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Cyclodextrin-Isolated Alkynylpyrenes as UV-Stable and Blue-Light-Emitting Molecules Even in Condensed States

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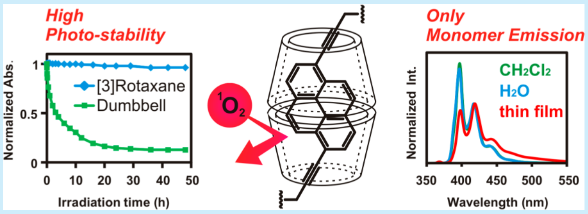
Ⓢ *Supporting Information*

ABSTRACT: Encapsulation of highly emissive alkynylpyrenes with permethylated α-cyclodextrin (PM-α-CD) followed by capping reaction yielded alkynylpyrene-based [3]rotaxanes. The [3]rotaxane emitted only blue light of monomeric pyrene under various circumstances such as lipophilic, hydrophilic, and even condensed states and exhibited extremely high stability for UV irradiation. These properties would result because PM-α-CD, like bulletproof glass, protected the alkynylpyrene core from the attack of another excited alkynylpyrene and singlet oxygen generated by the energy transfer from the excited alkynylpyrene.



Blue-light-emitting molecules occupy a cardinal position not only in optoelectronics but also in bioprobes, in which they act as a donor materials for other fluorescence acceptors of longer wavelengths.[1] Among the blue-light-emitting molecules, pyrene and its derivatives have long been attractive in terms of their chemical usability.[2] However, pyrene itself possesses a robust tendency to form an aggregated state, which dooms a substantially red-shifted emission as well as the fatal decrease for the luminescent efficiency particularly in aqueous and condensed conditions. These troublesome phenomena are seen not only for pyrenes but also for most organic hydrophobic fluorophores. More seriously, purely organic blue-light emitters still rquire improved optical stability due to their large HOMO−LUMO energy gaps.[3] Indeed, the prolonged irradiation usually results in photobleaching of the fluorophores mostly by the reaction with singlet oxygen generated by the energy transfer from the excited fluorophores to dissolved oxygen.

During the course of our studies for finding a new class of pyrene derivatives, we developed alkynylpyrenes possessing the advantage of high fluorescence quantum yields even in the presence of oxygen in water.[4] To reach the alkynylpyrenes near the ideal blue-emitting molecules, we additionally planned to encapsulate the fluorophore with “bulletproof glass” at the molecular level in order to no longer suffer from uncertainty due to its unfavorable aggregation even in the condensed states. This bulletproof glass isolated fluorescent core will also be protected from photochemically induced reactive species present outside.[5−7] Here, we report rotaxane-type alkynylpyrenes fully shielded with cyclodextrins (CDs), which show intensely blue-emitting characteristics under universal circumstances and extremely high stabilities against photoirradiation.

Bisalkynylpyrenes such as **1** exhibited strong blue emissions in the wavelength region ranging from 450 to 390 nm in dilute solutions. At high concentrations in lipophilic solvents and even at low concentrations in water, they showed yellow-green excimer emissions with maxima of at least 520 nm. For the construction of the desired blue-emitting fluorophore, we devised rotaxane-type alkynylpyrenes (Scheme 1). In the rotaxane, the single alkynylpyrene core is completely encapsulated within macrocycles, and the resulting complex is prohibited from dissociation by attaching appropriate stopper molecules. This encapsulation would prevent the alkynylpyrene core from aggregations of themselves and interactions with other photochemically induced reactive species of outside. CDs were selected as the macrocycle because of their well-known encapsulation ability for the hydrophobic fluorophores in aqueous solutions[8] and optical transparency above 200 nm. We recently reported a new class of [4]rotaxanes that exhibited strong circularly polarized luminescence in their excimer emission region, in which two alkynylpyrenes were threaded into two γ-CDs.[9] However, single alkynylpyrene-threaded [3]rotaxanes could not be synthesized not only in this study but also in any ones using α- and β-CDs. Indeed, unlike other fluorophores,[10] a few [3]rotaxanes have been prepared for pyrenes as an axle component.[11]

Returning to the basis, we thoroughly investigated the complex-forming ability of **1** with native and various chemically modified CDs by ^{1}H NMR titration experiments in D_2O. Among the CDs tested, permethylated α-CD (PM-α-CD) showed the

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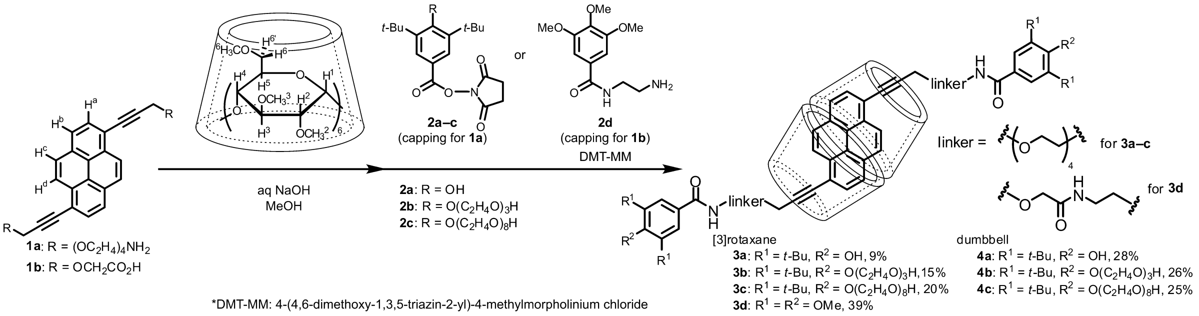
Scheme 1. Capping Reaction for Synthesizing [3]Rotaxane 3



*DMT-MM: 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride

most drastic changes in the ^{1}H NMR spectrum. In the ^{1}H NMR spectrum of **1a** (6×10^{-3} M) in D_2O, the aromatic proton signals shifted upfield compared to those in $CDCl_3$ because amphiphilic **1a** readily aggregates by using the large plane of the pyrene cores in an aqueous circumstance (Figure S1). Upon addition of PM-*α*-CD, a new set of signals appeared at a similar region in $CDCl_3$, indicating the existence of a nonaggregated alkynylpyrene independently on the ^{1}H NMR time scale against the aggregated species. When the molar ratio of PM-*α*-CD/**1a** increased up to 2, the original proton signal of **1a** disappeared completely and converged into the well-resolved four doublets of the nonaggregated alkynylpyrene. These NMR data including the DOSY spectrum (Figure S2) proved that one molecule of alkynylpyrene **1a** was included in the cavities of two PM-*α*-CD molecules to form a ternary complex, **1a**⊂(PM-*α*-CD)$_2$.

The capping reaction of **1a**⊂(PM-*α*-CD)$_2$ for constructing a [3]rotaxane needs to be performed at relatively low temperature because the inclusion complex will dissociate above 60 °C (Figure S3). The succinimidyl ester of bulky benzoic acid **2a** was selected as a stopper moiety of the [3]rotaxane, which could react with the terminal amines of **1a** at room temperature. When **2a** was added to a solution of **1a**⊂(PM-*α*-CD)$_2$ in water at 25 °C around pH 11, the reaction gave [3]rotaxane **3a** (9%) and PM-*α*-CD-free dumbbell **4a** (28%) (Scheme 1). To improve the water solubility of the stopper molecule, the hydroxyl group of **2a** was modified with oxyethylene chains to afford **2b** and **2c**. These stopper molecules fairly increased the isolated yields of the corresponding [3]rotaxanes, 15% and 20% for **3b** and **3c**, respectively, while those for both dumbbells **4b** and **4c** decreased to 26% and 25%. The formation of [2]rotaxanes that possess only one PM-*α*-CD was also confirmed on the basis of ESI-TOF-MS (Figure S4). All of the [3]rotaxanes **3** had good solubility not only in water but also in many organic solvents such as $CHCl_3$, MeOH, EtOH, NMP, DMF, DMSO, and MeCN. The good solubility for various solvents is essential for applying them to materials of optical interest using inkjet printing technology.[12] No unthreading of the dumbbell part was confirmed by heating a DMSO solution of **3a** at 100 °C for 18 h (Figure S5). A rather good yield was attained for a reversely connected [3]rotaxane **3d** synthesized from the carboxylic acid terminated alkynylpyrene **1b** and the stopper **2d** possessing an amino group in its reaction site. The short linkage in **3d** was introduced between the alkynylpyrene core and the stopper for reducing the flexibility of the axle in order to subject the rotaxane to X-ray structure analysis.

Various NMR experiments revealed the spatial correlation between the PM-*α*-CD wheels and the alkynylpyrene axle of the [3]rotaxane **3b** in solutions. COSY and long-range COSY spectra of **3b** allowed assignment of the aromatic protons (H^{a-d}) in the alkynylpyrene and the C−H protons (H$^{1-6}$) in the PM-*α*-CDs (Scheme 1 and Figures S6 and S7). Chemical shifts of the three methoxy protons ($CH_3^{2,3,6}O$) were decided by superimposing the ^{1}H NMR spectra of separately synthesized **3b** parallel to the corresponding PM-*α*-CDs possessing partially deuterated methoxy groups ($CD_3^{3,6}O$: Figure S8). The signals of CH_3^2O (−0.16 ppm) and CH_3^3O (−0.61 ppm) shifted upfield, whereas that of CH_3^6O (+0.03 ppm) shifted downfield in the ^{1}H NMR spectrum of **3b** at 400 MHz (Figures S8 and S9). Unexpectedly, not the inward substituent at C2 but the outward one at C3 largely shifted by the anisotropy from the inner alkynylpyrene, and this phenomenon was disclosed by the crystal structure of **3d** (vide infra). In the NOESY spectrum of **3b**, strong NOEs were detected between all the alkynylpyrene protons and the protons of H^3 and $CH_3^{2,3}O$ in the PM-*α*-CDs, while no NOEs for the narrow rim and outward protons of H$^{1,2,4-6,6'}$ and CH_3^6O (Figure S10). The NOEs illustrate that the alkynylpyrene moiety was fully encapsulated within the cavity of the two PM-*α*-CDs and located near wide rims of them as depicted in Scheme 1.

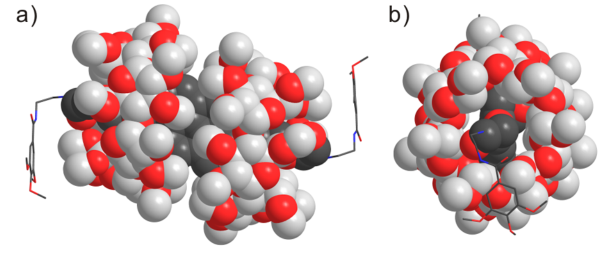


Figure 1. Structures of [3]rotaxane **3d** obtained from X-ray analyses: (a) side and (b) top views. The single crystal was obtained by recrystallization from DMSO and H_2O.

MacroModel-based Monte Carlo simulations followed by B3LYP/6-31G reinforced the spatial correlation, in which the two PM-*α*-CDs tightly wrapped the alkynylpyrene axle, and the stoppers were large enough to prevent unthreading in the designed manner (Figure S11). Conclusive proof for the rotaxane structure was obtained on the basis of the X-ray structure analysis for **3d** (Figure 1). The pyrene unit was largely separated from the neighboring pyrene units because of the wrapping CD units as well as the stopper moiety (Figure S12). The two PM-*α*-CDs were found to completely screen the inner alkynylpyrene fluorophore from the outer world in a head-to-head dimerization manner as speculated. There should not be enough space even for small reactive species to approach the inner alkynylpyrene. These structural aspects will guarantee the spatial isolation of the emitting core against other alkynylpyrenes and photochemically induced reactive species. The methoxy oxygen at C2 was located inward and that at C3 outward in PM-α-CD; on the other hand, the next methyl groups were reversed (Figure S13). This is a reason why the methoxy protons at C3 suffered more from the anisotropy by the inner alkynylpyrene in the ^{1}H NMR spectra of the rotaxanes (vide supra).

Optical properties of the [3]rotaxanes **3** proved to be highly independent of solvents used and of their concentrations because the emitting core was isolated from external environments by the two PM-α-CDs. A dilute solution of the dumbbell such as **4b** bearing an exposed alkynylpyrene moiety displayed sharp absorption bands at 388, 366, and 348 nm in organic solvents, which were attributed to the $\pi \rightarrow \pi^*$ transition of the alkynylpyrene core. However, **4b** easily aggregated by using the hydrophobic plane to afford the broad absorption band around 390 nm in an aqueous solution and in a thin film state (Figure S14a). By contrast, the absorption spectra of the [3]rotaxane **3b** were almost the same in CH_2Cl_2, EtOH, and water with absorption bands at 388 nm (Figure 2a). Noteworthy is that the shape of absorption band of **3b** remained unchanged even in its thin film state (Figure 2a, red line), which was verified by optical waveguide spectroscopy measurements (Figure S15). The notable insensitivity of **3b** against the measurement media most likely results from the spatial isolation of the emitting core by the optically transparent, amphiphilic PM-α-CD. This was also seen in other [3]rotaxanes, so that the spectra of **3a**−**d** in any media are similar (Figure S16).

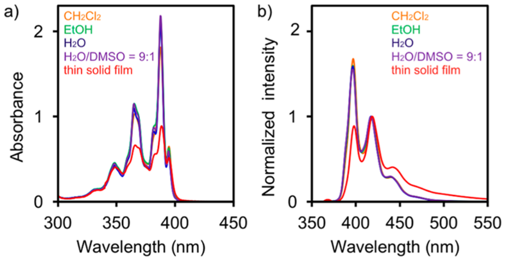


Figure 2. Electronic spectra of [3]rotaxane **3b** under various circumstances at 25 °C: (a) absorption (2.0×10^{-5} M) and (b) fluorescence spectra (2.0×10^{-6} M) in CH_2Cl_2 (orange), EtOH (green), H_2O (blue), and H_2O/DMSO = 9:1 (purple). Both of the red spectra were obtained in a thin film state. Excitation wavelengths are 366 and 388 nm for red and the others, respectively.

A striking difference between the [3]rotaxane **3** and the dumbbell **4** arose from their fluorescence spectra. For the dumbbell **4b**, blue light of monomeric pyrene was emitted in a dilute EtOH solution at 396 and 417 nm, while yellow-green emission became predominant around 520 nm in an aqueous solution and in a thin film state, which must originate from the excimer (Figure S14b). Hydrophilic and condensed environments should make the excited alkynylpyrene core easy to match up with another, usually that of the ground state. On the other hand, the rotaxane **3b** illuminated only blue light of monomeric pyrene not only in solvents but also in a thin film state (Figure 2b). The PM-α-CD shells would inhibit the alkynylpyrene core from crossing over to each other, so that the excited core no longer formed an excimer. Furthermore, the quantum yield of **3b** ($\Phi_f = 0.76$) was substantially higher than that of **4b** ($\Phi_f = 0.60$) in EtOH, being due to the reduced rate of nonradiative decay by restricting the rotation of the alkynylpyrene core within the PM-α-CD shells. Thus, the measurement media scarcely affected emitting efficiencies of all the [3]rotaxanes **3**, the quantum yields of which fell within the range from 0.77 to 0.60 in any media (Table S1). The fluorescence intensity of **3b** increased according to Beer's law up to a concentration of 5.5×10^{-6} M by monitoring at 397 nm, then began to saturate, and finally decreased above 1.1×10^{-5} M (Figure S17a). The deviation from Beer's law at higher concentrations would result not from the concentration quenching by aggregation but from the fluorescence reabsorption on **3b** by crosstalk between the absorption and emission bands ranging from 400 to 375 nm (Figure S17b). This was partly validated from the fact that the peak intensity of the shortest fluorescence band at 397 nm mainly weakened relative to the concentrations (Figure S17c). The thin film state of **3b** also emitted blue light, while the peak intensity at 397 nm was reduced because of the fluorescence reabsorption (Figure 2b, red line). Nevertheless, the [3]rotaxane **3b** proved to be an intensely blue-light-emitting molecule regardless of their circumstances. Other [3]rotaxanes **3a,c,d** also exhibited similar fluorescence properties (Figure S16).

The [3]rotaxanes **3** can be regarded as an "isolated" fluorophore by the encapsulation with visible-light-transparent shells. The shell, PM-α-CD, consists only of C−C, C−O, and C−H single bonds and never of unsaturated bonds that will be targets for reactive oxygen species as a major causative substance inducing photobleaching. Expecting that the shells will act as a bulletproof glass, we examined the stabilities of **3b** and **4b** under photoirradiation. While quartz cells containing air-saturated aqueous solutions of **3b** and **4b** were continuously irradiated with >300 nm light using a 150 W high-pressure mercury lamp with a UV-30 filter, the UV−vis spectra were monitored over time (Figure 3). The dumbbell **4b** was rapidly photobleached with the absorption decay half-life of 3 h, whereas the absorption spectrum of **3b** remained unchanged after 48 h irradiation. Anderson[6] and Smith[7] also reported photodurabilities of singly CD- and synthetic macrocycle-encapsulated fluorophores, respectively. Although those rotaxanes showed the increased photodurabilities compared with the corresponding dumbbells, most of them photobleached within several hours. The far superior photodurability of our rotaxanes is certainly caused by the doubly CD-encapsulated [3]rotaxane structure that near completely shields the fluorophore core from reactive oxygen species. On the other hand, the previous examples might be insufficient in terms of the shielding.

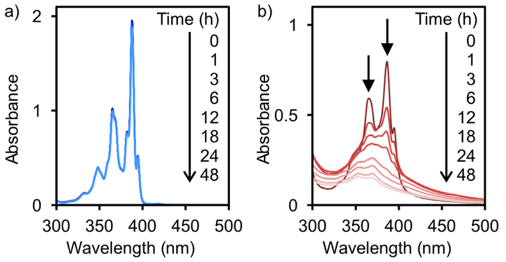


Figure 3. Absorption spectral changes of (a) [3]rotaxane **3b** (2.0×10^{-5} M) and (b) dumbbell **4b** (2.0×10^{-5} M) for 48 h at 25 °C in a mixed solvent (H_2O/MeOH = 6:4) under photoirradiation with >300 nm light using a 150 W high-pressure mercury lamp with a UV-30 filter.

Singlet oxygen is known to play a chief role in photobleaching for fluorescent aromatic hydrocarbons.[13] The bulletproof glass, PM-α-CD, seems to protect the alkynylpyrene core from the attack of singlet oxygen generated by the energy transfer from the excited alkynylpyrene to dissolved oxygen.[14] However, there, is still another possibility for the photodurability of the [3]-rotaxanes, i.e., no chance for the energy transfer because of the spatial separation between the alkynylpyrene core and oxygene by the bulletproof glass. To rule out this, trapping experiments were executed using 1,3-diphenylisobenzofuran (DPBF) that is known to rapidly react with singlet oxygen. The generation of singlet oxygen was evaluated from the decrease in the absorption band of DPBF during the photoirradiation.[15] Air-saturated aqueous solutions of **3b** and **4b** containing DPBF were irradiated by using a 150 W high-pressure mercury lamp with a UV-30 filter, located 10 cm away from the sample quartz cell, and the consumption of DPBF was monitored at 415 nm (Figure S18a). The absorption band of DPBF decreased quickly both in the presence of **3b** and **4b** with a decay half-life of around 120 s, which is 5 times as fast as that in the absence. The [3]rotaxane did generate singlet oxygen with the quantum yield for singlet oxygen generation of $\Phi_\Delta = 0.04$ (Figure S18b) but did not permit the approach of the singlet oxygen to its alkynylpyrene core by the bulletproof glass. From the other viewpoint of this experiment, the [3]rotaxane could be utilized as a highly stable singlet oxygen generator.

The blocking ability of PM-α-CD was reconfirmed by fluorescence quenching experiments through intermolecular photoinduced electron transfer (PET). For this purpose, we used maleimide that had been known to quench the fluorescence of alkynylpyrenes.[4a] From the Stern–Volmer plot of the dumbbell **4** in EtOH, the k_q value was estimated to be ca. 1.4×10^{10} M^{-1} s^{-1} that is larger than the diffusion-controlled rate constant (6.0×10^9 M^{-1} s^{-1}) in EtOH. Furthermore, the quenching efficiency increased by increasing the temperature of the solution, meaning the quenching being induced by intermolecular PET (Figure S19).[16] In contrast to the shell-free **4a**, the [3]rotaxane **3a** suffered little from the fluorescence quenching with the concentration up to as high as 0.2 M of maleimide. The PM-α-CD shell proved to inhibit the approach of maleimide to the alkynylpyrene core within the distance that allows the intermolecular PET.[17]

The present alkynylpyrene-based [3]rotaxanes only emitted blue light of monomeric pyrene under various circumstances and endured the UV irradiation for a long stretch of time. These photophysical characteristics resulted from the spatially isolated fluorophore by the rotaxane architecture. The [3]rotaxane could be a superior molecular basis of purely organic blue-light emitters and a highly stable singlet oxygen generator.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.6b00420.

Full experimental and characterization data for all compounds (PDF)

X-ray crystallographic data for compound **3d** (CIF)

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Notes

The authors declare no competing financial interest.

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