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Synthesis of fluorinated rhodamines and application for confocal laser scanning microscopy

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1-iodo-1H,1H,2H,2H-perfluorododecane (PubChem CID: 74886)
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ABSTRACT

Four different fluorinated fluorescence dyes were prepared by attaching perfluoroalkyl ponytails (including CH_2 or C_2H_4 spacer) to each of the two amine groups of rhodamine (Rh) and characterized with respect to their fluorescence properties in 2,2,2-trifluoroethanol (TFE), *N,N*-dimethylformamide (DMF), tetrahydrofuran (THF), and toluene. They showed an excellent quantum yield in TFE. Double staining with Rh-C_2H_4-$C_{10}F_{21}$ and Rh-DPPE was employed to visualize the distribution of perfluoropalmitic acid in mixed giant unilamellar vesicles with 1,2-dipalmitoyl-*sn-glycero*-3-phosphocholine (DPPC) observed by confocal laser scanning microscopy.

1. Introduction

Rhodamine (Rh) based fluorescence dyes have been employed frequently for all kinds of fluorescence microscopy [1]. In life sciences, Rh-based fluorescence dyes with one or two fatty acid chains are used since they incorporate selectively into the hydrophobic part of the double layer of cell or model membranes [2]. Fluorinated Rh dyes are synthesized since they have high quantum yields, and good photostability [3,4]. Additionally, it has been demonstrated that they exhibit some kind of fluorophilicity when functionalized with fluorous ponytails [5] since they tend to adsorb on fluorous silica gel in flash columns (F-SPE) when eluted with a fluorophobic solvent as e.g. aqueous methanol as reported by Kölmel et al. [6]. There seems to be some need for the detection of fluorine-rich domains by fluorescence techniques since more and more pharmaceutical active ingredients contain some perfluoroalkyl groups [7,8]. Furthermore, designer proteins employ highly fluorinated non-native amino acids [9], polar hydrophobic fluorinated sugars are introduced [10,11], fluorinated amphiphiles are synthesized for drug delivery [12], fluorinated compounds are used in medicinal chemistry [13,14], and fluorinated block copolymers are designed for specific membrane interactions [15,16].

Here, we describe the synthesis of four fluorinated rhodamines Rh-C_nH_{2n}-C_mF_{2m+1} (F-rhodamines with the m,n combinations of (1,3), (1,7), (2,8), and (2,10)). One F-ponytail (-C_nH_{2n}-C_mF_{2m+1}) is attached to each of the two amine groups of rhodamine. Then, UV–vis and fluorescence spectra are measured and the quantum yield is determined. Finally, the dye with the longest F-ponytails Rh-C_2H_4-$C_{10}F_{21}$ is employed together with Rh-DPPE to stain selectively fluorine-rich domains in mixed giant unilamellar vesicles (GUVs) of 1,2-dipalmitoyl-*sn-glycero*-3-phosphocholine (DPPC) and

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perfluoropalmitic acid (PFPA) as observed by confocal laser scanning microscopy (CLSM).

2. Results and discussion

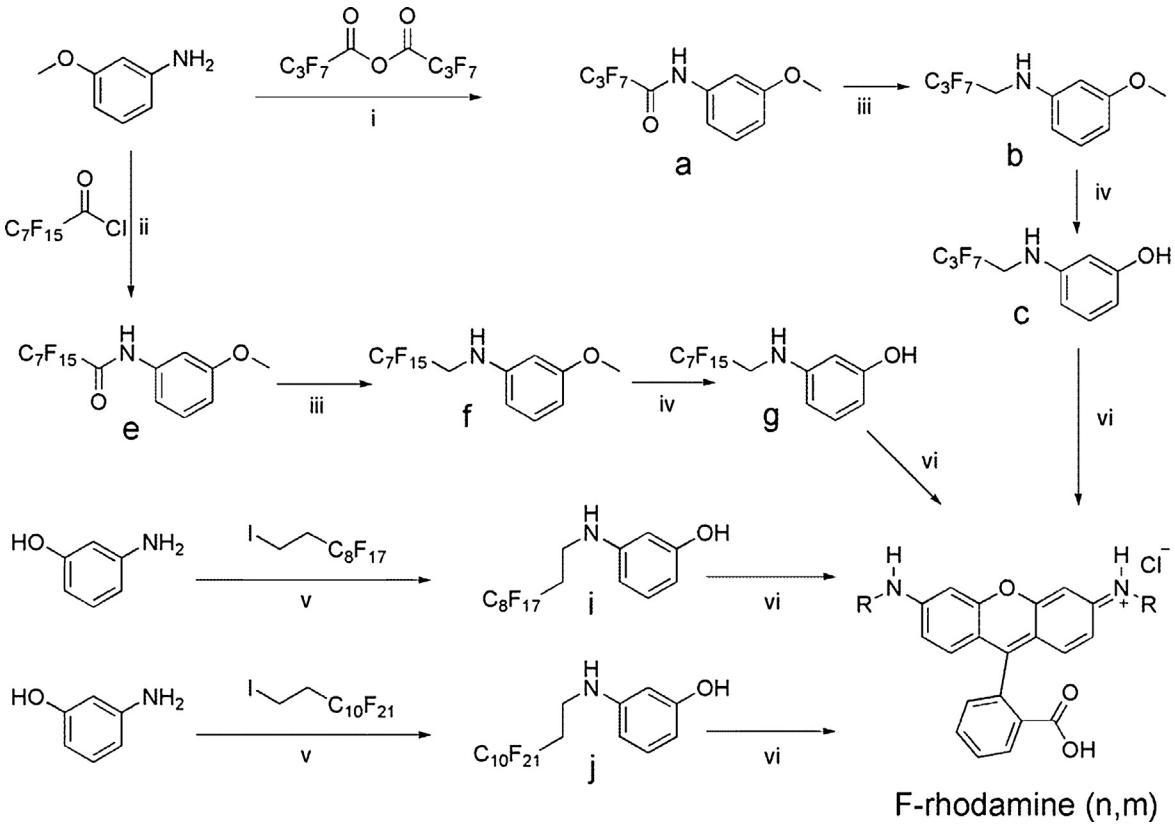
2.1. Synthesis and characterization

Four different rhodamines having partially fluorinated pony-tails as shown in Scheme 1 **d,h,k,l** were synthesized similarly to procedures applied by Belov and Hell et al. [3,17] and Kölmel et al. [6]. Compound **h** has been reported by Kölmel et al. synthesized in a different way. For the synthesis of Rh-CH_2-C_3F_7 heptafluorobutyric anhydride was reacted with *m*-anisidine in dichloromethane (DCM) in the presence of triethylamine (TEA) as an organic base for 12 h to obtain the C_3F_7-secondary amide intermediate (**a**). It was further reduced in the presence of lithium aluminum hydride ($LiAlH_4$) in THF under reflux for 12 h to obtain the C_3F_7-CH_2-secondary amine intermediate (**b**). It was demethylated by reacting with boron tribromide in DCM under reflux for 12 h to obtain the C_3F_7-CH_2-secondary amino phenol intermediate (**c**). It was then reacted with phthalic anhydride in the presence of *p*-toluenesulfonic acid monohydrate and an excess of propionic acid at 160 °C for 24 h to obtain the Rh-CH_2-C_3F_7 fluorescence dye (**d**) in a yield of 24%. Rh-CH_2-C_7F_{15} (**h**) was synthesized in a similar way to Rh-CH_2-C_3F_7 (**d**) with a main difference regarding the first synthesis step of the C_7F_{15}-secondary amide intermediate (**e**) in which pentadecafluorooctanoyl chloride was used to functionalize *m*-anisidine with a C_7F_{15}-alkyl group in the presence of pyridine. C_7F_{15}-CH_2-secondary amine intermediate (**f**) was synthesized similarly to (**b**) and the C_7F_{15}-CH_2-secondary amino phenol (**g**) was prepared in a similar way to (**c**). Compounds (**i**) and (**j**) were synthesized by reacting 3-aminophenol with the corresponding alkyl iodide bearing C_8F_{17} and $C_{10}F_{21}$ groups with two methylene spacers in 1-methyl-2-pyrrolidone (NMP) with ethyldiisopropylamine (DIPEA) as an organic base at 100 °C for 24 h to obtain the C_8F_{17}-C_2H_4-secondary amino phenol intermediates (**i**) and $C_{10}F_{21}C_2H_4$-secondary amino phenol (**j**), respectively. The last synthesis part yielding fluorinated fluorescence dyes Rh-CH_2-C_7F_{15}, Rh-C_2H_4-C_8F_{17}, and Rh-C_2H_4-$C_{10}F_{21}$ was a Friedel-Crafts condensation step [18] similar to the one used to synthesize Rh-CH_2-C_3F_7 (**d**). As an example, the characteristic ^1H and ^{19}F NMR spectra are given for Rh-C_2H_4-$C_{10}F_{21}$ (**l**) in Figs. 1 and 2, respectively. The NMR spectra of the other three F-rhodamines and their electrospray ionization time of flight (ESI-TOF) spectra are shown in the Supplementary data (Fig. S1–S10).

^{19}F NMR peaks were assigned according to data given by W. R. Dolbier [19].

2.2. UV–vis and fluorescence spectroscopy

The absorbance and emission of the four dyes was measured in 2,2,2-trifluoroethanol (TFE), *N*,*N*-dimethylformamide (DMF), tetrahydrofuran (THF), and toluene as a function of concentration in the dilute regime where the Lambert-Beer law is obeyed (absorbance less than 0.1, Supplementary data, Fig. S11–S22). Thus, all UV–vis and fluorescence measurements were done at concentrations less than 2 μM (equivalent to an absorption of less than 0.1 in TFE) to be safely within the linear regime. Quantum yields of the F-rhodamines Φ_x in TFE, DMF, THF, and toluene were calculated relative to a standard (fluorescein in 0.1 M NaOH aqueous solution with a quantum yield of $\Phi_{st} = 0.89$) as shown in Table 1 based on a protocol published by Würth et al. [20]. Absorbance *f* and emission flux *F* were measured at 490 and 491 nm for Rh-CH_2-C_3F_7 and Rh-CH_2-C_7F_{15}, respectively, in TFE,



Scheme 1. Synthesis of F-rhodamines Rh-C_nH_{2n}-C_mF_{2m+1}. i) DCM, TEA, 0 °C to RT, 12 h, ii) DCM, pyridine, 0 °C to RT, 12 h; iii) THF, $LiAlH_4$, reflux, 12 h; iv) DCM, BBr_3, reflux, 12 h; v) NMP, DIPEA, 100 °C, 24 h; vi) propionic acid, phthalic anhydride, *p*-toluenesulfonic acid monohydrate, 160 °C, 24 h. The following F-rhodamines (n,m) were synthesized **d** (1,3), **h**(1,7), **k**(2,8), and **l**(2,10).