

Synthèse, caractérisation et utilisation de sondes fluorogènes pour la nanoscopie

Synthesis, characterization and use of fluorogenic probes for nanoscopy

The CO2-SysChem research group showed in the last five years a growing interest in those fluorescent probes that reply to the following requests: **good cell permeability, specific staining of its target, high brightness, high photo-stability and suitable excitation and emission wavelengths.**[1] These molecules show an increase in fluorescence on binding to their target, alleviating the problem of a background signal. Most of the synthesized molecules were rhodamine and rhodamine-like fluorophores modified at the xantheno moiety or as amide derivatives (Figure 1A and B). The latter modifications increase cell permeability and rationally tune the dynamic equilibrium between two different forms: a **fluorescent zwitterion and a non-fluorescent, cell-permeable spirolactam.**[1,2] Due to an existing dynamic equilibrium between two forms (fluorescent amide and non-fluorescent, slightly colored spirolactam), such derivatives can be easily tuned in their emission. In particular, the presence of an electron-withdrawing group (EWG) on the amide shifts the equilibrium towards the fluorescent form. We have thus explored the synthesis and further characterized electron-deficient amides as xantheno derivatives, widening the scope to rhodamines, fluorescein and a class of less explored compounds, the rhodafluors, also known as rhodols,[3] to obtain probes exploitable in a wide range of wavelengths (Figure 1B).*

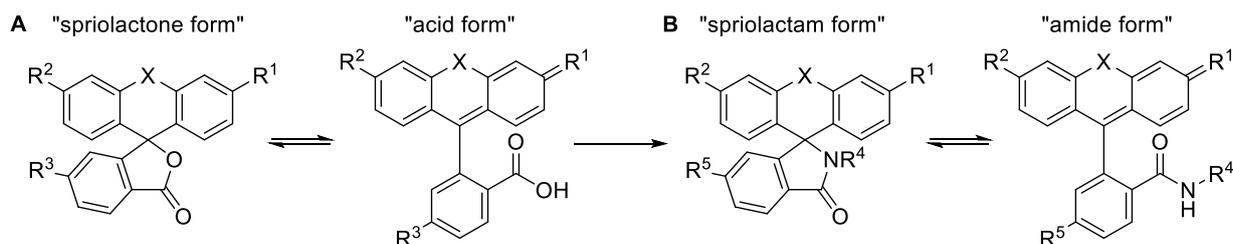
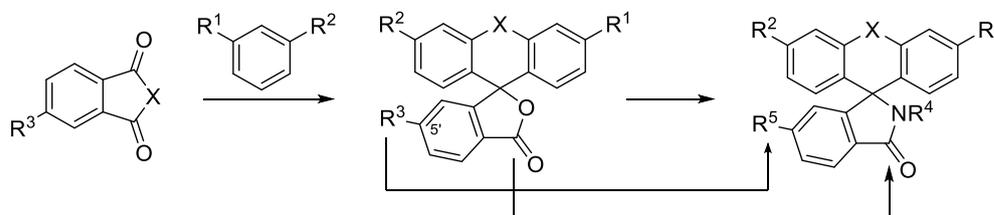


Figure 1. Generic structures of xanthenes under investigation. For example, R³ = Br or H, R⁴ =EWG group/ EWG clickable probe, R⁵ = H or lipophilic chain or hydrophilic group or clickable probe such as tetrazines

The objective of the proposed PhD program is to:

- find a new synthetic pathway to prepare xantheno derivatives functionalized at position 5'
- explore alternative synthetic methods for the synthesis of the "xantheno spirolactam form" from the spirolactone form, in order to have a double functionalization on the same probe using EWG or non-EWG amines



Scheme 1. Exploring the possible pathways for the preparation of xantheno derivatives. X can be O, Si, S, P, or CH₂

* C. Chieffo, PhD program 2019 – 2022

One of the main applications of the proposed research program is the functionalization in one of the position (5' or amide, see scheme 1) with a clickable probe, for example a tetrazine moiety.[4] The molecules prepared in this manner can be used, for example for studying the encapsulation of bio-molecules (DNA, RNA, short lipophilic peptides) in synthetic *ad hoc*-prepared membranes plausibly resembling prebiotic ones.[5]

A recent application of new xanthene derivatives, non-cytotoxic and all fluorogenic, is the use in confocal imaging of cells (in Figure 2, bone marrow cancer cells) to identify mitochondria among other organelles (Chieffo et al., 2022, article in preparation). This application opens the perspective of using synthesized molecules as mitochondrial trackers, a particular class of molecules able to stain mitochondria, rather than other cellular organelles.

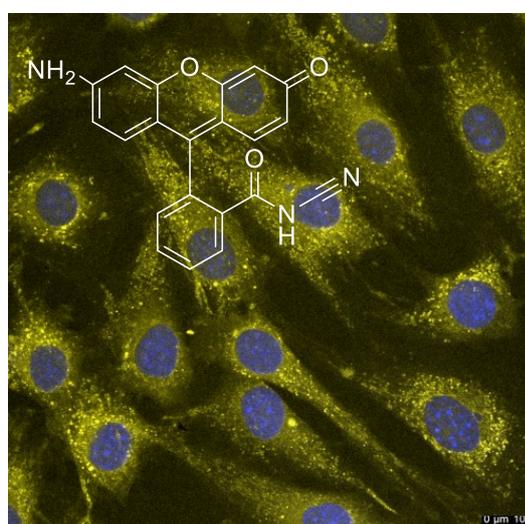


Figure 2. Confocal fluorescence image of bone marrow cancer cells, fixed with a new “rhodol” derivative (yellow-green staining from white molecular structure)

We look for a French or international candidate with a good background in organic synthesis and/or chemical physical synthesis. The candidates should have good skills in English (written and spoken), good propensity for team working and problem solving.

The interested students can write an email to the PhD supervisor and co-supervisor:

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