

**SYNTHESIS AND CHARACTERIZATIONS OF
CONJUGATED OLIGOMERS AND NANOPARTICLES
FOR OPTOELECTRONIC AND BIOLOGICAL
APPLICATIONS**

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By
Emre Köken
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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.

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ABSTRACT

SYNTHESIS AND CHARACTERIZATIONS OF CONJUGATED OLIGOMERS AND NANOPARTICLES FOR OPTOELECTRONIC AND BIOLOGICAL APPLICATIONS

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Advisor: Dönüş Tuncel

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This project firstly aims to develop water dispersible conjugated nanoparticles by reprecipitation method for FRET based white light emission. Preliminary NPs study with only OFT Pgy and only OFVBt N3 were done in order to determine size and distribution of NPs. Overlapping optical properties of donor (OFT Pgy) and acceptor (OFVBt N3) give possibility to FRET applications. By click reaction in water, terminal sites of oligomers are connected and white emission is sealed by keeping D-A pair close. FRET based white light is widely used in optoelectronic applications such as OLEDs or more specifically WOLEDs. Since the white light covers all visible spectrum, different color emissions are obtainable depending on excitation wavelength. Various biosensor and bioimaging applications are also possible with white light emitting NPs, since they are readily and stably dispersed in water.

In second part of the project, OFVBt N3 oligomer is cross-linked with di-sulfide containing crosslinker via copper catalyzed click reaction. NPs were synthesized in THF to obtain high click efficiency and redispersed in water, since the biological applications are targeted. OFVBt N3 oligomer is advantageous for bioimaging with its red emission close to IR region, since lower frequency emission overcomes the background auto-fluorescence and penetrates deeper in the body. Di-sulfide crosslinker, in addition to connecting the oligomer molecules and stabilizing NPs, provides possibility of drug delivery application. Since GSH (glutathione) or Trx

(thioredoxin) like thiol bearing bio-molecules subsist in higher concentrations in tumorous tissues, di-sulfide bond can be cleaved, releasing the loaded drug from NPs. Thus, crosslinked OFVBt N3 NPs is a theranostic agent with an advantageous emission color for bio-imaging and a cleavable di-sulfide bond for drug delivery & controlled release.

In last part of the study, white light emitting bi-oligomer nanoparticles were designed and obtained by using OFB Pgy and Porph N3. A quality white emission requires to cover all visible spectrum and overlapping optical properties of OFB Pgy (D) and Porph N3 (A) is used to white light emission by FRET. The purpose of clicking the oligomer pair is to stabilize the FRET efficiency. Moreover, using THF as the solvent is not only facilitated a better click chemistry, but also provided ease of applicability for solid state white light applications. Since THF evaporates easily, white light emitting NPs can form film on various surfaces. Thus, these NPs requires no host layer and can be applied directly to electrode surface when optoelectronic applications *e.g.* OLEDs or WOLEDs are considered.

Keywords: Conjugated oligomers, conjugated oligomer nanoparticles, FRET, click reaction, white light emission.

ÖZET

KONJUGE OLİGOMERLERİN VE NANOPARÇACIKLARIN OPTOELEKTRONİK VE BİYOLOJİK UYGULAMLARI İÇİN SENTEZİ VE KARAKTERİZASYONU

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Bu proje ilk olarak FRET temelli beyaz ışık emisyonu elde etmek için tekrar çöktürme metodu kullanarak suda dağılabilen konjuge nanoparçacıklar geliştirmeyi amaçlar. Boyut ve dağılımını belirleme amacıyla nanoparçacık ön çalışmaları sadece OFT Pgy ve OFVBt N3 ile gerçekleştirilmiştir. Vericinin (OFT Pgy) ve alıcının (OFVBt N3) örtüşen optikal özellikleri FRET uygulamalarını mümkün kılmaktadır. Suda gerçekleştirilen çıt-çıt reaksiyonu ile oligomerler uç noktaları birleştirilmiş ve V-A çifti yakın tutularak beyaz ışık emisyonu oluşumu kesinleştirilmiştir. FRET temelli beyaz ışık OLED ve özellikle WOLED gibi optoelektronik uygulamalarında yaygın olarak kullanılmaktadır. Beyaz ışık bütün görünür bölge tayfını kapsadığından uyarım dalga boyuna bağlı olarak farklı renk emisyonları elde edilebilir. Beyaz ışık saçan nanoparçacıkların suda kararlı kalabildiği ve kolayca dağılabildiği için çeşitli biyosensör ve biyogörüntüleme uygulamaları da mümkündür.

Projenin ikinci bölümünde, OFVBt N3 oligomer ile di-sülfid içeren çapraz bağlayıcı bakırla katalize edilmiş çıt-çıt reaksiyonu yoluyla çapraz bağlanmıştır. Nanoparçacıklar yüksek çıt-çıt verimi elde etme amacıyla THF içerisinde sentezlenmiş ve biyolojik uygulamalar amaçlandığından suda tekrar dağıtılmıştır. Düşük frekanslı emisyonlar oto-floresansın üstesinden geldiğinden ve vücut içinde daha derine penetre ettiğinden dolayı, OFVBt N3 oligomeri IR bölgesine yakın

kırmızı ışık emisyonu ile biyogörüntüleme için avantajlıdır. Di-sülfit çapraz bağlayıcısı, oligomer moleküllerini birbirine bağlamasının ve nanoparçacıkları stabilize etmesinin yanı sıra ilaç teslim uygulamasını mümkün kılar. Tiyo içeran GSH (glutatyon) ya da Trx (tiyoredoksin) gibi biyo-moleküller kanser dokularında yüksek konsantrasyonlarda bulunduğundan, di-sülfit bağı kırılabilir ve böylece nanoparçacıklardan yüklenmiş ilacın salınımına yol açar. Böylelikle, biyogörüntüleme için avantajlı emisyon rengi, ve ilacın gönderimi & kontrollü salınımı için açılabilir di-sülfit bağı ile çapraz bağlanmış OFVBt N3 nanoparçacıklar hem teşhis hem tedavi ajanıdır.

Çalışmanın son kısmında, beyaz ışın saçan bi-oligomer nanoparçacıkları tasarlandı ve OFB Pgy ve Porph N3 kullanılarak elde edildi. Kaliteli bir beyaz ışık emisyonunun bütün görülebilir tayfı kapsaması gerekmektedir ve OFB Pgy(D) ve Porph N3(A)'ün örtüşen optikal özellikleri FRET yoluyla beyaz ışık emisyonu elde etmekte kullanılmıştır. Çıt-çıt reaksiyonunun amacı FRET verimliliğini stabilize etmektir. Buna ek olarak, çözücü olarak THF kullanmak daha iyi çıt-çıt kimyası sağlamak dışında, katı durum aydınlatma uygulamaları için kolay uygulanabilirlik de sağlamıştır. THF kolay buharlaşabildiğinden, beyaz ışık saçan nanoparçalar çeşitli yüzeyler üzerinde film oluşturabilir. Böylelikle, bu nanoparçacıklar başka bir katman gerektirmemekte ve OLED ya da WOLED gibi optoelektronik uygulamalar göz önünde bulundurulduğunda elektrot yüzeyine direk olarak uygulanabilmektedir.

Anahtar kelimeler: Konjuge oligomerler, konjuge oligomerler nanoparçacıkları, FRET, çıt-çıt reaksiyonu, beyaz ışık emisyonu.

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Abbreviations

¹ H-NMR	Proton-Nuclear Magnetic Resonance spectroscopy
¹³ C-NMR	Carbon-Nuclear Magnetic Resonance spectroscopy
FTIR	Fourier Transform Infrared spectroscopy
HRMS/TOF	High Resolution Mass / Time-of-Flight
UV-Vis	Ultraviolet-Visible spectroscopy

PL	Fluorescence spectroscopy
DLS	Dynamic Light Scattering
SEM	Scanning Electron Microscope
TEM	Transmission Electron Microscope
TGA	Thermogravimetric Analysis
DSC	Differential Scanning Calorimetry
CDCl ₃	Deuterated Chloroform
THF	Tetrahydrofuran
TBAB	Tetrabutylammonium Bromide
CPs	Conjugated Polymers
NPs	Nanoparticles
D	Donor
A	Acceptor
D-A	Donor-Acceptor pair
FRET	Förster Resonance Energy Transfer
ET	Energy Transfer
M1	2-bromo-9,9-bis(3-bromopropyl)-9H-fluorene
M2	9,9-bis(3-bromopropyl)-2-vinyl-9H-fluorene
M3	9,9-bis(3-azidopropyl)-2-vinyl-9H-fluorene
OFB	1,4-bis(9,9-bis(3-bromopropyl)-9H-fluoren-2-yl)benzene
OFB Pgy	1,4-bis(9,9-bis(3-(prop-2-yn-1-yloxy)propyl)-9H-fluoren-2-yl)benzene
OFT	2,5-bis(9,9-bis(3-bromopropyl)-9H-fluoren-2-yl)thiophene
OFT Pgy	2,5-bis(9,9-bis(3-(prop-2-yn-1-yloxy)propyl)-9H-fluoren-2-yl)thiophene
OFVBt N3	4,7-bis((E)-2-(9,9-bis(3-azidopropyl)-9H-fluoren-2-yl)vinyl)benzo[c][1,2,5]thiadiazole
Porph Br	5,10,15,20-tetrakis((4-bromomethyl)phenyl)porphyrin
Porph N3	5,10,15,20-Tetrakis((4-azidomethyl)phenyl)porphyrin

Chapter 1

Introduction

1.1 Conjugated Materials

Conjugated polymers (CPs) are organic macromolecules which have alternating double and single-bonds in their backbones. Although first description of conjugated structure were investigated on polyaniline by Henry Letheby in mid-19th century, conjugated polymers got the attention of researchers after 1950s.[1]–[3] The first studies were focused on conductivity in organic materials by charge transfer complexes[4], [5] and halogen doping on poly(acetylene) (PA)[6], [7]. Then, the research on CPs was broadened in an interdisciplinary manner, focusing on organic semiconductors,[8] electrical conductivity,[7], [9] and organic superconductors[10] until mid-1980s. In 1977, the study on iodine doping on poly(acetylene) made a drastic change in conductivity of PA[7]. Alan J. Heeger, Alan G. MacDiarmid, and Hideki Shirakawa were the ones who shared the Nobel Prize in Chemistry 2000 *"for the discovery and development of conductive polymers"*. [11]

Conjugated materials are also called as conductive plastics due to delocalization of electrons in π -orbitals of the alternating backbone. The p_z -orbitals of the carbon atoms form the π -orbitals, which are orthogonal to the other sp^2 hybridized orbitals, resulting easier movement for electrons from one bond to the subsequent bond. Enhanced mobility of these π -electrons forms a one-dimensional electronic band along the chain. The term band gap denotes the energy difference between the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO). HOMO is in valence band while LUMO is in conduction band (Figure 1.1). Since the high mobility of electrons reduces the band gap and makes conjugated materials one-dimensional semiconductors with a band gap lower than 2 eV, conjugated materials show a similar behavior to those common commercial semiconductors, *e.g.* pure, electron-rich (n-type) or electron-poor (p-type) doped silicon.

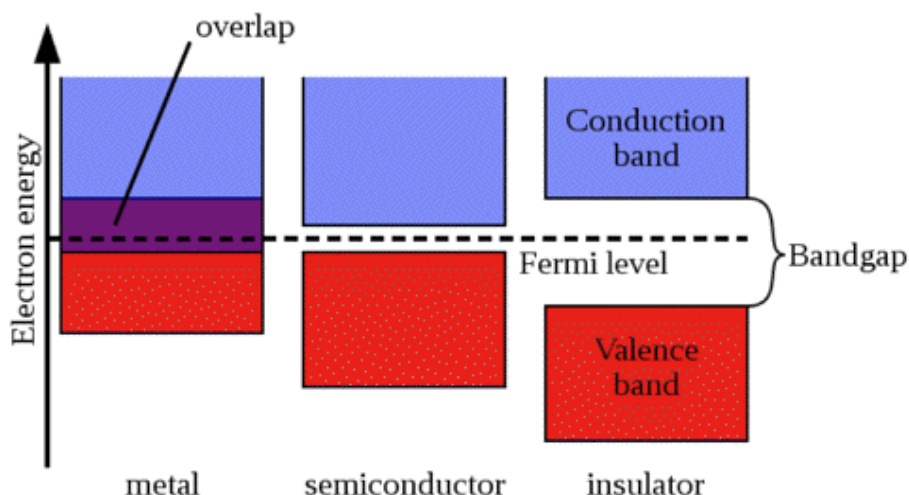


Figure 1.1: Schematic representation of band structure in metals, semiconductors and insulators

Considering delocalized electronic nature and excellence in conduction properties of conjugated materials, a wide range of applications is possible. Further investigations on conjugated materials revealed that they exhibit good mechanical stability along with the unique electro- and photo-luminescent properties. The ease of modification of conjugated materials with various functional groups provides numerous design options for the desired applications. Hence, optoelectronic devices such as organic light emitting diodes (OLEDs)[12] and organic field effect transistors (FETs)[13] are fabricated out of CPs. They have also promising properties such as light-amplify and light-harvest that makes them a strong candidate for interdisciplinary applications in chemistry, biology, and material sciences. Moreover, conjugated materials draw the attention in biology and biomedical sciences related applications such as biosensors,[14] bioimaging,[15] and drug delivery[16].

Conjugated materials research was first started and got the attention with the study on poly(acetylenes). However, the PA is accepted as the simplest alternating material among the current conjugated material research field. The structures of conjugated materials with aromatic cycles in backbone are on the spotlight in numerous practical applications. For instance, oligo- and poly(thiophene) derivatives are suitable for transistor and solar cell applications due to stability,

electronic potential, durability, and ease of side chain modifications.[17], [18] Fluorene derivatives is another sparkling conjugated material which has an increasing interest in academia and industries. Oligo- and poly(fluorene) derivatives have excellent thermal stability, high quantum efficiency, and susceptibility of functionalization at 9th position. The tunability over the whole visible part of electromagnetic spectrum provides a remarkable attention on fluorene-based applications and devices *i.e.* OLEDs,[19], [20] FETs,[21] solar cells,[22] and chemo-biosensors.[23], [24]

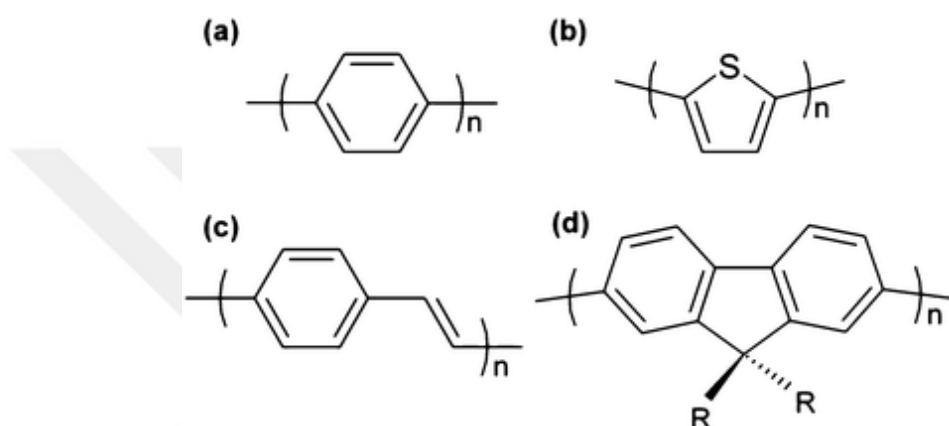


Figure 1.2: Molecular structures of some widely-studied conjugated polymers: (a) poly(p-phenylene) (PPP) (b) poly(thiophene) (PT), (c) poly(p-phenylene-vinylene) (PPV), (d) poly(9,9-dialkylfluorene) (PF), adapted with permission from ref [25]. Copyright 2013 Royal Society of Chemistry.

Conjugated materials have various possibility of color tuning by using different monomers. The band gap energy of the conjugated material depends on the monomers used in the backbone since each monomer has unique band gap by itself. Conjugation length of the backbone is governed by the design of these available monomers. Increasing number of delocalized electrons, which contribute to valence band, decreases the band gap of the material.[26] For instance, when benzothiadiazole, which is electron withdrawing monomer (low LUMO), is combined with a fluorene monomer, which has a higher HOMO energy, resulting conjugated oligomer or polymer has a narrow band gap energy. Additionally, it is possible to prevent aggregation induced quenching of the emission by different pendant groups that attached to 9th position of fluorene. Solubility increasing

pendant groups affect the conformity of the conjugated material in solution and prevents fluorescence decrease caused by the aggregation.[27]

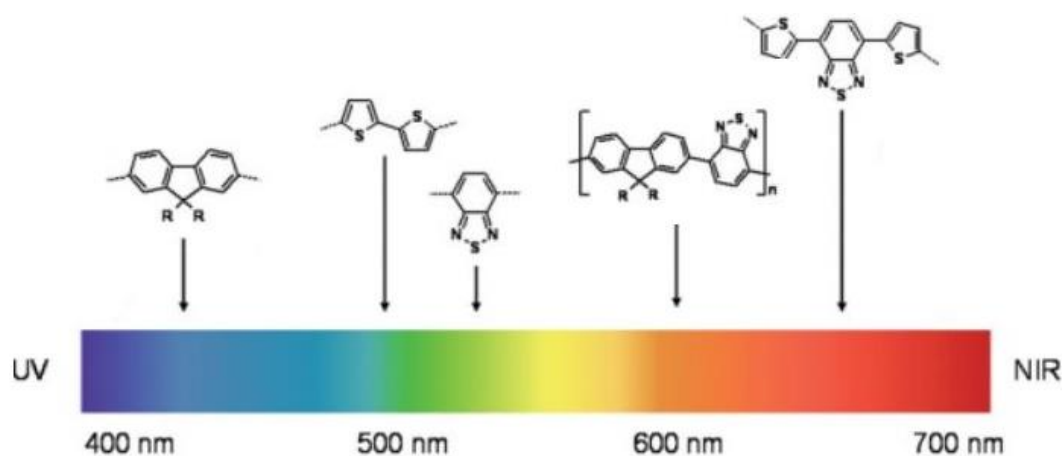


Figure 1.3: Fluorescence colors of several conjugated materials from blue to red, adapted with permission from Ref [28]. Copyright 2009 Wiley-VCH Verlag GmbH & Co. KGaA.

1.2 Synthesis of Conjugated Materials

Growing investments on conjugated materials related applications has improved the research interest to the topic. The excellence in properties of fluorescence efficiency, mechanical and thermodynamical stability, easy processability, and cost efficiency is getting brighter with every study. Common way to synthesize conjugated materials is to do coupling reactions.

1.2.1 Coupling Chemistry

Coupling reactions may be the best way to produce conjugated materials since they are the most rewarded reactions. The palladium catalyzed cross-coupling reactions occurs via palladium centered ligand like intermediate product. The intermediate brings the two molecules of interest to a close proximity, causing to form new C-C bond in between at the end. The reactions provide high compatibility with different monomers, various reaction conditions and solvents with a high reaction efficiency. Regarding to these advantages, Richard F. Heck, Ei-ichi Negishi, and Akira Suzuki were the ones who shared the Nobel Prize in Chemistry 2010 *"for palladium-catalyzed cross coupling in organic synthesis"*.[29]

In 1979, palladium-catalyzed cross coupling reaction was reported by Suzuki *et al.* Boronic acids or esters of vinyl and aryl halides was coupled in basic medium with the help of palladium catalyst.[30], [31] Suzuki coupling uses the organoborane compounds that makes the reaction advantageous over other organometallic coupling reactions in many aspects. First of all, organoborane compounds are non-toxic and environmental friendly, can tolerate various functionalities and be performed in aqueous media. It is prominent for the production of numerous industrial chemicals *i.e.* conjugated materials, agrichemicals, pharmaceuticals, and composite materials. Suzuki coupling is preferred in our study due to the mild reaction conditions, availability and eco-friendliness of organoborane compounds, and boronated side-products are easy to handle.

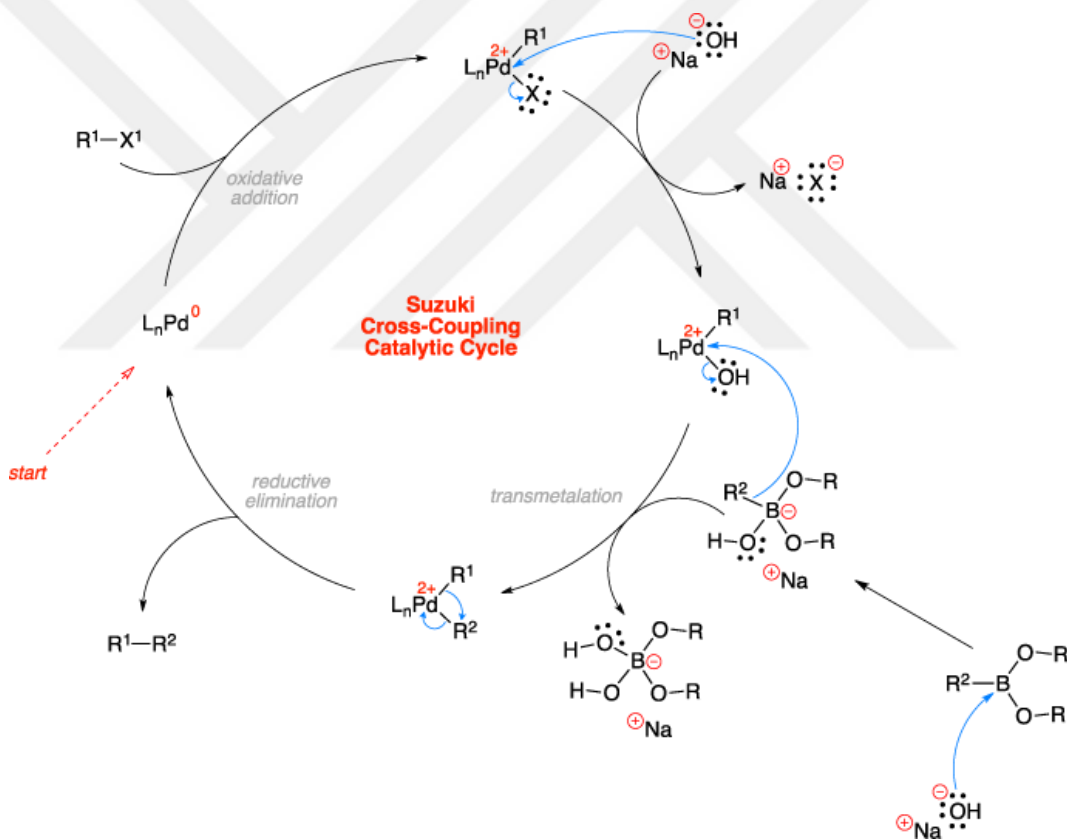


Figure 1.4: General mechanism of Suzuki cross-coupling reaction.

In the mechanism of Suzuki coupling reaction (Figure 1.4), organohalide complexes with $Pd(0)$ as the beginning. After oxidative addition, organoborane is added to the complex, while the base removes the halide. In transmetalation step,

borate leaves the complex with base, leaving Pd(II) complex with two R groups which later be joint together by newly created C-C bond in between. Reductive elimination step finalizes the product and regenerates the catalyst as Pd(0).

Richard F. Heck and co-workers was the first ones who reported the C-C bond formation via palladium catalytic cycle in 1972.[32] Heck coupling, also known as Heck-Mizoroki reaction, has great importance due to its capability of substitution on planar sp^2 hybridized carbon center, yielding trans product. Likewise Suzuki coupling, Heck coupling is also used for the synthesis of conjugated polymers and oligomers, natural and biological products. In oxidative addition step, organohalide-Pd(II) complex is formed. Then, coordination of alkene is followed by the migratory insertion of alkene to the palladium. Bond rotation results trans formation and β -hydride elimination yields the final product. Finally, Pd(II) is reduced to Pd(0) by the base (Figure 1.5).

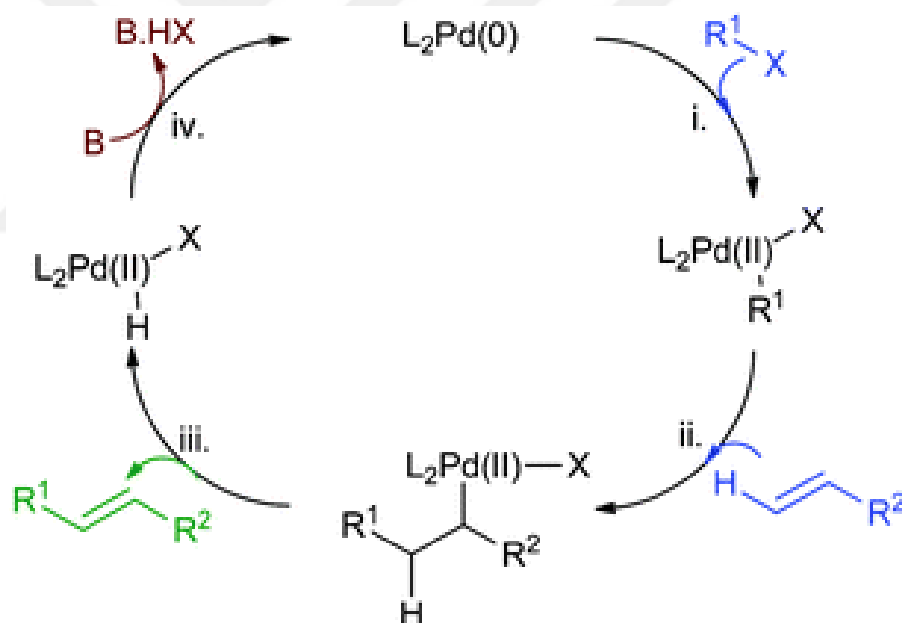


Figure 1.5: General mechanism of Heck coupling.

The Stille reaction is also another palladium-catalyzed coupling method that has been used for this study. The Stille coupling reaction makes use of organotin compounds to create C-C bond in the synthesis of conjugated oligomers and polymers. The reaction gained importance due to easy production of organotin compounds and tolerance to use numerous functional groups and electrophiles *e.g.*

triflates and halides.[34], [35] The main drawback of the reaction is the toxicity of tin containing products. The mechanism of Stille coupling reaction is similar to Suzuki mechanism with a nuance of using tin rather than boron, as shown in Figure 1.6.

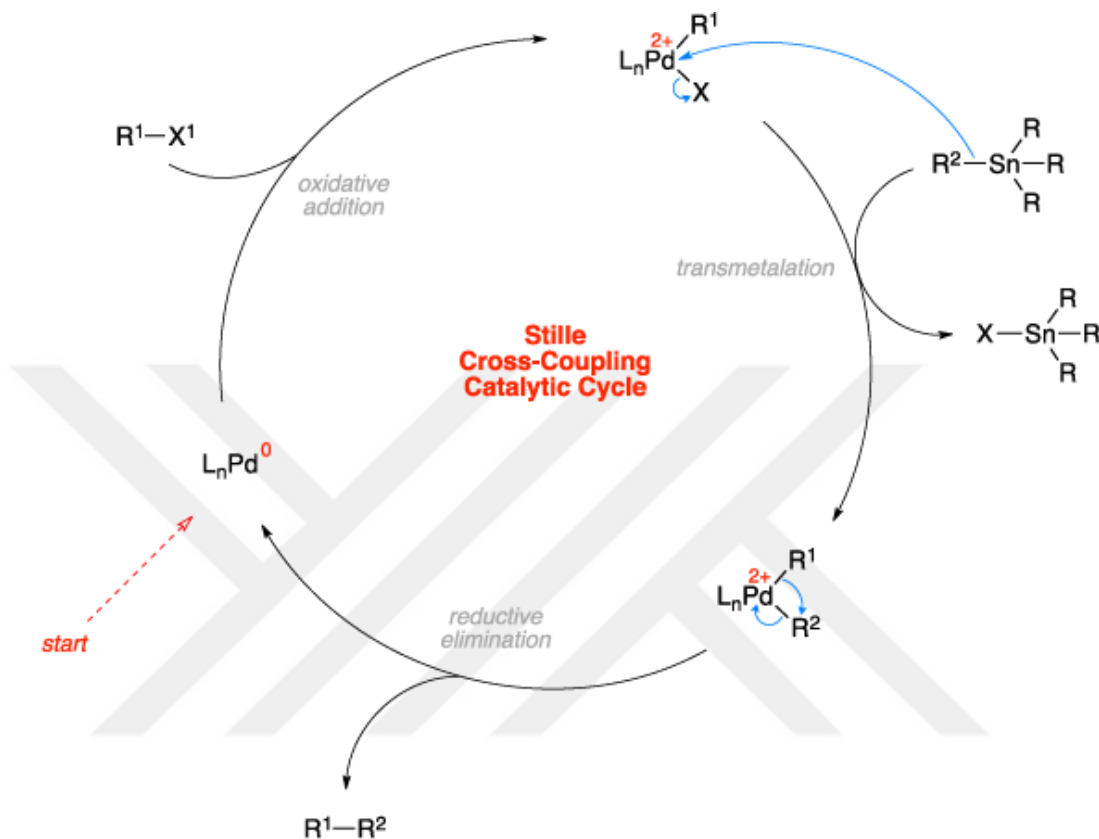


Figure 1.6: General mechanism of Stille coupling reactions.

1.2.2 Click Chemistry

The concept of click chemistry was first introduced by Karl B. Sharpless *et al.* in 2001.[36] The aim of the study is to join small molecules by heteroatom linking in a quick and reliable way. Click reaction has simple condition requirements and yields highly stereospecific products in a facile way. The advantage of click reaction is not only high yields of desired product, but also its eco-friendly byproducts and ease of product isolation without any chromatographic methods. Although the click chemistry includes many types of reactions namely cycloadditions, ring openings, Diels-Alders, Michael addition, thiol-ene and oximes,[37] the most widely studied one is copper catalyzed azide-alkyne

cycloadditions (CuAAC) reactions. CuAAC reaction mechanism (Figure 1.7) starts with the coordination of Cu(0) and alkyne group. Cu(I)acetylide complex combines with azide group to first form 6-membered intermediate and then recoordinate to 5-membered ring. Finally, Cu(0) is regenerated by the protonation of 5-membered pre-product which yields to 1,4-disubstituted 1,2,3-triazole.[38]

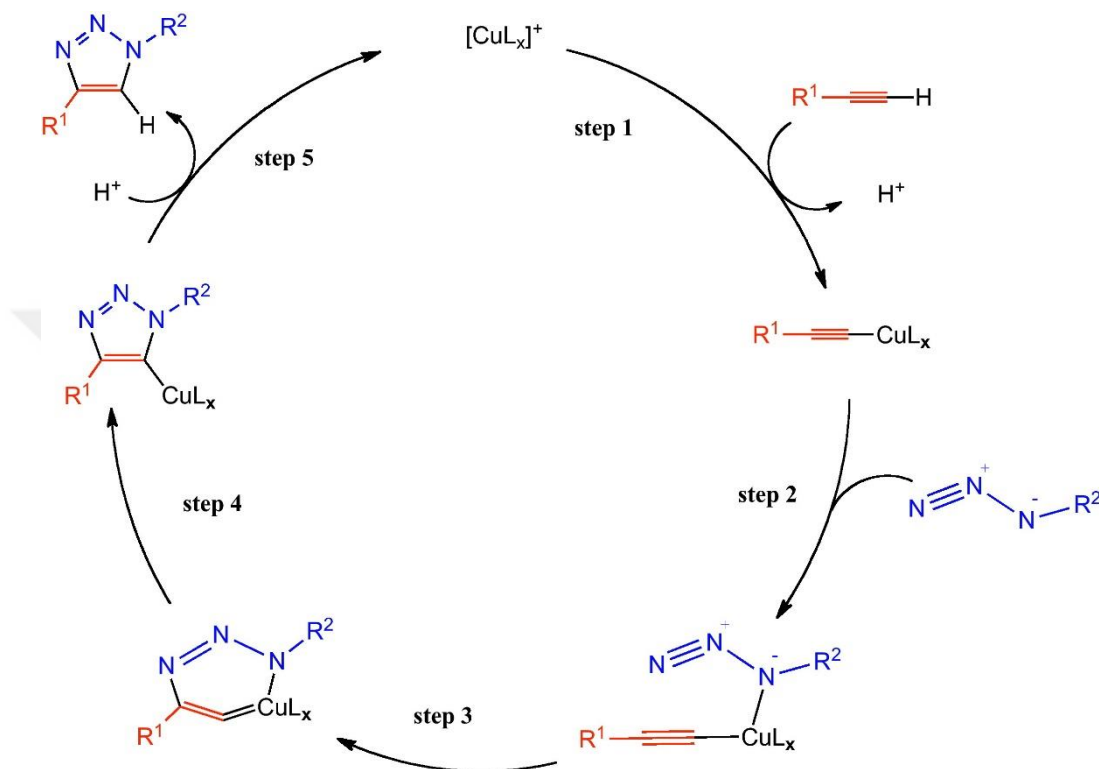
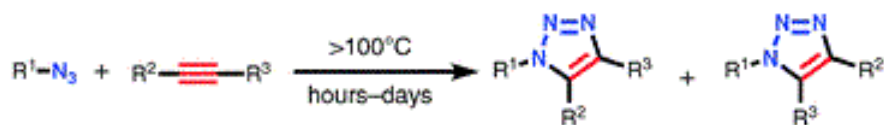


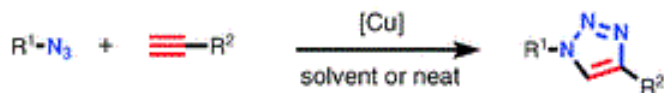
Figure 1.7: Mechanism of CuAAC reactions, adapted with permission from Ref [39]. Copyright 2011 Elsevier.

Another way to facilitate azide-alkyne fusion is the Huisgen's reaction.[40] Huisgen's reaction is not considered as one of the click reactions since it is slow and non-regioselective; resulting a mixture of 1,4- and 1,5-disubstituted 1,2,3-triazoles in equal amount (Figure1.8 A).[38] Additionally, Sharpless and co-workers also reported the other regioisomer (1,5-disubstituted triazole) selective click reaction, catalyzed by ruthenium cyclopentadienyl complexes (RuAAC) (Figure1.8 C).[41]

A. 1,3-Dipolar cycloaddition of azides and alkynes



B. Copper catalyzed azide-alkyne cycloaddition (CuAAC)



C. Ruthenium catalyzed azide-alkyne cycloaddition (RuAAC)

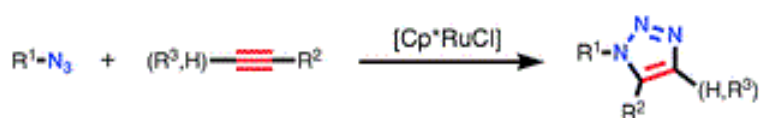


Figure 1.8: Comparison of (A) Huisgen's reaction, (B) CuAAC reaction and (C) RuAAC reaction in terms of regioselectivity, adapted with permission from Ref [42]. Copyright 2010 Royal Society of Chemistry.

High efficiency and great functional group tolerance of CuAAC based click chemistry took the attention of researchers in various fields. Although the polymerization and polymeric applications of click chemistry has been widely demonstrated,[38] first efficient polymerization of fluorene was reported by Steenis and co-workers.[43] The study showed highly efficient polymerization at room temperature, proving that CuAAC method is a robust way to synthesize conjugated materials. Functionalization problems of well-studied methods are overcome by click reaction. Especially the biological applications of organic materials where biomolecule attachment requires great effort. In 2010, Wu *et al.* reported the cellular target labeling by click reaction of conjugated materials.[44] Advantage of click chemistry in bioapplications are not only limited with easy modification biomolecules *e.g.* carbohydrates and nucleic acids, but also it is useful that click reaction can be carried out in aqueous media.[45], [46] Use of click chemistry has created the novel routes for design of bioimaging agents, biosensors and drug delivery applications based on conjugated materials.

1.3 Synthesis of Conjugated Material Nanoparticles

However the conjugated polymers and oligomers are desirable in many biology related applications, water dispersibility of the conjugated materials are quite limited to pendant group modifications such as ionic or hydrophilic groups. In order to achieve the water-solubility, conjugated material nanoparticles have been under investigation. Conjugated polymer and oligomer NPs have both the advantages of conjugated materials, *i.e.* quality of fluorescence efficiency, tunability of emission, and photostability, and nanoparticles such as small size.[47]–[49] To prepare conjugated material NPs, miniemulsion and reprecipitation methods are being frequently used.

1.3.1 Miniemulsion Method

As the name implies, miniemulsion method requires the use of a surfactant that keeps the conjugated materials within the droplets of emulsion. Conjugated materials first dissolved in water-immiscible organic solvent, then injected into the surfactant containing water whilst sonicating. Nanoparticle formation is sealed with the evaporation of organic solvent (see Figure 1.9). However numerous studies reported useful size and distributions of NPs,[48], [50] flocculence due to coalescence and Ostwald ripening is the main drawbacks of this method. NPs size range can be controlled by the choice of surfactant and average conjugated material amount within the droplets. Green *et al.* reported that conjugated polymer NPs shows 80-100 nm hydrodynamic size via PEG-lipid encapsulation,[51] while Liu and co-workers obtained 243-272 nm via PLGA encapsulation.[52]

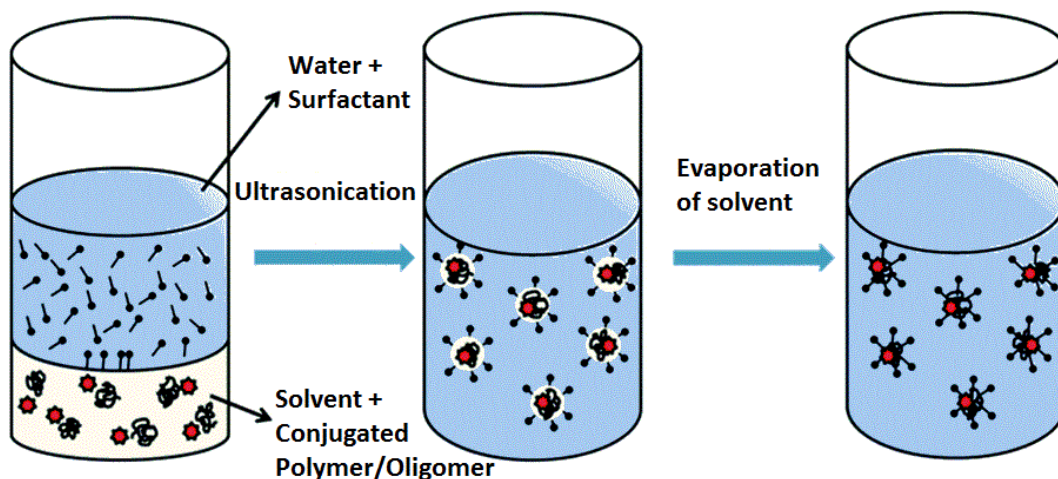


Figure 1.9: Preparation scheme of conjugated material nanoparticles by miniemulsion method, adapted with permission from Ref [53].

1.3.2 Reprecipitation Method

Conjugated material nanoparticles can be prepared by reprecipitation, also known as nanoprecipitation, method as described in Figure 1.10. The conjugated materials are dissolved in a good organic solvent *e.g.* THF that is miscible with water and then injected into deionized water while ultrasonicing or stirring to assist the NPs formation. The rapid injection of oligomers into the water drives the formation of nanoparticles by the hydrophobic effect. Since the conjugated materials have a hydrophobic backbone, the conjugated polymer or oligomer chains tend to avoid from water and so come together forming spherical nanoparticles. After conjugated material NPs are formed, the organic solvent is removed in order to prevent dissolution of NPs, thus NPs stabilization achieved. On the contrary to miniemulsion method, there is no requirement to use surfactants in reprecipitation method. This method applicable to many kinds of conjugated materials that are soluble in organic and water-miscible solvents. Additionally, size of conjugated material NPs depends on the concentration of conjugated materials. Keita *et al.* reported the multi-layered nanoparticle synthesis based on poly(fluorene) and poly(thiophene) derivatives by using reprecipitation method.[54] The study states that size of the white-emitting conjugated NPs changes from 98 nm to 118 nm when second layer of conjugated polymer is introduced to form bilayer NPs.

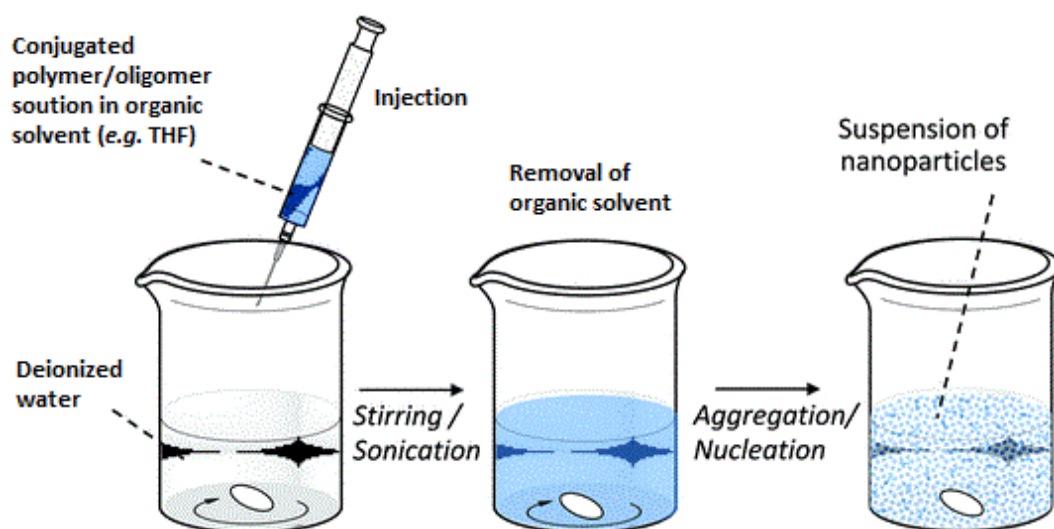


Figure 1.10: Preparation scheme of conjugated material nanoparticles by reprecipitation method, adapted with permission from Ref [55]. Copyright 2013 Royal Society of Chemistry.

1.4 Applications of Conjugated Material Nanoparticles

Conjugated materials attracting the applications of electronics and biology due to their promising properties. Ease of modification, processability, tunability over visible spectrum, flexibility in solid form, ease of printing to any surface and cost efficiency are the main reason that wide range application of optoelectronics *e.g.* OLEDs (organic light emitting diodes),[56] TFT (thin film transistors),[13] and organic solar cells.[57] Besides the electronics, bioapplication such as biosensors,[58] bioimaging,[59] and drug delivery[60] are promising research fields due to the water solubility via appropriate functionality, multi-targeting via anti-body functionality, and NIR tunability which is good for deep tissue penetration. Conjugated material nanoparticles has increasing interest on white-light generation and theranostic studies. FRET based applications are also under investigation for few decades and covering both bio- and electronic applications. Thus, white-light optoelectronic applications and theranostic applications are covered in next section starting from energy transfer.

1.4.1 Energy Transfer Applications

Fluorescence energy transfer (also known as Förster resonance energy transfer - FRET) is described as energy transfer between two chromophores. Spectral overlap

between the emission of donor fluorophore and the absorbance of acceptor fluorophore is an essential requirement for fluorescence energy transfer (FRET). When a donor molecule irradiated with light wavelength that absorbs, electrons from ground state are excited to higher electronic states. Excited electrons normally relaxes back to ground states by radiative ways *i.e.* fluorescence. In the case of FRET, non-radiative pathways have been taken by excited state electrons. In other words, energy relaxation occurs via the energy levels of acceptor chromophore. In Figure 1.11, FRET mechanism is described on Jablonski diagram that excited state energy of donor is transferred to energy states of acceptor. The distance between the chromophores is another requirement of FRET. Donor and acceptor molecules should be in a close proximity so that the energy transfer to be happen. Distance sensitivity of FRET efficiency is proportional to 10^{-6} distance in between (1-10 nm range).[61]

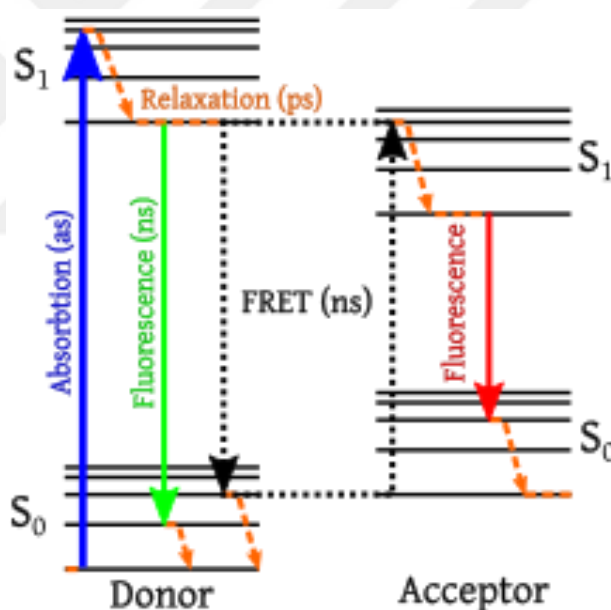


Figure 1.11: FRET mechanism is described on Jablonski diagram

FRET in nanoparticles is fundamental to various potential applications in optoelectronic devices and biomedical sciences. Emission wavelength or the color of nanoparticles can be tuned by FRET is gathering great attention in OLEDs, bioimaging, and biosensors.[63]–[65] FRET mechanism depends on the interactions of acceptor and donor molecules and it is typically dipole-dipole interactions. Keita *et al.* reported the multi-layered white emitting nanoparticle

synthesis based on poly(fluorene) and poly(thiophene) derivatives. White emission is facilitated by FRET within the NPs.[54] To obtain stable and shape persistent NPs, click reaction is used between acceptor and donor molecules. Click chemistry is also facilitated further interaction besides the dipole-dipole and FRET efficiency of NPs is increased by persistent close proximity of donor and acceptor. Wang *et al.* reported four different fluorene-based polymers (covering visible range blue, green, yellow, and red) for multicolor bioimaging and tumor cell detection.[66] Fluorene-based polymers and PSMA nanoparticles has multistep FRET and efficiency and multicolor emission was controlled by changing proportions of polymers in the nanoparticle. Tumor cell specificity of recognition was improved by using multi-antibody via modification of nanoparticles with different antibodies (Figure 1.12). Detection of tumor cell was improved by this way suggesting a better recognition than single-antibody modification.

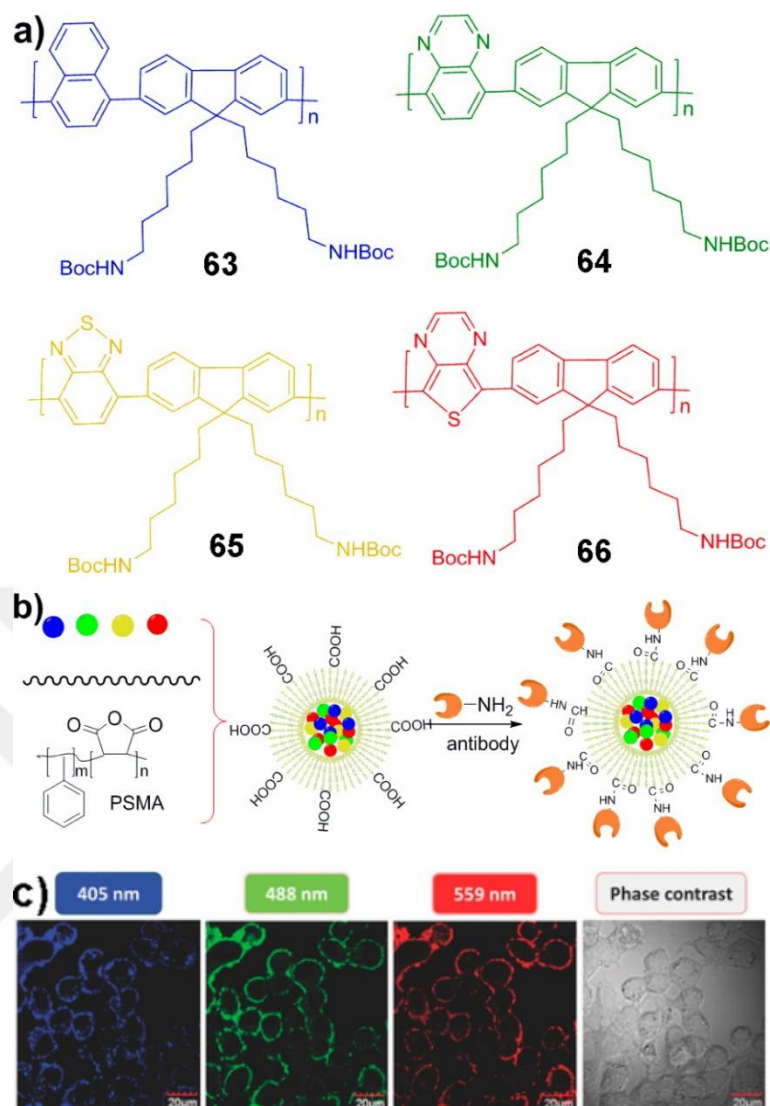


Figure 1.12: (a) Chemical structures of conjugated polymers, blue, green, yellow, and red. (b) Preparation of multicolor conjugated polymer nanoparticles and their modification with an antibody. (c) Multichannel fluorescence images of MCF-7 cells with the conjugated polymer nanoparticles. Excitation wavelengths are shown on top of each image. Adapted with permission from Ref [66]. Copyright 2014 Wiley-VCH Verlag GmbH & Co. KGaA.

1.4.2 Optoelectronic Applications

Organic light emitting diodes are the p-n junction diode type electronic compounds which has an organic electroluminescent layer. The thin film of organic layer is responsible of light generation through applied voltage between anode and cathode. Since it is a p-n type junction, application of electricity ejects electrons from

cathode to LUMO of organic layer and increase the number of electrons in anode which are from HOMO of organic layer. Thus, electron-hole pairs are created and recombination results a fluorescence type relaxation in organic layer. Fluorescence or emission of light depends on the band gap of the conductive material and it tunes the color or wavelength of light. In this case, conjugated polymers and oligomers are the organic conductive layers that has light emission at visible spectrum. In 1990, Burroughes *et al.* was the first one who reported a functioning conjugated polymer based OLED,[12] pioneered for flat displays. Flat displays or OLED screens are advantageous over LCD and LED screens in terms of flexibility, wide-angle view, power and brightness efficiency, lightweight, and slimness.

White emitting OLEDs (WOLEDs) have significant attention on solid state lighting as they cover all visible spectrum. First fabrication of WOLEDs was composed of a combination of three chromophores in a single electroluminescent layer, reported by Kido *et al.*[67], [68]. Recent devices has various approaches to generate white light emission such as single and multiple emissive layer, tandem white emission, and FP (fluorescent-phosphorescent) white emission.[69]–[72] Multicomponent WOLEDs, which has stacked components, has advantageous over color control. In single layer multicomponent WOLEDs, dopant contribution to white light is critique and dependent on the portion of each dye. Exciton (electron-hole pair) formation and recombination have many paths to occur; the contribution of each path *e.g.* direct recombination and ET recombination, defines the white light quality. Thus, concentration of each color component should be adjusted carefully so that the distribution of relaxation paths yields white light.[71]

Conjugated material nanoparticles are used to form electroluminescent layer of WOLEDs as possessing a better ET efficiency. Bi-polymer NPs are synthesized with blue and green emitting polymers showed a stable white emission in numerous studies.[63], [73] Efficiency of ET between blue-green polymer NPs is investigated by different preparation techniques such that core-shell and mixed preparations have better results than separate NPs mixtures.[54], [74]

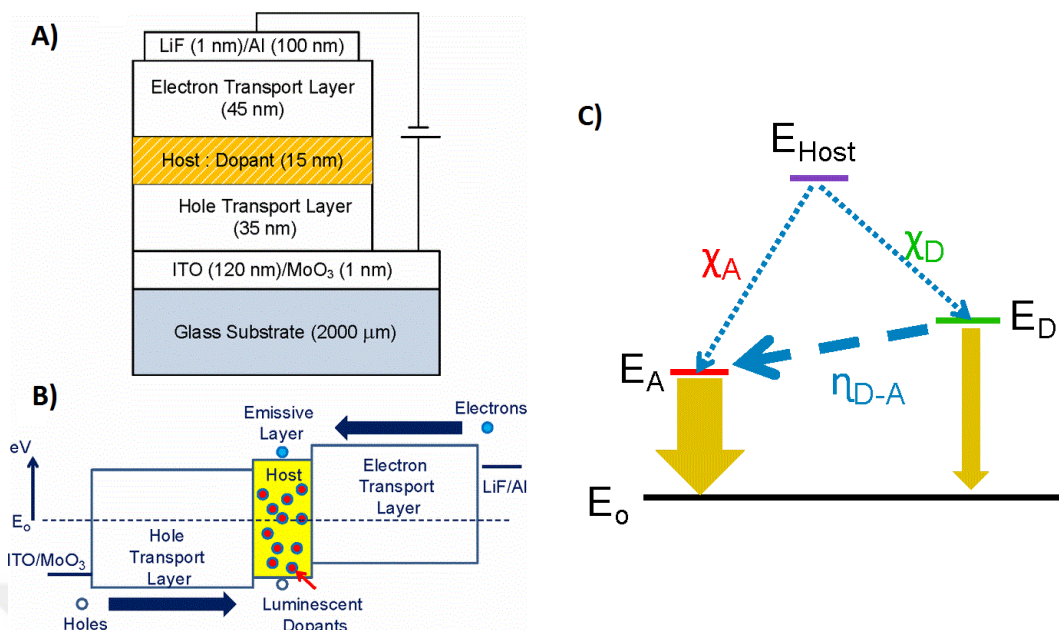


Figure 1.13: A) Schematic of a typical OLED device structure, B) Energy level diagram at equilibrium with representation of electrons and holes (excitons) in electroluminescent layer, C) Schematic of recombination paths in a two dopants system in a single layer. E_{Host} , E_A , E_D , E_0 , χ_A , χ_D and η_{D-A} represent the energy levels of host, acceptor, donor, and ground state, emission fraction of acceptor, donor, and energy transfer efficiency, respectively. Adapted with permission from Ref [71].

1.4.3 Biological Applications

Biological applications of conjugated materials have a growing attraction over several decades. Researchers focus shifted towards conjugated materials due to their fine photostability, quality in fluorescence brightness, minimal cytotoxicity, and ease of functionality altering modifications on the structure. Biocompatibility of conjugated materials expand the studies with cells and tissues both *in vivo* and *in vitro*. [75], [76] Variability of functionality modifications ensures both non-specific and specific bio-targeting applications such as cell labelling and sensing, cell tracking, imaging, and drug delivery. [50], [77], [78] Non-specific targeting is the direct use of conjugated polymers without having any bio targeting components, while specific imaging requires modifications of the structure with recognition components, *e.g.* peptide, sugar, protein, or anti-body (Figure 1.14).

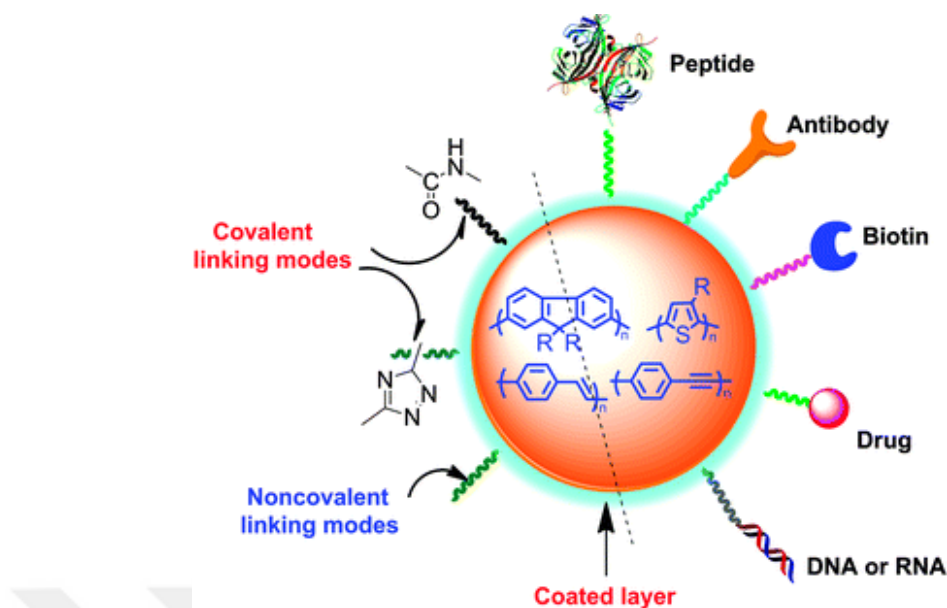


Figure 1.14: Schematic representation of different surface functionalization elements for bio-conjugated nanoparticles, reprinted with permission from Ref [79]. Copyright 2013 Royal Society of Chemistry.

Cellular internalization of conjugated materials has two endocytosis path: phagocytosis and pinocytosis. Phagocytosis is useful for larger particles, micron level, while pinocytosis is more applicable for NPs. Non-specific imaging generally targets the cytoplasm imaging via non-specific cellular uptake, while specific imaging targets organelles, molecules, nucleus and genetic material via receptor-mediated endocytosis (Figure 1.15). Hence, the size of the NPs is critique factor for cellular uptake in bio-applications. According to literature reports, 10-100 nm is the range that is optimal for *in vivo* studies, while the range of 40-80 nm is beneficial for *in vitro*. [80] Wang *et al.* developed four different color emitting poly(fluorene) NPs (blue, green, yellow, and red) for bio-analysis and imaging purposes. [81] Prepared NPs are 50-100nm in size and used for labelling of perinuclear region, successfully. On the hand, larger particle sizes could be useful for the therapeutic purposes. Tumor tissues have an abnormality in vascular distribution that it has higher permeability due to larger vessels. Lymphatic drainage has also damaged in cancerous sites, causing accumulation of macromolecules. Considering both factors, which are overall called enhanced permeability and retention (EPR effect),

therapeutic NPs concentration is higher and hardly disposed from tumorous site.[82]

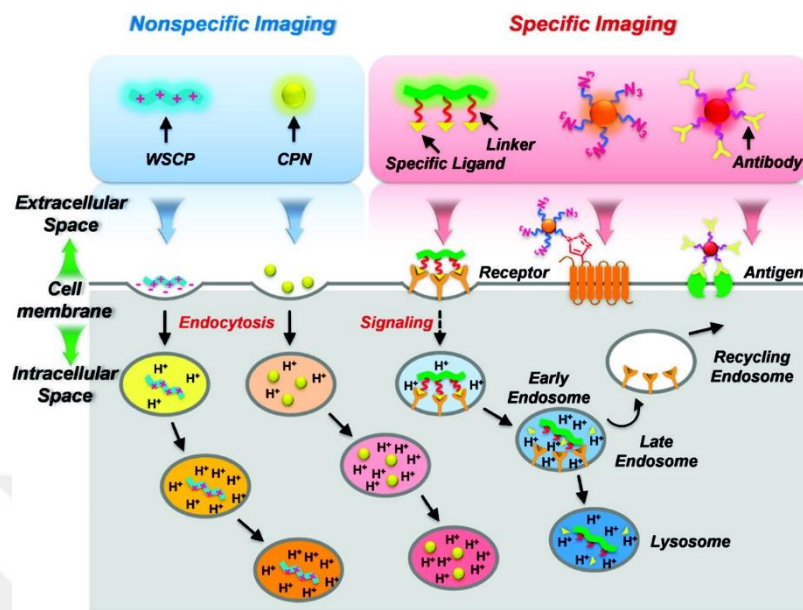


Figure 1.15: Representation of pinocytosis mechanism for non-specific and specific targeting, reprinted with permission from Ref [83]. Copyright 2012 American Chemical Society.

For bioimaging applications, variety of stains, such as chemical fluorophores,[84] fluorescent proteins,[85] and quantum dots,[86]–[88] are used to label the desired cells or organelles. However, high toxicity (*e.g.* heavy metal based quantum dots), rapid photobleaching, and low stability in aqueous media problems limit the applications of these agents. Although the linearity of the conjugated polymer molecules obstruct the uptake by the cells, recent studies on side chain design indicate improvement. Besides the biocompatible side chain design and hyperbranching method, conjugated polymer NPs have been developed by either reprecipitation or miniemulsion methods.[44], [89] These nanoparticles have both the excellent properties of conjugation of backbone, which are quality in fluorescence and brightness, low toxicity, and outstanding photostability, and ease of penetration through the cell membranes due to smaller sizes. Wang and co-workers reported an oligo-fluorene NPs for lysosome imaging with a specific amino group for targeting and PEG functionality for hydrodynamic stability.[90] NPs has

green emission (501nm) with quantum yield of 0.38 in PBS buffer. Lysosome targeting NPs showed low toxicity (85% viability in 24h with 1-20 μ M concentrations) and excellent photostability in living cell experiments.

An additional functionality is also possible with conjugated NPs due to valuable carrier potential. Encapsulation of drugs or covalently attachment of photosensitizers facilitates therapeutic applicability to conjugated materials. Photosensitizers, such as porphyrin, is covalently bound to a conjugated backbone so that close proximity yields an effective ET. Photosensitizer creates singlet oxygen, which damages cells, for photodynamic cancer therapy or anti-microbial effect, as in the case of poly(thiophene)-porphyrin dyad.[91], [92] Moreover, anti-cancer agents can be loaded into conjugated material NPs without any covalent attachment. Since the backbone of conjugated materials have hydrophobic effect when NPs are formed, doxorubicin or camptothecin like hydrophobic cancer drugs are easily encapsulated by NPs. The resulting drug-loaded NPs are used for treatment and diagnosis (*i.e.* targeted bioimaging) applications simultaneously, which is called theranostic applications. Chiu *et al.* prepared fluorene-based FRET pair in order to improve depth of penetration in tissues and reduce the background auto-fluorescence by red to near-IR region emission.[93] 15 nm average diameter sized NPs show a quantum yield of 0.56 in water at 650 nm emission maximum. This level of quantum yield (high when compared to similar dyes in water) and far-red emission was suggested to be cause of highly efficient energy transfer within NPs. Further functionalization with PEG and encapsulation of chlorotoxin (CTX) makes NPs targeted to neuroectodermal tumor. In animal experiment, the study provides beneficial results for diagnosis and treatment of clinical cancer sited on cerebellum of a mice.

1.5 Aim of the Thesis

This project firstly aims to develop water dispersible conjugated nanoparticles by using click chemistry for FRET based white light emission. Reprecipitation method was used in order to obtain water dispersible NPs. Preliminary NPs study with only OFT Pgy and only OFVBt N3 were done in order to determine size and distribution of NPs. Overlapping optical properties of donor (OFT Pgy) and acceptor (OFVBt

N3) give possibility to FRET applications. By click reaction in water, terminal sites of oligomers connected and white emission is sealed by keeping D-A pair close. FRET based white light is widely used in optoelectronic applications such as OLEDs or more specifically WOLEDs. Since the white light covers all visible spectrum, different color emissions are obtainable depending on excitation wavelength. Various biosensor and bioimaging applications are also possible with white light emitting NPs, since they are readily and stably dispersed in water.

In second part of the project, OFVBt N3 oligomer cross-linked with di-sulfide containing crosslinker via copper catalyzed click reaction. NPs were synthesized in THF due to higher click efficiency and redispersed in water, since the biological applications are targeted. OFVBt N3 oligomer has advantageous for bioimaging with its red emission close to IR region, since lower frequency emission overcomes the background auto-fluorescence and penetrates deeper in the body. Di-sulfide crosslinker connects the oligomer molecules and stabilizes NPs as well as providing possibility of drug delivery application. Since GSH (glutathione) or Trx (thioredoxin) like thiol bearing bio-molecules are presented in higher concentrations in tumorous tissues, di-sulfide bond can be cleaved, releasing the loaded drug from NPs. Thus, crosslinked OFVBt N3 NPs is a theranostic agent with an advantageous emission color for bio-imaging and a cleavable di-sulfide bond for drug delivery & controlled release.

In last part of the study, white light emitting bi-oligomer nanoparticles were designed and obtained by using OFB Pgy and Porph N3. Overlapping optical properties of OFB Pgy (D) and Porph N3 (A) is used to white light emission by FRET. The purpose of clicking the oligomer pair is to stabilize the FRET efficiency. Moreover, using THF as the solvent is provided ease of applicability for solid state white light applications. Since THF evaporates easily, white light emitting NPs can form film on various surfaces. Thus, these NPs requires no host layer and can be applied directly to electrode surface when optoelectronic applications e.g. OLEDs or WOLEDs are considered.

Chapter 2

Experimental

2.1 Materials

In all the experiments commercial grade reagents and solvents were used without further purification unless noted. Silica gel (Kieselgel 60, 0.063-0.0200 mm) was used to carry out column chromatography. Thin layer chromatography was carried out on silica gel plates (Kieselgel 60 F254, 1 mm).

2.2 Instrumentation

2.2.1 FT-IR Spectroscopy

The IR spectrum was recorded with Bruker Alpha Platinum ATR model FT-IR spectrometer. High sensitivity DTGS detector with a resolution of better than 2 cm^{-1} was used. All the samples were formed film on sampler of instrument by evaporation of solvent of sample solution. The FT-IR of the samples was recorded in the range $400\text{-}4000\text{ cm}^{-1}$ for 64 scans.

2.2.2 UV-VIS Spectroscopy

UV-VIS absorbance Spectrum was recorded with Cary 300 UV-Vis double beam spectrophotometer with spectral bandwidth down to 0.2 nm and Xenon Flash Lamp as the light source. The UV-Vis absorbance of the samples was recorded in solution using quartz cuvettes with 1 cm length within the range 200-800 nm.

2.2.3 Photoluminescence Spectroscopy

The photoluminescence spectrum was recorded with Cary Eclipse Varian Spectrophotometer and Xenon lamp as the light source. The photoluminescence of the samples was recorded in the solution using quartz cuvettes with 1 cm length within the range 200-800 nm. Slit width of 2.5-5 nm was used for fluorene based oligomers and their quantum yield measurements. All the samples were excited at their respective excitation wavelength.

2.2.4 ^1H -NMR and ^{13}C -NMR Spectroscopy

Both proton and carbon NMR were recorded with Bruker Avance DRX-400 MHz spectrometer. All the spectra were recorded in solution using deuterated solvents. The chemical shift values were expressed relative to tetramethylsilane as an internal standard.

2.2.5 Elemental Analysis

The elemental composition of the samples was determined using FLASH 2000 Organic Elemental / CHNS-O Analyzer. 2,5-Bis(5-tert-butyl-2-benzo-oxazol-2-yl) thiophene (BBOT) was used as a standard and vanadium (V) pentaoxide (V_2O_5) was used as a catalyst.

2.2.6 Mass Spectroscopy

The mass of the monomers and oligomers were determined with Agilent 6224 High Resolution Mass Time-of-Flight (TOF) LC/MS with Electrospray Ionization method.

2.2.7 Thermal Gravimetric Analysis (TGA)

TGA measurements were done on TGA Q500 at a heating rate of $10^\circ\text{C}/\text{min}$ from 35 to 700°C .

2.2.8 Differential Scanning Calorimetry (DSC)

DSC measurements were done on NETZSCH DSC 204 F1 at a heating rate of $2^\circ\text{C}/\text{min}$ from 30 to 200°C . Al pans and pierced lids were used as crucible.

2.2.9 Dynamic Light Scattering (DLS)

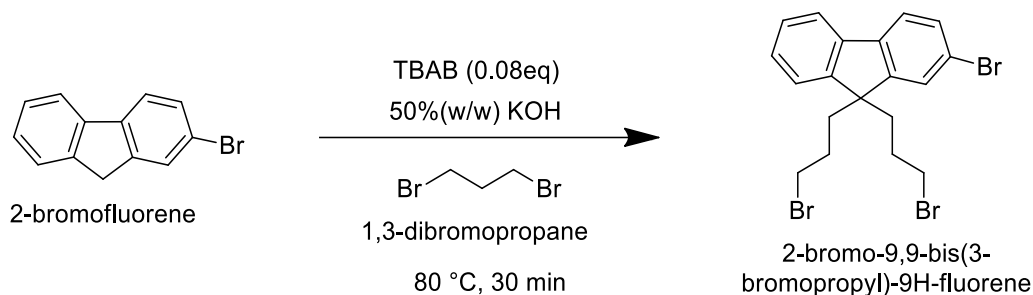
The sizes of oligomer nanoparticles were measured by DLS method using Zetasizer Nano-ZS instrument with 633 nm laser beam, for 13 scans. The measurements were taken at room temperature, using disposable DLS cuvettes for aqueous solutions and quartz cuvettes for organic solutions, with 1 cm width. The average particle size diameters were calculated in the software via Marquardt method.

2.2.10 Scanning Electron Microscopy (SEM)

The morphological characterizations of nanoparticles were done by SEM using Quanta 200 FEG instrument.

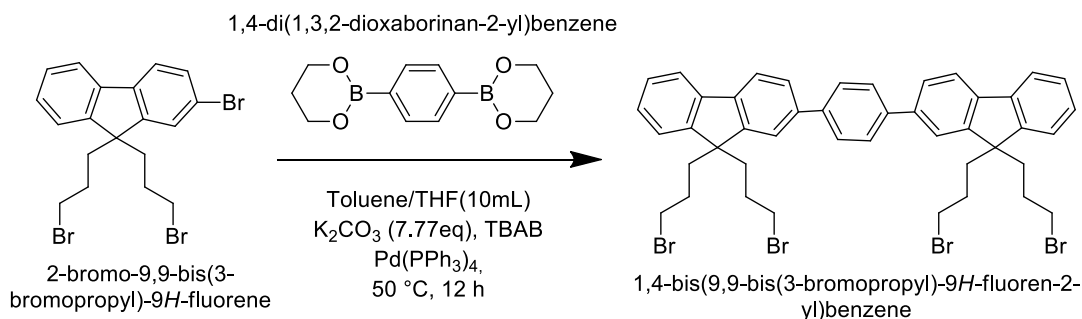
2.3 Experimental

2.3.1 Synthesis of 2-bromo-9,9-bis(3-bromopropyl)-9H-fluorene (M1)



50% KOH (15 g) aqueous solution prepared in 15 ml ddH₂O in a two-neck round-bottom flask. Tetra-*n*-butylammonium bromide (TBAB) (263 mg, 0.851 mmol) and 1,3-dibromopropane (41.145 g, 203.8 mmol) added to the solution while stirring. 2-bromofluorene (2.50 g, 10.19 mmol) added to the flask and heating started. Reaction took place at 80 °C for 45 minutes. The work-up was done to the reaction mixture by first water extraction with DCM. Organic layer further extracted with 2M HCl solution, water, and finally brine solution. Excess 1,3-dibromopropane removed by vacuum distillation. The product was further purified by silica column chromatography using cyclohexane as the eluent solvent. The solvent was evaporated under reduced pressure and yellow crystalline product was obtained. TLC using 8:2 (Cyclohexane:DCM) mobile phase. Yield: 2.0 g, 40%. ¹H NMR (400 MHz, CDCl₃, 25 °C) δ ppm: 1.11-1.22 (m, 4H), 2.11-2.26 (m, 4H), 3.12-3.16 (t, 4H), 7.37-7.71 (m, 7H).

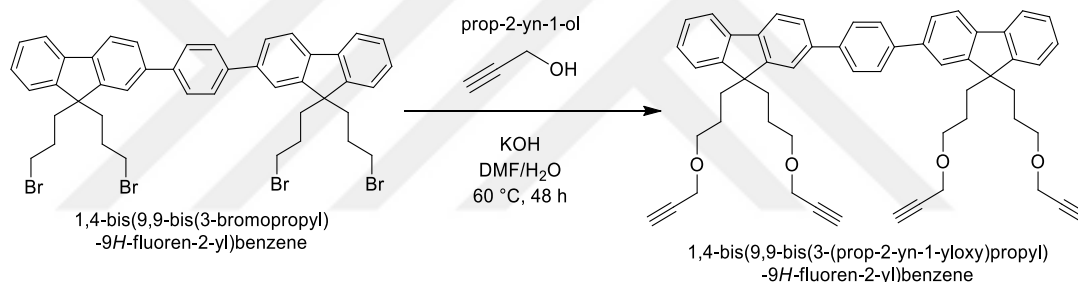
2.3.2 Synthesis of 1,4-bis(9,9-bis(3-bromopropyl)-9H-fluoren-2-yl)benzene (OFB)



2-bromo-9,9-bis(3-bromopropyl)-9H-fluorene (200 mg, 0.411 mmol) and 1,4-di(1,3,2-dioxaborinan-2-yl)benzene (50.5 mg, 0.205 mmol) were dissolved in 10

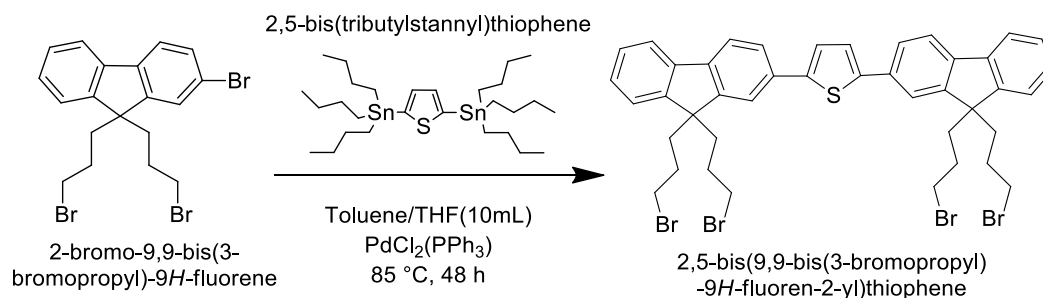
ml of degassed toluene/THF (1:1) mixture in a two-neck round bottom flask and stirred. Aqueous solution of K_2CO_3 (440.3 mg, 3.186 mmol), was prepared in 10 ml of degassed water, was added into the reaction flask followed by the addition of TBAB. The reaction mixture was subjected to freeze-thaw-pump cycle for 3 times to remove oxygen. Then a catalytic amount of $Pd(PPh_3)_4$ was added and heating started. The reaction took place at 50 °C for 12 h while stirring. After reaction was completed, the solvent was evaporated by rotary evaporator. DCM was used to dissolve the reaction mixture and extraction was done with deionized water. The solvent was evaporated and the product was dried under reduced pressure. Yield: 168.9 mg, 19%. ^1H NMR (400 MHz, $CDCl_3$, 25 °C) δ ppm: 1.16-1.19 (t, 8H), 2.14-2.24 (m, 8H), 3.12-3.15 (t, 8H), 7.35-7.60 (m, 18H).

2.3.3 Synthesis of 1,4-bis(9,9-bis(3-(prop-2-yn-1-yloxy)propyl)-9H-fluoren-2-yl)benzene (OFB Pgy)



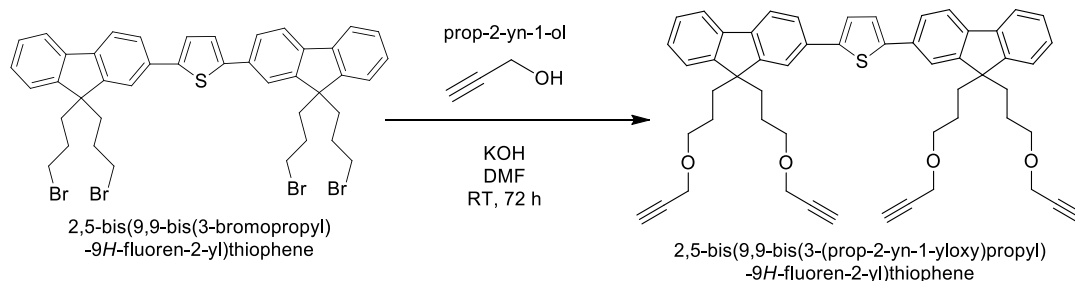
3 pellets of KOH was dissolved in 1 ml of dd. H_2O in a two-neck flask. 2 ml of DMF, 60 μ l of propargyl alcohol (57.76 mg, 1.031 mmol) and 1,4-bis(9,9-bis(3-bromopropyl)-9H-fluoren-2-yl)benzene (100 mg, 0.112 mmol) was added to the reaction flask. Then reaction mixture was heated up to 60 °C and reaction took place for 2 days. After completion of the reaction, the solvent was evaporated under reduced pressure. $CHCl_3$ was added to dissolve reaction mixture and extraction done with dd. H_2O . The product was further purified by silica filtration. Yield: 57.5 mg, 65%. ^1H NMR (400 MHz, $CDCl_3$, 25 °C) δ ppm: 1.16-1.19 (t, 8H), 2.14-2.24 (m, 8H), 3.12-3.15 (t, 8H), 7.35-7.60 (m, 18H). LC/MS HRMS-TOF: (MeCN, positive mode detection) M calculated= 790.38413 m/z, found= 790.402 m/z, (M+1) 791.406 (60.7%), (M+2) 792.409 (18.0%), (M+3) 793.412 (3.5%).

2.3.4 Synthesis of 2,5-bis(9,9-bis(3-bromopropyl)-9H-fluoren-2-yl)thiophene (OFT)



2-bromo-9,9-bis(3-bromopropyl)-9H-fluorene (936.0 mg, 1.922 mmol) and 2,5-bis(tributylstannyl)thiophene (636.4 mg, 0.961 mmol, 530 μ l) were placed in two-neck round bottom flask and dried under vacuum for 30 min. 10 ml of THF and 10 ml of toluene were injected and stirred to dissolve completely. Then reaction mixture was subjected to freeze-thaw-pump cycle for 3 times to remove oxygen. Catalytic amount of Pd(PPh₃)₄ was added under N₂ flow. The reaction flask heated up to 85 °C and reaction took place for 48 h under N₂ gas. After reaction was completed, the solvent was removed by using rotary evaporator. The crude product was purified by column chromatography using cyclohexane. Yield: 200 mg, 19%. ¹H NMR (400 MHz, CDCl₃, 25 °C) δ ppm: 1.20-1.27 (m, 8H), 2.25-2.29 (t, 8H), 3.14-3.17 (t, 8H), 7.35-7.70 (m, 16H). ¹³C-NMR (100 MHz, CDCl₃, 25 °C) δ ppm: 27.2, 29.7, 34.2, 38.7, 54.1, 119.8, 120.1, 120.5, 122.9, 124.2, 125.2, 127.6, 127.7, 133.7, 140.6, 140.7, 143.9, 149.0, 149.8.

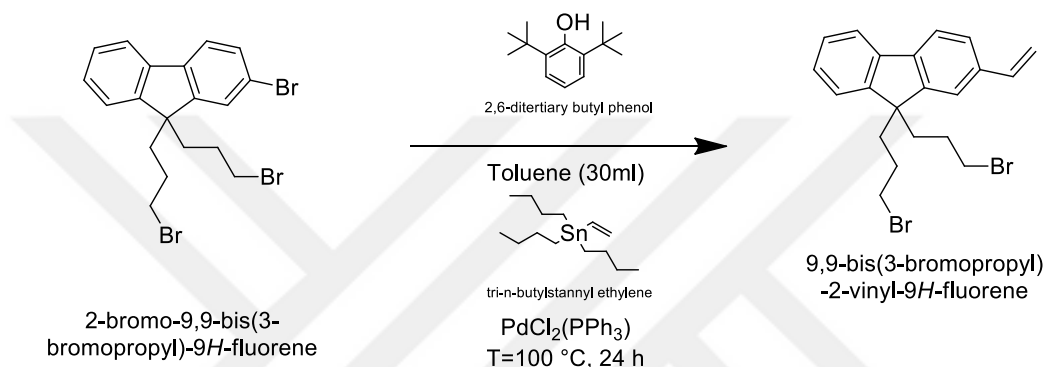
2.3.5 Synthesis of 2,5-bis(9,9-bis(3-(prop-2-yn-1-yloxy)propyl)-9H-fluoren-2-yl)thiophene (OFT Pgy)



2,5-bis(9,9-bis(3-bromopropyl)-9H-fluoren-2-yl)thiophene (110 mg, 0.124 mmol) was dissolved in 5 ml of degassed DMF. 2 ml (excess) of propargyl alcohol and 3 pellets of KOH were added to reaction flask. The reaction was carried out under N₂

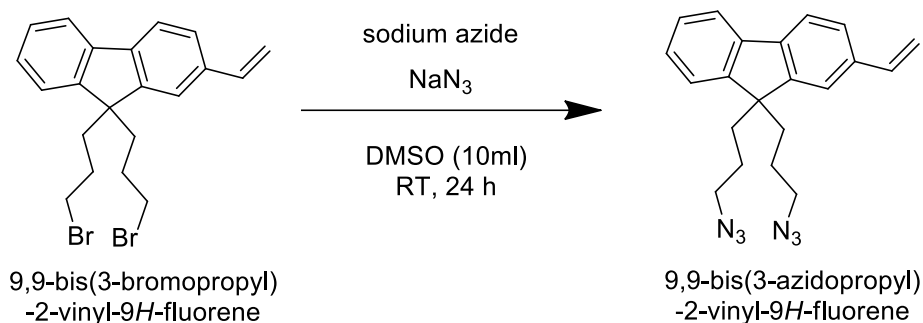
and at room temperature for 72 h. After completion of the reaction, the solvent was evaporated under reduced pressure. CHCl_3 was added to dissolve reaction mixture and extraction done with dd. H_2O several times. Yield: 90.2 mg, 92%. ^1H NMR (400 MHz, CDCl_3 , 25 °C) δ ppm: 0.98-1.07 (m, 8H), 2.15-2.19 (t, 8H), 2.36-2.37 (t, 4H), 3.26-3.29 (t, 8H), 3.98-3.99 (d, 8H), 7.31-7.74 (m, 16H). ^{13}C -NMR (100 MHz, CDCl_3 , 25 °C) δ ppm: 24.1, 29.7, 36.6, 54.6, 57.8, 70.0, 74.0, 80.0, 119.9, 120.0, 120.3, 123.0, 124.0, 124.9, 127.2, 127.4, 133.4, 140.7, 140.8, 144.0, 149.9, 150.6.

2.3.6 Synthesis of 9,9-bis(3-bromopropyl)-2-vinyl-9H-fluorene (M2)



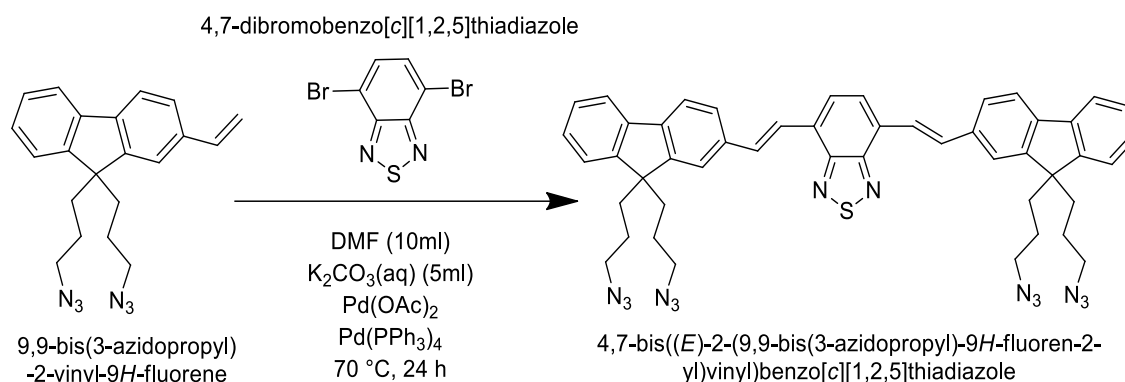
2-bromo-9,9-bis(3-bromopropyl)-9H-fluorene (2.00 g, 4.106 mmol) and 2,6-ditertiary butyl phenol (13.0 mg, 0.063 mmol) were placed in a two-neck round bottom flask and dried under vacuum for 30 min. 30 ml of degassed toluene addition was followed by tri-n-butylstannyl ethylene (738.0 mg, 2.468 mmol, 720 μl) addition into the flask and the mixture was stirred for a while to dissolve completely. Then reaction mixture was subjected to freeze-thaw-pump cycle for 3 times to remove oxygen. Catalytic amount of $\text{PdCl}_2(\text{PPh}_3)$ was added under N_2 flow. Reaction flask was refluxed (100 °C) for 24 h while constant stirring. After completion of the reaction, the solvent was removed by using rotary evaporator. The crude product was purified by column chromatography using cyclohexane and DCM (9:1). Yield: 1.65 g, 93%. ^1H NMR (400 MHz, CDCl_3 , 25 °C) δ ppm: 1.13-1.24 (m, 4H), 2.19-2.23 (m, 4H), 3.11-3.15 (t, 4H), 5.30-5.32 (d, 1H), 5.82-5.86 (d, 1H), 6.79-6.86 (m, 1H), 7.34-7.72 (m, 7H).

2.3.7 Synthesis of 9,9-bis(3-azidopropyl)-2-vinyl-9H-fluorene (M3)



NaN₃ (400.0 mg, 6.153 mmol) and 10 ml of DMSO were placed in a two-neck round bottom flask and stirred to complete dissolution. 9,9-bis(3-bromopropyl)-2-vinyl-9H-fluorene (600.0 mg, 1.382 mmol) was added into the flask. The reaction was completed after 24 h at room temperature. After completion of the reaction, the solvent was removed by using rotary evaporator. DCM was used to dissolve crude product and water extraction was done several times. Yield: 0.460 g, 93%. ¹H NMR (400 MHz, CDCl₃, 25 °C) δ ppm: 0.88-0.96 (m, 4H), 2.11-2.15 (t, 4H), 2.98-3.02 (t, 4H), 5.30-5.32 (d, 1H), 5.81-5.86 (d, 1H), 6.79-6.86 (m, 1H), 7.35-7.73 (m, 7H).

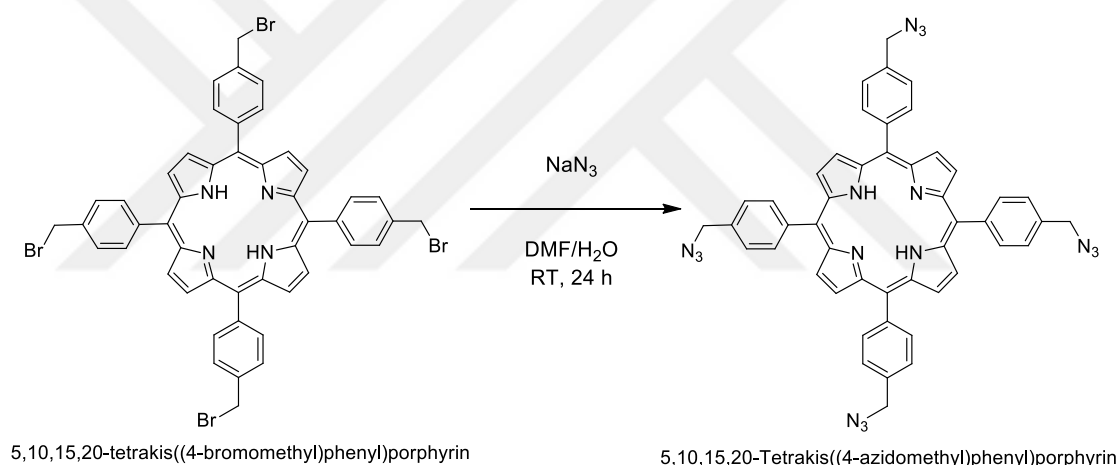
2.3.8 Synthesis of 4,7-bis((E)-2-(9,9-bis(3-azidopropyl)-9H-fluoren-2-yl)vinyl)benzo[c][1,2,5]thiadiazole (OFVBt N3)



9,9-bis(3-azidopropyl)-2-vinyl-9H-fluorene (172.0 mg, 0.480 mmol) and 4,7-dibromobenzo[c][1,2,5]thiadiazole (69.0 mg, 0.235 mmol) were placed in a two-neck round bottom flask and dried under vacuum for 30 min. 10 ml of degassed DMF was added and stirred to complete dissolution. Aqueous solution of K₂CO₃ (161.6 mg, 1.17 mmol) was added into the flask. The mixture was subjected to freeze-thaw-pump cycle for 3 times to remove oxygen entirely. Catalytic amount

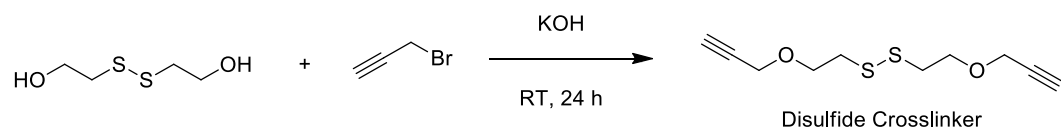
of Pd(OAc)₂ (25.3 mg, 0.113 mmol) and Pd(PPh₃)₄ (26 mg, 0.023 mmol) were added under N₂ flow and the reaction mixture was heated to 70 °C for 24 h while constant stirring and under N₂ flow. After completion of the reaction, the solvent was removed by vacuum distillation. The crude product was dissolved in DCM and extracted with water for several times. Organic phase was dried and further purified by washing with cyclohexane to remove excess catalyst. Yield: 141.0 mg, 35%. ¹H NMR (400 MHz, CDCl₃, 25 °C) δ ppm: 0.87-1.00 (m, 8H), 2.08-2.13 (m, 8H), 2.97-3.05 (d, 8H), 7.00-7.87 (m, 20H). LC/MS HRMS-TOF: (MeCN, positive mode detection) M calculated= 848.35698 m/z, found= 848.359 m/z, (M+1) 849.363 (51.9%), (M+2) 850.366 (13.2%), (M+3) 849.356 (5.2%).

2.3.9 Synthesis of 5,10,15,20-Tetrakis((4-azidomethyl)phenyl)porphyrin (Porph N3)



NaN₃ (24.0 mg, 0.369 mmol) and 3 ml of DMF/dd.H₂O (2:1) mixture were placed in a two-neck round bottom flask and stirred to complete dissolution. 5,10,15,20-tetrakis(4-bromophenyl)porphyrin[94] (30.0 mg, 0.030 mmol) was added into the flask. The reaction was completed after 24 h at room temperature. After completion of the reaction, the solvent was removed by using rotary evaporator. The crude product was washed with water for several times and the product was collected by suction filtration. Yield: 22.6 mg, 89%. ¹H NMR (400 MHz, CDCl₃, 25 °C) δ ppm: 4.75 (s, 8H), 7.73-7.75 (d, 8H), 8.25-8.27 (d, 8H), 8.85 (s, 8H). LC/MS HRMS-TOF: (MeCN, positive mode detection) M calculated= 835.32637 m/z, found= 834.315 m/z, (M+1) 835.319 (51.9%), (M+2) 836.322 (13.2%), (M+3) 835.312 (5.9%).

2.3.10 Synthesis of 1,2-bis(2-(prop-2-yn-1-yloxy)ethyl)disulfane



2,2'-disulfanediyldiethanol (1.261 g, 8.175 mmol) and 3-bromoprop-1-yne (5.340 g, 44.9 mmol) were placed in a one-neck round bottom flask. 2 pellets of KOH was added to stirring mixture and the flask was sealed with a septa. Reaction was carried out at room temperature for 24 h. After completion of the reaction, the solvent was evaporated under reduced pressure. CHCl_3 was added to dissolve reaction mixture and extraction done with dd. H_2O several times. ^1H NMR (400 MHz, CDCl_3 , 25 °C) δ ppm: 2.49 (t, 2H), 2.98 (t, 4H), 3.83 (t, 4H), 4.24 (d, 4H).

2.4 Nanoparticles synthesis

2.4.1 Synthesis and characterization of OFT Pgy NPs

OFT Pgy NPs were prepared by reprecipitation method. 0.1 mg (1.25×10^{-4} mmol) of OFT Pgy was measured from 0.5 g/l (0.627 mM) pre-prepared stock solution, diluted to 2 ml of THF and ultrasonicated for 10 min. The solution was then filtered through 0.45 μM syringe filter, injected into 20 ml of Milli-Q water (18.2 M Ω) and ultrasonicated for 30 min. THF was removed under reduced pressure at 40 °C to obtain stable NPs. DLS and SEM characterizations were done for NPs.

2.4.2 Synthesis and characterization of OFVBt N3 NPs

OFVBt N3 NPs were prepared by reprecipitation method. 0.5 mg (5.89×10^{-4} mmol) of OFVBt N3 was measured from 0.5 g/l (0.600 mM) pre-prepared stock solution, diluted to 2 ml of THF and ultrasonicated for 10 min. The solution was then filtered through 0.45 μM syringe filter, injected into 20 ml of Milli-Q water (18.2 M Ω) and ultrasonicated for 30 min. THF was removed under reduced pressure at 40 °C to obtain stable NPs. DLS and SEM characterizations were done for NPs.

2.4.3 Synthesis and characterization of OFT Pgy – OFVBt N3 NPs

Bioligomer NPs were designed by using OFT Pgy – OFVBt N3 due to the strong spectral overlap between the emission of donor (OFT Pgy) and the absorbance of acceptor (OFVBt N3). In this bioligomer NP design, 0.1 mg (1.25×10^{-4} mmol) of OFT Pgy was measured from 0.5 g/l (0.627 mM) pre-prepared stock solution,

diluted to 1 ml of THF and ultrasonicated for 10 min. And same procedure of dilution and ultrasonication was done for 0.02 mg (2.35×10^{-5} mmol) (20% by weight) of OFVBt N3 from 0.5 g/l (0.600 mM) pre-prepared stock solution, in a separate vial. The solutions were mixed, then filtered through 0.45 μ M syringe filter, injected into 20 ml of Milli-Q water (18.2 M Ω) and ultrasonicated for 30 min. After 30 min of sonication, THF was removed under reduced pressure at 40 °C to obtain stable NPs. Optical characterizations, DLS and SEM characterizations were done for NPs. After the observation of poor energy transfer in optical data, acceptor (OFVBt N3) amount increased and the particles with 45% by weight (OFT Pgy:OFVBt N3 ratio is 5:1) shows the best ET results. To obtain stable NPs, click reaction was facilitated with the addition of catalytic amount of copper sulfate and sodium ascorbate to the mixture while sonicating.

2.4.4 Synthesis and characterization of cross-linked OFVBt N3 NPs

Cross-linked OFVBt N3 NPs were prepared by click reaction with disulfide crosslinker (see section 2.2.10). 5 mg (5.88×10^{-3} mmol) of OFVBt N3 was weighed into a vial. 1.354 mg (5.88×10^{-3} mmol) of disulfide crosslinker was measured and added from 1 g/l (4.340 mM) pre-prepared stock solution into the vial. The ratio of OFVBt N3:disulfide crosslinker is 1:1 and pendant group ratio propargyl:N₃ is 1:2. The vial was filled with THF, making the total volume as 20 ml. After 10 min of ultrasonication, catalytic amount of sodium ascorbate and copper sulfate added and the solution was magnetically stirred at RT for 24 h. DLS and SEM characterizations were done for NPs. THF was removed under reduced pressure at 40 °C and NPs was dispersed in water. DLS measurement was done for samples.

As a second method, sonication was used for crosslinking experiment. 1.25 mg (1.47×10^{-3} mmol) of OFVBt N3 was dissolved in 5 ml of THF. 0.34 mg (1.47×10^{-3} mmol) of disulfide crosslinker was measured from pre-prepared stock solution and ultrasonicated for 10 min. The ratio of OFVBt N3:disulfide crosslinker is 1:1 and pendant group ratio propargyl:N₃ is 1:2. Catalytic amount of copper (I) iodide was added and ultrasonication continued for 2 h. Azide peak decrease was monitored by IR spectroscopy and DLS and SEM characterizations were done.

2.4.5 Synthesis and characterization of OFB Pgy – Porph N3 NPs

OFB Pgy – Porph N3 NPs were designed to obtain white emission by using their optical properties. In this bioligomer NP design, 1.397 mg (1.75×10^{-3} mmol, 40 equivolar) of OFB Pgy (D) was measured from 1.18 mM pre-prepared stock solution in THF, and 0.037 mg (4.43×10^{-5} mmol, 1 equivolar) (2.6% by weight) of Porph N3 (A) was measured from 0.30 mM pre-prepared stock solution in THF. The solution was ultrasonicated for 10 min to mix homogeneously. 0.041 mg (4.41×10^{-5} mmol, 1 equivolar) of copper (I) triphenylphosphine bromide, catalyst, was injected into the mixture while ultrasonication. Sonication was continued for 3 h and azide peak decrease was monitored by IR spectroscopy. Optical characterizations, DLS and SEM characterizations were done for NPs.

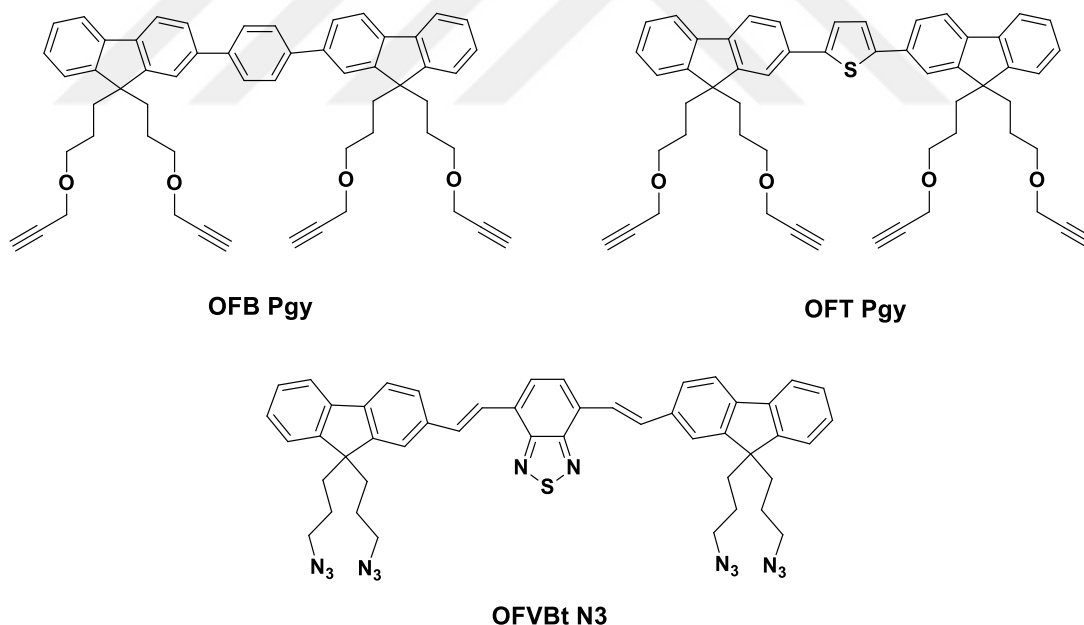
Chapter 3

Results and Discussion

This chapter is divided into two sections. Section one involves synthesis and characterization of conjugated oligomers. In section two, preparation and characterization of conjugated oligomer nanoparticles are discussed.

3.1 Synthesis and Characterization of Conjugated Oligomers

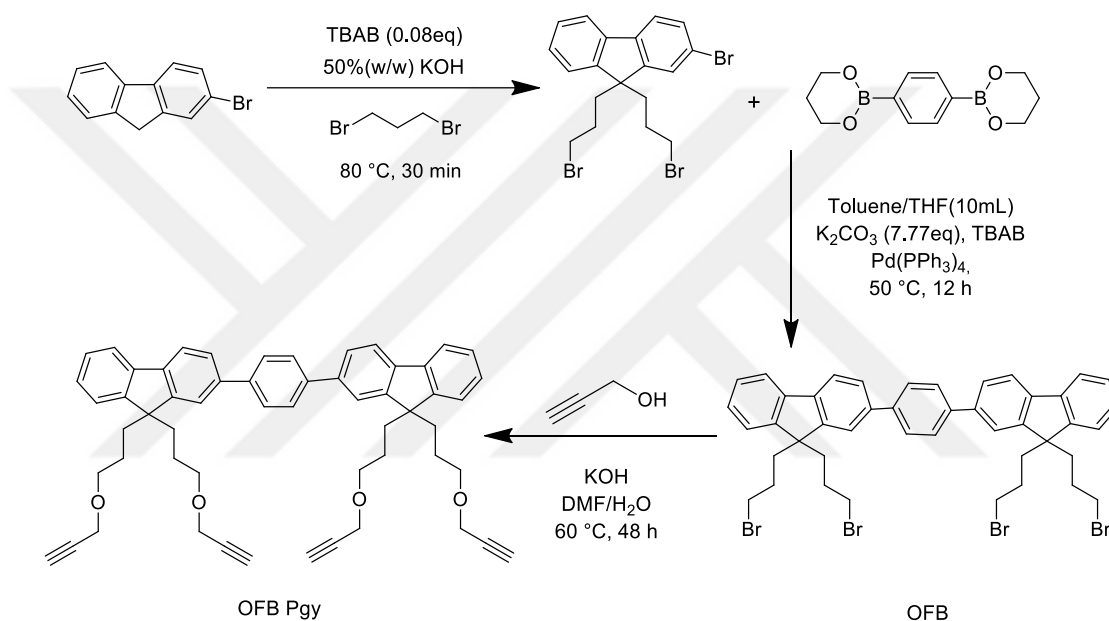
In order to obtain white emitting oligomer nanoparticles, fluorene based conjugated oligomers were synthesized and characterized. Fluorene derived monomers were coupled with benzene, thiophene, and benzothiadiazole in order to obtain variety of conjugation lengths. To form new C-C bonds between the fluorene monomers and aromatic compounds, three different coupling methods were used, namely Suzuki Coupling, Stille Coupling, and Heck Coupling. The optical properties of the oligomers were determined by the conjugation lengths of the monomer used.



Scheme 3.1: Structures of oligomers designed in this study

3.1.1 Synthesis of 1,4-bis(9,9-bis(3-(prop-2-yn-1-yloxy)propyl)-9H-fluoren-2-yl)benzene (OFB Pgy)

For the synthesis of OFB Pgy, M1 was first synthesized from 2-bromofluorene and 1,3-dibromopropane according to Scheme 3.2. The protons at 9th position of fluorene are weakly acidic (pKa: 22.6 in DMSO). Deprotonation gives the stable fluorenyl anion, which is aromatic. The anion is a nucleophile and most electrophiles react with it by adding to the 9th position. Nucleophilic substitution reaction was occurred with 1,3-dibromopropane by the help of strong base for abstraction of acidic protons.



Scheme 3.2: Synthesis mechanism of 1,4-bis(9,9-bis(3-(prop-2-yn-1-yloxy)propyl)-9H-fluoren-2-yl)benzene (OFB Pgy)

The ¹H-NMR spectrum of M1 is shown in Figure 3.1. The triplet peaks at 3.14 ppm belong to the protons near bromine ends of aliphatic chains of M1. Multiplets at 2.17 ppm and 1.16 ppm are due to CH₂ protons in the aliphatic chains. The characteristic aromatic peaks of M1 are shown between 7.37-7.71 ppm.

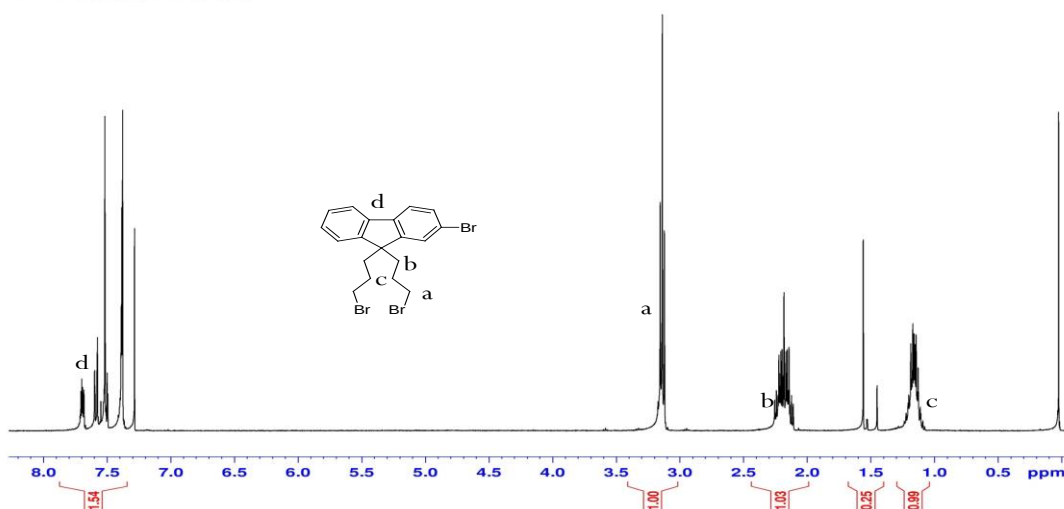


Figure 3.1: ^1H -NMR (400 MHz, CDCl_3 , 25 $^\circ\text{C}$) spectrum of M1

To obtain OFB, M1 and Benzene 1,4-(boronic acid) ethylene glycol ester were coupled through Suzuki cross-coupling reaction in THF and toluene mixture, as shown in Scheme 3.2.

OFB was characterized with ^1H -NMR spectroscopy and the spectrum shown in Figure 3.2. The ^1H -NMR spectrum shows multiplet at 2.19 ppm and triplet 1.17 ppm are due to CH_2 protons in the aliphatic chains. The triplet at 3.14 ppm belongs to the protons that are closest to the bromine ends of aliphatic part. The multiplets between 7.35-7.60 ppm are the characteristic aromatic peaks.

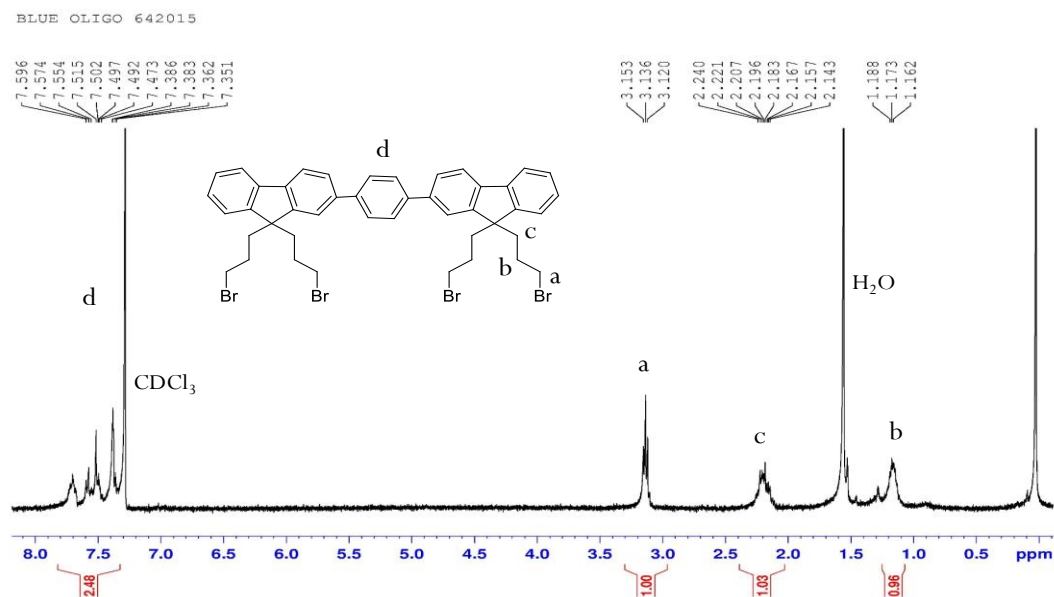


Figure 3.2: ^1H -NMR (400 MHz, CDCl_3 , 25 $^\circ\text{C}$) spectrum of OFB

OFB was further reacted with propargyl alcohol in DMF and H₂O mixture, as shown in Scheme 3.2, in order to obtain propargyl functionalization. After OFB Pgy was obtained, it was characterized with ¹H-NMR spectroscopy as shown in Figure 3.3. Appearance of the peaks labelled as ‘e’ and ‘f’ on the figure at 4.01 ppm and 2.38 ppm respectively are due to propargyl functionality. Downfield shift in proton belongs to the adjacent carbon, ‘a’, is the other expected result and conformation of successful propargylation reaction which is due to deshielding of electronegative oxygen. Other labelled peaks have negligibly small shifts as predicted.

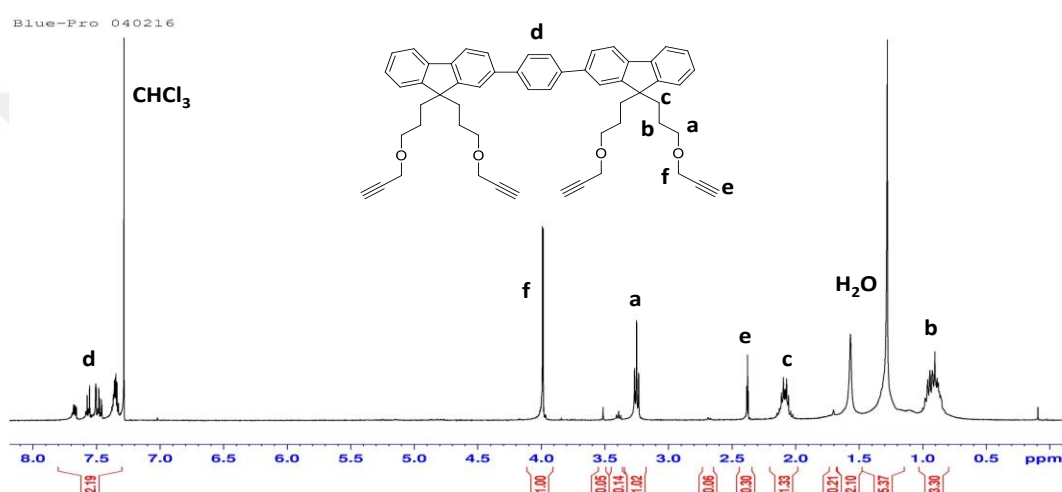


Figure 3.3: ¹H-NMR (400 MHz, CDCl₃, 25 °C) spectrum of OFB Pgy

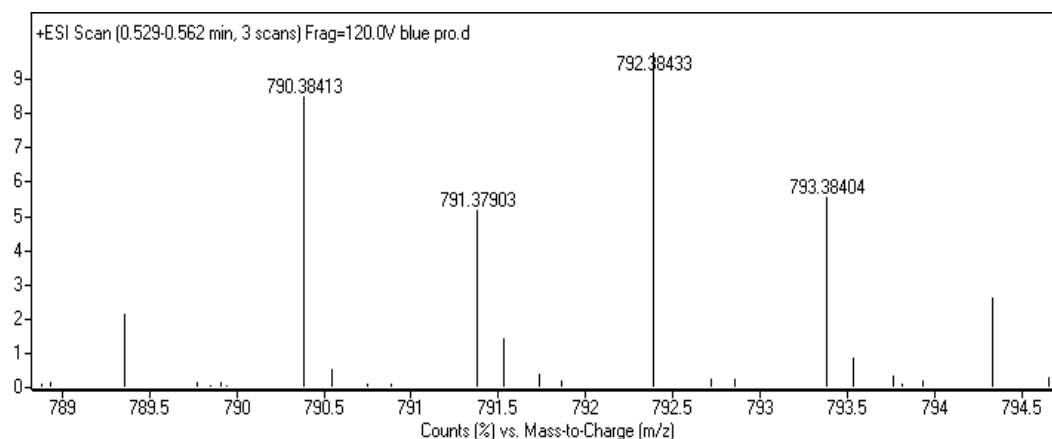


Figure 3.4: LC/MS HRMS-TOF spectrum of OFL Pgy

Figure 3.4 shows the LC/MS HRMS-TOF spectrum of OFL Pgy. The spectrum shows [M+H]⁺ m/z: 790.38413 peak that is matching to theoretical value, 790.40221 g/mol.

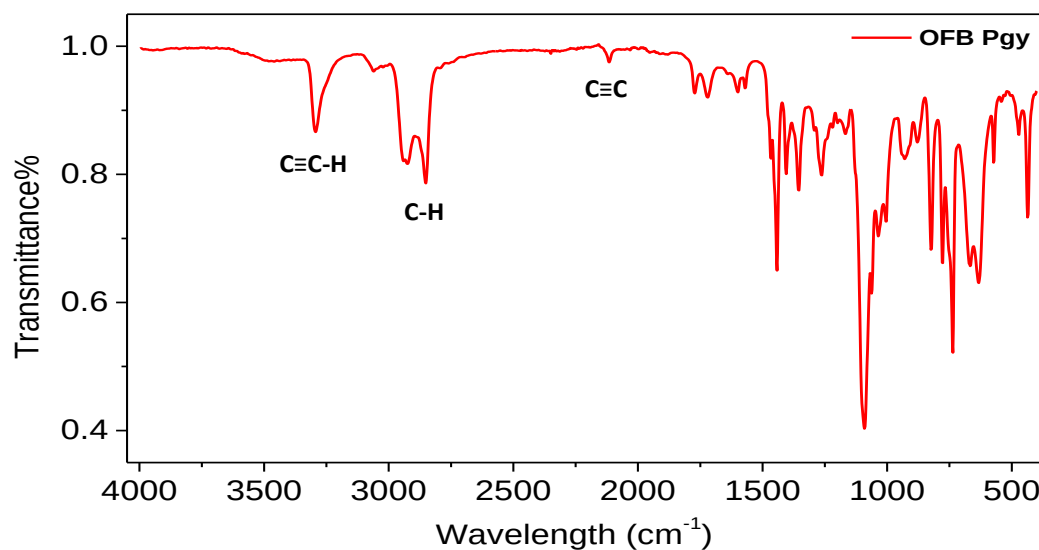


Figure 3.5: FTIR spectrum of OFB Pgy

The IR spectrum, shown in Figure 3.5, indicates characteristic peaks that are conforming OFB Pgy. The peak at 3293 cm^{-1} corresponds to C-H stretching in terminal alkynes. The weak peak at 2114 cm^{-1} is a typical $\text{-C}\equiv\text{C-}$ stretching. The peaks at 2851 cm^{-1} and 1441 cm^{-1} , indicating C-H stretching and bending respectively, belong to the aliphatic chains of OFB Pgy. The aromatic C-H stretchings are given by the peak at 2924 cm^{-1} .

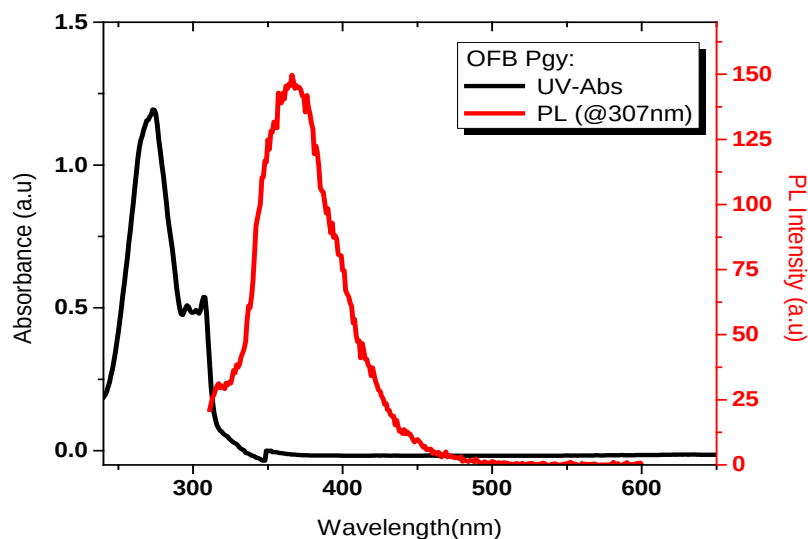


Figure 3.6: UV-Vis and fluorescence spectra of OFB Pgy in THF

OFB Pgy was optically characterized by UV-Vis and photoluminescence spectroscopy in THF, shown in Figure 3.6. Absorption maxima appear at 273 nm and 307 nm while the emission maximum appears at 366 nm.

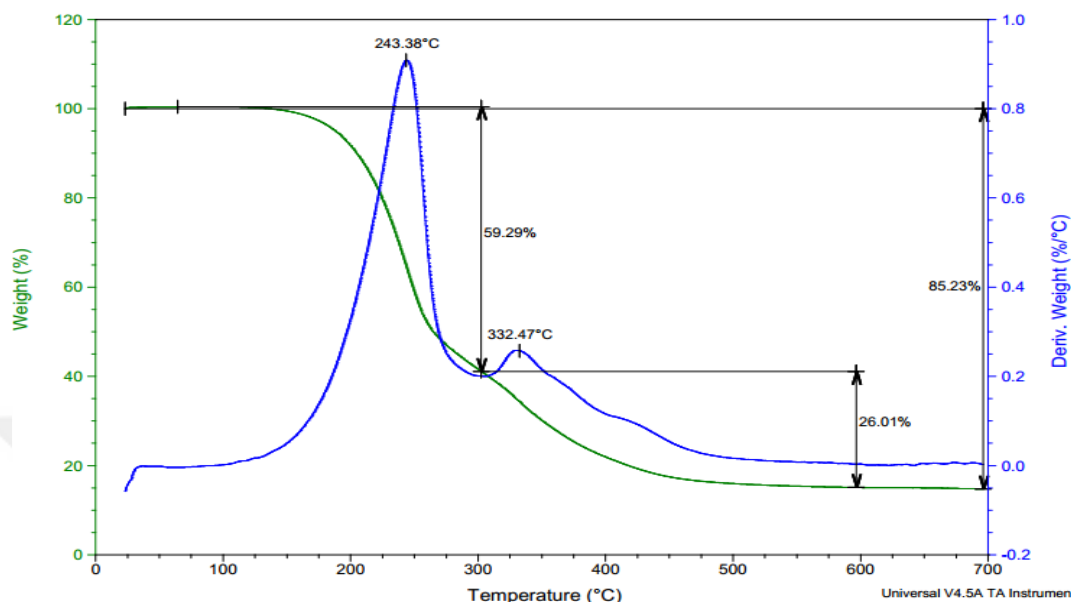
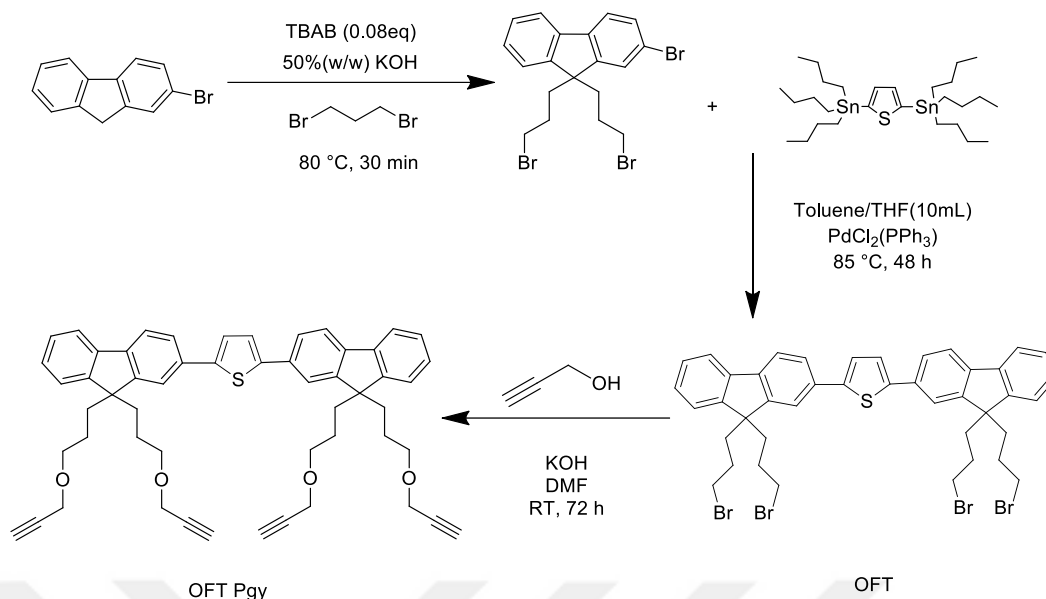


Figure 3.7: Thermogravimetric analysis (TGA) result of OFB Pgy

Figure 3.7 shows the TGA result of OFB Pgy. Green line indicates the raw (measurement) data, while the blue line is the derivation of it. The major weight loss, 59.29%, is attributed to the volatile components (i.e. moisture) and aliphatic chain decompositions. After 332 °C, aromatic backbone of the oligomer was decomposed and ~15% of material left as residual.

3.1.2 Synthesis of 2,5-bis(9,9-bis(3-(prop-2-yn-1-yloxy)propyl)-9H-fluorene-2-yl)thiophene (OFT Pgy)

For the synthesis of OFT Pgy, M1 was first synthesized according to Scheme 3.3 and synthesis and ^1H -NMR spectrum (see Figure 3.1) is discussed in section 3.1.1.



Scheme 3.3: Synthesis mechanism of 2,5-bis(9,9-bis(3-(prop-2-yn-1-yloxy)propyl)-9H-fluoren-2-yl)thiophene (OFT Pgy)

To obtain OFT, M1 and 2,5-bis(tributylstannyl)thiophene were coupled through Stille coupling reaction in THF and toluene mixture, as shown in Scheme 3.3.

OFT was characterized with ^1H -NMR spectroscopy and the spectrum shown in Figure 3.8. The ^1H -NMR spectrum shows multiplet at 1.24 ppm and triplet 2.27 ppm are due to CH_2 protons in the aliphatic chains. The triplet at 3.16 ppm belongs to the protons that are closest to the bromine ends of aliphatic part. The multiplets between 7.35–7.70 ppm are the characteristic aromatic peaks.

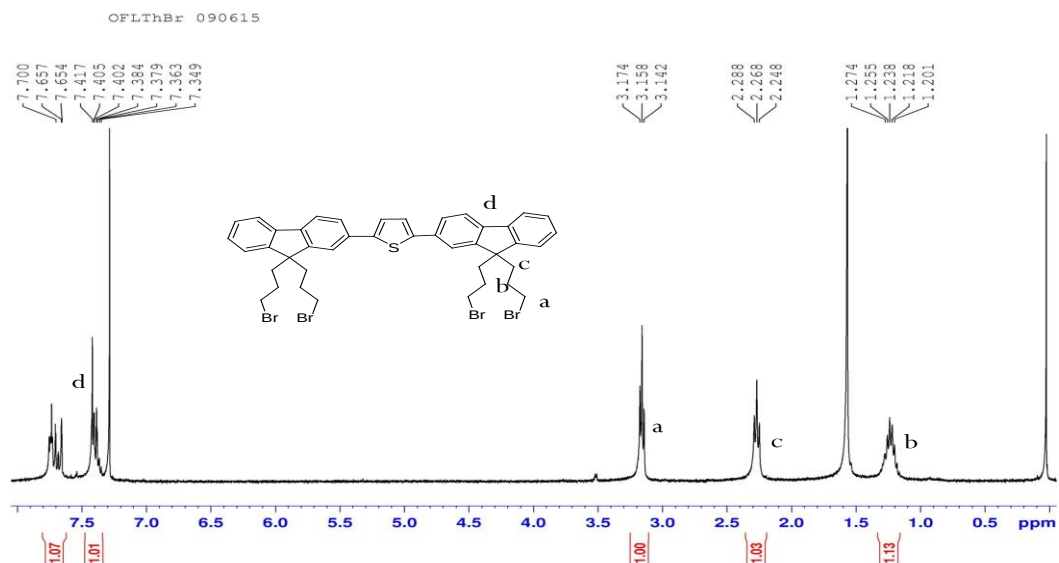


Figure 3.8: ^1H -NMR (400 MHz, CDCl_3 , 25 $^\circ\text{C}$) spectrum of OFT

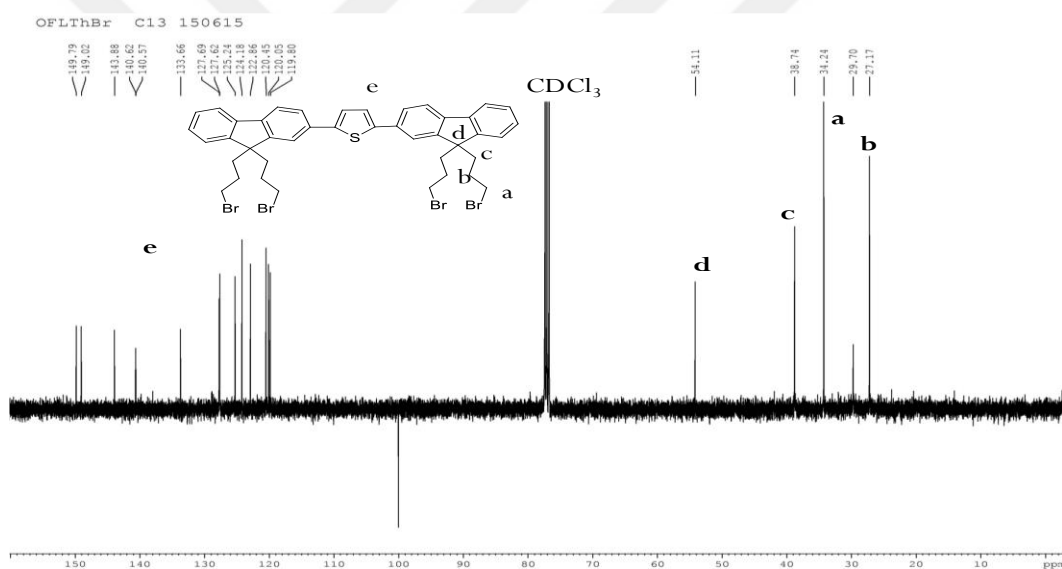


Figure 3.9: ^{13}C -NMR (400 MHz, CDCl_3 , 25 $^\circ\text{C}$) spectrum of OFT

^{13}C -NMR (400 MHz, CDCl_3 , 25 $^\circ\text{C}$) spectrum of OFT is shown in Figure 3.9. Aliphatic chain carbons are labelled as 'a' (at 34.2 ppm), 'b' (at 27.2 ppm), and 'c' (at 38.7 ppm) and they all are as expected. The 9th position carbon in fluorene is, labelled as 'd', at 54.1 ppm. All aromatic carbons signaled from 119.8 ppm to 149.8 ppm.

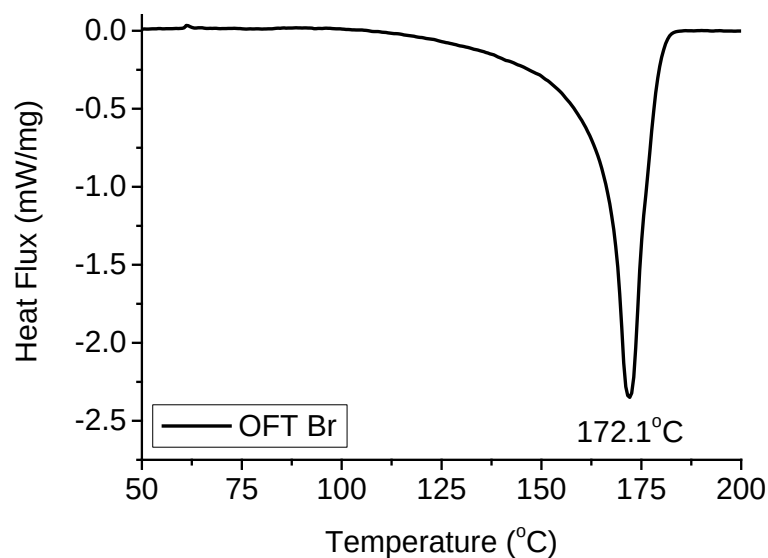


Figure 3.10: Differential Scanning Calorimetry (DSC) result of OFT

DSC characterization was done for OFT in order to determine thermal stability and the result is shown in Figure 3.10. Graph shows only an endothermic peak at 172.1 °C, indicating the melting point of the oligomer.

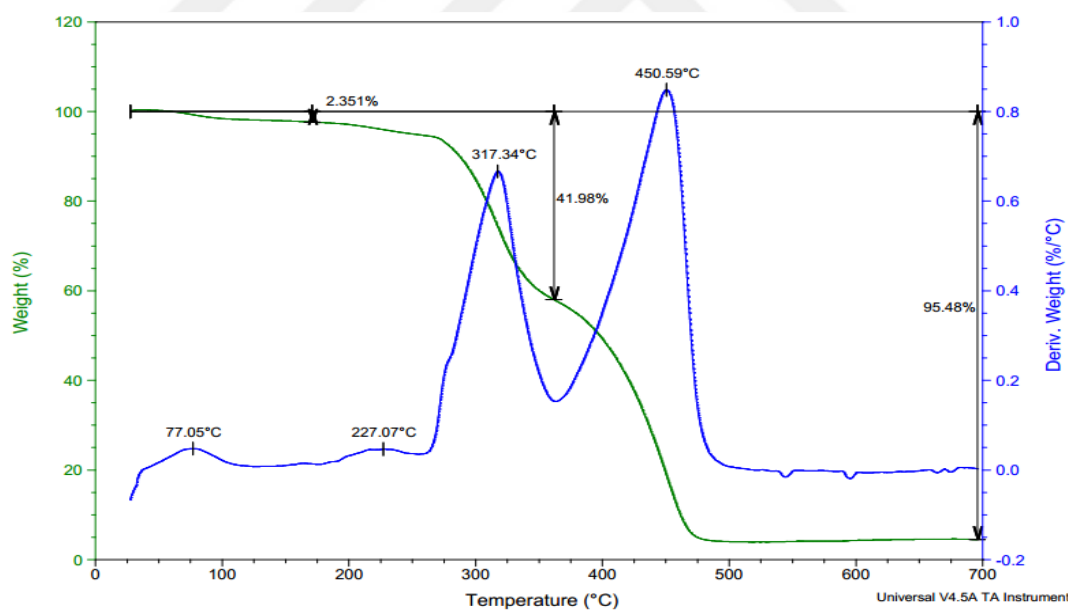


Figure 3.11: TGA result of OFT

Figure 3.11 shows the TGA result of OFT and the green line indicates the raw (measurement) data, while the blue line is the derivation of it. The first weight loss, 2.35%, is attributed to the volatile components i.e. moisture. Second weight loss is

41.98% and attributed to aliphatic chain decompositions. After 450 °C, the oligomer was completely decomposed and ~4.5% of material left as residual.

In order to obtain propargyl functionalization, OFT was further reacted with propargyl alcohol in DMF, as shown in Scheme 3.3. After OFT Pgy was obtained, it was characterized with ^1H -NMR spectroscopy as shown in Figure 3.12. The ^1H -NMR spectrum shows aromatic proton peaks between 7.31-7.74 ppm. The triplet at 2.36 ppm, labelled as 'a' on the figure, is due to the protons in the terminal alkyne. The presence of electronegative oxygen atoms deshields and causes a downfield shift of the peaks labelled as 'b' and 'c' to 4.0 ppm and 3.30 ppm respectively, which are the characteristics of terminal alkyne proton of propargyl group proves the successful propargylation of the oligomer.

Further characterization was done with ^{13}C -NMR (400 MHz, CDCl_3 , 25 °C) for OFT Pgy. In Figure 3.13, aliphatic chain carbons are labelled as 'b', at 24.1 ppm, and 'c', at 36.6 ppm, and the 9th position carbon in fluorene, labelled as 'd', at 54.5 ppm have slight shifts due to pendant group change, as expected. The carbon labelled as 'a' (at 70.0 ppm) has the strongest downfield shift, since it is the adjacent carbon. All aromatic carbons signaled from 119.9 ppm to 150.6 ppm. The peaks labelled as 'f', 'g', and 'h' are belong to the propargyl group which are signaled at 74.0 ppm, 76.4 ppm, and 57.8 ppm respectively.

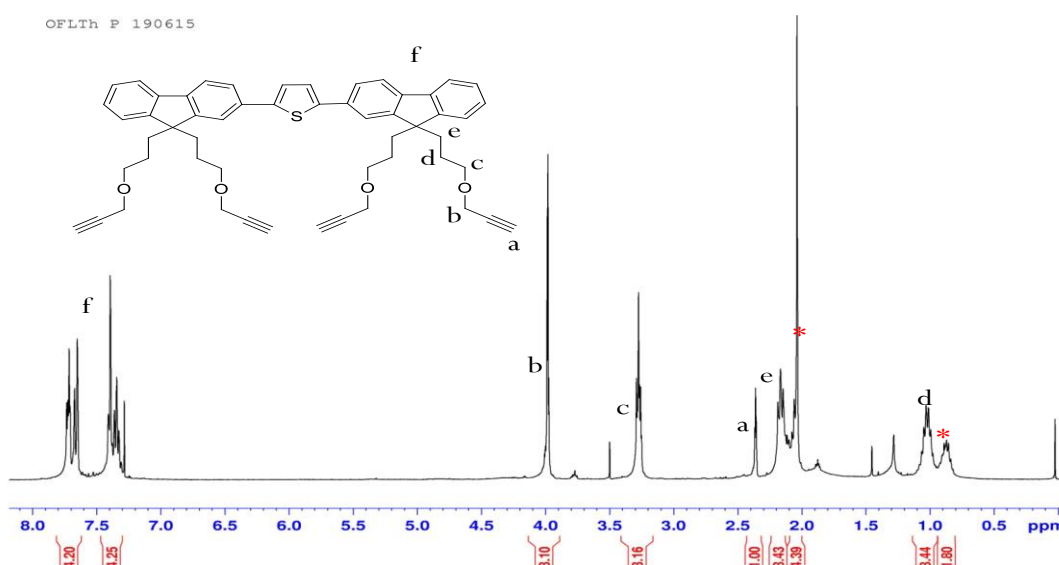


Figure 3.12: ^1H -NMR (400 MHz, CDCl_3 , 25 °C) spectrum of OFT Pgy

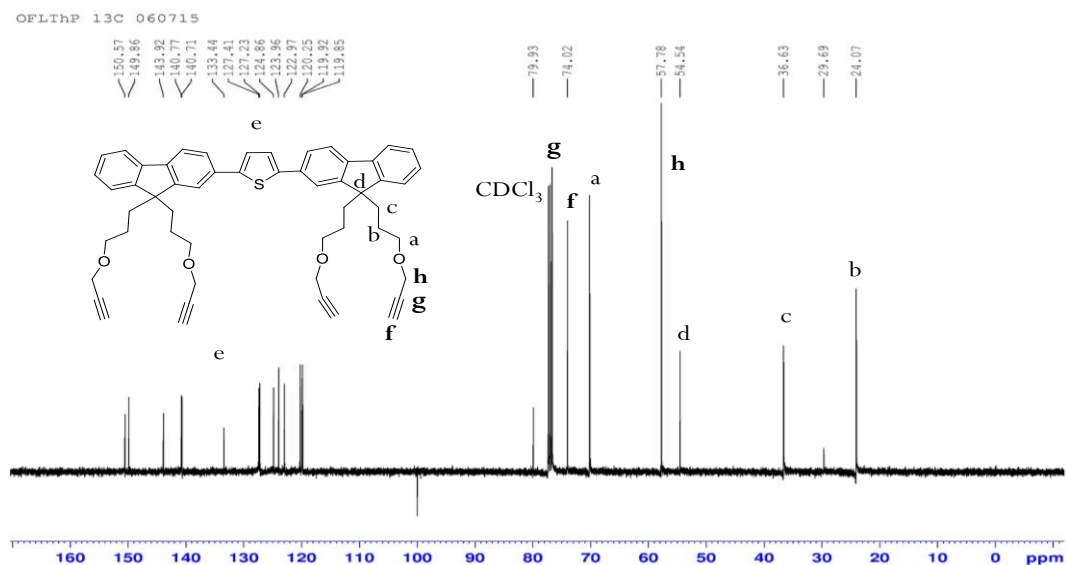


Figure 3.13: ^{13}C -NMR (400 MHz, CDCl_3 , 25 $^\circ\text{C}$) spectrum of OFT Pgy

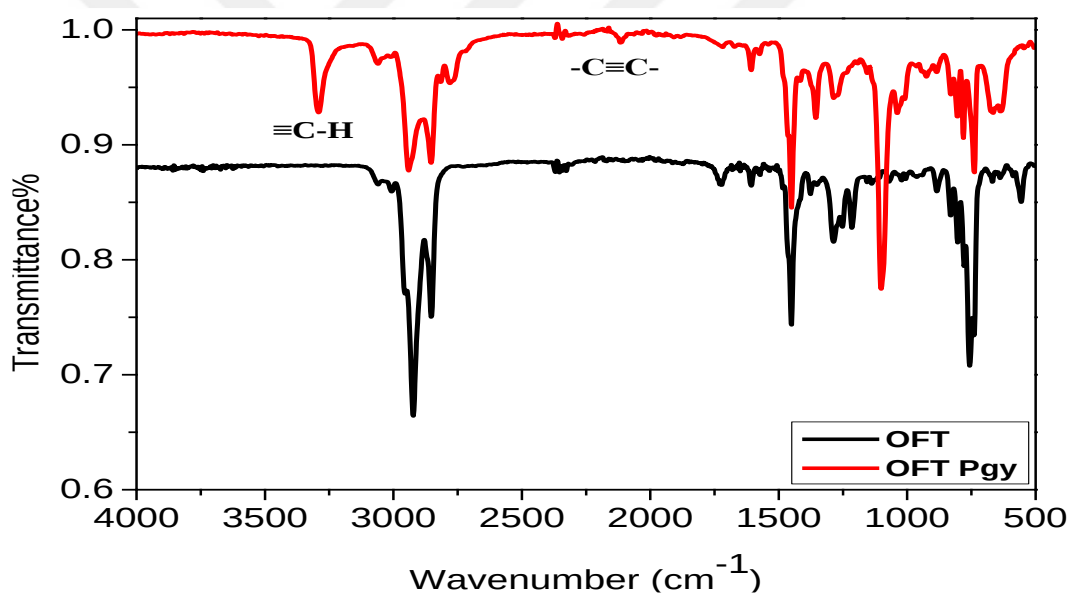


Figure 3.14: FTIR spectra of OFT and OFT Pgy

Further investigation of the structure was done with IR spectroscopy. In Figure 3.14, IR spectrum of OFT Pgy shows two characteristic peaks which are different than OFT spectrum. The signal at 3291 cm^{-1} indicates C-H stretching in terminal alkyne and a weak but more distinct peak at 2116 cm^{-1} is due to $\text{-C}\equiv\text{C-}$ stretching.

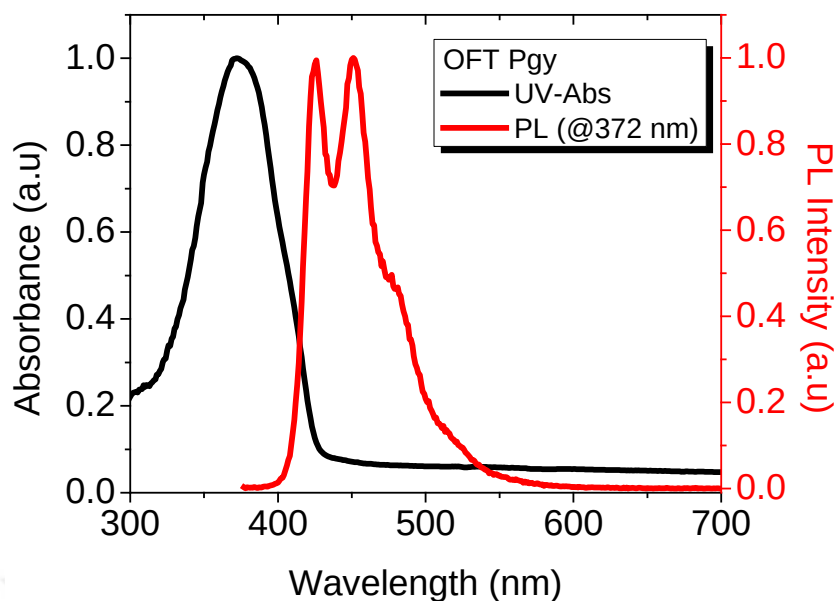
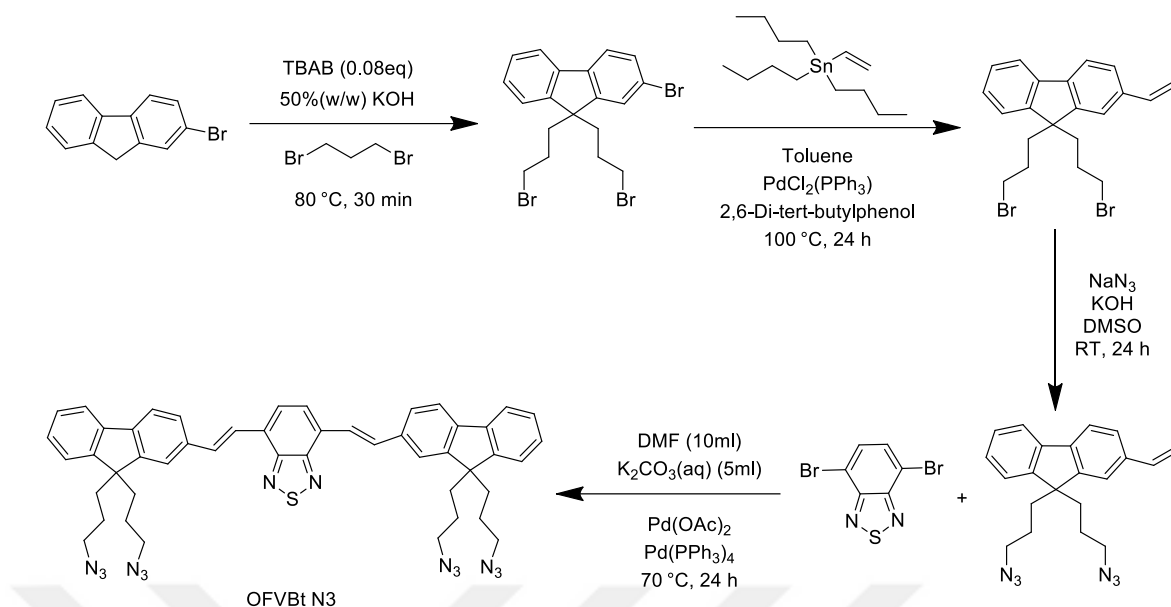


Figure 3.15: UV-Vis and fluorescence spectra of OFT Pgy

The optical properties of OFT Pgy were investigated with UV-Vis and Fluorescence spectroscopy. As shown in Figure 3.15, the absorption maximum appears at 372 nm and the emission maxima appear at 426 nm and 451 nm.

3.1.3 Synthesis of 4,7-bis((E)-2-(9,9-bis(3-azidopropyl)-9H-fluoren-2-yl)vinyl)benzo[c][1,2,5]thiadiazole (OFVBt N3)

For the synthesis of OFVBt N3, M1 was first synthesized according to Scheme 3.4 and synthesis and ^1H -NMR spectrum (see Figure 3.1) is discussed in section 3.1.1.



Scheme 3.4: Synthesis mechanism of 4,7-bis((E)-2-(9,9-bis(3-azidopropyl)-9H-fluoren-2-yl)vinyl)benzo[c][1,2,5]thiadiazole (OFVBt N3)

In order to obtain M2, M1 was reacted with tri-*n*-butyl(vinyl)tin in toluene, as shown in Scheme 3.4. The ¹H-NMR spectrum of M2 (Figure 3.16) shows the triplet peaks at 3.13 ppm belong to the protons near bromine ends of aliphatic chains. Multiplets at 2.21 ppm and 1.19 ppm are due to CH₂ protons in the aliphatic chains. The characteristic aromatic peaks of M2 are shown between 7.34-7.72 ppm. The other three signals, which are labelled as ‘d’, ‘e’, and ‘f’, are due to the vinyl group and proving the successful vinylation reaction. The doublets at 5.31 ppm and 5.84 ppm indicate the two terminal protons of vinyl, while the multiplet at 6.82 ppm showing the proton closest to aromatic part.

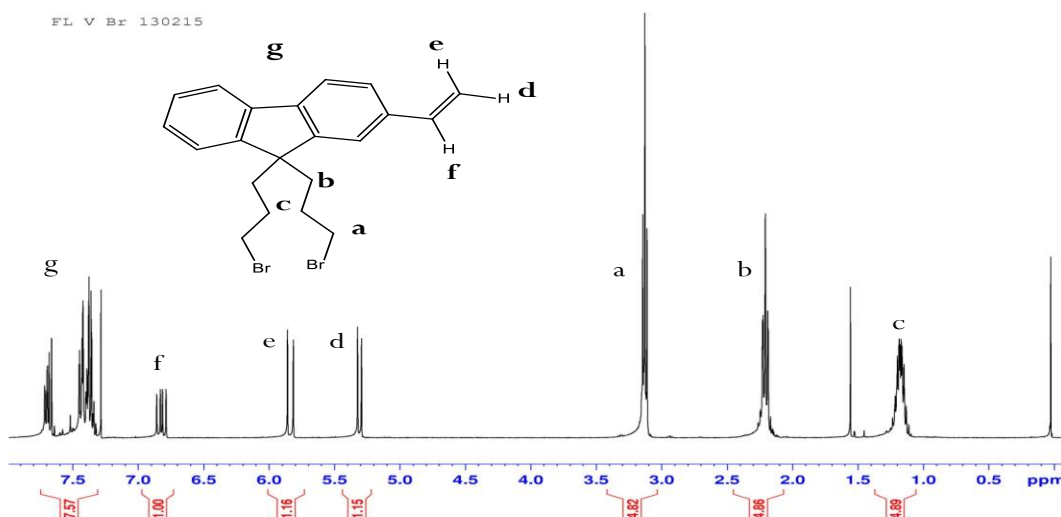


Figure 3.16: ^1H -NMR (400 MHz, CDCl_3 , 25 $^\circ\text{C}$) spectrum of M2

To add the azide functionality (M3), M2 was reacted with NaN_3 in DMSO and Figure 3.17 shows the ^1H -NMR spectrum of M3. The spectrum shows the triplet peaks at 3.00 ppm, labelled as 'a', which belong to the protons near azide ends of aliphatic chains, and multiplets at 2.13 ppm and 0.93 ppm, labelled as 'b' and 'c', which are due to CH_2 protons in the aliphatic chains. The aliphatic protons are shielded by azide group, which increases the electron density by electron donating, and so causes upfield shift in signals of these protons as compared to M2 spectrum, suggesting a successful azidation reaction. The characteristic aromatic peaks of M3 are shown between 7.35-7.73 ppm and multiplet at 6.82 ppm, doublets 5.83 ppm and 5.30 ppm are due to vinyl peaks, which all has no shift as expected.

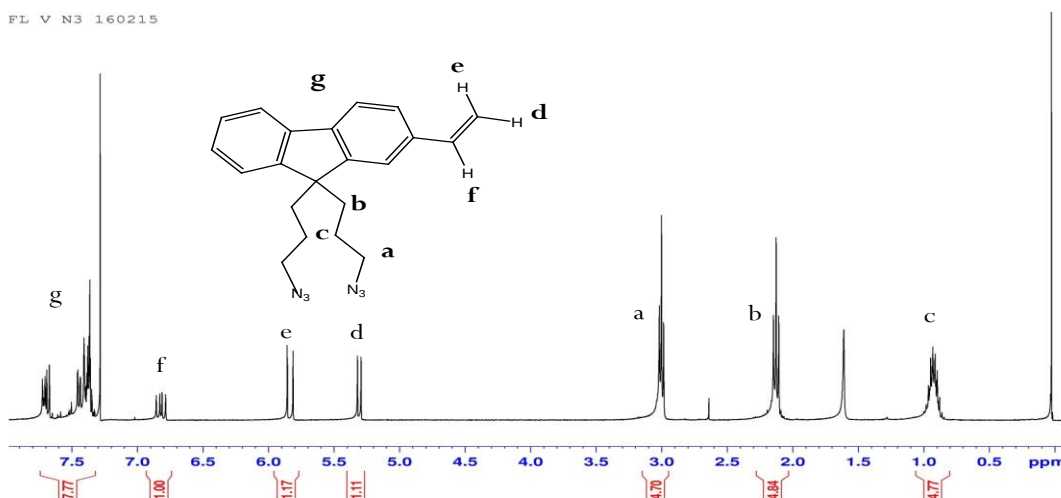


Figure 3.17: ^1H -NMR (400 MHz, CDCl_3 , 25 $^\circ\text{C}$) spectrum of M3

Further investigation for M3 was done with IR spectroscopy. In Figure 3.18, IR spectrum of M3 shows a characteristic sharp peak at 2096 cm^{-1} which is due to N_3 stretching. IR spectrum of M2 is also provided in same figure to compare presence of N_3 signal.

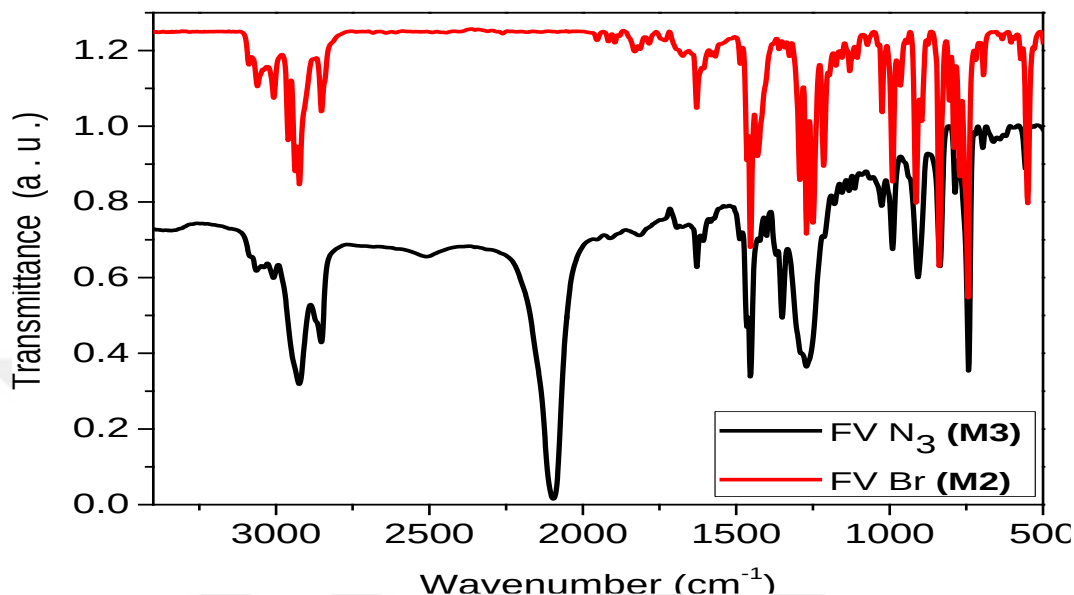


Figure 3.18: FTIR spectra of M2 and M3

To obtain OFVBt N3, M3 and 4,7-dibromobenzo[c][1,2,5]thiadiazole were coupled through Heck coupling reaction in DMF, as shown in Scheme 3.4.

OFVBt N3 was characterized with ^1H -NMR spectroscopy and the spectrum shown in Figure 3.19. The ^1H -NMR spectrum shows triplet at 3.02 ppm and multiplets at 0.94 ppm and 2.11 ppm belong to the aliphatic protons, which all have negligible shifts as expected compared to M3 spectrum. The multiplets between 7.38-7.88 ppm are the aromatic peaks. The disappearance of the three vinyl originated signals, which were between 5-7.00 ppm, is the proof of a successful coupling reaction. The vinyl proton signals of OFVBt N3 are downfield shifted to above 7.00 ppm and non-distinguishable among the aromatic peaks, integration increase in aromatic peaks is a clear indication.

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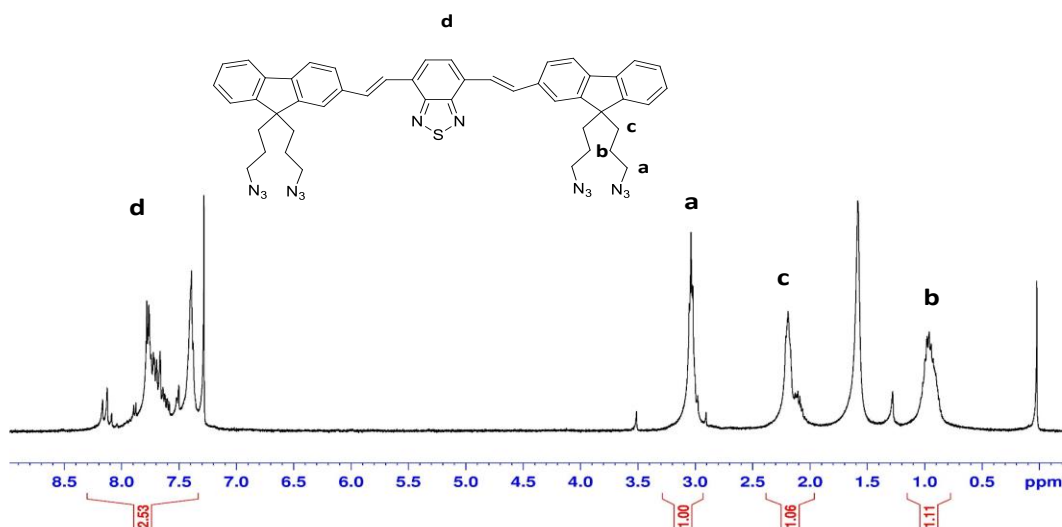


Figure 3.19: ^1H -NMR (400 MHz, CDCl_3 , 25 $^\circ\text{C}$) spectrum of OFVBt N3

The LC/MS HRMS-TOF spectrum of OFVBt N3 is shown in Figure 3.20. The spectrum shows $[\text{M}+\text{H}]^+$ m/z : 848.35698 peak and the theoretical mass trace of OFVBt N3 is perfectly matching with value of 848.35941 g/mol.

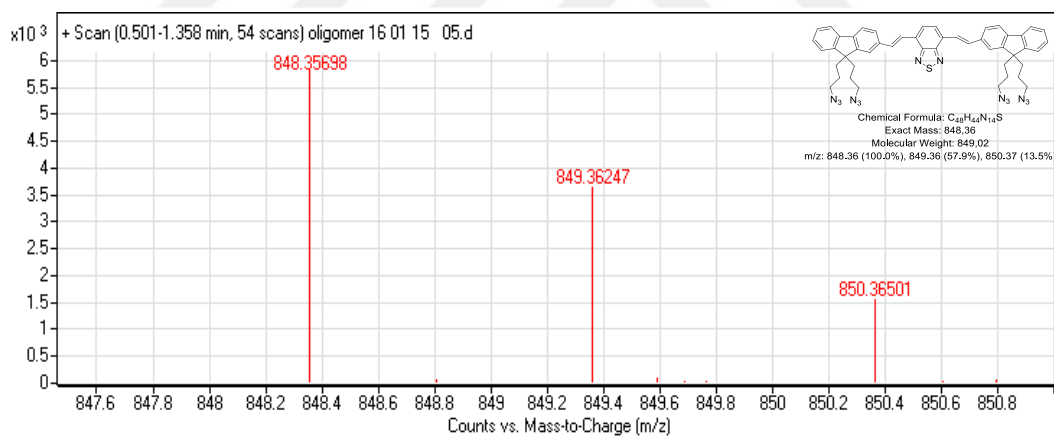


Figure 3.20: LC/MS HRMS-TOF spectrum of OFVBt N3

IR investigation of OFVBt N3 shows a sharp azide stretching peak at 2096 cm^{-1} in Figure 3.21. Characteristic azide peak has an increase when compared to IR spectrum of M3 (see Figure 3.18) due to doubled azide functionality in the oligomer.

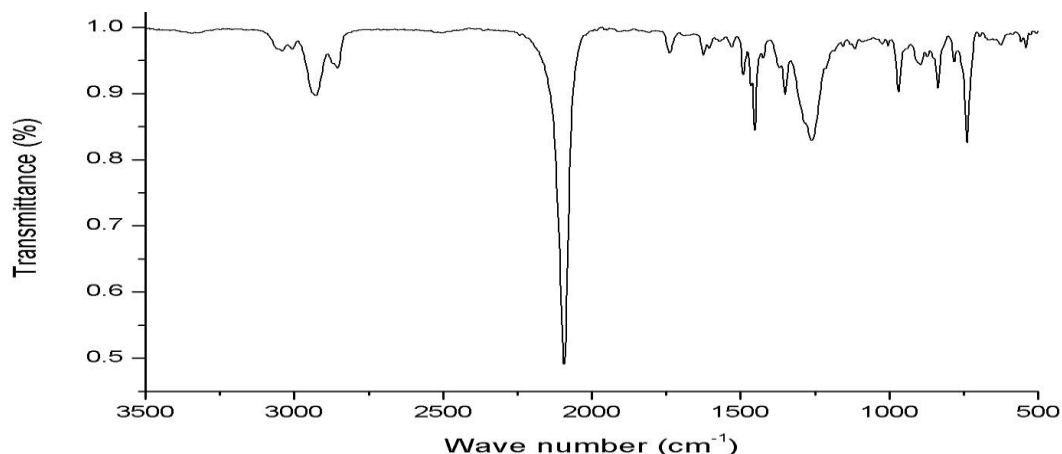


Figure 3.21: FTIR spectrum of OFVBt N3

The optical properties of OFVBt N3 were investigated with UV-Vis and Fluorescence spectroscopy. As shown in Figure 3.22, the absorption maxima appear at 349 nm and 480 nm, and the emission maximum appears at 588 nm. Two absorption bands are attributed to two different monomer derivatives in the backbone, which are fluorene and benzothiadiazole.

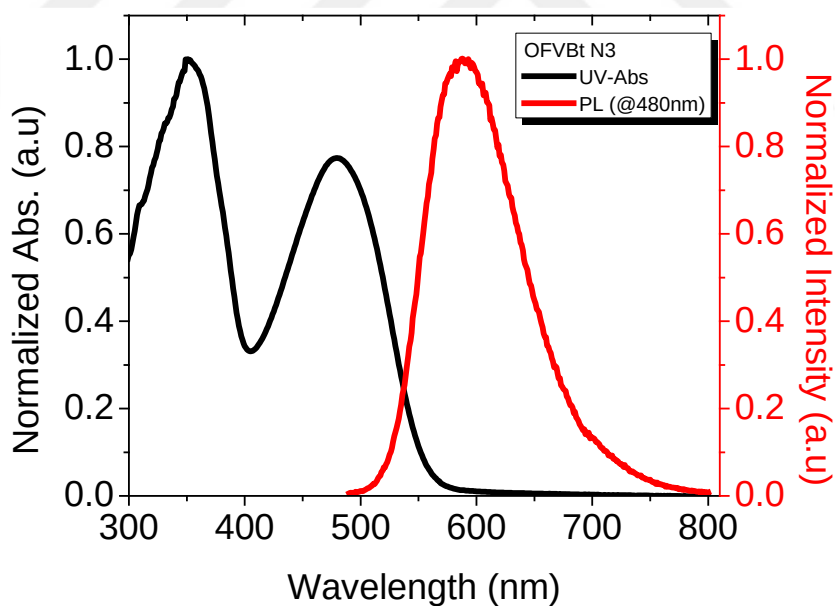


Figure 3.22: UV-Vis and fluorescence spectra of OFVBt N3

DSC result of OFVBt N3, shown in Figure 3.23, shows only an endothermic peak. The peak indicates the melting point of the oligomer that is 101.5°C.

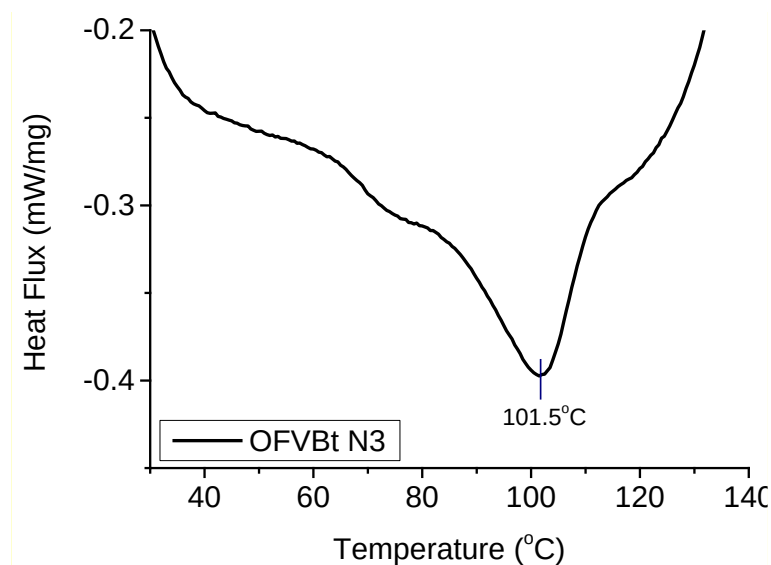


Figure 3.23: DSC result of OFVBt N3

Figure 3.24 shows the TGA result of OFVBt N3. Green line indicates the raw (measurement) data, while the blue line is the derivation of it. The first weight loss, almost 5% decrease, is attributed to the volatile components i.e. moisture. 12.77% weight loss is attributed to azide decomposition after 255 °C. Final weight loss is 38.37% and attributed to aliphatic chain decompositions. Residual ~60% of weight is non-decomposed aromatic part of the oligomer.

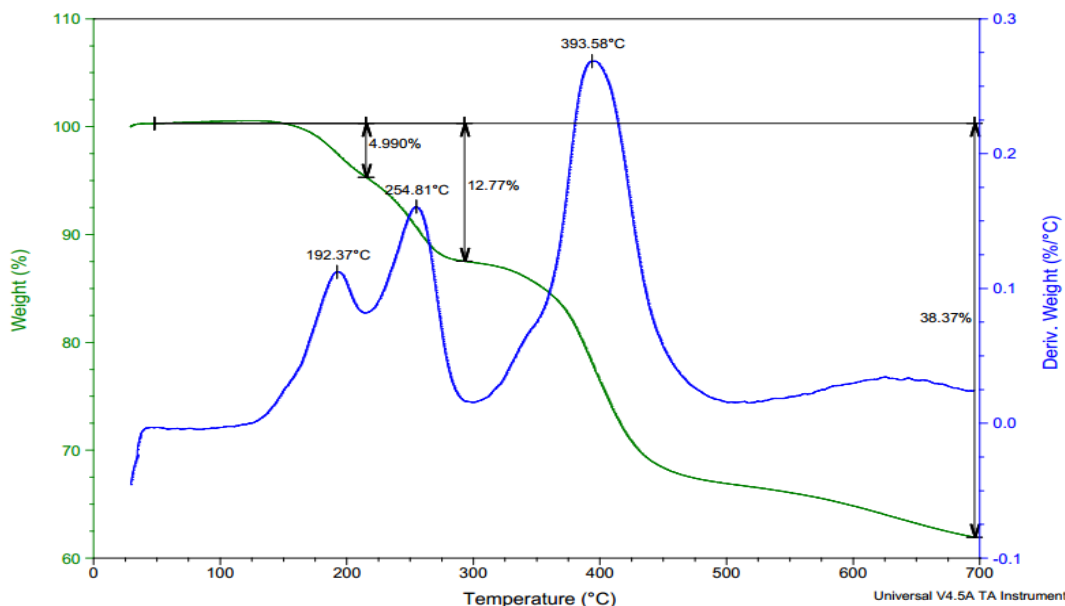
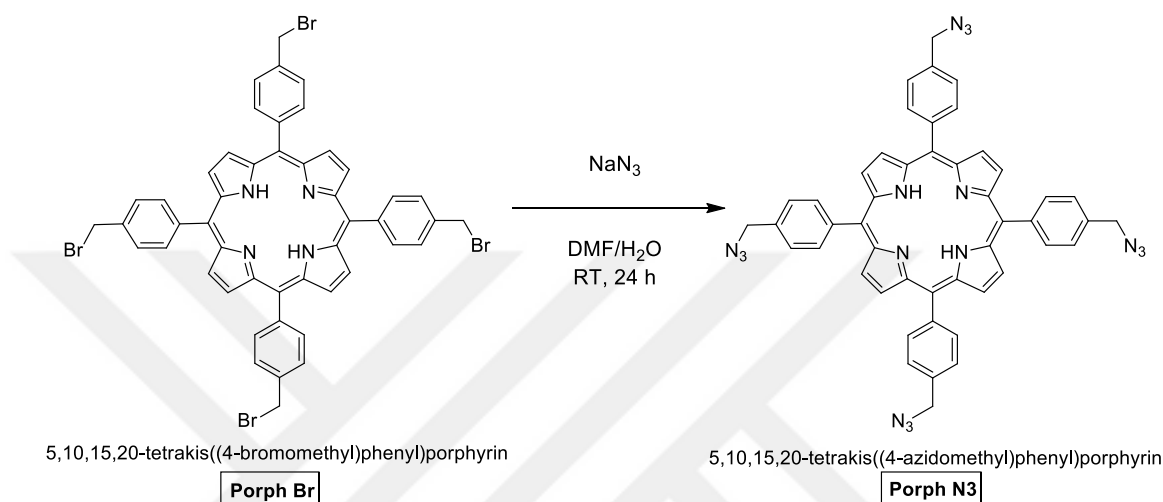


Figure 3.24: TGA result of OFVBt N3

3.1.4 Synthesis of 5,10,15,20-Tetrakis((4-azidomethyl)phenyl)porphyrin (Porph N3)

5,10,15,20-tetrakis((4-bromomethyl)phenyl)porphyrin (Porph-Br) was synthesized and well characterized by a previous group member, M. Idris. In order to obtain azide functionality, Porph-Br was reacted with NaN_3 in DMF, as seen in Scheme 3.5.



Scheme 3.5: Synthesis mechanism of 5,10,15,20-tetrakis((4-azidomethyl)phenyl)porphyrin (Porph N3)

Porph Br was characterized with ^1H -NMR spectroscopy and the spectrum shown in Figure 3.25. The ^1H -NMR spectrum shows four specific peak and all are labelled on structure in figure. The peak at 4.88 ppm belong to the protons adjacent to bromine terminals. Other three signals at 8.87 ppm, 8.21 ppm, and 7.82 ppm are attributed to conjugated aromatic protons.

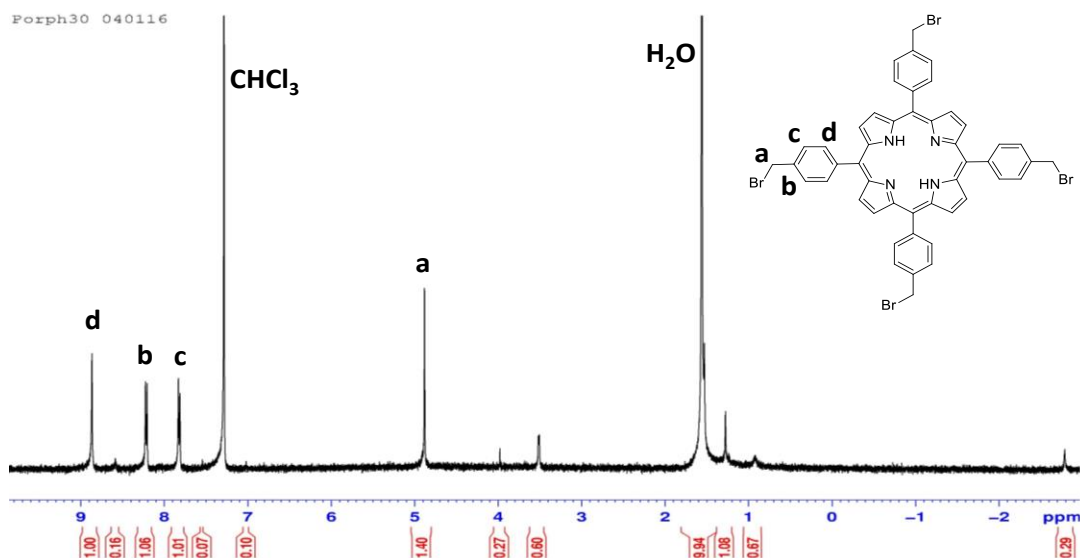


Figure 3.25: ^1H -NMR (400 MHz, CDCl_3 , 25 $^\circ\text{C}$) spectrum of Porph Br

Figure 3.26 shows the ^1H -NMR spectrum of Porph N3 and four specific proton signal is labelled as shown inset of the figure. The singlet peak at 4.75 ppm, labelled as 'a', belongs to the protons adjacent to azide functionality and it shows a upfield shift due to shielding by azide group, which is electron donating group compared to bromine. The upfield shift suggests successful azidation reaction. The other three peaks at 8.85 ppm, 8.26 ppm, and 7.74 ppm have negligible shifts compared to Porph Br spectrum.

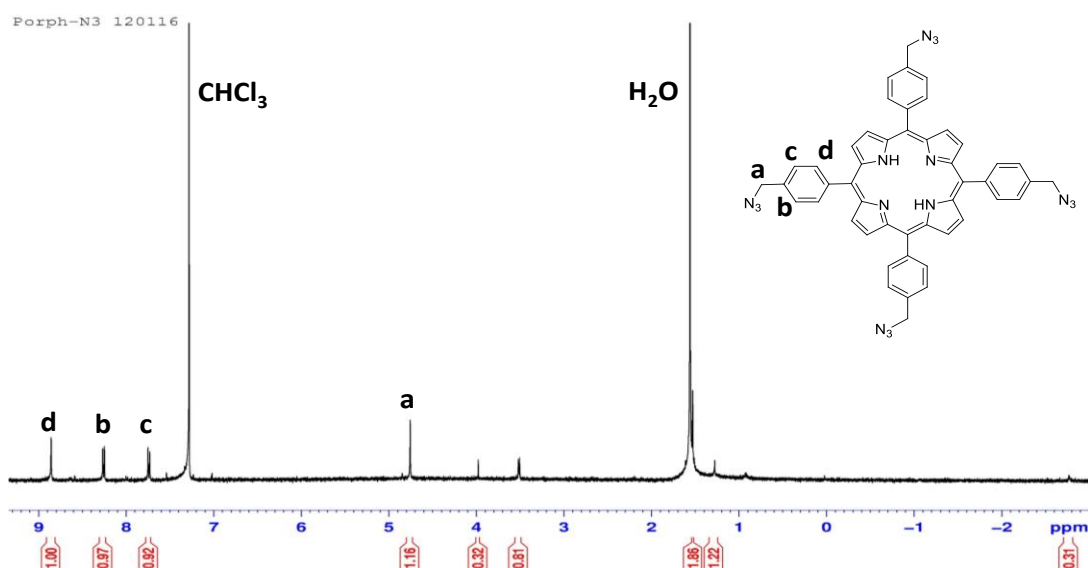


Figure 3.26: ^1H -NMR (400 MHz, CDCl_3 , 25 $^\circ\text{C}$) spectrum of Porph N3

The LC/MS HRMS-TOF spectrum of Porph N3, shown in Figure 3.27, shows $[M+H]^+$ m/z : 835.32637 peak with a great accuracy, the theoretical mass being 834.31524 g/mol.

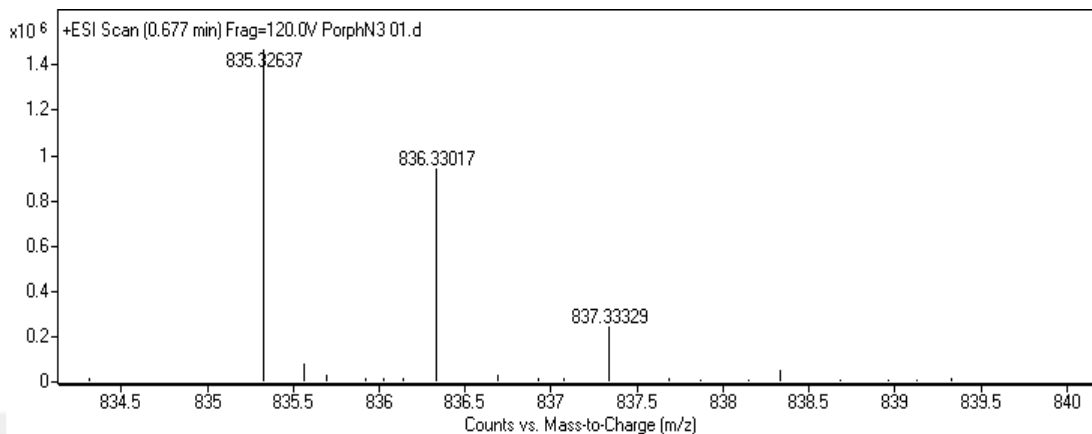


Figure 3.27: LC/MS HRMS-TOF spectrum of Porph N3

Further investigation was done with IR spectroscopy for Porph N3. In Figure 3.28, IR spectrum shows a characteristic sharp peak at 2098 cm^{-1} , indicating N_3 stretching. Sharp azide signal is another proof of successful azidation.

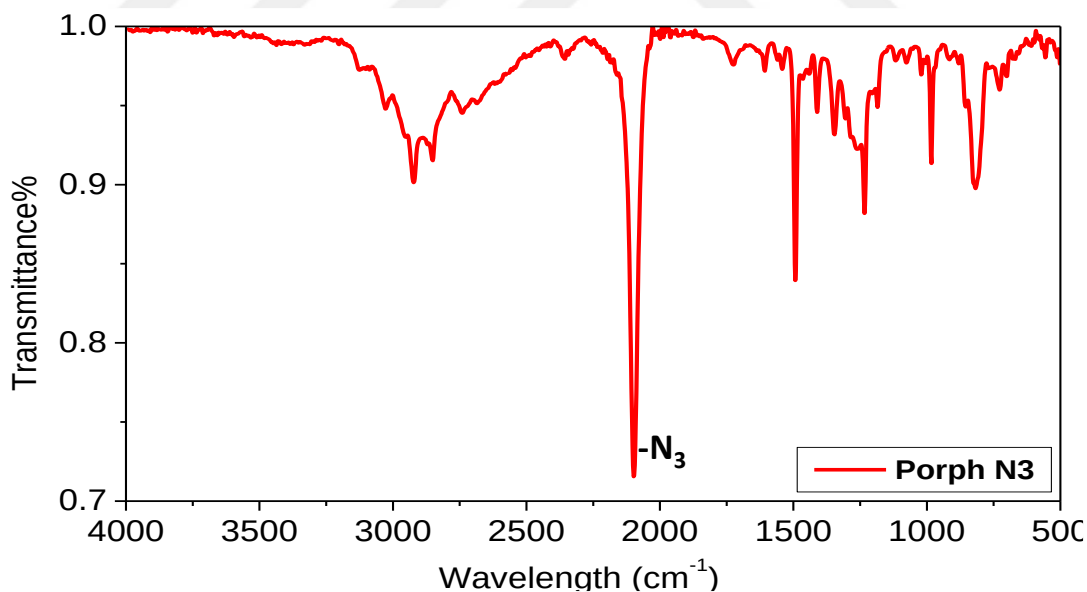


Figure 3.28: FTIR spectrum of Porph N3

3.2 Synthesis and Characterization of Conjugated Oligomer Nanoparticles

3.2.1 Synthesis and Characterization of Water Dispersible Conjugated Oligomer Nanoparticles

After preparation of oligomers with necessary functional groups, next step was the synthesis of water dispersible conjugated oligomer nanoparticles. Nanoparticles were prepared by the reprecipitation method as described in Figure 1.10 and discussed in section 1.3.2. The oligomers were dissolved in THF and injected into deionized water while sonicating. The rapid injection of oligomers into the water drives the formation of nanoparticles by hydrophobic effect. Since the oligomer backbone is hydrophobic, the oligomers tend to avoid from water and come together, forming spherical nanoparticles. Rotary evaporator was used to remove THF in order to prevent dissolution of NPs, thus stabilization achieved.

For the preparation of bioligomer NPs, same reprecipitation method and so hydrophobic effect was used with an additional click reaction. The click between propargyl groups of donor and azide groups of acceptor is facilitated by the addition of catalytic amounts of sodium ascorbate and copper sulfate in water. Control experiment was done without catalyst addition. The click reaction provides shape and size stability to bioligomer NPs as it forms stable triazole rings between oligomers. Triazole formation also ensures that the energy transfer (ET) between donor and acceptor is induced by bringing them close proximity.

Synthesis and characterization of OFT Pgy NPs

OFT Pgy NPs (D) were prepared by taking 0.1 mg of OFT Pgy from the stock solution, diluting it in 2 ml of THF and ultrasonicated for 10 min. Then the solution was injected into 20 ml of deionized water (18.2 M Ω) and ultrasonicated for 30 min. THF was removed to obtain stable NPs. The size of the NPs were determined by DLS as 71 nm, shown in Figure 3.29. The morphology of the NPs was determined by SEM as shown in Figure 3.30. SEM image reveals spherical nanoparticles with uniform distribution and no aggregation.

Z-Average (d.nm): 110,8
Pdl: 0,183
Intercept: 0,960
Result quality : Good

	Size (d.nm):	% Number:	St Dev (d.nm):
Peak 1:	71,11	100,0	21,41
Peak 2:	0,000	0,0	0,000
Peak 3:	0,000	0,0	0,000

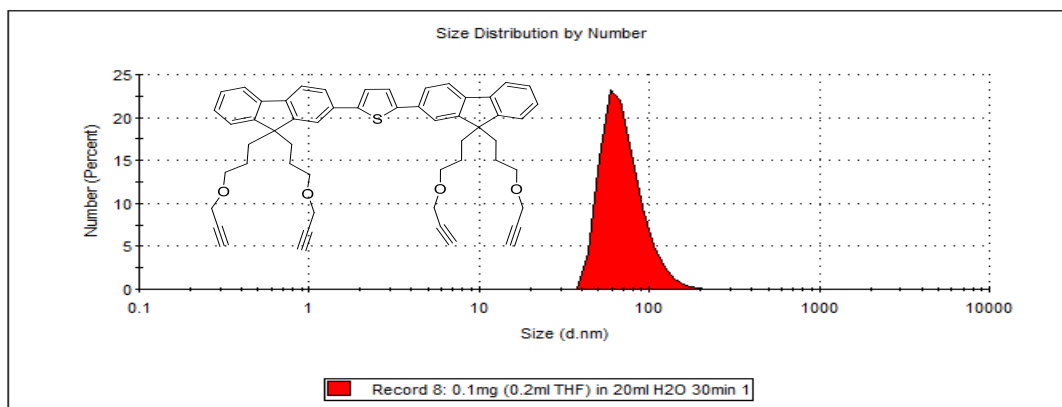


Figure 3.29: DLS result of OFT Pgy NPs, size distribution by number

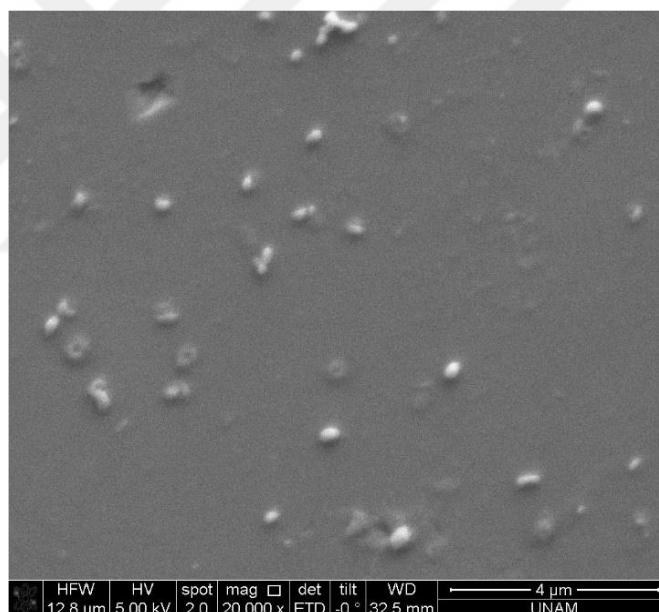


Figure 3.30: SEM image of OFT Pgy NPs

Synthesis and characterization of OFVBt N3 NPs

OFVBt N3 NPs (A) were prepared by taking 0.01 mg of OFVBt N3 from the stock solution, diluting it in 2 ml of THF and ultrasonicated for 10 min. Then the solution was injected into 20 ml of deionized water (18.2 M Ω) and ultrasonicated for 30 min. THF was removed to obtain stable NPs. The size of the NPs were determined by DLS as 91 nm, shown in Figure 3.31. The morphology of the NPs was

determined by SEM as shown in Figure 3.32. SEM image reveals spherical nanoparticles with uniform distribution and no aggregation.

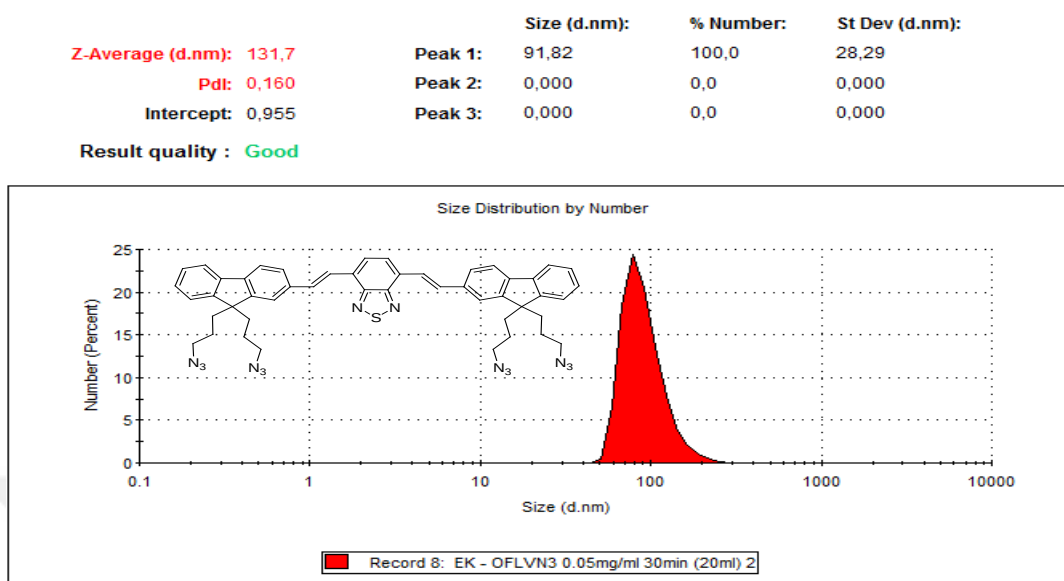


Figure 3.31: DLS result of OFVBt N3 NPs, size distribution by number

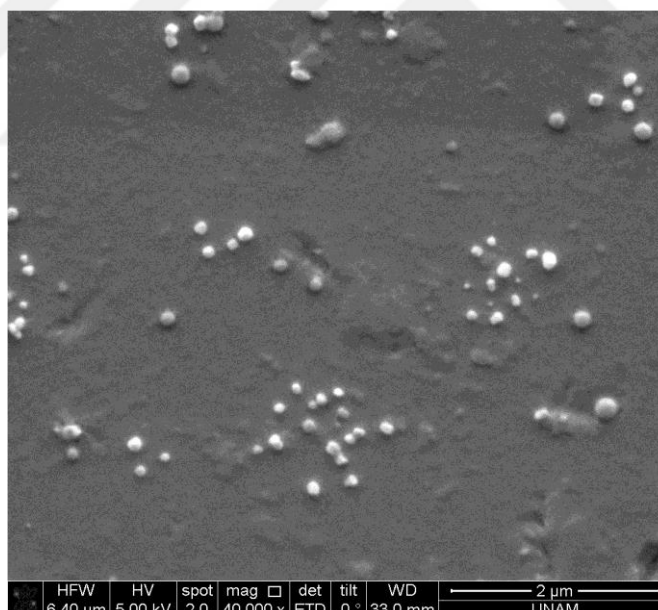


Figure 3.32: SEM image of OFVBt N3 NPs

Synthesis and characterization of OFT Pgy - OFVBt N3 NPs

Biologomer NPs were designed by using OFT Pgy - OFVBt N3 due to the strong spectral overlap. Spectral overlap between the emission of donor fluorophore and the absorbance of acceptor fluorophore is an essential requirement for fluorescence

energy transfer (FRET). FRET is fundamental to various potential applications in optoelectronic devices and biomedical sciences. Figure 3.33 shows the spectral overlap of OFT Pgy (D) and OFVBt N3 (A). The donor emission at 426 nm and 451 nm overlaps with broad absorbance band of acceptor at 477 nm. The strong spectral overlap indicates that FRET phenomenon is highly possible between donor and acceptor when conditions are met.

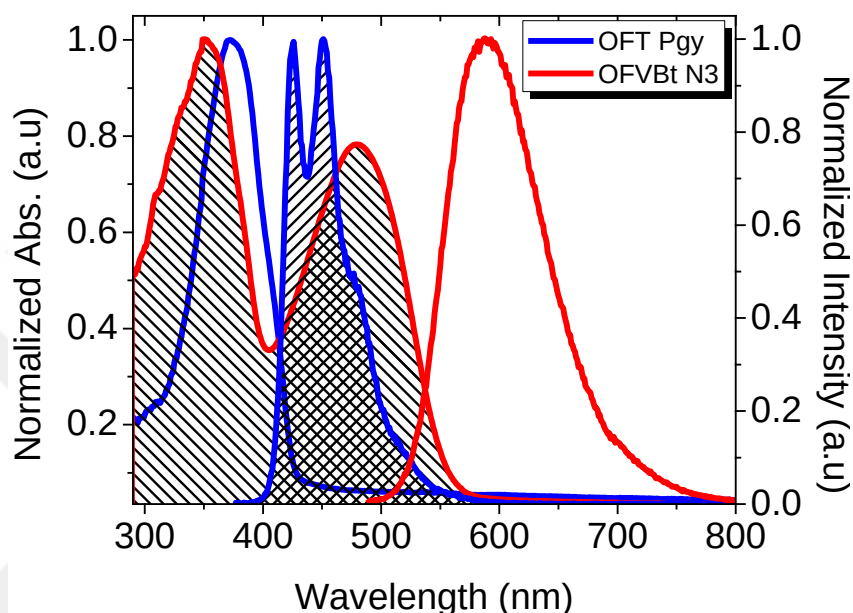


Figure 3.33: Spectral overlap between donor (OFT Pgy) and acceptor (OFVBt N3)

In this bioligomer NP design, 0.1 mg of OFT Pgy (D) and 0.02 mg of OFVBt N3 (A) (20% by weight) were measured from the stock solution, diluted in 1 ml of THF and ultrasonicated for 10 min in separate vials. Then the solutions were mixed and the mixture was injected into 20 ml of deionized water (18.2 M Ω) and ultrasonicated for 30 min. Then THF was removed to obtain stable NPs. Figure 3.34 shows the fluorescence spectroscopy results of the mixture (red line) and OFT Pgy NPs (black line) excited at 372 nm. Red line shows a decrease in emission peak of green (at 423 nm and 448 nm) compared to black line. Addition of acceptor quenches the emission of donor to some extent by ET. The red line also shows a hump in the region of emission peak of red (at 580 nm) which is due to the ET. Blue line indicates the emission from only red when mixture excited with 480 nm, where there is no ET. To sum up, the hump in red line indicates ET in mix and it is lower

than the peak in blue line, implying that not all acceptor fluorophore excited by ET due to low amount of acceptor and far proximity of donor-acceptor pairs.

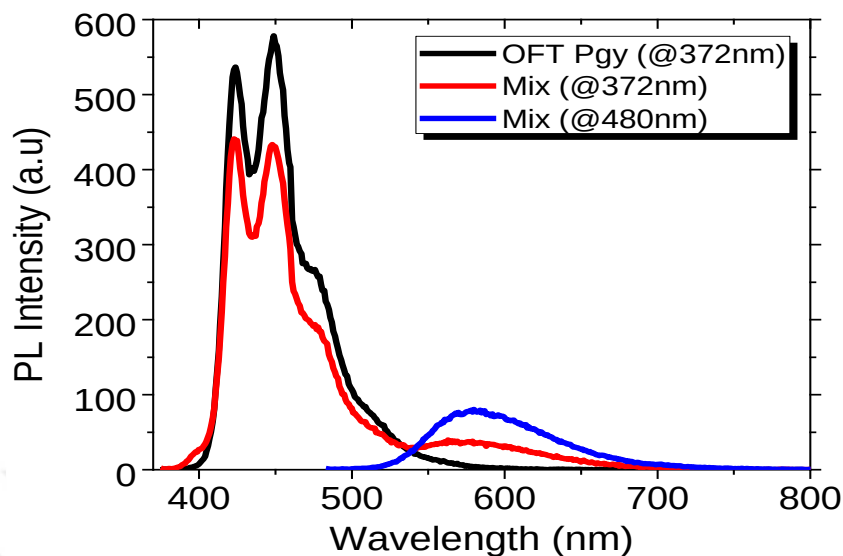


Figure 3.34: Photoluminescence spectra of OFT Pgy NPs (black) and Mix NPs (red and blue).

The 20% mix of donor and acceptor was dried under reduced pressure to obtain solid NPs, then was redispersed in THF. Figure 3.35 shows the 20% oligomer mix in both THF and Water to clarify that no ET in THF due to dissolution of NPs and so far proximity of oligomers when polar solvent is presented.

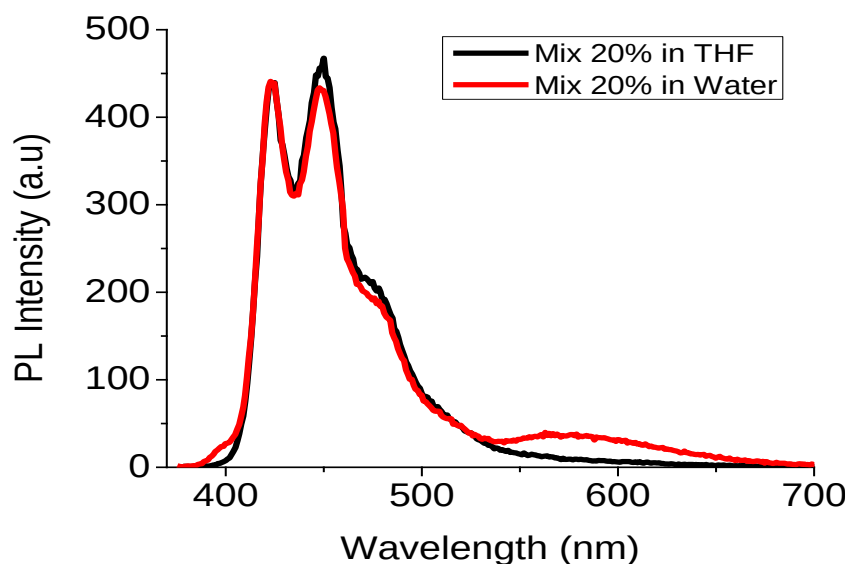


Figure 3.35: Photoluminescence spectra of mix 20% NPs in water (red) and in THF (black)

In order to increase ET, acceptor amount increased to 45% by weight. And to obtain stable NPs, that are not disintegrate even when polar solvents are presented, the click reaction was facilitated with the addition of catalytic amount of copper sulfate and sodium ascorbate to the mixture while sonicating. The click reaction between propargyl and azide groups leads the formation of triazole rings that crosslinks the oligomers and ensures stable and shape-persistent NPs. Figure 3.36 shows the optical result of 45% mixture NPs which were clicked. Intensity of both peaks, at 448 nm and 580 nm, are similar and emission covers all visible range (400-700 nm), yielding white emission as a result. Figure 3.37 shows the visual evidence of both only oligomer NPs and Mix 45% NPs under shortwave UV excitation (254 nm).

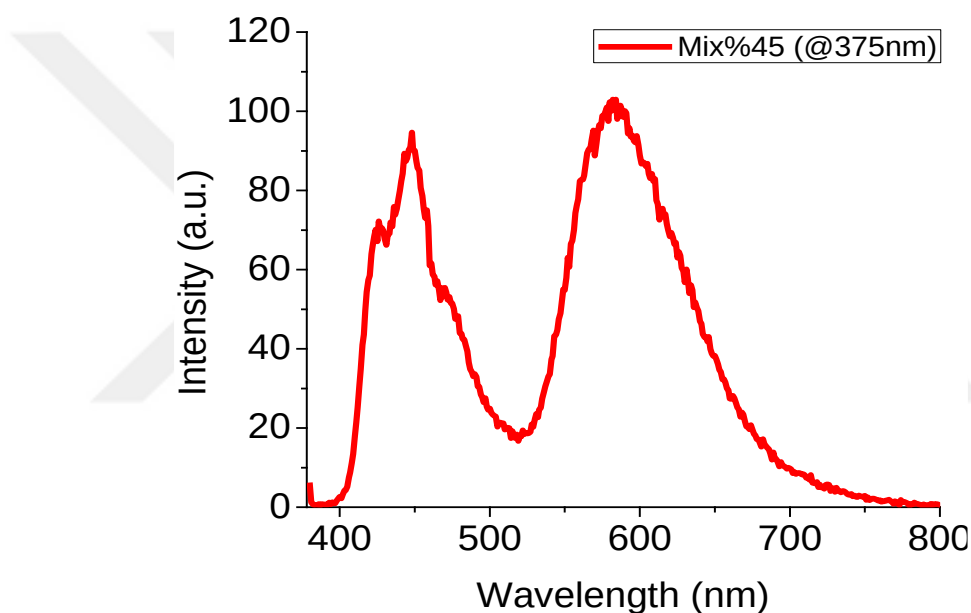


Figure 3.36: Photoluminescence spectrum of Mix 45% NPs in water.

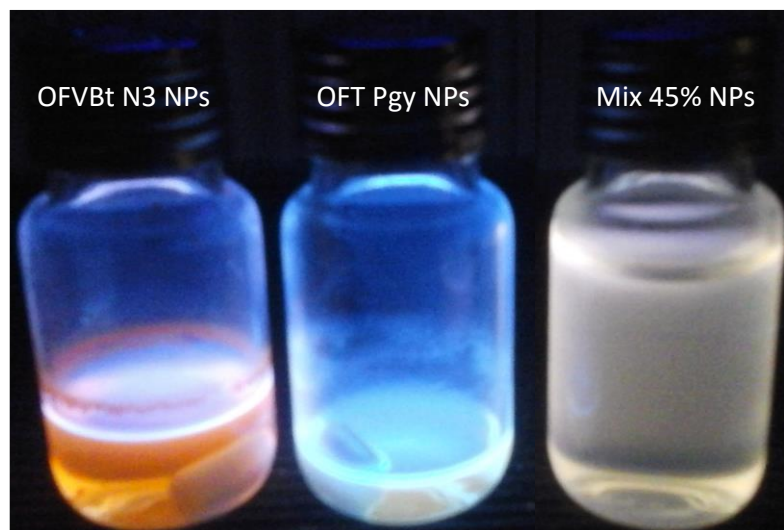


Figure 3.37: Acceptor, donor and Mix 45% NPs in water under UV (254 nm) illumination

The hydrodynamic size of the white emitting Mix 45% NPs were determined by DLS as 63 nm with a narrow size distribution, shown in Figure 3.38. The morphology of the NPs was determined by SEM as shown in Figure 3.39. SEM image reveals spherical nanoparticles with uniform distribution, no aggregation, and agrees with DLS results presented previously.

Z-Average (d.nm): 109.1 **Peak 1:** 63.24 100.0 21.22
Pdl: 0.129 **Peak 2:** 0.000 0.0 0.000
Intercept: 0.939 **Peak 3:** 0.000 0.0 0.000
Result quality : Good

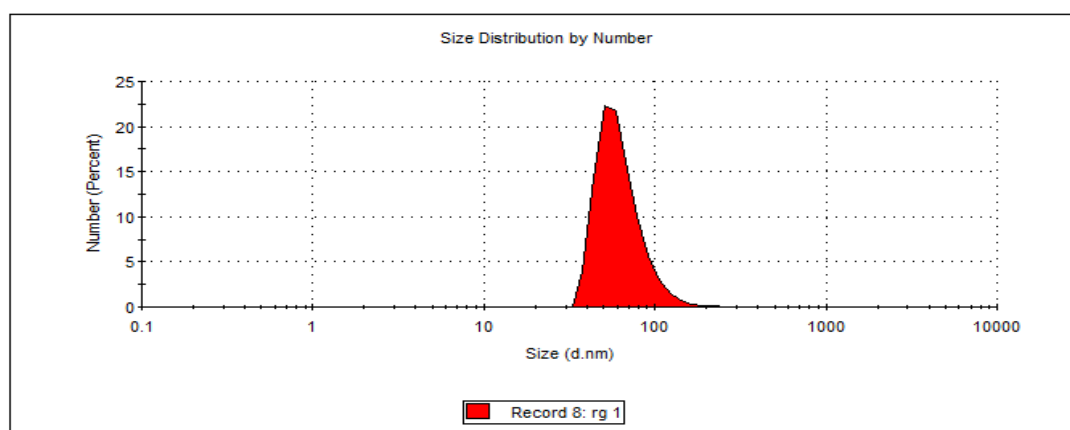


Figure 3.38: DLS result of Mix 45% NPs, size distribution by number

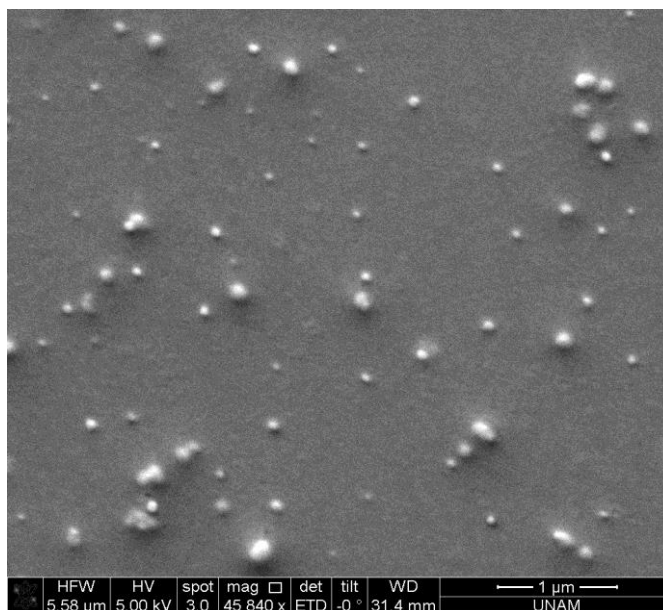


Figure 3.39: SEM image of Mix 45% NPs

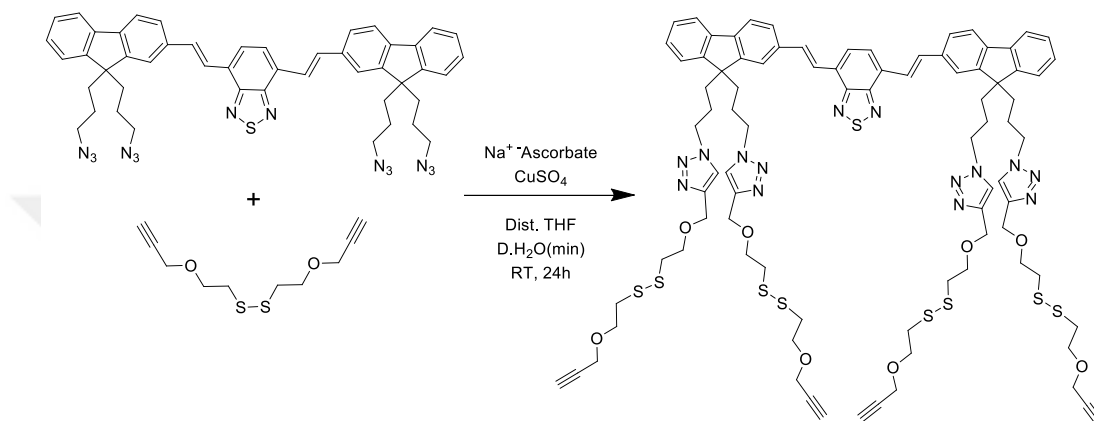
3.2.2 Synthesis and Characterization of Cross-linked Conjugated Oligomer Nanoparticles

Synthesis and characterization of cross-linked OFVBt N3 NPs

As it discussed before, OFVBt N3 NPs were successfully synthesized by reprecipitation method and characterized by DLS and SEM. Herein this study, a disulfide crosslinker with a propargyl terminations was used to crosslink the OFVBt N3 oligomer in THF. As it discussed for bioligomer NPs, click reaction yields triazole ring formation, making NPs crosslinked and stable.

The choice of oligomer and the crosslinker was critical for the purpose of bioapplications. The oligomer has chosen to be emit at the IR end of the visible spectrum so that it could overcome the auto-fluorescence caused by organic molecules within tissues. Besides the auto-fluorescence advantage, the lower emission energy increases the penetration depth in tissues, facilitating an efficient bioimaging. The crosslinker choice was also crucial for another bioapplication purpose. The role of crosslinker is not only stabilization of bioimaging purposed NPs by click reaction, but it has designed with di-sulfide bond so that it can cleave and release the load within the NPs. Disulfide bond is known as weak link and it can be ceased by both electrophiles and nucleophiles due to its polarizability.

Although disulfide bonds are stable in blood, they are efficiently cleaved by thiol bearing molecules like GSH (glutathione) and Trx (thioredoxin), which are presented in tumor cells with a higher concentration than normal tissues. Thus, drug delivery and controlled release from the crosslinked NPs is possible when they are loaded with cytotoxic drugs such as doxorubicin. As a result, the crosslinked NPs is an advantageous theranostic agent by the combination of the OFVBt N3 oligomer and disulfide crosslinker.



Scheme 3.6: Schematic representation of click reaction between OFVBt N3 and disulfide crosslinker.

In first method, 5 mg of OFVBt N3 oligomer was weighed into a vial. 1.354 mg of disulfide crosslinker was added from the stock solution and THF was filled up to 20 ml. After 10 min of ultrasonication, catalytic amount of sodium ascorbate and copper sulfate added and the solution was stirred at RT for 24h. THF was removed under reduced pressure at 40 °C and NPs were dispersed in water. DLS and SEM characterizations were done for NPs. Figure 3.40 shows the DLS result of NPs in THF, as 120 nm size determination. The morphology characterization was done by SEM, as shown in Figure 3.41. SEM image is conforming the DLS result by showing spherical nanoparticles without aggregation.

		Size (d.nm):	% Number:	St Dev (d.nm):
Z-Average (d.nm):	957,9	Peak 1:	120,3	100,0
Pdi:	0,753	Peak 2:	0,000	0,0
Intercept:	1,02	Peak 3:	0,000	0,0
Result quality : Refer to quality report				

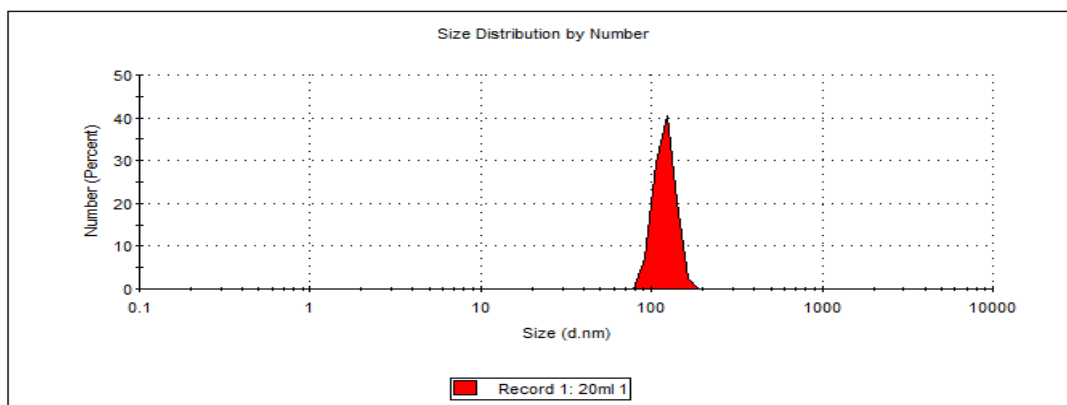


Figure 3.40: DLS result of cross-linked OFVBt N3 NPs in THF

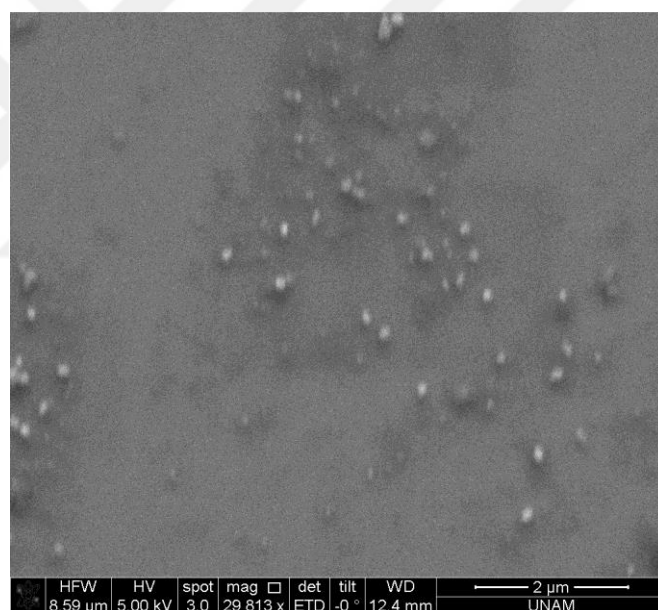


Figure 3.41: SEM image of cross-linked OFVBt N3 NPs

As a second method, 1.25 mg of OFVBt N3 was dissolved in 5 ml of THF. 0.34 mg of disulfide crosslinker was measured from pre-prepared stock solution and ultrasonicated for 10 min. Catalytic amount of copper (I) iodide was added and ultrasonication continued for 2h. Azide peak decrease was monitored by IR spectroscopy and DLS and SEM characterizations were done. Azide peak (at 2100 cm^{-1}) has a sharp decrease from initial to two hours of reaction, conforming the

click reaction occurrence, as shown in Figure 3.42. The size of the NPs were determined to be 153 nm, from the DLS result in Figure 3.43. The morphology characterization was done by SEM, as shown in Figure 3.44. SEM image is conforming the DLS result by showing spherical nanoparticles without aggregation and similar size distribution.

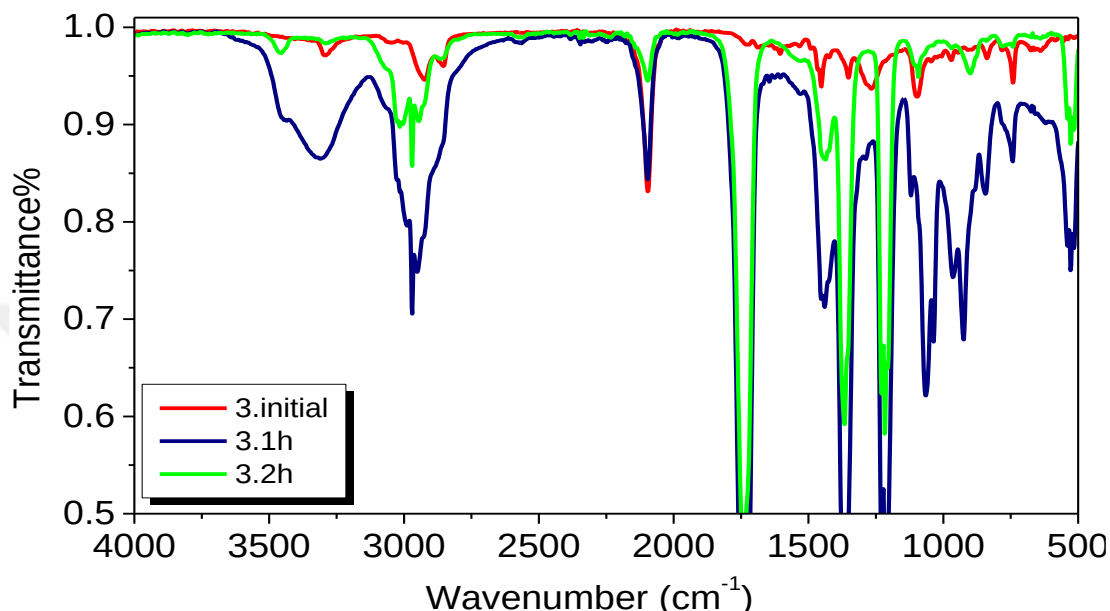


Figure 3.42: FTIR spectra of cross-linked OFVBt N3 NPs, as click reaction monitor.

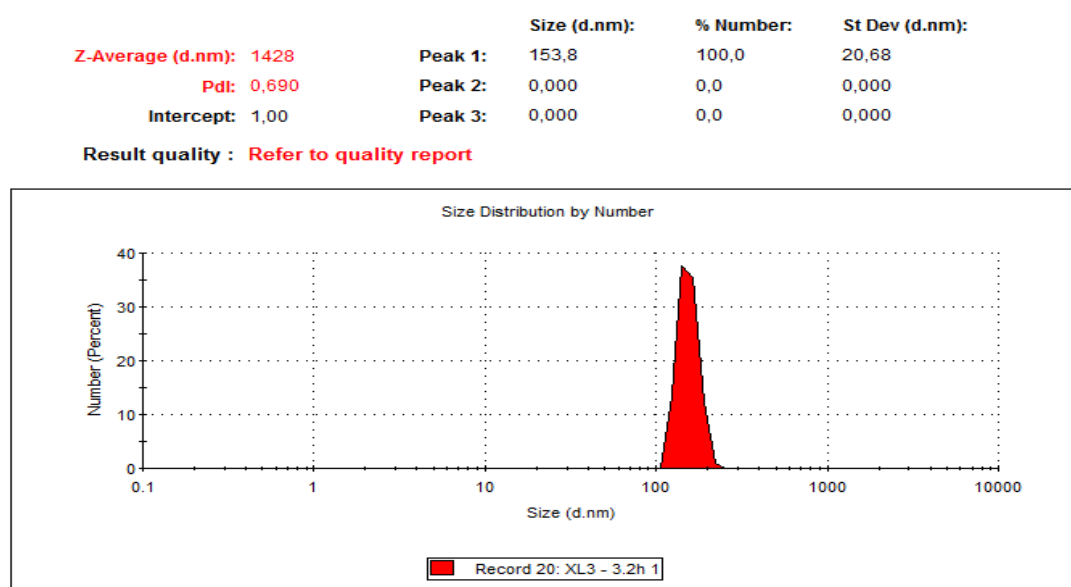


Figure 3.43: DLS result of cross-linked OFVBt N3 NPs in THF

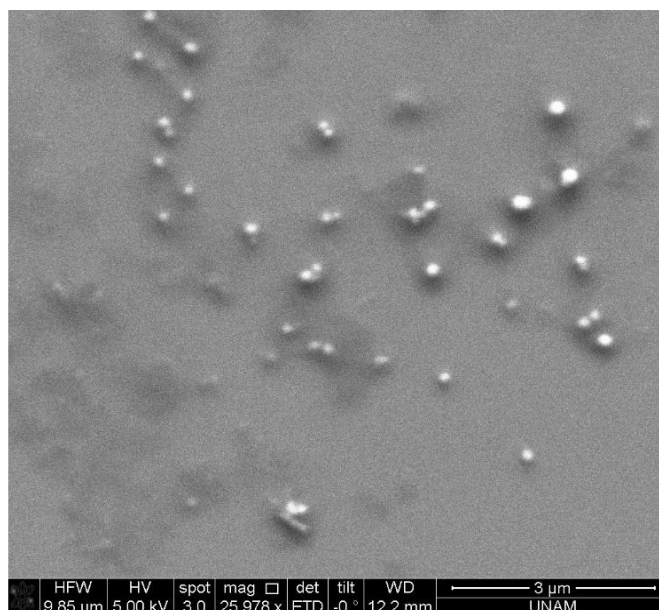


Figure 3.44: SEM image of cross-linked OFVBt N3 NPs

Water dispersed crosslinked NPs was characterized by DLS and SEM. In Figure 3.45, DLS result shows an increased size, 198 nm, when compared to in THF results. Size increase is likely observation due to NPs has hydrophobic backbone. Hydrophobic NPs came together when water presented, causing size increase. Another possible explanation to the size increase is that the crosslinked NPs were swelled up by water. Figure 3.46 shows SEM result of water dispersed NPs that is in line with DLS result when size distribution is considered.

	Size (d.nm):	% Number:	St Dev (d.nm):
Z-Average (d.nm): 668,5	Peak 1: 198,2	100,0	26,52
Pd: 0,662	Peak 2: 0,000	0,0	0,000
Intercept: 1,02	Peak 3: 0,000	0,0	0,000

Result quality : Refer to quality report

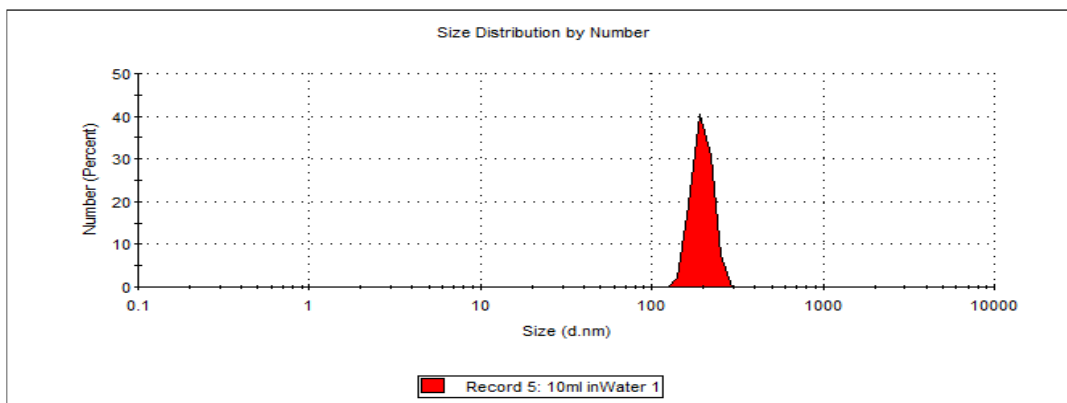


Figure 3.45: DLS result of cross-linked OFVBt N3 NPs in water

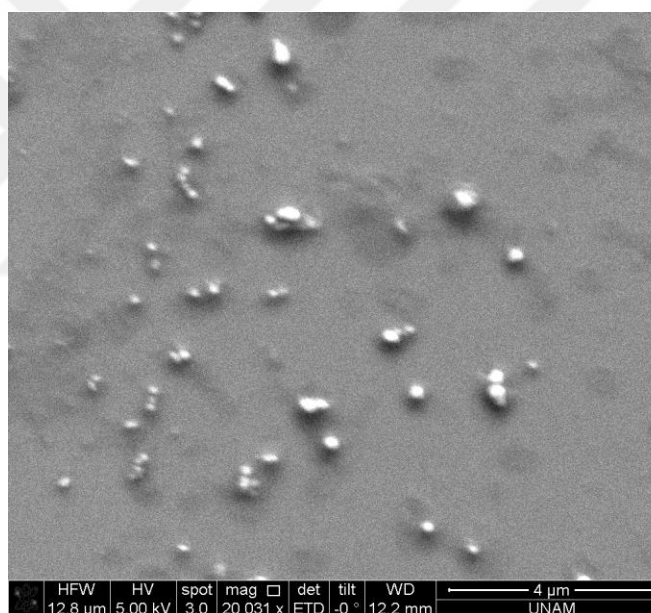


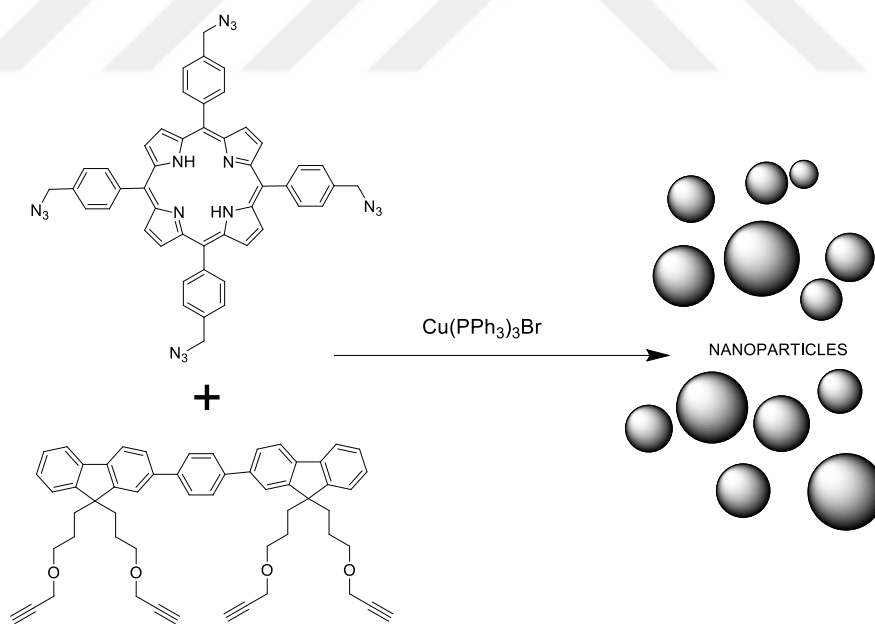
Figure 3.46: SEM image of cross-linked OFVBt N3 NPs (water dispersed)

3.2.3 Synthesis and Characterization of Bi-oligomer Nanoparticles

Bi-oligomer nanoparticles were designed and obtained by using OFB Pgy and Porph N3. The emission spectrum of OFB Pgy (D) and absorbance spectrum of Porph N3 (A) are highly overlapping and this overlap is used to obtain white light through FRET phenomenon. Bi-oligomer NPs are formed in THF by click reaction. The advantage of clicking the oligomer pair is to stabilize the FRET efficiency.

Moreover, using THF as the solvent is not only facilitated an effective click chemistry, but also rendered smooth applicability for solid state white light applications. Since THF can be removed easily, white light emitting NPs can form film on various surfaces like glasses, metals, or ceramics. Therefore, these NPs requires no host layer (or electroluminescent layer, see Figure 1.13) and can be applied directly to electrode surface when optoelectronic applications *e.g.* OLEDs or WOLEDs are considered.

OFB Pgy – Porph N3 NPs were designed to obtain white emission by FRET between oligomers. In this bi-oligomer NPs design, 1.397 mg of OFB (40 eq) Pgy and 0.037 mg (1 eq) (2.6% by weight) of Porph N3 were measured from the stock solutions and mixed in a vial. The solution was ultrasonicated for 10 min to mix homogeneously. Then, 0.041 mg (1 eq) of copper (I) triphenylphosphine bromide, catalyst, was injected into the mixture while ultrasonication. Sonication was continued for 3 h and the decrease in azide peak at 2100 cm^{-1} was monitored by IR spectroscopy during the sonication. Optical characterizations for white emission and DLS and SEM characterizations for size determination were done for NPs.



Scheme 3.7: Schematic representation of Bi-oligomer Nanoparticles synthesis

Figure 3.47 shows the stack plot of IR monitoring of the azide peak during the reaction. Since the Porph N3 is only 2.5% in the mixture, the azide signal is quite

small in comparison. Thus the bottom spectrum in Figure 3.48 is focused to azide peak (2100 cm^{-1}) area to clarify azide peak decrease over time. Azide peak was decreased for 2 hours of reaction and no decrease observed in third-hour of the reaction. Thus, it is concluded that no further reaction occurred after 2h and continued with the optical characterizations.

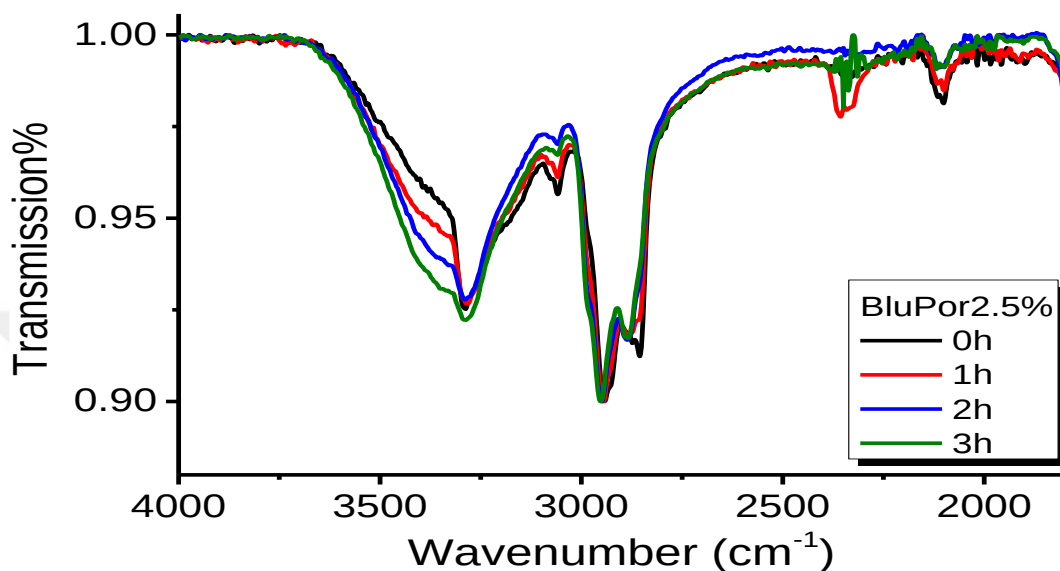


Figure 3.47: FTIR spectra of BluPor2.5% NPs, as click reaction monitor

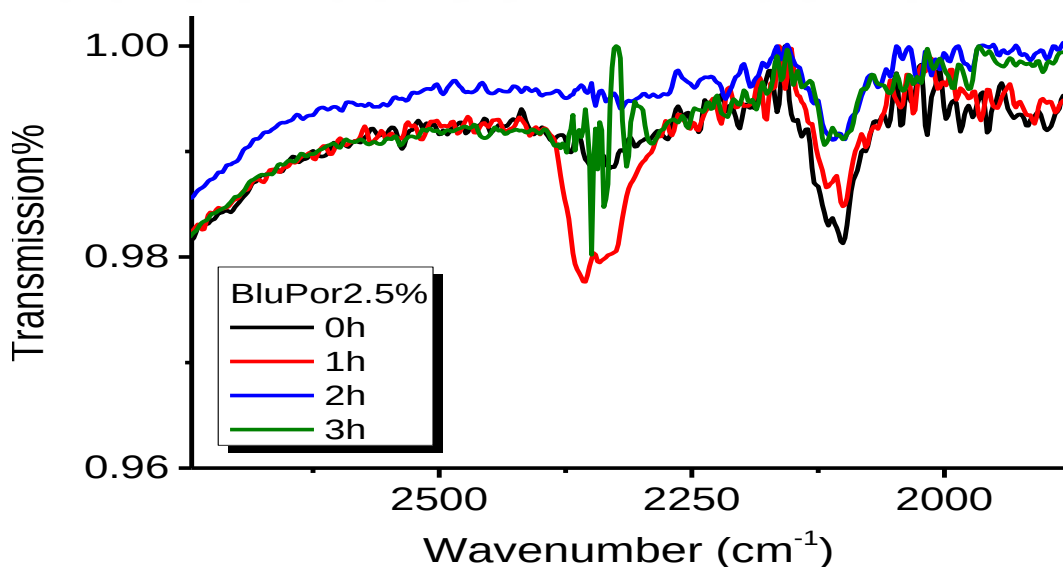


Figure 3.48: FTIR spectra of BluPor2.5% NPs, as click reaction monitor (azide peak focused version of Figure 3.47)

Optical characterizations for BluPor2.5% NPs were done by photoluminescence spectroscopy, shown in Figure 3.49. BluPor2.5% NPs has four different main peaks at 375 nm, 435 nm, 485 nm, and 550 nm, in blue line, which is the 10 slit-width data. 550 nm peak extends beyond the saturation point of detector, to show that peak completely, 5 slit-width data was obtained, shown in red line. The energy transfer from OFB Pgy to Porph N3 creates the peaks in the middle and with the shift of the main peaks of OFB Pgy and Porph N3, white emission was facilitated by these combinations of peaks. Figure 3.50 shows the visual evidence of white emission under UV illumination from BluPor2.5% NPs. Only OFB Pgy (on the left) and BluPor2.5% mixture without catalyst (on the right) solutions are also shown to compare with BluPor2.5% NPs. As shown in mixture without catalyst picture, there is no or not sufficient ET without click reaction. Although OFT Pgy is 40-fold in the composition, the mixture shows reddish color due to the domination Porph N3 in quantum efficiency.

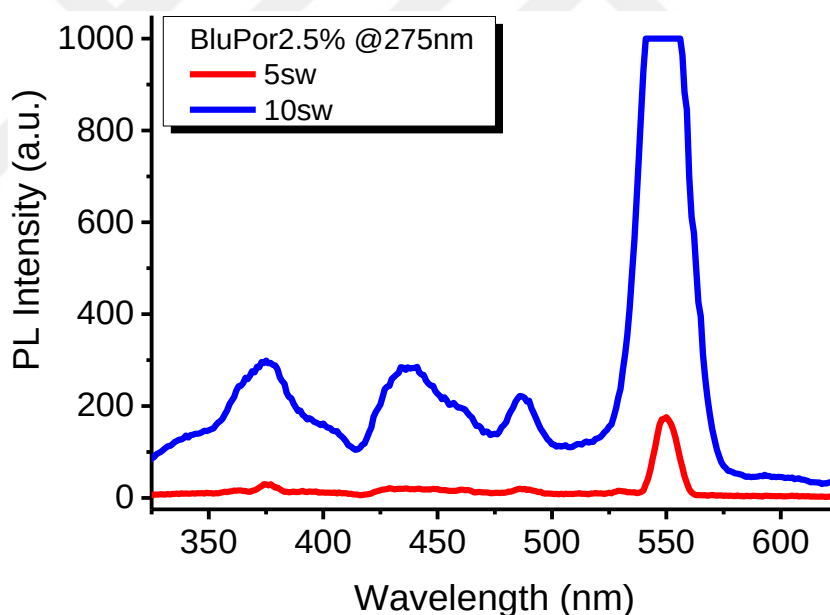


Figure 3.49: Photoluminescence spectra of BluPor2.5% NPs in THF

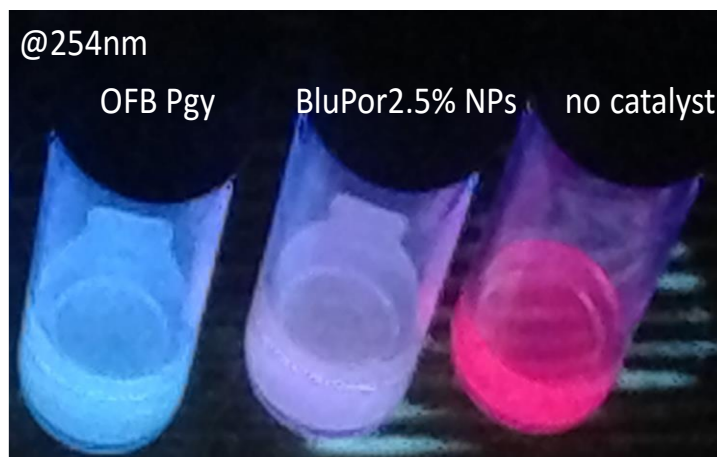


Figure 3.50: OFB Pgy stock solution, BluPor2.5% NPs, and control experiment (no click reaction) in THF under UV (254 nm) illumination

DLS measurement was done with the diluted sample to obtain a correct measurement, since the DLS instrument uses a red laser (633 nm) and the mixture has similar color (absorption), scattering of the light has problematic when concentrated sample is used. The size of BluPor2.5% NPs was determined to be 89 nm with a narrow size distribution, as DLS result shows in Figure 3.51. Morphological characterizations for BluPor2.5% NPs were done by SEM and TEM. Figure 3.52 shows SEM (on the left) and TEM (on the right) micrographs and they are in agreement with the DLS result by size determination and distribution.

	Size (d.nm):	% Number:	St Dev (d.nm):
Z-Average (d.nm): 157,7	Peak 1: 89,56	100,0	41,65
Pdl: 0,194	Peak 2: 0,000	0,0	0,000
Intercept: 0,728	Peak 3: 0,000	0,0	0,000

Result quality : Good

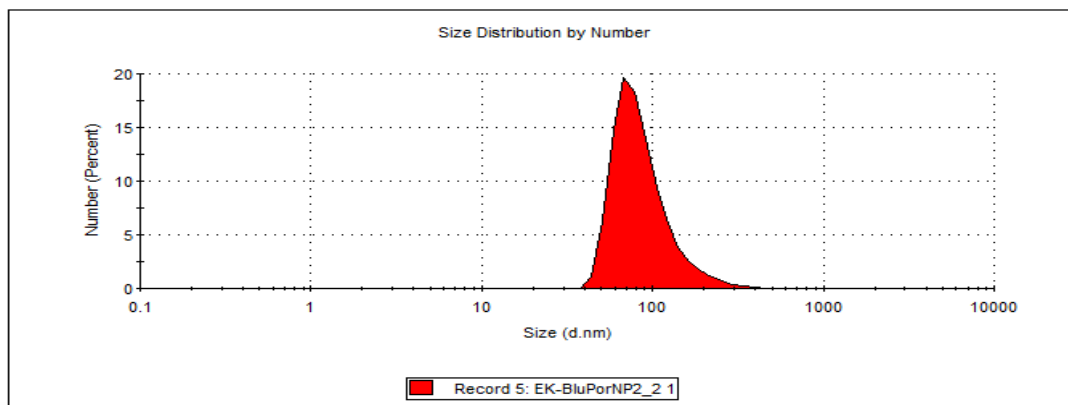


Figure 3.51: DLS result of BluPor2.5% NPs, size distribution by number

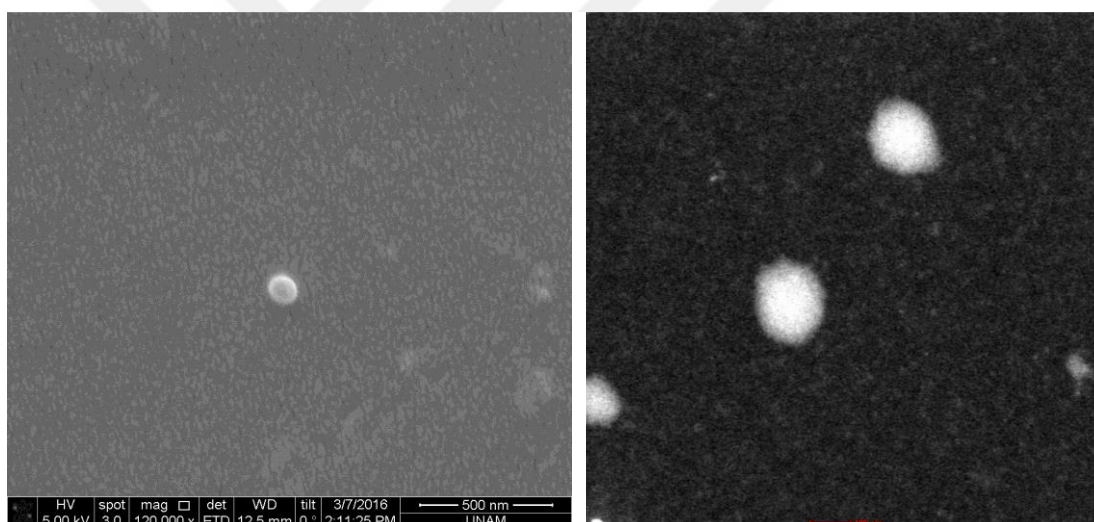


Figure 3.52: SEM (on the left) and TEM (on the right) images of BluPor2.5% NPs

Conclusion

In this project, water dispersed conjugated nanoparticles were synthesized by using click chemistry for the purpose of white light emission. All NPs were characterized by DLS and SEM in order to determine the particle size. For the bioligomer NPs, FRET measurement was done with fluorescence spectroscopy. Mix 45% NPs were showing white emission under UV light excitation and fluorescence spectrum confirms white emission by covering all visible spectrum, with two major peaks at 448 and 580 nm. FRET based white light is widely used in optoelectronic applications such as OLEDs or more specifically WOLEDs. Biosensor and bioimaging applications are also possible with white light emitting NPs due to dispersion in water. Hence, Mix 45% NPs are candidates for such applications.

Secondly, OFVBt N3 oligomer cross-linked with di-sulfide containing crosslinker via copper catalyzed click reaction in THF. Crosslinked NPs sizes were determined by DLS as 120 and 153 nm and SEM micrographs were confirming. NPs were redispersed in water since the biological applications are targeted. Although, water dispersed NPs were showing a higher size, 198 nm, it is still applicable when cellular uptake of tumors. OFVBt N3 oligomer emits at 588 nm, close to IR region, and it can be used for bioimaging purposes. Moreover, di-sulfide bond in NPs can be cleaved in tumor sites by thiol containing bio-molecules (GSH (glutathione) or Trx (thioredoxin)). Cleavage can be used for drug release from NPs. So, the crosslinked OFVBt N3 NPs is a promising theranostic agent with these properties. Finally, OFB Pgy and Porph N3 were clicked in THF for purpose of white light emission. Overlapping optical properties of OFB Pgy (D) and Porph N3 (A) were used to achieve FRET and BluPor2.5% NPs were yielding white light emission with main peaks at 375 nm, 435 nm, 485 nm, and 550 nm in fluorescence spectrum. Characterizations showed small NP size and relevant distribution, 89 nm. Using THF as the solvent is provided a possibility for solid state white light applications since THF evaporates easily. White light emitting NPs can form film on various surfaces, so, these NPs can be applied directly to electrode surface whilst optoelectronic applications *e.g.* OLEDs or WOLEDs are considered.

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Appendix A

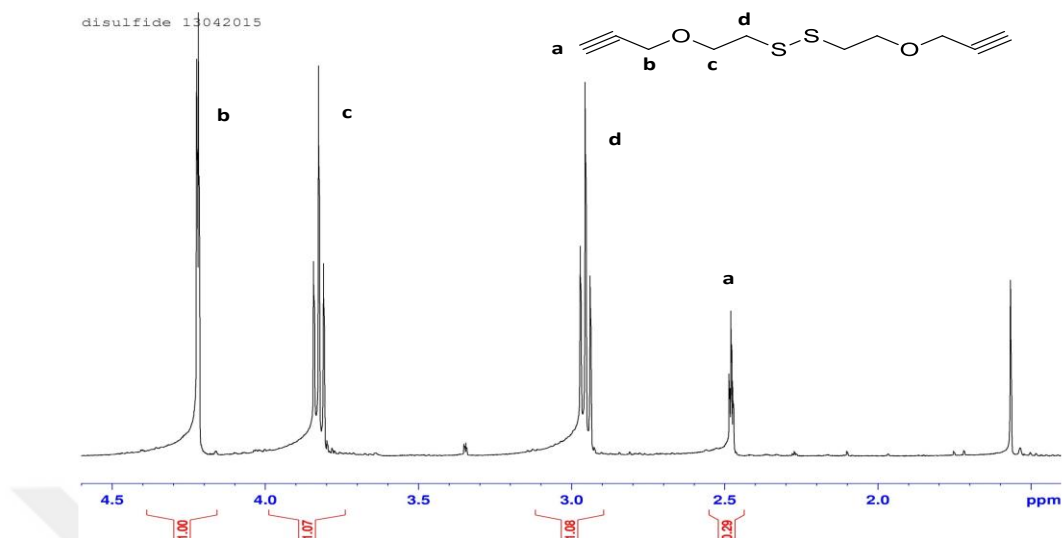


Figure A.1: ^1H -NMR (400 MHz, CDCl_3 , 25 $^\circ\text{C}$) spectrum of disulfide crosslinker

Table A.1: Molar absorptivity and quantum yield results of OFT Pgy

Solvent	$\lambda_{\text{abs}}(\text{nm})$	$\lambda_{\text{em}}(\text{nm})$	$\Phi_{\text{f}}(\%)$	$\epsilon(\text{cm}^{-1}\text{M}^{-1})$	Brightness($\text{cm}^{-1}\text{M}^{-1}$)
CHCl_3	375	423-450	34.35	53959.59	18346
THF	375	423-450	32.80	51338.52	17455

Table A.2: Molar absorptivity and quantum yield results of OFVBt N3

Solvent	$\lambda_{\text{abs}}(\text{nm})$	$\lambda_{\text{em}}(\text{nm})$	$\Phi_{\text{f}}(\%)$	$\epsilon(\text{cm}^{-1}\text{M}^{-1})$	Brightness($\text{cm}^{-1}\text{M}^{-1}$)
CHCl_3	480	592	47.38	26575	12591
THF	478	584	54.05	29369	15874
DMF	480	601	36.70	28795	10568
Water	517	600	25.45	16128	4106
THF (NPs)	481	584	59.77	32762	19583