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Convenient One-Step Synthesis of 5-Carboxy-Seminaphthofluoresceins

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**ABSTRACT**

The one-step synthesis and characterization of a series of regioisomerically pure 5-carboxy-seminaphthofluoresceins (5-carboxy-SNAFLs) is reported. The optical properties were determined in aqueous buffer at around biological pH, and highly pH sensitive, large Stokes-shift fluorophores with emission in the deep-red to near-infrared region were identified.

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**Keywords:**
5-carboxy-seminaphthofluoresceins
near-infrared-emitter
one-step synthesis
enhanced Stokes shifts

1. Introduction

The development and characterization of functional red and near-infrared-emitting (NIR) organic dyes with available anchoring sites is of great current interest especially in fields such as biomedical, biochemistry, and soft-matter science.\textsuperscript{1-4} Due to the low cost, reliability, and low invasiveness of fluorescent techniques in, for example, diagnostics and imaging, there is an ongoing search for new and better dyes. The importance placed on red-NIR dyes in this context is primarily due to the near-absence of biological autofluorescence in this spectral range, and the large penetration depth of NIR light in biological tissue.\textsuperscript{5-7}

5- and 6-carboxy-fluorescein are well-known functional dyes, with strongly pH sensitive optical properties and anchoring sites for target conjugation, and are suitable for a wide range of biological and materials science applications.\textsuperscript{8,9} The long history of their chemical modification of these dyes has broadened the scope of their use, however a fundamental limitation is the relatively high-energy emission, centered at approximately 520 nm. Developing robust approaches to substantially lowering the emission energy of this otherwise excellent class of dyes has thus been a topic of significant interest.\textsuperscript{5-13} One such approach has been to extend the fluorescein scaffold with additional benzoid rings to one or both sides, yielding seminaphthofluorescein (SNAFL) or naphthofluorescein respectively (Fig. 1). These represent fluorescein analogs of the well-known structurally related seminaphthorhodafluors (SNARF) rhodamine derivatives (see Fig. 1).

Furthermore, as a class of dyes they show tunable wavelengths, with emission ranging from the deep-red to the near-infrared region.\textsuperscript{5-13} This range of emission properties appear due to its dependence on both the geometry of the conjugated system and the position of the hydroxy group on the naphthalene moiety.\textsuperscript{5,7,13} The benzene unit can in general be positioned in three different ways termed type [a], [b] and [c] (Fig. 1). The importance of the benzanellation position has been studied both by semiempirical calculations and by synthesizing a large range of regioisomers of closely related seminaphthofluorene (SNAFR) derivatives.\textsuperscript{13} The structure of carboxy-SNAFLs diverges from SNAFRs by two additional carboxylic acids at the 3’ and 5’ or 6’
positions (Fig. 1). SNAFR derivatives thus lack the obvious linking sites provided by the 5’ or 6’ carboxylic acids. In general the synthesis of seminaphtho- and naphthofluorescein requires multiple steps and, in the cases where a carboxylic acid is present at the 5’ or 6’ positions, mixtures of the two regioisomers are formed. The fluorescence quantum yields are typically low in aqueous solutions (< 9%). In most cases an increase in quantum yield, Stokes shifts and maximum absorption/emission peak is observed in organic solvents.

In the present work we report the synthesis and optical properties of a number of 5-carboxy-SNAFL derivatives. The general synthetic approach uses the 5-carboxy-fluorescein scaffold and extends it by a benzene unit, enabling the use of well-known chemistry from 5-carboxy-fluorescein to synthesize the newly developed dyes. Depending on the direction of benzannulation, and in particular the position of the naphthol, the compounds exhibit deep-red to NIR emission with large Stokes shifts and $\kappa$ values around 8.

2. Results and Discussion

The synthetic approach described herein is based on our previous work, where we reported the condensation between 2- and 4-(2,4-dihydroxybenzoyl)isophthalic acid and a number of different resorcinol derivatives. The reaction between electron-rich dihydroxynaphthalene (naphthalene-1,3-diol) was found to proceed in a high yield and could easily be purified by precipitation to yield 5-carboxy-SNAFL-2.

To further investigate the scope of the reaction, the condensation between 4-(2,4-dihydroxybenzoyl)isophthalic acid (1) in methanesulfonic acid (or a 1:1 mixture with TFA), and the 10 regioisomers of dihydroxynaphthalene (Scheme 1 and Table 1) was examined. Through this procedure, 7 new benzannulated 5-carboxy-SNAFLs (2-8) were produced. To the best of our knowledge this constitutes the largest set of type [c] isomers of SNAFL (5-8) with the hydroxyl group located at 4 different positions. 2,7-Dihydroxynaphthalene gave a 1:1 mixture of regiosomeric 5-carboxy-SNAFLs (LCMS, not shown), however, the isomers were not separated. Only trace amounts of the desired product were observed using 1,2- and 1,7-dihydroxynaphthalene (LCMS).
As the pH is increased, the naphthol moiety is deprotonated, resulting in a red-shift of the absorption band, a loss of vibrational structure, and an increase in oscillator strength. This behavior is similar to that observed for carboxy-fluorescein derivatives. Clear isosbestic points are observed during the pH titration of the dyes, implying a simple two-species equilibrium over the pH range from approximately 5 to 10. We note that at very low pH the dyes exist largely as a colorless, likely lactone form, again analogous to carboxy-fluoresceins.

The extent of deprotonation at a given pH appears to depend on whether the dye can support a charge-resonance between the two oxygen substituents. In both 7 and 8 such charge-resonances are possible, effectively stabilizing the deprotonated form, which is reflected in a relatively low pK_a value. In contrast, 4 has a pK_a value of approximately 9, while it does not appear to be possible to deprotonate 3 in aqueous media as partial decomposition of the dye to a colorless product starts around pH 13. Transitions to charge-resonant states generally appear red-shifted, with the magnitude of the shift being related to the length of the resonance system involved. This is exemplified by, for example, the spectral behavior of cyanines. As 8 has a longer conjugated resonant system than 7, a stronger red-shift is predicted, in agreement with the spectra in Fig. 3.

The emission properties of the dyes investigated herein are surprisingly diverse. Neither 3, nor 4, nor 6 fluoresce appreciably in aqueous solution, regardless of pH, which is unexpected, considering the close structural analogy to the highly efficient carboxy-fluorescein fluorophores. The dye 5 and the charge-resonant species 7 and 8 are emissive in aqueous buffer, however, with 5 and 7 being moderately strong red emitters and 8 being a weak near-infrared emitter. The normalized absorption and emission spectra of these species are shown in Fig. 4. Compound 5 emits under acidic and neutral conditions, but is essentially non-emitting in alkaline media. Both charge-resonant species show substantially higher quantum yields and large Stokes shift in alkaline solution, however, the overall yield of 7 is in excess of 10 times larger than that of 8 regardless of environment. We note that 7 appear to be a rather strong excited-state proton donor. As a result, there is a noticeable contribution to the spectrum at approximately 630 nm from the oxynion form even at relatively low pH.

### Table 2. Optical properties of 5-carboxy-SNAFLs 3-8.

<table>
<thead>
<tr>
<th>SNAFL</th>
<th>λ_{max}^{abs} (nm) Naphthol&lt;sup&gt;a&lt;/sup&gt;</th>
<th>ε_{max} (M&lt;sup&gt;-1&lt;/sup&gt; cm&lt;sup&gt;1&lt;/sup&gt;)</th>
<th>λ_{max}^{abs} (nm) Oxyanion&lt;sup&gt;b&lt;/sup&gt;</th>
<th>ε_{max} (M&lt;sup&gt;-1&lt;/sup&gt; cm&lt;sup&gt;1&lt;/sup&gt;)</th>
<th>λ_{max}^{em} (nm) Naphthol&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Φ</th>
<th>λ_{max}^{em} (nm) Oxyanion&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Φ</th>
<th>pK_a</th>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>550</td>
<td>0.11</td>
<td>-</td>
<td>-</td>
<td>11</td>
</tr>
<tr>
<td>3</td>
<td>508</td>
<td>20300</td>
<td>-&lt;sup&gt;c&lt;/sup&gt;</td>
<td>-&lt;sup&gt;c&lt;/sup&gt;</td>
<td>-&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>-&lt;sup&gt;c&lt;/sup&gt;</td>
<td>&gt;&gt;10&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>4</td>
<td>517</td>
<td>20000</td>
<td>548</td>
<td>32500</td>
<td>-&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>-&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>-&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>538</td>
<td>43000</td>
<td>553</td>
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<td>18000</td>
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<td>19200</td>
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<td>&lt;0.01</td>
<td>748</td>
<td>&lt;0.01</td>
<td>7.9</td>
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</table>

All samples in aqueous phosphate/citric acid buffer containing 1% DMSO. <sup>a</sup>Data from Ref. 9. <sup>b</sup>Emission too weak to determine. <sup>c</sup>Not measurable in aqueous buffer. <sup>d</sup>Measured at pH 6. <sup>e</sup>Measured at pH 11.
Acknowledgments

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References and notes


16. General Procedure for the Synthesis of 5-Carboxy-SNAFL (5). Starting from 2,3-dihydroxynaphthalene (54 mg, 331 µmol) and 4-(2,4-dihydroxybenzoyl)isophthalic acid (I) (100 mg, 331 µmol), Purification by chromatography was necessary. Yield: 41 mg, 29%; Mp: 205-208 °C. 1H NMR (400 MHz, acetone-d6) δ 9.15 (broad s, 1D;O exchangeable OH), 9.10 (broad s, 1D;O exchangeable OH), 8.68 (dd, J = 9.5, 0.6 Hz, 1H), 8.34 (dd, J = 8.0, 1.5 Hz, 1H), 7.76 (d, J = 8.0 Hz, 1H), 7.52 (s, 1H), 7.42 (dd, J = 8.0, 0.6 Hz, 1H), 7.28 (dd, J = 8.0, 5.9, 2.0 Hz, 1H), 7.11 – 6.98 (m, 2H), 6.97 – 6.91 (m, 1H), 6.71-6.70 (m, 2H); 13C NMR (101 MHz, Acetone) δ 170.16, 167.15, 160.90, 160.65, 151.59, 146.64, 144.39, 138.34, 134.42, 133.53, 130.49, 126.63, 129.10, 128.07, 127.31, 126.48, 126.22, 125.84, 125.01, 115.19, 114.98, 112.53, 110.79, 103.97, 85.12; MS (ESI) m/z [M + H]+ caked for C26H14O7: 427.4, found 427.1. HR-MS (ESI): m/z [M + H]+ caked for C26H14O7: 427.0812, found 427.0825.