Electronic Supplementary Information (ESI)

Insight into aggregation-induced emission characteristics of red-emissive quinoline-malononitrile by cell tracking and real-time trypsin detection

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Contents

1. Experimental section

Materials and general methods

THF was distilled under argon atmosphere from sodium benzophenone ketyl immediately prior to use. All other solvents and chemicals were purchased commercially, and used as received without further purification. The ^{1}H and ^{13}C NMR spectra were obtained with a Bruker AM 400 spectrometer (relative to TMS). High resolution mass spectra measurements were carried out using a Waters LCT Premier XE spectrometer, and UV-vis spectra on a Varian Cary 500 spectrophotometer. Fluorescence spectra were recorded on a Varian Cary Eclipse fluorescence spectrophotometer. SEM micrographs were obtained on a JEOL JSM-6360 scanning electron microscope. TEM micrographs were obtained on a JEOL JEM-1400 instrument. Cell imaging was performed with an inverted FL microscope (Nikon Eclipse Ti). Dynamic light scatting (DLS) experiments were carried out with ALV5000 Laser Light Scattering Instrument. The X-ray diffraction intensity data were collected at room temperature on a Rigaku Ultima IV apparatus with Cu Kα radiation (λ = 0.15406 nm).

Assay procedures

BSA (20.0 mg/mL) of different volumes was added to PBS buffer solution (10.0 mM, pH 7.0) containing **EDS** (10.0 μM). The final concentrations of BSA were 0.0, 40.0, 80.0, 100.0, 130.0, 160.0, 240.0, 480.0, 800.0 μg/mL, respectively. Assay solution was kept at room temperature. The changes of fluorescence at 593 nm were recorded.

Trypsin (10.0 mg/mL) of different volumes was added to PBS buffer solution (10.0 mM, pH 7.0) containing **EDS** (10.0 μM) and BSA (800.0 μg/mL). The final concentrations of trypsin were 0.0, 1.0, 16.0, 40.0, 120.0, 160.0, 200.0 μg/mL, respectively. Assay solution temperature was kept at 37 ºC. The changes of fluorescence at 593 nm were recorded.

Trypsin inhibitor screening

Bisbenzenesulphonylimide (BBI) was selected as trypsin inhibitor. Different amounts of BBI were mixed with trypsin and the solutions were incubated at 4 ºC for 5 min. The final concentrations were 0.0, 2.0, 20.0, 80.0, 160.0 μg/mL. Assay solution containing **EDS** (10.0 μM) and BSA (800.0 μg/mL) was incubated at 37 ºC. The fluorescence intensity changes at 593 nm were recorded.

Calculation of the IC_{50} value

The definition of IC_{50} is the concentration of inhibitor required to achieve 50 % decrease of the enzyme activity, which has been widely used as a standard to evaluate the inhibition effects of enzyme inhibitors.[S1] The IC_{50} value could be obtained from the plot of the inhibition efficiency of BBI to trypsin *vs.* the concentration of BBI.

SEM and TEM

The samples for SEM observations were prepared by depositing several drops of the ensemble of **EDS** (10.0 μM) with BSA (800.0 μg/mL) and **EDPS** (10.0 μM) in 99% H_2O/THF solution onto the surface of cleaned glass separately, and the samples were air-dried at room temperature. The samples for TEM were prepared by dropping **EDS** (100.0 μM), the ensemble of **EDS** (100.0 μM) with BSA (8.0 mg/mL), **EDPS** (100.0 μM) in 99% H_2O/THF solution, the ensemble of **EDPS** (100.0 μM) with BSA (8.0 mg/mL) in 99% H_2O/THF solution onto carbon-coated copper grids, and the grids were dried at room temperature.

Cell imaging

Cell culture: The human cervical adencarcinoma HeLa cell line was supplied by the Institute of Cell Biology (Shanghai, China). They were cultured at 37 °C under a humidified 5% CO_2 atmosphere in RPMI-1640 medium (GIBCO/Invitrogen, Camarillo, CA, USA) supplemented with 10% fetal bovine serum (FBS) (Biological Industry, Kibbutz Beit Haemek, Israel) and 1% penicillin-streptomycin (10,000 U/ml penicillin and 10 mg/ml streptomycin, Solarbio life science, Beijing, China). The HeLa cells at 2×10^5 cells/ml were plated on 20 mm glass cover slips in a 6-well plates, and allowed to adhere overnight. The live cells were stained with either 5.0 μM of **EDS** (by adding 10 μL of a 500.0 μM stock solution in water to 1 mL culture medium) or **EDPS** (by adding 1 μL of a 5.0 mM stock solution in DMSO to 1 mL culture medium) for different times. Then the cells were washed three times with PBS buffer, and the medium was replaced with PBS buffer before imaging.

Microscopy and imaging methods: Imaging of cells was performed with a Nikon Eclipse Ti inverted fluorescence microscope. A 40 × objective lens was used. Excitation was carried out for **EDS** at 510-560 nm, and emission was collected at 590 nm. Excitation was carried out for **EDPS** at 465-495 nm, and emission was collected in the range of 515-555 nm. Hela cells were incubated with a PBS solution of

dye loading for 5 min, 15 min, 30 min, 45 min separately at 37 ºC. The stained cells were washed three times with PBS buffer. Then the treated cells were imaged by fluorescence microscopy.

Synthesis of EDS and EDPS

The intermediates of **1, 2** and **3** were prepared by the literature procedure.[S3]

Synthesis of EDS

Compound 2 (351.4 mg, 1.0 mmol) and 4-dimethylaminobenzaldehyde (149.2 mg, 1.0 mmol) were dissolved in 20 mL of acetonitrile with piperidine (1.0 mL) under argon protection at room temperature. Then the mixture was refluxed for 10 h. The solvent was removed under reduced pressure, and the crude product was purified by Al_2O_3 chromatography with dichloromethane/methanol (5:1) to afford a red solid (160.0 mg, 33.2% yield). ^{1}HNMR (400 MHz, DMSO-d_6, ppm): δ = 2.12 (m, 2H, -C***H***$_2$SO$_3^-$), 2.68 - 2.70 (m, 2H, -CH$_2$C***H***$_2$CH$_2$-), 3.01 (s, 6H, -N(C***H***$_3$)$_2$), 4.73 (m, J = 8.0 Hz, 2H, -NC***H***$_2$CH$_2$ -), 6.74 (d, J = 8.0 Hz, 2H, phenyl-H), 7.11 (s, 1 H, pyrrole -H), 7.38 (d, J = 16.0 Hz, 1H, alkene-H), 7.47 (d, J = 16.0 Hz, 1H, alkene-H), 7.58 (t, J = 8.0 Hz, 1H, phenyl-H), 7.79 (d, J = 8.0 Hz, 2H, phenyl-H), 7.88 (t, J = 8.0 Hz, 1H, phenyl-H), 8.21 (d, J = 8.0 Hz, 1H, phenyl-H), 8.91 (d, J = 8.0 Hz, 1H, phenyl-H). ^{13}C NMR (100 MHz, DMSO-d_6, ppm): δ = 5.32, 16.90, 29.68, 50.22, 50.88, 52.48, 52.85, 84.16, 84.40, 84.49, 110.65, 116.95, 119.30, 123.58, 125.85, 128.11, 129.94, 135.58, 138.66, 143.44, 146.36, 155.33, 156.68, 156.75. HRMS (TOF-ESI$^-$): *m/z* calcd for $C_{25}H_{23}N_4O_3S$ [M-Na$^+$] : 459.1491; found: 459.1490.

Synthesis of 4

Compound 3 (470.6 mg, 2.0 mmol) and 4-hydroxybenzaldehyde (244.2 mg, 2.0 mmol) were dissolved in 20 mL of acetonitrile with piperidine (1.0 mL) under argon protection at room temperature. Then the mixture was refluxed for 10 h. The solvent was removed under reduced pressure, and the crude product was purified by silica chromatography with dichloromethane/methanol (10 : 1) to afford an orange solid (400.0 mg, 58.9% yield). ^{1}HNMR (400 MHz, DMSO-d_6, ppm): δ = 1.41 (t, J = 8.0 Hz, 3H, -NCH$_2$C***H***$_3$), 4.57 (q, J = 8.0 Hz, 2H, -NC***H***$_2$CH$_3$), 6.85 (d, J = 8.0 Hz, 2H, phenyl-H), 7.02 (s, 1 H, pyrrole -H), 7.30 (d, J = 16.0 Hz, 1H, alkene-H), 7.37 (d, J = 16.0 Hz, 1H, alkene-H), 7.61 (t, J = 8.0 Hz, 1H, phenyl-H), 7.67 (d, J = 8.0 Hz, 2H, phenyl-H), 7.93 (t, J = 8.0 Hz, 1H, phenyl-H), 8.09 (d, J = 8.0 Hz, 1H, phenyl-H), 8.93 (d, J = 8.0 Hz, 1H, phenyl-H). ^{13}C NMR (100 MHz, DMSO-d_6, ppm): δ = 13.62, 43.73, 46.33, 54.87, 106.49, 115.71, 116.85, 118.08, 120.62, 124.87, 125.11, 126.29, 130.01, 133.62, 137.85,

139.98, 149.67, 152.12, 159.36. HRMS (TOF-ESI$^-$): *m/z* calcd for $C_{22}H_{16}N_3O$ [M-H$^+$] : 338.1293; found: 338.1294.

Synthesis of EDPS

Into a 50 mL round-bottom flask compound 4 (200.0 mg, 0.6 mmol) and 1,3-propanesultone (72.0 mg, 0.6 mmol) and 10 mL of ethanol were added. A mixture of NaOEt (82.0 mg, 1.2 mmol) in 10 mL of ethanol was added dropwise at room temperature, causing the orange solution to turn red. Then the mixture was refluxed for another 10 h under argon atmosphere. The crude product was collected by filtration and purified by recrystallization from methanol to afford an orange solid (138.1 mg, 47.6 % yield). ^{1}HNMR (400 MHz, DMSO-d_6, ppm): δ = 1.41 (t, J = 8.0 Hz, 3H, -NCH$_2$C***H***$_3$), 1.99-2.05 (m, 2 H, -CH$_2$C***H***$_2$CH$_2$-), 2.56 (t, J = 8.0 Hz, 2 H, -CH$_2$CH$_2$C***H***$_2$-), 4.14 (t, J = 8.0 Hz, 2 H, -C***H***$_2$CH$_2$CH$_2$-), 4.59 (q, J = 8.0 Hz, 2H, -NC***H***$_2$CH$_3$), 7.02 (d, J = 8.0 Hz, 2H, phenyl-H), 7.04 (s, 1H, pyrrole-H), 7.39 (d, J = 16.0 Hz, 1H, alkene-H), 7.43 (t, J = 16.0 Hz, 1H, alkene-H), 7.62 (t, J = 8.0 Hz, 1H, phenyl-H), 7.78 (d, J = 8.0 Hz, 2H, phenyl-H), 7.93 (t, J = 8.0 Hz, 1H, phenyl-H), 8.10 (d, J = 8.0 Hz, 1H, phenyl-H), 8.94 (d, J = 8.0 Hz, 1H, phenyl-H). ^{13}C NMR (100 MHz, DMSO-d_6, ppm): δ = 13.86, 21.22, 21.99, 24.38, 25.13, 44.89, 47.13, 47.51, 105.29, 111.60, 113.98, 118.25, 120.52, 122.79, 124.60, 124.86, 130.26, 133.29, 138.11, 141.02, 149.98, 151.32, 151.38. HRMS(TOF-ESI$^-$): *m/z* calcd for $C_{25}H_{22}N_3O_4S$[M-Na$^+$]: 460.1331; found: 460.1332.

2. Optical spectra of EDPS and EDS

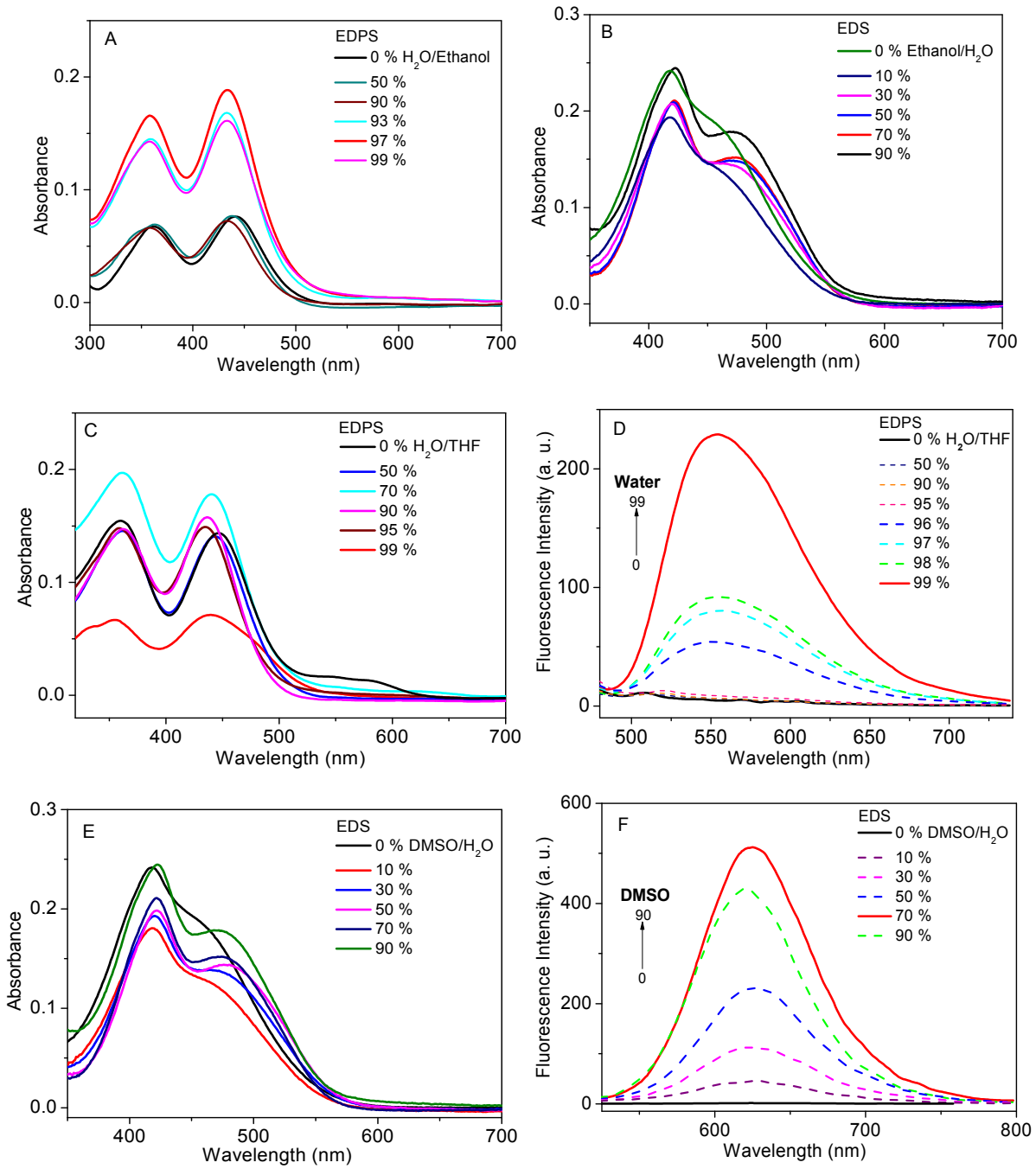


Fig. S1 Absorption spectra of (A) **EDPS** (10.0 μM) in different Ethanol/H_2O mixtures, (B) **EDS** (10.0 μM) in different H_2O/Ethanol mixtures, (C) **EDPS** (10.0 μM) in different THF/H_2O mixtures, (E) **EDS** (10.0 μM) in different H_2O/DMSO mixtures; PL spectra of (D) **EDPS** (10.0 μM) in different THF/H_2O mixtures and (F) **EDS** (10.0 μM) in H_2O/DMSO mixtures.

3. SEM images of EDPS and the ensemble of EDS and BSA

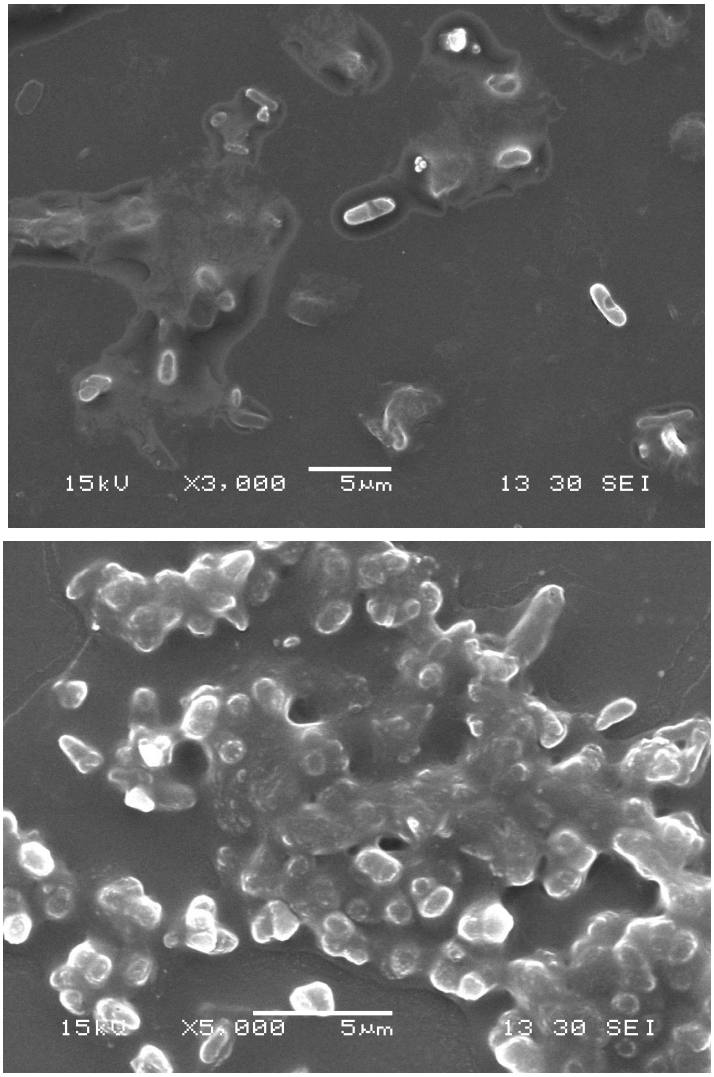


Fig. S2 SEM images of (Top) **EDPS** (10.0 μM) in 99% H_2O/ethanol mixture and (Bottom) the ensemble of **EDS** (10.0 μM) and BSA (800.0 μg/mL) in PBS buffer (10.0 mM, pH = 7.0).

4. The selectivity for evaluating the specificity of EDS to protein

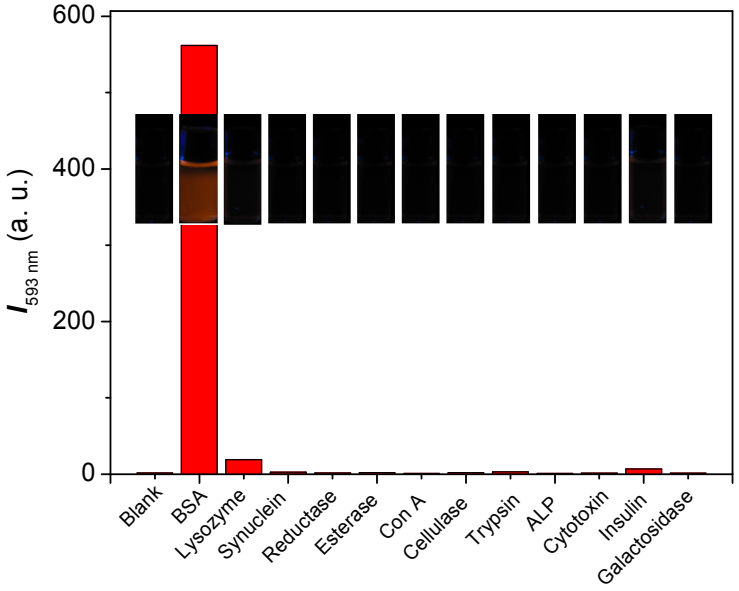


Fig. S3 The fluorescent intensity and images of **EDS** (10.0 μM) at 593 nm after addition of different proteins in PBS buffer solution (10.0 mM, pH =7.0); λ_{ex} = 460 nm. Note: among of these proteins, BSA as the model protein has the most efficient binding with EDS, along with exhibiting the strongest fluorescence enhancement.

5. Absorption of the ensemble of EDS and different concentration of BSA

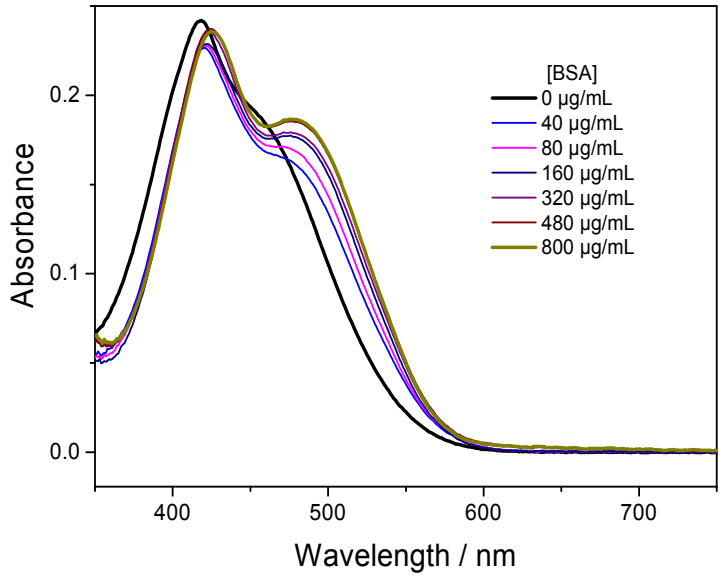


Fig. S4 Absorption spectra of **EDS** (10.0 μM) in the present of different concentration of BSA (0.0-800.0 μg/mL) incubated at room temperature.

6. The Linear plot for the detection limit of EDS to BSA

The detection limit of EDS to BSA is calculated according to the reference.[S2] Definitely, by recording the fluorescence change of EDS (10.0 μM) in PBS buffer solution (10.0 mM, pH 7.0) after addition different concentration of BSA (0.0-800.0 μg/mL), the plot of $(I-I_{min})/(I_{max}-I_{min})$ *vs.* Log([BSA]) can be obtained. Then a linear fit was used to the plot, and extending the line to X axis to get the minimum detection of EDS to BSA.

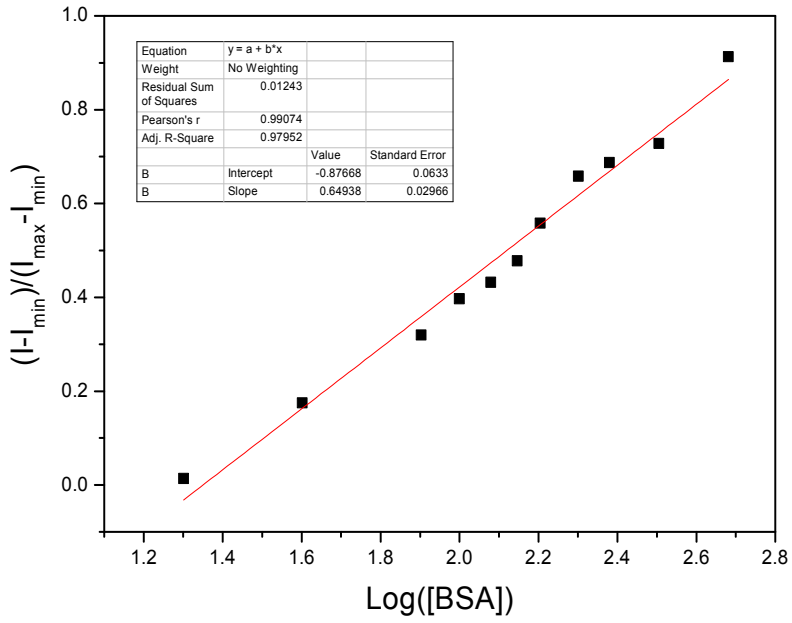


Fig. S5 The linear plot of $(I - I_{min}) / (I_{max} - I_{min})$ *vs.* Log([BSA])

7. Variation of the fluorescence intensity at 593 nm *vs.* the reaction time for EDS

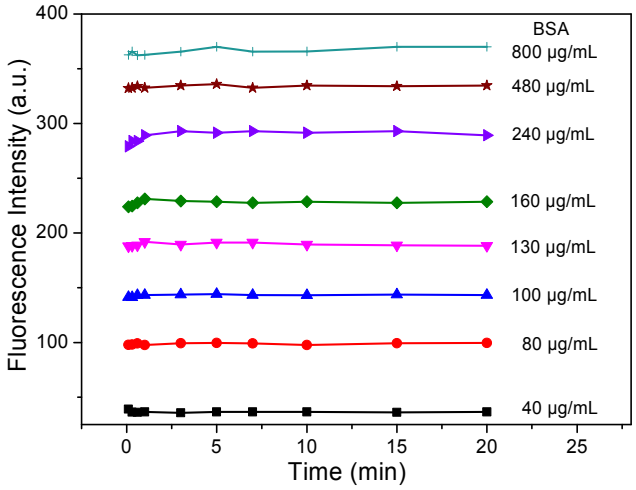


Fig. S6 Variation of the fluorescence intensity at 593 nm *vs.* the reaction time for **EDS** (10.0 μM) in PBS buffer solution (10.0 mM, pH 7.0) in the presence of different concentration of BSA (0.0-800.0 μg/mL); λ_{ex} = 460 nm.

8. TEM images of EDPS and the ensemble of EDPS and BSA

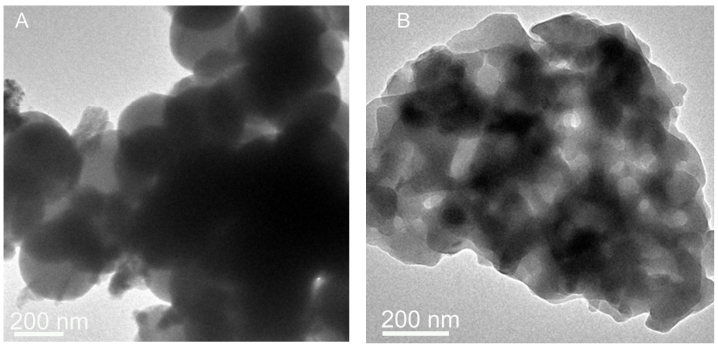


Fig. S7 TEM images of aggregates formed by (A) **EDPS** and (B) the ensemble of **EDPS** and BSA.

9. Powder X-ray diffraction patterns of EDS and EDPS

In X-ray diffraction curve, the difference in half-peak width and intensity can give information to the change of compound particle size. In XRD spectra, the diffraction curves of **EDS** display narrower half-peak width and stronger intensity than that of **EDPS**. These data imply that the particle size of **EDS** is larger than that of **EDPS** (Fig. S8). This further provides solid evidence for our previous speculation.

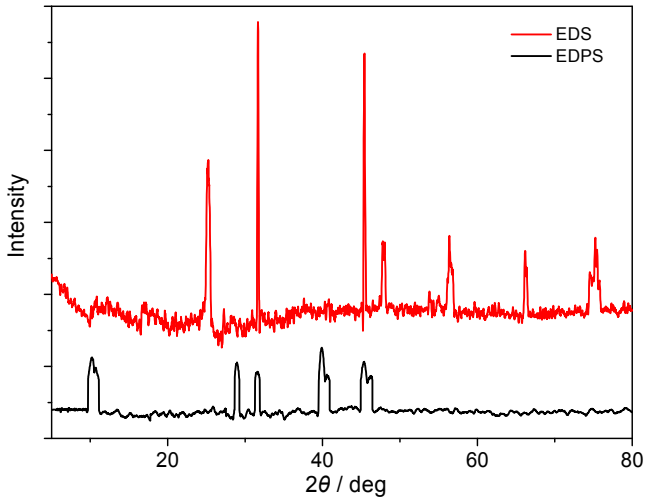


Fig. S8 Powder X-ray diffraction patterns of **EDS** and **EDPS.**

10. Fluorescent cell images of EDPS

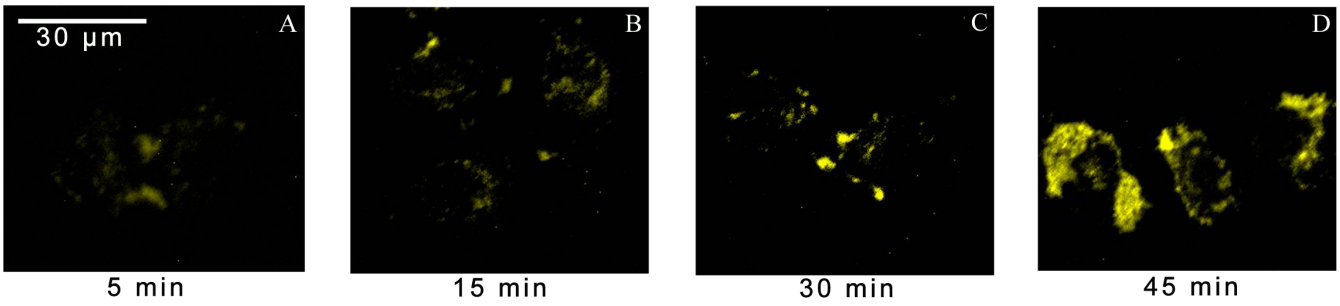


Fig. S9 Fluorescent cell images in HeLa cells incubated with a PBS solution of **EDPS** (10^{-5} M) for different time. From A to D: 5, 15, 30 and 45 min. Emission was collected at 515-555 nm.

11. Variation of the fluorescence intensity at 593 nm *vs.* the reaction time for the ensemble of EDS in PBS buffer solution and BSA in the presence of different concentration of trypsin

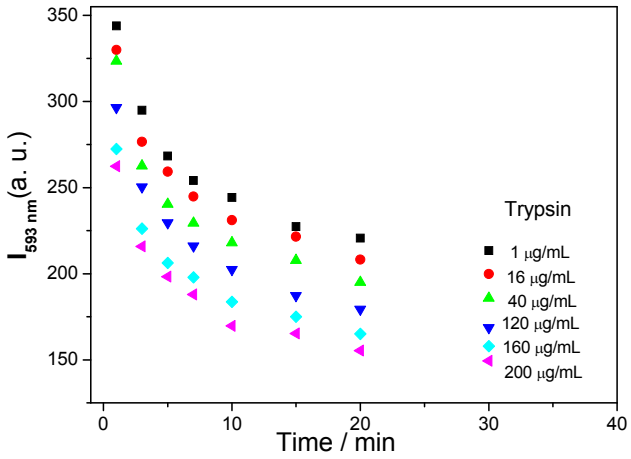


Fig. S10 Variation of the fluorescence intensity at 593 nm *vs.* the reaction time for the ensemble of **EDS** (10.0 μM) in PBS buffer solution (10.0 mM, pH 7.0) and BSA (800.0 μg/mL) in the presence of different concentration of trypsin (0.0-200.0 μg/mL); λ_{ex} = 460 nm.

12. Dynamic light scattering results for the ensemble of EDS and BSA in the presence of trypsin and the ensemble of EDPS and BSA

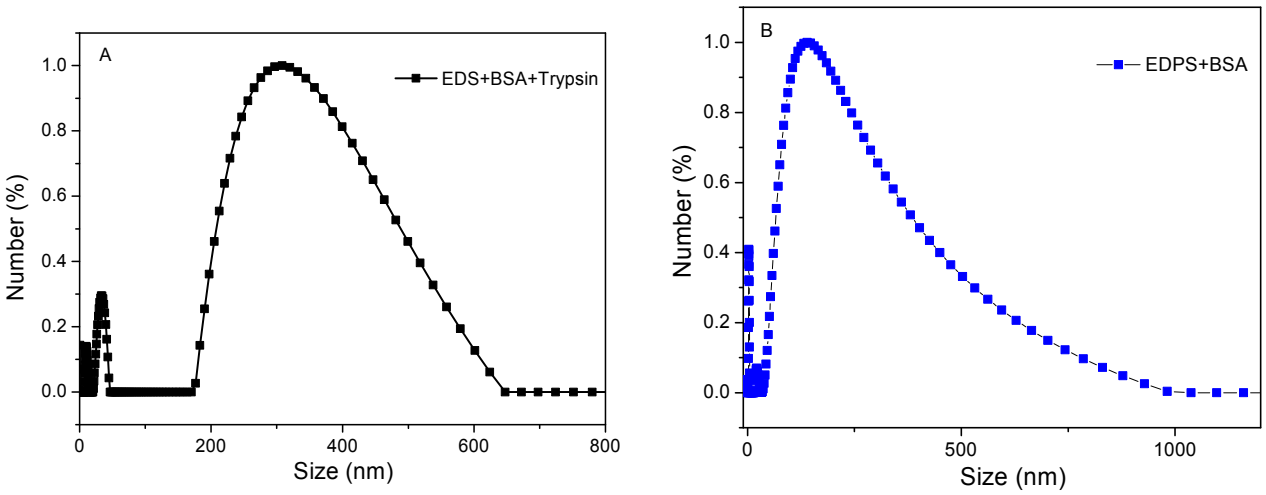


Fig. S11 Dynamic light scattering results for (A) **EDS** (10.0 μM) in PBS buffer solution (10.0 mM, pH =7.0) and BSA (800.0 μg/mL) in the presence of trypsin (200.0 μg/mL) after incubation for 30 min, (B) **EDPS** (10.0 μM) in 99% H_2O/ethanol mixture with BSA (1600.0 μg/mL).

13. Variation of the fluorescence intensity at 593 nm *vs.* the reaction time for the ensemble of EDS in PBS buffer solution, BSA and trypsin in the presence of different concentration of BBI

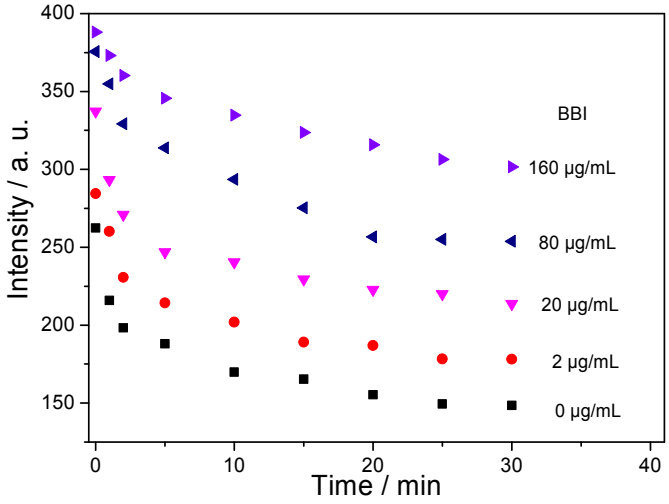


Fig. S12 Variation of the fluorescence intensity at 593 nm *vs.* the reaction time for the ensemble of **EDS** (10.0 μM) in PBS buffer solution (10.0 mM, pH 7.0), BSA (800.0 μg/mL) and trypsin (200.0 μg/mL) in the presence of different concentration of BBI (0.0 - 160.0 μg/mL); λ_{ex} = 460 nm.

14. Photoluminescence of the ensemble of EDS with different concentration of BSA

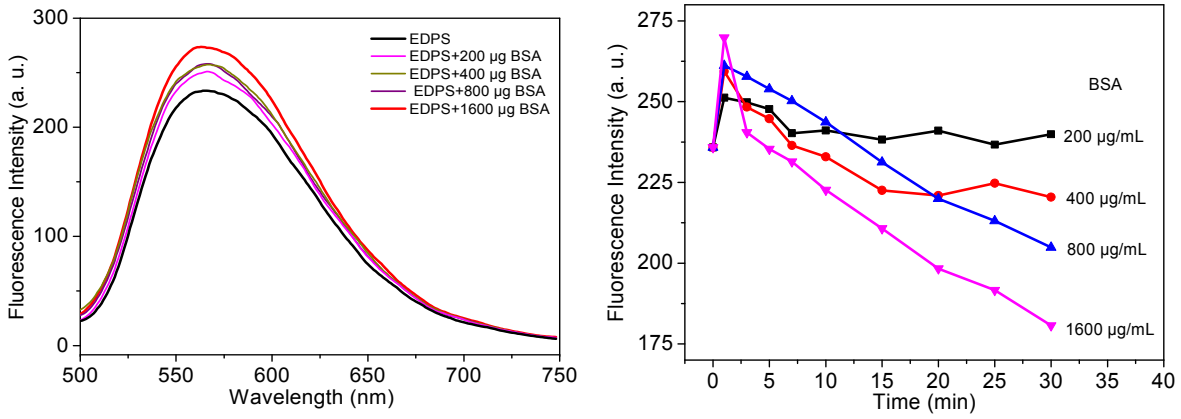


Fig. S13 (a) Photoluminescence (PL) spectra of **EDPS** in the presence of different concentration of BSA in 99% PBS buffer (10.0 mM, pH 7.0)/ethanol mixture incubated at room temperature. λ_{ex} = 450 nm; (b) Variation of the fluorescence intensity *vs.* the reaction time for **EDPS** in 99% PBS buffer (10.0 mM, pH 7.0)/ethanol mixture in the presence of different concentration of BSA; λ_{ex} = 450 nm.

15. Characterization of EDS and EDPS

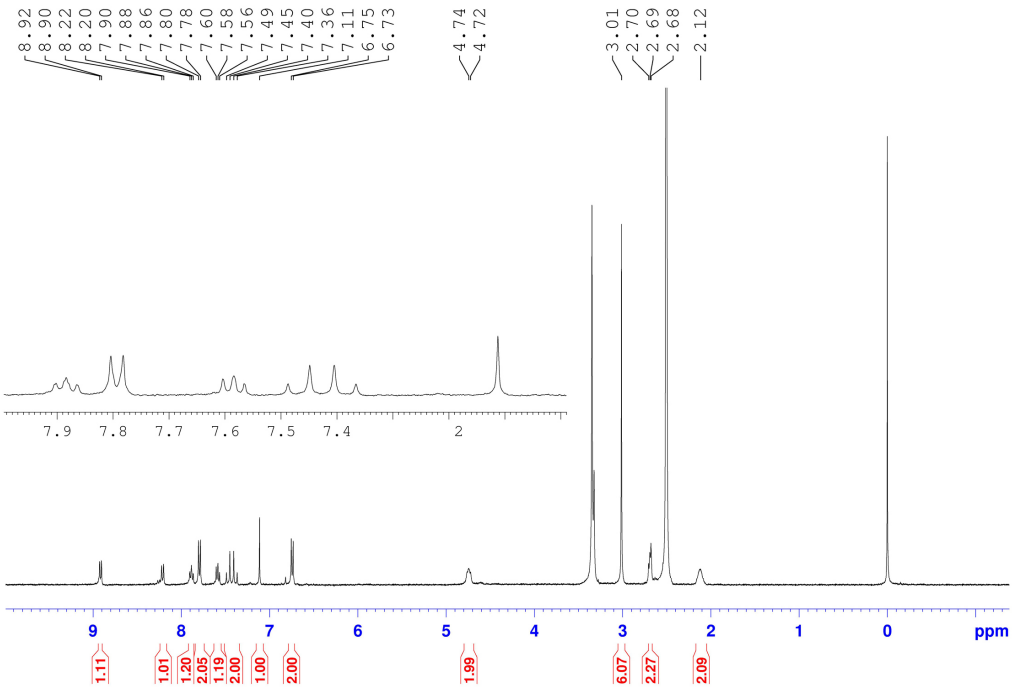
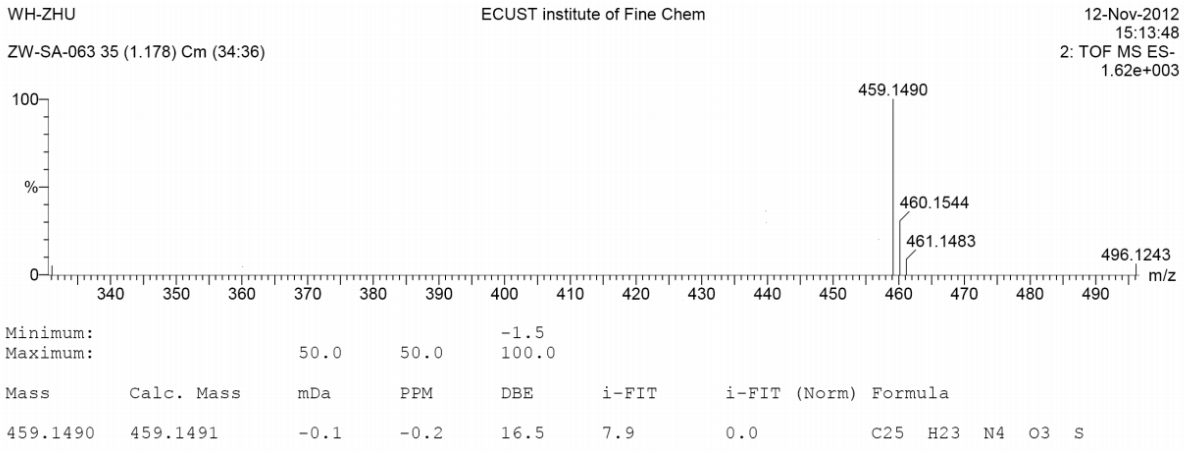


Fig. S14 ^{1}H NMR spectra of compound **EDS** in DMSO



Minimum:				-1.5			
Maximum:		50.0	50.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
459.1490	459.1491	-0.1	-0.2	16.5	7.9	0.0	C25 H23 N4 O3 S

Fig. S15 HR mass spectra of compound **EDS**

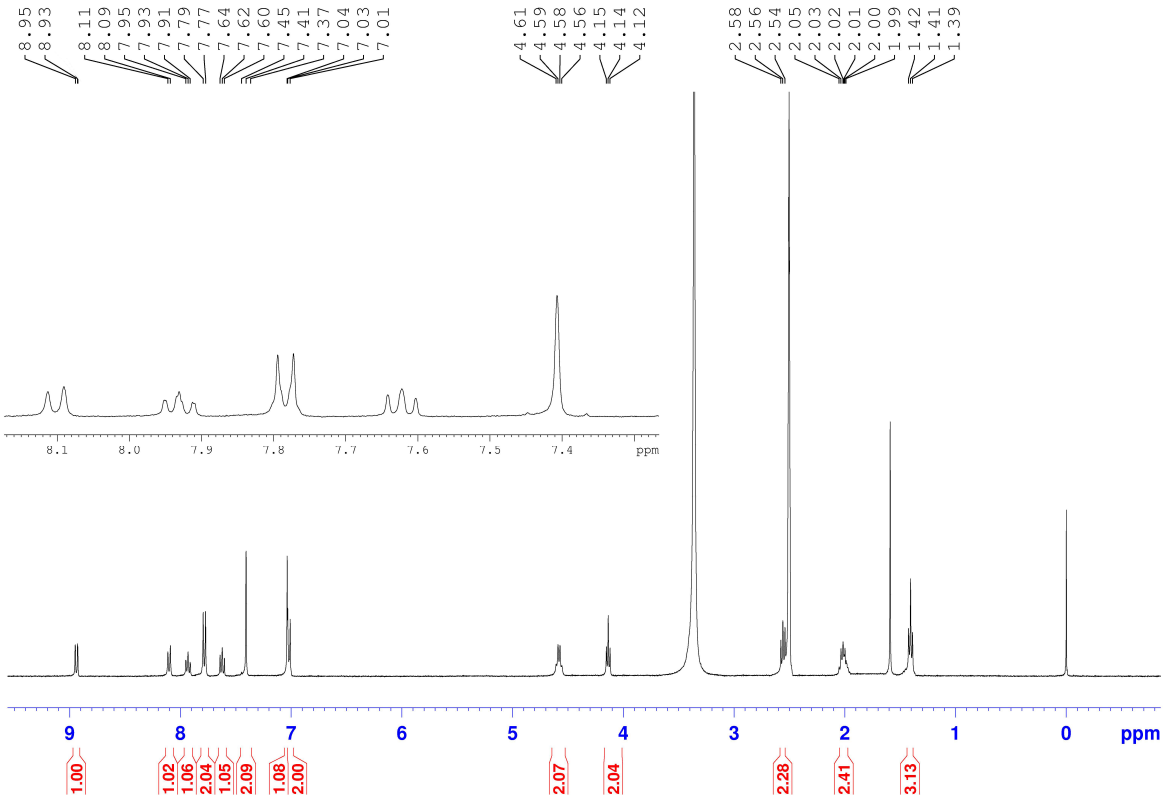
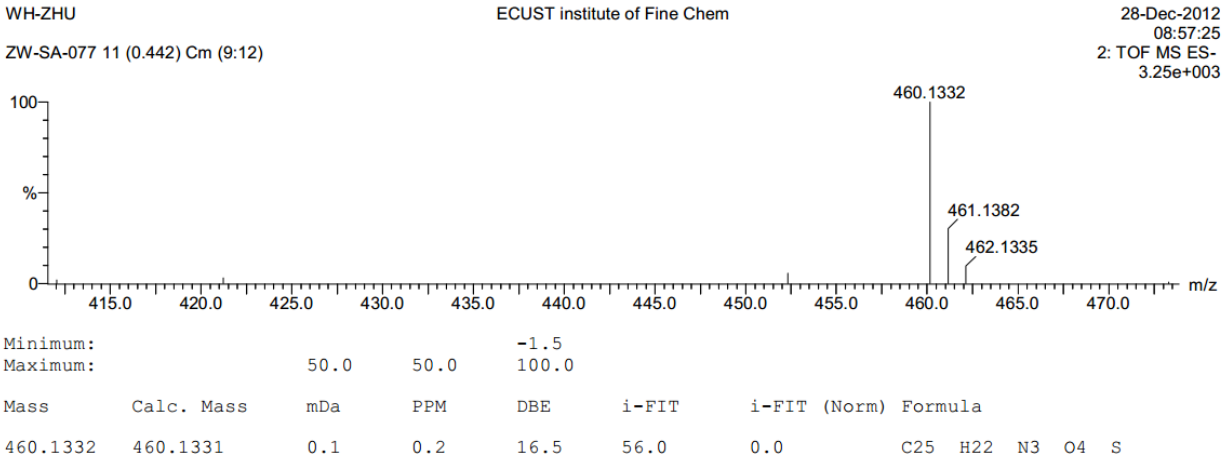


Fig. S16 [1]H NMR spectra of compound **EDPS** in DMSO



Minimum:				-1.5			
Maximum:		50.0	50.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
460.1332	460.1331	0.1	0.2	16.5	56.0	0.0	C25 H22 N3 O4 S

Fig. S17 HR mass spectra of compound **EDPS**

S1. J. C. Powers, J. L. Asgian, Ö. D. Ekici and K. E. James, *Chem. Rev.*, 2002, **102**, 4639.

S2. M. Shortreed, R. Kopelman, M. Kuhn and B. Hoyland, *Anal. Chem.,*1996, **68**, 1414.

S3. (a) L. Horwitz, *J. Am. Chem. Soc.,* 1955, **77**, 1687; (b) N. Narayanan and G. Patonay, *J. Org. Chem.*, 1995, **60**, 2391.