Supporting Information

Molecular luminogens based on restriction of intramolecular motions through host-guest inclusion for cell imaging

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Experimental Section

Materials and Instrumentations

Tetrahydrofuran (THF) was distilled from sodiumbenzophenone ketyl immediately prior to use. Cyclodextrins (CDs), diphenylmethane, *p*-bromobenzophenone andother chemicals were all purchased from Aldrichand used as received without further purification.

^{1}H, ^{13}C and 2D ROESY NMR spectra were measured on a Bruker ARX400 NMR spectrometer using dimethyl sulfoxide-d_6, chloroform-d or methanol-d_4 as solvent and tetramethylsilane (TMS) as internal reference. UV absorptionspectra were taken on a Milton Ray Spectronic 3000 arrayspectrophotometer. High-resolution mass spectra (HRMS) wererecorded on a Finnigan MAT TSQ 7000 Mass SpectrometerSystem operating in a MALDI-TOF mode. Photoluminescence (PL) spectra were recorded on a Perkin Elmer LS 55 spectrofluorometer. The solution fluorescence quantum yields (Φ_F) were estimated using quinine sulfate in 0.1 N sulfuric acid ($\Phi_F= 54.6\%$) as standard. The absorbance of the solutions was kept between 0.04 and 0.06 to avoid the internal filter effect. Time-resolved PL spectra were measured by using a Hamamatsu model C4334 streak camera coupled to a spectrometer. A femtosecond titanium-sapphire oscillator was used as the excitation source. A UV beam with a wavelength of 267 nm (third harmonic of the laser output at 800 nm) was used as the pumping source in the experiments. Pulse width and repetition rate of the laser were 200 fs and 76 MHz, respectively. Excitation power was about 0.3 mW. The time resolution was 20 ps. Circular dichroism measurements were recorded on a Jasco J-720 spectropolarimeter in 1 mm quartz cuvettes with a step resolution of 0.2 nm, a scan speed of 50 nm/min, a sensitivity of 0.1^o, and a response time of 9.5 s. Each spectrum was the average of 5−10 scans. The geometric structure obtained from a single crystal of β-CD was used to construct the schematic illustration of TPE-β-CD. The optimized tetraphenylethene molecule was fitted into the cavity of β-CD using Materials Studio software.

HeLa cells were cultured in minimum essential medium (MEM) containing 10% fetal bovine serum (FBS) and antibiotics (100 units/mL penicillin and 100 μg/mL streptomycin) in a 5% humidity incubator at 37 °C. The HeLa cells were grown overnight on a cover slid in a 35 mm Petri dish. A DMSO solution of TPE-β-CD with a concentration of 5 mg/mL was prepared and then diluted by 1000 times by adding 2μL of the solution into 2 mL aqueous buffer solution (MEM medium containing 10% FPS). The living cells were stained with such medium for 5h. The cells were imaged under an inverted fluorescence microscope (Nikon Eclipse TE2000-U); ex = 330−385 nm, diachronic mirror = 400 nm. The images of the cells were captured using a digital CCD camera.

Synthesis

1-(4-Bromophenyl)-1,2,2-triphenylethene (1). The compound was synthesized according to the synthetic route shown in Scheme S1. Typical procedures for its synthesis are shown as follows. To a solution of diphenylmethane (2.02 g; 12 mmol) in dry tetrahydrofuran (50 mL) was added 6.25 mL of a 1.6 M solution of *n*-butyllithium in hexane (10 mmol) at -78 °C under nitrogen. The resulting orange-red solution was stirred for 30 min at that temperature. To this solution was added 4-bromobenzophenone (2.35 g; 9 mmol). Afterwards, the reaction mixture was allowed to warm to room temperature and stirred for another 6 h. The reaction was quenched with the addition of an aqueous solution of ammonium chloride. The organic layer was then extracted with dichloromethane (3 × 50 mL). The organic layers were combined, washed with saturated brine solution and dried over anhydrous magnesium sulphate. After solvent evaporation, the resulting crude alcohol (containing excess diphenylmethane) was subjected to acid-catalyzed dehydration without further purification. The crude alcohol was dissolved in about 80 mL of toluene in a 100 mL Schlenk flask fitted with a Dean-Stark trap. A catalytic amount of *p*-toluenesulfonic acid (342 mg; 1.8 mmol) was

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added and the mixture was refluxed for 3−4 h. After the reaction mixture was cooled to room temperature, the toluene layer was washed with 10% aqueous $NaHCO_3$ solution (2 × 25 mL) and dried over anhydrous magnesium sulfate. Evaporation of the solvent under reduced pressure afforded the crude tetraphenylethene derivative, which was further purified by silica gel column chromatography using hexane as eluent. Yield 92%. ^{1}H NMR (400 MHz, DMSO-d_6), δ (TMS, ppm): 6.95−7.11 (m, 17 H, Ar−H), 7.32 (d, 2H, Ar−H).

4-(1,2,2-Triphenylvinyl)benzoic acid (TPE-CO$_2$H). To a solution of **1** (1.6 g; 3.89 mmol) in 30 mL dry THF was added dropwise 2.9 mL (4.64 mmol) of *n*-butyllithium (1.6 M in *n*-hexane) at -78 °C under stirring. The reaction mixture was stirred for 2 h to get a dark brown solution. To the obtained solution was then added dry ice pieces in small portions under nitrogen. The solution was allowed to warm to room temperature and stir for additional 12 h. The solvent was evaporated under reduced pressure. The crude product was purified on a silica-gel column using dichloromethane/methanol mixture (90/10 v/v) as eluent. Yield 85%. ^{1}H NMR (400 MHz, DMSO-d_6), δ (TMS, ppm): 6.95−7.11 (m, 17 H, Ar−H), 7.66 (d, 2H, Ar−H). ^{13}C NMR (400 MHz, CD$_3$OD), δ(TMS, ppm): 125.9, 126.8, 128.3 and 130.3 (Ar), 142.4 and 141.8 (C=C), 148.1 (Ar), 167.8 (CO$_2$H). HRMS (MALDF-TOF): *m/z* 376.1458 (M$^+$, calcd 376.1463).

TPE-β-CD. Into a 50 mL round-bottom flask were dissolved 0.188 g (0.5 mmol) of TPE-CO$_2$Hand 0.5675 g (0.5 mmol) of β-CD in 10 mL of anhydrous dimethylformamide (DMF) at 0 °C under nitrogen. After all the solids were dissolved, 0.103 g (0.5 mmol) of *N,N'*-dicyclohexylcarbodiimide (DCC) in 5 mL of anhydrous DMF was then added. The reaction mixture was allowed to warm to room temperature and stirred for 3 days under nitrogen. After filtration, the filtrate was added dropwise into copious ethyl ether under vigorous stirring. The solid was isolated by filtration and dried in vacuum at 40 °C overnight. Yield 70%. ^{1}H NMR (400 MHz, DMSO-d_6), δ (TMS, ppm): 3.28 (d, 7H,H$_4$), 3.33 (d, 7H, H$_2$),

3.55 (d, 7H, H_5), 3.60 (d, 12H,H_6), 3.64 (br, 7H,H_3), 4.44 (t, 6H, O_6H), 4.80 (d, 7H, H_1), 5.65 (s, 7H, O_3H), 5.70 (d, 7H, O_2H), 7.93 (s, 2H, Ar–H). ^{13}C NMR (400 MHz, DMSO-d_6),δ (TMS, ppm): 59.7 (C_6), 71.8(C_2), 72.3 (C_5), 72.9 (C_3), 81.3(C_4), 101.7 (C_1), 130.4 (Ar), 156.7 (C=C), 162.2 (C=O). HRMS (MALDF-TOF): m/z 1492.65 (M^+, calcd 1492.51).
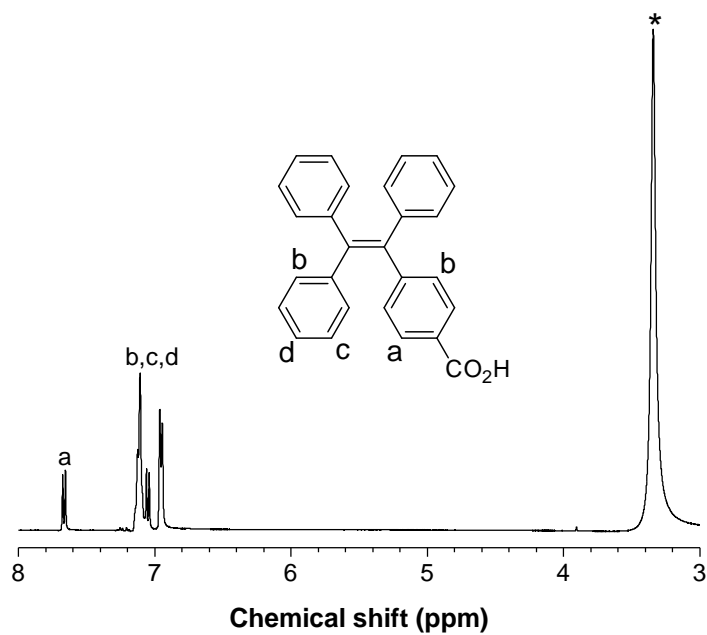
TPE-α-CD. It was synthesized from 0.188 g (0.5 mmol) of TPE-CO_2H and 0.486 g (0.5 mmol) of α-CD in 15 mL of anhydrous DMF at 0 °C under nitrogen using 0.103 g (0.5 mmol) of DCC as catalyst. The procedures were similar to those for TPE-β-CD. Yield 60%. ^1H NMR (400 MHz, DMSO-d_6), δ (TMS, ppm): 3.30 (m, 6H, H_4), 3.36 (m, 6H, H_2), 3.56 (d, 6H, H_5), 3.61(m, 10H,H_6), 3.75 (t, 6H,H_3), 4.46 (t, 5H, O_6H), 4.77 (d, 6H, H_1), 5.41 (d, 6H, O_3H), 5.50 (d, 6H, O_2H), 7.93 (s, 2H, Ar–H). ^{13}C NMR (400 MHz, DMSO-d_6),δ (TMS, ppm): 59.7 (C_6), 71.8(C_2), 72.3 (C_5), 72.9 (C_3), 81.3(C_4), 101.7 (C_1), 130.4 (Ar), 156.7 (C=C), 162.2 (C=O). HRMS (MALDF-TOF): m/z 1330.63 (M^+, calcd 1330.45).

TPE-γ-CD. It was synthesized from 0.188 g (0.5 mmol) of TPE-CO_2H, 0.648 g (0.5 mmol) of γ-CD and 0.103 g (0.5 mmol) of DCC in 10 mL of anhydrous DMF at 0 °C under nitrogen. The procedures were similar to those described above. Yield 52%. ^1H NMR (400 MHz, DMSO-d_6), δ (TMS, ppm): 3.28 (d, 8H,H_4), 3.33(d, 8H, H_2), 3.55 (d, 8H, H_5), 3.60(d, 16H,H_6), 3.64 (br, 8H,H_3), 4.44 (t, 7H, O_6H), 4.80 (d, 8H, H_1), 5.65 (s, 8H, O_3H), 5.70 (d, 8H, O_2H), 6.90–7.10 (m, 4H, Ar–H), 7.93 (s, 2H, Ar–H). ^{13}C NMR (400 MHz, DMSO-d_6): δ (TMS, ppm): 59.7 (C_6), 71.8(C_2), 72.3 (C_5), 72.9 (C_3), 81.3(C_4), 101.7 (C_1), 130.4 (Ar), 156.7 (C=C), 162.2 (C=O). HRMS (MALDF-TOF): m/z 1654.40 (M^+, calcd 1654.56).
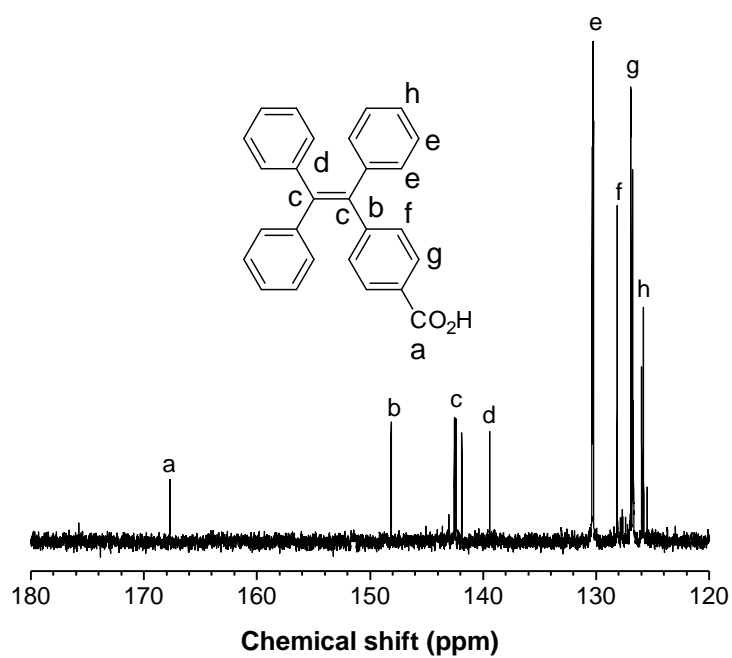
Ethyl 4-(1,2,2-triphenylvinyl)benzoate (TPE-C2). It was synthesized from 0.376 g (1 mmol) of TPE-CO_2H, 5 mL (2 mmol) of ethanol and 0.206 g (1 mmol) of DCC in 10 mL of anhydrous DMF at 0 °C under nitrogen.Yield 82%. ^1H NMR (400 MHz, $CDCl_3$), δ (TMS, ppm): 1.36 (t, 3H,CH_3), 4.32 (dd, 2H, C$H_2$$CH_3$), 6.90–7.10 (m, 19H, Ar–H), 7.93 (s, 2H, Ar–H). HRMS (MALDF-TOF): m/z 405.15 [$(M+1)^+$, calcd 404.18].
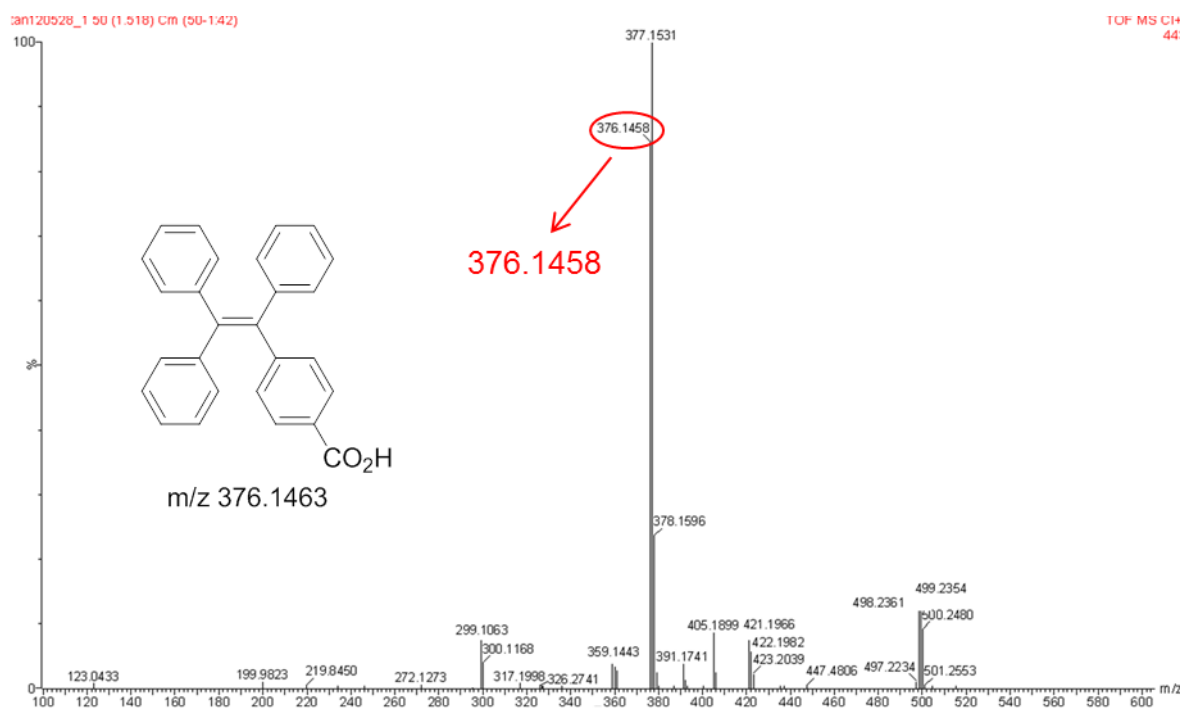
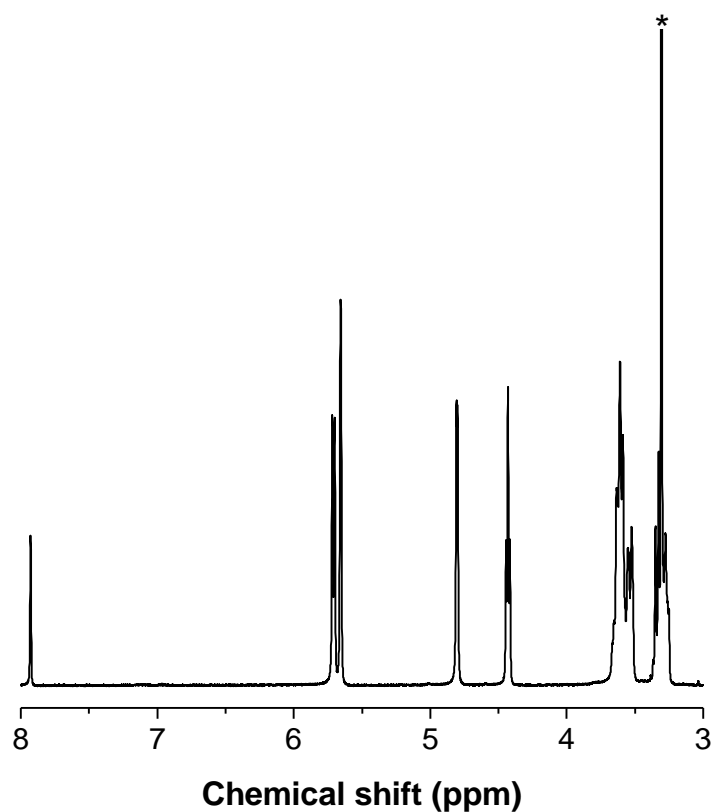**Scheme S1** Synthetic route for carboxylic acid-substituted tetraphenylethene.

Fig. S1 ^{1}H NMR spectrum of TPE-CO$_2$H in DMSO-d_6. The solvent peak was marked with an asterisk.
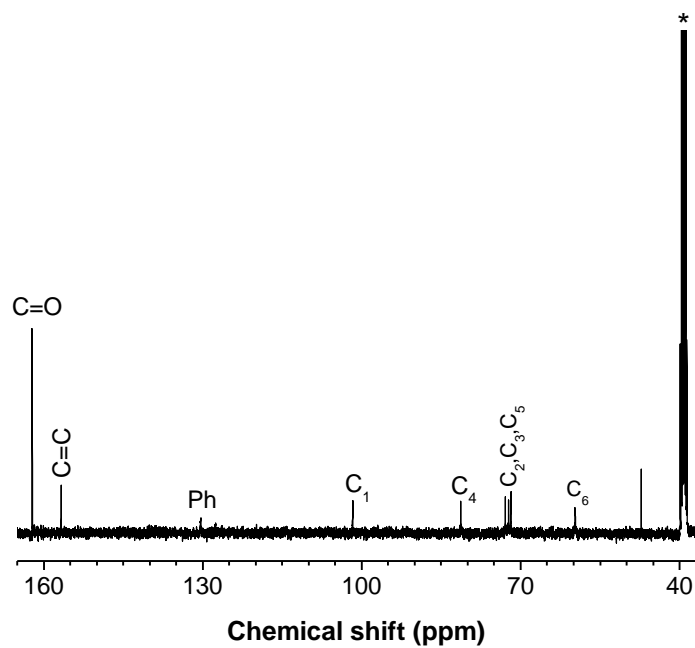
Fig. S2 ^{13}C NMR spectrum of TPE-CO$_2$H in DMSO-d_6.
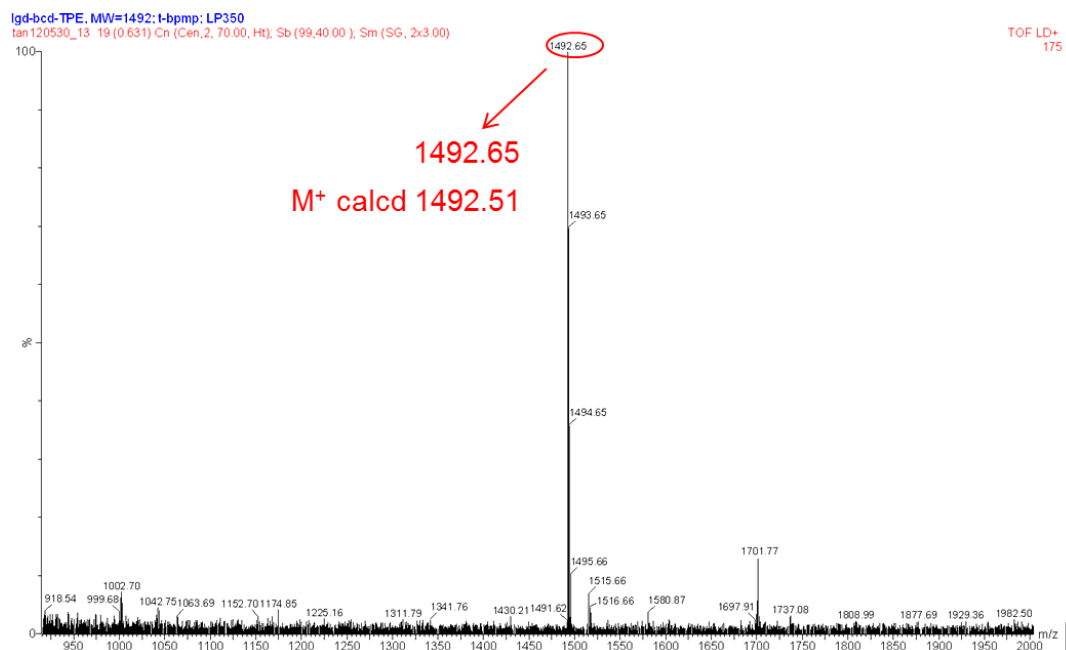

Fig. S3 High resolution mass spectrum of TPE-CO$_2$H.

Fig. S4 ^{1}H NMR spectrum of TPE-β-CD in DMSO-d_6. The solvent peak was marked with an asterisk. Solution concentration: 40 mM.
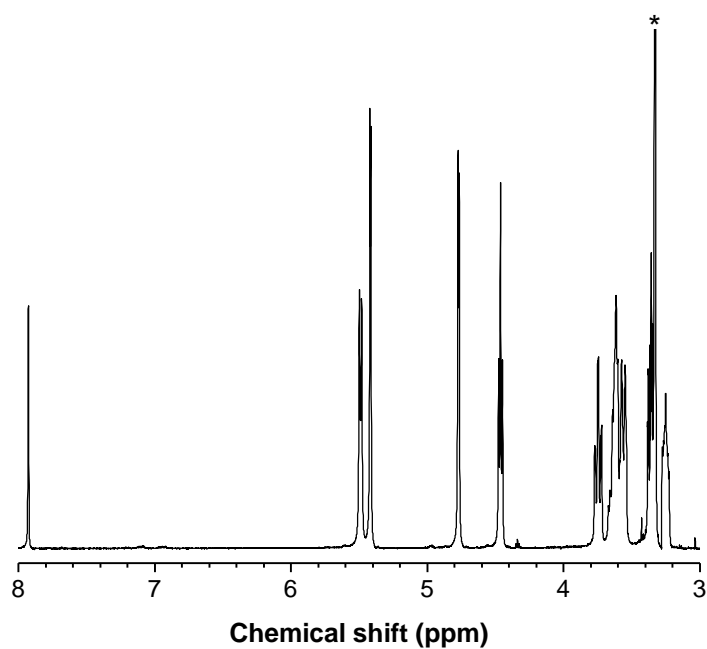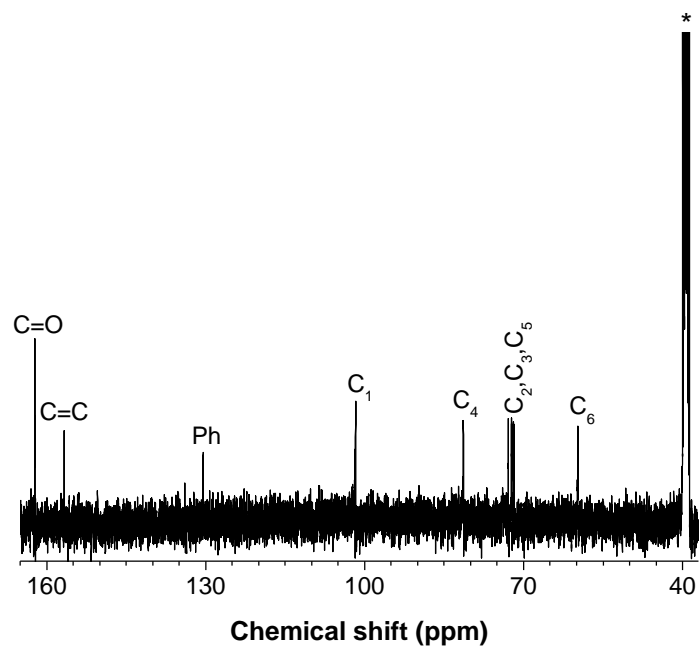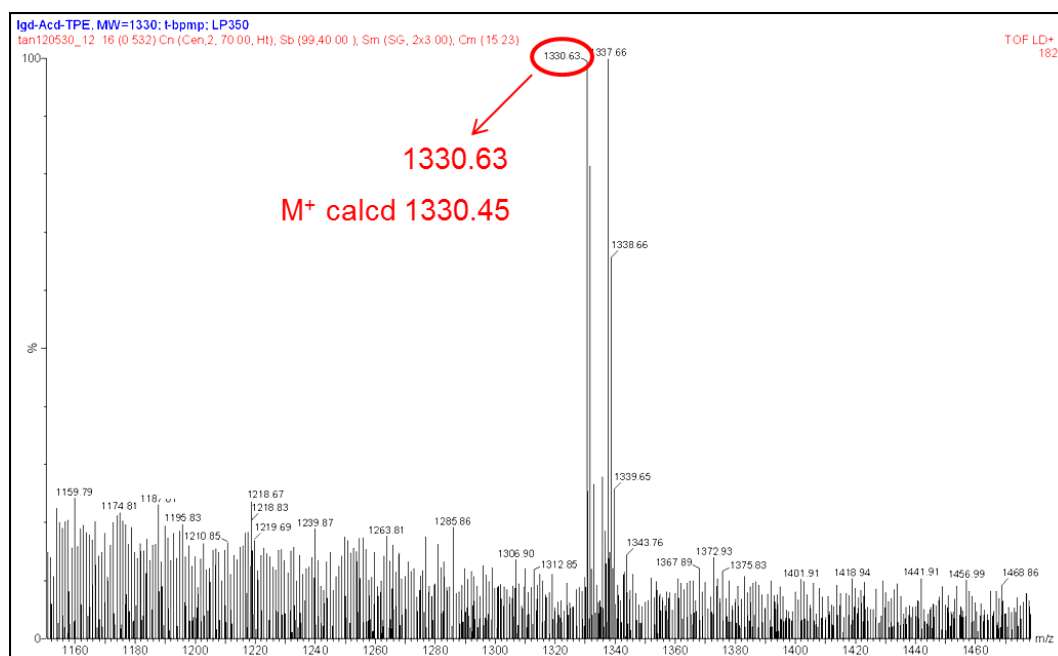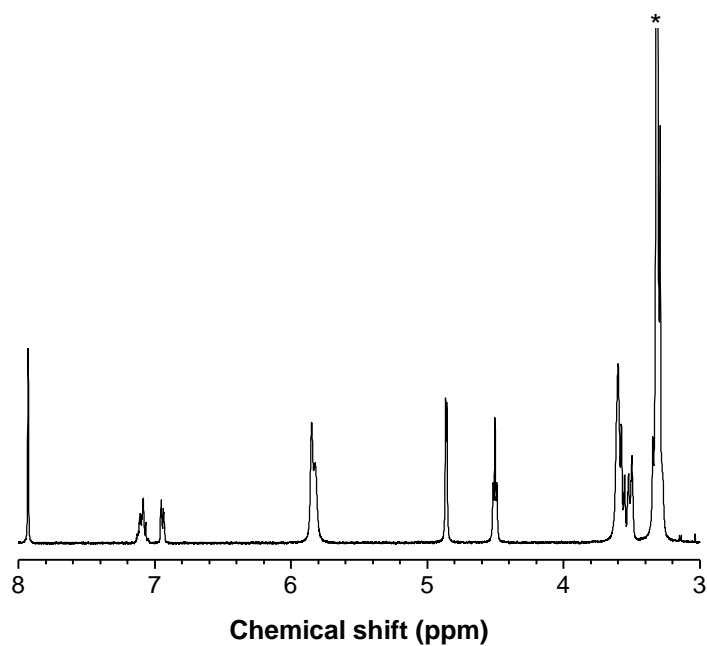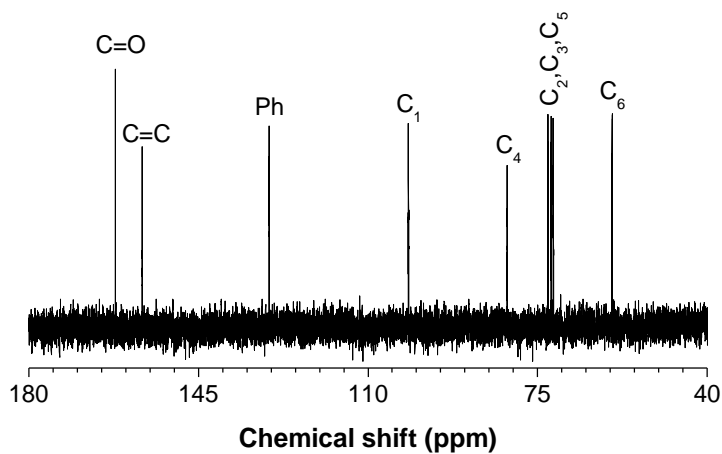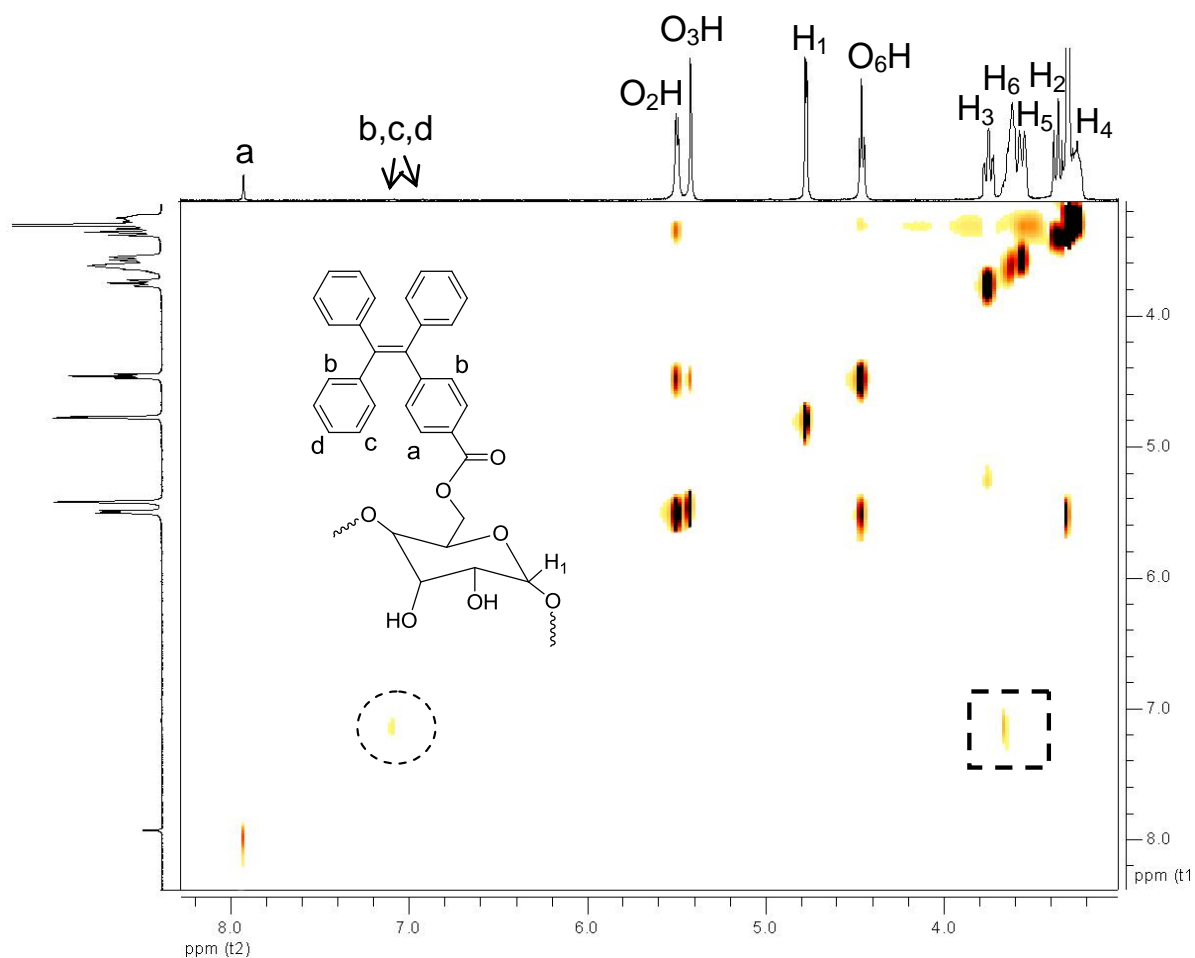

Fig. S5 ^{13}C NMR spectrum of TPE-β-CD in DMSO-d_6. The solvent peak was marked with an asterisk. Solution concentration: 40 mM.
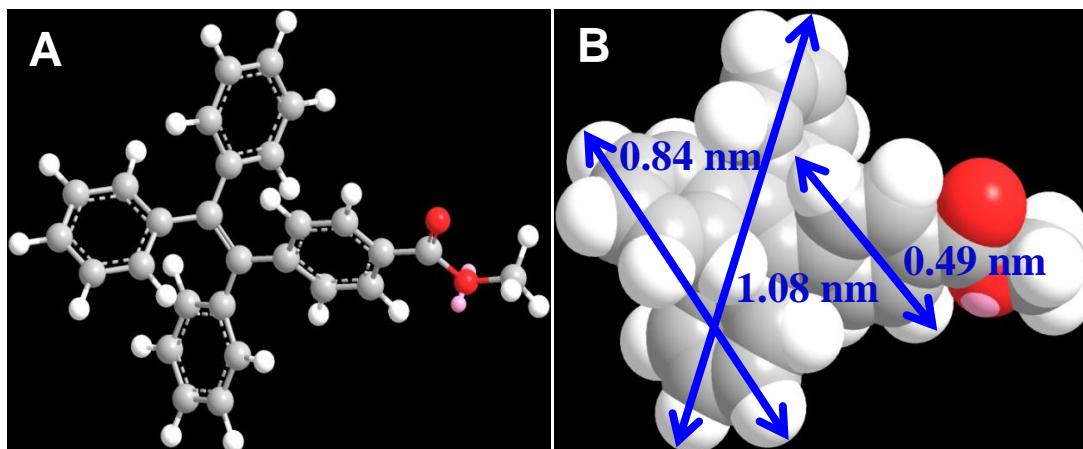
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Fig. S6 High resolution mass spectrum of TPE-β-CD.

Fig. S7 ^{1}H NMR spectrum of TPE-α-CD in DMSO-d_6. The solvent peak was marked with an asterisk. Solution concentration: 40 mM.

Fig. S8 ^{13}C NMR spectrum of TPE-α-CD in DMSO-d_6. The solvent peak was marked with an asterisk. Solution concentration: 40 mM.

Fig. S9 High resolution mass spectrum of TPE-α-CD.

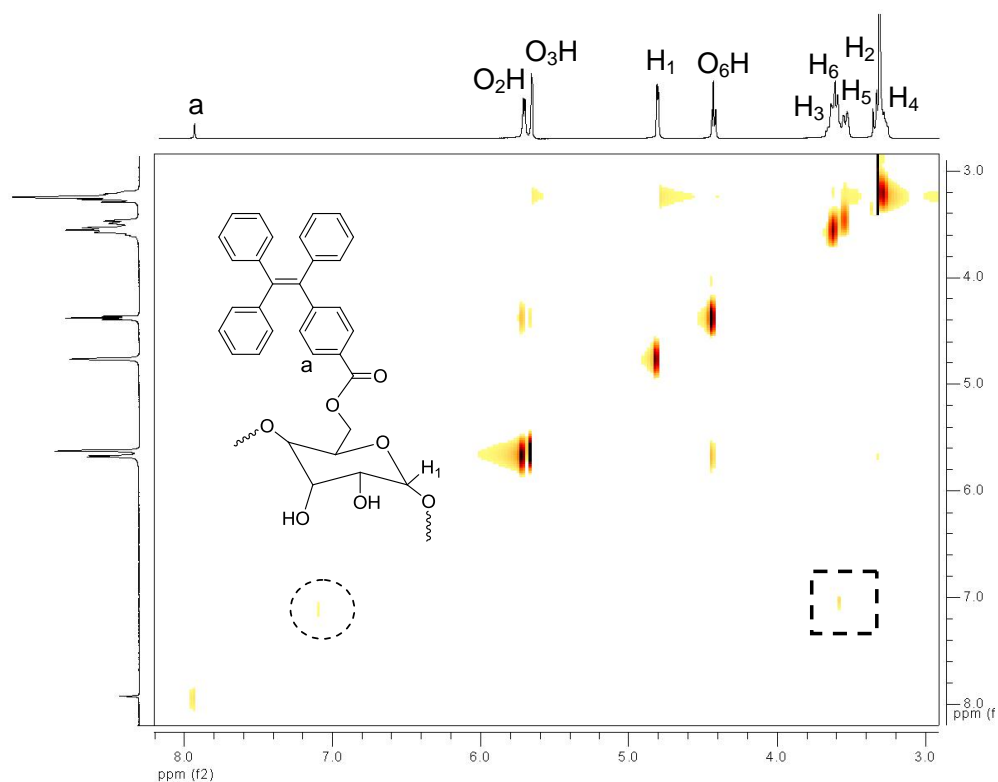**Fig. S10** ^{1}H NMR spectrum of TPE-γ-CD in DMSO-d_6. The solvent peak was marked with an asterisk. Solution concentration: 40 mM.

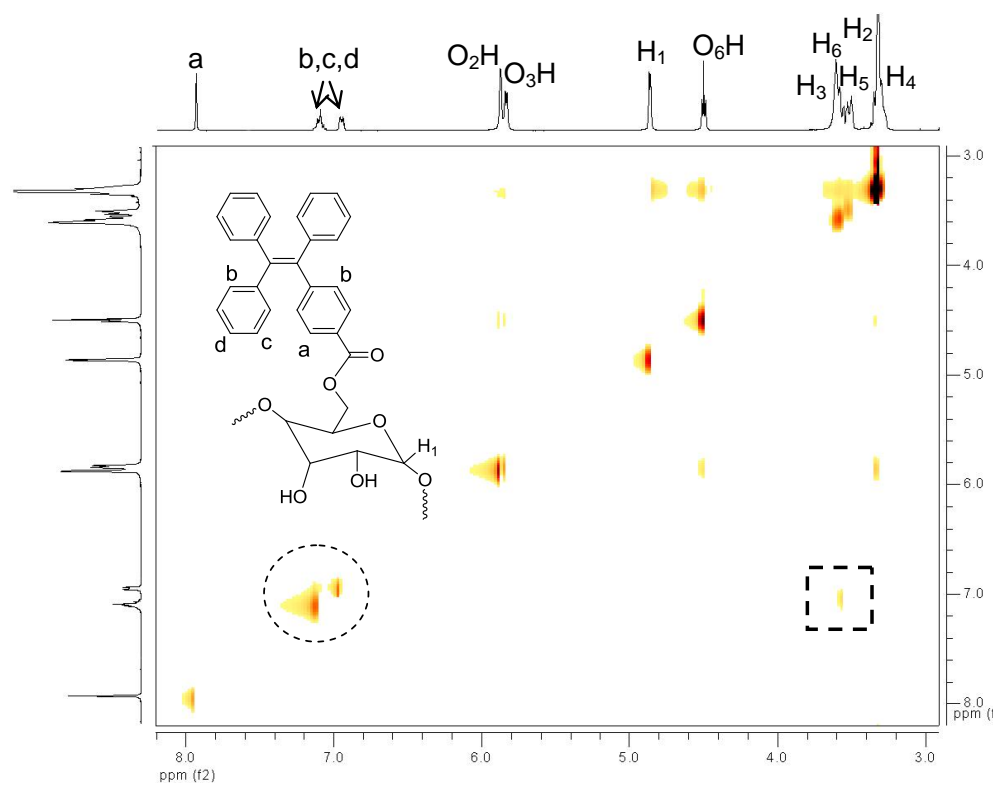**Fig. S11** ^{13}C NMR spectrum of TPE-γ-CD in DMSO-d_6. Solution concentration: 40 mM.

Fig. S12 2D ROESY NMR spectrum of TPE-β-CD in DMSO-d_6. Solution concentration: 2 mM.
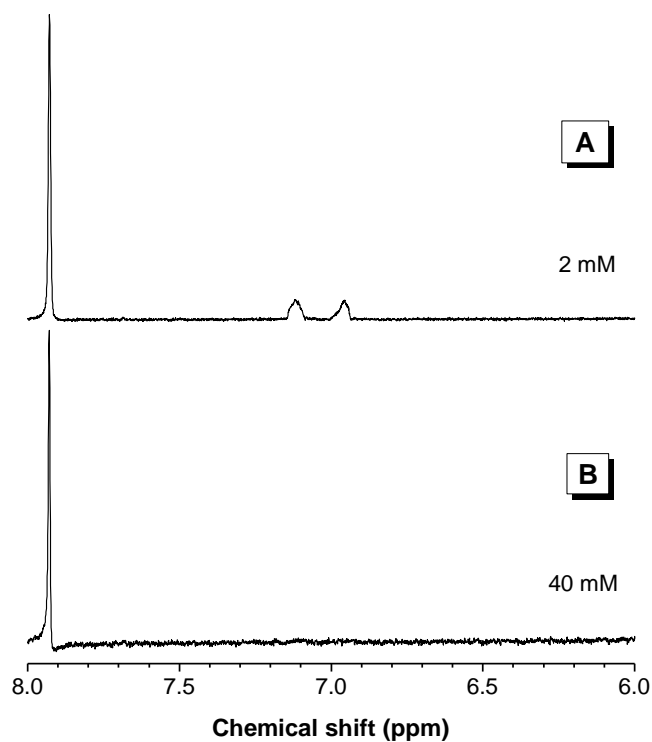

Scheme S2 Optimized chemical structure of TPE-CD in (A) rod-stick mode and (B) space fitting mode. For clarity, methyl group was used to stand for cyclodextrin.
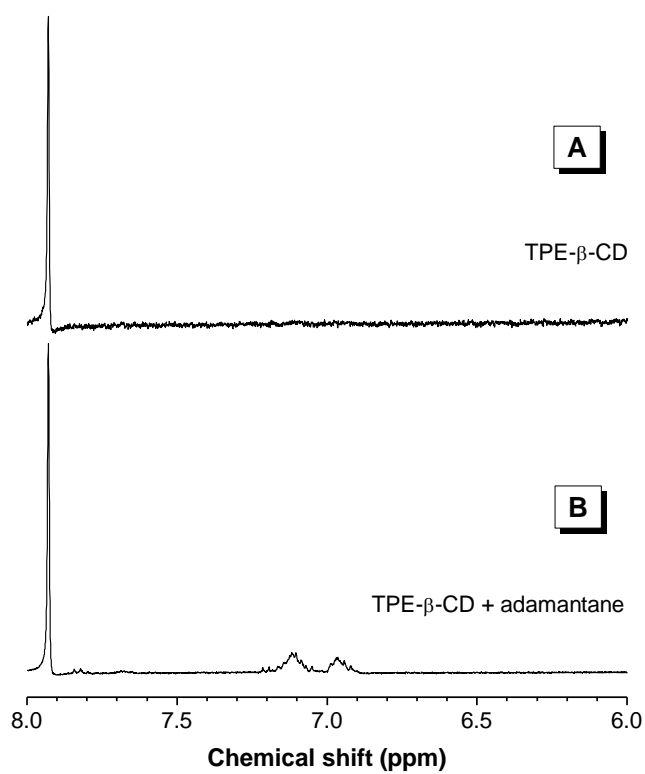
Fig. S13 2D ROESY NMR spectrum of TPE-α-CD in DMSO-d_6. Solution concentration: 2 mM.

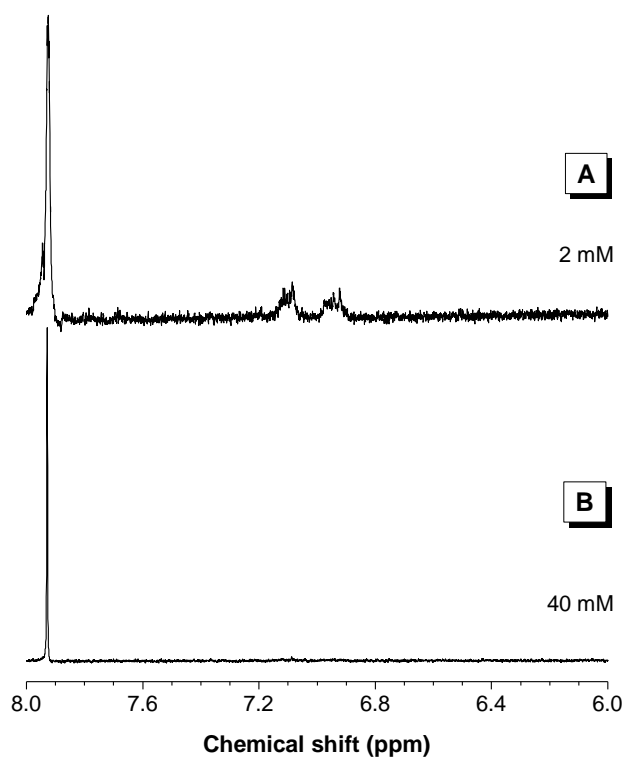**Fig. S14** 2D ROESY NMR spectrum of TPE-γ-CD in DMSO-d_6. Solution concentration: 2 mM.
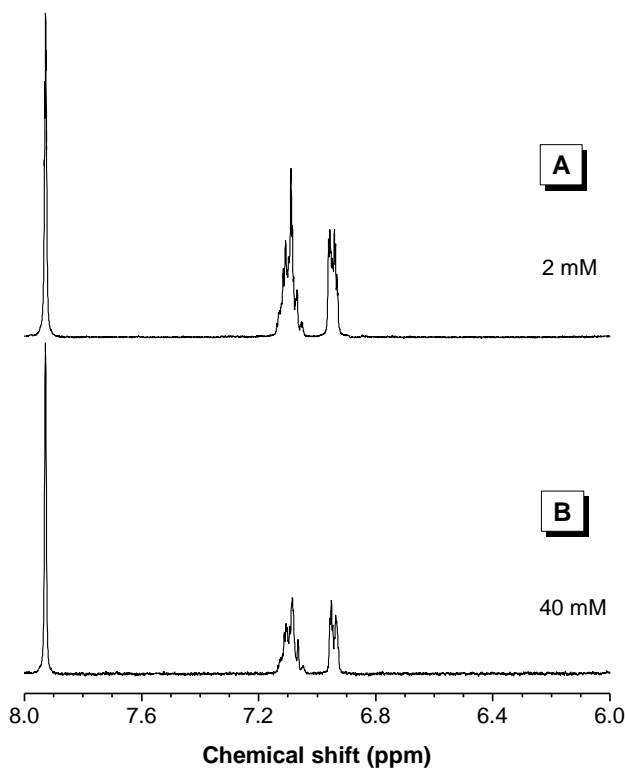
Fig. S15 ^{1}H NMR spectra of TPE-β-CD in DMSO-d_6 solutions with concentrations of (A) 2 mM and (B) 40 mM.

Fig. S16 ^{1}H NMR spectra of TPE-β-CD in DMSO-d_6 solutions (A) without and (B) with two equivalent molar concentration of adamantane. Concentration of TPE-β-CD: 40 mM.

Fig. S17 ^{1}H NMR spectra of TPE-α-CD in DMSO-d_6 solutions with concentrations of (A) 2 mM and (B) 40 mM.
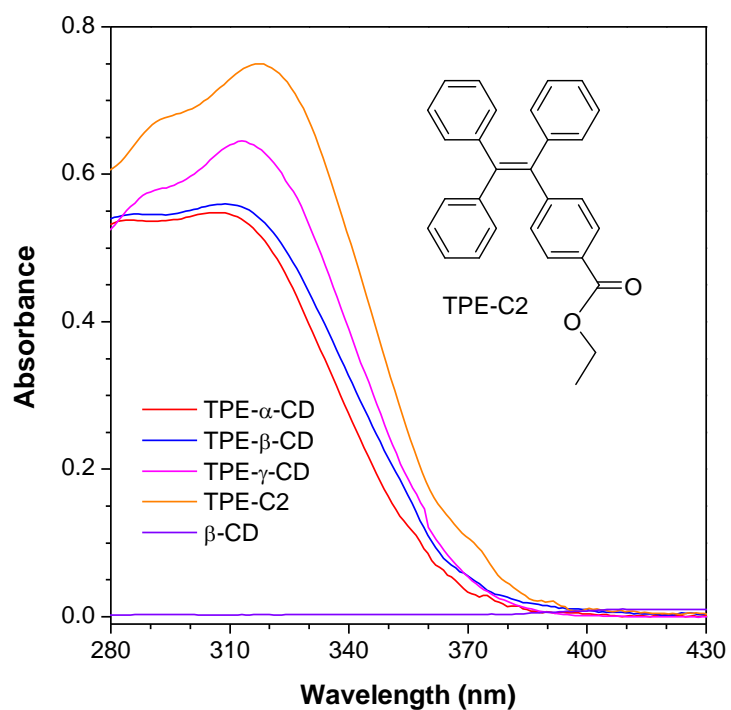

Fig. S18 ^{1}H NMR spectra of TPE-γ-CD in DMSO-d_6 solutions with concentrations of (A) 2 mM and (B) 40 mM.

Table S1 Cavity size of the cyclodextrins^a

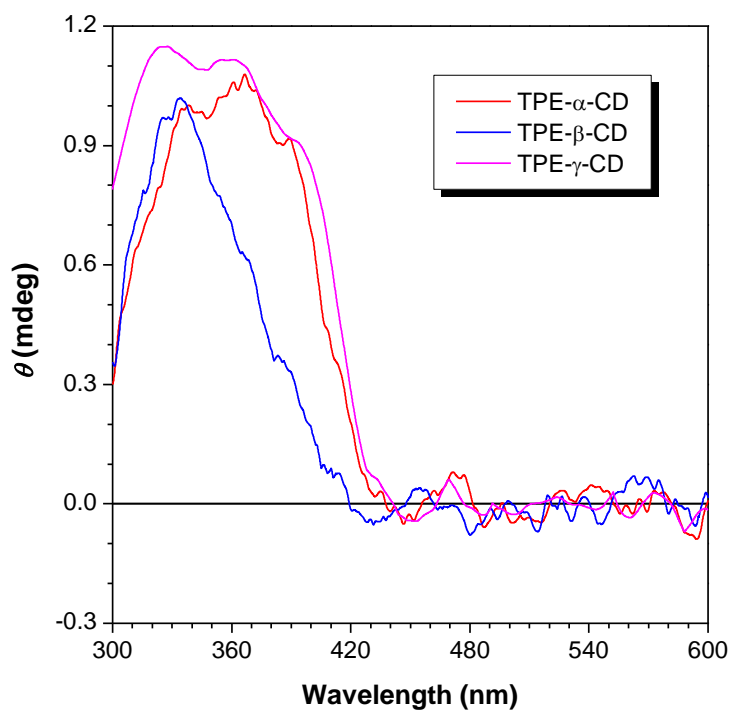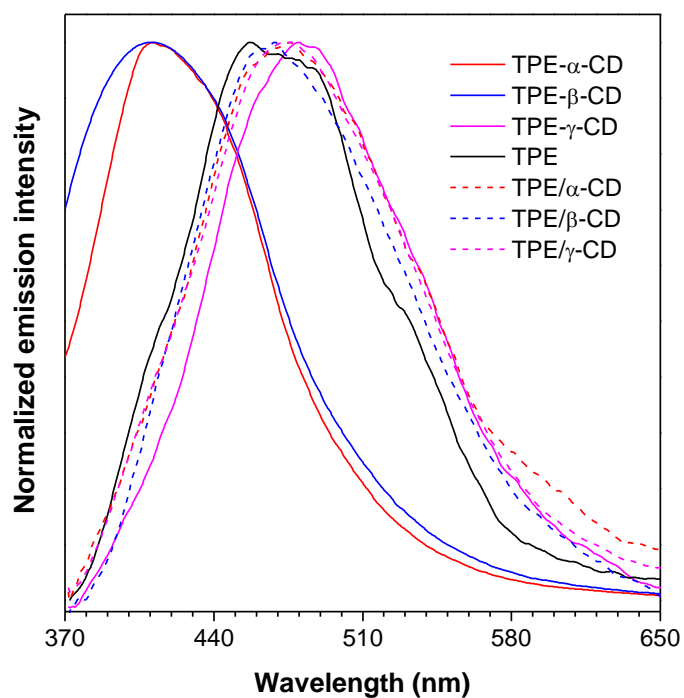| Compound	Inner size (nm)	Outer size (nm)
α-CD	0.57	1.37
β-CD	0.78	1.53
γ-CD	0.95	1.69

^{a} Depth of the cyclodextrins: 0.78 nm

Fig. S19 UV-vis spectra of TPE-CDs, TPE-C2 and β-CD in DMSO. Solution concentration:
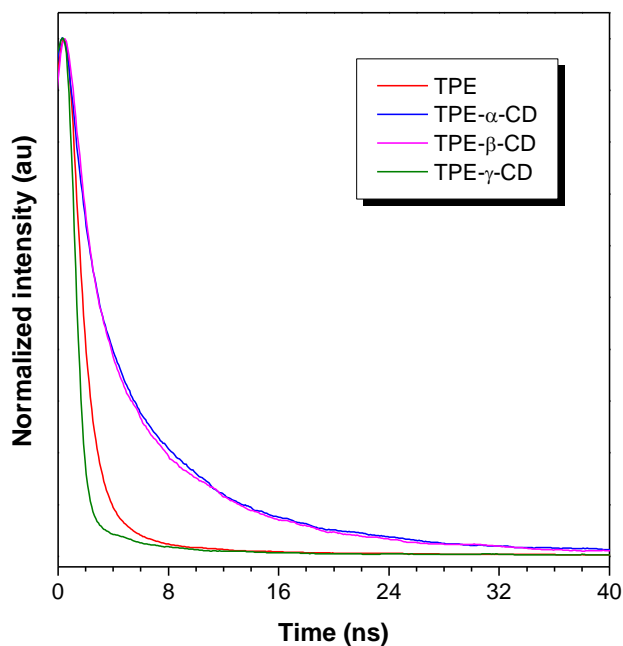
0.1 mM. Inset: chemical structure of TPE-C2.

Fig. S20 Circular dichroism of TPE-CDs in DMSO solutions. Solution concentration: 1 mM.

Fig. S21 Normalized emission spectra of TPE-CDs, bare TPE and TPE/CD mixtures in DMSO solutions. Solution concentration: 5 mM.

Fig. S22 Time-resolved fluorescence spectra of TPE-CDs and bare TPE in DMSO solutions.

Calculation of radiative and non-radiative decay rate constants:

Fluorescence typically follows first-order kinetics:[1]

$$[S] = [S]_0 e^{-t/\tau} \qquad (1)$$

[S] is the concentration of exited state molecules at time t, $[S]_0$ is the initial concentration

and τ is the fluorescence lifetime.

Decay rate (k) is the inverse of lifetime, consisting of radiative and non-radiative decay rate

constants:

$$k = k_{rad} + k_{nrad} \qquad (2)$$

where k_{rad} is the radiative decay rate constant and k_{nrad} is the nonradiative decay rate constant.

The quantum yield (QE) is defined as the fraction of emission process in which emission of light is involved:

$$QE = \frac{k_{rad}}{k_{rad}+k_{nrad}} \qquad (3)$$

The values of radiative and non-radiative rate constants of TPE and TPE-CDs were tabulated in Table S2.

Table S2 Emission lifetimes (τ), radiative decay rate constant (k_{rad}) and non-radiative decay rate constant (k_{nrad}) of TPE-CDs and bare TPE in DMSO

compound	τ (ns)	k_{rad} ($\times 10^7$ s^{-1})	k_{nrad} ($\times 10^7$ s^{-1})
TPE	1.41	0.355	70.6
TPE-α-CD	7.80	1.28	11.5
TPE-β-CD	8.23	1.94	10.2
TPE-γ-CD	0.85	0.706	117

References:

1 J. R. Lakowicz, *Principles of Fluorescence Spectroscopy*, Springer: New York, 2006, p:8-9.