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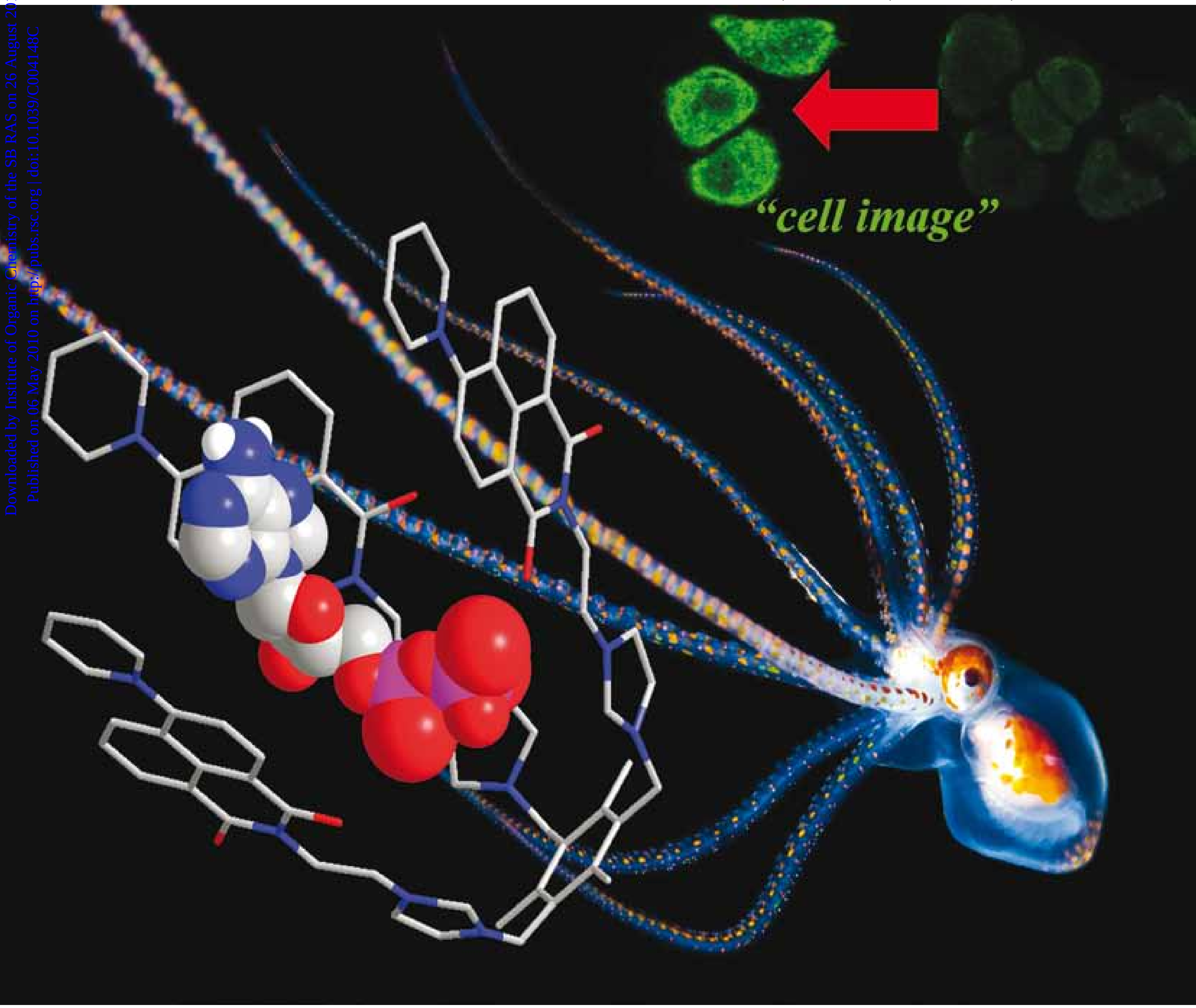

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COMMUNICATION
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PERSPECTIVE
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Chemistry of the cortistatins–a novel class of *anti*-angiogenic agents

Aminonaphthalimide-based imidazolium podands for turn-on fluorescence sensing of nucleoside polyphosphates†

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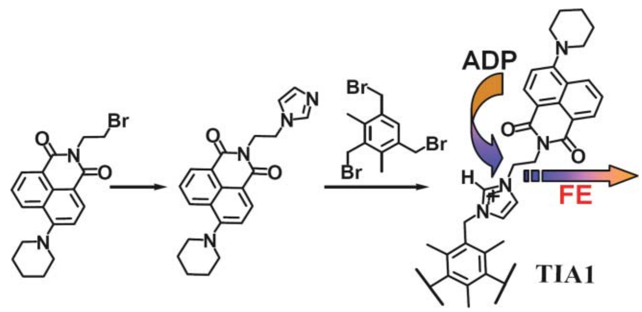
New aminonaphthalimide imidazolium podands, which worked as luminescence chemosensors for selectively sensing nucleoside polyphosphates through a "turn-on" manner, were prepared for fluorescent imaging of ADP and ATP in living cells.

Recently, a great deal of interest has been focused on the molecular recognition of nucleotide polyphosphates because they play a major role in understanding and evaluating several key biological processes.[1] While considerable efforts have been devoted to developing fluorescent chemosensors for various nucleotides like ATP,[2] GTP,[3] UTP/UDP[4] in the past decades. It is still not easy to find an example in which all of the twelve ribonucleoside polyphosphates were examined to evaluate the selectivity.[5] The distinguishability of ATP from adenosine di-phosphate (ADP) and adenosine mono-phosphate (AMP) is also urgently desired for an improved sensor, since ATP is made from ADP and AMP, and converted back into these precursors in metabolism.[6] In particular, ATP and ADP are basic and important components in bioenergetic conversion processes of living organisms, with their polyphosphate chains being the center for chemical energy storage and transfer.[7] Thus a fluorescent sensor, which can signal the concentration of ADP/ATP from the hindrance of other nucleoside polyphosphates is needed.

As a continuance of our research work on a tripodal receptor with arms comprising benzoimidazolium hydrogen bonding moieties,[8] herein, we report a new approach to the preparation of chemosensors that have the potential to distinguish ADP and ATP from other ribonucleoside polyphosphates, by incorporating 1,8-naphthalimide and imidazolium moieties into the preorganized tripodal receptors. Since amino-naphthalimide is a promising signaling subunit emitting in the green region ($\lambda \sim 540$–550 nm) with high quantum yields (Φ_f),[9] the chemosensors are also successfully applied to cells imaging for ADP and ATP, respectively.

Compound **TIA1** was synthesized by reaction of *N*-(2-(1*H*-imidazol-1-yl)ethyl)-4-piperidine-1,8-naphthalimide with 1,3,5-tris(bromomethyl)-2,4-dimethylbenzene in $CHCl_3$ and characterized by EA, ^{1}H NMR and MS (Scheme 1). **TIA1** exhibited a 1,8-naphthalimide characteristic absorption band at 425 nm (log $\varepsilon = 4.40$) in acetonitrile solution. The addition of all the twelve ribonucleotides did not cause any significant spectra variation. Free receptor **TIA1** exhibited a strong green emission at 548 nm (Fig. 1), assignable to the 1,8-naphthalimide ($\Phi_f = 0.04$)[10] upon excitation at 465 nm. The addition of ADP, caused fluorescence



Scheme 1 The aminonaphthalimide-based imidazolium podands **TIA1** for fluorescent sensing of ADP. FE is the fluorescence enhancement.

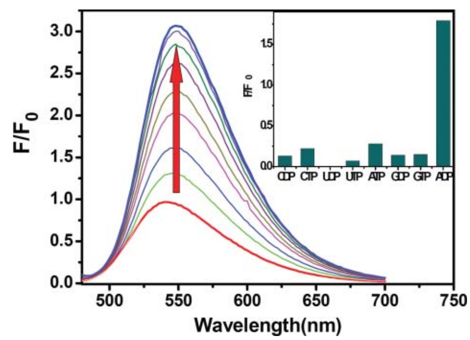


Fig. 1 Fluorescence responses of **TIA1** (20 μM) in acetonitrile solution upon addition of ADP (0 to 0.4 mM). Inset: fluorescence responses for several ribonucleotide di- and triphosphates (0.4 mM). The bars represent the value of $[(F - F_0)/F_0]$, where F and F_0 are the emission intensities in the presence and absence of the ribonucleotides, respectively. Intensity was recorded at 548 nm, excitation at 465 nm.

enhancement, and showed a steady and smooth increase until a plateau was reached ($\Phi_f = 0.12$). The Hill-plot profile of the titration curve suggested a 1 : 3 stoichiometry of the host–guest complexation species with the association constant (log K_{ass})[11] calculated as 10.44 (Fig. S5). Under the same conditions no significant fluorescence enhancements of **TIA1** (20 μM) were observed in the presence of other ribonucleotide di- and triphosphates. These results suggest that **TIA1** is a useful probe for the fluorescence detection of ADP with high selectivity and strong binding affinity.

Since the aminonaphthalimide absorbance band did not change upon the addition of increasing amounts of ADP, the enhancement of the fluorescence was attributed to the PET motif.[12] It seemed that the enhancement of the fluorescence was due to a reduced electron-charge density at the imidazole site after the binding with diphosphate of the ADP, which reduced the "push–pull" nature of the ICT excited state of **TIA1** (caused by the electron-donating amine and the electron-withdrawing imidazole).[13] The selectivity

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of the response with ADP over other adenosine polyphosphates was ascribed the suitable length of the linked group for the diphosphate group. The discrimination of ADP from other nucleotide diphosphates seems to come from the possible hydrogen bonding interactions between the adenosine and the receptor.

^{1}H NMR spectra of the receptor **TIA1** (1 mM) in the presence of ADP (1 mM) exhibited a significant downfield shift (*ca.* 0.15 ppm) of the $(C–H)^+$ proton H_1 on the imidazolium ring, and a chemical shift of the H_2 (0.04 ppm) compared to the free **TIA1** in the same experimental conditions, suggesting the formation of $(C–H)^+ \cdots O$ charged hydrogen bonds between the imidazolium of **TIA1** and phosphate groups in ADP (Fig. 2). While most flexible di- or tripods complexes contain rigid planar fluorescent groups an excimer effect may occur after the addition of guest,[14] however, our experiments did not support the possibility of the formation of the corresponding excimer. The small but significant upfield shifts of the signals corresponding to the aromatic protons within the 1,8-naphthalimide (*ca.* 0.03 pm), demonstrated the possible $\pi \cdots \pi$ stacking interaction between the adenosine and 1,8-naphthalimide groups. The downfield shifts of the H_{13} (0.02 ppm) and H_{12} from the adenine group during the host–guest interactions should be attributed to the additional hydrogen bonding interaction between the adenosine in ADP and **TIA1** receptor. It is thought that these complementary interactions are paramount for **TIA1** exhibiting selectivity toward ADP over other ribonucleotide diphosphate.

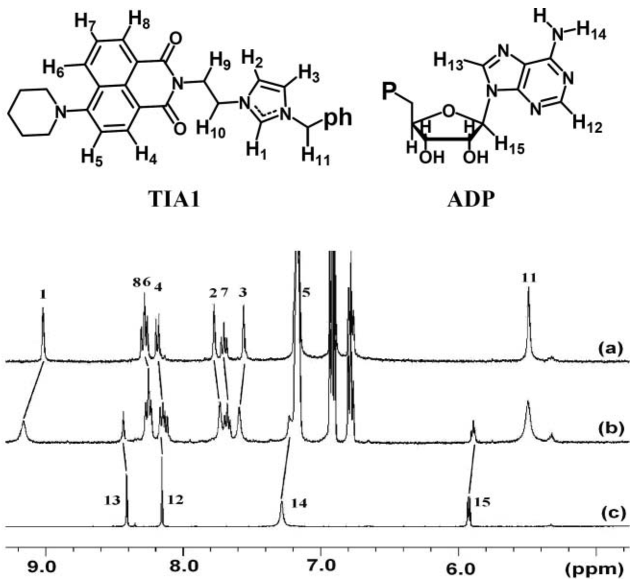
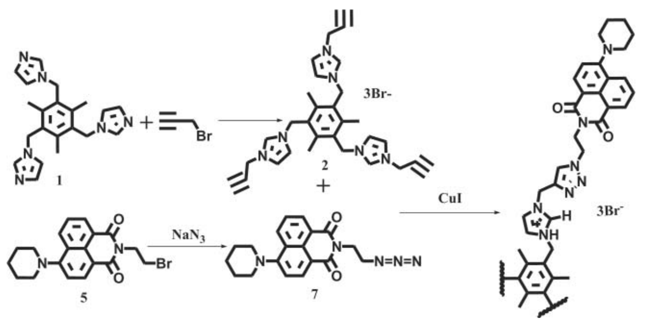


Fig. 2 Partial ^{1}H-NMR spectra for (a) **TIA1**, (b) **TIA1** + ADP (3 eq) and (c) pure ADP in d_6-DMSO, respectively.

Taking advantage of the off/on fluorescence sensing specifically of ADP by **TIA1**, this chemosensor was successfully applied to fluorescence imaging studies of intracellular ADP stores in living cells. HeLa cells incubated with **TIA1** (10 μM) for 30 min at room temperature showed a weak green intracellular fluorescence, which suggested that **TIA1** was cell permeable (Fig. S8). The cells remained viable and no apparent toxicity and side effects were observed throughout the imaging experiments. When cells stained with compound **TIA1** were further incubated with ADP (0.4 mM) in phosphate-buffered saline (PBS) for 30 min and washed, a remarkable enhancing of the green fluorescence intensity (Fig. S9) was observed, suggesting the successful application in the ADP stain experiments. When cells stained with compound **TIA1** were further incubated with ATP (0.4 mM) under the same experimental conditions, no remarkable fluorescence change was observed, suggesting the possibly distinguishability of ADP from ATP in the living cells.

To further investigate the selectivity of the different ribonucleotide polyphosphates and the effect of the linked groups on the sensitivity of the chemosensor **TIA1** toward nucleotide triphosphates, **TIA2** was designed and synthesized in multi-steps to enlarge the chain of each arm through the well-known "click" reaction with the aim to recognize the longer adenosine triphosphate (Scheme 2). The absorbance spectrum of **TIA2** showed a band about 415 nm ($\log\varepsilon = 4.41$), assignable to the ICT band of aminonaphthalimide.[13] Free receptor **TIA2** exhibited an emission band at 550 nm (Fig. 3), assignable to the 1,8-naphthalimide ($\Phi_f = 0.004$)[15] upon excitation at 395 nm. The addition of ATP caused a substantial fluorescence enhancement, which showed a steady and smooth decrease until a plateau was reached ($\Phi_f = 0.03$). The Hill-plot profile of the titration curve confirmed the presence of 1 : 2 stoichiometry of the **TIA2**–ATP complexation species with the association constant ($\log k_{ass}$) for ATP calculated as 8.75 (Fig. S6). Under the same conditions no significant fluorescence changes of **TIA2** were observed in the presence of adenosine polyphosphate. However, by addition of GTP and UTP, a fluorescence enhancement was observed with varying degree. The profile of the fluorescence titration also suggested a 1 : 2 stoichiometry for the complexation species.



Scheme 2 Synthetic procedure for **TIA2**.

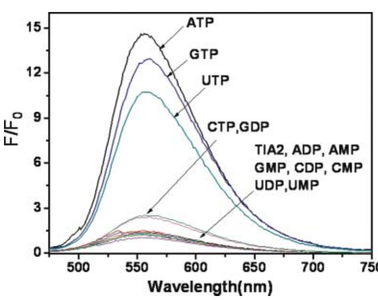


Fig. 3 Emission spectra of **TIA2** (20 μM) in aqueous solution upon addition of ribonucleotide polyphosphates (0.4 mM) (excitation at 396 nm).

^{1}H NMR titration of **TIA2** (1 mM) in a 8 : 2 d_6-DMSO/D_2O solution) upon the addition of ATP, GTP and UTP (3 mM) exhibited the disappearance of $(C–H)^+$ proton, suggesting the formation of $(C–H)^+ \cdots O$ hydrogen bonding between the imidazolium ring and the triphosphate groups. The protons of the nucleic bases and the aromatic rings corresponding to the 1,8-naphthalimide all exhibited small but significant upfield shifts, demonstrating the possible stacking interactions between the nucleic base and the aromatic moiety of 1,8-naphthalimide groups, like that reported by Yoon *et al*.[16]

The **TIA2** response with ATP in HeLa cells was examined (Fig. 4). HeLa cells incubated with **TIA2** (10 μM) for 30 min at room temperature showed a weak green intracellular fluorescence, which suggested that **TIA2** was cell permeable. The cells remained viable and no apparent toxicity and side effects were observed throughout the imaging experiments. Fluorescence enhancement was observed when cells stained were further incubated with ATP (0.4 mM) for 30 min, indicating the possible usage in fluorescence images involving nucleoside polyphosphates within living cells.

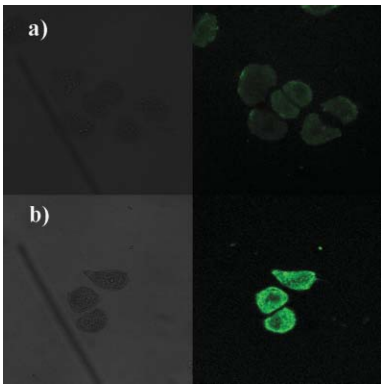


Fig. 4 Confocal fluorescence images of HeLa cells (λ_{ex} = 488 nm) incubated with (a) **TIA2** (10 uM) and (b) their images after further incubated with ATP (0.4 mM).

In summary, we have reported two new types of chemical sensors **TIA1** and **TIA2** for ADP and nucleotide triphosphates, respectively. **TIA1** exhibits a selective "turn-on" fluorescent property for ADP over other ribonucleotide polyphosphates. **TIA2** exhibits a same fluorescent property for ATP, GTP and UTP. These sensors are also successfully applied to cell imaging for the corresponding nucleotide polyphosphates.

Acknowledgements

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