NEW WAY TO GREEN BIOLOGICAL ASSAYS FLUORESCENT STEROIDS REPLACE RADIOISOTOPES

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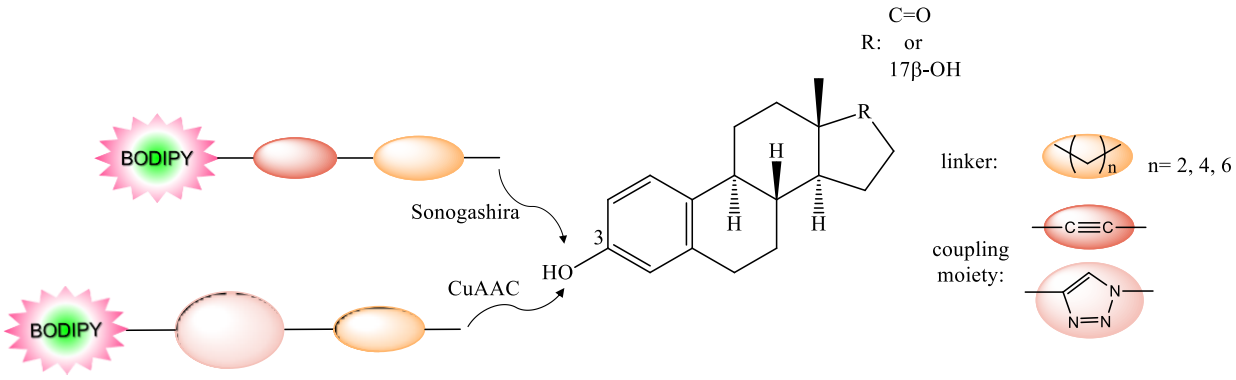
Abstract
Novel BODIPY–estrone and BODIPY–estradiol conjugates have been synthesized via Cu(I)-catalyzed azide-alkyne click (CuAAC) and/or Sonogashira reactions by selecting position C-3-*O* for labeling. The steroidal azides and/or bromides were reacted with BODIPY-based fluorescent dye bearing alkyne function. The new fluorescent estrone conjugates might replace radiolabeled compounds in certain biochemical assays.

Introduction
One of the primary goals of modern drug research is the use of environmentally friendly biochemical techniques that are based on the principles of green chemistry. Nowadays, methods based on the use of fluorescently labeled compounds are gaining prominence. There are only a few literature reports about the pharmacological effects of fluorescently labeled estrone derivatives.

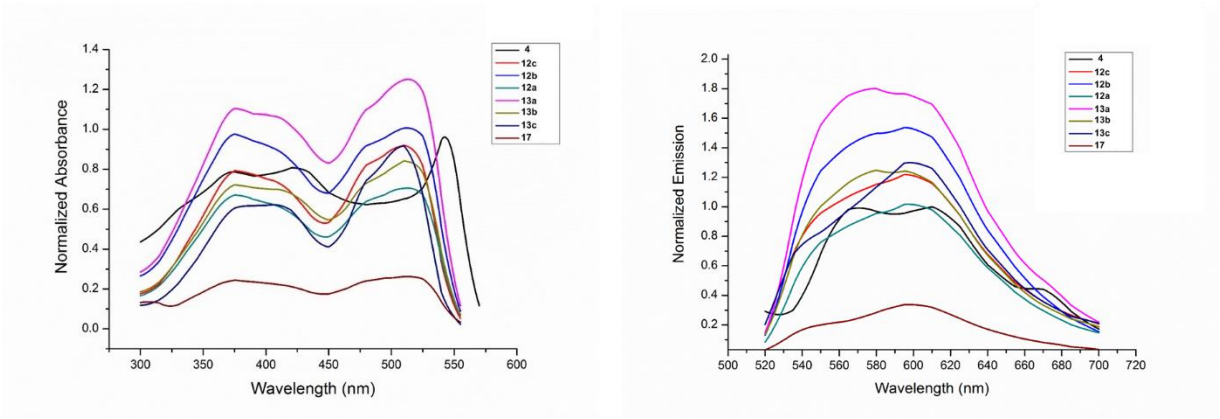
Experimental
The labeling was planned with a BODIPY derivative as a fluorescent moiety containing a terminal alkyne function, because it is stable under physiological conditions and offers many possibilities for its synthesis and functionalization. Conjugation of E1 and E2 via the chemically important C-3-*O* position was accomplished by 1,3-dipolar cycloaddition[2] (CuAAC) via triazole ring incorporation or Sonogashira coupling[3] (Figure 1). We aimed to synthesize the conjugates via inserting C_4–C_8 long linkers between the steroid and the dye.



3. Figure Synthesis of estrone-BODIPY and estradiol-BODIPY conjugates

Results and discussion

The synthesis of BODIPY alkyne was performed via our recently established efficient methodology[1]. Figure 2. shows the absorption and the fluorescence emission spectra of the non-conjugated BODIPY dye (4), the estradiol-BODIPY conjugates (12a–c, 13a–c), and the estrone–BODIPY conjugate (17).



4. Figure Normalized absorbance and emission spectra of the dye (4) and the conjugates (12a-c, 13a-c,17)

According to these data, these BODIPY-conjugated steroids have spectral characteristics similar to those of the non-conjugated BODIPY dye and may facilitate observations in living cells and tissues.

Conclusion

We have synthesized 6 new estrone-BODIPY and 6 new estradiol-BODIPY derivatives – conjugated with different chain length linkers – via CuAAC and/or Sonogashira reactions. The newly synthesized fluorescent estrone derivatives may serve as good candidates for the development of "green" biological assays. Additionally, thanks to the "visibility" of fluorescent estrogens, new important biological actions might also be identified.

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