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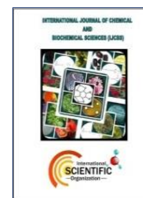

A review of synthesis of fluorescein based advanced materials

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Abstract

Xanthene dyes, including fluorescein, are a well-known class of fluorescent dyes having widespread applications in natural sciences. Fluorescein derivatives are important fluorescent probes widely used for detection and optical imaging of various objects. Fluorescein derivatives are usually prepared by introducing aldehydic groups or esterification reaction on fluorescein xanthene ring and benzene moiety. The present-day researches are focusing on connecting amino groups with fluorescein mono-aldehyde as these derivatives show high activity and can be complexed with the analytes to increase or decrease the fluorescence intensity. Therefore, the present review is designed to summarize the different synthesis methods, optical properties, possible mechanisms and applications of fluorescein probes. This article provides a reference for the screening of fluorescein probes with high sensitivity and effective biological detection. It further enhances its applications in sensing and detection of analytes and especially bio-imaging.

Key words: Fluorescein, xanthene, fluorescence intensity, bio-imaging, mono-aldehyde

Full length article *Corresponding Author, e-mail: farwa668@gmail.com

1. Introduction

Fluorescein ($C_{20}H_{12}O_5$) is a synthetic organic complex or a dye. Fluorescein is a dark red and orange powder having high solubility in alcohol and water. For many applications, the fluorescein is broadly used as a fluorescent tracer. Fluorescein is used in microscopy as a gain medium in a dye laser and also used in forensics and serology labs to detect bloodstains and dyes contents. The fluorescein shows maximum adsorption at 494 nm and maximum emission at 512 nm in water [1]. Xanthene dyes are present in fluorescent dyes which contain a three-membered ring in their molecular structure. The xanthenes show the central part of the natural compounds and are structurally similar to xanthone molecule [2].

2. History

The German chemist Johann Friedrich Wilhelm Adolf Baeyer (1835-1917), in 1883; corrected the chemical structure of indigo dye and selected the cis-form rather more accurate than trans-structure [3]. His other important contribution was to discover the phenolphthalein by the reaction between phthalic anhydride and phenol and this product is used as a pH indicator. Baeyer discovered the fluorescein by the reaction of resorcinol and phthalic anhydride in place of phenol. Ceresole was the first discovered rhodamine in 1887 by using N,N-diethyl-m-amino-phenol [4]. A new class of dyes fluorescein was discovered by Adolf Baeyer in June 1871 [5-6]. In 1885 the Adolf Baeyer was ennobled, becoming Adolf von Baeyer. In 1905 he was awarded the Nobel Prize in Chemistry for an appreciation of his services in the development of organic chemistry and the chemical industry, due to his work on hydro-aromatic compounds and organic dyes [7].

3. Synthesis of Fluorescein

The fluorescein can be synthesized by two different processes; first is the Friedel Craft's reaction (Fig.1) in which the reaction takes place between phthalic anhydride and resorcinol using a catalyst $ZnCl_2$ or methane-sulfonic acid. The catalyst; $ZnCl_2$ can catalyze the synthesis of fluorescein and during this reaction the concentrated HCl is used at high temperatures generally at 170-180°C. This synthesis was not appropriate for small-scale reactions due to the loss of significant amount of initial material during sublimation. The methane-sulfonic acid can be used as both; an appropriate solvent and acid catalyst in the reaction and give a better yield under minor conditions [8].

4. Applications of Fluorescein

The travel times of groundwater can be tracked by fluorescein. In current practice, the disodium salt of the fluorescein is uranine that can be discharged into a falling stream. From this stream, it flows through a drain system and reemerges at a spring. Charcoal detectors are located in the same positions and are checked every 3 months. The adsorbed fluorescein on the surface of charcoal can be removed by using a solution of sodium hydroxide in aqueous isopropanol and identified by using a scanning UV absorption at 254 nm [9].

In some applications, the uses of fluorescein as a tracker are not applicable. When the water is exposed to sunlight, it decolorizes the dye as it contains clay as natural adsorbing minerals. In these circumstances, alternative tracers can be used such as anions like bromide and radioactive tracers, some solid particles and bacteria or viruses, etc. St. Patrick's Day is celebrated on 17th of March because addition of fluorescein colored the Chicago River. Due to the ecotoxicological concerns, the vegetable dyes have an undisclosed identity and can be used for the replacement of fluorescein. The addition of fluorescein to foodstuff is another important application, which leads to fluorescent worms and these are particularly attractive to fish [10].

Fluorescein has found and still finding number of applications as a tracer and tracker for human beings especially in ophthalmology. Fluorescein staining is used to detect corneal and conjunctival epithelial damage [11]. Where there is damage at cell-cell junctions, fluorescein can rapidly diffuse into the cell and is detected readily due to its natural fluorescence. Fluorescein is widely used as an angiographic tracer, in intravenous injections and highlights the blood vessels at the back of eye. This can be applied for both diagnosis and guiding surgical intervention [12-13].

Different techniques of fluorescein such as fluorescent angiography (FAG), fluorescein angiography (FA) and fundus fluorescein angiography (FFA) can be used for observing the circulation of choroid which is a different fundus part and retina by using a specialized camera and fluorescent dye. Sodium fluorescein can be used to study the different parts of the eyes, it is added to the circulation system in which retina is illuminated at the wavelength of 490 nm by blue light and angiogram of the eye is obtained by fluorescent green light. In the dyes tracing method, the fluorescein can be controlled by intravenous fluorescein angiography (IVFA) and the oral in oral fluorescein angiography (OFA) [14].

The structure of probes which is based on metal ions can be modified into five different positions. The intensity and color of fluorescein can be changed by combining the probe with metal ions and the purpose of detection can be achieved by this process [15-16]. The fluorescein probes can be used to detect different metal ions like gold, iron, cadmium, magnesium, lead, zinc, silver, mercury and copper ions in living cells and different aqueous solutions. Detection of such ions has a high significance for the protection of the environment and human health [17].

In the biological process, biological macromolecules play a vital role and deficiency, or excess of some macromolecules will cause serious problems in physiology. The hydrazine can be detected by colorimeter because of fluorescein hydroxyl groups connected to yellow. In living cells, the hydrazine can be visualized successfully by F44 [18].

5. Synthesis of Fluorescein Materials

5.1 Synthesis of Fluorescein Modified Nano Carbon

Carbon nanotubes (CNT), glassy carbon (GC) and graphite are carbon-based materials having some important properties such as high chemical inertness, sensing ability, used in detection applications and surface chemistry due to these properties such carbon-based materials are used as solid supports in different modification process [19-20]. Conversely, carbon-based materials are still expensive. Graphite is used as an alternative because it possesses a poor surface than nanomaterials [21-22].

Carbon black (CB) nanoparticles are commercially available, containing a diameter of 14 nanometer and are used as alternatives due to their advanced properties like a high surface area to volume ratio, low cost and high spread availability as compared to carbon materials or substrate [23]. Carbon nanomaterials can combine with different fluorophores like fluorescein and form the visible fluorescein emission [24-27].

Carbon black can be modified by the physiosorption process of fluorescein on the surface of carbon black. Prepared a 50ml solution of 10 mM NaOH containing 2.5 mM fluorescein and then stirred a 50 mg of carbon black for 48 h, then isolate the mixture by centrifugation process and washed the final material by 10 mM NaOH or distilled water to remove all impurities. Washed material can be dried under vacuum and yield the final fluorescein–modified carbon black. Carbon black can be stirred in the solution of fluorescein, during this treatment the physiosorption process takes place on the surface of carbon black. The final product fluorescein can be used to detect the Pd(II) in the solution and during this process, the Fe(III) cannot interfere because latter it forms a complex with the salicylic acid. The fluorescein/carbon black synthesis is simple, environmentally friendly and produces

no hazardous chemicals during this process. The final sample can be characterized by cyclic voltammetry and x-ray photoelectron spectroscopy [28].

5.2 Synthesis of 5(6)-Carboxy-Fluorescein

The 5(6)–Carboxy-fluorescein can be activated by conjugation with N-hydroxy-succinimide (NHS) and dicyclohexylcarbodiimide (DCC). 5(6)-Carboxyfluorescein succinimidyl esters are more sensitive to change the pH so it can be used to check the internal pH in the cell of *Escherichia coli* [29-31].

5.2.1 Method 1

The (6)-Carboxyfluoresceins are synthesized by cyclodehydration method and Friedel-Crafts acylation in the presence of zinc chloride with 2 and 4-carboxyphthalic anhydride. The diester salts (6-carboxyfluorescein) are prepared by derivatization of (6)-Carboxy-fluoresceins with diisopropylamine (iPr_2NH) and pivalic anhydride (Pv_2O) then these salts are precipitate out in the presence of absolute ethanol. The crystallization of 5-carboxyfluorescein dipivalate takes place in the presence of nitro-methane. At the end diester salts such as 20% 5-Carboxyfluoresceins and 21% 6-carboxyfluorescein can be obtained after the hydrolysis process [32]. Both diester salts are not separate easily so pivaloyl chloride cab is used as an alternative of anhydride. The 5-carboxyfluorescein dipivalate cannot be obtained by crystallization but the ester salt of 6-carboxyfluorescein can easily be separated by this process (Fig.2) [33].

5.2.2 Method 2

In the second method methane sulfonic acid can be used as catalyst and dehydration solvent for the synthesis of 5 and 6-Carboxyfluorescein and form a mixture of 5(6)-carboxy fluorescein methane sulfonates [34]. The mixture of hexane and methanol can be used for the crystallization of two regio-isomers in which 5-carboxyfluorescein methane sulfonate can crystalize out firstly and then 6-carboxyfluorescein methanesulfonate are separated from the mixture. Pure 5- and 6-carboxyfluorescein can be separated by the treatment of NaOH and HCL with the 6-carboxy fluorescein methane sulfonate and 5-Carboxy fluoresceins methane sulfonate.

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5.3 Synthesis of Halogenated 5- and 6-carboxy Fluorescein

Different chemical and bioactive properties of the carboxy fluorescein can be diversified for various applications by the halogenation of carboxy fluorescein. While the carboxy fluorescein is an outstanding fluorescent dye and it shows limited diversity of fluorescent with different bioactive particles. The addition of halide atoms in the 5-carboxyfluorescein and 6-carboxy fluorescein can increase the emission and excitation wavelength to detect different target materials and decreases the absorption and emission bands for high selectivity.

5.3.1 Method 1

During this experiment the 4, 5, 6, 7, 2, 7-hexafluorofluorescein can be synthesized by the reaction of fluororesorcinol with tetra-fluoro-phthalic anhydride in the presence of methane sulfonic acid then this product can react with mercapto-acetic acid and form the end product [31] (Fig.4).

5.3.2 Method 2

5(6)-iodo-fluoresceins can be synthesized by the following method in which the diazotization of 5(6)-amino fluorescein takes place. After this, it is treated with potassium iodide by using cuprous bromide that can synthesize the bromo-analogous. The diester of halo-fluoresceins and regio-isomeric halo-fluoresceins are not easily separated by a simple fractional crystallization method because halogen groups are less polar than that of the nitro groups and carboxyl groups. The synthesis of 5(6)-Bromo fluorescein carried out by the treatment of 1 and 4 bromo phthalic anhydride and acetic anhydride in which the 5-bromofluorescein di-acetates can easily be separated by crystal formation (Fig.5) [35].

5.4 Synthesis of 5(6)-Hydroxy(amino)methylfluorescein

The synthesis of 5(6)-hydroxy methyl fluorescein can be carried out by the following method in which first form the 5(6)-carboxy fluorescein di-acetates by the treatment of acetic anhydride with 6-carboxyfluorescein and 5-carboxyfluorescein then 5(6)-carboxy fluorescein di-acetates react with ethyl chloro-formate in which sodium borohydride reduces and at the end form 5(6)-hydroxy methyl fluorescein di-acetates (Fig.6).

Regio isomeric di-acetates can be separated using flash chromatography. In methanol, the regio isomeric di-acetates can be treated with 4 dimethyl amino pyridine (DMAP) and form hydroxy methyl fluoresceins. In the second method it treated with diethyl azo-dicarboxylate (DEAD), dibenzyl imido-dicarbonate ($(BzOOC)_2NH$) and triphenyl phosphine (Ph_3P) in THF and form aminomethyl fluorescein. For immuno-histo-chemical analysis, 6-aminomethyl fluorescein and 5-aminofluorescein can widely use. For long storage, the 4-aminomethyl fluorescein is unstable in aqueous solutions because it easily decomposes in the retro-Mannich reaction [36].

5.5 Synthesis of 5(6)-amino fluorescein

5(6)-amino fluorescein can be synthesized by the reaction of fluorescein with 4-nitrophthalic acid. The regio isomers are separated by fractional crystallization method and then the nitro group reduced to a synthesized final product that is 5 and 6-aminomethyl fluorescein. The amino group has the ability to couple with activated carboxylic group of the target molecule so it can be used for transportation of lipid and metabolism, proper membrane structure, illumination of protein, analysis of nucleotides, lipids and nucleic acids (Fig.7) [37-38].

The mixture of 5-amino methyl fluorescein and 6-aminomethyl fluorescein are synthesized by the reduction of 5-nitrofluorescein and 6-nitrofluorescein using sodium sulfide and sodium hydrosulfide then 5 and 6-amino methyl fluorescein can be separated by crystallization method in dilute HCl. The amino group on both 5 and 6-aminofluorescein can act as functional group replacement and linker length increases as exemplified with the dye [39]. The amino group on both 5 and 6-amino fluorescein can act as functional group replacement and linker length increases as exemplified with the dye [31-40].

5.6 Synthesis of 5(6)-Azidofluorescein

Azide is the most important photoactive group that belongs to photo-affinity reagent [41-43]. The synthesis of 5-amino methyl fluorescein and azido fluorescein in Dimethyl formamide described by Rotman. This material can be acidified and azotized in which react with sodium azide and n-amylnitrite at 4ºC to synthesized 5-azidofluorescein [31]. 5-azidofluorescein diacetate can be synthesized by acylation of 5-azidofluorescein and overall yield is 42%. The 5-nitrenefluorescein can be synthesized from 5-azidofluorescein because 5-nitrenefluorescein can react with intracellular proteins and this detects at the wavelength of 3000 nm [31].

5.7 Synthesis of 5(6)-Isothiocyanatofluorescein

Under some slight conditions, the Isothiocyanate group can react with the amino group of the peptide bond and protein (Fig.8).

Isothiocyanatofluorescein are commonly used for the labeling of different biomolecules like proteins, immuno-globulins, peptides, oligosaccharides, lectins and polysaccharides etc. [44]. The derivatives of Isothiocyanatofluorescein can also be used for the analysis of biological molecules. It can be used as a fluorescent probe but contain some problems like quenching phenomenon caused by small stoke shift, large fluorescence dependence upon high light sensitivity and pH [36].

The properties of Isothiocyanatofluorescein can be improved by its conversion into 5(6)-isothiocyanato-2,7-dichlorofluorescein form. First, in the presence of zinc chloride, 4-chlorocresorcinol reacts with 4-nitrophthalic acid then it reduces by the reaction with sodium hydrosulfide and sodium sulfide, in methanol treatment with carbon disulfide, aqueous ferric chloride solution, and ammonium hydroxide. The final material can be purified by flash chromatography and crystallization in acetone [31]. The final compounds 5-isothiocyanato-2', 7'-dichloro-fluorescein and 6-isothiocyanato-2',7-dichlorofluorescein show brilliant labeling characteristics because their emitted and excited wavelengths are 10 nm longer than Isothiocyanatofluorescein and it improves their measurement by reducing the background interference. The fluorescent emission of 5 and 6-isothiocyanato-2',7'-dichlorofluorescein at pH 7-11 increases the range of its applications as compared to Isothiocyanatofluorescein emitting fluorescence at same pH range (Fig.9) [31].

5.8 Synthesis of 5(6)-Aminoxymethylfluorescein

In methanol and ammonium hydroxide solution the different reagents such as 5-hydroxy methyl fluorescein or 6-hydroxy fluorescein and N,N-bis-tertbutoxy carbonyl hydroxylamine react at ambient temperature then reflex the mixture in tetrahydrofuran and aqueous HCl under the mild condition and gain O-(5-fluoresceinmethyl) hydroxylamine hydrochloride and its 6-regioisomer (Fig.10).

The O-(5-fluoresceinmethyl) hydroxylamine hydrochloride can be used as the fluorescent reagent to detect the damaged part of the reacting material or nucleic acid because of the amino group of the O-(5-fluoresceinmethyl) hydroxylamine hydrochloride show specific reaction with damaged part of the reacting material or nucleic acid and also used as labeling material to label the oxo-steroid analogs for clinical immunoassays through oxime linkage [45].

5.9 Synthesis of 4'(5')-Alkylated Fluorescein

Three different methods are used to introduce a linker on fluorescein probes. In the first method, the linker is introduced into the starting chemical prior to the synthesis of dye. In the second process, the linker is introduced on the existing group of fluorescein dye which makes it more suitable for a specific conjugation with other material. The reactive protons of 4' and 5'-position on the surface of fluorescein are introduced to the linker. The reagent 2',7'-dichlorofluorescein are reduced with the formaldehyde and N-(2-methylaminoethyl)–bis (2-pyridylmethyl) amine through Mannich reaction. This material can be used to observe ATP and pyrophosphate in 100% aqueous solution which contains Mn_2 [46].

The 2',7'-dichloro-4',5'-bis-({[2-(bis-pyridine-2-ylmethylamino)-ethyl]-methylamino}-methyl) fluorescein can be synthesized by experiment in which the 2'-chloro-5'-formylfluorescein can react with 2-substituted-8-amino-quinolines to develop innovative fluorescein probes and in the cell, this probe can be used to track nitric oxide (Fig.12) [31].

The 5'-(2-substituted-8-aminoquinolino) is a new class of fluorescein probe that has been used for the analysis of metals like copper Cu(II), it also used in biochemistry and environmental analysis [47-48]. O-pinacolyl-methyl-phosphono-fluoridate and O-iso-propyl-methyl-phosphono fluoridate both are important warfare agents. Various studies have been used or concentrated on their decontamination, analysis and detection over the last five years [49-51]. A researcher recently reported that the chemical warfare agents have phosphoryl fluoride are detected by using fluorescein-4,5-dialdehyde with hydroxy-amine probe [31].

5.10 Synthesis of Mono-piperidyl and Di-piperidyl Fluorescein

Ultimately there are two different methods used for direct exchange of fluorescein into rhodamines and rhodols. The first methods are depending on $ZnCl_2$-catalysis in which the direct substitution of 3',6'-halogenated fluorescein with amines [52]. In the second process under the catalysis of the palladium-phosphine complex, the amination of mono and di-inflated fluorescein are formed using the Buchwald-Hartwig method. [53].

Tough reaction conditions give lower yields and the high cost of these dyes stimulated us to study the direct substitution mechanism on 3',6'-positions by the Nucleophilic process. The 3',6' demethylated fluorescein probe are synthesized by the Seidu-Larry process and it provides new methods for substitution on different positions. It also shows direct substitution with different amines. Fluorescein can react with different chemicals such as pyridine with 0.9 or 4 equivalents of methane sulfonyl chloride by the substitution method. In the mesylation process, two products such as mono-mesylate or di-mesylate fluoresceins are generated, after this process cool the mixture at 0°C, add some amine in excess quantity and this reaction performed in argon atmosphere Mono-piperidyl and di-piperidyl fluorescein also synthesized by this process. The mono-piperidyl/di-piperidyl can be synthesized by reaction with 0.9 or 4 equivalents of methane sulfonyl chloride. The uncontrollable mixtures of fluorescein such as mono, dipeptidyl fluorescein, and mono mesylate fluorescein or di-mesylate fluorescein were delivered to the next step. The preparative thin-layer chromatography is used for the analysis of this product and column chromatography is not used because it cannot afford this uncontaminated product (Fig.13).

5.11 Synthesis of Sulfonated Fluorescein

The sulfonated fluorescein can be synthesized by introducing the sulfo group on the surface of fluorescein and indirect substitution of different building blocks such as phenols by condensation with phthalic anhydride to develop sulfonated fluorescein. The sulfonation process can be simplified by direct electrophilic substitution on the surface of fluorescein and use interesting starting material for further modifications. The sulfonation process shifts the emission and absorption bands greater than ≈5 nm and spectral properties are not changed by using these groups [54]. Alternatively, the photo-stability and quantum yields of fluorescein can be increased by improving their solubility in aqueous media and it increased by using residues of sulfonic acid [55].

5.11.1 Di-sulfonation of Fluorescein on 4',5'-Positions

Prepared a solution of 30% SO_3^{-2} in H_2SO_4 Di-sulfonation of Fluorescein on 4',5'-Positions can be carried out in oleum then heat the mixture at 100°C for 8h and stirred for one night. The synthesized product are delivered to selective 4',5'-positions of fluorescein, which are recognized by ^1H NMR (Fig.14) [55].

5.11.2 Mono-sulfonation of Fluorescein on 4'- or 5'-Position

Mono-sulfonated fluorescein can be synthesized suing concentrated H_2SO_4 then heat the reaction mixture at 140°C for 6h. According to to^1H NMR, the reaction takes place selectively on the 4'-position and gives one product. The final product (30%) is yellow solid, separated by column chromatography and then store in the freezer at -24°C for one month after this it decomposes into red fluorescein. The second method was used to synthesize mono-sulfonated fluorescein as a sodium salt and a more

stable form towards the adverse decomposition (Fig.15) [56].

5.12 Synthesis of Taurine-Fluorescein

The taurine an endogenous *β*-amino sulfonic acid is most important for retinal, skeletal muscle and cardiovascular health. While it can be biochemically synthesized in the liver by cysteine but this way is not enough and at the end require dietary taurine [57]. The deficiency of dietary taurine can be caused by visual dysfunction in children and it decreases by increasing the taurine supplements. The taurine transporter (TauT) is used to transport exogenous taurine into the cell [58-60]. The taurine is used as an important component for proper functioning and structural maintenance of retina. The deficiency of taurine caused retinal degeneration, vision loss, retinitis and diabetic retinopathy [61-62].

The taurine-conjugated fluorescein (F–Tau) is synthesized by directly covalent attachment of taurine to the surface of fluorescein. The BRB-penetrating ability of F–Tau are investigated in adults by two accumulated monolayers of retina pigment epithelial cells (ARPE-19) and human retinal microvascular endothelial cells (hRMECs), two different cell lines stating TauT [63-64]. These experiments have examined the targeting potential of taurine Fluorescein (3.32 g, 10 mmol), dicyclohexylcarbodiimide (DCC, 2.10g, 10mmol) and *N*-hydroxy succinimide (1.17g, 10mmol) in anhydrous dimethyl formamide (DMF) then heated the mixture at 75°C for 1.5h. The final product is di-cyclo-hexyl-urea (DCU), it filtered at 0°C and reduces the pressure under the rotary evaporator and then the solvent can be evaporated [65]. Then the remaining mixture is purified through a silica column (Petroleum ether/Acetone, 3:1-1:1, *v*/*v*) and to give the final product, active ester in the form of red-orange solid, yielding1.29g (30%) [66].

Prepared a solution of anhydrous dimethylformamide which contains 0.65mL of tri-ethylamine (Et_3N, 4.66 mmol) and taurine (0.58g, 4.66 mmol), then dissolve active ester (1.00g, 2.33 mmol) in this solution. The final mixture is stirred for 36h at room temperature and then evaporated using a rotary evaporator at high vacuum pressure.

The final product can be purified to obtained final desired product that is F–Tau as a white powder, yielding 0.42g (41%) using different techniques such as ODS (octadecyl silane) column chromatography (methanol/H_2O), silica column (ethyl acetate/methanol, 4:1, *v*/*v*) and preparative high-performance liquid chromatography (HPLC) (acetonitrile/formic acid buffer). The HPLC (Agilent 1200, USA) combine with Phenomenex Synergi Polar-RP (250mm×4.6mm, 4μm) are used to determine the concentration of the F-Tau and fluorescein at 35°C. The detecting range of ultraviolet wavelengths can be set at 230nm and injection volume at 20μL. The mobile phase which consists of a phosphate buffer solution for phase A and phase B containing acetonitrile at pH 2.5 can be delivered at the flow rate of the 1.0 mL/min. The analyzation of fluorescein within 12 min takes place by the isocratic elution method at 50:50, *v*/*v*. The gradient elution method can be used to investigate the F–Tau by varying the percentages of phase A for 70:30 and phase B for 58:42 within 15 min.

For the synthesis of F–Tau (Fig.16), first step is to activate the fluorescein because it cannot react with amine easily such as taurine when the carboxyl group is present as masked. The activated fluorescein generated by reaction with *N*-hydroxy succinimide and then taurine can react with this active site under ambient temperature and alkali condition. The red-orange active ester solution obtained, after a reaction, it converts into the colorless solution and final product F–Tau as white solid can be isolated by high-performance liquid chromatography (HPLC). The consequential F–Tau is closed as a Spirolactam form [67]. This configuration of the final product is confirmed by the characteristic spirolactam peak at 64ppm in the C^{13}NMR spectrum [61-67]. After intravenous injection, by lactamase, the closed-Spirolactam form of F–Tau can be and this open form of the taurine conjugate by exposing the effective site of taurine. The activated newly-formed taurine conjugate is specifically recognized and transported to other sites by the taurine transporter, consequential is a penetration of the BRB.

There is a critical requirement for the development of enhanced treatments for many diseases of the retina. For treating the retina, the BRB can delays significantly the delivery of many systemically-controlled medications. The BRB permeability of different drugs can be enhanced by the investigation of effective retina-targeted ligands. The exogenous taurine is used as a neuro-protective agent in the retina where it functions as a neuro-protective agent and this compound used to evaluate its targeting efficiency. The results showed that the introduction of taurine can improve the solubility, increased residence time and reduction of log P of F–Tau on the cell as compared with fluorescein. Some transport studies also showed that the introduction of taurine presented significantly strong trans-epithelial permeability across the retinal cell barriers *in vitro*. Taurine may be used as a promising ligand for the delivery of retina-targeted medications [68].

5.13 Synthesis of New Samarium-Fluorescein

The synthesized metal-organic frameworks (MOFs) having lanthanide ions containing much attention

because of their versatile design and potential applications in gas storage, catalysis, sensor, magnetism and luminescence etc. The 4f-4f transitions are forbidden transition, they consist of electric dipole, magnetic dipole transitions and relatively have long excited states lifetime. 4f to 4f forbidden transition with high emitted light from colorimeter leads lanthanides as luminophores. In metal-organic frameworks (MOFs), show the property as large luminescence because the organic sensitizer that acts as ligands which absorbs the incident radiation and then directly populates to the f-excited state of lanthanides through antenna effect [69]. The lanthanides have very flexible environment and centers containing high variable coordination numbers. Therefore it constructs topological frameworks based on the organic ligands selection and obtained a variety of lanthanide complexes with different structures and properties. These complexes have brilliant luminescent properties and give intense emission lines [70].

Fluorescein can act as an important ligand with large oxygen atoms and shows high fluorescence yield and great absorption in the visible region. It can give different fluorescence spectra, absorbance spectra, quantum yield, exists different structural forms and high lifetime [71]. A large number of luminescent properties, characterization and synthesis of metal-organic frameworks (MOFs) can be reported with fluorescein and samarium [72].

Prepared a solution of 6.0 mL of DMF/H_2O (2:1) and dissolve 0.0664 g of fluorescein in it, then added 0.4 mL of 0.5M aqueous $SmCl_3$ solution with continuous stirring. Adjust the pH of the mixture at 7.0, then stirred for 1 h and transferred into a stainless steel Teflon lined autoclave. The reaction was continued at 393 K for 48 h and then cooled the mixture at room temperature to get red needle-shaped crystals arranged in a flower-like structure. The final crystals were filtered, washed with water and then dried. The proposed reaction mechanism is given (Fig.17).

The final crystal structure was characterized using single-crystal x-ray diffraction (XRD) analysis and the details of crystal data from collection parameters. The analysis exposes that the empirical formula of a compound has $C_{43}H_{30}N_{12}Sm$ with formula weight 903.03 and it shows a network structure in which at the center Sm_3^+ ion is coordinated with five different fluorescein molecules, a water molecule and giving pentagonal bi-pyramidal geometry. The asymmetric unit of the metal-organic framework and the coordination environment of the central Sm_3^+ ion are of great significance. The fluorescein molecule is coordinated to the Sm_3^+ ion by oxygen atoms of the carboxylate group and molecules of a second, the third fluorescein is connected to the Sm_3^+ ion through the oxygen atoms of keto groups. The fourth fluorescein molecule is coordinated through the OH group of COOH and the fifth one is coordinated through its OH group. The seventh coordination site is satisfied by a water molecule. The luminescence sensitization by the excitation of a ligand is effective than the direct excitation of the Sm_3^+ ion. The luminescence properties of rare earth complexes are influenced by intramolecular energy transfer from organic ligand to rare-earth ions [73].

5.14 Synthesis of Novel Fluorescein-Based Quinoline Glyco-Conjugates

The synthesis of novel molecular scaffolds (Fig.18) with unique structural and biological properties is an increasingly active area of current chemical research [74]. Over the past few years, the glyco-biology and its chemistry have gained much attention to understand the role played by carbohydrates in different biological events, like immune response, cell growth and inflammation [75-77]. Quinolines act as building blocks for different biological molecules and as functional materials, their derivatives play a vital role in organic chemistry as key structural units in different natural products and pharmaceutical products [78-80]. Consequently, the synthesis of quinoline derivatives bearing different substitution patterns has expected much attention [81-84].

We have explored the possibility of a single-pot multicomponent synthesis of fluorescein-based quinolone glyco-conjugates in the field of materials science and medicinal chemistry [85-86]. A new three-component reaction was developed in this article between a fluorescein-mono-aldehyde, an aromatic amine, 2-propyn-1-yl 2,3-di-O-acetyl-4,6-O-butylidene-β-D-gluco-pyranoside for the synthesis of a new class of fluorescein-based quinoline glyco-conjugates complex [87-90].

At first, the fluorescein-based quinoline glyco-conjugate was synthesized by using fluorescein mono-aldehyde 1,4-methoxyaniline and 2propyn-1-yl 2,3-di-O-acetyl-4,6-O-butylidene-β-D-glucopyranoside in the involvement of copper chloride (CuCl). The reaction was studied in many organic solvents, which are commercially available and use it without more purifying or drying to optimize the conditions. We found an extraordinary solvent effect with a copper chloride-catalyzed reaction of 30 mol percent at reflux temperature (80°C). The analysis showed that tetrahydrofuran, among many others, is the most appropriate solvent, such as acetonitrile, chloroform, dichloromethane, methanol and ethanol. When the design reaction was performed at room temperature, there was a decreased yield of only 32%. Moreover, whenever the reaction was catalyzed by 20 mol percent of copper chloride, the rate of reaction was extended to 6h and the required product was procured with a fluorescein-based quinoline glyco-conjugate yield of only 32 percent, whereas

no changeover of fluorescein-based quinoline glyco-conjugate 4a was attained without copper chloride. Therefore, the most appropriate reaction environment for the formation of fluorescein-based quinoline glyco-conjugate has been developed as a refluxing temperature (80°C) with tetrahydrofuran use as a solvent. Notably, the Lewis acid-catalyzed reaction continued efficiently without the exclusion of humidity or air. The quinoline glyco-conjugates based on fluorescein were observed to acquire progressive antioxidant activity.

To show the general statement of this technique, the procedure was evaluated using Fluorescein-based quinoline glyco-conjugates 4a–f for their capacity to prevent 1,1-diphenyl-2-picrylhydrazide (DPPH) peroxide radicals from forming in a peroxy-generating system as mentioned in the experimental portion. To show the general statement of this technique, the procedure was evaluated using Fluorescein-based quinoline glyco-conjugates 4a–f for their capacity to prevent 1,1-diphenyl-2-picrylhydrazide (DPPH) peroxide radicals from forming in a peroxy-generating system as mentioned in the experimental portion [91].

5.15 Synthesis of Fluorescein-Labeled Starch Maleate Nanoparticle

Minimum-cost, renewable and anti-toxic in behavior, polysaccharides such as starch, alginate, chitosan and dextran have received major interest as precursor/starting materials for the preparing of nanomaterials as they are available in abundance [92]. These nanoparticles based on fluorescent polysaccharides were used in different applications such as micro-imaging, chemical sensing, bio-sensing, drug delivery and pH sensing [93-96].

Fluorescein 5(6)-isothiocyanate-labeled starch maleate (FISM) nanomaterial have been effectively synthesized with starch maleate by strong covalently attaching fluorescein 5(6)-isothiocyanate (FITC). It was illustrated the possible application of fluorescein 5(6)-isothiocyanate-labeled starch maleate nanoparticles as delicate fluorescent samples for both the identification of heavy metal ions. Fluorescein 5(6)-isothiocyanate-labeled starch maleate nanoparticles could behave as detecting probes in anhydrous systems for the specific identification of Ag^+ and Pb^{2+} ions. The starch maleate was synthesized using a slightly modified method [97-99].

Approximately 2.0g native sago starch powder (12.34×10^{-3} mole anhydroglucose) was decomposed in N,N-dimethylformamide (DMF) 50mL. The mixture solution was stirring at 90°C for about 15–20 rpm per min. After which 3.63g of maleic anhydride (37.04×10^{-3} mole) and 30 micros per L Medroxyprogesterone acetate deposit is added into the mixture. The mixture was constantly stirred (900 rpm) for 4 hours at 90–95°C. The mixture was then refrigerated to ambient temperature and caused by extremely pure water. The precipitate was washed with ultrapure water several times to eliminate any unreacted salts. Fluorescein 5(6)-isothiocyanate-labeled starch maleate was produced by having to take 1.5g starch maleate at various levels of FITC (0.05, 0.25, 0.5, 1.0, 2.0 and 3.0mg/mL) in 10mL dimethyl sulfoxide at temperature 90–95°C for 6 hours. Additionally, 1.6mL of Dibutyltin dilaurate was applied as a catalyst to the solution. The resulting mixture solution, Fluorescein 5(6)-isothiocyanate-labeled starch maleate, was added dropwise into ethanol for the production of Fluorescein 5(6)-isothiocyanate-labeled starch maleate, nanoparticles via precipitation.

Fluorescein 5(6)-isothiocyanate-labeled starch maleate, nanoparticles were purified 24 hours before use for more study by dialyzing the specimens using dialysis membranes against ultrapure water. Nanoparticles from native sago starch grafted with maleic and fluorophore groups were produced from fluorescein 5(6)-isothiocyanate-labeled starch maleate. Starch was esterified and marked with FITC that use maleic anhydride. Throughout the synthesis of starch maleate (SM) and fluorescein-labeled starch maleate (FISM), dimethyl furan and dimethyl sulfoxide (DMSO) have been used as solvents in place of water, since this esterification reaction would release water as a byproduct. As the esterification reaction is reversible, its response could either proceed in the forward (left to right) or in the reverse direction depending on reaction conditions.

Enhancing the moisture content tends to push the reaction in the reverse reaction which is undesired as when the goal is to produce more starch maleate and starch maleate marked with fluorescein. As a result, dimethyl furan and dimethyl sulfoxide have been used as solvents to sustain the mixture with the lower output so that the equilibrium would offer a forward reaction to give higher product yield. The 4-Dimethylaminopyridine was a nucleophilic catalyst for anhydride esterification where it catalyzed the reaction of the starch group maleic anhydride and hydroxyl (OH) [100]. 4-Dimethylaminopyridine attacked the anhydrides to form optimal amide that is more reactive than that of the initial anhydride to the nucleophilic attack. Starch molecules have OH groups willing to act as nucleophiles, attacking the maleic anhydride's optimal amide or enabled carbonyl carbon to generate starch maleate (Fig.19).

Dibutyltin dilaurate has been used to catalyze the reaction among Fluorescein isothiocyanate molecules to starch maleate molecules by installing Fluorescein isothiocyanate molecules for the synthesis of fluorescein-labeled starch maleate. The OH starch maleate group

invaded this activated carbon isothiocyanate atom, which reacted by spreading the polymer to give thiocarbamate ester and regenerate the catalytic species [101].

The processes of esterification through Dimethylformamide/4-Dimethylaminopyridine and Dimethyl sulfoxide/Dibutyltin dilaurate systems were an efficient method of synthesizing starch maleate. The labeling reaction was based on the covalent binding here between the FITC group of isothiocyanates and the starch group OH. The degree of substitution (DS) of starch maleate was 1.64, while the extent of replacement of FITC with starch maleate was 0.63 percent. The degree of starch maleate substitution acquired from this study was greater than that previously reported at 0.03–0.21 [98].

This is because the 4-Dimethylaminopyridine used for this study was a more efficient catalyst than the NaOH used in the earlier study. New fluorescein isothiocyanate starch maleate nanoparticles have been effectively determined using the precipitation method with a mean particle size of around 87 nm. All such FISM nanoparticles showed a high rate of fluorescence in the pH 9 buffer solution. The results of the study showed that some of these fluorescein isothiocyanate starch maleate nanoparticles can be used as inexpensive, efficient fluorescent sensing probes for Ag^+ and Pb^{2+} ions with detection limits as low as 2.55×10^{-5} to 3.64×10^{-5} M for each [102].

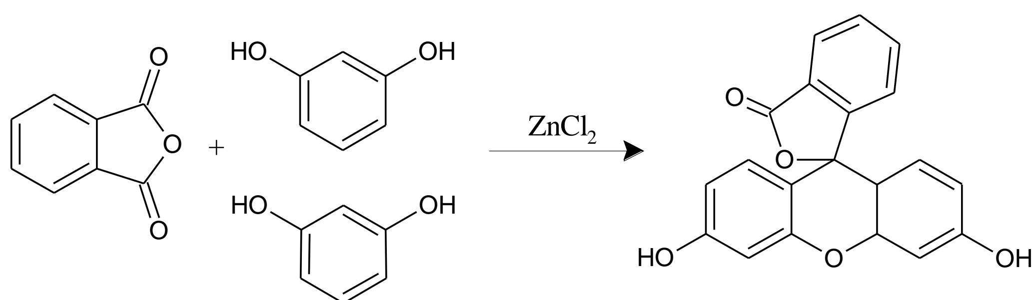

Fig.1 Synthesis of fluorescein

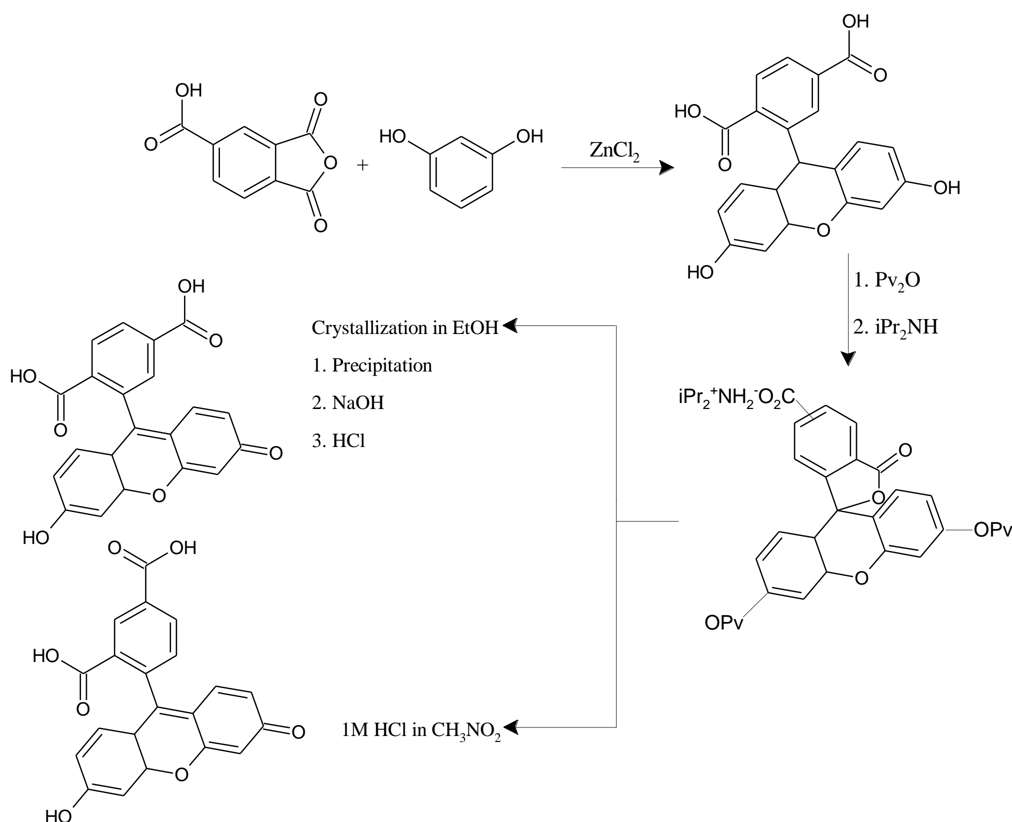

Fig.2 Synthesis and derivatization based separation of 5- and 6-carboxyfluorescein. Pv_2O, pivalic anhydride; iPr_2NH, diisopropylamine

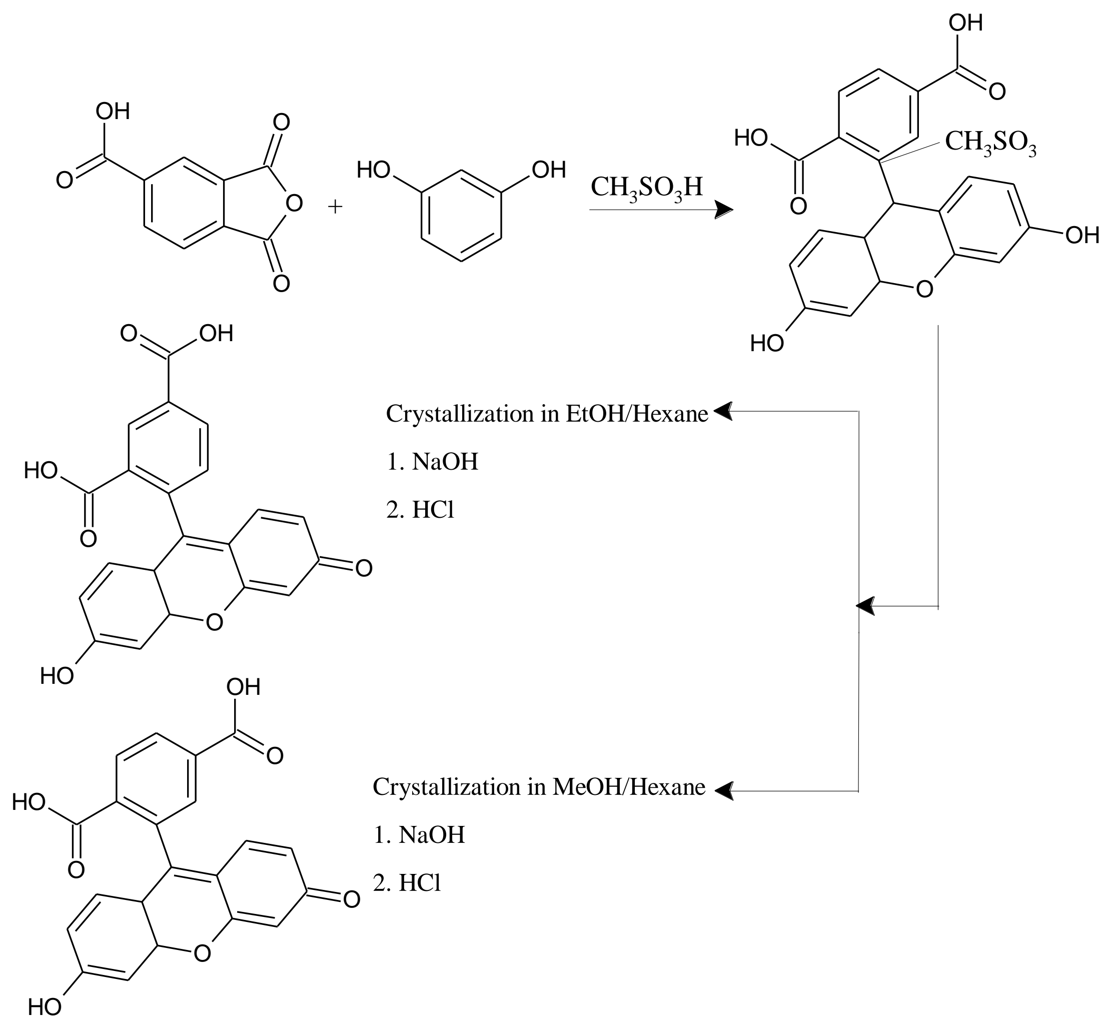

Fig.3 Methanesulfonic acid-catalyzed synthesis and separation of 5- and 6-carboxy fluoresceins

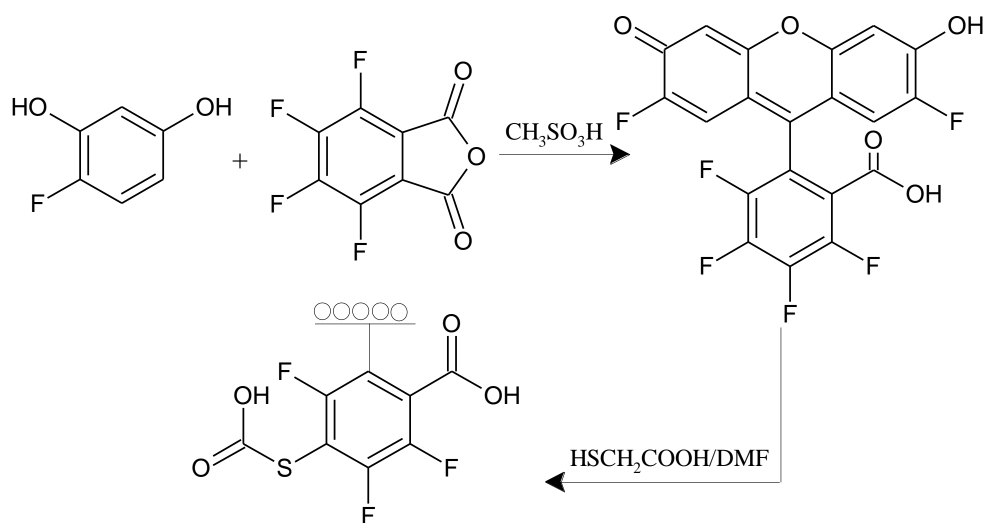

Fig.4 Synthesis of 6-carboxymethylthio-4, 5, 7, 2’, 7’-pentafluorofluorescein

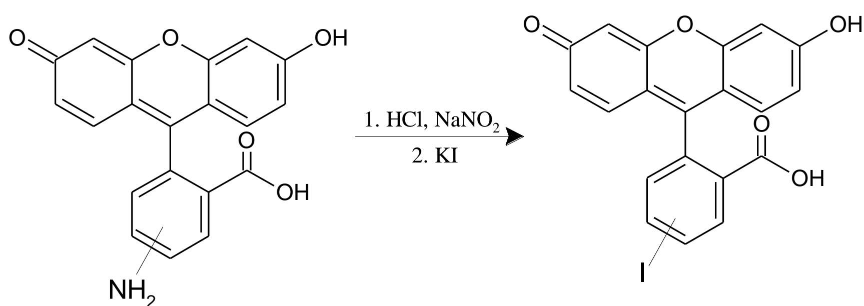

Fig.5 Synthesis of 5(6)-iodo fluoresceins

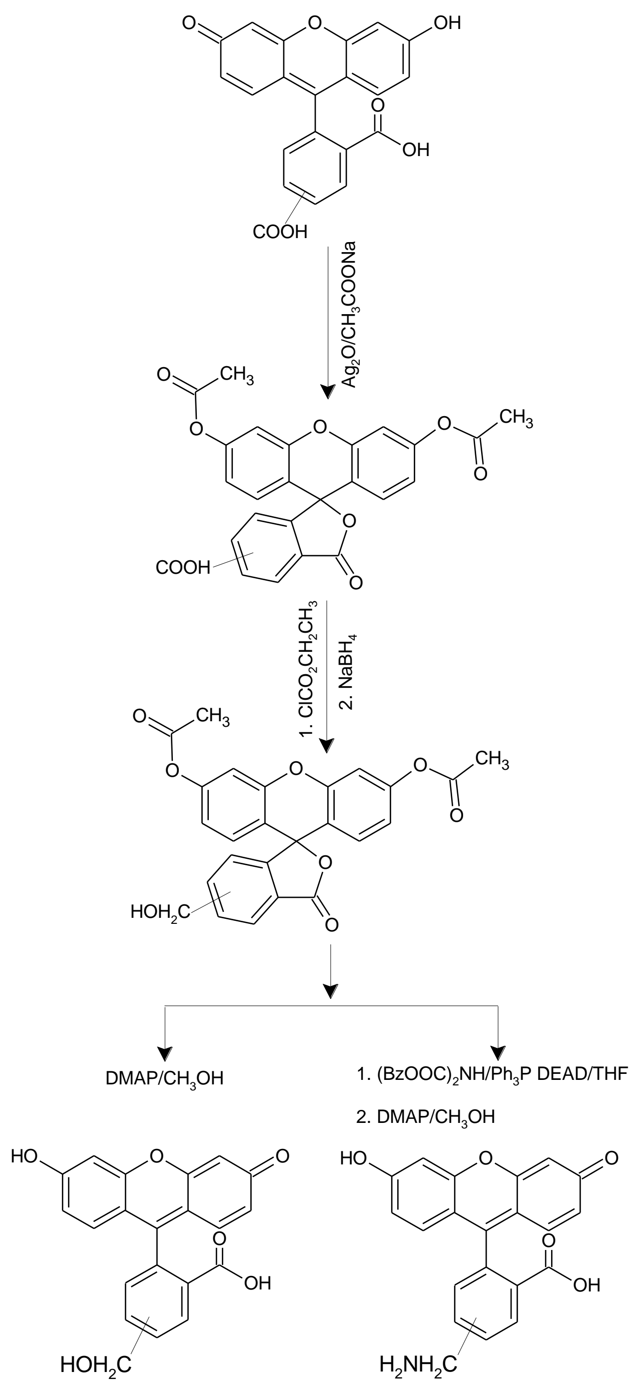

Fig.6 Synthesis of 5(6)-Hydroxy(amino)methyl fluorescein

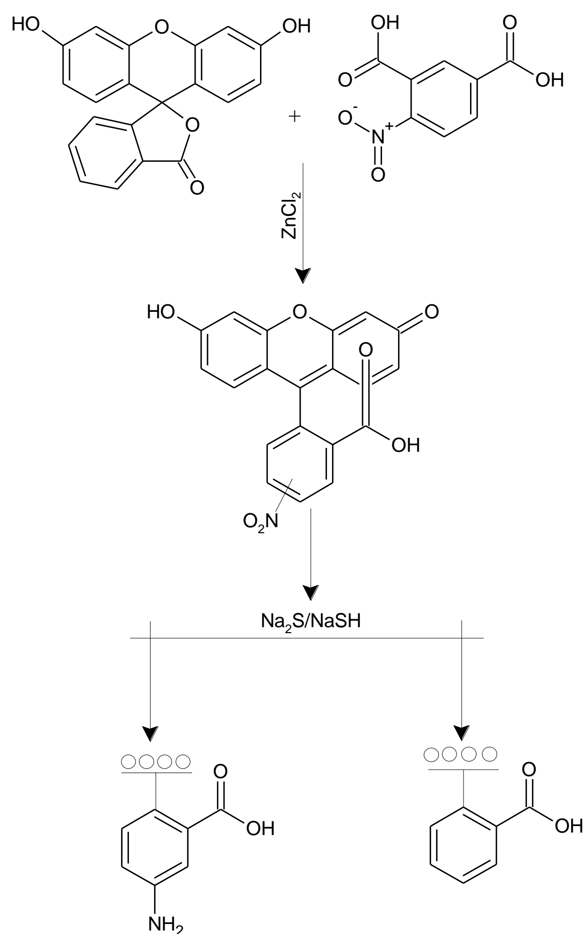

Fig.7 Synthesis and separation of 5(6)-amino fluorescein Ac_2O, acetic anhydride

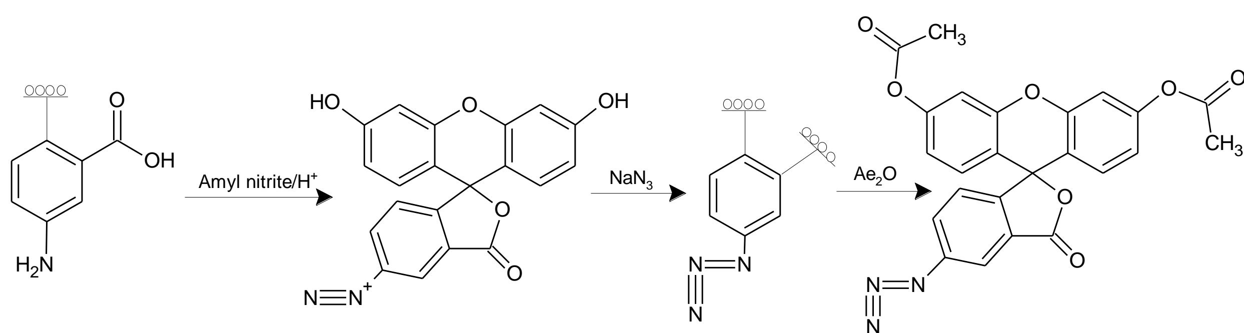

Fig.8 Synthesis of 5(6)-Isothiocyanatofluorescein

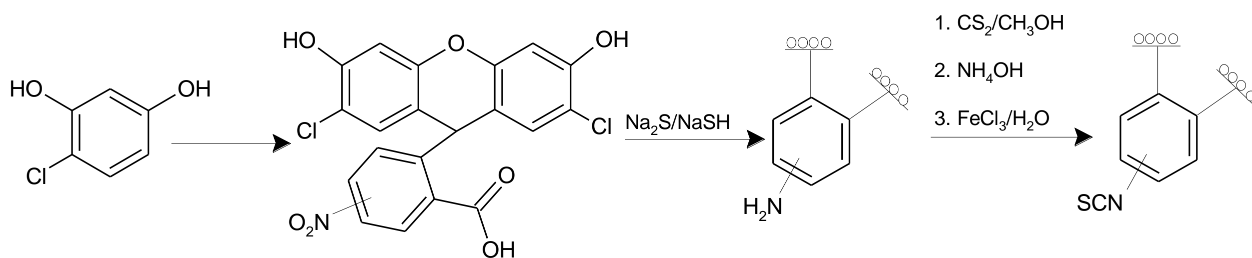

Fig.9 Conversion of Isothiocyanatofluorescein into 5(6)-isothiocyanato-2,7-dichlorofluorescein

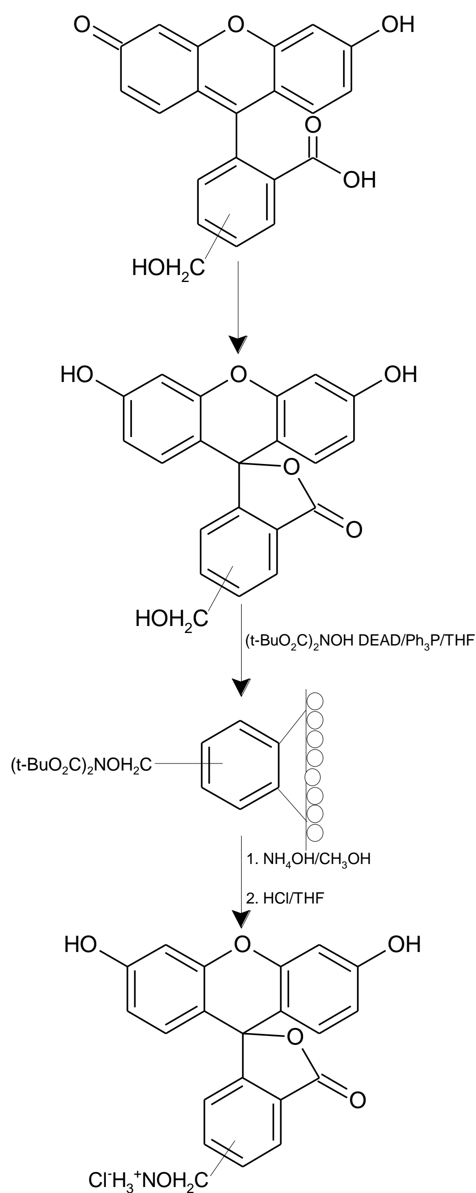

Fig.10 Synthesis of 5(6)-Aminoxymethylfluorescein

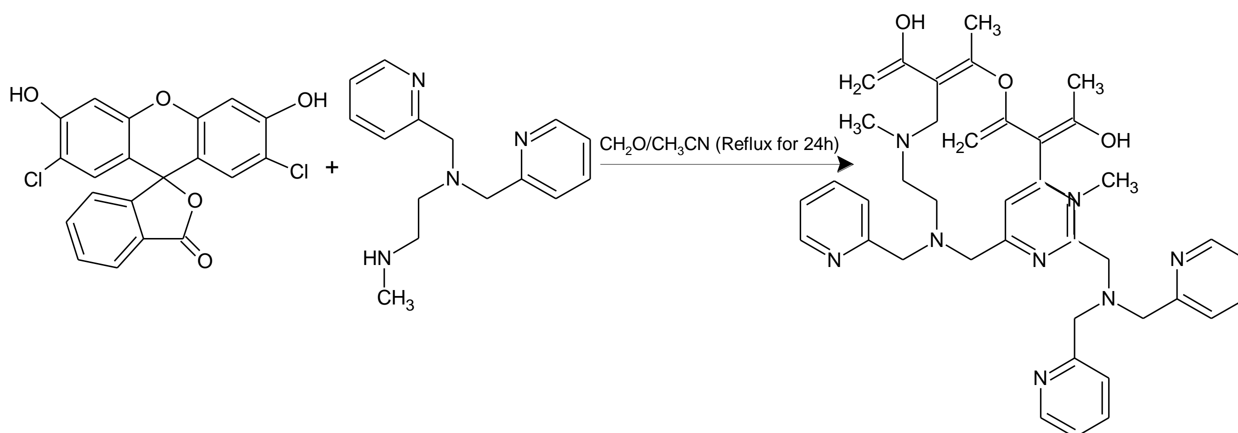

Fig.11 Synthesis of 2’,7’-dichloro-4’,5’-bis-({[2-(bis-pyridin-2-ylmethylamino)-ethyl]-methylamino}-methyl)fluorescein

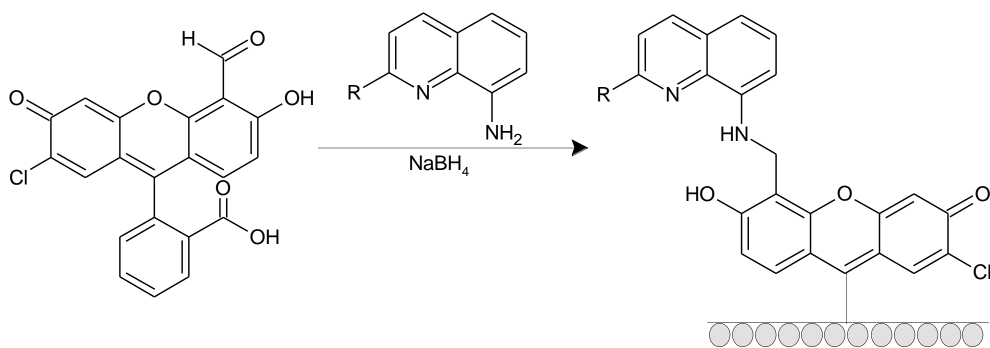

Fig.12 Synthesis of 5'-(2-substituted-8-aminoquinolino) fluorescein

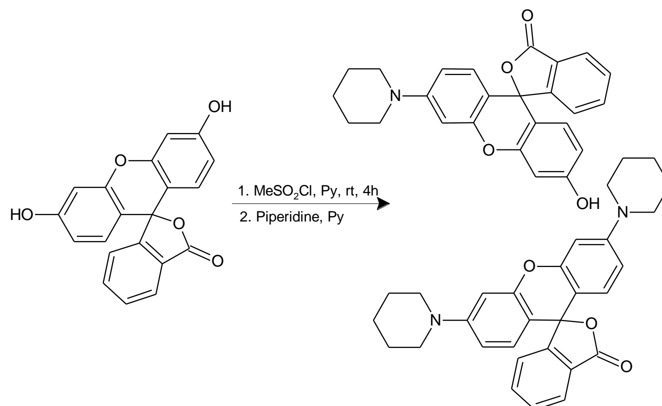

Fig.13 Synthesis of piperidyl fluores

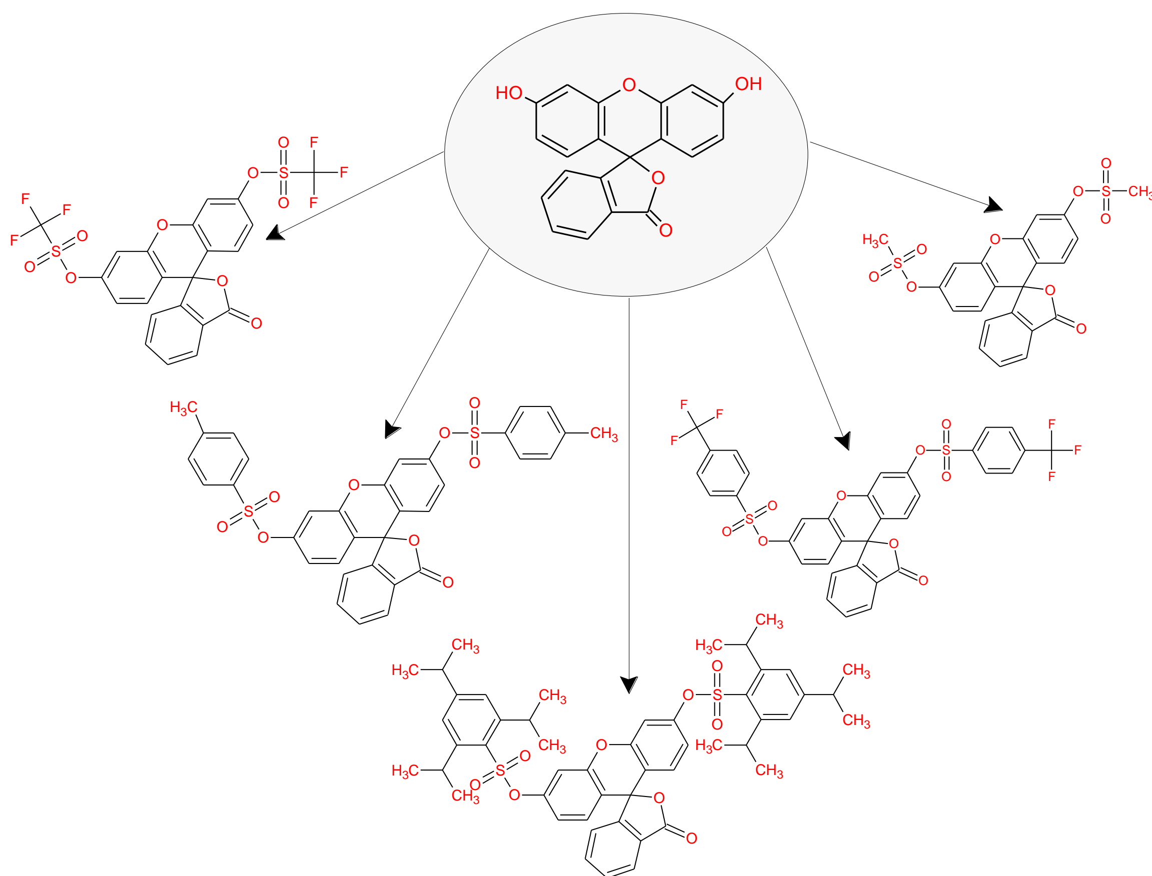

Fig.14 Di-sulfonation of Fluorescein on 4',5'-Positions

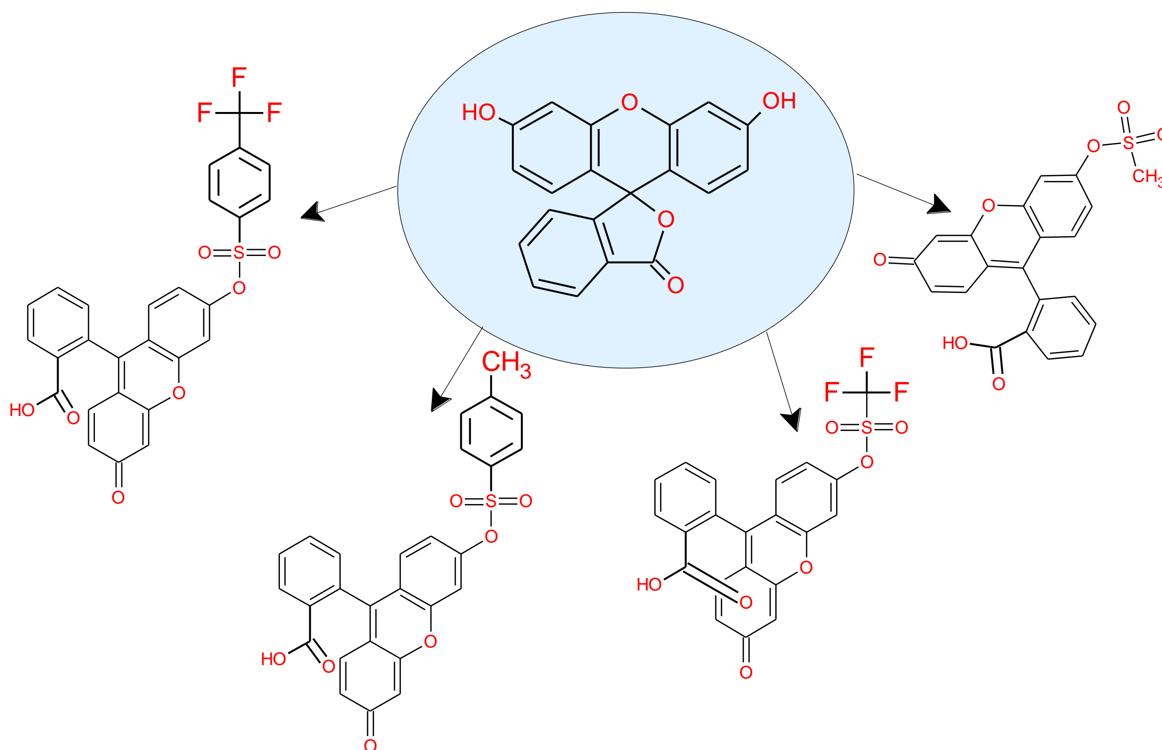

Fig.15 Mono-sulfonylation of fluorescein; Reagents and conditions

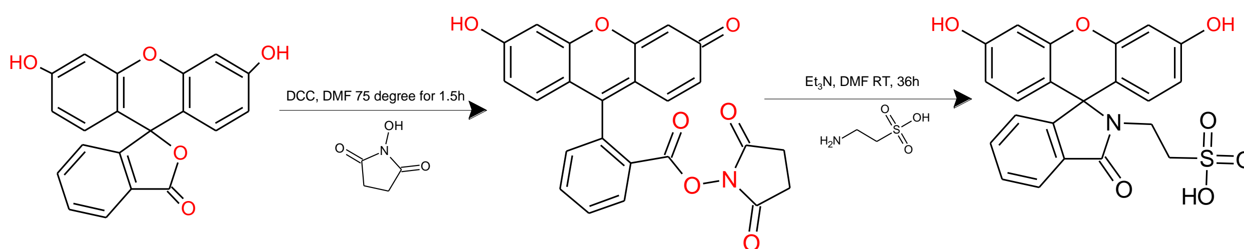

Fig.16 Synthesis of F-Tau

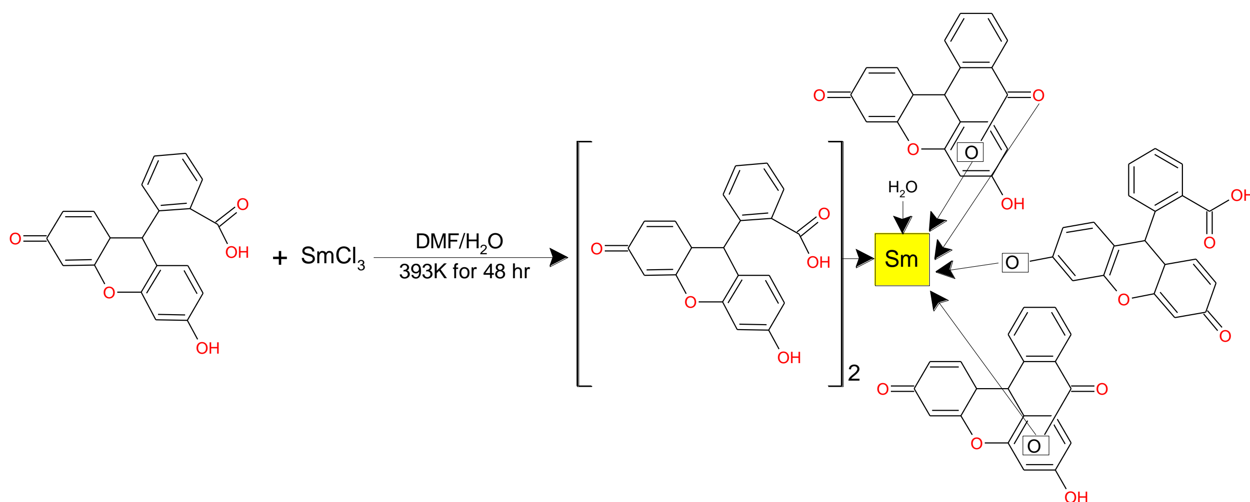

Fig.17 Proposed reaction mechanism

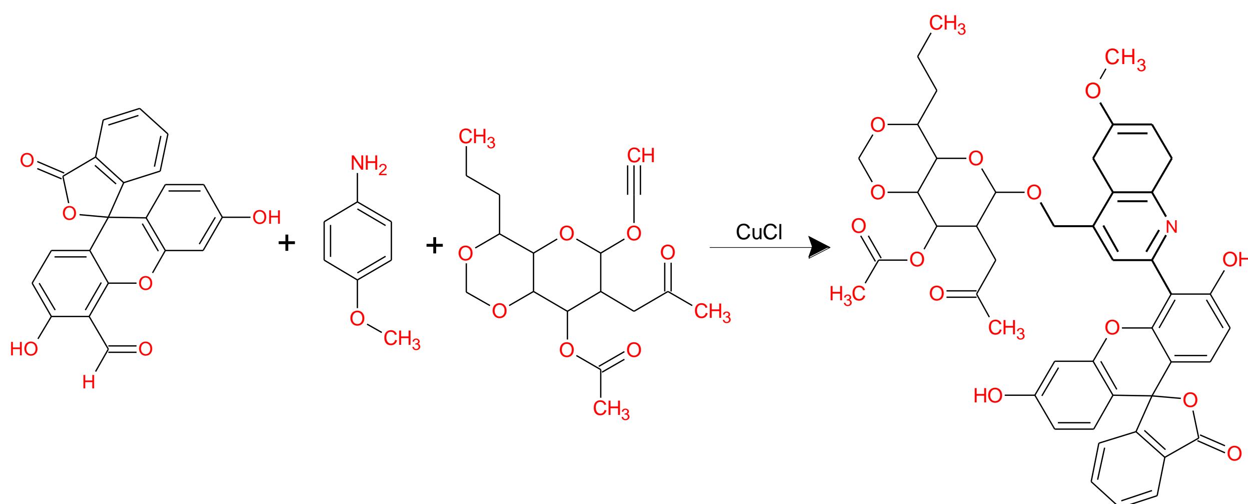

Fig.18 Synthesis of Novel Fluorescein-Based Quinoline Glyco-conjugates

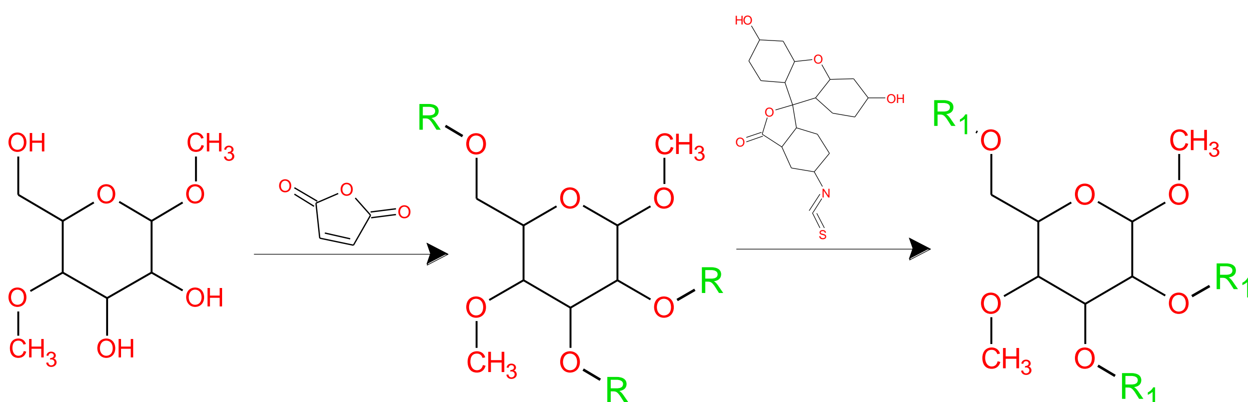

Fig.19 The reaction between starch and starch malate

Conclusion

The fluorescent probes play important roles in the life and environmental sciences. Having met various chemical and biological property requirements, fluorescein-based materials as a common class of fluorescent reagents have proven to be a sensitive, specific analytical tool in life sciences and chemical analyses as well as replacement of radioisotope uses. Since the synthesis of fluorescein in 1871, many novel fluorescein-based dyes have been synthesized via the utilization of new starting chemicals bearing a linker, modification of an existing group to a linker and introduction of a linker at different positions. Chemical, fluorescent and biological properties of fluorescein-based dyes are often improved via attachment of halogens and other substituents with unique properties such as chelation with metals. As the exciting applications of fluorescein materials continue to grow, the design and synthesis of novel fluorescein probes will certainly attract more attention, in addition to their high yield synthesis and efficient separation. At the same time, fluorescein probes still have some problems that limit their further applications, such as the recognition process that needs to be done in the solution with high organic solvent content. The toxicity of the organic solvent leads to the destruction of the biological system and the detection accuracy of the bonding signaling type probes is not as good as that of the reactive probes. Most of the detected analytes focus on the ions and the detection of organic small molecules, biological macromolecules are relatively few. Therefore, the direction of future research is to develop practical reactive probes that can be used for the detection of small molecules and biological macromolecules in water. It has important scientific significance and application prospects in the field of industrial production of disease diagnosis and treatment.

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