Electronic Supplementary Information for

Direct Validation of Restriction of Intramolecular Rotation Hypothesis *via* Synthesis of Novel *ortho*-Methyl Substituted Tetraphenylethenes and the Application in Cell Imaging†

Guo-Feng Zhang,[a] Ze-Qiang Chen,[a] Matthew P. Aldred,*[a] Zhe Hu,[a] Tao Chen,[a] Zhen-li Huang,[a] Xianggao Meng,*[b] and Ming-Qiang Zhu*[a]

[a] *Wuhan National Laboratory for Optoelectronics, School of Optical and Electronic Information, Huazhong University of Science and Technology, Wuhan, Hubei, China, 430074, China; Fax: 86 27 87793419; Tel: 86 27 87793419;*
E-mail: mqzhu@hust.edu.cn; mpaldred@hotmail.com.

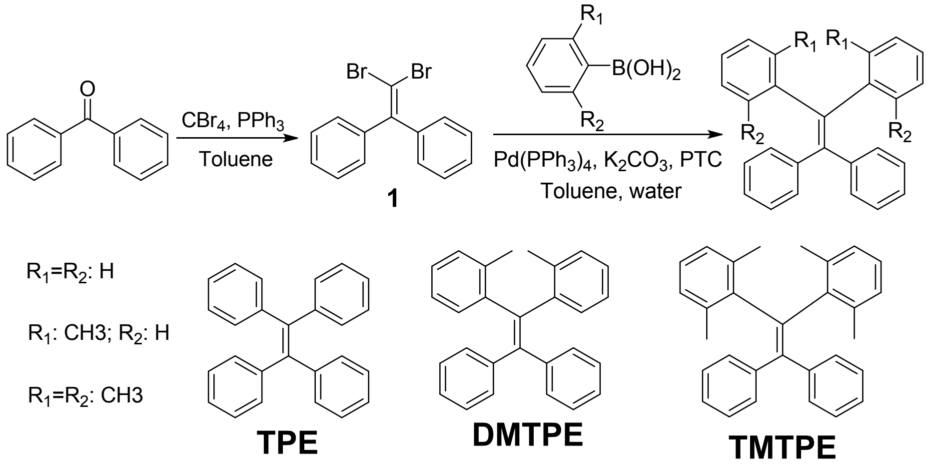
[b] *Key Laboratory of Pesticide and Chemical Biology of Ministry of Education, College of Chemistry, Central China Normal University, Wuhan, Hubei 430079, China.*
E-mail: mengxianggao@mail.ccnu.edu.cn

1. Experimental details

All commercially available starting materials, reagents and solvents were used as supplied, unless otherwise stated, and were purchased from Aladdin, Energy, and Sinopharm Chemical Reagent Co. Ltd. All reactions were carried out under a dry nitrogen atmosphere and the temperatures were measured externally. THF and toluene were dried using sodium wire and benzophenone as the indicator. Reported yields are isolated yields. Purification of all final products was accomplished by gravity column chromatography, using silica gel. For qualitative purity tests of all intermediates and final products, a single spot (visualised using UV-light at 254 nm and 365 nm) was obtained. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) in $CDCl_3$ were measured on a Bruker AV400 spectrometer, and reported in parts per million (PPM) relative to tetramethylsilane as an internal standard. UV-Vis and photoluminescence spectra were recorded on shimadzu UV-VIS-NIR spectrophotometer (UV-3600) and Edinburgh instruments (FLSP920 spectrometers), respectively. λ_{max} (abs) is the lowest energy absorption peak at concentration = 1 x 10^{-5} M, λ_{em} was measured using their respective λ_{max} (abs) as excitation wavelength, for λ_{em} of TMTPE THF solution, concentration = 1 x 10^{-5} M. The Mass Spectrometry was recorded on matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF) using Cyano-4-hydroxycinnamic Acid (CHCA) as the matrix or Agilent (1100 LC/MSD Trap). The energy levels of three compounds were obtained from the simulation based on Guassiun program. The relative quantum yield (Φ_F) was estimated against 9,10-diphenylanthracene (Φ_F = 0.9 in cyclohexane) as a standard.[1] For cell imaging, HeLa cells were cultured in Dublecco's Modified Eagle's Medium (DMEM, Invitrogen) containing 10% fetal bovine serum (FBS, Gibco) in a humidified incubator at 37ºC in 5% CO_2. HeLa cells were transferred into glass bottom petri dishes grown to 80% confluence. Before imaging, we co-incubated cells with our dye (at 1 uM) and LysoTracker Red (at 500 nM) for 30min. Confocal laser scanning microscope (CLSM) imagings were operated and carried out on the commercial LSM 710 system (Carl Zeiss Company, Germany).

For X-ray Crystallographic Studies, diffraction data were collected at room temperature or low temperature using a Apex Duo X-ray CCD diffractometer working with graphite-monochromatized Mokα X-radiation (λ = 0.71073 Å). Data collection, frame integration, data reduction using multi-scan method, and structure determination were carried out using APEX2 attached software. Empirical absorption corrections were applied to the data using the SADABS program. Structural refinements were performed by the full-matrix least-squares method on F^2 with SHELXTL-97 program. Anisotropic displacement parameters were refined for all nonhydrogen atoms. Hydrogens bonds to carbon atoms were placed at calculated positions with the appropriate AFIX instructions and refined using a riding model. Related parameters of some short contacts (such as $\pi\cdots\pi$ and C-H$\cdots\pi$) in the crystal structures are measured using PLATON,[2] Mercury and Diamond softwares. Dihedral angles between the neighbouring planes are calculated using Mercury software with all the atoms on one same aryl ring selected as a plane. With respect to X-ray powder diffraction, powder XRD patterns were obtained using a X'Pert PRO diffractometer from $2\theta = 5^o$ to 80^o with Cu-Kα radiation (λ = 1.5406 Å) under room temperature.

Synthesis:



Scheme S1. Synthesis route to *ortho*-methyl TPEs.

The start materials of *o*-tolylboronic acid[3] and (2,6-dimethylphenyl)boronic acid[4] were synthesized according to the published literatures.

Corey-Fuchs reaction for 1,1-dibromo-2,2-diphenylene (1): Typically, A mixture of benzophenone (3.04 g, 16.68 mmol), carbon tetrabromide (11.06 g, 33.36 mmol), and triphenyl phosphine (17.53 g, 66.84 mmol) in 250 ml of anhydrous toluene was run at 140^{o}C for 4 days. On cooling to room temperature, the mixture was filtered, washed with toluene and the filtrate was collected, washed with water. The organic layer was separated, dried with Na_2SO_4, and then the solvent was removed by rotary evaporator. After the residue was purified by column chromatography on silica gel using hexane as the eluent, the product was recrystallized in ethanol to afford 4.91 g of 1,1-dibromo-2,2-diphenylene as a light yellow needle-like crystalline solid (87.1% yield). ^{1}HNMR ($CDCl_3$, 400 MHz) δ (ppm): 7.25-7.34 (m, 10H). ^{13}CNMR ($CDCl_3$, 100 MHz) δ (ppm): 144.88, 141.41, 128.74, 128.34, 127.97, 90.30.

1,1,2,2-tetraphenylethene (**TPE**) : To a two-neck flask (100 ml), 1,1-dibromo-2,2-diphenylene (1) (0.67 g, 2 mmol), boronic acid (2) (0.31 g, 2.5 mmol), $Pd(PPh_3)_4$ (115 mg, 0.1 mmol), tetrabutyl-ammonium hydrogen sulfate (68 mg, 0.2 mmol), K_2CO_3 (828 mg, 6 mmol) in toluene (40 ml) and water (20 ml) were added and heated to 90^{o}C and stirred under nitrogen overnight. After cooling down to room temperature, the organic layer was separated and the water layer was extracted with dichloromethane (DCM). The combined organic solution was dried with Na_2SO_4 for several hours. After filtration, the resulting solution was concentrated by rotary evaporator. The residue was purified by column chromatography on silica gel using DCM/PE (v/v: 1:9) as the eluent to afford 1,1,2,2-tetraphenylethene (**TPE**) (0.60 g, 90.3 %). ^{1}HNMR ($CDCl_3$, 400 MHz) δ (ppm): 7.08-7.09 (m, 12H), 7.01-7.03 (m, 6H). ^{13}CNMR ($CDCl_3$, 100 MHz) δ (ppm): 143.744, 140.978, 131.347, 127.662, 126.426. ESI-MS: found: 332.1.

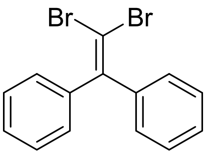
2,2'-(2,2-diphenylethene-1,1-diyl)bis(methylbenzene) (**DMTPE**) : To a two-neck flask (100 ml), 1,1-dibromo-2,2-diphenylene (1) (0.67 g, 2 mmol), *o*-tolylboronic acid (2) (0.34 g, 2.5 mmol), $Pd(PPh_3)_4$ (115 mg, 0.1 mmol), tetrabutyl-ammonium hydrogen sulfate (68 mg, 0.2 mmol), K_2CO_3

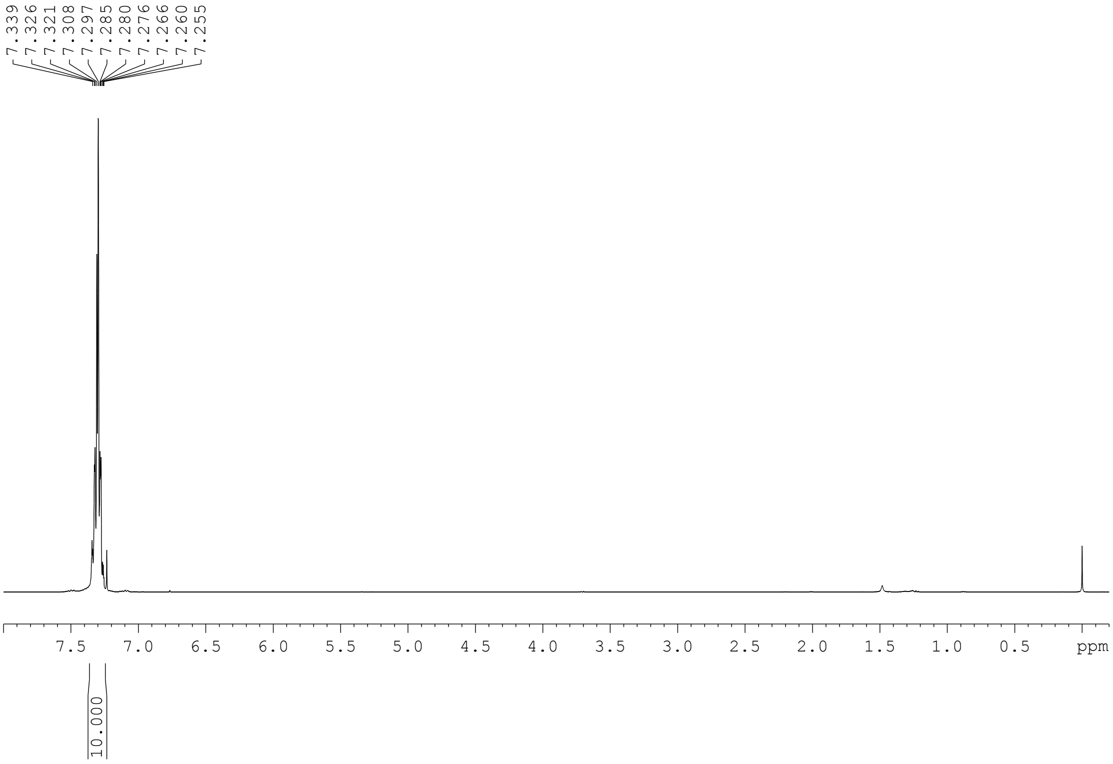
(828 mg, 6 mmol) in toluene (40 ml) and water (20 ml) were added and heated to 90^{o}C and stirred under nitrogen overnight. After cooling down to room temperature, the organic layer was separated and the water layer was extracted with dichloromethane (DCM). The combined organic solution was dried with Na_2SO_4 for several hours. After filtration, the resulting solution was concentrated by rotary evaporator. The residue was purified by column chromatography on silica gel using DCM/PE (v/v: 1:9) as the eluent to obtain the 2,2'-(2,2-diphenylethene-1,1-diyl)bis-(methylbenzene) **DMTPE** (0.58 g, 80.4 %). ^{1}HNMR ($CDCl_3$, 400 MHz) δ (ppm): 6.95-7.07 (m, 18H), 2.09 and 2.00 (d, br., 6H). ^{13}CNMR ($CDCl_3$, 100 MHz) δ (ppm): 142.97, 131.42, 131.04, 130.22, 127.37, 126.64, 126.58, 125.27, 20.21. MADLI-TOF: found: 360.394.

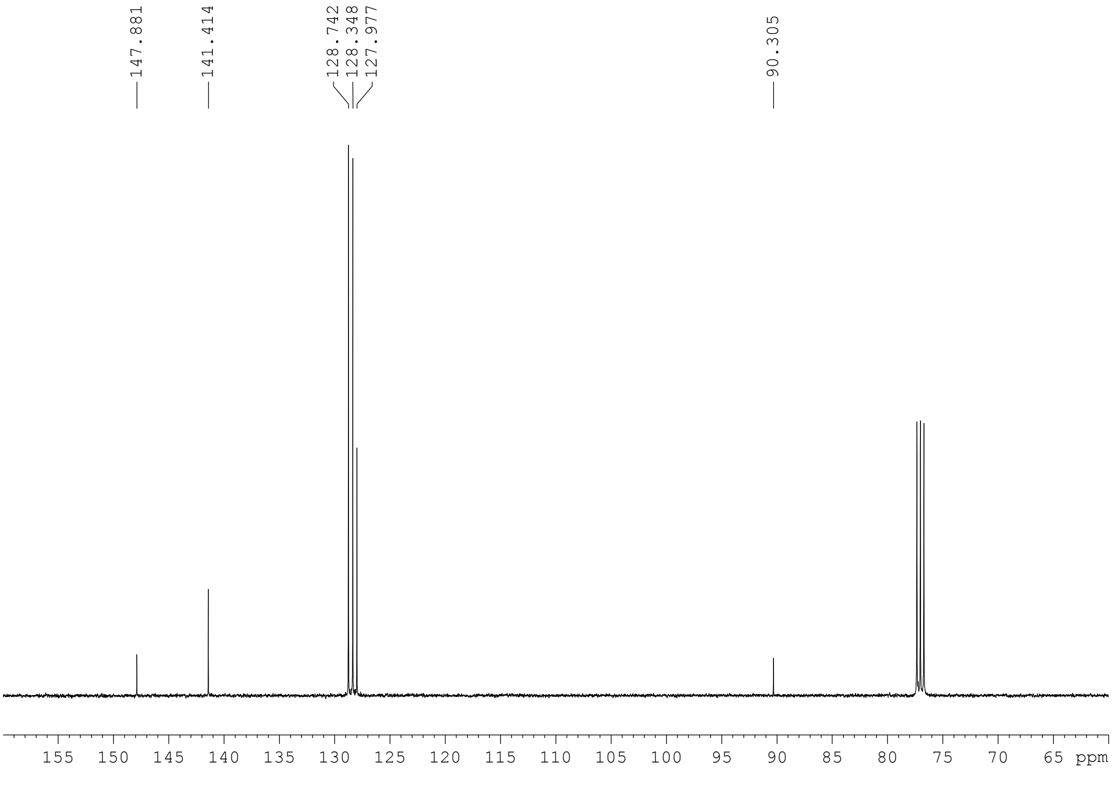
2,2'-(2,2-diphenylethene-1,1-diyl)bis(1,3-dimethylbenzene) (**TMTPE**): A mixture of 1,1-dibromo-2,2-diphenylene (1) (0.67 g, 2 mmol), (2,6-dimethylphenyl)boronic acid (3) (0.37 g, 2.5 mmol), $Pd(PPh_3)_4$ (115 mg, 0.1 mmol), tetrabutyl-ammonium hydrogen sulfate (68 mg, 0.2 mmol), K_2CO_3 (828 mg, 6 mmol) in toluene (40 ml) and water (20 ml) were added to a two-neck flask (100 ml), and heated to 90^{o}C and stirred under nitrogen overnight. After cooling down to room temperature, the organic layer was separated and the water layer was extracted with dichloromethane (DCM). The combined organic solution was dried with Na_2SO_4 for several hours. After filtration, the resulting solution was concentrated by rotary evaporator. The residue was purified by column chromatography on silica gel using DCM/PE (v/v: 1:9) as the eluent to obtain the 2,2'-(2,2-diphenylethene-1,1-diyl)bis(1,3-dimethylbenzene) **TMTPE** (0.53 g, 68.2 %). ^{1}HNMR ($CDCl_3$, 400 MHz) δ (ppm): 6.92-7.09 (m, 12H), 6.83 (d, J = 7.2 Hz, 2H), 6.74 (d, J = 7.6 Hz, 2H), 2.10 (s, 3H), 2.00 (s, 3H). ^{13}CNMR ($CDCl_3$, 100 MHz) δ (ppm):147.16, 143.40, 140.70, 138.11, 138.81, 136.34, 131.45, 128.47, 127.86, 127.02, 126.73, 21.87, 21.11. MADLI-TOF: found: 388.451.

2. NMR spectra.

Compound







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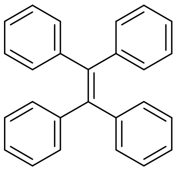

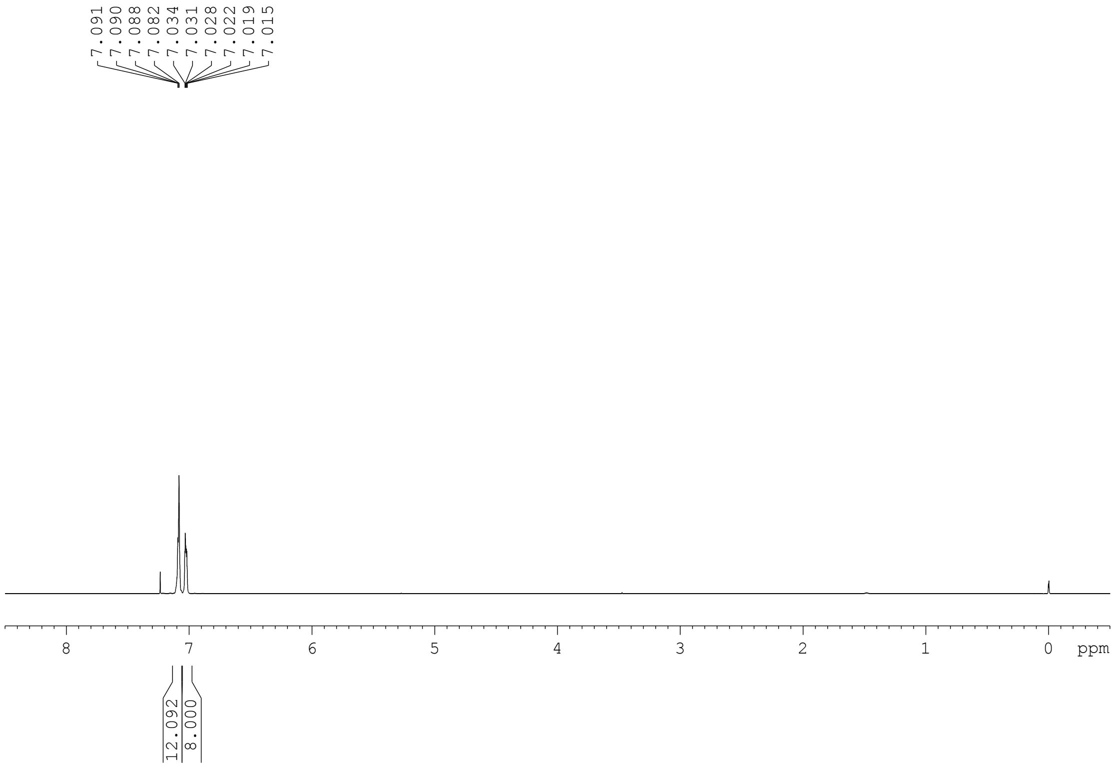
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7.088
7.082
7.034
7.031
7.028
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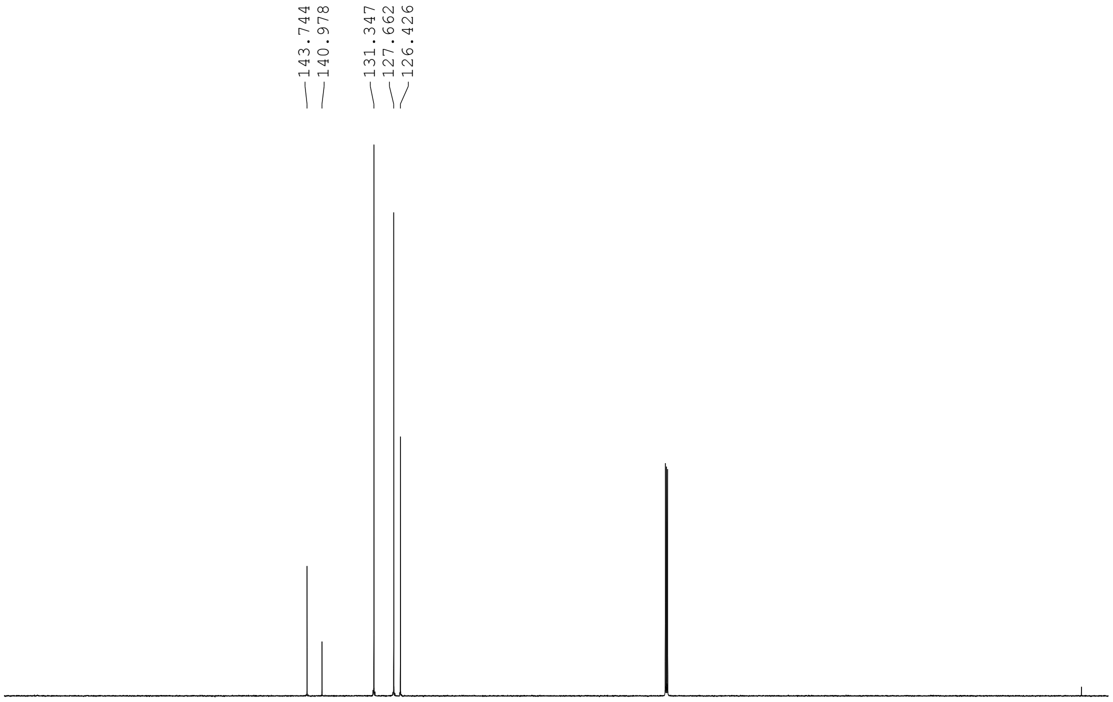
7
6
5
4
3
2
1
0
ppm
12.092
8.000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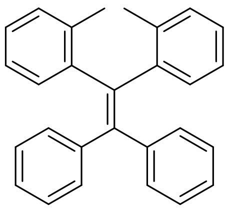
20
10
ppm



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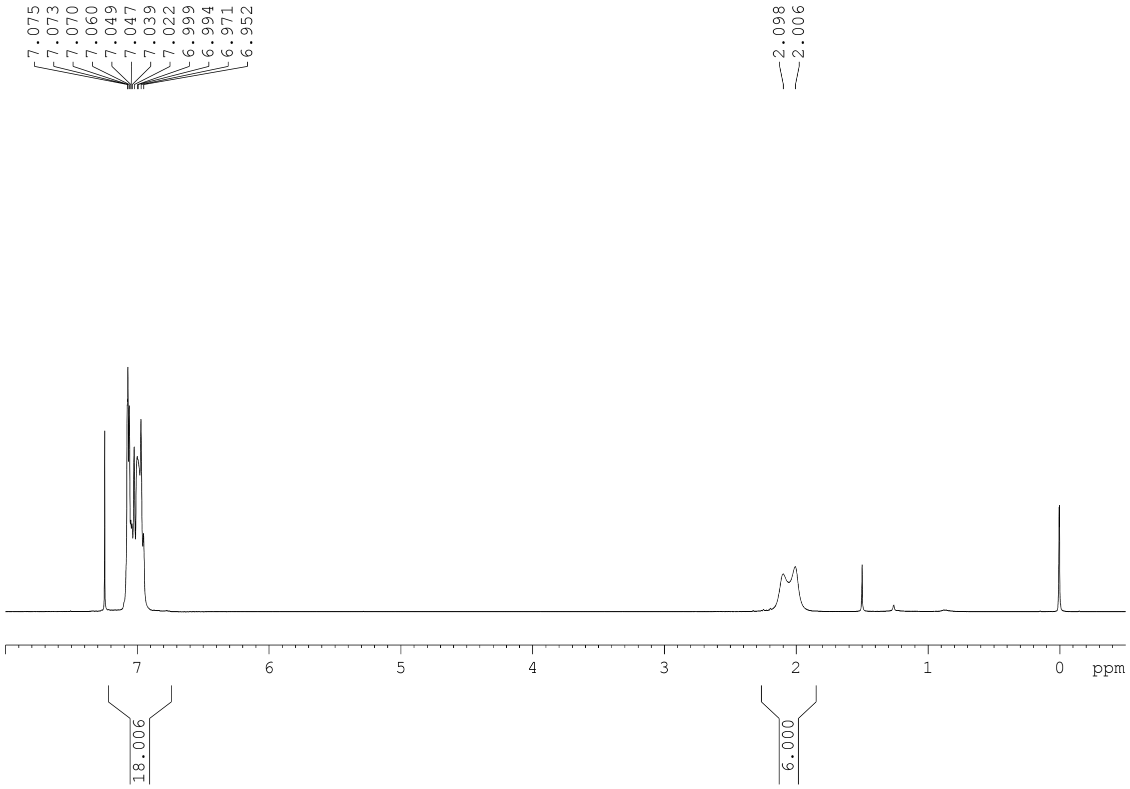
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7.047
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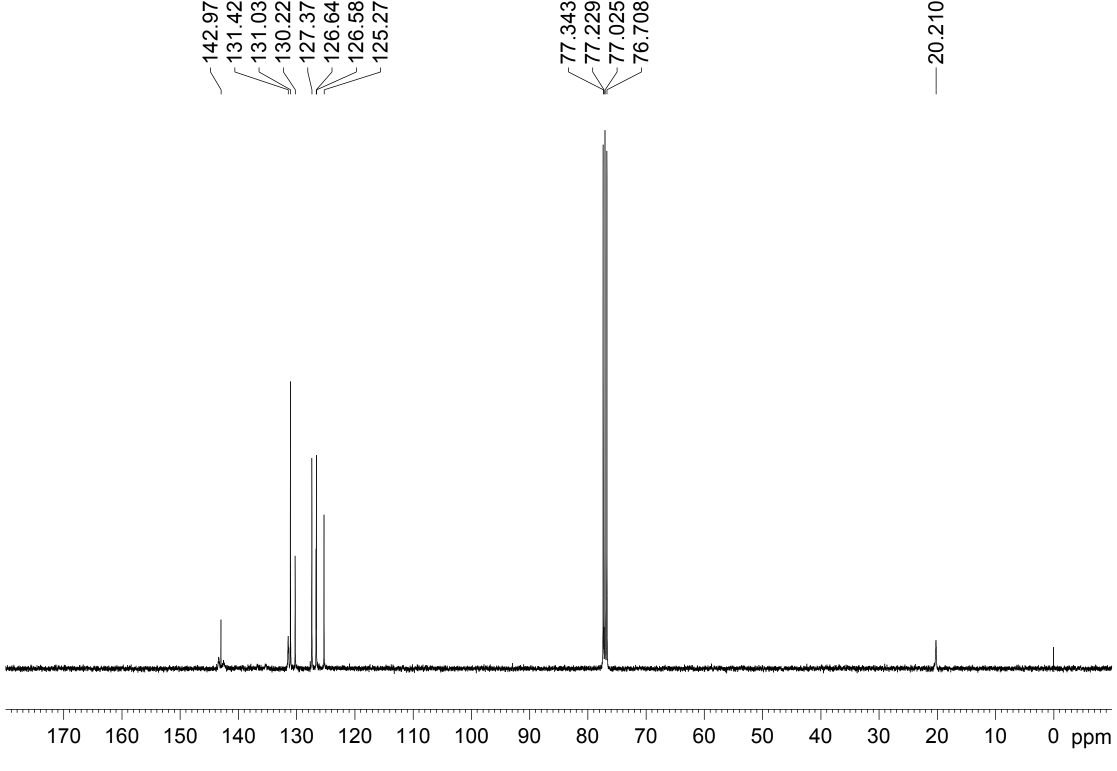
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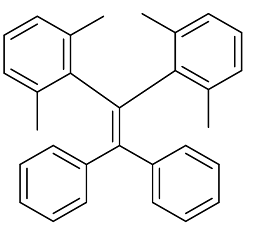
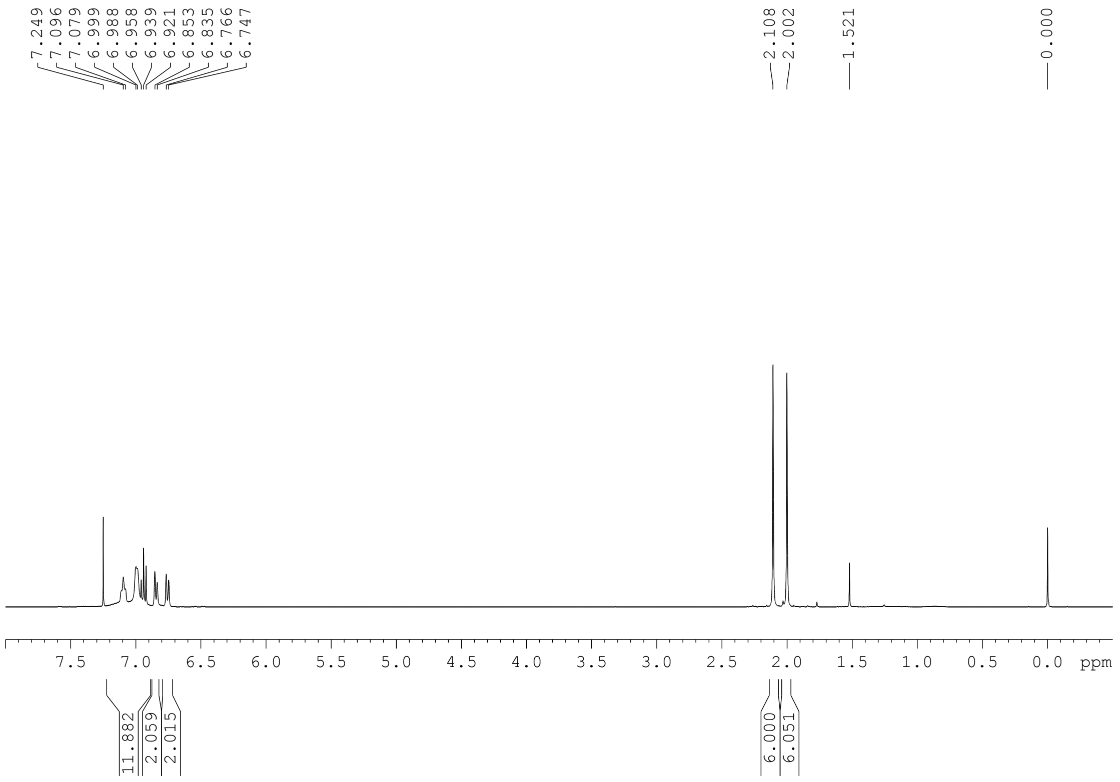


Compound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6.747
2.108
2.002
1.521
0.000
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
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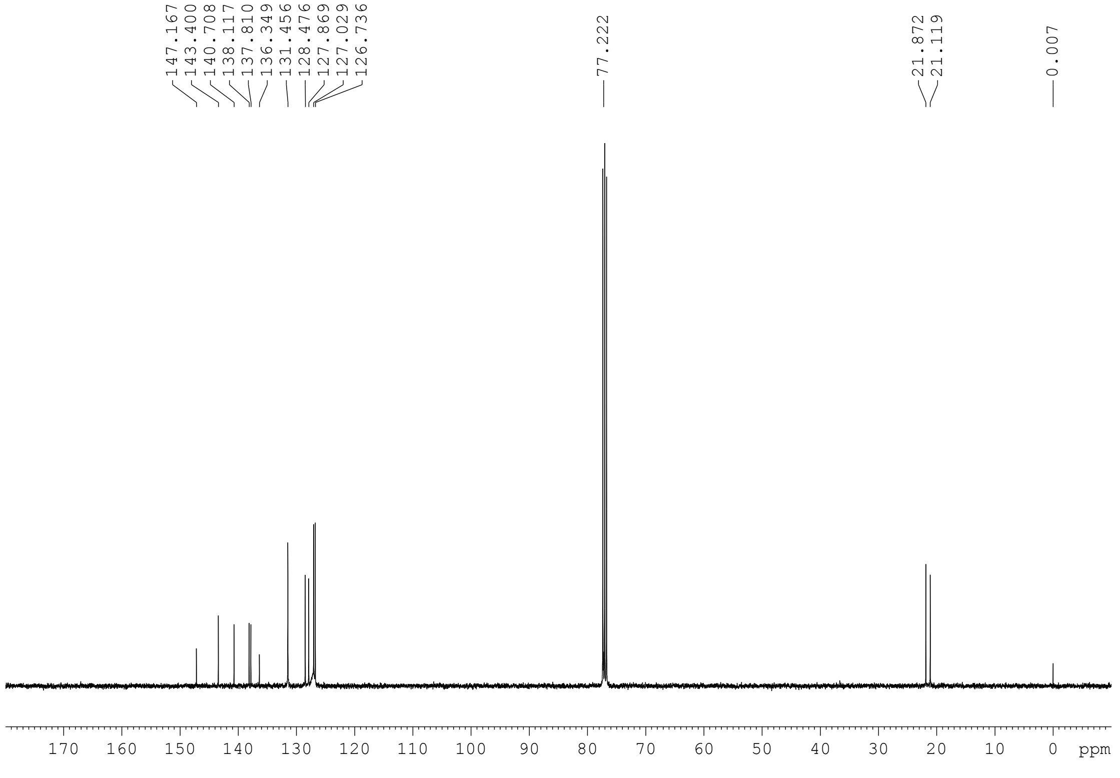
6.051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21.119
0.007
170
160
150
140
130
120
110
100
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80
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50
40
30
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0
ppm

3. PL spectrum of TPE in different THF-Water solution.

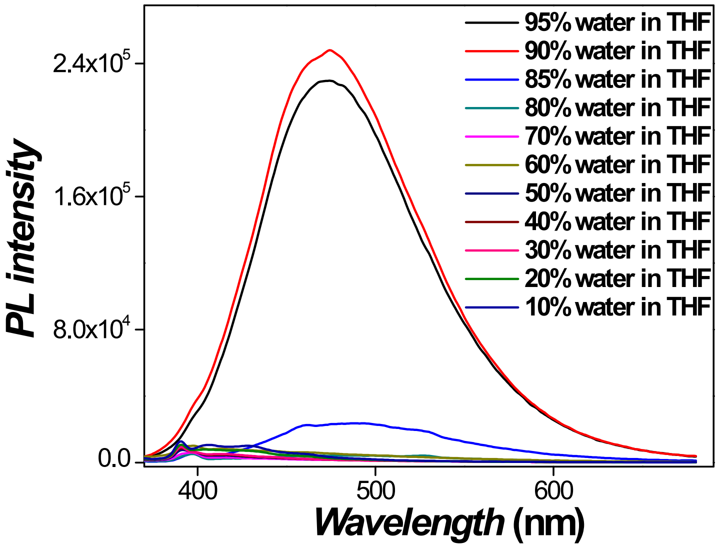

Fig. S1 PL spectrum of TPE in different THF-Water solution at concentration = 1 x 10^{-5} M.

4. PL spectrum of TMTPE in different THF-Water solution.

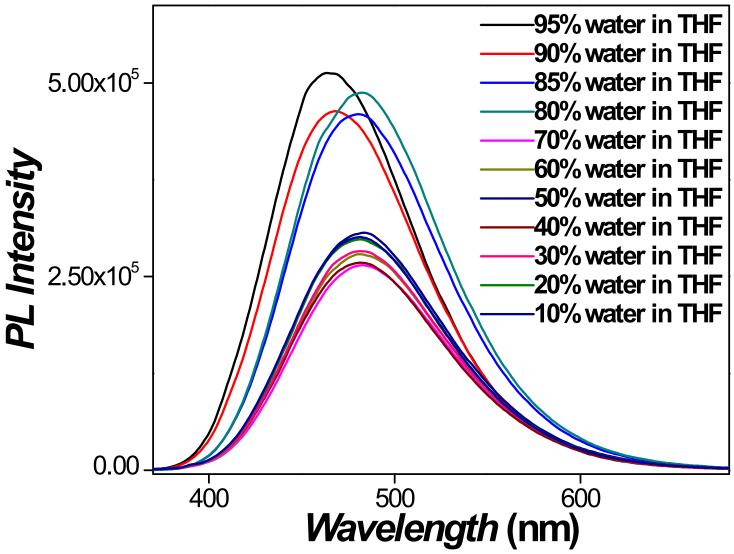

Fig. S2 PL spectrum of TMTPE in different THF-Water solution at concentration = 1 x 10^{-5} M.

5. XRD spectra of *ortho*-subtituted TPEs

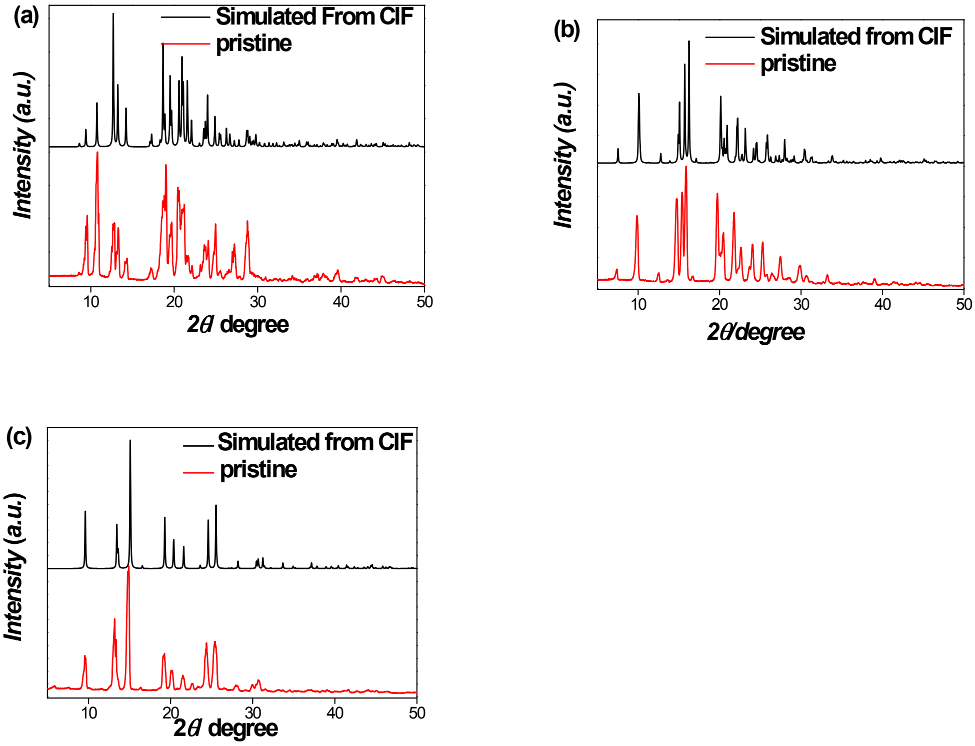

Fig. S3 XRD spectra of (a) TPE; (b) DMTPE; (c) TMTPE.

6. Fluorescence lifetime of TPE analogues

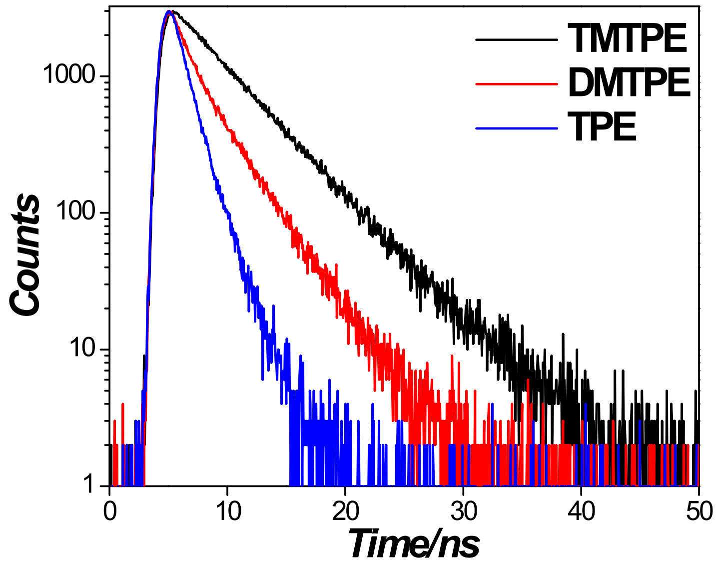

Fig. S4. Fluorescence lifetime spectrum of TPE, DMTPE and TMTPE.

7. Density functional theory (DFT) calculations

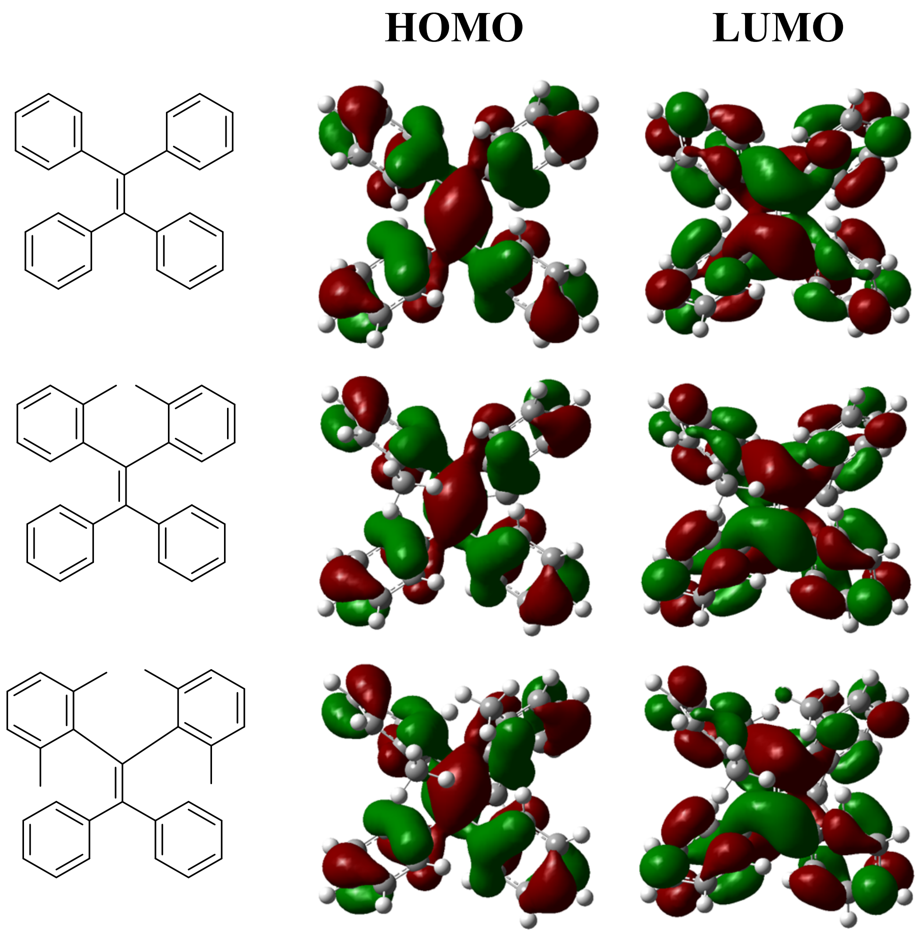

Fig. S5 HOMO-LUMO energy levels of ortho-substituted TPEs calculated using the B3LYP/6-31G(d) basis set.

8. Crystallographic data

Table S1 Crystallographic data of DMTPE and TMTPE

Structure	TPE	DMTPE	TMTPE
temperature (K)	298(2)	296(2)	100(2)
chemical formula	$C_{26}H_{20}$	$C_{28}H_{24}$	$C_{30}H_{28}$
crystal system	monoclinic	triclinic	tetragonal
space group	*P*2(1)	*P*-1	*P* 4/n
formula weight	332.42	360.47	388.52
a (Å)	9.8251(19)	9.036(19)	12.9991(19)
b (Å)	9.5020(18)	9.48(2)	12.9991(19)
c (Å)	10.709(2)	12.90(3)	6.5730(10)
α (°)	90	111.14(3)	90
β (°)	107.096(3)	98.54(5)	90
γ (°)	90	98.34(3)	90
V (Å^3)	955.6(3)	995(4)	1110.7(3)
D_c(gcm^{-3})	1.155	1.203	1.162
F (000)	352	384	416
Z	2	2	2
μ (mm^{-1})	0.065	0.068	0.065
R_1,[I > 2σ(I)]	0.0414	0.0571	0.0651
R_1, (all data)	0.0514	0.0728	0.0785
ωR_2, [I > 2σ(I)]	0.1167	0.1537	0.1656
ωR_2, (all data)	0.1263	0.1694	0.1827
R_{int}	0.0210	0.0361	0.0250
GOF	1.053	1.022	1.075

9. ORTEP structures

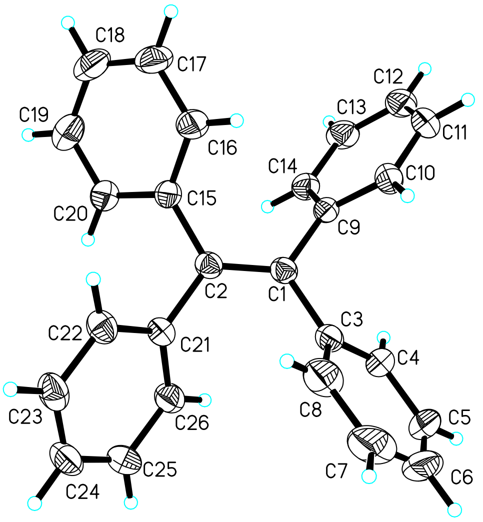

Fig. S6 ORTEP molecular structures of TPE shown as 30% thermal ellipsoid probability.

A specimen of $C_{26}H_{20}$, approximate dimensions 0.200 mm x 0.200 mm x 0.200 mm, was used for the X-ray crystallographic analysis. The X-ray intensity data were measured.

The integration of the data using a monoclinic unit cell yielded a total of 9662 reflections to a maximum θ angle of 30.00° (0.71 Å resolution), of which 5085 were independent (average redundancy 1.900, completeness = 99.7%, R_{int} = 2.10%, R_{sig} = 3.01%) and 4252 (83.62%) were greater than $2\sigma(F^2)$. The final cell constants of a = 9.8251(19) Å, b = 9.5020(18) Å, c = 10.709(2) Å, β = 107.096(3)°, volume = 955.6(3) $Å^3$, are based upon the refinement of the XYZ-centroids of reflections above 20 σ(I). The calculated minimum and maximum transmission coefficients (based on crystal size) are 0.9871 and 0.9871.

The structure was solved and refined using the Bruker SHELXTL Software Package, using the space group P 1 21 1, with Z = 2 for the formula unit, C26H20. The final anisotropic full-matrix least-squares refinement on F^2 with 235 variables converged at R1 = 4.14%, for the observed data and wR2 = 12.63% for all data. The goodness-of-fit was 1.053. The largest peak in the final difference electron density synthesis was 0.173 e-/$Å^3$ and the largest hole was -0.209 e-/$Å^3$ with an RMS deviation of 0.036 e-/$Å^3$. On the basis of the final model, the calculated density was 1.155 g/cm^3 and F(000), 352 e^-.

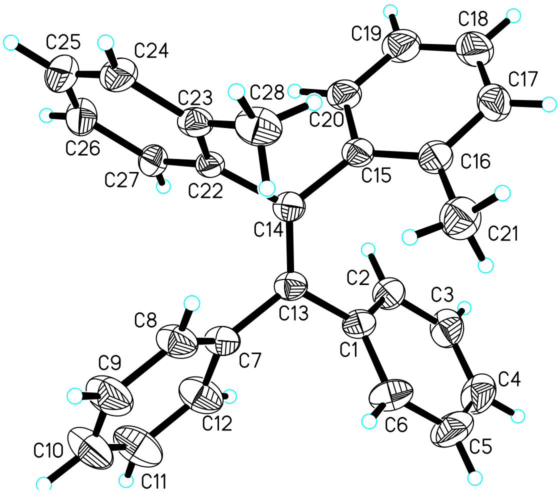

Fig. S7 ORTEP molecular structures of DMTPE shown as 30% thermal ellipsoid probability, and the disordered atoms C22’-C28’ were omitted for clarity.

A specimen of $C_{28}H_{24}$, approximate dimensions 0.100 mm x 0.100 mm x 0.120 mm, was used for the X-ray crystallographic analysis. The X-ray intensity data were measured.

The integration of the data using a triclinic unit cell yielded a total of 6919 reflections to a maximum θ angle of 25.00° (0.84 Å resolution), of which 3447 were independent (average redundancy 2.007, completeness = 98.2%, R_{int} = 3.61%, R_{sig} = 5.12%) and 2646 (76.76%) were greater than $2\sigma(F^2)$. The final cell constants of a = 9.036(19) Å, b = 9.48(2) Å, c = 12.90(3) Å, α = 111.14(3)°, β = 98.54(5)°, γ = 98.34(3)°, volume = 995.(4) Å3, are based upon the refinement of the XYZ-centroids of reflections above 20 σ(I). The calculated minimum and maximum transmission coefficients (based on crystal size) are 0.9919 and 0.9933.

The structure was solved and refined using the Bruker SHELXTL Software Package, using the space group *P* -1, with Z = 2 for the formula unit, $C_{28}H_{24}$. The final anisotropic full-matrix least-squares refinement on F2 with 297 variables converged at R1 = 5.58%, for the observed data and wR2 = 16.81% for all data. The goodness-of-fit was 1.023. The largest peak in the final difference electron density synthesis was 0.288 e-/Å^3 and the largest hole was -0.219 e-/Å^3 with an RMS deviation of 0.035 e-/Å^3. On the basis of the final model, the calculated density was 1.203 g/cm^3 and F(000), 384 e$^-$.

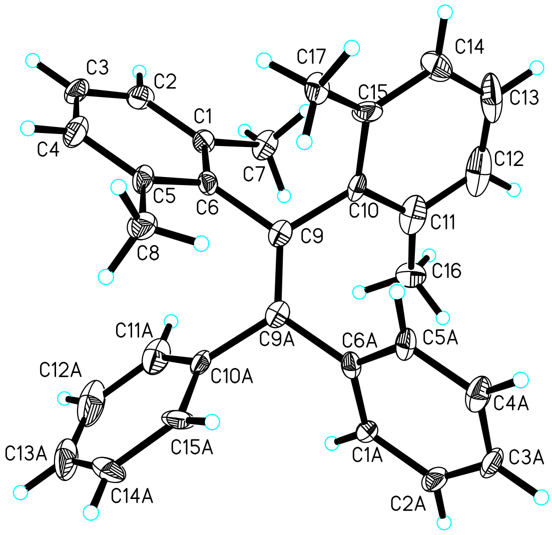

Fig. S8 ORTEP molecular structures of TMTPE shown as 30% thermal ellipsoid probability. Symmetry code: A = 1/2 - x, 1/2 - y, z. (The asymmetric unit of the compound TMTPE composed of one fourth of the molecule with all the atoms were symmetry-disordered by the four-fold axis. The occupancies of the methyl atoms were assigned to be 0.25 and 0.5 for the ethylene carbon atoms. All the aromatic atoms were assigned to be 0.5.)

A specimen of $C_{30}H_{28}$, approximate dimensions 0.200 mm x 0.200 mm x 0.200 mm, was used for the X-ray crystallographic analysis. The X-ray intensity data were measured.

The integration of the data using a tetragonal unit cell yielded a total of 11591 reflections to a maximum θ angle of 31.48° (0.68 Å resolution), of which 1838 were independent (average redundancy 6.306, completeness = 99.0%, R_{int} = 2.50%, R_{sig} = 1.68%) and 1492 (81.18%) were greater than $2\sigma(F^2)$. The final cell constants of a = 12.9991(19) Å, b = 12.9991(19) Å, c = 6.5730(10) Å, volume = 1110.7(3) Å3, are based upon the refinement of the XYZ-centroids of reflections above 20 σ(I). Data were corrected for absorption effects using the multi-scan method (SADABS). The ratio of minimum to maximum apparent transmission was 0.913. The calculated minimum and maximum transmission coefficients (based on crystal size) are 0.9871 and 0.9871.

The structure was solved and refined using the Bruker SHELXTL Software Package, using the space group *P* 4/n, with Z = 2 for the formula unit, $C_{30}H_{28}$. The final anisotropic full-matrix least-squares refinement on F2 with 132 variables converged at R1 = 6.51%, for the observed data and wR2 = 18.27% for all data. The goodness-of-fit was 1.075. The largest peak in the final difference electron density synthesis was 0.268 e-/Å^3 and the largest hole was -0.279 e-/Å^3 with an RMS deviation of 0.038 e-/Å^3. On the basis of the final model, the calculated density was 1.162 g/cm^3 and F(000), 416 e$^-$.

10 Confocal laser scanning microscope (CLSM) imaging of Hela cells visualized by TMTPE and LysoTracker Red.

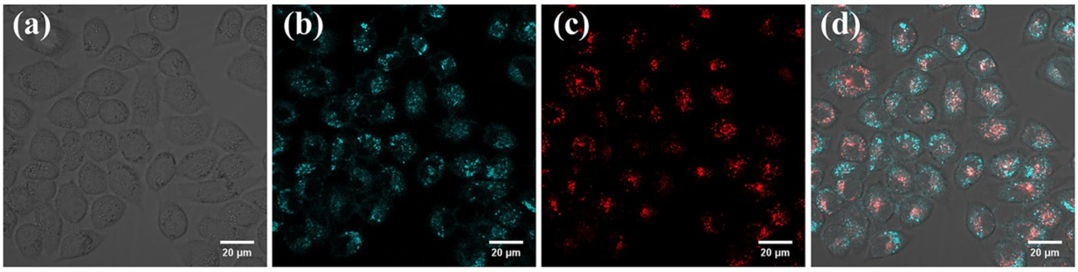

Fig. S9 The confocal laser scanning microscope (CLSM) imaging of Hela cells: (a) bright field; (b) incubated with TMTPE; (c) incubated with LysoTracker Red; (d) the merged imaging of (a), (b) and (c).

11. References

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