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# Fluorofluorophores: Fluorescent Fluorous Chemical Tools Spanning the Visible Spectrum

Ellen M. Sletten and Timothy M. Swager*

Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, United States

Supporting Information

**ABSTRACT:** "Fluoro" refers to both fluorescent and fluorinated compounds. Despite the shared prefix, there are very few fluorescent molecules that are soluble in perfluorinated solvents. This paucity is surprising, given that optical microscopy is a ubiquitous technique throughout the physical sciences and the orthogonality of fluorous materials is a commonly exploited strategy in synthetic chemistry, materials science, and chemical biology. We have addressed this shortage by synthesizing a panel of "fluorofluorophores," fluorescent molecules containing high weight percent fluorine with optical properties spanning the visible spectrum. We demonstrate the utility of these fluorofluorophores by preparing fluorescent perfluorocarbon nanoemulsions.

Highly fluorinated, or fluorous, compounds have gained considerable popularity due to their orthogonality to aqueous and organic species.[1] Fluorous molecules, often defined as those that contain ≥60 weight percent fluorine (wt% F) in C($sp^3$)−F bonds, phase-separate from polar and nonpolar compounds due to their disinclination to participate in van der Waals interactions.[2] This phenomenon, discovered by atomic scientists in the World War II era,[3] did not gain widespread recognition by chemists until 1994, when Rábai and Horváth coined the term "fluorous."[4] Today, the unique properties of perfluorinated compounds are exploited in both basic science and commercial applications. Fluorous materials are routinely employed for non-stick, self-cleaning, and anti-fouling coatings.[5] Fluorinated compounds have been reported as components in organic electronics.[6] Perfluorocarbon nanoemulsions have been used *in vivo* for oxygen delivery, MRI cell tracking, and ultrasound imaging and therapy.[7] Unique nano- and microstructures can be obtained from semifluorinated surfactants and block copolymers.[8] Fluorous phase synthesis facilitates simple purification schemes and greener chemical processes.[9] Additionally, chemical biology has benefited from the "fluorous effect,"[1] which has resulted in protein identification strategies, microarray assembly techniques, and extra-stable proteins.[10]

Despite the increasing interest in perfluorinated molecules, there are relatively few chemical tools available to study the fluorous phase. This shortage extends to fluorescent fluorous soluble small molecules, even though optical microscopy is prevalent throughout the physical sciences. Thousands of different fluorophores have been synthesized, but only a handful contain >50 wt% F.[11] Bräse and coworkers synthesized fluorous rhodamine dyes with up to 53 wt% F for solid-phase fluorous extractions.[12] Perylene bisimide dyes with significant fluorination have been reported for use in devices, but their fluorous properties have yet to be characterized.[6d,13] Porphyrins have been rendered soluble in perfluorocarbons for singlet oxygen generation and fluorous biphasic catalysis.[14] Prior to the naming of the "fluorous" phase, Matsui and coworkers synthesized fluorinated coumarin dyes for lasers (up to 52 wt% F) and found that fluorination resulted in increased photostability as well as altered absorption and photoluminescence spectra.[15] Recently, Sun et al. prepared highly fluorinated polyaromatics (60−70 wt% F), which also display improved photostability along with enhanced quantum yields of luminescence.[16] The latter fluorinated polyaromatics are the only organic fluorophores reported to date that have been fully characterized in perfluorocarbons.[17]

Here, we report a panel of highly fluorinated (54−61 wt% F) fluorescent compounds, termed "fluorofluorophores," in which "fluoro" refers to both fluorescence and fluorination. We employed aminophenol **1** as a common building block to access six fluorofluorophores traversing the visible spectrum in two or three steps from commercial materials. The array of absorption and emission properties of the fluorofluorophores will allow Förster resonance energy transfer (FRET)-based analyses and multicolor optical microscopy experiments to be performed in the fluorous phase. We demonstrate the utility and necessity of the fluorofluorophores by preparing highly luminescent perfluorocarbon nanoemulsions (Figure 1).

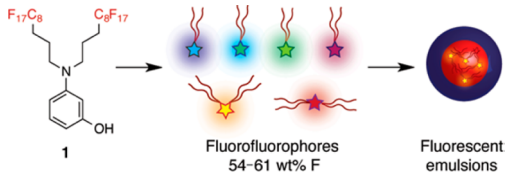


**Figure 1.** Aminophenol **1** as a building block for fluorous fluorophores and preparation of fluorescent perfluorocarbon emulsions.

We synthesized **1** through dialkylation of 3-aminophenol with (3-perfluorooctyl)propyl iodide and subjected **1** to aromatic substitution reactions with a variety of electrophiles (Scheme 1, SI). Coumarins **2** and **3** were prepared by Lewis acid-mediated Pechmann condensation of **1** and β-ketoesters. A three-component reaction between **1**, malononitrile, and benzaldehyde followed by oxidation with 2,3-dichloro-5,6-dicyanobenzo-

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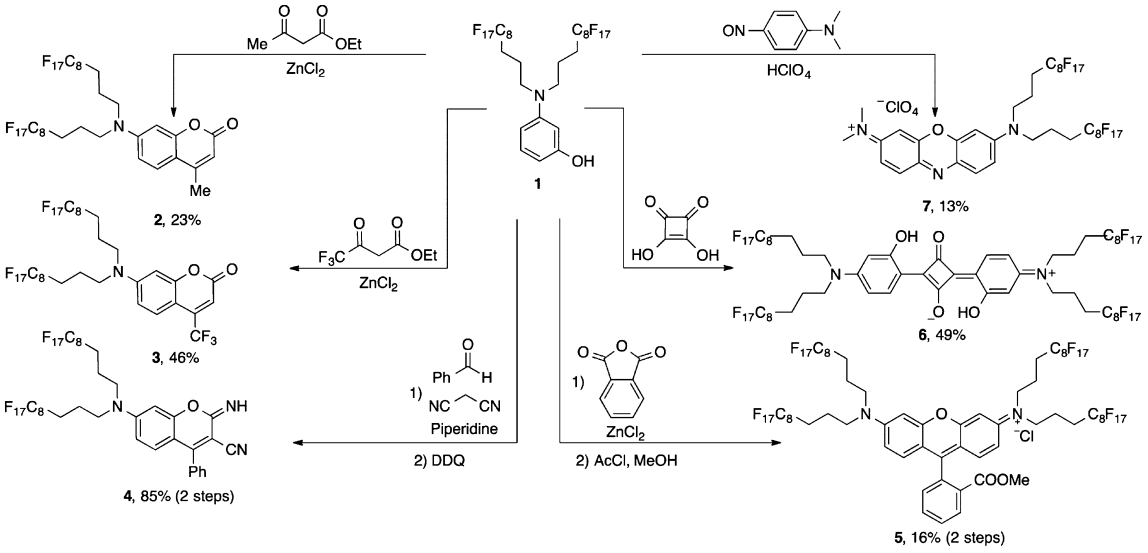
**Scheme 1. Synthesis of Fluorofluorophores 2−7 from Aminophenol 1**




quinone yielded chromene **4**. Fluorinated rhodamine **5** was synthesized through a Friedel−Crafts acylation and condensation with phthalic anhydride followed by esterification. Squaraine **6** and oxazine **7** chromophores were obtained by subjecting **1** to squaric acid or *N*,*N*-dimethyl-4-nitrosoaniline, respectively. Moderate to low yields of the fluorofluorophores are primarily due to loss of product during the final purification.[18] Despite the sub-optimal yields, the fluorofluorophores are prepared in two or three steps with no specialized techniques, facilitating their accessibility to many scientists.

The absorption (Abs) and photoluminescence (PL) of fluorofluorophores **2**−**7** extend across the visible spectrum (Figure 2A, Table 1). Coumarin **2** has the highest energy Abs and PL, displaying no color in solution and blue fluorescence upon excitation (Figure 2B). Coumarin **3** and chromene **4** have similar PL spectra, but **3** displays a more hypsochromically shifted Abs

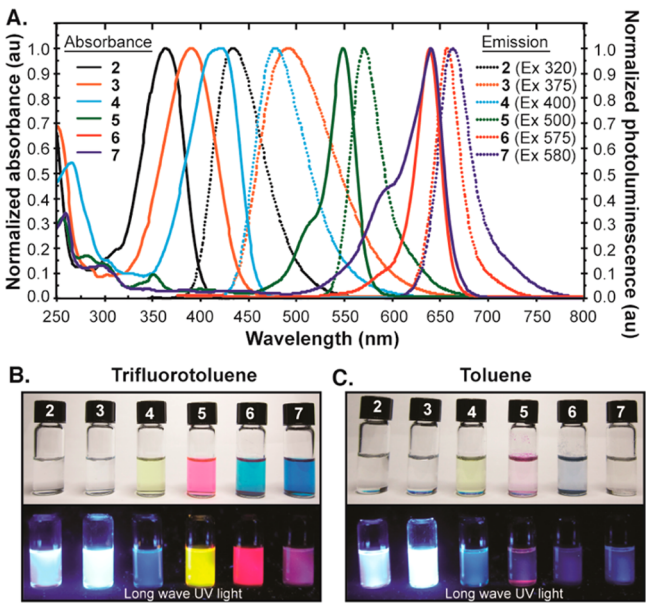



**Figure 2.** (A) Normalized absorption and emission spectra of **2**−**5** and **7** in ethanol and **6** in acetone. Below: Visible and UV light photographs of 0.1 M solutions of **2**−**7** in (B) trifluorotoluene and (C) toluene.

**Table 1. Photophysical Characterization of 2−7**

| | | absorption (EtOH) | | emission (EtOH) | |
|---|---|---|---|---|---|
| compd | wt% F[a] | $\lambda_{max}$ (nm) | $\varepsilon$ ($cm^{-1}$ $M^{-1}$) | $\lambda_{max}$ (nm) | $\Phi_F$ |
| **2** | 59 | 363 | 21 500 | 435 | 0.50 |
| **3** | 61 | 395 | 19 000 | 491 | 0.44 |
| **4** | 54 | 421 | 26 700 | 477 | 0.03 |
| **5** | 59 | 550 | 97 900 | 571 | 0.85 |
| **6**[b] | 60 | 639 | 168 000 | 657 | 0.89 |
| **7** | 56 | 641 | 71 900 | 664 | 0.16 |

[a]Wt% F calculations exclude counterion. [b]Squaraine **6** data in acetone.

and has the largest Stokes shift of the reported fluorofluorophores. Similar extinction coefficients ($\varepsilon$) are observed for **2**−**4**, but the quantum yield ($\Phi_F$) of **4** is very low. Compound **5** has characteristics consistent with rhodamine dyes,[12] including a large $\varepsilon$ and fluorescence $\Phi_F$ in the middle of the visible spectrum. Squaraine dye **6** is deep blue in solution, displays a brilliant red PL when excited, and has the highest $\varepsilon$ and $\Phi_F$ of the fluorofluorophores. Oxazine **7** spans a similar region of the electromagnetic spectrum as **6**, although its $\Phi_F$ is lower.

Next, we analyzed the solubility of fluorofluorophores **2**−**7** in solvents with a range of polarities and fluorophilicities. Standard solvents such as acetone, tetrahydrofuran, and dichloromethane solubilized most of the fluorofluorophores, with the exception being squaraine **6**, which has very limited solubility overall (Figure S1, Table S1). The best universal solvent for **2**−**7** was trifluorotoluene (Figure 2B), a solvent often employed in fluoroorganic synthesis.[19] The affinity of the fluorofluorophores for fluorine-containing solvents was immediately evident when comparing their solubility in trifluorotoluene and toluene, where in the latter solvent the PL of **4**−**7** is quenched by aggregation (Figure 2B/C). We assayed the solubility of **2**−**7** in six fluorous solvents: methoxyperfluorobutane, perfluorotripropylamine, perfluorooctyl iodide, perfluoromethylcyclohexane, perfluorodecalin, and perfluorohexanes (Figure S2, Table S2). The fluorofluorophores were moderately soluble in fluorous solvents containing a single heteroatom (O, N, or I); however, despite their significant wt% F, they were not readily dissolved in perfluoroalkanes, suggesting that the dispersion interactions of the $\pi$ system greatly impact the fluorophilicity.[2b]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Although the affinity of **2**–**7** for perfluoroalkanes is low, they can be rendered soluble in mixtures of fluorous solvents that contain >50% perfluoroalkane (*vida infra*).

We envision these fluorofluorophores will be valuable chemical tools for all scientists working with the fluorous phase. To showcase the utility of our new fluorophores, we prepared fluorescent perfluorocarbon (PFC) nanoemulsions. PFC emulsions were first developed in the 1960s as blood replacements, due to the high oxygen content in fluorous solvents.[20] Optical probes have only recently been added, as researchers strive to employ PFC nanoemulsions as scaffolds for multimodal imaging and therapy.[21] The absence of fluorous soluble fluorophores, particularly those with red emission, is evident when the details of optical imaging experiments with these emulsions are analyzed. Most strategies have involved introducing fluorescent molecules to the droplet surfaces either covalently[21a,b] or non-covalently,[7d,21c−d] which results in low loadings of fluorophore that are not protected within the droplets.[21e] The panel of fluorofluorophores reported herein should overcome these limitations and help facilitate the transition of PFC emulsions from oxygen delivery agents to multimodal theranostics.[22]

We prepared PFC nanoemulsions using a simplified version of Fluosol-DA, containing a 3:7 mixture of perfluorotripropylamine (PFTPA, **8**)/perfluorodecalin (PFD, **9**) (20 wt%) and Pluronic-F68 (**10**, 2.8 wt%) in phosphate-buffered saline (PBS).[23] Nanoemulsions were formed by ultrasonication and had a radius of ∼120 nm, as determined by dynamic light scattering (Figure S3). An array of fluorescent emulsions was prepared by predissolving **2**–**7** in PFD/PFTPA prior to ultrasonication (Figures 3A and S4). The rhodamine **5**-containing nanoemulsion was further analyzed by confocal microscopy (Figure 3B,C).[24]

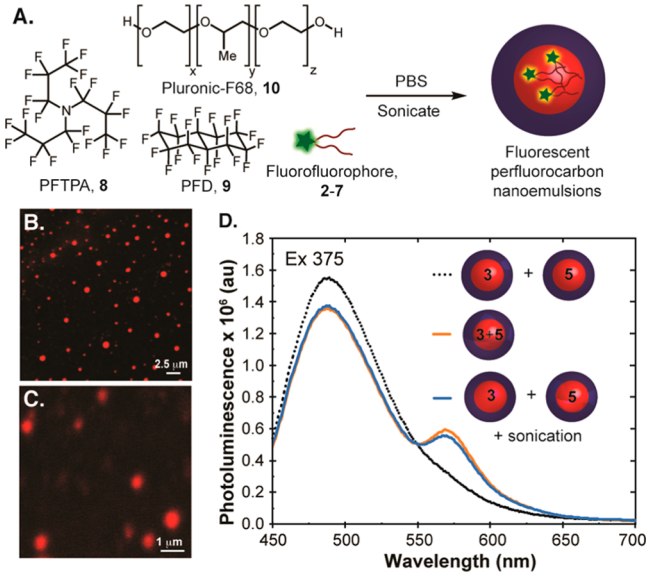


**Figure 3.** (A) Preparation of fluorescent perfluorocarbon nanoemulsions. (B,C) Confocal microscopy of nanoemulsion containing **5**. (D) Emulsions containing coumarin **3**, rhodamine **5**, or both **3** and **5** were prepared. A 1:1 mixture of nanoemulsions containing **3** or **5** was excited at 375 nm, and the PL was collected (black dashed line). A similar solution was sonicated for 15 min prior to taking the PL spectrum (blue solid line). Nanoemulsions containing both **3** and **5** were excited at 375 nm, and the PL was collected (orange solid line).

PFC nanoemulsions are kinetically stabilized, self-assembled colloids.[25] To gain insight into their solution dynamics, we prepared emulsions containing **3** and/or **5**. Fluorofluorophores **3** and **5** have appropriate spectral overlap such that they undergo FRET, and thus their presence in the same emulsion droplet should be evident through PL spectroscopy. A 1:1 mixture of nanoemulsions containing **3** or **5** excited at 375 nm did not exhibit energy transfer (black dashed line) until subjected to ultrasonication (blue solid line) (Figure 3D). In contrast, nanoemulsions containing both **3** and **5** in each droplet displayed emission from **5** when excited at 375 nm (orange solid line). Even after multiple days and in the presence of serum, no significant FRET was observed in a mixture of emulsions where droplets contained only **3** or **5** (Figure S5). Collectively, these experiments indicate that the fluorofluorophores are confined inside individual droplets and exchange between the droplets is minimal. Thus, despite the dynamic nature of nanoemulsions, adding perfluoroalkyl chains stabilizes the molecules inside the emulsions even when serum is present.

To further demonstrate that fluorination is essential for the long-term residence of fluorophores in the emulsions, we synthesized coumarin **11**, which contains fewer fluorine atoms than **3**, and coumarin **12**, which contains only a $CF_3$ group (Figure 4A, Schemes S2 and S3). Upon examining the

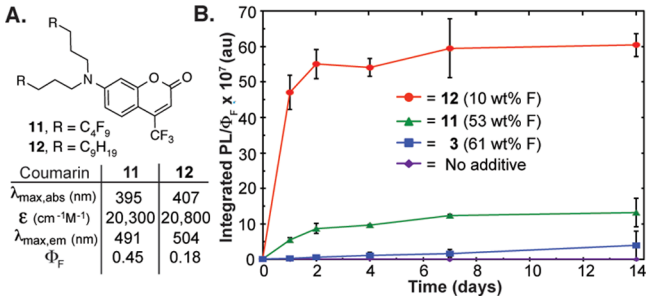


**Figure 4.** Perfluoroalkyl chains are essential for stability in the nanoemulsions. (A) Structure and photophysical data for coumarins **11** and **12**. (B) Nanoemulsions containing coumarins **3**, **11**, or **12** were prepared, diluted 10-fold in PBS, combined with 1-octanol, and continually agitated. The PL of the octanol (Ex 375) was measured over time. The integrated PL divided by the quantum yield of the coumarin (to account for photophysical differences in **3**, **11**, and **12**) was plotted vs time for each nanoemulsion. Error bars represent the standard deviation of three replicates.

photophysical properties of **11** and **12**, the effect of the perfluoroalkyl chains was evident. Coumarins **3** and **11** have very similar photophysics, but coumarin **12** displays bathochromically shifted spectra and a >2-fold reduction in quantum yield. These data are consistent with previous reports that indicate the electron-withdrawing effects of perfluoroalkyl groups can be felt through more than three methylene units[26] and the rigidity of fluorous moieties provides fewer opportunities for vibrational quenching.[16]

Solutions of coumarins **3**, **11**, or **12** in PFD/PFTPA (400 $\mu$M) were prepared and emulsified with Pluronic-F68. The resulting nanoemulsions, and a control without fluorophore, were agitated continually in the presence of octanol. The PL of the octanol, which contained coumarin expelled from the PFC nanoemulsion droplets, was measured periodically over 2 weeks. The data indicate (Figure 4B) that the fluorous chain has a large impact on the stability of the coumarin dyes inside the emulsions. The minimally fluorinated coumarin **12** readily displayed a large


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amount of PL in the octanol (red line), while coumarins **3** and **11** were both well retained in the nanoemulsions (blue and green lines, respectively). As anticipated, the more fluorinated **3** displayed the best stability inside the droplets, demonstrating the utility of highly fluorinated fluorescent molecules. These results also suggest that wt% F can be correlated to release times, which, if coupled with a cleavable linker, could provide an avenue for controlled drug delivery.

We have synthesized a panel of fluorinated fluorescent molecules, termed fluorofluorophores, which display photoluminescence spanning the visible spectrum. These compounds have the highest wt% F reported to date for their respective chromophore scaffolds and are most soluble in solvents containing fluorine atoms. Synthesis of the fluorofluorophores enabled preparation of highly fluorescent perfluorocarbon nanoemulsions, which are promising candidates for bright, non-toxic, *in vivo* imaging agents.[27] We demonstrated that fluorination was essential for the fluorophores to be retained in the nanoemulsion droplets. More broadly, the fluorofluorophores reported herein will allow scientists working with the fluorous phase to add multicolor fluorescence microscopy to their experimental toolbox.

## ■ ASSOCIATED CONTENT

### Supporting Information

Figures S1−S6, Tables S1 and S2, and Schemes S1−S3; experimental procedures; and characterization of **1**−**7**, **11**, and **12**. This material is available free of charge via the Internet at http://pubs.acs.org.

## ■ AUTHOR INFORMATION


### Corresponding Author

tswager@mit.edu


### Notes

The authors declare no competing financial interest.

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