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COMMUNICATION

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

Cite this: DOI: 10.1039/x0xx00000x

Received 00th January 2012,
Accepted 00th January 2012

DOI: 10.1039/x0xx00000x

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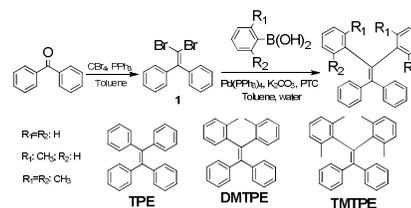
Guo-Feng Zhang,^a Ze-Qiang Chen,^a Matthew P. Aldred,^{*a} Zhe Hu,^a Tao Chen,^a Zhen-li Huang,^a Xianggao Meng,^{*b} and Ming-Qiang Zhu^{*a}

We demonstrate a novel synthetic approach to *ortho*-methyl substituted tetraphenylethene materials, which can be utilised for directly validating the restriction of intramolecular rotation hypothesis as the basic mechanism of aggregation-induced emission phenomenon and cell imaging.

During the recent decades, diverse attentions have been paid to design and synthesize novel organic fluorescent materials for the potential applications in sensors, optoelectronic devices and bioimaging, which is probably due to the variability and flexibility of chemical modifications.¹ Unfortunately, most fluorophores encounter with a common problem denoting “aggregation caused quenching (ACQ)” in solid state, because of the intrinsic intermolecular π - π stacking and/or self-absorption related to a small Stokes’s shift.² In 2001, an opposite and abnormal phenomenon named “aggregation-induced emission (AIE)” was observed by Tang’s group.³ A facile strategy to surmount the ACQ fluorophores is manifested by bonding them with AIE-active counterparts. Since then, various fluorophores with AIE characteristic have been reported and investigated.⁴ In the previous experimental observations and theoretical calculations, the proposed hypotheses which is accountable for the underlying mechanism of this newly AIE phenomenon are still plausible and ambiguous, such as the restriction of intramolecular rotation (RIR), *E/Z* isomerisation and *J*-aggregation formation.⁵ Therefore, it is urgently desirable to acquire novel molecules *via* simple modifications to directly validate the proposed hypotheses.

The most materials possessing AIE properties illustrated in the literatures are associated with silole or tetraphenylethene (TPE) motifs.^{2b,4} According to the molecular architecture designed by Tang and co-workers,⁶ the intramolecular rotation in hexaphenylsilole (HPS) can be extremely impeded *via* the introduction of the bulky isopropyl groups on phenyl rings with *ortho*-positions in silole regioisomers. With respect to TPEs, intensive endeavours, such as the construction of conjugated

microporous polymers, coordinative immobilization, locking the phenyl rings of TPE, host-guest inclusion, have been devoted to probing this intriguing AIE-active characteristics and the possible mechanism of the AIE phenomenon.⁷ However, the strategies to straightforward validation of RIR hypothesis for TPE analogues at molecular level are still seldom reported, which is probably due to the difficulty in the aspect of chemical syntheses and modifications.

Scheme 1 Synthetic route to novel *ortho*-methyl substituted TPEs.

Our aim was to directly verify the RIR hypothesis for the TPE derivatives at molecular level *via* facile chemical modifications, and investigate the influence of steric congestion between the contiguous phenyl rings in TPE unit. However, the well-developed approaches (Scheme S1): McMurry reaction⁸ and Rathore’s procedures,⁹ to producing TPE derivatives can hardly meet the increasing demands for diverse structural variations, which is not suitable for many substrates due to the impractical to access the requisite diaryl ketones. Therefore, it is imperative to introduce an alternative way to afford the *ortho*-substituted TPEs to directly validate the RIR hypothesis for AIE phenomenon by chemical modifications.

Herein, we report a versatile approach to the synthesis of *ortho*-methyl substituted TPE *via* Corey-Fuchs reaction and subsequent Suzuki coupling reaction.¹⁰ The synthetic route is outlined in Scheme 1, and the final products were characterized by NMR, MS, and X-ray crystallography. The resultant TPE, di(*ortho*-methyl)-substituted TPE (DMTPE) and tetra(*ortho*-methyl)-substituted TPE (TMTPE) were afforded with excellent yields, and can be dissolved in common solvents with

excellent solubility. The detailed procedures are depicted in ESI.

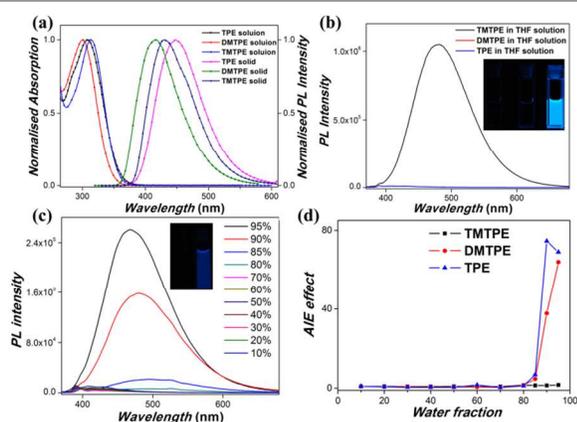


Fig. 1 (a) Normalised UV-Vis absorption of TPE, DMTPE and TMTPE in THF solution and PL spectra of TPE, DMTPE and TMTPE in solid state. (b) PL spectra of TPE, DMTPE and TMTPE in THF solution (10^{-5} M) under same circumstance (insert: their related fluorescence picture under 365 nm irradiation). (c) Emission spectra of DMTPE in THF-water mixtures (insert: fluorescence picture of DMTPE in 10% and 95 % water under 365 nm irradiation). (d) AIE effect ($\alpha_{AIE} = I/I_0$) of TPE, DMTPE and TMTPE.

The respective maximum UV-Vis absorption peaks (λ_{abs}) of TPE, DMTPE and TMTPE in THF solutions are located at 308 nm, 300 nm and 314 nm (Fig. 1a and Table 1). The individual maximum PL emission peaks (λ_{em}) of TPE, DMTPE and TMTPE in solid state are present at 448 nm, 417 nm and 429 nm. Measured at the same circumstance, the PL intensity of TMTPE in THF solution, with the QY up to 64.3%, is much higher than DMTPE and TPE (Fig. 1b). As shown in Figure 1b (insert picture), the THF solution of TMTPE (10^{-5} M) emits bright cyan-fluorescence under 365nm irradiation, while vague fluorescence can be observed *via* naked eyes for both of TPE and DMTPE. Similar to TPE (Fig. S1), DMTPE exhibits archetypal AIE characteristic. With the water fraction increased from 10% to 80%, the PL intensity display unnoticeable variations (Fig. 1c). The PL intensity is dramatically enhanced as the water fraction promoted from 80% to 95% (Fig. 1c, insert picture). On the contrary, TMTPE shows little AIE-active attribute (Fig. 1d and S2), and the profile alterations of PL spectra in different THF-water mixtures are probably due to the effect of agglomeration and morphology transition.¹¹

As we can envisage, the steric hindrance between the relevantly geminal phenyl rings are gradually enhanced with the number of *ortho*-methyl groups increased. With respect to TMTPE, due to the introduction of tetra(*ortho*-methyl) groups in TPE, the motion or vibration of the phenyl rings is extremely suppressed, which imparts the enhanced fluorescence in diluted solutions. In comparison of DMTPE, the rigidity of TMTPE molecule is probably improved as well, on account of the steric repulsion of tetra(*ortho*-methyl) groups in TPE, giving rising to fluorescence QYs boosted from 0.32% (DMTPE) to 64.3% (TMTPE) in THF solution (Table 1). Namely, by internal control through the introduction of *ortho*-methyl groups, the PL properties of the TPE derivatives can be

modulated, indicating that steric congestion of the relevant phenyl rings can fix the molecules and significantly inhibit the non-radiance annihilation process, thus TMTPE exhibit high luminescence even in dilute solution. Based on steric hindrance triggered by the introduction of *ortho*-methyl groups in TPE unit, RIR, as the fundamental mechanism of the intriguing AIE phenomenon, is directly verified through simply chemical modifications. Probably, the investigations on AIE phenomenon will be adequately emerging thanks to a definite understanding of the mechanism of AIE characteristics, which will be also beneficial for the novel molecular design.

Verified by powder X-ray diffraction (PXRD) showing several sharp peaks (Fig. S3), all the associated pristine states are indicative of crystalline. The fluorescence lifetime of these analogues are gradually increased from 1.37 ns (TPE), 2.74 ns (DMTPE) to 4.63 ns (TMTPE) (Fig. S4). Density functional theory (DFT) calculations were performed using Gaussian 09 program using B3LYP/6-31G(d) basis set, manifesting that the optimized structures of DMTPE and TMTPE are quite contorted. Accordingly, the HOMO and LUMO of these analogues are located on the whole molecules (Fig. S5). The energy level of TPE, DMTPE and TMTPE obtained from calculations are 4.12, 4.29 and 4.20 eV, respectively.

Table 1 optical and electrochemical properties.

	λ_{abs}/nm	λ_{em}/nm		Φ_F (%) ^a		α_{AIE} ^c	E_g ^d	τ/ns
		solution	solid	solution	aggregate ^b			
TPE	308	n.d.	448	0.20	13.8	69	3.48	1.37
DMTPE	300	n.d.	417	0.32	20.5	64	3.61	2.74
TMTPE	314	481	429	64.3	96.4	1.5	3.50	4.63

^a 9,10-diphenylanthracene ($\Phi_F = 0.9$ in cyclohexane) was used as a standard; ^b Aggregates formed in 95% water in THF mixture solvents; ^c $\alpha_{AIE} = I/I_0$; ^d Optical band gap (E_g) estimated from the onset wavelength (λ_{onset}) of the absorption spectra.

To further investigate intermolecular interactions between the adjacent phenyl rings in these TPE derivatives, single crystals were obtained *via* slowly solution evaporation process. Their individual ORTEP structures and detailed crystallographic data are shown in Fig.2 and S6-8 (ESI), respectively. Calculated by PLATON, no classical $\pi \cdots \pi$ interactions can be observable in all of them. On terms of TPE, C-H \cdots π interaction (Fig. 2d) with the distance of 2.95Å (H23 \cdots Cg_{C9-C14}, Cg is a centroid defined by some specified atoms) can be observed. For DMTPE, the phenyl group C22-C27 and methyl C28 atoms are disordered over two sites with the site occupancies of 0.70(1):0.30(1) for the major and minor components, respectively. Only one kind of C-H \cdots π interaction with the distance of 2.73Å (H26 \cdots Cg_{C15-C20}) presents. The two DMTPE molecules form a dimer in the molecular packing associated with C-H \cdots π interactions (Fig. 2e). With respect to TMTPE, the asymmetric unit composed of one fourth of the molecule with all the atoms are symmetry-disordered by the four-fold axis. No apparent $\pi \cdots \pi$ or C-H \cdots π can be observed in bulky crystal, instead the whole molecules packed *via* weak van der Waals interactions (Fig. 2d), which is probably due to the steric congestion of the methyl groups. Consequently, these

loose packing mode and propeller-like structures will be beneficial for the efficient photoluminescence in solid state.

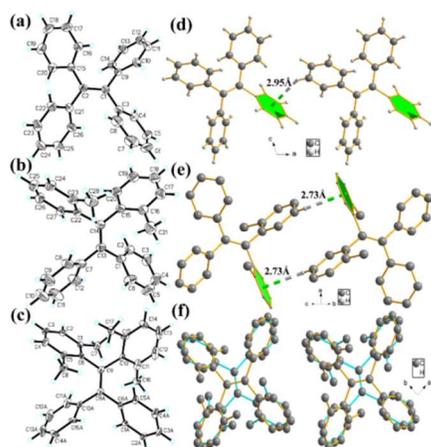


Fig. 2 ORTEP molecular structures of (a) TPE; (b) DMTPE; (c) TMTPE shown as 30% thermal ellipsoid probability. The respective molecular packing mode of single crystal: (d) TPE; (e) DMTPE; (f) TMTPE, symmetry code: A = 1/2 - x, 1/2 - y, z. The disordered atoms and non-related hydrogens are omitted for clarity.

Since TMTPE possess great fluorescence independent of solvents, both in solution or solid state, we utilised TMTPE with high quantum yield as a fluorescent probe for the application in biological imaging. The HeLa cells were co-incubated with TMTPE using 10% DMSO in water mixture solution. As the confocal laser scanning microscope (CLSM) imaging shown in Fig. 3 and S9 (ESI), the HeLa cells incubated with TMTPE and LysoTracker Red emitted cyan and red fluorescence upon UV irradiation at 405 nm, respectively, manifesting that TMTPE can infiltrate the cell membrane probably via endocytosis and be employed as a luminescent visualizer for cell imaging.

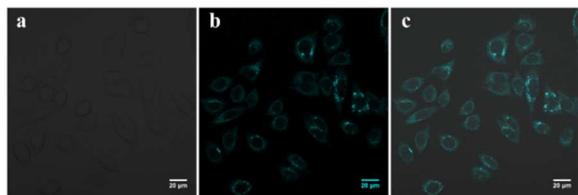


Fig. 3 Confocal laser scanning microscope (CLSM) imaging of HeLa cells: (a) bright field; (b) incubated with TMTPE; (c) the merged imaging of (a) and (b).

In conclusion, we have demonstrated a versatile approach to the synthesis of *ortho*-methyl substituted TPE *via* Corey-Fuchs and subsequent Suzuki coupling reactions. The introduction of *ortho*-methyl groups in DMTPE and TMTPE has a significant influence on the AIE effect, which is due to the enhancement of steric hindrance in the contiguous phenyl rings with *ortho*-groups. Through internal control *via* the introduction of *ortho*-groups, the RIR hypothesis as the basic mechanism for the AIE-active TPE derivatives is directly validated. Meanwhile, with further modifications, TPE derivatives with the introduction of bulky groups at the *ortho*-positions may be promising candidates for the fabrication of OLEDs and luminescent visualizers for cell imaging.

This work was supported by the NSFC (21174045), 973 Program of China (2013CB922104). We also thank Analytical and Testing Center of Huazhong University of Science and Technology.

Notes and references

^a Wuhan National Laboratory for Optoelectronics, School of Optical and Electronic Information, Huazhong University of Science and Technology, Wuhan, Hubei, China, 430074, China; Fax: 862787793419; Tel: 862787793419; 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.ccnucnu.edu.cn.

† Electronic Supplementary Information (ESI) available: experimental procedures/characterisation, optical spectra, molecular simulation and crystallographic information files (CCDC 1006150, CCDC 1006151; CCDC 1006152). See DOI: 10.1039/c000000x/

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