Electronic Supplementary Information (ESI)
for

Aggregation-induced emission and solid fluorescence of fluorescein derivatives

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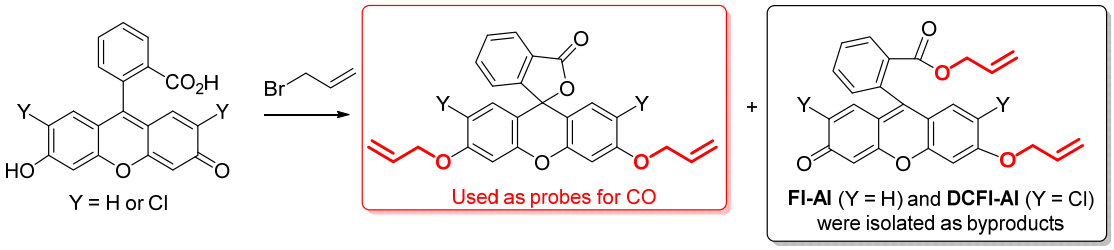
1. Experimental

1.1. Materials and instruments. All reagents and chemicals were purchased from commercial suppliers and used without further purification. **Fl-Al** and **DCFl-Al** have been synthesized in our previous work (Scheme S1. More details can be found in: S. Feng, D. Liu, W. Feng, G. Feng, *Anal. Chem.*, 2017, **89**, 3754−3760.)



Scheme S1. The synthesis of **Fl-Al** and **DCFl-Al**.

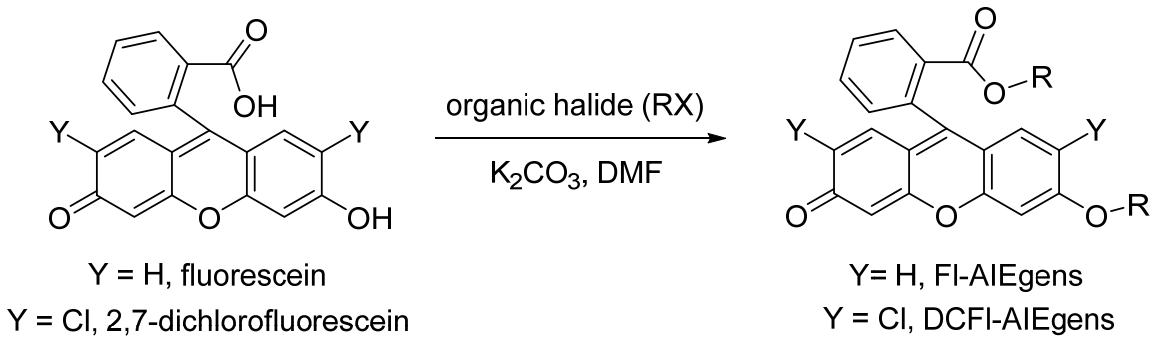
Melting points were determined using an X-4 apparatus and are not corrected. NMR spectra were recorded on a Varian Mercury 600 and a Bruker nuclear magnetic resonance 400 instrument. Mass spectra were collected on a Thermo Scientific DSQ II GC/MS spectrometer (for low-resolution mass spectra) and a Bruker micro TOF-Q instrument (for high-resolution mass spectra, HR-MS). Crystal data were recorded by an X-ray single crystal diffractometer (Bruker). Dynamic light scattering data for the particle sizes were recorded on a Nano laser particle potentiometer (model number: ZS3690, England Marlvin). The absolute fluorescence quantum yield (Φ) data were measured on an Edinburgh-steady/transient fluorescence spectrometer FLS1000 and the excitation wavelength was used at 460 nm. UV-Vis and fluorescence spectra were recorded on an Agilent Cary-100 UV-Vis spectrophotometer and an Agilent Cary Eclipse fluorescence spectrophotometer, respectively.

1.2. Optical Studies. Stock solutions of **Fl-AIEgens** (1 mM) and **DCFl-AIEgens** (1 mM) were prepared in HPLC grade THF and used freshly. In the optical studies, THF solutions of AIEgens (10 μM, 3 mL) with different fractions of water were prepared in quartz cuvettes. Their fluorescent spectra were then recorded at 25 °C. Unless otherwise noted, the excitation wavelength for **Fl-AIEgens** and **DCFl-AIEgens** was set at 450 and 460 nm, respectively. Slit width: d_{ex} = 2.5 nm, d_{em} = 5 nm.

The fluorescence spectra of solid **Fl-AIEgens** and **DCFl-AIEgens** were measured

on an Agilent Cary Eclipse fluorescence spectrophotometer. The **AIEgens** (~15 mg) were pressed to pancakes with a thickness of about 0.1 mm, respectively, and the fluorescence was measured with λ_{ex} = 460 nm, and the slit width was set at: d_{ex} = d_{em} = 5 nm.

1.3. Synthesis of Fl-AIEgens/DCFl-AIEgens



Scheme S2. Synthesis route for **Fl-AIEgens/DCFl-AIEgens.**

General synthetic procedure: To a solution of fluorescein or 2, 7-dichlorofluorescein (3.0 mmol) in DMF (5 mL) was added K_2CO_3 (1.46 g, 10.5 mmol, 3.5 eq) and the mixture was stirred at room temperature for 15 min. Organic halide (such as methyl iodide, 30 mmol, 10 equiv) was then added. After stirring at room temperature for 30 min, the resulting mixture was heated to 70 °C for several hours. After the reaction was completed (monitored by TLC), the mixture was cooled down and diluted with water (50 mL) and then extracted with ethyl acetate (50 mL × 4). The combined organic phase was washed with saturated $NaHCO_3$ solution and dried over sodium sulfate. The solvent was removed under reduced pressure and the crude solid product was purified by silica column chromatography to afford the target compound.

Fl-Me was synthesized from fluorescein and methyl iodide as an orange powder in 85% yield. Mp:198-202 ℃. TLC (silica plate): R_f ~ 0.13 (petroleum ether:ethyl acetate 2:1, v/v) ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.23 (d, J = 7.7 Hz, 1H), 7.72 (d, J = 7.4 Hz, 1H), 7.66 (d, J = 7.3 Hz, 1H), 7.30 (d, J = 7.3 Hz, 1H), 6.95 (d, J = 2.1 Hz, 1H), 6.87 (d, J = 8.9 Hz, 1H), 6.84 (d, J = 9.7 Hz, 1H), 6.73 (dd, J = 8.9, 2.2 Hz, 1H), 6.56 – 6.49 (m, 1H), 6.44 (s, 1H), 3.90 (s, 3H), 3.62 (s, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 185.67, 165.56, 164.04, 158.95, 154.25, 150.37, 134.55, 132.68, 131.09, 130.51, 130.21,

129.79, 129.65, 128.82, 117.47, 114.76, 113.43, 105.67, 100.27, 55.96, 52.38. MS (EI) m/z: 360.1 [M^+]. Crystals of **Fl-Me** were obtained by slowly evaporation of its solution in a mixture of ethyl acetate/chloroform at room temperature.

Fl-Pr was synthesized from fluorescein and bromopropane as orange powder in 82% yield. Mp:135-137 ℃. TLC (silica plate): R_f ~ 0.1 (petroleum ether:ethyl acetate 2:1, v/v). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.24 (d, *J* = 7.7 Hz, 1H), 7.71 (t, *J* = 7.1 Hz, 1H), 7.65 (t, *J* = 7.4 Hz, 1H), 7.29 (d, *J* = 7.3 Hz, 1H), 6.92 (s, 1H), 6.87 (dd, *J* = 12.4, 9.5 Hz, 2H), 6.72 (d, *J* = 8.8 Hz, 1H), 6.52 (d, *J* = 9.6 Hz, 1H), 6.43 (s, 1H), 4.01 (t, *J* = 6.4 Hz, 2H), 3.92 (dtd, *J* = 17.3, 10.8, 6.7 Hz, 2H), 1.84 (dd, *J* = 13.9, 6.9 Hz, 2H), 1.32 (dt, *J* = 13.9, 7.0 Hz, 2H), 1.04 (t, *J* = 7.3 Hz, 3H), 0.68 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 185.63, 165.42, 163.76, 158.74, 154.26, 150.41, 134.17, 132.50, 131.21, 130.70, 130.39, 130.25, 129.77, 129.59, 128.89, 117.63, 114.65, 113.80, 105.64, 100.61, 70.33, 67.12, 22.37, 21.50, 10.38, 10.17. MS (EI) m/z: 416.2 [M^+]. HR-MS (ESI): calcd. for $C_{26}H_{25}O_5^+$ $[M + H]^+$: 417.16965, found: 417.17097.

Fl-Bu was synthesized from fluorescein and n-butyl bromide as an orange powder in 83% yield. Mp: 122-126 ℃. TLC (silica plate): R_f ~ 0.15 (petroleum ether:ethyl acetate 2:1, v/v). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.26 (d, *J* = 7.7 Hz, 1H), 7.73 (t, *J* = 7.3 Hz, 1H), 7.67 (t, *J* = 7.5 Hz, 1H), 7.30 (d, *J* = 7.4 Hz, 1H), 6.94 (d, *J* = 1.8 Hz,1H), 6.90 (d, *J* = 9.0 Hz, 1H), 6.88 (d, *J* = 9.9 Hz, 1H), 6.74 (dd, *J* = 8.8, 1.8 Hz, 1H), 6.54 (d, *J* = 9.6 Hz, 1H), 6.45 (s, 1H), 4.07 (t, *J* = 6.3 Hz, 2H), 4.01 – 3.95 (m, 2H), 1.82 (dt, *J* = 13.7, 6.3 Hz, 2H), 1.51 (dq, *J* = 14.4, 7.1 Hz, 2H), 1.28 (dt, *J* = 14.1, 6.9 Hz, 2H), 1.06 (dq, *J* = 11.6, 6.8 Hz, 2H), 0.99 (t, *J* = 7.3 Hz, 3H), 0.77 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 181.56, 161.58, 159.72, 154.92, 150.29, 146.51, 130.12, 128.49, 127.26, 126.77, 126.41, 126.27, 125.80, 125.60, 124.93, 113.47, 110.68, 109.82, 101.66, 96.62, 64.62, 61.45, 26.66, 26.20, 15.18, 14.99, 9.54. MS (EI) m/z: 444.3 [M^+]. HR-MS (ESI) m/z: calcd. for $C_{28}H_{29}O_5^+$ $[M + H]^+$: 445.20095, found: 445.20150.

Fl-Do was synthesized from fluorescein and bromododecane and was isolated as a light-yellow powder in 83% yield. Mp: 83-85 ℃. TLC (silica plate): R_f ~ 0.15 (petroleum ether:ethyl acetate 2:1, v/v). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.22 (d, *J* = 7.2 Hz, 1H), 7.69 (s, 1H), 7.66 – 7.60 (m, 1H), 7.26 (d, *J* = 9.0 Hz, 1H), 6.89 (s, 1H), 6.87

– 6.80 (m, 2H), 6.70 (d, J = 8.8 Hz, 1H), 6.50 (d, J = 9.4 Hz, 1H), 6.41 (s, 1H), 4.02 (d, J = 3.7 Hz, 2H), 3.93 (s, 2H), 1.79 (s, 2H), 1.43 (s, 3H), 1.32 (s, 3H), 1.23 (s, 30H), 1.10 (s, 5H), 0.98 (s, 3H), 0.84 (d, J = 4.0 Hz, 6H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 181.58, 161.56, 159.64, 154.88, 150.27, 146.27, 130.13, 128.48, 127.26, 126.76, 126.42, 126.23, 125.81, 125.59, 124.91, 113.40, 110.68, 109.78, 101.68, 96.62, 64.92, 61.74, 27.89, 25.62, 25.33, 25.14, 24.89, 21.95, 21.83, 18.66, 10.17, 10.05. MS (EI) m/z: 668.6 [M^+]. HR-MS (ESI): calcd. for $C_{44}H_{61}O_5^+$ [M + H]$^+$: 669.45135, found: 669.45177.

DCFl-Me was synthesized from 2, 7-dichlorofluorescein and methyl iodide as an orange powder in 77% yield. Mp: 279-284 ℃. TLC (silica plate): R_f ~ 0.27 (petroleum ether:ethyl acetate 2:1, v/v). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.33 (d, J = 7.8 Hz, 1H), 7.81 (t, 1H), 7.75 (t, 1H), 7.32 (d, J = 8.2 Hz, 1H), 7.07 (s, 1H), 7.04 (s, 1H), 6.95 (s, 1H), 6.61 (s, 1H), 4.05 (s, 3H), 3.71 (s, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 177.73, 165.17, 159.26, 157.83, 152.53, 149.86, 135.15, 133.73, 133.11, 131.51, 130.38, 130.24, 129.84, 127.92, 127.30, 120.15, 117.55, 114.96, 105.64, 100.14, 56.96, 52.49. MS (EI) m/z: 428.8 [M^+]. HR-MS (ESI): calcd. for $C_{22}H_{15}O_5Cl_2^+$ [M + H]$^+$: 429.02911, found: 429.02926.

DCFl-Pr was synthesized from 2, 7-dichlorofluorescein and bromopropane as an orange-red powder in 92% yield. Mp: 188-191 ℃. TLC (silica plate): R_f ~0.37 (petroleum ether:ethyl acetate 2:1, v/v). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.23 (d, J = 7.5 Hz, 1H), 7.70 (t, J = 7.0 Hz, 1H), 7.65 (t, J = 7.3 Hz, 1H), 7.21 (d, J = 7.2 Hz, 1H), 6.96 (s, 1H), 6.93 (s, 1H), 6.87 (s, 1H), 6.50 (s, 1H), 4.04 (t, J = 6.4 Hz, 2H), 3.95 – 3.83 (m, 2H), 1.85 (dd, J = 13.9, 6.8 Hz, 2H), 1.37 (dd, J = 14.1, 7.0 Hz, 2H), 1.02 (t, J = 7.4 Hz, 3H), 0.70 (t, J = 7.4 Hz, 3H).^{13}C NMR (151 MHz, $CDCl_3$) δ 173.86, 161.02, 154.88, 153.75, 148.53, 146.00, 138.63, 131.10, 129.35, 128.96, 127.52, 126.35, 126.21, 123.96, 123.36, 116.49, 113.46, 110.75, 101.61, 96.62, 67.55, 63.17, 53.96, 47.77, 18.15, 17.65. MS (EI) m/z: 484.1[M^+]. HR-MS (ESI): calcd. for $C_{26}H_{23}O_5Cl_2^+$ [M + H]$^+$: 485.09171, found: 485.09131.

2. The optical data for Fl-AIEgens/DCFl-AIEgens

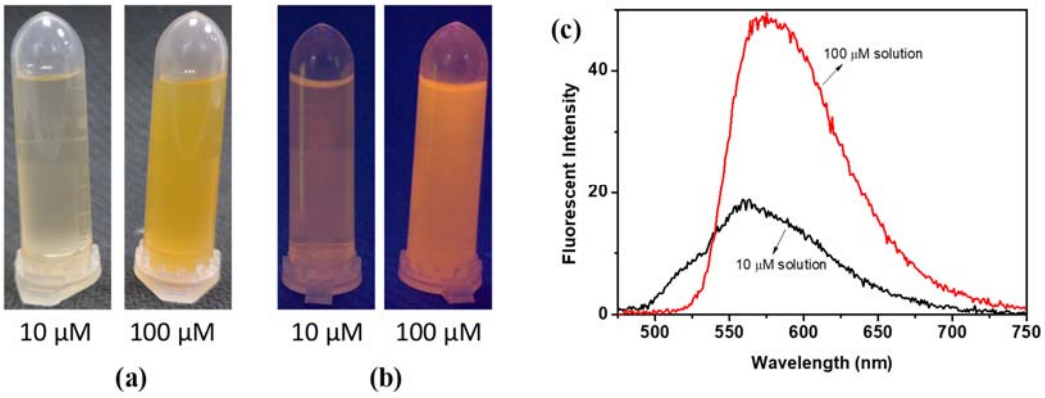


Fig. S1. (a) The solution of **DCFl-Al** (10 and 100 μM, respectively) in THF-water (1:99). (b) The fluorescence of **DCFl-Al** (10 and 100 μM, respectively) in THF-water (1:99) under a light of 365 nm. (c) The fluorescence spectra of **DCFl-Al** (10 and 100 μM, respectively) in THF-water (1:99) with λ_{ex} = 460 nm, slit width d_{ex} = 2.5 nm, d_{em} = 5 nm.

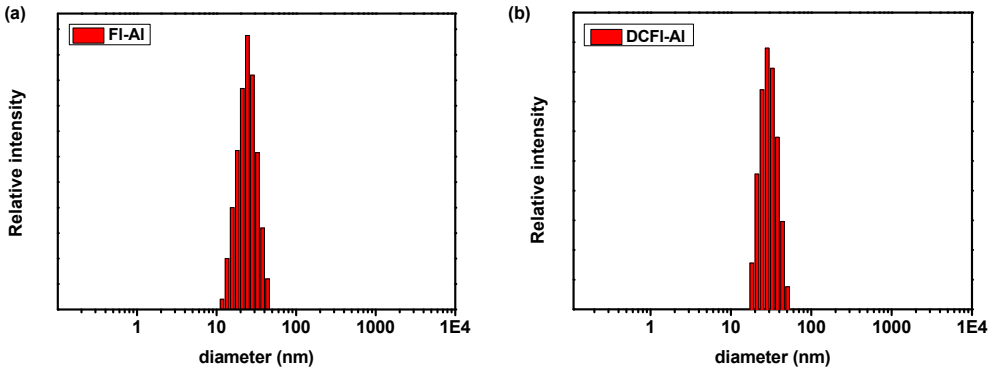


Fig. S2. The dynamic light scattering data of (a) **Fl-Al**, (b) **DCFl-Al** in H_2O containing 20% THF. Concentration of each AIEgen: 10 μM. Hydrated diameter: (a) **Fl-Al**: 26 nm, (b) **DCFl-Al**: 30 nm.

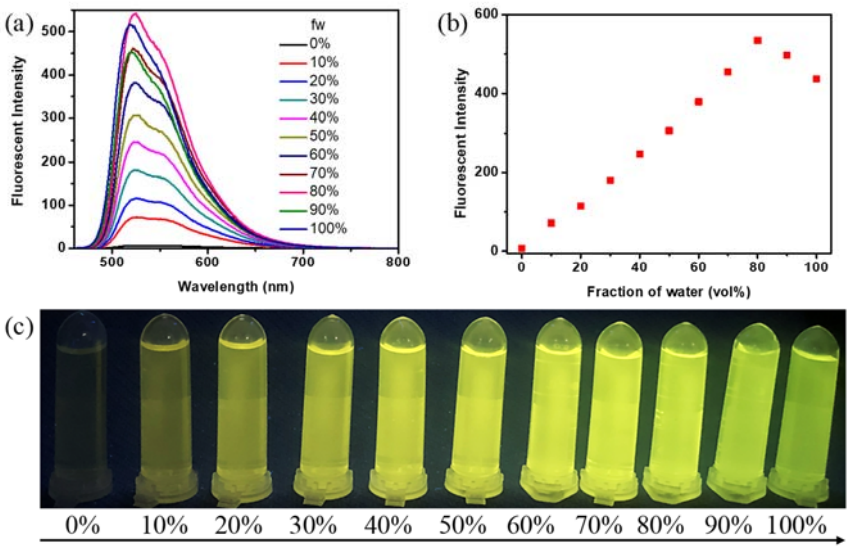


Fig. S3. (a) Fluorescent spectra of **Fl-Me** (10 μM) in THF with different fractions of water (fw). (b) Fluorescent intensity at 525 nm for **Fl-Me** with changes of fw. (c) The emission photographs of **Fl-Me** in THF under 365 nm with different fractions of water.

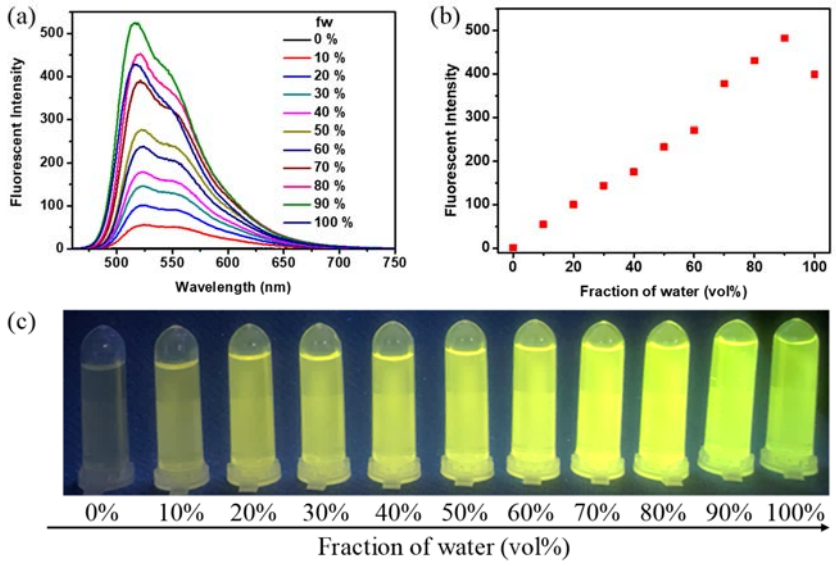


Fig. S4. (a) Fluorescent spectra of **Fl-Pr** (10 μM) in THF with different fractions of water (fw). (b) Fluorescent intensity at 525 nm for **Fl-Pr** with changes of fractions of water. (c) The emission photographs of **Fl-Pr** in THF under 365 nm with different fractions of water.

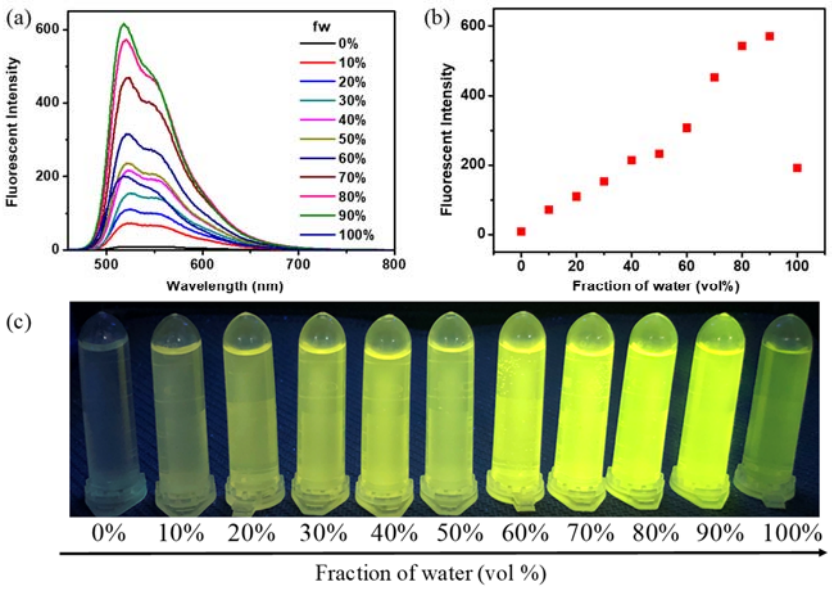


Fig. S5. (a) Fluorescent spectra of **Fl-Bu** (10 μM) in THF with different fractions of water (fw). (b) Fluorescent intensity at 525 nm for **Fl-Bu** with changes of fractions of water. (c) The emission photographs of **Fl-Bu** in THF under 365 nm with different fractions of water.

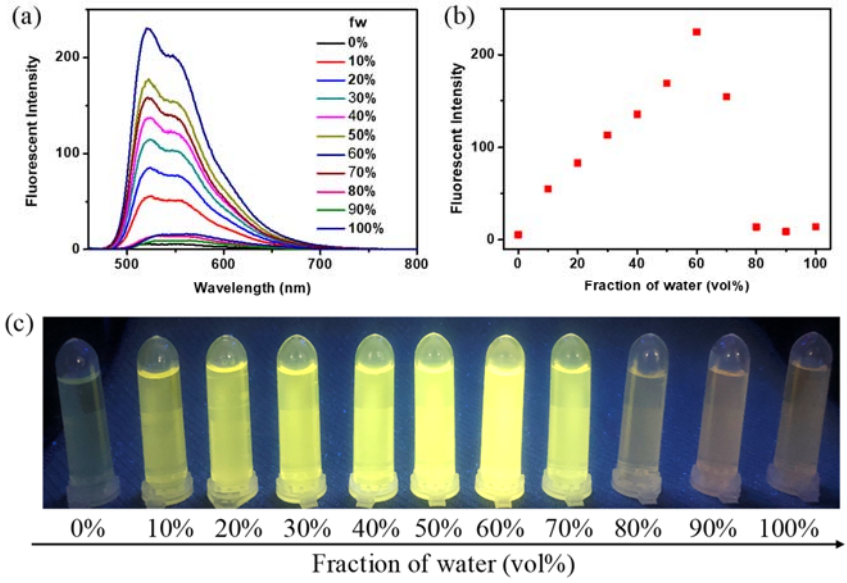


Fig. S6. (a) Fluorescent spectra of **Fl-Do** (10 μM) in THF with different fractions of water (fw). (b) Fluorescent intensity at 525 nm for **Fl-Do** with changes of fractions of water. (c) The emission photographs of **Fl-Do** in THF under 365 nm with different fractions of water.

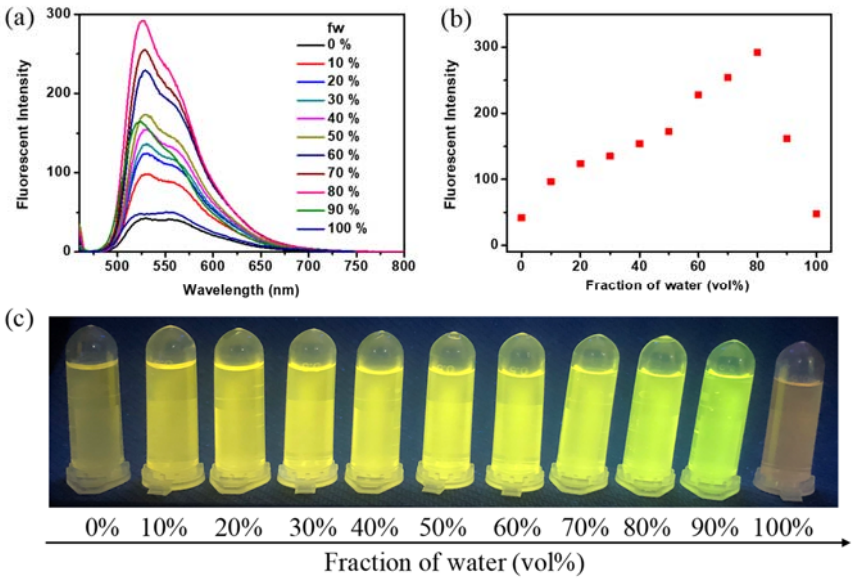


Fig. S7. (a) Fluorescent spectra of **DCFl-Me** (10 μM) in THF with different fractions of water (fw). (b) Fluorescent intensity at 525 nm for **DCFl-Me** with changes of fractions of water. (c) The emission photographs of **DCFl-Me** in THF under 365 nm with different fractions of water.

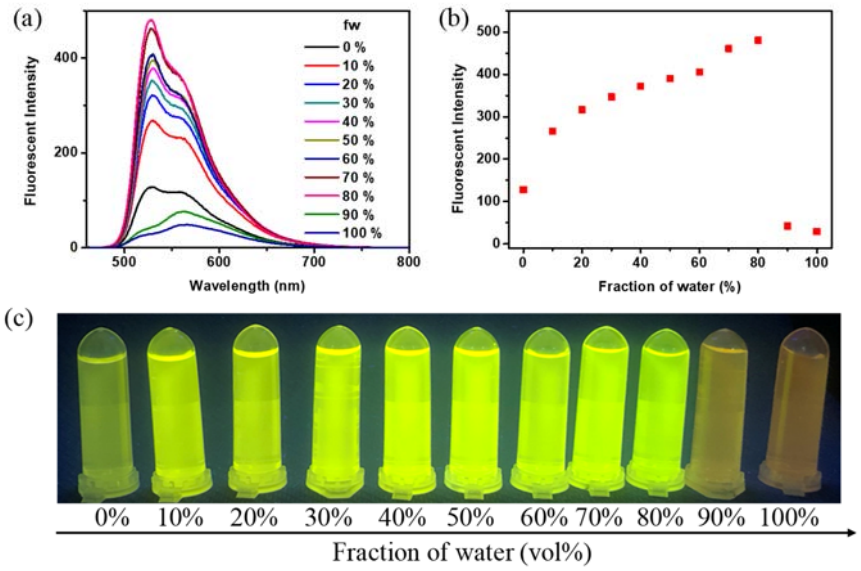


Fig. S8. (a) Fluorescent spectra of **DCFl-Pr** (10 μM) in THF with different fractions of water (f_w). (b) Fluorescent intensity at 525 nm for **DCFl-Pr** with changes of fractions of water. (c) The emission photographs of **DCFl-Pr** in THF under 365 nm with different fractions of water.

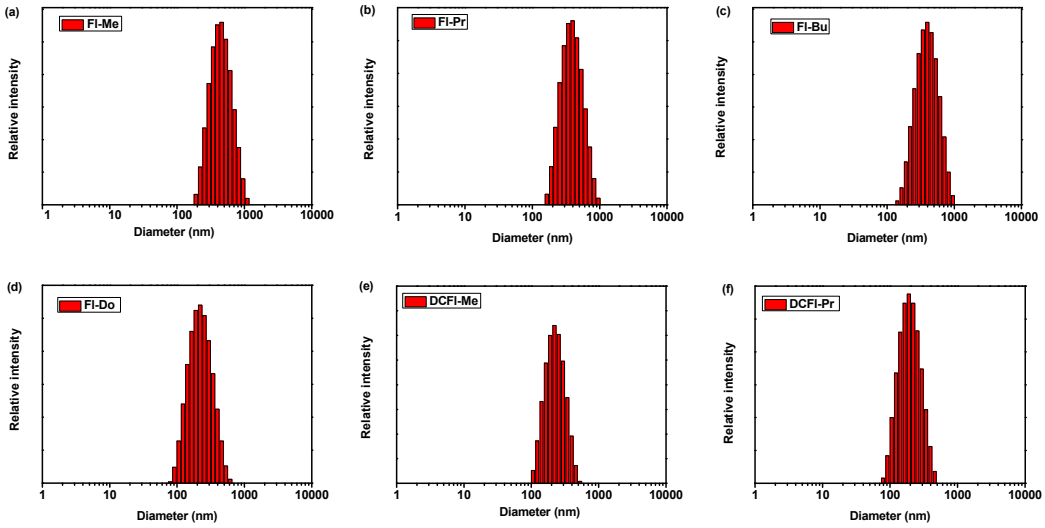


Fig. S9. The dynamic light scattering data of (a) **Fl-Me**, (b) **Fl-Pr**, (c) **Fl-Bu**, (d) **Fl-Do**, (e) **DCFl-Me**, and (f) **DCFl-Pr** in H_2O containing 1% THF. Concentration of each AIEgen: 10 μM. Hydrated diameter: (a) **Fl-Me**: 458 nm, (b) **Fl-Pr**: 396 nm, (c) **Fl-Bu**: 396 nm, (d) **Fl-Do**: 220 nm; (e) **DCFl-Me**: 396 nm, and (f) **DCFl-Pr**: 190 nm.

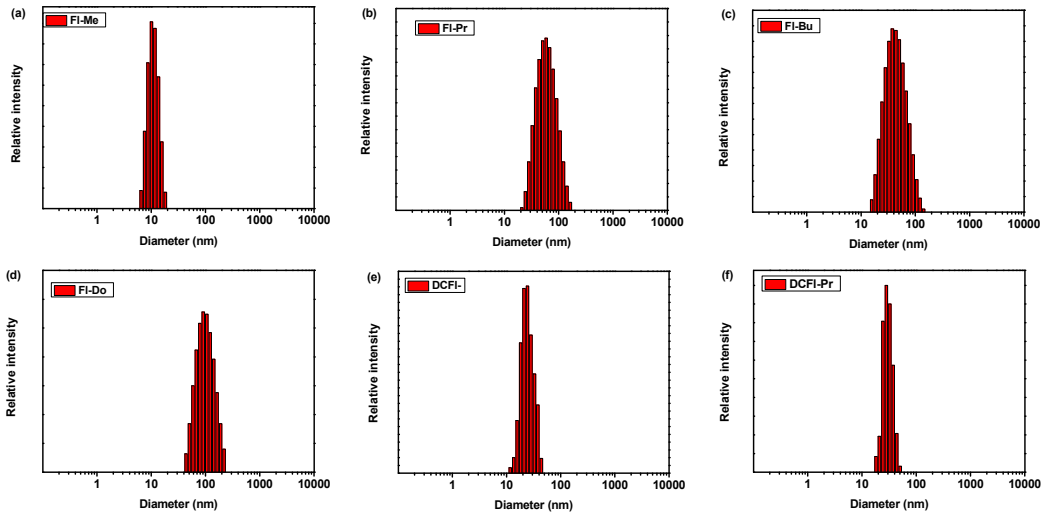


Fig. 10. The dynamic light scattering data of (a) **Fl-Me**, (b) **Fl-Pr**, (c) **Fl-Bu**, (d) **Fl-Do**, (e) **DCFl-Me**, and (f) **DCFl-Pr** in H_2O containing 20% THF. Concentration of each AIEgen: 10 μM. Hydrated diameter: (a) **Fl-Me**:11 nm, (b) **Fl-Pr**: 64 nm, (c) **Fl-Bu**: 47 nm, (d) **Fl-Do**: 106 nm; (e) **DCFl-Me**: 24 nm, and (f) **DCFl-Pr**: 30 nm.

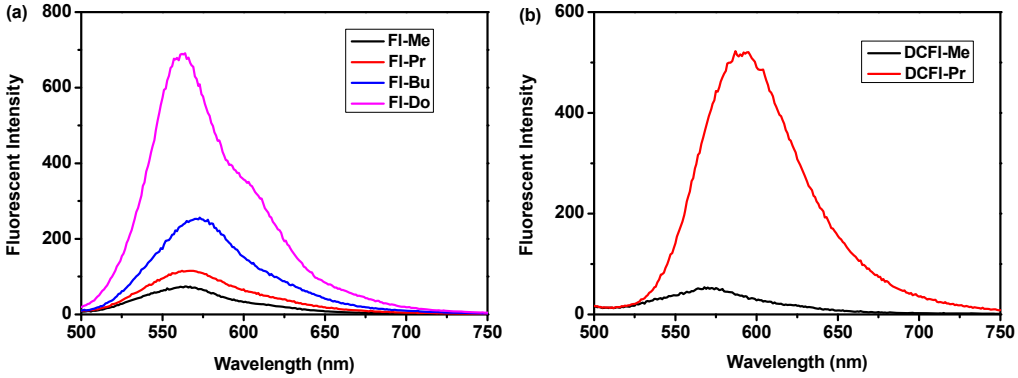


Fig. S11. Fluorescent spectra of (a) solid **Fl-AIEgens** and (b) solid **DCFl-AIEgens**.

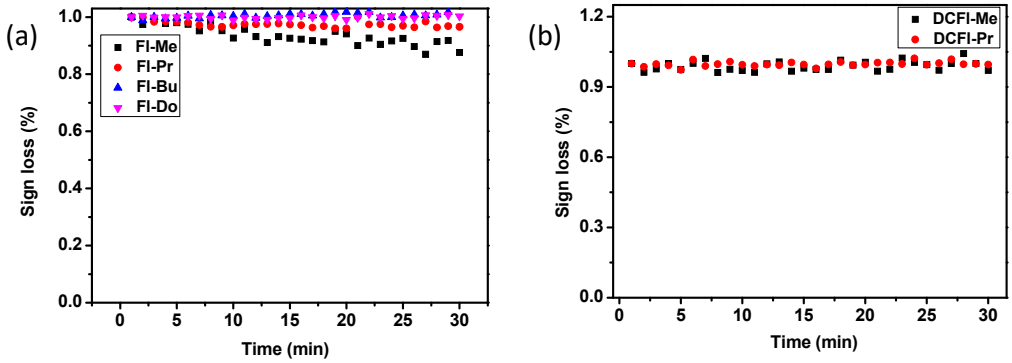


Fig. S12. The photostability of solid fluorescence of (a) **Fl-AIEgens** and (b) **DCFl-AIEgens**. Each data point shows the fluorescence of the solid **AIEgens** after a certain period of irradiation at 460 nm.

3. Crystallographic data

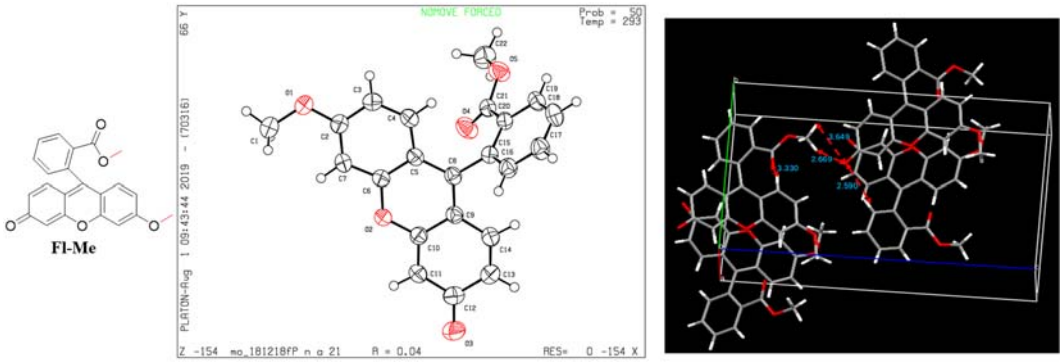


Fig. S13. Chemical and X-ray crystal structure of **Fl-Me** (CCDC 1970837 contains the supplementary crystallographic data, which can be obtained free of charge from the Cambridge Crystallographic Data Centre).

Crystal data for **Fl-Me**:

Bond precision:	C-C = 0.0050 A		Wavelength=0.71073
Cell:	a=8.5365(19)	b=10.915(2)	c=18.183(4)
	alpha=90	beta=90	gamma=90
Temperature:	293 K		

	Calculated	Reported
Volume	1694.2(6)	1694.2(6)
Space group	P n a 21	P n a 21
Hall group	P 2c -2n	P 2c -2n
Moiety formula	C22 H16 O5	C22 H16 O5
Sum formula	C22 H16 O5	C22 H16 O5
Mr	360.35	360.35
Dx,g cm-3	1.413	1.413
Z	4	4
Mu (mm-1)	0.100	0.100
F000	752.0	752.0
F000'	752.42	
h,k,lmax	10,12,21	10,13,21
Nref	2986[1545]	2957
Tmin,Tmax	0.986,0.990	0.632,0.745
Tmin'	0.985	

Correction method= # Reported T Limits: Tmin=0.632 Tmax=0.745
AbsCorr = MULTI-SCAN

Data completeness= 1.91/0.99 Theta(max)= 25.016

R(reflections)= 0.0354(2424) wR2(reflections)= 0.0942(2957)

S = 1.014 Npar= 246

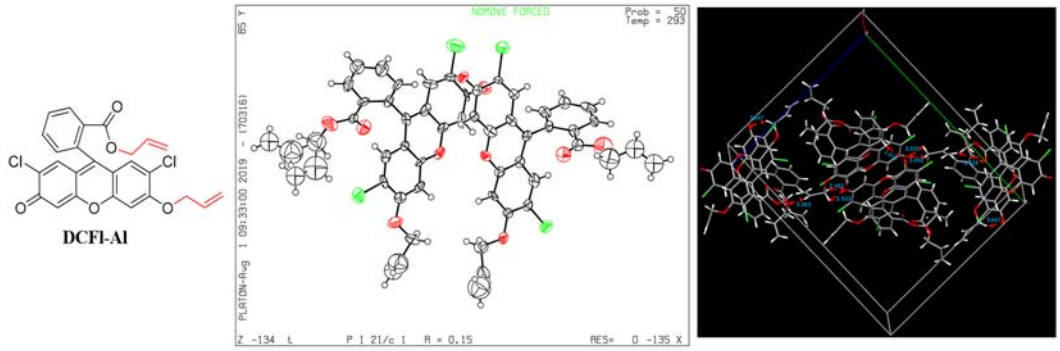


Fig. S14. Chemical and X-ray crystal structure of **DCFl-Al** (CCDC 1970840 contains the supplementary crystallographic data, which can be obtained free of charge from the Cambridge Crystallographic Data Centre).

Crystal data for **DCFl-Al**:

Bond precision:	C-C = 0.0266 A		Wavelength=0.71073
Cell:	a=10.613(7)	b=23.820(14)	c=19.202(14)
	alpha=90	beta=105.610(13)	gamma=90
Temperature:	293 K		

	Calculated	Reported
Volume	4675(5)	4675(5)
Space group	P 21/c	P 1 21/c 1
Hall group	-P 2ybc	-P 2ybc
Moiety formula	C26 H18 Cl2 O5	C26 H18 Cl2 O5
Sum formula	C26 H18 Cl2 O5	C26 H18 Cl2 O5
Mr	481.30	481.30
Dx,g cm-3	1.368	1.368
Z	8	8
Mu (mm-1)	0.313	0.313
F000	1984.0	1984.0
F000'	1987.30	
h k,lmax	9,21,17	9,21,17
Nref	3754	3629
Tmin,Tmax	0.984,0.988	0.477,0.745
Tmin'	0.984	

Correction method= # Reported T Limits: Tmin=0.477 Tmax=0.745
AbsCorr = MULTI-SCAN

Data completeness= 0.967 Theta(max)= 18.986

R(reflections)= 0.1472(2556) wR2(reflections)= 0.3592(3629)

S = 1.075 Npar= 585

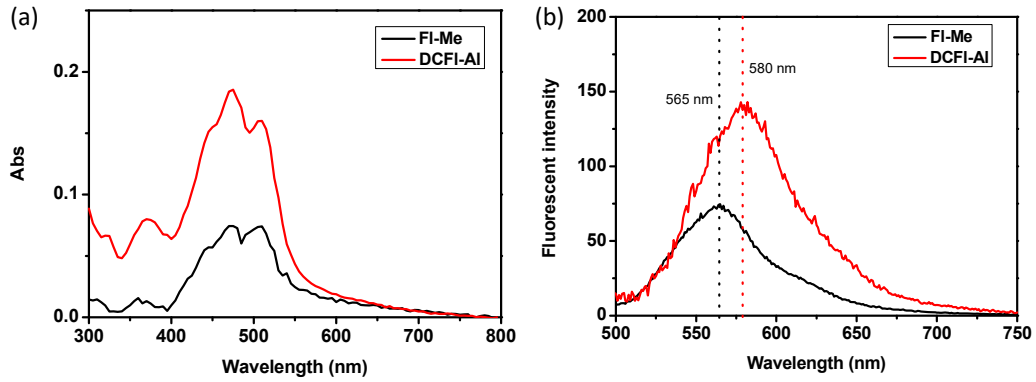
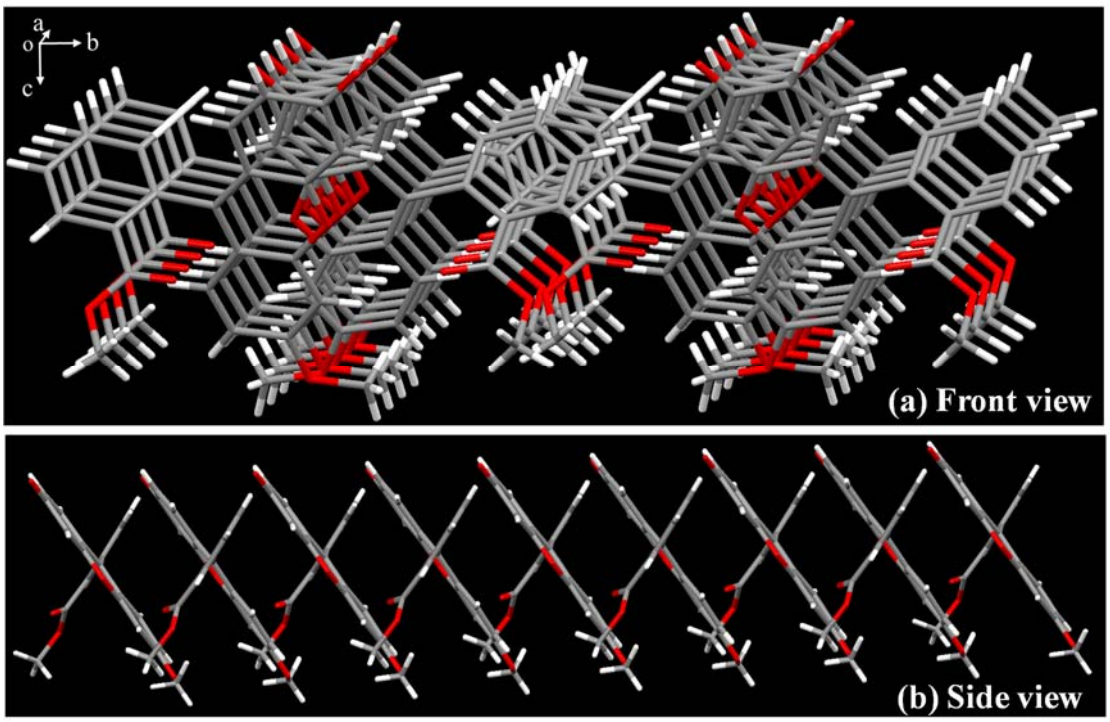


Fig. S15. (a) The absorption spectra of solid **Fl-Me** and **DCFl-Al**. (b) The fluorescence spectra of solid **Fl-Me** and **DCFl-Al** (λ_{ex} = 460 nm, slit width: d_{ex} = 5 nm, d_{em} = 5 nm).



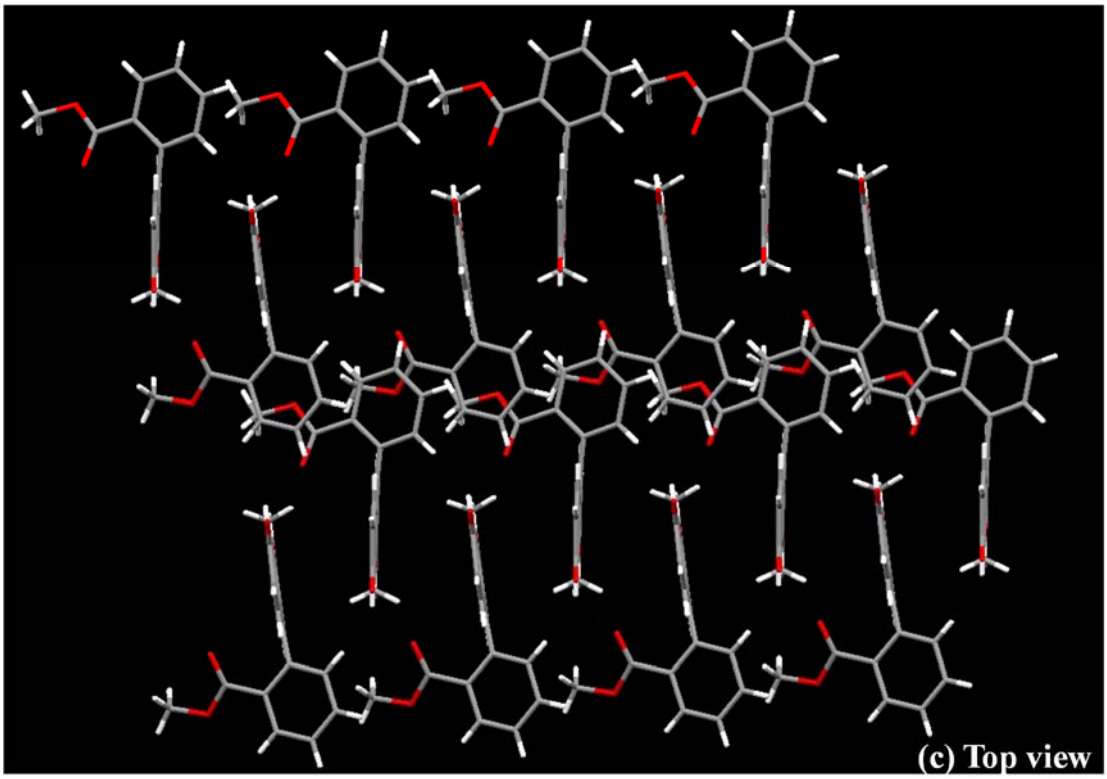


Fig. S16. Crystal packing and intermolecular stacking of **Fl-Me**.

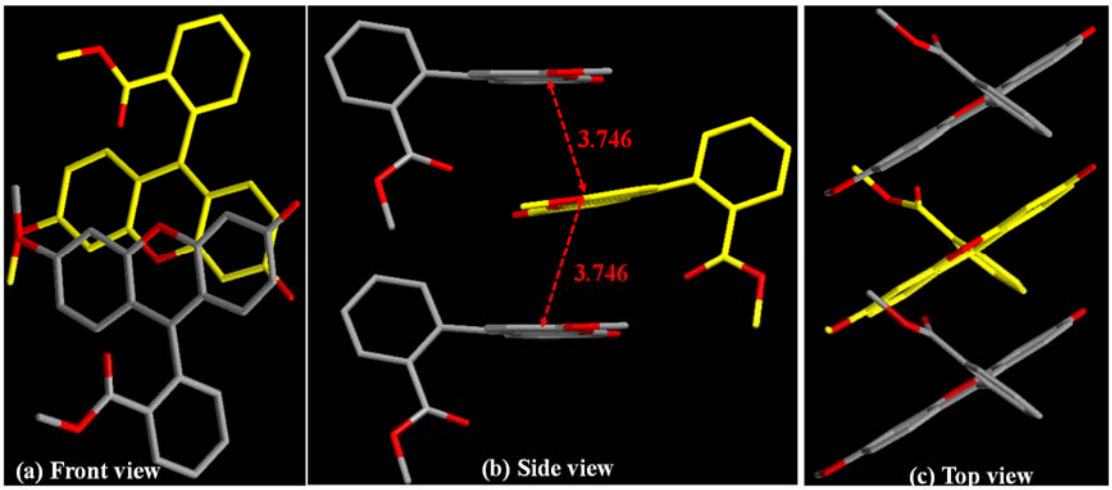


Fig. S17. The stacking of **Fl-Me** molecules in crystals. One can see from the front view (a), the adjacent two xanthenone planes (yellow and gray color, respectively) cannot form an effective face-to-face overlap due to the staggered arrangement, although the distance of the benzene rings is about 3.746 Å (b).

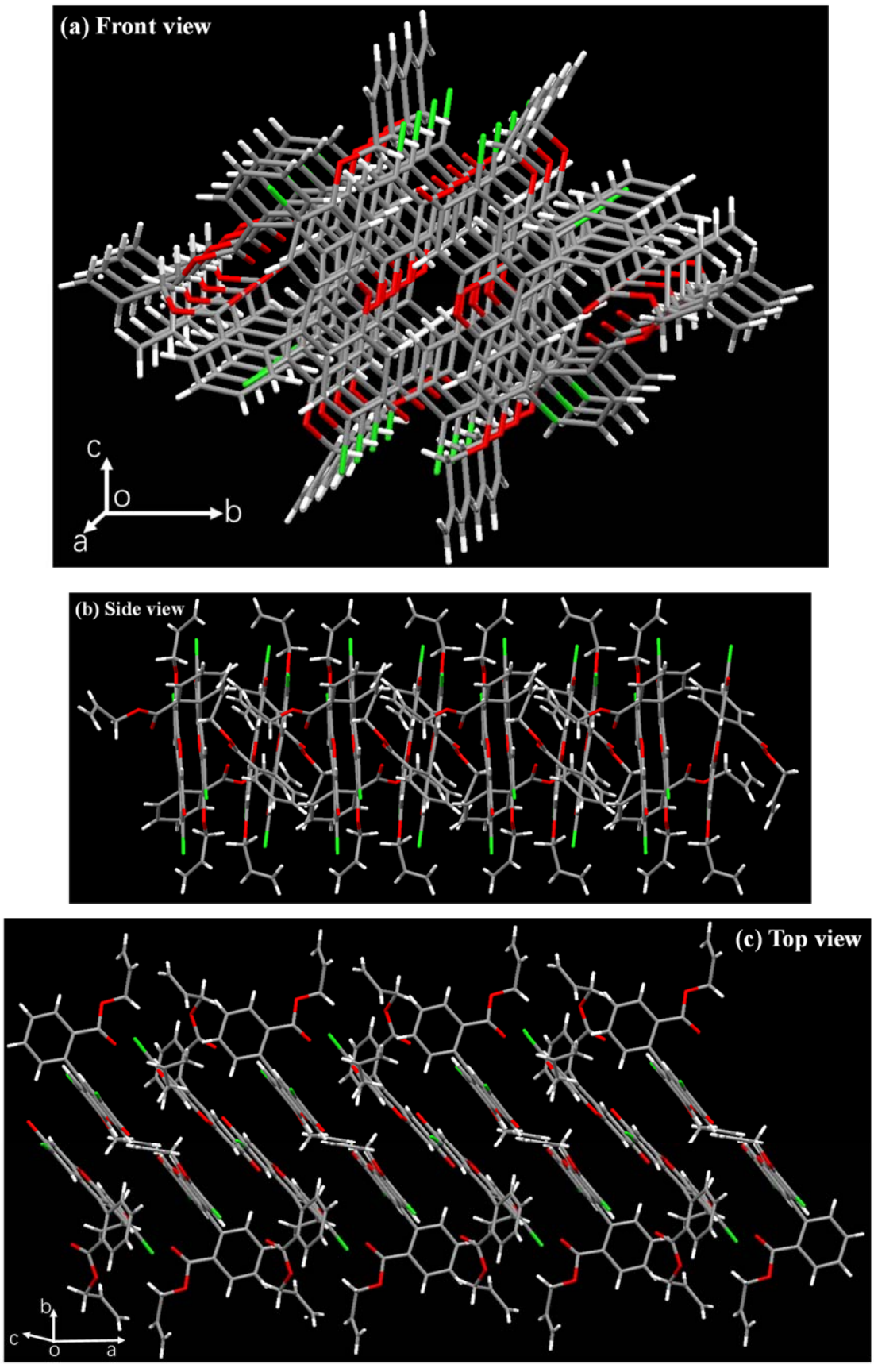


Fig. S18. Crystal packing and intermolecular stacking of **DCFl-Al**.

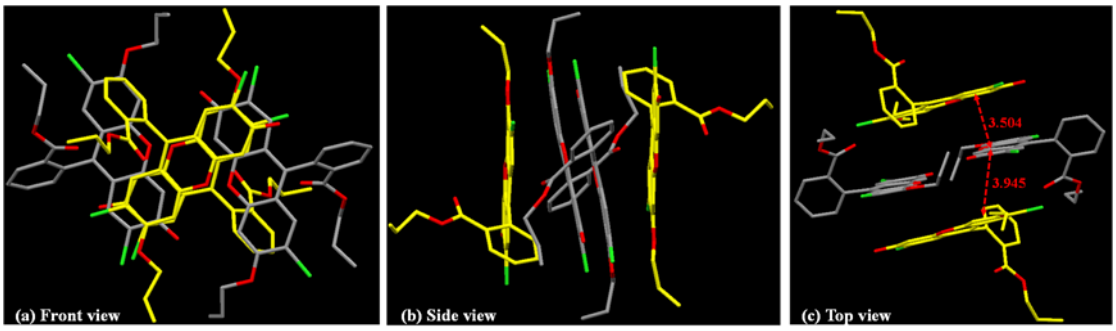
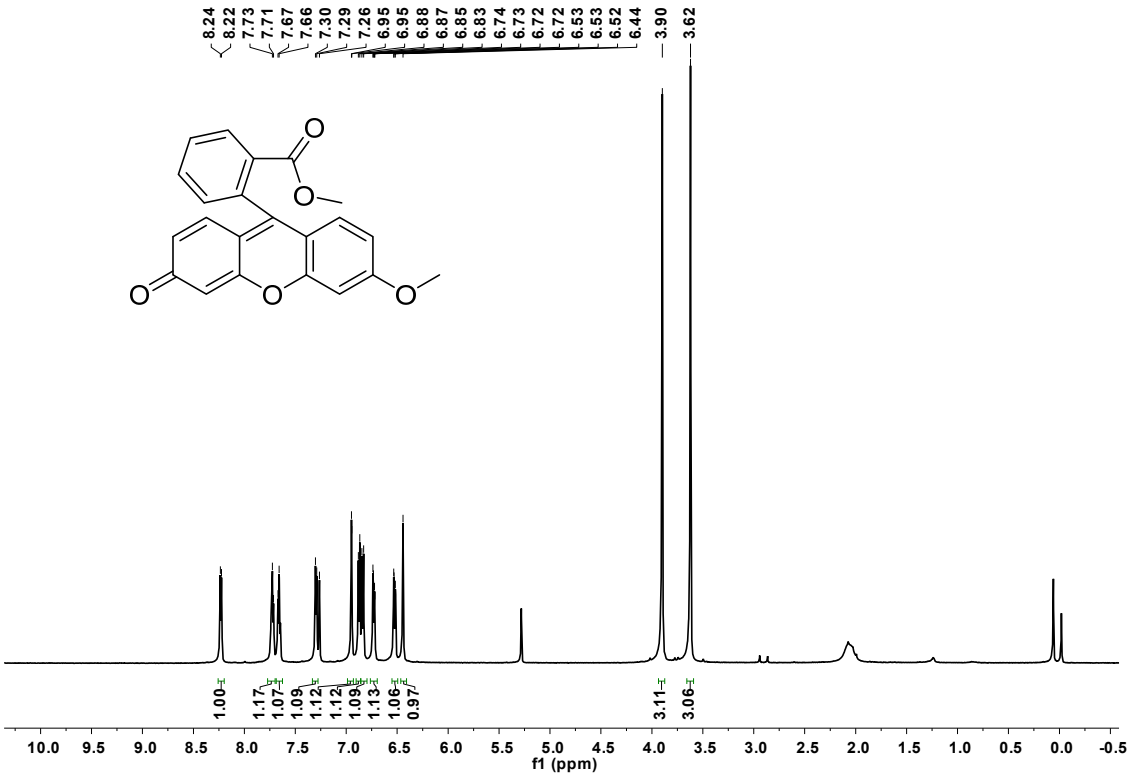
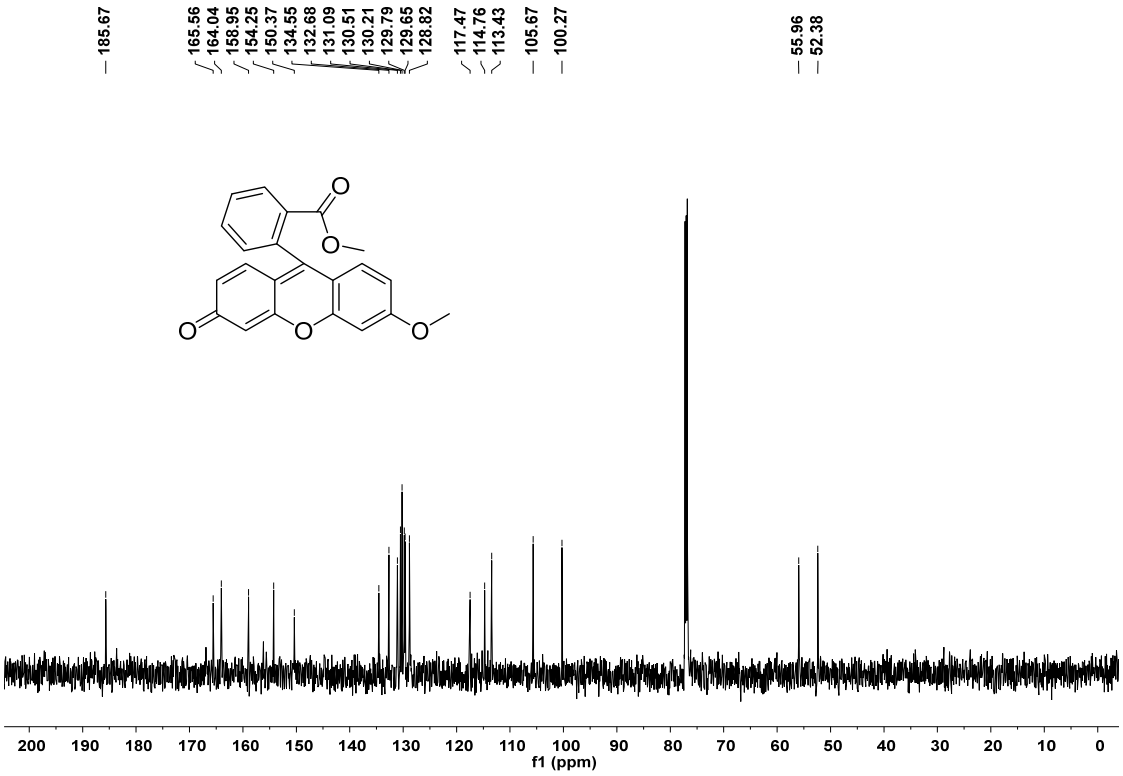


Fig. S19. The stacking of **DCFl-Al** molecules in crystals. One can see from the front view (a), the adjacent two xanthenone planes (yellow and gray color, respectively) cannot form an effective face-to-face overlap due to the staggered arrangement.

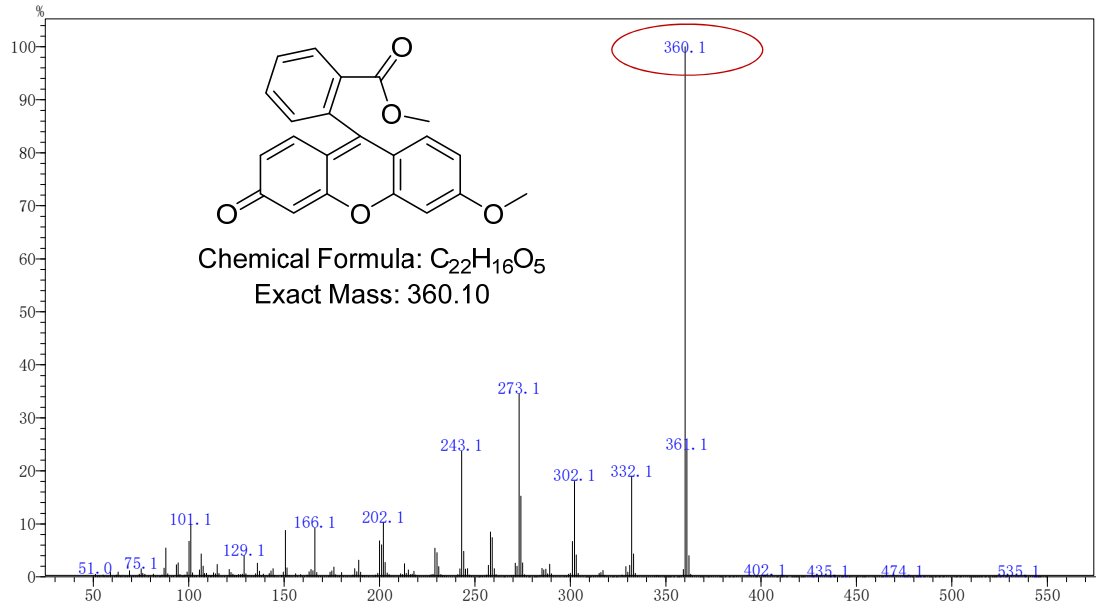
4. Structure characterizations.



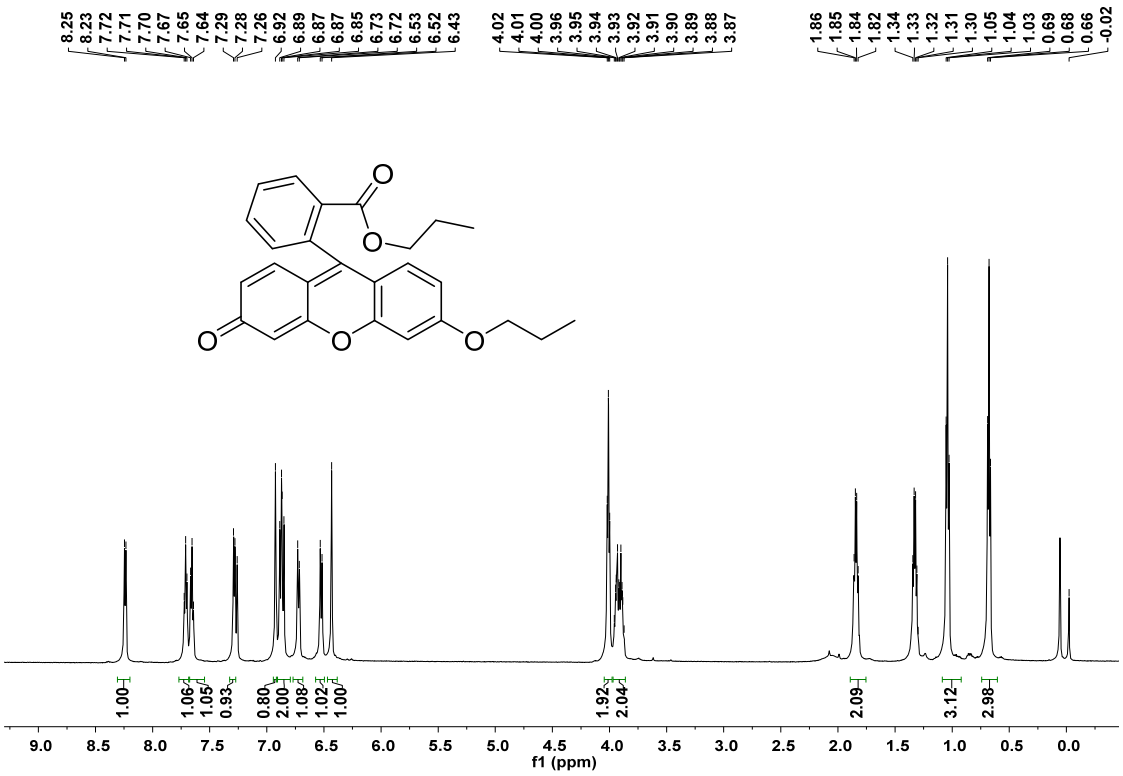
^{1}H NMR spectrum of **Fl-Me** in $CDCl_3$.



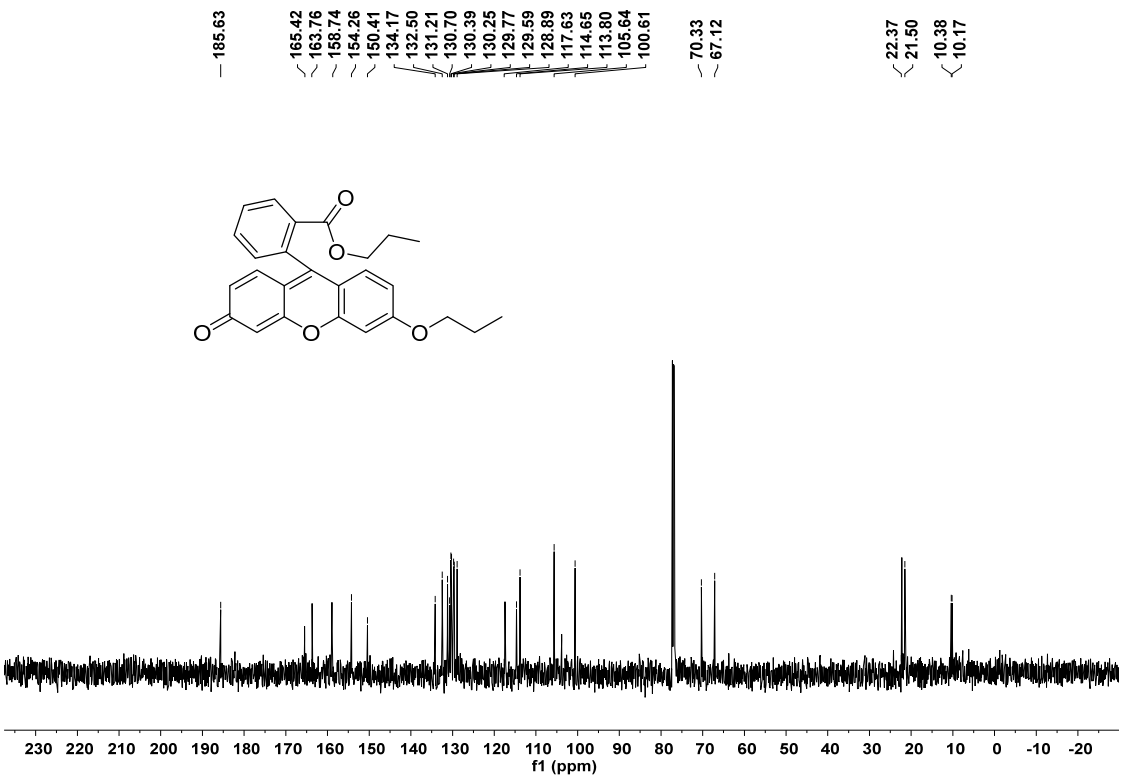
^{13}C NMR spectrum of **Fl-Me** in $CDCl_3$.



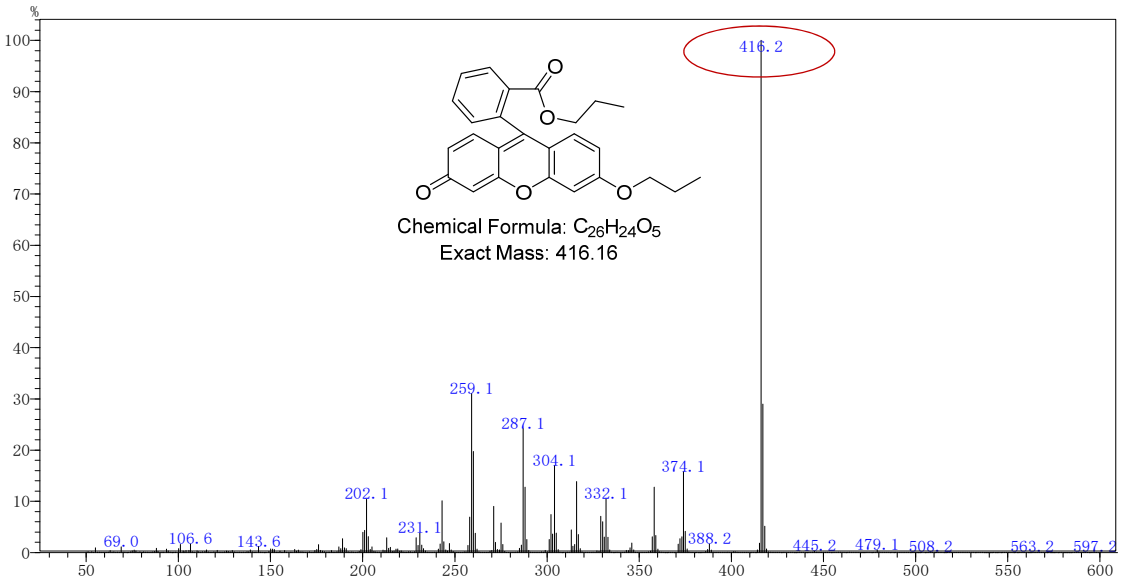
EI-MS spectrum of **Fl-Me**



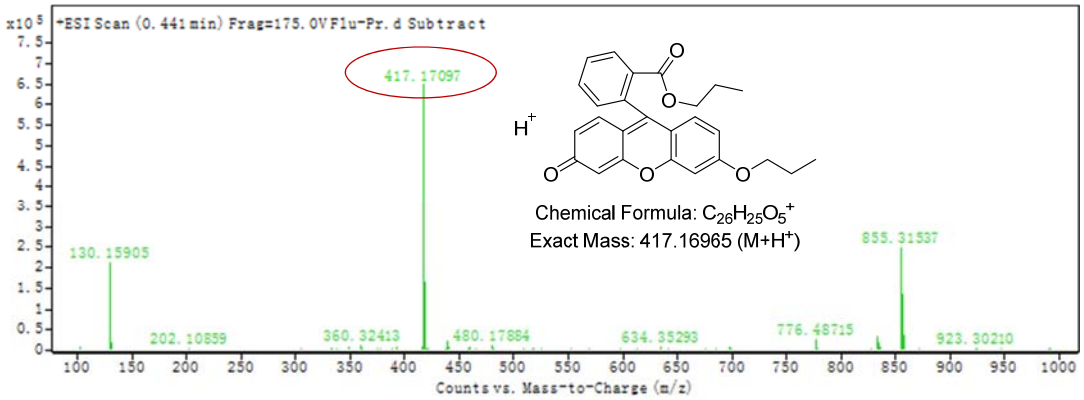
^{1}H NMR spectrum of **Fl-Pr** in $CDCl_3$.



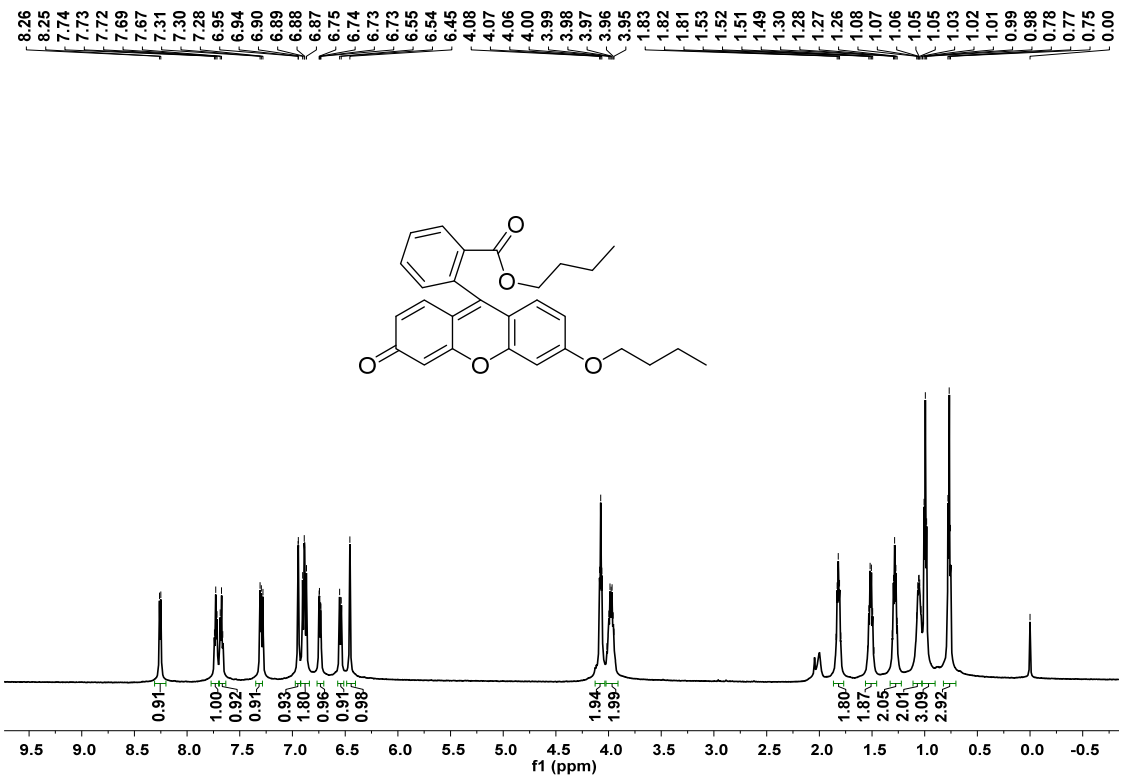
^{13}C NMR spectrum of **Fl-Pr** in $CDCl_3$.



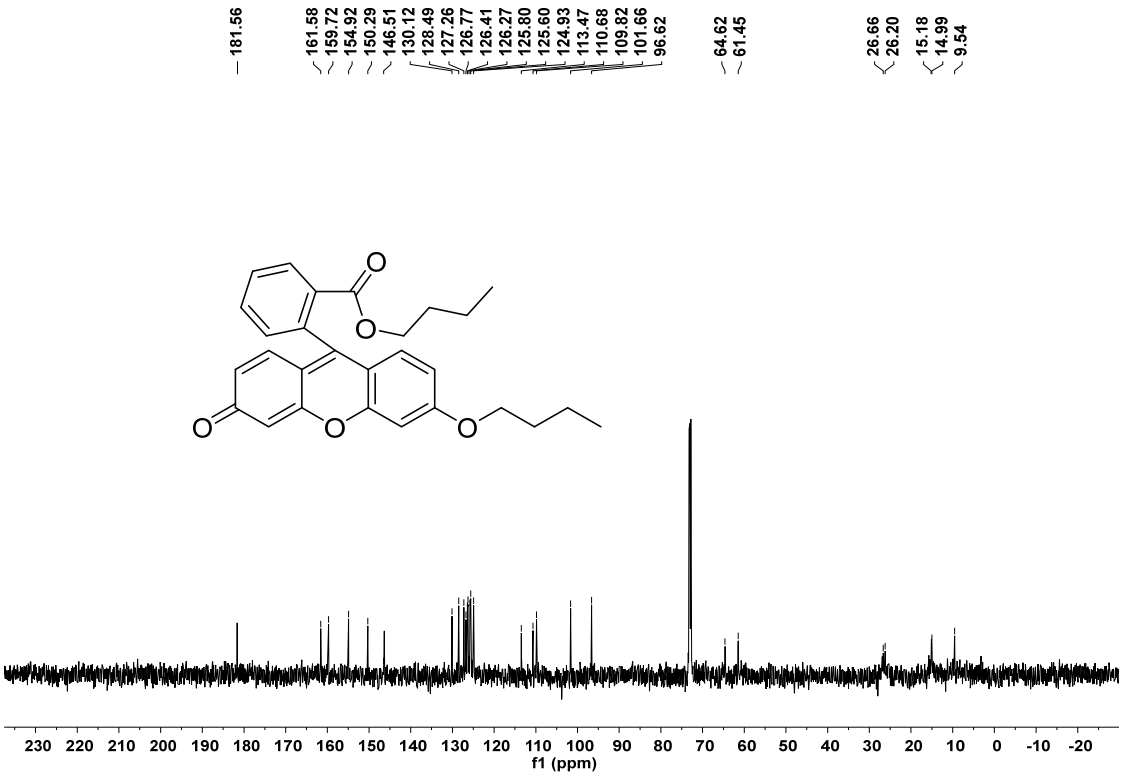
EI-MS spectrum of **Fl-Pr**.



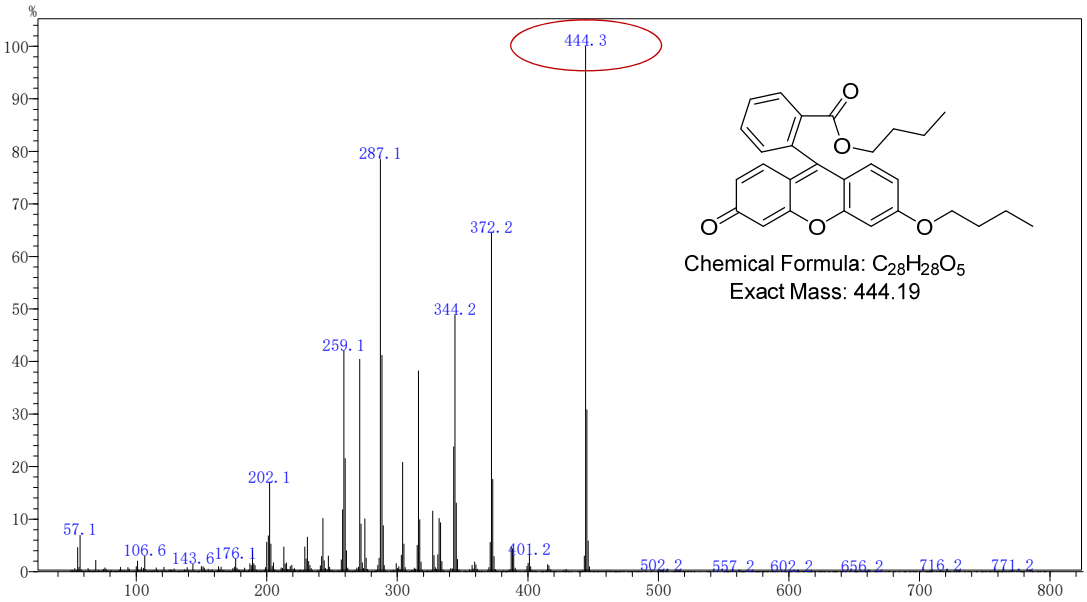
HR-MS spectrum of **Fl-Pr**.



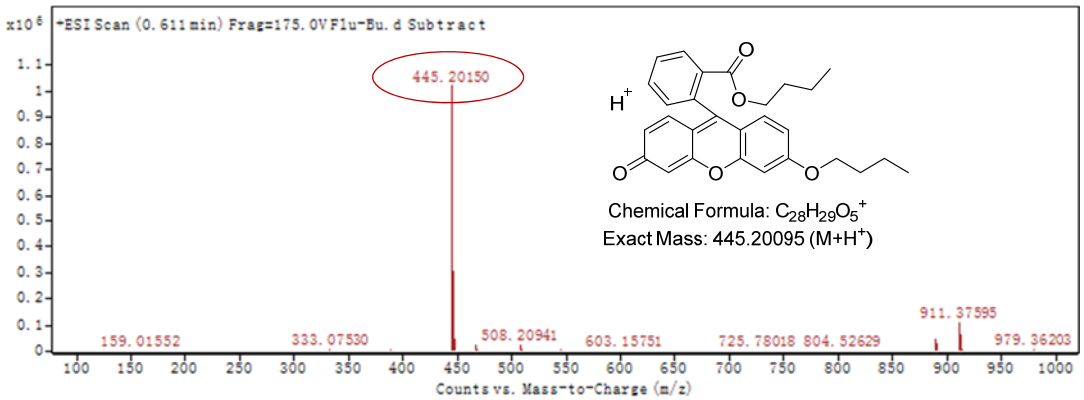
^{1}H NMR spectrum of **Fl-Bu** in $CDCl_3$.



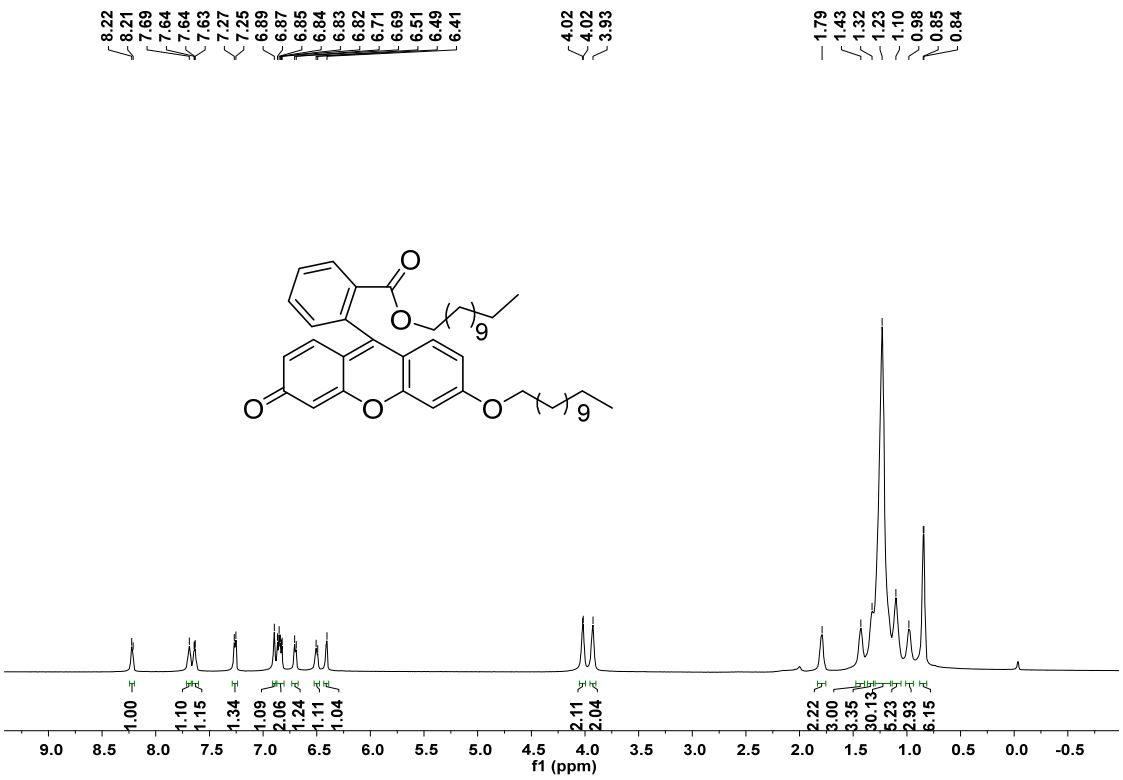
^{13}C NMR spectrum of **Fl-Bu** in $CDCl_3$.



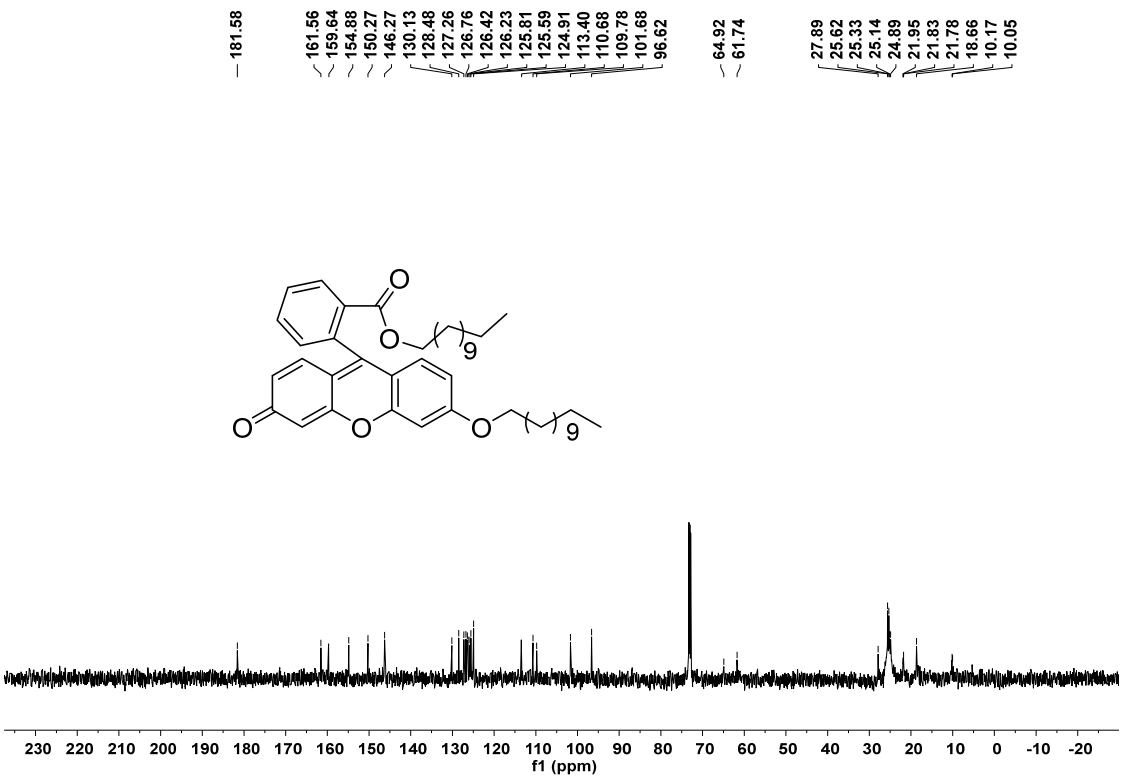
EI-MS spectrum of **Fl-Bu**.



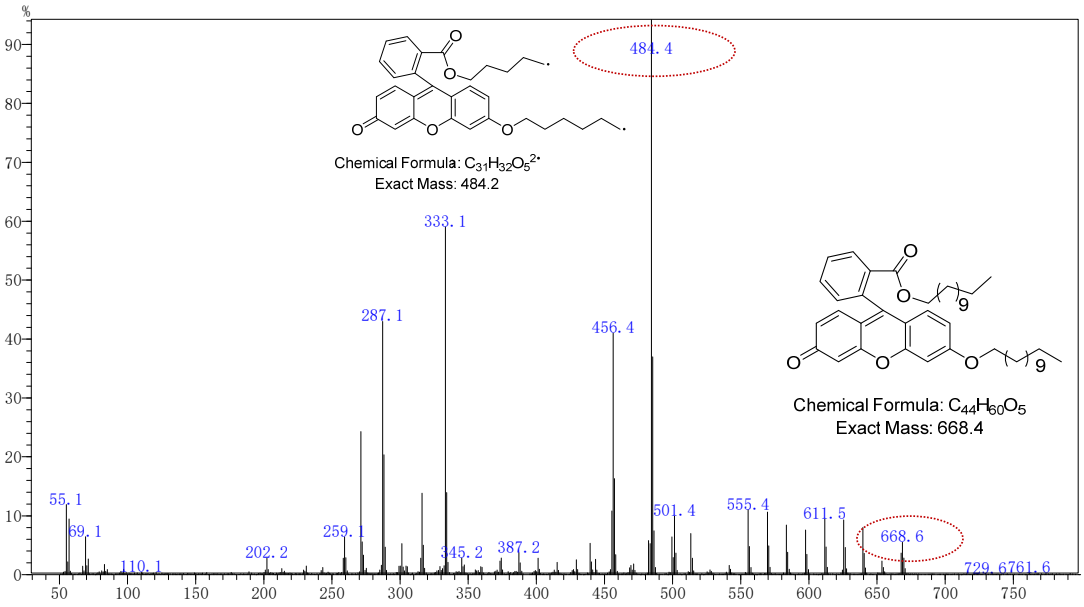
HR-MS spectrum of **Fl-Bu**.



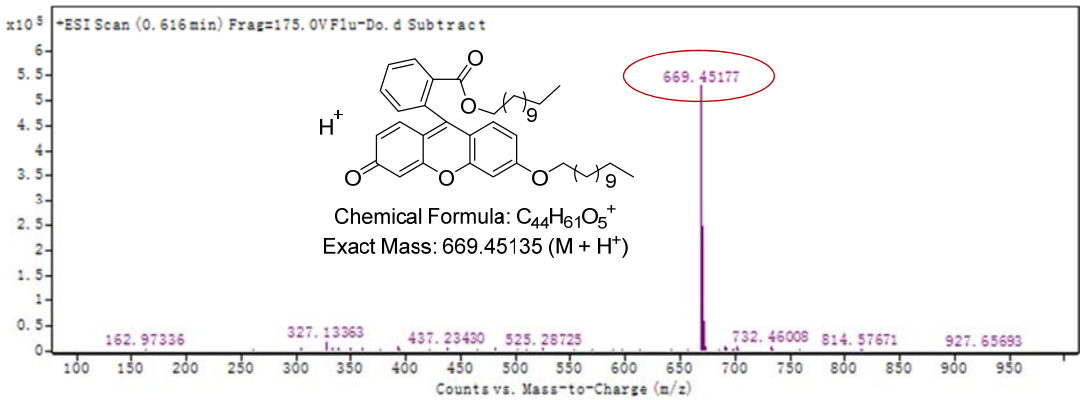
^{1}H NMR spectrum of **Fl-Do** in $CDCl_3$.



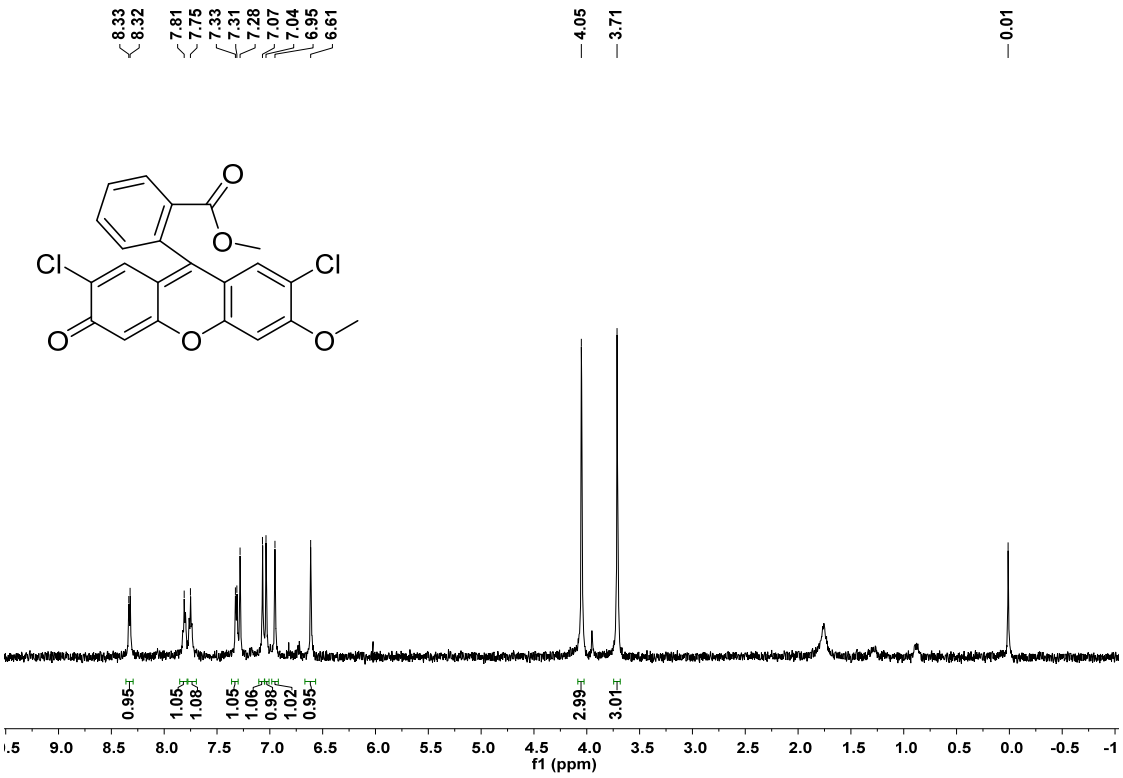
^{13}C NMR spectrum of **Fl-Do** in $CDCl_3$.



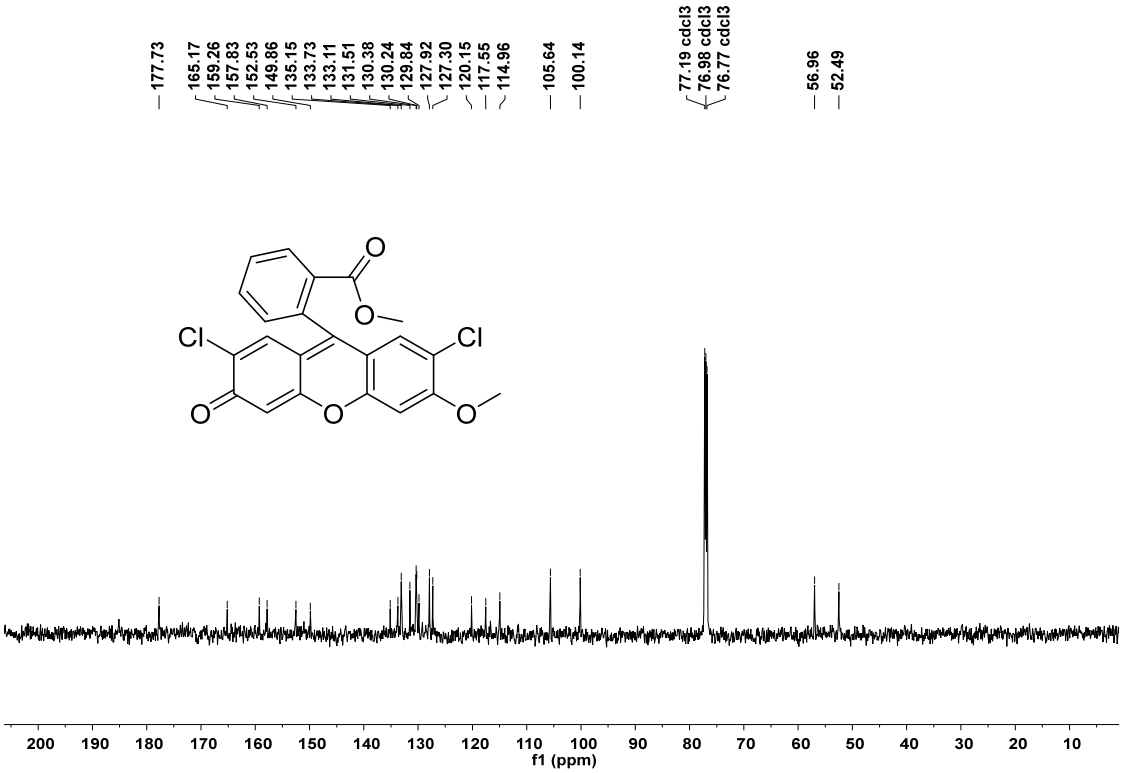
MS spectrum of **Fl-Do**.



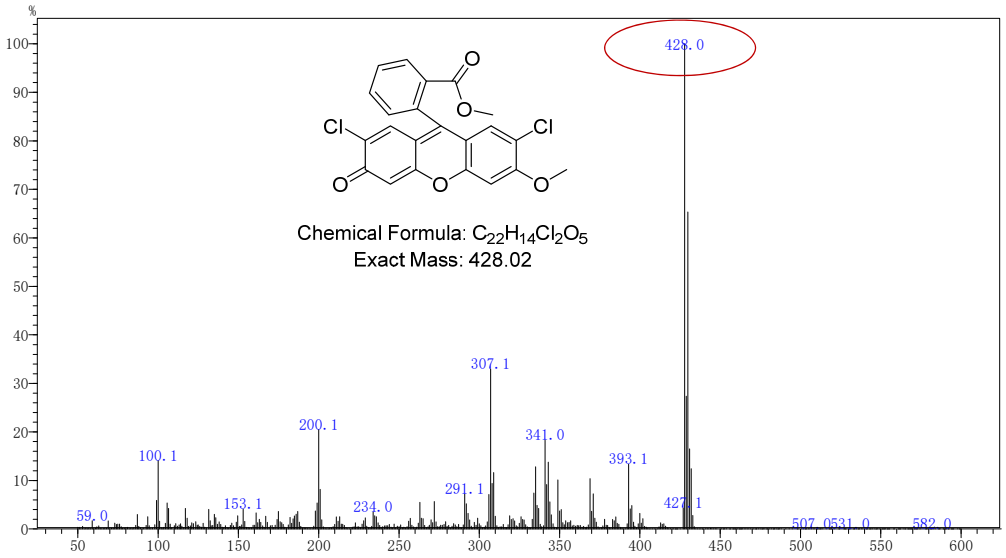
HR-MS spectrum of **Fl-Do**.



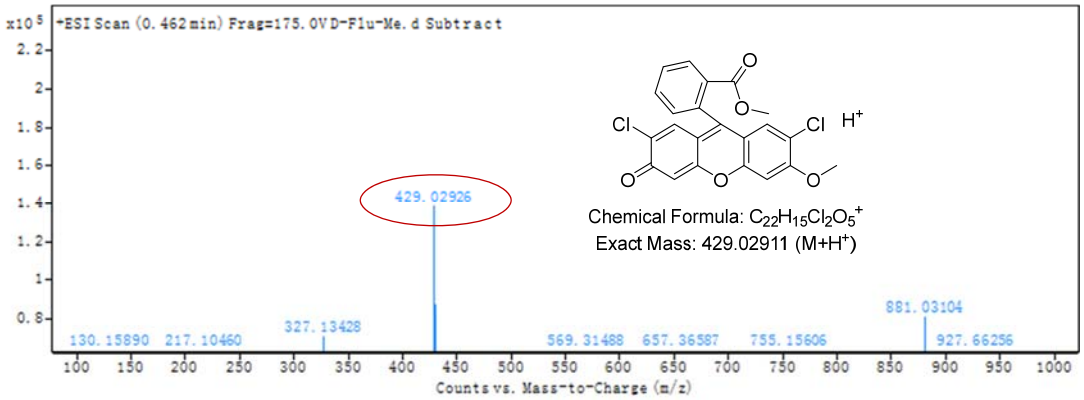
^{1}H NMR spectrum of **DCFl-Me** in $CDCl_3$.



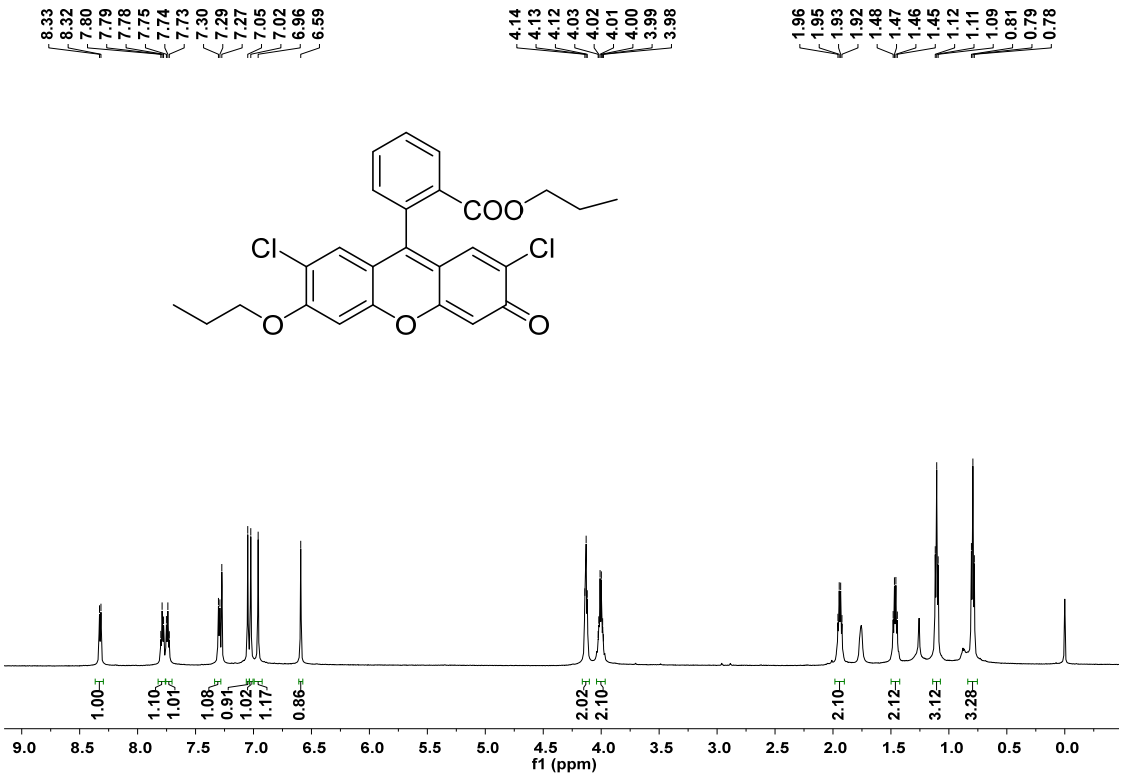
^{13}C NMR spectrum of **DCFl-Me** in $CDCl_3$.



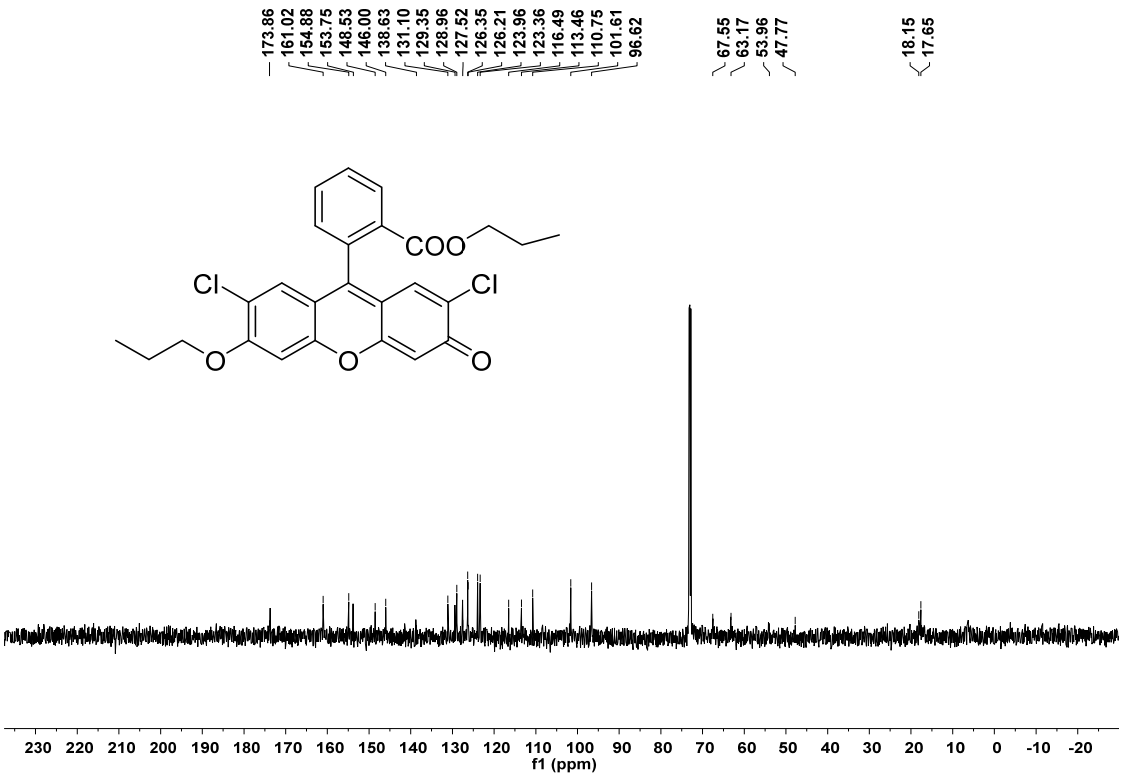
EI-MS spectrum of **DCFl-Me**



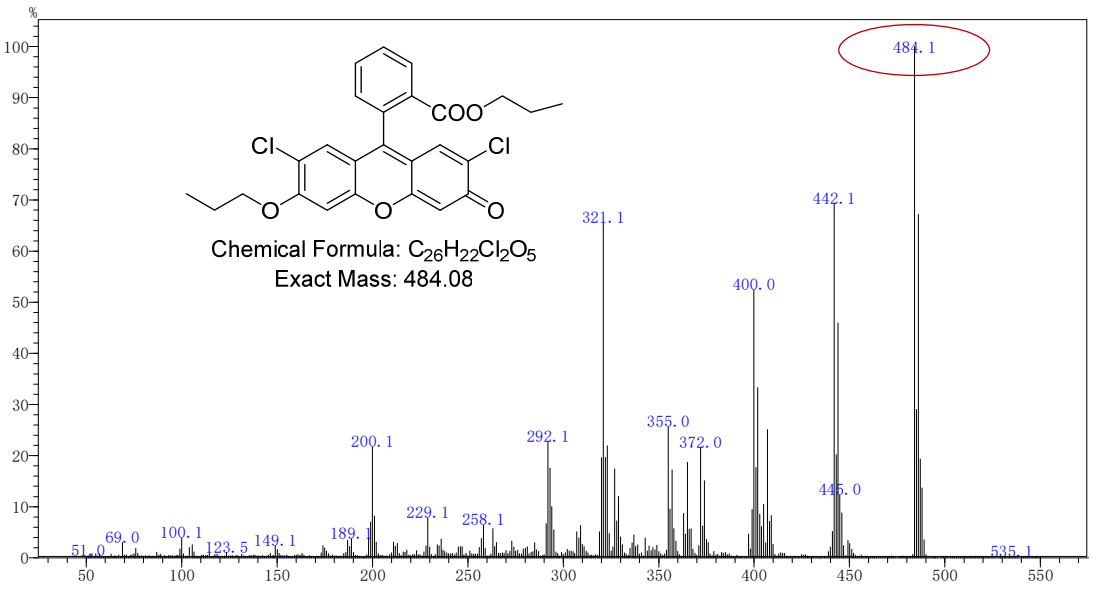
HR-MS spectrum of **DCFl-Me**



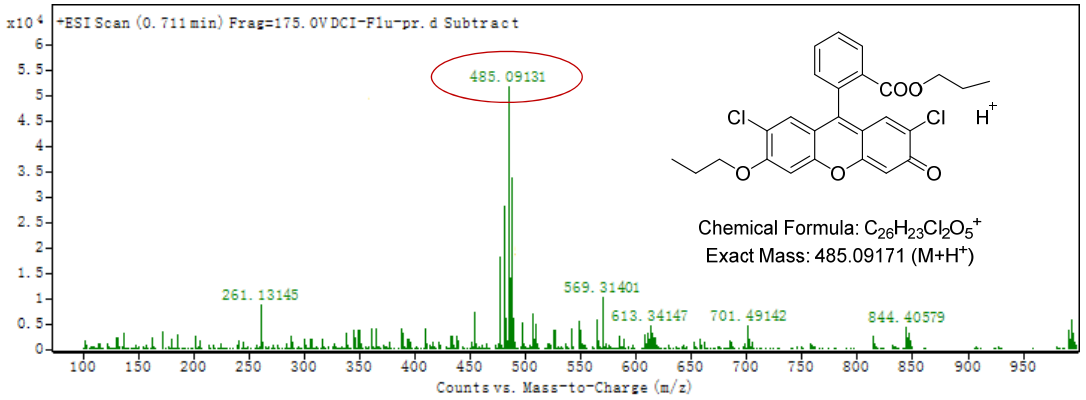
^{1}H NMR spectrum of **DCFl-Pr** in $CDCl_3$



^{13}C NMR spectrum of **DCFl-Pr** in $CDCl_3$.



EI-MS spectrum of **DCFl-Pr**



HR-MS spectrum of **DCFl-Pr**