

**SYNTHESIS OF BODIPY CONJUGATES TOWARDS
MULTIMODAL THERAPEUTIC APPLICATIONS**

A THESIS SUBMITTED TO
THE GRADUATE SCHOOL OF ENGINEERING AND SCIENCE
OF BILKENT UNIVERSITY
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR
THE DEGREE OF
MASTER OF SCIENCE
IN
CHEMISTRY

By
Darika Okeev
August 2016

SYNTHESIS OF BODIPY CONJUGATES TOWARDS MULTIMODAL
THERAPEUTIC APPLICATIONS

By Darika Okeev

August, 2016

We certify that we have read this thesis and that in our opinion it is fully adequate, in scope and in quality, as a thesis for the degree of Master of Science.

Engin Umut Akkaya (Advisor)

Bilge Baytekin

Mustafa Emrullahoğlu

Approved for the Graduate School of Engineering and Science:

Levent Onural

Director of the Graduate School

ABSTRACT

SYNTHESIS OF BODIPY CONJUGATES TOWARDS MULTIMODAL THERAPEUTIC APPLICATIONS

Darika Okeev

M.S. in Chemistry

Supervisor: Engin Umut Akkaya

August 2016

Photodynamic therapy (PDT) is a promising and developing strategy to treat various types of cancers. Either on its own, or combined with surgical interference, it provides easy treatment without destructive side effects of chemotherapy. Its success is straightly dependent on the presence of singlet oxygen (SO) in the tumor tissues. Upon the activation of the photosensitizer, SO gets produced, harming the surrounding tissues. The destructive effect can be increased by a simultaneous treatment with a cytotoxic cancer drug. In this project, we have combined a BODIPY photosensitizer and a Camptothecin (CPT) drug moiety with a (Z)-1,2-bis(alkyl-thio)ethane linker to produce a macromolecule with higher destructive power for tumors. Upon irradiation with a light source, it is expected of the BODIPY sensitizer to convert triplet ground state oxygen to singlet excited state, which then would react with the linker to release CPT.

Keywords: Photodynamic therapy, BODIPY, singlet oxygen, Camptothecin, linker.

ÖZET

MULTİMODLU TEDAVİ UYGULAMALARINA YÖNELİK BODIPY TÜREVLERİNİN SENTEZİ

Darika Okeev

Kimya Bölümü Yüksek Lisans Tezi

Tez Yöneticisi: Engin Umut Akkaya

Ağustos 2016

Fotodinamik terapi (PDT), yıkıcı yan etkileri olmayan ve değişik kanser türlerinin tedavisi için gelecek vaat eden bir yöntemdir. Tümör dokularındaki oksijen miktarı, bu yöntemin başarısını direkt olarak etkilemektedir. Fotosensitizörün aktivasyonu ile birlikte reaktif oksijen üretilir ve bunun sonucunda tümörlü dokularının yok edilmesi hedeflenir. Reaktif oksijenin hücreler üzerindeki etkisi, sitotoksik kanser ilacı ile birleştirilerek eş zamanlı muamele ile artırılabilir. Bu projede, çeşitli pozisyonlardan fonksiyonlandırılmış bir BODIPY sensitizörü ve Kamptotesin ilacı (Z)-1,2- bis(alkil-tio)etan bağlayıcı ile bir araya getirilmiştir. Bir ışık kaynağı ile uyarıldığında, bu BODIPY sensitizörünün temel seviyedeki oksijeni uyarılmış oksijen (singlet) haline çevirip bağlayıcı ile reaksiyona girerek hücre içinde Kamptotesin'in salınımının sağlanması beklenmektedir.

Anahtar Kelimeler: Fotodinamik terapi, BODIPY, singlet oksijen, Kamptotesin, bağlayıcı.



To you as a reader.

Acknowledgement

There are a lot people to thank for helping me through the period of my master studies. So many people made it a lot easier than I thought it would be.

First and most importantly, I would like to deeply thank my supervisor **Engin Umut Akkaya** for his patience, understanding and generous guidance through my studies. Thank you for teaching me the ability to look at a situation during research from different points of view, and the values of being a scientist.

I want to additionally thank the jury members **Bilge Baytekin** and **Mustafa Emrullahoğlu** for their presence and valuable discussion and opinions.

Secondly, I am very grateful to **Özlem Seven** for her valuable help and cooperation in every aspect of my studies, for teaching me never to give up. Your support was very essential to my success. I would like to thank **Sündüs Erbaş Çakmak** for finding time for me and providing indispensable advice and information from so far away. I owe many thanks to **Safacan Kölemen**, **Tuğba Özdemir Kütük**, and **Fazlı Sözmén** for their priceless advice during my research.

I would like to express my appreciation to all past and present members of the supramolecular chemistry laboratory, **Seylan Ayan**, **Yahya Fikry**, **Dilek Taşgın**, **İlke Şimşek Turan**, **Abdürrahman Türksoy**, **Esmá Uçar**, **Taha Bilal Uyar**, **Nisa Yeşilgül**, **Deniz Yıldız**, and the rest of the members.

Thank you, my dear friends, **Melek Baydar**, **Jose Luis Bila**, **Hale Bila**, **Ceren Çamur**, **Tuğçe Durgut**, and **Dielse Inroga**. Thank you so much, **Tuğçe Karataş** and **Cansu Kaya**, for the fun and crazy times, for teaching me so much. There are no words to express the immense gratitude I feel for knowing you. For all the amazing times, through laughs and tears, we had in these short yet beautiful years of our lives. You are my second family.

Last but not the least, I would like to thank my all members of my beloved family and **Jorge Berruga** and his family for always being there and supporting me through my good and bad times. It would not have been possible without your love, positive attitude, care and support.

List of Abbreviations

BODIPY	:	4,4-difluoro-4-bora-3a,4a-diaza-s-indacene
CHCl₃	:	Chloroform
CPT	:	Camptothecin
DCM	:	Dichloromethane
DMSO	:	Dimethyl Sulfoxide
EtOAc	:	Ethyl Acetate
FDA	:	Food and Drug Administration
HOMO	:	Highest Occupied Molecular Orbital
LUMO	:	Lowest Occupied Molecular Orbital
MS	:	Mass Spectroscopy
NMR	:	Nuclear Magnetic Resonance
PDT	:	Photodynamic Therapy
PEG	:	Polyethylene Glycol
PET	:	Photoinduced Electron Transfer
PS	:	Photosensitizer
RT	:	Room Temperature
TFA	:	Trifluoroacetic Acid
THF	:	Tetrahydrofuran
TLC	:	Thin Layer Chromatography
TMS	:	Tetramethylsilane

Table of Contents

1. INTRODUCTION.....	1
1.1. Photodynamic Therapy.....	1
1.1.1. Development of PDT	2
1.1.2. Major Components and Mechanism	6
1.1.2.1 Light and its Absorption	8
1.1.2.2 Intake and Localization	11
1.1.2.3 Requirements of Photosensitizers	12
1.1.2.4 Photosensitizers in Literature	14
1.1.2.5 BODIPY	17
1.1.3 Biological Response during PDT.....	19
1.1.4 Clinical Applications.....	20
1.2 Light Sensitive Linker.....	21
1.3 Camptothecin.....	22
1.4 Design of the BODIPY Conjugate Molecule.....	24
2. EXPERIMENTAL PROCEDURE.....	26
2.1 General.....	26
2.2 Experimental Part	26
2.2.1 Synthesis of Compound (1)	26
2.2.2 Synthesis of Compound (2)	27
2.2.3 Synthesis of Compound (3)	28
2.2.4 Synthesis of Compound (4)	29
2.2.5 Synthesis of Compound (5)	29
2.2.6 Synthesis of Compound (6)	30
2.2.7 Synthesis of Compound (7)	31
2.2.8 Synthesis of Compound (8)	32
2.2.9 Synthesis of Compound (9)	33
2.2.10 Synthesis of Compound (10)	34
2.2.11 Synthesis of Compound (11)	35
2.2.12 Synthesis of Compound (12)	36
2.2.13 Synthesis of Compound (13)	37
2.2.14 Synthesis of Compound (14)	38
3. RESULTS AND DISCUSSION	39

4. CONCLUSION.....	45
BIBLIOGRAPHY	46
APPENDIX A: ^1H NMR and ^{13}C NMR SPECTRA.....	53
APPENDIX B: MASS SPECTRA.....	79



List of Figures

Figure 1. Molecular Structure of Photofrin (Haemotoporphyrin Derivative).....	3
Figure 2. Jablonski's energy level diagram.....	6
Figure 3. Processes that take place during light penetration.....	8
Figure 4. Therapeutic window of the body.....	13
Figure 5. Structures of some photosensitizers in literature.....	17
Figure 6. BODIPY and the IUPAC numbering system.....	18
Figure 7. Comparison of singlet oxygen generation.....	19
Figure 8. 1, 4-cycloaddition of oxygen to conjugated dienes.....	21
Figure 9. Molecular structure of Camptothecin (CPT).....	22
Figure 10. Structures of Topotecan (<i>left</i>) and Irinotecan (<i>right</i>).....	23
Figure 11. Derivative drugs from CPT that are either commercially available or undergoing clinical trials.....	23
Figure 12. Design of the multimodal BODIPY conjugate.....	25
Figure 13. Synthesis of Compound 1.....	26
Figure 14. Synthesis of Compound 2.....	27
Figure 15. Synthesis of Compound 3.....	28
Figure 16. Synthesis of Compound 4.....	29
Figure 17. Synthesis of Compound 5.....	29
Figure 18. Synthesis of Compound 6.....	30
Figure 19. Synthesis of Compound 7.....	31
Figure 20. Synthesis of Compound 8.....	32
Figure 21. Synthesis of Compound 9.....	33
Figure 22. Synthesis of Compound 10.....	34
Figure 23. Synthesis of Compound 11.....	35
Figure 24. Synthesis of Compound 12.....	36
Figure 25. Synthesis of Compound 13.....	37
Figure 26. Synthesis of Compound 14.....	38
Figure 27. Basic representation of the overall aim.....	39
Figure 28. Positions of modification of a BODIPY core	40
Figure 29. Mesomeric effect of 2 and 6 positions of BODIPY.....	41

Figure 30. Cleavage reaction of the (Z)-1,2-bis(alkyl-thio) linker.....	42
Figure 31. Transesterification reaction of an alcohol with an ester.....	43
Figure 32. ¹ H-NMR spectrum of Compound 1.....	53
Figure 33. ¹³ C-NMR spectrum of Compound 1.....	54
Figure 34. ¹ H-NMR spectrum of Compound 2.....	55
Figure 35. ¹³ C-NMR spectrum of Compound 2.....	56
Figure 36. ¹ H-NMR spectrum of Compound 3.....	57
Figure 37. ¹³ C-NMR spectrum of Compound 3.....	58
Figure 38. ¹ H-NMR spectrum of Compound 4.....	59
Figure 39. ¹³ C-NMR spectrum of Compound 4.....	60
Figure 40. ¹ H-NMR spectrum of Compound 5.....	61
Figure 41. ¹³ C-NMR spectrum of Compound 5.....	62
Figure 42. ¹ H-NMR spectrum of Compound 6.....	63
Figure 43. ¹³ C-NMR spectrum of Compound 6.....	64
Figure 44. ¹ H-NMR spectrum of Compound 7.....	65
Figure 45. ¹³ C-NMR spectrum of Compound 7.....	66
Figure 46. ¹ H-NMR spectrum of Compound 8.....	67
Figure 47. ¹³ C-NMR spectrum of Compound 8.....	68
Figure 48. ¹ H-NMR spectrum of Compound 9.....	69
Figure 49. ¹³ C-NMR spectrum of Compound 9.....	70
Figure 50. ¹ H-NMR spectrum of Compound 10.....	71
Figure 51. ¹³ C-NMR spectrum of Compound 10.....	72
Figure 52. ¹ H-NMR spectrum of Compound 11.....	73
Figure 53. ¹³ C-NMR spectrum of Compound 11.....	74
Figure 54. ¹ H-NMR spectrum of Compound 12.....	75
Figure 55. ¹³ C-NMR spectrum of Compound 12.....	76
Figure 56. ¹ H-NMR spectrum of Compound 13.....	77
Figure 57. ¹³ C-NMR spectrum of Compound 13.....	78
Figure 58. Mass spectrum of Compound 1.....	79
Figure 59. Mass spectrum of Compound 2.....	79
Figure 60. Mass spectrum of Compound 3.....	80
Figure 61. Mass spectrum of Compound 4.....	80

Figure 62. Mass spectrum of Compound 5.....	81
Figure 63. Mass spectrum of Compound 6.....	81
Figure 64. Mass spectrum of Compound 7.....	82
Figure 65. Mass spectrum of Compound 8.....	82
Figure 66. Mass spectrum of Compound 9.....	83
Figure 67. Mass spectrum of Compound 10.....	83
Figure 68. Mass spectrum of Compound 11.....	84
Figure 69. Mass spectrum of Compound 12.....	84
Figure 70. Mass spectrum of Compound 13.....	85
Figure 71. Mass spectrum of Compound 14.....	85



List of Tables

Table 1: Clinically approved photosensitizers.....5

Table 2: Types of lasers available for use in PDT.....10



CHAPTER 1: INTRODUCTION

1.1. Photodynamic Therapy

Photodynamic therapy (PDT) is a recently clinically accepted promising method for treatment of various diseases, including skin actinic keratosis, several forms of malignant cancers and blindness caused by age-related macular degeneration.^{1,2} Photosensitizer and oxygen along with an appropriate suitable wavelength are the three core components of PDT.³

The two components of this type of therapy; a photosensitizer and light of a specific frequency are harmless on their own, but upon combination with oxygen they become lethal to the cells.⁴ The photosensitizer for PDT is an organic molecule that generates singlet oxygen by converting it from triplet to singlet state. The oxygen is in its ground triplet state in the cells, but upon irradiation of light of specific frequency that falls into the range of absorption of the photosensitizer, it gets converted to triplet state, which is deadly for cells. In order to achieve better penetration through tissues without causing harm, light is used in red or near IR range.⁵

The administration of the photosensitizer in PDT can be performed systematically, locally or topically.⁶ Upon administration of photosensitizers to the system and incubation for a specific amount of time, the drug selectively accumulates in the tissues depending on the hydrophilicity; the more hydrophobic photosensitizers accumulating in the membrane and the cationic ones in mitochondria.⁷

The properties of photosensitizers can be modified by attaching various groups to specific positions of the photosensitizer of use, creating possibilities for uses in different regions. PDT doesn't harm healthy tissues, making it an advantageous method with respect to radiotherapy and other invasive treatments.

1.1.1. Development of PDT

Since our planet's formation, Earth's development of life was and is still being fuelled by the light provided by our sun. From the beginning of the human race, the sun has always been attributed with magical energies which were considered in many early cultures and religions to imbue people with therapeutic effects. In ancient Egyptian, Chinese and Indian cultures the often considered godlike sun was believed to cure a wide assortment of diseases; from vitiligo, psoriasis, cancer to even psychosis.⁸ Whole-body sun exposure, first pioneered by Herodotus, also known as heliotherapy, was used to reinvigorate health and treat diseases. Treatments such as photochemotherapy, in which a photosensitive agent is used and later activated by light, and phototherapy, draw similarities that reach back to ancient civilizations. For example, using naturally occurring plant psoralens, ancient Indian and Egyptian cultures, treated skin conditions.⁹

At the turn of the 20th century, early literature on photodynamic therapy (PDT) was predominately in German, French, and Danish, due to the major bulk of pioneering work on PDT having been developed in Europe. Post World War II, PDT became familiar to English speaking countries. In 1900, while working for Professor Herman von Tappeiner in Munich, the German medical student Oscar Raab famously reported that light, after interacting with acridine red, could cause cell induced death on paramecium infusoria.¹⁰ This concept was an accidental discovery as the experiment was performed when lighting was unusual due to a thunderstorm. Acridine red alone, light alone, or acridine red exposed to light and then added to the paramecium revealed in subsequent experiments that the effects of the accidental experiment yielded greater effects. He postulated that in vitro toxicity occurred when the light transferred energy to the chemical causing fluorescence. Fluorescent substances in medicine were soon investigated in more detail after being predicted by Professor Von Tappeiner.

Von Tappeiner and dermatologist Jesionek reported the first medical use of topically applied eosin interacting with white light to treat skin tumours, after French neurologist Prime reported photosensitive reactions in sun exposed areas after a patient had been administered eosin parentally for epilepsy. This was during the same time of

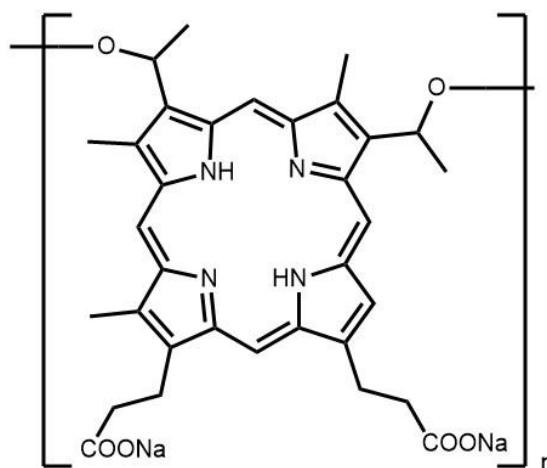


Figure 1: Molecular Structure of Photofrin (Haematoporphyrin Derivative).

Raab's observations. In 1907 the phrase "photodynamic action" was termed after Von Tappeiner and Jodlbauer identified that oxygen was an integral part in photosensitization reactions.^{11,12} All aspects related to PDT (mechanism of action, differing photosensitizers, clinically based applications, etc.) have been studied since its 1900 when it was incidentally discovered.

Haematoporphyrin was coined after Scherer heated blood with sulfuric acid that was washed free of iron and treated with alcohol in order to isolate a precipitate which had fluorescent properties. Half a decade later, Hausmann administered haematoporphyrin to mice and described photosensitivity reactions much in the same way that Friedrich Meyer - Betz had when he injected himself with 200mg of haematoporphyrin. He experienced pain and swelling in light exposed areas.¹³ In 1924, Policard described centralization of the porphyrins to malignant tissue; observing red fluorescence of haematoporphyrin in rat sarcomas when exposed to UV light. Later, the same centralization and fluorescence of remotely administered porphyrins in malignant tumours were described by Auler and Banzer in 1942 in Berlin.

The Department of Biophysics in the Norwegian Radium Hospital in Oslo is known for being at the cutting edge of PDT photosensitizer research since 1976. There, Professor Johan Moan's group and Biochemist Professor Claude Rimington have been working since 1984 to purify haematoporphyrin derivative (HpD) shown in *Figure 1* and other photosensitizers. After the assessment of the biochemical profiles

of isolated pure porphyrins those that were most likely to be effective in cancer treatment were selected to be tested further.^{14,15}

The use of fluorescence microscopy allowed for intracellular distribution of photosensitizers. Moan and Peng's groups helped pioneer a method for accurate readings of weakly fluorosensing fluorochromes even with rapid photobleaching of the photosensitizer by developing confocal laser scanning microscopy.¹⁶ The distribution of photosensitizer were studied and Moan's group discovered that the photosensitizers that localized the most to the vasculature and to the cancer cells have the strongest mechanism of action.^{16,17}

Singlet oxygen ($^1\text{O}_2$) played a vital role in toxicity during PDT processes. In 1979, a method of detecting the highly reactive singlet oxygen radical $^1\text{O}_2$ production when HpD was exposed to light using specific electron spin resonance was developed by Moan and Wold.¹⁸ The same group showed that decreasing oxygen concentrations meant that the PDT had less powerful effect. They found that oxygenating patients, so as to increase tumour oxygenation levels, would increase the effect of PDT since they found that hypoxic tumour cells are more resistant to PDT.¹⁹ They found that $^1\text{O}_2$ generated outside of the cell does not cause intracellular damage because $^1\text{O}_2$ diffused less than 0.05 μm from its site of origin before reaching with cellular targets or being quenched. They found that oxidative damage happens right in the close vicinity where the photosensitizer is localized.^{20,21}

The decision of light source depends basically on the depth of penetration.^{22,23} This depth increases in the visible and close infrared spectrum. Blue light (around 410 nm) is more effective with porphyrin sensitizer then red light because of their high extinction coefficient.²⁴ The wavelength has to match the absorption spectra of the photosensitizer that is being used. The best fit is the one that give maximum depth and maximum yield of $^1\text{O}_2$.

At first conventional lamps were used that gave off hot non-coherent light. Filters were often added to change the output.²⁵ Lasers are now the light source of choice due to their coherent light which is in phase and has a monochromatic wavelength. Optical fibres can be used for easy delivery too hard to reach treatment sites and the optic fibre

Name of Photosensitizer	Trade Name	λ (nm)	Area of Use
Porfirmer Sodium (HpD)	Photofrin	630	Cervical, lung, gastric, Bladder, Barret's oesophagus
Boronated Photofrin	BOPP	630	Brain carcinoma
Taporfin Sodium	Talaporfin	664	Solid tumours
Padoporfin (TOOKAD)	Bacteriochlorin	762	Prostate Cancers
ALA-PpIX	Levulan Kerastick	405-635	Actinic keratosis
mTHPC	Foscan	652	Head and neck tumours, prostate, pancreas, oesophagus tumours and mesothelioma
5-ALA	Levulan	635	Head and neck tumours, gynaecological and basal-cell tumours
Pd-bacteriopheophorbide	Tookad	762	Prostate cancers
BPD-MA	Verteporfin, Visudyne	689	Choroidal neovascularization
Silicon phthalocyanine-4	PC-4	672	Skin cancers

Table 1: Clinically approved photosensitizers.²⁶

tip can be adapted for irradiation of a target treatment site. The Argon dye laser was the most popular laser type to be used, because of the modifiable wavelengths, which can be changed depending on the absorption wavelengths of the photosensitizer being used. These lasers have been replaced with semi-conductor diode lasers with the advantage that they are cheaper and smaller in comparison to the argon dye lasers. They also do not require a separate power source not cooling system. They are mobile and remarkably reliable. The wavelength, however, cannot be modified therefore it must be matched for the photosensitizer being used.²⁵

PDT induces tumour necrosis with negligible effects to untreated areas.^{27,28} These first successful treatments using PDT laid the foundation for clinical based research which still continues to develop momentum. *Table 1* displays a list of currently clinically approved and used photosensitizers for malignant tumours.

1.1.2. Major Components and Mechanism

The core of photophysical and photochemical scientific applications is directly related to the interactions between matter and light.²⁹ Light induced photochemical processes usually damage biological systems, causing more damage especially when there are insufficient photoprotective mechanisms. However, this property can be taken advantage of for better cause. Thus, the ability to control this process depends on the good knowledge and understanding of the behavior of such interactions.^{30,31}

Photoactivation of fluorophore to produce reactive oxygen species is crucial for photodynamic therapy. Professor Aleksander Jabłoński, in 1933, first proposed a schematic illustration of fluorescence, which is shown in *Figure 2* along with generation of photon induced singlet oxygen ($^1\text{O}_2$).

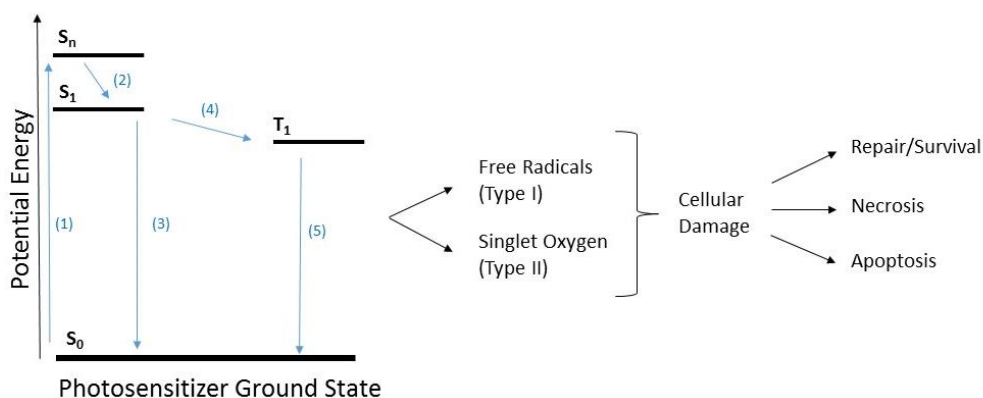


Figure 2: Jablonski's energy level diagram (adapted from C. H. Sibata et al, 2001)³²

During step 1, or initiation step, fluorophore is excited from the ground state to its first excited state with a photon, energy of which corresponds to exactly the difference of energy levels. From that point, the excited PS is very unstable and has a very short lifetime of nanoseconds, hence has two pathways to follow; either relax to its ground state producing fluorescence (step 3), or go through an action called intersystem crossing (step 4), a transition from singlet excited state to more stable and long-lived (microseconds) triplet excited state. The latter happens when the structure of the PS includes complexes of transition metals such as Pt, Ru, Ir, and Os, heavy atoms like iodine or bromine, or intramolecular spin convertors without heavy atoms (like

C₆₀).^{33,34} In cases where the intersystem crossing is favoured, triplet excited state energy is transferred to ground state, generating reactive single oxygen species from molecular oxygen.³⁰ This direct transition from the excited triplet state to singlet state, also known as phosphorescence, is spin-forbidden.³⁵

Besides the phosphorescence process, the excited triplet state of the photosensitizer can undergo Type I and Type II processes to form cytotoxic species.³⁶ Both of these processes can happen simultaneously, and the extent to which each reaction happens and in which proportion depends on the properties of the photosensitizer used, and the concentration of substrate with oxygen.^{37,38} When the activated photosensitizer reacts with an organic molecule in the cell membrane or the membrane itself, transporting a proton or an electron and creating a radical ion or cation, the process is named as Type I reaction. Then, singlet oxygen (¹O₂) is formed upon the reaction of these unstable radicals with molecular oxygen (³O₂) forms reactive oxygen species.³⁸ This generation of ROS is responsible for damage caused to mitochondria, lysosomal and nuclear membranes.^{36,38} In Type II reaction, the energy of the triplet photosensitizer is transferred to the triplet molecular oxygen in its ground state, resulting in singlet oxygen in excited state.³⁶

During Type I reaction, most of the time a superoxide anion is produced through monovalent reaction. This anion is not too reactive by itself, nor it can cause damage to the cells, but it rather undergoes a “dismutation” reaction where it reacts with itself to produce oxygen and hydrogen peroxide in the presence of superoxide dismutase enzyme.³⁶ Formation of these species leads to increase of oxidative stress in the cells.³⁹ Hydrogen peroxide penetrates easily through the cell membrane, and it is a one way process; it cannot be excluded from the cells. Superoxides then produce highly reactive hydroxyl radicals (HO[•]). This process includes reduction of metal ions by donation of an electron from the superoxide, and consequently catalyzation of breakage of the oxygen-oxygen bond in peroxide to form a hydroxyl radical (HO[•]) and a hydroxyl ion (HO⁻). A reaction between the superoxide and hydroxyl radical (HO[•]) result in production of singlet oxygen (¹O₂).³⁶ The reactive oxygen species (ROS) produced in Type I and Type II reactions react directly with various biological molecules such as amino acids, unsaturated lipids and DNA.⁴⁰ ROS affect membrane structure (making the membrane more labile to passage of different constituents), membrane integrity, folding and proper functioning of proteins, and normal functioning of DNA.^{41,42}

The important factor in this whole process is the proximity of production of singlet oxygen and hydroxyl radicals to the structures in the cells. Since ROS have a very short half-life and high reactivity, only the structures that are close to them get affected, which are the areas of photosensitizer localization (in the radius of 20 nm).⁴³

1.1.2.1 Light and its Absorption

Complete success of PDT can only be achieved if the light used is able to reach all the diseased tissues. The light used is directly related to its penetration depth in tissues. However, besides wavelength, other factors affect the ability of light to pass. Light can be absorbed, reflected, scattered or transmitted through tissue (*Figure 2*), depending on properties of tissue and light. Scattering and absorption are the most restricting factors for light penetration in tissues.³⁶ Also, in some cases phenomenon called “self-shielding” takes place, where the photosensitizer absorbs light very strongly at the treatment wavelength, limiting tissue light penetration.³⁶ A basic illustration of these processes are shown in *Figure 3*.

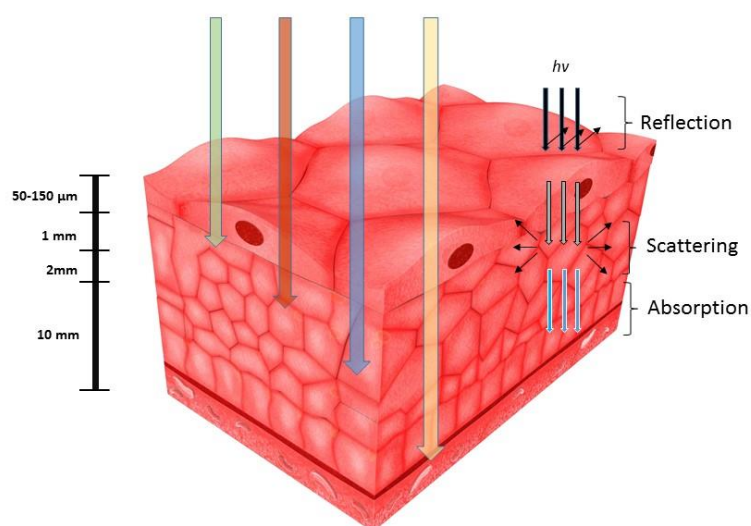


Figure 3: Processes that take place during light penetration.

Cells organelles, macromolecules and other organized cell structures scatter the light due to their turbidity, while molecules such as myoglobin, hemoglobin, melanin, cytochromes and water absorb the light. Turbidity, or in simpler words property of a matrix that is thick with suspended matter, causes the beam of light to scatter in different directions making it lose its directionality due to various optical properties of tissue matter.⁴⁴ When it comes to light absorption, it was seen that hemoglobin absorbs light at 425, 544 and 577 nm, and water absorbs light with wavelengths longer than 1200 nm.⁴⁵ The scattering and absorption effects are minimal between the wavelength values of 600 and 1200 nm, creating an “optical window of tissue”, meaning that red and infrared light is a more preferred choice for therapeutic use than blue light due to the reasons specified above.⁴⁶ On the contrary, above 800 nm, since the energy of photons is inversely proportional to the wavelength, the energy is not enough to trigger photodynamic action, and no singlet oxygen ($^1\text{O}_2$) can be produced.³² The typical penetration depth for light near 600 nm is 1 to 3 mm, but it almost doubles when light of 700 to 850 nm is used, so new photosensitizers with abilities to absorb at longer wavelengths are being developed.^{47,48}

The spectral characteristics of the light source used to illuminate the photosensitizer should overlap with the maximum absorption wavelength range of the photosensitizer. Other properties of the light source used should be chosen with respect to the disease characteristics such as size and location of the tumor and cost of use.^{49,50}

There are various light sources used in PDT, the choice of the source used can be done according to the absorption characteristics of the photosensitizer and the way it is delivered; systemically, orally or topically. In fact, there are more options for the laser source used than the amount of choices for photosensitizers for PDT. The light sources can be divided into mainly two categories: lasers and lamps. There are pros and cons for each type, yet there is no fundamental study on comparison between the light source usages for all tumor types.⁵¹

Lasers provide a very powerful monochromatic source of light which can deliver the energy needed in a short period of time with specific application. However, the monochromatic property makes the choice of lasers crucial, the laser should be chosen carefully with respect to the narrow absorption band of the photosensitizer. Since different photosensitizers absorb at different wavelengths, the laser can be used in combination with only one or very few of photosensitizers during the treatment.

Type	Wavelength(s)	Bandwidth	Light Delivery
Lasers			
Argon laser	488 and 514.5 nm	Monochrome	Direct or optical fiber
Dye laser pumped by argon laser	500–750 nm (depending on the dye)	5–10 nm	Direct or optical fiber
Metal vapor laser	UV or visible (depending on metal)	Monochrome	Direct or optical fiber
Dye laser pumped by metal vapor laser	500–750 nm (depending on the dye)	5–10 nm	Direct or optical fiber
Solid state	For a Nd:Yag 1064, 532, 355, 266 nm	Monochrome	Direct or optical fiber
Dye laser pumped by solid state laser	400–750 nm (depending on dye)	5–10 nm	Direct or optical fiber
Solid state optical parametric oscillator	250–2000 nm	Monochrome	Direct or optical fiber
Semiconductor diode lasers	600–950 nm	Monochrome	Optical fiber
Lamps			
Tungsten filament	400–1100 nm	10–100 nm (depending on filters used)	Direct or via liquid light guide
Xenon arc	300–1200 nm	10–100 nm (depending on filters used)	Normally liquid light guide
Metal halide	Depending on the metal, lines between 250–730 nm	10–100 nm (depending on filters used)	Direct or liquid light guide
Sodium (phosphor coated)	590–670 nm	10–80 nm (depending on filters)	Direct illumination
Fluorescent	400–450 nm	Approximately 30 nm	Direct illumination
Potential New Sources			
Solid state lasers for two photon PDT	Near infrared	Monochrome	Direct, scanned over the lesion
LED	Visible and infrared	5–10 nm	Direct

Table 2: Types of lasers available for use in PDT.⁵²

Lamps, on the other hand, provide a wide range of wavelengths. Even though the fluence rates of incandescent light sources are much lower, in order to avoid thermal effects, the time of incorporation is not much longer than the use of lasers. Therefore, a combination of different photosensitizers that absorb in the range of emission of the lamp can be used during treatment.⁵² Other sources like light-emitting diodes (LEDs) and femtosecond solid state lasers are being developed but there still are setbacks for their use. Generally, factors like minimal cost, easier installation and calibration features, and longer operational life make the semiconductor diode lasers more preferred types to other lasers. The development of lasers attached to fibers allows keeping track using imaging while delivering the light to nearly every type of tumor endoscopically.^{50,53} The summary of types of light sources (lamps, lasers and new potential sources) and their properties are listed in *Table 2*.

1.1.2.2 Intake and Localization

Upon introduction of the photosensitizer to the system, localization of the photosensitizer is a crucial factor. Reactive oxygen species (ROS) such as singlet oxygen and free radicals generated by the photosensitizer have short half-lives, therefore localization is an important factor for the efficiency of PDT. The other reason for importance of localization is that the ROS affect the site of generation, creating damage to all cells loaded with PS to some degree.⁵⁴ Understanding this process is important in order to choose an appropriate PS for intended area of use.

After the introduction of the photosensitizer to the cells, the localization happens mainly in mitochondria, plasma membrane, lysosomes, Golgi apparatus and endoplasmic reticulum.³⁶ This action can be monitored by either fluorescence resonance energy transfer (FRET) or introduction of specific probes with differing fluorescence and monitoring the damage after illumination.⁵⁵ The localization of the PS depends mainly on three structural features of cells; degree of hydrophilicity, net ionic charge with a range from (-4) to (+4) and degree of asymmetry in the molecule.³⁶ Sometimes, photosensitizers tend to distribute broadly among different parts of cells, an example of this is pyropheophorbide, which accumulates in endoplasmic reticulum, Golgi apparatus, mitochondria and lysosomes.⁵⁶

Out of the factors described above, the most pronounced one is hydrophilicity.⁵⁷ The selectivity of the photosensitizer is directly proportional to its lipophilic character. Hydrophobic sensitizers possessing two or less negative charges generally get attached to lipoproteins and transported to the tumour tissue. Hydrophobic sensitizers that have more than two negative charges lose their ability to pass through the cell membrane, so they are taken into cells by endocytosis, harming the cells mainly by direct interactions. The uptake can also be affected by the pH of the area, depending on the properties of the photosensitizer. Hydrophilic sensitizers are largely carried by serum proteins such as albumin, ending up within the interstitial and the vascular stroma of the diseased tissues, but they do not diffuse well from plasma membrane to cytoplasm.⁵⁸ These sensitizers harm the blood vessels of tumour cells while disrupting the supply of nutrients and oxygen, causing their death.⁵⁹

1.1.2.3 Requirements of Photosensitizers

In order for the photosensitizer to be a good therapeutic agent, it should satisfy specific requirements and have certain fundamental properties. The quality of the photosensitizer depends on its ability to generate reactive oxygen species, therefore it is important that it satisfies both physical and chemical criteria; it should be localized at the tumor tissue, absorb the suitable wavelength of light and generate ROS to trigger the biological response.⁶⁰

The ideal photosensitizer should (1) have high percentage of purity and stability, (2) be highly soluble, (3) aggregate well in the tissue, (4) have low levels of dark and administrative toxicity, such as allergic reactions or hypotension, (5) require the use of low amounts of PS by absorbing the light with high absorption coefficients, (6) absorb light of wavelength suitable for tissue penetration, (7) have an easy and reproducible synthesis process for large scale production, (8) localize in tumor tissue in maximum quantities in minimum time possible and be biodegradable, and (9) generate a high amount of the reactive oxygen species.⁴ During the photodynamic action as reactive oxygen species are generated, a degrading effect called *photobleaching* takes place.⁶¹ This factor makes the design of PS difficult, making the design to include

photostability as a factor. Thus, development of new molecules as photosensitizers is complicated due to these limiting specifications.

Singlet oxygen production occurs as the one-photon excitation takes place, some photosensitizers efficiently produce a triple photoexcited state. The photosensitizer must be able to convert the oxygen in its ground triplet state (S_0) to an excited singlet state (S_n) using the energy $h\nu$ of the photon. There are two low-lying singlet excited states for molecular oxygen with a difference in the π -antibonding orbitals having antiparallel spins; first excited state with 95 kJ mol^{-1} and second excited state with 158 kJ mol^{-1} above the ground state. The transition from the singlet first excited state to triplet ground state is spin-forbidden, therefore the lifespan of this species is higher than the spin-allowed transition from the second excited state to the ground state.⁶² For a more detailed representation refer to *Figure 1*. Despite being spin and symmetry forbidden, the transitions from the first excited state to the ground state are observed due to increased spin-orbit coupling. The coupling of spin angular momentum with orbital angular momentum mix the singlet and triplet states, making the triplet state obtain a certain singlet character, making its occurrence possible.^{63,64}

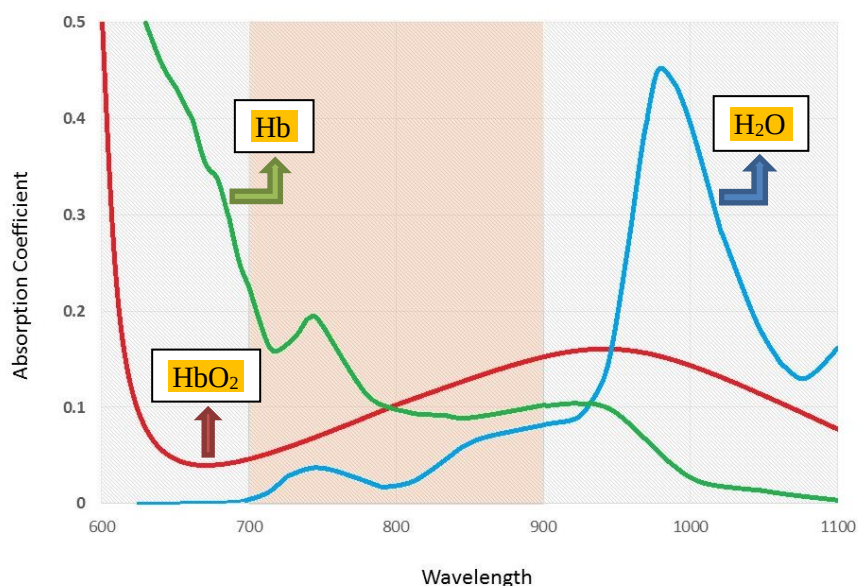


Figure 4: Therapeutic window of the body. (Absorbance of oxy-hemoglobin (HbO₂), deoxy-hemoglobin (Hb), and water (H₂O)).

Another important factor to consider when designing photosensitizers is the therapeutic window of the body. Different regions of the body have living organisms with absorbing molecules, and the penetration depth varies considerably depending on the region of administration and wavelength of light used. *Figure 4* shows a representation of the therapeutic window.

Light in the near-UV region gets absorbed mostly by certain aromatic compounds like amino acids tryptophan, tyrosine and phenylalanine, whereas the visible light gets absorbed by biomolecules such as melanin, collagens and hemoglobin. On the other side, light beyond 1150 nm gets absorbed by water molecules, which dramatically decreases the light penetration. Taking these factors into consideration, maximum penetration is considered to take place between 620 and 850 nm wavelengths of light, and is named the therapeutic or pharmaceutical window of the body. When designing a photosensitizer, its structure should be such that it absorbs light in the range described above and be able to then sufficiently produce reactive oxygen species.^{36,30}

Apart from the factors described above, an ideal photosensitizer should be biocompatible and have zero dark toxicity, meaning it should be inactive until introduced to a light source.

1.1.2.4 Photosensitizers in Literature

Most of the convenient photosensitizers have a common trait, a heterocyclic ring structure similar to that of heme in hemoglobin and chlorophyll. Synthesis of lots of photosensitizers happened between 1970 and 1990, nevertheless new types with new structures get developed regularly.⁶⁵

A photosensitizer suitable for photodynamic therapy must have four main properties; high absorption coefficient in the red region of the visible spectrum, a triplet state having enough energy to transfer to oxygen in the ground state, high quantum yield with long life-span, and photostability.⁶²

The photosensitizers for cancer therapy have been divided into three generations; haematoporphyrin derivatives and its analogues as *first generation*, structurally distinct compounds with long wavelength absorption as *second generation*, and second

generation PS attached to carriers for specific accumulation in tumors as *third generation*.

The first generation of photosensitizers, Haematoporphyrin derivatives (HpD) such as Porphyrin and Photoheme, were the first to be developed and clinically authorized. The first results of the applicability and efficacy of these compounds appeared in the 1970s and 1980s.⁶⁶ The active compounds of these PS are mixtures of porphyrin and oligomer dimers connected by ester, ether and C-C interporphyrin linkages. Even though these PS have been used clinically, there are downsides to them. They have very low selectivity (0.1-3% maximum can be found localized in the tissues).⁶² The compound can stay in the skin tissues up to 10 weeks, inducing photosensitivity in the patient. The penetration depth is very low due to the wavelength of 630 nm is used for excitation, which unfortunately is the weakest band of absorption for this PS. Even though this generation is difficult to reproduce due to it being complex mixtures without an isolated active compound, they have been a good starting point to development of second generation PS with enhanced properties.⁶²

The main idea in development of second generation photosensitizers was the ability to produce a single active compound with easy and high reproducibility. Also, they must have better selectivity and higher biodegradability in the human body.⁶² Absorption in the pharmaceutical window of 675-800 nm is preferred for deeper penetration. The obvious first step was to modify the existing first generation PS. One such group, a modified version of haematoporphyrin, or meso-Tetraphenylporphyrin can be used to substitute the phenyl groups with more hydrophilic groups. The downsides are the after effects such as skin sensitivity (ortho isomer) and tumor phototoxicity (met and para isomers).⁶⁷ A promising second generation PS for non-melanoma skin cancers called verteporfin is currently in trials for clinical use. It absorbs well in the band of 690 nm, and suits most of the desired properties described earlier. The body gets rid of it fast, meaning that the photosensitivity post administration does not last too long.⁶⁸

In order to find PS that absorb better in the red band, other PS types such as chlorins and bacteriochlorins were investigated. The derivatizations were based on the natural compounds chlorophyll *a* and bacteriochlorophyll *a*.⁶² The reduction of a pyrrole ring on porphyrin periphery yields chlorin, further reduction yields bacteriochlorin. Their absorption is suitable for PDT, with the former absorbing at 650-

670 nm, and the latter at 730-800 nm.⁶⁹ Preparation of these porphyrins had higher yields with better purity. They have high absorptivity and high tumor selectivity. The stability of these compounds upon storage needs further investigation. A large amount of various derivatives of these compounds are at different stages of research for use for PDT.^{36,70}

Another family of PS currently used for clinical trials is phthalocyanines and naphthocyanines. These compounds produce singlet oxygen with high quantum yields, with the high absorption band in the 650-850 nm range.³⁶ Due to their hydrophobic character, these compounds require a transporter during administration. For this purpose unilamellar liposomes like dipalmitoyl phosphatidyl choline (DPPC) are used, they prevent aggregation in the solution that happens due to the presence of four phenyl groups in the molecule.⁶² The localization in the tumor loci for these PS can be enhanced with the addition of polar groups (e.g. carboxylic acids, hydroxyls and sulfonic acids) to the hydrophobic main structure. It is important to maintain balance between these two groups. There are currently investigations for the use of sulfonated aluminum phthalocyanines derivatives for use against various cancers.^{67,4}

Third generation photosensitizers have the property of selective delivery enhanced by attachment to biomolecules (e.g. monoclonal antibodies). Biotin is an example for this. Another way of delivery is vesicles (e.g. adenovirus Type 2 capsid proteins).⁶² The surface antigens of cancer cells differ from those of normal cells, so if the delivery can be arranged according to this fact, very little to no harm will be done to the healthy tissues.

The use of singlet oxygen has big potential. Development of novel photosensitizers as well as improvement of current ones takes place every day. Better understanding of properties of photosensitizers is needed for their development, and factors such as photobleaching, quenching and localization must be improved even more.

Structures of some photosensitizers mentioned in literature are shown in *Figure 5*.

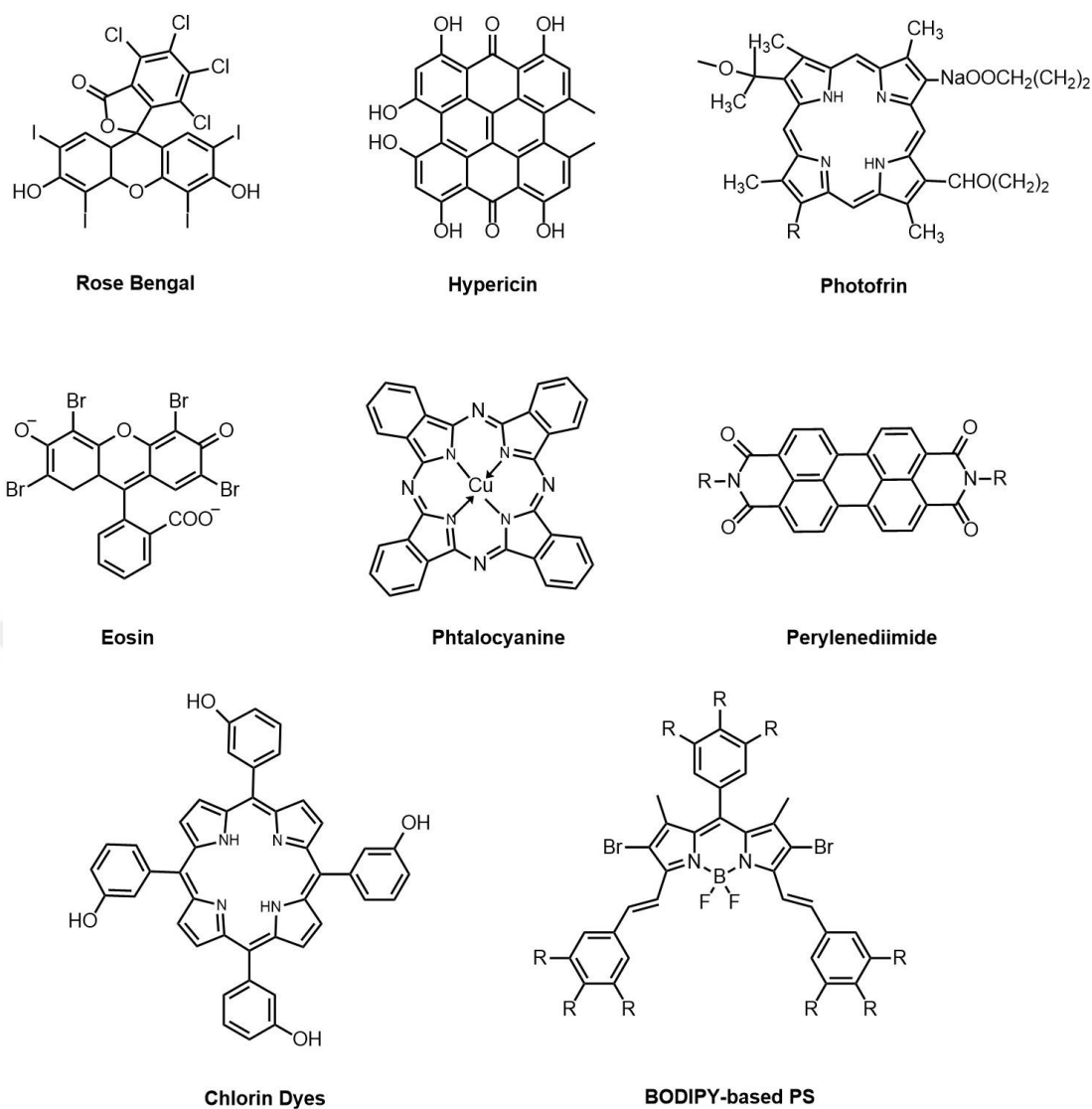


Figure 5: Structures of some photosensitizers in literature.

1.1.2.5 BODIPY

A novel type of photosensitizers that has not been mentioned in the previous section is a PS with 4,4-difluoro-4-bora-3a,4a-diaza-s-indacene (BODIPY). Its core structure is shown in *Figure 6*.

This photosensitizer gained its popularity in the past decade. It was first discovered in the year 1968 by Treibs and Kreuzer.⁷¹ Apart from being used in the areas like energy transfer cassettes⁷², light harvesting systems⁷³, ion sensing⁷⁴ and molecular logic gates⁷⁵, its properties make it an excellent agent for PDT.

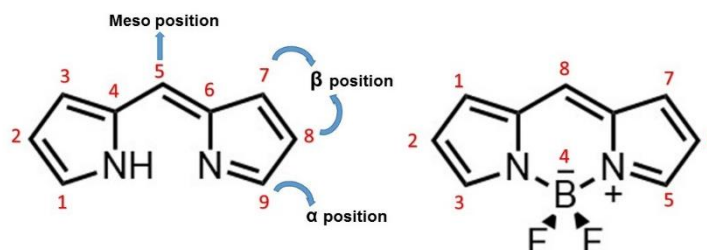


Figure 6: BODIPY and the IUPAC numbering system.

BODIPY dyes absorb light at wavelengths in the visible and near-IR range.⁷⁶ Their properties can be varied by modification of the specific positions. Akkaya et al., pioneers in this area, modified 1, 7, 3 and 5 positions of the BODIPY with Knoevenagel condensation reactions creating various styryl BODIPY's with emission at almost 840 nm.⁷⁶ Modification of 1 and 7 positions creates versatile products with narrow absorption and emission spectra. Other modifications include enhancement of selectivity taking into account that the tumor tissues have low pH and high concentration of Na^+ ion. Another good property is the insensitivity of these dyes to the polarity of the solvent and medium pH.^{75,77} These PS have high extinction coefficient, they are more resistant to photobleaching, and have higher light to dark toxicity ratios.⁷⁸

Even though BODIPY's have lots of characteristics that ideal PS would carry, there are yet some downsides that require further modification. High quantum yields are an example for this. A PS having high quantum yield is an undesirable property for fluorescence. In this case, much of the energy absorbed during excitation does not pass to triplet states. In order to promote the singlet-to-triplet intersystem crossing, fluorescence must be suppressed.⁷⁸ The solution for this problem is addition of heavy atoms to positions that would not disrupt the conjugation by ruining the planarity of

the PS. Differences in properties of BODIPY's with iodine atoms at various positions can be seen in *Figure 7*.

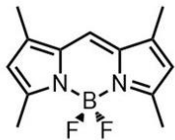
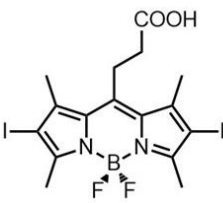
			
Solvent	MeOH	MeOH	EtOH
Quantum Yield	0.7	0.02	0.03
λ_{max} absorption (nm)	502	534	525
λ_{max} emission (nm)	508	548	540
$^1\text{O}_2$ Generation relative rate	0.48	23.9	26.4

Figure 7: Comparison of singlet oxygen generation.⁷⁸

The properties described above make BODIPY dyes very appealing for PDT. The modification and design strategies shall be described in the following chapters.

1.1.3 Biological Response during PDT

Post administration and activation of PS, the effects of cell damage can be seen almost instantly in the plasma membrane of the cells. This result can be observed with disruption of the usual behavior of processes like flaking of vesicles, swelling, and retardation of the activity of plasma membrane.⁵⁷ In PDT, cell death is induced by damaging the vascular structure and triggering the immune system. The response of cells to PDT depends on properties like type of PS used and its abundancy, light used and its intensity, amount of oxygen present in cells, and genetic properties of cells.⁷⁹ The localization of PS determines which tissues or organelles are going to be damaged first and to what extent. Due to the short lifespan of singlet oxygen and localization of PS in structures outside of nuclei, there are very little mutagenic effects on the cell. Following these factors, there can be three different reasons contributing to cell death. Direct death is caused by cytotoxicity of ROS resulting in apoptosis and/or

necrosis.^{80,81} Damage caused to the vascular system is the second cause of death due to the lack of nutrients and oxygen in the tumor loci.⁸² Third case consists of stimulation of immune system causing invasion of leukocytes, destroying the tumor even at remote locations.^{54,83} It is yet unknown which of the reasons described causes most damage, or the extent to which they affect each other, further research is needed.⁸³

Apoptosis is a genetically determined or “programmed” cell death. This homeostatic mechanism helps maintain the population of cells. It also occurs as a response to immune reactions or damage of cells by disease. Different stimuli might cause this. One of such effects is caused by administration and irradiation of the PS during PDT, causing DNA damage in cells leading to apoptosis.⁸⁴ Membrane and cytosolic proteins are responsible for this state. During apoptosis, cells are fragmented and enclosed into vesicles forming apoptotic bodies, which then undergo phagocytosis causing no inflammation.⁸²

The other mode, necrosis, is a more traumatic and unnatural way of death for cells. After the cell swells up, intracellular and plasma membranes break causing overall rupture of the cell. The ruptured tissues and organelles are released into surrounding tissues. This phenomenon can cause more harm to the surroundings, therefore is considered as the cytotoxic effect of PDT.⁸²

1.1.4 Clinical Applications

The first medical trial was performed in 1970s, and photodynamic therapy was used ever since to treat various diseases. More and more photosensitizers get developed day by day. However, only a number of them get approved, and are used in clinical studies, let alone be commercially produced. *Table 1* shows the latest most used photosensitizers and the diseases they are used to treat.⁸⁵

There have been more than 200 clinical studies over the past 30 years for different types of cancers. PDT has shown to have good response with low chance of cancers reoccurring and better cosmetic outcome.⁶⁰

Verteporfin is a photosensitizer approved for use in patients having age-related macular degeneration, the clinical trials were performed with placebo control.⁸⁶

Regressions in other types of tumours of eye tissue have been seen while using PDT.⁸⁷ PDT using Photofrin and Foscan is also a preferred type of treatment for head, neck and oral cavity cancers having higher curing rates when compared to radio and chemotherapy.⁸⁸ There is more control over precision during PDT treatment compared to surgical invasion, with lower risks of damaging nearby vital tissues.⁸⁹

PDT has shown positive results in animal trials for some cardiovascular diseases and gastroenterological, mesothelial and gynaecological cancers.⁶⁵ Other studies have shown that PDT has effect against viral diseases such as herpes.⁹⁰ It is hoped that advances in the properties of PDT agents will enable PDT to achieve its full potential in the nearest future.

1.2 Light Sensitive Linker

Connection between molecules can be done using various types of linkers. One such type is particularly interesting for PDT due to its sensitivity to singlet oxygen. The linker is (Z)-1,2-bis (alkyl-thio) ethane.

It has been reported by Breslow et al. that the double bond between the two carbon atoms can be simply cleaved by singlet oxygen in aqueous medium.⁹¹ The synthesis of this linker has been reported to be easy and having relatively high yields.⁹² Different types of reactions have been studied, but the 1,4-cycloaddition of oxygen to conjugated dienes has shown best properties. The cleavage reaction is shown in *Figure 8*.

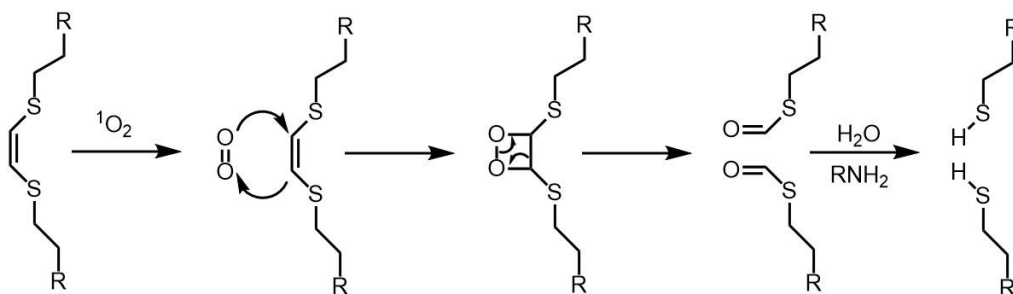


Figure 8: 1, 4-cycloaddition of oxygen to conjugated dienes.⁹²

The unstable dioxethane intermediates can easily rearrange to form more stable products.⁹³ The decomposition occurs through production of S-alkyl methanethionate. This compound further hydrolyzes to thiols in aqueous solutions and in the presence of amines.⁹⁴

1.3 Camptothecin

Most of plant defense systems contain many alkaloid structures that help them, protecting from being consumed by animals and microbes, and harmed by viruses.⁹⁵

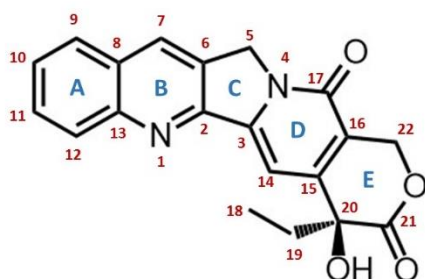


Figure 9: Molecular structure of Camptothecin (CPT).

These alkaloids are molecules with low molecular weight, and contain nitrogen in their structures. They can be found in a vast majority of plants. These compounds were used for traditional means for both warfare and treatment of diseases long before their structure was identified. There are different alkaloids that are extracted from plants, and are currently clinically used. One such example is an anti-cancer agent Camptothecin (CPT).⁹⁶ This compound was first isolated in 1958 by Monroe E. Wall and Mansukh C. Wani from a native Chinese tree *Camptotheca acuminata*. However, due to unavailability of sophisticated analytical techniques, the structure of CPT was determined through single-crystal X-ray analysis in 1966.⁹⁷ Its initial results showed promising properties in tumors of colonic and gastric origins. The molecular structure of CPT is shown in *Figure 9*.

CPT has very low solubility in water, so its salt-form analogues were first used for research. This type showed to be less efficient and more toxic to the body, further

studies have ceased until the mechanism of reaction was better understood.⁹⁸ In 1980's it was discovered that the primary target of CPT is topo I DNA topoisomerase.⁹⁹ Since then, CPT was modified to be more water-soluble, and other properties are currently being enhanced. There are derivatives that are clinically used, others are being developed.¹⁰⁰

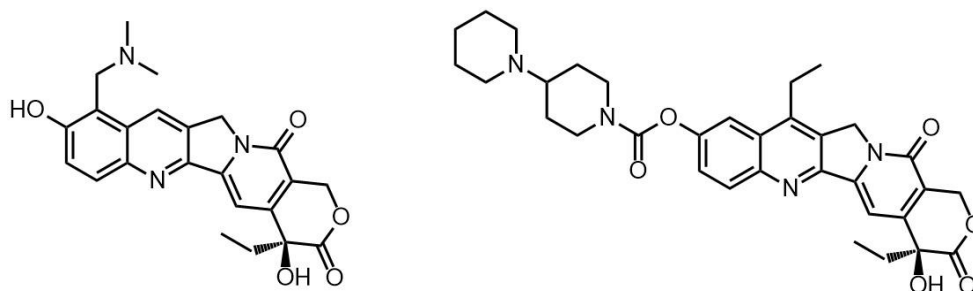


Figure 10: Structures of Topotecan (*left*) and Irinotecan (*right*).

Irinotecan and Topotecan are two first-generation CPT analogues that are FDA approved. They are used for treatment of ovarian, cervix, colon, and small and big cell lung cancers. Their structures are presented in *Figure 10*. Other derivatized drugs that are undergoing clinical trials are given in *Figure 11*.

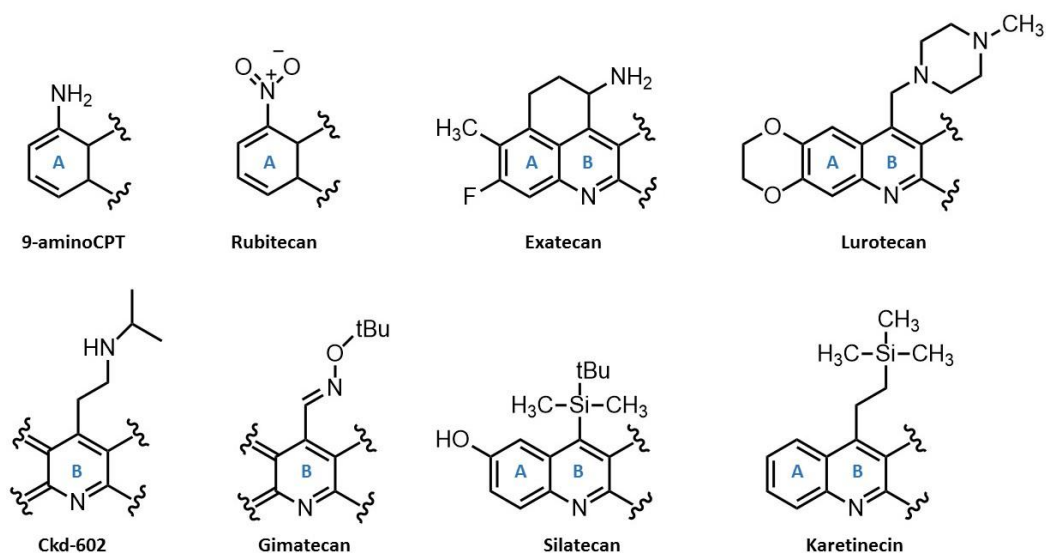


Figure 11: Derivative drugs from CPT that are either commercially available or undergoing clinical trials.

CPT is not currently used as it is in clinical trials due to low solubility and bad side effects. Modification of CPT is possible on the A and B rings. The activity of CPT depends greatly on the planarity of the molecule. The pyridine and lactone moieties along with 20-(S)-hydroxyl group¹⁰¹ on D and E rings restrict modification due to the risk of disabling activity of the drug. The 7, 9, 10 and 11 positions are derivatized to develop the properties.¹⁰² It has been found that changing or masking of the hydroxyl group at the 20 position can stabilize the drug while reducing its activity.¹⁰³

The main target of CPT is topo I topoisomerase. Topo I and topo II are enzymes that are responsible in DNA chromatin assembly, repair, transcription, recombination and replication. The main effect of CPT is poisoning topo I. Topo I breaks and reseals the photodiester bonds, changing the amount of linkages one DNA strand makes with the other. The mechanism of topo I can be summarized in four steps; (1) binding of enzyme with DNA, (2) cleavage of the single-strand of DNA by reverse transesterification, (3) single strand passage and (4) DNA strand relegation.¹⁰³ CPT inhibits the activity of type I topoisomerase by bonding interacting through the hydroxyl group at the 20 position.¹⁰⁴ The usually useful enzyme topo I gets transformed into cytotoxic poison, consequently causing cell death due to replication fork collision.¹⁰³

1.4 Design of the BODIPY Conjugate Molecule

The positive properties of BODIPY dyes were described in the previous sections in detail. Here, it is important to concentrate on how the molecule can be modified to reduce the disadvantages. As previously mentioned, there are two main problems with PDT, one of which is the hypoxic properties of tumor cells. The abundance of molecular oxygen is crucial for the success of PDT. To resolve this issue, some molecules have been synthesized with additional modes of action, such as the addition of an anti-cancer drug to the fluorophore. An example of such studies can be given of the group of Min Hee Lee¹⁰⁵. They have synthesized a theranostic drug that contains a fluorophore and a drug connected by a disulfide bridge. The design of the molecule for this project is shown in *Figure 12*.

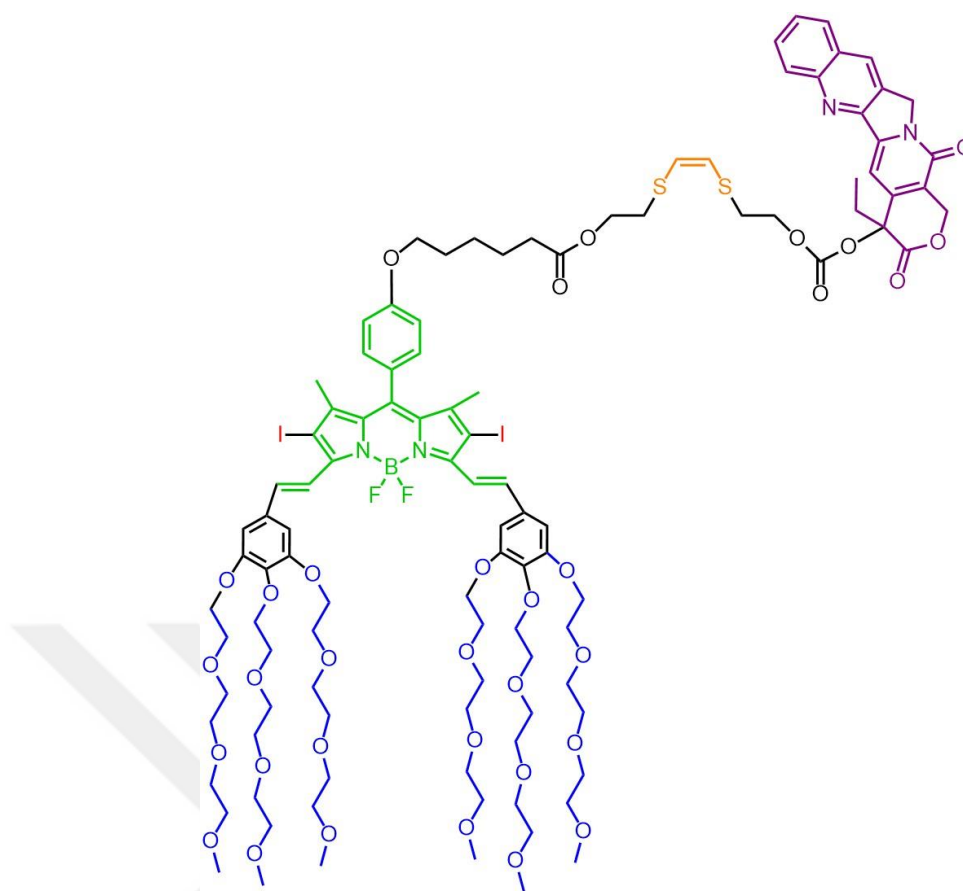


Figure 12: Design of the multimodal BODIPY conjugate.

The idea behind our design is that when the molecular oxygen is not enough to induce a complete destruction of the tumor cells, the drug would facilitate further destruction. As mentioned earlier, CPT poisons the topoisomerase, resulting in decrease of replication and death of cells, compensating for the negative sides of hypoxia. It was thought to be very useful to modify the PS with water soluble groups and heavy atoms to make it more convenient.

CHAPTER 2: EXPERIMENTAL PROCEDURE

2.1 General

All chemicals used for synthesis in this project were purchased from Aldrich without further purification. All ^1H NMR (100 MHz) and ^{13}C NMR (400 MHz) spectra were recorded using Bruker DPX-400 with the tetramethylsilane (TMS) peak as internal reference. The solvents used to obtain these spectra were mainly CDCl_3 or DMSO-d_6 . All of the recordings and characterizations were performed at 25 °C. Mass spectrometry values were measured by Bilkent University Agilent Technologies 6224 TOF LC/MS. All reactions were monitored through thin layer chromatography (Merck TLC Silica gel 60 F₂₅₄) together with column chromatography from Merck (Merck Silica Gel 60, pore size: 0.040 – 0.060 mm, 230-400 mesh ASTM).

2.2 Experimental Part

2.2.1 Synthesis of Compound (1)

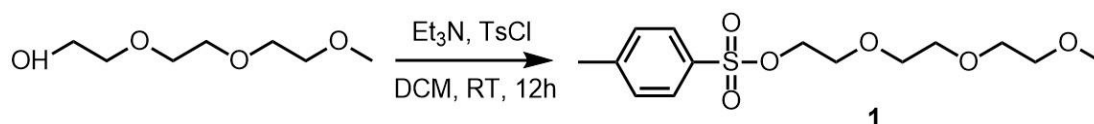


Figure 13: Synthesis of Compound 1.

Triethyleneglycol monomethyl ether (10 g, 61mmol) was dissolved in 100 ml DCM and 13 ml Et_3N . P-toluene sulfonyl chloride (12g, 63 mmol) was dissolved in 20 ml DCM in a dropper, and added dropwise to the previous solution while the reaction mixture was being cooled in an ice bath, and stirred for 12 hours until all the reactant was consumed. After the extraction with water, the organic layer was collected and dried with Na_2SO_4 , purified with silica column chromatography with DCM/MeOH

(98:2, v/v) as mobile phase and concentrated in vacuo to yield compound **1** as yellow oil. (17,3 g, 54 mmol, 89%)

^1H NMR (400 MHz, CDCl_3) δ : 7.81 (d, 2H, Ar-H), 7.35 (d, 2H, Ar-H), 4.17 (t, 2H, CH_2), 3.70 (t, 2H, CH_2), 3.70-3.58 (m, 6H, CH_2), 3.54 (t, 2H, CH_2), 3.38 (t, 3H, CH_3), 2.46 (t, 3H, CH_3). ^{13}C NMR (100 MHz, CDCl_3) δ : 190.77, 154.34, 131.98, 129.82, 114.74, 68.13, 33.76, 32.61, 28.88, 27.85, 25.21.

MS (TOF): m/z: Calculated: 319.12 g/mol $[\text{M}+\text{H}]^+$, Found: 319.1172 $[\text{M}+\text{H}]^+$, $\Delta=11.67$ ppm

2.2.2 Synthesis of Compound (2)

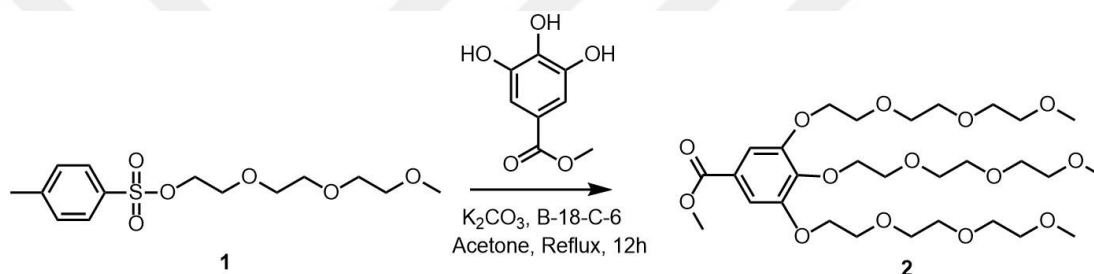


Figure 14: Synthesis of Compound 2.

Methyl-3,4,5-trihydroxybenzoate (2.75 g, 15 mmol), compound **1** (15 g, 47 mmol) and catalytic amount of benzo-18-crown-6 were dissolved in 60 ml of Acetone. Potassium carbonate (8.3 g, 60 mmol) was added to the reaction mixture, which was then refluxed overnight, and controlled with TLC until all methyl-3,4,5-trihydroxybenzoate was consumed. The mixture was filtrated and the solvent was removed under reduced pressure. The crude product was then extracted with EtOAc and brine. Organic layer was dried with Na_2SO_4 and evaporated under reduced pressure. The product was purified with silica column chromatography with EtOAc as mobile phase, concentrated in vacuo to yield compound **2** as colorless oil. (6 g, 9.6 mmol, 64%)

^1H NMR (400 MHz, CDCl_3) δ : 7.31 (s, 2H, Ar-H), 4.25-4.19 (m, 6H, $\text{CH}_2\text{O-Ar}$), 3.90 (s, 3H, CH_3), 3.79-3.60 (m, 24H, CH_2), 3.56-3.53 (m, 6H, CH_2), 3.39 (s, 9H, CH_3). ^{13}C NMR (100 MHz, CDCl_3) δ : 166.58, 152.32, 142.66, 124.95, 109.1, 72.41, 71.94, 70.84, 70.70, 70.69, 70.57, 70.56, 69.64, 68.88, 59.00, 58.99, 52.12.

MS (TOF): m/z: Calculated: 617.31 g/mol $[M+Na]^+$, Found: 645.3034 $[M+Na]^+$, $\Delta=8.98$ ppm.

2.2.3 Synthesis of Compound (3)

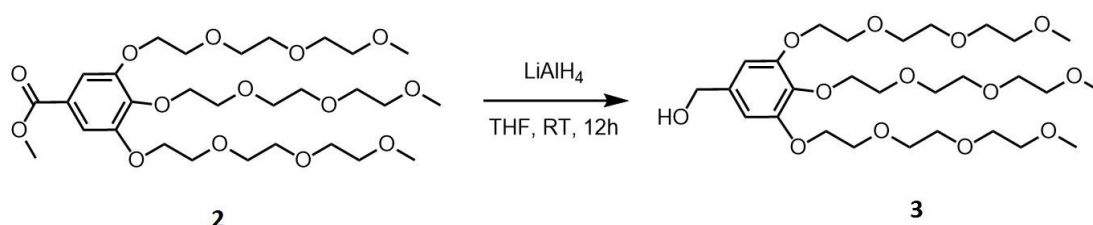


Figure 15: Synthesis of Compound 3.

Compound **2** (3 g, 4.8 mmol) was dissolved in 20 ml freshly distilled THF while the reaction mixture was cooled in an ice bath. $LiAlH_4$ (347 mg, 9.6 mmol) was added portionwise to this solution after which it was stirred for 12 hours at room temperature. Excess $LiAlH_4$ was then carefully quenched with cold water or pieces of ice. The mixture was then extracted with EtOAc and brine. Organic layer was dried with Na_2SO_4 and evaporated under reduced pressure. The product was purified with silica column chromatography using EtOAc/MeOH (90:10, v/v) as mobile phase. Fraction containing **3** was collected and the solvent was reduced in vacuo to yield colorless oil. (2.5 g, 4.2 mmol, 88%)

1H NMR (400 MHz, $CDCl_3$) δ : 6.64 (s, 2H, Ar-H), 4.58 (s, 2H, OCH_2), 4.18 (m, 6H, OCH_2), 3.85 (t, 4H, OCH_2), 3.80 (t, 2H, OCH_2), 3.73 (m, 6H, OCH_2), 3.65 (m, 12H; OCH_2), 3.56 (m, 6H; OCH_2), 3.39 (s, 9H; OCH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ : 212.98, 152.74, 137.74, 136.97, 136.64, 106.75, 72.27, 71.96, 71.94, 70.79, 70.72, 70.70, 70.55, 70.51, 69.83, 68.92, 66.25, 58.98.

MS (TOF): m/z: Calculated: 617.31 g/mol $[M+Na]^+$, Found: 617.3204 $[M+Na]^+$, $\Delta=-9.89$ ppm.

2.2.4 Synthesis of Compound (4)

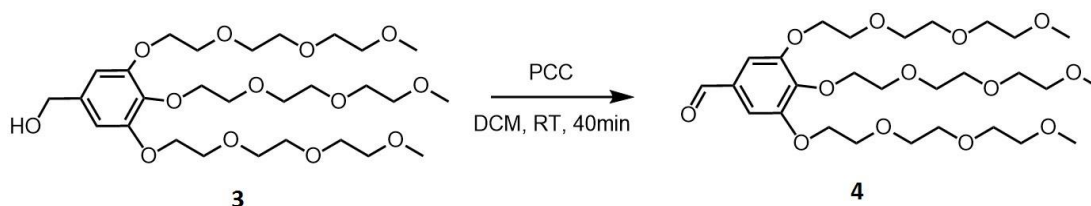


Figure 16: Synthesis of Compound 4.

Compound **3** (2.4 g, 4 mmol) was dissolved in 25 ml DCM. To this mixture, pyridinium chlorochromate (2.15 g, 10 mmol) was added, and stirred for 40 minutes at room temperature. The mixture was directly applied to silica column chromatography, and EtOAc/MeOH (95:5, v/v) was used as mobile phase. Fraction containing compound **4** was collected and concentrated in vacuo to yield colorless oil. (2.37 g, 4.00 mmol, 96%)

¹H NMR (400 MHz, CDCl₃) δ: 9.80 (s, 1H, CHO), 7.12 (s, 2H, Ar-H), 4.26-4.17 (m, 6H, OCH₂), 3.86 (m, 4H, OCH₂), 3.79 (m, 2H, OCH₂), 3.73-3.50 (m, 24H, OCH₂), 3.35 (s, 9H, OCH₃). ¹³C NMR (100 MHz, CDCl₃) δ: 190.89, 153.02, 152.29, 144.16, 131.57, 109.10, 109.02, 72.54, 71.92, 70.83, 70.68, 70.67, 70.59, 70.55, 70.50, 69.62, 69.01, 68.89, 58.97.

MS (TOF): m/z: Calculated: 615.29 g/mol [M+Na]⁺, Found: 615.3030 [M+Na]⁺, Δ=-7.11 ppm

2.2.5 Synthesis of Compound (5)

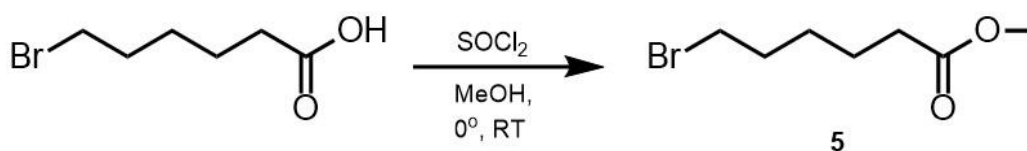


Figure 17: Synthesis of Compound 5.

15 ml of SOCl_2 were added dropwise to 6-bromohexanoic acid (15 g, 77 mmol) dissolved in 200 ml MeOH at 0°C . The reaction mixture was allowed to come to room temperature and stirred for 5 hours. After the reaction was finished, the solvent was removed in vacuo and the residue was dissolved in 100 ml of EtOAc. The mixture was then extracted with water, NaHCO_3 , and brine. The organic layer was evaporated, yielding compound **5** as yellow oil. (14.6 g, 0.07 mol, 91%).

^1H NMR (400 MHz, CDCl_3) δ : 3.60 (s, 3H), 3.34 (t, $J = 6.8$, 2H), 2.26 (t, $J = 7.5$, 2H), 1.87-1.75 (m, 2H), 1.66-1.53 (m, 2H), 1.47- 1.36 (m, 2H). ^{13}C NMR (101 MHz, CDCl_3) δ : 173.68, 51.34, 33.63, 33.24, 32.20, 27.47, 23.88.

MS (TOF): m/z : Calculated: 208.00 g/mol $[\text{M}+\text{H}]^+$, Found: 209.0172 $[\text{M}+\text{H}]^+$, $\Delta = -7.56$ ppm

2.2.6 Synthesis of Compound (6)

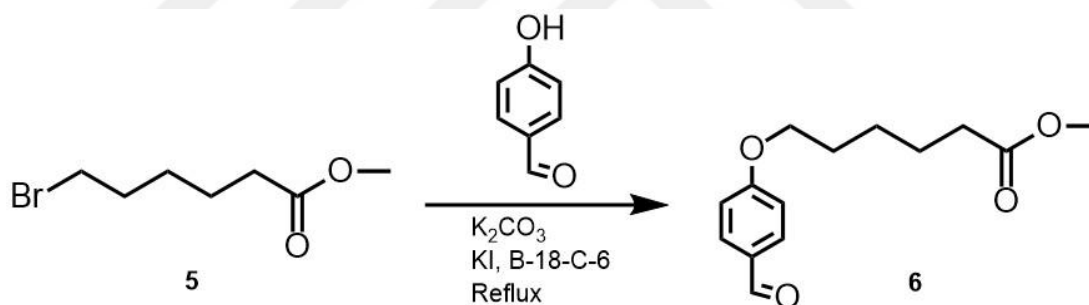


Figure 18: Synthesis of Compound 6.

4-hydroxybenzaldehyde (374 mg, 3.06 mmol), K_2CO_3 (1 g, 7.65 mmol) and compound **5** (800 mg, 3.84 mmol) were dissolved in 25 ml of acetonitrile. A pinch of KI and B-18-C-8 were added to the solution. The solution was monitored with TLC, until all the reactant was consumed. The product was filtered, and the solvent was removed in vacuo. The residue was diluted with EtOAc (150 ml) and washed with brine (100ml x 2). Organic phase was dried with MgSO_4 , filtered, and concentrated. The compound was then purified with silica column chromatography with DCM as mobile phase to yield compound **6**. (641 mg, 2.56 mmol, 67 %)

^1H NMR (400 MHz, CDCl_3) δ : 9.88 (s, 1H), 7.82 (dd, $J = 8.8, 2.1$ Hz, 2H), 6.98 (dd, $J = 8.8, 2.1$ Hz, 2H), 4.04 (td, $J = 6.4, 2.0$ Hz, 3H), 3.67 (d, $J = 2.0$ Hz, 2H), 2.35 (dd, $J = 7.5, 2.0$ Hz, 2H), 1.87 – 1.83 (m, 2H), 1.72 – 1.67 (m, 2H), 1.53 (dd, $J = 7.7, 2.0$ Hz, 2H). ^{13}C NMR (100 MHz, CDCl_3) δ : 190.54, 173.72, 163.91, 131.75, 129.63, 114.53, 67.83, 51.29, 33.69, 28.53, 25.36, 24.39.

MS (TOF): m/z : Calculated: 250.12 g/mol $[\text{M}+\text{H}]^+$, Found: 251.1278 $[\text{M}+\text{H}]^+$, $\Delta = -6.03$ ppm

2.2.7 Synthesis of Compound (7)

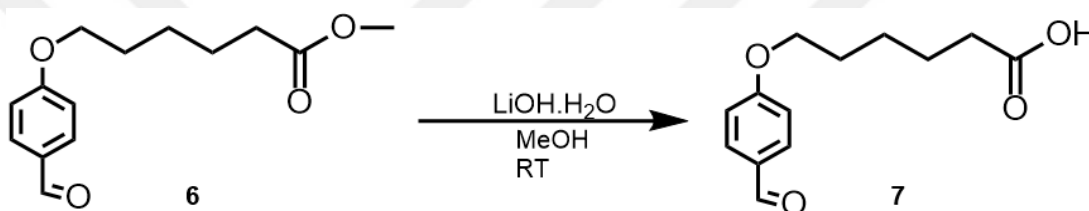


Figure 19: Synthesis of Compound 7.

Compound **6** (612 mg, 2.45 mmol) and $\text{LiOH}\cdot\text{H}_2\text{O}$ (205 mg, 4.89 mmol) were dissolved in 15 ml MeOH. The reaction was left to take place at room temperature, overnight. After all the reactant was consumed, MeOH was removed in vacuo. The remaining solid was dissolved in minimum amount of DCM. 50ml of H_2O with 2ml HCl mixture was added, and the pH was monitored to be lower than 2. The mixture was extracted with DCM. Organic phase was dried with MgSO_4 , filtered, and concentrated. The compound was then purified with silica column chromatography with DCM as mobile phase to yield compound **7**. (312 mg, 1.32 mmol, 60%)

^1H NMR (400 MHz, CDCl_3) δ : 9.81 (s, 1H), 7.76 (d, $J = 7.9$ Hz, 2H), 6.92 (d, $J = 8.3$ Hz, 2H), 3.98 (t, $J = 6.2$ Hz, 2H), 2.35 (t, $J = 7.2$ Hz, 2H), 1.90 – 1.73 (m, 2H), 1.71 – 1.57 (m, 2H), 1.53 – 1.37 (m, 2H). ^{13}C NMR (100 MHz, CDCl_3) δ : 190.62, 176.01, 131.83, 114.57, 99.83, 76.53, 67.84, 33.11, 28.58, 25.36, 24.22.

MS (TOF): m/z : Calculated: 236.27 g/mol $[\text{M}+\text{H}]^+$, Found: 237.1158 $[\text{M}+\text{H}]^+$, $\Delta = -15.29$ ppm

2.2.8 Synthesis of Compound (8)

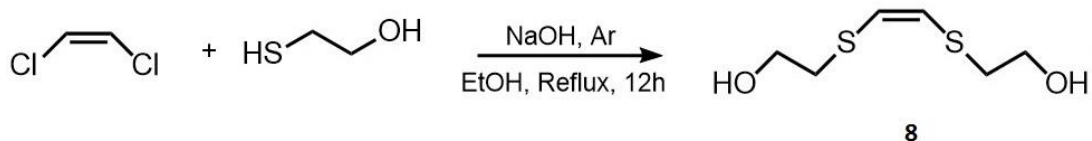


Figure 20: Synthesis of Compound 8.

A mixture of 2-mercaptoethanol (3 g, 38.39 mmol) and NaOH (1.535 g, 38.39 mmol) were dissolved in 15 ml EtOH and stirred at 0°C for 30 minutes. Then, cis-1,2-dichloroethylene (1.86 g, 19.19 mmol) was dissolved in 2 ml EtOH and added dropwise to the mixture. The resulting solution was heated to 80°C for 18 hours. The mixture was then cooled and diluted with water (20 ml), and washed with diethyl ether (3 x 10). The organic phase was dried with Na₂SO₄ and the solvent was removed under reduced pressure. The crude product was then purified with silica column chromatography using EtOAc/Hex (2:1, v/v) as mobile phase. The fraction containing compound **8** was then concentrated in vacuo, resulting in yellowish oil. (2.07 g, 11.5 mmol, 60 %)

¹H NMR (400 MHz, CDCl₃) δ: 6.16 (s, 2H), 4.01 – 3.54 (m, 4H), 3.10 – 2.69 (m, 4H), 2.22 (d, 2H). ¹³C NMR (100 MHz, CDCl₃) δ: 124.51, 61.12, 37.00.

MS (TOF): m/z: Calculated: 203.01 g/mol [M+Na]⁺, Found: 203.0170 [M+Na]⁺, Δ= 11.44 ppm

2.2.9 Synthesis of Compound (9)

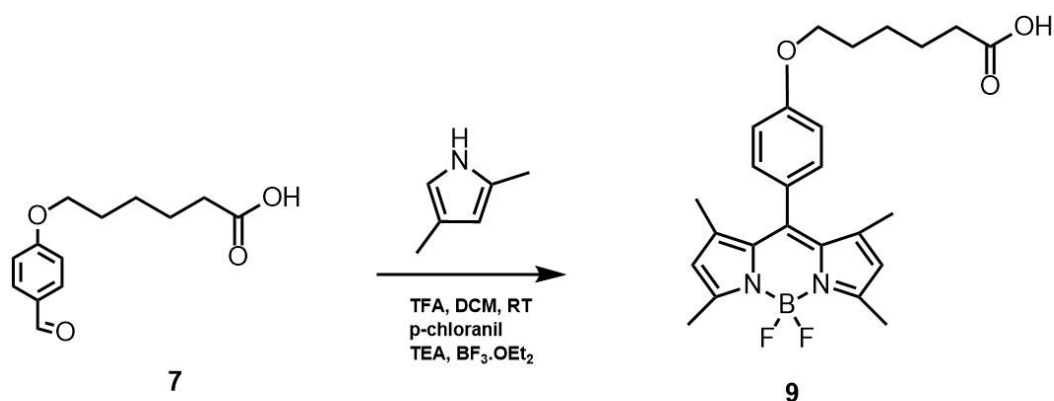


Figure 21: Synthesis of Compound 9.

300 ml of DCM was purged with Ar for 30 minutes. To this solution, pyrrole (0.258 ml, 2.71 mmol) and compound 7 (270 mg, 1.14 mmol) were added, along with 2 drops of TFA. The solution was left to spin overnight in room temperature. After that, p-chloranil (440 mg, 1.79 mmol) was added, and the solution was stirred for 2 more hours. Then 2 ml of triethylamine and 2 ml of BF₃·OEt₂ were added with intervals of 30 minutes. The mixture was extracted with water, dried with Na₂SO₄ and the solvent was removed under reduced pressure. The crude was purified with silica column chromatography using DCM/MeOH (95:5, v/v) as mobile phase. Fractions containing compound 9 were concentrated under vacuo. (145 mg, 0.32 mmol, 28%)

¹H NMR (400 MHz, CDCl₃) δ: 7.08 (d, *J* = 8.4 Hz, 2H), 6.92 (d, *J* = 8.5 Hz, 2H), 5.90 (s, 2H), 3.94 (t, *J* = 6.4 Hz, 2H), 2.60 – 2.23 (m, 4H), 2.35 (t, *J* = 7.4 Hz, 2H), 1.79 (dt, *J* = 14.4, 6.6 Hz, 2H), 1.68 (p, *J* = 7.4 Hz, 2H), 1.57 – 1.49 (m, 2H), 1.36 (s, 6H). ¹³C NMR (100 MHz, CDCl₃) δ: 177.87, 159.42, 155.08, 143.00, 141.75, 131.70, 129.12, 126.81, 120.92, 114.89, 67.57, 33.49, 28.77, 25.46, 24.32, 14.41.

MS (TOF): *m/z*: Calculated: 453.22 g/mol [M+H]⁺, Found: 454.23 [M+H]⁺, Δ=-3.22 ppm

2.2.10 Synthesis of Compound (10)

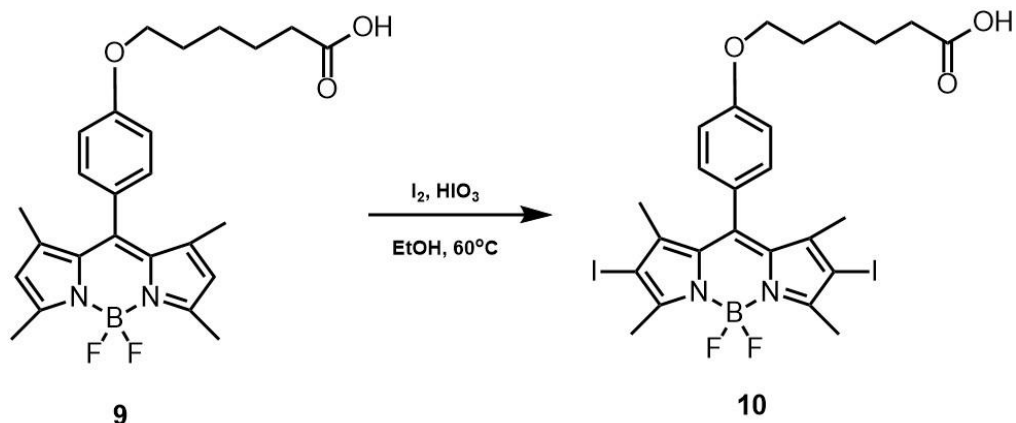


Figure 22: Synthesis of Compound 10.

Compound **9** (140 mg, 0.306 mmol) was dissolved in 95 ml ethanol with I_2 (236.09 mg, 0.78 mmol). HIO_3 was dissolved in several drops of water and then added to the mixture. The reaction was set to reflux for several hours until all reactant was consumed. After the mixture cooled to ambient temperature, 47.5 ml sodium thiosulfate solution was added to it, and stirred for additional 30 minutes. The extraction was performed with DCM and water. The organic layer was dried with Na_2SO_4 and the solvent was removed under reduced pressure. The crude was purified with silica column chromatography using DCM/MeOH (95:5, v/v) as mobile phase. Fractions containing compound **10** were concentrated under vacuo.

1H NMR (400 MHz, $CDCl_3$) δ : 7.05 (d, $J = 8.6$ Hz, 2H), 6.94 (d, $J = 8.6$ Hz, 2H), 3.96 (t, $J = 6.4$ Hz, 2H), 2.57 (s, 6H), 2.37 (t, $J = 7.4$ Hz, 2H), 1.86 – 1.73 (m, 2H), 1.73 – 1.63 (m, 2H), 1.58 – 1.43 (m, 2H), 1.38 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ : 159.86, 156.40, 145.22, 128.94, 115.21, 67.69, 33.35, 29.54, 28.74, 25.46, 24.32, 17.02, 15.85.

MS (TOF): m/z : Calculated: 578.11 g/mol $[M+H]^+$, Found: 579.12 $[M+H]^+$, $\Delta = -2.60$ ppm

2.2.11 Synthesis of Compound (11)

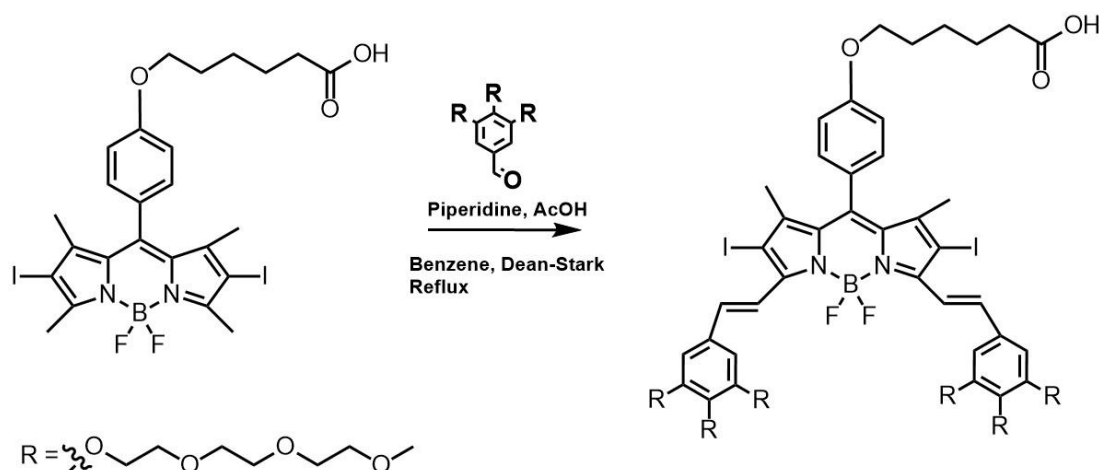


Figure 23: Synthesis of Compound 11.

Compound **9** (131 mg, 0.186 mmol), compound **4** (243 mg, 0.372 mmol) were dissolved in 45 ml benzene, to which 0.44 ml piperidine and 0.44 ml acetic acid were added. The reaction was set to reflux with Dean-Stark apparatus until all the reactants were consumed. The reaction was monitored by TLC. The mixture was extracted with DCM and water, organic layer was dried with Na₂SO₄, the crude was concentrated under reduced pressure, then purified with silica gel chromatography with DCM/MeOH (90:10, v/v). Fraction containing compound **11** was concentrated in vacuo.

¹H NMR (400 MHz, CDCl₃) δ: 7.86 (d, *J* = 16.3 Hz, 2H), 7.39 (d, *J* = 16.6 Hz, 2H), 7.10 (d, *J* = 8.5 Hz, 2H), 6.97 (d, *J* = 8.4 Hz, 2H), 6.78 (s, 6H), 4.15 – 4.10 (m, 12H), 3.98 (t, *J* = 6.3 Hz, 2H), 3.71 – 3.62 (m, 12H), 3.61 – 3.52 (m, 36H), 3.50 – 3.43 (m, 12H), 3.31 (d, *J* = 1.3 Hz, 9H), 3.29 (d, *J* = 1.3 Hz, 9H), 2.37 (t, 2H), 2.32 (t, *J* = 1.3 Hz, 1H), 1.89 – 1.76 (m, 2H), 1.73 – 1.65 (m, 2H), 1.58 – 1.49 (m, 2H), 1.45 (s, 6H).
¹³C NMR (100 MHz, CDCl₃) δ: 152.73, 115.26, 107.63, 99.83, 72.35, 71.80, 71.78, 70.65, 70.53, 70.52, 70.41, 70.38, 69.59, 68.98, 58.85, 58.83, 33.11, 28.76, 25.48, 24.36, 17.66.

MS (TOF): m/z: Calculated: 1876.61 g/mol [M+H]⁺, Found: 1876.62 [M+H]⁺, Δ=-8.21 ppm

2.2.12 Synthesis of Compound (12)

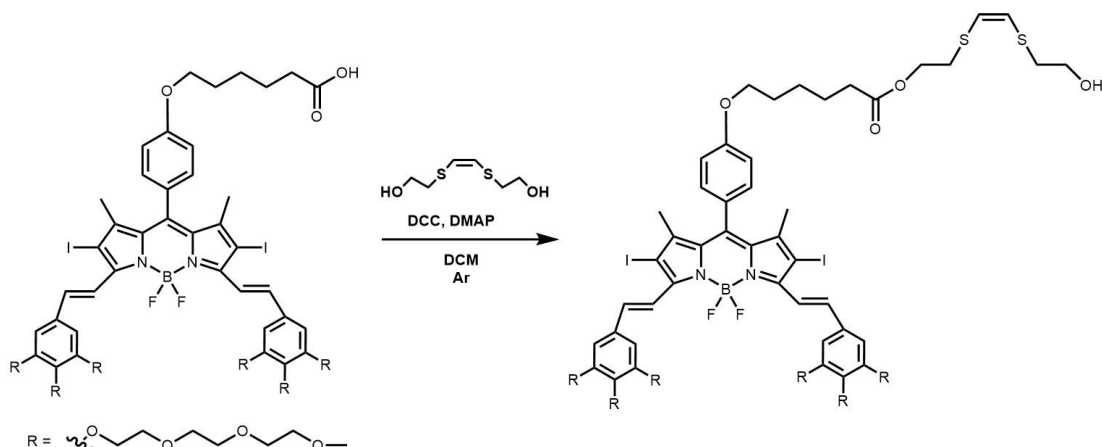


Figure 24: Synthesis of Compound 12.

Compound **11** (0.167 mg, 0.09 mmol), compound **8** (162 mg, 0.90 mmol) and 0.1 equivalent DMAP (1.1 mg, 0.9 μmol) were dissolved in 30 ml DCM, argon was purged for 15 minutes. 1 equivalent of DCC (19 mg, 0.09 mmol) was added at 0°C, then stirred overnight at room temperature. The solvent was then removed under reduced pressure, and the crude was subjected to silica column chromatography with DCM/MeOH (90:10, v/v) as mobile phase. Fraction containing compound **12** was concentrated in vacuo. (50 mg, 0.024 mmol, 27%)

^1H NMR (400 MHz, CDCl_3) δ : 7.94 (d, $J=16.4$ Hz, 2H), 7.47 (d, $J=16.4$ Hz, 2H), 7.19 (d, $J=8.8$ Hz, 2H), 7.06 (d, $J=8.8$ Hz, 2H), 6.87 (s, 4H), 4.41 (t, $J=6.8$ Hz, 2H), 4.25-4.20 (m, 12H), 4.07 (t, $J=6.4$ Hz, 2H), 3.87 (t, $J=5.6$ Hz, 8H), 3.83 (t, $J=5.2$ Hz, 4H), 3.77-3.73 (m, 12H), 3.69-3.64 (m, 24H), 3.59-3.53 (m, 12H), 3.40 (s, 6H), 3.38 (s, 12H), 2.97 (t, $J=6.8$ Hz, 2H), 2.94-2.90 (m, 2H), 2.43-2.41 (m, 2H), 2.31 (2H), 1.94-1.84 (m, 2H), 1.84 (2H), 1.64-1.60 (m, 2H), 1.54 (s, 6H). ^{13}C NMR (100 MHz, CDCl_3) δ : 159.92, 152.70, 139.48, 131.99, 124.70, 124.52, 124.10, 115.24, 107.56, 72.33, 71.78, 71.76, 70.65, 70.63, 70.53, 70.51, 70.49, 70.40, 70.36, 69.59, 69.56, 68.94, 61.12, 37.13, 33.90, 29.53, 28.74, 25.50, 24.48, 17.66.

MS (TOF): m/z : Calculated: 2016.63 g/mol $[\text{M}+\text{H}]^+$, Found: 2016.64 $[\text{M}+\text{H}]^+$, $\Delta=19.03$ ppm

[illegible]

Compound **12** (22.9 mg, 0.011 mmol) was dissolved in 5 ml DCM, and the solution was purged with argon for 10 minutes. To this mixture, 9 μ l triethylamine and nitrophenylchloroformate (6.86 mg, 34.05 μ mol) were added. The reaction was stirred at room temperature overnight. Solvent was reduced in volume, and crude was applied directly to silica column with Hexane/Acetone (3:2, v/v) as mobile phase. Fraction containing compound **13** was concentrated in vacuo. (10 mg, 4.58 μ mol, 36 %)

MS (TOF): m/z: Calculated: 2181.64 g/mol [M+H]⁺, Found: 2181.59 [M+H]⁺, Δ= 12.43 ppm

2.2.14 Synthesis of Compound (14)

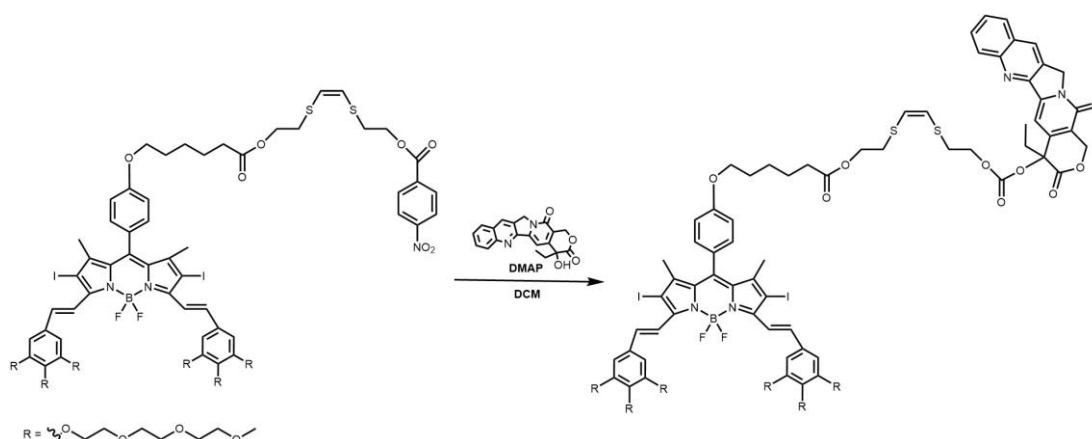


Figure 26: Synthesis of Compound 14.

Compound **13** (19.9 mg, 9.12 μmol) was dissolved in minimum amount of DCM. After addition of 20-S-Camptothecin (3.7 mg, 10.68 μmol) and DMAP (1.3 mg, 10.68 μmol) the reaction mixture was stirred overnight. After the starting material was consumed, the mixture was evaporated under reduced pressure and residue was subjected to silica column chromatography using DCM as mobile phase. (3 mg, 1.25 μmol , 13%).

MS (TOF): m/z : Calculated: 2389.72 g/mol $[\text{M}+\text{H}]^+$, Found: 2390.73 $[\text{M}+\text{H}]^+$, $\Delta = -12.54$ ppm

CHAPTER 3: RESULTS AND DISCUSSION

When chemotherapy is used together with a cytotoxic drug, and combined with surgery, they yield grand promising therapeutic results. As previously mentioned, chemotherapy used alone has negative side effects on the whole body, it is not selective to tumors, is toxic and does a lot of damage to other tissues other than the target tumor tissue. Development of this strategy would include increasing the selectivity of the agent and minimizing side effects. Based on this theory, various target delivery systems with different carrying agents have been and are still being developed.

The aim of this project was to choose a fluorophore and a drug, and connect them with a cleavable linker that can be activated by a trigger. Upon administration, the drug would build up in the tumor tissues, and with the help of a light source it would be activated, cleaving the cytotoxic drug. The rough representation is shown in *Figure 27*.

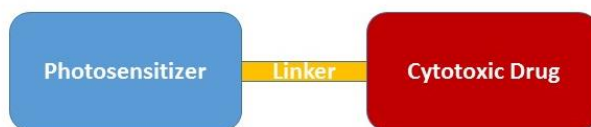


Figure 27: Basic representation of the overall aim.

The choice of the BODIPY sensitizer was made considering its unique chemical and physical properties. These photosensitizers have high extinction coefficients in the visible and near IR range with low light-dark toxicity ratios. They are very thermal and photostable, and soluble in organic solvents, easing the progress of purification during synthesis. The absorption of BODIPY's can be shifted towards longer wavelengths via modifications at specific positions. The BODIPY core can be modified in several positions, they are shown in *Figure 28*.

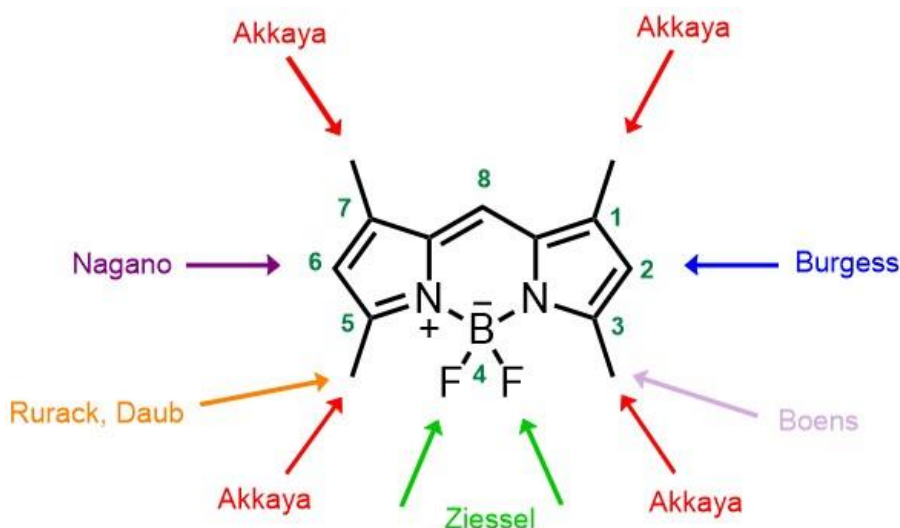


Figure 28: Positions of modification of a BODIPY core.

Properties of BODIPY change with respect to the number, position and the nature of the group attached. The *meso*-, or position 8 can be modified during the acid-catalyzed condensation reaction of pyrroles with acid chlorides, anhydrides, and aldehydes. Addition of a substitute at this position usually enhances stability of the dye when compared to an unsubstituted cores. The 1, 3, 5, and 7 positions of the core can be modified through Knoevenagel condensation reactions with aldehydes. Positions 2 and 6 are usually favored for electrophilic iodination or bromination.

For this project, the first step was to decide on the aldehyde, which would be in the meso position of the BODIPY. It was important to provide space between the sensitizer and the drug and decrease the chance of steric hindrance, so an organic molecule with a long chain was chosen for best results, 6-bromohexanoic acid. During its synthesis, even though it was possible to have a reaction from 6-bromohexanoic acid with 4-hydroxybenzaldehyde to produce compound **6**, the pathway was chosen to be extended through a Fischer esterification process, or esterification of carboxylic acids with alcohols catalyzed by an acid to produce better yields. After compound **6** was obtained, it was proceeded to the synthesis of the BODIPY core with compound **6** as aldehyde.

The 2 and 6 positions of the BODIPY core bear the least positive charge (*Figure 29*), which consequently makes them susceptible to electrophilic attacks. Heavy atoms

like bromine and iodine decrease the quantum yield through the heavy-atom effect described earlier.

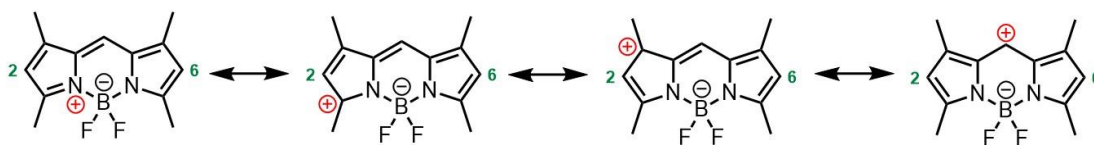


Figure 29: Mesomeric effect of 2 and 6 positions of BODIPY.

BODIPY cores have low solubility in aqueous media, and in order to eliminate this, very water soluble polyethylene glycol (PEG) or triethylene glycol (TEG) groups can be attached to the 1, 3, 5, and 7 positions of the sensitizer. Since we already had a bulky benzene ring at the *meso*- position, it was logical to choose to modify only the 3 and 5 positions with TEG moieties with the Knoevenagel reaction. The addition occurs through the aromatic moiety, which helps create a red-shift in the optical spectra with respect to the unsubstituted molecule to the near-IR range. The synthesis of TEG groups began with the reflux reaction of 3,4,5-trihydroxybenzoate and triethylene glycol methyl ether tosylate in acetone, where K_2CO_3 and 18-crown-6 are added for catalysis, resulting in compound **2**. This process was then followed by reduction with $LiAlH_4$, and then consequent oxidation by PCC, resulting in compound **4**, which then replaces the acidic methyl groups at 3 and 5 positions.

The second part of the project was choosing a drug suitable for this kind of therapy and attaching it to the BODIPY. After doing some research about novel promising drugs, 20(S)-camptothecin (CPT) was chosen as a good candidate. It consists of five rings, and can be modified through more than one position. Even though CPT was mostly modified through A and B rings, ring E seemed to be a reasonable place for attachment. CPT is unstable due to the 20-hydroxy lactone, which under the conditions with neutral pH gets hydrolyzed to produce an inactive carboxylate.¹⁰⁶ The hydroxyl moiety plays a crucial role in the stability and activity of CPT. Masking this molecule results in reduced activity of the drug and increased stability of the lactone ring.¹⁰³ Another positive property that comes up after the masking is the increase of solubility of CPT, which is almost insoluble in aqueous and organic media.

After it was decided on the photosensitizer and the drug, it was important to find a proper linker that would (1) link the two parts together and (2) be able to release both parts upon activation without deviating the initial properties and purpose. It was essential to use the property of BODIPY dyes and the generation of $^1\text{O}_2$. In theory, as singlet oxygen is generated, the (Z)-1,2-bis(alkyl-thio)ethane goes through 1,4-cycloaddition to oxygen, resulting in intramolecular cyclization and the release of both the sensitizer and the drug in its initial state. The representation of this process is shown in *Figure 30*.

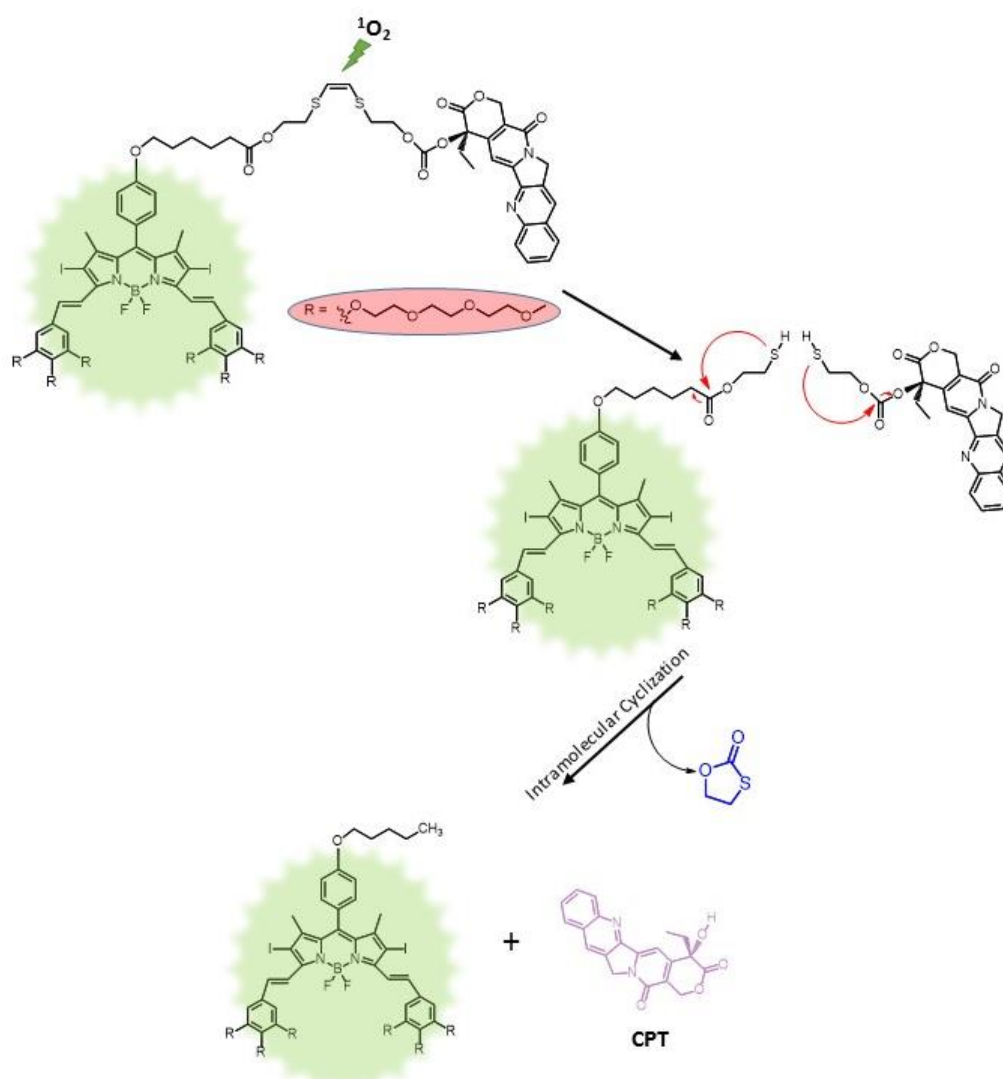


Figure 30: Cleavage reaction of the (Z)-1,2-bis(alkyl-thio) linker.

The usage of this linker would not only protect the purpose of the sensitizer and the drug, but also the unstable dioxethane would easily hydrolyze post cleavage to corresponding thiols. The resulting two molecules would harm the tissues by both the production of cytotoxic singlet oxygen, and the CPT would regain its activity and cytotoxicity, harming the cells.

After compound **11** was synthesized, it was mixed with compound **8** in the ratio of 10 to 1 in the presence of DMAP and DCC, to eliminate the di-substituted linker. After that, nitrochloroformate was used to attach compound **12** to CPT. The downside of this process is purification of the product. The important point in this step was not to use an alcohol during the silica column chromatography due to the high possibility of transesterification process, process of which is shown in more detail in *Figure 31*. Methanol is used very frequently during purification due to its polarity, and its absence created difficulties during the purification step. Other solvents like triethylamine were used, but they were not as efficient.

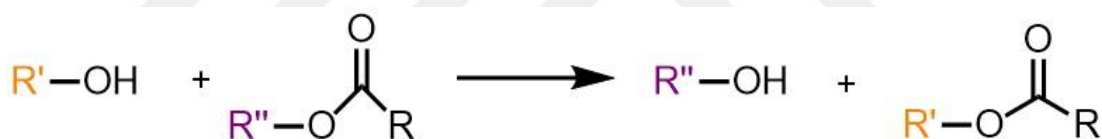


Figure 31: Transesterification reaction of an alcohol with an ester.

The colour of the products does not change after the production of compound **11**, and the molecules become very large. This fact created difficulties during the distinguishing process of the last three compounds on TLC plates. Also, the compounds were not very stable, and decomposition took place, yielding even more side products.

We were able to obtain clear mass spectra for all the compounds, up until the last step. However, the NMR spectra was not as clear for the last step. The decomposition and the large size of the molecule made it difficult to characterize. Considering the fact that the BODIPY part is relatively stable, we concentrated on the aromatic region of

the compound. Even though there was a lot of interference in the aliphatic region, the amount of aromatic protons should have increased from 16 to 18 if not considering the protons of the linker. NMR shifts for compounds **1-10** were compared with similar compounds in the literature.^{107,108} The indicator peaks, apart from the amount of protons in the aromatic area, were the protons of the carbon-carbon double bond of the linker, which occur around 6.16 – 6.25 ppm for compound **13**. Their presence, along with the increase in MS data with the Δ below the value of ± 20.00 ppm. The four proton peaks of nitrochloroformate upon addition were also seen on the NMR spectra. All values for mass spectra were consistent with the calculated predictions.

It was confirmed by the mass spectra that compound **14** was successfully synthesized. For future work, it would be possible to add more properties to the molecule. The compound can be further modified with targeting moieties such as biotin at 3 and 5 positions, whereas the fluorescence of a molecule can be blocked while it is attached to the drug, and triggered upon release for imaging. However, in this case it would be more convenient to use a linker with properties other than sensitivity to singlet oxygen, but rather a light source. It is possible to synthesize a theranostic compound, combining the therapeutic effects and imaging capability in one.

CHAPTER 4: CONCLUSION

In this project, we focused on PDT, its major problems, and the synthesis and development of a BODIPY based multimodal therapeutic compound. Photodynamic theory includes three major components, where a photosensitizer in a presence of light produces singlet oxygen, it is cytotoxic to the surrounding tissue. The aim was to utilize that singlet oxygen and make a compound more deadly by adding an additional drug used to treat various cancers.

A BODIPY sensitizer was chosen as a sensitizer, and modified to be more compatible with the body. Its properties were adjusted so that it would be more water soluble, and its emission would be in the longer wavelengths. Camptothecin was chosen to be the second part of the compound. This drug has a very high antitumor activity, and its main property is the inhibition of topoisomerase I during cell division. BODIPY and CPT were then attached by a (Z)-1,2-bis(alkyl-thio) moiety, a linker that undergoes a cleavage reaction in the presence of singlet oxygen. When CPT is modified through the 20-hydroxy position, its activity vastly decreases. When the drug is released, it regains its activity, making this compound a better option with respect to chemotherapy.

All compounds were synthesized and characterized by mass and NMR spectra. The future work includes the design and synthesis of derivatives with even more properties. Mainly, the efficacy of the compound can be enhanced by modification of 3 or 5 positions with moieties that promote localization in the tumor tissues, with BODIPY having a water soluble group in one of the two positions, and the localizing group at the other.

BIBLIOGRAPHY

1. M. Triesscheijn, P. Baas, J. Schellens and F. Stewart, "Photodynamic therapy in oncology," *Oncologist*, vol. 11, pp. 1034–44, 2006.
2. J. Cabrera-rivero, "Delamanid for Multidrug-Resistant Pulmonary Tuberculosis," *New England Journal of Medicine*, vol. 366, pp. 2151–2160, 2012.
3. J. F. Lovell, T.W. B. Liu, J. Chen and G. Zheng, "Activatable Photosensitizers for Imaging and Therapy," *Chem. Rev.* vol. 110, pp. 2839–2857, 2010.
4. W. M. Sharman, C. M. Allen and J. E. Van Lier, "Photodynamic therapeutics: Basic principles and clinical applications," *Drug Discov. Today*, vol. 4, pp. 507–517, 1999.
5. K. Szaciłowski, W. Macyk, A. Drzewiecka-Matuszek, M. Brindell and G. Stochel, "Bioinorganic Photochemistry: Frontiers and Mechanisms," *Chem. Rev.* vol. 105, pp. 2647–2694, 2005.
6. Y. N. Konan, R. Gurny and E. Allemann, "State of the art in the delivery of photosensitizers for photodynamic therapy," *J. Photochem. Photobiol. B.*, vol. 66, pp. 89–106, 2002.
7. A. Castano, P. Mroz and M. Hamblin, "Photodynamic therapy and anti-tumour immunity," *Nat. Rev. Cancer*, vol. 6, pp. 535–545, 2006.
8. R. Ackroyd, C. Kelty, N. Brown and M. Reed, "The history of photodetection and photodynamic therapy," *Photochem. Photobiol.* vol. 74, pp. 656–669, 2001.
9. T. B. Fitzpatrick, and M. A. Pathak, "Considerations of the Psoralens," *J. Invest. Dermatol.* vol. 32, pp. 229–231, 1958.
10. O. Raab, "Ueber die Wirkung Fluorescierenden Stoffe auf Infusorien," *Z. Biol.*, vol. 39, pp. 524–546, 1904.
11. H. Von Tappeiner and H. Jesionek, "Therapeutische Versuche mit fluoreszierenden Stoffen," *Munch. Med. Wschr.*, vol. 50, pp. 2042–2044, 1903.
12. H. Von Tappeiner, "Uber die Wirkung fluoreszierender Stoffe auf Infusorien nach Versuchen von O. Raab," *Muench Med Wochenschr.*, vol. 47, pp. 23–24, 1900.
13. W. Meyer, "The surgery of the pulmonary artery," *Ann Surg.*, vol. 58, pp. 188–205, 1913.
14. J. F. Evensen, S. Sommer, C. Rimington and J. Moan, "Photodynamic therapy of C3H mouse mammary carcinoma with haematoporphyrin di-ethers as sensitizers," *Br J Cancer*, vol. 55, 483–486, 1987.
15. J. Moan, C. Rimington and S. Sommer, "Cellular uptake and photosensitizing properties of hematoporphyrin di-ethers with similar chromatographic properties as the tumorlocalizing fraction of hematoporphyrin derivative," *Cancer Letters*, vol. 34, pp. 283–289, 1987.
16. Q. Peng, J. Moan, G. Farrants, H. E. Danielsen and C. Rimington, "Localization

- of potent photosensitizers in human by means of laser scanning microscopy tumor LOX," *Cancer Letters*, vol. 53, pp. 129–139, 1990.
17. J. Moan, T. Christensen and S. Sommer, "Retention and photodynamic effects of haematoporphyrin derivative in cells after prolonged cultivation in the presence of porphyrin." *Br J Cancer*, vol. 15, pp. 35–43, 1982.
 18. J. Moan and E. Wold, "Detection of singlet oxygen production by ESR," *Nature*, vol. 279, pp. 450–451, 1979.
 19. J. Moan and S. Sommer, "Oxygen dependence of the photosensitizing effect of hematoporphyrin derivative in NHIK 3025 cells," *Cancer Res*, vol. 45, pp. 1608–1610, 1985.
 20. J. Moan and K. Berg, "The photodegradation of porphyrins in cells can be used to estimate the lifetime of singlet oxygen," *Photochem. Photobiol.* vol. 53, pp. 549–53, 1991.
 21. J. Moan, "On the diffusion length of singlet oxygen in cells and tissues," *J. Photochem. Photobiol. B Biol.* vol. 6, pp. 343–344, 1990.
 22. V. Iani, J. Moan and L. W. Ma, "Measurements of light penetration into human tissues in vivo," *P Soc Photo-Opt Ins*, vol. 2625, pp. 378–383, 1996.
 23. J. Moan, Q. Peng, R. Sorensen, V. Iani and J. Nesland, "The biophysical foundations of photodynamic therapy," *J. Chem. Inf. Model.* vol. 30, pp. 387–391, 1998.
 24. J. Moan, V. Iani and L. W. Ma, "Choice of the proper wavelength for photochemotherapy," *P Soc Photo-Opt Ins*, vol. 2625, pp. 544–549, 1989.
 25. C. Whitehurst, K. Byrne and J. V. Moore, "Development of an alternative light source to lasers for photodynamic therapy: 1. Comparative in vitro dose response characteristics," *Lasers Med. Sci.* vol. 8, pp. 259–267, 1993).
 26. T. C. Zhu, and J. C. Finlay, "The role of photodynamic therapy (PDT) physics," *Med. Phys.* vol. 35, pp. 3127–3128, 2008.
 27. A. G. Wile, A. Dahlman and R. G. Burns, "Laser Photoradiation Therapy of Cancer Following Hema toporphyrin Sensitization," *Lasers Surg Med.*, vol. 168, pp. 163–168, 1982.
 28. J. F. Kelly, M. E. Snell and M. C. Berenbaum, "Photodynamic destruction of human bladder carcinoma," *Br. J. Cancer*, vol. 31, pp. 237–44, 1975.
 29. V. Balzani, A. Credi and M. Venturi, "Photoprocesses," *Curr. Opin. Chem. Biol.* vol. 1, pp. 506–513, 1997.
 30. K. Szaciłowski, W. Macyk, A. Drzewiecka-matuszek and M. Brindell, "Bioinorganic Photochemistry : Frontiers and Mechanisms," *Chem. Rev.*, vol. 105, pp. 2647–2694. 2005.
 31. J. R. Lakowicz, "Principles of Fluorescence Spectroscopy," Kluwer/Plenum, 1999.
 32. C. H. Sibata, V. C. Colussi, N. L. Oleinick and T. J. Kinsella, "Photodynamic therapy in oncology," *Expert Opin. Pharmacother.* vol. 2, pp. 917–927, 2001.

33. N. J. Turro, V. Ramamurthy and J. C. Scaiano, "Principles of Molecular Photochemistry: An Introduction," University Science Books: Sausalito, 2009.
34. J. Zhao, W. Wu, J. Sun and S. Guo, "Triplet photosensitizers: from molecular design to applications," *Chem. Soc. Rev.* vol. 42, pp. 5323–5351, 2013.
35. J. F. Lovell, T. W. B. Liu, J. Chen and G. Zheng, "Activatable Photosensitizers for Imaging and Therapy," *Chem Rev.*, vol. 110, pp. 2839–2857, 2010.
36. A. P. Castano, T. N. Demidova and M. R. Hamblin, "Mechanisms in photodynamic therapy: part one—photosensitizers, photochemistry and cellular localization," *Photodiagnosis Photodyn. Ther.* vol. 1, pp. 279–293, 2004.
37. M. Ochsner, "Photophysical and photobiological processes in the photodynamic therapy of tumours," *J Photochem Photobiol B*, vol. 39, pp. 1-18, 1997.
38. C. A. Robertson, D. H. Evans and H. Abrahamse, "Photodynamic therapy (PDT): A short review on cellular mechanisms and cancer research applications for PDT," *J. Photochem. Photobiol. B Biol.* vol. 96, pp. 1–8, 2009.
39. A. Wright, W. A. Bubb, C. L. Hawkins and M. Davies, "Singlet oxygen-mediated protein oxidation: evidence for the formation of reactive side chain peroxides on tyrosine residues," *Photochem Photobiol*, vol. 76, pp. 35–46, 2002.
40. A. W. Girotti, "Mechanisms of lipid peroxidation," *J. Free Radicals Biol. Med.* vol. 1, pp. 87–95, 1985.
41. W. A. Prütz, "Hypochlorous acid interactions with thiols, nucleotides, DNA, and other biological substrates," *Arch. Biochem. Biophys.* vol. 332, pp. 110–120, 1996
42. E. Buytaert, M. Dewaele and P. Agostinis, "Molecular effectors of multiple cell death pathways initiated by photodynamic therapy," *Biochim. Biophys. Acta - Rev. Cancer*, vol. 1776, pp. 86–107, 2007.
43. J. Moan and K. Berg, "The Photodegradation of Porphyrins in Cells Can Be Used To Estimate the Lifetime of Singlet Oxygen," *Photochem. Photobiol.* vol. 53, pp. 549–553, 1991.
44. B. C. Wilson, W. P. Jeeves and D. M. Lowe, "In vivo and post mortem measurements of the attenuation spectra of light in mammalian tissues." *Photochem. Photobiol.* vol. 42, pp. 153–162. 1985.
45. R. R. Allison and C. H. Sibata, "Oncologic photodynamic therapy photosensitizers: A clinical review," *Photodiagnosis Photodyn. Ther.* vol. 7, pp. 61–75, 2010.
46. P. Agostinis *et al.* "Photodynamic Therapy of Cancer: An Update," *CA Cancer J Clin*, vol. 61, pp. 250–281, 2011.
47. B. C. Wilson, W. P. Jeeves and D. M. Lowe, "In vivo and post mortem measurements of the attenuation spectra of light in mammalian tissues," *Photochem. Photobiol.* vol. 42, pp. 153–62, 1985.
48. L. O. Svaasand, "Optical dosimetry for direct and interstitial photoradiation therapy of malignant tumors," *Prog. Clin. Biol. Res.* vol. 170, pp. 91–114, 1984.

49. B. W. Henderson, T. M. Busch and J. W. Snyder, "Fluence Rate as a Modulator of PDT Mechanisms," *Lasers Surg Med*, vol. 493, pp. 489–493, 2006.
50. R. M. Szeimies, C. A. Morton, A. Sidoroff and L. R. Braathen, "Photodynamic therapy for non-melanoma skin cancer," *Acta Derm. Venereol*, vol. 85, pp. 483–90, 2005.
51. A. M. Soler *et al.*, "Photodynamic Therapy of Superficial Basal Cell Carcinoma with 5-Aminolevulinic Acid with Dimethylsulfoxide and Ethylendiaminetetraacetic Acid: A Comparison of Two Light Sources," *Photochem Photobiol*, vol. 71, pp. 724–729, 2000.
52. L. Brancalion and H. Moseley, "Laser and Non-laser Light Sources for Photodynamic Therapy," *Lasers Med Sci*, vol. 17, pp. 173–186, 2002.
53. B. C. Wilson and M. S. Patterson, "The physics, biophysics and technology of photodynamic therapy," *Phys. Med. Biol.*, vol. 53, pp. R61–109, 2008.
54. M. C. Pazos and H. B. Nader, "Effect of photodynamic therapy on the extracellular matrix and associated components," *Brazilian J. Med. Biol. Res.*, vol. 40, pp. 1025–35, 2007.
55. D. Kessel, Y. Luo, Y. Deng and C. K. Chang, "The role of subcellular localization in initiation of apoptosis by photodynamic therapy," *Photochem. Photobiol*, vol. 65, pp. 422–6, 1997.
56. X. Sun, and W. N. Leung, "Photodynamic therapy with pyropheophorbide-a methyl ester in human lung carcinoma cancer cell: efficacy, localization and apoptosis," *Photochem. Photobiol.*, vol. 75, pp. 644–51, 2002.
57. K. Kalka, H. Merk and H. Mukhtar, "Photodynamic therapy in dermatology," *J. Am. Acad. Dermatol.*, vol. 42, pp. 389–413, 2000.
58. K. Berg, J. C. Bommer and J. Moan, "Evaluation of sulfonated aluminum phthalocyanines for use in photochemotherapy. Cellular uptake studies," *Cancer Lett.*, vol. 44, pp. 7–15, 1989.
59. Z. Luksiene, "Photodynamic therapy: mechanism of action and ways to improve the efficiency of treatment," *Medicina (Kaunas)*, vol. 39, pp. 1137–50, 2003.
60. M. R. Detty, S. L. Gibson and S. J. Wagner, "Current clinical and preclinical photosensitizers for use in photodynamic therapy," *J. Med. Chem.*, vol. 47, pp. 3897–915, 2004.
61. J. D. Spikes and J. C. Bommer, "Photosensitizing properties of mono-L-aspartyl chlorin e6 (NPe6): a candidate sensitizer for the photodynamic therapy of tumors," *J. Photochem. Photobiol. B.*, vol. 17, pp. 135–43, 1993.
62. M. C. Derosa and R. J. Crutchley, "Photosensitized singlet oxygen and its applications," *Coordination Chemistry Reviews*, vol. 233-234, pp. 351-371, 2002.
63. N. E. Schore, "Modern molecular photochemistry," *J. Chem. Educ.*, vol. 58, p. 312, 1981.
64. M. A. El-Sayed, D. S. Tinti and D. V. Owens, "Spectroscopic determination of the most probable intersystem crossing route in phosphorescing molecules,"

- Chem. Phys. Lett.*, vol. 3, pp. 339–342, (1969).
65. Z. A. Huang, "Review of Progress in Clinical Photodynamic Therapy," *Technol. Cancer Res. Treat.*, vol. 4, pp. 283–293, 2005.
 66. T. J. Dougherty, "Photosensitizers: therapy and detection of malignant tumors," *Photochem. Photobiol.*, vol. 45, pp. 879–89, 1987.
 67. R. Bonnett, "Photosensitizers of the porphyrin and phthalocyanine series for photodynamic therapy," *Chem. Soc. Rev.* vol. 24, p. 19, 1995.
 68. G. I. Stables and D. V. Ash, "Photodynamic therapy," *Cancer Treat. Rev.* vol. 21, pp. 311–23, 1995.
 69. M. C. Berenbaum, R. Bonnett, E. B. Chevretton, S. L. Akande-Adebakin and M. Ruston, "Selectivity of meso-tetra(hydroxyphenyl)porphyrins and chlorins and of photofrin II in causing photodamage in tumour, skin, muscle and bladder. The concept of cost-benefit in analysing the results," *Lasers Med. Sci.*, vol. 8, pp. 235–243, 1993.
 70. J. Ma and L. Jiang, "Photogeneration of singlet oxygen (1O_2) and free radicals (Sen^{*-} , O_2^{*-}) by tetra-brominated hypocrellin B derivative," *Free Radic. Res.*, vol. 35, pp. 767–77, 2001.
 71. A. Treibs and F. H. Kreuzer, "Difluoroboryl Komplexe von Di- und Tripyrrylmethenen," *Justus Liebigs Ann. Chem.*, vol. 718, pp. 208–223, 1968.
 72. Z. Kostereli, T. Ozdemir, O. Buyukcakil and E. U. Akkaya, "Tetrastaryl-BODIPY-Based Dendritic Light Harvester and Estimation of Energy Transfer Efficiency," *Org. Lett.* vol. 14, pp. 3636–3639, 2012.
 73. M. D. Yilmaz, O. A. Bozdemir and E. U. Akkaya, "Light Harvesting and Efficient Energy Transfer in a Boron-dipyrrin (BODIPY) Functionalized Perylenediimide Derivative," *Org. Lett.*, vol. 8, pp. 2871–2873, 2006.
 74. M. Yuan *et al.*, "A colorimetric and fluorometric dual-model assay for mercury ion by a molecule," *Org. Lett.*, vol. 9, pp. 2313–6, 2007.
 75. H. Kim, A. Burghart, M. B. Welch, J. Reibenspies and K. Burgess, "Synthesis and spectroscopic properties of a new 4-bora-3a,4a-diaza-s-indacene (BODIPY) dye," *Chem. Commun.*, vol. 64, pp. 1889–1890, 1999.
 76. O. Buyukcakil, O. A. Bozdemir, S. Kolenen, S. Erbas and E. U. Akkaya, "Tetrastaryl-Bodipy Dyes: Convenient Synthesis and Characterization of Elusive Near IR Fluorophores," *Org. Lett.*, vol. 11, pp. 4644–4647, 2009.
 77. J. Bañuelos Prieto, F. López Arbeloa, V. Martínez Martínez, T. Arbeloa López and I. López Arbeloa, "Photophysical Properties of the Pyrromethene 597 Dye: Solvent Effect," *J. Phys. Chem.*, vol. 108, pp. 5503–5508, 2004.
 78. A. Kamkaew *et al.*, "BODIPY dyes in photodynamic therapy," *Chem. Soc. Rev.* vol. 42, pp. 77–88, 2013.
 79. A. C. Moor, "Signaling pathways in cell death and survival after photodynamic therapy," *J. Photochem. Photobiol. B.*, vol. 57, pp. 1–13, 2000.
 80. A. Juzeniene and J. Moan, "The history of PDT in Norway. Part II. Recent

- advances in general PDT and ALA-PDT," *Photodiagnosis Photodyn. Ther.*, vol. 4, pp. 80–87, 2007.
81. J. J. Schuitmaker, *et al.*, "Photodynamic therapy: a promising new modality for the treatment of cancer," *J. Photochem. Photobiol. B.*, vol. 34, pp. 3–12, 1996.
 82. B. W. Henderson and T. J. Dougherty, "How does photodynamic therapy work?" *Photochem. Photobiol.*, vol. 55, pp. 145–157, 1992.
 83. D. Nowis *et al.*, "Direct tumor damage mechanisms of photodynamic therapy," *Acta Biochim. Pol.*, vol. 52, pp. 339–52, 2005.
 84. S. Elmore, "Apoptosis: a review of programmed cell death," *Toxicol. Pathol.*, vol. 35, pp. 495–516, 2007.
 85. A. Graczykova, "Photodynamic method of diagnosis and therapy of tumors (in Polish)". Dom Wydawniczy Bellona, 1999.
 86. N. M. Bessler, "Verteporfin therapy in age-related macular degeneration (VAM): an open-label multicenter photodynamic therapy study of 4,435 patients," *Retina*, vol. 24, pp. 512–20, 2004.
 87. A. D. Singh, "Photodynamic therapy of circumscribed choroidal haemangioma," *Br. J. Ophthalmol.*, vol. 88, pp. 1414–1418, 2004.
 88. J. Feyh, "Photodynamic treatment for cancers of the head and neck," *J. Photochem. Photobiol. B.*, vol. 36, pp. 175–7, 1996.
 89. W. Stummer, H. J. Reulen, A. Novotny, H. Stepp and J. C. Tonn, "Fluorescence-guided resections of malignant gliomas--an overview," *Acta Neurochir. Suppl.*, vol. 88, pp. 9–12, 2003.
 90. D. R. Kearns, "Physical and chemical properties of singlet molecular oxygen," *Chem. Rev.*, vol. 71, pp. 395–427, 1971.
 91. S. D. P. Baugh, Z. Yang, D. K. Leung, D. M. Wilson and R. Breslow, "Cyclodextrin Dimers as Cleavable Carriers of Photodynamic Sensitizers," *J. Am. Chem. Soc.*, vol. 123, pp. 12488–12494, 2001.
 92. A. Rotaru and A. Mokhir, "Nucleic acid binders activated by light of selectable wavelength," *Angew. Chemie - Int. Ed.*, vol. 46, pp. 6180–6183, 2007.
 93. D. R. Kearns, "Physical and chemical properties of singlet molecular oxygen," *Chem. Rev.*, vol. 71, pp. 395–427, 1971.
 94. N. V. Voigt *et al.*, "Single-molecule chemical reactions on DNA origami," *Nat. Nanotechnol.*, vol. 5, pp. 200–203, 2010.
 95. M. Wink, "Evolution of secondary metabolites from an ecological and molecular phylogenetic perspective," *Phytochemistry*, vol. 64, pp. 3–19, 2003.
 96. I. Raskin *et al.*, "Plants and human health in the twenty-first century," *Trends Biotechnol.*, vol. 20, pp. 522–31, 2002.
 97. M. E. Wall *et al.*, "Plant Antitumor Agents. I. The Isolation and Structure of Camptothecin, a Novel Alkaloidal Leukemia and Tumor Inhibitor from *Camptotheca acuminata*," *J. Am. Chem. Soc.*, vol. 88, pp. 3888–3890, 1966.

98. J. F. Pizzolato and L. B. Saltz, "The camptothecins," *Lancet*, vol. 361, pp. 2235–2242, 2003.
99. Y. H. Hsiang, R. Hertzberg, S. Hecht and L. F. Liu, "Camptothecin induces protein-linked DNA breaks via mammalian DNA topoisomerase I," *J. Biol. Chem.*, vol. 260, pp. 14873–8, 1985.
100. R. Garcia-Carbonero and J. G. Supko, "Current perspectives on the clinical experience, pharmacology, and continued development of the camptothecins," *Clin. Cancer Res.*, vol. 8, pp. 641–61, 2002.
101. X. Wang, X. Zhou and S. M. Hecht, "Role of the 20-Hydroxyl Group in Camptothecin Binding by the Topoisomerase I–DNA Binary Complex," *Biochemistry*, vol. 38, pp. 4374–4381, 1999.
102. M. R. Redinbo, L. Stewart, P. Kuhn, J. J. Champoux and W. G. Hol, "Crystal structures of human topoisomerase I in covalent and noncovalent complexes with DNA," *Science*, vol. 279, pp. 1504–13, 1998.
103. V. J. Venditto, K. Allred, C. D. Allred and E. E. Simanek, "Intercepting the synthesis of triazine dendrimers with nucleophilic pharmacophores: a general strategy toward drug delivery vehicles," *Chem. Commun.*, vol. 37, 5541–2, 2009.
104. X. Hu, J. Tian, T. Liu, G. Zhang and S. Liu, "Photo-triggered release of caged camptothecin prodrugs from dually responsive shell cross-linked micelles," *Macromolecules*, vol. 46, pp. 6243–6256, 2013.
105. M. H. Lee, J. L. Sessler and J. S. Kim, "Disulfide-Based Multifunctional Conjugates for Targeted Theranostic Drug Delivery," *Acc. Chem. Res.*, vol. 48, pp. 2935–2946, 2015.
106. N. Pessah *et al.*, "Bioactivation of carbamate-based 20(S)-camptothecin prodrugs," *Bioorganic Med. Chem.*, vol. 12, pp. 1859–1866, 2004.
107. S. D. Topel, G. T. Cin and E. U. Akkaya, "Near IR excitation of heavy atom free Bodipy photosensitizers through the intermediacy of upconverting nanoparticles," *Chem. Commun.*, vol. 50, pp. 8896–8899, 2014.
108. J. Li and S. Q. Yao, "'Singapore Green': A New Fluorescent Dye for Microarray and Bioimaging Applications," *Organic Letters*, vol. 11, pp. 405–408, 2009.

APPENDIX A: ^1H NMR and ^{13}C NMR SPECTRA

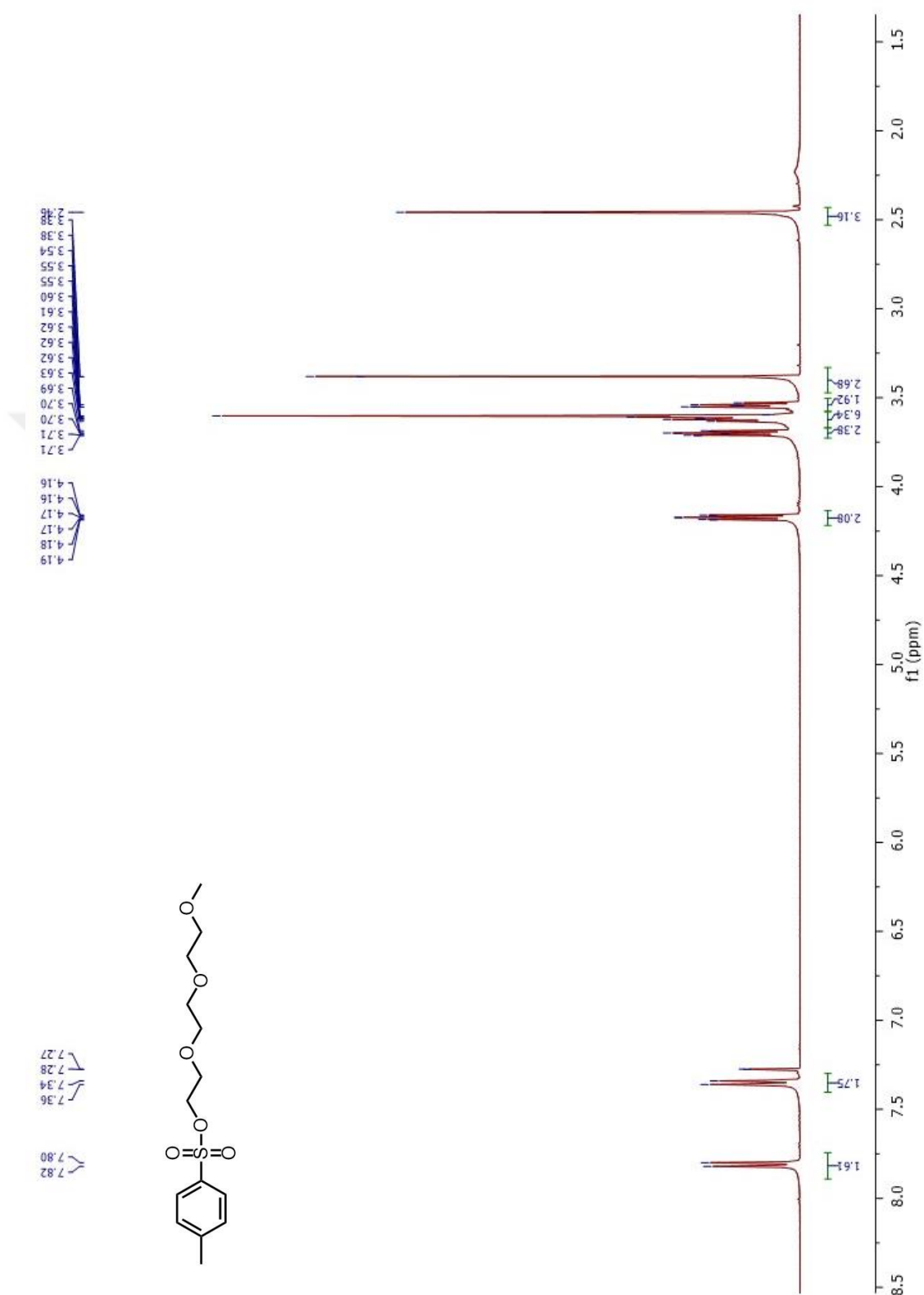


Figure 32: ^1H -NMR spectrum of Compound 1.

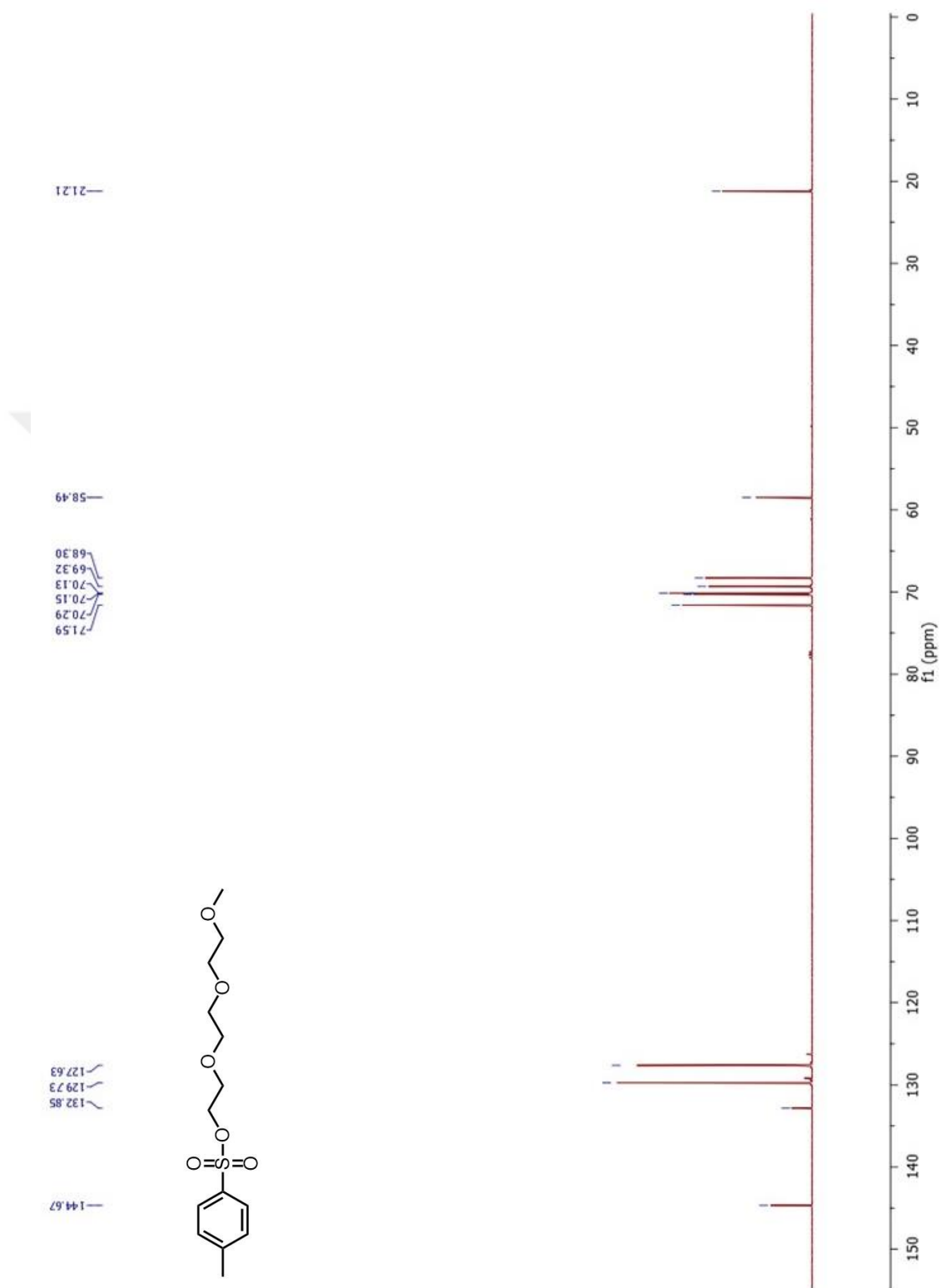
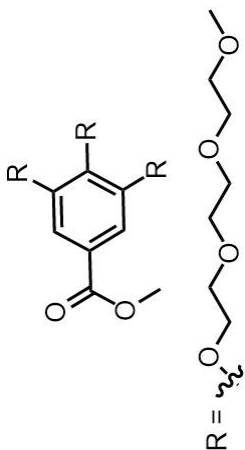
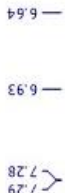


Figure 33: ^{13}C -NMR spectrum of Compound 1.



55



57

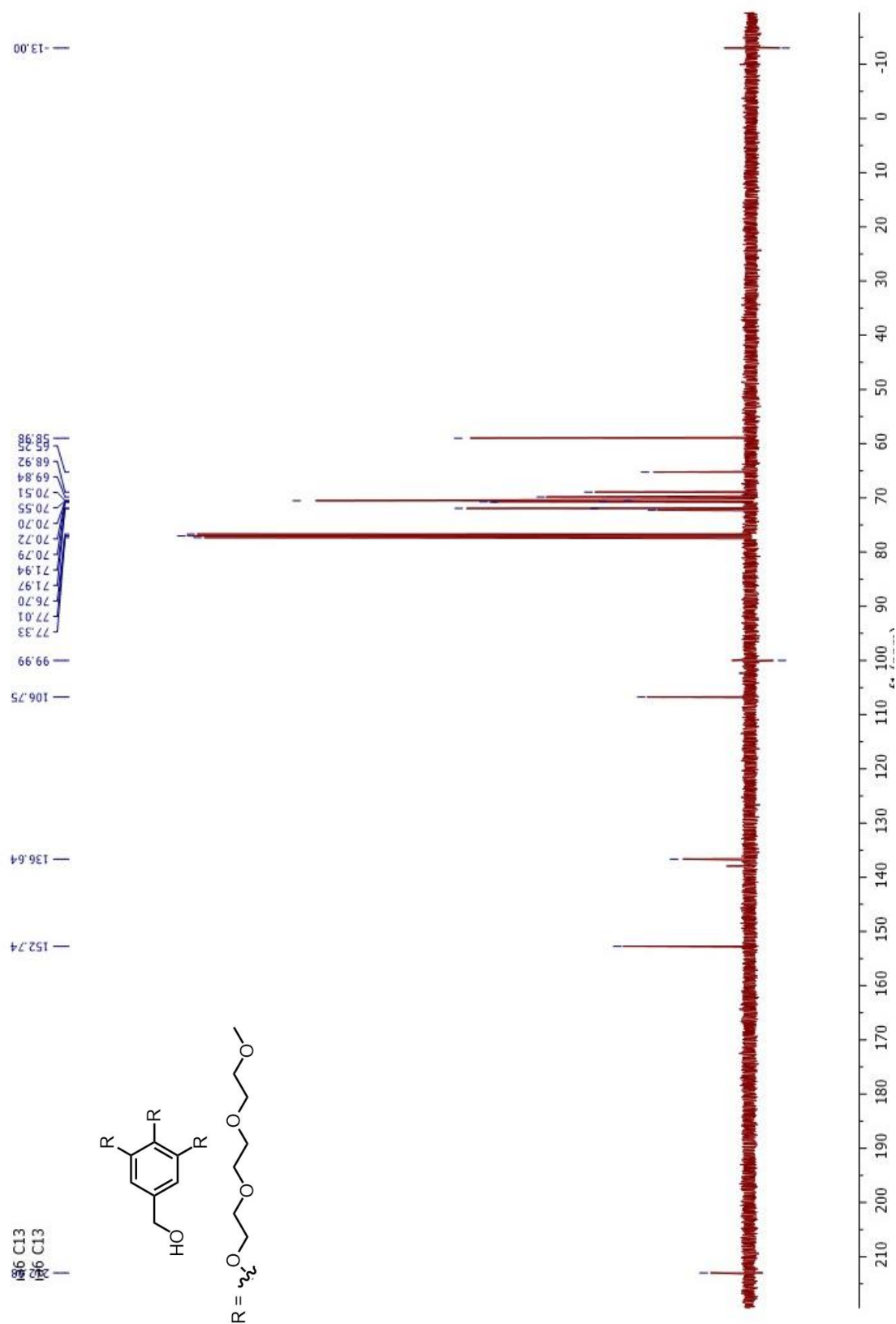


Figure 37: ^{13}C -NMR spectrum of Compound 3.

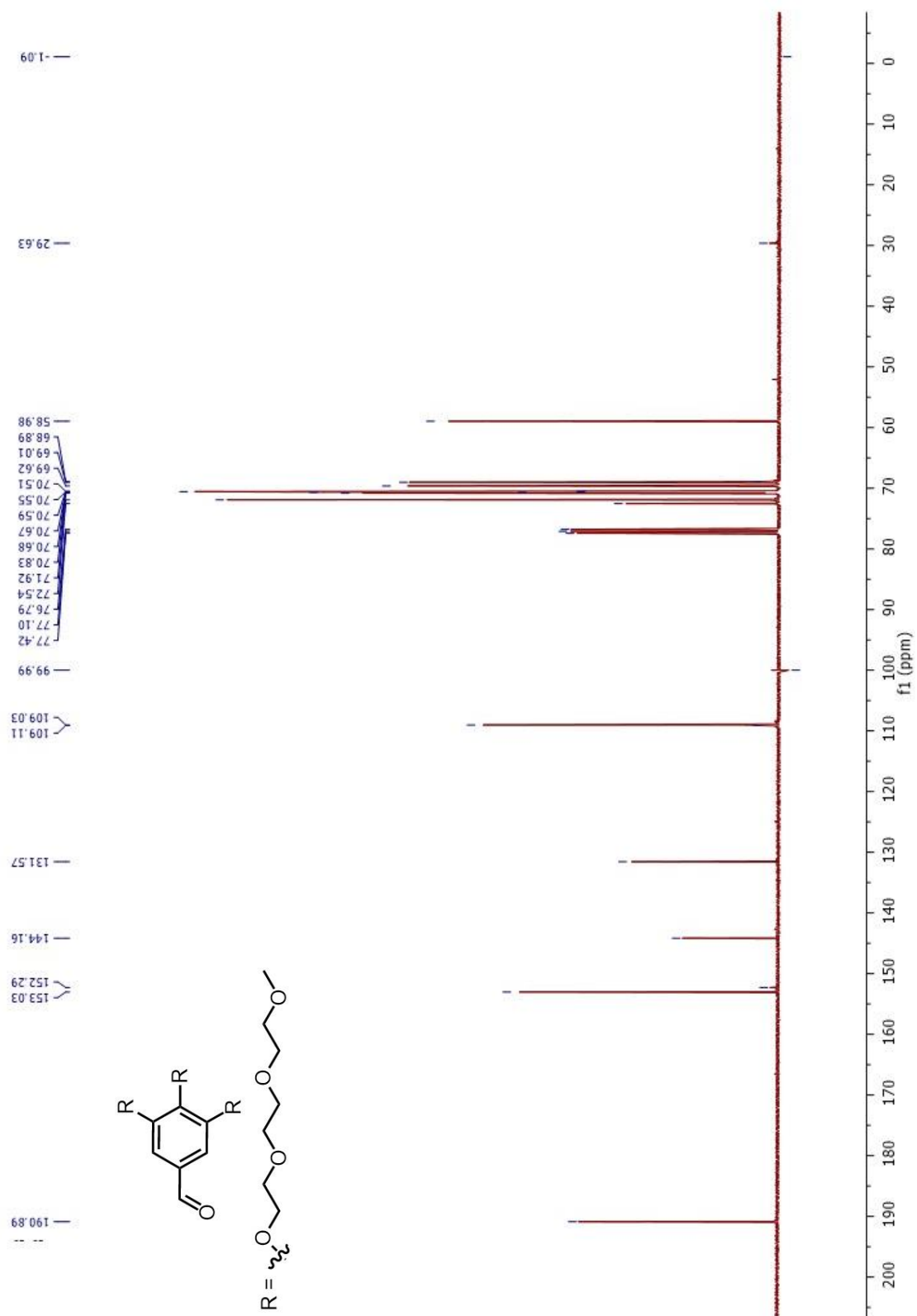


Figure 39: ¹³C-NMR spectrum of Compound 4.

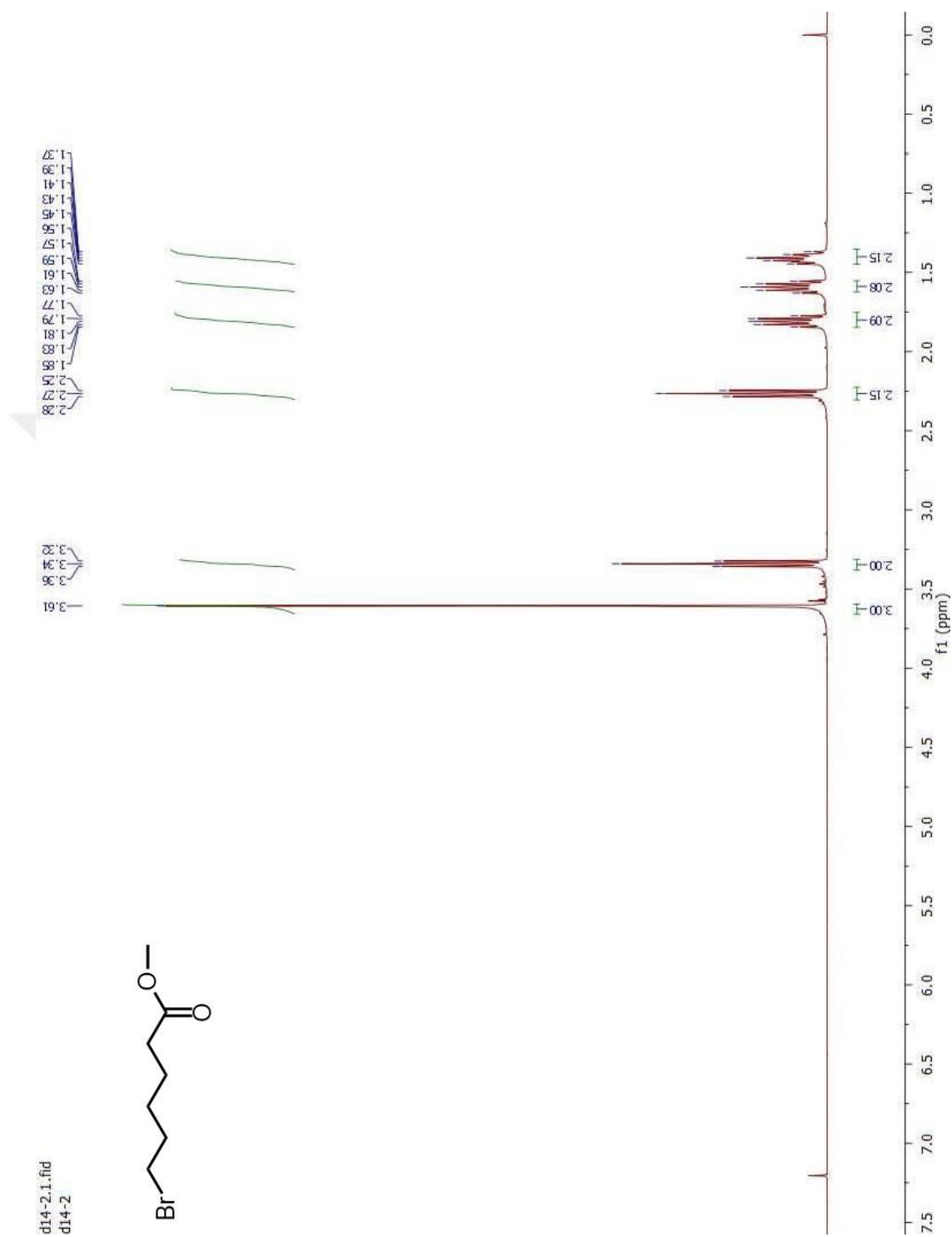


Figure 40: ¹H-NMR spectrum of Compound 5.

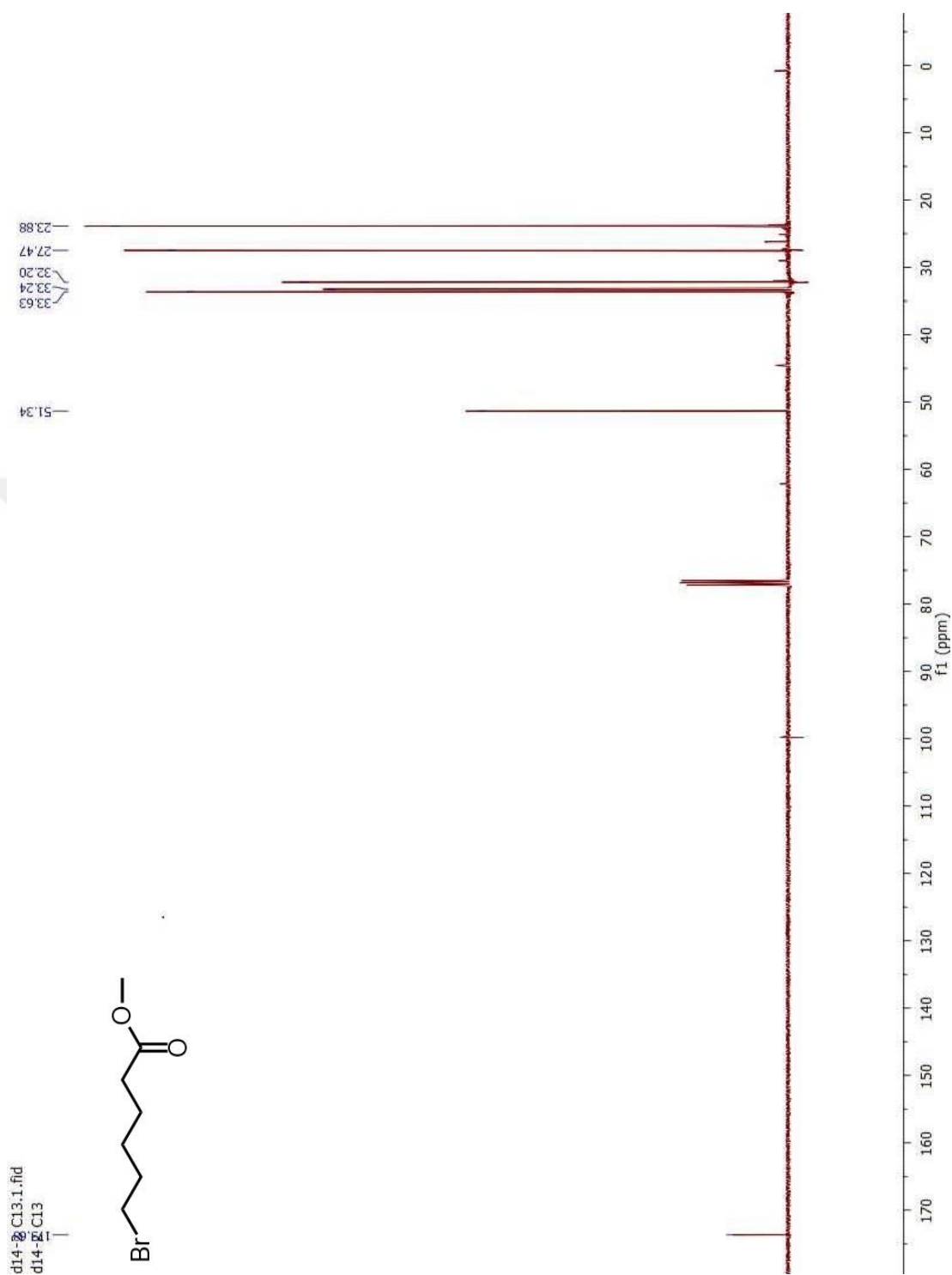


Figure 41: ¹³C-NMR spectrum of Compound 5.

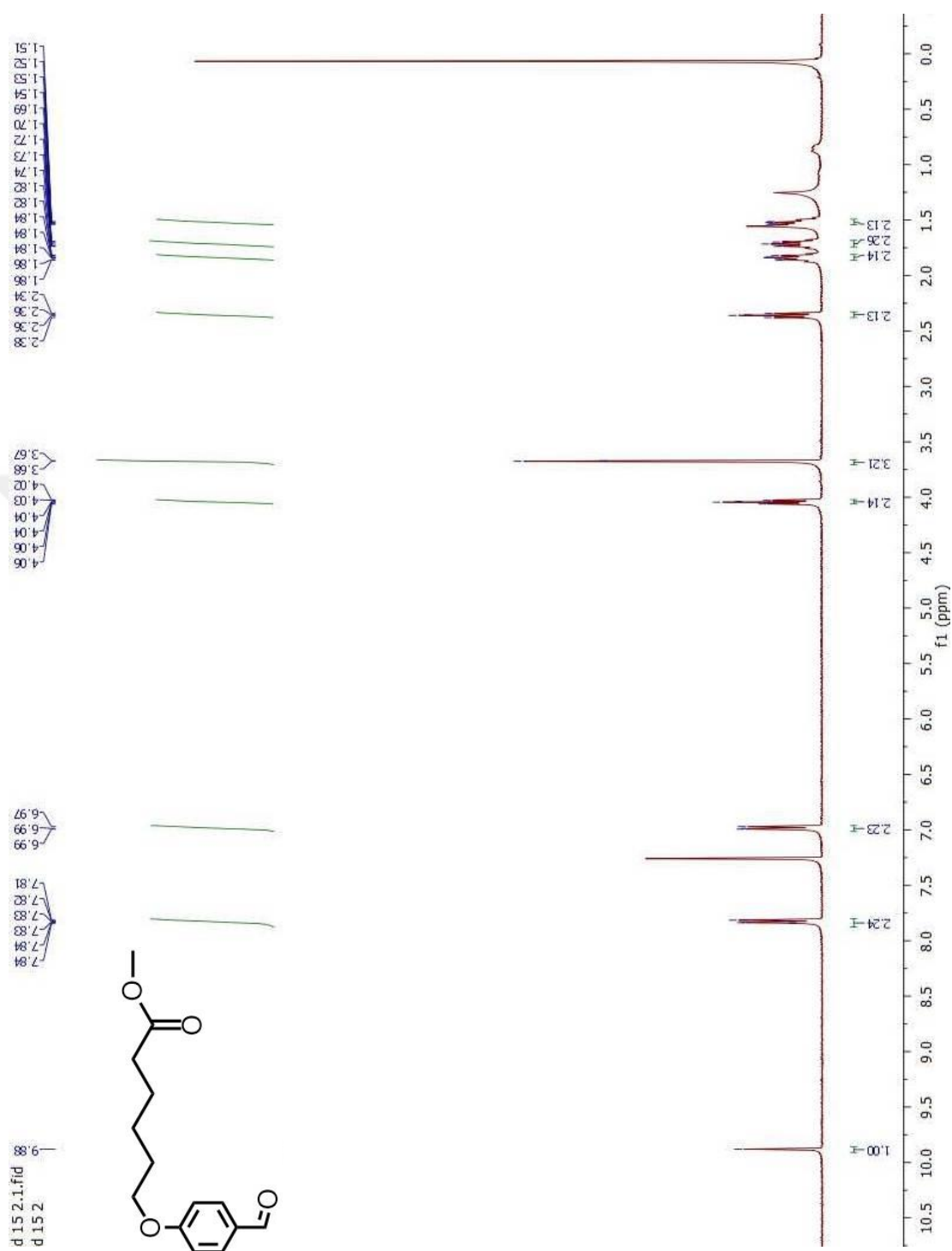


Figure 42: ¹H-NMR spectrum of Compound 6.

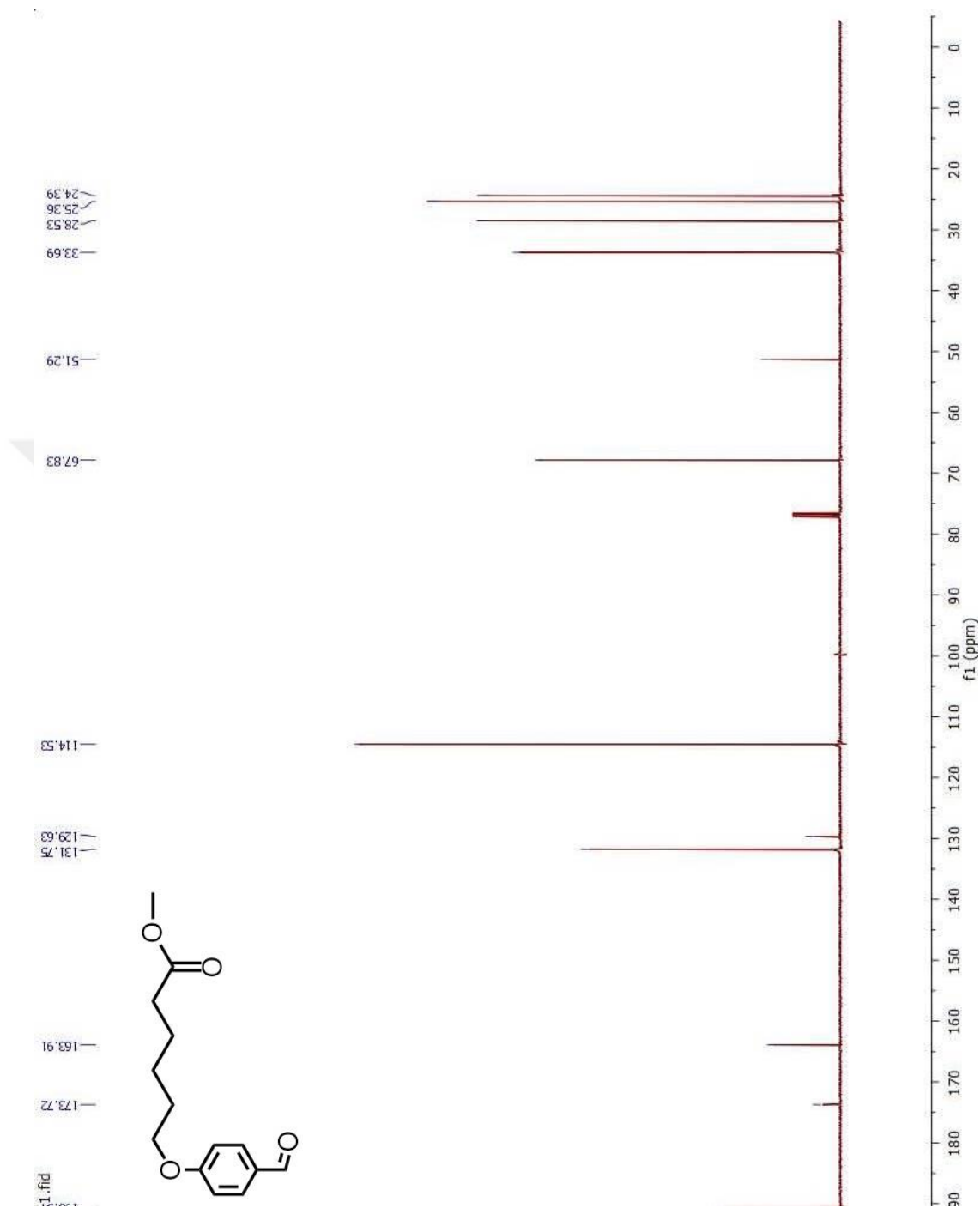


Figure 43: ^{13}C -NMR spectrum of Compound 6.

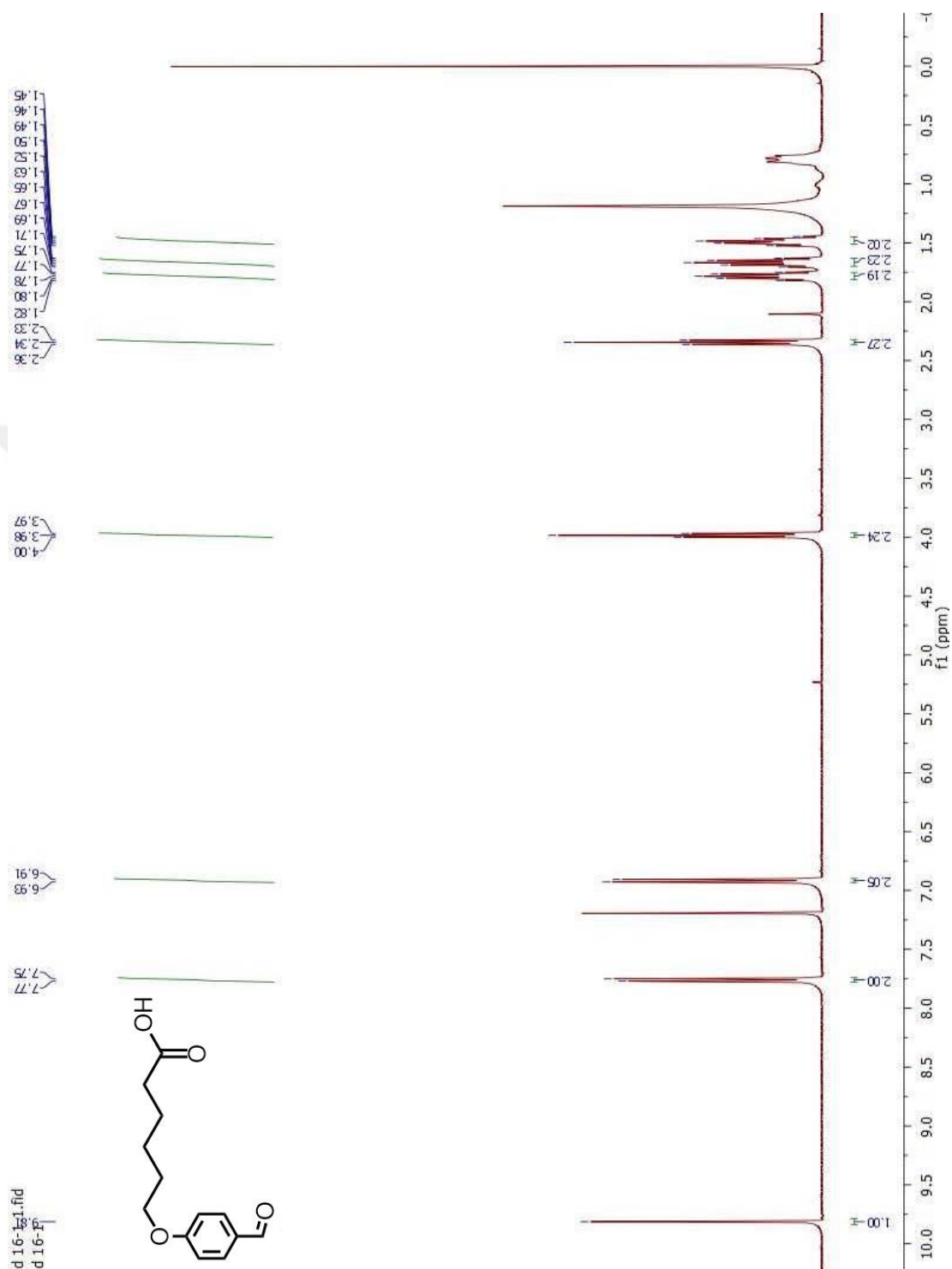


Figure 44: ^1H -NMR spectrum of Compound 7.

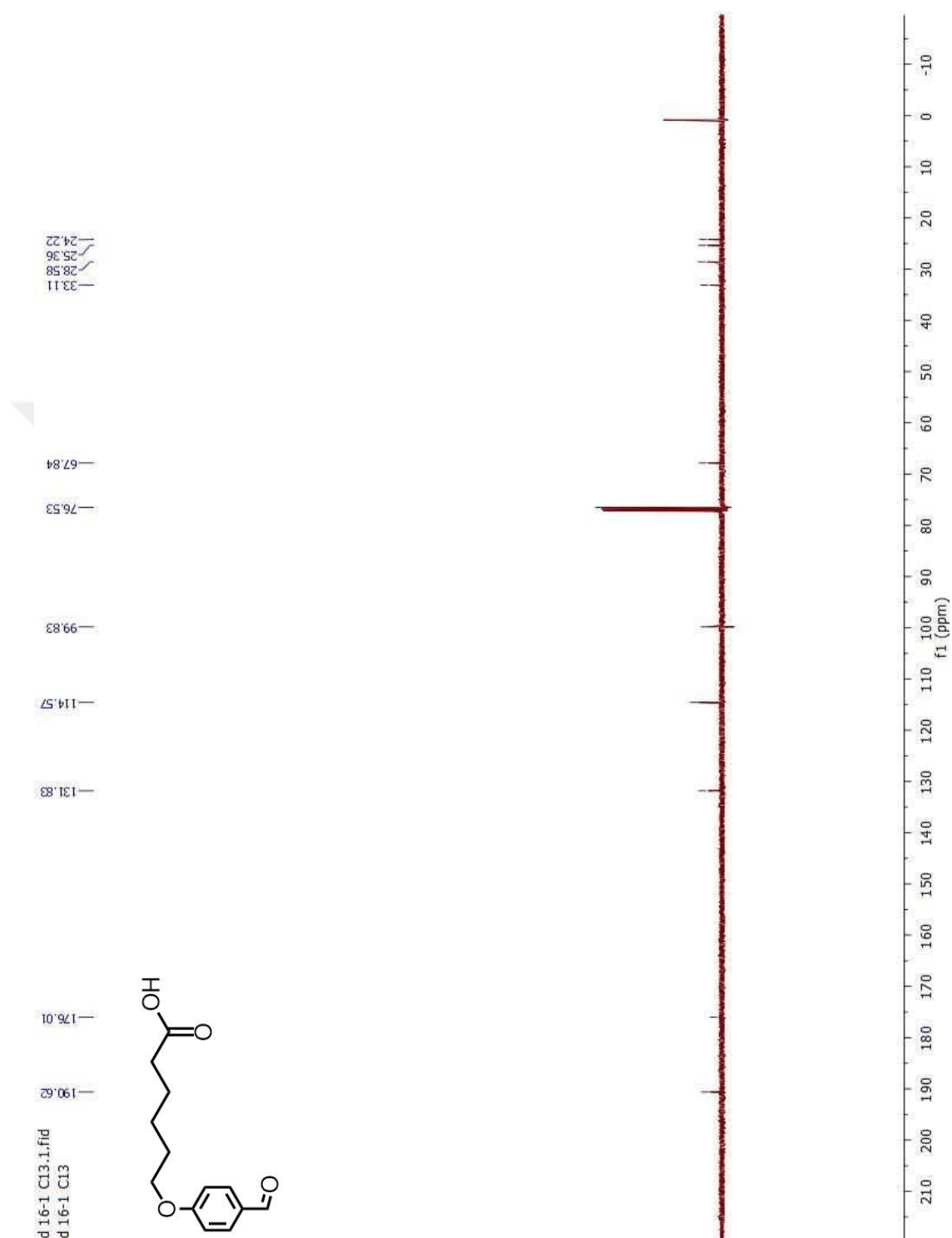


Figure 45: ^{13}C -NMR spectrum of Compound 7.

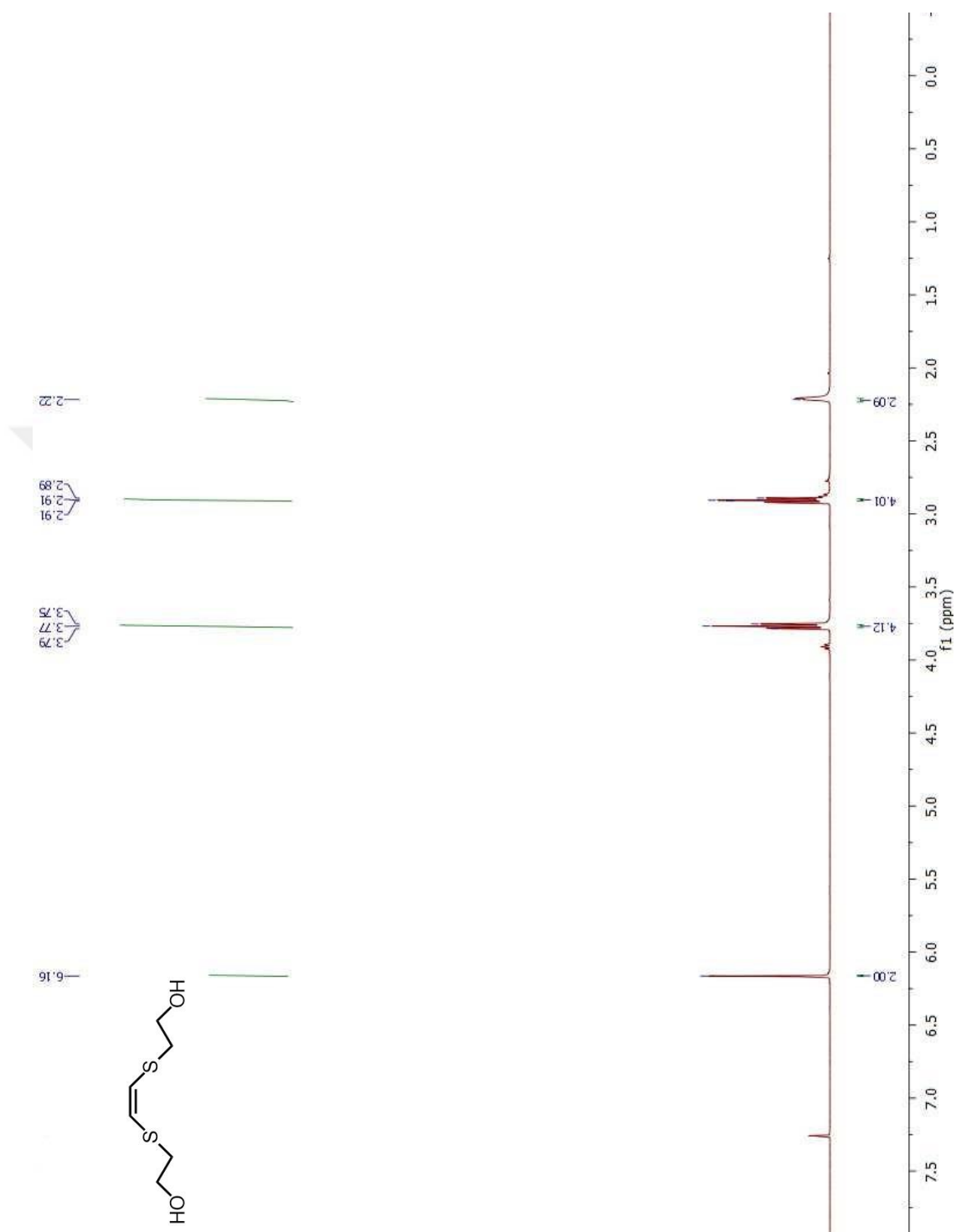


Figure 46: ^1H -NMR spectrum of Compound 8.

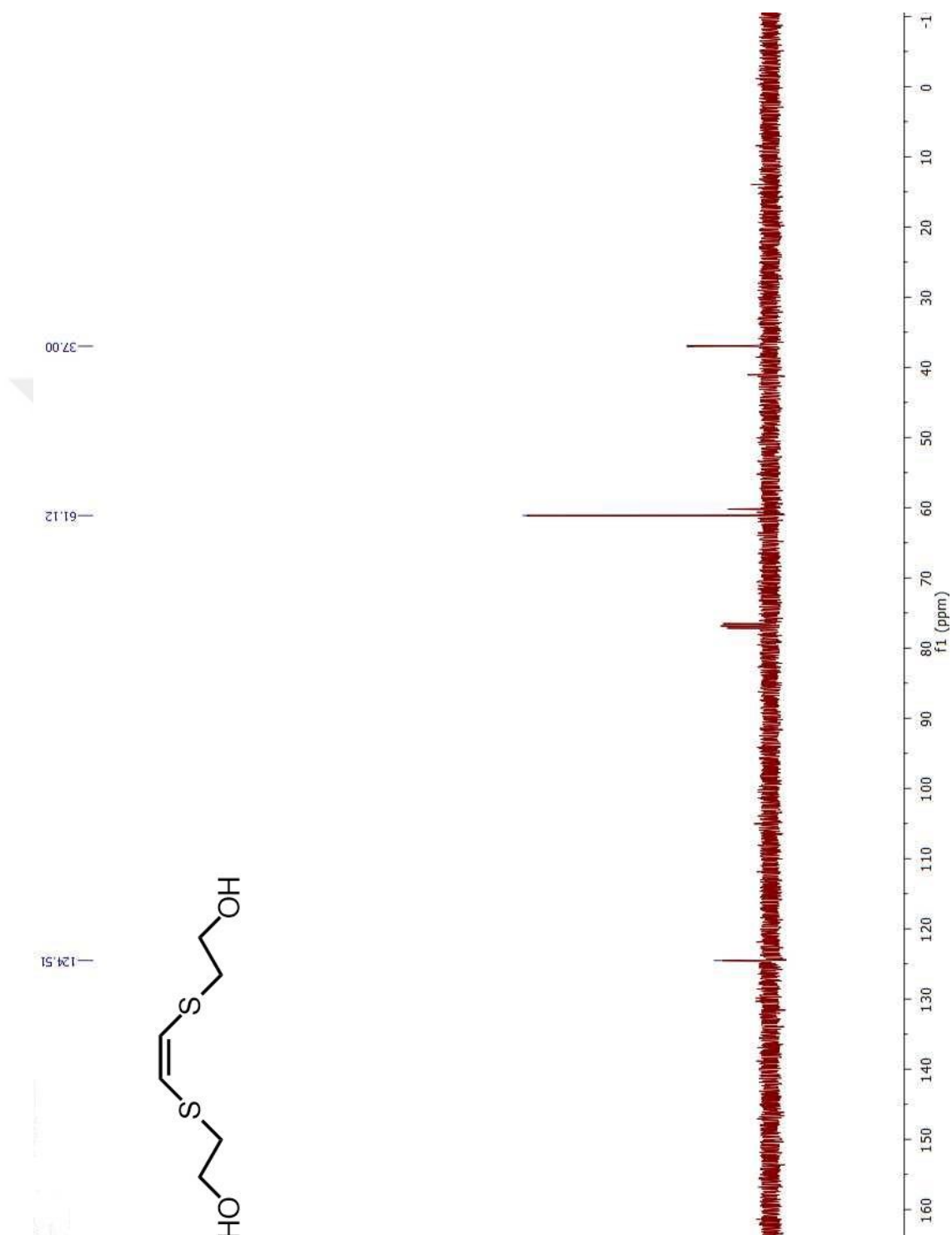


Figure 47: ^{13}C -NMR spectrum of Compound 8.

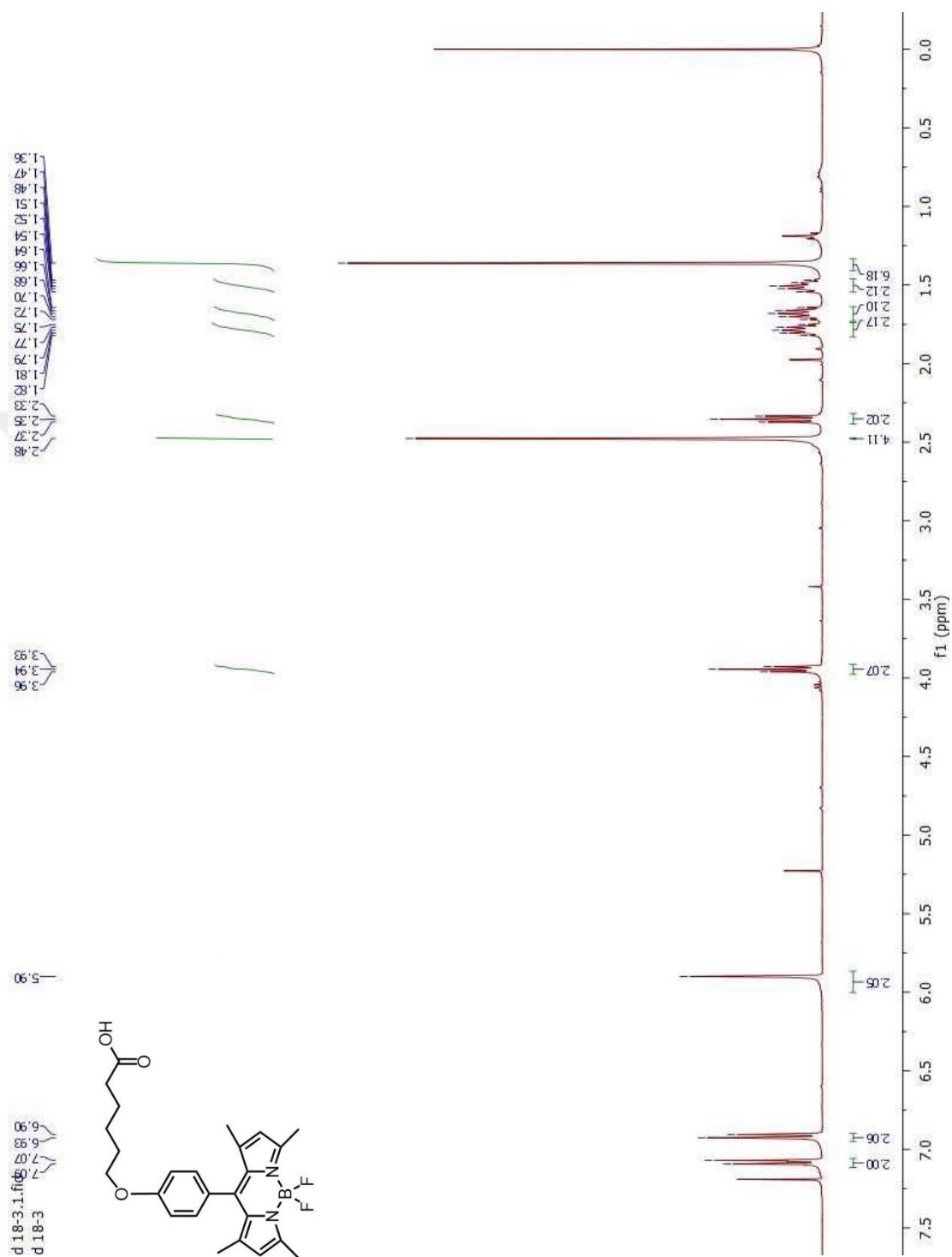


Figure 48: ¹H-NMR spectrum of Compound 9.

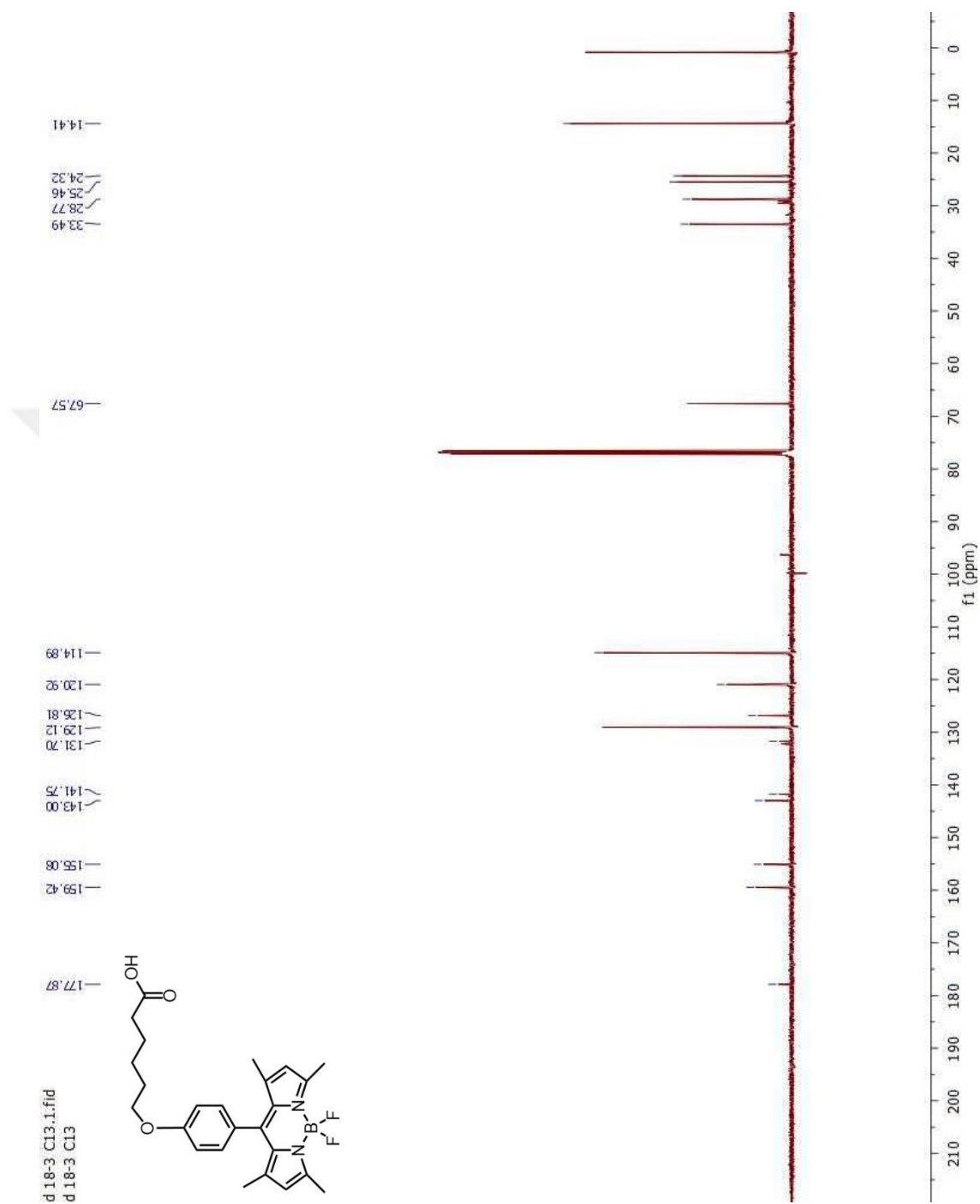


Figure 49: ¹³C-NMR spectrum of Compound 9.

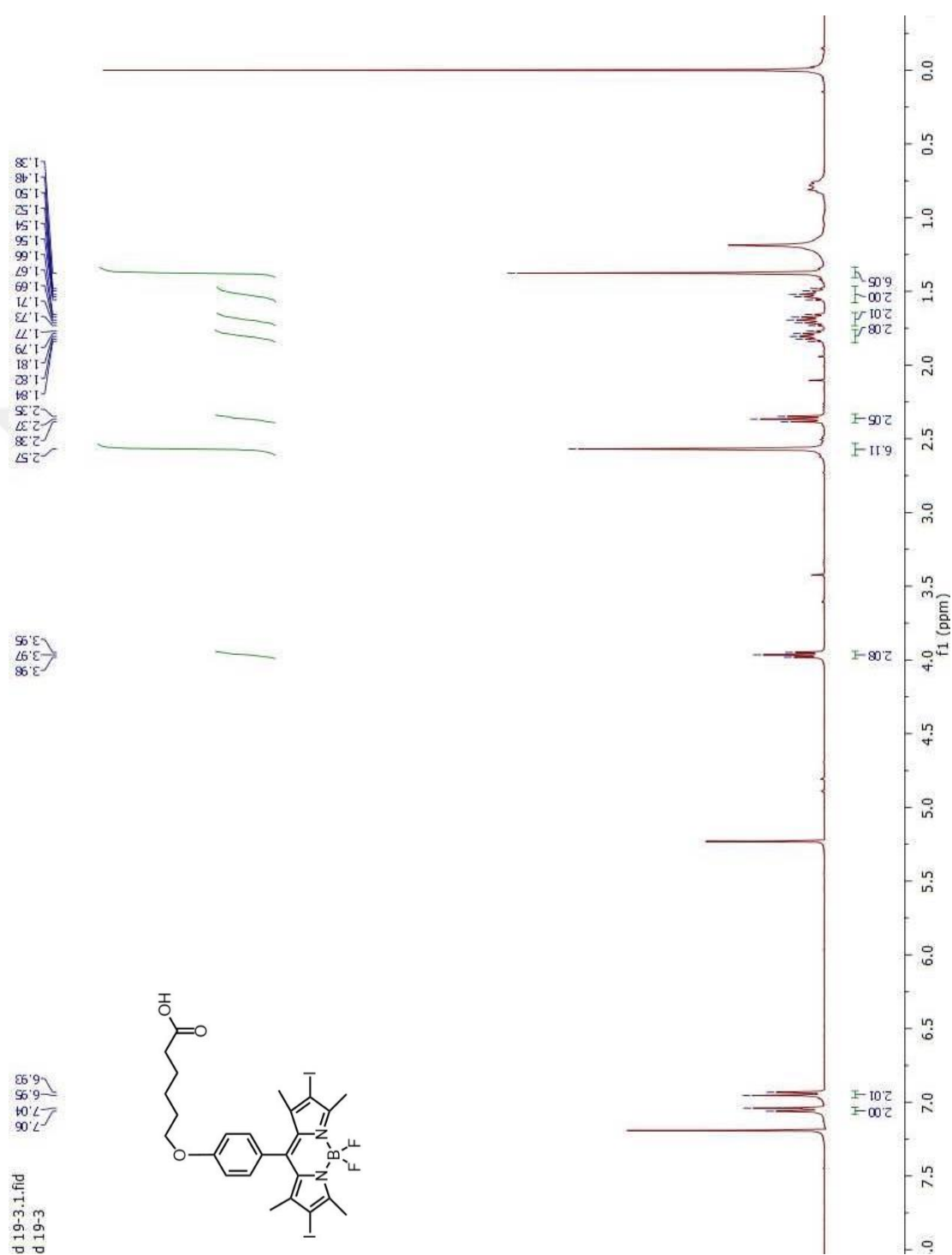


Figure 50: ¹H-NMR spectrum of Compound 10.

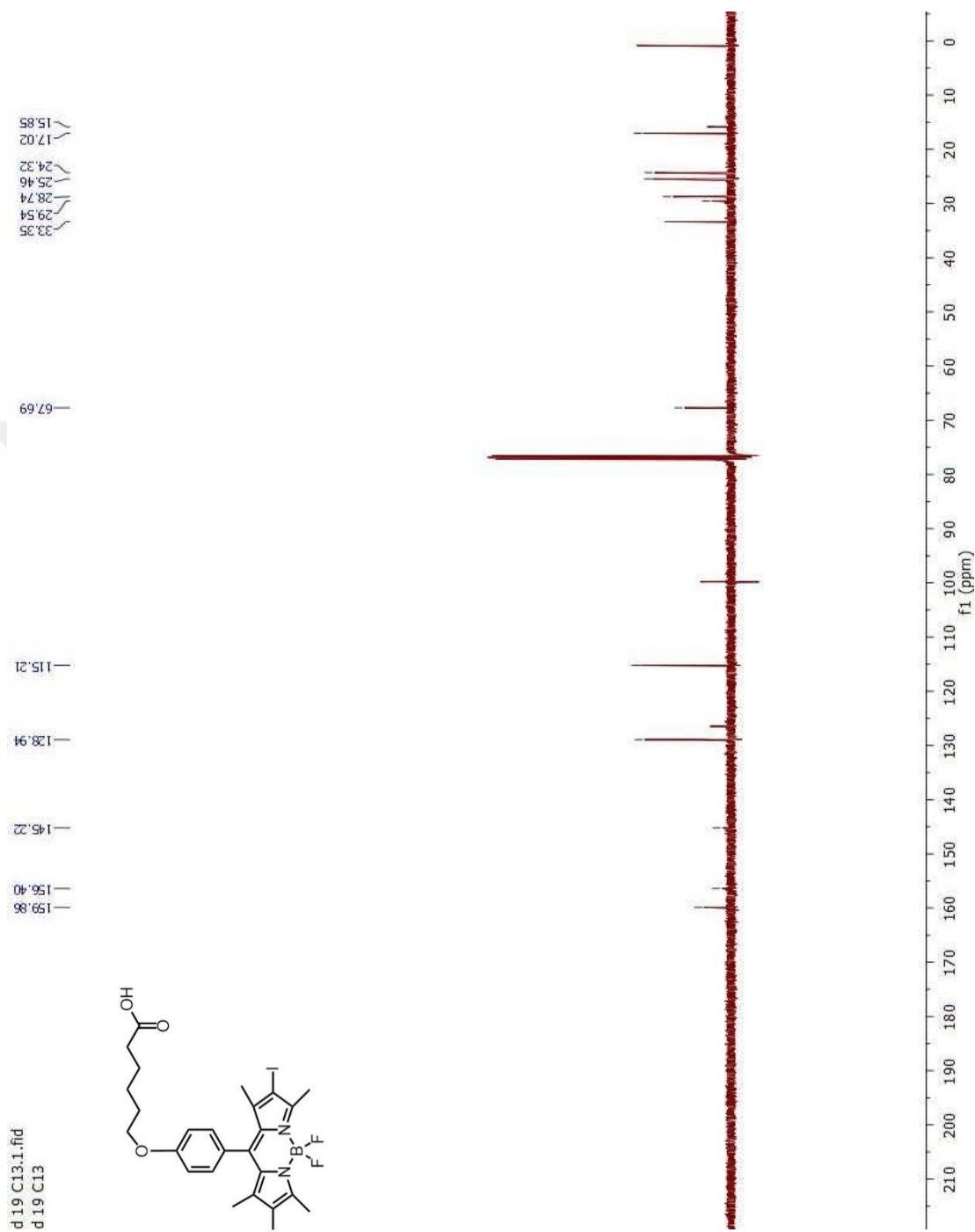


Figure 51: ¹³C-NMR spectrum of Compound 10.

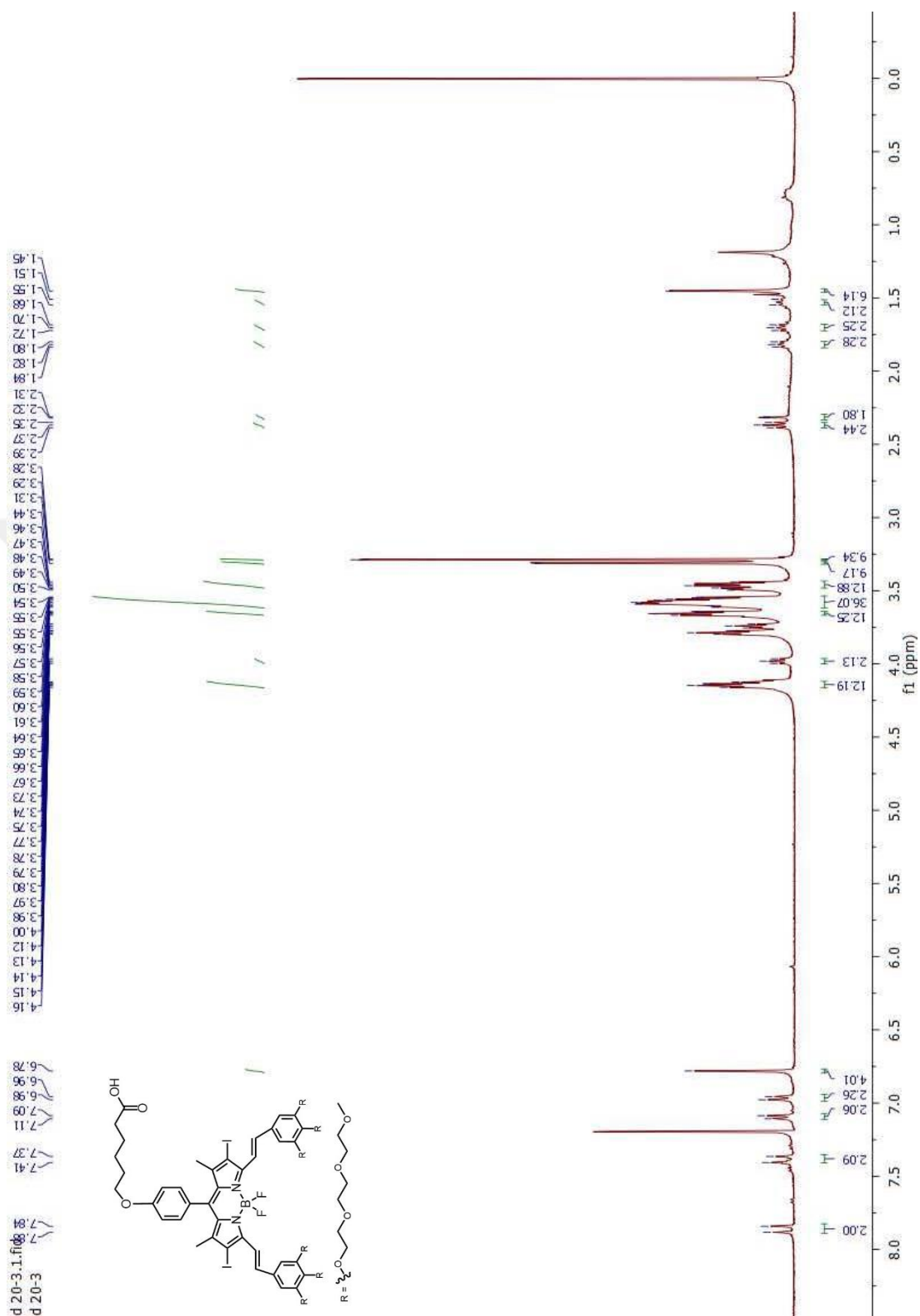


Figure 52: $^1\text{H-NMR}$ spectrum of Compound 11.

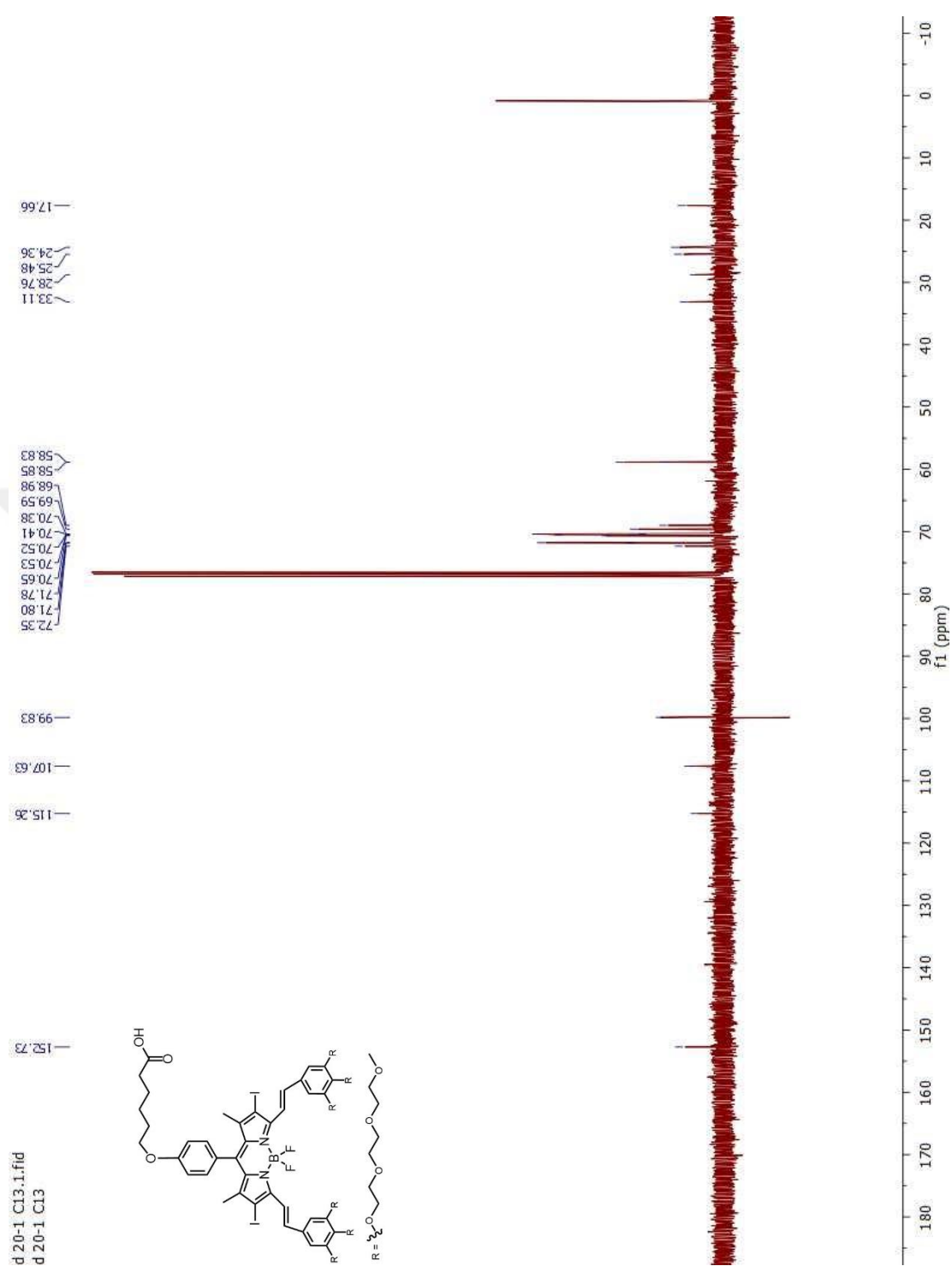
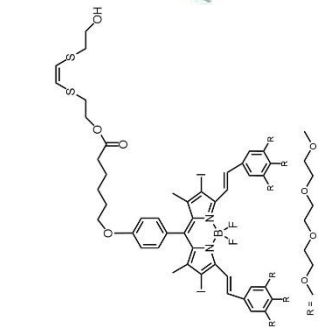


Figure 53: ¹³C-NMR spectrum of Compound 11.



75



77

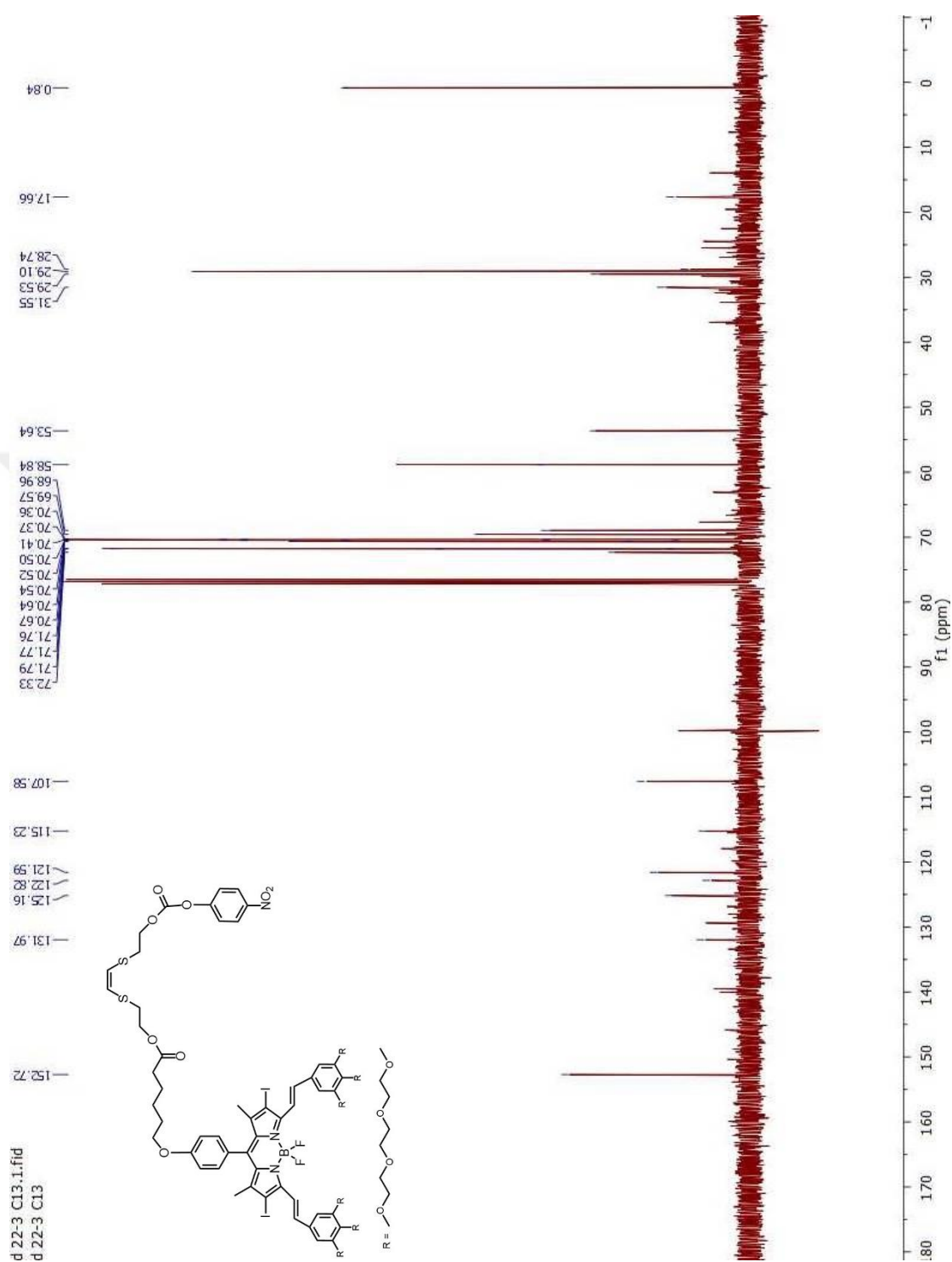


Figure 57: ¹³C-NMR spectrum of Compound 13.

APPENDIX B: MASS SPECTRA

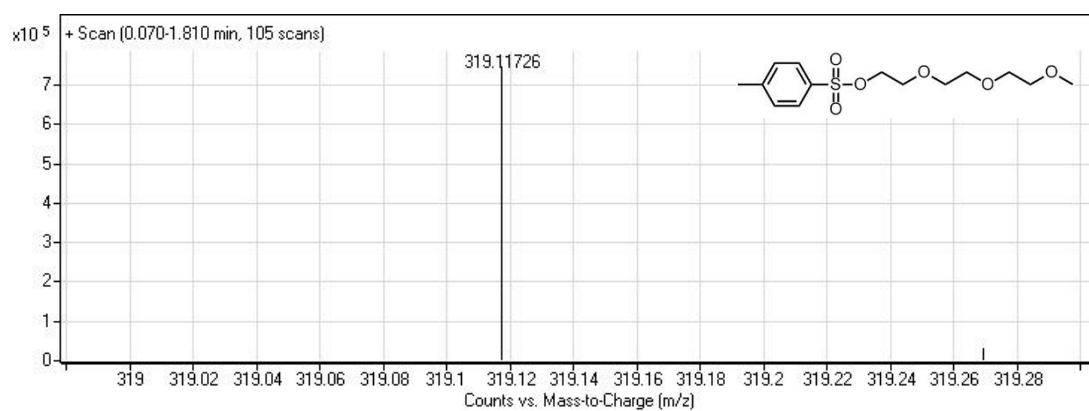


Figure 58. Mass spectrum of Compound 1.

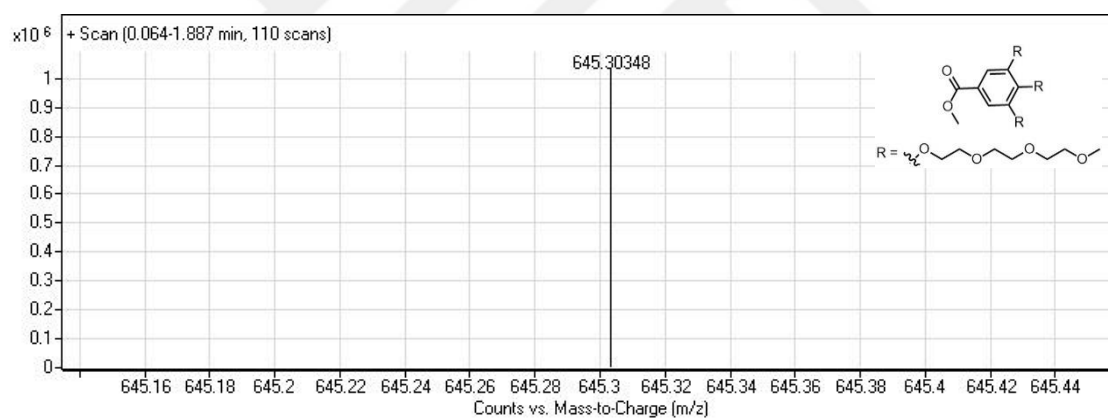


Figure 59. Mass spectrum of Compound 2.

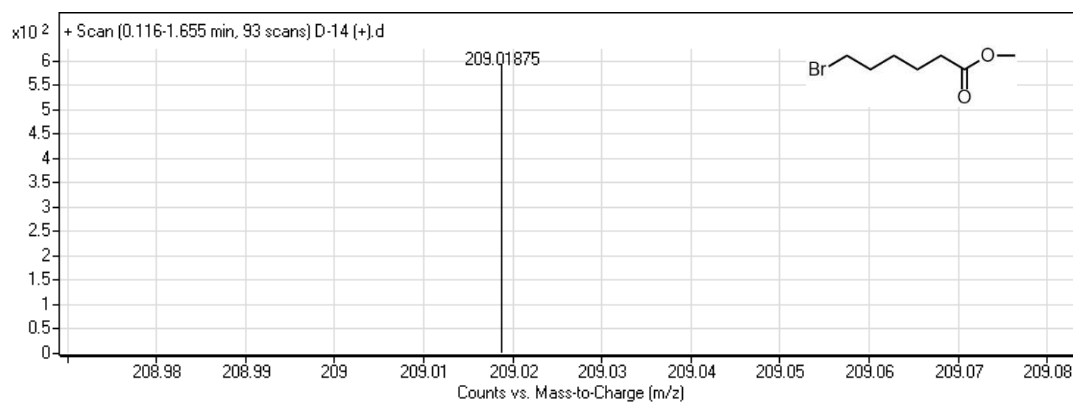


Figure 62. Mass spectrum of Compound 5.

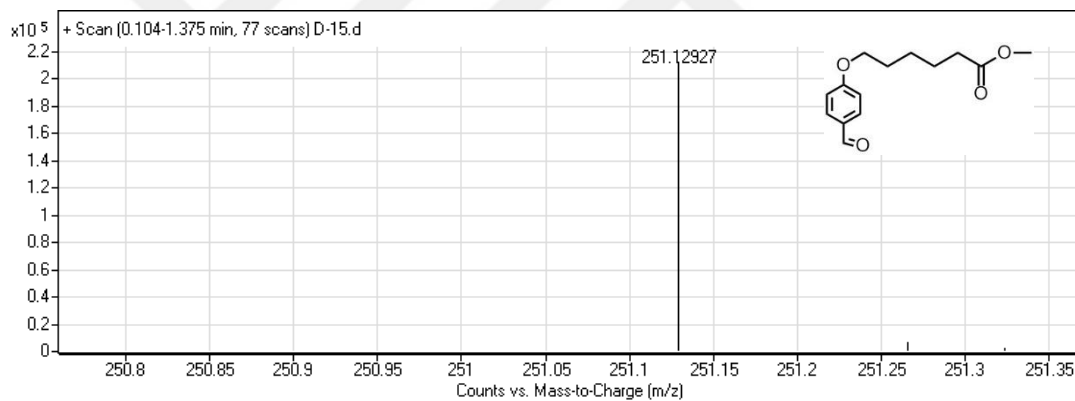


Figure 63. Mass spectrum of Compound 6.

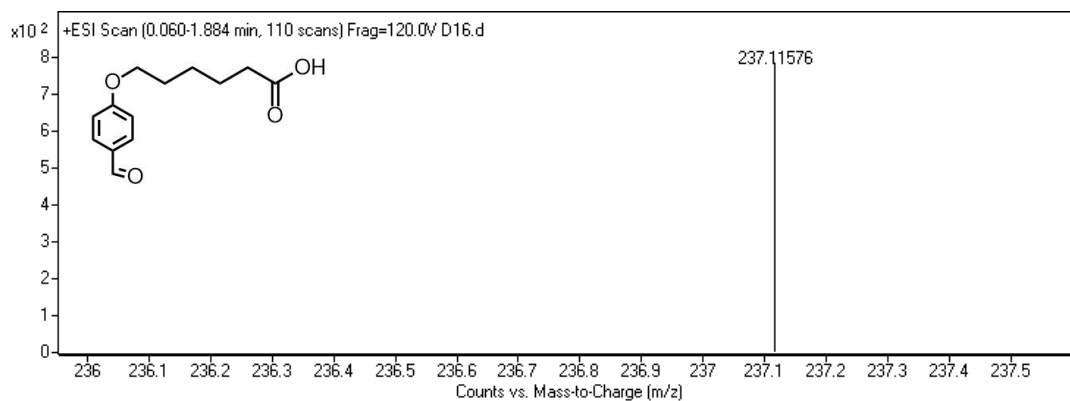


Figure 64. Mass spectrum of Compound 7.

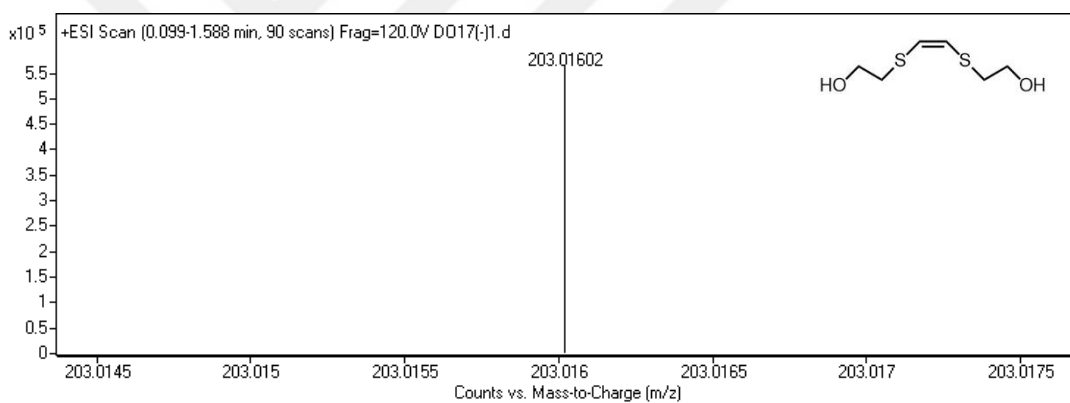


Figure 65. Mass spectrum of Compound 8.

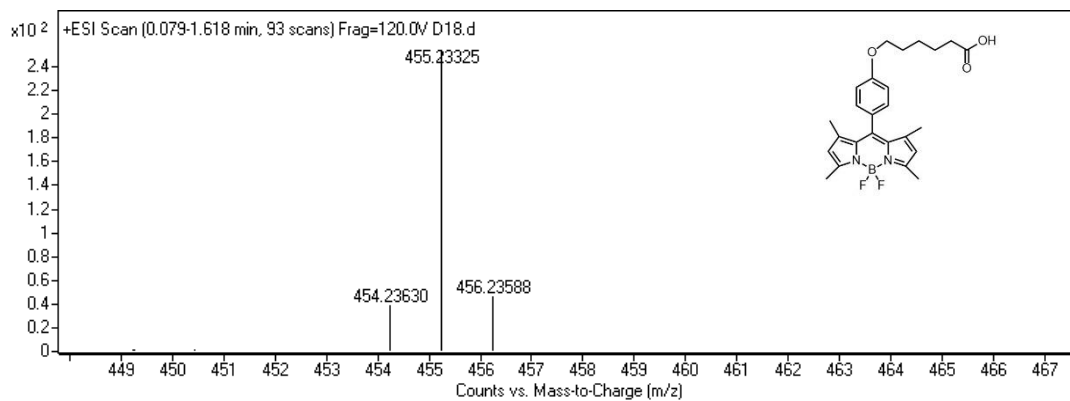


Figure 66. Mass spectrum of Compound 9.

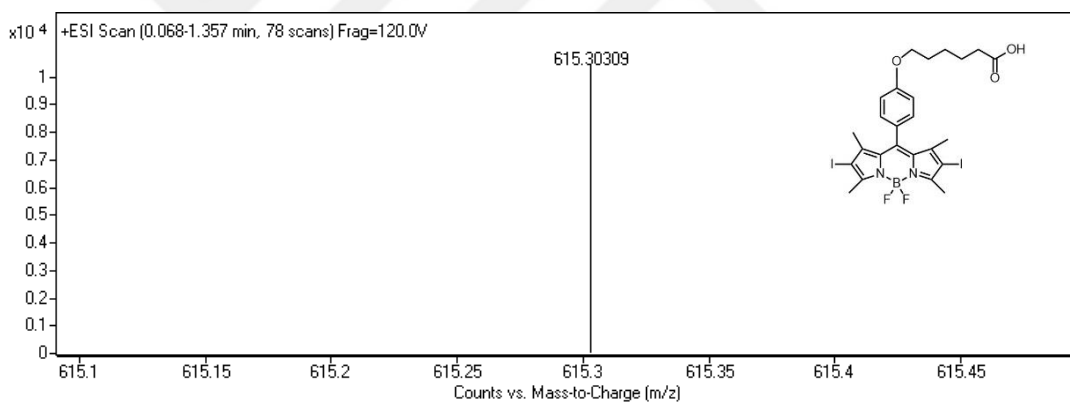


Figure 67. Mass spectrum of Compound 10.

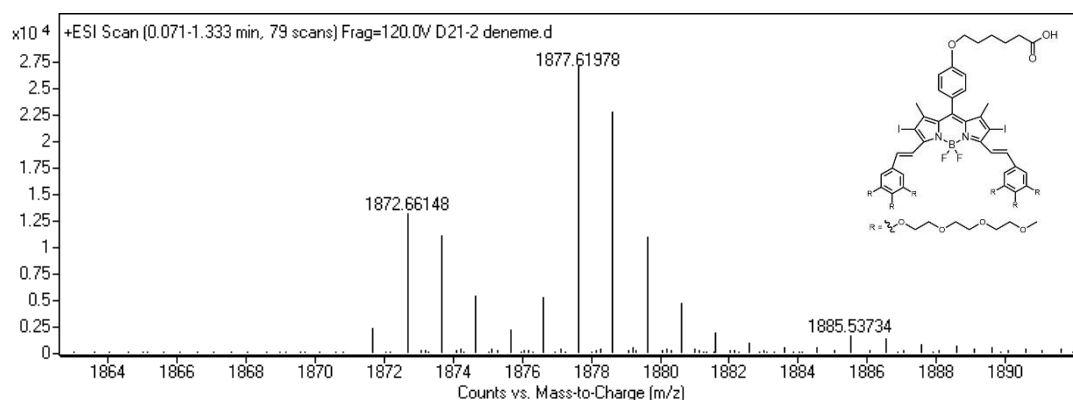


Figure 68. Mass spectrum of Compound 11.

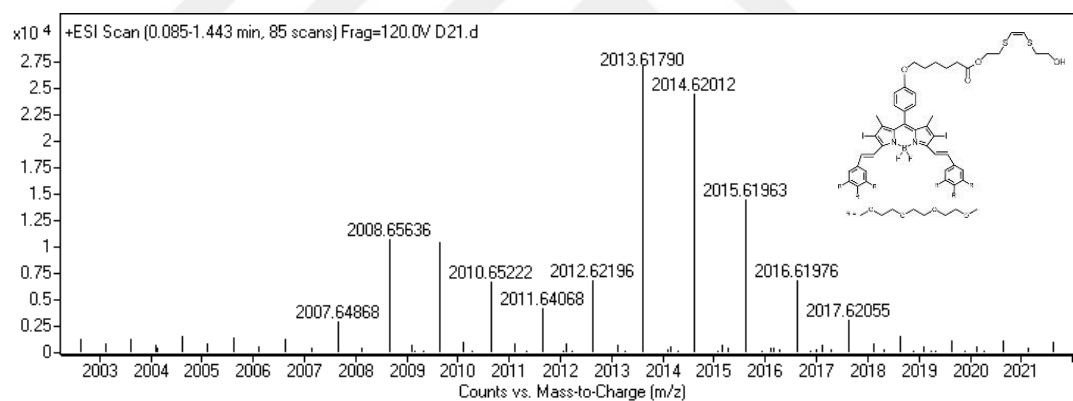


Figure 69. Mass spectrum of Compound 12.

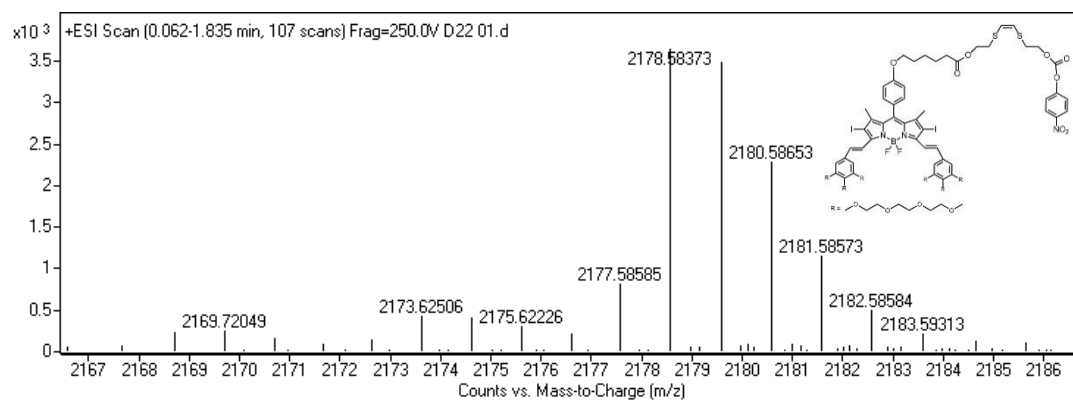


Figure 70. Mass spectrum of Compound 13.

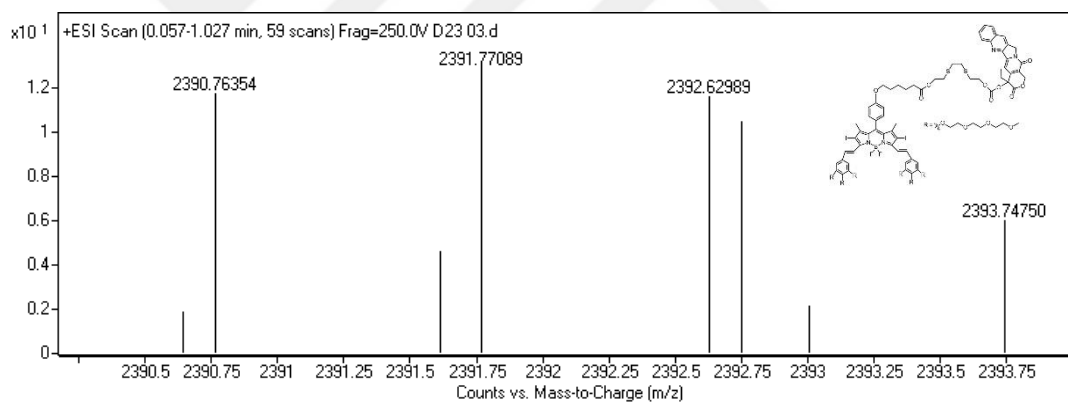


Figure 71. Mass spectrum of Compound 14.