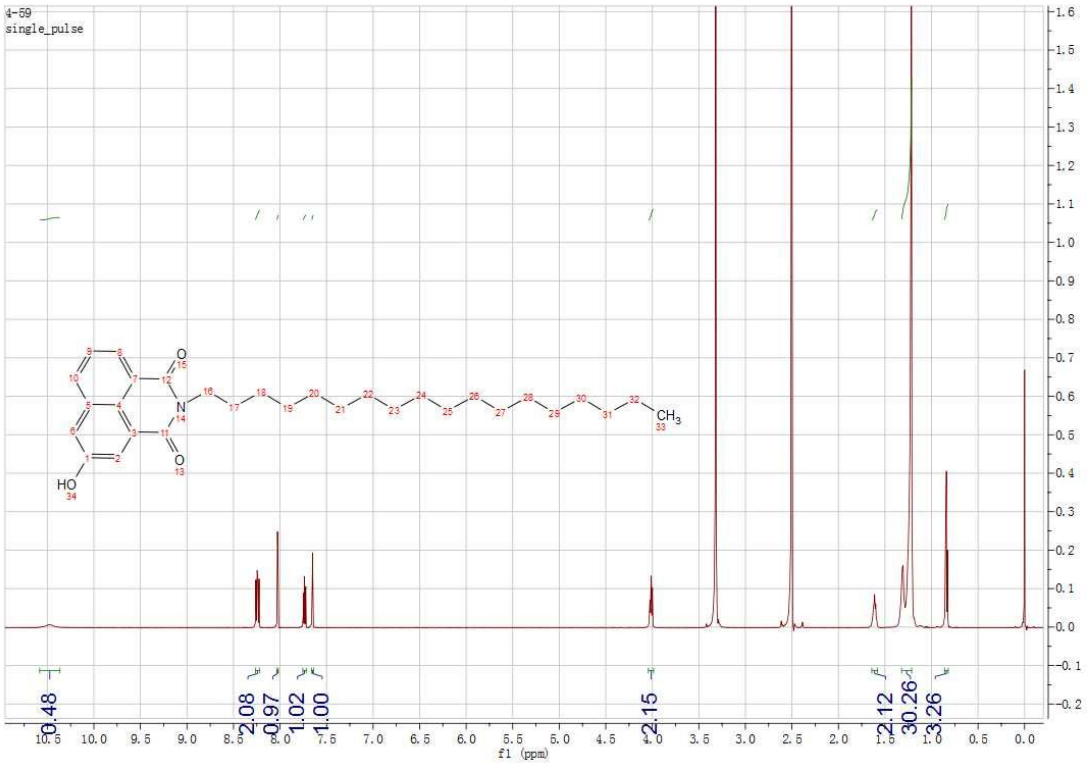


Fig. S4 ^{1}HNMR spectrum of N-18 in DMSO-d_6

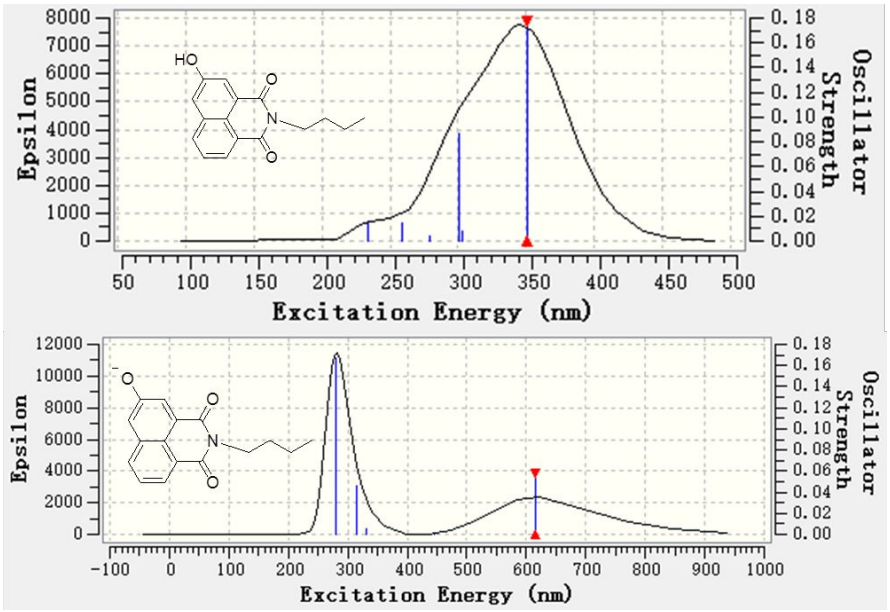


Fig. S5 The emission spectra of **N-4** and deprotonated **N-4** obtained from the DFT calculation.

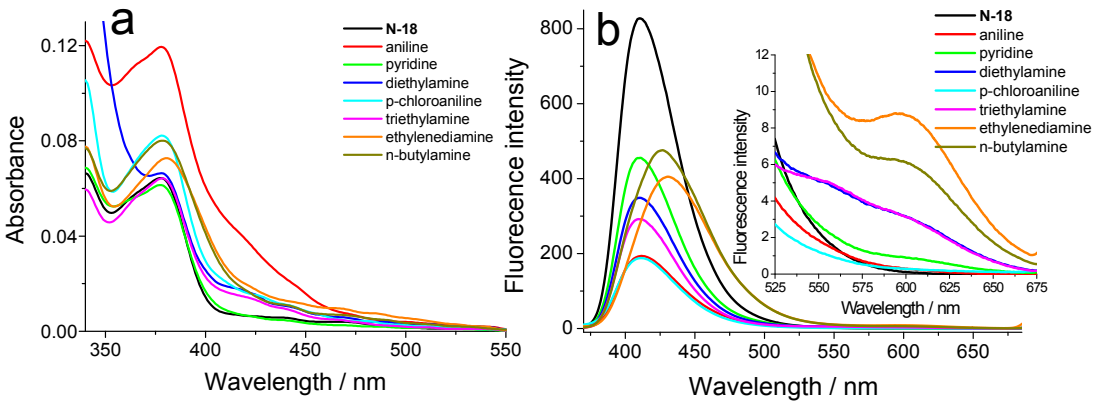


Fig. S6 UV-vis absorption (a) and fluorescence emission (b) change of **N-18** acetonitrile solution (10 μmol/L) under the addition of different amines. The addition amount of amines was 88 μL of amine acetonitrile solution with the concentration of 1M.

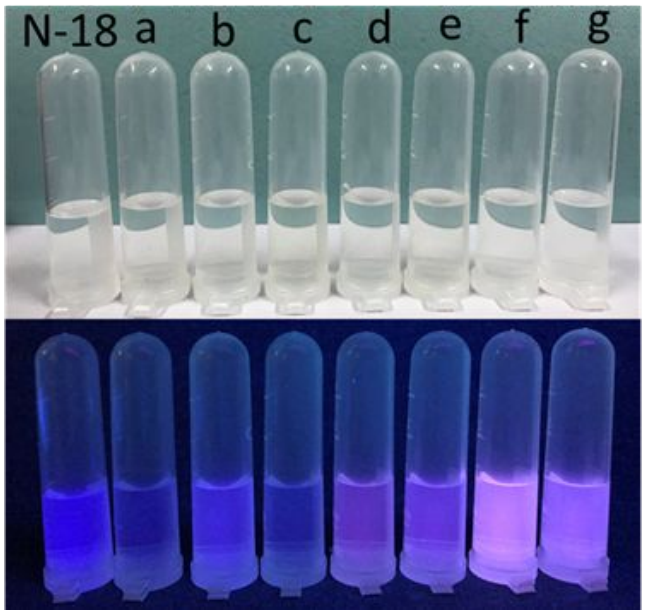


Fig. S7 Images of **N-18** solution in acetonitrile with addition of different organic amines: a) for 4-chloroaniline; b) for pyridine; c) for aniline; d) for TEA; e) for diethylamine; f) for ethylenediamine; g) for n-butylamine. The uppers were under daylight. The lowers were under 365 nm light.

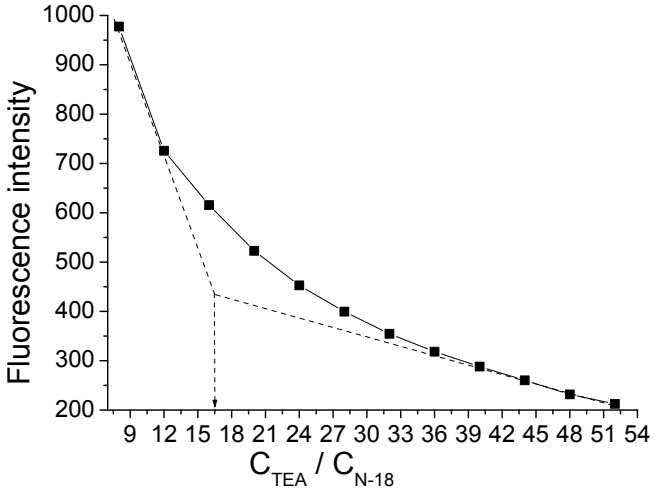


Fig. S8 Fluorescence intensity changes at 410 nm with different molar ratio of TEA and **N-18** in acetonitrile.

Limit of detection calculation

Table S2 Detection limit of compound **N-18** toward TEA in acetonitrile by fluorescence intensity changes at 409 nm

n	1	2	3	4	5	6	7	8	9	10	11
Intensity (Xn)	826.1	826.1	826.2	826.1	826.1	826.1	826.1	826.1	826.1	826.0	826.1

The limit of detection (LOD) was determined with the following equation: $LOD = 3\sigma/b$. The σ was the standard deviation of 11 blank samples (10^{-5} M of **N-18**), and b was the slope between the ratio of emission intensity versus TEA concentration.

$X_{average} = 826.1$ $\sigma_{wb} = sqrt(\sum(X_n - X_{average})^2/n) = 0.0018$

The detection limit: $[TEA] = 3\sigma/b = 2.23\times10^{-6}$ M

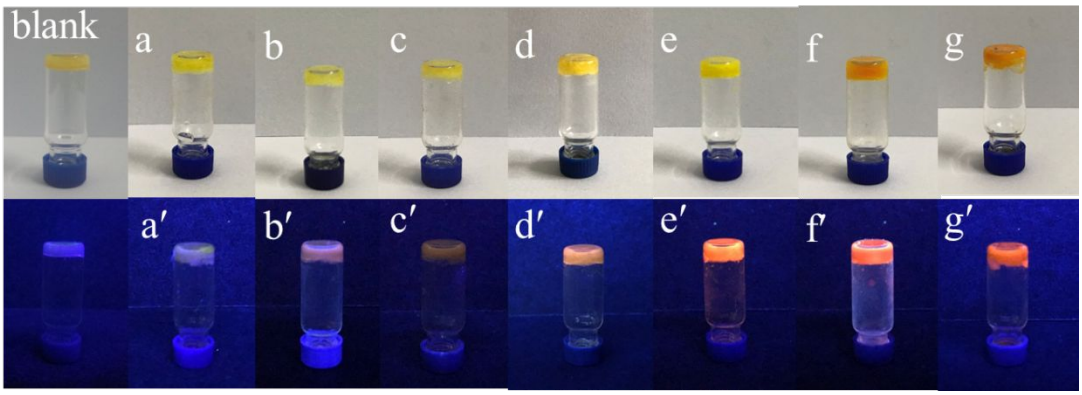


Fig. S9 Images of organogels **N-18** in acetonitrile with addition of different organic amines: a) for 4-chloroaniline; b) for pyridine; c) for aniline; d) for TEA; e) for diethylamine; f) for ethylenediamine; g) for n-butylamine. The uppers were under daylight. The lowers were under 365 nm light.

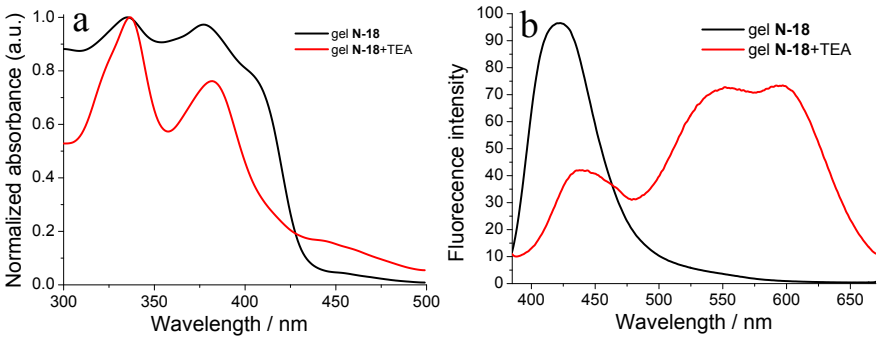


Fig. S10 UV-vis absorption (a) and fluorescence emission (b) change of organogel **N-18** in acetonitrile before and after addition of TEA (10 μL).

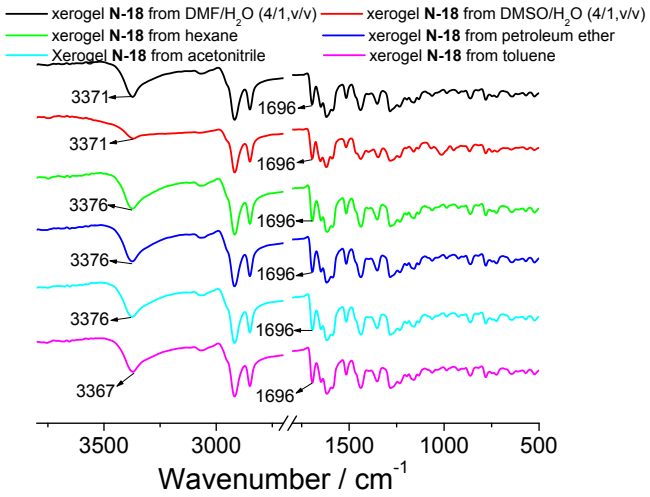


Fig. S11 FTIR spectra of xerogels **N-18** from DMF/H_2O (4/1, v/v), DMSO/H_2O (4/1, v/v), n-hexane, petroleum ether, acetonitrile and toluene.

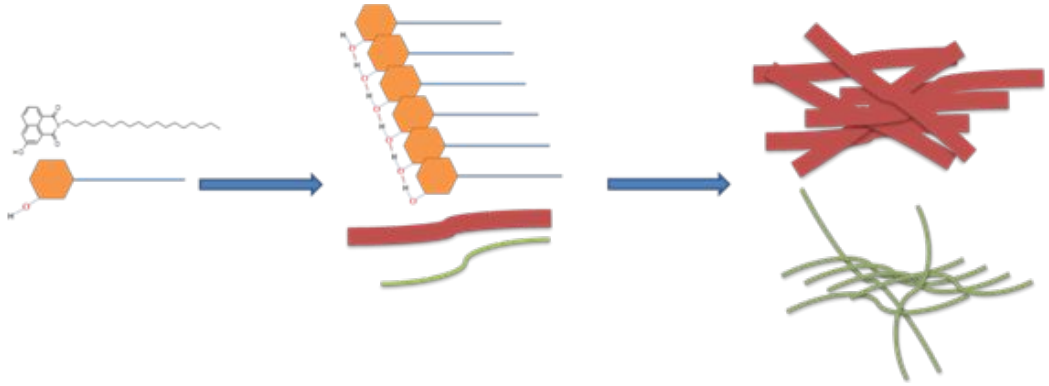


Fig. S12 The probable self-assembly mode of molecule **N-18**.