SYNTHESIS OF RED-EMITTING FLUOROPHORES

Tamás Hlogyik[1], Erzsébet Mernyák[1]

[1]Department of Organic Chemistry, University of Szeged, H-6720 Szeged, Dóm tér 8, Hungary
e-mail: hlogyik@chem.u-szeged.hu

Abstract
Understanding the mechanism of action of biologically active compounds is of great interest in medicine, including cancer research. Chemical labeling of molecules, i.e. making them "visible" under the right conditions, is a widespread and intensively researched field of science. The fluorescent labeling of compounds offers several advantages over other methods. It might be considered as an environmentally friendly alternative to radioisotope labeling. It is highly important that fluorophores used in biomedical applications meet certain criteria, including high photostability, good cell permeability and stability under physiological conditions. However, the accessibility of the fluorophores is often limited owing to their high price and complex synthetic strategies. Here we synthesized *aza*-BODIPY fluorophores, which are red-emitting altervatives to BODIPY derivatives. The syntheses were carried out considering the principles of green chemistry. One-pot and/or microwave-assisted syntheses were performed in order to minimize the number of reaction steps and time and use of solvents.

Introduction
The labeling of biologically active compounds is necessary in biomedical applications, however it proposes several problems [1]. Radioisotope labeling in radiotherapy requires extreme caution because of its potential risks to the living organism and the environment from inappropriate dosing, and the potential for radioisotopes to be released into the atmosphere through improper handling. Activities involving radiation exposure are subject to strict safety regulations [2].
Today, intensive research is being carried out to develop techniques that could replace radioisotope tracer-based methods. One such promising technique is the use of fluorescent dyes. This imaging technique makes visible the biological processes involved in early carcinogenesis and may therefore allow the detection of small tumours at an early stage. Fluorescence imaging is based on the visualisation of fluorophores excited by light of a specific wavelength depending on the excitation spectra. The light is then re-emitted at lower energy but at longer wavelengths. The depth of penetration depends on the wavelengths tested, ranging from a few hundred micrometres to a few millimetres [3].
The extremely high cost of fluorescent dyes is encouraging researchers to develop more efficient processes for synthesizing these materials. BODIPY-based (4,4-difluoro-4-boro-3a,4a-diaza-s-indacene) compounds display a very favourable compound group for this application (Fig. 1.). In addition to their excellent suitability for fluorescent labeling of biologically active compounds due to their strong absorption in the UV range, they are characterized by narrow absorption and emission bands and high molar coefficient values. They also have the advantage of being highly photostable and insensitive to the pH of their environment, making them suitable for use under physiological conditions. As their framework can easily be modified chemically (Fig. 2.), thus their optical properties can be manipulated, and their emission can be extended to higher wavelengths [4].

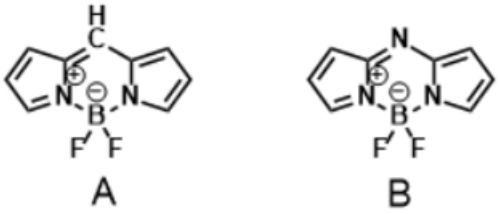


Figure 1. BODIPY (**A**) and *aza*-BODIPY (**B**) framework

The *aza*-BODIPY core (**B**), in which the carbon atom at *meso*-position is replaced by a nitrogen atom, has even more advantageous properties to those of compound **A** (Fig. 1.). A*za*-derivatives (**B**) show significant batokromic shifts. Accordingly, their emission spectra are shifted towards the red region [5]. These favourable optical properties allow their widespread biological application, especially because the radiation emitted is non-invasive, i.e. the lower energy emission does not damage cells. In addition, the light emission is also better separated from the autofluorescence of the cells (which is typically below 600 nm), which further improves the usability of the fluorophore [6].
Encouraged by the promising optical, chemical and biological properties of the *aza*-BODIPY derivatives, here we aimed to synthesize novel fluorophores based on framework **B**. Functional groups capable of later conjugation to biomolecules were introduced *via* postfunctionalization. The enhancement of water solubility was also an important aspect of our strategy. In order to consider the principles of green chemistry, one-pot and/or microwave-assisted syntheses were planned in order to minimize the number of reaction steps and time, and use of solvents.

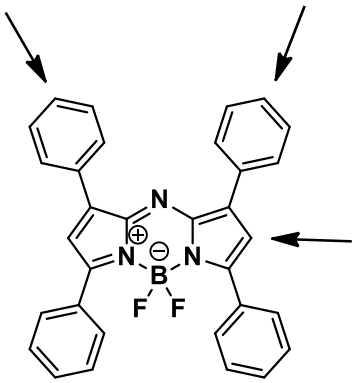
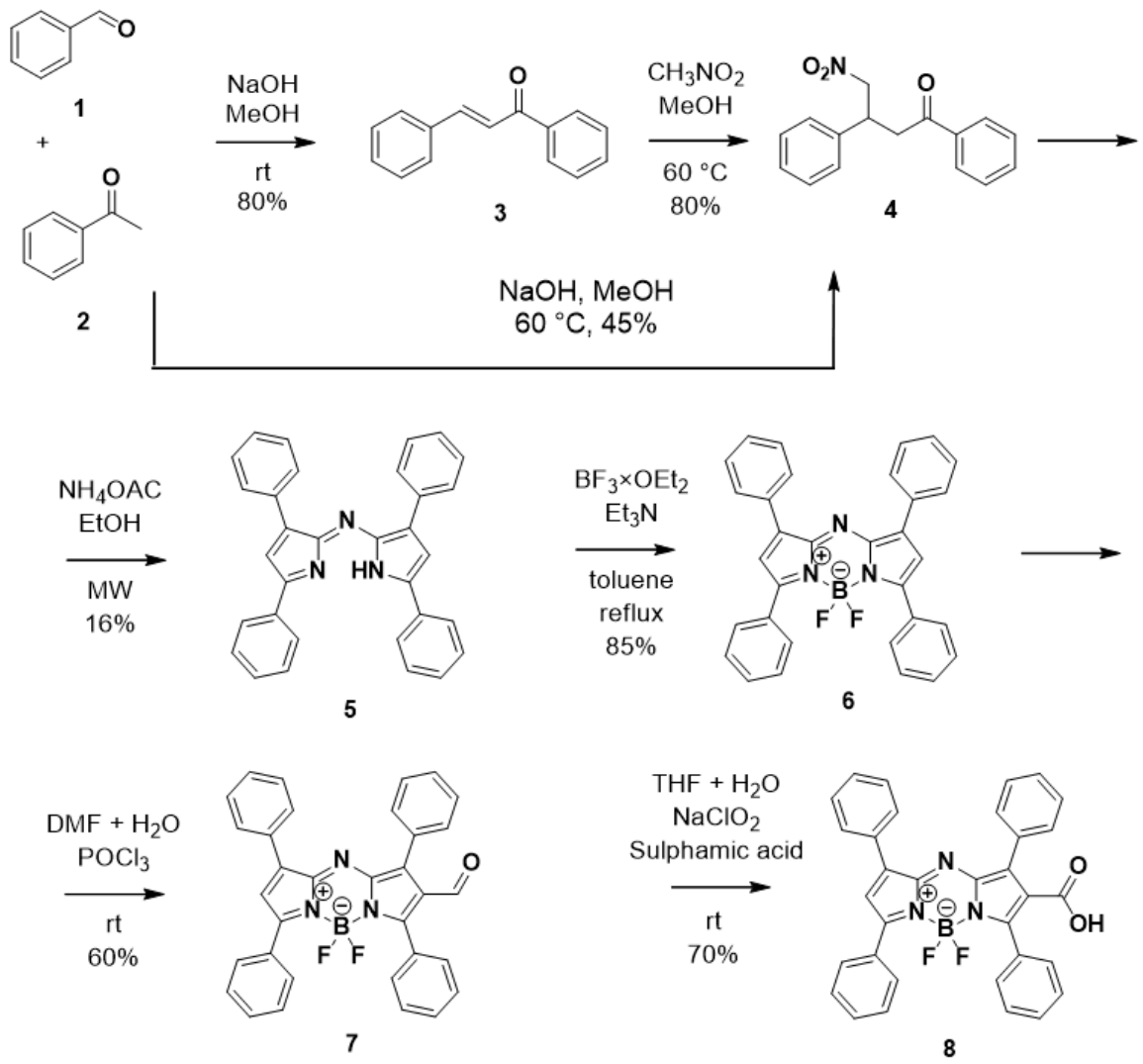


Figure 2. 1,3,5,7-tetraphenyl-*aza*-BODIPY and the possible positions for postfunctionalization

Results and discussion

The reaction sequence is depicted on Scheme 1. The base-catalyzed condensation of the benzaldehyde (**1**) and acetophenone (**2**) resulted in the corresponding chalcone (**3**). Michael addition using nitromethane as reagent was carried out on the α,β-unsaturated ketone (**3**), leading to the nitromethyl derivative (**4**) in high yield. It should be noted that the first two reaction steps were achievable in one-pot also, but the yield was slightly worse. The dipirromethene (**5**) was synthesized using NH_4OAc in EtOH solvent, under microwave irradiation. This step is the critical point of the whole reaction sequence, according to its extremely poor yield. After complexation with $BF_3.OEt_2$, the pyrrole rings were selectively formylated in a Vilsmeier-Haack reaction, leading to the monoformyl compound (**7**). The last step was the oxidation of the aldehyde, which resulted in the formation of a carboxylic acid

derivative **8**. The reaction conditions applied for the oxidation did not affect other moieties of the dye.



Scheme 1. Synthesis of the carboxylic acid derivative (**8**)

Conclusion

An efficient strategy was elaborated for the preparation of *aza*-BODIPY framework and the carboxylic acid derivative (**8**). The tetraphenyl derivative provides further opportunities for postfunctionalization, including enhancement of its water solubility. Our future plans involve determination of the optical properties of the newly synthesized dyes and their conjugation to certain biomolecules. The biological investigations will be carried out in cooperation.

Acknowledgements

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References

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