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COMMUNICATIONS

Published on Web 01/14/2003

Combinatorial Approach to Organelle-Targeted Fluorescent Library Based on the Styryl Scaffold

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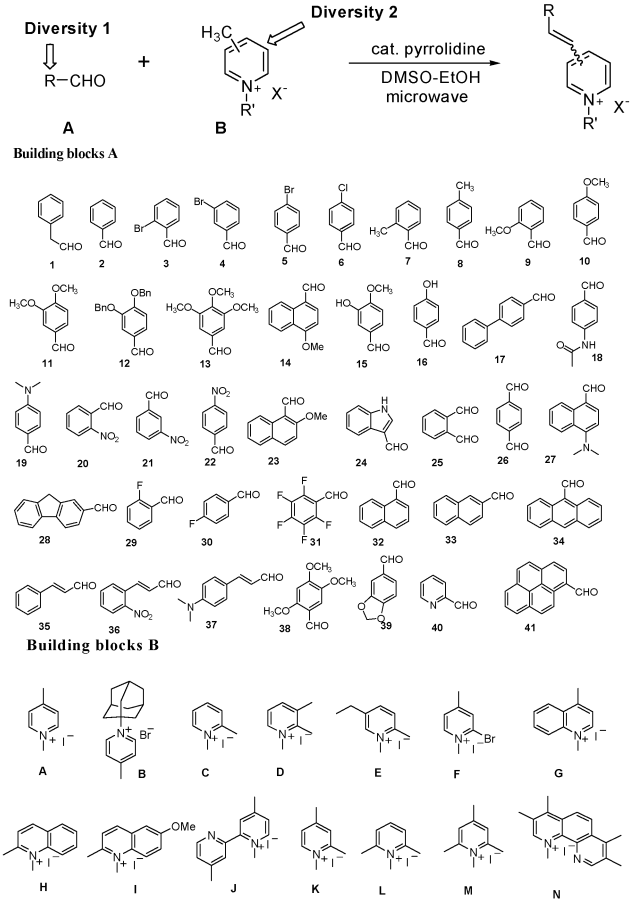
Fluorescent compounds have attracted attention due to their broad application[1] coupled with highly sensitive and specific detection methods.[2] Styryl dyes are a class of fluorescent, lipophilic cations that have been used as mitochondrial labeling agents and membrane voltage-sensitive probes of cellular structure and function.[3] Because of the electrochemical potential across the mitochondrial inner membrane, these lipophilic cations can accumulate in mitochondria according to the Nernst equation.[4] Nevertheless, the affinity of some styryl molecules for cellular macromolecules such as DNA prompts the question as to what structural or physicochemical features of these molecules can actually confer specific mitochondrial localization, and whether certain derivatives could be developed as probes for other organelles or macromolecules.[5]

While rational design of compounds with specific emission wavelengths and high quantum yields is difficult, the combinatorial approach has been reported to be quite powerful in developing fluorescent libraries.[6] For microscopic imaging and flow cytometry applications, it is often desirable to obtain fluorescent compounds that excite or emit in a specific color range. Nevertheless, the spectral properties and potential applications of the reported combinatorial fluorescent libraries are still limited. In this communication, we report the first combinatorial wide-color range fluorescent toolbox and its potential application as organelle-specific probes.

Our fluorescent library is based on the styryl scaffold, synthesized by the condensation of 41 aldehydes (**A**) and 14 pyridinium (2- or 4-methyl) salts (**B**) (Scheme 1). For building blocks, we chose commercially available aldehydes (**A**) containing functionalities of various sizes, conjugation lengths, and electron-donating or -withdrawing capabilities. *N*-methyl pyridinium iodide compounds (**B**) were synthesized by the methylation of commercially available 2- or 4-methylpyridine derivatives using methyl iodide.[7] The condensation of **A** and **B** with a secondary amine catalyst[8] was performed in 96-well plates, and the dehydration reaction was accelerated by microwave irradiation for 5 min to give 10−90% conversion. The resulting library compounds were analyzed by LC−MS equipped with diode array and fluorescence detectors, and a fluorescence plate-reader to determine the absorption and emission maximum (λ_{ex} and λ_{em}), and the emission colors are summarized in Table 1.

It can be easily visualized that this styryl dye library covers a broad color range from blue to long red, representing practically all the visible colors! This should be attributed to the structural diversity as discussed above. It is noteworthy that further purification is not required for primary analysis, as the fluorescent properties of the products are easily distinguishable from those of left-over building blocks **A** and **B** (weak fluorescence or much shorter λ_{ex} and λ_{em}). Furthermore, the synthesis was designed so that the reaction mixture can be used directly in biological screening; toxic

Scheme 1. Synthesis of styryl dyes



catalysts (such as strong acid, base, metals) were avoided, and most of the low-boiling point solvent and catalyst (pyrrolidine) were removed during the microwave reaction, leaving only DMSO, the common solvent for biological sample preparation.

Without further purification, the library compounds were incubated with live UACC-62 human melanoma cells growing on glass bottom 96-well plates, and the localizations of the different compounds in the cells were determined using an Axiovert (Zeiss) microscope (λ_{ex} = 405, 490, and 570 nm; λ_{em} > 510 nm) with a 100× Zeiss oil immersion objective. It was found that 119 out of 276 fluorescent compounds localize to specific subcellular compartments (i.e., mitochondria, ER (endoplasmic reticulum), vesicles, nucleoli, chromatin, cytoplasm, or granules). The images in Figure 1 show cells stained with selected fluorescent compounds. Previous studies have established that there is large voltage between the inside of the mitochondria and the cytosol, and compounds with strong polarizability and charged compounds can interact strongly with the mitochondrial membrane. Considering that our library com-

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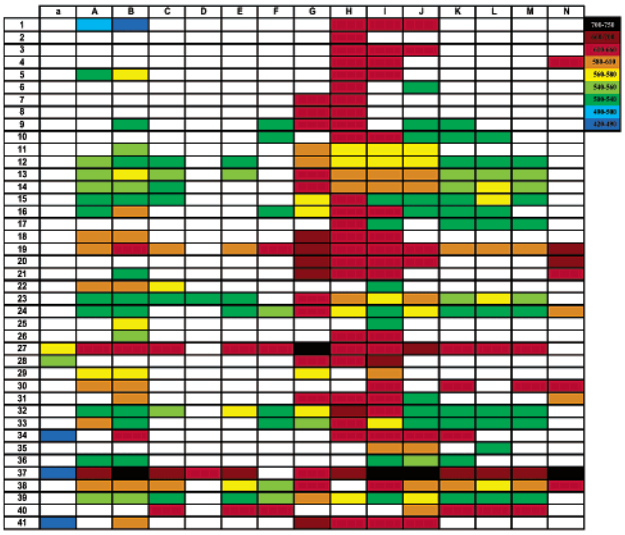
10.1021/ja027587x CCC: $25.00

Table 1. Emission Colors of the Fluorescent Compounds from the Styryl Dye Library [(a) Components in Building Block A, (b) Components in Building Block B][a]



[a] Row a is aldehyde only.

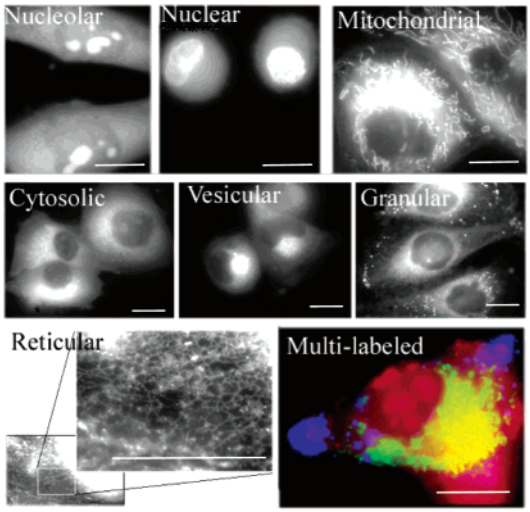


Figure 1. Images of representative localizations (bar = 10 μm): nucleolar (I19); nuclear (H28); mitochondria (A12); cytosolic (I37); vesicular (H12); granular (B41); reticular (J37); multilabeled: nucleolar (I19, red), granular (34, blue), mitochondrial (B24, green).

pounds are positively charged, it is not surprising that 64 out of 119 selected compounds localize specifically to mitochondria. Again, owing to the diversity of the molecular structure, some compounds targeted organelles other than mitochondria. This encrypted interesting structure-localization relationships (SLR), which can lead to rational design of molecular probes for cellular components and opened the chance of multicolor labeling using our toolbox (Table 2).

Combinatorial chemistry has grown to be one of the most powerful tools in new drugs and materials discovery, and our combinatorial approach for organelle-targeted fluorescent dyes further demonstrates its prowess. In addition to organelle-specific

Table 2. Localization Distribution of the Organelle Specific Styryl Dyes [(#) Nuclear, (*) Nucleolar, (♦) Mitochondria, (●) Cytosolic, (×) Endoplasmic Reticular (ER), (■) Vesicular, (▲) Granular][a]

	a	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1		•	•						♦	♦	♦				
3									♦	♦	♦				
4										▲					
7								♦							
8								♦							
9								♦	♦		♦	♦			
10									♦	■ •	♦	♦	♦		
11			♦												
12		♦	×	♦ ×		× ■		♦ *	■	■	■	×	×	×	
14		♦	×					♦	■	■		♦	♦ ×	♦	
15									♦		■				
16			♦						*	■*	■				
17									■ *	■		×	×	×	
18								♦	♦ *	*					
19		♦ *	♦ ×	♦		♦		♦	*	*	♦	♦	♦	♦	*
20								♦	*	*	■				*
21								♦	♦ *	■*					
22			♦												
23		♦ •	•	•	•	•		■	■*			•	•	•	
24		•	♦				■	♦	•			♦			•
25			•												
27	▲	•	♦ ×					♦	♦	*		♦		♦	
28									#	*					
30										♦					
31			♦					♦ *	♦#	♦#•	♦				
32		♦• ■	♦					♦	♦ *	♦#*	♦	♦	♦	♦	
33		♦• ■						♦ •	♦#• ■	♦#■	♦	♦	♦	♦	
34	▲														
35										•	•		♦		
36			♦												
37			♦		♦	♦ *				•	×	■			♦ *
38			♦#					■•#		•	♦				
39			♦						•		♦	♦		♦	
41			▲												

[a] Row a is aldehyde only.

binding in a larger view, some compounds may be DNA, RNA, or protein-specific binding probes. Further studies will be carried out on the compounds that bind specifically to these macromolecules and those compounds that are sensitive to the cellular environment, to elucidate structural features of the styryl molecules responsible for their organelle selectivity and optical properties.

Supporting Information Available: All experimental procedures and chemical and biological data (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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JA027587X