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Functionalized bispyridoneannelated BODIPY – Bright long-wavelength fluorophores

Yuriy V. Zatsikha, Viktor P. Yakubovskyi, Mykola P. Shandura, Yuriy P. Kovtun*

Institute of Organic Chemistry, National Academy of Sciences of Ukraine, 5 Murmanska Str., 02094 Kyiv, Ukraine

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ABSTRACT

A simple approach to the modification of BODIPY nucleus has been developed. The method is based on the reaction of monofunctionalized acetaldehyde-substituted dye with primary amines or anilines followed by cyclization of enamine intermediates. This procedure allowed preparing a series of new stable intensive long-wavelength BODIPY derivatives (λ_{em} = 680–720 nm) with two functional substituents useful for various practical purposes, including bioconjugation and other biomedical applications.

1. Introduction

Dyes derived from boron dipyrromethene (4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene, BODIPY, BDP) have been attracting considerable attention over the past two decades. The ever-increasing interest in this type of very important compounds stems from their excellent thermal, chemical, and photochemical stability, high molar absorption coefficients, high fluorescence quantum yields, general insensitivity to both solvent polarity and pH, large two-photon cross-section for multiphoton excitation, the lack of ionic charge, a good solubility, etc [1]. Often the dyes which will be used need to be appropriately functionalized (with the carboxyl, amino, azido groups, etc.). We have recently found a new approach to this problem *via* monopyridoneannelated compounds of type **1** [2]. In this case, the series of luminophores (λ_{em} = 615 nm) with functional substituents useful for various practical purposes, including bioconjugation and other biomedical applications, can be readily obtained. Moreover, symmetrical bispyridoneannelated dye of type **7** emitting at the region of so-called "phototherapeutic window" (R = *i*-Am, λ_{em} = 683 nm) was synthesized too [3]. Compounds of this family are intensive long-wavelength dyes and from this point of view are of interest for various applications ranging from materials science to biology and medicine [1h,1i,4]. Thus, the goal of current research was to develop the convenient method of preparation of unsymmetrical dyes **7** with two different practically important substituents. This approach could lay the foundation for a new combinatorial strategy in the family of long-wavelength BODIPY dyes, which is now considered to be of great importance in fluorescent probe development [5].

2. Results and discussion'

In order to achieve the goal the recently described BODIPY **2** [6] was used as a starting material (Scheme 1). The N-butyl-substituted compound was chosen due to its optimal solubility. Some characteristics of this aldehyde are similar to observed ones in the case of simpler analogs [7]. For example, formation of enamine **4** occurs by the reaction with tertiary amines.

This time we have managed to isolate intermediate compound **3** which is enough stable to be characterized. While heating, especially with excess of amine, the Hofmann-type decomposition with ethylene evolution is easily observed. Quite expectedly, *peri*-condensed heterocyclic system **5** was obtained after the treatment of compound **2** with boron trifluoride etherate solution.

On the other hand, high reactivity of aldehyde **2** also leads to undesirable side processes, such as self-condensation or deformylation. In order to minimize these processes during the synthesis of enamines **6** the acetic acid should be added and the excess of amine

* Corresponding author.
E-mail address: kovtun@ioch.kiev.ua (Y.P. Kovtun).

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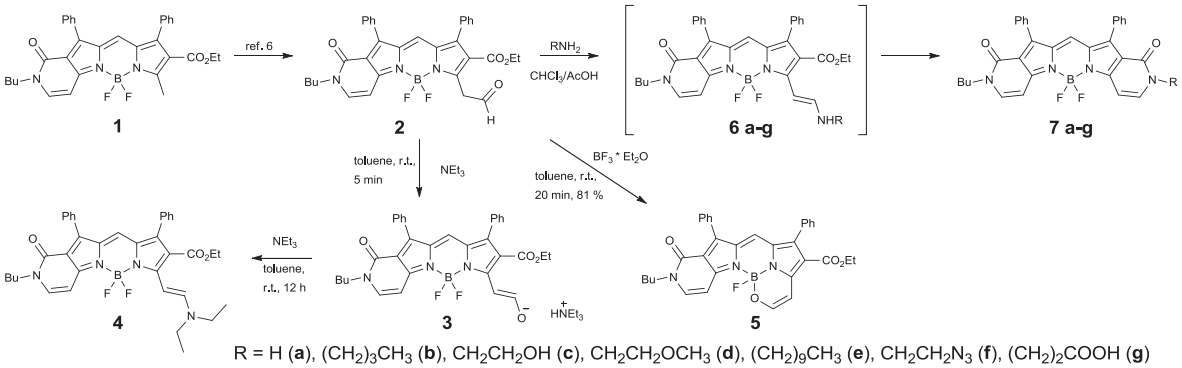


Scheme 1. Chemical properties of aldehyde **2**.

should be avoided. However, this is not enough to stop the reaction at enamine stage in all cases, as a further cyclization into pyridone derivatives **7** occurs, especially in the case of electron donating substituents. Since the non-fluorescent intermediates **6** were not of our interest, the reaction mixture was left for a few hours at room temperature to finish the reaction. In order to synthesize compound **7a** (R = H) ammonium acetate in ethanol was used.

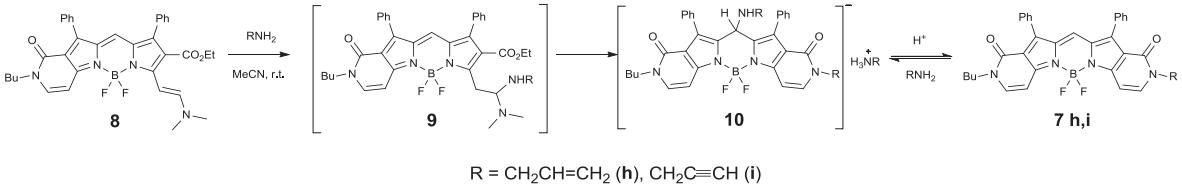
At the same time, the reaction with amines, which contain electron-withdrawing substituents (for example, allylamine or propargylamine) did not proceed so readily. That is why, the modification of the basic method which will include the use of enamine **8** [6] as a staring compound was developed (Scheme 2).

This compound is essentially less reactive than appropriate aldehyde, therefore the time of reaction was longer – about 24 h in acetonitrile with significant excess of amine (4 equivalents). The reaction was carried out at room temperature, because the heating, especially higher than 60 °C, facilitates the side processes more substantially. Thus, dyes **7h** and **7i** were synthesized in about 80% yield. The advantage of this method is that enamine **8** is a precursor of aldehyde **2**, i.e. more available compound. Moreover, in this case it is very convenient to monitor visually the end of reaction as the reaction mass becomes colorless due to adduct **10** formation. Dipyrromethane **10** is a stable compound and quantitatively returns into dipyrromethene form after treatment with acid. The dipyrromethanes' salt-like forms were firstly described by the founder of BODIPY chemistry Alfred Treibs [8]. We have also observed the formation of such compounds earlier [2]. However, this process was possible only in the presence of alkali. Due to the electron-withdrawing influence of two annelated pyridone rings in the compounds of type **7**, the nucleophilic attack at the *meso*-position of dye is considerably facilitated, and can occur even after the treatment with amines.

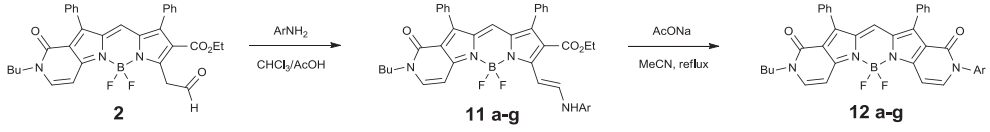
Anilides **11** are formed by the reaction of aldehyde **2** with anilines or aminoheterocycles. In contrast to enamines **6**, they are stable compounds. Their cyclization into the compounds **12** require more severe conditions, namely continued refluxing in acetonitrile in the presence of sodium acetate (Scheme 3). It should be noted, that attempts to cyclize monoanilide derivatives of BODIPY of this range were unsuccessful [2].

Obtained bispyridoneannelated dyes **7a–i** and **12a–g** have intensive absorption at 650–660 nm and fluorescence at 670–680 nm with high quantum yields (~70%, Table 1). Obviously, in terms of the brightness it is one of the highest result for this spectral region [4]. The dyes can be used in diverse applications, depending on the introduced substituent. For example, BODIPY dye **7e** containing a hydrophobic decyl group is well soluble in hexane, so presumably it should be well soluble in lipid systems and thus useful for cell membrane studies. Compound **7g** is functionalized with carboxyalkyl group suitable for bioconjugation reactions, e.g. for the fluorescent labeling of peptides, proteins and other amine-containing biomolecules *via* the formation of amide bond. Dyes **7f,i** are modified with azide and acetylene function, respectively, to be used as reagents in "click chemistry". Alkene fragment was introduced into compound **7h** – either for polymerization or further modification of the dye *via* the oxidation of ethylene bond.

Arylsubstituted derivatives **12** possess similar spectroscopic properties to alkylsubstituted ones. However, in the case of anilides **11**, some unexpected effects are observed. Due to PET process, free conjugated terminal amino groups in BODIPY most often lead to fluorescence quenching [9]. This takes place with enamines **6**, but



Scheme 2. Synthesis of compounds **7h,i** *via* enamine **8**.



Scheme 3. Synthesis of anilinosubstituted dyes **11–12a–g**.