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A 3-hydroxychromone with dramatically improved fluorescence properties

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Abstract—A new 3-hydroxychromone derivative, 2-(6-diethylaminobenzo[*b*]furan-2-yl)-3-hydroxychromone, has been synthesized by a concise route. Possessing dual emission common for 3-hydroxyflavones, it exhibits strong red shifts of both absorption and fluorescence spectra, which makes it the longest wavelength fluorescent dye among all known chromones. It also demonstrates a significant increase in fluorescence quantum yield in aprotic solvents and shift in solvent-polarity-dependent switch between normal and tautomer emissive forms. This derivative offers new possibilities in designing novel molecular sensors. © 2001 Elsevier Science Ltd. All rights reserved.

The presence of two well-separated fluorescence bands belonging to excited-state normal (N*) and tautomer (T*) forms is a characteristic feature of 3-hydroxyflavone (**A**, Fig. 1) derivatives.[1,2] Introduction of an electron donor group in the 4′-position increased the charge-transfer nature of N* form and made the distribution between these forms in emission very sensitive to solvent polarity. This property resulted in a variety of applications of substituted 3-hydroxyflavones as probes in the studies of organized ensembles such as micelles,[3,4] phospholipid vesicles,[5,6] protein molecules[7] and polymers.[8] However, this unique property has remained unexplored in applications of fluorescence microscopy to living tissues and cells, which requires new fluorescent sensors. In order to decrease photo-damage, remove self-fluorescence and use common light sources in the visible region, the probe has to be excited at 450 nm, or longer wavelengths, and its fluorescence quantum yield should not be lower than that of the commonly used dyes. During our previous studies,[9] we replaced the 2-phenyl group of 3-hydroxyflavone with 2-benzo[*b*]furanyl and, in having extended conjugation, the product, 2-(2-benzo[*b*]furanyl)-3-hydroxychromone (**B**), exhibited better fluorescence properties than 3-hydroxyflavone (**A**). In the next step, we have made an attempt to increase the charge-transfer character of 3-hydroxychromone in the normal excited state (N*). In analogy with 4′-diethylamino-3-hydroxyflavone (**C**), a π-electron donor group, diethylamino, was introduced to the 6-position of benzofuran ring. Our results show that this group has dramatically improved the fluorescence properties of 2-(2-benzo[*b*]furanyl)-3-hydroxychromone (**B**). To our best knowledge, this novel product, 2-(6-diethylaminobenzo[*b*]furan-2-yl)-3-hydroxychromone (**D**), has the longest wavelength of absorption and fluorescence among all the known flavones and chromones to date.

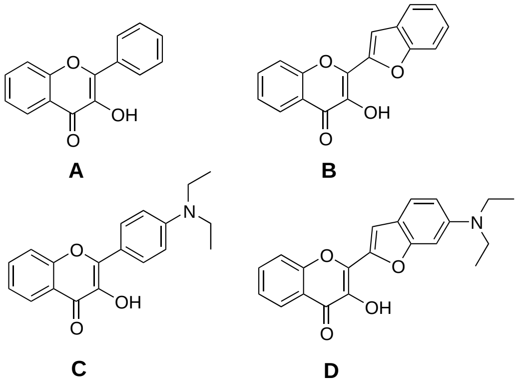

Figure 1. 3-Hydroxychromones **A**–**D**.

2-(6-Diethylaminobenzo[*b*]furan-2-yl)-3-hydroxychromone (**D**) was synthesized in four steps starting from

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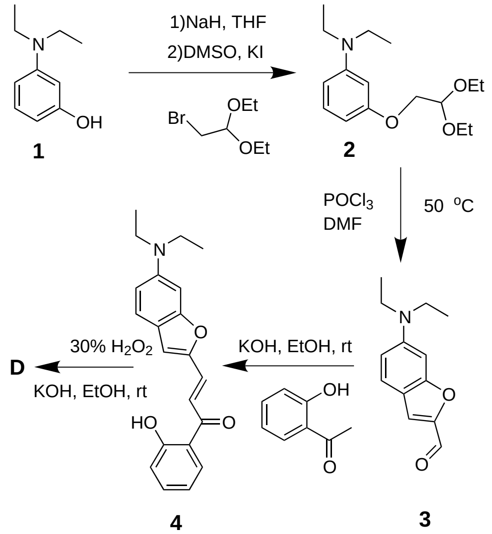
3-diethylaminophenol **1** (Scheme 1). It was transformed to a sodium salt with sodium hydride, and then reacted with bromoacetaldehyde diethylacetal in DMSO at 50°C, using KI as a catalyst. The ether **2** was converted to the aldehyde **3**[10] in $POCl_3$ and DMF at 50°C, so that the synthesis of this highly functionalized aldehyde, 6-diethylaminobenzo[*b*]furan-2-carbaldehyde **3**, has been achieved in two steps. To our best knowledge, this is a new and concise route leading to such an aldehyde. Condensation of the aldehyde **3** with 2-hydroxyacetophenone in the presence of excess KOH in aqueous ethanol yielded 2-propen-1-one **4**, which was then, applying Algar–Flynn–Oyamada reaction,[11] treated with 30% hydrogen peroxide to form chromone **D**.[12]

Absorption and fluorescence properties of 3-hydroxychromone **D** were studied with respect to parent compounds **A**–**C** in six solvents of different polarity: hexane, toluene, ethyl acetate, chloroform, acetonitrile and ethanol.[13] In all the solvents, chromone **D** shows significant red shift of absorption spectra, compared with **A**–**C** (Fig. 2), approaching ca. 450 nm. Comparing the absorption maxima of **A** with **C**, and **B** with **D** (Table 1), it can clearly be noted that the red shift, produced by introduction of π-electron donor, diethylamino, to benzofuran ring of **B** (84 nm, 5177 cm^{-1} in toluene) is considerably higher than in the case of the introduction of the same donor to the phenyl ring of **A** (65 nm, 4645 cm^{-1} in toluene). The 40 nm difference between absorption maxima of **C** and **D** is considered to be an effect of the chromophore extension. Low solvent sensitivity of absorption spectra is observed for all four compounds. The increase of molar extinction coefficient of **D** up to 1.3 times (Table 1), compared with **B** and **C**, is the result of gathering their extended conjugation and π-electron donor group, respectively, in one molecule.

The most remarkable effects are observed in fluorescence (Fig. 3). First, in all the solvents, chromone **D** demonstrates record long-wavelength fluorescence of both N* and T* forms. Second, introduction of diethylamino group in 6-position of the benzofuran ring leads to dramatic long-wavelength shifts—up to 180 nm in ethanol for N* form and up to 66 nm in toluene for T* form (Table 1). This is significantly higher than the effect of the same group in 4′-position of 3-hydroxyflavone (**A**)—up to 123 nm in ethanol for N* form and up to 40 nm for T* form in toluene. Such a strong effect of the donor group on the fluorescence profile of **B** can provide an opportunity to design new sensitive fluorescent sensors of ions.[14,15]



Scheme 1.

As a result of increase in charge transfer character of normal excited state, an extremely high sensitivity of **D** to solvent polarity is observed. The emission band of **N*** form shifts from 460 nm in hexane to 600 nm in ethanol, displaying strong positive solvatofluorochromy (140 nm, 5072 cm^{-1}), which is significantly higher than that of the analog **C**, with shorter conjugation (73 nm, 3102 cm^{-1}) (Table 1). It is important to note that non-substituted **A** and **B** show very small positive solvatofluorochromy. Another excellent feature of chromone **D** is that the ratio of intensities of two forms, N*/T*, changes from 0.1 to $\sim$5 with the growth of solvent polarity from hexane ($E_T[30]=31.0$)[16] to ethyl acetate ($E_T[30]=38.1$) (Fig. 4). On the other hand, chromones **A**–**C** show predominantly emission of the T* form in this range of solvents. In the more polar solvent, acetonitrile ($E_T[30]=45.6$), the T* form emission of **D** is already not observed. These dramatic changes of partition between emission of T* and N* forms with such a small variation in the polarity of non-polar solvents is a unique feature of **D** among 3-hydroxychromones, which normally display significant spectral changes in a range of more polar solvents. Considering the sequence of chromones **A**, **C** and **D**, it could be concluded that both the introduction of donor group and increase of the dipole length favor the charge transfer character of the excited state of 3-hydroxychromones and shift dramatically the sensitivity of charge trans-

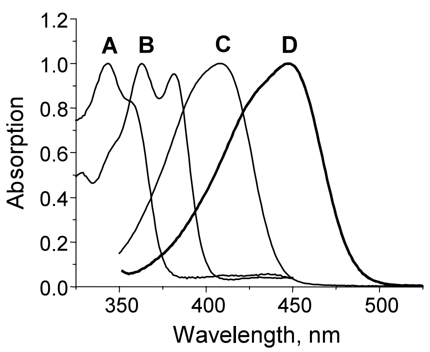


Figure 2. Normalized absorption spectra of chromones **A**–**D** in toluene.

Table 1. Spectroscopic properties of compounds **A**–**D**[a]

Solvent		λ_{max} abs (nm)	λ_{max} fl N* (nm)	λ_{max} fl T* (nm)	φ	$\varepsilon \times 10^{-3}$
Hexane	C	403	450	553	0.12	33.2
	D	**439**	**458**	**586**	**0.22**	**44.8**
Toluene	A	343	–	528	0.29[c]	14.4[c]
	B	364	414	547	0.39[d]	29.9
	C	408	452	568	0.12	30.7
	D	**446**	**508**	**613**	**0.24**	**37.6**
AcOEt	C	401	474	571	0.039	31.1
	D	**437**	**537**	**618**	**0.18**	**37.3**
$CHCl_3$	C	413	482	562	0.11	31.5
	D	**453**	**545**	**611**[b]	**0.36**	**37.5**
CH_3CN	A	339	390	325	0.046[c]	14.3[c]
	B	358	418	542	0.18[d]	29.9
	C	404	509	571	0.10	31.1
	D	**437**	**582**	–	**0.23**	**36.6**
EtOH	A	343	400	532	0.024[c]	13.3[c]
	B	366	423	544	0.12[d]	29.4
	C	412	523	–	0.52[e]	31.5
	D	**444**	**600**	–	**0.10**	**35.9**

[a] λ_{max} abs, positions of absorption maxima, λ_{max} fl N* and λ_{max} fl T*, positions of fluorescence maxima of N* and T* forms (dashes signify that corresponding maxima are not resolved). φ is the fluorescence quantum yield determined with **C** as the reference (φ=0.52 in ethanol), and ε-molar coefficient of extinction (ml mol^{-1} cm^{-1}).

[b] Band appears as a shoulder.

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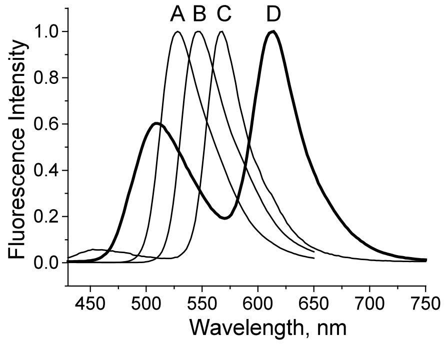

Figure 3. Normalized fluorescence spectra of chromones **A**–**D** in toluene.

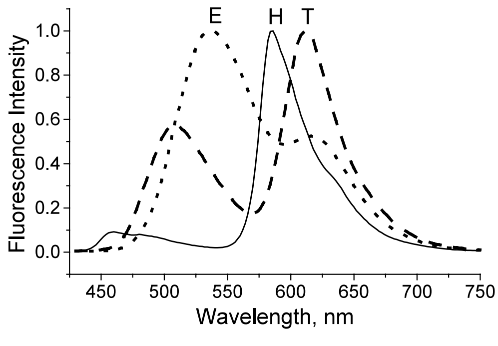

Figure 4. Normalized fluorescence spectra of **D** in ethyl acetate (E), hexane (H), and toluene (T).

fer–proton transfer switch to less polar solvents. Thus, **A** displays significant solvent sensitivity of partition between N* and T* forms in the range of polar solvents, acetonitrile–ethanol–trifluoroethanol,[17] so are **C** in the solvents of medium polarity, ethyl acetate–acetonitrile–ethanol, and **D** in low polar solvents, hexane–toluene–ethyl acetate (Fig. 4).

In polar aprotic solvents, chromone **D** shows much higher fluorescence quantum yield than **A**–**C** (Table 1). The higher quantum yield of benzo/naphthofuryl-3-hydroxychromones, non-substituted at 3-position of furan ring, was previously reported with respect to 3-hydroxyflavone.[9] Presumably, this is due to their more planar structure in steady state compared with 3-hydroxyflavones, which are known to be non-planar[18,19] with an angle of ca. 28° between chromone and phenyl units.

In conclusion, this work is not only confined to showing the unique fluorescence sensitivity of **D** to solvents. In a more general sense, we have demonstrated the fine tuning in switching between two well-resolved emissive states with almost ideal 'all-or-none' switchability by environmental stimuli. By designing substituents at the site of electron-donor, diethylamino group, in line with similar research based on substituted 3-hydroxyflavones,[14,15] new sensing properties can be induced with better prospects. In this work we also report, for the first time, a concise route to 6-diethylaminobenzo[*b*]furan-2-carbaldehyde, which can provide an easy access to the synthesis of various new compounds.

Acknowledgements

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10. 6-diethylaminobenzo[*b*]furan-2-carbaldehyde, IR ν 1655 cm^{-1} (HC=O); ^{1}H NMR (200 MHz, CDCl$_3$) 1.21 (6H, t, *J* 7.1 Hz), 3.33 (3H, q, *J* 7.1 Hz), 6.70 (1H, d, *J* 2.3 Hz), 6.76 (1H, dd, *J* 8.8, 2.3 Hz), 7.40 (1H, s), 7.49 (1H, d, *J* 8.8 Hz), 9.61 (1H, s); EI *m*/*z* 217.1 (M$^+$), 202.1, 193.1, 174.0, 159.0, 145.0, 118.0, 89.0.
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