Dyes and Pigments 142 (2017) 161–166

Contents lists available at ScienceDirect

Dyes and Pigments

journal homepage: www.elsevier.com/locate/dyepig

Lavender violet, blue and pink: A new type of benzo[*a*]phenoxazine-based dipolar, red-emitting dyes

Mithun Santra, Yong Woong Jun, Ye Jin Reo, Sourav Sarkar, Kyo Han Ahn*

Department of Chemistry, Pohang University of Science and Technology (POSTECH), 77 Cheongam-Ro, Nam-Gu, Pohang, Gyungbuk 37673, Republic of Korea

ARTICLE INFO

Article history:
Received 2 March 2017
Received in revised form
13 March 2017
Accepted 13 March 2017
Available online 16 March 2017

Keywords:
Benzo[*a*]phenoxazine derivatives
Dipolar dyes
Fluorescence
Cellular imaging

ABSTRACT

A new type of the red-emitting benzo[*a*]phenoxazine-based dyes, dipolar analogues of Meldola's Blue, are synthesized by condensation of *p*-nitrosoanilines or *p*-nitrosophenol with 7-(alkylamino)naphthalen-2-ol in good yields. Their photophysical properties are characterized by the donor substituents introduced, offering dyes of lavender violet, blue, and pink colors. These dyes exhibit high absorptivity, marginal-to-good optical brightness, good photostability, and show large Stokes shifts in aqueous solution. One of the dyes emits bright red fluorescence inside cells, as observed by confocal laser scanning microscopy (CLSM). The new dyes thus have potential to be used for staining purposes including cellular imaging.

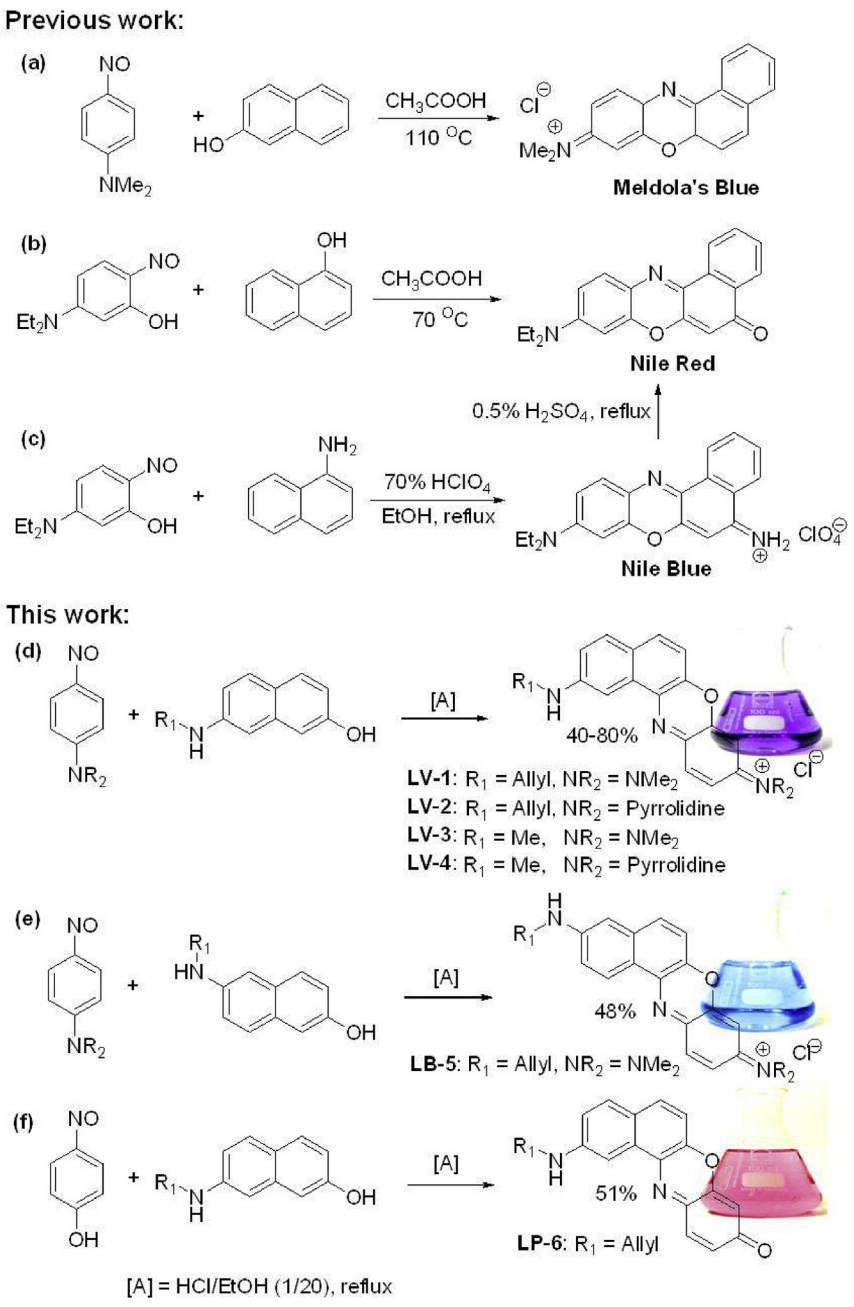
1. Introduction

Optical imaging with the aid of fluorescent dyes is essential in studying various biological processes in living systems [1–5]. Accordingly, the development of new dyes with desirable optical properties has attracted continuous research interest [6,7]. In particular, fluorophores that emit in the red or near-infrared wavelength region (620–1000 nm) have great potential for bioimaging applications [8] since they offer deeper tissue penetration, suppressed photodamage and photobleaching, and alleviate autofluorescence interference from intrinsic biomolecules such as NADH and riboflavins [9,10]. In general, expanding the π-conjugation to reduce HOMO–LUMO gap is the most straightforward strategy to obtain red/NIR dyes. Such π-extension, on the other hand, enhances the dyes' susceptibility to light and chemicals, and also lowers their water-solubility [11]. Therefore, compact molecules based on minimal π-conjugation systems that emit in the red/NIR region are in great demand. Benzophenoxazine dyes are often recognized as a class of red/NIR emitting dyes having intense colors. Meldola's Blue, the first benzophenoxazine-based dye synthesized in 1879 by condensation of (*p*-*N*,*N*-dimethylamino)nitrosobenzene with 2-naphthol (Scheme 1a) [12], is weakly fluorescent and hence has not been used as a fluorescent dye. Later, Nile Red, a benzo[*a*]phenoxazinone dye which is structurally related to Meldola's Blue except the added keto group (Scheme 1b), was developed through a different synthetic route that involved an (*o*-hydroxy)nitrosobenzene. Owing to the donor-acceptor feature (dipolar nature) of Nile Red, it is highly fluorescent in less polar organic media. A drawback of Nile Red is its poor solubility in aqueous media (<1 μg/mL water) [13]. On the other hand, Nile Blue, a benzophenoxazin-imium dye synthesized starting from 1-aminonaphthalene, instead of 1-naphthol, by following the synthetic route to Nile Red, has a positively charged iminium group instead of the keto group in Nile Red (Scheme 1c) and thus is more water-soluble. Nile Blue is weakly emissive in aqueous media, although its fluorescence is highly dependent on the medium pH. Accordingly, various strategies have been investigated to synthesize modified derivatives of Nile Red and Nile Blue with improved water solubility as well as photophysical properties [14–16]. Overall, such benzophenoxazine-based fluorophores represent an interesting expansion of the fluorescent dye toolbox. We have realized that another type of dipolar analogues of Meldola's Blue, which are structurally distinguished from Nile Blue and Nile Red, are missing. Herein, we report novel synthetic routes to the missing dipolar benzophenoxazine-based dyes that include the Lavender Violet, Blue, and Pink, and characterization of their photophysical properties.

The new benzophenoxazin-iminium or benzophenoxazinone dyes shown in Scheme 1(d–f) can be readily synthesized by condensation of 4-(dialkylamino)nitrosobenzene with

* Corresponding author.
E-mail address: ahn@postech.ac.kr (K.H. Ahn).

http://dx.doi.org/10.1016/j.dyepig.2017.03.031
0143-7208/



Scheme 1. Structurally related benzophenoxazine dyes.

7-(alkylamino)naphthalen-2-ol in refluxing ethanol using 5% (*v*/*v*) HCl as a catalyst, which afforded intense violet-colored products in 40–80% yields (Scheme 1d). These fluorophores were termed Lavender Violets (**LV-1**, **LV-2**, **LV-3**, and **LV-4**) to reflect their absorption color. Similarly, condensation of 4-(*N*,*N*-dimethylamino) nitrosobenzene with 6-(allylamino)naphthalen-2-ol produced a faint blue dye in moderate yield (48%) (Scheme 1e), which was named Lavender Blue (**LB-5**). On the other hand, condensation of 7-(allylamino)naphthalen-2-ol with 4-nitrosophenol as a nitroso component formed a benzophenoxazinone with pink color (Scheme 1f), which was named Lavender Pink (**LP-6**). The **LV** series and **LB-5** having iminum acceptors can be structurally compared with Nile Blue, similarly **LP-6** which has the keto acceptor can be compared with Nile Red. It should be noted that almost all the known synthetic procedures for benzophenoxazine-based dyes are based on the condensation methods at high temperature with very low yields. The synthetic procedures disclosed here provide the novel benzophenoxazine-based dyes in moderate to good yields, which manifests its potential for the synthesis of other structural derivatives.

2. Experimental section

2.1. Materials and methods

All experiments that were sensitive to moisture or air were performed under Ar atmosphere in flame-dried glassware equipped with a rubber septum. Solvent and liquid reagents were transferred using syringes or cannulaes after flushing with argon. Unless otherwise noted, commercial reagents were purchased from