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Synthesis of Nucleoside Derivatives Containing Benzophenoxazinone Moiety

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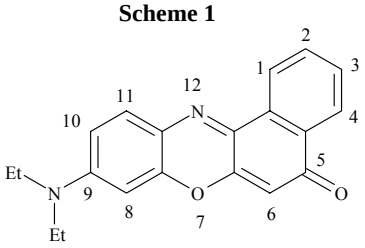
Abstract: Two new nucleoside derivatives containing benzophenoxazinone moiety were synthesized. Their luminescence spectra show that they have strong near infrared fluorescence. Our study provides a new method for direct introduction of near infrared fluorescent probe to bioactive molecules.

Keywords: Synthesis, nucleoside, near-infrared fluorescence, benzophenoxazinone.

In recent years, fluorescence spectroscopy is becoming an extremely sensitive analytical technique due to the advances in instrumentation. As a result, the use of fluorescent probes for bioactive molecules such as DNA, proteins and drugs has grown remarkably[1,2]. A successful fluorescent label must operate in or near the far visible-near infrared region (600-1000 nm), in this area, interference from absorption scattering and from fluorescence of biological molecules is minimum[3,4].

Scheme 1



Nile Red

Benzophenoxazinone, such as Nile Red (**Scheme 1**), has strong long-wavelength fluorescence maxima with high quantum yield and large molar absorptivity and is suitable for biolabeling[5,6]. In order to allow coupling of the fluorescent probe to biomolecules, a reactive carboxylic acid group was introduced to the benzophenoxazinone moiety to obtain compound **3**. **6a**[7] and **6b**[8] were prepared by condensation of **3** with protected uridine and adenosine nucleosides in the presence of DCC (N, N′-dicyclohexylcarbodiimide) (**Scheme 2**).

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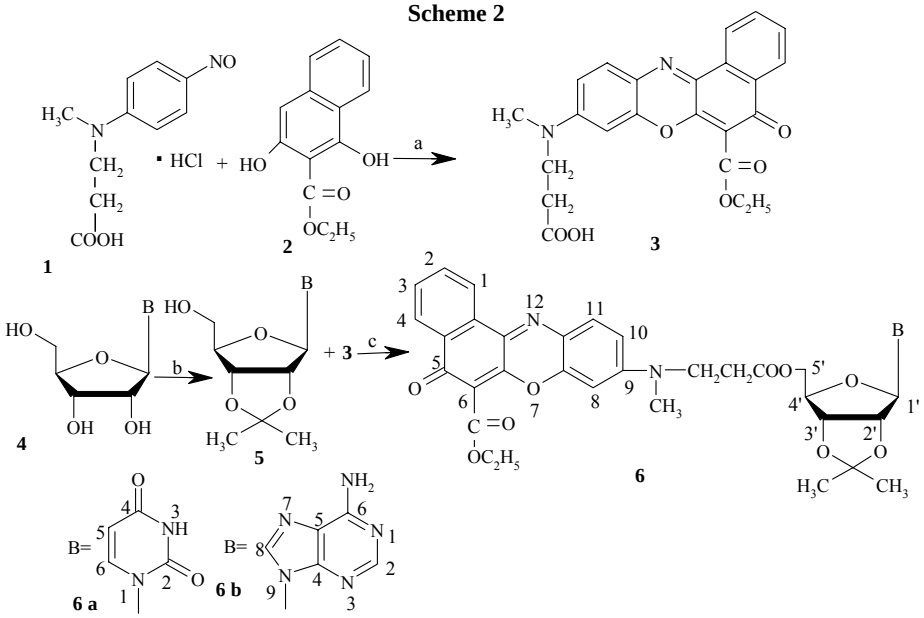
Scheme 2



a) acetonitrile, reflux, 45% yield; b) acetone, *p*-toluenesulfonic acid, RT, 82% yield;
c) N,N′- dicyclohexylcarbodiimide, ethylene glycol dimethylether, RT, 73% yield.

Results and Discussion

Nile Red analogues can be prepared by oxidation of phenoxazinone derivatives[9], as well as by the addition of N, N′-disubstituted amino-4-nitrosoaniline hydrochlorides to a solution of ethyl 1, 3-dihydroxy naphthoate in boiling ethanol[10]. In order to introduce a carboxyl group to the fluorophore, compound **3** was prepared from N-methyl-N′-carboxyethyl amino-4-nitrosoanline[11]. The -COOH group of **3** can couple readily with hydroxyl groups of nucleoside ribose to form ester bond. However, as expected, coupling may occur on any of the three hydroxyl groups at 2′, 3′ and 5′ positions of the ribose. To avoid the difficulty in separation and purification, hydroxyl groups at 2′ and 3′ positions were protected by conversion of ribonucleosides to 2′, 3′-*O*-isopropylidene derivatives[12]. The subsequent coupling reaction with DCC proceeded mildly to afford the desired product **6a** and **6b**.

The maxima of the absorption, excitation and fluorescence emission spectra of compounds **3**, **6a** and **6b** in chloroform and ethanol were summarized in **Table 1**. Under given circumstance, the data of **6a** and **6b** are virtually the same, indicating the structure of the heterocyclic bases of the nucleoside affect the photophysical properties slightly. The spectra of **3**, however, differ from that of **6a** and **6b**. All of the absorption, excitation and emission maxima of **6a** and **6b** appear at shorter wavelength than that of **3**. The maxima of the fluorescence emissions of **6a** and **6b** in ethanol solution appear at 618 and 619 nm in the near infrared region, where there is inherently low in biological interferences. Solvent effect is observed, comparing the emission spectra in chloroform and ethanol solution. Red shifts of the emission maxima are recorded as the polarity of solvent increases, because more polar surrounding

environment stabilizes the excited state more effectively[13,14].

In summary, nucleoside derivatives **6a** and **6b** were synthesized and found to have strong near infrared fluorescence. Our work may serve as a preliminary study of the introduction of fluorescent probe to bioactive molecules. Further investigation is in progress.

Table 1 Photophysical data of compounds **3**, **6a** and **6b**

Entry	λ_{max} (Abs) /nm in ethanol	λ_{max} (Ex) /nm in chloroform	λ_{max} (Ex) /nm in ethanol	λ_{max} (Em) /nm in chloroform	λ_{max} (Em) /nm in ethanol
3	550	573	581	599	627
6a	544	562	574	589	618
6b	543	563	575	589	619

Abs: UV-Vis absorption; Ex: fluorescence excitation; Em: fluorescence emission

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7. ^{1}H-NMR (200 MHz) data for **6a** (DMSO-d_6, δ ppm): 1.30-1.52 (m, 9 H, -CH_2CH_3, -$C(CH_3)_2$), 2.61-2.73 (m, 2 H, -NCH_2CH_2-), 3.05 (s, 3 H, -NCH_3), 3.83-3.95 (m, 2 H, -NCH_2CH_2-), 4.15-4.42 (m, 5 H, -OCH_2CH_3, 2×H-5′, H-4′), 4.73-4.82 (m, 1 H, H-3′), 5.04-5.14 (m, 1 H, H-2′), 5.62 (d, 1 H, *J* =7.6 Hz, base H-5), 5.70 (s, 1 H, base H-3), 5.75 (d, 1 H, *J* =2.3 Hz, H-1′), 6.68 (d, 1 H, *J* =2.0 Hz, H-8), 6.98 (dd, 1 H, *J* =9.7, 2.2 Hz, H-10), 7.66-7.82 (m, 4 H, H-2, H-3, H-11,base H-6), 8.14 (d, 1 H, *J* =7.5 Hz, H-4), 8.60 (d, 1 H, *J* =7.3 Hz, H-1);
   MS (FAB) data for **6a** (*m/z*): 687.3 (M+1).
8. ^{1}H-NMR (200 MHz) data for **6b** (DMSO-d_6, δ ppm): 1.33-1.51 (m, 9 H, -CH_2CH_3, -$C(CH_3)_2$), 2.58-2.72 (m, 2 H, -NCH_2CH_2-), 3.02 (s, 3 H, -NCH_3), 3.78-3.86 (m, 2 H, -NCH_2CH_2-), 4.06-4.38 (m, 5 H, -OCH_2CH_3, 2×H-5′, H-4′), 4.98-5.04 (m, 1 H, H-3′), 5.40-5.47 (m, 1 H, H-2′), 6.17 (d, 1 H, *J* =1.9 Hz, H-1′), 6.62 (d, 1 H, *J* =2.2 Hz, H-8), 6.94 (dd, 1 H, *J* =9.5, 2.1 Hz, H-10), 7.29 (s, 2 H, base -NH_2), 7.64-7.83 (m, 3 H, H-2, H-3, H-11), 8.11-8.14 (m, 2 H, H-4, base H-2), 8.28 (s, 1 H, base H-8), 8.57 (d, 1 H, *J* =7.3 Hz, H-1);
   MS (FAB) data for **6b** (*m/z*): 710.3 (M+1).
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