Stony Brook University

The Ongoing Synthesis of a Tetrahedral Chromophore

A Thesis Presented

by

Rosemarie Salerno

to

The Graduate School

in Partial Fulfillment of the

Requirements

for the Degree of

Master of Science

in

Chemistry

Stony Brook University

May 2009

Stony Brook University

The Graduate School

Rosemarie Salerno

We, the thesis committee for the above candidate for the
Master of Science degree, hereby recommend
acceptance of this thesis.

Dr. Nancy S. Goroff, Thesis Advisor
Department of Chemistry

Dr. Kathlyn A. Parker, Chairperson of Defense
Department of Chemistry

Dr. **Daniel P. Raleigh**, **Third Member**
Department of Chemistry

This thesis is accepted by the Graduate School

Lawrence Martin
Dean of the Graduate School

Abstract of the Thesis

The Ongoing Synthesis of a Tetrahedral Chromophore

by

Rosemarie Salerno

Master of Science

in

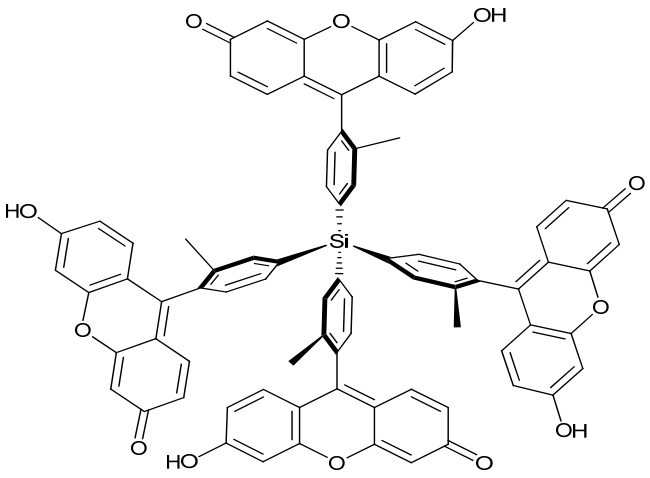
Chemistry

Stony Brook University

2009

Förster Resonance Energy Transfer (FRET) is a process used for many biological applications, such as for measurements of distances on macromolecules. FRET's efficiency is determined by the distance, spectral overlap and angular orientation of two fluorescent chromophores at the time of energy transfer. Unfortunately, the angular orientation factor introduces an uncertainty in FRET measurements, especially if the donor and acceptor are unable to rotate freely. A tetrahedral array of Tokyo Green chromophores attached to a tetraaryl silane core has been targeted to reduce the angular dependence of FRET. The tetrahedral compound is isotropic, meaning that it has equal absorption and emission in every direction. The target compound is shown in Figure 1. We have prepared the protected xanthene moiety bis-TBS xanthone, and used it to prepare Tokyo Green. Coupling of the xanthene moiety and the tetraarylsilane core has also been

attempted in order to build the tetrahedral chromophore. The tetraarylsilane core is the compound tetrakis(4-bromo-2-methylphenyl)silane and it has also been synthesized.



1

Figure 1. A tetrahedral assembly of Tokyo Green.

Table of Contents

List of Schemes

List of Figures

List of Abbreviations

Å: Angstrom unit

n-BuLi: n-Butyl lithium

t- BuLi: t-Butyl lithium

°C: degree Celsius

Cos: cosine

^{13}C NMR: Carbon-13 Nuclear Magnetic Resonance

d: doublet

DNA: Deoxyribonucleic acid nucleic acid

DMF: dimethylformamide

DMSO: dimethylsulfoxide

E: efficiency of energy transfer in FRET

eV: electronvolt

Ex/Em: Excitation/ Emission

f_D : donor fluorescence

FRET: Förster Resonance Energy Transfer

fs: femtosecond

HCl: hydrocloric acid

^{1}H NMR: Proton Nuclear Magnetic Resonance

i-PrMgCl- isopropyl- magnesium chloride

J_{da}: spectral overlap integral of the donor's emission and acceptor's absorption

m: multiplet

MeOH: methanol

MEM: methoxyethoxymethyl

MHz: megahertz

min: minute

mL: millimeter

mmol: millimoles

MOM: methoxymethyl

n : index of refraction of the solvent

nm: nanometers

PM3: parameterized model number 3

ppm: parts per million

R : distance between the donor and acceptor chromophores

R_o : Förster radius

RBF: round-bottom flask

s: singlet

Sin: sine

t: triplet

TBS: t-butyldimethylsilane

TFA: trifluoroacetic acid

THF: tetrahydrofuran

TIPS: triisopropylsilyl

TLC: thin layer chromatography

κ^2 : angular orientation factor

λ: wavelength

Φ : fluorescence quantum yield

ε_A : molar extinction coefficient of the acceptor

θ_T: angle between the donor and acceptor during FRET

I. Introduction

Förster Resonance Energy Transfer (FRET) is a distance dependent interaction between the excited states of two fluorescent chromophores (the donor and acceptor), which usually occurs between 10 and 100 Å, as shown in Figure 2.[1-2] There are many biological applications for FRET; for example it can be used to measure distances on macromolecules, improving our understanding of how they function. FRET is also used to get information about the structure and conformation of proteins, as a sensor of protein-protein interactions in living cells, in automated DNA sequencing, and for tumor imaging.[1-3] Both *in vivo* and *in vitro* energy transfer can be measured.

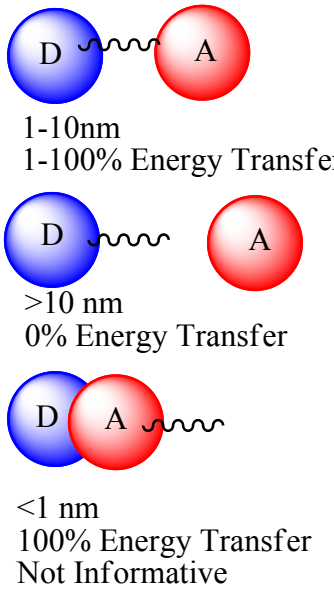


Figure 2: FRET is a distance dependent interaction between the excited states of two fluorescent chromophores.[2]

A chromophore is a molecule that absorbs light in the visible region, such as an organic dye. In FRET, the donor chromophore absorbs light at certain wavelengths to form an excited state and transfers the excitation by long range dipole-dipole interactions (without the emission of a photon).[1] Excited fluorophores behave like oscillating dipoles which create electric fields. The acceptor receives the excitation by direct electrodynamic interactions and it will emit light at a longer wavelength.[4] The fluorescence intensity and excited-state lifetime of the donor are reduced, while the emission intensity of the acceptor is increased. Photobleaching, which is the photochemical degradation of the fluorophore, can occur after the fluorophore has been excited.[1] Some fluorophores have a greater resistance to photobleaching than others. Since this energy transfer is non-radiative, the acceptor does not need to be fluorescent, but when it is, it can be referred to as Fluorescence Resonance Energy Transfer. The mechanism is shown in Figure 3.

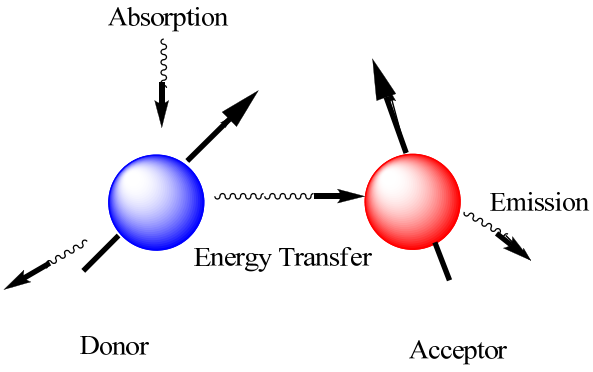


Figure 3: Schematic of the FRET mechanism between two chromophores.

The equation governing FRET was developed by Theodor Förster, who found that the efficiency of energy transfer (E) depends on the inverse sixth power of distance between the donor and acceptor (R) (Equation 1).[1]

$$E = R_0^6 / (R^6 + R_0^6) \quad (1)$$

He defined the distance R_0, now referred to as the Förster radius (R_0), as the distance at which energy transfer is 50% efficient (when 50% of excited donors are deactivated by FRET). The magnitude of R_0 is dependent on the spectral properties of the donor and acceptor dyes.[5] R_0 can be determined by using Equation (2), where κ^2 is the angular orientation factor, n is the index of refraction of the solvent which can range from 1.33-1.40, Φ_d is the fluorescence quantum yield of the donor, and J_{da} is the spectral overlap integral between the donor and the acceptor.

$$R_0^6 = (8.79 \times 10^{23}) \kappa^2 n^{-4} \Phi_d J_{da} \quad (2)$$

For many fluorophores, many of these values have already been measured; for example when blue fluorescent protein (BFP) and green fluorescent protein (GFP) are the donor and acceptor, respectively, R_0 = 40.3 Å, and when the donor and acceptor are fluorescein and tetramethylrhodamine, R_0 = 49 - 55 Å.[1] Figure 4 illustrates the exponential relationship between the efficiency of energy transfer and the distance between the donor and acceptor molecules. As the distance, R, decreases below R_0 the energy transfer rapidly reaches 100%, so that FRET can only provide an upper limit in the distance. The energy transfer also rapidly

reaches 0% as the separation increases, in which case FRET provides a lower limit on energy transfer.

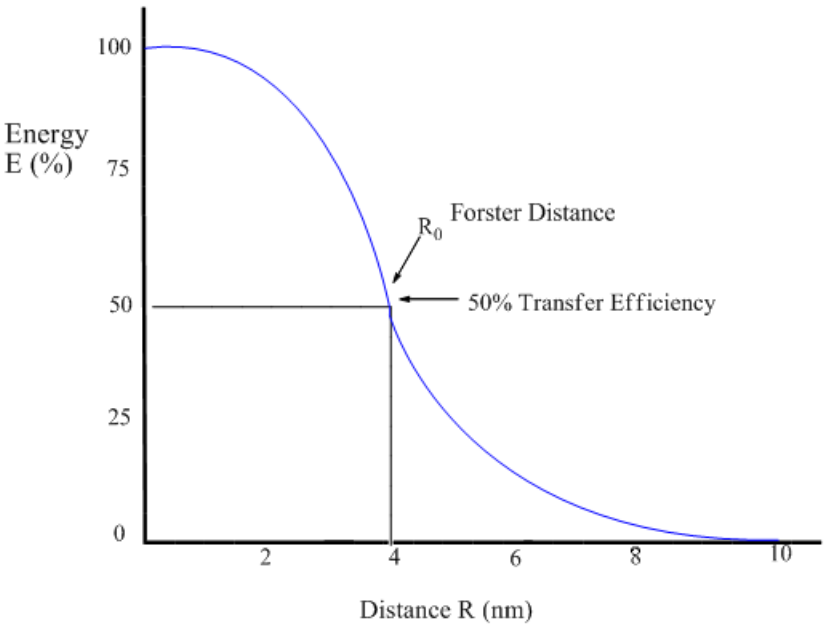


Figure 4: The efficiency of energy transfer between the donor and acceptor, E, is exponentially related to the distance, R, between them. R_0 is 4 nm in the example above.

The fluorescence quantum yield, or quantum efficiency of a fluorophore (Φ_d) is the number of light quanta emitted by a donor divided by the total number of quanta it absorbs.[1] This number must be large (near one) for efficient energy transfer. Φ_d is determined by comparing its fluorescence intensity relative to a known reference such as rhodamine B. Many of these values have already been measured and are listed in "*Resonance Energy Transfer*" by Andrews and Demidov.[1] Φ_d can be difficult to measure because its value depends on the environment of the probes when it is bound to a protein. It is also important to

note that Φ_d can be affected by pH. Some fluorophores are more sensitive than others depending on whether the molecule can exist in both a protonated and deprotonated form. Fluorescein has a Φ_d of 0.85 at a pH of 13 but 0.30 at a pH of 3.4. Tokyo Green has similar Φ_d values but rhodamine B is insensitive to pH.[6]

The spectral overlap (J_{da}) of the donor/ acceptor pair is also an important parameter in determining FRET efficiency as shown in Figure 5. The emission spectrum of the donor must overlap the absorption spectrum of the acceptor for resonance energy transfer to occur. The more the spectra overlap, the stronger the FRET efficiency, but an overlap of at least 30% is needed.[1] If the spectra overlap too much, acceptor bleed-through and crosstalk can occur. Acceptor bleed-through happens when the acceptor absorbs at the wavelengths used to excite the donor. This causes the direct photo excitation of the acceptor. Crosstalk refers to donor emission detected in the acceptor emission channel.[4] J_{da} is determined by Equation 3 where f_D (λ) is the donor fluorescence per unit wavelength interval, and ε_A (λ) is the molar extinction coefficient of the acceptor at wavelength λ which is a measure of how strongly a substance absorbs light. When the fluorophore is irradiated by the laser, it must be able to absorb at that wavelength. The most common lasers are the argon and krypton-argon ion laser which emit at several wavelengths from 400-600 nm.[4] For measuring short distances of less than 2 nm, the donor and acceptor pair must have a short Förster radius. In order for R_0 to be small the pair must have small overlap, a low quantum yield, and a small extinction coefficient.

$$J_{da} = \int f_D(\lambda)\, \varepsilon_A(\lambda)\, \lambda^4\, d\lambda \,/\, \int f_D(\lambda)\, d\lambda \qquad (3)$$

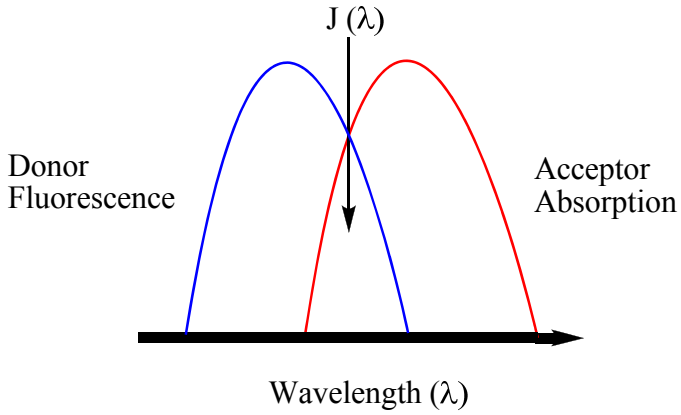


Figure 5: A representation of the donor and acceptor absorption and emission spectra.[5]

The angular orientation factor (κ^2) describes the relative orientations of the two fluorescent chromophores at the time of energy transfer. κ^2 introduces an uncertainty in FRET measurements, especially if the donor and acceptor are unable to rotate freely (Figure 6), for example when they are linked to a biomolecule. If the donor and acceptor probes are not free to undergo unrestricted rotation, the value of κ^2 can range between 0 and 4. κ^2 will have a value of 4 when the donor and acceptor are parallel to r, and a value of 0 when either the donor (or the acceptor) is perpendicular to the electric field produced by the acceptor (or donor). There is a higher probability of the value being closer to 0 so, for randomly oriented chromophores, $\langle\kappa^2\rangle = 2/3$. If the donor and acceptor probes are free to undergo unrestricted isotropic motion on the timescale of the measurement, $\kappa^2 = 2/3$ over time. The angular dependence is determined by

Equation 4, which relates κ^2 to the angles of the donor and acceptor chromophores transition dipoles relative to the line (r) connecting them at the time of energy transfer. θ_T is the angle between the donor (D) and acceptor (A), θ_D is the angle between the donor and r; θ_A is the angle between the acceptor and r; and Φ is the angle between the projections of D and A on a plane perpendicular to r.[1]

$$\kappa^2 = (\cos\theta_T - 3\cos\theta_D \cos\theta_A)^2 = (\sin\theta_D \sin\theta_A \cos\Phi - 2\cos\theta_D \cos\theta_A)^2 \qquad (4)$$

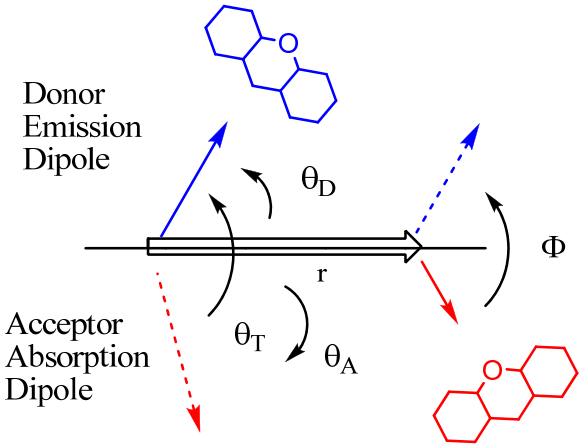


Figure 6: The angular orientation factor (κ^2) introduces an uncertainty in FRET measurements.[4]

FRET's efficiency is determined by the distance, spectral overlap and angular orientation of the two fluorescent chromophores. The goal of this research is the synthesis of a tetrahedral chromophore that will reduce the uncertainties and ensure a κ^2 value of 2/3. We plan to prepare chromophore arrays on tetrahedral scaffolds. An appropriate core for this chromophore assembly will separate individual chromophores enough to prevent electronic coupling, so that each one maintains its absorption and emission energies. The core will also place the

chromophores close enough for rapid intramolecular FRET to occur. Nancy Goroff performed semi-empirical quantum calculations (PM3) and found that using silicon as a core would situate the chromophores close enough for rapid FRET to occur and achieve isotropicity. The chromophores will still be far enough apart, that they each will remain electronically independent. Figure 7 shows the tetrahedral scaffold that we will be using.

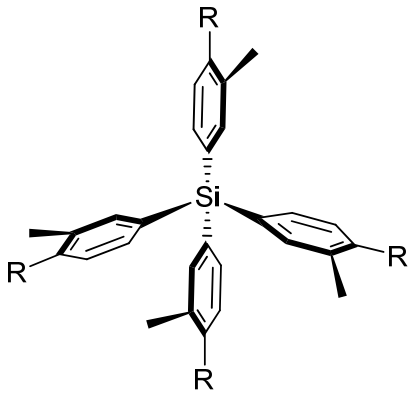


Figure 7: Tetrahedral Silane Chromophore Array.

Our project is based on studies done by Goodson and coworkers who have synthesized organic dendrimers.[7] In our tetrahedral arrays, each individual chromophore is in very close proximity to the others. The chromophores may interact by an intra-array FRET mechanism or true quantum coupling. When one of the chromophores within the array absorbs a photon, the excitation to the other chromophores happens on a subpicosecond timescale (880 fs).[7] The issue is whether the energy levels change as a result of the coupling or not. It was found that the energy transfer will follow an intra-array FRET mechanism as opposed to

quantum coupling. They used an adamantane core distyrylbenzene tetramer (tetrakis(3,5-di-tert-t-butylstyrylstillbene)methane) as shown in Figure 8. This rapid intra-array FRET mechanism will occur when one of the chromophores is excited, so the array will appear as a single, spherical chromophore. The appearance is similar to a single planar chromophore, such a fluorescein, rapidly rotating, which will ensure the maintenance of an isotropic system.

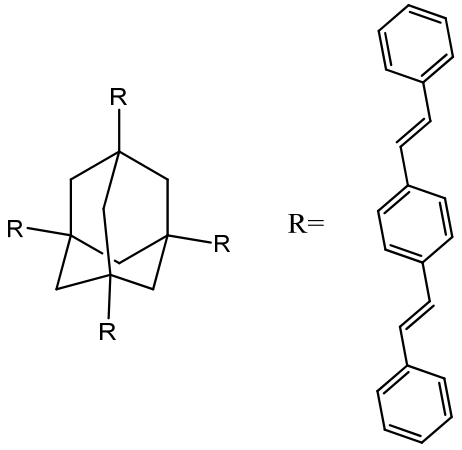


Figure 8: Adamantane cored distrylbenzene tetramer used to show that an inter-array FRET mechanism occurs on a femtosecond timescale.[7]

The individual chromophores in this project are organic dyes which are highly fluorescent xanthene derivatives, as shown in Figure 9. Fluorescein is the most widely used fluorophore for labeling and sensing biomolecules due to its high fluorescence quantum yield in aqueous solution.[8] It is also somewhat resistant to photobleaching and can be excited about 10,000 times before being destroyed by photodestruction.[1]

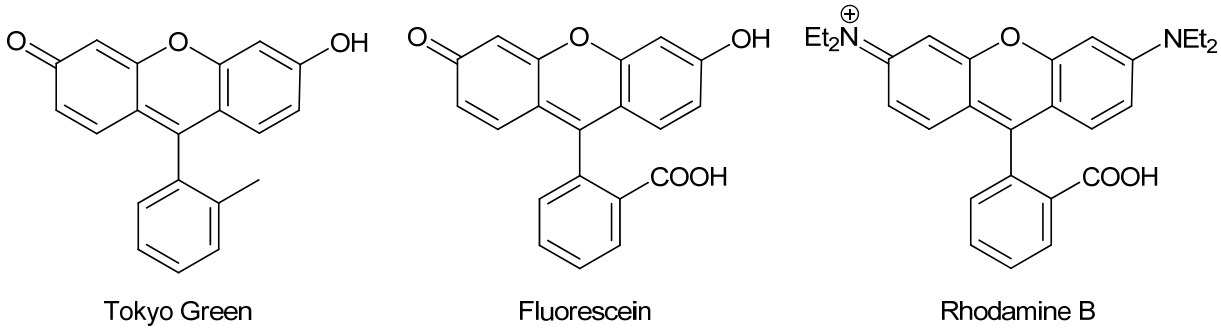


Figure 9: Organic dyes.

Our tetrahedral array is large compared to an individual chromophore and in order to use our arrays in FRET studies, the size must not add any new uncertainties. Nancy Goroff's calculations predict that this assembly will have a diameter of 1.5-1.8 nm. The diameter of an individual fluorescein along its dipole is measured to be 0.9 nm, but this difference in size should not have a big effect. When the array is rotating, the cross-section is similar to the size of an individual rotating chromophore, which is 1.7 nm. If the chromophores experience any quantum coupling their emission and absorption energies may be changed, making them unsuitable for studies using FRET. Using a pseudotetrahedral array with rhodamine B chromophore units, Nancy Goroff found there is no electronic coupling between the individual chromophores and almost no electron density was observed on the benzene moieties. The calculated HOMO-LUMO energy gap on an individual rhodamine B in the array is 0.27 eV, whereas an isolated rhodamine B is 0.26 eV.

II. Synthesis

A. Previous work

A previous group member, Lu Zhou, attempted the synthesis of a tetrahedral assembly of rhodamine B chromophores.[9] Her first efforts focused on trying to build the chromophores directly on the central silane core. This procedure however was unsuccessful, perhaps because rhodamine B exists in several charge states as well as two different isomers. Zhou also attempted to couple rhodamine B directly to $SiCl_4$. This approach involved synthesizing the chromophores individually and attaching them to the core all at once. There was difficulty in finding protecting groups that would withstand the n-BuLi used for this reaction, so fluorescein was used. Fluorescein can be protected using different groups that will not be cleaved by n-BuLi. Both reactions were unsuccessful.

n-BuLi is such a harsh chemical that very few protecting groups can withstand it. Triisopropylsilyl (TIPS) ether does not withstand the n-BuLi but methoxyethoxymethyl (MEM) and t-butyl-dimethylsilyl (TBS) groups have been found to work well.[6,10] A few other possible protection groups that can be tested include: methyl ethers, silyl ethers such as trimethylsilyl (TMS), and acetals such as methoxymethyl (MOM) ether.[11]

A new synthetic route was developed that involves the synthesis of a xanthene moiety coupled to a tetraaryl silane core, tetrakis(4-bromo-2-methylphenyl) silane (**8**). Since previous members have encountered difficulties

with the synthesis of the tetrahedral array using rhodamine B or fluorescein, as described above, this project now focuses on Tokyo Green, which has been found to have very similar emission and absorption and fluorescence efficiency to fluorescein.[6] Tokyo Green does not have the carboxylic acid group which had caused complications in previous studies (Figure 10).[6,12] The excitation wavelengths correspond well with argon and krypton ion laser wavelengths.

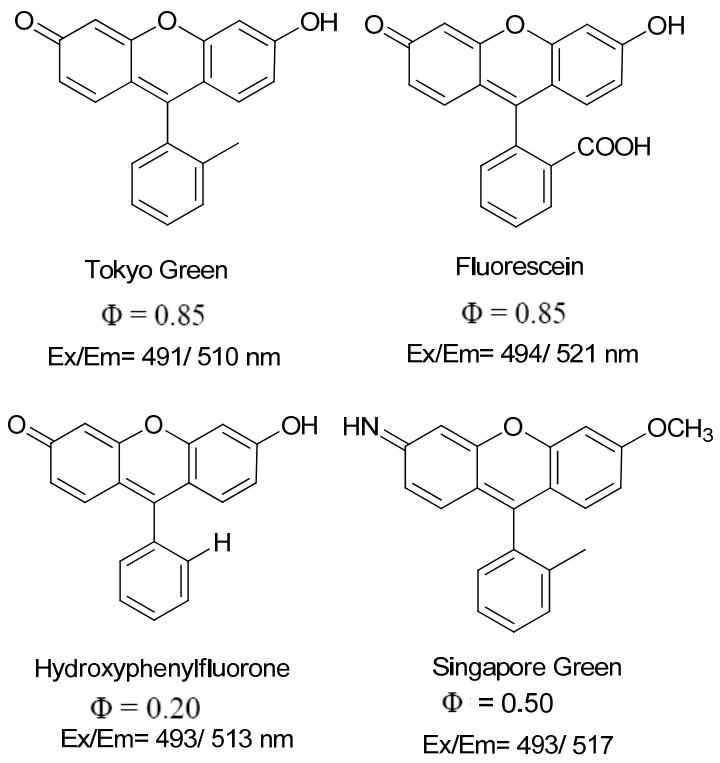


Figure 10: The fluorescence quantum yield and excitation/emission wavelengths of different chromophores.[6]

The carboxylic acid group of fluorescein was first thought necessary for the fluorescence of a compound, and removal was thought to decrease Φ_{fl}.

Kamiya and co-workers later found that the carboxylic acid was important only in keeping the xanthone (the fluorophore) and the benzene moieties orthogonal to each other. They came to this conclusion by synthesizing derivatives of fluorescein and comparing the fluorescence quantum yields. There is little or no interaction between the two parts. The carboxylic acid can therefore be replaced by a different functional group, in this case a methyl group (Figure 11), as long as the steric effect is similar. Figure 10 shows that as long as there is a functional group present, Φ_d is not greatly affected by the nature of the group. Tokyo Green and fluorescein both have Φ_d values of 0.85 but hydroxyphenylfluorone has a Φ_d value of 0.20. Derivatives with benzene moieties that have electron-deficient rings, and a higher oxidation potential (greater than 1.7 eV) are more fluorescent. Fluorescence also depends on the HOMO energy of the benzene moiety. Due to all these factors Tokyo Green is a very fluorescent molecule making it suitable for use in FRET.

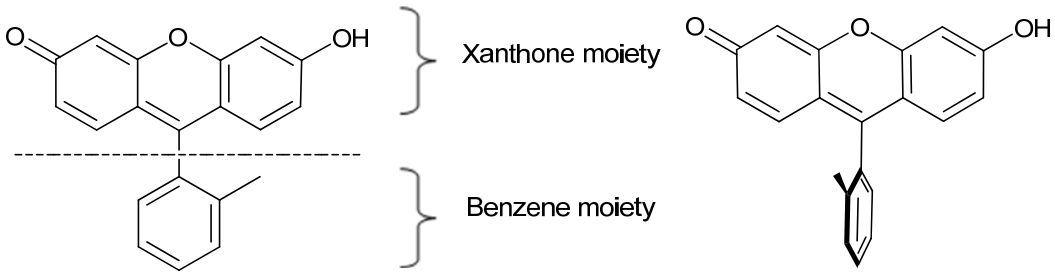
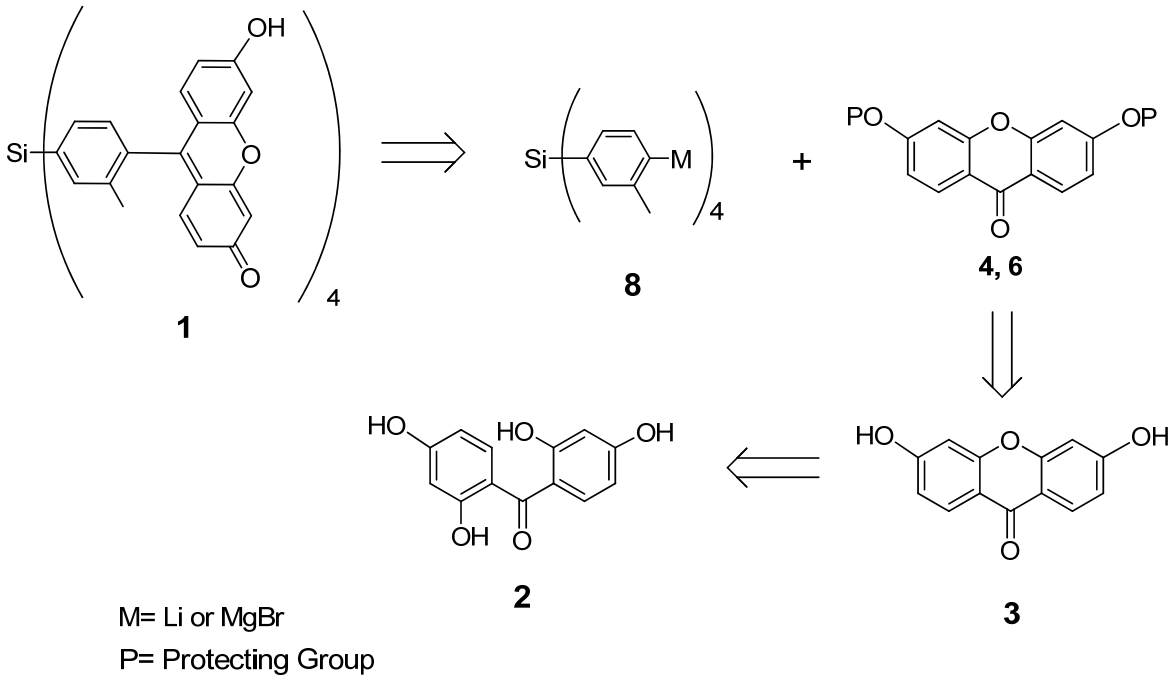


Figure 11: Tokyo green is composed of a xanthene moiety and a benzene moiety orthogonal to each other.[6]

In the current route (Scheme 1), the tetraaryl silane core and chromophore units are synthesized individually before forming the tetrahedral array. The chromophores are connected to the core by reaction of an aryl lithium or Grignard species (**8**) with the protected xanthone (**4** or **6**).



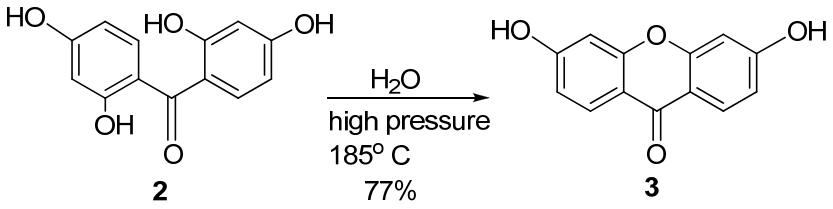
Scheme 1: Planned synthetic route.

B. Synthesis of the xanthone moiety

To produce the xanthone moiety,[13] 2,2',4,4'-tetrahydroxybenzophenone (**2**)**,** which is an orange powdery solid, was heated with water to about 185°C under high pressure (in a pressure tube) for approximately 20 hours producing the orange frothy solid 3,6-dihydroxyxanthone (**3**). The literature procedure calls for reaction for 2 hours at 220-220°C but these conditions were found to produce low

yields. Previous group member Lu Zhou found that when compound **2** reacts with water overnight a higher yield was obtained, so the literature synthesis was modified.[9]

The starting material is soluble in hot water but when one filters the product under vacuum and washes the solid with hot water, some unreacted starting material remains. The ^{1}H NMR shows peaks corresponding to the starting materials as well as the product. The 3, 6-dihydroxyxanthone (**3**) can be purified by heating the solid under reflux in water. This procedure produced pure product but in low yields (about 25%). One reason may be that some of the product undergoes the reverse reaction back to the starting material. We carried out a series of solubility tests to find a solvent that could dissolve the starting material, but not the product. The solvent that worked the best was ethyl acetate. Using this solvent in an Erlenmeyer flask with heating to dissolve the starting material from the crude product, and then filtering again, the yield was increased to 77%. The xanthone was kept in a vacuum oven overnight to make sure it stayed dry. This procedure was repeated a few times to obtain more material and it was recently found that using a new bottle of the starting material, 2,2',4,4'-tetrahydroxybenzophenone (**2**)**,** produces a pure product in which no further purification steps were needed, in 85.5% yield.



Scheme 2: Synthesis of the Xanthone moiety.

C. Synthesis of Tokyo Green (Grignard Reaction)

The two hydroxyl groups on the xanthone need to be protected in order to go on to the next step, and Peterson and coworkers reported that methoxyethoxymethyl groups (MEM) worked well in preparing the fluorescein derivative Pennsylvania Green (Figure 12).[10] Adopting this method to the synthesis of Tokyo Green (**5**) (Scheme 3), the xanthone (**3**) was dissolved in dry THF under argon, forming an orange solution. The solution was cooled, sodium hydride was added and the reaction mixture was stirred for 30 minutes. 2-Methoxyethoxymethyl chloride, MEM-Cl; was added, and the reaction mixture was stirred overnight at room temperature. NH_4Cl was used to quench the reaction, producing a large amount of bubbling, followed by extraction with ethyl acetate. After flash column chromatography was used to purify it, the solvent was removed *in vacuo* to give a white solid (**4**), in 63.4% yield.

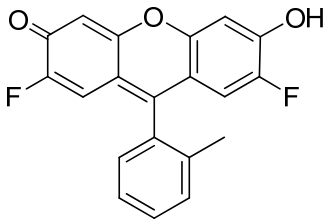
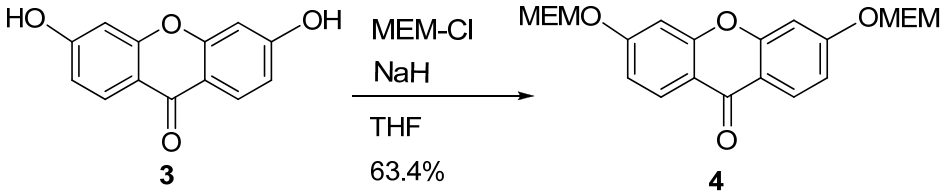
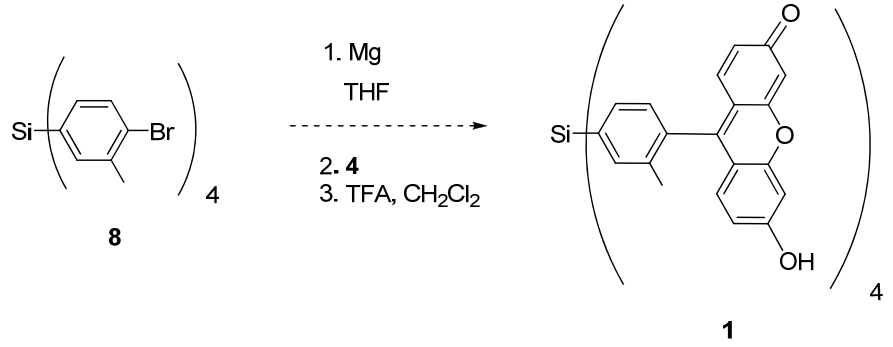


Figure 12: The Pennsylvania Green Fluorophore.[10]



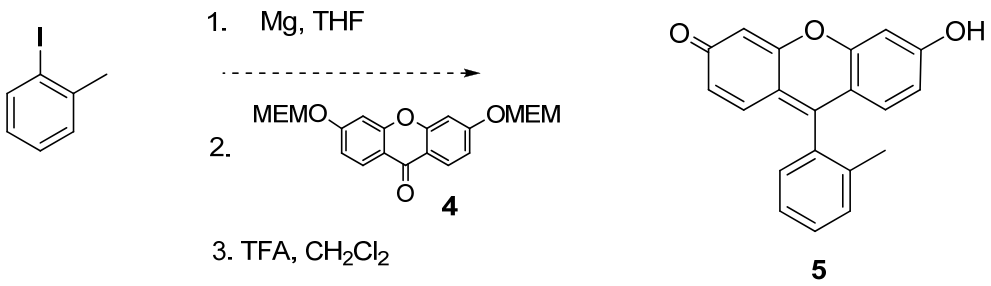
Scheme 3: Synthesis of bis-MEM xanthone.[10]

The first attempt at coupling the xanthene moiety to the tetraaryl core used a Grignard reaction (Scheme 4). A previous group member Sarah Richards had attempted this reaction repeatedly but obtained no informative results.



Scheme 4: Coupling of the xanthene moiety to the tetraaryl core using a Grignard reaction.[14]

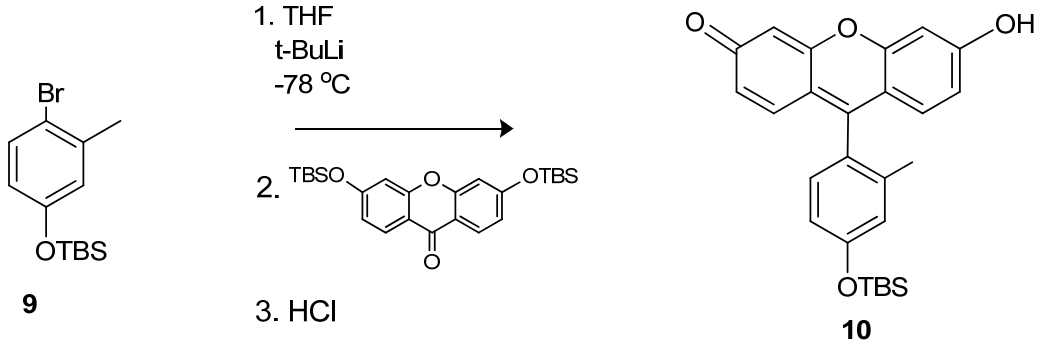
To test the methodology, a model experiment was carried out using Yasuteru Urano's reported synthesis of the chromophore Tokyo Green (**5**).[6] Grignards work well with aryl iodides so this route was modified to use iodotoluene instead of bromotoluene. This reaction is outlined in Scheme 5. In the future, the silane core could be modified to tetrakis(4-iodo-2-methylphenyl) silane. Special care was taken to make sure no water entered the system due to the Grignards sensitivity to it. Dry magnesium ribbon, THF, and 2-iodotoluene were heated under reflux. Bis-MEM xanthone (**4**) dissolved in THF, was added after the flask was cooled. After 20 hours, this mixture was quenched with MeOH. The result was a green solid which did not dry fully while on the rotary evaporator. After the xanthone was deprotected with trifluoroacetic acid, the solid turn dark red. The solid was purified by flash column chromatography. ^{1}H NMR was taken in DMSO but no peaks were observed. Richards had also carried out this reaction, and obtained a crude product mixture that appeared to contain Tokyo Green, but she was unable to isolate the product.



Scheme 5: Attempt at coupling 2-iodotoluene to bis-MEM xanthone to synthesize Tokyo Green.[14]

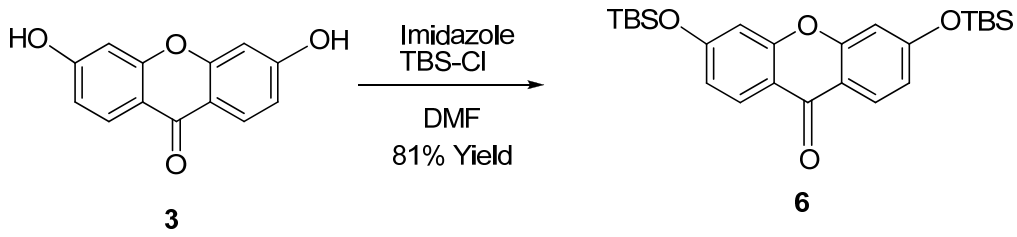
D. Synthesis of Tokyo Green (Butyl Lithium Reaction)

The Grignard reaction proved difficult, so a second route to Tokyo Green was attempted using t-Butly Lithium instead. t-BuLi lithiates 2-bromotoluene, enabling reaction of the benzene moiety with the protected xanthone. This method was reported by Kamiya, et.al who used a similar method to prepare fluorescein derivative **10** successfully from compound **9** (Scheme 6).[15] The reaction is less sensitive to water and is faster overall than the Grignard reaction.



Scheme 6: The model reaction used for the synthesis of Tokyo Green.[15]

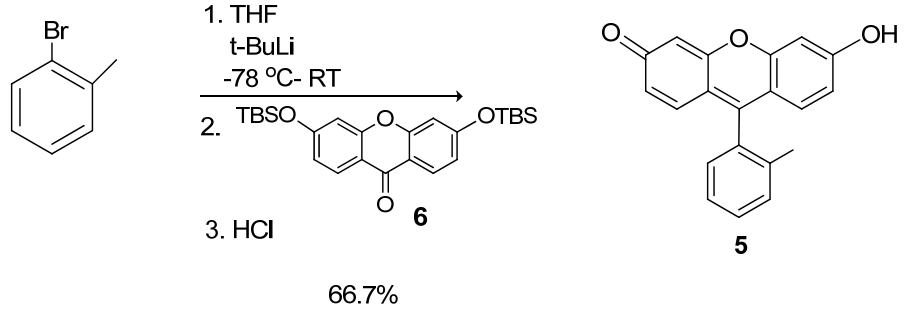
t-Butyldimethylsilane (TBS) was found to be a good protecting group for this molecule.[16] Bis-TBS xanthone (**6**) was prepared following a literature procedure modified by Sarah J. Richards.[14] Xanthone (**3**) was added to a flask and was left under high vacuum for 15 minutes. Imidazole was then added to the same flask. The mixture was again pumped for 15 minutes. TBS-Cl that had been under high vacuum for 15 minutes was then added to the mixture. Each reagent was added separately, while pumping, to make sure no water interfered with the reaction. The solids were dissolved in dry DMF, making a cloudy orange mixture. After the work up, shiny white crystals (**6**) were produced in 81.5% yield. This procedure was repeated a few times to obtain more material.



Scheme 7: Synthesis of bis-TBS xanthone.[16]

To prepare Tokyo Green from protected xanthone (**6**), the aryl lithium reagent was prepared from 2-Bromotoluene. Special care was taken to make sure water did not interfere with the system. t-BuLi was added dropwise to form 2-lithiumtoluene. To make sure that t-butyl lithium was reacting with 2-bromotoluene to form the lithium complex, the solution was warmed to room

temperature. The mixture was cooled back down to -78°C and protected xanthone (**6**), dissolved in THF was added to the reaction mixture, which was warmed again to ensure reaction. HCl was added to quench the reaction, cleaving the protecting groups. The color darkened and the liquid started to glow green slightly. An orange solid crashed out of solution and was filtered out (66.8% yield). ^{1}H NMR showed that this compound was pure Tokyo Green (**5**). When dissolved in methanol this orange solid glows bright green.

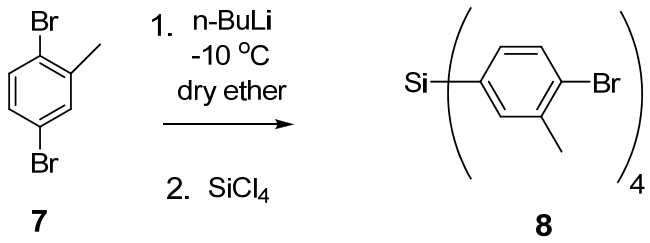


Scheme 8: Synthesis of Tokyo Green.

This reaction was also carried out using n-BuLi, but produced a lower yield of 45%. MEM-protected xanthone was also used and also gave a lower yield. Deprotection of the MEM groups with HCl gave a 25% yield, but the literature procedure, which calls for deprotection with TFA in dichloromethane[10], gave a crude product which was a red solid that did not completely dry. This product has not been identified.

E. Synthesis of the tetraaryl silane core

The success in preparing Tokyo Green from bromotoluene suggests that a bromoaryl core should be effective for making the target array. Fournier and coworkers have reported the synthesis of tetrakis(4-bromophenyl) silane.[17] Their procedure was adapted to make compound **8**. Fournier used 1,4-bromobenzene but our procedure uses 2,5-dibromotoluene (7) as shown in Scheme 9.



7 **8**

Scheme 9: Tetraaryal silane core.[17]

The 2,5-dibromotoluene (**7**) was dissolved in dry ether. Dropwise, n-butyl lithium was added, and the solution stayed a slightly cloudy white color. $SiCl_4$ was added dropwise, and the mixture turned completely white and cloudy. We found that to maintain the activity of $SiCl_4$, it is important to store the reagent at room temperature rather than in the freezer, where condensed water can make it hydrolyze. After quenching with HCl and the work-up, the crude product is a yellow oil. This oil was recrystallized from $CHCl_3$/ethanol several times to produce shiny white crystals (**8**) (13%), pure by ^1H and ^{13}C NMR. This reaction was repeated several times to obtain more material, but we have not been able to

obtain a higher yield.[9] Figure 13 depicts the X-ray structure of the core, determined by a crystal prepared by Lu Zhou. This reaction was also carried out in THF, but that attempt was unsuccessful.[9]

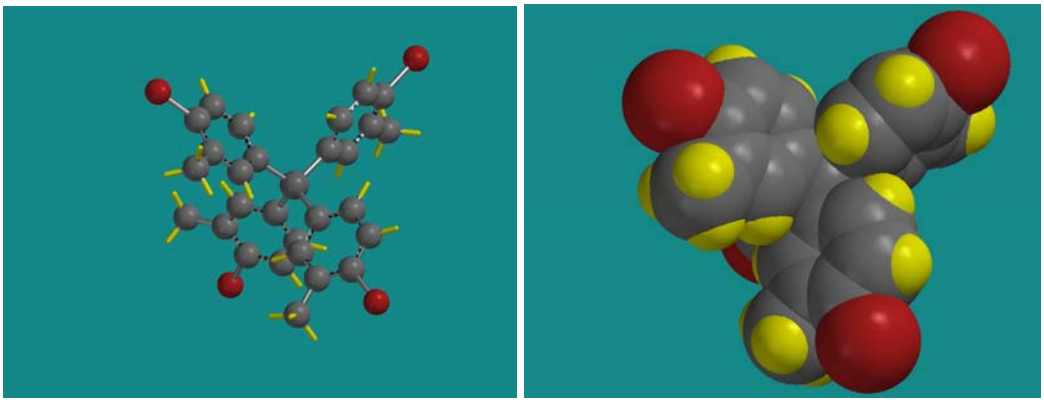
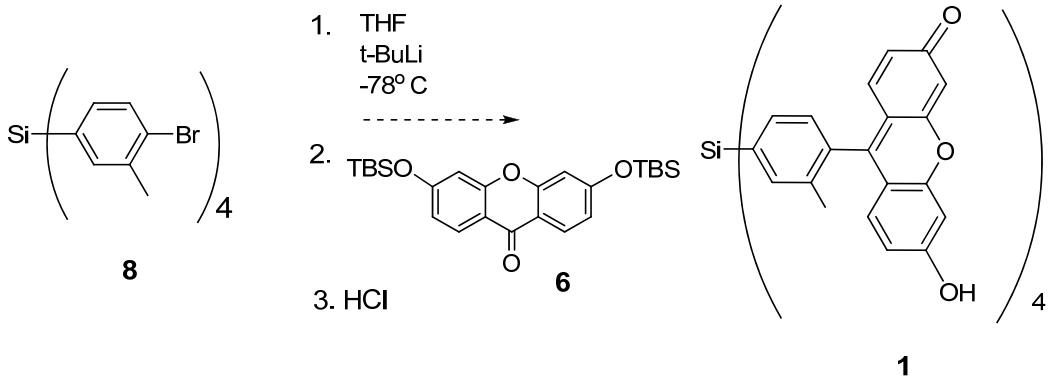


Figure 13: Crystal structure of tetrakis(4-bromo-2-methylphenyl)silane 8.[9]

F. Attempted Synthesis of Silyl Tetrachromophore Array

Having success in preparing Tokyo Green by Kamiya's method and with the tetraaryl core in hand, we turned to the synthesis of the tetrafluoresceinyl silane itself (Scheme 10).[17] To a solution of Tetrakis(4-bromo-2-methylphenyl)silane **8** dissolved in dry THF, t-BuLi was added drop wise and this solution immediately turned pink. The color darkened to a light purple which might be due to the t-BuLi reacting with the core. A solution of xanthone bis-TBS ether (**6**) was added and the solution turned orange. HCl was added to quench the reaction and cleave the protecting groups. A yellow solid formed, determined by ^{1}H NMR to be the starting xanthone bis-TBS ether (**6**). The solution glowed green

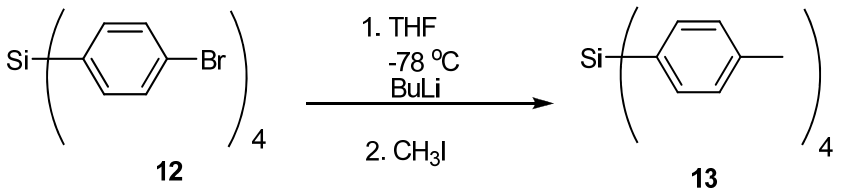
slightly suggesting the production of a small amount of chromophore. Thus the desired reaction may take place in low yield.



Scheme 10: Proposed synthetic method of Tokyo Green chromophores built up on a silane core.

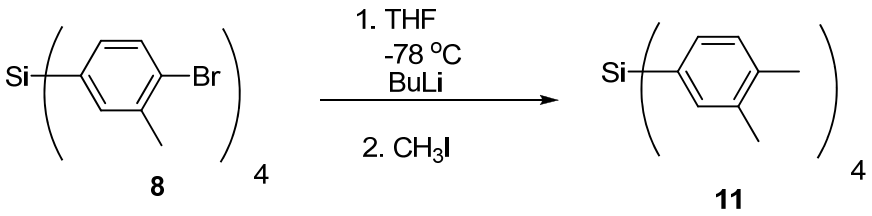
This procedure was repeated a few times, yielding a red solid each time. It was observed that when a yellow solid formed, either the THF was not dry enough or the t-BuLi was not reactive enough. When a fresh THF still or fresh bottle of t-BuLi was used this red solid formed. ^{1}H NMR again shows unreacted starting materials. A TLC was taken in ethyl acetate and showed a spot corresponding to the starting TBS protected xanthone indicating the reaction was not successful. Multiple attempts provided no more success. The reaction time was varied, the reaction mixture was left to run over night, but with no visible effect. In another attempt, methanolic HCl was used to quench the reaction instead of the aqueous HCl, but again, this change made no difference.

To make sure that the initial lithiation step occurred as desired, we carried out the methylation reaction shown in Scheme 11.[17] This procedure was reported by Fournier.



Scheme 11: Fournier's synthesis of tetrakis(4-methylphenyl)silane.[17]

Although compound **13** was synthesized in a low yield, we modified the reaction and used our silane core (**8**) which is shown in Scheme 12.



Scheme 12: Model reaction of the silane core.

Tetrakis(4-bromo-2-methylphenyl)silane (**8**), dissolved in dry THF was cooled to -78°C. n-BuLi was added dropwise, and this solution immediately turned pink. The color slowly disappeared. CH_3I was added dropwise to the solution, which was then allowed to stirr overnight. The solution looked yellow and cloudy. Saturated $Na_2S_2O_3$ was added to react with any iodine in the solution. The yellow/brown solid was recrystallized from toluene to form a yellow solid which ^1H NMR demonstrated to be compound **11**, although in a small yield

(14%)**.** We can conclude that the pink color after the addition of the n-BuLi indicates that in our previous reaction, we were achieving lithiation of the silane core.

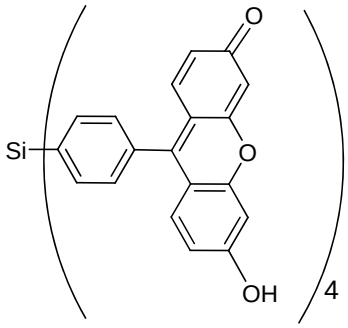
Since the above reaction worked we concluded that the bromine at that site is able to react with butyl lithium. Although a methyl group is much smaller than the xanthone moiety needed for the tetraaryl reaction, the effort to make the tetrachromophore silane was repeated. n-BuLi was added drop wise to a solution of Tetrakis(4-bromo-2-methylphenyl)silane (**8**) which immediately turned the solution pink. After 45 minutes the color disappeared. This observation was seen in the procedure outlined in Scheme **12**. Xanthone bis-TBS ether (**6**) was added in the same fashion as called for in the Tokyo Green reaction reported by Kamiya. This solution turned orange. After reacting for approximately 20 hours the solution turned bright yellow and HCl was added to quench the reaction turning it to a darker yellow/orange color. The aqueous and organic layers were separated (both were yellow and glowing slightly). After the solvent was removed *in vacuo* the organic layer produced a yellow solid which ^{1}H NMR showed to be a mixture of starting materials, showing unknown peaks in the aryl region. This reaction was repeated several times and similar results were obtained. The final product has not yet been isolated or demonstrated to be present in the mixture.

A similar reaction was also attempted using MEM protected xanthone instead of TBS- protected xanthone. A red solid formed after deprotection with

TFA in CH_2Cl_2, and TLC on this material indicated the presence of protected and unprotected xanthone, as well as an orange spot at the base line which did not move. A column was run to try to separate these unwanted starting materials. The NMR showed a mixture of peaks in the aryl region. Since bis-MEM xanthone did not produce a high yield in making Tokyo Green, it was decided that the bis-TBS protected xanthone was the better choice.

The reaction conditions involved using one equivalent of the silane core to eight equivalents of n-BuLi and eight equivalents of bis-TBS xanthone. Kamiya's procedure, on the other hand, calls for the protected xanthone to be the limiting reagent. Therefore, the next time this reaction was repeated, a 1:4 ratio of the silane core:bis-TBS xanthone was used. After the workup a yellow solid formed and NMR showed a mixture of peaks in the aryl region.

The reaction was also carried out using Fournier's tetraaryl silane to try to produce compound **14**. As the n-BuLi was added to the solution of the tetraaryl silane dissolved in THF, the color turned pink. Bis-TBS xanthone was added and this mixture was left overnight. After quenching with HCl the color was yellow-orange and there was a slight green glow. A red solid was produced. Again the NMR showed a mixture of peaks in the aryl region.



14

Figure 14: A tetrahedral assembly of hydroxyphenylfluorone.

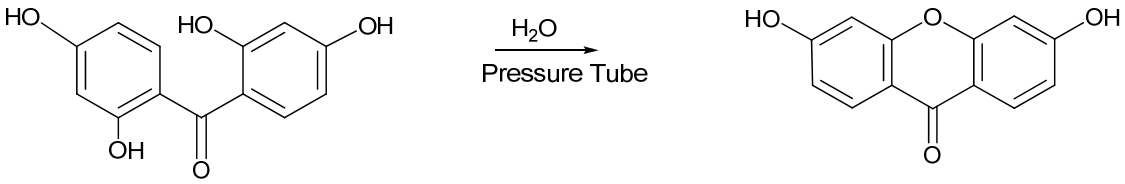
III. Future Work:

The methylation reaction outlined in Scheme 12 was completed successfully but with a low yield. This result indicates that lithiation of the core takes place but not as completely as desired. More work may have to be done to try to increase this yield before we can carry out the reaction outlined in Scheme **10** to obtain the desired product in good yields. Once the conditions for the methylation have been optimized, the synthesis of the tetrachromophore array will be attempted again.

After the final product (**1**) has been synthesized, the physical, chemical, and optical properties will be studied. The absorption and emission spectra will be measured, as well as the water solubility and stability. We will also confirm that the tetrafluoreceinly silane is an isotropic absorber and emitter. To use this array for FRET studies we will have to modify the core with a linker to provide a way of attaching the chromophore array to biomolecules of interest.

IV. Experimental:

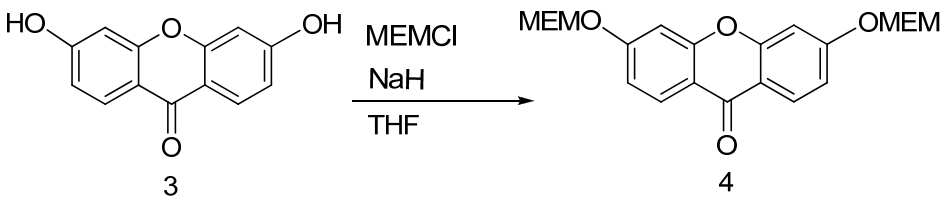
3,6-Dihydroxyxanthone:



2 3

3.0025 g of 2,2',4,4'-tetrahydroxybenzophenone **2** and 25 mL of deionized water were put into a 50 mL pressure tube. The tube was put in an oil bath heated to ~190°C. The reaction mixture was stirred at this temperature overnight (approximately 20 hours). The solid was filtered off by gravity filtration and washed with hot water (200 mL, 65 °C). To purify the product, ~10-15 mL of ethyl acetate was added to the solid in a 250 mL Erlenmeyer flask and filtered. What remained was a red-brown solid **3**, (2.3836 mg, 77%). Mp > 350 °C. ^{1}H NMR (400 MHz, DMSO) δ 10.82 (s, 2H), 7.98 (d, 2H, J = 8.8 Hz), 6.82-6.88 (m, 4H).[13]

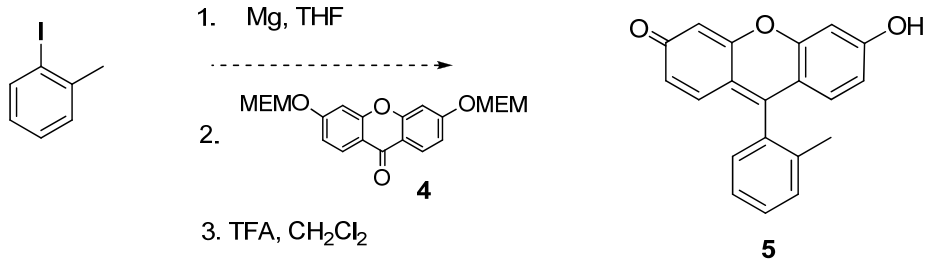
3,6-Bis-(2-methoxy-ethoxymethoxy)-xanthen-9-one:



3 4

3,6-Dihydroxyxanthone **3** (103 mg, 0.425 mmol) was added to dry THF (10 mL) while stirring under argon. This was put in an ice bath and cooled to 4°C. 82.6 mg (2.065 mmol) NaH was added. This solution was stirred for 30 minutes and 2-methoxyethoxymethyl chloride, MEM-Cl; (0.2357 mL, 2.065 mmol) was added. This reaction mixture was left to warm to room temperature and stirred overnight, approximately 20 hours. NH_4Cl (5 ml) was used to quench the reaction followed by extraction with ethyl acetate (3 x 100 mL). The organic layer was washed with saturated aqueous NaCl (20 mL) and dried with sodium sulfate. The solvent was removed *in vacuo*. The orange solid was dissolved in dichloromethane/methanol and flash column chromatography (1:1, hexanes/ethyl acetate) was used to purify. A white solid (**4**) was produced (110 mg, 63.4% yield) as white crystals. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.23 (d, J = 8.8 Hz, 2H), 7.05 (d, J = 7.8 Hz, 2H), 6.99 (dd, J = 8.4, 5.7 Hz, 2H), 5.35 (s, 4H), 3.85-3.87 (m, 4H), 3.54 (m, 4H), 3.38 (s, 6H).[14]

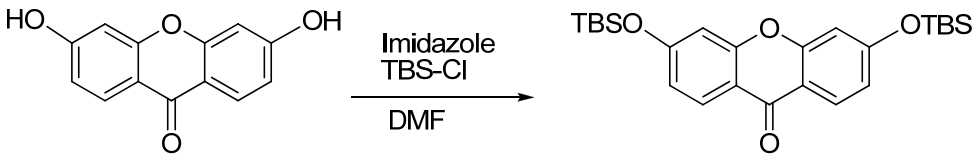
Model reaction 1, Tokyo Green:



Dry magnesium ribbon (87.1261 mg, 3.58 mmol) (which was dried in a vacuum oven for 2 days at 80°C with fresh P_2O_5) was added to a flame dried round bottom flask under argon. Special care was taken to make sure no water entered the system. THF (4 mL), and 2-iodotoluene (0.15 mL, 1.182 mmol) were added and the reaction mixture was refluxed for 2 hours. The temperature rose too high (140°C) which may have burned the magnesium and ruined the Grignard reaction. While this was being cooled in ice after the 2 hours, the second flask was being prepared. It was also flame dried under vacuum and flooded with argon. Bis-MEM xanthone **4** (100 mg, 0.239 mmol) was added along with THF (5 mL). A flame dried cannula was used to transfer the contents of the second RBF into the first one. The reaction was allowed to warm to room temperature over 20 hours and then quenched with MeOH (5 mL). The solvent was removed *in vacuo* and the result was a green solid but it did not fully dry. To deprotect this compound, CH_2Cl_2 (10 mL) and TFA (1 mL) were added. This step made the solid turn dark red. After stirring for 3 hours and the solvent was removed *in vacuo.* The solid

was purified by flash column chromatography (1:20 $MeOH/CH_2Cl_2$). ^1H NMR was taken in DMSO but no peaks were present.

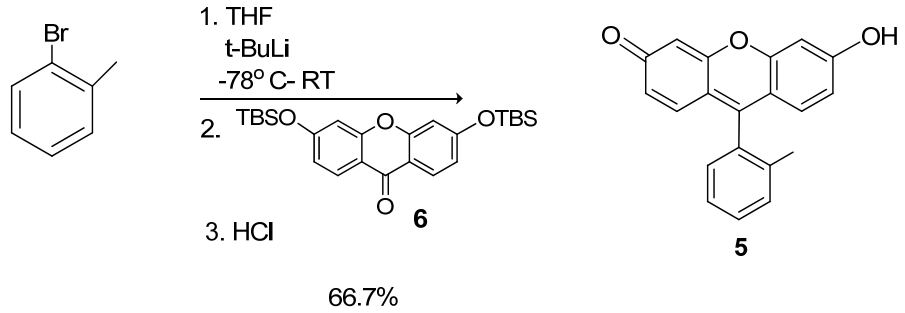
Xanthone bis-TBS ether:



3 6

To a flame dried RBF (while pumping) 3,6-dihydroxyxanthone **3** (605 mg, 2.73 mmol) was added. Imidazole (2.025 g, 29.55 mmol) was added and this was pumped for 5 minutes before TBS-Cl (2.6 g, 17.25 mmol) was added. The reaction mixture was again pumped for 5 minutes and dry DMF (40 mL) was added. This solution was stirred overnight under argon. Then the reaction mixture was extracted with toluene (50 mL), washed with water (2 x 60 mL), and dried with magnesium sulfate. The solvent was evaporated *in vacuo* yielding an orange solid. This solid was recrystallized from chloroform/ cold reagent alcohol to give shiny white crystals **6** (937.8 mg, 81.5%). MP 152-154 °C. ^1H-NMR (400 MHz, $CDCl_3$), δ 0.29 (s, 12 H), 1.01 (s, 18 H), 6.83-6.86 (m, 4H), 8.19-8.21 (m, 2H, J = 9.2 Hz).[15]

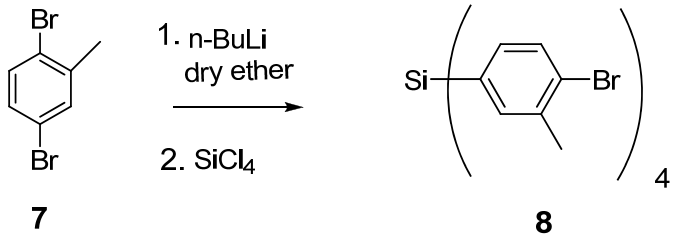
Model reaction 2, Tokyo Green:



66.7%

2-Bromotoluene (0.06 mL, 0.46 mmol) and THF (1 mL) were added to a flame dried RBF filled with argon and cooled to -78°C. t- Butyllithium (0.9 mL, 1.35mmol) was added drop wise to form 2-lithiumtoluene. This solution was warmed to room temperature and then cooled back down to -78°C. Xanthone bis-TBS ether **6** (106.4 mg, 0.23 mmol) was dissolve in 2 ml THF in a second flame dried RBF and added to the reaction mixture which was stirred for 30 minutes and warmed to room temperature. The solution was cooled back down to 0°C and ~3-4 mL HCl was added to quench the reaction. A yellow solid formed and the flask was left to warm for another half hour. The color darkened and the yellow-orange solid was filtered out (**5**) (50 mg, 66.8%). ^{1}H-NMR (400 MHz, CD_3OD), δ 7.495-7.647 (m, 5H), 7.32-7.34 (m, 3H), 7.202- 7.224 (dd, J = 9, 2 Hz, 2H), 2.056 (s, 3H).[6]

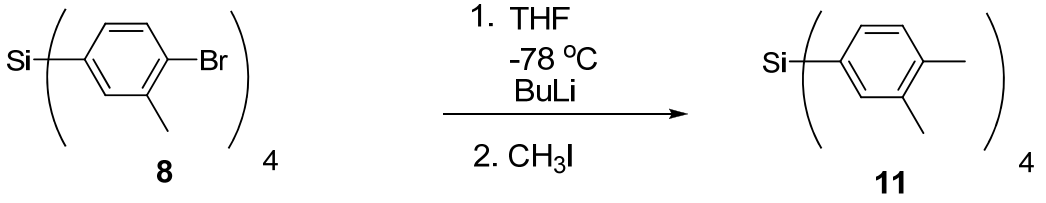
Tetrakis(4-bromo-2-methylphenyl)silane:[3]



7 **8**

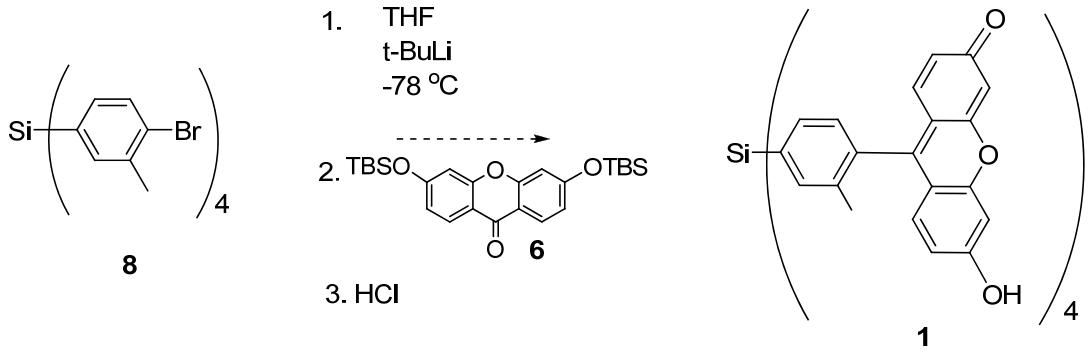
In a flask filled with argon, 2,5-dibromotoluene **7** (4.14 mL, 30.0 mmol) and dry ether (60 mL) were cooled to -10 °C. Dropwise, n-butyl lithium (18.78 mL, 30.0 mmol) was added, and the solution was stirred at -10 °C for 30 min. $SiCl_4$ (1 mL, 7.5 mmol) was added dropwise, and the mixture was kept at -10 °C for an additional 45 min. The reaction mixture was warmed to room temperature for 3 hours. 10 mL Cold HCl (1 M, aq) was added to quench the reaction. The mixture was then extracted with ether (3 x 50 mL), washed with brine (100 mL) and water (100mL) and dried over anhydrous magnesium sulfite. The solvent was removed *in vacuo* and the residue left was a yellow oil. This solid was recrystallized from CHCl3/ethanol several times to yield shiny white crystals (**8**) (703.8 mg, 13.3 %). MP 174-186 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.55-7.58 (d, 4H, J = 7.5 Hz), 7.32-7.33 (d, 4H, J = 0.8 Hz), 7.15-7.17 (m, 4H), 2.38 (s, 12H), ^{13}C NMR (400 MHz, $CDCl_3$) δ 138.3, 137.7, 135.0, 132.3, 132.2, 127.8, 23.0.[16]

Tetrakis(3,4-dimethylphenyl)silane:



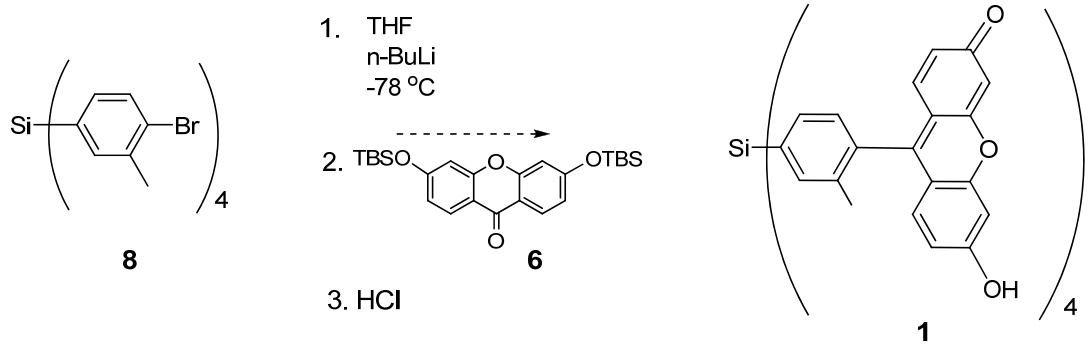
Tetrakis(4-bromo-2-methylphenyl)silane **8** (50 mg 0.07 mmol) dissolved in 10 mL dry THF was cooled to -78°C. n-BuLi (0.45 mL) was added dropwise. This solution was left to react for 45 minutes and the color dissappeared. 0.1 mL CH_3I was added dropwise to the solution which was then stirred overnight. The solution looked yellow and cloudy. Saturated $Na_2S_2O_3$ was added to react with unreacted iodine in the solution and the solvent was removed *in vacuo* and extracted with ethyl acetate. The yellow/brown solid was recrystallized from toluene to form a yellow solid (7 mg 14% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.287 (s, 12H), 2.35 (s, 12H), 7.21-7.18 (d, 4H, J = 7.5 Hz), 7.37-7.36 (d, 4H, J = 2 Hz), 7.40 (s, 4H).[9]

Tetrafluoresceinyl silane:



In an RBF filled with argon, tetrakis(4-bromo-2-methylphenyl)silane **8** (52.7 mg, 0.0762 mmol) was dissolved in 0.3 mL dry THF. This solution was cooled to -78°C and 0.4 mL (0.57 mmol) t-BuLi was added dropwise. This mixture was kept at -78°C for 15 minutes. While this solution was stirring, a solution of 253 mg (0.57 mmol) xanthone bis-TBS ether **6** and 5 mL THF was being prepared in a separate RBF filled with argon. The first solution was warmed to room temperature for 15 minutes to make sure the silane core was lithiated by the t-BuLi, then it was cooled back down to -78°C and the second solution was added. This mixture was stirred for 30 minutes and warmed to room temperature. The solution was cooled back down to 0°C and ~3-4 mL HCl was added to quench the reaction and this solution was warmed to room temperature and a yellow solid formed (187 mg) which was found to be xanthone bis-TBS ether **6**.

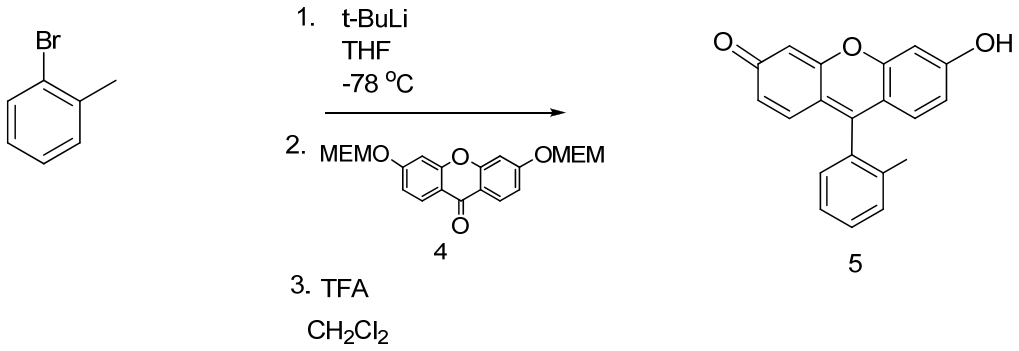
Tetrafluoresceinyl silane:



100 mg of Tetrakis(4-bromo-2-methylphenyl)silane **8** dissolved dry THF (10 mL) was cooled to -78°C. This was a clear solution. n-BuLi (0.45 mL, 1.6M in Hexanes) was added dropwise and allowed to react for 45 minutes. While this solution was stirring, a solution of 500 mg (1 mmol) xanthone bis-TBS ether **6** in 10 mL THF was prepared in a separate RBF filled with argon. The first solution was warmed to room temperature for 15 minutes to make sure the silane core was lithiated by the n-BuLi, then it was cooled back down to -78°C and the second solution was added dropwise. This solution was stirred for 2 hours at -78°C and warmed to room temperature overnight. After reacting for approximately 20 hours the solution was cooled back down to 0°C and 10 mL of cold HCl was added to quench the reaction and the color darkened to a yellow/orange. This mixture was warmed to room temperature, and left to react for about an hour. The aqueous and organic layers were separated. After the solvent was removed *in vacuo*, the

organic layer produced a yellow solid which proved to be a mixture of starting materials by ^{1}H NMR.

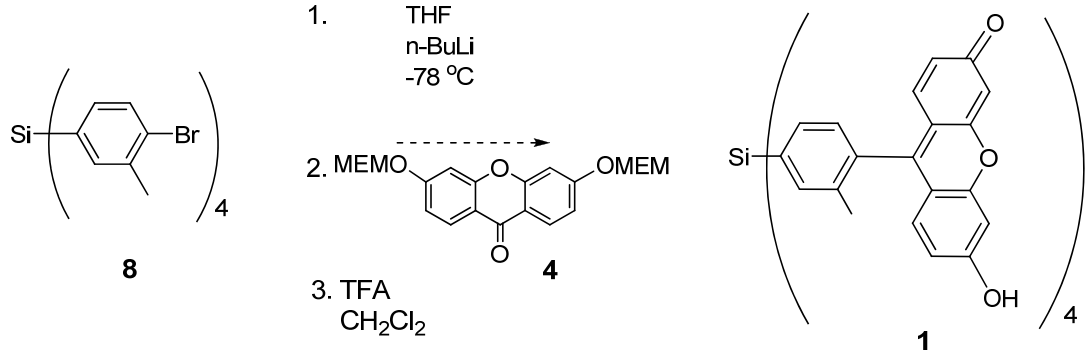
Tokyo Green:



2-Bromotoluene (0.12 mL, 0.92 mmol) and THF (2 mL) were added to a flame dried round bottem flask filled with argon and cooled to -78°C. t- Butyllithium (1.68 mL, 2.70 mmol) was added drop wise to form 2-lithiumtoluene. This solution was warmed to room temperature and then cooled back down to -78°C. Bis-MEM xanthone **4** (200 mg, 0.46 mmol) was dissolve in 4 ml THF in a second flame dried RBF and added to the reaction mixture which was stirred for 30 minutes and warmed to room temperature. The solution was cooled back down to 0°C where MeOH was added to quench. The solvent was removed *in vacuo* and the result was a red solid but it did not fully dry. To deprotect this compound, CH_2Cl_2 (10 mL) and TFA (1 mL) were added. The solvent was removed *in vacuo*

and crude Tokyo green (an orange liquid/solid) formed. ^{1}H-NMR (300 MHz, CD_3OD), d 7.495- 7.647 (m, 5H), 7.32-7.34 (m, 3H), 7.202- 7.224 (dd, J = 9, 2 Hz, 2H), 2.056 (s, 3H).[6] An accurate yield could not be calculated.

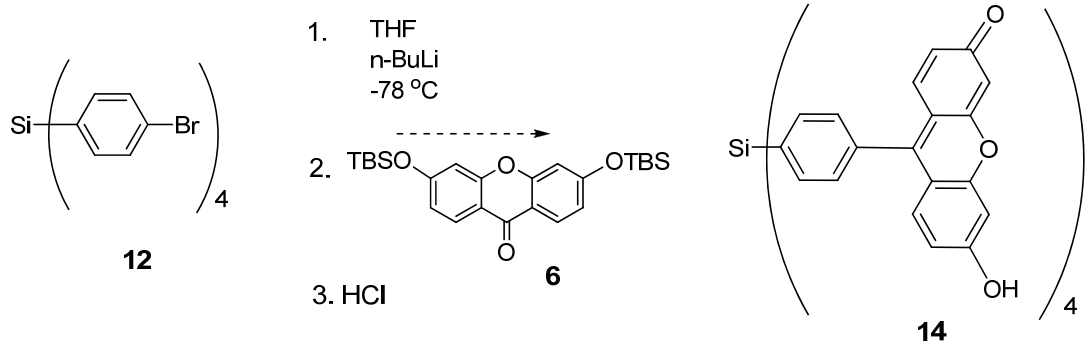
Tetrafluorescinyl silane:



100 mg of Tetrakis(4-bromo-2-methylphenyl)silane **8** dissolved dry THF (10 mL) was cooled to -78°C. n-BuLi (0.45 mL, 1.6M in hexanes) was added drop wise and this was left to react for 45 minutes. While this solution was stirring, a solution of Bis-MEM xanthone **4** in 10 mL THF was prepared in a separate RBF filled with argon. The first solution was warmed to room temperature for 15 minutes to make sure the silane core was lithiated by the n-BuLi, then it was cooled back down to -78°C and the second solution was added dropwise. This mixture was stirred for 2 hours at -78°C and warmed to room temperature overnight. After reacting for approximately 20 hours the solution was cooled back

down to 0°C and quenched with MeOH (5 mL). The solvent was removed *in vacuo* and the result was a red solid but it did not fully dry. To deprotect this compound, CH_2Cl_2 (10 mL) and TFA (1 mL) were added. After stirring for 3 hours the solvent was removed *in vacuo.* ^{1}H NMR was taken of this red wet solid but it showed to be a mixture of starting materials and had unknown peaks in the aryl region.

Model reaction of a tetrahedral assemble of chromophores:



55 mg (0.07 mmol) of compound 13 was dissolved in 1 mL of THF. This was cooled to -78°C. 0.4 mL of n-BuLi (1.5 M in hexanes) was added drop wise and this solution was left to react for a half hour. This solution was warmed to room temperature and cooled back to -78°C. 250 mg (0.57 mmol) of xanthone bis-TBS ether **6** was dissolved in 5 ml THF and added to the solution drop wise. This

solution was left to react overnight. Cold HCl was added and a red solid formed which proved to be a mixture of starting materials by ^{1}H NMR.

V. References

1. Andrews, D, L., and Demidov, A.A., *Resonance Energy Transfer*, John Wiley and Sons: New York, NY, 1999.

2. Bhat**,** R. A., Lahaye, T., and Panstruga, R., **"**The Visible Touch: *In Planta* Visualization Of Protein-Protein Interactions By Fluorophore-Based Methods" *Plant Methods* 2006, date accessed April 7, 2008 <http://www.plantmethods.com/content/2/1/12/ >

3. Ha, T., Enderle, T., Ogletree, D.F., Chemla, D. S., Selvin, P. R., and Weiss, S., "Probing The Interaction Between Two Single Molecules: Fluorescence Resonance Energy Transfer Between a Single Donor and a Single Acceptor", *Proc. Natl. Acad. Sci. USA*, **1996**, *93* 6264.

4. Herman, B., Centonze Frohlich, V.E.; Lakowicz, J.R., Fellers, T.J., Davidson, M.W., "Applications in Confocal Microscopy: Fluorescence Resonance Energy Transfer (FRET) Microscopy", Olympus Confocal Microscopes Resource Center <http://www.olympusfluoview.com/applications/fretintro.html>

5. Invitrogen Corporation 2007. Date accessed April 7, 2008 <http://probes.com/handbook/boxes/0422.html>

6. Kamiya, M., Urano, Y., Kojiro, K., Ueno, T., Hirose, K., and Nagano, T., "Evolution of Fluorescein as a Platform for Finely Tunable Fluorescence Probes", *J. Am. Chem. Soc.* **2005,** *127* 4888-4894.

7. Vamavski, O.P., Ostrowski, J.C.; Sukhomlinova, L., Twieg, R.J., Bazan, G.C., Goodson, T., "Coherent Effects In Energy Transport In Model Dendritic Structure Investigated By Ultrafast Fluorescence Anisotropy Spectroscopy", *J. Am. Chem*. *Soc*. **2002,** *124*, 1736.

8. Tanaka, K., Miura, T., Umezawa, N., Urano, Y., Kikuchi, K., Higuchi, T., Nagano, T., "Rational Design of Fluorescein-Based Fluorescence Probes: Mechanism-Based Design of a Maximum Fluorescence Probe for Singlet Oxygen", *J. Am. Chem. Soc,* **2001,** *123* 2530.

9. Zhou, Lu. “Synthesis of Different Tethered Compounds for Cylindrically Conjugated Organic Belts and Synthesis of Three-Dimensional Assemblies of Chromophores for FRET Studies.” PhD Dissertation, SUNY Stony Brook, Chemistry Dept., 2006.

10. Peterson, B., Mottram, L.F., Boonyarattanakalin, S., Kovel, R.E., “The Pennsylvannia Green Fluorophore: A Hybrid of Oregon Green and Tokyo Green for the Construction of Hydrophobic and pH-Insensitive Molecular Probes”, *Org. Lett*. **2006**, *8*, 581-584.

11. Greene, T. W., Wuts, P. G., “Protective Groups in Organic Synthesis”, John Wiley and Sons, New York, NY **1999.**

12. Li, Junqi and Yao, Shao Q., “Singapore Green: A New Fluorescent Dye For Microarray And Bioimaging Applications”, Org. Lett., **2009**, 11 (2), 405-408.

13. Shi, J., Zhang, X.P., and Neckers, D.C., “Xanthenes: Fluorone Derivatives”, *J. Org. Chem*. **1992**, *57*, 4418.

14. Richards, Sara J., “Synthesis of Tetrahedral Chromophore Arrays For Applications in FRET Studies” State University Of New York at Stony Brook, Masters Thesis, 2007.

15. Kamiya, M., Kobayashi, H., et.al., “An Enzymatically Activated Fluorescence Probe For Targeted Tumor Imaging” *J. Am. Chem. Soc.* **2007**, 129, 3918-3929.

16. Minta, A., Kao, J. P.Y., Tsein, R.Y., “Fluorescent Indicators for Cytosolic Calcium Based on Rhodamine and Fluorescein Chromophores”, *J. Bio. Chem*. **1989**, *26*4, 8171-8178.

17. Fournier, J.-H., Wang, X., and Wuest, J. D., “Derivatives Of Tetraphenylmethane And Tetraphenylsilane: Synthesis Of New Tetrahedral Building Blocks For Molecular Construction”, *Can J. Chem*. **2003,** *81*, 376.

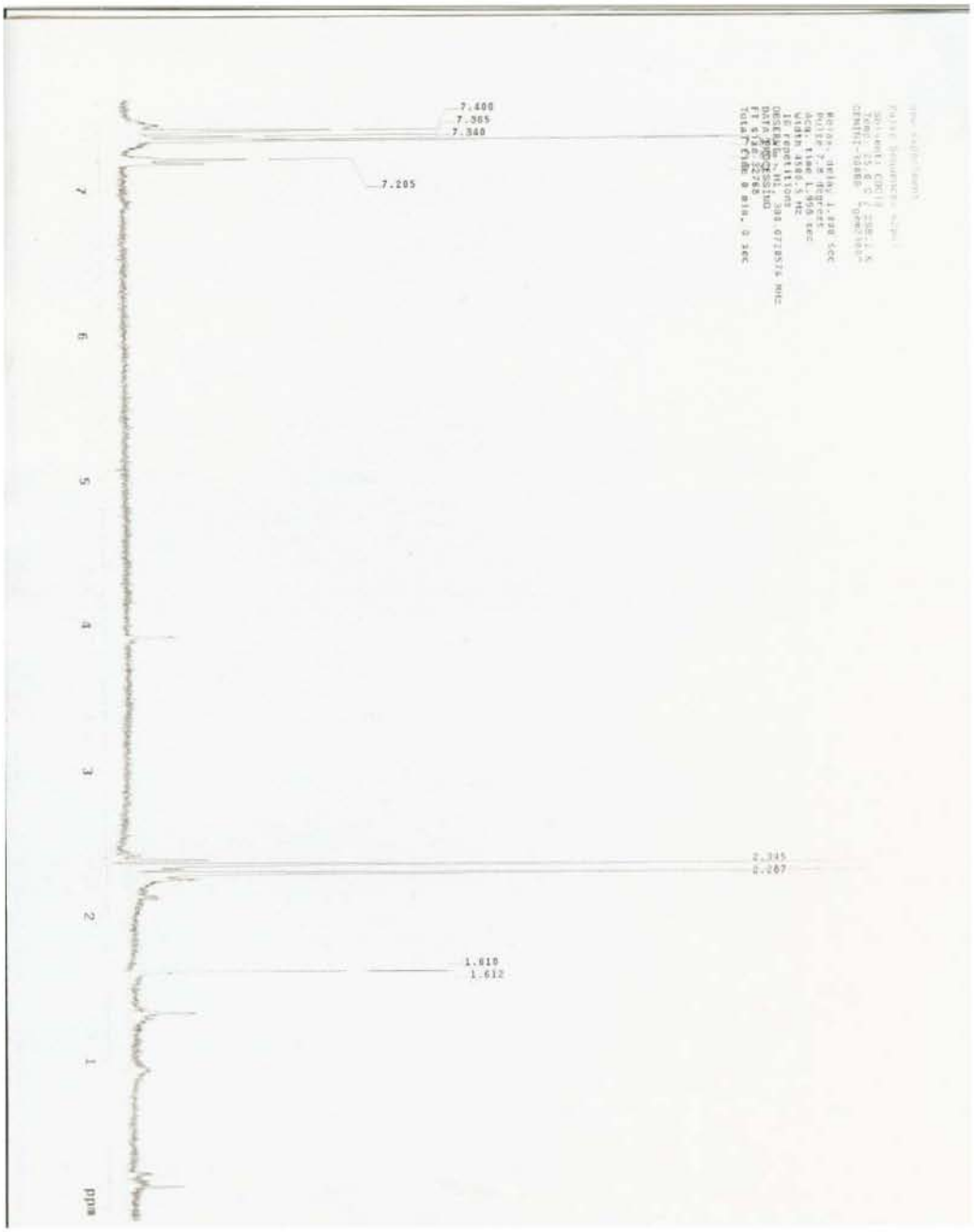
18. Schuler, B., Lipman, E. A., and Eaton, W. A., "Probing The Free-Energy Surface For Protein Folding With Single-Molecule Fluorescence Spectroscopy", *Natur*e, **2002**, *419*, 743.

19. Wu, P. and Brand, L., "Resonance Energy Transfer: Methods And Applications", *Anal Biochem*, **1994**, *218*, 1, 1-13.

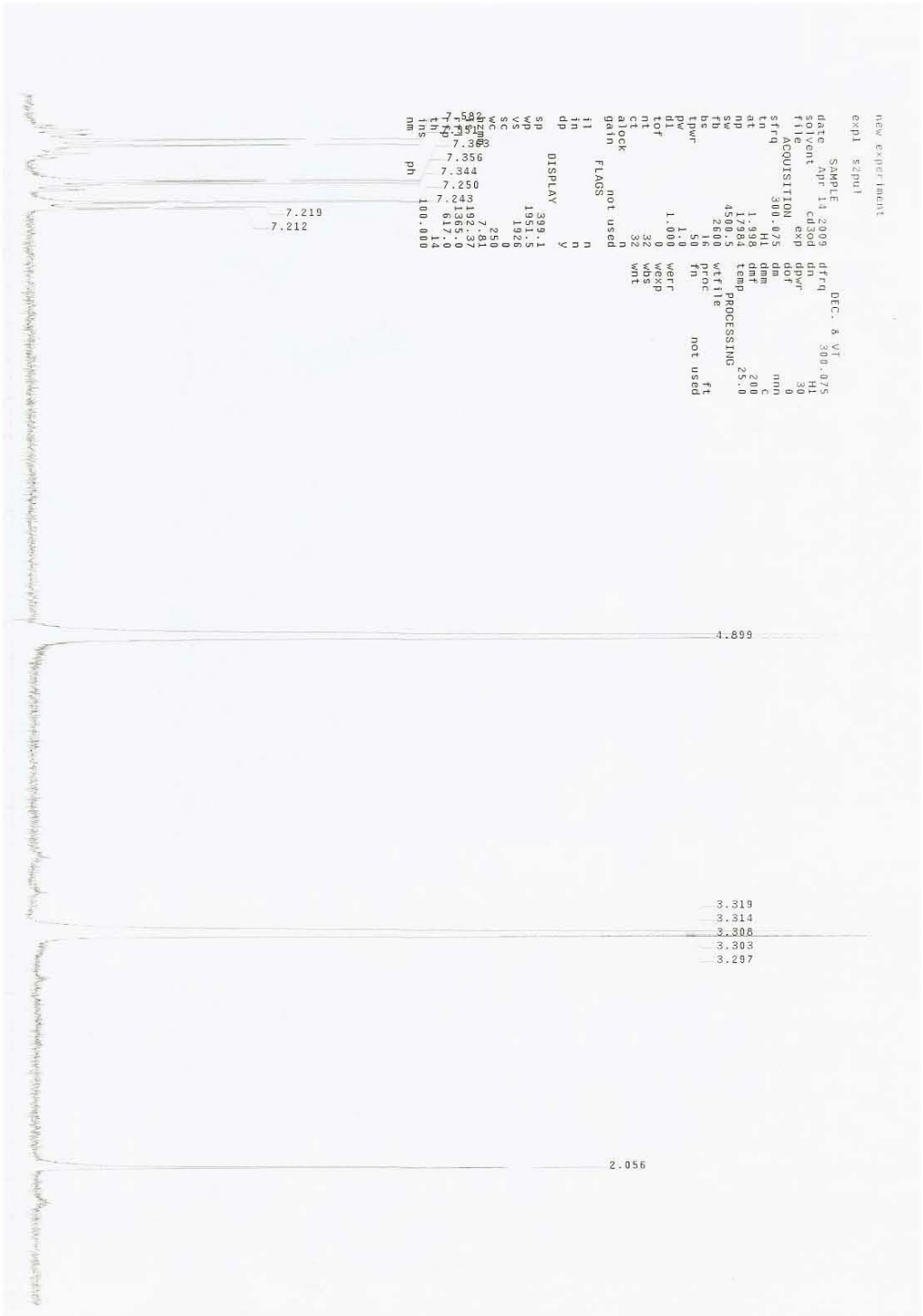
20. Giepmans, B. N. G., Adams, S. R., Ellisman, M. H., Tsien, R. Y., "The Fluorescent Toolbox For Assessing Protein Location And Function", *Science* **2006**, *312*, 217.

21. Xu, Q.-H., Wang, S., Korystov, D., Mikhailovsky, A., Bazan, G.C., Moses, D., Heeger, A.J., "The Fluorescence Resonance Energy Transfer (FRET) Gate: A Time-Resolved Study", *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 530.

VI.Appendix

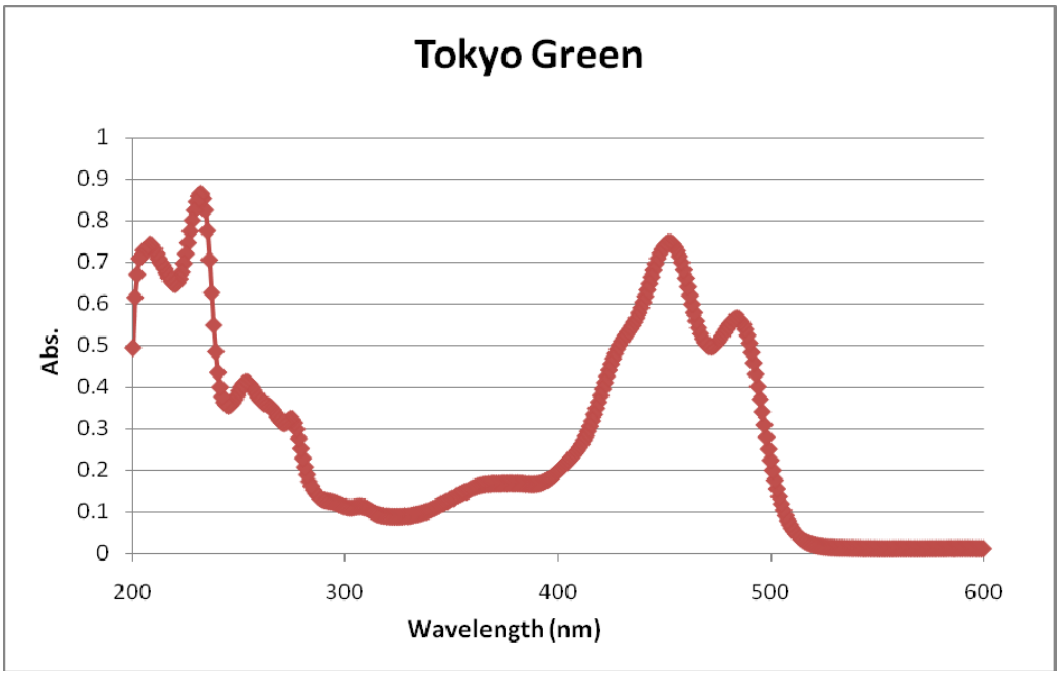


^{1}H NMR (300 MHz, $CDCl_3$): Tetrakis(3,4-dimethylphenyl)silane **11**

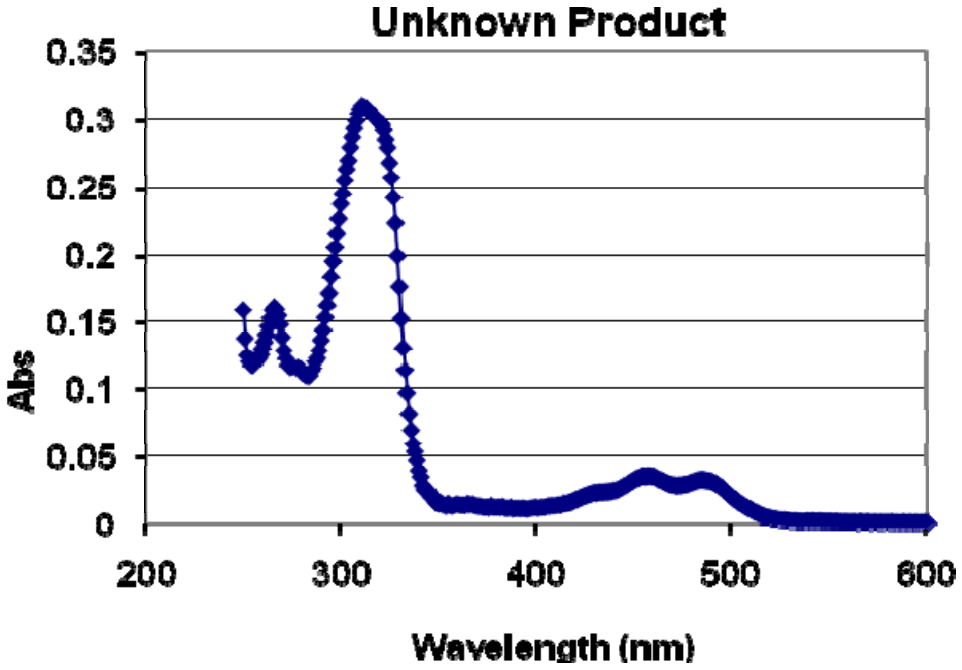


^{1}H NMR (300 MHz, CD_3OD): Tokyo Green **5**

UV spectra of Tokyo Green



UV spectra of Unknown red solid



UV Spectra Comparing Tokyo Green and Unknown Solid