Synthesis of squaraine dyes with potential application in protein detection by fluorescence spectroscopy

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INTRODUCTION
Squaraines are interesting organic molecules due to the strong absorption in the visible region ($\varepsilon \sim 10^6$ M$^{-1}$·cm$^{-1}$), intense fluorescence emission and good photoconductivity presented in these dyes. These features make them useful in a variety of applications in the area of imaging, nonlinear optics, photodynamic therapy and photovoltaic cells. Furthermore, squaraine dyes are suitable for fluorescence detection of proteins at long-wavelength excitation and labels in biological assays due their unique photochemical and photophysical properties namely light absorption in the visible red and near-infrared regions.

This work describes the synthesis of squaraine dyes prepared by the condensation of heteroaromatic compounds with a dialkyl ester derived from squaric acid in a one-step reaction.

RESULTS AND DISCUSSION
Symmetrical squaraine 6 was synthesized as shown in Scheme 1. The reaction of squaric acid (1) with excess of $n$-butanol afforded the dibutyl ester (2), which was subsequently treated with 1.0 equivalent of malononitrile to give the intermediate (3) in 85% yield. Then, 2,3,3-trimethylindolenine (4) was converted in the quaternary ammonium salt (5) by coupling of 4 with excess of iodoctane in 75% yield. The subsequent base-catalyzed condensation of 2.0 equivalents of $n$-iodoindolium salt 5 and 1.0 equivalent of 3 under reflux in a $n$-butanol:pyridine mixture, afforded the symmetrical squaraine dye 6 in 39% yield.

Squaraine dye 10 was obtained by a similar synthetic pathway using 2-aminophenol (7) as starting material (Scheme 2).

Condensation reaction of iodide 5 with dicyanomethylene 3 in the presence of triethylamine, afforded the intermediate mono-squaraine 11 (Scheme 3). Subsequent reflux of crude product containing mainly 11 with ammonium salt 7 in a $n$-butanol:benzene mixture led to the unsymmetrical squaraine dye 12 in 25% yield from quaternized indolenine 5.

CONCLUSION
The dyes 6, 10 and 12 were synthesized by the base-catalysed condensation of heterocyclic ammonium salts with dicyanomethylene 3. These compounds exhibit interesting photophysical properties for application in protein detection by fluorescence spectroscopy.

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REFERENCES