



4'-Rhodamin Derivates as Fluorescent Dyes

Technology Offer

File no.: MI0104-5931

Background

As a result of the evermore-enhanced super resolution microscopic techniques that allow observation of biological processes up to the molecular level in living cells the demand for fluorescent dyes owning the capability of specific binding to molecules like proteins as label for visualization aspects is increased.

The search for a suitable fluorescent dye that fulfills all requirements of an ideal candidate for the use in biological environments is still challenging:

- (i) high photostability
- (ii) good brightness – high extinction coefficient and quantum yield
- (iii) derivatization possibility of the fluorescent dye molecule
- (iv) no unspecific interaction with other biomolecules
- (v) high membrane permeability

Several approaches already based on derivatives of rhodamine fluorophores. The most prominent derivates in this area are carbopyronines (CP) or silicone-rhodamine (SiR).

Due to the fact that an equilibrium between their non-fluorescent spirolactone and fluorescent zwitterionic form exist one can push the preferred form by introducing electron-withdrawing or electron-donating groups into the structure. In this context it should be mentioned that the spirolactone form is the more hydrophobic one and shows better membrane permeability. Several studies attempted to switch the equilibrium towards spirolactone form by introducing the electron-withdrawing groups in the xanthene core or in the benzoic acid substituent. However, all these approaches result in a bulkier core structure and fast altering process of physicochemical properties of the dyes.

Technology

To overcome the aforementioned drawbacks a new class of rhodamine derivates that meets all requirements could be developed in the group of Prof. Stefan Hell, Max Planck Institute for Biophysical Chemistry in Göttingen by introducing an amide group in the 4' position shifting the equilibrium to the lactone form and hence improving the membrane permeability (Fig. 1a).

In such 4'-isomers of rhodamines a superposition of inductive and steric effects and hydrogen bonding due to neighbouring groups in the benzene ring of the fluorophore occurs that provokes a so called neighbouring group effect (NGE). This effect is observed in 4'-isomers of rhodamines only.

Rhodamine 4'-isomers containing NGE show significant NMR chemical shift of the interacting atoms that are covalently bonded and show increased HPLC retention times. Importantly, this results in not so far observed increased cell membrane permeability compared to widely used 5'-isomers or 6'-isomers while keeping all photophysical properties almost unchanged.

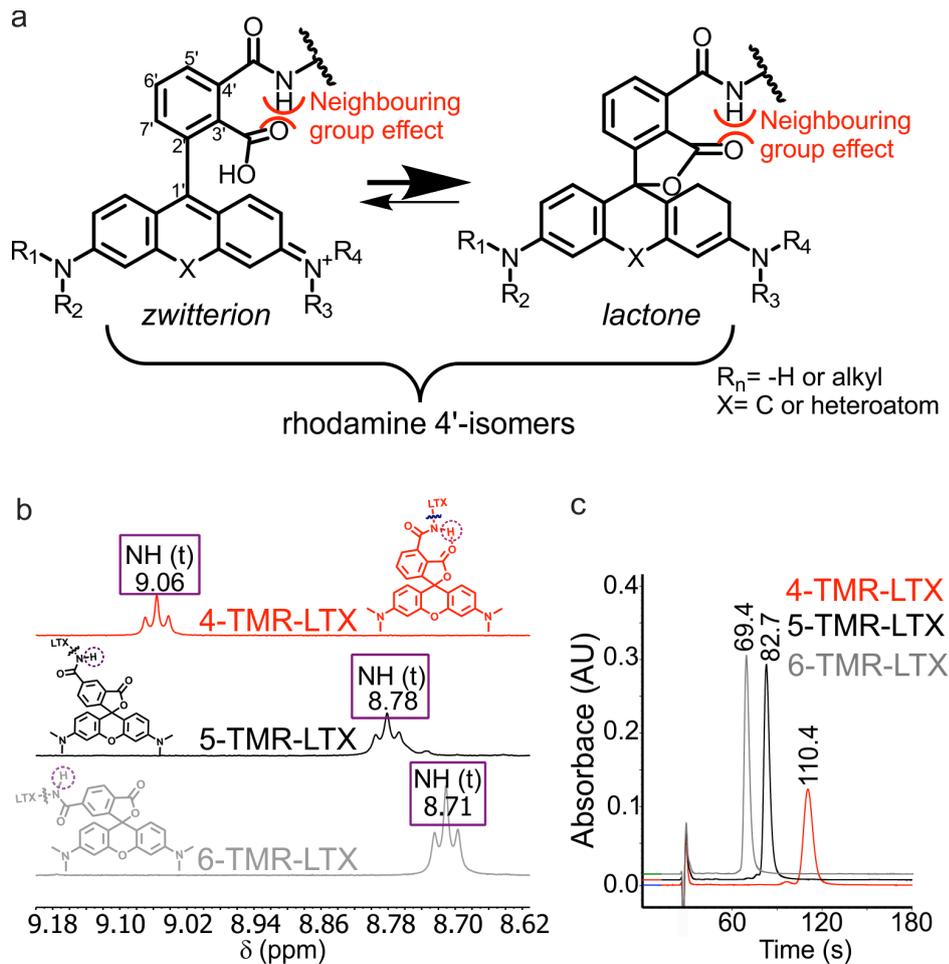


Figure 1. Occurrence of neighbouring group effect in the 4'-isomers of rhodamines. **a**, Neighbouring group effect shifts spirolactone-zwitterion equilibrium of rhodamines **b**, Chemical shift differences of amide proton of TMR-LTX (tetramethylrhodamine-larotaxol) regioisomeric probes; 4'-isomers, 5'-isomers and 6'-isomers. **c**, Comparison of retention times of TMR-LTX regioisomeric probes in HPLC analysis with SB-C18 column and isocratic elution conditions (75:25 MeOH : H₂O 25mM HCOONH₄ pH = 3.6).

Advantages

- good brightness
- specific binding
- no interaction with other biomolecules
- stable against a various of environmental factors: pH, ion concentration, enzyme, tension force, local microenvironment polarity or light
- membrane permeability – no need of injection step
- simply incubation the cells with fluorescent probe

Patent Information

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