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Synthesis of fluorinated rhodamines and application for confocal laser scanning microscopy

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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

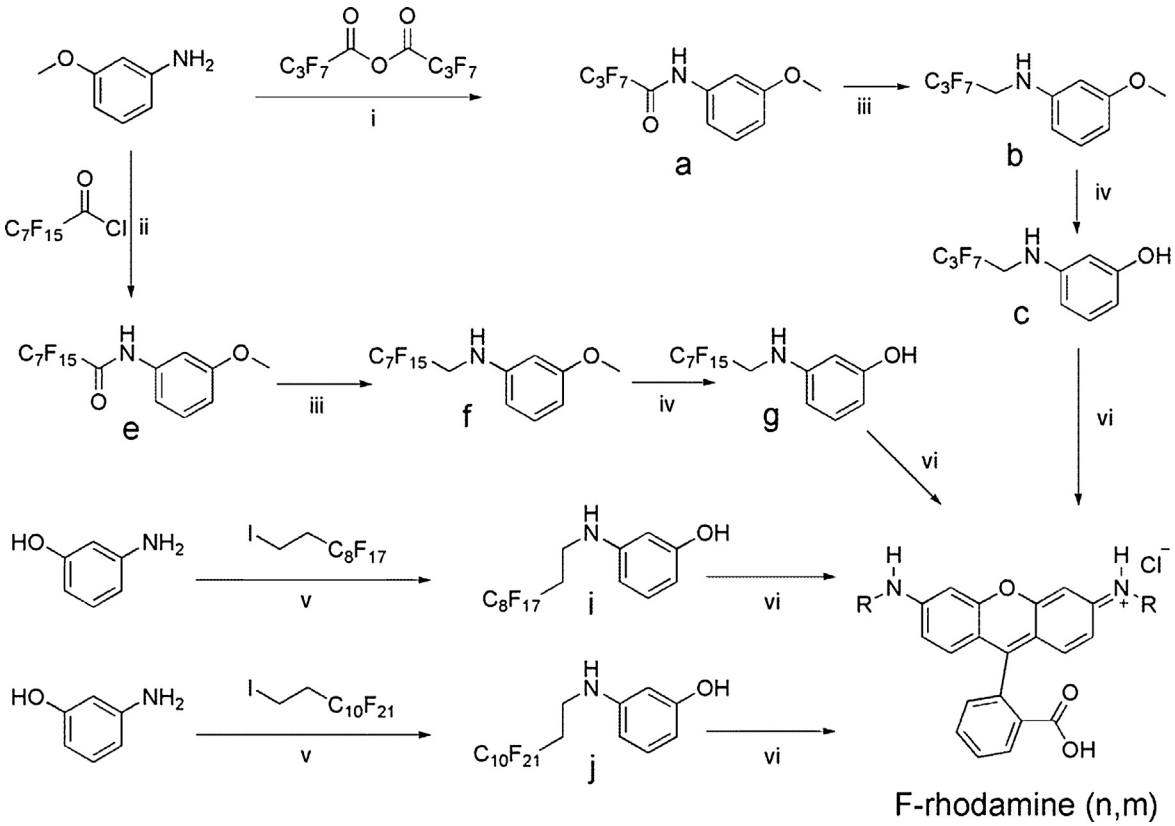
2.1. Synthesis and characterization

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).

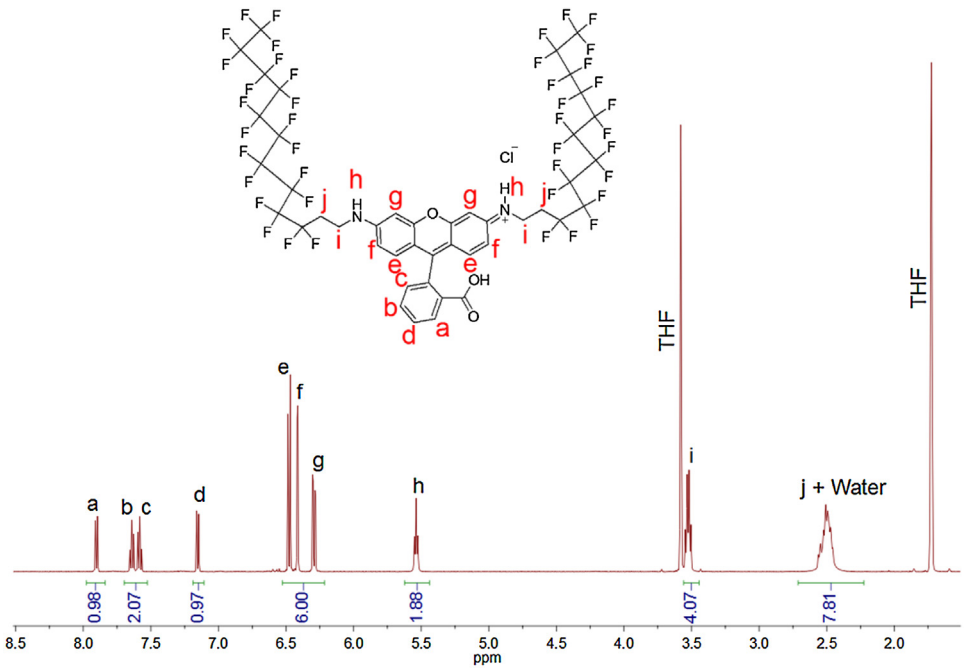


Fig. 1. ^{1}H NMR spectrum (500 MHz, THF-d_8) of Rh-C_2H_4-$C_{10}F_{21}$ (**I**) measured at 27 °C.

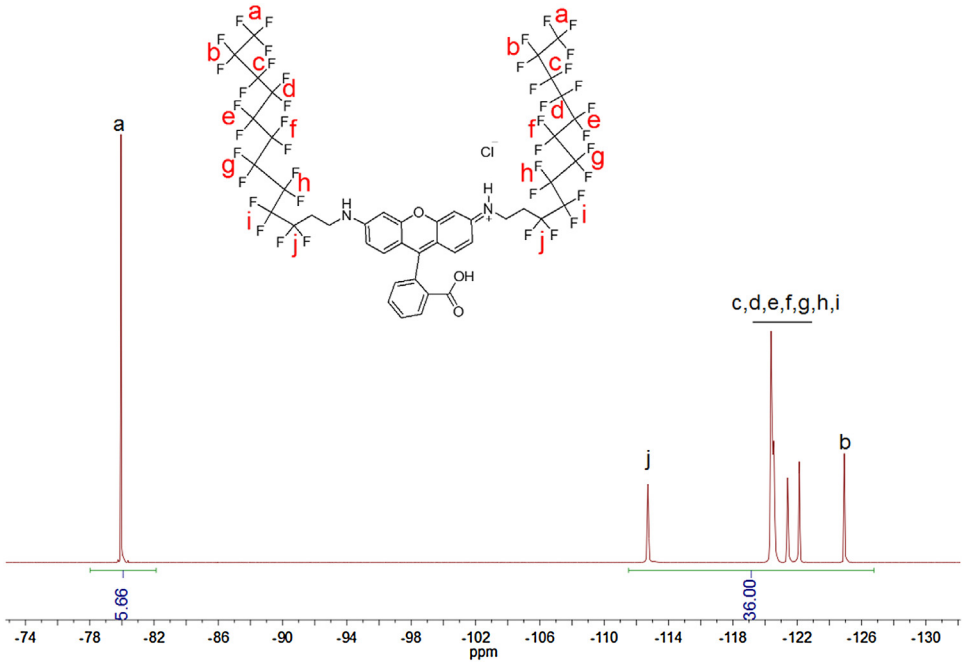


Fig. 2. ^{19}F NMR spectrum (470 MHz, THF-d_8) of Rh-C_2H_4-$C_{10}F_{21}$ (**I**) measured at 27 °C.

Table 1
Relative quantum yields of F-rhodamines in TFE, DMF, THF, and toluene measured against a fluorescein standard in 0.1 M NaOH as solvent at 20 °C.

Quantum Yield Φ	Rh-CH_2-C_3F_7	Rh-CH_2-C_7F_{15}	Rh-C_2H_4-C_8F_{17}	Rh-C_2H_4-$C_{10}F_{21}$
TFE	0.91[a]	0.93[b]	0.88[c]	0.87[c]
DMF	0.14[a]	0.10[b]	0.19[d]	0.26[d]
THF	0.06[a]	0.08[b]	0.22[d]	0.20[d]
Toluene	0.26[a]	0.12[b]	0.18[d]	0.21[d]

[a]Emission spectra were recorded at 490 nm, [b]491 nm, [c]496 nm, and [d]504 nm excitation.

DMF, THF, and toluene. The quantum yields of Rh-C_2H_4-C_8F_{17} and Rh-C_2H_4-$C_{10}F_{21}$ were determined at 496 nm excitation in TFE and at 504 nm excitation in DMF, THF, and toluene. Eq. (1) was used to calculate the relative quantum yields of the synthesized F-rhodamines

$$\Phi_x = \Phi_{st} \cdot \frac{F_x}{F_{st}} \cdot \frac{f_{st}}{f_x} \cdot \frac{n_x^2(sodium\ line)}{n_{st}^2(sodium\ line)} \quad (1)$$

where the subscript *x* refers to the values of the F-rhodamines and *st* to the values of the fluorescein standard. For the F-rhodamine solutions the refractive index of their corresponding solvents were used and the refractive index of 0.1 M NaOH aqueous solution was used for the fluorescein standard. The relative quantum yields are summarized in Table 1.

As observed from Table 1, the F-rhodamines display a high quantum yield in TFE in contrast to DMF, THF, and toluene where the quantum yields were significantly lower. Simultaneously, the UV–vis absorption and emission spectra differ drastically between TFE solutions and the other organic solvents used.

The absorption peaks in the 400–500 nm range visible in the TFE solutions (Fig. 3a) disappear when the F-rhodamines are dissolved in THF (Fig. 4a) and a strong absorption at 285 nm is observed due to the shift in equilibrium towards the non-fluorescent spirolactone "closed form" [3]. The "open form" exhibits strong fluorescence and the spirolactone is non-fluorescent as illustrated in Scheme 2. The strong emission observed for the F-rhodamines in TFE (Fig. 3b) is dramatically reduced in THF (Fig. 4b) and almost vanished for Rh-CH_2-C_3F_7 and Rh-CH_2-C_7F_{15}. The appearance of the elastic scattering Rayleigh peaks observed at the excitation wavelengths indicates the very weak fluorescence in 1 μM THF solutions (Fig. 4b). The situation is similar for DMF and toluene where the fluorescence is practically switched off as seen in Fig. S23–26 in comparison with the emission peak when TFE is used as a solvent.

The synthesized dyes have similar absorption and fluorescence properties in TFE as shown in Fig. 3a and b. A significant red shift of about 13 nm in the absorption and emission spectra is observed when the length of the spacer increases from one to two methylene groups. This is mainly caused by a decrease of the electron withdrawing effect of the fluorinated alkyl group on the nitrogen of the amine group with increasing spacer length [21]. The 22 nm Stokes shift, i.e. the difference between the maximum absorption wavelength ($\lambda_{max,abs}$) and the maximum emission wavelength ($\lambda_{max,em}$) remains constant for all four synthesized F-rhodamines. Thus, it depends neither on the length of the spacer nor on the length of the fluorinated ponytail. The quantum yields measured in TFE were similar to other rhodamines measured in other alcohols. F-rhodamines have a high resistance to photo bleaching and can be used as laser dyes such as in confocal laser scanning microscopy (CLSM) [3,4,18].

2.3. Confocal laser scanning microscopy

The fluorophilic character of the synthesized fluorescence dye bearing the longest fluorinated ponytails (Rh-C_2H_4-$C_{10}F_{21}$) was tested in GUVs formed by the hydration of a DPPC/PFPA 10:1 (mol %) mixture including 0.1 mol% Rh-C_2H_4-$C_{10}F_{21}$ and 0.1 mol% Rh-DPPE on agarose [22]. The chemical structures of both dyes are given on top of Fig. 5. It should be noted that Rh-C_2H_4-$C_{10}F_{21}$ is not able to incorporate into pure DPPC GUVs. An equatorial image of the obtained GUVs (Fig. 5A) shows that the two dyes in use are incorporated into the mixed lipid bilayer but not homogeneously. This is even more evident in Fig. 5B (z-stacking). The red, middle channel of Fig. 5B shows the DPPC-rich continuous phase stained by Rh-DPPE. This is not surprising since the fluorescence dye and the phospholipid have identical acyl chain residues. But the yellow color of the overlay image indicates that also the F-rhodamine dye incorporates into the continuous phase. The dark spots in the middle, red image represent regions where Rh-DPPE is excluded but the green channel, left image and the overlay, right image strongly indicate an enrichment of the fluorophilic dye Rh-C_2H_4-C_{10}-F_{21}. The exclusion of Rh-DPPE from the ordered gel phase of DPPC is due to the very large head group of the dye compared with the head group of the lipid [2,23]. Obviously, PFPA is also located in the dark domains indicated by the fluorescence of the F-rhodamine. Thus the double staining is a suitable tool to analyze the internal structure of the hydrophobic part of the double layer of the GUVs.

3. Conclusion

We synthesized four fluorinated rhodamine fluorescence dyes which were characterized by their UV–vis and fluorescence spectra and showed excellent quantum yields in TFE. As an example Rh-C_2H_4-$C_{10}F_{21}$ was used as CLSM fluorescence dye and proved to be stable and resistant to photobleaching after repetitive laser scans. Rh-C_2H_4-$C_{10}F_{21}$ was successfully used to stain fluorinated moieties partitioned into DPPC/PFPA mixed GUVs (obtained by hydration on agarose). This is a proof of principle for the possibility of using highly fluorinated fluorescence dyes to selectively stain fluorine-rich domains in model membranes.

4. Experimental

4.1. Materials

m-Anisidine, heptafluorobutyric anhydride, perfluoropalmitic acid (PFPA), and phthalic anhydride were purchased from Alfa Aesar. Triethylamine (TEA), pyridine, anhydrous dichloromethane (DCM), anhydrous THF, anhydrous DMF, toluene, agarose super LM, *tert*-butyl methyl ether (MTBE), diethyl ether, *n*-hexane, ethanol,

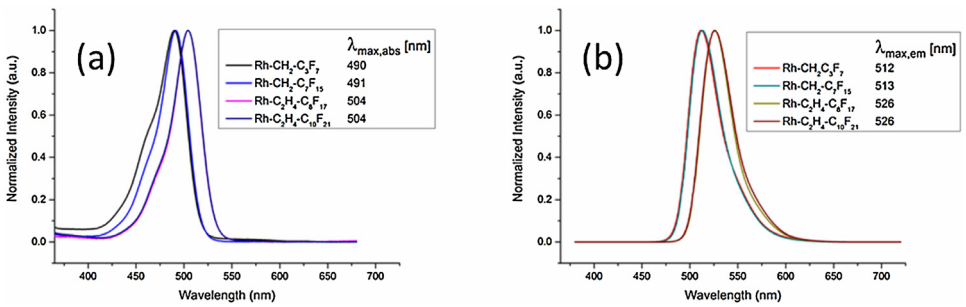


Fig. 3. Normalized absorption (a) and emission (b) spectra of 1 μM F-rhodamines in TFE at 20 °C.

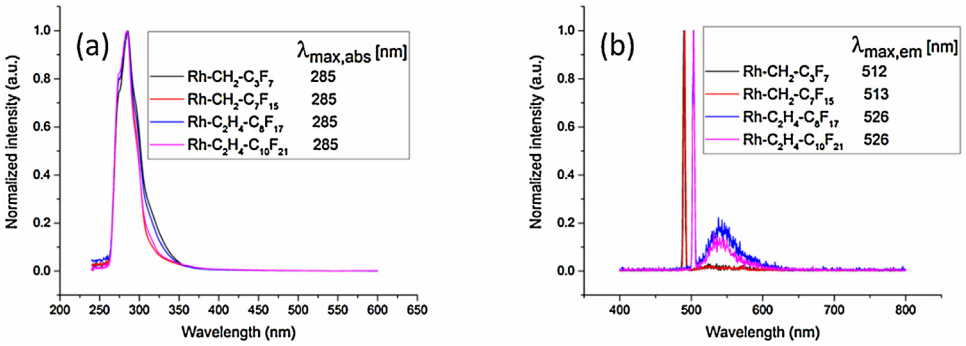
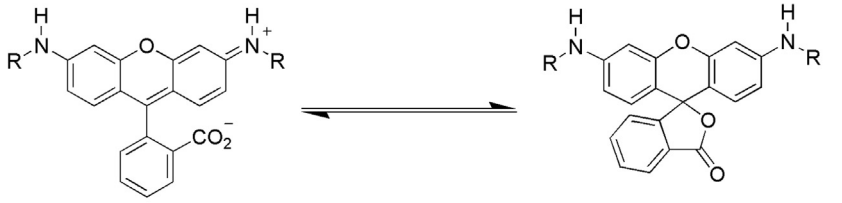


Fig. 4. Normalized absorption (a) and emission (b) spectra (Rh-CH_2-C_3F_7 and Rh-CH_2-C_7F_{15} were excited at 490 and 491 nm respectively; Rh-C_2H_4-C_8F_{17} and Rh-C_2H_4-$C_{10}F_{21}$ were both excited at 504 nm) of 1 μM F-rhodamines in THF at 20 °C.



Scheme 2. Equilibrium between the fluorescent (open) and the non-fluorescent (closed) spirolactone.

and chloroform were purchased from Carl Roth. Lithium aluminum hydride [2 M] THF solution, boron tribromide [1 M] dichloromethane solution, propionic acid, *p*-toluenesulfonic acid monohydrate, pentadecafluorooctanoyl chloride, 3-aminophenol, 1-iodo-1*H*,1*H*,2*H*,2*H*-perfluorodecane, 1-iodo-1*H*,1*H*,2*H*,2*H*-perfluorododecane, anhydrous 1-methyl-2-pyrrolidone (NMP), ethyldiisopropylamine (DIPEA), and nonafluorobutyl methyl ether (HFE-7100) were purchased from Sigma-Aldrich. 1,2-dipalmitoyl-*sn*-*glycero*-3-phosphocholine (DPPC) was purchased from Avanti Polar Lipids. Rhodamine B 1,2-dihexadecanoyl-*sn*-*glycero*-3-phosphoethanolamine (Rh-DPPE) was purchased from Thermo Fisher Scientific. Deuterated solvents for solution NMR spectroscopy ($CDCl_3$, CD_3OD, DMSO-d_6, and THF-d_8) were purchased from Armar Chemicals. All chemicals were used without further purification.

4.2. Methods

Solution NMR spectra were recorded at 27 °C by Agilent Technologies 400 MHz ^{1}H VNMRS spectrometer or Agilent Technologies 500 MHz ^{1}H DD2 spectrometer. Samples were dissolved in deuterated solvents ($CDCl_3$, CD_3OD, DMSO-d_6, or THF-d_8). ^{1}H and ^{13}C NMR spectra were reported relative to TMS, ^{19}F spectra relative to $CFCl_3$, and ^{13}C NMR spectra were proton decoupled.

ESI-TOF measurements were performed on a Focus micro ToF by Bruker Daltonics. The samples were dissolved in 2,2,2-trifluoroethanol to a final concentration of 30 μM and directly infused (180.00 μL/h, positive mode).

Varian-Cary 4000 double beam UV–vis spectrometer was used for UV–vis measurements. BRAND® UV micro single-use cuvettes (mfr. no. BRAND® 7592 20) with center height 15 mm having a 1 cm path length were filled with 500 μL 2,2,2-trifluoroethanol (TFE) solutions. UV–vis measurements were done with baseline correction (subtraction) at a temperature of 20 °C. Fluorescence was measured by a FluoroMax 2 spectrometer (Jobin-Yvon). 200 μL solutions were placed in a Hellma SUPRASIL® cuvette (105.250-QS) with center height 15 mm. For fluorescence emission spectra, $\lambda_{abs,max}$ was used to excite the corresponding F-rhodamines. Rh-CH_2-C_3F_7 and Rh-CH_2-C_7F_{15} were excited at 490 and 491 nm, respectively, whereas Rh-C_2H_4-C_8F_{17} and Rh-C_2H_4-$C_{10}F_{21}$ were excited at 504 nm. The calibration of the FluoroMax 2 spectrometer was checked with the water Raman peak at 397 nm before and after the measurement of each sample. All fluorescence measurements were done at 20 °C. Emission spectra were recorded with 0.5 nm increments, 0.1 s integration time, and 2 nm excitation and emission slits. The emission spectra were recorded as counts per second (cps) as a function of the wavelength (nm) using S/R (signal/reference) detection mode to compensate the aging effect of the xenon lamp.

50 μl of a 20 mM DPPC chloroform solution containing 0.1 mol% Rh-DPPE were mixed with 50 μl of a 2 mM PFPA acid solution of diethyl ether/nonafluorobutyl methyl ether 1:1 (v/v) containing 0.1 mol% Rh-C_2H_4-$C_{10}F_{21}$. 5 μl of the homogeneous organic solution were applied as a thin layer on an agarose coated cover glass, dried for 10 min at RT, 1 min at 50 °C, followed by gentle hydration in deionized water for 2 h at 45 °C [23]. Double staining was realized with Rh-DPPE and Rh-C_2H_4-$C_{10}F_{21}$. A Leica TCS SP2 DM IRE2 confocal microscope equipped with an HCX PL APO lbd.BL 63 × 1.2 W CORR (water immersion) objective (Leica Microsystems, Wetzlar, Germany) was used [24]. Commercially available Rh-DPPE (0.1 mol%) was used to stain the GUVs, excited with the 543 nm laser and a detection range of 580–620 nm (red channel). The F-rhodamine Rh-C_2H_4-$C_{10}F_{21}$ (0.1 mol%) was excited with the 514 nm laser and a detection range of 525–540 nm (green channel). Imaging of the obtained GUVs was done at room temperature (RT). Z-stacking was performed from bottom to top.

4.3. Synthesis of fluorescence dyes

4.3.1. Rh-CH_2-C_3F_7 synthesis (***a–d***)

Secondary amide intermediate (**a**): 5 g (40.6 mmol) *m*-anisidine, 19.98 g (48.72 mmol) heptafluorobutyric anhydride, and 0.493 g (4.872 mmol) triethylamine were added to a Schlenk flask equipped with a magnetic stirrer, containing 50 mL anhydrous

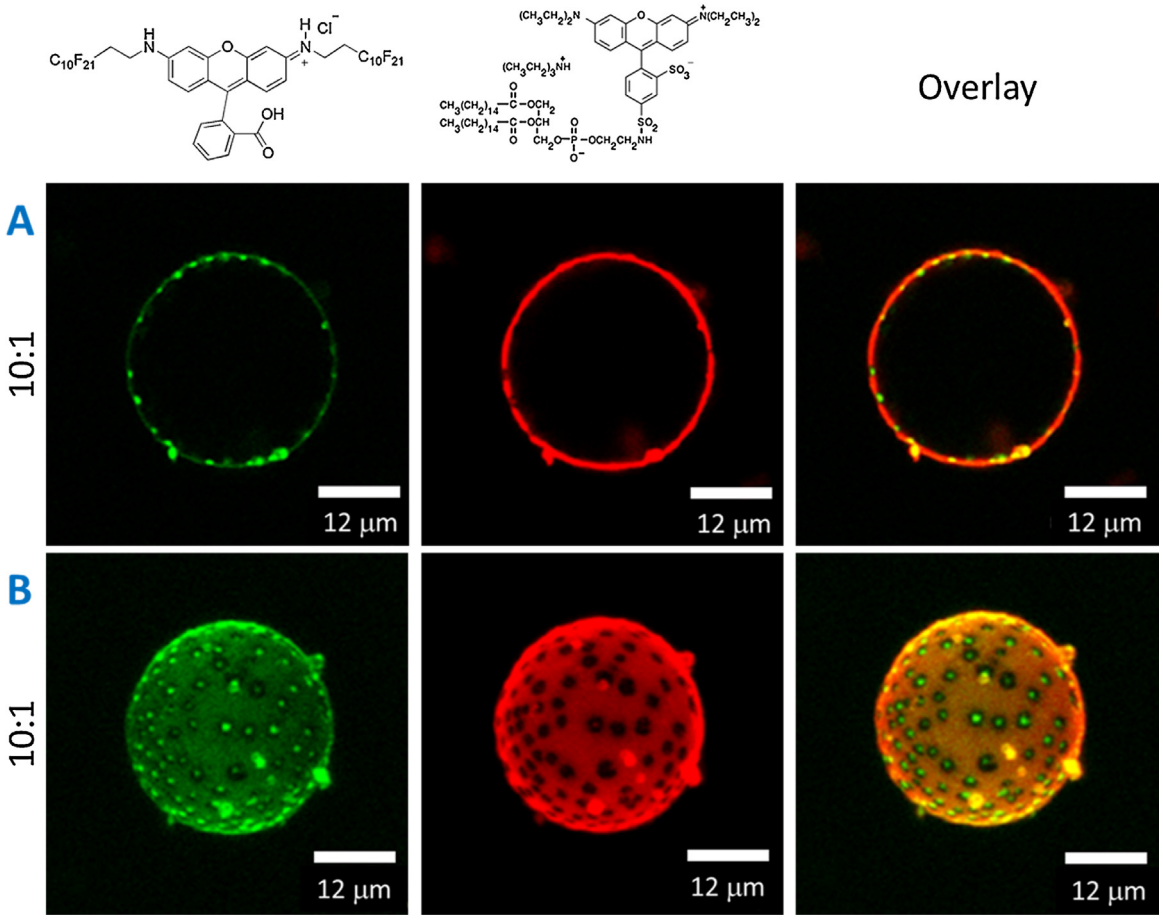


Fig. 5. The left chemical structure gives the Rh-C_2H_4-$C_{10}F_{21}$ employed for CLSM and the right chemical structure represents Rh-DPPE. CLSM equatorial image (A) and z-stacking image (B) of GUVs made from DPPC/PFPA 10:1 (mol%) binary mixture. The Rh-C_2H_4-$C_{10}F_{21}$ dye (0.1 mol%) is observed through the green (left) channel, the Rh-DPPE dye (0.1 mol%) is observed through the red (middle) channel, and the right images show the overlay of both channels. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

DCM maintained at 0 °C in an ice-water bath. The reaction was left under continuous stirring for 1 h in the ice-water bath then removed and left under constant stirring at RT overnight. The reaction was opened to the atmosphere, and the organic phase was further diluted with 100 mL DCM. The DCM phase was washed three times with 5% $NaHCO_3$ aqueous solution, one time with water, three times with 0.01 M HCl aqueous solution, and three times with sodium chloride brine. The DCM phase was dried over sodium sulfate, filtered and concentrated at 30 °C using a rotary evaporator under reduced pressure. 150 mL *n*-hexane were added to the round bottom flask and heated to boiling. The clear warm *n*-hexane phase was decanted into a beaker, and the product was left to crystallize at 4 °C overnight. The product was filtered with a Büchner funnel, washed with portions of cold *n*-hexane and dried under vacuum at RT. Recrystallization from *n*-hexane was repeated a second time to give compound (**a**) as a white solid with 54% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.86 (s, 1H), 7.33–7.27 (m, 2H), 7.07–7.01 (m, 1H), 6.83–6.78 (m, 1H), 3.83 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 160.34 (s), 136.17 (s), 130.10 (s), 112.46 (s), 112.36 (s), 106.20 (s), 55.42 (s). ^{19}F NMR (376 MHz, $CDCl_3$) δ −80.51 (t, J = 8.8 Hz, 3F), −120.06–120.50 (m, 2F), −126.54–126.87 (m, 2F).

*4.3.2. Secondary amine intermediate (**b**)*

2 g (6.27 mmol) C_3F_7-sec-amide (**a**) were transferred into a two-neck round-bottom flask equipped with a magnetic stirrer, reflux condenser and a drying tube filled with calcium chloride. 40 mL THF were added followed by 7.84 mL (15.68 mmol) of a 2 M lithium aluminum hydride in THF solution. The reaction was refluxed for 12 h, left to cool down slowly to RT, and quenched with 1 mL water. The THF phase was filtered, diluted with 200 mL DCM, and filtered a second time. The organic phase was washed two times with water, twice with 5% $NaHCO_3$ aqueous solution and twice with NaCl brine. The organic phase was dried over sodium sulfate, filtered and evaporated at 30 °C using a rotary evaporator under reduced pressure. The product was purified by silica gel column chromatography with mobile phase composition cyclohexane/ethyl acetate 4:1 (v/v) to obtain compound (**b**) as a slightly yellow viscous liquid with 61% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.12 (t, J = 8.1 Hz, 1H), 6.37 (dd, J = 8.2, 2.3 Hz, 1H), 6.30 (dd, J = 8.1, 2.1 Hz, 1H), 6.24 (t, J = 2.3 Hz, 1H), 3.92–3.79 (m, 3H), 3.78 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 160.83 (s), 147.76 (s), 130.19 (s), 106.08 (s), 104.12 (s), 99.58 (s), 55.13 (s), 44.18 (t, J = 23.4 Hz). ^{19}F NMR (376 MHz, $CDCl_3$) δ −80.74 (t, J = 9.4 Hz, 3F), −118.89–119.22 (m, 2F), −127.54–127.73 (m, 2F).

*4.3.3. Secondary amino phenol intermediate (**c**)*

1 g (3.277 mmol) C_3F_7-CH_2-sec-amine (**b**) was transferred to a two-neck round-bottom flask equipped with a magnetic stirrer, reflux condenser and a drying tube filled with calcium chloride. 20 mL DCM were added followed by 4.92 mL (4.92 mmol) of 1 M boron tribromide solution in DCM. The reaction was refluxed for 12 h, left to cool down slowly to RT, and quenched with 1 mL water. The DCM phase was added to 100 mL 5% $NaHCO_3$ aqueous solution and the pH was checked with pH paper to be around 8. The product

was extracted with portions of DCM, and the organic portions were combined and washed three times with NaCl brine, dried over sodium sulfate and filtered. DCM was removed on a rotary evaporator at 30 °C under reduced pressure. The product was purified by silica gel column chromatography with mobile phase composition cyclohexane/ethyl acetate 3:1 (v/v) to obtain compound (**c**) as a brownish waxy solid with 58% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.03 (s, 1H), 6.93–6.82 (m, 1H), 6.19–6.11 (m, 2H), 6.09–6.05 (m, 1H), 6.05–5.98 (m, 1H), 3.96–3.81 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 158.60 (s), 149.46 (s), 129.99 (s), 105.16 (s), 104.21 (s), 100.07 (s), 43.28 (t, J = 22.5 Hz). ^{19}F NMR (376 MHz, DMSO-d_6) δ −80.29 (t, J = 9.4 Hz, 3F), −117.46–117.76 (m, 2F), −127.05–127.40 (m, 2F).

*4.3.4. Rh-CH_2-C_3F_7 (**d**)*

246.6 mg (0.8469 mmol) C_3F_7-CH_2-amino-phenol (**c**), 100.4 mg (0.6778 mmol) phthalic anhydride, 564.6 mg (7.621 mmol) propionic acid, and 12.08 mg (0.0635 mmol) *p*-toluenesulfonic acid monohydrate were added to a Schlenk flask equipped with a magnetic stirrer. The reaction was carried out in a 160 °C thermostated oil bath for 24 h under a nitrogen atmosphere and continuous stirring. The reaction was left to cool down, and the Schlenk flask was opened to the atmosphere. The products were dissolved in chloroform/ethanol 10:1 (v/v) mixture, filtered and washed six times with 0.1 M HCl aqueous solution. The organic phase was dried over sodium sulfate and filtered. The product was purified by silica gel column chromatography with a chloroform/methanol 3:1 (v/v) mobile phase and later precipitated from a concentrated acetone solution into excess cold *n*-hexane. The product was filtered using a Büchner funnel and washed with portions of cold *n*-hexane then put under vacuum at RT to yield Rh-CH_2-C_3F_7 (**d**) as an orange solid with 24% yield. ^{1}H NMR (400 MHz, CD_3OD) δ 8.16 (d, J = 7.5 Hz, 1H), 7.79 (td, J = 7.4, 1.2 Hz, 1H), 7.74 (td, J = 7.5, 1.0 Hz, 1H), 7.32 (d, J = 7.3 Hz, 1H), 6.94–6.86 (m, 4H), 6.79–6.75 (m, 2H), 4.18 (t, J = 15.7 Hz, 4H). ^{19}F NMR (376 MHz, CD_3OD) δ −82.33 (t, J = 9.7 Hz, 6F), −119.19–119.47 (m, 4F), −128.45–129.00 (m, 4F). ESI-TOF, *m*/*z*: calculated for $C_{28}H_{17}F_{14}N_2O_3$ 695.101 $[M]^+$; found 695.0288.

*4.3.5. Rh-CH_2-C_7F_{15} synthesis (**e–h**)*

Secondary amide intermediate (**e**): 5 g (11.56 mmol) pentadecafluorooctanoyl chloride, 1.186 g (9.631 mmol) *m*-anisidine, and 0.9525 g (12.04 mmol) pyridine were added to a Schlenk flask equipped with a magnetic stirrer, containing 50 mL DCM maintained at 0 °C in an ice water bath. The reaction was left under continuous stirring for one hour in the ice-water bath then at RT overnight. The reaction was opened to the atmosphere, and the organic phase was further diluted with 100 mL DCM and filtered to remove part of the precipitated pyridinium chloride. The DCM phase was washed three times with 0.01 M HCl aqueous solution, one time with water, three times with 5% $NaHCO_3$, three times with NaCl brine, dried over sodium sulfate, and filtered. The DCM phase was concentrated with a rotary evaporator at 30 °C under reduced pressure until the onset of turbidity. Approximately 250 mL *n*-hexane were added to the DCM concentrate and the solution was heated to boiling. The upper clear organic phase was decanted into a beaker and allowed to crystallize at RT overnight to yield the product C_7F_{15}-secondary amide (**e**) as a white solid with 59% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.87 (s, 1H), 7.32–7.27 (m, 2H), 7.04 (ddd, J = 8.1, 2.0, 0.6 Hz, 1H), 6.80 (ddd, J = 8.4, 2.4, 0.7 Hz, 1H), 3.82 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 160.35 (s), 136.19 (s), 130.09 (s), 112.47 (s), 112.35 (s), 106.20 (s), 55.41 (s). ^{19}F NMR (470 MHz, $CDCl_3$) δ −80.77 (tt, J = 10.1, 2.4 Hz, 3F), −119.21–119.34 (m, 2F), −121.31–121.55 (m, 2F), −121.80–122.04 (m, 2F), −122.15–122.32 (m, 2F), −122.56–122.81 (m, 2F), −126.02–126.15 (m, 2F).

*4.3.6. Secondary amine intermediate (**f**)*

1 g (1.926 mmol) C_7F_{15}-secondary amide (e) was transferred into a two-neck round-bottom flask equipped with a magnetic stirrer, reflux condenser, and a drying tube filled with calcium chloride. 30 mL THF were added followed by 2.4 mL (4.8 mmol) of a 2 M lithium aluminum hydride solution in THF. The reaction was refluxed for 12 h, left to cool down slowly to RT and quenched with 0.5 mL water. The THF phase was filtered to remove insoluble salts, diluted with 200 mL diethyl ether, and filtered. The organic phase was washed one time with water, twice with 5% $NaHCO_3$ aqueous solution and twice with sodium chloride brine. The organic phase was dried over sodium sulfate and filtered. The organic solvents were evaporated at 30 °C using a rotary evaporator under reduced pressure to obtain compound (**f**) as a yellowish viscous liquid with 68% yield. No further purification steps were needed. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.12 (t, J = 8.1 Hz, 1H), 6.39–6.36 (m, 1H), 6.32–6.29 (m, 1H), 6.25 (t, J = 2.3 Hz, 1H), 3.97–3.80 (m, 3H), 3.78 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 160.84 (s), 147.76 (s), 130.18 (s), 106.06 (s), 104.11 (s), 99.56 (s), 55.11 (s), 44.40 (t, J = 23.2 Hz). ^{19}F NMR (470 MHz, $CDCl_3$) δ −80.79 (tt, J = 10.1, 2.4 Hz, 3F), −117.90–118.37 (m, 2F), −121.63–121.90 (m, 2F), −121.91–122.21 (m, 2F), −122.56–122.87 (m, 2F), −123.11–123.53 (m, 2F), −125.90–126.34 (m, 2F).

*4.3.7. Secondary amino phenol intermediate (**g**)*

0.5 g (0.99 mmol) C_7F_{15}-CH_2-secondary amine (f) was transferred into a two-neck round-bottom flask equipped with a magnetic stirrer, reflux condenser and a drying tube filled with calcium chloride. 20 mL DCM were added followed by 1.5 mL (1.5 mmol) 1 M boron tribromide solution in DCM and refluxed for 12 h. The reaction was left to cool down slowly to RT and quenched with 0.25 mL water. The DCM phase was added to 100 mL 5% $NaHCO_3$ aqueous solution and the pH was checked with pH paper to be around 8. The product was extracted with portions of DCM, and the organic portions were combined and washed three times with NaCl brine, dried over sodium sulfate and filtered. DCM was removed on a rotary evaporator at 30 °C under reduced pressure to obtain compound (**g**) as a brownish waxy solid with 73% yield. No further purification steps were needed. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.06 (t, J = 8.1 Hz, 1H), 6.28 (d, J = 2.3 Hz, 1H), 6.27 (d, J = 2.3 Hz, 1H), 6.20 (t, J = 2.3 Hz, 1H), 4.78 (s, 1H), 3.96–3.76 (m, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 156.67 (s), 148.03 (s), 130.40 (s), 106.11 (s), 106.07 (s), 100.23 (s), 44.33 (t, J = 23.3 Hz). ^{19}F NMR (470 MHz, $CDCl_3$) δ −80.80 (tt, J = 10.1, 2.4 Hz, 3F), −117.84–118.42 (m, 2F), −121.57–121.90 (m, 2F), −121.91–122.21 (m, 2F), −122.57–122.93 (m, 2F), −123.17–123.47 (m, 2F), −125.96–126.29 (m, 2F).

*4.3.8. Rh-CH_2-C_7F_{15} (**h**)*

300 mg (0.6107 mmol) C_7F_{15}-CH_2-phenol (g), 72.39 mg (0.4887 mmol) phthalic anhydride, 407.3 mg (5.498 mmol) propionic acid, and 8.716 mg (0.04582 mmol) *p*-toluenesulfonic acid monohydrate were added to a Schlenk flask equipped with a magnetic stirrer. The reaction was carried out in a 160 °C thermostated oil bath for 24 h under a nitrogen atmosphere and continuous stirring. The reaction was left to cool down, and the Schlenk flask was opened to the atmosphere. The products were dissolved in chloroform/ethanol 10:1 (v/v) mixture, filtered and washed six times with 0.1 M HCl aqueous solution. The organic phase was dried over sodium sulfate and filtered. The product was purified by silica gel column chromatography with a chloroform/methanol 5:1 (v/v) mobile phase and later precipitated from a concentrated acetone solution into excess cold *n*-hexane. The product was filtrated using a Büchner funnel and washed with portions of cold *n*-hexane then put under vacuum at RT to obtain Rh-CH_2-C_7F_{15} (**h**) as an orange solid with 19% yield. ^{1}H NMR (500 MHz, CD_3OD) δ 8.02 (d, J = 7.4 Hz, 1H), 7.74 (td, J = 7.5, 1.2 Hz, 1H), 7.68 (td, J = 7.5, 1.0 Hz, 1H), 7.24 (d, J = 7.5 Hz, 1H), 6.72–6.68 (m,

4H), 6.61–6.57 (m, 2H), 4.08 (t, J = 15.8 Hz, 4H). ^{19}F NMR (470 MHz, CD_3OD) δ −82.40 (tt, J = 10.1, 2.4 Hz, 6F), −118.41–118.87 (m, 4F), −122.59–122.88 (m, 4F), −122.89–123.21 (m, 4F), −123.59–123.92 (m, 4F), −124.12–124.43 (m, 4F), −127.14–127.53 (m, 4F). ESI-TOF, m/z: calculated for $C_{36}H_{17}F_{30}N_2O_3$ 1095.0755 $[M]^+$; found 1094.9658.

*4.3.9. Rh-C_2H_4-C_8F_{17} synthesis (**i**,**k**)*

Secondary amino phenol intermediate (**i**): 861.2 mg (7.892 mmol) 3-aminophenol, 10 g (17.42 mmol) 1-Iodo-1*H*,1*H*,2*H*,2*H*-perfluorodecane, 20 mL NMP, and 2.559 g (19.80 mmol) DIPEA were transferred into a Schlenk flask equipped with a magnetic stirrer. The reaction mixture was bubbled with nitrogen for 30 min then transferred to a 100 °C thermostated oil bath and kept under stirring for 24 h. The Schlenk flask was removed from the oil bath and left to cool down to RT then opened to the atmosphere. The reaction was diluted with 300 mL MTBE and filtered to remove the precipitated DIPEA.HI salt. The organic phase was washed ten times with a 10% NaCl aqueous solution (w/w), dried over sodium sulfate, and filtered. The MTBE phase was evaporated on a rotary evaporator at 40 °C under reduced pressure, and the product was purified by silica gel column chromatography with an *n*-hexane/MTBE 1:1 (v/v) mobile phase yielding C_8F_{17}-C_2H_4-phenol (**i**) as an off-white solid with 61% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.05 (t, J = 8.0 Hz, 1H), 6.25–6.17 (m, 2H), 6.14–6.08 (m, 1H), 4.59 (s, 1H), 3.79 (s, 1H), 3.52 (t, J = 7.1 Hz, 2H), 2.53–2.26 (m, 2H). ^{19}F NMR (376 MHz, $CDCl_3$) δ −80.76 (t, J = 10.0 Hz, 3F), −113.49–114.13 (m, 2F), −121.24–122.21 (m, 6F), −122.44–122.92 (m, 2F), −123.17–123.70 (m, 2F), −125.75–126.36 (m, 2F).

*4.3.10. Rh-C_2H_4-C_8F_{17} (**k**)*

0.6 g (1.081 mmol) C_8F_{17}-C_2H_4-phenol (i), 128.1 mg (0.8648 mmol) phthalic anhydride, 0.721 g (9.73 mmol) propionic acid, and 15.42 mg (81.06 μmol) *p*-toluenesulfonic acid monohydrate were added to a Schlenk flask equipped with a magnetic stirrer. The reaction was carried out in a 160 °C thermostated oil bath for 24 h under nitrogen atmosphere and continuous stirring. The reaction was left to cool down, and the Schlenk flask was opened to the atmosphere. The products were dissolved in chloroform/THF/ethanol 10:1:1 (v/v/v) mixture, filtered and washed six times with 0.1 M HCl aqueous solution. The organic phase was dried over sodium sulfate and filtered. Organic solvents were evaporated on a rotary evaporator at 45 °C under reduced pressure and silica gel column chromatography with gradient elution starting from diethyl ether/THF 25:1 (v/v) and slowly increasing the THF portion reaching a final mobile phase composition of diethyl ether/THF 1:1 (v/v) was used to obtain the Rh-C_2H_4-C_8F_{17} (k) as a red solid. The product was dissolved in a small quantity of acetone and precipitated into excess cold *n*-hexane, filtered using a Büchner funnel and dried under vacuum at RT to obtain Rh-C_2H_4-C_8F_{17} (**k**) as a red solid with 23% yield. ^{1}H NMR (500 MHz, THF-d_8) δ 7.91 (d, J = 7.6 Hz, 1H), 7.65 (td, J = 7.5, 1.1 Hz, 1H), 7.59 (td, J = 7.5, 0.7 Hz, 1H), 7.16 (d, J = 7.6 Hz, 1H), 6.48 (d, J = 8.6 Hz, 2H), 6.42 (d, J = 2.3 Hz, 2H), 6.30 (dd, J = 8.7, 2.3 Hz, 2H), 5.55 (t, J = 6.1 Hz, 2H), 3.56–3.48 (m, 4H), 2.58–2.45 (m, 4H). ^{19}F NMR (376 MHz, THF-d_8) δ −81.81 (−81.81 (t, J = 10.1 Hz, 6F), −114.37–114.78 (m, 4F), −121.84–122.92 (m, 12F), −123.06–123.58 (m, 4F), −123.69–124.35 (m, 4F), −126.55–127.18 (m, 4F). ESI-TOF, m/z: calculated for $C_{40}H_{21}F_{34}N_2O_3$ 1223.1004 $[M]^+$; found 1222.9859.

*4.3.11. Rh-C_2H_4-$C_{10}F_{21}$ synthesis (**j**,**l**)*

Secondary amino phenol intermediate (**j**): 184 mg (1.686 mmol) 3-aminophenol, 2.5 g (3.709 mmol) 1-Iodo-1*H*,1*H*,2*H*,2*H*-perfluorododecane, 15 mL NMP, and 0.545 g (4.217 mmol) DIPEA were transferred into a Schlenk flask equipped with a magnetic stirrer. The reaction mixture was bubbled with nitrogen for 30 min then transferred to a 100 °C thermostated oil bath and kept under stirring for 24 h. The Schlenk flask was removed from the oil bath and left to cool down to RT then opened to the atmosphere. The reaction was diluted with 200 mL MTBE and filtered to remove the precipitated DIPEA.HI salt. The organic phase was washed six times with a 10% NaCl aqueous solution (w/w), dried over sodium sulfate and filtered. The MTBE phase was evaporated on a rotary evaporator at 40 °C under reduced pressure, and the product was crystallized twice from a clear boiling *n*-hexane solution at RT to yield $C_{10}F_{21}$-C_2H_4-secondary amino phenol (**j**) as an off-white solid with 70% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.06 (t, J = 8.0 Hz, 1H), 6.25 (ddd, J = 8.0, 2.3, 0.7 Hz, 1H), 6.21 (ddd, J = 8.1, 2.2, 0.7 Hz, 1H), 6.14 (t, J = 2.3 Hz, 1H), 3.80 (s, 1H), 3.57–3.46 (m, 2H), 2.49–2.32 (m, 2H). ^{19}F NMR (470 MHz, $CDCl_3$) δ −80.76 (t, J = 10.0 Hz, 3F), −113.65–113.98 (m, 2F), −121.23–122.11 (m, 10F), −122.49–122.86 (m, 2F), −123.20–123.73 (m, 2F), −125.82–126.34 (m, 2F).

*4.3.12. Rh-C_2H_4-$C_{10}F_{21}$ (**l**)*

458 mg (0.699 mmol) $C_{10}F_{21}$-C_2H_4-secondary amino phenol (j), 82.83 mg (0.5592 mmol) phthalic anhydride, 466 mg (6.290 mmol) propionic acid and 9.97 mg (52.4 μmol) *p*-toluenesulfonic acid monohydrate were added to a Schlenk flask equipped with a magnetic stirrer. The reaction was carried out in a 160 °C thermostated oil bath for 24 h under nitrogen atmosphere and continuous stirring. The reaction was left to cool down, and the Schlenk flask was opened to the atmosphere. The products were dissolved in HFE-7100/THF/ethanol 10:2:1 (v/v/v) mixture, filtered and washed six times with 0.1 M HCl aqueous solution. The organic phase was dried over sodium sulfate and filtered. Organic solvents were evaporated on a rotary evaporator at 45 °C under reduced pressure. Silica gel column chromatography with gradient elution starting from MTBE/THF 10:1 (v/v) and slowly decreasing the MTBE portion reaching pure THF was used to obtain Rh-C_2H_4-$C_{10}F_{21}$ (l) as a red solid. The product was dissolved in a small quantity of acetone and precipitated into excess cold *n*-hexane, filtered using a Büchner funnel and dried under vacuum at RT to obtain Rh-C_2H_4-$C_{10}F_{21}$ (**l**) as a red solid with 20% yield. ^{1}H NMR (500 MHz, THF-d_8) δ 7.90 (d, J = 7.6 Hz, 1H), 7.64 (td, J = 7.4, 1.1 Hz, 1H), 7.58 (td, J = 7.4, 0.7 Hz, 1H), 7.15 (d, J = 7.6 Hz, 1H), 6.48 (d, J = 8.6 Hz, 2H), 6.42 (d, J = 2.3 Hz, 2H), 6.29 (dd, J = 8.7, 2.3 Hz, 2H), 5.54 (t, J = 6.1 Hz, 2H), 3.56–3.49 (m, 4H), 2.64–2.35 (m, 4H). ^{19}F NMR (470 MHz, THF-d_8) δ −79.95 (t, J = 10.0 Hz, 6F), −112.54–112.89 (m, 4F), −120.09–120.90 (m, 20F), −121.23–121.62 (m, 4F), −121.94–122.35 (m, 4F), −124.78–125.13 (m, 4F). ESI-TOF, m/z: calculated for $C_{44}H_{21}F_{42}N_2O_3$ 1423.0876 $[M]^+$; found 1422.9367.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jfluchem.2016.07.025.

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