Microwave synthesis of [6-Diethylamino-9-(2-octylcarbamoyl-phenyl)-xanthen-3-ylidene]-diethyl-amine and its isomer

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Abstract:

The title compound, [6-Diethylamino-9-(2-octylcarbamoyl-phenyl)-xanthen-3-ylidene]-diethyl-amine was synthesized in high yield by an amidation with N-octylamine in a CEM Discover microwave at 60°C for 2 hrs. Its structure was characterized by Ultraviolet-Visible and fluorescence spectral analysis.

Introduction:

Rhodamine B is a well characterized water soluble dye which has been used as an immunofluorescent marker and spectroscopic sensor [1, 2, 3]. The ionic structure of Rhodamine B allows for good water solubility however limits its absorbance into lipids and fats [4]. This work sought to increase fat solubility by amidation of the carboxylic acid with N-octylamine. There are labile portions of the synthesized dye that can bind macromolecules, including specific hydrophobic proteins. Thus, this dye has the potential to track the utilization of specific biomolecules by microorganisms. The octyl amide derivative also allows for improved adhesion to cellular membranes and surfaces [5, 6]. This study provides preliminary information in determining fluorescent probes that can be utilized in tracing metabolic processes of microorganisms [7, 8, 9]. Further testing will use this product as a determinate for lipid uptake in white nose syndrome, which is a putative fungal pathogen responsible for the current mass mortality of several species of North American cave bats.
Materials and Methods:

All reagents were purchased from Fisher Scientific and used without further purification. UV-Visible Spectroscopic data used a Genesys 6 system (Thermo Scientific) with the Visionlite software package. Partition coefficients were determined at the absorbance maximum. The fluorescence spectrum was taken with a SLM spectrofluorometer. The fluorescent product was diluted in 95% methanol, placed into a quartz cuvette, excited at 25 nm intervals (200-700 nm), and emission scanning range was 200-800 nm. Excitation peaks were not removed and are visible in the given spectra.

Results and Discussion:

\[
\begin{align*}
\text{(1)} & \quad \text{Octyl Amidation of Rhodamine B} \\
\text{(2)} & \quad \text{Microwave} \\
\text{(3)} & \quad \text{H}_2\text{O}
\end{align*}
\]

Figure 1. Octyl Amidation of Rhodamine B

Synthesis of [6-Diethylamino-9-(2-octylcarbamoyl-phenyl)-xanthen-3-ylidene]-diethyl-amine:

Rhodamine B was weighed out (0.25 g) and a molar excess of octylamine (0.20 g) was added. The mixture was reacted at 60°C for 2 hrs in a CEM Discover microwave (Matthews, NC) (Figure 1). The crude product was allowed to cool to room temperature. The product was isolated using an ethyl acetate: water (liquid/liquid) extraction. The organic solvent was evaporated under vacuum affording a light brown solid (3). Initial purity was identified by thin layer chromatography. The liquid/liquid isolation of the product incorporated the understanding that Rhodamine B is not soluble in ethyl acetate to isolate the lipid soluble product. This ensures that only the lipid soluble Rhodamine B amide product will be isolated.

The UV-Visible spectra of the product (3) showed some interesting characteristics when compared to the parent compound of Rhodamine B. A photochromic shift of the wavelength maximum was seen in molecule 3 with a 300 nm peak (N-octanol) when compared to
Rhodamine B 555 nm peak (water). The partition coefficient of 0.0767 for the octyl derivative versus 1.724 for Rhodamine B clearly shows a shift towards hydrophobicity. In line with the product formation of (3) very little absorbance was seen in water.

![UV-Visible Spectra of the Octyl Derivative (3)](image)

Figure 2. UV-Visible Spectra of the Octyl Derivative (3) in water and N-octanol

Conjugation of Rhodamine B uses the salt formation to assist in increased resonance across the benzene rings (Figure 3a). However, the new derivative (3) shows strong absorbance in the benzene rings with little absorbance deeper in the visible range indicating a conversion to a new isoform (Figure 3b). This is also indicated with the absorbance spectra of the new derivative (3) when dissolved in a non-polar solvent (not shown). Although we see a strong peak in the ultra-violet region of the spectra for (3) we also see some possible conjugation with a peak between 450 and 550 nm in N-octanol. This may indicate that there are two isoforms that are present in the final product. The major product (Figure 3b) may be indicated with the large absorbance peak ~300 nm, while the minor conjugated product has an absorbance peak between 450-550 nm. The exact configuration of the second isoform (Figure 3a) has not been determined.
Figure 3. Possible isoforms of the Rhodamine B derivative

The fluorescence spectrum showed two emission peaks indicating that two compounds were present (Table 1). This seems to be in line with two isoforms being produced. The first peak excitation range is between 325-400 nm while the second larger peak is excited between 425 to 550 nm. Based on the absorbance data obtained from the UV/Vis spectrum the first peak in the fluorescence spectrum is the non-conjugated isomer (Figure 3b) and the second peak is the conjugated isomer (Figure 4). Conjugation is indicated through the strong emission spectra found in Figure 4 between 500 and 620 nm.

Figure 4. Fluorescence Contour of Octyl Amide Derivative (3)
Conclusion:

We were able to synthesize a fat soluble derivative of Rhodamine B as a mechanistic biomarker. Improved hydrophobicity was seen with a decrease value for the partition coefficients of the octyl amide derivative (3). The UV-Visible and fluorescence data indicated that there were 2 isoforms present with one being strongly fluorescent.

References:

Data

Partition Coefficient Equation = Absorbance in water / Absorbance in N-octanol

Partition Coefficient (Rhodamine B 555 nm) = $\frac{2.594 \, \text{A}}{1.504 \, \text{A}} = 1.724$

Figure 5. UV-Visible Spectra of Rhodamine B in water and N-octanol

Partition Coefficient (Rhodamine B N-Octyl amide 485 nm) = $\frac{0.033 \, \text{A}}{0.430 \, \text{A}} = 0.0767$

Figure 6. UV-Visible Spectra of Rhodamine B octyl amide (3) in water and N-octanol
Table 1. Excitation wavelength and emission peaks of product.

<table>
<thead>
<tr>
<th>Excitation Wavelength (nm)</th>
<th>Excitation Peak Intensity</th>
<th>Emission Intensity Peak 1 (425 nm)</th>
<th>Emission Intensity Peak 2 (550 nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>350</td>
<td>0.013</td>
<td>0.026</td>
<td>0.017</td>
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<tr>
<td>375</td>
<td>0.081</td>
<td>0.185</td>
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<td>400</td>
<td>1.080</td>
<td>0.300</td>
<td>0.346</td>
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<tr>
<td>425</td>
<td>1.580</td>
<td>0.084</td>
<td>0.849</td>
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Figure 7. Fluorescent spectrum of [6-Diethylamino-9-(2-octylcarbamoyl-phenyl)-xanthen-3-ylidene]-diethyl-ammonium chloride excited at 350 nm.
Figure 8. Fluorescent spectrum of [6-Diethylamino-9-(2-octylcarbamoyl-phenyl)-xanthen-3-ylidene]-diethyl-ammonium chloride excited at 350, 375, 400, and 425 nm.