

EXPANDING THE SPECTRUM OF PHOTSENSITIZERS FOR POTENT
PHOTODYNAMIC THERAPY AGAINST BRAIN CANCER

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submitted by **OSMAN KARAMAN** in partial fulfillment of the requirements for the
degree of **Doctor of Philosophy in Chemistry, Middle East Technical University**
by,

Prof. Dr. Naci Emre Altun
Dean, **Graduate School of Natural and Applied Sciences**

Prof. Dr. Ali Çırpan
Head of the Department, **Chemistry**

Assoc. Prof. Dr. Gökem Günbaş
Supervisor, **Chemistry, METU**

Examining Committee Members:

Prof. Dr. Cihangir Tanyeli
Chemistry, METU

Assoc. Prof. Dr. Gökem Günbaş
Chemistry, METU

Assoc. Prof. Dr. Salih Özçubukçu
Chemistry, METU

Assoc. Prof. Dr. Yunus Emre Türkmen
Chemistry., Bilkent Uni.

Assoc. Prof. Dr. Safacan Kölemen
Chemistry, Koç Uni.

Date: 30.05.2024



I hereby declare that all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

Name Last name : Osman Karaman

Signature :

ABSTRACT

EXPANDING THE SPECTRUM OF PHOTSENSITIZERS FOR POTENT PHOTODYNAMIC THERAPY AGAINST BRAIN CANCER

Karaman, Osman
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Cancer remains one of the foremost global health concern, since effective treatment modalities for a range of cancer types are still elusive. While significant progress has been made in cancer prevention and treatment across various types of cancer, brain tumors persist as an unresolved clinical challenge. Three fundamental issues stand at the forefront of the long-standing challenges of brain cancer treatment: a high percentage of inoperable brain tumors, the limited number of diagnostic and therapeutic drugs can pass through the blood-brain barrier and absence of effective, targeted conventional therapies. Considering the mortality rate, all these challenges in the diagnosis and treatment of brain cancer emphasize the urgent need for alternative, targeted treatment modalities.

Photodynamic Therapy (PDT) is an alternative treatment method for diverse cancer types and has attained attention due to minimally invasive nature and fewer side effects compared to current treatment methods. Despite, all these advantages of PDT, limited tissue penetration of light that initiate the activation of photosensitizer hinders the widespread utilization of PDT. To address these challenges, near-infrared (NIR) absorbing, low molecular weighted, brain cancer targeted photosensitizers have emerged.

Here, a wide range of advanced fluorophores and photosensitizers with strong Far-Red/NIR absorption, low molecular weight and functionalized with targeting moieties have been developed. Novel synthetic approaches for their synthesis, investigation of their photophysical properties and evaluation of their *in vitro* efficacy as theranostic PDT agents have been demonstrated. Specifically, photosensitizers based on resorufin and Si-fluorescein exhibited absorption maxima in the red region at 572 nm and 598 nm, respectively. The Si-rhodamine analogue displayed a further red-shifted absorption maximum at 640 nm. Moreover, both resorufin and Si-fluorescein, as well as Si-rhodamine derivatives, demonstrated selectivity towards various brain tumors in *in vitro* studies. Furthermore, photosensitizers based on phenoselenazine exhibited absorption maxima in the NIR region (>650 nm), and their application in *in vitro* studies revealed their potential as promising candidates for the treatment of brain cancer.

Keywords: Photodynamic Therapy, Photosensitizer, Brain Cancer, Near Infrared

ÖZ

BEYİN KANSERİNE KARŞI ETKİN FOTODİNAMİK TERAPİ İÇİN IŞIĞA DUYARLILAŞTIRICILARIN SPEKTRUMUNUN GENİŞLETİLMESİ

Karaman, Osman
Doktora, Kimya
Tez Yöneticisi: Doç. Dr. Görkem Günbaş

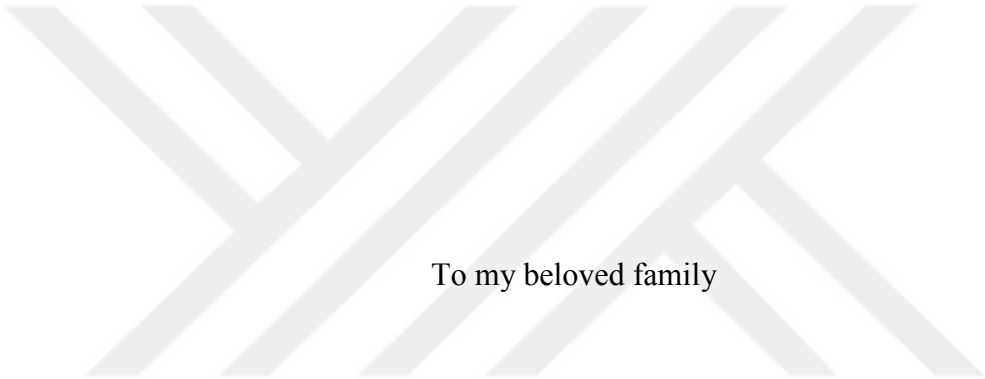
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Kanser, bir dizi kanser türü için etkili tedavi yöntemlerinin hala bulunması zor olduğundan, en önemli küresel sağlık sorunlarından biri olmaya devam etmektedir. Çeşitli kanser türlerinde kanserin önlenmesi ve tedavisinde önemli ilerlemeler kaydedilmiş olsa da, beyin tümörleri çözülmemiş bir klinik sorun olmaya devam etmektedir. Beyin kanseri tedavisinin uzun süredir devam eden zorluklarının ön saflarında üç temel konu yer almaktadır: ameliyat edilemeyen beyin tümörlerinin yüksek bir yüzdesi, sınırlı sayıda teşhis ve tedavi ilacının kan-beyin bariyerini geçebilmesi ve etkili, hedefe yönelik geleneksel tedavilerin yokluğu. Ölüm oranı göz önüne alındığında, beyin kanserinin tanı ve tedavisindeki tüm bu zorluklar, alternatif, hedefe yönelik tedavi yöntemlerine olan acil ihtiyacı vurgulamaktadır.

Fotodinamik Terapi (PDT), çeşitli kanser türleri için alternatif bir tedavi yöntemi olup, minimal invaziv yapısı ve mevcut tedavi yöntemlerine göre daha az yan etkisi nedeniyle dikkat çekmektedir. PDT'nin tüm bu avantajlarına rağmen, ışıığa duyarlılaştırıcının aktivasyonunu başlatan ışığın dokuya sınırlı nüfuz etmesi, PDT'nin yaygın kullanımını engellemektedir. Bu zorlukların üstesinden gelmek için yakın kızılötesi (NIR) emici, düşük moleküler ağırlıklı, beyin kanserini hedef alan ışıığa duyarlılaştırıcılar ortaya çıktı.

Burada, güçlü Uzak-Kırmızı/NIR absorpsiyonuna sahip, düşük moleküler ağırlığa sahip ve hedefleme parçalarıyla işlevselleştirilmiş çok çeşitli gelişmiş floroforlar ve ışığa duyarlılaştırıcılar geliştirilmiştir. Bunların sentezi için yeni sentetik yaklaşımlar, fotofiziksel özelliklerinin araştırılması ve teranostik PDT ajanları olarak *in vitro* etkinliklerinin değerlendirilmesi gösterilmiştir. Spesifik olarak, resorufin ve Si-floresein bazlı ışığa duyarlılaştırıcılar, kırmızı bölgede sırasıyla 572 nm ve 598 nm'de maksimum emilim sergiledi. Si-rodamin analogu, 640 nm'de bir başka kırmızıya kayan emilim maksimumu sergiledi. Ayrıca hem resorufin hem de Si-floresein ve ayrıca Si-rodamin türevleri, *in vitro* çalışmalarda çeşitli beyin tümörlerine karşı seçicilik göstermiştir. Ayrıca, fenoselenazine dayalı ışığa duyarlılaştırıcılar, NIR bölgesinde (>650 nm) maksimum emilim sergilemiştir ve bunların *in vitro* çalışmaları, beyin kanseri tedavisi için umut verici adaylar olarak potansiyellerini ortaya çıkarmıştır.

Anahtar Kelimeler: Fotodinamik Terapi, Işığa Duyarlılaştırıcı, Beyin Kanser, Yakın Kızılötesi



To my beloved family

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LIST OF ABBREVIATIONS

ABBREVIATIONS

β -gal	β -galactosidase
9-BBN	9-Borabicyclo(3.3.1)nonane
A549	lung carcinoma from human
AA	astrocytoma
ADMDA	9,10- antracenediyl-bis(methyene)dimalonic acid
Aliquat 336	<i>N</i> -methyl- <i>N,N,N</i> -triethylammonium chloride
ALA	5-aminolevulinic acid
APAF-1	Apoptotic protease activating factor-1
APN	aminopeptidase-N
ATP	adenosine triphosphate
BBB	blood brain barrier
BINAP	([1,1'-binaphthalene]-2,2'-diyl)bis(diphenylphosphane)
BODIPY	4,4-difluoro-4-bora-3a,4a-diaza-s-indacene
BTMA	benzyltrimethylammonium
CAN	Ceric ammonium nitrate
Ce6	chlorin e6

CT	computed tomography
DDQ	2,3-dichloro-5,6-dicyano-1,4-benzoquinone
DMEM	Dulbecco's Modified Eagle Medium
DNA	deoxyribonucleic Acid
DNBS	dinitrobenzenesulfonate
EEDQ	2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline
FasL	Fas ligand
FBS	Fetal Bovine Serum
FR	far red
FDA	Food and Drug Administration
FITC	fluorescein isothiocyanate
GMB	glioblastoma multiforme
GSH	glutathione
HeLa	cervical carcinoma from human
HOMO	highest occupied molecular orbital
Hp	hematoporphyrin
HpD	hematoporphyrin derivative
HPLC	high-performance liquid chromatography
HRMS	high-resolution mass spectrometry
IBX	2-iodoxybenzoic acid
ICT	intramolecular charge transfer
IC50	half-maximal inhibitory concentration

ICG	indocyanine green
ISC	intersystem crossing
L929	mouse fibroblast cell line
LAP	leucine aminopeptidase
LED	light-emitting diode
LDL	low-density lipoprotein
LUMO	lowest unoccupied molecular orbital
MCF-7	human breast cancer cell line
mCPBA	<i>meta</i> -Chloroperoxybenzoic acid
MDA MB 231	human breast adenocarcinoma
m-IDH-1	mutated isocitrate dehydrogenase
MRI	magnetic resonance imaging
MTT	3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide
NADPH	nicotinamide adenine dinucleotide phosphate
NBS	N-bromosuccinimide
NCS	N-chlorosuccinimide
NHS	N-hydroxysuccinimide
NIR	near infrared
NIS	N-iodosuccinimide
NMR	nuclear magnetic resonance
NTR	nitroreductase

PBS	phosphate buffered Saline
PCC	pyridinium chlorochromate
PET	positron emission tomography
PDT	photodynamic therapy
PMB	<i>para</i> -methoxybenzyl
PS	photosensitizer
ROS	reactive oxygen species
SDS	sodium dodecyl sulfate
SH-SY5Y	neuroblastoma cell line
SIT	structure intrinsic targeting
S _N 2	bimolecular nucleophilic substitution
S _N Ar	nucleophilic aromatic substitution
TBAF	tetra- <i>n</i> -butylammonium fluoride
TBDMS	<i>tert</i> -butyldimethylsilyl
TICT	twisted intramolecular charge transfer
TM	Tokyo Magenta
TMRM	tetramethyl rhodamine methyl ester
TN-C	extracellular matrix glycoprotein tenascin C
TNF _∞	tumor necrosis factor
TRAIL	TNF-related apoptosis inducing ligand
TTA	triplet - triplet annihilation
U118 MG	human brain glioma cells

U87 MG	human glioblastoma cells
XPhos	dicyclohexyl[2 <i>¢</i> ,4 <i>¢</i> ,6 <i>¢</i> -tris(propan-2-yl)[1,1 <i>¢</i> -biphenyl]-2-yl]phosphane



LIST OF SYMBOLS

SYMBOLS

λ_{abs}	Maximum Absorption Wavelength
λ_{fl}	Maximum Fluorescence Wavelength
Φ_{F}	Fluorescence Quantum Yield
Φ_{Δ}	Singlet Oxygen Quantum Yield

CHAPTER 1

INTRODUCTION

1.1 What is Cancer?

Cancer simply known as immense group of diseases that can be appear in almost in all tissue or organ of the body in which cell can divide uncontrollably and sweep into nearby normal cells. Cancer may also march into other tissues and organs via bloodstream or lymph system.¹ Usually, normal cells grow and undergoes cell division when body needs new cells. When they aged or damaged their lifecycle ends and new cells take their places. But sometimes, without any signaling to grow, abnormal or damaged cells start to grow and divide to form lumps of tissue which is known as tumor. Tumors could be either benign or cancerous. Cancerous tumors can invade adjacent cells and travel far from the original tissue/organ to form new tumors which is known as metastasis.²

The underlying cause of cancer is the formation of genetic disorders, especially genes that play a role in cell functions, such as cell growth or death. Several factors could cause defects in genes. They are usually caused by errors occurring in cell division or due to DNA damage caused by harmful substances in the environmental like chemicals in tobacco, UV ray from sun. Additionally, these genetic disorders could be inherited from previous generations. Before cancer develops, the body typically eliminates cells with damaged DNA. However, as we age, the body's ability to do so decreases, resulting in a proportional increase in the risk of cancer.³

1.1.1 Are We Losing Battle Against Cancer?

The first written record of human cancer incidence documented in ancient Egypt which is discovered in 19th century. However, it pursues us from pre-historic ages. The oldest evidence of tumor in mammals founded in fossilized dinosaurs that lived approximately 70 million years ago and human bones from pre-historic ages.⁴ One of the most detailed analyses conducted with several species of dinosaurs by fluoroscopy to find evidence of tumor and abnormalities further evaluated with computerized tomography and only one kind of dinosaur accommodate tumors but only 0.2% of specimens determined as malignant tumor.⁵ Most cancer types emerged in the latter decades of human life. Before 19th century, lifetime expectancy was quiet low. Since the middle of the 19th century, lifetime expectancy began to increase onwards due to improvements in health science, such as the discovery of vaccines and antibiotics. With these discoveries, most infectious diseases eliminated as a leading cause of death. Nowadays, death mostly has been attributed to old age and diseases either unique for old age or fatal only in old people declared as a main cause of death. Arterial diseases and cancer are the leading cause of death in latest century.⁶ Even arterial diseases still the main reason of death in our population, cancer now become the major health problem of the world.⁷ Although the mortality rate of cancer has become more common than that arterial diseases in high income countries,⁸ reduced modifiable risk factors (cancer monitoring, more successful treatments, prevention, ceasing smoking) have had a positive effect on incidence and mortality rate of cancer. In the United States overall rate of death caused by cancer decreased by 26% in between 1991 and 2015.⁹ However, globally, cancer rate continues to increase. The type and rate of cancer differ across countries and depending on lifestyle and the level of economic development in each country.¹⁰ In high-income level countries, rate of incidence is the highest for all cancers including breast, lung, prostate and colorectal cancer but rates of mortality are declining. However, in low-income countries, both incidence and mortality rate remain high including liver, cervical, esophageal cancers due to lack of access to early diagnosis and treatment.¹¹

1.1.2 How Can We Cope with Cancer?

Cancer is the number two in the list of the most leading causes of death behind the arterial diseases. Today, in the United States, almost half of the men and women will be diagnosed with cancer during their lifetimes. However, cancer is not a new phenomenon, affecting people all around the world. Evidence from prehistoric times and documents from ancient Egypt reveal that cancer has been present since the beginning of human history. During those times, only palliative treatment methods were applied to address cancer.¹²

Since ancient times, people devised theories to explain sources of cancer. Hippocrates believed that the human body consists of four bodily fluids known as humor: blood, phlegm, yellow bile and black bile. According to the humoral theory, an excess amount of black bile in a particular organ led to cancer. Diseases were also believed to result from an imbalance between these fluids. This belief was accepted until the end of the Middle Ages. During those times, knowledge about cancer was limited due to religious reasons. In 17th century, people supported the Lymph theory which is suggested that cancer forms from liquid known as lymph that constantly expelled by the blood. In the 19th century, scientists revealed that cancer is not composed of lymph but of cells. These cells were found to be derived from other cells, like all cells. Later, several theories developed about cause of cancer including chronic irritation, trauma or spread from parasite. After the discovery of DNA helical structure by the middle of the 20th century by Watson and Crick who were received Nobel prize for that discovery¹³, scientists learned how genes works and how mutations could damage them. Scientists realized that chemicals, radiations could cause cancer. Also, inheritable from ancestor. In 1970s two important genes family were discovered: oncogenes and tumor suppressor genes. Oncogenes are formed by the mutated normal genes of the cell, known as protooncogenes. They cause uncontrolled growth of the normal cells. On the other hand, tumor suppressor genes control cell division, DNA repair and send signal to the cell about when to die.

Dysfunction of these genes leads to cancer. After discovery of these gene families, several gene correlated with cancer also discovered.¹²

The first cancer monitoring method was developed by George Papanicolaou. Originally, Pap test developed for understanding the menstrual cycle, but he realized its potential for early detection of cervical cancer. After that, mammography was developed in the 1960s for monitoring breast cancer. Later, many techniques were developed and clinically tested for the early detection of most types of cancer.¹⁴

In ancient times, tumors were attempted to be removed from the body by surgery. Usually, cancer would come back after removal. Even today, many people think that many types of cancer are incurable. After discovery of anesthesia, surgeons began to remove the whole tumor along with lymph nodes. However, the limitations of surgery were realized after an understanding of cancer metastasis emerged. In the 1970s, sonography, computed tomography (CT scans), positron emission tomography (PET scan), magnetic resonance imaging (MRI scans) began to replace surgery. Also, in the late 20th century, surgery was combined with chemotherapy and radiotherapy. Even though chemotherapy exhibited successful results in the treatment of cancer over the years, several new approaches are being studied to eliminate side effects.¹⁵

As our understanding of cancer biology deepens, significant advances had been made in prevention, early cancer detection and treatment in recent years. Currently, cancer research is advancing across multiple fronts. Disciplines including antiangiogenic chemotherapy, RNA expression profiling, proteomics, nanotechnology and more targeted treatment modalities such as: antisense oligodeoxynucleotides and small interfering RNA are assuming pivotal roles in the landscape of cancer research.¹²

Despite numerous advances in cancer prevention, early detection and treatment in recent decades, a universal cure remains elusive. Many researchers are dedicated to achieving ultimate victory over cancer following numerous battles. Cancer is a multifaceted disease, requiring comprehensive approaches to conquer it's all aspects.

1.1.3 Overcoming the Obstacles in the Treatment of Brain Cancer

While significant progress has been made in cancer prevention, early detection, and treatment across various types of cancer, brain tumors persist as an unresolved clinical challenge. Despite their lower incidence compared to many other types of cancer, brain tumors are among the most feared due to their high mortality rates¹⁶. Among the pediatric solid tumors, brain cancers are also the most prevalent and most fatal type of cancer. Furthermore, several adverse effect of medical operations such as surgery, chemotherapy and/or radiotherapy are often observed in people who have suffered from brain tumor and have survived in their childhood¹⁷.

Benign and malignant brain tumors exhibit broad heterogeneity concerning sex, age, ethnicity, leading to high mortality rate¹⁸. Additionally, their highly sensitive location limits the neurosurgeons' ability to access tumors. Furthermore, the blood brain barrier (BBB) poses another obstacle to treatment with systematic chemotherapy. Both conventional and new treatment modalities remain inadequate in addressing persistent brain cancer due to the brain's unique propagation, microenvironmental conditions and genetic and epigenetic features¹⁹⁻²¹. Moreover, compared to other types of cancer, the rarity of brain cancer and along with the aforementioned challenges, makes it unattractive toward pharmaceutical companies and most of the researchers^{22,23}.

Among all types of brain tumors, glioblastoma is the most aggressive. More than 65% of diagnosed individuals will die within two years after being diagnosis with glioblastoma²⁴. For all malignant brain cancer types, the five-year relative survival rate is reported to be around 35% and the ten-year relative survival rate is reported to be around 29%²⁵. On the other hand, the five-year relative survival rate is documented to be around 94% for pilocytic astrocytoma, while glioblastoma's five-year relative survival rate is reported as only 5%²⁵.

Three fundamental issues stand at the forefront of the long-standing challenges of brain cancer treatment. The first issue pertains to the high percentage of inoperable

brain tumors. The proximity of tumors to vital cells impedes the complete resection of the tumor during neurosurgery²⁶. Additionally, difficulties in detecting tumors at early stages restricts the surgical options²⁷. Secondly, both diagnostic and therapeutic drugs must penetrate the BBB. The BBB consist of tightly fused cerebral capillary endothelium junctions, forming a continuous lipid layer to prevent uncontrolled movement into and out of the brain^{17,22}. Most drugs are inadequate for penetrating BBB, as it only allows small, lipophilic, low molecular weight (up to 500 Da) and electrically neutral molecules to pass¹⁶. The final issue lies in the absence of effective, targeted conventional therapies. While there are over 10 targeted therapies for lung, breast and colon cancer - three of the ten most common cancer types - only one targeted therapy exists for malignant brain cancer (Avastin). Unfortunately, Avastin has not shown superiority over chemotherapy and radiation in terms of extending survival²⁴. Considering mortality rate, all these challenges in the diagnosis and treatment of brain cancer emphasize the urgent need for further research and investment.

1.2 Photodynamic Therapy

Photodynamic Therapy (PDT) is an alternative treatment method for diverse cancer types and has attained attention due to minimally invasive nature and fewer side effects compared to current treatment methods.²⁸

1.2.1 A Brief History of Photodynamic Therapy

The utilization of light as a therapeutic modality has garnered increasing attention since the early 20th century and continues to do so today. Nonetheless, awareness of the therapeutic efficacy of light was prevalent in antiquity. In civilizations such as Egypt, Greece, India, and China, sunlight was commonly employed in the treatment of various dermatological conditions. Nevertheless, the significance of light therapy remained largely overlooked until the advent of the 20th century.²⁹

The golden age of modern light therapy commenced with the utilization of light to treat lupus vulgaris by Niels Finsen, who was awarded the Nobel Prize in Physiology or Medicine in 1903.³⁰ In 1900, Oscar Raab and Prof. Herman von Tappeiner reported the first scientific explanation of photosensitized reaction. They described the fatal effect of acridine red and light combination on protozoa, marking the first reported instance of cell death induced by photochemical reaction.³¹ Building upon this research, von Tappeiner and Jesionek successfully treated the skin tumor with the topically applied eosin and white light. Subsequently, von Tappeiner identified the final crucial component of photosensitized reactions. Following the recognition of oxygen's significance in photosensitized reactions, the term “photodynamic action” was introduced by von Tappeiner in 1907 to describe this phenomenon.³²

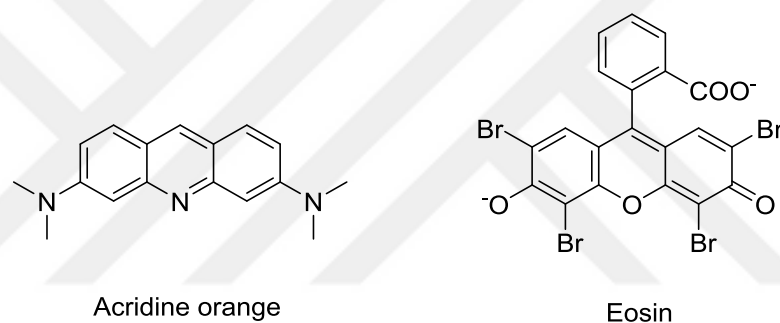


Figure 1.1. Structure of Acridine orange and Eosin.

After the recognition of effectiveness of hematoporphyrin by W. Hausmann in 1911,³³ the first human trial of photodynamic action was conducted by F. Meyer-Betz. He described the effects of photochemical reactions on himself after injection of hematoporphyrin.³⁴

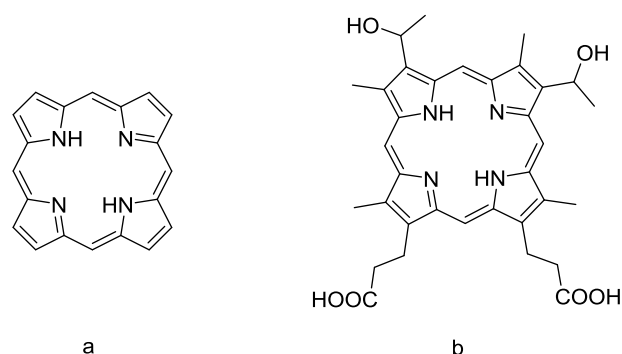


Figure 1.2. (a): Structure of porphyrin ring. (b): Structure of Hematoporphyrin.

In 1960, R. Lipson and S. Schwartz made a notable discovery regarding the diagnostic and therapeutic effect of a hematoporphyrin derivative (HpD) by injecting.³⁵ Subsequently, in the 1970s, significant advancements unfolded in PDT. Initially, T. Dougherty achieved the inhibition of mammary tumor growth in mice via administration of HpD.³⁶ This was closely followed by J. F. Kelly's demonstration of bladder tumors eradication in mice utilizing PDT.³⁷ Collaboratively with M. E. Snell, Kelly delved deeper into investigating the photodynamic effect of HpD on bladder cancer and culminating the first PDT application to humans with HpD in 1976.³⁸ Advancements in the PDT application of hematoporphyrin continued with the treatment esophageal cancer by J. S. McCaughan,³⁹ and the treatment of gastric carcinoma by Y. Hayata.⁴⁰ Furthermore, HpD purified by Dougherty and his colleagues, leading to production of the first US Food and Drug Administration (FDA) approved photosensitizer, Photofrin. Since then, PDT has continued to evolve and expand its clinical application areas.⁴¹

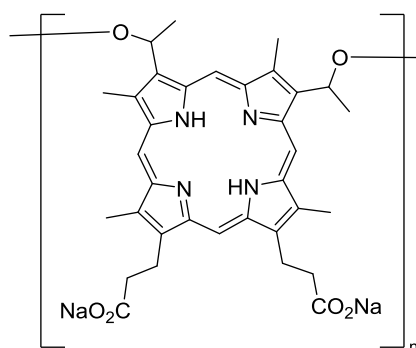


Figure 1.3. Structure of Photofrin.

1.2.2 Working Principles of PDT

The photodynamic action only occurs with the cooperation of a photosensitizer (PS), light, and molecular oxygen. This combination results in tumor necrosis or apoptosis. The photodynamic action begins with the irradiation of the PS in the target tissue with appropriate light. After irradiation, the PS is transformed into the singlet excited state ($^1\text{PS}^*$) from its singlet ground state (^1PS).⁴² This excited state is short-lived with a half-life ranging between 10^{-6} to 10^{-9} seconds and very unstable.⁴³ The PS can decay to a more stable ground state (^1PS) either by losing energy through fluorescence or by converting this energy to heat (internal conversion). Additionally, the singlet excited PS could transition to the long-lived, more stable, and energetically lower excited state, the triplet excited state ($^3\text{PS}^*$), by intersystem crossing. To undergo intersystem crossing, PS should convert spin orbit coupling at higher energy state. In this state, PS could release energy to return ground state through phosphorescence. Additionally, triplet excited state provides longer duration (up to ten nanoseconds) for the direct transfer of energy to molecular oxygen (O_2).⁴⁴ This energy transfer termed as Type II reaction and resulted in generation of highly toxic type of reactive oxygen species (ROS), singlet oxygen ($^1\text{O}_2$) and returns the PS to its fundamental state. The aggressive nature of $^1\text{O}_2$ can induce oxidative stress, ultimately resulting in cell death.⁴⁵ Additionally, excited PS may undergo an alternative reaction pathway. Specifically, it can interact directly with biomolecules through electron transfer to generate $\text{HO}_2^\cdot$ and superoxide anion radical, $\text{O}_2^{\cdot-}$. Subsequently, $\text{O}_2^{\cdot-}$ can undergoes further transformation, yielding hydrogen peroxide (H_2O_2), a precursor to the highly reactive hydroxyl radical ($\text{OH}^\cdot$). The latter exhibits pronounced reactivity towards a wide array of biomolecules, rendering it a promising agent for combating hypoxia-related conditions. This kind of reaction known as Type I and may occurs simultaneously with Type II reactions.⁴⁶ The relative occurrence of these reactions depends on the factors such as the PS substrate type, oxygen level and the affinity of the photosensitizer for the substrate. During PDT, Type II reactions typically predominate over Type I reactions and singlet oxygen is mainly

responsible for inflicting biological damage.^{42,47} The mechanism of PDT is illustrated in the Jablonski diagram which is depicted in Figure 1.4.

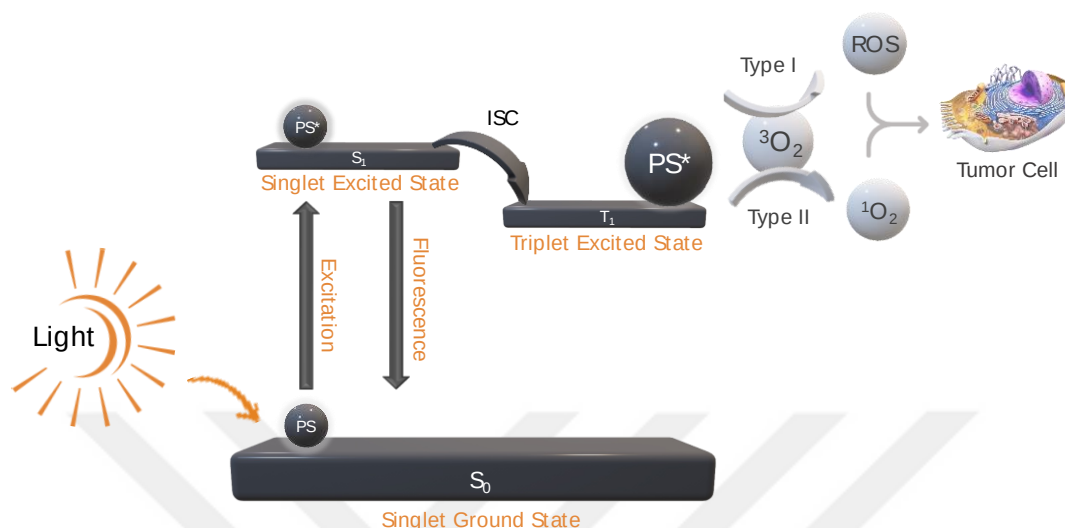


Figure 1.4. Schematic illustration of PDT including the Jablonski diagram²⁸

The effectiveness of PDT action also depends on the singlet oxygen quantum yield (Φ_Δ) which is determined by its triplet excited state's quantum yield and lifetime. ROS exert influence primarily on cells proximal to the localization of PS, owing to their short half-life and high reactivity. This ROS characteristic enhances the tumor selectivity of PDT.⁴⁸

Several other factors also contribute to the influence area of PDT. Extension of damage and cytotoxicity relies on factors such as PS type, intra- and extracellular location, concentration of drug, light fluence and its rate, oxygen concentration and incubation time following light irradiation.⁴⁷

The application of PDT in deeper and hypoxic cancers poses greater challenges due to lack of oxygen and poor penetration ability of light into the tissue. Conversely, in superficial applications and oxygen-rich tissues, ROS generation is greater, thereby leading to enhanced PDT therapy.⁴⁹

1.2.3 Effects of PDT on Tumors

While designing a potent PS, it is crucial to consider possible cell death mechanisms and to understand how PDT works. In PDT, cancer cells can be eliminated directly through apoptosis or non-apoptotic pathways including necrosis and autophagy. Additionally, indirect damage to the tumor vasculature, which serves as a vital source of nutrients and oxygen for tumors can cause cell death. Furthermore, PDT has the capacity to evoke an immune response against cancer cells.⁵⁰

All these mechanisms are induced by various signaling pathways, depending on possible factors such as the type of PS, its localization and dosage, the genotype of the cell, protocol utilized, oxygen concentration among others. For instance, mitochondrial localization typically induces apoptosis, whereas plasma membrane or lysosomal localization may lead to necrosis.⁵⁰

Both programmed (apoptotic) and non-programmed (necrotic) pathways can cause cell death in PDT based mediation. Apoptosis is considered the principal cell death mechanism. It is observed that low-intensity light can trigger an energy dependent, genetically encrypted and programmed mechanism, apoptosis while high-intensity light can lead to the necrosis, which is defined as uncontrolled, sudden and violent cell death over a broad area.⁵¹

Condensation of chromatin, cleavage of chromosomal DNA into internucleosomal compartments, shrunken cells, wrinkled membrane and the absence of membrane breakdown during formation of apoptotic bodies are characteristics features of apoptosis. Since there is no toxic chemical leakage, effect or immune response is not expected. The apoptosis process could be initiated through either intrinsic or extrinsic pathways.⁵² In extrinsic pathway, death receptors are activated and binding of the ligands such as FasL, TRAIL, TNF- α to their receptors, which exist on the cell surface, can trigger the extrinsic apoptotic pathway. These death receptors form clusters and induce signaling that engages procaspases 8 and 10. These initiator procaspases dimerize and activate effector procaspases 3 and 7, resulting in

apoptosis.⁵³ In the intrinsic pathway, the released cytochrome c from the mitochondrial membrane initiates apoptosis. Several proapoptotic proteins regulate apoptosis. Mitochondria plays a crucial role in the intrinsic pathway. The cytosolic release of cytochrome c or other proapoptotic molecules, which are stored in mitochondria, activates death stimuli through different signaling pathways and increases mitochondrial membrane permeability. The released cytochrome c binds to the apoptotic protease-activating factor (Apaf-1), which then binds to the caspase 9 in the presence of ATP to form the apoptosome. Activated caspase 9 activates caspase 3 and 7. These activated effector caspases cleave cellular substrates, subsequently result in cell death.⁵⁴

Nucleus pyknosis, swelling of cytoplasm, cellular fragmentation and material release into extracellular environments due to progressively disintegrated cytoplasmic membrane represent the characteristic features of necrosis. High-intensity light and elevated PS concentration lead to cell necrosis and suppress apoptosis as a result of more damage to the membrane. A high degree of ROS rather than singlet oxygen in PDT is the main cause of necrosis. Necrosis mainly occurs when PS localizes in the plasma membrane. For instance, well known PDT agent, Photofrin, also localizes in the plasma membrane and induces vascular endothelial damage under hypoxic conditions and leading to thrombosis subsequent ischemic tumor necrosis. Following this, an immune response ensues, which is associated with intense inflammation.⁵⁵

In the absence of apoptotic machinery, the autophagic pathway could lead to cell death.⁵⁶ In PDT, autophagy either increases the cell survival rate or kills the cells. Generally, autophagy assists in damaged organelles and protein recycling and acting as a self-survival mechanism. In PDT, type of PS and ROS, presence of apoptotic machinery and extended photodamage could influence whether autophagy will be cytotoxic or cytoprotective.⁵⁷ The mitochondrial, lysosomal or endoplasmic reticulum localization of PS could induce cell death through the autophagic pathway. Autophagy in apoptosis-related cells could play a regenerative role and could trigger cell death in non-apoptotic cells. The mechanism of autophagy remains ambiguous regarding whether it triggers cell death or survival and further research is needed to

elucidate the mechanism of autophagy and to determine the immune response after PDT.⁵⁸

In addition to direct pathways, the vascular effect of PDT also contributes to the tumor destruction. Tumor microvasculature can be destroyed through PDT. PS localized to the endothelial cells of the vascular system can generate ROS after irradiation with proper light. The generated ROS could disrupt the vascular wall, obstructing tumor feeding such as, oxygen and nutrient, eventually, resulting in death of starved tumors cells. In terms of long-term efficacy, the vascular effect of PDT appears to be very important and could be enhanced when PSs are localized to the vasculature through usage of short drug-light interval. Vascular system-localized PSs could be rapidly evacuated from organism, minimizing photosensitivity. These advantages give higher long-term efficacy over direct pathways in a short period of time.⁵⁹

Unless the general considerations for many years that PDT is a tumor localized treatment modality, the recent studies revealed that PDT can influence disparately the immune system through stimulating or suppressing the immune response. It is obvious that induced immune system has a great impact on PDT efficacy. Combined direct effect of PDT on the tumor and its vasculature with upregulated immune system provide long-term tumor control.⁶⁰

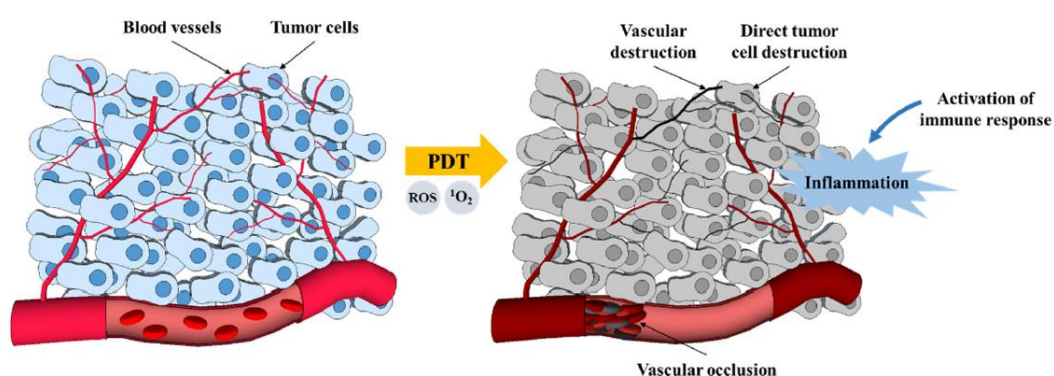


Figure 1.5. PDT mechanism for tumor destruction.⁴⁸

1.2.4 Building Blocks of PDT

Photodynamic Therapy stands out as one of the most promising treatment modalities for diverse array of diseases, including both carcinogenic and non-carcinogenic afflictions, as well as infectious diseases. Over the past two decades, PDT has significantly broadened its application areas demonstrating efficacy in tumor eradication within tissues, combating fungal, bacterial and viral infections, including notorious COVID 19.⁴⁸ Achieving optimal efficacy in PDT necessitates the comprehensive consideration of its three fundamental constituents. The dynamic interactions between PS, precisely tuned light of a specific wavelength and molecular oxygen is pivotal in generating ROS essential for the targeted destruction of tumor tissue.⁶¹ Improvements across all three critical elements are imperative to yield more promising outcomes in our enduring battle against the enemy, cancer.

1.2.4.1 Light

Light is one of the most crucial components of PDT. Various types of light sources are employed in PDT applications, such as lasers, laser-emitting diodes (LEDs), incandescent light even daylight.⁶² The choice of light source and delivery mode may vary for depending on the targeted tissue type. While lasers offer precise dosimetry calculations, their use is often accompanied by high costs and necessitates optical system to increase the influence area of the light beam to irradiate larger tissues. Conventional light sources are typically avoided due to their potential thermal hazards. LED light sources are widely used in PDT applications due to their cost effectiveness, simplicity, wide illumination coverage, availability of diverse arrays, minimal hazard and absence of thermal side effects. Additionally, natural light finds utility in skin cancer treatment. Its reduced photosensitivity and ease of applicability render it preferable for topological PDT applications.⁴⁸

The penetration of light into tissue is very complicated process influenced by reflection, scattering or absorption. These processes can vary depending on the type

of tissue and wavelength of light employed. Endogenous chromophores naturally present in tissues, such as hemoglobin, myoglobin and cytochromes absorb light, competing with PS and resulting in reduced photodynamic activity. The use longer wavelengths can mitigate light absorption by tissues, facilitating deeper tissue penetration. The term “tissue optical window” denotes the optimal range for tissue penetration, typically spanning from 600 nm to 1200 nm. Light wavelengths shorter than this ranges are associated with more absorption of light by chromophores and reduced tissue penetration, leading to high skin photosensitivity. Conversely, wavelengths exceeding 850 nm may not provide sufficient energy to excite PS to its triplet energy state, where interaction with molecular oxygen generates ROS. Optimal tissue penetration occurs within the range 600-850 nm and light sources for PDT are predominantly selected from this interval, known as the “phototherapeutic window”.

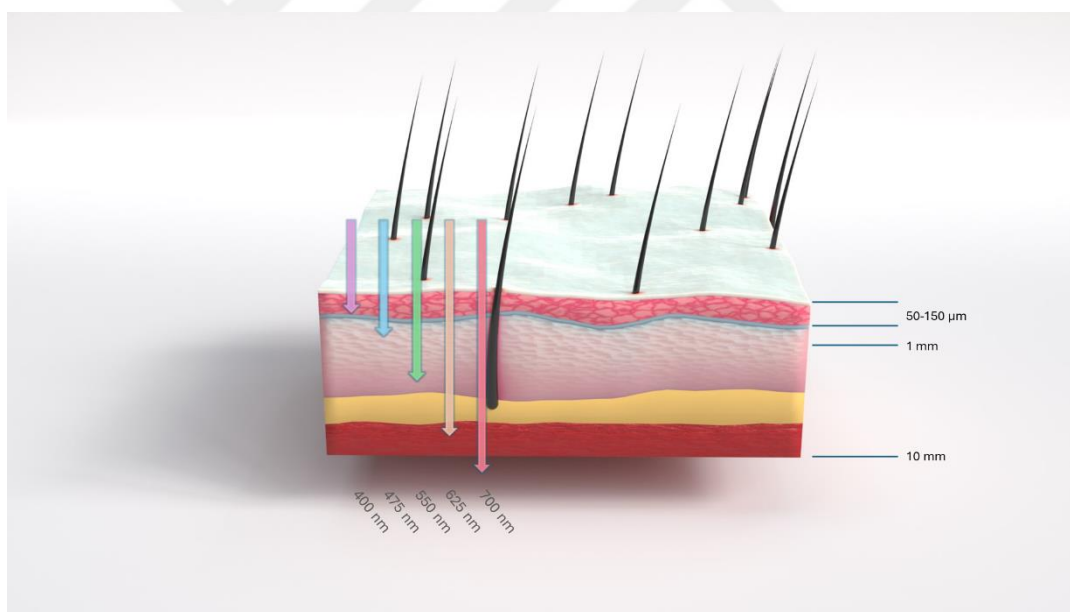


Figure 1.5. Penetration depth of light in human tissues.

The efficacy of PDT in clinical applications depends on the accurate delivery of light to the tumor tissue and the appropriate dosage of light. Modifying the dosimetry of light, including parameter such as total light dose, exposure time, delivery method, can enhance the clinical effectiveness of PDT. The fluence of light is typically

expressed in J/cm^2 , which defines the total amount of energy over cross-sectional area of the illuminated region. Higher fluence level can adversely affect normal tissues and may induce hyperthermia.⁴⁷ In PDT applications, a fluence around $200 \text{ J}/\text{cm}^2$ is commonly utilized, with a slower fluence rate, (expressed in W/cm^2) being favorable. The fluence rate denotes the instant energy delivered to a cross-sectional area of illuminated spot per second. At high-rate fluence, cellular oxygen may become depleted, leading to reduced photo-degradation of the PS. Additionally, the fluence rate has a significant impact on the type of cell death induced. A low fluence rate tends to result in apoptotic cell death, which is the dominant and preferred mechanism in PDT, as opposed to necrotic cell death. Necrosis can lead to the uncontrolled burst of cell content integrity, inflammation and edema.⁶³

Several alternative light dosing strategies are currently under investigation to mitigate oxygen depletion and other adverse effects. Fractionation, metronomic PDT and low dose PDT represent promising alternatives; however, none have been widely adopted to date. In fractionation, to maintain oxygen sources during PDT, intermittent dark periods are introduced. Unfortunately, this approach may lead to increased tumor resistance and clinical relapse, even though it may be more effective than single dose treatments. On the other hand, metronomic PDT involves delivery of PS and light to the tumor area over an extended period at slow rates or in fractions, aimed at minimizing oxygen depletion. While this technique has yet to be widely implemented in human subjects, encouraging preclinical results support its potential efficacy.⁶²

1.2.4.2 Oxygen

Another crucial element of Photodynamic Therapy (PDT) is oxygen. The efficacy of this treatment is highly contingent upon the concentration of oxygen within the targeted tumor. In PDT, activated PS can engage in reaction with nearby molecules via two pathways: Type I or Type II. In the former pathway, electrons or hydrogen are directly exchanged between PS and nearby biomolecules, resulting in the

generation of free radicals. In Type II, triplet excited PS ($^3\text{PS}^*$) and molecular oxygen ($^3\text{O}_2$) undergo a triplet-triplet annihilation (TTA) interaction to produce singlet oxygen ($^1\text{O}_2$).⁶⁴ PDT heavily relies on oxygen, as the Type II mechanism predominates in the cell death mechanism. $^1\text{O}_2$ is an exceptionally reactive and prominent type of ROS with a short lifetime of only a few microseconds. It exerts cytotoxic effects on various organic molecules under physiological conditions within the range of 200 nm by oxidizing them. Therefore, availability of molecular oxygen serves as the rate-determining factor in ROS generation via Type II mechanism.⁶⁵ Regrettably, it is widely acknowledged that tumor tissues often suffer from oxygen deficiency, primarily due to inadequate vasculature and the rapid, aggressive growth rate of tumors, which in turn prolongs the path of oxygen diffusion, exacerbating oxygen deprivation. The term used to delineate this oxygen deficiency is "hypoxia," defined as cellular oxygen concentrations below 1 μM , which is recognized as indicative of hypoxic conditions. Given the indispensable role of oxygen in PDT, addressing this issue is imperative for optimizing its efficacy.⁶⁶

In addition to hypoxic condition of tumor tissues, PDT also induces to oxygen depletion. Illumination of tumor tissue with high fluence rate results in temporary oxygen depletion in the irradiated area. When the consumption of oxygen by PDT exceeds the rate of oxygen diffusion, oxygen deficiency arises in the irradiated tumor tissue, impeding the effectiveness of the treatment. Moreover, PDT can diminish blood flow to the tumor area by obstructing the vasculature, thereby exacerbating hypoxia.⁴⁵

To address the challenges arising from hypoxic condition of tumor tissues and to enhance the PDT efficacy, numerous strategies have been reported. One approach involves maintaining the appropriate light dose by adjusting the fluence rate, often facilitated by the use of several imaging dyes. Before and during PDT, assessing the tissue oxygen concentration enables the attainment of optimal therapeutic outcomes. However, real-time monitoring of the oxygen concentration remains as challenge, primarily due to the limited reports on the combined usage of PS and imaging dyes. Moreover, various techniques have been developed to increase oxygen level in tumor

tissues, categorized into two main approaches: direct and indirect oxygen introduction. Indirect methods aim to elevate intracellular oxygen concentrations by decomposing intracellular pre-oxygen sources, such as hydrogen peroxide, manganese oxide, calcium peroxide. Conversely, oxygen can be directly introduced to the cell through oxygen carriers such as hemoglobin, perfluorocarbons and red blood cells.⁶⁷

1.2.4.3 Photosensitizers

The final key element of PDT are the photosensitizers (PSs). In addition to light and oxygen, PSs offers additional avenues for enhancing PDT efficiency thorough modifications. The term “photosensitizer” refers to dyes that can be activated by appropriate light to initiate photochemical or photophysical reactions. Photosensitizers can be classified into three subgroups based on their evolutionary timeline which reflects the emerging needs for enhanced PDT applications. Apart from its generation, all PSs must exhibit certain characteristics to be considered as ideal for PDT applications. These includes high purity, chemical stability, photosensitivity only upon illumination with appropriate light, high reactivity, activation within therapeutic window (600-850 nm), minimal dark toxicity, solubility in bodily fluids, high selectivity for tumor tissues over healthy tissues and ease of synthesis and availability.⁶⁸

1.2.4.3.1 Pioneering Photosensitizers: First Generation Innovations

Following the rediscovery of the importance of light therapy in the early 1900s, photosensitizers entered the realm of treatment on a commercial scale in the 1970s. Dougherty and his colleagues conducted tests using hematoporphyrin derivative (HpD) comparing it with hematoporphyrin (Hp), the first porphyrin derivative employed as a photosensitizer.³⁶ HpD exhibited enhanced tumor tissue selectivity, greater hydrophilicity and reduced photosensitivity on the skin compared to Hp,

rendering it promising candidate for clinical applications. Consequently, HpD was commercialized as a PDT agent under the market name “Photofrin”. Even though being the pioneer commercial PDT agent, Photofrin, now known as sodium porfimer, has remained the most frequently utilized PS in clinical applications. However, despite its widespread use, Photofrin limitations, including low chemical purity, inadequate tissue penetration ($\lambda_{\text{max}} = 630 \text{ nm}$), post-treatment photosensitivity, have constrained its clinical utility. These deficiencies have spurred researchers to explore new molecules and develop the second-generation PSs.⁶⁹

1.2.4.3.2 Upgraded Photosensitizers: Second Generation Improvements in Purity, Reactivity, and Tissue Penetration

In order to address the limitations of first-generation PSs, efforts to develop next generation PSs commenced in the early 1980s. Although numerous new molecules exhibiting potential photosensitizer characteristics were synthesized, only a select few progressed to clinical stage. Among them, an even smaller subset obtained official approval for clinical utilization as anti-cancer PDT agent.⁷⁰

In addition to hematoporphyrin derivatives, synthetic PSs, including benzoporphyrins, texaphyrins, chlorin, bacteriochlorin, thiopurines and 5-aminolevulinic acid (ALA) can be classified as second-generation PSs. Among these, ALA functions as a prodrug and serves as a precursor to protoporphyrin IX. The active form of ALA, protoporphyrin IX, acts as an active PS and finds place in clinical applications including topical or oral applications.⁷¹

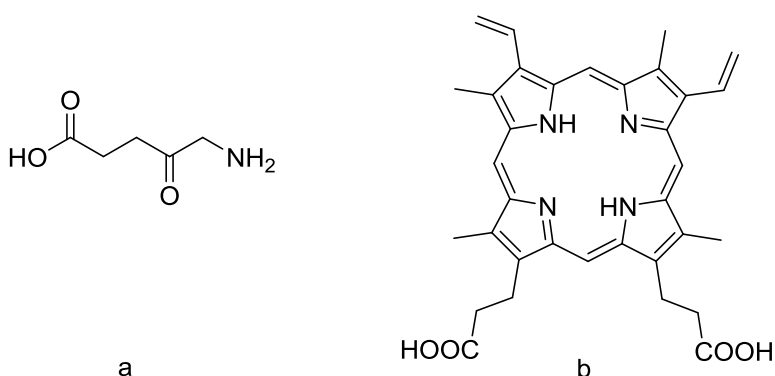


Figure 1.6. a) Structure of ALA, b) Structure of protoporphyrin IX

Higher chemical purity, increased singlet oxygen quantum yield, enhanced tissue penetration, red-shifted absorption maxima (650-800 nm), greater tumor selectivity, reduced adverse effects, and faster removal from the body are the characteristics features of second-generation PSs. However, the low hydrophilicity of these PSs limits their clinic applicability.⁶¹

1.2.4.3.3 Advanced Photosensitizers: Third Generation Breakthroughs in Tumor-Targeting and Drug Delivery

In this generation of PSs, researchers have concentrated on enhancing the selectivity and accumulation of PS within the tumors to reduce the necessary drug dosage for treatment. To achieve this object, various strategies have been devised, including incorporation of targeting groups, LDL lipoproteins, or monoclonal antibodies with second-generation PSs to increase their specificity toward particular tumor tissues.⁷²

Moreover, to facilitate administration of PSs, novel drug delivery approaches have been developed to improve the bioavailability of PDT applications. Techniques such as nanotechnology, the utilization of liposomes or lipoproteins and electroporation have been integrated with PDT to address emerging challenges in drug delivery.⁷³

1.2.5 Enhancing Therapeutic Impact: Photosensitizers Demonstrating Efficacy Within the Therapeutic Window

Tissue penetration of light is highly complex, since light can either be absorbed or scattered upon reaching to the tissue. The type of tissue and wavelength of light are crucial parameters affecting the extent of PDT. At shorter wavelengths, light may be absorbed by endogenous chromophores. Conversely, although light penetration into the tissue may be deeper into at longer wavelengths, it could be scattered or interfered through water absorption at wavelengths exceeding 1300 nm. These limitations have prompted researchers to introduce the concept “optical window”.⁷⁴

In PDT, heightened skin photosensitivity occurs at shorter wavelengths (<600 nm) due to poor light penetration and high light absorption by chromophores. Conversely, wavelengths longer than 850 nm cannot provide sufficient energy to excite ground state PS to its triplet excited state, where cytotoxic singlet oxygen is generated upon interaction of PS with molecular oxygen. Considering these limitations, the optimal tissue penetration interval is defined within the range of 600-850 nm, referred to as the therapeutic window for photochemical reactions in PDT applications.⁷⁵

In recent years, increased attention has been directed toward exploration of near infrared (NIR) shifted PSs for facilitating deep tissue PDT. The NIR region (700-1000 nm), also recognized as the biologically transparent window, exhibits minimal photodamage to healthy cells and allows for deeper tissue penetration compared to UV-Vis light. Given these favorable attributes of NIR light, considerable efforts have been undertaken to expand the clinical application of PDT by developing NIR-absorbing PSs.⁷⁰

1.2.5.1.1 Porphyrin

Porphyrins and their derivatives consist of 18 conjugated π -electrons macrocycles formed from tetrapyrrole structures connected by alternating double bonds. Due to strong excitation and emission bands, long triplet state lifetimes and high quantum

yields of ROS, porphyrin derivatives have been extensively investigated as a PS. Indeed, the first clinical PDT agent, Photofrin belongs to this family. Porphyrin and its derivatives demonstrate strong tumor selectivity and high cytotoxicity toward tumor. However, certain derivatives suffer from poor hydrophilicity, inadequate tumor localization, requirements for multiple light excitations and aggregation reduce PDT efficiency.⁷⁶ To address these challenges, continuous efforts have been devoted to the design of porphyrin-based PSs since the 1970s. As a result, several subgroups of porphyrins have been developed.⁴⁵ Chlorin and bacteriochlorin are porphyrin derivatives obtained through the reduction of one or two double bond of pyrrole respectively. Chlorin e6 (Ce6) stands out as a promising porphyrin derivative due to its reduced dark toxicity and elevated singlet oxygen quantum yield. In response to the challenges of PDT, numerous approaches have been proposed based on chlorin e6.⁷⁷

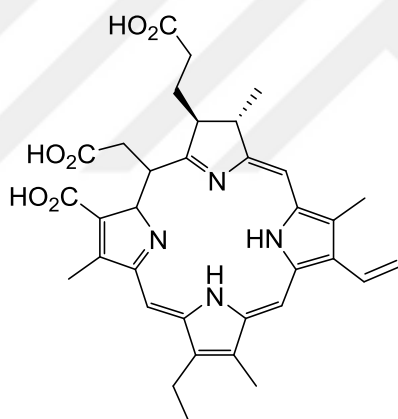


Figure 1.7. Structure of Chlorin e6.

1.2.5.1.2 Phthalocyanine

Phthalocyanines are derivative of porphyrins. Similar to porphyrins, they comprise four isoindole rings on an aromatic heterocycle and possess 18 conjugated π -electrons. Their notable physicochemical stability stems from the uniformly distributed electron density throughout the conjugated rings, resulting in very similar C-N bonds lengths. Phthalocyanines exhibit high extinction coefficients and absorb

light in the red and NIR regions. Furthermore, upon coordination with diamagnetic ions such as Zn^{2+} , Al^{3+} , Si^{4+} , they demonstrate a high singlet oxygen quantum yield. These advantageous characteristics of phthalocyanines have been evaluated in clinical PDT applications. Further research endeavors have aimed to enhance efficacy by focusing on tumor selectivity and cancer inhibition within the tumor area.⁷⁸

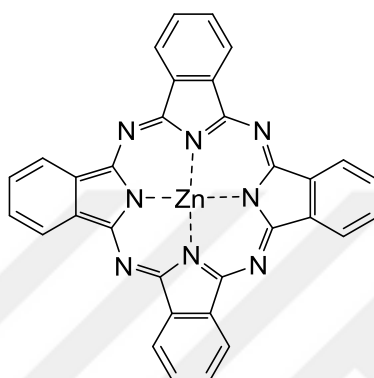


Figure 1.8. Structure of Liposomal Zn-Phthalocyanine derivative.⁷⁹

1.2.5.1.3 Cyanine

Cyanine dyes are distinguished by several attributes, notably high extinction coefficients, bright emission in *in vitro* applications, adjustable absorption maxima spanning from the visible to the NIR region, outstanding biocompatibility, minimal dark toxicity have rendered them the most extensively studied dyes bioimaging applications. Furthermore, researcher have recently verified the suitability of cyanine dyes as PS in PDT applications.

The well-established heptamethine cyanine derivatives, including IR825, IR808, IR783, IR780 and indocyanine green (ICG) have found extensive application in cancer imaging research. Notably, indocyanine green has obtained approval from FDA for clinical cancer bioimaging investigations and have been employed as PS in PDT. However, its low singlet oxygen quantum yield (~ 0.08) has resulted in poor photocytotoxicity. Subsequent research has indicated that halogenated cyanine

derivatives demonstrate improved singlet oxygen quantum yield. Nonetheless, the emergence of dark toxicity arises from introduction of heavy atoms has constrained their potential in clinical PDT applications.⁸⁰

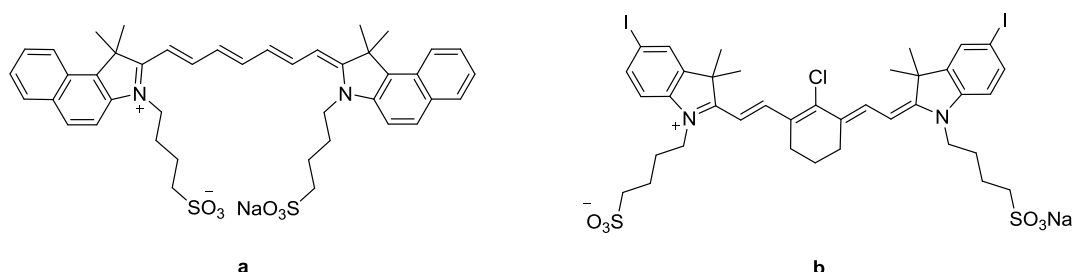


Figure 1.9. a) Structure of indocyanine green. b) Structure of halogenated cyanine derivative.⁸¹

1.2.5.1.4 BODIPY

Boron dipyrromethene (BODIPY) fluorophore family represents another widely utilized group in research endeavors. Prominence for their high fluorescence quantum yield, robust photostability and other advantageous photophysical properties, BODIPY dyes have garnered significant attention from researchers over several decades. Beyond their numerous outstanding photophysical features, the facile adjustability of absorption and emission maxima along with their intersystem crossing ability emphasize their potential as promising PS.

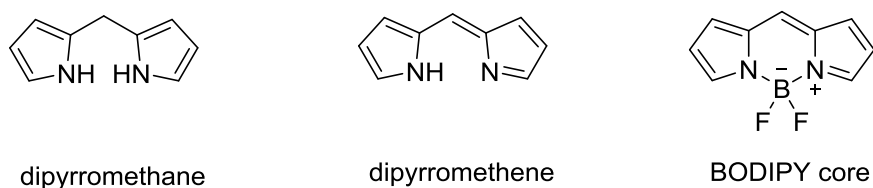


Figure 1.10. Structure of BODIPY core and its precursors.

Several strategies have been devised to optimize the BODIPY core as a NIR absorbing PS. The incorporation of halogens or chalcogens at the 2 and 6 positions of the core stands out as the predominant approach to enhance singlet oxygen quantum yield. Numerous derivatives, incorporating iodine, bromine, sulfur and

selenium or transition metals have demonstrated pronounced efficacy in singlet oxygen generation. However, heavy atom incorporation can lead to dark toxicity. In response, current research is focused on developing heavy atom-free BODIPY PSs. Orthogonal BODIPY dimers or trimers have emerged as particularly promising, showing higher triplet state lifetime with reduced dark toxicity.⁸²

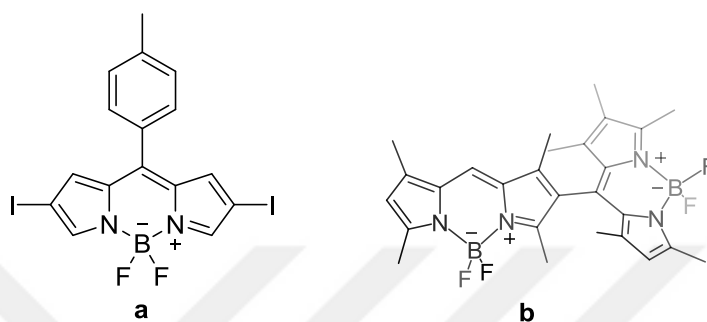


Figure 1.11. a) Structure of iodinated BODIPY PS derivative.⁸³ b) Structure of halogen-free BODIPY PS derivative.⁸⁴

Despite the high triplet excited state lifetime exhibited by BODIPY PSs, inadequate absorption and emission maxima hinder their *in vitro* and *in vivo* applications. Consequently, various structural modifications have been explored. These include fused ring expanded BODIPY, styryl substitution at positions 1-, 3-, 5- and 7- of the BODIPY core or aza-nitrogen substitution of the meso carbon atom (aza-BODIPY). Such alterations induce a red shift in their absorption and emission maxima, thereby affording BODIPY PS with NIR absorbing properties.⁸⁵

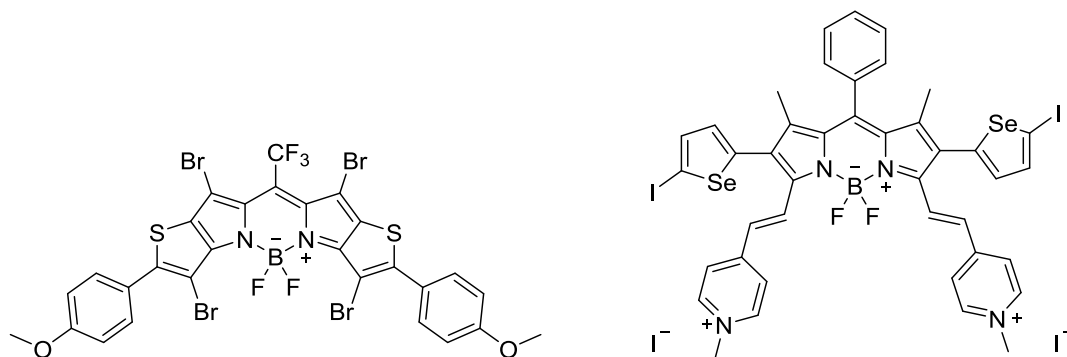


Figure 1.12. a) Structure of fused ring expanded BODIPY PS derivative.⁸⁶ b) Structure of styryl substituted BODIPY PS derivative⁸⁷

1.2.5.1.5 Phenothiazine

Methylene blue and Nile blue derivatives represent widely employed phenothiazine derivatives. These derivatives feature absorption maxima in the NIR region and their highly hydrophilic, cationic nature facilitates their utilization in both *in vitro* and *in vivo* applications.⁶⁹

The first entirely synthetic PS is Methylene blue. Primarily recognized as an imaging dye, it has found application in diverse fields including antidepressant therapy, anti-malarial treatment, antidote administration, anti-microbial interventions and ant PDT against cancer. Its high solubility in bodily fluids, compact structure and relatively elevated singlet oxygen quantum yield ensure its clinical relevance as a PDT agent. Nevertheless, the absence of tumor selectivity results in significant adverse effects, thereby limiting its potential in PDT applications. In response to this challenge, various structural modifications have been devised. Introducing variety of targeting groups to the core, often through reduction of C-N double bond, has shown promise in enhancing the effectiveness of PDT treatment in *in vitro* applications. However, the *in vivo* efficacy of activatable Methylene blue has yet to be demonstrated.⁸⁸

Nile blue, a derivative of benzophenoxazine, has been employed in bio-imaging studies as a stain for lysosome and lipid. It possesses higher fluorescence and greater stability in comparison to methylene blue. However, lack of heavy atom hinders its utility in PDT area. The substitution of oxygen atom with heavier counterparts such as sulfur or selenium results in significant enhancement in triplet state lifetime and subsequent ROS generation. In recent years, numerous activatable Nile blue analogs incorporating tumor-targeting or self-oxygen generating moieties have been developed. *In vitro* studies have revealed their potential as NIR light activatable PS.⁸⁹

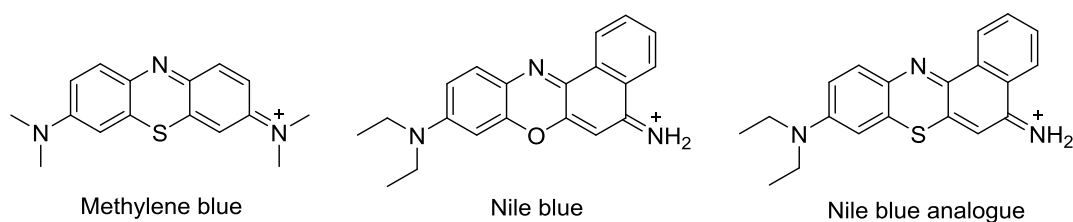


Figure 1.13. Structures of Methylene blue,⁸⁸ Nile blue and Nile blue analogue.⁸⁹

1.2.5.1.6 Squaraine

The dis-condensation reaction of electron rich squaric acid yields squaraine, characterized by sharp and condensed absorption with low energy, resulting in strong fluorescence. Strong charge transfer interactions and tendency toward aggregation contribute to the broad absorption band observed in the solid state. Squaraine dyes shared electronic similarities with cyanine dyes, although squaraines are electrically neutral. Furthermore, their nonlinear optical behavior and reversible redox capacities further increase their potential applications across diverse areas. In addition to their desirable optical properties, the flexibility of starting materials expands the scope of squaraine family.⁹⁰

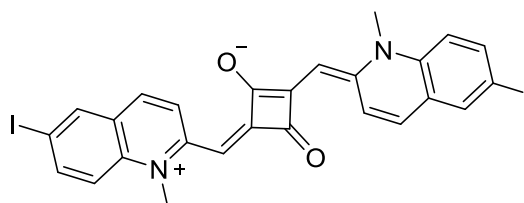


Figure 1.14. Structure of halogenated squaraine derivative.⁹¹

The potential of squaraine dyes, as highlighted above has been explored across various domains including data storage, photoconductivity, solar cells, fluorescent probes and PDT agents. Several halogenated squaraine derivatives have been employed in PDT applications owing to their intense absorption and emission maxima within the therapeutic window. Despite the synthesis of numerous squaraine-based PS, only a limited number have undergone testing *in vitro* tumor cells or *in vivo* animal studies.⁹⁰

1.2.6 Xanthene Dyes for Cancer Imaging and Treatment

One of the most important families of synthetic dyes and photosensitizers comprises xanthenes. While many derivatives, particularly classical xanthene dyes, suffer from short absorption and emission maxima, various strategies have been devised to shift their absorption and emission maxima into the therapeutic window.²⁸

Xanthenes can be described fundamentally as anthracene derivative where the 10-position is replaced with an oxygen atom. They can be categorized into three groups based on the heteroatoms present at the 3- and 6-positions: fluoresceins, rhodamines and rhodols.²⁸

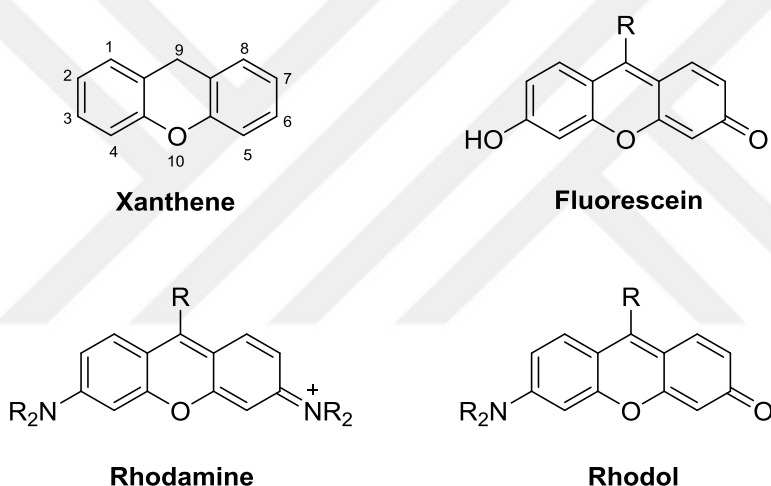


Figure 1.15. General structures of xanthenes, fluoresceins, rhodamines, and rhodols²⁸

1.2.6.1 Classical Xanthene Dyes

Classical xanthene dyes encompassing fluorescein, rhodamine and rhodols, display exceptional characteristics including high extinction coefficients, distinguishable singlet oxygen and fluorescence quantum yields, robust photostability, high hydrophilicity and facile modification toward target tumor applications.⁹²

1.2.6.1.1 Fluorescein

The spirolactonized fluorescein derivative, 3-carboxyfluorescein, was first synthesized in 1871 through condensing resorcinol with phthalic anhydride in the presence of Lewis acid. In aqueous media, 3-carboxyfluorescein exhibits four analogues with charge changing +1 to -2 under varying pH conditions. The dianionic form of 3-carboxyfluorescein displays absorption and emission maxima at 492 and 517 nm respectively. This pH dependent behavior presents opportunities for the utilization of spirolactonized fluorescein derivatives across diverse applications, including pH sensing, clinical imaging and water monitoring.⁹³ Furthermore, some derivatives have received approval from FDA for use in angiography to analyze blood flow due to their low toxicity. Moreover, fluoresceins finds wide application in cytology and immunohistochemistry as imaging agents and fluorescein isothiocyanate (FITC), 5(6)-carboxyfluorescein, 5(6)-carboxyfluorescein succinimidyl ester ranking among the most preferred fluorescein derivatives.⁹⁴

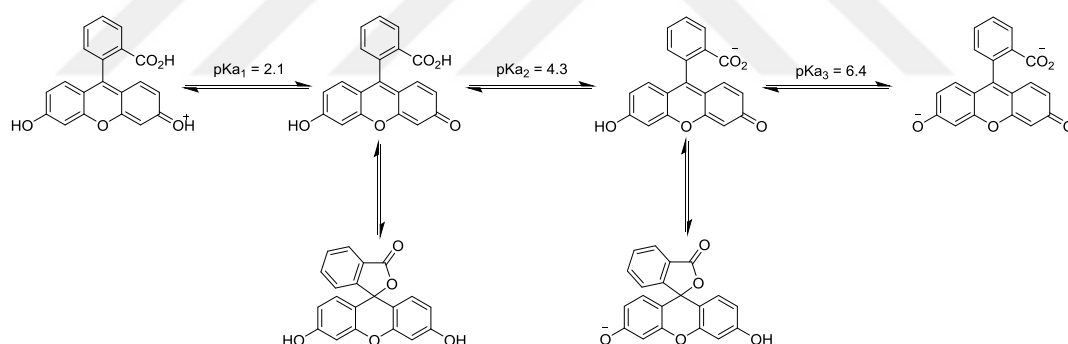


Figure 1.16. pH dependent equilibrium reaction of 3-carboxyfluorescein.⁹³

In addition to numerous fluorescence imaging-guided applications of fluorescein and its structurally modified derivatives, they have undergone further modifications with halogens to investigate their potential for PDT. Several derivatives, including Rose Bengal,⁹⁵ erythrosine B,⁹⁶ eosin Y,⁹⁷ have been halogenated and their photophysical properties along with the in vitro applicability as PS were tested. Rose Bengal generates $^1O_2$ with an almost 100% quantum yield. This remarkable attribute has been subjected to extensive evaluation across various disease models, revealing

notable in vitro damage to rabbit corneal and retinal pigment epithelial cells. However, despite the promising $^1\text{O}_2$ quantum yield demonstrating its potential as a PS, its clinical applicability remains constrained due to low absorption and emission maxima, rapid removal after injection and subsequent accumulation in the liver. Furthermore, Rose Bengal has been assessed for its diagnostic utility in ophthalmological conditions and its effectiveness in PDT interventions targeting diverse microorganisms.⁹⁵ Erythrosine B and eosin Y have also been enlisted as PS in PDT applications against bacteria and yeast.⁹⁶

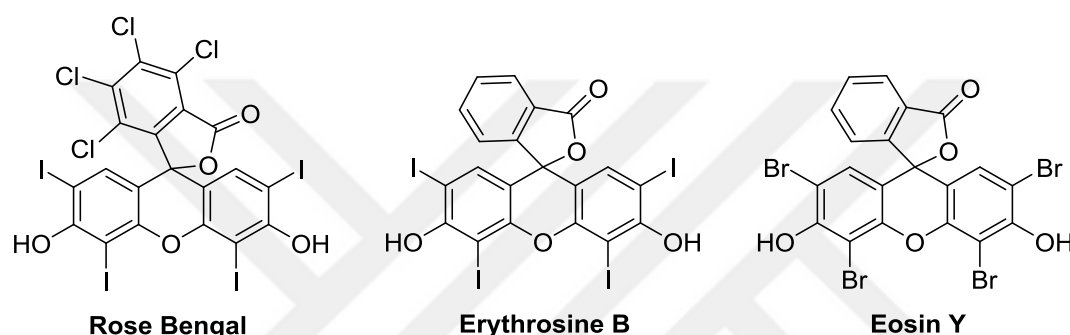


Figure 1.17. Structures of Rose Bengal,⁹⁵ Erythrosine B⁹⁶ and Eosin Y

1.2.6.1.2 Rhodamine

Rhodamine derivatives of the xanthene core structurally differ from fluorescein solely by the replacement of the oxygen atom with either cyclic or acyclic amino groups at the 3- and 6- positions. Rhodamines were discovered long ago, around the same time as fluorescein and have since been widely utilized across diverse fields. These applications include their use as fluorescent marker or small molecule detector in biotechnology, as reference dye in fluorescence spectrophotometry and laser dye applications.^{98,99}

In contrast to fluorescein, rhodamine analogues exhibit longer absorption and emission maxima. Furthermore, rhodamines are typically found as cationic ammonium salts, which confer high hydrophilicity, good biocompatibility and high selectivity for mitochondria. Owing to these excellent characteristics, numerous

rhodamine derivatives have been commercialized as bioimaging dyes. Examples include Rhodamine 6G, Rhodamine 800,¹⁰⁰ Rhodamine 123¹⁰¹ and tetramethyl rhodamine methyl ester (TMRM).¹⁰² Notably, TMRM and Rhodamine 123 are employed as mitochondrial stains in live cells and are marketed under the MitoTracker brand name.¹⁰¹

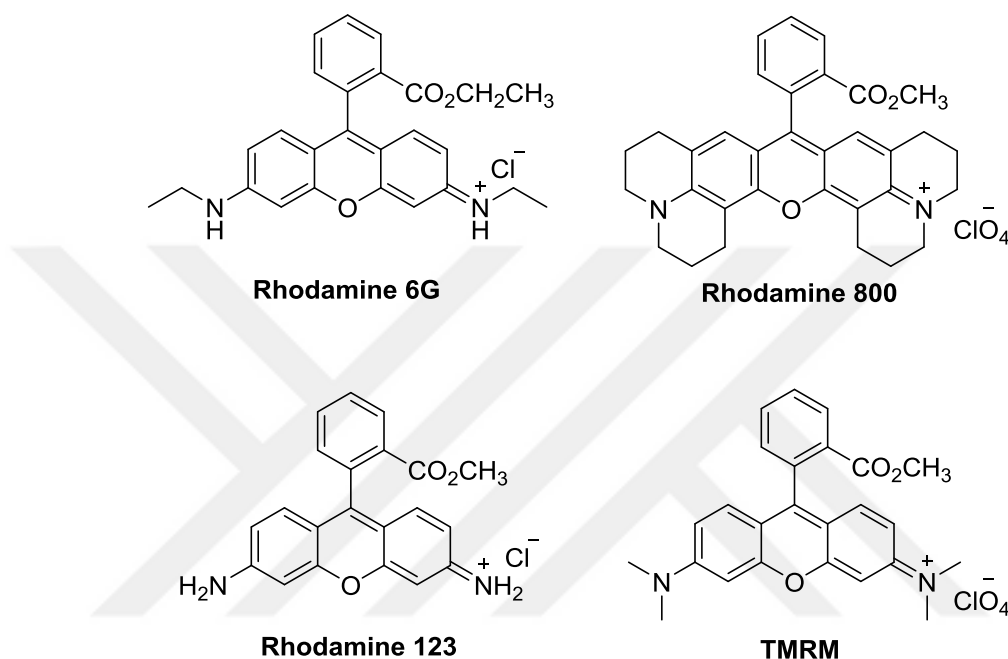


Figure 1.18. Structures of rhodamine derivatives; Rhodamine 6G, Rhodamine 800,¹⁰⁰ Rhodamine 123¹⁰¹ and TMRM.¹⁰²

The absence of efficient tumor targeting of PS limits their clinical applications. Rhodamine dyes, owing to their cationic nature, can be categorized as structure intrinsic targeting (SIT) dyes. The higher membrane potential of cancer cells compared to normal cells ensures accumulation of the cationic molecules bearing constantly delocalized positive charge within tumor tissue. Furthermore, cationic rhodamine dyes exhibit inherent propensity to accumulate within the mitochondria of tumor tissue, increasing the efficacy of PDT based treatment.¹⁰³

Due to these outstanding characteristics of rhodamine dyes, efforts have been directed towards their structural modification to enhance triplet excited state lifetime and photostability. To this end, rhodamine dyes have been either integrated with

luminescent transition metal, including Re (I), Pt (II), Ir (III) and Rh (III)¹⁰⁴ or halogens such as -I, -Br are introduced to classical rhodamine dyes, thereby converting them into potent photosensitizer. These strategic endeavors have yielded anticipated outcomes, manifesting prolonged triplet excited state lifetimes and consequently, higher ROS generation, along with enhanced tumor selectivity.^{105,106}

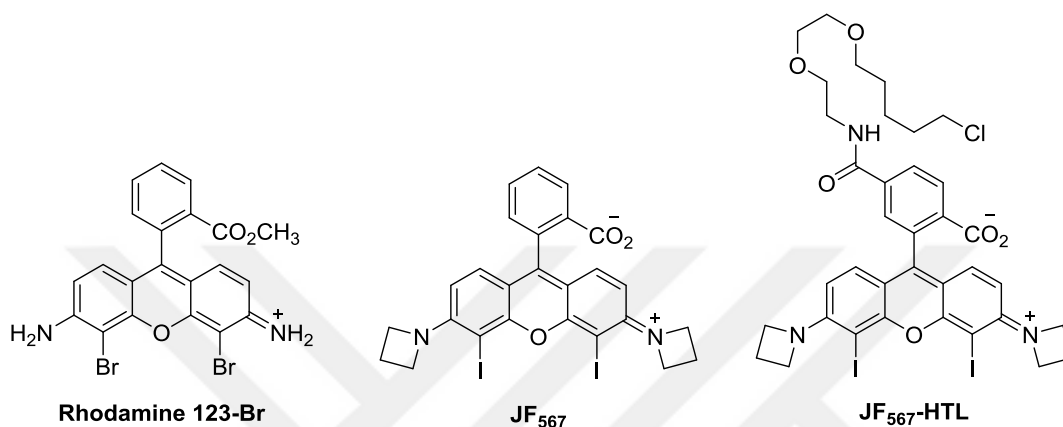


Figure 1.19. Structures of rhodamine-based PSs; Rhodamine 123-Br,¹⁰⁶ JF₅₆₇, JF₅₆₇-HTL¹⁰⁵

1.2.6.1.3 Rhodol

In addition to fluoresceins and rhodamines, rhodols represent another family of xanthene dyes. They constitute hybrid versions of rhodamines and fluoresceins. Their absorption maxima range between 490 and 550 nm and their emission maxima range between 520 and 580 nm in aqueous media, contingent upon the substituents present. The preparation rhodols entails Friedel-Crafts type reaction occurs between para-amino-substituted ortho-hydroxybenzophenone with resorcinol derivatives. Rhodols distinguish themselves from other xanthene derivatives through solvatochromic properties. They can exist in both neutral and zwitterionic forms, depending on solvent polarity, leading to fluctuations in band intensity and minor alterations in absorption maxima.¹⁰⁷

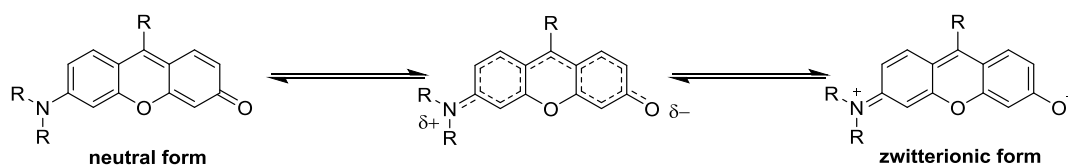


Figure 1.20. Resonance structures of rhodols.

Although the discovery of rhodols dates back to the 19th century, their significance was notably recognized in the late 20th century. Since then, utilization of rhodols as fluorescent probes has experienced a dramatic surge. Enhanced comprehension of the relationships between structure and photophysical properties has facilitated more efficacious probe design. Rhodols offer greater flexibility in structural modulation compared to other xanthene families. Substitution from O- group transforms them into colorless spiro form while the presence of the O- terminal group allows for fluorescent on/off control owing to spiro ring. Functionalizing amino R groups further enables the development of more precisely targeted probes.¹⁰⁸

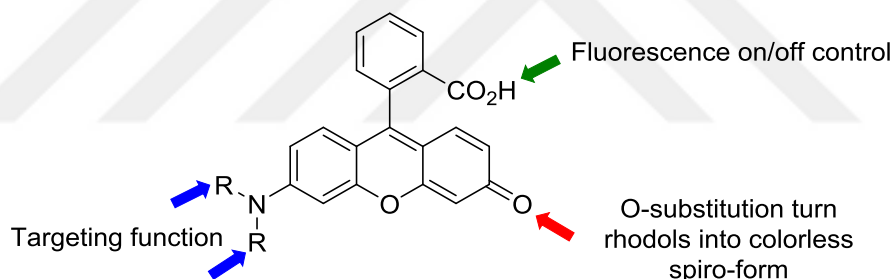


Figure 1.21. Importance of structure-photophysical properties relationship.

Despite the increased research focus on rhodols, their utilization in PDT applications remains minimal. However, existing research demonstrates the efficacy of rhodols in singlet oxygen generation capacity.¹⁰⁹

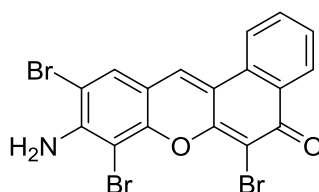


Figure 1.22. Structure of rhodol PS derivative.¹⁰⁹

1.2.6.2 Hybrid Xanthene Dyes

The classical xanthene dyes, particularly fluoresceins and rhodamines have historically been the most widely utilized fluorophores. However, their clinical bioimaging applications and employment as PS in PDT applications are limited due to their low absorption and emission maxima. As a result, red-shifted fluorophores and photosensitizers are emerged due to various advantages of far-red (FR) and near-infrared (NIR) absorbing and emitting dyes.¹¹⁰

FR/NIR imaging offers several benefits including less photon scattering, reduced autofluorescence from tissue, low levels of photo-absorption. Additionally, FR/NIR bioimaging enables the acquisition of more distinct deep-tissue images with an enhanced signal/noise ratio. Furthermore, it can be employed *in vivo* for bio- or photoacoustic imaging.¹¹¹

In the context of *in vivo* deep-tissue PDT applications, FR/NIR absorbing PSs are essentials. Light penetration to the tissues is limited in the visible region. whereas, in the FR/NIR region, light can penetrate deeper, thereby enhancing PDT efficiency. Therefore, the utilization of FR/NIR light is crucial for achieving improved deep-tissue PDT outcomes in future clinical trials.⁹³

Structural modifications of xanthene dyes facilitate the enhancement of absorption and emission maxima without compromising their other desired photophysical properties. Various strategies have been devised to shift xanthene dyes into therapeutic window and broaden their applicability in *in vivo* bioimaging and PDT treatment areas. The most commonly employed approaches can be categorized into three subsections: extension of the π -conjugation system, N-substituted group modification and changing the central oxygen with other elements.^{93,112}

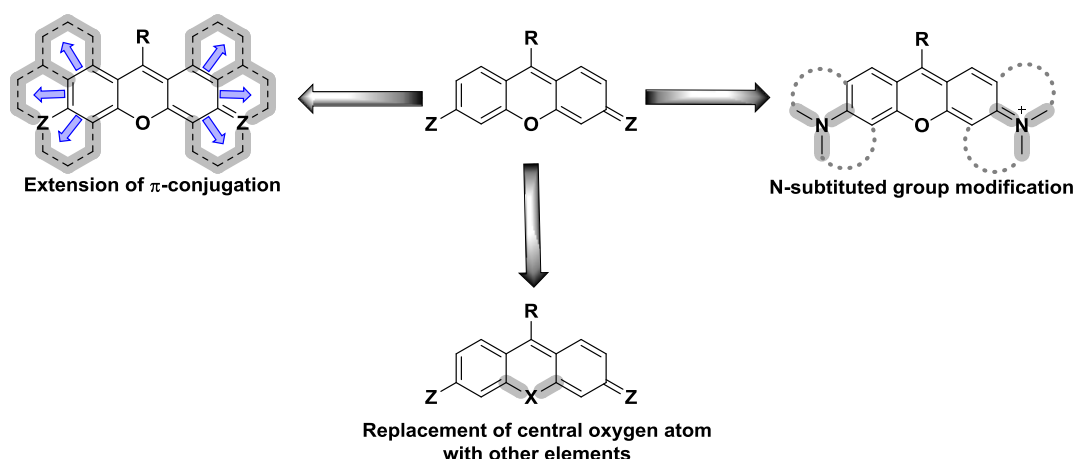


Figure 1.23. The most utilized strategies to develop hybrid xanthene dyes.

To extend the π -conjugation system within the xanthene family, numerous novel xanthene derivatives have been developed. The extension of π -conjugation system lowers the gap between the highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO), consequently resulting in a bathochromic shift in the absorption and emission maxima.⁹³ The initial endeavor to produce FR/NIR absorbing/emitting xanthene derivatives was undertaken by Lee and colleagues in 1989. They pioneered the design and synthesis of naphtho-fluoresceins, which were subsequently subjected to *in vitro* analysis (Figure 1.25, molecule a). This modification yielded a noteworthy bathochromic shift in both absorption and emission maxima upon variations in pH ($\lambda_{\text{abs}} = 609 \text{ nm}$, $\lambda_{\text{em}} = 665 \text{ nm}$ at pH = 9.5 in aqueous media, $\lambda_{\text{abs}} = 524 \text{ nm}$, $\lambda_{\text{em}} = 570 \text{ nm}$ at pH = 5.2 in aqueous media).¹¹³

Following this seminal investigation, several xanthene derivatives with extended π -conjugation systems have been developed with successful reports of bathochromic shift. Notably, seminaphtho-rhodol derivatives were developed by Stongin and colleagues in 2017. One of these rhodol derivatives exhibited substantial bathochromic shift, with absorption and emission maxima at 582 and 622 nm, respectively (Figure 1.25, molecule b).¹¹⁴ However, another rhodol derivative demonstrated emission maxima in aqueous media lower than expected due to electrostatic repulsion induced by an increased dihedral angle of naphthyl group at elevated pH levels (Figure 1.25, molecule c). This challenge was addressed through

the stabilization of intramolecular charge transfer (ICT) via solvation, resulting in the observation of expected emission maxima at 630 nm.¹¹⁵ Subsequently, this approach was applied by Ahn and colleagues to develop naphtho-rhodamine derivative yielding to shifted absorption and emission maxima at 655 and 785 nm, respectively (Figure 1.25, molecule d).¹¹⁶

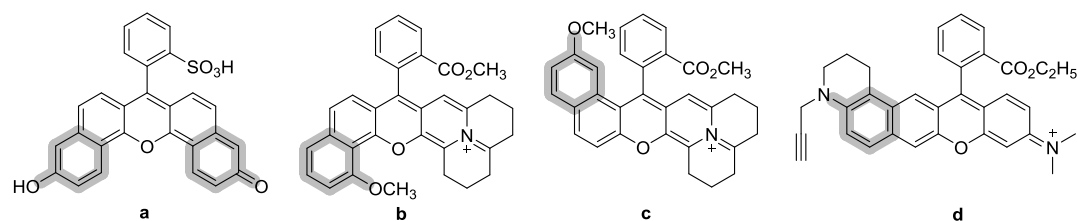


Figure 1.24. Structures of π -conjugation extended xanthene derivatives.

Another approach to tune the absorption and emission maxima of xanthene derivatives involves the N-substituted group modification. This approach has been applied to the rhodamines and rhodols to enhance their photophysical properties. For instance, incorporating a julolidine ring or quinoline ring induces a bathochromic shift in both absorption and emission maxima, while also enhancing the fluorescence quantum yield compared to classical analogues. Furthermore, the introduction of electron-rich amino groups leads to larger Stokes shift, rendering them as NIR-emitting rhodamines.¹¹² For example, Scott and colleagues developed hybrid rhodamines by conjugating pyrrole, indole or indolizine to the rhodamine π -electron system. Pyrrole and indole derivatives demonstrate absorption and emission maxima ranging from 660 to 720 nm. Notably the indolizine derivative displays absorption and emission maxima in NIR-II region, specifically at 925 and 1051 nm, respectively.¹¹⁶

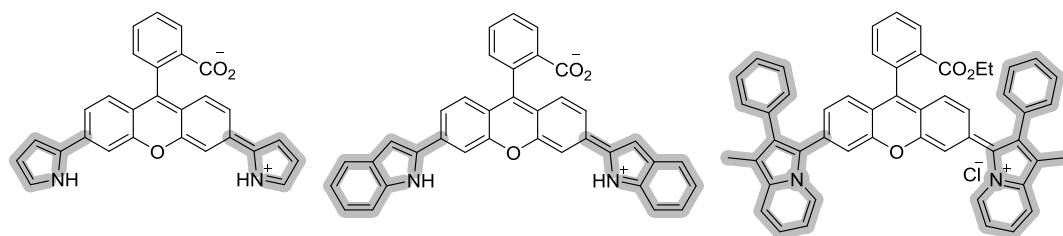


Figure 1.25. Structures of pyrrole, indole and indolizine modified rhodamine derivatives.

In addition to the enhancing π -electron system of xanthene dyes by substituting amino group, which has led to the development of hybrid xanthene dyes exhibiting exceptional brightness and photostability along with significant bathochromic shifts. Various research endeavors have focused on changing the type of cyclic amine to mitigate the formation of twisted intramolecular charge transfer (TICT). The TICT state arises from steric repulsion between the xanthene ring and acyclic, dialkyl amino moieties leading to diminished emission of the molecule and enhanced reactivity. To inhibit steric repulsion and enhance emission and photostability, cyclic amines are employed.¹¹⁷ For instance, Lavis and colleagues introduced the azetidine moiety through Buchwald-Hartwig cross coupling reactions. Compared to its dimethyl amino analogue, the rhodamine bearing the azetidine moiety demonstrated exceptional brightness with an enhanced quantum yield.¹¹⁸

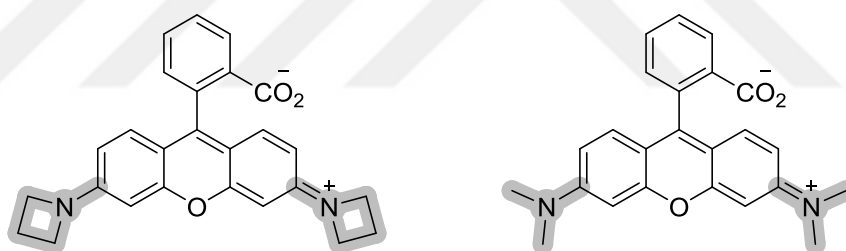


Figure 1.26. Structures of azetidinyl rhodamine and tetramethyl rhodamine derivatives.

The modification of oxygen at the 10th position of xanthene dyes with other elements, including group 14 elements (C, Si, Ge, Sn), group 15 elements (P, Bi) and chalcogens (S, Se, Te) not only enhances absorption and emission maxima, but also some of them facilitate intersystem crossing ability of xanthene dyes. Replacing the central oxygen atom with these elements generally induces a bathochromic shift, resulting in absorption maxima above 600 nm. This modification leads to the stabilization of LUMO level due to the $\sigma^* \rightarrow \pi^*$ interaction between the σ^* of the introduced element and the π^* of the nearby carbon of the fluorophore.¹¹⁹ For

instance, replacing the oxygen atom in fluorescein with a gem-dimethyl carbon moiety accounted for more than 50 nm bathochromic shift while its difluoro analogue even shifted more. In addition to the Stokes shift, fluorine atoms enhanced the photostability and brightness.¹²⁰ Moreover, gem-dimethyl silicon analog was prepared although none of these analogues reached the therapeutic window.¹²¹

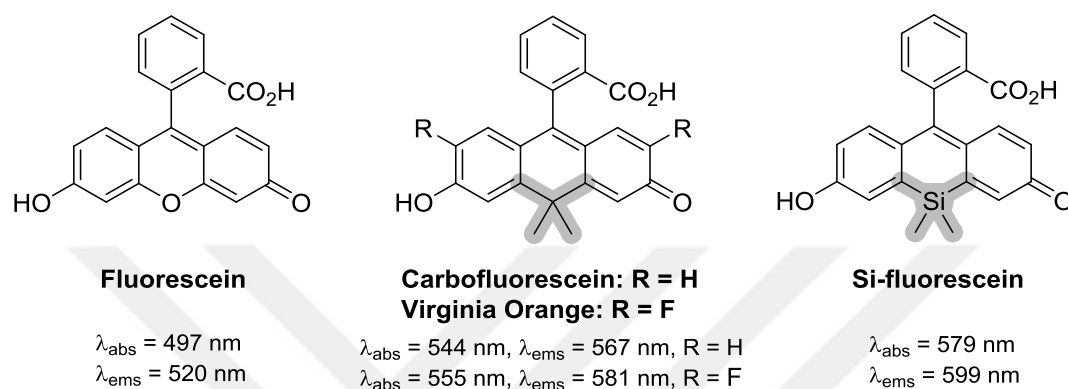


Figure 1.27. Structures of fluoresceins, carbofluorescein, Virginia Orange and Si-fluorescein.

Modification of rhodamine derivatives with gem-dimethyl carbon or silicon affords greater flexibility in tuning their photophysical properties, owing to additional effect of substituting nitrogen with either cyclic or acyclic alkyl groups. For instance, carborhodamine 110 displays a bathochromic shift of more than 50 nm compared to rhodamine 110¹²² and silicon rhodamine demonstrates further shifted absorption and emission maxima. Moreover, further modification of the nitrogen atom results in additional shifts absorption and emission maxima.^{123,124} Furthermore, carbon and silicon-bearing analogues have greater tendency to adopt the closed form, thereby enhancing cell permeability.

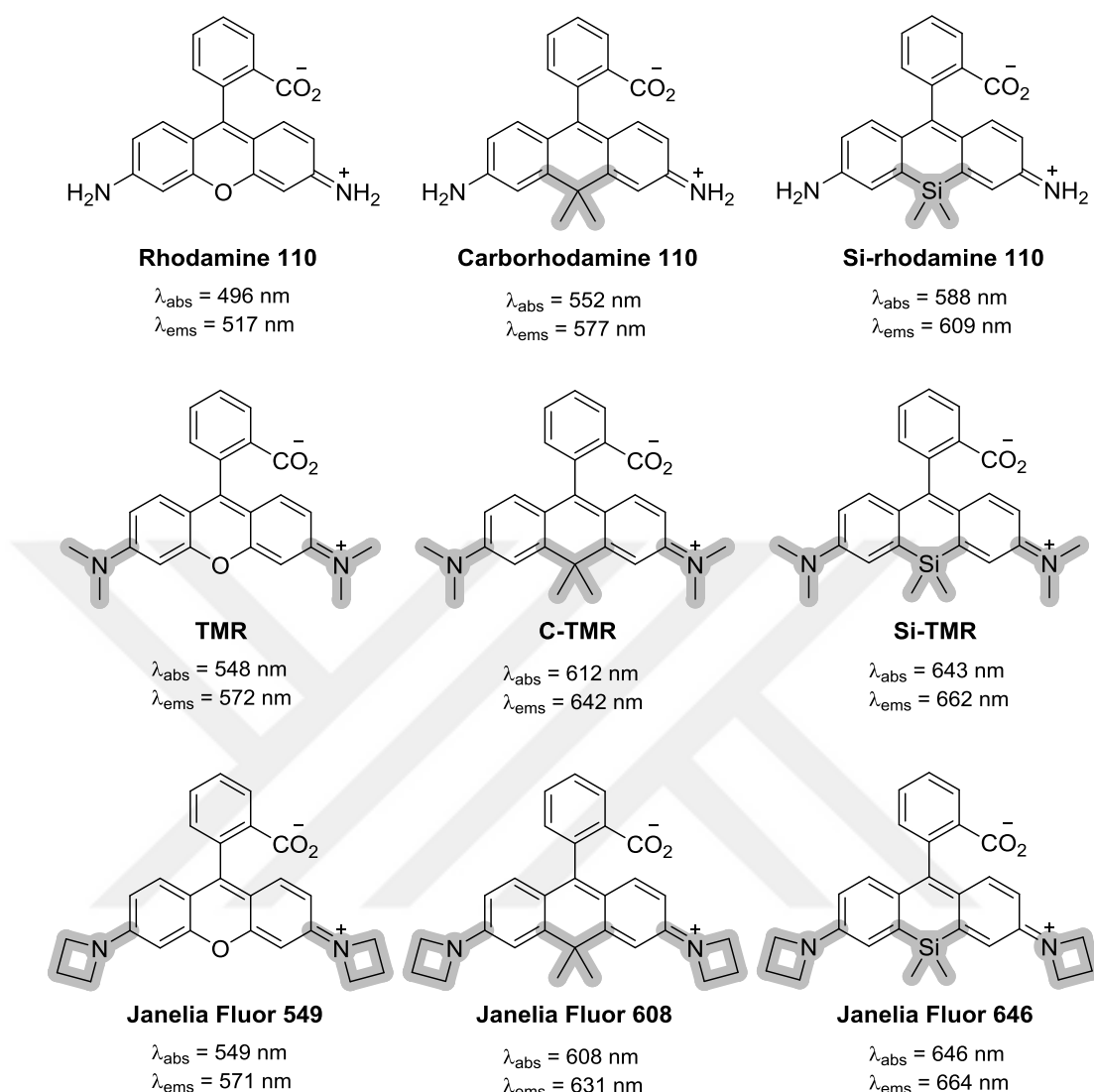


Figure 1.28. Structures of rhodamine, carborhodamine and Si-rhodamine derivatives.

While Si-rhodamines have succeeded in reaching the therapeutic window, their fluorescein analogues remain inadequate in absorbing or emitting light in the FR/NIR regions, thus limiting their *in vivo* applicability. Introducing phosphorous element instead of central oxygen atom leads to a significant decrease in the LUMO level, attributable to the $\sigma^* \rightarrow \pi^*$ interaction. Further oxidation of the phosphorous atom enhances the electron-accepting ability, resulting in a narrower gap between the HOMO and LUMO levels compared to silicon analogues. Consequently, P-

fluoresceins exhibit greater bathochromic shifts and can absorb and emit light within therapeutic window. Furthermore, numerous P-rhodamine derivatives have also been developed, exhibiting a similar pattern to that observed in fluorescein derivatives. Their absorption and emission maxima range from 660 to 775 nm and 690 to 820 nm, respectively, owing to the flexibility of modifications in both the alkylation of the nitrogen atom and the side group of the phosphorous atom.²⁸

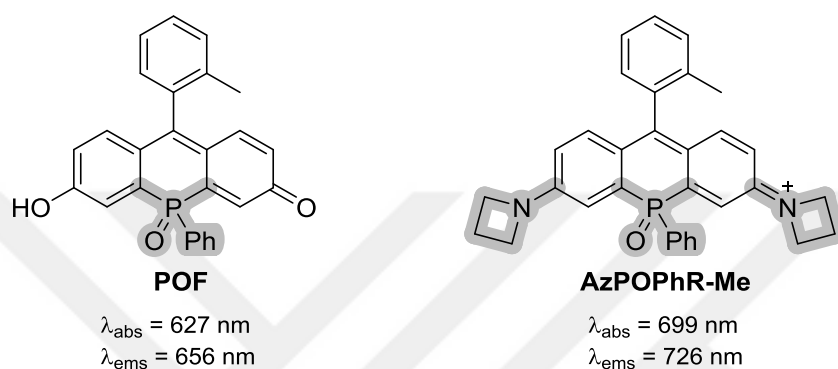


Figure 1.29. Structures of P-fluorescein and P-rhodamine derivatives.

Given the great impact of central atom modification with silicon or phosphorous on bioimaging applications, their adoption in PDT-based treatment remains uncommon. Our collaborative effort has resulted in the development of the first Si-fluorescein based PDT agent. Tetra-iodinated Si-fluorescein (SF-I) demonstrates exceptional singlet oxygen quantum yield and has undergone testing for efficacy in *in vitro* models of triple negative breast cancer and colon cancer. SF-I exhibited red-shifted absorption and emission maxima at 614 and 630 nm, respectively, compared to its Si-fluorescein counterpart. Despite achieving a high singlet oxygen quantum yield through heavy atom incorporation, SF-I retains significant fluorescence, rendering it a promising candidate for image-guided PDT applications.¹²⁵

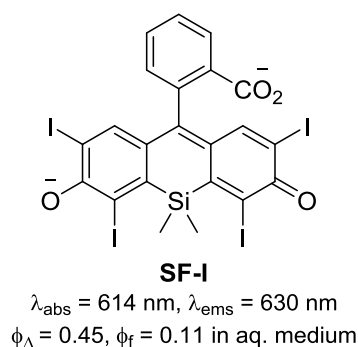


Figure 1.30. Structure of SF-I, the first Si-fluorescein photosensitizer.

In addition to the red-shifted xanthene derivatives resulting from the incorporation of 3rd row elements such as Si and P instead of central oxygen, the introduction of 4th row elements (Se, Te) has yielded prolonged triplet excited state lifetime alongside a notable bathochromic shift. The incorporation of heavy atoms into the center of the xanthene core facilitates the generation of ROS, thereby rendering them as promising candidates for PDT applications.²⁸

In 2015, Kryman and colleagues developed selenorhodamine derivatives of PSs bearing amide or thioamide functionalities. These PSs denoted as JSeR exhibited red-shifted absorption and emission maxima at 626 and 660 nm, respectively, alongside notable singlet oxygen quantum yields (0.64 for amide and 0.51 for thioamide derivatives in MeOH). The synthesized selenorhodamine derivatives have demonstrated their potential as effective PSs in the PDT based treatment of Colo-26 cells *in vitro*.¹²⁶

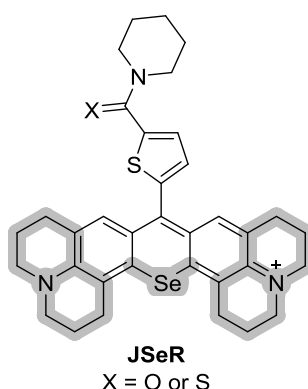


Figure 1.31. Structure of JSeR.

Following this work, Piao and collaborators introduced another selenorhodamine derivative PS. 2,6-dimethylphenyl bearing photosensitizer (SeR) which exhibited relatively modest absorption and emission maxima at 533 and 577 nm, respectively. SeR underwent further modification with an azo group to yield the desired PS, AzoSeR which notably exhibited an elevated bathochromic shift in absorption up to 630 nm without emission characteristics. Furthermore, *in vitro* investigations revealed the selective cleavage of azo group under hypoxic conditions, wherein the activated PS demonstrated a pronouncedly high singlet oxygen quantum yield ($\Phi_{\Delta}=0.56$) particularly in tumor tissues.¹²⁷

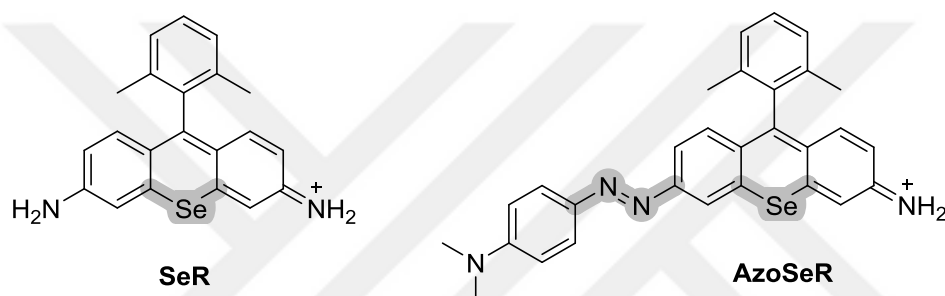


Figure 1.32. Structures of SeR and AzoSeR.

Kryman and colleagues also reported a series of tellurorhodamine derivatives and their corresponding telluroxide analogues. The tellurorhodamine derivatives, including TeR, 2-Me-TeR, MTeR, and JTeR exhibit non-emissive characteristic with absorption maxima around 600 nm. Among these, TeR has the lowest singlet oxygen quantum yield ($\Phi_{\Delta}=0.43$) due to the free rotation of the 9-phenyl substituent and JTeR demonstrates the highest efficiency owing to steric hinderance induced from 2,4,6-trimethylphenyl substitution at the 9th position. These derivatives were further subjected to modification via photo-oxidation reactions to develop their telluroxide analogues, namely TeOR, 2-Me-TeOR, MTeOR, and JTeOR. The telluroxide derivatives demonstrate a significant bathochromic shift of up to 72 nm. However, their capacities for singlet oxygen generation decreased dramatically.¹²⁸

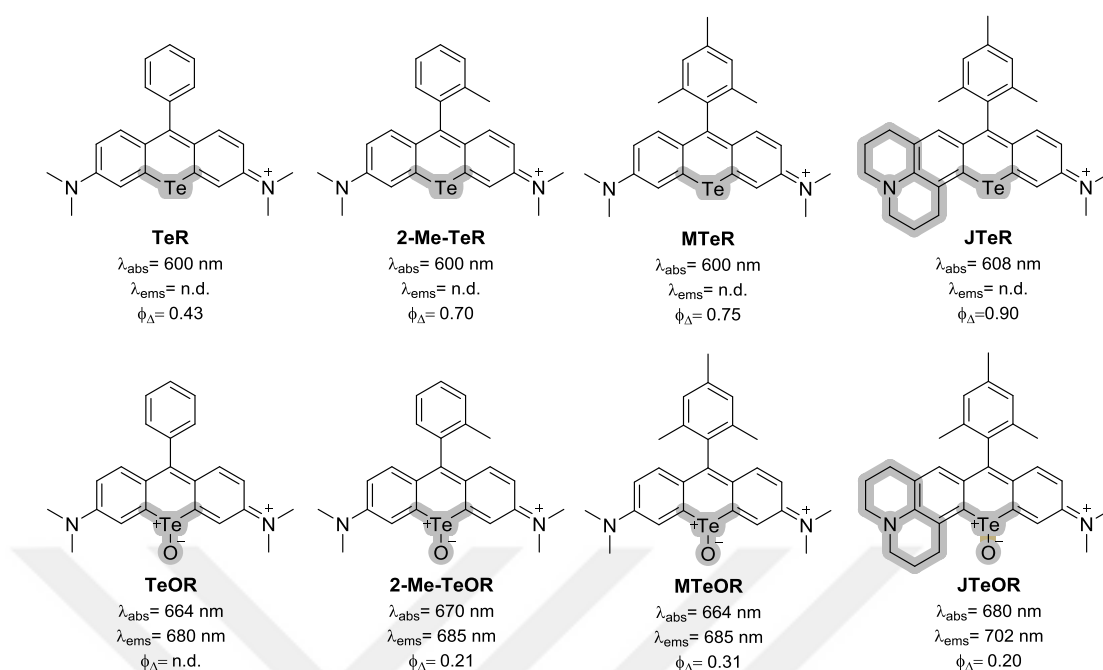


Figure 1.33. Structures of tellurorhodamine derivatives and their corresponding telluroxide derivatives.

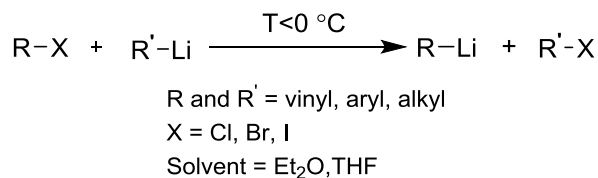
1.2.7 Key Synthetic Strategies for FR/NIR Absorbing Photosensitizer Synthesis

While designing the synthetic approaches to develop FR/NIR absorbing photosensitizers, utilization of some strategies is inevitable. Particularly, lithium-halogen exchange reactions, Buchwald Hartwig cross coupling reactions and aromatic halogenation reactions are the most employed synthetic strategies in the development of FR/NIR absorbing PS synthesis.

1.2.7.1 Lithium-Halogen Exchange Reaction

Organometallic reactions, particularly organolithium reactions, have significantly influenced modern synthetic chemistry. Following the recognition of lithium-halogen exchange reactions by Wittig and Gilman in the early 1900s, organolithium

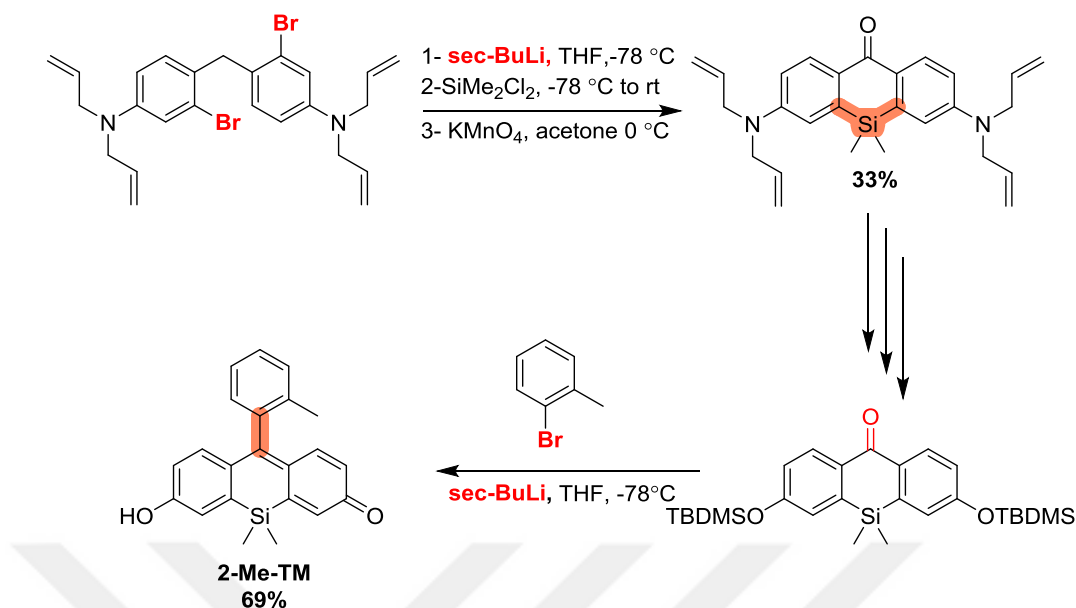
compounds have been extensively utilized in organic synthesis and polymerization reactions both at the lab scale and industrial applications.¹²⁹



Scheme 1. 1. General Lithium-Halogen Exchange reaction scheme.

As a lithium source, generally butyllithiums, including n-, sec-, tert-butyl lithium, are typically employed. These reagents exhibit high reactivity and can become pyrophoric in the presence of oxygen or moisture. Organolithium compounds serve as proficient nucleophiles and strong bases owing to the high polarity of C-Li bond. Due to the electron deficiency of organolithium compounds, electron-donating solvents such as diethyl ether or THF are predominantly utilized for lithium coordination. Additionally, amines are commonly employed alongside alkyllithiums as complexing agent. The exchange rate primarily depends on the carbanion intermediate stability ($sp > sp^2 > sp^3$) and the type of halogen and follows the $\text{Cl} < \text{Br} < \text{I}$ trend. Alkyl fluorides or aryl fluorides are commonly unreactive toward exchange reactions.¹³⁰

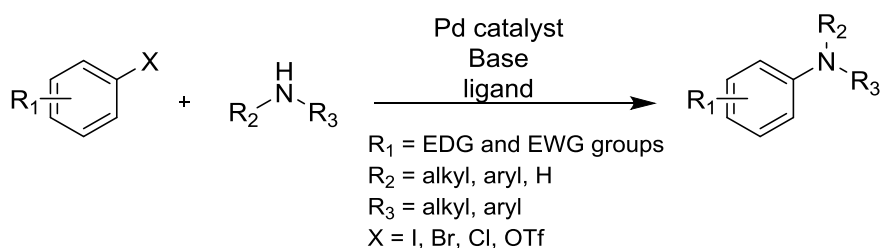
In the synthesis of new generation FR/NIR PSs, especially in the hybrid xanthene-based PS synthesis lithium-halogen exchange reactions extensively utilized. The reactions play a crucial role in both the incorporation of elements such as Si and P in place of central oxygen and the introduction of phenyl derivatives to the 9th position of the xanthene core. The lithium-halogen exchange strategy is widely utilized in these processes.¹³¹



Scheme 1. 2. Utilization of lithium-halogen exchange strategy in the synthesis of 2-Me-TM.

1.2.7.2 The Buchwald-Hartwig Amination Reaction

The formation of a bond between sp^2 hybridized carbon and an amine is widely utilized method across diverse synthetic areas. Prior to the discovery of Buchwald-Hartwig reaction, synthesis of aniline derivatives typically relied on either copper mediated reactions such as Goldberg and Ullmann type reactions or nucleophilic aromatic substitution reactions (S_NAr) type reactions had been employed. However, the necessity for harsh conditions and the limited substrate scope restricted their applicability, rendering them inadequate in many fields.¹³²



Scheme 1. 3. General reaction scheme of the Buchwald Hartwig amination.

Reaction of Pd-catalyzed aryl halides with amines have been advanced by the utilization of novel phosphaligands including Xphos, Xantphos, BINAP and dppf. These ligands expand the scope of substrate and enhance the efficiency of reaction.¹³²

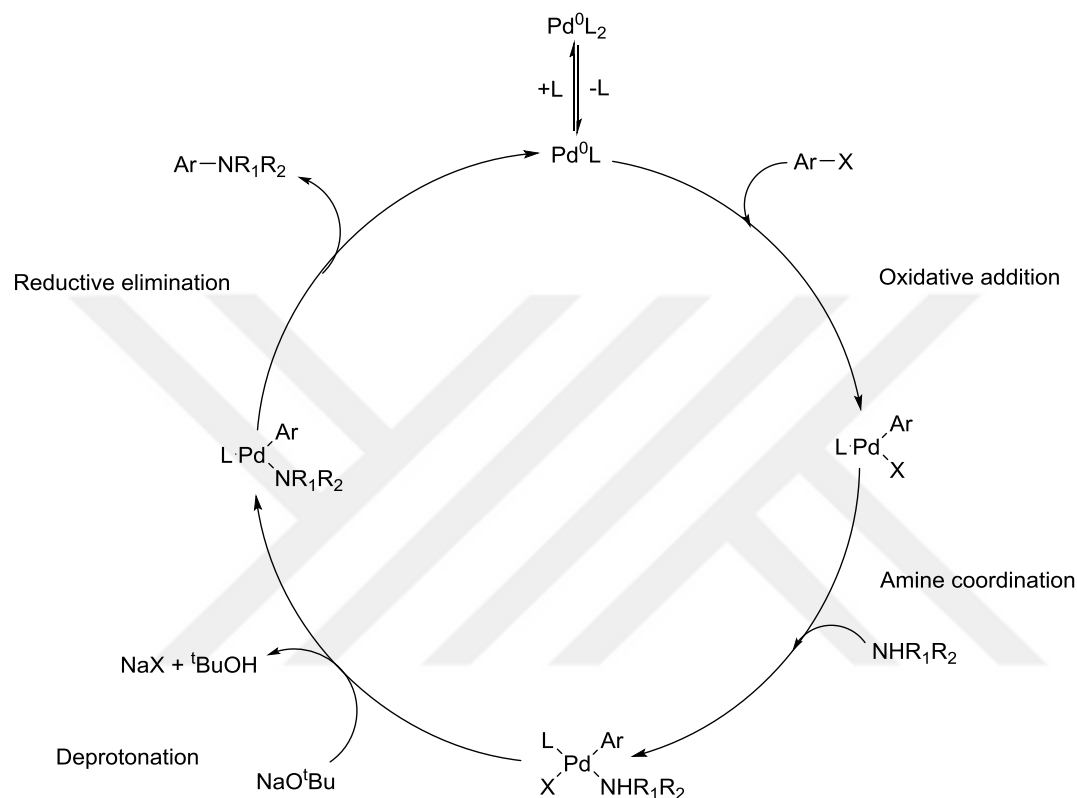
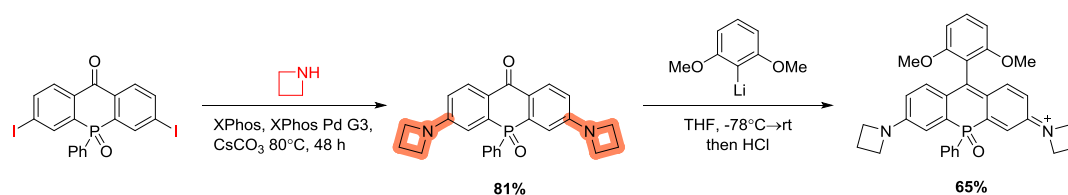


Figure 1.34. The catalytic cycle of the Buchwald Hartwig amination.

The Buchwald Hartwig amination offers a more facile and more efficient synthesis of aniline derivatives. Since anilines serve as essential building blocks for numerous organic and biologically active compounds, the Buchwald Hartwig amination has become one of the most important organic synthetic methods widely used in both research laboratories and in industry. Furthermore, in the synthesis of new-generation FR/NIR absorbing photosensitizer, especially in the synthesis of hybrid rhodamine derivatives, this methodology is also widely employed for shifting absorption maxima via the N-substituent modification approach.¹³³



Scheme 1. 4. Reaction scheme of N-substituent modified via Buchwald Hartwig amination phospharhodamine derivative.

1.2.7.3 Aromatic Halogenation Reactions

The halogenation of arenes facilitates the synthesis of various compounds. Despite the existence of numerous reagents developed for this purpose, the utilization of N-halosuccinimides, Sand-Mayer type halogenation reactions and halogenation with molecular halogen, notably iodine stands out as the preferred approaches. Preference of iodine stems from the difficulties associated with handling gaseous Cl_2 and highly toxic Br_2 . These methodologies are extensively employed in the synthesis of bioimaging dyes and photosensitizers.¹³⁴

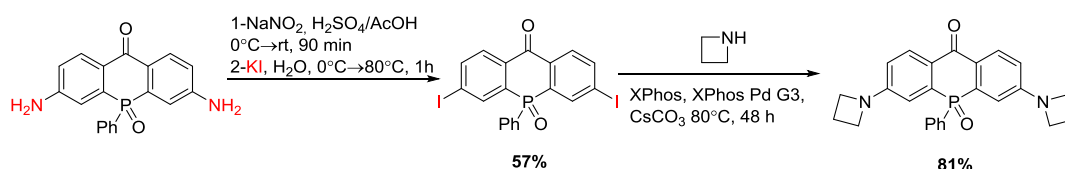
N-halosuccinimides including N-chloro-, N-bromo- and N-iodosuccinimide (NCS, NBS and NIS respectively) can introduce halogen to the activated arenes under mild conditions regioselective and chemoselective electrophilic halogenation reaction. Owing to environmentally friendly conditions, facile synthesis and variety of substrate advances the utilization of this methodology over other electrophilic halogenation reactions.¹³⁴

N-halosuccinimides can be activated through either electrophilic or nucleophilic pathways. In the electrophilic pathway, the activation of N-halosuccinimides involves Brønsted or Lewis acids. This pathway operates efficiently at room temperature and non-nucleophilic solvents such as acetonitrile, toluene, dichloromethane and chloroform are typically employed. On the other hand, in the nucleophilic activation pathway, the σ -hole of the halogen on the succinimide interacts with the nucleophilic atom. This σ -hole arises from the electron

withdrawing ability of carbonyl moiety on succinimide, which draw electron density away from the halogen. The utilization of these pathways enables the electrophilic aromatic halogenation reactions to proceed even in the presence of acid or base sensitive functional groups.¹³⁵

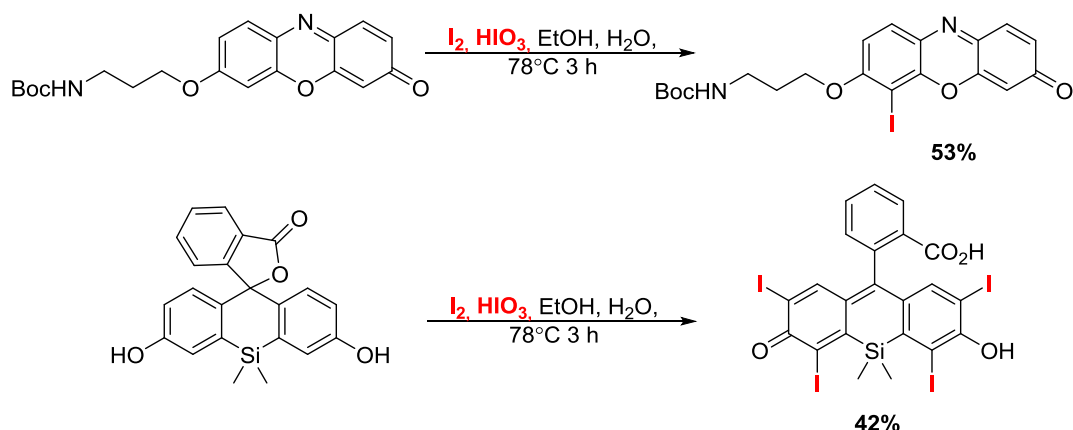
Electrophilic aromatic halogenation reactions employing N-halosuccinimides have a crucial role in the synthesis of PSs. These reactions are not only instrumental in introducing heavy atoms, but also prove valuable in the synthesis of precursors for Buchwald Hartwig amination.

The Sand-Mayer type halogenation represents another commonly utilized methodology in the synthesis of bioimaging dyes and photosensitizers. The conversion of 2,6-amino-xanthenes to corresponding halogenated xanthone derivatives facilitates the modification of rhodamines or rhodols with various amino groups. In the Sand-Mayer reaction, the process commences with the preparation of a diazonium salt, which is subsequently replaced with various nucleophilic groups, including iodine. This conversion enables the coupling xanthone core with a wide range of amino groups via Buchwald Hartwig amination reaction.¹³³



Scheme 1. 5. Reaction scheme of the phosphorodiamine derivative which subjected to Sand Mayer type iodination reaction.

In the synthesis of recent PSs, especially concerning the incorporation of heavy atom, the widespread utilization of molecular iodine in the presence of a catalytic iodic acid (HIO₃) is notable. While iodinating fully conjugated xanthene systems with molecular iodine poses challenges, lactonized xanthene derivatives undergo facile iodination using this system, yielding multiple iodinated derivatives.¹³⁶



Scheme 1. 6. Incorporation of heavy atom(s) to the xanthene-based PSs through molecular iodine.

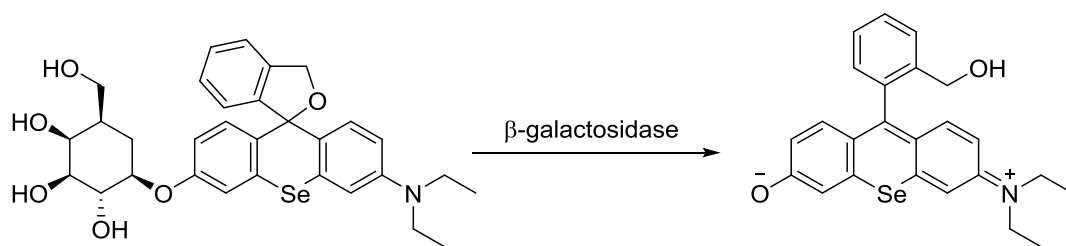
1.2.8 Effective Tumor Targeting

Targeted drug delivery represents one of the primary challenges in PDT. The desired PS should selectively accumulate within the tumor cell while sparing normal cells from harm. The core principle of PDT revolves around effectively treating cancer with a minimal adverse effect. Following the discovery of DNA and the initial expression of the human genome, there has been extensive exploration into personal and tumor genomes facilitated by enhanced DNA sequencing methods. These advancements have propelled researchers toward investigating personalized medicine to treat various diseases, notably cancer.¹³⁷

During the past few decades, significant efforts have been devoted to the development of targeted PDT systems. The intracellular localization of PS within mitochondria, endoplasmic reticulum (ER), plasma membrane, lysosome plays a pivotal role in the mechanism of cell death. Among these organelles, mitochondria are considered the most critical, as dysfunctional mitochondria are implicated in programmed cell death and tumor progression. Consequently, many FDA-approved PDT drugs have been specifically designed to accumulate within mitochondria.¹³⁸

In addition to the subcellular localization, microenvironment of tumor is also taken into consideration while designing PSs. To achieve this, the latest generation of PSs is covalently linked to the handle groups to enhance treatment selectivity. These handle group modified PSs transition from an inactive state to an active state upon cleavage of the handle group. Such PSs are known as “activatable photosensitizers”.¹³⁷ Targeting the acidic environment of tumor area is the most utilized strategy in activatable PS design.¹³⁹ Another common strategy involves targeting glutathione (GSH), which is often elevated in cancer cells. To target GSH conventional PSs decorated with bio-thiols. For instance, in 2014, a GSH-activatable zinc phthalocyanine derivative was developed by modification with 2,4-dinitrobenzenesulfonate (DNBS). In the presence of the DNBS, photo-induced electron (PeT) predominates over ISC, resulting in inhibition of singlet oxygen generation. However, upon cleavage of DNBS by GSH, the PS begins to generate singlet oxygen efficiently due to enhanced ISC. In addition to this research, Ng and colleagues have developed a zinc phthalocyanine derivative that is activatable by both pH and GSH to further increase the selectivity of PDT. This PS only initiates singlet oxygen generation in the presence of both variables.¹⁴⁰

As comprehension of cancer biology has advanced and the crucial roles of various enzymes in cancer have become apparent, activatable PSs have been modified with enzyme activatable moieties to target overexpressed enzymes in tumor area. Enzymes such as B-galactosidase, nitroreductase, cathepsin B, caspase-3 have been targeted with PSs for PDT evaluation.¹³⁷ For instance, a B-galactosidase activatable selenorhodol derivative has been developed. B-galactosidase is predominantly overexpressed in ovarian cancer cells. The prodrug remains colorless until enzymatic cleavage of the cage group occurs due to its spirolactonized structure. Upon localization in the tumor, the cage group is removed, resulting in a red-shifted active PS capable of generating singlet oxygen upon irradiation. While there exist numerous examples, enzyme activatable PSs remain relatively rare and their potential warrants further evaluation with the aid of new generation PDT drug cores.¹⁴¹



Scheme 1. 7. An enzyme activatable selenorhodol based PDT agent.

PDT has also been utilized in the treatment of malignant brain tumors such as glioblastoma multiforme (GBM) and anaplastic astrocytoma (AA). In most cases, first-generation porphyrin and chlorin derivatives have been employed as a drug (Table 1.1) in combination with postoperative radio chemotherapy. However, despite the existence of numerous targeting moieties known for various cancers, specific targeting moieties tailored for brain tumors to facilitate targeted activatable PDT remain unidentified.¹⁴²

Table 1.1 Summary of the history of photodynamic therapy for the treatment of malignant glioma¹⁴²

<i>Author</i>	<i>No of patients</i>	<i>GBM</i>	<i>AA</i>	<i>PS</i>	<i>Dose</i>	<i>Light density</i>	<i>Median survival (months)</i>	
							<i>Overall</i>	<i>Primary</i>
Perria et al. (1980)	8	2	4	HpD	5 mg/kg	720-2400 J	6.9	
Stylli et al (2005)	145	78	67	HpD	5 mg/kg	70-240 J/cm ²		14
Muller and Wilson (2006)	96	43	24	HpD	2 mg/kg	58±17		7.6
Kostron et al. (2006)	52	26		Temoporfin	0.15 mg/kg	20 J/cm ²		9
Eljamel (2010)	27	13		HpD	2 mg/kg	100 J/cm ²		13.2
Akimoto et al. (2012)	14	10	4	Talaporfin sodium	40 mg/m ²	27 J/cm ²		26
Muragaki et al. (2013)	22	13		Talaporfin sodium	40 mg/m ²	27 J/cm ²	27.9	24.8

CHAPTER 2

AIM OF THE STUDY

Early, precise diagnosis and treatment are crucial for cancer patients. However, conventional diagnostic and therapeutic methods remains inadequate for treating a wide range of cancer types. The treatment of brain tumors in particular, persists as an unresolved clinical challenge.¹⁴³ Three primary issues contribute to the enduring difficulties in brain cancer treatment: a high percentage of brain tumors are inoperable due to their proximity to critical areas of the brain,¹⁴⁴ a limited number of diagnostic and therapeutic drugs can cross the blood-brain barrier which is one of the most tightly regulated gateways in the human body,¹⁴⁵ and absence of effective, targeted conventional therapies.¹⁴⁶ Given the mortality rate and significant challenges in diagnosis and treatment of brain cancer, there is an urgent need for alternative, targeted treatment modalities.

Photodynamic therapy represents a potential alternative treatment modality for diverse cancer types and holds potential for overcoming the barriers of the brain cancer treatment. Nevertheless, its applicability remains limited to a small group of cancers due to the poor penetration of the requisite light to trigger singlet oxygen generation through human tissue and the lack of selective targeting of cancer cells.⁴⁷

Additionally, designing an ideal PS for PDT presents another challenge for effective PDT. An ideal photosensitizer should possess several characteristics, including high purity, high tumor selectivity, strong absorption along with a high extinction coefficient in the therapeutic window (600-850 nm), high singlet oxygen generating capacity, minimal dark toxicity, rapid removal after use, a straightforward synthetic route, and high water solubility.⁷⁰ Furthermore, in addition to aforementioned properties of an ideal PS, for the treatment of brain cancer employing PDT also

requires controlled lipophilicity and possessing low molecular weight to enhance the chances of crossing the BBB. A maximum molecular weight of 700 Da is generally considered the limit for brain cancer-targeted PS. To increase the probability of PS crossing the BBB, a lower molecular weight, preferably below 400 Da, is commonly accepted.¹⁴⁷

Despite the development of numerous fluorophores in recent decades, only a small number of have been modified into PSs due to the absence of absorption maxima in the therapeutic window, inapplicability in aqueous media and difficulties in structural modification. However, in the last two decades, different fluorophore cores have been decorated with heavy atoms and converted into PDT agents. For instance, porphyrins, BODIPY and phthalocyanine dyes have been utilized in important and pioneering studies. Nevertheless, their hydrophobic nature and poor photostability, respectively limit their applicability as PDT agents. Furthermore, the absence of cancer cell targeting moieties and high skin sensitivity after treatment diminish interest in these dyes.^{148–150} Another group of PDT agents based on squaraine dyes absorbs light in the red region, but they are highly reactive, posing significant challenges for clinical applications.¹⁵¹ Fluorescein dyes have tissue penetration issues due to their comparatively high band-gap; their absorption and emission maxima are generally fall within the green region of the spectrum, limiting their utility in *in vivo* studies.¹⁵² Similarly, cyanine dyes suffer from poor photostability, hindering their widespread adoption.¹⁵³

Given the challenges encountered, the necessity for the development of near-infrared (NIR) absorbing, low molecular weight photosensitizers with facile modification routes for targeting brain cancer has emerged. The promising properties of xanthene based dyes, such as water solubility, photostability and ease of modification motivates us to investigate improvements on classical xanthene dyes such as fluorescein and rhodamine towards the realization of NIR absorbing/emitting dyes with highly cancer selective activation for imaging and treatment opportunities.

Previously, extending conjugation within the molecule has been favored methodology to shift absorption toward the NIR region. This ultimately results in higher molecular weight. Thus, ineffective for brain cancer treatment. On the other hand, modification on certain parts of the xanthene core is also effective for shifting the absorption maximum toward NIR region without significantly affecting the molecular weight.^{131,154} For instance, substituting sp^2 carbon with nitrogen induces 79 nm red shift,¹⁵⁵ attributable to the higher electronegativity of nitrogen, thereby lowering the LUMO level and yielding to one of the lowest molecular weight fluorophore with an absorption maximum above 550 nm, resorufin.¹⁵⁶ Similarly, replacing the central oxygen atom to dimethyl silyl accounts for 89 nm red shift towards NIR region, yielding to Si-fluorescein, or selenium can shift the absorption maximum by up to 31 nm facilitated by effective $\sigma^*-\pi^*$ conjugation, consequently lowering the LUMO level while maintaining similar HOMO level.¹³¹ Additionally, substituting with phosphine oxide induces 134 nm bathochromic shift in absorption maximum, yielding to P-fluorescein. In addition to the electronic modifications of π -conjugated systems due to the orbital interaction between π -skeleton and significant perturbation of the electronic structure, the oxidation of the phosphorus atom enhanced the electron-accepting ability and chemical stability of the π -skeleton and resulted in remarkable red shift in absorption maximum.¹⁵⁴ Furthermore, introducing nitrogen at the 3- and 6- positions instead of oxygen also induces a red shift in absorption maximum (Figure 2.1).

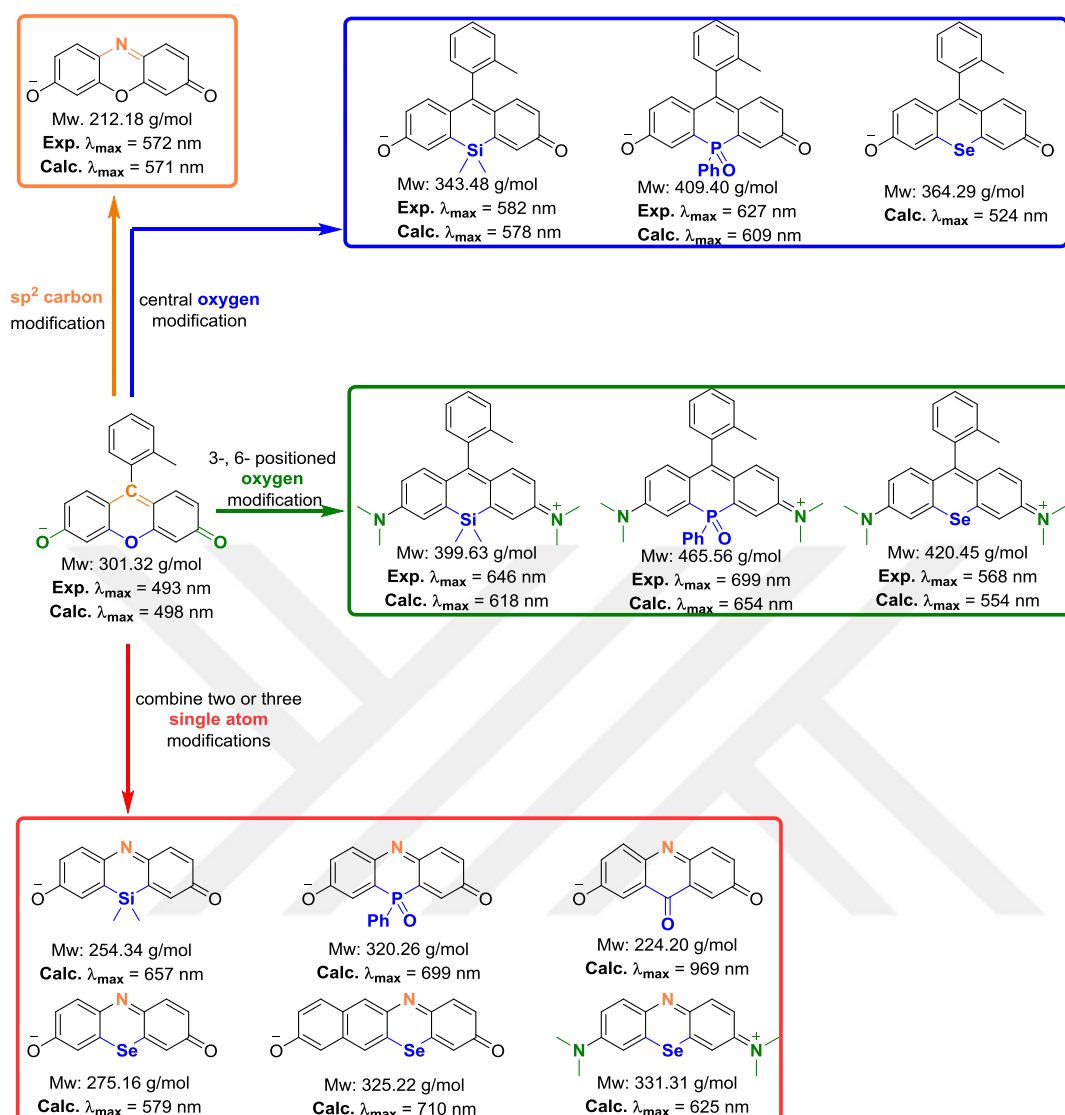


Figure 2.1. Design strategy for FR/NIR absorbing fluorophores and photosensitizer with low molecular weight.

The main question to be addressed in this thesis is whether these effect are additive? To test the feasibility of our hypothesis, several dyes that combine two or three different modifications were designed and their absorption maxima theoretically calculated as well as those of known compounds, using commonly utilized model, SMD/TD-B3LYP/6-311++G(d,p)¹⁵⁵ by Marion group.

The calculated values for the known compounds including fluorescein, resorufin, Si-fluorescein and Si-rhodamine were strongly correlated to previously published

experimental results, supporting the consistency of our calculations and demonstrating that the effects were almost entirely additive (Figure 2.1).

In addition to designing low molecular weight and NIR absorbing PSs for brain cancer treatment, effective targeting of brain tumors is crucial for PDT based applications. Targeting approaches in PDT based treatments function differently and the most important constituent of targeting is the “handle”. In a typical design of PS with a handle group, it is activated when the handle is cleaved and PS goes from an OFF to an ON state. The singlet oxygen generation occurs only when a PS in an ON state is illuminated. These handles are designed to be cleaved by a small biomolecules or enzymes that are differentially expressed by cancer cells, or preferably specific to the type of cancer, ensuring cleavage occurs at the tumor while sparing healthy tissue.¹³⁷

Despite the availability of handles for a variety of cancers, brain tumor-specific handles for targeted activatable PDT remains elusive. To effectively target brain tumors, designed PSs were modified with known handle groups that are cleavable with enzymes which are commonly upregulated in cancer cells, such as, β -galactosidase (β -Gal), nitroreductase (NTR), cathepsin B and aminopeptidase-N (APN) activatable handle groups. Additionally, they will be decorated with a novel handle for one of the most promising and studied potential targets for human malignant glioblastoma (GBM), mutated isocitrate dehydrogenase 1 (m-IDH1). Targeting this enzyme is critical for brain cancer treatment because the mutation is highly specific to brain cancers, enabling treatment at significantly early stages. Since IDH1 mutation is an early event in glioma-genesis, it has significant implications for glioma progression and tumor behavior.¹⁵⁷ The designed handle groups and activation mechanisms upon interaction with the corresponding enzyme are illustrated in Figure 2.2.

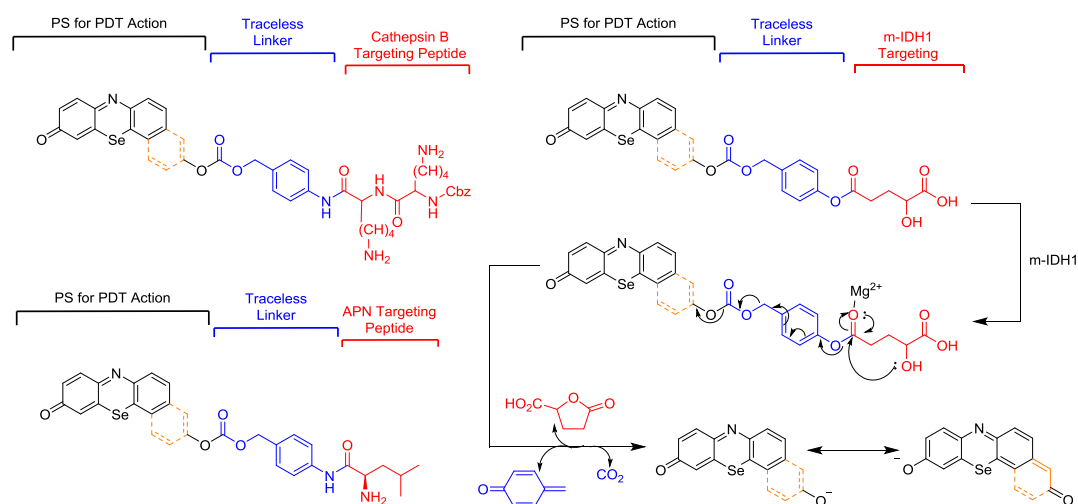


Figure 2.2. Cathepsin B, Aminopeptidase N and m-IDH1 targeting PS design and release mechanism of m-IDH1 targeting PS.

In the first three parts of the thesis, the objective was to convert single modified fluorophores into corresponding PSs through introduction heavy atoms, along with brain cancer targeted handle groups. This aims to create novel PSs and demonstrate the effectiveness these handle groups in targeting brain tumors *in vitro*.

In the fourth part of the thesis, phenoselenazine based PSs were designed by combination two or three aforementioned modifications. Although selenium modifications result in lower absorption maxima compared to dimethyl silyl and phosphine oxide analogues, selenium atom facilitates synthesis by eliminating the need for a halogenation step and reduces dark toxicity compared to halogen containing derivatives. Additionally, since the selenium acts as both bridging group and heavy atom, NIR absorbing PSs with much lower molecular weight can be achieved.

In the final two parts of the thesis, acridone and acridine based fluorophores and PSs were designed, using approaches similar to those employed for phenoselenazine based PSs to shift absorption maxima further into deep NIR region.

CHAPTER 3

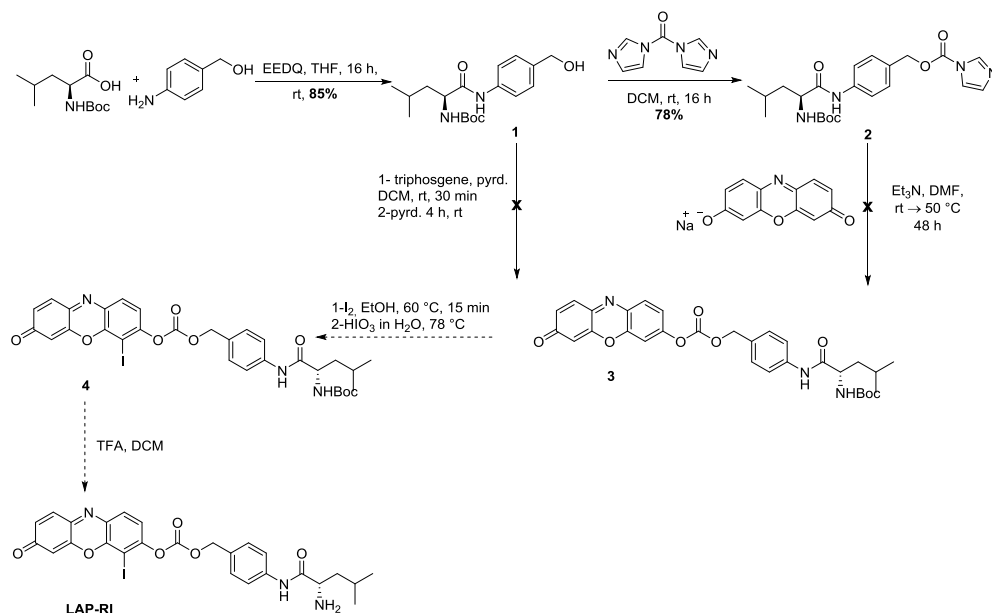
RESULTS AND DISCUSSION

3.1 Synthesis of the Photosensitizers

3.1.1 Resorufin Based Photosensitizers

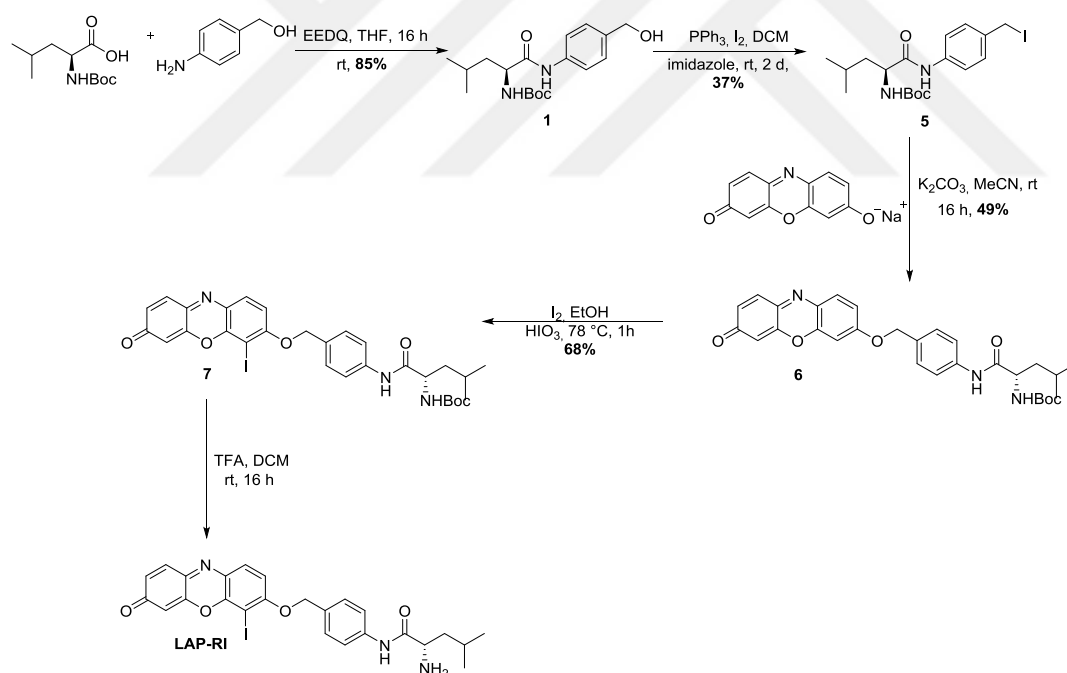
In this part of the thesis, to explore the impact of single atom modification on PDT applications, resorufin, one of the lowest molecular weight fluorophore with absorption maximum above 550 nm, was modified with heavy atoms, either iodine or selenophene and decorated with brain cancer targeted handle groups. The synthetic efforts to realize resorufin based PSs are discussed in detail.

3.1.1.1 Synthesis of LAP Activatable Iodo-Resorufin PS (LAP-RI)



Scheme 3. 1. Initial synthetic route for **LAP-RI**.

For the synthesis of **LAP-RI**, initially proposed synthetic pathway is illustrated in scheme 3.1. Compound **1** was synthesized via an amide coupling reaction between commercially available (tert-butoxycarbonyl)-L-leucine and 4-aminobenzyl alcohol in the presence of a stoichiometric amount of 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (EEDQ), with 85% yield. Subsequently, an attempt was made to modify compound **1** with an imidazole moiety to convert the hydroxyl group into a better leaving group. For this purpose, compound **1** underwent a reaction with commercially available carbonyldiimidazole to yield compound **2**. Although compound **2** was obtained with a high yield, it did not react with commercially available resorufin sodium salt, to give compound **3**. As an alternative, compound **1** was attempted to be converted to compound **3** with the aid of triphosgene. However, this strategy also proved unsuccessful. Consequently, alternative methods and structural modifications were designed for the synthesis of **LAP-RI**.

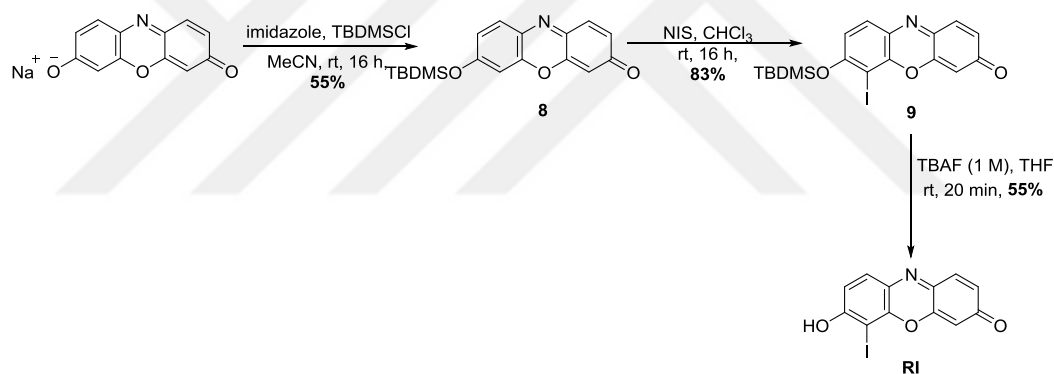


Scheme 3. 2. Final synthetic route for **LAP-RI**.

To synthesize LAP activatable resorufin based PS, modifications were made to the linker group between resorufin and leucine.¹⁵⁸ Due to challenges encountered in synthesizing a carbamate bearing linker group, structural simplicity was prioritized.

In the revised synthetic pathway, benzylic hydroxyl group of compound **1** was converted into iodine yielding compound **5** with an acceptable yield. Subsequently, compound **5** underwent an S_N2 type reaction with commercially available resorufin sodium salt. Obtained compound **6** was iodinated using molecular iodine with the assistance of iodic acid, resulting in compound **7** with a yield of 68%. Finally, the Boc protective group was removed from the leucine, yielding the target PS, **LAP-RI**. Prior to investigating its photophysical properties and conducting *in vitro* cell studies, **LAP-RI** was purified using reverse phase HPLC to achieve purity above 95% before investigating. Furthermore, synthesized PS, **LAP-RI**, was characterized with ¹H, ¹³C NMR and HRMS.

3.1.1.2 Synthesis of Iodinated Resorufin PS (RI)

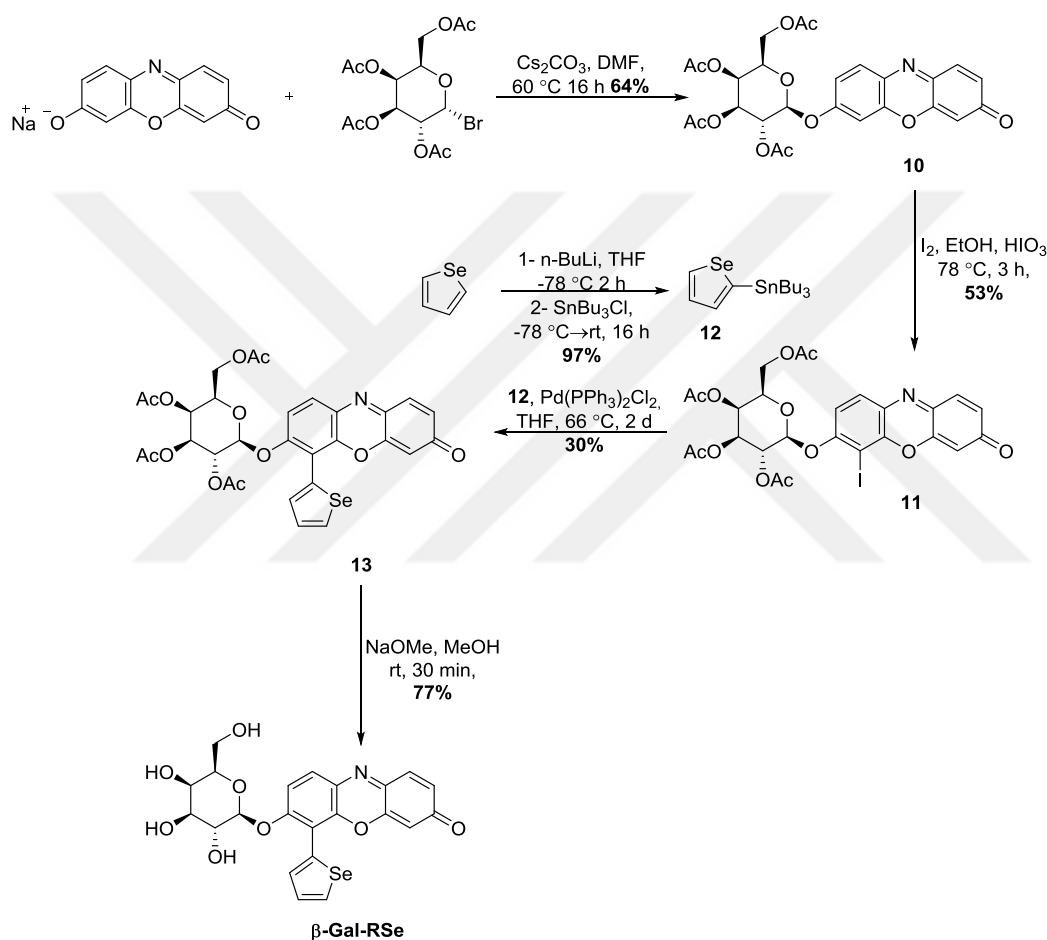


Scheme 3. 3. Synthetic route for **RI**.

To compare activatable PS, **LAP-RI** with its active form, **RI** was prepared over three steps. Initially, commercially available resorufin sodium salt protected with TBDMS protective group. Then, compound **8** iodinated with NIS and compound **9** was obtained with 83% yield. Finally, TBDMS protective group removed with 1 M TBAF solution and desired product **RI** obtained with 55% yield.¹³⁶

3.1.1.3 Synthesis of β -Gal Activatable Seleno-Resorufin PS (β -Gal -RSe)

To further extend the conjugation and shift the absorption into the NIR region, a modification with selenophene ring was envisioned. Additionally, following promising results with the LAP activatable moiety, the introduction of β -galactosidase activatable handle group was proposed.

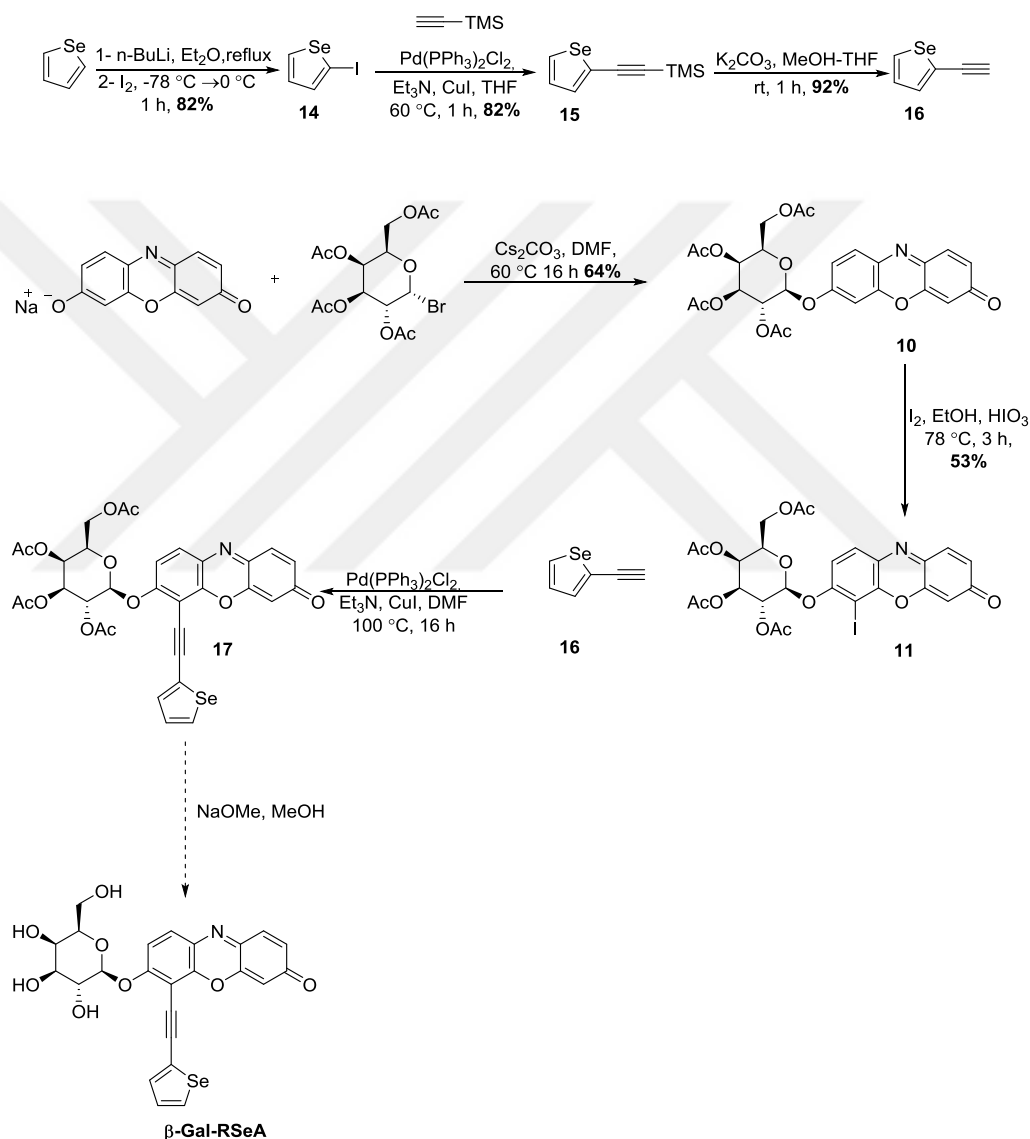


Scheme 3. 4. Synthetic route for β -Gal -RSe.

For the synthesis of β -Gal-RSe, the proposed synthetic pathway is illustrated in scheme 3.4.¹⁵⁹ Synthesis of compounds **10** and **11** were conducted by Kolemen group. the selenophene moiety was incorporated into compound **11** via Stille cross-coupling methodology. Compound **12**, the precursor of **13**, was synthesized from commercially available selenophene through a stannylation reaction with SnBu_3Cl

with a high yield. Finally, the acetyl protective groups removed from the β -Gal moiety, yielding the target PS, **β -Gal-RSe**. Synthesized PS, **β -Gal-RSe**, was characterized with ^1H , ^{13}C NMR and HRMS.

3.1.1.4 Synthesis of β -Gal Activatable Seleno-Resorufin PS (**β -Gal -RSeA**)



Scheme 3. 5. Synthetic route for **β -Gal -RSeA**.

Due to inadequate incorporation of selenophene into the π -electron system in **β -Gal -RSe**, singlet oxygen quantum yield and absorption maxima fell below anticipated levels (Table 3.4, Scheme 3.61). To enhance the optical properties and triplet lifetime of **β -Gal -RSe** further modification were undertaken to increase planarity and extend the alternating π -electron system. Specifically, the introduction of an acetylene group between the resorufin and selenophene derivatives was conceived.

The introduction of the acetylene group to the selenophene proceeded through a three steps process. Initially, molecular iodine introduced to the selenophene following lithiation, resulting in compound **14** with a high yield. Subsequently, compound **14** underwent Sonogashira coupling with commercially available TMS-acetylene to yield compound **15**. Finally, the TMS protective group was removed using a mild base, K_2CO_3 and compound **16** freshly utilized in another Sonogashira coupling with the previously synthesized compound **11**. However, the low yield of the coupling reaction and challenges encountered during the purification hindered the progress in the synthesis of the target PS, **β -Gal -RSeA**. Optimization studies for the synthesis of compound **17** are presently underway.

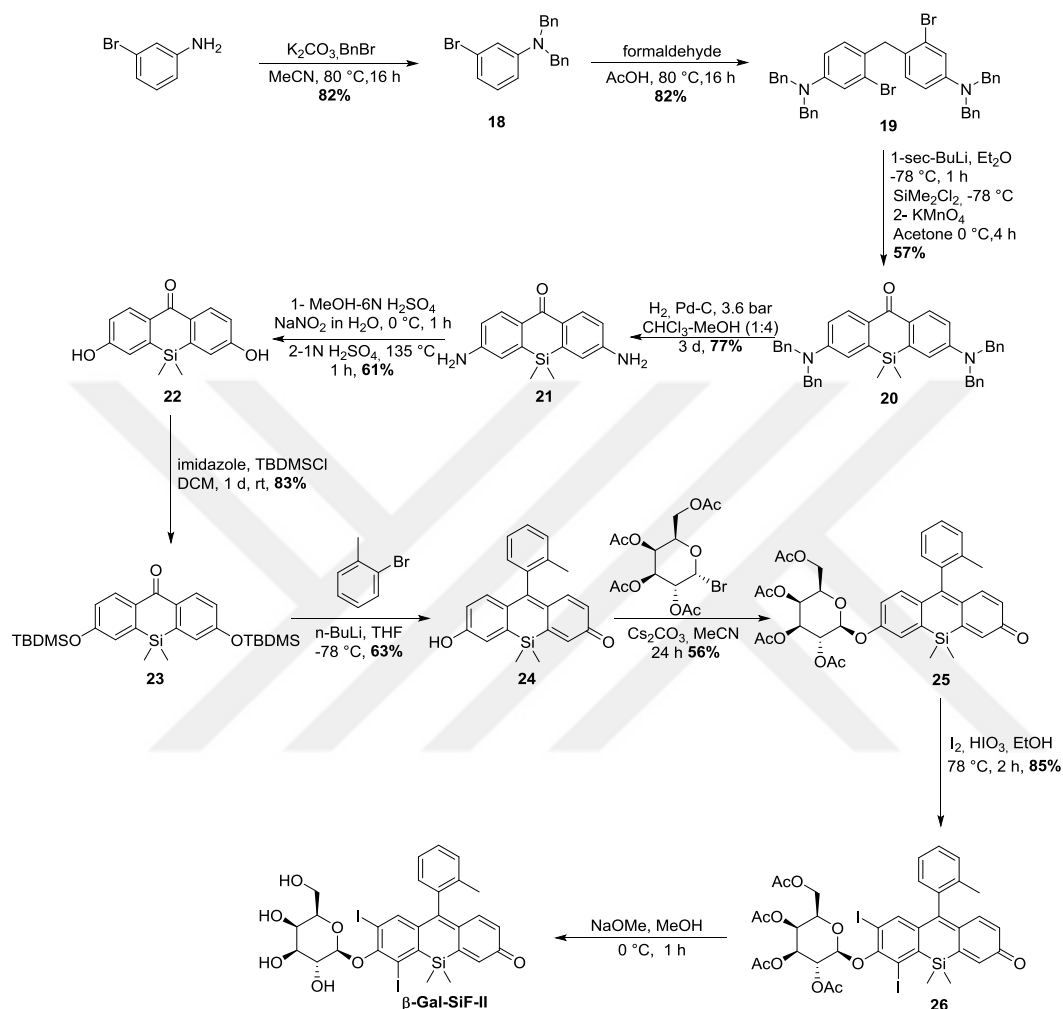
3.1.2 Si-Fluorescein Based Photosensitizers

Previously, the synthesis of Si-fluorescein-based fluorophores typically commenced with the protection of 3-bromoaniline using an allyl protective group.¹⁵⁴ However, this synthetic approach presents several drawbacks. Firstly, allyl groups were employed as protecting groups, necessitating stoichiometric amounts of $Pd(PPh_3)_4$ for their deprotection, which is not cost-effective. Secondly, following the formation of the carbon bridge, oxidation of the carbon center to a ketone is required. Oxidation of sp^3 carbon is generally more challenging compared to standard oxidation of alcohols, leading to a diminished overall yield of the synthesis. The third drawback arises from utilizing an aniline moiety as the starting material for the synthesis of fluorescein derivatives. Upon formation of the silicon or phosphorus bridge, the Sandmeyer reaction is employed to introduce phenolic moieties. These additional

steps in the synthesis increase both the synthetic effort and cost. To address the aforementioned issues, an attempt was made to initiate the carbon-bridged system using 3-bromophenol, as pursued by Dr. Gizem Atakan Alkan in her PhD thesis study.¹⁶⁰ However, she encountered difficulties in establishing the carbon bridge starting from 3-bromoaniline. Consequently, we opted to address this issue by exploring alternative protective groups, specifically by substituting the allyl protective group with a benzyl protective group while maintaining the remainder of the pathway unchanged.



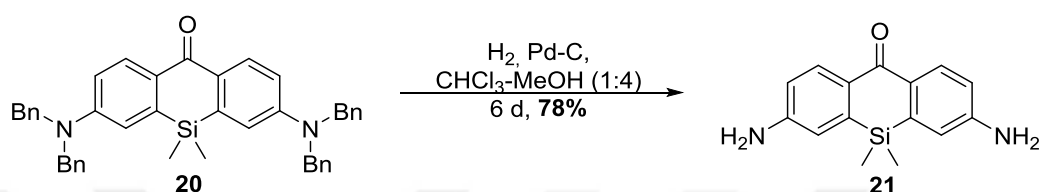
3.1.2.1 Synthesis of β -Gal Activatable Diiodo-Si-Fluorescein PS (β -Gal-SiF-II)



Scheme 3. 6. Synthetic route for β -Gal-SiF-II.

For the synthesis of β -Gal-SiF-II, the proposed synthetic pathway is illustrated in Scheme 3.6. To initiate the synthesis, commercially available 3-bromo aniline was protected with a benzyl moiety yielding a dibenzyl protected aniline derivative, compound **18**, with an 82% yield. Subsequently, compound **18** underwent condensation with formaldehyde to form carbon bridge within the xanthene core. Upon successful synthesis of compound **19**, silicon bridge was introduced to the core through a lithium-halogen exchange reaction facilitated by sec-BuLi. Following the

establishment of the silicon bridge, the crude product was dissolved in acetone and dibenzylic position was oxidized with KMnO_4 , resulting in compound **20**. Benzyl deprotection constituted the most time-consuming aspect of the synthesis. Initially, attempts were made to remove the four benzyl groups through hydrogenation reaction at 1 atmospheric pressure. However, the completion of the reaction required 6 days.

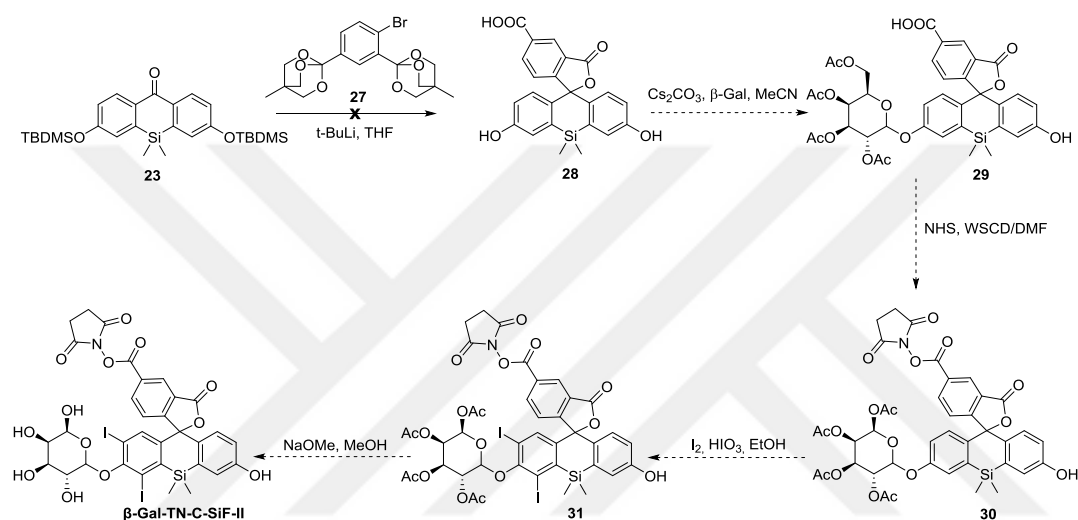


Scheme 3. 7. Synthesis of compound **21** by utilizing 1 atm H_2 .

Following the implementation of the reaction in Parr reactor with a 3.6 bar H_2 gas, the reaction time was reduced to three days, while maintaining a comparable yield. However, the use of higher pressure to further decrease the reaction time was avoided due to the presence of highly sensitive groups within the xanthone core, such as dimethyl silane. After the completion of optimization studies for the synthesis of compound **21**, the amino groups were converted into phenolic hydroxyl groups via the Sandmeyer reaction, with moderate yield. Subsequently, the hydroxyl groups were protected with the TBDMS protective group in high yield, resulting in compound **23**. Following the synthesis of Si-xanthone core, commercially available o-bromo toluene was successfully introduced at the 9th position of the Si-xanthone core. The resulting compound **24**, also known as Tokyo Magenta (TM) in the literature, has been extensively utilized in numerous imaging studies owing to its outstanding photophysical properties. Compound **24** was further modified with a β -galactose derivative to serve as an activatable PS. Heavy atom incorporation into the PS was achieved through the iodination of compound **25** with molecular iodine in the presence of HIO_3 with a high yield. Finally, the desired PS, **β -Gal-SiF-II** was obtained by the removal of the acetyl protective groups from the β -Gal moiety. Prior to investigating its photophysical properties and conducting in vitro cell studies,

β -Gal-SiF-II was also purified using reverse phase HPLC to achieve purity above 95% before investigating. Furthermore, synthesized PS, **β -Gal-SiF-II**, was characterized with ^1H , ^{13}C NMR and HRMS.

3.1.2.2 Synthesis of β -Gal and TN-C Activatable Diiodo-Si-Fluorescein PS (**β -Gal-TN-C-SiF-II**)

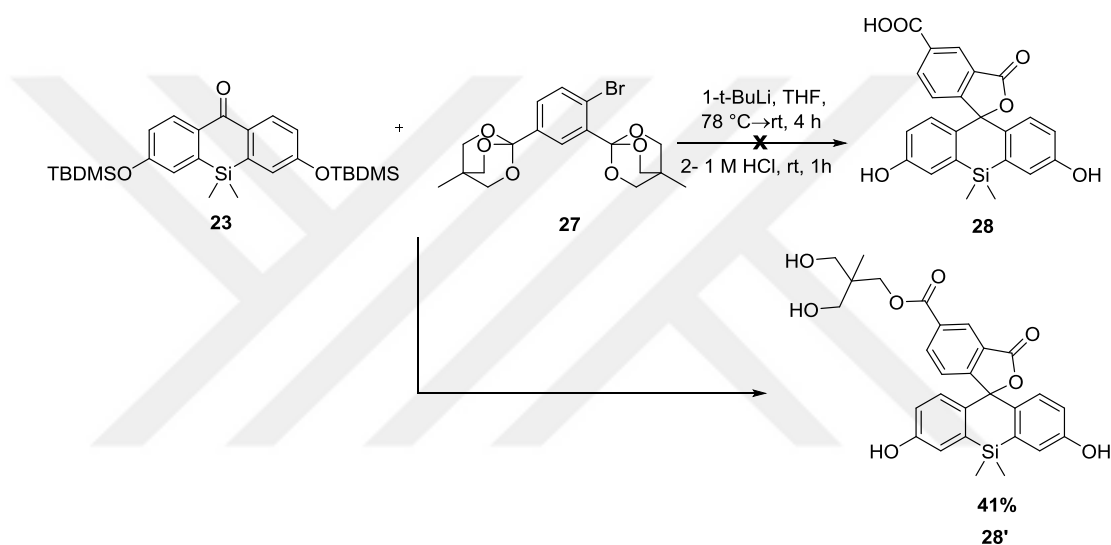


Scheme 3. 8. Initial synthetic route for **β -Gal-TN-C-SiF-II**

To enhance selectivity for brain tumors, particularly gliomas, further modifications on **β -Gal-TM-II** was envisioned. The extracellular matrix glycoprotein tenascin-C (TN-C), known to be overexpressed in malignant gliomas was targeted for selective binding using a N-hydroxysuccinimide (NHS) moiety. This moiety was intended to be introduced to the PS from the dicarboxylic acid analogue of **β -Gal-TM-II**.¹²³

For the synthesis of **β -Gal-TN-C-SiF-II**, initially proposed synthetic pathway is illustrated in scheme 3.8.¹²³ Compound **27** was synthesized from commercially available 2-bromo-5-carboxybenzoic acid by Kolen group. Subsequently, compound **27** underwent lithium halogen exchange reaction and was then introduced to the previously synthesized compound **23**. According to the designed synthetic route, after the introduction of dicarboxybenzene, one of the phenolic hydroxyl

group would be modified with a β -Gal group which will serve as a cage group. Following this, NHS would be introduced to the carboxylic acid at the 5th position of benzene, serving as a handle group to increase efficacy of treatment. Finally, after halogenation and deprotection steps, the target activatable PS, **β -Gal-TN-C-SiF-II**, was aimed to synthesized (Scheme 3.8). However, in the synthesis of compound **28**, one of the protective group on carboxylic acid could not be completely cleaved. Due to the challenges encountered, this pathway was abandoned and a new strategy for the introduction of the NHS moiety was developed.



Scheme 3. 9. Synthesis of compound **28'**.

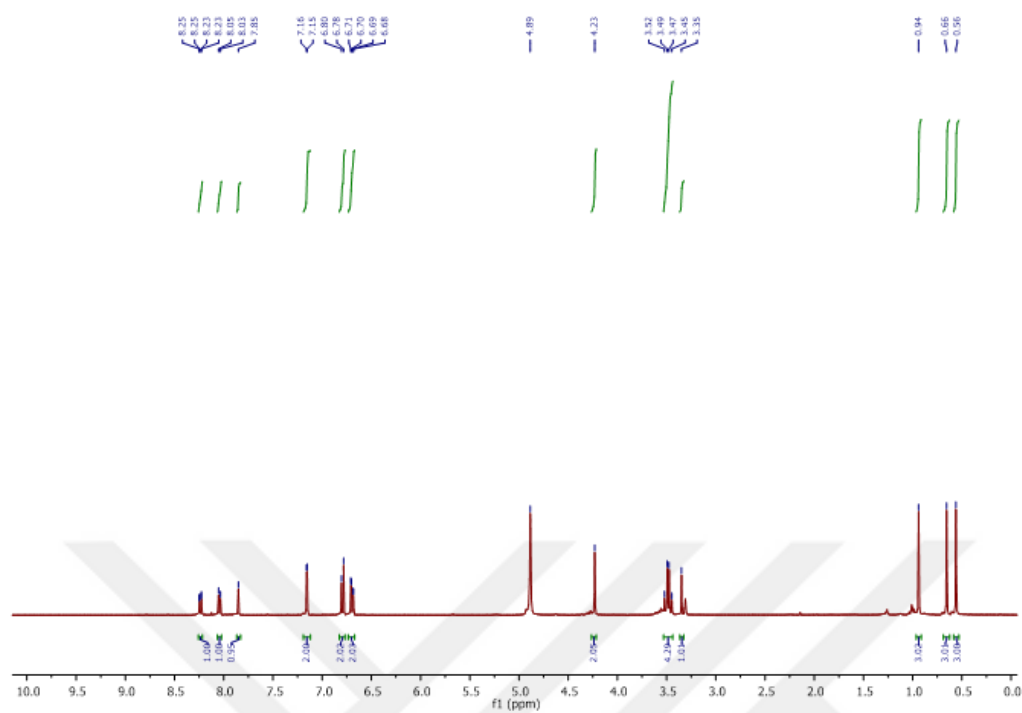


Figure 3.1. ¹H NMR spectrum of compound **28'** in MeOD.

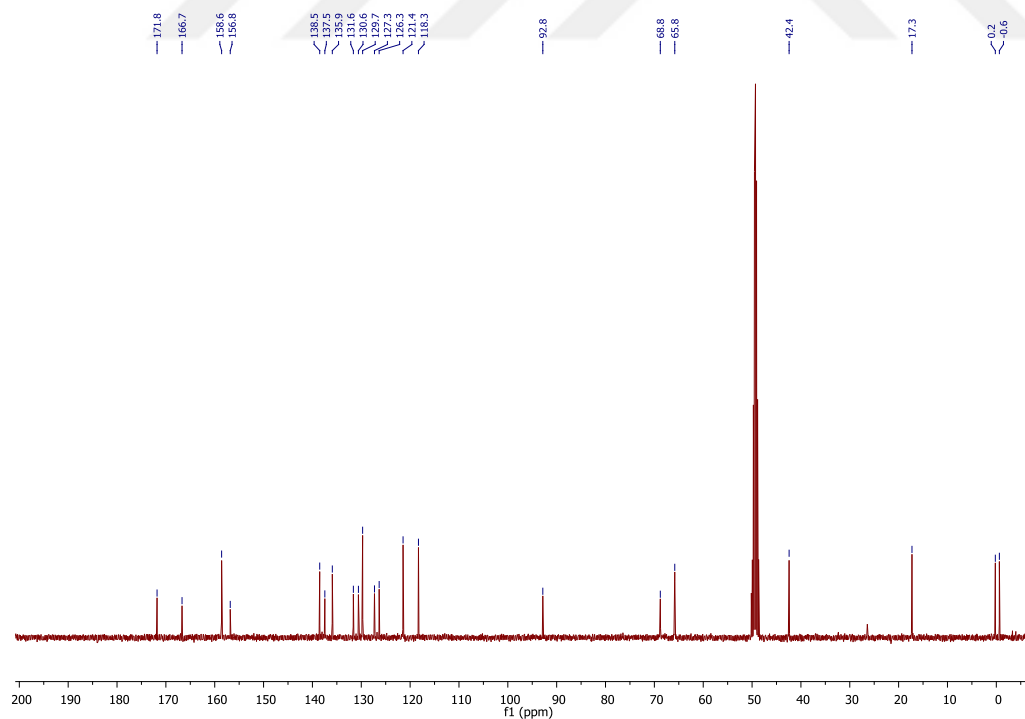
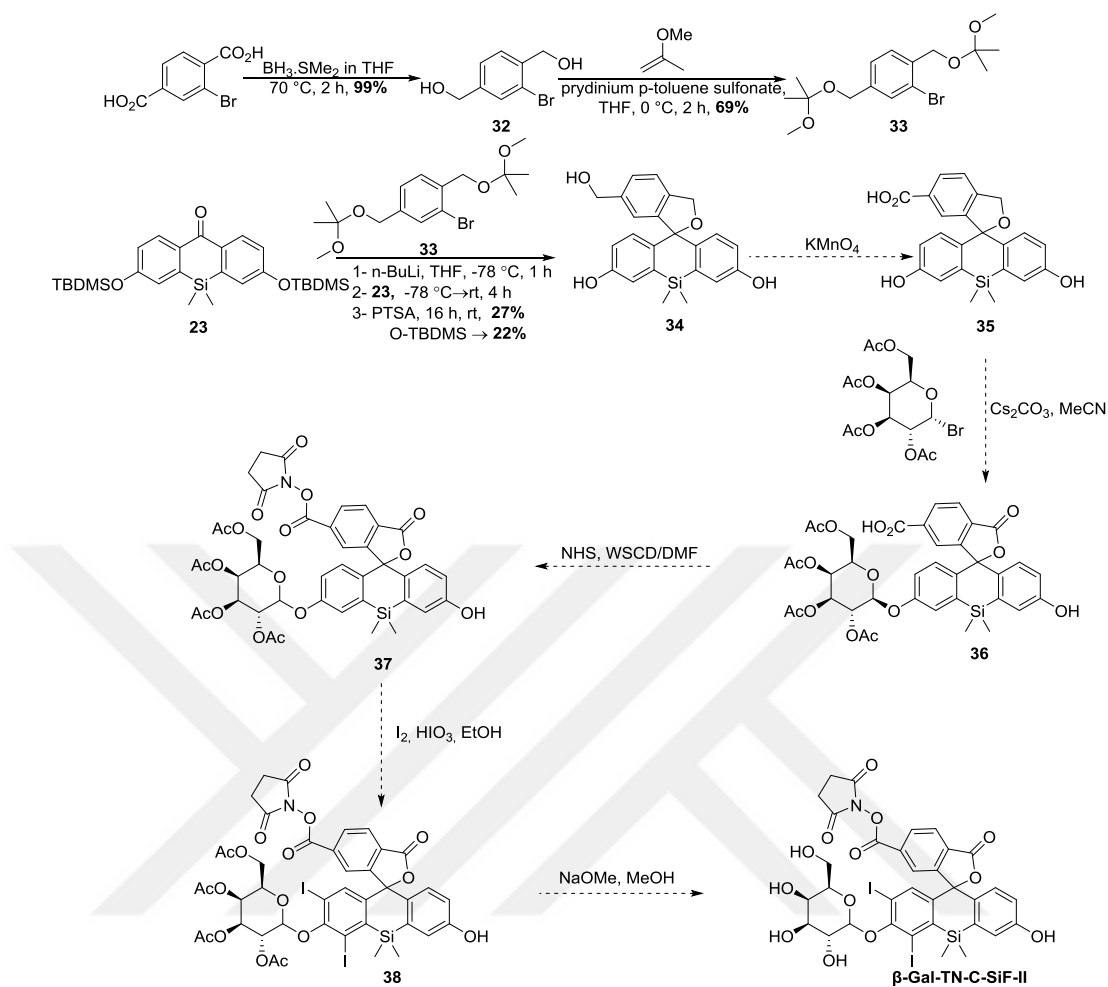


Figure 3.2. ¹³C NMR spectrum of compound **28'** in MeOD.



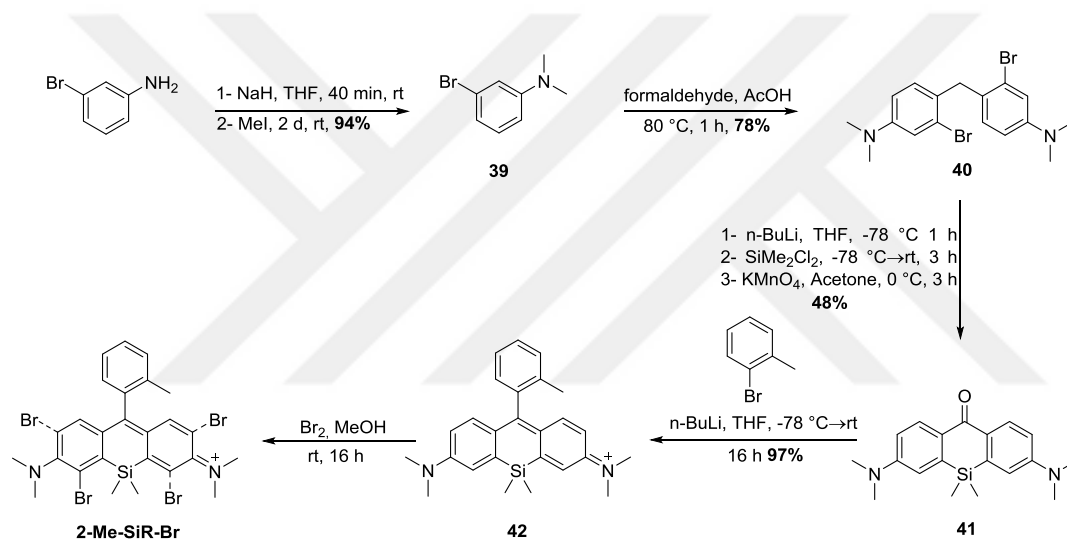
Scheme 3. 10. Alternative synthetic route for β -Gal-TN-C-SiF-II.

Due to challenges encountered during the deprotection of carboxylic acid, an alternative pathway involving the introduction of 1,4-benzyl alcohol derivative to a Si-xanthone was pursued.¹⁶¹ For this purpose, compound **33** was synthesized in two steps. Initially, carboxylic acid moieties were reduced to the corresponding alcohols with the aid of $\text{BH}_3 \cdot \text{SMe}_2$ with a reasonable yield. Subsequently, compound **32** was protected with 2-methoxyprop-1-ene in the presence of catalytic pyridinium PTSA, resulting in compound **33** with a yield of 99%. The prepared compound **33** underwent lithium halogen exchange and was subsequently incorporated into the previously synthesized compound **23**. Unfortunately, compound **34** was obtained with a low yield of 27%. Additionally, a TBDMS-protected analogue was obtained as a side product with a 22% yield. In the proposed pathway, following the oxidation

of benzylic alcohol to the corresponding carboxylic acid, a similar approach to the former method was intended. However, due to the low yield in the synthesis of compound **34** and challenges encountered in the oxidation step (to be addressed in synthesis of Si-rhodamine based PSs, Scheme 3.22), the synthesis of dual-targeted Si-fluorescein based PS, **β -Gal-TN-C-SiF-II** was postponed as a future work.

3.1.3 Si-Rhodamine Based Photosensitizers

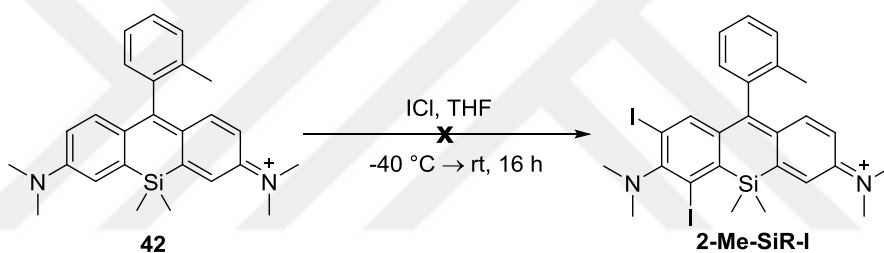
3.1.3.1 Synthesis of Tollyl Bearing Si-Rhodamine PS (2-Me-SiR-Br)



Scheme 3. 11. Initial synthetic route for **2-Me-SiR-Br**.

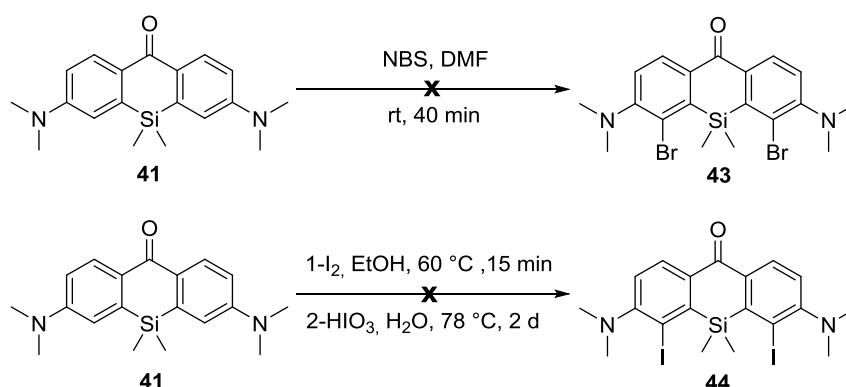
For the synthesis of **2-Me-SiR-Br**, the initially proposed synthetic pathway is illustrated in scheme 3.11.¹²¹ Initially, commercially available 3-bromoaniline was methylated with MeI after deprotonation with NaH, resulting in compound **39** with nearly complete conversion. Compound **39** was then condensed with formaldehyde solution to establish the carbon bridge of the xanthene core. Subsequently, compound **40** underwent lithium halogen exchange, followed by introduction of dimethylsilane. After the establishment of the Si bridge, the 9th position of the crude product was oxidized with KMnO₄. This series of reactions resulted in compound **41** with an overall yield of 48%. Following this, commercially available o-

bromotoluene underwent lithium halogen exchange and then o-tolylolithium was introduced to the compound **41** from the carbonyl moiety on the 9th position of the Si- xanthone core. Resulting compound **42**, also known as 2-Me-SiR650, was synthesized with a high yield. 2-Me-SiR650 has been utilized in numerous bioimaging studies over the last decades. However, its utilization as a PS for PDT applications was not explored. Consequently, the synthesis of the first Si-rhodamine based PS for PDT was attempted through heavy atom modification. The synthesized compound **42** was brominated with molecular bromine in MeOH. However, a mixture of mono-, di-, tri-, tetra- brominated derivatives of compound **42** was obtained. Due to the challenges encountered in the purification step, the mixture of these derivatives could not be separated. Consequently, heavy atom introduction was attempted with several other reagents, both in this step and in former steps.



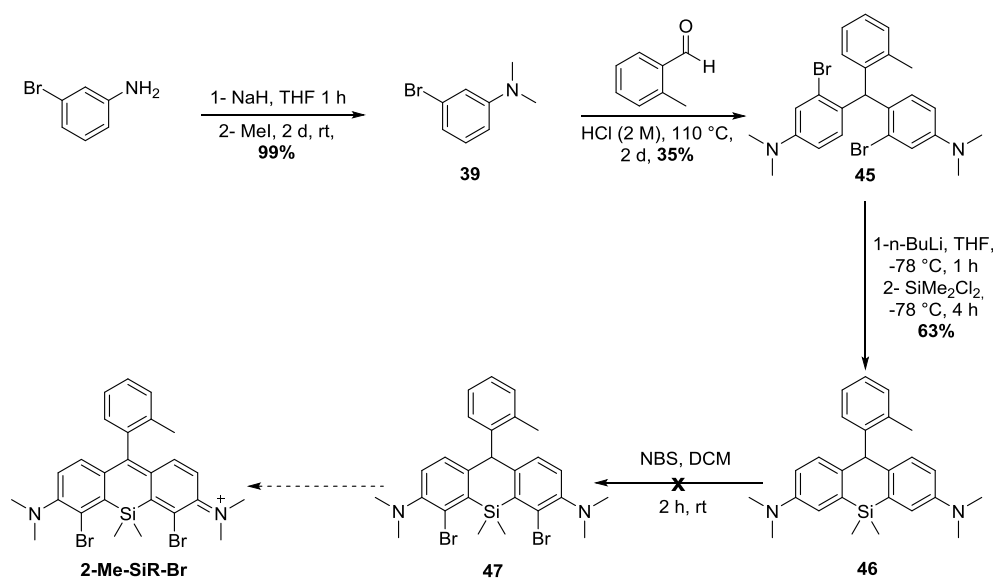
Scheme 3. 12. Scheme of heavy atom introduction attempt to compound **42**.

Following the attempted bromination of compound **42** with molecular bromine, an attempt was made to incorporate iodine into compound **42**. ICl was employed for iodine incorporation; however, instead of obtaining **2-Me-SiR-I**, a complex mixture was obtained.



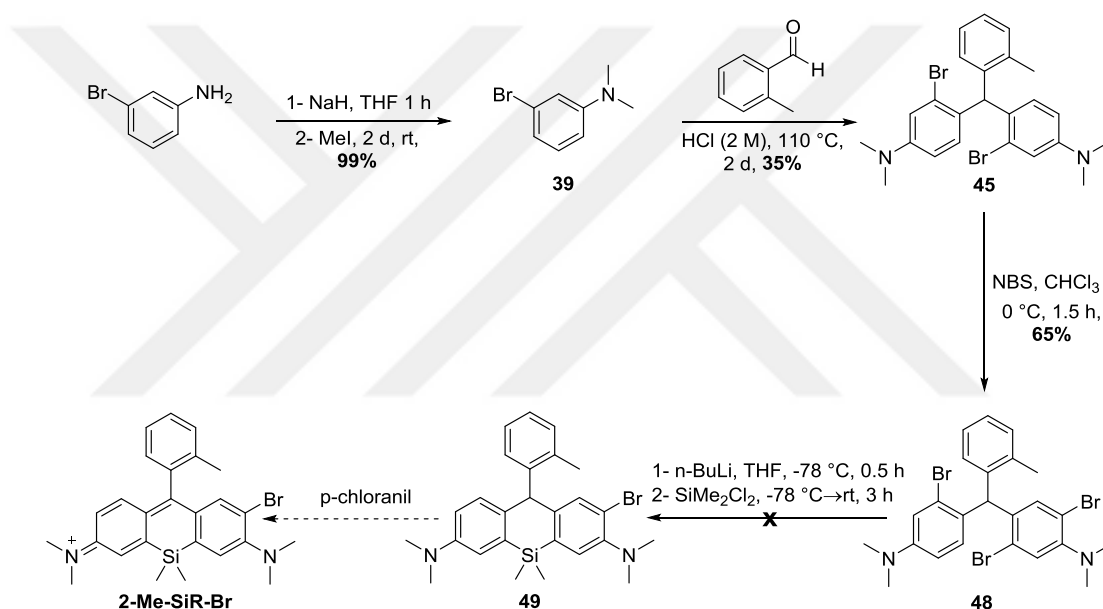
Scheme 3. 13. Scheme of heavy atom introduction attempt to compound **41**.

New approaches were then developed for heavy atom incorporation. It is well documented that halogenation reactions of fully conjugated systems pose challenges. Therefore, heavy atom incorporation was attempted in a previous step using the synthesized Si-xanthone core, compound **41**, which was subjected to halogenation with various reagents. Initially, compound **41** underwent bromination reaction with NBS. However, the desired product could not be separated from complex mixture. Subsequently, an iodination reaction with molecular iodine, aided by HIO₃ was attempted. Similar to the bromination reaction, a complex mixture was obtained (scheme 3.13).



Scheme 3. 14. Alternative synthetic route for **2-Me-SiR-Br**.

Following the failure of previous approaches for the synthesis of **2-Me-SiR-Br**, a new synthetic route was devised. The new synthetic approach is illustrated in Scheme 3.14. The previously synthesized compound **39** was condensed with commercially available o-tolylbenzaldehyde under acidic conditions to yield compound **45**. Subsequently, the Si bridge was established with a comparable yield. The synthesized compound **46** was then subjected to halogenation with bromine using NBS. However, instead of desired product, compound **42** was obtained. Further attempts were made using molecular iodine, albeit concluded with similar results.



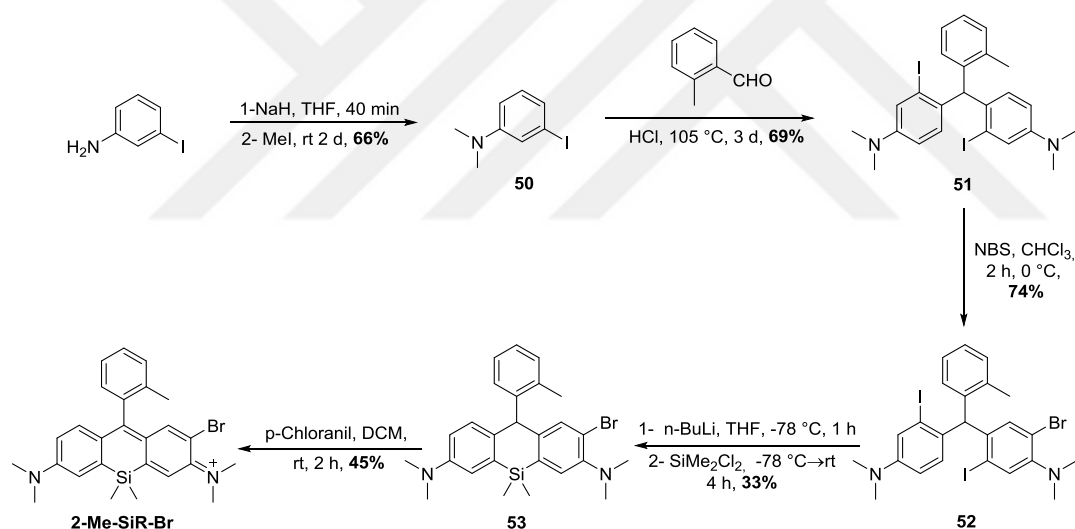
Scheme 3. 15. Alternative synthetic route for **2-Me-SiR-Br**.

In the next approach, the previously synthesized compound **45** was subjected to bromination with NBS, resulting in compound **48**. Initially, in the synthesis of compound **49** and subsequently the final product **2-Me-SiR-Br**, a lithium halogen exchange reaction was attempted with n-BuLi, followed by the introduction of dimethylsilane. However, unexpectedly, undesirable bromine underwent exchange with lithium. Theoretically, electron density at the meta position of the amino group should be lower than the ortho analogue, which could stabilize the existence negative

charge better. Unfortunately, ortho analogue underwent the reaction, resulting in failure to incorporate dimethylsilane. Optimization studies were conducted to alter selectivity but, in each cases, exchange at the ortho position with respect to amine was observed. The attempts are listed in Table 3.1.

Table 3.1 Reaction conditions for the synthesis of compound **49**

Entry	<i>n</i> -BuLi/eq.	SiMe ₂ Cl ₂ (eq)	Result
1	n- / 2.1	1.1	ortho bromo elimination
2	n- / 2.4	5	ortho bromo elimination
3	sec- / 2.1	1.1	ortho bromo elimination
4	sec- / 2.4	5	ortho bromo elimination

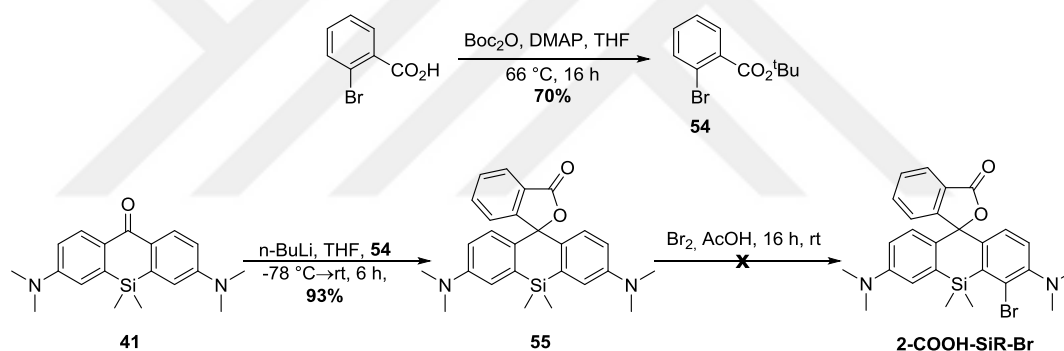


Scheme 3. 16. Final synthetic route for **2-Me-SiR-Br**.

The final synthetic approach for the synthesis of **2-Me-SiR-Br**, takes advantage of the selectivity between iodine and bromine in the lithium halogen exchange reaction. To achieve this, commercially available 3-iodoaniline was methylated with MeI. Subsequently, the synthesized compound **50** underwent a condensation reaction with commercially available o-tolylaldehyde in acidic conditions, yielding compound **51** with a reasonable yield of 69%. Following this, compound **51** was brominated with

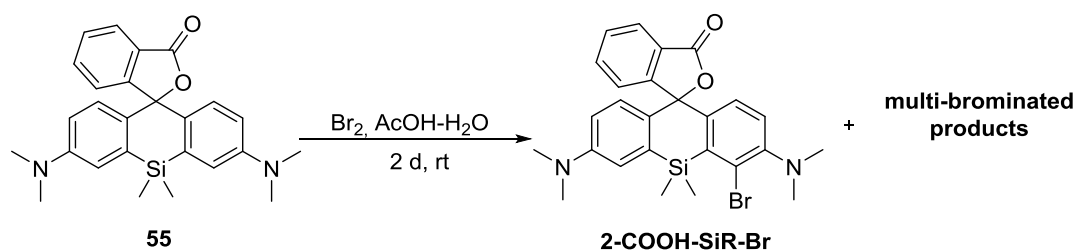
NBS, resulting in compound **52** with a yield of 52%. In the next step, compound **52** underwent lithium halogen exchange with *n*-BuLi, selectively eliminating iodines from the core structure and subsequently dimethylsilane was introduced. This reaction resulted in compound **53** with an acceptable yield of 33%. Finally, compound **53** oxidized with *p*-chloranil to yield the target PS, **2-Me-SiR-Br**. Prior to investigating its photophysical properties and conducting in vitro cell studies, **2-Me-SiR-Br** was purified using reverse phase HPLC to achieve pharmaceutical purity grade (> 95%) before investigating. Furthermore, synthesized PS, **2-Me-SiR-Br**, was characterized with ^1H , ^{13}C NMR and HRMS.

3.1.3.2 Synthesis of Benzoic Acid Bearing Si-Rhodamine PS (2-COOH-SiR-Br/I)



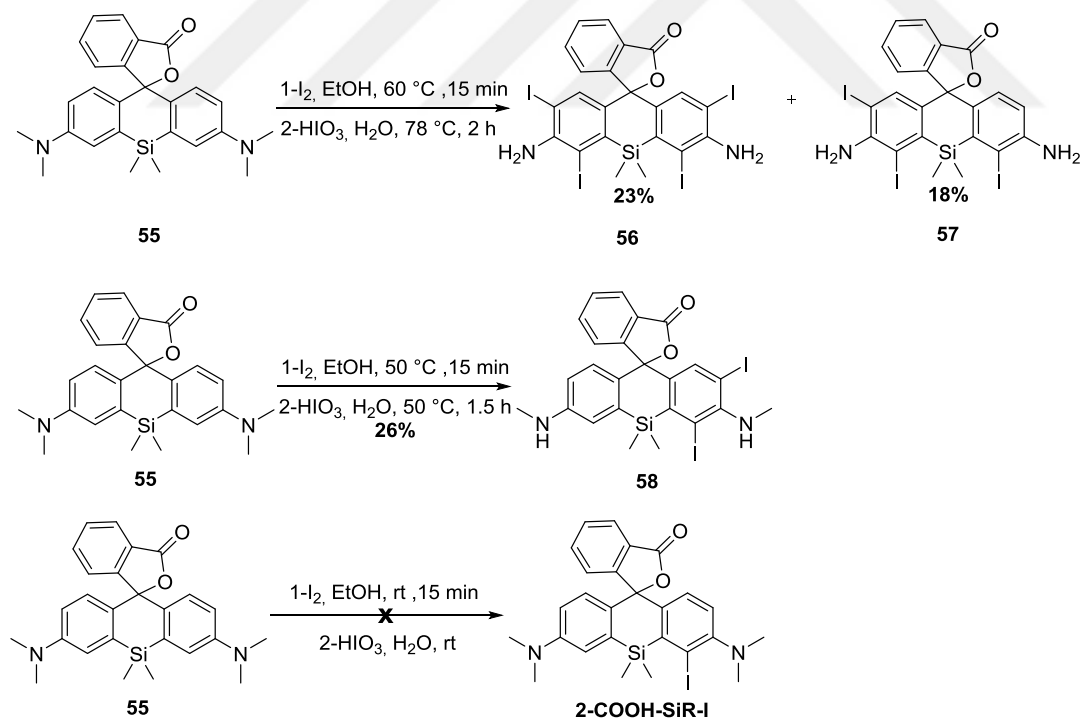
Scheme 3. 17. Synthetic route for **2-COOH-SiR-Br**.

To synthesize a carboxylic acid bearing derivative of **2-Me-SiR-Br**, with the potential to enhance tumor selectivity via its pH-dependent lactone formation capability, commercially available 2-bromobenzoic acid was initially protected with the Boc group.¹⁶² Subsequently, the Boc-protected 2-bromobenzoic acid, denoted as compound **54**, underwent a lithium halogen exchange reaction, followed by its incorporation into the previously synthesized compound **41**. This protocol resulted in the successful synthesis of compound **55**. In attempts to introduce heavy atom(s) to compound **55**, molecular bromine in acetic acid was employed. However, the target PS, **2-COOH-SiR-Br** could not be attained.



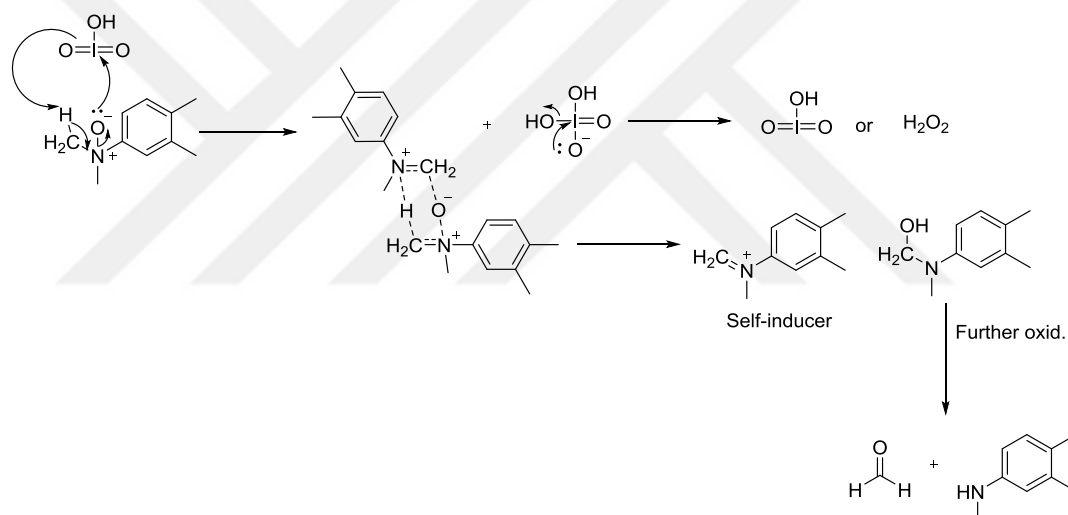
Scheme 3. 18. Alternative bromination condition for the synthesis of **2-COOH-SiR-Br**.

For the incorporation of heavy atoms, reaction conditions were attempted to be optimized. In the second trial of bromination, an AcOH-H₂O (1:1) solution was employed. Although the equivalency of bromine (2 eq.) remained consistent with the former attempt, the reaction duration extended to two days until complete consumption of the starting material. However, a mixture of mono and multi-brominated products was obtained. Due to the challenges encountered in the purification step, this approach was not pursued further.



Scheme 3. 19. Iodination attempts of compound **55**.

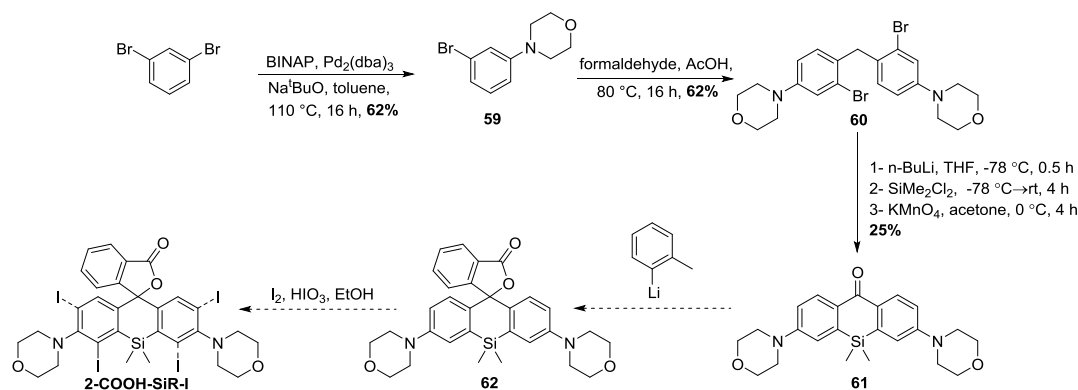
Following the unsuccessful bromination of compound **55**, an iodination endeavor was pursued. Molecular iodine in the presence of iodic acid was employed for the iodination reaction. Despite the successful iodination, oxidative demethylation of tertiary amines was observed. Consequently, both a tetra-iodinated and completely demethylated product alongside its tri-iodinated analogue were synthesized. In the second trial, the temperature was reduced to 50 °C to mitigate potential oxidative demethylation. However, this adjustment yielded mono demethylation of each amine in tandem with double iodination. Encouraged by the promising outcomes of the second trial, the reaction was undertaken at ambient room temperature. Regrettably, due to the compound **55**'s limited solubility under these conditions, the desired target PS, **2-COOH-SiR-I**, could not be realized.



Scheme 3. 20. Proposed reaction mechanism for oxidative demethylation of tertiary amines in **2-COOH-SiR-I**.

The demethylation observed during the halogenation reaction of compound **55** was elucidated through the proposed mechanism illustrated in Scheme 3.20. In the presence of an oxidant such as I_2 and HIO_3 , the formation of N-oxide may ensue. Subsequently, facilitated by HIO_3 , a Mannich intermediate was generated, accompanied by the formation of H_2IO_4 as a byproduct, which may further decompose into HIO_3 and H_2O_2 . The resultant imine intermediate could act as a self-

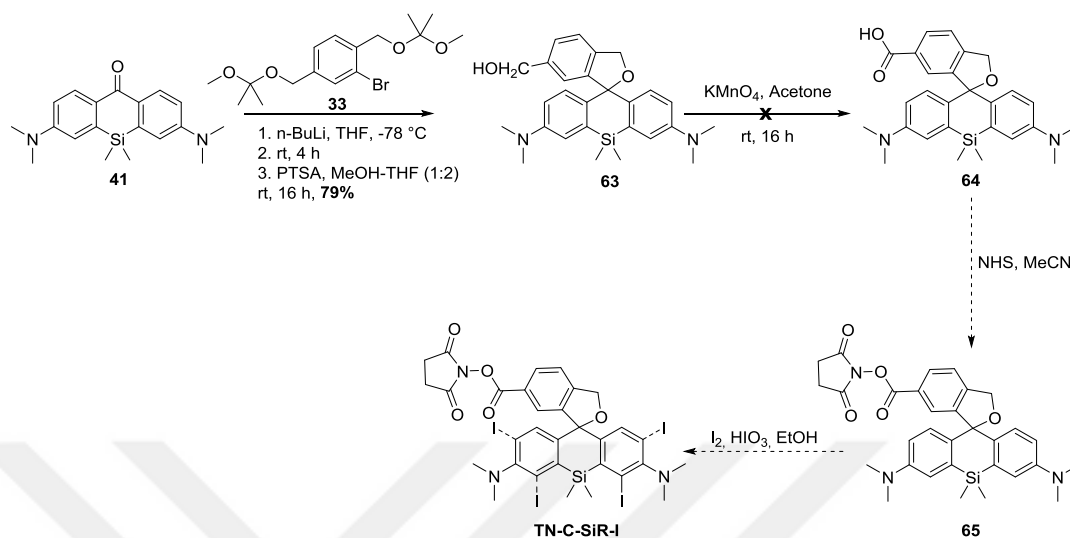
inducer, catalyzing the oxidation of the methyl group and leading to the subsequent elimination of methyl as formaldehyde.^{163–166}



Scheme 3. 21. Synthetic route for morpholine bearing **2-COOH-SiR-I**.

To mitigate the oxidative demethylation of tertiary amines, an alternative approach has been developed. It was postulated that the incorporation of morpholine moieties instead of dimethyl groups could effectively attenuate oxidative demethylation.¹⁶⁷ This involved the utilization of commercially available 1,3-dibromobenzene in a mono Buchwald-Hartwig amination reaction with morpholine. Subsequently, the resultant compound **59** underwent condensation with formaldehyde under acidic conditions. Following this, a silicon bridge was established through the introduction of dimethylsilane moiety to the lithium-halogen-exchanged compound **60** intermediate, followed by oxidation of the 9th position of the xanthene core with KMnO₄. Nonetheless, the synthesis of compound **61** resulted in a low yield, impeding progress towards the intended target PS, **2-COOH-SiR-I**. Owing to the encountered synthetic challenges associated with compound **61**, the synthesis of **2-COOH-SiR-I** was postponed to the future work.

3.1.3.3 Synthesis of TN-C Targeted Si-Rhodamine PS (TN-C-SiR-I)

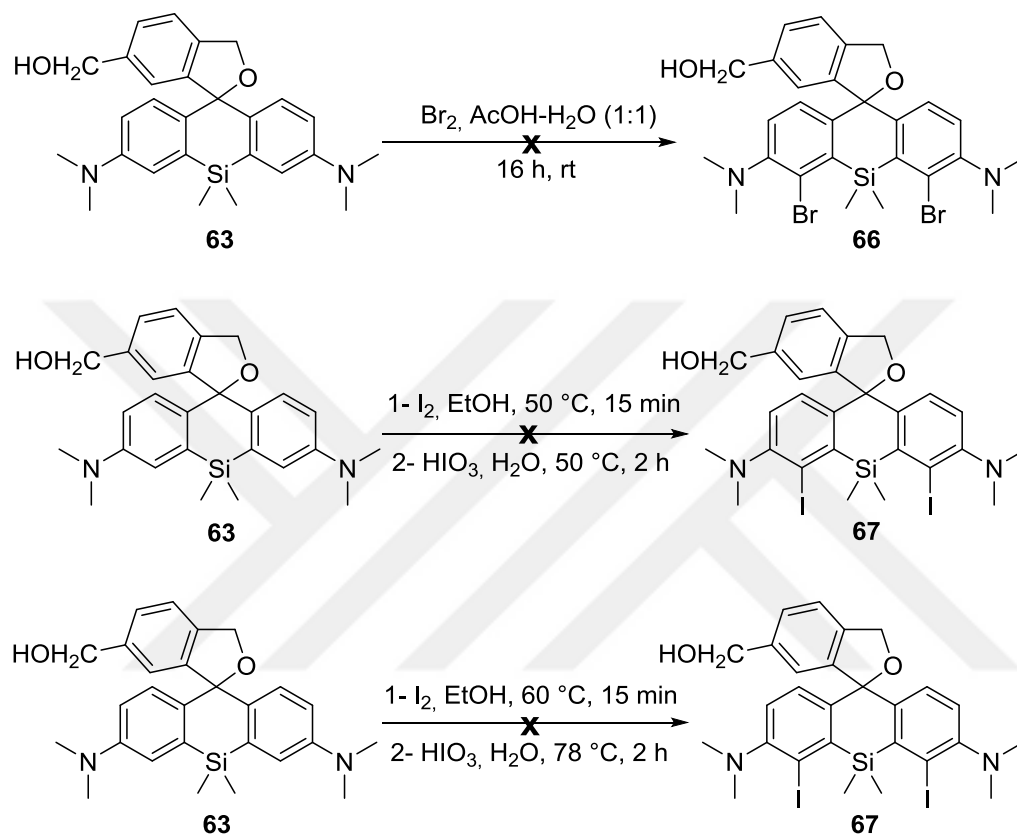


Scheme 3. 22. Synthetic route for **TN-C-SiR-I**.

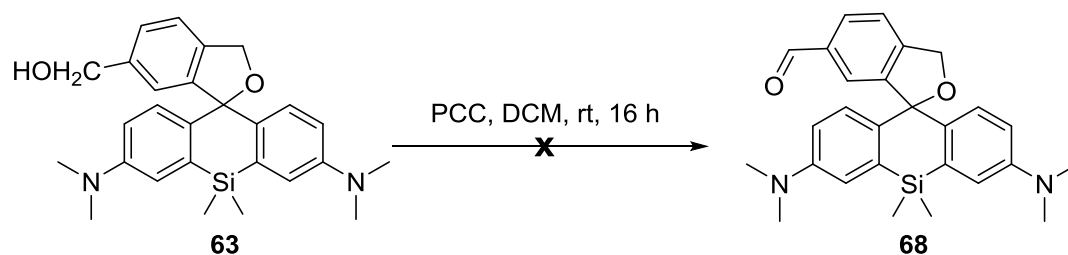
For the synthesis of the extracellular matrix glycoprotein tenascin-C (TN-C) targeted silicon rhodamine-based photosensitizer,¹⁶⁸ **TN-C-SiR-I**, compound **33** was incorporated with a yield of 79% into the previously synthesized compound **41**. Subsequently, KMnO₄ was utilized as an oxidizer in the following step; however, the targeted compound **64** could not be obtained, resulting in the recovery of compound **63**. Following the unsuccessful synthesis of compound **64**, numerous attempts were made to successfully synthesize the target photosensitizer, **TN-C-SiR-I**.

For the synthesis of **TN-C-SiR-I**, halogenation reaction was conducted with compound **63**. Initially, molecular bromine was utilized as the halogenating agent in an AcOH-H₂O solvent system. However, the target product, compound **66**, could not be obtained and compound **63** was recovered. Subsequently, molecular iodine was employed with the presence of HIO₃. In the initial attempt, temperature was maintained at 50 °C to control multi-iodination, but in addition to mono-iodinated product, starting material and multi-iodinated products were obtained. As a

subsequent endeavor, temperature was raised to the reflux to mitigate side product formation. Nonetheless, the desired product could not be isolated from the mixture. Due to the challenges encountered during purification, halogenation step was deferred to latter stages of the synthesis.

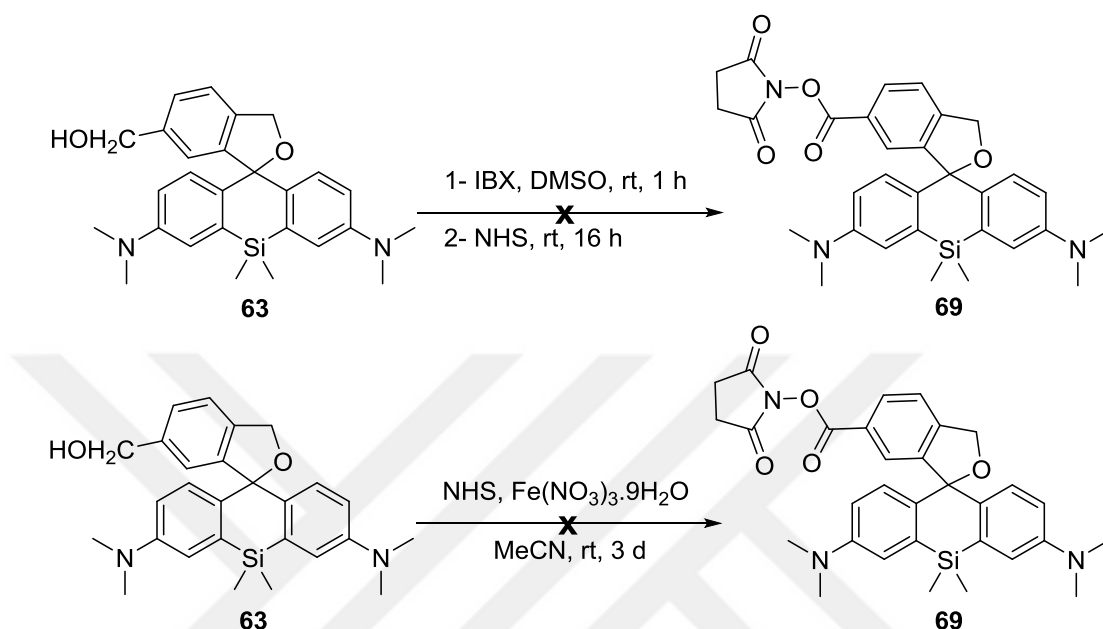


Scheme 3. 23. Attempts for the halogenation of compound **63**.



Scheme 3. 24. Attempt for the oxidation of compound **63** to compound **68**.

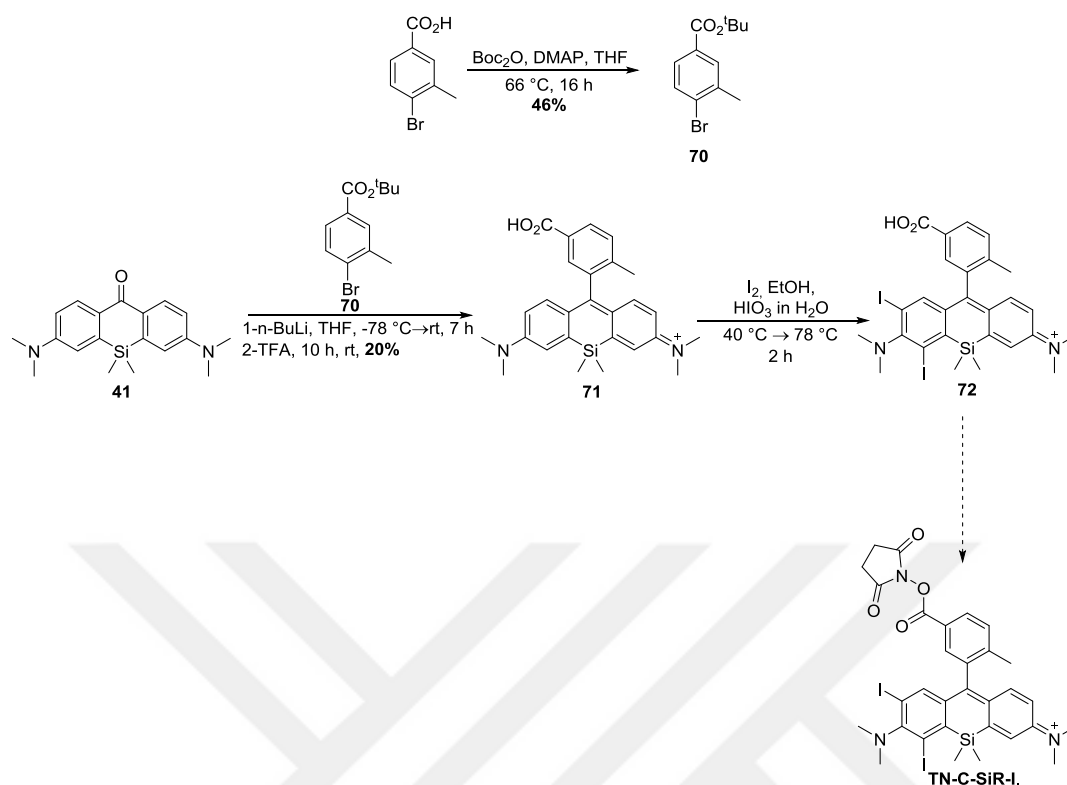
After attempting the oxidation of compound **63** with KMnO_4 , PCC was utilized to oxidize benzyl alcohol to its corresponding aldehyde.^{161,169} However, the formation of complex mixture was observed.



Scheme 3. 25. Attempts for the direct introduction of NHS to the compound **63**.

Following the unsuccessful oxidation attempts, an in-situ oxidation coupled with the incorporation of NHS was undertaken (Scheme 3.25). Initially, IBX utilized as an oxidizing agent, followed by the introduction of NHS. Regrettably, the target product compound **69** remained elusive.¹⁷⁰ In a subsequent attempt, NHS was employed along with $\text{Fe(NO}_3)_3 \cdot 9\text{H}_2\text{O}$ to synthesize compound **69**.^{169,171} Unfortunately, the formation of complex mixture was observed.

Given the series of unsuccessful attempts to develop a TN-C targeted silicon rhodamine-based PS, a new approach was devised. The latest methodology is illustrated in Scheme 3.26.¹²³



Scheme 3. 26. Alternative route for the synthesis of **TN-C-SiR-I**.

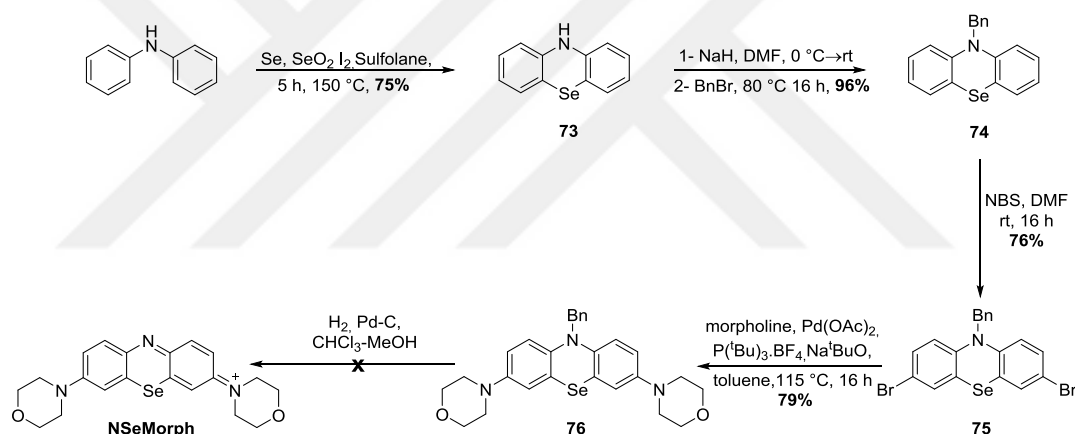
For the synthesis of modified **TN-C-SiR-I**, commercially available 4-bromo-3-methylbenzoic acid was protected with a *tert*-butyl moiety, yielding compound **70**. Subsequently, compound **70** underwent a lithium halogen exchange reaction and was then introduced to the previously synthesized compound **41**, resulting in the synthesis of compound **71** with moderate yield. Following this step, heavy atom introduction was performed using molecular iodine in the presence of HIO_3 and successive halogenation was proven by NMR analysis of crude product. Efforts to

purify compound **72** are currently underway. Upon successful isolation of compound **72**, an NHS moiety will be introduced to yield the target PS, **TN-C-SiR-I**.

3.1.4 Phenoselenazine Based Photosensitizers

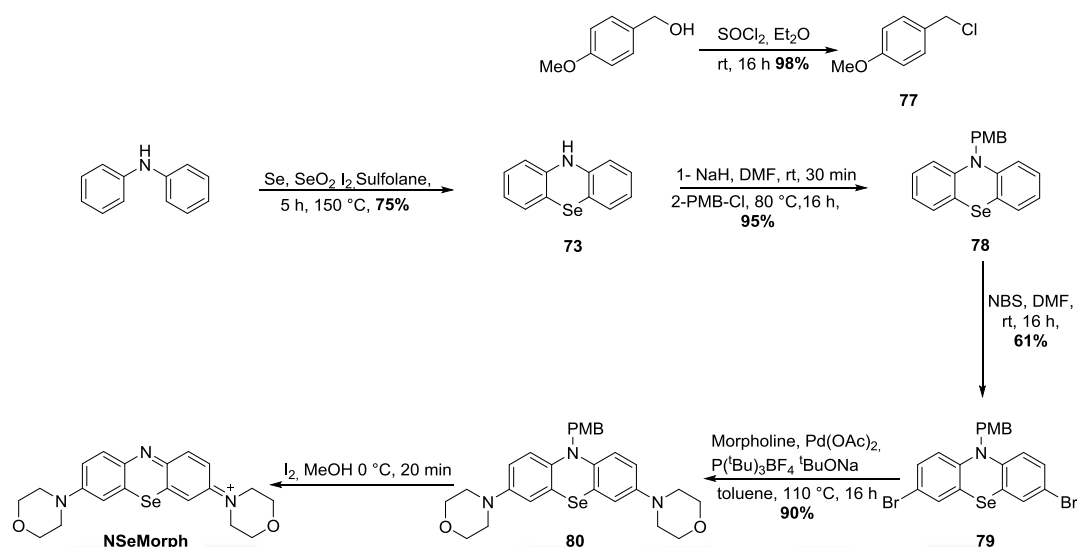
To realize a new series of NIR-absorbing, novel and halogen-free photosensitizers based on phenoselenazine core were designed. This sections will elucidate the attempts made in their synthesis.

3.1.4.1 Synthesis of Morpholine Bearing Phenoselenazine Based PS (NSeMorph)



Scheme 3. 27. Initial synthetic route for **NSeMorph**.

For the synthesis of **NSeMorph**, to commercially available diphenylamine, selenium atom was introduced with good yields.¹⁷² Following this, compound **73** was protected with a benzyl group, yielding compound **74**. Subsequently, the para positions relative to nitrogen were brominated with NBS. The morpholine moiety was then incorporated via Buchwald-Hartwig amination reaction into compound **75**, resulting in compound **76** with a 79% yield. Finally, the deprotection of the benzyl group was attempted through hydrogenation to obtain the target PS. Unfortunately, complex mixture was obtained (Figure 3.27).

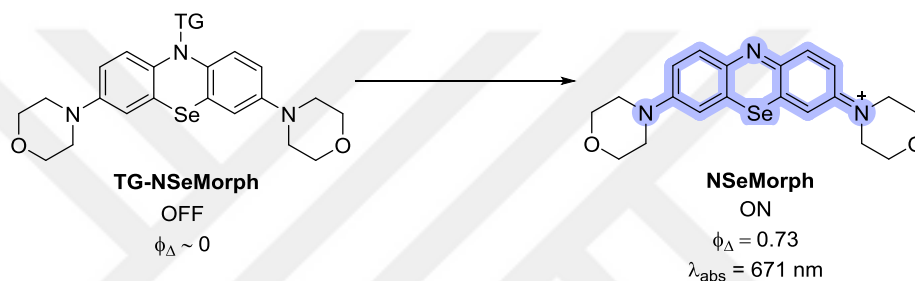


Scheme 3.28. Final synthetic route for **NSeMorph**.

Given the encountered challenges in the deprotection of benzyl group in the preceding approach, a substitution of the protective group on the amine was pursued (Scheme 3.28). P-methoxybenzyl (PMB) protective group was chosen to facilitate the synthesis of the target PS, **NSeMorph**. To this end, p-methoxybenzyl chloride, denoted as compound **77**, was synthesized from commercially available anisyl alcohol with thionyl chloride. Subsequently, the synthesized compound **77** was introduced to the compound **73**, affording compound **78** with an excellent yield of 95%. Next, the para positions were brominated with NBS, resulting in compound **79** with 61% yield. Compound **79** underwent a Buchwald-Hartwig amination reaction with under conditions identical to those employed in the synthesis of compound **76**, resulting in the formation of compound **80** with an improved yield of 90%. Ultimately, the target PS, **NSeMorph** was obtained from oxidative cleavage of the PMB protective group via utilization of molecular iodine. Prior to investigating its photophysical properties and conducting *in vitro* cell studies, **NSeMorph** was purified using reverse phase HPLC to achieve purity above 95% before investigating. Furthermore, synthesized PS, **NSeMorph** was characterized with ^1H , ^{13}C NMR and HRMS.

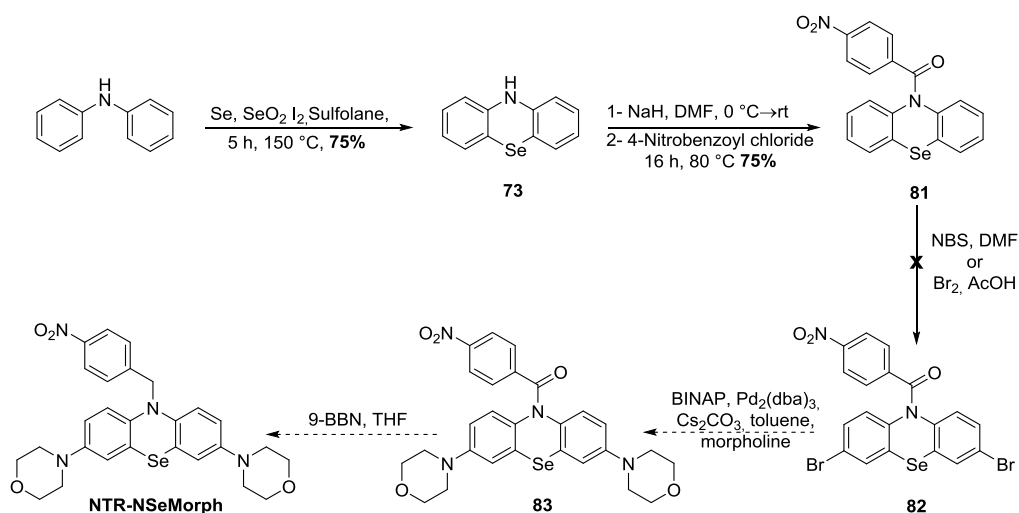
3.1.4.2 Synthesis of Activatable Morpholine Bearing Phenoselenazine Based PS (TG-NSeMorph)

To enhance the selectivity of **NSeMorph** toward various types of cancer, numerous activatable **NSeMorph** based PSs were developed. Although **NSeMorph** is an active drug, it has exhibited good tumor selectivity over healthy cells across various cancer cell lines (Section 3.3.4). To further enhance this characteristics and direct its efficacy towards specific cancer types, several targeting-group bearing **NSeMorph** derivatives were designed and their synthesis were attempted.



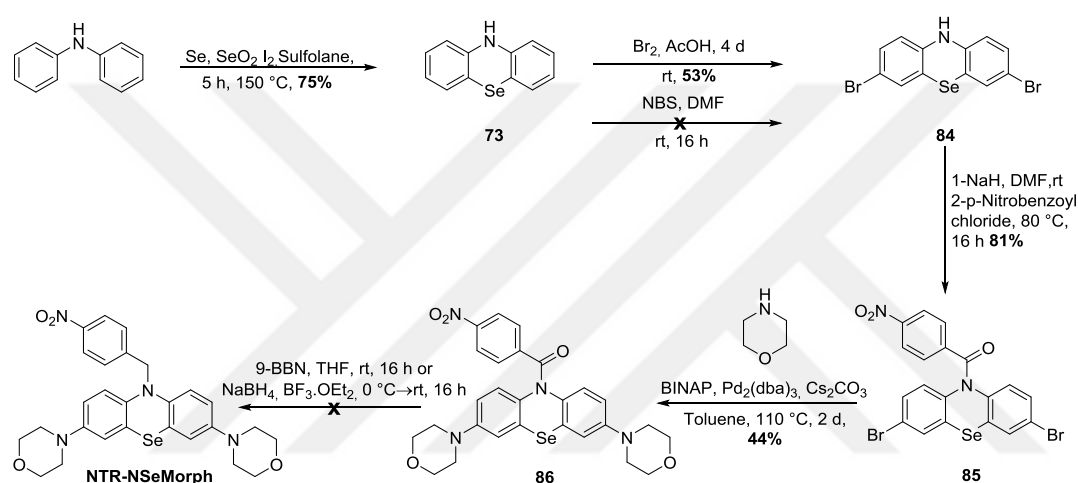
Scheme 3. 29. The On-Off stage of **NSeMorph** after modification with targeting groups.

3.1.4.2.1 Nitroreductase Activatable NSeMorph (NTR-NSeMorph)



Scheme 3. 30. Initial synthetic route for **NTR-NSeMorph**.

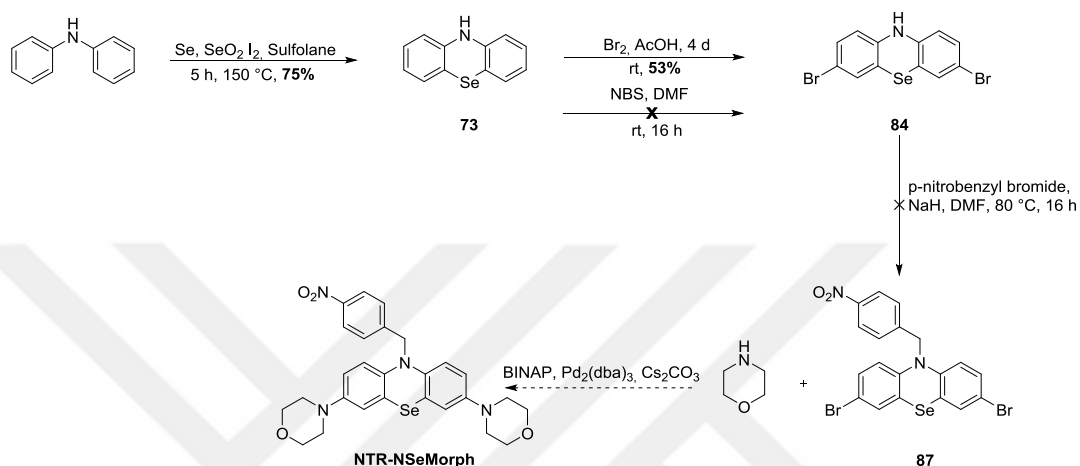
To synthesize nitroreductase activatable **NSeMorph**, commercially available 4-nitrobenzoyl chloride was incorporated with the previously synthesized compound **73**, resulting in compound **81** with a 75% yield. To introduce morpholine moieties to the para positions relative to nitrogen through Buchwald-Hartwig amination, bromination of compound **81** was attempted. In the initial trial, NBS was utilized as the bromine source. However, due to the diminished electron density of the ring, the reaction did not occur, leading to recovery of compound **81**. Additionally, this reaction was also attempted with Br₂ in acetic acid, albeit with no success.



Scheme 3. 31. Alternative synthetic route for **NTR-NSeMorph**.

Due to challenges encountered in the bromination step, a new approach has emerged. The introduction of p-nitrobenzoyl group diminishes the reactivity of the ring leading to failures in the bromination step. Initially, halogenation of compound **73** with NBS was performed, but the desired compound could not be attained. Subsequently, molecular bromine was utilized under the acidic conditions, resulting in the synthesis of the target product with a low yield. To enhance the yield of the reaction, fresh, degassed glacial acetic acid was employed. After optimization efforts, the target compound **84** was obtained with a moderate yield of 53%. Following this, a p-nitrobenzoyl moiety was incorporated into compound **84** successfully. Subsequently, compound **85** underwent Buchwald-Hartwig amination reaction with morpholine, resulting in compound **86** with a yield of 44%. To obtain the desired PS, **NTR-**

NSeMorph, selective reduction of amide to the corresponding amine was attempted. Initially, 9-borabicyclo[3.3.1]nonane (9-BBN) was utilized as a amide selective reducing agent but the target PS could not be attained.¹⁷³ Following this, NaBH₄ in the presence of BF₃·OEt₂ was employed. However, both efforts resulted in the recovery of starting material, compound **86**.



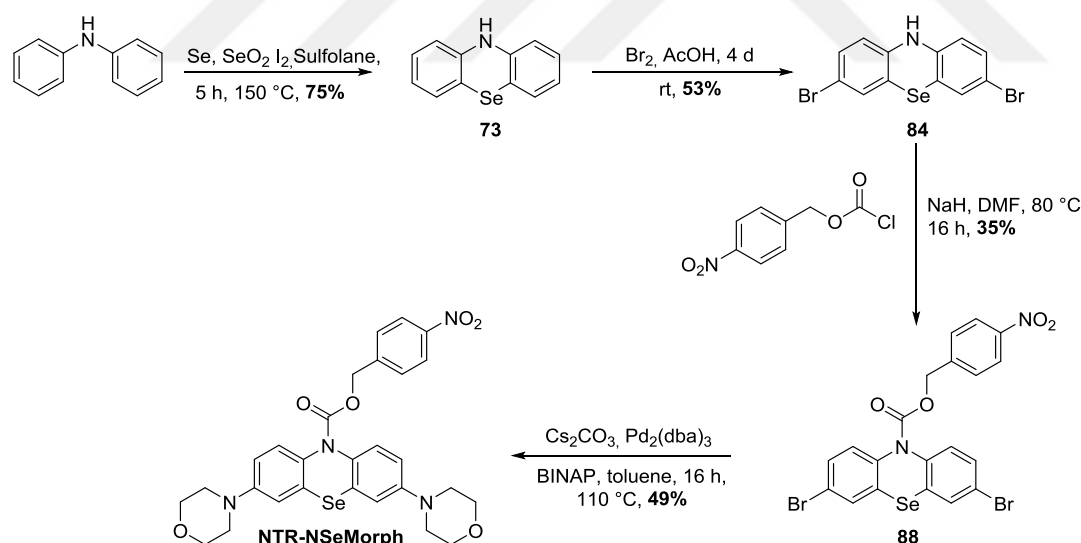
Scheme 3. 32. Another alternative route for **NTR-NSeMorph**.

Direct introduction of the *p*-nitrobenzyl group was also explored. To incorporate the *p*-nitrobenzyl moiety into compound **84**, NaH was initially utilized as a base in DMF. Subsequent to the deprotonation of amine, *p*-nitrobenzyl bromide introduced into the reaction medium and subjected to heating at 80 °C, followed by overnight stirring at that temperature. Although all starting material were consumed, none of the resulting compounds was compound **87**. Following this, K₂CO₃ was employed as a base in the following three attempts. Initially, the reaction was conducted in MeCN with a catalytic amount of KI and stirred overnight at reflux conditions. Nevertheless, the desired reaction between compound **84** and *p*-nitrobenzyl bromide failed to proceed, leading to the recovery of starting materials. To circumvent solubility issues, DMF was employed as a solvent and the reaction was replicated under the same conditions as entry 2 (Table 3.2); however, results were similar. In a further attempt, KI was added in stoichiometric amount, maintaining the remaining parameters consistent with those of entry 2. In this instance, compound **84** was consumed, yet the resulting

compound could not be identified. In the final endeavor, Cs_2CO_3 was employed as a base and catalytic amount of KI was utilized in DMF. Due to previous unsuccessful efforts, the reaction was conducted via microwave irradiation at 880 W. Regrettably, the reaction failed to proceed, resulting in the recovery of starting materials.

Table 3.2 Reaction conditions for the synthesis of compound **87**.

Entry	Base	Solvent	Reagent	Temperature (°C)	Time (h)	Result
1	NaH	DMF	-	80	16	Decomposition
2	K_2CO_3	MeCN	KI (cat)	82	16	No rxn
3	K_2CO_3	MeCN	KI (1 eq.)	82	16	Decomposition
4	K_2CO_3	DMF	KI (cat)	80	16	No rxn
5	n-BuLi	THF	-	0	2	No rxn
6	Cs_2CO_3	DMF	KI (cat)	100	1 (880 w)	No rxn



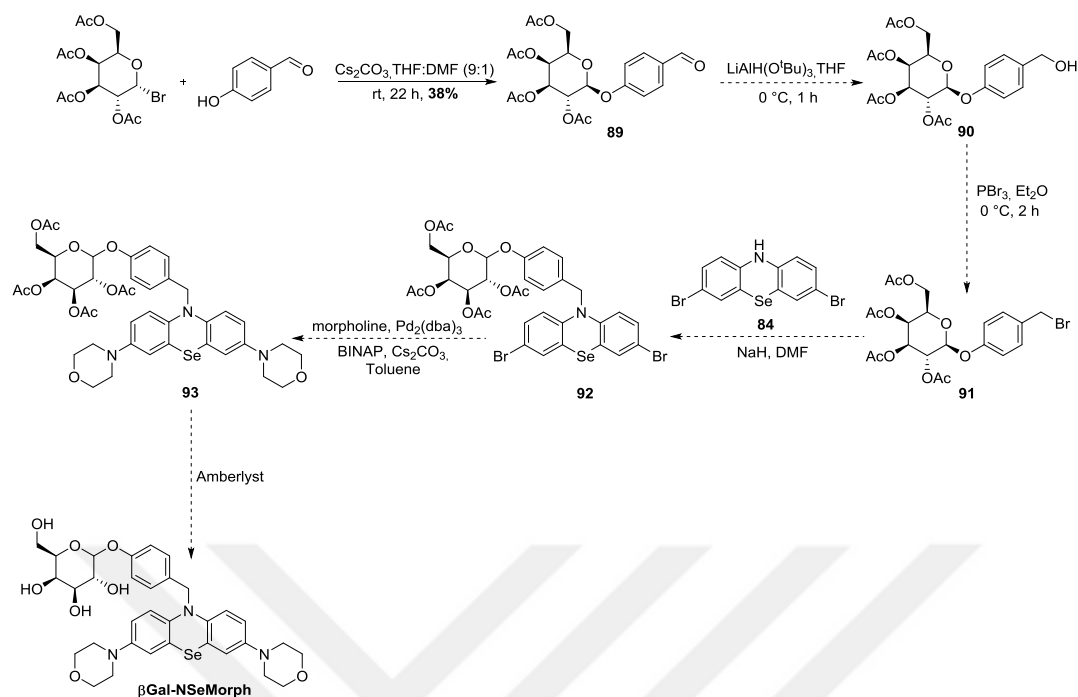
Scheme 3. 33. Final synthetic route for **NTR-NSeMorph**.

In the final endeavor for the synthesis of the target nitroreductase activatable PS, **NTR-NSeMorph**, commercially available p-nitrobenzyl chloroformate was

incorporated into the previously synthesized compound **84** with an acceptable yield. Subsequently, modification with morpholine was accomplished through a Buchwald-Hartwig amination reaction, resulting in the target activatable PS, **NTR-NSeMorph**, with a moderate yield of 49%. Prior to investigating its photophysical properties and conducting *in vitro* cell studies, the high purity of **NTR-NSeMorph** (>95%) was confirmed through reverse phase HPLC analysis. Furthermore, the synthesized PS, **NTR-NSeMorph** was characterized with ^1H , ^{13}C NMR and HRMS.

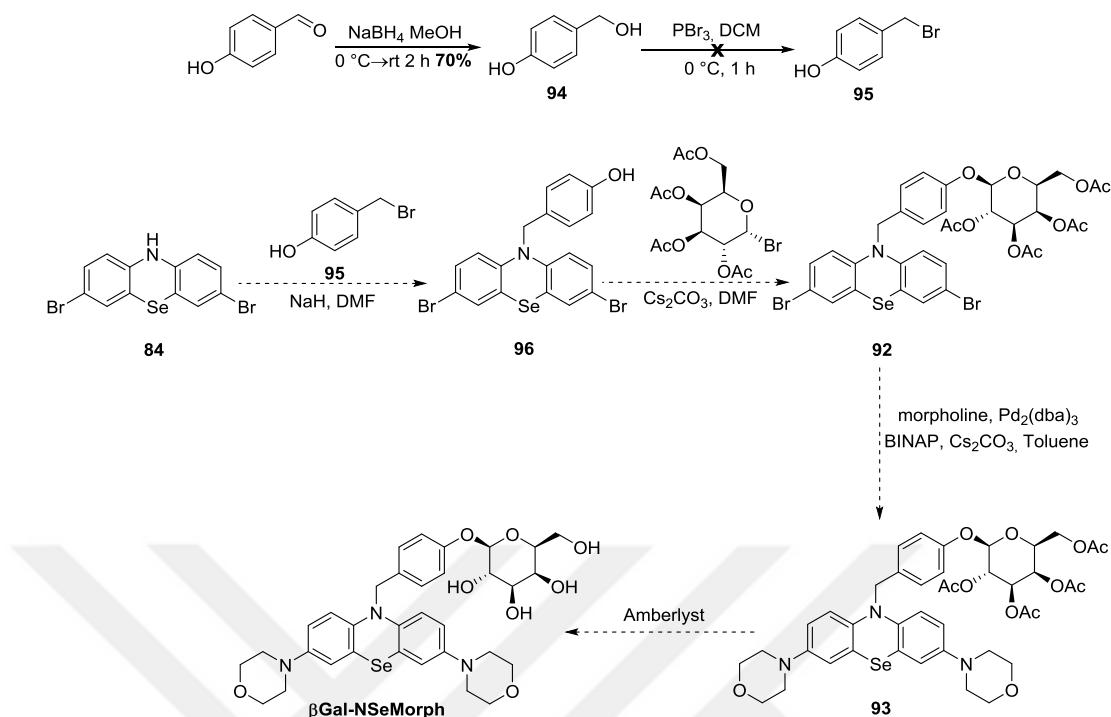
3.1.4.2.2 β -Galactosidase Activatable NSeMorph (β -Gal-NSeMorph)

To assess the activity of **NSeMorph** in another cancer cell line, a β -galactosidase activatable handle bearing **NSeMorph** was envisioned. β -galactosidase enzyme is predominantly overexpressed in ovarian cancer, but significant amounts of the enzyme are also present in gliomas. To target gliomas, β -galactosidase activatable **NSeMorph** was developed and the initial synthetic route is illustrated in Scheme 3.34.¹⁵⁹



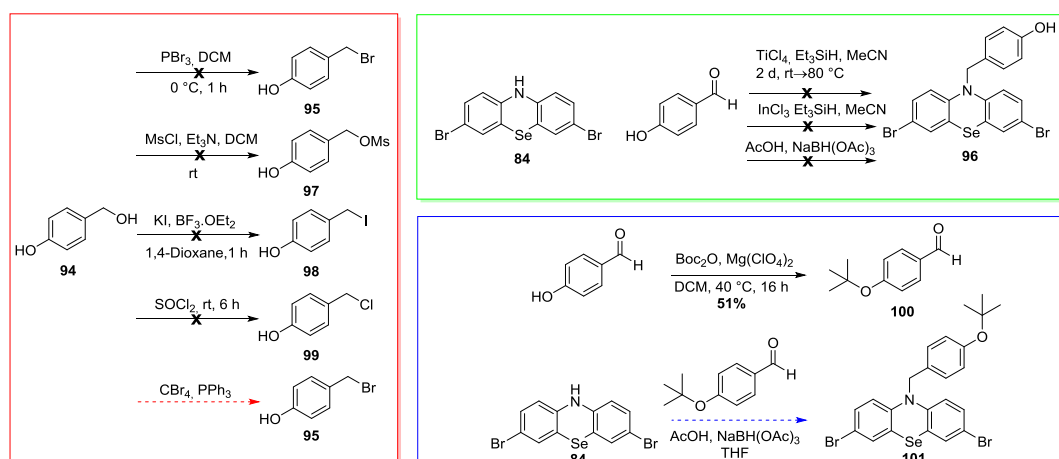
Scheme 3. 34. Initial synthetic route for **β-Gal-NSeMorph**.

For the synthesis of the **β-Gal-NSeMorph**, the strategy involved introducing a galactose derivative via traceless linker to facilitate the cleavage of galactose moiety in the presence of β -galactosidase enzyme. Initially, the objective was to incorporate the galactose derivative into a traceless linker. Subsequently, this resulting compound was to be integrated into the previously synthesized compound **84**, followed by the introduction of morpholine to this compound through Buchwald-Hartwig amination method (Scheme 3.34). To this end, commercially available acetobromo- α -D-galactose was coupled with commercially available *p*-hydroxybenzaldehyde to yield compound **89**. However, the low yield of this reaction and high cost associated with acetobromo- α -D-galactose necessitated the exploration of alternative synthetic routes for the target activatable PS, **β-Gal-NSeMorph**.



Scheme 3. 35. Alternative synthetic route for **β-Gal-NSeMorph**.

As an alternative to the previous approach, the introduction of a traceless linker to compound **84** was pursued. For this purpose, commercially available p-hydroxybenzaldehyde was reduced into the corresponding benzyl alcohol, compound **94**, with a satisfactory yield, using NaBH₄. Subsequently, efforts were made to convert benzylic alcohol into benzyl bromide to facilitate integration of traceless linker into compound **84**. Following this reaction, the sequential introduction of acetobromo-α-D-galactose and morpholine to the core was planned. Finally, the deprotection of acetyl groups was anticipated to yield the target activatable PS, **β-Gal-NSeMorph**. Several attempts were made for the synthesis of the precursor and incorporation into compound **84**, as illustrated in Scheme 3.36, albeit with no success.

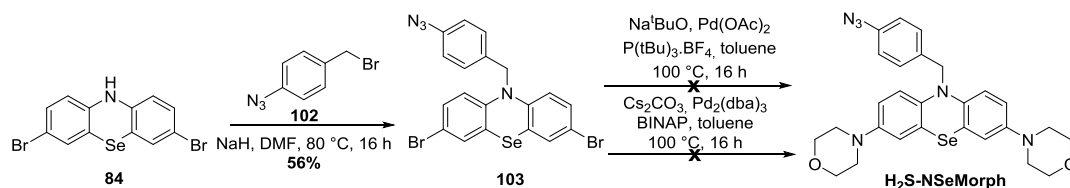


Scheme 3. 36. Synthetic efforts for the incorporation of a traceless linker into compound **84**.

Attempts to convert benzylic alcohol into a leaving group on compound **94** to facilitate an S_N2 type reaction with compound **84** were unfruitful (Scheme 3.36, red box). Future work will include Appel reaction and a protection/deprotection approach.

Another approach was also developed to incorporate a traceless linker into compound **84**, employing a reductive amination methodology (Scheme 3.36, green and blue boxes). Initially, Et_3SiH was employed as a reducing agent with catalytic amounts of TiCl_4 . The reaction was initially maintained at room temperature but the poor solubility of compound **84** in MeCN precludes the reaction. The temperature was then raised to 80 °C and reaction was allowed to proceed for an additional day. However, most of the starting materials remained and new formed compounds could not be separated from the mixture. The same reaction was also attempted with catalytic amount of InCl_3 , yielding in a similar results to the previous attempt. Furthermore, reductive amination was attempted using $\text{NaBH}(\text{OAc})_3$ under acidic conditions, but the target compound could not be isolated. Lastly, the phenolic hydroxyl group was protected with tertbutyl moiety to mitigate side reactions in the reductive amination reactions. This protected compound, **100**, will be utilized with compound **84** with the aid of $\text{NaBH}(\text{OAc})_3$ to obtain the target compound **101**.

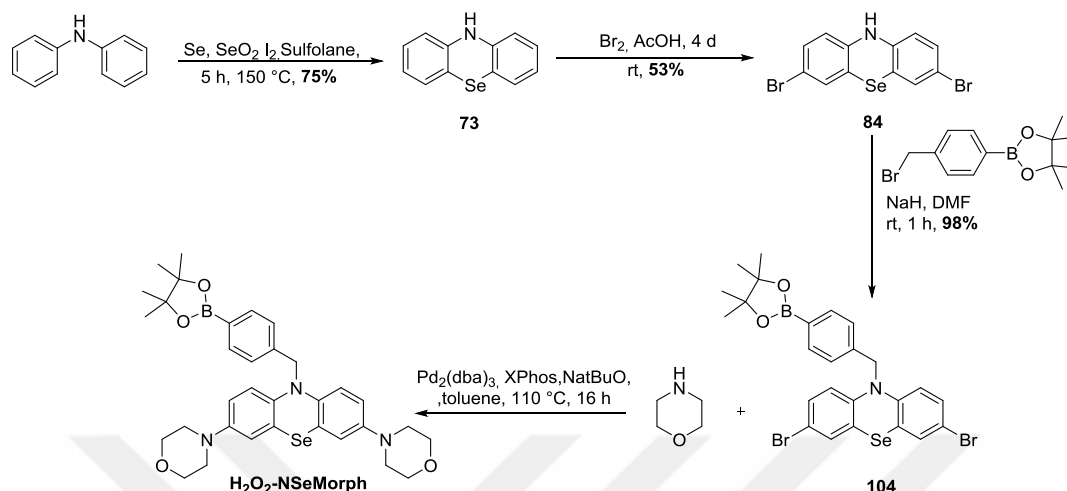
3.1.4.2.3 H₂S Activatable NSeMorph (H₂S -NSeMorph)



Scheme 3. 37. Synthetic route for **H₂S-NSeMorph**.

For the synthesis of **H₂S-NSeMorph**, proposed synthetic pathway is illustrated in Scheme 3.37.¹⁷⁴ Compound **102**, derived from was commercially available p-aminobenzyl alcohol through two steps synthesis by the Kolenen group, was incorporated into the previously synthesized compound **84**, with a moderate yield of 56%. Subsequent efforts focused on the introduction of a morpholine moiety into the resulting compound **103** via the Buchwald-Hartwig amination method, employing various catalyst-ligand-base systems. Initially, the reaction was executed using Pd(OAc)₂, P(tBu)₃.BF₄ and Na^tBuO trio in toluene. However, after 16 hours, all starting materials was remained unreacted. Consequently, due to the absence of the desired product in the reaction mixture, the reaction was terminated and the starting materials were recovered. Following this, the reaction was replicated using Pd₂(dba)₃, BINAP, Cs₂CO₃ trio. Unfortunately, this endeavor yielded similar results to the former attempt. To obtain H₂S activatable **NSeMorph**, **H₂S-NSeMorph**, alternative Buchwald-Hartwig kits will be utilized. Additionally, adjustments in temperature and reaction duration will be undertaken to reveal the optimal conditions for the synthesis of the target activatable PS, **H₂S-NSeMorph**.

3.1.4.2.4 H₂O₂ Activatable NSeMorph (H₂O₂ -NSeMorph)



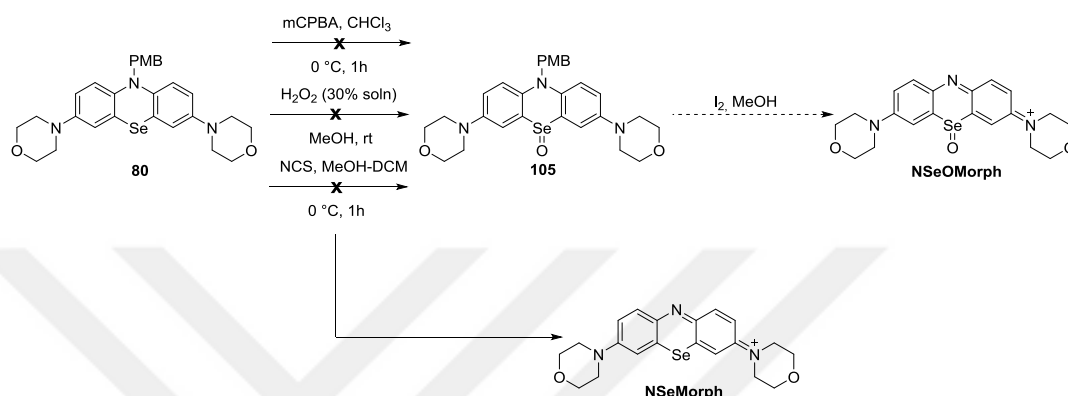
Scheme 3. 38. Synthetic route for **H₂O₂ -NSeMorph**.

For the synthesis of H₂O₂ activatable NSeMorph based PS, **H₂O₂ -NSeMorph** proposed synthetic pathway is illustrated in Scheme 3.38.¹⁷⁵ Initially, commercially available 4-bromomethylphenylboronic acid pinacol ester was incorporated into previously synthesized compound **84**, yielding a commendable 98%. Subsequently, the introduction of a morpholine moiety to compound **104** was affected through Buchwald-Hartwig amination method, culminating in the target H₂O₂ activatable PS, **H₂O₂ -NSeMorph**. Rigorous purification endeavors are currently underway to attain purity over 95%, a prerequisite preceding the investigation of its photophysical properties and *in vitro* cell studies.

3.1.4.3 Synthesis of Morpholine and Selenium Oxide Bearing Phenoselenazine Based PS (NSeOMorph)

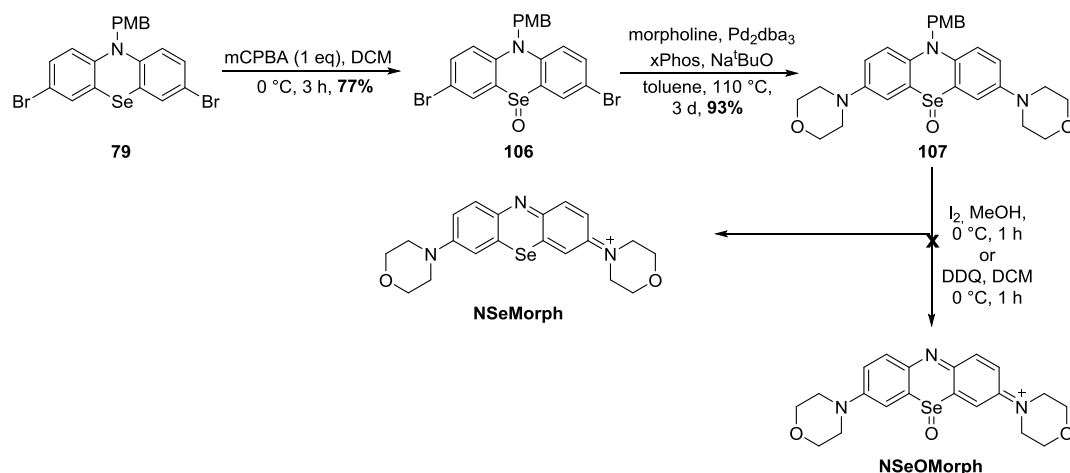
To explore the impact of selenium atom oxidation on **NSeMorph**, the derivative **NSeOMorph** was conceived and extensive efforts were undertaken to synthesize the target PS. The oxidation of the tellurium atom led to a notable bathochromic shift accompanied by a reduction in the singlet oxygen quantum yield in telluroxide

analogues of tellurorhodamine. To validate this concept for selenium-based PSs, a selenium oxide derivative of **NSeMorph** was designed and initially pursued via a two-step synthesis involving oxidation of compound **80** and subsequent cleavage of protective group to yield **NSeOMorph**. The various synthesis strategies employed for **NSeOMorph**, as delineated in Scheme 3.39, are elaborated upon.¹²⁸



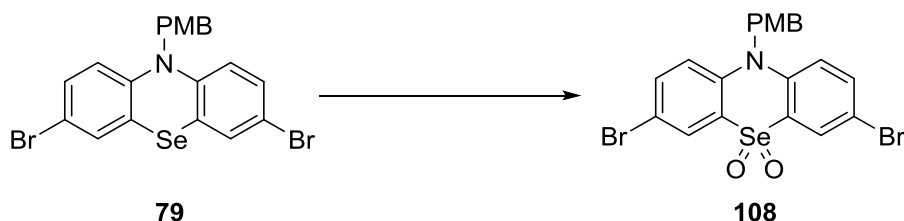
Scheme 3. 39. Initial synthetic route for **NSeOMorph**.

For the synthesis of **NSeOMorph**, initially proposed pathway involves the oxidation of the previously synthesized compound **80**, followed by oxidative cleavage of PMB protective group to yield the target PS, **NSeOMorph**. In the initial attempt, compound **80** was subjected to oxidation reaction using m-CPBA at 0 °C. After one hour, the reaction was terminated due to the consumption of all the starting materials. However, the obtained product was characterized as **NSeMorph** (Scheme 3.39). Following this, H₂O₂ and N-chlorosuccinimide (NCS) were employed as an oxidation system. Nevertheless, in each instance, **NSeMorph** was obtained instead of compound **105**.



Scheme 3. 40. Alternative synthetic route for **NSeOMorph**.

For the synthesis of **NSeOMorph**, an alternative synthetic pathway was devised in response to encountered challenges. In this refined approach, the selenium atom on previously synthesized compound **79** underwent successful oxidation with the aid of m-CPBA. Subsequently, resulting compound **106** was subjected to Buchwald-Hartwig amination reaction with a morpholine moiety, resulting in the synthesis of the target compound **107** with a high yield. Following this, attempts were made at the oxidative cleavage of the PMB protective group. Initially, molecular iodine was employed for this purpose. However, the desired product, **NSeOMorph** could not be attained. Subsequent attempt using DDQ similarly proved unsuccessful. In both instances, **NSeMorph** was the obtained product.



Scheme 3. 41. Reaction scheme of compound **108**.

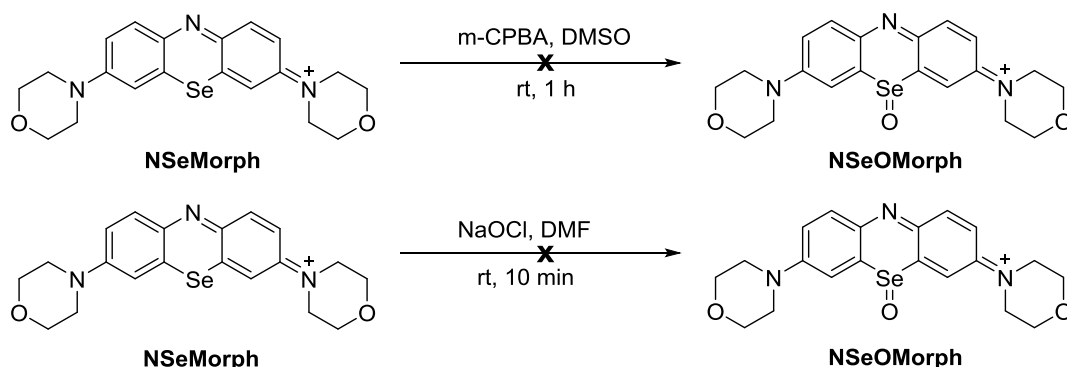
Given the challenges encountered in achieving successful oxidative cleavage, a strategy involving the double oxidation of the selenium atom was devised to ensure retention of at least one oxide moiety on the selenium atom following “oxidative”

cleavage. To pursue this strategy, the synthesis of compound **108** was undertaken, as depicted in Scheme 3.41. The endeavors made towards the synthesis of compound **108** are listed in Table 3.3.

Table 3.3 List of endeavors for the synthesis of compound **108**.

Entry	Reagent	Temperature (°C)	Time (h)	Result
1	m-CPBA (3 eq.)	r.t.	16	Compound 106
2	m-CPBA (4 eq.)	0	4	Complex mix.
3	m-CPBA (4 eq.)	r.t	4	Complex mix.
4	H ₂ O ₂ (15 eq.)	90	16	Decomposition

The synthesis of compound **108** underwent several iterations. Initially, m-CPBA served as the oxidizing agent. Compound **79** was subjected to 3 eq. m-CPBA and was stirred for 16 hours at ambient temperature. However, only the mono-oxidized derivative, compound **106** was successfully isolated. Subsequent adjustments were made by increasing the m-CPBA equivalence to 4; as well as increasing the temperature however, two fold oxidation did not occur. Following these attempts, H₂O₂ was employed. The reaction mixture was stirred overnight at 90 °C. Unfortunately, the desired compound **108** remained elusive.

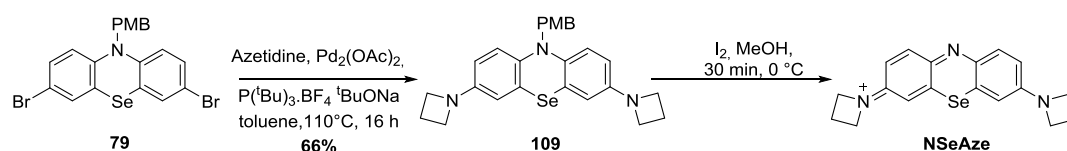


Scheme 3. 42. Alternative synthetic approach for the synthesis of **NSeOMorph**.

For the synthesis of **NSeOMorph**, given the unsuccessful outcomes of preceding methodologies, the oxidation of **NSeMorph** to **NSeOMorph** was pursued. Initially, *m*-CPBA was employed as the oxidizing agent, yet the reaction failed to proceed, resulting in recovery of starting material. Following this, NaOCl was utilized. However, after a duration of 10 minutes, the reaction resulted in C-Se bond cleavage. Subsequent endeavors will entail meticulous adjustments to the reaction parameters aimed at establishing the requisite optimal conditions for the successful synthesis of **NSeOMorph**.

3.1.4.4 Synthesis of Azetidine Bearing Phenoselenazine Based PS (**NSeAze**)

The introduction of azetidine to rhodamine dyes stands as a commonly utilized methodology for fine tuning of the fluorescence characteristics of the fluorophore. The substitution of cyclic amine moieties instead of acyclic analogues typically induces a bathochromic shift in both absorption and emission maxima accompanied by enhanced photostability and intensified emission owing to prolonged lifetime of the singlet excited state. Despite these advantageous, the utilization of azetidine in the realm of photosensitizer development remains underexplored. Recognizing the potential of azetidine modification, a novel class of phenoselenazine-based PSs incorporating azetidine, denoted as **NSeAze**, was devised to explore the impact of azetidine modification on both photophysical properties and *in vitro* efficacy of the resultant PS. The proposed synthetic pathway for **NSeAze**, is illustrated in Scheme 3.43.¹⁶⁷



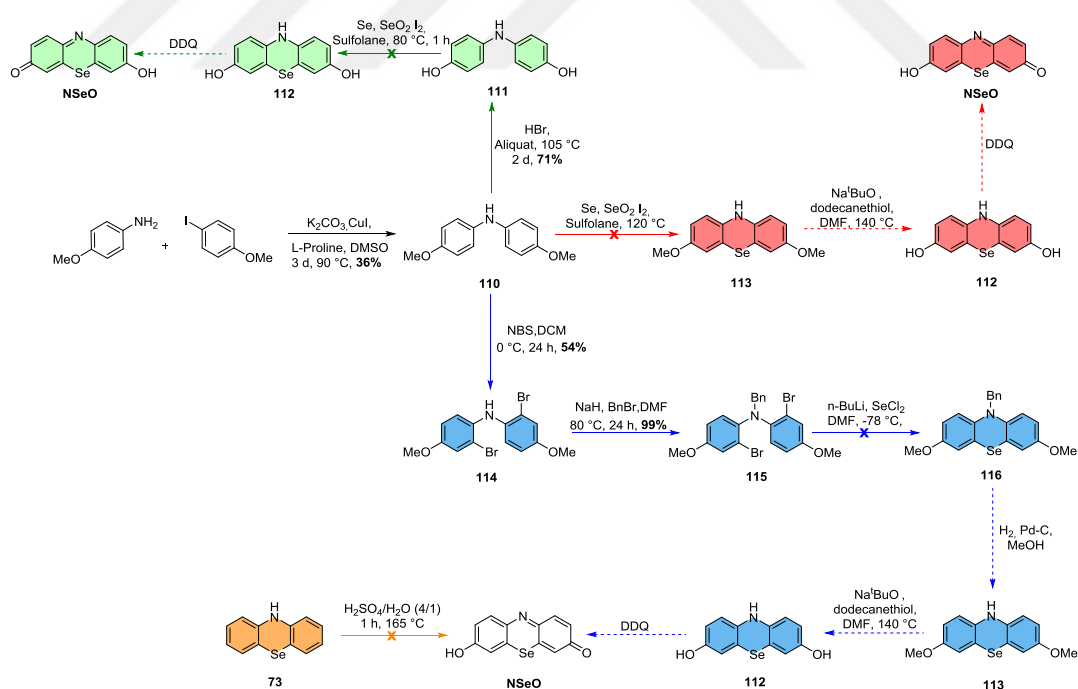
Scheme 3. 43. Synthetic route for **NSeAze**.

Previously synthesized compound **79** underwent a Buchwald-Hartwig amination reaction with azetidine, employing the similar conditions to those utilized for

morpholine incorporation. This process resulted in compound **109** with a satisfactory yield of 66%. Subsequently, compound **109** subjected to oxidative cleavage reaction facilitated by molecular iodine as the oxidizing agent, leading to the successful synthesis of the target PS, **NSeAze**. Prior to investigating its photophysical properties and conducting *in vitro* cell studies, **NSeAze** was also purified using reverse phase HPLC to achieve high purity over 95% before investigating. **NSeAze**, was characterized with ^1H , ^{13}C NMR and HRMS.

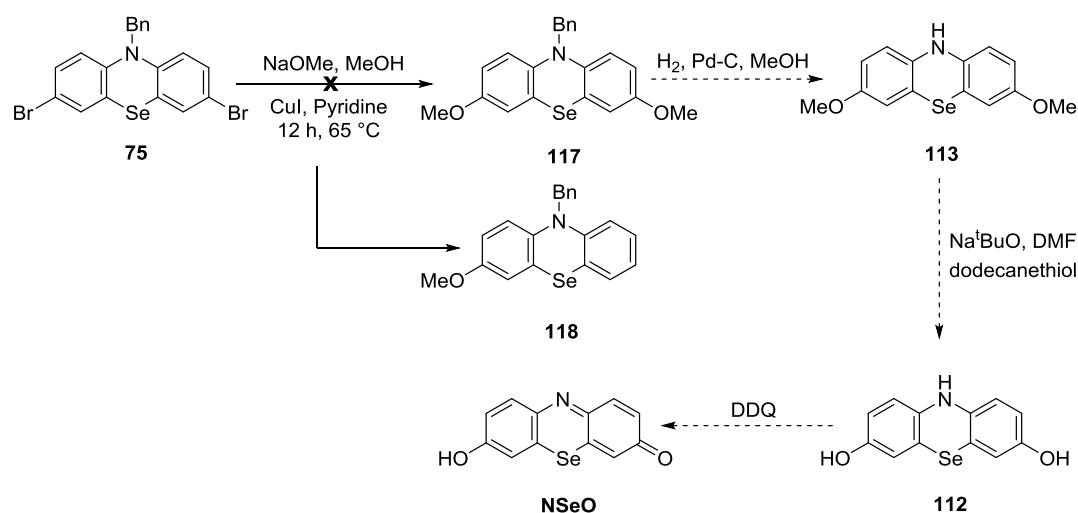
3.1.4.5 Synthesis of Oxygen Bearing Phenoselenazine Based PS (NSeO)

To facilitate integration of a targeting group into the phenoselenazine core, an oxygen bearing analogue of **NSeMorph** has emerged. Numerous synthetic approaches were devised for the synthesis of **NSeO**. These approaches are briefly outlined in Scheme 3.44, with each pathway depicted using distinct colors to enhance clarity.



Scheme 3. 44. Various synthetic routes for **NSeO**, differentiated with different colors (black, red, blue, green, orange)

For the synthesis of **NSeO**, compound **110** was initially synthesized from commercially available *p*-iodoanisole and *p*-anisidine via the Buchwald-Hartwig reaction. This compound served as the starting compound both three pathways (green, red, blue). In the green pathway, methyl cleavage was performed utilizing HBr in aliquot, resulting in the desired product, compound **111**. Subsequently, attempts were made to introduce a selenium atom to compound **111** using previously established system. However, the target compound **112** could not be obtained (green pathway). A similar approach was employed for the methoxy bearing derivative, but the selenium bearing derivative, compound **113** could not be observed (red pathway). In the blue pathway, compound **110** was brominated with NBS, resulting in compound **114**. Compound **114** underwent a subsequent protection reaction with benzyl bromide, yielding compound **115**. Following this, compound **115** underwent lithium halogen exchange reaction with *n*-BuLi, followed by addition of freshly prepared SeCl₂. Unfortunately, the formation of a complex mixture was observed and the desired product could not be isolated. Alternatively, previously synthesized compound **73** vigorously refluxed with an aqueous H₂SO₄ solution in a one-step reaction to obtain the target PS, **NSeO**.¹⁷⁶ However, the target PS could not be isolated from the mixture (orange pathway).

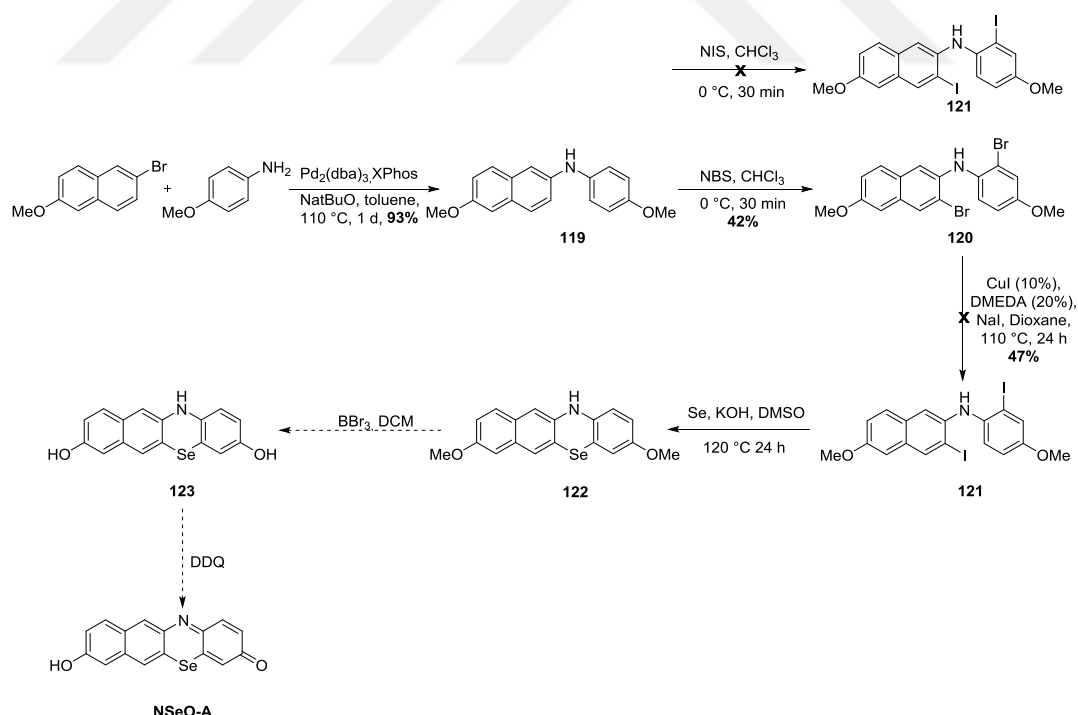


Scheme 3. 45. Alternative synthetic route for **NSeO**.

In addition to the aforementioned approaches, a new methodology has been developed for the synthesis of **NSeO** (Scheme 3.45). Initially, previously synthesized compound **75** was subjected to a copper-catalyzed ipso-substitution reaction to yield compound **117**. However, only mono substitution was observed in this instance with a loss of the bromine atom. To realize the synthesis of the desired PS, **NSeO**, the reaction parameters will be adjusted to achieve optimal conditions for the di-substitution with methoxide.

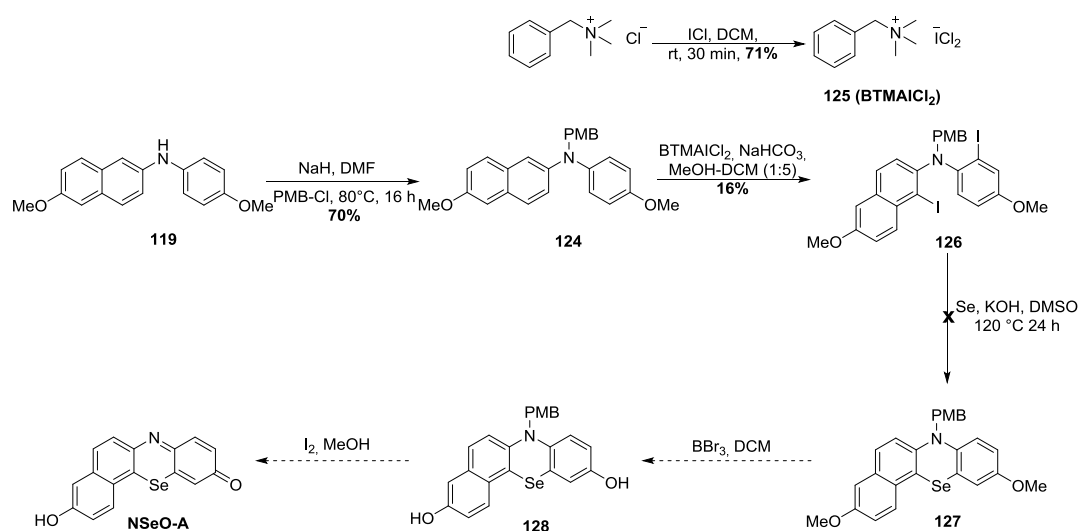
3.1.4.6 Synthesis of Oxygen Bearing Annulated Phenoselenazine Based PS (**NSeO-A**)

To shift the absorption and emission maxima of **NSeO** (Calc. λ_{max} = 579 nm, Figure 2.1), its annulated derivative has been designed through extension of conjugation. For the synthesis of **NSeO-A**, initially devised synthetic pathway is illustrated in Scheme 3.46.



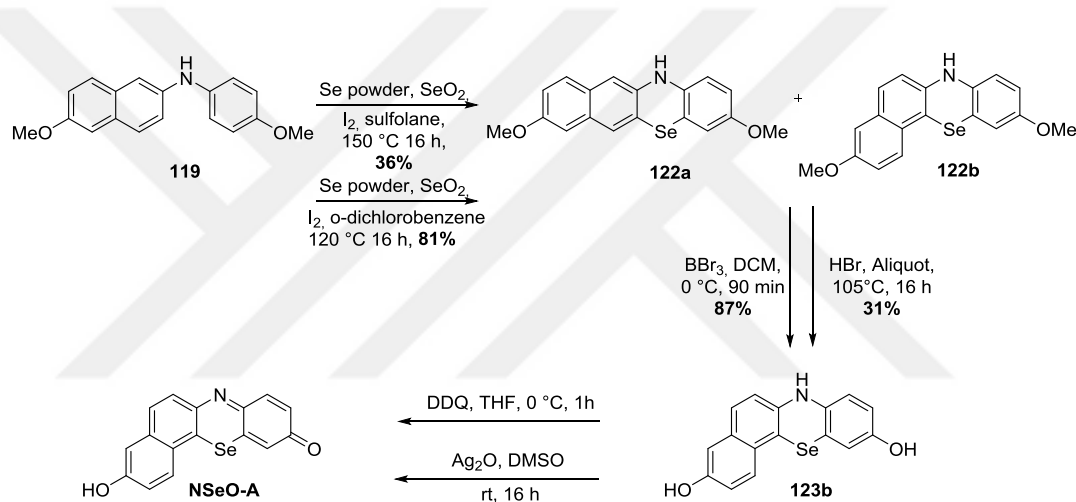
Scheme 3. 46. Initial synthetic route for **NSeO-A**.

For the synthesis of **NSeO-A**, initially, ring closure with selenium atom was attempted using Se powder, base and DMSO combination. According to literature this reaction proceeds only in the presence of iodine atoms.¹⁷⁷ To achieve **NSeO-A**, a commercially available 2-bromo-6-methoxynaphthalene and p-anisidine were subjected to a Buchwald-Hartwig reaction to yield compound **119** with a notably high yield of 93%. Subsequently, bromination of compound **119** was attempted with NBS, resulting in compound **120** with a moderate yield of 42% and bromination of other possible position was not observed. Compound **120** was characterized through NMR analysis. According to literature, introduction of halogen or lithiation reactions of 2-substituted naphthalene has varying regioselectivity depending upon the specific substrate, directing group, base and solvent selection.¹⁷⁸ Simultaneously, compound **119** was subjected to iodination reaction with NIS but the desired compound **121** could not be obtained. Therefore, compound **120** underwent aromatic Finkelstein reaction to yield its iodinated analogue. Following the successful synthesis of compound **121**, a ring closure reaction with selenium atom was attempted. Despite the consumption of all compound **121**, the resulting products did not include the desired product and the byproducts could not be characterized.



Scheme 3. 47. Alternative synthetic route for **NSeO-A**.

Due to the challenges encountered in the ring closure reaction involving selenium atom, efforts were made to mitigate the formation of byproducts. To this end, the amine moiety on the previously synthesized compound **119** was protected with a PMB group, resulting in compound **124** with an acceptable yield. Subsequently, compound **124** underwent iodination reaction using benzyltrimethylammonium dichloriodate (**125**, BTMAICl₂) which was freshly synthesized from commercially available benzyltrimethylammonium chloride using ICl. Although compound **126** was obtained with low yield, it was utilized in subsequent reaction without further optimization. Nonetheless, similar to the previous approach, the target compound could not be obtained.



Scheme 3. 48. Final synthetic route for **NSeO-A**.

For the synthesis of **NSeO-A**, a new approach has been devised, similar to the strategy employed for **NSeMorph**. The initial step involved the planned ring closure with a selenium atom, as illustrated in Scheme 3.48. Initially, the incorporation of selenium atom into the previously synthesized compound **119** was achieved using sulfolane as a solvent at 150 °C, yielding a diastereomeric mixture of compound **122a** and **122b** with a yield of 36%. Before proceeding with subsequent reactions, efforts were made to optimize the reaction yield by utilizing o-dichlorobenzene as a solvent at 120 °C, resulting in enhanced reaction yield of 81%. Subsequently, the methyl groups of mixture were cleaved using HBr in aliquot. Surprisingly, only **123b**, the product of **122b**, was isolated from the reaction mixture with a yield of

31%. To further optimize the reaction yield, BBr₃ was employed, leading to an increased yield of 87%. Finally, compound **123b** oxidized using various oxidant including DDQ and Ag₂O, to yield the target PS, **NSeO-A** according to HRMS and NMR analyses of the crude product. Ongoing efforts are focused on purifying **NSeO-A** to obtain the target PS in high purity over 95%.

3.1.5 Acridone Based Photosensitizers

To synthesize series of NIR-absorbing, novel acridone based imaging agents and photosensitizers were developed. This sections will elucidate the attempts made in their synthesis.

3.1.5.1 Synthesis of Morpholine Bearing Acridone Based imaging Agent and PS (NCMorph)

Acridone-based fluorophores are extensively utilized in bioimaging applications within the visible/red regions.^{179,180} Nevertheless, their interrupted conjugation constrains their utility in deeper tissues.

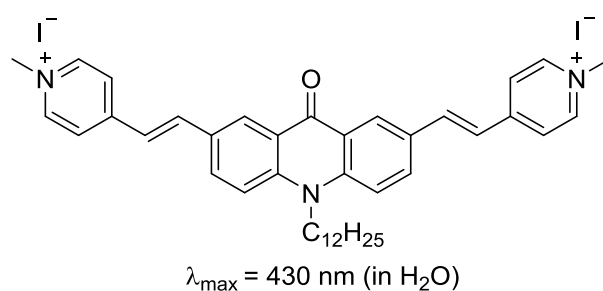
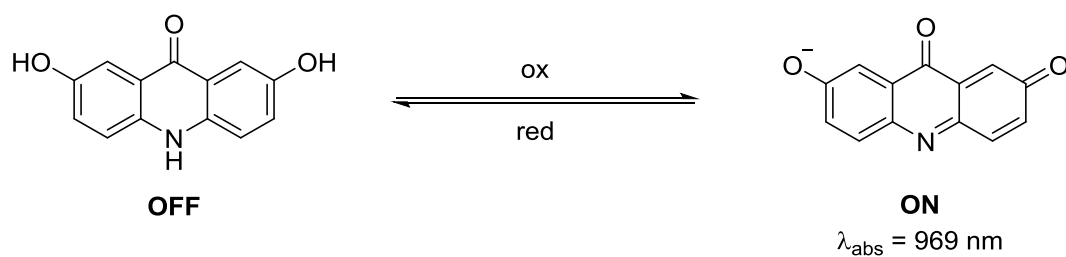


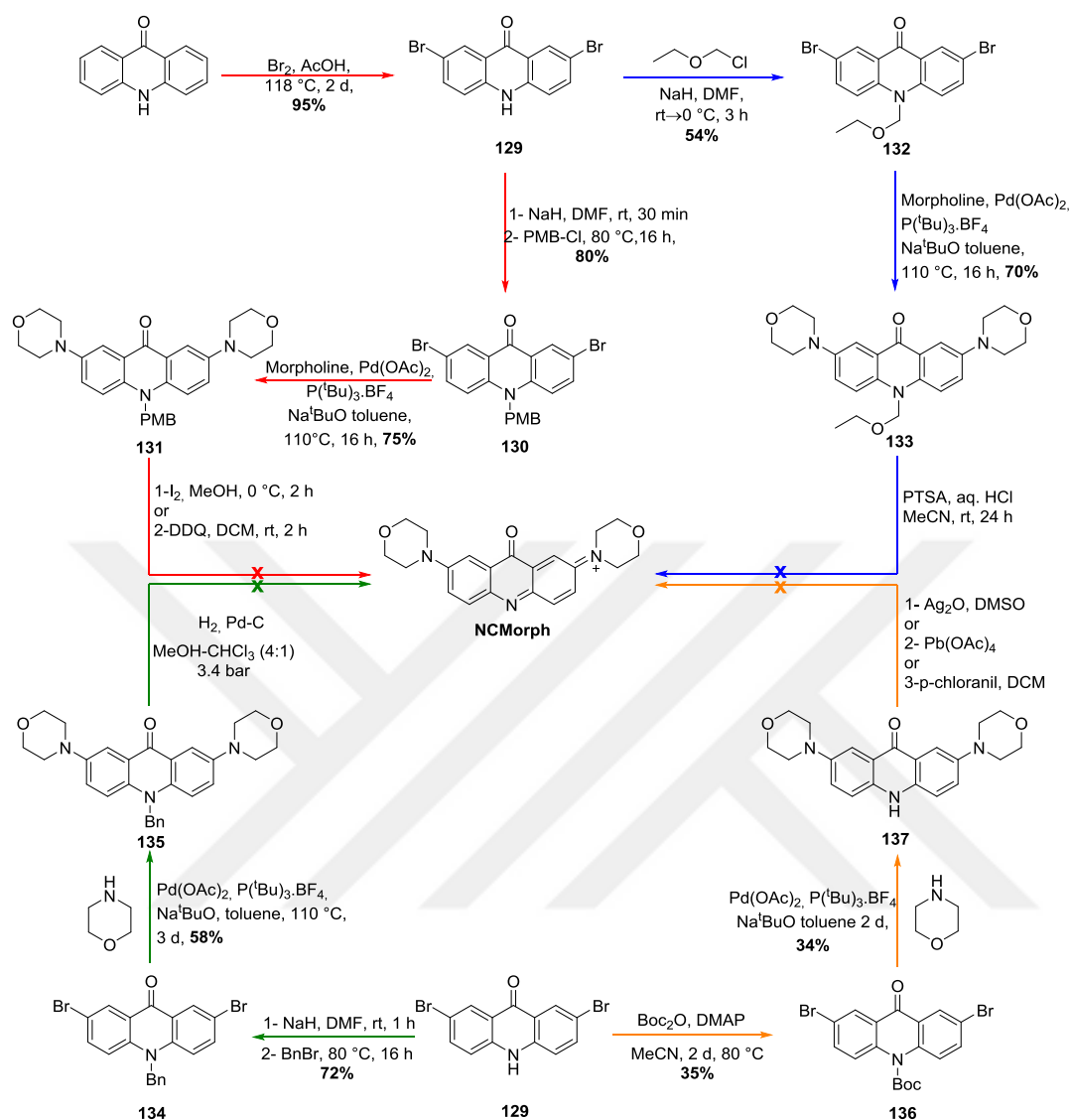
Figure 3.3. Structure of acridone based fluorophore and its absorption maximum in H₂O.¹⁸⁰

Additionally, their application in PDT applications has yet to be explored. The prospect of their effectiveness in deep tissue imaging and their potential for *in vitro/in vivo* PDT applications motivates the development of NIR absorbing and emitting acridone-based imaging agents and photosensitizers. Theoretical

calculations were also conducted to validate the hypothesis with the oxygen bearing analogue. however, the straightforward synthetic route of amine bearing analogues direct our attention toward the realization of **NCMorph**.



Scheme 3. 49. OFF-ON state of **NCO** and its theoretical on state absorption maximum.



Scheme 3. 50. Synthetic routes for **NCMorph**.

For the synthesis of **NCMorph**, several methodologies employing diverse protective groups have been developed and are illustrated in Scheme 3.50. Initially, commercially available acridone underwent bromination reaction using molecular bromine, affording compound **129** with a remarkable yield of 95%. Subsequently, compound **129** was engaged in various pathways indicated by distinct colors (red, blue, green and orange).

In the red pathway, the amine moiety of compound **129** was protected with PMB protective group with a notable yield of 80%. Thereafter, morpholine was

incorporated into compound **130** via a Buchwald-Hartwig amination reaction, leading to formation of compound **131**. Finally, compound **131** was subjected to oxidative cleavage of the PMB protective group to furnish the target dye, **NCMorph**. Initially molecular iodine was employed for this purpose; unfortunately, the reaction failed to proceed and compound **131** was recovered. In the second attempt, DDQ was utilized as an oxidizing agent, yielded analogous outcomes.

In addition to the PMB protection, ethoxymethyl ester protection was explored. Ethoxymethyl chloride was incorporated to compound **129** with a moderate yield of 54%. Subsequently, compound **132** underwent a Buchwald-Hartwig reaction with morpholine, yielding compound **133** with a 70% yield. In the blue pathway's final endeavor, the removal of ethoxymethyl group was attempted via catalytic PTSA in an aqueous HCl solution, followed by oxidation to yield the target dye, **NCMorph**. Regrettably, this approach also failed to proceed and compound **133** was recovered.

In the green pathway, compound **129** was protected with a benzyl protective group, affording a product with a satisfactory yield of 72%. The resulting compound **134** was then coupled with morpholine via the Buchwald-Hartwig method, yielded compound **135** with a moderate yield of 58%. Subsequently, efforts to remove the benzyl group using H₂ gas in the presence of Pd-C were undertaken. Despite initial attempts with 1 bar of H₂ gas and subsequent escalation to 3.4 bars due to the lack of consumption of compound **135**, the benzyl protective group remained intact, resulting in the recovery of compound **135**.

In the final attempt, a relatively mild protective group, the Boc protective group, was incorporated into compound **129** with a quite low yield. Subsequently, a Buchwald-Hartwig coupling method was utilized to incorporate morpholine moieties into compound **136**. Although, morpholine moieties were successfully incorporated into compound **136**, cleavage of the Boc protective group was observed. Unfortunately, despite attempts employing various utilization of various oxidizing agents including Ag₂O, Pb(OAc)₄, p-chloranil, oxidation of the resulting compound could not be achieved.

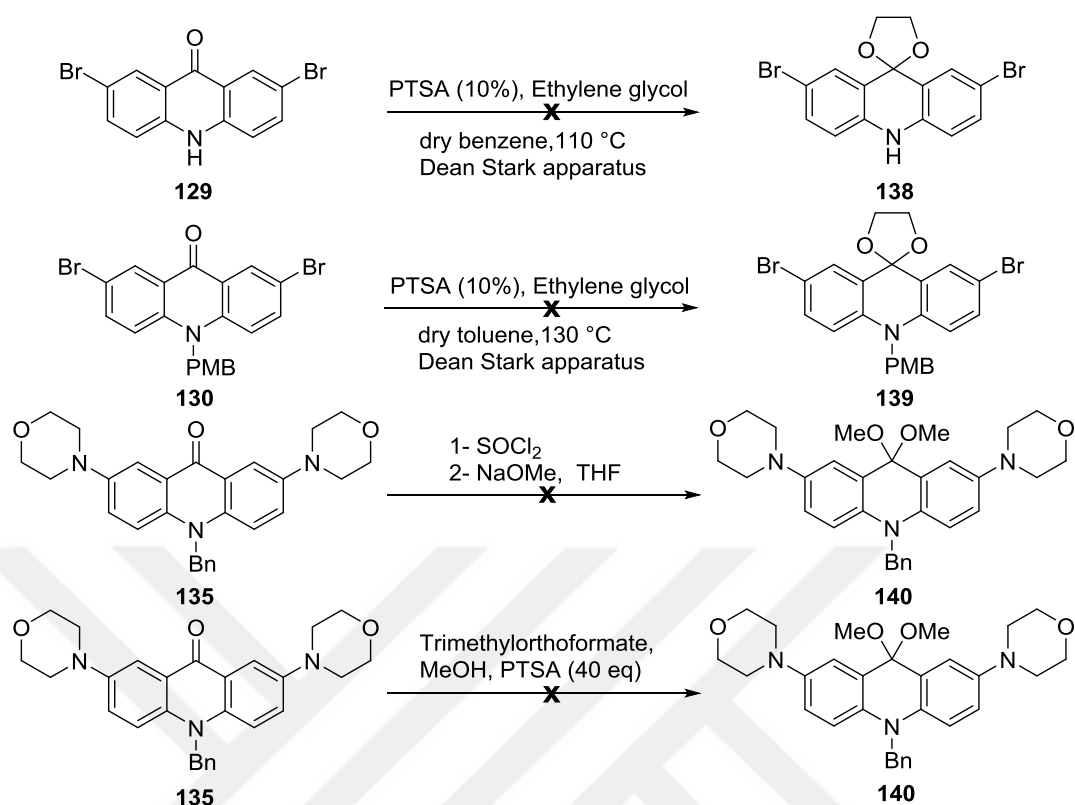
It is well documented in the literature that, the substitution of the central oxygen atom within the xanthene core with other atoms bearing electron withdrawing ability such as $\text{PPh}=\text{O}$, results in a reduction of the LUMO energy level. Consequently, this reduction narrows the band gap between the HOMO and LUMO energy levels, thereby facilitating a bathochromic shift in both absorption and emission maxima.¹⁵⁴ Specifically, the introduction of a carbonyl moiety at the 10th position of xanthene scaffold is posited to induce a remarkable bathochromic shift. This conjecture was rigorously examined through with theoretical calculations. Notably the oxygen bearing analogue of **NCMorph** demonstrated an absorption maximum at 969 nm whereas its central oxygen bearing analogue, resorufin exhibited the absorption maximum at 572 nm, thereby validating the hypothesis.



Scheme 3. 51. Resonance structure of compound **137**.

Despite extensive efforts, the synthesis of the final product remained elusive. The inability to achieve oxidation can be attributed to the diminished electron donating capability of the amine group, stemming from its proximity to the highly electron withdrawing carbonyl moiety. The resonance structure of compound **137** is illustrated in Scheme 3.51.

In response to this challenge, various methodologies were conceived focusing on the protection of the carbonyl group. The proposed synthetic pathways for carbonyl group protection in **NCMorph** are elucidated in Scheme 3.52.



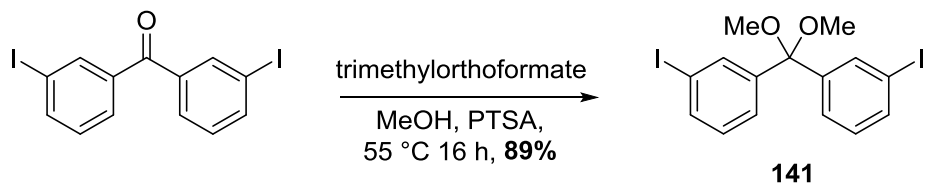
Scheme 3. 52. Synthetic efforts for the protection of carbonyl moiety.

For the protection of the carbonyl moiety, an initial attempt involved the reaction of compounds **129** and **130** with ethylene glycol in the presence of a catalytic amount of PTSA with the aid of Dean Stark apparatus to enhance reaction efficiency. Regrettably, the target compound **138** and **139** could not be obtained.

In an alternative approach, acetal conversion of the carbonyl moiety in compound **135** to a dimethoxy acetal using SOCl_2 , followed by subsequent addition of NaOMe was pursued. However, this reaction also failed to proceed, resulting in the recovery of starting material. Following this, trimethylorthoformate was employed in the carbonyl protection reaction with excess PTSA. Nevertheless, this attempt also proved unsuccessful, yielding similar outcomes to the previous results.

These unsuccessful attempts can be attributed to the presence of the resonance structure, as illustrated in scheme 3.51. To ascertain the validity of this hypothesis, bis(3-iodophenyl)methanone was subjected to acetalization reaction with

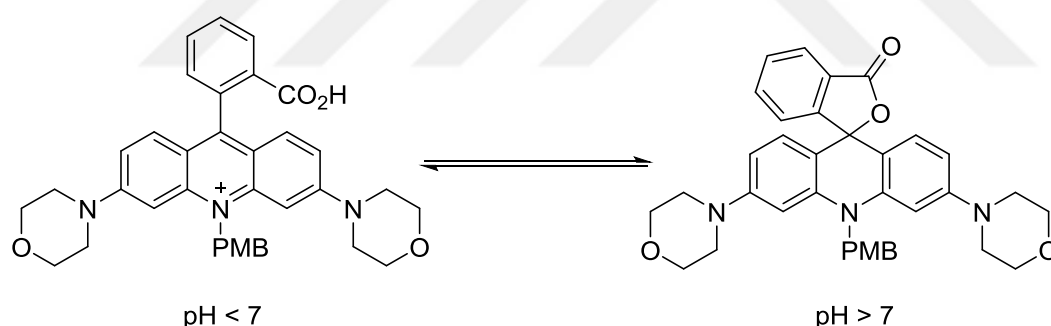
trimethylorthoformate, leading to synthesis of the dimethoxy acetal with a notable yield of 89% (Scheme 3.53).



Scheme 3. 53. Synthesis of compound **136**.

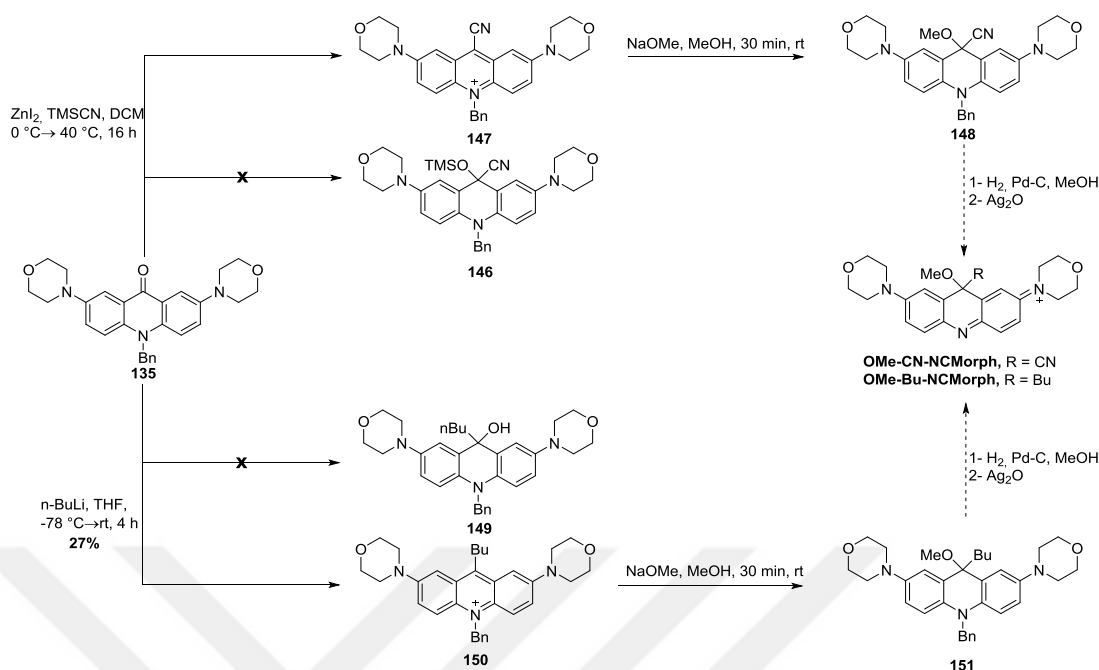
To mitigate the effect of extensive electron withdrawing ability of carbonyl moiety, new approaches has emerged, which involved introduction of various strong nucleophiles via addition reaction to the carbonyl group. Initial approach for the synthesis of acridone based dyes and PSs is illustrated in Scheme 3.54.

LaCl₃.2LiCl to the mixture prior to the addition of compound **131** at -78 °C. After the addition of compound **131**, the mixture was stirred at room temperature for 4 hours. Following the incorporation of compound **54** into compound **131**, tertbutyl protective group was removed using TFA to yield the target compound **142**. However, an unexpected ring opening was observed under acidic conditions (Scheme 3.55). To mitigate this, the crude product was treated with mild base for two hours and compound **142** was obtained with a yield of 29%. Subsequently, the PMB protective group was cleaved using molecular iodine, resulting in the desired dye, **2-COOH-NCMorph**. Efforts to purify the target dye are underway. Additionally, attempts were made to synthesize **2,5-CH₂OH-NCMorph** using the same synthetic approach employed in the synthesis of **2-COOH-NCMorph**. Upon completion of the purification efforts, the photophysical properties of these dyes will be investigated and cell culture studies will be performed. Furthermore, heavy atom bearing derivatives of these imaging dyes have been designed and synthetic efforts toward the development of these PS are ongoing.



Scheme 3. 55. pH dependency of compound **142**.

Compound **142** exhibits different colors in response to changes in pH and oxidative/reductive conditions of the environment, attributable to shifts in the absorption maximum due to resonance preferences. This distinctive characteristic of compound **142** makes it suitable for use in dual selective imaging applications. Further investigation into photophysical properties and *in vitro* response of compound **142** is envisioned.



Scheme 3. 56. Alternative synthetic route for **NCMorph** derivatives.

In addition to the preceding approach, a strategy involving the incorporation of diverse functional groups into the carbonyl moiety within the compound **135** was devised. These functional groups not only have the capability to inhibit the electron withdrawing effect of the carbonyl group but also hold the potential to induce red or blue shifts in absorption and emission maxima relative to **NCMorph**. The synthesis of novel **NCMorph** derivatives intended for bioimaging studies coupled with an exploration of the influence of different functional groups on photophysical properties, necessitates the deliberate incorporation of various electron withdrawing and donating groups into the **NCMorph** core. Initial objective was the integration of -OTMS and -CN moieties. TMSCN was selected for this purpose, in the presence of catalytic ZnI_2 , to synthesize the target compound **146**. However, the desired compound could not be obtained, resulting in the characterization of the crude product as compound **147**. Drawing upon insights into the behavior of compound **142** across diverse pH ranges, compound **147** underwent treatment with NaOMe,

yielding compound **148**. Current efforts are focused on purifying compounds **147** and **148**.

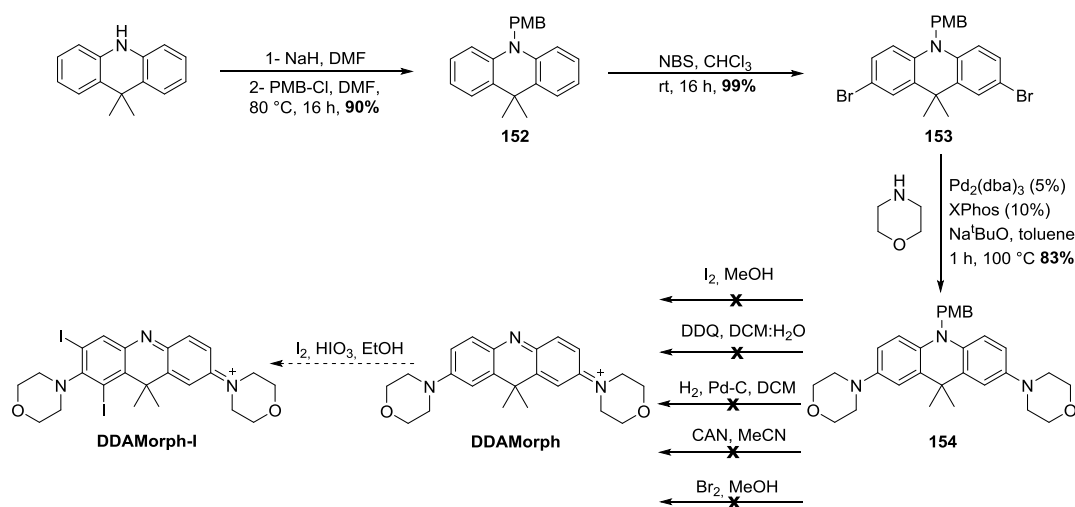
Subsequently, compound **135** underwent treatment with n-BuLi at 78 °C with the anticipation of yielding compound **149**. However, compound **150** was obtained instead. Treatment of compound **150** with NaOMe successfully yielded compound **151**. Upon completion of purification efforts, the obtained products will undergo deprotection reactions, followed by oxidation, to yield the target products **OMe-CN-NCMorph** and **OMe-Bu-NCMorph** respectively. Considering the investigation of photophysical properties of **NCMorph** derivatives, the introduction of heavy atom will be pursued to realize related PDT agent(s).

3.1.6 Acridine Based Photosensitizers

Acridine based fluorophores have found extensive application as fluorescent probe in the red to NIR regions for detecting various enzyme activities, including β -galactosidase, sulfatase, carboxylesterase.^{182–184} However, their exceptional photophysical characteristics have not yet been evaluated for PDT applications. To address this gap, several acridine based fluorophores and PSs were designed and synthesized.

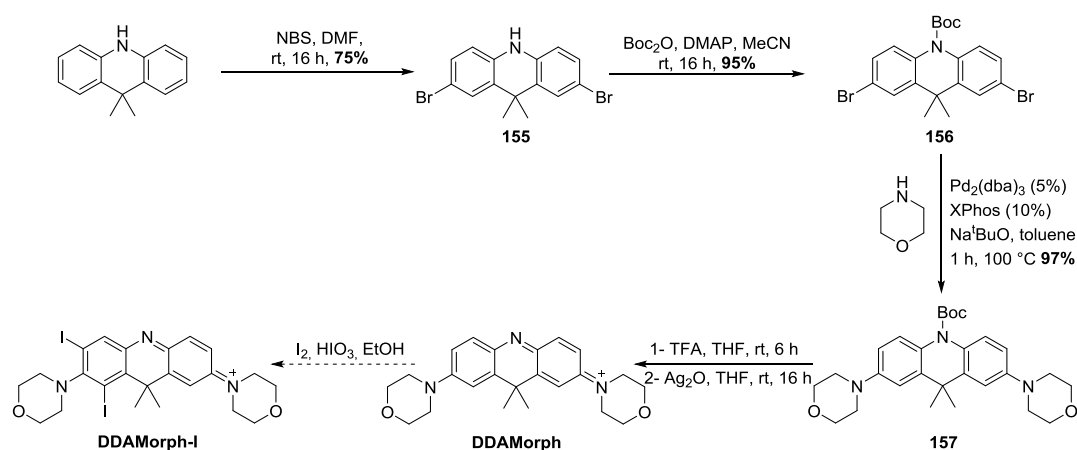
3.1.6.1 Synthesis of Morpholine Bearing Acridine Based Imaging Agent and PS (DDAMorph & DDAMorph-I)

In the initial design of acridine based imaging and PDT agents, morpholine bearing at 2- and 7- position of acridine core was designed. The proposed synthetic pathway is illustrated in Scheme 3.57.



Scheme 3. 57. Initial synthetic route for **DDAMorph** and **DDAMorph-I**.

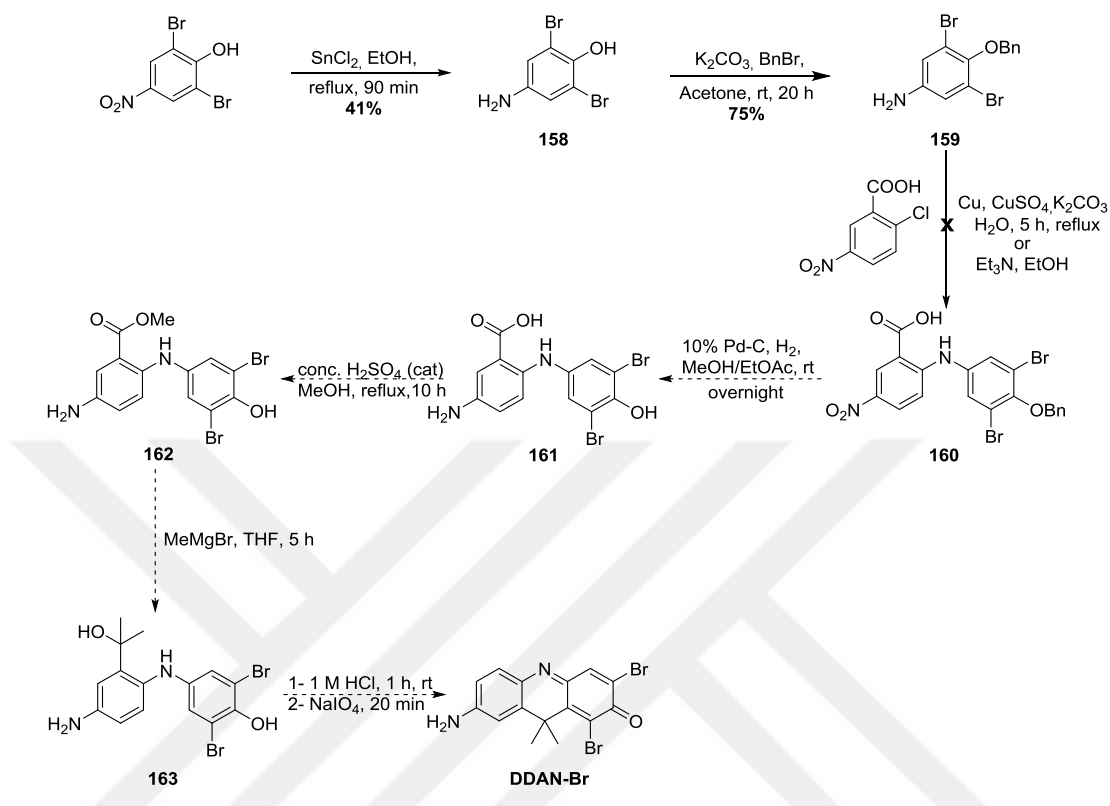
For the synthesis of **DDAMorph** and its heavy atom bearing analogue **DDAMorph-I**, initially, commercially available 9,9-dimethyl-9,10-dihydroacridine was protected with PMB group, yielding compound **152** with a remarkable yield of 90%. Subsequently, compound **152** was brominated with NBS, resulting in compound **153** with nearly complete conversion. Following this, morpholine moieties were incorporated into compound **153** through a Buchwald-Hartwig amination reaction with a high yield of 83%. Finally, oxidative cleavage of PMB protective group was attempted with molecular iodine, but the target fluorophore could not be obtained, resulting in the recovery of starting material. Despite extensive efforts to remove the PMB protective group, including the use of DDQ, H_2 , CAN and molecular bromine, the target fluorophore could not be obtained in these trials and compound **154** was recovered.



Scheme 3. 58. Alternative synthetic route for **DDAMorph** and **DDAMorph-I**.

For the synthesis of **DDAMorph** and **DDAMorph-I**, an alternative synthetic approach has emerged due to the challenges encountered in the oxidative deprotection of the PMB group. The proposed alternative synthetic pathway is illustrated in Scheme 3.58. Initially, commercially available 9,9-dimethyl-9,10-dihydroacridine was brominated with NBS with a notable yield of 75%. Subsequently, the amine group in compound **155** was protected with a Boc protective group, yielding compound **156** with a notably high yield of 95%. Following this, morpholine moieties were incorporated into compound **156** via a Buchwald-Hartwig amination reaction, resulting in compound **157** with a remarkable yield of 97%. To synthesize the target fluorophore **DDAMorph**, Boc protective group was cleaved from compound **157** by employing TFA. Subsequently, the crude product was oxidized with Ag_2O to yield the target fluorophore, **DDAMorph**. Current efforts are focused on purifying **DDAMorph**. Upon completion of purification efforts, the photophysical properties of **DDAMorph** will be investigated and cell culture studies will be conducted. On the other hand, heavy atom introduction will be performed, employing molecular iodine in the presence of HIO_3 to obtain the target PS, **DDAMorph-I**.

3.1.6.2 Synthesis of 7-amino Bearing Acridine Based PS (DDAN-Br)

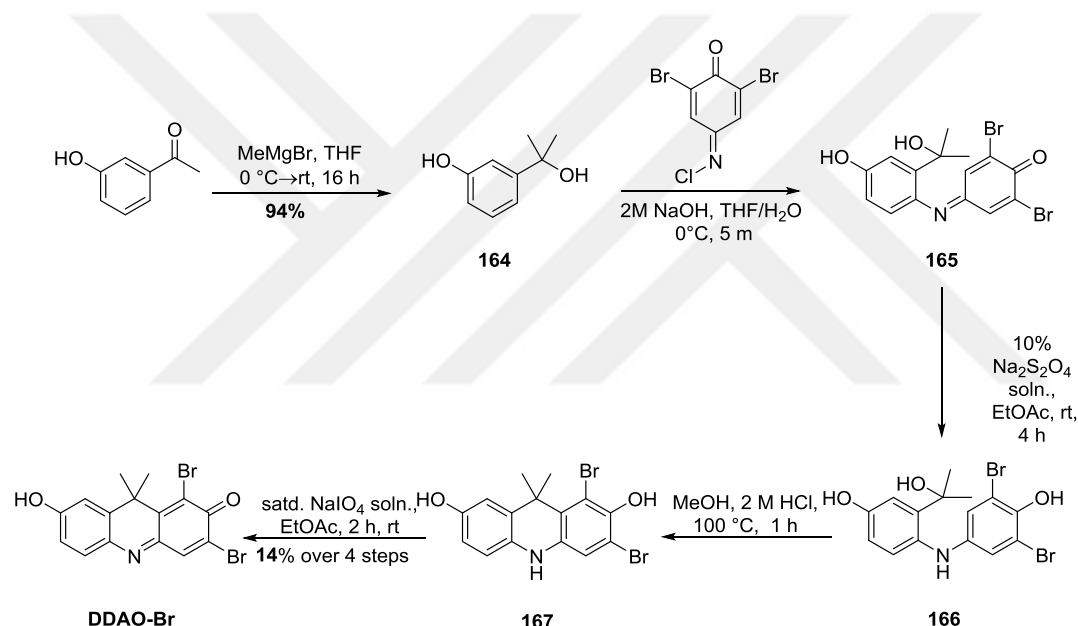


Scheme 3. 59. Synthetic route for **DDAN-Br**.

For the synthesis of rhodol-like acridine based photosensitizer, a synthetic route has been developed and illustrated in Scheme 3.59. Initially, commercially available 2,6-dibromo-4-nitrophenol underwent reduction using SnCl_2 , yielding compound **158** with a moderate yield of 41%. Following this, the phenolic hydroxyl group was protected with benzyl moiety with a notable yield of 75%. Subsequently, compound **159** and commercially available 2-chloro-5-nitrobenzoic acid underwent a Cu-catalyzed Ullmann amine cross-coupling reaction to yield compound **160**. However, the desired compound could not be obtained. Although the obtained side products were not characterized, in order to mitigate self-reaction of compound **159** and initiate nucleophilic aromatic substitution reaction ($\text{S}_{\text{N}}\text{Ar}$) Et_3N was employed as a base and the reaction was replicated. However, formation of complex mixture was observed. Current efforts are focused on the optimization of condition for the

synthesis of compound **160**. Upon succeeding in the synthesis of compound **160**, the proposed synthetic route will be followed (Scheme 3.59). After completing the synthesis of the target PS, **DDAN-Br** will be decorated with glioma targeted activatable group. Furthermore, both active PS and Activatable PS will undergo investigations of their photophysical properties, followed by *in vitro* cell culture studies will be conducted to explore efficacy of **DDAN-Br** and its activatable analogue as potential PDT agents.

3.1.6.3 Synthesis of 7-Hydroxy Bearing Acridine Based PS (DDAO-Br)



Scheme 3. 60. Synthetic route for **DDAO-Br**.

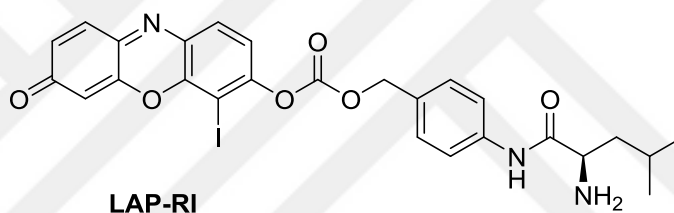
For the synthesis of **DDAO-Br**, the proposed synthetic route is illustrated in Scheme 3.60. Initially, commercially available 3-hydroxyacetophenone underwent a Grignard reaction with MeMgBr , yielding compound **164** with a remarkable yield of 94%. Subsequently, compound **164** underwent a rapid reaction with commercially available 2,6-dibromoquinonechlorimide under basic conditions, forming compound **165**. The resulting deep, blue-colored solid, crude compound **165**, was then reduced with 10% $\text{Na}_2\text{S}_2\text{O}_4$ solution, yielding the purple-colored compound **166**. Without

further purification, compound **166** underwent an acid catalyzed cyclization reaction, resulting in compound **167**. Finally, the target PS, **DDAO-Br** was obtained through an oxidation reaction using a saturated NaIO₄ solution. After extensive effort, **DDAO-Br** was obtained with a yield of 14% over 4 steps of synthesis. Ongoing efforts are focused on purifying **DDAO-Br** to obtain the target PS in >95% purity.

3.2 Investigation of Photophysical Properties of Photosensitizers

3.2.1 Resorufin Based Photosensitizers

3.2.1.1 LAP Activatable Iodo-Resorufin PS (LAP-RI)



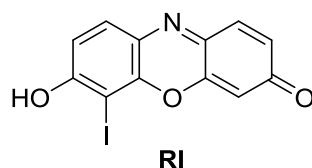
Photophysical properties of synthesized leucine aminopeptidase (LAP) enzyme activatable resorufin based photosensitizer, **LAP-RI**, were investigated by our group member Büşra Asena Sayın within her thesis. Summary of the results are given below.

The concentration-dependent activation of the **LAP-RI** photosensitizer was investigated. Treatment of the target photosensitizer with LAP enzyme in a range of 0-5 units resulted in the conversion of **LAP-RI** into its active form, **RI**. The increasing absorption peak, with a maximum at 572 nm, correlated with the increasing concentration of LAP enzyme, indicating the successful conversion of the **LAP-RI** photosensitizer into **RI**.

Following the successful cleavage of the LAP moiety, the absorption and emission maxima of the resultant photosensitizer were recorded at 572 nm and 587 nm,

respectively. The absorbance and emission spectra were documented after the incubation of **LAP-RI** with 1 unit of LAP enzyme at 37 °C for 1 hour, demonstrating the complete conversion of **LAP-RI** into **RI**.

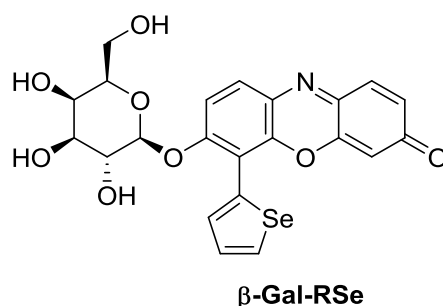
3.2.1.2 Iodinated Resorufin PS (**RI**)



The investigation of **RI**'s photophysical properties was done by Toghrul Almammadov from our collaborating group, Kölemen lab within his thesis study. Summary of the results are given below.

The fluorescence quantum yield of **RI** was calculated as 6.8% in PBS buffer containing 1% DMSO at 37 °C, using resorufin sodium salt as a reference in PBS ($\Phi_F = 0.74$). Moreover, the singlet oxygen quantum yield of **RI** was calculated as 54% in PBS buffer containing 1% DMSO at 37 °C, using methylene blue as a reference in PBS ($\Phi_\Delta = 0.52$).

3.2.1.3 β -Gal Activatable Seleno-Resorufin PS (β -Gal -RSe)



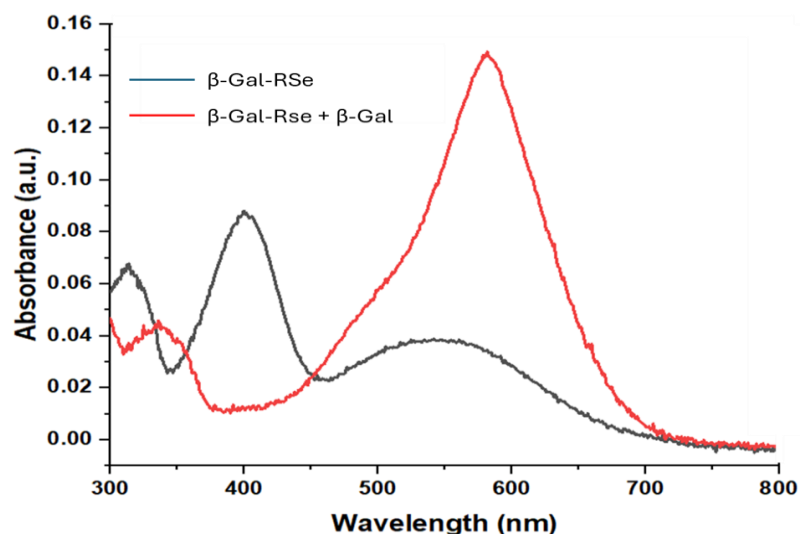


Figure 3.4. Absorption spectra of **β-Gal-RSe** in PBS buffer (10 μ M, 1% DMSO, pH:7.4) were measured both with and without incubation with 5 μ L β -Gal enzyme at 37 $^{\circ}$ C.

The absorbance spectrum of 10 μ M **β-Gal-RSe** (dark blue) and 5 μ L β -Gal enzyme treated **β-Gal-RSe** at 37 $^{\circ}$ C were represented in Figure 3.7. exhibited absorption maximum at 542 nm Inactive photosensitizer, **β-Gal-RSe**, exhibited an absorption maximum at 542 nm which is 52 nm red shifted compared to its iodo analogue owing to extended conjugation. However, upon cleavage of β -Gal moiety absorption maximum was observed at 582 nm which is same as its iodo analogue (Table 3.4). This clearly indicates that phenoxide resonance became predominant upon the cleavage of β -Gal moiety (Scheme 3.62).

Table 3.4. Absorption maxima of **β-Gal-RSe** and **β-Gal-RI** and their absorption maxima after treatment with β -Gal enzyme.

PS	<i>β-Gal-RSe</i>	<i>RSe</i>	<i>β-Gal-RI</i>	<i>RI</i>
λ_{abs} (nm)	542	582	490	582

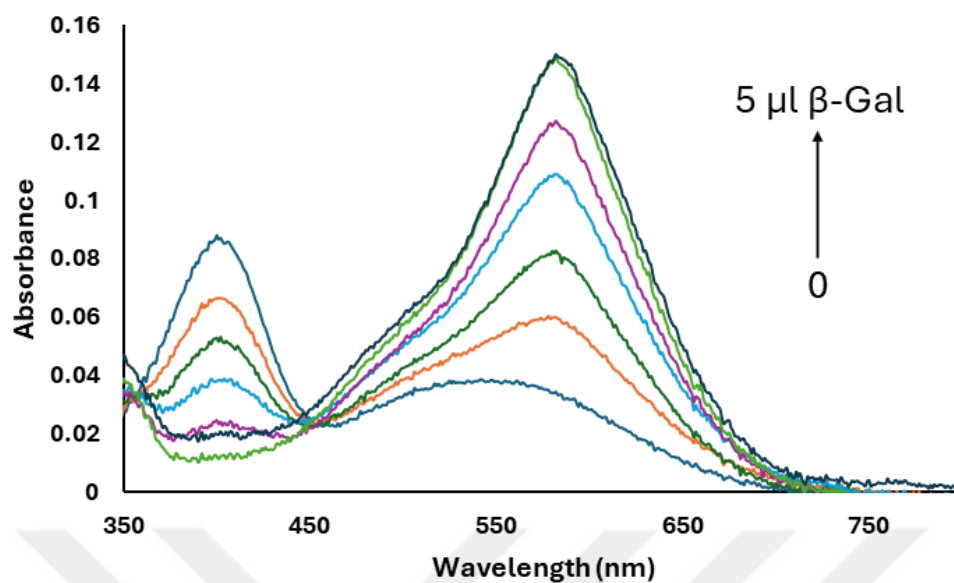
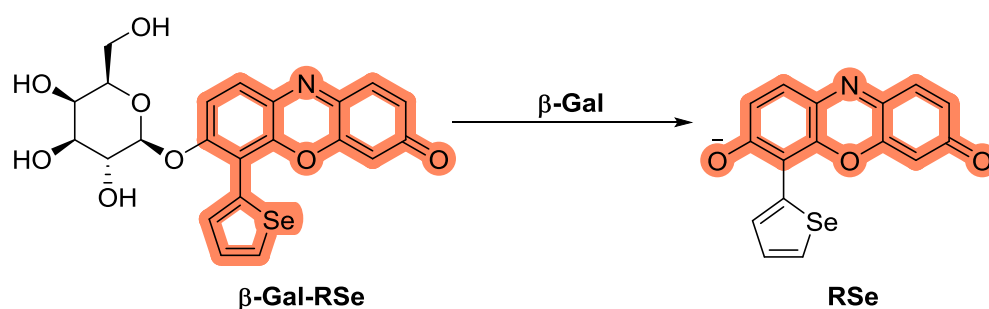


Figure 3.5. β -Gal concentration dependent absorption spectra of **β -Gal-RSe** (10 μ M)

Furthermore, besides the successful cleavage of the β -Gal moiety in the presence of 5 μ L β -Gal enzyme, various amounts of β -Gal enzyme, ranging from 0 to 5 μ L, were employed to investigate the cleavage of the handle group. Figure 3.8 illustrates the dependence of β -Gal enzyme on the conversion of **β -Gal-RSe** into **RSe**. Nearly complete conversion was observed in the presence of 5 μ L β -Gal enzyme.



Scheme 3. 61. Resonance preference of **β -Gal-RSe** before and after cleavage of handle group in the presence of β -Gal enzyme.

3.2.2 Si-Fluorescein Based Photosensitizers

3.2.2.1 β -Gal Activatable Diiodo-Si-Fluorescein PS (β -Gal-SiF-II)

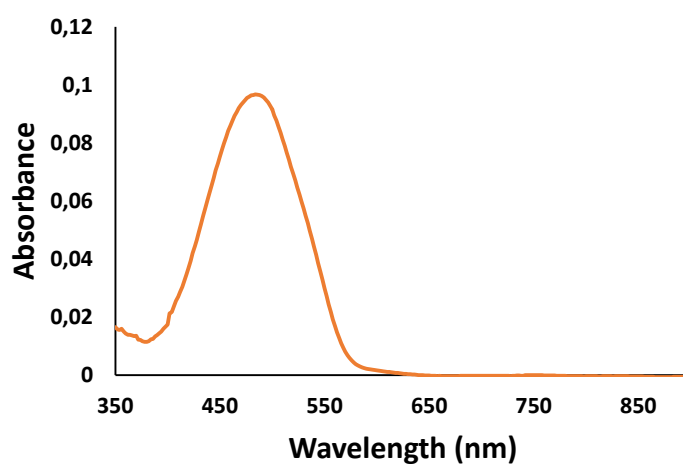
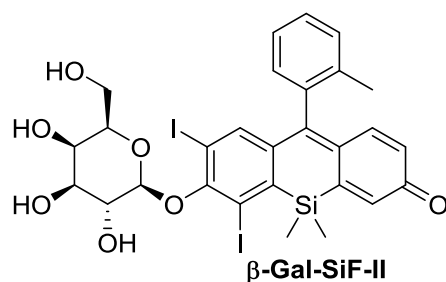


Figure 3.6. Absorption spectrum of β -Gal-SiF-II (10 μ M) in PBS buffer (pH 7.4, 1% DMSO)

The absorbance spectrum of 10 μ M β -Gal-SiF-II which was prepared in the PBS buffer containing 1% DMSO was recorded at pH 7.4. β -Gal-SiF-II exhibited absorption maximum at 486 nm.

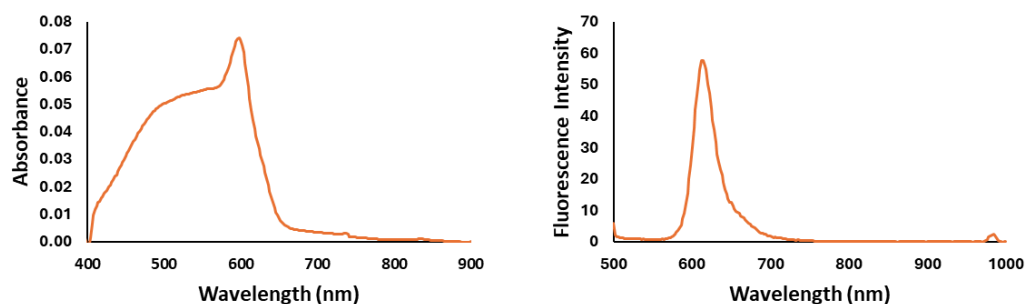


Figure 3.7. Absorption spectrum of **β -Gal-SiF-II** (10 μ M) in PBS buffer (pH 7.4, 1% DMSO) upon incubation with β -galactosidase (5 U) for 30 min (37 $^{\circ}$ C) (left). Fluorescence spectrum of **β -Gal-SiF-II** (10 μ M) in PBS buffer (pH 7.4, 1% DMSO) upon incubation with β -Galactosidase (5 U) for 30 min (37 $^{\circ}$ C) (right).

The absorption and emission spectra of the 10 μ M **β -Gal-SiF-II** which was prepared in PBS buffer containing 1% DMSO at pH 7.4 was recorded after the incubation of **β -Gal-SiF-II** with 5 units of β -galactosidase enzyme at 37 $^{\circ}$ C for 1 hour as depicted in Figure 3.10, demonstrating the complete conversion of **β -Gal-SiF-II** into **SiF-II**. **SiF-II** exhibited absorption and emission maxima at 598 nm and 613 nm, respectively.

Table 3.5 Photophysical properties of **β -Gal-SiF-II** and **SiF-II**.

PS	λ_{abs} (nm)	λ_{ems} (nm)	ϕ_F (%)
β-Gal-SiF-II	486	n.d.	n.d.
SiF-II	598	613	6.4

3.2.3 Si-Rhodamine Based Photosensitizers

3.2.3.1 Tollyl Bearing Si-Rhodamine PS (2-Me-SiR-Br)

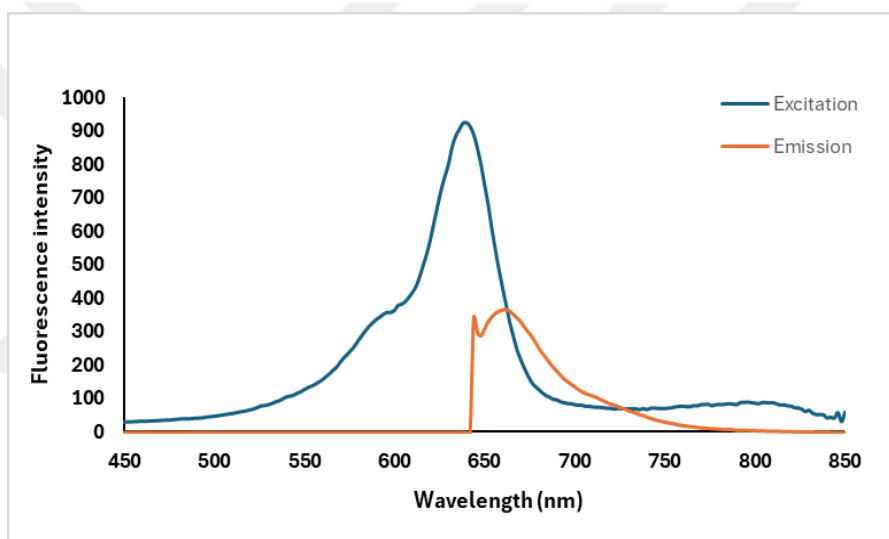
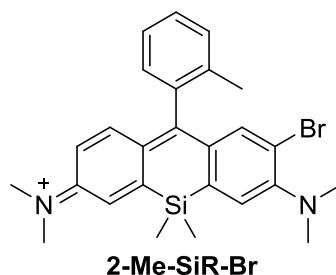


Figure 3.8. Absorption and emission spectra of **2-Me-SiR-Br** (50 μ M) in PBS buffer (pH 7.4, 1% DMSO)

The UV-VIS spectrum of **2-Me-SiR-Br** was obtained and its absorption maximum was recorded at 640 nm. Upon excitation of **2-Me-SiR-Br** with a wavelength of 640 nm the emission spectrum was obtained, resulting in a maximum at 662 nm (Table 3.6).

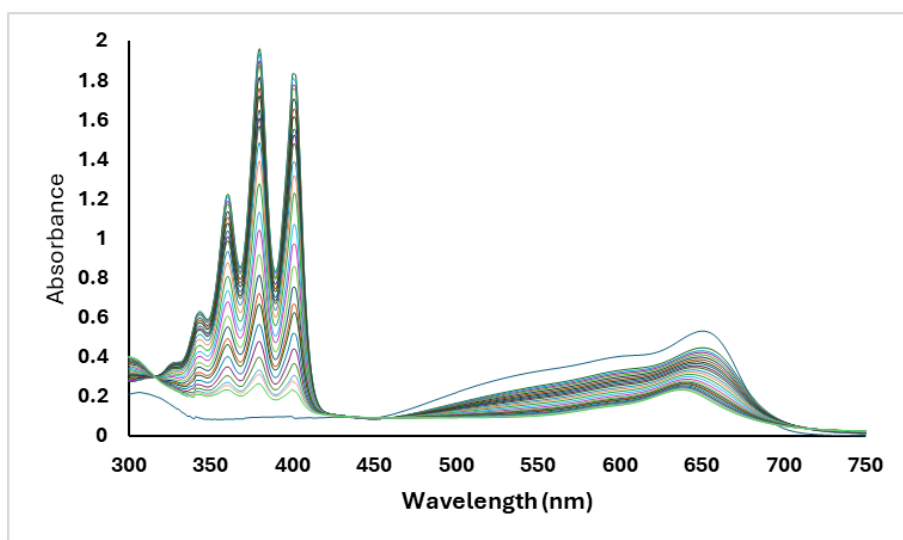


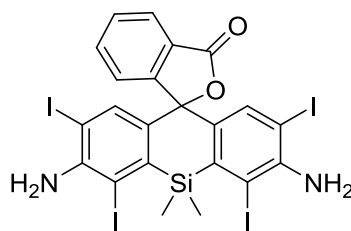
Figure 3.9. Decrease in the absorption signal of ADMDA at 380 nm with respect to time.

The singlet oxygen trap experiment was conducted with **2-Me-SiR-Br** to investigate singlet oxygen generation capacity of photosensitizer in PBS buffer. To calculate the singlet oxygen quantum yield of **2-Me-SiR-Br** 2,2'-(anthracene-9,10-diyl)bis(methylene)dimalonic acid (ADMDA) was employed as a singlet oxygen trap molecule. Upon irradiation of **2-Me-SiR-Br** with 650 nm laser light (20%), a time dependent decrease in the absorption maximum of the ADMDA at 380 nm was observed due to the cycloaddition reaction between photosensitized singlet oxygen and the anthracene ring of ADMDA (Figure 3.15). The singlet oxygen quantum yield of the **2-Me-SiR-Br** was calculated as 45% in PBS buffer containing 1% DMSO with respect to methylene blue ($\Phi_{\Delta} = 0.52$ in PBS buffer) (Table 3.6).

Table 3.6 Photophysical properties of **2-Me-SiR-Br**

PS	λ_{abs} (nm)	λ_{ems} (nm)	Φ_{Δ} (%)
2-Me-SiR-Br	640	662	45

3.2.3.2 Benzoic Acid Bearing Si-Rhodamine PS (Compound 56)



56

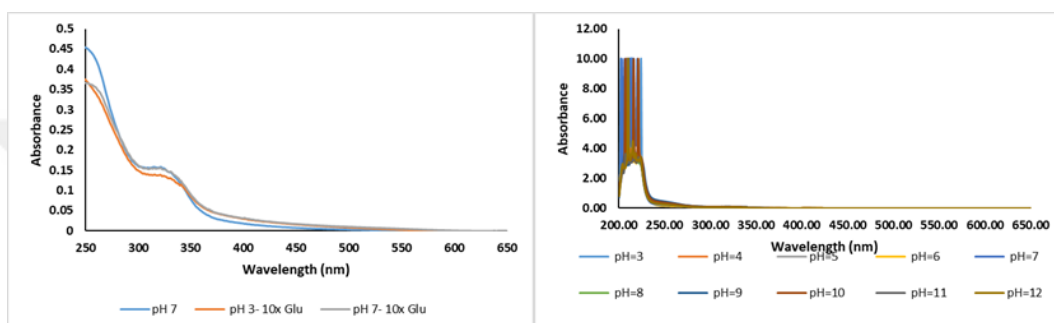


Figure 3.10. Absorbance spectra of compound **56** upon treatment with glutathione (GSH) (left) or at varying pH ranging from 3 to 12 (right)

Photophysical properties of synthesized compound **56** were investigated. Oxidative demethylation of amine groups resulted in photo-bluening in the absorption spectrum in aqueous media. Various treatments were applied to facilitate ring opening and induce a bathochromic shift. First, glutathione (GSH) was introduced, as the opening conformation could be predominant in the presence of GSH upon conjugation.¹⁸⁵ However, no bathochromic shift in absorption was not observed at various pH levels. Subsequently, the absorption spectra of compound **56** at various pH levels were examined across a pH range of 3-12, but no shift were detected.

3.2.4 Phenoselenazine Based Photosensitizers

3.2.4.1 Morpholine Bearing Phenoselenazine Based PS (NSeMorph)

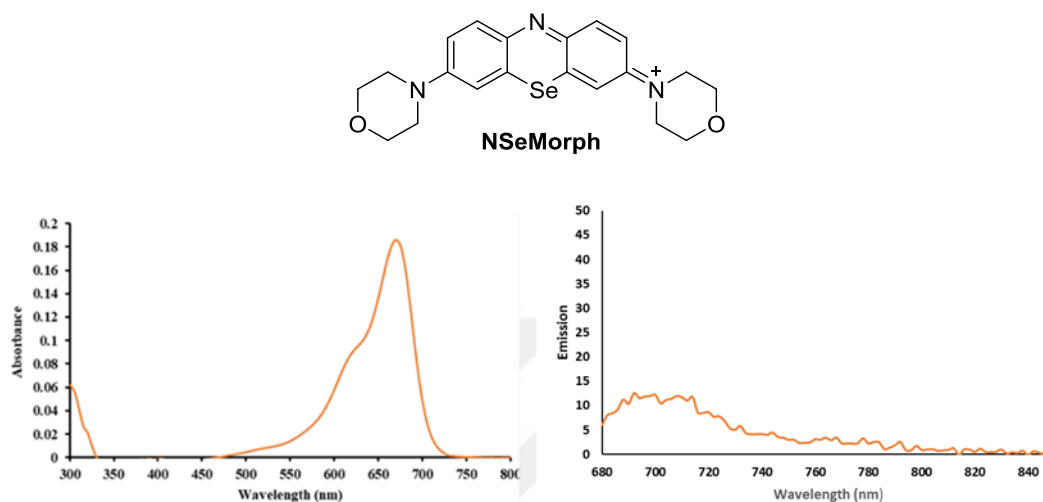


Figure 3.11. Absorption (left) and emission (right) spectrum of **NSeMorph** (20 μ M) in PBS buffer (pH 7.4, 1% DMSO).

The UV-VIS spectrum of **NSeMorph** was obtained and its absorption maximum was recorded at 671 nm. Upon excitation of **NSeMorph** with a wavelength of 671 nm the emission spectrum was obtained, resulting in a maximum at 695 nm (Table 3.7, Figure 3.16).

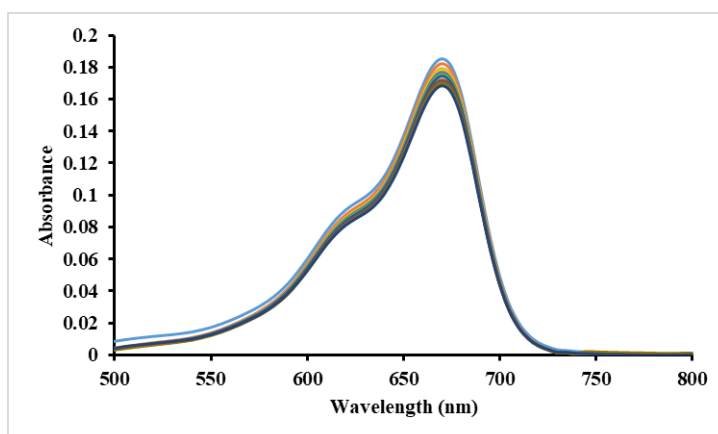


Figure 3.12. Photostability of **NSeMorph** (20 μ M) in PBS buffer (pH 7.4, 1% DMSO) upon irradiation with laser light (650 nm, 20%, 10 s irradiation time).

Prior to conducting the singlet oxygen trap experiment, the photostability of **NSeMorph** was assessed upon irradiation with light using a laser light source with a wavelength of 650 nm. Absorbance spectra of both **NSeMorph** was obtained after 10 seconds of light exposure. No significant photodecomposition was observed in **NSeMorph** for up to 100 seconds of illumination (Figure 3.17).

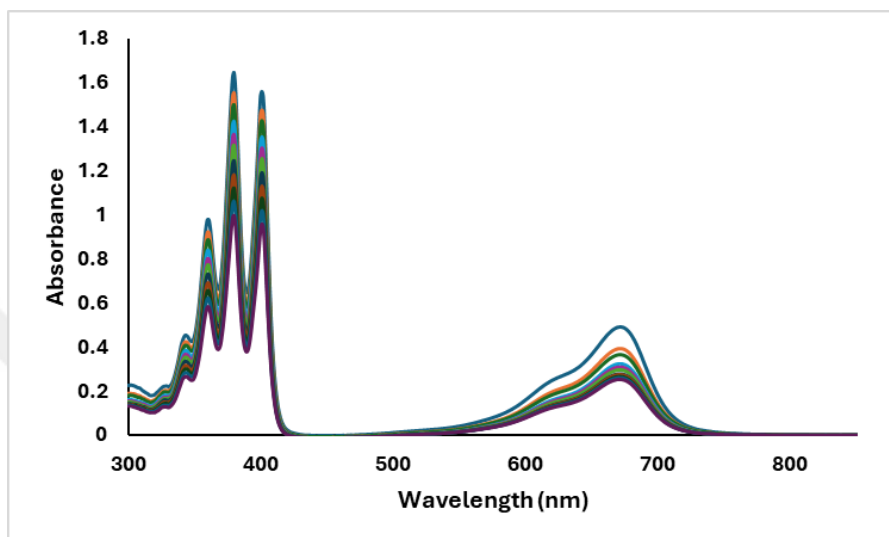


Figure 3.13. Decrease in the absorbance of ADMDA in PBS (pH 7.4, 1% DMSO) upon irradiation of **NSeMorph** (20 μ M).

The singlet oxygen trap experiment was conducted with **NSeMorph** to investigate singlet oxygen generation capacity of photosensitizer in PBS buffer. To calculate the singlet oxygen quantum yield of **NSeMorph** 2,2'-(anthracene-9,10-diyl)bis(methylene)dimalonic acid (ADMDA) was employed as a singlet oxygen trap molecule. Upon irradiation of **NSeMorph** with 660 nm LED light, a time dependent decrease in the absorption maximum of the ADMDA at 380 nm was observed due to the cycloaddition reaction between photosensitized singlet oxygen and the anthracene ring of ADMDA (Figure 3.18). The singlet oxygen quantum yield of the **NSeMorph** was calculated as 75% in PBS buffer containing 1% DMSO with respect to methylene blue ($\Phi_{\Delta} = 0.52$ in PBS buffer) (Table 3.7).

Table 3.7 Photophysical properties of **NSeMorph**

PS	λ_{abs} (nm)	λ_{ems} (nm)	ϕ_F (%)	ϕ_A (%)
NSeMorph	671	695	n.d.	75

3.2.4.1.1 Nitroreductase Activatable NSeMorph (NTR-NSeMorph)

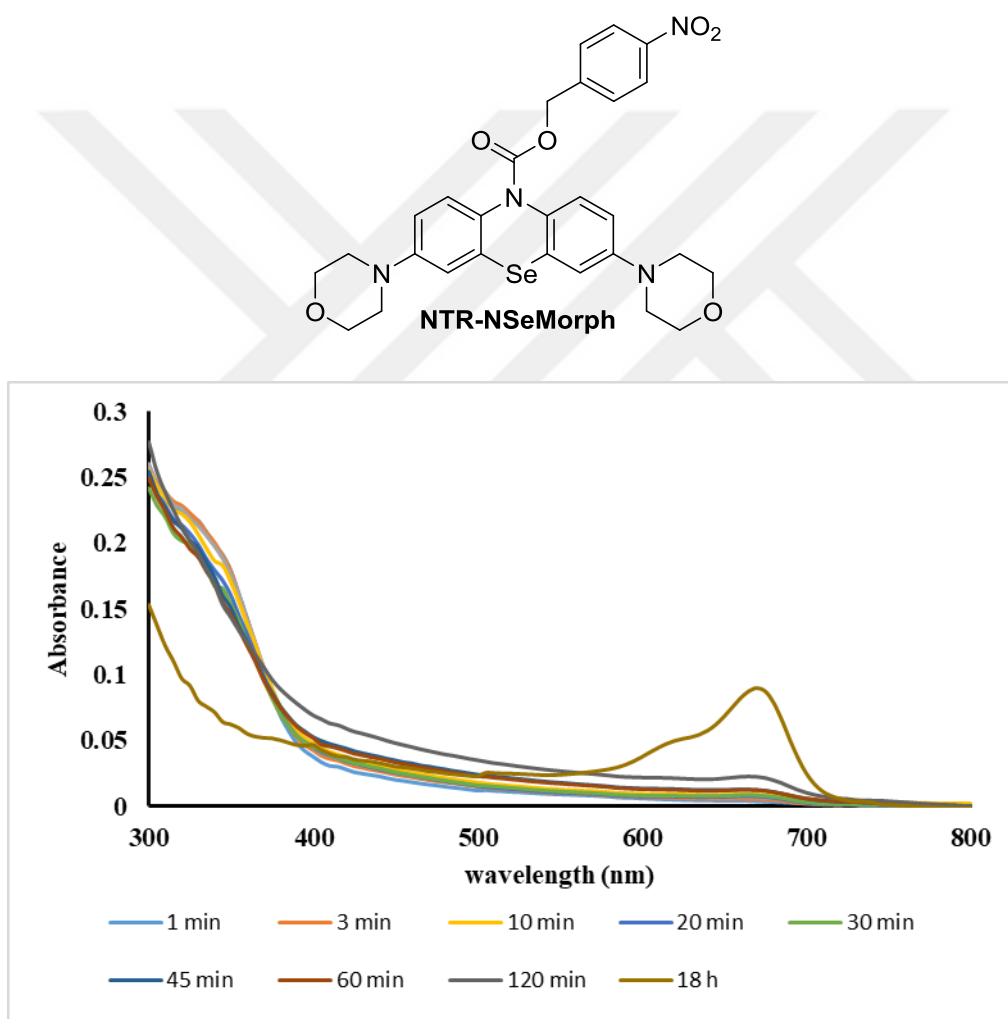


Figure 3.14. UV-vis absorption spectra responses of **NSeMorph-NTR** (20 μ M) in the presence of NADPH (5 μ M) and Nitroreductase (5 u) upon various incubation times. Detection condition: PBS buffer, pH = 7.4, T = 37°C.

The time-dependent conversion of the **NSeMorph-NTR** photosensitizer into **NSeMorph**, utilizing a constant concentration of Nitroreductase enzyme (5 U) with the aid of NADPH (5 μ M) was investigated. Initially, the effect of 5 units of Nitroreductase enzyme and NADPH on **NSeMorph-NTR** was monitored over a period of 1 to 120 minutes by obtaining UV-VIS spectra. However, cleavage of the handle group from the target photosensitizer slightly observed. Following the 18 hours incubation time, successive cleavage of Nitroreductase activatable moiety was observed.

3.2.4.2 Azetidine Bearing Phenoselenazine Based PS (**NSeAze**)

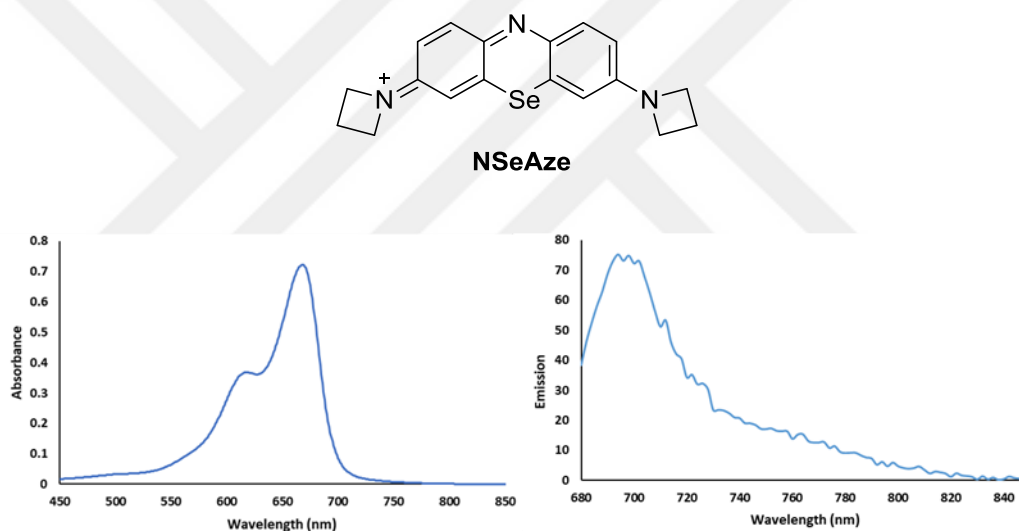


Figure 3.15. Absorption (left) and emission (right) spectra of **NSeAze** (20 μ M) in PBS buffer (pH 7.4, 1% DMSO).

The UV-VIS spectrum of **NSeAze** was obtained and its absorption maximum was recorded at 671 nm, same as **NSeMorph**. Upon excitation of **NSeAze** with a wavelength of 671 nm the emission spectrum was obtained, resulting in a maximum at 695 nm with a 7-fold intensified fluorescence emission than **NSeMorph** (Table 3.9).

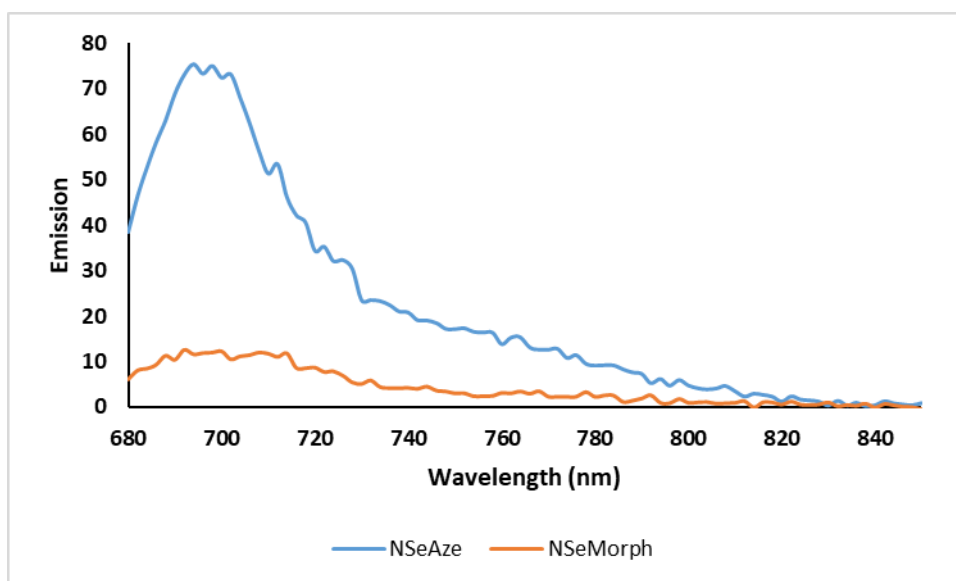


Figure 3.16. Emission spectra of **NSeAze** (20 μ M) and **NSeMorph** in PBS buffer (pH 7.4, 1% DMSO).

Prior to conducting the singlet oxygen trap experiment, the photostability of **NSeAze** was assessed upon irradiation with light using a laser light source with a wavelength of 660 nm. Absorbance spectra of both **NSeAze** was obtained after 20 seconds of light exposure. No significant photodecomposition was observed in **NSeAze** for up to 100 seconds of illumination (Figure 3.23)

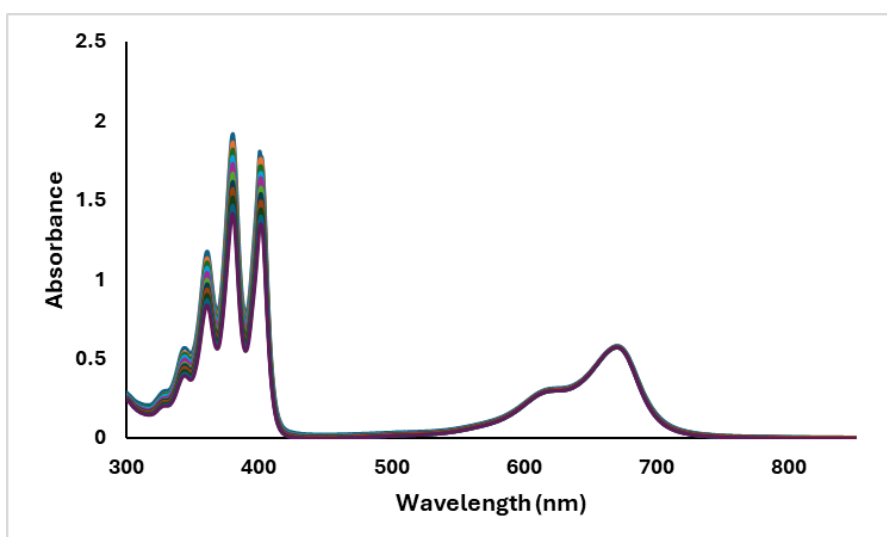


Figure 3.17. Decrease in the absorbance of ADMDA in PBS (pH 7.4, 1% DMSO) upon irradiation of **NSeAze** (20 μ M).

The singlet oxygen trap experiment was conducted with **NSeAze** to investigate singlet oxygen generation capacity of photosensitizer in PBS buffer. To calculate the singlet oxygen quantum yield of **NSeAze** 2,2'-(anthracene-9,10-diyl)bis(methylene)dimalonic acid (ADMDA) was employed as a singlet oxygen trap molecule. Upon irradiation of **NSeAze** with 660 nm LED light, a time dependent decrease in the absorption maximum of the ADMDA at 380 nm was observed due to the cycloaddition reaction between photosensitized singlet oxygen and the anthracene ring of ADMDA (Figure 3.24). The singlet oxygen quantum yield of the **NSeAze** was calculated as 31% in PBS buffer containing 1% DMSO with respect to methylene blue ($\Phi_{\Delta} = 0.52$ in PBS buffer) (Table 3.9).

Table 3.8 Photophysical properties of **NSeAze**

PS	$\lambda_{abs} (nm)$	$\lambda_{ems} (nm)$	$\phi_F (%)$	$\phi_{\Delta} (%)$
NSeAze	671	695	n.d.	31

3.2.4.3 Oxygen Bearing Annulated Phenoselenazine Based PS (NSeO-A)

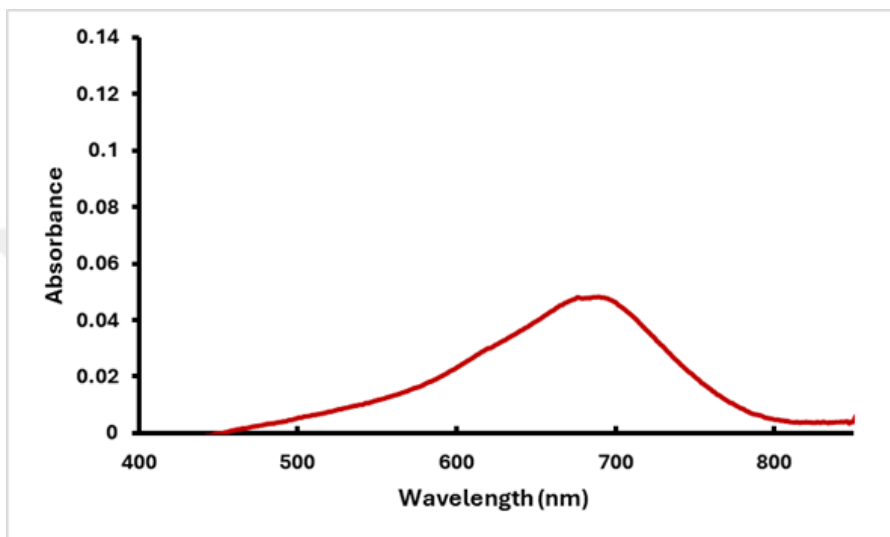
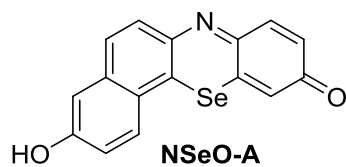
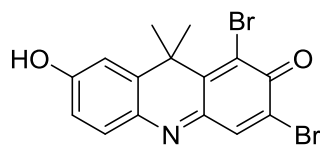


Figure 3.18. Absorption spectra of **NSeO-A** in PBS buffer under basic conditions (pH > 7).

The UV-VIS spectrum of **NSeO-A** was obtained and its absorption maximum was recorded at 689 nm, upon treatment with NaOH.

3.2.4.4 7-Hydroxy Bearing Acridine Based PS (DDAO-Br)



DDAO-Br

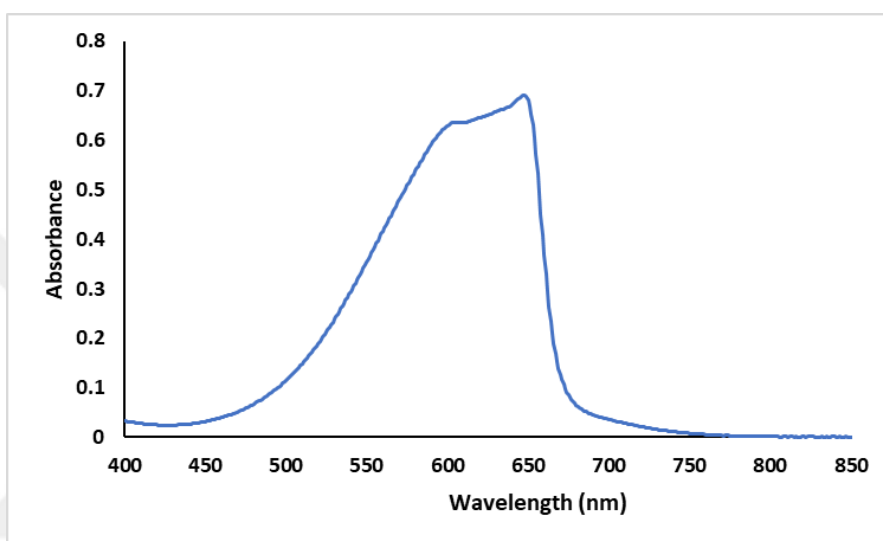


Figure 3.19. Absorption spectra of **DDAO-Br** in PBS buffer under basic conditions (pH > 7).

The UV-VIS spectrum of **DDAO-Br** was obtained and its absorption maximum was recorded at 648 nm, upon treatment with NaOH.

3.3 Cell Culture Studies

3.3.1 Resorufin Based Photosensitizers

3.3.1.1 LAP Activatable Iodo-Resorufin PS (LAP-RI)

In vitro cell culture studies of **LAP-RI** were conducted by our group members Dilay Kepil and Dr. Zübeyir Elmazoğlu.

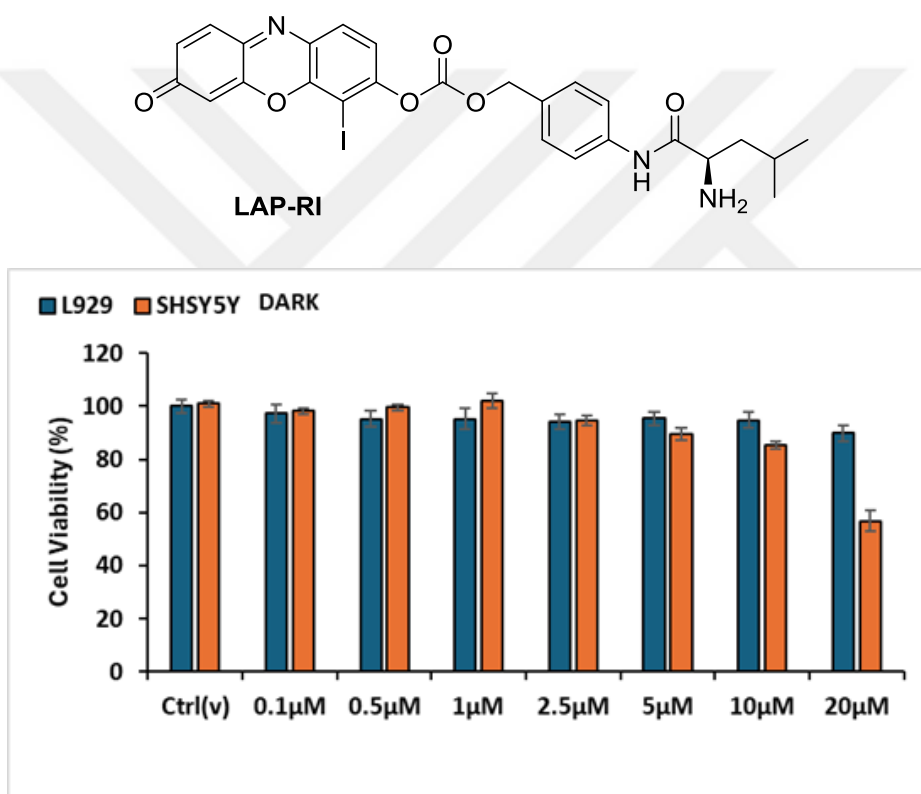


Figure 3.20. Cell viabilities of L929 and SHSY5Y cells. For dark toxicity analysis, cells were treated with the increasing concentrations (0.1-20 μM) of **LAP-RI** for 24 h at dark in a humidified 37°C, 5% CO₂ incubator.

Following promising results that obtained from investigation of photophysical properties, effectiveness of **LAP-RI** was tested in healthy L929 fibroblast cell line and in SHSY5Y neuroblastoma cell line. According to MTT assay, photosensitizer

does not exhibit dark toxicity across both healthy and malignant cell lines up to 20 μM **LAP-RI** concentrations.

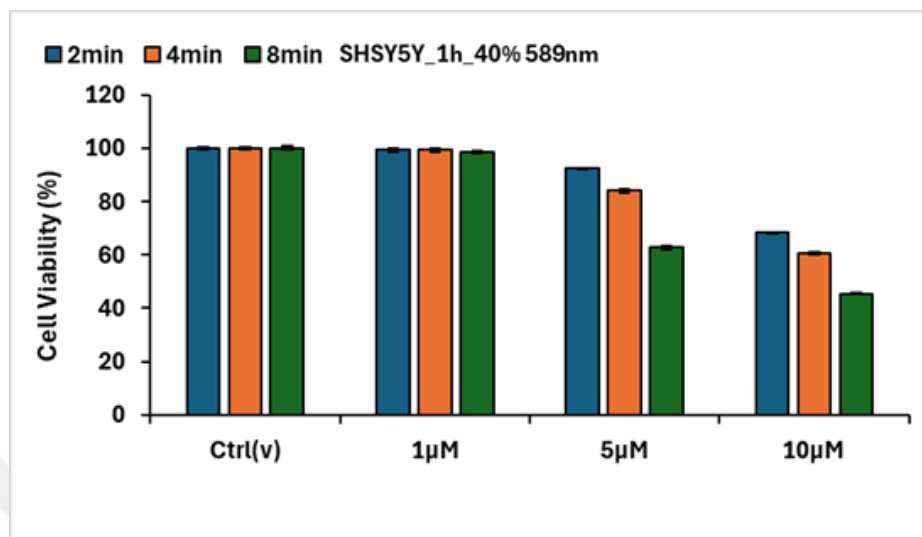


Figure 3.21. Cell viabilities of L929 and SHSY5Y cells. Cells were treated with the increasing concentrations (0.1-20 μM) of **LAP-RI** for 24 h at dark and 1h dark incubation followed by 2, 4, 8 minutes laser light illumination (589 nm, 40%). followed by dark incubation up to 24 h in incubator (n=5-6).

After the evaluation of dark toxicity of **LAP-RI** on L929 and SHSY5Y cell lines, its phototoxicity was evaluated by employing laser light source with a wavelength of 589 nm. Initially, **LAP-RI** treated cells incubated at dark for 24 hours, then incubated 1 hour more before illuminating them by 2, 4, 8 minutes laser light (589 nm, 40%). The IC₅₀ value for neuroblastoma was achieved at 8.95 μM after 8 min laser irradiation. The IC₅₀ value for rest of the times could not be obtained up to μM **LAP-RI** concentration.

3.3.2 Si-Fluorescein Based Photosensitizers

3.3.2.1 β -Gal Activatable Diiodo-Si-Fluorescein PS (β -Gal-SiF-II)

In vitro cell culture studies of β -Gal-SiF-II were conducted by our group member Dilay Kepil.

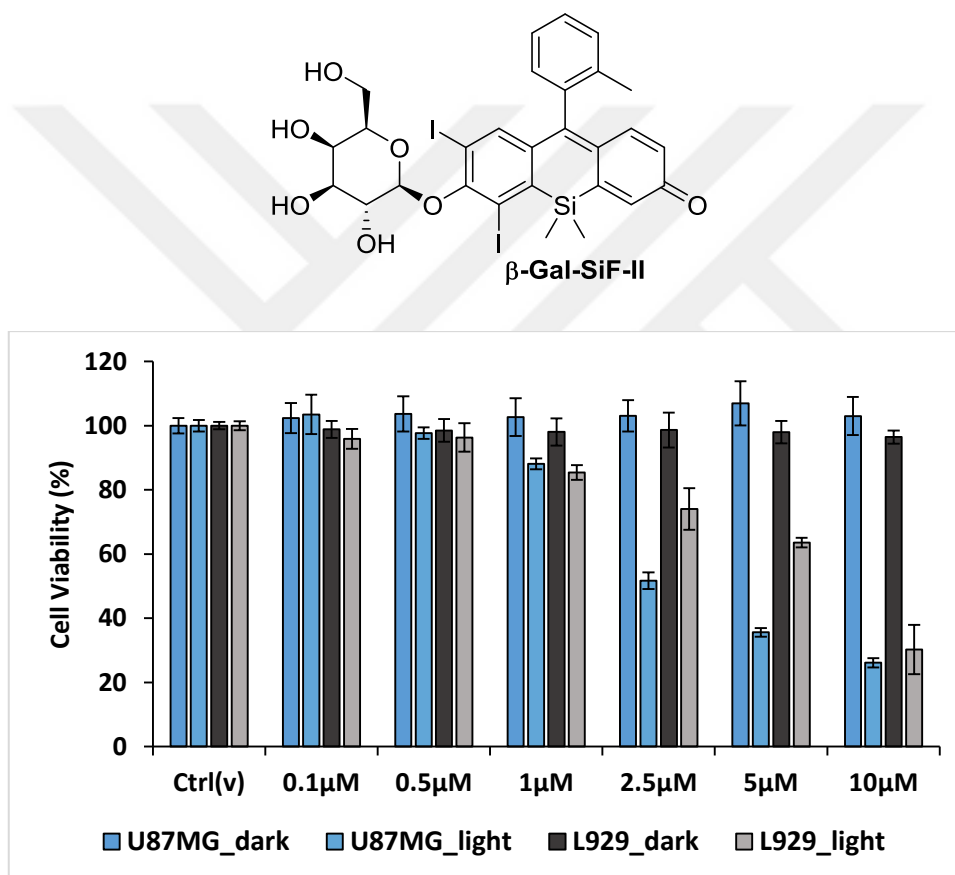


Figure 3.22. MTT assays of β -Gal-SiF-II treated (0-10 μ M) U87MG and L929 cells after 24 h dark incubation and 1 h dark incubation followed by 2 h light illumination (595 nm, 9.38 mW/cm²).

The photodynamic therapy (PDT) efficacy of β -Gal-SiF-II was investigated in U87 MG glioblastoma and L929 healthy cell lines. A dose-dependent decrease in cell viability was observed upon treatment with β -Gal-SiF-II. According to MTT assay,

β -Gal-SiF-II does not exhibit dark toxicity across both healthy and malignant cell lines up to 10 μ M **β -Gal-SiF-II** concentrations. Following the incubation of **β -Gal-SiF-II** treated both cell lines 24 hours at dark, they were incubated 1 hour more at dark before illuminating them with LED light (595 nm, 9.38 mW/cm²) for 2 hours. Subsequently, the IC₅₀ values for U87MG and L929 cells were determined to be 3.369 μ M and 6.270 μ M, respectively, showing notably higher values in healthy cells, indicating an enhanced selectivity of **β -Gal-SiF-II** towards cancerous cells, specifically for glioblastomas.

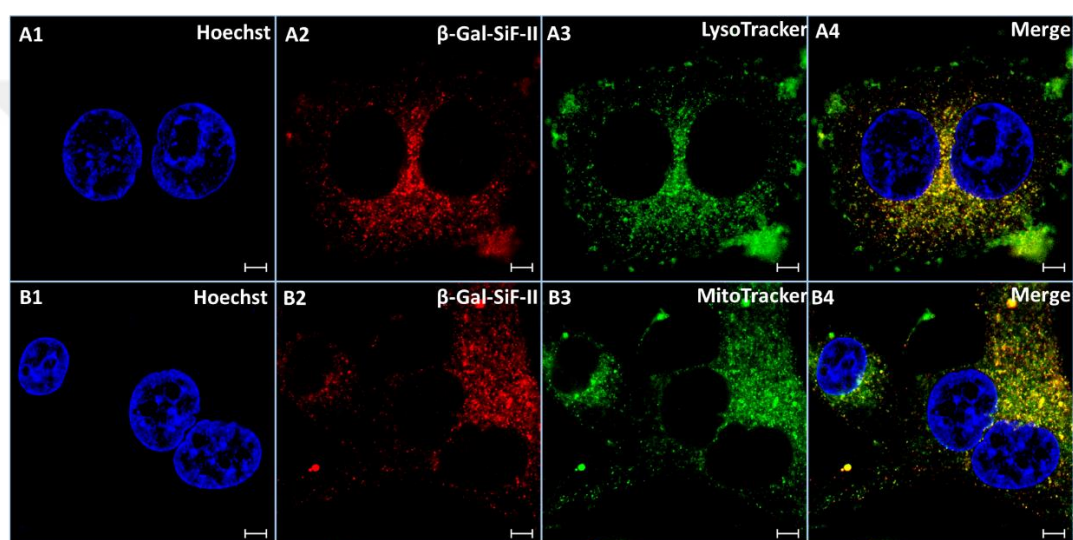


Figure 3.23. The subcellular localization of **β -Gal-SiF-II** (1 μ M, 1 h) in U87MG cells using LysoTracker Yellow (A1-4) or MitoTracker Green FM (B1-4). Scale bar: 5 μ m.

Furthermore, the intracellular localization of **β -Gal-SiF-II** in glioblastoma cells was investigated using MitoTracker and LysoTracker dyes. Following a 1-hour incubation period with **β -Gal-SiF-II**, activation was detected within both mitochondria and lysosomes, as illustrated in Figure 3.30. This dual intracellular localization highlights the potential multifaceted roles of **β -Gal-SiF-II**, suggesting its viability as a promising phototheranostic agent within glioblastoma therapy.

3.3.3 Si-Rhodamine Based Photosensitizers

3.3.3.1 Tollyl Bearing Si-Rhodamine PS (2-Me-SiR-Br)

In vitro cell culture studies of **2-Me-SiR-Br** were conducted by our group member Naz Özoğul.

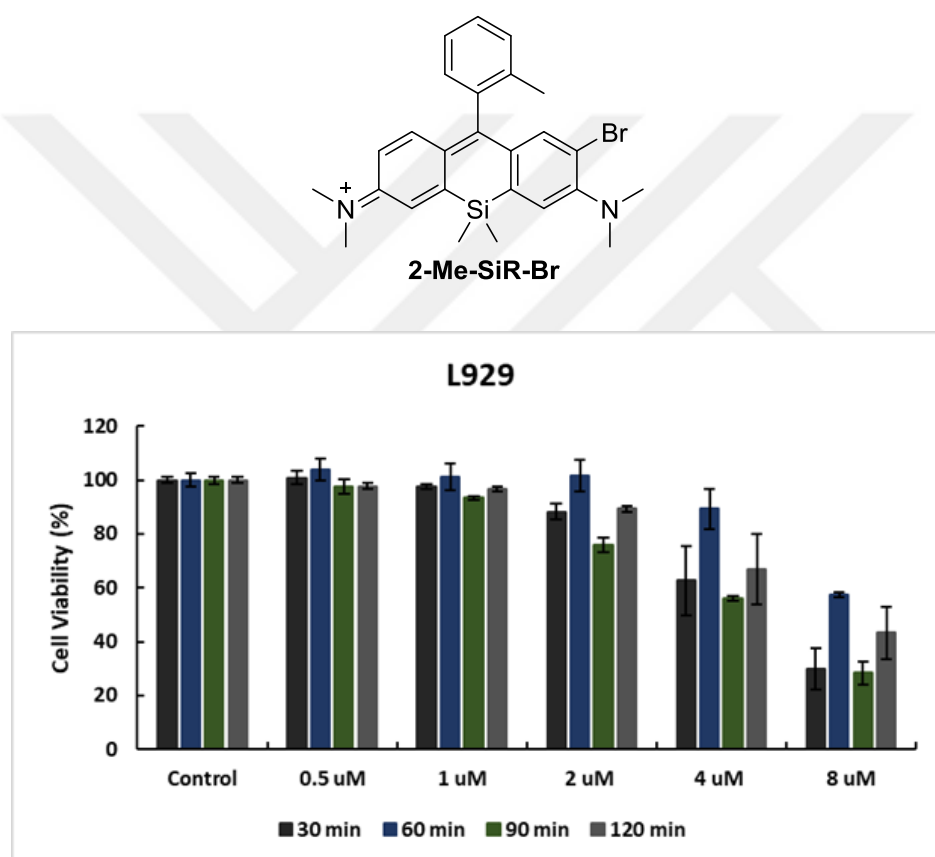


Figure 3.24. *In vitro* cytotoxicity of **2-Me-SiR-Br** towards L929 fibroblast cell lines. The cytotoxicity of the drug was measured with MTT assay. Cells were exposed to various concentrations of drug for different incubation times under both dark and light irradiated ($364,8 \text{ mW/cm}^2$, 650 nm) conditions. (n=6)

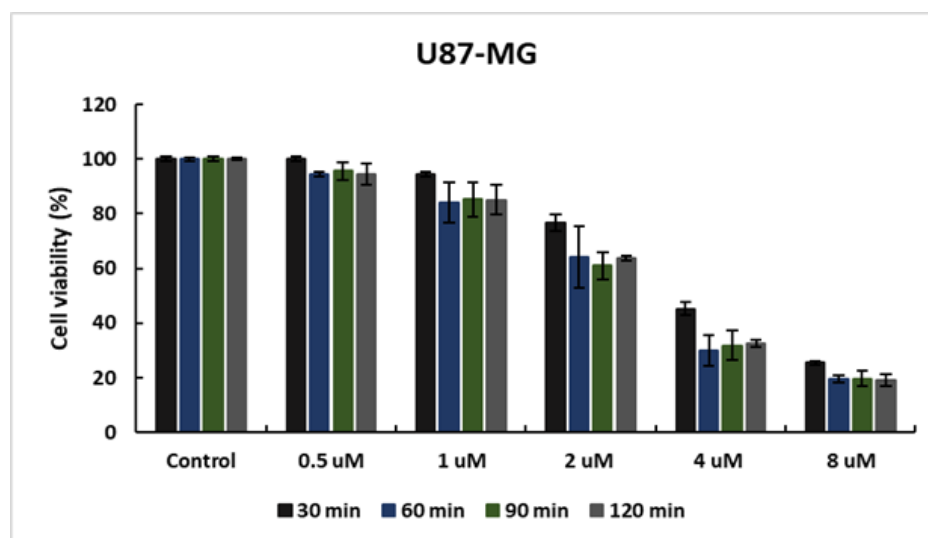


Figure 3.25. In vitro cytotoxicity of **2-Me-SiR-Br** towards U87-MG human glioblastoma cell lines. The cytotoxicity of the drug was measured with MTT assay. Cells were exposed to various concentrations of drug for different incubation times under both dark and light irradiated ($364,8 \text{ mW/cm}^2$, 650 nm) conditions. ($n=6$)

The photodynamic therapy (PDT) efficacy of **2-Me-SiR-Br** was investigated in healthy L929 and U87 MG glioblastoma cell lines. A dose-dependent decrease in cell viability was observed upon treatment with **2-Me-SiR-Br**. According to MTT assay, **2-Me-SiR-Br** does not exhibit dark toxicity across both healthy and malignant cell lines up to $8 \mu\text{M}$ **2-Me-SiR-Br** concentrations. Following the incubation of **2-Me-SiR-Br** treated both cell lines 24 hours at dark, they were incubated 1 hour more at dark before illuminating them with laser light ($364,8 \text{ mW/cm}^2$, 650 nm) for various time range (0.5-2 h). Subsequently, the IC_{50} values for U87MG cells was determined to be $2.51 \mu\text{M}$, exhibiting no significant effect on healthy cells at that concentration range. Despite the active drug profile of **2-Me-SiR-Br**, the notable disparity in IC_{50} values between healthy and malignant cells indicates the high affinity of **2-Me-SiR-Br** for cancer cells. This characteristic offers a promising avenue for effective cancer treatment through PDT.

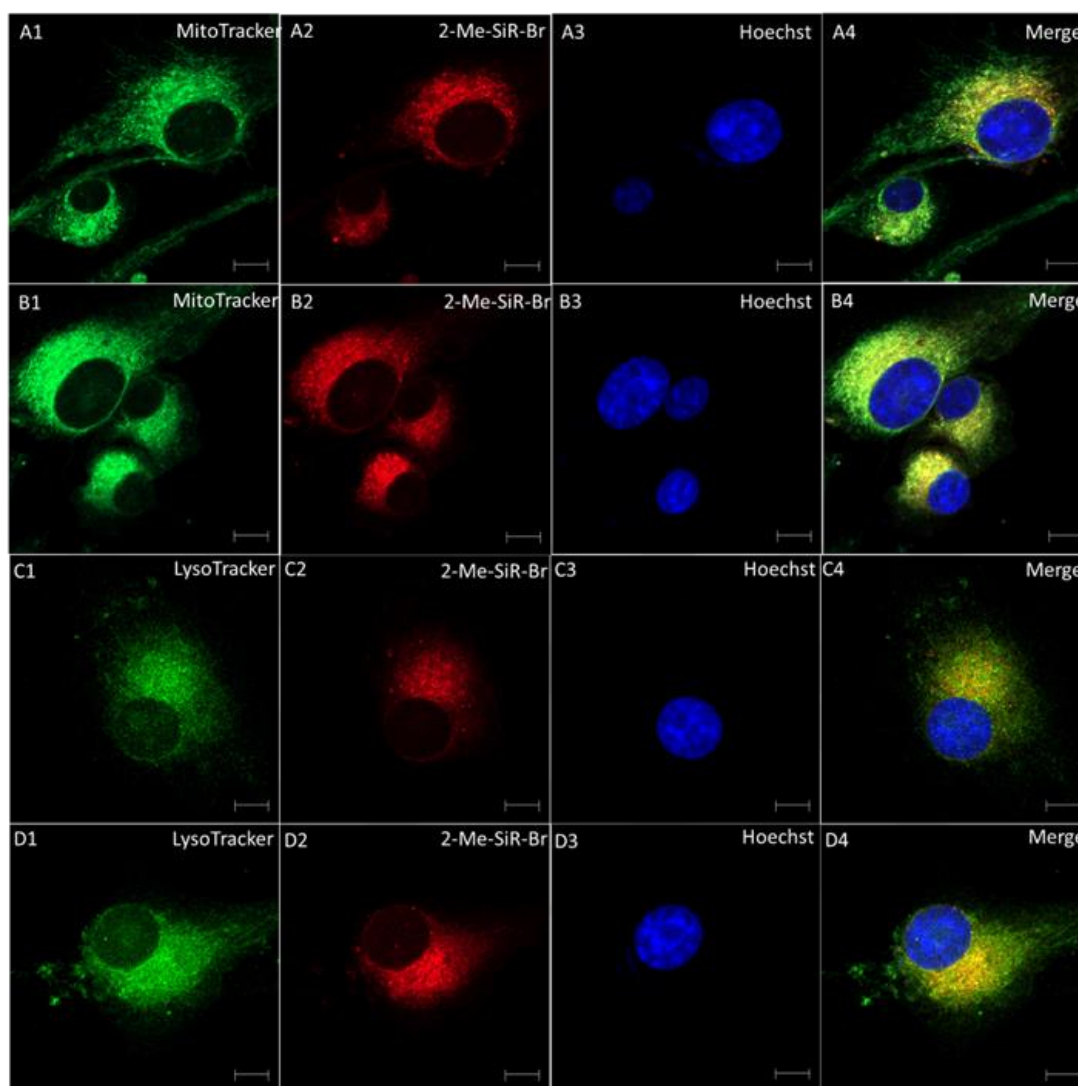


Figure 3.26. Sublocalization of **2-Me-SiR-Br** in U87-MG cells. Mitochondria (A1,B1), lysosome (C1,D1) and nuclei (A3-D3) were labeled with MitoTracker (green), LysoTracker (green) and Hoechst (blue), respectively. **2-Me-SiR-Br** (A2-D2) is shown here as red. Scale bar: 10 μ m.

Furthermore, the intracellular localization of **2-Me-SiR-Br** in glioblastoma cells was investigated using MitoTracker and LysoTracker dyes. Following a 1-hour incubation period with **2-Me-SiR-Br**, localization was detected within both mitochondria and lysosomes, as illustrated in Figure 3.33.

3.3.3.2 Benzoic Acid Bearing Si-Rhodamine PS (Compound **56** and **57**)

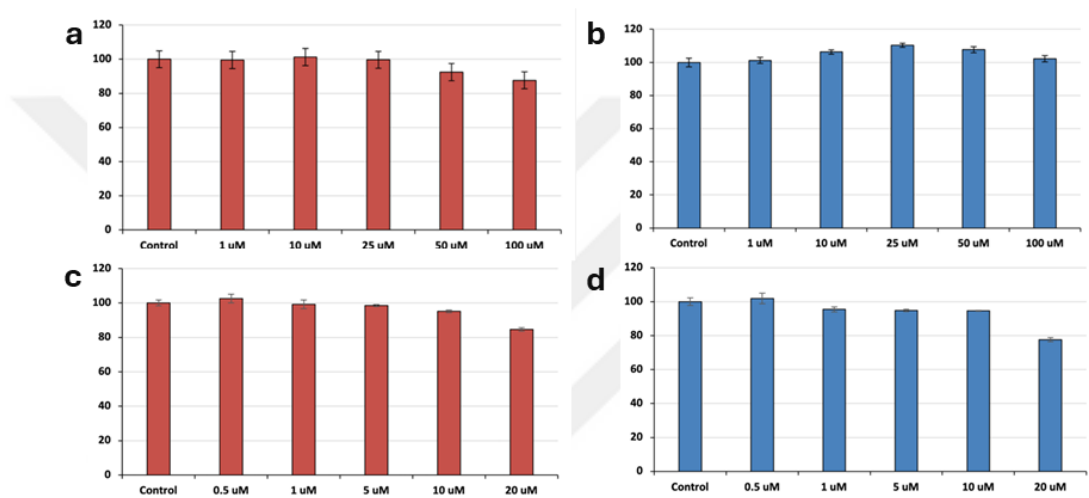
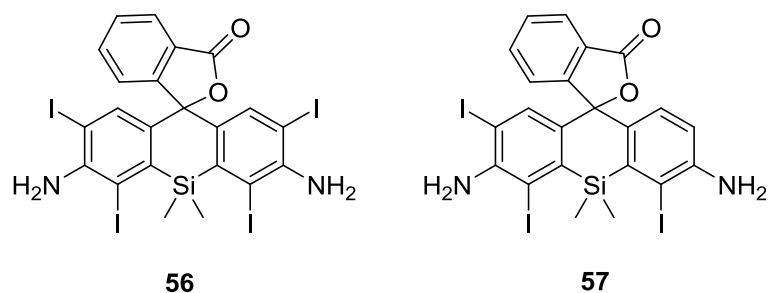


Figure 3.27. **a,b** cell viabilities of L929 cells treated with increasing concentrations (1-100 μM) of compound **56** and **57**, respectively for 24 h dark in a humidified 37°C, 5% CO₂ incubator (n=6) **c,d**. Cell viabilities of SH-SY5Y cells treated with increasing concentrations (1-20 μM) of compound **56** and **57**, respectively then 4 h incubation at dark followed by 5 min 589 nm (450 mW/cm², 50%) laser irradiation and then 20 h at dark (n=3-4)

The PDT efficacies of compounds **56** and **57** were tested in healthy L929 and SH-SY5Y neuroblastoma cell line and as it expected no toxicity toward either healthy or tumor cells were observed due to incompatibility of light and absorption maxima of PS.

3.3.4 Phenoselenazine Based Photosensitizers

3.3.4.1 Morpholine Bearing Phenoselenazine Based PS (NSeMorph)

In vitro peripheral cell culture studies of **NSeMorph** were conducted by Dr. Zübeyir Elmazoğlu.

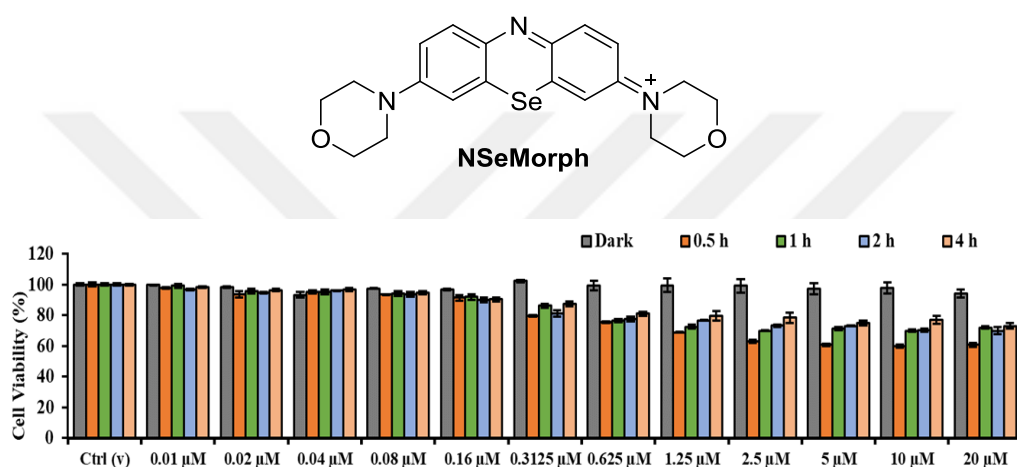


Figure 3.28. Cell viabilities of L929 cell. For dark toxicity analysis, cells were treated with the increasing concentrations (0.01-20 µM) of **NSeMorph** for 24 h at dark in a humidified 37°C, 5% CO₂ incubator. For PDT application, cells were treated at the same concentrations then directly exposed with 660 nm LED light source (1 W chip LED, 15 pcs) at a distance 2 cm for 0.5, 1, 2 or 4 h followed by dark incubation up to 24 h in incubator (n=5-6).

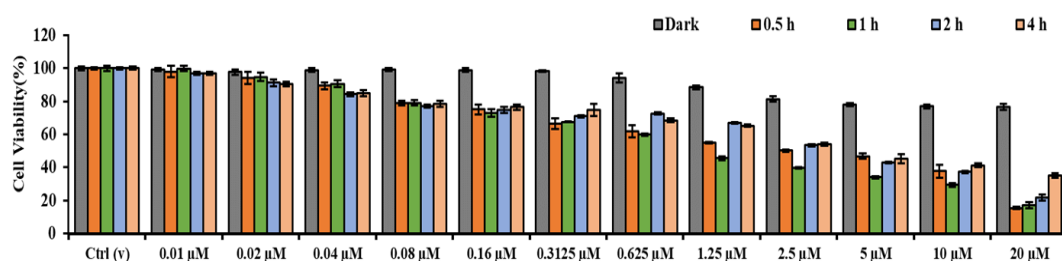


Figure 3.29. Cell viabilities of MDAMB-231 cell. For dark toxicity analysis, cells were treated with the increasing concentrations (0.01-20 μM) of **NSeMorph** for 24 h at dark in a humidified 37°C, 5% CO₂ incubator. For PDT application, cells were treated at the same concentrations then directly exposed with 660 nm LED light source (1 W chip LED, 15 pcs) at a distance 2 cm for 0.5, 1, 2 or 4 h followed by dark incubation up to 24 h in incubator (n=5-6).

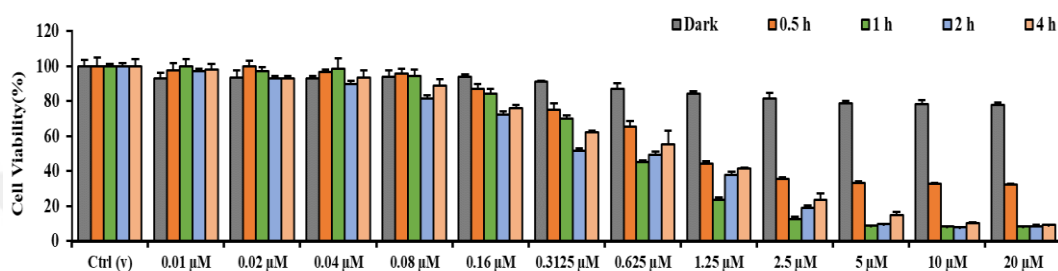


Figure 3.30. Cell viabilities of MCF-7 cell. For dark toxicity analysis, cells were treated with the increasing concentrations (0.01-20 μM) of **NSeMorph** for 24 h at dark in a humidified 37°C, 5% CO₂ incubator. For PDT application, cells were treated at the same concentrations then directly exposed with 660 nm LED light source (1 W chip LED, 15 pcs) at a distance 2 cm for 0.5, 1, 2 or 4 h followed by dark incubation up to 24 h in incubator (n=5-6).

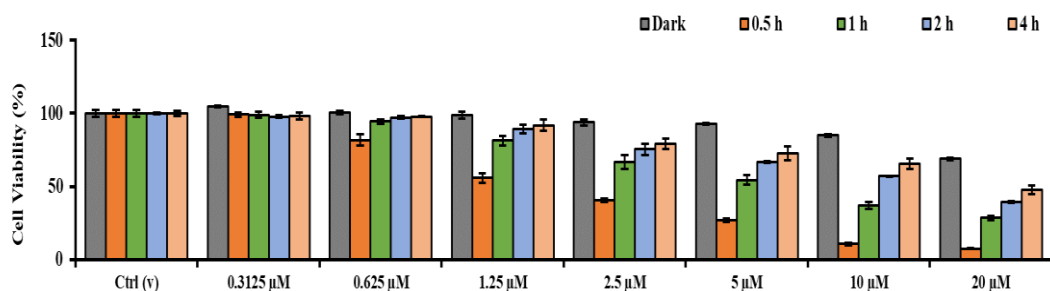


Figure 3.31. Cell viabilities of HeLa cell. For dark toxicity analysis, cells were treated with the increasing concentrations (0.01-20 μM) of **NSeMorph** for 24 h at dark in a humidified 37°C, 5% CO₂ incubator. For PDT application, cells were treated at the same concentrations then directly exposed with 660 nm LED light source (1 W chip LED, 15 pcs) at a distance 2 cm for 0.5, 1, 2 or 4 h followed by dark incubation up to 24 h in incubator (n=5-6).

The photodynamic therapy (PDT) efficacy of **NSeMorph** was investigated in healthy L929 and various peripheral cell lines including triple negative breast cancer (MDAMB-231), breast cancer (MCF-7) and cervix cancer cell (HeLa). A dose-dependent decrease in cell viability was observed upon treatment with **NSeMorph**. According to MTT assay, **NSeMorph** does not exhibit dark toxicity across both healthy and malignant cell lines up to 20 μM **NSeMorph** concentrations. Following the incubation of **NSeMorph** treated both cell lines 24 hours at dark, they were incubated 1 hour more at dark before illuminating them with 660 nm LED light (1 W chip LED, 15 pcs) for various time range (0.5-4 h). Subsequently, the IC₅₀ values for MDAMB-231 cells were determined for each illumination time and for 1 h illumination determined to be 1.17 μM , exhibiting no significant effect on healthy cells at that concentration range. For the MCF-7 and HeLa cells the IC₅₀ values were determined to be 0.57 μM and 6.01 μM , respectively. Despite the active drug profile of **NSeMorph**, the notable disparity in IC₅₀ values between healthy and malignant cells indicates the high affinity of **NSeMorph** for cancer cells. This characteristic offers a promising avenue for effective cancer treatment through PDT.

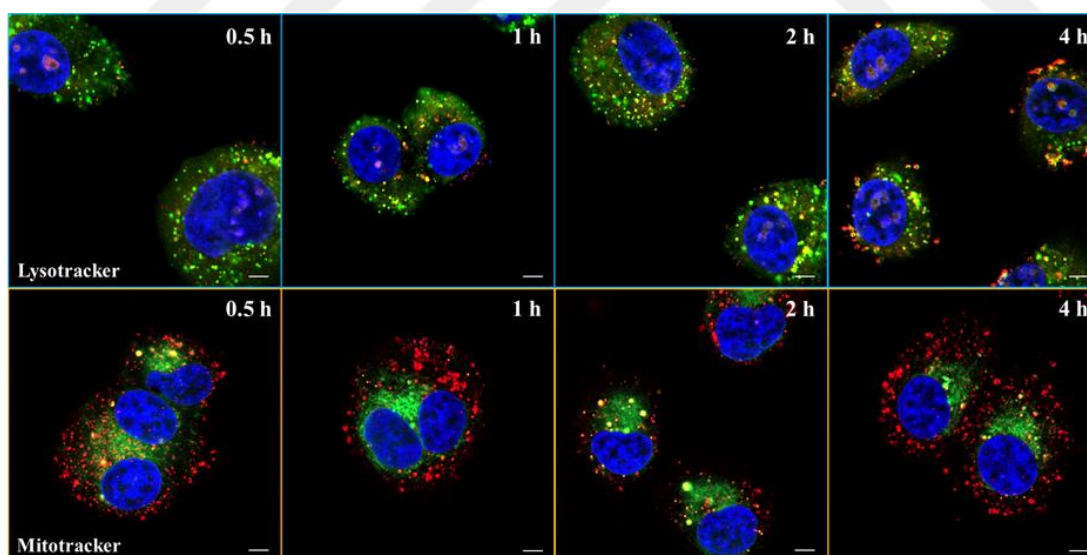


Figure 3.32. Time dependent subcellular localization of **NSeMorph** (2 μM) in MDAMB-231 cells. Blue: Hoechst 33342; Green: Lysotracker (top) or Mitotracker (bottom); Red, **NSeMorph**. Scale bar: 5 μm .

Following the remarkable efficacy of **NSeMorph** on peripheral cell lines, its subcellular localization was investigated in MDAMB-231 in order to realize the underlying reason for high affinity toward cancer cells. The time dependent intracellular localization of **NSeMorph** in MDAMB-231 cells was investigated using MitoTracker and LysoTracker dyes. Following the specified incubation period with **NSeMorph**, localization was detected within both mitochondria and lysosomes, predominantly lysosomal localization, as illustrated in Figure 3.38 and Figure 3.39.

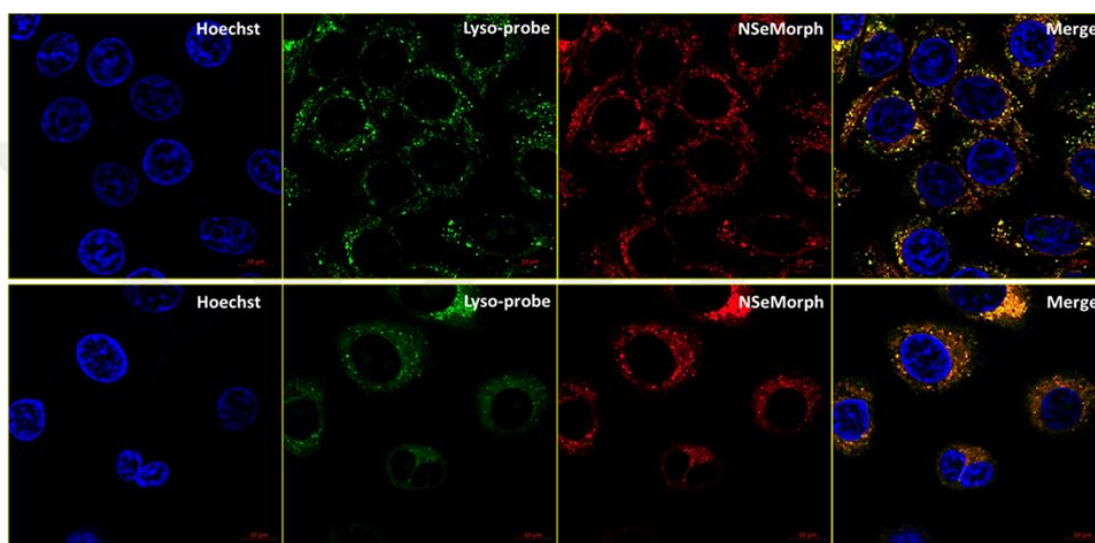


Figure 3.33. Subcellular localization of **NSeMorph** (2 μ M) in HeLa (top) and A549 (bottom) cells. Blue: Hoechst 33342; Green: LysoTracker, Red, **NSeMorph**. Scale bar: 5 μ m.

Furthermore, intracellular localization of **NSeMorph** was investigated in additional cell lines, to verify the consistency of its lysosomal localization affinity. To achieve this, **NSeMorph** treated HeLa and A549 cell lines underwent a localization experiment, revealing a predominant lysosomal localization similar to that observed in MDAMB-231 cells.

In vitro brain cancer cell culture studies of **NSeMorph** were conducted by our group member Elif Yeşilçimen.

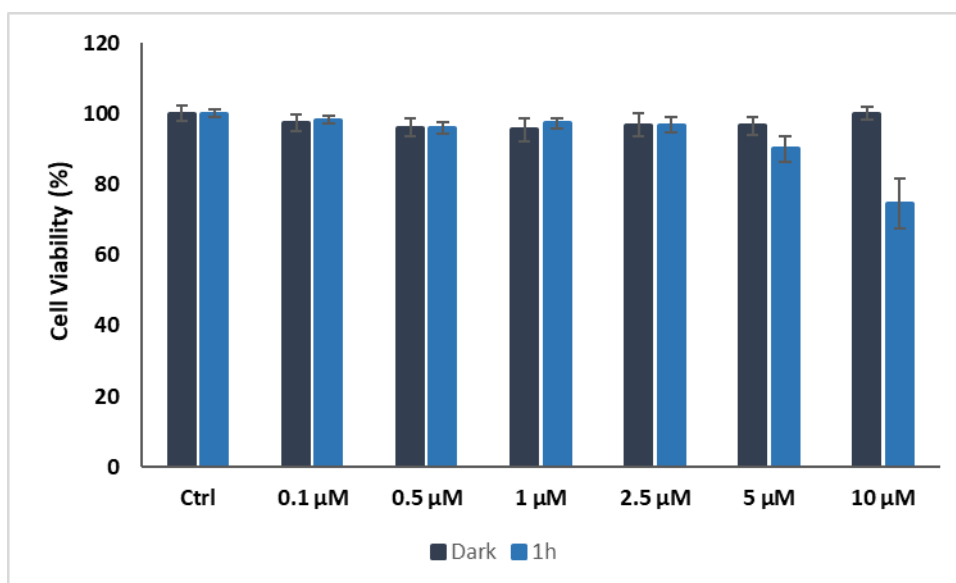


Figure 3.34. Changes in the cell viabilities of L929 treated with varying concentrations of **NSeMorph** (0.1-10 μM). Cells kept in the dark for 24 h for dark toxicity. Cells incubated 1 h in the dark, then irradiated for 2 h for light application (n=6).

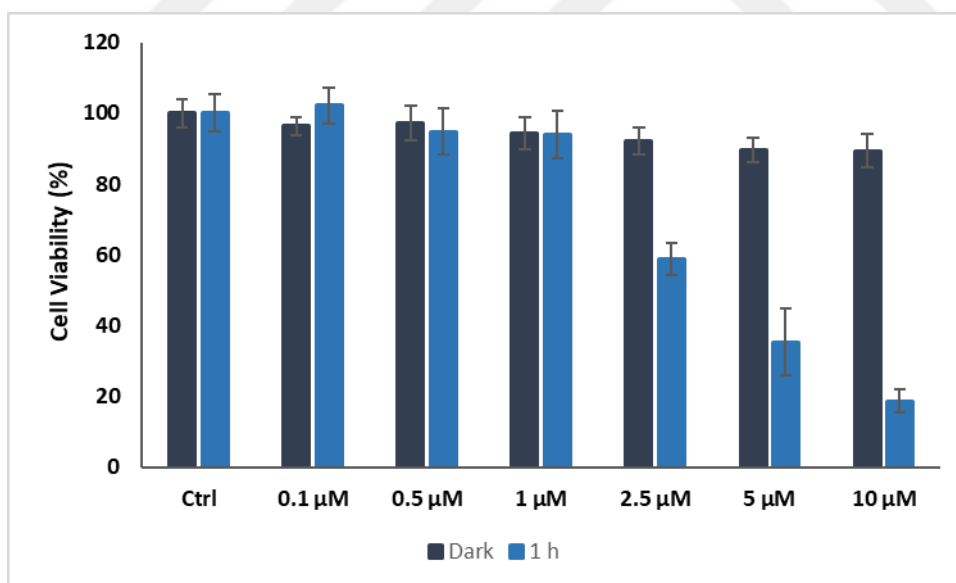


Figure 3.35. Changes in the cell viabilities of U-118 MG treated with varying concentrations of **NSeMorph** (0.1-10 μM). Cells kept in the dark for 24 h for dark toxicity. Cells incubated 1 h in the dark, then irradiated for 2 h for light application (n=6).

After obtaining promising results in peripheral cell lines, PDT efficacy of **NSeMorph** was tested in glioblastoma cell line, U-118 MG. A dose-dependent decrease in cell viability was observed upon treatment with **NSeMorph**. According to MTT assay, **NSeMorph** does not exhibit dark toxicity across both healthy and malignant cell lines up to 10 μM **NSeMorph** concentrations. Following the incubation of **NSeMorph** treated both cell lines 24 hours at dark, they were incubated 1 hour more at dark before illuminating them with 660 nm LED light (1 W chip LED, 15 pcs) for 1 hour. Subsequently, the IC₅₀ values for U-118 MG and U-87 MG cells were determined to be 10.29 μM and 3.47 μM , respectively. Notably exhibiting no significant effect on healthy cells at that concentration range, showing the wide range efficacy of **NSeMorph** on various cancer types. Despite the active drug profile of **NSeMorph**, it exhibited outstanding affinity toward diverse cancer cell lines, offering a promising avenue for effective cancer treatment through PDT.

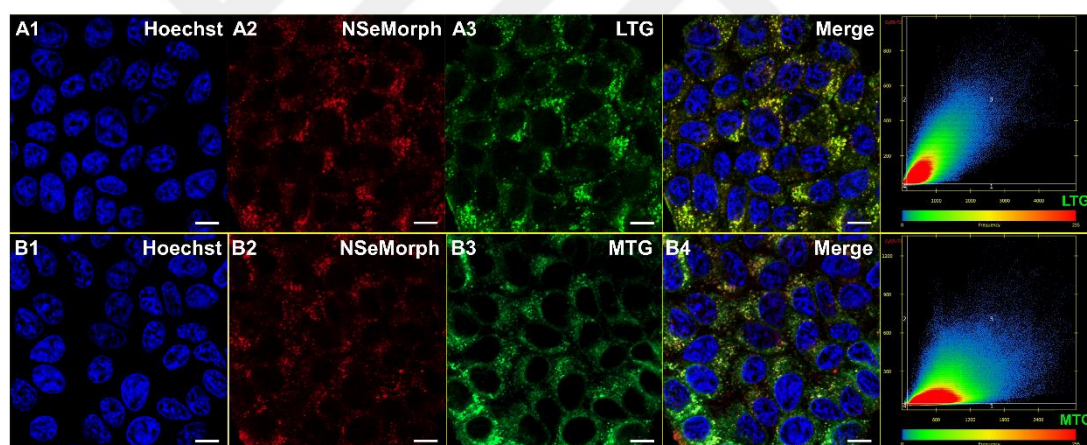


Figure 3.36. The subcellular localization of **NSeMorph** (1 μM , 1 h) in U118 MG cells using LysoTracker Yellow (A1-4) or MitoTracker Green FM (B1-4) and their corresponding Pearson's correlation graphs. Blue: Hoechst 33342; Green: LysoTracker (top) or MitoTracker (bottom); Red, **NSeMorph**. Scale bar: 10 μm

The subcellular localization of **NSeMorph** was investigated in U-118 MG cell line, to verify the consistency of its lysosomal localization affinity. Predominant lysosomal localization was also proven in glioblastoma cell line. The percentage of lysosomal and mitochondrial localization were determined by Pearson's correlation

diagram and coefficients were calculated for mitochondrial localization as 0.58 and for lysosomal localization as 0.81.

3.3.4.1.1 Nitroreductase Activatable NSeMorph (NTR-NSeMorph)

In vitro cell culture studies of **NTR-NSeMorph** were conducted by Dr. Zübeyir Elmazoğlu.

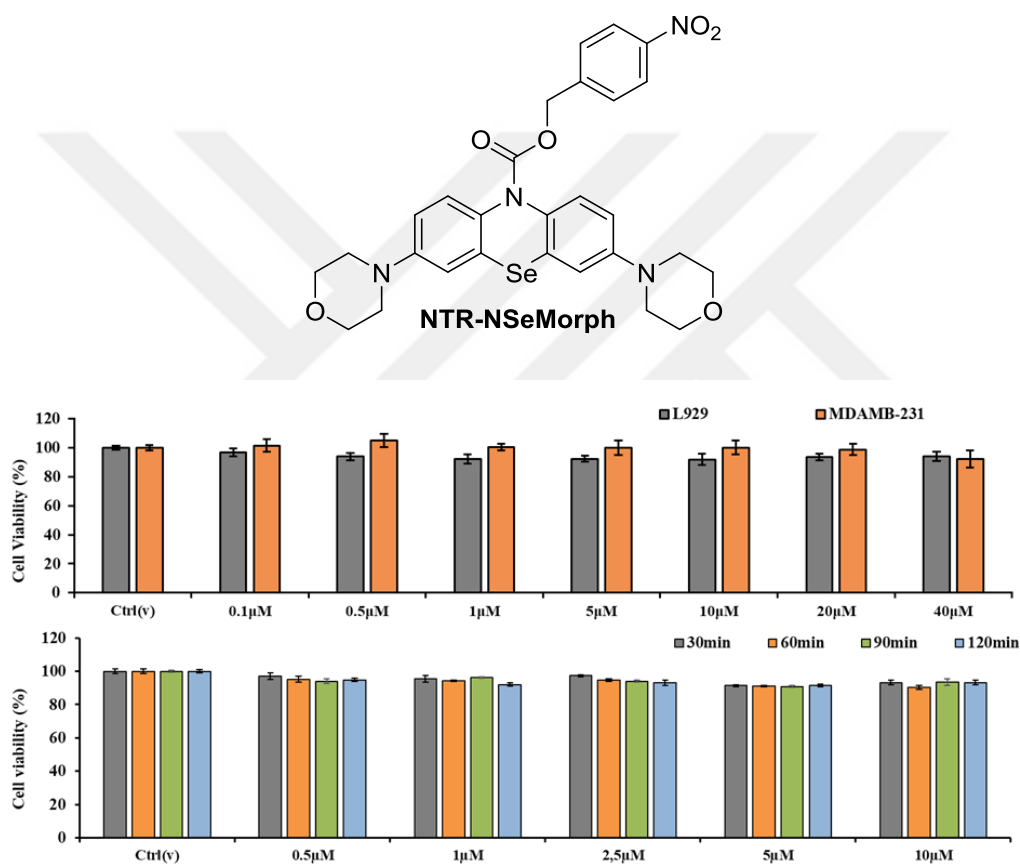


Figure 3.37. Cell viabilities of L929 and MDAMB-231 cells treated with various concentrations (0.1-40 μ M) of **NTR-NSeMorph** for 24 h at dark. (n=6) (top). 30, 60, 90, 120 min incubation at dark followed by 5 min 650 nm (450 mW/cm², 50%) laser irradiation of L929 cells treated with **NTR-NSeMorph** and then 20 h at dark (n=3-4) (bottom).

The cell viabilities of healthy L929 fibroblast cell line and MDAMB-231 triple negative breast cancer was examined in the presence of photosensitizer. Any toxicity toward either cell line was not observed. It is well known that Nitroreductase enzymes overexpress at hypoxic condition. To mimic these conditions and enhance efficacy of photosensitizer in *in vitro* studies our efforts continue.

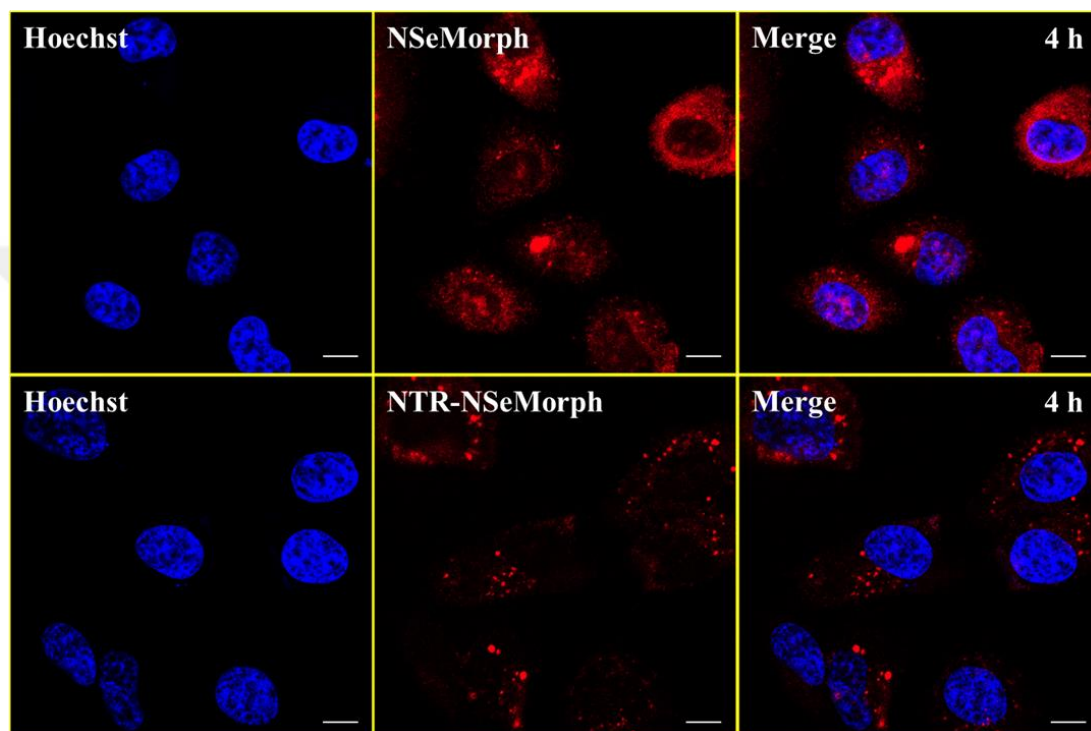


Figure 3.38. Confocal images of **NSeMorph** (2 μ M) and **NTR-NSeMorph** (2 μ M) in MDAMB-231 cells after 4 h incubation. Blue: Hoechst 33342; Red, NSeMorph. Scale bar: 10 μ m.

Furthermore, the intracellular localization of **NTR-NSeMorph** in MDAMB-231 cells was investigated using Hoechst nucleus dye to detect cleavage of handle group in cellular medium. The bare activation of **NTR-NSeMorph** was detected by confocal images.

3.3.4.2 Azetidine Bearing Phenoselenazine Based PS (NSeAze)

In vitro brain cancer cell culture studies of NSeAze were conducted by our group member Elif Yeşilçimen.



Figure 3.39. Changes in the cell viabilities of L929 treated with varying concentrations of NSeAze (0.1-10 μ M). Cells kept in the dark for 24 h for dark toxicity. Cells incubated 1-4 h in the dark, then irradiated for 30 min for light application in fresh medium (n=4,6).

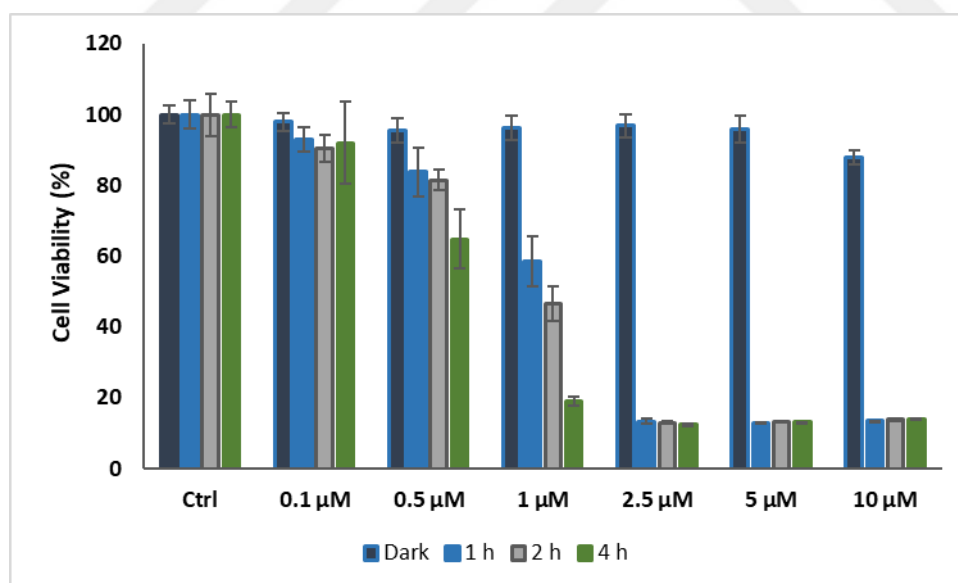


Figure 3.40. Changes in the cell viabilities of U-118 MG treated with varying concentrations of NSeAze (0.1-10 μ M). Cells kept in the dark for 24 h for dark toxicity. Cells incubated 1-4 h in the dark, then irradiated for 30 min for light application in fresh medium (n=4,6).

PDT efficacy of **NSeAze** was tested in healthy L929 and glioblastoma cell line, U-118 MG. A dose-dependent decrease in cell viability was observed upon treatment with **NSeAze**. According to MTT assay, **NSeAze** does not exhibit dark toxicity across both healthy and malignant cell lines up to 10 μM **NSeAze** concentrations. Following the incubation of **NSeAze** treated both health and glioma cell lines 24 hours at dark, they were incubated 1 hour more at dark before illuminating them with 660 nm LED light (1 W chip LED, 15 pcs) for 0.5 hours. Subsequently, the IC₅₀ values for L929, U-118 MG cells were determined to be 1.14 μM and 3.44 μM respectively. Although **NSeAze** exhibited lower singlet oxygen quantum yield than **NSeMorph**, it demonstrated lower IC₅₀ values than **NSeMorph** in gliomas, unfortunately also in healthy cells. To understand these phenomena, efforts are focused on cell death mechanism both for **NSeMorph** and **NSeAze** mediated PDT. To enhance the glioma selectivity of **NSeAze** the introduction of glioma selective handle group was pursued.

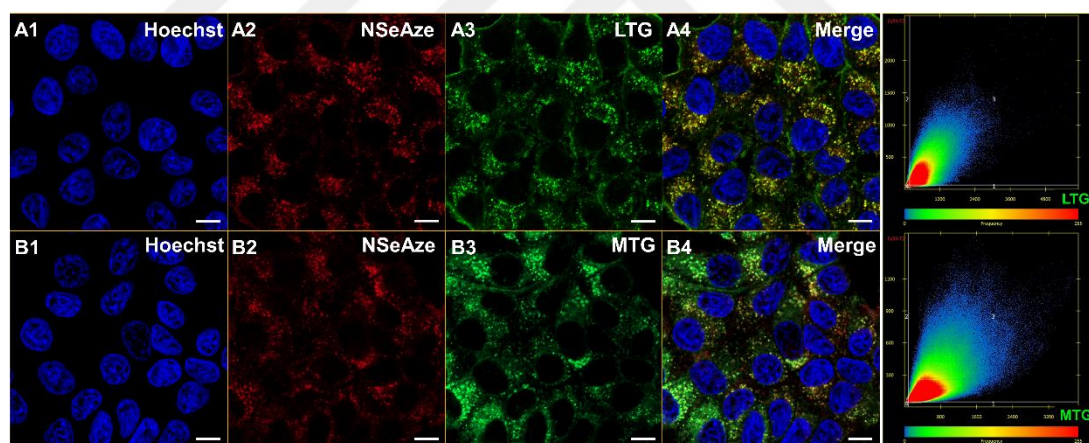


Figure 3.41. The subcellular localization of **NSeAze** (1 μM , 1 h) in U118 MG cells using LysoTracker Yellow (A1-4) or MitoTracker Green FM (B1-4) and their corresponding Pearson's correlation graphs. Blue: Hoechst 33342; Green: LysoTracker (top) or MitoTracker (bottom); Red, **NSeAze**. Scale bar: 10 μm

The subcellular localization of **NSeAze** in U-87 MG cell line was investigated using MitoTracker and LysoTracker dyes. Following a 0.5 hour incubation period with **NSeAze**, the percentage of lysosomal and mitochondrial localization were

determined by Pearson's correlation diagram and coefficients were calculated for mitochondrial localization to be 0.69 and for lysosomal localization as 0.78.



CHAPTER 4

CONCLUSION

In this thesis, the objective was to realize novel fluorophores and photosensitizers capable of absorbing and emitting in the FR/NIR region for effective photodynamic action-based treatment of brain cancer. To achieve this, several materials were developed and supported by theoretical calculations.

In the first part of the thesis, heavy atom bearing and brain cancer targeted resorufin based PSs were designed and synthesized to evaluate the effectiveness of brain cancer targeting handle groups, along with enhanced PDT action facilitated by heavy atoms such as iodine or selenophene moieties. Initially, Leucine aminopeptidase activatable iodinated resorufin, **LAP-RI**, was developed and its active form RI was synthesized to compare efficacy of prodrug with its active analogue. After several synthetic modifications, **LAP-RI** was successfully synthesized and its photophysical properties were investigated, followed by *in vitro* studies. These efforts led to the realization of LAP activatable resorufin based PS with a high affinity for neuroblastomas over healthy cells. Following the promising results with **LAP-RI**, a β -galactosidase activatable, selenophene bearing analogue, **β -Gal-RSe**, was developed and synthesized to achieve a red shifted absorbance maximum and enhanced singlet oxygen generation yield. However, the anticipated results were not obtained in the investigation of photophysical properties. To address this, an acetylene bearing analogue was developed with current efforts focused on the development of **β -Gal-RSeA**.

In the second part of the thesis, realization of the first activatable Si-fluorescein based PSs were aimed. Initially, the first β -galactosidase activatable, iodinated Si-fluorescein based photosensitizer, **β -Gal-SiF-II** was synthesized and employed in *in vitro* cell culture studies utilizing glioblastoma cell lines. These studies demonstrated

selective tumor elimination and localization within the mitochondria and lysosome. This dual intracellular localization underscores the potential multifaceted roles of **β -Gal-SiF-II**, suggesting its viability as a promising theranostic agent within glioblastoma therapy. Following the promising results obtained with **β -Gal-SiF-II**, efforts were directed towards developing its dual activatable analogue, **β -Gal-TN-C-SiF-II**. Current endeavors are concentrated on realizing this target photosensitizer to enhance the glioma selectivity of Si-fluorescein based photosensitizers.

In the third part of the thesis, the objective was to realize the first Si-rhodamine based photosensitizers for PDT applications. To achieve this goal, several Si-rhodamine based photosensitizers, decorated with heavy atoms were developed. Initially, an o-toly-bearing, brominated Si-rhodamine based PS was synthesized. The efficacy of the obtained active PSs was evaluated through *in vitro* cell culture studies employing both healthy fibroblast cell lines and malignant glioblastoma cell lines. Despite absence of any tumor targeting moiety on **2-Me-SiR-Br**, it exhibited remarkable glioma selectivity over healthy cell lines. Additionally, the realization of **2-COOH-SiR-I** and **TN-C-SiR-I** PSs was pursued and several attempts were made for their synthesis. Current endeavors are focused on the development of these PSs to elevate the affinity of Si-rhodamine based PSs toward tumor cells.

The promising results observed with single modified xanthene-based PSs such as resorufin, Si-fluorescein and Si-rhodamine along with the successive utilization of brain cancer targeted handle groups, led to the development of their multi-modified analogues to verify the feasibility of our main hypothesis. To achieve this, phenoselenazine based PSs were developed. Initially, **NSeMorph** was synthesized and exhibited one of the highest singlet oxygen quantum yields in aqueous medium reported in the literature. Furthermore, it demonstrated widespread selective effect on various cancer types, including peripheral cell lines and gliomas. To enhance selectivity towards specific cancer type, several activatable **NSeMorph** derivatives were designed, such as **NTR-NSeMorph**, **β -Gal-NSeMorph**, **H₂S-NSeMorph**, **H₂O₂-NSeMorph**. among these, **NTR-NSeMorph** was successfully synthesized

and employed in photophysical investigations and in vitro cell culture studies. However, the expected results were not yet achieved and optimization studies are ongoing. Furthermore, the selenium oxide analogue of **NSeMorph**, **NSeOMorph** was developed to further shift the absorption maximum and efforts are being made to synthesize the target PS. Current efforts are concentrated on developing **NSeOMorph**. Additionally, an azetidine modified phenoselenazine based PS, **NSeAze** was devised and successfully synthesized to fine-tune the photophysical properties of its morpholine bearing analogue, **NSeMorph**. This modification resulted in a 7-fold enhancement in fluorescence intensity along with significant reduction in singlet oxygen generation capacity was obtained. However, **NSeAze** showed elevated cytotoxicity toward gliomas which is currently under investigation. Unfortunately, **NSeAze** also showed activity toward healthy cells. To improve tumor selectivity, a glioma selective **NSeAze** analogue will be pursued. In addition to morpholine and azetidine bearing phenoselenazine derivatives, an oxygen bearing analogue was developed to facilitate incorporation of handle group into the PS. Synthetic efforts are currently underway for **NSeO**. Due to the possible lower range absorption and emission maxima expectations of **NSeO**, its annulated derivative, **NSeO-A** has been developed to shift absorption and emission maxima towards FR/NIR region through extension of conjugation, synthesis of **NSeO-A** was achieved successfully and it exhibited absorption maximum at 689 nm, consisted with preliminary theoretical calculations. Ongoing efforts are focused on purifying **NSeO-A** to obtain the target PS in high purity.

Following the phenoselenazine based PSs, acridone based multi-modified fluorophores and PSs were developed and their synthesis were attempted. Initially, instead of selenium atom, carbonyl bearing analogue, **NCMorph**, was devised. Despite several attempts were made to realize the target fluorophore, **NCMorph** could not be obtained due to the high electron withdrawing effect of the carbonyl moiety. To mitigate this effect and explore the influence of functional groups on photophysical properties, various fluorophores and PSs were designed. Significant efforts have been directed towards the synthesizing these derivatives, with current

work focusing on the purification of the target fluorophores **OMe-CN-NCMorph** and **OMe-Bu-NCMorph**. Once the desired photophysical properties were achieved, halogen bearing derivatives will be developed for PDT applications. Following this, in the line with the methodologies used for the synthesizing Si-fluorescein and Si-rhodamine analogues, benzoic acid or 2,5-benzyl alcohol bearing compounds were introduced to the carbonyl moiety within the acridone core. Fluophores **2,5-CH₂OH-NCMorph** and **2-COOH-NCMorph** were synthesized successfully and currently efforts concentrated on purification to obtain the target fluorophores in a high purity and development of their iodinated analogues.

In the final part of the thesis, similar to the acridone derivatives, several carbon dimethyl bearing analogues were devised. Initially, a di-morpholine bearing acridine based fluorophore and PS were targeted. Currently, purification studies ongoing to obtain the target fluorophore, **DDAMorph**. Once the **DDAMorph** is developed, it will be halogenated to realize the target PS, **DDAMorph-I**. Additionally, attempts were made to synthesize a rhodol-like derivative, **DDAN-Br** but, to realize the target PS, **DDAN-Br**, new approaches have emerged. Finally, another acridine based PS, **DDAO-Br** was devised and synthesized and it exhibited absorption maximum at 648 nm, consistent with preliminary theoretical calculations.

In conclusion, a wide range of FR/NIR absorbing photosensitizer for the treatment of brain cancer have been developed and their PDT efficacy have been demonstrated in *in vitro* cell culture studies and several more PSs are currently underway and their potential as diagnostic and therapeutic agents will be evaluated in due course.

CHAPTER 5

EXPERIMENTAL

5.1 General Information

The starting materials and solvents were purchased from Sigma Aldrich, abcr, TCI, and Merck. Solvents used for column chromatography, Hexane, EtOAc, and DCM, were distilled prior to use over CaCl₂. All reactions were conducted under a nitrogen atmosphere, unless otherwise specified. Reaction solvents (Diethyl ether, THF, DCM, Toluene, and DMF) were directly used from MBraun MBSPS5 solvent drying system. The reactions were monitored by thin-layer chromatography (TLC) (Merck Silica Gel 60 F254) and visualized by UV light at 245 nm and 366 nm. Column chromatography of all products was performed using Merck Silica Gel (particle size: 0.040-0.063 mm, 230-400 mesh ASTM). Microwave reactions were conducted using Biotage initiator systems.

Reverse phase HPLC analyses were conducted using Agilent Technologies 1260 Infinity II series HPLC systems with DAD detector. All the analyses were performed by using gradient elution with different Milli Q (0.1% TFA) and acetonitrile (0.8% TFA) systems as mobile phase and the column compartment temperature was 40°C. HPLC purifications were performed using a Phenomenex Kinetex 5 µm C18 100 A semi-preparative column with a flow rate of 1.5 mL/min. And for the purities, an Agilent Technologies Poroshell 120 EC-C18 analytical column was used with a flow rate of 0.5 ml/min.

The structural analysis of the synthesized compounds was conducted using NMR and HRMS. ¹H and ¹³C nuclear magnetic resonance spectra of the compounds were recorded in deuterated solvents with Bruker Avance III Ultrashield 400 Hz NMR spectrometer. The chemical shifts were stated in parts per million (ppm) with tetramethylsilane (TMS) as an internal reference. Spin multiplicities were indicated

as s (singlet), d (doublet), dd (doublet of doublet), t (triplet), tt (triplet of triplet), m (multiplet), and coupling constants (J) were reported as in Hz (Hertz). ^1H and ^{13}C NMR spectra of compounds were given in Appendix A. NMR spectra were processed with MestReNova program. High-Resolution Mass Spectra (HRMS) were processed for the novel compounds using Time of Flight mass analyzer with Water Synapt MS System. The HRMS of synthesized compounds are given in Appendix B.

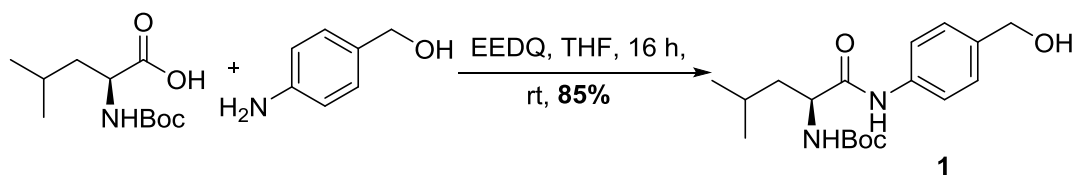
The photophysical analyses of the final compounds were examined through absorbance and fluorescence measurements. Absorption spectra were collected using a double beam Jasco V-730 (Easton, MD, USA) UV-vis spectrophotometer. A Cary Eclipse fluorescence spectrophotometer (Santa Clara, CA, USA) equipped with a Cary single cell peltier (Agilent Technologies) temperature controller was used to record the fluorescence emission spectra. All measurements were performed at room temperature using 10 mm quartz (3.5 mL, 111-QS, Hellma).

Lithium-halogen exchange, Buchwald-Hartwig amination, and aromatic halogenation reactions are well-documented in the literature, with numerous examples available. The fundamental principles and literature examples for their application in dye and PS synthesis are utilized in this study extensively. These reactions, as adopted from the literature, are summarized in Section 1.2.7.

5.2 Synthesis of Resorufin Based Photosensitizers

5.2.1 Synthesis of LAP Activatable Iodo-Resorufin PS (LAP-RI)

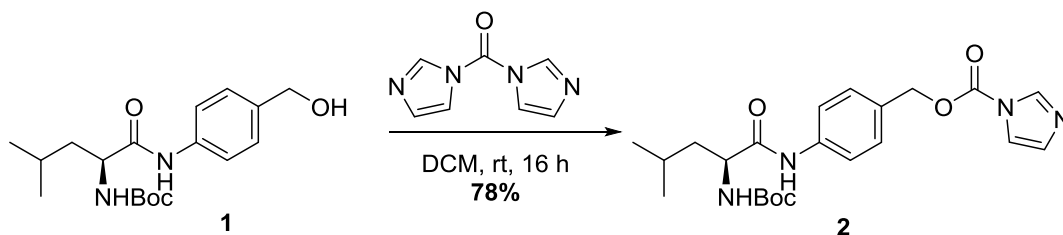
5.2.1.1 Synthesis of Compound 1



Scheme 5. 1. Synthetic route for compound 1.

To a 50 mL two-necked round-bottomed flask, commercially available N-(tert-butoxycarbonyl)-L-leucine (0.50 g, 2.0 mmol) and 4-aminobenzyl alcohol (0.12 g, 2.1 mmol, 1.1 eq.) were added, and vacuum- N_2 was applied. Then, mixture was dissolved with dry THF (14 mL). Subsequently, commercially available N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ, 0.52 g, 2.1 mmol, 1.1 eq.) was added and resulting mixture stirred for 16 hours at room temperature. After completion of the reaction, THF was removed under vacuum. The mixture was then quenched with distilled water (50 mL) and extracted with DCM. Collected organic phases dried over Na_2SO_4 , filtered and solvent evaporated. The crude product purified with column chromatography (silica, MeOH:DCM 5:95). Target product obtained as white solid (0.57 g, 85%). ^1H NMR (400 MHz, $CDCl_3$) δ 9.62 (s, 1H), 7.34 (d, J = 8.2 Hz, 2H), 6.99 (d, J = 8.1 Hz, 2H), 5.91 (s, 1H), 4.47 (s, 2H), 4.09 (s, 1H), 1.73 (ddd, J = 14.2, 10.9, 5.3 Hz, 2H), 1.60 (dd, J = 15.1, 6.5 Hz, 1H), 1.38 (s, 9H), 0.96 (d, J = 6.5 Hz, 3H), 0.93 (d, J = 6.4 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.4, 156.7, 137., 136.45, 127.5, 119.9, 80.2, 64.5, 54.0, 41.5, 28.4, 24.8, 23.2, 21.7.

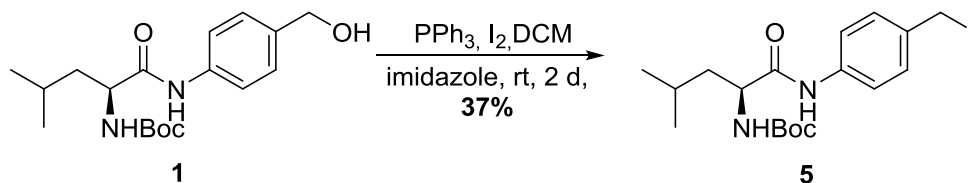
5.2.1.2 Synthesis of Compound 2



Scheme 5. 2. Synthetic route for compound 2.

To a 50 mL Schlenk tube, compound 1 (0.27 g, 0.8 mmol) and commercially available carbonyldiimidazole (0.17 g, 1.0 mmol, 1.3 eq.) were added. and vacuum- N_2 was applied. Then, mixture was dissolved with dry DCM (7 mL) and stirred for 16 hours at room temperature. After completion of reaction, solvent was evaporated and the crude product was purified with column chromatography (silica, MeOH:DCM, 3:97). The target product was obtained as white solid. (0.26 g, 78%).

5.2.1.3 Synthesis of Compound 5

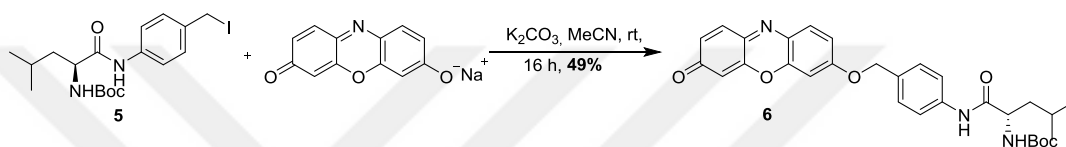


Scheme 5. 3 Synthetic route for compound 5.

To a 50 mL Schlenk tube, PPh_3 (0.61 g, 2.3 mmol, 1.5 eq.) and I_2 (0.60 g, 2.3 mmol, 1.5 eq.) were added and vacuum- N_2 was applied. Then, mixture was dissolved with dry DCM (7 mL) and stirred for 10 minutes at room temperature. Following this, imidazole (1.72 g, 1.1 mmol, 0.7 eq.) was added to the mixture and stirred 10 more minutes. Subsequently compound 1 (0.52 g, 1.5 mmol) was added to the reaction mixture and stirred for 2 days at room temperature. After completion of the reaction, mixture quenched with distilled water (25 mL) and extracted. Collected organic phases dried over Na_2SO_4 , filtered and solvent evaporated. The crude product

purified with column chromatography (silica, MeOH:DCM 3:97). Target product, compound **5**, obtained as white solid (0.26 g, 37%). ^1H NMR (400 MHz, CDCl_3) δ 9.38 (s, 1H), 7.38 (d, $J = 8.1$ Hz, 2H), 7.17 (d, $J = 7.9$ Hz, 2H), 5.63 (s, 1H), 4.42 (s, 3H), 1.82 – 1.73 (m, $J = 13.0, 6.5$ Hz, 1H), 1.71 – 1.61 (m, $J = 12.9, 6.9$ Hz, 2H), 1.41 (s, 9H), 0.95 (dt, $J = 11.4, 6.5$ Hz, 6H). ^{13}C NMR (100 MHz, CDCl_3) δ 171.9, 156.7, 137.8, 134.7, 129.4, 120.1, 80.5, 54.1, 41.2, 28.5, 24.9, 23.2, 21.8.

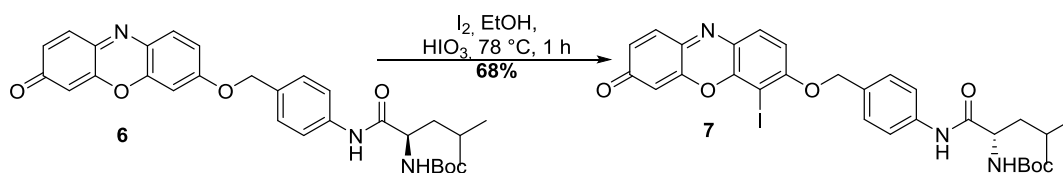
5.2.1.4 Synthesis of Compound 6



Scheme 5. 4. Synthetic route for compound **6**.

To a 25 mL Schlenk tube, compound **5** (70.0 mg, 0.20 mmol, 1.25 eq.) and commercially available resorufin sodium salt (26.4 mg, 0.12 mmol) were added and vacuum- N_2 was applied. Then, mixture was dissolved with dry DMF (5 mL). To this solution K_2CO_3 (34.3 mg, 0.24 mmol, 2 eq.) was added. The resulting suspension was stirred 16 hours at room temperature. After completion of reaction, mixture was quenched with distilled water (25 mL) and extracted with EtOAc (50 mL). Collected organic phases washed with brine solution (50 mL), then dried over Na_2SO_4 , filtered and solvent evaporated. The crude product purified with column chromatography (silica, MeOH:DCM 5:95). Target product, compound **6**, obtained as orange solid (32.0 mg, 49%). ^1H NMR (400 MHz, CDCl_3) δ 8.69 (s, 1H), 7.69 (d, $J = 8.9$ Hz, 1H), 7.56 (d, $J = 7.9$ Hz, 2H), 7.41 (d, $J = 9.8$ Hz, 1H), 7.34 (d, $J = 7.5$ Hz, 2H), 6.98 (d, $J = 10.5$ Hz, 1H), 6.83 (d, $J = 7.8$ Hz, 2H), 6.31 (s, 1H), 5.09 (s, 2H), 5.05 (d, $J = 7.8$ Hz, 1H), 4.28 (s, 1H), 1.78 – 1.70 (m, 3H), 1.45 (s, 10H), 0.96 (t, $J = 6.5$ Hz, 7H). ^{13}C NMR (100 MHz, CDCl_3) δ 186.5, 171.1, 162.8, 156.5, 149.9, 145.8, 145.7, 138.4, 134.8, 134.4, 131.7, 131.1, 128.6, 128.5, 120.2, 114.4, 106.9, 101.2, 80.8, 70.7, 53.9, 40.5, 36.6, 31.6, 28.5, 24.9, 23.1, 22.1.

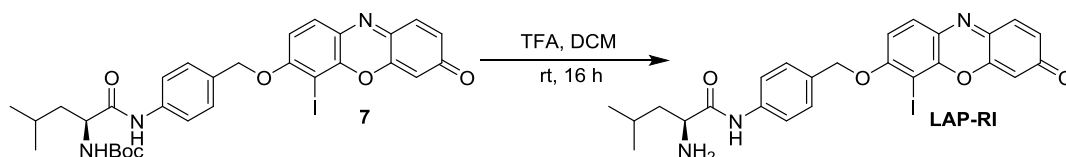
5.2.1.5 Synthesis of Compound 7



Scheme 5. 5. Synthetic route for compound 7.

To a 25 mL Schlenk tube, compound 6 (32.0 mg, 0.06 mmol), was added and vacuum- N_2 was applied. Then, mixture was dissolved with EtOH (7 mL). Subsequently, molecular iodine (38.2 mg, 0.15 mmol, 2.50 eq.) was added to the reaction mixture and temperature was raised to the $60\text{ }^\circ\text{C}$. Resulting mixture was stirred for 10 minutes at that temperature. Following this, HIO_3 solution (5.30 mg, 0.03 mmol, 0.50 eq. in 0.50 mL H_2O) was added dropwise to the reaction mixture. After completion of the addition, temperature was raised to the $78\text{ }^\circ\text{C}$ and stirred 1 more hour at that temperature. Then, EtOH was evaporated and quenched with 10% $Na_2S_2O_3$ solution (10 mL) and extracted with EtOAc. Collected organic phases dried over Na_2SO_4 , filtered and solvent evaporated. The crude product purified with column chromatography (silica, MeOH:DCM 5:95). Target product obtained as dark orange solid (27.0 mg, 68%). ^1H NMR (400 MHz, $CDCl_3$) δ 8.95 (s, 1H), 7.73 (d, $J = 8.8$ Hz, 1H), 7.57 (d, $J = 7.8$ Hz, 2H), 7.41 (d, $J = 9.7$ Hz, 1H), 7.33 (d, $J = 7.0$ Hz, 2H), 7.02 (d, $J = 9.7$ Hz, 2H), 6.98 (s, 1H), 5.23 (d, $J = 7.8$ Hz, 1H), 5.10 (s, 2H), 4.37 (s, 1H), 1.80 – 1.74 (m, 2H), 1.68 – 1.61 (m, 1H), 1.47 (s, 9H), 0.98 (dt, $J = 8.4$, 6.1 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 180.5, 171.1, 163.0, 150.8, 145.7, 144.1, 138.2, 134.5, 131.9, 131.3, 130.5, 128.5, 128.3, 119.9, 119.6, 115.0, 100.9, 85.1, 80.6, 70.6, 53.8, 40.5, 29.6, 28.2, 24.7, 22.9, 21.7.

5.2.1.6 Synthesis of LAP-RI

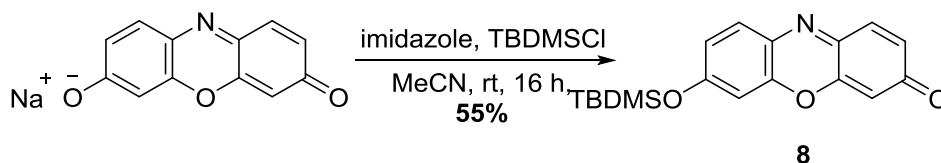


Scheme 5. 6. Synthetic route for **LAP-RI**.

To a 25 mL Schlenk tube, compound **7** (27.0 mg, 0.05 mmol) was added and vacuum-N₂ was applied. Then, compound **7** was dissolved with dry DCM (5 mL). Subsequently, TFA (0.30 mL) was added slowly and mixture stirred for 16 hours at room temperature. After completion of reaction, DCM and TFA was evaporated and the crude product was purified with column chromatography (silica, MeOH:DCM 5:95). Target product obtained as dark orange solid. In order to utilize LAP-RI in in vitro studies, **LAP-RI** was further purified with reverse phase HPLC. ¹H NMR (400 MHz, CDCl₃) δ 9.66 (s, 1H), 7.76 (d, *J* = 9.5 Hz, 1H), 7.68 (d, *J* = 8.4 Hz, 2H), 7.48 – 7.37 (m, 3H), 7.09 – 7.04 (m, 2H), 7.03 (d, *J* = 9.7 Hz, 1H), 5.17 (s, 2H), 5.00 (d, *J* = 18.8 Hz, 1H), 1.49 – 1.39 (m, 2H), 1.35 (s, 1H), 0.98 (dt, *J* = 10.3, 6.2 Hz, 6H).

5.2.2 Synthesis of Iodinated Resorufin PS (RI)

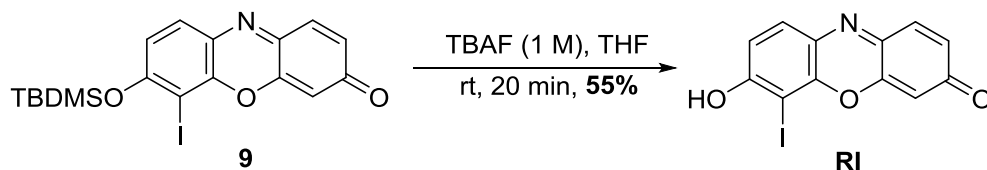
5.2.2.1 Synthesis of Compound 8



Scheme 5. 7. Synthetic route for compound **8**.

To a 100 mL two-necked round-bottomed flask, commercially available resorufin sodium salt (0.12 g, 0.6 mmol) was added and vacuum-N₂ was applied. Then resorufin sodium salt was dissolved with dry MeCN (20 mL), followed by addition

5.2.2.3 Synthesis of Compound **RI**

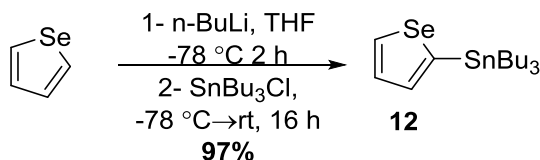


Scheme 5. 9. Synthetic route for compound **RI**

To a 25 mL Schlenk tube, compound **9** (49.0 mg, 0.11 mmol) was added and vacuum–N₂ was applied. After that, it was dissolved with dry THF (5 mL). Subsequently, 1 M TBAF (0.12 mL, 0.12 mmol, 1.10 eq.) was added to the mixture dropwise and stirred for 20 minutes at room temperature. Upon completion of reaction, mixture quenched with 1 M, HCl (50 mL) and extracted with CHCl₃ (50 mL). The collected organic phases dried over Na₂SO₄, filtered and solvent evaporated. The crude product purified with column chromatography (silica, MeOH:DCM 5:95). The target product, **RI**, obtained as orange solid (20.0 mg, 55%). ¹H NMR (400 MHz, MeOD) δ 7.75 (d, *J* = 8.9 Hz, 1H), 7.55 (d, *J* = 9.6 Hz, 1H), 6.97 (d, *J* = 9.6 Hz, 2H), 6.89 (d, *J* = 2.3 Hz, 1H).

5.2.3 Synthesis of β-Gal Activatable Seleno-Resorufin PS (β-Gal -RSe)

5.2.3.1 Synthesis of Compound **12**

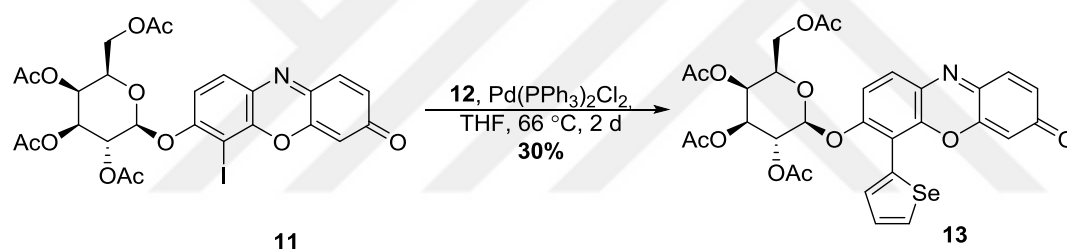


Scheme 5. 10. Synthetic route for compound **12**

To a 50 mL two neck reaction flask, selenophene (1.0 g, 7.6 mmol) was added and dissolved in dry THF (25 mL) under argon atmosphere. Then, solution cooled to -78 °C and n-BuLi (3.4 mL, 8.50 mmol, 2.5 M in hexane) was added dropwise over 40

minutes. Reaction mixture was stirred for 1.5 h at -78 °C. Tributyltin chloride (2.5 mL, 9.2 mmol) was added dropwise over 20 minutes at -78 °C. Then, reaction mixture warm to the room temperature and stirred 16 hours. Then, mixture quenched with distilled water and aqueous phase was extracted with DCM (100 mL). The organic layer was dried over anhydrous Na₂SO₄ and the solvent was evaporated under reduced pressure. The crude product was obtained as brown oil (3.0 g, 95%) and used without making any further purification. ¹H NMR (400 MHz, CDCl₃) δ: 8.40 – 8.32 (m, 1H), 7.57 – 7.45 (m, 2H), 1.63 – 1.52 (m, 6H), 1.42 – 1.29 (m, 6H), 1.14 – 1.08 (m, 6H), 0.95 – 0.86 (m, 9H) ¹³C NMR (100 MHz, CDCl₃) δ: 143.5, 137.9, 135.3, 130.6, 29.0, 27.3, 13.7, 11.1.

5.2.3.2 Synthesis of Compound 13

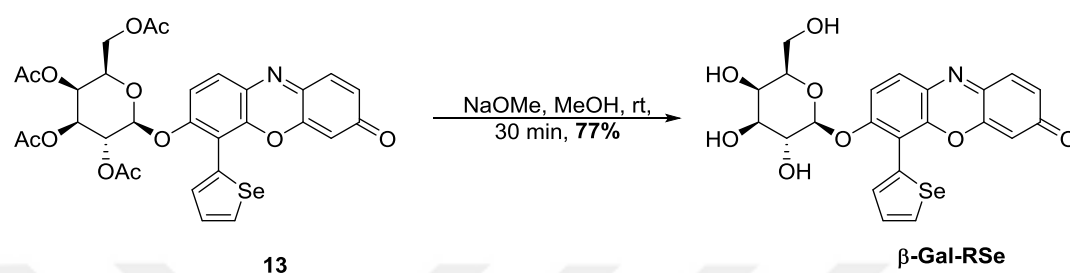


Scheme 5. 11. Synthetic route for compound **13**

To a 25 mL Schlenk tube, compound **11** (50.0 mg, 0.08 mmol) was added and vacuum-N₂ was applied. Then, compound **12** (63.0 mg, 0.15 mmol, 2.00 eq.), Pd(PPh₃)₂Cl₂ (5.20 mg, 10%) were added and dissolved with dry THF (5 mL). Subsequently, reaction mixture heated to 66 °C and stirred for 2 days. Upon completion of reaction, THF was removed and the crude product was purified with column chromatography (silica, MeOH:DCM, 1:99) to yield the target product, compound **13**, as a white solid (15.0 mg, 30%). ¹H NMR (400 MHz, CDCl₃) δ 8.51 (d, *J* = 3.3 Hz, 1H), 8.30 (d, *J* = 5.1 Hz, 1H), 7.79 (d, *J* = 8.8 Hz, 1H), 7.52 (dd, *J* = 5.8, 4.1 Hz, 1H), 7.45 (s, 1H), 7.11 (s, 1H), 7.07 (dd, *J* = 8.8, 2.4 Hz, 1H), 7.00 (d, *J* = 9.7 Hz, 1H), 5.56 (dd, *J* = 10.4, 7.9 Hz, 1H), 5.51 (d, *J* = 2.7 Hz, 1H), 5.26 (d, *J* =

7.9 Hz, 1H), 5.18 (dd, $J = 10.4, 3.3$ Hz, 1H), 4.22 (m, 3H), 2.21 (s, 3H), 2.11 (s, 3H), 2.04 (s, 3H), 2.02 (s, 3H).

5.2.3.3 Synthesis of Compound β -Gal -RSe

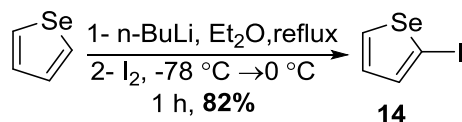


Scheme 5. 12. Synthetic route for compound β -Gal -RSe

To a 25 mL Schlenk tube, compound **13** (45.0 mg, 0.07 mmol) was added, and vacuum- N_2 was applied. Then, it was dissolved with MeOH (7 mL). To this solution, NaOMe (6.71 mg, 0.29, 4.4 eq.) in MeOH (1.0 mL) solution added dropwise and stirred for 30 minutes. Upon completion of reaction, mixture neutralized with amberlyst, then filtered. Collected filtrate evaporated and the crude product purified with preparative HPLC. The target product, β -Gal -RSe, obtained as dark orange solid (26.0 mg, 77%). ^1H NMR (400 MHz, DMSO) δ 8.58 (d, $J = 4.0$ Hz, 1H), 8.36 (d, $J = 5.8$ Hz, 1H), 7.88 (d, $J = 8.8$ Hz, 1H), 7.60 (d, $J = 9.7$ Hz, 1H), 7.53 (s, 1H), 7.52 – 7.47 (m, 1H), 7.21 (dd, $J = 8.7, 2.1$ Hz, 1H), 6.99 (s, 1H), 5.33 (s, 1H), 5.13 (d, $J = 7.7$ Hz, 1H), 4.96 (s, 1H), 4.77 (s, 1H), 4.62 (s, 1H), 3.75 (s, 2H). ^{13}C NMR (100 MHz, DMSO) δ 183.3, 161.5, 145.1, 144.8, 143.0, 135.1, 134.6, 134.5, 132.8, 131.1, 130.9, 129.3, 128.7, 115.7, 112.3, 103.3, 101.1, 76.1, 73.3, 70.2, 68.2, 60.5.

5.2.3.4 Synthesis of β -Gal Activatable Seleno-Resorufin PS (β -Gal -RSeA)

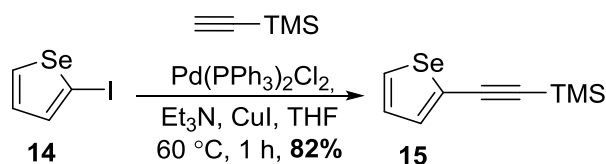
5.2.3.5 Synthesis of Compound 14



Scheme 5. 13. Synthetic route for compound **14**

To a solution of Selenophene (1.0 g, 7.6 mmol) in dry Et₂O (25 mL), n-BuLi (3.0 mL, 7.6 mmol, 2.5 M in hexane) was added dropwise in rt, over 25 min. Then solution heated to reflux (34.6 °C) for 10 min. After being cooled to -78 °C, to this solution, solution of iodine (1.9 g, 7.6 mmol) in dry Et₂O (40 mL) was added dropwise over 50 min. The solution was allowed to warm 0 °C stirred 1 hour at that temperature. After being warmed to rt, reaction mixture poured into ice-cold water and extract with Et₂O. The organic layer was separated and wash with saturated NaHSO₃ (50 mL) and brine (50 mL). The organic layer dried over MgSO₄ and the solvent was evaporated under reduced pressure. The crude product purified by flash column chromatography (silica, petroleum ether) to yield target the product, compound **14**, as light yellow oil (1.6 g, 82%). ¹H NMR (400 MHz, CDCl₃) δ : 8.09 (d, 1H, J=7.0 Hz), 7.53 (d, 1H, J=3.7 Hz), 6.97 (dd, 1H, J= 5.9, 3.8 Hz), ¹³C NMR (100 MHz, CDCl₃) δ : 140.1, 137.2, 131.4.

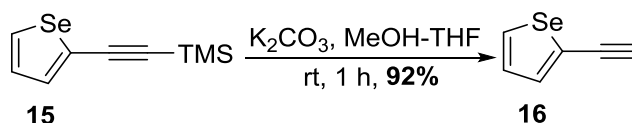
5.2.3.6 Synthesis of Compound 15



Scheme 5. 14. Synthetic route for compound **15**

Solution of compound **14** (0.100, 0.39 mmol), trimethylsilylacetylene (0.053, 0.54 mmol), PdCl₂(PPh₃)₂ (2.50 mg, 0.01 mmol), CuI (1.00 mg, 0.01 mmol) and NEt₃ (0.5 mL) in dry THF (2 mL) heated to 80 °C. After 30 min stirring, the TLC showed that the reaction was completed. Then reaction mixture was allowed to cool rt and 1 N HCl (0.5 mL) was added and extracted with ethyl acetate. The organic layer was separated and washed with brine (25 mL) and saturated NaHCO₃ (25 mL). The organic layer dried over Na₂SO₄ and solvent was evaporated under reduced pressure. The crude product purified by flash column chromatography (silica, petroleum ether) to yield target the product as yellow oil (73.0 mg, 82%). ¹H NMR (400 MHz, CDCl₃) δ: 7.96 (d, 1H, J=5.6 Hz), 7.43 (d, 1H, J=3.7 Hz), 6.97 (dd, 1H, J=5.6, 3.8 Hz), 0.25 (s, 9H) ¹³C NMR (100 MHz, CDCl₃) δ: 135.1, 133.3, 129.5, 127.6, 100.7, 99.8, 0.0

5.2.3.7 Synthesis of Compound 16



Scheme 5. 15. Synthetic route for compound **16**

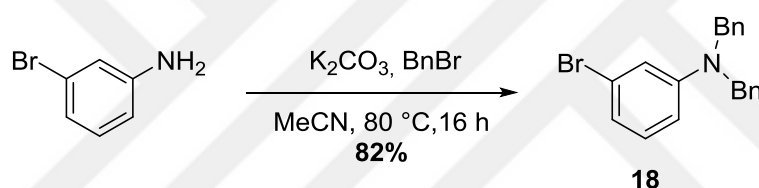
Compound **15** (0.87 g, 3.8 mmol) was dissolved in MeOH-THF mixture (50 mL, 1:1) and K₂CO₃ (1.3 g, 9.5 mmol, 2.5 eq.) was added to solution and was stirred at rt. Reaction monitored by TLC and upon completion after 1 hour, the reaction mixture diluted with Et₂O and washed with saturated NH₄Cl and brine respectively. The organic layer dried over MgSO₄ and concentrated under reduced pressure. The

crude product purified by flash column chromatography (silica, hexane) to yield target the product as yellow oil (0.55 g, 92%). ^1H NMR (400 MHz, CDCl_3) δ : 7.99 (d, 1H, $J=6.6$ Hz), 7.48 (d, 1H, $J=3.7$ Hz), 7.21 (dd, 1H, $J=5.6, 3.8$ Hz), 3.52 (s, 1H) ^{13}C NMR (100 MHz, CDCl_3) δ : 135.6, 133.5, 129.4, 126.4, 83.0, 79.1.

5.3 Si-Fluorescein Based Photosensitizers

5.3.1 Synthesis of β -Gal Activatable Diiodo-Si-Fluorescein PS (β -Gal-SiF-II)

5.3.1.1 Synthesis of Compound 18

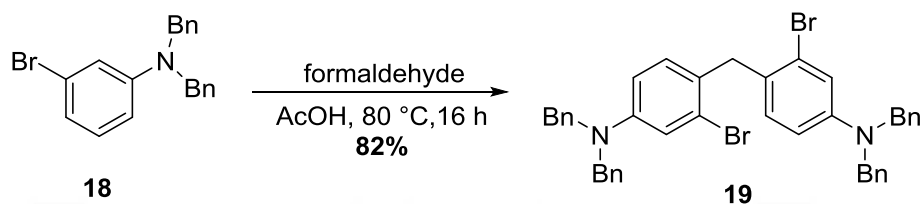


Scheme 5. 16. Synthetic route for compound **18**.

To a 250 mL Schlenk tube, vacuum- N_2 was applied. Then, commercially available 3-bromoaniline (8.10 g, 47.1 mmol) and K_2CO_3 (18.88 g, 136.6 mmol, 2.9 eq.) were suspended with dry MeCN (110 mL). To a suspension, BnBr (18.8 mL, 160.1 mmol, 3.4 eq.) was added slowly and then, mixture heated to 80°C stirred for 16 hours. After completion of reaction, MeCN evaporated and the crude product quenched with distilled water (80 mL) and extracted with EtOAc (80 mL). Collected organic phases dried over Na_2SO_4 , filtered and solvent evaporated. The crude product redissolved with EtOAc (5 mL) and added to cold hexane dropwise to remove excess BnBr and obtain the pure product as white precipitate. After completion of addition, hexane removed by decantation and precipitate dried under vacuum. The target product, compound **18**, was obtained as white solid (13.6 g, 82%). ^1H NMR (400 MHz, CDCl_3) δ 7.35 (t, $J = 7.3$ Hz, 4H), 7.29 (d, $J = 7.1$ Hz, 2H), 7.23 (d, $J = 7.3$

Hz, 4H), 7.01 (t, $J = 8.1$ Hz, 1H), 6.90 (s, 1H), 6.82 (d, $J = 7.7$ Hz, 1H), 6.64 (dd, $J = 8.3, 2.0$ Hz, 1H), 4.63 (s, 4H). ^{13}C NMR (100 MHz, CDCl_3) δ 150.6, 137.9, 130.6, 128.9, 127.2, 126.7, 123.6, 119.7, 115.15, 111.2, 54.1.

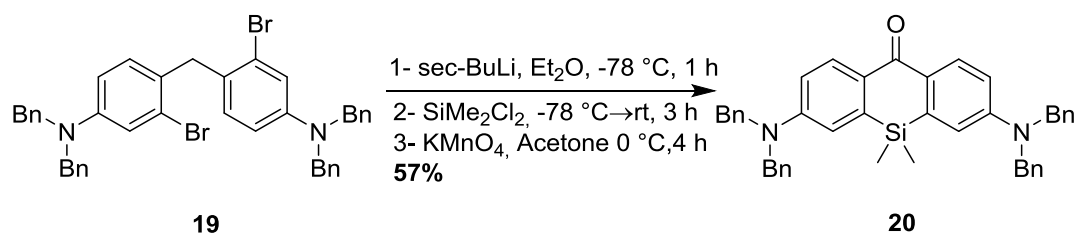
5.3.1.2 Synthesis of Compound 19



Scheme 5. 17. Synthetic route for compound **19**

To a 250 mL Schlenk tube, compound **18** (4.85 g, 13.8 mmol, 2 eq.) and formaldehyde solution (2.07 g, 6.90 mmol, 30%) were placed and dissolved with AcOH (40 mL). Then mixture heated to 80°C and stirred for 16 hours at that temperature. After that, mixture cooled to room temperature and neutralized with satd. NaHCO_3 and satd. NaOH solutions and then extracted with CHCl_3 . Collected organic phases dried over Na_2SO_4 , filtered and solvent evaporated. The crude product purified with column chromatography (silica, hexane:EtOAc 6:1). The target product, compound **19**, was obtained as white solid (4.05 g, 82%). ^1H NMR (400 MHz, CDCl_3) δ 7.24 (t, $J = 7.1$ Hz, 8H), 7.18 (d, $J = 6.5$ Hz, 4H), 7.13 (d, $J = 7.4$ Hz, 8H), 6.88 (d, $J = 2.0$ Hz, 2H), 6.70 (d, $J = 8.5$ Hz, 2H), 6.47 (dd, $J = 8.4, 2.2$ Hz, 2H), 4.50 (s, 8H), 3.86 (s, 2H). ^{13}C NMR (100 MHz, CDCl_3) δ 148.8, 138.1, 131.1, 128.8, 127.4, 127.1, 126.7, 125.8, 125.8, 116.0, 111.8, 54.1, 39.9

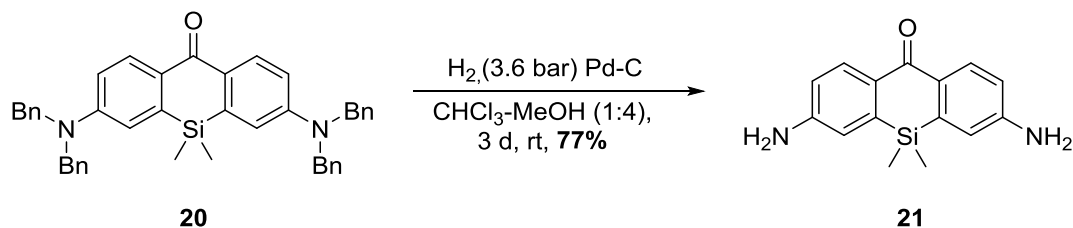
5.3.1.3 Synthesis of Compound 20



Scheme 5. 18. Synthetic route for compound **20**

To a 100 mL Schlenk tube, compound **19** (2.00 g, 2.79 mmol) was added and vacuum-N₂ was applied. After that, it was dissolved with dry THF (12 mL) and cooled to -78°C. Then, sec-BuLi (6.50 mL, 7.95 mmol, 1.4 M, 2.85 eq.) was added dropwise and stirred at that temperature for 1 hour. 1.9 mL SiMe₂Cl₂ from stock solution ((stock solution: 1.9 mL SiMe₂Cl₂ + 3.8 mL THF), 5.30 mmol, 1.9 eq.) was added dropwise then reaction mixture warmed to room temperature slowly. After reaching to the room temperature, mixture stirred at that temperature for 3 h. After that, reaction mixture quenched with 1 N HCl (1 mL) then neutralized with NaHCO₃ (25 mL) and extracted with CHCl₃. Collected organic phases dried over Na₂SO₄, filtered and solvent evaporated. The crude product placed in 100 mL two necked round-bottomed balloon and dissolved with acetone (26 mL) and cooled to 0°C. To reaction mixture KMnO₄ (1.22 g, 7.67 mmol, 2.75 eq.) in portions over 1.5 hours and stirred for 2.5 hours more at that temperature. After that, reaction mixture filtered through celite and washed with CHCl₃. The crude product purified with column chromatography (neutral alumina hexane:CHCl₃ 1:2). The target product, compound **20**, obtained as yellow solid (0.99 g, 57%). ¹H NMR (400 MHz, CDCl₃) δ 8.28 (d, *J* = 9.0 Hz, 2H), 7.34 – 7.28 (m, 8H), 7.23 (d, *J* = 7.3 Hz, 12H), 6.86 (dd, *J* = 9.0, 2.7 Hz, 2H), 6.80 (d, *J* = 2.7 Hz, 2H), 4.71 (s, 8H), 0.14 (s, 6H). ¹³C NMR (100 MHz, CDCl₃) δ 185.1, 150.7, 140.6, 137.8, 131.8, 130.4, 128.9, 127.3, 126.7, 115.2, 113.7, 54.2, -1.4.

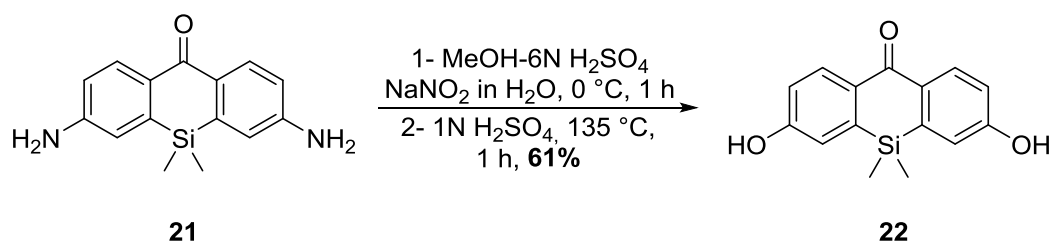
5.3.1.4 Synthesis of Compound 21



Scheme 5. 19. Synthetic route for compound **21**

To a parr reactor, compound **20** (0.60 g, 1.0 mmol) was placed and dissolved with MeOH:CHCl₃ mixture (30 mL, 4:1). To this solution Pd-C (0.12 g, 20% by mass) was added then air removed by vacuum. Reactor refilled with H₂ gas (3.6 bar) and stirred for 3 days. After completion of reaction, mixture filtered through celite and washed with MeOH. The crude product purified with column chromatography (silica, MeOH:DCM, 1:9). The target product, compound **21**, obtained as white solid (0.20 g, 77%). ¹H NMR (400 MHz, MeOD) δ 8.13 (d, *J* = 8.7 Hz, 2H), 6.87 (d, *J* = 2.4 Hz, 2H), 6.75 (dd, *J* = 8.7, 2.4 Hz, 2H), 0.40 (s, 6H). ¹³C NMR (100 MHz, MeOD) δ 187.6, 153.1, 142.8, 132.9, 131.1, 118.6, 116.7, -1.2.

5.3.1.5 Synthesis of Compound 22

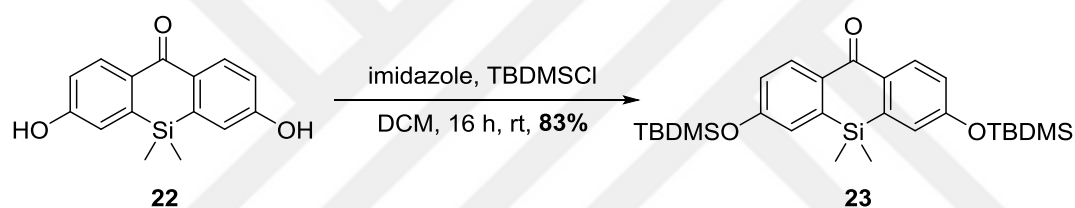


Scheme 5. 20. Synthetic route for compound **22**

To a 250 mL two-necked round bottomed flask, compound **21** (0.10 g, 0.4 mmol) was added and vacuum-N₂ was applied. Then, dissolved with MeOH : 6 N H₂SO₄ (92 mL, 1:1) and cooled to 0 °C. Solution of NaNO₂ (0.17 g, 2.5 mmol, 6.7 eq.) in H₂O (4 mL) was added dropwise and stirred for 1 hour at that temperature. After

that, mixture was added dropwise to the boiling 1 N H₂SO₄ (135 °C, 100 mL) over 1 hour. Then stirred 10 more minutes at that temperature before cooling to room temperature. After completion of reaction, mixture extracted with CHCl₃. Collected organic phases washed with brine, dried over Na₂SO₄, filtered and solvent evaporated. The crude product purified with column chromatography (silica, MeOH:DCM 5:95). The target product, compound **22**, obtained as white solid (61 mg, 61%). ¹H NMR (400 MHz, MeOD) δ 8.22 (d, *J* = 8.8 Hz, 2H), 7.03 (d, *J* = 2.6 Hz, 2H), 6.91 (dd, *J* = 8.8, 2.6 Hz, 2H), 0.39 (s, 6H). ¹³C NMR (100 MHz, MeOD) δ 187.8, 162.3, 143.2, 133.9, 133.5, 120.2, 118.5, -1.4.

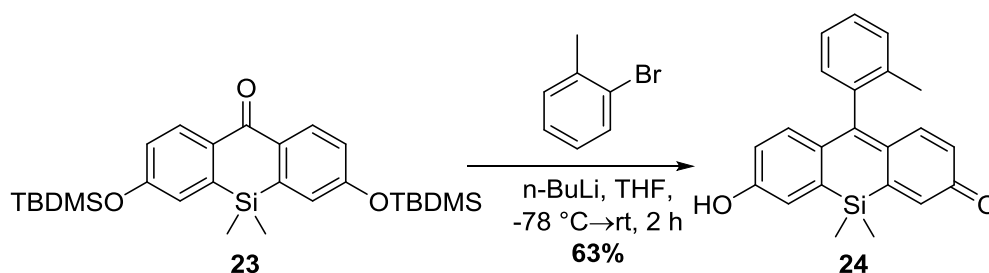
5.3.1.6 Synthesis of Compound 23



Scheme 5. 21. Synthetic route for compound **23**

To a 250 mL two-necked round bottomed flask, compound **22** (100 mg, 0.37 mmol) and imidazole (260 mg, 3.83 mmol, 10.4 eq.) were added and vacuum-N₂ was applied. Then, dissolved with dry DCM (55 mL). To this solution, solution of TBDMSCl (562 mg, 3.74 mmol, 10.1 eq.) in dry DCM (13 mL) was added slowly and stirred for 16 hours at room temperature. After completion of reaction, mixture quenched with distilled water (50 mL) and extracted. Collected organic phases washed with brine, dried over Na₂SO₄, filtered and solvent evaporated. The crude product purified with column chromatography (silica, hexane:DCM 1:8). The target product, compound **23**, obtained as white solid (153 mg, 83%). ¹H NMR (400 MHz, CDCl₃) δ 8.38 (d, *J* = 8.7 Hz, 2H), 7.05 (d, *J* = 2.2 Hz, 2H), 6.99 (dd, *J* = 8.7, 2.5 Hz, 2H), 1.01 (s, 18H), 0.47 (s, 6H), 0.27 (s, 12H). ¹³C NMR (100 MHz, CDCl₃) δ 186.1, 158.9, 141.3, 134.7, 132.4, 123.8, 121.9, 25.8, 18.4, -1.4, -4.2.

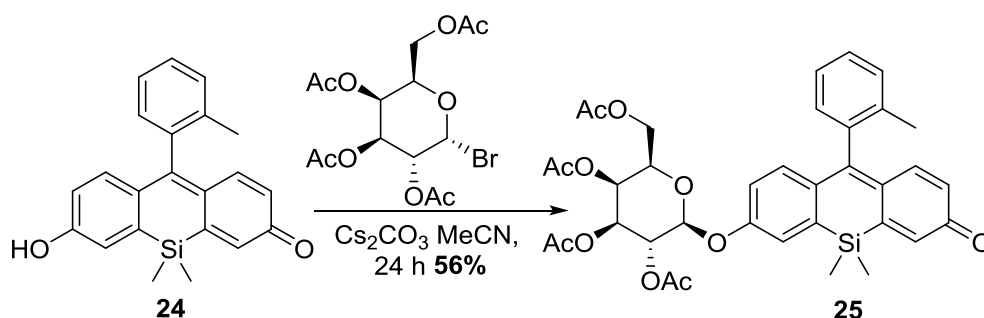
5.3.1.7 Synthesis of Compound 24



Scheme 5. 22. Synthetic route for compound **24**

To a 100 mL two necked round bottom flask, commercially available o-bromotoluene (343 mg, 2.00 mmol, 5 eq.) was added and vacuum-N₂ was applied. Then, dissolved with dry THF (30 mL) and cooled to -78°C. After that, n-BuLi (0.80 mL, 2.0 mmol, 2.5 M, 5 eq.) was added dropwise and stirred for 30 minutes at that temperature. Then, compound **23** (200 mg, 0.40 mmol) in dry THF (5 mL) was added to the reaction mixture dropwise and let the mixture to warm room temperature slowly and stirred 1.5 hours more at that temperature. After completion of reaction, mixture quenched with 1 M HCl (12 mL) and extracted with DCM (50 mL). Collected organic phases washed with brine, dried over Na₂SO₄, filtered and solvent evaporated. The crude product purified with column chromatography (silica, MeOH:CHCl₃ 1:18). The target product, compound **24**, obtained as dark orange solid (86 mg, 63%). ¹H NMR (400 MHz, CDCl₃) δ 7.38 – 7.34 (m, *J* = 7.4 Hz, 1H), 7.29 (d, *J* = 8.2 Hz, 2H), 7.09 – 7.04 (m, 3H), 6.94 (d, *J* = 9.4 Hz, 2H), 6.56 (dd, *J* = 9.4, 2.1 Hz, 2H), 3.49 (s, 1H), 2.03 (s, 3H), 0.43 (s, 3H), 0.41 (s, 3H). ¹³C NMR (100 MHz, CDCl₃) δ 172.6, 161.5, 146.0, 140.5, 139.3, 135.9, 130.2, 129.9, 129.2, 129.0, 128.5, 125.7, 122.1, 19.6, -1.2, -1.5.

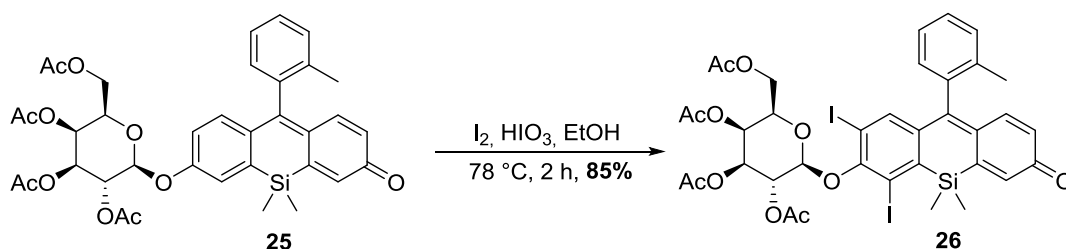
5.3.1.8 Synthesis of Compound 25



Scheme 5. 23. Synthetic route for compound **25**

To a 100 mL two necked round bottom flask, compound **24** (69 mg, 0.20 mmol) and CsCO_3 (326 mg, 1.00 mmol, 5 eq.) were added and vacuum- N_2 was applied. Then, dissolved with dry MeCN (45 mL). After that, acetobromo- α -D-galactose (348 mg, 1.00 mmol, 5 eq.) was added and stirred for 16 hours. After completion of reaction, MeCN removed from reaction mixture with reduced pressure and mixture dissolved in CHCl_3 (50 mL) and extracted with distilled water (50 mL) and washed with brine (50 mL). Collected organic phases dried over Na_2SO_4 , filtered and solvent evaporated. The crude product purified with column chromatography (silica, MeOH: CHCl_3 1:18). The target product, compound **25**, obtained as orange solid (75 mg, 56%). ^1H NMR (400 MHz, CDCl_3) δ 7.39 – 7.33 (m, 1H), 7.33 – 7.27 (m, 2H), 7.24 (d, J = 2.1 Hz, 1H), 7.06 (t, J = 6.5 Hz, 1H), 6.94 (d, J = 10.1 Hz, 1H), 6.85 – 6.76 (m, J = 9.2, 5.6 Hz, 3H), 6.22 (dd, J = 10.1, 1.9 Hz, 1H), 5.53 – 5.42 (m, 2H), 5.16 – 5.08 (m, 2H), 4.20 – 4.04 (m, 3H), 2.16 (s, 3H), 2.04 (dd, J = 6.7, 1.1 Hz, 6H), 2.00 – 1.97 (m, 6H), 0.46 (t, J = 6.0 Hz, 6H). ^{13}C NMR (100 MHz, CDCl_3) δ 184.4, 170.3, 170.2, 170.1, 169.4, 157.0, 157.0, 154.9, 146.8, 141.6, 140.6, 139.1, 137.2, 136.1, 136.0, 135.7, 135.0, 130.3, 130.2, 129.2, 128.4, 128.0, 125.9, 125.9, 122.9, 116.6, 98.2, 71.2, 70.7, 68.4, 66.7, 61.3, 61.3, 53.5, 20.8, 20.6, 20.6, 19.5, -1.2, -1.2, -1.4, -1.5.

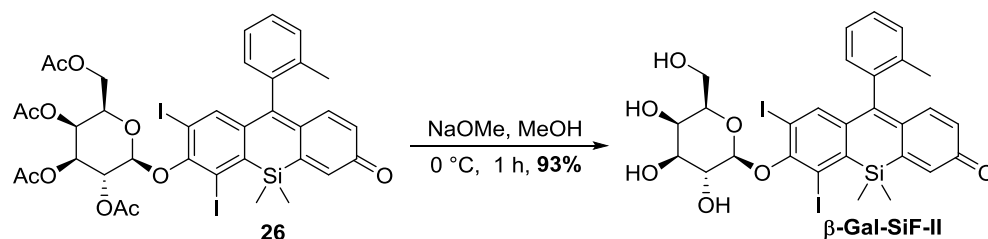
5.3.1.9 Synthesis of Compound 26



Scheme 5. 24. Synthetic route for compound **26**

To a 50 mL Schlenk tube, compound **25** (64 mg, 0.10 mmol) was added, and vacuum- N_2 was applied. Then, compound **25** was dissolved with EtOH (10 mL). After that, I_2 (48 mg, 0.19 mmol, 2 eq.) was added and mixture heated to $60\text{ }^\circ\text{C}$ stirred for 15 minutes. Then, HIO_3 (33.4 mg, 0.19 mmol, 2 eq.) solution in H_2O (1.0 mL) was added dropwise to the reaction mixture and temperature raised to $78\text{ }^\circ\text{C}$ stirred for 2 hours. After consumption of all starting material, EtOH evaporated, and mixture quenched with 10% $Na_2S_2O_3$ solution (10 mL) and extracted with EtOAc. Collected organic phases dried over Na_2SO_4 , filtered and solvent evaporated. The crude product purified with column chromatography (silica, MeOH:DCM 1:18). Target product obtained as dark orange solid (75 mg, 85%). ^1H NMR (400 MHz, $CDCl_3$) δ 7.74 (s, 1H), 7.41 (t, $J = 7.2$ Hz, 1H), 7.36 – 7.29 (m, 2H), 7.24 (s, 1H), 7.06 (t, $J = 6.7$ Hz, 1H), 6.80 (s, 2H), 5.50 (dd, $J = 10.3, 8.0$ Hz, 1H), 5.45 (d, $J = 3.2$ Hz, 1H), 5.18 (d, $J = 7.7$ Hz, 1H), 5.13 (dd, $J = 10.4, 3.2$ Hz, 1H), 4.21 – 4.08 (m, 3H), 2.17 (s, 3H), 2.06 (s, 3H), 2.04 (s, 3H), 2.02 – 1.98 (m, $J = 4.8$ Hz, 6H), 0.78 (t, $J = 5.6$ Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.9, 170.1, 170.0, 169.9, 169.2, 158.1, 157.3, 151.4, 151.3, 143.0, 138.9, 135.6, 135.5, 134.2, 131.9, 130.3, 128.8, 128.8, 125.9, 123.0, 118.2, 116.6, 98.0, 98.0, 97.7, 71.2, 70.5, 68.3, 66.6, 61.2, 61.2, 20.6, 20.5, 20.4, 19.5, 19.5, -1.2, -1.2, -1.3, -1.3.

5.3.1.10 Synthesis of Compound β -Gal-SiF-II

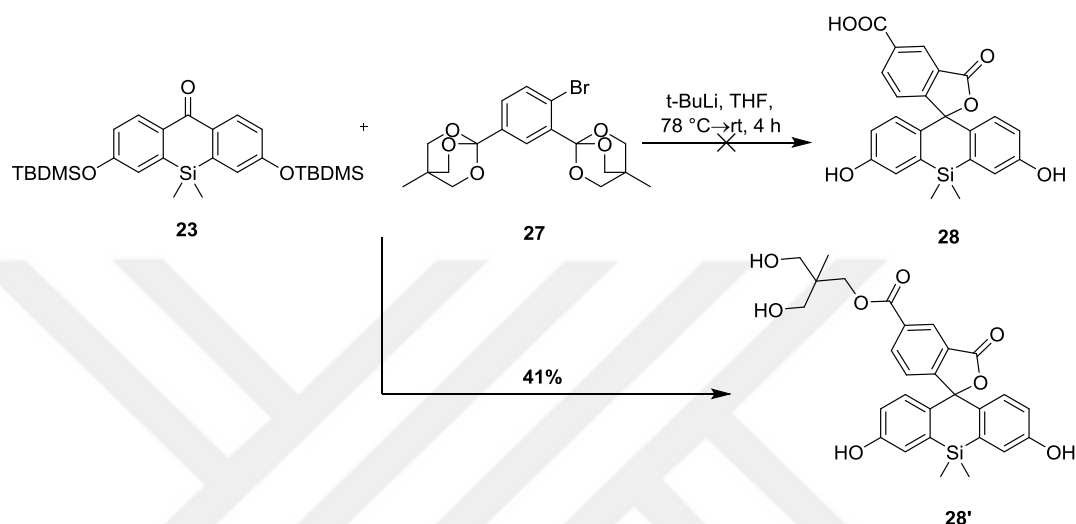


Scheme 5. 25. Synthetic route for compound β -Gal-SiF-II

To a 25 mL Schlenk tube, compound **26** (75 mg, 0.1 mmol) was added, and vacuum–N₂ was applied. Then, compound **26** was dissolved with MeOH (7 mL) and cooled to 0°C. To this solution, NaOMe (10 mg, 0.4, 4.4 eq.) in MeOH (1.1 mL) solution added dropwise and stirred for 1 hour at that temperature. After completion of reaction, mixture neutralized with amberlyst, then filtered. Collected filtrate evaporated and the crude product purified with preparative HPLC. The target product, β -Gal-SiF-II, obtained as dark orange solid (57 mg, 93%). ¹H NMR (400 MHz, MeOD) δ 7.82 (s, 1H), 7.55 – 7.48 (m, J = 7.5, 5.1 Hz, 2H), 7.47 – 7.38 (m, J = 14.3, 7.1 Hz, 2H), 7.17 (d, J = 7.4 Hz, 1H), 7.05 – 6.99 (m, 1H), 6.90 (d, J = 9.1 Hz, 1H), 5.03 (dd, J = 7.7, 5.1 Hz, 2H), 3.91 (d, J = 3.3 Hz, 1H), 3.85 (dd, J = 9.5, 7.9 Hz, 1H), 3.79 – 3.74 (m, 2H), 3.62 (dd, J = 9.7, 3.2 Hz, 2H), 2.07 (s, 3H), 0.84 (dd, J = 5.3, 3.5 Hz, 6H). ¹³C NMR (100 MHz, MeOD) δ 172., 160.2, 160., 159.9, 152.7, 151.9, 143.0, 139.0, 136.0, 135.4, 132.7, 131.1, 130.0, 128.6, 125.6, 122.7, 122.6, 117.5, 117.4, 115.8, 100.4, 100.4, 99.9, 95.3, 75.9, 73.2, 70.5, 68.7, 61.0, 18.0, -2.6, -2.7, -2.7, -2.8. HRMS (ESI/MS) m/z : [M+H]⁺ Calcd. for C₂₂H₁₈I₂O₂Si⁺ 596.9200; Found 596.9240

5.3.2 Synthesis of β -Gal and TN-C Activatable Diiodo-Si-Fluorescein PS (β -Gal-TN-C-SiF-II)

5.3.2.1 Synthesis of Compound **28'**

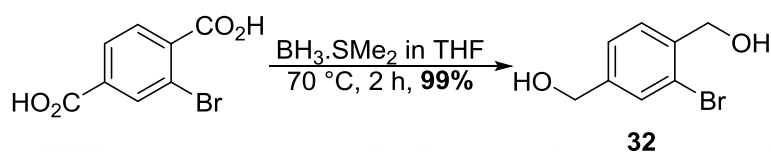


Scheme 5. 26. Synthetic route for compound **28** and **28'**

To a 100 mL two necked round bottom flask, compound **27** (415 mg, 1.20 mmol, 5 eq.) was added and vacuum- N_2 was applied. Then, dissolved with dry THF (20 mL) and cooled to $-78\text{ }^\circ\text{C}$. After that, t-BuLi (1.20 mL, 2.40 mmol, 1.7 M, 10 eq.) was added dropwise and stirred for 30 minutes at that temperature. Then, compound **23** (100 mg, 0.24 mmol) in dry THF (10 mL) was added to the reaction mixture dropwise and let the mixture to warm room temperature slowly and stirred 4 hours more at that temperature. After completion of reaction, 1 M HCl (20 mL) added to the mixture and stirred 1 hour more. After that mixture quenched with satd. NaHCO_3 (20 mL) and extracted with EtOAc (50 mL). Collected organic phases washed with brine, dried over Na_2SO_4 , filtered and solvent evaporated. The crude product purified with column chromatography (silica, MeOH:DCM 1:9). The side product, compound **28'**, obtained as white solid (42.0 mg, 41%). ^1H NMR (400 MHz, MeOD) δ 8.24 (dd, $J = 8.0, 1.2$ Hz, 1H), 8.04 (d, $J = 8.0$ Hz, 1H), 7.85 (s, 1H), 7.16 (d, $J = 2.7$ Hz, 2H), 6.79 (d, $J = 8.8$ Hz, 2H), 6.70 (dd, $J = 8.8, 2.7$ Hz, 2H), 4.23 (s, 2H),

3.49 – 3.45 (m, , 4H), 0.94 (s, 3H), 0.66 (s, 3H), 0.56 (s, 3H). ^{13}C NMR (100 MHz, MeOD) δ 171.5, 166.4, 158.3, 156.6, 138.2, 137.2, 135.7, 131.3, 130.3, 129.4, 127.0, 126.0, 121.2, 118.0, 92.6, 68.6, 65.6, 42.1, 17.0, -0.1, -0.9. HRMS (ESI/MS) m/z : $[\text{M}+\text{H}]^+$ Calcd. for $\text{C}_{28}\text{H}_{29}\text{O}_8\text{Si}^+$ 521.1600; Found 521.1628

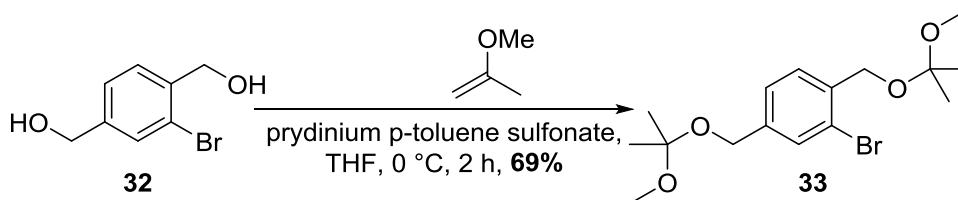
5.3.2.2 Synthesis of Compound 32



Scheme 5. 27. Synthetic route for compound **32**

To a 50 mL Schlenk tube, 2-bromo terephthalic acid (1.00 g, 4.08 mmol) was added and vacuum- N_2 was applied. Then, dissolved with dry THF (13 mL) and cooled to 0°C . Then, $\text{BH}_3\cdot\text{SMe}_2$ (6.12 mL, 12.24 mmol, 3 eq. 2 M) was added slowly. The mixture heated to 70°C and stirred for 2 hours at that temperature. After that, mixture cooled to 0°C and quenched with dropwise addition of water (10 mL) and extracted with EtOAc (50 mL) and washed with brine (50 mL). Collected organic phases dried over Na_2SO_4 , filtered and solvent evaporated. The target product, compound **32**, obtained without further purification as white solid. (0.88 g, 99%) ^1H NMR (400 MHz, Acetone- d_6) δ 7.57 – 7.51 (m, 2H), 7.34 (d, $J = 7.6$ Hz, 1H), 4.65 (d, $J = 5.7$ Hz, 2H), 4.62 (d, $J = 5.9$ Hz, 2H), 4.45 (t, $J = 5.7$ Hz, 1H), 4.35 (t, $J = 5.8$ Hz, 1H). ^{13}C NMR (100 MHz, Acetone- d_6) δ 144.2, 140.3, 130.9, 128.8, 126.4, 122.0, 64.1, 63.6.

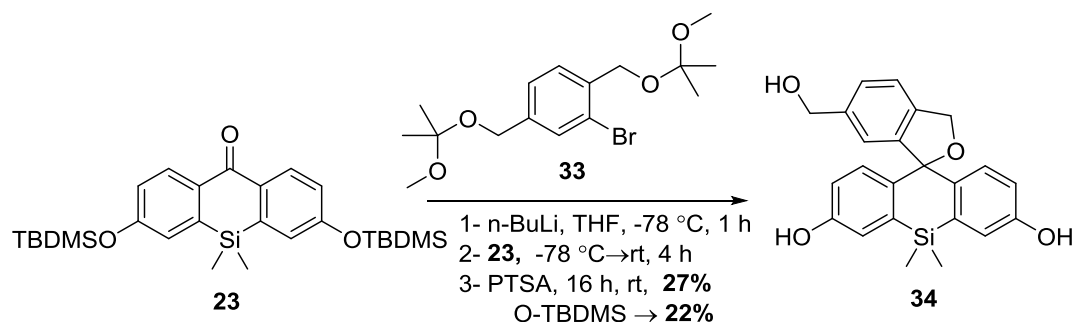
5.3.2.3 Synthesis of Compound 33



Scheme 5. 28. Synthetic route for compound **33**

To a 50 mL Schlenk tube, compound **32** (0.77 g, 3.6 mmol) was added and vacuum- N_2 was applied. Then, dissolved with dry THF (13 mL) and cooled to 0 °C. Then, 2-methoxypropene (3.4 mL, 2.55 g, 35.50 mmol, 10 eq.) and pyridinium-PTSA (20.0 mg, 0.07 mmol, 0.02 eq.) were added and stirred at that temperature for 2 hours. After that, mixture quenched with satd. $NaHCO_3$ (50 mL) and extracted with EtOAc (50 mL). Collected organic phases dried over Na_2SO_4 , filtered and solvent evaporated. The target product, compound **33**, obtained without further purification as colorless oil. (0.89 g, 69%) ^1H NMR (400 MHz, $CDCl_3$) δ 7.54 (s, 1H), 7.50 (d, J = 7.9 Hz, 1H), 7.28 (d, J = 7.1 Hz, 1H), 4.53 (s, 2H), 4.44 (s, 2H), 3.24 (s, 2H), 3.23 (s, 2H), 1.45 (s, 6H), 1.42 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 139.9, 137.4, 131.3, 128.6, 126.5, 122.4, 100.7, 100.6, 62.4, 62.1, 48.9, 48.9, 24.7, 24.7.

5.3.2.4 Synthesis of Compound 34



Scheme 5. 29. Synthetic route for compound **34**

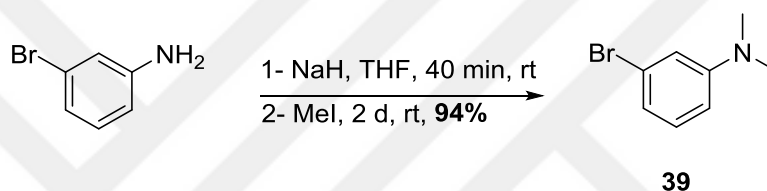
To a 50 mL Schlenk tube, compound **33** (345 mg, 0.96 mmol, 5 eq.) was added and vacuum-N₂ was applied. Then, dissolved with dry THF (5 mL) and cooled to -78 °C. Then, n-BuLi (0.45 mL, 0.96 mmol, 5 eq., 2.5 M) was added dropwise to a solution of compound **33** and stirred for 1 hour at that temperature. After that, compound **23** (95 mg, 0.19 mmol) was added dropwise to the reaction mixture and slowly warmed to room temperature and stirred for 4 hours more. After completion of reaction, reaction quenched with distilled water (10 mL) and extracted with CHCl₃ (50 mL). Collected organic phases dried over Na₂SO₄, filtered and solvent evaporated. The crude product dissolved with THF:MeOH mixture (6 mL, 2:1). To a solution, PTSA (40 mg, 0.25 mmol, 1.25 eq.) and stirred for 16 hours. After completion of reaction, reaction mixture quenched with water (10 mL) and extracted with CHCl₃ (50 mL). Collected organic phases dried over Na₂SO₄, filtered and solvent evaporated. The crude product purified with column chromatography (silica, MeOH:DCM, 5:95). Target product, compound **34**, obtained as an off-white solid (20 mg, 27% for **34** and 26 mg, 22% for TBDMS protected). For compound **34**: ¹H NMR (400 MHz, MeOD) δ 7.34 – 7.29 (m, 1H), 7.22 (d, *J* = 7.8 Hz, 1H), 7.08 (d, *J* = 8.7 Hz, 2H), 7.04 (d, *J* = 2.6 Hz, 2H), 6.90 (s, 1H), 6.69 (dd, *J* = 8.7, 2.7 Hz, 2H), 5.39 (s, 2H), 4.49 (s, 2H), 0.61 (s, 3H), 0.48 (s, 3H). ¹³C NMR (100 MHz, MeOD) δ 156.9, 149.0, 143.0, 142.5, 137.7, 136.0, 130.5, 128.0, 127.3, 122.9, 122.3, 119.9, 117.9, 93.9, 74.3, 64.8, -0.3. For TBDMS protected **34**: ¹H NMR (400 MHz, CDCl₃) δ 7.21 (s, 1H), 7.18 (s, 1H), 7.02 (d, *J* = 8.7 Hz, 2H), 7.00 (d, *J* = 2.6 Hz, 2H), 6.91 (s, 1H), 6.64 (dd, *J* = 8.7, 2.7

Hz, 2H), 5.27 (s, 2H), 4.54 (s, 2H), 0.90 (s, 18H), 0.54 (s, 3H), 0.44 (s, 3H), 0.11 (s, 12H). ^{13}C NMR (100 MHz, CDCl_3) δ 154.3, 147.0, 143.3, 140.6, 138.1, 135.7, 129.1, 126.4, 124.4, 123.0, 122.6, 121.5, 121.3, 92.4, 73.2, 65.3, 25.9, 18.4, 0.2, -0.7, -4.2.

5.4 Si-Rhodamine Based Photosensitizers

5.4.1 Synthesis of Tollyl Bearing Si-Rhodamine PS (2-Me-SiR-Br)

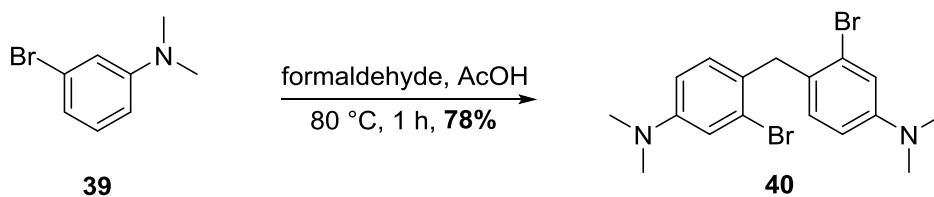
5.4.1.1 Synthesis of Compound 39



Scheme 5. 30. Synthetic route for compound **39**

To a 50 mL Schlenk tube, 3-bromoaniline (2.00 g, 11.63 mmol) was added, and vacuum- N_2 was applied. Then, 3-bromoaniline was dissolved in dry THF (15 mL) and cooled to 0 °C. Afterward, NaH (1.14 g, 28.5 mmol, 2.5 eq., 60%) was added and warmed to room temperature, then stirred for 40 min at that temperature. Subsequently, MeI (4.05 g, 28.5 mmol, 2.5 eq.) was added slowly. After completion of addition, reaction mixture was stirred for 2 days. After that, quenched with water and extracted with EtOAc. Collected organic phases were dried over Na_2SO_4 before filtration. The collected organic phases were then removed under reduced pressure. The crude product was purified by column chromatography (silica, hexane:EtOAc, 6:1), yielding the target product, compound **39**, as a colorless oil (2.19 g, 94%). ^1H NMR (400 MHz, CDCl_3) δ 7.11 (t, J = 8.2 Hz, 1H), 6.86 (d, J = 7.9 Hz, 2H), 6.66 (dd, J = 7.7, 1.6 Hz, 1H), 2.96 (s, 6H). ^{13}C NMR (100 MHz, CDCl_3) δ 151.6, 130.2, 123.3, 119.0, 115.0, 110.8, 40.3.

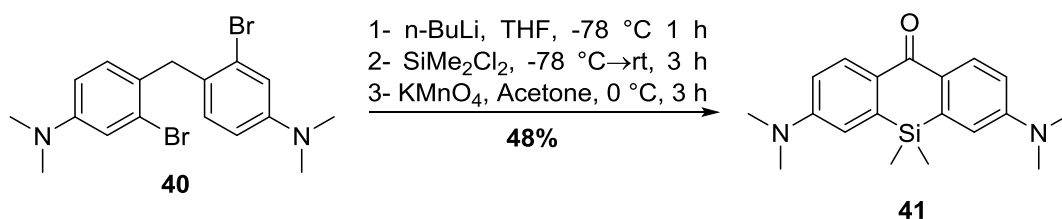
5.4.1.2 Synthesis of Compound 40



Scheme 5. 31. Synthetic route for compound **40**

To a 50 mL Schlenk tube, compound **39** (1.00 g, 5 mmol, 2 eq.) and formaldehyde solution (75 mg, 2.5 mmol, 30%) were placed and dissolved with AcOH (15 mL). Then mixture heated to 80 °C and stirred for 1 hour at that temperature. After that, mixture cooled to room temperature and neutralized with satd. NaHCO₃ and satd. NaOH solutions and extracted with CHCl₃. Collected organic phases dried over Na₂SO₄, filtered and solvent evaporated. The crude product purified with column chromatography (silica, hexane:EtOAc 6:1). The target product, compound **40**, obtained as white solid (0.80 g, 78%). ¹H NMR (400 MHz, CDCl₃) δ 7.01 (d, *J* = 2.1 Hz, 2H), 6.93 (d, *J* = 8.4 Hz, 2H), 6.64 (dd, *J* = 8.2, 2.1 Hz, 2H), 4.08 (s, 2H), 2.96 (s, 12H). ¹³C NMR (100 MHz, CDCl₃) δ 150.0, 130.8, 127.0, 125.6, 116.2, 111.9, 40.5, 39.9.

5.4.1.3 Synthesis of Compound 41

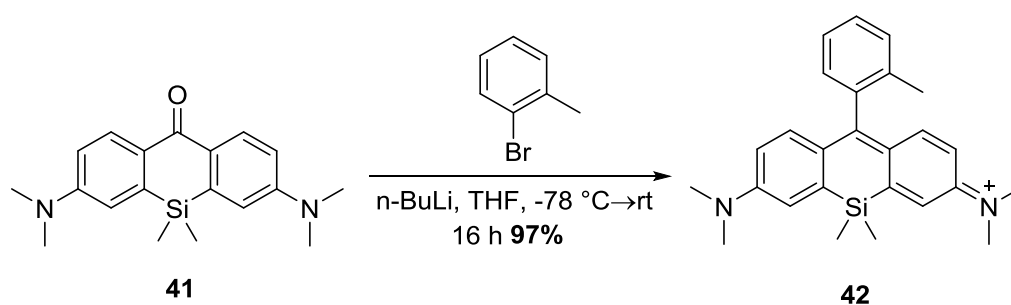


Scheme 5. 32. Synthetic route for compound **41**

To a 25 mL Schlenk tube, compound **40** (0.41 g, 1 mmol) was added and vacuum–N₂ was applied. After that, compound **40** was dissolved with dry THF (7 mL) and cooled to -78 °C. Then, n-BuLi (1.14 mL, 2.85 mmol, 2.5 M, 2.85 eq.) was added

dropwise and stirred at that temperature for 1 hour. 0.7 mL SiMe₂Cl₂ from stock solution ((stock solution: 1.9 mL SiMe₂Cl₂ + 3.8 mL THF), 1.1 mmol, 1.1 eq.) was added dropwise then reaction mixture warmed to room temperature slowly. After reaching to the room temperature, mixture stirred at that temperature for 3 hour. After that, reaction mixture quenched with 1 N HCl (0.5 mL) then neutralized with NaHCO₃ (15 mL) and extracted with CHCl₃. Collected organic phases dried over Na₂SO₄, filtered and solvent evaporated. The crude product placed in 100 mL two necked round-bottomed balloon and dissolved with acetone (15 mL) and cooled to 0 °C. To reaction mixture KMnO₄ (0.44 g, 2.75 mmol, 2.75 eq.) in portions over 1 h and stirred for 2 hour more at that temperature. After that, reaction mixture filtered through celite and washed with CHCl₃. The crude product purified with column chromatography (neutral alumina hexane:CHCl₃ 2:1). The target product, compound **41**, obtained as yellow solid (150 mg, 48%). ¹H NMR (400 MHz, CDCl₃) δ 8.41 (d, *J* = 8.9 Hz, 2H), 6.83 (dd, *J* = 9.0, 2.8 Hz, 2H), 6.80 (d, *J* = 2.7 Hz, 2H), 3.08 (s, 12H), 0.48 (s, 6H). ¹³C NMR (100 MHz, CDCl₃) δ 185.4, 151.6, 140.6, 131.7, 129.8, 114.3, 113.2, 40.0, -0.9.

5.4.1.4 Synthesis of Compound 42

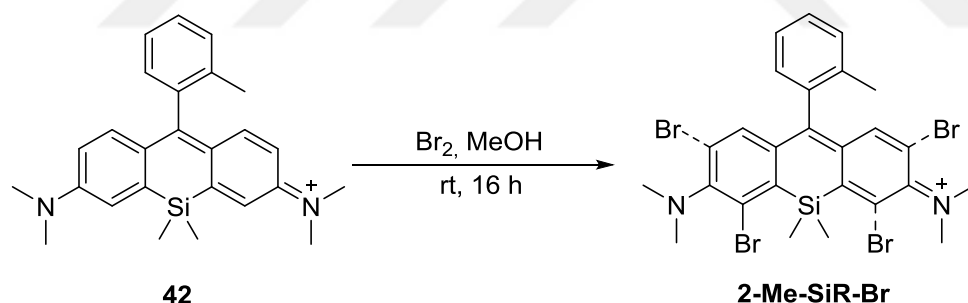


Scheme 5. 33. Synthetic route for compound **42**

To a 50 mL Schlenk tube, o-bromotoluene (158 mg, 0.92 mmol, 3 eq.) was added and vacuum-N₂ was applied. After that, 1-bromotoluene was dissolved with dry THF (10 mL) and cooled to -78 °C. Then, n-BuLi (0.4 mL, 0.92 mmol, 2.5 M, 3 eq.) was added dropwise and stirred at that temperature for 1 h. After that, compound **41**

(100 mg, 0.31 mmol) which was dissolved in dry THF (6 mL) was added to the reaction mixture dropwise. After completion of the addition, reaction mixture warmed to room temperature slowly and stirred at that temperature for 16 hour. Then, reaction quenched with 2 M HCl (0.5 mL) and stirred for 10 more minutes. Reaction mixture extracted with CHCl₃ and washed with distilled water. Collected organic phases dried over Na₂SO₄, filtered and solvent evaporated. The crude product purified with column chromatography (silica, MeOH:DCM, 5:95). The target product obtained as dark blue solid (120 mg, 97%). ¹H NMR (400 MHz, CDCl₃) δ 7.44 – 7.38 (m, *J* = 7.5 Hz, 1H), 7.34 – 7.29 (m, 2H), 7.19 (d, *J* = 2.7 Hz, 2H), 7.06 (d, *J* = 9.5 Hz, 3H), 6.62 (dd, *J* = 9.6, 2.7 Hz, 2H), 3.39 (s, 12H), 2.01 (s, 3H), 0.61 (d, *J* = 8.2 Hz, 6H). ¹³C NMR (100 MHz, CDCl₃) δ 170.0, 154.3, 148.6, 141.7, 138.6, 135.8, 130.4, 129.0, 129.0, 127.7, 125.8, 121.0, 114.2, 41.3, 19.5, -0.5, -0.9.

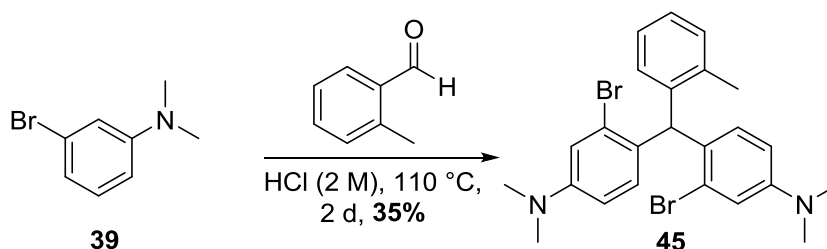
5.4.1.5 Synthesis of Compound 2-Me-SiR-Br



Scheme 5. 34. Synthetic route for compound **2-Me-SiR-Br**

To a 25 mL Schlenk tube, compound **42** (30 mg, 0.08 mmol) was added, and vacuum-N₂ was applied. Then, compound **42** was dissolved with MeOH (5 mL). Then 0.1 mL Br₂ solution in MeOH ((stock solution 0.2 mL Br₂ + 0.8 mL MeOH), 0.35 mmol, 4.6 eq.) was added slowly and stirred for 16 h. After consumption of all starting material, solvent evaporated and column chromatography was applied (silica, EtOAc:Hexane, 4:1). Desired product could not be isolated.

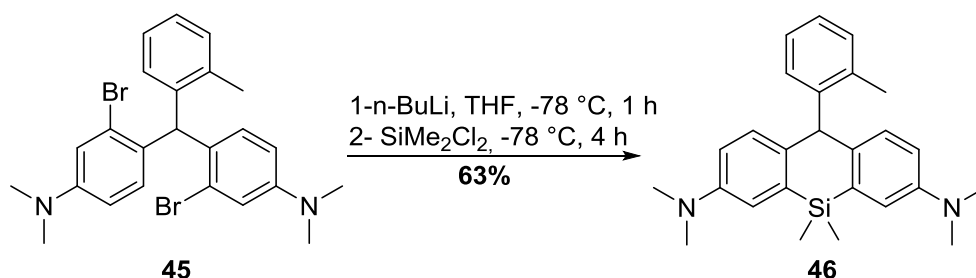
5.4.1.6 Synthesis of Compound 45



Scheme 5. 35. Synthetic route for compound 45

To a 100 mL Schlenk tube, compound **39** (2.00 g, 10 mmol, 2 eq.) was added. Then, 2-methylbenzaldehyde (0.60 g, 5 mmol) was added and suspended with 2 M HCl (20 mL). Afterwards, reaction mixture heated up to 110°C and stirred at that temperature for 2 days. After completion of the reaction, mixture cooled to room temperature and neutralized with NaHCO₃ and extracted with DCM. The collected organic phases washed with brine and dried over Na₂SO₄ before filtration. The collected organic phases were then removed under reduced pressure. The crude product was purified by column chromatography (silica, hexane:EtOAc, 10:1), yielding the target product, compound **45**, as a white solid (0.88 g, 35%). ¹H NMR (400 MHz, CDCl₃) δ 7.19 – 7.12 (m, 2H), 7.11 – 7.05 (m, 1H), 6.95 (d, *J* = 2.6 Hz, 2H), 6.73 (d, *J* = 7.5 Hz, 1H), 6.64 (d, *J* = 8.6 Hz, 2H), 6.54 (dd, *J* = 8.7, 2.6 Hz, 2H), 5.97 (s, 1H), 2.92 (s, 12H), 2.22 (s, 3H). ¹³C NMR (100 MHz, CDCl₃) δ 149.9, 141.6, 137.0, 131.0, 130.3, 129.5, 128.7, 126.5, 126.2, 125.6, 116.5, 111.2, 51.4, 40.4, 19.6.

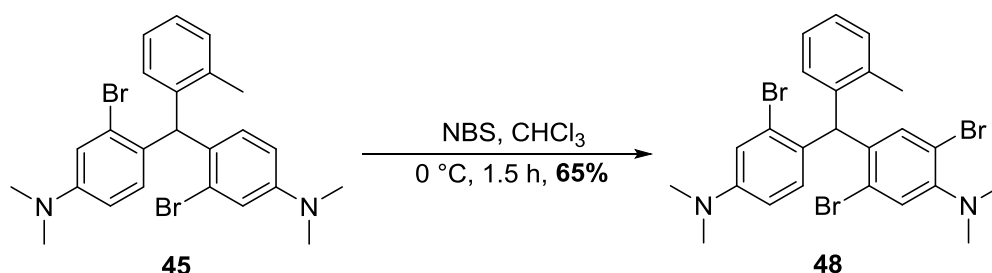
5.4.1.7 Synthesis of Compound 46



Scheme 5. 36. Synthetic route for compound **46**

To a 25 mL Schlenk tube, compound **45** (200 mg, 0.40 mmol) was added and vacuum- N_2 was applied. After that, compound **45** was dissolved with dry THF (6 mL) and cooled to -78°C . Then, n-BuLi (0.46 mL, 1.0 mmol, 2.5 M, 2.5 eq.) was added dropwise and stirred at that temperature for 1 hour. 0.3 mL SiMe_2Cl_2 from stock solution ((stock solution: 1.9 mL SiMe_2Cl_2 + 3.8 mL THF), 0.44 mmol, 1.1 eq.) was added dropwise then reaction mixture warmed to room temperature slowly. After reaching to the room temperature, mixture stirred at that temperature for 3 hours. After completion of reaction, mixture quenched with distilled water and extracted with chloroform. Collected organic phases were dried over Na_2SO_4 before filtration. The collected organic phases were then removed under reduced pressure. The crude product was purified by column chromatography (silica, hexane:EtOAc, 10:1), yielding the target product, compound **46**, as a white solid (100 mg, 63%). ^1H NMR (400 MHz, CDCl_3) δ 7.20 – 7.13 (m, 2H), 7.13 – 7.11 (m, 2H), 6.98 (d, J = 2.7 Hz, 2H), 6.92 (d, J = 8.7 Hz, 2H), 6.69 (dd, J = 8.7, 2.7 Hz, 2H), 5.61 (s, 1H), 2.96 (s, 12H), 2.28 (s, 3H), 0.64 (s, 3H), 0.48 (s, 3H). ^{13}C NMR (100 MHz, CDCl_3) δ 147.8, 146.0, 137.3, 135.5, 133.8, 131.1, 130.9, 129.8, 126.0, 125.7, 116.3, 114.6, 49.6, 40.7, 20.5, -0.3, -0.9. HRMS (ESI/MS) m/z : $[\text{M}+\text{H}]^+$ Calcd. for $\text{C}_{26}\text{H}_{33}\text{N}_2\text{Si}^+$ 401.2413; Found 401.2419

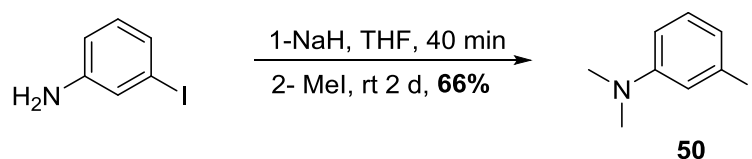
5.4.1.8 Synthesis of Compound 48



Scheme 5. 37. Synthetic route for compound **48**

To a 25 mL Schlenk tube, compound **45** (200 mg, 0.40 mmol) was added and vacuum-N₂ was applied. After that, compound **45** was dissolved with CHCl₃ (4 mL) and cooled to 0 °C. Then NBS (72 mg, 0.41 mmol, 1.02 eq.) in CHCl₃ (3 mL) was added dropwise. Reaction mixture stirred at that temperature for 90 minutes. After completion of reaction, mixture quenched with distilled water and extracted. Collected organic phases washed with brine then dried over Na₂SO₄, filtered and solvent evaporated. The crude product purified with column chromatography (silica, hexane:EtOAc 10:1). The target product, compound **48** obtained as white solid (150 mg, 65%). ¹H NMR (400 MHz, CDCl₃) δ 7.28 (s, 1H), 7.21 – 7.17 (m, *J* = 6.3 Hz, 2H), 7.15 – 7.09 (m, *J* = 11.0, 4.9 Hz, 1H), 7.00 (s, 1H), 6.97 (d, *J* = 2.5 Hz, 1H), 6.74 (d, *J* = 7.6 Hz, 1H), 6.64 (d, *J* = 8.6 Hz, 1H), 6.56 (dd, *J* = 8.7, 2.5 Hz, 1H), 6.00 (s, 1H), 2.93 (s, 6H), 2.81 (s, 6H), 2.23 (s, 3H). ¹³C NMR (100 MHz, CDCl₃) δ 151.0, 150.0, 140.3, 137.9, 136.8, 135.2, 130.8, 130.4, 128.5, 127.9, 126.6, 126.4, 125.8, 124.8, 124.7, 117.5, 116.3, 111.2, 51.6, 44.0, 40.2, 19.5.

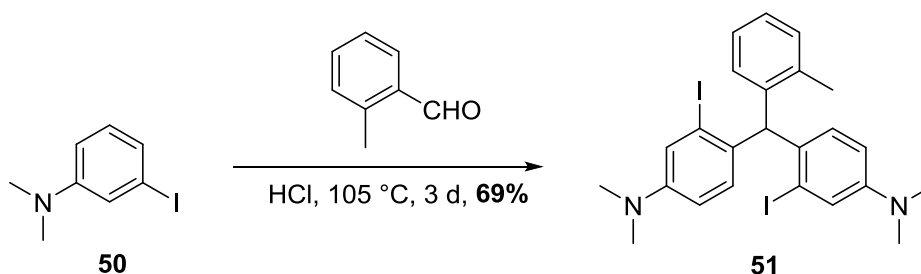
5.4.1.9 Synthesis of Compound 50



Scheme 5. 38. Synthetic route for compound **50**

To a 50 mL Schlenk tube, 3-iodoaniline (2.00 g, 9.13 mmol) was added, and vacuum-N₂ was applied. Then, compound 3-iodoaniline was dissolved in dry THF (15 mL) and cooled to 0 °C. Afterward, NaH (0.91 g, 22.83 mmol, 2.5 eq., 60%) was added and warmed to room temperature, then stirred for 40 min at that temperature. Subsequently, MeI (3.24 g, 22.83 mmol, 2.5 eq.) was added slowly. After completion of addition, reaction mixture heated to 50 °C and stirred for 2 days. After that, mixture cooled to room temperature and quenched with water and extracted with EtOAc. Collected organic phases were dried over Na₂SO₄ before filtration. The collected organic phases were then removed under reduced pressure. The crude product was purified by column chromatography (silica, hexane:EtOAc, 6:1), yielding the target product, compound **50**, as a colorless oil (1.49 g, 66% yield). ¹H NMR (400 MHz, CDCl₃) δ 7.03 (d, J = 7.0 Hz, 2H), 6.94 (t, J = 8.2 Hz, 1H), 6.70 – 6.63 (m, 1H), 2.93 (s, 6H). ¹³C NMR (100 MHz, CDCl₃) δ 151.6, 130.5, 125.3, 121.2, 111.7, 95.7, 40.5.

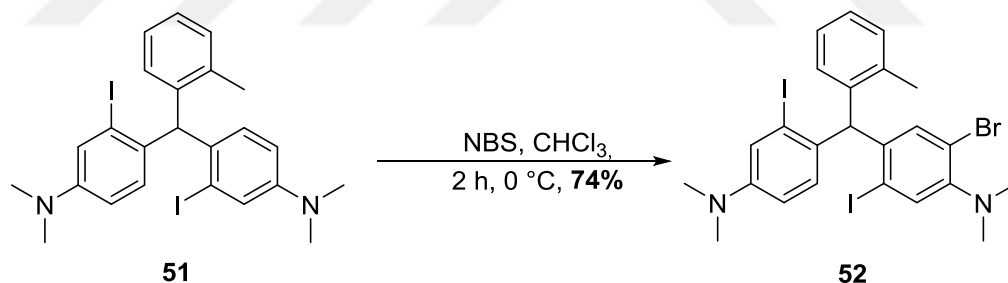
5.4.1.10 Synthesis of Compound 51



Scheme 5. 39. Synthetic route for compound **51**

To a 25 mL Schlenk tube, compound **50** (1.30 g, 5.26 mmol, 3 eq.) and o-tolylaldehyde (0.26 g, 1.73 mmol) were added and vacuum-N₂ was applied. After that, 2 M HCl (13 mL) was added and heated to 105 °C and stirred for 3 days. After completion of reaction mixture cooled to room temperature and neutralized with conc. NaOH solution and then extracted with DCM. Collected organic phases were dried over Na₂SO₄ before filtration. The collected organic phases were then removed under reduced pressure. The crude product was purified by column chromatography (silica, hexane:EtOAc, 6:1), yielding the target product, compound **51**, as a white solid (0.71 g, 69% yield). ¹H NMR (400 MHz, CDCl₃) δ 7.25 – 7.19 (m, 2H), 7.18 – 7.11 (m, 2H), 7.09 – 7.02 (m, 1H), 6.65 (d, J = 7.4 Hz, 1H), 6.56 (t, J = 9.9 Hz, 4H), 5.59 (s, 1H), 2.91 (s, 12H), 2.20 (s, 3H). ¹³C NMR (100 MHz, CDCl₃) δ 149.8, 142.0, 137.4, 132.9, 130.6, 130.3, 129.1, 126.4, 125.7, 123.4, 112.1, 104.4, 60.8, 40.5, 20.0. HRMS calculated for C₂₄H₂₇N₂I₂: 597.0264. Found: 597.0254.

5.4.1.11 Synthesis of Compound 52

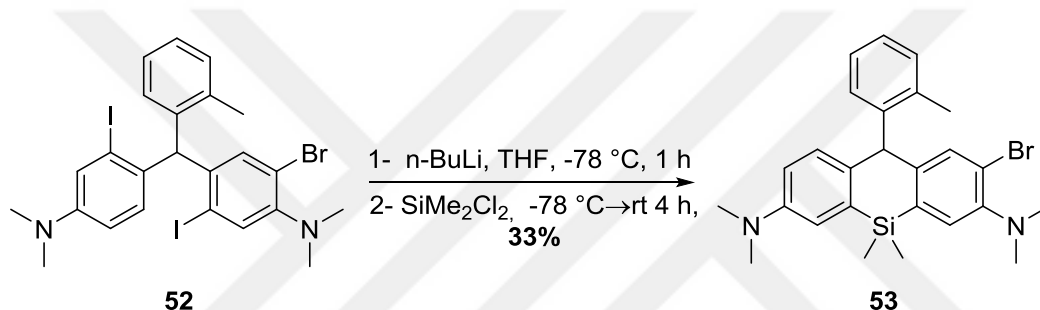


Scheme 5. 40. Synthetic route for compound **52**

To a 25 mL Schlenk tube, compound **51** (310 mg, 0.520 mmol) was added and dissolved with CHCl₃ (5 mL) and cooled to 0 °C. Then, *N*-Bromo succinimide (NBS) (94 mg, 0.530 mmol, 1.02 eq.) which was dissolved in chloroform (3 mL) was added dropwise into the reaction mixture. After completion of reaction, mixture quenched with distilled water and extracted. Collected organic phases were dried over Na₂SO₄ before filtration. The collected organic phases were then removed under reduced pressure. The crude product was purified by column chromatography (silica, 10

hexane : 1 EtOAc) and compound **52** obtained as a white solid (260 mg, 74%). ^1H NMR (400 MHz, CDCl_3) δ 7.50 (s, 1H), 7.26 – 7.21 (m, 1H), 7.18 (d, J = 3.8 Hz, 2H), 7.10 (dd, J = 7.5, 4.8 Hz, 1H), 6.87 (s, 1H), 6.63 (d, J = 7.4 Hz, 2H), 6.53 (d, J = 8.3 Hz, 1H), 5.59 (s, 1H), 2.93 (s, 6H), 2.79 (s, 6H), 2.19 (s, 3H). ^{13}C NMR (100 MHz, CDCl_3) δ 151.1, 150.0, 141.4, 140.8, 137.4, 135.0, 131.7, 130.6, 130.5, 129.0, 126.8, 126.0, 123.4, 119.0, 112.3, 104.4, 101.9, 101.9, 61.1, 44.3, 40.5, 20.0. HRMS calculated for $\text{C}_{24}\text{H}_{26}\text{N}_2\text{BrI}_2$: 674.9369. Found: 674.9370.

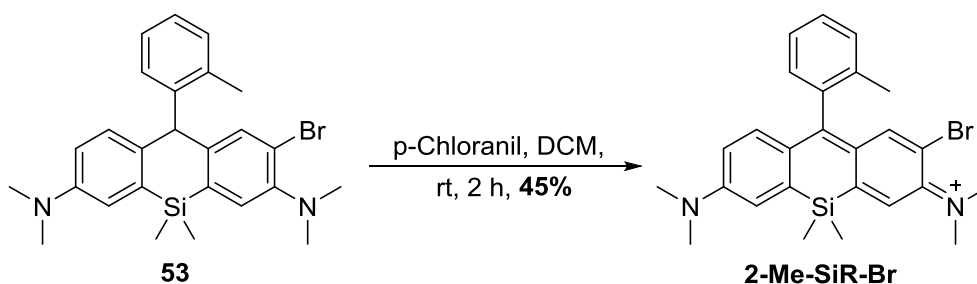
5.4.1.12 Synthesis of Compound 53



Scheme 5. 41. Synthetic route for compound **53**

To a 25 mL Schlenk tube, compound **52** (150 mg, 0.22 mmol) was added and vacuum- N_2 was applied. After that, compound **52** was dissolved with dry THF (5 mL) and cooled to -78°C . Then, $n\text{-BuLi}$ (0.20 mL, 0.44 mmol, 2.5 M, 2 eq.) was added dropwise and stirred at that temperature for 1 hour. 0.3 mL SiMe_2Cl_2 from stock solution ((stock solution: 0.3 mL SiMe_2Cl_2 + 2.7 mL THF), 0.24 mmol, 1.1 eq.) was added dropwise then reaction mixture warmed to room temperature slowly. After reaching to the room temperature, mixture stirred at that temperature for 4 hours. after completion of reaction, mixture quenched with distilled water and extracted with chloroform. Collected organic phases were dried over Na_2SO_4 before filtration. The collected organic phases were then removed under reduced pressure. The crude compound **53** was used without further purification.

5.4.1.13 Synthesis of Compound 2-Me-SiR-Br

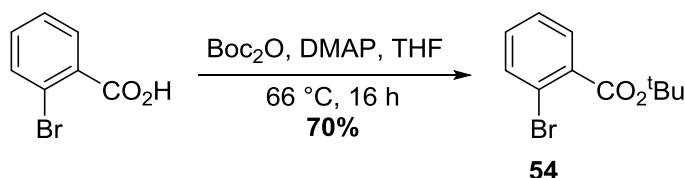


Scheme 5. 42. Synthetic route for compound **2-Me-SiR-Br**

To a 25 mL Schlenk tube, compound **53** (45 mg, 0.09 mmol) was added and dissolved with chloroform (5 mL). Then, p-chloranil (23 mg, 0.09 mmol, 1.95 eq.) was added and stirred for 1 hour. After completion of reaction, chloroform removed from the mixture and the crude product purified with HPLC and the target product, **2-Me-SiR-Br**, obtained as dark blue solid. (19.4 mg, 45%) ^1H NMR (400 MHz, CDCl_3) δ 7.54 (s, 1H), 7.44 (t, $J = 7.5$ Hz, 1H), 7.37 – 7.32 (m, 2H), 7.20 (s, 2H), 7.13 (d, $J = 9.7$ Hz, 1H), 7.05 (d, $J = 7.4$ Hz, 1H), 6.81 (d, $J = 9.0$ Hz, 1H), 3.62 (s, 6H), 3.21 (s, 6H), 2.02 (s, 3H), 0.66 (s, 3H), 0.64 (s, 3H). ^{13}C NMR (100 MHz, CDCl_3) δ 167.9, 156.1, 154.8, 151.3, 143.9, 143.7, 143.5, 137.7, 135.7, 132.0, 130.6, 129.5, 129.0, 125.9, 124.6, 124.1, 116.3, 113.3, 100.1, 43.7, 29.8, 19.7, -0.6, -0.9. HRMS calculated for $\text{C}_{26}\text{H}_{30}\text{N}_2\text{SiBr}$: 477.1362. Found: 477.1317.

5.4.2 Synthesis of Benzoic Acid Bearing Si-Rhodamine PS (2-COOH-SiR-Br/I)

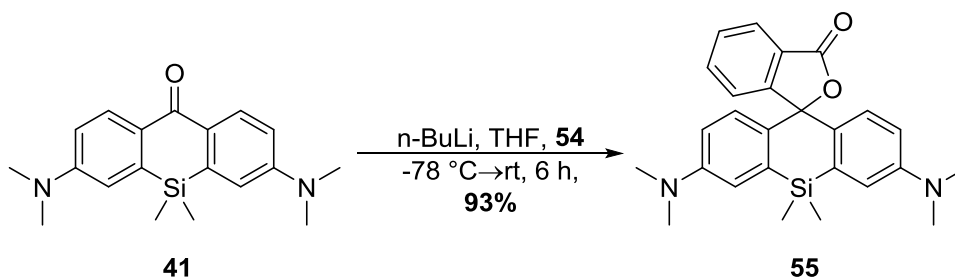
5.4.2.1 Synthesis of Compound **54**



Scheme 5. 43. Synthetic route for compound **54**

To a 250 mL Schlenk tube, compound o-bromobenzoic acid (5.65 g, 28.3 mmol) was added, and vacuum- N_2 was applied. Then, compound o-bromobenzoic acid was dissolved with dry THF (40 mL). Then Boc_2O (8.65 g, 39.5 mmol, 1.4 eq.), DMAP (0.77 g, 6.27 mmol, 0.22 eq.) were added and mixture heated to $66\text{ }^\circ\text{C}$ stirred for 16 hours. After completion of reaction, mixture quenched with saturated NaHCO_3 and extracted with EtOAc. Collected organic phases washed with brine then dried over Na_2SO_4 , filtered and solvent evaporated. The crude product purified with column chromatography (silica, hexane:EtOAc, 10:1). The target product, compound **54**, obtained as colorless oil (5.01 g, 70%). ^1H NMR (400 MHz, CDCl_3) δ 7.69 (dd, $J = 7.6, 1.6\text{ Hz}$, 1H), 7.61 (d, $J = 7.9\text{ Hz}$, 1H), 7.33 (t, $J = 7.2\text{ Hz}$, 1H), 7.27 (td, $J = 7.5, 1.4\text{ Hz}$, 1H), 1.62 (s, 9H). ^{13}C NMR (100 MHz, CDCl_3) δ 165.6, 134.2, 133.9, 131.7, 130.7, 127.0, 120.8, 82.4, 28.0.

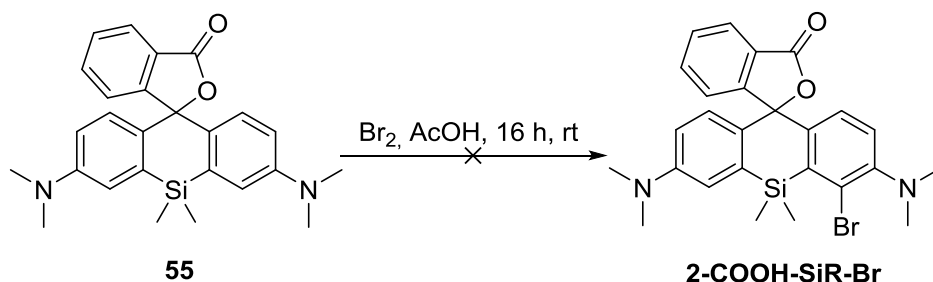
5.4.2.2 Synthesis of Compound 55



Scheme 5. 44. Synthetic route for compound **55**

To a 50 mL Schlenk tube, compound **54** (515 mg, 2.00 mmol, 5 eq.) was added, and vacuum- N_2 was applied. Then, compound **54** was dissolved with dry THF (7 mL) and cooled to $-78\text{ }^{\circ}\text{C}$. *n*-BuLi (0.9 mL, 2.00 mmol, 5 eq.) was added dropwise and stirred at that temperature for 1 hour. Then, solution of compound **41** (130 mg, 0.40 mmol) in dry THF (8 mL) was added to the reaction mixture dropwise. The final mixture warmed to room temperature slowly and stirred 6 hours more. After that, mixture quenched with 2 M HCl (2 mL) and stirred 10 minutes more before neutralizing with NaHCO_3 (25 mL). Aqueous mixture extracted with CHCl_3 and collected organic phases washed with brine then dried over Na_2SO_4 , filtered and evaporated. The crude product dissolved with TFA (2 mL) and stirred for 10 h more. After removal of TFA, EtOAc was added and extracted with NaHCO_3 . Collected organic phases washed with brine then dried over Na_2SO_4 , filtered and solvent evaporated. The crude product purified with column chromatography (basic alumina, hexane: CHCl_3 , 2:1). The target product, compound **55**, obtained as white solid (160 mg, 93%). ^1H NMR (400 MHz, CDCl_3) δ 7.98 (d, $J = 7.6$ Hz, 1H), 7.65 (t, $J = 7.9$ Hz, 1H), 7.55 (t, $J = 7.5$ Hz, 1H), 7.33 (d, $J = 7.7$ Hz, 1H), 7.00 (d, $J = 2.7$ Hz, 2H), 6.81 (d, $J = 8.9$ Hz, 2H), 6.57 (dd, $J = 8.9, 2.8$ Hz, 2H), 2.97 (s, 12H), 0.67 (s, 3H), 0.64 (s, 3H). ^{13}C NMR (100 MHz, CDCl_3) δ 170.8, 154.5, 149.3, 137.1, 133.8, 132.1, 128.8, 128.2, 127.1, 125.7, 124.7, 116.7, 113.4, 92.0, 40.3, 0.5, -1.4.

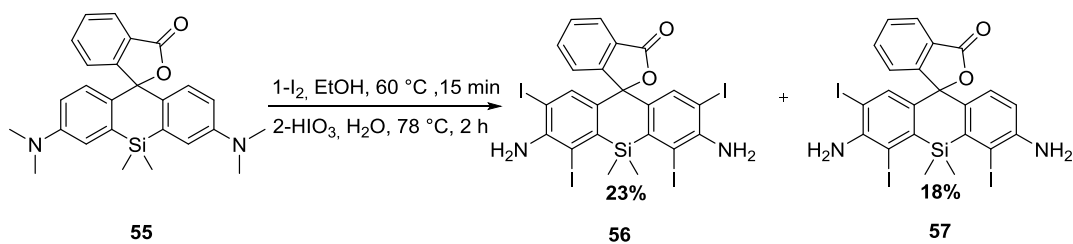
5.4.2.3 Synthesis of Compound 2-COOH-SiR-Br



Scheme 5. 45. Synthetic route for compound **2-COOH-SiR-Br**

To a 50 mL two-necked round-bottom flask, compound **55** (35 mg, 0.08 mmol) was added, and vacuum-N₂ was applied. Then, compound **55** was dissolved with AcOH (5 mL). Then solution of Br₂ in AcOH (1.0 mL from stock solution (0.2 mL Br₂ + 2.3 mL AcOH), 0.16 mmol, 2 eq.) was added slowly and stirred overnight. Then reaction mixture quenched with 10% aq. Na₂SO₃ solution (10 mL) and extracted with CHCl₃. Collected organic phases washed with brine then dried over Na₂SO₄, filtered and solvent evaporated. The crude product tried to be purified with column chromatography (basic alumina, hexane:CHCl₃, 2:1). Desired product could not be isolated.

5.4.2.4 Synthesis of Compounds **56** and **57**



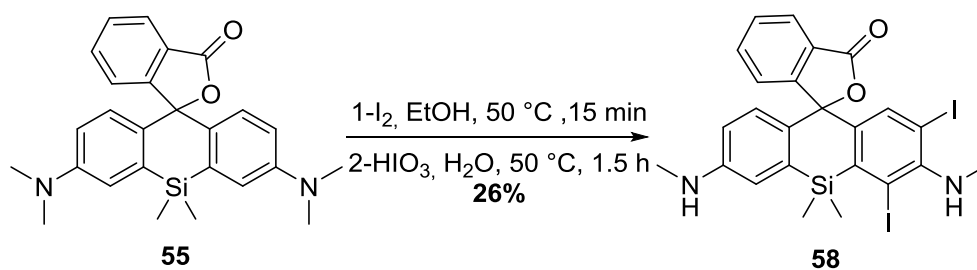
Scheme 5. 46. Synthetic route for compounds **56** and **57**

To a 25 mL Schlenk tube, compound **55** (65 mg, 0.15 mmol) was added, and vacuum-N₂ was applied. Then, compound **55** was dissolved with EtOH (10 mL). Then I₂ (77 mg, 0.30 mmol, 2.2 eq.) was added and mixture heated to 60 °C stirred

for 15 minutes. Then, HIO_3 (53 mg, 0.30 mmol, 2 eq.) solution in H_2O (1.0 mL) was added dropwise to the reaction mixture and temperature raised to 78°C stirred for 2 hours. After consumption of all starting material, EtOH evaporated, and mixture quenched with 10% $\text{Na}_2\text{S}_2\text{O}_3$ solution (10 mL) and extracted with EtOAc. Collected organic phases dried over Na_2SO_4 , filtered and solvent evaporated. The crude product purified with column chromatography (neutral alumina, hexane:DCM, 2:1). Compound **56** = 30 mg, 23%, Compound **57** = 20 mg, 18%. ^1H NMR (400 MHz, CDCl_3) δ 7.90 (d, $J = 7.7$ Hz, 1H), 7.54 – 7.49 (m, 1H), 7.50 – 7.47 (m, $J = 2.3$ Hz, 2H), 7.43 (t, $J = 7.4$ Hz, 1H), 6.99 (d, $J = 7.6$ Hz, 1H), 4.85 (s, 4H), 1.19 (s, 3H), 0.99 (s, 3H). (Compound **56**). HRMS calculated for $\text{C}_{22}\text{H}_{17}\text{N}_2\text{O}_2\text{SiI}_4$: 876.7238. Found: 876.7201.

^1H NMR (400 MHz, CDCl_3) δ 7.83 (d, $J = 7.6$ Hz, 1H), 7.48 (t, $J = 7.4$ Hz, 1H), 7.39 (t, $J = 7.5$ Hz, 1H), 7.12 (d, $J = 10.5$ Hz, 2H), 7.01 (d, $J = 7.7$ Hz, 1H), 6.84 (s, 1H), 4.66 (s, 4H), 0.75 (s, 3H), 0.64 (s, 3H). (Compound **57**). HRMS calculated for $\text{C}_{22}\text{H}_{18}\text{N}_2\text{O}_2\text{SiI}_3$: 750.8272 Found: 750.8267.

5.4.2.5 Synthesis of Compound **58**

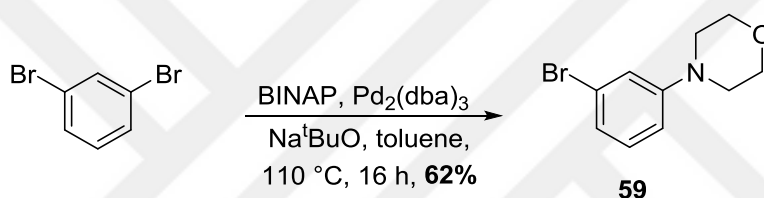


Scheme 5. 47. Synthetic route for compounds **58**

To a 25 mL Schlenk tube, compound **55** (55 mg, 0.13 mmol) was added, and vacuum- N_2 was applied. Then, compound **55** was dissolved with EtOH (10 mL). Then I_2 (65 mg, 0.28 mmol, 2.2 eq.) was added and mixture heated to 50°C stirred for 15 minutes. Then, HIO_3 (45 mg, 0.26 mmol, 2 eq.) solution in H_2O (1.0 mL) was added dropwise to the reaction mixture and temperature raised to 50°C stirred for

90 minutes. After consumption of all starting material, EtOH evaporated, and mixture quenched with 10% Na₂S₂O₃ solution (10 mL) and extracted with EtOAc. Collected organic phases dried over Na₂SO₄, filtered and solvent evaporated. The crude product tried to be purified with column chromatography (neutral alumina, hexane:DCM, 2:1). The target product obtained as white solid (22 mg, 26%). ¹H NMR (400 MHz, CDCl₃) δ 8.00 (d, *J* = 7.6 Hz, 1H), 7.71 (t, *J* = 7.5 Hz, 1H), 7.60 (d, *J* = 7.5 Hz, 1H), 7.30 (d, *J* = 7.7 Hz, 1H), 7.15 (s, 2H), 6.74 (s, 2H), 4.34 (s, 2H), 2.94 (s, 6H), 0.66 (s, 3H), 0.62 (s, 3H).

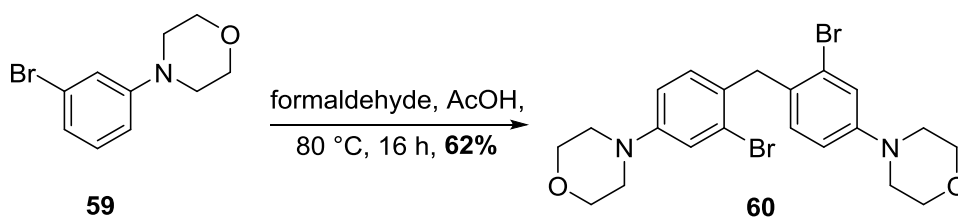
5.4.2.6 Synthesis of Compound 59



Scheme 5. 48. Synthetic route for compounds **59**

To a 25 mL Schlenk tube, 1,3-dibromobenzene (1.17 g, 5.00 mmol) was added and vacuum–N₂ was applied. Then, BINAP (0.16 g, 0.25 mmol, 5%), Pd₂(dba)₃ (0.23 g, 0.25 mmol, 5%), Na^tBuO (0.58 g, 6.00 mmol, 1.2 eq.), and morpholine (0.44 g, 5.00 mmol, 1 eq.) were added and dissolved with dry toluene (10 mL). The resulting mixture was heated to 110°C and stirred for 16 hours. After completion of reaction, mixture filtered through the celite and washed with EtOAc. The crude product was purified with column chromatography (silica, CHCl₃). The target product, compound **59**, was obtained as colorless oil (0.75 g, 62%). ¹H NMR (400 MHz, CDCl₃) δ 7.12 (t, *J* = 8.1 Hz, 1H), 7.03 (s, 1H), 6.99 (d, *J* = 7.8 Hz, 1H), 6.81 (dd, *J* = 8.3, 1.6 Hz, 1H), 3.86 – 3.80 (m, 4H), 3.15 – 3.10 (m, 4H). ¹³C NMR (100 MHz, CDCl₃) δ 152.5, 130.4, 123.3, 122.5, 118.3, 114.0, 66.7, 48.8.

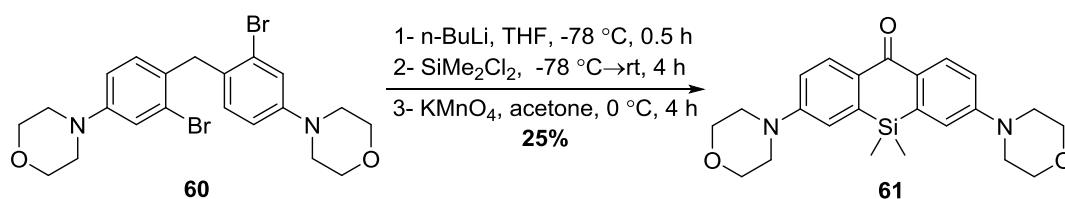
5.4.2.7 Synthesis of Compound 60



Scheme 5. 49. Synthetic route for compounds **60**

To a 50 mL Schlenk tube, compound **59** (0.73 g, 3.04 mmol, 2 eq.) and formaldehyde solution (46 mg, 1.52 mmol, 30%) were placed and dissolved with AcOH (9 mL). Then mixture heated to 80 °C and stirred for 16 hours at that temperature. After that, mixture cooled to room temperature and neutralized with satd. NaHCO₃ and satd. NaOH solutions and extracted with CHCl₃. Collected organic phases dried over Na₂SO₄, filtered and solvent evaporated. The crude product purified with column chromatography (silica, hexane:EtOAc 6:1). The target product, compound **60**, obtained as white solid (0.47 g, 62%). ¹H NMR (400 MHz, CDCl₃) δ 7.12 (d, *J* = 2.5 Hz, 2H), 6.88 (d, *J* = 8.5 Hz, 2H), 6.76 (dd, *J* = 8.5, 2.5 Hz, 2H), 4.03 (s, 2H), 3.85 – 3.82 (m, 8H), 3.13 – 3.10 (m, *J* = 4.7 Hz, 8H). ¹³C NMR (100 MHz, CDCl₃) δ 150.8, 131.0, 130.4, 125.7, 119.6, 114.9, 66.9, 49.1, 40.3.

5.4.2.8 Synthesis of Compound 61



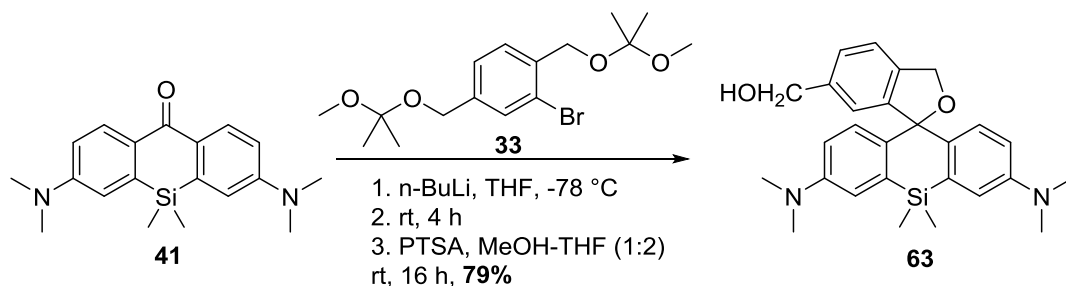
Scheme 5. 50. Synthetic route for compounds **61**

To a 25 mL Schlenk tube, compound **60** (0.38 g, 0.76 mmol) was added and vacuum–N₂ was applied. After that, compound **60** was dissolved with dry THF (5 mL) and cooled to -78 °C. Then, n-BuLi (0.9 mL, 2.17 mmol, 2.5 M, 2.85 eq.) was added

dropwise and stirred at that temperature for 30 minutes. 0.5 mL SiMe_2Cl_2 from stock solution ((stock solution: 1.9 mL SiMe_2Cl_2 + 3.8 mL THF), 0.83 mmol, 1.1 eq.) was added dropwise then reaction mixture warmed to room temperature slowly. After reaching to the room temperature, mixture stirred at that temperature for 4 hours. After that, reaction mixture quenched with 1 N HCl (0.5 mL) then neutralized with NaHCO_3 (15 mL) and extracted with CHCl_3 . Collected organic phases dried over Na_2SO_4 , filtered and evaporated. The crude product placed in 100 mL two necked round-bottomed balloon and dissolved with acetone (10 mL) and cooled to 0 °C. To reaction mixture KMnO_4 (0.33 g, 2.08 mmol, 2.75 eq.) in portions over 1 h and stirred for 3 hours more at that temperature. After that, reaction mixture filtered through celite and washed with CHCl_3 . The crude product purified with column chromatography (neutral alumina hexane: CHCl_3 2:1). The target product, compound **61**, obtained as yellow solid (77 mg, 25%). ^1H NMR (400 MHz, CDCl_3) δ 8.40 (d, J = 8.7 Hz, 2H), 7.06 – 6.98 (m, J = 10.5 Hz, 4H), 3.92 – 3.86 (m, 8H), 3.41 – 3.27 (m, 8H), 0.47 (s, 6H). ^{13}C NMR (100 MHz, CDCl_3) δ 185.4, 152.6, 140.6, 132.4, 131.7, 117.2, 115.9, 66.8, 47.8, -1.0.

5.4.3 Synthesis of TN-C Targeted Si-Rhodamine PS (TN-C-SiR-I)

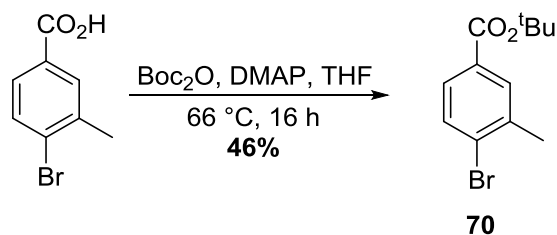
5.4.3.1 Synthesis of Compound 63



Scheme 5. 51. Synthetic route for compounds **63**

To a 50 mL Schlenk tube, compound **33** (557 mg, 1.54 mmol, 5 eq.) was added and vacuum-N₂ was applied. Then, dissolved with dry THF (4 mL) and cooled to -78 °C. Then, n-BuLi (0.73 mL, 1.54 mmol, 5 eq., 2.5 M) was added dropwise to a solution of compound **33** and stirred for 1 hour at that temperature. After that, compound **41** (100 mg, 0.31 mmol) was added dropwise to the reaction mixture and slowly warmed to room temperature and stirred for 4 hours more. After completion of reaction, reaction quenched with distilled water (10 mL) and extracted with CHCl₃ (50 mL). Collected organic phases dried over Na₂SO₄, filtered and solvent evaporated. The crude product dissolved with THF:MeOH mixture (3 mL, 2:1). To a solution, PTSA (0.07 g, 0.38 mmol, 1.25 eq.) and stirred for 16 hours. After completion of reaction, reaction mixture quenched with water (10 mL) and extracted with CHCl₃ (50 mL). Collected organic phases dried over Na₂SO₄, filtered and solvent evaporated. The crude product purified with column chromatography (neutral alumina, hexane:DCM, 2:1). Target product obtained as blue solid (108 mg, 79%). ¹H NMR (400 MHz, CDCl₃) δ 7.25 – 7.19 (m, 2H), 7.09 (d, *J* = 8.8 Hz, 2H), 7.03 (d, *J* = 2.8 Hz, 2H), 7.00 (s, 1H), 6.66 (dd, *J* = 8.9, 2.8 Hz, 2H), 5.32 (s, 2H), 4.47 (s, 2H), 2.95 (s, 12H), 0.72 (s, 3H), 0.63 (s, 3H). ¹³C NMR (100 MHz, CDCl₃) δ 148.6, 147.0, 140.6, 138.6, 138.1, 134.9, 128.6, 126.0, 122.5, 121.1, 116.5, 114.1, 92.4, 72.5, 64.8, 40.5, 0.4, -0.7.

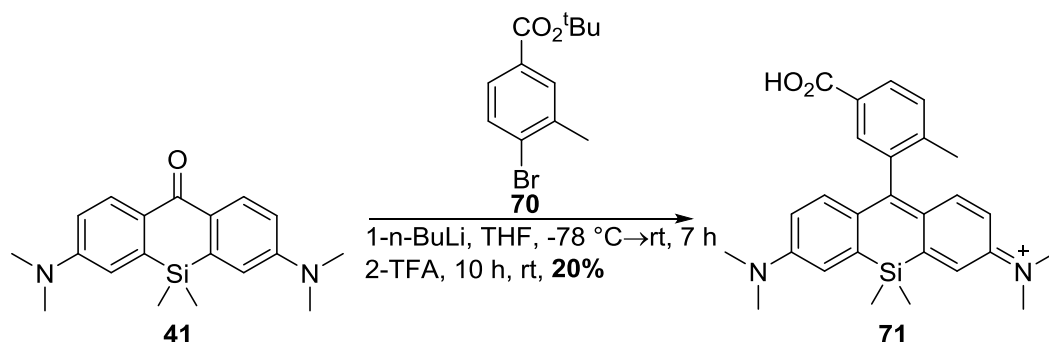
5.4.3.2 Synthesis of Compound 70



Scheme 5. 52. Synthetic route for compounds **70**

To a 250 mL Schlenk tube, 4-bromo-3-methylbenzoic acid (6.00 g, 28.0 mmol) was added, and vacuum–N₂ was applied. Then, it was dissolved with dry THF (40 mL). Then Boc₂O (8.58 g, 39.2 mmol, 1.4 eq.), DMAP (0.76 g, 6.16 mmol, 0.22 eq.) were added and mixture heated to 66 °C stirred for 16 hours. After completion of reaction, mixture quenched with saturated NaHCO₃ and extracted with EtOAc. Collected organic phases washed with brine then dried over Na₂SO₄, filtered and solvent evaporated. The crude product purified with column chromatography (silica, hexane:EtOAc, 10:1). The target product, compound **70**, obtained as colorless oil (3.49 g, 46%). ¹H NMR (400 MHz, CDCl₃) δ 7.82 (s, 1H), 7.63 (dd, *J* = 8.3, 1.5 Hz, 1H), 7.54 (d, *J* = 8.3 Hz, 1H), 2.41 (s, 3H), 1.57 (s, 9H). ¹³C NMR (100 MHz, CDCl₃) δ 165.3, 138.0, 132.4, 131.6, 131.2, 129.9, 128.3, 81.4, 28.3, 23.0.

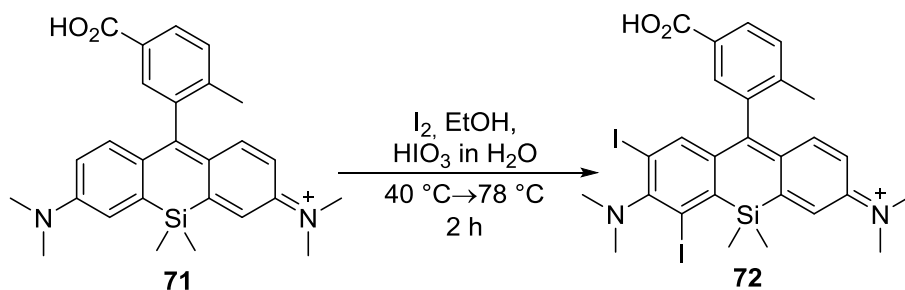
5.4.3.3 Synthesis of Compound 71



Scheme 5. 53. Synthetic route for compounds **71**

To a 50 mL Schlenk tube, compound **41** (0.40 g, 1.48 mmol, 5 eq.) was added, and vacuum–N₂ was applied. Then, compound **54** was dissolved with dry THF (5 mL) and cooled to -78 °C. n-BuLi (0.66 mL, 1.48 mmol, 5 eq.) was added dropwise and stirred at that temperature for 1 hour. Then, solution of compound **41** (96 mg, 0.30 mmol) in dry THF (5 mL) was added to the reaction mixture dropwise. The final mixture warmed to room temperature slowly and stirred 6 hours more. After that, mixture quenched with 2 M HCl (2 mL) and stirred 10 minutes more before neutralizing with NaHCO₃ (25 mL). Aqueous mixture extracted with CHCl₃ and collected organic phases washed with brine then dried over Na₂SO₄, filtered and evaporated. The crude product dissolved with TFA (2 mL) and stirred for 10 h more. After removal of TFA, EtOAc was added and extracted with NaHCO₃. Collected organic phases washed with brine then dried over Na₂SO₄, filtered and solvent evaporated. The crude product purified with column chromatography (basic alumina, hexane:CHCl₃, 2:1). The target product, compound **55**, obtained as white solid (26 mg, 20%). ¹H NMR (400 MHz, MeOD) δ 7.91 (s, 1H), 7.87 (d, *J* = 7.4 Hz, 1H), 7.31 (d, *J* = 2.3 Hz, 2H), 7.04 (d, *J* = 7.9 Hz, 1H), 7.01 (d, *J* = 9.6 Hz, 2H), 6.69 (dd, *J* = 9.5, 2.3 Hz, 2H), 3.28 (s, 12H), 1.97 (s, 3H), 0.57 (s, 3H), 0.55 (s, 3H). ¹³C NMR (100 MHz, MeOD) δ 170.7, 155.9, 149.6, 142.4, 139.1, 136.7, 132.6, 132.3, 130.0, 129.9, 128.4, 127.8, 122.3, 115.4, 41.1, 19.7, -0.9, -1.2. HRMS (ESI/MS) *m/z*: [M+H]⁺ Calcd. for C₂₇H₃₃N₂O₂Si⁺ 445.2311; Found 445.2282

5.4.3.4 Synthesis of Compound 72



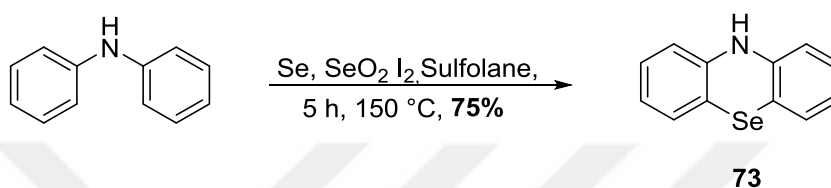
Scheme 5. 54. Synthetic route for compounds **72**

To a 25 mL Schlenk tube, compound **71** (44 mg, 0.10 mmol) was added, and vacuum- N_2 was applied. Then, it was dissolved with EtOH (7 mL). Then I_2 (51 mg, 0.20 mmol, 2.0 eq.) was added and mixture heated to $40\text{ }^\circ\text{C}$ stirred for 15 minutes. Then, HIO_3 (35 mg, 0.2 mmol, 2 eq.) solution in H_2O (1.0 mL) was added dropwise to the reaction mixture and temperature raised to $78\text{ }^\circ\text{C}$ stirred for 2 hours. After consumption of all starting material, EtOH evaporated, and mixture quenched with 10% $\text{Na}_2\text{S}_2\text{O}_3$ solution (10 mL) and extracted with EtOAc. Collected organic phases dried over Na_2SO_4 , filtered and solvent evaporated. The crude product tried to be purified with column chromatography (neutral alumina, hexane:DCM, 2:1). Efforts are focused on purification.

5.5 Benzoselenazine Based Photosensitizers

5.5.1 Synthesis of Morpholine Bearing Phenoselenazine Based PS (NSeMorph)

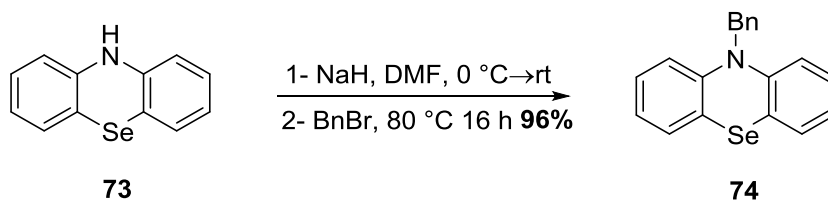
5.5.1.1 Synthesis of Compound 73



Scheme 5. 55. Synthetic route for compounds **73**

To a 25 mL Schlenk tube, diphenylamine (4.32 g, 25.58 mmol, 2 eq.) was added, and vacuum-N₂ was applied. Then, selenium powder (1.00 g, 12.79 mmol, 1 eq.), SeO₂ (1.70 g, 15.35 mmol, 1.2 eq.), and I₂ (162.6 g, 1.28 mmol, 0.1 eq.) were added and dissolved in sulfolane (5 mL). The reaction mixture was heated to 150 °C and stirred at that temperature for 16 hours. After the conclusion of the reaction, the mixture was cooled to room temperature and filtered through celite, then washed with CHCl₃. The crude product was purified with column chromatography (silica, hexane:EtOAc, 5:1), and the target product, compound **73**, was obtained as an off-white solid (2.54 g, 75% yield). ¹H NMR (400 MHz, DMSO) δ 8.62 (s, 1H), 7.09 (d, J = 7.4 Hz, 2H), 7.03 (t, J = 7.6 Hz, 2H), 6.77 (d, J = 8.4 Hz, 4H). ¹³C NMR (100 MHz, DMSO) δ 142.2, 128.9, 127.9, 122.2, 115.2, 111.6.

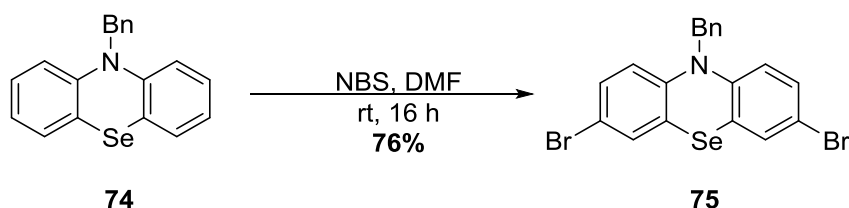
5.5.1.2 Synthesis of Compound 74



Scheme 5. 56. Synthetic route for compounds **74**

To a 25 mL Schlenk tube, compound **73** (0.50 g, 2.03 mmol) was added, and vacuum–N₂ was applied. Then, it was dissolved in dry DMF (8 mL) and cooled to 0 °C. Afterward, NaH (0.16 g, 4.06 mmol, 2 eq., 60%) was added and warmed to room temperature, then stirred for 30 min at that temperature. Subsequently, BnBr (0.70 g, 4.06 mmol, 2 eq.) was added, and the mixture was heated to 80 °C and stirred for 16 hours. After the completion of the reaction, the reaction mixture was cooled to room temperature, quenched with water, and extracted with CHCl₃. The organic phase was washed with 10% Na₂S₂O₃ (100 mL), then collected and dried over Na₂SO₄ before filtration. The collected organic phases dried over Na₂SO₄, filtered and solvent evaporated. The crude product was purified by column chromatography (silica, hexane:DCM, 2:1), yielding the target product, compound **74**, as an off-white solid (0.66 g, 96%). ¹H NMR (400 MHz, CDCl₃) δ 7.42 (d, J = 7.5 Hz, 2H), 7.39 – 7.32 (m, J = 7.4 Hz, 4H), 7.31 – 7.26 (m, 1H), 7.11 (t, J = 7.7 Hz, 2H), 6.95 (t, J = 7.4 Hz, 2H), 6.88 (d, J = 8.1 Hz, 2H), 5.18 (s, 2H). ¹³C NMR (100 MHz, CDCl₃) δ 145.0, 137.2, 129.7, 128.7, 127.6, 127.1, 123.2, 120.5, 117.1, 53.7.

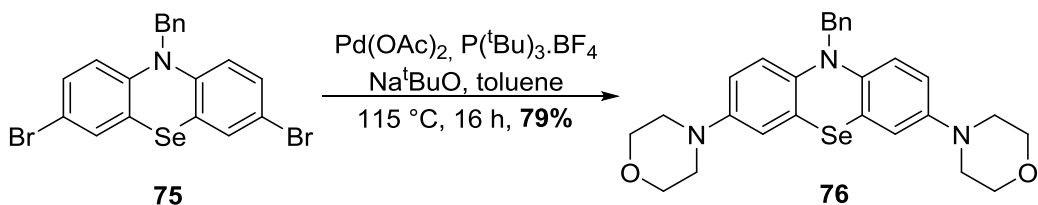
5.5.1.3 Synthesis of Compound 75



Scheme 5. 57. Synthetic route for compounds **75**

To a 50 mL two-necked round-bottom flask, compound **74** (1.8 g, 5.35 mmol) was added, and vacuum–N₂ was applied. Then, it was dissolved in dry DMF (20 mL). Subsequently, NBS (2.38 g, 13.38 mmol, 2.5 eq.) was added in portions over 1 hour. After the completion of the addition, the reaction mixture was stirred for 16 hours at room temperature. Then, the crude product was quenched with water and extracted with Et₂O. The collected organic phases dried over Na₂SO₄, filtered and solvent evaporated. The crude product was purified by column chromatography (silica, hexane:EtOAc, 10:1), yielding the target product, compound **75** as a white solid (2.00 g, 76%). ¹H NMR (400 MHz, CDCl₃) δ 7.36 (d, J = 2.3 Hz, 2H), 7.30 – 7.26 (m, 4H), 7.24 – 7.20 (m, 1H), 7.12 (dd, J = 9.1, 1.9 Hz, 2H), 6.62 (d, J = 8.7 Hz, 2H), 5.01 (s, 2H). ¹³C NMR (100 MHz, CDCl₃) δ 144.0, 136.2, 131.8, 130.5, 128.9, 127.5, 127.0, 122.1, 118.3, 115.8, 53.9.

5.5.1.4 Synthesis of Compound 76

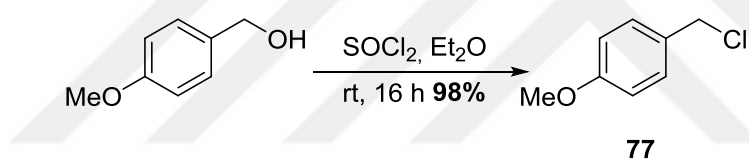


Scheme 5. 58. Synthetic route for compounds **76**

To a 25 mL Schlenk tube, compound **3** (0.20 g, 0.41 mmol), Pd(OAc)₂ (2.0 mg, 2%), P(^tBu)₃BF₄ (3.5 mg, 3%), Na^tBuO (0.12 g, 1.20 mmol, 2.95 eq.) were added, and

vacuum–N₂ was applied. Then, toluene (5 mL) was added and degassed for 1 hour. After that, morpholine (0.12 g, 1.42 mmol, 3.5 eq.) was added and heated to 115 °C and stirred for 16 hours. After the completion of the reaction, the reaction mixture was cooled to room temperature and filtered through celite, then washed with CHCl₃. The crude product was purified with column chromatography (basic alumina, hexane:DCM, 3:2), and the target product, compound **76**, was obtained as an off-white solid (0.16 g, 79%). ¹H NMR (400 MHz, CDCl₃) δ 7.33 – 7.28 (m, 4H), 7.23 – 7.20 (m, J = 7.1 Hz, 1H), 6.90 (d, J = 2.6 Hz, 2H), 6.71 (d, J = 8.9 Hz, 2H), 6.62 (dd, J = 8.8, 2.4 Hz, 2H), 5.04 (s, 2H), 3.81 (t, 8H), 3.02 (t, 8H). ¹³C NMR (100 MHz, CDCl₃) δ 147.2, 137.6, 129.2, 128.7, 127.1, 127.0, 121.3, 117.5, 117.2, 115.7, 67.0, 53.6, 50.3.

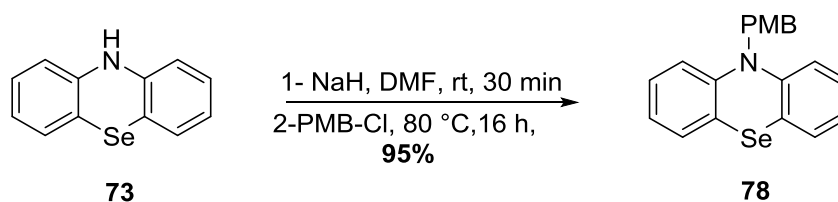
5.5.1.5 Synthesis of Compound **77**



Scheme 5. 59. Synthetic route for compounds **77**

To a 250 mL two-necked round-bottom flask, anisyl alcohol (4.00 g, 28.9 mmol) was added, and vacuum–N₂ was applied. Then, anisyl alcohol was dissolved in dry Et₂O (60 mL). Subsequently, SOCl₂ (6.85 g, 57.8 mmol, 2 eq.) was added dropwise and stirred for 16 hours at room temperature. After completion of reaction, reaction mixture quenched with water and extracted. Collected organic phases dried over MgSO₄, filtered and solvent evaporated. The crude product was purified by column chromatography (silica, hexane:DCM, 1:1), yielding the target product, compound **77**, as a colorless oil (4.44 g, 98%). ¹H NMR (400 MHz, CDCl₃) δ 7.27 (d, J = 8.6 Hz, 2H), 6.84 (d, J = 8.7 Hz, 2H), 4.52 (s, 2H), 3.74 (s, 3H). ¹³C NMR (100 MHz, CDCl₃) δ 159.8, 130.2, 129.8, 114.2, 55.3, 46.4.

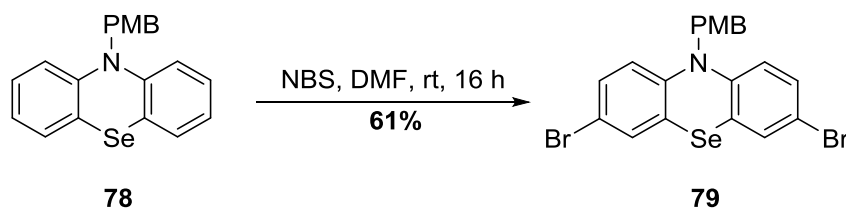
5.5.1.6 Synthesis of Compound 78



Scheme 5. 60. Synthetic route for compounds **78**

To a 50 mL Schlenk tube, compound **73** (1.27 g, 5.16 mmol) was added, and vacuum-N₂ was applied. Then, it was dissolved in dry DMF (20 mL) and cooled to 0°C. Afterward, NaH (0.41 g, 10.32 mmol, 2 eq., 60%) was added and warmed to room temperature, then stirred for 30 min at that temperature. Subsequently, PMB-Cl (1.61 g, 4.06 mmol, 2 eq.) was added, and the mixture was heated to 80 °C and stirred for 16 hours. After the completion of the reaction, the reaction mixture was cooled to room temperature, quenched with water, and extracted with Et₂O. The collected organic phases dried over MgSO₄, filtered and solvent evaporated. The crude product was purified by column chromatography (alumina, hexane:DCM, 2:1), yielding the target product, compound **78**, as a white solid (1.65 g, 95%). ¹H NMR (400 MHz, CDCl₃) δ 7.30 (dd, J = 12.7, 8.1 Hz, 4H), 7.08 (t, J = 7.7 Hz, 2H), 6.90 (t, J = 7.4 Hz, 2H), 6.84 (dd, J = 8.5, 2.5 Hz, 4H), 5.08 (s, 2H), 3.77 (s, 3H). ¹³C NMR (100 MHz, CDCl₃) δ 158.4, 144.9, 129.5, 128.9, 128.1, 127.4, 123.0, 120.5, 117.0, 113.9, 55.1, 52.9. HRMS calculated for C₂₀H₁₇NOSe: 367.0475. Found: 367.0475. HRMS (ESI/MS) *m/z*: [M+H]⁺ Calcd. for C₂₀H₁₇NOSe⁺ 367.0475; Found 367.0475.

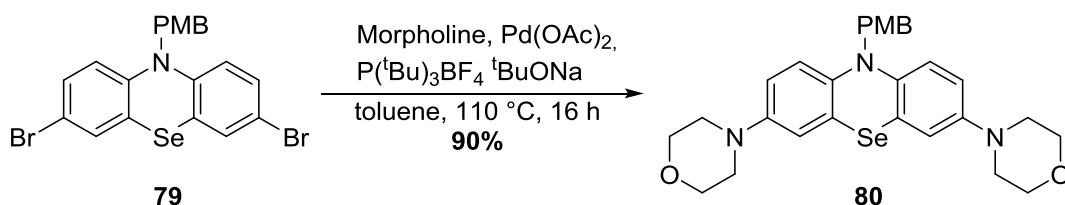
5.5.1.7 Synthesis of Compound 79



Scheme 5. 61. Synthetic route for compounds **79**

To a 50 mL two-necked round-bottom flask, compound **78** (0.61 g, 1.81 mmol) was added, and vacuum–N₂ was applied. Then, it was dissolved in dry DMF (20 mL). Subsequently, NBS (0.81 g, 4.53 mmol, 2.5 eq.) was added in portions over 1 hour. After the completion of the addition, the reaction mixture was stirred for 16 hours at room temperature. Then, the crude product was quenched with water and extracted with Et₂O. The collected organic phases dried over MgSO₄, filtered and solvent evaporated. The crude product was purified by column chromatography (alumina, hexane:DCM, 4:1), yielding the target product, compound **79**, as a white solid (0.58 g, 61%). ¹H NMR (400 MHz, CDCl₃) δ 7.38 (s, 2H), 7.22 (d, J = 8.1 Hz, 2H), 7.15 (d, J = 8.5 Hz, 2H), 6.84 (d, J = 8.0 Hz, 2H), 6.65 (d, J = 8.6 Hz, 2H), 4.95 (s, 2H), 3.77 (s, 3H). ¹³C NMR (100 MHz, CDCl₃) δ 158.8, 143.9, 131.7, 130.4, 128.2, 127.9, 122.2, 118.4, 115.7, 114.2, 55.3, 53.2. HRMS calculated for C₂₀H₁₅NOSeBr₂: 524.8665. Found: 524.8665

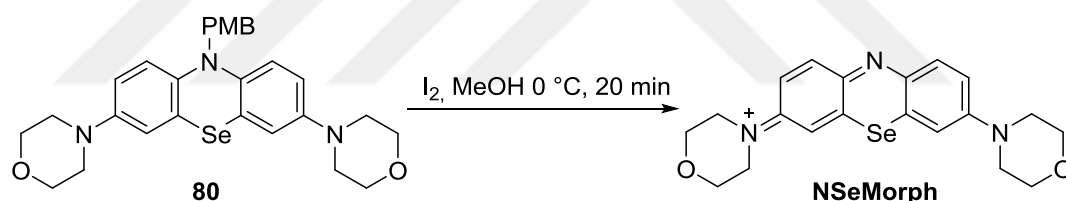
5.5.1.8 Synthesis of Compound 80



Scheme 5. 62. Synthetic route for compounds **80**

To a 25 mL Schlenk tube, compound **79** (0.17 g, 0.32 mmol), Pd(OAc)₂ (1.5 mg, 2%), P(^tBu)₃BF₄ (2.8 mg, 3%), Na^tBuO (0.09, 0.96 mmol, 2.95 eq.) and morpholine (0.10, 1.12 mmol, 3.5 eq.) were added, and vacuum–N₂ was applied. Then, toluene (5 mL) was added and degassed for 1 hour. After that, morpholine (0.09 g, 0.97 mmol, 3.5 eq.) was added and heated to 115 °C and stirred for 16 hours. After the completion of the reaction, the reaction mixture was cooled to room temperature and filtered through celite, then washed with CHCl₃. The crude product was purified with column chromatography (alumina, hexane:EtOAc, 10:1), and the target product, compound **80**, was obtained as an off-white solid (0.16 g, 90% yield). ¹H NMR (400 MHz, MeOD) δ 7.83 (d, J = 2.6 Hz, 2H), 7.49 (dd, J = 11.5, 6.1 Hz, 4H), 7.00 (d, J = 8.7 Hz, 2H), 6.82 (d, J = 8.8 Hz, 2H), 5.63 (s, 2H), 3.89 – 3.82 (m, 8H), 3.70 (s, 3H), 3.22 – 3.15 (m, 8H).

5.5.1.9 Synthesis of Compound NSeMorph

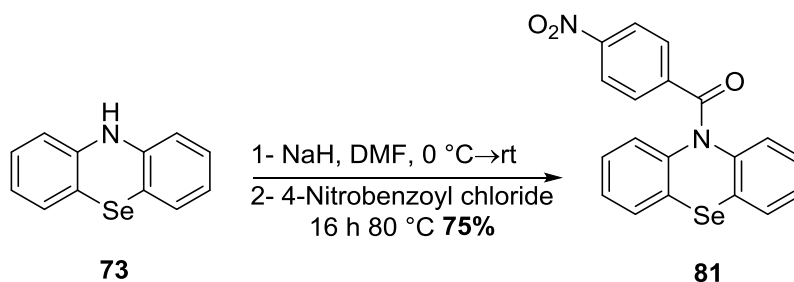


Scheme 5. 63. Synthetic route for compounds **NSeMorph**

To a 50 mL two-necked round-bottom flask, compound **80** (55 mg, 0.10 mmol) was added, and vacuum–N₂ was applied. Then, it was dissolved in MeOH (10 mL). After that, reaction mixture was cooled to 0 °C and subsequently I₂ (50 mg, 0.20 mmol, 2 eq.) and stirred at that temperature for 20 min. After completion of reaction, mixture filtered and washed with hexane (200 mL) and greenish black solid was obtained. The target product, **NSeMorph**, purified with preparative HPLC. ¹H NMR (400 MHz, DMSO) δ 8.07 (d, J = 2.2 Hz, 2H), 8.00 (d, J = 9.6 Hz, 2H), 7.63 (dd, J = 9.7, 2.3 Hz, 2H), 3.87 – 3.83 (m, J = 4.6 Hz, 8H), 3.81 – 3.78 (m, J = 4.4 Hz, 8H). ¹³C NMR (100 MHz, DMSO) δ 152.7, 139.9, 136.4, 135.4, 118.8, 110.8, 65.9, 47.8. HRMS calculated for C₂₀H₂₂N₃O₂Se: 416.0877. Found: 416.0877

5.5.1.10 Nitroreductase Activatable NSeMorph (NTR-NSeMorph)

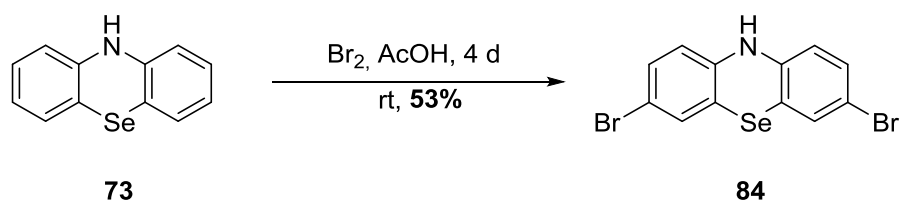
5.5.1.10.1 Synthesis of Compound 81



Scheme 5. 64. Synthetic route for compounds **81**

To a 25 mL Schlenk tube, compound **73** (0.50 g, 2.03 mmol) was added, and vacuum-N₂ was applied. Then, it was dissolved in dry DMF (7 mL) and cooled to 0 °C. Subsequently, NaH (0.16 g, 4.06 mmol, 2 eq. 60%) was added and mixture warmed to the room temperature. After 30 minutes stirring, 4-nitrobenzoyl chloride (0.75 g, 4.06 mmol, 2 eq.) was added and mixture stirred for 16 hours after heated to 80 °C. Upon completion, mixture quenched with distilled water (50 mL) and extracted with CHCl₃ (50 mL). The collected organic phases dried over Na₂SO₄, filtered and solvent evaporated. The crude product was purified by column chromatography (neutral alumina, hexane:CHCl₃, 2:1), yielding the target product, compound **81** as a white solid (0.60 g, 75%). ¹H NMR (400 MHz, CDCl₃) δ 8.06 (d, *J* = 8.8 Hz, 2H), 7.61 (dd, *J* = 7.2, 2.0 Hz, 2H), 7.52 (d, *J* = 8.8 Hz, 2H), 7.39 (s, 2H), 7.23 – 7.13 (m, 4H). ¹³C NMR (100 MHz, CDCl₃) δ 167.0, 148.5, 141.5, 138.9, 130.5, 129.7, 128.2, 127.9, 127.7, 127.4, 123.4.

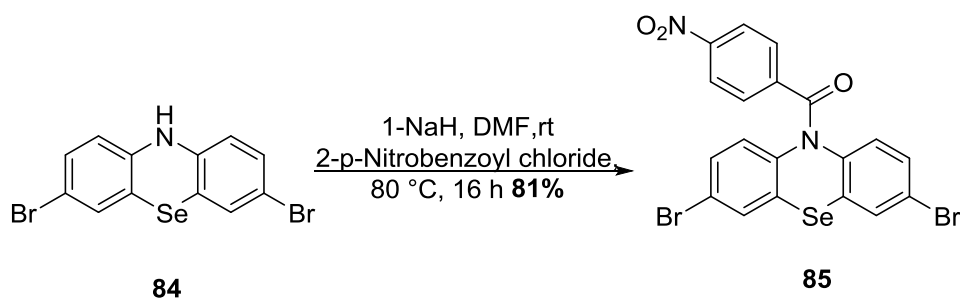
5.5.1.10.2 Synthesis of Compound **84**



Scheme 5. 65. Synthetic route for compounds **84**

To a 50 mL two necked round-bottomed flask compound **73** (0.4 g, 1.63 mmol) was added and suspended with fresh glacial acid (15 mL). Then molecular bromine (0.4 mL, 8.12 mmol, 5 eq.) was added dropwise and stirred for 4 days. Upon completion of reaction, quenched with 10% Na₂SO₃ (20 mL) and stirred 1 hour more. After that, mixture poured into ice-cold water (100 mL) and filtered. The crude product was purified with column chromatography (silica, hexane:EtOAc 3:1) to yield the target product, compound **84**, as gray solid (0.35 g, 53%). ¹H NMR (400 MHz, DMSO) δ 8.84 (s, 1H), 7.33 (s, 2H), 7.18 (dd, J = 8.3, 1.7 Hz, 2H), 6.66 (d, J = 8.4 Hz, 2H).

5.5.1.10.3 Synthesis of Compound **85**

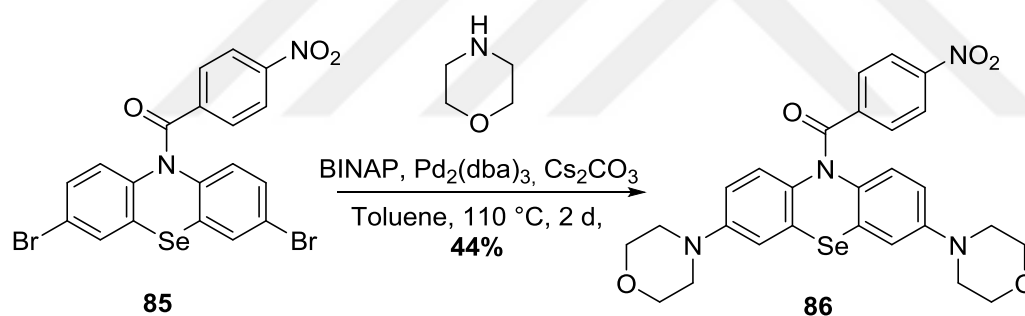


Scheme 5. 66. Synthetic route for compounds **85**

To a 25 mL Schlenk tube, compound **84** (0.20 g, 0.50 mmol) was added, and vacuum–N₂ was applied. Then, it was dissolved in dry DMF (5 mL) and cooled to 0 °C. Subsequently, NaH (0.04 g, 0.99 mmol, 2 eq. 60%) was added and mixture warmed to the room temperature. After 30 minutes stirring, 4-nitrobenzoyl chloride

(0.18 g, 0.99 mmol, 2 eq.) was added and mixture stirred for 16 hours after heated to 80 °C. Upon completion, mixture quenched with NaHCO₃ (50 mL) and extracted with CHCl₃ (50 mL). The collected organic phases dried over Na₂SO₄, filtered and solvent evaporated. The crude product was purified by column chromatography (basic alumina, hexane:DCM, 2:1), yielding the target product, compound **85** as a white solid (0.22 g, 81%). ¹H NMR (400 MHz, CDCl₃) δ 8.12 (d, *J* = 8.7 Hz, 2H), 7.76 (d, *J* = 2.0 Hz, 2H), 7.52 (d, *J* = 8.7 Hz, 2H), 7.34 (d, *J* = 7.5 Hz, 2H), 7.24 (s, 2H). ¹³C NMR (100 MHz, CDCl₃) δ 166.7, 148.8, 140.7, 137.9, 133.1, 131.1, 130.9, 129.8, 129.0, 123.7, 121.1. HRMS calculated for C₁₉H₁₁N₂O₃SeBr₂: 554.8281. Found: 554.8284

5.5.1.10.4 Synthesis of Compound **85**

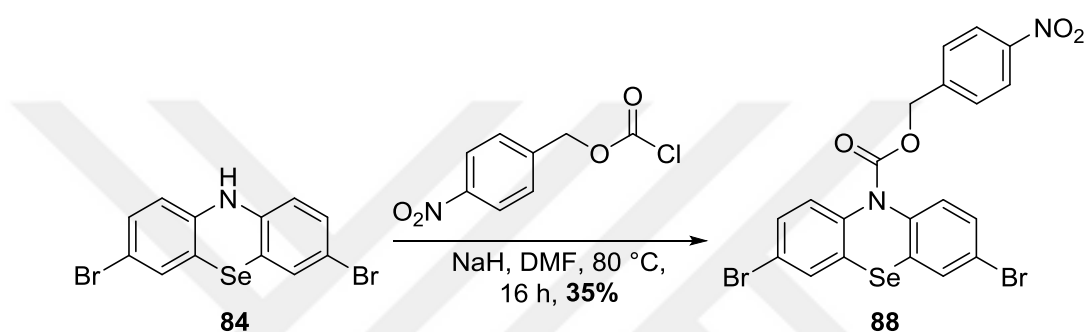


Scheme 5. 67. Synthetic route for compounds **86**

To a 25 mL Schlenk tube, compound **86** (100 mg, 0.18 mmol), Pd₂(dba)₃ (8.3 mg, 5%), BINAP (4.1 mg, 10%), Cs₂CO₃ (0.29, 0.91 mmol, 5 eq.) and morpholine (0.08, 0.91 mmol, 5 eq.) were added, and vacuum–N₂ was applied. Then, toluene (5 mL) was added and degassed for 1 hour. After that, morpholine (0.09 g, 0.97 mmol, 3.5 eq.) was added and heated to 110 °C and stirred for 2 days. After the completion of the reaction, the reaction mixture was cooled to room temperature and filtered through celite, then washed with CHCl₃. The crude product was purified with column chromatography (alumina, CHCl₃), and the target product, compound **86**, was

obtained as an off-white solid (20 mg, 44% yield). ^1H NMR (400 MHz, CDCl_3) δ 7.93 (d, $J = 8.5$ Hz, 2H), 7.37 (d, $J = 8.5$ Hz, 2H), 7.11 (s, 2H), 6.92 (d, $J = 2.2$ Hz, 2H), 6.64 – 6.38 (m, $J = 32.5, 18.4$ Hz, 2H), 3.71 – 3.63 (m, 8H), 3.02 – 2.91 (m, 8H). ^{13}C NMR (100 MHz, CDCl_3) δ 167.1, 150.1, 148.3, 142.2, 129.7, 128.3, 128.2, 127.9, 123.4, 116.4, 114.6, 66.8, 49.0.

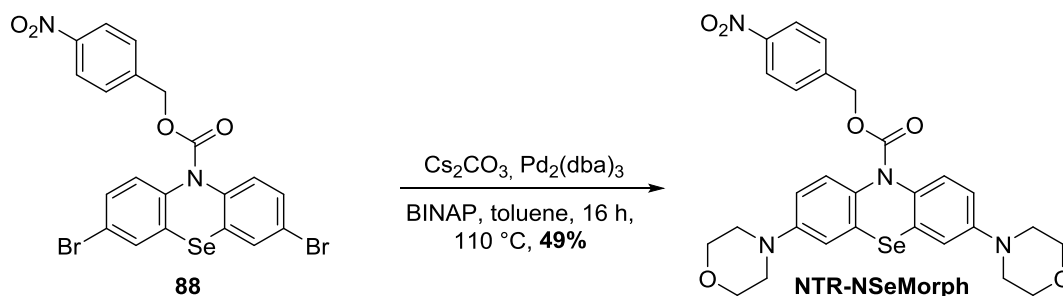
5.5.1.10.5 Synthesis of Compound 88



Scheme 5. 68. Synthetic route for compounds **88**

To a 25 mL Schlenk tube, compound **84** (200 mg, 0.50 mmol) was added, and vacuum- N_2 was applied. Then, it was dissolved in dry DMF (5 mL) and cooled to 0 °C. Subsequently, NaH (38 mg, 0.99 mmol, 2 eq. 60%) was added and mixture warmed to the room temperature. After 30 minutes stirring, 4-nitrobenzyl chloroformate (213 mg, 0.99 mmol, 2 eq.) was added and mixture stirred for 16 hours after heated to 80 °C. Upon completion, mixture quenched with NaHCO_3 (50 mL) and extracted with CHCl_3 (50 mL). The collected organic phases dried over Na_2SO_4 , filtered and solvent evaporated. The crude product was purified by column chromatography (basic alumina, hexane:DCM, 2:1), yielding the target product, compound **88** as a white solid (100 mg, 35%). ^1H NMR (400 MHz, CDCl_3) δ 8.20 (d, $J = 8.7$ Hz, 2H), 7.68 (d, $J = 1.9$ Hz, 2H), 7.46 (dd, $J = 8.6, 2.0$ Hz, 2H), 7.42 – 7.38 (m, 4H), 5.30 (s, 2H). ^{13}C NMR (100 MHz, CDCl_3) δ 153.1, 147.9, 142.9, 137.4, 132.7, 131.0, 129.1, 128.2, 124.0, 120.8, 67.0.

5.5.1.10.6 Synthesis of Compound NTR-NSeMorph

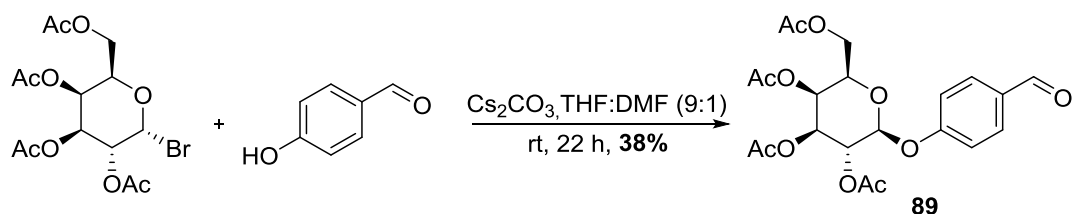


Scheme 5. 69. Synthetic route for **NTR-NSeMorph**

To a 25 mL Schlenk tube, compound **88** (100 mg, 0.17 mmol), $\text{Pd}_2(\text{dba})_3$ (7.8 mg, 5%), BINAP (10.6 mg, 10%), Cs_2CO_3 (279 mg, 0.91 mmol, 5 eq.) and morpholine (75 mg, 0.86 mmol, 5 eq.) were added, and vacuum- N_2 was applied. Then, toluene (5 mL) was added and degassed for 1 hour and then heated to 110°C and stirred for 16 hours. After the completion of the reaction, the reaction mixture was cooled to room temperature and filtered through celite, then washed with CHCl_3 . The crude product was purified with column chromatography (alumina, CHCl_3), and the target product, **NTR-NSeMorph**, was obtained as an off-white solid (50 mg, 49% yield). ^1H NMR (400 MHz, CDCl_3) δ 8.18 (d, $J = 8.7$ Hz, 2H), 7.42 (d, $J = 8.7$ Hz, 4H), 7.03 (d, $J = 2.0$ Hz, 2H), 6.86 (dd, $J = 8.8, 2.3$ Hz, 2H), 5.29 (s, 2H), 3.91 – 3.82 (m, $J = 4.7$ Hz, 8H), 3.20 – 3.09 (m, 8H). ^{13}C NMR (100 MHz, CDCl_3) δ 154.1, 149.8, 147.6, 143.7, 131.3, 130.4, 127.9, 123.9, 116.4, 115.1, 66.8, 66.5, 49.4. HRMS calculated for $\text{C}_{28}\text{H}_{29}\text{N}_4\text{O}_6\text{Se}$: 597.1252. Found: 597.1253

5.5.1.11 B-Galactosidase Activatable NSeMorph (β -Gal-NSeMorph)

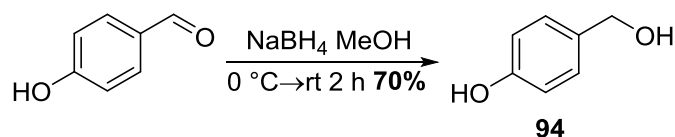
5.5.1.11.1 Synthesis of Compound 89



Scheme 5. 70. Synthetic route for **89**

To a 25 mL Schlenk tube, Cs_2CO_3 (1.35 g, 4.15 mmol, 4.05 eq.) was added and suspended with DMF-THF solution (0.5:4.5 mL). Then to a suspension, solution of 4-hydroxybenzaldehyde (0.13 g, 1.02 mmol) in DMF-THF solution (0.4:3.1 mL) was added. Subsequently, solution of acetobromo- α -D-galactose (0.50 g, 1.22 mmol, 1.2 eq.) in DMF-THF solution (0.4:3.1 mL) was added and stirred for 22 hours at room temperature. Upon completion of reaction mixture quenched with satd. NaHCO_3 solution (50 mL) and extracted with CHCl_3 (50 mL). The collected organic phases washed with brine (50 mL), then dried over Na_2SO_4 , filtered and solvent evaporated. The crude product was purified by column chromatography (silica, hexane:EtOAc, 4:3), yielding the target product, compound **89** as a white solid (0.18 g, 38%). ^1H NMR (400 MHz, CDCl_3) δ 9.87 (s, 1H), 7.81 (d, J = 7.8 Hz, 2H), 7.07 (d, J = 7.9 Hz, 2H), 5.49 (d, J = 9.6 Hz, 1H), 5.43 (s, 1H), 5.16 (d, J = 7.8 Hz, 1H), 5.10 (d, J = 10.4 Hz, 1H), 4.19 – 4.05 (m, 3H), 2.14 (s, 3H), 2.02 (s, 6H), 1.97 (s, 3H). ^{13}C NMR (100 MHz, CDCl_3) δ 190.6, 170.2, 170.0, 169.9, 169.2, 161.1, 131.7, 116.6, 98.3, 71.1, 70.5, 68.2, 66.6, 61.2, 20.6, 20.5, 20.4.

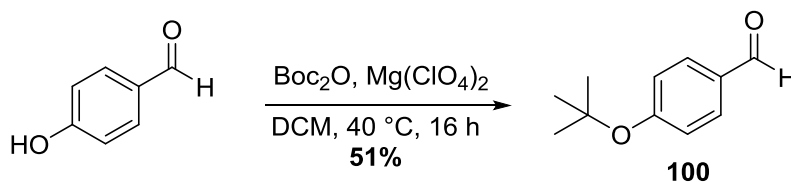
5.5.1.11.2 Synthesis of Compound 94



Scheme 5. 71. Synthetic route for **94**

To a 50 mL two necked, round bottom flask, 4-hydroxybenzaldehyde (1.00 g, 8.19 mmol) was added and vacuum-N₂ was applied. Then it was dissolved with MeOH (10 mL). Subsequently, solution cooled to 0 °C and NaBH₄ (0.46 g, 12.27 mmol, 1.5 eq.) was added to this solution in portions. The resulting mixture stirred at that temperature for 2 hours. Upon completion of reaction, mixture quenched with satd. NH₄Cl solution (20 mL) then MeOH was evaporated. Following this, EtOAc (20 mL) was added and extracted. The collected organic phases dried over Na₂SO₄, filtered and solvent evaporated. The crude product was purified by column chromatography (silica, MeOH:DCM, 5:95), yielding the target product, compound **94** as a white solid (0.71 g, 70%). ¹H NMR (400 MHz, Acetone-d₆) δ 8.23 (s, 1H), 7.18 (d, *J* = 8.5 Hz, 2H), 6.78 (d, *J* = 8.5 Hz, 2H), 4.50 (d, *J* = 5.8 Hz, 2H), 4.02 (t, *J* = 5.8 Hz, 1H). ¹³C NMR (100 MHz, Acetone-d₆) δ 157.3, 134.1, 129.1, 115.7, 64.5.

5.5.1.11.3 Synthesis of Compound 100

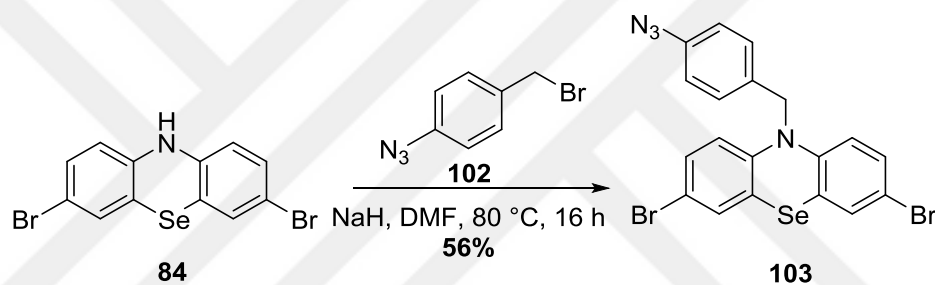


Scheme 5. 72. Synthetic route for **100**

To a 50 mL Schlenk tube, 4-hydroxybenzaldehyde (1.22 g, 10.00 mmol) and Mg(ClO₄)₂ (0.22 g, 1.00 mmol, 0.1 eq.) were added and vacuum-N₂ was applied. Then it was dissolved with dry DCM (10 mL). Then Boc₂O (5.02 g, 23 mmol, 2.3

eq.) was added and mixture heated to 40 °C and stirred for 16 hours at that temperature. Upon completion of reaction, mixture cooled to the room temperature and quenched with distilled water (20 mL) and extracted. The collected organic phases dried over Na₂SO₄, filtered and solvent evaporated. The crude product was purified by column chromatography (silica, MeOH:DCM, 1:99), yielding the target product, compound **100** as a white solid (0.90 g, 51%). ¹H NMR (400 MHz, CDCl₃) δ 9.93 (s, 1H), 7.82 (d, *J* = 8.6 Hz, 2H), 7.12 (d, *J* = 8.6 Hz, 2H), 1.46 (s, 9H). ¹³C NMR (100 MHz, CDCl₃) δ 191.2, 161.8, 133.9, 131.3, 122.5, 80.1, 29.0.

5.5.1.11.4 Synthesis of Compound **103**

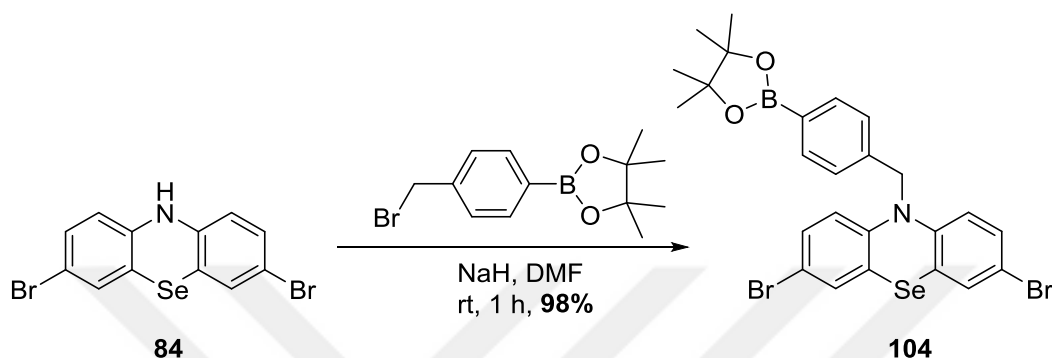


Scheme 5. 73. Synthetic route for **103**

To a 50 mL Schlenk tube, compound **84** (0.50 g, 1.24 mmol) was added, and vacuum–N₂ was applied. Then, it was dissolved in dry DMF (10 mL) and cooled to 0 °C. Subsequently, NaH (0.10 g, 2.48 mmol, 2 eq. 60%) was added and mixture warmed to the room temperature. After 30 minutes stirring, compound **102** (0.53 g, 2.48 mmol, 2 eq.) was added and mixture stirred for 16 hours after heated to 80 °C. Upon completion, mixture quenched with NaHCO₃ (50 mL) and extracted with CHCl₃ (50 mL). The collected organic phases dried over Na₂SO₄, filtered and solvent evaporated. The crude product was purified by column chromatography (neutral alumina, hexane:DCM, 2:1), yielding the target product, compound **103** as a white solid (0.37 g, 56%). ¹H NMR (400 MHz, CDCl₃) δ 7.40 (d, *J* = 2.3 Hz, 2H), 7.30 (d, *J* = 8.6 Hz, 2H), 7.17 (dd, *J* = 8.7, 2.3 Hz, 2H), 6.96 (d, *J* = 8.5 Hz, 2H), 6.64 (d, *J* =

8.7 Hz, 2H), 5.00 (s, 2H). ^{13}C NMR (100 MHz, CDCl_3) δ 143.9, 139.3, 132.9, 132.0, 130.5, 128.6, 122.7, 119.6, 118.4, 116.0, 53.2.

5.5.1.11.5 Synthesis of Compound **104**

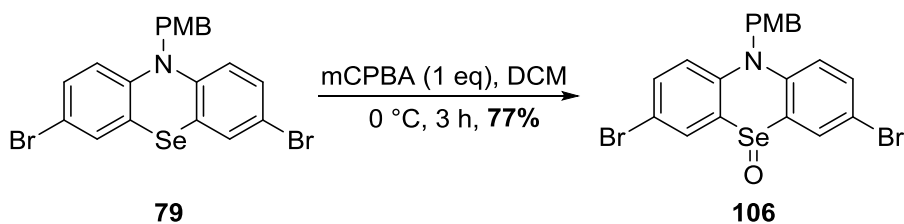


Scheme 5. 74. Synthetic route for **104**

To a 25 mL Schlenk tube, compound **84** (0.15 g, 0.37 mmol) was added, and vacuum- N_2 was applied. Then, it was dissolved in dry DMF (5 mL) and cooled to 0 °C. Subsequently, NaH (0.02 g, 0.56 mmol, 1.5 eq. 60%) was added and mixture warmed to the room temperature. After 40 minutes stirring, 4-bromomethylphenylboronic acid pinacol ester (0.17 g, 1.5 mmol, 1.5 eq.) was added and mixture stirred for 1 hour at room temperature. Upon completion, mixture quenched with distilled water (50 mL) and extracted with Et_2O (50 mL). The collected organic phases dried over MgSO_4 , filtered and solvent evaporated. The crude product was purified by column chromatography (neutral alumina, hexane:DCM, 2:1), yielding the target product, compound **104** as a white solid (0.24 g, 98%). ^1H NMR (400 MHz, CDCl_3) δ 7.75 (d, J = 8.0 Hz, 2H), 7.38 (d, J = 2.3 Hz, 2H), 7.30 (d, J = 7.9 Hz, 2H), 7.12 (dd, J = 8.7, 2.2 Hz, 2H), 6.61 (d, J = 8.7 Hz, 2H), 5.04 (s, 2H), 1.33 (s, 12H). ^{13}C NMR (100 MHz, CDCl_3) δ 143.9, 139.4, 135.35, 131.8, 130.5, 128.4, 126.4, 122.0, 118.3, 115.8, 84.0, 54.1, 25.0.

5.5.2 Synthesis of Morpholine and Selenium Oxide Bearing Phenoselenazine Based PS (NSeOMorph)

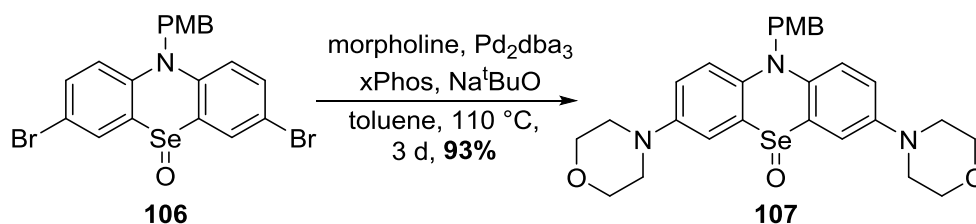
5.5.2.1 Synthesis of Compound 106



Scheme 5. 75. Synthetic route for **106**

To a 25 mL Schlenk tube, compound **79** (0.20 g, 0.4 mmol) was added and vacuum-N₂ were applied. Then, it was dissolved with dry DCM (10 mL). Then, the mixture cooled to 0 °C and mCPBA (0.07 g, 0.4 mmol, 1eq) was added. The mixture stirred under N₂ atmosphere and room temperature for 2 hours. Upon completion of reaction, mixture quenched with distilled water (20 mL) and extracted. The collected organic layers were dried over NaSO₄, filtered and solvent was evaporated. The crude product was purified by column chromatography (neutral alumina, MeOH:DCM, 2:98), yielding to the target product, compound **106** as a white solid (0.16 g, 77%). (158.5 mg, 77%). ¹H NMR (400 MHz, CDCl₃) δ 7.91 (d, *J* = 2.3 Hz, 2H), 7.46 (dd, *J* = 9.0, 2.3 Hz, 2H), 7.04 (dd, *J* = 8.8, 3.4 Hz, 4H), 6.86 (d, *J* = 8.6 Hz, 2H), 5.23 (s, 2H), 3.76 (s, 3H). ¹³C NMR (100 MHz, CDCl₃) δ 159.2, 138.6, 135.7, 132.9, 127.2, 126.3, 125.1, 119.3, 115.1, 114.7, 55.4, 54.0.

5.5.2.2 Synthesis of Compound 107

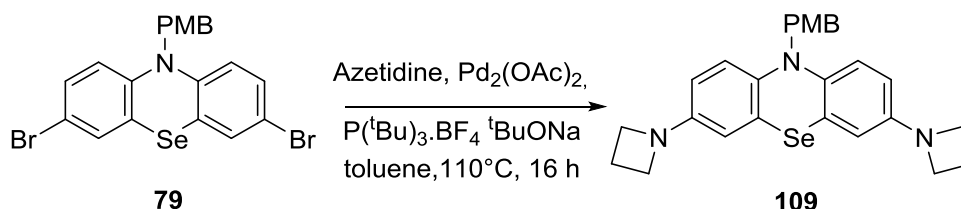


Scheme 5. 76. Synthetic route for **107**

To a 25 mL Schlenk tube, compound **4** (0.16 g, 0.3 mmol) was added and vacuum- N_2 were applied. Then, $\text{Pd}_2(\text{dba})_3$ (13.5 mg, 0.015 mmol, 5%), xPhos (14.0 mg, 0.03 mmol, 10%), and Na^tBuO_4 (0.10 g, 1.0 mmol, 3.5 eq.) were added and dissolved in with dry toluene (10 mL). The resulted mixture degassed with N_2 for 1 hour. Then, morpholine (0.09 g, 1.0 mmol, 3.5 eq.) was added and reaction mixture heated to $110\text{ }^\circ\text{C}$ and stirred for 3 days. Upon completion of reaction, mixture cooled to the room temperature and filtered through celite and washed with EtOAc. The crude product purified by column chromatography (neutral alumina, Hexane:EtOAc, 5:1). yielding to the target product, compound **106** as a white solid (0.18 g, **93%**). ^1H NMR (400 MHz, CD_3CN) δ 7.27 (d, $J = 8.7$ Hz, 2H), 6.94 (d, $J = 2.7$ Hz, 2H), 6.83 (d, $J = 3.4$ Hz, 2H), 6.80 (d, $J = 3.2$ Hz, 2H), 6.69 (dd, $J = 8.8, 2.7$ Hz, 2H), 4.94 (s, 2H), 3.72 – 3.68 (m, $J = 4.8$ Hz, 12H), 2.98 – 2.94 (m, 8H). ^{13}C NMR (100 MHz, CD_3CN) δ 159.6, 148.5, 139.6, 131.0, 129.5, 123.2, 117.9, 116.0, 114.6, 67.4, 55.8, 50.6. HRMS calculated for $\text{C}_{19}\text{H}_{11}\text{N}_2\text{O}_3\text{SeBr}_2$: 554.8281. Found: 554.8284

5.5.3 Synthesis of Azetidine Bearing Phenoselenazine Based PS (NSeAze)

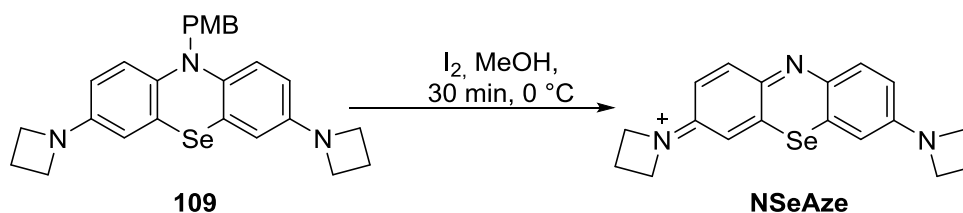
5.5.3.1 Synthesis of Compound 109



Scheme 5. 77. Synthetic route for **109**

To a 25 mL Schlenk tube, compound **79** (0.2 g, 0.38 mmol), Pd(OAc)₂ (4.0 mg, 2%), P(^tBu)₃BF₄ (6.0 mg, 3%), Na^tBuO (0.11, 1.13 mmol, 2.95 eq.) were added, and vacuum–N₂ was applied. Then, toluene (5 mL) was added and degassed for 1 hour. After that, azetidine (0.08 g, 1.34 mmol, 3.5 eq.) was added and heated to 110 °C and stirred for 16 hours. After the completion of the reaction, the reaction mixture was cooled to room temperature and filtered through celite, then washed with CHCl₃. The crude product was purified with column chromatography (alumina, hexane:CHCl₃ 2:1), and the target product, compound **109**, was obtained as an off-white solid used in next step without further purification.

5.5.3.2 Synthesis of Compound NSeAze



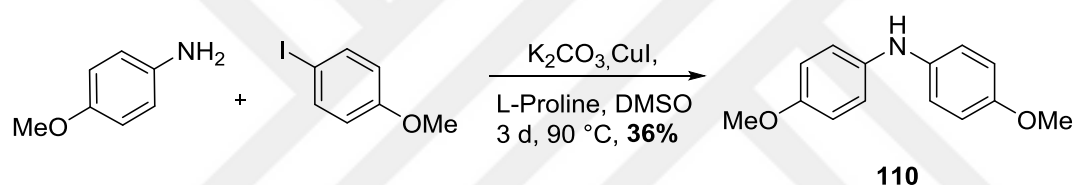
Scheme 5. 78. Synthetic route for **NSeAze**

To a 50 mL two-necked round-bottom flask, compound **109** (50 mg, 0.10 mmol) was added, and vacuum–N₂ was applied. Then, it was dissolved in MeOH (10 mL). After

that, reaction mixture was cooled to 0 °C and subsequently I₂ (51 mg, 0.20 mmol, 2 eq.) and stirred at that temperature for 30 min. After completion of reaction, mixture filtered and washed with hexane (200 mL) and greenish black solid was obtained. The target product, **NSeAze**, purified with preparative HPLC. ¹H NMR (400 MHz, DMSO) δ 7.86 (d, *J* = 9.3 Hz, 2H), 7.38 (d, *J* = 2.3 Hz, 2H), 6.96 (dd, *J* = 9.3, 2.3 Hz, 2H), 4.33 (t, *J* = 7.3 Hz, 8H), 2.49 – 2.43 (m, 4H). ¹³C NMR (100 MHz, DMSO) δ 152.2, 139.7, 134.7, 134.4, 116.5, 108.5, 52.4, 15.4.

5.5.4 Synthesis of Oxygen Bearing Phenoselenazine Based PS (NSeO)

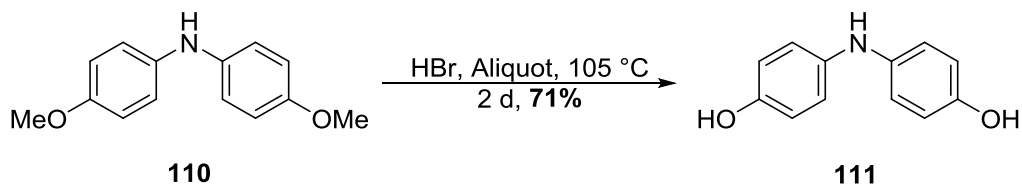
5.5.4.1 Synthesis of Compound **110**



Scheme 5. 79. Synthetic route for **110**

To a 25 mL Schlenk tube, p-anisidine (2.46 g, 20.00 mmol), iodoanisole (7.00 g, 30.00 mmol, 1.5 eq.), K₂CO₃ (5.53 g, 40 mmol 2 eq.), CuI (0.38 g, 2.00 mmol, 0.1 eq.), L-proline (0.46 g, 4.00 mmol, 0.2 eq.) were added, and vacuum–N₂ was applied. The resulting mixture suspended with DMSO (3 mL) and heated to 90 °C and stirred for 3 days. Upon completion, the reaction mixture was cooled to room temperature and filtered through celite, then washed with CHCl₃. The crude product was purified with column chromatography (silica, hexane:EtOAc 6:1), and the target product, compound **110**, was obtained as a white solid (1.65 g, 36%). ¹H NMR (400 MHz, CDCl₃) δ 6.95 (d, *J* = 8.9 Hz, 4H), 6.83 (d, *J* = 8.9 Hz, 4H), 5.30 (s, 1H), 3.79 (s, 6H). ¹³C NMR (100 MHz, CDCl₃) δ 154.3, 138.1, 119.7, 114.8, 55.8.

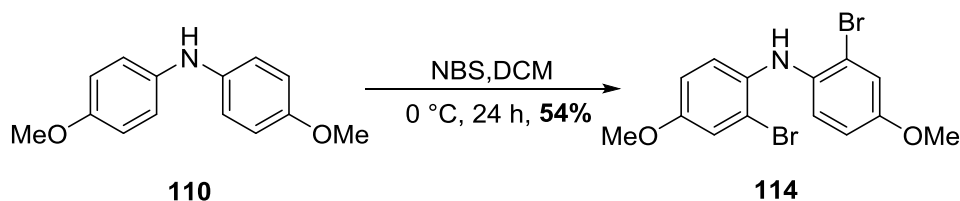
5.5.4.2 Synthesis of Compound **111**



Scheme 5. 80. Synthetic route for **111**

To a 25 mL Schlenk tube, compound **110** (0.80 g, 3.49 mmol) was added and vacuum–N₂ was applied. Then, Aliquot (0.20 mL) and HBr (1.8 Ml) were added and mixture heated to 105 °C and stirred for 2 days. Upon completion, the reaction mixture was cooled to room temperature and quenched with distilled water (50 mL) and extracted with EtOAc (50 mL). The collected organic layers were dried over NaSO₄, filtered and solvent was evaporated. The crude product redissolved with MeOH (2 mL) and added dropwise to the cold hexane (250 mL) and stirred for 1 hour. The resulting precipitates filtered and dried under vacuo. Compound **111** was obtained as white solid and used in following reaction without further purification. (0.50 g, 71%) ¹H NMR (400 MHz, MeOD) δ 6.83 (d, *J* = 8.8 Hz, 4H), 6.67 (d, *J* = 8.8 Hz, 4H).

5.5.4.3 Synthesis of Compound **114**

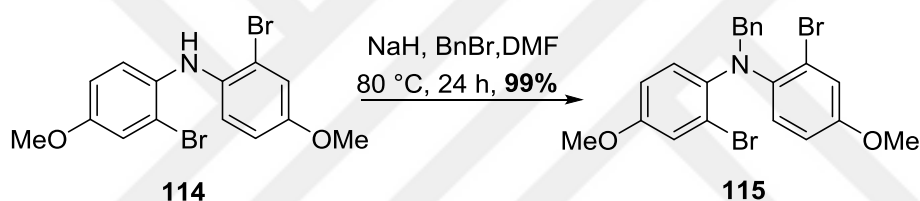


Scheme 5. 81. Synthetic route for **114**

To a 50 mL two-necked round-bottom flask, compound **110** (1.0 g, 4.36 mmol) was added, and vacuum–N₂ was applied. Then, it was dissolved in dry DCM (15 mL). After that, reaction mixture was cooled to 0 °C and subsequently NBS (1.94 g, 10.90

mmol, 2.5 eq.) was added in portions over 1 hour and stirred at that temperature for 23 hours. After completion of reaction, mixture quenched with distilled water (50 mL) and extracted. The collected organic layers were dried over NaSO₄, filtered and solvent was evaporated. The crude product was purified with column chromatography (silica, hexane:DCM 1:1), and the target product, compound **114**, was obtained as a white solid (0.91 g, 54%). ¹H NMR (400 MHz, CDCl₃) δ 7.14 (d, *J* = 2.8 Hz, 2H), 7.02 (d, *J* = 8.9 Hz, 2H), 6.79 (dd, *J* = 8.9, 2.8 Hz, 2H), 5.82 (s, 1H), 3.78 (s, 6H). ¹³C NMR (100 MHz, CDCl₃) δ 154.8, 135.0, 119.9, 118.3, 115.2, 114.5, 56.0.

5.5.4.4 Synthesis of Compound 115



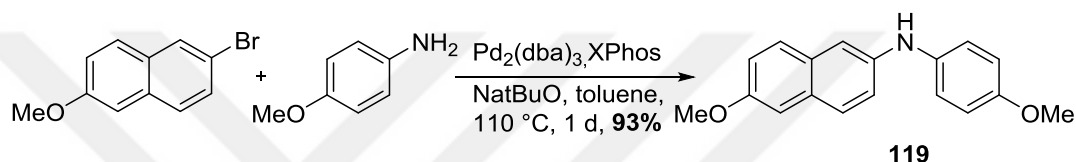
Scheme 5. 82. Synthetic route for **115**

To a 25 mL Schlenk tube, compound **114** (0.50 g, 1.29 mmol) was added, and vacuum–N₂ was applied. Then, it was dissolved in dry DMF (5 mL) and cooled to 0 °C. Afterward, NaH (0.10 g, 2.58 mmol, 2 eq., 60%) was added and warmed to room temperature, then stirred for 1 hour at that temperature. Subsequently, BnBr (0.44 g, 4.06 mmol, 2 eq.) was added, and the mixture was heated to 80 °C and stirred for 24 hours. After the completion of the reaction, the reaction mixture was cooled to room temperature, quenched with water (50 mL), and extracted with EtOAc (50 mL). The organic phase was washed with 10% Na₂S₂O₃ (100 mL), then collected and dried over Na₂SO₄ before filtration. The collected organic phases dried over Na₂SO₄, filtered and solvent evaporated. The crude product was purified by column chromatography (silica, hexane:EtOAc, 6:1), yielding the target product, compound **74**, as an off-white solid (0.61 g, 99%). ¹H NMR (400 MHz, CDCl₃) δ 7.43 (d, *J* = 7.4 Hz, 2H), 7.17 (t, *J* = 7.3 Hz, 2H), 7.09 (d, *J* = 7.0 Hz, 1H), 7.05 (d, *J* = 2.6 Hz,

2H), 6.79 (d, $J = 8.8$ Hz, 2H), 6.61 (dd, $J = 8.7, 2.7$ Hz, 2H), 4.62 (s, 2H), 3.64 (s, 6H). ^{13}C NMR (100 MHz, CDCl_3) δ 156.3, 140.3, 138.2, 128.4, 127.8, 127.0, 125.6, 122.0, 119.5, 113.6, 57.4, 55.7.

5.5.5 Synthesis of Oxygen Bearing Annulated Phenoselenazine Based PS (NSeO-A)

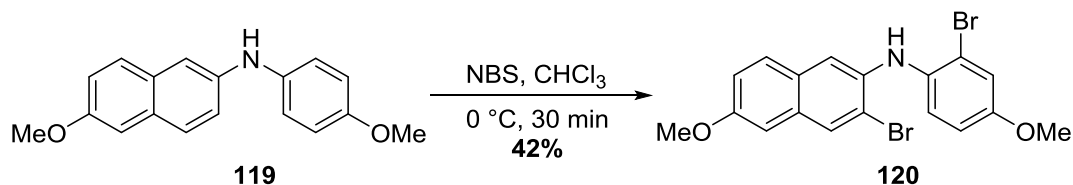
5.5.5.1 Synthesis of Compound 119



Scheme 5. 83. Synthetic route for **119**

To a 250 mL Schlenk tube, commercially available 2-bromo-6-methoxynaphthalene (2.00 g, 8.44 mmol), commercially available p-Anisidine (1.14 g, 9.28 mmol, 1.1 eq), $\text{Pd}_2(\text{dba})_3$ (0.16 g, 0.17 mmol, 0.02 eq.), XPhos (0.16 g, 0.34 mmol, 0.04 eq.) and NatBuO (1.14 g, 11.80 mmol, 1.4 eq) were added and vacuum- N_2 was applied. Then, they were dissolved with dry toluene (45 mL) and degassed for 45 minutes. Reaction mixture heated to 110°C and stirred for 16 hours. Upon completion of reaction, mixture is filtered through celite and washed with CHCl_3 . Obtained crude product purified by column chromatography (silica, CHCl_3 :Hexane 1:1). The target product, compound **119** was obtained as white solid (2.23 g, 93%). ^1H NMR (400 MHz, CDCl_3) δ 7.63 (d, $J = 8.7$ Hz, 1H), 7.53 (d, $J = 8.7$ Hz, 1H), 7.24 (s, 1H), 7.11 (dd, $J = 14.4, 10.5$ Hz, 5H), 6.90 (d, $J = 8.0$ Hz, 2H), 5.63 (s, 1H), 3.90 (s, 3H), 3.83 (s, 3H). ^{13}C NMR (100 MHz, CDCl_3) δ 155.9, 155.2, 140.9, 136.3, 130.1, 129.6, 128.0, 127.9, 121.8, 119.89, 119.1, 114.8, 110.5, 106.1, 55.7, 55.4.

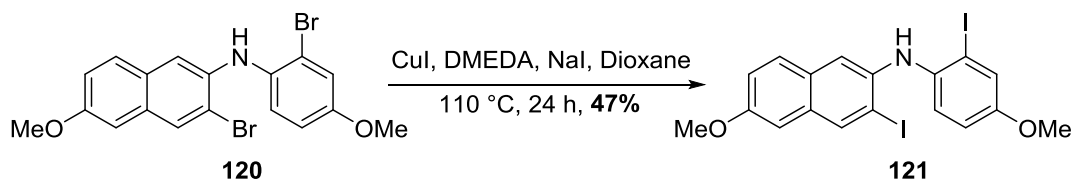
5.5.5.2 Synthesis of Compound 120



Scheme 5. 84. Synthetic route for **120**

To a 50 mL Schlenk tube, compound **119** (200 mg, 0.72 mmol) was added, and vacuum–N₂ was applied. Then, it was dissolved in CHCl₃ (7 mL) and cooled to 0 °C. Afterward, NBS (255 mg, 1.43 mmol, 2 eq.) in CHCl₃ (3 mL) was added dropwise and stirred 30 minutes at that temperature. Upon completion of reaction, mixture quenched with distilled water (50 mL) and extracted. The collected organic layers were dried over NaSO₄, filtered and solvent was evaporated. The crude product was purified with column chromatography (silica, hexane:DCM 1:1), and the target product, compound **120**, was obtained as a white solid (130 mg, 42%). ¹H NMR (400 MHz, CDCl₃) δ 8.05 (d, *J* = 9.2 Hz, 1H), 7.55 (d, *J* = 8.9 Hz, 1H), 7.22 (s, 1H), 7.21 – 7.19 (m, 2H), 7.06 (d, *J* = 2.0 Hz, 1H), 6.84 (dd, *J* = 8.8, 2.8 Hz, 1H), 6.32 (s, 1H), 3.91 (s, 3H), 3.80 (s, 3H). ¹³C NMR (100 MHz, CDCl₃) δ 156.4, 155.8, 137.9, 133.8, 130.7, 128.6, 127.5, 127.1, 122.4, 120.2, 118.4, 118.2, 117.5, 114.5, 109.3, 106.6, 56.0, 55.6. ¹³C NMR (100 MHz, DEPT90, CDCl₃) δ 127.3, 126.9, 122.2, 120.0, 118.2, 118.0, 114.3, 106.4. ¹³C NMR (100 MHz, DEPT135, CDCl₃) δ 127.3, 126.93, 122.2, 120.0, 118.2, 118.0, 114.3, 106.4, 55.8, 55.4.

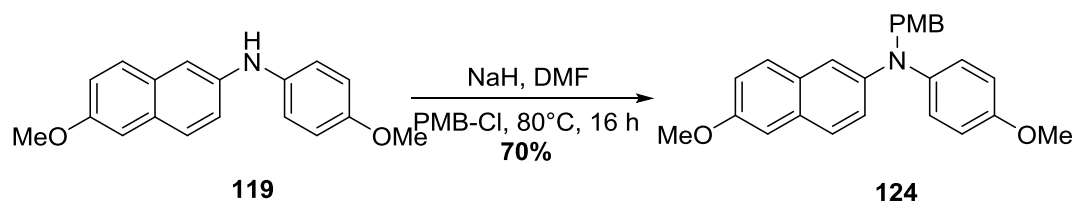
5.5.5.3 Synthesis of Compound 121



Scheme 5. 85. Synthetic route for **121**

To a 10 mL Schlenk tube, compound **120** (0.20 g, 0.46 mmol), CuI (87 mg, 0.46 mmol, 1 eq.), DMEDA (16.2 mg, 0.18 mmol, 0.4 eq.) and NaI (0.41 g, 2.75 mmol, 6 eq.) were added, and vacuum-N₂ was applied. Then, they were dissolved in dioxane (4 mL) and heated to 110 °C and stirred 24 hours at that temperature. Upon completion of reaction, mixture quenched with distilled water (20 mL) and extracted with DCM (20 mL). The collected organic layers were dried over NaSO₄, filtered and solvent was evaporated. The crude product was purified with column chromatography (silica, hexane:DCM 1:1), and the target product, compound **120**, was obtained as a white solid and used in next step without further purification (0.11 g, 47%). ¹H NMR (400 MHz, CDCl₃) δ 7.96 (d, *J* = 9.2 Hz, 1H), 7.52 (d, *J* = 8.9 Hz, 1H), 7.41 (d, *J* = 2.8 Hz, 1H), 7.17 (dd, *J* = 9.2, 2.6 Hz, 1H), 7.10 (d, *J* = 8.7 Hz, 1H), 7.04 (d, *J* = 8.9 Hz, 1H), 7.02 (d, *J* = 2.5 Hz, 1H), 6.86 (dd, *J* = 8.8, 2.8 Hz, 1H), 6.21 (s, 1H), 3.89 (s, 3H), 3.78 (s, 3H). ¹³C NMR (100 MHz, CDCl₃) δ 156.3, 156.0, 141.6, 137.0, 132.6, 131.1, 130.5, 128.3, 124.4, 122.1, 120.3, 117.8, 115.4, 106.5, 94.4, 89.7, 55.9, 55.5.

5.5.5.4 Synthesis of Compound **124**

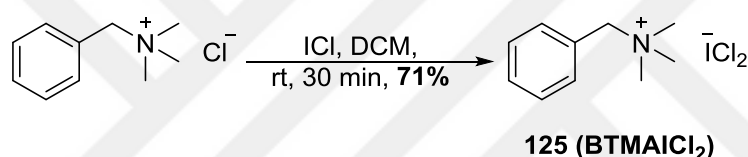


Scheme 5. 86. Synthetic route for **124**

To a 25 mL Schlenk tube, compound **119** (210 mg, 0.76 mmol) was added, and vacuum-N₂ was applied. Then, it was dissolved in dry DMF (5 mL) and cooled to 0 °C. Afterward, NaH (46 mg, 1.14 mmol, 1.5 eq., 60%) was added and warmed to room temperature, then stirred for 1 hour at that temperature. Subsequently, PMB-Cl (180 mg, 1.14 mmol, 1.5 eq.) was added, and the mixture was heated to 80 °C and stirred for 16 hours. After the completion of the reaction, the reaction mixture was cooled to room temperature, quenched with water (50 mL), and extracted with Et₂O

(50 mL), then collected and dried over MgSO_4 filtered and solvent evaporated. The crude product was purified by column chromatography (silica, hexane:DCM, 2:1), yielding the target product, compound **124**, as a white solid (213 mg, 70%). ^1H NMR (400 MHz, CDCl_3) δ 7.58 (d, $J = 9.6$ Hz, 1H), 7.52 (d, $J = 8.8$ Hz, 1H), 7.33 (d, $J = 8.6$ Hz, 2H), 7.21 – 7.13 (m, $J = 7.7, 5.5$ Hz, 4H), 7.12 – 7.04 (m, $J = 11.8, 3.0$ Hz, 2H), 6.89 (dd, $J = 8.7, 6.8$ Hz, 4H), 4.99 (s, 2H), 3.90 (s, 3H), 3.82 (s, 3H), 3.80 (s, 3H). ^{13}C NMR (100 MHz, CDCl_3) δ 158.5, 155.9, 155.7, 145.0, 141.6, 131.2, 130.1, 129.0, 128.1, 128.0, 127.4, 125.3, 120.7, 118.9, 114.8, 114.0, 112.4, 105.9, 56.3, 55.5, 55.4, 55.3.

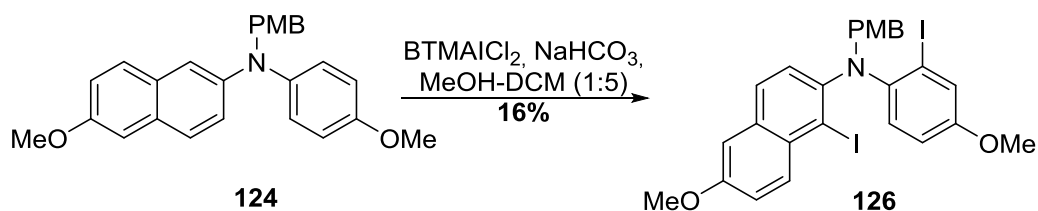
5.5.5.5 Synthesis of Compound 125



Scheme 5. 87 Synthetic route for **125**

To a 250 mL, two-necked, round-bottom flask, ICl (2.63 g, 16.2 mmol, 1 eq.) was added and dissolved with DCM (40 mL). To this solution, BTMACl (3.00 g, 16.2 mmol) in distilled water (20 mL) was added dropwise and resulted mixture stirred for 30 minutes at room temperature. Upon completion, mixture extracted. The collected organic phases were dried over NaSO_4 , filtered and solvent was evaporated. The crude product was purified by recrystallization ($\text{DCM}:\text{Et}_2\text{O}$, 3:1, 40 mL). The target product, compound **125**, obtained as yellow solid (4.00 g, 71%). ^1H NMR (400 MHz, DMSO) δ 7.54 (s, 5H), 4.52 (s, 2H), 3.03 (s, 9H).

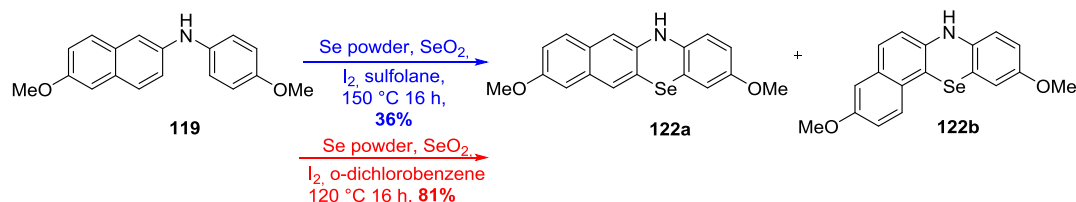
5.5.5.6 Synthesis of Compound 126



Scheme 5. 88. Synthetic route for **126**

To a 25 mL Schlenk tube, compound **124** (200 mg, 0.50 mmol), compound **125** (348 mg, 1.00 mmol, 2 eq.) and NaHCO_3 (630 mg, 7.50 mmol, 15 eq.) were added, and vacuum- N_2 was applied. Then, it was dissolved in MeOH:DCM (6 mL 1:5) and stirred 16 hours at room temperature. Upon completion of reaction, mixture quenched with distilled water (50 mL) and diluted with DCM (45 mL) and extracted. The collected organic layers were dried over NaSO_4 , filtered and solvent was evaporated. The crude product was purified with column chromatography (silica, hexane:DCM 2:1), and the target product, compound **126**, was obtained as a white solid (53 mg, 16%). ^1H NMR (400 MHz, CDCl_3) δ 8.14 (dd, $J = 9.3, 2.1$ Hz, 1H), 7.54 (d, $J = 8.7$ Hz, 1H), 7.32 – 7.26 (m, $J = 7.2, 1.8$ Hz, 3H), 7.16 (d, $J = 9.4$ Hz, 1H), 7.03 (s, 1H), 6.76 (dd, $J = 8.6, 1.9$ Hz, 2H), 6.64 (dd, $J = 9.0, 2.1$ Hz, 2H), 6.56 – 6.49 (m, 2H), 4.78 (s, 2H), 3.83 (s, 3H), 3.68 (s, 3H), 3.63 (s, 3H). ^{13}C NMR (100 MHz, CDCl_3) δ 158.6, 158.1, 152.5, 142.6, 140.7, 134.0, 131.2, 130.1, 128.8, 128.3, 127.6, 126.9, 126.4, 119.8, 116.2, 114.5, 113.9, 106.2, 56.1, 55.7, 55.5, 55.3.

5.5.5.7 Synthesis of Compounds 122a and 122b

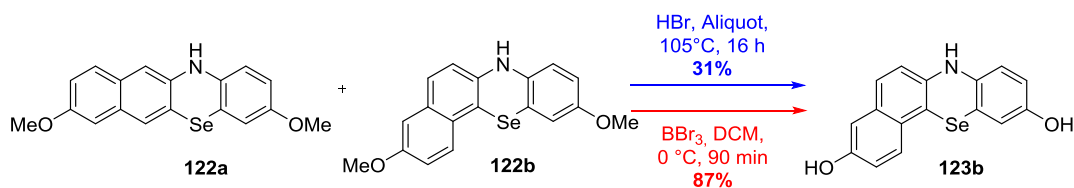


Scheme 5. 89. Synthetic routes for **122a** and **122b**

Blue pathway: To a 50 mL Schlenk tube, Compound **119** (400 mg, 1.43 mmol, 2.0 eq) was added and vacuum–N₂ was applied. Then, Se powder (57 mg, 0.72 mmol), SeO₂ (96 mg, 0.86 mmol, 1.2 eq), I₂ (9.0 mg, 0.07 mmol, 0.1 eq), and sulfolane (6 mL) were added. The reaction mixture was heated to 150 °C and was stirred for 16 hours. Upon completion of reaction, mixture is filtered through celite and washed with chloroform. The obtained crude product was purified with column chromatography (silica, Hexane:EtOAc, 5:1). Compound **122a** and **122b** were obtained as gray solid as a diastereomeric mixture (92 mg, 36%).

Red pathway: To a 50 mL Schlenk tube, Compound **119** (300 mg, 1.07 mmol, 2.0 eq) was added and vacuum–N₂ was applied. Then, Se powder (43 mg, 0.54 mmol), SeO₂ (71 mg, 0.64 mmol, 1.2 eq), I₂ (6.7 mg, 0.05 mmol, 0.1 eq), and o-dichlorobenzene (5 mL) were added. The reaction mixture was heated to 120 °C and was stirred for 16 hours. Upon completion of reaction, mixture is filtered through celite and washed with chloroform. The obtained crude product was purified with column chromatography (silica, Hexane:DCM, 1:2). Compound **122a** and **122b** were obtained as gray solid as a diastereomeric mixture (160 mg, 81%). ¹H NMR (400 MHz, CDCl₃) δ 7.89 (d, *J* = 9.2 Hz, 1H), 7.82 (d, *J* = 9.1 Hz, 1H), 7.70 (d, *J* = 9.0 Hz, 1H), 7.49 (d, *J* = 9.0 Hz, 1H), 7.24 (s, 1H), 7.17 (d, *J* = 1.3 Hz, 1H), 7.11 (dd, *J* = 9.1, 2.3 Hz, 1H), 7.05 (d, *J* = 8.8 Hz, 2H), 6.96 (d, *J* = 2.2 Hz, 1H), 6.90 (d, *J* = 2.4 Hz, 1H), 6.81 (d, *J* = 8.8 Hz, 2H), 6.70 (d, *J* = 9.0 Hz, 1H), 6.57 (s, 1H), 6.45 – 6.42 (m, 1H), 3.90 (s, 3H), 3.85 (s, 3H), 3.75 (s, 3H), 3.71 (s, 3H). ¹³C NMR (100 MHz, CDCl₃) δ 155.8, 155.7, 134.8, 129.5, 127.9, 126.2, 124.5, 123.6, 119.9, 118.7, 118.1, 117.2, 114.5, 113.3, 107.0, 106.2, 55.5, 55.4, 55.3, 55.3. HRMS calculated for C₁₈H₁₅NO₂Se: 357.0268 Found: 357.0280

5.5.5.8 Synthesis of Compound **123b**

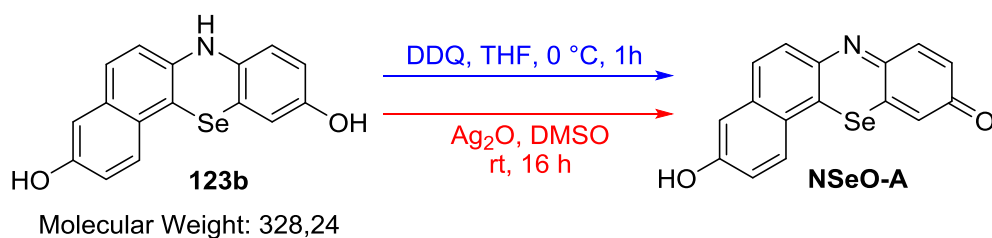


Scheme 5. 90. Synthetic route for **123b**

Blue pathway: To a 25 mL Schlenk tube, mixture of compound **122a** and **122b** (30 mg, 0.08 mmol) was added and vacuum–N₂ was applied. Then, aliquot (0.1 mL), and HBr (1.5 mL) were added, respectively. Reaction mixture was heated to 105 °C and stirred for 16 hours. Upon completion of reaction, mixture quenched with distilled water (20 mL) and extracted with EtOAc (30 mL). The collected organic layers were dried over NaSO₄, filtered and solvent was evaporated. The crude product was purified with column chromatography (silica gel, DCM:MeOH 97:3). The target product, compound **123b** was obtained as dark gray solid (9 mg, 31%).

Red pathway: To a 25 mL Schlenk tube, mixture of compound **122a** and **122b** (100 mg, 0.28 mmol) was added and vacuum–N₂ was applied. Then, they dissolved in dry DCM (5 mL) and cooled to 0 °C. To the resulting mixture 1 M BBr₃ solution (1.30 mL, 1.28 mmol, 4.6 eq.) was added dropwise and stirred for 90 minutes at that temperature. Upon completion of reaction, mixture quenched with MeOH (5 mL) and stirred for 15 minutes more. Then solvent evaporated and the crude product was purified with column chromatography directly. The target compound **123b**, was obtained as dark gray solid. (40 mg, 87%). ¹H NMR (400 MHz, MeOD) δ 7.47 (d, *J* = 8.8 Hz, 1H), 7.42 (d, *J* = 8.8 Hz, 1H), 7.13 (s, 1H), 7.08 (dd, *J* = 8.8, 2.1 Hz, 1H), 7.02 (d, *J* = 8.7 Hz, 2H), 6.98 (d, *J* = 2.0 Hz, 1H), 6.94 (dd, *J* = 8.8, 2.3 Hz, 1H), 6.75 (d, *J* = 8.7 Hz, 2H).

5.5.5.9 Synthesis of Compound NSeO-A



Scheme 5. 91. Synthetic route for NSeO-A

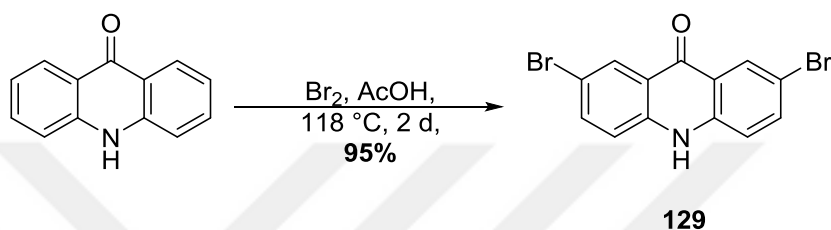
Blue pathway: To a 25 mL Schlenk tube, compound **123b** (9 mg, 0.03 mmol) was added and vacuum-N₂ was applied. Then, it was dissolved in dry THF (5 mL) and cooled to 0 °C. To the resulting mixture DDQ (9 mg, 0.04 mmol, 1.5 eq.) was added and stirred for 1 hour at that temperature. Upon completion of reaction, solvent evaporated and the crude product was purified with column chromatography (silica, MeOH:DCM, 8:92) to yield the target product, NSeO-A, as black solid.

Red pathway: To a 25 mL Schlenk tube, compound **123b** (9 mg, 0.03 mmol) was added and vacuum-N₂ was applied. Then, it was dissolved in DMSO (2 mL). To the resulting mixture Ag₂O (9 mg, 0.04 mmol, 1.5 eq.) was added and stirred for 16 hours at that temperature. Upon completion of reaction, solvent evaporated and the crude product was purified with column chromatography (silica, MeOH:DCM, 8:92) to yield the target product, NSeO-A, as black solid. ¹H NMR (400 MHz, DMSO) δ 10.64 (s, 1H), 7.99 (d, *J* = 9.0 Hz, 1H), 7.92 (s, 1H), 7.81 (dd, *J* = 16.1, 8.8 Hz, 1H), 7.77 – 7.71 (m, *J* = 9.2 Hz, 1H), 7.37 (d, *J* = 2.1 Hz, 1H), 7.31 (dd, *J* = 12.6, 3.6 Hz, 1H), 6.90 (dd, *J* = 9.8, 2.1 Hz, 1H). HRMS calculated for C₁₆H₁₀NO₂Se: 327.9877 Found: 327.9900

5.6 Acridone Based Photosensitizers

5.6.1 Synthesis of Morpholine Bearing Acridone Based imaging Agent and PS (NCMorph)

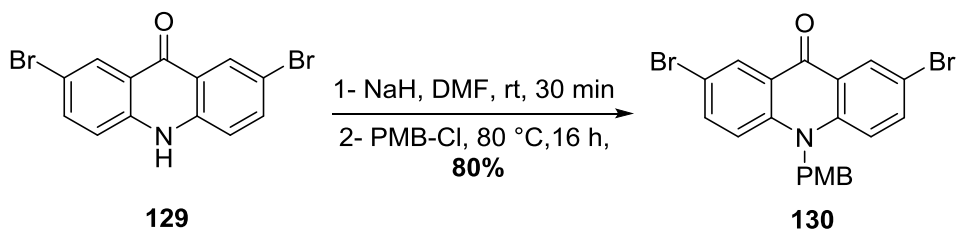
5.6.1.1 Synthesis of Compound 129



Scheme 5. 92. Synthetic route for **129**

To a 50 mL Schlenk tube, acridin-9(10H)-one (1.0 g, 5.12 mmol) was added and vacuum-N₂ was applied. Then, it was dissolved with acetic acid (20 mL). Separately, Br₂ (2.0 mL, 38.4 mmol, 7.5 eq.) was dissolved in acetic acid (5 mL) and added dropwise to this solution. Then the reaction mixture was heated to 118 °C and refluxed under nitrogen atmosphere at that temperature for 2 days. After the reaction was completed, it was cooled in ice bath and subsequently, the precipitates were filtered and washed with NaHCO₃ (100 mL) & 20% (w/v) NaHSO₄ (50 mL). Compound **129** was obtained as yellow solid (2.12 g, **95%**) ¹H NMR (400 MHz, DMSO) δ 12.08 (s, 1H), 8.26 (d, *J* = 1.6 Hz, 2H), 7.88 (dd, *J* = 8.9, 2.2 Hz, 2H), 7.51 (d, *J* = 8.9 Hz, 2H). ¹³C NMR (100 MHz, DMSO) δ 174.9, 140.0, 136.7, 128.3, 122.0, 120.6, 114.0.

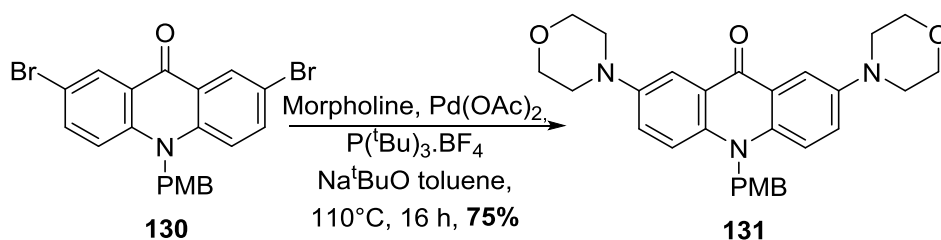
5.6.1.2 Synthesis of Compound 130



Scheme 5. 93. Synthetic route for **130**

To a 50 mL Schlenk tube, compound **129** (250 mg, 0.71 mmol) was added and vacuum–N₂ was applied. Then, it was dissolved with dry DMF (15 mL). Then mixture cooled to 0 °C and NaH (71 mg, 1.77 mmol, 2.5 eq.) was added and the reaction mixture warmed to room temperature and stirred for 30 min. Following this, PMB-Cl (280 mg, 1.77 mmol, 2.5 eq.) was added and mixture was stirred for 16 hours at 80°C. Upon completion of reaction, mixture quenched with distilled water (50 mL) and extracted with Et₂O (50 mL). The collected organic layers were dried over MgSO₄, filtered and solvent was evaporated. The crude product was purified with column chromatography (silica gel, DCM:Hexane 3:1). The target product, compound **130**, was obtained as yellow solid (270 mg, 80%). ¹H NMR (400 MHz, CDCl₃) δ 8.50 (d, *J* = 2.4 Hz, 2H), 7.60 (dd, *J* = 9.1, 2.5 Hz, 2H), 7.17 (d, *J* = 9.2 Hz, 2H), 7.00 (d, *J* = 8.6 Hz, 2H), 6.82 (d, *J* = 8.7 Hz, 2H), 5.43 (s, 2H), 3.73 (s, 3H). ¹³C NMR (100 MHz, CDCl₃) δ 175.7, 159.3, 141.0, 137.0, 126.6, 126.1, 123.5, 117.3, 115.3, 114.7, 55.2, 50.4.

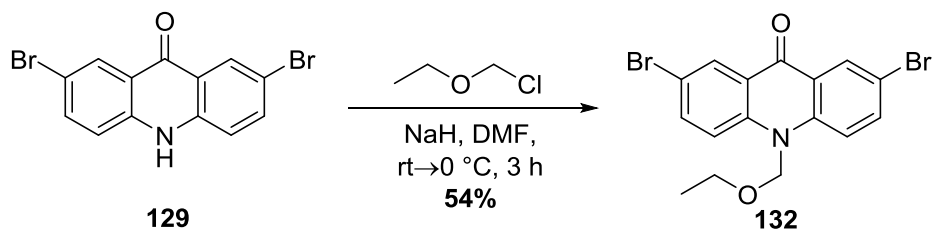
5.6.1.3 Synthesis of Compound 131



Scheme 5. 94. Synthetic route for **131**

To a 25 mL Schlenk tube, compound **130** (250 mg, 0.53 mmol), Pd(OAc)₂ (24.2 mg, 0.03 mmol, 0.05 eq.), P(^tBu)₃.BF₄ (25.2 mg, 0.05 mmol, 0.1 eq.), NaO^tBu (0.18 g, 1.85 mmol, 3.5 eq.) was added and vacuum–N₂ was applied. Then, it was dissolved with dry toluene (10 mL) were added. Then, the reaction mixture was degassed for 40 minutes. After that, morpholine (162 mg, 1.85 mmol, 3.5 eq.) was added and mixture heated to 100°C and stirred for 16 hours. Upon completion of reaction, mixture filtered through celite and washed with chloroform. The crude product was purified by using column chromatography (silica gel, MeOH:DCM, 2:98) the target product, compound **131**, was obtained as yellow solid (289 mg, 75%) ¹H NMR (400 MHz, CDCl₃) δ 8.04 – 7.97 (m, 2H), 7.39 – 7.30 (m, 4H), 7.08 (d, *J* = 8.6 Hz, 2H), 6.85 (d, *J* = 8.6 Hz, 2H), 5.30 (s, 2H), 3.95 – 3.87 (m, *J* = 4.1 Hz, 8H), 3.77 (s, 3H), 3.35 – 3.11 (m, 8H). ¹³C NMR (100 MHz, CDCl₃) δ 177.2, 159.0, 145.6, 136.5, 127.6, 126.8, 124.4, 122.3, 116.3, 114.5, 110.9, 66.8, 55.2, 49.7, 49.7.

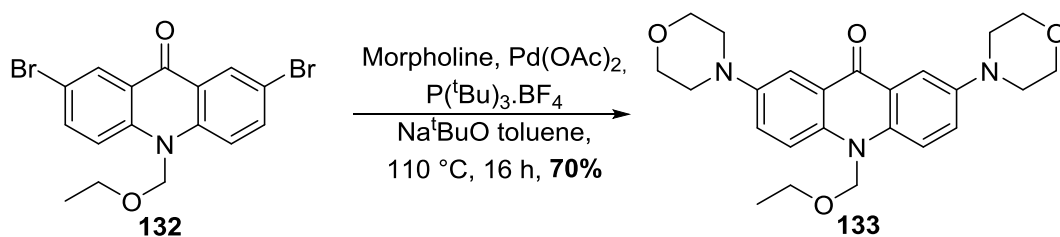
5.6.1.4 Synthesis of Compound 132



Scheme 5. 95. Synthetic route for **132**

To a 50 mL Schlenk tube, compound **129** (410 mg, 1.16 mmol) was added and vacuum-N₂ was applied. Then, it was dissolved with dry DMF (10 mL). Then mixture cooled to 0 °C and NaH (116 mg, 2.90 mmol, 2.5 eq.) was added and the reaction mixture warmed to room temperature and stirred for 30 min. Following this, mixture cooled to 0 °C and chloromethyl ethyl ether (350 mg, 3.71 mmol, 3.2 eq.) was added slowly and mixture was stirred for 3 hours at 0°C. Upon completion of reaction, mixture quenched with distilled water (10 mL) and, resulting precipitates filtered and washed with hexane (150 mL). The target product, compound **132**, was obtained as yellow solid (259 mg, 54%). ¹H NMR (400 MHz, DMSO) δ 8.38 – 8.28 (m, 2H), 8.03 – 7.92 (m, *J* = 7.4 Hz, 2H), 7.89 (d, *J* = 9.2 Hz, 2H), 5.85 (s, 2H), 3.69 (q, *J* = 13.6, 6.8 Hz, 2H), 1.15 (t, *J* = 6.7 Hz, 3H).

5.6.1.5 Synthesis of Compound 133

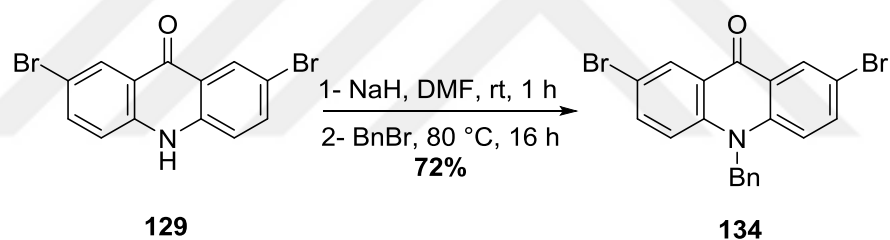


Scheme 5. 96. Synthetic route for **133**

To a 25 mL Schlenk tube, compound **132** (200 mg, 0.49 mmol), Pd(OAc)₂ (5.5 mg, 0.02 mmol, 0.05 eq.), P(^{*t*}Bu)₃.BF₄ (13.9 mg, 0.05 mmol, 0.1 eq.), NaO^{*t*}Bu (171 mg,

1.72 mmol, 3.5 eq.) was added and vacuum-N₂ was applied. Then, it was dissolved with dry toluene (10 mL) were added. Then, the reaction mixture was degassed for 40 minutes. After that, morpholine (162 mg, 1.85 mmol, 3.5 eq.) was added and mixture heated to 100°C and stirred for 16 hours. Upon completion of reaction, mixture filtered through celite and washed with chloroform. The crude product was purified by using column chromatography (silica gel, MeOH:DCM, 2:98) the target product, compound **133**, was obtained as yellow solid (151 mg, 70%). ¹H NMR (400 MHz, MeOD) δ 7.79 – 7.73 (m, 4H), 7.60 – 7.55 (m, 2H), 5.80 (s, 2H), 3.94 – 3.85 (m, 8H), 3.73 (q, 2H), 3.24 – 3.18 (m, *J* = 4.5 Hz, 8H), 1.24 (t, *J* = 7.0 Hz, 3H). ¹³C NMR (100 MHz, MeOD) δ 179.3, 147.8, 137.9, 126.5, 122.8, 118.2, 110.5, 76.8, 67.9, 64.8, 50.9, 15.5.

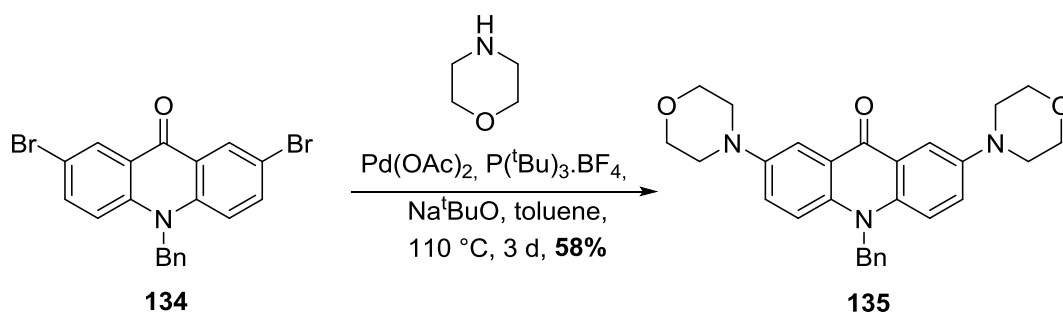
5.6.1.6 Synthesis of Compound **134**



Scheme 5. 97. Synthetic route for **134**

To a 50 mL Schlenk tube, compound **129** (0.75 g, 2.96 mmol) was added and vacuum-N₂ was applied. Then, it was dissolved with dry DMF (20 mL). Then mixture cooled to 0 °C and NaH (0.29 g, 7.40 mmol, 2.5 eq.) was added and the reaction mixture warmed to room temperature and stirred for 1 hour. Following this, BnBr (1.27 g, 7.40 mmol, 2.5 eq.) was added and mixture was stirred for 16 hours at 80°C. Upon completion of reaction, mixture quenched with distilled water (50 mL) and extracted with Et₂O (50 mL). The collected organic layers were dried over MgSO₄, filtered and solvent was evaporated. The crude product was purified with column chromatography (silica gel, DCM:Hexane 3:1). The target product, compound **134**, was obtained as yellow solid (0.94 g, 72%).

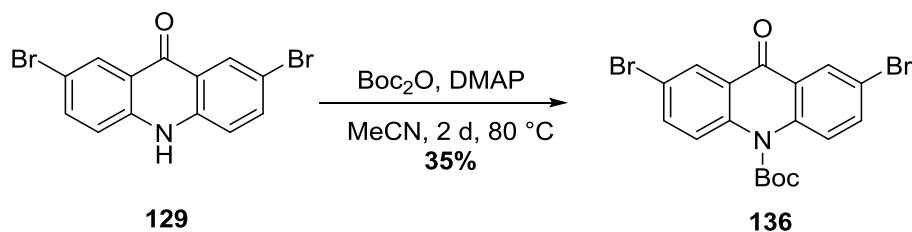
5.6.1.7 Synthesis of Compound 135



Scheme 5. 98. Synthetic route for **135**

To a 25 mL Schlenk tube, compound **134** (300 mg, 0.68 mmol), $\text{Pd}(\text{OAc})_2$ (7.6 mg, 0.03 mmol, 0.05 eq.), $\text{P}(\text{tBu})_3 \cdot \text{BF}_4$ (9.9 mg, 0.03 mmol, 0.05 eq.), NaO^tBu (0.19 g, 2.00 mmol, 2.95 eq.) was added and vacuum- N_2 was applied. Then, it was dissolved with dry toluene (12 mL) were added. Then, the reaction mixture was degassed for 40 minutes. After that, morpholine (206 mg, 2.37 mmol, 3.5 eq.) was added and mixture heated to 110°C and stirred for 3 days. Upon completion of reaction, mixture filtered through celite and washed with chloroform. The crude product was purified by using column chromatography (alumina, DCM) the target product, compound **135**, was obtained as yellow solid (180 mg, 58%). ^1H NMR (400 MHz, CDCl_3) δ 7.91 (s, 2H), 7.27 – 7.17 (m, 7H), 7.07 (d, $J = 7.0$ Hz, 2H), 5.46 (s, 2H), 3.86 – 3.76 (m, 8H), 3.19 – 3.08 (m, 8H). ^{13}C NMR (100 MHz, CDCl_3) δ 177.3, 145.7, 136.5, 135.9, 129.1, 127.7, 125.6, 124.5, 122.4, 116.3, 111.0, 66.9, 50.2, 49.7.

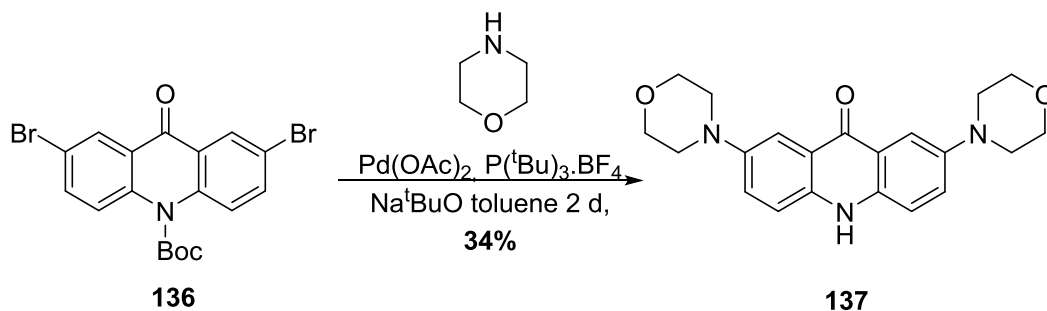
5.6.1.8 Synthesis of Compound 136



Scheme 5. 99. Synthetic route for **136**

To a 25 mL Schlenk tube, compound **129** (0.50 g, 1.42 mmol) was added, and vacuum–N₂ was applied. Then, it was dissolved with dry MeCN (8 mL). Then Boc₂O (0.46 g, 2.12 mmol, 1.5 eq.), DMAP (0.26 g, 2.12 mmol, 1.5 eq.) were added and mixture heated to 80 °C stirred for 2 days. After completion of reaction, mixture quenched with saturated NaHCO₃ and extracted with EtOAc. The collected organic phases washed with brine then dried over Na₂SO₄, filtered and evaporated. The crude product purified with column chromatography (silica, MeOH:DCM, 2:98). The target product, compound **136**, obtained as yellow solid (0.23 g, 35%) ¹H NMR (400 MHz, CDCl₃) δ 8.52 (d, *J* = 2.3 Hz, 2H), 7.75 (dd, *J* = 9.1, 2.4 Hz, 2H), 7.51 (d, *J* = 9.0 Hz, 2H), 1.67 (s, 9H). ¹³C NMR (100 MHz, CDCl₃) δ 176.5, 151.2, 138.4, 136.6, 129.9, 124.4, 120.3, 117.3, 87.2, 27.9.

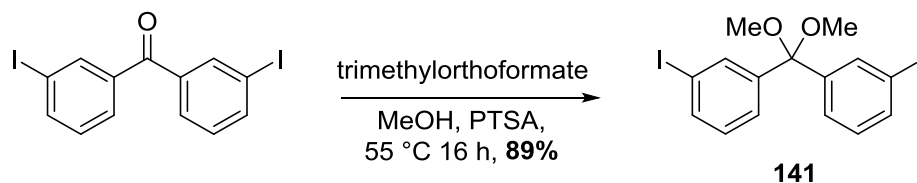
5.6.1.9 Synthesis of Compound 137



Scheme 5. 100. Synthetic route for **137**

To a 25 mL Schlenk tube, compound **136** (110 mg, 0.24 mmol), Pd(OAc)₂ (2.7 mg, 0.01 mmol, 0.05 eq.), P(^tBu)₃.BF₄ (3.6 mg, 0.01 mmol, 0.05 eq.), NaO^tBu (0.07 g, 0.71 mmol, 2.95 eq.) was added and vacuum–N₂ was applied. Then, it was dissolved with dry toluene (12 mL). Then, the reaction mixture was degassed for 40 minutes. After that, morpholine (0.08 g, 0.84 mmol, 3.5 eq.) was added and mixture heated to 110 °C and stirred for 2 days. Upon completion of reaction, mixture filtered through celite and washed with chloroform. The crude product was purified by using column chromatography (silica, MeOH:DCM, 5:95) the target product, compound **137**, was obtained as yellow solid (30 mg, 34%). ¹H NMR (400 MHz, DMSO) δ 11.58 (s, 1H), 7.60 – 7.52 (m, 4H), 7.46 (d, *J* = 8.8 Hz, 2H), 3.82 – 3.75 (m, 8H), 3.17 – 3.09 (m, 8H). ¹³C NMR (100 MHz, DMSO) δ 175.6, 145.4, 134.9, 124.8, 120.1, 118.3, 108.1, 66.1, 49.4.

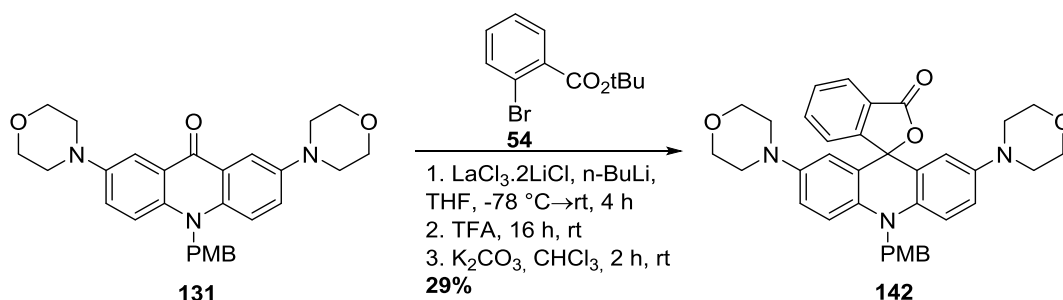
5.6.1.10 Synthesis of Compound **141**



Scheme 5. 101. Synthetic route for **141**

To a 25 mL Schlenk tube, compound bis(3-iodophenyl)methanone (0.26 g, 0.60 mmol) was added and vacuum–N₂ was applied. Then, it was dissolved with MeOH (5 mL). To this solution PTSA (41 mg, 0.24 mmol, 0.4 eq.) and trimethylorthoformate (0.96 mL, 12.00 mmol, 20 eq.) were added respectively. The resulting solution heated to 55 °C and stirred for 16 hours. Upon completion of reaction, mixture quenched with distilled water (50 mL) and extracted with DCM (50 mL). The collected organic phases washed with brine then dried over Na₂SO₄, filtered and evaporated. The crude product purified with column chromatography (silica, MeOH:DCM, 2:98). The target product, compound **141**, obtained as white solid (0.26 g, 89%). ¹H NMR (400 MHz, CDCl₃) δ 7.92 (d, *J* = 1.2 Hz, 2H), 7.58 (d, *J* = 8.3 Hz, 2H), 7.38 (d, *J* = 7.9 Hz, 2H), 7.03 (t, *J* = 7.8 Hz, 2H), 3.11 (s, 6H). ¹³C NMR (100 MHz, CDCl₃) δ 144.1, 136.9, 135.7, 130.1, 126.4, 101.4, 94.4, 49.6.

5.6.1.11 Synthesis of Compound 142

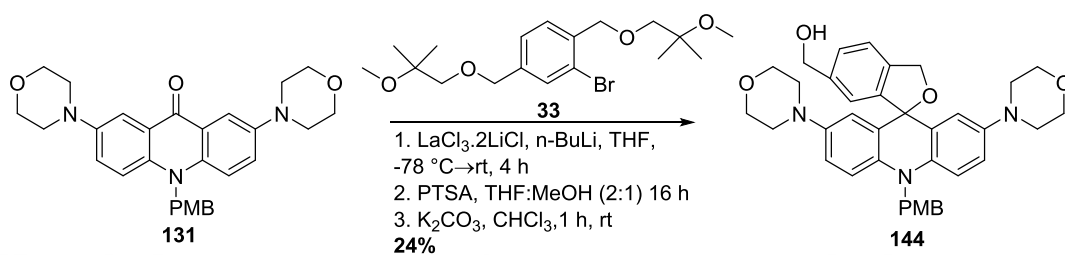


Scheme 5. 102. Synthetic route for **142**

To a 25 mL Schlenk tub, compound **131** (92 mg, 0.19 mmol) was added and vacuum- N_2 was applied. Then, it dissolved in dry THF (3 mL). In another 50 mL Schlenk tube, compound **54** (0.30 g, 1.17 mmol, 6 eq.) was added and vacuum- N_2 was applied. Then, it dissolved in THF (5 mL). The solution of compound **54** was cooled to $-78\text{ }^\circ\text{C}$ and $n\text{-BuLi}$ (0.47 mL, 1.17 mmol, 2.5 M, 6 eq.) and $\text{LaCl}_3 \cdot 2\text{LiCl}$ (1.95 mL, 1.17 mmol, 0.6 M, 6 eq.) were added dropwise respectively. After stirring for 40 min at $-78\text{ }^\circ\text{C}$, compound **131** solution was added to the mixture dropwise. After stirred for 40 min, the reaction mixture was warmed to room temperature. After 3 hours stirring, the reaction was completed. Then reaction mixture quenched with distilled water (20 mL) and extracted with chloroform (20 mL). The collected organic layers were dried over Na_2SO_4 , filtered and solvent evaporated. Then the crude product dissolved in TFA (3 mL) and stirred 16 hours at room temperature. Upon completion of reaction, TFA evaporated and mixture extracted with chloroform (20 mL) and distilled water (20 mL). The collected organic layers were dried over Na_2SO_4 filtered and K_2CO_3 (32 mg, 0.38 mmol, 2 eq.) was added to the organic layer and stirred at room temperature for 2 hours. Then solution washed with distilled water (20 mL) and the crude product purified with column chromatography (silica, 1.8:0.1:0.05 CHCl_3 :MeOH: Et_3N) to yield the target product, compound **142**, as a red solid (30 mg, 29%) ^1H NMR (400 MHz, CDCl_3) δ 8.00 (d, $J = 7.5$ Hz, 1H), 7.65 (t, $J = 7.3$ Hz, 1H), 7.59 (t, $J = 7.4$ Hz, 1H), 7.31 (d, $J = 7.5$ Hz, 1H), 7.07 (d, $J = 8.4$ Hz, 2H), 6.95 – 6.84 (m, $J = 9.4, 5.6$ Hz, 4H), 6.81 (d, $J = 8.6$ Hz, 2H), 6.30

(d, $J = 2.3$ Hz, 2H), 5.24 (s, 2H), 3.73 (s, 3H), 3.70 – 3.64 (m, 8H), 2.84 – 2.77 (m, 8H).

5.6.1.12 Synthesis of Compound 144

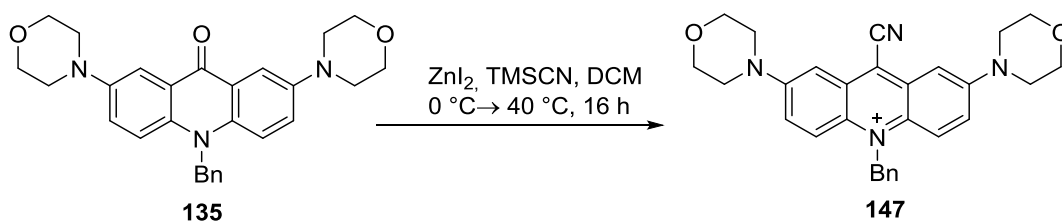


Scheme 5. 103. Synthetic route for **144**

To a 25 mL Schlenk tub, compound **131** (50 mg, 0.10 mmol) was added and vacuum- N_2 was applied. Then, it dissolved in dry THF (7 mL). In another 50 mL Schlenk tube, compound **33** (186 mg, 0.52 mmol, 5 eq.) was added and vacuum- N_2 was applied. Then, it dissolved in THF (5 mL). The solution of compound **33** was cooled to -78°C and $n\text{-BuLi}$ (0.25 mL, 0.52 mmol, 2.5 M, 6 eq.) and $\text{LaCl}_3 \cdot 2\text{LiCl}$ (0.85 mL, 0.52 mmol, 0.6 M, 5 eq.) were added dropwise respectively. After stirring for 1 hour at -78°C , compound **131** solution was added to the mixture dropwise. After stirred for 1 hour, the reaction mixture was warmed to room temperature. After 3 hours stirring, the reaction was completed. Then reaction mixture quenched with distilled water (20 mL) and extracted with chloroform (20 mL). The collected organic layers were dried over Na_2SO_4 , filtered and solvent evaporated. Then the crude product dissolved in THF-MeOH mixture (2:1, 6 mL) and PTSA (0.02 g, 0.13 mmol, 1.25 eq.) stirred 16 hours at room temperature. Upon completion of reaction, solvent evaporated and mixture extracted with chloroform (20 mL) and distilled water (20 mL). The collected organic layers were dried over Na_2SO_4 filtered and K_2CO_3 (18 mg, 0.13 mmol, 1.25 eq.) was added to the organic layer and stirred at room temperature for 1 hour. Then solution washed with distilled water (20 mL) and the crude product purified with column chromatography (silica, 1.8:0.1:0.05

CHCl₃:MeOH:Et₃N) to yield the target product, compound **142**, as a red solid and used in next step without further purification(15 mg, 24%).

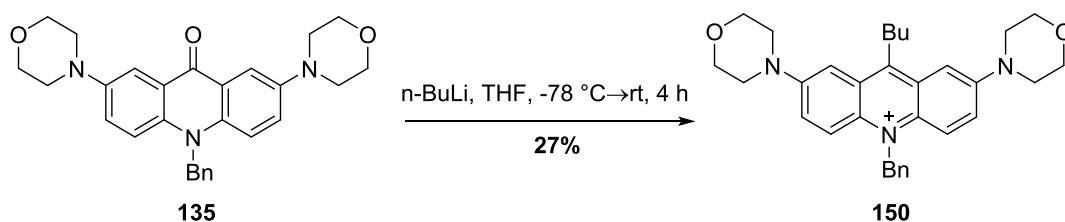
5.6.1.13 Synthesis of Compound 147



Scheme 5. 104. Synthetic route for **144**

To a 25 mL Schlenk tube, compound **135** (46 mg, 0.10 mmol) and ZnI₂ (2 mg, 0.005 mmol, 0.5 eq.) were added and vacuum–N₂ was applied. Then, it dissolved in dry DCM (3 mL) and cooled to 0 °C. Then, to this solution, TMSCN (40 mg, 0.40 mmol, 4 eq.) was added slowly. After that, temperature raised to 40 °C and stirred 16 hours. After completion of reaction mixture quenched with distilled water (20 mL) and diluted with DCM (20 mL) and extracted. The collected organic layers were dried over Na₂SO₄ filtered and solvent evaporated. The crude product purified with column chromatography (silica, MeOH:DCM, 5:95) and the target product, compound **147**, obtained as purple solid and used in next step without further purification. ¹H NMR (400 MHz, CDCl₃) δ 8.51 (dd, *J* = 10.0, 2.3 Hz, 2H), 8.02 (d, *J* = 9.3 Hz, 2H), 7.33 (d, *J* = 5.7 Hz, 2H), 7.29 (dd, *J* = 7.6, 4.5 Hz, 3H), 7.20 (dd, *J* = 6.7, 2.6 Hz, 2H), 6.93 (s, 2H), 3.96 – 3.87 (m, 8H), 3.60 – 3.49 (m, 8H).

5.6.1.14 Synthesis of Compound 150



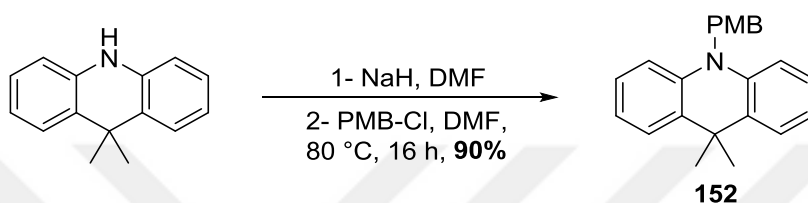
Scheme 5. 105. Synthetic route for **150**

To a 25 mL Schlenk tube, compound **135** (50 mg, 0.11 mmol) was added and vacuum- N_2 was applied. Then, it dissolved in dry THF (3 mL) and cooled to $-78\text{ }^{\circ}\text{C}$ and $n\text{-BuLi}$ (0.25 mL, 0.55 mmol, 5 eq., 2.5 M) was added dropwise. After 1 hour, mixture cooled to the room temperature and stirred 3 more hours at that temperature. Upon completion of reaction, mixture quenched with distilled water (20 mL) and extracted with DCM (20 mL). The collected organic layers were dried over Na_2SO_4 filtered and solvent evaporated. The crude product purified with column chromatography (silica, $\text{MeOH}:\text{DCM}$, 5:95) and the target product, compound **150**, obtained as purple solid (15 mg, 27%). ^1H NMR (400 MHz, CDCl_3) δ 8.47 (d, $J = 10.0$ Hz, 2H), 7.97 (dd, $J = 9.9$, 2.3 Hz, 2H), 7.33 (d, $J = 2.2$ Hz, 2H), 7.28 (dd, $J = 4.9$, 1.1 Hz, 3H), 7.24 – 7.18 (m, 2H), 7.01 (s, 2H), 3.94 (t, 8H), 3.42 (t, 8H), 1.68 (q, 2H), 1.25 (s, 4H), 1.11 (t, $J = 7.3$ Hz, 3H). ^{13}C NMR (100 MHz, CDCl_3) δ 154.1, 149.4, 134.9, 134.1, 129.9, 129.4, 128.5, 127.7, 126.1, 121.1, 103.5, 66.6, 54.8, 48.2, 32.2, 29.3, 23.6, 14.1.

5.7 Acridine Based Photosensitizers

5.7.1 Synthesis of Morpholine Bearing Acridine Based Imaging Agent and PS (DDAMorph & DDAMorph-I)

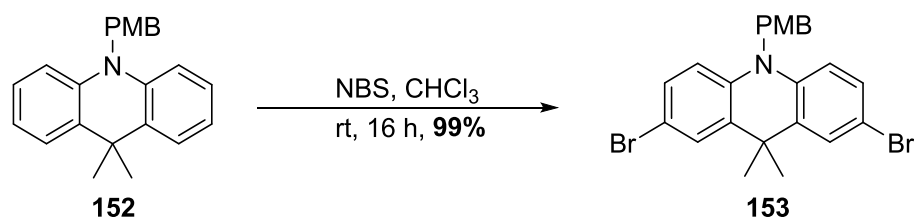
5.7.1.1 Synthesis of Compound **152**



Scheme 5. 106. Synthetic route for **152**

To a 25 mL Schlenk tube, 9,9-dimethyl-9,10-dihydroacridine (0.56 g, 2.68 mmol) was added and vacuum-N₂ was applied. Then, it was dissolved with dry DMF (7 mL). Then mixture cooled to 0 °C and NaH (0.21 g, 5.35 mmol, 2 eq.) was added and the reaction mixture warmed to room temperature and stirred for 30 min. Following this, PMB-Cl (0.85 g, 5.35 mmol, 2 eq.) was added and mixture was stirred for 16 hours at 80°C. Upon completion of reaction, mixture quenched with distilled water (50 mL) and extracted with Et₂O (50 mL). The collected organic layers were dried over MgSO₄, filtered and solvent was evaporated. The crude product was purified with column chromatography (silica gel, Hexane:EtOAc 6:1). The target product, compound **152**, was obtained as white solid (0.79 g, 90%). ¹H NMR (400 MHz, CDCl₃) δ 7.47 (d, *J* = 7.7 Hz, 2H), 7.16 – 7.12 (m, 2H), 7.09 (d, *J* = 8.1 Hz, 2H), 6.98 (t, *J* = 7.5 Hz, 2H), 6.87 (d, *J* = 8.6 Hz, 2H), 6.78 (d, *J* = 8.1 Hz, 2H), 5.17 (s, 2H), 3.80 (s, 3H), 1.63 (s, 6H). ¹³C NMR (100 MHz, CDCl₃) δ 158.5, 140.7, 132.1, 128.8, 127.5, 126.7, 124.4, 120.7, 114.2, 113.3, 55.3, 50.7, 36.3, 29.5.

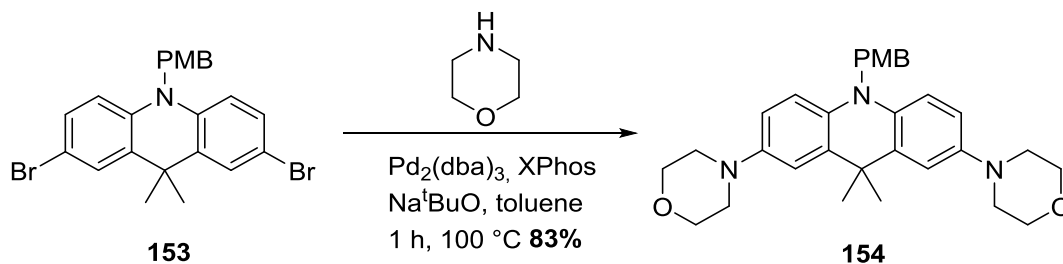
5.7.1.2 Synthesis of Compound **153**



Scheme 5. 107. Synthetic route for **153**

To a 50 mL two-necked round-bottom flask, compound **153** (0.40 g, 1.21 mmol) was added, and vacuum-N₂ was applied. Then, it was dissolved in CHCl₃ (25 mL). Subsequently, NBS (0.45 g, 2.55 mmol, 2.1 eq.) was added in portions over 1 hour. After the completion of the addition, the reaction mixture was stirred for 16 hours at room temperature. Then, the crude product was quenched with distilled water (50 mL) and extracted. The collected organic phases dried over Na₂SO₄, filtered and solvent evaporated. The crude product was purified by column chromatography (silica gel, Hexane:EtOAc 6:1), yielding the target product, compound **153**, as a white solid (0.59 g, 99%). ¹H NMR (400 MHz, CDCl₃) δ 7.50 (d, *J* = 2.2 Hz, 2H), 7.17 (dd, *J* = 8.7, 2.1 Hz, 2H), 7.04 (d, *J* = 8.5 Hz, 2H), 6.85 (d, *J* = 8.6 Hz, 2H), 6.62 (d, *J* = 8.7 Hz, 2H), 5.09 (s, 2H), 3.79 (s, 3H), 1.55 (s, 6H). ¹³C NMR (100 MHz, CDCl₃) δ 158.8, 139.5, 133.7, 129.6, 127.5, 127.3, 127.3, 115.1, 114.4, 113.6, 55.3, 50.6, 36.6, 29.0.

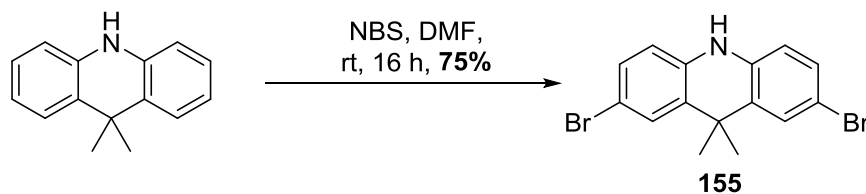
5.7.1.3 Synthesis of Compound **154**



Scheme 5. 108. Synthetic route for **154**

To a 25 mL Schlenk tube, compound **153** (200 mg, 0.41 mmol), $\text{Pd}_2(\text{dba})_3$ (19.0 mg, 5%), XPhos (20.0 mg, 10%), Na^tBuO (0.14, 1.44 mmol, 3.5 eq.) were added, and vacuum- N_2 was applied. Then, toluene (10 mL) was added and degassed for 1 hour. After that, morpholine (125 mg, 1.44 mmol, 3.5 eq.) was added and heated to 100°C and stirred for 1 hour. After the completion of the reaction, the reaction mixture was cooled to room temperature and filtered through celite, then washed with CHCl_3 . The crude product was purified with column chromatography (silica, $\text{MeOH}:\text{DCM}$ 5:95), and the target product, compound **154**, was obtained as a white solid (171 mg, 83%). ^1H NMR (400 MHz, CD_3CN) δ 7.08 (ö, 2H), 7.04 (d, $J = 8.6$ Hz, 2H), 6.83 (d, $J = 8.7$ Hz, 2H), 6.69 – 6.57 (m, 4H), 5.03 (s, 2H), 3.77 – 3.72 (m, 8H), 3.72 (s, 3H), 3.05 – 2.92 (m, 8H), 1.54 (s, 6H). ^{13}C NMR (100 MHz, CD_3CN) δ 159.6, 146.9, 135.9, 133.3, 130.6, 128.7, 115.6, 114.9, 114.7, 114.3, 67.7, 55.9, 51.6, 37.8, 29.7.

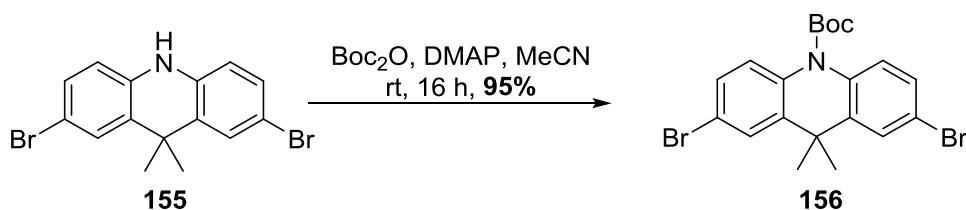
5.7.1.4 Synthesis of Compound **155**



Scheme 5. 109. Synthetic route for **155**

To a 50 mL two-necked round-bottom flask, 9,9-dimethyl-9,10-dihydroacridine (0.20 g, 0.96 mmol) was added, and vacuum- N_2 was applied. Then, it was dissolved in dry DMF (6 mL). Subsequently, NBS (0.36 g, 2.01 mmol, 2.1 eq.) was added in portions over 1 hour. After the completion of the addition, the reaction mixture was stirred for 16 hours at room temperature. Then, the crude product was quenched with distilled water (50 mL) and extracted with Et_2O (50 mL). The collected organic phases dried over $MgSO_4$, filtered and solvent evaporated. The crude product was purified by column chromatography (silica gel, Hexane:EtOAc 6:1), yielding the target product, compound **155**, as a white solid (0.27 g, 75%). ^1H NMR (400 MHz, $CDCl_3$) δ 7.44 (d, J = 1.2 Hz, 2H), 7.20 (dd, J = 8.3, 1.8 Hz, 2H), 6.57 (d, J = 8.3 Hz, 2H), 6.17 (s, 1H), 1.53 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 137.2, 130.8, 129.8, 128.6, 115.3, 113.0, 36.7, 30.5.

5.7.1.5 Synthesis of Compound **156**

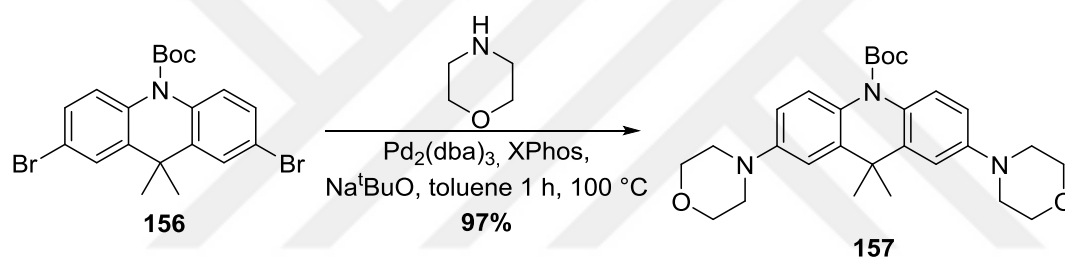


Scheme 5. 110. Synthetic route for **156**

To a 25 mL Schlenk tube, compound **155** (0.20 g, 0.54 mmol) was added and vacuum- N_2 was applied. Then, it was dissolved in dry MeCN (8 mL). Subsequently,

DMAP (0.20 g, 1.63 mmol, 3 eq.) and Boc_2O (0.35 g, 1.63 mmol, 3 eq.) were added and resulting solution stirred for 16 hours at room temperature. Upon completion of reaction, mixture quenched with distilled water (50 mL) and extracted with DCM (50 mL). The collected organic phases dried over Na_2SO_4 , filtered and solvent evaporated. The crude product was purified by column chromatography (silica gel, Hexane:EtOAc 6:1), yielding the target product, compound **156**, as a white solid (0.24 g, 95%). ^1H NMR (400 MHz, CDCl_3) δ 7.50 – 7.46 (m, J = 8.6, 2.7 Hz, 4H), 7.36 (dd, J = 8.6, 2.1 Hz, 2H), 1.52 (s, 6H), 1.51 (s, 9H). ^{13}C NMR (100 MHz, CDCl_3) δ 152.1, 142.9, 137.3, 129.1, 127.2, 126.4, 119.0, 82.7, 38.0, 28.3, 26.6.

5.7.1.6 Synthesis of Compound 157

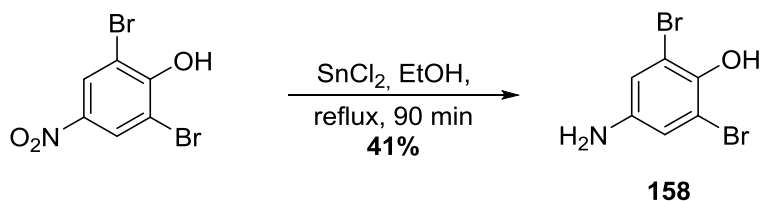


Scheme 5. 111. Synthetic route for **157**

To a 25 mL Schlenk tube, compound **156** (0.20 g, 0.43 mmol), $\text{Pd}_2(\text{dba})_3$ (20.0 mg, 5%), XPhos (20.4 mg, 10%), Na^tBuO (0.14, 1.50 mmol, 3.5 eq.) were added, and vacuum- N_2 was applied. Then, toluene (10 mL) was added and degassed for 1 hour. After that, morpholine (0.13 g, 1.50 mmol, 3.5 eq.) was added and heated to 100 °C and stirred for 1 hour. After the completion of the reaction, the reaction mixture was cooled to room temperature and filtered through celite, then washed with CHCl_3 . The crude product was purified with column chromatography (silica, MeOH:DCM 5:95), and the target product, compound **157**, was obtained as an off-white solid (0.20 g, 97%). ^1H NMR (400 MHz, CDCl_3) δ 7.50 (d, J = 8.8 Hz, 2H), 6.93 (d, J = 2.4 Hz, 2H), 6.79 (dd, J = 8.8, 2.5 Hz, 2H), 3.90 – 3.84 (m, 8H), 3.16 – 3.10 (m, 8H), 1.54 (s, 6H), 1.51 (s, 9H). ^{13}C NMR (100 MHz, CDCl_3) δ 153.0, 149.0, 142.0, 131.9, 126.0, 113.7, 110.9, 81.3, 67.1, 50.3, 38.3, 28.4.

5.7.2 Synthesis of 7-amino Bearing Acridine Based PS (DDAN-Br)

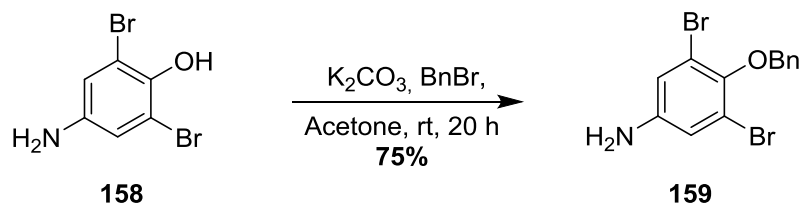
5.7.2.1 Synthesis of Compound 158



Scheme 5. 112. Synthetic route for **158**

To a 250 mL Schlenk tube, 2,6-dibromo-4-nitrophenol (1.64 g, 5.52 mmol) was added and vacuum-N₂ was applied. Then, it was dissolved in EtOH (54 mL). Subsequently, SnCl₂ (5.24 g, 13.8 mmol, 2.5 eq.) was added and mixture heated to 78 °C and stirred for 90 minutes. After the completion of the reaction, the reaction mixture was cooled to room temperature and quenched with EtOAc : satd. NaHCO₃ solution (80 mL, 1:1). Then, more satd. NaHCO₃ was added to adjust pH = 9. The resulting suspension was filtered and filtrate extracted. The collected organic phases dried over Na₂SO₄, filtered and solvent evaporated. The crude product was purified by column chromatography (silica gel, Hexane:EtOAc 4:1), yielding the target product, compound **158**, as a pale yellow solid (0.60 g, 41%). ¹H NMR (400 MHz, DMSO) δ 8.69 (s, 1H), 6.76 (s, 2H), 5.02 (s, 2H). ¹³C NMR (100 MHz, DMSO) δ 144.2, 140.5, 117.1, 113.5.

5.7.2.2 Synthesis of Compound 159

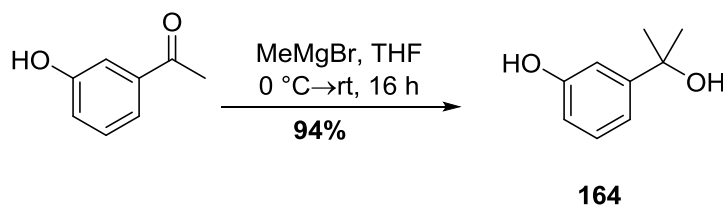


Scheme 5. 113. Synthetic route for **159**

To a 100 mL two-necked round-bottom flask, compound **158** (1.00 g, 3.75 mmol) and K_2CO_3 (0.55 g, 3.78 mmol, 1.01 eq.) were added, and vacuum- N_2 was applied. Then, they were suspended in acetone (20 mL). Subsequently, $BnBr$ (0.61 g, 3.56 mmol, 0.95 eq.) was added to this suspension slowly and stirred for 20 hours at room temperature. Upon completion of reaction, acetone evaporated and mixture quenched with distilled water (50 mL) and extracted with DCM (50 mL). The collected organic phases dried over Na_2SO_4 , filtered and solvent evaporated. The crude product was purified by column chromatography (silica gel, MeOH:DCM 2:98), yielding the target product, compound **159**, as a white solid (1.00 g, 75%). ^1H NMR (400 MHz, $CDCl_3$) δ 7.60 (d, $J = 7.3$ Hz, 2H), 7.41 (t, $J = 7.2$ Hz, 2H), 7.38 – 7.33 (m, 1H), 6.85 (s, 2H), 4.94 (s, 2H), 3.64 (s, 2H).

5.7.3 Synthesis of 7-Hydroxy Bearing Acridine Based PS (DDAO-Br)

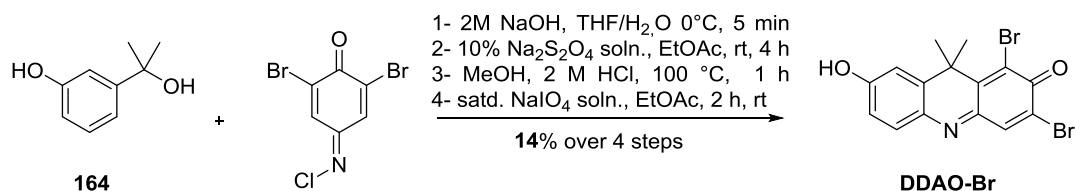
5.7.3.1 Synthesis of Compound 164



Scheme 5. 114. Synthetic route for **164**

To a 100 mL Schlenk tube, 3-hydroxyacetophenone (1.00 g, 3.34 mmol) was added and vacuum–N₂ was applied. Then, it was dissolved in dry THF (20 mL) and cooled to 0 °C. To this solution, MeMgBr (7.40 mL, 21.95 mmol, 6.5 eq.) was added dropwise. Subsequently, resulting mixture warmed to the room temperature and stirred 16 hours. Upon completion of reaction, mixture quenched with distilled water and THF was evaporated. Then, extracted with DCM (50 mL). The collected organic phases dried over Na₂SO₄, filtered and solvent evaporated. The crude product was purified by column chromatography (silica gel, hexane:EtOAc 1:1), yielding the target product, compound **164**, as a white solid (1.05 g, 94%). ¹H NMR (400 MHz, DMSO) δ 9.21 (s, 1H), 7.06 (t, *J* = 7.8 Hz, 1H), 6.89 (s, 1H), 6.84 (d, *J* = 7.6 Hz, 1H), 6.56 (dd, *J* = 7.7, 1.7 Hz, 1H), 4.92 (s, 1H), 1.37 (s, 6H). ¹³C NMR (100 MHz, DMSO) δ 156.9, 152.2, 128.7, 115.3, 112.7, 111.8, 70.6, 32.0.

5.7.3.2 Synthesis of DDAO-Br



Scheme 5. 115. Synthetic route for **DDAO-Br**

To a 25 mL Schlenk tube, compound **164** (0.30 g, 2.00 mmol) and 2,6-dibromoquinonechlorimide (0.58 g, 1.95 mmol, 0.98 eq.) were added and vacuum-N₂ was applied. Then, they dissolved in THF-H₂O solution (3:2, 5 mL) and cooled to 0 °C. To this solution, 2 M NaOH solution (2.1 mL) was added dropwise. Upon completion of the addition, mixture quenched with satd. NH₄Cl solution (20 mL) and extracted with EtOAc (30 mL). Then, to a collected organic phase 10% Na₂S₂O₄ solution (50 mL) was added and stirred vigorously for 4 hours at room temperature. After completion of reaction, distilled water (50 mL) added and extracted. The collected organic phases dried over Na₂SO₄, filtered and solvent evaporated. The crude product was added to the 100 mL two-necked, round-bottom flask, and dissolved with sufficient amount of MeOH (8 mL). Following this, 2 M HCl (15 mL) was added and resulting mixture heated to 100 °C and stirred for 1 hour at that temperature. Upon completion of reaction, mixture cooled to the room temperature and extracted with EtOAc (50 mL). Then, to a collected organic phase, satd. NaIO₄ solution (30 mL) was added and stirred for 2 hours at room temperature. After that, phases separated and the collected organic phases dried over Na₂SO₄, filtered and solvent evaporated. The crude product was purified by column chromatography (silica gel, MeOH:DCM 5:95), yielding the target product, compound **DDAO-Br**, as a red solid (0.11 g, 14%). ¹H NMR (400 MHz, DMSO) δ 10.85 (s, 1H), 8.07 – 7.88 (m, 1H), 7.50 (d, *J* = 8.5 Hz, 1H), 7.06 (s, 1H), 6.85 (d, *J* = 8.4 Hz, 1H), 1.84 (s, 6H).

5.8 Fluorescence Quantum Yield

Fluorescence quantum yields of the samples were investigated using a fluorescence spectrometer (FLS 1000, Edinburgh Instruments) equipped with an integrating sphere accessory. A continuous-wave xenon lamp served as the excitation source, and the emitted fluorescence was detected with a standard photomultiplier (PMT-900) covering a wavelength range of 200-800 nm. During measurements, the PMT was cooled to -20 °C using a built-in housing to reduce undesired dark current noise.

For quantum yield measurement, an integrating sphere (Edinburgh Instruments) was placed inside the spectrometer's sample compartment. The internal cavity of the sphere was coated with a PTFE-like material, enabling a reflectance of approximately >99% (>95%) over the wavelength ranges of 400-1500 nm and 250-2500 nm, respectively. The sphere had two ports positioned 90° apart. The excitation beam was directed to the sample through the excitation port, and the fluorescence was collected from the emission port. The excitation port of the sphere included a lens to effectively focus the beam on the sample, while the emission port was an open aperture.

Prior to the experiments with the PSs, blank spectra were measured using the reference solvents (PBS, pH 7.4, 1% DMSO). For both measurements (blank and sample), two identical quartz cuvettes with equal volumes were used. Initially, the reference sample was placed inside the sphere, and the emission/excitation slits were adjusted to the excitation wavelength to ensure the PMT's response remained linear during measurements. To cover a scattering range, the emission scans started 20 nm below the actual excitation wavelengths and ended at 900 nm. Additionally, the step size and integration time of the measurements were set to 1 nm and 0.2 seconds, respectively.

Upon completing all emission measurements of the samples and references, the quantum yields of the samples were determined using the Fluoracle software. The built-in analysis tool calculates the quantum yield (QY) as:

$$QY=(Es-EB)/(SB-Ss) ,$$

where *Es* (*EB*) and *Ss* (*SB*) are the selected areas for the emitted and scattered signals of the sample (blank).

5.9 Singlet Oxygen Trap Experiment

Singlet oxygen quantum yields were calculated using the following equation and methylene blue as the reference fluorophore. The singlet oxygen trapping experiment for methylene blue was performed at pH 7.4 in phosphate saline buffer, with a reported yield of 0.52 from the literature. The relative quantum yields were determined with reference to methylene blue in phosphate saline buffer. Oxygen-saturated PBS was prepared by bubbling molecular oxygen through the solution for 10 minutes. The cuvette was then filled with a 5 μ M photosensitizer solution, corresponding to an absorbance of approximately 0.2-0.3. 9,10-Anthracenediyl-bis(methylene)dimalonic acid (ADMDA) was added as a singlet oxygen trap, and its absorbance was adjusted to between 0.6 and 1.9.

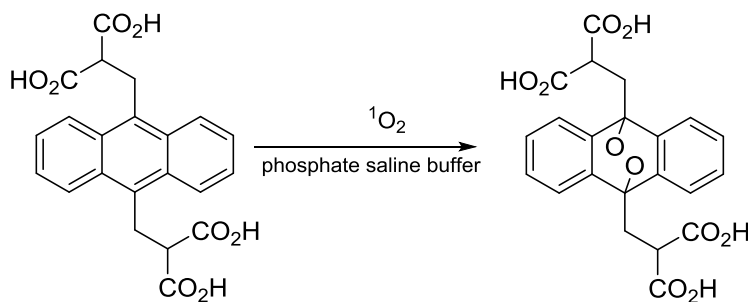


Figure 5.1. Reaction between photosensitized $^1\text{O}_2$ and ADMDA.

Several measurements were taken in the dark to stabilize the trap's solubility. Subsequently, the cuvette was exposed to light of an appropriate wavelength. Each irradiation was performed from a distance of 10 cm for variable durations. The decrease in the trap's absorbance at 380 nm was recorded and repeated multiple times. The slope for each photosensitizer was calculated by plotting the trap's

absorbance at 380 nm versus time. Singlet oxygen quantum yields were then calculated according to the following equation:

$$\Phi_{\Delta sample} = \Phi_{\Delta standard} \left(\frac{1 - 10^{-A_{std}}}{1 - 10^{-A_{sam}}} \right) \left(\frac{m_{sample}}{m_{standard}} \right)$$

Here, "sample" and "standard" represent photosensitizer derivatives and methylene blue, respectively. m is the slope for the photosensitizer derivatives, indicating the variation of the trap's absorbance at 380 nm with irradiation time, A_{sam} and A_{std} represents FPF for PS and methylene blue respectively, F is the correction factor, which is given by $F = 1 - 10^{-OD}$ (OD at absorption maxima of irradiation wavelength), and PF is absorbed photonic flux in $\mu\text{Einstein dm}^{-3}\text{s}^{-1}$.

5.10 Cell Culture and MTT Assay

Cancer cells (SHSY5Y, U87 MG, U118 MG, MDAMB 231, MCF 7, HeLa) healthy cells (L929) were grown under standard conditions (in 10% FBS high glucose DMEM, which includes 1% penicillin/streptomycin, 0.5% amphotericin B, and 2 mM glutamine at 37°C with 5% CO₂). The cells were passaged upon reaching 80-90% confluency every three days. For photodynamic therapy application, cells were exposed to increasing concentrations of PS (0-20 μM) for variable time in the dark, followed by illumination with LED or laser light with proper wavelength for various illumination time. Cells were kept in the dark for 24 hours for dark toxicity, and then an MTT assay was applied. Cytotoxicity analysis: Cells were seeded in a 96-well plate in 10% FBS high glucose phenol red-free DMEM. Upon following cell morphology, cells were treated with PS as described above. Following the incubation time, cells were kept in a fresh medium containing 0.5mg/mL MTT. After forming formazan crystals (2-4 hours), 10% SDS in PBS (0.01 N HCl) was added to each well and incubated at 37°C overnight. Absorbance values of each well were measured at 490 nm and 570 nm wavelengths by using a MultiskanSky microplate

reader (Thermo Scientific, USA). The results were determined as percentages relative to the control samples treated with DMSO (0.2%). IC₅₀ values were determined using concentration-normalized response curves derived from non-linear regression analysis (GraphPad Prism 8.02, GraphPad Software). (n=5-8)

5.11 Subcellular Localization

Cells were plated onto glass-bottom confocal dishes and incubated with PS with variable concentration in the incubator. After varying time of incubation, cells were washed with 1X PBS three times. Then, cells were treated with Hoechst 33342 (0.5 µg/ml in PBS) and MitoTracker Green FM (100 nM) or LysoTracker TM Yellow HCK-123 (75 nM) for 45 min at 37 °C. After three washes, cells were fixed using 4% paraformaldehyde at room temperature for 30 min. After incubation time, cells were rinsed three times, and confocal images were taken at diverse excitation and emission wavelengths for PS, Hoechst, MitoTracker Green FM and LysoTracker TM Yellow HCK-123 by Zeiss LSM 900 CLSM, respectively (63X) (n=3)

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APPENDICES

A. NMR Spectra

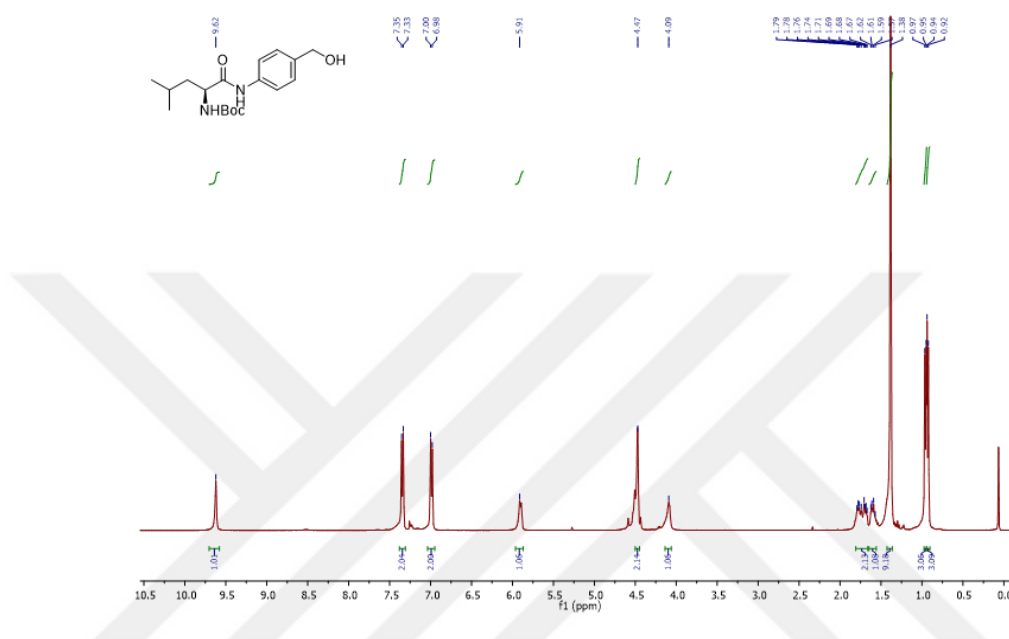


Figure A.1. ¹H NMR spectrum of compound **1** in CDCl₃.

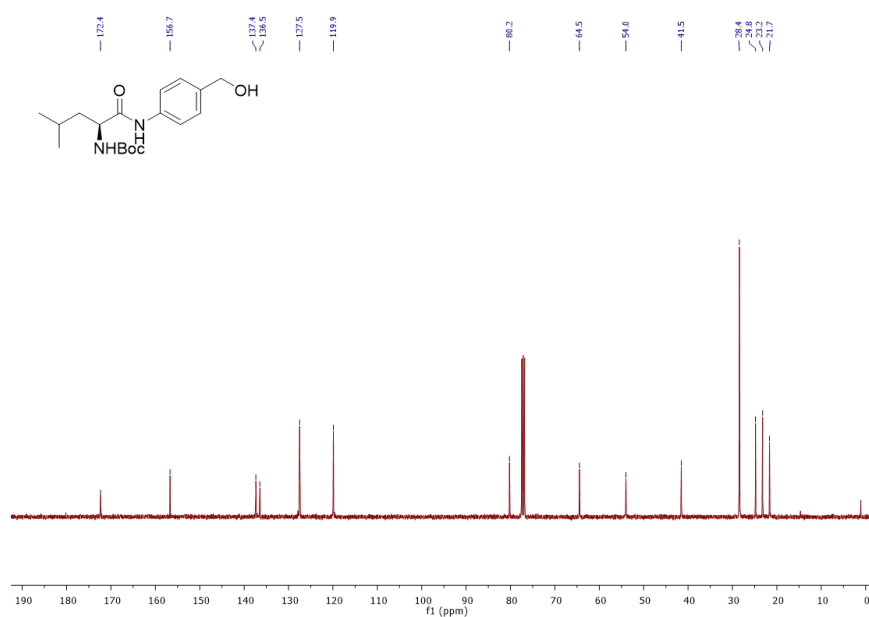


Figure A.2. ^{13}C NMR spectrum of compound **1** in CDCl_3 .

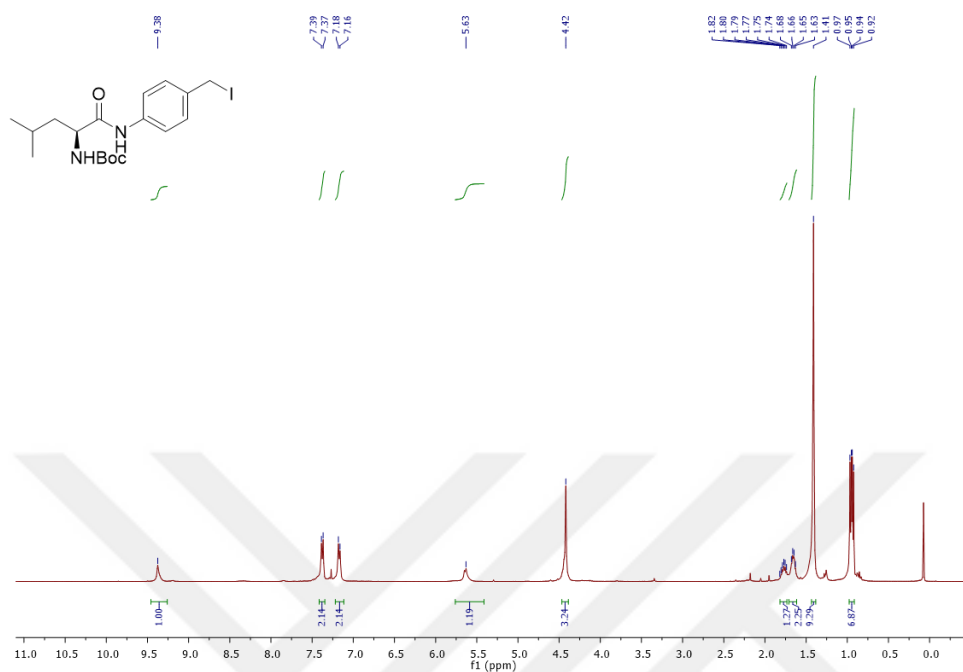


Figure A.3. ^1H NMR spectrum of compound **5** in CDCl_3 .

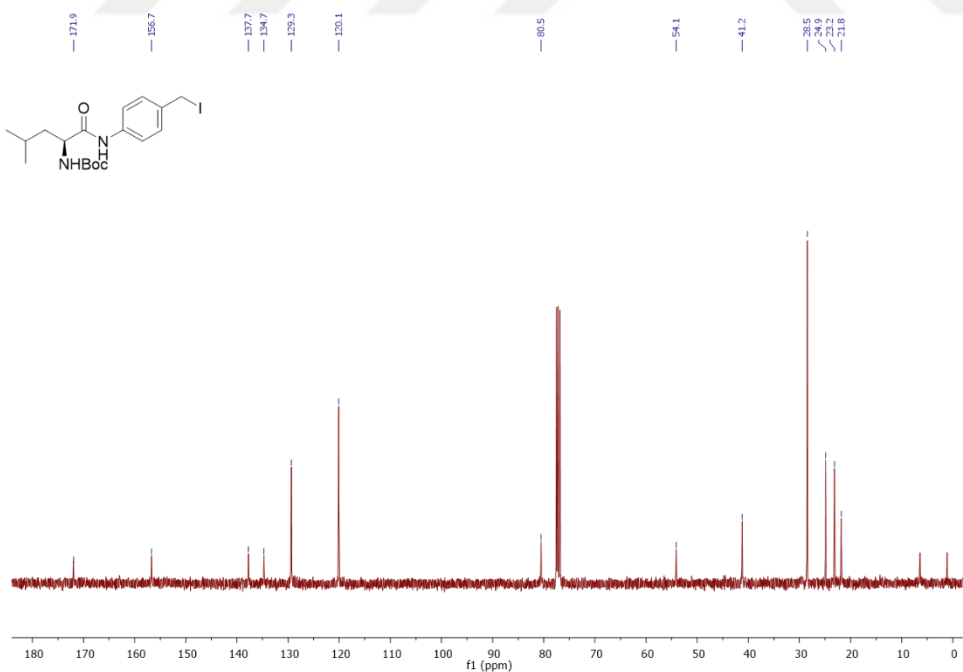


Figure A.4. ^{13}C NMR spectrum of compound **5** in CDCl_3 .

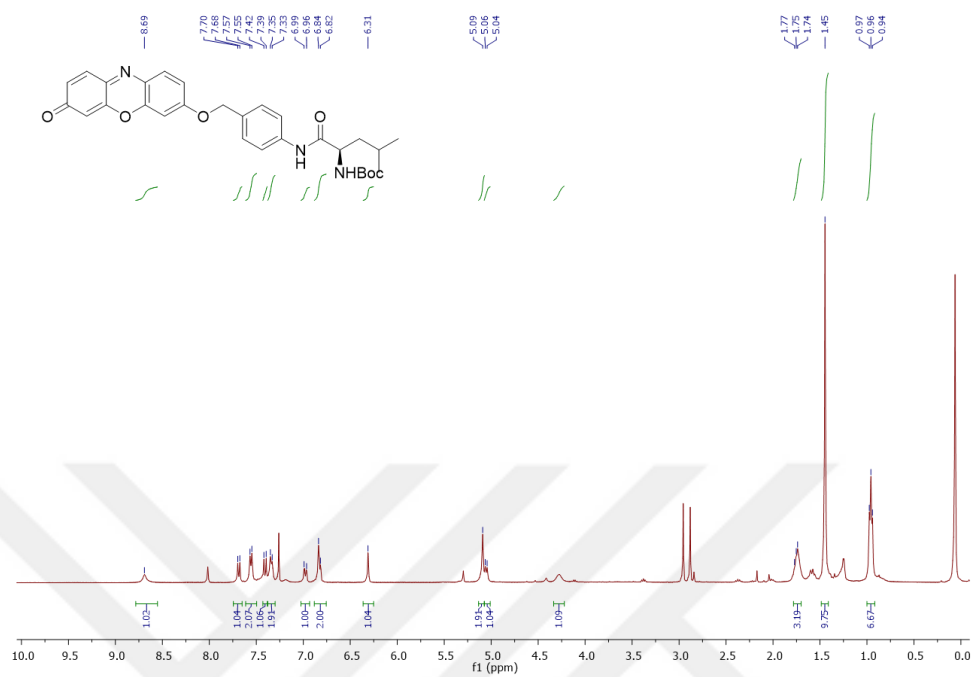


Figure A.5. ¹H NMR spectrum of compound **6** in CDCl₃.

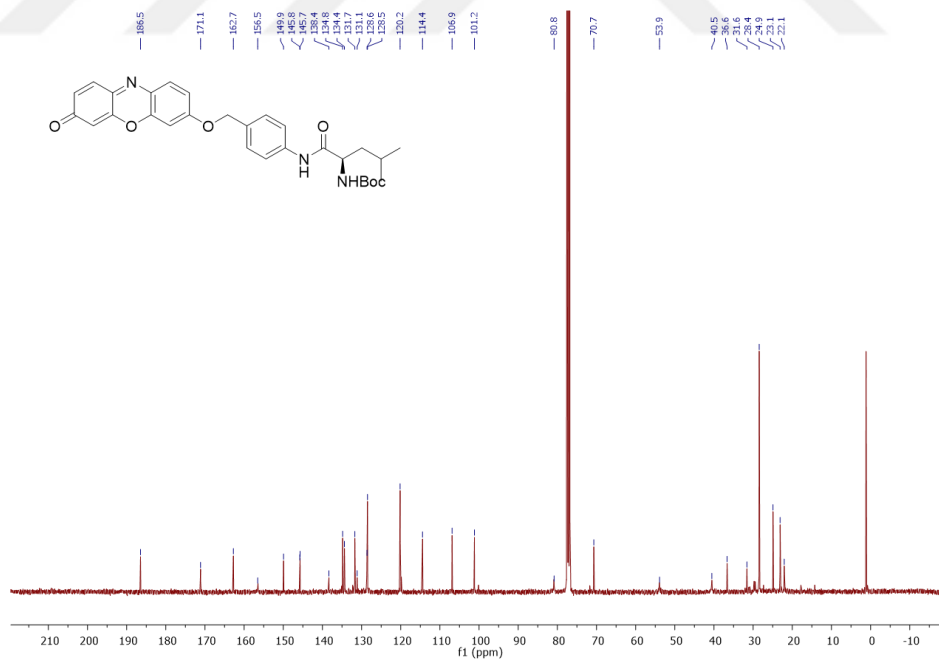


Figure A.6. ^{13}C NMR spectrum of compound **6** in CDCl_3 .

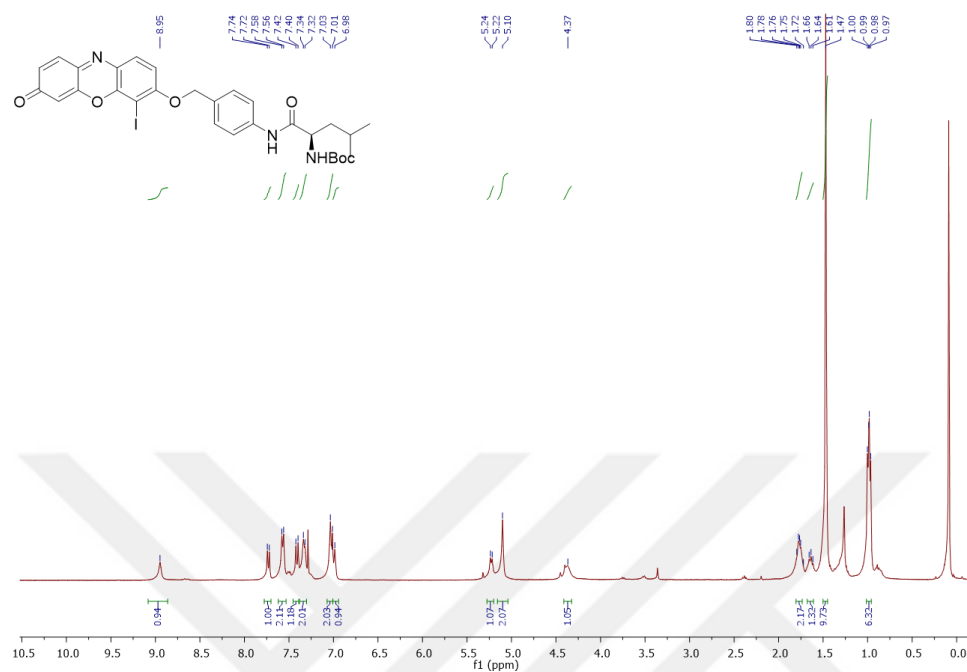


Figure A.7. ^1H NMR spectrum of compound **7** in CDCl_3 .

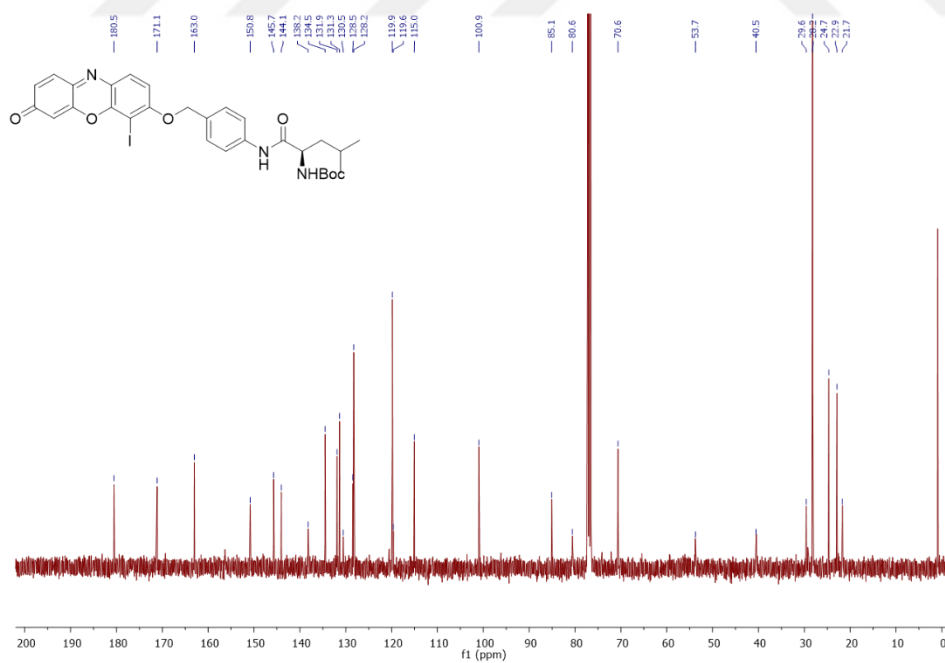


Figure A.8. ^{13}C NMR spectrum of compound **7** in CDCl_3 .

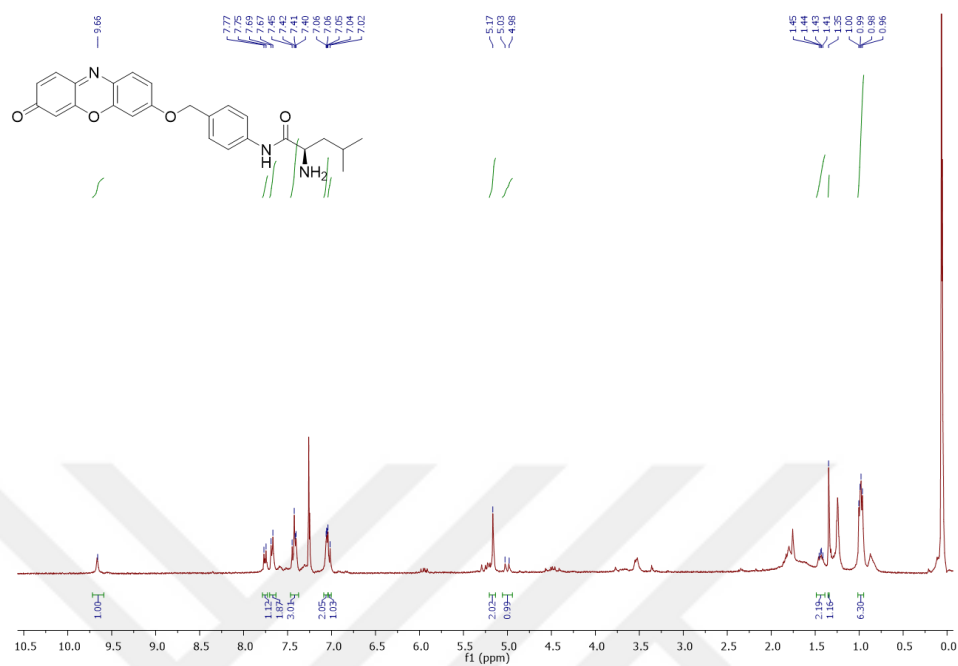


Figure A.9. ¹H NMR spectrum of **LAP-RI** in CDCl₃.

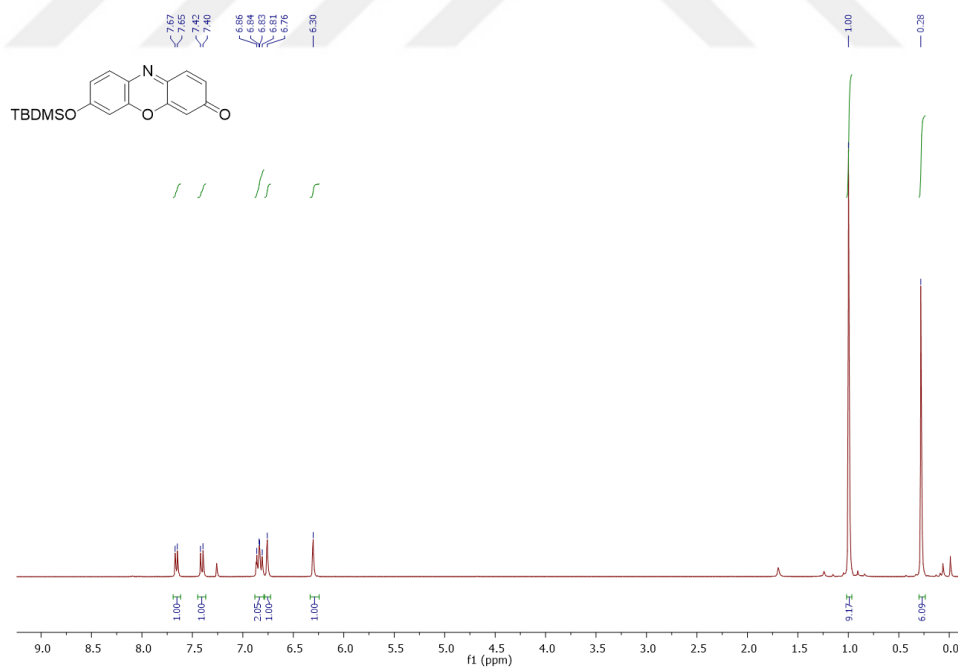


Figure A.10. ¹H NMR spectrum of compound **8** in CDCl₃.

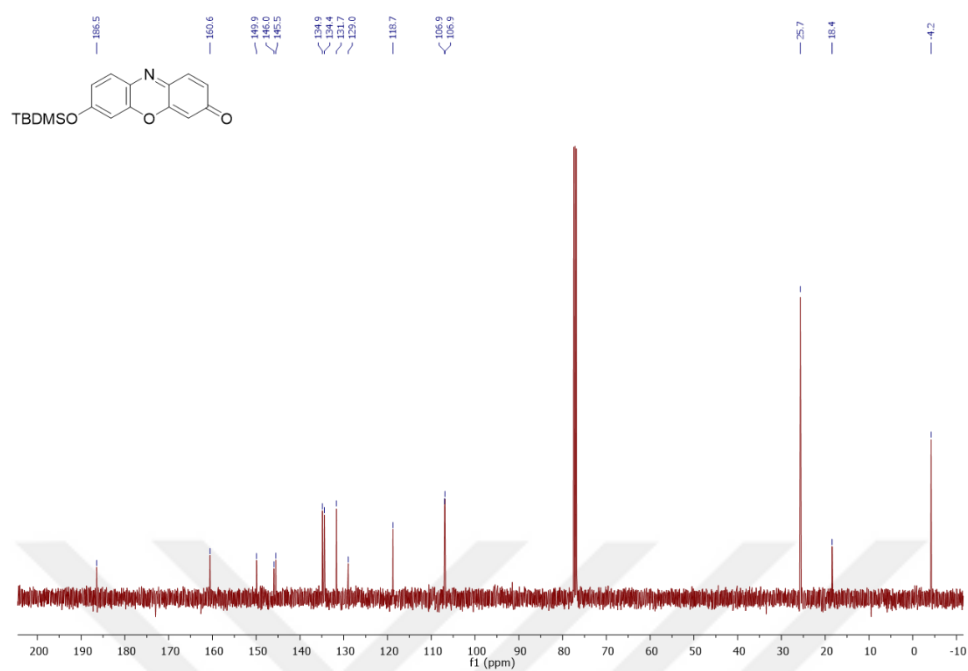


Figure A.11. ^{13}C NMR spectrum of compound **8** in CDCl_3 .

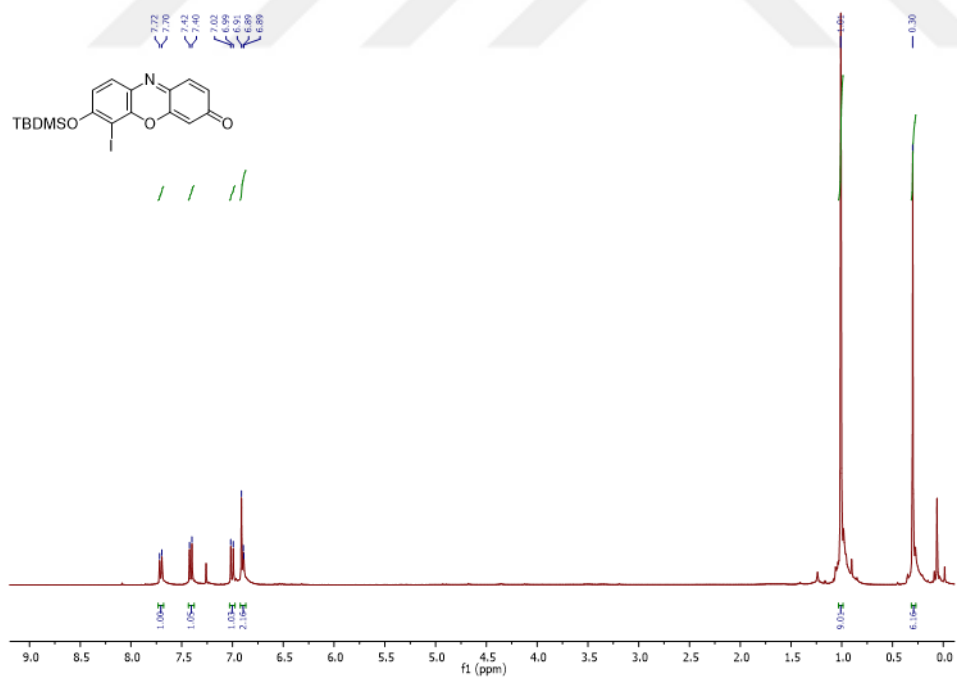
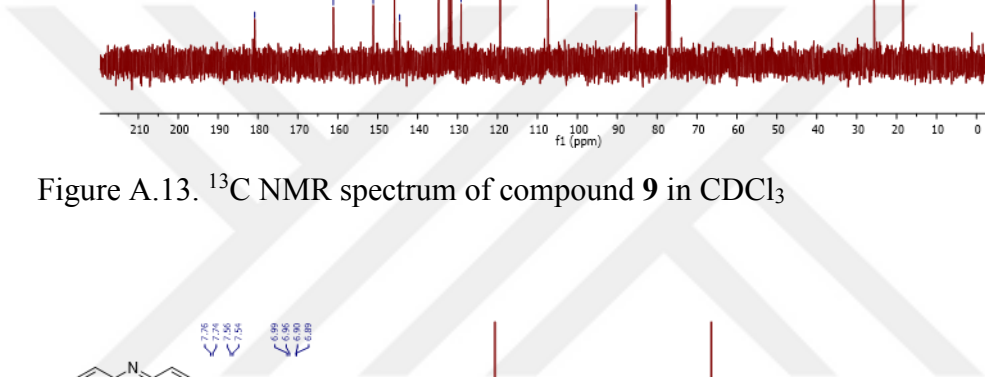
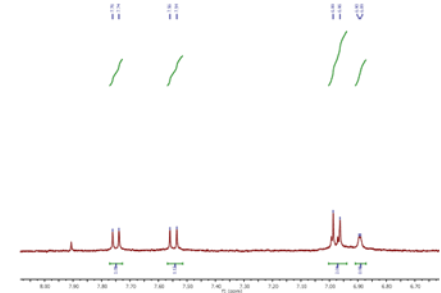
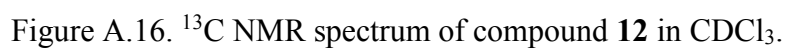
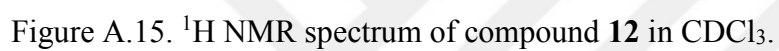


Figure A.12. ^1H NMR spectrum of compound **9** in CDCl_3 .



Chemical structure of 1,2,3,4-tetrahydro-1H-benzothiazole is shown with NMR peaks labeled: 7.76, 7.74, 7.56, 7.54, 6.99, 6.90, 6.89 ppm.





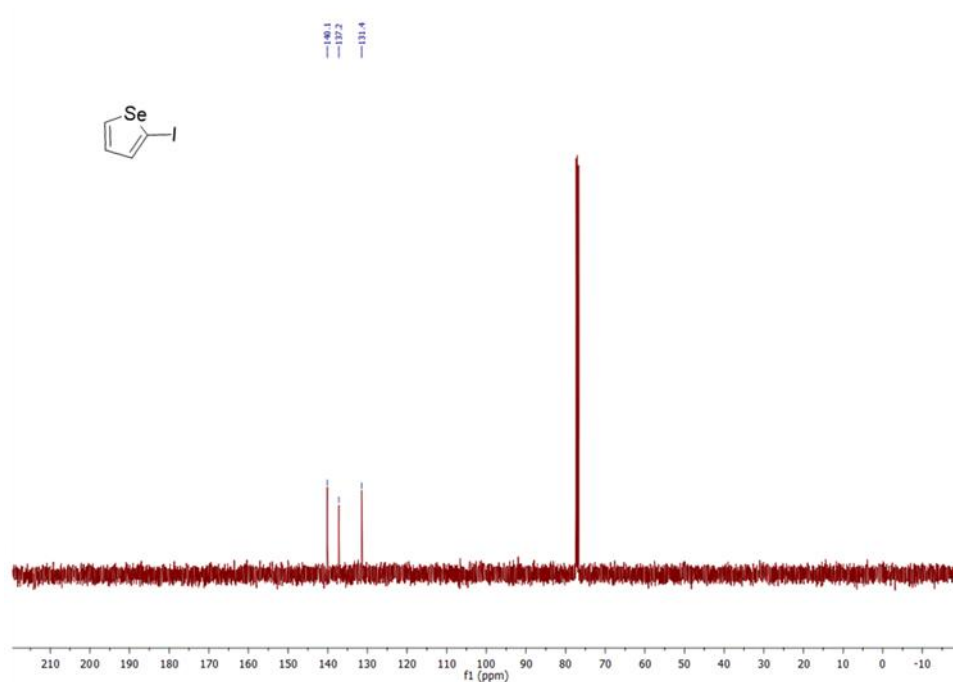


Figure A.19. ^{13}C NMR spectrum of compound **14** in CDCl₃.

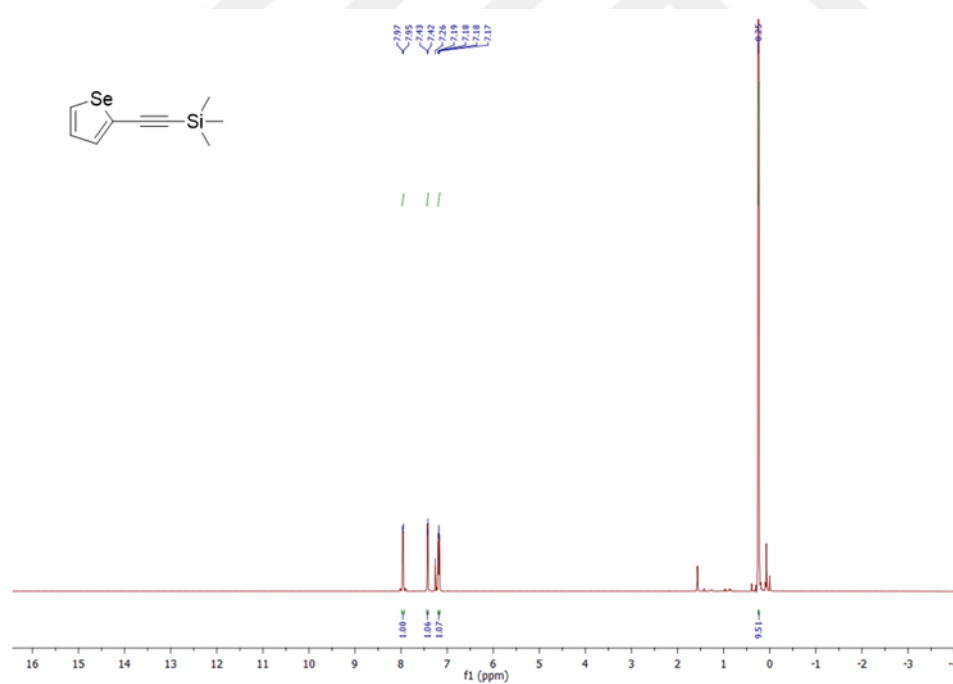


Figure A.20. ^1H NMR spectrum of compound **15** in CDCl₃.

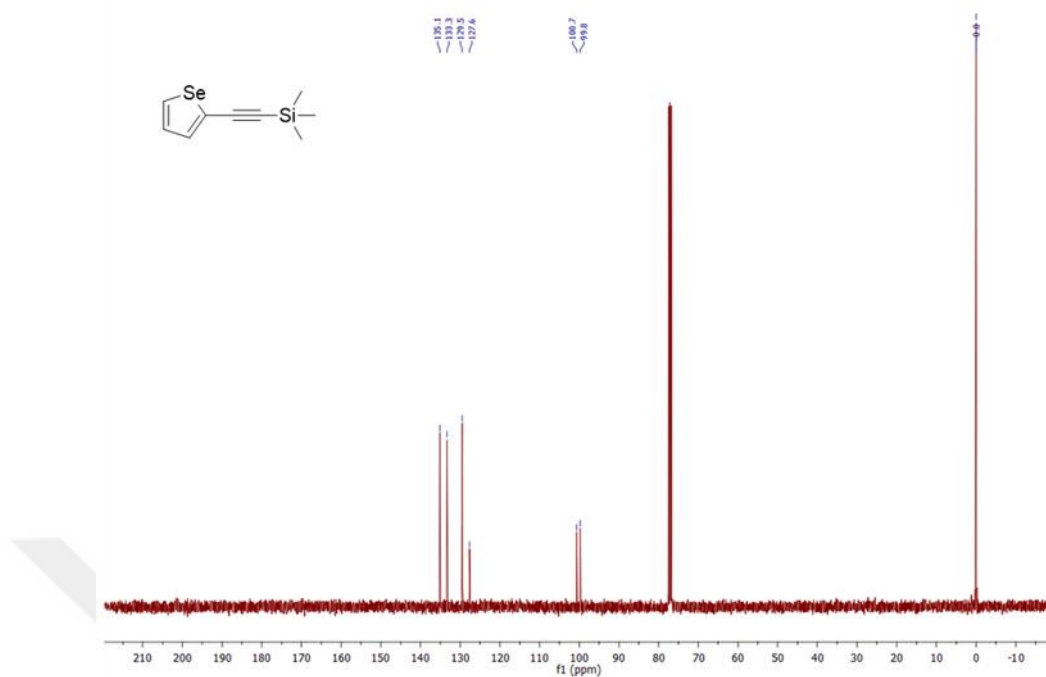


Figure A.21. ^{13}C NMR spectrum of compound **15** in CDCl₃

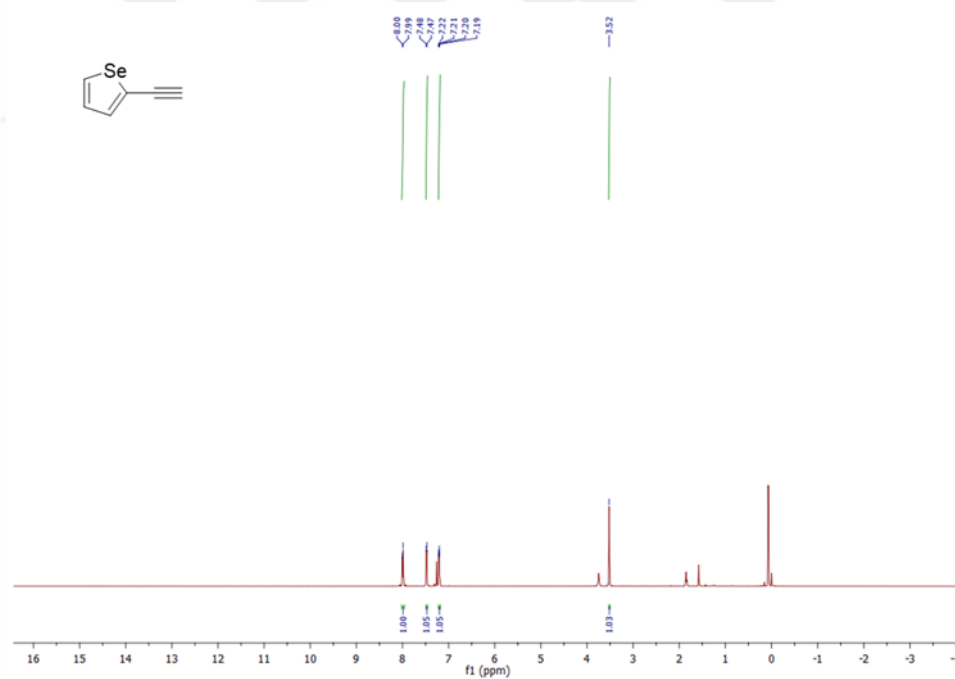


Figure A.22. ^1H NMR spectrum of compound **16** in CDCl₃.

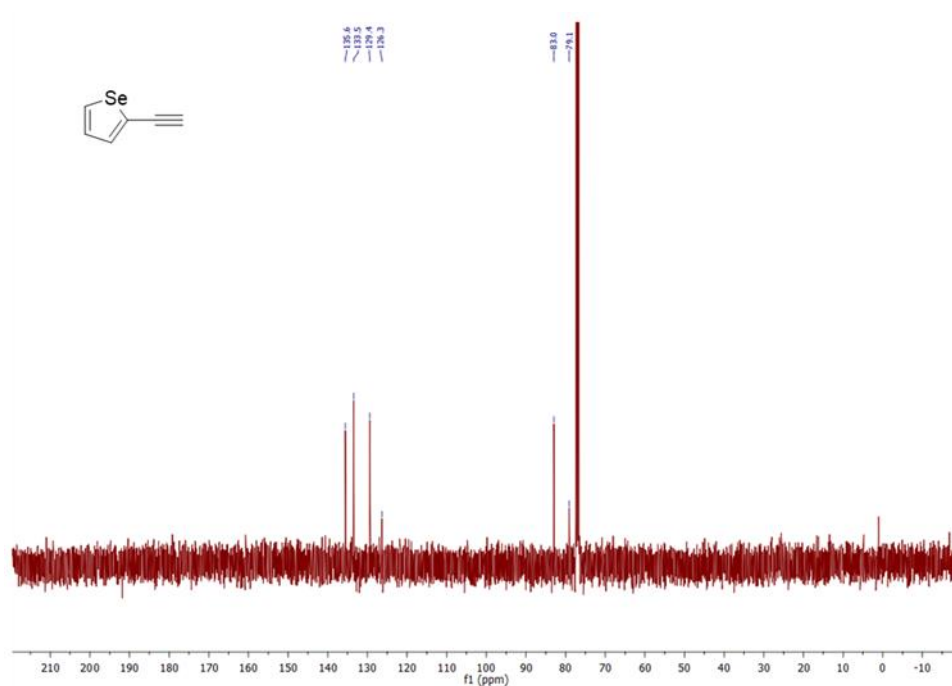


Figure A.23. ^{13}C NMR spectrum of compound **15** in CDCl₃

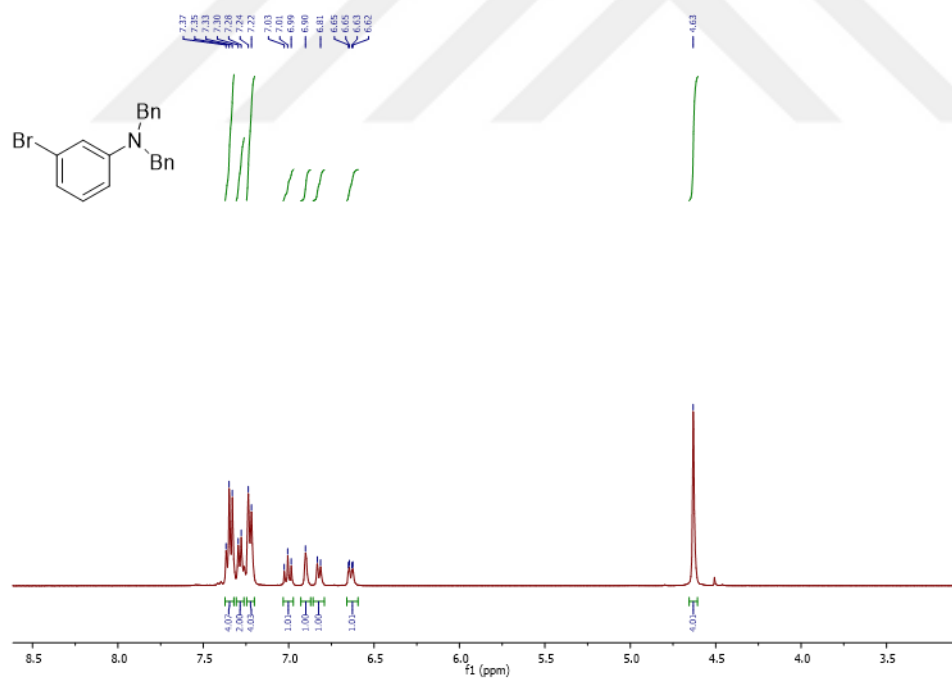


Figure A.24. ^1H NMR spectrum of compound **18** in CDCl₃.

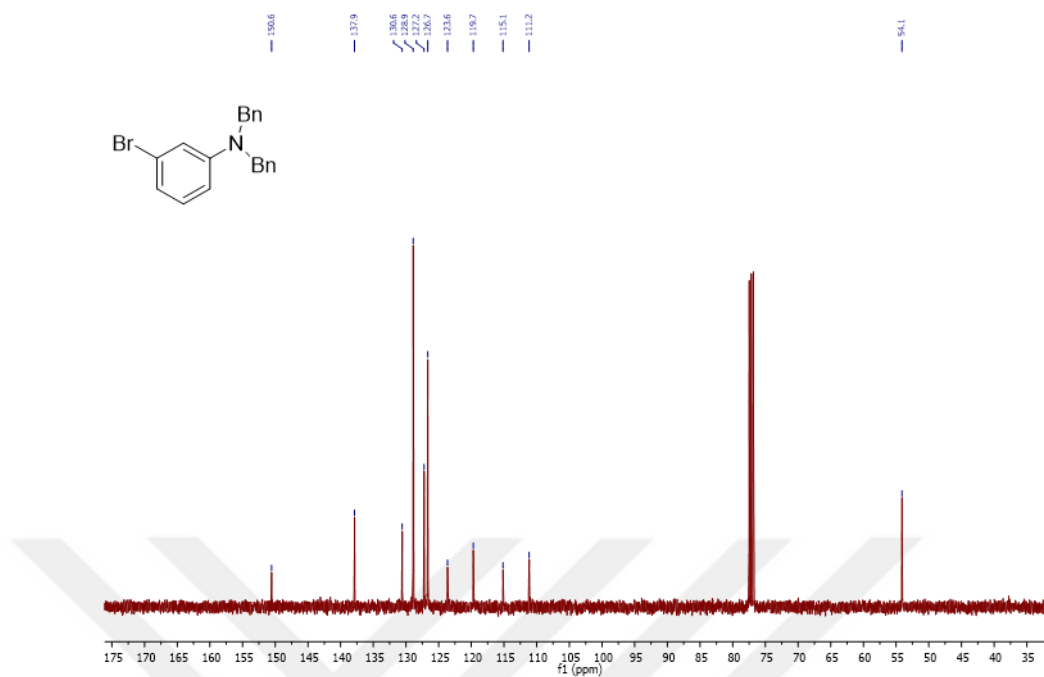


Figure A.25. ^{13}C NMR spectrum of compound **18** in CDCl_3 .

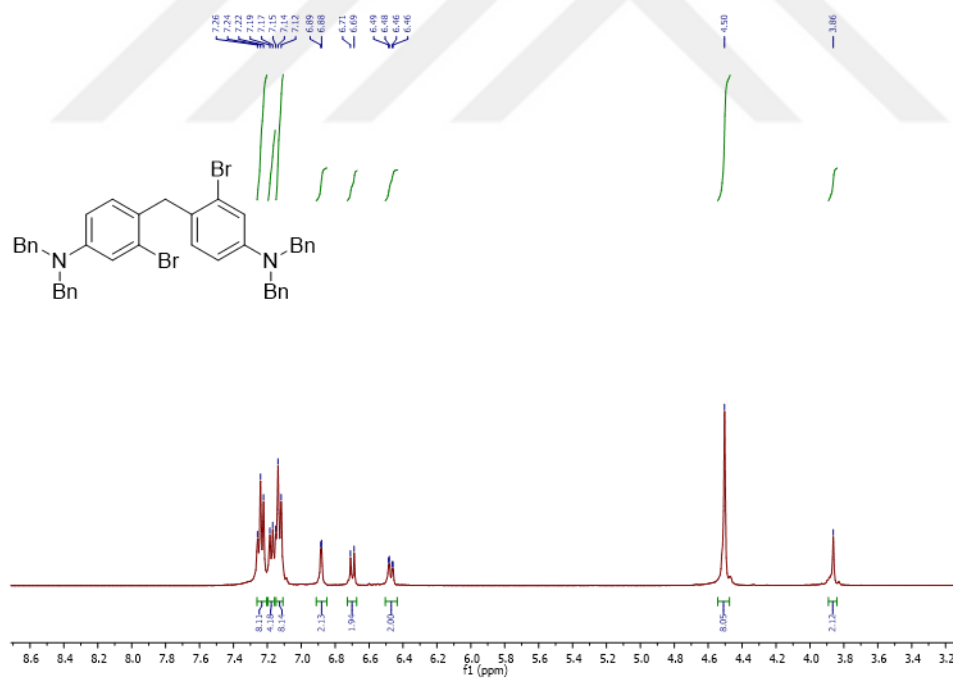


Figure A.26. ^1H NMR spectrum of compound **19** in CDCl_3 .

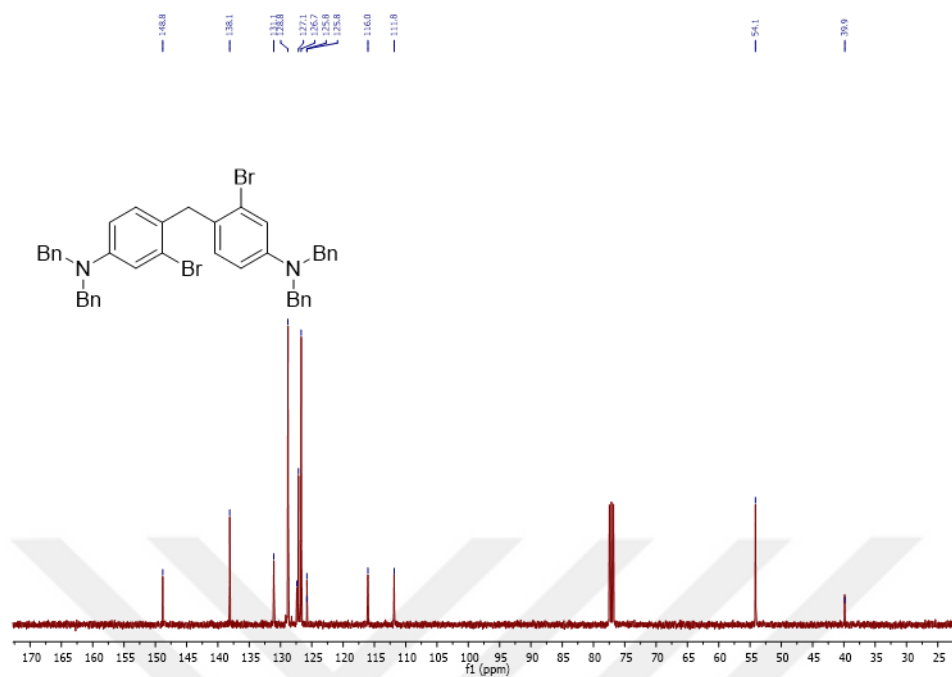


Figure A.27. ¹³C NMR spectrum of compound **19** in CDCl₃.

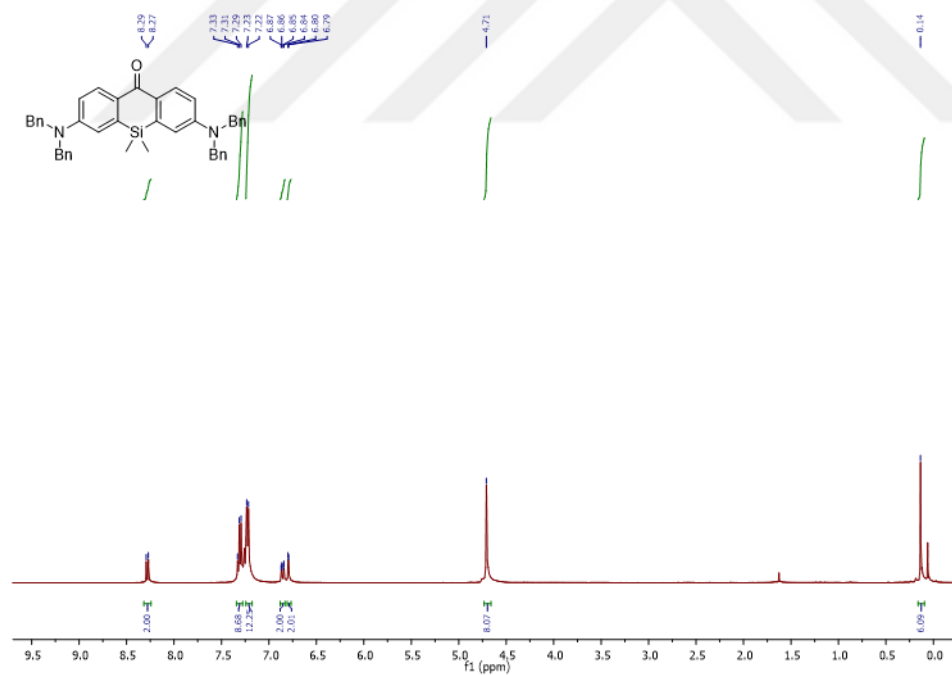


Figure A.28. ¹H NMR spectrum of compound **20** in CDCl₃.

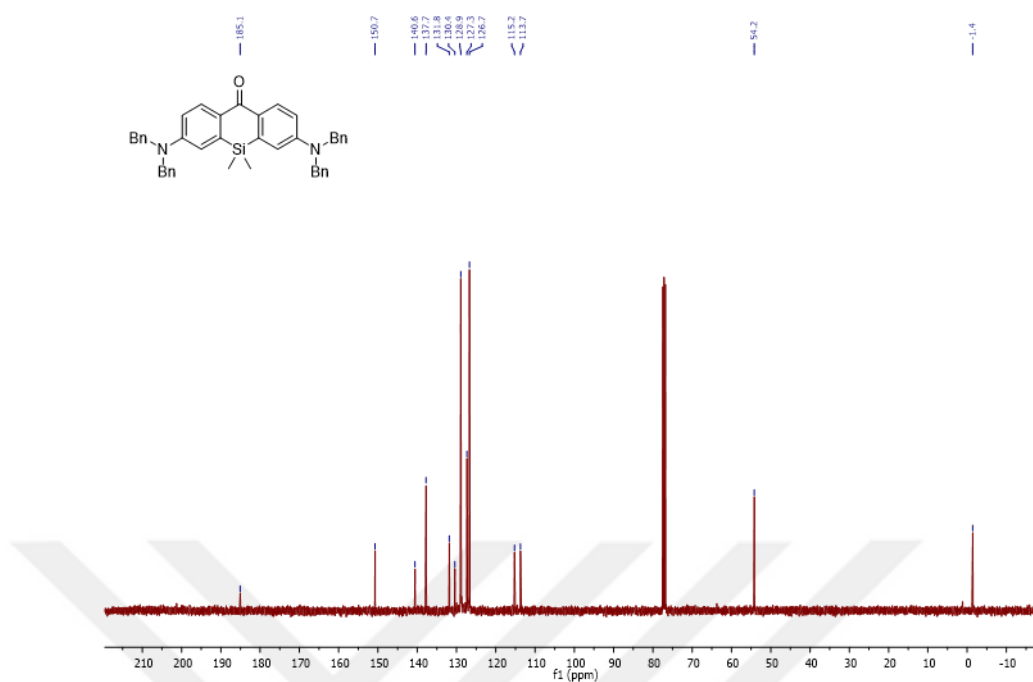


Figure A.29. ¹³C NMR spectrum of compound **20** in CDCl₃.

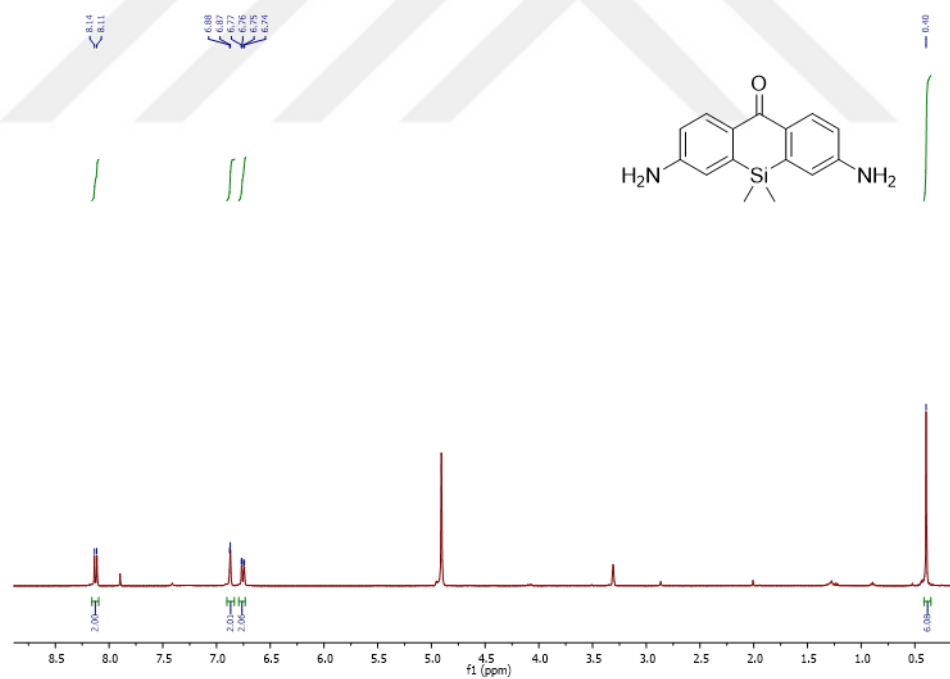


Figure A.30. ¹H NMR spectrum of compound **21** in MeOD.

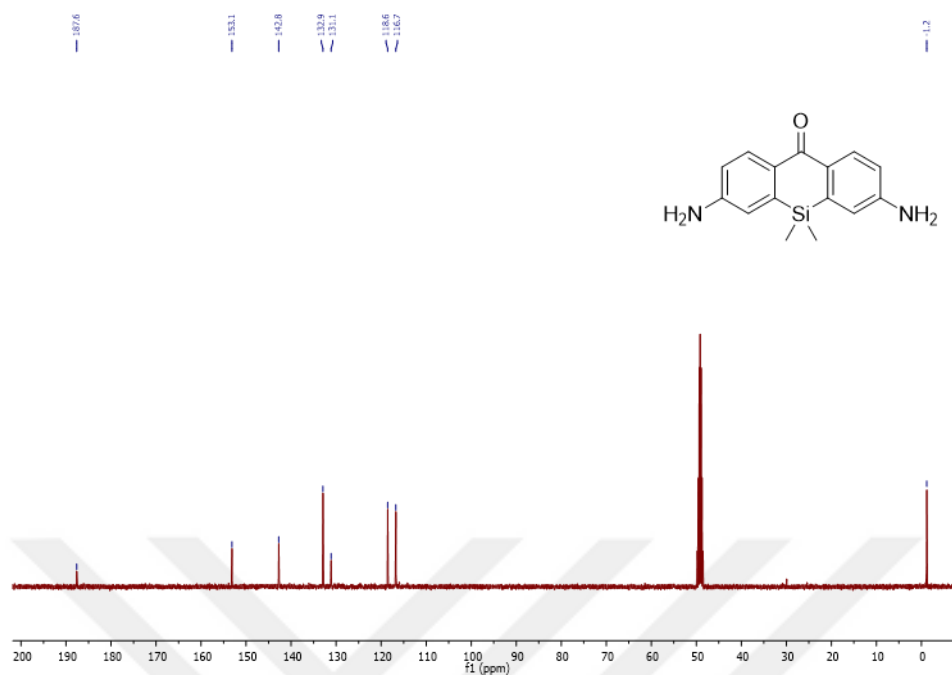


Figure A.31. ¹³C NMR spectrum of compound **21** in MeOD.

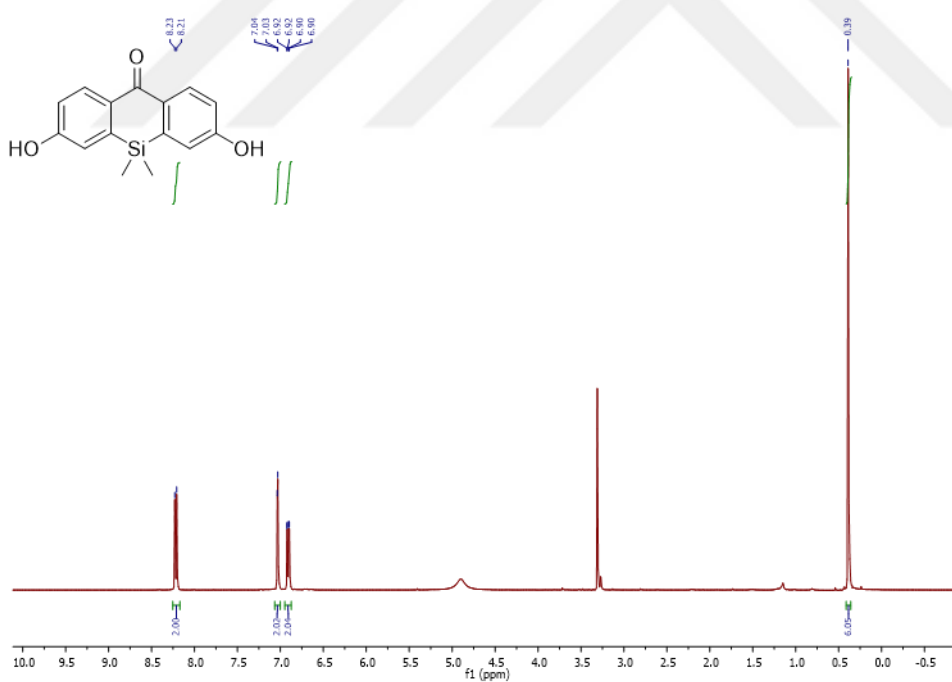


Figure A.32. ¹H NMR spectrum of compound **22** in MeOD.

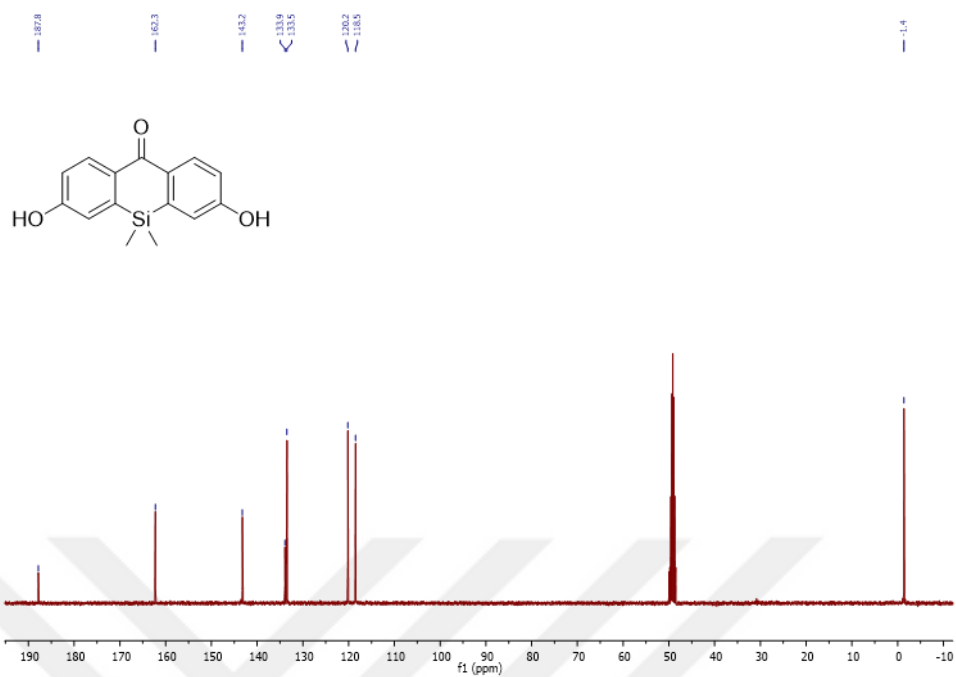


Figure A.33. ¹³C NMR spectrum of compound **22** in MeOD.

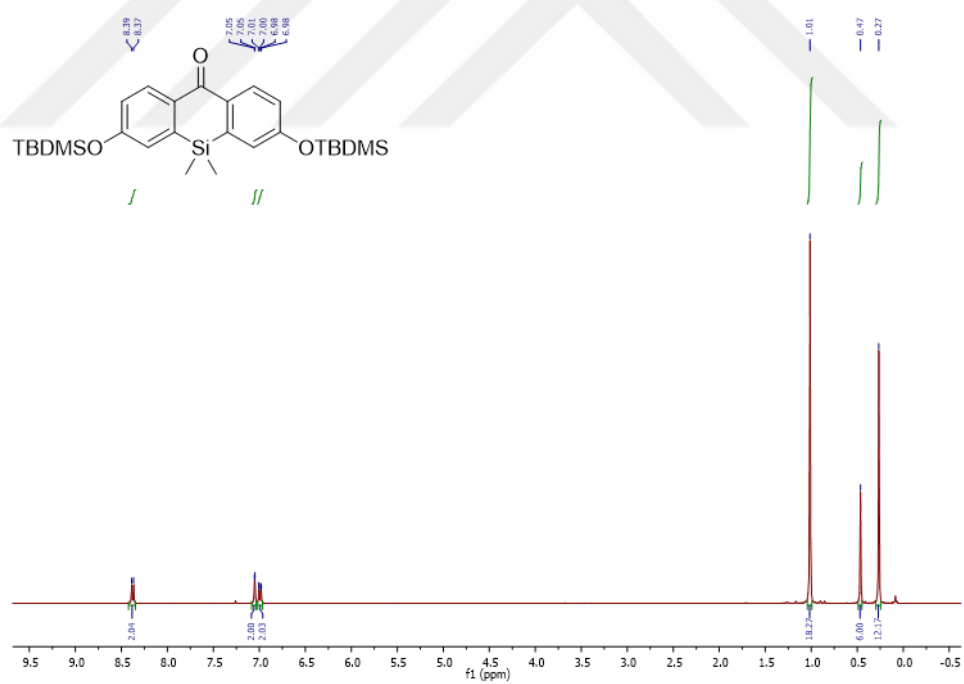


Figure A.34. ¹H NMR spectrum of compound **23** in CDCl₃.

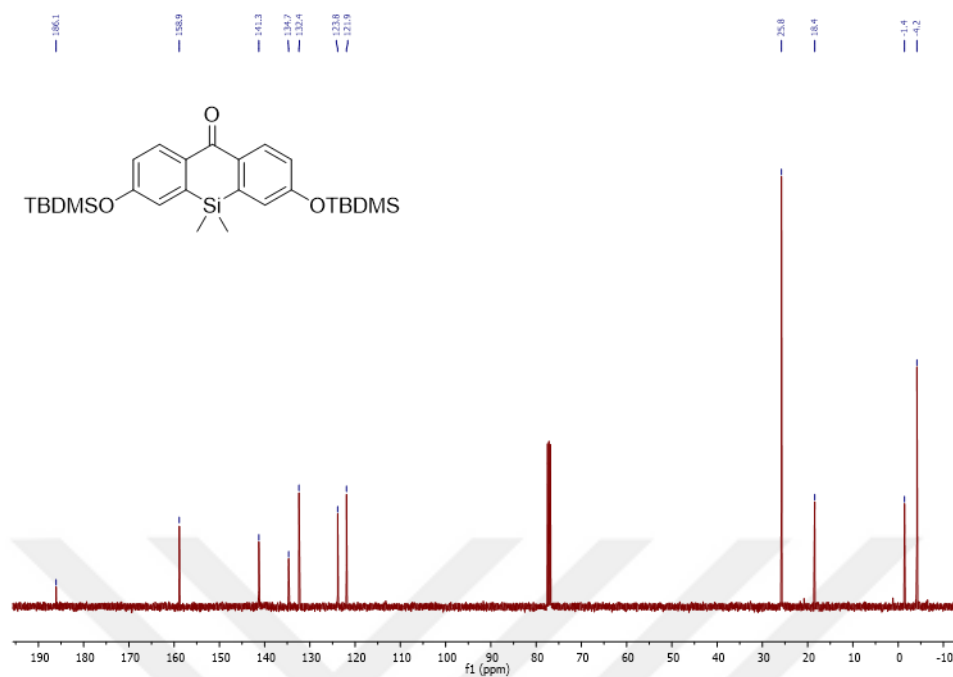
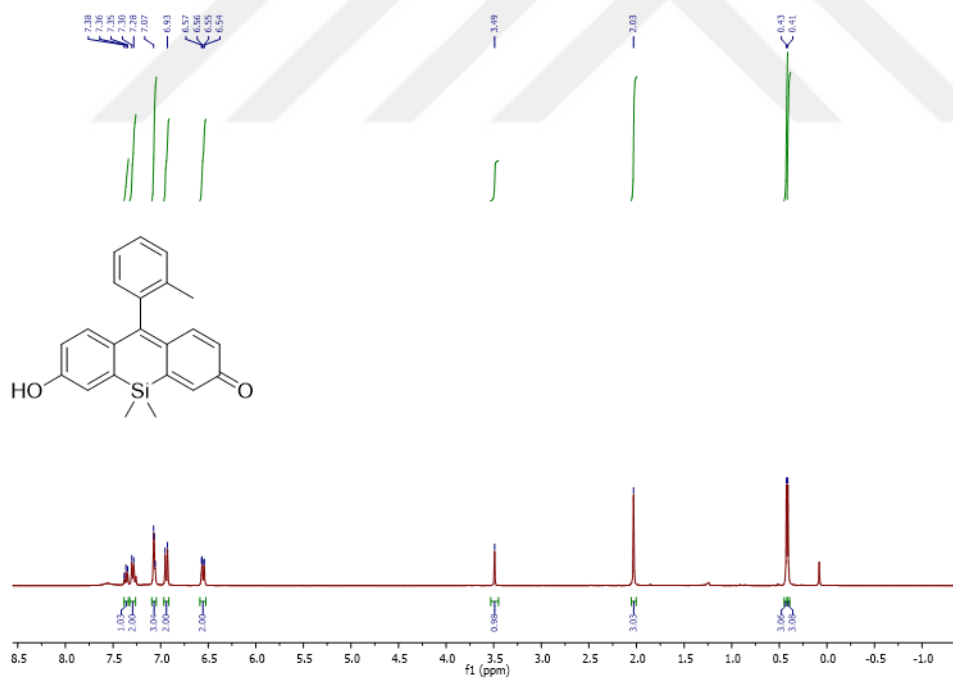


Figure A.35. ¹³C NMR spectrum of compound **23** in CDCl₃.



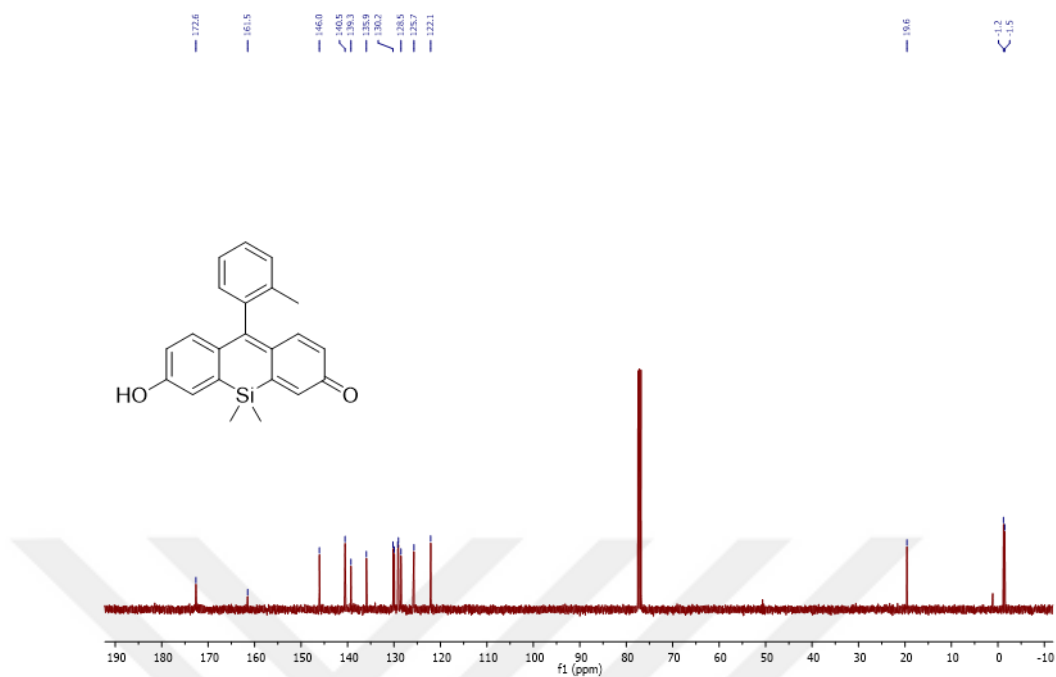


Figure A.37. ¹³C NMR spectrum of compound **24** in CDCl₃.

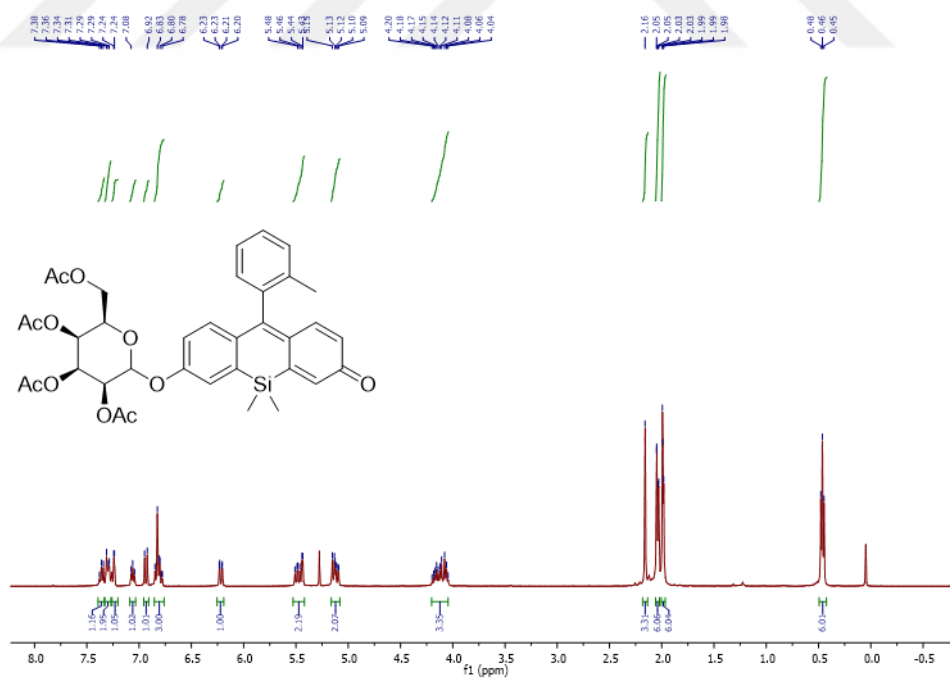


Figure A.38. ¹H NMR spectrum of compound **25** in CDCl₃.

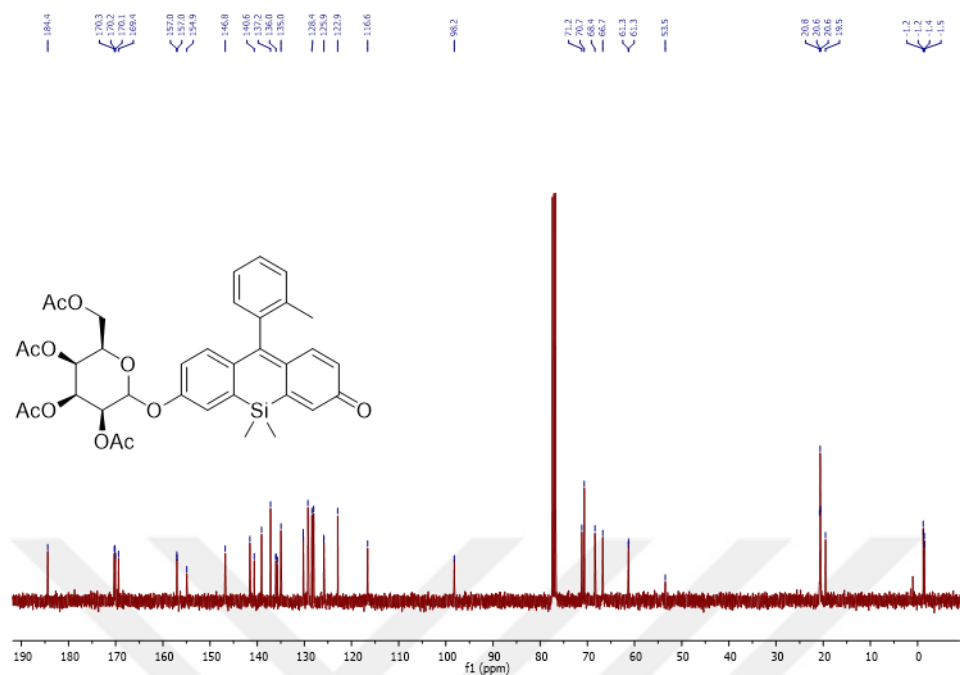


Figure A.39. ^{13}C NMR spectrum of compound **25** in CDCl_3 .

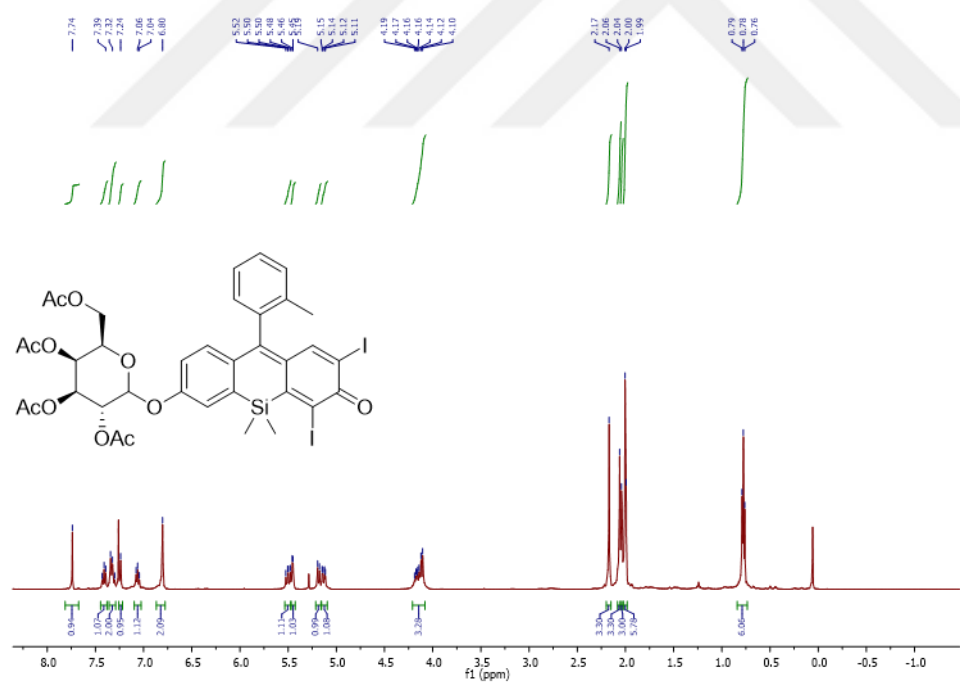


Figure A.40. ^1H NMR spectrum of compound **26** in CDCl_3 .

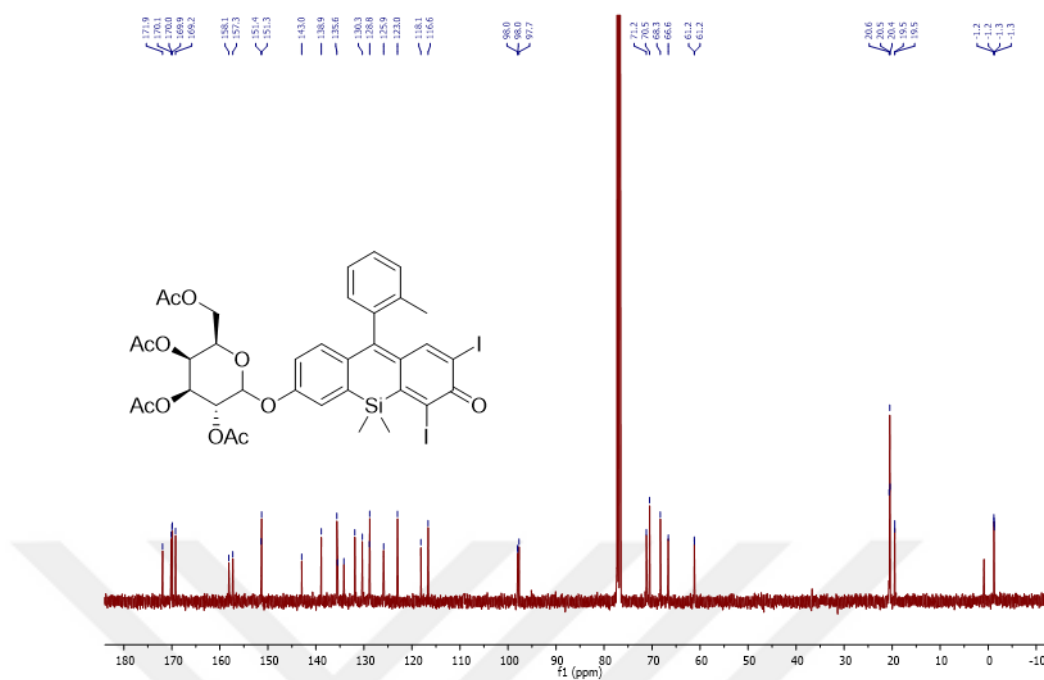


Figure A.41. ¹³C NMR spectrum of compound **26** in CDCl₃.

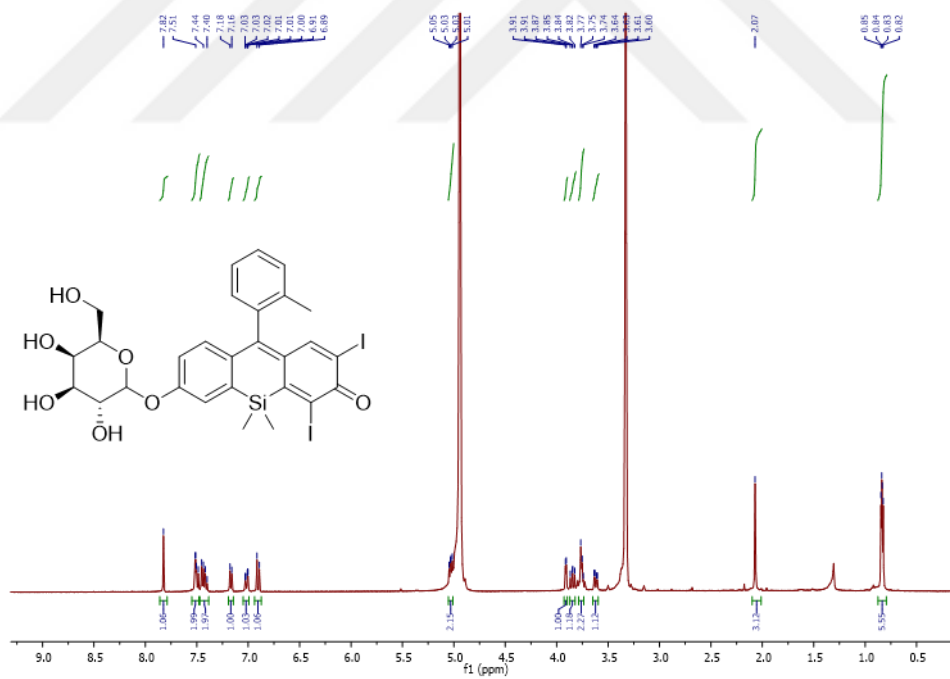


Figure A.42. ¹H NMR spectrum of β -Gal-SiF-II in MeOD.

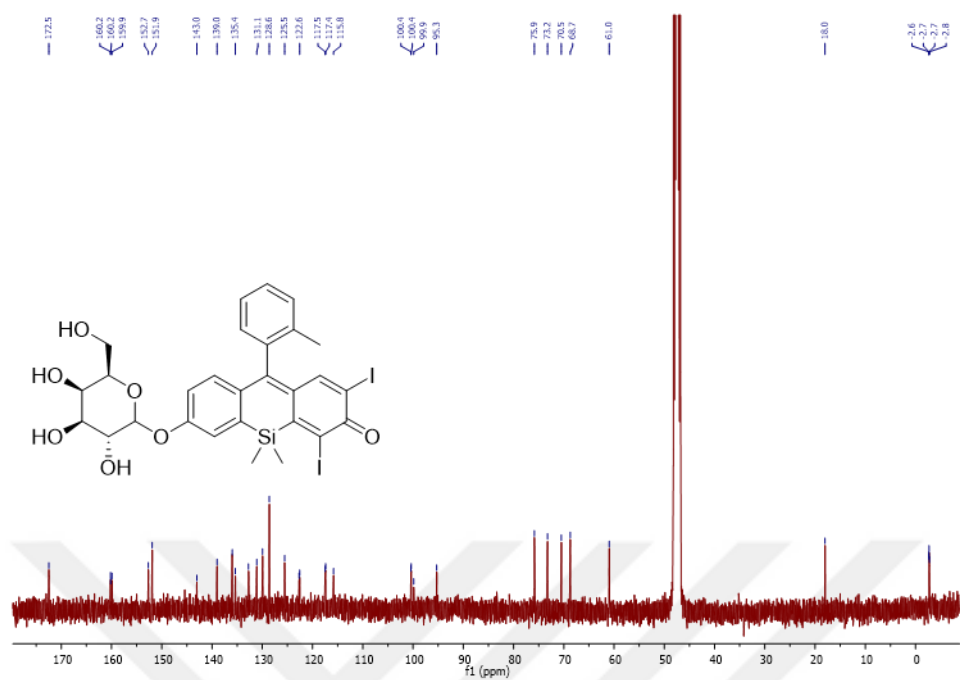


Figure A.43. ^{13}C NMR spectrum of β -Gal-SiF-II in MeOD.

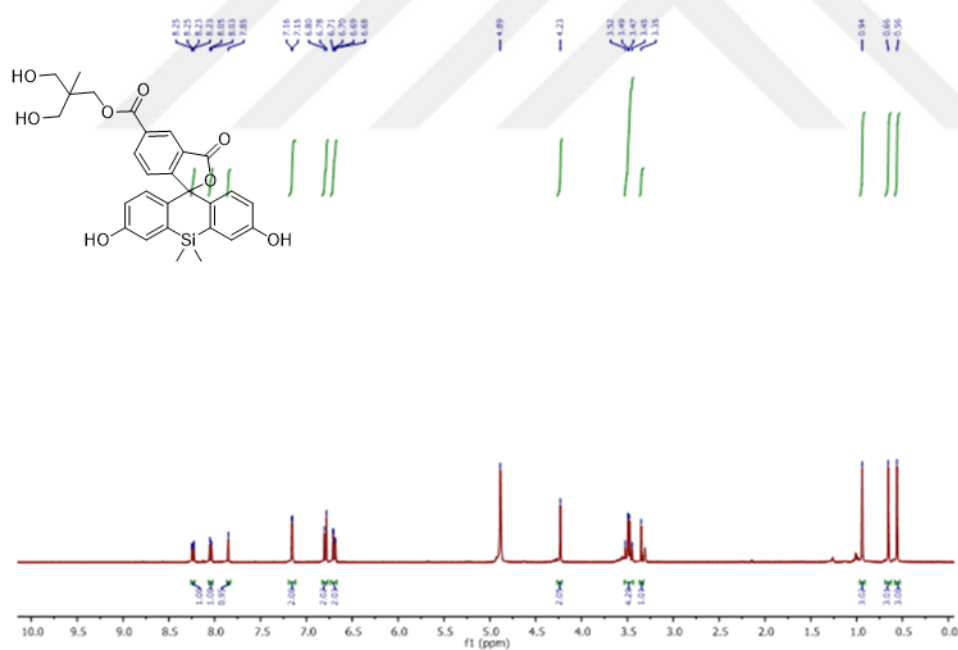


Figure A.44. ^1H NMR spectrum of compound **28'** in MeOD.

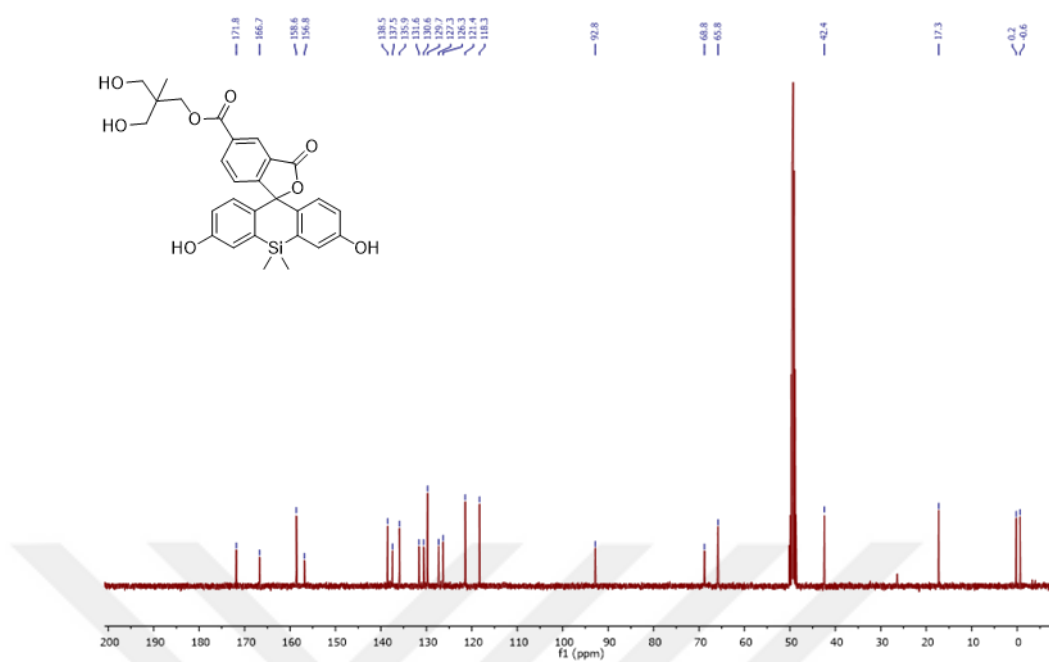


Figure A.45. ^{13}C NMR spectrum of compound **28'** in MeOD.

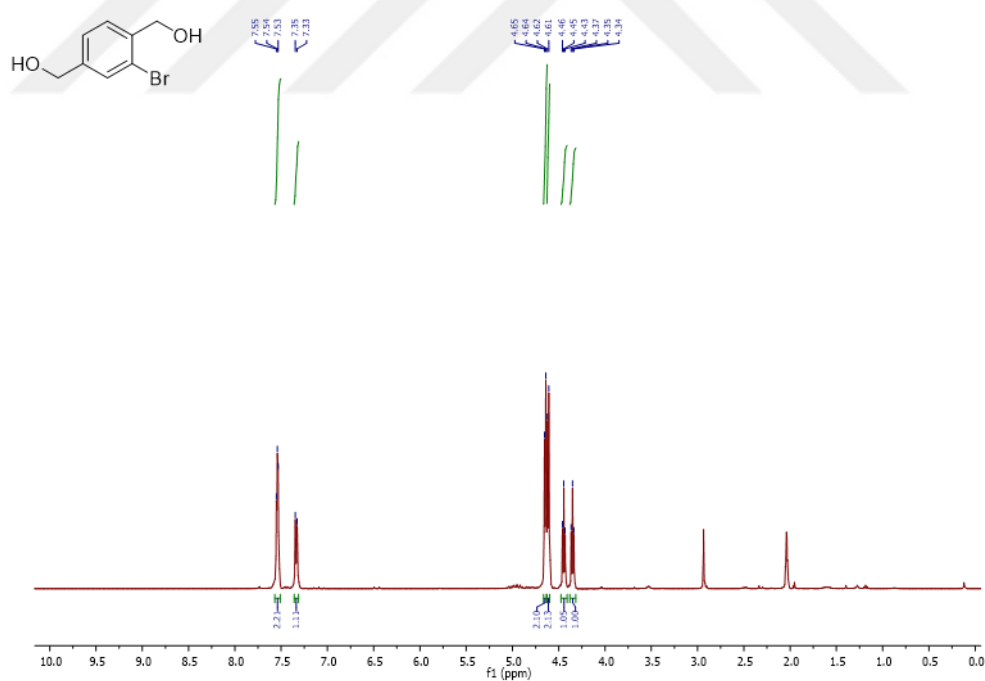


Figure A.46. ^1H NMR spectrum of compound **32** in Acetone- d_6 .

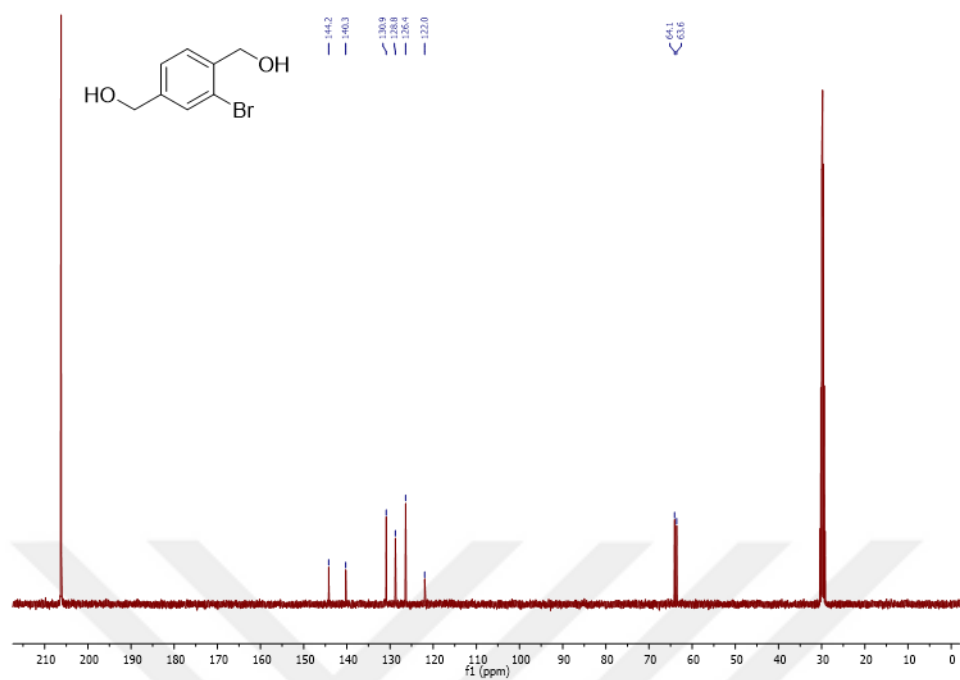


Figure A.47. ¹³C NMR spectrum of compound **32** in Acetone-d₆.

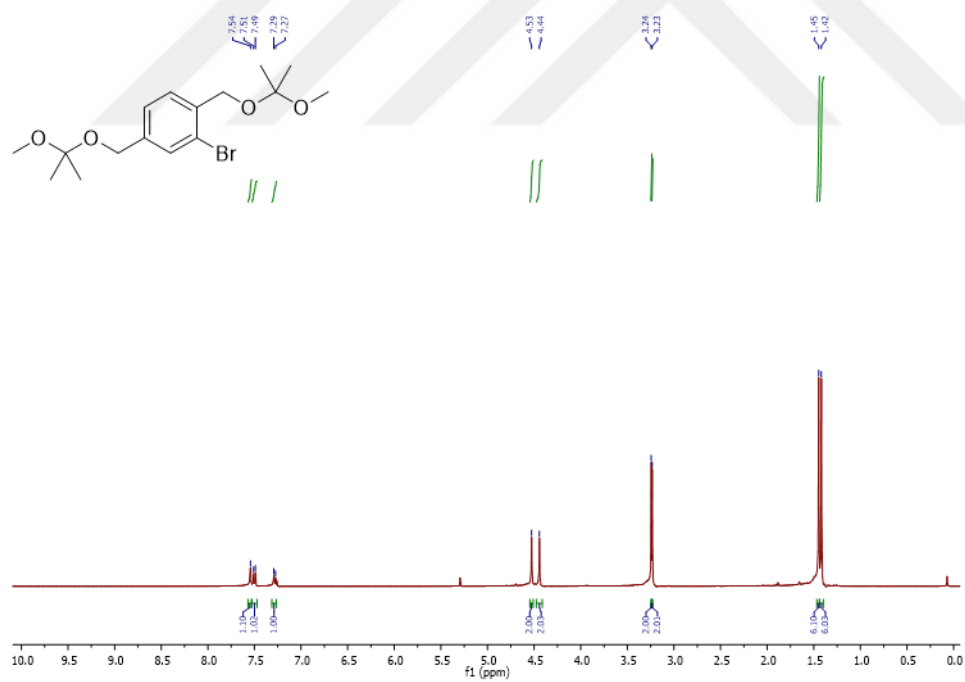


Figure A.48. ¹H NMR spectrum of compound **33** in CDCl₃.

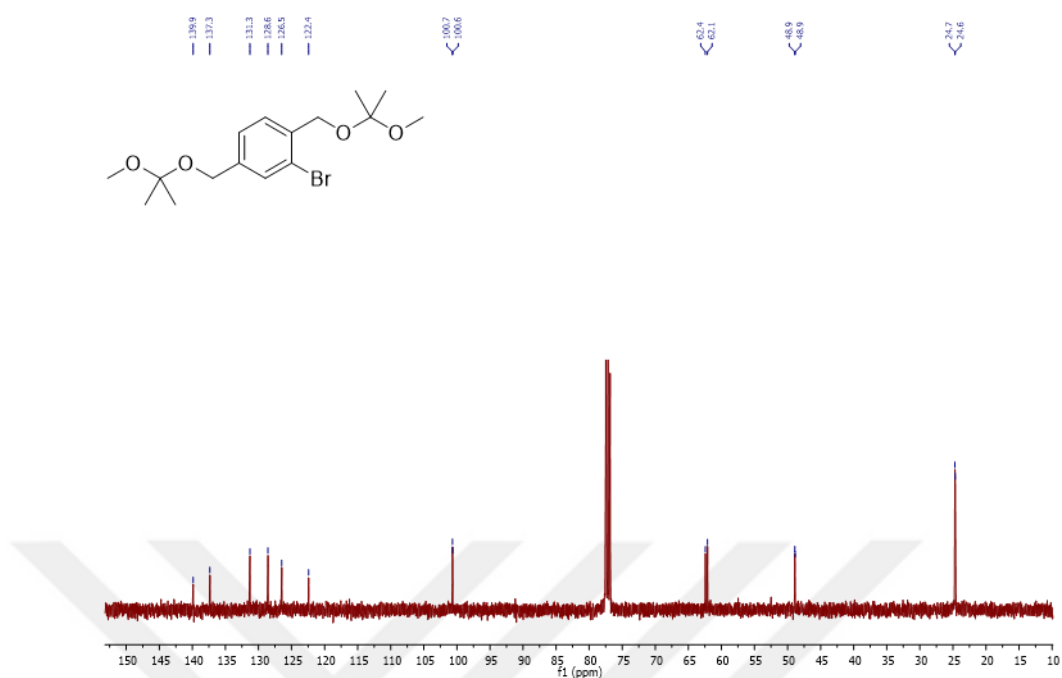


Figure A.49. ^{13}C NMR spectrum of compound **33** in CDCl₃.

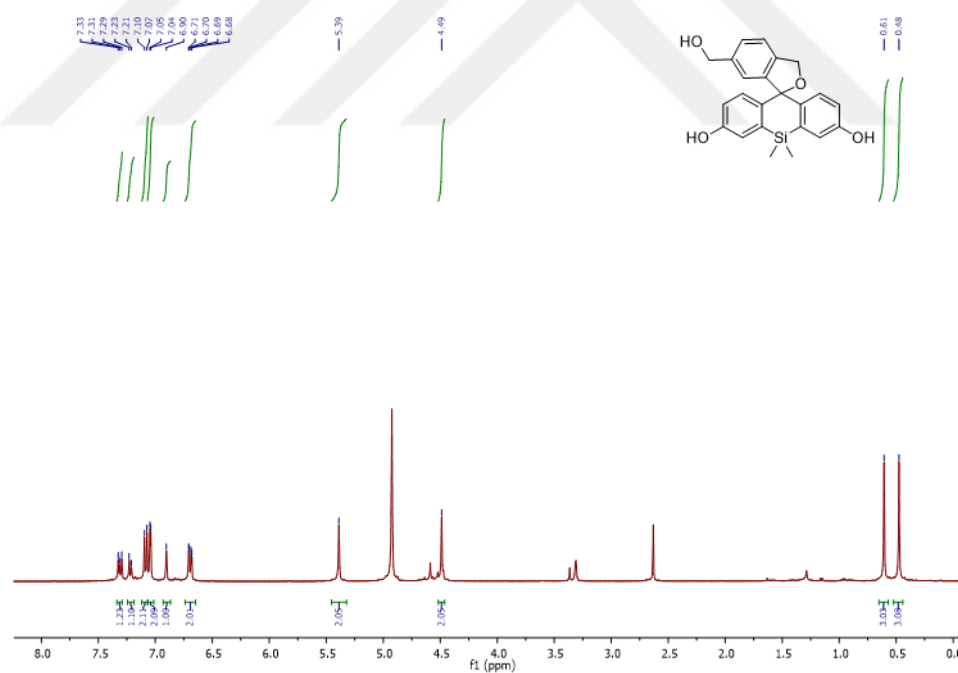


Figure A.50. ^1H NMR spectrum of compound **34** in MeOD.

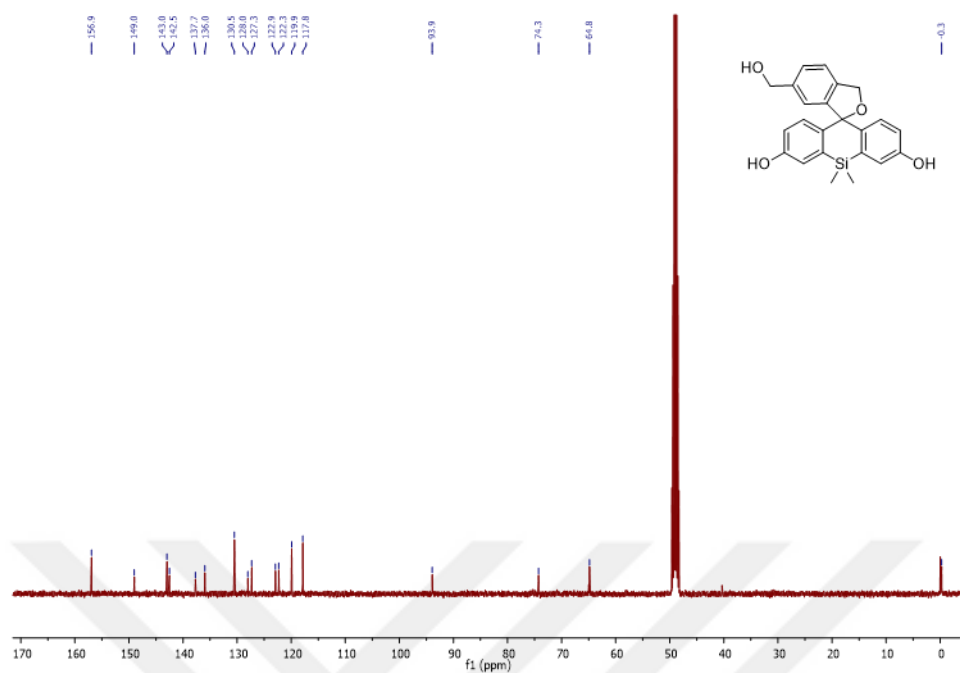


Figure A.51. ¹³C NMR spectrum of compound **34** in MeOD

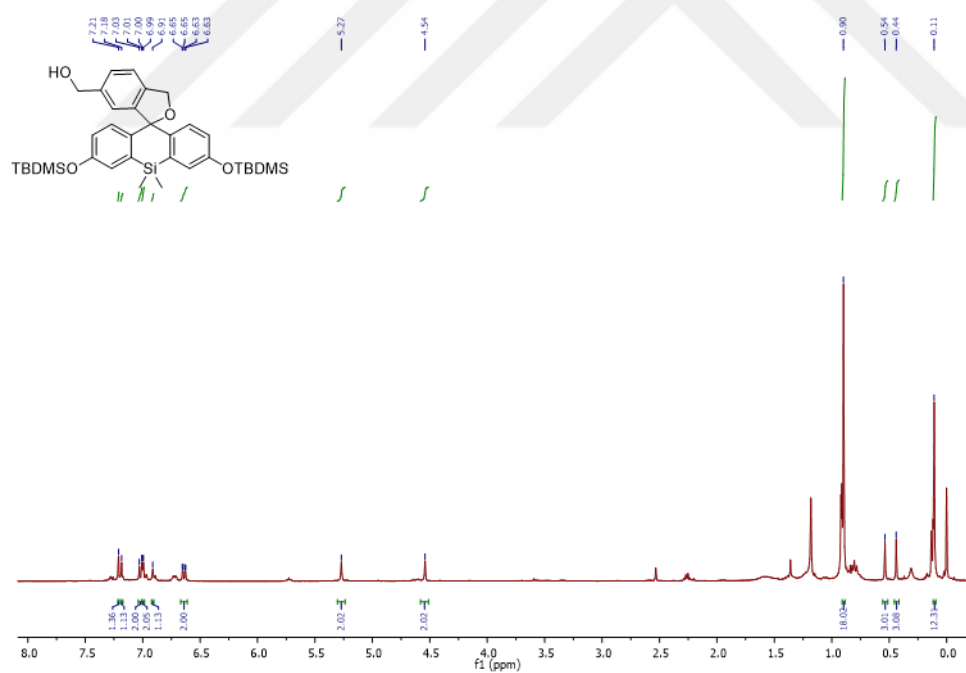


Figure A.52. ¹H NMR spectrum of compound **34** (TBDMS) in CDCl₃.

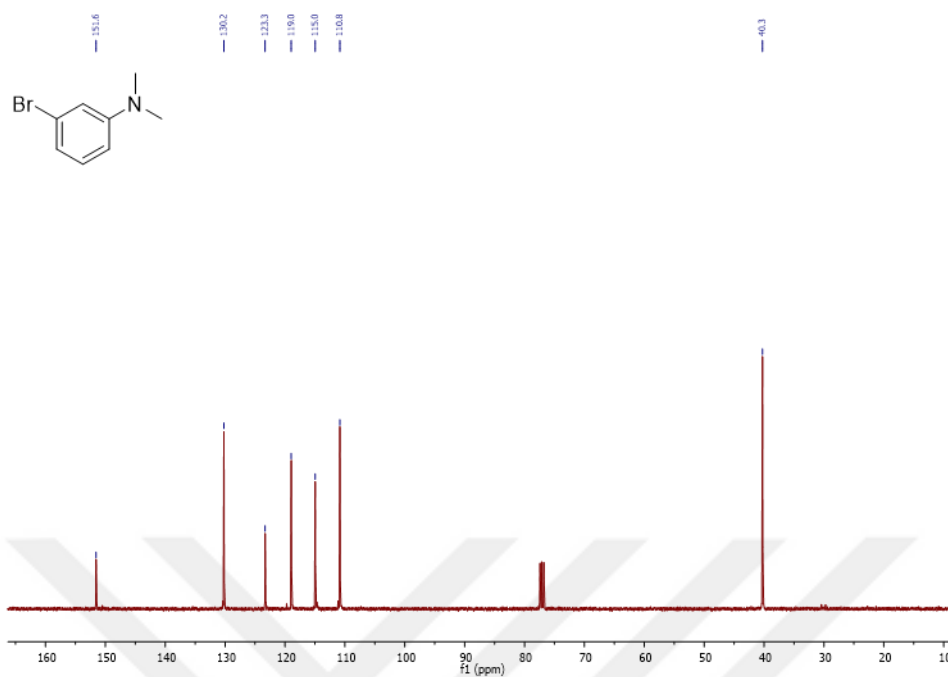


Figure A.55. ¹³C NMR spectrum of compound **39** in CDCl₃.

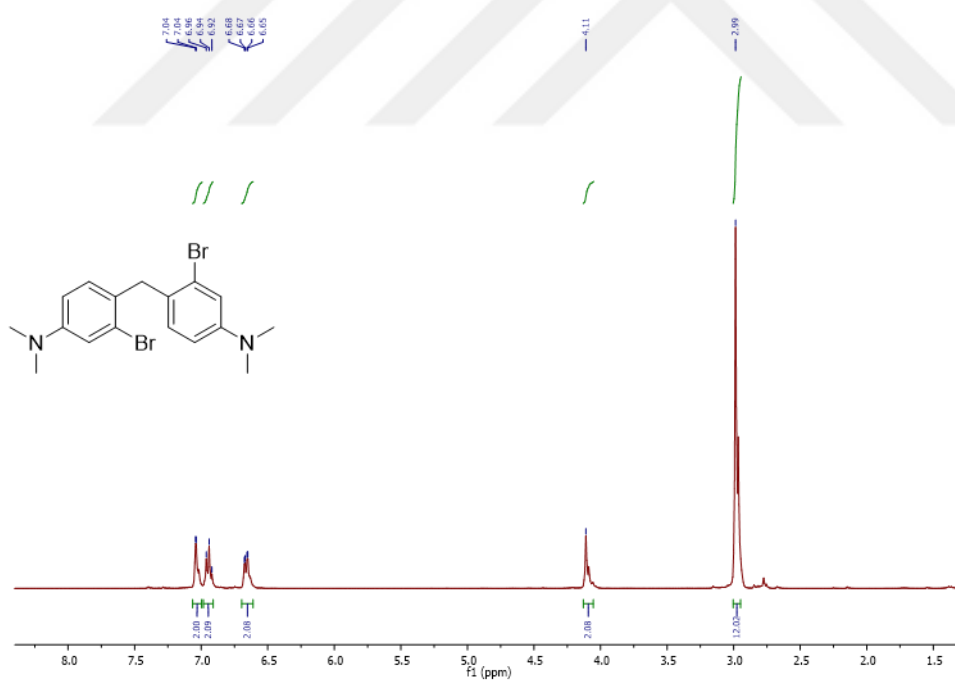


Figure A.56. ¹H NMR spectrum of compound **40** in CDCl₃.

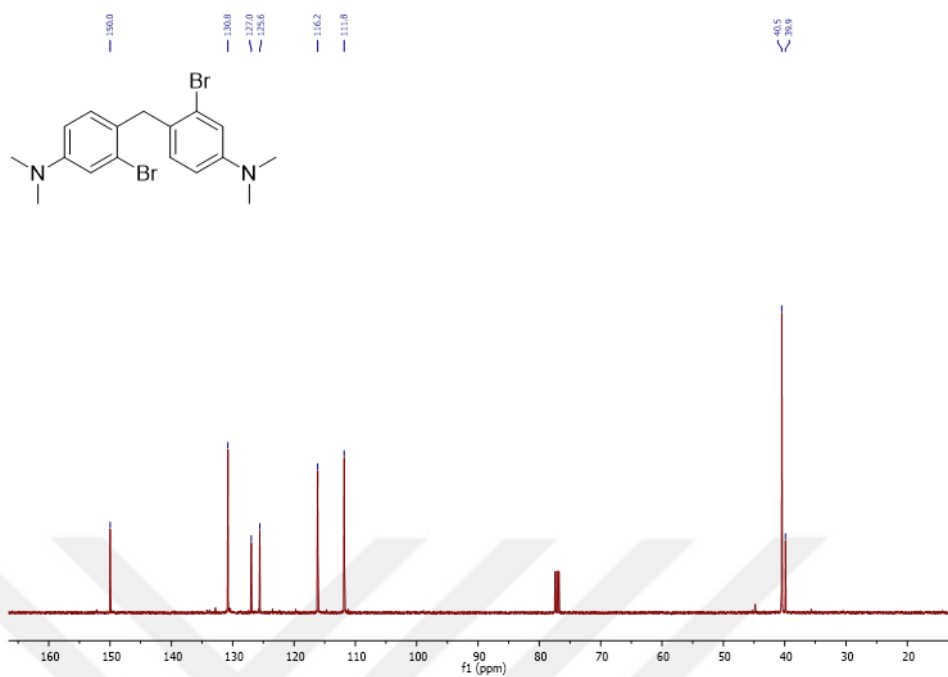


Figure A.57. ^{13}C NMR spectrum of compound **40** in CDCl₃.

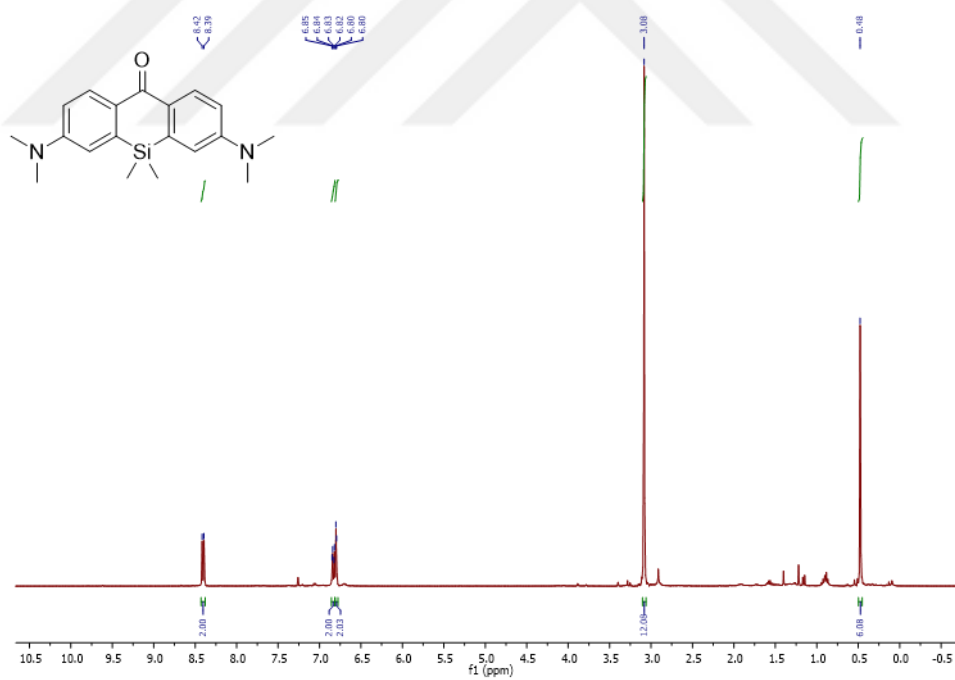


Figure A.58. ^1H NMR spectrum of compound **41** in CDCl₃.

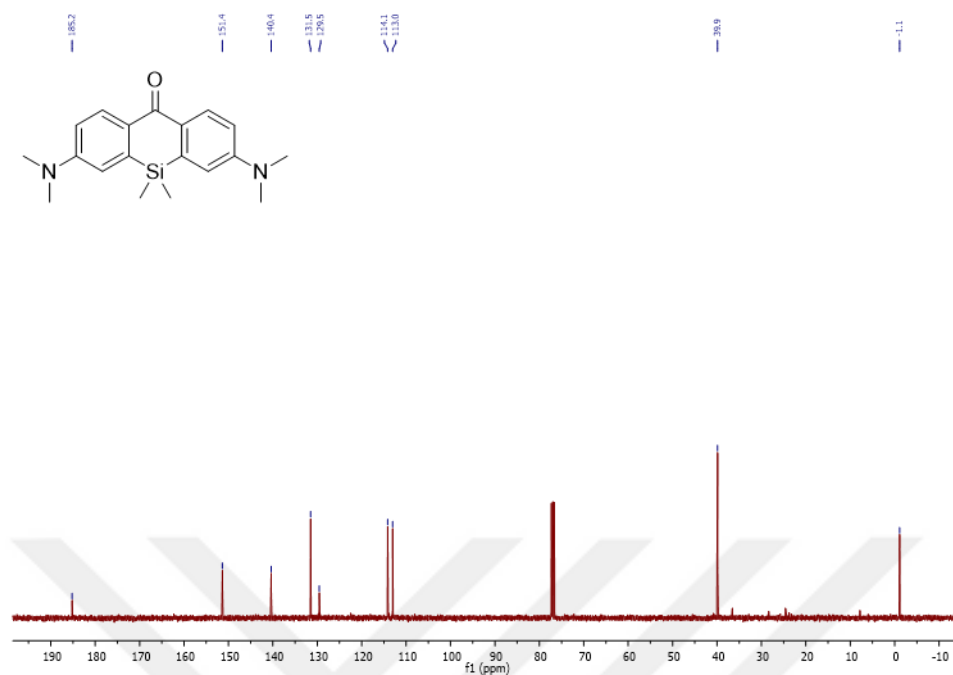


Figure A.59. ^{13}C NMR spectrum of compound **41** in CDCl_3 .

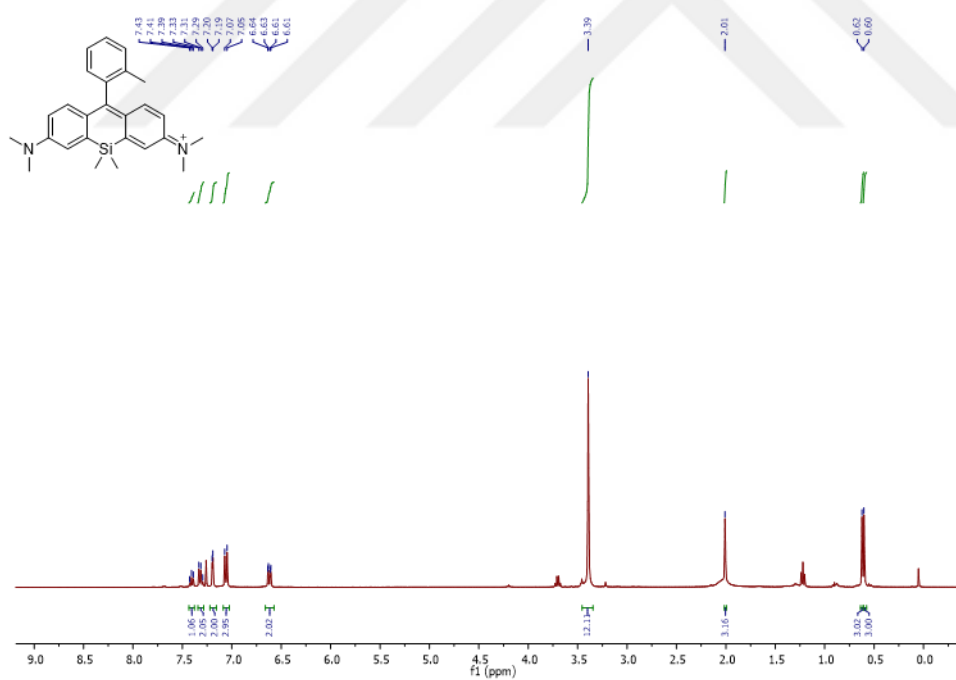


Figure A.60. ^1H NMR spectrum of compound **42** in CDCl_3 .

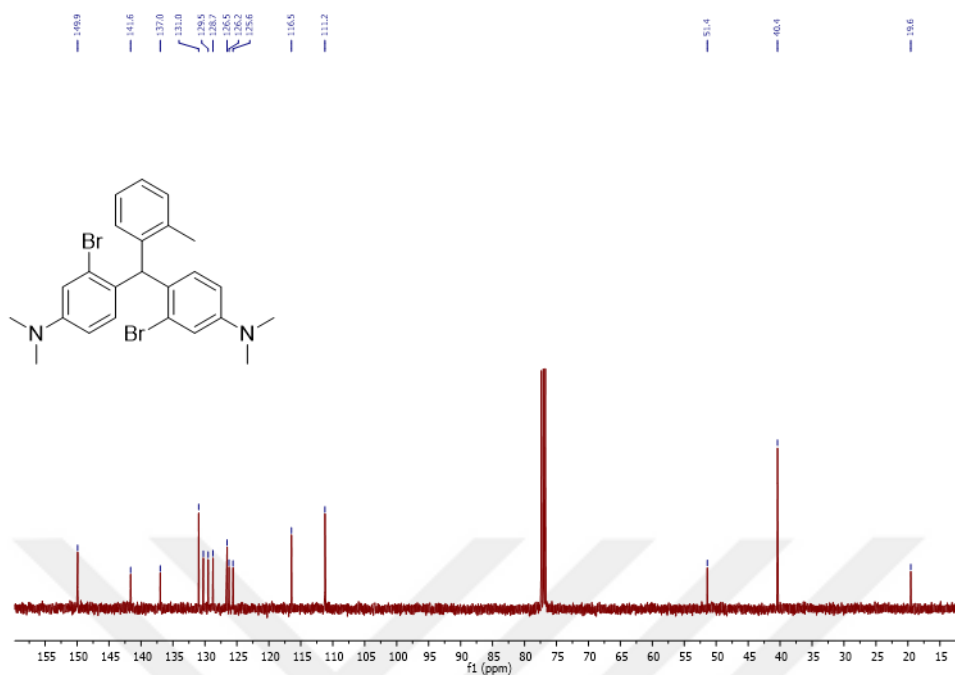


Figure A.63. ¹³C NMR spectrum of compound **45** in CDCl₃.

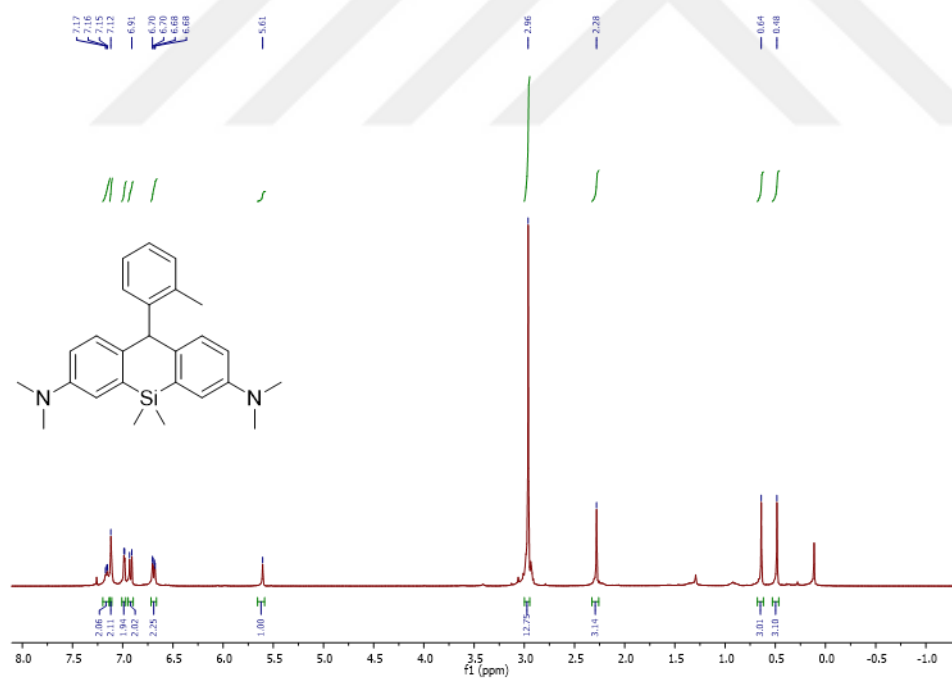


Figure A.64. ¹H NMR spectrum of compound **46** in CDCl₃.

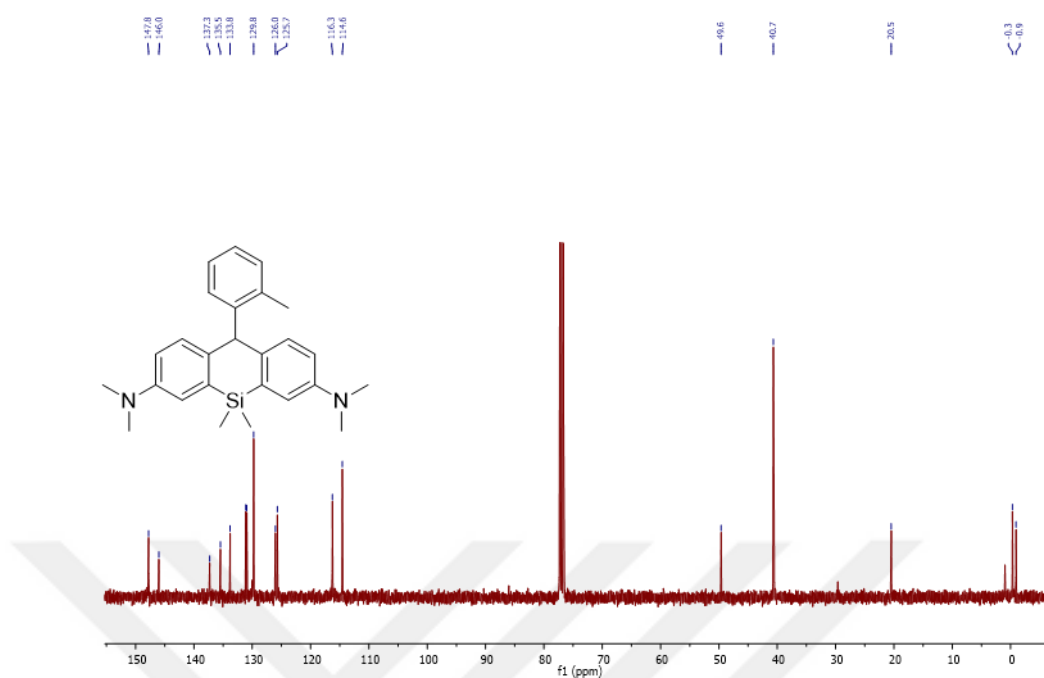


Figure A.65. ^{13}C NMR spectrum of compound **46** in CDCl_3 .

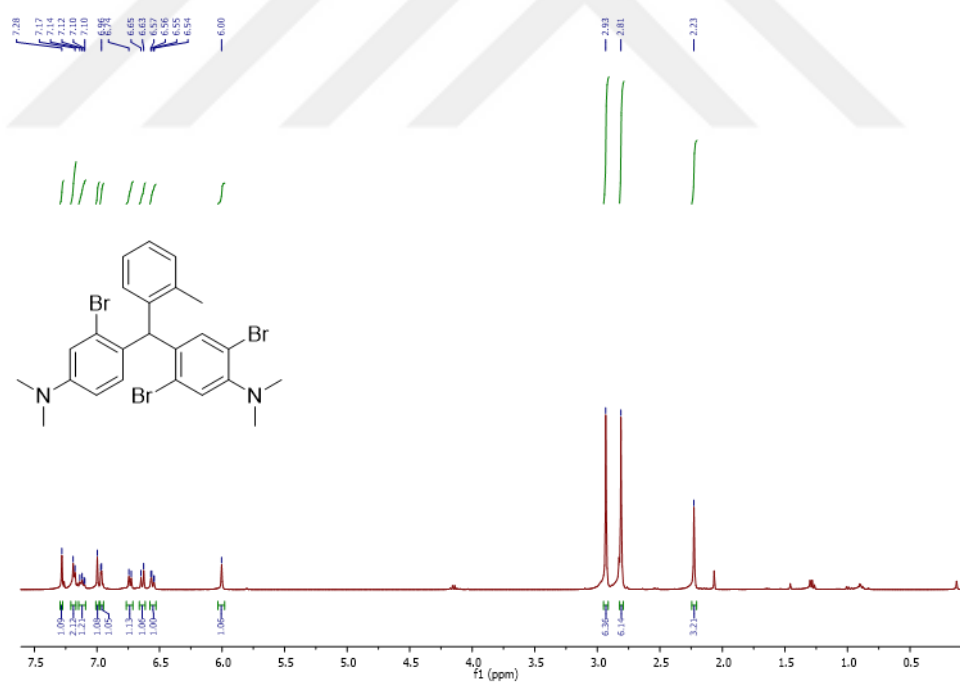


Figure A.66. ^1H NMR spectrum of compound **48** in CDCl_3 .

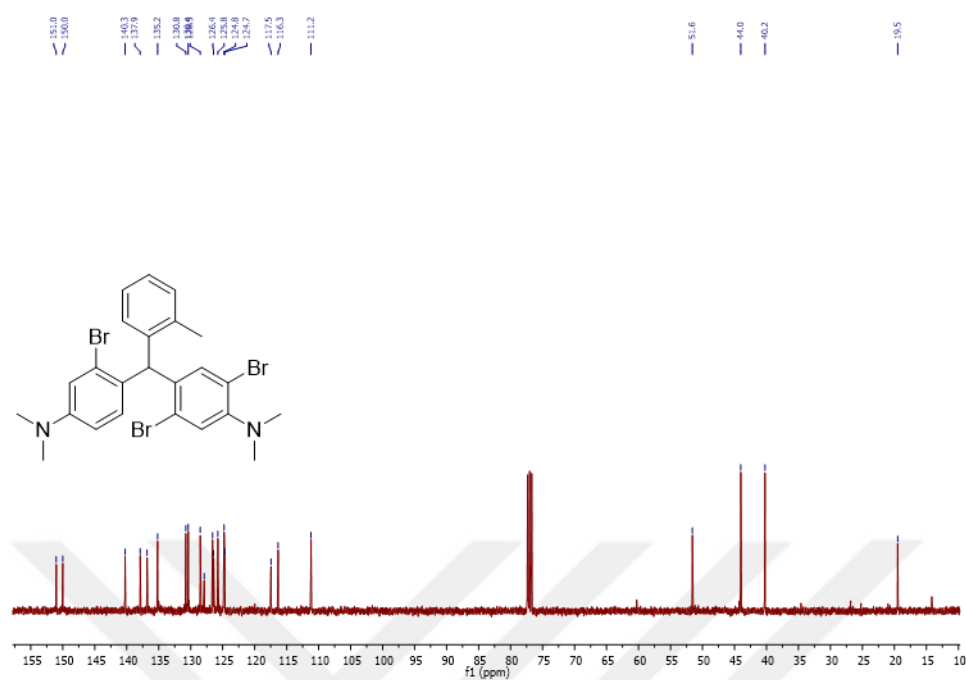


Figure A.67. ¹³C NMR spectrum of compound **48** in CDCl₃.

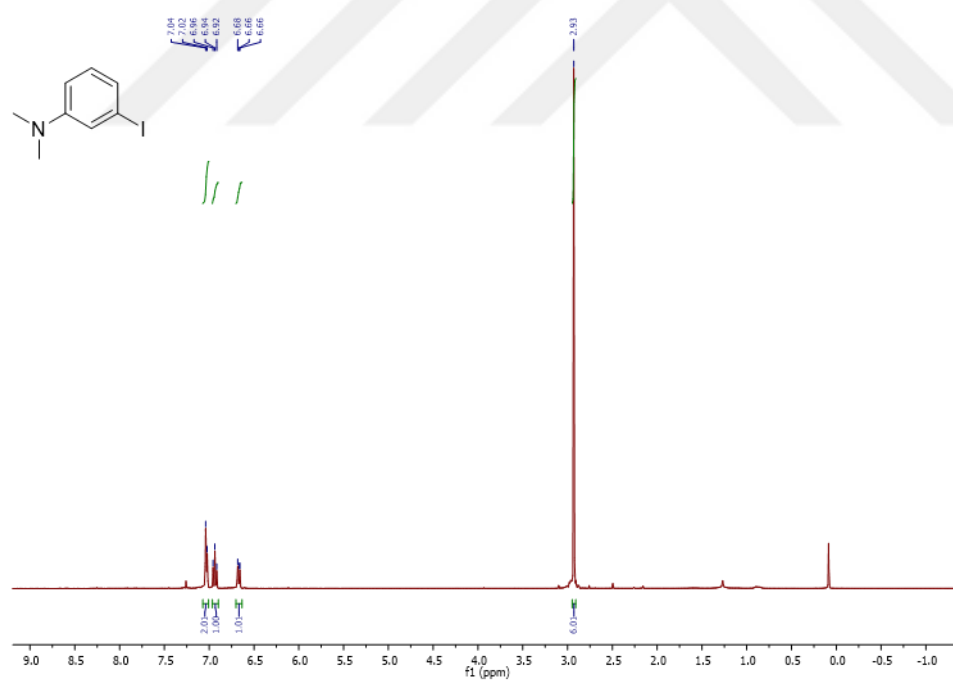


Figure A.68. ¹H NMR spectrum of compound **50** in CDCl₃.

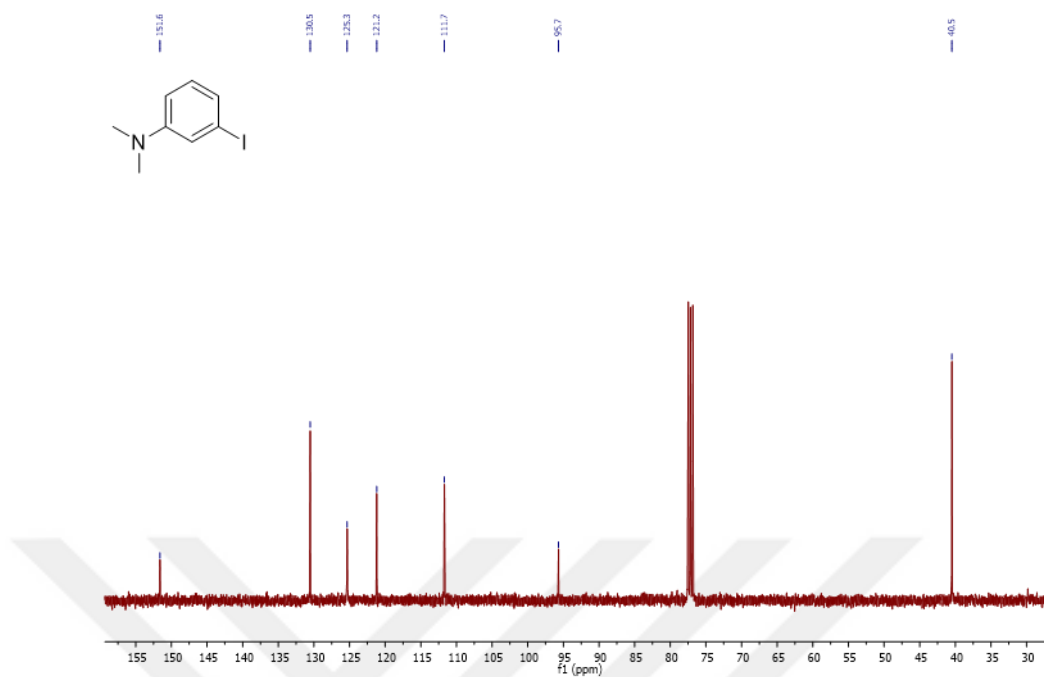


Figure A.69. ^{13}C NMR spectrum of compound **50** in CDCl_3

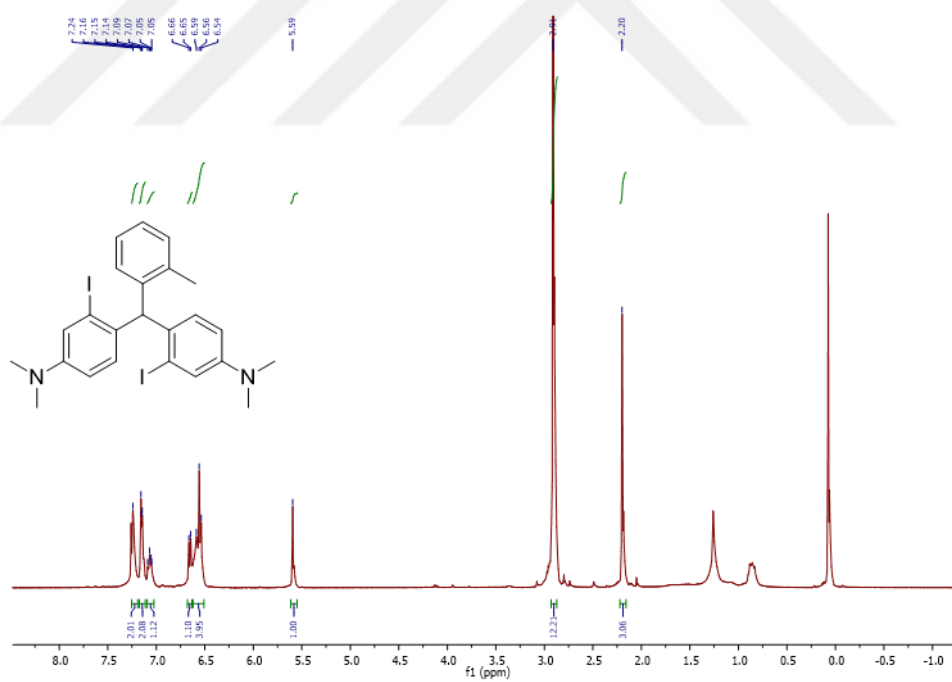


Figure A.70. ^1H NMR spectrum of compound **51** in CDCl_3 .

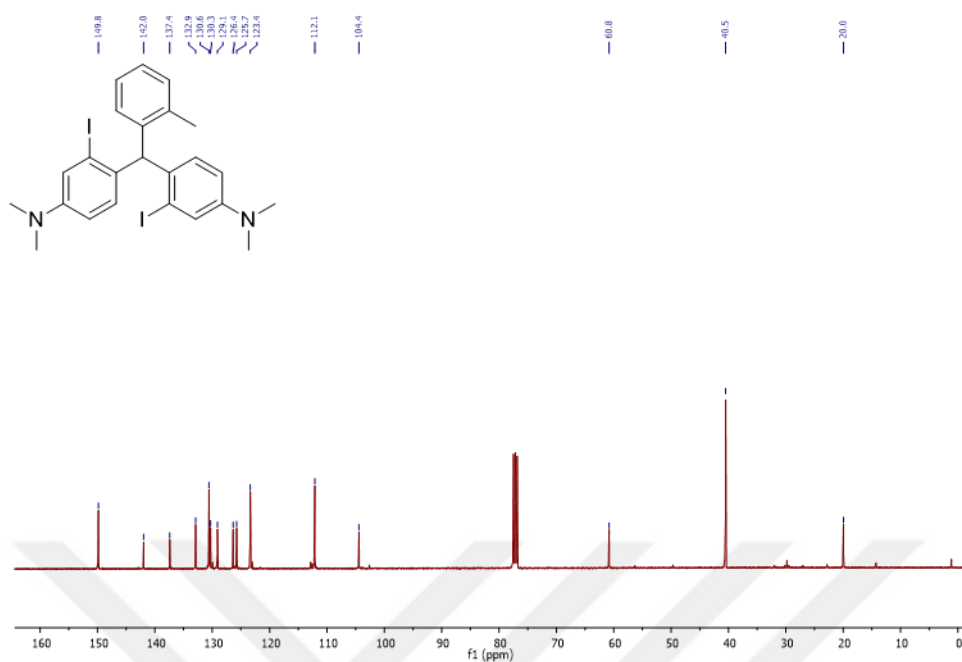


Figure A.71. ^{13}C NMR spectrum of compound **51** in CDCl_3

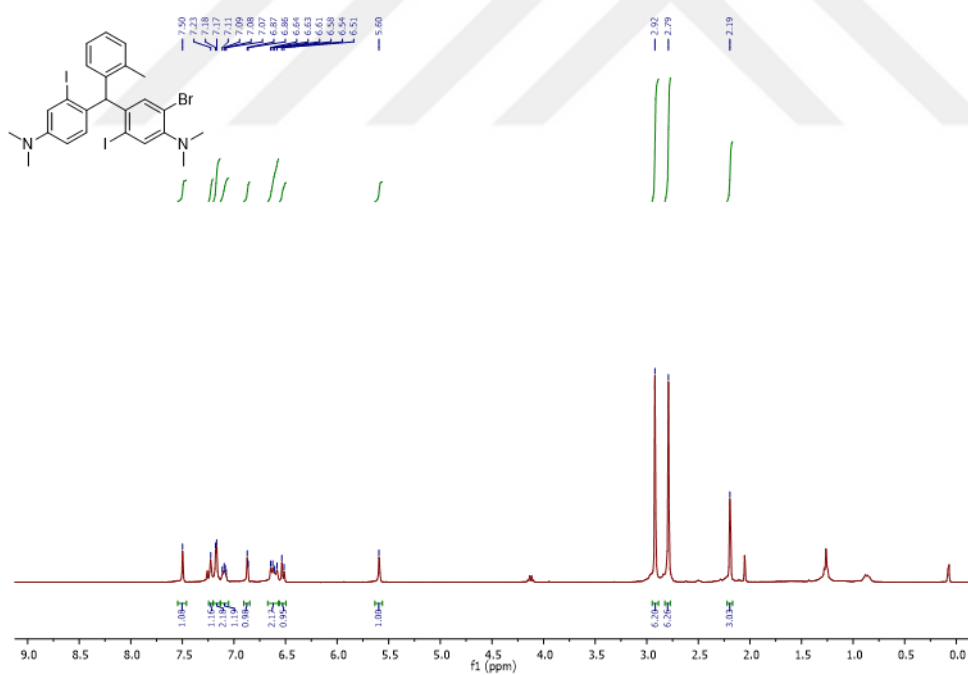


Figure A.72. ^1H NMR spectrum of compound **52** in CDCl_3 .

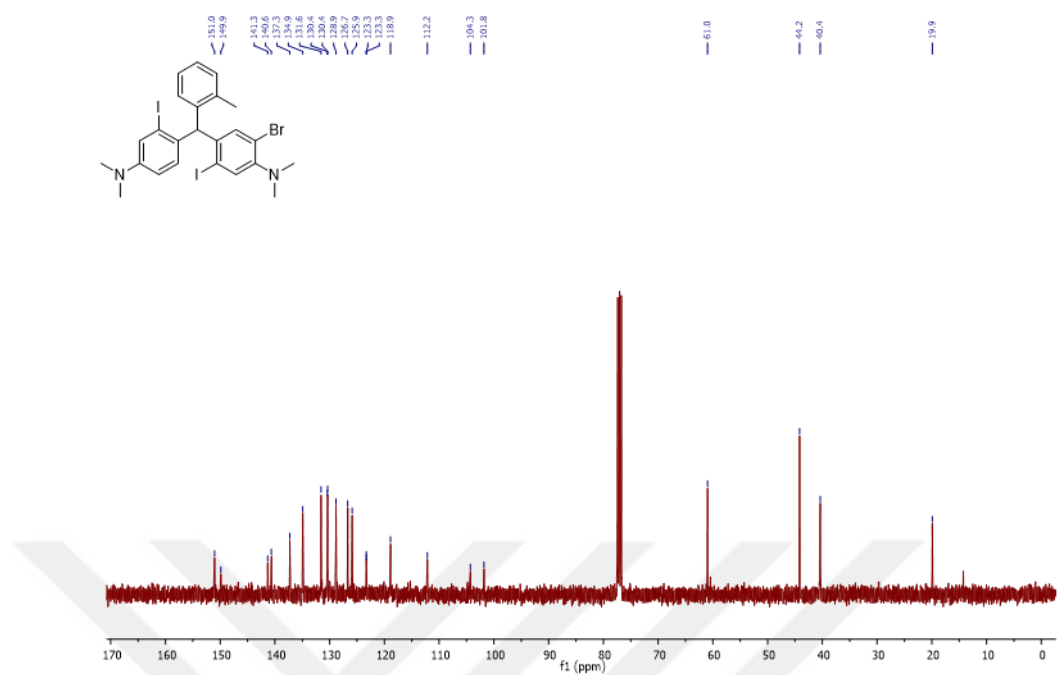


Figure A.73. ¹³C NMR spectrum of compound **53** in CDCl₃

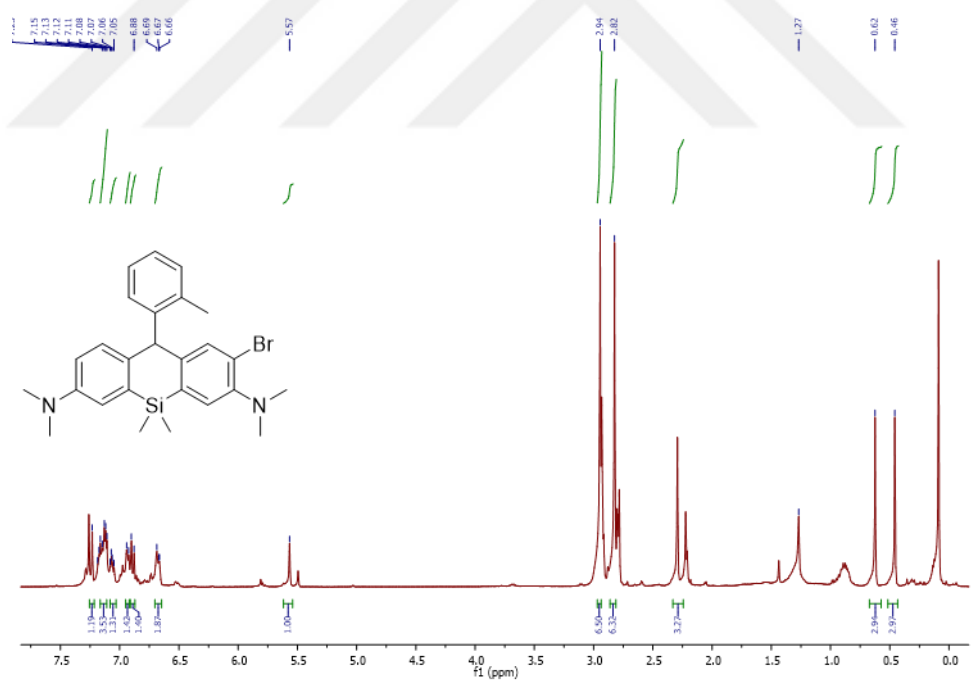


Figure A.74. ¹H NMR spectrum of compound **53** in CDCl₃.

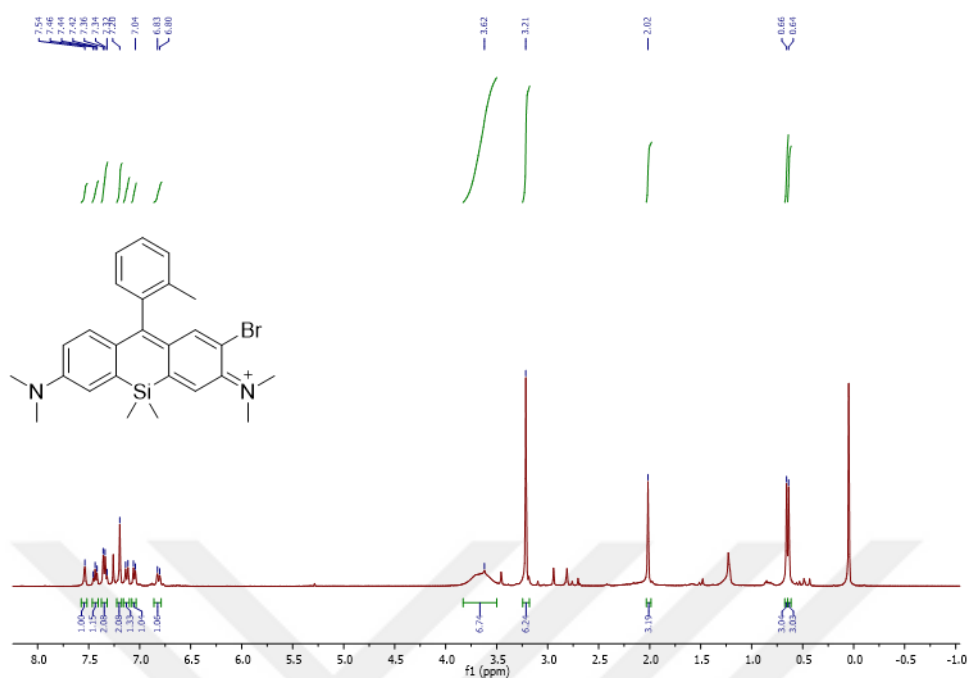


Figure A.75. ¹H NMR spectrum of compound **2-Me-SiR-Br** in CDCl₃.

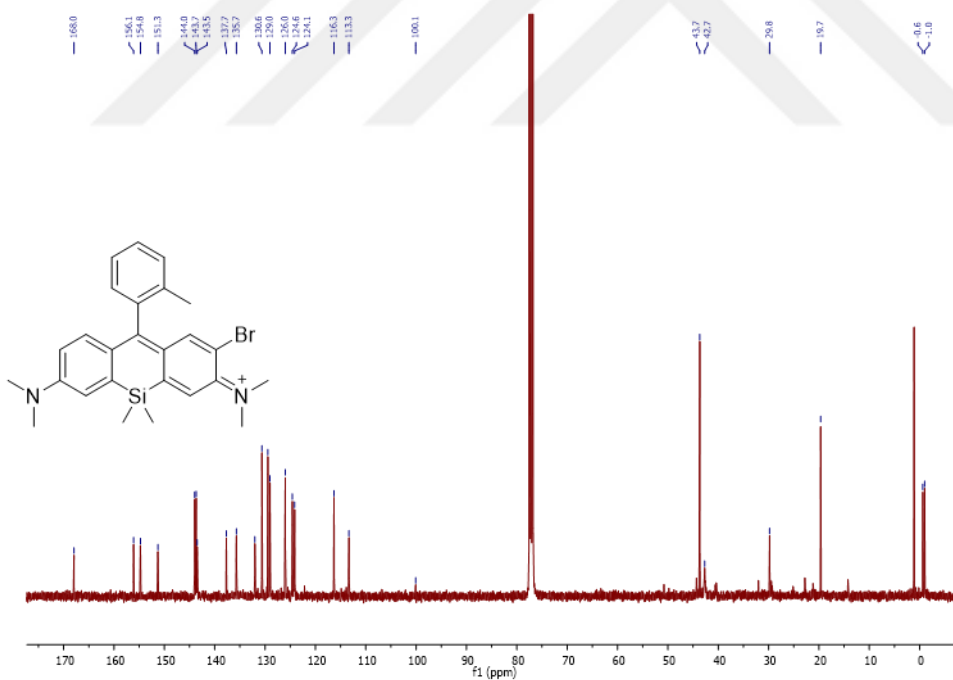


Figure A.76. ¹³C NMR spectrum of compound **2-Me-SiR-Br** in CDCl₃.

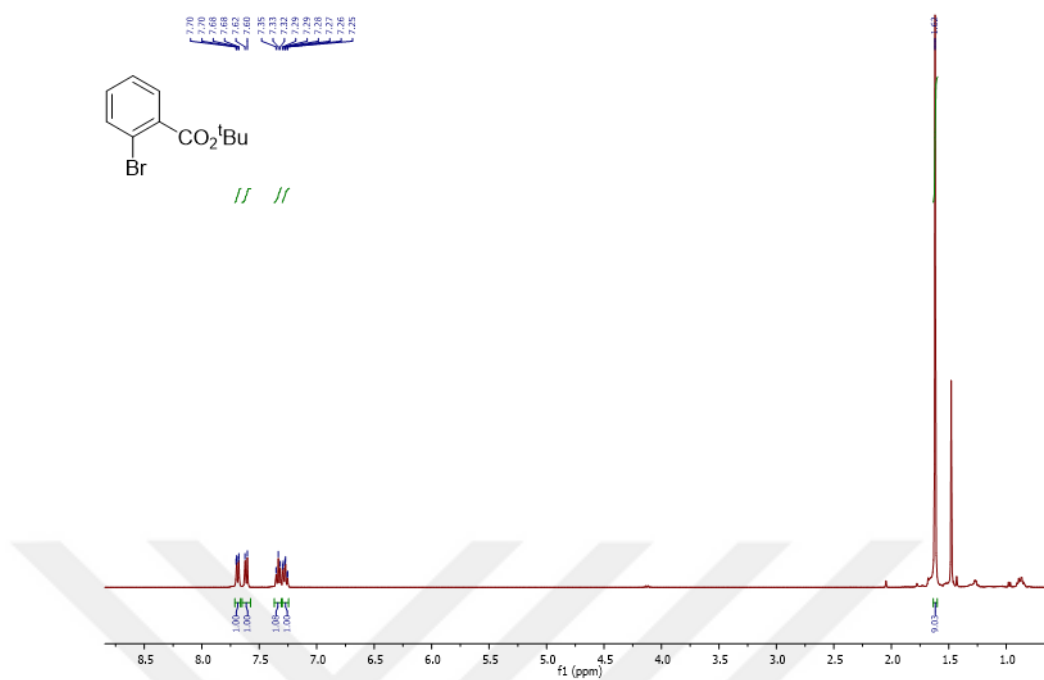


Figure A.77. ¹H NMR spectrum of compound **54** in CDCl₃.

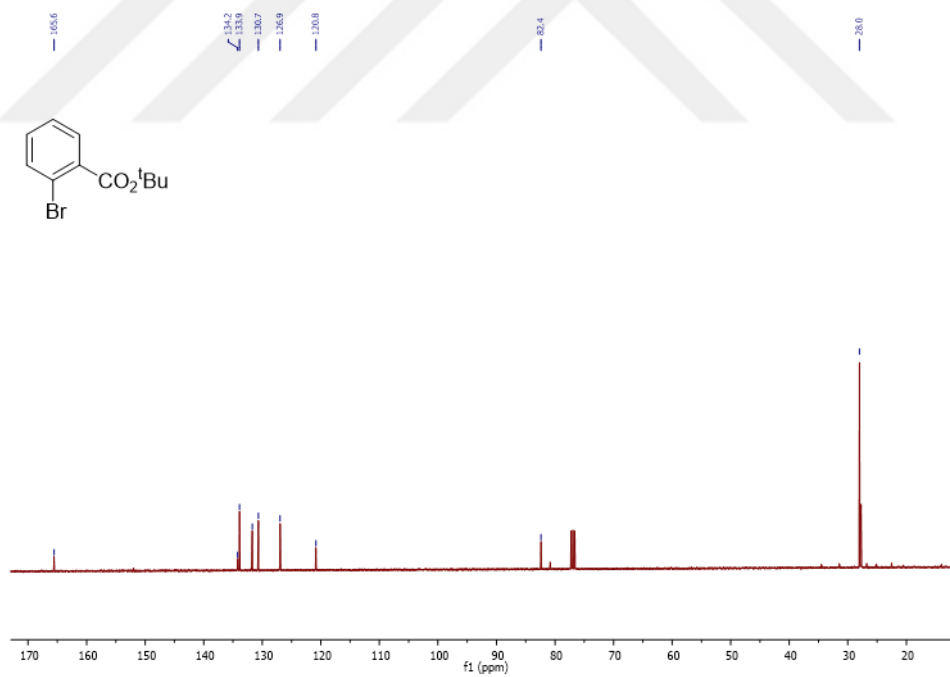


Figure A.78. ¹³C NMR spectrum of compound **54** in CDCl₃.

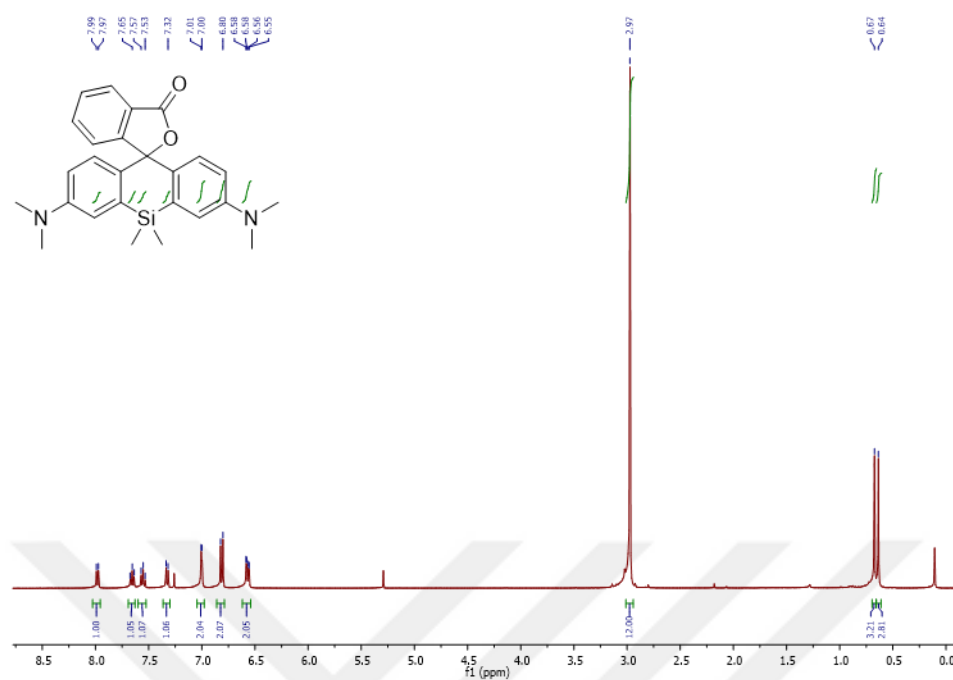


Figure A.79. ^1H NMR spectrum of compound **55** in CDCl_3 .

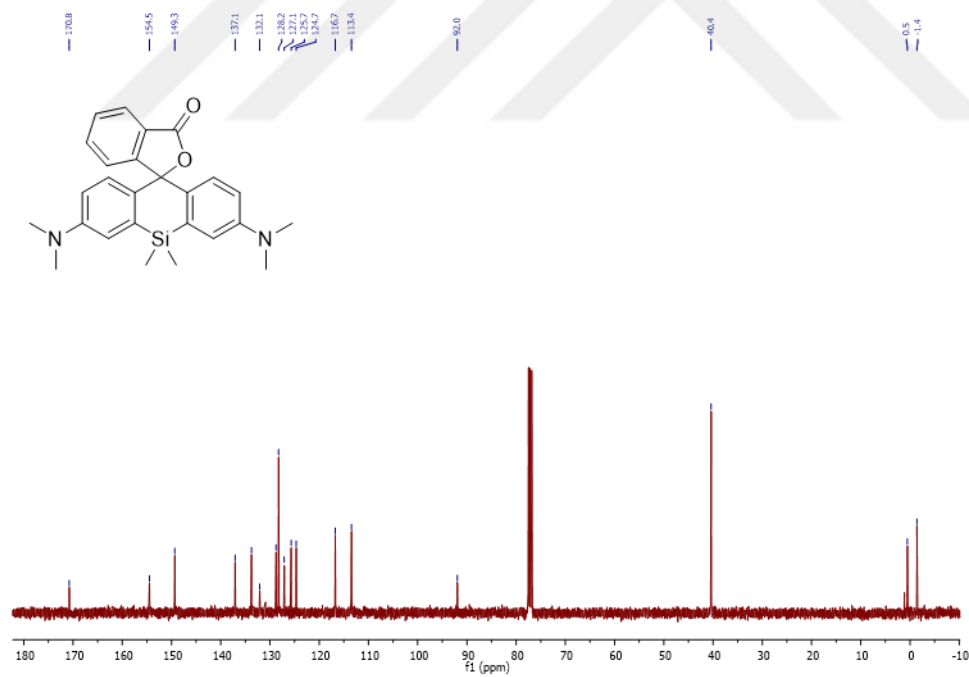


Figure A.80. ^{13}C NMR spectrum of compound **55** in CDCl_3 .

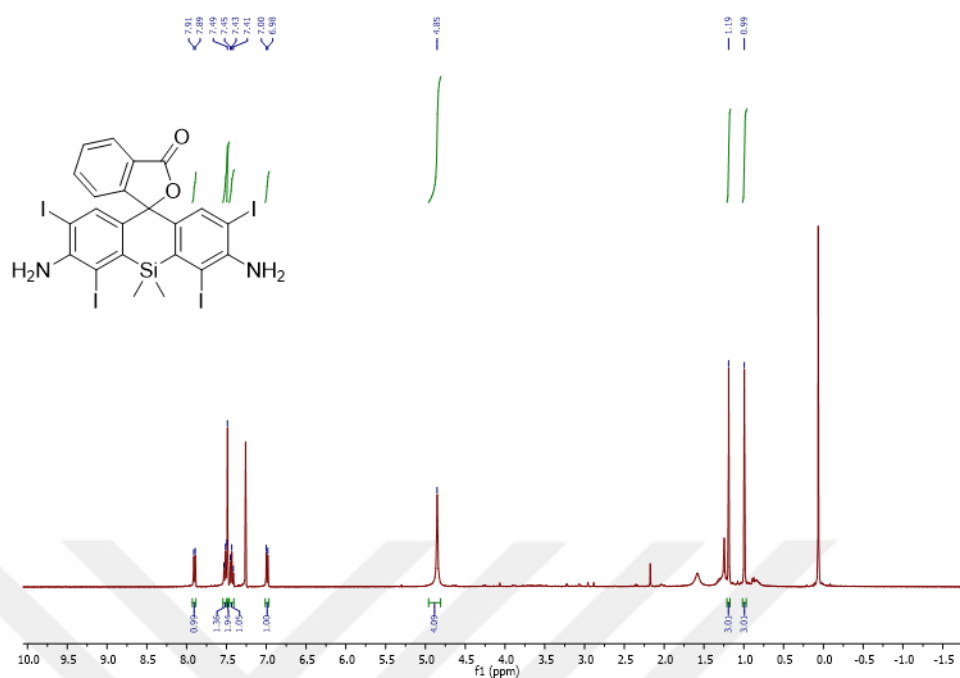


Figure A.81. ^1H NMR spectrum of compound **56** in CDCl_3 .

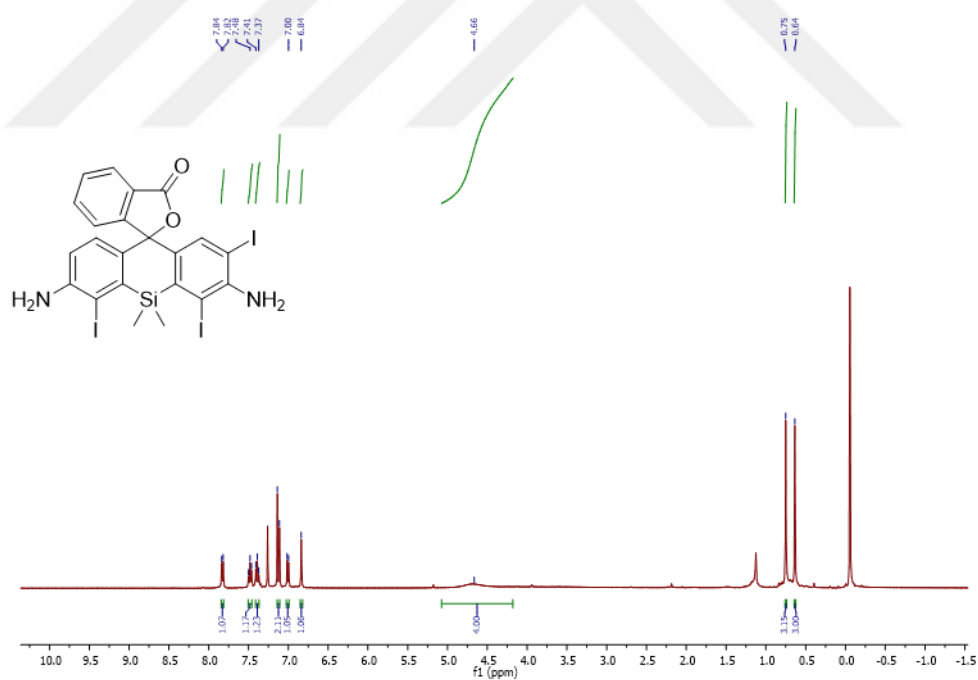


Figure A.82. ^1H NMR spectrum of compound **57** in CDCl_3 .

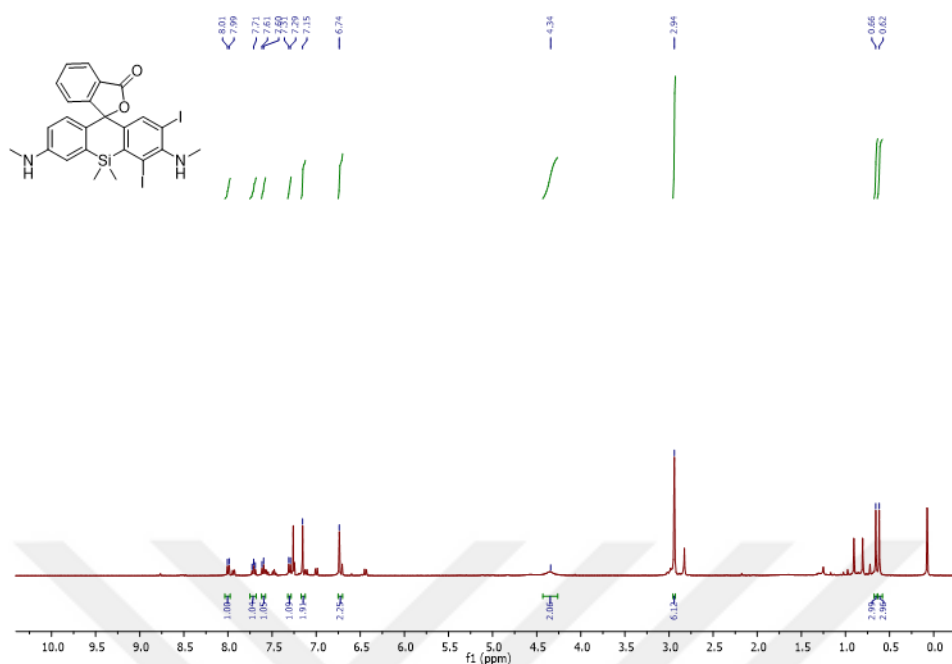


Figure A.83. ^1H NMR spectrum of compound **58** in CDCl_3 .

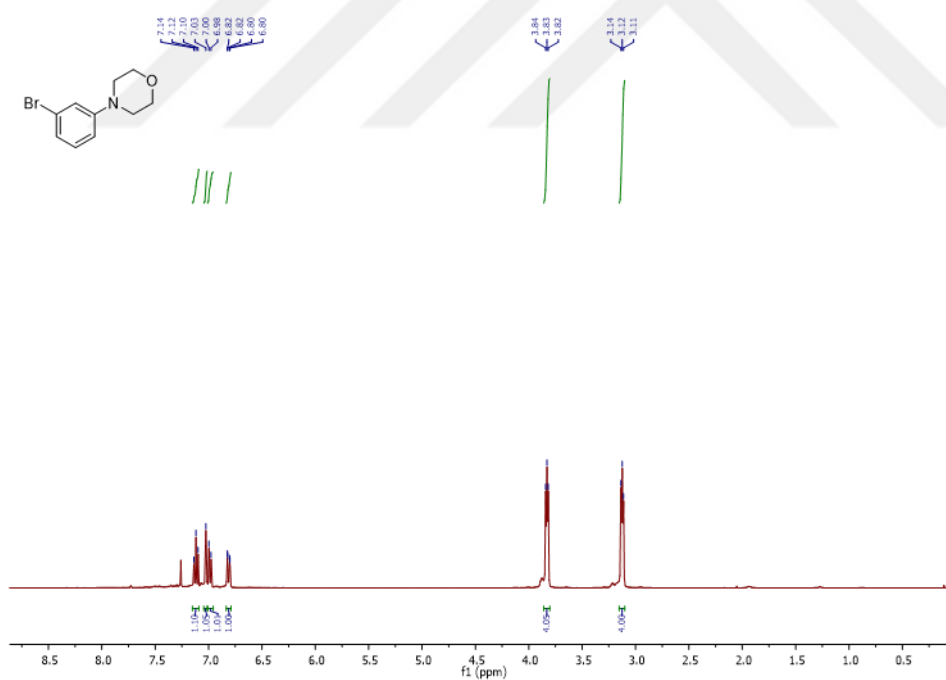


Figure A.84. ^1H NMR spectrum of compound **59** in CDCl_3 .

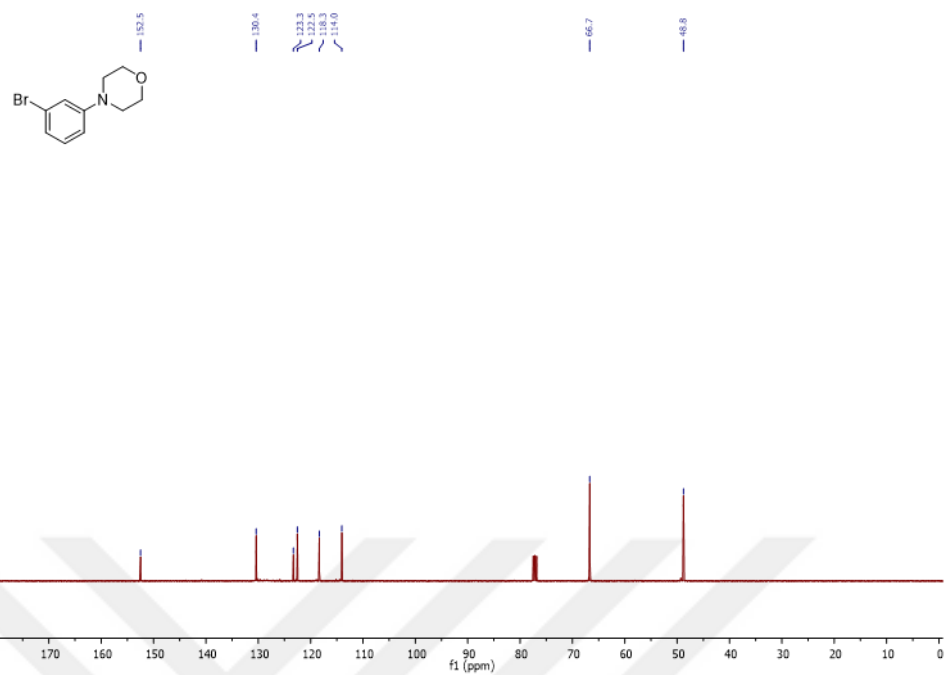


Figure A.85. ^{13}C NMR spectrum of compound **59** in CDCl_3 .

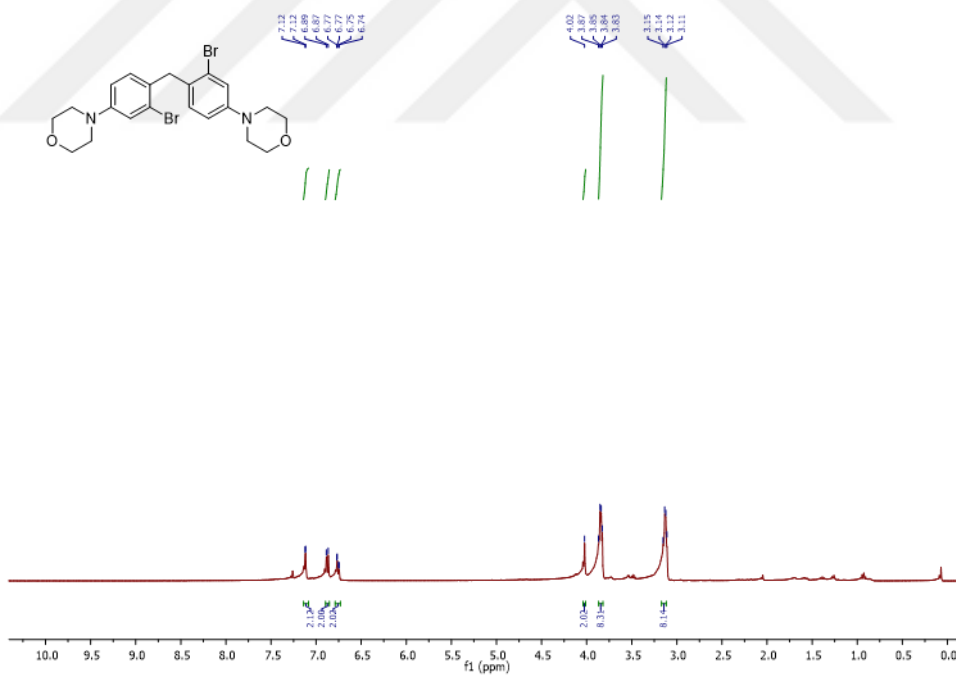


Figure A.86. ^1H NMR spectrum of compound **60** in CDCl_3 .

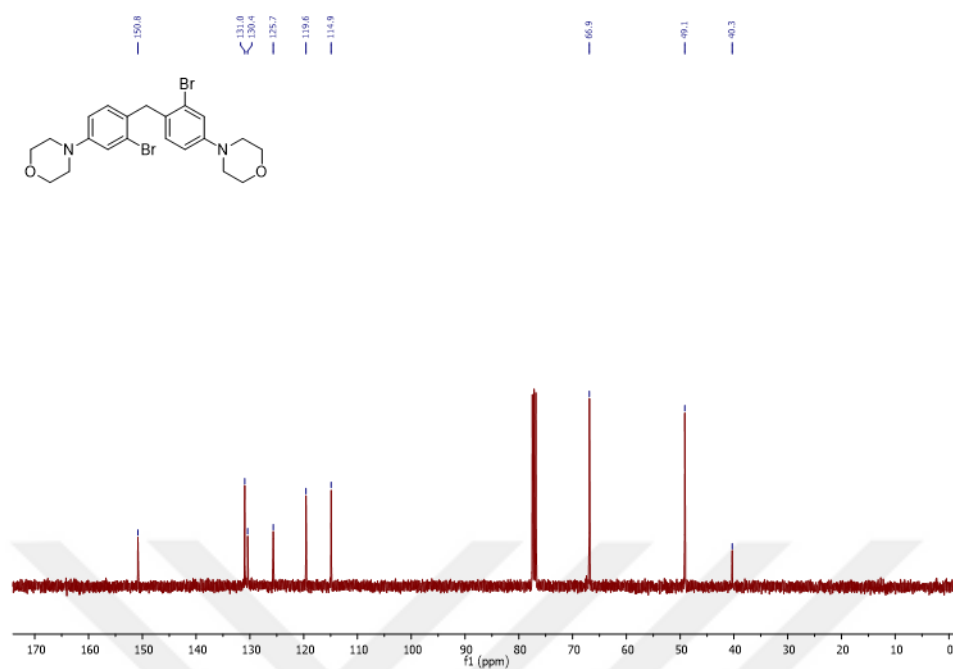


Figure A.87. ¹³C NMR spectrum of compound **60** in CDCl₃.

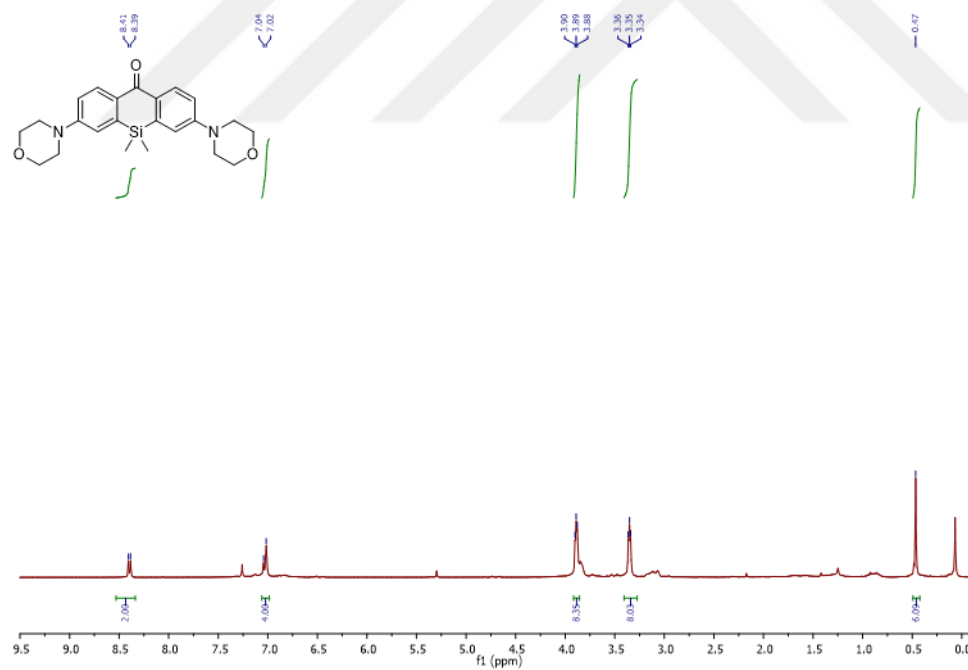


Figure A.88. ¹H NMR spectrum of compound **61** in CDCl₃.

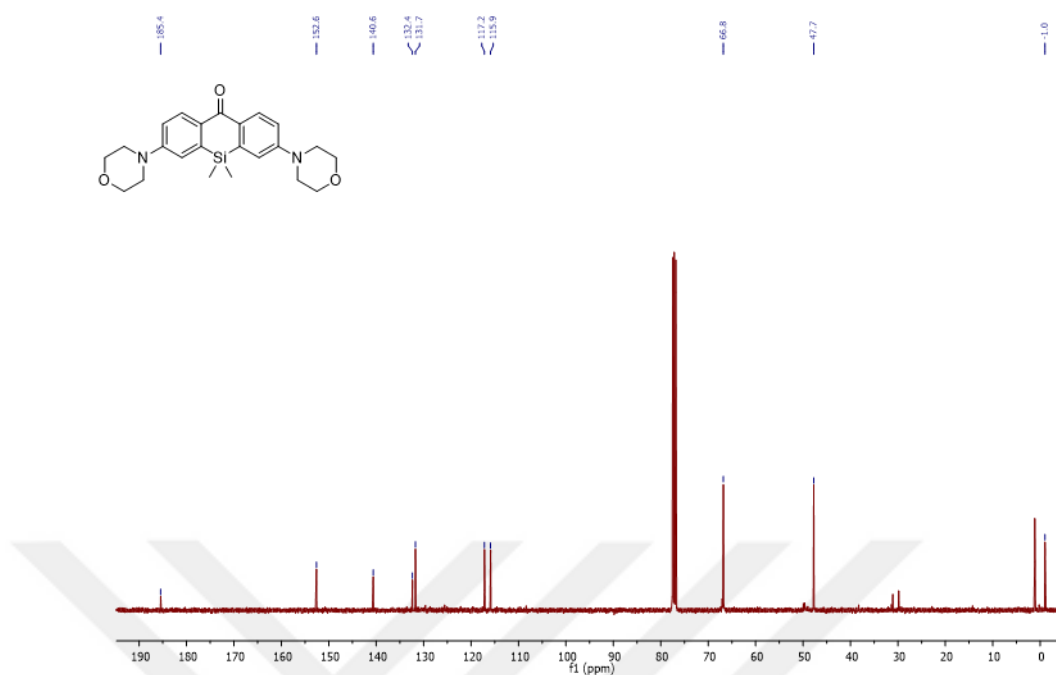


Figure A.89. ¹³C NMR spectrum of compound **61** in CDCl₃.

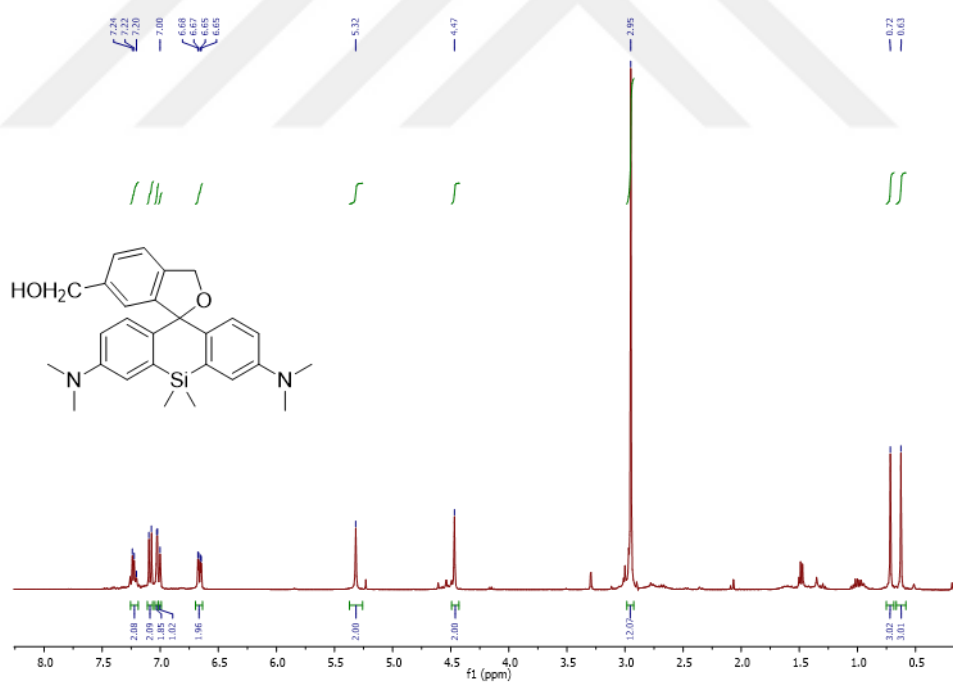


Figure A.90. ¹H NMR spectrum of compound **63** in CDCl₃.

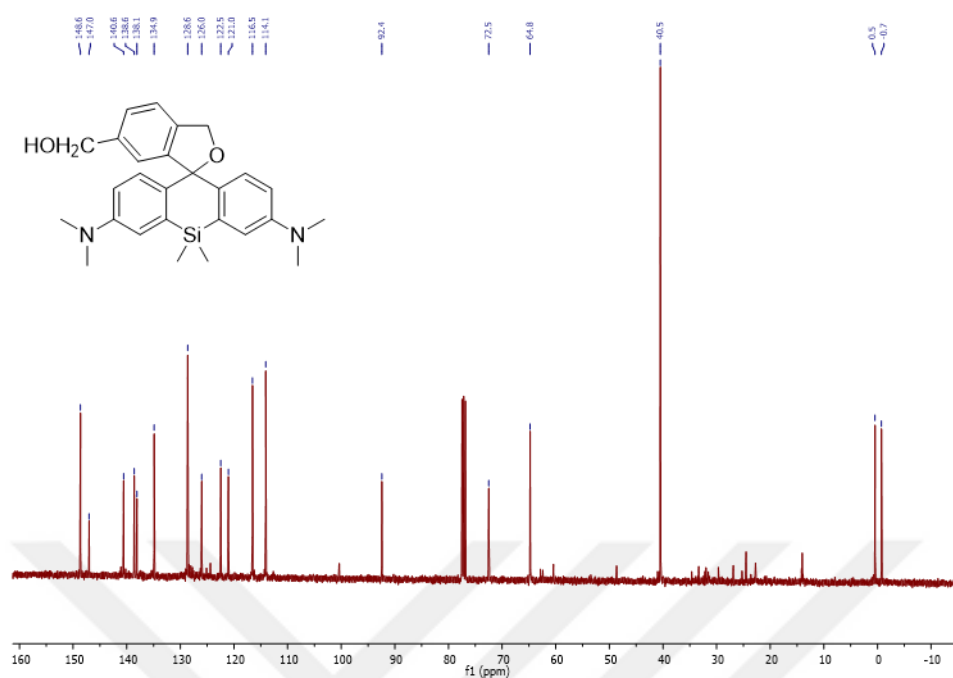


Figure A.91. ¹³C NMR spectrum of compound **63** in CDCl₃.

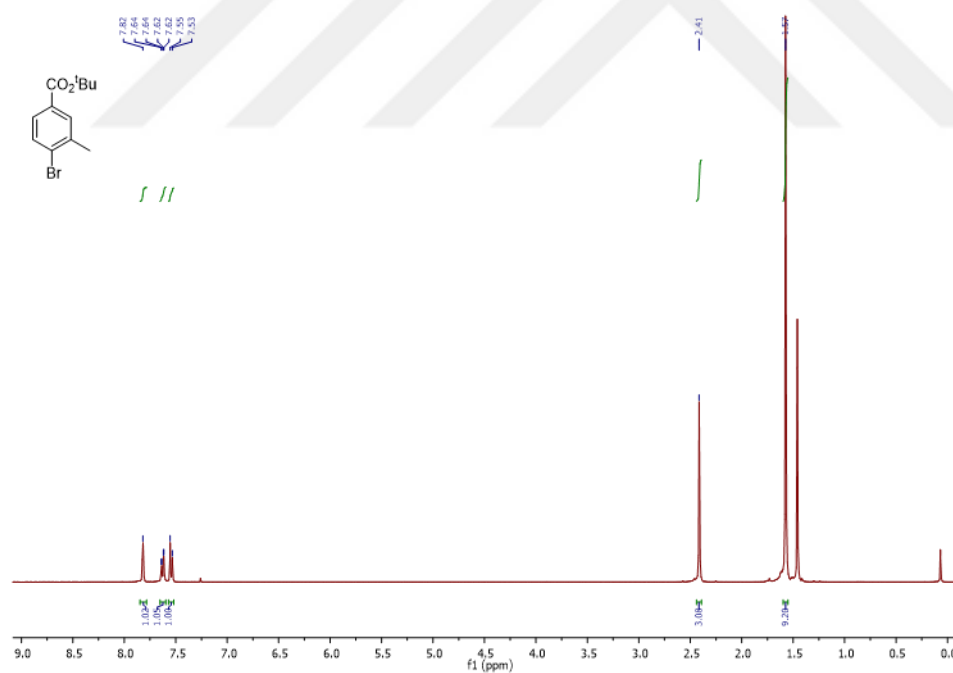


Figure A.92. ¹H NMR spectrum of compound **70** in CDCl₃.

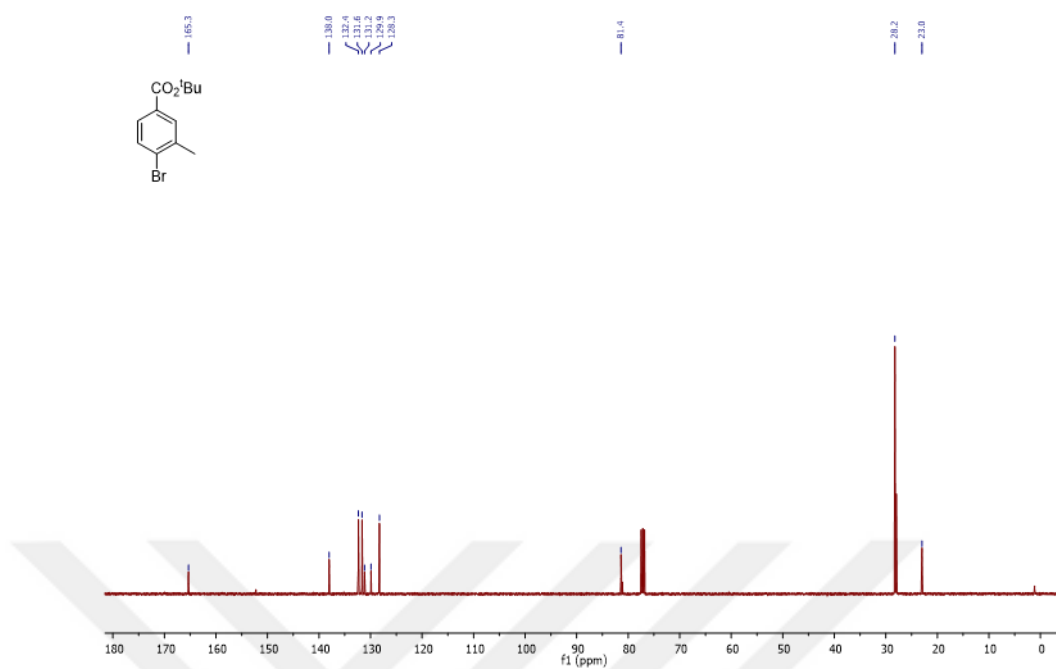


Figure A.93. ¹³C NMR spectrum of compound **70** in CDCl₃.

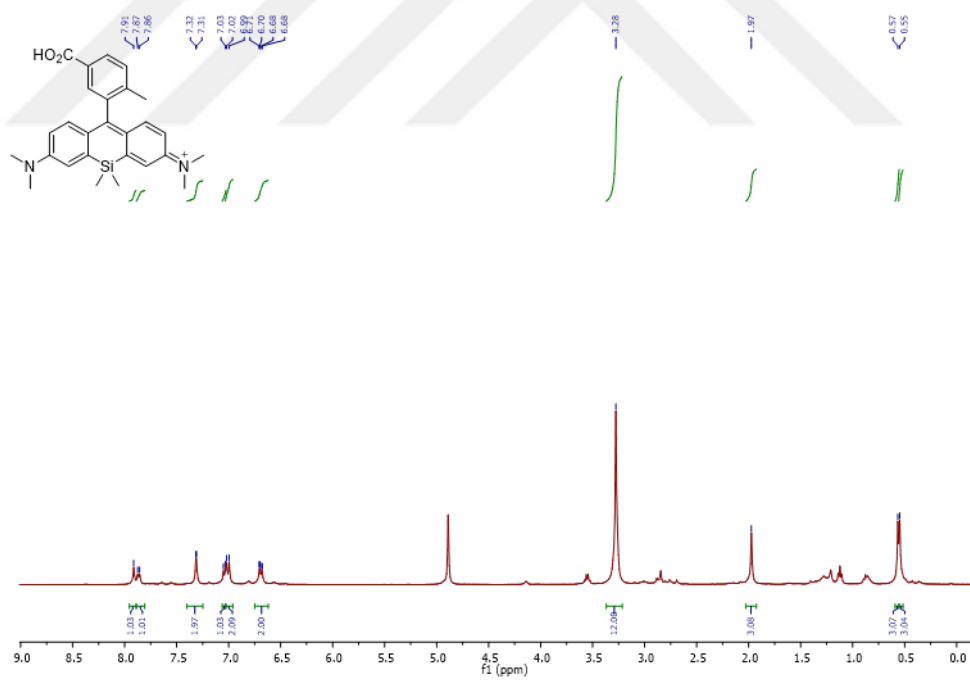


Figure A.94. ¹H NMR spectrum of compound **71** in CDCl₃.

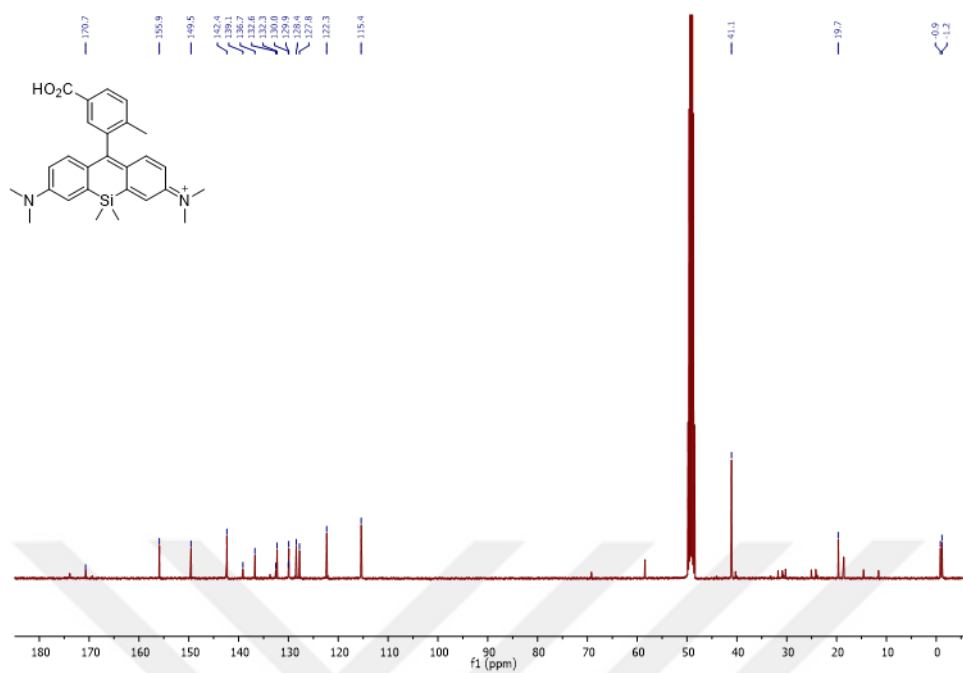


Figure A.95. ¹³C NMR spectrum of compound **71** in CDCl₃.

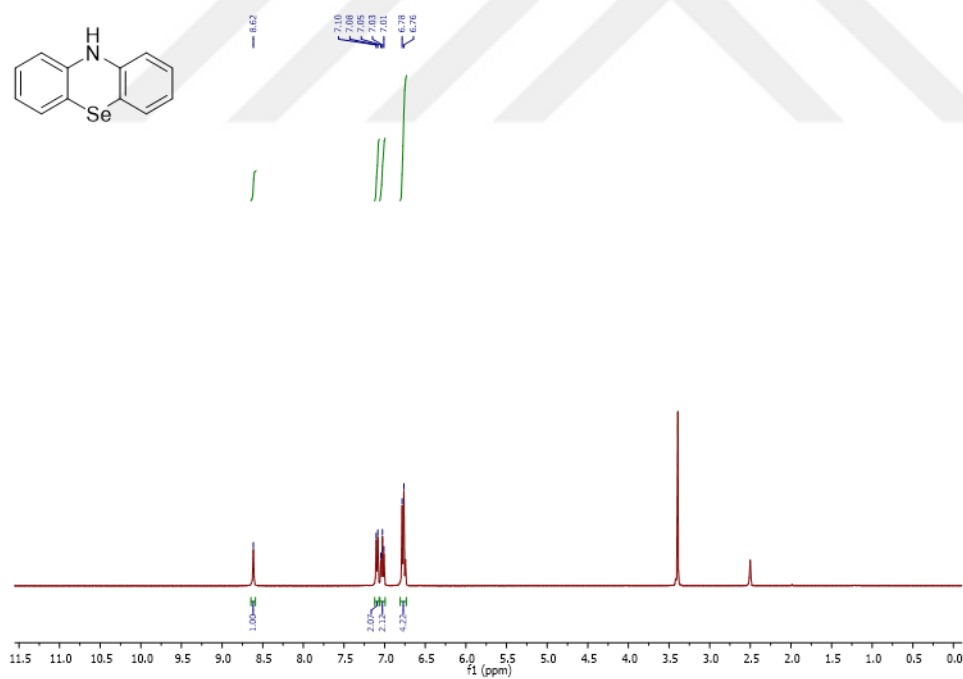


Figure A.96. ¹H NMR spectrum of compound **73** in DMSO

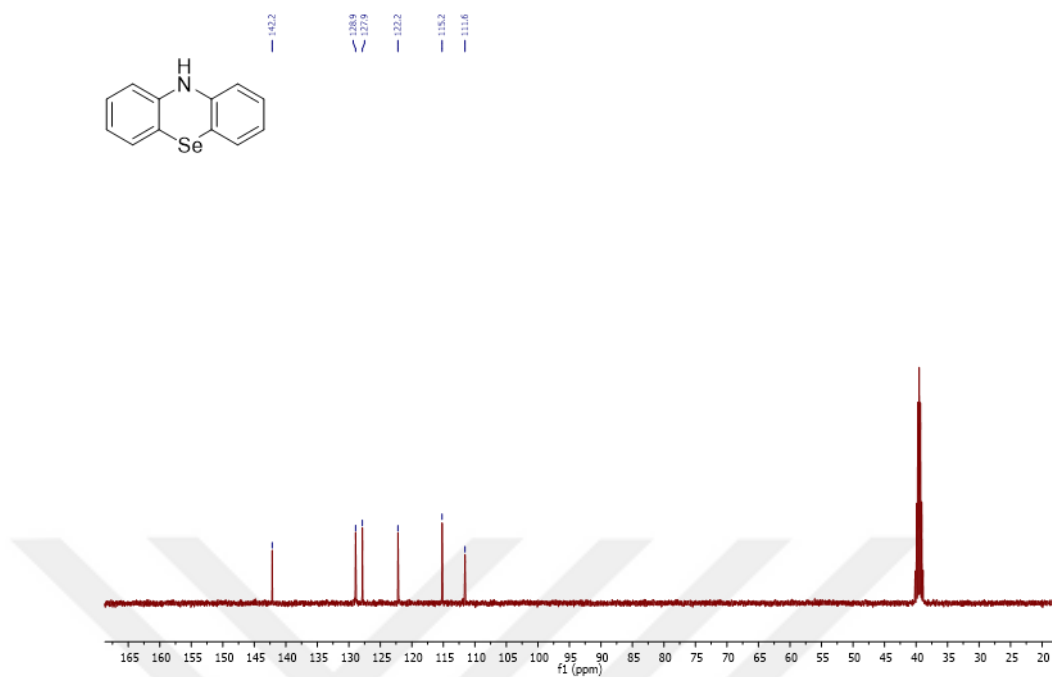


Figure A.97. ¹³C NMR spectrum of compound **73** in DMSO

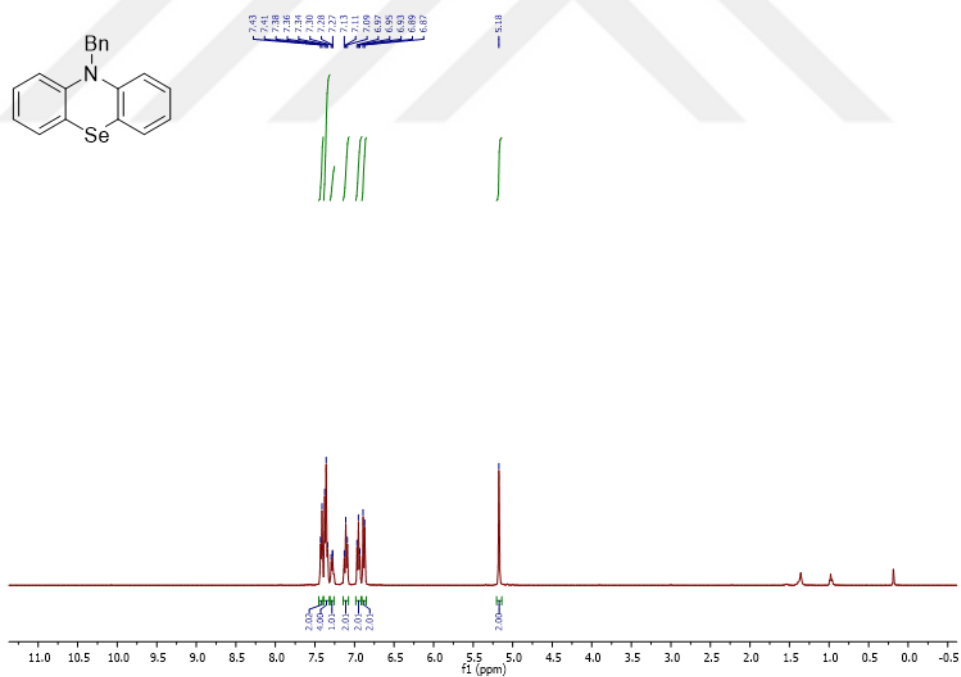


Figure A.98. ¹H NMR spectrum of compound **74** in CDCl₃

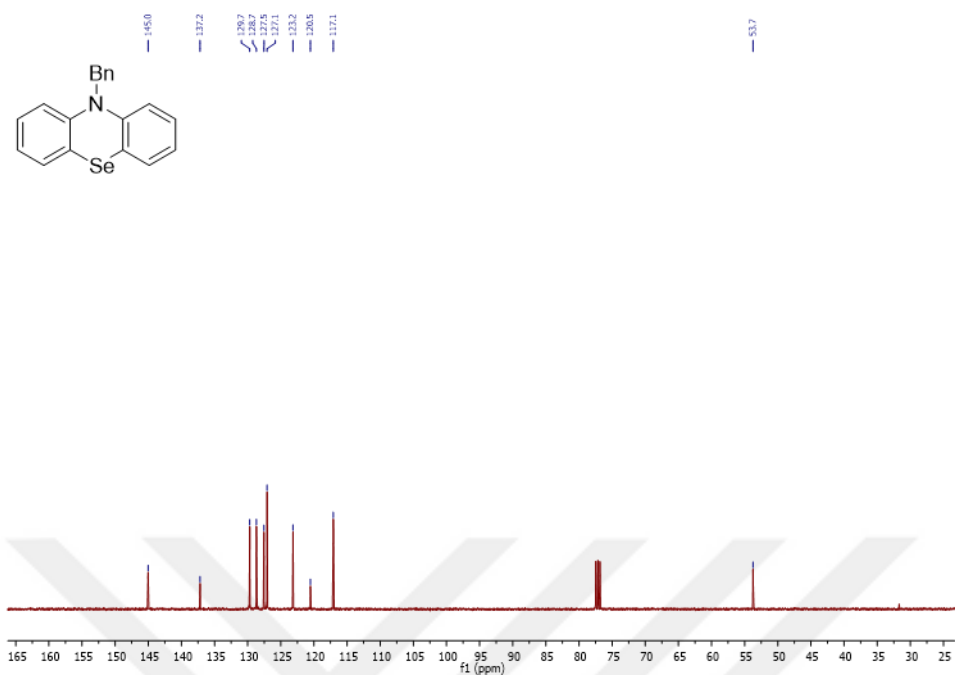


Figure A.99. ¹³C NMR spectrum of compound **74** in CDCl₃.

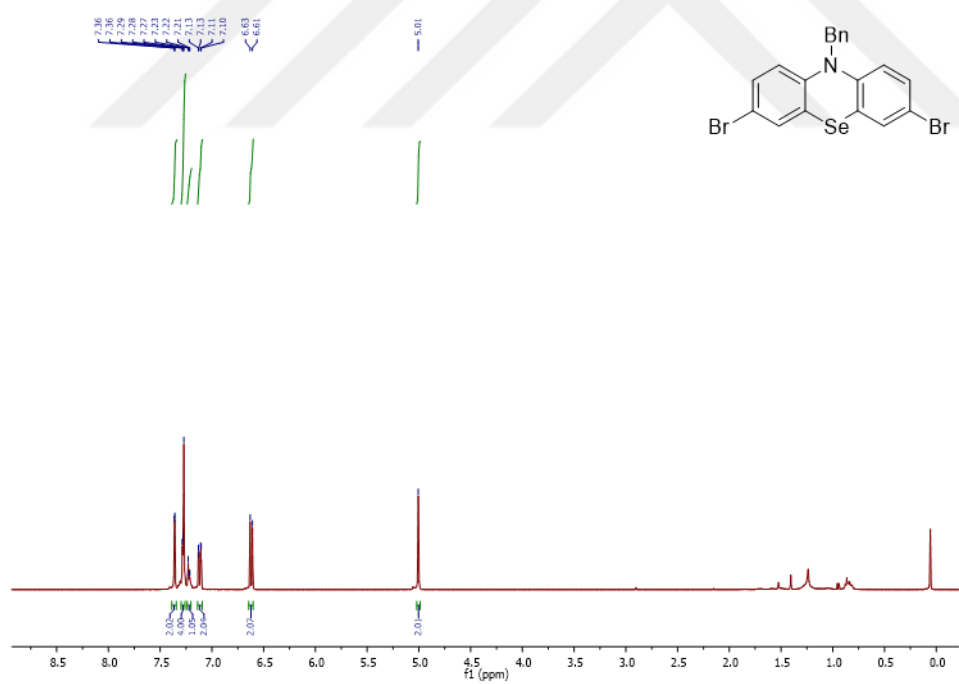


Figure A.100. ¹H NMR spectrum of compound **75** in CDCl₃.

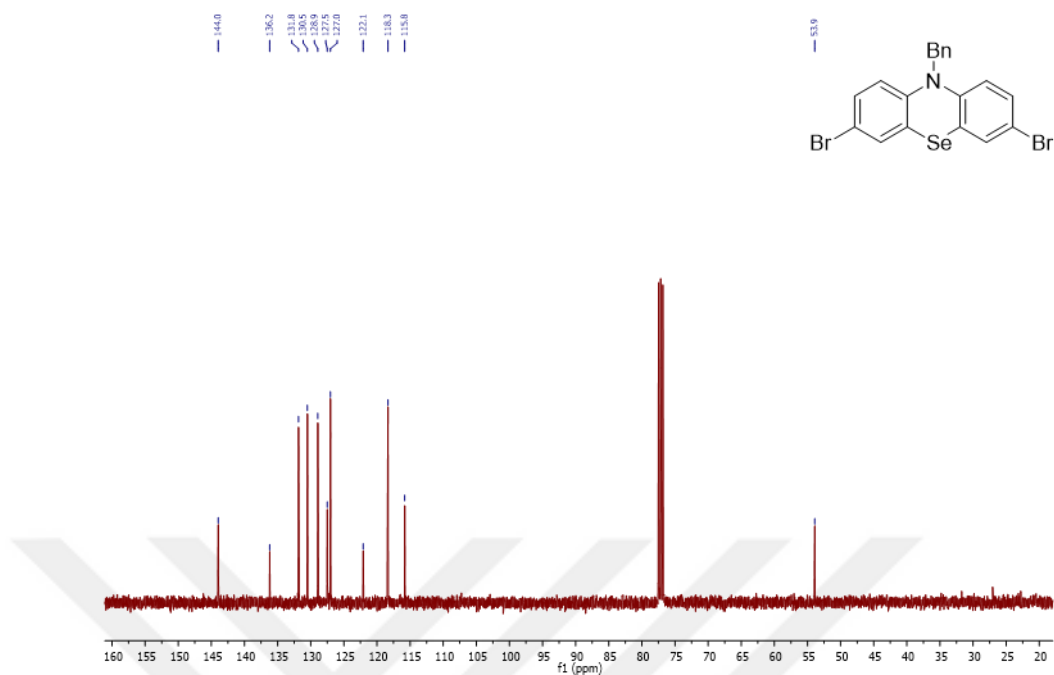


Figure A.101. ^{13}C NMR spectrum of compound **75** in CDCl_3 .

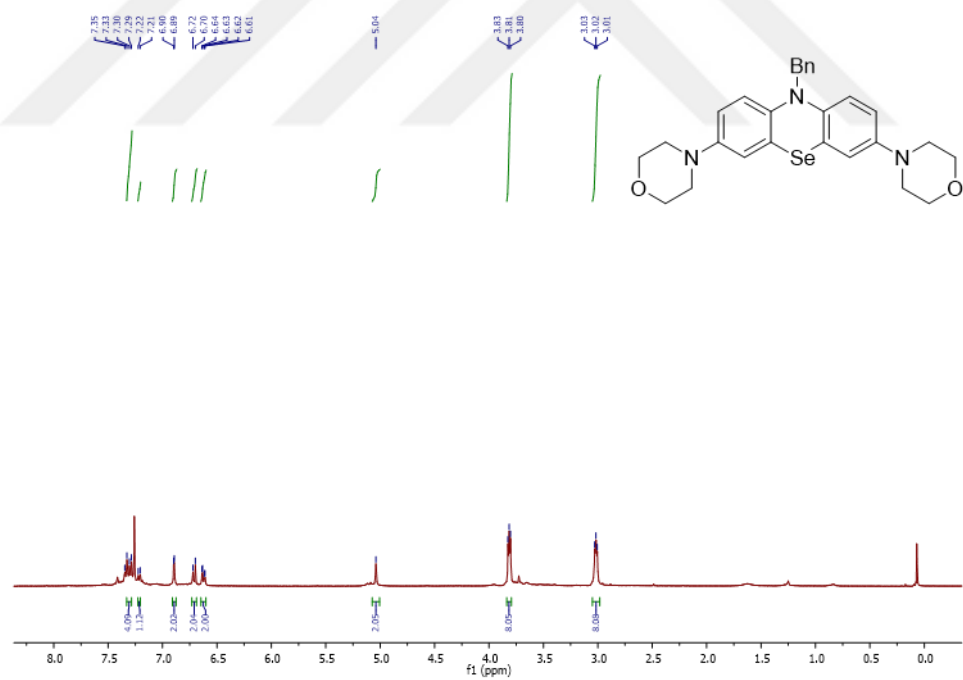


Figure A.102. ^1H NMR spectrum of compound **76** in CDCl_3 .

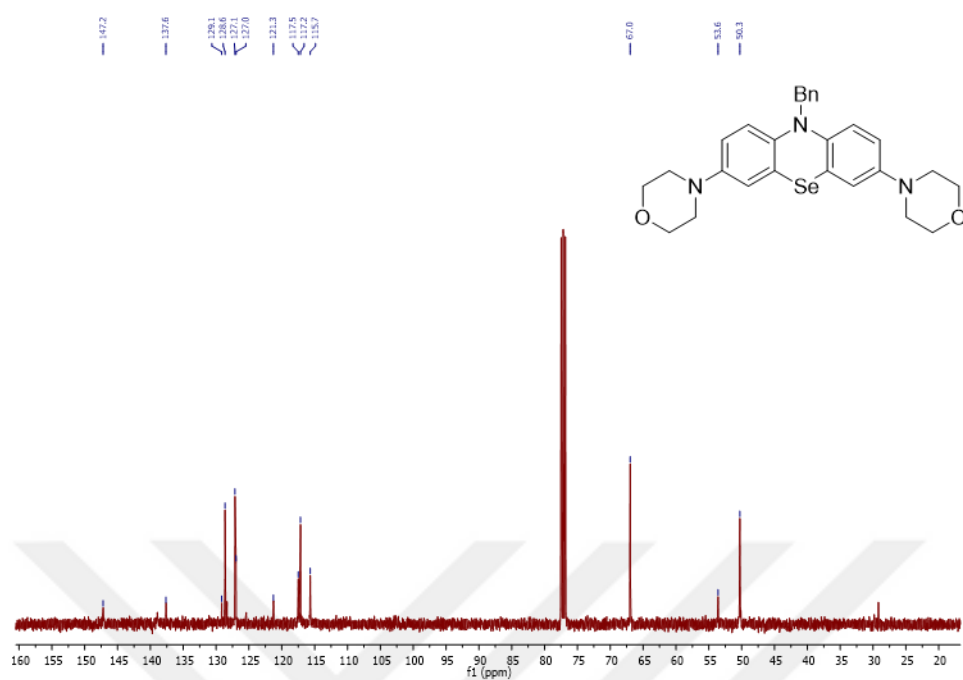


Figure A.103. ¹³C NMR spectrum of compound **76** in CDCl₃.

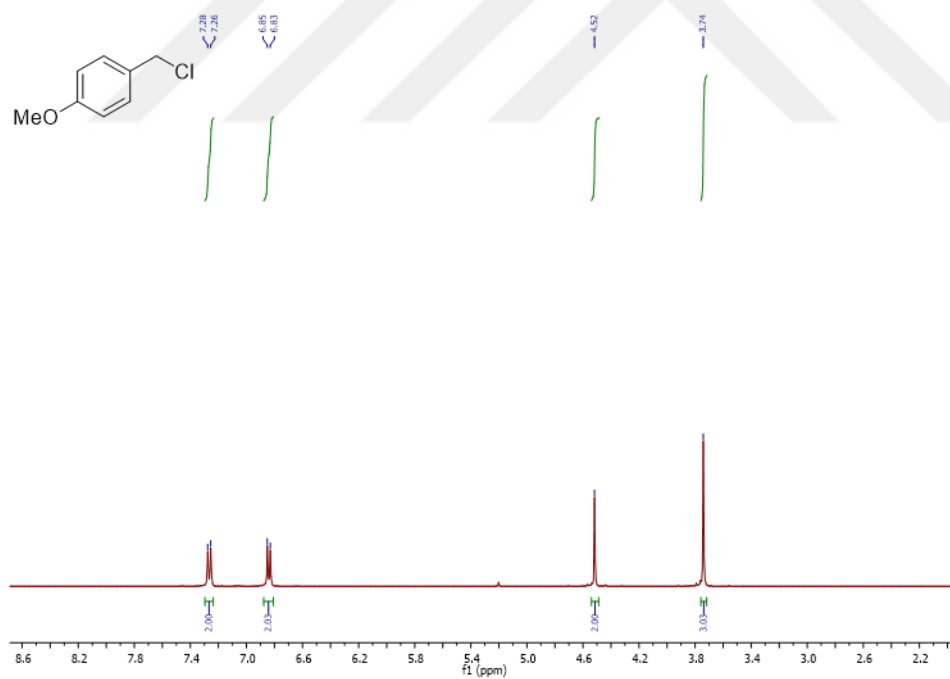


Figure A.104. ¹H NMR spectrum of compound **77** in CDCl₃.

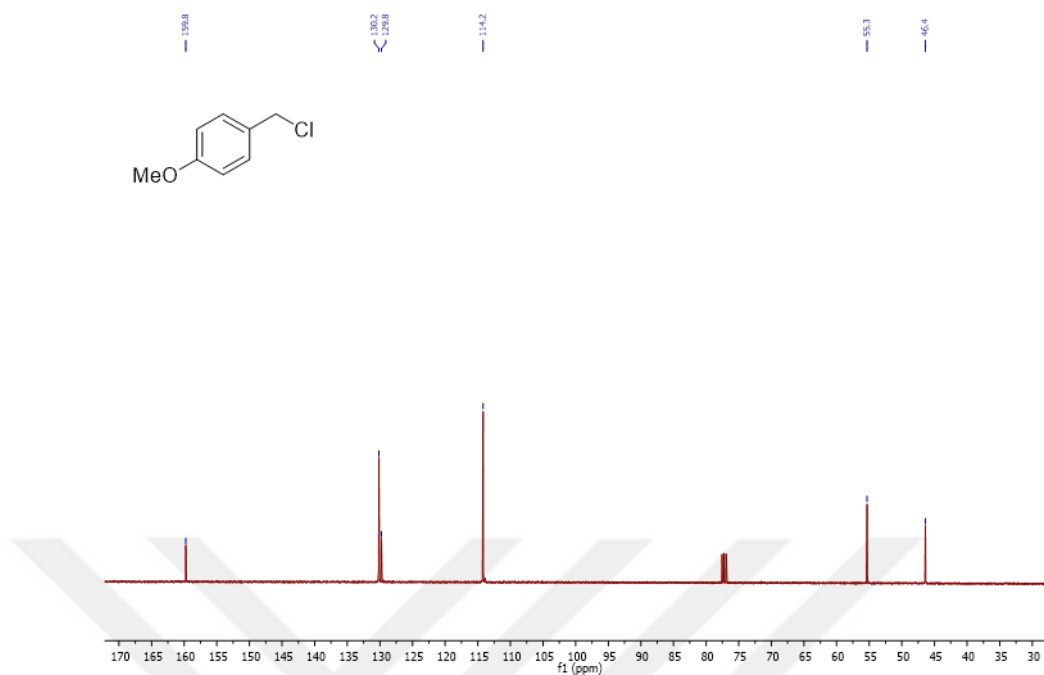


Figure A.105. ^{13}C NMR spectrum of compound **77** in CDCl₃

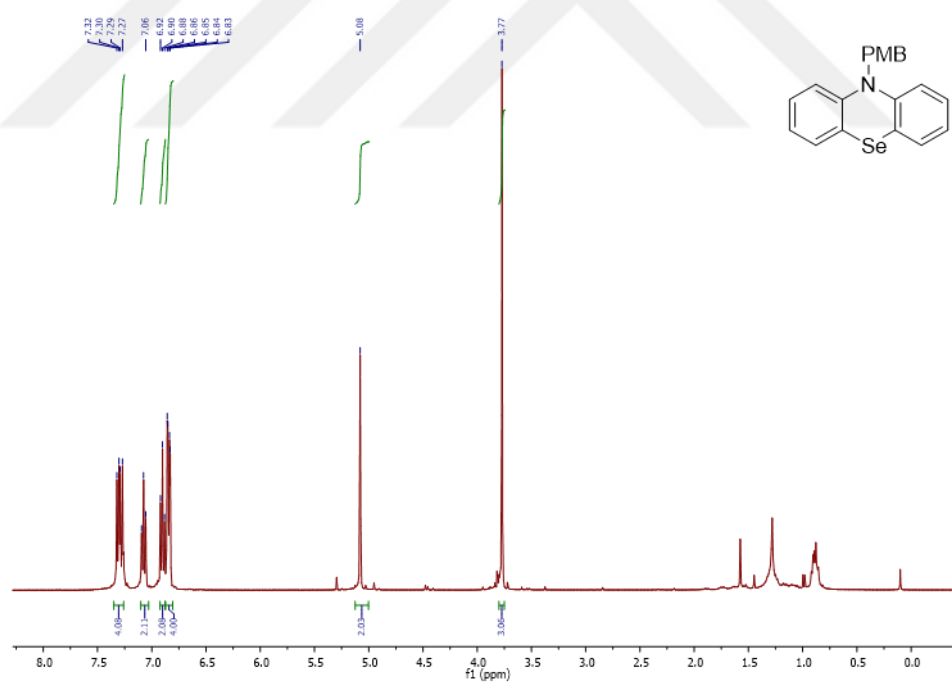


Figure A.106. ^1H NMR spectrum of compound **78** in CDCl₃

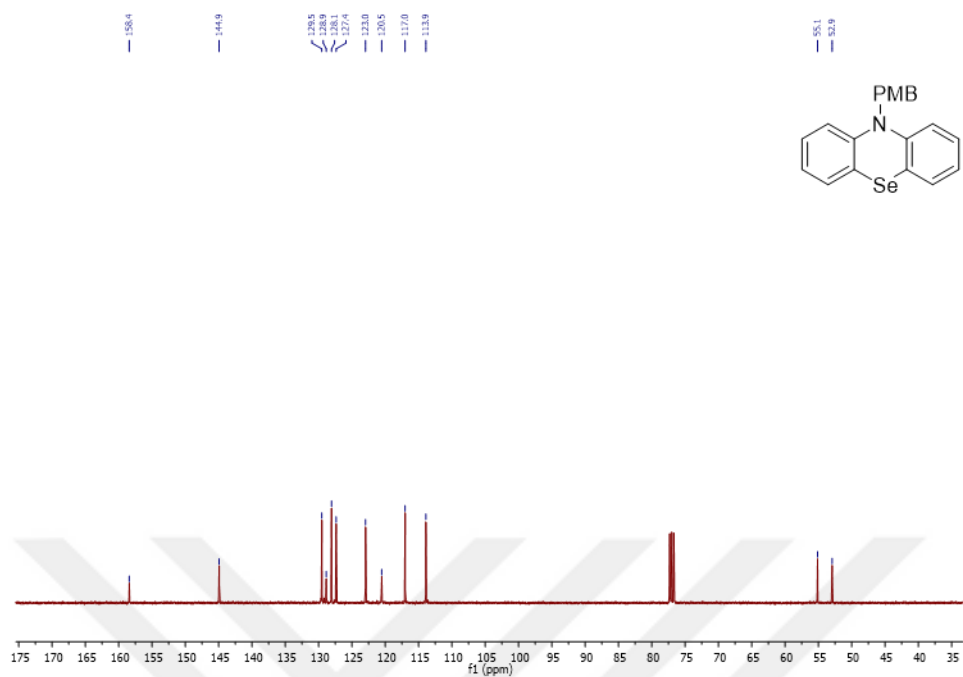


Figure A.107. ¹³C NMR spectrum of compound **78** in CDCl₃

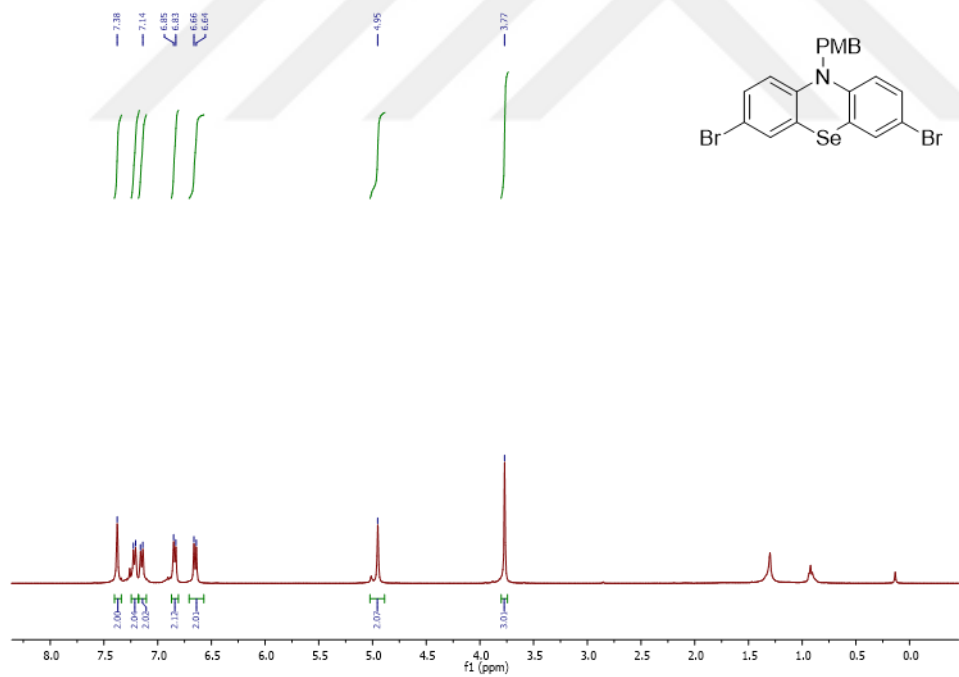


Figure A.108. ¹H NMR spectrum of compound **79** in CDCl₃

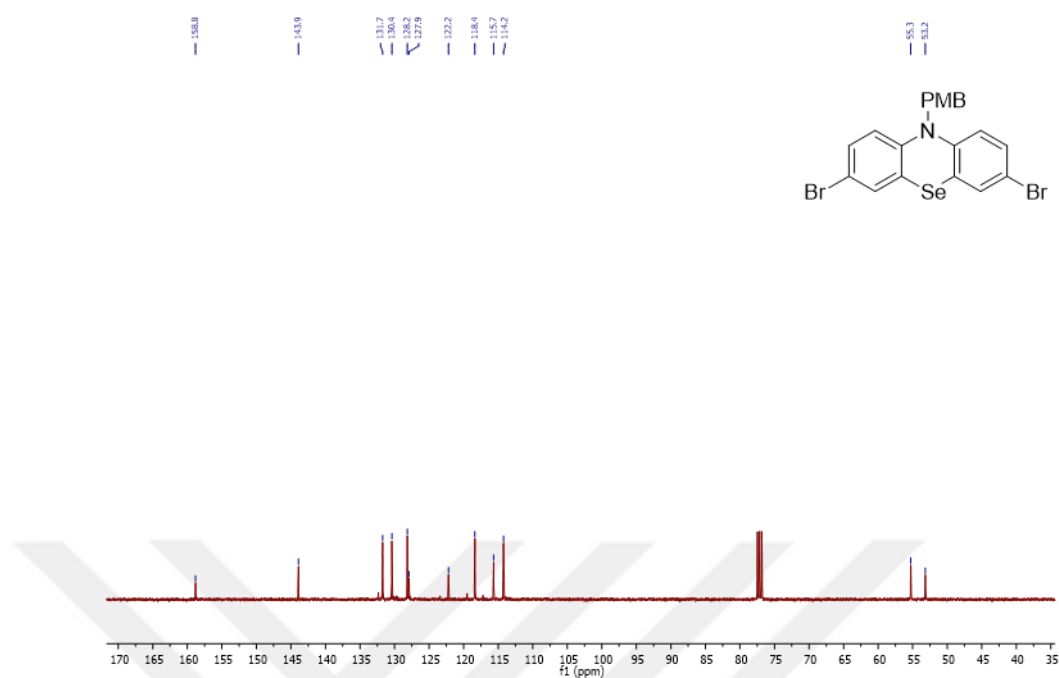


Figure A.109. ¹³C NMR spectrum of compound **79** in CDCl₃

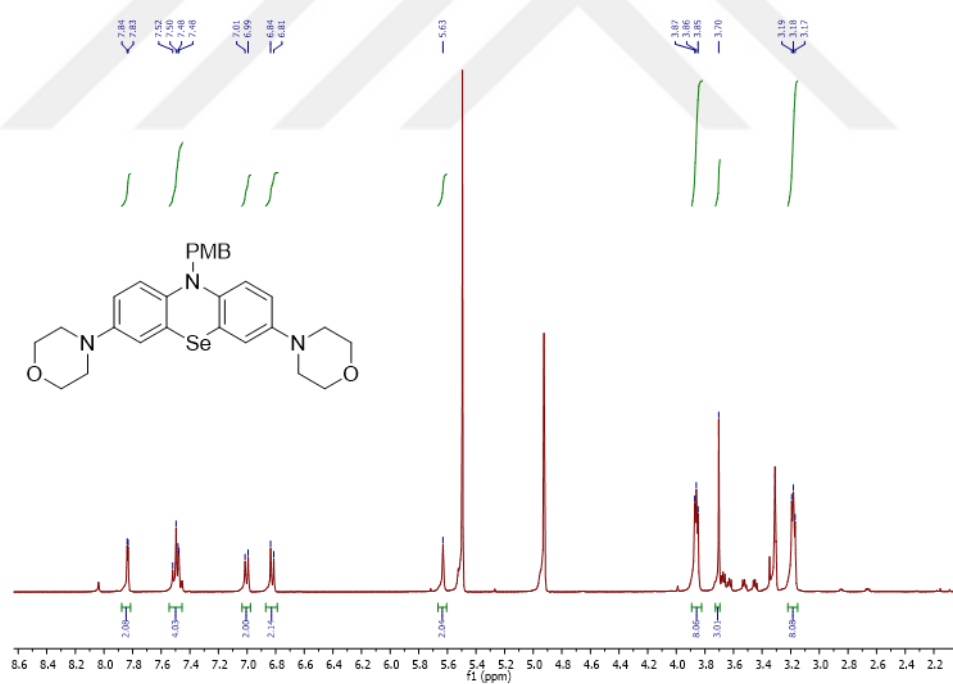


Figure A.110. ¹H NMR spectrum of compound **79** in Acetonitrile-d₃

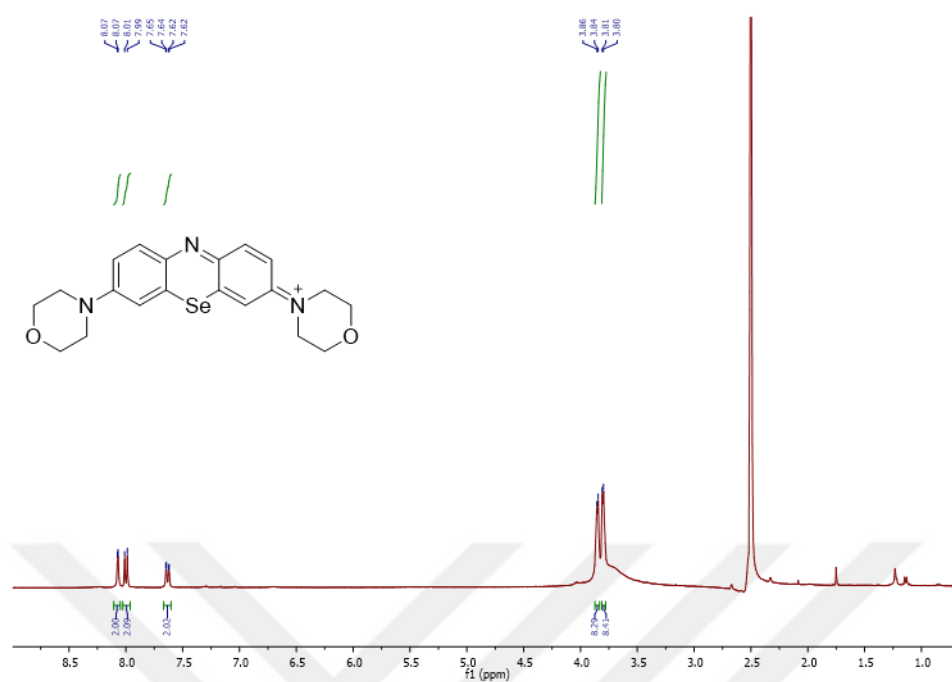


Figure A.111. ¹H NMR spectrum of NSeMorph in DMSO.

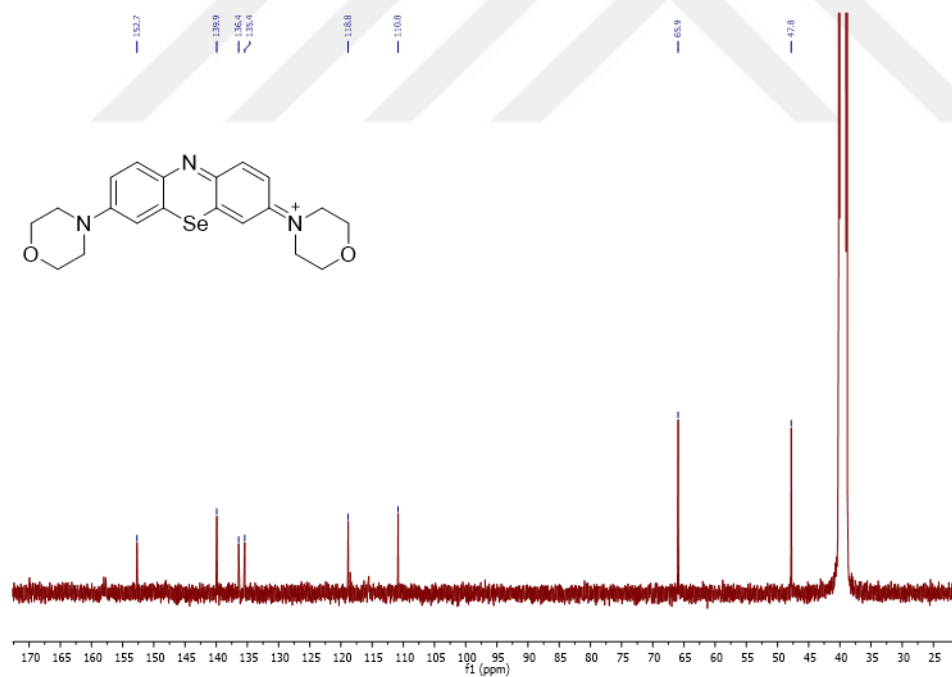


Figure A.112. ¹³C NMR spectrum of NSeMorph in DMSO.

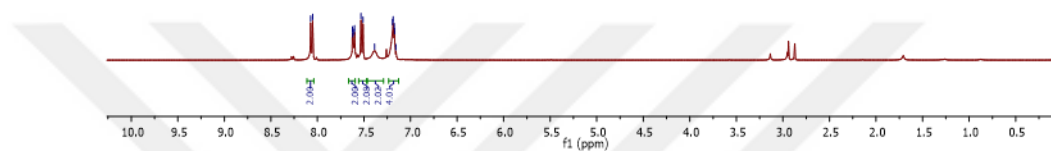
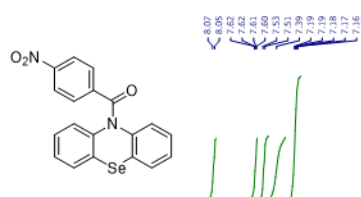


Figure A.113. ^1H NMR spectrum of compound **81** in CDCl_3

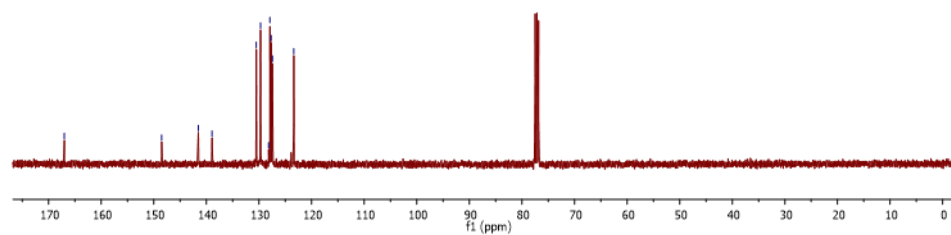
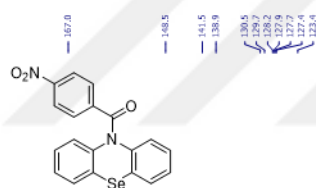


Figure A.114. ^{13}C NMR spectrum of compound **81** in CDCl_3

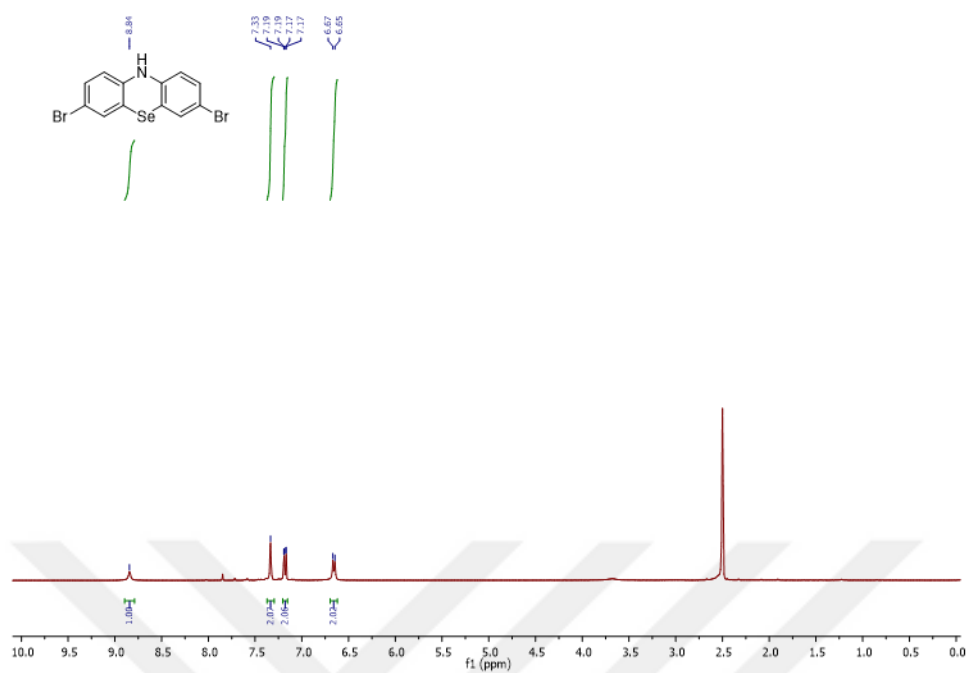


Figure A.115. ^1H NMR spectrum of compound **84** in DMSO.

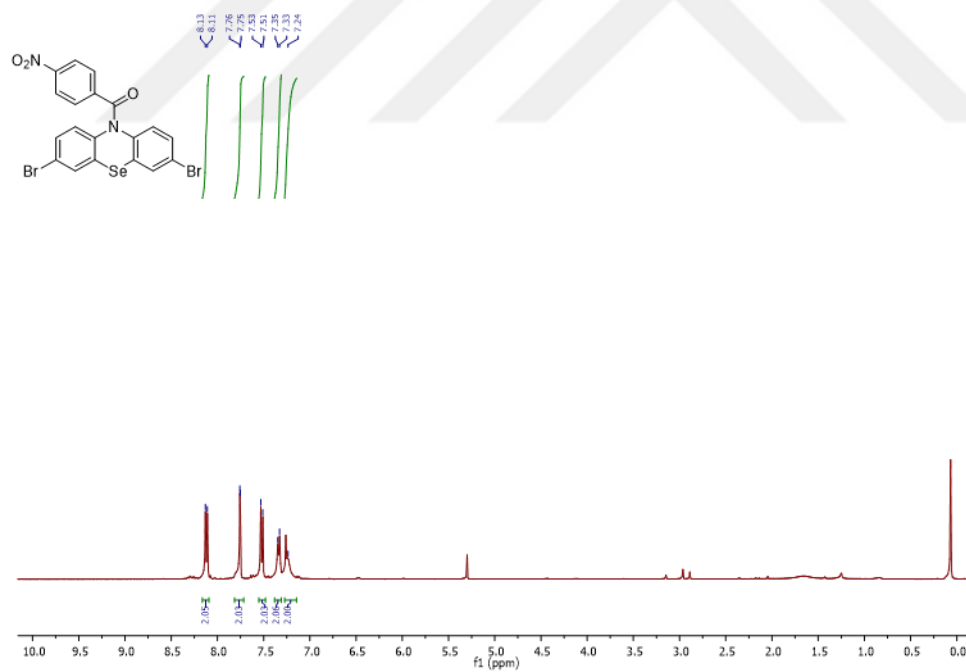


Figure A.116. ^1H NMR spectrum of compound **85** in CDCl_3

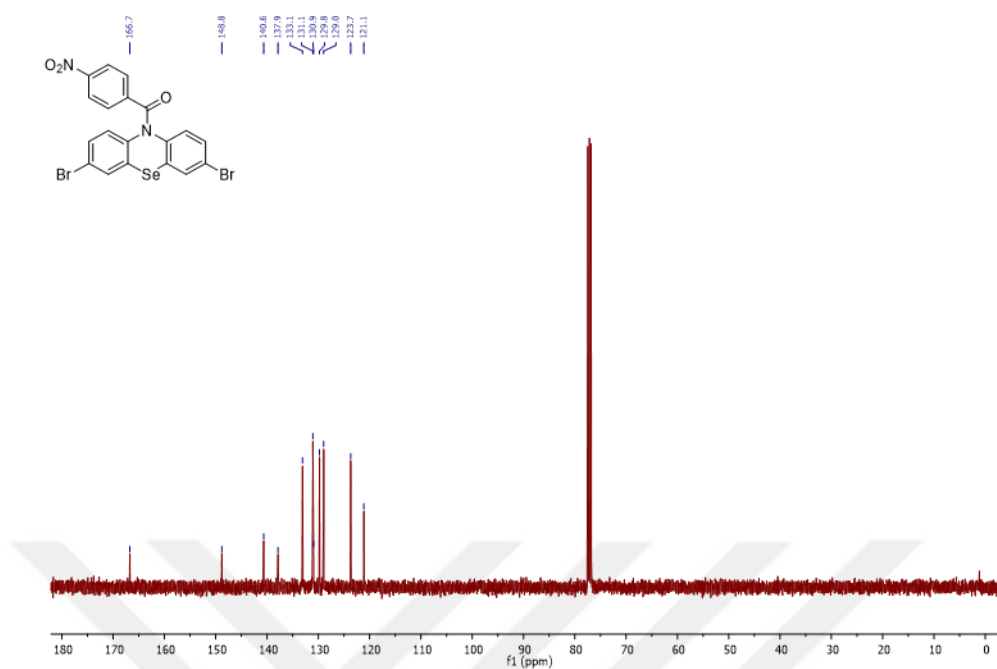


Figure A.117. ^{13}C NMR spectrum of compound **85** in CDCl_3

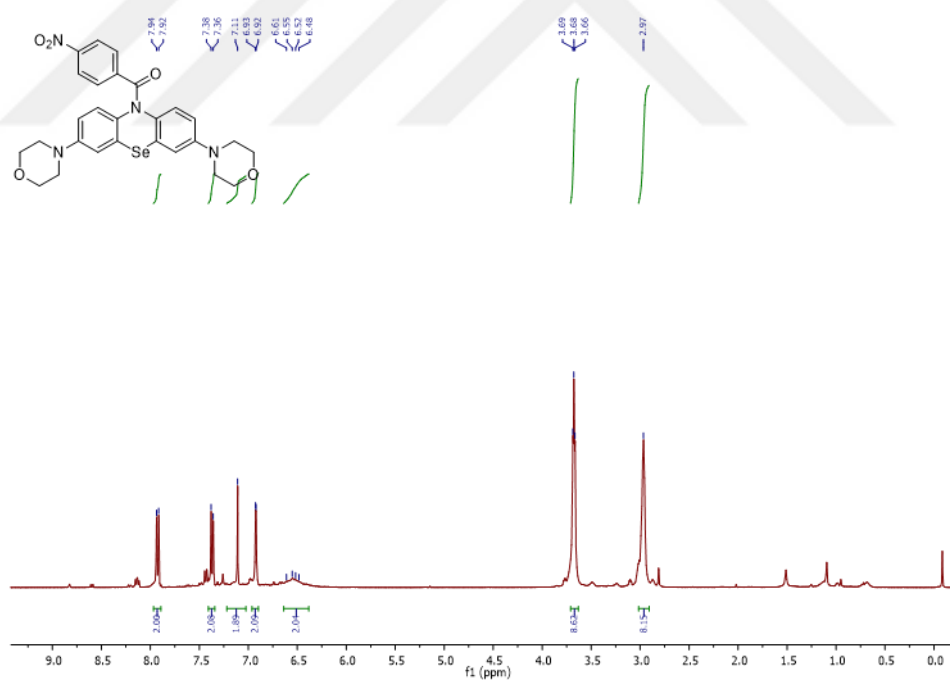


Figure A.118. ^1H NMR spectrum of compound **86** in CDCl_3

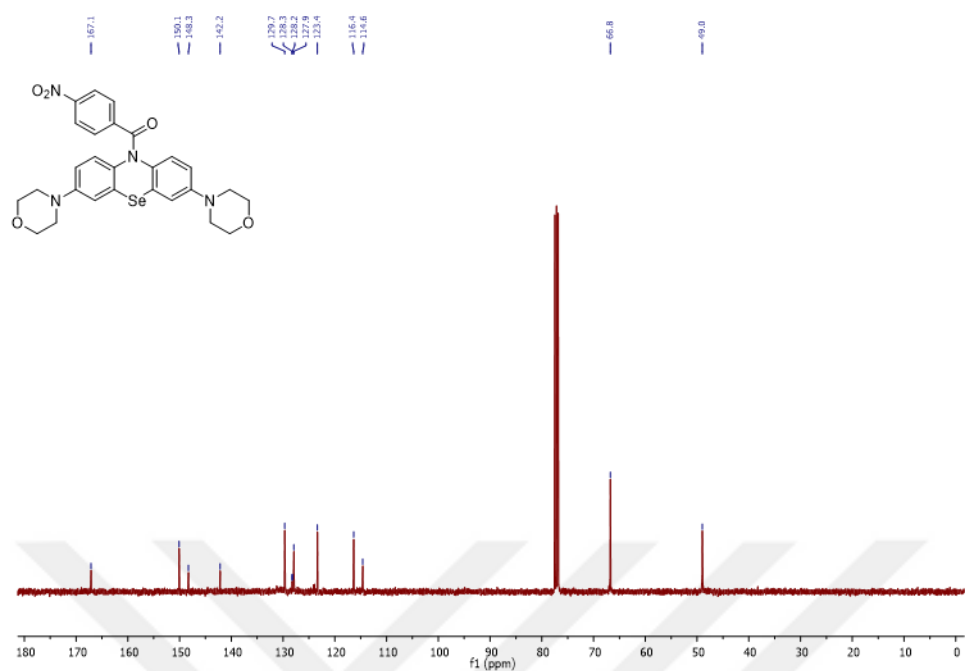


Figure A.119. ¹³C NMR spectrum of compound **86** in CDCl₃

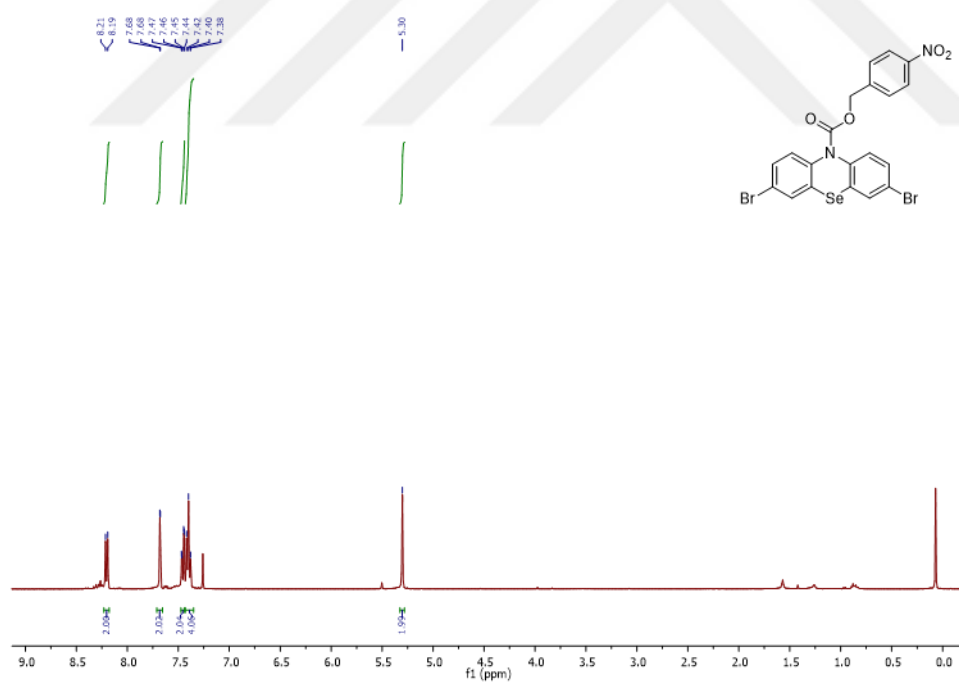


Figure A.120. ¹H NMR spectrum of compound **88** in CDCl₃

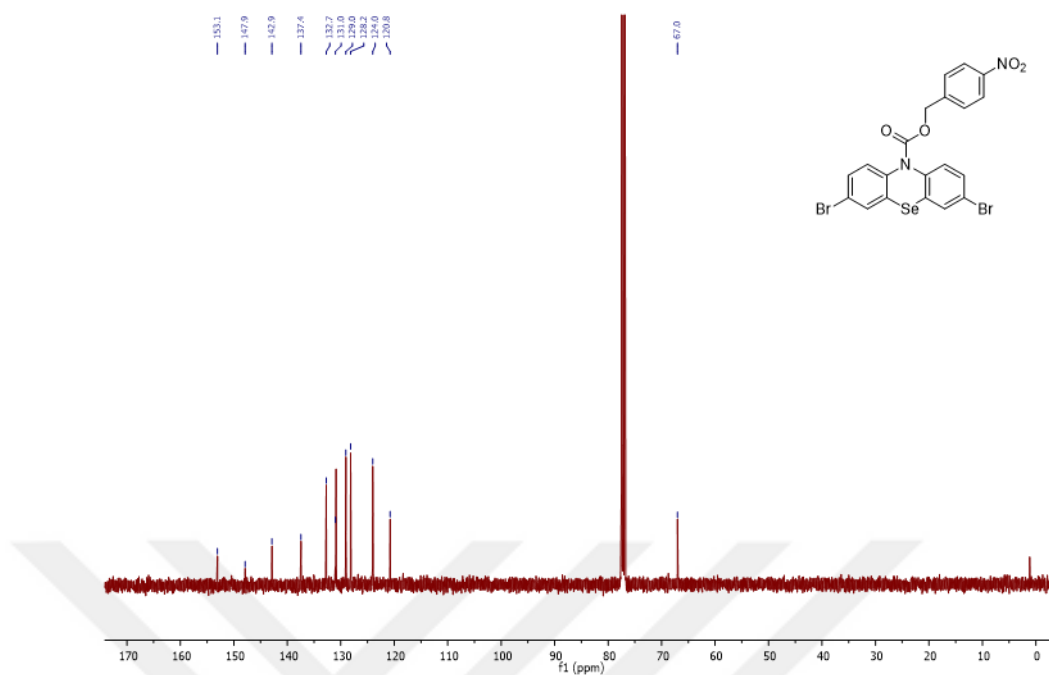


Figure A.121. ¹³C NMR spectrum of compound **88** in CDCl₃

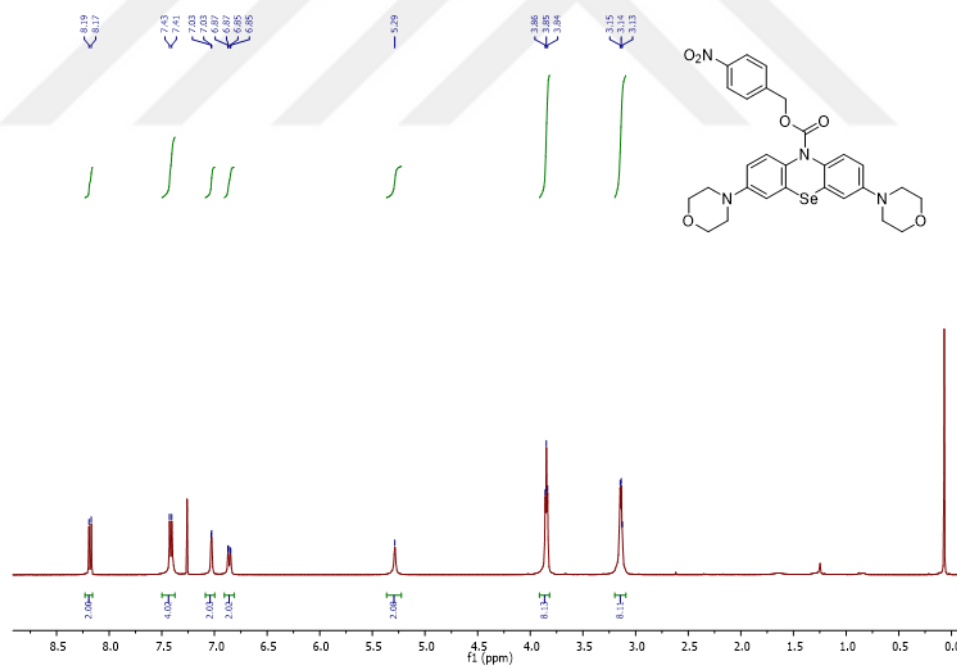


Figure A.122. ¹H NMR spectrum of NTR-NSeMorph in CDCl₃

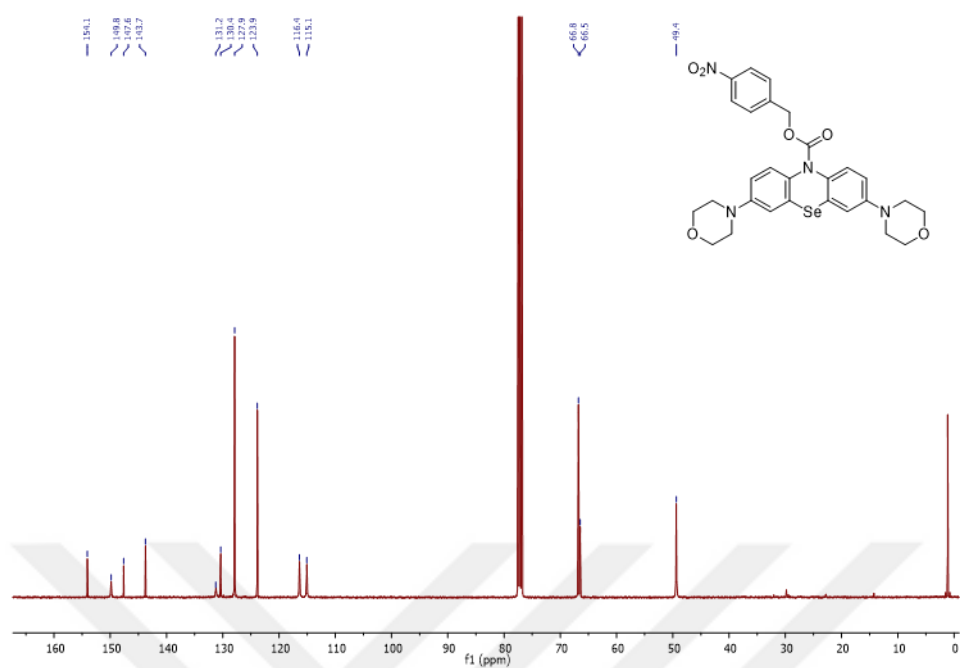


Figure A.123. ¹³C NMR spectrum of NTR-NSeMorph in CDCl₃

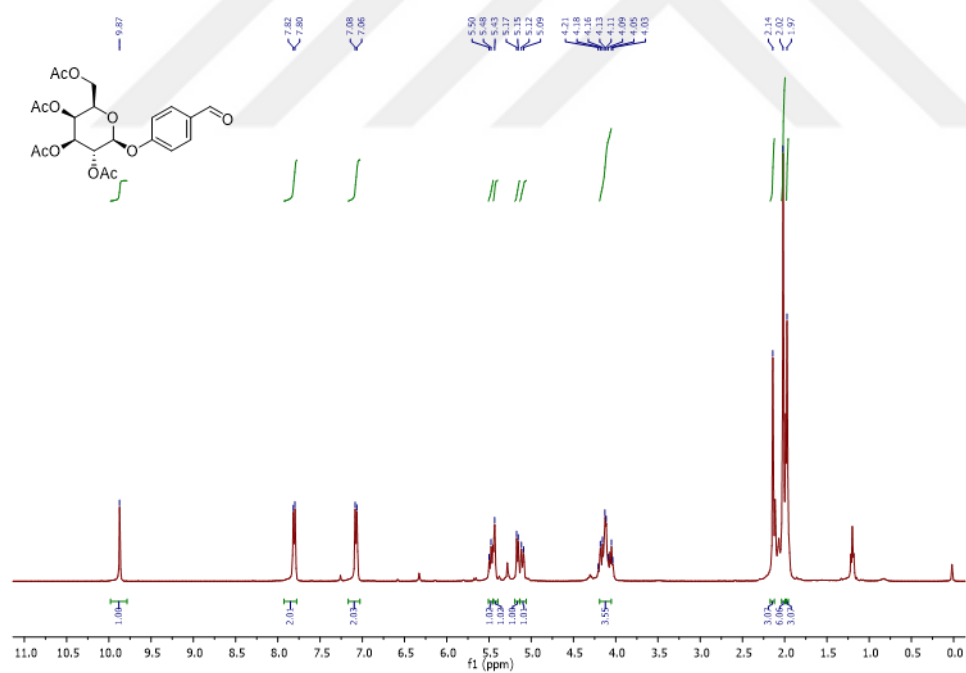


Figure A.124. ¹H NMR spectrum of compound **89** in CDCl₃

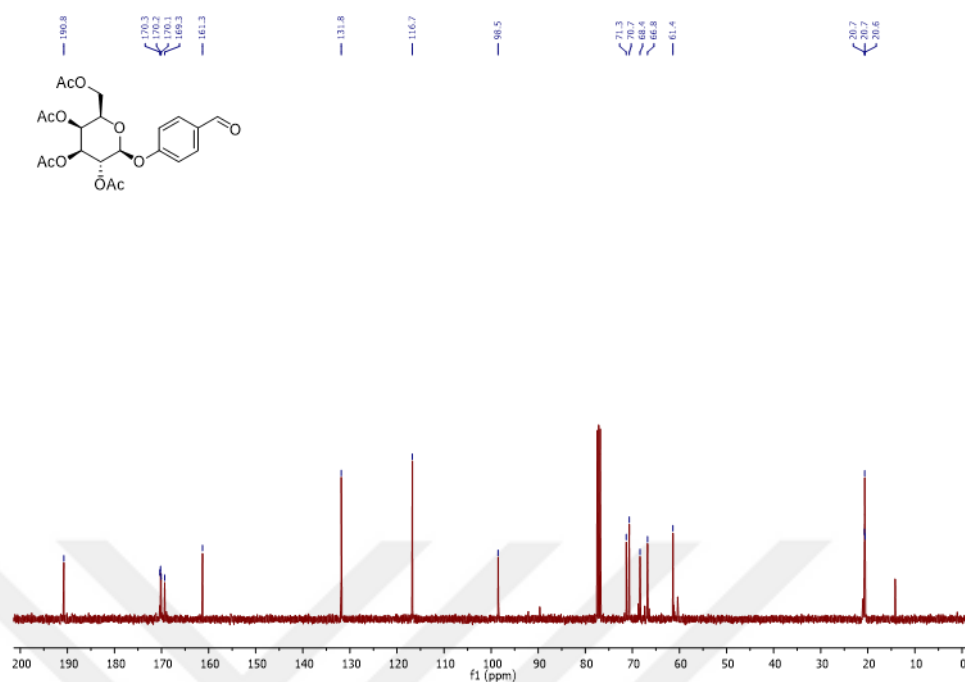


Figure A.125. ^{13}C NMR spectrum of compound **89** in CDCl_3

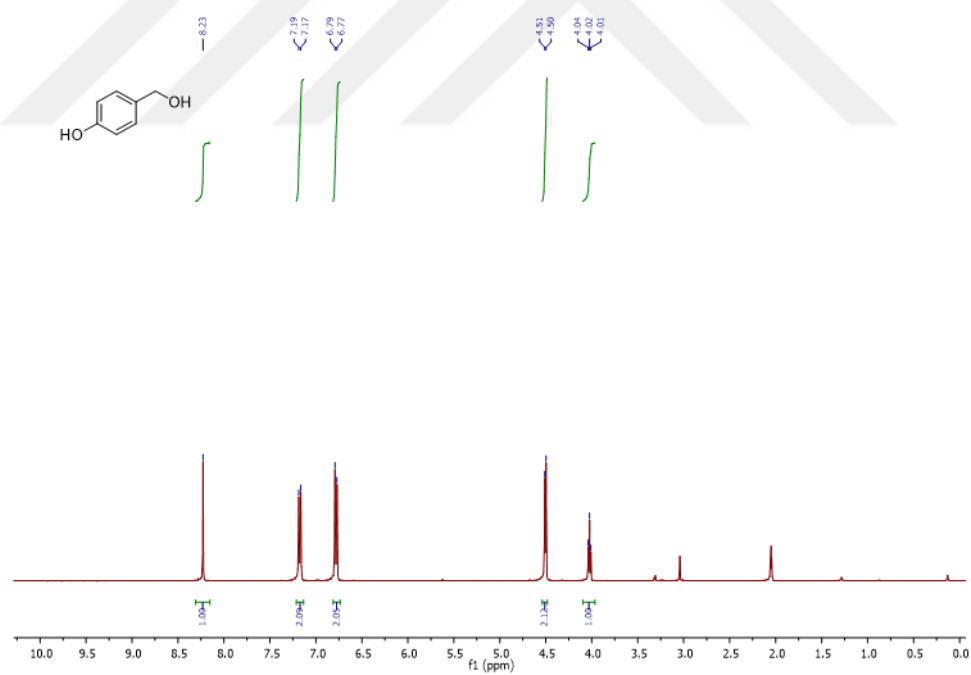


Figure A.126. ^1H NMR spectrum of compound **94** in Acetone-d_6

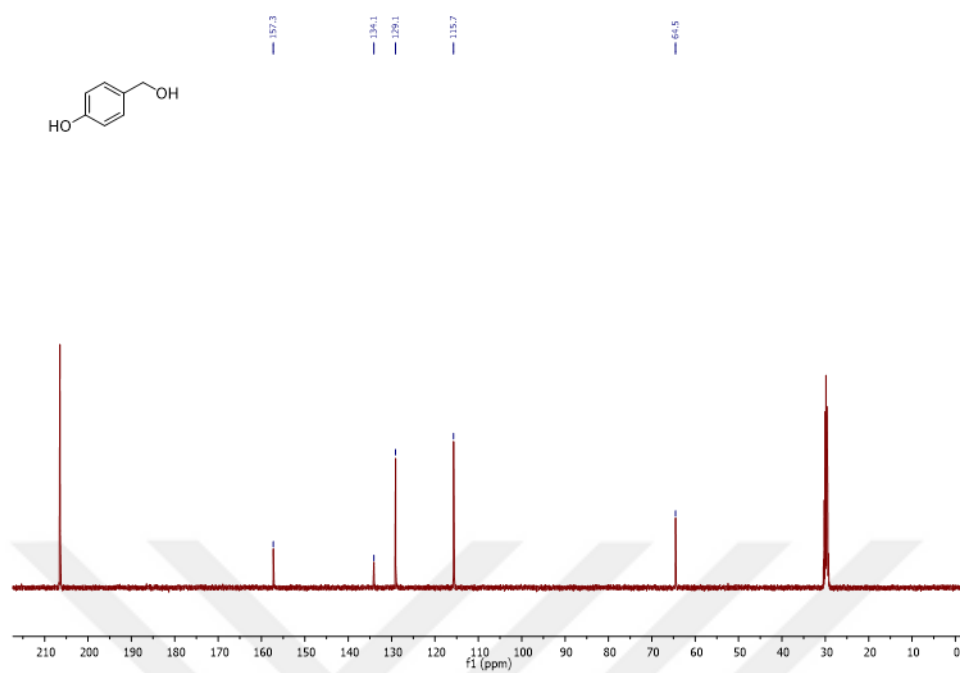


Figure A.127. ^{13}C NMR spectrum of compound **94** in Acetone- d_6

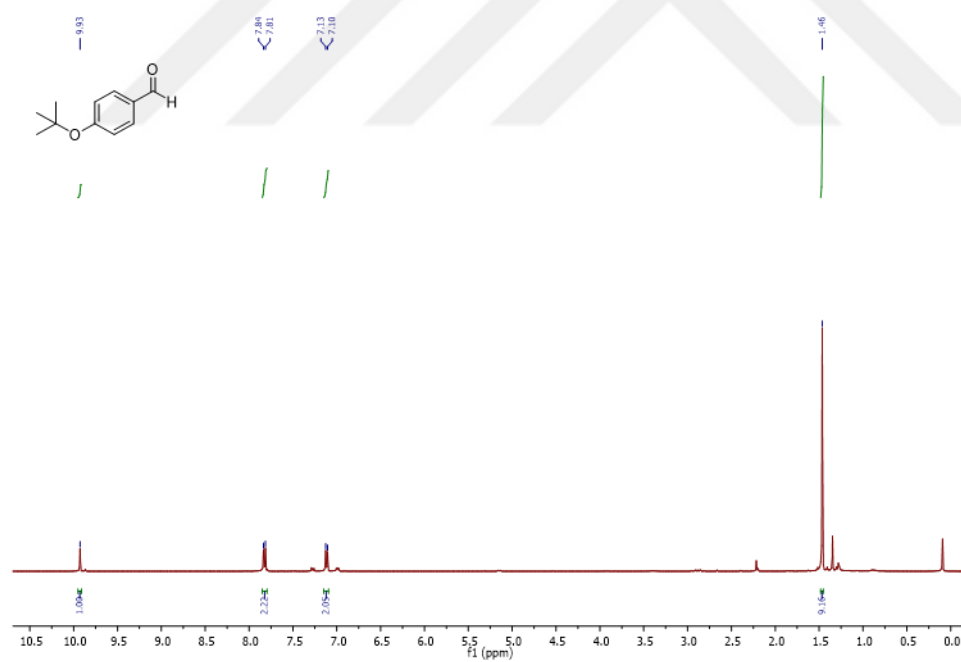


Figure A.128. ^1H NMR spectrum of compound **100** in CDCl_3

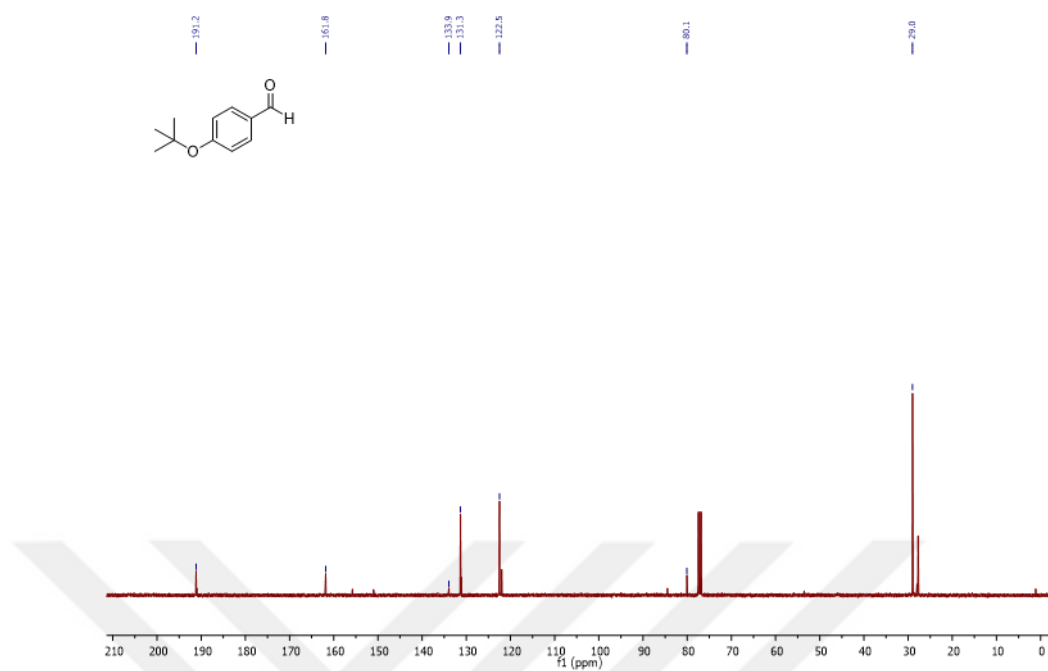


Figure A.129. ^{13}C NMR spectrum of compound **100** in CDCl₃

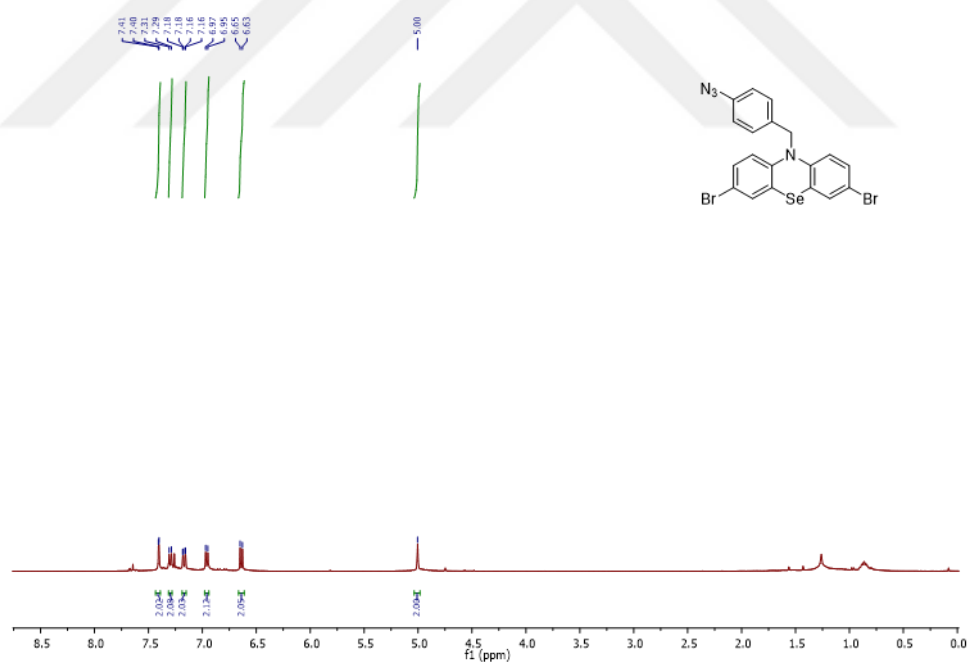


Figure A.130. ^1H NMR spectrum of compound **103** in CDCl₃

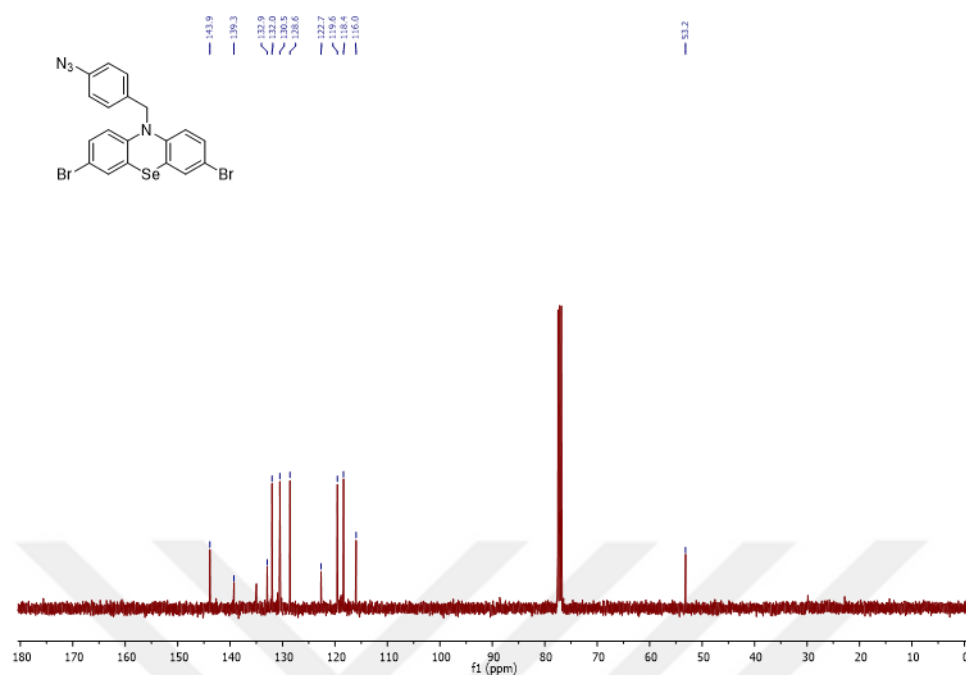


Figure A.131. ¹³C NMR spectrum of compound **103** in CDCl₃

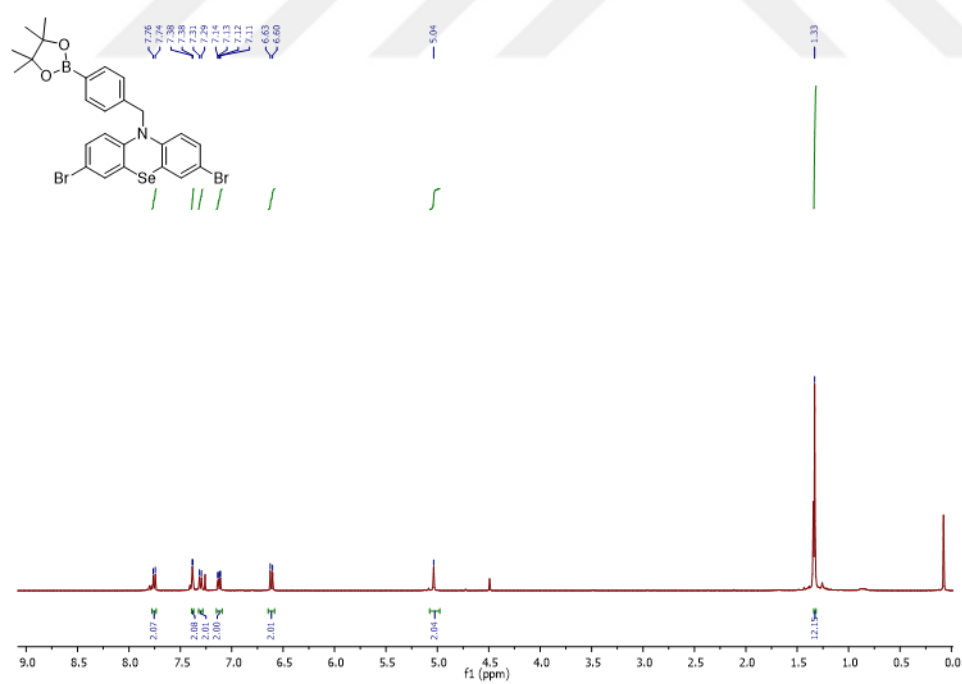


Figure A.132. ¹H NMR spectrum of compound **104** in CDCl₃

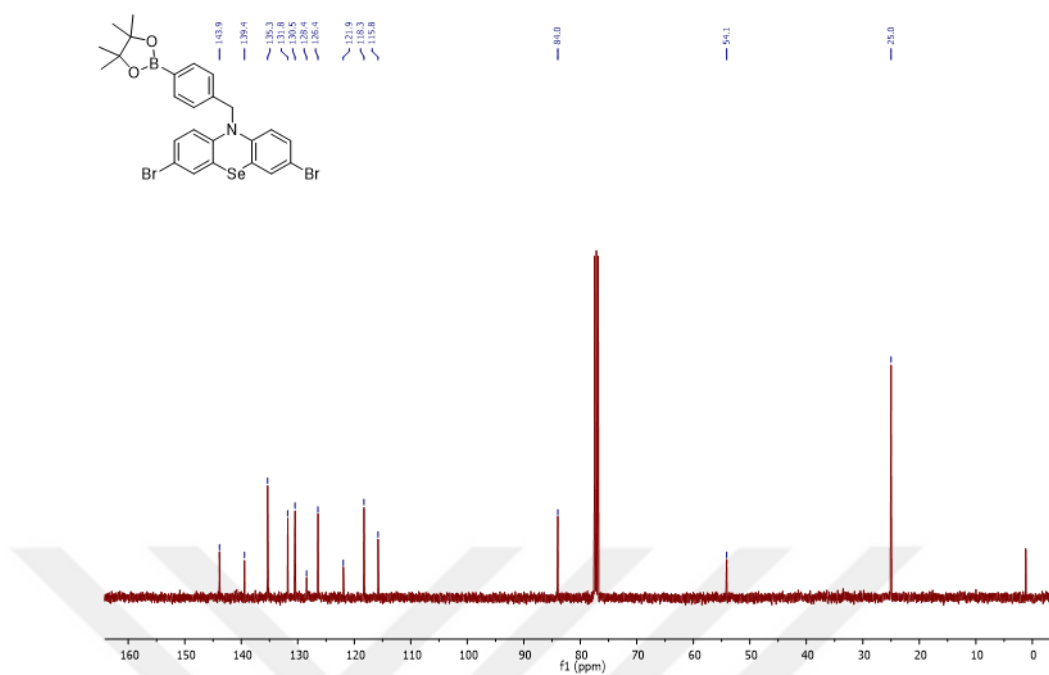


Figure A.133. ^{13}C NMR spectrum of compound **104** in CDCl_3

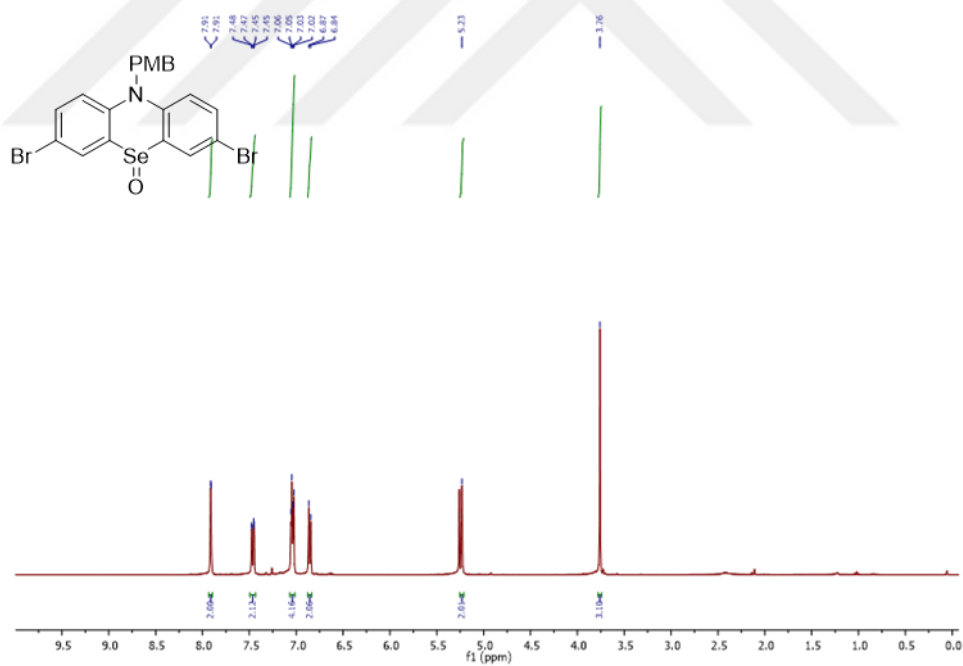


Figure A.134. ^1H NMR spectrum of compound **106** in CDCl_3

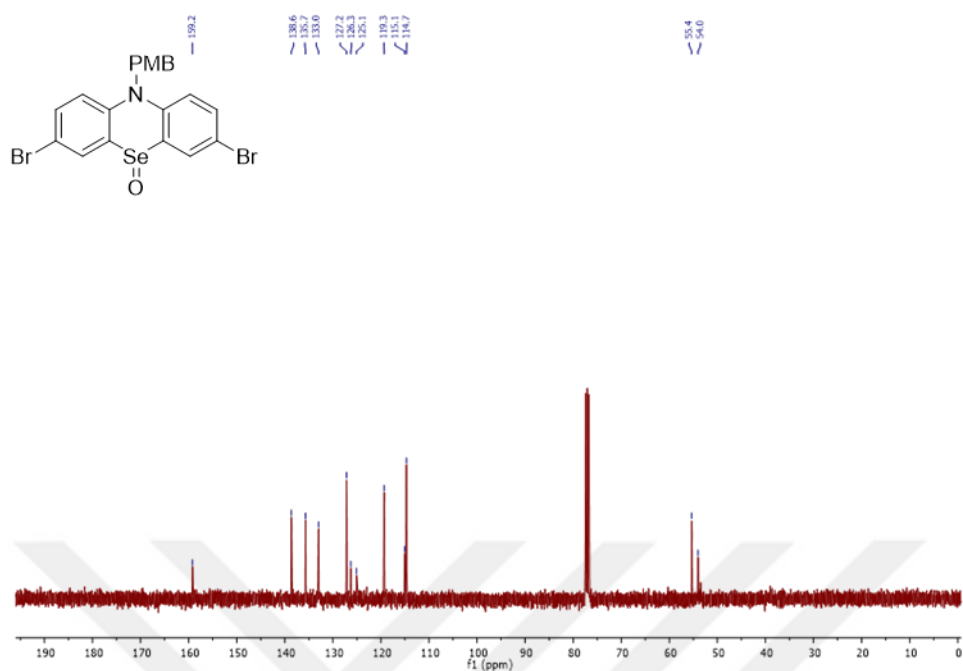


Figure A.135. ^{13}C NMR spectrum of compound **106** in CDCl_3

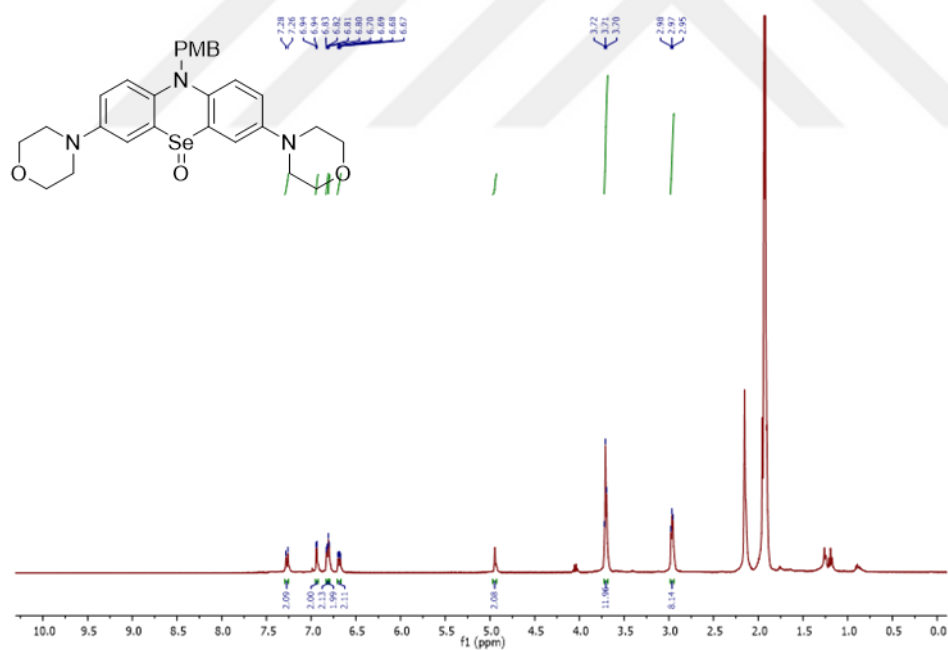


Figure A.136. ^1H NMR spectrum of compound **107** in CD_3CN

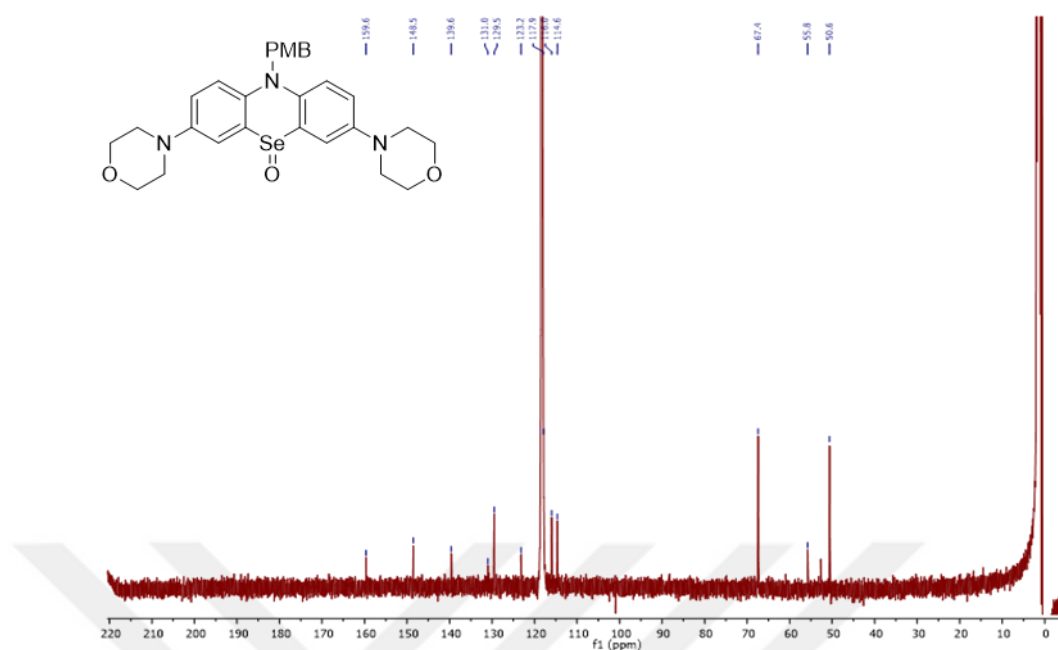


Figure A.137. ^{13}C NMR spectrum of compound **107** in CD_3CN

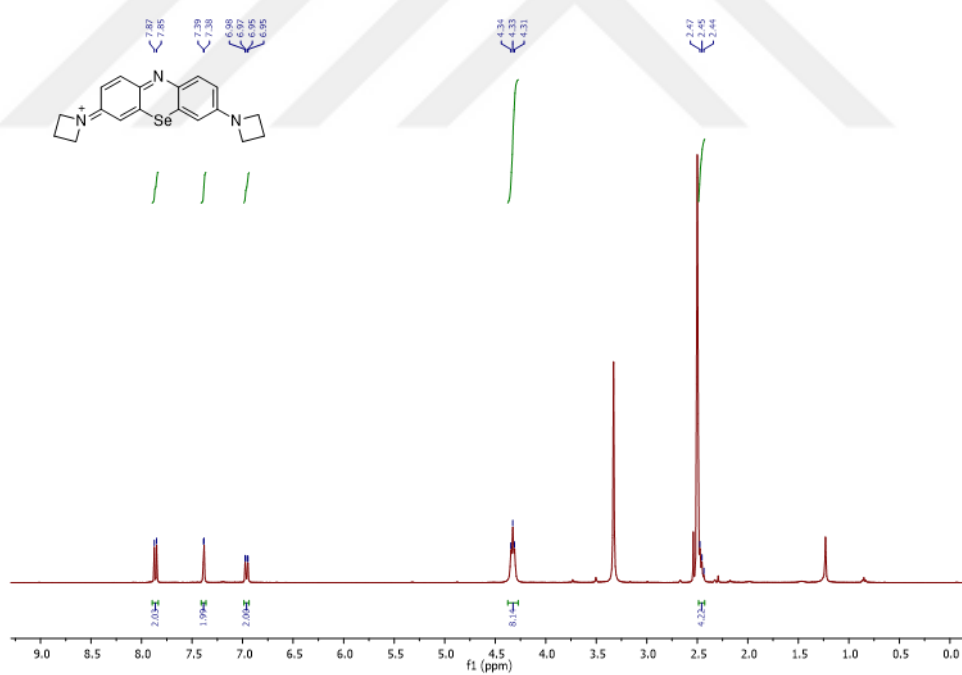


Figure A.138. ^1H NMR spectrum of **NSeAze** in DMSO

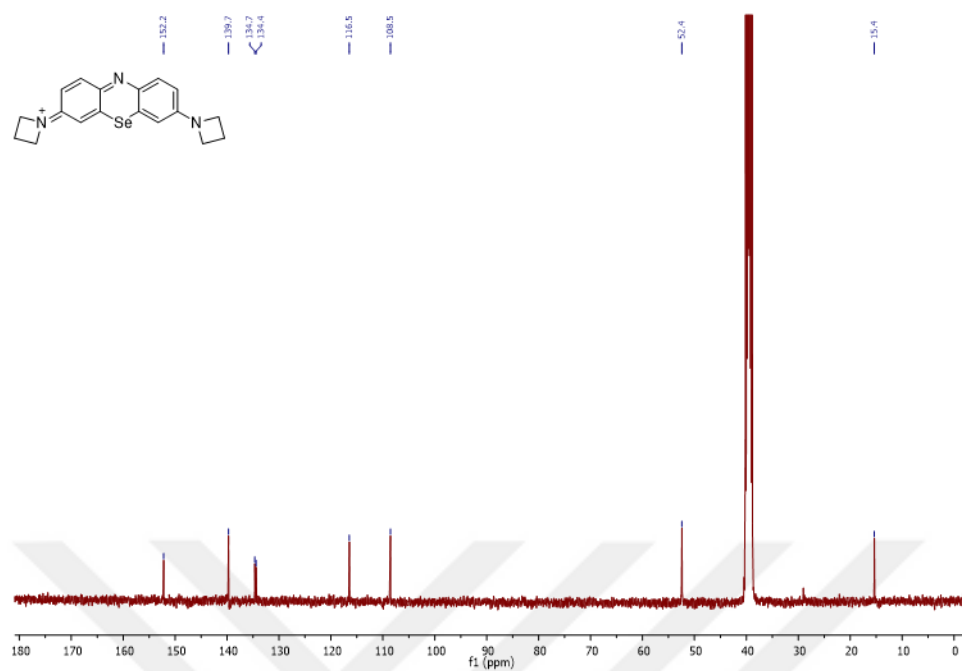


Figure A.139. ¹³C NMR spectrum of NSeAze in DMSO

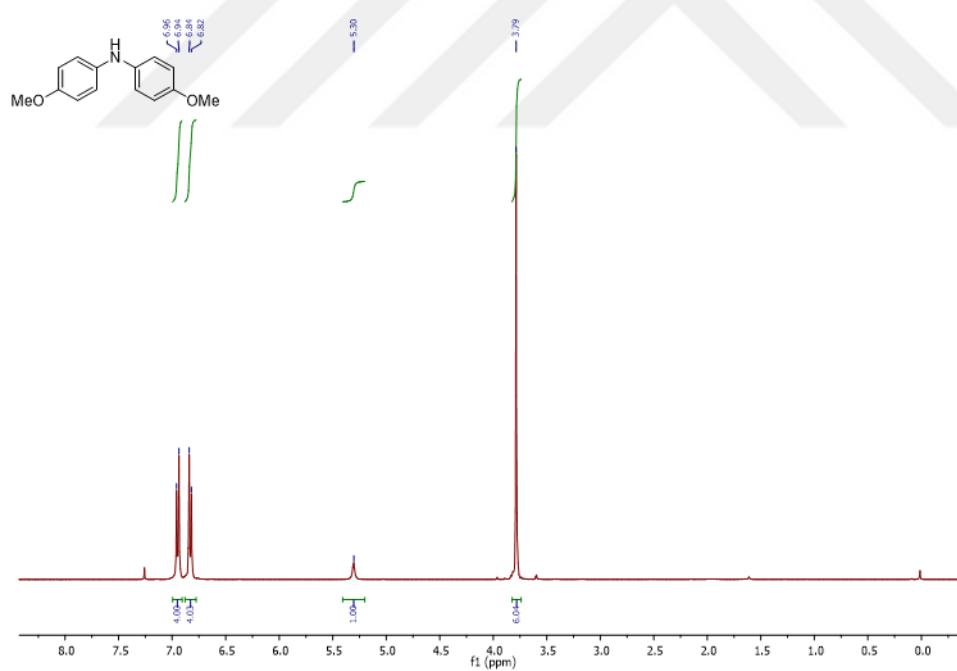


Figure A.140. ¹H NMR spectrum of compound **110** in CDCl₃

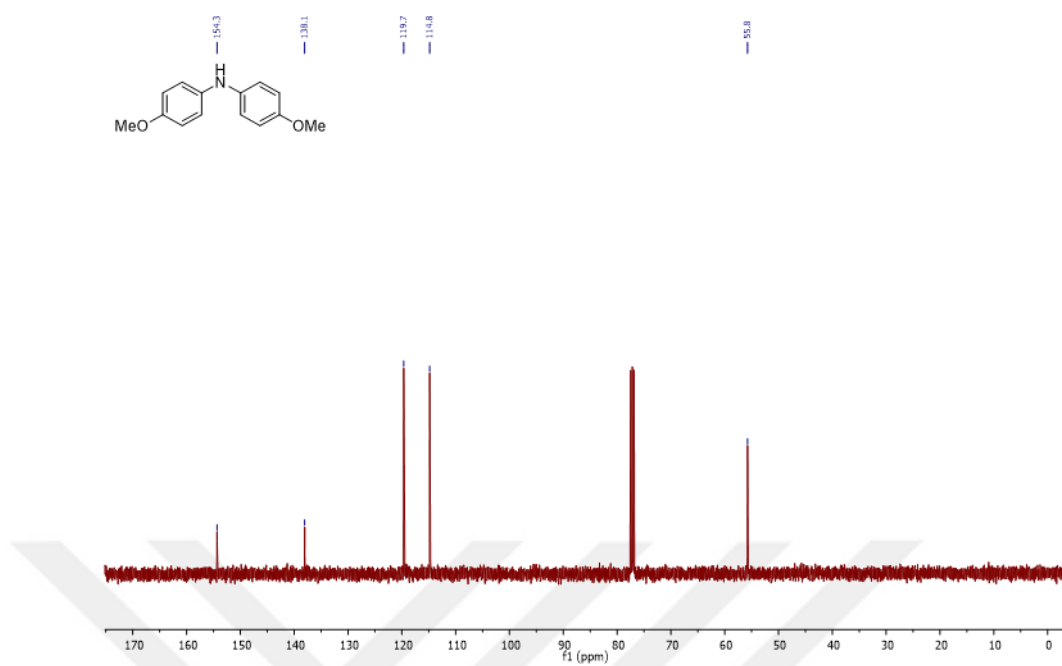


Figure A.141. ^{13}C NMR spectrum of compound **110** in CDCl_3

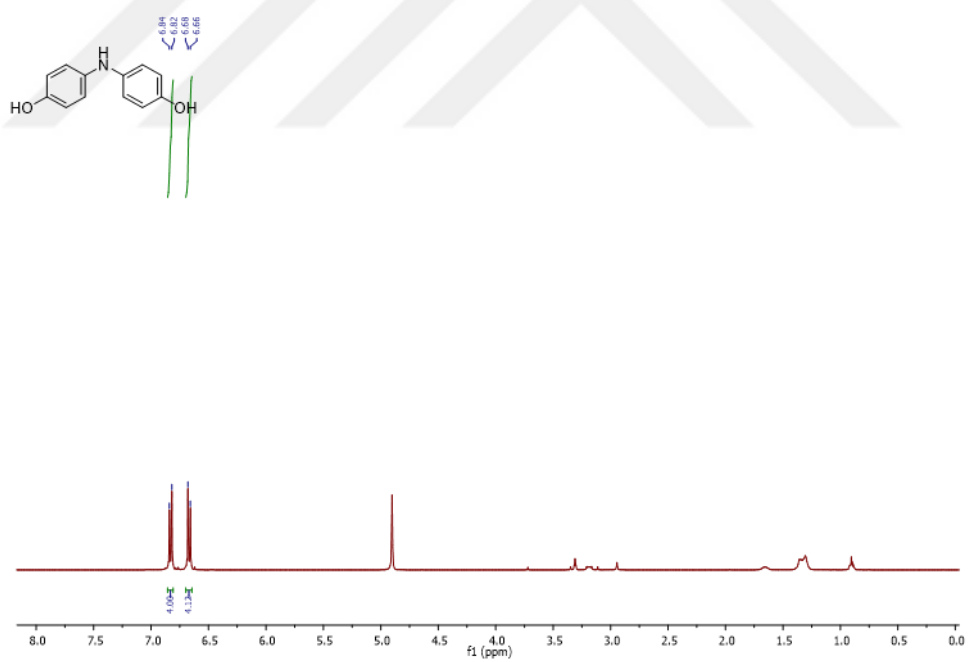


Figure A.142. ^1H NMR spectrum of compound **111** in MeOD

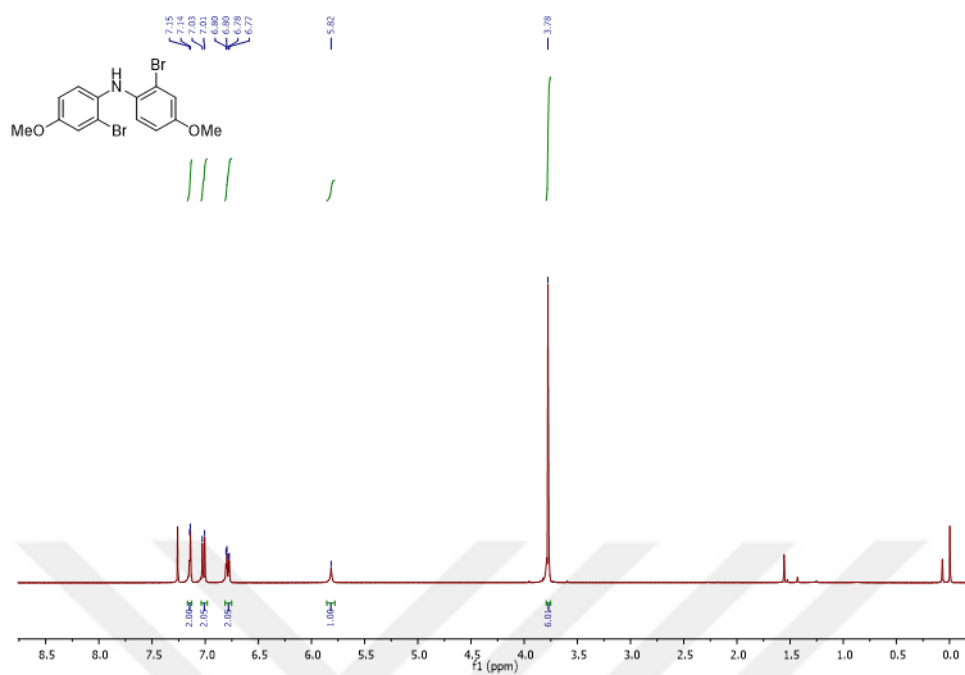


Figure A.143. ^1H NMR spectrum of compound **114** in CDCl_3

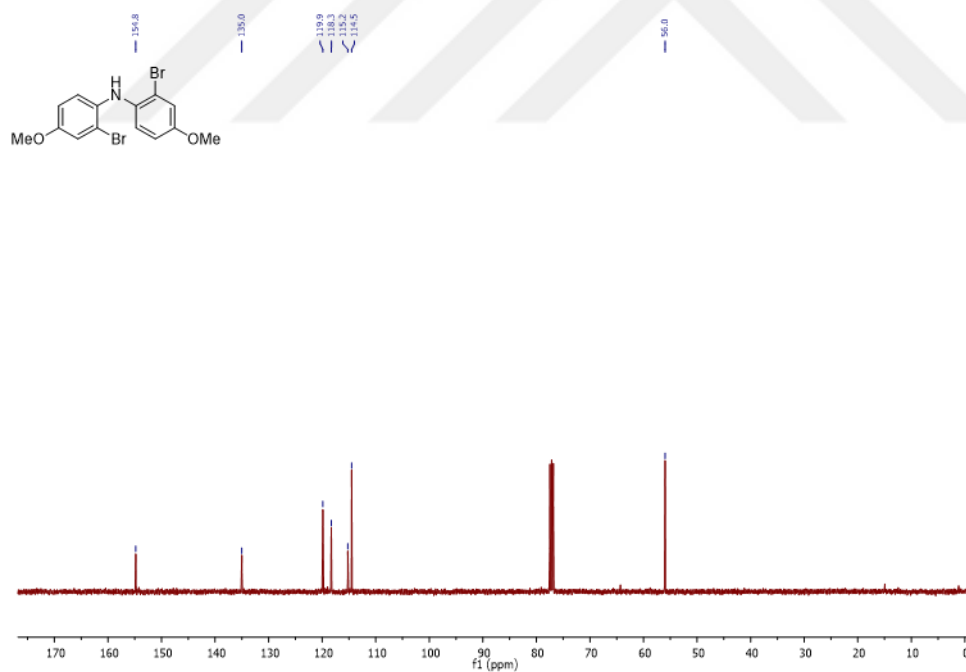


Figure A.144. ^{13}C NMR spectrum of compound **114** in CDCl_3

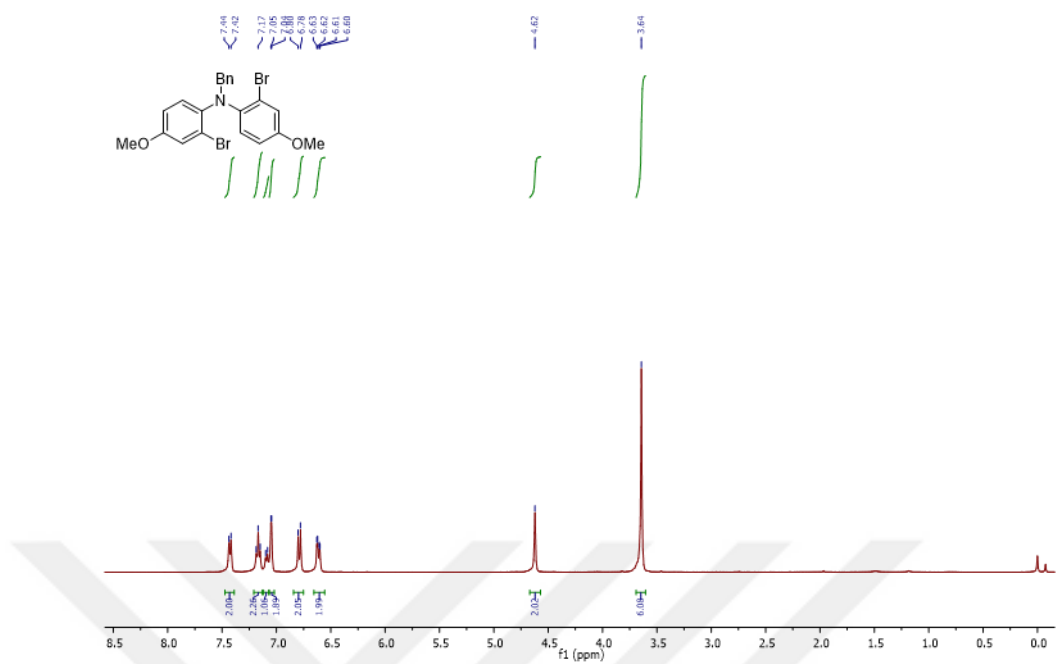


Figure A.145. ^1H NMR spectrum of compound **115** in CDCl_3

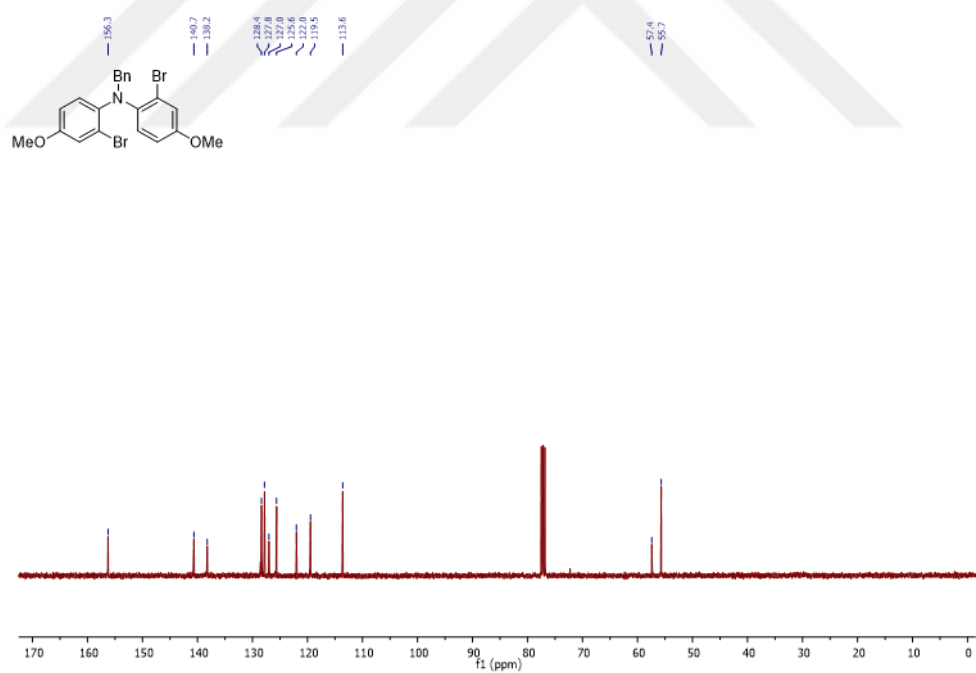


Figure A.146. ^{13}C NMR spectrum of compound **115** in CDCl_3

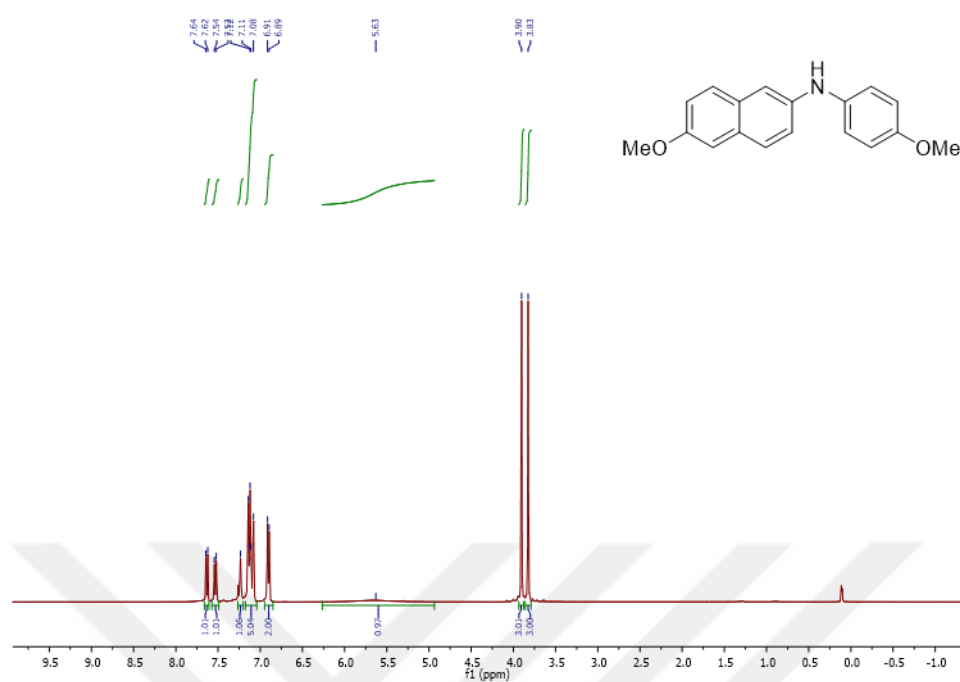


Figure A.147. ¹H NMR spectrum of compound **119** in CDCl₃

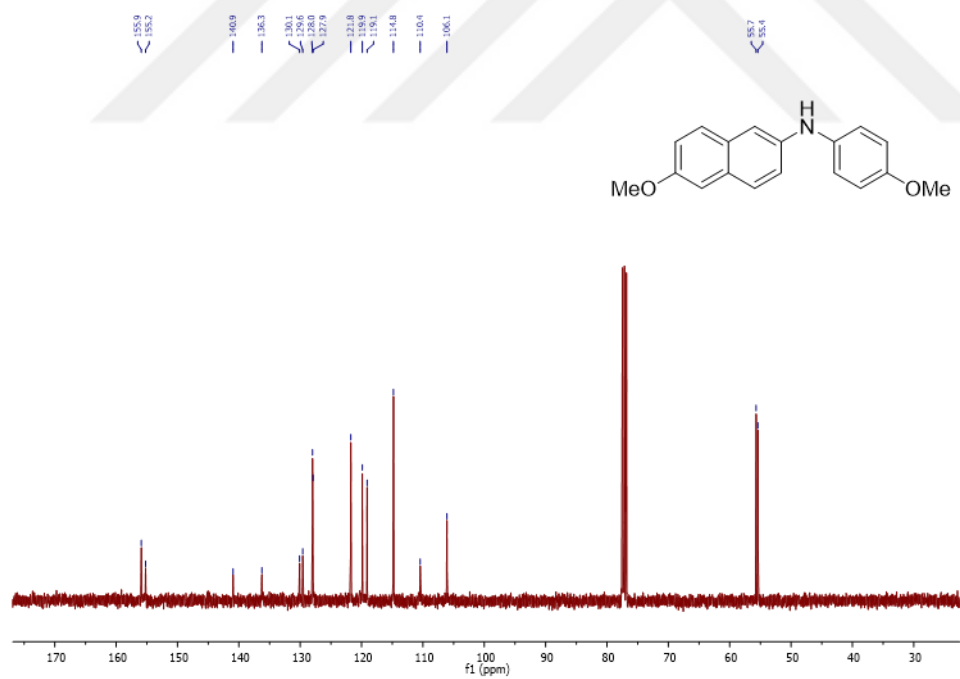


Figure A.148. ¹³C NMR spectrum of compound **119** in CDCl₃

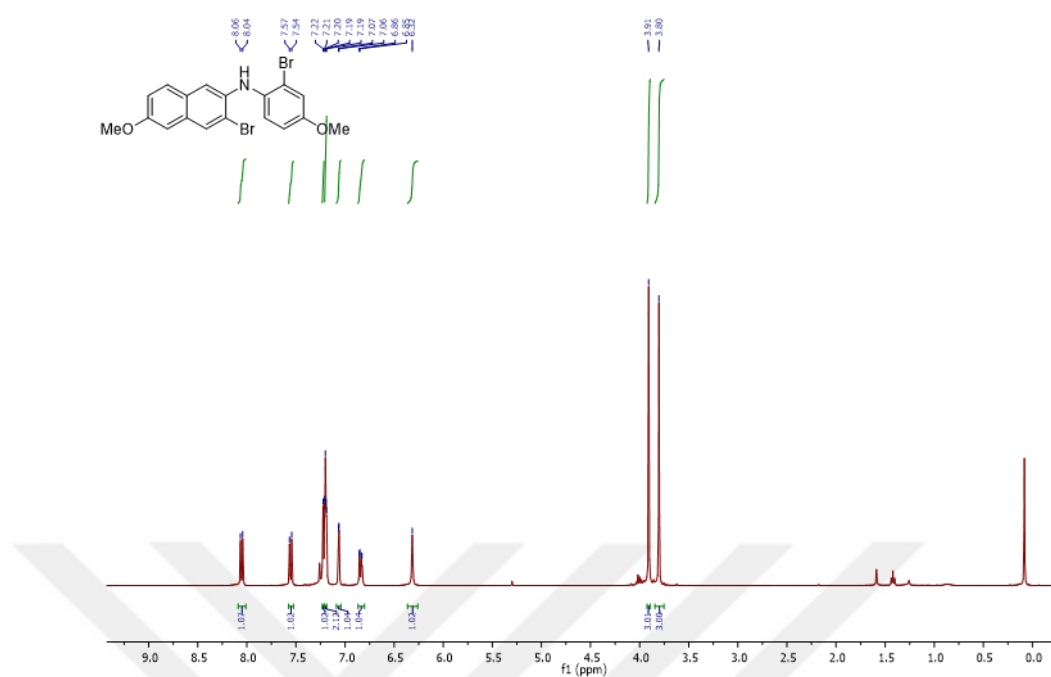


Figure A.149. ¹H NMR spectrum of compound **120** in CDCl₃

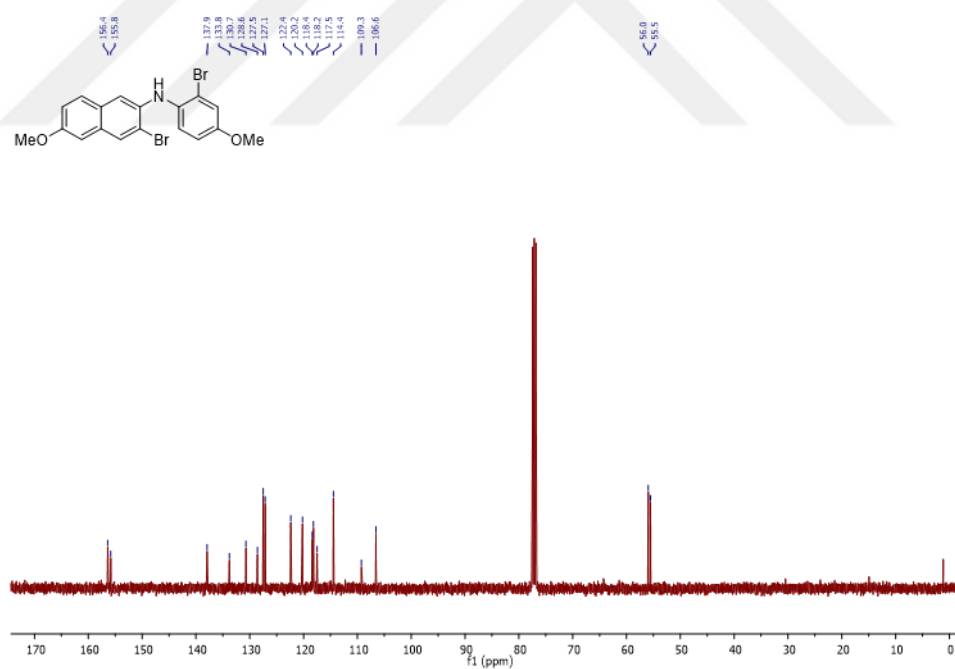


Figure A.150. ¹³C NMR spectrum of compound **120** in CDCl₃

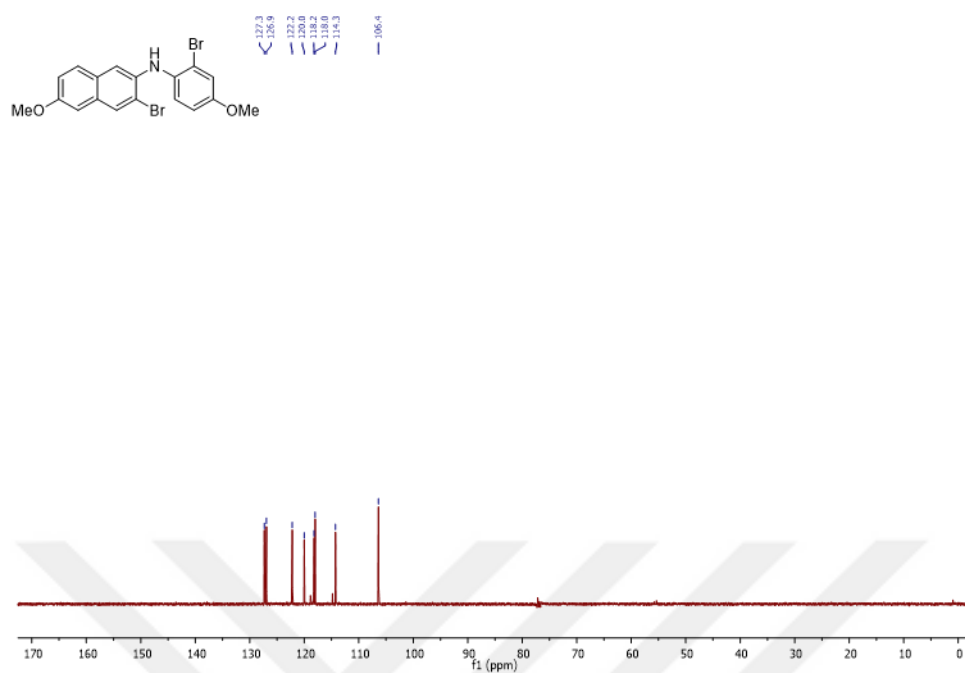


Figure A.151. DEPT90 NMR spectrum of compound **120** in CDCl_3

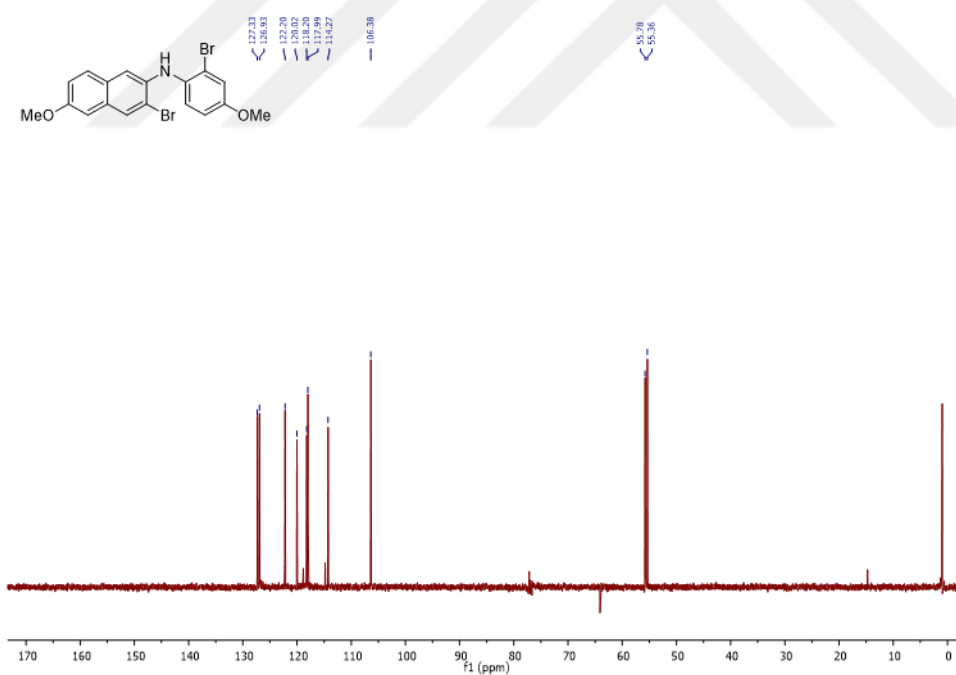


Figure A.152. DEPT135 NMR spectrum of compound **120** in CDCl_3

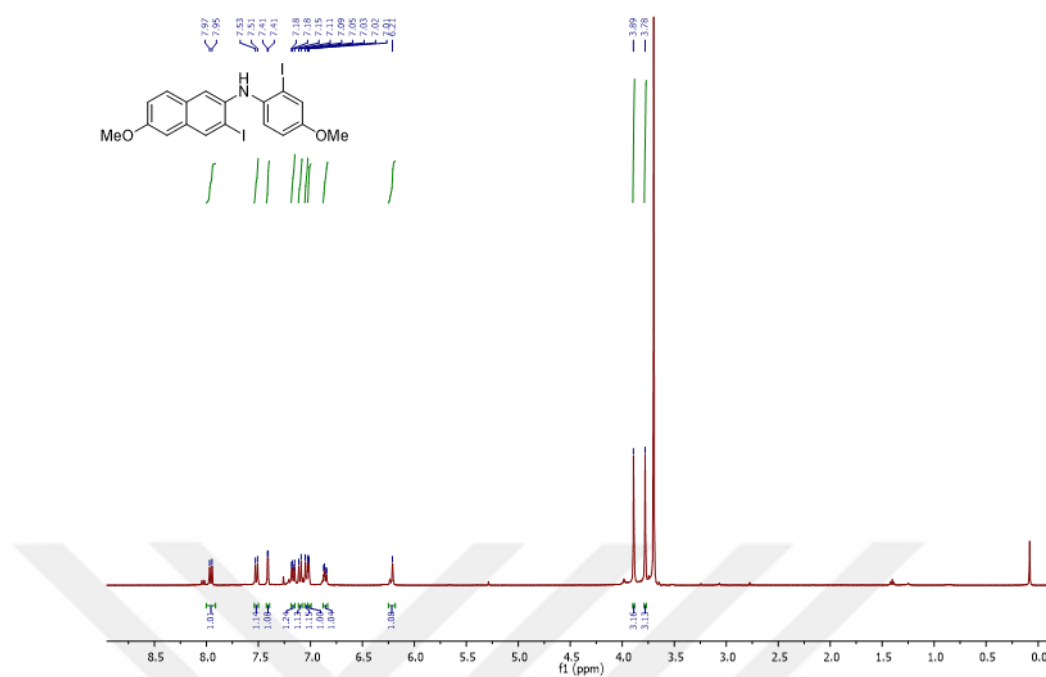


Figure A.153. ¹H NMR spectrum of compound **121** in CDCl₃

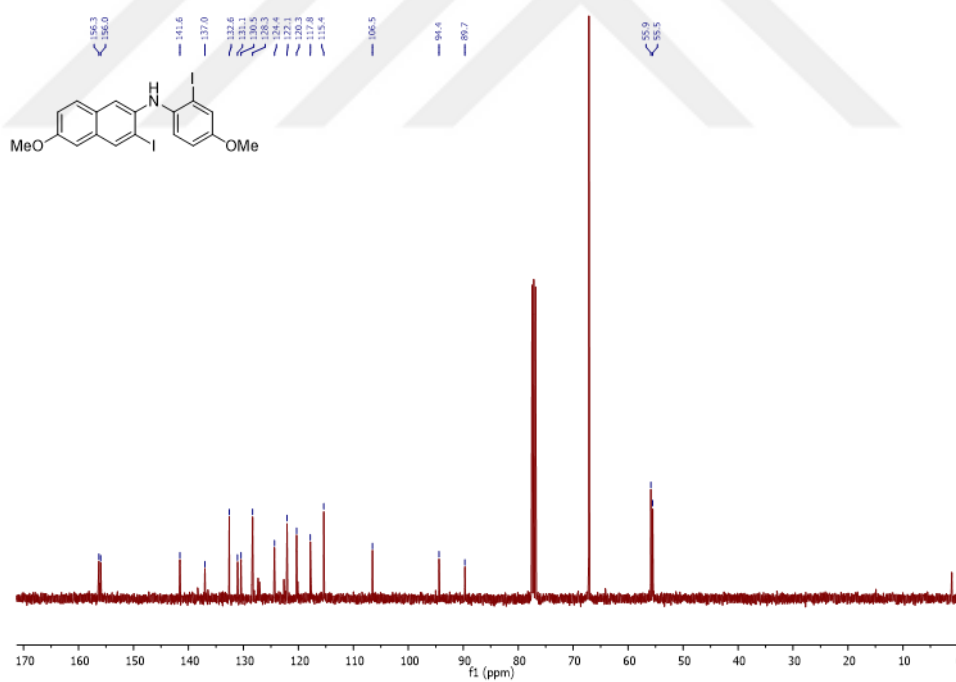


Figure A.154. ¹³C NMR spectrum of compound **121** in CDCl₃

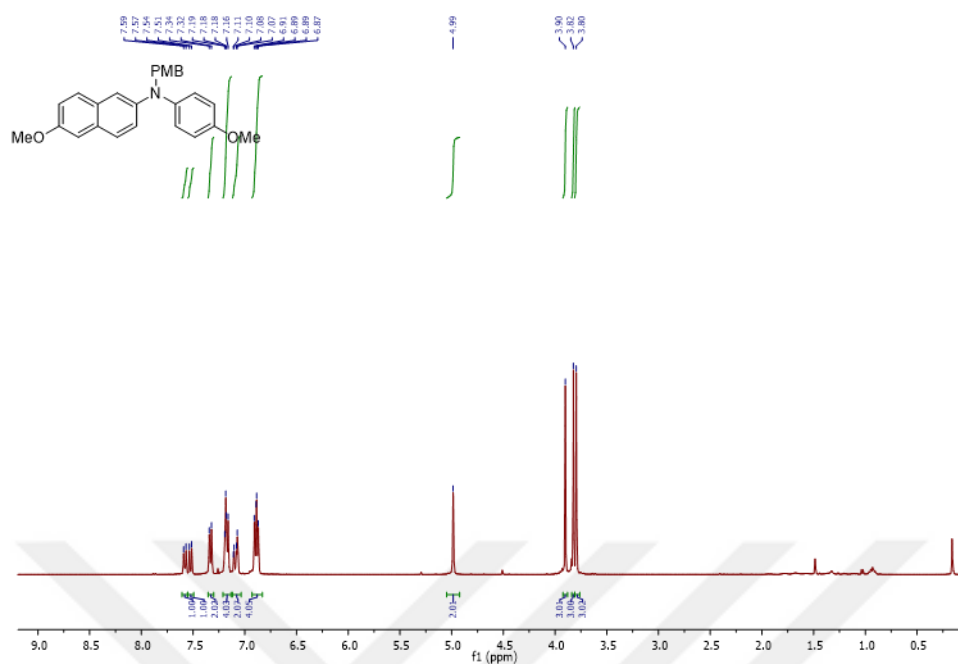


Figure A.155. ^1H NMR spectrum of compound **125** in CDCl_3

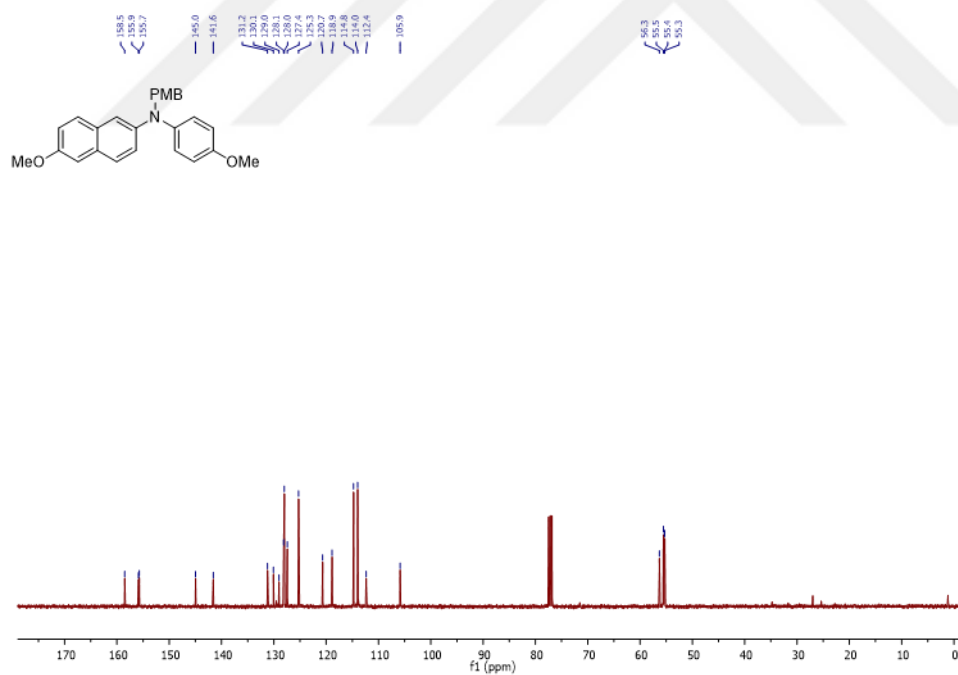
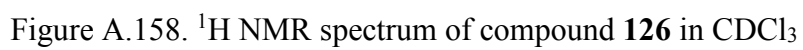
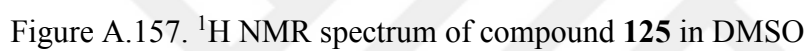
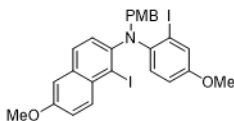


Figure A.156. ^{13}C NMR spectrum of compound **125** in CDCl_3





¹H NMR spectrum (CDCl₃) of 6,6'-dimethoxy-2,2'-bis(phenyl)diselenane. The spectrum displays aromatic signals in the range of 6.5–8.0 ppm and methoxy singlets at approximately 3.8 ppm. Integration values are provided below the peaks. Chemical structures of the reactants are shown above the spectrum.

[illegible]

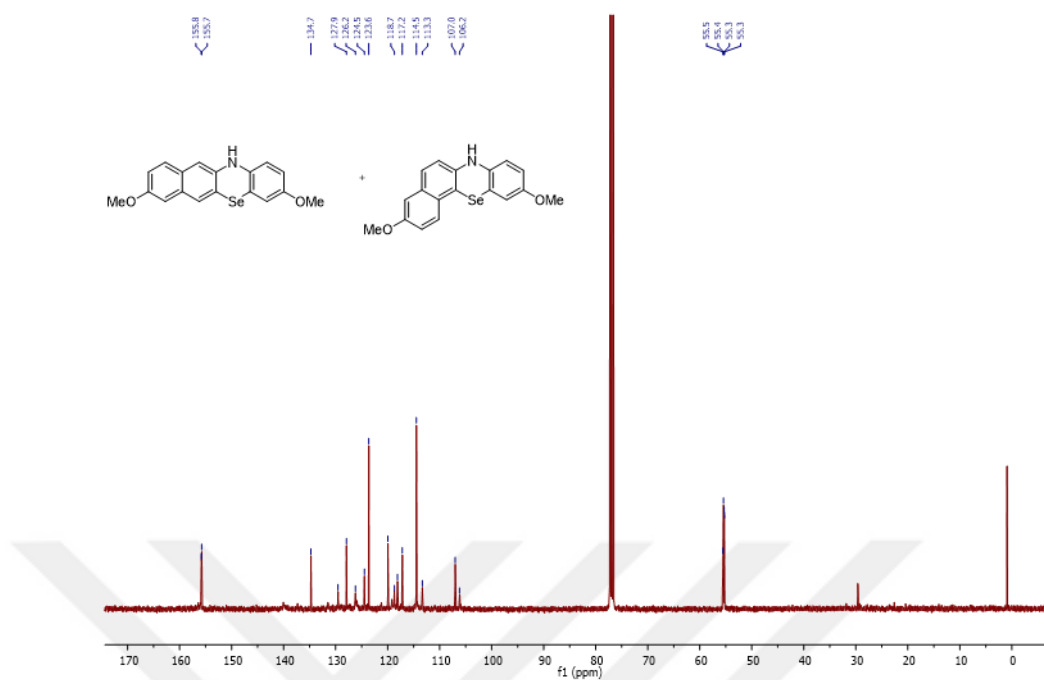


Figure A.161. ^{13}C NMR spectrum of compounds **122a** and **122b** in CDCl_3

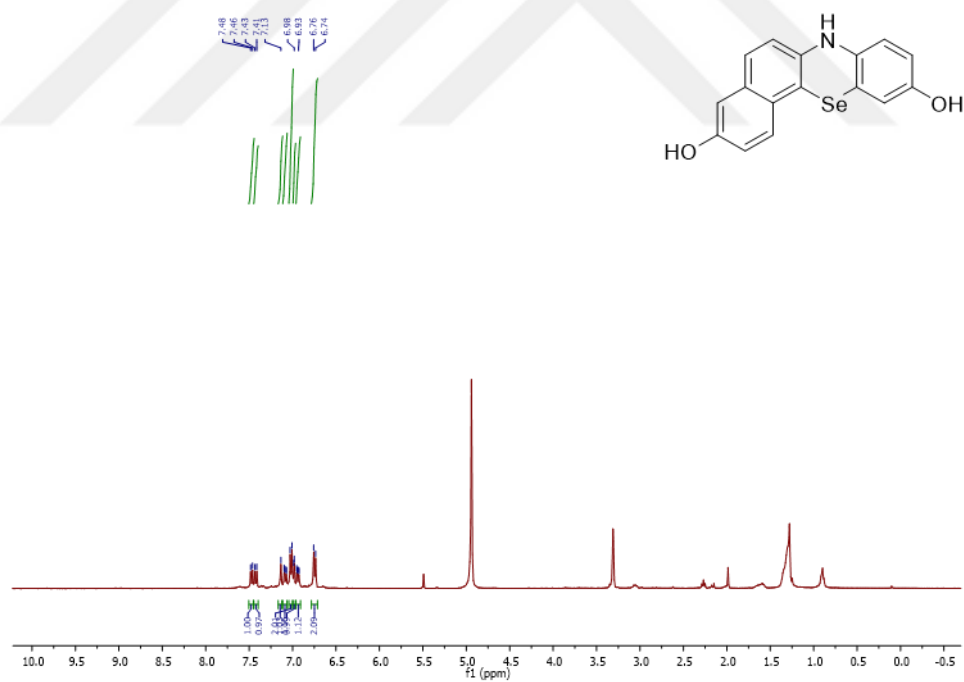


Figure A.162. ^1H NMR spectrum of compound **123b** in CDCl_3

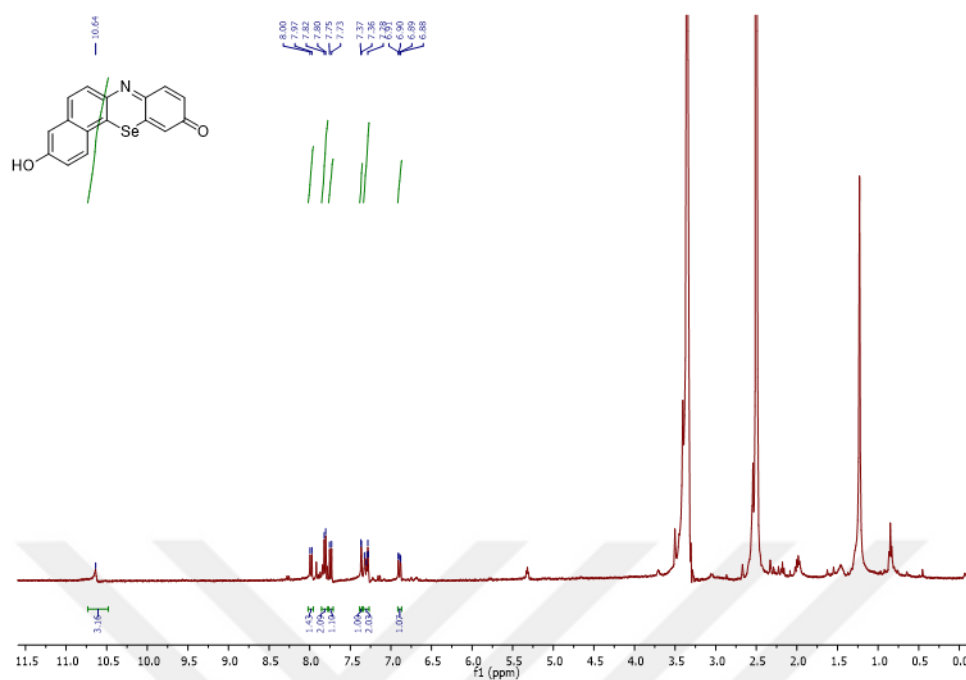


Figure A.163. ^1H NMR spectrum of compound **NSeO-A** in DMSO

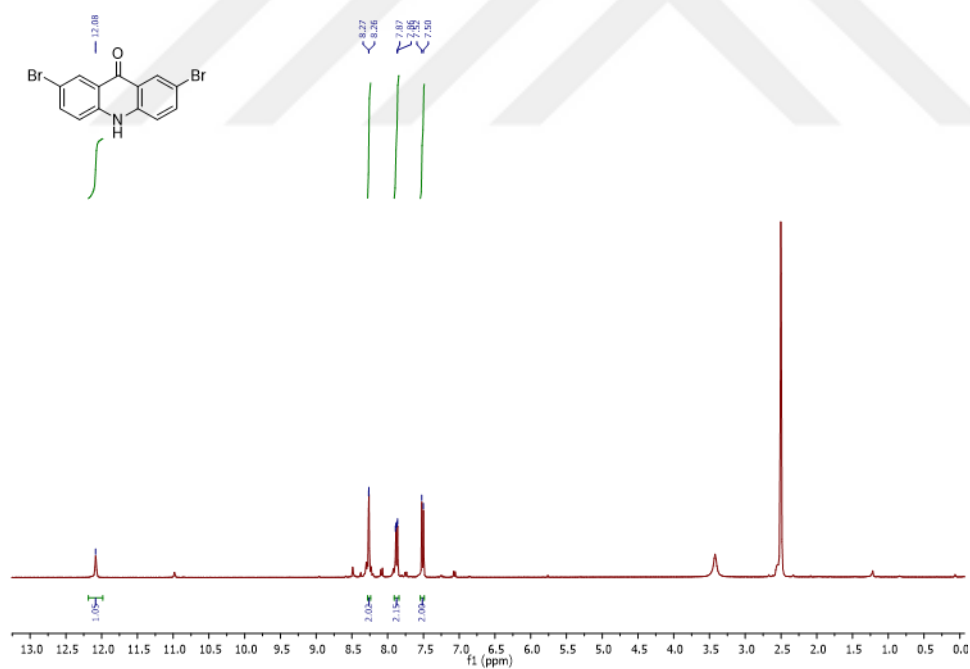


Figure A.164. ^1H NMR spectrum of compound **129** in DMSO

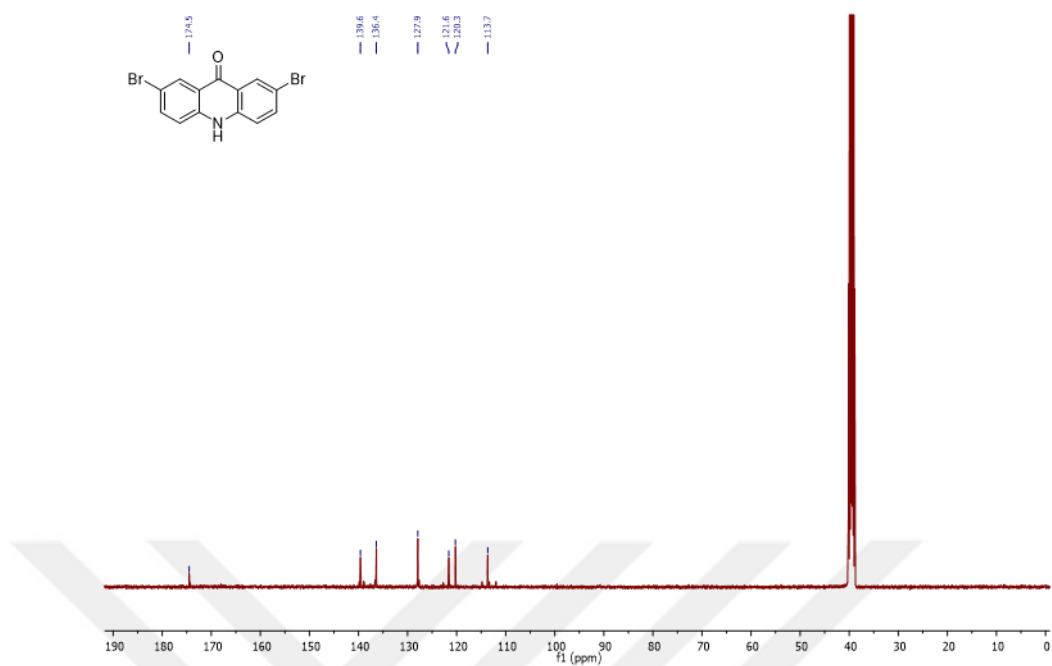


Figure A.165. ^{13}C NMR spectrum of compound **129** in DMSO

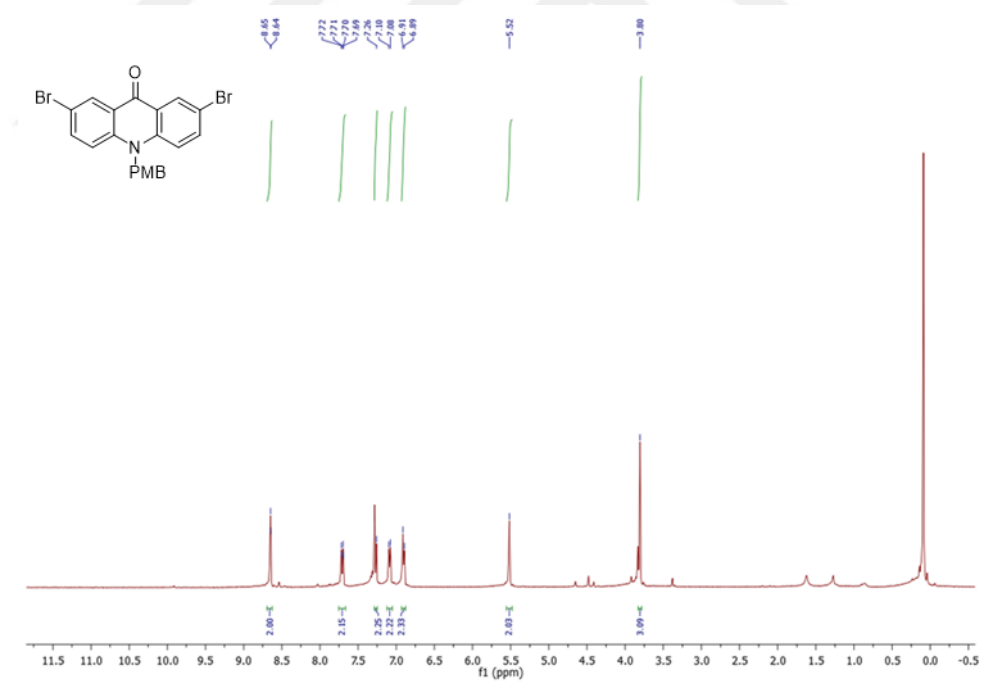
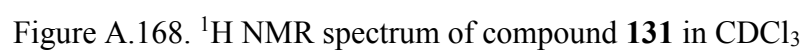


Figure A.166. ^1H NMR spectrum of compound **130** in CDCl_3



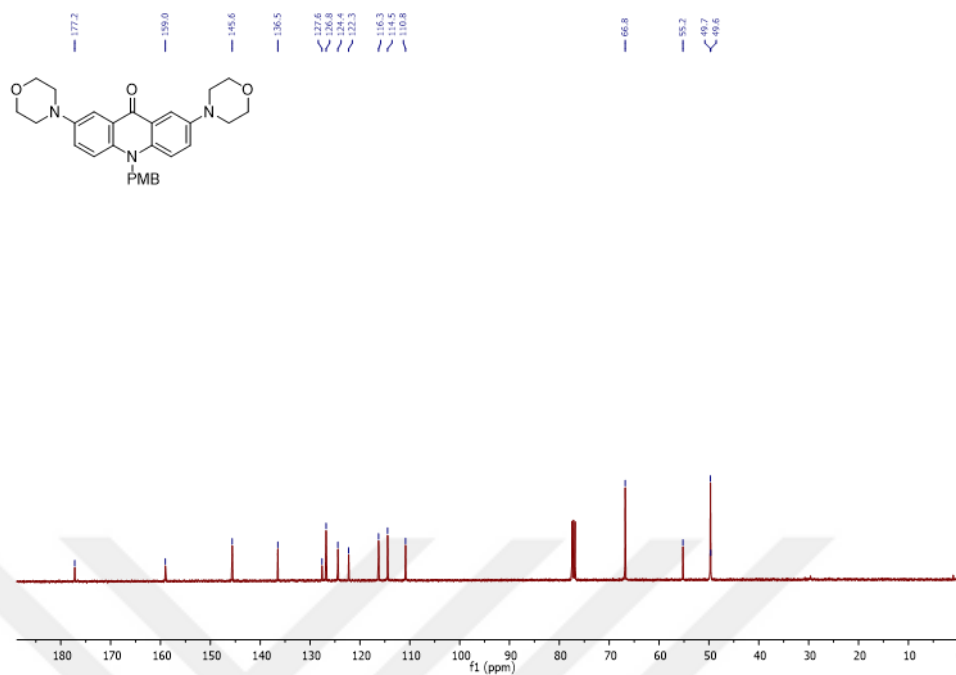


Figure A.169. ^{13}C NMR spectrum of compound **131** in CDCl_3

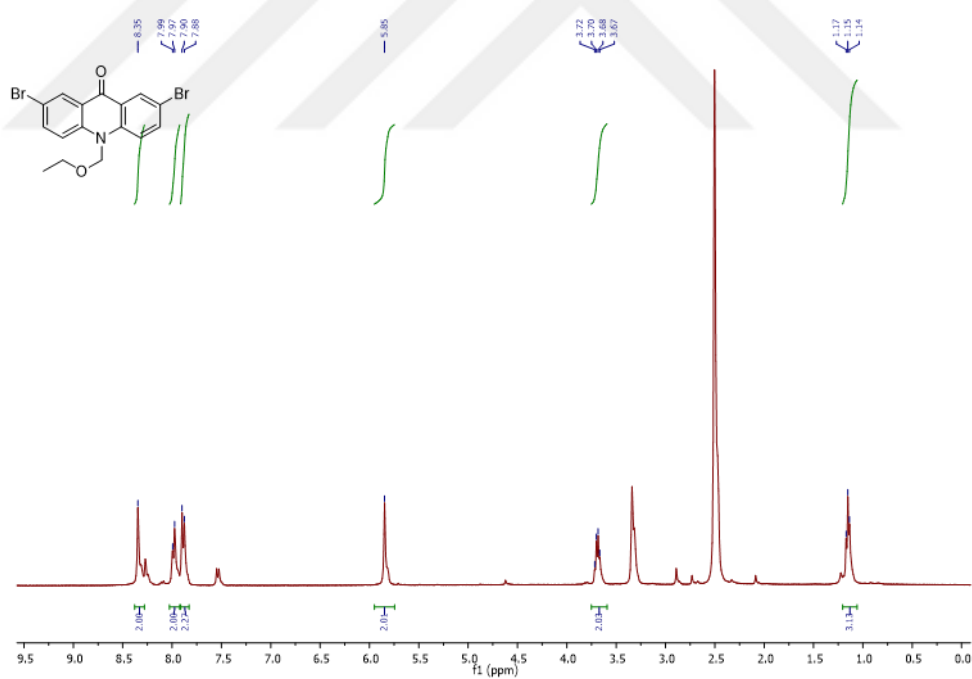


Figure A.170. ^1H NMR spectrum of compound **132** in CDCl_3

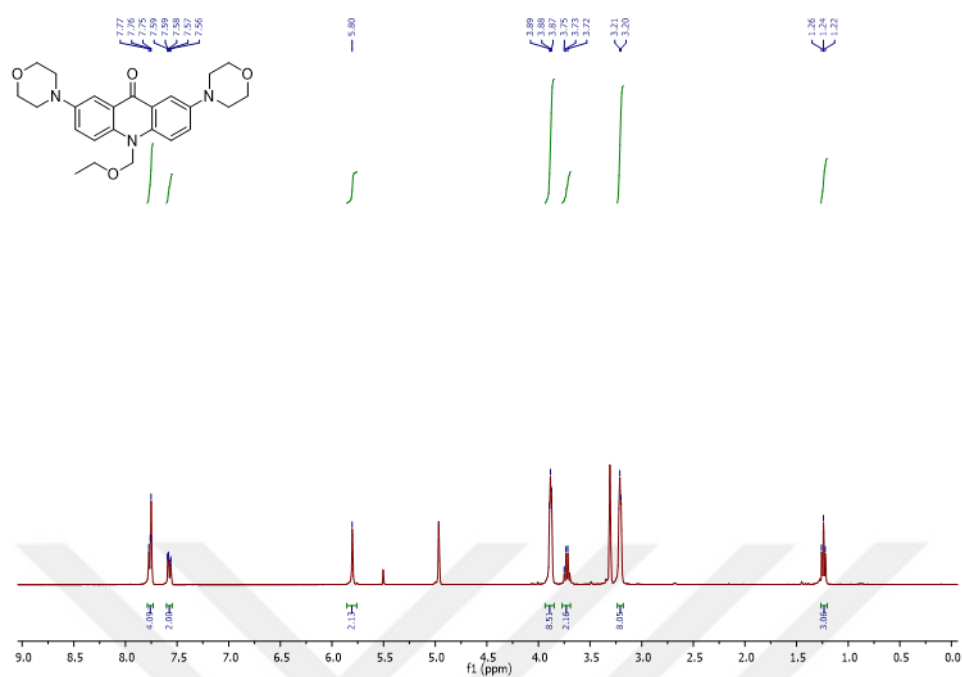


Figure A.171. ^1H NMR spectrum of compound **133** in MeOD

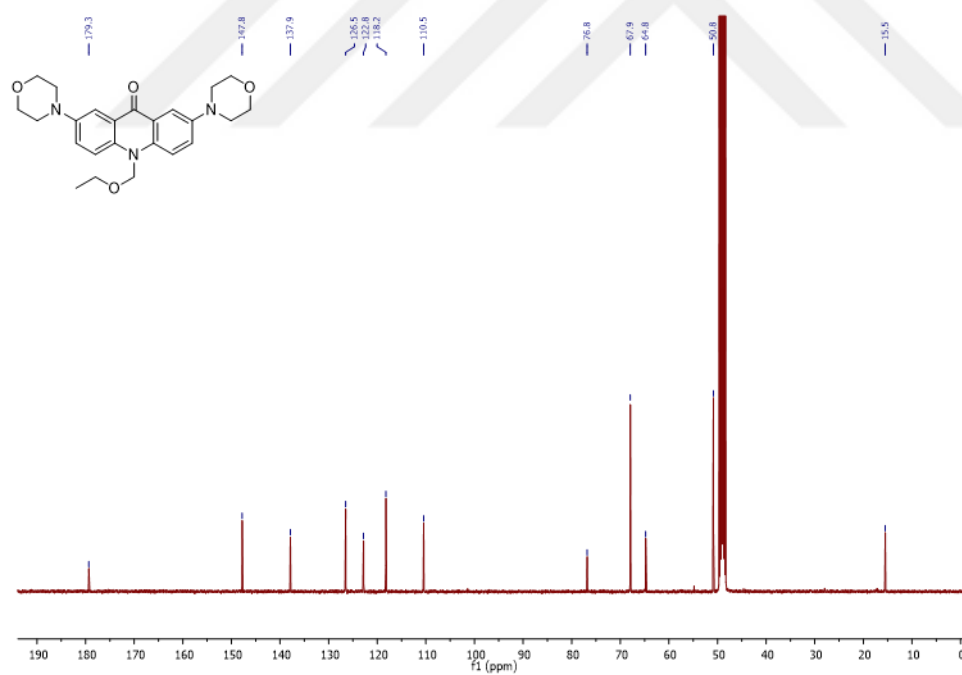


Figure A.172. ^{13}C NMR spectrum of compound **133** in MeOD

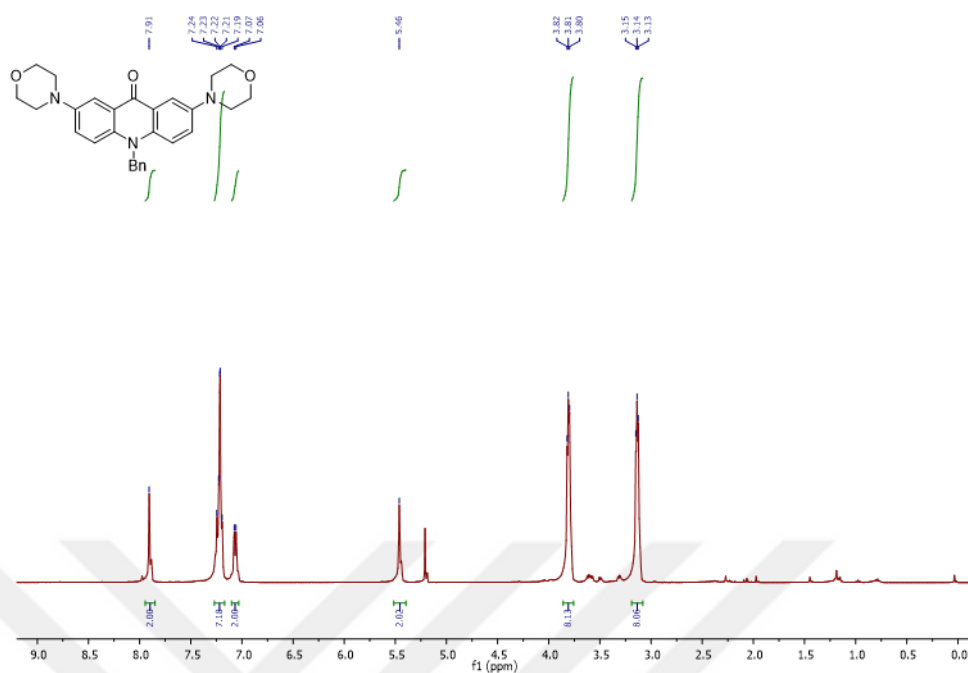


Figure A.173. ^1H NMR spectrum of compound **135** in CDCl₃

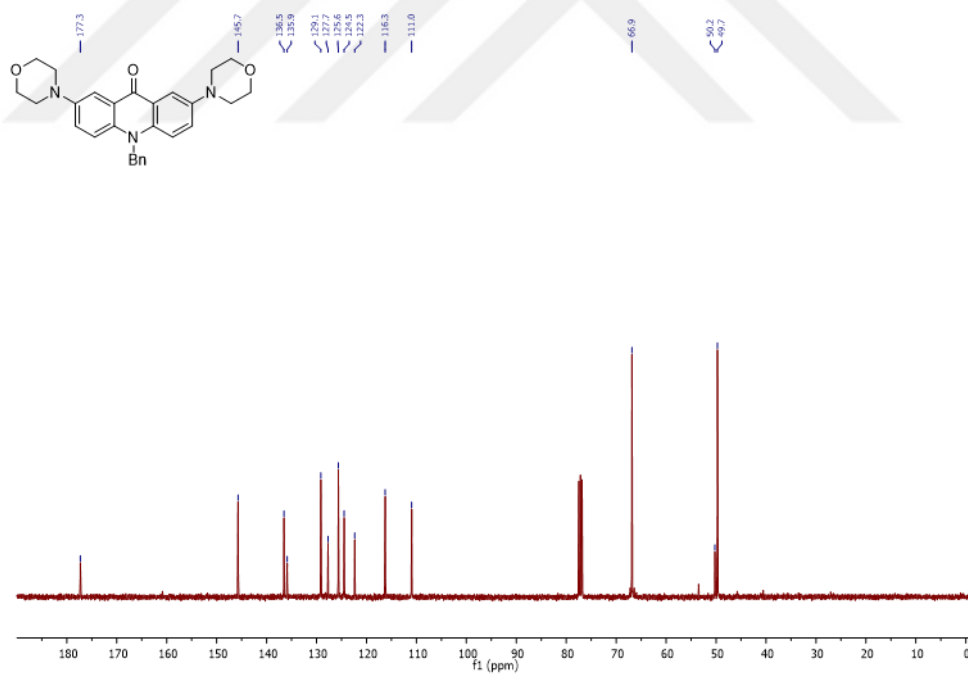


Figure A.174. ^{13}C NMR spectrum of compound **135** in CDCl₃

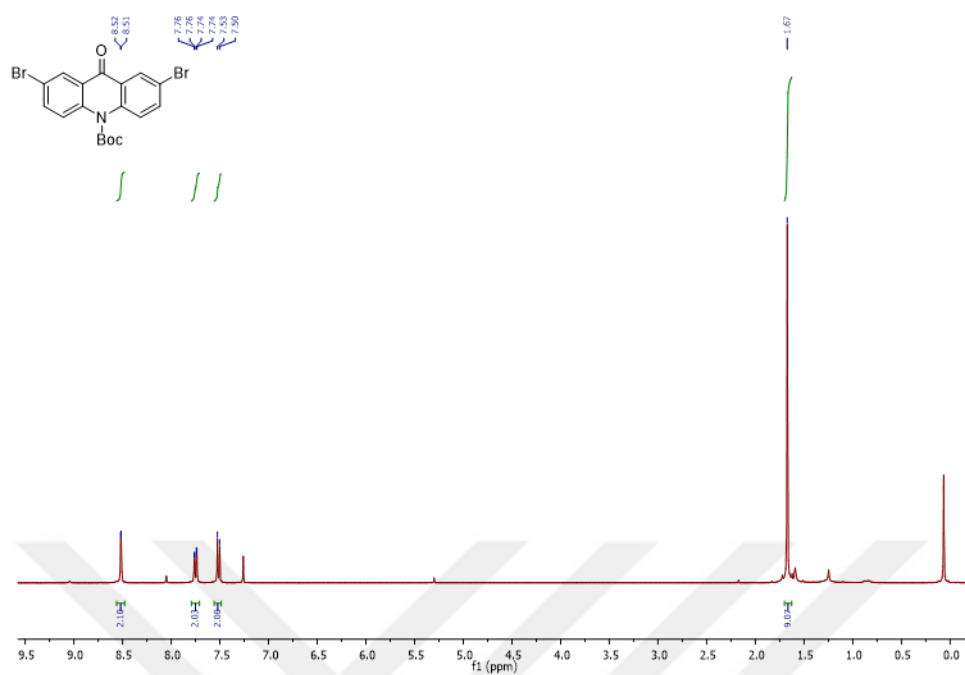


Figure A.175. ^1H NMR spectrum of compound **136** in CDCl_3

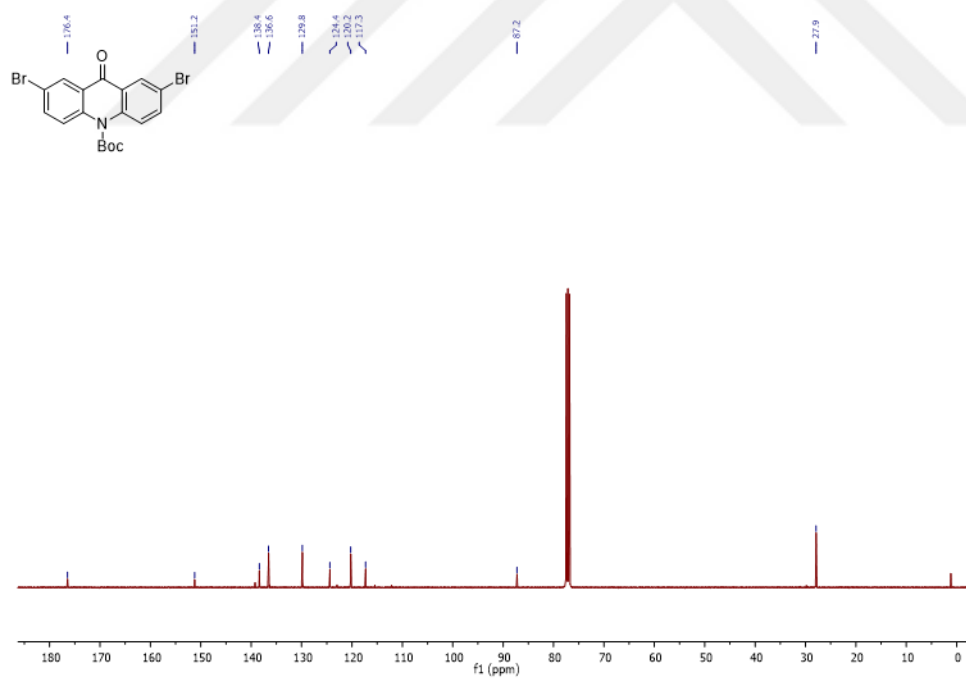


Figure A.176. ^{13}C NMR spectrum of compound **136** in CDCl_3

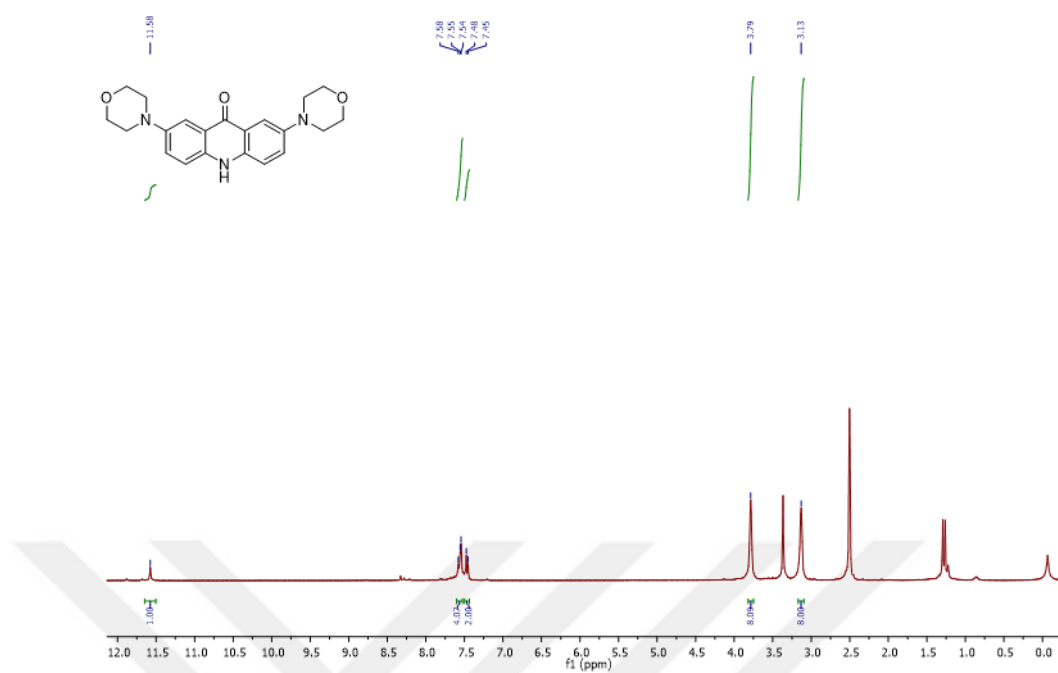


Figure A.177. ¹H NMR spectrum of compound **137** in DMSO

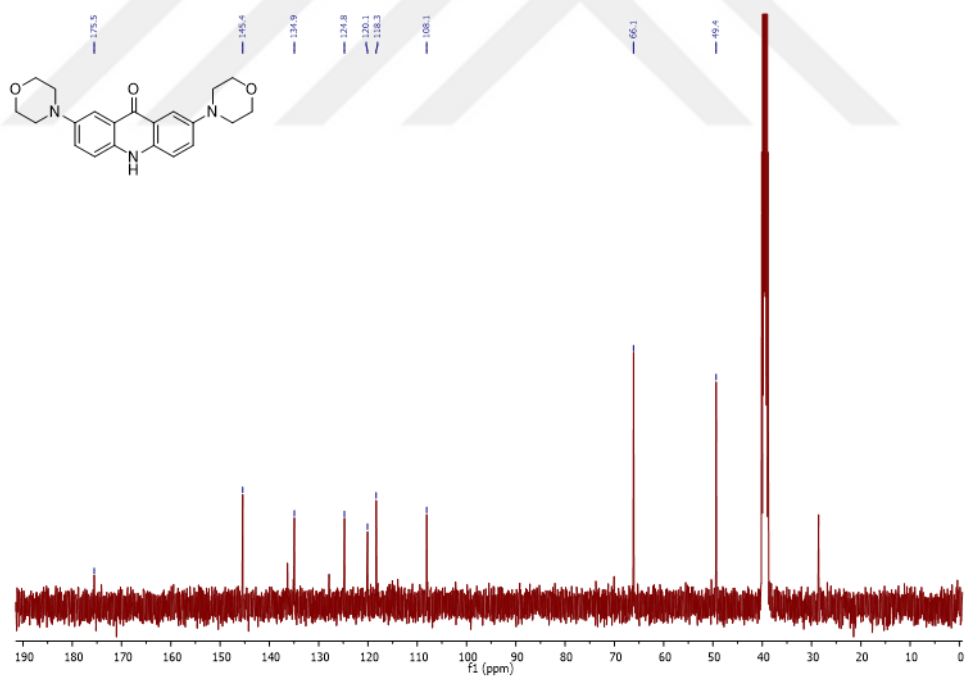


Figure 6.178. ¹³C NMR spectrum of compound **137** in DMSO

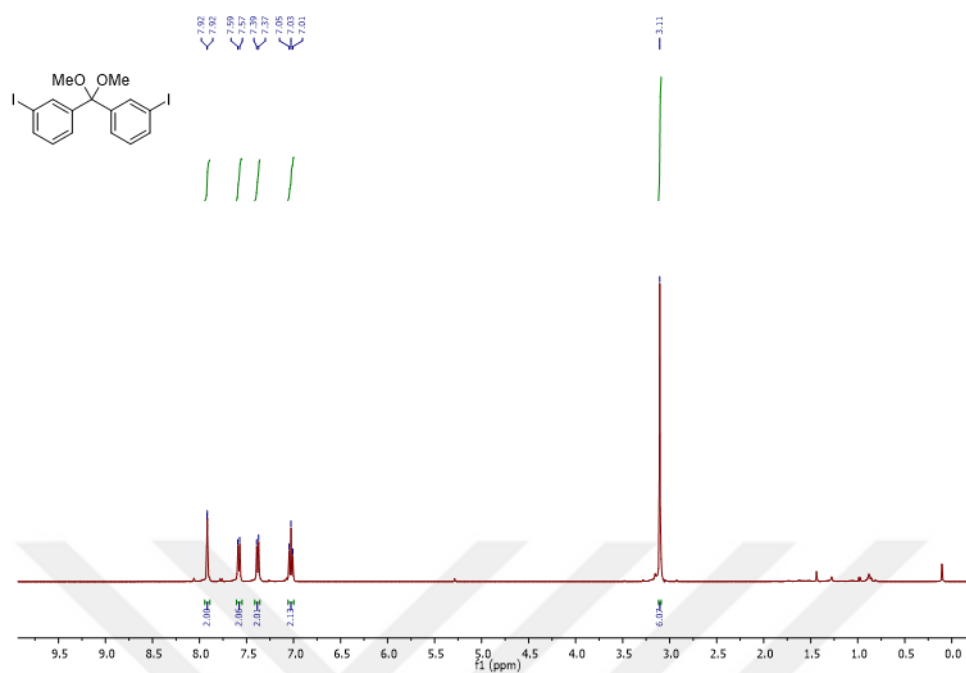


Figure A.179. ^1H NMR spectrum of compound **141** in CDCl₃

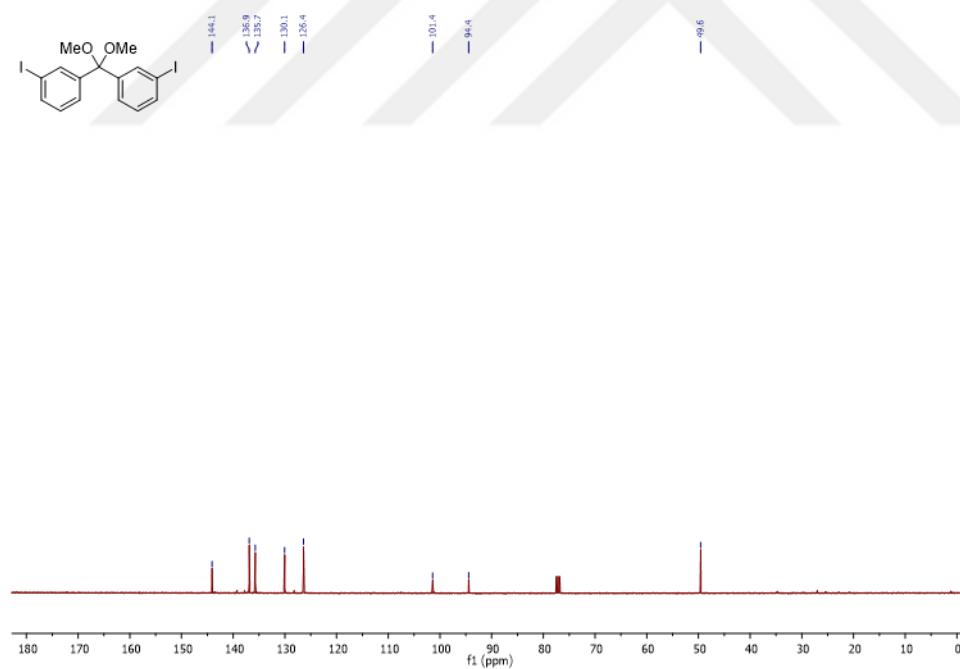


Figure A.180. ^{13}C NMR spectrum of compound **141** in CDCl₃

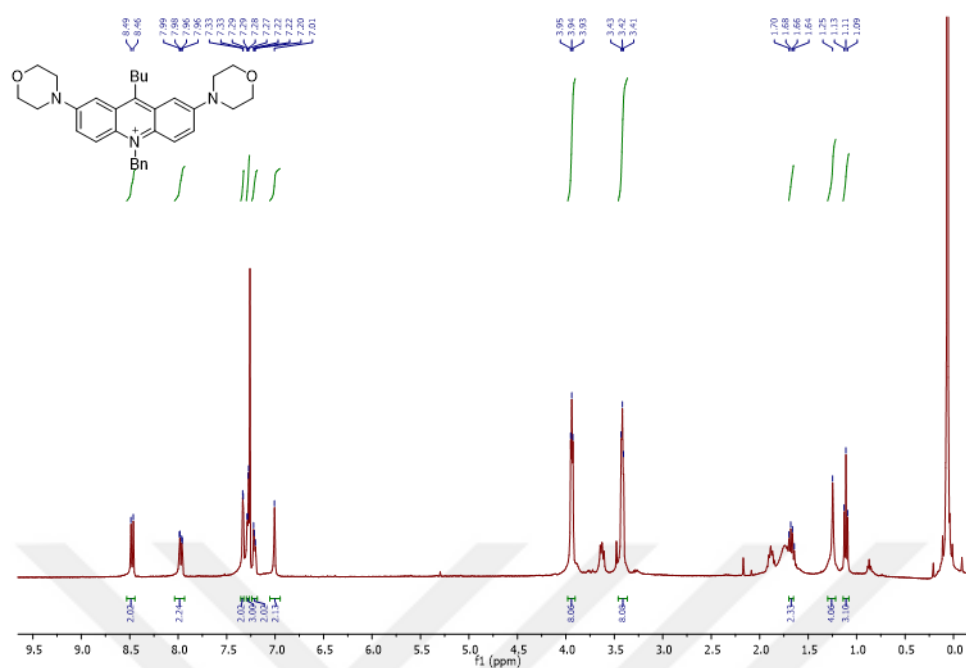


Figure A.183. ¹H NMR spectrum of compound **150** in CDCl₃

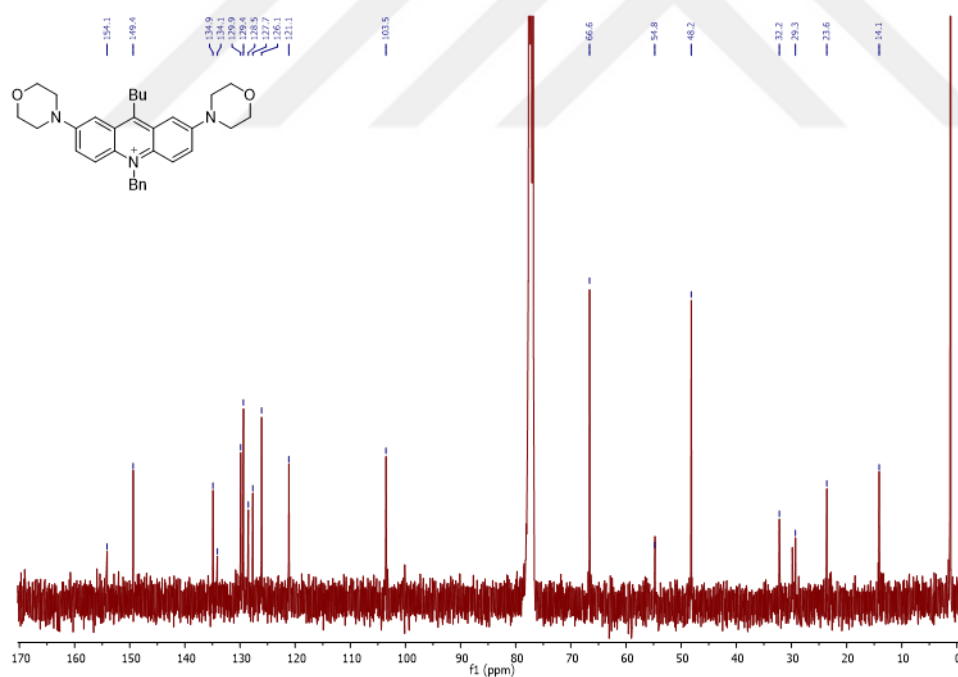


Figure A.184. ¹³C NMR spectrum of compound **150** in CDCl₃

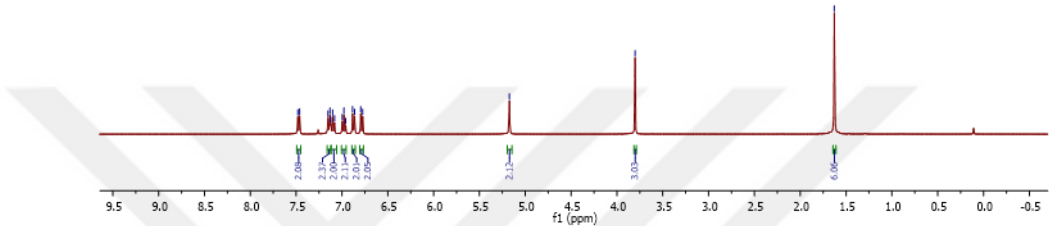


Figure A.185. ^1H NMR spectrum of compound **152** in CDCl_3

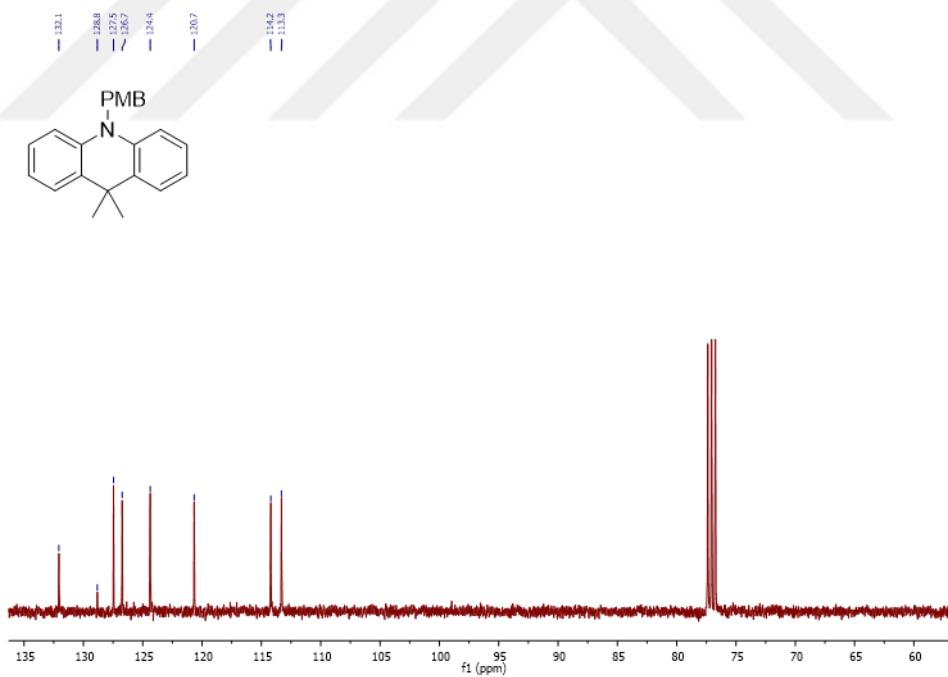


Figure A.186. ^{13}C NMR spectrum of compound **152** in CDCl_3

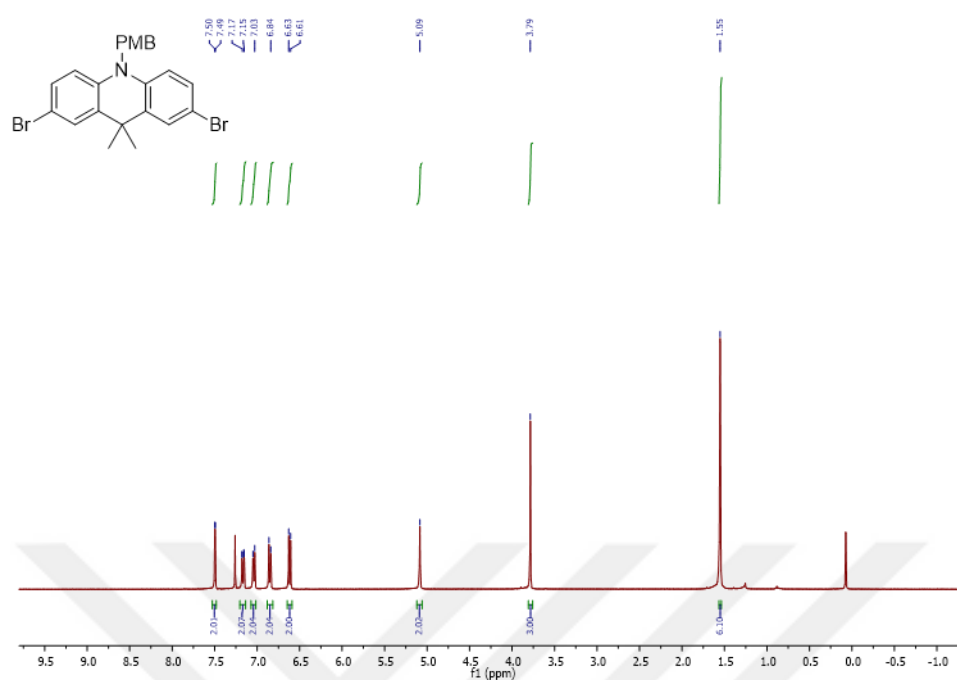


Figure A.187. ^1H NMR spectrum of compound **153** in CDCl_3

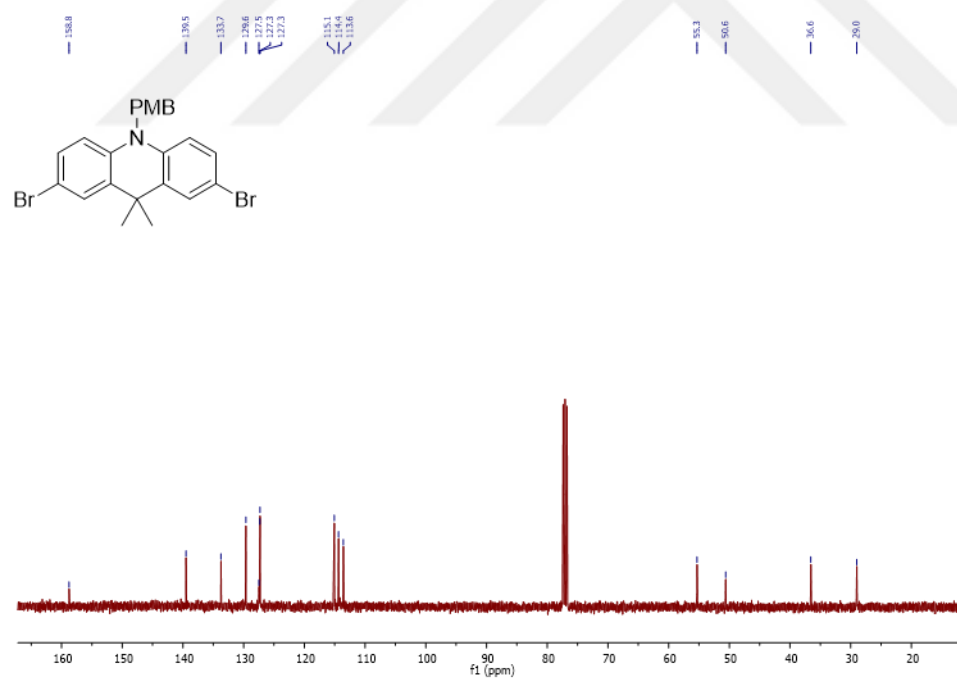


Figure A.188. ^{13}C NMR spectrum of compound **153** in CDCl_3

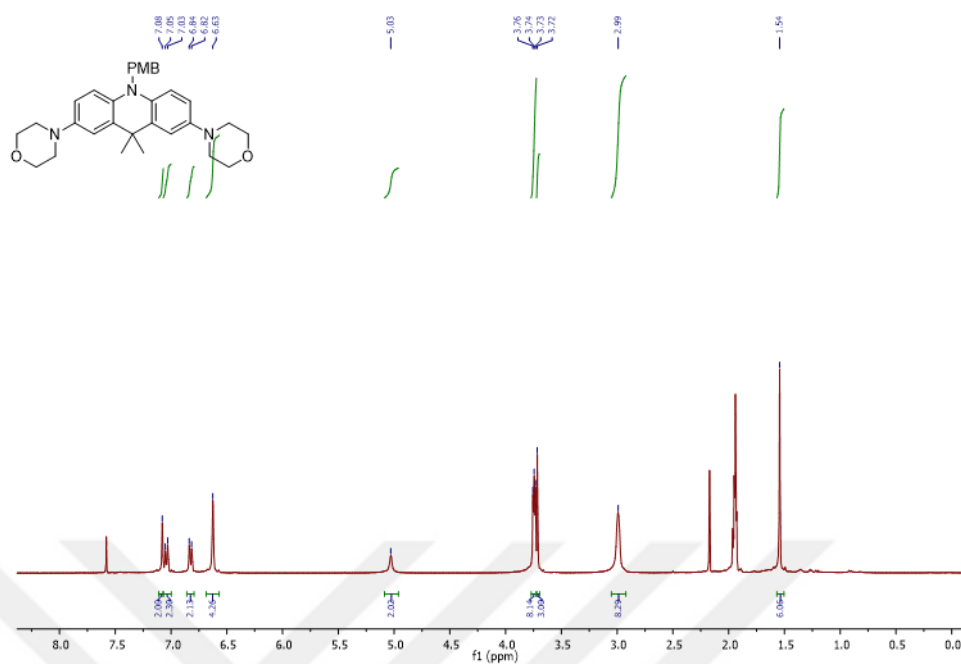


Figure A.189. ¹H NMR spectrum of compound **154** in CD₃CN

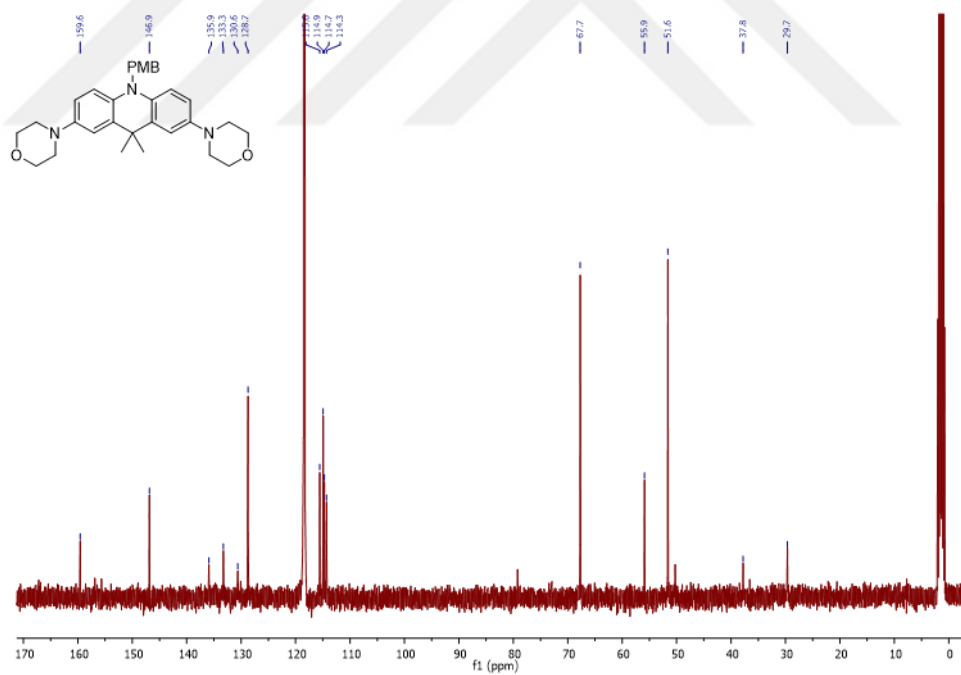


Figure A.190. ¹³C NMR spectrum of compound **154** in CD₃CN

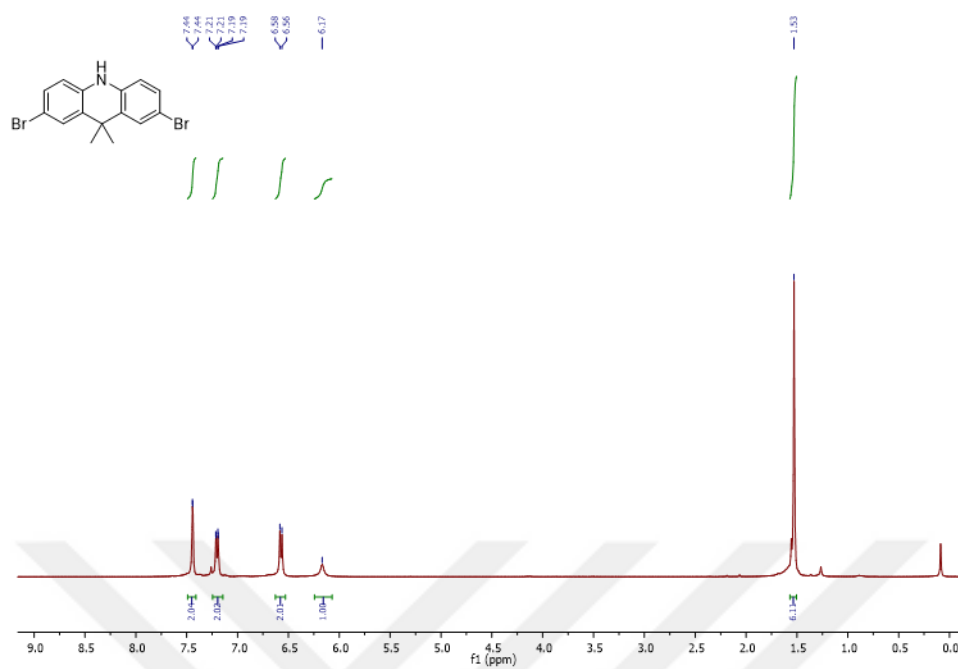


Figure A.191. ^1H NMR spectrum of compound **155** in CDCl_3

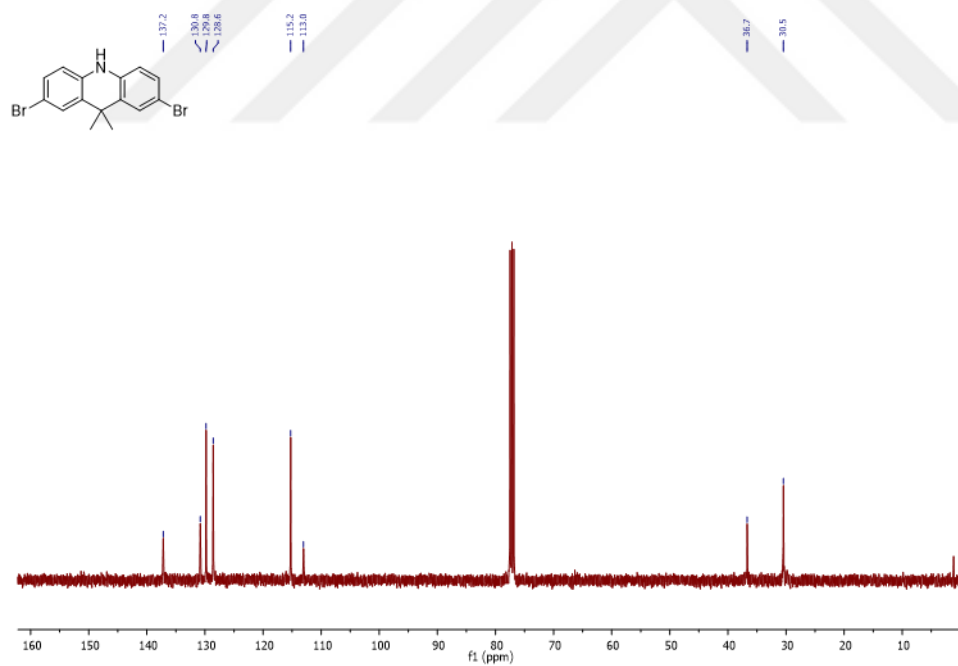


Figure A.192. ^{13}C NMR spectrum of compound **155** in CDCl_3

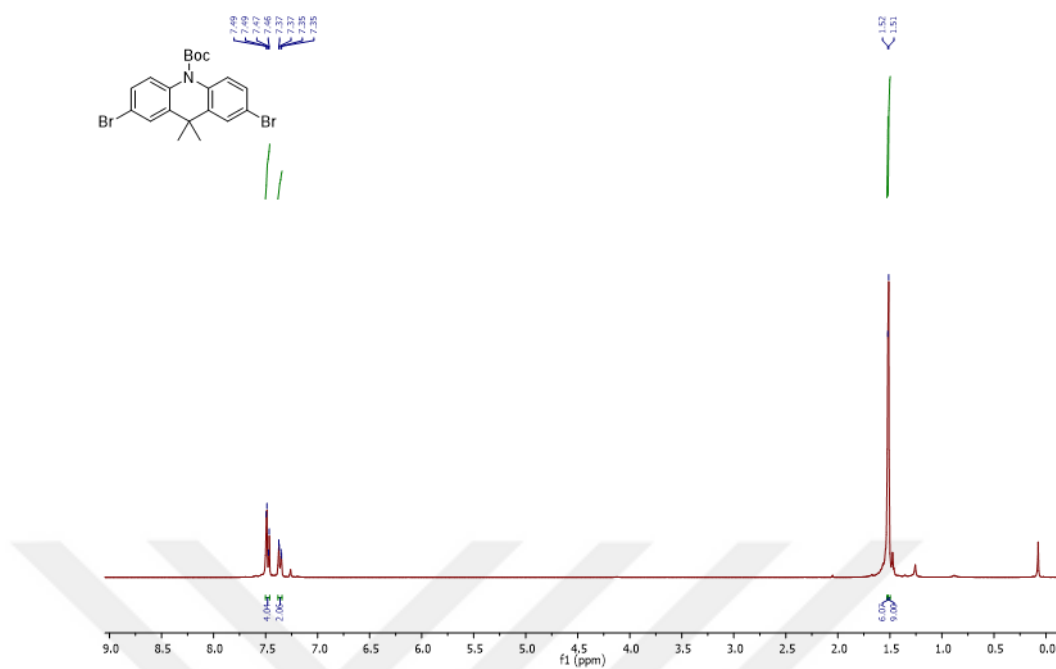


Figure A.193. ¹H NMR spectrum of compound **156** in CDCl₃

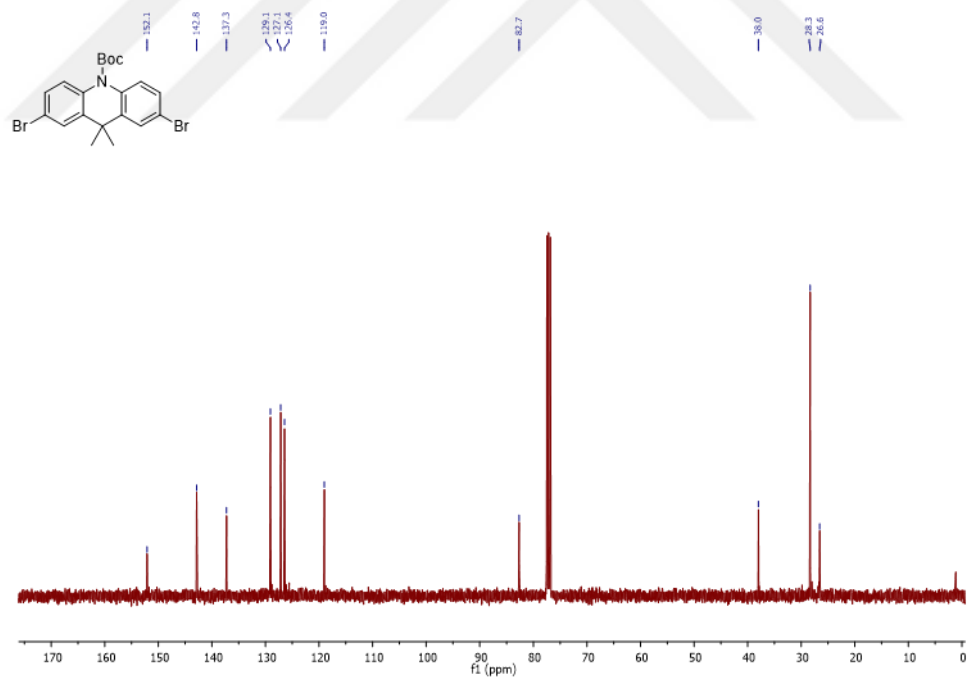


Figure A.194. ¹³C NMR spectrum of compound **156** in CDCl₃

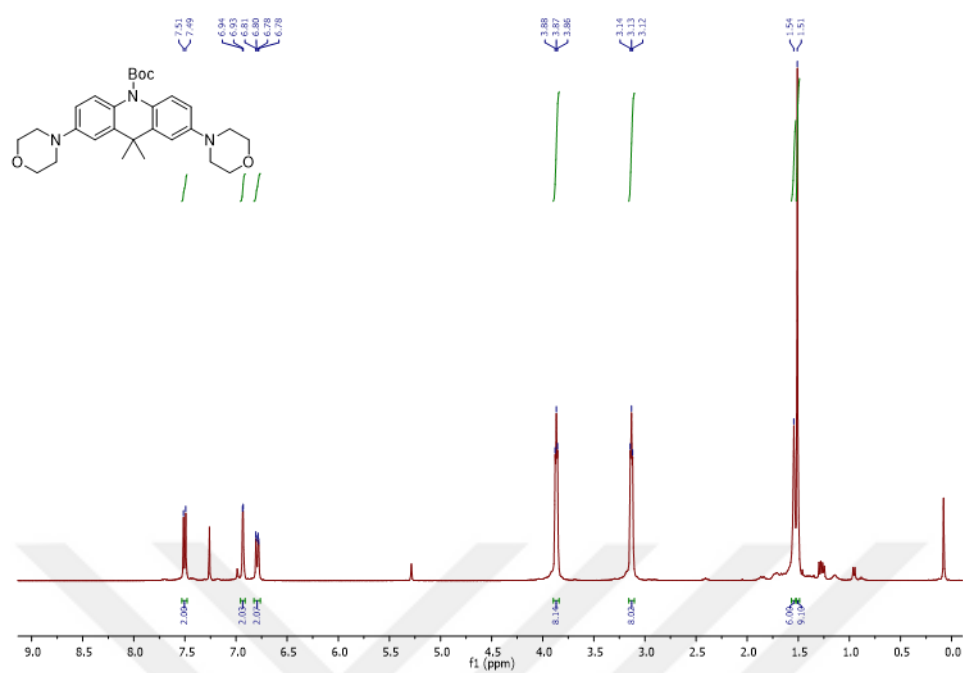


Figure A.195. ¹H NMR spectrum of compound **157** in CDCl₃

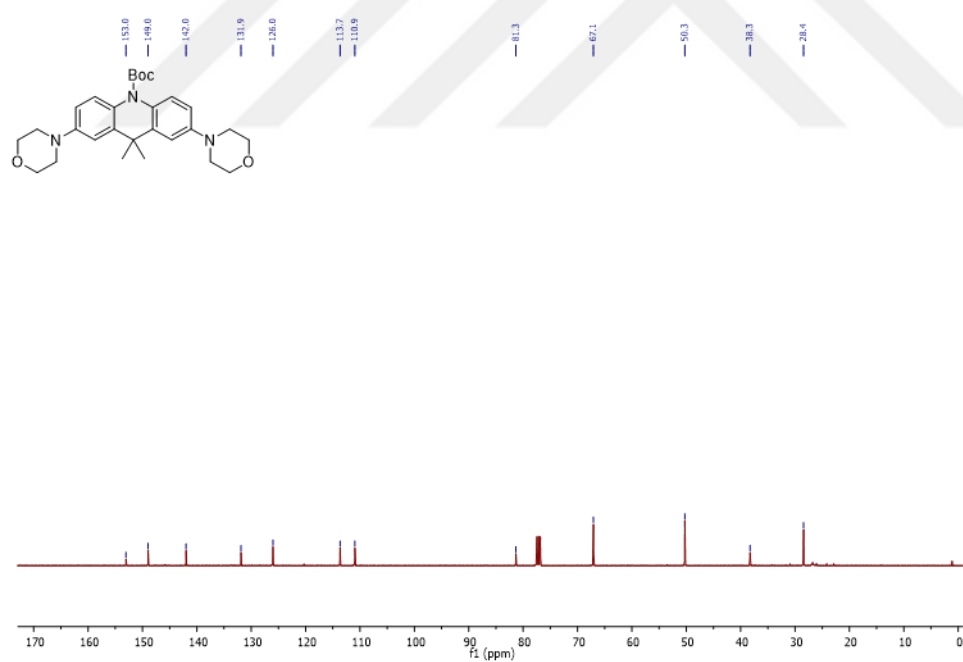


Figure A.196. ¹³C NMR spectrum of compound **157** in CDCl₃

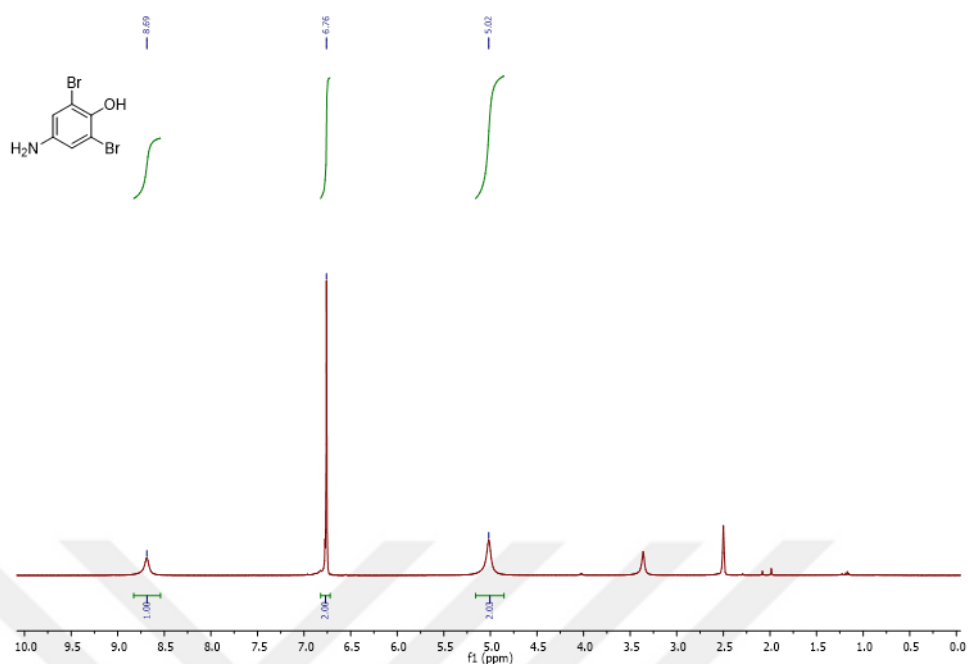


Figure A.197. ^1H NMR spectrum of compound **158** in DMSO

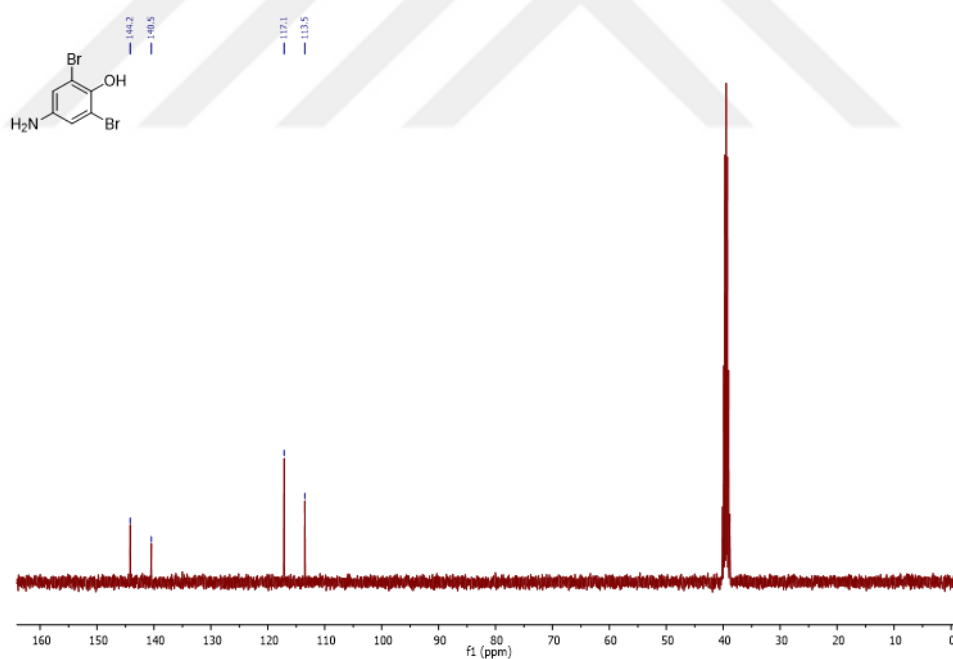


Figure A.198. ^{13}C NMR spectrum of compound **158** in DMSO

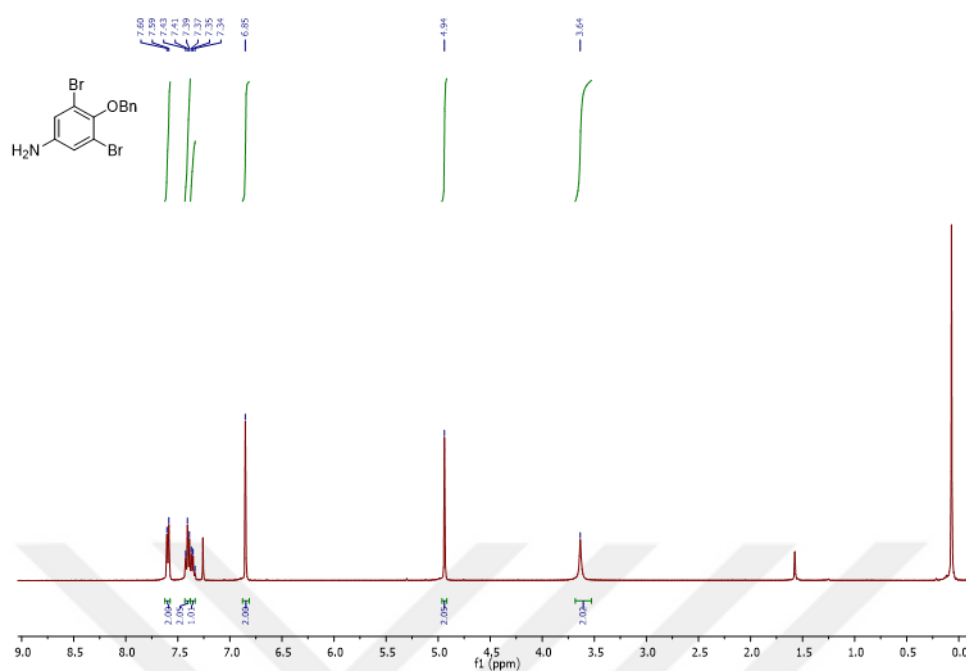


Figure A.199. ¹H NMR spectrum of compound **159** in CDCl₃

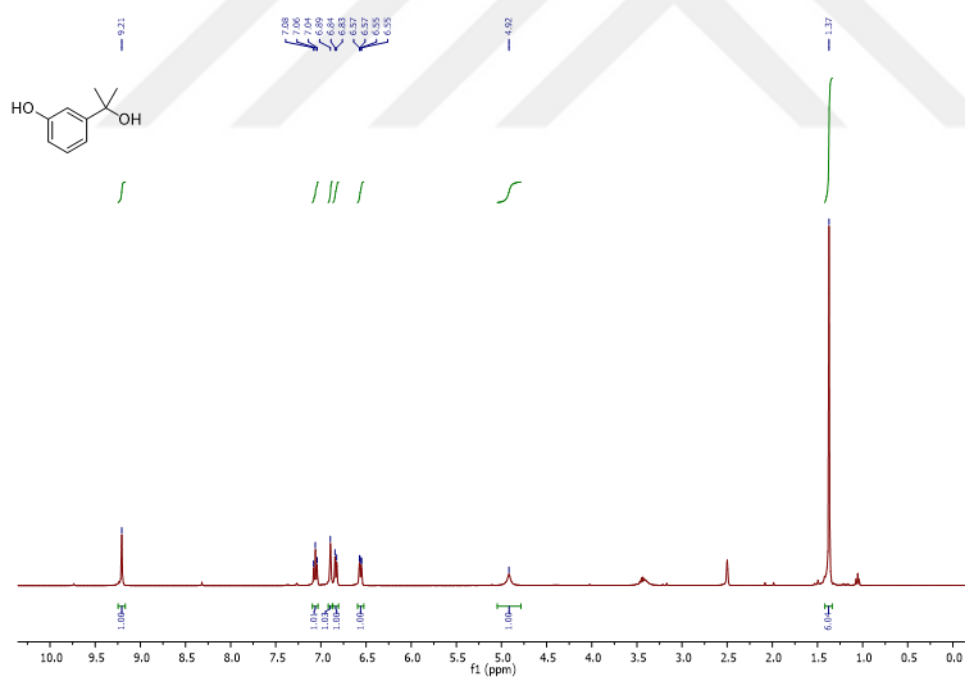


Figure A.200. ¹H NMR spectrum of compound **164** in DMSO

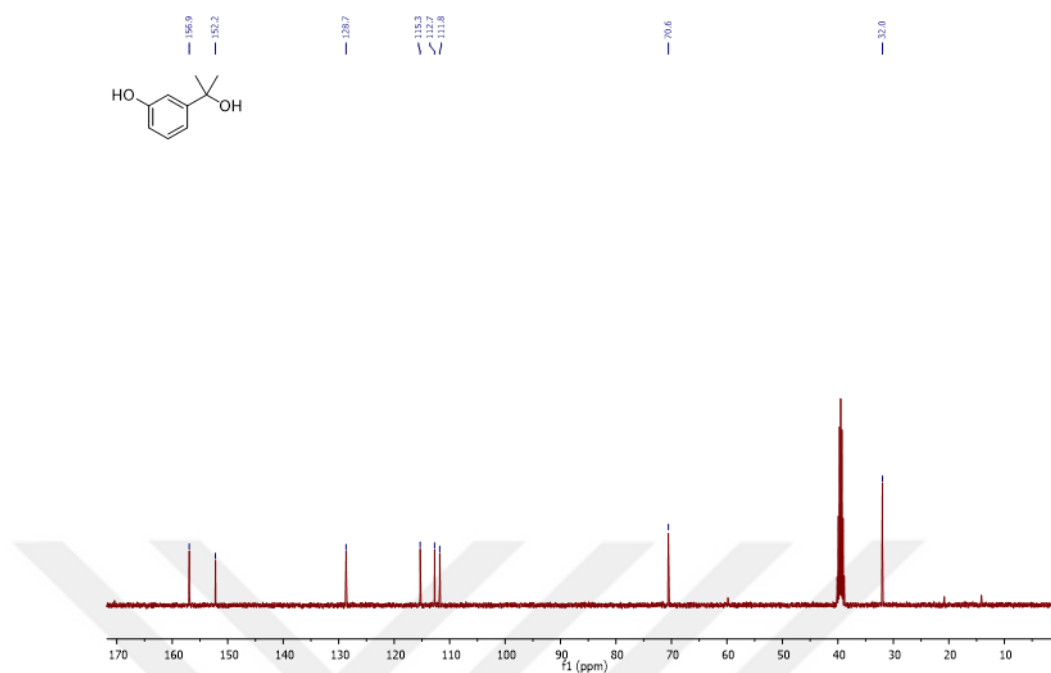


Figure A.201. ^{13}C NMR spectrum of compound **164** in DMSO

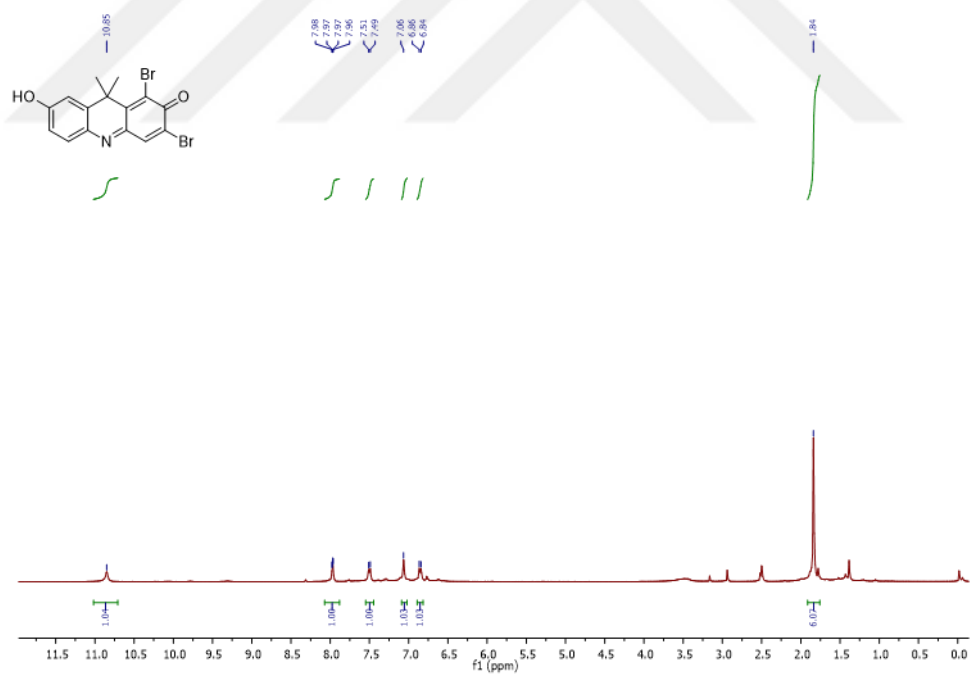


Figure 6.202. ^1H NMR spectrum of compound **DDAN-Br** in DMSO



B. HRMS Spectra

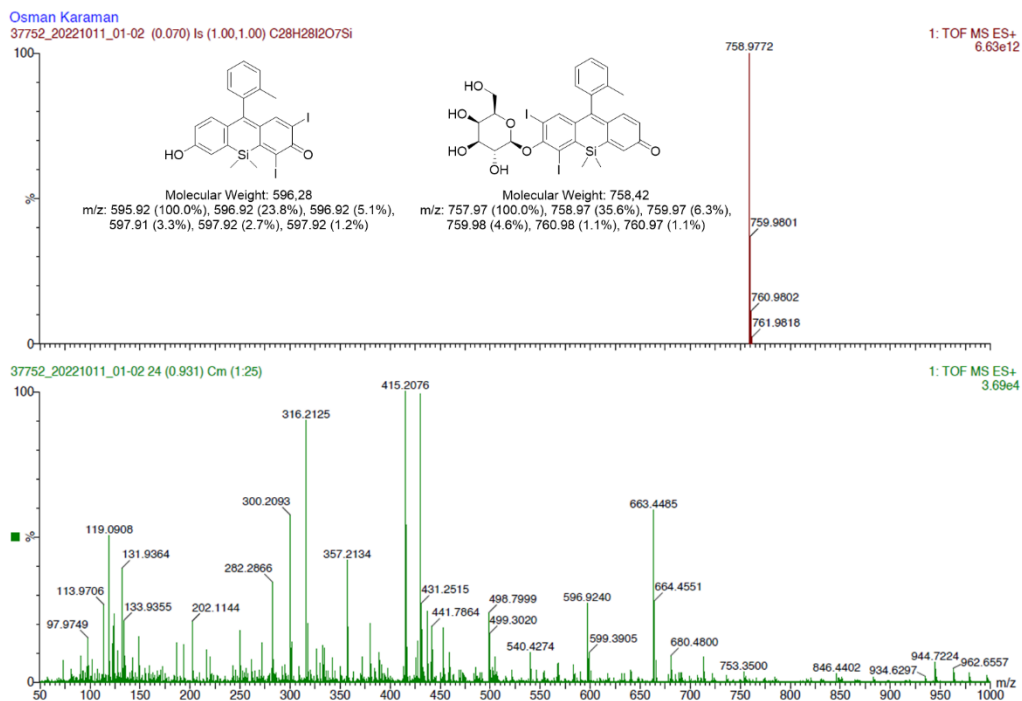


Figure B.203. HRMS of SiF-II

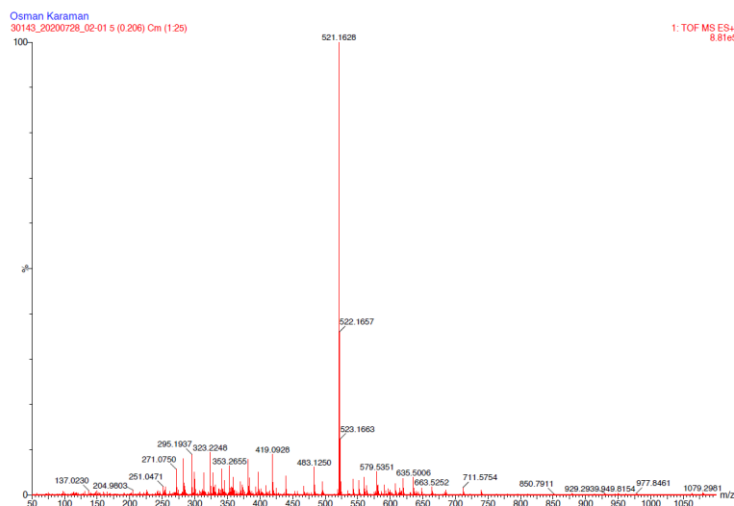


Figure B.204. HRMS of compound 28'

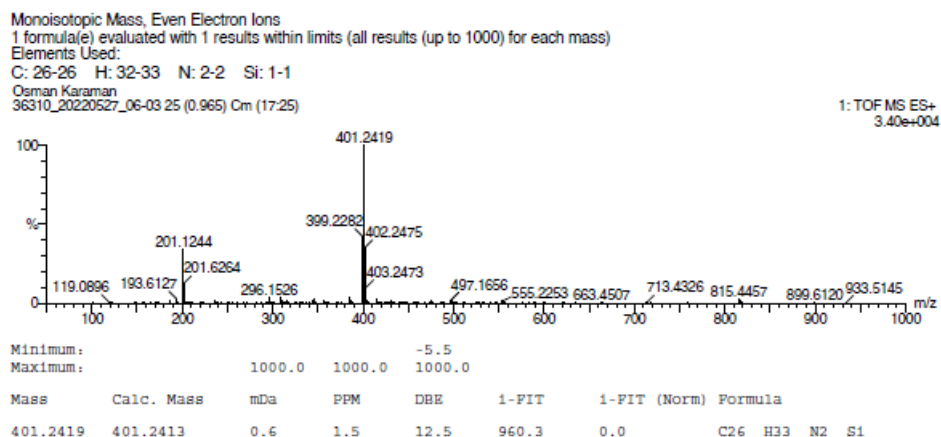


Figure B.205. HRMS of compound **46**

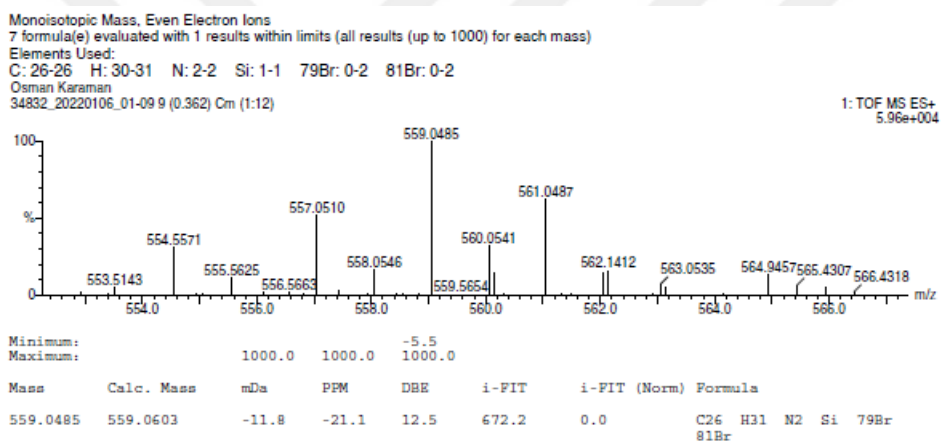


Figure B.206. HRMS of compound **47**

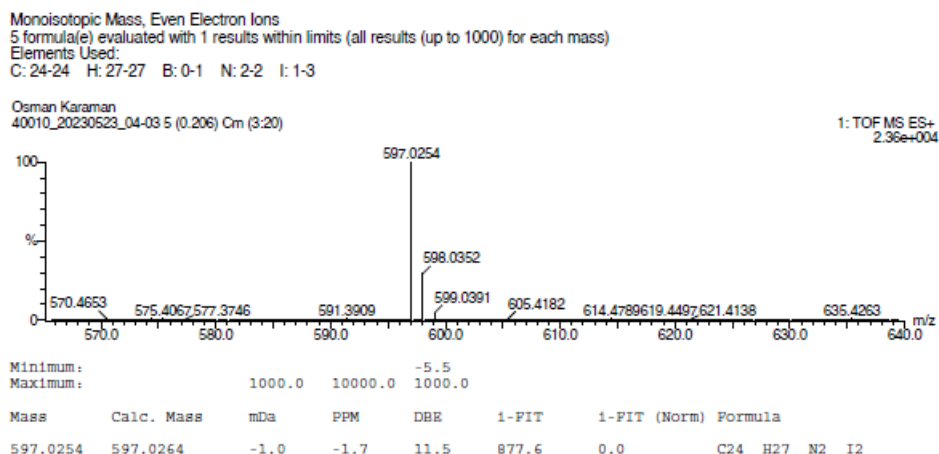


Figure B.207. HRMS of compound **51**

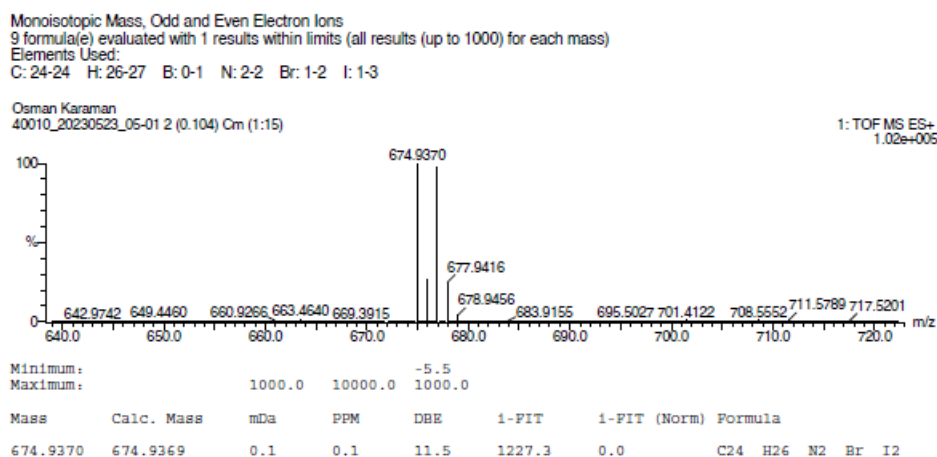


Figure B.208. HRMS of compound **52**

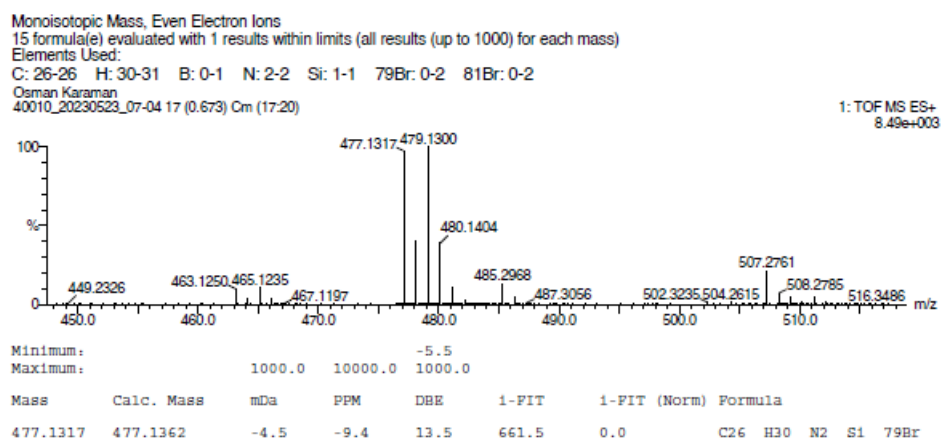


Figure B.209. HRMS of **2-Me-SiR-Br**

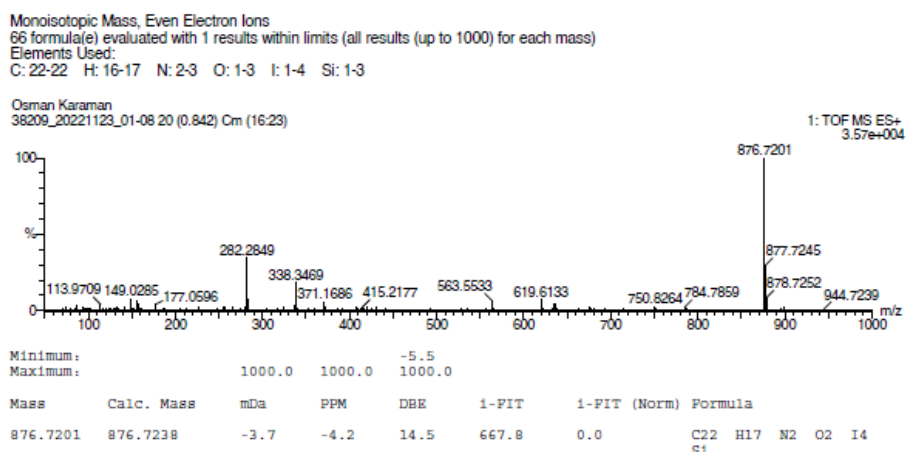


Figure B.210. HRMS of compound **56**

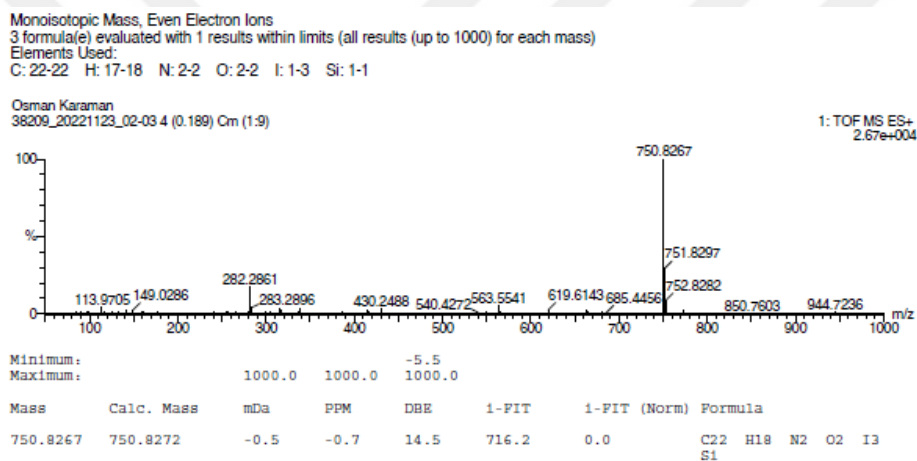


Figure B.211. HRMS of compound **57**

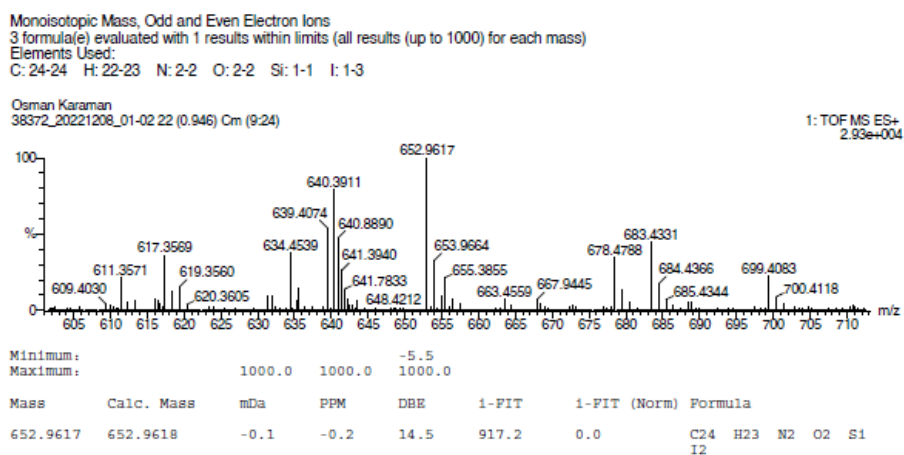


Figure B.212. HRMS of compound **58**

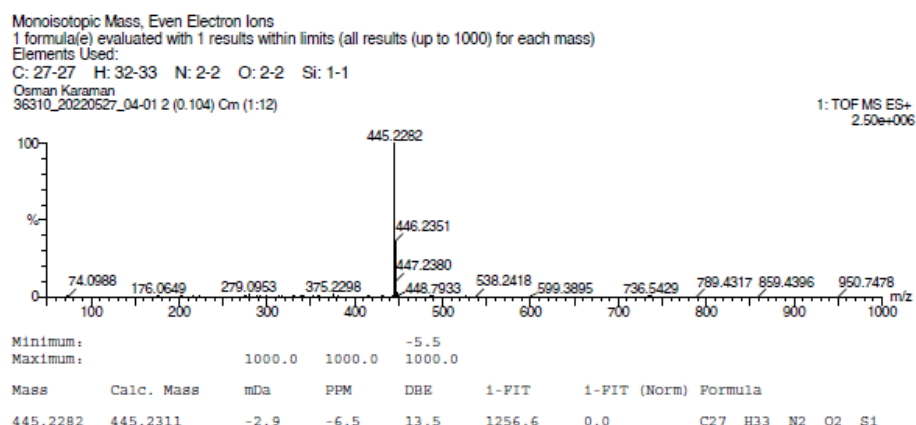


Figure B.213. HRMS of compound **71**

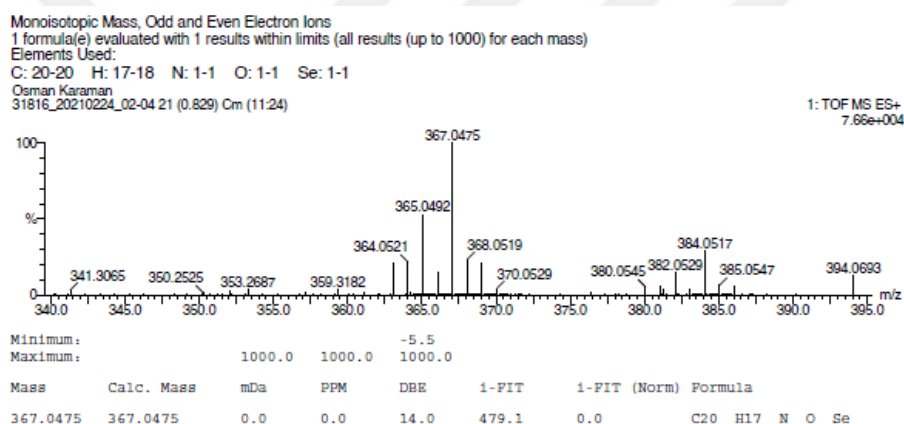


Figure B.214. HRMS of compound **78**

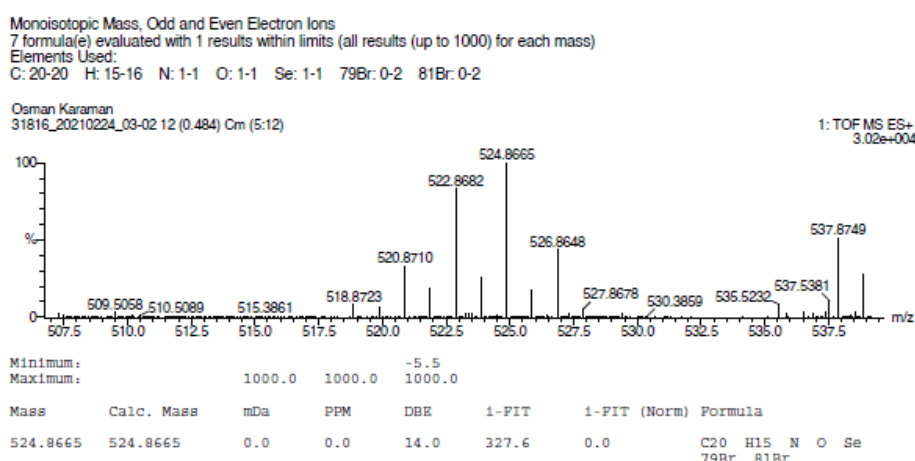


Figure B.215. HRMS of compound **79**

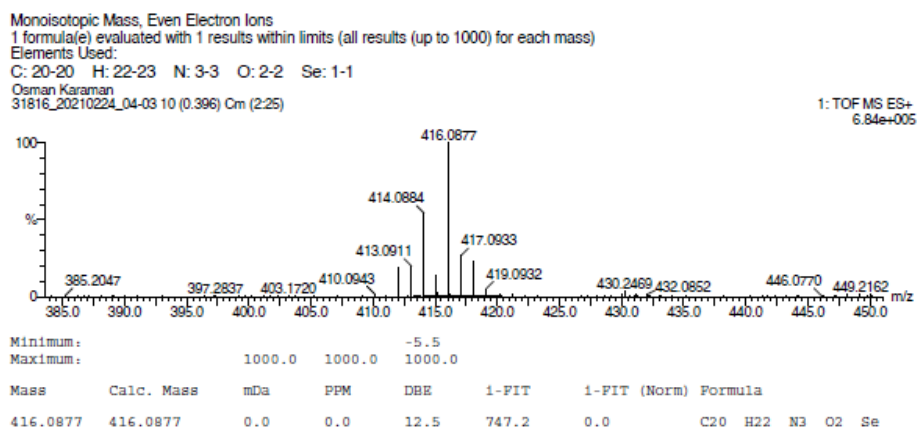


Figure B.216. HRMS of NSeMorph

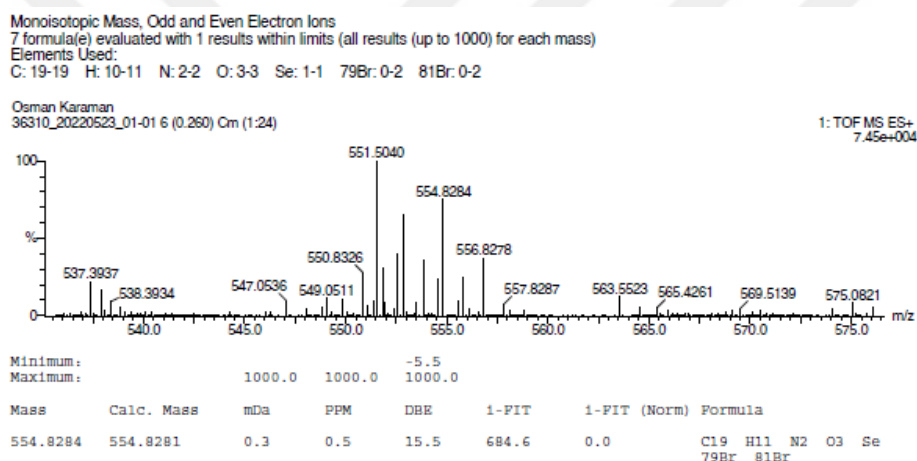


Figure B.217. HRMS of compound **85**

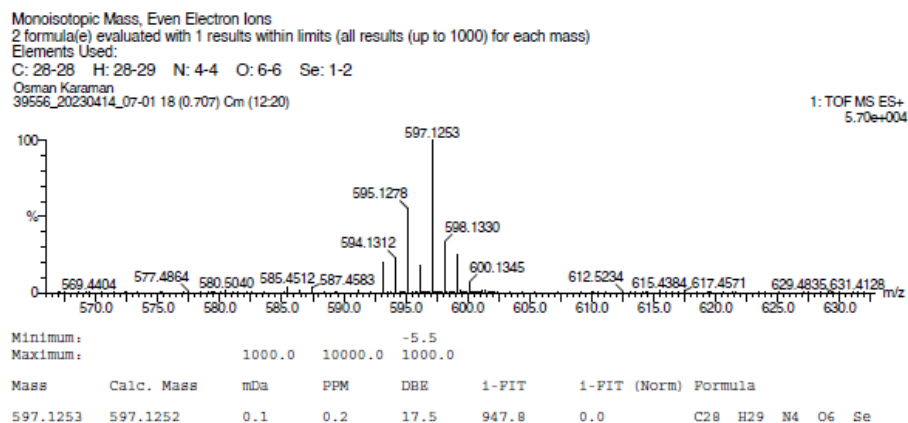


Figure B.218. HRMS of NTR-NSeMorph

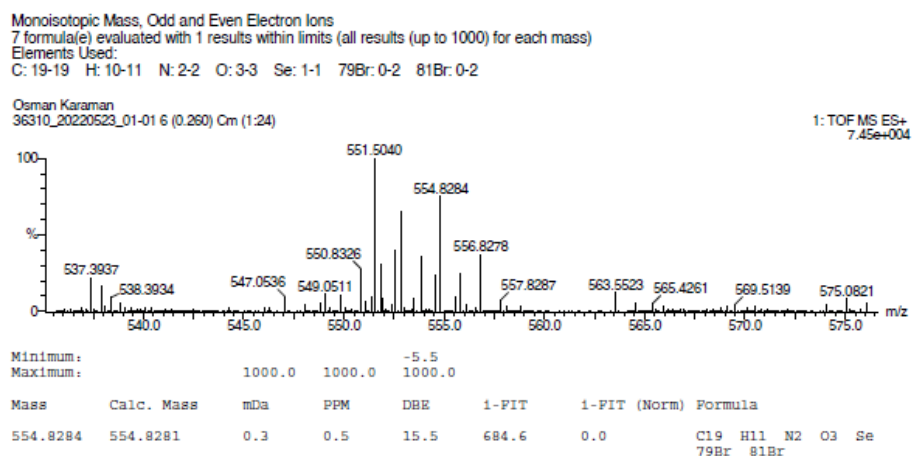


Figure B.219. HRMS of compound **107**

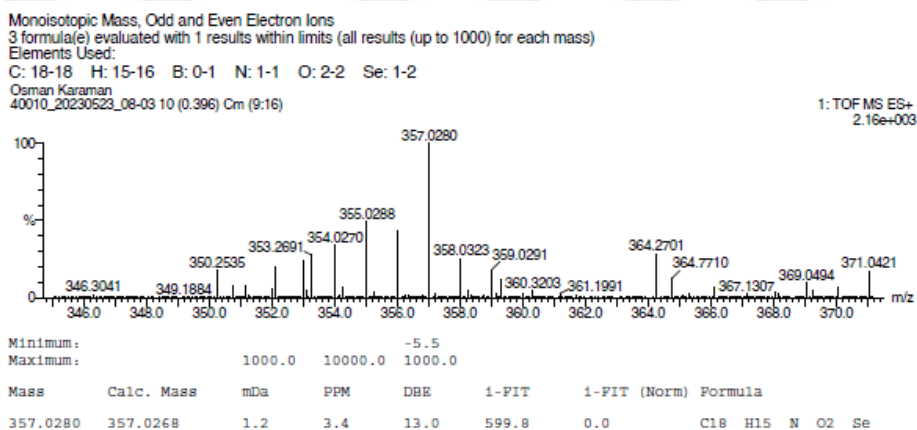


Figure B.220. HRMS of compounds **122a** and **122b**

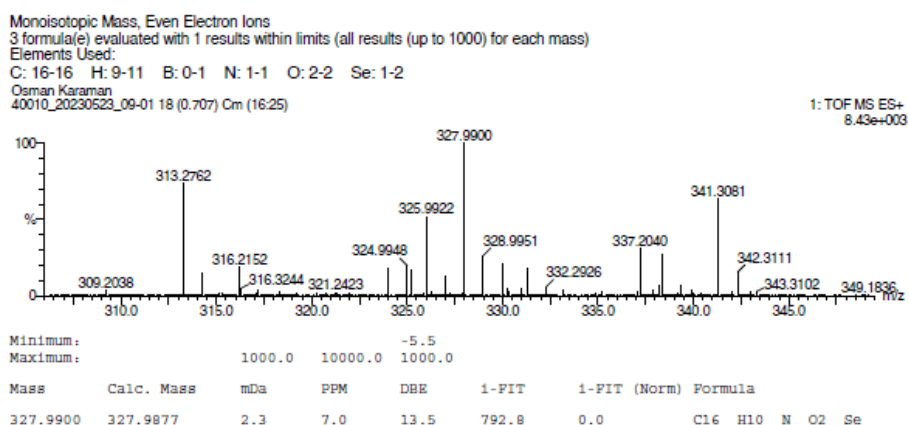


Figure B.221. HRMS of **NSeO-A**

C. HPLC Spectra

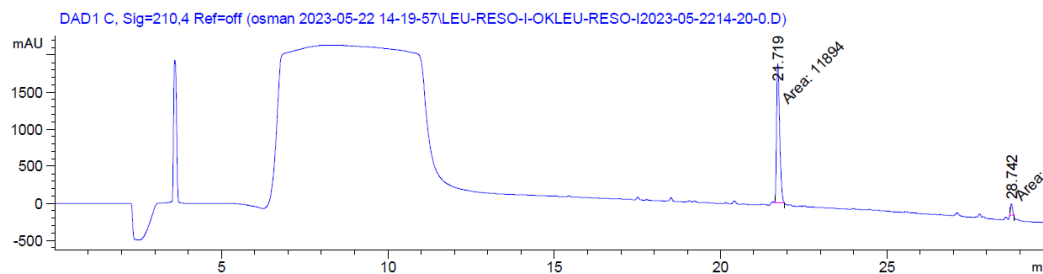


Figure C.222. HPLC spectrum of **LAP-RI**.

Table C.1 HPLC retention times and peak areas of **LAP-RI**.

Peak	Ret. Time (min)	Area (%)
1	21.719	95.2642
2	22.83	4.7358

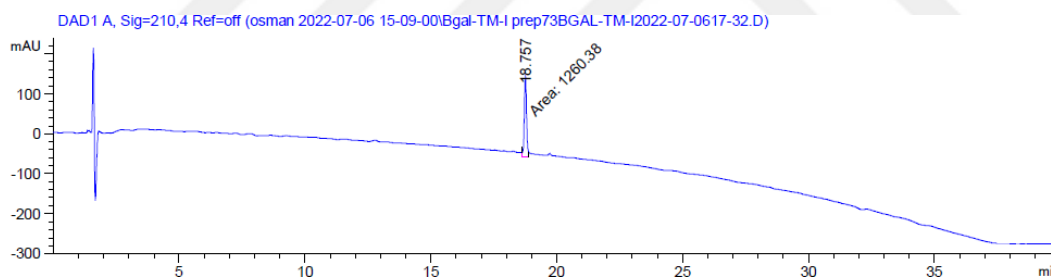


Figure C.223. HPLC spectrum of **β-Gal-SiF-II**.

Table C.2 HPLC retention times and peak areas of **β-Gal-SiF-II**.

Peak	Ret. Time (min)	Area (%)
1	18.757	100.0

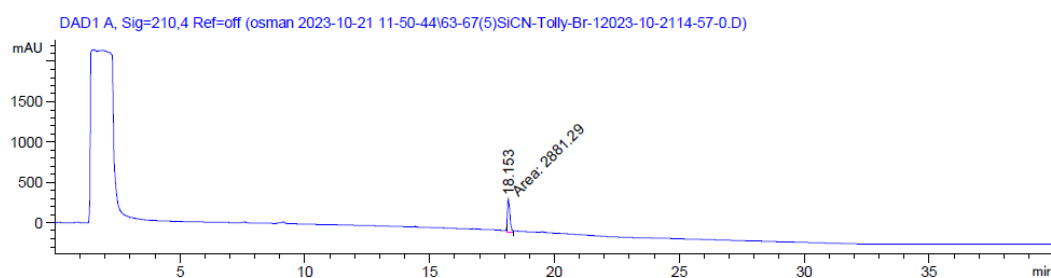


Figure C.224. HPLC spectrum of **2-Me-SiR-Br**.

Table C.3 HPLC retention times and peak areas of **2-Me-SiR-Br**.

Peak	Ret. Time (min)	Area (%)
1	18.153	100.0

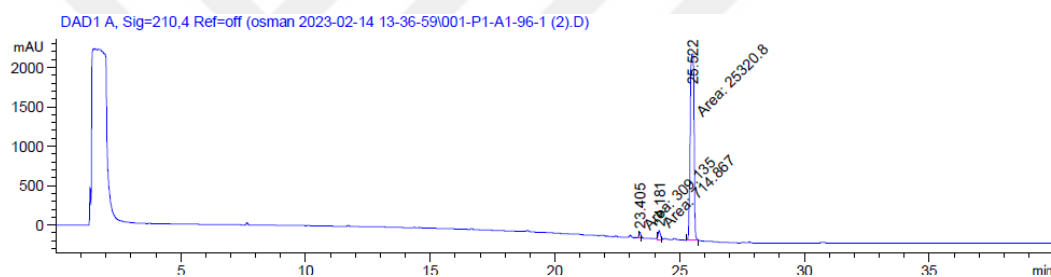


Figure C.225. HPLC spectrum of compound **56**.

Table C.4 HPLC retention times and peak areas of compound **56**.

Peak	Ret. Time (min)	Area (%)
1	23.405	1.1734
2	24.181	2.7135
3	25.522	96.1131

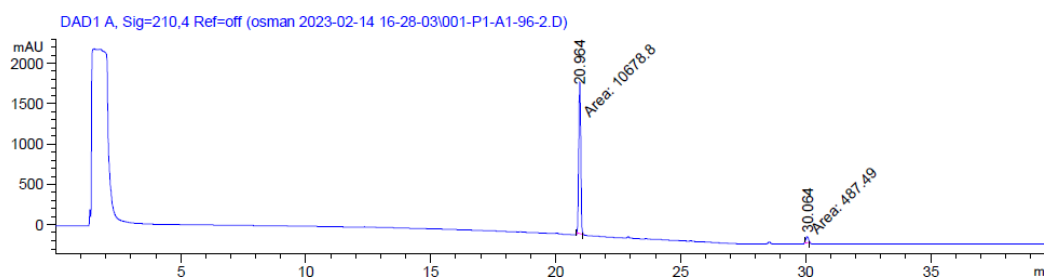


Figure C.226. HPLC spectrum of compound **56**.

Table C.5 HPLC retention times and peak areas of compound **56**.

Peak	Ret. Time (min)	Area (%)
1	20.964	95.6343
2	30.064	4.3657

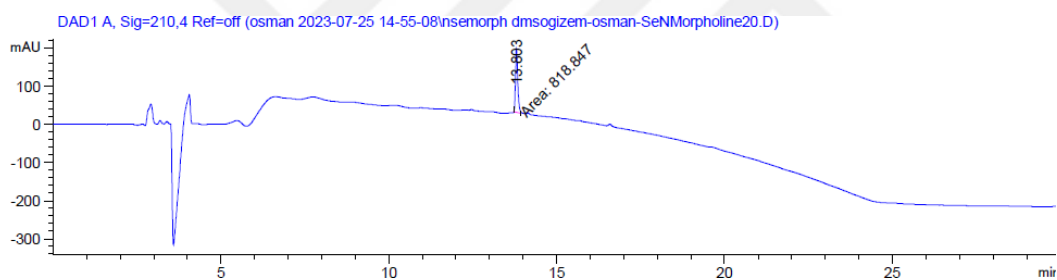


Figure C.227. HPLC spectrum of **NSeMorph**.

Table C.6 HPLC retention times and peak areas of **NSeMorph**.

Peak	Ret. Time (min)	Area (%)
1	13.803	100.0

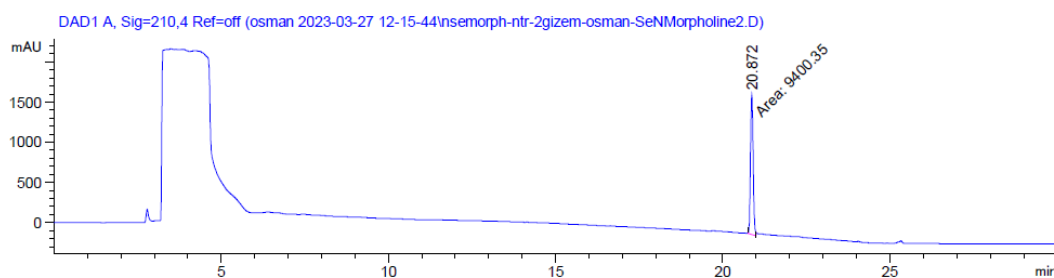


Figure C.228. HPLC spectrum of **NSeMorph-NTR**.

Table C.7 HPLC retention times and peak areas of **NSeMorph-NTR**.

Peak	Ret. Time (min)	Area (%)
1	20.872	100.0

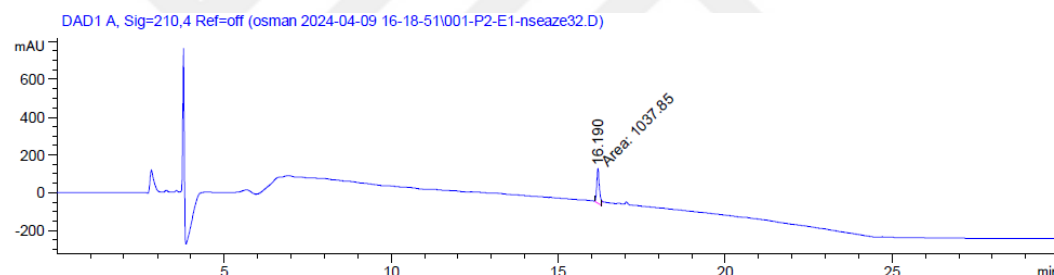


Figure C.229. HPLC spectrum of **NSeAze**.

Table C.8 HPLC retention times and peak areas of **NSeAze**.

Peak	Ret. Time (min)	Area (%)
1	16.190	100.0

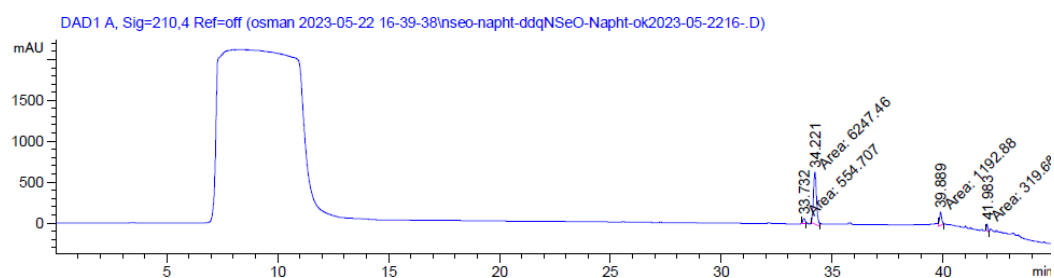


Figure C.230. HPLC spectrum of crude **NSeO-A**.

Table C.9 HPLC retention times and peak areas of crude **NSeO-A**.

Peak	Ret. Time (min)	Area (%)
1	33.732	6.6714
2	34.221	75.1373
3	39.889	14.3466
4	41.983	3.8448

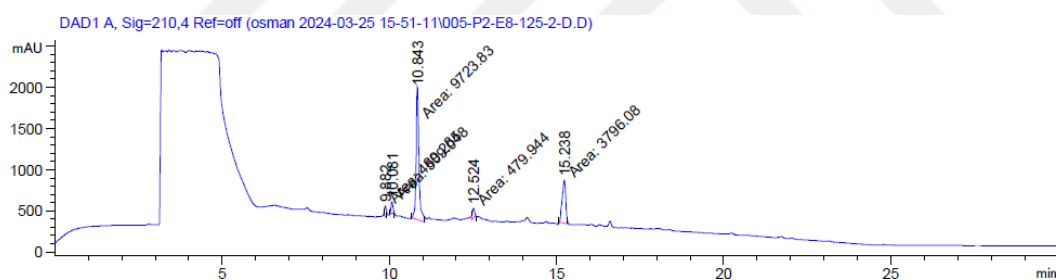


Figure C.231. HPLC spectrum of crude **DDAMorph**.

Table C.10 HPLC retention times and peak areas of crude **DDAMorph**.

Peak	Ret. Time (min)	Area (%)
1	9.882	3.2623
2	10.081	3.3941
3	10.843	64.8333
4	12.524	3.2000
5	15.238	25.3103

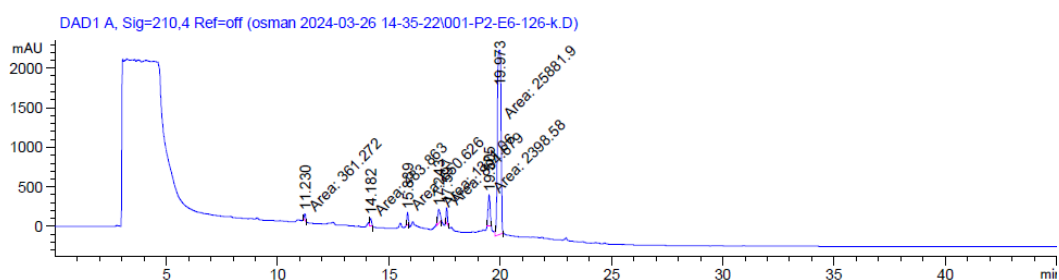


Figure C.232. HPLC spectrum of crude **DDAO-Br**.

Table C.11 HPLC retention times and peak areas of crude **DDAO-Br**.

Peak	Ret. Time (min)	Area (%)
1	11.230	1.1321
2	14.182	1.5162
3	15.839	2.0388
4	17.243	4.1710
5	17.597	2.5216
6	19.505	7.5162
7	19.973	81.1041



CURRICULUM VITAE

Surname, Name: Karaman, Osman

EDUCATION

Degree	Institution	Year of Graduation
MS	METU Chemistry	2019
BS	METU Mechanical Engineering	2017
High School	Famagusta Turkish Maarif College, Cyprus	2011

FOREIGN LANGUAGES

Advanced English

PUBLICATIONS

1. **Karaman, O.**, Alkan, G. A., Kizilenis, C., Akgul, C. C., & Gunbas, G. (2023). Xanthene dyes for cancer imaging and treatment: A material odyssey. *Coordination Chemistry Reviews*, 475, 214841.
2. Ozvural Sertcelik, K. N., **Karaman, O.**, Almammadov, T., Gunbas, G., Kolemen, S., Yagci Acar, H., & Onbasli, K. (2022). Superior Photodynamic Therapy of Colon Cancer Cells by Selenophene- BODIPY- Loaded Superparamagnetic Iron Oxide Nanoparticles. *ChemPhotoChem*, 6(10), e202200104.
3. Ozketen, A. C., **Karaman, O.**, Ozdemir, A., Soysal, I., Kizilenis, C., Nteli Chatzioglou, A., ... & Gunbas, G. (2022). Selenophene-modified boron dipyrromethene-based photosensitizers exhibit photodynamic inhibition on a broad range of bacteria. *ACS omega*, 7(38), 33916-33925.
4. Cetin, S., Elmazoglu, Z., **Karaman, O.**, Gunduz, H., Gunbas, G., & Kolemen, S. (2021). Balanced Intersystem Crossing in Iodinated Silicon-Fluoresceins Allows New Class of Red Shifted Theranostic Agents. *ACS medicinal chemistry letters*, 12(5), 752-757.

5. **Karaman, O.**, Almammadov, T., Emre Gedik, M., Gunaydin, G., Kolemen, S., & Gunbas, G. (2019). Mitochondria- Targeting Selenophene- Modified BODIPY- Based Photosensitizers for the Treatment of Hypoxic Cancer Cells. *ChemMedChem*, 14(22), 1879-1886.

6. Igci, C., **Karaman, O.**, Fan, Y., Gonzales III, A. A., Fenniri, H., & Gunbas, G. (2018). Synthesis of N-bridged pyrido [4, 3-d] pyrimidines and self-assembly into twin rosette cages and nanotubes in organic media. *Scientific Reports*, 8(1), 15949.

Swimming, Golf, Movies, Football

