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Multi-stimuli-responsive high contrast fluorescence molecular controls with a far-red emitting BODIPY-based [2]rotaxane

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ABSTRACT

A novel fluorescent switchable [2]rotaxane **NIR4** composed of two different molecular stations and rotaxane arms terminated with far-red boron-dipyrromethene (BODIPY) fluorophores and its derivatives were synthesized by CuAAC click chemistry. The molecular shuttling motion of mechanically interlocked molecules (MIMs) could be addressed by the fluorescence signal transduction *via* distance dependent photo-induced electron transfer process of [2]rotaxane **NIR4** triggered by external chemical stimuli of acid/base. Moreover, the flexible arms of triazolium moiety in [2]rotaxane **NIR4** and its axle **NIR2** exhibited impressive selectivity and sensitivity toward complementary anionic analyte ($H_2PO_4^-$), where the specific mechanical molecular motion was supported by quantum mechanical calculations. The development of [2]rotaxane **NIR4** with a high level of structural complexity can be utilized for novel dual sensory detections of acid-base and dihydrogen phosphate ($H_2PO_4^-$) anion. Importantly, the host of **NIR2** and [2]rotaxane **NIR4** could be applied for the *vitro* imaging and clarify the distribution of $H_2PO_4^-$ at subcellular levels.

1. Introduction

Inspired by naturally occurring biological machines, such as ATPase rotary motors, chaperonins and myosin linear motor systems [1], canvassers have tried to develop a variety of artificial mechanically interlocked molecules (MIMs) [2–6], especially unidirectional rotors, switches, scissors, artificial muscles and molecular elevators [7–11]. The unique structural features of rotaxanes (with typical mechanically interlocked architectures) have been widely employed as crucial forerunners and building blocks for the fabrication of advanced and invigorated molecular switches via supramolecular chemistry [12]. As a manipulation platform for functional groups, the structural complexities of MIMs considerably provide abundant possibilities in the design of novel functional molecular machines. Hence, the construction and efficient synthesis of controllable molecular motions with high structural complexities have received considerable attention but still remain significant challenges [13–17]. This is motivated by the promise of their potential applications, such as unique three-dimensional topological cavities formed in situ by virtue of mechanical bonding in rotaxanes and catenanes, which are capable of binding specific anions owing to their co-conformational changes [18–21]. However, the preorganized geometries in interlocked molecules are driven by a variety of light, chemical and redox stimuli [22]. Whereas, the rotaxanes or catenanes are widespread in the literature [23], systems that use anions as an external stimuli to produce co-conformational changes and high degrees of selectivities have been gained much interest in the field of anion supramolecular chemistry [24–26].

Over the years, allured by Cu(I)-catalyzed Huisgen [27–29] and alkyne-azide 1,3-dipolar cycloaddition (CuAAC “click” chemistry) [30,31] the synthetic community has witnessed a revolutionary change in mechanical interlocked molecules. In addition to their high efficiencies, the 1,2,3-triazole unit with the tolerance to the sensitive functional group, mild reaction condition, technical simplicity and unique dipole nature can be easily converted to the triazolium ion, which is useful as a viable synthetic tool to produce expedient anion receptors and secondary binding site for macrocycle [32–34]. Pandey et al. developed such a cyclic and acyclic bile acid-based 1,2,3-triazolium receptors, which showed high affinities and selectivities toward phosphate anions [35]. Flood et al. also reported triazolophanes as ideal size-selective anion binding hosts [36]. Successively, Coutrot et al. also

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extensively explored rotaxane using the macrocycle transporter of crown ether-based molecular switches according to the triazolium cation and macrocycle electrostatic interactions [37,38]. Therefore, designing and employing of MIMs-based molecular sensors (such as rotaxanes and catenanes) with suitable fluorophores have drawn particular attention to exploit the unusual dynamic properties of shuttling and conformational switching induced by anion species. For instance, Qu et al., also described a fluoride selective and acid/base controllable photo-induced electron transfer process between ferrocene and morpholin-napathalimide fluorophore [39]. Likewise, Beer et al. demonstrated a selective sensor towards sulfate anion naphthalene-based fluorescent [3]rotaxane host [40]. In this track, far-red (boron-dipyrromethene) derivatives were also recognized as one of the most important and simple organic fluorophore due to their special absorptions, emission properties, high quantum yields and good photostabilities [41,42]. Moreover, receptors with far-red rather than normal visible emission renders more favorable properties for chemosensors and biological imaging by means of minimum photo-damages to biological samples and good tissue penetrations [43,44].

More recently, a range of pseudorotaxane, rotaxane and catenane host systems have been reported towards sensing of alkali metal cations and halogen species [45–48]. Chiu and co-workers have reported the sodium-templated interpenetration of squarine or antroquinone dyes within a bis-dibenzo-crown-6 derived macrocycle, which led to a high contrast fluorescence output [49]. In a similar manner, our group also successfully developed diketopyrrolopyrrole (DPP) based rotaxane which can selectively sense fluoride anion via a fluorescence response [50]. However, to the best of our knowledge the far-red emitting BODIPY involved a rotaxane-based molecular host with a high fluorescence output has not been explored yet. Interestingly, phosphates and its derivatives are widely employed in our daily life and played important roles in two important biopolymers (i.e., DNA and RNA) as well as predominant equilibrium species of inorganic phosphates at physiological pH values [51–53]. Among the biologically relevant anions, the dihydrogen phosphate is physiologically the most abundant and hold important roles in signal transduction and energy storage living systems. Therefore, the selective recognition and sensing of phosphates are of great interests to general researchers [54]. Nevertheless, the far-red emitting based rotaxane with acid/based and $H_2PO_4^-$ responses has not been reported so far, especially those incorporating MIMs-based fluorogenic functionalities of the host remain rarely challenged.

In the present study, we demonstrate the molecular construction and efficient synthesis of a new type of fluorescent axles **NIR1** and **NIR2** and [2]rotaxanes **NIR3** and **NIR4** as depicted in Fig. 1, which are symmetrically terminated at the end of the axle with a far-red emitting BODIPY fluorophore. In addition, [2]rotaxanes **NIR3** and **NIR4** consist of a macrocycle (i.e., DB24C8) as a wheel and a dumb bell-shaped axle. Accordingly, the dynamic interlocked [2]rotaxane **NIR4** revealed that the molecular shuttling behavior over the axle under the external stimuli of acid/base, which has been verified by the NMR and fluorescence investigations. However, in the presence of acid/base and $H_2PO_4^-$ no conformational changes occurred in interlocked [2]rotaxane **NIR3** owing to the absence of triazolium $(C{-}H)^+$ unit. Moreover, to understand the experimental results of molecular motion and host-guest interactions at the molecular level, we also carried out the quantum mechanical calculations. Finally, axle **NIR2** and [2]rotaxane **NIR4** were successfully applied to detect $H_2PO_4^-$ in live cell images which could be used as efficient fluorescent sensor materials.

2. Experimental

2.1. Materials and instrumentations

Unless otherwise stated, all solvents and reagents were purchased from Aldrich and used without further purification. ^1H NMR and ^{13}C NMR spectra were measured on Agilent-NMR400–vnmrs400 series in $CDCl_3$ and CD_3CN. Chemical shifts (δ) were expressed in parts per million from low to high fields and coupling constants (J) in Hz. The detailed NMR assignments of target molecules were done with 2D TOCSY (Varian Inova 500). Electronic UV–vis spectra were measured on a Jasco UV-600 spectrometer (1 cm quartz cell). Fluorescence spectra were recorded on HITACHI 7000 spectrometer (1 cm quartz cell). Infrared spectroscopy data were recorded using Perkin Elmer IR spectrophotometer.

2.2. Stock solutions

Standard solutions of axles **NIR1** and **NIR2** along with [2]rotaxanes **NIR3** and **NIR4** (10 μM) were prepared in CH_3CN solvent. The solutions of acid (TFA) and base (NaOH or DBU) were prepared in deionized water (1×10^{-3} M). The solutions of all anions were prepared by dissolving the respective tetra-butyl ammonium (TBA) salts F^-, CN^-, HPO_4^{2-}, HSO_4^-, ClO_4^-, Br^-, $H_2PO_4^-$, SCN^-, $P_2O_7^{4-}$, NO_3^-, I^- AcO^- and Cl^- in deionized water (1×10^{-3} M).

2.3. Cell culture for Hela cells

The cell line Hela cells was provided by the Food Industry Research and Development Institute (Taiwan). Hela cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) at 37 °C under an atmosphere of 5% CO_2. Cells were plated on 18 mm glass coverslips and allowed to adhere for 24 h.

2.4. Fluorescence imaging of **NIR2** and **NIR4** in living cells of $H_2PO_4^-$

Experiments to assess dihydrogen phosphate $H_2PO_4^-$ ion uptakes were performed in the tris-buffered saline (TBS) with 10 μM TBA-$H_2PO_4^-$. The cells treated with 2 μL of 10 mM $H_2PO_4^-$ ion (final concentration: 10 μM) were dissolved in sterilized TBS (pH 7.4) and incubated for 30 min at 37 °C. The treated cells were washed with TBS (3×2 mL) to remove remaining TBA-$H_2PO_4^-$ ion. The culture medium (2 mL) was added to the cell culture, which was treated with a solution of **NIR2** and **NIR4** (10 mM, 2 μL) to have a final concentration of 10 μM dissolved in DMSO. The samples were incubated at 37 °C for 30 min. The culture media were removed, and the treated cells were washed with PBS (3×2 mL) before observation. The confocal fluorescence images of cells were performed with a Leica TCS SP5 X AOBS confocal fluorescence microscope, and a 63 x oil-immersion objective lens was used. The cells were excited with a blue light laser at 480 nm, and emission was collected at 500 ± 550 nm.

2.5. Synthetic procedures and characterization

2.5.1. Synthesis of compound **S2**

TFA (0.1 mL) was added to the solution of 4-(4-azidobutoxy)benzaldehyde (1.2 g, 5.47 mmol) and 2,4-dimethylpyrrole (1.14 g, 12.04 mmol) in CH_2Cl_2 (100 mL) under N_2 atmosphere. After the solution was stirred for 6 h, TLC analysis revealed complete conversion of starting materials to the dipyrromethane. To the reaction mixture, DDQ (1.49 mg, 6.56 mmol) dissolved in CH_2Cl_2 (50 mL) was added. Then, the solution was stirred for further 1 h; TLC analysis revealed the complete disappearance of dipyrromethane and formation of the desired dipyrromethene. Triethyl amine (16.0 mL) and $BF_3{\cdot}Et_2O$ (20.0 mL) were added to the reaction mixture and stirring was continued for further 5 h. Reaction mixture was washed with water (50 mL) by three times and the organic layer was dried over anhydrous $MgSO_4$. The solvent was removed under reduced pressure and the crude product was purified by column chromatography (eluent: EA/hexane = 1:9 v/v) to give the compound **S2** as a red solid. Yield: 1.78 g (74%); ^1H **NMR** (400 MHz, $CDCl_3$): δ (ppm) = 7.16 (d, 2H, J = 8.8 Hz), 6.99 (d, 2H, J = 8.8 Hz), 5.97 (s, 2H), 4.04 (t, 2H, J = 5.6 Hz), 3.39 (t, 2H, J = 6.8 Hz), 2.54 (s, 6H), 1.92-1.80 (m, 4H), 1.43 (s, 6H). ^{13}C **NMR**