SYNTHESIS OF FLUORESCENT DYES FOR BIOIMAGING

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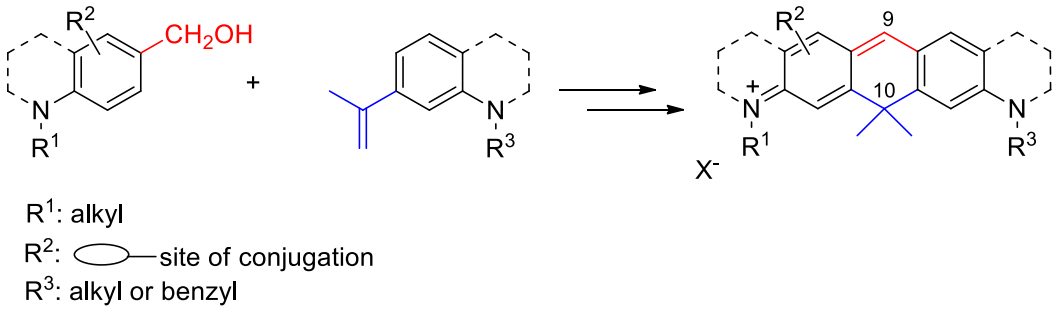
Abstract
Super-resolution fluorescence microscopy offers good sensitivity and high temporal resolution for characterizing cellular structures and biomolecules. Red-emittig fluorescent dyes are ideal fluorophores for biomolecule labeling, due to reduced absorbance and autofluorescence of cells in far-red or near-red window. Here we synthesized novel fluorescent dyes based on carbopyronine core. The dyes were synthesized from two fragments based on cyclocondensation and/or Friedel-Crafts alkylation strategies.

Introduction
The majority of the biochemical assays exist based on radioisotope labeling; however, there is an increasing demand for friendlier and greener bioanalytical methods. Fluorescent labeling could serve as a more favorable alternative.[1,2] Nevertheless, the fluorescent labeling might greatly influence the original biological behavior of the biomolecule. The position of labeling, the spacing between the labeled compound and the dye, and the coupling moiety might all determine biological applicability. The ideal fluorophore would have high photostability, far-red excitation and emission wavelenghts, and could be attached to the biomolecule without affecting its original biological function.[3] Here we aimed to synthesize red-emitting dyes based on carbopyronine core.

Results and discussion
The syntheses of the carbopyronine dyes were carried out according to the strategy described on Scheme 1. One fragment was a benzyl alcohol derivative, the other contained the isopropenyl function. The methylene bridges were established via electrophilic aromatic substitution reactions, catalyzed by Lewis- or Brønsted-acids. Functional groups suitable for the late-stage conjugation of the dyes to certain biomolecules were introduced onto the first fragment. The structures of the dyes were characterized by one- and two-dimensional ^{1}H and ^{13}C NMR methods. The conjugation of the fluorophores to neurosteroids is planned in near future.



Scheme 1. Synthesis of fluorophores based on carbopyronine core

Conclusion

In conclusion, we have synthesized fluorescent dyes suitable for the labeling of biomolecules. The labeling of neurosteroids with red-emitting dyes allows the investigation of the conjugates by super-resolution fluorescence microscopy in living cells.

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References

[1] L. Wang, M.S. Frei, A. Salim, K. Johnsson, J. Am. Chem. Soc., **2019**, *141*, 2770–81.
[2] K. Kolmakov, E. Hebisch, T. Wolfram, L. A. Nordwig, C. A. Wurm, et al. Chemistry **2015**, *21*, 13344–56.
[3] V.N. Belov, S. Stoldt, F. Rüttger, M. John, M., et al. J. Org. Chem. **2020**, *85*, 7267–75.