Synthesis and fluorescence of the new environment-sensitive fluorophore 6-chloro-2,3-naphthalimide derivative†

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Convenient and efficient synthesis of a new environmentally sensitive chlorine-substituted 2,3-naphthalimide-based fluorophore is reported. Benzotriazole carboxyl group activation of the 6-chloro-fluorophore enabled quick labeling of free and Fmoc-protected amino acids. The photophysical properties of the compounds obtained include high quantum yields in solvents of different polarity: water, methanol, acetonitrile and hexane.

Introduction

Fluorescence spectroscopy is a valuable tool for biochemical research[1] on ions,[2] biosensors,[3] and processes such as protein folding,[4] protein–protein interactions,[5,6] and phosphorylation.[7] While many fluorescence applications rely on the use of intrinsic fluorophores, the development of new extrinsic fluorophores remains an essential element for the design of new fluorescent probes.[8]

Organic fluorophores based on a naphthalene nucleus, usually with an amino substituent, have aroused the interest of photochemists and photobiologists because of their sensitivity to solvent effects.[9–12] Substantial changes in the fluorescence spectrum, quantum yield, and lifetime are often observed with solvent changes or as a result of binding to a substrate.

Environment-sensitive fluorophores as a class demonstrate spectroscopic behavior dependent on the physicochemical properties of the surrounding environment.[13] 6-Propionyl-2-dimethylaminonaphthalene (PRODAN) **1**,[14] 4-dimethylaminophthalimide (4-DAP) **2**,[15] and 4-amino-1,8-naphthalimide[16] **3** (Fig. 1) are particularly useful solvatochromic fluorophores, which generally exhibit a low quantum yield in aqueous solution, but become highly fluorescent in nonpolar solvents or when bound to hydrophobic sites in proteins or membranes.

Environmentally sensitive fluorophores frequently possess an electron donor and an electron acceptor substituent attached to the same aromatic ring system; the effects are maximized when these groups are far apart. In naphthalenes, fluorescence is most intense when donor and acceptor groups are attached to the 2 and 6 positions of the ring. The fluorescence of unsubstituted aromatic hydrocarbons is insensitive to the environment due to the high degree of symmetry between the ground and lowest singlet excited states.[17] The electron donating and accepting groups on the aromatic ring confer a low energy excited state with marked charge transfer character. The most important potential donor groups are amino or alkylamino, while sulfonyl and carbonyl groups are among the best acceptors.[14]

Recently, Vazquez *et al.* have synthesized (Scheme 1) and studied the fluorescence properties of the new environmentally sensitive fluorophore, 6-*N*,*N*-dimethylamino-2,3-naphthalimide (6DMN) (**4**).[18] The fluorescence quantum yield of this chromophore changes from $\Phi = 0.225$ in chloroform to $\Phi = 0.002$ in water.

The selective substitution of chlorine for aromatic hydrogen changes significantly the photophysical properties of organic compounds.[19] Ge *et al.* have found that chlorine substitution of fluoresceins at the 4 or 7 position significantly increases their fluorescence quantum yield.[19] In another study the chlorinated fluoresceins were used for labeling of proteins.[20]

We have previously labeled amino acids,[21,22] peptides[22] and sugars[23] with benzotriazole activated coumarin fluorophores.[24]

We have now synthesized a novel environmentally sensitive chlorine substituted naphthalimide-based fluorophore which can be utilized for the labeling of amino acids. The photochemical properties of the new fluorophore and the labeled amino acids are also reported.

Results and discussion

Synthesis of the 6-chloro-2,3-naphthalimide-based fluorophore 15

p-Chlorobenzaldehyde (**5**) and malonic acid (**6**) were condensed to give 3-(4-chlorophenyl)acrylic acid (**7**) which on bromination

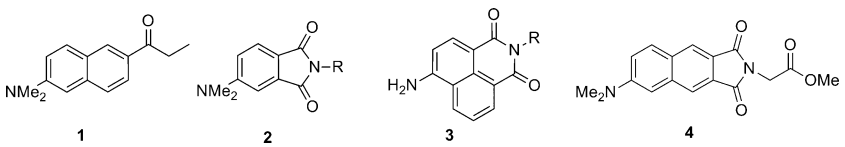

Fig. 1 Environment-sensitive fluorophores.

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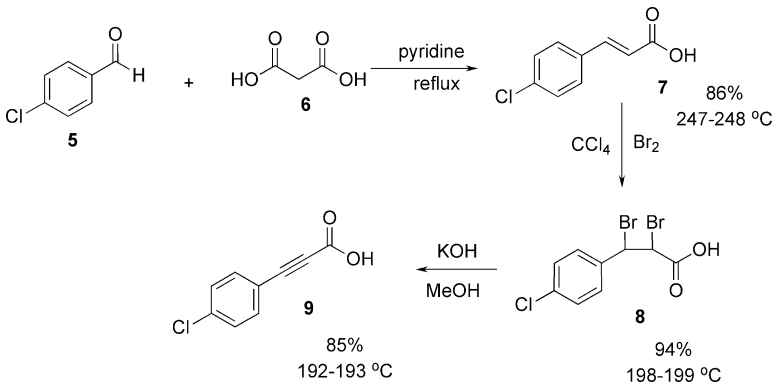
† Electronic supplementary information (ESI) available: Experimental details for compounds **7–9**, **11–13**, **20** and emission and UV spectra for **19**, **23** and **24**. See DOI: 10.1039/c000684j



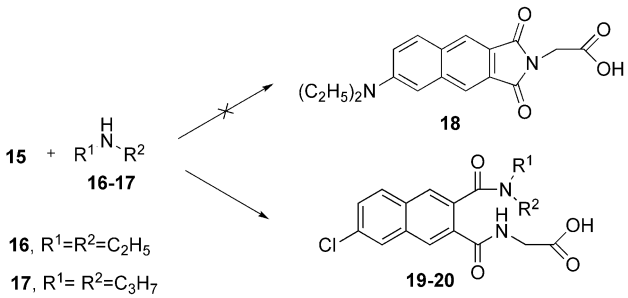
Scheme 1

afforded 2,3-dibromo-3-(4-chlorophenyl)-propionic acid (**8**) in 94% yield. Elimination of the bromine groups from **8** by potassium hydroxide in methanol gave **9** in 85% yield (Scheme 1).

3-(4-Chlorophenyl)propiolic acid (**9**) reacted with propiolic acid (**10**) in acetic anhydride to form 6-chloronaphtho[2,3-*c*]furan-1,3-dione 31% (**11**) together with 55% of 6-chloro-4-(4-chlorophenyl)naphtho[2,3-*c*]furan-1,3-dione (**12**) by self addition of **9** (Scheme 2).

To avoid the formation of **12**, 3-(4-chlorophenyl)propiolic acid (**9**) was reacted with thionyl chloride giving 3-(4-chlorophenyl)propioloyl chloride (**13**) (93%) which was refluxed in benzene with propiolic acid (**10**) to obtain, as the single product, 6-chloronaphtho[2,3-*c*]furan-1,3-dione (**11**) in 85% yield. Compound **11** was then heated under reflux with glycine (**14**) in the presence of Et_3N in toluene for 4 h to yield the desired 2-(6-chloro-1,3-dioxo-1*H*-benzo[*f*]isoindol-2(3*H*)-yl)acetic acid (**15**) in 91% yield (Scheme 3).

Attempted substitution of the chlorine with diethylamine (**16**), gave ring-opening to 2-(7-chloro-3-diethylcarbamoyl-2-naphthamido)acetic acid (**19**) (73%) (Scheme 4). To test the scope and limitations of this ring opening reaction, **15** was reacted with diisopropylamine (**17**) under the same reaction conditions and indeed 2-(7-chloro-3-(diisopropylcarbamoyl)-2-naphthamido)acetic acid (**20**) was obtained in 79% yield.



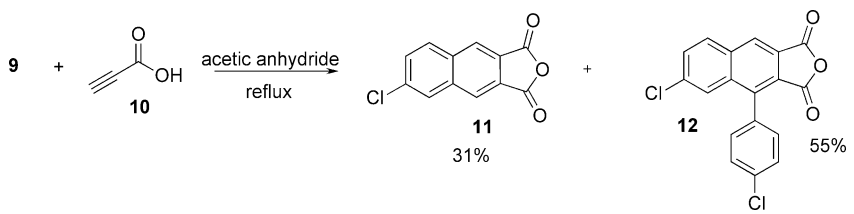
Scheme 4

Labeling of amino acids using 6-chloro-2,3-naphthalimide-based fluorophore 15

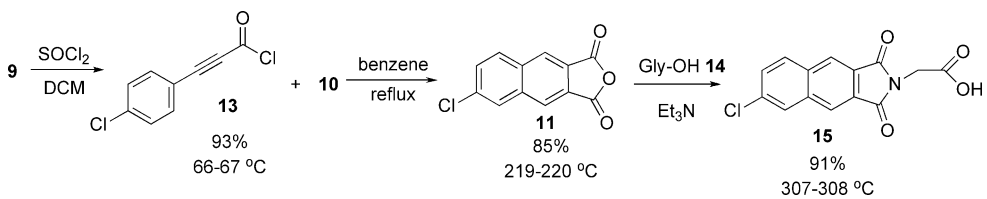
For labeling of the amino acids, **15** was treated with 1*H*-benzotriazole and thionyl chloride in tetrahydrofuran at 20 °C to give **21** as a stable compound in 85% yield which was then coupled with glycine (**14**) and Fmoc$^{\alpha}$-Lys-OH **22** to form **23** and **24** in 76% and 72% yield, respectively (Scheme 5).

Photophysics of 6-chloro-2,3-naphthalimide-based fluorophore and labeled amino acids

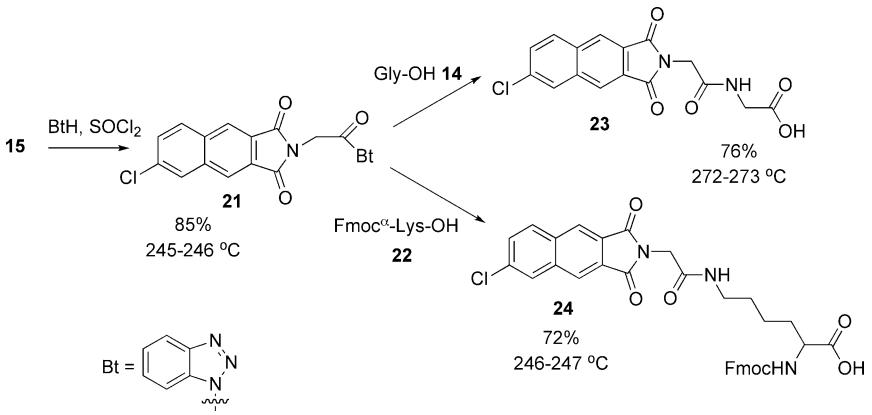
The fluorescence properties of compounds **15**, **19**, **23** and **24** were investigated in solvents of diverse polarity including acetonitrile,



Scheme 2



Scheme 3



Scheme 5

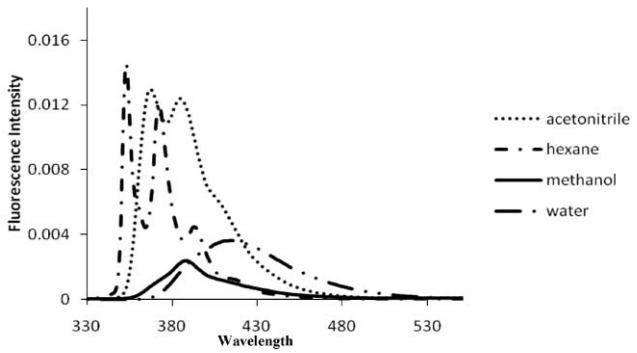


Fig. 2 Emission spectrum of **15**.

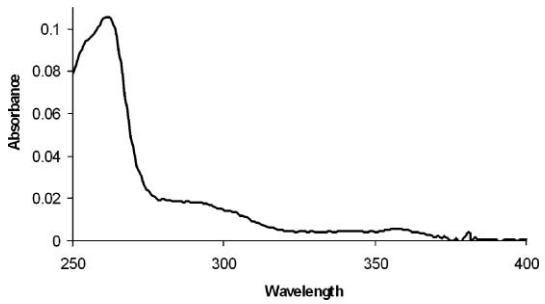


Fig. 3 UV absorption spectrum of **15** (1.45 μM) in methanol.

hexane, methanol and water (Table 1, Fig. 2 and 3). The wavelength of absorption maxima (λ_{abs}), the wavelength of fluorescence emission maxima (λ_{em}), molar extinction coefficients (ε) and fluorescence quantum yields (Φ) for compounds **15**, **19**, **23** and **24** are summarized in Table 1. UV absorption spectra and fluorescence emission spectra for **19**, **23** and **24** are given in the Supporting Information. In the UV spectra the absorption maxima of all the studied compounds in all four solvents are at 260–268 nm. In the emission spectra, the maxima are at 353–408 nm. As the polarity increases, bathochromic shifts are observed due to stabilization of LUMO by polar solvents. Compounds **15**, **19**, **23** and **24** were excited at 260 nm and naphthalene was used as the standard in quantum yield calculations. The quantum yields of these environmentally sensitive fluorophores were usually found to be highest in acetonitrile and lowest in methanol.

Conclusions

A facile synthesis to access the new chlorine-substituted 2,3-naphthalimide based environment-sensitive fluorophore is outlined. This new building block can be successfully incorporated

Table 1 Photophysical properties of **15**, **19**, **23** and **24**

No.	λ_{ab}/nm	λ_{ex}/nm	λ_{em}/nm	ε/mol cm^{-1} × 10^4	Φ	Solvent
15	263	260	384	7.16	0.11	Methanol
	262	260	370, 383	5.24	0.65	Acetonitrile
	260	260	352, 371, 390	2.86	0.43	Hexane
	267	260	408	5.22	0.27	Water
19	262	260	387	5.55	0.24	Methanol
	262	260	366, 383	4.25	0.85	Acetonitrile
	260	260	353, 372, 396	1.22	0.31	Hexane
	267	260	408	5.86	0.49	Water
23	263	260	384	3.14	0.72	Methanol
	263	260	370, 383	2.82	0.84	Acetonitrile
	261	260	358, 373	0.36	0.47	Hexane
	268	260	408	3.03	0.68	Water
24	263	260	386	9.07	0.29	Methanol
	263	260	366, 382	8.81	0.53	Acetonitrile
	261	260	361, 375	2.48	0.74	Hexane
	265	260	408	6.73	0.15	Water

into amino acids using standard protocol benzotriazole methodology.

Experimental

2-(6-Chloro-1,3-dioxo-1*H*-benzo[*f*]isoindol-2(3*H*)-yl)acetic acid (15)

Glycine (160 mg, 2.14 mmol) and triethylamine (22 mg, 0.214 mmol) were added to *p*-chloronaphtho[2,3-*c*]furan-1,3-dione (**11**) (500 mg, 2.14 mmol) in toluene (30 mL) and heated under reflux for 4 h. The precipitate was filtered off and washed with dichloromethane to give the pure 2-(6-chloro-1,3-dioxo-1*H*-benzo[*f*]isoindol-2(3*H*)-yl)acetic acid (**15**) (550 mg, 91%). mp 307–308 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.63 (s, 1H), 8.56 (s, 1H), 8.43 (s, 1H), 8.32 (d, J = 8.5 Hz, 1H), 7.84 (d, J = 8.5 Hz, 1H), 4.32 (s, 2H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 168.9, 166.7, 166.5, 136.0, 134.3, 133.6, 132.4, 129.9, 129.0, 128.2, 127.5, 125.0, 124.1, 32.3. Anal. Calcd for $C_{14}H_8ClNO_4$: C, 58.05; H, 2.78; N, 4.84; Found C, 57.70; H, 2.63; N, 4.67%.

2-(7-Chloro-3-(diethylcarbamoyl)-2-naphthamido)acetic acid (19)

Diethylamine (190 mg, 2.56 mmol) was added to 2-(6-chloro-1,3-dioxo-1*H*-benzo[*f*]isoindol-2(3*H*)-yl)acetic acid (**15**) (500 mg, 1.7 mmol) in DMF (10 mL) and refluxed for 1 h. The solution was cooled and 2-(7-chloro-3-(diethylcarbamoyl)-2-naphthamido)acetic acid (**19**) precipitated and was washed with dichloromethane. (400 mg, 73%). mp 238–239 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.30 (br s, 1H), 8.53 (s, 1H), 8.51 (s, 1H), 8.38 (s, 1H), 8.27 (d, J = 8.7 Hz, 1H), 7.78 (dd, J = 8.7 Hz, J = 2.1 Hz, 1H), 3.96 (d, J = 6 Hz, 2H), 2.95–2.75 (m, 4H), 1.40–1.05 (m, 6H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 168.2, 167.4, 167.2, 136.0, 134.0, 133.7, 132.4, 129.7, 129.3, 129.0, 128.6, 124.2, 123.5, 42.2, 41.3, 11.4. Anal. Calcd for $C_{18}H_{19}ClN_2O_4$: C, 59.59; H, 5.28; N, 7.72; Found C, 59.31; H, 5.32; N, 7.65%.

2-(2-(1*H*-Benzo[*d*][1,2,3]triazol-1-yl)-2-oxoethyl)-6-chloro-1*H*-benzo[*f*]isoindole-1,3(2*H*)-dione (21)

Thionyl chloride (220 mg, 1.9 mmol) was added to benzotriazole (820 mg, 6.8 mmol) in THF (10 mL) at 0 °C. The mixture was stirred for 40 min. 2-(6-Chloro-1,3-dioxo-1*H*-benzo[*f*]isoindol-2(3*H*)-yl)acetic acid (**15**) (500 mg, 1.7 mmol) was then added slowly and followed by stirring for an additional 2 h at room temperature. The solvent was evaporated and dichloromethane was added to the residue. Undissolved solid was filtered off and the solution was washed with saturated sodium carbonate (25 mL × 2) and sodium chloride solution (25 mL × 2). The solvent was evaporated to give pure 2-(2-(1*H*-benzo[*d*][1,2,3]triazol-1-yl)-2-oxoethyl)-6-chloro-1*H*-benzo[*f*]isoindole-1,3(2*H*)-dione (**21**) (560 mg, 85%). mp 245–246 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.71 (s, 1H), 8.64 (s, 1H), 8.46 (s, 1H), 8.36–8.31 (m, 2H), 8.18 (d, J = 8.1 Hz, 1H), 7.88–7.80 (m, 2H), 7.66 (t, J = 7.5 Hz, 1H), 5.62 (s, 2H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 166.7, 166.4, 145.6, 136.2, 134.5, 133.8, 132.6, 131.5, 130.7, 130.2, 129.3, 128.3, 127.6, 127.1, 125.4, 124.6, 120.6, 113.8, 41.3. Anal. Calcd for $C_{20}H_{11}ClN_4O_3$: C, 61.47; H, 2.84; N, 14.34; Found C, 61.45; H, 2.69; N, 13.96%.

2-(2-(6-Chloro-1,3-dioxo-1*H*-benzo[*f*]isoindol-2(3*H*)-yl)acetamido)acetic acid (23)

2-(2-(1*H*-Benzo[*d*][1,2,3]triazol-1-yl)-2-oxoethyl)-6-chloro-1*H*-benzo[*f*]isoindole-1,3(2*H*)-dione (**21**) (480 mg, 1.2 mmol) in THF (5 mL) was added dropwise into a solution of glycine (110 mg, 1.4 mmol) and triethylamine (120 mg, 1.2 mmol) in acetonitrile–water (7 : 3) and was stirred for 1 h at room temperature. To the reaction mixture aqueous 4 M HCl (1 mL) was added and the solvent was removed under vacuum. The residue was dissolved in ethyl acetate (30 mL) and washed twice with 4 M HCl (20 mL × 2) and twice with saturated NaCl solution (20 mL × 2). The solvent was evaporated to give the pure 2-(2-(6-chloro-1,3-dioxo-1*H*-benzo[*f*]isoindol-2(3*H*)-yl)acetamido)acetic acid (**23**) (320 mg, 76%). mp 272–273 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.63–8.61 (m, 2H), 8.55 (s, 1H), 8.43 (s, 1H), 8.32 (d, J = 8.7 Hz, 1H), 7.83 (d, J = 8.7 Hz, 1H), 4.31 (s, 2H), 3.79 (d, J = 5.7 Hz, 2H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 170.9, 166.9, 166.3, 136.0, 134.1, 133.5, 132.3, 129.8, 129.0, 128.7, 128.0, 124.6, 123.8, 40.8, 40.3. Anal. Calcd for $C_{16}H_{11}ClN_2O_5$: C, 55.43; H, 3.20; N, 8.08; Found C, 55.26; H, 3.53; N, 7.89%.

2-(((9*H*-Fluoren-9-yl)methoxy)carbonylamino)-6-(2-(6-chloro-1,3-dioxo-1*H*-benzo[*f*]isoindol-2(3*H*)-yl)acetamido)hexanoic acid (24)

2-(2-(1*H*-Benzo[*d*][1,2,3]triazol-1-yl)-2-oxoethyl)-6-chloro-1*H*-benzo[*f*]isoindole-1,3(2*H*)-dione (**21**) (350 mg, 0.9 mmol) in THF (5 ml) was added dropwise to a solution of Fmoc$^{\alpha}$-Lysine-OH·HCl (360 mg, 0.9 mmol) and triethylamine (180 mg, 1.8 mmol) in acetonitrile–water (7 : 3) and the mixture was stirred for 1 h at room temperature. To the reaction mixture aqueous HCl (1 mL, 4 M) was added and the solvent was removed under vacuum. The residue was dissolved in ethyl acetate (30 ml) and washed twice with 4 M HCl (20 mL × 2) and saturated NaCl solution (20 mL × 2). The solvent was dried and evaporated to give the pure 2-(((9*H*-fluoren-9-yl)methoxy)carbonylamino)-6-(2-(6-chloro-1,3-dioxo-1*H*-benzo[*f*]isoindol-2(3*H*)-yl)acetamido)-hexanoic acid (**24**) (420 mg, 72%). mp 246–247 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.58 (s, 1H), 8.52 (s, 1H), 8.40 (s, 1H), 8.30 (d, J = 8.7 Hz, 1H), 7.87 (d, J = 7.5 Hz, 2H), 7.80 (dd, J = 8.7 Hz, J = 2.0 Hz, 1H), 7.72 (d, J = 7.5 Hz, 2H), 7.63 (s, J = 7.7 Hz, 1H), 7.41 (t, J = 7.5 Hz, 2H), 7.34–7.30 (m, 3H), 4.28–4.24 (m, 4H), 3.92 (s, 1H), 3.08 (s, 2H), 1.2-1.8 (m, 6H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 173.9, 166.9, 166.8, 165.6, 156.1, 143.7, 140.6, 135.8,133.9, 133.4, 132.2, 129.6, 128.9, 128.6, 127.9, 127.6, 127.0, 125.2, 124.4, 123.7, 120.0, 65.6, 53.7, 46.6, 40.4, 38.5, 30.3, 28.5, 23.0. Anal. Calcd for $C_{35}H_{30}ClN_3O_7\cdot H_2O$: C, 63.88; H, 4.90; N, 6.38; Found C, 63.97; H, 4.33; N, 6.51%.

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