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Combinatorial Discovery of Full-Color-Tunable Emissive Fluorescent Probes Using a Single Core Skeleton, 1,2-Dihydropyrrolo[3,4-β]indolizin-3-one

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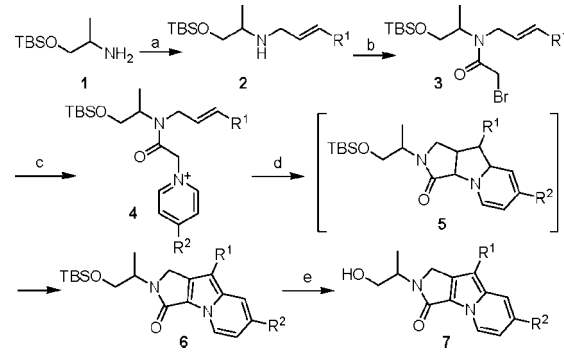
Fluorescent probes have been used extensively as research tools in biological science, clinical diagnosis, and drug discovery because of their high sensitivity and ease of handling.[1] In addition to their applications in biomedical research, the study of fluorescent materials has become a popular research area because of their industrial application as organic light-emitting diodes (OLEDs).[2] Despite the high demand of fluorophores, there exists only a limited number of fluorescent core skeletons with flexibility in their synthetic strategies for tunable emission wavelengths.[3] Many strategies for the discovery of fluorescence probes have been empirically pursued, one at a time lacking flexibility. In addition, the rational design of fluorescent probes with specific emission wavelengths and high quantum yields is still difficult owing to the complexity of the underlying photophysical phenomena.[4] In this paper, we report the de novo design and discovery of novel fluorescent core skeletons with full-color-tunable emissive properties using the combinatorial approach.[5]

The discovery of this novel core skeleton was based on our original attempt for synthetic pathway development of a novel core skeleton using diversity-oriented synthesis,[6] and we identified a novel molecular framework **6** which was partially formed via spontaneous aromatization from cycloadducts **5**. This fluorescent core skeleton **6** was designed to be synthesized with excellent substituent-pending potentials for the construction of diverse fluorescent compounds. Prior to the construction of a fluorescent-compound library, we pursued a rational design through theoretical calculation for the identification of electronic perturbation positions on our core skeleton. The orbital shapes of the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO) of our fluorophore were examined;[10] the R^1 position at the LUMO has a significantly smaller lobe than that at the HOMO, whereas their difference at the R^2 position is less pronounced. On the basis of this calculation, we reasoned that the introduction of an electron-donating group (EDG) on the R^1 position could trigger a bathochromic shift in our fluorescent compounds, while it was difficult to systematize the electronic perturbation of the substituents on R^2 position onto the influence on emissive properties.[7] Through the deliberation of computational studies, the construction of various fluorophores in a single molecular framework was carried out using the combinatorial approach with five α,β-unsaturated aldehydes (R^1, A−E) and five pyridine derivatives (R^2, 1−5) of different electronic properties: A series of several straightforward reactions yield azomethine ylide precursor **4**. The crude products of **4** were then subjected to 1,3-dipolar cycloaddition[8a] in toluene in the presence of DBU. The resulting cycloaddition products **5** were successfully transformed into our desired products **6**, 1,2-dihydropyrrolo[3,4-β]indolizin-3-one, via the subsequent oxidative aromatization with DDQ in a one-pot fashion with reasonable yields (63% in three-step yields, Scheme 1).[8b] Overall, a total of 24 novel fluorescent compounds with a single core skeleton were successfully synthesized by solution-phase parallel synthesis.

Scheme 1. Synthetic Scheme of Fluorescent Core Skeleton[a]



[a] Reagents and conditions: (a) α,β-unsaturated aldehydes, AcOH, Na_2SO_4, CH_2Cl_2, room temp; then $NaBH_4$ in MeOH, 0 °C; (b) bromoacetyl bromide, TEA, CH_2Cl_2, −78 °C; (c) pyridines; (d) DBU in toluene; then DDQ; (e) HF/pyridine/THF (v:v:v = 5:5:90), then TMSOMe.

With the compilation of the fluorescent-compound library, we evaluated the photochemical properties of each individual fluorophore in our library. As shown in Table 1, the overlaid fluorescent emission spectra highlight the development of full-color-tunable emissive fluorophores.[10] It is unprecedented to achieve a dramatic change in the fluorescence properties within a single molecular framework simply by changing the substituents at just two variation points of the fluorophore. The direct comparison of the electronic properties of the R^1 substituents clearly demonstrates the tunablity of this core skeleton. The electron-donating potentials are increased in the following order: methyl (**A5**), phenyl (**B5**), 2-methoxyphenyl (**C5**), 2-thiophenyl (**D5**), and 4-dimethylamino phenyl (**E5**) on a single skeleton with fixed R^2 substituent such as acetyl group. The corresponding emission wavelengths of **A5**, **B5**, **C5**, **D5**, and **E5** are 471, 507, 508, 540, and 613 nm, respectively. The bathochromic shift of emission wavelengths is consistent with our initial prediction based on our quantum mechanics calculation. Changes of the substituents on R^2 position also trigger the notable bathochromic shift, which required further investigations for the rationalization.

Among our novel fluorophores, we chose **B5** for direct comparison with the well-known fluorescent molecule fluorescein since **B5** has a comparable quantum yield [Φ_F: 0.74] and considerably larger Stokes shift that enables intense fluorescent signals with the minimal background.[11] In addition, **B5** is more resistant to photobleaching than fluorescein. Fluorescein is well-known to have multiple ionization equilibria, which leads to pH-dependent absorption and emission, whereas, because of its novel molecular framework, the fluorescence intensity and maximum emission wavelength of **B5** are not influenced by pH value in the range 4−9 (physiological range of in vivo system), that is an important element in bioimaging and bioapplication.[12] To confirm the bioapplicability of our fluorophores, we employed them for protein modification. Because our novel fluorescent molecules were designed with hydroxy functionality, the functional-group inter-

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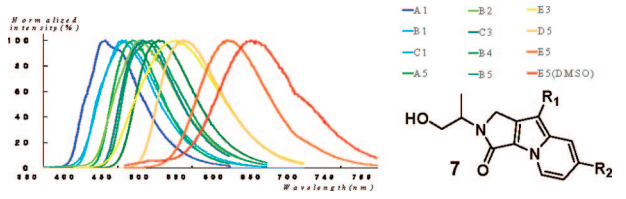
Table 1. Photophysical Data of All Fluorophores (**A1**−**E5**)



Cpd	R^1	R^2	clogP	λ_{abs} (nm)[a]	λ_{em} (nm)[b]	gap (eV)[c]	Φ_F[d]
A1	methyl	hydrogen	1.59	326	434	2.92	0.41
A2	methyl	methoxy	1.61	–	–	2.71	–
A3	methyl	phenyl	3.48	349	433	2.55	0.19
A4	methyl	nitrile	1.06	342	460	2.53	0.76
A5	methyl	acetyl	1.12	396	471	2.27	0.82
B1	phenyl	hydrogen	2.97	298	420	2.80	0.57
B2	phenyl	methoxy	2.99	320	481	2.64	0.27
B3	phenyl	phenyl	4.87	350	490	2.47	0.83
B4	phenyl	nitrile	2.45	388	497	2.41	0.65
B5	phenyl	acetyl	2.50	403	507	2.17	0.74
C1	*o*-methoxy phenyl	hydrogen	2.34	320	461	2.82	0.37
C2	*o*-methoxy phenyl	methoxy	2.36	323	465	2.64	0.26
C3	*o*-methoxy phenyl	phenyl	4.23	381	489	2.46	0.69
C4	*o*-methoxy phenyl	nitrile	1.82	391	493	2.39	0.55
C5	*o*-methoxy phenyl	acetyl	1.87	404	508	2.15	0.71
D1	thiophenyl	hydrogen	2.84	322	481	2.55	0.08
D2	thiophenyl	methoxy	2.85	335	500	2.46	0.03
D3	thiophenyl	phenyl	4.72	283	515	2.30	0.11
D4	thiophenyl	nitrile	2.31	398	529	2.13	0.15
D5	thiophenyl	acetyl	2.36	349	540	1.92	0.35
E1	*p*-dimethylaminophenyl	hydrogen	3.15	298	495	2.53	0.10
E2	*p*-dimethylaminophenyl	methoxy	3.16	311	480	2.44	0.03
E3	*p*-dimethylaminophenyl	phenyl	5.04	403	530	2.17	0.21
E4	*p*-dimethylaminophenyl	nitrile	2.63	428	509	2.03	0.13
E5	*p*-dimethylaminophenyl	acetyl	2.67	440	613	1.78	0.15

[a] Only the longest absorption maxima are shown. [b] Excited at the maximum excitation wavelength. [c] Value of calculated energy gap between the HOMO and LUMO. [d] Absolute fluorescence quantum yield. Absolute quantum yields of known fluorescent dyes in various wavelengths were measured to confirm the reliability of the system [anthracene: $\Phi_F = 0.27$ (reported: 0.27);[9a] fluorescein: $\Phi_F = 0.76$ (reported: 0.79);[9b] cresyl violet: $\Phi_F = 0.48$ (reported: 0.54)[9c]].

conversions from hydroxy to either azide or amine were straightforward, efficient, and orthogonal to the photochemical properties of our fluorescent core skeleton.[10]

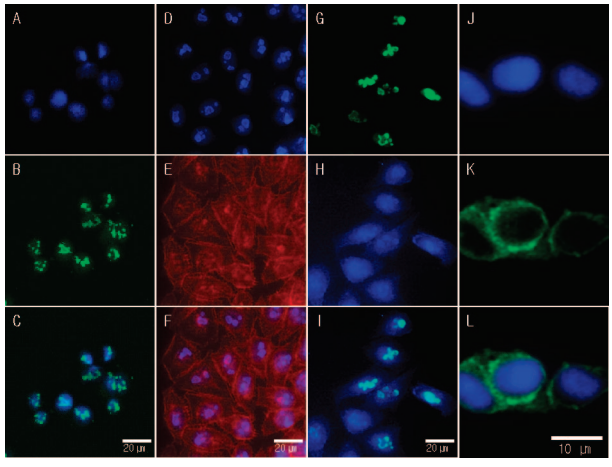


Figure 1. Immunofluorescence image of HeLa cell. Nucleus stained by Hoechst (A, J); nucleol targeted by antinucleol 1°Ab and visualized by **B5**-labeled antimouse 2°Ab (B, G) or **B1**-labeled antimouse 2°Ab (D); Actin visualized by TRITC-labeled phalloidin (E); EGFR targeted by Erbitux and visualized by **B1**-labeled antihuman 2°Ab (H); EGFR visualized by **B5**-labeled Erbitux (K). Panels C, F, I, and L are merged images of A and B, D and E, G and H, and J and K, respectively.[10]

The representative fluorophores (**B1** and **B5**) with amine moiety were then charged with a maleimide group for the specific conjugation with thiol moiety of biopolymers.[10] As shown in Figure 1, our fluorophores were labeled on protein-of-interests and successfully applied in immunocytochemistry via direct labeling of 1°Ab (Erbitux) or labeling of 2°Ab to visualize nucleol and epidermal growth factor receptor (EGFR) as model systems.

In summary, we have developed a full-color-tunable fluorescent core skeleton, 1,2-dihydropyrrolo[3,4-β]indolizin-3-one, by complexity-generating one-pot reactions. This core skeleton can accommodate various emission maxima simply by changing substituents at the R^1 and R^2 positions, having different electronic properties. These novel fluorophores have excellent photophysical and photochemical properties, resistance to photobleaching, moderate to excellent quantum yields, pH-independent fluorescence, large Stokes shifts, and druglike lipophilicity for membrane permeability. Further, we have successfully demonstrated the bioapplication of our fluorophore in the immunofluorescence.

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Supporting Information Available: Experimental procedures, complete spectroscopic characterization data of all new compounds, and results of computational studies. This material is available free of charge via the Internet at http://pubs.acs.org.

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