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Convenient One-Step Synthesis of 5-Carboxy-Seminaphthofluoresceins

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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 biomedicine, biochemistry, and soft-matter science. 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.

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. 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. 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. This range of emission properties appears due to its dependence on both the geometry of the conjugated system and the position of the hydroxy group on the naphthalene moiety. The benzene unit can in general be positioned in three different ways termed type [a], [b] and [c] (Fig. 1). The importance of the benzannulation position has been studied both by semiempirical calculations and by synthesizing a large range of regioisomers of closely related seminaphthofluorene (SNARF) derivatives. 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 pK_a values around 8.

2. Results and Discussion

\[ \text{Scheme 1. Synthesis of type [a], [b] and [c] 5-carboxy-SNAFLs 2-8.} \]

The synthetic approach described herein is based on our previous work, where we reported the condensation between 2- and 4-(2,4-dihydroxynbenzoyl)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.
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 pKₐ value. In contrast, 4 has a pKₐ 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 oxanion form even at relatively low pH.

3. Conclusion

The synthesis, structure, and optical properties of a series of 5'-carboxy-SNAFL dyes. The synthetic approach is quite flexible, and may be generalized to yield a broad range of “fluorescein-type” dyes with diverse optical properties. The SNAFL dyes are water soluble and can be conjugated to a variety of targets using standard amide-coupling protocols or by subsequent formation of the N-hydroxysuccinimide ester. Of the SNAFL species investigated, the charge-resonant compounds 7 and 8 prove most interesting due to reasonably efficient fluorescence. Dye 7 in particular displayed emission of comparable strength to many red/NIR emitting dyes, and may find use in biological sensing due to its relatively low pKₐ and strong optical response to pH. The emission of 8 is comparatively weak, and in practice only appreciable in a weakly alkaline environment. The NIR emission and large Stokes shift of the oxanion form however results in a dye that is highly resistant to autofluorescence background and scattering. It might therefore, despite its relatively low emission yield, prove useful in applications where background rejection rather than excitation/emission power is the major concern.

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

<table>
<thead>
<tr>
<th>SNAFL</th>
<th>λ_{abs}^{max} (nm)</th>
<th>ε_{max} (M⁻¹ cm⁻¹)</th>
<th>λ_{abs}^{max} (nm)</th>
<th>ε_{max} (M⁻¹ cm⁻¹)</th>
<th>λ_{em}^{max} (nm)</th>
<th>Φ</th>
<th>λ_{em}^{max} (nm)</th>
<th>Φ</th>
<th>pKₐ</th>
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<tbody>
<tr>
<td>2⁻</td>
<td>508</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<td>0.11</td>
<td>-</td>
<td>-</td>
<td>11</td>
</tr>
<tr>
<td>3</td>
<td>508</td>
<td>20300</td>
<td>-²</td>
<td>-²</td>
<td>-²</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>&gt;&gt;10⁰</td>
</tr>
<tr>
<td>4</td>
<td>517</td>
<td>20000</td>
<td>548</td>
<td>32500</td>
<td>-³</td>
<td>-</td>
<td>-³</td>
<td>-</td>
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<tr>
<td>5</td>
<td>534</td>
<td>17100</td>
<td>590</td>
<td>12200</td>
<td>595</td>
<td>0.02</td>
<td>-³</td>
<td>-</td>
<td>8.5</td>
</tr>
<tr>
<td>6</td>
<td>518</td>
<td>16200</td>
<td>545</td>
<td>11000</td>
<td>-³</td>
<td>-</td>
<td>-³</td>
<td>-</td>
<td>8.5</td>
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<tr>
<td>7</td>
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<td>24500</td>
<td>538</td>
<td>43000</td>
<td>553</td>
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<td>632</td>
<td>0.11</td>
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<tr>
<td>8</td>
<td>527</td>
<td>18000</td>
<td>590</td>
<td>19200</td>
<td>600</td>
<td>&lt;0.01</td>
<td>748</td>
<td>&lt;0.01</td>
<td>7.9</td>
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</tbody>
</table>

All samples in aqueous phosphate/citric acid buffer containing 1% DMSO. ²Data from Ref. 9. ³Emission too weak to determine. 'Not measurable in aqueous buffer. ⁴Measured at pH 6. ⁵Measured at pH 11.
References and notes


General Procedure for the Synthesis of 5-Carboxy-SNAFL: 4-(2,4-Dihydroxybenzoyl)isophthalic acid (I) (500 mg, 1.65 mmol) and the appropriate dihydroxynaphthalene (275 mg, 1.75 mmol) were dissolved in methanesulfonic acid (10 mL) or a mixture of TFA (5 mL) and methanesulfonic acid (5 mL), and stirred at room temperature overnight. The reaction was quenched with H2O (25 mL) and the resulting dark purple precipitate collected by centrifugation. After decantation, the precipitate was dissolved in NaOH(aq) (2 M, 15 mL) and precipitated with HCl(aq) (2 M, 20 mL). After decantation, the precipitate was washed with H2O (2 x 35 mL), dissolved in EtOH (10 mL) and precipitated with H2O. After decantation and washing with H2O (2 x 35 mL), the compound was dried in vacuo yielding a dark purple powder. If required, further purification by silica gel dry column vacuum chromatography (2% AcOH in CH2Cl2/MeOH with 5% increments) was performed by dissolution of the compound in MeOH and NaOH(aq) (12 M, 2 drops) and evaporation onto Celite in vacuo.

Analytical data for a typical 5-carboxy-SNAFL (3). 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, δ-D-O exchangeable OH), 9.10 (broad s, δ-D-O exchangeable OH), 8.68 (dd, J = 1.5, 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, 129.63, 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] calculated for C25H17O14 742.4, found 742.1. HR-MS (ESI): m/z [M + H] calculated for C25H17O14 742.0812, found 742.0825.