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〈Communication〉

Synthesis of Novel Fluorescent Dye Based on Fluorescein

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Abstract: The functional materials have been developed as a promising research topic toward the end uses for organic materials and applications. In this study, fluorescein based dye was synthesized by three step reaction. We have designed and synthesized the colorimetric dye through the reactions of fluorescein and methoxy group and ethylene diamine and squaric acid. The structure of the non-fluorescent spirolactam was elucidated by ^{1}H-NMR, LC-Mass and FT-IR analyzes. Further studies are in progress to understand the effects of various substituent during the recognition process and to develop fluorescein based sensors for cations or anions.

Keywords: *fluorescein, squaric acid, spiolactam, fluorescein methyl ester, xanthene dye*

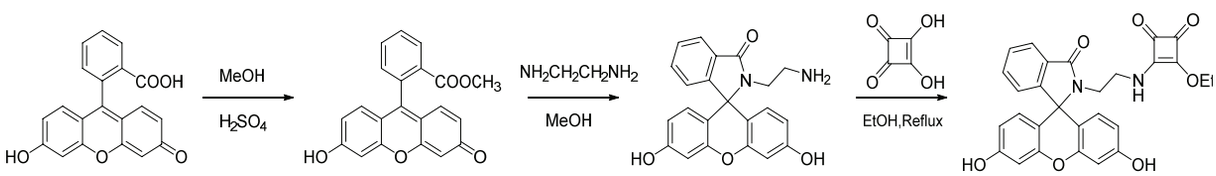
1. Experimental

Since researchers synthesis in the long time, various fluorescent dyes have been utilized in many chemical and biotechnological applications[1,2]. Among the fluorescent dyes developed, xanthenes, including rhodamine and fluoresce in derivatives, are highly favorable because of their excellent photophysical properties, such as high extinction coefficients, quantum yields, photostability and relatively long emission wavelengths[3,4].

Fluorescein of typical xanthene dye was first synthesized by von Bayer in 1871. Some of the advantageous features of the fluorescein fluorophores are good water solubility, absorption and emission and maximum brightness at physiological pH[5]. These fluorescein dyes can be applied into the useful tool of chemosensor probes for the determination of solvent polarity and harmful ions.

In particular, these types of dye might be utilized as the potential direct tools for the fluorogenic sensor. In this context, the initial study of this novel dye compounds, namely synthesis and related analysis needs to be rapidly communicated and discussed with reviewers and readers. Shortly afterward, the application and the useful array making results will be introduced to this journal. Due to the many researches, fluorescein dye has been viewed to exist in two forms. This is related to spirolactam ring system in the structure of fluorescein derivative[6,7].

Generally, fluorescein derivatives shows a color change and fluorescence in basic solution by activation of a carbonylgroup(C=O) in a spirolactam



Scheme 1. The synthetic route for fluorescein squaraine dye.

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moiety[8,9](Scheme 1).

Fluoresecin have been frequently used as ratio metric fluoroionophores due to their characteristic spirolactam ring, which can "open-close" forms with an "on-off" fluorescence response[10]. Similarly, fluorescein squaraine dye is non-fluorescent when they exist in the spirolactam ring form and than spirolactiam ring opened form can induce color changes and fluorescence enhancements[11].

All the reagents and solvents, used for synthesis of fluorescein squaraine dye, were purchased form Aldrich and used without further purification. ^{1}H-NMR spectra were recorded with JNM-AL400 spectrometer operated at 400MHz NMR. Mass spectra were recorded on a JEOL MStation[JMS-700].

2. Discussion

Reaction 1 : Fluorescein methyl ester

Fluorescein methyl ester is prepared according to the literature method[12-14]. Fluorescein methyl ester was synthesized by an improvement concentrated sulfuric acid(H_2SO_4, 6ml) was added dropwise to a suspension of fluorescein(3.34g, 10mmol) in methanol(40ml).

The resulting suspension was refluxed for 14h with an addition funnel packed with oven dried molecular sieves between the pot and condenser to continuously dry the cooled methanol. The mixture was then cooled and ice was added followed by sodium bicarbonate($NaHCO_3$). The suspension was filtered and washed with water three times.

The resulting red precipitate was suspended in aqueous sodium bicarbonate(100ml) filtered and washed with water. The washing process was repeated four additional times the solid was resupended in 1% acetic acid and finally washed with water. Drying at room temperature for 5h yield a red solid(60%). ^{1}H-NMR(DMSO-d_6): δ 8.22-8.20(d, 1H), 7.89-7.85(t, 1H), 7.80-7.77(t, 1H), 7.50-7.48(d, 1H), 6.86-6.83 (d, 2H), 6.65-6.60 (m, 4H), 3.58(s, 3H)(Figure 1).

Reaction 2 : Fluorescein ethylenediamine

Reaction of fluorescein methyl ester with ehtylenedaimne in MeOH at ambient temperature at 100℃ for 14h resulted

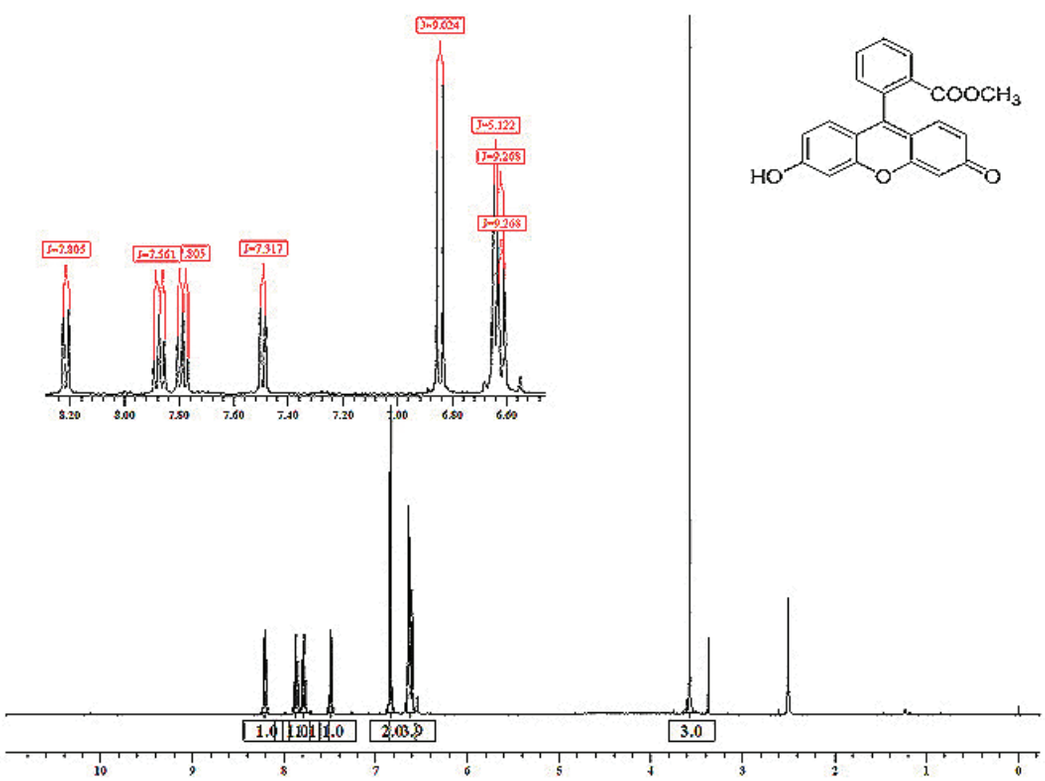

Figure 1. ^{1}H-NMR spectrum of fluorescein methyl ester in DMSO-d_6.

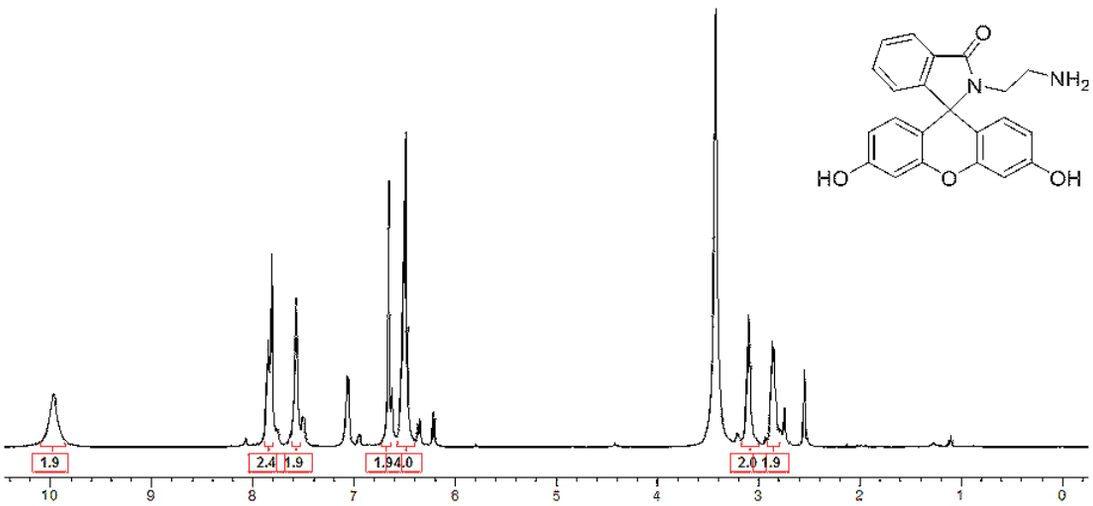

Figure 2. ^{1}H-NMR spectrum of fluorescein ethylenediamine in DMSO-d_6.

in decolorizatin of the intensely red-orange solution.

Unexpectedly, non-fluorescent white or off-white solids were isolated by column chromatography ($CHCl_3$/MeOH)[15-17]. ^{1}H-NMR(DMSO-d_6): δ 9.97(s, 1H), 7.87-7.81(dd, 2H), 7.58-7.57(t, 2H), 6.66-6.65(d, 2H), 6.53-6.47(m, 4H), 3.12-3.08(t, 2H), 2.89-2.85(t, 2H) (Figure 2).

Reaction 3 : Fluorescein squaraine dye

Fluorescein ethylenediamine and squaric acid were dissolved in absolute EtOH. The mixture solution was then refluxed for 14hrs.

The resulting precipitate was filtered and washed by three times with ethanol. The precipitate product was purified by silica gel column chromatography using $CHCl_3$/MeOH as eluent, which resulted in fluorescein squaraine product as a white or yellowish-white solid[18-21]. ^{1}H-NMR(DMSO-d_6): δ 10.42(s, 1H), 9.89(s, 2H), 7.79-7.77(t, 1H), 7.50-7.48 (t, 2H), 6.97-6.95 (t, 1H), 6.57-6.52(t, 2H) 6.44-6.38(t, 4H), 4.61-4.56 (m, 2H), 3.46-3.42(t, 2H), 3.26-3.23(t, 2H), 1.33-1.30 (t, 3H); LC-Mass(m/z) : 499.1 $[M+H]^{+}$; IR(film,cm^{-1})

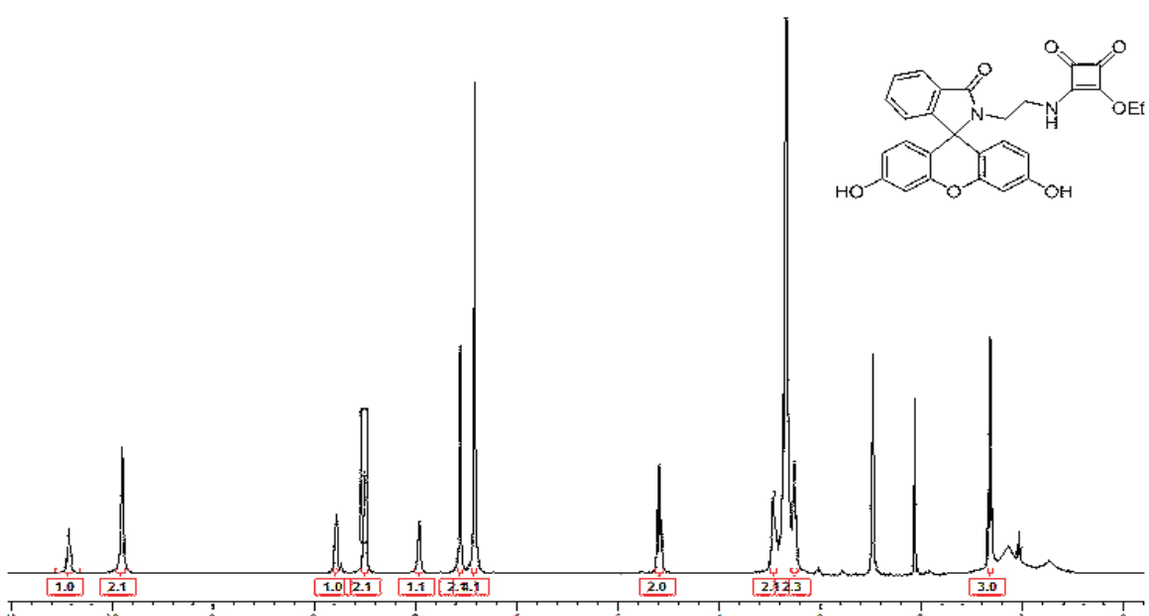

Figure 3. ^{1}H-NMR spectrum of fluorescein squaraine dye in DMSO-d_6.

: 3448.65(-OH), 2929.86(-NH), 1784.15 (C=O), 1552.69 (C=C), 1367.53(C-N)(Figure 3).

3. Conclusions

In this study, fluorescein dye based molecule was synthesized through the reaction of through the reaction of fluorescein and methoxy group and ethylenediamine and squaric acid. The structure of fluorescein-squaraine dye with spirolactam was identified by a combination of mass spectrometry, NMR spectroscopy and FT-IR analyzes(Figures 4, 5).

Further studies for the submission afterward are in progress to check the sensing properties for selective recognition among various metal cations and to develop fluorescein-based chemosensors for important harmful ions. Moreover, this novel fluorescein derivative should find great utility in various chemical and biotechnological applications. Therefore, the application studies using fluorescein derivative will be published after measuring and monitoring the optical properties of fluorescein derivative.

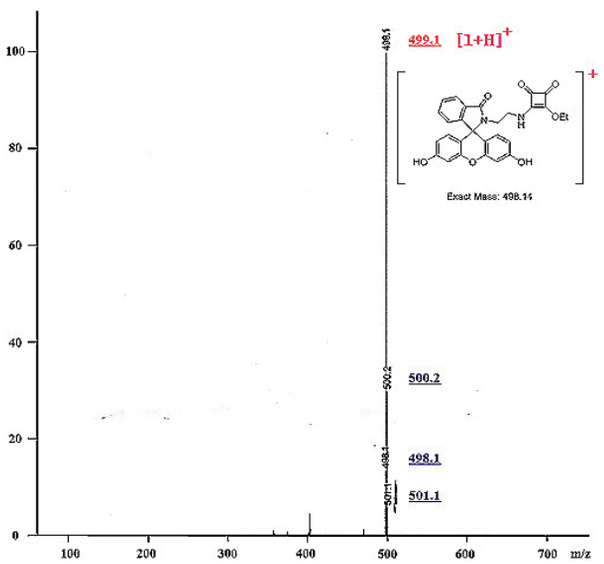

Figure 4. LC-Mass spectrum of fluorescein squaraine dye.

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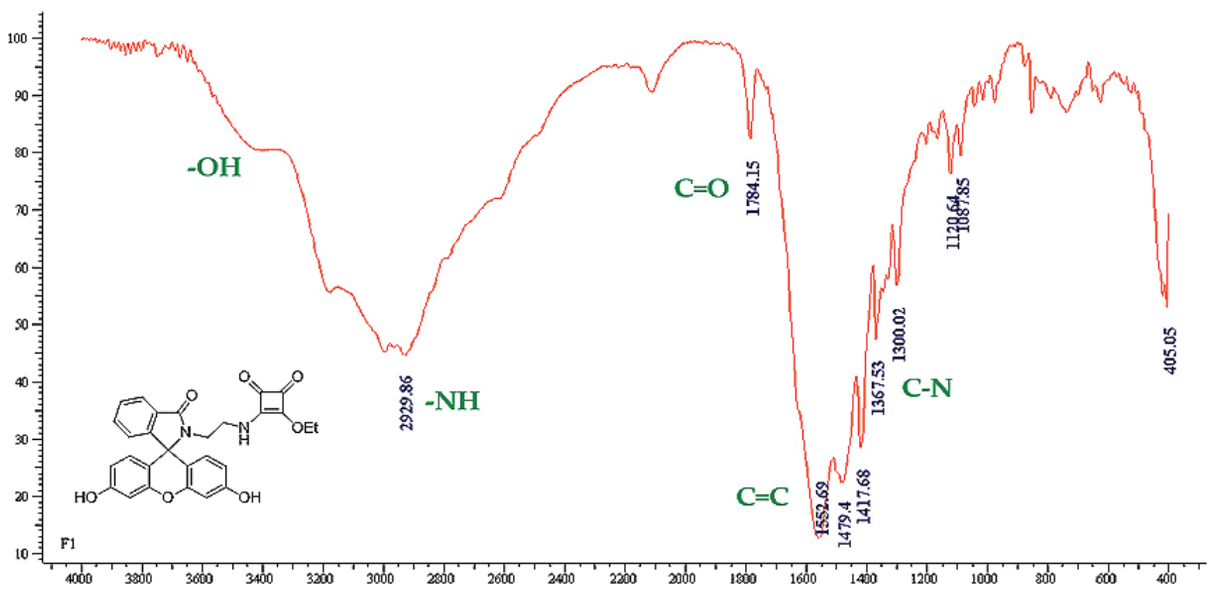

Figure 5. FT-IR spectrum of fluorescein squaraine dye.

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