Diels-Alder Reactions of Phencyclone and Coumarin Derivatives and Click Chemistry of Novel BODIPY Dyes for Fluorescence Detection

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Engin Aytaç AYDIN

aus Istanbul, Türkei

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„Our true mentor in life is science. “

„Hayatta en hakiki mürşit ilimdir, fendir.“

Mustafa Kemal ATATÜRK

(1881-1938)

„Science is the search for truth - it is not a game in which one tries to beat his opponent, to do harm to others.“

Linus Pauling

(1901-1994)

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1. Gutachter: *Prof. Dr. Hans-Josef Altenbach* (BU Wuppertal, D)

2. Gutachter: *Prof. Dr. Ullrich Scherf* (BU Wuppertal, D)

Abstract

Fluorescent dyes have been used frequently to label biomolecules for imaging and detection applications. Besides, non-fluorescent substances that present fluorescence emission after incorporation into click reactions, so called “fluorogenic click reactions”, are very useful in biological and chemical applications. The synthesis and investigation of the properties of novel dyes have been studied. Moreover, the click chemistry reactions with the purpose of fluorogenic applications were also investigated. The thesis starts with an introduction to the click reaction types applied to the fluorescence labeling and the fluorogenic reactions based on Huisgen 1,3-dipolar and Diels-Alder cycloadditions with the most important examples published in literature.

In this thesis, fluorogenic Diels-Alder reaction based on novel phencyclone derivatives have been developed and the optical properties of Diels-Alder adducts are discussed in detail. To the best of our knowledge, this is the first example of a fluorogenic Diels-Alder reaction, in which the diene part of the reaction is on the fluorogenic substance and the reaction between diene and dienophile brings the conjugation of the fluorogenic substance to provide “turn-on” fluorescence at room temperature. The crucial properties of this method are that the reaction allows to monitor Diels-Alder reaction by color change and up to 33-fold of fluorescence increase was achieved. Some nucleoside derivatives containing maleimide groups in order to incorporate into this fluorogenic Diels-Alder reaction were also developed and these “bio”-components were used as models and as a starting point for the development of bioorthogonal applications of fluorogenic Diels-Alder reaction.

Furthermore, new BODIPY dyes were designed with the purpose of using them in fluorogenic Huisgen 1,3-dipolar cycloaddition. Ortho-, meta- and para-azido substituted BODIPY derivatives have been synthesized and as expected, the ortho-azido-substituted BODIPY derivative presented relatively less fluorescence compared to the others. The click reaction with alkyne modified thymidine derivative promoted the fluorescence of this BODIPY derivative. The fluorescence enhancement was 14-fold. Moreover, the new synthetic approach of these BODIPY fluorophores permits to synthesize quite higher amounts of azidophenyl-substituted BODIPYs compared to literature procedures.

During the study to invent fluorogenic BODIPY derivatives, novel BODIPY dyes carrying nitro and amino groups in the BODIPY core were also produced. The synthetic approach and the investigation of optical properties of these new BODIPY derivatives are described. A nitro group is known for its fluorescence quenching properties, however, it is not able to quench BODIPY fluorescence in every situation, instead, it provides new fluorescence properties.

In conclusion, Diels-Alder cycloaddition and Huisgen 1,3-dipolar cycloaddition as click reactions were investigated for fluorescence detection. Moreover, novel phencyclone derivatives and new BODIPY based fluorophores were synthesized and their optical properties were studied.

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1 General Introduction

Between countless chemical reactions, a number of them, called click reactions, draw the attention with versatility, efficiency, good chemoselectivity, high yields and bioorthogonality. Among these reactions, the copper catalyzed Huisgen 1,3-dipolar cycloaddition reaction between azides and terminal alkynes has emerged as a powerful tool in chemical and biological applications. Also the Diels-Alder reaction has collected great interest in recent years as its click type properties and number of applications in chemical, biological and materials processes are increasing.

The important aspects of these reactions are their orthogonal and/or even bioorthogonal applications. Click reactions are orthogonal to each other, meaning two or more reaction types can be done in one pot without interfering or disrupting. The bioorthogonal term is used for chemical reactions that neither interact nor interfere with a biological environment.[1]

Fluorescence spectroscopy, one of the most informative and sensitive analytical techniques has a great importance in modern research. Investigation of biological substances via fluorescence labeling enlightens the inner working of biomolecules, cells and organisms.[2] Nowadays, click chemistry is the mostly preferred reaction for fluorescence labeling as it is very efficient and practical. For selective labeling of biomolecules, click chemistry offers high yields under biological conditions and biorthogonality, because of the non-reactivity of the precursors with common functional groups present in biological systems.[3]

Fluorogenic probes are normally non-fluorescent and emissive fluorescence only after reaction with the target substance. On the basis of reducing background noise, fluorogenic reactions are an impressive solution.[4] The fluorogenic reactions are depending on mostly the formation of a triazole ring via Huisgen 1,3-dipolar cycloaddition or the quenching effect of the maleimide group over PET (photoinduced electron transfer) mechanism. Fluorogenic application of different types of click reactions have been studied frequently and it will be for sure a hot topic for quite a long time.

Fluorogenic or not, new derivatives of fluorescence dyes are always needed with improved properties to be used for developments in the field of diagnostics in medicine and in the area of organic electroluminescent devices. Beyond numerous fluorescent dyes, the BODIPY (4,4-difluoro-4-bora-3a,4a-diaza-s-indacene) family has been attracted great attention over the past two decades because of their versatile properties.[5] Adaptable and easy synthetic procedures

of BODIPY dyes provide the opportunity to adjust desired optical and physical (solubility, stability … etc.) properties.[6]

1.1 Introduction of Click Reactions

Click reactions, that offer the efficiency, simplicity and selectivity together, have been collecting considerably interest, especially, in the new century. The click term was first defined by Sharpless et al.[7] to express the opportunity to focus only on the combination of two components via fast and efficient reactions, instead of considering complicated bond formations. After creation of the click chemistry concept, the reactions attributed to it, have been applied to a wide range of research areas, including materials science,[8-9] supramolecular chemistry[10], pharmaceutical sciences[11], and in *vitro* and *vivo* labeling[12]. There are mainly four category of reactions which are taking place under the name of click reactions:

(i) Cycloaddition reactions: Huisgen 1,3-dipolar cycloaddition,[7, 13-14] and Diels–Alder reaction,

(ii) ring-opening reactions of strained heterocyclic electrophiles (epoxides, aziridines and aziridinium ions, …) via nucleophilic addition,[15]

(iii) "non-aldol" carbonyl chemistry (for example, the formation of ureas, oximes and hydrazones),[16-17]

(iv) addition reactions to carbon–carbon multiple bonds (most importantly thiol–ene chemistry and also Michael additions).[18-20]

Among these four major classes, in the literature, the "click" term is mostly used to address the Cu(I) catalyzed azide-alkyne cycloadditions,[9] also some Diels–Alder reactions surely fulfill the different requirements of click chemistry.[21-22] In fact, the increasing number of publications, especially in materials science, are refering "click" term not only for Huisgen 1,3-dipolar cycloaddition, but also for other categories explained by Sharpless et al. It is very useful to combine click reactions to functionalize materials selectively without destroying other functional groups on the structure, because they are orthogonal to each other. This allows chemists precisely to position functional groups by parallel or cascade reactions in one-pot.[23]

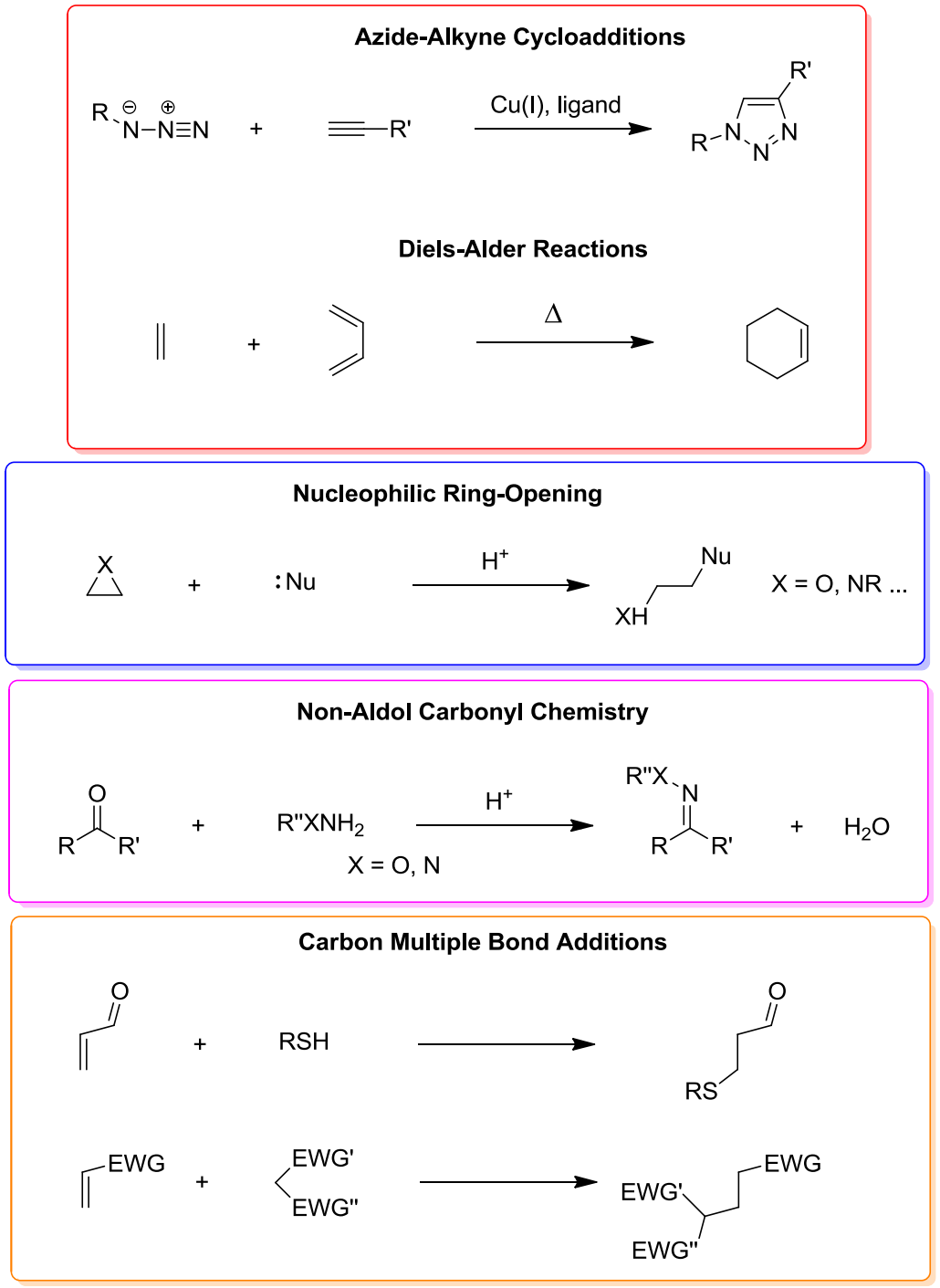


Figure 1: Main classes of click reactions (Nu = nucleophile; EWG = electron-withdrawing group).[11]

1.1.1 1,3-Dipolar Cycloadditions

1.1.1.1 Copper(I)-catalyzed Azide-Alkyne Cycloaddition (CuAAC)

The most preferred example of click reactions is the copper(I)-catalyzed azide-alkyne cycloaddition (CuAAC) reaction.[13-14] Indeed, [3+2] cycloadditions of alkynes and azides have been known since A. Michael, firstly, synthesized 1,2,3-triazole by reaction of phenyl

azide and diethyl acetylenedicarboxylate in 1893.[24] However, detailed studies about this reaction were made mainly by Huisgen and coworkers in 1960s.[25-26] Because of its highly exothermic (ΔH^0 between -50 and -65 kcal mol^{-1}) character and its high activation barrier (as an example, reaction between methyl azide and propyne has activation barrier about 25 kcal mol^{-1} [27]), the reaction takes place only at high temperatures. In addition, especially in the reaction of unsymmetrically substituted alkyne derivatives, a mixture of regioisomeric 1,2,3-triazoles is generally observed; because HOMO-LUMO energy levels for both reactants (azides and alkynes) are similar to each other and dipole-HOMO- and dipole-LUMO-controlled mechanism of cycloadditions are taking place at the same time (Figure 2).

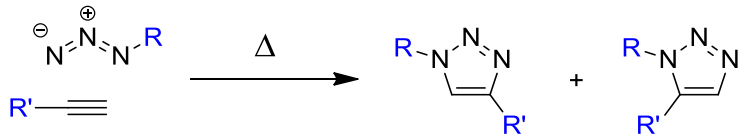
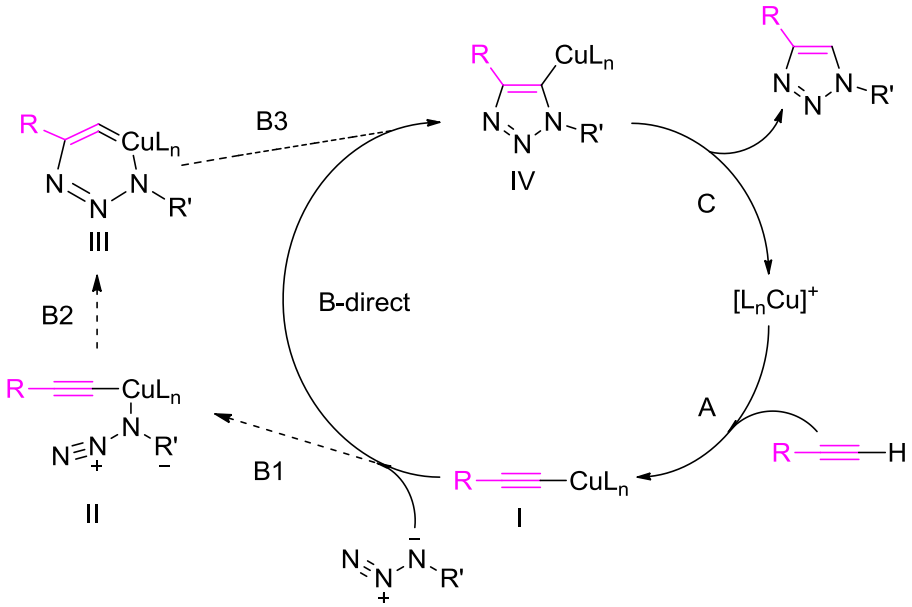


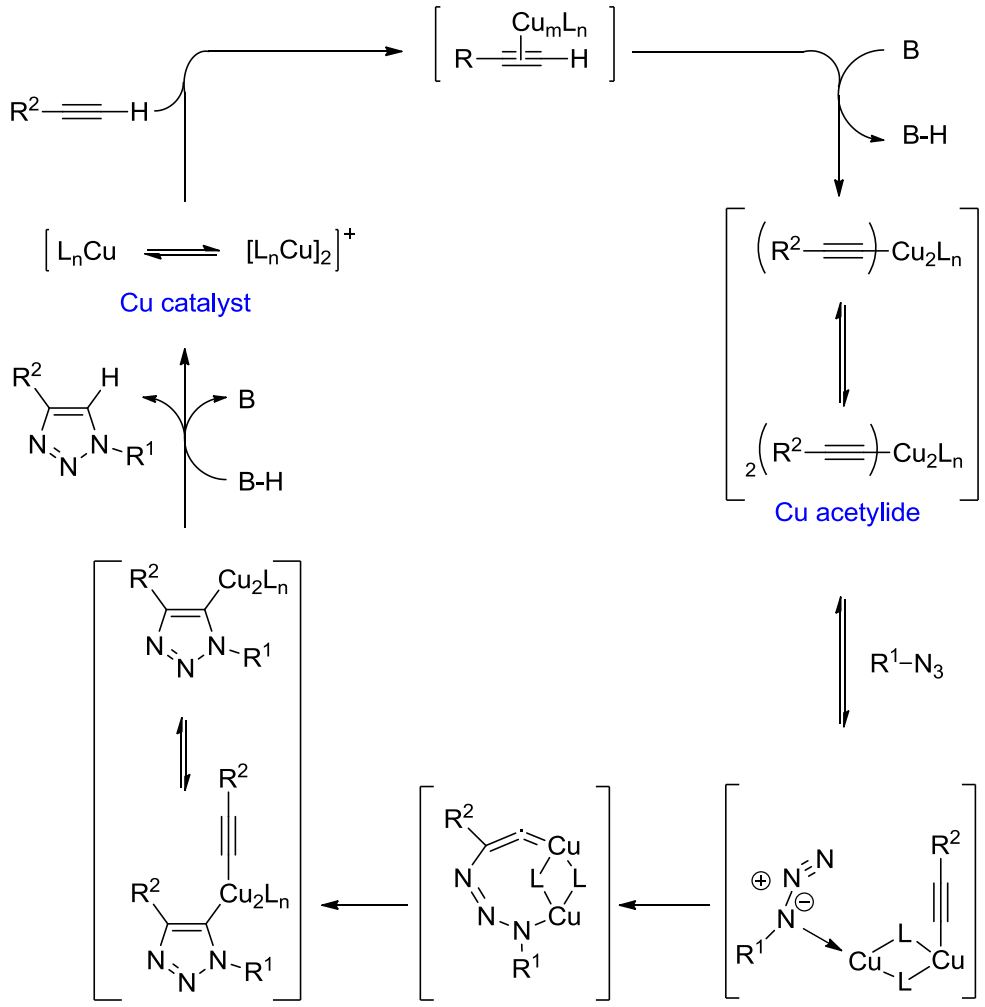
Figure 2: Formation of two products: 1,4- and 1,5-regioisomers.

In 2002, Meldal *et al.*[13] and Sharpless *et al.*[14] separately presented the copper(I) catalyzed reaction of terminal alkynes leading to faster and more efficient formation of 1,4-substituted triazoles at mild temperatures. In their work, Sharpless *et al.* prepared the Cu(I) catalyst in situ by reduction of Cu(II) salts ($CuSO_4 \cdot 5H_2O$ works very good) with ascorbic acid and/or sodium ascorbate acting as the reducing agent. At 0.25-2 mol% catalyst loading, one could generate a wide variety of 1,4-substituted triazoles in high yields.[14] They suggested a reaction mechanism, which starts with the formation of the copper(I) acetylide **I.** Then, there are two possibilies of cycloaddition (Scheme 1). In the first proposal, the B-direct route, a direct [2+3] cycloaddition occurs, however, after examination of extensive density functional theory calculations they have added another mechanism to the scheme which is a stepwise sequence (B1→B2→B3), also named "ligation" pathway (Scheme 1). According to the calculations, the energy need of this route is 12-15 kcal less than in the B-direct route.[14]



Scheme 1: Copper(I)-catalyzed mechanism of Huisgen 1,3-dipolar cycloaddition proposed by Sharpless et. al.[14, 28]

Later, the mechanism was changed by Finn *et al.*[29-30], approved by Sharpless *et al.* via computational methods[28] and in 2006 a revised mechanism was proposed by Bock *et al.*[31] (Scheme 2). According to the mechanism of Sharpless et al., the rate of the catalytic process is first order in copper and the acetylide formed is supposed to form an acetylide-azide complex immediately. However, the mechanism of Bock et al. expresses the formation of the acetylide group as a second order catalytic process with respect to copper; this means, the copper acetylide must carry a second copper component to be able to activate the azide functionality as a dimer and yielding a copper acetylide-azide complex. This complexation surely reduces the alkyne electron density which smooths the cyclization process. The process of cyclization triggers the azide to make a nucleophilic attack to the acetylide carbon to generate a metallocycle. Related to this mechanism, some literature data prove that the electron-withdrawing groups on the alkyne speed up the copper(I) catalyzed alkyne–azide cycloadditions.[13, 32] Then, the metallocycle transforms quickly into a triazole-copper derivative; and lastly, the triazole-copper molecule is protonated and the product separated such as the catalyst (Scheme 2).

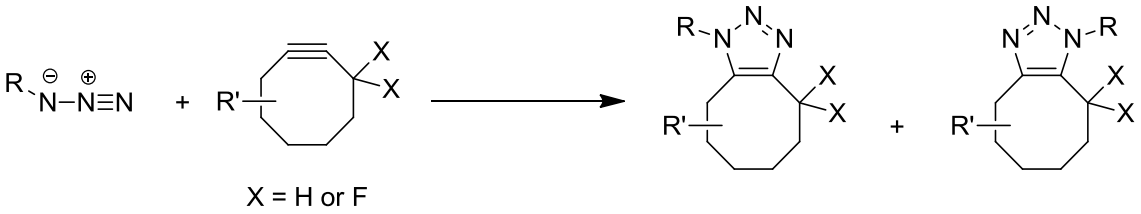


Scheme 2: Proposed mechanism of copper(I)-catalyzed Huisgen 1,3-dipolar cycloaddition by Bock *et al.*[31]

The usage of copper(I) catalysis in Huisgen 1,3-dipolar cycloaddition increases the reaction rate up to 10^7 times,[33] allows mild reaction temperatures and gives higher yields in a variety of solvents, including water, over a wide range of pH values. Moreover, work-up and purification needed after reaction is minimal. In this respect, CuAAC is an ideal click reaction. As a result, numerous applications have been published in the fields of dendrimer and polymer grafting,[34-36] bioconjugations,[37-39] combinatorial drug discovery,[40-41] pharmaceutical sciences,[11, 42] modification of DNA,[43] *etc*. Besides, CuAAC is exceedingly suitable for biological applications due to its reaction conditions which are compatible with biological environments such as aqueous medium or low temperatures. Also, azide and alkyne groups are quite rare or in many cases absent in the biological environments and this gives the opportunity of chemoselective functionalizations or ligations for biological structures.[44]

1.1.1.2 Copper-Free Azide–Alkyne Cycloadditions

CuAAC has several applications in materials, chemical and biological sciences, however, the toxicity of copper is restricting the possibility of applications, in vivo and in vitro.[45-46] As a solution of this problem, Bertozzi and co-workers developed strain-promoted [3+2] azide-alkyne cycloaddition by using cyclooctyne derivatives which do not need copper catalysis for a fast reaction (Scheme 3).[46-47] Indeed, in 1961, Wittig and Krebs discovered that cyclooctyne and phenyl azide react "like an explosion" to yield a single triazole product.[48] Differently from a linear [3 + 2] cycloaddion, a large part of ring-strain energy of cyclooctyne (18 kcal/mol) releases during the strain-promoted alkyne-azide cycloaddition (SPAAC) accelerating the rate of reaction.[49]



Scheme 3: General presentation of strain-promoted cycloadditions.

Bertozzi et al. first synthesized a cyclooctyne derivative, which contains a carboxylic acid side chain, starting from dibromocyclopropane.[46] They named this first-generation reagent OCT.[47] It is assumed that the lowest unoccupied molecular orbital (LUMO) of the alkyne and the highest occupied molecular orbital (HOMO) of the azide are entangled during the formation of the triazole ring. To this end, lowering the LUMO of the alkyne via substitution with electron withdrawing groups results in an increase of the reaction rate.[50] Addition of two fluorine atom to the ring of cyclooctyne (DIFO) accelerates the reaction 60 times more than OCT.[51] The poor soluability of DIFO directed Bertozzi et al. to produce dimethoxy-azacyclooctyne (such as DIMAC) by modification with heteroatoms in the ring.[52] Meanwhile, Boons and coworkers were also working on developing derivatives of cyclooctynes and they synthesized a functionalized derivative of dibenzocyclooctyne to increase the strain-energy.[53] These dibenzocyclooctynes also give triazole products with azides almost as fast as DIFO.[50] In 2010, Bertozzi and coworkers obtained a great reaction rate by addition of an amide bond to the structure of DIBO to yield a biarylazacyclooctynone (BARAC).[54] In Table 1 and Figure 3, the different examples of cyclooctyne derivatives and their reaction rates are summarized.

Name	Solvent	k(x10^{-3} M^{-1} s^{-1})	Ref.
OCT	CD_3CN	2.4	[46]
DIFO	CD_3CN	76	[51]
DIFO2	CD_3CN	42	[55]
DIFO3	CD_3CN	52	[55]
ALO	CD_3CN	1.3	[56]
MOFO	CD_3CN	4.3	[56]
NOFO	CD_3CN	1.2	[56]
DIBO1	CH_3OH	57	[57]
DIBO2	CH_3OH	76	[57]
BARAC	CD_3CN	960	[54]
DIBAC	CD_3OD	310	[58]
BCN	$CD_3CN:D_2O$ (3:1)	140	[59]
DIMAC	CD_3CN	3	[52]

Table 1: Reactivity of Functionalized Cyclooctynes.

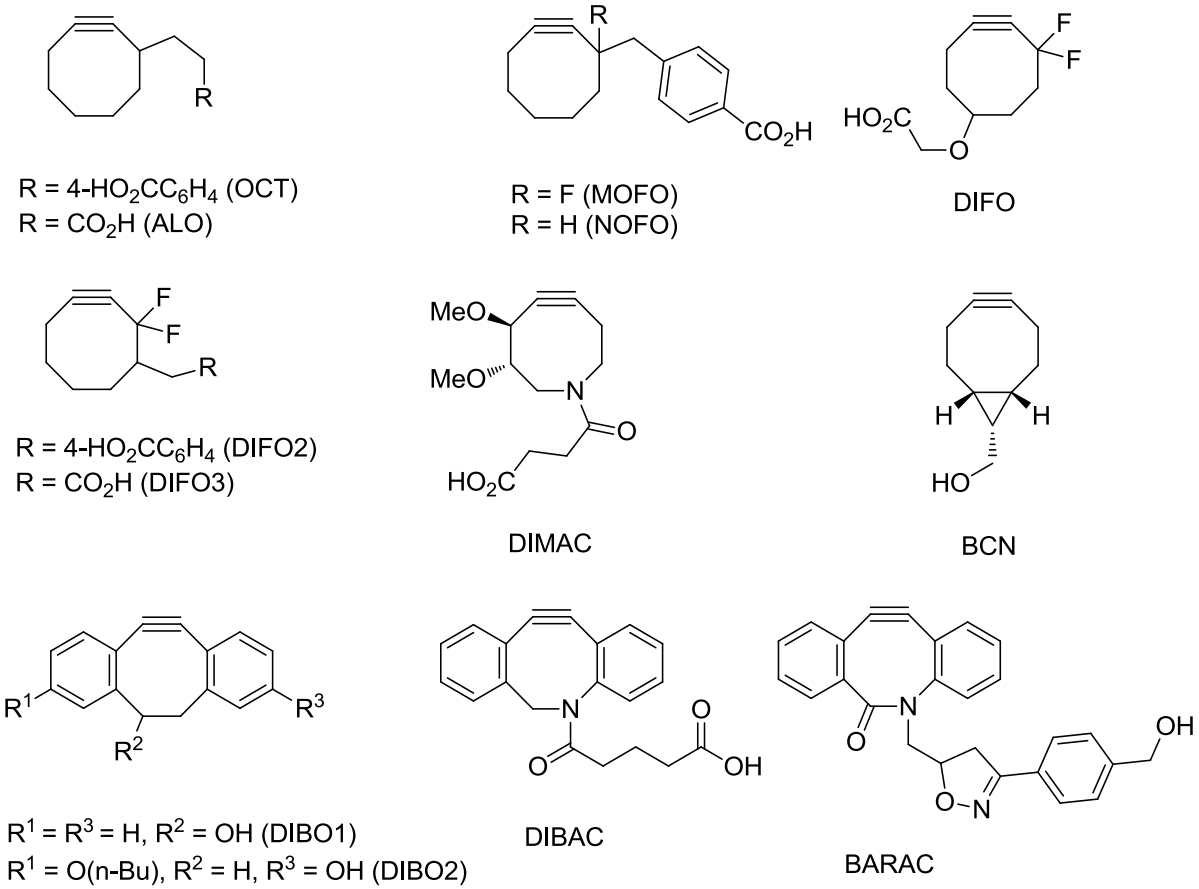
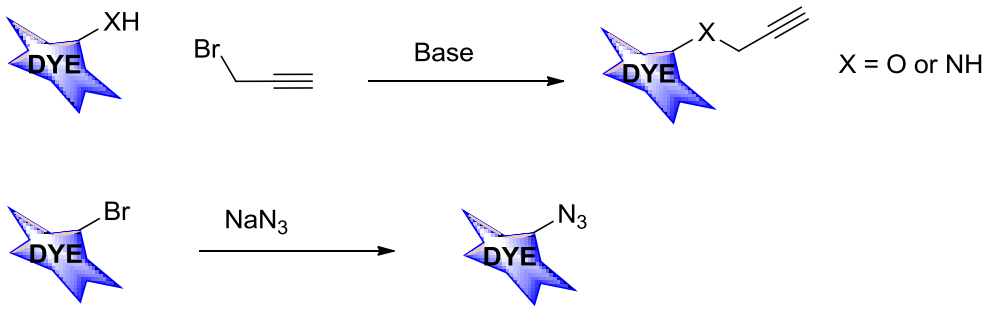


Figure 3: Functionalized Cyclooctynes.

1.1.1.3 Azide-Alkyne Cycloadditions for Fluorescent Labeling

Azide-alkyne click reactions have been employed to label different biomolecules for different applications. Applications in cells[45, 60-62] and even animals[63-64] have been also published several times. The bioorthonogality of this click chemistry and the small size of the azide and alkyne groups allow the modification and fluorescence labeling of amino acids,[65] nucleosides,[66-68] monosaccharide[51], proteins,[69] and carbohydrates.[70] Many dyes have been modified with an azide and/or alkyne function for such labeling-applications.[62, 71] The easiest and fastest modification of a substance with a alkyne function, as seen in these examples, is using propargyl bromide in a nucleophilic substitution reaction. Most of the known and popular dyes can be modified by nucleophilic substitution between their free –OH or $–NH_2$ groups and propargyl bromide. Addition of the $–N_3$ group is commonly done by conversion of the bromide group into an azido group (Scheme 4). These modifications are not applied only to the dyes, but also to the substances that have to be labeled.



Scheme 4: Preparation of dyes for click applications.

Furthermore, instead of substitution of fluorophores with functional groups as shown in Scheme 4, another possibility to functionalize the dye is using readily alkyne and/or azide substituted starting materials to synthesize fluorophores. In order to design a wide variety of fluorophores, various retro-synthetic approaches can be envisioned. Numerous methods and modifications for the synthesis of click derivatives have been presented in the literature. In Table 2, commonly prefered organic reactions for the introduction of alkyne and azide functional groups are presented.[43]

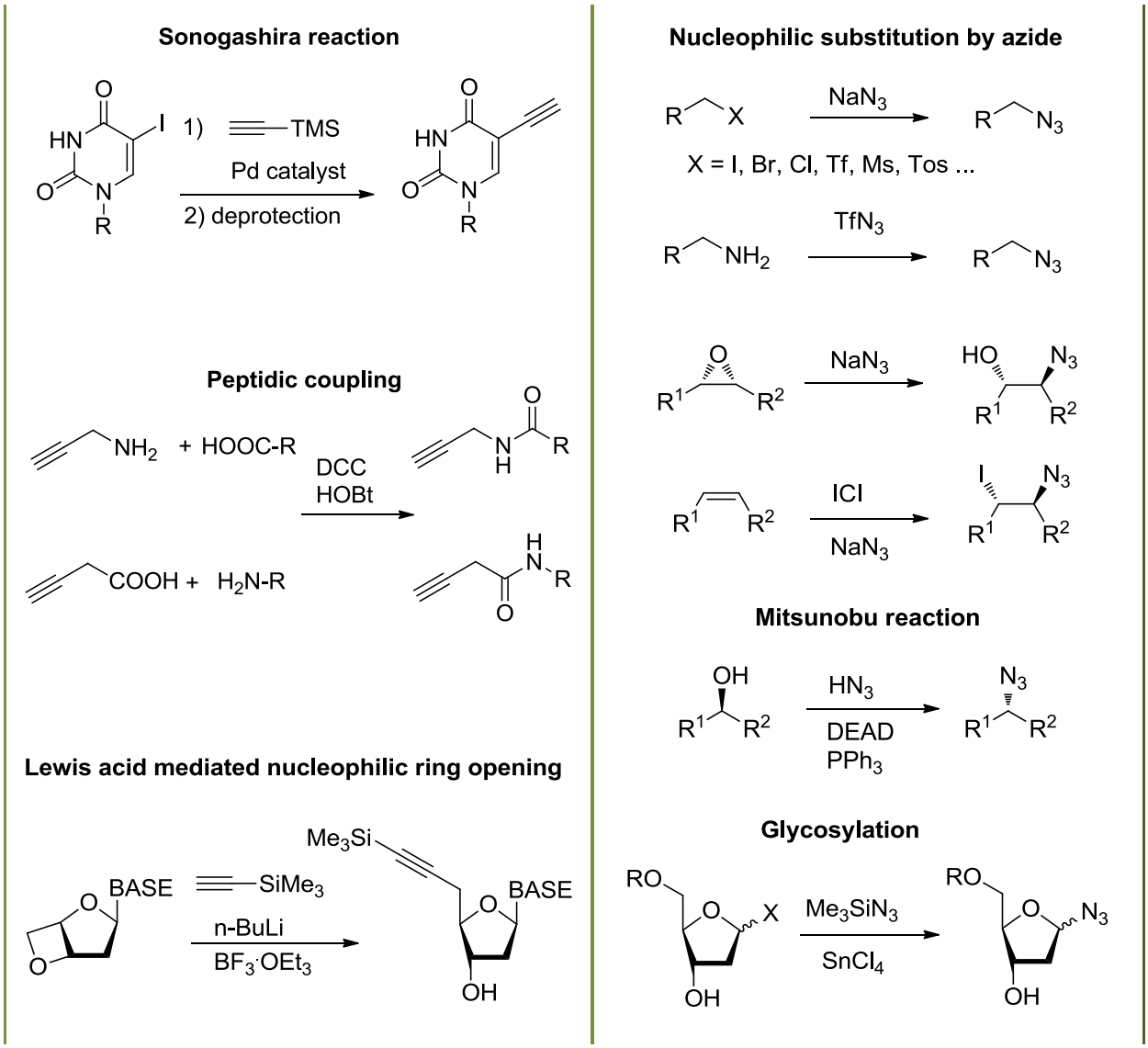


Table 2: Most common organic reactions to introduce alkyne and azide functional groups.

1.1.2 Conjugations via Diels-Alder Cycloadditions

1.1.2.1 Diels-Alder Reaction

The Diels-Alder (DA) reaction is one of the most notable and utilized cycloadditions in organic chemistry. It was first discovered by Otto Diels and Kurt Alder in 1928 and this invention brought them the Nobel Prize in 1950.[72] The reaction takes place via a suprafacial [π4s+π2s] cycloaddition of a diene and a dienophile to form a six-membered cyclohexene ring (Figure 4).

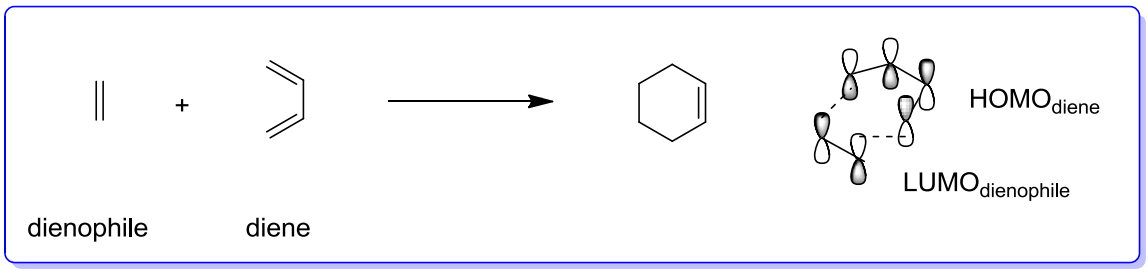


Figure 4: The Diels-Alder (DA) reaction.

The Diels-Alder reactions can be classified according to the properties and positions of the substituents in normal electron demand and inverse electron demand Diels-Alder cycloadditions. In a normal electron demand Diels-Alder reaction, electron donating substituents are necessary on the diene and electron withdrawing substituents on the dienophile. These substituents are promoting the reaction and determining the rate of it. Contrary, by inverse electron demand cycloaddition, the electron withdrawing substituents are on the diene and electron donating substituents are connected to the dienophile.

The effect of substituents on the reaction rate can be explained by Frontier Molecular Orbital (FMO) theory, introduced by Woodward and Hoffmann[73] and, independently, by Fukui[74]. The FMO theory explains the reactions with the intractions of the molecular orbitals of the reactants and according to the theory, the efficiency and rate of reaction are best when the molecular orbitals of reactants are closest in energy to get highest overlapping. One of the reactants carries two electrons in its Highest Occupied Molecular Orbital (HOMO) and these electrons interact with other reactant's Lowest Unoccupied Molecular Orbital (LUMO).

In normal electron demand Diels-Alder reaction, a dienophile has an electron withdrawing group (EWG) decreasing its LUMO energy. Hence, the lower difference between energies of the HOMO of the diene and the LUMO of the dienophile makes the reaction rate increase.[75-78] However, in inverse electron demand Diels-Alder cycloaddition, an electron donating group (EDG) substituted on the dienophile accelerates the reaction by lowering dienophile's HOMO to provide donation of its electrons to the LUMO of the diene (Figure 5).

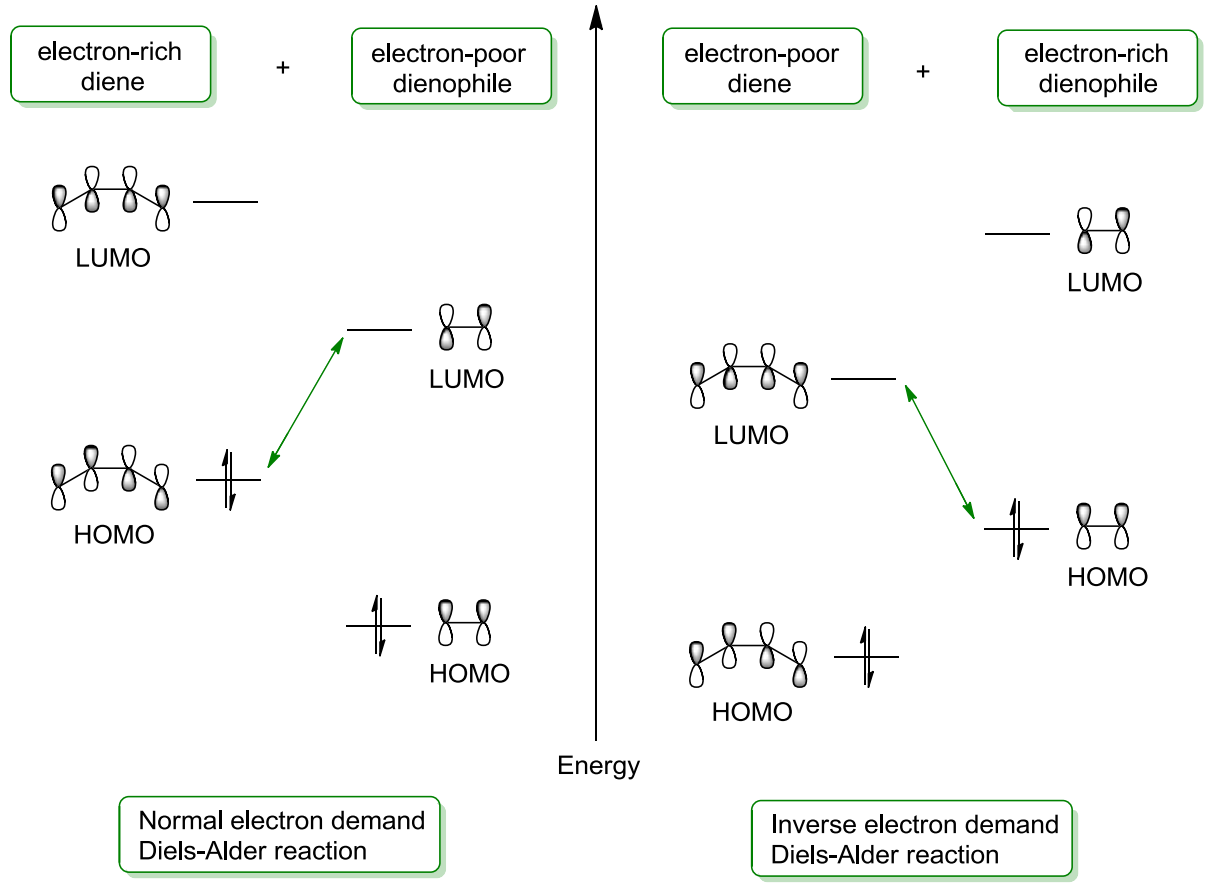


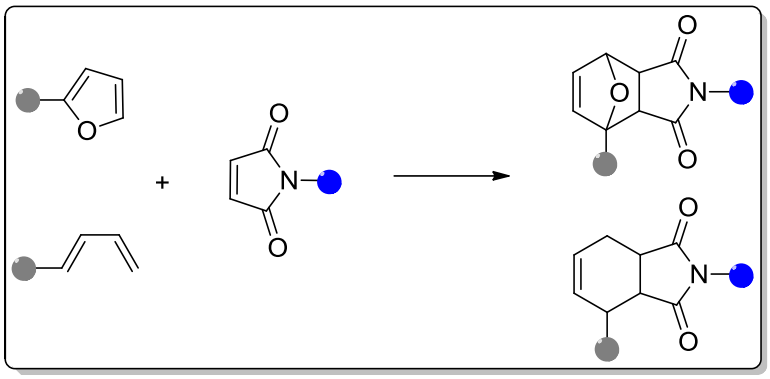
Figure 5: Orbital interactions for the Diels-Alder reaction.

The Diels-Alder reaction is a fascinating organic reaction that allows the formation of not only carbon–carbon bonds but also carbon-heteroatom and heteroatom–heteroatom bonds (hetero-Diels–Alder[79], HDA). The reaction is stereospecific, atom-economic and gives little or no by-products, in addition to these properties, also being chemoselective, catalyst-free and bioorthogonal. So the Diels-Alder reaction fulfills clearly the conditions of "click" chemistry. As a result of this, like its several applications in classical synthetic organic chemistry,[80] DA has also been used for numerous applications in materials science,[81] in the synthesis of dendrimers and polymer,[22] and the conjugation of biomolecules[82].

1.1.2.2 Diels-Alder Reaction in Fluorescence Labeling

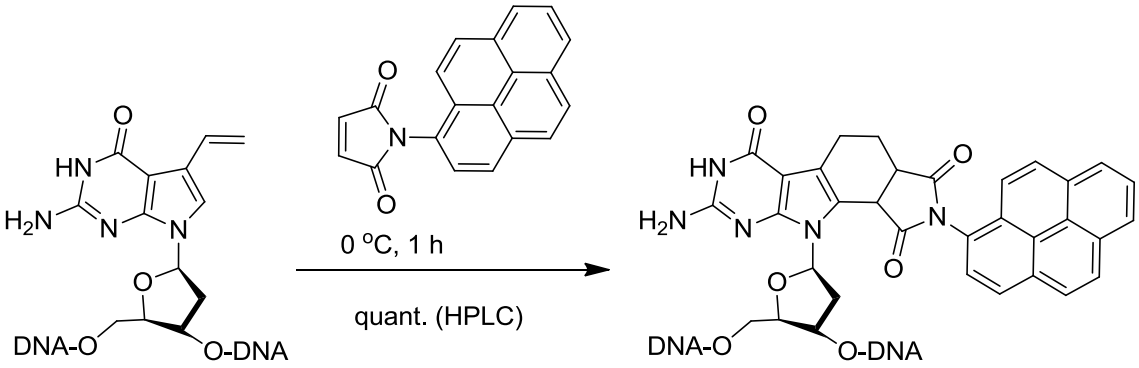
The best alternative of the classical azide-alkyne click reaction is obviously a Diels-Alder reaction by being efficient, atom-economical and fast. Many times, both normal and inverse electron demand Diels-Alder cycloadditions have been used for labeling or functionalization applications. In recent years, rather than electron demand Diels-Alder reaction, an increasing amount of tetrazine based inverse electron demand Diels-Alder addition studies can be found in the literature, because this reaction type is much faster and furthermore precludes the

problem of reversibility which is not wanted in many biological applications.[83] The studies of fluorescent tagging with normal Diels-Alder reactions, generally, are based on the reaction of maleimide dye and a diene moiety, mostly a furan or a butadiene structure (Scheme 5).



Scheme 5: Schematic presentation of commonly preferred normal electron demand Diels-Alder by fluorescence tagging.

In 2001, Hill and coworkers published a post-synthetic route for modified oligonucleotides and conjugated them with different maleimide-substituted dyes via Diels-Alder reaction.[84] In the next year, an interesting approach was studied by Okamoto et al.: Oligonucleotides involving 7-vinyl-7-deaza-2'-deoxyguanosine 3'-phosphoramidite underwent the reaction with maleimide modified pyrene (Scheme 6).[85]



Scheme 6: Diels-Alder addition of vinyl modified deoxyguanosine derivative with N-(1-pyrenyl)maleimide.

In 2002, in a study of Graham and his coworkers, a furan side chain attached to the 5'-end of a synthetic oligonucleotide furnished conjugation with a benzotriazole dye maleimide in the presence of copper(II)nitrate to create active signals in surface enhanced resonance Raman scattering (SERRS). The addition of a catalyst provided shorter reaction time, which is less than 1 hour.[86-87] Then, they modified 5-iodo-2'-deoxyuridine with an acetylenic furan derivative via palladium catalyzed Sonogashira coupling in order to use it in oligonucleotide

labeling.[88] Moreover, Tona and Haner introduced suitable dye maleimides into a 1,3-butadiene-modified DNA hairpin mimic via Diels-Alder reaction. At 20 ^{o}C, after seven days reaction time, the structure of DNA hairpin retained stable.[89-90]

Labeling of peptides and proteins via Diel-Alder addition was introduced by Araujo et al.[91-92] Avidin and/or streptavidin were incorporated with a hexadienyl ester and reacted with different maleimide-activated dyes (dansyl- and fluorescein-maleimide derivatives).

After introduction of tetrazine based inverse electron demand Diels-Alder additions into bioconjugation reactions,[83, 93] the topic of Diels-Alder [4+2] cycloaddition labeling was shifted into this new area. In 2010 an orthogonal system involving both normal and inverse electron demand Diels-Alder reaction was demonstrated by Willems et al. leading to a two-step activity-based labeling of enzymes.[94] A β1-specific proteasome inhibitor was modified by different bioorthogonal click reactions and a BODIPY maleimide derivative was incorporated into the system by normal electron demand Diels-Alder reaction as a fluorescent tag.

1.2 Fluorogenic Conjugations by Formation of Covalent Bonds

Fluorogenic reactions, with the formation of covalent bonds by click reactions have gained great interest in recent years. Especially, azide-alkyne cycloadditions, thiol-ene and Diels-Alder reactions are the most commonly studied bioorthogonal reaction types,[23, 50, 95] used as fluorogenic reactions. Fluorogenic Cu(I) catalyzed Huisgen 1,3-dipolar cycloadditions take place between alkynes and azides of two non-fluorescent molecules to produce highly fluorescent triazole complexes.[4, 96-98] The main disadvantage of this method is the need to use copper(I), which is toxic and not suitable for *in vivo* biological applications.[56] Recently, Jewett *et al.* synthesized a cyclooctyne-fused coumarin derivative which has the ability to make fluorogenic Cu-free click reaction.[99] Another fluorogenic reaction, the thiol-ene click reaction mainly depends on the maleimide group which is added to the structure of dye, directly or with a spacer. The maleimide group acts as an acceptor and it quenches the fluorescence of the dye (donor) by PET (photoinduced electron transfer) mechanism. The Michael addition between the –RSH groups and C=C bond of maleimide inhibits the quenching effect of maleimide moiety and restores the fluorescence.[100-101] A new structural example of this group, based on chromenoquinoline, has been published recently and ICT (intramolecular charge-transfer) was given as a reason for the non-fluorescent effect of maleimide.[102]

1.2.1 Fluorogenic Copper Catalyzed Azide-Alkyne Cycloadditions

The fluorogenic dyes based on the Cu(I) catalyzed azide-alkyne cycloaddition (CuAAC) depend on the quenching effect of the azide group (electron-donating group) or an alkyne group (electron-withdrawing group) connected to the fluorophore. These "click-on" reactions form a triazole ring creating a conjugation to delocalize the local electrons and restores fluorescence.[4] Coumarin,[103-106] 1,8-naphthalimide,[107] anthracene,[108] benzothiazole,[4, 97] BODIPY[98] derivatives as fluorogenic dyes have been synthesized up to date (Figure 6).

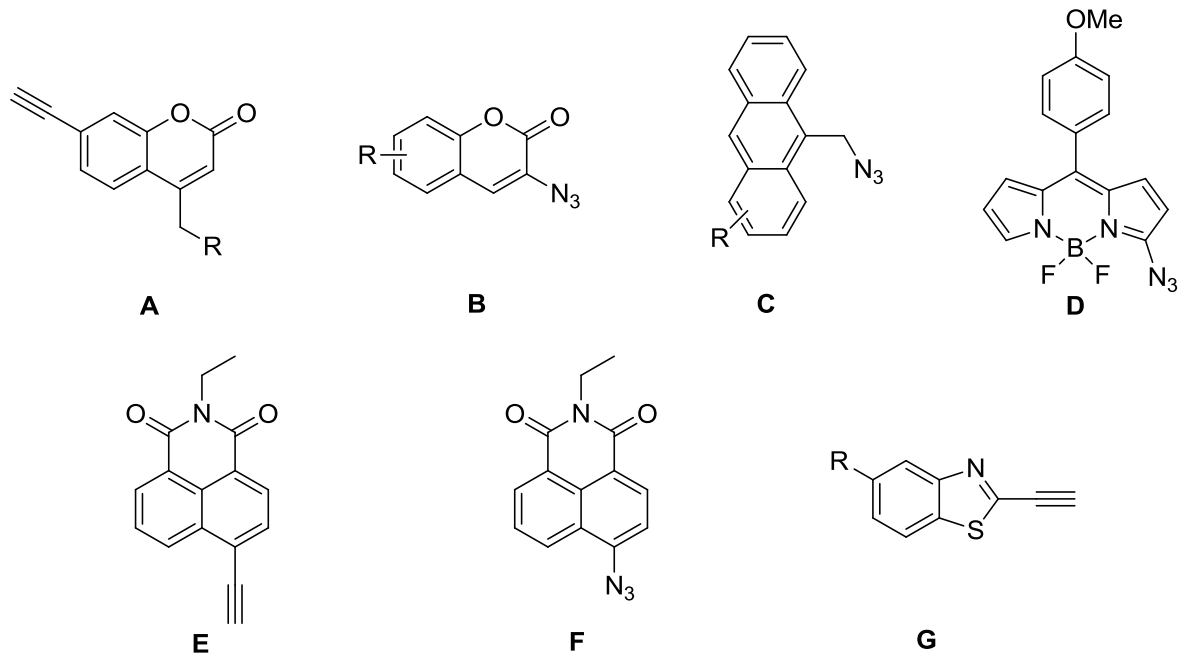


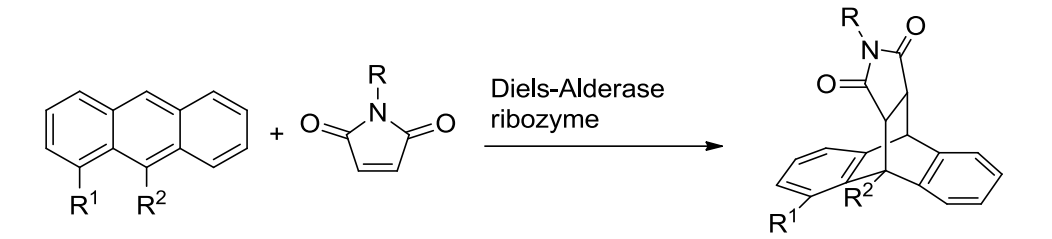
Figure 6: „Click-on" type fluorogenic dyes.

Easy synthesis of alkynes and azides, bioorthogonality and mild conditions are the main advantages of fluorogenic CuAAC and these features provide the ideal conditions for in vitro and in vivo bioconjugation applications. Moreover, fluorogenic CuAAC reactions have been demonstrated to be a versatile tool in the application of combinatorial synthesis of fluorescence dyes, protein labeling, cell imaging and labeling of DNA.[96, 105]

1.2.2 Fluorogenic and/or Fluorescence Quenching Diels-Alder Reaction by Carbon-Carbon Bond Formations

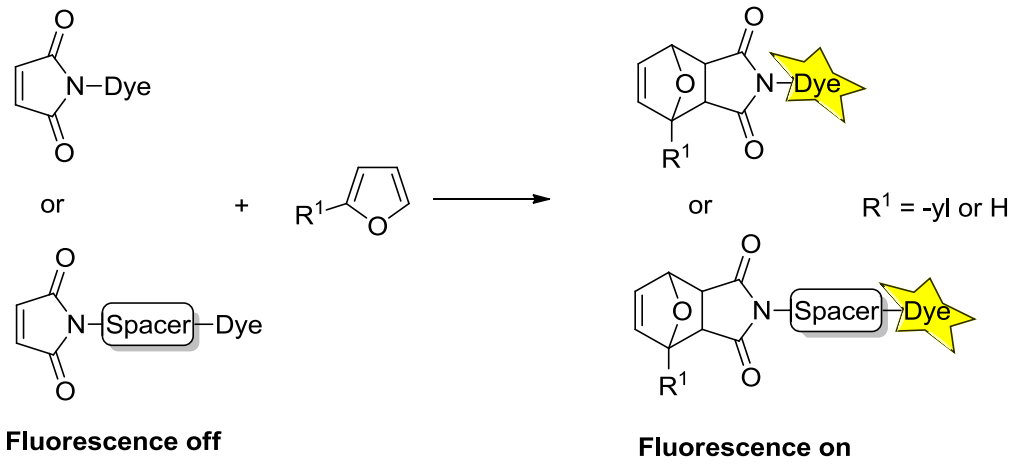
In fluorogenic or fluorescent quenching detections, due to carbon-carbon bond formation, the Diels-Alder reaction has been used several times for catalyst screening,[109-110] the detection of carbon-carbon bond formation,[111-112] studies of fluorescent molecular thermometers[113] and for monitoring of reaction progresses[114-115].

According to these examples, the fluorogenic reactions occur between anthracenes or furans as dienes and N-substituted maleimides as dienophiles. By the work of Nierth et al., the Diels-Alder cycloaddition between anthracene modified BODIPYs and N-pentylmaleimide, catalyzed by Diels-Alderase ribozyme, was used to break the conjugated π-system of anthracene (Scheme 7). After destroying the conjugation of anthracene, fluorescence enhancement was observed. They explained this effect with the strong electron reduction potential of anthracene causing reductive photoinduced electron-transfer (PET) which quenches the fluorescence of the BODIPY.[109]



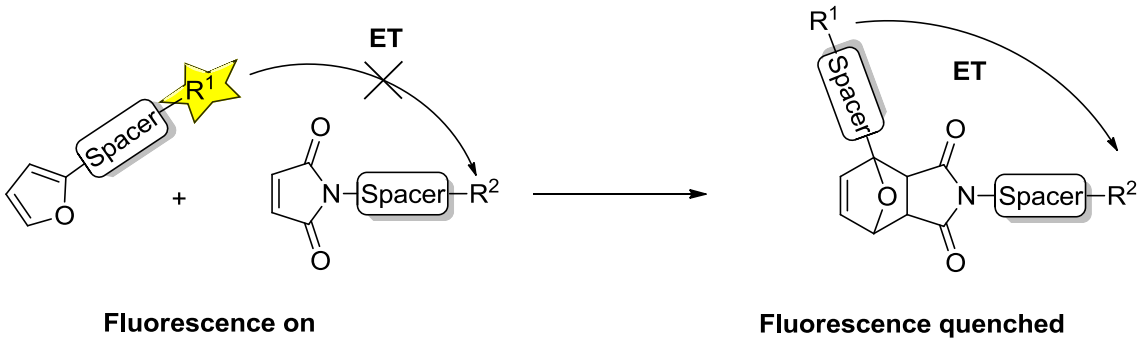
Scheme 7: Fluorogenic ribozyme-catalyzed Diels-Alder reaction between the probes, anthracen-yl-BODIPYs and N-pentylmaleimide.[109-110]

The maleimides' fluorescence quenching effect over PET or ICT was another commonly used phenomenon to create fluorogenic Diels-Alder cycloadditions (Scheme 8). As an interesting example, in 2004, Zhang et al. published the first study of the retro-Diels-Alder reaction between maleimides and furan bringing reversible fluorescence and at the same time, reversible aggregation.[115-116] They used tris(4-maleimidophenyl) amine (TMPA) which is non-fluorescent due to ICT mechanism between donor core and acceptor maleimides. At room temperature, the Diels-Alder reaction between TMPA and furan takes place and the adduct exhibits a strong fluorescence. By heating up to 60 °C, the retro-Diels-Alder reaction is achieved which forms the C=C bond of maleimide and quenches the fluorescence again.



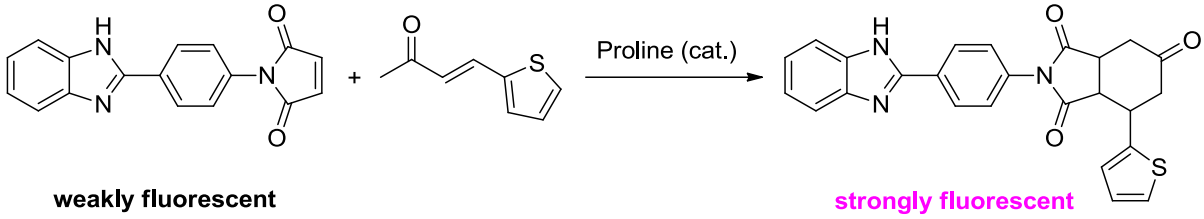
Scheme 8: Schematic representation of fluorogenic Diels-Alder reaction induced by destroying acceptor property of maleimide derivatives connected to a dye (directly or with a spacer).

Other examples were based on combination of two substances to create energy transfer (ET) through space with the intention of fluorescence quenching. In Scheme 9, this method is schematically illustrated to explain the studies of He at el.[111] and Liu et al.[114]. He and coworkers developed a strategy for the detection of a Diels-Alder reaction by quenching the fluorescence of polyfluorene via Diels-Alder reaction of a maleimide modified electron quencher, 1-methyl-4,4-bipyridinium iodide (M–MV^{2+}). One year later, from the same institute, similar work was published by Liu et al. In this work, hybrid nanomaterials consisting of gold nanoparticles (GNPs) and conjugated polymers (CP) were combined to fabricate hybrid nanomaterials. During Diels-Alder addition, they observed spectacular photophysical properties by a quenching effect created by electron transfer and proposed the possibility to use this procedure to monitor the process of the Diels-Alder reaction.



Scheme 9: Quenching of fluorescence through an electron transfer mechanism by formation of Diels-Alder adducts.[111, 114]

Differently, in 2003, Tanaka et al. found that the Diels-Alder addition between the fluorophore possessing a maleimide group and the α,β-unsaturated ketone in the presence of catalytic amount of proline, yields a fluorescent Diels-Alder adduct (Scheme 10). In the publication, it is suggested to use this system to monitor C-C bond formation in the Diels-Alder reaction.[112] In terms of cycloaddition, this approach is interesting, because of usage of a catalyst and an α,β-unsaturated ketone as the diene. The possibility to use wide variety of dienes enables chemists to consider new applications of fluorogenic reactions.

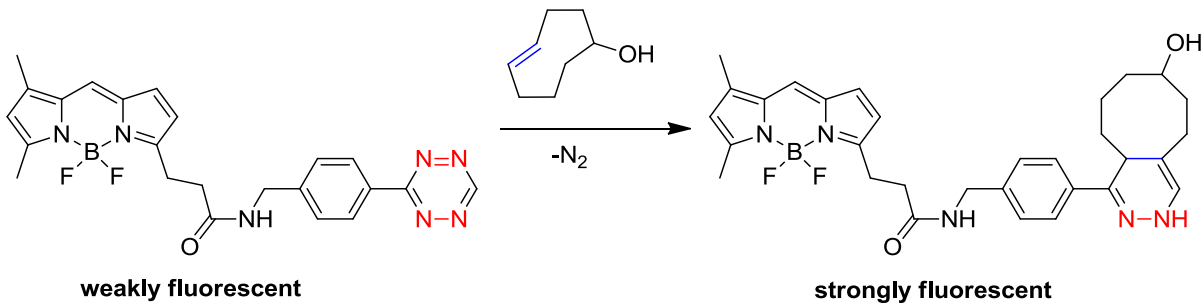


Scheme 10: Fluorogenic detection method to monitor C-C bond formation developed by Tanaka et al.

1.2.3 Fluorogenic and/or Fluorescence quenching by Heteroatom-Carbon Bond Formations via Diels-Alder Reaction

The inverse electron demand Diels-Alder cycloaddition between 1,2,4,5-tetrazines and strained dienophiles (norbornene, cyclooctyne, and trans-cyclooctene, etc.) has gained immense importance after discovery of its bioorthogonal coupling applications in 2008.[83, 93] These reactions are crucially fast compared to the other bioorthogonal coupling reactions which also do not need catalyst. Moreover, it is easy to synthesize the starting agents and the reaction can be performed in aqueous environments.[117]

Additionally to these essential features, Weissleder and coworkers recognized the tetrazines' quenching effect of some covalently attached fluorophores. The fluorescence is regenerated after reaction with trans-cyclooctene (Scheme 11).[118]



Scheme 11: The inverse electron demand Diels-Alder cycloaddition between tetrazine-BODIPY FL and trans-cyclooctenol.[118]

Tetrazine conjugates of commercial dyes, BODIPY FL, BODIPY TMR-X, and Oregon Green 488 showed quite effective quenching. After reaction with trans-cyclooctene, between 15- and 20-fold enhancements of fluorescence were observed. The result of these studies was that the dyes emitting between 510 and 570 nm, like the examples, can efficiently be quenched by tetrazine.[117]

1.3 Introduction to BODIPY Dyes

4,4-Difluoro-4-bora-3a,4a-diaza-s-indacene (BODIPY) dyes were first discovered in 1968 by Treibs and Kreuzer.[119] Ever since, the importance of these versatile organic dyes was recognized by chemists and biochemists because of their magnificent properties, like high molar extinction coefficients, excellent fluorescence quantum yields and narrow peaks of absorption and emission at visible light. Due to these properties, BODIPY dyes have been widely used in the application of monitoring biomolecules in living environments.[120] Moreover, several studies were carried out by attaching BODIPY derivatives to proteins,[121-122] lipids, DNA,[123-124] carbohydrates,[125] etc. Except biological applications, also numerous applications for materials sciences have been studied in the field of optoelectronic devices,[126-128] light-harvesting materials,[129-130] and optical chemosensors.[131-134]

1.3.1 General Properties of BODIPY Derivatives

BODIPY dyes consist of difluoroboron chelated half porphyrin structure and this chelation provides rigidity of the structure (Figure 7).[135]

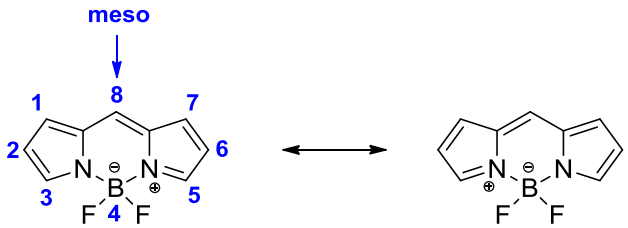


Figure 7: The IUPAC numbering system of BODIPY and its delocalization.

The general optical properties of BODIPY dyes are influenced strongly by the substituents attached to the core. Especially 3-,5- and meso positions are very sensitive to the substituent type.[96, 135] It is very easy to change BODIPY's properties including fluorescence quantum yield (λ_F), fluorescence lifetime (τ_F), molar absorption coefficient (ε_{max}), fluorescence (λ_{fl}) and absorption maximum (λ_{abs}) by electron withdrawing and/or donating groups.

Another important feature of BODIPYs is that the self-aggregation of BODIPY dyes in solution is very little and negligible, compared to other organic dyes such as rhodamine 6G.

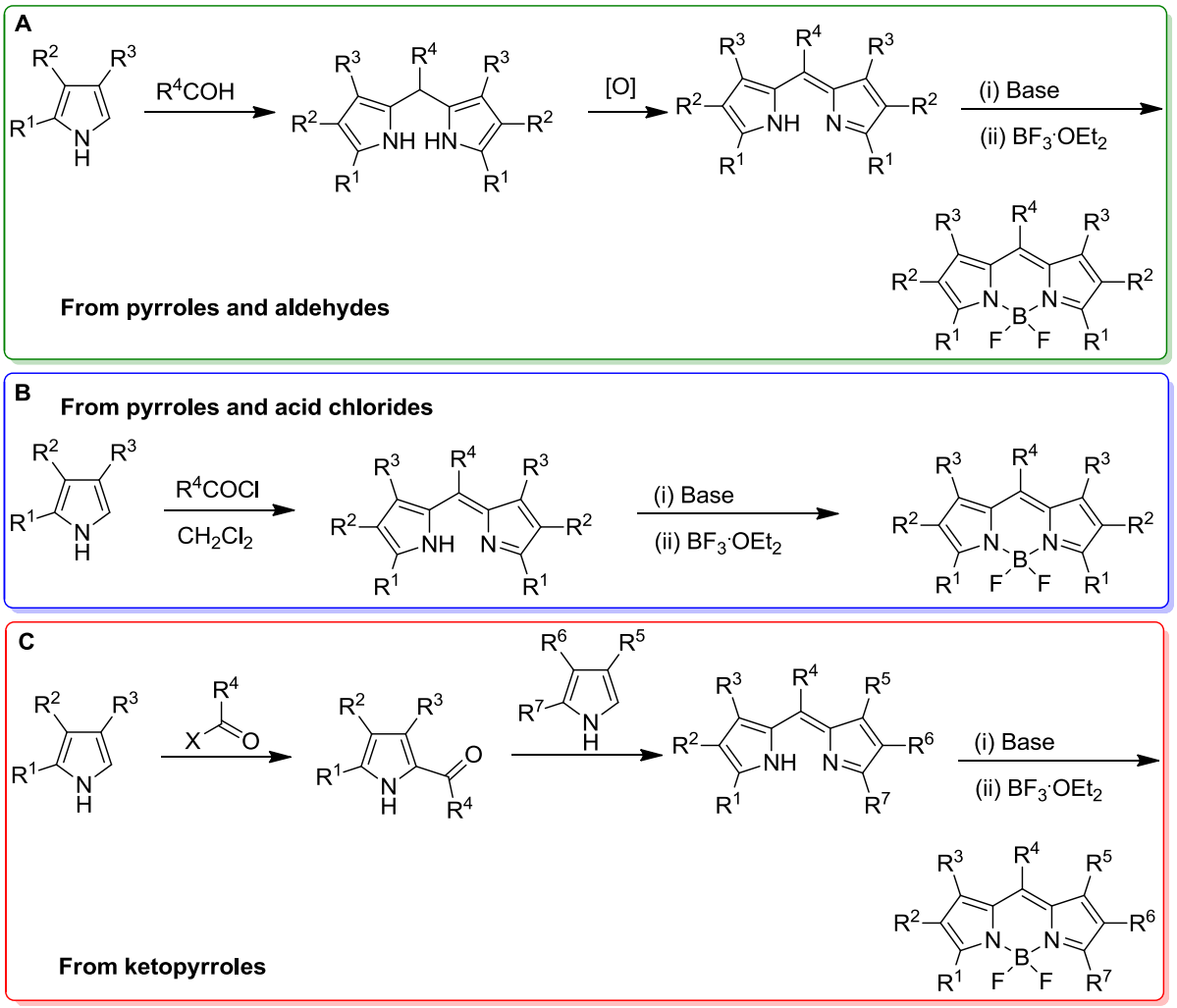
BODIPY fluorophores disclose generally narrow absorption and emission peaks. Both λ_{abs} and λ_{fl} values are usually greater than 500 nm. The increasing number of studies for creating BODIPY derivatives that have higher values than 600 nm provides adequate options for biological labeling. Moreover, generally, BODIPY derivatives present close values of fluorescence and absorption maxima that means small Stokes shifts.

1.3.2 General Synthesis of BODIPY Dyes

The synthetic procedure of BODIPY core starts with a condensation reaction of a simple pyrrole and a highly electrophilic carbonyl substance such as an aldehyde, an acid anhydride or an acyl chloride. However, due to the unstable character of unsubstituted dipyrromethenes, 2-substituted pyrroles are preferred to avoid rapid polymerization or porphyrin formation. There are three main synthetic procedures for the BODIPY family: i) starting from pyrroles and aldehydes, ii) from pyrroles and acid chlorides and iii) from ketopyrroles.[136]

The first method to synthesize BODIPYs contains the condensation of aromatic aldehydes with pyrroles and oxidation to form dipyrromethenes. Mostly in the same pot (one-pot process), the addition of a tertiary amine, followed by $BF_3{\cdot}OEt_2$ (boron trifluoride diethyl etherate) gives BODIPYs (Scheme 12-A). Commonly, DDQ (2,3-dichloro5,6-dicyano-p-benzoquinone) is chosen as the oxidizing agent. For example, with this method, α,β-unsubstituted BODIPYs can be prepared under mild conditions.[137]

The second method to synthesize a BODIPY derivative depended on the condensation of an acyl chloride with a pyrrole to give dipyrromethene and addition of Et_3N, followed by $BF_3{\cdot}OEt_2$ to yield BODIPY.[136] Today, this is another popular method to obtain the BODIPY structures (Scheme 12-B). 8-Substituted BODIPY dyes can be prepared easily with this approach.



Scheme 12: General synthetic approaches of BODIPY dyes.

The first two synthetic approaches allow the preparation of symmetrically substituted BODIPYs. For asymmetrically substituted BODIPY derivatives, it is wise to modify pyrroles to yield ketopyrroles and then incorporate these structures into a Lewis acid mediated condensation with another pyrrole derivative. After obtaining dipyrromethene structure, reaction with $BF_3{\cdot}OEt_2$ in the presence of Et_3N furnishes asymmetric BODIPYs (Scheme 12-C).

1.4 Aims of the Thesis

Fluorogenic dyes are difficult to design, because of the lack of knowledge in the logic of these systems. The fluorogenic approaches are mostly achieved by several trials and it can be said that luck is an effective factor. This is the main reason that only a few number of fluorogenic dye families are available. However, fluorogenic dyes are used to avoid background noise of fluorescence measurements and to get rid of some purification steps in analytical methods.

In this thesis, the synthesis of novel phencyclone derivatives incorporating fluorogenic Diels-Alder reactivity was envisioned, as there are not many examples of Diel-Alder based fluorogenic reactions. Due to its versatile, efficient and selective character, the search for a fluorogenic Diels-Alder reaction is a major need for bioorthogonal applications. The photophysical and spectroscopic properties of the Diels-Alder adducts will be investigated in detail.

BODIPY dyes are one of the most important classes of organic fluorophores and their visible light absorption and emission characters allow in vivo and in vitro applications. Fluorogenic BODIPY derivatives can be very effective agents for monitoring biological systems. The synthesis of ortho-, meta-, para-azido substituted BODIPY derivatives was another aim of the thesis. A new synthetic approach should be introduced to achieve these derivatives. Some ortho-substituted phenyl BODIPY derivatives are known which demonstrate fluorogenic reactions. With this in mind, the ortho-azido substituted BODIPY derivative has the possibility to show lowest fluorescence quantum efficiency and moreover, the triazole formation via click reaction with alkyne carrying substances should enhance the fluorescence of this substance.

The search for BODIPY derivatives should also include another class of BODIPY dyes: nitro and amino BODIPYs. It is known that the character of the substituent at the 3- or 5-position of the BODIPYs has efficient influence on the fluorescence properties of the fluorophore. Therefore 2-nitro, 3-nitro and amino substituted BODIPY derivatives should be synthesized and their optical properties examined.

2 Diels-Alder Reaction of Furo[3,4-c]coumarin and Phencyclone Derivatives

2.1 Introduction and Motivation

Fluorophores contain fused aromatic groups or planar multiple π bonds. The conjugation of these connected p-orbitals with delocalized electrons allows fluorophores to absorb and re-emit light upon light excitation. Destroying of the conjugation also disrupts fluorescence of the fluorophore. For example, rhodamine, widely used as a fluorescent probe, presents reversible non-fluorescent (lactone form) to fluorescent (zwitterionic form) conversion by lactone ring opening causing conjugation of the chromophoric groups (Figure 8)[138].

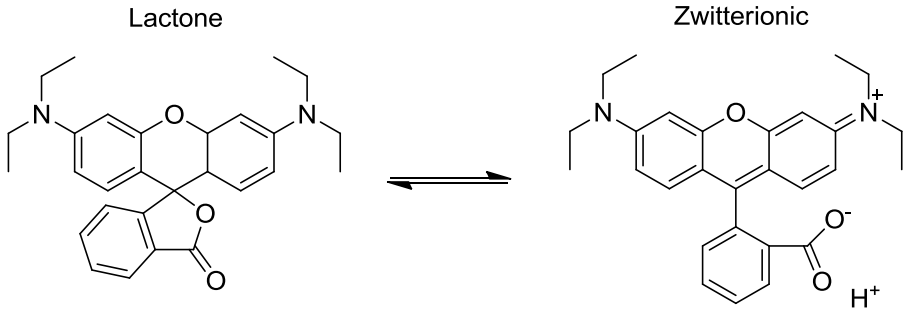


Figure 8: Two forms of rhodamine B as an example of lactone ring opening of rhodamines.

The same logic can be also visualized with the Diels-Alder reaction of anthracene. There are generally two possibilities; loss of fluorescence by Diels-Alder reaction and generation of fluorescence by retro-Diels-Alder reaction. By Diels-Alder addition, in one of the simplest fluorophore, anthracene's central ring reacts as a diene, because the resulting products has two fully aromatic benzene rings on both sides (Figure 9). The Diels-Alder reaction destroys the conjugation of anthracene and the fluorescence of it is not observed anymore.

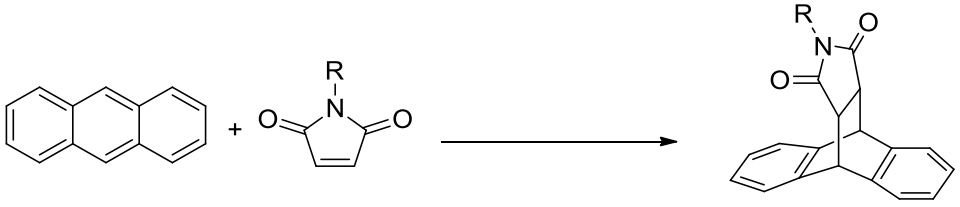


Figure 9: The schematic presentation of Diels-Alder reaction of anthracene.

Moreover, the retro-Diels-Alder provides the restoration of fluorescence. An interesting example of this process is the release of nitroxyl (HNO) from an anthracene based prodrug via

retro-Diels-Alder reaction catalyzed by the catalytic antibody, Ab-9D9 (Figure 10)[139-140]. The fluorescence release of anthracene derivative allows the detection of HNO by screening of fluorescence in cell culture.

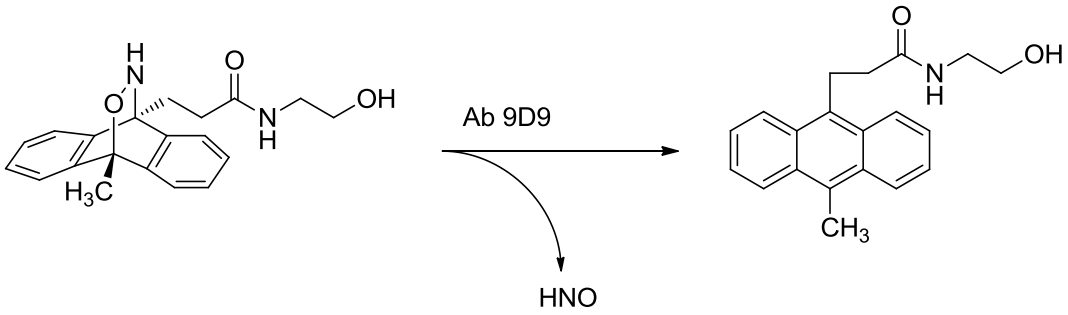


Figure 10: An example of application of fluorogenic retro-Diels-Alder reaction.

The main idea of this chapter is to create a system for "conjugation on-fluorescence on" types of fluorogenic Diels-Alder reaction. A diene directly connected to the core of a fluorophore can be used to serve as a an auxiliary for fluorogenic Diels-Alder reaction (Figure 11). The Diels-Alder reaction leads to completion of conjugation. For this purpose, furan as the diene connected to the core of coumarin derivatives is used in the first part of this chapter. The Diels-Alder reactions of coumarin based dienes are executed and their characterizations and optical properties are discussed. In the second part, the synthesis of novel phencyclone derivatives that serve as dienes in successful fluorogenic Diels-Alder reaction is described. In the same part, also, maleimide side-chain containing nucleosides are introduced as models for dienophiles in this fluorogenic Diels-Alder with potential use for biological applications. The complicated NMR-spectroscopic analysis are explained in detail. UV-vis spectroscopy, fluorescence spectroscopy and quantum efficiency measurements as optical properties of the Diels-Alder adducts also were studied.

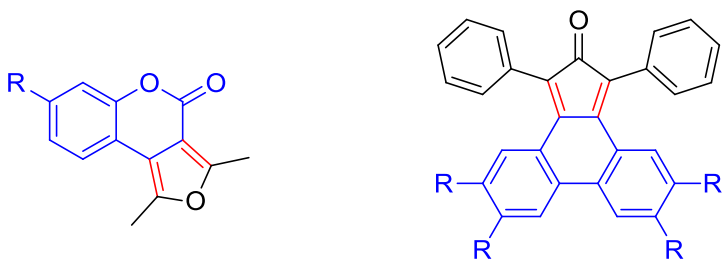
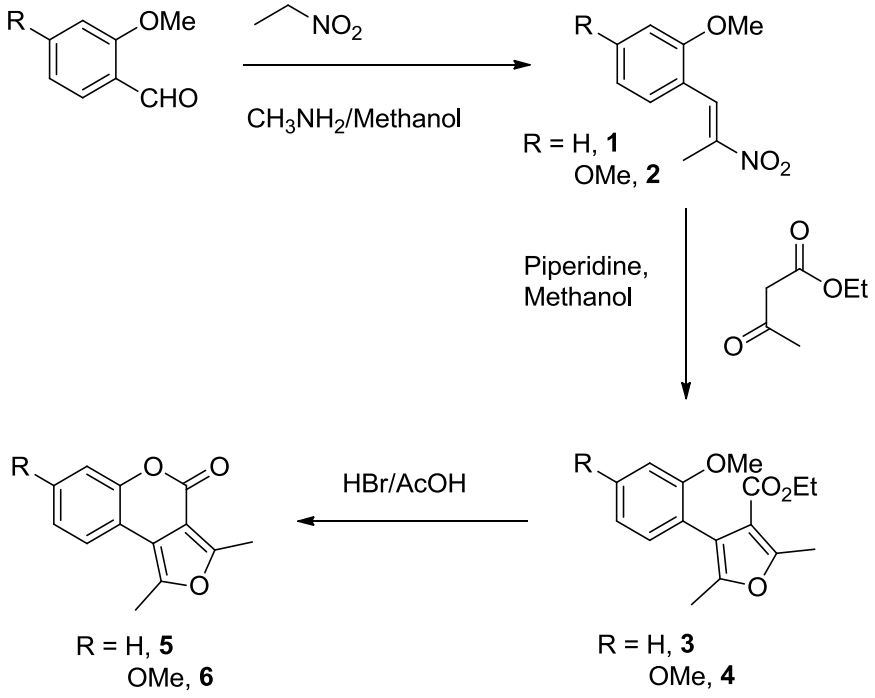


Figure 11: The dienes as the precursors of Diels-Alder additions in order to increase the conjugation of the fluorophore cores.

2.2 Coumarin Derivatives

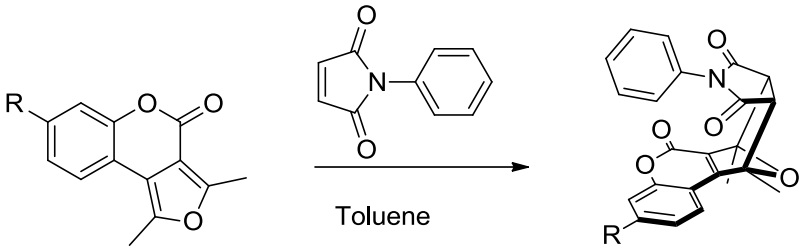
2.2.1 Synthesis of Furo[3,4-c]coumarins and Their Diels-Alder Adducts

The main goal of this study is to create some coumarin derivatives acting as dienes to see the effect of the Diels-Alder reaction which changes the molecular structure of the fluorescence core. The best choice in order to achieve a coumarin structure having a diene function fused with the lactone ring of coumarin, seemed to be furo[3,4-c]coumarins. Brahmbhatt et al.[141] have already published a synthetic route to such derivatives (Scheme 13). According to their strategy, the first step is the synthesis of 1-aryl-2-nitro-prop-1-ene derivatives. In a simple reaction procedure, equivalent amounts of phenylnitromethane and methoxy-benzaldehyde derivatives were mixed in a flask in the presence of methanolic methylamine solution and the flask was placed in the refrigerator for 15 h.[142] The crystal product was filtered, washed with ether, dried and the product was pure enough for the next step. The compound **1** was achieved in 93% yield and compound **2** in 83% yield. 3-Substituted-4-ethoxycarbonyl furans were then formed by the base catalyzed Nef reaction between the corresponding 1-aryl-2-nitro-prop-1-ene and ethyl acetoacetate in refluxing methanol containing catalytic amount of piperidine, giving compound **3** in 40% and compound **4** in 44% yield. Finally, by a demethylation-cyclization reaction of the 3-substituted-4-ethoxycarbonyl furans led to the desired furo[3,4-c]coumarins, for compound **5** in 50% and compound **6** in 46% yield.



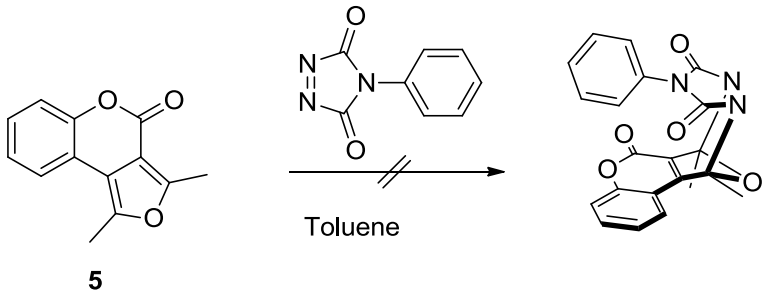
Scheme 13: Synthesis of furo[3,4-c]coumarins.

To test the Diels-Alder reactivity of the furo[3,4-c]coumarins as dienes, their reactions with N-phenylmaleimide as dienophile were examined. We first synthesized compound **5,** the easiest synthesized and cheapest derivative of these coumarins. In order to increase the expected fluorescence of these structures, the methoxy derivative **6** was also used. As depicted in Scheme 14, the reaction between furo[3,4-c]coumarins and N-phenylmaleimide leads to the adducts in toluene, approximately in half an hour and quantitively. Isolation of the products could be achieved by the addition of hexane causing precipitation of product.



Scheme 14: The Diels-Alder reactions of furo[3,4-c]coumarins and N-phenylmaleimide.

However, in the same reaction, 4-phenyl-1,2,4-triazole-3,5-dione ,PTAD, induced only some decomposition products (Scheme 15), despite the fact that PTAD is one of the strongest dienophiles.[143] But it is known that it reacts in some situations in an unexpected way. Jones, D.W.[144] explained the Diels-Alder reaction between 1,3-dimesitylisobenzofuran and PTAD as a "foiled" Diels-Alder addition, because the resulting compound was a zwitterion. As an assumption, this could also be the case in Diels-Alder reaction between the furo[3,4-c]coumarins and PTAD.



Scheme 15: The schematic presentation of Diels-Alder reaction of furo[3,4-c]coumarin **5** and PTAD which induced only some decomposition products.

The products of the reactions of furo[3,4-c]coumarins and N-phenylmaleimide were investigated by mass spectrometry and NMR spectroscopy. The ^{1}H-NMR spectrum of the compound **7** in $CDCl_3$ shows two singlets for the methyl groups (protons H-3 and H-4) at δ =

2.25 and 2.13 ppm (Figure 12). The doublets at 3.24 and 3.15 with ^{3}J = 6.6 Hz can be assigned to the protons H-1 and H-2. Moreover, beside the aromatic protons of the phenyl group and the coumarin core, the protons H-5 and H-6 can be observed more downfield shifted due to their high electron density environment. The ^{13}C-NMR of the compound **7** reveals the primary methyl carbons at δ = 16.87, 14.66 ppm, carbons C-1 and C-2 at δ = 52.84, 52.40 ppm and the aromatic carbon atoms' peaks at δ = 156.19, 155.52, 132.72, 132.27, 131.58, 129.21, 128.94, 126.47, 124.80, 124.01, 117.95, 114.45 ppm and two peaks for the carbonyl groups at δ = 172.22, 159.97 ppm, as expected. Additionally, the two quaternary carbons arise at δ = 87.82, 86.95 ppm and proved by Dept-135. The molecular integrity of **7** is also verified by mass spectrometric investigation with a molar peak as [M + Na]$^{+}$ at m/z = 410.09.

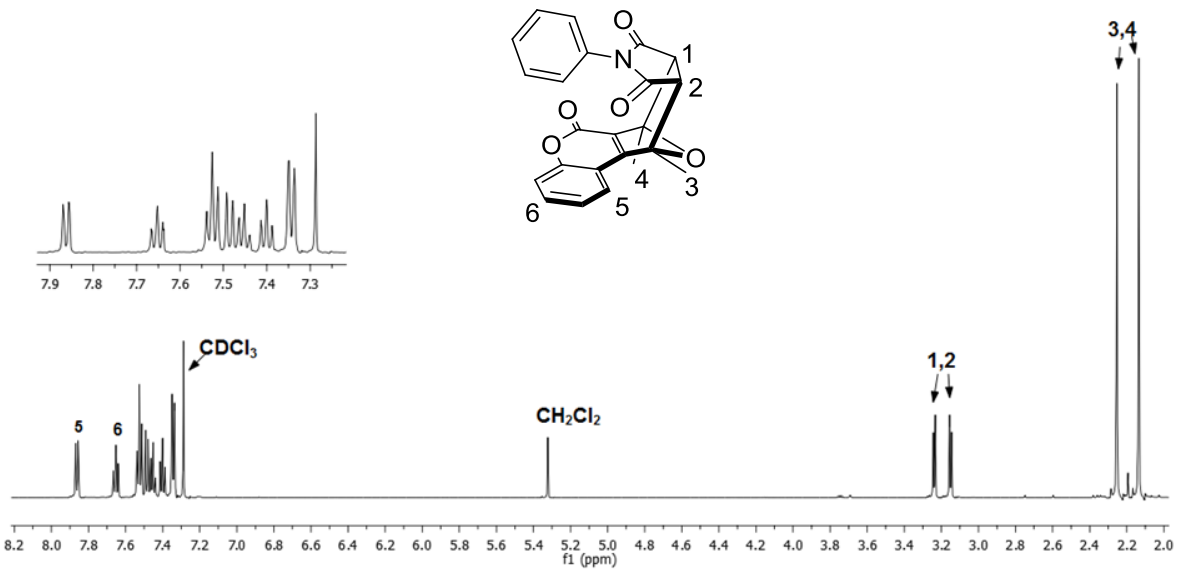


Figure 12: ^{1}H-NMR of **7** in $CDCl_3$.

In the ^{1}H-NMR spectrum of the compound **8** in $CDCl_3$, two singlets for the methyl protons H-3 and H-4 can be seen at δ = 2.21 and 2.11 ppm and two dublets of protons H-1 and H-2 at δ = 3.23 and 3.12 ppm with ^{3}J = 6.6 Hz, the same as **7** (Figure 13). Here, as the main structural difference, the -OCH_3 protons can be observed at δ = 3.93 ppm as a singlet. The electron-donating properties of the methoxy group makes the aromatic neighbour protons more upfield shifted and protons H-6 and H-7 are found at δ = 6.96 ppm. Moreover, H-5 can be assigned to a multiplet at δ = 7.78 – 7.71 ppm and the other aromatic protons are found between these two signals as different multiplets, as expected. The ^{13}C-NMR of the compound **8** shows the primary methyl carbons at δ = 16.84, 14.72 ppm, carbons C-1 and C-2 at δ = 53.08, 52.47 ppm, aromatic carbon atoms' peak at δ = 160.25, 157.65, 156.51, 131.61, 129.18, 128.89,

128.39, 126.47, 124.94, 113.28, 107.80, 101.69 ppm and two peaks for carbonyl groups at δ = 172.60, 172.39, 163.48 ppm, as expected. The methoxy groups' primary carbon can be associated with a signal at δ = 55.89 ppm. Additionally, the two quaternary carbons arise at δ = 87.73, 86.87 ppm and proved by Dept135. Moreover, the high resolution mass analysis discloses a correct molecular peak at $[M + Na]^+$ at m/z = 440.1105.

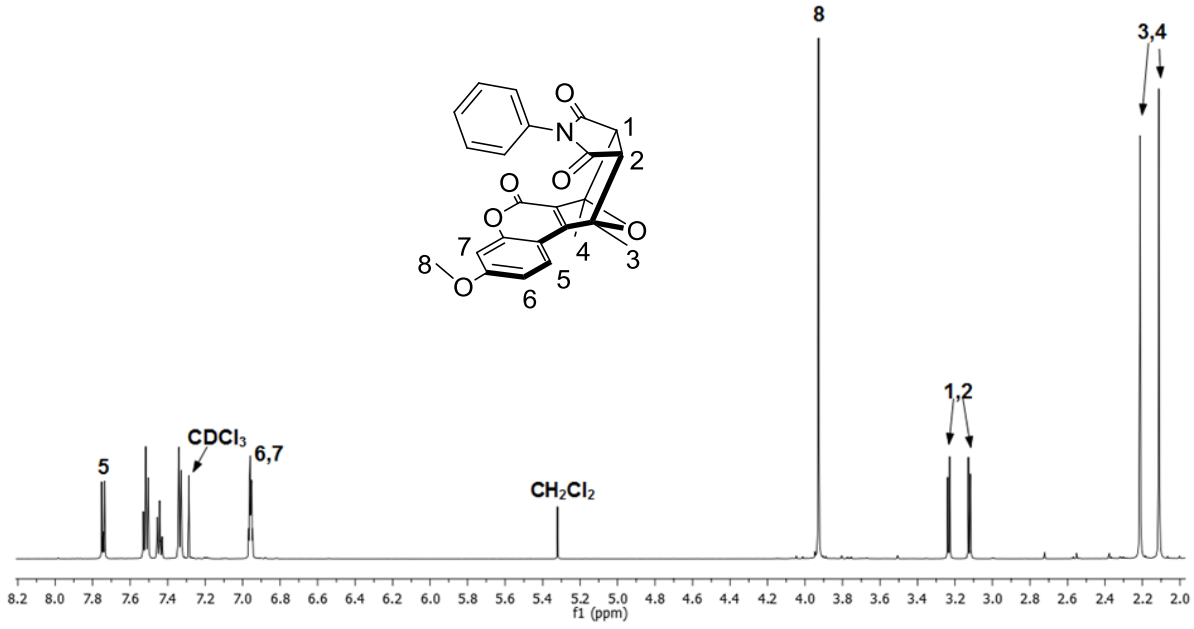


Figure 13: ^{1}H-NMR of **8** in $CDCl_3$.

2.2.2 Optical Properties of Furo[3,4-c]coumarin Adducts

The pre-investigation was made both during and after reactions via TLC under a handheld UV-lamp, but the results were not as expected. Unfortunately, the furo[3,4-c]coumarin derivatives **5** and **6** were also fluorescent and after the Diels-Alder reaction, the adducts **7** and **8** were not stronger fluorescent than the starting compounds **5** and **6** by naked eye observation. Moreover, unexpectedly, both reagents and adducts show really weak fluorescent under UV radiation. The addition of the electron-donating group, -OCH_3 at 7-position of coumarin derivatives should increase the fluorescence efficiency.[145] However, here, it did not show an effective difference and even derivative **8** barely exhibited fluorescence under UV lamp.

Even so, the optical properties of two different systems – the dienes and the Diels-Alder adducts were worth to investigate. The optical properties of these molecules were evaluated in CH_2Cl_2.

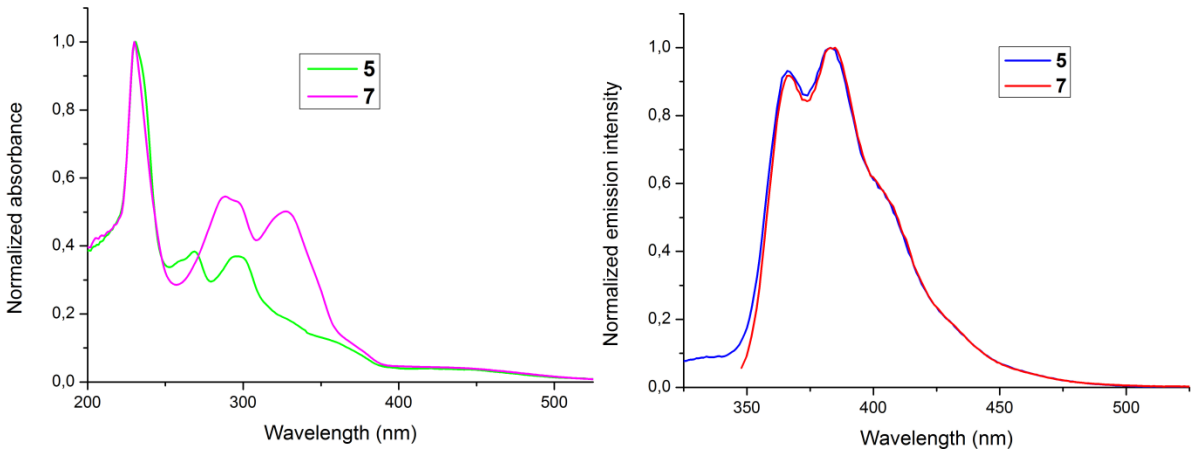


Figure 14: Normalized absorption spectra of **5** and **7** (left) and their emission spectra (λex = 266 nm) (right) at 1.0 x 10^{-5} M.

Generally speaking, λ_{max} of absorbance and emission spectra of the synthesized compounds are at shorter wavelength compared to the general characteristics of coumarin derivatives.[146] As seen in the UV-vis spectra of **5** and **7**, the Diels-Alder addition of N-phenylmaleimide caused a bathochromic shift which is explained by extended conjugation of the system (Figure 14). The same situation is also available in the spectra of **6** and **8**, too (Figure 15).

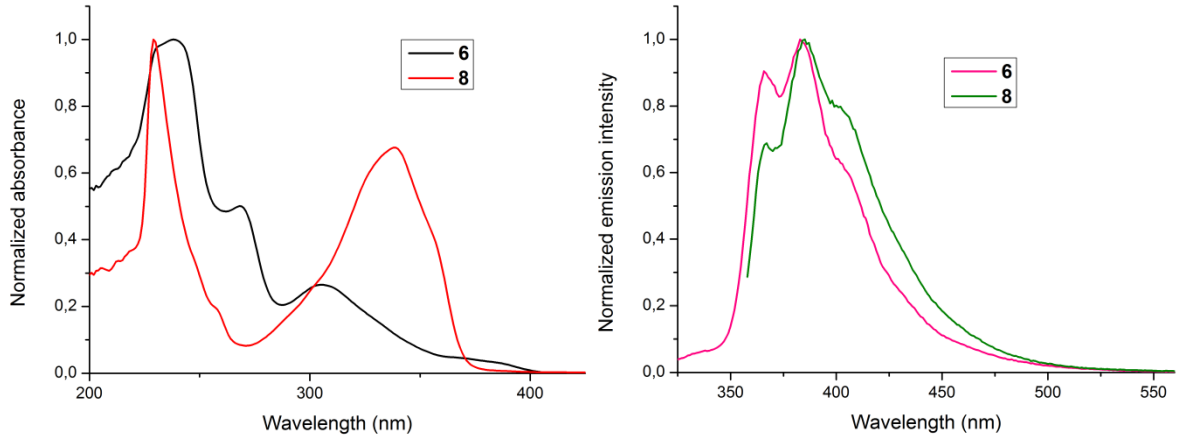


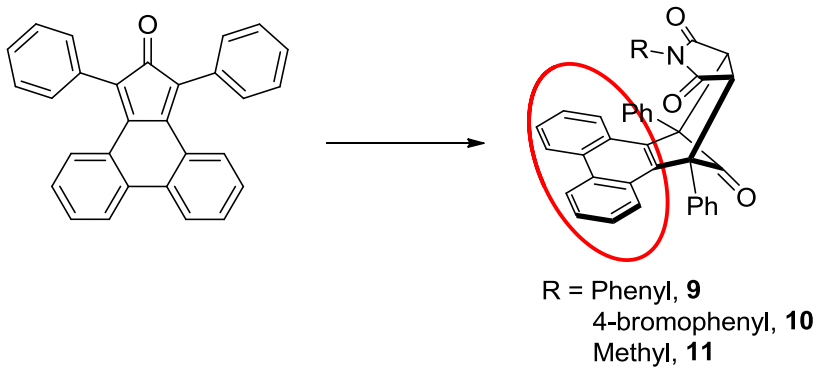
Figure 15: Normalized absorption spectra of **6** and **8** (left) and their emission spectra (λex = 266 nm) (right) at 1.0 x 10^{-5} M.

However, in the emission spectra of both groups, there is not a distinctive difference between the spectra of furocoumarins and their Diels-Alder adducts (Figure 14 and Figure 15).

2.3 Phencyclone Derivatives

After the unexpected optical results of Diels-Alder adducts of the synthesized coumarin derivatives, we started to search for another alternative in order to create fluorogenic Diels-Alder reaction. The most appropriate alternative would be phencyclone derivatives due to their convenient structures and properties.

Phencyclone is a strong diene in Diels–Alder reactions, and can react via [4+2] cycloaddition with a wide range of dienophiles. Both electron-poor (normal Diels-Alder reaction) as well as electron-rich dienophiles (inverse-electron-demand Diels–Alder reaction)[147] can react with phencyclone, leading to Diels-Alder adducts of phencyclone that contain a phenanthrene core (Scheme 16).



Scheme 16: The Diels-Alder reaction of phencyclone.

In this part, our purpose is to obtain phenanthrene derivatives as fluorescence markers and phencyclone derivatives are the most appropriate candidates to use as a Diels-Alder reaction based fluorescent markers. However, the phencyclone, itself, shows no fluorescent and its Diels-Alders adducts exhibit extremely weak fluorescence (Figure 16).

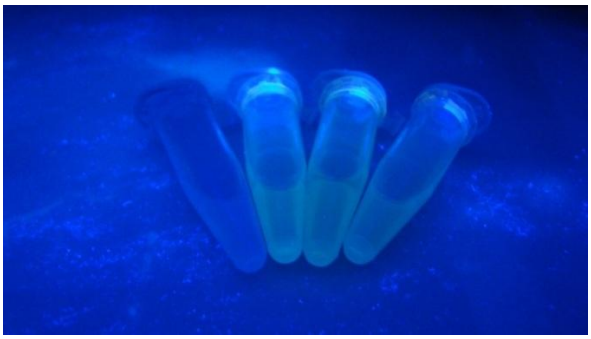
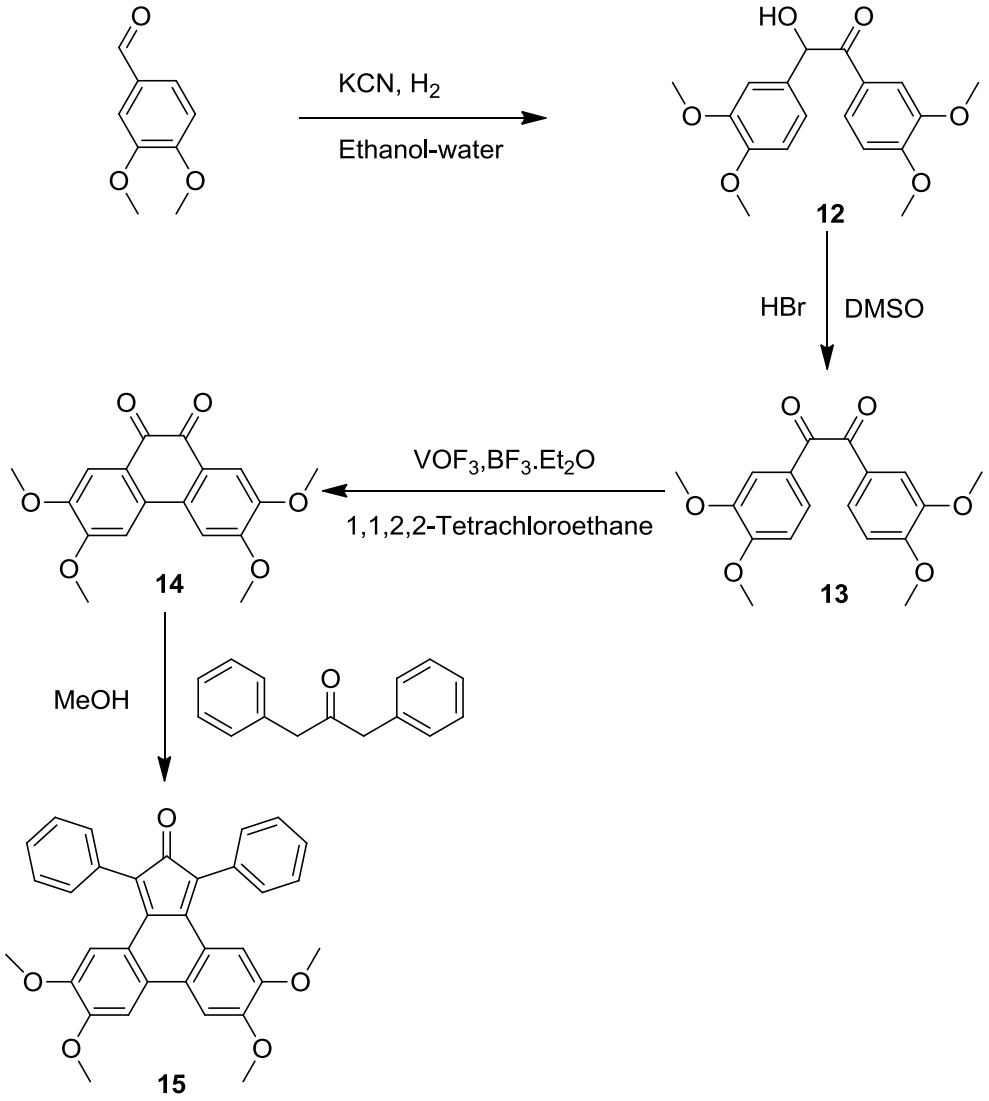


Figure 16: The picture of phencyclone adducts under UV light (in CH_2Cl_2).

The addition of –R groups at 2,3 and 6,7-positions of phencyclone was expected to give better fluorescence properties and some derivatives were synthesized.

2.3.1 Synthesis of Phencyclone Derivatives and Their Diels-Alder Adducts

The synthetic route of compound **15** can be seen in Scheme 17. The reaction sequence started with the acyloin reaction of veratraldeyhde and KCN in ethanol-water mixture as solvent. The reaction mixture was bubbled with H_2 gas while it was heated to reflux for 5 h to obtain veratroin **12** in 50% yield.[148] The addition product was further oxidized with DMSO/HBr to obtain compound **13** in 90% yield.[149] For the intramolecular coupling reaction, we used the procedure of Mohr, et.al.[150] In the paper, it was proved that the usage of 1,1,2,2-tetrachloroethane as a solvent was necessary to increase the yield for the oxidative reaction between VOF_3, $BF_3.Et_2O$ and compound **13** leading compound **14** in 79% yield. The Knoevenagel condensation reaction between compound **14** and 1,3-diphenylacetone[151] finally generated the target compound **15** as a dark green solid in 34% yield.



Scheme 17: Synthesis of compound **15**.

In the ^{1}H-NMR spectrum of **15**, two impurity peaks in the aliphatic region could not be removed even after several precipitations. Nevertheless, the respective peaks for compound **15** can be clearly observed. As shown in Figure 17, the protons H-1 and H-2 of the methyl groups of **15** can be attributed to the peaks (δ = 3.98 and 3.33 ppm) in the aliphatic region of the ^{1}H-NMR spectrum. In the aromatic region, the more upfield shifted two singlets obviously belong to protons H-3 (δ = 6.96 ppm) and H-4 (δ = 7.03 ppm), due to the electron-donating effect of neighboring methoxy groups. Protons H-3 are more downfield shifted compared to protons H-4 because of deshielding by the aromatic rings. Lastly, the ten phenyl protons can be associated to the multiplet between δ = 7.47-7.33 ppm. In the ^{13}C-NMR of the compound **15**, two peaks for the methyl groups can be observed at δ = 55.99, 55.11 ppm. The electron-donating methoxy groups are differentiating the neighbouring carbons C-3 and C-4 compared to other aromatic peaks as more upfielded at δ = 111.38, 106.49 ppm. Other aromatic carbons can be observed at δ = 151.63, 134.14, 130.30, 128.51, 127.85, 121.79, 121.43 ppm. At last, the carbonyl carbon can be attributed to the peak at δ = 200.51 ppm.

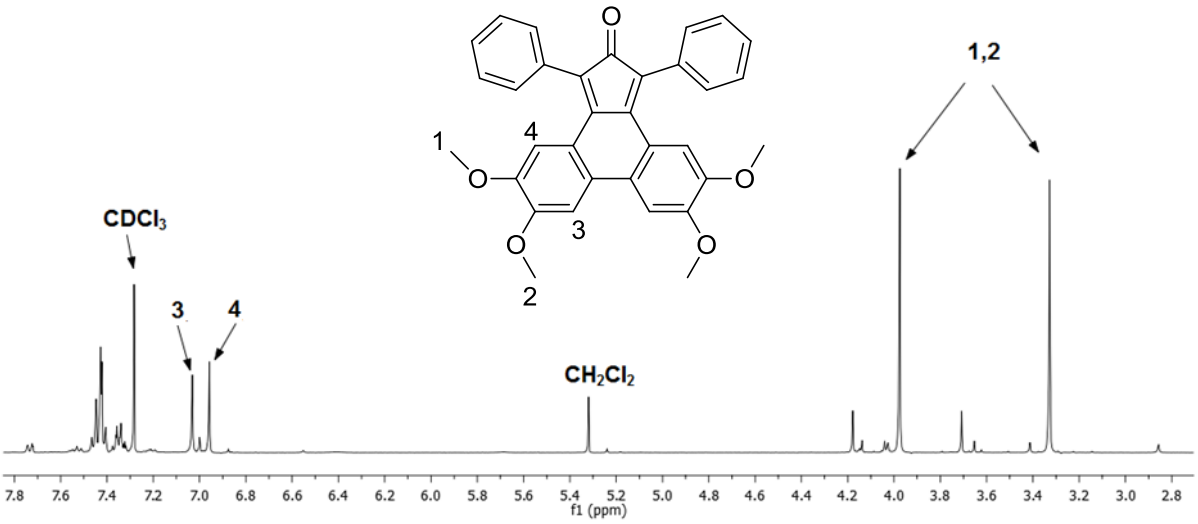
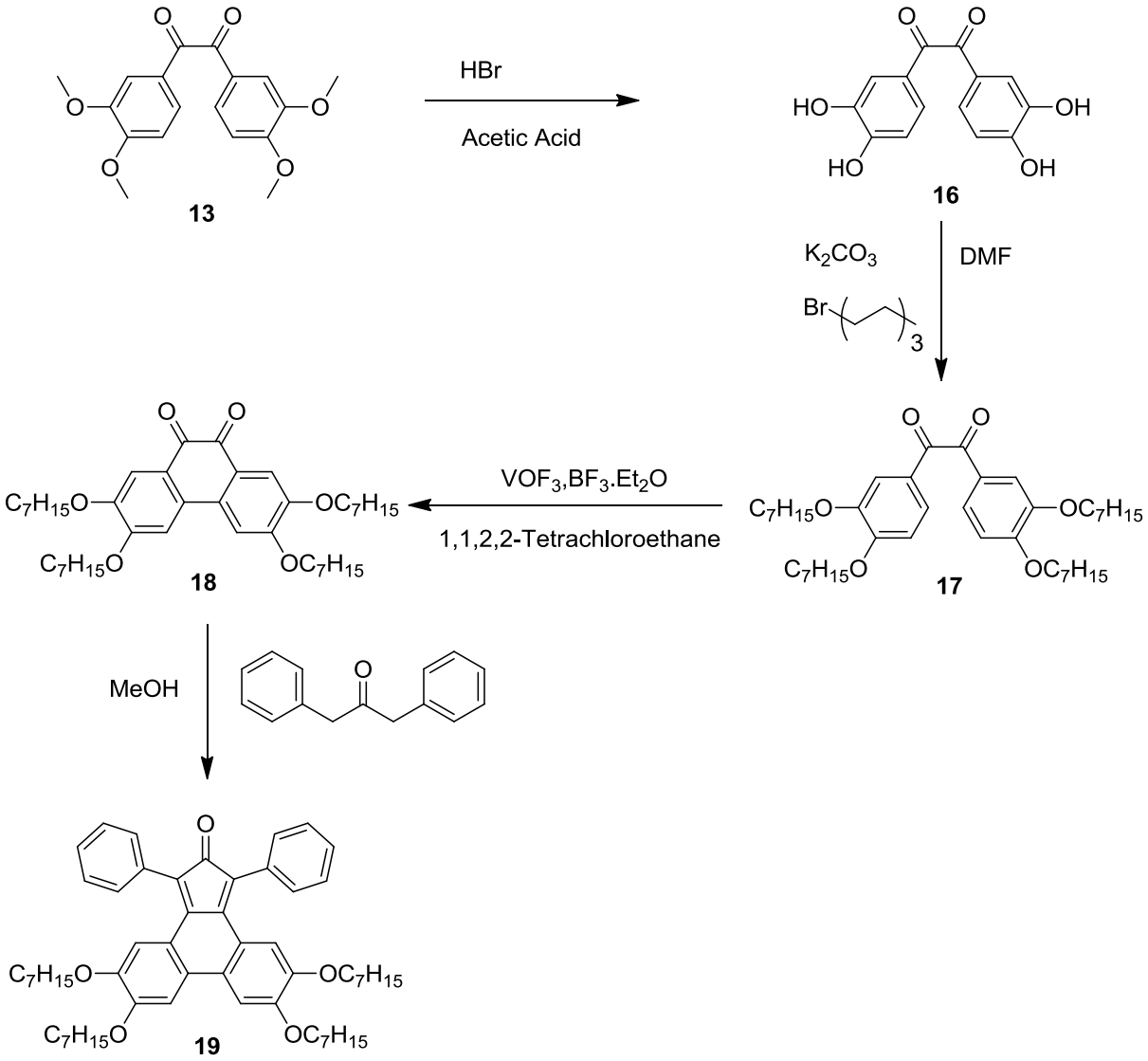


Figure 17: ^{1}H-NMR of **15** in $CDCl_3$.

The variation of –R groups connected to the phencyclone core are done according to the synthetic procedure in Scheme 18. In order to remove methyl groups to have free –OH groups, we tried several different procedures, but the one published by Voit et.al.[152] gave the best results for easy purification and high yield. According to it, compound **13** was heated to reflux in a optimized mixture of aqueous HBr, HBr in acetic acid and glacial acetic acid for demethylation. In the next step, the alkylation of compound **16** with 1-bromoheptane and K_2CO_3 in DMF leads to **17**. For the intramolecular coupling, the same procedure as in the production of compound **14** was used, the oxidative reaction by VOF_3 and $BF_3.Et_2O$, in order

to obtain **18**. However, this time the reaction was performed in CH_2Cl_2 as solvent instead of 1,1,2,2-tetrachloroethane and still high yield was obtained, 75%. The intention was to avoid the more toxic and cancerogenic solvent, 1,1,2,2-tetrachloroethane. In the final step, the Knoevenagel condensation reaction between compound **11** and 1,3-diphenylacetone[151] gives **19** as a dark green solid in 51% yield.



Scheme 18: Synthesis of compound **19**.

The ^{1}H-NMR spectrum of compound **19** reveals that the protons with the chemical shifts in the range of $\delta = 0.97 - 0.89$ ppm can be assigned to protons H-1 and H-2 (Figure 18). The other clearly observed group of protons are H-3 and H-4, which can be associated to the peaks at $\delta = 4.10$ and 3.38 ppm. The peaks between these two groups obviously belong to the protons of aliphatic chains. In the aromatic region, here again, two peaks H-5 and H-6 are more upfield shifted due to electron-donating effect of neighbouring –OR groups. Protons H-5

and H-6 can be seen at δ = 7.02, 6.93 ppm as singlets, respectively. Lastly, the phenyl protons are in the range of δ = 7.66 – 7.11 ppm as multiplet for ten protons. From the analysis of the ^{13}C-NMR spectrum and Dept 135, the carbons C-1 and C-2 can be seen as primary carbons at δ = 14.07, 14.03 ppm. Other alkyl chains' protons can be assigned to the secondary carbons at δ = 31.92, 31.80, 31.76, 29.43, 29.34, 29.21, 29.13, 29.03, 28.89, 28.82, 28.73, 26.04, 25.99, 25.92, 25.80, 25.64, 22.66, 22.58, 22.55 ppm and the carbons directly connected to the oxygen atoms are at δ = 69.52, 68.12 ppm. There is high electron density around the two groups of carbons C-5 and C-6, due to the electron-donating effect of –OR group shifting them more upfield compared to the other aromatic carbons. They can be seen at δ = 109.13, 112.85 ppm, respectively. Carbons C-5 are much more downfield shifted due to extra effect of neighbouring deshielding area of benzene rings. The phenyl groups' tertiary carbons can be attributed to the peaks at δ = 130.27, 128.42, 127.79, 127.61 ppm. There are quartenary carbons at δ = 151.55, 148.84, 148.40 ppm for the carbons directly connected to the –OR groups, at δ = 132.97 ppm for phenyls' quartenary carbons and δ = 121.66, 121.15 ppm for the rest of the quarternary carbons in the compound. The peak at δ = 200.49 ppm can be assigned to the carbonyl carbon.

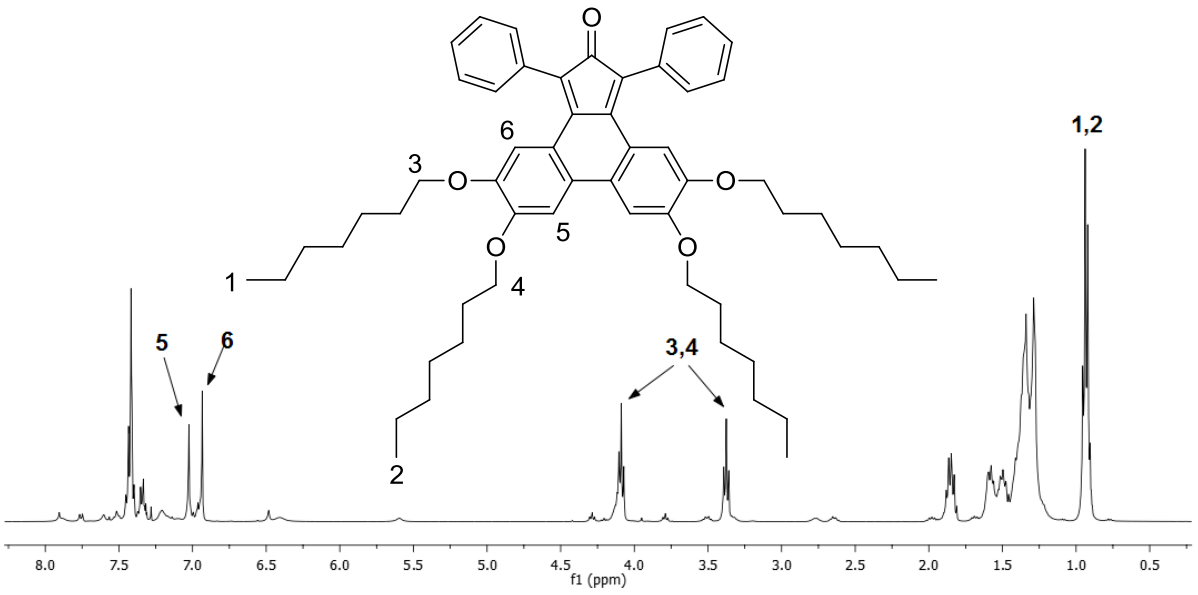
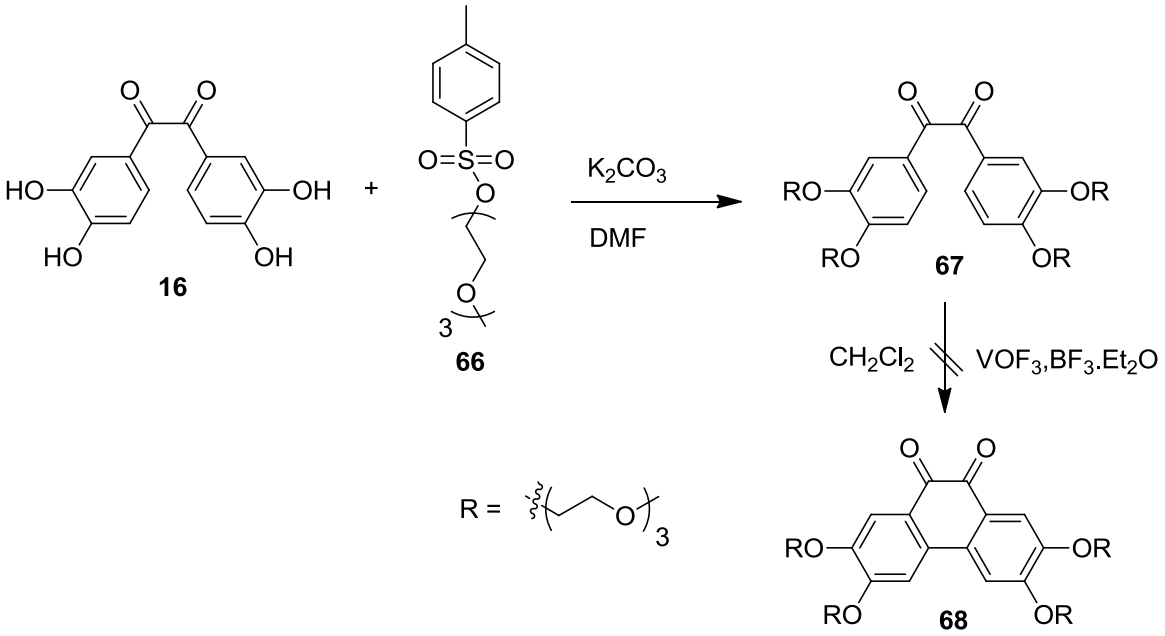


Figure 18: ^{1}H-NMR of **19** in $CDCl_3$.

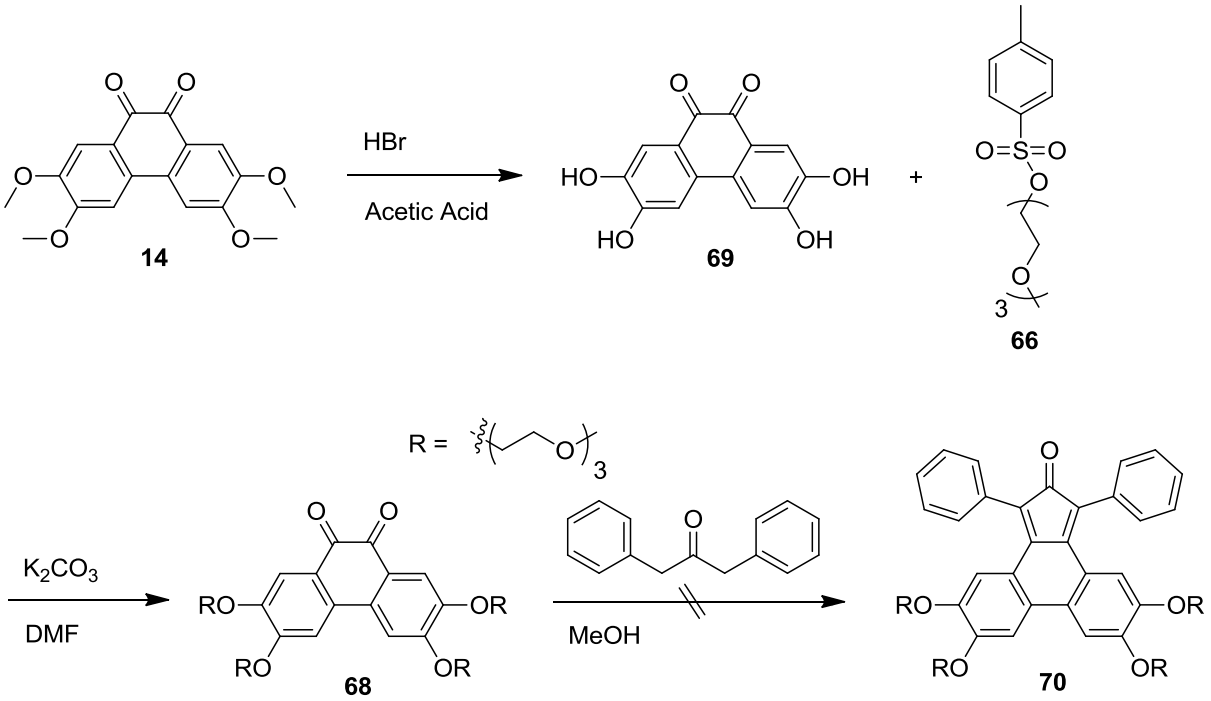
The triethylene glycol monomethyl ether has a strong hydrophilic nature and its incorporation as side chains of phencyclone would enhance the enthalpic interactions of the structure with water and make it better water soluble. Therefore, in order to bring water solubility to the phencyclone structure, we tried to modify it with –R groups consisting of triethylene glycol monomethyl ether. For this purpose, triethylene glycol monomethyl ether tosylate **66** was

synthesized according to the published procedure[153] and it was added to the mixture of compound **16** and K_2CO_3 in DMF. After 20 h of reaction time at 100 ^oC, the reaction led to the compound **67**, in 76% yield, which was incorporated to the oxidative reaction with VOF_3 and $BF_3.Et_2O$ in CH_2Cl_2 in order to yield compound **68**. Unfortunately, after standard reaction conditions, the desired compound **68** could not be isolated (Scheme 19).



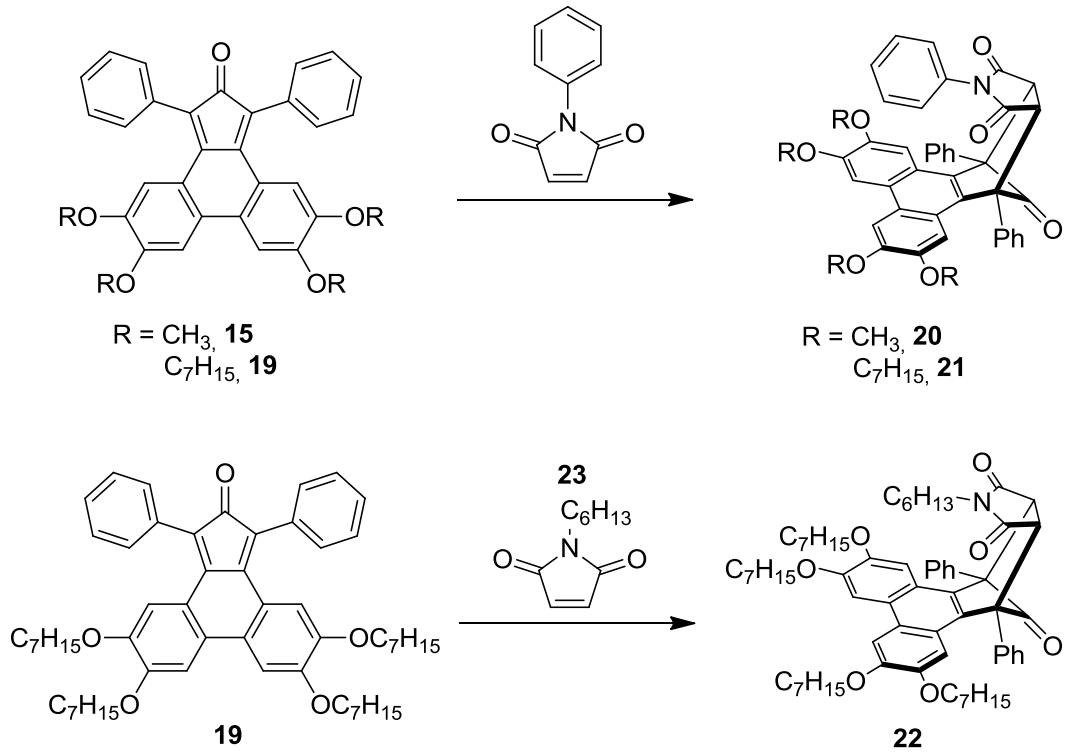
Scheme 19: The first synthetic approach of compound **68**.

Therefore, the synthetic route was modified and the oxidative intramolecular coupling reaction was executed before addition of triethylene glycol monomethyl ether side chains. In order to synthesize **69**, the removal of methyl groups of compound **14** was achieved by reflux in an optimized mixture of aqueous HBr, HBr in acetic acid and glacial acetic acid.[152] Then, the crude product **69** was subjected to nucleophilic substitution with triethylene glycol monomethyl ether tosylate **66** in DMF to afford compound **68** in 76% yield. Unfortunately, after the Knoevenagel condensation reaction between compound **68** and 1,3-diphenylacetone,[151] the desired compound **70** could not be isolated.



Scheme 20: The synthetic approach of compound **70**.

The aim of the synthesis of modified phencyclone derivatives is to put them into the fluorogenic Diels-Alder reaction with some maleimide derivatives. For this purpose, commercially available N-phenylmaleimide and easily synthesized N-hexylmaleimide were used. N-hexylmaleimide was synthesized according to the procedure of Wang, et.al.[154] with small modifications. Briefly, maleic anhydride and hexylamine were reacted in THF in the same pot for maleimide ring formation, followed by addition of $ZnBr_2$ and hexamethyl disilazane. Purification of the mixture gave N-hexylmaleimide in good yield. These maleimides and modified phencyclones were reacted in a 1:1 mol ratio in CH_2Cl_2 and reaction continued for 0.5 h at room temperature to give Diels-Alder adducts in high yields (Scheme 21).



Scheme 21: Diels-Alder reaction between modified phencyclone derivatives and maleimide derivatives.

The Diels-Alder addition takes place approximately in 30 minutes in the situation of 1:1 molar ratio of both substances, however, trials proved that excess addition of N-substituted maleimides permits the reaction to be completed much faster (approximately between 10-15 min). On TLC, the reaction seems quite clean, however, a short column chromatography was performed to eliminate minor fluorescent side products to avoid interference in fluorescence measurements.

To understand the ^{1}H-NMR spectra of the Diels-Alder adducts, as an example, the ^{1}H-NMR spectrum of compound **21**, depicted in Figure 19, is discussed. In the aliphatic region of spectrum, the peaks of the side chains can be found. Protons H-1 and H-2 can be assigned to the peaks at δ = 0.94 ppm. Other easily discernible group of protons at δ = 3.13, 3.44 (H-4) and protons at between δ = 4.26 – 4.09 ppm (H-3) are downshifted due to the inductive effect from the neighboring oxygen atom. In between these two significant groups of protons, other H atoms of side chains can be depicted. Besides, the protons H-7 can be associated to the singlet at δ = 4.54 ppm. In the aromatic region, the usual low field position for protons H-5 in phenanthrene systems can be again seen here (at δ = 7.77 ppm). Interestingly protons H-6 take place at δ = 6.59 ppm due to the upfielding shift effect of the benzene ring above these protons H-6. These two groups of peaks are singlets and according to the COSY spectrum,

these peaks are not interacting with others. This result indicates that the most downfielded peak at δ = 8.44 ppm belongs to the other aromatic region hydrogens. Comparisons with other derivatives of Diels-Alder adducts synthesized with other maleimide derivatives proof that this peak can be assigned to one of the aromatic signals for phenyl groups of phencyclone. From the analysis of ^{13}C-NMR spectrum and Dept 135, the carbons C-1 and C-2 can be observed as primary carbons at δ = 14.10, 14.05 ppm. Other alkyl chains' carbons can be assigned to the secondary carbons at δ = 31.84, 31.79, 29.27, 29.10, 29.04, 28.77, 26.02, 25.80, 22.63, 22.57 and oxygen neighboring carbons at δ = 69.53, 67.71, 63.24. The protons C-7 are a bit more downfield shifted and take place at δ = 44.94. In the aromatic region of the ^{13}C-NMR, carbons C-5 and C-6 are upfield shifted as they are the characteristic peaks of phencyclone derivatives which are shielded by the electronic effect of the core (δ = 107.14, 105.71 ppm, respectively). The tertiary carbons can be seen for the phencyclone core and the phenyl groups at δ = 148.97, 148.34, 134.50, 131.11, 125.46, 120.82 ppm. Moreover, the peak at δ = 173.45 ppm belongs to carbonyl carbons of the former maleimide group and the one at δ = 196.63 ppm to the carbonyl carbon of phencyclone. Additionally, the molecular structure was confirmed by mass spectrometry with a correct molar peak at $[M + Na]^+$ at m/z = 1034.5905.

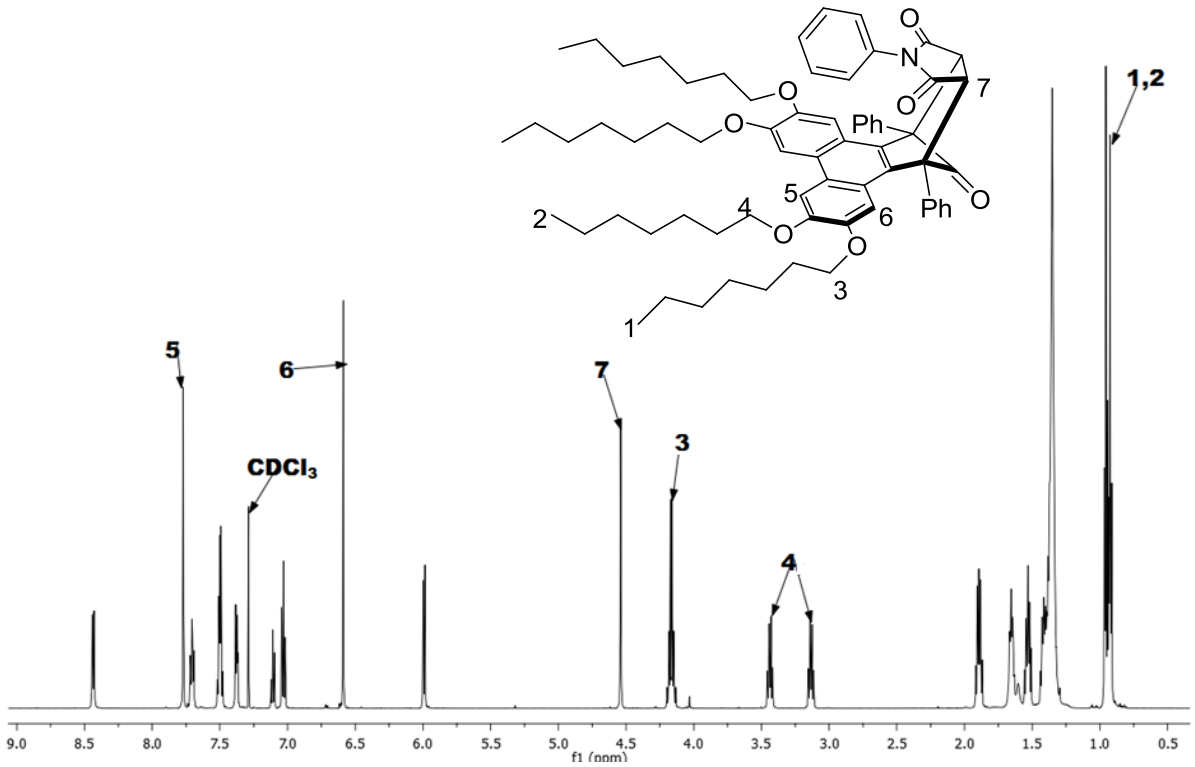
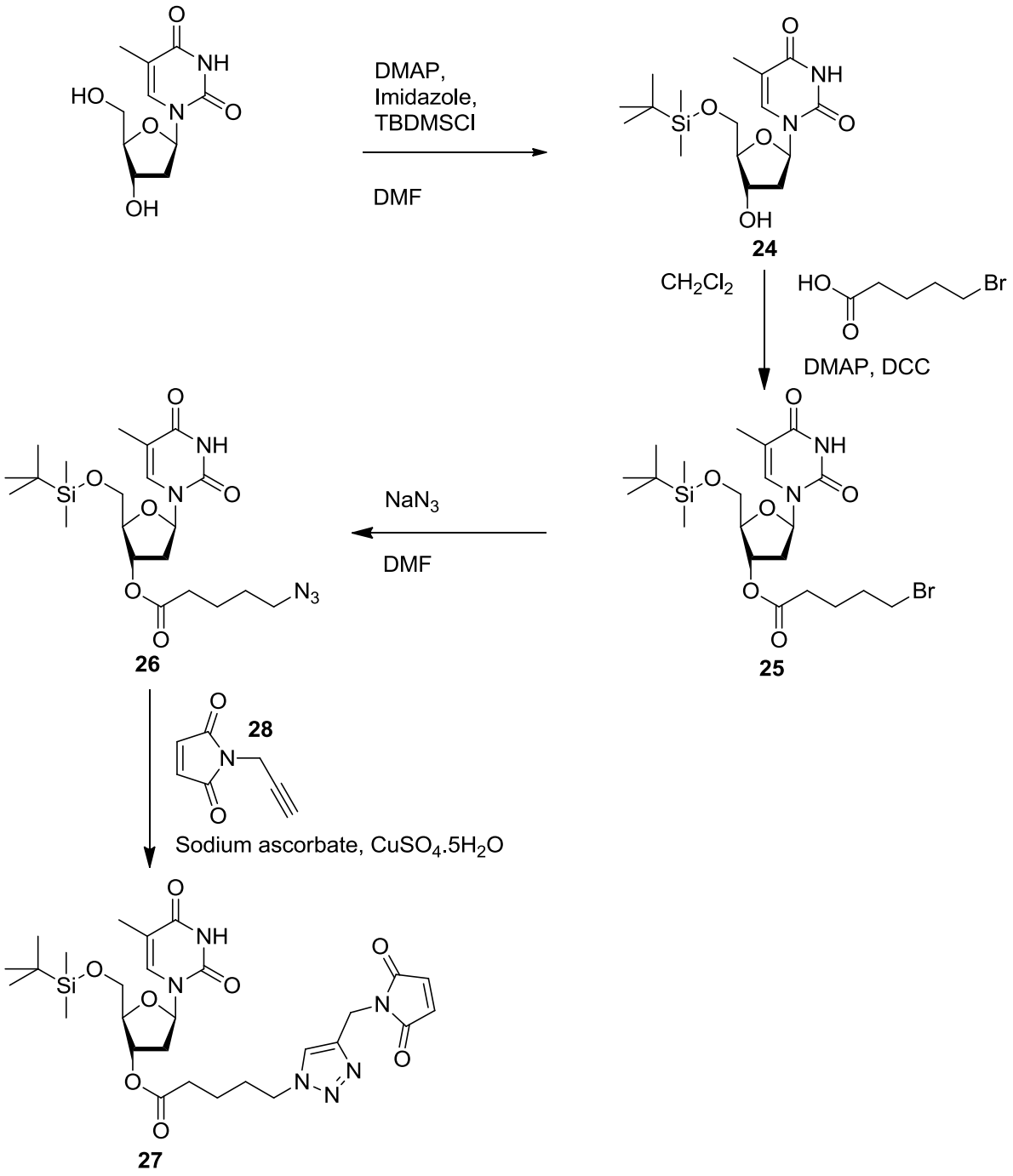


Figure 19: ^{1}H-NMR of **21** in $CDCl_3$.

2.3.2 Synthesis of Maleimide Bearing Nucleosides and Their Diels-Alder Adducts

The modification of nucleosides for fluorescent labelling is an important tool in biological and analytical research.[155-157] Therefore, modification of nucleosides with a flexible alkyl spacer bearing a maleimide group would be a good choice to use as precursors for a bioorthogonal click reaction.

N-Propargylmaleimide was previously used for click modification of fluorescence substances, which have azide group in the side chains, to acquire thiol-reactive labels.[158] By the same logic, we modified thymidine derivative **26**[159] with N-propargylmaleimide **28** via click reaction in order to use it in fluorogenic Diels-Alder reaction. First, the 5'-OH of deoxythymidine was protected with *tert*-butyl-dimethylsilylchloride (TBDMSCl) in the presence of imidazole and catalytic amount of DMAP (4-dimethylaminopyridine) in DMF in 85% yield.[160] Then 5-bromovaleric acid was reacted with the 3′-OH group of the TBDMS-protected thymidine **24** by Steglich esterification in the presence of DCC (dicyclohexylcarbodiimide) and catalytic amount of DMAP to give **25** in 70% yield. Nucleophilic substitution of the terminal bromine in **25** with sodium azide yielded **26** in 80% yield. And finally, **27** was produced by click reaction of N-propargylmaleimide **28** and azido functionalized thymidine **26** with the catalytic system of Cu^{2+}/ascorbic acid in water:THF (1:1 mixture) in 62% yield (Scheme 22).



Scheme 22: The synthetic route to **27**.

The structure of maleimide side chain thymidine derivative **27** was analyzed by mass spectrometry and NMR spectroscopy. For ^{1}H-NMR spectrum of **27**, the IUPAC numbering of thymidine derivative[161] was used and for the rest of the structure, noteworthy peaks were marked by letters (Figure 20). According to the ^{1}H-NMR spectrum of **27**, the first three singlets, two at δ = 0.14 ppm and one at δ = 0.93 ppm can be assigned to the protons of thymidine's protecting group, *tert*-butyldimethylsilyl. The proton of thymidine base H-6 can be seen at δ = 7.54 ppm, the –NH proton at δ = 9.28 ppm and the methyl group of the base

interfering with one of the $–CH_2$ group of side chain at between δ = 1.98 – 1.90 ppm as a multiplet. The deoxyribose's protons of thymidine can be associated to H-1′ at δ = 6.34 ppm, protons H-2′ at δ = 2.11 ppm and interfering with one of the $–CH_2$ group of side chain at δ = 2.39 ppm as α and β, proton H-3′ downfield shifted due to neighboring ester group at δ = 5.25 ppm, proton H-4′ at δ = 4.07 ppm and protons H-5′ at δ = 3.91 ppm as oxygen neighboring hydrogens. The other significant signal is the singlet of maleimides two protons H-a at δ = 6.73 ppm and the singlet for protons H-b at δ = 4.82 ppm, triazole ring's proton H-c as singlet at δ = 7.58 ppm, side chains protons H-d as triplet at δ = 4.35 ppm and protons H-e interfering with proton H-2' at δ = 2.39 ppm. According to the analysis of ^{13}C-NMR and DEPT-135 analysis, three primary at δ = -5.56, -5.45, 25.84 ppm and one quaternary at δ = 18.24 ppm are the protons of the TBDMS protecting group. The carbon of methyl group of thymidine base can be attributed to the primary carbon at δ = 12.40 ppm and the other peaks of thymidine, which can be significantly chosen, quarternary carbon C-5 at δ = 111.20 ppm, secondary carbon C-2' at δ = 37.94 ppm, the carbonyl carbons at δ = 163.70, 150.46 ppm. The other specific carbons of the system are the maleimide's tertiary carbons C-a at δ = 134.25 ppm, the triazole's quarternary carbon at δ = 142.56 ppm and the carbonyl carbons of ester and the maleimide at δ = 172.42 and 169.96 ppm, respectively. Besides, the molecular integrity of the system was also determined by high resolution mass measurements with a correct molar peak as $[M + H]^+$ at m/z = 617.2750.

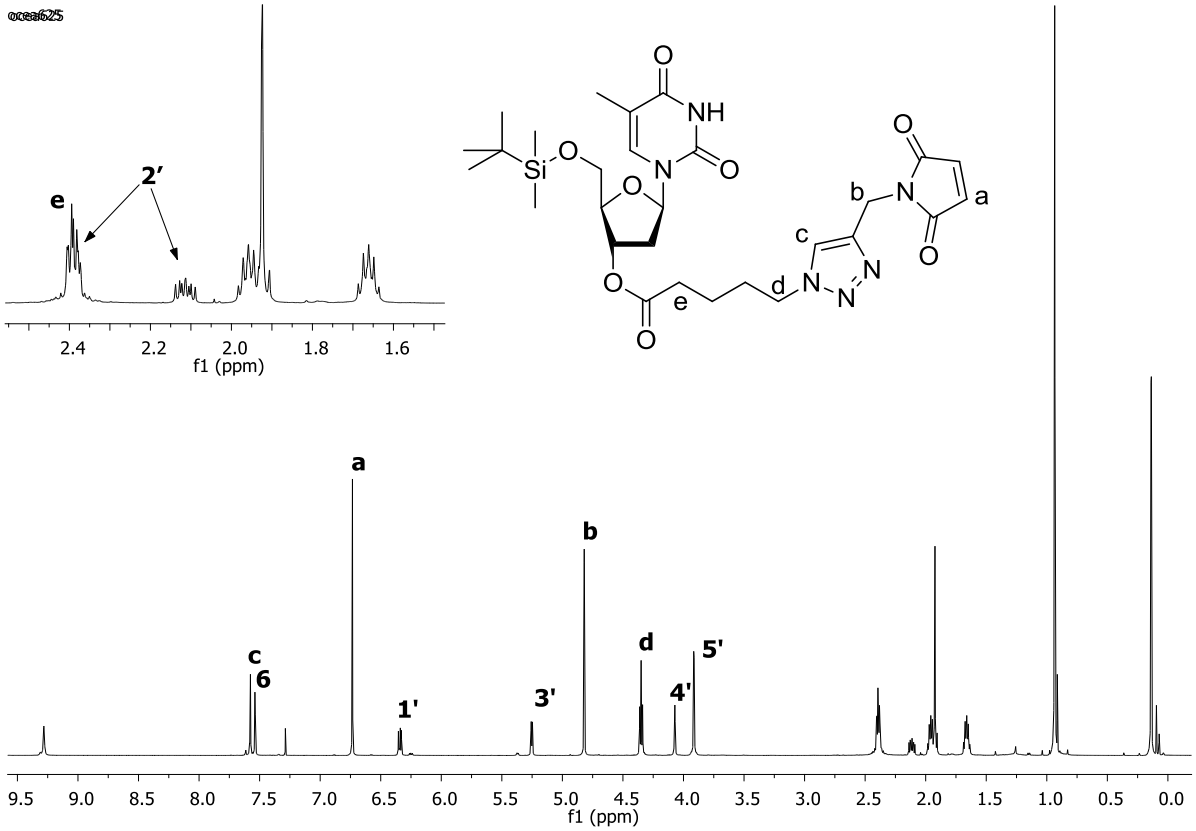
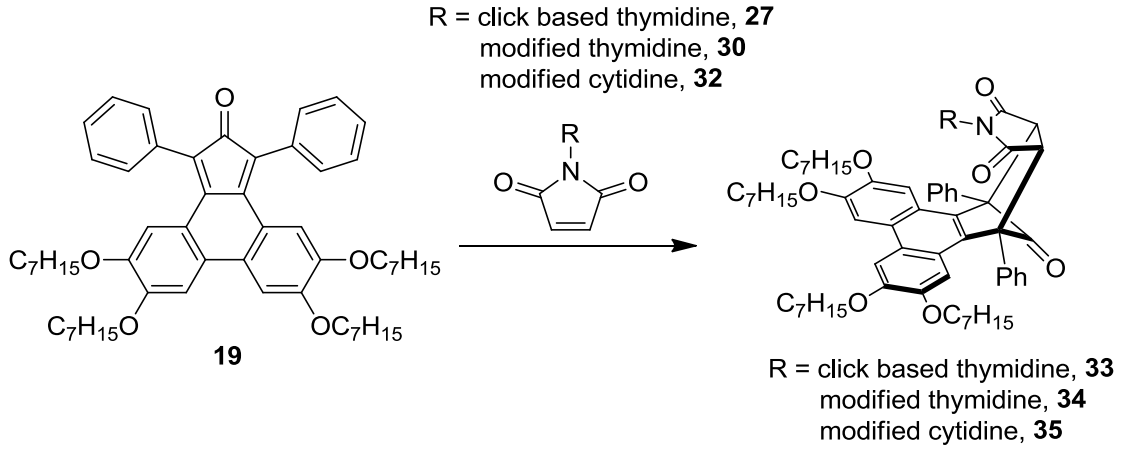


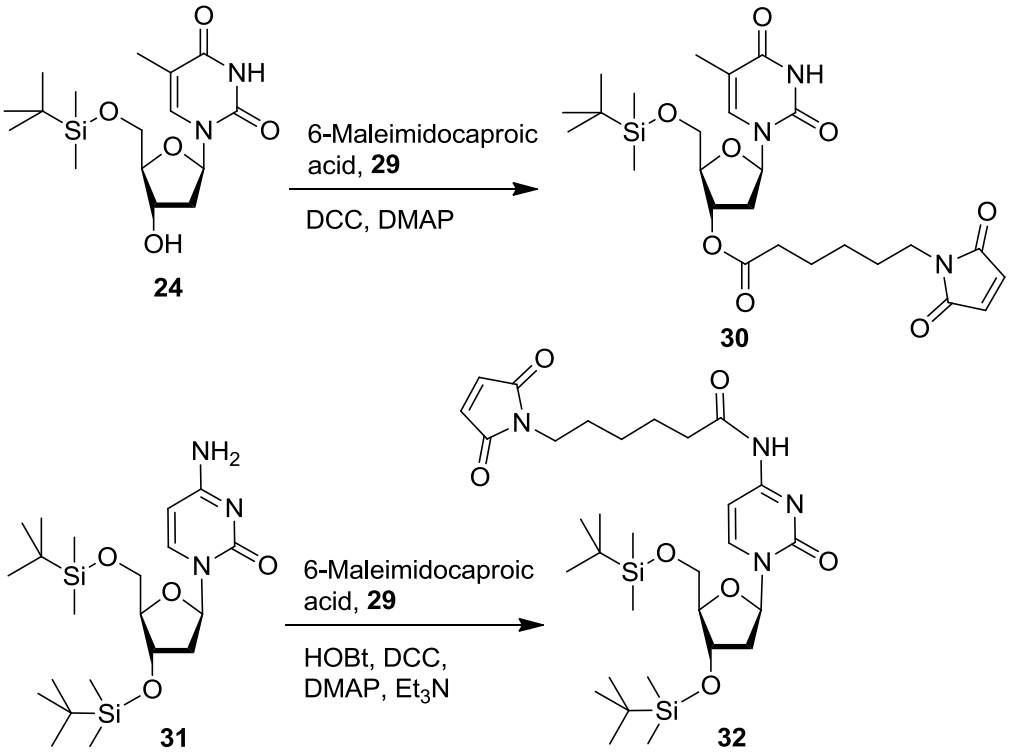
Figure 20: ^{1}H-NMR of **27** in $CDCl_3$.

The Diels-Alder addition of modified thymidine **27** was performed under argon at room temperature in CH_2Cl_2 (Scheme 23). The starting material was consumed in half an hour, as usual, however, the reaction gave multiple fluorescence side products which have very close R_f values and column chromatography was inefficient to purify the desired product. Nevertheless, the product was isolated in sufficient amount for NMR-measurements after several column chromatography purifications. This problem coerced us to find alternative side chain modification methods.



Scheme 23: Diels-Alder additions of modified nucleosides and **19**.

6-Maleimido caproic acid **29** was chosen for the coupling reaction with nucleoside derivatives to introduce the maleimide moiety (Scheme 24). It was reacted with the 3′-OH group of the TBDMS-protected thymidine derivative by a Steglich esterification in the presence of dicyclohexylcarbodiimide (DCC) and a catalytic amount of DMAP to give **30** in 20% yield. Similarly, the deoxycytidine's free –OH groups were first protected[162] and used for the synthesis of derivative **32** by DCC/HOBt amide coupling in 56% yield.



Scheme 24: Synthesis of maleimide modified nucleosides.

The Diels-Alder additions of these derivatives **30** and **32** were also performed under previously explained conditions (Scheme 23). The products were quite pure, however, a short column chromatography was applied to eliminate poor side products. **34** was isolated in 77% and **35** in 79% yield, and as mentioned previously the purified yield of click modified thymidine **33** was really low with only 35% yield.

2.3.3 Optical Properties of Phencyclone Derivatives and Their Adducts

The optical properties of phencyclone derivatives and their adducts were evaluated in CH_2Cl_2. In UV-vis absorption spectra, major differences between the phencyclone derivatives and their adducts can be observed (Figure 21). Generally, all UV-vis spectra of the adducts are almost the same and the phencyclone derivatives have quite different UV-vis spectra compared to the adducts.

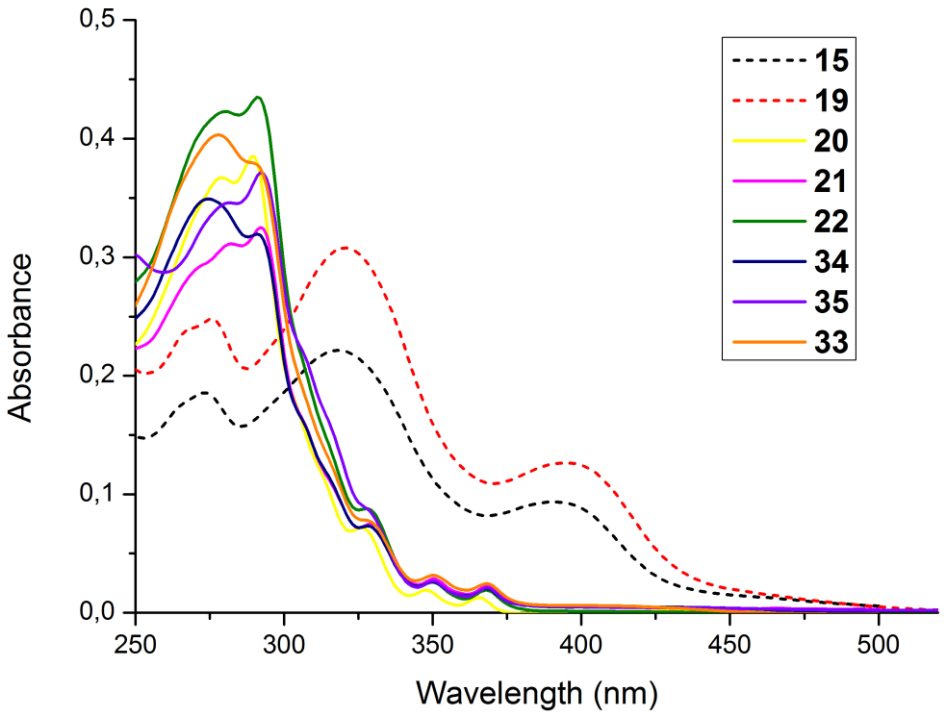


Figure 21: UV-Vis spectra of phencyclone derivatives and their adducts at 1.0 x 10^{-5} M.

The phencyclone derivatives have distinct peaks over 400 nm which disappear after the Diels-Alder reaction. This phenomena allows the process of the reaction to be determined by color change. The color of the reaction turns from dark green to colorless. It is clearly seen in the UV-Vis spectra that the peak in the visible range disappears (Figure 21 and Figure 22).

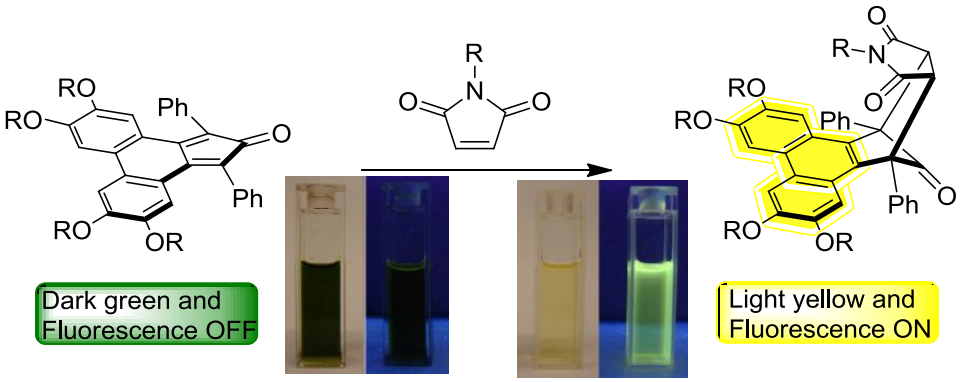


Figure 22: Illustrative presentation of colorimetric and fluorogenic Diels-Alder reaction.

In emission spectra, the substances display two broad emission maxima at approx. 372 nm and 390 nm. Moreover, the phencyclone derivatives have almost no fluorescence emission. As it is seen, Diels-Alder reactions between these phencyclone derivatives and N-substituted maleimides are fluorogenic (Figure 23). A direct indication of the occurance of the Diels-Alder reaction can also be observed by a handheld UV lamp during the fluorogenic conversion (Figure 22).

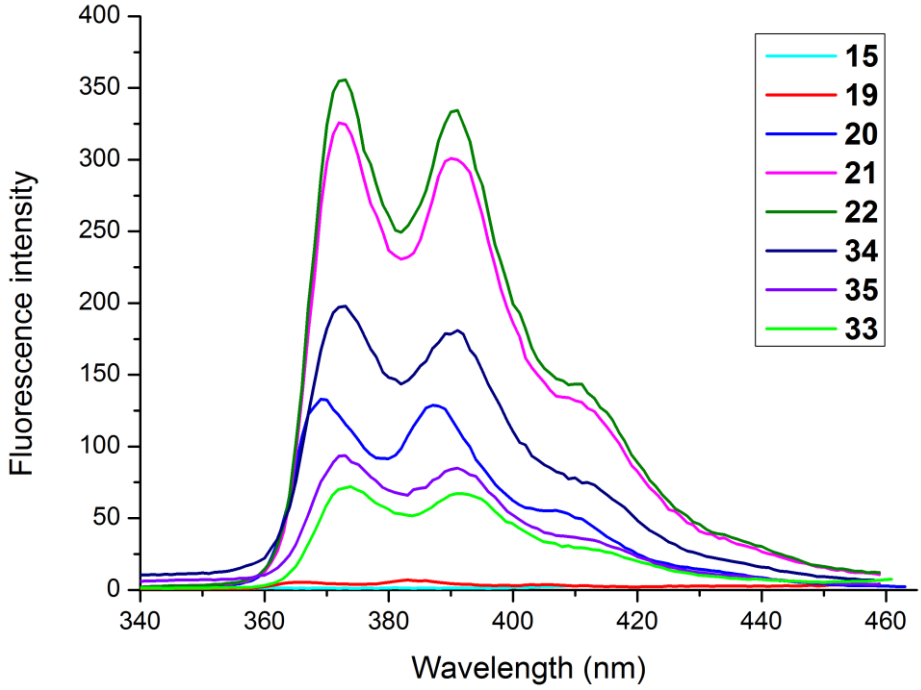


Figure 23: Fluorescence spectra of phencyclone derivatives and their adducts at 1.0 x 10^{-5} M (λex = 266 nm).

By measuring the magnitude of this fluorogenic effect, we achieved the fluorescence quantum yields of phencyclone derivative **19**, as $\Phi_f \sim 0.0011$ and its Diels-Alder adduct **22**, as $\Phi_f \sim 0.037$. The enhancement of fluorescence is 33-fold for this example. The amount of fluorescence enhancements varies according to the substituent of maleimide derivatives (Table 3).

Compound	R[a]	R_1[b]	Φ_f[c]
20	CH_3	Ph	0.015
21	C_7H_{15}	Ph	0.033
22	C_7H_{15}	C_6H_{13}	0.037
34	C_7H_{15}	thymidine deriv.	0.021
35	C_7H_{15}	cytidine deriv.	0.011

[a]The substituent of side-chain of phencyclone derivatives. [b]The R_1 group of maleimide part. [c]Determined using anthracene (Φ_f = 0.27 in ethanol)[163]

Table 3: Fluorescence quantum efficiencies of Diels-Alder adducts.

2.4 Conclusion

In this chapter, we focused on the fluorogenic Diels-Alder reactions. First experiments were performed by Diels-Alder reaction of the synthesized furo[3,4-c]coumarins. The unexpected optical results from these experiments directed us to investigate other fluorophores that can be possible candidates for fluorogenic Diels-Alder reaction. Phencyclone derivatives were chosen and in the second part of this chapter, the fluorogenic Diels-Alder reaction of these derivatives were discussed.

In the first part of this chapter, synthesis of furo[3,4-c]coumarins and their Diels-Alder additions were performed. However, these first trials did not disclose the desired results. The Diels-Alder reactions between N-phenylmaleimide and furan part of these coumarins were not fluorogenic. Then, we also examined the Diels-Alder reactions of synthesized furo[3,4-c]coumarins with PTAD, however, they resulted in decomposition of the dienes.

In the second study, phencyclone derivatives for fluorogenic Diels-Alder addition were successfully synthesized. After realizing that the Diels-Alder adducts of N-substituted maleimides and phencyclone itself emit actually very week fluorescence, we synthesized systems with electron-donating –OR groups. With these phencyclone derivatives, in Diels-Alder addition reactions, up to 33-fold of fluorescence enhancements were achieved. Very high yields and low reaction times at room temperature fulfill the click chemistry conditions. Moreover, the dark green color of phencyclone derivatives disappears by the Diels-Alder reaction and the reaction mixture becomes colorless. This phenomena allows us to follow the reaction by naked eye observations.

In that respect, we have successfully developed a new fluorogenic system in order to monitor Diels-Alder reactions. This fluorogenic and colorimetric method reveals a new perspective of usage of Diels-Alder reaction in fluorogenic labeling. The modification of thymidine and deoxycytidine derivatives with maleimide side chain and their usage in fluorogenic Diels-Alder reaction could be tested as a first example. Unfortunately, due to the lack of water solubility of the structures, in vitro and/or in vivo applications were not possible.

3 BODIPY Derivatives

3.1 Introduction and Motivation

During the research of fluorogenic click reactions, we encountered the impressive studies of BODIPY derivatives and the idea of development of new BODIPY derivatives to create fluorogenic click reaction came into question. Moreover, the mostly studied fluorogenic click reaction type is the copper catalyzed alkyne-azide click reaction (CuAAC) and with this in mind, we wanted to use the fluorescence quenching effect of azido group by substituting it into suitable positions of BODIPYs. In this third chapter of the thesis, the novel BODIPY derivatives and their CuAAC ligations are discussed. Besides, during this research, some BODIPY derivatives were isolated because of their potential interesting properties and at the end of chapter, their synthesis and optical properties are explained.

Because of their excellent properties, 4,4-difluoro-4-bora-3a,4a-diaza-s-indacenes (BODIPYs) are commonly preferred to be designed as fluorescent probes. The optical features of BODIPY derivatives can be easily adjusted by desired modification of positions of either the BODIPY core or connected substituents.[164] From literature examples, it is known that an azido group has a fluorescence quenching effect[96] and the ortho-substitution of 8-phenyl BODIPYs can be effective to quench the fluorescence of BODIPYs by PET mechanism. For this reason, the fluorogenic effect by changing the position of azido group on phenyl substituent was investigated. In this respect, the BODIPY dyes containing ortho-, meta- and para-azidophenyl at the 8-position were synthesized. In the first part of the chapter, the synthesis and investigation of properties of these 8-(azidophenyl) BODIPYs are discussed.

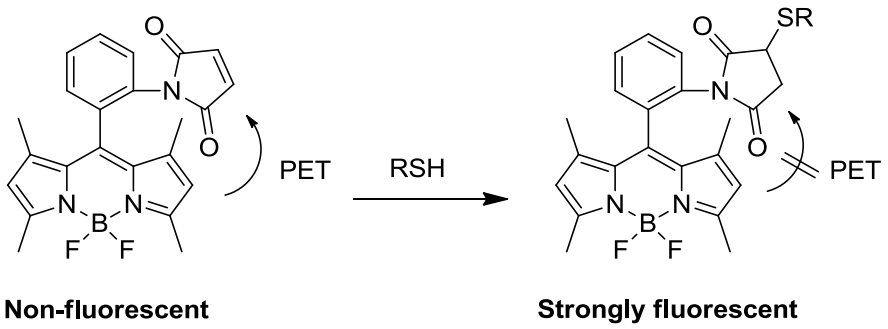
In the second section of this chapter, the synthesis and investigation of properties of nitro and amino BODIPYs are presented. The nitro group is also known for its fluorescence quenching effect[165] and this phenomenon is investigated by addition of it to the BODIPY core.

3.2 8-Azidophenyl BODIPYs

For the purpose of CuAAC reaction, the introduction of an azido group to the benzene moiety at the 8-position of the BODIPY fluorophore was examined. There are a few examples in the literature that present only 8-(4-azidophenyl)BODIPY derivatives. To the best of our knowledge, the ortho- and meta-azido substitution at the 8-position of the BODIPY dyes have

not been published. Burgess and his coworkers have synthesized the 8-(4-azidophenyl)BODIPY derivatives by reduction of the nitro group at the benzene moiety of BODIPY which was then converted to the azide by addition of NaN_3.[166-168]

In 2007, Matsumoto et al. published their results on ortho-substituted maleimide derivatives of BODIPY disclosing their fluorogenic behaviours in the presence of thiols (Scheme 25). By donor-excited photoinduced electron transfer (d-PeT) from BODIPY to the electron acceptor maleimide, the fluorescence of the dye is quenched. Reaction with thiols disrupts d-PeT and regenerates fluorescence with an enhancement of 350-fold. By the same logic, Guo et al. developed another BODIPY derivative by changing the electron acceptor for the PET mechanism. They functionalized the –OH group at the ortho-position of the benzene ring with 2,4-dinitrobenzenesulfonyl (DNBS) to quench fluorescence and by cleavage of DNBS with thiols, a 300-fold of fluorescence enhancement was observed.[169]

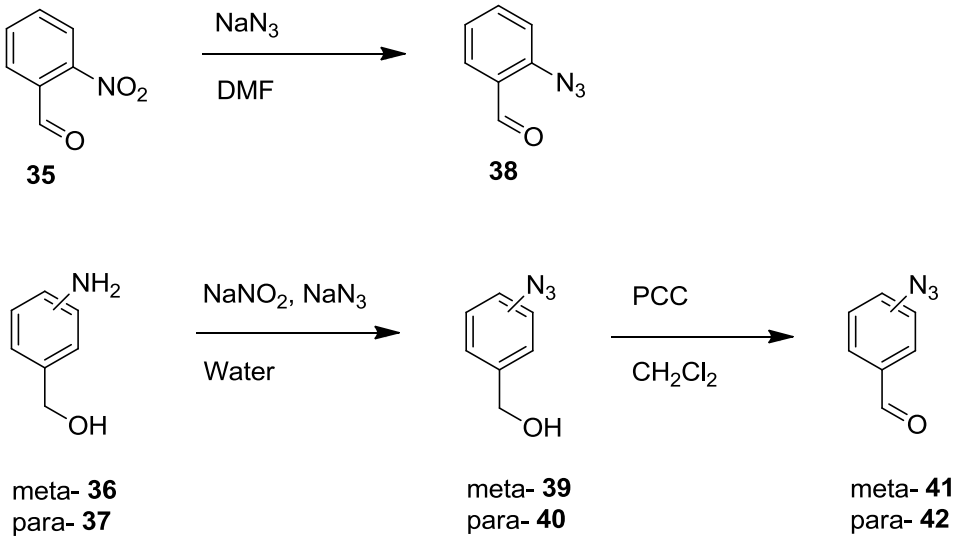


Scheme 25: The schematic presentation of thiol-reactive fluorogenic BODIPY probe.

With these examples in mind, we have designed a new short synthetic route to achieve high yields of 8-(azidophenyl) BODIPYs by pre-synthesizing of azidobenzaldehydes and then using these educts in conventional one-pot BODIPY synthesis.

3.2.1 Synthesis of Azidobenzaldehydes

The synthesis of 2-azidobenzaldehyde **38** was easily established by nucleophilic aromatic substitution of 2-nitrobenzaldehyde **35** using NaN_3.[170] As Pelkey and Gribble explained in their related work, HMPA as solvent gives much higher yields and the reaction can be done at room temperature. Due to the carcinogenic effect of HMPA, however, we preferred DMF as the solvent. The reaction mixture is heated to 60 ^oC for one day to get 65% yield of 2-azidobenzaldehyde (Scheme 26).[171]



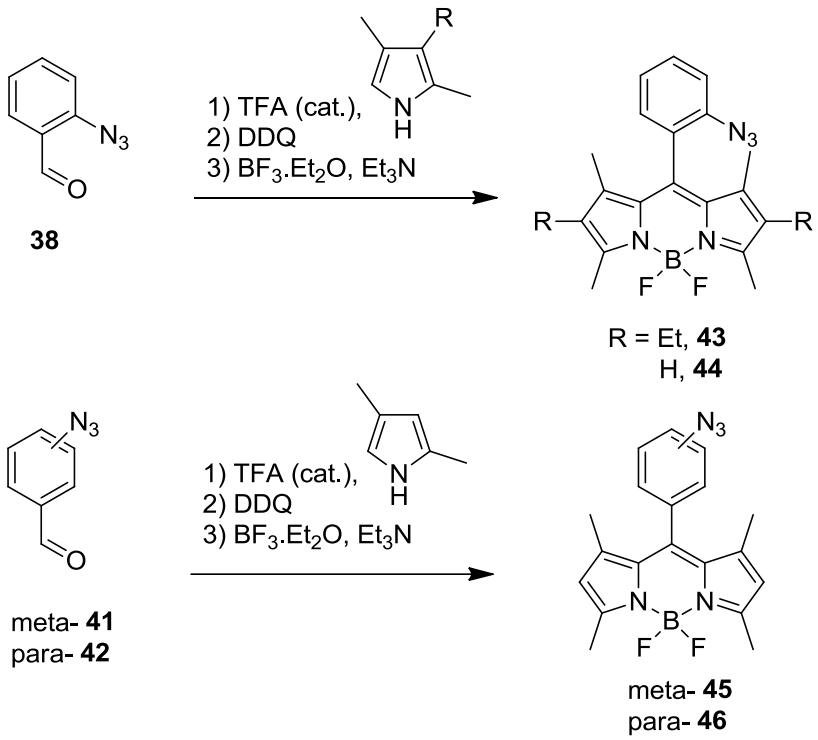
Scheme 26: Synthesis of azidobenzaldehydes.

Unfortunately, nucleophilic aromatic substitution of nitrobenzaldehydes with NaN_3 is only working with the activation of carbonyl group at ortho-position. Therefore, meta- and para-azidobenzaldehydes were obtained by conversion of aminobenzylalcohols **36** and **37** to the azides by diazotization, followed by displacement with NaN_3. Then, azido alcohols **39** and **40** were oxidized to the aldehydes **41** and **42** with PCC (pyridinium chlorochromate), respectively (Scheme 26).[172] All steps gave very high yields.

3.2.2 Synthesis of 8-(Azidophenyl) BODIPYs

The azido BODIPYs were synthesized by a one-pot procedure in good yield. Azidobenzaldehydes were reacted with pyrroles (2,4-dimethylpyrrole or 2,4-dimethyl-3-diethyl pyrrole) in a 1:2 molar ratio and with TFA as an acid catalyst to give the corresponding dipyrromethanes. Then, these structures were oxidized by adding 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ) into dipyrromethenes and after addition of triethylamine and reaction with $BF3{\cdot}OEt_2$ (boron trifluoride etherate), the desired BODIPYs were formed as red solids (Scheme 27).

2,4-Dimethyl-3-diethyl pyrrole were also used to be able to evaluate the effects of substitution in the BODIPY core regarding yield and optical properties. All yields are quite good and there is no yield difference between **43** and **44**, the approximate yield being 35%. As expected, meta and para substituted BODIPYs **45** and **46** have higher yields, 42% and 46%, respectively.



Scheme 27: One-pot synthesis of azido BODIPYs.

In the literature, only para substituted azido BODIPY **46** is known.[167] In the published procedure, the yield of this azido BODIPY is nearly equal, however, on small scale procedure, the amount of the product is less then even 50 mg. In other examples of the same group, similar structures of para substituted azido BODIPYs were synthesized and again, it is obvious that the increase of product formation is difficult. All examples gave less than 50 mg of products.[166, 168] However, with the pre-synthesized azidobenzaldehydes the procedure is much more flexible for scale up. Minimum 10 times higher starting amounts yielded satisfactory amounts of BODIPYs. The conversion of the –NH2 group into the $–N_3$ group via conventional ways is more difficult on the BODIPY nucleus, thus processing this step in the starting material allowed as to achieve much higher amounts of BODIPYs.

To proof the structure of the synthesized BODIPY fluorophores, NMR and mass spectroscopy was used. As an example, the ^{1}H-NMR spectrum of **45** can be seen in Figure 24. In the ^{1}H-NMR spectrum of **45** in $CDCl_3$, the protons H-1 and H-2 of methyl groups of BODIPY core can be assigned to the singlets in the aliphatic region δ = 1.46 and 2.58 ppm, respectively. The singlet at δ = 6.02 ppm obviously belongs to the protons H-3 of BODIPY. The peaks at δ = 7.51, 7.18, 7.13 – 7.07, 7.02 – 6.96 ppm belong to the aromatic protons of benzene moiety. From the analysis of ^{13}C-NMR spectrum and Dept 135, the carbons C-1 and C-2 can be recognized as primary carbons at δ = 14.43, 14.56 ppm. The rest of the aromatic carbons can be observed as tertiary carbons at δ = 131.15, 130.64, 124.64, 121.40, 119.40, 118.82

ppm and the quaternary carbons more downfield at δ = 155.93, 142.87, 141.33, 140.01, 136.82 ppm. Moreover, the molecular integrity of **45** is also verified by mass spectrometric investigation with a correct molar peak as $[M + H]^+$ at m/z = 366.1696.

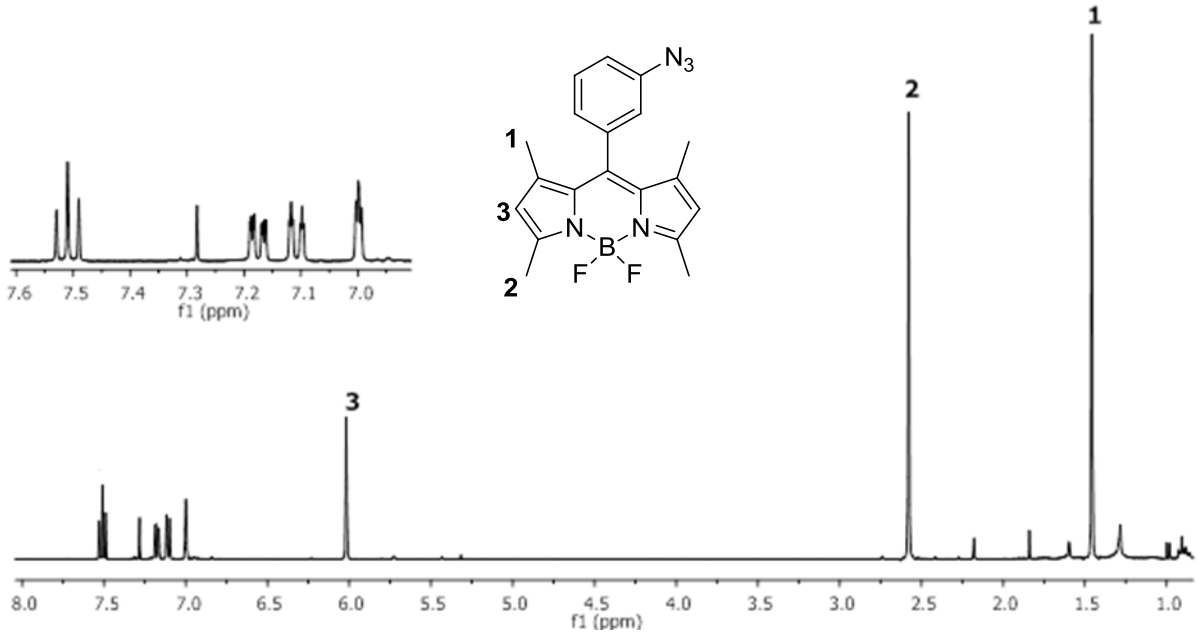
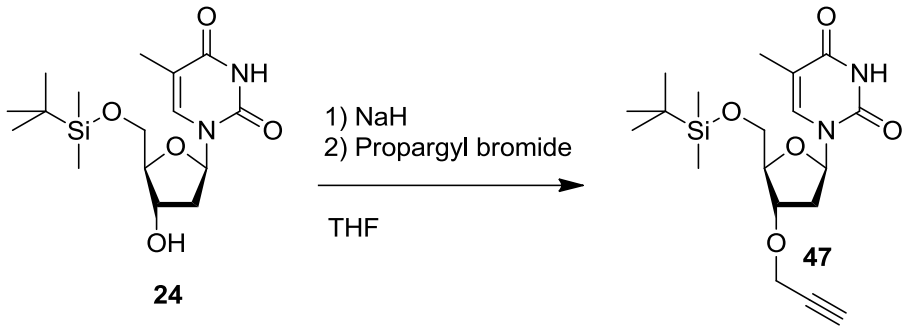


Figure 24: ^{1}H-NMR of **45** in $CDCl_3$.

3.2.3 Click Reaction of Azido BODIPYs

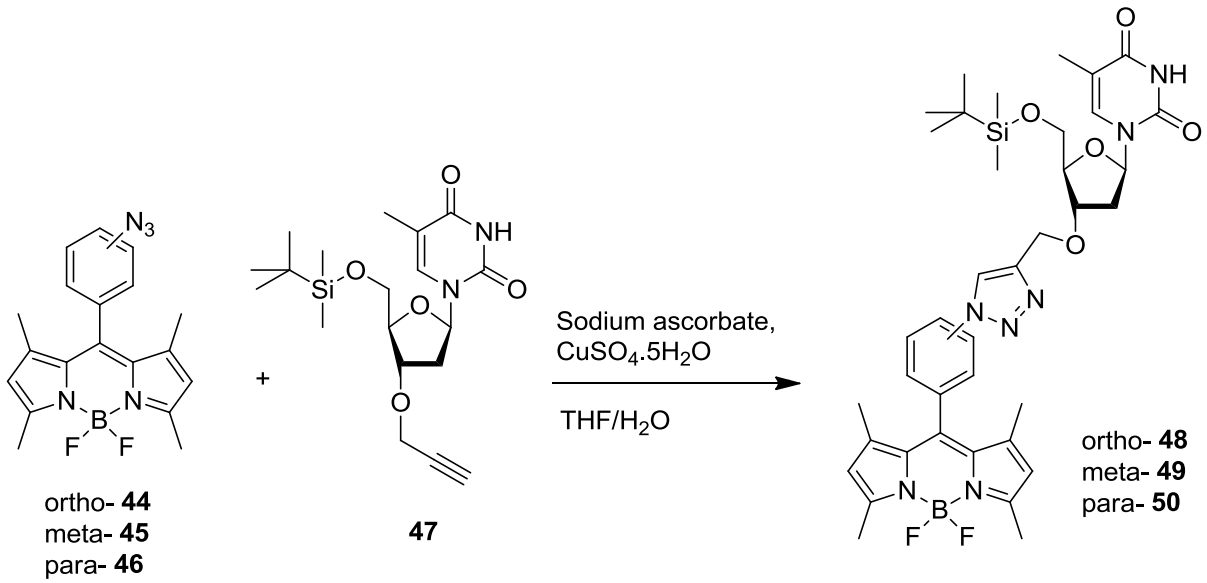
Nucleotides and nucleosides are taking place in numerous essential biochemical processes like gastrointestinal tract repair after damage, immune response, iron absorption in the gut and metabolism of fatty acids.[173] Moreover, there are very practical ways to modify nucleotides with an alkyne group. For these reasons, we wanted to carry out the click reaction between synthesized azido BODIPYs and alkyne modified nucleosides.

5'-O-TBDMS-protected thymidine **24** can be alkylated with propargyl bromide in the presence of NaH. To avoid side products by alkylating –NH group in the base part of the nucleoside, the additions were made dropwise at 0 °C.[174] Eventually, thymidine derivative **47**[175] was isolated as alkyne modified "bio"-substance for click applications (Scheme 28).



Scheme 28: Synthesis of alkyne modified thymidine.

The aim of the work was to develop fluorogenic substances on the basis of azido BODIPYs for click reactions. Optimization of click conditions or minimizing catalyst amounts were not investigated. The most common and easily feasible CuAAC would be convenient. Therefore, for click reaction, a standard procedure was preferred (Scheme 29).[13-14] Sodium ascorbate and $CuSO_4.5H_2O$ were used to generate Cu(I) in situ. The propargyl thymidine and an azido BODIPY derivative were reacted in a 1.25:1 molar ratio, respectively. To obtain high amounts of products and for convenient preparation of a reaction, high amounts of the catalysts were adjusted according to the published literature ($CuSO_4.H_2O$, 20 mol% and sodium ascorbate, 60 mol %).[176] Catalyts and reagents were dissolved in 1:1 (v/v) mixture of THF/H_2O and the mixture was stirred overnight at room temperature. After purification, except ortho-azido BODIPY derivative **44**, all derivatives yielded very high yields. For meta-azido BODIPY derivative **45**, the yield is 94% and for para-azido BODIPY derivative **46**, it is 97%.



Scheme 29: The click reaction of 8-(azidophenyl)BODIPYs and propargyl thymidine.

The chemical structures of the compounds were determined by mass and NMR investigations. To understand the main aspects of the analysis, compound **50** can be seen in Figure 25. The numbering of protons and carbons were made by using IUPAC numbering system of BODIPY[6] and thymidine[161] (to differentiate the numbers for BODIPY "B" was used in front of numbers, others belong to thymidine). From starting aliphatic region, first, the singlets can be attributed to the protons H-i at δ = 0.13 and 0.14 ppm as two singlets and protons H-h at δ = 0.94 ppm for nine protons of the tertiary butyl group connected to the silicon atom. The methyl group protons of BODIPY core can be observed as singlets; protons H-e at δ = 1.46 ppm and protons H-f at δ = 2.58 ppm. Thymidine's methyl group protons H-g are at δ = 1.94 ppm. α,β-protons of H-2' of thymidine present different values due to their conformational diversity as broad multiplets at between δ = 2.08 – 2.01 and 2.55 – 2.48 ppm. Other protons of deoxyribose can be determined; H-5′ at δ = 3.87 ppm, ; H-4′ at δ = 4.20 ppm, H-3′ at δ = 4.33 ppm and H-1′ at δ = 6.36 ppm . The methylene protons H-d at the connection point of nucleoside and dye can be associated with the peak at δ = 4.79 ppm. The β-pyrrolic protons of BODIPY H-2 and H-6 are both shifted at δ = 6.03 ppm. The protons in para substituted benzene moiety exhibit a "roofing" effect at δ = 7.51 ppm (protons H-b) and δ = 7.96 ppm (protons H-a). The signal of protons H-b is interfering with thymidine base's proton H-6 which is shifted between δ = 7.54 – 7.48 ppm. Moreover, triazole's proton H-c can be observed at δ = 8.15 ppm as a singlet which can be found in ^{1}H-^{1}H-COSY analysis without any interaction. Lastly, of course the most downfield shifted singlet belongs to the –NH of thymidine's base. In ^{13}C-NMR spectrum and DEPT-135 of **50**, three primary carbons at δ = -5.38, -5.51, 25.88 ppm and one quaternary carbon at δ = 18.31 ppm belong to the TBDMS protecting group. The primary carbons of BODIPY's methyl groups can be seen at δ = 14.68, 14.58 ppm as well as the thymidine base's primary carbon at δ = 12.47 ppm. The other signals of the thymidine derivative can be attributed as follows: secondary carbons occur at δ = 37.90, 62.80, 63.68 ppm and four tertiary at δ = 135.18, 84.97, 84.93, 80.02 ppm. Other tertiary carbons of the system are found at δ = 129.85, 121.57, 120.88, 120.65 ppm and quaternary at δ = 150.34, 145.80, 142.75, 139.50, 137.39, 135.68, 131.19, 111.01 ppm. Finally, two quaternary peak at δ = 163.63, 156.14 ppm can be associated with the carbonyl groups of thymidine nucleus. Moreover, the molecular structure was also proved by the mass spectrometry with a correct molar peak as $[M + Na]^{+}$ at m/z = 782.3440.

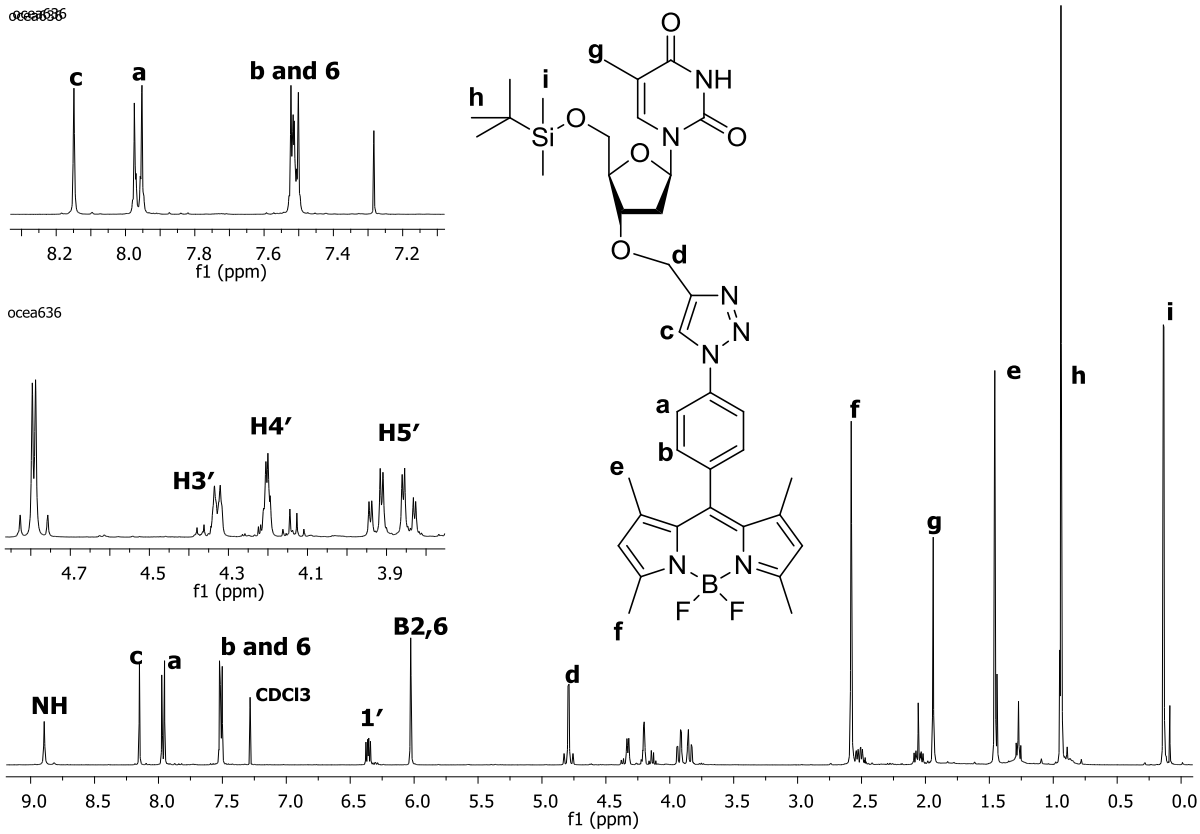
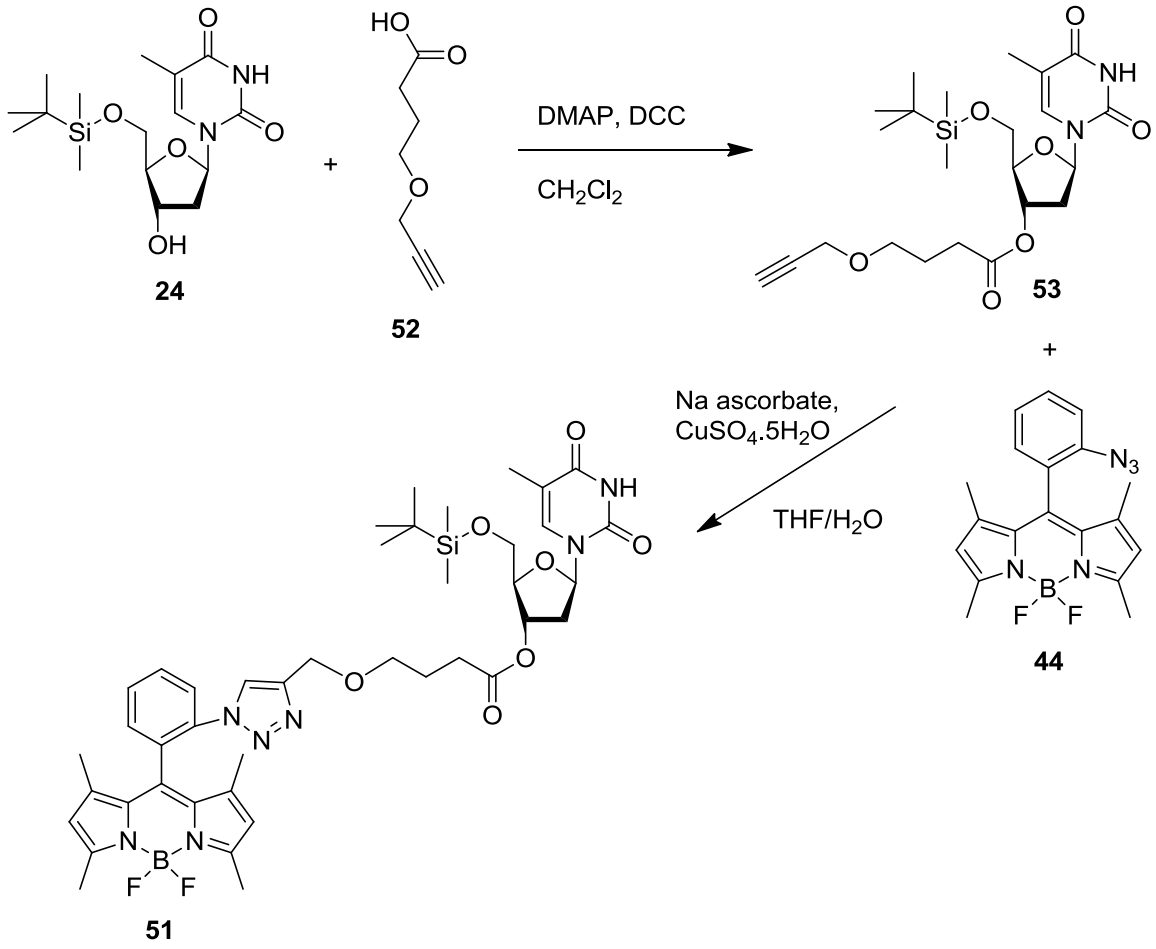


Figure 25: ^{1}H-NMR spectrum of **50** in $CDCl_3$.

The yields with meta- and para- azidophenyl substituted BODIPYs are very good. However, the yield with the ortho azido derivative **44** was not satisfactory. This can be due to steric hindrance of BODIPY's methyl groups. It is known that the bulky groups, in the 8-position (meso) and/or at the 1- and 7-positions of BODIPYs or both, reveal steric hindrance.[177-178] Therefore, the 35% yield of click reaction between ortho-azido derivatized BODIPY **44** and propargyl thymidine derivative **47** is not astonishing.

To check the influence of thymidine itself as a bulky group in this reaction, we also synthesized a long-side chain alkyne substituted thymidine (Scheme 30). First, 4-propargyloxybutanoic acid **52** was synthesized starting from γ-butyrolactone via a synthetic approach for a similar structure published in the literature.[179] The ring opening was achieved by addition of NaOH to yield 4-hydroxybutanate and to this structure propargyl bromide was added in the presence of NaH as base in DMF. Then, the coupling reaction was executed between the 3′-OH group of the TBDMS-protected thymidine and 4-propargyloxybutanoic acid by Steglich esterification in the presence of DCC and a catalytic amount of DMAP. After purification, the yield of the product was 78% and this 4-propargyloxybutanoyl thymidine derivative was incorporated into the click reaction with the 8-(2-azidophenyl)BODIPY derivative **44** to yield ligation product **51**. As expected, there was a

decent improvement by the yield, being 63%. This result led us to think that the low yields of these ligations are also related with the bulky group, TBDMS-protected thymidine. However, the yield of click reaction was still not competitive to the meta- and/or para-azido BODIPYs. Therefore, it can be expressed that steric compulsion of triazole group and BODIPY causes the system to have a fragile structure and even by soft interventions decomposition takes place. It is highly possible that during the flash column chromatography, the product decomposes. However, column chromatography is mandatory to get rid of minor but highly fluoroscent side products.



Scheme 30: The CuAAC reaction of long chain alkyne modified thymidine **53** and azidophenyl substituted BODIPY **44**.

3.2.4 Optical Properties of 8-(Azidophenyl) BODIPYs and Their Click Ligations

The optical properties of the synthesized 8-(azidophenyl) BODIPYs and their click ligation products were evaluated and the spectroscopic data collected in Table 4. All measurements were executed in CH_2Cl_2.

The aim of our investigation was to achieve a BODIPY structure that can undergo fluorogenic click reaction. None of the synthesized azido derivatized BODIPY structures were completely non-fluorescent. But, the quenching efficiency of $-N_3$ group was enough to decreace fluorescence of the ortho-azidophenyl BODIPY derivative **44** around 10-fold compared to the other derivatives (Figure 26).

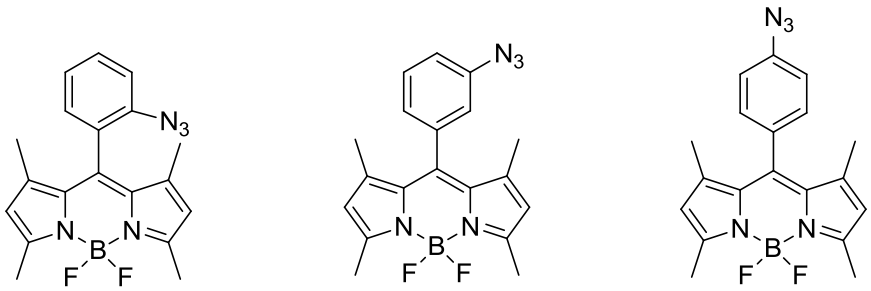


Figure 26: Structures and the fluorescence quantum yields of compounds **44**, **45** and **46**.

Generally speaking, all compounds show a strong absorption band between $\lambda \approx 503\text{-}531$ nm with an absorption coefficient in the 17200-82500 $M^{-1}cm^{-1}$ range that is corresponding to the $S_0 \rightarrow S_1$ transition of the BODIPY core.[180-181] The shoulder on the high-energy side, attributed to the 0–1 vibrational band of the same transition can be also observed.[164] Besides, a significantly weaker, broad absorption band at about 375 nm for BODIPY derivatives can be assigned to the $S_0 \rightarrow S_2$ transition.[180-181] This transition band can not be clearly observed by the click ligation products.

The BODIPY dyes are generally known for their close absorption and emission region, thus, disclosing a problem of the reabsorption/reemission process (self-quenching).[182] Unfortunately, both the azidophenyl BODIPY and their click ligation products exhibit quite small Stokes stifts (the difference between the peak excitation and peak emission wavelengths). Their Stokes shifts range from 7 nm to 12 nm.

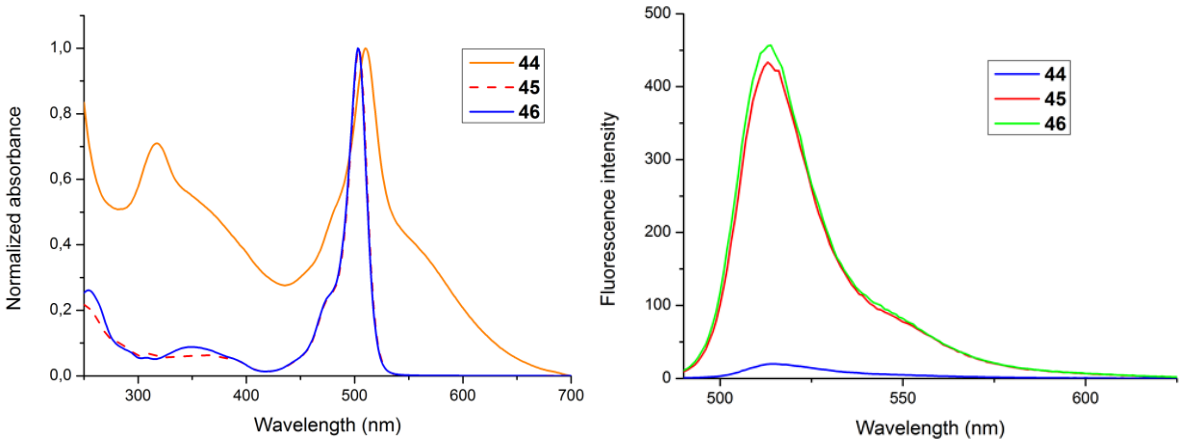


Figure 27: The normalized UV-Vis spectra at 1.0 x 10^{-5} M in CH_2Cl_2 (left) and the emission spectra at 1.0 x 10^{-6} M in CH_2Cl_2 (λ_{exc} = 470 nm) (right) of the 8-(azidophenyl) BODIPYs.

For the 8-(azidophenyl) BODIPYs, the absorption maximum of **44**, **45** and **46** are λ_{max} = 508, 504 and 503 nm, respectively (Figure 27). The absorbance of ortho-azidophenyl BODIPY derivative **44** is much less then other derivatives (Table 4). Fluorescence quantum yields of these substances are 0.055, 0.63 and 0.56, respectively. To examine the effect of substitution on the core of the ortho-azidophenyl based BODIPY derivatives, we also investigated the optical properties of 2,6-diethyl substituted BODIPY derivative **43** (Figure 28). Introducing ethyl groups made the absorbance and emission maxima red-shifted and fluorescence quantum efficiency is increased (Table 4). Similar changes in properties have been published for unsubstituted phenyl derivatives of this BODIPY and its 2,6-diethyl BODIPY derivative.[183]

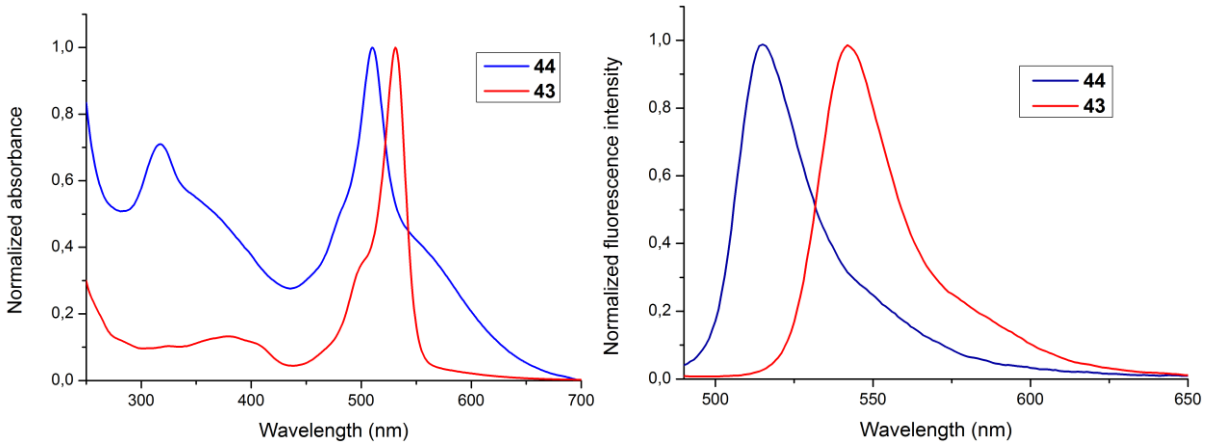


Figure 28: The normalized UV-Vis spectra at 1.0 x 10^{-5} M in CH_2Cl_2 (left) and the normalized emission spectra at 1.0 x 10^{-6} M in CH_2Cl_2 (λ_{exc} = 470 nm) (right) of **43** and **44**.

The UV-vis spectra of click ligation products of BODIPY derivatives are shown in Figure 29. The general appearance of the absorbance spectra is almost the same in all cases. The

absorbance maxima of ortho-azidophenyl derivatized BODIPY compounds **48** and **51** are the same, as well as meta- and para-azidophenyl BODIPYs (510 nm for **48** and **51**, 505 nm for **49** and **50**).

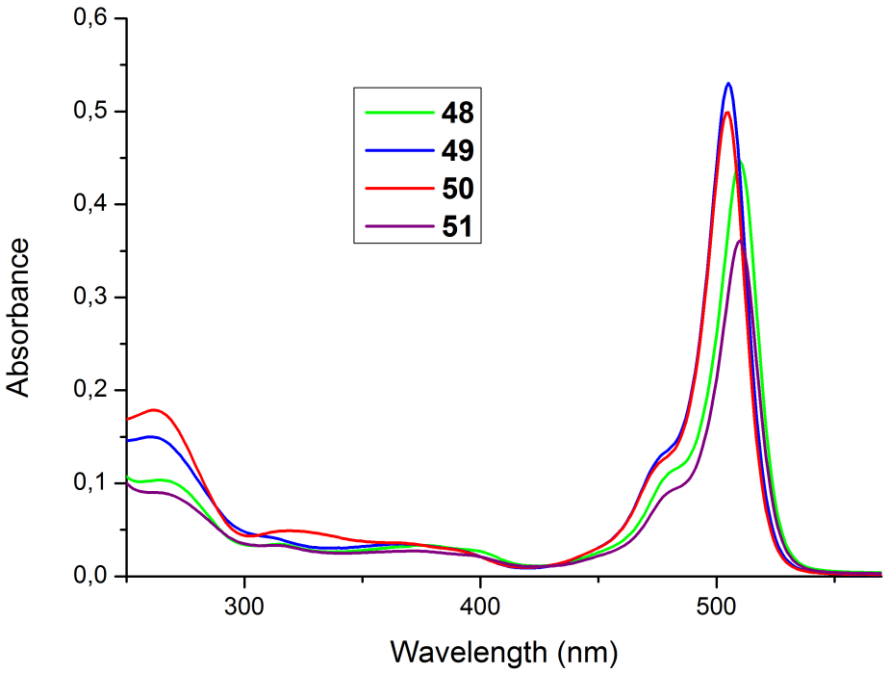


Figure 29: The UV-Vis spectra of click ligations of 8-(azidophenyl) BODIPYs at 1.0 x 10^{-5} M in CH_2Cl_2.

Fluorescence emission spectra of the BODIPYs **44**, **45**, **46** and their click ligation products show that all compounds have similar spectral pattern (Figure 30). Their emission maxima are almost the same at $\lambda \approx 515$ nm.

The azido group in the ortho-position of phenyl quenches the fluorescence of the BODIPY compound by increasing the electron transfer to the excited BODIPY core (photoinduced electron transfer). The click reaction with an alkyne side chain causes restoration of fluorescence due to the decreased electron effect of nitrogen by the formation of triazole ring.[96] Its low fluorescence quantum efficiency exhibits a 14-fold enhancement after click reaction with thymidine derivative **47.** The fluorescence quantum yield of **44** is 0.055 and after the formation of click ligation product **48**, it raises to 0.78 (Figure 31). Moreover, compared to this derivative, the click ligation product of ortho-azidophenyl BODIPY derivative **44** and the TBDMS-protected thymidine derivative **53**, which has longer side chain with terminal alkyne, has lower fluorescence quantum yield as 0.61.

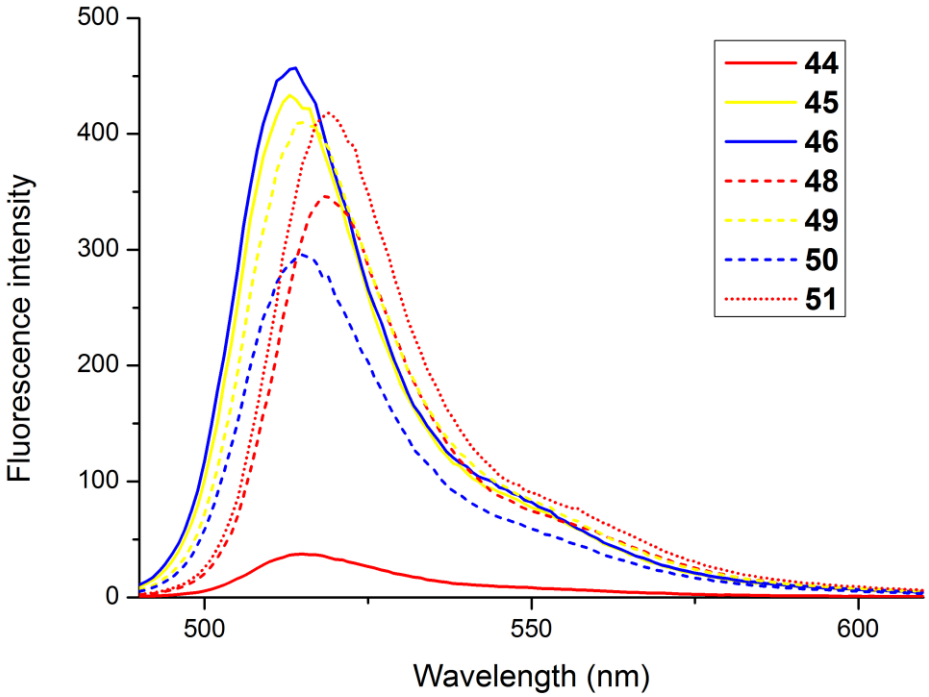


Figure 30: The emission spectra at 1.0 x 10^{-6} M in CH_2Cl_2 (λ_{exc} = 470 nm) of the 8-(azidophenyl) BODIPYs and their click ligations.

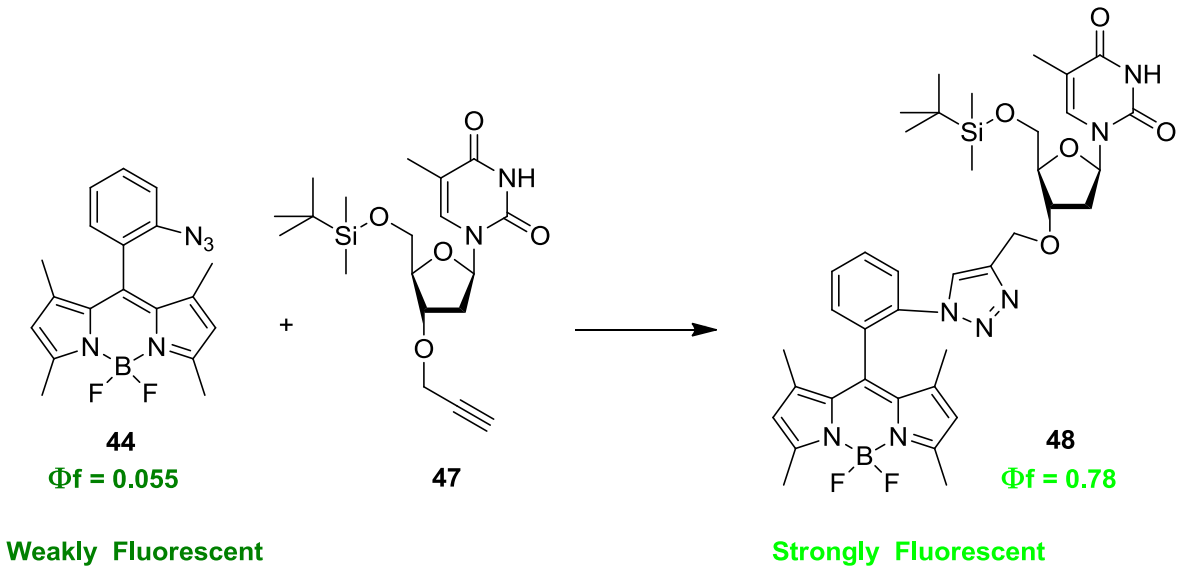


Figure 31: The schematic presentation of fluorescence enhancement of BODIPY **44** after click reaction.

As seen in Table 4, in comparison to meta-azido phenyl BODIPY derivative **45**, the para-derivative **46** has lower fluorescence quantum efficiency. This situation can be explained by the rotation of the aryl-ring, causing a nonradiative deactivation of the excited-state.[184] It is worth noting that the click ligation product of derivative **50** discloses the lowest fluorescence quantum efficiency between all derivatives except ortho-azidophenyl BODIPY derivatives **43** and **44**. Additional groups might conduce the rotation of the aryl-ring.

Compound	Absorbance λ_{max} [nm]	Emission λ_{max} [nm]	ε (M^{-1} cm^{-1})	Φ_f[a]	Stoke shift cm^{-1}
44	508	515	17200	0.055	267
43	531	543	17200	0.202	416
45	504	513	58800	0.63	348
46	503	513	56300	0.56	387
48	510	518	82500	0.78	303
49	505	515	80900	0.79	384
50	505	515	72700	0.32	384
51	510	519	70500	0.61	340

[a]Fluorescence quantum yields for BODIPY derivative **43** (λ_{exc} = 500 nm), other BODIPYs **44**-**46** and click ligation products **48**-**51**(λ_{exc} = 470 nm) were calculated using rhodamine 6G (0.95 in ethanol)[185] as standard.

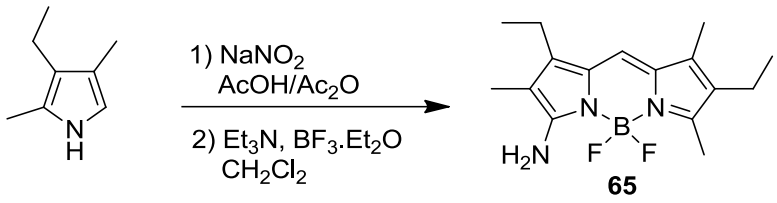
Table 4: Optical properties of the of the synthesized 8-(azidophenyl) BODIPYs and their click ligation products.

3.3 Nitro and Amino BODIPY Derivatives

During the studies for fluorogenic click derivatives of BODIPYs, the synthesis of azido functionalized BODIPY compounds in the position 2 and 3, with the purpose of using fluorescence quenching effect of azido groups, was also our intention. In order to get these structures, one of the key reaction in organic chemistry, the synthesis of aromatic azides from the corresponding amines would be convenient. However, despite several attempts, this approach was not successful for the synthesis of the desired compounds.

Nevertheless, the outcome of these experiments gave some novel BODIPY derivatives that exhibit interesting optical properties. According to a long literature search, to the best of our knowledge, BODIPY derivatives containing NO_2 groups at position 2 and 3 are not available. We managed to synthesize these derivatives successfully. Moreover, we also succeeded in obtaining 3-amino BODIPY derivative and again to the best of our knowledge, there is only one example of 3-amino BODIPY in the literature,[186-187] prepared unexpectedly during the trials of developing 8-aza-BODIPY derivative in order to use it as laser dye. The one-pot synthesis started by treatment of 2,4-dimethyl-3-ethylpyrrole with aqueous $NaNO_2$ in a mixture of acetic acid/acetic anhydride; and then, after evaporation of the solvent, the crude

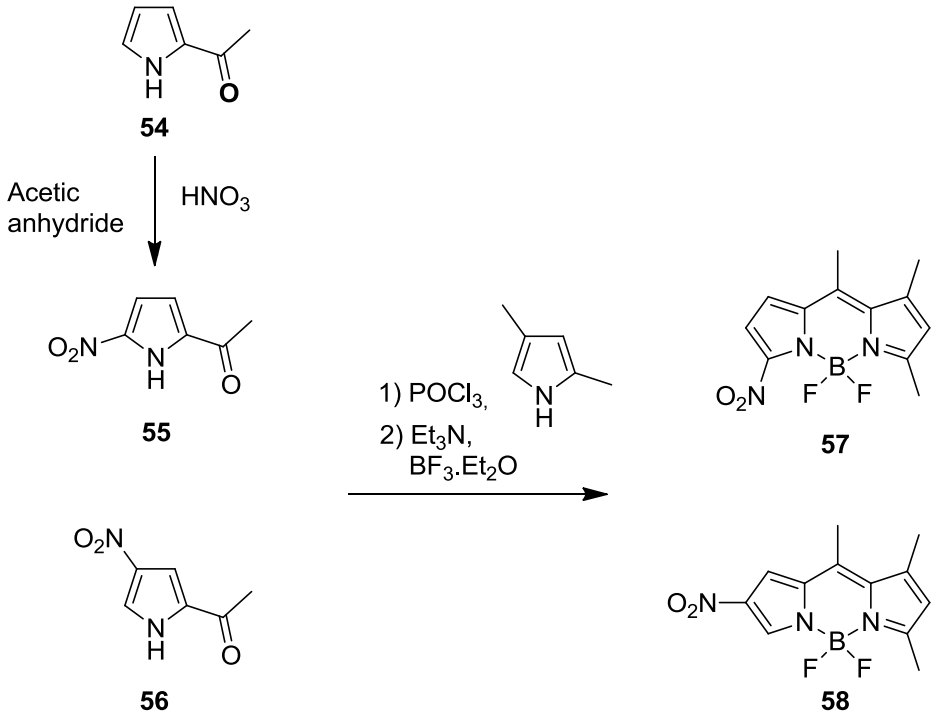
mixture was dissolved in CH_2Cl_2 and the synthesis was completed by addition of Et_3N and $BF_3.Et_2O$ (Scheme 31). When trying to purify the reaction product, 3-amino BODIPY derivative **65** could be isolated only after double column chromatography (that is required in supporting information of the paper).



Scheme 31: Published synthesis of 3-amino BODIPY.

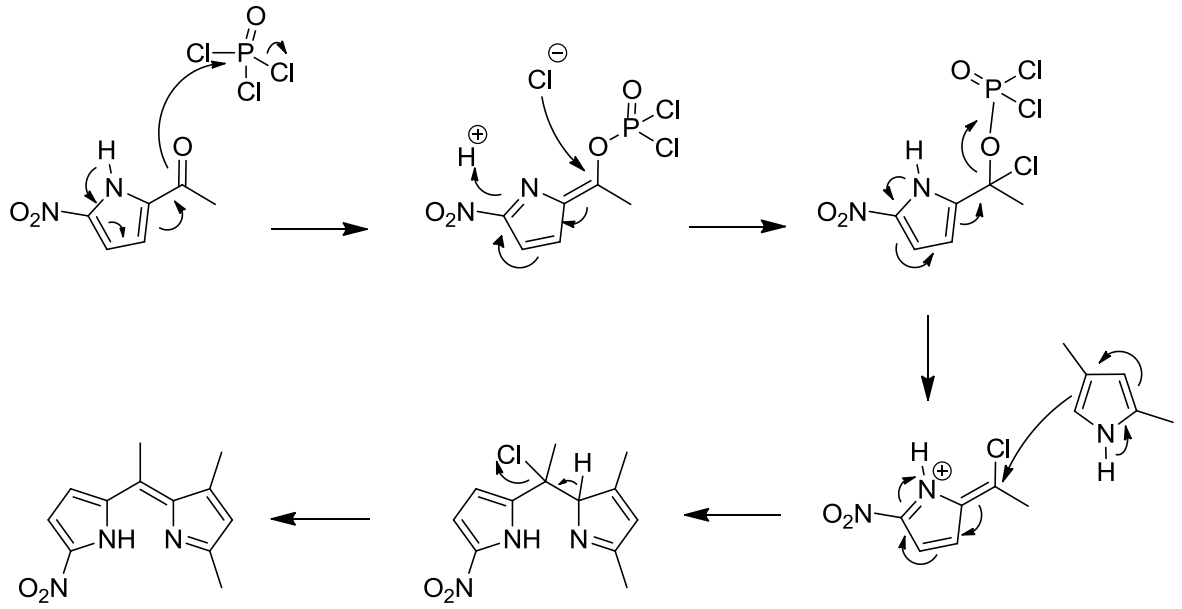
3.3.1 Synthesis of Nitro BODIPYs

One of the synthetic approaches of BODIPY derivatives is to start from pyrrolic ketones. This approach permits chemists to achieve asymmetrical BODIPYs.[136] Moreover, the postsynthetic modification of pyrrole would be a wise idea to avoid possible decomposition of BODIPY during the reaction and also multiple substitutions. Therefore, we did start our process by synthesizing nitro pyrrole derivatives (Scheme 32). According to the published procedure,[188] aqueous nitric acid was added to a solution of 2-acetyl pyrrole in acetic anhydride to give a mixture of 5- and 4-nitro-2-acetylpyrrole (**55** and **56**) in 35% and 50% yield, respectively. Then, **55** and **56** were incorporated separately to the reaction with 2,4-dimethyl pyrrole to furnish BODIPY derivatives **57** and **58** in 55% and 25% yield, respectively. For this common synthetic procedure, the pyrroles are dissolved in CH_2Cl_2 and one equivalent of $POCl_3$ was added. After a certain time, the formed dipyrromethene was converted into BODIPY systems by addition of excess triethylamine and boron trifluoride etherate. Column chromatograpy was executed to purify the Nitro-BODIPYs.



Scheme 32: The synthetic route to Nitro-BODIPYs.

The formation of asymmetrical Nitro-BODIPYs starts with the condensation of ketopyrroles and 2,4-dimethylpyrrole in the presence of $POCl_3$. The possible reaction mechanism of this formation is shown in Scheme 33,[189] with 2-acetyl-5-nitropyrrole as an example just for schematic presentation. The lewis acid $POCl_3$ attacks to ketopyrrole and forms a vinylic Vilsmeier-Haack reagent. Then, electrophilic attack to the 2. position of 2,4-dimethylpyrrole takes place, followed by elimination of HCl to give the dipyrromethene product.



Scheme 33: Proposed mechanism of formation of dipyrromethene.

The molecular structures of nitro BODIPYs were confirmed by NMR and mass spectrometry. The ^{1}H-NMR spectrum of 3-nitro BODIPY **57** reveals that the singlets at δ = 2.72, 2.63 and 2.51 ppm belong to the methyl groups of the system (Figure 32). In the aromatic region, the only singlet at δ = 6.42 ppm can be attributed to the proton H-6. The protons H-1 and H-2 can be associated to the doublets at δ = 6.93, 7.20 ppm, respectively, with the equal J-value (^{3}J = 4.2 Hz). H-2 is more deshielded by the electron withdrawing nitro group and because of this, it is more downfield shifted. In the ^{13}C-NMR of 3-nitro BODIPY **57**, the aliphatic carbons are at δ = 16.22, 14.93 and 14.76 ppm. In the aromatic region, C-3 should have been much more downfield shifted due to the electronic distribution, however, because of directly substituted nitro group, it is less downfield shifted than C-5. These two carbons are at δ = 168.49 and 148.31 ppm. The rest of the aromatic carbons are at δ = 139.41, 136.81, 133.88, 126.24, 117.59, 113.58 ppm. Besides, the high resolution mass measurements gave a correct molar peak at $[M + H]^{+}$ at m/z = 278.0918.

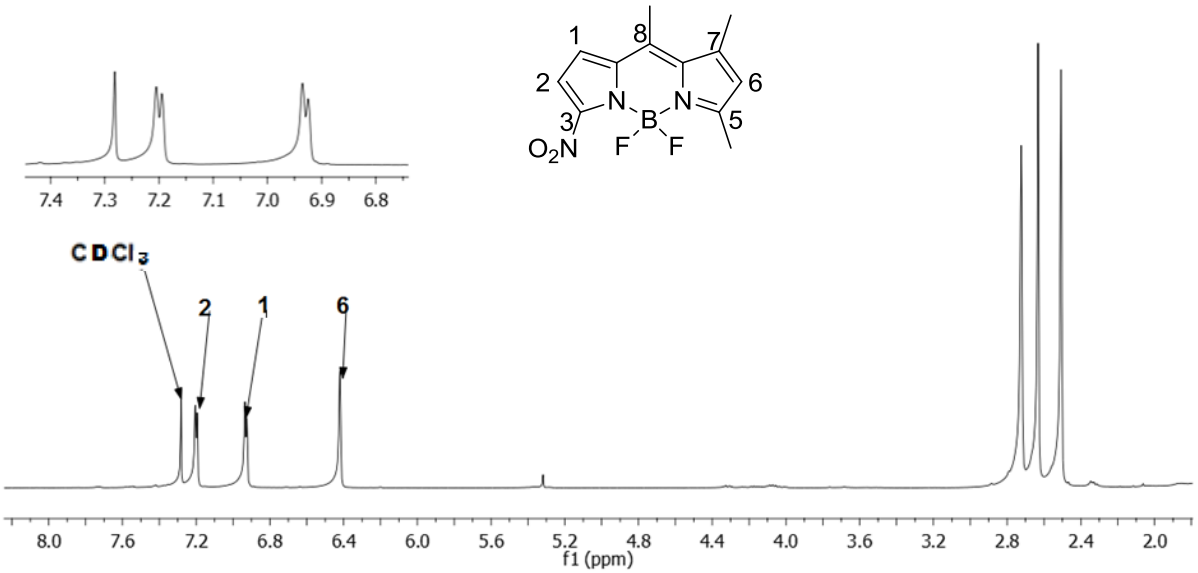


Figure 32: ^{1}H-NMR spectrum of 3-nitro BODIPY **57**.

The positional change of the electron withdrawing NO_2 group causes differences in the aromatic region of ^{1}H-NMR spectrum (Figure 33). In the ^{1}H-NMR spectrum of 2-nitro BODIPY **58**, it is observed that all aromatic peaks are singlets as expected. The proton H-6 is again in a similar position at δ = 6.40 ppm, however, the nitro group induces H-1, in both spectra, to a downfield shift at δ = 7.48 ppm. The proton H-2 is covered by electronegative atoms and electron withdrawing group, so it is highly downfield shifted and the peak at δ = 8.15 ppm can be associated with H-2. Moreover, the molecular integrity of **58** was also

proven by mass spectrometric analysis with a correct molar peak at $[M + Na]^+$ at m/z = 302.0883.

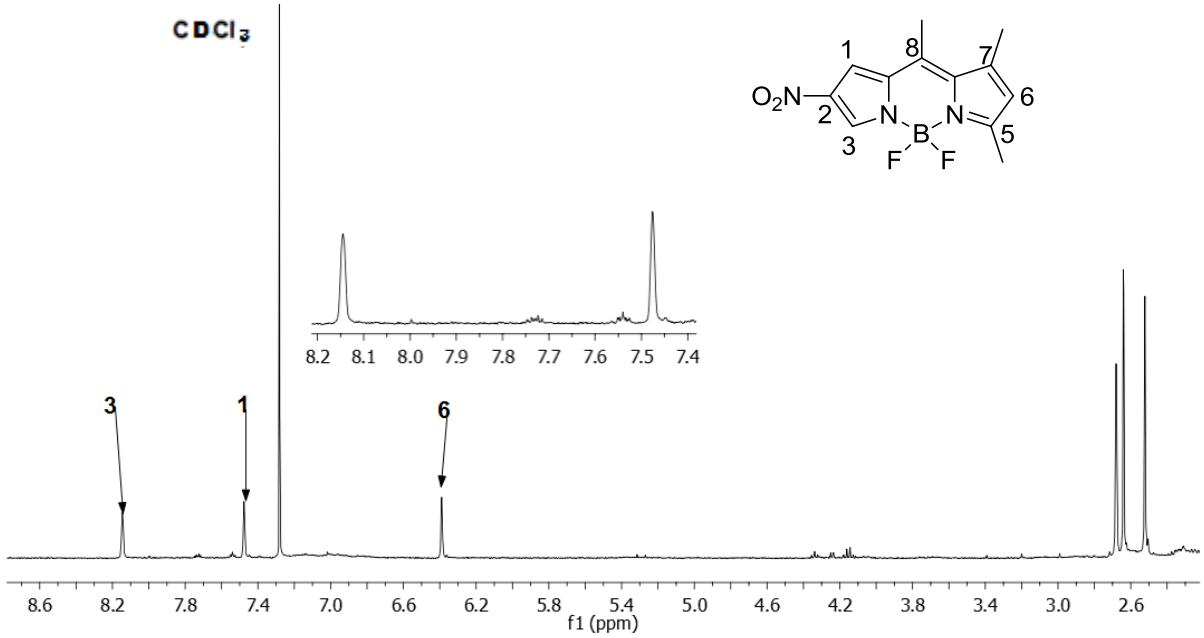
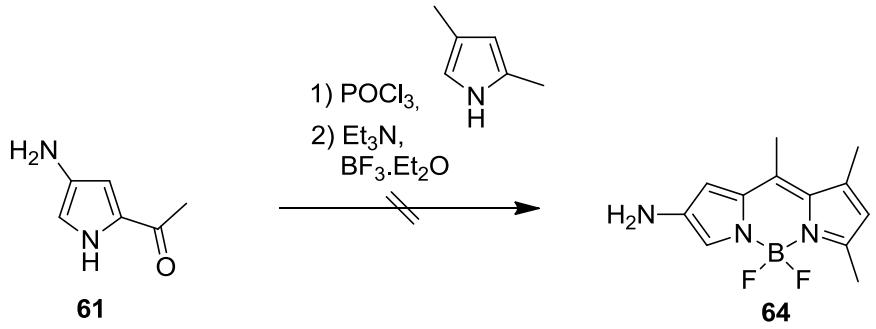


Figure 33: ^{1}H-NMR spectrum of 3-nitro BODIPY **58**.

3.3.2 Synthesis of 3-Amino BODIPY

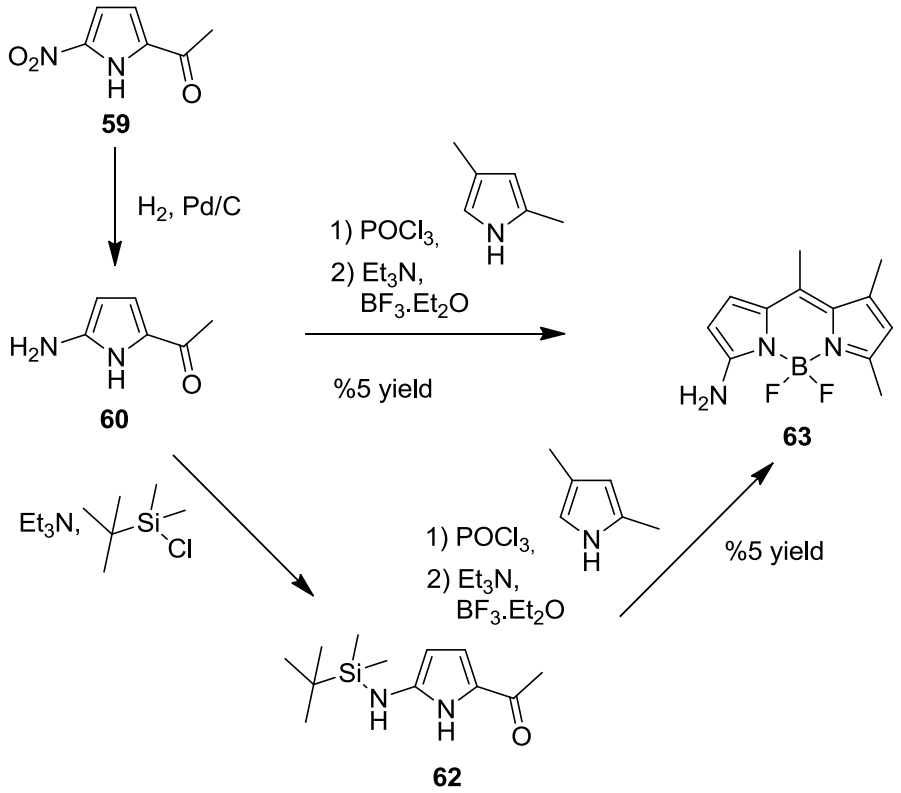
Nitro groups can be readily reduced into amino groups and it was naturally the first idea to synthesize amino BODIPYs after obtaining nitro BODIPYs. The first experiments were based on again modification of pyrroles with the desired group before formation of BODIPY (Scheme 35). For this purpose, reduction of 2-nitropyrrole was carried out by hydrogenation in the presence of Pd/C catalyst in methanol. But, there is a problem in the BODIPY synthesis, as phosphorus oxychloride definitely reacts with the free amino group. However, it would be worth to look at the competition between the two active groups reacting with $POCl_3$. To a mixture of 2-acetyl-5-aminopyrrole **60** and 2,4-dimethylpyrrole, $POCl_3$ was added and after some time by the addition of Et_3N and $BF_3.Et_2O$, 3-amino BODIPY **63** was formed. However, the yield was quite low with only 5%. Purification of the product is not a problem because, the structure is fluorescent and due to this, it can easily isolated by column chromatography.

The same route was also studied in order to synthesize 2-amino BODIPY derivative with starting 2-acetyl-4-aminopyrrole **61**, but unfortunately, the product **64** was not isolated (Scheme 34).



Scheme 34: The synthetic route of 2-amino BODIPY derivative.

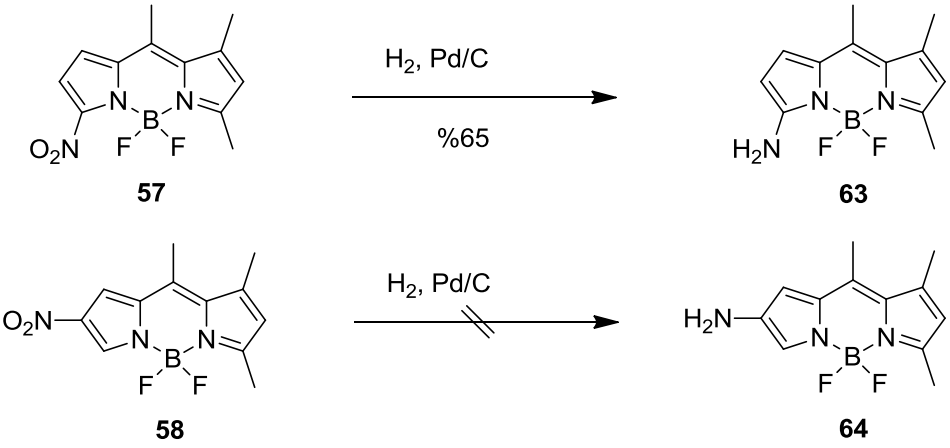
In the literature, there are a lot of examples of very low yields in the formation of BODIPYs, nevertheless, the idea of protecting amino group before BODIPY formation would be a wise solution. The TBDMS-protected pyrrole derivative **62** seemed to be a good choice, but the isolation of TBDMS-protected BODIPY was not successful (Scheme 35). Again, unprotected 3-amino BODIPY derivative **63** was obtained, unfortunately, in only 5% yield. The low yield of BODIPY formation and the extra reaction step for protection of pyrrole derivative is indeed causing this second idea impossible to use.



Scheme 35: Synthesis of 3-amino BODIPY via amino-acetylpyrrole.

Direct reduction of nitro BODIPYs into amino BODIPYs were also studied. Hydrogenation were executed with H_2 gas and Pd/C catalyst at room temperature. After 2 hours of stirring,

the desired derivative **63** could be isolated in a quite high yield (65%). The 2-amino BODIPY derivative **64**, however, could not be isolated (Scheme 36).



Scheme 36: Hydrogenation of nitro BODIPYs.

This surprising result gave us the opportunity to use this new route to increase the amount of 3-amino BODIPY **63** and to investigate its optical and physical properties.

3.3.3 Optical Properties of 2- and 3-Nitro BODIPYs and 3-Amino BODIPY

The optical properties of the synthesized nitro and amino BODIPY derivatives were evaluated and the spectroscopic data collected in Table 5. The measurements were executed for **57** and **58** in CH_2Cl_2 and for **63** in CH_2Cl_2 and also in EtOH.

All compounds show a strong absorption band between $\lambda \approx 464$-507 nm with an absorption coefficient in the 10749-39163 $M^{-1}cm^{-1}$ range that is corresponding to the $S_0 \rightarrow S_1$ transition of the BODIPY core.[180-181]

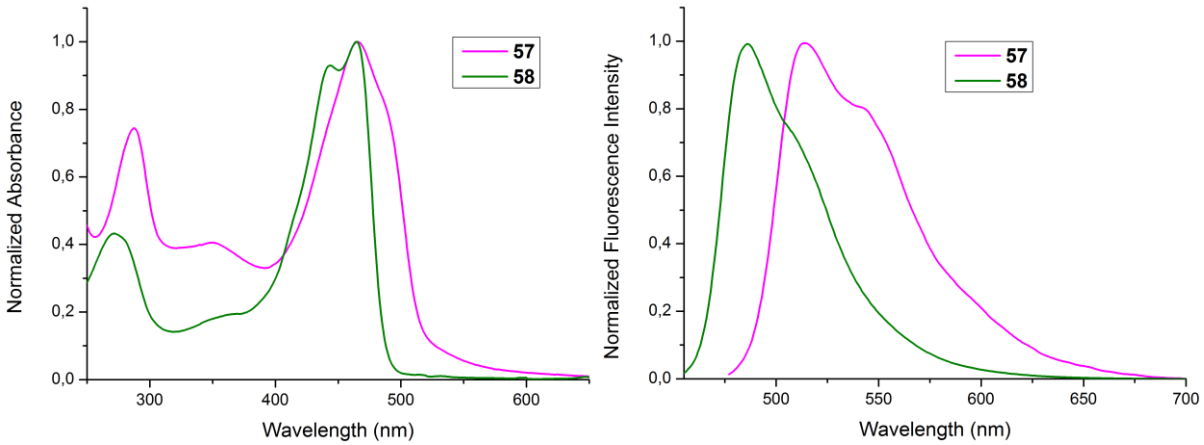


Figure 34: The normalized UV-Vis spectra at 1.0 x 10^{-5} M in CH_2Cl_2 (left) and the normalized emission spectra at 1.0 x 10^{-6} M in CH_2Cl_2 (right) of **57** (λ_{exc} = 470 nm) and **58** (λ_{exc} = 445).

Figure 34 shows normalized absorbance (left) and fluorescence (right) spectra for compounds **57** and **58**. In the absorbance spectrum of **57**, one of the characteristic peaks of BODIPY derivatives, the 0-1 vibrational band of the $S_0 \rightarrow S_1$ transition (the shoulder on the high-energy side of it) can not be observed and interestingly, in the absorbance spectrum of **58**, it is significantly strong at λ = 444 nm. The structural change, the different position of the electron withdrawing NO_2 group might be the reason of this phenomenon. Moreover, both **57** and **58** have large Stokes shifts, 2050 and 1018 cm^{-1} (normally, BODIPYs have small Stokes shifts in the range of 500 cm^{-1}). The bathochromic shift in the fluorescence band of 3-nitro BODIPY **57** is higher and the absorption bands are almost the same giving rise to a larger Stokes shift than **58**.

In the absorbance spectra of nitro BODIPY derivatives **57** and **58**, the peaks around 350 nm can be assigned to the $S_0 \rightarrow S_2$ transition. They come out at higher energy region than the generally observed area for BODIPYs (around at $\lambda \approx$ 375 nm).[180-181]

Figure 35 shows the normalized UV-Vis spectra (left) and the normalized emission spectra (right) of 3-amino BODIPY **63** in both CH_2Cl_2 and EtOH. Its absorption and emission are dependent on solvent polarity. The absorption EtOH maximum 507 nm in CH_2Cl_2 and it is 7 nm blue shifted (hypsochromic shift) in EtOH, and also the fluorescence emission maximum shows 4 nm blue shift in EtOH compared to DCM solution (Table 5).

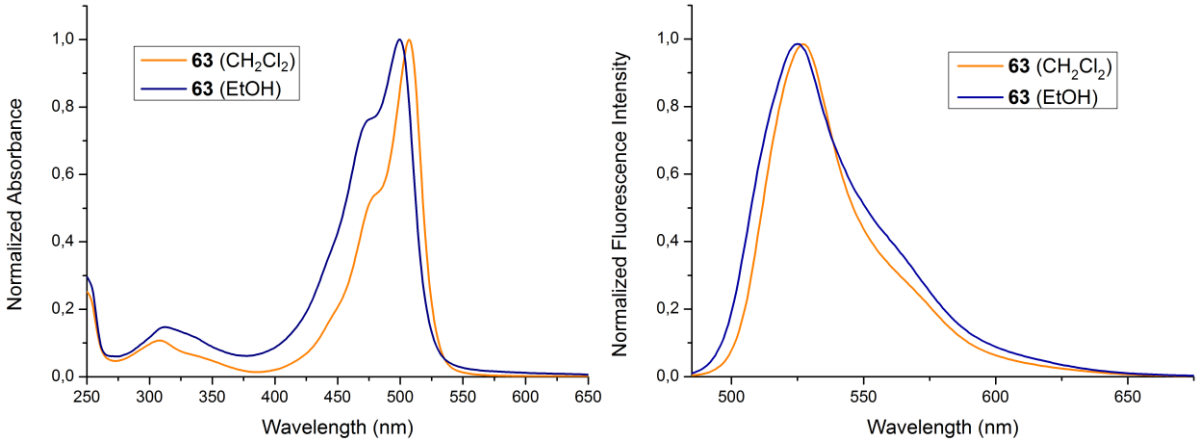


Figure 35: The normalized UV-Vis spectra at 1.0 x 10^{-5} M (left) and the normalized emission spectra at 1.0 x 10^{-6} M (right) of **63** in CH_2Cl_2 and EtOH.

Moreover, compared to the nitro BODIPY derivatives, the introduction of an amine group into position 3 of BODIPY core induces large bathochromic shifts of the absorption and emission maxima. This bathochromic shifts occur due to extented delocalization of the π – system by the amino group.[186] Furthermore, lower Stokes shifts, in both CH_2Cl_2 and EtOH, are observed (748 and 879 cm^{-1}, respectively). From all nitrogen substituted BODIPYs, the

highest fluorescence quantum efficiency and molar extinction coefficient are obtained by 3-amino BODIPY **63**. It also means, it is the brightest derivative (Table 5).[190]

The fluorescence quantum yield of the 3-amino BODIPY **63** alters according to the solvent, too. The polar solvent (ethanol), the quantum yield of **63** decreases, it is 0.81 in DCM and 0.58 in EtOH solution. This might be due to the aggregation of the dye, because in the study of Banuelos et al., the fluorescence quantum yield of amino BODIPY derivative (introduced in chapter 3.3) is nearly solvent independent.[186]

Compound	Absorbance λ_{max} [nm]	Emission λ_{max} [nm]	ε (M^{-1} cm^{-1})	Φ_f[a]	Stoke shift cm^{-1}
57	465	514	10700	0.10	2050
58	444, 464	487	25500	0.74	1018
63 (CH_2Cl_2)	507	527	39100	0.81	748
63 (EtOH)	500	523	25000	0.58	879

[a]Fluorescence quantum yields for nitro BODIPY derivatives **57** and **58** (λ_{exc} = 470 nm) were calculated using rhodamine 6G (0.95 in ethanol)[185] as standard while for amino BODIPY **63** (λ_{exc} = 445 nm) was calculated using acridine yellow (0.47 in ethanol)[191] as reference.

Table 5: Optical properties of the of the synthesized nitro BODIPYs **57**, **58** and amino BODIPY derivatives **63**.

3.3.4 Conclusion

During this work, the influence of the substitution of position 2 and 3 with nitrogen containing groups have been investigated. For this purpose, 2- and 3-nitro BODIPY **57**, **58** and 3-amino BODIPY derivatives **63** have been synthesized. The synthesis of these derivatives start with the nitration of 2-acetyl pyrrole and after separation of the two nitro compound, they were separately introduced into the conventional one-pot synthesis of BODIPY derivatives with 2,4-dimethylpyrrole to yield **57** and **58** in 55% and 25%, respectively. Besides, the reduction of 5- and 4-nitro-2-acetylpyrroles **55** and **56** in the presence of Pd/C gave amino pyrroles **60** and **61**. However, only 3-amino BODIPY derivative **63** starting from 2-acetyl-5-aminopyrrole **60** was successfully achieved in 5% yield. Indeed, direct reduction of 3-nitro BODIPY **57** allows to obtain the 3-amino BODIPY derivative **63** in 65% yield.

The investigation of the optical properties of nitro BODIPY derivatives showed that the position of the nitro group of BODIPY derivatives have significant influence on the

photophysical properties of the compounds. The main difference is found in the fluorescence spectra of these compounds. Compared to **58**, 3-nitro BODIPY **57** exhibits a bathochromic shift in the fluorescence band and this is the reason of its larger Stokes shift. However, the fluorescence quantum efficiency of 3-nitro BODIPY **57** is not satisfactory (0.10). This is understandable, because the substitution at the positions 3 and 5 of the BODIPYs are strongly influencing the fluorescence properties of the system and it is known that a nitro group is inherently an effective fluorescence quencher. Interestingly, the fluorescence quantum yield of 2-nitro BODIPY **58** is 0.74.

The 3-amino BODIPY also presented interesting optical properties. The functionalization of position 3 of BODIPY exposed large bathochromic shifts of the absorption and emission maxima. Its molar extinction coefficient is higher than nitro derivatives, but the Stokes shift is not improved than the BODIPY dyes.

4 Summary

In the beginning of this thesis, the aim was to develop new fluorogenic click reactions. During the studies, we managed to achieve fluorogenic Diels-Alder reactions of novel phencyclone derivatives and fluorogenic Huisgen 1,3-dipolar cycloaddition of a 8-(2-azidophenyl) BODIPY derivative. Moreover, other outcomes of these studies were some new BODIPY derivatives with interesting optical properties.

With the aim of obtaining fluorogenic Diels-Alder reaction, novel phencyclone derivatives were synthesized. In a short time and convenient reaction conditions, the conversion of Diels-Alder adducts were observed by color-change and up to 33-fold enhancement of fluorescence. The modification of thymidine and deoxycytidine derivatives with maleimide side chain and their usage in fluorogenic Diels-Alder reaction is also reported. From the results, it is also obvious that the adducts present relatively lower quantum efficiencies than most of the studied fluorescent dyes and their low excitation and emission maxima are not satisfactory for biological applications. Further studies will allow the improvement of optical properties of this fast and efficient fluorogenic Diels-Alder reaction.

We then moved onto the investigation of BODIPY based fluorogenic reactions. The ortho, meta, and para azido substituted meso-phenyl ring of BODIPYs have been studied to examine the substituent effect in the optical properties of BODIPYs. The ortho-substitution by an azide group successfully quenched the fluorescence of azidophenyl BODIPY derivative **44** compared to other derivatives. The fluorescence quantum efficiency of **44** is 0.055, and after its copper(I) catalyzed alkyne-azide cycloaddition with TBDMS-protected thymidine derivative **47**, a 14-fold of fluorescence enhancement was observed.

The properties of other 8-(azidophenyl) BODIPY derivatives and their click ligation products with TBDMS-protected thymidine derivatives were also investigated. The fluorescence quantum yield of 8-(3-azidophenyl) BODIPY derivative **45** is improved by click reaction (from 0.63 to 0.79). However, interestingly, a decrease was observed by formation of the click ligation product of the 8-(4-azidophenyl) BODIPY derivative **46** (from 0.56 to 0.32).

Moreover, the click reaction of 8-(2-azidophenyl) BODIPY derivative **44** with TBDMS-protected thymidine derivative **47** was quite low and the solution to increase it was to increase the distance of BODIPY derivative and TBDMS-protected thymidine. For this purpose, a terminal alkyne carrying longer chain was used to synthesize a new TBDMS-protected

thymidine derivative **53** and usage of this derivative into the CuAAC reaction with 8-(2-azidophenyl) BODIPY derivative **44** nearly doubles the reaction yield.

In addition to these studies, some interesting novel nitro and amino BODIPYs, emerged during search of the fluorogenic click reactions, were also investigated. The pre-synthetic modification of pyrrole derivatives ensure the one-pot synthesis of nitro BODIPYs **57** and **58**. However, for the amino BODIPY **63**, this method did not work quite effective, so it was synthesized by direct reduction of nitro BODIPY **57** in 65% yield.

The fluorescence quantum yield 3-nitro BODIPY **57** was quite poor, however, by 2-nitro BODIPY **58**, the quenching effect of nitro group was not so efficient and the fluorescence quantum efficiency was 0.74. Additionally, these two compound's Stokes shifts were relatively higher than common BODIPY dyes. Besides, the optical properties of 3-amino BODIPY **63** was examined in CH_2Cl_2 and also in EtOH. Higher extinction coefficients were obtained and the brightest derivative between all three BODIPYs was determined as 3-amino BODIPY **63** due to its high extinction coefficient and fluorescence quantum yield in CH_2Cl_2.

5 Experimental

5.1 Chemicals and Materials

All chemical reagents were purchased from Sigma-Aldrich, Acros and ABCR and used without purification. All solvents were applied in commercial p.a. quality and dried/ distilled prior to use. All experiments were carried out under standard Schlenk conditions and argon atmosphere.

5.2 Equipments

NMR Spectroscopy

The ^{1}H and ^{13}C {1H} NMR spectra were recorded on an ARX 400 or Avance III 600 NMR spectrometer from Bruker, using deuterated solvents. The chemical shift is given in ppm. The coupling constant J is expressed in Hz. As a reference, the ^{1}H- and ^{13}C-signal of the deuterated solvent used.

The measured values are given in the following order:

NMR experiment (measurement frequency, solvent, temperature): δ [ppm] = value (spin multiplicity; if necessary number of cores; optionally coupling constant; optionally assignment).

Spin-spin couplings are abbreviated as follows:

s= Singlet, d = Doublet, t = Triplet, q = Quartet, m = Multiplet, dd = Doublet of Doublets, dt = Doublet of Triplet. Additionally, b is used for „broad“ (for example, bs ≡ broad Singlet).

Mass Spectrometry

The GC-MS analysis were performed on a GC 17A Shimadzu QP 5050. Electron impact (EI) ionization was used, and the ionization energy was 70 eV.

The ESI-measurements were performed by a Bruker Daltronic micrOTOF.

UV/Vis Spectroscopy

The UV/Vis spectra were recorded with a V-670 spectrometer from Jasco.

Fluorescence Spectroscopy

The Fluorescence spectra were recorded with a Varian CARY Eclipse F2500 Fluorescence spectrometer.

A 10 mm quartz cuvette was used in all measurements. For all measurements, the slit width was constant. Fluorescence quantum efficiencies of the products were determined by comparing the areas under the emission spectra of the samples and a solution of reference in ethanol excited at the same wavelength of products. For derivatives of phencyclone, anthracene in ethanol (Φ_f = 0.27 in ethanol)[163] and for BODIPY derivatives, rhodamine 6G in ethanol (Φ_f = 0.95)[185] was used as references. Quantum yields were determined using:

$$\varphi_x = \varphi_{st}\left(\frac{I_x}{I_{st}}\right)\left(\frac{A_{st}}{A_x}\right)\left(\frac{n_x^2}{n_{st}^2}\right)$$

where φ_{st} is the reported quantum yield of the standard, I is the area of the integrated emission spectra, A is the absorbance at the excitation wavelength and η is the refractive index of the solvent. The subscripts "x" and "st" denote sample and standard, respectively.

Thin layer chromatography

Thin layer chromatography was applied by silica gel plates, SIL ® ALUGRAM G/UV254 from Macherey-Nagel (slice thickness 0.2 mm). The visualization was performed using a UV lamp working at 254 and 366 nm.

Column Chromatography

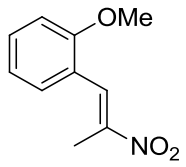
By preparative chromatography, Kieselgel 60 (0.040 – 0.063 mm) from Merck was used.

5.3 Synthesis

5.3.1 General Synthesis of 1-Aryl-2-nitro-prop-1-ene Derivatives

To a mixture of 0.05 mol of nitromethane and 0.05 mol of aldehyde, 2.5 ml of methylamine solution (33% in ethanol) was added dropwise until solution occurred. The solution was placed in the refrigerator for fifteen hours. The resultant bright yellow crystals were filtered, washed with ether and dried in the vacuum. The product was pure enough for the next step.

5.3.1.1 (E)-1-Methoxy-2-(2-nitroprop-1-en-1-yl)benzene (1) [142]



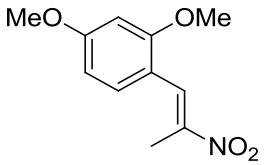
3.75 g. (0.05 mol) of nitromethane and 6.81 g. (0.05 mol) of o-anisaldehyde were reacted according to the general procedure (5.3.1). Yield: 8.9 g (93 %) of yellow crystals.

^{1}H-NMR (600 MHz, $CDCl_3$) : δ [ppm] = 8.30 (s, 1H, CH), 7.47 – 7.39 (m, 1H, H_{Ar}), 7.32 (dd, *J* = 7.7, 1.3 Hz, 1H, H_{Ar}), 7.07 – 7.01 (m, 1H, H_{Ar}), 6.98 (d, *J* = 8.3 Hz, 1H, H_{Ar}), 3.90 (s, 3H, OCH_3), 2.40 (s, 3H, CH_3).

^{13}C-NMR (151 MHz, $CDCl_3$) : δ [ppm] = 158.06, 147.47, 131.48, 129.91, 129.72, 121.44, 120.43, 110.80, 55.50, 14.04.

GC-MS (EI, 70 eV): t_R = 7,3 min, m/z = 193 $[M]^+$.

5.3.1.2 (E)-2,4-Dimethoxy-1-(2-nitroprop-1-en-1-yl)benzene (2)[142]



3.75 g. (0.05 mol) of nitromethane and 8.31 g. (0.05 mol) of 2,4-dimethoxybenzaldehyde were reacted according to the general procedure (5.3.1). Yield: 9.3 g (83%) of yellow crystals.

^{1}H-NMR (400 MHz, $CDCl_3$) : δ [ppm] = 8.32 (s, 1H, CH), 7.30 (d, *J* = 8.6 Hz, 1H, H_{Ar}), 6.57 (dd, *J* = 8.6, 2.4 Hz, 1H, H_{Ar}), 6.51 (d, *J* = 2.4 Hz, 1H, H_{Ar}), 3.88 (s, 3H, OCH_3), 2.42 (d, *J* = 1.0 Hz, 3H, CH_3).

^{13}C-NMR (101 MHz, $CDCl_3$) : δ [ppm] = 162.86, 159.84, 145.68, 131.06, 129.54, 114.28, 104.96, 98.36, 55.60, 55.49, 14.26.

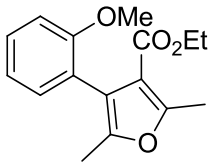
GC-MS (EI, 70 eV): t_R = 8,4 min, m/z = 223 $[M]^+$.

5.3.2 General Synthesis of Furan Carboxylate Derivatives

To a solution of the appropriate 1-aryl-2-nitro-prop-1-ene (0.026 mol) in 40 mL of methanol, 3-4 drops of piperidine were added at room temperature. After that, ethyl acetoacetate (3.37 g, 3.27 ml, 0.026 mol) was also added dropwise. The reaction mixture was then refluxed for 4 h. Methanol was evaporated by rotary and the residue was poured into water and extracted with chloroform (3 x 30 mL). The chloroform extract was washed with dil. HCl and water

successively. Organic layers were collected and dried over Na_2SO_4. After removal of chloroform, the resulting *oily* mixture was purified by column chromatography on silica gel (3:1 Hexane/EtOAc) to afford the furans as thick oils.

5.3.2.1 Ethyl 4-(2-methoxyphenyl)-2,5-dimethylfuran-3-carboxylate (3)[141]



5 g (0.026 mol) (E)-1-methoxy-2-(2-nitroprop-1-en-1-yl)benzene was used in the reaction according to the general procudure (5.3.2). Yield: 2.85 g (40%) of colourless thick oil.

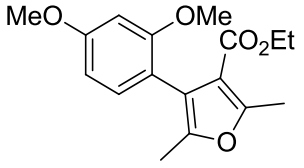
R_f = 0.45 (3:1 Hexane/EtOAc)

^{1}H-NMR (600 MHz, $CDCl_3$) : δ [ppm] = 7.35 – 7.30 (m, 1H, H_{Ar}), 7.17 (dd, *J* = 7.4, 1.7 Hz, 1H, H_{Ar}), 6.99 (td, *J* = 7.4, 1.0 Hz, 1H, H_{Ar}), 6.95 – 6.90 (m, 1H, H_{Ar}), 4.10 (q, *J* = 7.1 Hz, 2H, OCH_2), 3.78 (s, 3H, OCH_3), 2.58 (s, 3H, CH_3), 2.20 (s, 3H, CH_3), 1.06 (t, *J* = 7.1 Hz, 3H, CH_3).

^{13}C-NMR (151 MHz, $CDCl_3$) : δ [ppm] = 164.49, 157.43, 156.58, 147.12, 131.08, 128.45, 122.52, 120.09, 117.32, 114.50, 110.29, 77.21, 77.00, 76.79, 59.45, 55.27, 13.86, 13.78, 11.75.

GC-MS (EI, 70 eV): t_R = 7,9 min, m/z = 274 $[M]^+$.

5.3.2.2 Ethyl 4-(2,4-dimethoxyphenyl)-2,5-dimethylfuran-3-carboxylate (4)[141]



5.8 g (0.026 mol) (E)-2,4-dimethoxy-1-(2-nitroprop-1-en-1-yl)benzene was used in the reaction according to the general procudure (5.3.2). Yield: 3.5 g (44%) of colourless thick oil.

R_f = 0.49 (3:1 Hexane/EtOAc)

^{1}H-NMR (400 MHz, $CDCl_3$) : δ [ppm] = 7.06 (dd, *J* = 7.7, 0.9 Hz, 1H, H_{Ar}), 6.52 (d, *J* = 7.7 Hz, 2H, H_{Ar}), 4.11 (q, *J* = 7.1 Hz, 2H, CH_2), 3.86 (s, 3H, OCH_3), 3.75 (s, 3H, OCH_3), 2.57 (s, 3H, CH_3), 2.18 (s, 3H, CH_3), 1.10 (t, *J* = 7.1 Hz, 3H, CH_3).

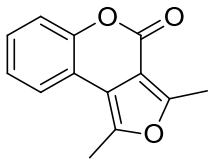
^{13}C-NMR (101 MHz, $CDCl_3$) : δ [ppm] = 162.86, 159.84, 145.68, 131.06, 129.54, 114.28, 104.96, 98.36, 55.60, 55.49, 14.26.

HR-MS (ESI): calc. 327.1208; found 327.1203 $[M + Na]^+$.

5.3.3 General Synthesis of Furo[3,4-c]coumarins

Furan carboxylate derivative (0.01 mol) and 45 ml of 33% HBr solution in acetic acid are added into a 100 ml round bottom flask and heated at 130 ^oC for 4 h. After cooling down to room temperature, the reaction mixture was poured into crushed ice, extracted with chloroform (3 x 30 mL), washed with saturated sodium bicarbonate (3 x 30 mL), water (2 x 30 mL) and brine (2 x 30mL). The chloroform layer was dried over Na_2SO_4, filtered and the solvent was evaporated in vacuum. The crude product was purified by column chromatography using silica gel (3:1 Hexan/EtOAc).

5.3.3.1 1,3-Dimethyl-4H-furo[3,4-c]chromen-4-one (5)[141]



2.74 g of 4-(2-methoxyphenyl)-2,5-dimethylfuran-3-carboxylate was used in the reaction according to the general procudure (5.3.3). Yield: 1.08 g (50%) of white solid.

R_f = 0.44 (3:1 Hexane/EtOAc)

M.p. 172^oC (Lit.[141] 175 ^oC)

^{1}H-NMR (600 MHz, $CDCl_3$) : δ [ppm] = 7.64 (dd, J = 7.8, 1.5 Hz, 1H, H_{Ar}), 7.36 – 7.18 (m, 3H, H_{Ar}), 2.70 (s, 3H, CH_3), 2.66 (s, 3H, CH_3).

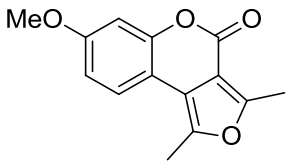
^{13}C-NMR (151 MHz, $CDCl_3$) : δ [ppm] = 158.58, 157.53, 151.39, 144.15, 128.08, 124.28, 123.39, 117.66, 116.00, 114.35, 107.92, 13.75, 13.38.

HR-MS (ESI): calc. 215.0703; found 215.0630 $[M + H]^+$.

UV/Vis (DCM): λ [nm] = 230, 269, 295.

FL λ_{Exc} = 266 nm (DCM): λ_{max} = 366, 383 nm.

5.3.3.2 7-Methoxy-1,3-dimethyl-4H-furo[3,4-c]chromen-4-one (6)[141]



3.04 g of 4-(2-methoxyphenyl)-2,5-dimethylfuran-3-carboxylate was used in the reaction according to the general procudure (5.3.3). Yield: 1.12 g (46%) of white solid.

R_f = 0.60 (1:1 Hexane/EtOAc)

M.p. 164°C (Lit.[141] 168 °C)

^{1}H-NMR (400 MHz, $CDCl_3$) : δ [ppm] = 7.51 (d, J = 8.5 Hz, 1H, H_{Ar}), 6.84 – 6.71 (m, 2H, H_{Ar}), 3.83 (s, 3H, OCH_3), 2.67 (s, 3H, CH_3), 2.60 (s, 3H, CH_3).

^{13}C-NMR (101 MHz, $CDCl_3$) : δ [ppm] = 159.49, 158.69, 157.46, 152.41, 142.60, 124.04, 114.23, 111.38, 108.70, 107.47, 102.35, 55.46, 13.53, 13.36.

HR-MS (ESI): calc. 245.0736; found 245.0808 $[M + H]^+$.

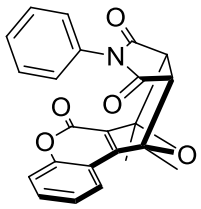
UV/Vis (DCM): λ [nm] = 238, 268, 305.

FL λ_{Exc} = 266 nm (DCM): λ_{max} = 366, 382 nm.

5.3.4 General Reaction of Diels-Alder Adducts of Coumarin Derivatives

N-Phenylmaleimide (1 eq.) and furo[3,4-c]coumarin (1 eq.) were placed in a reaction flask and dissolved in 10 ml toluene. Reaction mixture was heated at 100 °C for 2 h and cooled down to room temperature. Hexane was added until precipitation of a solid substance. Filtration and washing with a small amount of hexane gave the product as a white solid.

5.3.4.1 Diels-Alder reaction of 1,3-dimethyl-4H-furo[3,4-c]chromen-4-one and N-phenylmaleimide (7)



0.12 g (0.69 mmol) of 1,3-dimethyl-4H-furo[3,4-c]chromen-4-one and N-phenylmaleimide (0.12 g, 0.69 mmol) were used in the process explained in 5.3.4. Yield: quant.

R_f = 0.48 (1:1 Hexane/EtOAc)

M.p. 119°C

^{1}H-NMR (600 MHz, $CDCl_3$) : δ [ppm] = 7.86 (d, *J* = 7.9 Hz, 1H, H_{Ar}), 7.70 – 7.59 (m, 1H, H_{Ar}), 7.57 – 7.32 (m, 7H, H_{Ar}), 3.24 (d, *J* = 6.6 Hz, 1H, CH), 3.15 (d, *J* = 6.6 Hz, 1H, CH), 2.25 (s, 3H, CH_3), 2.13 (s, 3H, CH_3).

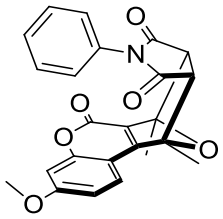
^{13}C-NMR (151 MHz, $CDCl_3$) : δ [ppm] = 172.22, 159.97, 156.19, 155.52, 132.72, 132.27, 131.58, 129.21, 128.94, 126.47, 124.80, 124.01, 117.95, 114.45, 87.82, 86.95, 77.21, 77.00, 76.79, 52.84, 52.40, 16.87, 14.66.

HR-MS (ESI): calc. 410.1004; found 410.0999 $[M + Na]^+$.

UV/Vis (DCM): λ [nm] = 230, 288, 327.

FL λ_{Exc} = 500 nm (DCM): λ_{max} = 366, 384 nm.

5.3.4.2 Diels-Alder reaction of 7-methoxy-1,3-dimethyl-4H-furo[3,4-c]chromen-4-one and N-phenylmaleimide (8)



0.15 g (0.615 mmol) of 7-methoxy-1,3-dimethyl-4H-furo[3,4-c]chromen-4-one N-phenyl-maleimide (0.106 g, 0.615 mmol) were used in the process explained in 5.3.4. Yield: quant.

R_f = 0.42 (1:1 Hexane/EtOAc)

^{1}H-NMR (600 MHz, $CDCl_3$) : δ [ppm] = 7.78 – 7.71 (m, 1H, H_{Ar}), 7.55 – 7.48 (m, 2H, H_{Ar}), 7.47 – 7.41 (m, 1H, H_{Ar}), 7.36 – 7.32 (m, 2H, H_{Ar}), 6.96 (dq, *J* = 4.9, 2.5 Hz, 2H, H_{Ar}), 3.93 (s, 3H, OCH_3), 3.23 (d, *J* = 6.6 Hz, 1H, CH), 3.12 (d, *J* = 6.6 Hz, 1H, CH), 2.21 (s, 3H, CH_3), 2.11 (s, 3H, CH_3).

^{13}C-NMR (151 MHz, $CDCl_3$) : δ [ppm] = 172.60, 172.39, 163.48, 160.25, 157.65, 156.51, 131.61, 129.18, 128.89, 128.39, 126.47, 124.94, 113.28, 107.80, 101.69, 87.73, 86.87, 55.89, 53.08, 52.47, 16.84, 14.72.

HR-MS (ESI): calc. 440.1110; found 440.1105 $[M + Na]^+$.

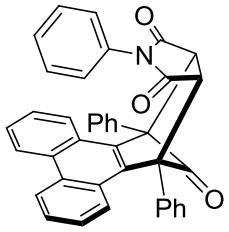
UV/Vis (DCM): λ [nm] = 229, 338.

FL λ_{Exc} = 266 nm (DCM): λ_{max} = 367, 385 nm.

5.3.5 Synthesis of Phencyclone and Maleimide Adducts

Maleimide derivative (1 eq.) and phencyclone (1 eq.) were placed in a reaction flask and dissolved in 10 ml toluene. Reaction mixture was heated to reflux for 1 h and cooled down to room temperature. Hexane was added in order to precipitate solid substance. Filtration and washing with a small amount of hexane gave the product as a white solid.

5.3.5.1 Phencyclone and N-phenylmaleimide adduct (9)



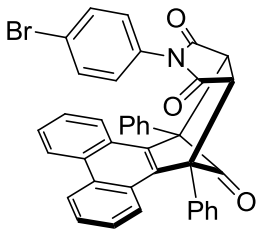
According to the reaction procedure (5.3.5), phencyclone (0.05 g, 0.1307 mmol) and N-phenylmaleimide (0.023 g, 0.1307 mmol) were reacted. 0.065 g of product was isolated. Yield: 90%.

^{1}H-NMR (400 MHz, $CDCl_3$) : δ [ppm] = 8.70 (d, *J* = 8.4 Hz, 2H, H_{core}), 8.39 (d, *J* = 7.8 Hz, 2H, H_{core}), 7.74 (t, *J* = 7.6 Hz, 2H, H_{core}), 7.56 (t, *J* = 7.4 Hz, 4H, H_{Ar}), 7.47 (t, *J* = 7.6 Hz, 2H, H_{core}), 7.37 – 7.14 (m, 6H, H_{Ar}), 7.06 (t, *J* = 7.4 Hz, 1H, H_{Ar}), 6.97 (t, *J* = 7.7 Hz, 2H, H_{Ar}), 5.91 (d, *J* = 7.9 Hz, 2H, H_{Ar}), 4.62 (s, 2H, CH).

^{13}C-NMR (101 MHz, $CDCl_3$) : δ [ppm] = 196.94, 173.35, 133.72, 133.41, 131.23, 130.93, 129.36, 129.12, 128.71, 128.65, 128.46, 127.17, 126.82, 126.28, 125.85, 125.62, 123.02, 63.58, 44.76.

HR-MS (ESI): calc. 578.1732; found 578.1727 $[M + Na]^+$.

5.3.5.2 Phencyclone and N-(4-Bromophenyl)maleimide Adduct (10)



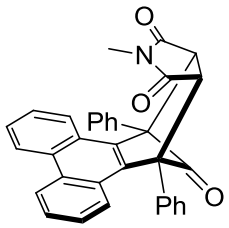
According to the reaction procedure (5.3.5), phencyclone (0.05 g, 0.1307 mmol) and N-(4-bromophenyl)maleimide (0.033 g, 0.1307 mmol) were reacted. 0.124 g of product was isolated. Yield: 95%.

^{1}H-NMR (600 MHz, $CDCl_3$) : δ [ppm] = 8.69 (d, *J* = 8.3 Hz, 2H, H_{core}), 8.36 (d, *J* = 7.8 Hz, 2H, H_{core}), 7.75 (td, *J* = 7.7, 1.2 Hz, 2H, H_{core}), 7.62 – 7.53 (m, 4H, H_{Ar}), 7.48 (td, *J* = 7.7, 1.2 Hz, 2H, H_{core}), 7.31 (d, *J* = 7.9 Hz, 2H, H_{Ar}), 7.25 (ddd, *J* = 8.1, 6.9, 1.0 Hz, 2H, H_{Ar}), 7.20 (dd, *J* = 8.4, 1.1 Hz, 2H, H_{Ar}), 7.12 – 7.08 (m, 2H, H_{Ar}), 5.88 – 5.73 (m, 2H, H_{Ar}), 4.62 (s, 2H, CH).

^{13}C-NMR (151 MHz, $CDCl_3$) : δ [ppm] = 197.00, 173.03, 133.57, 133.34, 131.93, 131.20, 130.90, 129.41, 128.99, 128.72, 128.54, 127.29, 127.10, 126.87, 126.21, 125.81, 123.06, 63.58, 44.74.

HR-MS (ESI): calc. 656.0837; found 656.0832 $[M + Na]^+$.

5.3.5.3 Phencyclone and N-methylmaleimide Adduct (11)



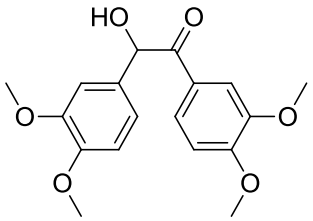
According to the reaction procedure (5.3.5), phencyclone (0.05 g, 0.1307 mmol) and N-methylmaleimide (0.0145 g, 0.1307 mmol) were reacted. 0.12 g of product was isolated. Yield: 94%.

^{1}H-NMR (600 MHz, $CDCl_3$) : δ [ppm] = 8.67 (d, *J* = 8.3 Hz, 2H, H_{core}), 8.36 (d, *J* = 7.8 Hz, 2H, H_{core}), 7.75 (td, *J* = 7.7, 1.1 Hz, 2H, H_{core}), 7.56 (m, 4H, H_{Ar}), 7.45 (td, *J* = 7.7, 1.1 Hz, 2H, H_{core}), 7.27 – 7.21 (m, 4H, H_{Ar}), 7.18 (dd, *J* = 8.4, 1.0 Hz, 2H, H_{Ar}), 4.46 (s, 2H, CH), 2.24 (s, 3H, CH_3).

^{13}C-NMR (151 MHz, $CDCl_3$) : δ [ppm] = 196.71, 174.26, 133.79, 133.24, 131.15, 130.93, 129.37, 129.08, 128.59, 128.39, 127.04, 126.56, 126.06, 125.83, 122.96, 63.33, 44.71, 24.86.

HR-MS (ESI): calc. 516.1576; found 516.1570 $[M + Na]^+$.

5.3.6 3,3',4,4'-Tetramethoxybenzoin (12)[148]



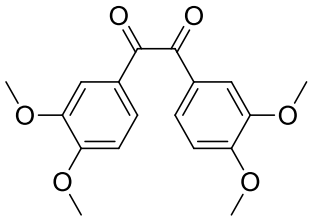
15 gr (0.09 mol) veratraldeyhde and 3 gr KCN (0.046 mol) are dissolved in 45 ml ethanol-15 ml water mixture. The mixture is heated to reflux under H_2 bubbling for 5 h. After cooling, dry ice is added until the water is saturated then it is extracted with ether, dried with Na_2SO_4 and evaporated. The compound is purified by coloumn chromatography (Diethylether : MeOH, 100 : 1) and 7.47 g of product is obtained as light yellow solid. Yield: 50%

^{1}H-NMR (400 MHz, $CDCl_3$) : δ [ppm] = 7.54 (m, 2H, H_{Ar}), 6.96 – 6.71 (m, 4H, H_{Ar}), 5.86 (s, 1H, CH), 4.57 (s, 1H, OH), 3.90 (s, 3H, CH_3), 3.89 (s, 3H, CH_3), 3.84 (s, 6H, CH_3).

^{13}C-NMR (101 MHz, $CDCl_3$) : δ [ppm] = 197.27, 153.84, 149.46, 149.19, 148.98, 132.27, 126.35, 124.16, 120.44, 111.36, 111.11, 110.24, 110.06, 75.54, 55.99, 55.88, 55.85, 55.80.

HR-MS (ESI): calc. 355.1158; found 355.1152 $[M + Na]^+$.

5.3.7 3,3',4 ,4'-Tetramethoxybenzil (13)[148]



To a solution of 3,3',4,4'-tetramethoxybenzoin (2.94 g, 8.83 mmol) in 10 ml DMSO, HBr (48 wt % in water, 2.4 mL, 44.17 mmol) was added dropwise during 1 h. The solution was stirred at 50 ^oC for 3 h, after that heated to 90 ^oC and continued mixing overnight at this temperature. After cooling to room temperature, the mixture was poured into water whereupon a yellow solid precipitated, filtrated and washed with water and dried to obtain 2.62 g of product pure enough for next step. Yield: 90%

R_f = 0.64 (70:1 DCM/MeOH)

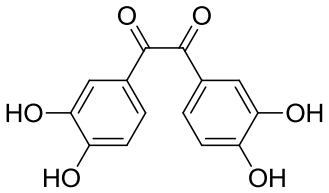
M.p. 223^{o}C (Lit.[192] 225)

^{1}H-NMR (600 MHz, $CDCl_3$) : δ [ppm] = 7.61 (d, *J* = 2.0 Hz, 2H, H_{Ar}), 7.50 (dd, *J* = 8.4, 2.0 Hz, 2H, H_{Ar}), 6.91 (s, 1H, H_{Ar}), 6.90 (s, 1H, H_{Ar}), 3.97 (d, *J* = 2.0 Hz, 12H, CH_3).

^{13}C-NMR (151 MHz, $CDCl_3$) : δ [ppm] = 193.43, 154.82, 149.54, 126.47, 126.22, 110.36, 110.33, 56.18, 56.06.

HR-MS (ESI): calc. 353.1001; found 353.0996 $[M + Na]^+$.

5.3.8 3,3',4, 4'-Tetrahydroxybenzil (16)[192]



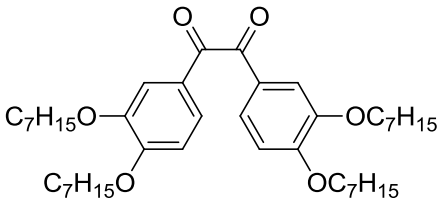
In a 250 ml three-neck flask, 3,3',4,4'-tetramethoxybenzil (4 g, 12.12 mmol), 25 ml of 48% aqueous HBr, 25 ml of a 33% solution of HBr in acetic acid, and 40 ml of glacial acetic acid was added and the mixture was heated to reflux for 12 h. The solution was poured into water and extracted in diethylether. Drying on Na_2SO_4, filtration and evaporation of the solvent gave the wanted crude sticky yellow product which could be used in next step without further purifications. Yield: quant.

M.p. 216-218°C

R_f = 0.67 (8:2 DCM/MeOH)

HR-MS (ESI): calc. 297.0375; found 297.0370 $[M + Na]^+$.

5.3.9 3,3',4, 4'-Tetraheptyloxybenzil (17)



To a 100-mL round-bottom flask, 3,3',4,4'-tetrahydroxybenzil (0.5 g, 1.82 mmol), 1-bromoheptane (1.44 g, 8.02 mmol), K_2CO_3 (2.52 g, 18.23 mmol) and 15 ml of DMF were added. This suspension was heated for 12 h at 80 °C under a nitrogen atmosphere. Then, DMF was removed under reduced pressure. The mixture was extracted with water (2 times), the organic phase was dried with Na_2SO_4 and evaporation of the solvent gave 1.14 g of the product as a yellow solid. Yield: 94%

R_f = 0.60 (DCM)

M.p. 89-91°C

^{1}H-NMR (600 MHz, $CDCl_3$) : δ [ppm] = 7.59 (s, 2H, H_{Ar}), 7.46 (d, *J* = 8.4 Hz, 2H, H_{Ar}), 6.87 (d, *J* = 8.4 Hz, 2H, H_{Ar}), 4.08 (td, *J* = 6.5, 3.3 Hz, 8H, CH_2), 1.95 – 1.79 (m, 8H, CH_2), 1.55 – 1.44 (m, 8H, CH_2), 1.39 (dd, *J* = 17.7, 10.2 Hz, 8H, CH_2), 1.34 (dd, *J* = 10.5, 6.3 Hz, 16H, CH_2), 0.95 – 0.89 (m, 12H, CH_3).

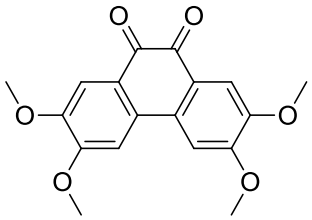
^{13}C-NMR (151 MHz, $CDCl_3$) : δ [ppm] = 193.78, 154.99, 149.30, 126.21, 126.07, 112.34, 111.62, 69.23, 69.10, 31.77, 31.73, 29.06, 29.02, 28.96, 28.92, 25.92, 25.85, 22.57, 22.55, 14.05, 14.03.

HR-MS (ESI): calc. 689.4757; found 689.4752 $[M + Na]^+$.

5.3.10 General Synthesis of 2,3,6,7-Tetrakis(alkyloxy)phenanthrene-9,10-diones[150]

3,3',4,4'-Tetrakis(alkyloxy)benzyl (1 eq.) was placed in a 100 ml three neck flask, dry dichloromethane (approx. 100 mL) was added and the flask was purged with argon. VOF_3 (3.45 eq.) was also added under argon atmosphere. Then, $BF_3.Et_2O$ (2.25 eq.) was added via syringe and the reaction mixture was stirred for 1 hour before being quenched with an aqueous citric acid solution (7 eq.). The mixture was extracted with dichloromethane (3 times). The extracts were washed with water, dried with Na_2SO_4, filtered and evaporated. Column chromatography (silica gel) with dichloromethane was done to purify the product.

5.3.10.1 2,3,6,7-Tetramethoxyphenanthrene-9,10-dione (14)[150]



3,3',4 ,4'-tetramethoxybenzil (2 g, 6.05 mmol), VOF_3 (2.59 g, 20.89 mmol), $BF_3.Et_2O$ (1.93 g, 1.68 ml, 13.62 mmol), citric acid solution (8.14 g, 42.38 mmol in 50 ml water) and 40 ml 1,1,2,2-tetrachloroethane (instead of dichloromethane) were used in the reaction process according to the general procedure (5.3.10). 1.57 g of product was isolated. Yield: 79%

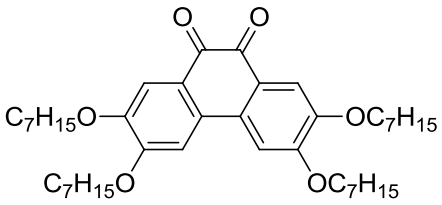
R_f = 0.28 (70:1 DCM/MeOH)

^{1}H-NMR (600 MHz, $CDCl_3$) : δ [ppm] = 7.48 (s, 2H, H_{Ar}), 7.09 (s, 2H, H_{Ar}), 4.10 (s, 6H, CH_3), 3.97 (s, 6H, CH_3).

^{13}C-NMR (151 MHz, $CDCl_3$) : δ [ppm] = 178.94, 155.49, 149.53, 131.12, 111.55, 105.36, 56.36, 56.17.

HR-MS (ESI): calc. 329.1025; found 329.1020 $[M + H]^+$.

5.3.10.2 2,3,6,7-Tetrakis(heptyloxy)phenanthrene-9,10-dione (18)



3,3',4 ,4'-tetraheptyloxybenzil (1.11 g, 1.66 mmol), VOF_3 (0.71 g, 5.73 mmol), $BF_3.Et_2O$ (0.53 g, 0.461 ml, 3.73 mmol), citric acid solution (2.23 g, 11.62 mmol in 20 ml water) in 25 ml dichloromethane were reacted according to the general procedure (5.3.10). 0.825 mg of product was isolated. Yield: 75%

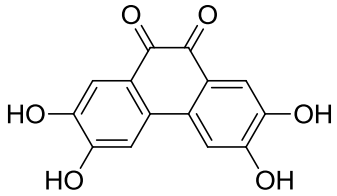
R_f = 0.29 (DCM)

^{1}H-NMR (400 MHz, $CDCl_3$) : δ [ppm] = 7.39 (s, 2H, H_{Ar}), 7.00 (s, 2H, H_{Ar}), 4.17 (t, *J* = 6.4 Hz, 4H, CH_2), 4.01 (t, *J* = 6.4 Hz, 4H, CH_2), 1.99 – 1.78 (m, 8H, CH_2), 1.59 – 1.28 (m, 32H, CH_2), 0.92 (dt, *J* = 6.9, 3.4 Hz, 12H, CH_3).

^{13}C-NMR (101 MHz, $CDCl_3$) : δ [ppm] = 179.04, 155.47, 149.28, 130.96, 124.30, 112.76, 106.93, 69.41, 68.98, 31.75, 29.11, 29.03, 25.94, 25.88, 22.54, 14.01.

HR-MS (ESI): calc. 665.4781; found 665.4776 $[M + H]^+$.

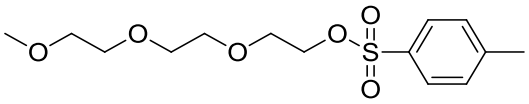
5.3.11 2,3,6,7-Tetrahydroxyphenanthrene-9,10-dione (69)



In a 250 ml three-neck flask, 2,3,6,7-tetramethoxyphenanthrene-9,10-dione (1.25 g, 3.81 mmol), 7 ml of 48% aqueous HBr, 7 ml of a 33% solution of HBr in acetic acid and 11 ml of glacial acetic acid was added and the mixture was heated to reflux for 12 h. The solution was poured into water and extracted in diethyl ether. Drying on Na_2SO_4, filtration and evaporation of the solvent gave the crude product as a sticky yellow solid which was used in next step without further purification. Yield: 87%.

HR-MS (ESI): calc. 273.0399; found 273.0394 $[M + H]^+$.

5.3.12 Triethylene Glycol Monomethyl Ether Tosylate (66)



NaOH (0.8 g, 20 mmol) was dissolved in 5 ml of water and added to solution of triethylene glycol monomethyl ether (2.3 g, 10 mmol) in 5 ml of THF. The reaction flask was placed in an ice bath and cooled down. Then, a solution of p-toluene sulfonyl chloride (2.5 g, 10 mmol) in 5 ml of THF was added dropwise over 1 hour and the reaction mixture was stirred at ice bath temperature for an additional 1h. The mixture was poured into the ice and extracted with CH_2Cl_2. The combined organic layers were washed with water and brine, dried over Na_2SO_4 and the solvent was removed under vacuum to give 2.7 g of clear oil. Yield: 85%.

R_f = 0.45 (EtOAc)

^{1}H-NMR (600 MHz, $CDCl_3$) : δ [ppm] = 7.80 (d, J = 8.3 Hz, 2H, H_{Ar}), 7.34 (d, J = 8.0 Hz, 2H, H_{Ar}), 4.18 – 4.14 (m, 2H, CH_2), 3.71 – 3.66 (m, 2H, CH_2), 3.63 – 3.56 (m, 6H, CH_2), 3.55 – 3.51 (m, 2H, CH_2), 3.37 (s, 3H, CH_3), 2.45 (s, 3H, CH_3).

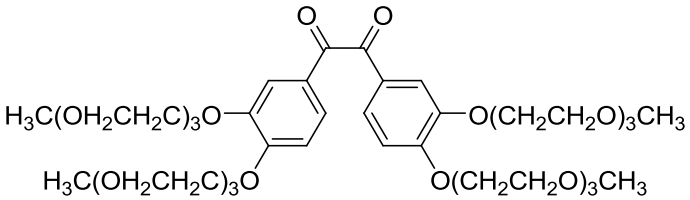
^{13}C-NMR (151 MHz, $CDCl_3$) : δ [ppm] = 144.68, 132.99, 129.72, 127.86, 71.81, 70.64, 70.45, 69.15, 68.57, 58.89, 21.51.

HR-MS (ESI): calc. 341.1035; found 341.1029 $[M + Na]^+$.

5.3.13 General Synthesis of Triethylene Glycol Monomethyl Ether modified Compounds

Tetrahydroxy compound (1 eq) and anhydrous K_2CO_3 (16 eq) were mixed in dry DMF and stirred at 100 ^oC for 20 min. Then triethylene glycol monomethyl ether tosylate (5 eq) was added dropwise. The reaction mixture was stirred at 100 ^oC for 20 h. After evaporation of solvent under vacuum, the residue was dissolved in ethyl acetate and extracted with water (3 times). The organic layer was dried over Na_2SO_4 and the solvent was evaporate. The column chromatography (8:2 EtOAc/MeOH as eluent) was used to purify the product as yellowish oil.

5.3.13.1 1,2-Bis(3,4-bis(2-(2-(2-methoxyethoxy)ethoxy)ethoxy)phenyl)ethane-1,2-dione (67)

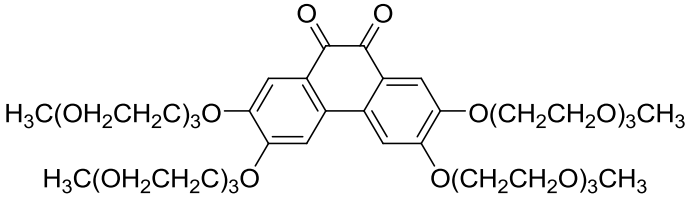


3,3',4,4'-Tetrahydroxybenzil (0.3 g, 1.09 mmol), K_2CO_3 (2.42 g, 17.5 mmol), in 10 ml DMF and triethylene glycol monomethyl ether tosylate (1.74 g, 5.47 mmol) were reacted according to the general procedure (5.3.13). Yield: 76%

^{1}H-NMR (400 MHz, $CDCl_3$) : δ [ppm] = 7.58 (d, J = 1.9 Hz, 2H, H_{Ar}), 7.45 (d, J = 2.0 Hz, 1H, H_{Ar}), 7.43 (d, J = 2.0 Hz, 1H, H_{Ar}), 6.91 (s, 1H, H_{Ar}), 6.89 (s, 1H, H_{Ar}), 4.21 (dd, J = 10.2, 5.6 Hz, 8H, CH_2), 3.88 (dd, J = 7.1, 2.9 Hz, 8H, CH_2), 3.73 (dt, J = 5.4, 3.7 Hz, 8H, CH_2), 3.68 – 3.60 (m, 16H, CH_2), 3.56 – 3.49 (m, 8H, CH_2), 3.36 (s, 6H, CH_3), 3.35 (s, 6H, CH_3).

^{13}C-NMR (101 MHz, $CDCl_3$) : δ [ppm] = 193.32, 154.62, 148.96, 126.47, 126.14, 113.17, 112.35, 71.83, 71.81, 70.84, 70.79, 70.58, 70.57, 70.45, 70.44, 69.43, 69.26, 68.76, 68.59, 58.89.

5.3.13.2 2,3,6,7-Tetrakis(2-(2-(2-methoxyethoxy)ethoxy)ethoxy)phenanthrene-9,10-dione (68)



2,3,6,7-Tetrahydroxyphenanthrene-9,10-dione (130 mg, 0.48 mmol), K_2CO_3 (1.06 g, 7.64 mmol), in 5 ml DMF and triethylene glycol monomethyl ether tosylate (760 mg, 2.39 mmol) were reacted according to the general procedure (5.3.13). Yield: 76%

R_f = 0.17 (8:2 EtOAc/MeOH)

^{1}H-NMR (600 MHz, $CDCl_3$) : δ [ppm] = 7.59 (s, 2H, H_{Ar}), 7.24 (s, 2H, H_{Ar}), 4.38 (t, J = 4.9 Hz, 4H, CH_2), 4.29 – 4.23 (m, 4H, CH_3), 3.99 – 3.94 (m, 4H, CH_2), 3.93 – 3.88 (m, 4H, CH_2), 3.79 (dd, J = 5.6, 4.0 Hz, 4H, CH_2), 3.76 (dd, J = 5.6, 4.0 Hz, 4H, CH_2), 3.70 – 3.64 (m, 16H, CH_2), 3.58 – 3.55 (m, 4H, CH_2), 3.55 – 3.52 (m, 4H, CH_2), 3.38 (s, 6H, CH_3), 3.36 (s, 6H, CH_3).

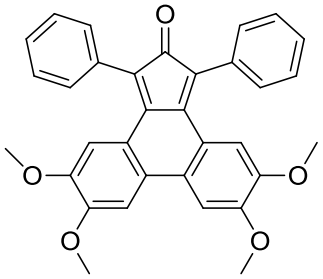
^{13}C-NMR (151 MHz, $CDCl_3$) : δ [ppm] = 178.96, 155.36, 149.31, 131.31, 124.84, 113.70, 108.37, 71.92, 71.88, 70.93, 70.89, 70.67, 70.65, 70.55, 70.50, 69.58, 69.45, 69.23, 68.70, 58.97, 58.95.

HR-MS (ESI): calc. 879.3990; found 879.3985 $[M + Na]^+$.

5.3.14 General Synthesis of Knoevenagel Condensation Adducts

To a dry 50 ml flask, 2,3,6,7-Tetrakis(alkyloxy)phenanthrene-9,10-dione (1 eq.), 1,3-diphenylpropan-2-one (1.1 eq.), potassium hydroxide (1.25 eq.) and methanol were added under argon atmosphere. The reaction mixture was heated to reflux with stirring for 1 h. A dark green precipitate appeared during the reaction and it was filtered off, washed with ethanol and dried to give the product, pure enough for the next step.

5.3.14.1 5,6,9,10-Tetramethoxy-1,3-diphenyl-2H-cyclopenta[*l*]phenanthren-2-one (15)



2,3,6,7-tetramethoxyphenanthrene-9,10-dione (0.56 g, 1.72 mmol), 1,3-diphenylpropan-2-one (0.4 g, 1.89 mmol), potassium hydroxide (0.12 g, 2.15 mmol) in 25 ml methanol were reacted according to the general procedure (5.3.14) and 0.293 g dark green product was obtained. Yield: 34%

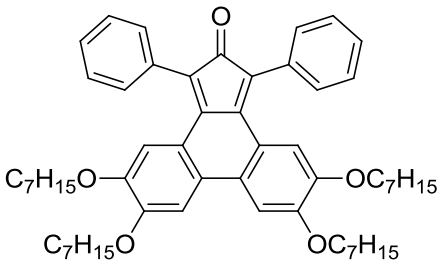
R_f = 0.54 (70:1 DCM/MeOH)

^{1}H-NMR (400 MHz, CDCl3) : δ [ppm] = 7.47 – 7.33 (m, 10H, H_{Ar}), 7.03 (s, 2H, H_{Ar}), 6.96 (s, 2H, H_{Ar}), 3.98 (s, 6H, CH_3), 3.33 (s, 6H, CH_3).

^{13}C-NMR (101 MHz, $CDCl_3$) : δ [ppm] = 200.51, 151.63, 134.14, 130.30, 128.51, 127.85, , 121.79, 121.43 111.38, 106.49, 55.99, 55.11.

UV/Vis (DCM): λ [nm] = 273, 318, 391.

5.3.14.2 5,6,9,10-Tetrakis(heptyloxy)-1,3-diphenyl-2H-cyclopenta[*l*]phenanthren-2-one (19)



2,3,6,7-tetrakis(heptyloxy)phenanthrene-9,10-dione (0.825 g, 1.24 mmol), 1,3-diphenylpropan-2-one (0.287 g, 1.36 mmol), potassium hydroxide (87 mg, 1.55 mmol) in 20 ml methanol were reacted according to the general procedure (5.3.14) and 0.53 g dark green product was obtained. Yield: 51%

R_f = 0.24 (1:1 DCM/Hexane)

^{1}H-NMR (400 MHz, CDCl3) : δ [ppm] = 7.66 – 7.11 (m, 10H, H_{Ar}), 7.02 (s, 2H, H_{Ar}), 6.93 (s, 2H, H_{Ar}), 4.10 (dd, *J* = 12.1, 5.6 Hz, 4H, CH_2), 3.38 (t, *J* = 6.8 Hz, 4H, CH_2), 1.86 (dd, *J* = 14.3, 7.2 Hz, 4H, CH_2), 1.64 – 1.55 (m, 4H, CH_2), 1.54 – 1.45 (m, 4H, CH_2), 1.43 – 1.25 (m, 28H, CH_2), 0.97 – 0.89 (m, 12H, CH_3).

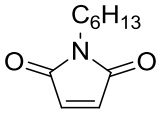
^{13}C-NMR (101 MHz, $CDCl_3$) : δ [ppm] = 200.49, 151.55, 148.84, 148.40, 132.97, 130.27, 128.42, 127.79, 127.61, 121.66, 121.15, 112.85, 109.13, 69.52, 68.12, 31.92, 31.80, 31.76, 29.43, 29.34, 29.21, 29.13, 29.03, 28.89, 28.82, 28.73, 26.04, 25.99, 25.92, 25.80, 25.64, 22.66, 22.58, 22.55, 14.07, 14.03.

UV/Vis (DCM): λ [nm] = 276, 321, 395.

FL λ_{Exc} = 266 nm (DCM): λ_{max} = 367, 383 nm.

5.3.15 Synthesis of Maleimide Derivatives

5.3.15.1 N-Hexylmaleimide (23)[193]



The solution of n-octylamine (0.94 g, 1.22 ml, 9.27 mmol) in 15 ml of benzene was added to maleic anhydride (1 g, 10.20 mmol) suspension in 25 ml benzene and the mixture was stirred at 30 °C for 1 h. Then, $ZnBr_2$ (2.3 g, 10.20 mmol), followed by hexamethyl disilazane (2.09 g, 2.69 ml, 12.98 mmol) in 20 ml of benzene were added. The mixture was refluxed for 2 h, cooled down to room temperature and poured into 75 ml of 0.5 M HCl. The benzene phase

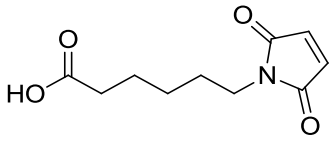
was separated and the aqueous phase was extracted with EtOAc (2x100 ml). The combined organic layers were washed with saturated aq. $NaHCO_3$ (2x50 ml), brine, and dried over Na_2SO_4. It was then filtered, dried and evaporated. Column chromatography, with dichloro-methane as eluent, gave the product as white solid (1.54 g). Yield: 92%

R_f = 0.56 (DCM)

^{1}H-NMR (600 MHz, $CDCl_3$) : δ [ppm] = 6.65 (s, 2H, CH), 3.56 (t, 2H, CH_2), 1.62 – 1.42 (m, 2H, CH_2), 1.33 – 1.11 (m, 6H, CH_2), 0.83 (t, 3H, CH_3).

^{13}C-NMR (151 MHz, $CDCl_3$) : δ [ppm] = 170.70, 133.89, 37.74, 31.13, 28.33, 26.23, 22.32, 13.80.

5.3.15.2 6-Maleimidocaproic acid (29)[194]



6-Aminocaproic acid (1 g, 7.62 mmol) was added to a stirred solution of maleic anhydride (747.54 mg, 7.62 mmol) in acetic acid. After mixing 3 h at room temperature, precipitated white solid was collected by filtration. Without further purification, the solid was dissolved in 10 ml acetic anhydride and sodium acetate (0.32 g, 3.88 mmol) was added. The mixture was heated to 90^{o}C for 2 h. The solvent was removed under reduced pressure and the remaining solid was dissolved in ethyl acetate (30 ml) and extracted with water (30 ml), followed by brine (30 ml). The ethyl acetate phase was dried over Na_2SO_4 and filtrated. After evaporation of the solvent, the white solid was purified by column chromatography (ethyl acetate as eluent) to give 6-maleimidocaproic acid as a white solid. Yield: 70%

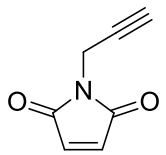
R_f = 0.46 (EtOAc)

^{1}H-NMR (400 MHz, $CDCl_3$) : δ [ppm] = 10.57 (s, 1H, OH), 6.69 (s, 2H, CH=CH), 3.51 (t, *J* = 7.2 Hz, 2H, CH_2), 2.34 (t, *J* = 7.4 Hz, 2H, CH_2), 1.75 – 1.49 (m, 4H, CH_2), 1.34 (m, 2H, CH_2).

^{13}C-NMR (101 MHz, $CDCl_3$) : δ [ppm] = 179.42, 170.81, 134.00, 37.49, 33.68, 28.05, 26.02, 23.99.

HR-MS (ESI): calc. 234.0742; found 234.0737 $[M + Na]^+$.

5.3.15.3 N-Propargylmaleimide (28)[158]



2 g of maleic anhydride (0.02 mol, 1 eq) was dissolved in 15 ml of acetone and the mixture was heated to reflux. To this solution, 1.37 ml of propargylamine (1.99 g, 0.02 mol, 1 eq) was added dropwise. Then, it was refluxed for 1 h, cooled and the solvent was removed under vacuo. A red solid precipitated and was recrystallized, by dissolving in ether and addition of methanol, to yield 1.84 g maleic acid N-propargylmonoamide as colorless crystals. Yield: 60%

^{1}H-NMR (400 MHz, d-DMSO) : δ [ppm] = 9.30 (s, 1H, NH), 6.28 (q, *J* = 12.4 Hz, 2H, CH=CH), 3.97 (d, *J* = 1.6 Hz, 2H, CH_2), 3.17 (t, *J* = 2.5 Hz, 1H, CH).

^{13}C-NMR (101 MHz, d-DMSO) : δ [ppm] = 166.25, 164.64, 132.29, 130.36, 80.00, 73.66, 28.24.

To close the ring, 1.35 g of maleic acid N-propargylmonoamide (8.82 mmol, 1 eq) and 1. 92 g of $ZnBr_2$ (8.82 mmol, 1 eq) were added into 15 ml of THF and 2.55 ml of hexamethyl disilazane (1.99 g, 12.34 mmol, 1.4 eq) was added dropwise. The resulting suspension was refluxed for 2 h and cooled to room temperature. It was poured into 50 ml of 0.5 M HCl and extracted with EtOAc. The water phase was extracted with EtOAc (2 x 50 ml) and the combined organic layers were washed with $NaHCO_3$ (2 x 50 ml) and brine. After drying over Na_2SO_4, the solvent was evaporated under vacuo. A short column chromatography using CH_2Cl_2 gave 1.07 g of product as a white solid. Yield: 90%

R_f = 0.56 (DCM)

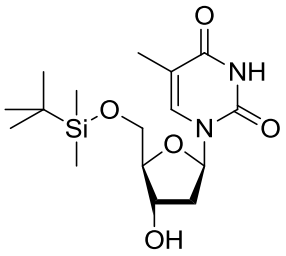
M.p. 54°C (Lit.[158] 54°C)

^{1}H-NMR (400 MHz, $CDCl_3$) : δ [ppm] = 6.76 (s, 2H, HC=CH), 4.27 (d, J = 2.5 Hz, 2H, CH_2), 2.21 (t, J = 2.5 Hz, 1H, CH).

^{13}C-NMR (101 MHz, $CDCl_3$) : δ [ppm] = 169.16, 134.38, 71.46, 26.68.

5.3.16 Synthesis of Nucleoside Derivatives

5.3.16.1 5'-O-(*tert*-Butyldimethylsilyl)thymidine (24)[160]



To a solution of thymidine (0.48 g, 2 mmol, 1 eq), imidazole (0.409 g, 6 mmol, 3 eq), and DMAP (24.4 mg, 0.2 mmol, 0.1 eq) in 10 ml of DMF was added dropwise a solution of TBDMSCl (*tert*-butyldimethylsilyl chloride, 0.3 g, 2 mmol, 1 eq) in 5 ml of DMF at 0 °C. The mixture was stirred at room temperature for 1 h, quenched using 10 ml of saturated aqueous solution of ammonium chloride and extracted with ethyl acetate (3 x 20 ml). The combined organic layers were dried over Na_2SO_4, concentrated under vacuo and TBDMS-thymidine was purified as a white solid by flash chromatography using 3:1 EtOAc/hexane solvent mixture. Yield: 85%.

R_f = 0.2 (3:1 EtOAc/Hexane)

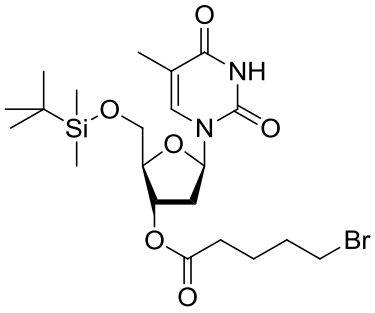
M.p. 197°C (Lit.[160] 198-200°C)

^{1}H-NMR (400 MHz, $CDCl_3$) : δ [ppm] = 9.84 (s, 1H, NH), 7.55 (d, J = 1.2 Hz, 1H, H6), 6.40 (dd, J = 8.3, 5.6 Hz, 1H, H1′), 4.44 (dt, J = 5.6, 1.9 Hz, 1H, H3′), 4.08 (d, J = 2.3 Hz, 1H, H4′), 3.87 (m, 2H, H5′), 2.42 (ddd, J = 13.3, 5.6, 2.0 Hz, 1H, H2′), 2.07 (ddd, J = 8.3, 7.1, 3.5 Hz, 1H, H2′), 1.91 (d, J = 1.1 Hz, 3H, CH_3), 0.92 (s, 9H, $SiC(CH_3)_3$), 0.11 (s, 3H, $SiCH_3$), 0.10 (s, 3H, $SiCH_3$).

^{13}C-NMR (101 MHz, $CDCl_3$) : δ [ppm] = 164.17, 150.73, 135.54, 110.88, 87.43, 85.07, 72.41, 63.59, 41.04, 25.86, 18.28, 12.44, -5.49.

HR-MS (ESI): calc. 379.1665; found 379.1660 $[M + Na]^+$.

5.3.16.2 5'-O-(tert-Butyldimethylsilyl)-3'-O-(5-bromopentanoyl)-thymidine (25)[159]



5'-O-tert-butyl-dimethylsilyl-thymidine (0.55 g, 1.5 mmol), DMAP (19 mg, 0.15 mmol) and DCC (0.318 g, 1.5 mmol) were dissolved in 15 ml CH_2Cl_2 and cooled to 0 °C. A solution of 5-bromovaleric acid (0.42 g, 2.3 mmol) in 5 ml CH_2Cl_2 were added and the mixture was stirred for 3 hours. After filtration through Celite, the solvent was evaporated in vacuo and the product was purified by column chromatography with a solvent mixture of EtOAc/hexane yielding the product as a colourless solid. Yield: 70%.

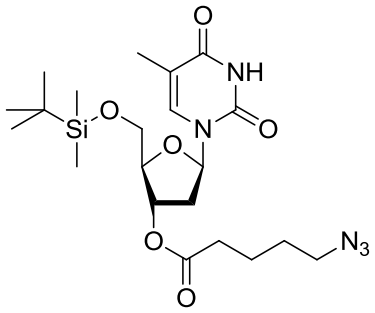
R_f = 0.64 (2:1 EtOAc/Hexane)

^{1}H-NMR (400 MHz, $CDCl_3$) : δ [ppm] = 8.93 (s, 1H, NH), 7.57 (d, *J* = 1.2 Hz, 1H, H6), 6.37 (dd, *J* = 9.2, 5.2 Hz, 1H, H1'), 5.28 (d, *J* = 6.1 Hz, 1H, H3'), 4.10 (d, *J* = 1.5 Hz, 1H, H4'), 3.94 (d, *J* = 1.4 Hz, 2H, H5'), 3.44 (t, *J* = 6.5 Hz, 3H, CH_2Br), 2.41 (m, 3H, H2'+CH_2), 2.19 – 2.08 (m, 1H, H2'), 1.96 – 1.93 (m, 5H, CH_2 and CH_3), 1.85 – 1.80 (m, 2H, CH_2), 0.95 (s, 9H, $SiC(CH_3)_3$), 0.16 (s, 6H, $SiCH_3$).

^{13}C-NMR (101 MHz, $CDCl_3$) : δ [ppm] = 172.76, 163.78, 150.31, 135.13, 111.19, 85.49, 84.74, 75.43, 63.57, 38.04, 33.66, 33.15, 32.78, 31.88, 25.90, 23.29, 18.31, 12.44, -5.39, -5.50.

HR-MS (ESI): calc. 541.1345; found 541.1340 $[M + Na]^+$.

5.3.16.3 5'-O-(tert-Butyldimethylsilyl)-3'-O-(5-azidopentanoyl)-thymidine (26)[159]



5'-O-(tert-Butyldimethylsilyl)-3'-O-(5-bromopentanoyl)-thymidine (533 mg, 1.03 mmol) and NaN_3 (333 mg, 5.13 mmol) in 15 ml of DMF were stirred overnight at 80 °C. Then, DMF was removed under vacuum and the residue was dissolved in diethyl ether and extracted with sat.

$NaHCO_3$ solution. The separated aqueous layer was extracted with diethyl ether (3 x 30 ml) and the collected organic layers were dried over Na_2SO_4. After removal of solvent in vacuo, the product was purified by flash column chromatography with a solvent mixture of EtOAc/hexane yielding the product as a white solid. Yield: 80%.

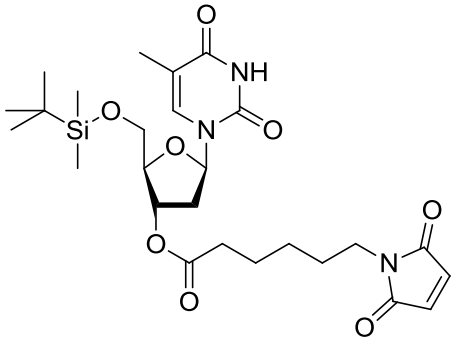
R_f = 0.70 (3:1 EtOAc /Hexane)

^{1}H-NMR (400 MHz, $CDCl_3$) : δ [ppm] = 9.75 (bs, 1H, NH), 7.52 (d, J = 1.2 Hz, 1H, H6), 6.36 (dd, J = 9.2, 5.2 Hz, 1H, H1'), 5.25 (d, J = 6.0 Hz, 1H, H3'), 4.06 (d, J = 1.4 Hz, 1H, H4'), 3.90 (d, J = 1.2 Hz, 2H, H5'), 3.30 (t, J = 6.6 Hz, 2H, CH2N3), 2.39 (m, 3H, H2'+CH2), 2.16 – 2.04 (m, 1H, H2'), 1.91 (d, J = 1.1 Hz, 3H, CH3), 1.75 – 1.59 (m, 4H, CH2), 0.92 (s, 9H, $SiC(CH_3)_3$), 0.12 (s, 6H, $SiCH_3$).

^{13}C-NMR (101 MHz, $CDCl_3$) : δ [ppm] = 172.68, 163.88, 150.60, 134.81, 111.17, 85.31, 84.55, 77.32, 77.00, 76.68, 75.31, 63.46, 50.86, 37.88, 33.38, 28.11, 25.78, 21.81, 18.18, 12.35, -5.53, -5.64.

HR-MS (ESI): calc. 504.2254; found 504.2249 $[M + Na]^+$.

5.3.16.4 5'-O-(tert-Butyldimethylsilyl)-3'-O-(6-maleimidohexanoyl)-thymidine (30)



120 mg (0.336 mmol, 1 eq) of 5'-O-*tert*-butyl-dimethylsilyl-thymidine, 71 mg of 6-maleimidocaproic acid (0.336 mmol, 1 eq) and 4.12 mg of DMAP (0.034 mmol, 0.1 eq) were dissolved in 10 ml of dry CH_2Cl_2 and cooled down to 0 ^oC. 69.5 mg of DCC (0.336 mmol, 1 eq), dissolved in 5 ml of dry CH_2Cl_2, was added dropwise within 1 h, and the solution stirred at room temperature for 1 day. The solution was filtered and evaporated in vacuo. The residue was chromatographed on a silica gel column (hexane/ethyl acetate 1:3) to afford the desired product as a colorless solid. Yield: 20%.

R_f = 0.5 (3:1 EtOAc/Hexane)

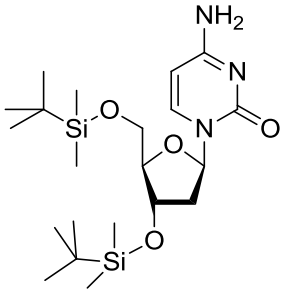
^{1}H-NMR (400 MHz, $CDCl_3$) : δ [ppm] = 9.30 (s, 1H, NH), 7.54 (d, *J* = 1.2 Hz, 1H, H6), 6.70 (s, 2H, CH), 6.36 (dd, *J* = 9.2, 5.3 Hz, 1H, H1′), 5.25 (d, *J* = 6.1 Hz, 1H, H3′), 4.07 (d, *J* = 1.5

Hz, 1H, H4′), 3.95 – 3.90 (m, 2H, H5′), 3.52 (t, *J* = 7.2 Hz, 2H, CH_2), 2.40 (dd, *J* = 13.5, 5.4 Hz, 1H, H2′), 2.34 (t, *J* = 7.4 Hz, 2H, CH_2), 2.16 – 2.05 (m, 1H, H2′), 1.92 (d, *J* = 1.1 Hz, 3H, CH_3), 1.64 (m, 4H, CH_2), 1.38 – 1.28 (m, 2H, CH_2), 0.93 (s, 9H, $SiC(CH_3)_3$), 0.13 (s, 6H, $SiCH_3$).

^{13}C-NMR (101 MHz, $CDCl_3$) : δ [ppm] = 173.04, 170.75, 163.76, 150.46, 134.96, 134.01, 111.15, 85.42, 84.63, 77.32, 77.00, 76.68, 75.21, 63.53, 37.94, 37.48, 33.83, 28.10, 26.09, 25.85, 24.14, 18.25, 12.41, -5.45, -5.56.

HR-MS (ESI): calc. 572.2404; found 572.2399 $[M + Na]^+$.

5.3.16.5 2'-Deoxy-3',5'-bis-O-(*tert*-butyldimethylsilyl) cytidine (31)[162]



2'-deoxycytidine monochlorid (1.58 g, 12.0 mmol), TBDMSCl (3.96 g, 26 mmol) and imidazole (3.60 g, 52 mmol) are dissolved in 6 ml dry DMF. The mixture was stirred at room temperature for 24 h. Then, 30 ml of water was added and this solution was extracted with CH_2Cl_2 (3 x 20 ml). The combined organic layers were washed with water, dried over Na_2SO_4 and evaporated under vacuo. A short column chromatography was carried out in order to obtain product as white solid. Yield: 65%.

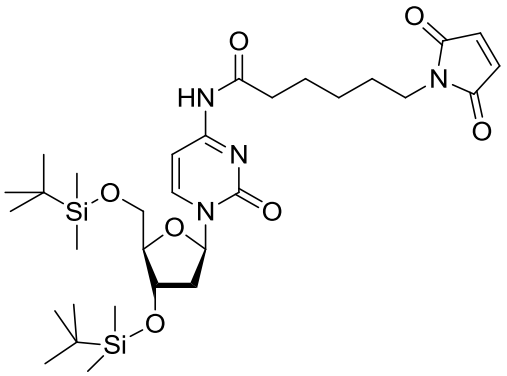
R_f = 0.2 (19:1 DCM/MeOH)

^{1}H-NMR (400 MHz, CDCl3) : δ [ppm] = 7.89 (d, *J* = 7.4 Hz, 1H, H6), 6.25 (t, *J* = 5.8 Hz, 1H, H1'), 5.75 (d, *J* = 7.4 Hz, 1H, H5), 4.36 (dd, *J* = 10.4, 5.5 Hz, 1H, H4'), 3.92 – 3.83 (m, 2H, H5'), 3.75 (dd, *J* = 12.1, 3.2 Hz, 1H, H3'), 2.37 (ddd, *J* = 13.3, 6.3, 5.5 Hz, 1H, H2'), 2.09 – 1.98 (m, 1H, H2'), 0.92 – 0.89 (m, 9H, $SiC(CH_3)_2$), 0.89 – 0.84 (m, 9H, $SiC(CH_3)_2$), 0.09 (two singlets, 6H, $Si(CH_3)_2$), 0.05 (s, 6H, $Si(CH_3)_2$).

^{13}C-NMR (101 MHz, $CDCl_3$) : δ [ppm] = 165.81, 155.87, 140.76, 94.34, 87.16, 85.69, 70.38, 61.97, 50.28, 42.03, 25.81, 25.63, 18.25, 17.85, -4.67, -5.00, -5.56, -5.64.

HR-MS (ESI): calc. 456.2714; found 456.2708 $[M + H]^+$.

5.3.16.6 2'-Deoxy-3',5'-bis-O-(tert-butyldimethylsilyl)-N^4-(6-maleimidohexanoyl)-cytidine (32)



To a solution of 82 mg of 6-maleimidocaproic acid (0.38 mmol, 2.2 eq), 59 mg of HOBT, (1-hydroxybenzotriazole, 0.38 mmol, 2.2 eq) and 2.14 mg of DMAP (0.175 mmol, 0.1 eq) in dry CH_2Cl_2, 76 mg of DCC (0.37 mmol, 2.1 eq) in dry CH_2Cl_2 was added dropwise and the mixture stirred for half an hour at room temperature. Then, undissolved material was filtrated removed by filtration. The mixture of the filtrate and Et_3N (49 mml, 0.35 mmol, 2 eq) were added dropwise into a solution of 2'-deoxy-3',5'-bis-O-(*tert*-butyldimethylsilyl)-cytidine in dry CH_2Cl_2 over 30 min at room temperature under stirring. The mixture was extracted with brine and separated organic phase was dried over Na_2SO_4, evaporated in vacuo, and applied to a silica gel column to obtain the product as a colorless solid. Yield: 56%.

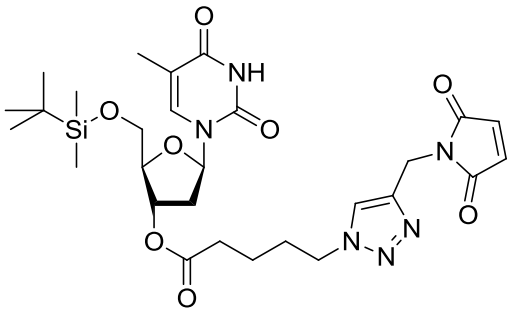
R_f = 0.21 (30:1 DCM/MeOH)

^{1}H-NMR (400 MHz, CDCl3) : δ [ppm] = 9.30 (s, 1H, NH), 8.41 (d, J = 7.4 Hz, 1H, H6), 7.39 (d, J = 7.4 Hz, 1H, H5), 6.69 (s, 2H, CH=CH), 6.32 – 6.15 (m, 1H, H1′), 4.40 (m, 1H, H4′), 4.02 – 3.89 (m, 2H, H5′), 3.79 (m, 1H, H3′), 3.52 (t, J = 7.2 Hz, 2H, CH_2), 2.57 – 2.42 (m, 3H, CH_2 and H2′) , 2.17 (dd, J = 7.4, 3.9 Hz, 1H, H2′), 1.80 – 1.67 (m, 2H, CH_2), 1.63 (m, 2H, CH_2), 1.36 (m, 2H, CH_2), 0.94 (s, 9H), 0.89 (s, 9H), 0.13 (d, J = 4.4 Hz, 6H), 0.07 (d, J = 5.1 Hz, 6H).

^{13}C-NMR (101 MHz, $CDCl_3$) : δ [ppm] = 170.77, 161.98, 144.96, 134.04, 95.97, 87.80, 86.81, 69.91, 61.70, 42.28, 37.49, 37.29, 28.20, 26.07, 25.88, 25.66, 24.06, 18.34, 17.90, -4.61, -4.96, -5.48, -5.53.

HR-MS (ESI): calc. 649.3453; found 649.3447 $[M + H]^+$.

5.3.16.7 CuAAC between N-propargyl maleimide and 5'-O-(*tert*-butyldimethylsilyl)-3'-O-(5-azidopentanoyl)-thymidine (27)



5'-O-(tert-Butyldimethylsilyl)-3'-O-(5-azidopentanoyl)-thymidine (200 mg, 0.415 mmol), N-propargylmaleimide (70.14 mg, 0.519 mmol), sodium ascorbate (49.36 mg, 0.249 mmol) and $CuSO_4.5H_2O$ (20.74 mg, 0.083 mmol) were dissolved in 1:1 mixture of water and THF. After stirring overnight, THF was evaporated under vacuum and the water phase was extracted with dichloromethane (3 x 20 ml). The combined organic layers were dried over Na_2SO_4 and filtrated. The solvent was evaporated under vacuo and flash column chromatography (EtOAc as eluent) was performed to obtain the desired product. Yield: 62%.

R_f = 0.26 (EtOAc)

^{1}H-NMR (600 MHz, $CDCl_3$) : δ [ppm] = 9.28 (s, 1H, NH), 7.58 (s, 1H, CH), 7.54 (d, J = 1.1 Hz, 1H, H6), 6.73 (s, 2H, HC=CH), 6.34 (dd, J = 9.3, 5.2 Hz, 1H, H1′), 5.25 (d, J = 6.0 Hz, 1H, H3′), 4.82 (s, 2H, CH_2), 4.35 (t, J = 7.1 Hz, 2H, CH_2), 4.07 (d, J = 1.2 Hz, 1H, H4′), 3.91 (d, J = 1.8 Hz, 2H, H5′), 2.39 (m, 3H, H2′+CH_2), 2.11 (ddd, J = 13.9, 9.3, 6.1 Hz, 1H, H2′), 1.98 – 1.90 (m, 5H, CH_2+CH_3), 1.69 – 1.62 (m, 2H, CH_2), 0.93 (s, 9H, $SiCCH_3$), 0.14 (two s, 6H, $SiCH_3$).

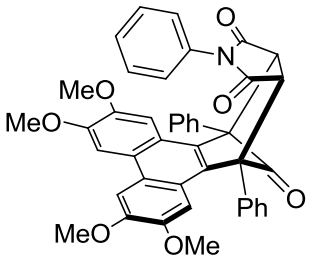
^{13}C-NMR (151 MHz, $CDCl_3$) : δ [ppm] = 172.42, 169.96, 163.70, 150.46, 142.56, 134.90, 134.25, 122.57, 111.20, 85.27, 84.58, 75.48, 63.52, 49.81, 37.94, 33.19, 32.78, 29.42, 25.84, 21.56, 18.24, 12.40, -5.45, -5.56.

HR-MS (ESI): calc. 617.2755; found 617.2750 $[M + H]^+$.

5.3.17 General Synthesis of Diels Alder Adducts of Phencyclone Derivatives

Diels Alder educt (1 eq.) and maleimide derivative (1 eq.) were added in 10 ml dichloromethane in a dry flask under argon. The reaction was stirred half an hour at room temperature and then the solvent was evaporated. The residue was purified by coloum chromatography to give desired product.

5.3.17.1 5,6,9,10-Tetramethoxy-1,3-diphenyl-2H-cyclopenta[*l*]phenanthren-2-one and N-phenylmaleimide adduct (20)



According to the general reaction procedure (5.3.17), 5,6,9,10-tetramethoxy-1,3-diphenyl-2H-cyclo-penta[*l*]phenanthren-2-one (0.164 g, 0.32 mmol) and N-phenylmaleimide (56.5 mg, 0.32 mmol) were reacted. 0.183 g of product was isolated. Yield : 87%.

R_f = 0.57 (70:1 DCM/MeOH)

^{1}H-NMR (600 MHz, $CDCl_3$) : δ [ppm] = 8.43 (d, *J* = 7.8 Hz, 2H, H_{Ar}), 7.74 (s, 2H, H_{core}), 7.72 – 7.67 (m, 2H, H_{Ar}), 7.54 – 7.47 (m, 4H, H_{Ar}), 7.40 – 7.35 (m, 2H, H_{Ar}), 7.11 (t, *J* = 7.5 Hz, 1H, H_{Ar}), 7.04 (t, *J* = 7.8 Hz, 2H, H_{Ar}), 6.63 (s, 2H, H_{core}), 6.00 (d, *J* = 7.6 Hz, 2H, H_{Ar}), 4.51 (s, 2H, CH), 4.04 (s, 6H, CH_3), 3.32 (s, 6H, CH_3).

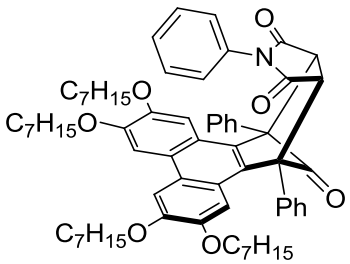
^{13}C-NMR (151 MHz, $CDCl_3$) : δ [ppm] = 196.47, 173.47, 149.40, 148.34, 134.37, 131.24, 130.22, 129.41, 129.17, 128.84, 128.78, 128.47, 128.27, 125.88, 125.61, 120.81, 106.24, 103.03, 63.27, 55.93, 55.00, 44.92.

HR-MS (ESI): calc. 698.2155; found 698.2149 $[M + Na]^+$.

UV/Vis (DCM): λ [nm] = 279, 289.

FL λ_{Exc} = 266 nm (DCM): λ_{max} = 369, 387 nm.

5.3.17.2 5,6,9,10-Tetrakis(heptyloxy)-1,3-diphenyl-2H-cyclopenta[*l*]phenanthren-2-one and N-phenylmaleimide adduct (21)



According to the general reaction procedure (5.3.17), 5,6,9,10-tetrakis(heptyloxy)-1,3-diphenyl-2H-cyclopenta[*l*]phenanthren-2-one (0.174 g, 0.21 mmol) and N-phenylmaleimide (35.9 mg, 0.21 mmol) were reacted. 0.193 g of product was isolated. Yield: 91%.

R_f = 0.4 (3:1 DCM/Hexane)

^{1}H-NMR (600 MHz, $CDCl_3$) : δ [ppm] = 8.44 (d, *J* = 7.8 Hz, 2H, H_{Ar}), 7.77 (s, 2H, H_{core}), 7.74 – 7.63 (m, 2H, H_{Ar}), 7.56 – 7.44 (m, 4H, H_{Ar}), 7.42 – 7.33 (m, 2H, H_{Ar}), 7.11 (ddd, *J* = 6.8, 4.0, 1.0 Hz, 1H, H_{Ar}), 7.03 (dd, *J* = 10.8, 4.9 Hz, 2H, H_{Ar}), 6.59 (s, 2H, H_{core}), 6.05 – 5.91 (m, 2H, H_{Ar}), 4.54 (s, 2H, CH), 4.26 – 4.09 (m, 4H, CH_2), 3.44 (dt, *J* = 9.3, 7.0 Hz, 2H, CH_2), 3.13 (dt, *J* = 9.3, 7.0 Hz, 2H, CH_2), 1.96 – 1.80 (m, 4H, CH_2), 1.65 (dd, *J* = 13.9, 6.9 Hz, 4H, CH_2), 1.53 (dt, *J* = 15.2, 7.3 Hz, 4H, CH_2), 1.47 – 1.30 (m, 28H, CH_2), 0.94 (dt, *J* = 18.5, 7.0 Hz, 12H, CH_3).

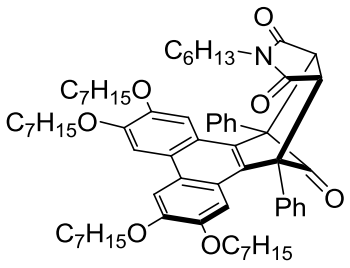
^{13}C-NMR (151 MHz, $CDCl_3$) : δ [ppm] = 196.63, 173.45, 148.97, 148.34, 134.50, 131.27, 131.11, 130.00, 129.39, 129.09, 128.78, 128.71, 128.38, 128.14, 125.92, 125.46, 120.82, 107.14, 105.71, 69.53, 67.71, 63.24, 44.94, 31.84, 31.79, 29.27, 29.10, 29.04, 28.77, 26.02, 25.80, 22.63, 22.57, 14.10, 14.05.

HR-MS (ESI): calc. 1034.5911; found 1034.5905 $[M + Na]^+$.

UV/Vis (DCM): λ [nm] = 282, 292.

FL λ_{Exc} = 266 nm (DCM): λ_{max} = 372, 390 nm.

5.3.17.3 5,6,9,10-Tetrakis(heptyloxy)-1,3-diphenyl-2H-cyclopenta[*l*]phenanthren-2-one and N-hexylmaleimide adduct (22)



According to the general reaction procedure (5.3.17), 5,6,9,10-tetrakis(heptyloxy)-1,3-diphenyl-2H-cyclopenta[*l*]phenanthren-2-one (0.12 g, 0.143 mmol) and N-hexylmaleimide (25.92 mg, 0.143 mmol) were reacted. And column chromatography was used (1:3 Hexane/DCM) to purified 0.125 g of product. Yield : 86%.

R_f = 0.4 (3:1 DCM/Hexane)

^{1}H-NMR (400 MHz, $CDCl_3$) : δ [ppm] = 8.41 (d, J = 7.3 Hz, 2H, H_{Ar}), 7.79 – 7.64 (m, 4H, H_{core} and H_{Ar}), 7.55 – 7.42 (m, 4H, H_{Ar}), 7.38 – 7.29 (m, 2H, H_{Ar}), 6.56 (s, 2H, H_{core}), 4.35 (s, 2H, CH), 4.13 (t, J = 6.6 Hz, 4H, CH_2), 3.49 (dt, J = 9.1, 6.8 Hz, 2H, CH_2), 3.23 – 3.08 (m, 2H, CH_2), 2.95 – 2.79 (m, 2H, CH_2), 1.95 – 1.82 (m, 4H, CH_2), 1.68 (d, J = 6.3 Hz, 4H, CH_2),

1.55 – 1.46 (m, 4H, CH_2), 1.34 (m, 28H, CH_2), 0.96 (t, J = 6.8 Hz, 6H, CH_3), 0.91 (t, J = 6.8 Hz, 6H, CH_3), 0.88 – 0.80 (m, 2H, CH_2), 0.71 (t, J = 7.2 Hz, 3H, CH_3), 0.64 (dd, J = 15.1, 7.4 Hz, 2H, CH_2), 0.48 (dd, J = 15.3, 7.8 Hz, 2H, CH_2), -0.16 – -0.33 (m, 2H, CH_2).

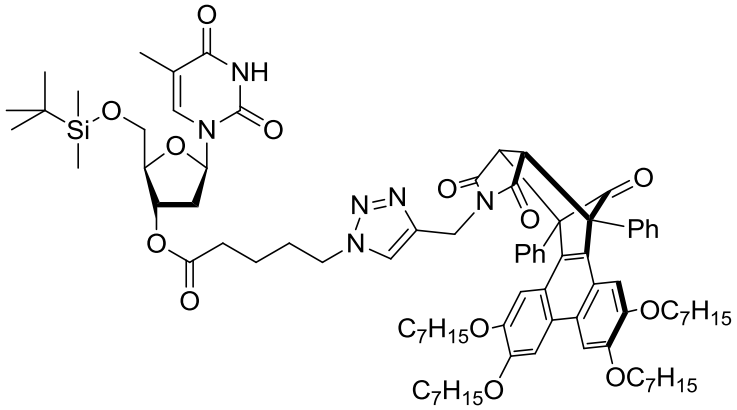
^{13}C-NMR (101 MHz, $CDCl_3$) : δ [ppm] = 196.63, 174.30, 148.88, 148.13, 134.65, 131.27, 129.91, 129.42, 129.06, 128.68, 128.08, 125.51, 120.88, 107.20, 105.34, 69.36, 67.77, 63.04, 44.58, 38.70, 31.87, 31.80, 31.09, 29.25, 29.10, 28.79, 26.38, 26.04, 25.82, 25.69, 22.66, 22.57, 21.94, 14.14, 14.05, 13.98.

HR-MS (ESI): calc. 1042.6531; found 1042.6537 $[M + Na]^+$.

UV/Vis (DCM): λ [nm] = 280, 291.

FL λ_{Exc} = 266 nm (DCM): λ_{max} = 373, 391 nm.

5.3.17.4 5,6,9,10-Tetrakis(heptyloxy)-1,3-diphenyl-2H-cyclopenta[*l*]phenanthren-2-one and click modified maleimide thymidine adduct (27)



According to the general reaction procedure (5.3.17), 5,6,9,10-tetrakis(heptyloxy)-1,3-diphenyl-2H-cyclopenta[*l*]phenanthren-2-one (41 mg, 0.049 mmol) and click derivatized maleimide thymidine (30 mg, 0.049 mmol) were reacted. Column chromatography (1:1 hexane/EtOH) gave 24.5 mg of pure product. Yield: 35%.

^{1}H-NMR (400 MHz, CDCl3) : δ [ppm] = 8.60 (s, 1H, NH), 8.39 (d, *J* = 7.6 Hz, 2H, H_{Ar}), 7.80 – 7.59 (m, 4H, 2H_{Ar} and 2H_{core}), 7.54 (d, *J* = 1.2 Hz, 1H, H6), 7.52 – 7.37 (m, 5H, H_{Ar} and CH), 7.25 (d, *J* = 7.5 Hz, 2H, H_{Ar}), 6.53 (s, 2H, H_{core}), 6.32 (dd, *J* = 9.0, 5.2 Hz, 2H, H1′), 5.24 (d, *J* = 5.9 Hz, 1H, H3′), 4.44 (s, 2H, CH), 4.21 – 4.04 (m, 7H, CH_2), 3.98 (dd, *J* = 10.8, 4.4 Hz, 2H, H4′), 3.92 (d, *J* = 1.2 Hz, 2H, H5′), 3.45 (dd, *J* = 11.6, 4.6 Hz, 2H, CH_2), 3.21 – 3.04 (m, 2H, CH_2), 2.42 – 2.30 (m, 3H, H2′ and CH_2), 2.17 – 2.03 (m, 1H, H2′), 1.93 (t, *J* = 3.2 Hz, 3H, CH_3), 1.87 (dd, *J* = 14.6, 6.8 Hz, 4H, CH_2), 1.56 (m, 12H, CH_2), 1.44 – 1.31 (m, 32H, CH_2), 0.98 – 0.89 (m, 21H, $SiC(CH_3)_3$ and CH_3), 0.15 (d, *J* = 1.1 Hz, 6H, $SiCH_3$).

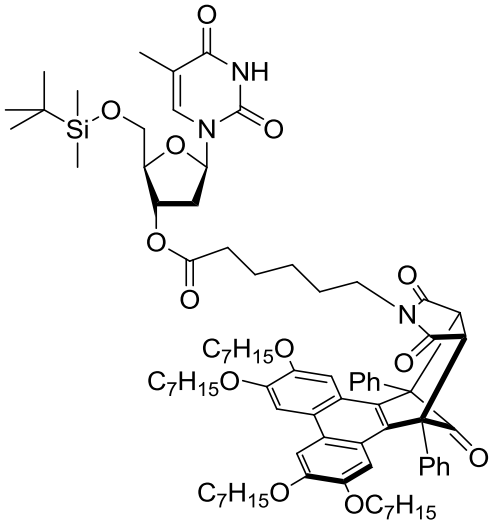
^{13}C-NMR (101 MHz, $CDCl_3$) : δ [ppm] = 195.90, 173.82, 172.41, 163.42, 148.94, 148.23, 134.93, 134.37, 131.21, 129.98, 129.38, 129.09, 128.64, 128.10, 125.18, 120.57, 111.15, 107.26, 105.51, 85.36, 84.65, 77.32, 77.00, 76.68, 75.49, 69.54, 67.79, 63.56, 63.00, 49.62, 45.03, 37.98, 33.18, 31.84, 31.77, 29.65, 29.38, 29.30, 29.09, 29.04, 28.78, 26.04, 25.88, 25.81, 22.63, 22.55, 21.54, 18.28, 14.11, 14.04, 12.43, -5.43, -5.53.

HR-MS (ESI): calc. 1455.8291; found 1455.8286 $[M + H]^+$.

UV/Vis (DCM): λ [nm] = 278.

FL λ_{Exc} = 266 nm (DCM): λ_{max} = 373, 391 nm.

5.3.17.5 5,6,9,10-Tetrakis(heptyloxy)-1,3-diphenyl-2H-cyclopenta[*l*]phenanthren-2-one and maleimide thymidine adduct (34)



According to the general reaction procedure (5.3.17), 5,6,9,10-tetrakis(heptyloxy)-1,3-diphenyl-2H-cyclopenta[*l*]phenanthren-2-one (25 mg, 0.030 mmol) and maleimide thymidine derivative (16.4 mg, 0.030 mmol) were reacted. Column chromatography was used (2:1 hexane/ EtOAc) to give 32 mg of pure product. Yield: 77%.

R_f = 0.3 (2:1 Hexane/EtOAc)

^{1}H-NMR (400 MHz, $CDCl_3$) : δ [ppm] = 8.62 (s, 1H, NH), 8.41 (s, 1H, H_{Ar}), 8.39 (s, 1H, H_{Ar}), 7.75 (two singlets, 2H, H_{core}), 7.72 – 7.63 (m, 2H, H_{Ar}), 7.55 (d, J = 1.2 Hz, 1H, H6), 7.51 – 7.40 (m, 4H, H_{Ar}), 7.30 (dd, J = 5.4, 3.6 Hz, 2H, H_{Ar}), 6.56 (s, 2H, H_{core}), 6.33 (dd, J = 9.2, 5.3 Hz, 1H, H1′), 5.18 (d, J = 6.0 Hz, 1H, H3′), 4.35 (s, 2H, CH), 4.01 (d, J = 1.4 Hz, 1H, H4′), 3.91 (d, J = 1.7 Hz, 2H, H5′), 3.49 (dt, J = 13.8, 6.8 Hz, 2H, CH_2), 3.14 (dt, J = 9.3, 7.1 Hz, 2H, CH_2), 2.89 (t, J = 7.5 Hz, 2H, CH_2), 2.35 (dd, J = 13.4, 5.4 Hz, 1H, H2′), 2.15 – 2.07 (m, 1H, H2′), 1.93 (d, J = 1.0 Hz, 3H, CH_3), 1.87 (m, 6H, CH_2), 1.68 (dd, J = 8.5, 4.8 Hz, 4H,

CH_2), 1.55 – 1.45 (m, 4H, CH_2), 1.43 – 1.18 (m, 28H, CH_2), 0.96 – 0.88 (m, 21H, $SiC(CH_3)_3$ and CH_3), 0.56 – 0.38 (m, 2H, CH_2), 0.15 (d, J = 1.3 Hz, 6H, $SiCH_3$), 0.01 – -0.19 (m, 2H, CH_2).

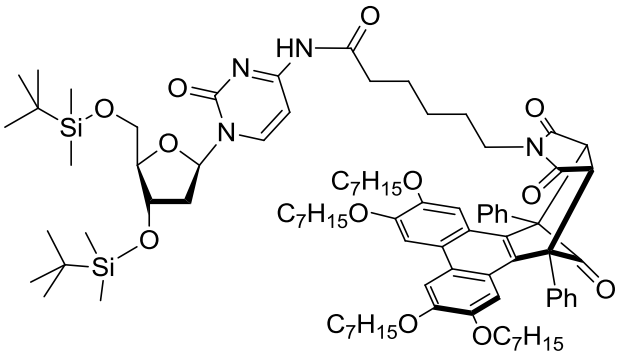
^{13}C-NMR (101 MHz, $CDCl_3$) : δ [ppm] = 196.47, 174.29, 172.92, 163.48, 150.14, 148.93, 148.14, 135.02, 134.58, 131.24, 129.92, 129.39, 129.05, 128.65, 128.06, 125.47, 120.82, 111.07, 107.16, 105.39, 85.44, 84.66, 75.05, 69.36, 67.74, 63.57, 63.01, 44.60, 38.37, 37.95, 33.47, 31.84, 31.77, 29.26, 29.12, 29.06, 28.78, 26.16, 26.05, 26.03, 25.89, 25.79, 25.49, 23.97, 22.64, 22.55, 18.29, 14.12, 14.04, 12.43, -5.43, -5.51.

HR-MS (ESI): calc. 1410.7940; found 1410.7935 $[M + Na]^+$.

UV/Vis (DCM): λ [nm] = 275, 291.

FL λ_{Exc} = 266 nm (DCM): λ_{max} = 373, 391 nm.

5.3.17.6 5,6,9,10-Tetrakis(heptyloxy)-1,3-diphenyl-2H-cyclopenta[*l*]phenanthren-2-one and maleimide cytidine adduct (35)



According to the general reaction procedure (5.3.17), 5,6,9,10-tetrakis(heptyloxy)-1,3-diphenyl-2H-cyclopenta[*l*]phenanthren-2-one (20 mg, 0.024 mmol) and maleimide cytidine derivative (16 mg, 0.024 mmol) were reacted. Column chromatography was used (2:1 hexane/ EtOAc) to give 28 mg of pure product. Yield: 79%.

R_f = 0.35 (1:1 Hexane/EtOAc)

^{1}H-NMR (400 MHz, $CDCl_3$) : δ [ppm] = 8.40 (d, J = 7.7 Hz, 2H, H_{Ar}), 8.36 (d, *J* = 7.4 Hz, 1H, H6), 7.78 (s, 2H, H_{core}), 7.71 (t, *J* = 6.9 Hz, 2H, H_{Ar}), 7.53 – 7.42 (m, 4H, H_{Ar}), 7.36 – 7.28 (m, 3H, H_{Ar} and H5), 6.56 (s, 2H, H_{core}), 6.31 – 6.20 (m, 1H, H1′), 4.40 (d, *J* = 4.8 Hz, 1H, H3′), 4.36 (s, 2H, CH), 4.16 (d, *J* = 5.1 Hz, 4H, CH_2), 4.03 – 3.92 (m, 2H, H5′), 3.84 – 3.75 (m, 1H, H4′), 3.57 – 3.42 (m, 2H), 3.15 (dd, *J* = 15.1, 7.9 Hz, 2H), 2.93 (d, *J* = 6.7 Hz, 2H), 2.55 (m, 1H, H2′), 2.18 – 2.07 (m, 1H, H2′), 1.85 (m, 6H), 1.68 (d, *J* = 4.6 Hz, 4H), 1.47 (d, *J* = 15.9 Hz, 4H), 1.37 (m, 28H), 1.07 (d, *J* = 5.8 Hz, 2H), 0.95 (t, *J* = 3.2 Hz, 18H), 0.90

(d, *J* = 5.1 Hz, 18H), 0.38 (s, 2H), 0.13 (d, *J* = 3.3 Hz, 6H), 0.08 (d, *J* = 1.7 Hz, 6H), -0.06 (s, 2H).

^{13}C-NMR (101 MHz, $CDCl_3$) : δ [ppm] = 196.47, 174.38, 148.97, 148.19, 134.55, 131.22, 129.97, 129.41, 129.10, 128.66, 128.09, 125.52, 120.85, 107.23, 105.61, 87.76, 86.77, 70.07, 69.53, 69.48, 67.81, 63.03, 61.78, 44.64, 42.33, 38.73, 38.21, 36.99, 33.90, 31.85, 31.77, 29.25, 29.10, 29.06, 28.78, 26.22, 26.01, 25.87, 25.80, 25.67, 25.31, 24.92, 23.79, 22.65, 22.56, 18.33, 17.91, 14.12, 14.05, -4.61, -4.97, -5.49, -5.55.

HR-MS (ESI): calc. 1487.8989; found 1487.9062 $[M + H]^+$.

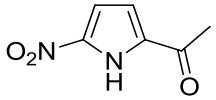
UV/Vis (DCM): λ [nm] = 282, 292.

FL λ_{Exc} = 266 nm (DCM): λ_{max} = 373, 391 nm.

5.3.18 General Synthesis of Nitro Pyrrole Derivatives

65% nitric acid (4.12 ml, 91.64 mmol, 2 eq) was added dropwise to a solution of 2-acetyl pyrrole (5 g, 45.82 mmol, 1 eq) in 30 ml of acetic anhydride, at - 40 ^oC over 30 min. The mixture was warmed to room temperature with stirring over 2 h, and then poured into ice-cooled water. It was extracted with ethyl acetate (3x50 ml) and the combined organic layers were dried over anhydrous Na_2SO_4, filtered and evaporated. Flash column chromatography of the residue with dichloromethane-ethyl acetate (19:1) gave 2- or 3-nitro pyrrole derivatives as product.

5.3.18.1 2-Acetyl-5-nitropyrrole (55)[188]



The product was synthesized according to the general reaction procedure (5.3.18) and 2.48 g of light yellow product was isolated. Yield: 35%

R_f = 0.49 (19:1 DCM/EtOAc)

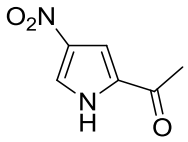
M.p. 232^{o}C (Lit.[195] 234^{o}C)

^{1}H-NMR (600 MHz, $CDCl_3$) : δ [ppm] = 10.42 (br s, 1H, NH), 7.10 (dd, *J* = 4.2, 2.7 Hz, 1H), 6.89 (dd, *J* = 4.2, 2.7 Hz, 1H), 2.56 (s, 3H, CH_3).

^{13}C-NMR (151 MHz, $CDCl_3$) : δ [ppm] = 188.78, 132.55, 115.31, 110.46, 25.85.

HR-MS (ESI): calc. 153.0300; found 153.0306 $[M - H]^+$.

5.3.18.2 2-Acetyl-4-nitropyrrole (56)[188]



After the general procedure explained in 5.3.19, 3.54 g of orange coloured product was obtained. Yield: 50%

R_f = 0.38 (19:1 DCM/EtOAc)

^{1}H-NMR (600 MHz, $CDCl_3$) : δ [ppm] = 12.94 (s, 1H, NH), 8.07 (dd, *J* = 3.7, 1.6 Hz, 1H), 7.61 (dd, *J* = 2.5, 1.7 Hz, 1H), 2.44 (s, 3H, CH_3).

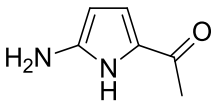
^{13}C-NMR (151 MHz, $CDCl_3$) : δ [ppm] = 188.26, 136.64, 131.42, 125.00, 110.97, 25.68.

HR-MS (ESI): calc. 153.0300; found 153.0306 $[M - H]^+$.

5.3.19 General Synthesis of Amino Pyrrole Derivatives

Nitro pyrrole derivative (1 eq.) in methanol was reduced over 10% palladium on carbon (0.05 eq.) under hydrogen (1 atm) at room temperature for 2 h. Filtration on Celite and evaporation of the solvent under reduced pressure gave the product as a solid.

5.3.19.1 2-Acetyl-5-aminopyrrole (60)



2-Acetyl-5-nitropyrrole (1.56 g, 10.12 mmol) and 10% palladium on carbon (0.54 g, 0.507 mmol) in 30 ml methanol were reacted according to the general reaction procedure (5.3.19). 1.18 g of product was obtained as a dark green solid. Yield: 94%

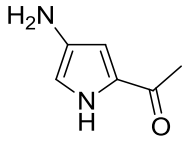
R_f = 0.23 (19:1 DCM/MeOH)

^{1}H-NMR (400 MHz, $CDCl_3$) : δ [ppm] = 6.96 (d, *J* = 4.2 Hz, 1H), 5.43 (d, *J* = 4.2 Hz, 1H), 4.89 (s, broad, 3H, NH_2 and NH), 2.23 (s, 3H, CH_3).

^{13}C-NMR (101 MHz, $CDCl_3$) : δ [ppm] = 183.57, 150.15, 125.41, 125.31, 95.95, 23.60

HR-MS (ESI): calc. 125.0637; found 125.0709 $[M - H]^+$.

5.3.19.2 2-Acetyl-4-aminopyrrole (61)



2-Acetyl-4-nitropyrrole (2 g, 12.98 mmol) and 10% palladium on carbon (0.69 g, 0.649 mmol) in 35 ml methanol was used according to the general reaction procedure (5.3.19). 1.56 g of product was obtained as a dark green solid. Yield: 97%

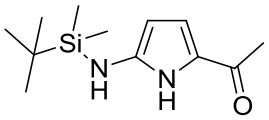
R_f = 0.25 (19:1 DCM/MeOH)

^{1}H-NMR (400 MHz, $CDCl_3$) : δ [ppm] = 10.96 (s, 1H, NH), 6.44 (dd, *J* = 2.6, 1.9 Hz, 1H), 6.31 (dd, *J* = 2.6, 1.9 Hz, 1H), 3.92 (br s, 2H, NH_2), 2.24 (s, 3H, CH_3).

^{13}C-NMR (101 MHz, $CDCl_3$) : δ [ppm] = 185.68, 134.65, 129.33, 111.85, 106.02, 25.32.

HR-MS (ESI): calc. 125.0637; found 125.0709 $[M - H]^+$.

5.3.20 5-Acetyl-2-(tert-butyldimethylsilyl)amino-1H-pyrrole (62)



To a solution of 2-acetyl-5-aminopyrrole (0.3 g, 2.42 mmol) in 15 ml dry THF, Et_3N (0.506 ml, 3.62 mmol) was added dropwise and the reaction mixture was stirred for half an hour. Then, it was cooled to 0 ° C, followed by addition of a solution of $ClSiMe_2{}^tBu$ (TBDMS-Cl) (0.364 g, 2.42 mmol) in 10 ml THF. The reaction mixture was allowed to warm to room temperature and stirred for a further 4 h. After that, THF was evaporated and the residue was dissolved in CH_2Cl_2 and extracted with water (2x100 ml). The organic phase was then dried with Na_2SO_4, filtered and evaporated in vacuo. 0.348 gr of beige product was isolated by column chromatography with DCM/MeOH (19:1). Yield: 60%

R_f = 0.64 (19:1 DCM/MeOH)

^{1}H-NMR (400 MHz, $CDCl_3$) : δ [ppm] = 11.13 (br s, 1H, NH), 6.89 (dd, *J* = 4.0, 2.5 Hz, 1H), 5.42 (dd, *J* = 4.0, 2.5 Hz, 1H), 5.23 (br s, 1H, SiNH), 2.29 (s, 3H, CH_3), 1.01 (s, 9H, CH_3), 0.26 (s, 6H, CH_3).

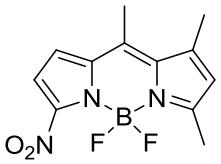
^{13}C-NMR (101 MHz, $CDCl_3$) : δ [ppm] = 182.30, 148.69, 124.04, 122.95, 94.81, 25.97, 23.77, 17.56, -4.75.

5.3.21 General Synthesis of Amino and Nitro BODIPY Derivatives

Pyrrole derivative (1 eq.) and 2,4-dimethylpyrrole (1 eq.) were dissolved in CH_2Cl_2 under argon atmosphere. The mixture was then cooled to 0 °C in an ice bath, followed by the addition of $POCl_3$ (1 eq.) and the solution was stirred at room temperature for an appropriate time. Meanwhile the mixture turned dark. Triethylamine (10 eq.) was added and the mixture was stirred for 10 minutes while being cooled to 0 °C. Then, borontrifluoride etherate

($BF_3.Et_2O$) (1.1 eq.) was added dropwise and the reaction mixture is than stirred at room temperature for 1 h. After that, CH_2Cl_2 was added to the reaction mixture and extracted with water (3 × 200 ml). The organic phase was dried over Na_2SO_4, filtered and evaporated on rotary. To obtain pure product column chromatography was used.

5.3.21.1 3-Nitro-5,7,8-trimethyl-4,4-difluoro-4-bora-3a,4a-diaza-s-indacene (57)



2-Acetyl-5-nitropyrrole (0.25 g, 1.62 mmol), 2,4-dimethylpyrrole (0.154 g, 0.17 ml, 1.62 mmol) in 5 ml CH_2Cl_2 and $POCl_3$ (0.249 g, 0.151 ml, 1.62 mmol), triethylamine (2.26 ml, 16.2 mmol), $BF_3.Et_2O$ (2.26 ml, 17.84 mmol) were used according to the general reaction procedure (5.3.21). After column chromatography with dichloromethane, 0.248 g of a brown solid was obtained. Yield: 55%

R_f = 0.18 (DCM)

M.p. >250°C

^{1}H-NMR (400 MHz, $CDCl_3$) : δ [ppm] = 7.20 (d, *J* = 4.2 Hz, 1H, H_{core}), 6.93 (d, *J* = 4.2 Hz, 1H, H_{core}), 6.42 (s, 1H, H_{core}), 2.72 (s, 3H, CH_3), 2.63 (s, 3H, CH_3), 2.51 (s, 3H, CH_3).

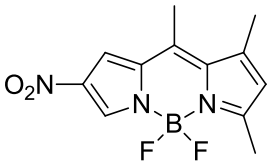
^{13}C-NMR (101 MHz, $CDCl_3$) : δ [ppm] = 168.49, 148.31, 139.41, 136.81, 133.88, 126.24, 117.59, 113.58, 16.22, 14.93, 14.76.

HR-MS (ESI): calc. 278.0912; found 278.0918 $[M + H]^+$.

UV/Vis (DCM): λ_{max} [nm] = 465.

FL λ_{Exc} = 470 nm (DCM): λ_{max} = 514 nm.

5.3.21.2 2-Nitro-5,7,8-trimethyl-4,4-difluoro-4-bora-3a,4a-diaza-s-indacene (58)



2-Acetyl-4-aminopyrrole (0.886 g, 5.75 mmol), 2,4-dimethylpyrrole (0.547 g, 0.592 ml, 5.75 mmol) in 15 ml CH_2Cl_2 and $POCl_3$ (0.881 g, 0.536 ml, 5.75 mmol), triethylamine (8.02 ml, 57.49 mmol), $BF_3.Et_2O$ (8.01 ml, 63.24 mmol) were reacted according to the general reaction

procedure (5.3.21). After column chromatography with dichloromethane, 0.4 g of a light brown solid was obtained. Yield: 25%

R_f = 0.45 (DCM)

M.p. 252°C (dec.)

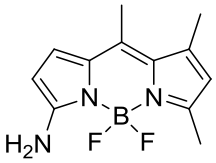
^{1}H-NMR (600 MHz, $CDCl_3$) : δ [ppm] = δ 8.15 (s, 1H), 7.48 (s, 1H), 6.40 (s, 1H), 2.68 (s, 3H), 2.64 (s, 3H), 2.52 (s, 3H).

HR-MS (ESI): calc. 302.0888; found 302.0883 $[M + Na]^+$.

UV/Vis (DCM): λ_{max} [nm] = 444, 464.

FL λ_{Exc} = 445 nm (DCM): λ_{max} = 487 nm.

5.3.21.3 3-Amino-5,7,8-trimethyl-4,4-difluoro-4-bora-3a,4a-diaza-s-indacene (63)



Route A

2-Acetyl-5-aminopyrrole (0.331 g, 2.67 mmol), 2,4-dimethylpyrrole (0.253 g, 0.274 ml, 2.67 mmol) in 20 ml CH_2Cl_2 and $POCl_3$ (0.409 g, 0.248 ml, 2.67 mmol), triethylamine (3.72 ml, 26.6 mmol), $BF_3.Et_2O$ (3.72 ml, 29.33 mmol) were reacted according to the general reaction procedure (5.3.21). After column chromatography with dichloromethane, 34 mg of a dark red solid was obtained. Yield: 5%

Route B

2-Acetyl-5-(tert-butyldimethylsilyl)aminopyrrole (0.311 g, 1.30 mmol) and 2,4-dimethyl-pyrrole (0.124 g, 0.134 ml, 1.30 mmol) in 15 ml CH_2Cl_2 and $POCl_3$ (0.2 g, 0.122 ml, 1.30 mmol), triethylamine (1.82 ml, 13.05 mmol), $BF_3.Et_2O$ (1.82 ml, 14.35 mmol) were reacted according to the reaction procedure (5.3.21). After column chromatography with dichloromethane, 24 mg of a dark red solid was obtained. Yield: 5%

Route C

3-Nitro-5,7,8-trimethyl-4,4-difluoro-4-bora-3a,4a-diaza-s-indacene (50 mg, 0.179 mmol, 1 eq.) in 5 ml methanol was reduced over 10% palladium on carbon (9.53 mg, 0.009 mmol, 0.05 eq.) under hydrogen (1 atm) at room temperature for 1 h. The mixture was filtrated on

Celite and the solvent was evaporated under reduced pressure. After column chromatography with dichloromethane, 29 mg of a dark red solid was obtained. Yield: 65%

R_f = 0.71 (19:1 DCM/MeOH)

M.p. 168 °C (dec.)

^{1}H-NMR (600 MHz, $CDCl_3$) : δ [ppm] = 7.18 (d, *J* = 4.7 Hz, 1H), 5.95 (s, 1H), 5.93 (d, *J* = 4.7 Hz, 1H), 5.41 (br s, 2H, NH_2), 2.49 (s, 3H, CH_3), 2.43 (s, 3H, CH_3), 2.38 (s, 3H, CH_3).

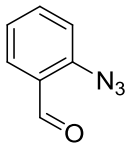
^{13}C-NMR (151 MHz, $CDCl_3$) : δ [ppm] = 158.60, 149.84, 145.99, 132.51, 130.87, 120.42, 117.90, 112.83, 108.40, 99.58, 15.96, 15.74, 13.90.

HR-MS (ESI): calc. 249.1249; found 249.1358 $[M + H]^+$.

UV/Vis λ_{max} [nm] = 507 (DCM), 500 (EtOH).

FL λ_{Exc} = 470 nm : λ_{max} = 527 nm (DCM), 523 nm (EtOH).

5.3.22 2-Azidobenzaldehyde (38)[171]



To a stirring solution of 2-nitrobenzaldehyde (2.5 g, 16.5 mmol) in DMF (40 mL) was added NaN_3 (2.145 g, 33 mmol) at 0 °C. Then, the mixture was heated to 60 °C for 24 h. After that, it was was poured onto ice and extracted with methyl tert-butyl ether (100 ml). The organic phase was washed with water (2×100 ml), dried over Na_2SO_4, filtered and the solvent was evaporated. Column chromatography with hexane/EtOAc (7:3) was used to give 1.58 g of pure product. Yield: 65%

R_f = 0.60 (7:3 Hexane/EtOAc on Et_3N-treated SiO_2 plate)

^{1}H-NMR (600 MHz, $CDCl_3$) : δ [ppm] = 10.36 (s, 1H, HCO), 7.89 (dd, J = 8.0, 2.0 Hz, 1H, H_{Ar}), 7.63 (m, 1H, H_{Ar}), 7.29 (d, J = 7.9 Hz, 1H, H_{Ar}), 7.24 (t, J = 7.9 Hz, 1H, H_{Ar})

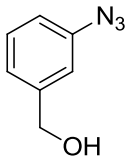
^{13}C-NMR (151 MHz, $CDCl_3$) : δ [ppm] = 188.72 , 143.36 , 135.55, 129.35, 127.01, 124.95, 119.11.

5.3.23 Synthesis of 3- and 4-Azidobenzyl Alcohol[196]

Aminobenzyl alcohol (2.50 g, 20.3 mmol) was dissolved in 6.0 M HCl (21 mL) at 0 °C. 1.2 M $NaNO_2$ solution (25 mL, 30.4 mmol, 2.07 g) was added dropwise over 20 min and the mixture was stirred at 0 °C for 20 min, followed by the addition of 1.6 M NaN_3 solution (5.2 g

NaN_3, 50 ml, 81.1 mmol). The mixture was stirred at 0 °C for 30 min. The solution was extracted with diethylether (3x60 ml). The organic layer was washed with brine, saturated $NaHCO_3$ solution and brine and dried over Na_2SO_4, filtrated and concentrated. The residue was purified by column chromatography with AcOEt/ hexane (1:3).

5.3.23.1 3-Azidobenzyl alcohol (39)[196]



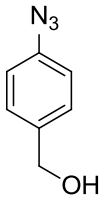
3-Aminobenzyl alcohol was used and 2.9 g of product was obtained as a yellow oil according to the general reaction procedure (5.3.23). Yield : 96%

R_f = 0.25 (3:1 Hexane/EtOAc)

^{1}H-NMR (600 MHz, $CDCl_3$) : δ [ppm] = 7.31 (t, *J* = 7.9 Hz, 1H, H_{Ar}), 7.07 (d, *J* = 7.6 Hz, 1H, H_{Ar}), 6.97 (s, 1H, H_{Ar}), 6.93 (dd, *J* = 7.9, 2.0 Hz, 1H, H_{Ar}), 4.58 (s, 2H, CH_2), 3.56 (s, 1H, OH).

^{13}C-NMR (151 MHz, $CDCl_3$) : δ [ppm] = 142.68, 139.97, 129.61, 123.00, 117.82, 117.04, 64.06.

5.3.23.2 4-Azidobenzyl alcohol (40)[172]



4-Aminobenzyl alcohol was used and 2.88 g of product was obtained as a yellow oil according to the general reaction procedure (5.3.23). Yield: 95%

R_f = 0.21 (3:1 Hexane/EtOAc)

^{1}H-NMR (600 MHz, $CDCl_3$) : δ [ppm] = 7.26 (d, *J* = 8.8 Hz, 2H, H_{Ar}), 6.96 (d, *J* = 8.8 Hz, 2H, H_{Ar}), 4.53 (s, 2H, CH_2), 3.74 (s, 1H, OH).

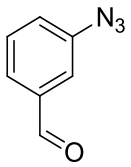
^{13}C-NMR (151 MHz, $CDCl_3$) : δ [ppm] = 138.89, 137.34, 128.18, 118.73, 63.89.

5.3.24 Synthesis of 3- and 4-Azidobenzaldehyde[172]

PCC (3.67 g, 17.08 mmol) was added in one portion to a solution of azidobenzyl alcohol (1.5 g, 10.05 mmol) in 40 ml CH_2Cl_2. Then, the mixture was stirred at room temperature for 1 h,

followed by filtration over a pad of silica gel on a fritted funnel. The solvent was removed on rotary, affording pure product as a thick oil.

5.3.24.1 3-Azidobenzaldehyde (41)[196]



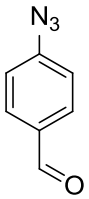
3-Azidobenzyl alcohol (1.5 g, 10.05 mmol) was used in the general reaction process (5.3.24) and 1.38 g of product was obtained as yellow oil. Yield : 93%

R_f = 0.26 (3:1 Hexane/EtOAc)

^{1}H-NMR (600 MHz, $CDCl_3$) : δ [ppm] = 10.01 (s, 1H, CH), 7.66 (dd, *J* = 7.6, 0.9 Hz, 1H, H_{Ar}), 7.56 (s, 1H, H_{Ar}), 7.54 (d, *J* = 7.7 Hz, 1H, H_{Ar}), 7.28 (ddd, *J* = 7.9, 1.5, 0.9 Hz, 1H, H_{Ar}).

^{13}C-NMR (151 MHz, $CDCl_3$) : δ [ppm] = 191.13, 141.44, 137.83, 130.44, 126.58, 124.83, 118.97.

5.3.24.2 4-Azidobenzaldehyde (42)[172]



4-Azidobenzyl alcohol (1.5 g, 10.05 mmol) was used in the general reaction process (5.3.24) and 1.32 g of product was obtained as yellow oil. Yield: 89%

R_f = 0.22 (3:1 Hexane/EtOAc)

^{1}H-NMR (600 MHz, $CDCl_3$) : δ [ppm] = 9.94 (s, 1H, CH), 7.88 (d, *J* = 8.5 Hz, 2H, H_{Ar}), 7.16 (d, *J* = 8.5 Hz, 2H, H_{Ar}).

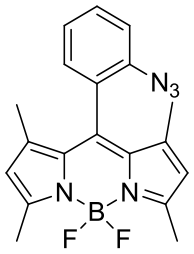
^{13}C-NMR (151 MHz, $CDCl_3$) : δ [ppm] = 190.43, 146.18, 133.20, 131.44, 119.39.

5.3.25 General Synthesis of 8-(Azidophenyl)-BODIPY Derivatives

In a 1000 mL flask, equipped with septum, azidobenzaldehyde (1 eq.) and 2,4-dimethyl-pyrrole (or 2,4-dimethyl-3-diethyl pyrrole) (2 eq.) were dissolved in CH_2Cl_2 and the solution was purged with argon (bubbling). One drop of TFA was added and the mixture was stirred at room temperature for 3 h. After that, a solution of (1 eq.) of DDQ (2,3-dichloro-5,6-dicyano-p-benzoquinone) in CH_2Cl_2 was added. After 3 h, Et_3N (10 eq.) was added and followed by

$BF_3.OEt_2$ (11 eq.). The reaction mixture was washed with water (3x100 ml), dried over Na_2SO_4 and concentrated on rotary. Then crude product was purified by silica gel column chromatography hexane/CH_2Cl_2 (1:1).

5.3.25.1 8-(2-Azidophenyl)-1,3,5,7-tetramethyl-4,4-difluoro-4-bora-3a,4a-diaza-s-indacene (44)



2-Azidobenzaldehyde (0.3 g, 2.04 mmol), 2,4-dimethylpyrrole (0.39 g, 0.42 mL, 4.08), in 300 ml CH_2Cl_2, DDQ (0.463 g, 2.04 mmol), Et_3N (2.85 mL, 20.4 mmol), $BF_3.OEt_2$ (2.84 mL, 22.43 mmol) were reacted according to the general procedure (5.3.25). After purification, 0.26 g of dark red solid was obtained. Yield: 35%

R_f = 0.25 (1:1 Hexane/CH_2Cl_2)

M.p. 128-130^{o}C (dec.)

^{1}H-NMR (400 MHz, $CDCl_3$) : δ [ppm] = 7.55 (ddd, *J* = 8.2, 7.3, 1.7 Hz, 1H, H_{Ar}), 7.28 (ddd, *J* = 11.5, 9.2, 4.9 Hz, 3H, H_{Ar}), 6.01 (s, 2H, H_{core}), 2.58 (s, 6H, CH_3), 1.45 (s, 6H, CH_3).

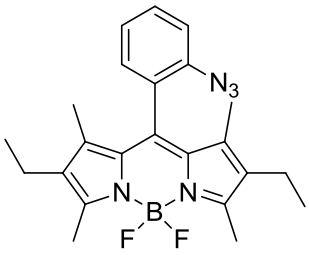
^{13}C-NMR (101 MHz, $CDCl_3$) : δ [ppm] = 155.81, 142.45, 138.36, 130.76, 130.04, 126.42, 125.65, 121.26, 118.78, 14.60, 13.82.

HR-MS (ESI): calc. 366.1702; found 366.1696 $[M + H]^+$.

UV/Vis (DCM): λ_{max} [nm] = 508.

FL λ_{Exc} = 470 nm (DCM): λ_{max} = 515 nm.

5.3.25.2 8-(2-Azidophenyl)-2,6-diethyl-1,3,5,7-tetramethyl-4,4-difluoro-4-bora-3a,4a-diaza-s-indacene (43)



2-Azidobenzaldehyde (0.4 g, 2.72 mmol), 2,4-dimethyl-3-diethyl pyrrole (0.67 g, 5.44), in 350 ml CH_2Cl_2, DDQ (0.617 g, 2.72 mmol), Et_3N (3.79 mL, 27.2 mmol), $BF_3.OEt_2$ (3.79 mL, 29.9 mmol) were reacted according to the general procedure (5.3.25). After purification, 0.386 g of red solid was obtained. Yield: 35%

R_f = 0.32 (1:1 Hexane/CH_2Cl_2)

M.p. 161-163°C (dec.)

^{1}H-NMR (600 MHz, $CDCl_3$) : δ [ppm] = 7.55 (ddd, *J* = 8.2, 7.5, 1.8 Hz, 1H, H_{Ar}), 7.34 – 7.24 (m, 3H, H_{Ar}), 2.56 (s, 6H, CH_3), 2.34 (q, *J* = 7.6 Hz, 4H, CH_2), 1.36 (s, 6H, CH_3), 1.03 (t, *J* = 7.6 Hz, 6H, CH_3).

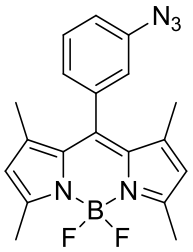
^{13}C-NMR (151 MHz, $CDCl_3$) : δ [ppm] = 154.11, 138.55, 137.68, 135.80, 132.80, 130.55, 130.37, 127.31, 125.56, 118.72, 17.09, 14.58, 12.54, 11.08.

HR-MS (ESI): calc. 444.2147; found 444.2142 $[M + Na]^+$.

UV/Vis (DCM): λ_{max} [nm] = 531.

FL λ_{Exc} = 470 nm (DCM): λ_{max} = 543 nm.

5.3.25.3 8-(3-Azidophenyl)-1,3,5,7-tetramethyl-4,4-difluoro-4-bora-3a,4a-diaza-s-indacene (45)



3-Azidobenzaldehyde (0.4 g, 2.72 mmol), 2,4-dimethylpyrrole (0.517 g, 0.56 mL, 5.44), in 350 ml CH_2Cl_2, DDQ (0.617 g, 2.72 mmol), Et_3N (3.79 mL, 27.2 mmol), $BF_3.OEt_2$ (3.79 mL, 29.9 mmol) were reacted according to the general procedure (5.3.25). After purification, 0.417 g of red solid was obtained. Yield: 42%

R_f = 0.25 (1:1 Hexane/CH_2Cl_2)

M.p. 168-171°C (dec.)

^{1}H-NMR (400 MHz, $CDCl_3$) : δ [ppm] = 7.51 (t, *J* = 7.9 Hz, 1H, H_{Ar}), 7.18 (ddd, *J* = 8.1, 2.3, 1.0 Hz, 1H, H_{Ar}), 7.13 – 7.07 (m, 1H, H_{Ar}), 7.02 – 6.96 (m, 1H, H_{Ar}), 6.02 (s, 2H, H_{core}), 2.58 (s, 6H, CH_3), 1.46 (s, 6H, CH_3).

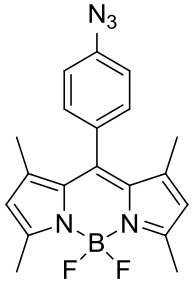
^{13}C-NMR (101 MHz, $CDCl_3$) : δ [ppm] = 155.93, 142.87, 141.33, 140.01, 136.82, 131.15, 130.64, 124.64, 121.40, 119.40, 118.82, 14.56, 14.43.

HR-MS (ESI): calc. 366.1702; found 366.1696 $[M + H]^+$.

UV/Vis (DCM): λ_{max} [nm] = 504.

FL λ_{Exc} = 470 nm (DCM): λ_{max} = 513 nm.

5.3.25.4 8-(3-Azidophenyl)-1,3,5,7-tetramethyl-4,4-difluoro-4-bora-3a,4a-diaza-s-indacene (46)



3-Azidobenzaldehyde (0.4 g, 2.72 mmol), 2,4-dimethylpyrrole (0.517 g, 0.56 mL, 5.44), in 350 ml CH_2Cl_2, DDQ (0.617 g, 2.72 mmol), Et_3N (3.79 mL, 27.2 mmol), $BF_3.OEt_2$ (3.79 mL, 29.9 mmol) were reacted according to the general procedure (5.3.25). After purification, 0.45 g of red solid was obtained. Yield: 46%

R_f = 0.26 (1:1 Hexane/CH_2Cl_2)

M.p. 148-150^{o}C (dec.)

^{1}H-NMR (600 MHz, $CDCl_3$) : δ [ppm] = 7.29 (d, *J* = 8.5 Hz, 2H, H_{Ar}), 7.18 (d, *J* = 8.5 Hz, 2H, H_{Ar}), 6.01 (s, 2H, H_{core}), 2.58 (s, 6H, CH_3), 1.45 (s, 6H, CH_3).

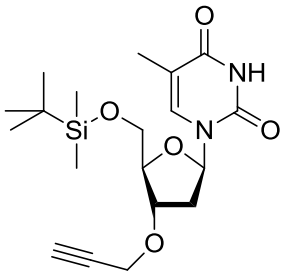
^{13}C-NMR (151 MHz, $CDCl_3$) : δ [ppm] = 155.72, 142.88, 141.04, 140.55, 131.51, 131.46, 129.64, 121.34, 119.71, 14.55, 14.52.

HR-MS (ESI): calc. 388.1521; found 388.1516 $[M + Na]^+$.

UV/Vis (DCM): λ_{max} [nm] = 503.

FL λ_{Exc} = 470 nm (DCM): λ_{max} = 513 nm.

5.3.26 5'-O-*tert*-Butyldimethylsilyl-3'-O-propargylthymidine (47)[175]



A solution of 0.5 g of 5'-O-(*tert*-butyldimethylsilyl)thymidine (1.37 mmol) in 10 ml of dry THF was cooled down to 0°C and a 60% dispersion of NaH in oil (29 mg, 0.73 mmol) was added. After stirring at 0°C for 1 h, 0.32 ml of propargyl bromide (3.61 mmol) was added dropwise and the mixture was stirred at room temperature for 24 h. Then, extra 0.29 g of NaH (7.17 mmol) was added. Stirring was continued for another 4 h and the reaction was quenched by addition of water. After extraction with diethyl ether (3 x 50 ml), the combined organic phases were collected and washed with brine. The organic layer was dried over Na_2SO_4 and evaporated under vacuum. The product was isolated by flash column chromatography (95:5 DCM/MeOH as eluent) as an colorless oil. Yield: 45%

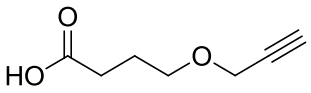
R_f = 0.45 (95:5 DCM/MeOH)

^{1}H-NMR (400 MHz, $CDCl_3$) : δ [ppm] = 9.74 (s, 1H, NH), 7.48 (d, J = 1.2 Hz, 1H, H6), 6.33 – 6.15 (m, 1H, H1′), 4.34 (dt, J = 6.0, 1.9 Hz, 1H, H3′), 4.19 (dt, J = 5.8, 3.2 Hz, 2H, CH_2), 4.13 – 4.09 (m, 1H, H4′), 3.84 (m, 2H, H5′), 2.57 – 2.39 (m, 2H, C=CH and H2′), 1.98 (ddd, J = 13.8, 10.9, 6.6 Hz, 1H, H2′), 1.91 (t, J = 3.8 Hz, 3H, CH_3), 0.95 – 0.87 (m, 9H, $SiC(CH_3)_3$), 0.11 (two singlets, 6H, $SiCH_3$).

^{13}C-NMR (101 MHz, $CDCl_3$) : δ [ppm] = 164.06, 150.45, 135.15, 110.79, 84.84, 84.77, 79.05, 78.41, 74.94, 63.37, 56.24, 37.51, 25.79, 18.21, 12.39, -5.50, -5.60.

HR-MS (ESI): calc. 417.1822; found 417.1816 $[M + Na]^+$.

5.3.27 4-Propargyloxybutanoic acid (52)[179]



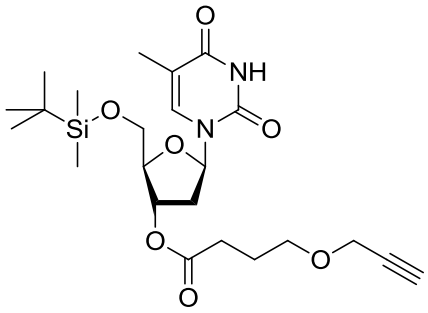
Into a 50 ml flask, γ-butyrolactone (2 g, 23 mmol, 1 eq) and an aqueous solution of NaOH (1.07 g, 27 mmol, 1.15 eq) was added at room temperature and the mixture was stirred for 24 h. The reaction mixture was evaporated to dryness and the residue was washed with ethanol to yield sodium 4-hydroxybutanate as a white solid. The product was used in next step without further purification. Yield: 85%

In the next step, sodium 4-hydroxybutanate (1 g, 7.93 mmol, 1 eq) was dissolved in DMF and a 60% dispersion of NaH in oil (952 mg, 24 mmol, 3 eq) was added at room temperature. The reaction mixture was stirred for 24 h and propargyl bromide (943 mg, 684 mml, 7.93 mmol, 1 eq) was added dropwise. After stirring another 24 h, the mixture was evaporated to dryness under vacuo. The residue was dissolved in 25 ml of water and extracted with diethyl ether in order to remove by-product. The aqueous phase was then neutralized by 1 M HCl, causing precipitation of a solid which was filtrated, extracted with 25 ml of ethyl acetate and washed by water. The combined organic phases were concentrated under vacuum to yield 4-propargyloxybutanoic acid as a brown oil. Yield: 25%

^{1}H-NMR (400 MHz, $CDCl_3$) : δ [ppm] = 10.11 (s, 1H, OH), 4.12 (t, J = 3.1 Hz, 2H, CH_2), 3.58 – 3.53 (m, 2H, CH_2), 2.48 – 2.41 (m, 3H, CH_2 and CH), 1.94 – 1.86 (m, 2H, CH_2).

^{13}C-NMR (101 MHz, $CDCl_3$) : δ [ppm] = 179.23, 79.55, 74.35, 68.60, 57.91, 30.60, 24.40.

5.3.28 5'-O-(*tert*-Butyldimethylsilyl)-3'-O-(4-propargyloxybutanoyl)-thymidine (53)



85 mg (0.24 mmol, 1 eq) of 5'-O-*tert*-butyldimethylsilyl thymidine, 34 mg of 4-propargyloxybutanoic acid (0.24 mmol, 1 eq) and 2.92 mg of DMAP (0.023 mmol, 0.1 eq) were dissolved in 10 ml of dry CH_2Cl_2 and cooled down to 0 ^{o}C. 49.2 mg of DCC (0.24 mmol, 1 eq), dissolved in 5 ml of dry CH_2Cl_2, was added dropwise within 1 h and the solution stirred at room temperature for 24 h. The solution was filtered over Celite and evaporated in vacuo. The residue was chromatographed on a silica gel column (ethyl acetate/hexane 3:1) to afford the desired product as colorless solid. Yield: 78%

R_f = 0.56 (3:1 EtOAc/Hexane)

^{1}H-NMR (400 MHz, $CDCl_3$) : δ [ppm] = 9.67 (s, 1H, NH), 7.52 (d, J = 1.2 Hz, 1H, H6), 6.34 (dd, J = 9.2, 5.3 Hz, 1H, H1′), 5.23 (d, J = 6.1 Hz, 1H, H3′), 4.10 (d, J = 1.8 Hz, 2H, CH_2), 4.08 – 4.05 (m, 1H, H4′), 3.94 – 3.81 (m, 2H, H5′), 3.54 (t, J = 6.0 Hz, 2H, CH_2), 2.46 – 2.36 (m, 4H, CH_2, H2′, CCH), 2.13 – 2.03 (m, 1H, H2′), 1.93 – 1.88 (m, 5H, CH_3 and CH_2), 0.90 (s, 9H, $SiC(CH_3)_3$), 0.11 (s, 6H, $SiCH_3$).

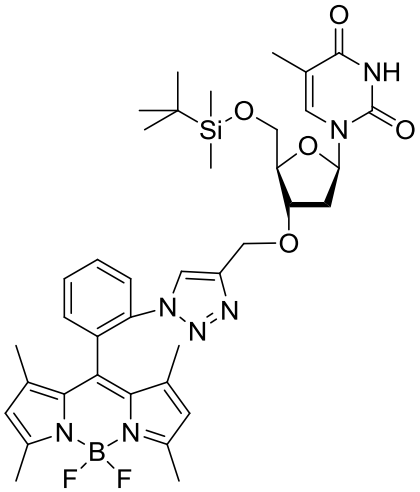
^{13}C-NMR (101 MHz, $CDCl_3$) : δ [ppm] = 172.97, 163.87, 150.51, 134.86, 111.07, 85.23, 84.58, 79.57, 75.20, 74.33, 68.61, 63.47, 57.94, 37.87, 32.50, 30.90, 30.69, 26.08, 25.77, 24.61, 18.17, 12.34, -5.53, -5.64.

HR-MS (ESI): calc. 503.2189; found 503.2184 $[M + Na]^+$.

5.3.29 General Synthesis of Click Derivatives

Azido BODIPY derivative (1 eq), alkyne modified thymidine (1.25 eq), sodium ascorbate (0.6 eq) and $CuSO_4.5H_2O$ (0.2 eq) were dissolved in a 1:1 mixture of water and THF in a small test tube. After stirring overnight, THF was evaporated in vacuo and the water phase was extracted with ethyl acetate (3 x 10 ml). The combined organic layers were dried over Na_2SO_4 and filtrated. The solvent was evaporated in vacuum and a flash column chromatography was performed to obtain the desired product.

5.3.29.1 Click Reaction of 8-(2-Azidophenyl)BODIPY and 5'-O-(*tert*-Butyldimethyl-silyl)- 3'-O-propargyl-thymidine (48)



8-(2-Azidophenyl)-2,6-diethyl-1,3,5,7-tetramethyl-4,4-difluoro-4-bora-3a,4a-diaza-s-indacene (30 mg, 0.082 mmol), 5'-O-*tert*-butyldimethylsilyl-3'-O-propargyl-thymidine (40.5 mg, 0.103 mmol), sodium ascorbate (9.76 mg, 0.049 mmol) and $CuSO_4.5H_2O$ (4.1 mg, 0.017 mmol) were reacted according to the general procedure (5.3.29). After purification, 21.8 mg of red solid was isolated. Yield: 35%

R_f = 0.4 (3:1 EE/Hexane)

M.p. 82-84°C

^{1}H-NMR (400 MHz, $CDCl_3$) : δ [ppm] = 8.74 (s, 1H, NH), 7.89 (d, J = 7.8 Hz, 1H, CH), 7.69 (dt, J = 21.7, 7.5 Hz, 2H, H_{Ar}), 7.59 (s, 1H, H6), 7.54 – 7.41 (m, 2H, H_{Ar}), 6.24 (dd, J = 8.3, 5.7 Hz, 1H, H1′), 6.00 (s, 2H, H_{core}), 4.58 (m, 2H, CH_2), 4.36 (t, J = 7.0 Hz, 1H, H3′), 4.06 (d,

J = 2.0 Hz, 1H, H4′), 3.86 (dd, J = 11.4, 2.4 Hz, 1H, H5′), 3.75 (dd, J = 11.3, 2.1 Hz, 1H, H5′), 2.52 (s, 6H, CH_3), 2.34 (dd, J = 13.4, 5.6 Hz, 1H, H2′), 1.96 (d, J = 9.0 Hz, 1H, H2′), 1.93 (s, 3H, CH_3), 1.49 (s, 6H, CH_3), 0.92 (s, 9H, $SiC(CH_3)_3$), 0.10 (s, 6H, $SiCH_3$).

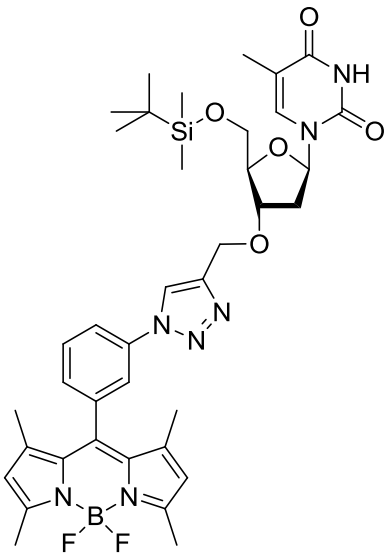
^{13}C-NMR (101 MHz, $CDCl_3$) : δ [ppm] = 163.68, 156.86, 156.71, 150.19, 144.90, 142.38, 142.28, 135.42, 135.29, 135.21, 131.05, 130.84, 130.20, 130.09, 128.64, 126.39, 123.00, 122.01, 110.80, 84.97, 84.83, 79.64, 63.52, 62.58, 37.86, 29.64, 29.30, 25.89, 18.30, 14.62, 13.96, 12.44, -5.43, -5.51.

HR-MS (ESI): calc. 782.3445; found 782.3440 $[M + Na]^+$.

UV/Vis (DCM): λ_{max} [nm] = 510.

FL λ_{Exc} = 470 nm (DCM): λ_{max} = 518 nm.

5.3.29.2 Click Reaction of 8-(3-Azidophenyl)BODIPY and 5'-O-(*tert*-Butyldimethyl-silyl)- 3'-O-propargyl-thymidine (49)



8-(3-Azidophenyl)-2,6-diethyl-1,3,5,7-tetramethyl-4,4-difluoro-4-bora-3a,4a-diaza-s-indacene (20 mg, 0.055 mmol), 5'-O-*tert*-butyldimethylsilyl-3'-O-propargyl-thymidine (27 mg, 0.068 mmol), sodium ascorbate (6.51 mg, 0.033 mmol) and $CuSO_4.5H_2O$ (2.7 mg, 0.011 mmol) were reacted according to the general procedure (5.3.29). After purification, 39.3 mg of red solid was isolated. Yield: 94%

R_f = 0.34 (3:1 EE/Hexane)

M.p. 212-214^{o}C (dec.)

^{1}H-NMR (400 MHz, $CDCl_3$) : δ [ppm] = 8.58 (s, 1H, NH), 8.09 (s, 1H, CH), 7.96 (ddd, J = 8.1, 2.2, 1.0 Hz, 1H, H_{Ar}), 7.79 (t, J = 1.8 Hz, 1H, H_{Ar}), 7.71 (t, J = 7.9 Hz, 1H, H_{Ar}), 7.51 (d, J = 1.2 Hz, 1H, H6), 7.46 – 7.41 (m, 1H, H_{Ar}), 6.33 (dd, J = 8.7, 5.4 Hz, 1H, H1′), 6.03 (s, 2H,

H_{core}), 4.77 (s, 2H, CH_2), 4.31 (d, J = 5.8 Hz, 1H, H3′), 4.19 (q, J = 2.3 Hz, 1H, H4′), 3.92 (dd, J = 11.3, 2.7 Hz, 1H, H5′), 3.83 (dd, J = 11.3, 2.5 Hz, 1H, H5′), 2.58 (s, 6H, CH_3), 2.54 – 2.46 (m, 1H, H2′), 2.05 – 1.98 (m, 1H, H2′), 1.93 (d, J = 1.1 Hz, 3H, CH_3), 1.47 (s, 6H, CH_3), 0.93 (s, 9H, $SiC(CH_3)_3$), 0.13 (s, 3H, $SiCH_3$), 0.12 (s, 3H, $SiCH_3$).

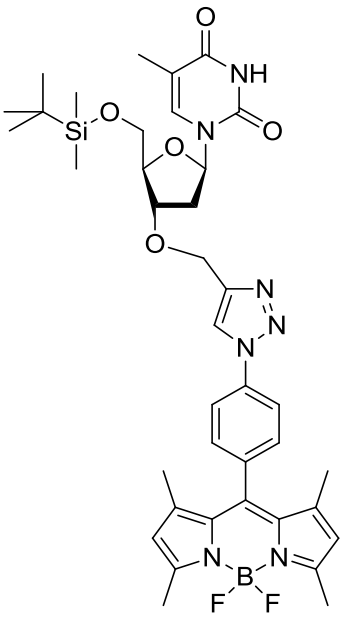
^{13}C-NMR (101 MHz, $CDCl_3$) : δ [ppm] = 163.51, 156.28, 150.19, 142.71, 137.72, 136.98, 135.19, 130.79, 128.58, 122.15, 121.63, 120.78, 120.54, 120.12, 110.95, 84.99, 84.95, 80.13, 63.68, 62.84, 37.87, 25.88, 18.30, 14.69, 14.60, 12.47, 12.29, -5.39, -5.50.

HR-MS (ESI): calc. 782.3445; found 782.3440 $[M + Na]^+$.

UV/Vis (DCM): λ_{max} [nm] = 505.

FL λ_{Exc} = 470 nm (DCM): λ_{max} = 515 nm.

5.3.29.3 Click Reaction of 8-(4-azidophenyl)BODIPY and 5'-O-(tert-Butyldimethyl-silyl)- 3'-O-propargyl-thymidine (50)



8-(4-Azidophenyl)-2,6-diethyl-1,3,5,7-tetramethyl-4,4-difluoro-4-bora-3a,4a-diaza-s-indacene (20 mg, 0.055 mmol), 5'-O-*tert*-butyldimethylsilyl-3'-O-propargyl-thymidine (27 mg, 0.068 mmol), sodium ascorbate (6.51 mg, 0.033 mmol) and $CuSO_4.5H_2O$ (2.7 mg, 0.011 mmol) were reacted according to the general procedure (5.3.29). After purification, 40.5 mg of red solid was isolated. Yield: 97%

R_f = 0.32 (3:1 EE/Hexane)

M.p. 118-120^{o}C (dec.)

^{1}H-NMR (400 MHz, $CDCl_3$) : δ [ppm] = 8.89 (s, 1H, NH), 8.15 (s, 1H, CH), 7.96 (d, J = 8.6 Hz, 2H, H_{Ar}), 7.54 – 7.48 (m, 3H, H_{Ar} and H6), 6.36 (dd, J = 8.7, 5.4 Hz, 1H, H1′), 6.03 (s, 2H, H_{core}), 4.79 (d, J = 3.3 Hz, 2H, CH_2), 4.33 (d, J = 5.8 Hz, 1H, H3′), 4.20 (dd, J = 4.4, 2.4 Hz, 1H, H4′), 3.89 – 3.84 (m, 2H, H5′), 2.58 (s, 6H, CH_3), 2.55 – 2.48 (m, 1H, H2′), 2.08 – 2.01 (m, 1H, H2′), 1.94 (d, J = 1.1 Hz, 3H, CH_3), 1.46 (s, 6H, CH_3), 0.94 (s, 9H, $SiC(CH_3)_3$), 0.14 (s, 3H, $SiCH_3$), 0.13 (s, 3H, $SiCH_3$).

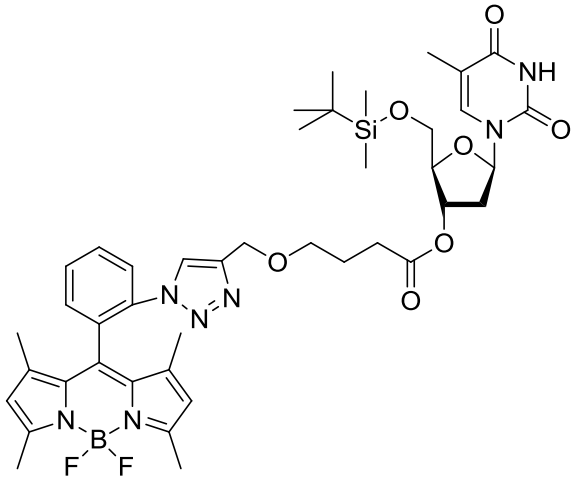
^{13}C-NMR (101 MHz, $CDCl_3$) : δ [ppm] = 163.63, 156.14, 150.34, 145.80, 142.75, 139.50, 137.39, 135.68, 135.18, 131.19, 129.85, 121.57, 120.88, 120.65, 111.01, 84.97, 84.93, 80.02, 63.68, 62.80, 37.90, 25.88, 18.31, 14.68, 14.58, 12.47, -5.38, -5.51.

HR-MS (ESI): calc. 782.3445; found 782.3440 $[M + Na]^+$.

UV/Vis (DCM): λ_{max} [nm] = 505.

FL λ_{Exc} = 470 nm (DCM): λ_{max} = 515 nm.

5.3.29.4 Click Reaction of 8-(2-Azidophenyl)BODIPY and 5'-O-(tert-Butyldimethyl-silyl)- 3'-O-(4-propargyloxybutanoyl)-thymidine (51)



8-(2-Azidophenyl)-2,6-diethyl-1,3,5,7-tetramethyl-4,4-difluoro-4-bora-3a,4a-diaza-s-indacene (15 mg, 0.041 mmol), 5'-O-(tert-butyldimethylsilyl)-3'-O-(4-propargyloxybutanoyl)-thymidine (24.7 mg, 0.051 mmol), sodium ascorbate (4.88 mg, 0.025 mmol) and $CuSO_4.5H_2O$ (2.05 mg, 0.008 mmol) were reacted according to the general procedure (5.3.29). After purification, 22 mg of red solid was isolated. Yield: 63%

R_f = 0.32 (3:1 EE/Hexane)

M.p. 75-77^{o}C

^{1}H-NMR (400 MHz, $CDCl_3$) : δ [ppm] = 8.53 (d, J = 28.1 Hz, 1H, NH), 7.90 (d, J = 7.7 Hz, 1H, CH), 7.72 (td, J = 7.8, 1.5 Hz, 1H, H_{Ar}), 7.66 (td, J = 7.6, 1.0 Hz, 1H, H_{Ar}), 7.60 – 7.56

(m, 2H, H_{Ar} and H6), 7.47 (dd, J = 7.5, 1.4 Hz, 1H, H_{Ar}), 6.35 (dd, J = 9.2, 5.3 Hz, 1H, H1′), 6.00 (s, 2H, H_{core}), 5.27 (d, J = 6.1 Hz, 1H, H3′), 4.57 (s, 2H, CH_2), 4.10 (d, J = 1.4 Hz, 1H, H4′), 3.96 – 3.88 (m, 2H, H5′), 3.37 (t, J = 6.1 Hz, 2H, CH_2), 2.53 (s, 6H, CH_3), 2.42 (dd, J = 13.8, 5.3 Hz, 1H, H2′), 2.38 (t, J = 7.4 Hz, 2H, CH_2), 2.12 (ddd, J = 13.9, 9.2, 6.2 Hz, 1H, H2′), 1.94 (d, J = 0.7 Hz, 3H, CH_3), 1.87 – 1.83 (m, 2H, CH_2), 1.49 (s, 6H, CH_3), 0.95 (s, 9H, $SiC(CH_3)_3$), 0.16 (2s, total 6H, $SiCH_3$).

^{13}C-NMR (101 MHz, $CDCl_3$) : δ [ppm] = 173.10, 163.47, 156.77, 150.15, 142.39, 135.40, 135.30, 135.10, 131.06, 130.82, 130.12, 130.07, 128.63, 126.40, 122.81, 121.95, 111.05, 85.41, 84.73, 75.22, 68.86, 63.85, 63.59, 38.71, 38.00, 31.88, 30.93, 29.65, 29.32, 25.90, 24.75, 22.65, 18.30, 14.62, 14.07, 13.96, 12.43, -5.41, -5.50.

HR-MS (ESI): calc. 868.3813; found 868.3807 $[M + Na]^+$.

UV/Vis (DCM): λ_{max} [nm] = 510.

FL λ_{Exc} = 470 nm (DCM): λ_{max} = 519 nm.

6 Appendix

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Specially, I am very thanking to my parents, I am very happy to have their love. They have always shared their great knowledge and experiences to make everything much easier. I am also very much thankful to my brother who is always beside me.

6.2 List of Symbols and Abbreviations

AcOH	Acetic acid
ALO	Arylless cyclooctyne
aq.	Aqueous
BARAC	biarylazacyclooctynone
BCN	Bicyclononyne
Bu	Butyl
cat.	Catalyst
COSY	Correlation spectroscopy
CuAAC	Copper(I)-catalyzed Azide-Alkyne Cycloaddition
d	day
DCM	Dichloromethane
DEPT	Distortionless Enhancement by Polarization Transfer
DDQ	2,3-dichloro-5,6-dicyanobenzoquinone
DIBAC	Dibenzoazacyclooctyne
DIBO	Dibenzocyclooctyne
DIFO	Difluorocyclooctyne (1st, 2nd, 3rd generation)
DIMAC	Dimethoxyazacyclooctyne
DMAP	4-Dimethylaminopyridine
DMF	*N*,*N*-Dimethylformamide
DMSO	Dimethylsulfoxide
eq	equivalent
ESI	Electrospray ionization
EtOAc	Ethylacetat
EtOH	Ethanol
GC	Gas chromatography

h	hour
H_{Ar}	Aromatic Hydrogen
H_{core}	Hydrogen at core structure
HMPA	Hexamethylphosphoramide
HOMO	highest occupied molecular orbital
HR	High resolution
Hz	Hertz
IR	Infrared
K	Kelvin
LUMO	lowest unoccupied molecular orbital
M	Molarity
MeOH	Methanol
MOFO	Monofluorinated cyclooctyne
MS	Mass spectroscopy
M_w	Molecular weight
N	Normality
NIR	Near Infraret
NMR	Nuclear Magnetic Resonance
NOFO	Nonfluorocyclooctyne
OCT	Cyclooctyne (1st generation)
PCC	Pyridinium chlorochromate
Pd/C	Palladium on carbon
PET	Photoinduced electron transfer
Ph	Phenyl
ppm	parts per million
Rf	Retention time
RT	Room temperature

T	Temperature
TBDMS-Cl	*tert*-Butyldimethylsilyl chloride
THF	Tetrahydrofuran
TLC	Thin Layer Chromatography
UV/Vis	Ultraviolet/visible spectroscopy
λ	Wavelength
λ_{Exc}	Excitation wavelength
δ	Chemical shift [ppm]
Φ	Fluorescence quantum efficiency
ε	Molar extinction coefficient

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