Synthesis of Fluorescent Ligands in Humid Conditions for the Purposes of a Biosensor

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Honors Program

Table of Content (Projected)

Abstract

Background

The study of synthetic polymer chemistry is to alter and control the order and structure of polymeric chains, therefore manipulating their function. These alterations can be used in applications such as: information storage, self-assembly, and microelectronics.[1] With such promising application, the field of synthetic polymer chemistry has progressed significantly in the past decades. The focus for this study will be to synthesize Self-Assembled Monolayers in order to construct a biological sensor. Given their presence in most biological processes and biomolecules, anions are an important compound to select for detection. Currently, ELISA and IDA are commonly used anion sensors but they are there expensive and have inefficient sample times.[5] Tests such as these typically use dyes in their turn-off signal which often diminish the sensitivity of the sensor due to self-quenching.[5] Photoluminescence is a potential solution to eliminate these undesired signals.[5] Using cost effective material and quick formation methods, supramolecular assembly and photoluminescence will be employed to create a device sensitive enough to detect biologically relevant anions. This will be accomplished by using short oligomers to form a cooperative catalytic system.[1] Although longer chains have a higher degree of organization, spaced out the ligands allows for more control of the interchain interactions. [1] Similarly, a short chain length is preferred when a fluorescent probe is attached to the ligand.[2] Although longer chains allow for a higher surface coverage which increases the abundance of fluorophores, logically to would increase the intensity of the fluorescence signal. Instead, the longer chain's ability to compact causes allows for more interactions between neighboring chains which causes fluorescent self-quenching.[4] Due to the self-quenching experienced in longer chains, shorter chain lengths produce a significantly stronger signal of fluorescence.[4]

While shorter chains circumvent issue of self-quenching, other problems arise with adsorption on gold surface. When monitored, shorter intermolecular distance increase the speed of an important cleavage reaction because it has a transition state with a lower activation energy.[3] This cleavage reaction occurs just before surface adsorption.[2,3] Thiols will be used because they provide a faster than average cleavage reaction time than most other substituents.[2] Utilizing this information, the SAMs need fluorescent probes in order to monitor the mechanism steps of alkythoil synthesis.

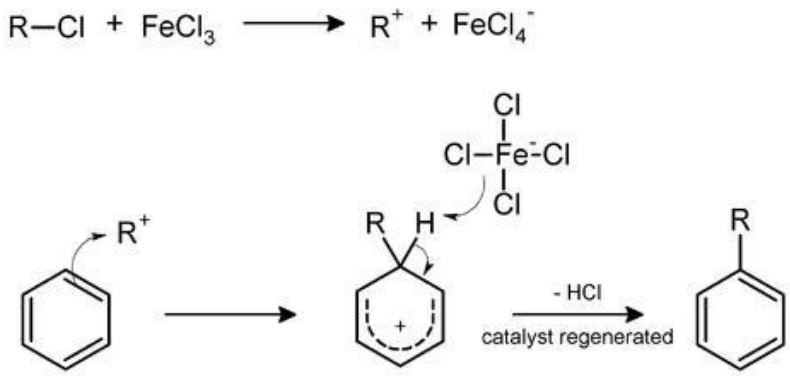
- It relates to the ability for recognition, selective binding, rigidity, constrictions, and tensions, as well as dynamics, reorientations, transport, and transformations. Hydrogen bonding, ion pairing, □-□ dispersion interactions, van-der-Waals attractions, ion-dipole coupling, and solvophobic phenomena are the intermolecular forces holding molecular complexes together. Life depends on these weak, delicate interactions, which require much less energy than covalent bonds require. Host-guest chemistry is a branch in that field concerned with small organic or inorganic entities having a hydrophobic core of approximately 100 to 1000 $Å^3$ –hosts– and able to interact with ions or small compounds –guests–. In Life Sciences, host-guest chemistry is attractive since it involves complementary stereoelectronic arrangement of binding sites as well as non-covalent interactions such as those observed in biological systems, but presenting the advantage of avoiding the complexities associated with them. Organic host entities are relatively simple to synthesize, as the process is commonly based on simple oligomerization and cyclization of one single unit. Due to geometrical limitations derived from atomic orbital hybridization modes, only a certain level of complexity can be achieved without increasing the synthetic demands or the host's size. It originates from the notion that hydrophobic long hydrocarbon

chains tend to aggregate on aqueous solution mainly due to induced dipole interactions known as London forces. If functionalized with groups such as thiols or sulfides and exposed to a clean metal surface, the chains assemble in an organize manner over the metal as a monolayer. From a supramolecular perspective, the formation of SAM offers an opportunity to define hosts from a different perspective. The "host" is now a hydrophobic pocket within a SAM, where functionalized hydrocarbon chain units assemble around a given space previously blocked by a selected organic compound, here defined as an SMC. Replacing an oligomerization by a self-assembling process permits the simple addition of functionalized elements to the chains or core molecular compound while preserving the size of its hydrophobic cavity and the flexibility of the system, one of the principal drawbacks of the more classical host models. Each element involved is synthesized separately, simplifying dramatically the synthetic effort.

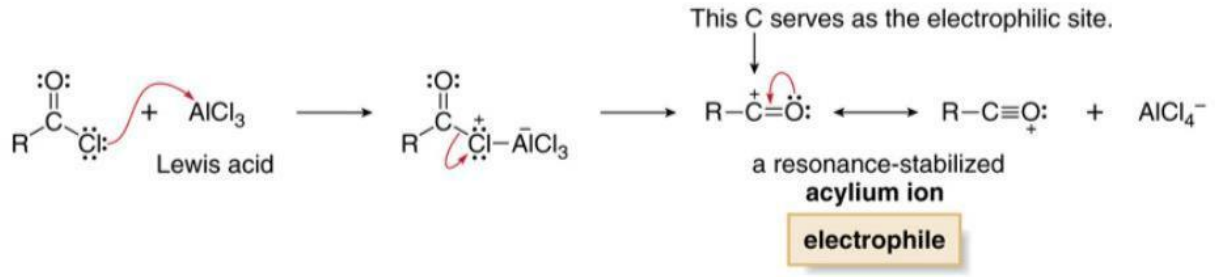
- Biosensors design for earlier diagnosis and prognosis of Alzheimer's Disease. The design of surface molecular containers (SMC) is an innovation that aims to emulate biological entities involved in hormone/receptor interactions. Metabolites are chemical species of endogenous origin and low molecular weight (approx. <1200 Da) found in biofluids such as urine, saliva or blood. They are the intermediates and final products of thousands of enzyme-catalyzed cascade reactions occurring continuously within a cell or organism. Unfortunately, even when the actual technological advances in this field (NMR, MS) have facilitated its application to multiple fields, they handle biofluids' complexity from a conceptual approach that appears increasingly inadequate with respect to its implementation in clinical practice. High-cost facilities, specific requirements for the collection and transportation and stability of biosamples, or the need for highly

specialized technicians in the central facilities compromise the assimilation of metabolomics into the health care system. Once a biomarker system has been identified by metabolomics means, a biosensor will be designed for the selected metabolites. I will focus on the development of biosensors based on mixed SAMs, organized over a metal surface (see Scheme 1). The monolayer will be organized as multiple, precisely located receptor areas, each capable of coordinating selectively with different components of the metabolomics biomarker system, forming an intelligent surface.These intelligent surfaces, acting as receptors, are recognition elements of a biosensor system. The recognition and quantification of a singular metabolite on an *ad hoc*-designed set of receptors will occur by transducing the signal into a series of fluorescence on/off switches, and the metabolite's concentration will be calculated by matrices solution of the generated linear system –each fluorescence spot's intensity is related to the metabolite concentration and its binding constant to the set of receptors. This experiment is associated with Alzheimer's. We aim to create a surface covered with self-assembling monolayers with pores able to encapsulate specific metabolite biomarkers of the disease.

- General Friedel-Craft Alkylation

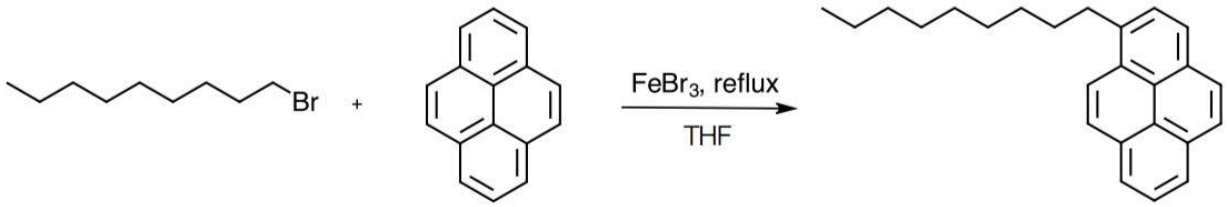


- General Friedel-Craft Acylation



Methods

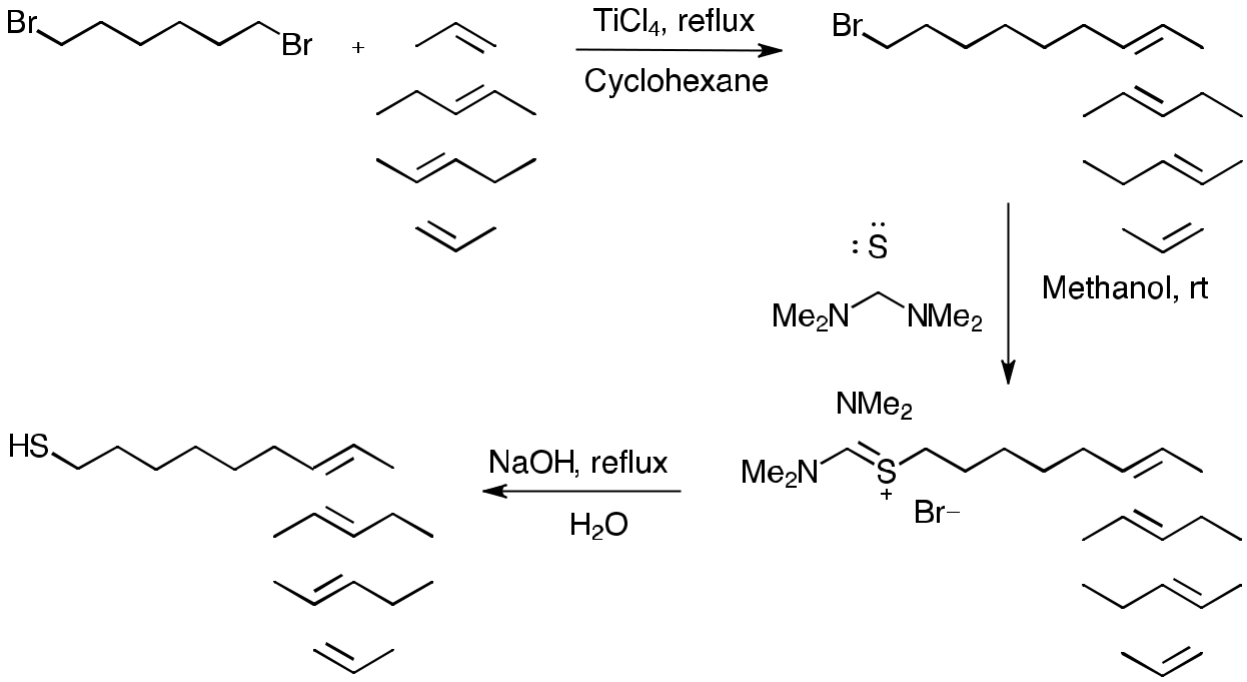
- 1st Synthesis: Friedel-Craft Alkylation of Pyrene (FeBr3)



Instructions

1. Obtain 200mg of pyrene and dissolve them in 5ml dried THF (10ml round bottom flask).
2. Add one equivalent of 1-bromononane and stir the mixture.
3. Add carefully 0.05mg of $FeBr_3$ (catalytic amount).
4. ATTENTION: Be extremely careful adding the $FeBr_3$ as it is corrosive & highly pigmented and may stain clothes/skin.
5. Reflux the reaction mixture, checking the reaction media as it progresses by TLC, *i.e.*, silica gel as stationary phase, hexane as eluent).
6. Cool down the mixture to room temperature once the reaction is completed.
7. Destroy the $FeBr_3$ by adding 3ml of H_2O.
8. Decant your organic layer and let it dry over Na_2SO_4.

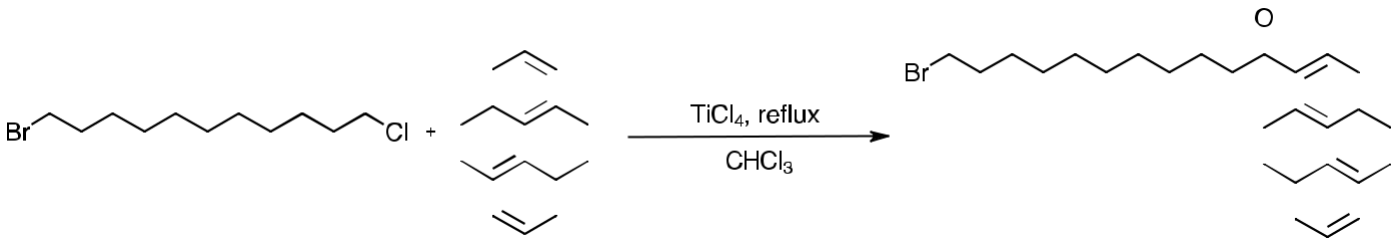
- 2nd Synthesis: Friedel-Craft Acylation of Pyrene (TiCl4, THF)



Instructions

1. Obtain 1ml of acyl chloride 1.0M solution and add it to 6ml cyclohexane (10ml round-bottom flask).
2. Add one equivalent of pyrene while stirring the mixture.
3. Add carefully 0.05mg of Lewis Acid ($AlCl_3$ or $TiCl_4$; catalytic amount, do not weight it). ATTENTION: Be extremely careful adding the LA as it is very hazardous in case of skin contact, ingestion or inhalation. Add it in the presence of your PI.
4. Reflux the reaction mixture, checking the reaction media as it progresses using TLC, *e.g*, silica gel stationary phase, CH_2Cl_2 as eluent.
5. Cool down the mixture to room temperature once the reaction is completed.
6. Destroy the Lewis acid by adding 2ml of H_2O.
7. Decant your organic layer and let it dry over Na_2SO_4.
8. Filtrate and rotavap the solution, leaving the sample in a pre-weighted vial.

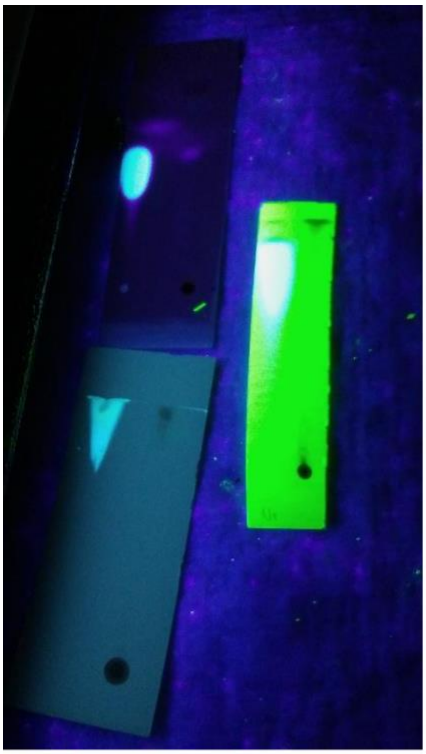
- 3rd Synthesis: Friedel-Craft Acylation of Pyrene (TiCl4, CHCl3)

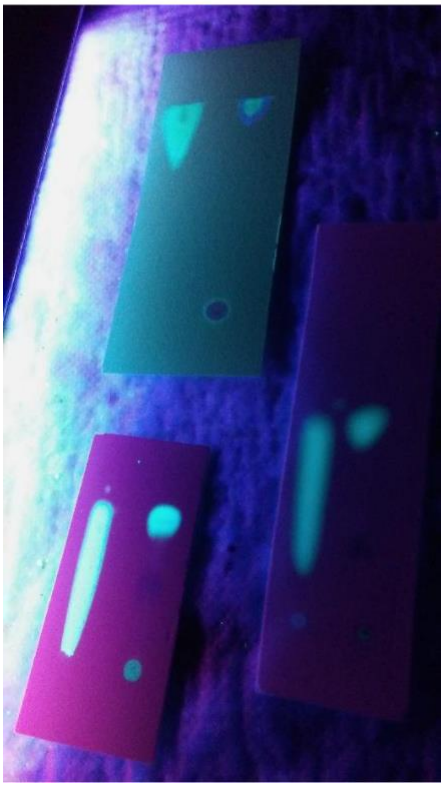


Instructions

1. Set up a refluxing system in the hood, using a 100ml round bottom flask (do not forget the stirring magnet).
2. Add 0.990g of 11-bromoundecanoic acid to 10ml dried $CHCl_3$.
3. Add 0.250g of imidazole.
4. Add 0.35ml thionyl chloride in the flask at room temperature.
5. If no precipitate is formed, reflux for one hour (imidazolium chloride should precipitate).
6. Let it cool to room temperature.
7. Add 750mg of pyrene and stir the solution to dissolve it.
8. Add 0.410ml $TiCl_4$.
9. Stir the reaction for ½ hour, checking the reaction media as it progresses using TLC, *e.g,* silica gel stationary phase, hexane as eluent. If no reaction, reflux it.
10. Cool down the mixture to room temperature once the reaction is completed.
11. Destroy the $TiCl_4$ by adding 25ml of H_2O.
12. Decant your organic layer and let it dry over Na_2SO_4.
13. Filtrate and rotavap the solution, leaving the sample in a pre-weighted vial.

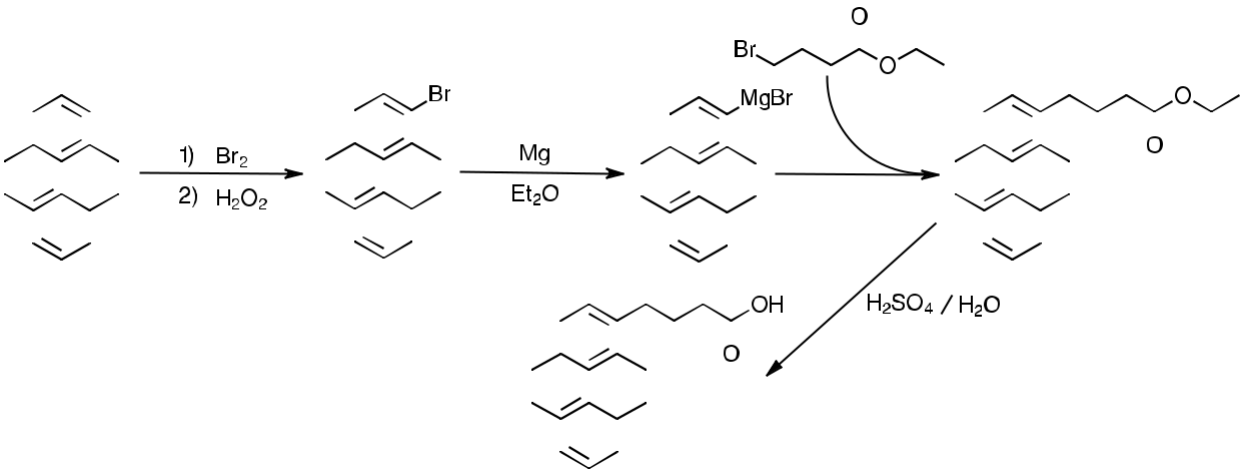
Results





Conclusion

- Friedel-Crafts alkylation reaction. This reaction consists in the activation of an alkyl halide by the presence of a Lewis Acid, followed by an electrophilic attach (E2) to an aromatic compound. It does not require setting up a refluxing system since the alkyl halide is activated, but it can generate a significant amount of side products due to rearrangements of the intermediate carbocation and multiple substitutions on the ring.
- This reaction has some disadvantages; for example, the product is more nucleophilic than the reactant due to the positive inductive effect of new electron donating alkyl-chain. Therefore, the substitution of another aromatic hydrogen is plausible, leading to over-alkylation of the aromatic compound. Only one equivalent of the alkyl halide should be use to avoid multi-substituted side-products in high amount.
- Discuss Friedel-Craft Acylation
- Other Possible Mechanisms: Grignard Reaction
- Talk to Cesar



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(6) Manual…

(7) Synthesis)of)1-Bromopyrene)and)1-Pyrenecarbaldehyde…

(8) An Improved Preparation of a Grignard Reagent…