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Labeling of nucleosides with fluorescent 6-chloro-2,3-napthalimide

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ABSTRACT

The synthesis and fluorescence properties of new highly fluorescent nucleosides are reported. 6-Chloro-2,3-napthalimides activated with benzotriazole and chlorine label nucleosides quickly and efficiently in yields of 70–82%: the products exhibit quantum efficiencies of 10–94% in solvents of diverse polarity.

Fluorescently labeled nucleosides, nucleotides and nucleic acids are important as reagents for the detection and biological assay of nucleic acid hybridization,[1] DNA sequencing,[2,3] nucleic acid–protein interactions[4] and other medical diagnostic applications.[5] Base-modified nucleosides have been applied as antiviral tools against VZV and HIV, in the evaluation and study of DNA damage, as well as in the anti-sense approach and in DNA-probe technology with fluorescence properties.[6–10]

Fluorescence studies of parent heterocyclic bases, nucleosides, nucleotides, and their polymers date back to the early 20th century.[11–13] The fluorescence of nucleic acids is weak while that of nucleic acids containing certain naturally occurring fluorophores has been of analytical use. Direct fluorescent labeling has been achieved by covalent attachment using various enzymatic[14] or chemical methods[15,16] through the accessible sites on the bases, sugars (3′ and 5′) or phosphate units of oligonucleotides[17–19] often attached at the end of spacer arms or side chains of nucleobases and sugars. Polymerase chain reaction (PCR) applications involve modification of the 5′-end of the nucleosides. Nucleosides bearing side arms terminating in groups like $-NH_2$ have often been used as linkers for post-synthesis modifications.[20]

Most fluorescent nucleic acid labeling studies have utilized organic fluorescent dye molecules (e.g., fluorescein and rhodamine); but a few fluorescent metal chelates that have a long-lived time-resolvable signals (e.g., europium chelates[21]) on various organic (e.g., carbon nanotubes[22]) and inorganic (e.g., quantum dots, gold particles, and fluorescent mineral[23]) particles. Many previous fluorescent labels for nucleic acids were for immunoassay.[23] Factors in the choice of a fluorophore include fluorescence quantum yield, the Stokes shift, fluorescence emission spectrum (including time-resolvability and the ability to use several fluorophore labels, simultaneously), susceptibility to photobleaching, and reduction of background interference (provided by time-resolved fluorescent labels of lanthanide chelates and metal chelate-based IR labels).[23]

The covalent binding of oligonucleotides to coumarin, naphthalene[24] and pyrene units,[25] enhances the stability of duplexes with the complementary target sequences.[26] The only fluorescent labeling of nucleosides was reported recently by Staudinger ligation[26] with coumarin and ferrocene derivatives. The labeled nucleosides were obtained in 41–64% yield from uridine-2′-yl and thymidine-5′-yl, but only in max 15% yield from uridine-5′-yl and not at all from thymidine-3′-yl.[26]

2′-Deoxyribosyl-2-aminopurine (2-AP), a mimic of 2′-deoxyadenosine, is the most popular fluorescent base.[27,28] However, 2-AP displays severe limitations related to the dramatic drop (up to 100-fold) of its quantum yield (0.68)[29] when incorporated in oligonucleotides.[30,31] Other fluorescent nucleoside analogues when incorporated into ODNs are usually quenched, destabilized or show limited sensitivity to environmental changes.[23,30,31] As a consequence, there is a strong demand for new fluorescent nucleoside analogues with improved spectroscopic properties.[32,33]

Previously we utilized benzotriazole activated coumarin fluorophores to label amino acids, peptides[34,35] and sugars.[35–37] More recently we synthesized a novel fluorophore 2-(6-chloro-1,3-dioxo-1*H*-benzo[*f*]isoindol-2(3*H*)-yl)acetic acid **1**, studied its photophysical properties and utilized it for labeling of free and protected amino acids.[38] We now report a convenient and efficient preparation of fluorescent labeled nucleosides utilizing a 2,3-naphthalimide-based fluorophore.

2-(6-Chloro-1,3-dioxo-1*H*-benzo[*f*]isoindol-2(3*H*)-yl)acetic acid[38] **1** fulfills the requirements of a fluorophore to be used for

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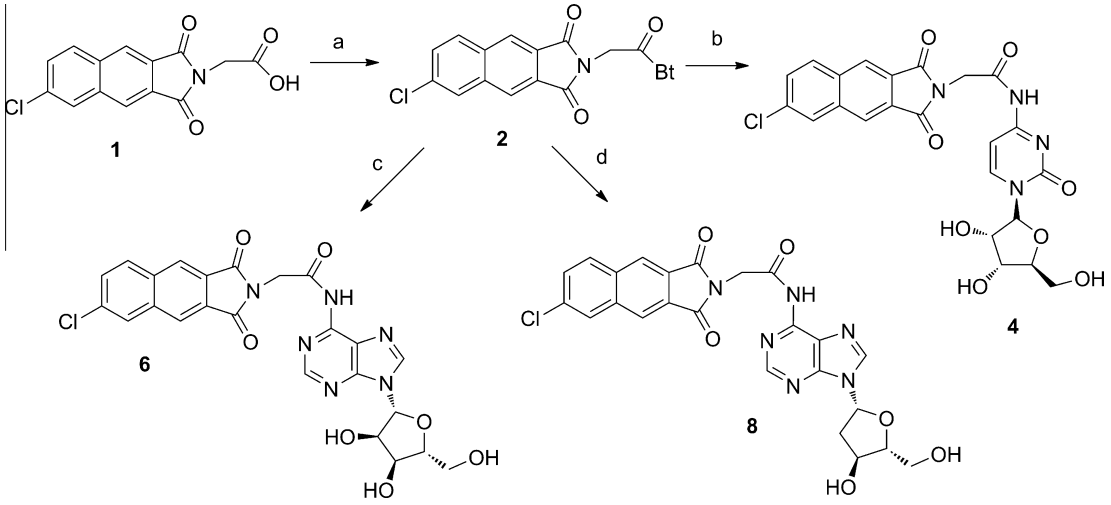
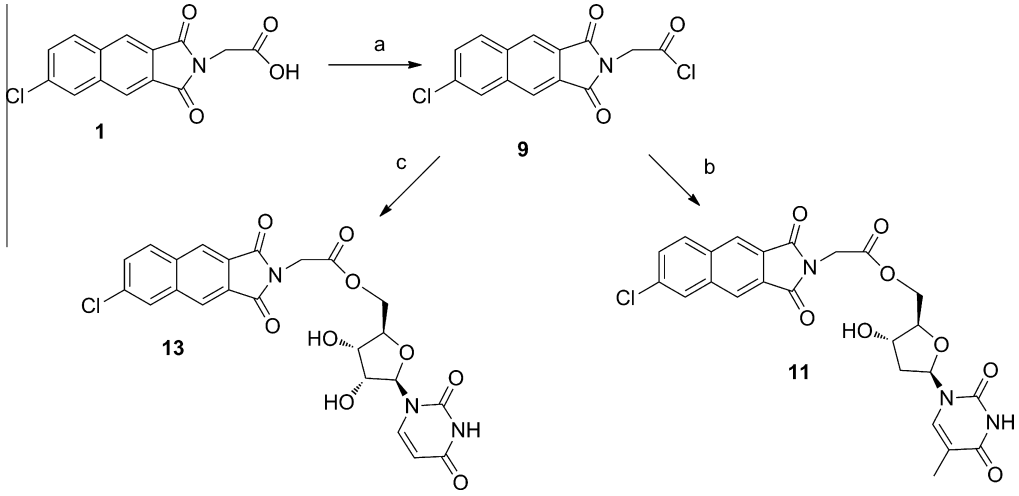


Scheme 1. Reagents and conditions: (a) BtH, $SOCl_2$, THF, rt, 2 h (85%); (b) cytidine **3**, Et_3N, rt, 24 h (82%); (c) adenosine **5**, Et_3N, rt, 24 h (76%); (d) deoxyadenosine **7**, Et_3N, rt, 24 h (73%).



Scheme 2. Reagents and conditions: (a) $SOCl_2$, CH_2Cl_2, reflux, 10 h (95%); (b) thymidine **10**, 65 °C, 3 h (75%); (c) uridine **12**, 65 °C, 3 h (71%).

labeling of nucleosides by possessing a good quantum yield (0.11–0.65) in solvents of different polarity, showing a large Stokes shift 120–130 nm and a low excitation wavelength (260 nm).

For labeling of nucleosides 2-(6-chloro-1,3-dioxo-1*H*-benzo[*f*]isoindol-2(3*H*)-yl)acetic acid[38] **1** was converted into the corresponding benzotriazole derivative[38] **2** and then coupled with cytidine **3**, adenosine **5** and deoxyadenosine **7** at rt for 24 h to form **4**, **6**, and **8**[39] as single products in 82%, 76%, and 73% yields, respectively (Scheme 1).

Our attempts to couple **2** with thymidine **10** or uridine **12** under various conditions failed. For this reason, fluorophore **1** was chlorinated with thionyl chloride to give intermediate **9** which was then reacted immediately with thymidine **10** and uridine **12** under reflux for 3 h to form 5*O*′-acylated products **11** and **13**[40] in 75% and 71% yield, respectively (Scheme 2).

The fluorescence properties of **4**, **6**, **8**, **11**, and **13** were investigated in water and methanol. 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 **4, 6, 8, 11**, and **13** are summarized in Table 1. The quantum yield of the chlorine-substituted 2,3-napthalimide-based fluorophore **1** of 0.11 increases from 0.41 (for **11**) to 0.93 (for **4**) in methanol. The quantum yields in water of 0.27 decreases slightly for the labeled deoxyadenosine **8** to 0.10, remains almost unchanged for the labeled adenosine **6** and increases for the labeled cytidine **4**, thymidine **11** and uridine **13**.

Table 1
Photophysical properties of **1**, **4**, **6**, **8**, **11**, and **13**

	λ_{ab} (nm)	λ_{ex} (nm)	λ_{em} (nm)	ε (mol/cm^{-1} × 10^4)	Φ	Solvent
1	267	260	407	5.69	0.27	Water
	263	260	384	7.16	0.11	Methanol
4	265	260	412	4.86	0.64	Water
	263	260	384	8.07	0.93	Methanol
6	267	260	409	4.21	0.21	Water
	263	260	384	8.30	0.69	Methanol
8	263	260	408	3.62	0.10	Water
	261	260	384	8.22	0.59	Methanol
11	268	260	411	5.84	0.46	Water
	263	260	382	6.93	0.41	Methanol
13	268	260	409	2.80	0.43	Water
	265	260	382	5.06	0.66	Methanol

In summary, the chlorine-substituted 2,3-naphthalimide-based fluorophore **1** possesses a high quantum yield in solvents of different polarities (0.11–0.65), shows large Stokes shifts (120–130 nm), and low excitation wavelength (260 nm). When this group is

incorporated into nucleosides the quantum yield either remains unchanged for labeled nucleosides or increases from 0.27 to 0.64 in water on from 0.11 to 0.93 in methanol for compound **4**. However with other fluorophores, for example, the most popular fluorescent base 2′-deoxyribosyl-2-aminopurine (2-AP)[27,28] a dramatic drop of quantum yield (from 0.68 to 0.017 in water) is reported when incorporated in oligonucleotides.[29–31]

Our mild labeling strategy with simple reaction and work-up conditions gives good yields (70–82%), whereas the only reported method[26] provides yields ranging from 10% to 64% and in some cases no product was isolated.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.06.100.

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39. *General preparation of* **4**, **6**, *and* **8**: Nucleoside (cytidine, adenosine or deoxyadenosine) (1.6 mmol) was added to 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 **2** (0.6 g, 1.6 mmol) in DMF (5 mL). Et_3N (0.16 g, 1.6 mmol) was added and the mixture stirred for 24 h at room temperature. The product was precipitated from water, filtered off and dried to give the pure product.
2-(6-Chloro-1,3-dioxo-1H-benzo[f]isoindol-2(3H)-yl)-N-(1-((2R,3S,4R,5S)-3,4-dihydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-2-oxo-1,2-dihydropyrimidin-4-yl)acetamide (**4**): 82%. Mp 224–225 °C. ^{1}H NMR (DMSO-d_6) δ 8.58 (s, 1H), 8.52 (s, 1H), 8.45 (d, J = 7.2 Hz, 1H), 8.34 (s, 1H), 8.28 (d, J = 8.7 Hz, 1H), 7.79 (s, 1H), 7.03 (s, 1H), 5.78 (s, 1H), 5.50 (s, 1H), 5.15 (s, 1H), 5.07 (s, 1H), 4.59 (s, 2H), 3.97–3.90 (m, 3H), 3.75–3.60 (m, 3H). ^{13}C NMR (DMSO-d_6) δ 167.6, 166.6, 161.9, 154.5, 145.8, 135.9, 134.1, 133.5, 132.3, 129.8, 128.9, 128.3, 127.6, 124.8, 124.0, 95.2, 90.2, 84.2, 74.5, 68.6, 59.9, 41.4. Anal. Calcd for $C_{23}H_{19}ClN_4O_8\ H_2O$: C, 51.84; H, 3.97; N, 10.51. Found: C, 51.80; H, 3.96; N, 10.25.
2-(6-Chloro-1,3-dioxo-1,3-dihydro-benzo[f]isoindol-2-yl)-N-[9-(3,4-dihydroxymethyl-tetrahydro-furan-2-yl)-9H-purin-6yl]-acetamide (**6**): Colorless microcrystals (76%); Mp 252–254 °C. ^{1}H NMR (DMSO-d_6) δ 8.66 (s, 1H), 8.60 (s, 1H), 8.43 (s, 1H), 8.37–8.35 (m, 2H), 7.86 (s, 1H), 7.84 (dd, J = 1.8 Hz, J = 8.7 Hz, 1H), 7.40 (s, 1H), 5.95–5.90 (m, 2H), 5.74–5.67 (m, 1H), 5.40–5.38 (m, 1H), 4.97–4.90 (m, 1H), 4.72 (s, 1H), 4.66–4.64 (d, J = 6.Hz, 2H), 4.22 (br s, 1H), 3.71–3.59 (m, 2H). ^{13}C NMR (DMSO-d_6) δ 166.9, 166.4, 156.2, 152.4, 149.1, 139.8, 135.9, 134.2, 133.5, 132.3, 129.9, 129.0, 128.1, 127.3, 125.0, 124.2, 119.3, 87.3, 83.3, 75.2, 71.7, 61.5. Anal. Calcd for $C_{24}H_{19}ClN_6O_7$: C, 53.49; H, 3.55. Found: C, 53.20; H, 3.47.
2-(6-Chloro-1,3-dioxo-1,3-dihydro-benzo [f]isoindol-2-yl)-N-[9-(4-hydroxy-5-hydroxymethyl-tetrahydro-furan-2-yl)-9H-purin-6yl]-acetamide (**8**): Colorless microcrystals (73%); Mp 246–247 °C. ^{1}H NMR (DMSO-d_6) δ 8.65–8.24 (m, 5H), 8.22 (d, J = 9 Hz, 1H), 7.37–7.32 (m, 2H), 6.40 (br s, 1H), 5.45 (br s, 2H), 4.62–4.32 (m, 4H), 4.51 (s, 2H), 4.15–4.11 (m, 1H), 3.97–3.94 (m, 1H), 3.72–3.70 (m, 2H). ^{13}C NMR (DMSO-d_6) δ 166.9, 166.5, 156.2, 152.5, 149.1, 139.8, 135.9, 134.2, 133.6, 132.3, 129.9, 129.0, 128.1, 127.4, 125.0, 124.2, 119.2, 87.3, 83.3, 75.1, 71.7, 61.5. Anal. Calcd for $C_{24}H_{19}ClN_6O_6$: C, 55.13; H, 3.66. Found: C, 55.25; H, 3.47.
40. *General preparation of* **11** *and* **13**: Thionyl chloride (1.02 g, 8.75 mmol) was added to a solution of 2-(6-chloro-1,3-dioxo-1*H*-benzo[*f*]isoindol-2(3*H*)-yl)acetic acid (0.25 g, 0.87 mmol) in dry dichloromethane and the mixture was refluxed till a clear solution was formed. After evaporation of solvent nucleoside (thymidine, uridine) (0.85 mmol) was added in dry THF and the mixture was refluxed for 3 h. The solvent was evaporated and the residue was washed with dichloromethane. The undissolved solid was purified with recrystallization technique using methanol/hexane (8:2).
((2R,3S,5R)-3-Hydroxy-5-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)tetrahydrofuran-2-yl)methyl 2-(6-chloro-1,3-dioxo-1H-benzo[f]isoindol-2(3H)-yl)acetate (**11**): Colorless microcrystals (75%), ^{1}H NMR (DMSO-d_6) δ 11.3 (s, 1H), 8.63 (s, 1H), 8.57 (s, 1H), 8.42 (s, 1H), 8.31 (d, J = 8.7 Hz, 1H), 7.83 (dd, J = 8.7 Hz, J = 1.5 Hz, 1H), 7.42 (s, 1H), 6.18 (m, 1H), 5.92–5.98 (m, 1H), 5.44 (br s, 1H), 4.54–4.58 (m, 2H), 4.25–4.57 (m, 2H), 3.93–3.94 (m, 1H), 2.16–2.08 (m, 2H), 1.79 (s, 3H). ^{13}C NMR (DMSO-d_6) δ 168.7, 167.4, 163.6, 150.3, 135.9, 135.7, 134.2, 133.5, 132.3, 129.9, 128.9, 128.0, 127.3, 125.0, 124.2, 109.8, 83.7, 83.3, 70.1, 65.3, 12.1. Anal. Calcd for $C_{24}H_{20}ClN_3O_8$: C, 56.09; H, 3.92. Found: C, 56.25; H, 3.62.
((2R,3S,4R,5R)-5-(2,4-Dioxo-3,4-dihydropyrimidin-1(2H)-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl 2-(6-chloro-1,3-dioxo-1H-benzo[f]isoindol-2(3H)-yl)acetate (**13**): Colorless microcrystals (71%). Mp 169–170 °C. ^{1}H NMR (DMSO-d_6) 11.4 (s, 1H), 8.65 (s, 1H), 8.59 (s, 1H), 8.44 (s, 1H), 8.33 (d, J = 8.7 Hz, 1H), 7.85 (dd, J = 1.8 Hz, J = 8.7 Hz, 1H), 7.62 (d, J = 7.9 Hz, 1H), 5.71(d, J = 4.8 Hz, 1H), 5.69 (dd, J = 2.1 Hz, J = 8.1 Hz, 1H), 4.58 (d, J = 2.7 Hz, 2H), 4.45 (dd, J = 11.9 Hz, J = 2.8 Hz, 1H), 4.32 (dd, J = 12.2 Hz, J = 5.4 Hz, 1H), 4.10–3.96 (m, 3H), 3.60–3.56 (m, 2H). ^{13}C NMR (DMSO-d_6) 166.9, 166.5, 156.2, 152.5, 139.8, 135.9, 134.2, 133.6, 132.3, 129.9, 129.0, 128.1, 127.4, 125.0, 124.2,, 119.2, 87.3, 83.3, 75.1, 71.7, 61.5. Anal. Calcd for $C_{23}H_{18}ClN_3O_9{\cdot}H_2O$: C, 51.74; H, 3.40; N, 7.87. Found: C, 51.59; H, 3.36; N, 7.47.