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Novel Fluorescent Sensors for Cations with Additional PET Pathways to Suppress Signals from Protons

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NOVEL FLUORESCENT SENSORS FOR CATIONS WITH ADDITIONAL PET PATHWAYS TO SUPPRESS SIGNALS FROM PROTONS

By

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ABSTRACT

A large number of fluorescent sensors for cations that use a photoinduced electron transfer (PET) process to signal cation binding have been developed over the past three decades.[1] The PET process of some of these sensors depends on having a tertiary nitrogen atom as a part of the receptor for cations. While these sensors work well as cation sensors, they also generate a fluorescent signal due to protonation of these receptors. The goal of this project is to design a fluorescent sensor that uses the same cation receptors but would not generate a signal for protons. Our new sensor has an additional PET pathway that is triggered when the tertiary nitrogen of the receptor is protonated.

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ACKNOWLEDGEMENTS

I take this opportunity to express gratitude to the Chemistry and Biochemistry Department faculty members for all their help and support in completing this project. I am especially grateful to Dr. de Silva for sharing his expertise and invaluable guidance extended to me. I would also like to thank all the other members of my committee for their assistance and provisions in writing the thesis. Finally, I would like to thank my friends and family for all their support.

LIST OF FIGURES AND SCHEMES

INTRODUCTION

Fluorescent photoinduced electron transfer (PET) sensors have been widely used as chemosensors for cations over the past few decades and their application have been expanding to other areas such as engineering and computer science.[1,2] Fluorescent PET sensors are an ideal choice for developing new sensors since they follow a common design (Figure 1). A PET will occur if the oxidation potential of the receptor is smaller in magnitude than that of the fluorophore.[3] In these systems, the fluorophore is not restricted to being an electron acceptor or donor. The direction of the PET process depends on the reduction and oxidation potentials of the excited and ground states. A molecule in its excited state is a better oxidizer or reducer than in its ground state. Typically, sensors start in a nonfluorescent, or weakly fluorescent, state and binding of an analyte will cause a fluorescent response ("On" state).

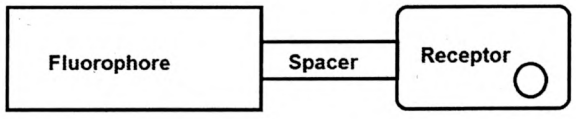
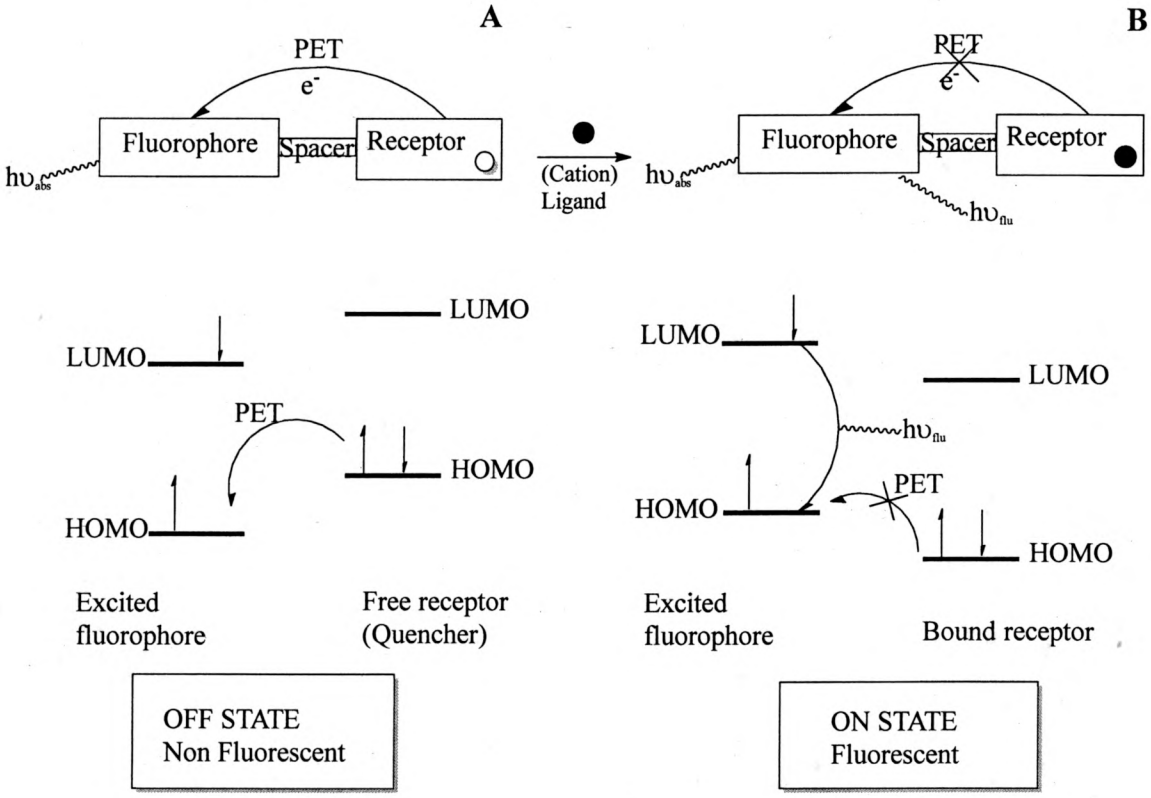


Figure 1: The Fluorophore-Spacer-Receptor Principle.[3]

A compound will experience a change in fluorescence when the cation binding inhibits the receptor-fluorophore electron transfer. Because this transfer occurs after the molecule absorbs light, it is considered a photoinduced electron transfer. These are either On-Off or Off-On systems. These systems, or switches, are signaling processes that depend on the PET process being inhibited or vice versa upon molecular recognition.

This event can result in fluorescent emission or quenching. Scheme 1 summarizes the PET process of an Off-On state.



Scheme 1: Schematic diagram and molecular orbital diagram illustrating the PET process of an "Off-On" state.[4]

In sensors that behave as Off-On switches, the fluorophore gets excited to a higher energy state due to photon absorbance and an electron transfer will occur between the HOMO of the receptor and the HOMO of the excited fluorophore, thus quenching the fluorophore. Since the excitation energy is used by the PET process, fluorescence is not observed. This is seen on part A of Scheme 1.

If a cation is bound in the receptor, the PET process would be disrupted. When bound, the receptor's HOMO energy level is lower than the fluorophore's HOMO energy level. Thus, the PET process is inhibited and the photon absorbed is emitted as a fluorescent photon ($h\nu_{flu}$). This is illustrated on part B of Scheme 1.

The change in energy for photoinduced electron transfers is defined by the Rehm-Weller equation.[4]

$$\Delta G = E_{red}\,(D^{+}/D) - E_{red}\,(A/A^{-}) - \Delta G_{00} - e^2 / \epsilon d$$

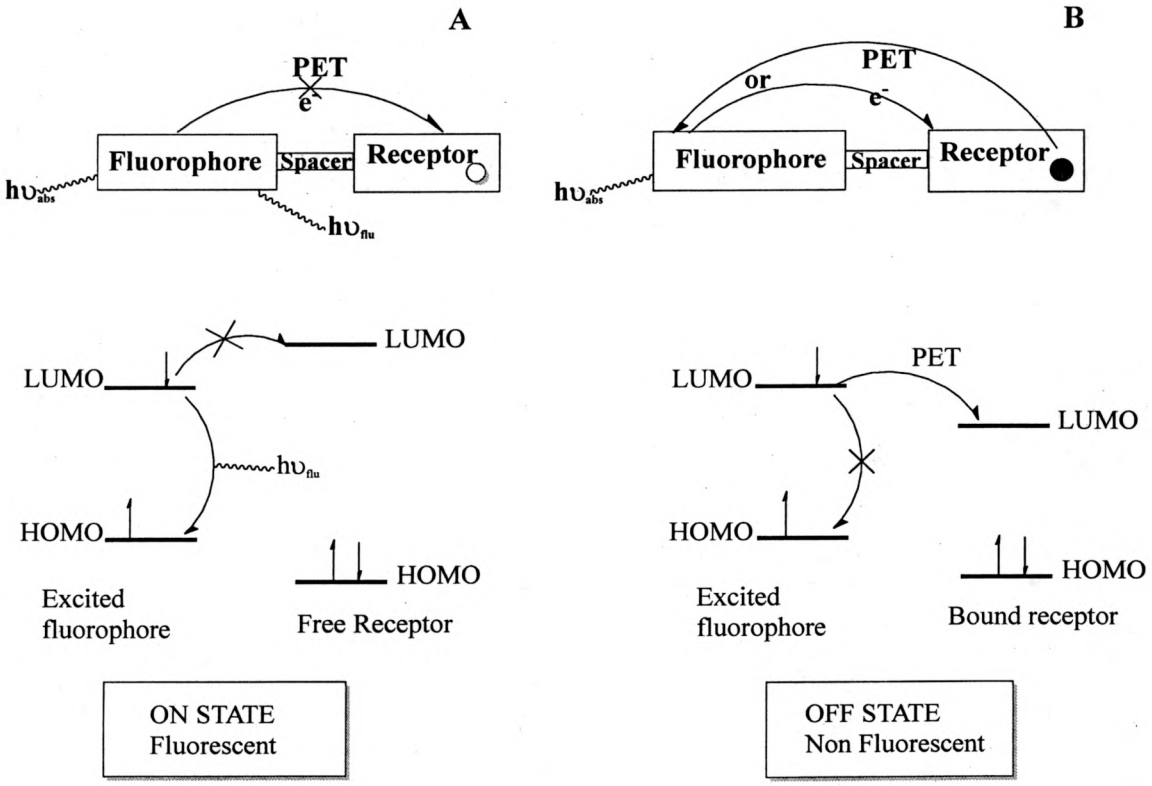
The change in energy for a PET process is between an electron donor (D) and electron acceptor (A) where either one may be excited. The first two terms refer to the reduction potentials of the donor and acceptor. The third term refers to the change in energy of the ground transition state (S_o) and the excited state (S_1) of the fluorophore. Finally, the last term accounts for the coulombic attraction energy experienced by the ion pair following the electron transfer reaction.

In the Off-On switch, without cation binding the electron transfer between the excited fluorophore and receptor is exothermic. The receptor donor has a higher propensity to donate an electron to the ground state of the fluorophore. This is especially true if the sensor has an electron deficient moiety in its structure. With cation binding, the PET becomes endothermic and the energy is released by the excited fluorophore falling back to its ground state with the emission of light as fluorescence.

An On-Off switch will work the opposite way of an Off-On sensor with minor variations. The fluorophore starts in an "On" phase due to the difference in energy of the free receptor and fluorophore. There is less of a difference in energies between the

fluorophore's excited and ground states than any other energy levels on the receptor. The light absorbed by the fluorophore is emitted as fluorescent light. It is thermodynamically unfavorable for the fluorophore or receptor to make any electron transfers. This is shown in Part A of Scheme 2.

When the receptor binds a cation, the reduction potential of the receptor increases. This in turn decreases the change in energy so a PET may occur between the fluorophore's LUMO state and the receptor's HOMO state. This is the "Off" state seen in Part B of Scheme 2.



Scheme 2: Schematic diagram and molecular orbital diagram illustrating the PET process of an "On-Off" state.[4]

Many sensors that can multiple cation binding events with multiple PET processes have been developed over the past two decades.[1] These sensors can be

classified as primary, secondary or tertiary depending on the number of PET processes that could occur in these sensors. The maximum number of PET processes reported in current literature for these types of sensors is three and examples of higher generation sensors are shown in Figure 2.[6] In the second and third generation sensors there are two types of nitrogen atoms that promote different on and off systems. The second generation sensor shown here can function as a sensor for zinc ions by generating a fluorescent signal with Zn^{2+} binding. It also exhibits a dual fluorescent switch (Off-On-Off) with pH due to protonation of the tertiary nitrogen and the pyridine rings at different pH values.[3,5,6]

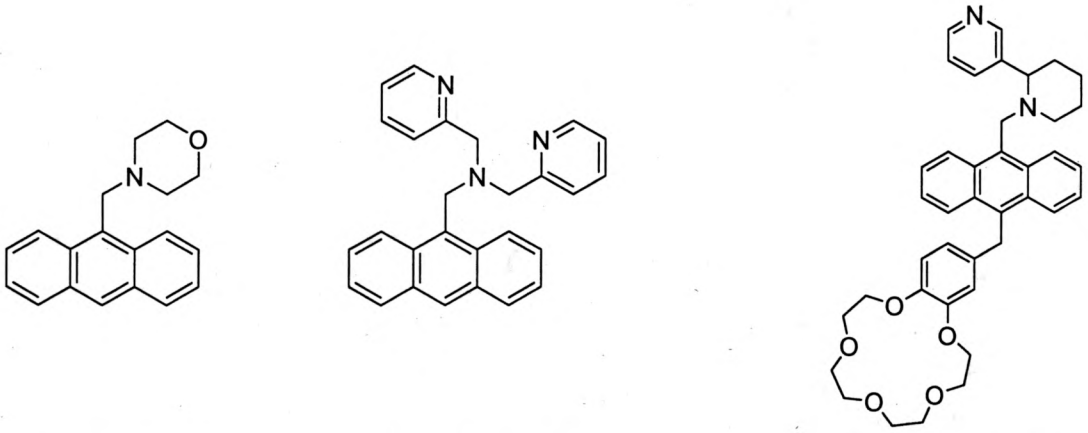


Figure 2: Evolution of PET sensors in the last few decades.[3,5,7]

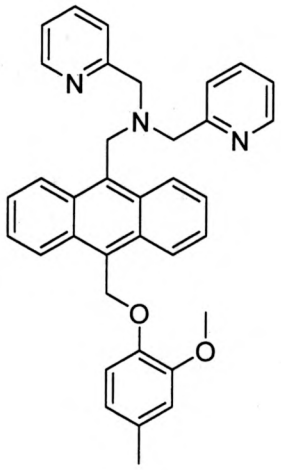
Fluorescence is an ideal technique for the detection of cations in biological systems. Fluorescence is highly sensitive partly because no reference sample is needed

prior to measuring fluorescence sample. The signals from low concentrations can be distinguished easier this way, so much that a single cation binding is measurable.

Most of the cations studied with these PET sensors are biologically linked and exist intracellularly. Ergo, the PET sensors described here has the potential to operate in living cells[3]. Zinc is an important ion that is monitored in neurophysiology in the study of degenerative diseases. It is a challenging ion to detect because of its low concentration in comparison to other ions present in cells. Recent findings are able to detect zinc ions with fluorescence in the picomolar range.[4,10]

GOAL

The goal of this project was to find the best method to synthesize *N-((10-((2-methoxy-4methylphenoxy)methyl)anthracen-9-yl)methyl)(pyridin-2-yl)-N-((pyridin-2-yl)methyl)methanamine* (**11**). This is a third generation fluorescent PET sensor that is designed to be responsive to zinc but not to protons. This is achieved by attaching an alkoxy phenol to the anthracene that would quench the fluorescence due to protonation by generation of an additional PET process.



11

RESULTS AND DISCUSSION

Previous attempts to prepare compound **11** starting with 9,10-bis(chloromethyl)anthracene have not been successful due to the low solubilty of the starting material and the formation of disubstituted products.[11] Therefore, in this study, we decided to start with a differently substituted anthracene ring that is also commercially available. Two similar approaches were used to make compound **11** where the only difference was the order of alkylations. There were several synthetic routes attempted in preparing compound **11**. The different approaches will be discussed separately.

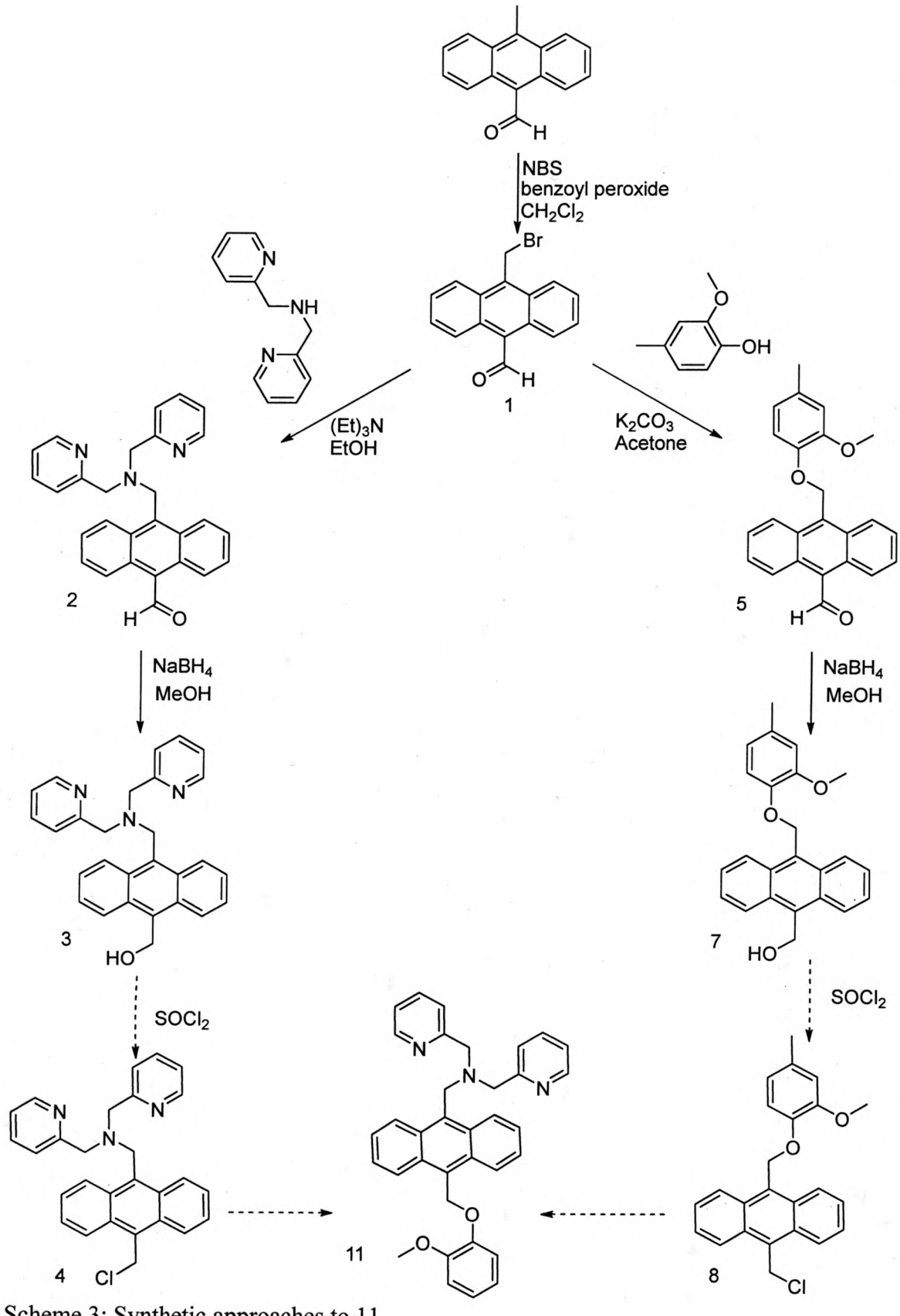
Two similar approaches were used to make compound **11**. The only variation was the order of alkylations. Both routes use the common intermediate, **1**, which was prepared by the bromination of the commercially available 10-methylanthracene-9-carboxaldehyde with NBS. The brominated product was formed in 87% yield and the ^{1}H NMR shows the aldehyde and the methylene group at 11.5 and 5.51 ppm respectively in a 1:2 ratio.

Attempts to substitute the bromine of **1** with an amine or a phenol first were both successful. The methoxy phenyl ether, **5**, was prepared by a Williamson ether synthesis by alkylating 2-methoxy-4-methylphenol with **1**. The ^{1}H NMR spectrum of **5** shows that the aldehyde group is not affected in this reaction. The reduction of **5** with sodium borohydride gave the expected alcohol, **7**, which was isolated in 63% yield.

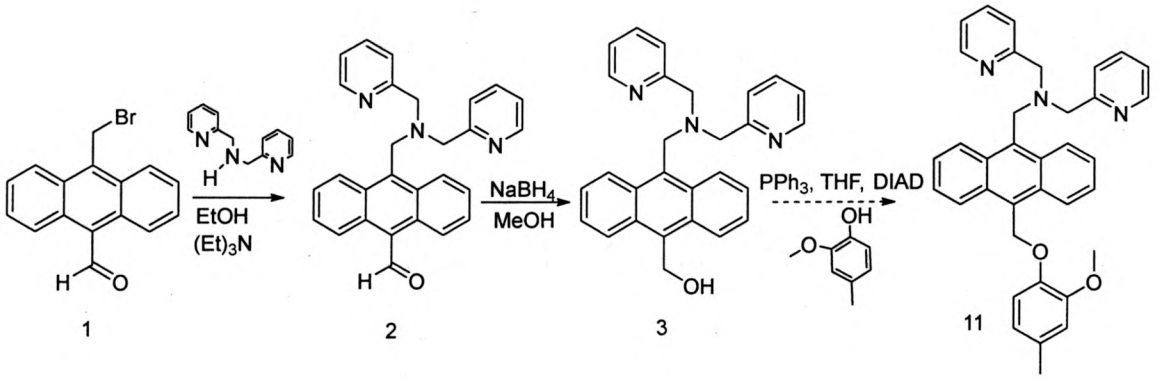
In the second method we were able to carry out a substitution reaction of **1** with bis(2-picolyl)amine to produce **2**. Again, the ^{1}H NMR of the product confirmed that the

aldehyde group is not affected during the reaction. The aldehyde **2** was reduced with sodium borohydride to give the expected alcohol, **3**, which was isolated in 90% yield.

Attempts to chlorinate both alcohols, **3** and **7**, with thionyl chloride resulted in a complex mixture of products. We suspect that these reaction conditions resulted in the cleavage of the amine or alkoxy phenyl groups. Alternatively, a bromination reaction with compound **7** was tried with carbon tetrabromide and triphenylphosphine in dichloromethane. After two hours, the NMR spectrum and mass spectroscopy analysis showed very little of the desired product. This reaction requires further investigation and may reach higher yields if left overnight. It may also benefit to react the carbon tetrabromide and triphenylphosphine in DCM prior to adding the starting material.

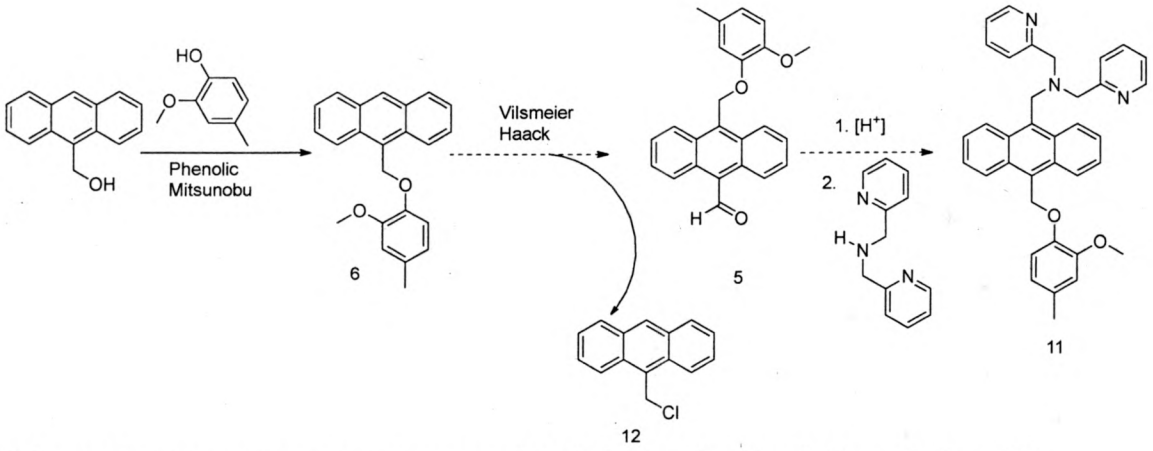


Scheme 3: Synthetic approaches to 11



Scheme 4: Phenolic Mitsunobu approach to 11

An alternative approach was sought out by trying to eliminate the halogenation of the hydroxyl group. Lepore and He reported phenolic Mitsunobu reactions to couple sterically hindered phenols in high concentrations in combination with sonication.[11] Several trial runs were run with 9-anthracenemethanol and 2-methoxy-4methylphenol to produce the ether **6**. Due to the high concentrations in our experiments, sonication provided a more efficient method of mixing over magnetic or mechanical stirring.[12] The reaction had to maintain a specific concentration (between 1.0 M – 3.0 M) for good yields. Trial runs verified the reaction should work with compound **3** and the optimal reacting time for a 1.0 g scale to be 3 hours. The NMR spectrum for 15 minutes sonication contained too many solvent traces and the overnight sonication showed very little improvement over the three hour reaction. The yields for the 15 minute, 3 hour and overnight reactions were 27%, 53%, and 51% respectively.



Scheme 5: Attempt to prepare compound 5 via Vilsmeier Haack formylation of 6

Finally, the initial approaches were revisited and attempts were made to prepare the aldehyde, **5**, by a Vilsmeier-Haack on **6** as shown in scheme 5. The ^{1}H NMR spectrum of the reaction mixture did not show an aldehyde. We believe that these reaction conditions result in the cleavage of the ether leading to the formation of 9-chloromethylanthracene. This conclusion is the result of an NMR analysis of the reaction mixture as shown in Figure 3. It was observed that the methylene group of **6** which is at 5.97 ppm disappears and a new signal appears at 5.64 ppm. The new signal appears exactly at the same position as the methylene group of 9-chloromethyl anthracene as shown in Figure 3.

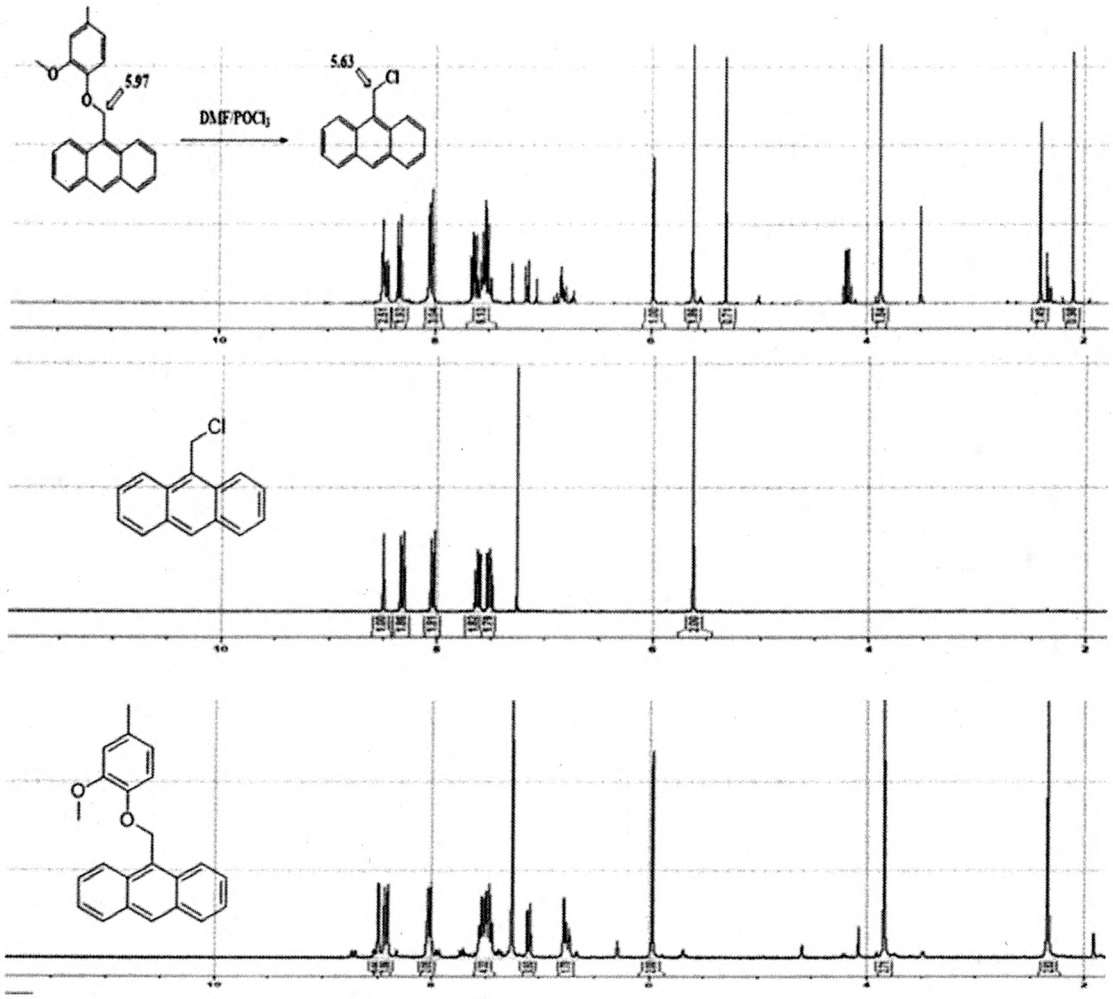


Figure 3: A Comparison of NMR data

One possibility is the Vilsmeier reagent, formed from $POCl_3$ and DMF, reacts with the starting material as shown in Figure 4 leading to the cleavage of the ether. In the literature, there is no record of a Vilsmeier reagent cleaving ethers albeit it can cleave and chlorinate compounds with hydroxyl groups.[13]

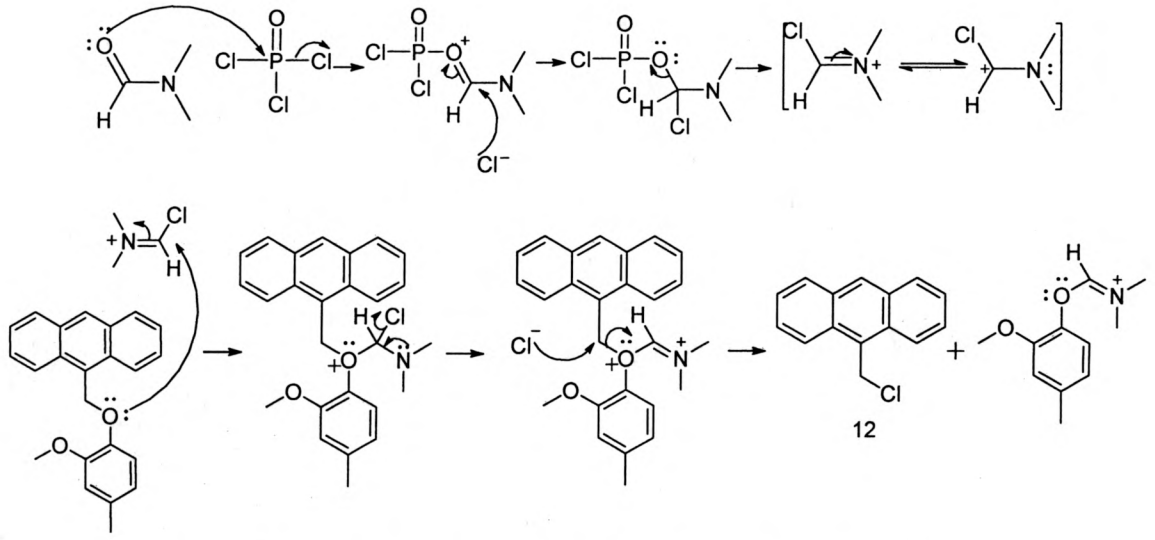


Figure 4: Proposed mechanism for cleavage of 6 with the Vilsmeier reagent

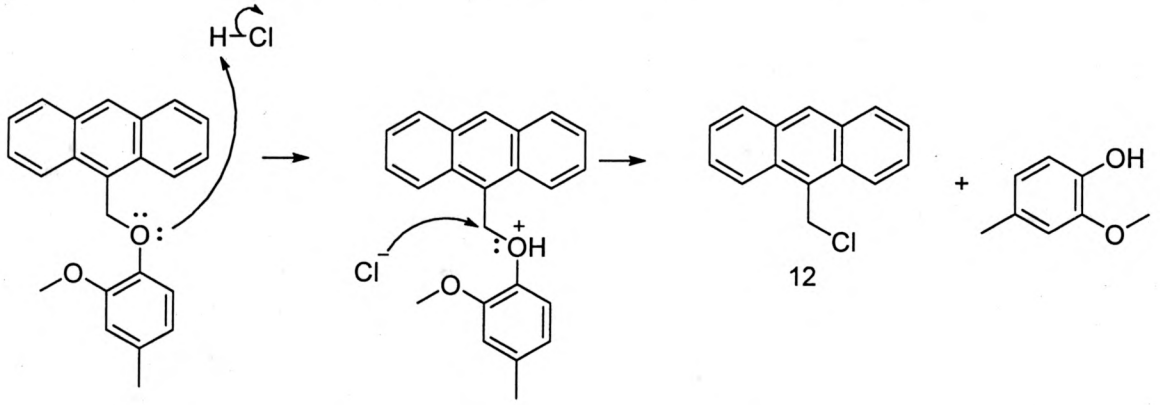
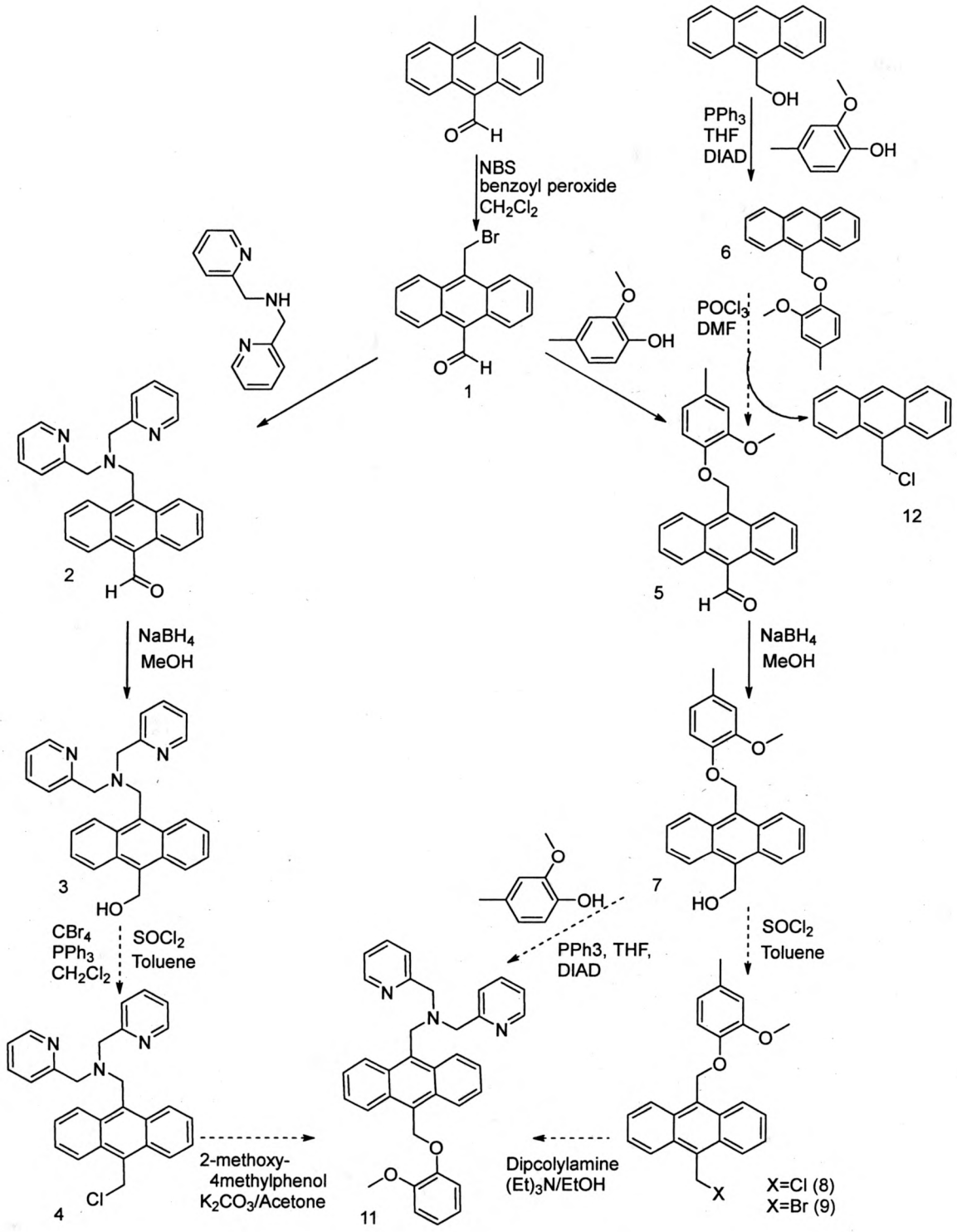


Figure 5: Proposed mechanism for cleavage of 6 with HCl

Alternatively, since it is possible that there could be some acid in the reaction mixture, the direct cleavage with HCl is also possible as shown in Figure 5. Whether it was due to an acidic environment can be verified by running the reaction under similar Vilsmeier Haack conditions with an absence of DMF. The reaction (without DMF) would not be a Vilsmeier Haack reaction but it would clarify whether the chloride comes from hydrochloric acid from the $POCl_3$.

CONCLUSION

Scheme 6 summarizes our synthetic approaches to the sensor **11**. Currently, we have the two alcohols, **3** and **7**. These two alcohols have to be chlorinated of brominated under mild conditions to carry out the final substitution reaction as shown in Scheme 3. We have also found that it is possible to cleave an ether under Vilsmeier-Haack reaction conditions. Further investigations of this reaction are currently in progress.



Scheme 6: Synthetic routes to compound **11**.

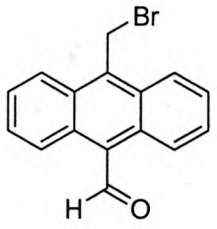
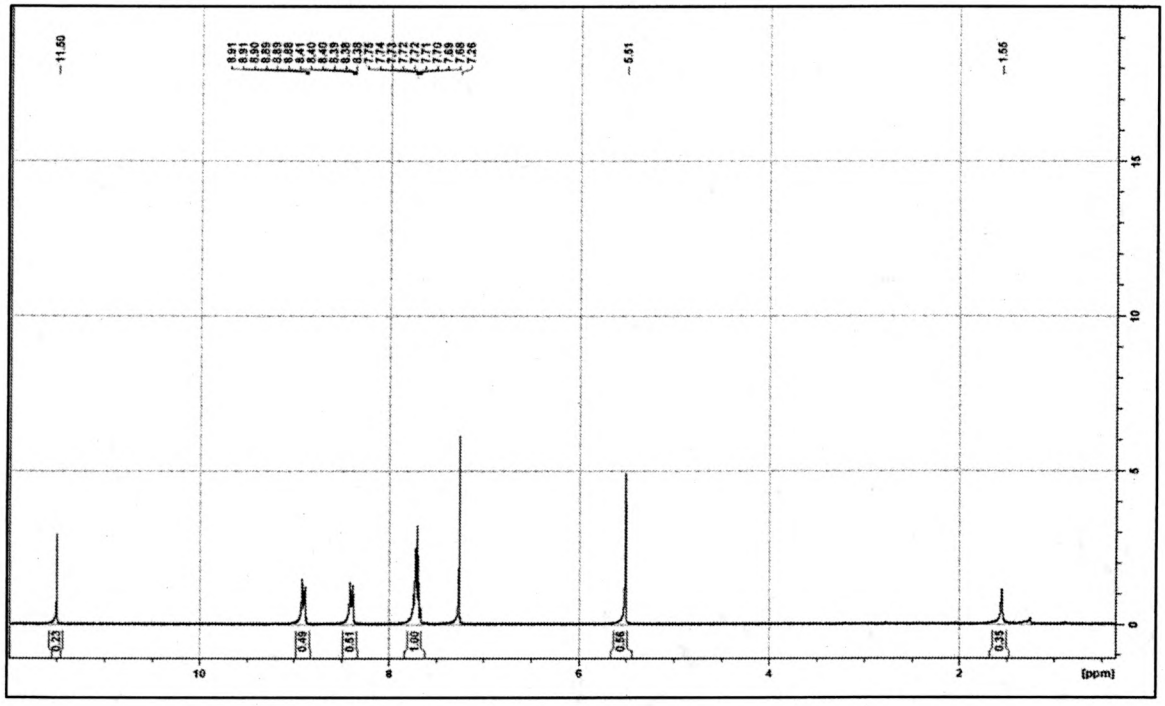
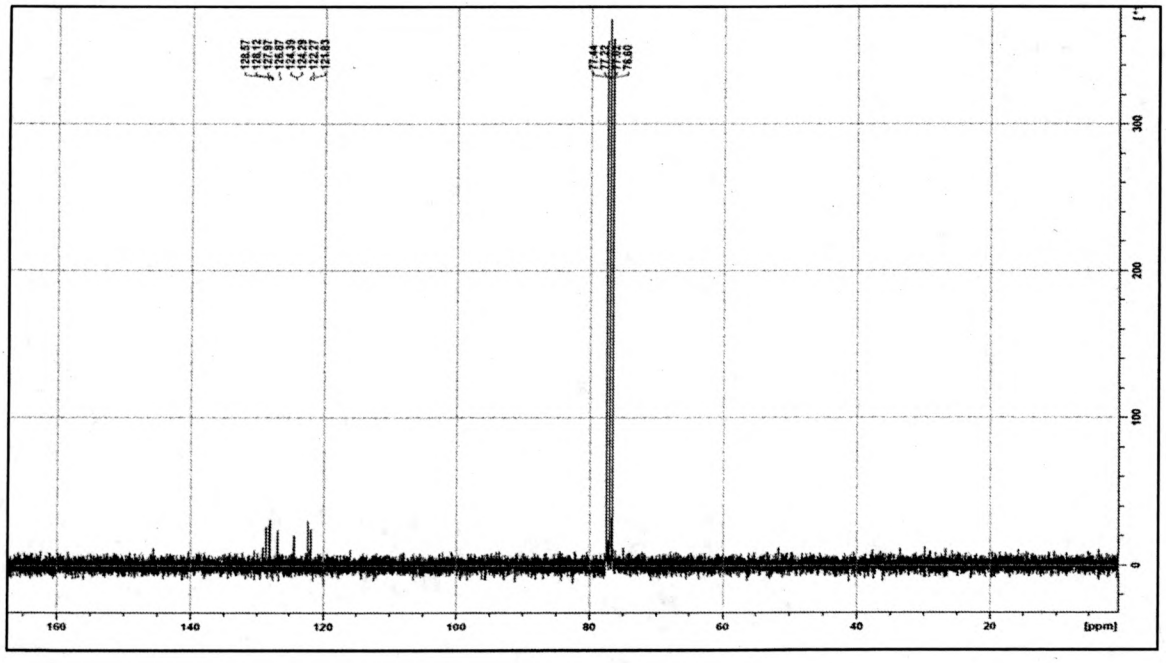


Figure 6: ^{1}H and ^{13}C NMR spectra for Compound **1**





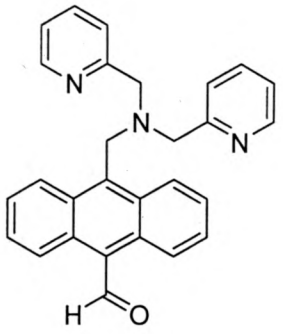
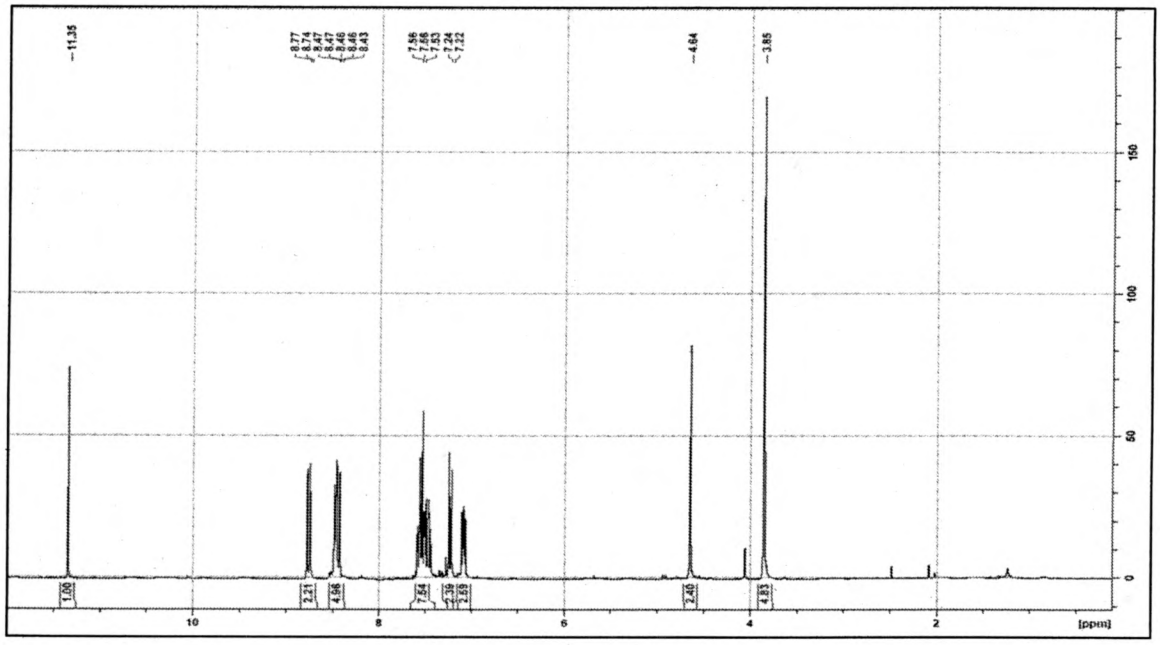
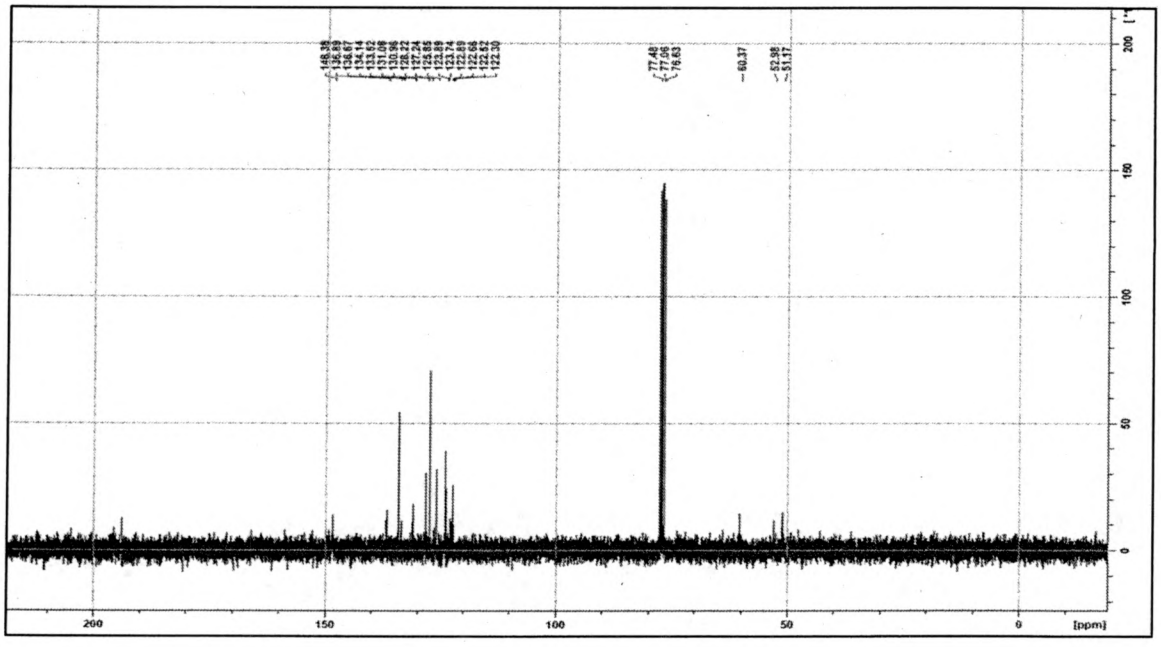


Figure 7: ^{1}H and ^{13}C NMR spectra for Compound **2**





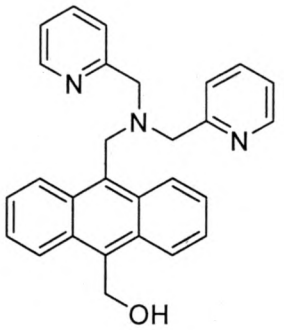
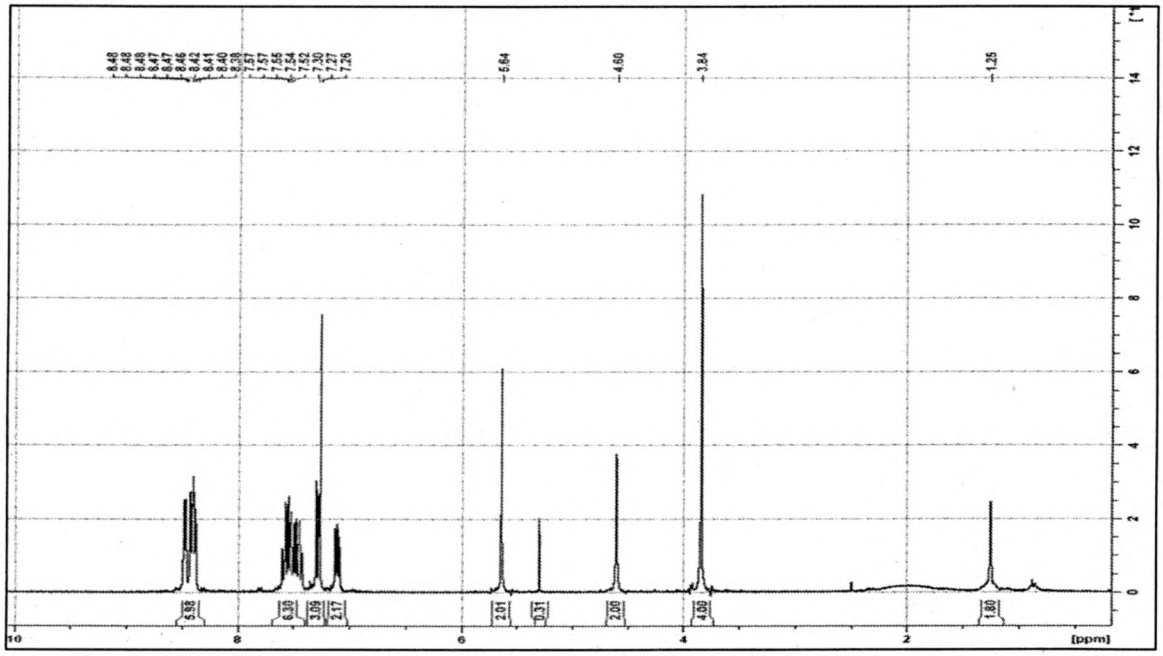
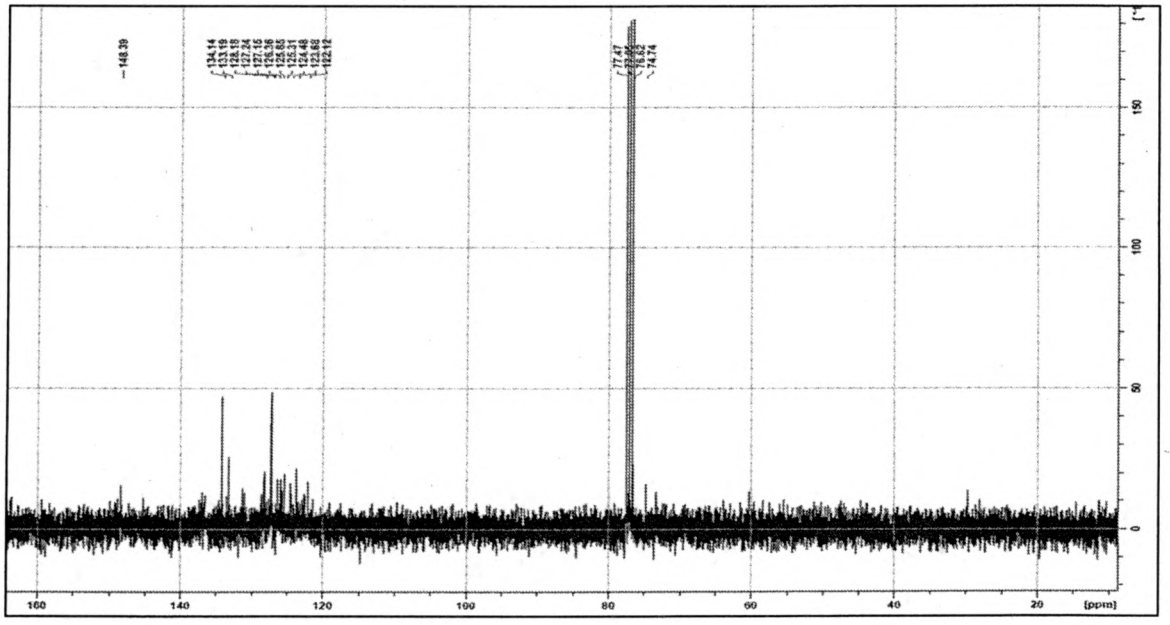


Figure 8: ^{1}H and ^{13}C NMR spectra for Compound **3**





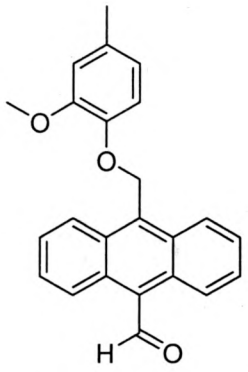
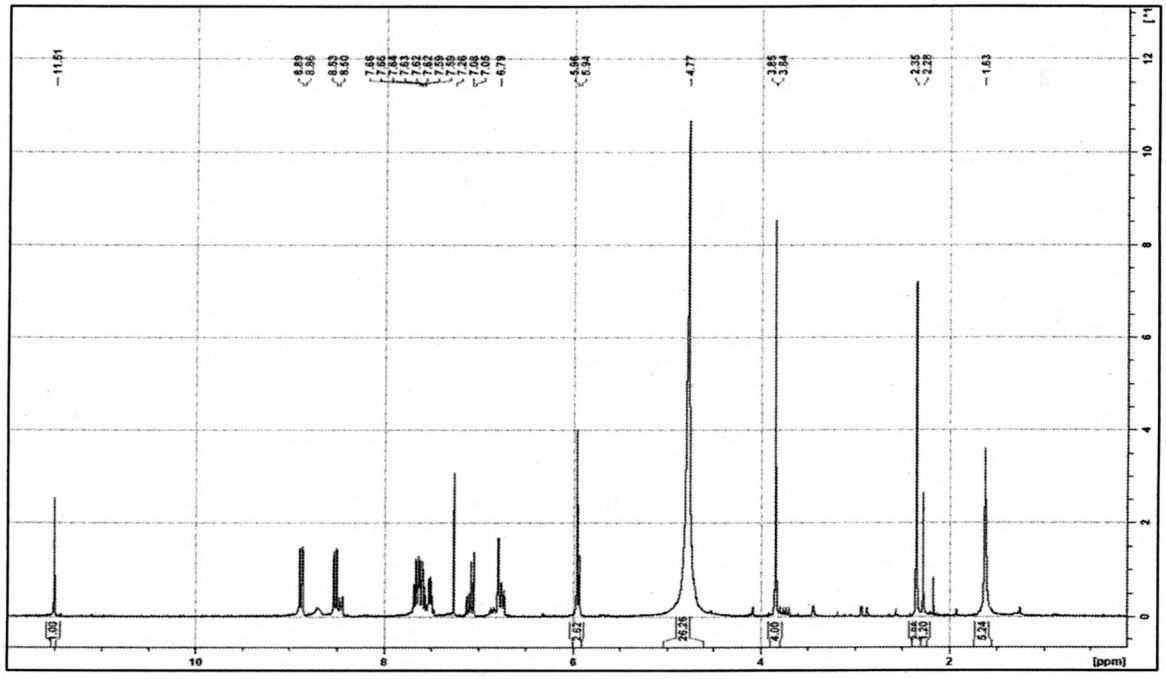
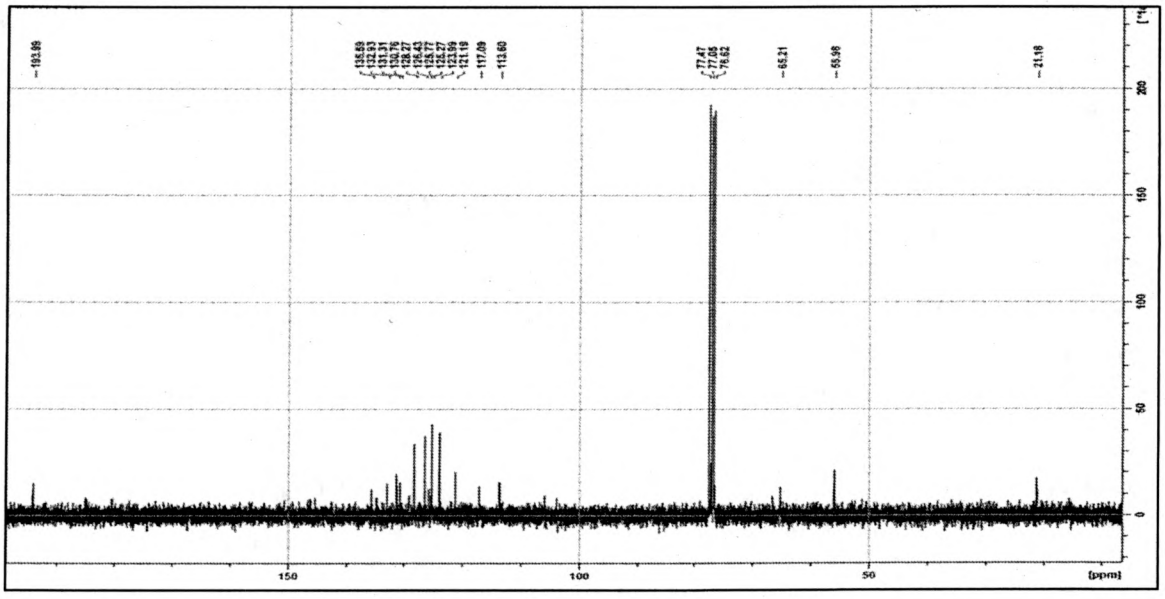


Figure 9: ^{1}H and ^{13}C NMR spectra for Compound **5**





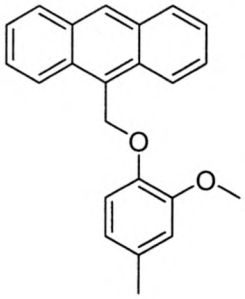
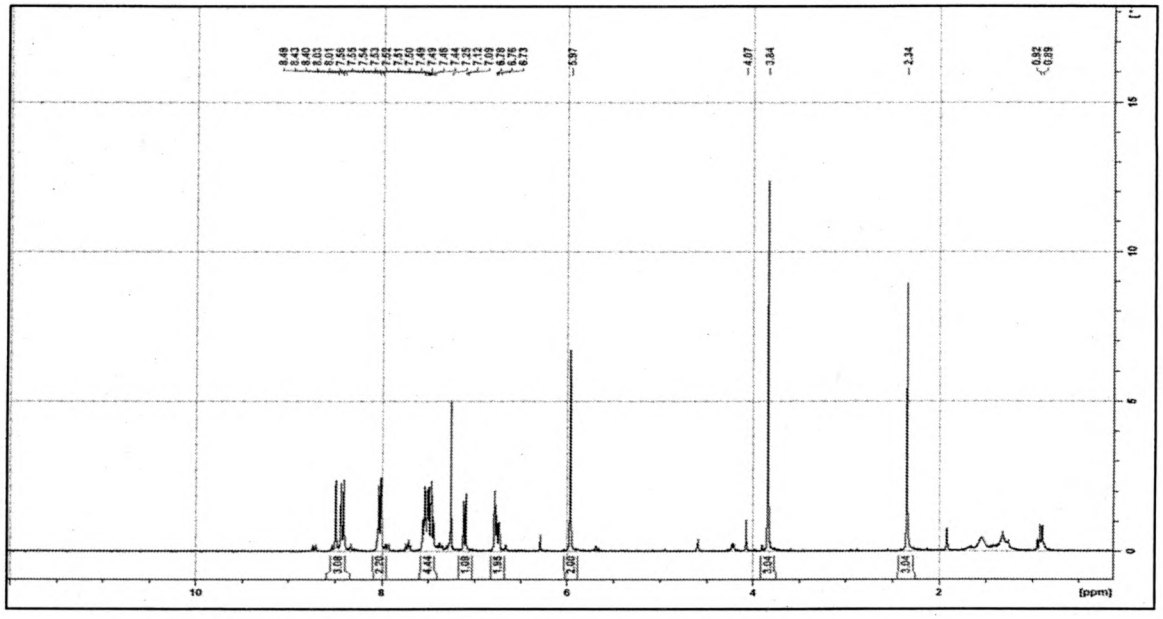
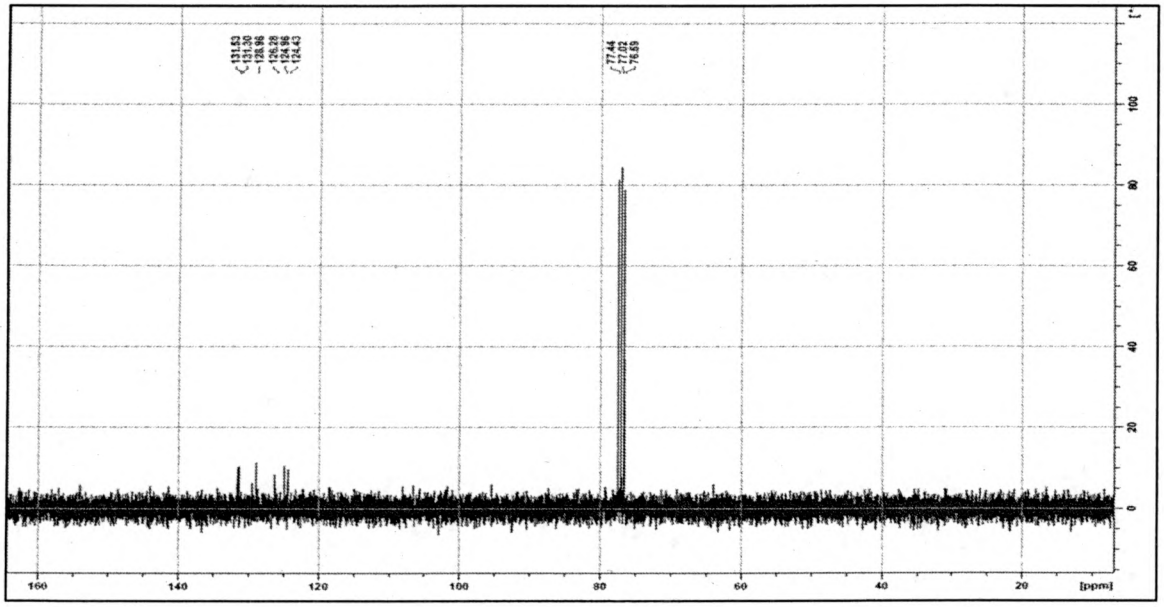


Figure 10: ^{1}H and ^{13}C NMR spectra for Compound **6**





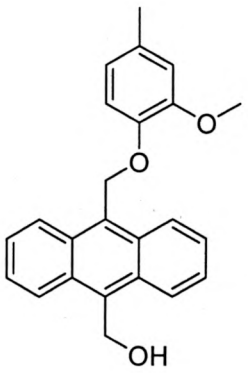
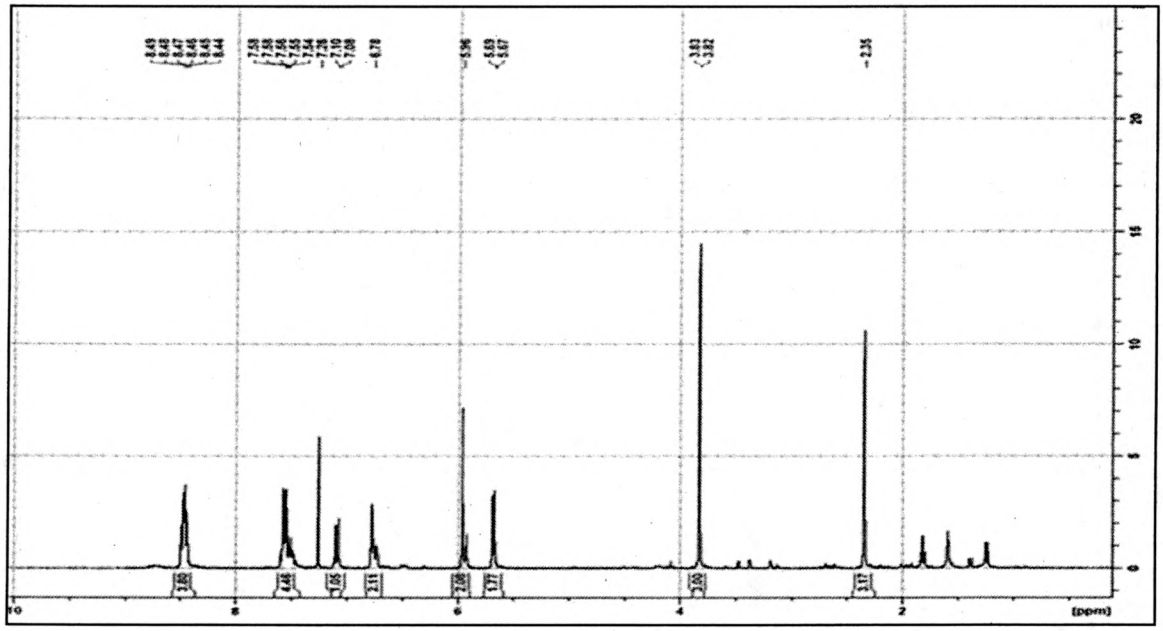


Figure 11: ^{1}H and ^{13}C NMR spectra for Compound **7**



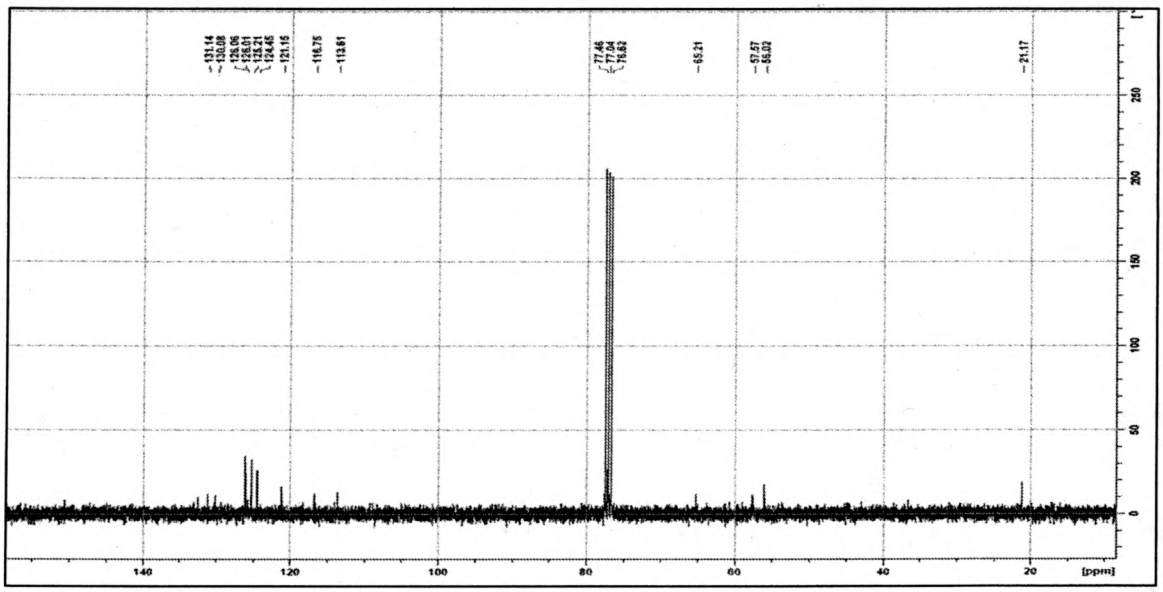
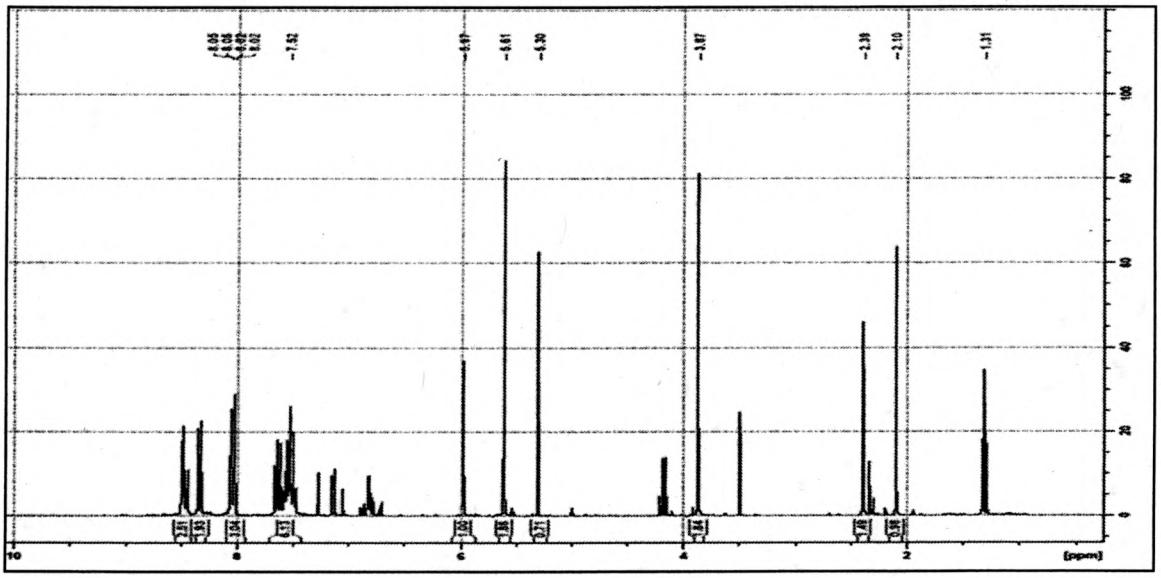


Figure 12: ^{1}H NMR spectrum of the Vilsmeier Haack reaction mixture of compound 6 [Scheme 5]



EXPERIMENTAL

Nuclear magnetic resonance spectra were recorded on Bruker Avance 300 FT-NMR Spectrometer. All samples were prepared in $CDCl_3$ and chemical shifts are reported in δ values (ppm) relative to TMS. Mass spectrometry was obtained from a Shimadzu LCMS-2020.

A mixture of 10-methylanthracene-9-carboxaldehyde (1.00 g, 4.54 mmol), N-bromosuccinimide (0.80 g, 4.49 mmol), and bezoyl peroxide (0.056 g, 0.23 mmol) in 20.0 mL dichloromethane was refluxed for 2 hours. Post cooling, 20.0 mL methanol was added and a yellow fluffy product (**1**) was obtained (1.18 g, 87%). Characterization for product **1**: ^{1}H 11.50 (s), 8.91-8.8.88 (m), 8.40-8.38 (m), 7.75-7.68 (m), 5.51 (s). ^{13}C 128.57-127.97, 126.87, 124.39-124.29, 122.27-121.83. Calculated mass of product **1**: 299.15 g/mol Found m/z+1: 300 g/mol

Product **1** (0.38 g, 1.27 mmol) was mixed with 2,2'-dpicolylamine(0.25 g, 1.25 mmol) and trimethylamine (0.19 g, 1.87 mmol) in 15.0 mL ethanol. The solution mixture refluxed overnight. Product **2** was extracted with 4M hydrochloric acid and dichloromethane. The aqueous layer was neutralized and the solution (0.4346 g, 82%) was concentrated and stored in the cold for slow crystallization. Characterization for product **2**: ^{1}H 11.35 (s), 8.77-8.8.73 (m), 8.47-8.43 (m), 7.76-7.22 (m), 4.64 (s), 3.85 (s). ^{13}C 148.38-136.67, 134.14-130.96, 128.22, 125.85, 123.89-122.30, 60.37, 51.17. Calculated mass of product **2**: 417.15 g/mol Found m/z+1: 419 g/mol

Product **2** (0.35 g, 0.838 mmol) was then reduced with sodium borohydride (0.06 g, 1.59 mmol) in excess and methanol (10.0 mL). Product **3** was poured over ice for crystallization. The crude product (**3**) was collected via vacuum filtration. It was then worked up with 25.0 mL DCM and concentrated to give a golden yellow solid (0.22 g, 63%). Characterization for product **3**: ^{1}H 11.51 (s), 8.48-8.46 (m), 7.57-7.52 (m), 7.30-6.81 (m), 5.64 (s), 4.60 (s), 3.80 (s). ^{13}C 148.39, 134.14-133.19, 128.18-126.36, 125.85-122.12. Calculated mass of product **3**: 417.15 g/mol Found m/z+1: 419 g/mol

Alternatively, product **1** (0.24 g, 1.09 mmol) was added to a mixture of 2-methoxy-4-methylphenol, potassium carbonate (0.17 g, 1.23 mmol) and acetone (10.0 mL) and left to reflux overnight. The bright yellow solid product (**5**) was then crystallized and vacuum filtered (0.25 g, 87%). Characterization for product **5**: ^{1}H 11.51 (s), 8.89-8.50 (m), 7.66-7.59 (m), 7.08-6.79 (m), 5.94 (s), 3.85-3.84 (d), 2.35 (s). ^{13}C 193.99, 135.59-130.76, 128.27-121.19, 117.09-113.60, 65.21, 55.98, 21.18. Calculated mass of product **5**: 356.14 g/mol. Found m/z+1: 357 g/mol

Product **5** (0.20 g, 0.56 mmol) was reduced with sodium borohydride (0.42 g, 11.10 mmol) in excess and methanol (25.0 mL). Product **7** was poured over ice for crystallization. The crude product (**7**) was collected via vacuum filtration (0.18 g, 90%). Characterization for product **7**: ^{1}H 8.49-8.44 (m), 7.58-7.54 (m), 7.10-6.78 (m), 5.96 (s), 5.69 (s), 3.83 (s), 2.35 (s). ^{13}C 131.14, 130.08, 126.06, 125.21, 124.45, 116.75, 65.21, 56.02, 21.17. Calculated mass of product **7**: 356.14 g/mol Found m/z+1: 357 g/mol

9-anthracenemethanol (1.0 g, 4.80 mmol) was sonicated for 5 minutes with 2-methoxy-4-methylphenol (0.62 g, 4.49 mmol), triphenylphosphine (1.20 g, 4.58 mmol) and tetrahydrofuran (1.39 g, 19.3 mmol). Diisopropyl azodicarboxylate, 40% wt soln, was then added (2.0 mL, 4.80 mmol) dropwise and the solution was sonicated for an extra 3 hours. 10.0 mL hexanes was added and stirred to remove byproduct. The hexane layer was pipetted out and 5.0 mL methanol was added, just enough to dissolve the product. Product **6** was left covered overnight and concentrated to give a pale yellow solid (0.83 g, 53%). Characterization for product **6**: ^{1}H 8.49-8.01 (m), 7.56-7.44 (m), 7.25-7.09 (m), 6.78-6.73 (m), 5.97 (s), 3.84 (s), 2.34 (s). ^{13}C 131.53, 131.30, 128.96, 126.28, 124.96, 124.43. Calculated mass: 328.15 g/mol. Found m/z+1: 329 g/mol

Compound **12** was synthesized by mixing compound 6 (0.21 g, 0.64 mmol), phosphoryl chloride (0.26 g, 1.69 mmol), and dimethylformamide (0.12 g, 1.64 mmol) in 5.0 mL methylene chloride stirring at room temperature for 1 hour. Characterization for product **12**: ^{1}H 8.50-8.41 (m), 8.05-8.02 (m), 7.56-7.50 (m), 6.78-6.73 (m), 5.97 (s), 5.51(s), 5.30 (s), 3.87 (s), 2.39 (s), 2.10 (s).

Synthesis of compound **11** via phenolic Mitsunobu

N-((10-((2-methoxy-4-methylphenoxy)methyl)anthracen-9-yl)methyl)(pyridin-2-yl)-N-((pyridin-2-yl)methyl)methanamine (**11**)

Product **3** was sonicated with 2-methoxy-4-methylphenol (0.05 g, 3.62 mmol), and triphenylphosphine (0.06 g, 228.76 mmol) in THF (0.75 g, 10.40 mmol). While sonicating, diethylazodicarboxylate (DIAD), 40% by wt. Soln in toluene, (0.10 mL, 0.24

mmol) was added dropwise to the solution. The reaction remained in a Branson Ultrasonic 50/60 Hz sonication bath for a total of three hours. 3.0 mL hexanes was added to the mixture and removed after 10 minutes. Methanol was also added and left sitting overnight. An oily black product was purified through a column. Calculated mass of compound 11: 539.68 g/mol Found m/z+1: 557 g/mol

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