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Electronic Supplementary Information

Bimodal detection of carbon dioxide using a fluorescent molecular aggregates

Rakesh K. Mishra,*[ab] Samiyappan Vijayakumar,[a] Arindam Mal,[ac] Varsha Karunakaran,[cd] Jith C. Janardhanan,[a] Kaustabh Kumar Maiti,[cd] Vakayil K. Praveen,*[ac] and Ayyappanpillai Ajayaghosh*[ac]

[a]Photosciences and Photonics Section, Chemical Sciences and Technology Division, CSIR-National Institute for Interdisciplinary Science and Technology (CSIR-NIIST), Thiruvananthapuram-695019, India.
Email: vkpraveen@niist.res.in; aajayaghosh@niist.res.in

[b]Department of Sciences and Humanities, National Institute of Technology, Uttarakhand (NITUK), Srinagar (Garhwal)-246174, India. Email: rkmishra@nituk.ac.in

[c]Academy of Scientific and Innovative Research (AcSIR), Ghaziabad- 201002, India

[d]Organic Chemistry Section, Chemical Sciences and Technology Division, CSIR-NIIST, Thiruvananthapuram-695019, India.

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1. General Experimental Details

1.1. Synthesis-General Procedures

Unless otherwise stated, all starting materials and reagents were purchased from commercial suppliers and used without further purification. Acetone and C_2H_5OH were distilled prior to use. The reactions were monitored using thin layer chromatography on silica gel 60 F_{254} (0.2 mm; Merck). Visualization was accomplished using UV light (254 and 365 nm). Column chromatography was performed on glass columns of different sizes hand packed with 100-200 mesh silica gel (Merck).

1.2. Synthesis-Characterization Techniques

^1H and ^{13}C NMR spectra were measured on a 500 MHz Bruker Avance DPX spectrometer using TMS (0 ppm for ^1H NMR) or $CDCl_3$ (77 ppm for ^{13}C NMR) as an internal reference. Melting points were determined with Stuart SMP 30 melting point apparatus. FT-IR spectra were recorded on a Shimadzu IRPrestige-21 Fourier Transform Infrared Spectrophotometer. Electrospray ionization (ESI) high-resolution mass spectra were recorded on using Thermo Scientific Exactive mass spectrometer.

1.3. Measurements and Methods

The electronic absorption spectra were recorded on a Shimadzu spectrophotometer UV-2100. The fluorescence spectra were recorded on a SPEX-Fluorolog-3 FL3−221 spectrofluorimeter. Optical studies in solution-state were carried out in a 1 cm quartz cuvette. Fluorescence lifetime measurements were carried out using Horiba (model DeltaFlex) time-correlated single photon counting system. The lifetime values (τ), relative amplitude (*a*) and average lifetime (τ_{av}) were determined using EzTime decay analysis software. The equation used for the calculation of τ_{av} of triexponential decay is

$\alpha_1\tau_1+\alpha_2\tau_2+\alpha_3\tau_3$, where α is normalized pre-exponential value. The quality of the fit has been judged by the fitting parameters such as χ^2 (<1.1) as well as the visual inspection of the residuals. SEM images were obtained using a Zeiss EVO 18cryo SEM with an accelerating voltage of 20 kV. Samples were prepared by drop casting on a Silicon wafer and dried in air. DLS analyses were carried out with a Zetasizer Nano S from Malvern Instruments at 25 °C and an average of three measurements were taken.

For biological studies, dulbecco's modified eagle's medium (DMEM), Trypsin-EDTA, Penicillin-Streptomycin, dimethylsulfoxide (DMSO) for cell culture were purchased from Himedia. Fetal bovine serum (FBS) was purchased from Invitrogen. The fluorescence imaging of cells were performed in Inverted Microscope TS-100FLED (Nikon, Tokyo, Japan) with specialized epifluorescence objectives using blue excitation filters (450-490nm)

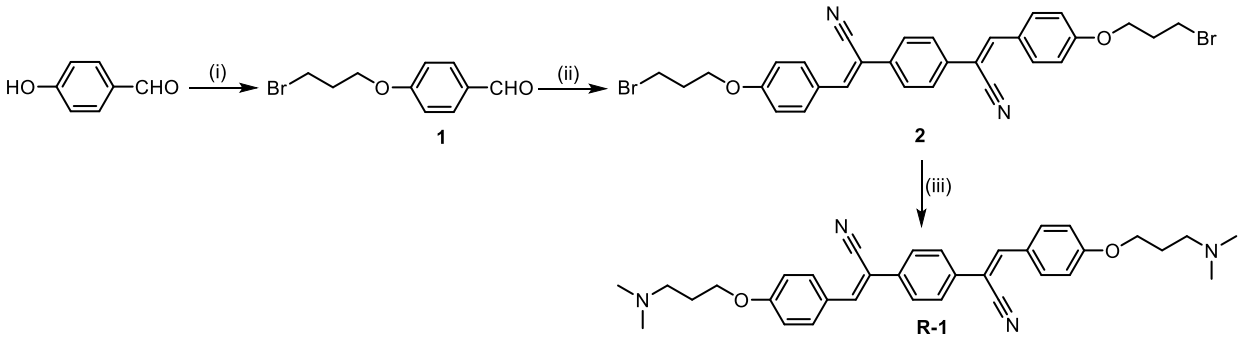
1.3.1. Cell and Cell Culture

The human lung adenocarcinoma cell line A549 was obtained from the American Type Culture Collection (ATCC). The cells were cultured in T25 culture flask in DMEM medium containing 10% FBS and 1% Penicillin-Streptomycin antibiotic at 37 °C in a humid CO_2 incubator with 5% CO_2. Cells were cultured up to 70-80% confluence as a monolayer and were detached using trypsin-EDTA (1X). Sterile conditions have been maintained throughout the cell culture work.

For the fluorescence microscopy analysis, 100 µL of cell suspension were seeded at a density of 10000 cells/well in a 96-well plate and left for an overnight attachment. **R-1** aggregates were added at a final concentration of 100 µM for 1 h at normal atmospheric condition (0.038% CO_2) at 25 °C. Following the appearance of

fluorescence, the cells were kept in a CO_2 incubator with 5% CO_2 for 2 h and the reduction in fluorescence was monitored.

2. Scheme for the Synthesis



Scheme S1 Reagents and condition: **(i)** K_2CO_3, acetone, reflux, 24 h; **(ii)** 1,4-phenylenediacetonitrile, EtONa, EtOH, 80 °C, 3 h; **(iii)** dimethylamine, THF, 60 °C, 6 h.

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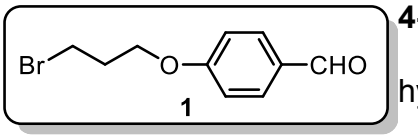
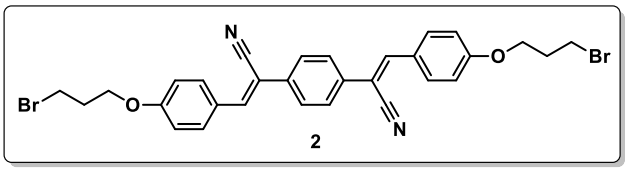
3. Synthesis and Characterization



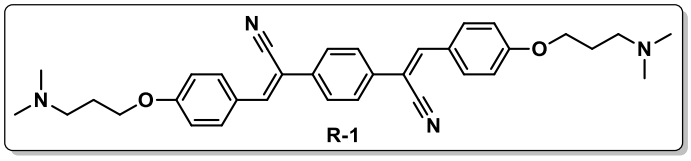
4-(3-bromopropoxy)benzaldehyde (1) : A mixture of *p*-hydroxybenzaldehyde (2.44 g, 20 mmol), 1,3-dibromopropane (6.12 mL, 60 mmol), K_2CO_3 (2.76 g, 20 mmol) and tetrabutyl ammonium bromide (0.1 g) in acetone (50 mL) was refluxed in a double-necked round-bottom flask (100 mL) equipped with a condenser for 10 h. After this time, the reaction mixture was cooled and the solvent was evaporated under reduced pressure and the resulting residue was redissolved in chloroform (150 mL) and washed with water. The organic layer was then dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The crude product thus obtained was purified by column chromatography on silica gel (*n*-hexane and ethyl acetate, 1:2) afforded **1** as a white solid in 80% yield (3.9 g).

^{1}H NMR (500 MHz, $CDCl_3$): δ = 9.90 (s, 1H), 7.86-7.84 (m, 2H), 7.03-7.01 (m, 2H), 4.21 (t, *J* = 5.7 Hz, 2H), 3.62 (t, *J* = 6.5 Hz, 2H), 2.39-2.34 (m, 2H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 190.7, 163.7, 132.0, 130.0, 114.7, 65.6, 32.0, 29.7 ppm.



(2Z,2'Z)-2,2'-(1,4-phenylene)bis(3-(4-(3-bromopropoxy) phenyl)acrylonitrile) **2**: To a mixture of 4-(3-bromopropoxy)benzaldehyde (3.97 g, 12.8 mmol), 1,4-phenylenediacetonitrile (1.00 g, 6.42 mmol) and EtOH (50 mL) was added equivalent amount of sodium ethoxide. The resulting solution was then stirred at room temperature for 3 h. The resulting yellow precipitate was collected by filtration, and washed with EtOH successively to give **2** as a yellow solid (4.28 g, 90%).

^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.91 (d, *J* = 9 Hz, 4H), 7.72 (s, 4H), 7.52 (s, 2H), 7.00 (d, *J* = 8.5 Hz, 4H), 4.19 (t, *J* = 5.7 Hz, 4H), 3.62 (t, *J* = 6.2 Hz, 4H), 2.38-2.33 (m, 4H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 160.7, 142.1, 135.1, 131.4, 126.5, 126.2, 114.9, 107.8, 99.9, 65.5, 32.1, 29.7 ppm; HRMS: *m*/*z* calcd. for $[M+Na]^+$: $C_{30}H_{26}Br_2N_2O_2Na$; 629.0238; found: 629.0247.



Z,2'Z)-2,2'-(1,4-phenylene)bis (3(4-(3-(dimethylamino)propoxy) phenyl) acrylonitrile) R-1: A 100 mL round bottom flask equipped with a magnetic stir bar, condenser and **2** (0.61 g, 1.0 mmol) was flushed with N_2, followed by addition of dimethyl amine (2M solution in THF, 65 mL). The reaction mixture was heated to 60 °C while stirring for 6 h. A yellow solid product

was obtained, which was filtered and washed with EtOH several times to afford **R-1** (0.407 g, 88%).

mp: 303-304 °C; FT-IR (KBr): ν_{max} = 3045, 2951, 2812, 2760, 2214, 1595, 1516, 1467, 1377, 1301, 1257, 1182, 1051, 837, 596, 536 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6): δ = 8.07 (s, 2H) 7.98 (d, J = 9 Hz, 4H), 7.84 (s, 4H), 7.12 (d, J = 9 Hz, 4H), 4.15 (t, J = 6 Hz, 4H), 3.18 (t, J = 7.7 Hz, 4H), 2.77 (s, 12H), 2.16-2.10 (m, 4H) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): δ = 160.7, 143.2, 134.3, 131.8, 126.8, 126.5, 118.6, 115.4, 106.8, 65.6, 54.6, 42.9, 24.5 ppm; HRMS: m/z calcd. for $[M+H]^+$: $C_{34}H_{39}N_4O_2$; 535.3073; found: 535.3070.

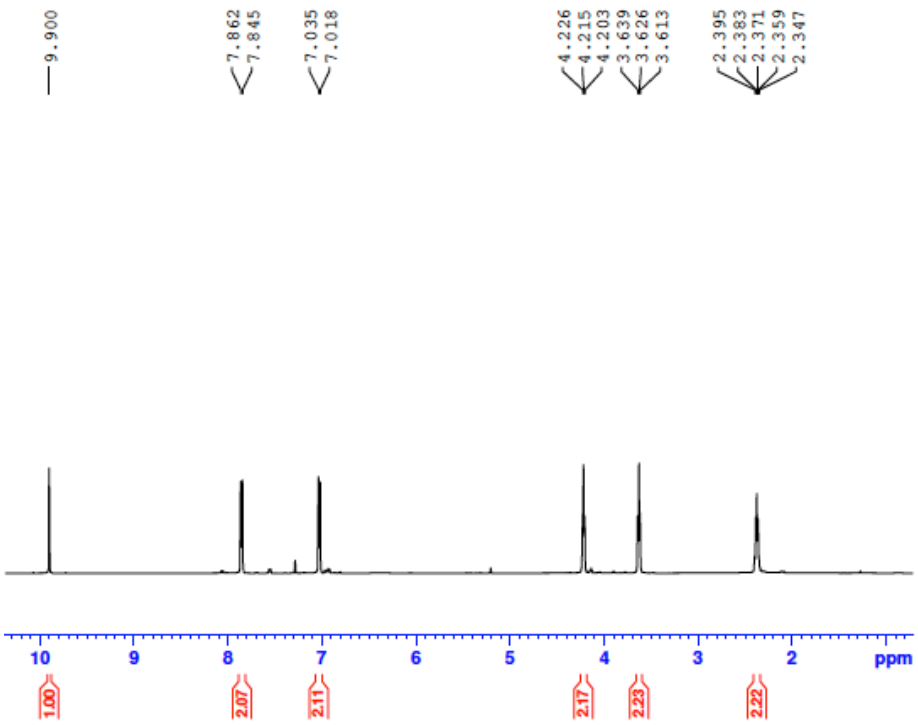


Fig. S1 ^{1}H NMR (500 MHz) spectrum of **1** in $CDCl_3$.

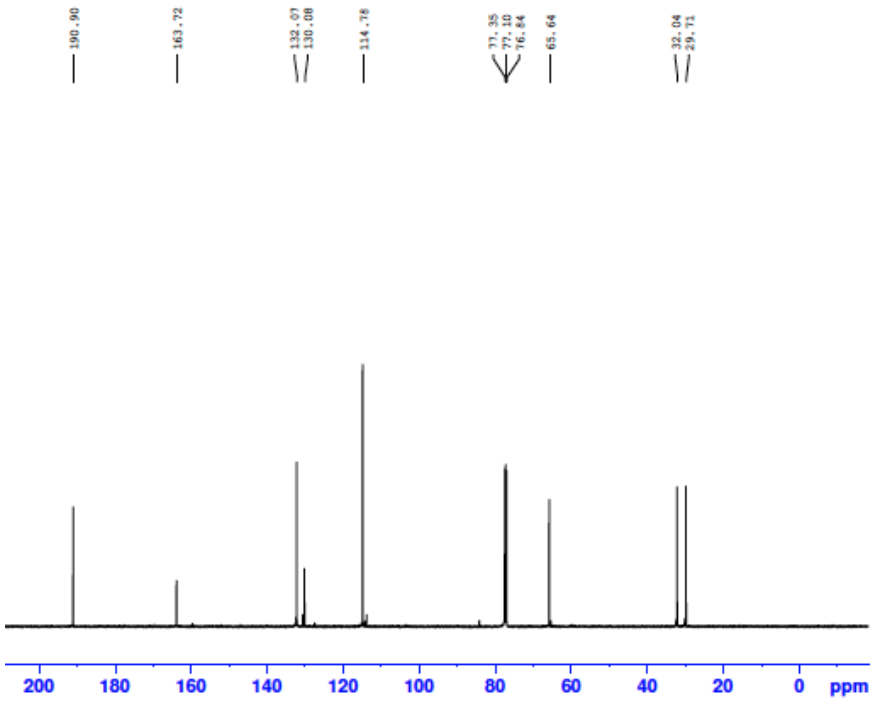


Fig. S2 ^{13}C NMR (125 MHz) spectrum of **1** in $CDCl_3$

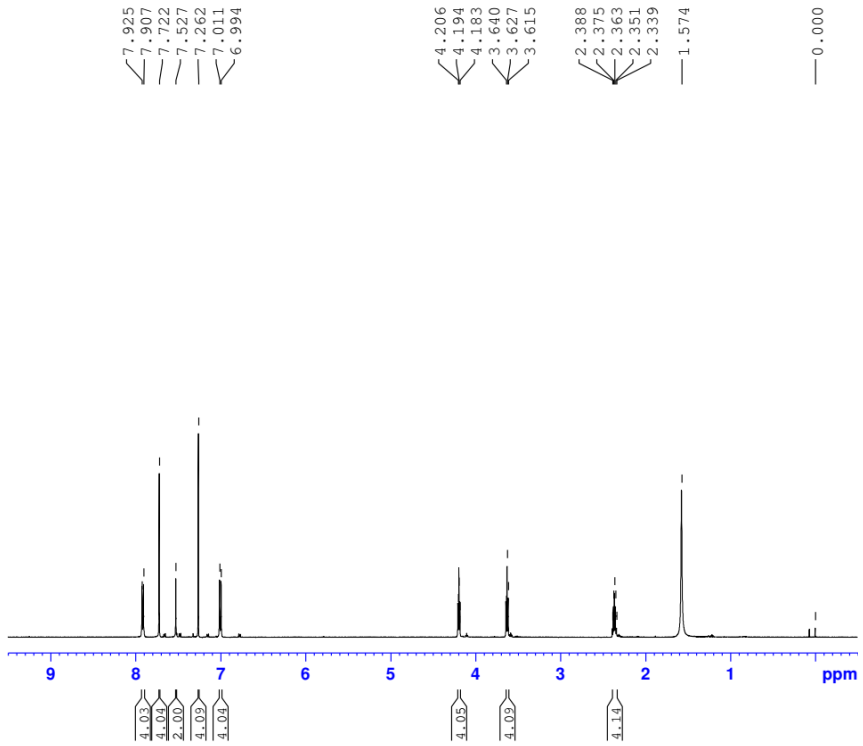


Fig. S3 ^{1}H NMR (500 MHz) spectrum of **2** in $CDCl_3$.

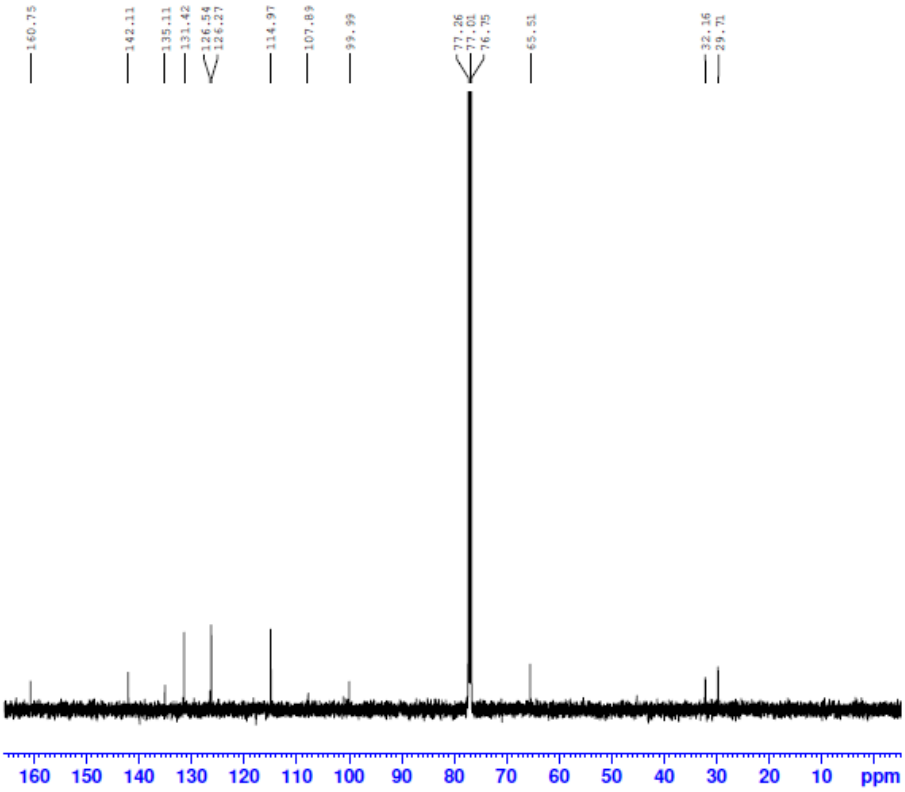


Fig. S4 ^{13}C NMR (125 MHz) spectrum of **2** in $CDCl_3$.

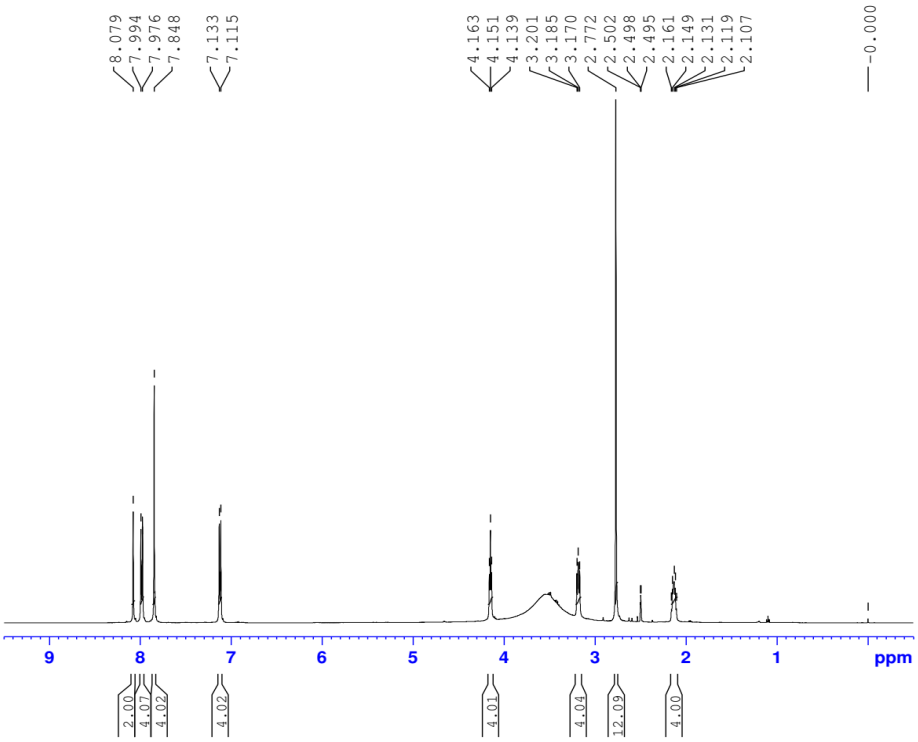


Fig. S5 ^{1}H NMR (500 MHz) spectrum of **R-1** in DMSO-d_6.

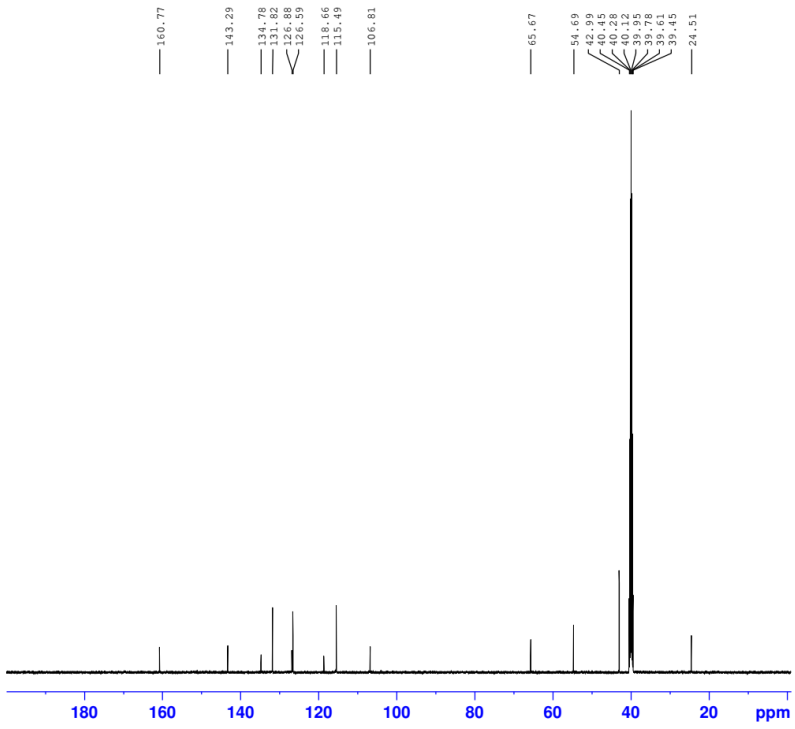


Fig. S6 ^{13}C NMR (125 MHz) spectrum of **R-1** in DMSO-d_6.

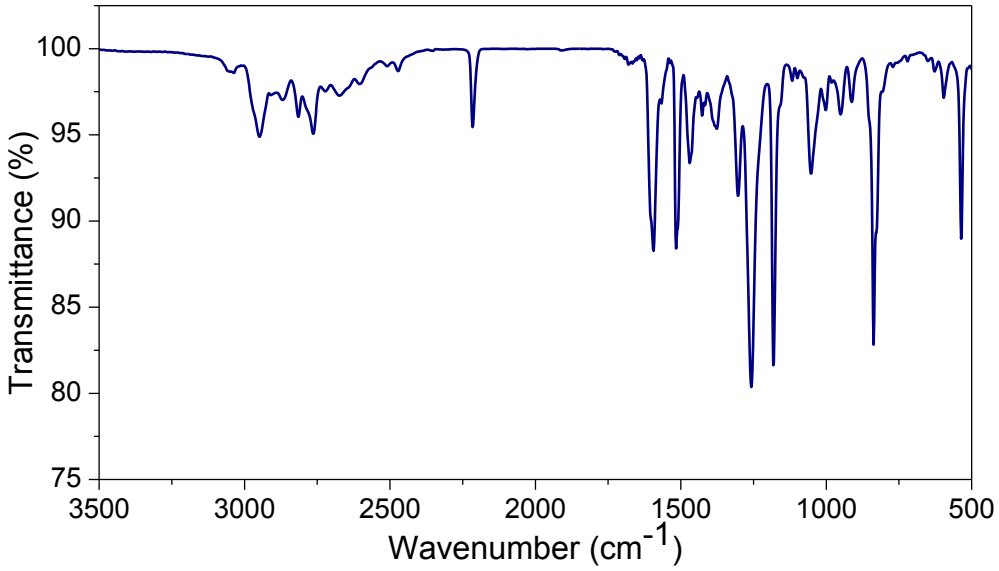


Fig. S7 FT-IR spectrum of **R-1**.

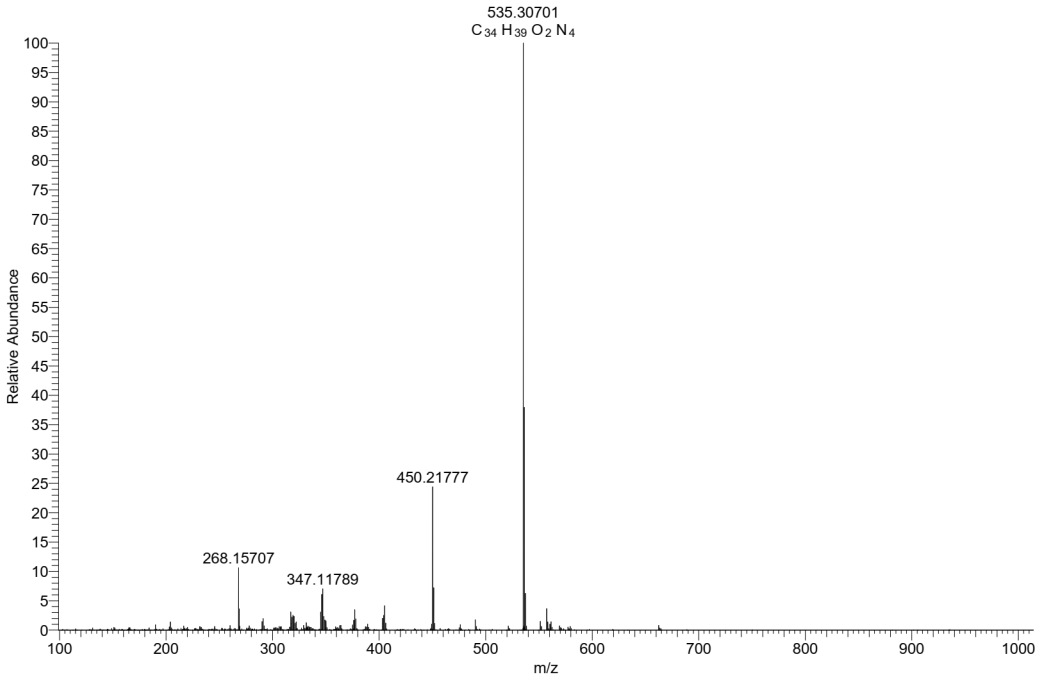


Fig. S8 HRMS spectrum of **R-1**.

5. Aggregation Studies

To examine whether **R-1** is an AIE-active molecule, its fluorescence property is investigated in DMSO/water solvent mixtures. As shown in Fig. S9, **R-1** is weakly emissive in pure DMSO (λ_{max} = 465 nm), while increasing the water content in DMSO/water mixtures from 0 to 99%, the fluorescence intensity was almost remains unchanged up to 90% composition of water. Further addition of water into the mixture leads to a sharp and significant enhancement in fluorescence intensity at 550 nm. At 99% of water content, the fluorescence intensity is nearly 60-fold higher than that in the pure DMSO indicating the AIE character of **R-1** aggregates.

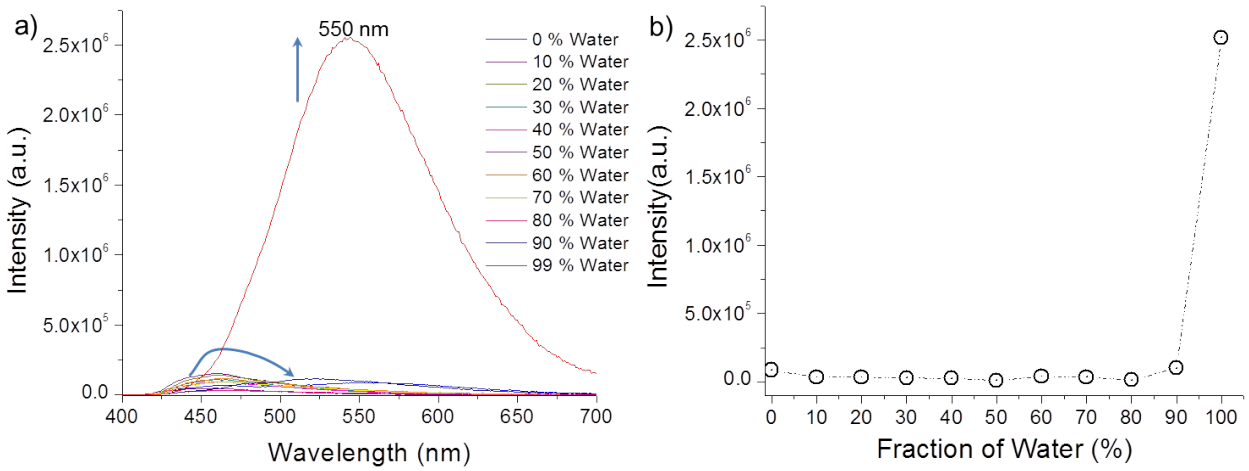


Fig. S9 (a) Fluorescence spectra of **R-1** in DMSO/water mixtures (5×10^{-5} M, λ_{ex} = 360 nm). (b) Corresponding changes in emission intensity at 550 nm as fraction of water content in DMSO/water mixtures.

6. Fluorescence Based Calibration Curve

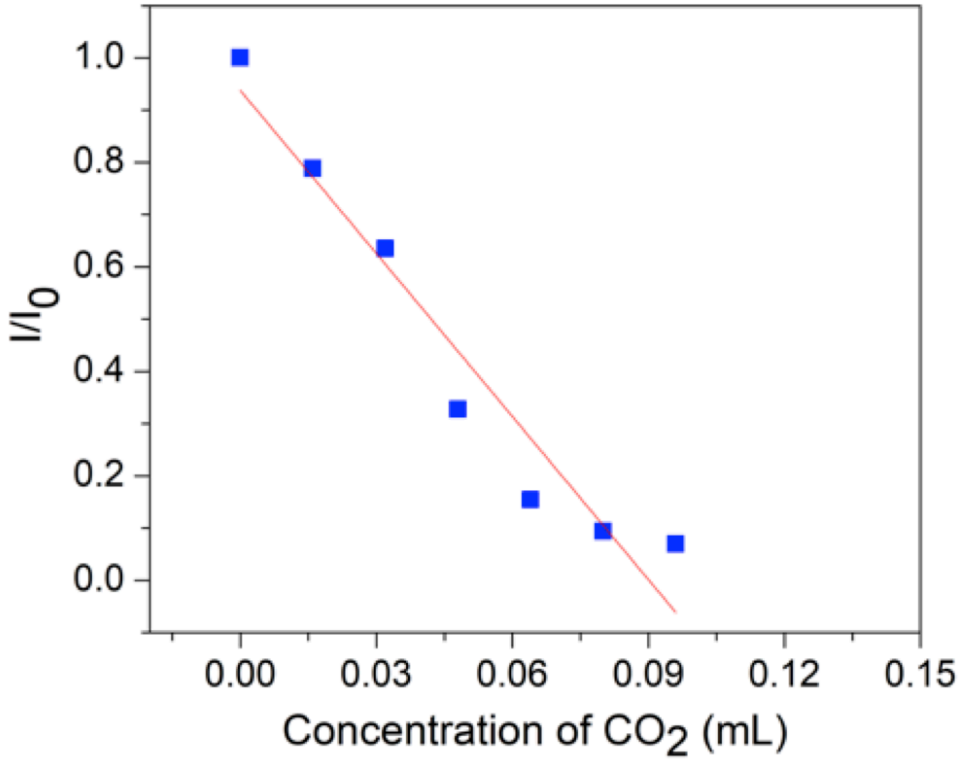


Fig. S10 Calibration curve based relative changes in fluorescence intensity of **R-1$_{Agg}$** (1×10^{-4} M) in aqueous medium (water/DMSO, 99/1 v/v) after purging with different volume of CO_2.

7. Table S1 Lifetime values of **R-1$_{Agg}$** in aqueous medium (water/DMSO, 99/1 v/v) with increasing amount of CO_2 (λ_{ex}= 375 nm and λ_{em}= 550 nm) and **R-1** in DMSO and in water/DMSO, 90/10 v/v (λ_{ex}= 375 nm and λ_{em}= 465 nm).

Sl. No.	Experiments	τ_1 (a_1) ns	τ_2 (a_2) ns	τ_3 (a_3) ns	τ_{av}
1	**R-1$_{Agg}$**	5.12 (29.05)	13.07 (65.79)	0.75 (5.16)	5.67
2	**R-1$_{Agg}$ +** CO_2 (0.016 mL)	3.66 (27.13)	11.72 (57.17)	0.49 (15.70)	2.28
3	**R-1$_{Agg}$ +** CO_2 (0.048 mL)	1.37 (36.95)	8.02 (38.60)	0.17 (24.45)	0.58
4	**R-1$_{Agg}$ +** CO_2 (0.33 mL)	0.97 (51.61)	4.40 (14.3)	0.14 (34.09)	0.33
5	**R-1$_{Mono}$** (DMSO)	0.00584 (100)	-	-	0.00584
6	**R-1$_{Agg}$**(water/DMSO, 90/10 v/v)	0.65 (28.47)	0.08 (62.08)	3.39 (9.45)	0.12

8. Dynamic Light Scattering (DLS) and Tyndall Effect Studies

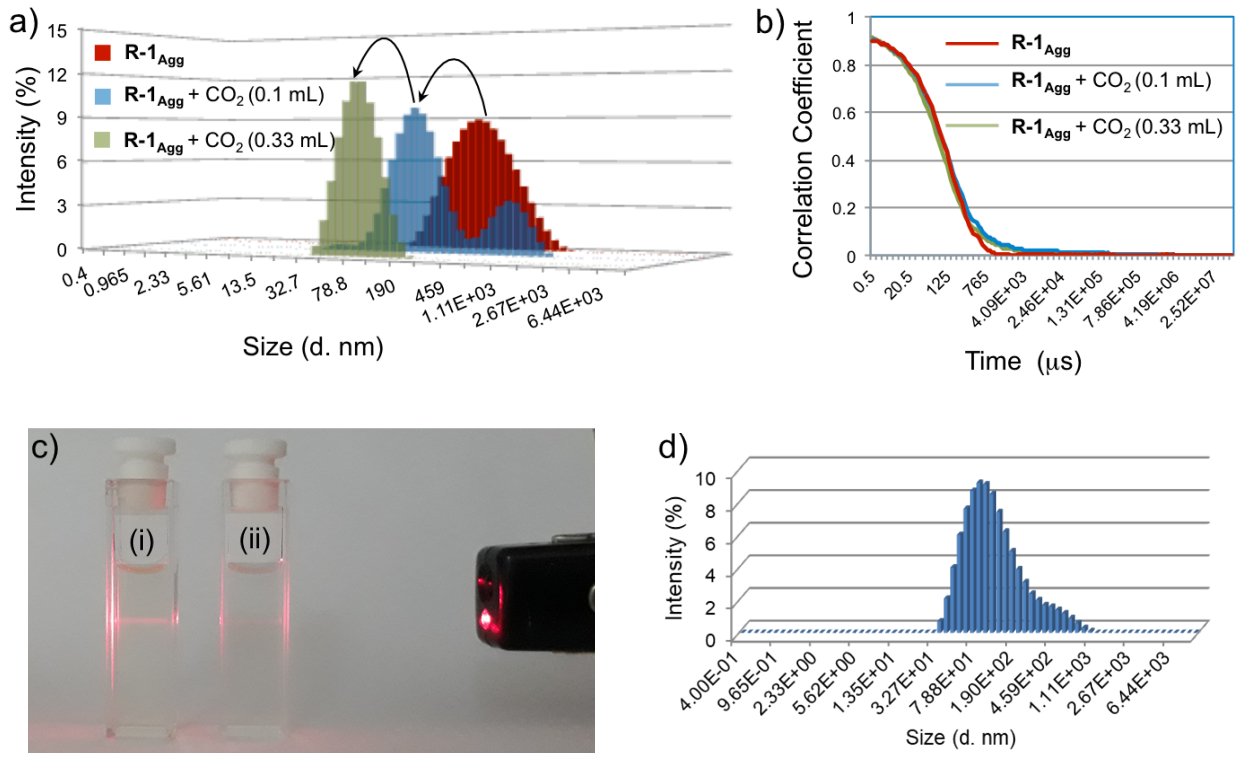


Fig. S11 (a) DLS size distribution of an aqueous solution of **R-1$_{Agg}$** (1×10^{-4} M, water/DMSO, 99/1 v/v) and in presence of different amount of CO_2. (b) Corresponding correlogram. (c) Photographs of Tyndall effect of an aqueous solution of **R-1$_{Agg}$** in the (i) absence and (ii) presence of CO_2 (0.33 mL). (d) DLS size distribution for the **R-1** in water/DMSO (90/10 v/v).

9. Table S2 pH dependent Lifetime values of **R-1$_{Agg}$** (λ_{ex}= 375 nm and λ_{em}= 550 nm).

Sl. No.	pH	τ_1 (a_1) ns	τ_2 (a_2) ns	τ_3 (a_3) ns	τ_{av}
1	8.0	2.33 (29.66)	12.51 (52.94)	0.26 (17.4)	1.21
2	7.0	2.09 (31.75)	12.12 (46.26)	0.25 (21.99)	0.94
3	6.0	1.52 (42.36)	9.06 (25.29)	0.24 (32.35)	0.60
4	5.0	1.44 (48.63)	6.54 (23.86)	0.19 (27.51)	0.57
5	4.0	1.42 (48.37)	6.31 (19.8)	0.21 (31.82)	0.56
6	3.0	1.34 (47.23)	6.04 (20.2)	0.22 (32.56)	0.55

10. pH Dependent Fluorescence Studies of R-1$_{Agg}$

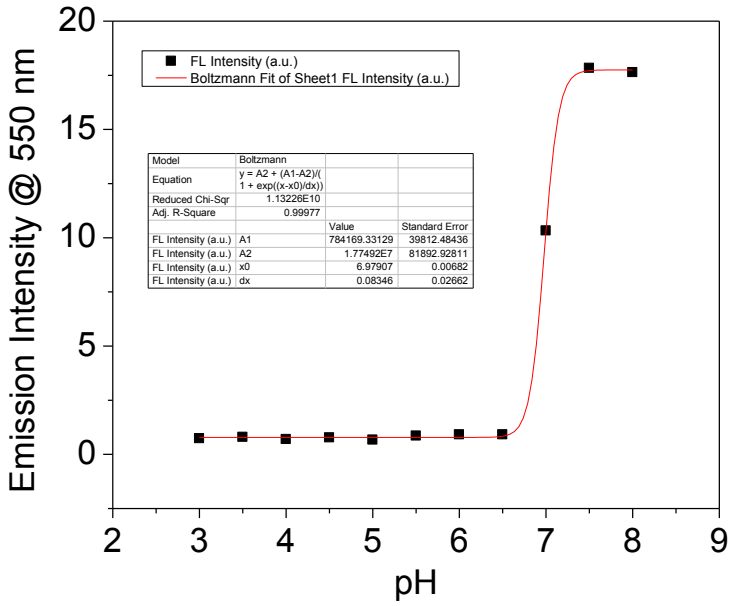


Fig. S12 pH dependent emission intensity changes at 550 nm spectra of **R-1$_{Agg}$** (1 × 10^{-4} M, λ_{ex} = 360 nm) in aqueous medium (water/DMSO, 99/1 v/v). pka value calculated by nonlinear curve-fitting of the emission intensity at the respective pH.

11. Reversible On-Off Fluorescence Changes for R-1$_{Agg}$

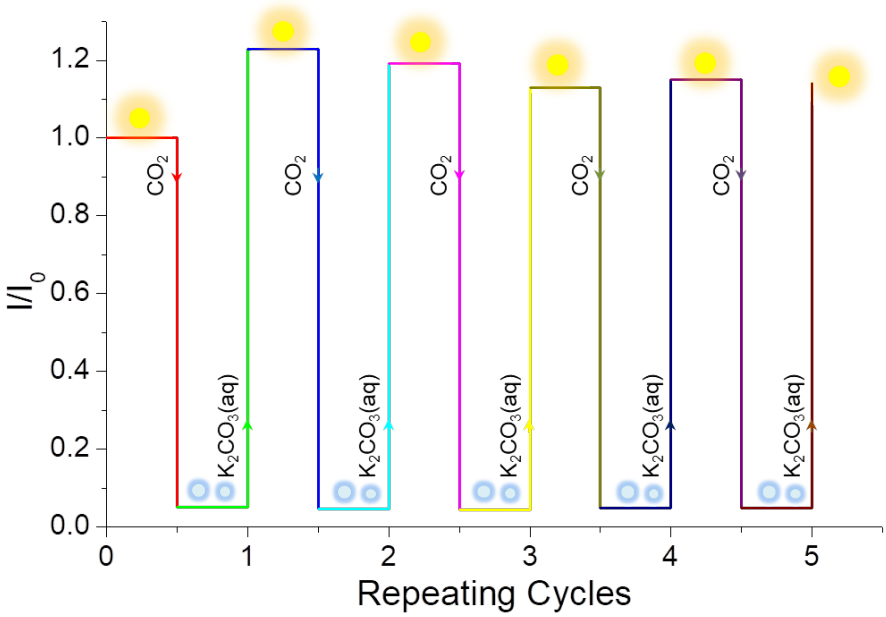


Fig. S13 Fluorescence response of the **R-1$_{Agg}$** (λ_{em} = 550 nm) towards CO_2 and after addition of K_2CO_3 in repeating cycles.

12. MTT Assay

General Procedure: The cytotoxic properties of compound **R-1** were evaluated using A549 (human lung carcinoma) obtained from American Type Culture Collection (ATCC). The cells were seeded at a density of 10000 cells/ well in 100 µL complete medium (DMEM, Himedia) supplemented with 10% FBS (Himedia) and antibiotics (Antibiotics Antimycotic 100 x diluted to 1 x, Himedia) in a 96-multiwell flat bottom microtiter plates and incubated for 24 h at 37 °C, 5 % CO_2 for cells to adhere. Cells were further treated with 100 µL compounds (diluted in plain DMEM medium) of concentrations between 1 and 200 µM followed by further incubation for 3 h. 100 µL MTT solution (0.5 mg/mL in HBSS, Sigma) was added to each well and incubated for 3 h. The solution was subsequently removed from wells and resulting formazan crystals solubilized in 100 µL DMSO. Culture plates were rocked gently for 20 min to solubilize before optical density was measured spectrophotometrically using a microplate reader (BioTek) at 570 nm. The relative cell viability in percent was calculated as: Absorbance of treated/Absorbance of control × 100.

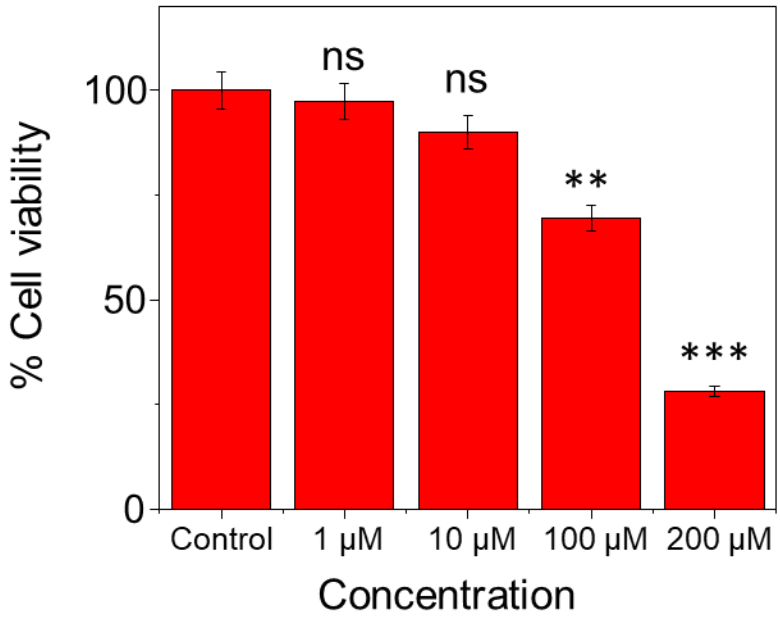


Fig. S14 Cell viability of **R-1** in A549 cells. Data are the mean ± SD of six independent experiments; ** p< 0.01; ***p < 0.001; ns: not significant relative to control.

13. Localization Experiment

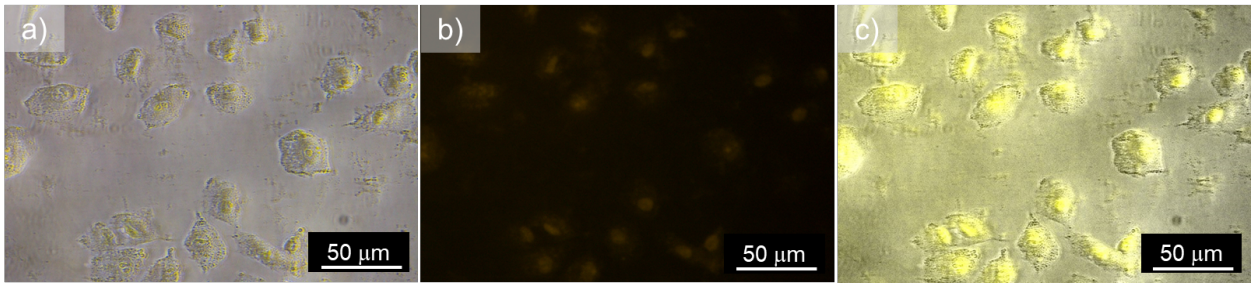


Fig. S15 Initial localization of the **R-1$_{Agg}$** inside the nucleus in A549 lung cancer cells after 1 h (a) bright field (b) fluorescence and (c) merged images.

14. Control Experiment

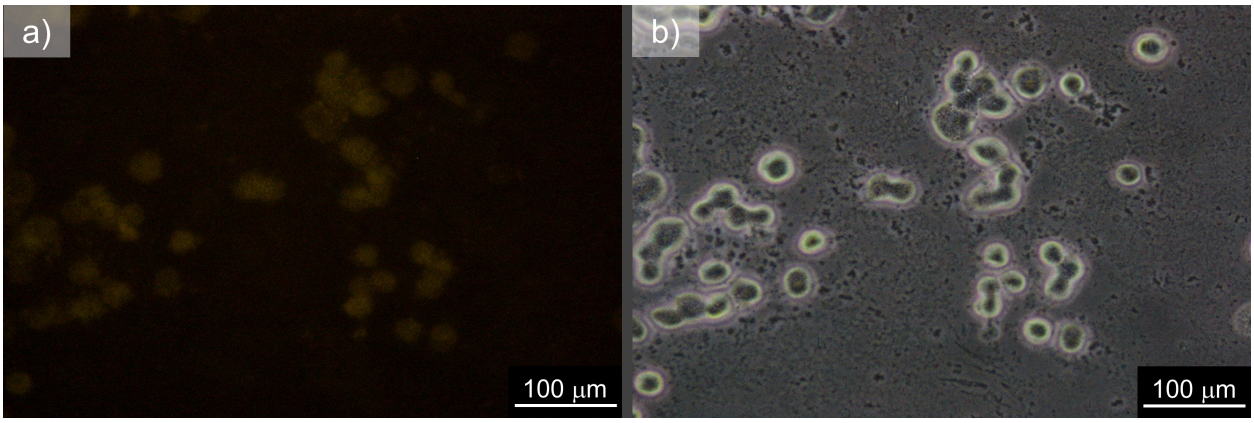


Fig. S16 (a) Fluorescence and (b) bright field images of **R-1$_{Agg}$** in A549 lung cancer cells after keeping the sample for 2 h under ambient condition without treating with 5% CO_2.