A new class of long-wavelength fluorophores: strong red fluorescence, convenient synthesis and easy derivation

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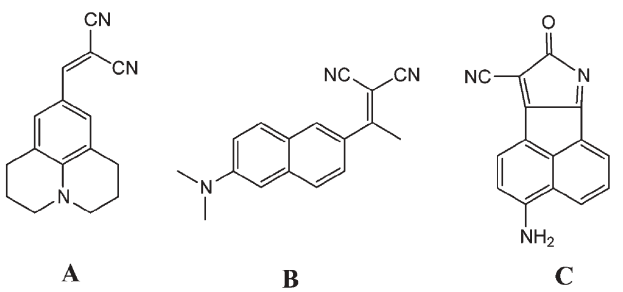
A new class of structurally simple fluorophores with strong long-wavelength emission have been developed through a very convenient procedure.

Recently, fluorescent dyes used as powerful detection tools in biological fields[1] have been studied intensively. For example, in 2003 there were about 60 relevant investigations reported in *J. Am. Chem. Soc.*, and about 70 in *Chem. Commun.*, respectively. Despite the multitude of available fluorophores, new fluorophoric systems are hotly sought for more challenging applications including single molecule imagining.[2] The background emission (generally in the short-wavelength range) from biological systems might cause interference in their accurate detection, so fluorophores emitting in the relatively long-wavelength range are more desirable. It is popular to extend the π-conjugation system of some widely used fluorophore precursors, such as fluorescein,[3] rhodamine,[4] BODIPY,[5] xylene[6] *etc.*, to get new analogs of longer-wavelength emission, although their synthesis and further modification may become difficult and laborious. Relatively less attention has been paid to the development of novel, structurally smaller fluorophores[7] with simplified synthetic approaches and excellent properties.

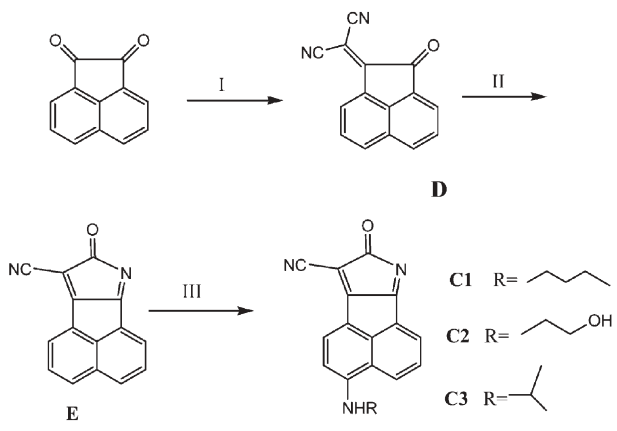
On the other hand, it is known that strongly electron-withdrawing moieties such as multi-cyano groups, particularly malononitrile (and analogs), are often incorporated easily *via* condensation reactions, into π-conjugation systems to fabricate electron-optic materials.[8] Some of these molecules are fluorescent, *e.g.*, fluorophores **A**[8c] and **B**,[8d] shown in Scheme 1. Considering their small conjugation systems, they emit at relatively long wavelengths (*e.g.* for **B**, $\lambda_{flo} \approx 600$ nm in acetonitrile), which is ascribed to their strong ICT (intramolecular charge transfer) nature.[9] However, their fluorescence in solutions, is very weak. For these fluorophores, one of the important decay paths for the excited state is the out-of-plane rotation of the biscyano-vinyl parts.[8c,8d] We assume that, if a rigid ring-framework is fabricated to block the rotation, this decay path could be inhibited greatly and so the fluorescence could possibly be strengthened. To confirm this, 3-amino-8-oxo-8*H*-acenaphtho[1,2-b]pyrrol-9-carbonitrile **C**, is designed.



Scheme 1 The structures of fluorophores.

The **C** derivatives were synthesized very efficiently *via* a convenient 3-step procedure. As shown in Scheme 2, the starting material acenaphthalenequinone undergoes Knoevenagel condensation[10] with malononitrile to give the monoadduct **D**,[10b] and following cyclization catalyzed by anhydrous K_2CO_3, the precursor **E** was obtained almost quantitatively.† Then, at room temperature, the highly electron-deficient and so highly reactive precursor **E** readily underwent nucleophilic substitution of aromatic hydrogen (S_NAr^H) by primary amines to give the fluorophore **C** with moderate yield. All three steps were very easy and under mild conditions no special and expensive reagent was used. Particularly, the third step was useful and noteworthy, because it is the first example of malononitrile promoted substitution of aromatic hydrogen; most other reported systems were nitroarenes and nitrohetrocyclic compounds.[11] The high reactivity of **E** toward nucleophiles, such as amines, make it a universal parent for the derivation to get fluorophores with side chains containing different functional groups.

Compounds **C1**–**C3** exhibited brilliant purple color and strong orange-red fluorescence. The absorption and emission spectra of the three compounds were similar in various solvents, and those of **C1** in acetonitrile, as typical examples, are displayed in Fig. 1. The fundamental optical properties of compounds **C1**–**C3** are listed in



Reagents and conditions:
I: Malononitrile, acetonitrile, reflux, yield>95%
II: Anhydrous K_2CO_3, acetonitrile, reflux, yield>95%
III: Corresponding amines, acetonitrile, r.t. yield: 40-45%

Scheme 2 Synthesis of the new fluorophores.

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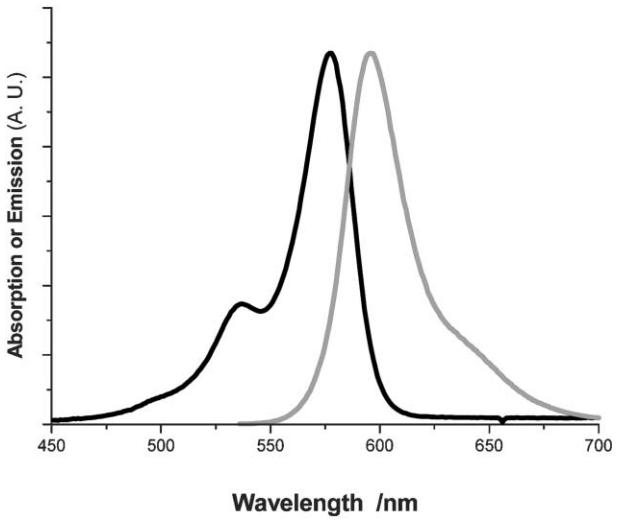

Fig. 1 Normalized absorption and emission spectra of **C1** in actonitrile.

Table 1 Spectroscopic data of **C1**–**C3** in three solvents

	Solvent	λ_{abs}/nm	log ε	λ_{flo}/nm	Φ^a	τ/ns
	Dichloromethane	574	4.84	592	0.59	7.65
C1	Acetononitrile	574	4.90	597	0.58	8.34
	Ethanol	577	4.94	596	0.57	8.13
	Dichloromethane	570	4.10	591	0.81	7.65
C2	Acetononitrile	574	4.11	596	0.95	8.25
	Ethanol	577	4.24	597	0.88	7.96
	Dichloromethane	574	4.73	591	0.64	7.17
C3	Acetononitrile	575	4.83	596	0.62	7.91
	Ethanol	577	4.81	597	0.60	7.47

[a] Determined by comparison with rhodamine B in ethanol (Φ = 0.49, according to ref. 12).

Table 1. These fluorophores show high fluorescence quantum yields (0.55–0.95), moderate Stokes shifts (18–23 nm), relatively long fluorescence lifetimes (7.2–8.4 ns) and relatively long-wavelength absorption and emission with maxima around 575 and 595 nm respectively. The spectra were not greatly influenced by solvents of differing polarities: both the absorption and the emission spectra of compounds **C1**–**C3** showed small spectral shifts (just several nanometres). The fluorescence quantum yields of each compound were of similarly high values, and also the lifetime values did not change greatly in the three solvents. When small amounts of water (<10% volume, if more, precipitation would appear) were added to the acetonitrile solution, the spectral properties of **C1**–**C3** were not influenced greatly, either. These data indicate that the new fluorophores have stable spectral properties. Due to the insolubility in aqueous media, the influence of pH on the photophysical properties could not be recorded accurately. Instead, similar studies were carried out in organic solution. When solutions of compounds **C1**–**C3** in ethanol, acetonitrile or dichloromethane, were acidified carefully with small amounts of trifluoroacetic acid, neither any apparent spectral shift, nor fluorescence quench could be observed which indicated that the spectral properties of **C1**–**C3** are insensitive to acidic media, unlike the often used fluorescein-type fluorophores whose fluorescence would be quenched greatly in acidic solution.

The strong fluorescence of the new fluorophores could be ascribed mainly to two factors: one is the strong rigidity, and the other is the efficient intramolecular charge transfer (ICT) nature—they contained both strongly electron-withdrawing groups and electron-donating groups along the axis of the π-conjugation systems.

The strongly fluorescent **C** derivatives might be potent fluorophore candidates for biological applications. Although their spectra have not entered the near infrared range, they have relatively long wavelengths compared with most available flurophores in the UV-vis range, *e.g.* fluorescein ($\lambda_{flo} \approx$ 520 nm), BODIPY ($\lambda_{flo} \approx$ 520 nm), 4-amino-1,8-naphthalimide ($\lambda_{flo} \approx$ 530 nm), amino-NBD ($\lambda_{flo} \approx$ 550 nm), Cy3($\lambda_{flo} \approx$ 570 nm), tetramethylrhodamine ($\lambda_{flo} \approx$ 570 nm), *etc*. They are smaller molecules than other long-wavelength fluorophores, and so they might not suffer from aggregation so much. Their optical properties are stable in different chemical environments and acidic media, they are electronically neutral unlike those cation or anion-type fluorophores, and these properties facilitate cellular membrane permeation. The most outstanding advantage is the simplicity of synthesis or derivation at room temperature. This is valuable for their competition with other fluorophores, because the synthetic method involved the parent compound **E** which is very electron-deficient and readily undergoes S_NAr^H reactions under very mild conditions without a conventional leaving-group like a halide atom.

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Notes and references

† Selected characterization data.

E: mp 275–277 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 8.71–8.69 (d, J = 8.0 Hz, 1H), 8.67–8.65 (d, J = 7.6 Hz, 1H), 8.64–8.62 (d, J = 8.0 Hz, 1H), 8.42–8.40 (d, J = 7.6 Hz, 1H), 8.04–8.08 (t, J = 8.0 Hz, 1H), 7.99–7.95 (t, J = 7.8 Hz, 1H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 177.48, 138.26, 137.73, 134.40, 132.72, 131.82, 131.37, 128.91, 127.94, 127.37, 126.13, 122.22, 119.72, 113.82, 113.38; IR (KBr) ν/cm^{-1}: 2231, 1643, 1577; ESI-MS: m/z = 253, (M + Na)$^+$; HRMS: m/z calcd. for $C_{15}H_{16}N_2$ 230.0480, found 230.0477.

C1: mp >300 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 9.60 (br s, –N*H*–, 1H), 8.95–8.93 (d, J = 7.6 Hz, 1H), 8.60–8.58 (d, J = 7.2 Hz, 1H), 7.98–7.96 (d, J = 8.8 Hz, 1H), 7.88–7.92 (t, J = 7.8 Hz, 1H), 7.04–7.02 (d, J = 9.2 Hz, 1H), 3.60–3.59 (br s, –NHC*H*$_2$CH$_2$–, 2H), 1.75–1.71 (m, –NHCH$_2$C*H*$_2$CH$_2$–, 2H), 1.47–1.43 (m, –CH$_2$CH$_2$C*H*$_2$CH$_3$, 2H), 0.94–0.98 (t, J = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 176.45, 155.79, 138.66, 132.35, 130.99, 129.63, 127.98, 126.95, 125.49, 121.97, 116.12, 114.33, 111.30, 108.13, 103.95, 43.42, 30.05, 19.68, 13.66; IR (KBr) ν/cm^{-1}: 3284, 2217, 1619, 1562, 1529; ESI-MS: m/z = 300, (M − H)$^-$; HRMS: m/z calcd. for $C_{19}H_{15}N_3O$ 301.1215, found 301.1223.

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