



One-step assembly of Pd-Keggin-polyoxometalates for catalytic benzothiadiazole generation and derived cell-imaging probe application

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ABSTRACT

One-step assembly of organic-ligand modified Pd-Keggin-POMs has been rarely reported, so as for their applications in catalytic benzothiadiazole generation and derived cell-imaging probing. Herein, three Pd-Keggin-POMs (compounds **1–3**) have been successfully synthesized via a one-step assembly strategy. Thus-obtained Pd-Keggin-POMs with well-defined structures and heterogeneous properties enable highly efficient catalytic benzothiadiazole generation. Specifically, compound **3** showed outstanding catalytic activities in Suzuki-Miyaura coupling reactions for the generation of benzothiadiazole derivatives (yields, 90%–97%) and was represented as one of the best catalysts reported to date. Consequently, the obtained benzothiadiazoles were used as the bio-probe for tracking lipid droplets in living-cells and exhibited large Stokes shifts (130 nm), low cytotoxicity and good targeting, which could be also applied to mark the distribution of LDs in living HeLa cells. Systematic investigations clearly decipher the functions of Pd-Keggin-POMs toward finding novel bio-probe materials, highlighting a new insight into the generation of sustainable materials in life-science.

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2,1,3-Benzothiadiazole is a kind of essential structural motif in the fabrication of luminescent, electronic and biological derivatives [1–4], in which they have intriguing optical properties owing to their strong electron-withdrawing capacity, strong light absorption efficiency and tailorable band potentials, *etc.* [5–7]. The as-generated multifunctional materials have attracted considerable attention in the field of biological imaging [8], organic light emitting diodes [9], sensitizers for dye-sensitized solar cells (DSC) [10], two-photon absorption (2PA) chromophores [11] and organic photovoltaic cells [12]. Traditionally, 2,1,3-benzothiadiazole derivatives have been successively synthesized in the presence of Pd(dppf)Cl₂, Pd(PPh)₄ or prepared Pd-complexes [13–16]. However, the recy-

cling utilization of these noble metal catalysts remains a challenge due to the homogenous nature, thus these synthesis processes are generally expensive and unmet for the criteria of sustainable chemistry. Therefore, it is of paramount importance to explore efficient heterogeneous catalysts that enable the sustainably catalytic generation of functionalized 2,1,3-benzothiadiazole derivatives.

Polyoxometalates (POMs), as a family of metal oxide clusters incorporating early transition metals, have emerged as a brand-new platform materials and undergone explosive growth for many suitable applications including catalysis [17–23], magnetism [24,25], energy [26] and materials science [27–29], *etc.* Generally, the combination of reactive transition metal-complexes units into POM anions with favourable catalytic properties has received considerable interest in the catalytic reactions [30–32]. Specifically, the assembly of POMs with Pd cations produces a family of binary Pd-POMs, which not only possess the diverse

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structures, but also have significant catalytic applications in construction of C-C bonds owing to the catalytic properties of Pd [33]. However, so far, only a few organic ligand-modified Pd-POMs have been synthesized and efforts to extend this research realm of Pd-POMs have been of limited success. In the early work, Mizuno's group proposed two Pd-POMs $[(en)Pd(4,4'-bpy)]_2[\alpha-SiW_{12}O_{40}] \cdot 8DMSO \cdot 4DMF$ and $[(en)Pd(OH_2)_2]_2[\alpha-SiW_{12}O_{40}]$ with structurally well-defined clusters [34]. Later, Neumann revealed the synthetic strategy of $Pd^{II}(15-crown-5-phen)Cl_2 \cdot H_5PV_2Mo_{10}O_{40}$ (phen = 1,10-phenanthroline) with high catalytic activity in the Wacker oxidation [35]. Besides, Sun's group synthesized a binary POMs@Pd-cage complex with excellent desulfurization properties [36]. Additionally, Kortz's group reported that the successful introduction of Pd metals in POMs skeleton could not only develop the precise local structure determination but also explore the integration of intrinsic catalytic properties [37–39]. In these cases, the aforementioned synthetic strategy of Pd-POMs usually requires the pre-synthesis or modification of specially designed organic ligands, which makes the preparation process complicated, expensive and time-consuming [40]. As far as we know, the one-pot syntheses of organic ligand modified Pd-POMs with defined structures have been rarely reported, which encourages us with great interest in developing powerful strategy to meet the requirements. Hence, the exploration of novel one-pot synthetic strategy for the preparation of Pd-POMs that can be applicable in the catalytic production of 2,1,3-benzothiadiazole derivatives is highly desirable yet largely unmet.

In this work, we firstly adopted the readily available liquid imidazoles as N-ligands and three imidazole-based Pd-POMs, $[Pd(1-eIM)_4]HPW_{12}O_{40} \cdot DMSO$ (**1**), $[Pd(1-pIM)_4]_2PMo_{11}VO_{40}$ (**2**) and $[Pd(1-mIM)_4]_2HPMo_{10}V_2O_{40} \cdot 4DMSO$ (**3**) were successfully obtained through a facile one-step reaction and fully characterized by single crystal X-ray diffraction (SXRD), powder X-ray diffraction (PXRD), Fourier transform infrared spectroscopy (FT-IR) and elemental analysis (EA), etc. The above strategy is considerably simple and straightforward in the synthesis of binary Pd-POMs, owing to the complicated synthesis or modification procedures of organic ligands can be omitted. Interestingly, compound **3** exhibited extraordinary catalytic performance in the construction of 2,1,3-benzothiadiazole derivatives under mild conditions without any additional co-catalysts. Furthermore, these Pd-POMs can be fully recycled and reused with maintained catalytic activities. Moreover, in parallel to our research on the catalytic synthesis of 2,1,3-benzothiadiazole derivatives, a novel bio-probe NBDS-N has been designed with effective D- π -A structure and minimal biotoxicity, which exhibited sufficiently sensitive properties to lipid droplets (Fig. 1). The work paves a new way to design efficient POMs based catalysts for life science applications.

In this work, compounds **1–3** were synthesized by the reaction of $Pd(OAc)_2$, Keggin POMs and imidazole derivatives in the absence or presence of CF_3COOH . The synthetic pathway of binary Pd-POMs is elaborated in Fig. 2: Firstly, reacting N-donor im-

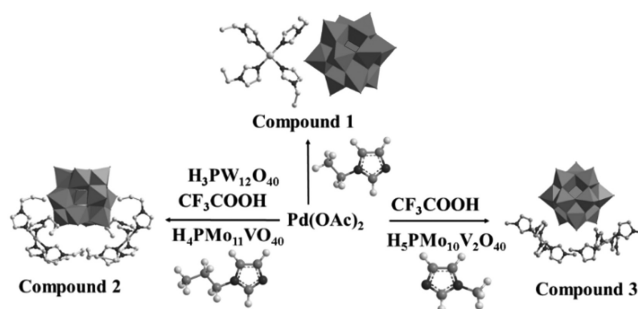


Fig. 2. Schematic representation of the formation of four binary Pd-POMs.

idazole ligands with $Pd(OAc)_2$ to substitute the acetate and give Pd-imidazole complexes in the DMSO, and then followed by the method of ion exchange reaction between Pd-imidazole complexes and three Keggin-type POMs in DMSO to yield three Pd-POMs **1–3** (Fig. 2). In the reaction, the reaction environment caused by inherent basic of different imidazoles were slightly different, and the solubility of three Pd-POMs was also different in DMSO solution. To obtain the crystals of compounds **2** and **3**, different amounts of CF_3COOH were added into the reaction to increase the solubility of Pd-POMs in DMSO and the amounts of CF_3COOH in the syntheses of compounds **2** and **3** were 30 μL and 70 μL , respectively.

SXRD shows that compound **1** crystallizes in the monoclinic space groups P21/n and is composed of one α -Keggin-type $[PW_{12}O_{40}]^{3-}$ polyanion and one $(Pd(1-eIM)_4)^{2+}$ cations (Fig. 2). In $(Pd(1-eIM)_4)^{2+}$ cations, the four 1-eIM nitrogens [N(2), N(2A), N(4) and N(6)] respectively coordinate to Pd(1) with Pd-N distances of 1.96(2)–2.00(2) Å and form nearly square-planar coordination geometry. The distance of Pd-N bonds is close to those found in $Pd(F_6acac)_2(4-ClC_5H_4N)_4$ (2.019(5)–2.034(5) Å) [41]. The Keggin $[PW_{12}O_{40}]^{3-}$ polyanion has an approximate *Td* symmetry based on a central PO_4 tetrahedron surrounded by 12 WO_6 octahedra arranged in four groups of three edge-shaped octahedra, W_3O_{13} [42].

When $[PW_{12}O_{40}]^{3-}$ was replaced by $[PMo_{11}VO_{40}]^{4-}$ and $[PMo_{10}V_2O_{40}]^{5-}$, compounds **2** and **3** are achieved, respectively (Fig. 2). Compounds **2** and **3** are isostructural, thus the structure of compound **3** is described here as an example. Compound **3** crystallizes in the Triclinic space groups P-1 and consisted of two Pd^{2+} cations and one Keggin $PMo_{10}V_2O_{40}^{5-}$ polyoxoanion. The P-O and Mo-O lengths are in the range of 1.455(19)–1.58(19) Å, and 1.635(11)–2.504(19) Å, respectively [43,44]. In compound **3**, each Pd center also displays a four-coordinated geometry and is completed by four imidazole donors. The corresponding bond lengths and angles of compound **3** are given in Table S1 (Supporting information). The Pd-N bond lengths (2.000(10)–2.019(11) Å, Table S2 in Supporting information) are close to those found in compound **1** and the reported $Pd_{24}L_{24}$ (2.02–2.03 Å; L = tris(4-(pyrimidin-5-yl)phenyl)amine) [45]. Although compounds **2** and **3** were pre-

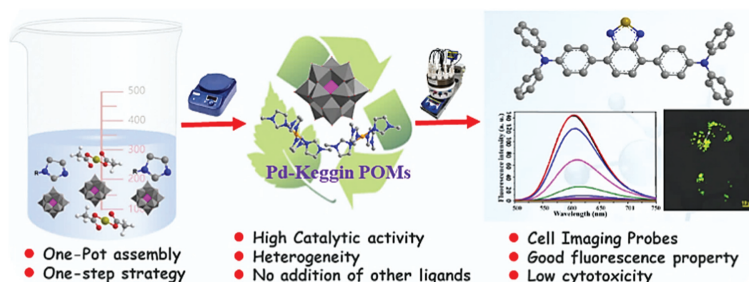
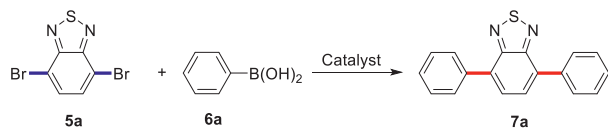


Fig. 1. Schematic representation of the one-step assembly of Pd-Keggin POMs for catalytic benzothiadiazole generation and derived cell-imaging probe application.



Scheme 1. The model reaction of Suzuki-Miyaura coupling reaction.

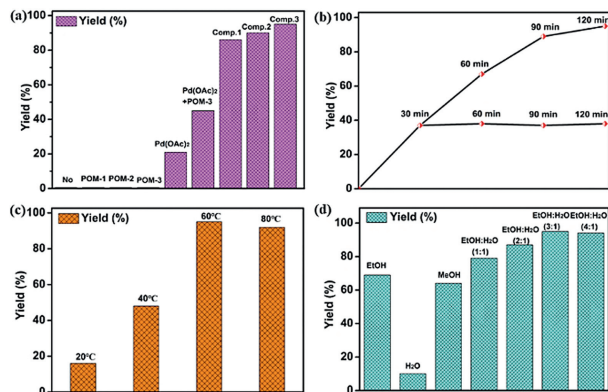


Fig. 3. The optimization conditions of Suzuki-Miyaura coupling reaction of **5a** and **6a**. Reaction conditions: **5a** (0.25 mmol), **6a** (0.55 mmol), K₂CO₃ (0.75 mmol), solvent (4 mL), catalyst (0.5 mol%), temperature 2 h. (a) Scanning the effect of different catalysts at 60°C. (b) Kinetics and leaching experiment catalyzed by compound **3**. (c) The effect of different temperature and (d) different solvents. POMs-1: H₃PW₁₂O₄₀; POMs-2: H₄PMo₁₁VO₄₀; POMs-3: H₅PMo₁₀V₂O₄₀.

pared similarly to **1**, the structures of them were still obviously differed from that of **1** (Fig. 2). The structural difference reveals that polyanion plays a crucial role in the assembly of Pd-Keggin POMs.

Given the fact that the 2,1,3-benzothiadiazole derivatives were one of the key organic functionalized materials and the Pd-POMs encompassed potential catalytic centers in the formation of these functionalized molecules [46–48], we decided to evaluate the catalytic activities of these Pd-POMs in Suzuki-Miyaura coupling reactions (Scheme 1). Our catalytic experiment was initially performed on the coupling reaction of 4,7-dibromobenzo[2,1,3]thiadiazole **5a** and phenylboronic acid **6a** as a model substrate with the conditions of 0.5 mol% catalysts, **5a** (0.25 mmol), phenylboronic acid **6a** (0.55 mmol), and K₂CO₃ (0.75 mmol) in 4 mL solvent at 60°C for 2 h and the optimum reaction results were listed in Fig. 3. Preliminary screenings indicated that employing H₃PW₁₂O₄₀, H₄PMo₁₁VO₄₀ and H₅PMo₁₀V₂O₄₀ as the catalysts at 60°C afforded a trace amount of the desired product as well as the result of no catalyst. Then, we investigated the palladium catalyst in terms of this transformation and the desired product **7a** was achieved in 17% yield. Replacement of Pd(OAc)₂ with the mixture of Pd(OAc)₂ and H₅PMo₁₀V₂O₄₀ gave the desired product in 40% yield. Interestingly, when compounds **1–3** were applied as the catalysts, the yields of product **7a** were dramatically increased, indicating the intramolecular cooperation between cation and anion in compounds **1–3** might play vital role in the catalytic process. Specifically, an excellent yield of 93% was obtained using the compound **3** as the catalyst (Fig. 3a). This preliminary result implied that the combination of Pd complexes and Keggin-POMs H₅PMo₁₀V₂O₄₀, *i.e.*, compound **3**, can dramatically enhance the catalytic activities of cross-coupling reactions of aryl dibromides with phenylboronic acid owing to the stronger oxidation properties of H₅PMo₁₀V₂O₄₀. Additionally, the effect of reaction time on the Suzuki-Miyaura coupling were also monitored at different time intervals and found that model reaction of **5a** and **6a** gave the corresponding coupling product in 93% yield in 120 min (Fig. 3b). Besides, attempts to increase or lower the reaction tem-

perature led to the decrease of reaction efficiency (Fig. 3c). Further investigation on the reaction solvents showed that EtOH-H₂O (3:1) was the optimal solvent for this transformation (Fig. 3d). Thus, the optimal reaction conditions were eventually determined to include **5a** (0.25 mmol) and **6a** (0.55 mmol) in EtOH-H₂O (3:1) at 60°C for 2 h in the presence of compound **3** (0.5 mol%) and it could achieve a 93% yield in the formation of product **7a**.

With the optimal conditions, we explored the substrate scope of this Suzuki-Miyaura coupling reaction. As shown in Table 1, a series of phenylboronic acid derivatives, regardless of electron-donating or -withdrawing group on the phenyl ring, could efficiently induce the coupling reaction to produce the desired products in excellent yields, and compound **3** also afforded the corresponding biaryl products in excellent yields (90%–97%) (Table 1, entries 1–7). In addition, sterically hindered *meta*-methylphenylboronic acid or *meta*-chlorophenylboronic acid gives a relatively lower yield compared to its *para*-counterparts (Table 1, entries 6 and 7). Furthermore, the reaction of 4-bromo-7-nitro-2,1,3-benzothiadiazole and arylboronic acid underwent cross-coupling smoothly and afforded the desired products in 94%–97% yields (Table 1, **7h–7j**). Noteworthy, compound **3** as heterogeneous catalyst displays superior performance to the previous homogeneous and heterogeneous catalysts (*e.g.*, nano-sized Pd₆L₈ (L = 1,3,5-tris(4'-pyridyloxadiazone)-2,4,6-triethylbenzene) [49], guar gum supported Pd catalyst [50], *etc.*) reported to date.

To determine the heterogeneous nature of Pd-POMs clusters in the coupling reaction between 4,7-dibromobenzo[2,1,3]thiadiazole and PhB(OH)₂, the leaching experiment was investigated. The solid catalyst **3** was filtrated from the reaction after 1 h under the optimum conditions and the filtrate was subsequently reacted at 60°C for additional 1 h, no yield change of **7a** was detected, indicating the reaction was indeed a heterogeneous catalytic process (Fig. 3b). In addition, atomic absorption analysis showed that there were no palladium, vanadium and molybdenum ions in the filtrate of the reaction system, which further proved that the catalytic system using **3** was heterogeneous in the coupling reactions. Next, the reusability of the hybrid catalyst was also evaluated in the coupling of 4,7-dibromobenzo[2,1,3]thiadiazole and phenylboronic acid. After the reaction, the catalyst **3** was separated by centrifugation, washed with ethyl acetate three times, and then subjected to the second run under the same conditions. The catalyst **3** could be recycled and reused for three times with remained catalytic activity [yields of **7a**: 93% (first run), 92% (second run), 92% (third run)] (Fig. 4a). In addition, there were almost no changes detected

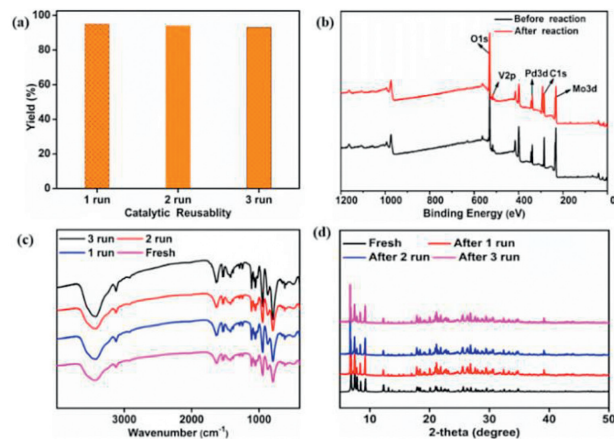
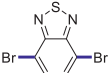

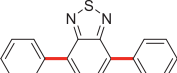
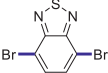

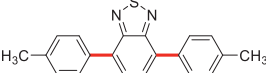
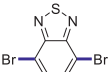

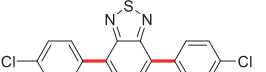
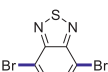

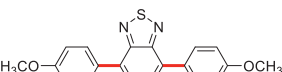
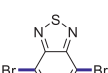

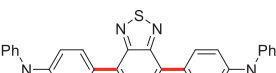
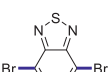

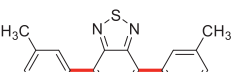
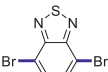

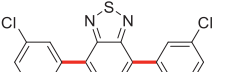
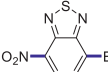

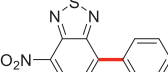
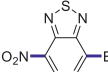

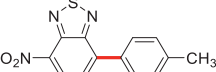
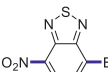

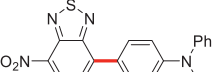


Fig. 4. (a) The three run recycle experiments for of the reaction of **5a** and **6a** catalyzed by compound **3**. (b) The XPS of compound **3** before and after the Suzuki-Miyaura coupling. (c) The IR of compound **3** before and after three runs reaction. (d) The PXRD of compound **3** before and after the reaction.

Table 1
The scope of synthesis of benzo[2,1,3]thiadiazole derivatives.^a

Entry	5	6	Products 7	Yield ^b (%)
1				93
2				95
3				92
4				96
5				97
6 ^c				91
7 ^d				90
8 ^e				94
9 ^e				96
10 ^e				97

^a Reaction conditions: **5** (0.25 mmol), **6** (0.55 mmol), catalyst **3** (0.5 mol%), K₂CO₃ (0.75 mmol), 60 °C, 2.0 h, EtOH:H₂O(v/v = 3/1, 4.0 mL).

^b Isolated yield

^c 4.0 h.

^d 80 °C, 4.0 h.

^e **6** (0.375 mmol), K₂CO₃ (0.50 mmol).

in the XPS, FT-IR and PXRD tests of the recycled compound **3** (Figs. 4b-d).

Lipid droplets are an important dynamic subcellular organelle in the cell for storing intracellular lipids, and lipid homeostasis has important implications for cellular physiology and pathophysiology [51,52]. It has been demonstrated that lipid droplets are able to participate in the transport of a wide range of enzymes and proteins and thus in the dynamic physiological homeostasis of the cell [53,54]. Abnormalities in intracellular lipid droplet levels may lead to disturbances in the cellular physiological environment and thus induce the development of related diseases, such as cardiovascular disease, cancer [55,56]. Therefore, achieving intracellular tracking and imaging of LDs is important for monitoring the normal physiological environment within cells.

In addition to the above research work, an organic bio-probe NBDS-N (**FF**) for tracking and intracellular imaging of lipid droplets was also designed and synthesized, which was consisted of a triphenylamine chain segment as an electron donor group and a benzothiadiazole main body as an electron acceptor group, forming a typical D-π-A molecular structure. Immediately afterwards, the spectral properties of the probe NBDS-N were studied. Firstly, the absorption and emission spectra of the probe NBDS-N in different solvents including hexane, toluene, dichloromethane, tetrahydrofuran (THF), ethanol (EtOH), acetone, acetonitrile, *N,N*-dimethylformamide (DMF), methanol (MeOH), ethylene glycol, dimethyl sulfoxide (DMSO) were investigated. As shown in Fig. 5a, for the probe NBDS-N, the results demonstrated that the absorption spectra of probe NBDS-N exhibited a small shift in organic

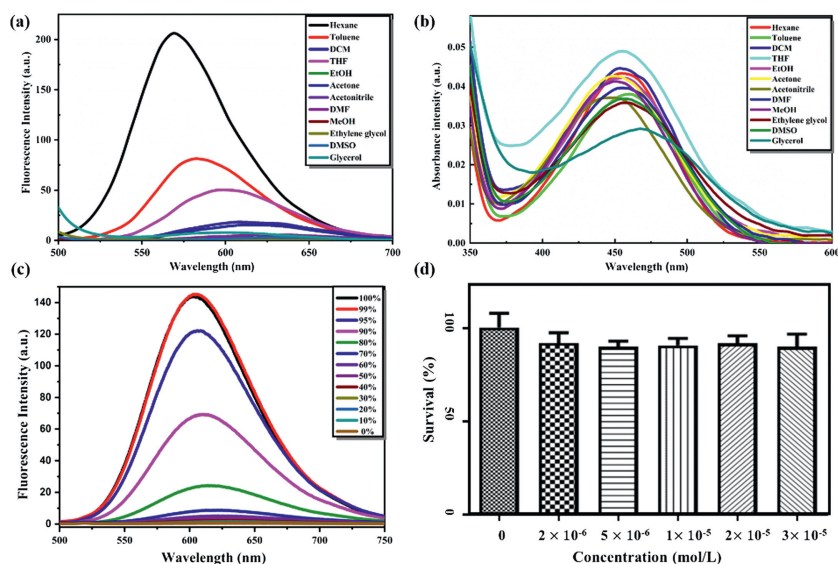


Fig. 5. (a) The fluorescence and (b) the absorbance spectra of probe NBDS-N (10^{-5} mol/L) in different solvents ($\lambda_{\text{ex}} = 450$ nm, slit = 5 nm, voltage = 400 V). (c) the fluorescence spectra of probe NBDS-N (10^{-5} mol/L) in different ratios of EtOAc/MeOH ($\lambda_{\text{ex}} = 450$ nm, slit = 5 nm, voltage = 400 V). (d) Cytotoxicity assays of probe NBDS-N at different concentrations for HeLa cells.

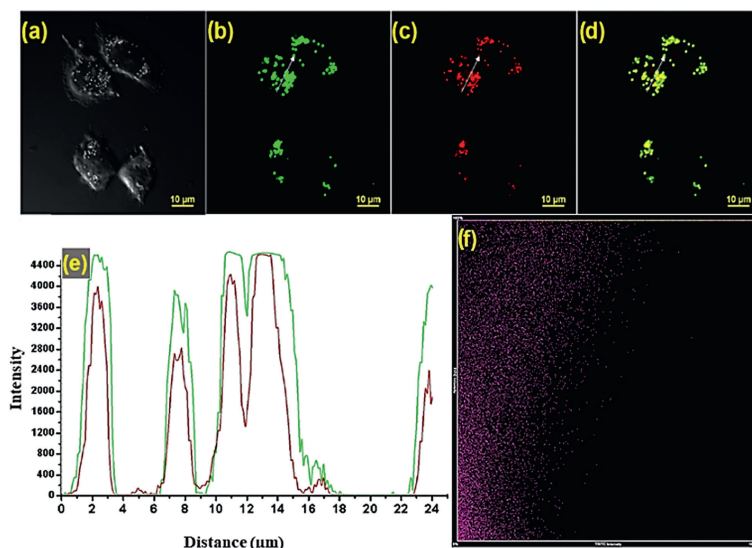


Fig. 6. The co-localization cell images of probe NBDS-N in living HeLa cells. (a) Bright field of HeLa cells, (b) probe NBDS-N (10^{-5} mol/L) stain, (c) Nile red ($5.0 \mu\text{mol/L}$) stain, (d) merged image of (b) and (c), (e) co-localization curve diagram, (f) the intensity scatter plot of (b) and (c).

solvents around at 450 nm with almost same absorption intensity. The emission spectra of probe NBDS-N showed an increasing fluorescence intensity in liposoluble solvents especially in hexane, toluene and THF. The results indicated that probe NBDS-N could be applied for monitoring LDs in living cells (Fig. 5b). Secondly, we investigated the optical properties of the probe NBDS-N in MeOH with different concentrations of ethyl acetate. As shown in Fig. 5c, the result showed that when the ethyl acetate content enhanced in the mixed solution system, the fluorescence intensity increased considerably and accompanied by a slight blue shift from 620 nm to 605 nm with a large Stokes shift (130 nm). These apparent fluorescence intensity change indicated that the probe NBDS-N possessed a significant detection effect on LDs.

According to the significant variation in fluorescence intensity exhibited by probe NBDS-N in different viscous and polar solvents, the cellular biotoxicity of the probe NBDS-N was veri-

fied by MTT assay (Fig. 5d). It was shown that the cell viability of the probe NBDS-N could still reach more than 90% after co-incubation with HeLa cells for 24 h. This data indicated that the probe could be used as a practical tool for marking LDs under complex biological environments with low biotoxicity. Subsequently, the co-localization fluorescence imaging of the probe NBDS-N was accomplished in living HeLa cells due to the different emission wavelengths of the probe NBDS-N and the commercial organic dye Nile Red. We found that green fluorescence imaging was conducted by probe NBDS-N (Fig. 6). Nile red was also applied to incubate the same cells, which exhibited red fluorescence imaging as depicted in Fig. 6c. The merge image was also shown in Fig. 6d. Moreover, the parameters of co-localization image were obviously depicted in Fig. 6e, indicating that the red and green channel was overlapped largely with Pearson correlation coefficient up to 0.91. The above results suggested that this novel probe NBDS-N could be used for LDs marker in living bio-samples.

In summary, a series of imidazole modified binary Pd-POMs with defined structures have been prepared through a facile and rarely reported one-step approach and successfully applied to synthesize 2,1,3-benzothiadiazoles by Suzuki-Miyaura coupling reaction. Specifically, compound **3** exhibits outstanding heterogeneous catalytic performance for Suzuki-Miyaura coupling reaction of aryl dibromides (excellent yields up to 97%) and it can be reused with remained high activity and durability. Subsequently, a novel organic bio-probe NBDS-N was constructed for LDs cell imaging by using a benzothiadiazole derivative as the main body and it exhibited good properties including large Stokes shift (130 nm), good LDs distribution, and a low biological toxicity. Furthermore, bio-probe NBDS-N successfully labelled the distribution of LDs in living HeLa cells. The work might shed light on the design of efficient POMs based catalysts for the derived potential applications in life science.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ccl.2022.07.035.

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