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Combinatorial Synthesis of Benzimidazolium Dyes and Its Diversity Directed Application toward GTP-Selective Fluorescent Chemosensors

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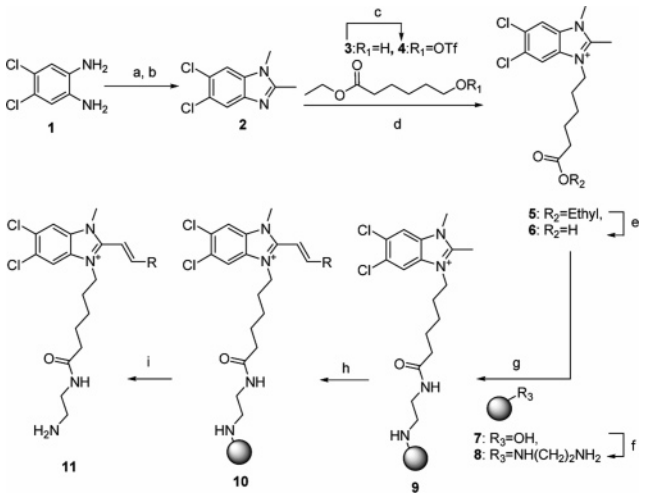
Fluorescent chemosensors are dye molecules whose fluorescence excitation/emission changes in response to the surrounding medium or through specific molecular recognition events.[1] Due to their simplicity and high sensitivity, fluorescent sensors have been widely utilized as popular tools for chemical, biological, and medical applications.[1,2] The most general strategy for fluorescent sensor design is to combine fluorescence dye molecules with designed receptors for specific analytes, in hopes that the recognition event between receptor and analyte will lead to a fluorescence property change of the dye moiety. Although many fluorescent sensors have been successfully developed through this approach, each individual development requires a major effort in both the design and synthesis of the sensors. Also, the sensor's scope of application is limited to the selected specific analytes that the sensor was rationally designed for, so-called *Analyte Directed Sensors*.[3,4] Combinatorial dye library synthesis offers one of the most promising alternatives as *Diversity Directed Sensors*, once an efficient synthetic route can be developed for a diverse set of dyes. Combinatorial chemistry is now widely being used in the chemical biology and medicinal/pharmaceutical fields for the discovery of biologically active molecules or drug candidates, yet the application of this method to fluorescent dyes is only in its infancy.[5] Herein, we report a synthesis of a combinatorial benzimidazolium dye library and the discovery of the first turn-on fluorescent GTP sensor reported thus far.

Nucleotide anion detection has long intrigued researchers and witnessed continuous growth.[6,7] Assuming the cationic hemicyanine dye is a potential receptor of nucleotides due to electrostatic interactions, we chose the benzimidazolium motif as the library scaffold of the fluorescent sensors. Condensation of benzimidazolium ring with 96 aromatic aldehydes provides extended conjugation and structural diversity. To achieve longer wavelengths of the final fluorophore, which may be more useful for possible biological application, we introduced two Cl groups to the benzimidazolium ring (green−red range of emission) rather than using an unsubstituted benzimidazolium ring (UV−blue range of emission). It is noteworthy that the diversity elements (from aldehydes) constitute part of the conjugation system of the dye products and will also serve as recognition motifs for analyte binding. Without linking two separate motifs, as in common analyte directed sensors, our diversity directed sensors can be smaller in size and may respond more directly to their conformational change upon analyte binding.

To facilitate the synthetic procedure, securing high purity compounds without further purification, we developed a novel solid-phase synthesis pathway for the benzimidazolium library. The optimized synthetic procedure is described in Scheme 1. The benzimidazolium scaffold with linker was prepared in solution phase and loaded onto ethylenediamine derivatized 2-chlorotrityl polystyrene solid support. Various lengths of the linker were tested and optimized for best loading of the benzimidazolium compound onto the resin. Aromatic aldehyde building blocks were then coupled to the benzimidazolium ring on solid support, and final products were collected by acidic cleavage. Due to the structural diversity, various excitation/emission wavelengths were observed (see Supporting Information).

Scheme 1. Synthesis of Benzimidazolium Dyes[a]



[a] Reagents and conditions: (a) triethyl orthoacetate, H^+, toluene, reflux; (b) KOH, MeI, acetone; (c) Tf_2O, poly(4-vinylpyridine), DCM; (d) **4**, DCM; (e) 48% HBr, 65 °C; (f) 2-chlorotrityl alcohol resin sequentially treated with thionyl chloride in DCM and ethylenediamine in DCM; (g) **8**, HATU, DIPEA, 30% DMF/DCM; (h) R−CHO (96 aromatic aldehydes, Supporting Information Chart S1), pyrrolidine, NMP; (i) 5% TFA/DCM.

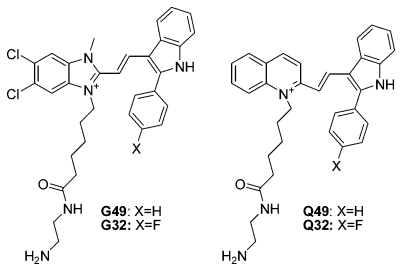


Figure 1. Structure of G32 and G49 and their quinolinium analogues.

For a primary screening, the synthesized dye compounds were tested at 20 μM for 100 μM of AMP, ADP, ATP, UTP, CTP, and GTP in 10 mM HEPES buffer (pH = 7.4) with 1% DMSO in 384-well microplates using a fluorescence plate reader. Two structurally related compounds (G32 and G49, Figure 1) showed dramatically increased fluorescence upon addition of GTP, while not responding to other nucleotides. Although GTP plays an important role in biological processes,[8] very little work has been done on the development of fluorescent sensors for it.[7] Thus far, the best reported GTP sensor, which was designed rationally, showed around 90% quenching response at around mM concentration of GTP,[7b] and most of the known GTP sensors compete with ATP to some extent. To our knowledge, no turn-on fluorescent sensors for GTP have been reported yet.

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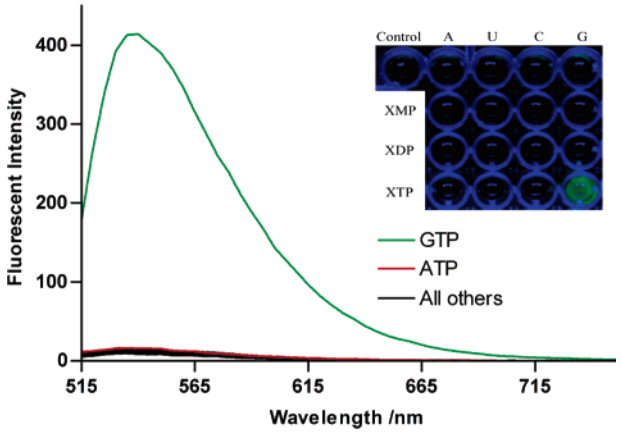

Figure 2. Fluorescence emission spectra (excitation = 480 nm, cutoff = 515 nm) of G49 (1 μM) with 100 μM of GTP (green), ATP (red), all other 14 analytes and blank control (all in black) in 10 mM HEPES buffer (pH = 7.4) with 1% DMSO; 96-well picture was taken using 5 μM of G49 for better visualization, otherwise in the same condition, under 365 nm UV lamp light.

To fully check the selectivity of the two hit compounds, all the nucleosides (adenosine, uridine, cytidine, guanosine) and nucleotides (XNP, where X = A, U, C, G, and N = mono, di, tri) were tested systematically in a 96-well plate. High selectivity of both G49 and G32 only to GTP was clearly exhibited without any obvious cross response to any of other nucleotides or nucleosides (Figure 2 for G49 and Figure S9 in Supporting Information for G32). As we observed, G32 suffered from significant photobleaching under strong irradiation light, so we decided to focus on G49 for further analysis. Upon addition of GTP (100 μM) to G49 (1 μM), a red shift for both λ_{ex} (from 450 to 480 nm) and λ_{em} (from 520 to 540 nm) was observed (the full titration curve is available in Supporting Information). When excited at 480 nm, an approximately 80-fold fluorescence increase at an emission wavelength of 540 nm was observed only for GTP, while only two (ATP) or fewer fold changes were observed for all other analytes. In the same condition, dGTP showed a little weaker (70-fold increase) but almost similar response to that of GTP. This indicates that the 2′-hydroxyl group of GTP is crucial for the molecular interaction with G49. The quantum yields (Φ) of G49 before and after addition of GTP were 0.003 and 0.074, respectively.[9] A visual distinction was also possible when 5 μM of G49 was used (picture in Figure 2).

The quinolinium analogues of G32 and G49 were also prepared as control compounds, and neither of them showed any fluorescence change in the presence of GTP. Combined with the fact that none of the other 94 library members that originated from different aldehyde groups showed strong fluorescent response to GTP, we postulate that both the imidazolium and the 2-phenylindole moiety are important for selective GTP recognition.

In conclusion, we discovered the first turn-on GTP fluorescent sensor thus far from a semi-designed diversity directed sensor approach. On the basis of this unprecedented high selectivity of G49 to GTP and its visual green fluorescence increase, we propose to dub this compound "GTP Green." The details of molecular recognition between GTP Green and GTP, structure−activity relationships, and biological application studies are in progress.

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Supporting Information Available: Complete experimental details; spectral data for G32, G49, and precursors thereof; fluorescence emission spectra of G32 upon addition of 16 analytes; fluorescent titration of G32 and G49 upon GTP. This material is available free of charge via the Internet at http://pubs.acs.org.

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