A. Discovery of novel reactivity under the Sonogashira reaction conditions

B. Synthesis of functionalized BODIPYs and BODIPY-sugar conjugates

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ABSTRACT

A. During our attempts to synthesize substituted enediynes, coupling reactions between terminal alkynes and 1,2-*cis*-dihaloalkenes under the Sonogashira reaction conditions failed to give the corresponding substituted enediynes. Under these conditions, terminal alkynes underwent self-trimerization or tetramerization. In an alternative approach to access substituted enediynes, treatment of alkynes with trisubstituted (*Z*)-bromoalkenyl-pinacolboronates under Sonogashira coupling conditions was found to give 1,2,4,6-tetrasubstituted benzenes instead of Sonogashira coupled product. The reaction conditions and substrate scopes for these two new reactions were investigated.

B. BODIPY core was functionalized with various functional groups such as nitromethyl, nitro, hydroxymethyl, carboxaldehyde by treating 4,4-difluoro-1,3,5,7,8-pentamethyl-2,6-diethyl-4-bora-3a,4a-diaza-*s*-indacene with copper (II) nitrate trihydrate under different conditions. Further, BODIPY derivatives with alkyne and azido functional groups were synthesized and conjugated to various glycosides by the Click reaction under the microwave conditions. One of the BODIPY–glycan conjugate was found to form liposome upon rehydration. The photochemical properties of BODIPY in these liposomes were characterized by fluorescent confocal microscopy.

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Ac	acetyl
AcOH	acetic acid
BODIPY	boron dipyrromethene
$CDCl_3$	deuterated chloroform
d	doublet
DBU	1,8-diazabicyclo [5 4 0] unde-7ene
DMF	dimethylformamide
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
EI	electron impact
ESI	electrospray ionization
EtOAc	ethyl acetate
FAB	fast atom bombardment
Glc	glucose
GlcNAc	*N*-acetyl glucosamine
h	hour
Lac	lactose
m	multiplet
M	molar concentration
MeOH	methanol
t-BuOH	tertiary butanol
min	minute
NMR	nuclear magnetic resonance

Ph	phenyl
$^i Pr_2EtN$	*N*, *N*-diisopropyl ethylamine
t	triplet
THF	terahydrofuran

Chapter 1

Enediynes as an effective DNA-cleaving motif

1.1 Enediyne-containing natural products

In the late 1980s, the discovery of new enediyne natural products generated enormous research interest in the scientific community in both academia and industry. These compounds were isolated from bacteria and proved to have powerful antibiotic and anti-cancer properties.[1,2,3] Afterwards, researchers from various disciplines have shown increased attention to its chemistry, biology and potential medicinal applications. So far nearly 40 enediyne-containing compounds have been isolated from various natural sources, with a few of them exhibiting activities a few orders of magnitude higher than the existing antitumor and antibiotics. For example, calicheamicin-γ1 and shishijimicin A are nearly 4,000 and 13,000 times, respectively, more potent than the widely known antitumor agent adriamycin. The commonly known potent antitumor and antimicrobial enediynes (**Figure 1-1**) include calicheamicin,[4] esperamicin,[1] dynemicin,[5] neocarzinostatin,[6] kedarcidin,[7] namenamicin,[8] maduropeptin,[9] N1999A2[10] and shishijimicin,[11] and the recently found uncialamycin.[12] Among these natural enediynes, calicheamicin and neocarzinostatin are well studied and approved for clinical uses. The other enediyne natural products also proved to have anticancer properties in *in vitro* studies.

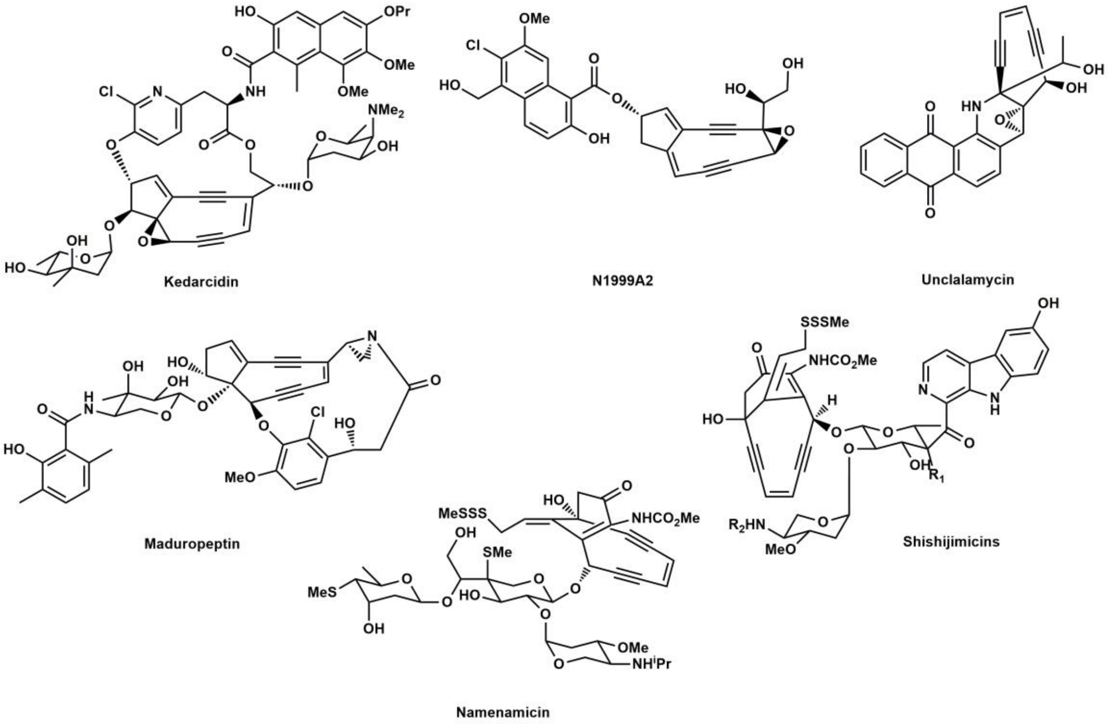


Figure 1-1: Natural enediyne compounds

These enediyne antibiotics and antitumour compounds attracted interest from researchers not only for their structural complexity, but also their remarkable biological activities. These compounds, however, do not exhibit cell specificity, which typically lead to severe toxicity as therapeutic agents. To address the specificity problem, polymer-based and antibody-conjugated derivatives of enediyne natural products have been developed.[13,14,15] For example, calicheamicin-antibody (IgG4) conjugate was approved in the US in 2000 for treating acute myloid leukemia (AML),[14] and marketed under the trade-name Mylotarg®. The antibody portion of Mylotarg binds specifically to the CD33 antigen, a sialic acid-dependent adhesion protein found on the surface of leukemic blasts and immature normal cells of myelomonocytic lineage, but not on normal hematopoietic stem cells.[16] A poly(styrene-co-maleic acid)-neocarzinostatin conjugate was also approved in 1993 in Japan and marketed to treat hepatoma.[17] These examples demonstrated the potential to improve target specificity of enediynes through appropriate drug delivery systems.

1.2 Mechanism of action of enediynes

Enediyne-containing natural products are very diverse in structure, but they also share some common structural and functional features. Three distinct structural motifs are commonly found in these compounds. One part of the structure is responsible for binding to the target such as DNA, while the second part acts as a molecular trigger to activate the molecule at the target site. These two regions are quite different in the chemical composition among enediyne natural products. The third part of the molecule is enediyne, which is responsible for their potent biological activity through a reaction called Bergman cyclization as shown in Figure 1-2. Bergman cyclization was first reported in 1972,[18] where *Z*-enediynes were activated by heating to cyclize to form a biradical intermediate, which then abstracts hydrogen atoms from a donor to generate a benzene ring.

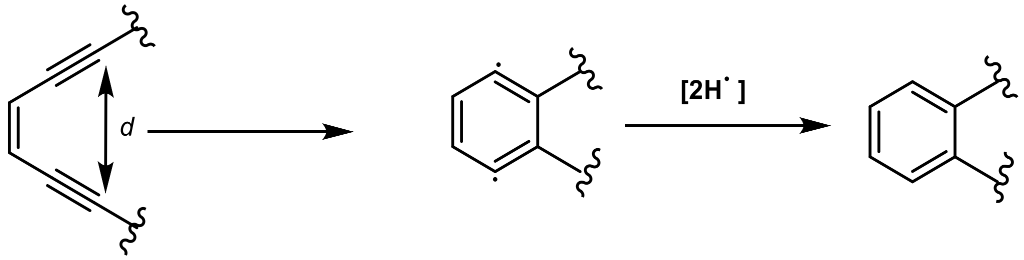


Figure 1-2: Bergman cyclization

The mode of action of natural enediyne molecules is explained below using calicheamicin as an example. Calicheamicin consists of three distinct structural regions (**Figure 1-3**). The larger of three, coloured in green, consists of four monosaccharide units joined by unusual linkages such as a thioester and a hydroxylamine glycosidic linkage.

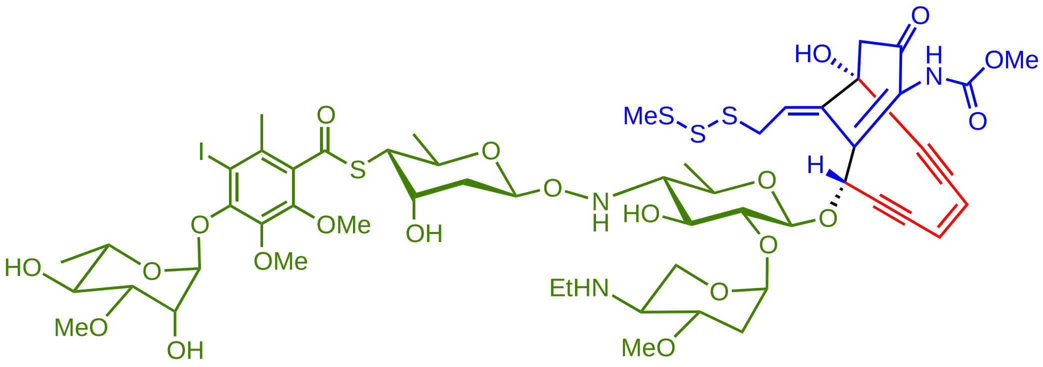


Figure 1-3: Structure of calicheamicin

The oligosaccharide motif, highlighted in green, presumably binds to the minor groove of DNA duplex in a sequence specific manner.[19,20] It was found that the carbohydrate tail in calicheamicin determines the sequence specificity for cytosine- containing oligopyrimidine tracts such as TCCT, TCCC, TCCA, ACCT, TCCG, GCCT, CTCT, and TCTC.[21] After docking the drug into the minor groove of the DNA, activation of calicheamicin occurs by the reaction of the triggering moiety and an external substance. In calicheamicin a trisulfide functionality acts as a triggering device. Thus, an attack by nucleophiles, such as glutathione, on the trisulfide activates the enediyne functionality in the molecule by a series of steps (**Figure 1-4**).[22]

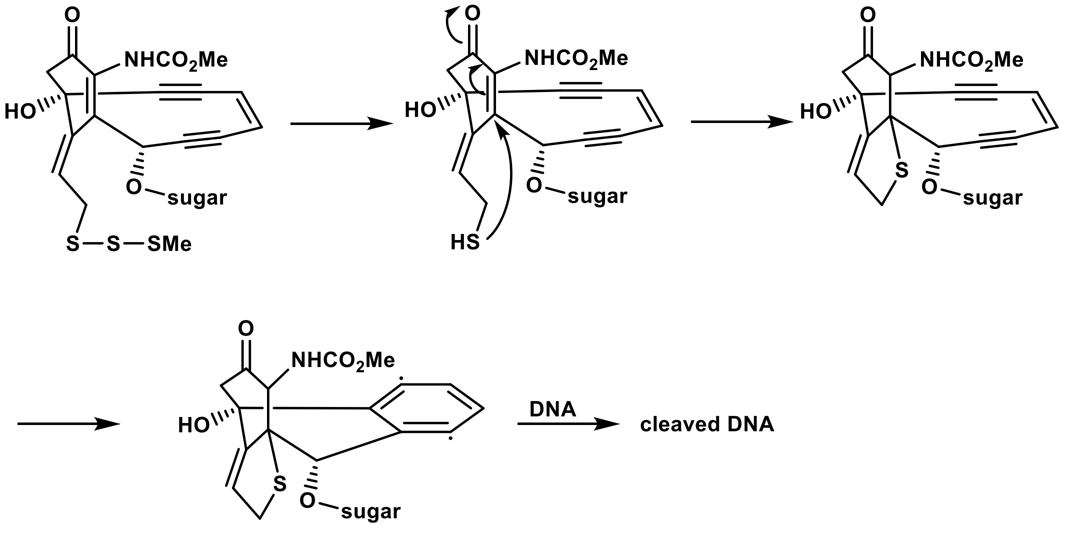


Figure 1-4: The activation and DNA cleavage mechanism of Calicheamicin.[22]

In this activation process an external nucleophile attacks the central sulfur atom of trisulfide, leading to the formation of nucleophilic thiolate or thiol, which subsequently undergoes a Micheal addition on the unsaturated carbonyl group. As a result of this reaction, the β-carbon of the unsaturated carbonyl is converted from sp^2 to sp^3 hybridization, giving rise to strain in the 10-membered ring that contains the enediyne functionality. This strain is relieved by bringing the two alkyne moieties closer, which then lowers the activation energy of the enediyne for the Bergman cyclization. Upon cyclization, a highly reactive species 1,4-benzenoid biradical is formed.[18] The newly formed benzenoid radicals are positioned in the DNA such that they can abstract two hydrogen atoms from the sugar (either at C4', C5' or C1') backbone of DNA, which ultimately leads to strand breakages (**Figure 1-5**).[23, 24, 25, 26]

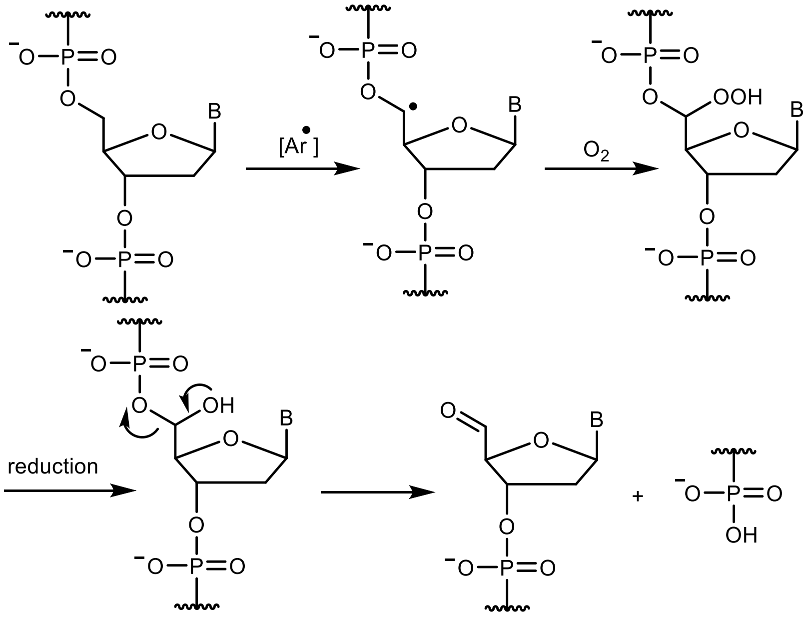


Figure 1-5: DNA cleavages by abstracting the 5'-H of sugar backbone

1.3 Synthetically prepared enediynes and their biological activities

Due to the limited availability of enediyne compounds from natural sources, chemical synthesis of enediynes has been pursued by a number of academic laboratories. To date, only a few successful total syntheses of naturally occurring enediynes have been reported, however, synthesis of a number of model systems have been accomplished that led to better understanding of the enediyne chemistry. As discussed previously, the active functional group in naturally occurring enediyne is *Z*-enediyne. As shown in a 1972 publication by Bergmann *et al.*,[18] *Z*-enediyne reacts to give benzene-related products upon heating. It was found that a highly reactive benzenoid radical species was formed during the reaction, which, upon abstraction of hydrogen atoms from a hydrogen atom source, led to the formation of benzene derivatives. Although this reaction is useful for making substituted benzene derivatives, it did not attract much interest in the synthetic chemist community due to the availability of several other methods for making substituted benzenes. Interest in the Bergman cyclization was revived, however, after the discovery of enediyne natural products where the heart of their activity mainly depends on the enediyne cyclization. The combination of experimental and computational investigations revealed the factors that affect the reactivity of enediynes.[27] In this respect, higher activities of enediynes are always attained when 1) the distance between two terminal acetylene carbons is shorter, 2) a higher order of strain is present in the enediyne, or 3) a higher concentration of trapping solvent, *i.e.* source of hydrogen atoms, is present. Several enediynes were synthesized for the better understanding of the mechanism of action of naturally occurring enediynes, and to find the substitution effects and metal complexation on the cylization temperatures. Enediynes that can be activated by various triggers were also synthesized.

K. C. Nicolaou *et al*. synthesized the first generation of enediynes **1**, which have a simple cyclic structure (**Figure 1-6**).[28] A compound with 10-membered cyclic structures (n = 2) has the ability to undergo Bergman cyclization at room temperature while the larger rings required higher temperatures. The same research group also prepared water soluble cyclic enediyne **2**, which could undergo cyclization at 37°C.[28]

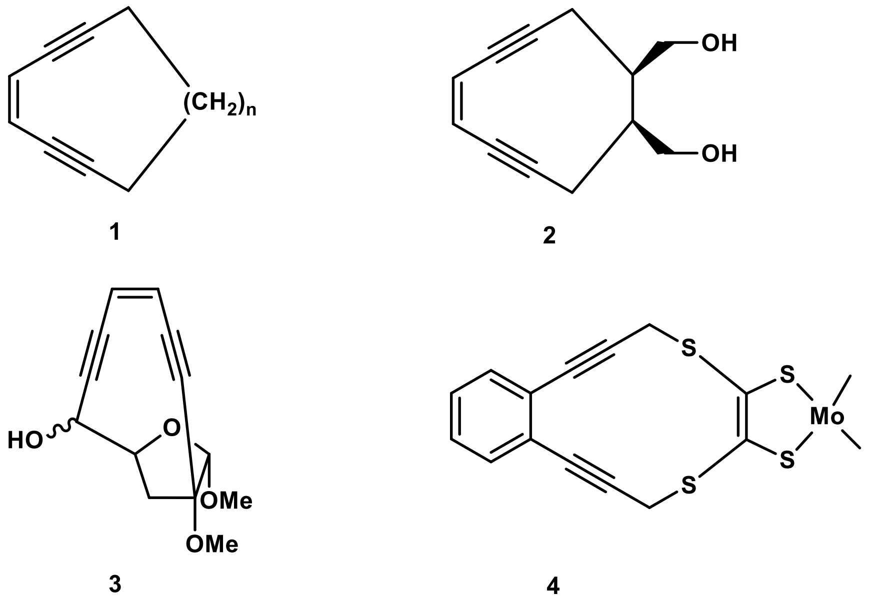
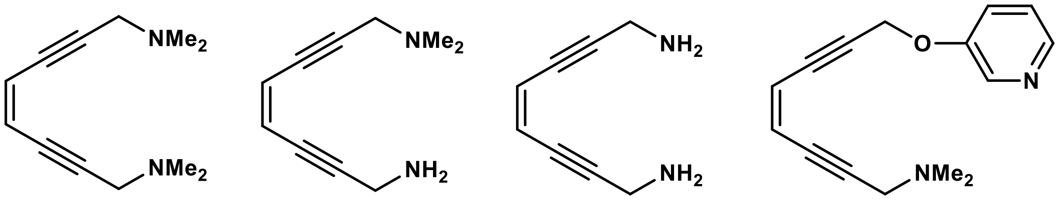


Figure 1-6: First generation of synthetic enediynes

These compounds were found to cleave DNA in *in vitro* experiments. Maier *et al*. synthesized bicycle derivative **3**, which is stable at room temperature but undergoes Bergman cyclization at elevated temperatures (80°C).[29] The stereochemistry of the isolated compound **3** was not assigned. The molybdenum complex of enediyne compound **4** prepared by Bhattacharyya *et al.* was found to undergo Bergman cyclization more readily than the free enediyne.[30] Work on these first generation enediynes motivated further design and synthesis of new enediynes. There are two broad classes of chemically synthesized enediynes: cyclic and acyclic. In addition, thermal or photo-triggers were also incorporated into these enediynes. Research on these synthetic enediynes will be discussed here. Some of these enediynes have been evaluated for their anticancer properties as well.

To identify the role of steric bulk at the terminal carbons of the acetylene bonds in enediyne in the cyclization, a series of enediyne compounds were synthesized and their cyclization temperatures were analyzed through differential scanning calorimetry (DSC). When the enediyne undergoes cyclization, the radical intermediate undergoes polymerization in the absence of a source of hydrogen atoms that can be readily abstracted. The polymerization reaction is exothermic, thus DSC can be used to monitor heat produced from Bergman cyclization at a range of temperatures.[31] A peak in DSC at a provided temperature is considered to be the starting point of cyclization or temperature required for the cyclization. A series of enediynes with amino functionality (**Figure 1-7**) at both termini of alkynes were prepared to examine the effect of steric bulk on the cyclization temperature.[32]



5 DSC:186 °C

6 DSC:139 °C

7 DSC:106 °C

8 DSC:149 °C

Figure 1-7: Enediynes with substituted amine and the corresponding cyclization temperature

Based on the DSC measurement, enediyne with dimethyl amine at both termini of alkynes (**Compound 5**) requires the highest cyclization temperature, but when the number of methyl substitutions decreases (**Compounds 6** and **7**), cyclization temperature decreases.[32] There is a nearly 80°C difference in the temperature required for Bergman cyclization between the fully methylated **5** and non-methylated enediyne

7. Another enediyne **8** with an aromatic ring was also shown to require higher temperature for cyclization to occur.[32] This study provided insights of the steric effect on Bergman cyclization. The lower onset temperature requirement of the free amine-containing enediyne was thought to be useful for designing therapeutically important metal enediyne complexes.

Enediynes-containing aromatic and hetero aromatic groups were synthesized and their biological activities were evaluated against selected cancer cell lines. Lin *et al*. reported thermally stable enediynes with aromatic substitutions at both termini of the two alkynyl groups (**Figure 1-8**).[33]

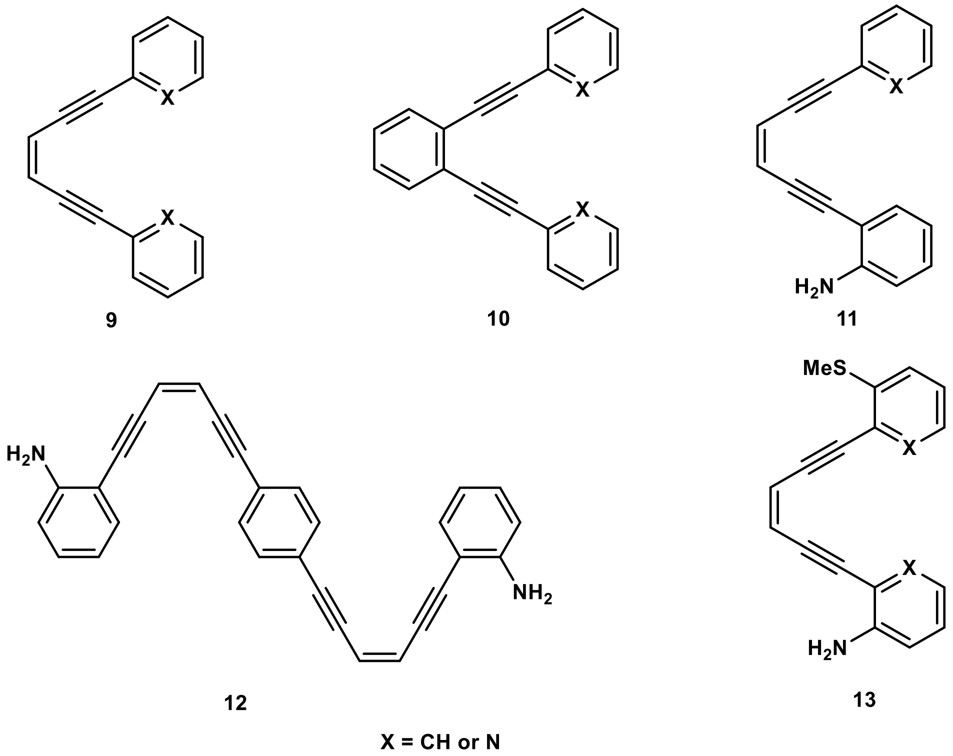
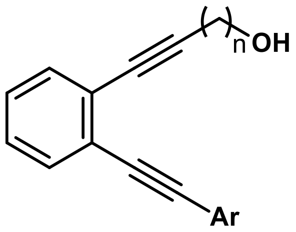


Figure 1-8: Enediynes with aromatic and hetero aromatic substitutions

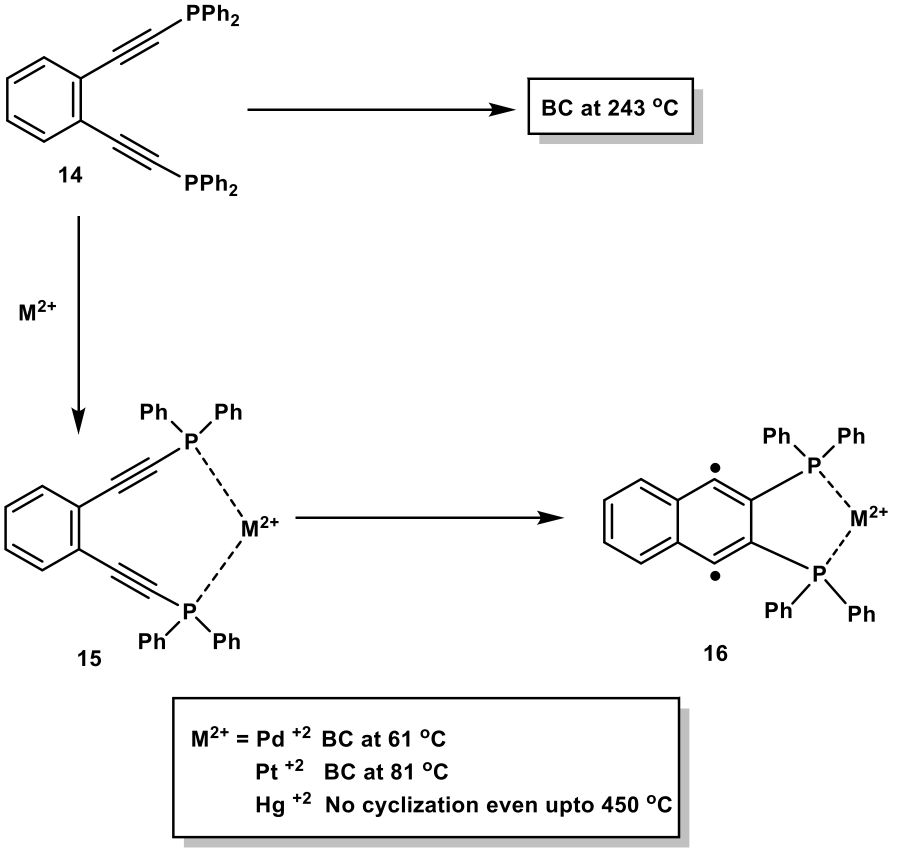
In the cell cycle analysis, all these compounds (**9-13**) showed moderate to high apoptotic induction. Compounds **9** and **10** were shown to inhibit growth on 60 cancer cell lines with average IC_{50} values ranging from 0.01 to 96.9 m*M*.[34,35] Another enediyne **13**, THDA (2-(6-(2-thieanisyl)-3(*Z*)-hexen-1,5-diynyl)aniline), was found to be active against human leukemia K562 cells. Enediynes with a terminal OH group have been prepared and shown to have good cytotoxic activity against cancer cells (**Figure 1-9**).[36] Among this group of enediynes, the compound with 4-trifluoromethyl phenyl was shown as the most potent growth inhibitor against tumor cell lines at low concentrations, and blocked the cell cycle at G2/M phase via controlling cyclin A and Cdc25C expression.[36]



n = 1,2,4, Ar = 2-MeO-C_6H_4, pyridine-2-yl, thiophen-2-yl, 2-Mes-C_6H_4, 2-CF_3-C_6H_4,3-Mes-C_6H_4, 3-HO-C_6H_4, 3-CF_3-C_6H_4, 4-OH-C_6H_4, 4-CF_3-C_6H_4, 4NC-C_6H_4

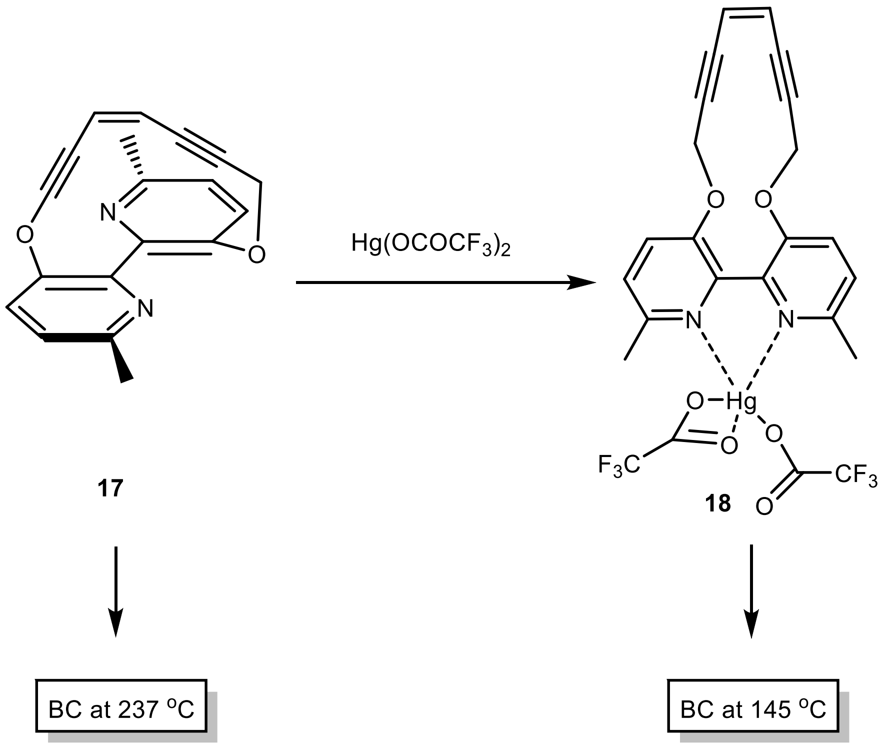
Figure 1-9: Enediynes with a hydroxyl side chain

Inspired by the robust anticancer agent cisplatin, a few metal complexes of enediynes have been prepared. One active research area in the enediynes focuses on the use of metal ions to promote the Bergmann cyclization. The enediynes designed for metal complexation should have a chelating functionality. In most cases, metal ion complexation lowers the Bergmann cyclization temperature, however, enediyne – metal complexes with higher cyclization temperatures were also observed (**Scheme 1-1**). The enediyne with phosphine groups (**14**) undergoes Bergman-cyclization at 243°C but the complexes formed with Pd^{2+} and Pt^{2+} ions (**15**) showed a dramatic decrease in the cyclization temperature to give clyclized product **16**.[37] In the case of complex formed with Hg^{2+} there was no observed cyclization, even at 450°C.[37] These experiments clearly suggest that electronic factors have an important role in the cyclization of enediynes.



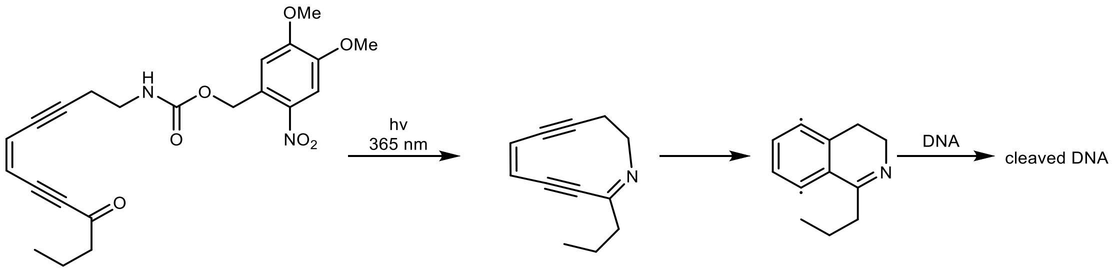
Scheme 1-1: enediynes with phosphine ligands

Bipyridyl-based macrocyclic enediyne (**Scheme 1-2**) was synthesized and their cyclization temperatures determined.[38,39] Although it was proposed that the cyclic network can reduce the cyclization temperature, the conformation of the cyclic structure is also important. In bipyridyl-based enediynes, two pyridyl moieties are in the transoid form (**17**) due to steric repulsions, but are converted to the cisoid conformation (**18**) upon metal ion complexation, and undergo cyclization at lower temperatures.



Scheme 1-2: Enendiynes with bypiridyl ligands

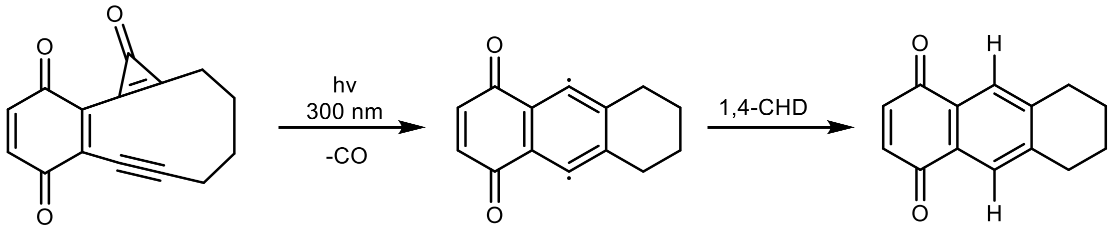
In addition to metal-ion complexation, another widely explored strategy to activate enediynes towards Bergman cyclization is photo irradiation.[40] In this strategy, an enediyne locked from cyclization is activated through exposure to a light source. For example, in compound **19 (Scheme 1-3)**, the amino group is masked with a photo labile protecting group. Upon exposure to light at 365 nm, the protecting group is removed to generate a free amine, which then reacts with the ketone to form an imine and a cyclic enediyne (**20**).[41] The cyclic enediyne, in turn, undergoes Bergman cyclization to form a biradical compound (**21**) that cleaves plasmid DNA at ambient temperatures.



19 **20** **21**

Scheme 1-3: DNA cleavages by photo enediyne activated by deprotection of a photolabile amino protecting group

Popik *et al*. reported an enediyne in which one of the acetylene bonds was masked by a cyclopropenone (**22**) (**Scheme 1-4**).[42] Irradiation of this compound led to unmasking of the acetylene bond through the release of carbon monoxide, consequently generating an enediyne which spontaneously underwent Bergman cyclization to produce aromatic radicals (**23**). These aromatic radicals abstracted hydrogen atoms from hydrogen donors, such as 1,4-cyclohexadiene (1,4-CHD), to give compound **24**.

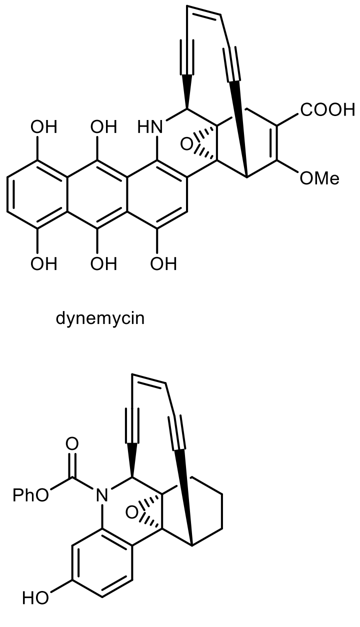


22 **23** **24**

Scheme 1-4: DNA cleavage by enediyne activated photochemically

Nicolaou *et al*. synthesized model analogs of a naturally occurring enediyne, dynemicin (**Figure 1-10, 25-28**), in order to understand the essential features of this natural product for the activation of cycloaromatization.[43] One of the proposed pathways for the activation of dynemicin suggests that donation of the lone pair electrons from the nitrogen atom leads to the opening of the epoxide ring, introducing a great deal of strain in the molecule that is then rapidly relieved by cycloaromatization of enediyne core.

This proposed pathway was verified through studies of dynemycin analogues, asserting that the electron density on the nitrogen atom indeed participates in the activation of dynemicin through the opening of the epoxide ring.



dynemycin 25 26

27 28

Figure 1-10: Analogues of dynemycin

The synthesized model compounds **26** and **27**, which are very labile, underwent cycloaromatization very rapidly at ambient temperatures due to the electron-pushing ability of nitrogen in **26** and oxygen in **27**. While compound **25** was stable under thermal conditions, it was activated under acidic conditions, as the opening of epoxide occurs under acidic conditions. The understanding of the importance of the electron density on the nitrogen in activating the enediyne core for the aromatic cyclization led to the design of a 2-(phenylsulfonyl)ethyl carbamate **28**.[44] The nitrogen-protecting group in compound **28** was shown to be easily removed under mild basic conditions and to undergo slow deprotection at physiological pH. Further, the same authors showed that compound **26** that contain a free amine can be used as a prodrug.

Compound **28** has an IC_{50} of 2.0×10^{-14} *M* against the MOLT-4 leukemia cell line, demonstrating it to be a potent *in vitro* antitumor agent.[44]

Another interesting enediyne compound conjugated with the pyrrolo[2,1-c][1,4]benzodiazepine (PBDs) was prepared.[45] PBDs are a class of naturally occurring compounds, such as anthramycin, DC-81, tomaymycin and sibiromycin, isolated from various *Streptomyces* species and proved to have potent antitumor activity.[52] PBDs are DNA minor groove binders. Mechanistic studies revealed that PBDs form irreversible covalent bond with DNA through alkylation of N-2 of guanine nucleotide as shown in **Figure 1-11**. This reaction leads to DNA lesions and inhibit gene function.

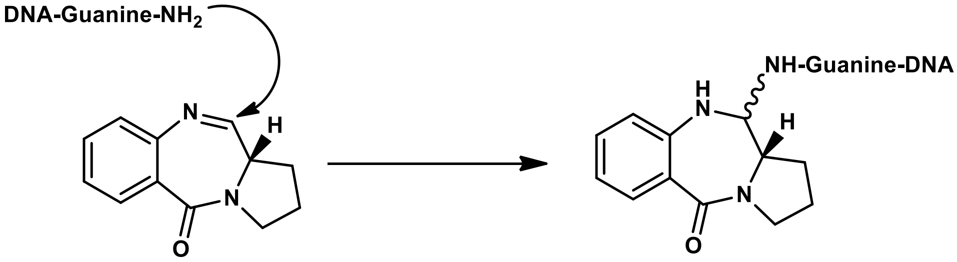
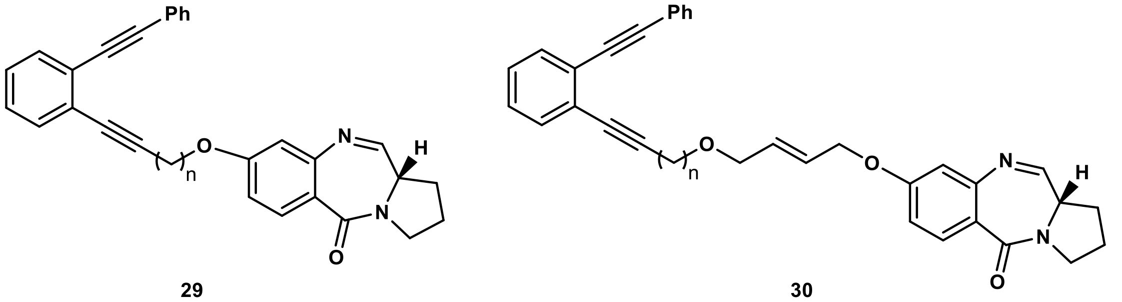


Figure 1-11: Covalent bond formation between DNA and a PBD

This ability of PBDs to bond to DNA became attractive to medicinal chemists in designing molecules that target DNA. Since enediyne molecules are known to target and cleave DNA, attachment of a DNA binding molecule to the enediyne may improve the target specificity. On this basis, a few enediynes–PDB conjugates were synthesized (**Figure 1-12**: **29** and **30**) and proved to have higher cytotoxicity than PDB against human cancer cell lines.[45]



29 30

Figure 1-12: PBDs conjugated enediynes

Another interesting note is that enediynes have been incorporated into the biologically active molecule Combretastatin A-4 (CA-4) **31**. [46] The structurally simple CA-4 was found to be cytotoxic against cancer cells.[47,48,49] The toxicity of this molecule was attained by inhibiting the tubulin polymerization. One of its water-soluble phosphate derivatives (CA-4P) **31** entered Phase-II clinical trial in 2005. Several chemical modifications have been introduced to the natural Combretastatin to modulate its activity. Provot *et al.* was inspired by the biological action of Combretastatin and synthesized Combretastatin derivatives containing enyne and enediyne in order to understand the structure–activity relationship on inhibiting the tubulin polymerization (**Figure 1-13**: **32** and **33**).[56] The tubulin polymerization was inhibited only by two of these derivatives with IC_{50} values ranging from 60–70 µM which is much lower than the activity of Combretastatin, while other compounds did not exhibit any activity. The results of this study revealed that incorporation of enyne and endiyne into combretastatin does not have any effect on improving its anticancer activity.[46]

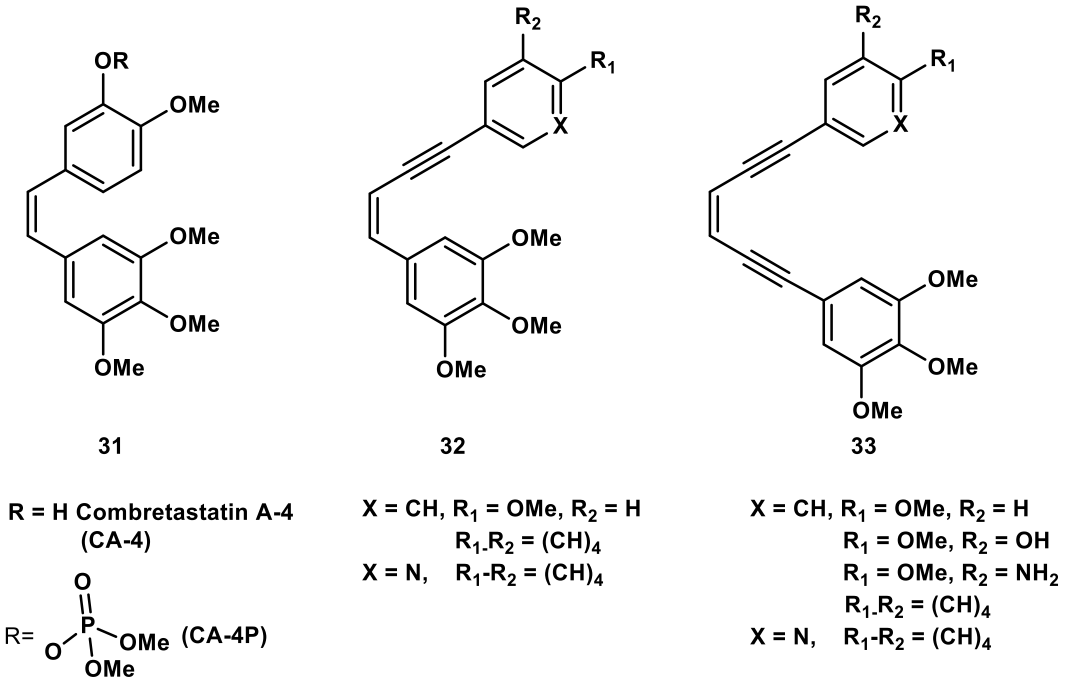


Figure 1-13: Enediynes with similar structure of Combretastatin

1.4 Potential of enediyne as site-specific DNA cutters

Although the DNA-cleaving ability of the enediyne functionality has been explored for its anticancer and antimicrobial proprieties, there is a potential to develop this functionality as DNA cutters or site-specific DNA cleaving agents, which can be very useful in DNA manipulation. Currently, DNA manipulation employs the use of restriction enzymes and ligases.[50] This method cannot be applied to site-specific manipulation of genomes, as restriction enzymes recognise 4-8 base pair sequences, site-specific cleavages of large DNAs, such as genomes, by these enzymes cannot be achieved as these cleavages typically lead to the formation of a large number of fragments. Thus, there is a need for a tool that can cleavage large DNAs in a site-specific fashion. Several chemistry-based artificial DNA cutters have been reported in the literature.[51] In these DNA cutters, a DNA-cutting molecule is combined with the sequence-recognising molecule such as the oligonucleotide sequence. Since the scission site in the target DNA strand is recognised by the DNA cutters through Watson-Crick base pairs, site-specific cleavage within a given region in a long DNA sequence can be accomplished. DNA cutters cleave target DNA, single stranded or double stranded, either by oxidative or hydrolytic cleavage of the phosphodiester backbone. Chu and Orgel covalently attached EDTA to a 16-mer deoxyoligonucleotide sequence at the 5'-terminus through an ethylene diamine linker (**Figure 1-14**).[52] This sequence acted as a hybridization-dependent DNA cutter, cleaving a 37-mer single stranded target DNA in the presence of Fe^{2+}, dithiothreitol, and oxygen. The Fe^{2+} complex of EDTA acts as a radical initiator responsible for the oxidative cleavage of target DNA. The cleavage site in the target 37-mer strand has approximately four residues on each side of the terminal phosphate of the 16-mer DNA cutter.

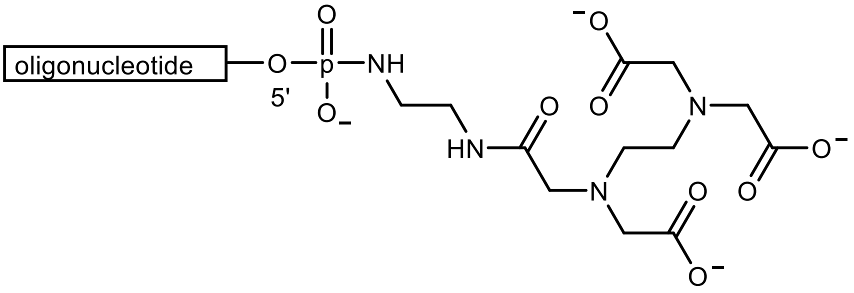


Figure 1-14: EDTA conjugated oligo nucleotide

Dervan *et al.* reported a single stranded DNA-cutter in which a 19-mer deoxyolionucleotide containing a metal chelator EDTA covalently attached to the thymidine residue at the 10th position of the 19-mer (**Figure 1-15**).[53] This 19-mer DNA cutter was able to specifically cleave the 167-nt target site in the presence of Fe^{2+}, dithiothreitol, and oxygen. The cleavage site in the target strand is within a range of 16-nt at the site of hybridization.

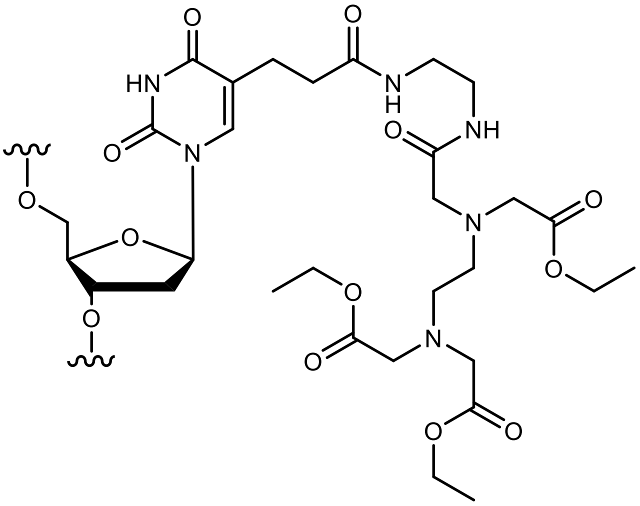


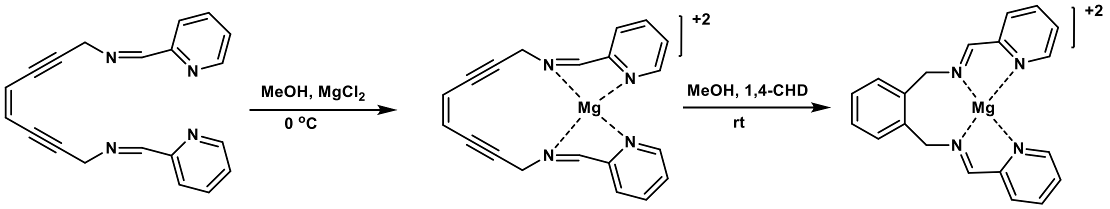
Figure 1-15: EDTA containing thymidine

Unlike single-stranded DNA-cutters, double-stranded DNA-cutters need to be constructed with specific types of recognition elements such as triple-helix-forming oligonucleotides, and minor groove binders that recognize the double strand.[54,55] For example, the oligonucleotide of a homopyrimidine sequence can be used as a recognition element in cutters for double stranded DNA. This sequence recognizes the homopurine/homopyrimidine sequence in the target double-stranded DNA by forming a triple helix structure. Recognition element homopyrimidine sequence attached to either the Fe^{2+}/EDTA complex[56] or Cu^{2+}/phenanthroline complex[57] have been reported to perform site-specific cleavage of double stranded DNA through oxidative reaction.

1.5 General perspectives and objectives of this project

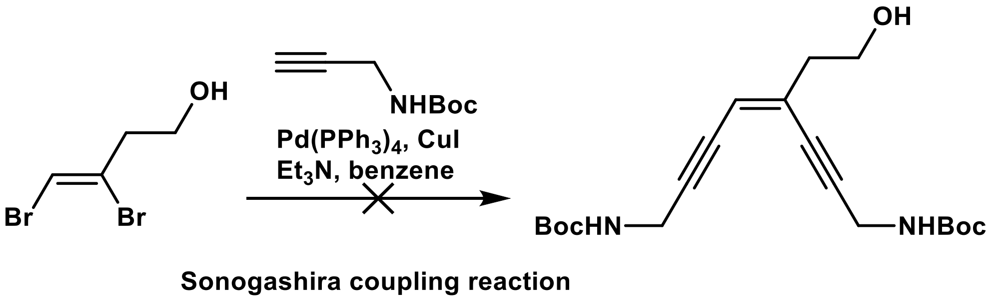
1.5.1 Synthesis and conjugation of enediyne functionality to oligonucleotides

As discussed above, the enediyne functionality is known to be the pharmacophore in a number of natural products that exhibit anti-cancer and antimicrobial properties. Our objectives for this project are to attach the enediyne functionality to an oligonucleotide and investigate its ability to cleave DNA in a site-specific fashion. As an enediyne activating functionality, a Schiff base will be introduced in the designed enediyne, which activates the enediyne for cyclization upon binding to metal cations. It was already reported that an enediyne with Schiff base attached at the terminal carbons of acetylene bonds would undergo Bergman cyclization at room temperature in the presence of magnesium chloride (**Scheme 1-5**).[58] Enendiyne **34** containing Schiff bases first form a complex **35** with magnesium ions, lowering the activation energy for the cyclization. This enediyne-magnesium complex then undergoes Bergman cyclization at room temperature, and in the presence of a hydrogen donor, gives aromatized product **36**.



Scheme 1-5: Enendiyne cyclization at room temperature in the presence of Mg^{2+} ions

A synthetic scheme was designed to synthesize the desired enediyne, which involves Sonogashira coupling reaction as a key step as shown in **Scheme 1-6**.[59]



Scheme 1-6: Key synthetic step for the preparation of desired enediyne

Many attempts of this Sonogashira coupling reaction were unsuccessful in our hands, but careful analysis of the products formed during this reaction led to the discovery of a reaction where alkynes, one of the substrates of the Sonogashira reaction, undergo self oligomerization (dimerizarion, trimerization and tetramerization).

1.5.2 Investigation of the self-oligomerization of terminal alkyne

The reaction discovered while attempting the Sonogashira coupling reaction has applications for the synthesis of branched enediynes. Our objectives for this newly discovered reaction are

i. To investigate the reaction components responsible for this reaction

ii. To optimize reaction conditions for the trimerized products

iii. To investigate its substrate scope.

1.5.3 Reaction between (*Z*)-bromoalkenylpinacol boronates and terminal alkyne

Since attempts of Sonogashira coupling reaction failed to generate the desired enediyne, another substrate, trisubstituted (*Z*)-bromoalkenylpinacol boronate, was prepared for sequential Sonogashira and Suzuki couplings. Surprisingly, attempts towards the sequential Sonogashira and Suzuki coupling reactions with these substrates led to the discovery of another interesting reaction, that is, a reaction between (*Z*)-bromoalkenylpinacol boronates and alkynes to give an aromatized tetrasubstituted benzene. The objectives of this discovered reaction are

i. To investigate the reaction components responsible for this reaction

ii. To optimize reaction conditions for the aromatized products

iii. To investigate the substrate scope

iv. To investigate the reaction mechanism.

Chapter 2

BODIPY as a versatile fluorophore

2.1 Introduction to fluorophores

Fluorescence is a molecular property that allows molecules to emit light at lower frequencies upon exposure to higher frequencies of light.[60] Fluorescence has a wide variety of applications in various research fields, such as molecular biology, biochemistry, medicine, and clinical diagnostics.[61] The attachment of a fluorophore to a given biomolecule of interest, such as proteins and nucleic acids, can be used to image the accumulation of the molecule in specific organs of animals and human subjects.[62] Imaging of peptides or proteins can be performed by expressing fluorescent proteins (FP), which is achieved through the introduction of a gene encoding the fluorescent protein into the vectors.[63,64,65,66,67] However, using FP is burdened by a major disadvantage: the larger size of the introduced FPs may interfere with the intrinsic function of target proteins. In this arena, small fluorescent molecules are considered as potential candidates for imaging processes.

A large number of small molecule fluorescent probes have been employed to investigate functions of biomolecules in the interior and exterior of cells. Small molecule fluorescent probes have certain advantages over large FPs, such as ease of functionalization, and easy tuning of emitting wavelength and intensity, which can be accomplished via chemical modifications.[68] In addition, fluorescent molecules can be tuned to emit fluorescence in near infrared (NIR) regions, an alternative that can be used to avoid background fluorescence stemming from amino acids and other luminescent biomolecules.[68] In addition to these advantages, temporal resolution can be attained by conjugating a fluorophore to the target of interest through application of a bio-orthogonal conjugation chemistry method.[69,70] Considering the many applications and advantages of small molecule fluorophores, it is not surprising that there are many reports available on the literature concerning these compounds. Some of the most widely used small molecule fluorophores include coumarin, fluorescein, BODIPY (4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene), rhodamine, and cyanine dyes (**Figure 2-1**).[71,72]

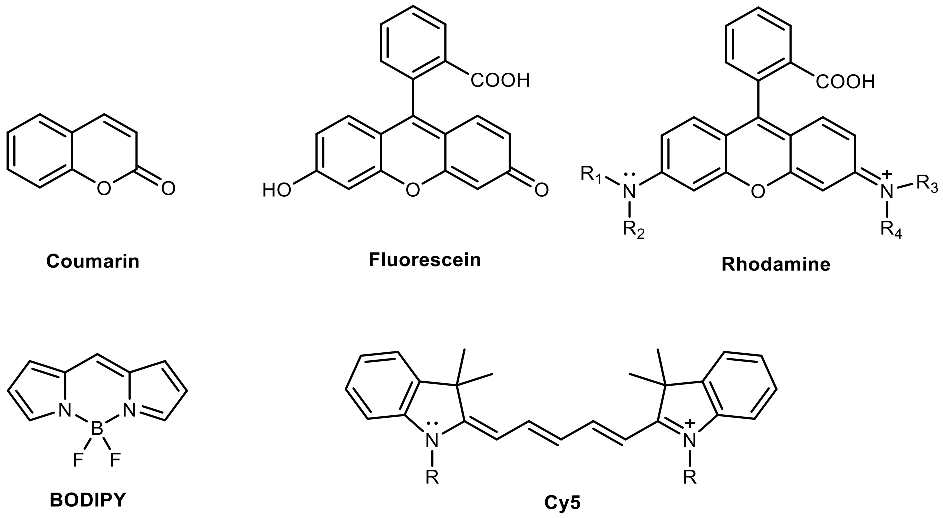


Figure 2-1: Structures of fluorescent dyes

In live-cell imaging applications, negatively charged fluorophores, such as fluorescein, are generally not considered as good candidates, as they lack the necessary cell membrane permeability needed to enter the cell. For such applications, cationic dyes such as rhodamine are generally considered as a better choice, as they generally possess good cell membrane permeability.[73] However, the cationic and hydrophobic nature of rhodamine makes the compound prone to accumulation in the mitochondria or bind non-specially to proteins and lipids.[73] Cyanine dyes, on the other hand, can be very useful in tissue imaging applications, as these molecules absorb NIR light, which can penetrate deeper into tissues without causing significant damage to the organism under study.[74] In addition, the use of NIR fluorescence can also prevent the occurrence of background fluorescent interference related to tissue auto-fluorescence.[74] However, while cyanine dyes contain good photo-physical properties, these molecules are characterized by lower photo-stability in comparison to rhodamine. As all fluorophores are characterized by their own application-specific advantages and disadvantages, the development of new fluorophores is still a growing field of study. Within this context, BODIPY is considered a very useful skeleton for development of new fluorophores, as it possesses a number of advantages over other fluorescent molecules.

2.2 BODIPY as a fluorophore

BODIPY fluorophores are a class of molecules often applied for various imaging applications; as such, a considerable amount of academic attention has been given to the development of BODIPY-based fluorescent probes. The structure of the BODIPY core is shown in **Figure 2-2**. The IUPAC name of BODIPY is 4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene, a nomenclature similar to the all-carbon tricyclic *s*-indacene ring system. Rules for numbering any substituents on the BODIPY core structure are the same as for the carbon polycyclic structure.[75]

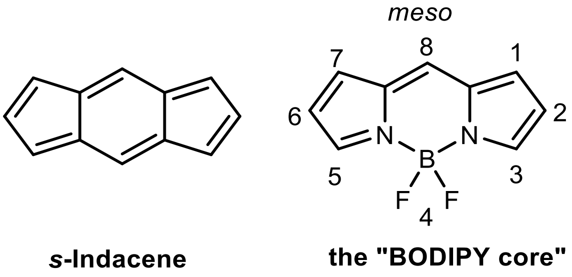


Figure 2-2: Numbering scheme for BODIPY framework derived from indacene

BODIPY-based fluorophores exhibit extraordinary photophysical properties, such as high molar extinction coefficients, high fluorescent quantum yields, and sharp excitation and emission peaks. BODIPYs are also relatively stable thermally, chemically, and photo-chemically, and are relatively insensitive to solvent polarity and pH. Due to these intriguing photophysical and photochemical properties, within the last two decades, tremendous research interest has been given to the creation of BODIPY-based fluorophores and BODIPY derivatives for a variety of applications such as laser dyes,[76] fluorescent indicators, photodynamic therapy,[77] and labeling of biomolecules.[78] Today, several BODIPY probes are commercially available, such as ER tracker Green and Lyso Tracker Red (**Figure 2-3**).

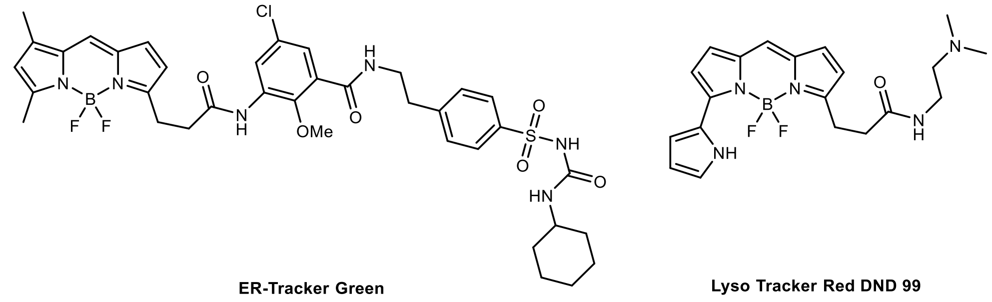
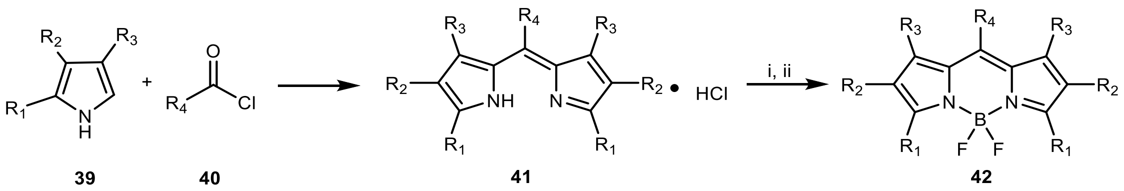


Figure 2-3: Commercially available BODIPY probes from Molecular Probes.

2.3 Methodologies for the synthesis of BODIPY

Due to its superior properties, the BODIPY skeleton has increasingly garnered the interest of synthetic chemists, leading to the development of several synthetic methods for the preparation of numerous BODIPY derivatives. Today, the majority of BODIPY compounds reported in the literature are fully substituted, mostly due to the instability of dipyrromethene, the precursor for unsubstituted BODIPY. A fully unsubstituted BODIPY core was first reported in 2009 by three independent groups.[79,80,81]

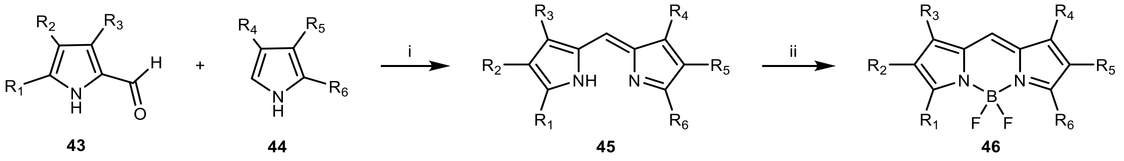
The most commonly used method for the synthesis of BODIPY derivatives involves the treatment of acid chlorides (**40**) with pyrroles (**39**), yielding the unstable dipyrromethene hydrochloride salt (**41**), which further complexes with $BF_3{\bullet}OEt_2$ in the presence of a base such as Hünig’s base or 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) to give the desired BODIPY (**42**) (**Scheme 2-1**).[82, 83]



Scheme 2-1: *Reagents and conditions*: i) NEt_3, *i*-Pr_2EtN or DBU; (ii) $BF_3{\cdot}Et_2O$, toluene or CH_2Cl_2.

This method is suited for the synthesis of symmetrical BODIPYs. Using the same method, other electrophiles such as acid anhydrides and aldehydes have also been used as alternatives to the acid chlorides.[84] Although isolation of unstable intermediates such as dipyrromethene hydrochlorides is quite difficult, the stability of this intermediate can be improved by increasing the number of C-substituents. Nevertheless, isolation of these intermediates is not necessary, and the intermediates can be treated with borontrifluoride dietherate directly for the boron complexation step.

Synthesis of unsymmetrically-substituted BODIPYs should be followed by a different approach, in which condensation of carbonyl pyrrole (**43**) is treated with another pyrrole (**44**) having an unsubstituted α-position to give the corresponding dipyrromethene **45**, which in turn is further treated with $BF_3{\cdot}OEt_2$ in the presence of a base to give the desired BODIPY (**46**) (**Scheme 2-2**).[85]



Scheme 2-2: *Reagents and conditions*: i) H^+, CH_2Cl_2; ii) NEt_3, $BF_3{\cdot}Et_2O$

While BODIPYs have certain advantages, they are also associated with a few drawbacks that limit their applications, such as narrow Stoke's shifts, poor water solubility, and lack of functional groups for conjugation.[86,87,88,89] While these issues may be addressed with functionalized BODIPYs, major challenges exist to access those functionalized BODIPYs, especially due to the limited availability of functionalized pyrroles. As such, the introduction of functional groups into the BODIPY core is an attractive way to access functional BODIPYs. The structural features of the BODIPY core, such as the positions of the substituent, π-electron conjugation, and structural rigidity, largely influence the fluorescent properties of the molecule. An increase in the π-electron conjugation of the BODIPY core leads to enhanced fluorescence emission, red-shifts in excitation and emission wavelengths. Increasing structural rigidity or steric constrains can aid in improving the quantum yields of the BODIPY compound, as smaller entropic losses are observed from the absorbed light energy.

2.4 Functionalization of the BODIPY core

2.4.1 Electrophilic substitution reactions

Several functional groups have been introduced into the BODIPY core through electrophilic substitution reactions. Considering the mesmeric structures of the BODIPY core, the 2- and 6-positions possess the least positive charges, thus more prone to electrophilic attack (**Figure 2-4**). However, no definitive studies have been undertaken regarding the regioselectivity of these electrophilic substitution reactions, owing to the instability of BODIPY precursors.

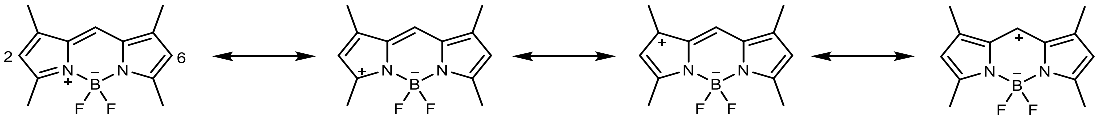
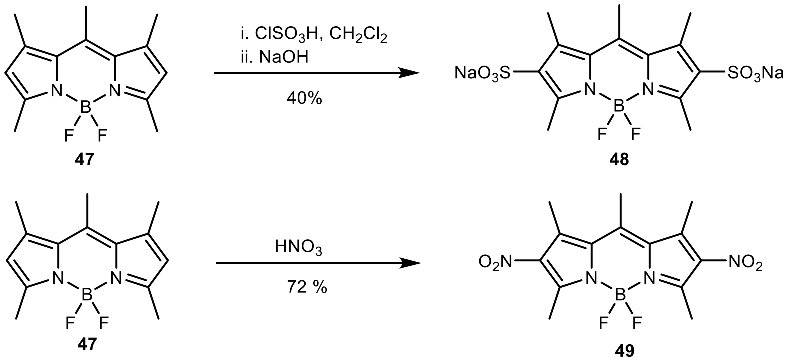


Figure 2-4: Mesomeric structures of BODIPY core

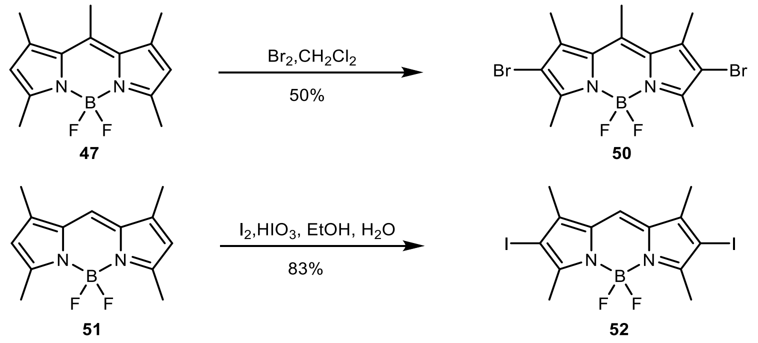
Electrophilic substitution reactions have been reported with the BODIPY core including sulfonation, nitration and halogenation. Sulfonation of pentamethyl BODIPY

(**47**) was performed by treatment with chlorosulfonic acid, followed by neutralization with a base (**Scheme 2-3**).[82] This sulfonated BODIPY dye (**48**) was shown to exhibit strong fluorescence in water or MeOH, and is more stable than parent BODIPYs. Nitration of BODIPY was performed with nitric acid; in this work, the introduction of nitro groups was observed to quench the fluorescence of BODIPY (**47**), and drastically reduce quantum yields (Quantum yield of 0.73 for **47**, 0.31 for **49**).[90]



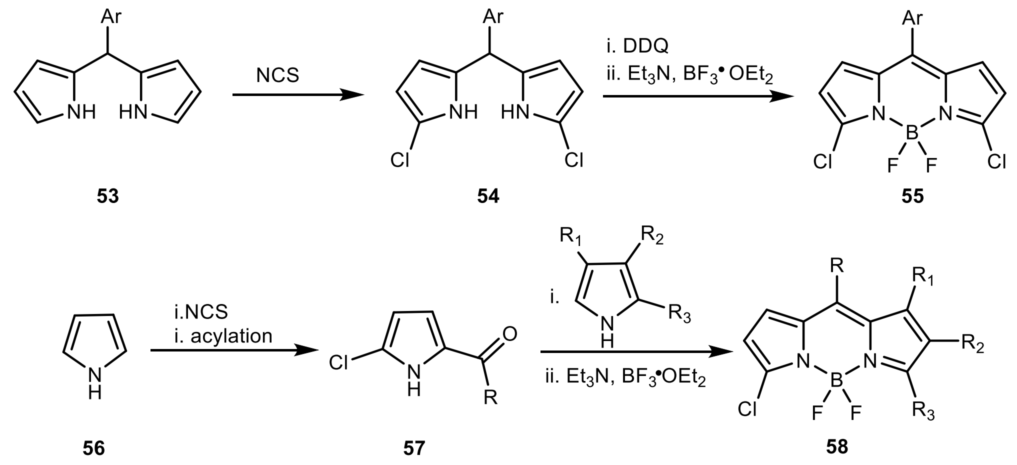
Scheme 2-3: Sulfonation and nitration of BODIPY

Electrophilic bromination of the penta-substituted BODIPY (**47**) was performed with bromine (**Scheme 2-4**). The brominated compound (**50**) was shown to exhibit a red shift in the emission spectra, as well as lower quantum yields due to the heavy atom effect.[91] An iodo-BODIPY was obtained by treating BODIPY (**51**) with iodine in the presence of HIO_3. The resulting iodo-BODIPY (**52**) was shown to be highly resistant to photo bleaching and was found to be an efficient photosensitizer.[92]



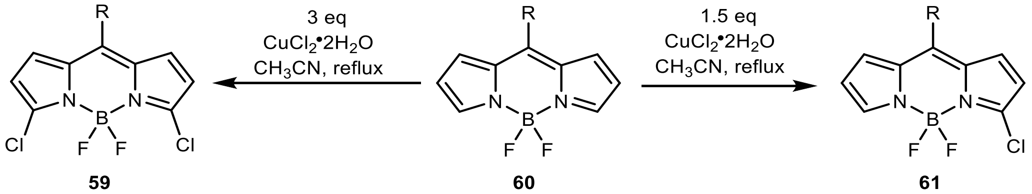
Scheme 2-4: Halogenation of BODIPY

Halogenated BODIPYs are of particular interest, as these compounds allow for the introduction of a variety of functionalities onto the BODIPY core.[93] Halogenated BODIPYs are also versatile precursors for several palladium-based cross-coupling reactions such as Stille, Negishi, Heck, Suzuki, and Sonogashira. Fine tuning of the optical properties of BODIPY can be achieved by introducing functional groups with the aid of nucleophilic substitutions on halogenated BODIPYs. A 2,6-halogenated BODIPY core can be obtained with the use of various reagents, such as *N*-chlorosuccinimide (NCS), *N*-bromosuccinimide (NBS), *N*-iodosuccinimide (NIS), Br_2, I_2 through electrophilic substitution reactions.[94,95] In comparison to the other positions, such as 3, 5, 7, 8, halogenations at the 2, 6 positions can be easily accomplished due to the least positive charges present in the latter positions (**Figure 2-3**). BODIPYs halogenated at 3, 5, 7, 8 can be obtained from halogenated dipyrromethane core or halogenated pyrroles. For example Dehaen and Boens developed a method to obtain 3- and/or 5-chloro BODIPYs (**55**, **58**) either by the treatment of dipyrromethane core (**53**) with NCS to give the chlorinated dipyrromethane (**54**) and followed by oxidation and boron complexation, or by taking the chlorinated pyrrole precursors (**57**) which were obtained by the chlorination of pyrrole (**56**) with NCS (**Scheme 2-5**).[96,97,98,99]



Scheme 2-5: Synthesis of 3, 5-chloro substituted BODIPYs

Recently, Zhou *et al.* reported regioselective halogenation by treatment of *meso*-aryl, *meso*-H, and *meso*-alkyl substituted pyrroles (**60**) with copper halides (**Scheme 2-6**). The number of substitutions (mono- or di-) was controlled by the equivalents of the copper halide reagent.[100] While the treatment of BODIPY with $CuCl_2$ gives the 3, 5-chloro substitutions (**59, 61**), treatment of BODIPY with $CuBr_2$ gives the 2, 6-bromo substitutions. This difference in the regioselectivity of the halogenation with different types of copper halides is attributed to the different mechanistic pathways of the halogenation process.[100]

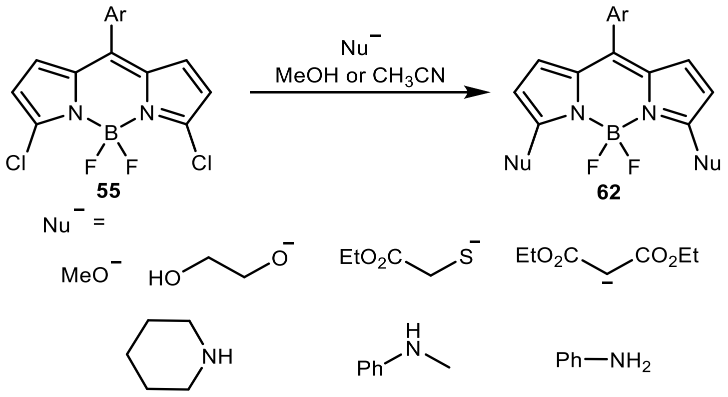


Scheme 2-6: 3, 5 selective chlorination of BODPY with $CuCl_2$

Mechanistic investigations performed to understand the chlorination reaction revealed that chlorination of BODIPY likely occurs through a cation radical pathway in the single-electron transfer (SET) process followed by a nucleophilic addition by the chloride anion. The bromination reaction, on the other hand, is believed to operate through the generation of molecular bromine from the oxidation of bromide anion, leading to the electrophilic substitution reaction to give brominated BODIPY derivatives. Attempts for chlorination of BODPY with other metal chlorides such as includes $ZnCl_2$, $MgCl_2$, $FeCl_3$, $AlCl_3$, and $TiCl_4$ did not lead to the formation of any chlorinated BODIPY. [100]

2.4.2 Nucleophilic substitutions of halogenated BODIPYs

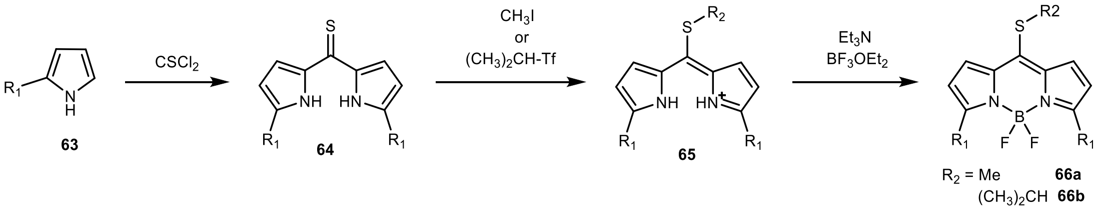
Halogenated BODIPYs (**55**) have been used for further functionalization through nucleophilic substitution reactions by nucleophiles such as alkoxides, thioalkoxides, amines (piperidine, aniline, *N*-methyl aniline), and diethylmalonate (O, N, S, and C centered nucleophiles) (**Scheme 2-7**).[97,98]



Scheme 2-7: Nucleophilic substitution of 3 and 5-chlorinated BODIPY

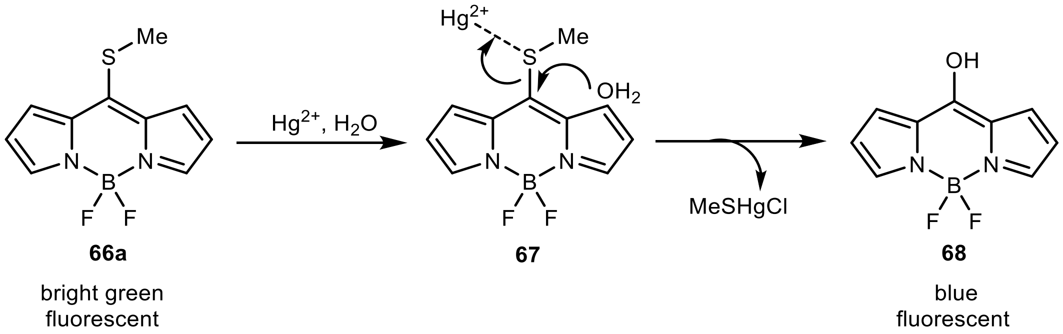
These nucleophilic substitution reactions can be used to obtain either mono- or di-substituted BODIPYs (**62**) by altering the reaction conditions. While stirring the dihalo BODIPY with two equivalents of nucleophile at room temperature gives the monosubstituted product, treatment of dihalo BODIPY with four equivalents of nucleophile under reflux in acetonitrile provided the di-substituted product. Two different nucleophiles can be introduced one after another using similar conditions. Nucleophilic substitutions at the 3, 5 positions can effectively alter the absorption and emissions spectra of parent dihalo-BODIPY (**55**). For example, amino- and thio-substituted BODIPYs have been shown to exhibit a red shift in both absorption and emission spectra. One of the BODIPY derivatives synthesized by sequential nucleophilic substitution with methoxide and azacrown ether act as a potassium selective ratiometric fluorescent indicator.[98]

Nucleophilic substitution reactions have also been explored at the *meso*-position of BODIPY. Biellmann *et al.* reported BODIPY derivatives with sulfur- containing leaving group at the *meso*-position for the nucleophilic substitution reactions (**Scheme 2-8**).[101] The synthesis of these sulfur-containing BODIPYs was performed by the treatment of pyrroles (**63**) with thiophosgene to provide the thioketone (**64**) which was then treated with electrophilic reagents such as methyl iodide or isopropyl triflate to give the corresponding dipyrromethene (**65**). Subsequent complexation of dipyrromethene (**65**) with $BF_3 \cdot OEt_2$ in the presence of Et_3N afforded the final thio-containing BODIPY derivative (**66**).



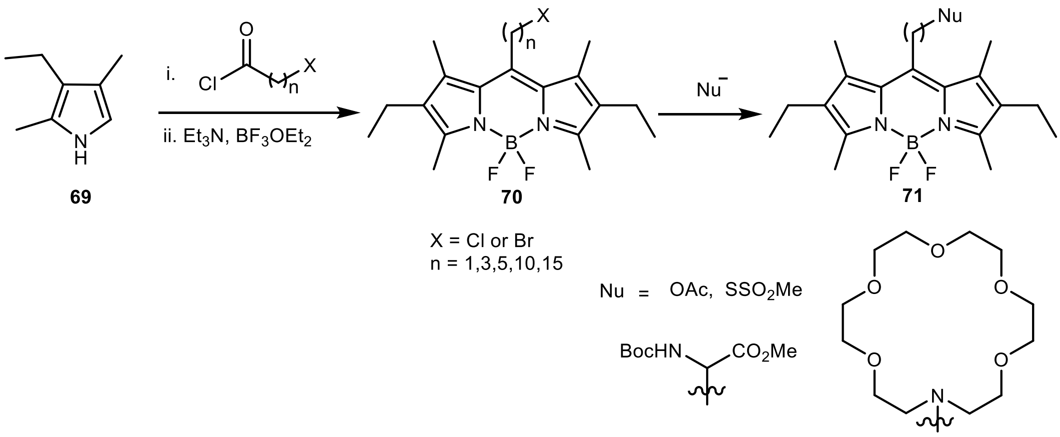
Scheme 2-8: Synthesis of 8-alkylthio-BODIPY

These thio BODIPY derivatives were treated with aniline for the nucleophilic substitution reaction to give 8-arylamino-BODIPY compounds.[101] In an interesting study 8-methylthio-BODIPY (**66a**) was shown to have ratiometric fluorescent behavior towards mercury ions. Thus, fluorescence emission from a solution of 8-methylthio-BODIPY (**66a**) changed from bright green to blue upon addition of $HgCl_2$. This fluorescent shift was explained by the Hg^{2+} promoted displacement of methylthio with water to convert the 8-methylthio- to 8-hydroxy-BODIPY (**68**) through an intermediate compound **67**, which will leads to the changes in both the absorption and emission wavelengths of the 8-methylthio BODIPY (**Scheme 2-9**).[102]



Scheme 2-9: Mechanism of mercury ion detection by 8-methylthio-BODIPY

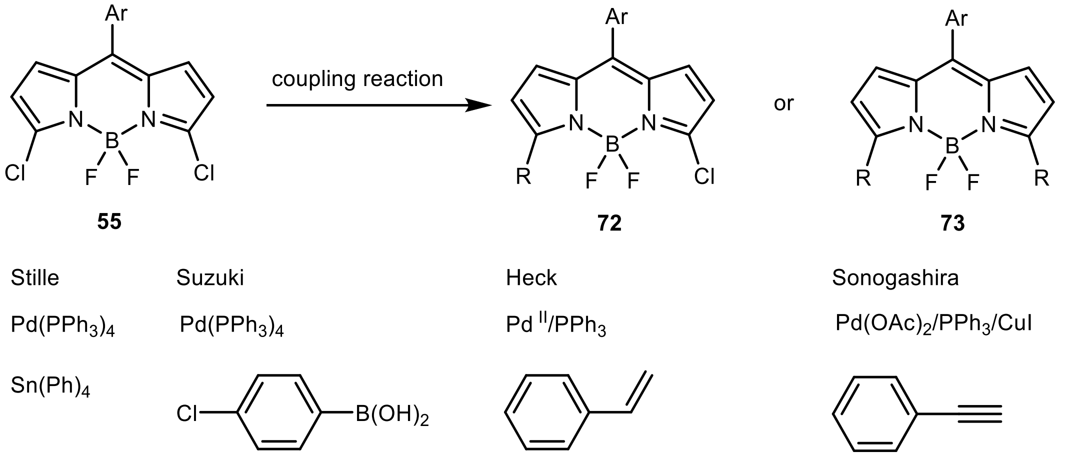
Nucleophilic substitutions have also been performed on the halogenated side chain of the BODIPY (**70**) at the *meso*-position (**Scheme 2-10**).[103,104] Thus, halogenated BODIPYs (**70**), which can be readily prepared by the treatment of pyrrole (**69**) with halogenated acyl chloride, have been treated with several nucleophiles to give various substituted BODIPYs (**71**). Some of the most notable compounds made in this approach include azacrown ether conjugated BODIPY compounds, which are known to act as metal ion sensors by the PET mechanism.[103]



Scheme 2-10: Nucleophilic substitutions on the halogenated side chain at the *meso*-position of BODIPY

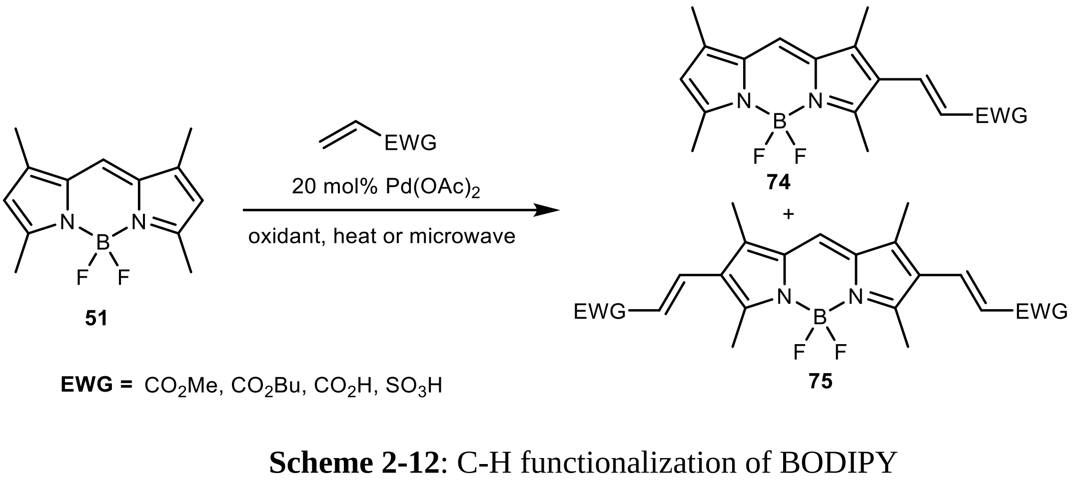
2.4.3 Palladium catalyzed functionalization of BODIPY compounds

Halogenated BODIPY derivatives (**55**) have been successfully used as precursors for palladium catalyzed coupling reactions, allowing access to functionalized BODIPY compounds.[105] This approach opens up new pathways towards the derivatization of the BODIPY core with various functional groups such as aryl, ethenylaryl, and ethynylaryl through the use of various coupling reactions such as Stille, Suzuki, Heck, and Sonogashira couplings (**Scheme 2-11**). BODIPY compounds **72** and **73** derived by these coupling reactions are characterized by extended conjugations, and contain a range of absorption and emissions wavelengths. Mono-substituted BODIPYs can be further derivatized into unsymmetrical BODIPYs either by nucleophilic substitutions or by another palladium coupling reaction.[105]



Scheme 2-11: Coupling reaction of halogenated BODIPYs

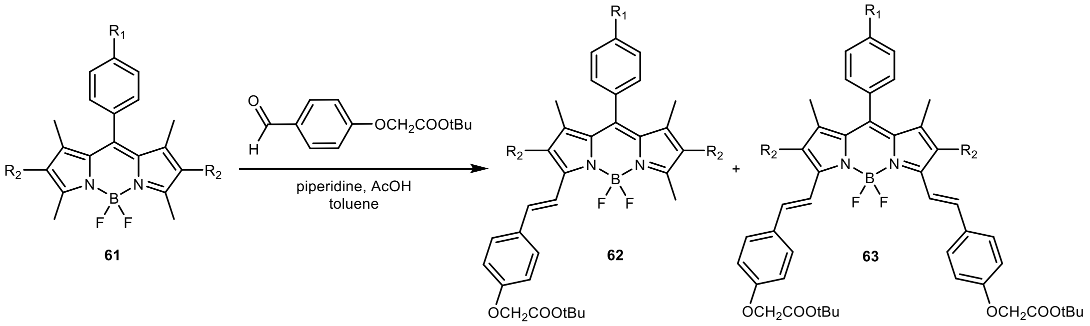
Palladium-catalyzed coupling reactions have also been used for the C-H functionalization of the BODIPY core at the 2, 6 positions (**Scheme 2-12**). This method allows for direct access to functionalized BODIPY derivatives, without the need for use of halogenated BODIPY precursors.[106] The BODIPY compound **51** was derivatized with unsaturated esters, acids, sulfonic acids by a palladium catalyzed oxidative coupling to give either mono- or di-substituted BODIPY derivatives **74** and **75**, respectively.[106]



Scheme 2-12: C-H functionalization of BODIPY

2.4.4 Condensation reactions

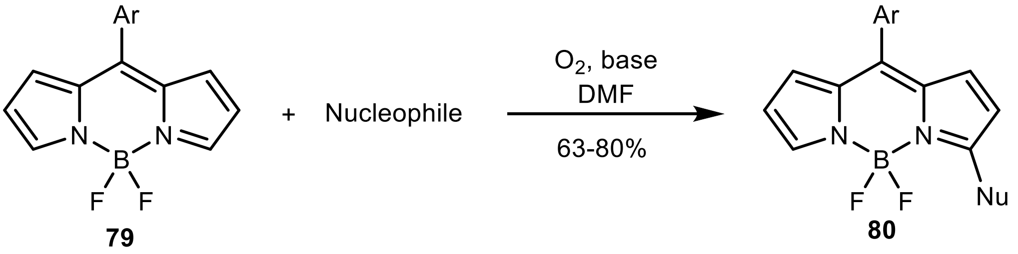
Methyl substitutions at the 3, 5 positions of BODIPY are known to have enough acidity to participate in nucleophilic addition and condensation reactions, such as Knoevenagel reactions. Accordingly, the reactivity of these methyl groups can be utilized to make styryl compounds through application of condensation of BODIPY with aromatic aldehydes. Akkaya *et al.* synthesized styryl substituted BODIPYs (**77**, **78**) by the condensation of 3, 5-methyl groups of BODIPY **76** with aromatic aldehyde (**Scheme 2-13**).[107] This condensation method is very useful for the derivatization of BODIPY to extend the conjugation and to tune the absorption and emission wavelengths of BODIPY core.



Scheme 2-13: Condensation of BODIPY with an aldehyde

2.4.5 C-H activation

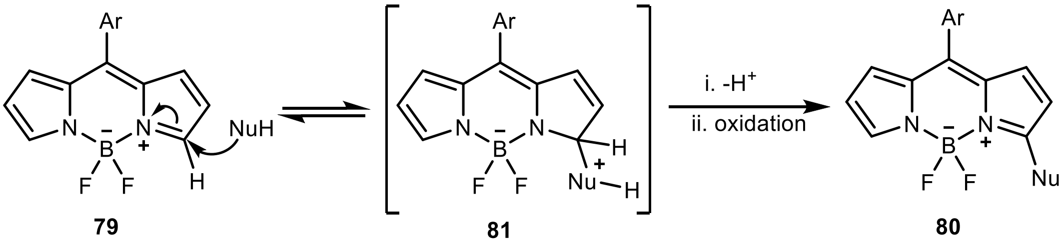
In addition to various methods, C-H activation at 3,5-positons is an alternative method for the functionalization of the BODIPY core. Dehaen *et al.* reported functionalization of BODIPY dyes by oxidative nucleophilic hydrogen substitution at the 3,5-positions (**Scheme 2-14**). Thus, BODIPY **79** was treated with C and N nucleophiles followed by oxidation by oxygen to give the corresponding substituted BODIPY derivatives **80** in excellent yields.[108]



Nucleophile = $BuNH_2$, $DodecylNH_2$, $BnNH_2$, piperidine, $(MeOOC)_2CH_2$, $(tBuOOC)_2CH_2$, $PhCOCH_3$

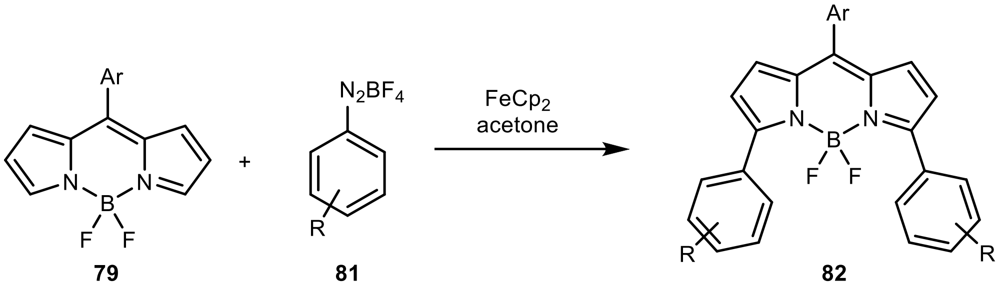
Scheme 2-14: Oxidative nucleophilic hydrogen substitution of BODIPY

While the nucleophilic substitution can be easily performed with 3,5-halogenated BODIPYs as halogens act as leaving groups, it is not possible to carry out nucleophilic substitution reactions with non-halogenated BODIPYs without an oxidation step. Generally electron poor aromatic rings of BODIPYs are prone to undergo nucleophilic attack at activated positions by forming a σ-H adduct (**81**). Since hydride in this adduct is a poor leaving group, oxidation is required to re-establish the aromaticity (**Scheme 2-15**).



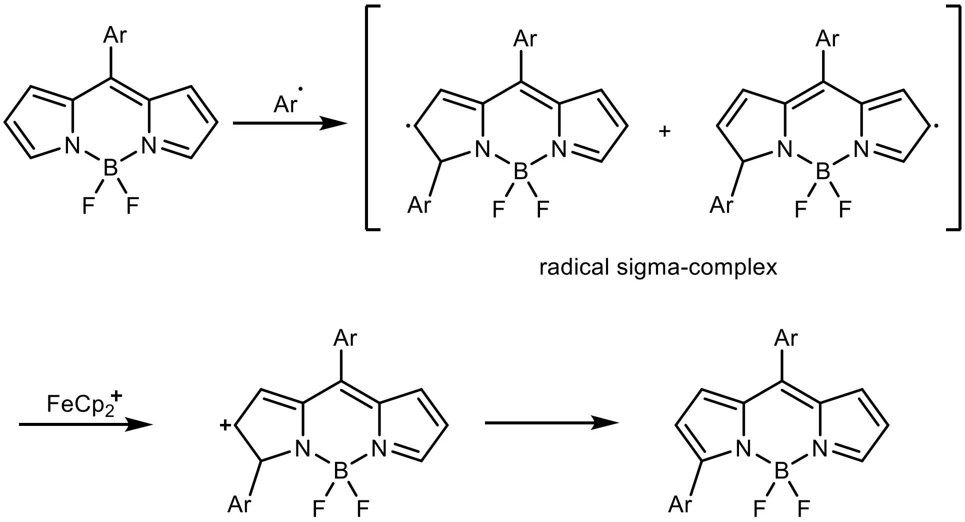
Scheme 2-15: Mechanism for the oxidative nucleophilic substitutions

In another report Dehaen *et al.* showed a radical based C-H arylation of the BODIPY core with aryl diazonium salts which are known to generate radicals through heterolytic dediazoniation mechanism (**Scheme 2-16**). Thus, reactions between *meso*-arylated BODIPY (**79**) and various aryldiazonium salts (**81**) in the presence of a catalyst $FeCp_2$ yielded exclusively 3- or/and 5-aryl substituted BODIPYs (**82**).[109]



Scheme 2-16: C-H arylation of BODPYs with aryldiazonium salts

The catalyst was found to lose its activity due to the arylation of newly formed ferricinium ions, resulting in the formation of phenylferrocene and diphenylferrocene. To overcome this problem the catalyst was replenished by continuous addition at a flow rate 0.2 mmol/h to maintain its presence in the reaction mixture. The selectivity for the 3, 5-positions was attributed to the stability of the radical sigma complex formed after the reaction of BODIPY and aryl radical. The addition of aryl radical at the 3-position of one of the pyrrole would generate a radical stabilized by conjugation over several double bond and the electron withdrawing effect of imine in the other pyrrole due to the complexation to the strongly polarized BF_2 (**Scheme 2-17**).



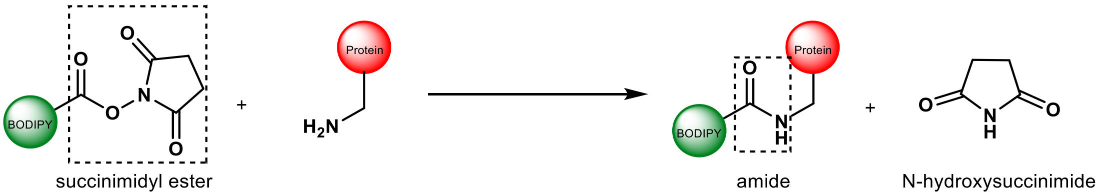
Scheme 2-17: Mechanism for C-H arylation of BODIPY

2.5 Functional groups on the BODIPY core for conjugation purposes

BODIPY derivatives synthesized for conjugating to the biomolecules such as proteins have found many applications in the imaging of biological systems such as localizing proteins and small molecules of interest in living cells. For example Meijler *et al*. used an aminooxy-BODIPY to localize an intracellular bacterial receptor involved in cell-to-cell communication in *Pseudomonas aeruginosa* cells.[110] In another study a fluorogenic tetrazine-BODIPY was used to track an anticancer drug taxol in live cell imaging.[111] Due to the number of interesting bio-imaging applications of BODIPY-conjugates, many BODIPY derivatives with functional groups, such as succinimidyl esters, sulfonyl chlorides, isothiocyanates, alkynyls, and azides, have been synthesized to conjugate a target of interest, leading to the formation of amide, sulphonamide, thiourea and other conjugates. Depending on the available functional group on the BODIPY and the target, several methods may be available for the conjugation of BODIPY to the desired molecule.

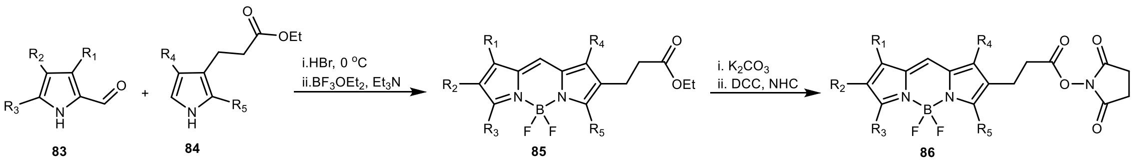
2.5.1 Succinimidyl esters

N-Hydroxysuccinimide (NHS) ester is the most commonly used functionality for conjugation of BODIPYs to amine-containing target compounds such as proteins. This methodology has been widely used for the labelling of proteins with various targets, including radioactive isotopes such as iodine-125, tritium, and carbon-14, as well as fluorescent molecules such as fluorescein and BODIPY.[112,113,114] Succinimidyl esters react with amines, forming an amide bond and producing *N*-hydroxy succinimide as a leaving group (**Scheme 2-18**). These conjugation reactions are generally quite efficient, requiring 30 min to 4 hrs in water–DMSO at room temperature for conjugation to complete.



Scheme 2-18: Amine labelling with succinimidyl ester

Numerous BODIPY derivatives containing the succinimidyl ester reacting group have been synthesized by both academic and industrial research groups. The first synthesis of the BODIPY-succinimidyl ester was reported in a 1988 patent by Molecular Probes (now part of Life Technologies - Thermo Fisher Scientific).[91] Synthesis of the BODIPY-succinimidyl ester was performed by the coupling of an ester-substituted pyrrole (**84**) with a pyrrole carboxyaldehyde (**83**) to give a dipyrromethene, which was further complexed with $BF_3{\bullet}OEt_2$ to generate a BODIPY derivative having an ethyl ester (**85**). This ester was hydrolyzed in the presence of a base to give a carboxylic acid, and subsequently coupled with *N*-hydroxy succinimide (NHS) to yield NHS conjugated BODIPY derivatives (**86**) (**Scheme 2-19**).

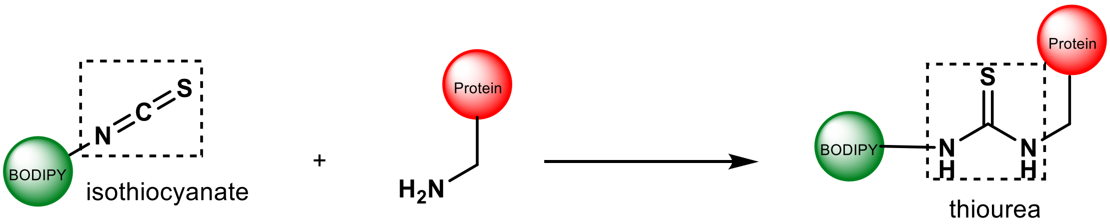


Scheme 2-19: Synthesis of BODIPY-succinimidylester

In an application of BODIPY-succinimidyl ester, a conjugated protein was used to measure the activity of proteases by fluorescent assay. [115] Initially, the BODIPY was conjugated to a small protein casein, which is a substrate for several endoproteases. Labelling of casein with multiple BODIPY can lead to “autoquenching” by the adjacent fluorophores. Upon cleavage of casein by protease, the initially linked fluorophore is released, enhancing the signal. The degree of signal enhancement can then be used as a measurement for protease activity.

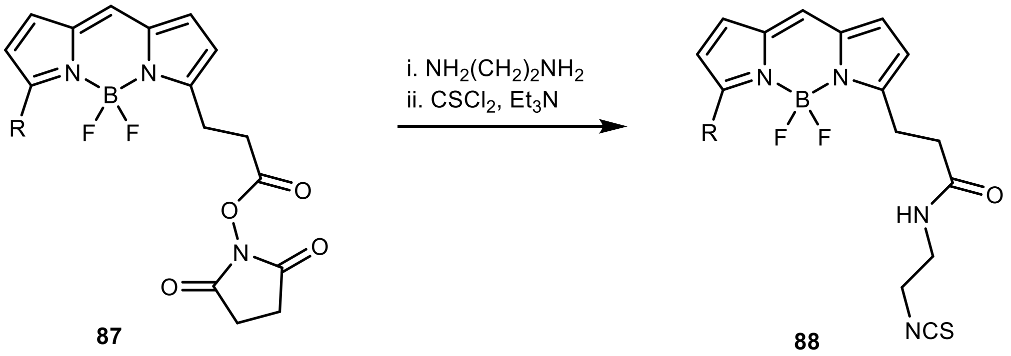
2.5.2 Isothiocyanate BODIPY dyes

Owing to the electrophilic nature of center carbon, isothiocyanate is very reactive towards nucleophiles such as amines. The nucleophilic attack of an amine on isothiocyanate results in the formation of a thio-urea linkage (**Scheme 2-20**).



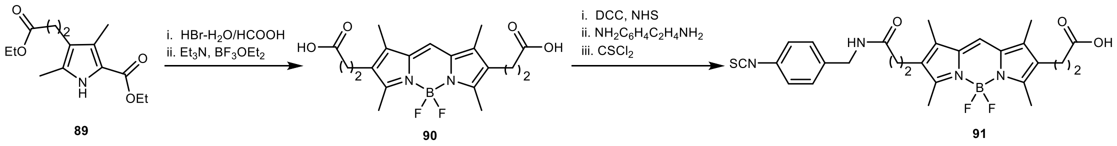
Scheme 2-20: Amine labelling with isothiocyanate

Similar to the BODIPY-succinimidyl ester, the first synthesis of the BODIPY-isothiocyanate derivative was reported in a Molecular Probes patent in 1988.[91] In this patent, the BODIPY-succinimidyl ester was reported as a starting material for the synthesis of the BODIPY-isothiocyanate. Thus, the BODIPY-succinimidyl ester (**87)** was reacted with ethylenediamine to yield the amino-amido-BODIPY, which was converted to BODIPY-isothiocyanate (**88**) with the use of thiophosgene (**Scheme 2-21**).



Scheme 2-21: Synthesis of BODIPY-isothiocyanate

Following a similar approach, Meltola *et al.* synthesized a BODIPY-succinimidyl ester having an aromatic thiocyanate group (**Scheme 2-22**).[116] For this purpose, a BODIPY (**90**) consisted of two propionic acid substituents was first obtained from condensation of tetrasubstituted pyrrole (**89**). One of the free carboxylic acid groups was activated by treatment with *N*-hydroxysuccinamide. The activated carboxylic acid was then reacted with 4-(2-aminoethyl)-aniline to yield a BODIPY derivative with a free amino group. The resulting compound was subsequently treated with thiophosgene in a basic medium to give the BODIPY dye with isothiocyanate functionality (**91**)

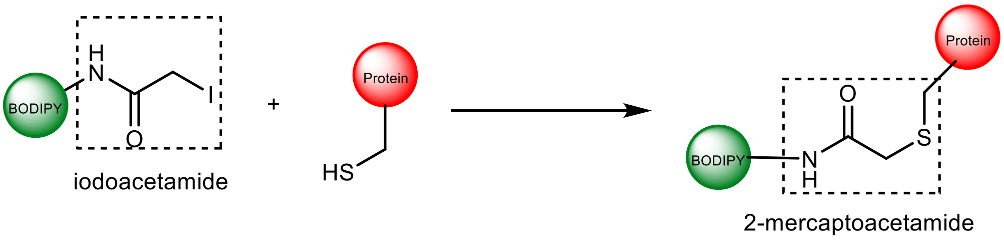


Scheme 2-22: Synthesis of BODIPY-isothiocyanate

This synthesized BODIPY-isothiocyanate was then conjugated to IgG antibody by incubating a 10-fold excess of BODIPY-isothiocyanate with IgG. In this application, the conjugation of isothiocyanate derivatives was noted to be less efficient in comparison to conjugation with succinimidyl esters.

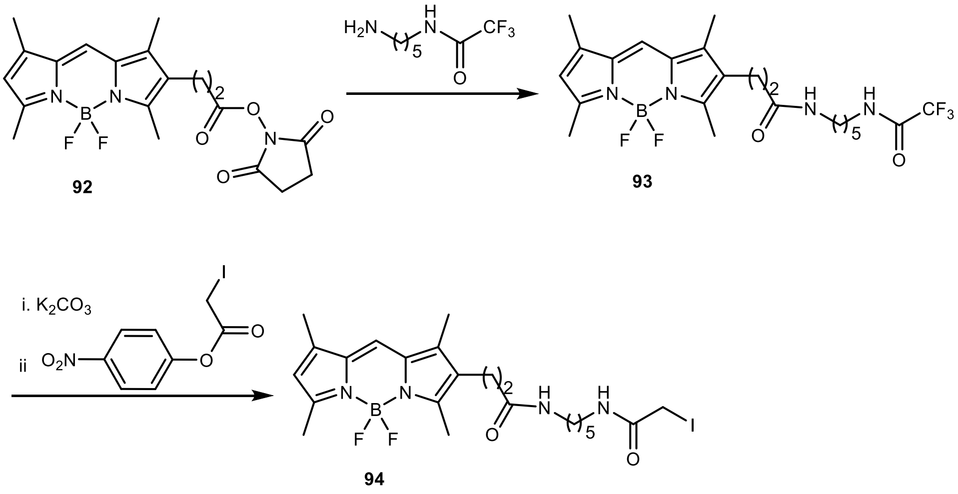
2.5.3 Iodoacetamide BODIPY dyes

Iodoacetamides are well-known compounds in conjugation chemistry, and are widely recognized to be very reactive towards the sulfhydryl groups.[117] Since the methylene group in iodoacetamide is attached to a carbonyl and an iodide, it reacts in an electrophilic manner with nucleophiles (**Scheme 2-23**).



Scheme 2-23: Labeling of thiol with iodoacetamide

Although iodoacetamides are very reactive with the sulfhydryl groups of cysteine, they are also reactive with other functionalities present in amino acids side chains, in the following decreasing order of reactivity: imidazolyl of histidines, thioether of methionines and amines from lysines. Selectivity of iodoacetamides for sulfhydryl groups can be improved by reducing the amount of iodoacetamide, or by increasing the basicity of the reaction mixture to an alkaline pH. Synthesis of BODIPY-iodoacetamide derivatives was performed in a manner similar to the synthesis of isothiocyanates (**Scheme 2-24**). First, a BODIPY-succinimide ester **92** was reacted with a free amine from cadaverine to yield a BODIPY containing a trifluoroacetamide function (**93**). The trifluoroacetamide was then converted to a free amine, and reacted with a *para*-nitrophenyl iodoacetate to give the BODIPY-iodoacetamide derivative (**94**).

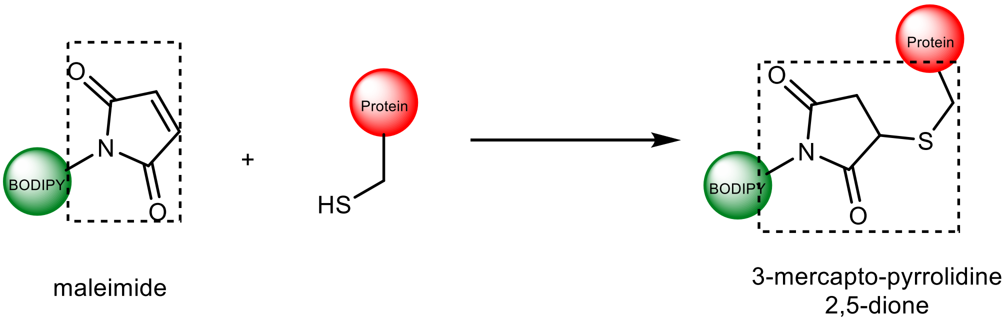


Scheme 2-24: Synthesis of BODIPY iodoacetamides

The obtained BODIPY-iodoacetamide derivative **94** was then used to label the protein plasminogen activator inhibitor I, which modulates fibrinolytic activity in human blood.[118] This allowed for the discovery and further study of important interaction sites responsible for the inhibition

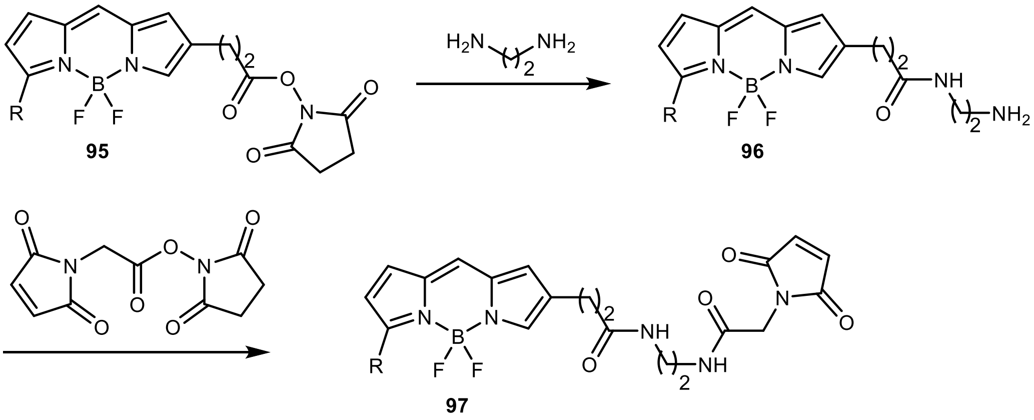
2.5.4 Maleimide BODIPY dyes

Maleimide is a well-known functional group in conjugation chemistry. Maleimides are very reactive Michael acceptors; they react rapidly and specifically with sulfhydryl groups through Michael addition reactions, in spite of the presence of amines and hydroxyls (**Scheme 2-25**).[119] Due to this useful reactivity, BODIPY-maleimides have been used extensively to label the cysteine residues of proteins.[120]



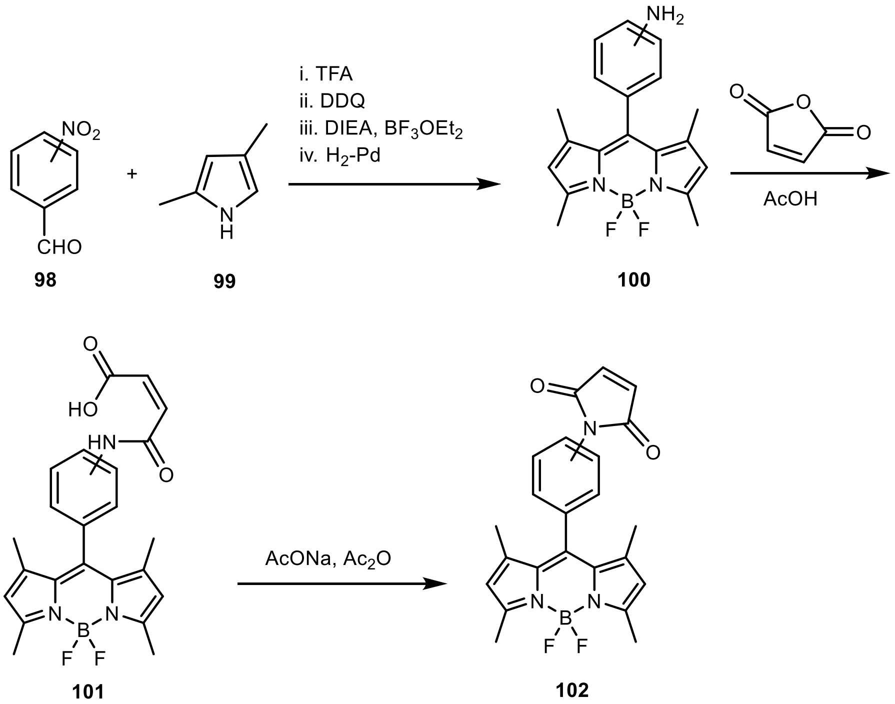
Scheme 2-25: Labelling of thiol with maleimide

The synthesis of the BODIPY-maleimide derivative was first initiated by reacting a BODIPY-succinimidyl ester (**95**) with ethylenedimine (**Scheme 2-26**). Next, the free amino group was reacted with a cross linking agent, *N*-succinimidyl maleimidoacetate to give the BODPY-maleimide **97**.[121] BODIPY-maleimide derivatives such as **97** have been applied in the development of a fluorimetric assay used to quantify reactive thio groups of proteins such as glyceraldehyde-3-phosphate dehydrogenase (GAPDH). In this study, incubation of the BODIPY-maleimide derivative **81** with GAPDH in an ice bath for 4 hours in a phosphate buffer resulted in a BODIPY conjugated GAPDH.[122]



Scheme 2-26: Synthesis of BODIPY-maleimide derivative

In another approach, three isomeric BODIPY-maleimide derivatives were synthesized by using BODIPYs containing aniline at the *meso*-position (**Scheme 2-27**).[123] BODIPY-aniline derivatives (**100**) were obtained from reduction of nitro BODIPYs, which were obtained using a classical BODIPY synthesis process. This process included the treatment of 2,4-dimethylpyrrole (**99**) with nitrobenzaldehyde (**98**), followed by aromatization with 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ) and complexation with BF_3•OEt_2. BODIPY-derived aniline (**100**) was reacted with maleic anhydride to form BODIPY-maleic acid (**101**), which was subsequently converted to BODIPY-maleimide (**102**) by dehydrative cyclization. The same method was used for the synthesis of three substitutional isomers of BODIPY derivatives.[123]

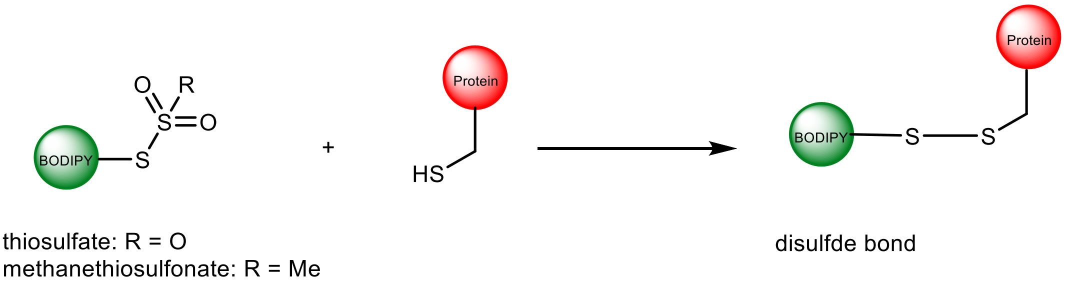


Scheme 2-27: Synthesis of BODIPY-maleimide derivatives

Among the three substitutional isomers of **102**, the *ortho* compound has been shown to have the lowest fluorescence, probably due to the photo-induced electron transfer phenomenon. Interestingly, a product from the reaction of this *ortho* isomer of BODIPY-maleimide with free thiols was shown to restore BODIPY fluorescence. This is likely due to loss of the double bond in maleimide, which prevents electron transfer, thus restoring fluorescence.

2.5.5 Thiosulfate and thiosulfonate BODIPY dyes

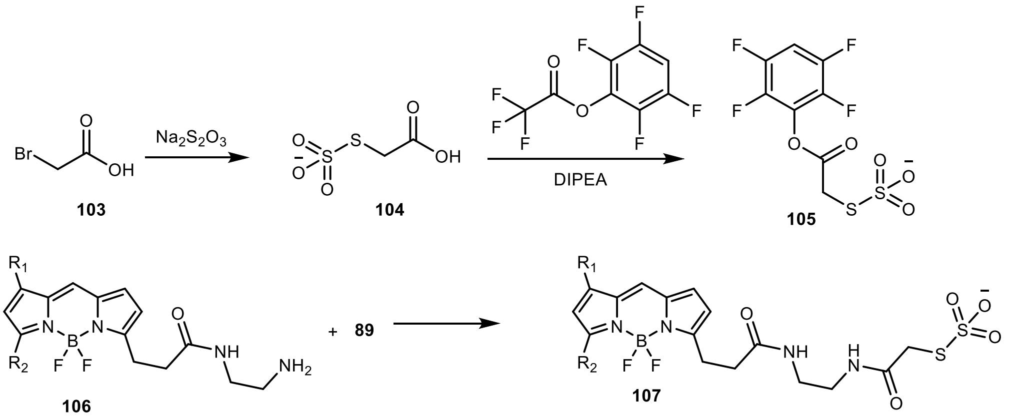
Thiosulfate and thiosulfonate functional groups have been widely used to conjugate proteins with fluorescent probes.[124,125] Thiosulfates and thiosulfonates differ from sulfates and sulfonates; in the former compounds, one of the oxygen atoms is replaced with a sulfur, making the compound more reactive towards nucleophilic attack from compounds such as thiols (**Scheme 2-28**). An interesting feature of these functional groups is the reversibility of the disulfide bond generated after conjugation to the protein through the sulfhydryl moiety of cysteine.



Scheme 2-28: Labelling of thiols with thiosulfate and thiosulfonates

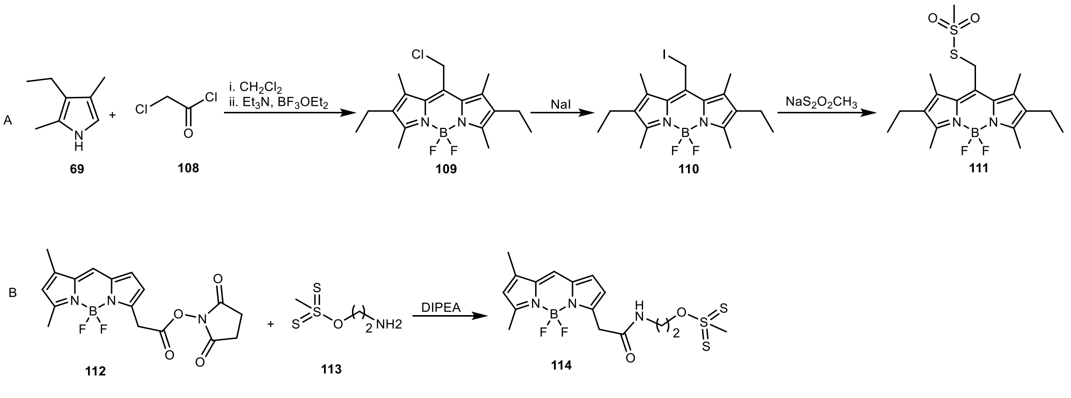
These disulfide bond can be cleaved (reduced) by reducing agents such as dithiothreitol (DTT) and tris(2-carboxyethyl)phosphine (TCEP). The synthetic strategy followed for the synthesis of BODIPY-thiosulfate derivatives is shown in **Scheme 2-29**.[126] A carboxylic acid with thiosulfate functionality (**104**) was obtained through a reaction of bromoacetic acid (**103**) and sodium thiosulfate, and subsequently reacted with 1,2,4,5-tetrafluorophenyl ester DIPEA, yielding a thiosulfate transferring reagent (**105**). A

BODIPY bearing an amino group (**106**) was then reacted with the thiosulfate transferring reagent to attain the final BODIPY-thiosulfate (**107**).[126]



Scheme 2-29: Synthesis of BODIPY-thiosulfate

Several methods have been reported to prepare BODIPY-methanethiosulfonate derivatives. For example, a trisubstituted pyrrole (**69**) was treated with chloroacetyl chloride (**108**) to yield dipyrromethene, which was then complexed with BF$_3$•OEt$_2$ in the presence of triethylamine to give a chloro BODIPY (**109**). The chlorine was replaced with iodine by treatment with sodium iodide, yielding an iodo-BODIPY (**110**), which was then reacted with sodium thiosulfonate to attain the BODIPY methanethiosulfonate (**111**) (**Scheme 2-30 A**).[127]



Scheme 2-30: Synthesis of BODIPY-methanethiosulfonate

In another method, a BODIPY bearing *N*-hydroxysuccinimide (**112**) was treated with 3-aminopropyl methanethiosulfonate hydrobromide (**113**) in the presence of diisopropylethylamine (DIPEA), resulting in the formation of BODIPY-methanethiosulfonate (**114**) (**Scheme 2-30 B**).[128]

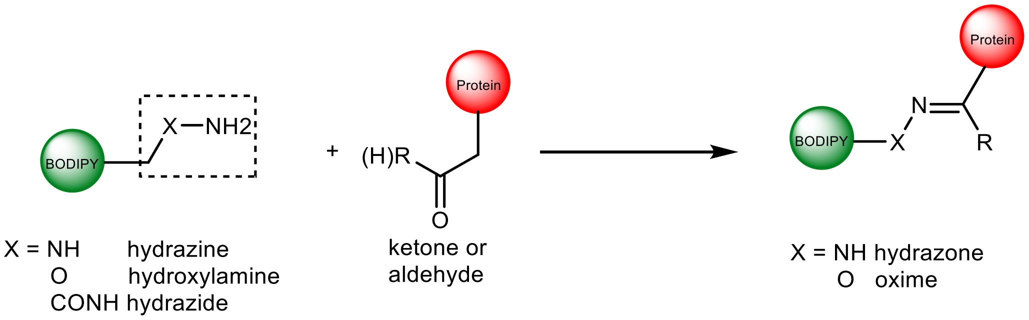
BODIPY-methanethiosulfonate has been used in many applications of protein labelling. For instance, this fluorescent probe was attached to the photosynthetic complex CP29, with the aim to study the structure of the *N*-terminal domain of the complex with the use of fluorescence resonance energy transfer (FRET). [129] For this purpose, several proteins with single amino acid mutations were synthesized by replacing each amino acid in the *N*-terminal domain with cysteine residues. After conjugation with BODIPY-methaenthiosulfonate, FRET experiments were carried out to study the interactions of labelled amino acid with other amino acids in order to constitute the structure of *N*-terminal domain.

2.6. Bio-orthogonal labeling of biomolecules

In addition to classical conjugating functionalities, which can be used to react with amino acids of proteins, bio-orthogonal conjugation has become a valuable approach for protein modification. In this approach, a specific functional group is incorporated into the protein to react with a specific functional group present on the other conjugating partner, such as a fluorophore.

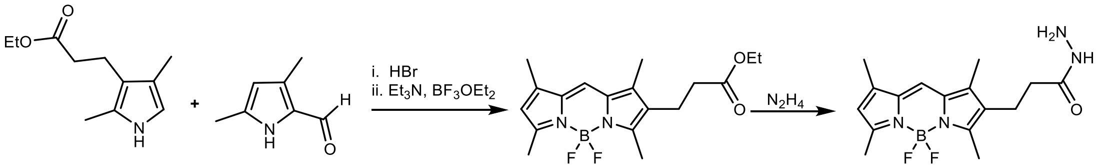
2.6.1 BODIPY-hydrazine, hydrazide or aminooxy compounds

An extensively explored bio-orthogonal approach involves the conjugation of a nitrogen nucleophile, such as hydrazine, hydrazide, or hydroxylamine, to the carbonyl functionalities (**Scheme 2-31**).[130] These carbonyl functionalities can be incorporated into proteins either via chemical modifications, or through genetic manipulation, such as insertion of *para*-acetyl-phenylalanine or post-translational enzymatic modification of glycine to formaldehyde. While the reaction of carbonyls with hydrazine and hydrazide generates hydrazine linkages, the reaction with hydroxylamine generates oxime linkages.[131]



Scheme 2-31: Labelling of aldehydes or ketones with hydrazide, hydroxylamine or hydrazide

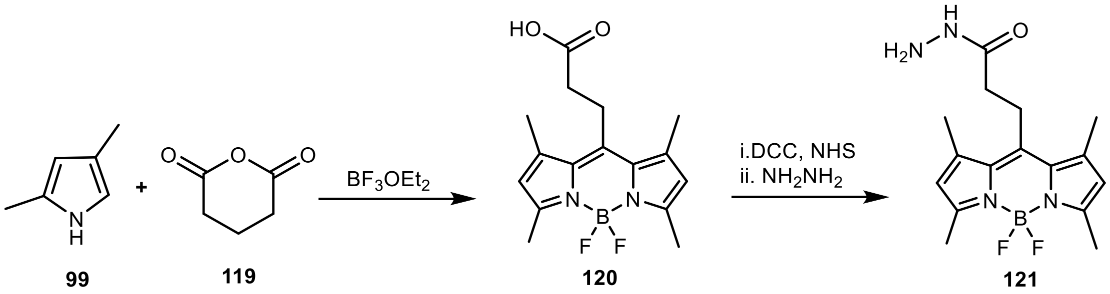
BODIPY-hydrazide derivative (**118**) was synthesized by treating hydrazine hydrate with BODIPY ethyl ester (**117**), which was accessible by the reaction of pyrrole-2-carboxaldehyde (**116**) and a pyrrole containing ester functionality (**115**), followed by complexation with borontrifluoride (**Scheme 2-32**).[91]



115 **116** **117** **118**

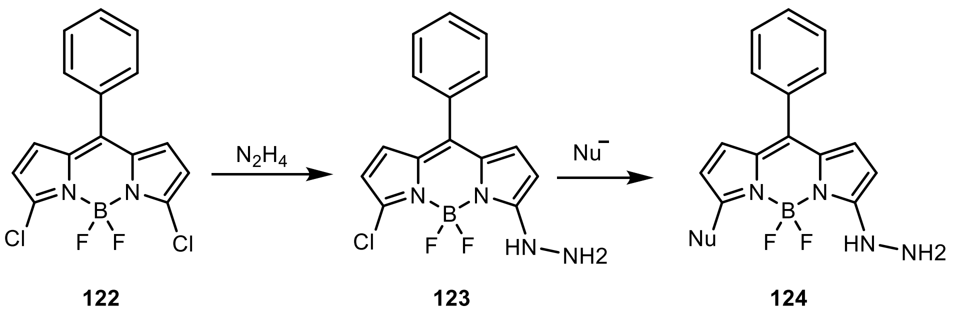
Scheme 2-32: Synthesis of BODIPY-hydrazide

In another method, BODIPY-hydrazide (**121**) was synthesized by inducing condensation of hydrazine with the BODIPY-carboxylic acid derivative (**120**), which was obtained from the reaction of dimethyl pyrrole (**99)** with a cyclic anhydride (**119**) (**Scheme 2-33**).[132]



Scheme 2-33: Synthesis of BODIPY-hydrazide

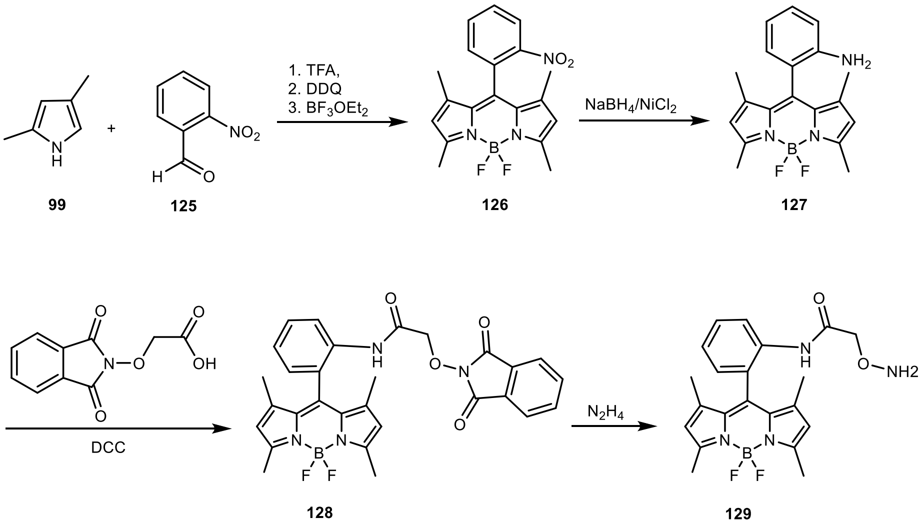
BODIPY-hydrazine derivatives (**124**) have been synthesized by nucleophilic substitution of chlorinated BODIPY (**122**) with hydrazine.[133] Depending on the equivalents of hydrazine present for nucleophilic substitution, either mono- or dihydrazine BODIPYs will be obtained. The mono hydrazine-substituted chloro-BODIPY (**123**) was treated with various nucleophiles in order to obtain a library of BODIPY-hydrazine derivatives (**Scheme 2-34**).[133]



Scheme 2-34: Synthesis of BODIPY-hydrazide

A hydroxylamine BODIPY (**129**) was synthesized by using an amine-containing BODIPY precursor.[134] The BODIPY precursor used in this case was obtained through reduction of nitro BODIPY (**126**), which was in turn prepared using the condensation of dimethyl pyrrole (**99**) and nitro aldehyde (**125**). Next, an amino-BODIPY (**127**) was coupled to the phthalimido-aminooxyacetic acid through an amine.

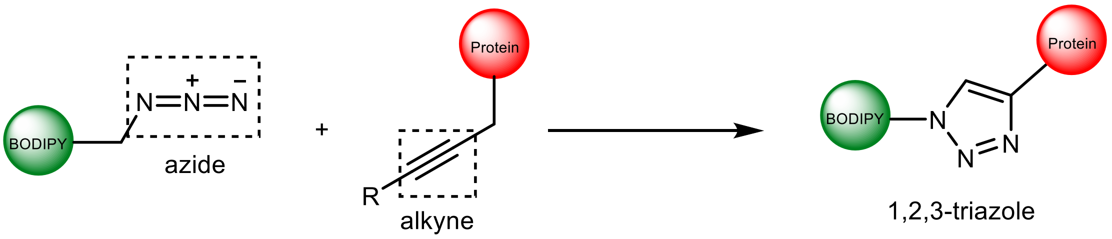
Finally, the phthalimide group of compound **128** was deprotected to give the hydroxylamino-BODIPY (**129**) (**Scheme 2-35**).[134]



Scheme 2-35: Synthesis of BODIPY-hydroxylamine

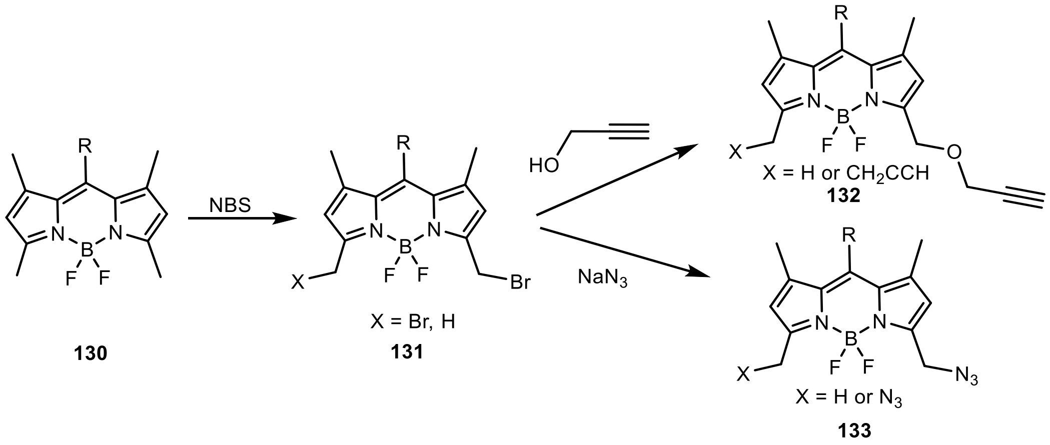
2.6.2 Azide alkyne click reaction

Another important bioorthogonal strategy in conjugation chemistry involves the cycloaddition of azide and alkynes. This cycloaddition reaction, often termed a Click reaction or Huisgen cycloaddition, occurs via a concerted mechanism to generate a stable 1,2,3-triazole linkage (**Scheme 2-36**). Click reaction has been widely applied in the field of bio-imaging for various applications.[135,136]



Scheme 2-36: Conjugation of BODIPYs through alkyne-azide click reaction

Generally, this reaction is performed by using a metal-catalyst such as CuI. However catalysts can be eliminated by using highly reactive alkynes such as strained cyclooctyne. There are several ways to incorporate either alkynes or azides into BODIPYs; the method chosen is dependent on the purpose of its application. The most commonly used methods to generate BODIPY alkynes (**132**) or azides (**133**) include the use of BODIPY substrates with halogenated side chains (**131**), which can be obtained through a reaction of BODIPY (**130**) with NBS. Nucleophilic displacement of the halide with either sodium azide or propargyl alcohol can then be used to generate azido and alkyno BODIPYs (**Scheme 2-37**).[137]

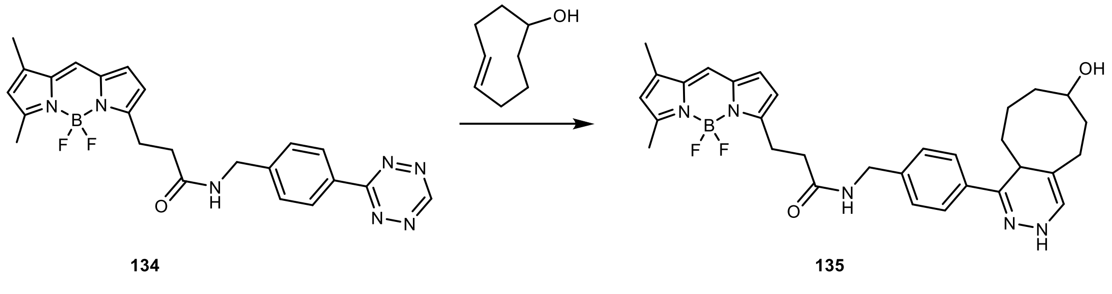


Scheme 2-37: Synthesis of BODIPY-alkyne and azides

2.6.3 Tetrazene-alkene click reaction

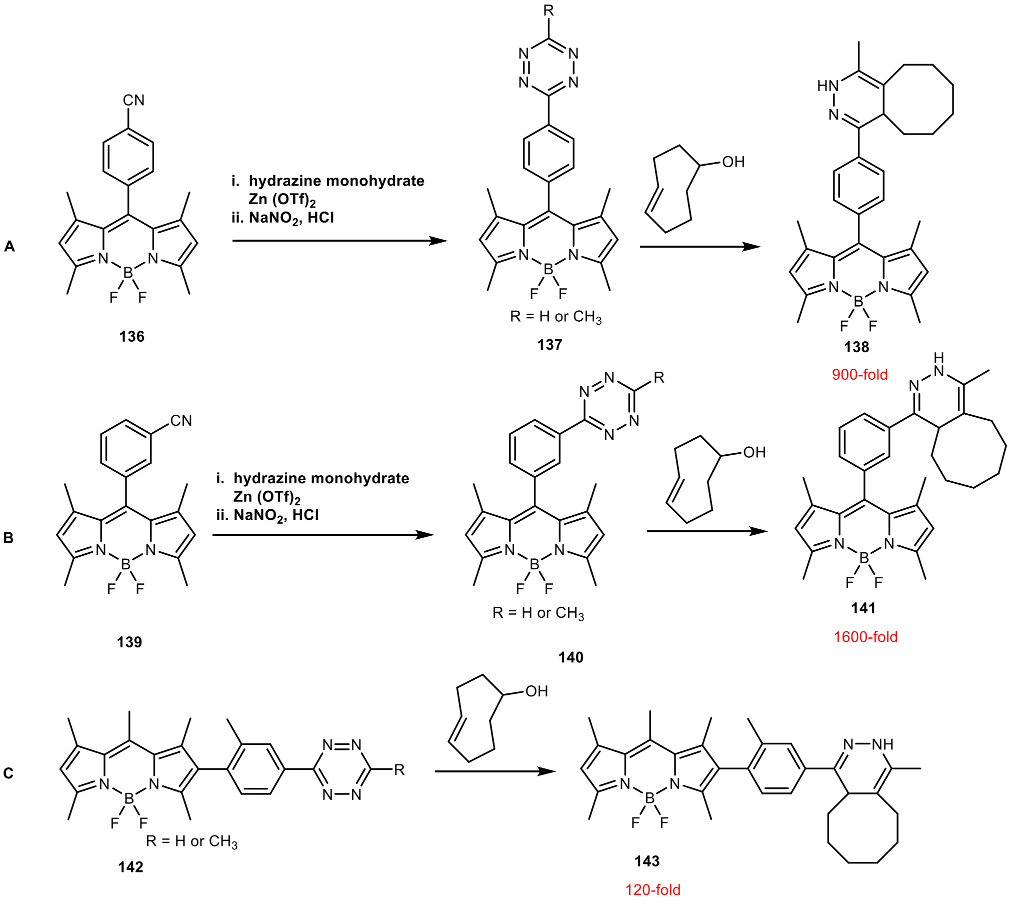
Inverse-electron-demand Diels–Alder (IEDDA) tetrazene cycloaddition is yet another click reaction introduced for bioorthogonal conjugation. Unlike normal Diels-Alder reactions, in IEDDA, the reaction occurs between electron-poor diene and electron-rich dienophile.[138,139] BODIPY-tetrazine can be efficiently conjugated to various partners having functional groups such as 1-methylcyclopropenes, norboranes, *trans*-cyclooctene, and cyclooctynes through IEDDA cycloaddition.

Tetrazene moieties act as chromophores as they absorb light in the visible region. As such, when tetrazenes are attached to fluorophores such as BODIPYs, they act as a fluorescent quencher. Depending on how the tetrazene is attached to the BODIPY, fluorescence quenching of the BODIPY can occur by energy transfer through space, namely fluorescence resonance energy transfer (FRET), through a bond, as well as by both. However, upon reaction of BODIPY-tetrazene with an alkene, fluorescence is restored due to changes that occur in both the electronic and structural properties of BODPY-tetrazene. Thus, BODIPY-tetrazene can be used as a turn-on fluorescent probe. A green-light-emitting BODIPY (**134**) having a tetrazene through a spacer exhibited 15-fold increased fluorescence upon reaction with *trans*-cyclooctene to form compound **135**.[140,141]



Scheme 2-38: Reaction of BODIPY-tetrazine with cyclooctene

In another report, non-fluorescent BODIPY molecules (**137**, **140** and **142**) were synthesized with their fluorescence quenched by through bond resonance energy transfer. Compounds **137** and **140** were synthesized through a reaction of hydrazine hydrate with the corresponding cyanophenyl-BODIPY (**136** and **139**) in the presence of zinc triflate, followed by oxidation with sodium nitrate and hydrochloric acid. Compound **142** was synthesized by coupling of a tetrazine derivative with 2-iodo-pentamethyl BODIPY (**Scheme 2-39**).[142]



Scheme 2-39: Reaction of substitutional isomers of BODIPY-tetrazine with *trans*-cyclooctene

Conjugation of these compounds with *trans*-cycloocetene exhibited dramatic fluorescence enhancement upon the formation of **138**, **141** and **143**. Reaction of compound **136** with *trans*-cyclooctenol in water gave a 900-fold turn-on ratio of fluorescence, while its substitutional isomer compound **140** yielded a 1600 fold turn-on ratio. Surprisingly, compound **142** was shown to only exhibit a 120-fold turn-on ratio.[142]

2.7 General perspectives and objectives of the present work

The development of new methods capable of introducing particular functionalities into the BODIPY core can aid in improved access to BODIPYs with desired properties. In this arena, we discovered and developed mild reaction conditions to introduce various useful functional groups into the BODIPY core by treatment of BODIPY with cupric nitrate pentahydrate. The work presented in this thesis includes the following objectives:

i. To optimize conditions for the introduction of a particular functional group, such as hydroxyl, aldehyde, nitro, or nitromethane

ii. To investigate the effect of water content of the reaction mixture on the outcome of reaction

iii. To broaden the scope of this chemistry by treatment with various metal salts, especially copper salts

iv. To synthesize BODIPY-sugar conjugates through the click chemistry

v. To characterize the liposomes formed by the BODIPY-sugar conjugates.

Chapter 3
Results and Discussions

3.1 Homotrimerization and tetramerization of terminal alkynes

Enediyne functionality is known to be the pharmacophore in several natural products, and has been shown to target DNA, leading to their anti-microbial and anti-cancer properties (discussed in Chapter 1). The aim of the current project is to attach enediyne functionality to DNA in order to investigate its site-specific DNA cleavage properties. We have hypothesized that a single strand DNA having enediyne functionality would be useful for site-specific cleavage of target DNA. When the DNA strand containing enediyne functionality ("guide sequence") binds to the target DNA through Watson-Crick base pairing, the enediyne functional group comes into close proximity to the target DNA. The activation of enediyne then leads to the formation of benzenoid radicals by a Bergmann cyclization mechanism. The newly formed benzenoid radicals are capable of stripping hydrogen atoms from the sugar moieties of the target stand, leading to cleavages of the target DNA (**Figure 3-1**).

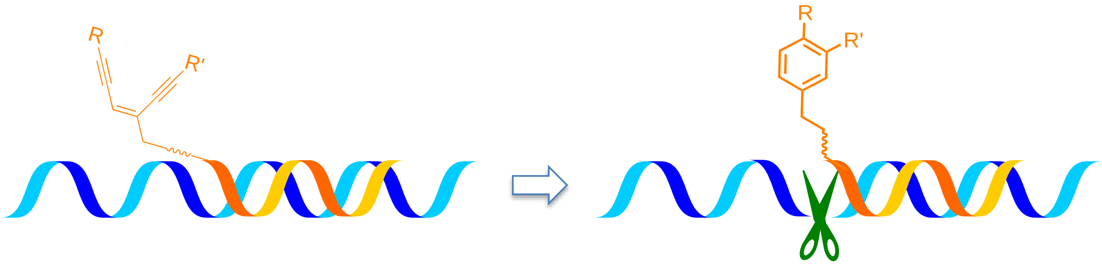
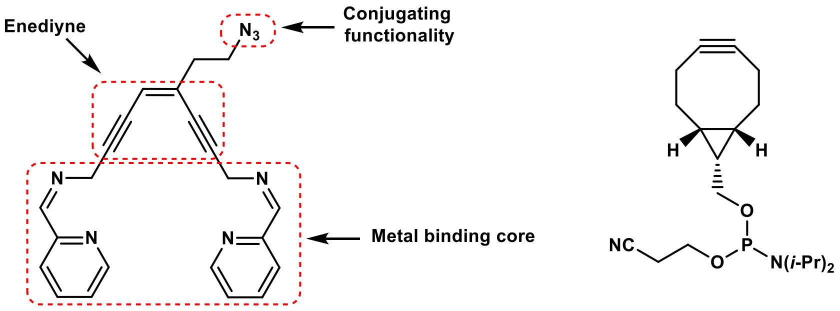


Figure 3-1: Cleavage of target DNA by enediyne

Since enediyne is preorganized in a specific nucleobase position of DNA through the sequence complementarity between the target and guide sequences, it will be possible to achieve site-specific cleavages of DNA. The attachment of enediyne functionality to DNA can be achieved through post-synthetic modifications. For instance, an oligonucleotide having a cyclooctyne at 5' terminus will be synthesized by coupling the bicyclo[6.1.0]nonyne (BCN) phosphramdite (**145**) as a final coupling cycle during the solid-phase synthesis of oligonucleotide.[143] After purification, the 5'-cyclooctyne modified oligonucleotide will be treated with compound **144** to incorporate an enediyne at the 5'-terminums of the oligonucleotide through an azide-alkyne cycloaddition (click reaction).[143]



144

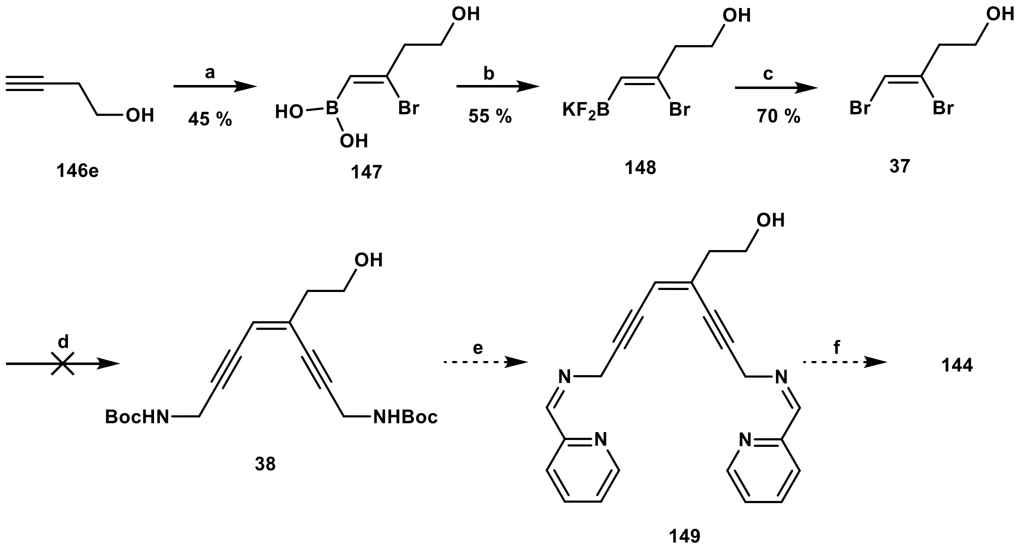
145

Figure 3-2: Designed enediyne phosphoramidite

As highlighted in compound **144 (Figure 3-2)**, each functionality of the molecule has specific purpose. The azido functional group on the side chain of enediyne is needed for the incorporation of this molecule into DNA through Click reaction. The enediyne functional group is required for the cleavage of the target DNA strand. Another important functional group that has been designed into this molecule is an aldimine (Schiff base). Here, the Schiff base formed by the amine and pyridine-2-aldehyde would serve as a trigger for the activation of enediyne for cyclization. Upon binding of the Schiff base to a metal ion, the distance between the two acetylene bonds in the enediyne is decreased, lowering the activation barrier of the Bergman cyclization temperature to room temperature. Zaleski *et al*. reported an enediyne containing a Shiff base at both termini of the acetylene bonds which underwent for the Bergmann cyclization at room temperature in the presence of magnesium chloride[58]with a half-life of cyclization reaction of 2.2 h as estimated by NMR. This literature observation serves as the basis for our approach towards the Bergman cyclization-mediated DNA cleavages.

3.1.1. Synthesis of the designed substituted enediynes

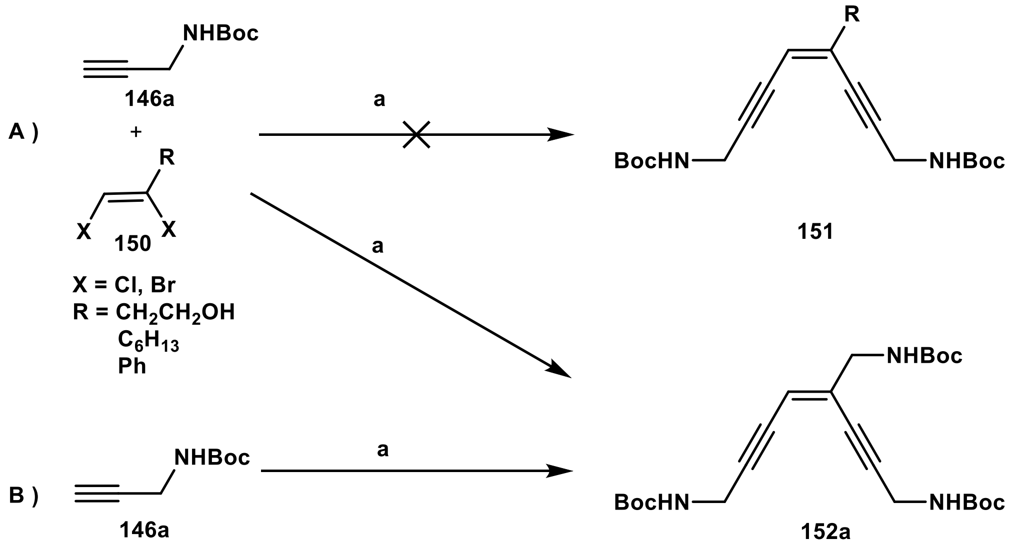
The strategy for the synthesis of the desired substituted enediyne is shown in **Scheme 3-1**. The synthesis was initiated through treatment of 3-buyne-1-ol **146e** with boron trimbromide, followed by hydrolysis to produce bromovinylboronic acid **147** in 45% yield. The bromovinylboronic acid **147** was then converted to potassium salt **148** in 55% yield by the treatment with potassium dihydrofluoride. The resulting potassium trifluoroborate **148** was then subjected to bromodeborination by treatment with tetrabutylammoniumtribromide (TBAT) to produce *cis*-dibromoalkene **37** in 70% yield.



Scheme 3-1: *Reagents and conditions*: a). i. BBr_3, Dry DCM, 0°C–rt, 3 h. ii. H_2O, rt; b). KHF_2, MeOH–H_2O, rt, 3 h; c). TBAT, THF-H_2O, rt, 3h; d). *N*-Boc-propargylamine,

$Pd(PPh_3)_4$, CuI, dry benzene, 45 °C, 10 h; e). i. TFA, ii. pyridine-2-aldehyde, AcOH. f). i. MsCl, pyridine, ii. NaN_3, DMF.

The next key step in this strategy involved the introduction of two alkyne substituents to *cis*-dibromoalkene **37** in a single step under Sonogashira coupling reaction conditions (Pd-catayst, CuI, and a base). Unfortunately, all attempts to produce the desired enediyne from the reaction between dibromoalkene (**37**) and *N*-Boc-propargylamine under various conditions were unsuccessful. While there are several reports in the literature detailing the use of the Sonogashira coupling reaction for the vicinal dihalides,[144,145,146] most of the reports used aromatic halides as substrates, with very few reports detailing the successful use of vinyl halides, especially aliphatic halides having side chains.[147,148,149] In order to investigate the reasons behind the unsuccessful attempts, reactions were performed between *N*-Boc propargylamine **146a** and dihaloalkenes with various side chains **150** (**Scheme 3-2A**). None of these reactions produced desired Sonogashira-coupled products. As a starting point, it was speculated that the hydroxyl group on the side chain might bind to and inactivate the catalysts, leading to unsuccessful Sonogashira coupling reactions. To investigate this hypothesis, the hydroxyl group was protected with TBDMS, and the coupling reaction was attempted. However, the attempt failed to yield Sonogashira-coupled product **151**. In the next attempt, two dihaloalkenes, one with a hexyl side chain (**150**, R = C_6H_{13}) and another with a phenyl side chain (**150**, R = Ph), were synthesized to compare the potential influence of functional groups of side chains in the coupling reaction. These two attempts also failed to yield the desired Sonogashira coupling products. Further, a *trans*-dichloroalkene with a hexyl side chain was synthesized and submitted to the Sonogashira coupling reaction with *N*-Boc propargylamine, which also failed to give the desired coupling products, indicating that stereochemistry of the double bond does not present hindrance for the reaction. Sonogashira coupling reactions were also attempted between a *cis*-1,2-dibromoalkene with a hydroxyethyl side chain and various alkynes, such as propargyl alcohol and propargyl bromide, but none of these attempts gave the Sonogoshira coupling product. On the other hand, it is not surprising that, when the Sonogashira coupling reaction was performed between *cis*-dichloroethene, which does not have side chains on the alkene, and *N*-Boc propargylamine, the Sonogashira coupled product was produced in 70% yield, as was reported in the literature.[59]

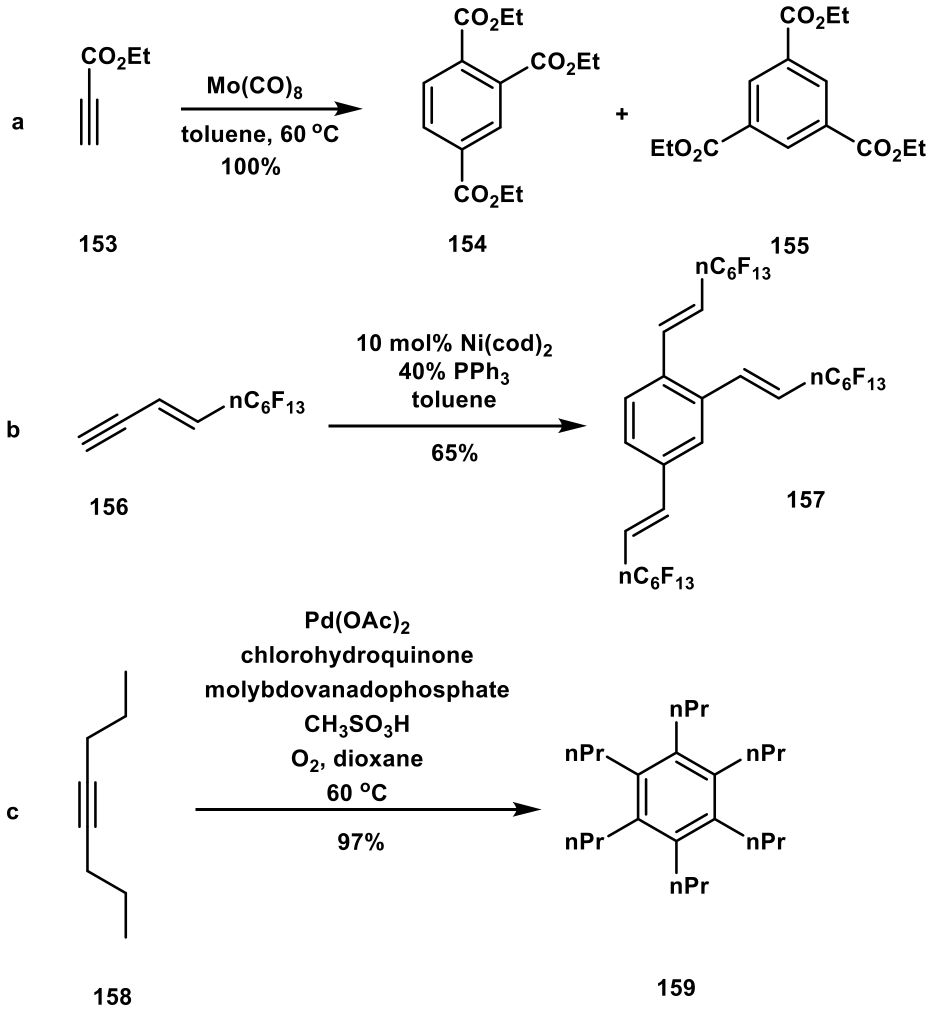


Scheme 3-2: *Reagents and conditions* a). Pd(PPh$_3$)$_4$, CuI, Et$_3$N, dry benzene, 45°C, overnight.

In a control experiment (**Scheme 3-2B**), *N*-Boc-propargylamine **146a** alone was treated under the same Sonogashira reaction conditions. Surprisingly, the product observed in this control reaction had the same R_f with the product observed in the previous Sonogashira coupling reaction between dihaloalkenes and *N*-Boc-propargylamine (**Scheme 3-2A**). The product from this control reaction was isolated, and its structure characterized by NMR and mass spectroscopy. To our surprise, the product from this control reaction was shown to be the trimer of the *N*-Boc-propargylamine **146a**. As expected, a dimer of the *N*-Boc-propargylamine was also shown to be present in the reaction mixture, which is a common by-product formed in Sonogashira coupling reactions.[150]

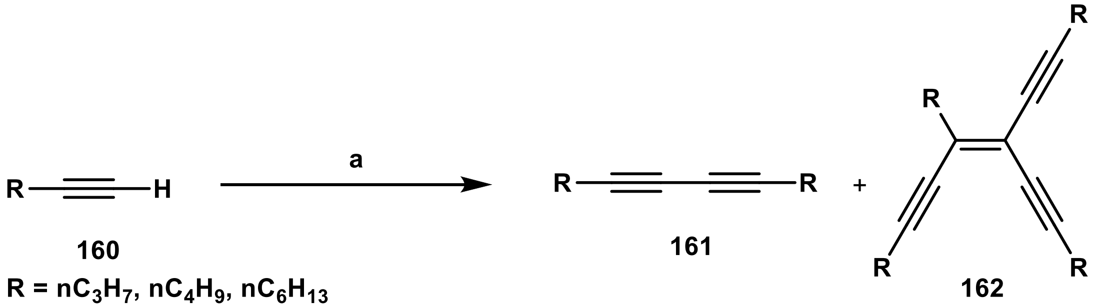
3.1.2 Homocoupling of terminal alkynes

Alkyne dimerizarion is a well-known side reaction under Sonogashira reaction conditions.[150] Similarly, several reports can be found in the literature on the metal catalyzed [2+2+2] trimerizarion of alkynes, which have found utility in the rapid construction of substituted benzene derivatives.[151] Examples for such type of trimerization reactions are shown below (**Scheme 3-3**).



Scheme 3-3: Metal calayzed [2+2+2] trimerizarion of alkynes

As shown in Scheme 3-3a, a molybdenum (0) comlex catalyzed cycloaromatazation of a terminal alkyne, ethylpropionate (**153**), gave a mixture of 1,2,4- and 1,3,5-carboxyethoxybenzenes (**154** and **155**) in a 2:1 ratio.[152] Yamamoto and co-workers reported a cyclotrimerization of 1-perfluoroalkylenynes (**156**) in the presence of a nickel catalyst $Ni(PPh_3)_4$, prepared from $Ni(cod)_2$ and PPh_3 (Scheme 3-3b).[153] This cycloaromatization reaction proceeded in a highly regioselective manner to give 1,2,4-regioismer (**157**) together with trace amount of 1,3,5-isomer. In another report a multi catalytic system containing $Pd(OAc)_2$, chlorohydroquinone (HQ-Cl) molybdovanadophosphate (NPMoV) in the presence of dioxygen was used for the cycloaromatization of internal alkynes (Scheme 3-3c).[154] An alkyne **158**, for example, underwent cycloaromatization under these conditions to give a fully substituted benezene derivative **159**. Self-trimerization and tetramerization of alkynes to form branched products under cross-coupling reaction conditions, however, are very rare. To the best of our knowledge, there are only three such examples reported in the literature. Rossi *et al.* first reported the formation of dimerized **161** and tetramerized **162** products when aliphatic alkynes **160** were treated under Sonogashira conditions in the presence of chloroacetone as a reoxidant (**Scheme 3-4**).[155] Two other similar reactions catalyzed by palladium (0) or zirconocene in the presence of an oxidant such as *p*-chloranil or dichlorodicyanobenzoquinone were reported.[156, 157]



Scheme 3-4: *Reagents and conditions*: $Pd(PPh_3)_4$, CuI, benzene, $ClCH_2COCH_3$, Et_3N.

The homo trimerization of terminal alkynes, on the other hand, has not been observed to date under the Sonogashira reaction conditions used in our investigations, which stimulated us to further explore this reaction for its substrate scope. Prior to investigating the substrate scope of this reaction, the roles of reaction components such as dihaloalkene, Pd-catalyst, CuI, and a base in the trimerization of alkyne wereinvestigated. As a starting point, a control reaction was performed by eliminating dihaloalkene from the reaction components, which produced the alkyne trimer, indicating that alkenes did not play a role in the trimerization of alkyne. In another set of three control reactions, performed in the absence of Pd-catalyst, CuI, or base, no trimer product formation was observed (entries 2, 3, and 4, Table 1). While virtually no reaction was observed in the absence of CuI, only the homocoupled alkyne dimer was isolated in the absence of palladium catalysts. The observations made from these three control experiments confirmed the roles of all three components in the trimerization reaction.

3.1.3 Optimization of the reaction conditions for the self-trimerization of terminal alkynes

The reaction conditions were optimized for the self-trimerization of *N*-Boc-propargylamine (**Scheme 3-2B**). Results are summarized in **Table 3-1**. Varying in the percentage of catalyst, solvent, and base, as well as reaction temperature were shown to influence the yield of the trimerized product.

Table 3-1: Optimization of reaction conditions described in **Scheme 3-2B**

Entry	**Reaction temperature and time**	**Solvent**	**Base**	**Catalyst**	**Isolated yield**
1	45°C, 15 h	Benzene	*n*-butylamine	$Pd(PPh_3)_2Cl_2$ (5 mol%)	40 %

				CuI (5 mol%)	
2	45ºC, 15 h	Benzene	No base	$Pd(PPh_3)_2Cl_2$ (5 mol%) CuI (5 mol%)	n/o
3	45ºC,15 h	Benzene	*n*-butylamine	CuI (5 mol%)	n/o
4	45ºC, 15 h	Benzene	*n*-butylamine	$Pd(PPh_3)_2Cl_2$ (5 mol%) CuI (5 mol%)	n/o
5	45ºC, 15 h	Benzene	*n*-butylamine	$Pd(PPh_3)_2Cl_2$ (15 mol%) CuI (15 mol%)	37 %
6	RT, 15 h	Benzene	*n*-butylamine	$Pd(PPh_3)_2Cl_2$ (5 mol%) CuI (5 mol%)	30 %
7	75 ºC, 15 h	Benzene	*n*-butylamine	$Pd(PPh_3)_2Cl_2$ (5 mol%) CuI (5 mol%)	n/o
8	45 ºC, 3h	THF	*n*-butylamine	$Pd(PPh_3)_2Cl_2$ (5 mol%) CuI (5 mol%)	28 %
9	45 ºC, 15 h	MeOH	*n*-butylamine	$Pd(PPh_3)_2Cl_2$ (5 mol%) CuI (5 mol%)	10 %
10	45 ºC, 15 h	DMF	*n*-butylamine	$Pd(PPh_3)_2Cl_2$ (5 mol%) CuI (5 mol%)	14 %
11	45 ºC, 15 h	1,4-Dioxane	*n*-butylamine	$Pd(PPh_3)_2Cl_2$ (5 mol%) CuI (5 mol%)	36 %
12	45 ºC, 15 h	Benzene	DBU	$Pd(PPh_3)_2Cl_2$ (5 mol%) CuI (5 mol%)	20 %
13	45 ºC, 15 h	Benzene	Hünig's base	$Pd(PPh_3)_2Cl_2$ (5 mol%) CuI (5 mol%)	10 %

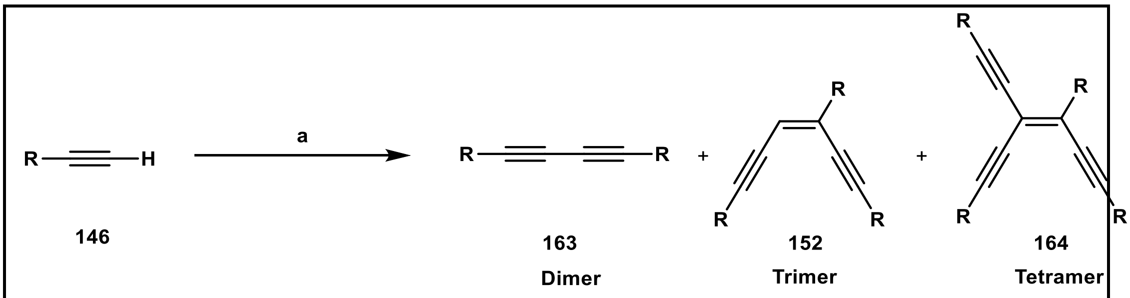
n/o: not observed

Two palladium catalysts, $Pd(PPh_3)_2Cl_2$ and $Pd(PPh_3)_4$, were shown to give similar yields of the trimerized product. The initial conditions (**entry 1**) with 5 mol% of Pd-catalyst and 5 mol% CuI led to the formation of the trimerized product in a yield of 40 %. Increasing the catalyst loading to 15 mol% was shown to have very little effect on the yield (**entry 5**). A lower yield was observed when the reaction was performed at room temperature instead of 45 °C (**entry 6**), however, no detectable trimerized product was observed by TLC when the reaction was carried out at 75°C (**entry 7**). Lower yields were also observed for reactions performed in polar solvents such as THF, MeOH, DMF, and dioxane (**entries 8, 9, 10,** and **11**) in comparison to those performed in benzene. When bases such as DBU and DIPEA, which are characterized by higher basicity but lower nucleophilicity compared with butylamine, were used, lower yields of product formation were also observed (**entries 12** and **13**).

3.1.4 Exploring the substrate scope for the self-trimerization of terminal alkynes

The substrate scope of the alkyne trimerization reaction was explored with various commercially available alkynes (**146 a-h**) such as propargyl alcohol, *N*-acetyl-propargylamine, 3-butyne-1-ol, *N*-Boc-1-amino-3-butyne, phenyacetylene, and 1-octyne. The results of these reactions are summarized in **Table 3-2**. With the exception of phenylacetylene (**146g**) and 1-octyne (**146h**), trimerized products (**152**), together with the corresponding homodimers (**163**), were formed from all other alkyne substates. Reactions with phenylacetylene (**146g**) and 1-octyne (**146h**) did not lead to the formation of corresponding trimerized products, possibly due to the absence of a heteroatom in these alkyne substrates which might be important for catalyst binding. Surprisingly, reactions of alkynes with a -CH_2CH_2- spacer between acetylene and the heteroatom yielded tetramerized products (**164 e, f**) in addition to the homodimer and homotrimer. Considering that trimerized and tetramerized products were only formed when alkynes with heteroatoms were used for the reaction, we hypothesized that presence of a heteroatom in the alkyne substrate is a requirement for homotrimerization and tetramerization.

Table 3-2: Homocoupling products of terminal alkynes. *Reagents and conditions*: a). Pd(PPh$_3$)$_4$, CuI, Et$_3$N, dry benzene, 45°C, overnight.



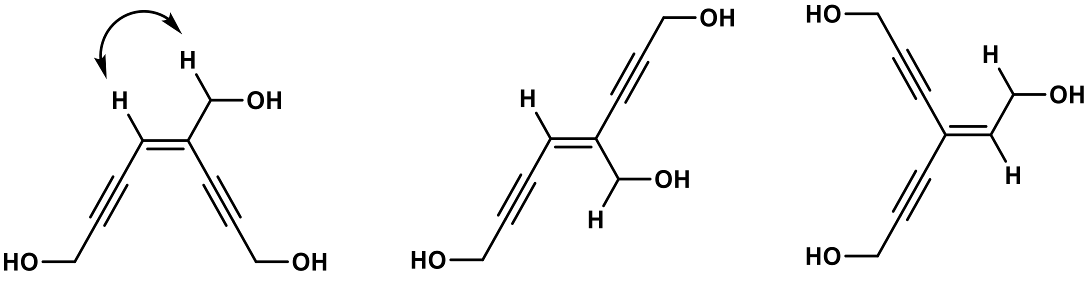
Entry	Substrate	Product yield (%)		
	R =	**Dimer**	**Trimer**	**Tetramer**
1	CH$_2$NHBoc (a)	10	40	n/o
2	CH$_2$OH (b)	53	31	n/o
3	CH$_2$NHAc (c)	20	61	n/o
4	(CH$_2$)$_2$NHBoc (d)	60	10	10
5	(CH$_2$)$_2$OH (e)	20	30	23
6	CH$_2$OCH$_3$ (f)	n/o	n/o	n/o
7	Ph (g)	69	n/o	n/o
8	(CH$_2$)$_4$CH$_3$ (h)	40	n/o	n/o

n/o: not observed.

Since the trimerized and tetramerized products are Bergmann cyclization precursors, we were curious to see whether these compounds undergo Bergmann cyclization upon heating to produce substituted benzene derivatives. For this purpose, two trimerized products of *N*-Boc-propargylamine and propargyl alcohol were investigated. The compounds were individually dissolved in either benzene or methanol and heated under reflux for two days. Unfortunately, these attempts did not result in Bergmann cyclization. The failure might be due to higher temperature requirements for the cyclization to occur.

3.1.5 Characterization of the trimerized alkyne products by NOE and HMBC NMR spectroscopy

The structures of the trimerized products were confirmed by NOESY and HMBC NMR spectroscopy. Structural assignment of the trimerized product (**152b**) from propargyl alcohol is shown below. Three possible geometric isomers (**Figure 3-3**) were examined against the NOE and HMBC spectra of the isolated product. As the structure of the trimer cannot be confirmed by ^{1}H and ^{13}C NMR alone, NOE and 2D NMR spectra were used to assign the correct structures of the trimers.



152 b1 152 b2 152 b3

Figure 3-3: Possible structures of trimerized product **152 b**.

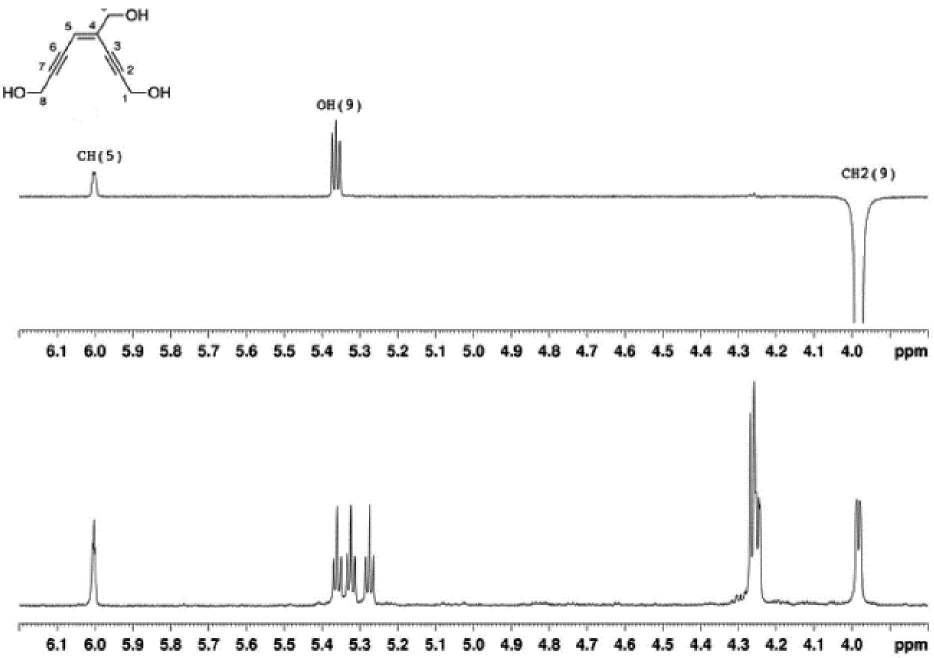


Figure 3-4: 1D NMR spectra of compound **152 b** in DMSO-d$_6$. Top: NOE spectrum of compound **152 b** excited at 3.98 ppm (CH$_2$(9-)); bottom: ^{1}H NMR spectrum of **152b**.

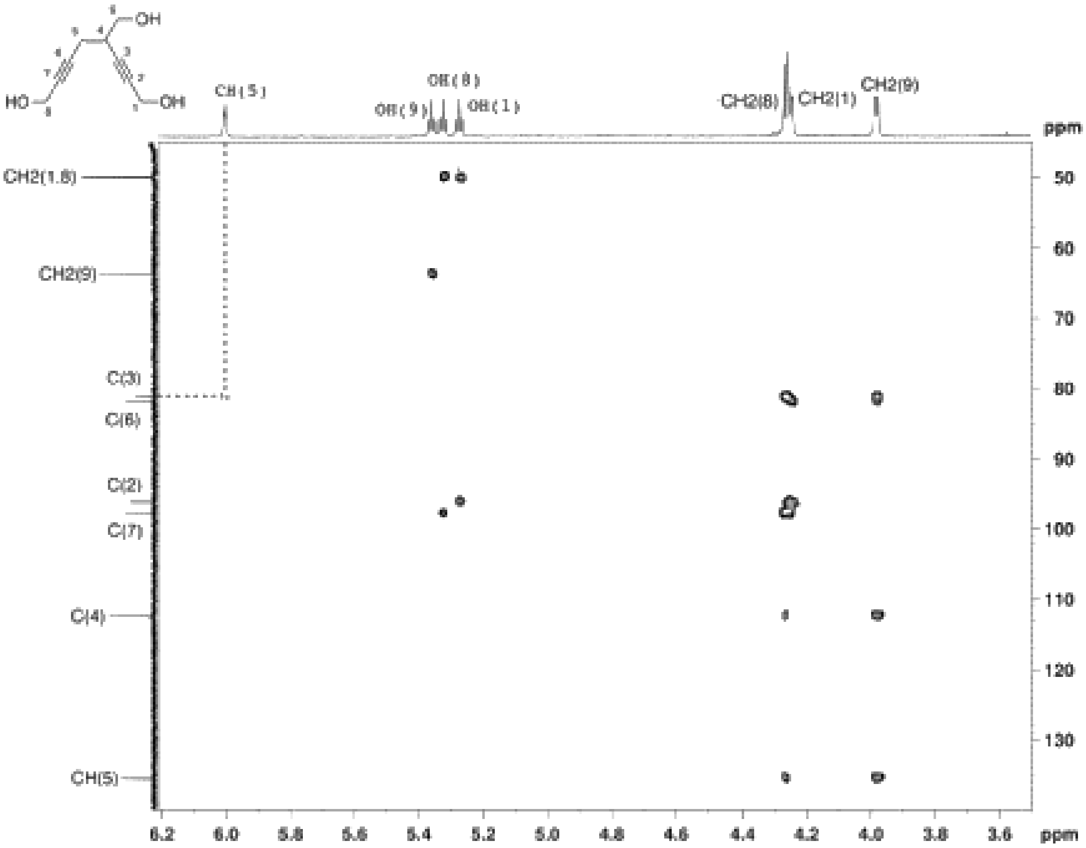


Figure 3-5: HMBC spectrum of compound **152b** in DMSO-d$_6$.

In a NOE experiment, the presence of a NOE signal (**Figure 3-4**) between the two protons indicated by the arrow in **152b1** (**Figure 3-4**) excludes **152b2** as the product, whereas the cross-peak identified in the HMBC spectrum (**Figure 3-5**) between CH(5) and C(3), but not between CH(5) and C(6), confirms **152b1** as the correct structure.

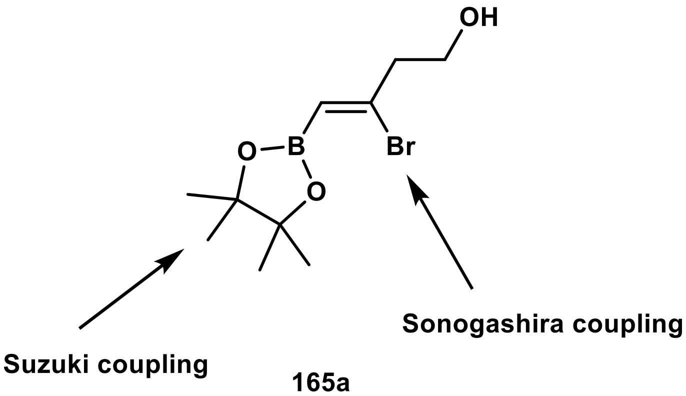
3.1.6 Conclusion

In conclusion, various attempts to synthesize the enedyine compund **38** by the treatment of substituted dibromoalkenes with *N*-Boc-propargylamine under Sonogashira reaction conditions were unsuccesful. Sonogashira reactions between various dihaloalkenes and alkynes were also unsuccessful in yielding the desired enediyne product. A careful structure analysis of the product formed under Sonogashira conditions led to the discovery of homo trimerization and tetramerization of terminal alkynes. The newly discovered reaction was optimized and further investigated for its substrate scope.

3.2 Reactions of terminal alkynes with trisubstituted (*Z*)-bromoalkenyl pinacol boronates towards the synthesis of 1,2,4,6-tetrasubstituted benzenes

3.2.1 Synthesis of pinacolboronate and bromoalkene

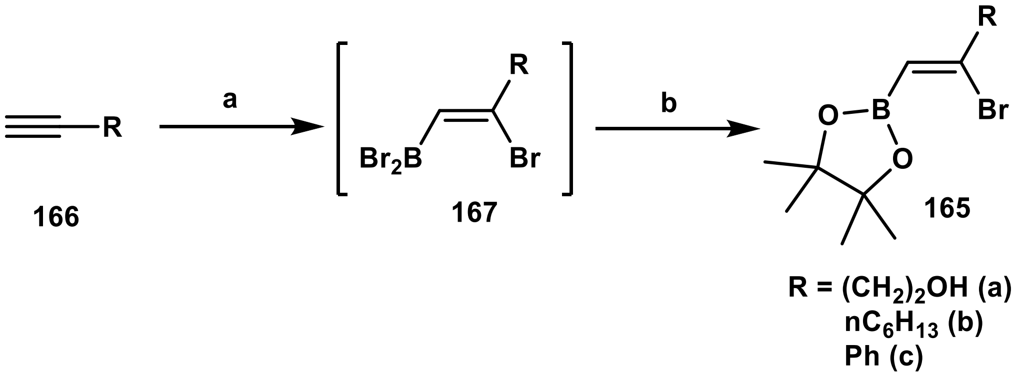
After many unsuccessful attempts at producing the desired enediynes by Sonogashira coupling reactions with dihaloalkenes, focus was switched to another class of alkene substrate, bromoalkene boronate **165a** (**Figure 3-6**).



165a

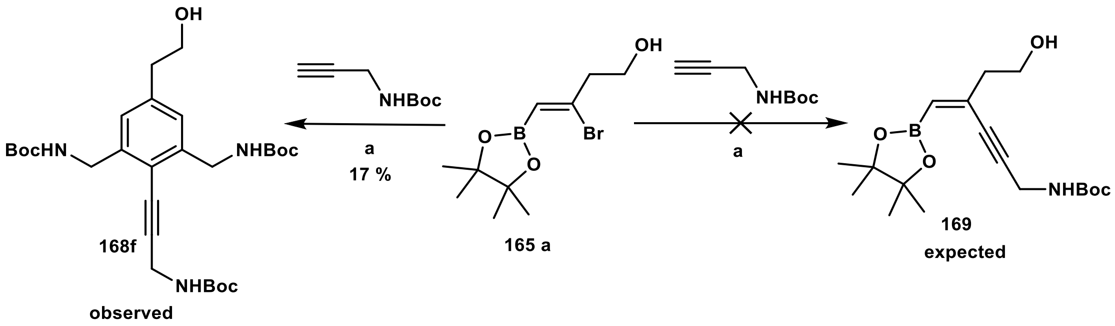
Figure 3-6: Bromoboronate alkene designed for sequential Sonogashira and Suzuki coupling reactions

This substrate was designed with the aim of performing sequential Sonogashira and Suzuki coupling reactions to synthesize the desired enediyne. The bromoalkene boronate **165a** was synthesized by the treatment of alkyne **166**a with boron tribromide to generate the (*Z*)-2-bromo-1-alkenyldibromoborane **167a**, followed by the treatment with pinacol in one-pot (**Scheme 3-5**) with an overall yield of 37%. This type of alkene substrate has been shown to be useful in sequential Negishi and other palladium-catalyzed coupling reactions.[158]



Scheme 3-5: *Reagents and conditions*: a). BBr_3, dry DCM, 0 °C–rt; b). Pinacol, dry DCM, rt.

The bromoalkene boronate substrate (**165a**) was then subjected to Sonogashira coupling reaction with *N*-Boc-propargylamine. Surprisingly, the expected Sonogashira coupled product **169** did not form. Careful analysis of the product formed in the reaction led to the discovery of a novel reaction between bromoboronate alkene and alkyne, producing the tetrasubstituted benzene **168f** (**Scheme 3-6**).



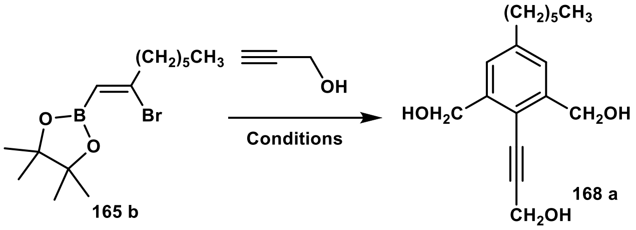
Scheme 3-6: *Reagents and conditions*: $Pd(PPh_3)_4$, CuI, DIPEA, 45°C, 15 h.

Polysubstituted benzenes are known to be useful synthetic intermediates, and can be found in many natural products. A considerable amount of literature is available regarding the synthesis of these polysubstituted aromatics. Along with other approaches, these substituted aromatic compounds are often generated through annulation of smaller building blocks.[159] Among these, the preparation of tetrasubstituted benzenes has been accomplished by various methodologies.[160,161] However, challenges remain towards regiospecific synthesis of aromatics of certain substitution patterns. As such, our newly discovered reaction can be applied as a novel way of synthesizing tetrasubstituted benzene derivatives in a regioselective manner.

3.2.2 Optimization of the reaction conditions for the aromatization reaction

Following the discovery of the novel aromatization reaction, conditions were optimized for the reaction between bromoalkenyl boronate with an alkyl side chain (**165b**) and propargyl acohol. The reaction was performed at various temperatures and with the use of different catalysts, bases, and solvents. The results are summarized in **Table 3-3**. In the absence of Pd-catalyst, CuI, or base (**entries 2, 3,** and **4**), the reactions did not produce aromatized products, indicating that all three reaction components are required for the reaction to produce an aromatic product. Lower yields were observed when lower molar percentages of the palladium catalyst were used, and upon changing the catalyst from $Pd(PPh_3)_4$ to $Pd(PPh_3)_2Cl_2$ (**entries 5** and **6**). Decreasing the temperature from 45°C to room temperature, or increasing the temperature to 75°C also resulted in lower yields (**entries 7** and **8**). The reaction was also performed in a few solvents such as THF, acetonitrile, benzene, methanol, and dioxane. While the use of THF produced the aromatized product, other solvents failed to produce any observable amount of aromatized product (**entries 9 10 11 12 and 13**). Substituting the base from triethylamine to DIPEA also resulted in lower yields of the product (**entry 14**). Use of other bases, such as DBU or Cs_2CO_3, failed to produce any aromatized product. The best yield (43%) for the aromatization reaction was obtained under the following conditions: 5 mol% $Pd(PPh_3)_4$, 20 mol % CuI, DIPEA as base, DMF as solvent, and reaction temperature set at 45°C.

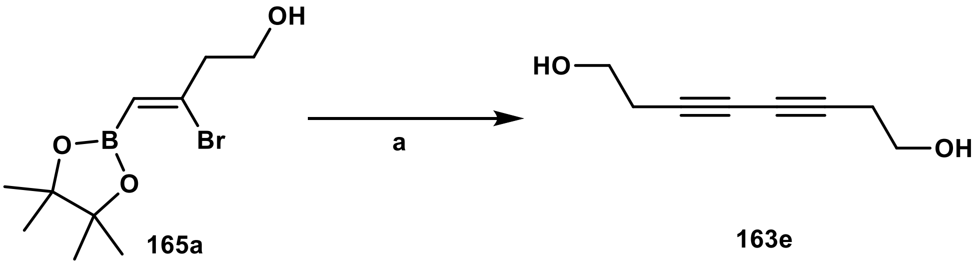
Table 3-3: Optimization of aromatization conditions



Entry	Catalyst	CuI Mol%	Base (1 equiv.)	Temperature	Solvent	Yield (%)
1	Pd(PPh$_3$)$_4$ (5mol%)	20	DIPEA	45 °C	DMF	43%
2	-----	20	DIPEA	45 °C	DMF	n/o
3	Pd(PPh$_3$)$_4$ (5mol%)	-	DIPEA	45 °C	DMF	n/o
4	Pd(PPh$_3$)$_4$ (5mol%)	20	-	45 °C	DMF	n/o
5	Pd(PPh$_3$)$_2$Cl$_2$ (5mol%)	20	DIPEA	45 °C	DMF	23%
6	Pd(PPh$_3$)$_4$ (1mol%)	20	DIPEA	45 °C	DMF	35%
7	Pd(PPh$_3$)$_4$ (5mol%)	20	DIPEA	rt °C	DMF	28%
8	Pd(PPh$_3$)$_4$ (5mol%)	20	DIPEA	75 °C	DMF	31%
9	Pd(PPh$_3$)$_4$ (5mol%)	20	DIPEA	45 °C	THF	9%
10	Pd(PPh$_3$)$_4$ (5mol%)	20	DIPEA	45 °C	CH$_3$CN	n/o
11	Pd(PPh$_3$)$_4$ (5mol%)	20	DIPEA	45 °C	Benzene	n/o
12	Pd(PPh$_3$)$_4$ (5mol%)	20	DIPEA	45 °C	CH$_3$OH	n/o
13	Pd(PPh$_3$)$_4$ (5mol%)	20	DIPEA	45 °C	Dioxane	n/o
14	Pd(PPh$_3$)$_4$ (5mol%)	20	Et$_3$N	45 °C	DMF	11%
15	Pd(PPh$_3$)$_4$ (5mol%)	20	DBU	45 °C	DMF	n/o
16	Pd(PPh$_3$)$_4$ (5mol%)	20	CS$_2$CO$_3$	45 °C	DMF	n/o

n/o: not observed

While the reactions described in **Table 3-3** are typically rather clean as judged by TLC, isolated yields for the aromatization products range from low to moderate, as can be seen in **Table 3-3**. Given the lower than expected yields, the trisubstituted (*Z*)-bromoalkenyl-pinacolboronates **165** were suspected to be prone to side reactions under the reaction conditions described in entry 1. To verify this, a control reaction was performed with compound **165a**.



Scheme 3-7: Side reaction observed under the conditions of entry 1 in table **3-3**

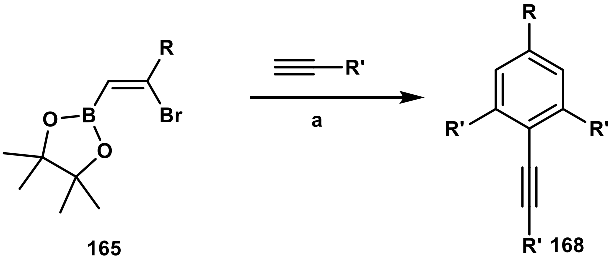
Thus, treatment of (*Z*)-bromoalkenyl-pinacolboronate **165a** alone with Pd(PPh_3)$_4$, CuI, and Hünig’s base (in the absence of terminal alkynes) was found to lead to the formation of alkyne **163e** (**Scheme 3-7**). This side reaction might be one of the factors leading to the rather low yield of the aromatized reaction. Conditions that suppress the formation of the observed side product may help to improve the yield of the aromatized product.

3.2.3 Exploring the substrate scope for the aromatization reaction

The substrate scope of this reaction was also explored by treating various bromoalkene boronates with different side chains and various terminal alkynes under the optimized conditions for aromatization (**Entry 1, Table 3-3**). Three bromoalkene boronates (**165**

a-c) with different side chains were synthesized using the procedure described previously (**Scheme 3-5**). Purification of the bromoalkene boronate with a phenyl side chain (**165c**) by column chromatography resulted in decomposition of the product.

Table 3-4: Substrate scope of the aromatization reaction. *Reagents and conditions*: a). Same as entry 1 in Table 3-3.



165	168	Yield %
a: R = -CH_2CH_2OH	a: R = CH_2-CH_2-OH, R' = CH_2OH	43
	b: R = CH_2-CH_2-OH, R' = CH_2NHBoc	16
	c: R = CH_2-CH_2-OH, R' = CH_2NHAc	24
	d: R = CH_2-CH_2-OH, R' = Ph	n/o
b: R = -$(CH_2)_5CH_3$	e: R = CH_3-$(CH_2)_5$-, R' = CH_2OH	37
	f: R = CH_3-$(CH_2)_5$-, R' = CH_2NHBoc	16
	g: R = CH_3-$(CH_2)_5$-, R' = CH_2NHAc	39
	h: R = CH_3-$(CH_2)_5$-, R' = Ph	n/o
c: R = -Ph	i: R= Ph, R' = CH_2OH	20
	j: R = Ph, R' = CH_2NHBoc	27
	k: R = Ph, R' = CH_2NHAc	14
	l: R = Ph, R' = Ph	n/o

n/o: not observed

Yields calculated for the aromatization reaction performed by this particular alkene (**165c**) were based on the crude starting material. Structures of the compounds produced by the attempted substrates (various bromoalkene boronates and alkynes) are shown in **Figure 3-7**. Aromatization reactions performed with phenylacetylene as the alkyne component failed to yield any aromatized product, possibly due to the steric hindrance of the phenyl group.

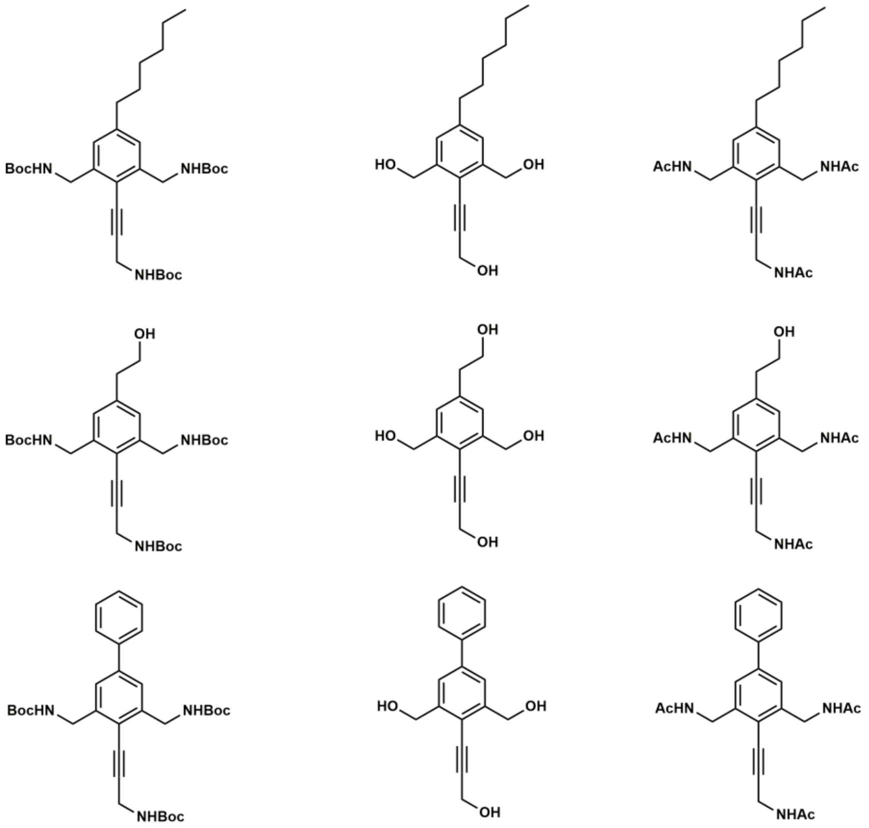
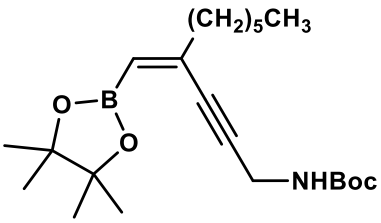


Figure 3-7: Products generated in the aromatization reactions with various alkene and alkyne substrates

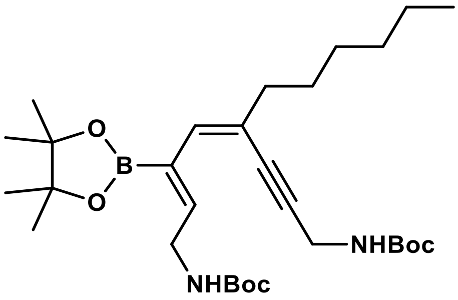
3.2.4 Mechanistic investigations of the aromatization reaction between a bromoalkene boronate and an alkyne

The product of an aromatization reaction between a bromoalkene boronate and an alkyne contains one alkene and three alkyne units. This reaction can be either a single-step or multi-step process. In the current work, attempts were made to investigate the mechanistic aspects of this newly discovered reaction. In an attempt to elucidate reaction pathways, the aromatization reaction was quenched two hours after the reaction commenced. Two intermediates, not found in the products when the reaction was allowed to proceed to completion, were isolated. One of the intermediates (**170**) (**Figure 3-8**) was confirmed by NMR and mass spectrometry to be the Sonogashira coupling product **170**, while the other intermediate (tentatively assigned as **171** but could not be confirmed by spectroscopy) apparently resulted from two rounds of coupling of alkene with *N*-Boc-propargylamine; however, the exact structure of the product could not be established at this time. These two intermediates were individually subjected to reactions with *N*-Boc-propargylamine under the same reaction conditions used for the aromatization reaction, but neither could produce the aromatized product.

Based on these observations, we hypothesized that the catalytic cycle of the reaction contains these two isolated intermediates but in a form that is co-coordinated to either palladium or copper; upon isolation, the intermediates formed in the reaction mixture lose their co-ordination with the metal, thus losing the ability to continue the catalytic cycle. In another attempt to investigate the reaction pathway, intermediate **170** was treated with an alkyne dimer; however, no aromatized products were observed to form, possibly for the same reasons as explained above.



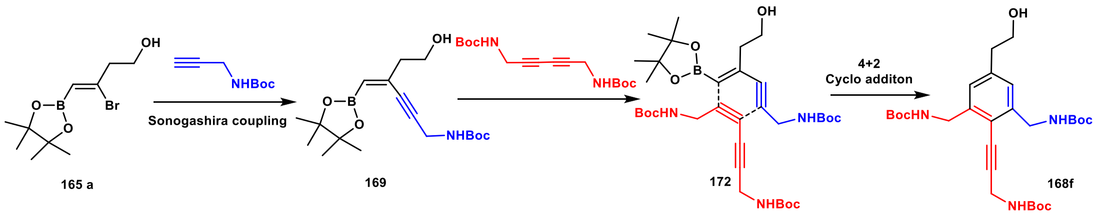
170



171

Figure 3-8: Two intermediates isolated in the aromatization reaction

Since the structure of the second intermediate could not be identified, we proposed a mechanism for the aromatization reaction based on the first intermediate. In the first step of the proposed mechanism, Sonogashira coupling occurs at the quaternary carbon of bromoalkene boronate to give the Sonogashira-coupled product **169**. This may be followed a [4+2] cyclization with a dimerized diyne to produce the final aromatized product **168f** (**Scheme 3-8**).



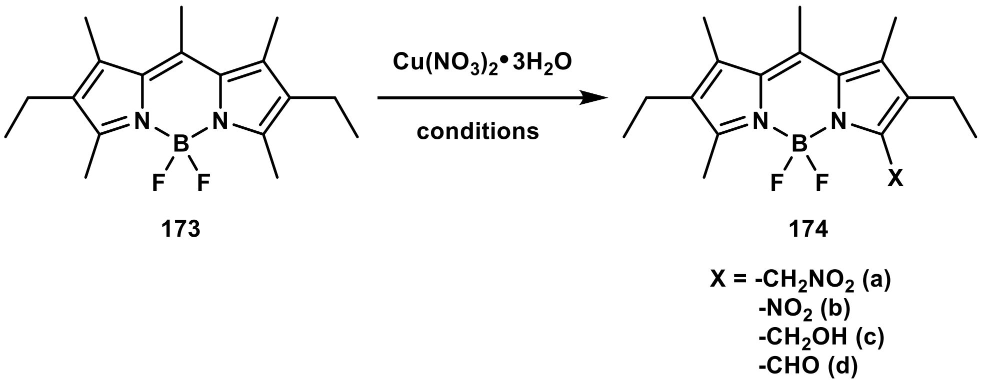
Scheme 3-8: Proposed mechanism for the aromatization reaction

3.2.5 Conclusion

In conclusion, bromoalkene pinacoboronate, intended for sequential Sonogashira and Suzuki coupling reactions, failed to produce the desired coupling product. The failed attempts led to the discovery of a new reaction, where bromoalkene pinacoboronate and terminal alkynes reacted to produce tetrasubstituted benzene under Sonogashira conditions. This reaction has the potential to be used for the synthesis of highly substituted benzene derivatives. The optimized reaction conditions and substrate scope of the aromatization reaction were demonstrated. A few attempts were made to understand the reaction pathway of the reaction.

3.3 Reactions of BODIPY with cupric nitrate

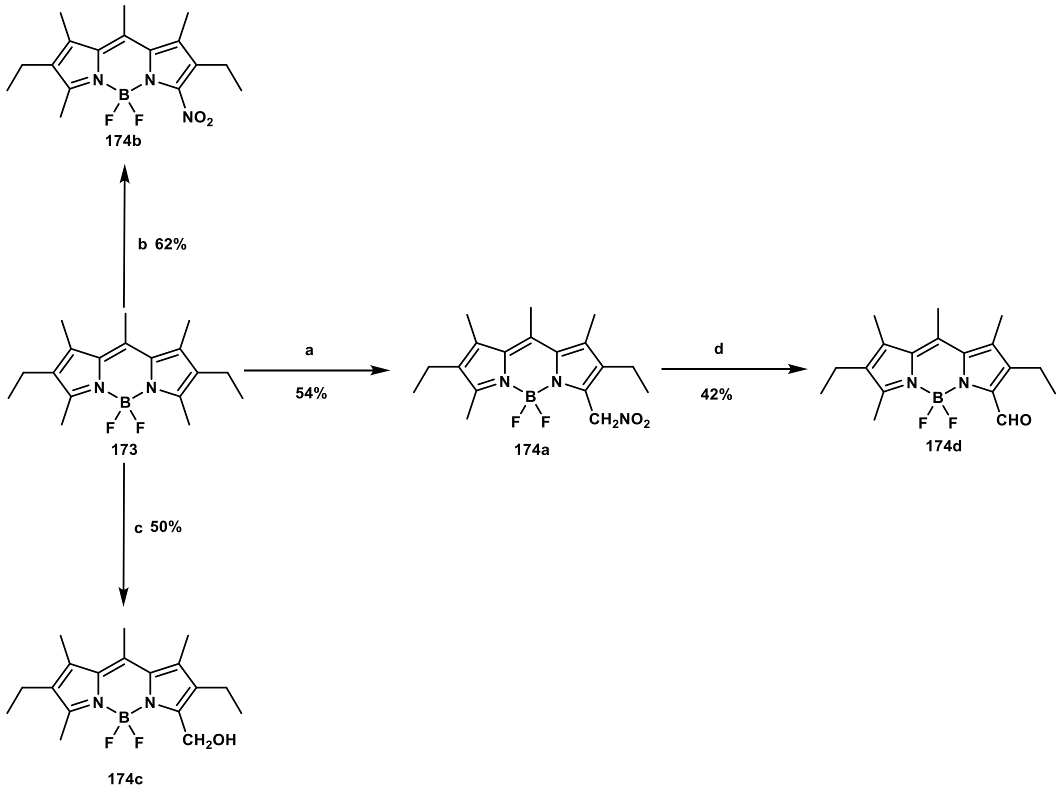
BODIPY fluorophores have found increasing applications in various areas such as biological and material sciences; as such, synthesis of functionalized BODIPYs is an active research topic in the synthetic community (discussed in chapter 2).[62] Although there are a number of reports in the literature for the synthesis of functionalized BODIPYs, the introduction of functional groups to BODIPYs that allow for further transformations remains a challenging task. To this end, we have introduced new chemical transformations of 4,4-difluoro-1,3,5,7,8-pentamethyl-2,6-diethyl-4-bora-3a,4a-diaza-*s*-indacene (**173**) upon treatment with cupric nitrate trihydrate in acetonitrile (**Scheme 3-9**).



Scheme 3-9: Transformations of BODIPY with $Cu(NO_3)_2 \cdot 3H_2O$

3.3.1 Various transformations of BODIPY with $Cu(NO_3)_2 \cdot 3H_2O$

The outcome of the transformations described in **Scheme 3-9** was found to depend on the molar equivalents of cupric nitrate trihydrate and the water content in the reaction mixture. Variations in these conditions could produce different BODIPYs products characterized by a variety of functional groups such as nitro-, nitromethyl-, carboxaldehyde, and hydroxymethyl functionalities (**174 a-d**). Thus, when 4,4-difluoro-1,3,5,7,8-pentamethyl-2,6-diethyl-4-bora-3a,4a-diaza-*s*-indacene (**173**) was treated with a half molar equivalent of cupric nitrate trihydrate in wet acetonitrile and dichloromethane (containing 0.004% water), 4,4-difluoro-3-nitromethyl-1,5,7,8-tetramethyl-2,6-diethyl-4-bora-3a,4a-diaza-*s*-indacene (**174a**) was isolated in 54% yield after column chromatography.



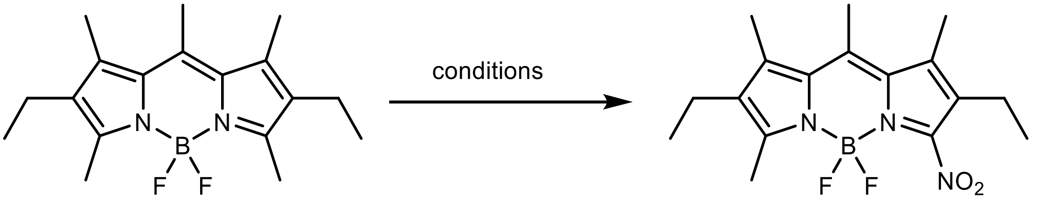
Scheme 3-10: *Reagents and conditions*: a). $Cu(NO_3)_2{\cdot}3H_2O$ (0.5 eq.), wet CH_2Cl_2, wet MeCN; b). $Cu(NO_3)_2{\cdot}3H_2O$ (5 eq.), dry CH_2Cl_2, dry MeCN; c). $Ca(NO_3)_2{\cdot}4H_2O$ (10 eq.), wet CH_2Cl_2, wet MeCN; d). $Cu(NO_3)_2{\cdot}3H_2O$ (5 eq.), wet CH_2Cl_2, wet MeCN.

Treatment of 4,4-difluoro-1,3,5,7,8-pentamethyl-2,6-diethyl-4-bora-3a,4a-diaza-*s*-indacene (**173**) with five molar equivalents of cupric nitrate trihydrate in dry acetonitrile and dichloromethane, however, led to the formation of a mixture of 4,4-difluoro-3-nitro-1,5,7,8-tetramethyl-2,6-diethyl-4-bora-3a,4a-diaza-*s*-indacene (**174b**) and 4,4-difluoro-1,5,7,8-tetramethyl-2,6-diethyl-4-bora-3a,4a-diaza-*s*-indacene-3-carboxyaldehyde (**174d**) in an approximate 6:1 ratio, as determined by NMR spectroscopy. It was further observed that treatment of 4,4-difluoro-1,3,5,7,8 pentamethyl-2,6-diethyl-4-bora-3a,4a-diaza-*s*-indacene (**173**) with ten molar equivalents of calcium nitrate in the presence of 0.1 molar equivalent of cupric nitrate trihydrate in a mixture of acetonitrile and dichloromethane containing 0.02% water led to the formation of 4,4-difluoro-3-hydroxymethyl-1,5,7,8-tetramethyl-2,6-diethyl- 4-bora-3a,4a-diaza-*s*-indacene (**174c**) in 50% isolated yield (**Scheme 3-10**).

3.3.2 Optimization of the reaction conditions for the synthesis of 3-nitro BODIPY 174b

In order to optimize the yield and to provide mechanistic insights, the reaction for the formation of 4,4-difluoro-3-nitro-1,5,7,8-tetramethyl-2,6-diethyl-4-bora-3a,4a-diaza-*s*-indacene (3-nitro BODIPY, **174b**) was carried out under different conditions. Thus BODIPY **173** was first treated with varied equivalents of cupric nitrate trihydrate in the presence or absence of oxygen. The highest yield (62%) for 3-nitro BODIPY **174b** was obtained when BODIPY **173** was treated with five molar equivalents of $Cu(NO_3)_2{\cdot}3H_2O$ in dry ACN/DCM (**table 3-5: entry 1**). Increasing the molar equivalents of cupric nitrate trihydrate from 5 to 10 equivalents lowered the yield to 50% (**table 3-5: entry 2**). In an effort to understand the role of solvents in this transformation, BODIPY **173** was treated with $Cu(NO_3)_2{\cdot}3H_2O$ (10 molar equivalents) in dry DMF, THF, DMSO, and acetone (**table 3-5: entries 3, 4, 5,** and **6**). No reaction was observed under these conditions. These observations seem to suggest that hydration of cupric nitrate plays a role in this transformation. Considering that the donor strengths or Lewis basicity of DMF, THF, DMSO, and acetone are greater than those of dichloromethane and acetonitrile, it is conceivable that water co-ordinated to the metal center might have been displaced in solvents such as DMF, THF, DMSO, and acetone; however, the full extent of this hypothesis is not understood at this time.[162]

Table 3-5: Optimization of conditions for the synthesis of 3-nitro BODIPY 65b



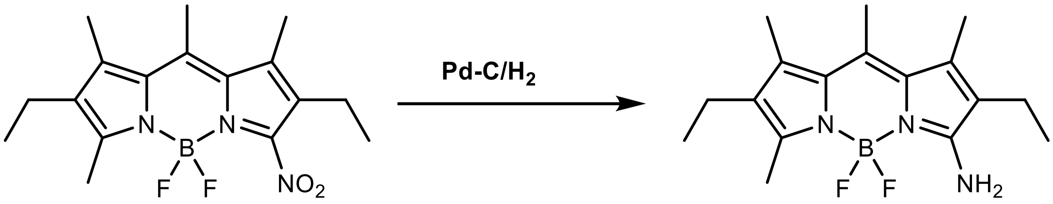
173 **174b**

	metal salts	Solvents	air	174a	174b	174c	175d	Note
	$Cu(NO_3)_2{\cdot}3H_2O$ mol. equiv.							
1	5	Dry DCM/ACN	Y	trace	62%	-	11%	
2	10	Dry DCM/ACN	Y	trace	50%	-	10%	
3	10	Dry DMF	Y	-	-	-	-	n/o
4	10	Dry THF	Y	-	-	-	-	n/o
5	10	Dry DMSO	Y	-	-	-	-	n/o
6	10	Dry Acetone	Y	-	-	-	-	n/o
7	10	Dry ACN	N	9%	12%	-	15%	
8	$Ca(NO_3)_2{\cdot}4H_2O$ 12 mol. equiv.	Dry ACN	Y	-	-	-	-	n/o
10	$AgNO_3$ 12 mol. equiv.	Dry ACN	Y	-	-	-	-	n/o

n/o: not observed

It was also speculated that oxygen could be involved in the conversion of methyl- into the nitro- functional group in this transformation. To verify this, a control reaction was performed between BODIPY **173** and cupric nitrate trihydrate in the absence of oxygen. Degassing was performed by first freezing a solution of BODIPY **173** in acetonitrile with liquid nitrogen, and then evacuating the reaction flask. After repeating the procedure three times in order to ensure that oxygen was completely removed from the reaction flask, $Cu(NO_3)_2{\cdot}3H_2O$ was added to the mixture and again degassed the reaction mixture for three times. The reaction mixture was then brought to room temperature and stirred for 30 min. As expected, in the absence of oxygen, a drastic difference in the yield of 3-nitro BODIPY **174b** (12% versus 62% in the presence of air) was observed (**table 3-5: entry 7**), clearly indicating that oxygen is involved in the transformation of methyl- into the nitro functional group. Once the involvement of oxygen in this reaction was confirmed, the possibility of the reaction occurring through radical intermediates was investigated. For this purpose, an excess of radical quencher (2,2,6,6-tetramethylpiperidine-1-oxyl, TEMPO) was added to the solution prior to the addition of $Cu(NO_3)_2 \cdot 3H_2O$. Virtually no difference was found for this transformation, compared with the reaction carried out in the absence of TEMPO. It is possible that either the reaction does not go through radical intermediates, or the half-life of those radicals is so short that the radicals cannot be quenched by TEMPO before the actual transformation occurs. Use of nitrates such as $Ca(NO_3)_2$ and $AgNO_3$ in this reaction, instead of $Cu(NO_3)_2 \cdot 3H_2O$, did not lead to formation of 3-nitro BODIPY. Although the mechanism involving the conversion of the methyl group of the BODIPY to the nitro group is not clear at this time, the possibility of introducing other functional groups with the use of a similar method was explored. Thus, reactions were performed with various copper salts such as CuI, $CuCl_2$, $Cu(OAc)_2$, and $Cu(SO_4)_2$, but none of these reactions resulted in respective functional group transformations, that is, iodination, chlorinaton, sulfation, and acylation. Future experiments will be required to provide a better understanding of the mechanism of the transformation.

The introduction of functional groups, such as nitro, nitromethyl, carboxaldehyde, and hydroxymethyl, into the BODIPY core with the use of $Cu(NO_3)_2 \cdot 3H_2O$ can be useful for further transformations, while the obtained modified BODIPY compounds can also be used towards the preparation of fluorescent labeling agents. One compound, 3-nitro BODIPY **174b**, is of particular interest. While the fluorescence of this compound is virtually quenched ($\Phi = 0.01$), upon reduction by catalytic hydrogenation (**Scheme 3-11**), the resulting 3-amino BODIPY **175** becomes rather fluorescent ($\Phi = 0.40$). This compound is anticipated to be useful as a fluorescent turn-on sensor for reductive environments.



174b
non-fluoroscent

175
fluorescent

Scheme 3-11: Reduction of compound **65b** to amino BODIPY

3.3.3 Conclusion

Various functional groups such as 3-nitro, nitromethyl, carbaxaldehyde, and hydroxymethyl have been introduced into the BODIPY core under mild conditions with the use of a single reagent $Cu(NO_3)_2{\cdot}3H_2O$. Variations in the reaction conditions, such as changing the equivalents of $Cu(NO_3)_2{\cdot}3H_2O$ and altering water content, resulted in the formation of different BODIPYs containing one of the functionalities described above. These functionalized BODIPY compounds are useful for conjugating BODIPYs to several classes of molecules of interest. While 3-nitro BODIPY **174b** is virtually non-fluorescent, fluorescence is restored upon reduction of nitro- to amino group, and thus this modification has the potential to be useful as a turn-on fluorescence probe for the reductive environment.

3.4 Microwave-assisted synthesis of BODIPY-sugar conjugates through Click chemistry and assembly of the conjugate into liposomes

In recent years, the fluorescent labeling of glycans has gathered increased popularity in applications such as fluorescent imaging of systems involving glycans, and as fluorescent substrates for glycan-processing enzymes. The use of fluorescently labeled glycans can aid in the study of glycan trafficking and localization, and glycosytransferases and glycosidases.[163] Research in our laboratory has been focused on the 4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene (BODIPY) fluorophore due to its practicle properties, such as relatively high photochemical stability, high fluorescent quantum yields, and relative independence of fluorescence on pH. The currently presented project aimed at conjugating four glycans to modified BODIPY fluorophores.

Glycan: beta-Glc (176)
beta-GlcNAc (177)
beta-Lac (178)

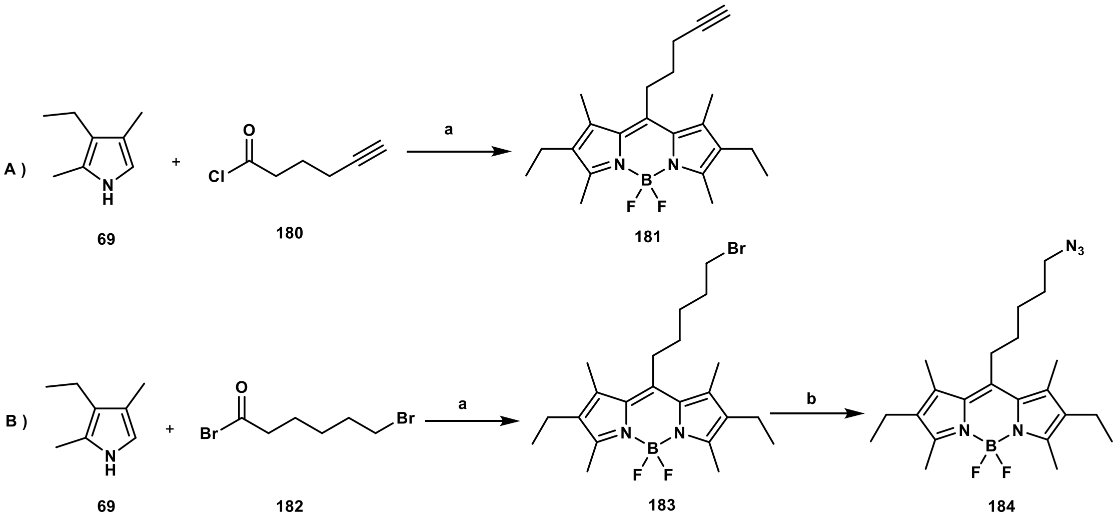
Glycan: beta-GlcNAc (179)

Figure 3-9: Glycans with either an alkyne or azide

These glycans, gifted to us by the Sussex Research Laboratories in Ottawa, include glucoside (Glc) **176**, *N*-acetyl-glucosamine glycoside (GlcNAc) **177** and **179**, and lactoside **178**, and have either azido or alkynyl functional groups at the terminal position of the PEG spacer covalently *β*-linked to sugars at the anomeric position (**Figure 3-9**). Since glycan substrates contain either alkyne or azido functional groups attached through a spacer, click chemistry was chosen as the method of choice for the conjugation of these sugar molecules to the BODIPY, as click chemistry is a well-known method in organic synthesis, and is often chosen as a suitable technique for conjugation chemistry due to its compatibility with many functional groups and lack of byproduct formation.

3.4.1 Synthesis of BODIPY derivatives with alkyne and azido functional groups

In order to perform conjugations with carbohydrates using Click chemistry, an azido or terminal alkynyl functionality needed to be incorporated into BODIPY. For this purpose, alkynyl BODIPY **181** was obtained through the sequential treatment of pyrrole **69** with acid chloride **180**, boron trifluoride diethyl etherate, and triethylamine to give the product in 37 % yield (**Scheme 3-12A**). Acid chloride **180** was prepared through the treatment of the corresponding acid with oxalyl chloride in the presence of DMF as a catalyst, using dichloromethane as a solvent. The reaction mixture was distilled to give acid chloride **180**. The azido BODIPY compound **184** was synthesized using a two-step process.



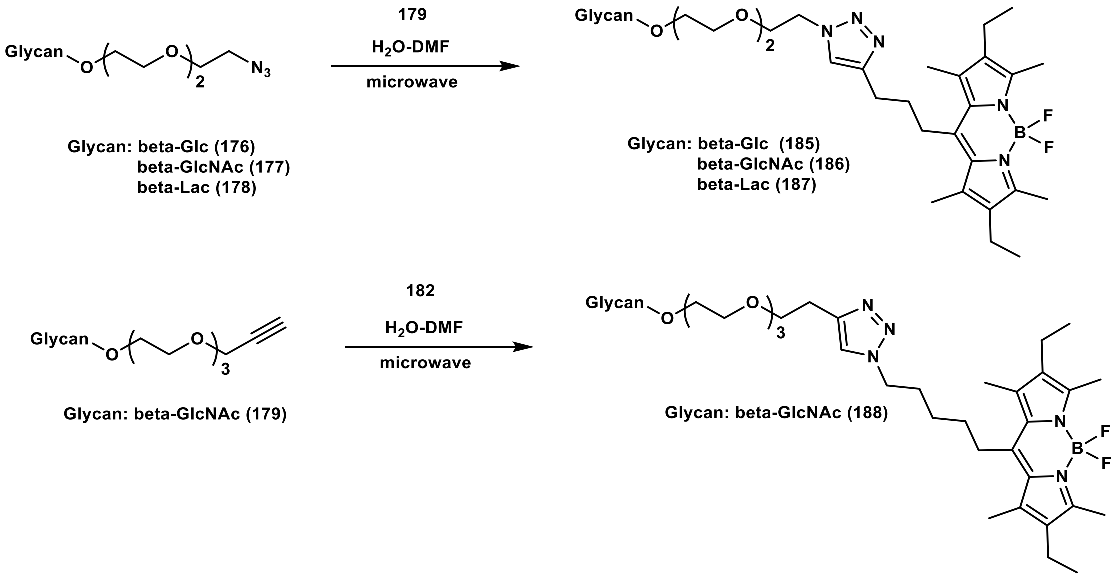
Scheme 3-12: *Reagents and conditions*: a). i. Dry DCM, reflux, 3h, ii. Et_3N, $BF_3{\cdot}OEt_2$, reflux 3 h. b). NaN_3, H_2O-DMF, 10 min, 80°C, MW.

First, bromo BODIPY **183** was synthesized by the same method used for the synthesis of alkynyl BODIPY **181**. Next, bromo BODIPY **183** was treated with sodium azide in a water-DMF mixture under microwave irradiation for 10 min to produce the azido BODIPY **184** in 90% yield (**Scheme 3-12B**). It is worth noting that while the microwave-assisted transformation from bromo BODIPY **183** to azido BODIPY **184** proceeded smoothly and was completed in five minutes, an attempt at the same transformation under conventional heating in DMF in oil-bath and stirring for 3 hrs resulted in a much lower yield (<30%).

3.4.2 Synthesis of BODIPY-sugar conjugates through click reaction

Synthesis of BODIPY sugar conjugates was attempted by using click reaction conditions. Alkynyl BODIPY **181** and azido sugar **178** were treated with 2 mol% $CuSO_4 \cdot 5H_2O$ and 10 mol% sodium ascorbate in a H_2O – *t*-BuOH mixture at room temperature for 2 days. Surprisingly, on the TLC no product spot was observed under these conditions. The same reaction mixture was loaded with an additional 10 mol% $CuSO_4$ and 20 mol% sodium ascorbate and stirred for another two days under reflux, no formation of the product spot was observed either. While it was speculated that the solubility of the nonpolar BODIPY in polar solvents might have led to the failure of the reaction, the same reaction was performed with 10 mol% $CuSO_4$ and 20 mol% sodium ascorbate in DMF to increase the solubility of the BODIPY substrate. Surprisingly, however, these attempts also failed to yield the desired product. The reaction was also carried out in several other solvents such as MeOH and DMSO, and at elevated temperatures up to 160°C with the use of an oil-bath, however, all these attempts failed to give the desired click chemistry coupling product. Similarly, all attempts for the click reaction between azido BODIPY **184** and alkynyl sugar **179** failed using the reaction conditions described above.

Finally, the click reaction was performed under microwave (MW) irradiation at 80 °C. The reactions were complete in 15 min to produce four BODIPY-sugar conjugates **185**, **186**, **187**, and **188** in yields ranging from 30-35 % (**Scheme 3-13**). One of the starting materials (sugar, as the limiting reagent) of the reaction mixture was fully consumed, but lower isolated yields were observed. It is possible that the sugar starting material partially degraded upon microwave heating and reasoned for lower yields. To verify this possibility, shortening of microwave exposure time from 15 to 3 min led to the isolation of click reaction products in much higher yields (70-80%). All BODIPY-sugar conjugates were purified by C18 reverse-phase FPLC with an acetonitrile-water mixture as an eluting solvent.



Scheme 3-13: Synthesis of sugar-BODIPY conjugates

It was interesting to observe the drastic differences in yield obtained for the click reaction between alkynyl (or azido)-BODIPY and azido (or alkynyl) sugar under oil-bath heating, in contrast to microwave heating. It is possible that owing to the extreme contrast in hydrophobicity/polarity between glycan and BODIPY compounds, the reaction between azido and alkynyl functionalities is entropically disfavored. Although there have been debates on the existence of “microwave-specific effect”,[164, 165] it has been suggested in the literature that the presence of an electric field may affect the orientation of dipolar molecules, and thus could affect the entropic aspect of certain reactions.[165, 166]

Although regioselectivity for the click reaction in the presence $CuSO_4$ has been known to favor the formation of 1,4-substituted triazoles, since our click reaction was performed under MW irradiation, we set out to investigate the regioselectivity outcome of the BODIPY-sugar conjugated products by NOESY spectra. As expected, products of the click reaction under MW irradiation exhibited selectivity exclusively for 1, 4-substitution. The confirmation for the 1,4-substitution was obtained through an evaluation of NOE signals between the triazole C-H proton and methylene protons in the NOESY spectra of the click reaction products of sugar **179** and BODIPY **184** substrates (**Figure 3-10**).

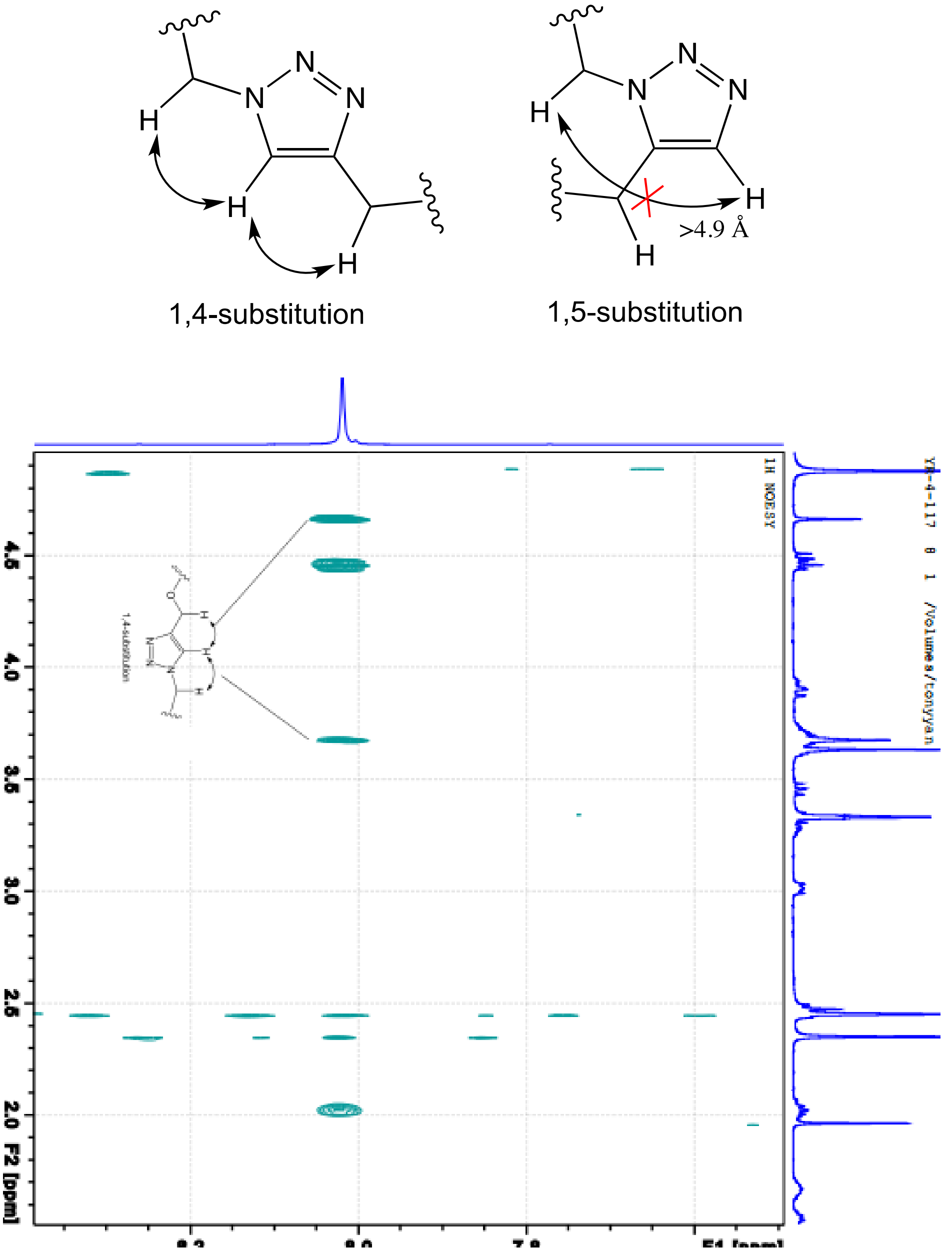


Figure 3-10: NOESY spectra of click reaction product

3.4.3 Formation of liposomes by the BODIPY-sugar conjugate

Upon dissolution of conjugate **183** in water, the initially clear solution turned turbid over time. This observation led us to speculate that these molecules have the ability to form liposomes. Since BODIPY-sugar conjugates are characterized by an amphiphilic nature, we hypothesized that the conjugate in the water solution reorganized into ordered structures commonly observed in liposomes. To verify the ability of these BODIPY-sugar molecules to form liposomes, a standard protocol for the preparation of liposomes from lipids was followed.[167] For the purpose of this investigation, a BODIPY – β-Glc conjugate **185** was dissolved in chloroform, and the solvent evaporated to form a thin layer around the round bottom flask. Preheated water was then added to the mixture to break the thin layer into hydrated sheets. The mixture was then further mixed by grinding with glass beads. The resulting solution was heated to 70°C, and then brought to room temperature. Bright field microscopy (Figure 3-11A) confirmed the formation of spherical structures. The ability of this conjugate to form a bilayer liposome structure is consistent with its overall organization of hydrophobicity/polarity, where the presence of both hydrophobic and polar moieties in the same molecule in a given conjugate renders the molecule amphiphilic, a property that allows for its assembly into bilayers in a manner similar to lipids, as represented in **Figure 3-11**.

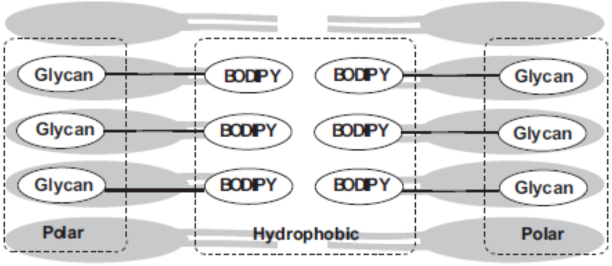


Figure 3-11: A cartoon illustrating the bilayer formed by the BODIPY–sugar conjugates in aqueous solutions

Interestingly, our attempts to generate liposomes from the other three conjugates under the same conditions were unsuccessful, presumably due to differences in the overall size and geometry of these conjugates.

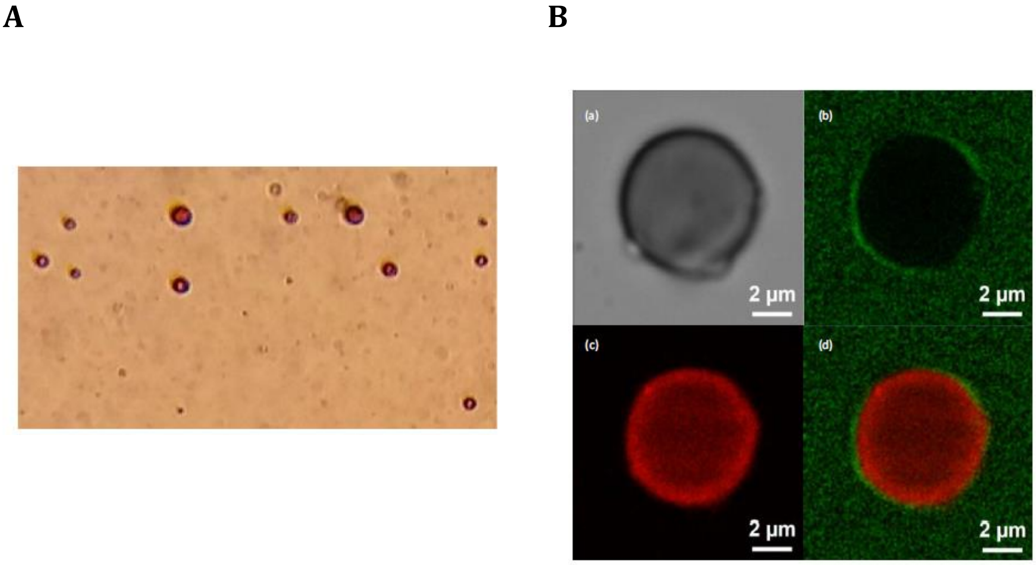


Figure 3-12: A. Bright field microscope image of a cluster of liposomes formed from BODIPY-conjugate of sugar **185**; B. Microscope images of a single liposome recorded with a Carl Zeiss Axio Observer. Z1 inverted light/epifluorescence microscope with ApoTome.2 optical sectioning. (a) Bright field, (b) green fluorescence (ex. 488 nm, em. 509 nm), (c) red fluorescence (ex. 587 nm, em. 610 nm), and (d) overlay of the green and red fluorescence channels (emission spectra obtained by a specific channel to collect around the emission values mentioned above)

Fluorescent microscope images of the obtained liposomes are shown in **Figure 3-12**. A closer look at a single liposome-like structure, depicted in **Figure 3-12A**, is provided in **Figure 3-12B**. The bright field image (**Figure 3-12A**) corroborates with the fluorescence images of the same “giant” liposome (**Figure 3-12 Ba to d**), with a diameter of *ca*. 6 μm. Surprisingly, when the preparation of liposomes was excited at 488 nm, *i.e*. at the green fluorescence channel, there was no detectable fluorescence inside the liposomes at emission wavelength 509 nm, while the solution outside the liposomes fluoresced green (**Figure 3-12Ba**). However, when the liposome preparation was excited at 587 nm, i.e. at the red fluorescence channel, a strong red fluorescence was observed on the periphery of the liposomes (**Figure 3-12Bc**). Overlay of the two images obtained from the green and red fluorescence channel excitation of liposome exhibited different fluorescence properties inside and outside of the liposome (**Figure 3-12Bd**). The red shift of BODIPY emission observed on the periphery of the liposomes might be due to the high effective concentration of fluorophore (BODIPY) in the liposome bilayer, leading to the formation of BODIPY excimers that emit at longer wavelengths, as suggested in the literature.[168]

3.4.4 Conclusion

Click reactions between BODIPY and sugars were unsuccessful to give the respective conjugates under various conditions when heated in an oil-bath. To our surprise, when the reaction was performed in the presence of microwaves at 80°C for 3 min, the BODIPY-sugar conjugates were obtained at moderate to high yields. One of the BODIPY-sugar conjugate (**183**) was observed to form liposomes upon rehydration. The photophysical properties of these liposomes were characterized by fluorescence microscopy. Typically BODIPY emits green fluorescence, however, BODIPY present in the liposomes exhibits red fluorescence, most probably due to the higher effective concentration of BODIPY that leads to excimer formation.

Chapter 4
Experimental Section

4.1 General Experimental Procedures

All chemicals were purchased from Aldrich or TCI America and used without further purification unless stated otherwise. Glycans used for conjugating to the BODIPYs were gifts from Sussex Research Laboratory Inc, Ottawa, Canada. Solvents were distilled: DMF, *i*Pr_2EtN, Et_2N and pyridine from CaH_2; CH_2Cl_2 from P_2O_5; MeOH from magnesium methoxide; THF, Benzene and Toluene from Na/benzophenone. Desican 230–400 mesh silica gel 60 was used for flash column chromatography. Thin layer chromatography was performed on Silicycle SiliaPlate F-254 TLC plates using the following solvent system:

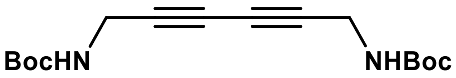
A: hexanes
B: hexane-dichloromethane (70:30, v/v)
C: hexane-ethylacetate (90:10, v/v)
D: hexane-ethylacetate (60:40, v/v)
E: hexane-ethylacetate (50:50, v/v)
F: dichloromethane
G: dichloromethane-methanol (94:6, v/v)
H: dichloromethane-methanol (92:8, v/v)
I: dichloromethane-methanol (90:10, v/v)
J: dichloromethane-methanol (88:12, v/v)
K: dichloromethane-methanol-water (40:10:1, v/v)

NMR spectra were obtained on a Bruker Avance 300, 400 or 600 MHz instrument and are referenced to the residual proton signal of the deuterated solvent for ^1H spectra, and to the carbon multiplet of the deuterated solvent for ^{13}C spectra.

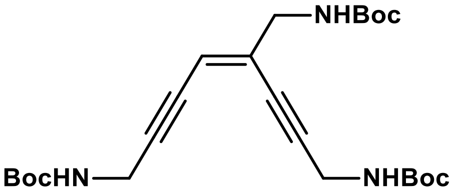
Tetramethylsilane was used as an internal standard. Spectroscopic data are reported as follows: (multiplicity, number of protons, coupling constant), where s = singlet, d = doublet, t = triplet, q = quartet, dd = doublet of doublets, and m = multiplet. Chemical shifts are reported in ppm and *J* values are given in Hz. Low and high resolution mass spectra were obtained with Kratos Concept 1S high resolution mass spectrometer using electron impact sources interfaced with DART 32 bit acquisition system through a Sun Sparcstation 10 and Mach 3 software.

4.2 Detailed Experimental Procedures

4.2.1 Coupling reaction of *N*-Boc-propargylamine



163a



152a

To a solution of *N*-Boc-propargylamine (0.200 g, 1.29 mmol) in dry benzene (6.0 mL) were added $Pd(PPh_3)_2Cl_2$ (18 mg, 2 mol%) and *n*-butylamine (2 mL). The solution was frozen followed by addition of CuI (5 mg, 2 mol%). While frozen, the reaction mixture was evacuated and then purged with argon. After this degassing procedure was repeated two more times, the mixture was stirred at 45°C for 15 h in an argon atmosphere. After the reaction mixture was evaporated under reduced pressure, the residue was taken up with dichloromethane (50 mL) and extracted with saturated aqueous sodium hydrogen carbonate (10 mL). The layers were separated and the organic layer was dried ($MgSO_4$) and evaporated under reduced pressure. The residue was purified by column chromatography on silica gel. The appropriate fractions, which were eluted with ethyl acetate–hexanes (30:70 v/v), were combined and evaporated under reduced pressure to give the trimerized product **152a** as a white solid (80 mg, 40%). Homo coupling dimer **163a** was also isolated as a white solid (20 mg, 10%).

Dimer: **163a**

*R*f = 0.73 (System E).

M.p. 106–108°C (ethanol).

δ_H ($CDCl_3$): 1.46 (18 H, s), 4.00 (4 H, d, *J* = 4.6), 4.77 (2 H, s, br).

δ_C ($CDCl_3$): 28.3, 31.0, 67.3, 74.8, 80.3, 155.1.

MS (ESI^+) found 331.2, $C_{16}H_{24}N_2NaO_4^+$ requires 331.16.

Trimer: **152a**

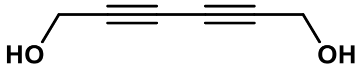
*R*f = 0.59 (System E).

δ_H ($CDCl_3$): 1.44, 1.45, 1.46 (three singlets integrate 27 H, br), 3.80 (2 H, d, *J* = 5.5), 3.86 (4 H, br), 4.89 (1 H, s, br), 5.00 and 5.08 (2 H, br), 5.78 (1 H, s).

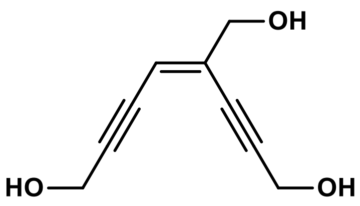
δ_C ($CDCl_3$): 28.4, 31.3, 44.9, 79.8, 79.9, 80.2, 92.4, 93.9, 114.9, 132.0, 155.4, 155.5.

MS (ESI^+) found 464.2741,$C_{22}H_{38}N_3O_6^+$ requires 464.2761

4.2.2 Coupling reaction of propargyl alcohol



163b



152b

To a solution of propargyl alcohol (0.100 g, 1.78 mmol) in dry benzene (9.0 mL) were added $Pd(PPh_3)_2Cl_2$ (119 mg, 10 mol%) and *n*-butylamine (2 mL). The mixture was frozen followed by addition of CuI (32 mg, 10 mol%). The mixture was evacuated and purged with argon. After this degassing procedure was repeated two more times, the mixture was stirred at 45°C overnight in an argon atmosphere. The solvents were removed under reduced pressure and the residue was purified by column chromatography on silica gel without aqueous work up. The appropriate fractions, which were eluted with dichloromethane–methanol (93:7 v/v), were combined and evaporated under reduced pressure to give the trimerized product **152b** as a light yellow oil (30 mg, 31%). Homo coupling product **163b** was also obtained as a light yellow solid (50 mg, 53%).

Dimer: **163b**

R_f = 0.55 (System H).

δ_H (DMSO-d$_6$): 4.17 (s, 4 H), 5.40 (2 H, s, br, ex).

δ_C (DMSO-d$_6$): 49.8, 68.4, 80.0.

HRMS (EI) found 110.03661, $C_6H_6O_2$ requires 110.03678.

Trimer: **152b**

R_f = 0.38 (System H).

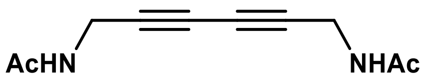
δ_H (DMSO-d$_6$): 3.98 (2 H, dd, *J* = 5.8 and 1.7), 4.25 (2 H, dd, *J* = 5.9 and 1.7), 4.26 (2 H, d, *J* = 5.8), 5.27 (1 H, t, *J* = 5.9, ex), 5.32 (1 H, t, *J* = 5.9, ex), 5.36 (1 H, t, *J* = 5.9, ex), 6.00 (1 H, t, *J* = 1.9).

δ_C (DMSO-d$_6$): 49.9, 50.0, 63.7, 81.2, 81.8, 96.2, 97.7, 112.4, 135.5.

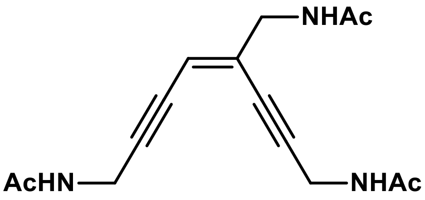
Mass spectrometric analysis of this trimer was unsuccessful, however, upon acetylation, the corresponding triacetyl analogue gave correct mass.

HR-EI found 292.07806, $C_{15}H_{16}O_6$ requires 292.09469.

4.2.3 Coupling reaction of *N*-acetyl propargylamine



163c



152c

To a solution of *N*-acetyl propargylamine (200 mg, 2.06 mmol) in dry benzene (6 mL) were added $Pd(PPh_3)_2Cl_2$ (71 mg, 5 mol%) and *n*-butylamine (2 mL). The solution was frozen followed by addition of CuI (19 mg, 5 mol%). The mixture was evacuated and purged with argon. The degasing procedure was repeated two more times, and the mixture was stirred at 45°C overnight in an argon atmosphere. The products were evaporated under reduced pressure and the residue was purified by column chromatography on silica gel. The appropriate fractions, which were eluted with dichloromethane–methanol (92:8 v/v), were combined and evaporated under reduced pressure to give the trimerized product **152c** as a light yellow solid (120 mg, 61%). Homo coupling dimer product **163c** was also isolated as light yellow solid (40 mg, 20%).

Dimer: **163c**

R_f = 0.38 (System H).

δ_H (DMSO-d_6): 1.82 (6 H, s), 3.95 (4 H, d, *J* = 5.1), 8.35 (2 H, s, br).

δ_C (DMSO-d_6): 22.7, 28.9, 66.2, 76.8, 169.5.

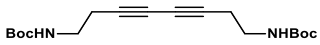
Trimer: **152c**

R_f = 0.28 (System H).

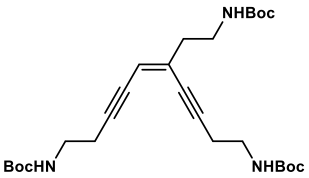
δ_H ($CDCl_3$): 2.02 (3 H, s), 2.04 (3 H, s), 2.05 (3 H, s), 3.89 (2 H, d, *J* = 5.8), 4.16 (2 H, dd, *J* = 5.2 and 1.7), 4.18 (2 H, d, *J* = 5.2), 5.78 (1 H, s), 6.79 (1 H, s), 7.30 (1 H, s), 7.44 (1 H, s).

δ_C ($CDCl_3$): 22.92, 22.93, 23.1, 30.1, 30.2, 43.6, 80.0, 80.1, 92.2, 93.7, 115.7, 131.8, 170.7.

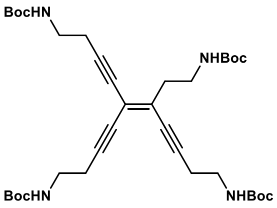
4.2.4 Coupling reaction of *N*-Boc-amino-1-butyne



163d



152d



164d

To a solution of *N*-Boc-amino-1-butyne (100 mg, 0.59 mmol) in dry benzene (3 mL) were added $Pd(PPh_3)_2Cl_2$ (20 mg, 5 mol%) and *n*-butylamine (0.5 mL). The solution was frozen followed by addition of CuI (5.6 mg, 5 mol%). The frozen mixture was evacuated and purged with argon. This degassing procedure was repeated two more times and the mixture was stirred at 45°C overnight in an argon atmosphere. The products were evaporated under reduced pressure and the residue was purified by column chromatography on silica gel. The appropriate fractions, which were eluted with ethyl acetate–hexanes (40:60 v/v), were combined and evaporated under reduced pressure to give the trimerized product **152d** as a light yellow solid (10 mg, 10%).

Tetramerized **164d** and dimer **163d** coupling products were also isolated as a light yellow solid (15 mg, 10%) and white solid (60 mg, 60%), respectively.

Dimer: **163d**

R_f: 0.68 (System E).

M.p. 130–132°C (ethanol).

δ_H ($CDCl_3$): 1.45 (18 H, s), 2.46 (4 H, t, *J* = 6.3), 3.27 (4 H, dt, *J* = 5.5 and 6.2), 4.91 (2 H, s, br).

δ_C ($CDCl_3$): 20.8, 28.4, 39.2, 66.4, 75.1, 79.6, 155.6.

MS: $C_{18}H_{28}N_2NaO_4$ requires 359.2, ESI-MS$^+$ found 359.2.

Trimer: **152d**

R_f: 0.47 (System E).

δ_H ($CDCl_3$): 1.45 (17 H, br), 2.36 (2 H, t, *J* = 5.8), 2.59 (2 H, t, *J* = 5.9), 2.62 (2 H, *J* = 5.9), 3.31–3.36 (6 H, m), 4.77 (1 H, s, br), 5.00 (1 H, s, br), 5.20 (1 H, s, br), 5.64 (1 H, s).

δ_C ($CDCl_3$): 21.2, 21.3, 28.4, 37.3, 39.1, 39.5, 79.3, 79.5, 79.9, 80.4, 92.6, 95.6, 115.9, 131.9, 155.7, 155.8.

MS: $C_{27}H_{44}N_3NaO_6$ requires 528.3, ESI-MS$^+$ found 528.3.

Tetramer: **164d**

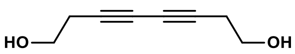
R_f: 0.35 (System E).

δ_H ($CDCl_3$): 1.45 (36 H, s, br), 2.59–2.66 (8 H, m), 3.31–3.36 (8 H, m), 4.93, 5.04, 5.23 (4 H, br, m).

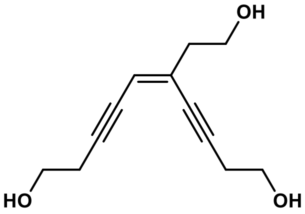
δ_C ($CDCl_3$): 21.2, 21.7, 28.4, 29.7, 35.1, 39.1, 39.4, 40.5, 78.1, 79.4, 80.1, 81.6, 90.7, 95.5, 110.8, 135.3, 155.7, 155.9.

MS: $C_{36}H_{56}N_4NaO_8$ requires 695.4, ESI-MS$^+$ found 695.5.

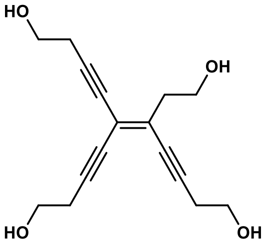
4.2.5 Coupling reaction of 3-butyne-1-ol



163e



152e



164e

To a solution of 3-butyne-1-ol (100 mg, 1.42 mmol) in dry benzene (6 mL) were added $Pd(PPh_3)_2Cl_2$ (24 mg, 2.5 mol%) and *n*-butylamine (1.5 mL). The solution was frozen followed by addition of CuI (6 mg, 2.2mol%). The mixture was evacuated and purged with argon. This degasing procedure was repeated two more times and the mixture was stirred at 45°C for 45 min in an argon atmosphere. The products were evaporated under reduced pressure and the residue was purified by column chromatography on silica gel. The appropriate fractions, which were eluted with dichloromethane–methanol (92:8 v/v), were combined and evaporated under reduced pressure to give the trimerized product **152e** as a light yellow oil (30 mg, 30%). Tetramerized **164e** and dimer **163e** coupling products were also isolated as a colorless solid (20 mg, 20%) and light yellow oil (23 mg, 23%), respectively.

Dimer: **163e**

R$_f$: 0.50 (System H).

δ_H (DMSO-d_6): 2.40 (4 H, t, *J* = 6.6), 3.49 (4 H, dt, *J* = 6.6 and 5.5), 4.89 (2 H, d, *J* = 5.5).

δ_C (DMSO-d_6): 23.4, 59.8, 66.4, 76.6.

HRMS (EI) found 138.06795, $C_8H_{10}O_2$ requires 138.06808.

Trimer: **152e**

R$_f$: 0.40 (System H).

δ_H ($CDCl_3$): 2.46 (2 H, t, *J* = 5.9), 2.68 (2 H, dt, *J* = 1.9 and 5.9), 2.70 (2 H, t, *J* = 5.9), 3.77 (2 H, t, *J* = 5.8), 3.79 (2 H, t, *J* = 5.9), 3.83 (2 H, t, *J* = 6.0), 5.74 (1 H, s).

δ_C ($CDCl_3$): 24.1, 39.9, 60.6, 60.7, 60.9, 80.9, 81.6, 92.3, 94.9, 116.6, 131.7.

MS (ESI$^+$) found 209.2, $C_{12}H_{17}O_3^+$ requires 209.1.

Tetramer: **164e**

R$_f$: 0.26 (System H).

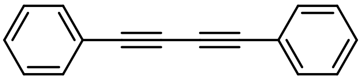
δ_H ($CDCl_3$): 2.67 (2 H, t, *J* = 6.0), 2.69 (2 H, t, *J* = 6.0), 2.73 (2 H, t, *J* = 5.8), 2.75 (2 H, t, *J* = 5.8), 3.78 and 3.79 and 3.80 (three sets of triplets, total 6 H, *J* = 5.8), 3.87 (2 H, t, *J* = 6.0).

δ_H (DMSO-d_6): overlapping signals with DMSO solvent residuals, 3.48–3.56 (8 H, m), 4.63 (1 H, t, *J* = 5.4, ex), 4.84 and 4.85 and 4.87 (three sets of triplets, total 3 H, *J* = 5.6, ex).

δ_C (DMSO-d_6): 23.8, 23.9, 24.3, 38.3, 59.6, 60.0, 60.1, 60.2, 78.1, 79.9, 81.5, 91.3, 95.9, 99.9, 110.2, 135.5.

MS (EI) found 276, $C_{16}H_{20}O_4$ requires 276.

4.2.6 Coupling reaction of phenyl acetylene



163g

To a solution of phenylacetylene (100 mg, 0.98 mmol) in dry benzene (5 mL) were added $Pd(PPh_3)_2Cl_2$ (68 mg, 10 mol%) and *n*-butylamine (1 mL). The solution was frozen followed by addition of CuI (18 mg, 10 mol%). The mixture was evacuated and purged with argon. The degassing procedure was repeated two more times and the mixture was stirred at 45°C for 45 min in an argon atmosphere. The products were evaporated under reduced pressure and the residue was purified by column chromatography on silica gel. The appropriate fractions, which were eluted with ethyl acetate–hexanes (10:90 v/v), were combined and evaporated under reduced pressure to give homodimer coupling product **163g** as a white solid (70 mg, 69%).

R_f: 0.48 (System A).

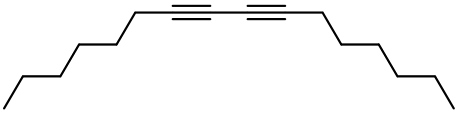
M.p. 96–97°C (Ethanol).

δ_H ($CDCl_3$): 7.34–7.42 (6 H, m), 7.56–7.59 (4 H, m).

δ_C ($CDCl_3$): 74.0, 81.6, 121.8, 128.5, 129.2, 132.5.

HRMS (EI) found 202.07790, $C_{16}H_{10}$ requires 202.06825.

4.2.7 Coupling reaction of 1-octyne



163h

To a solution of 1-octyne (500 mg, 4.54 mmol) in dry benzene (16 mL) were added $Pd(PPh_3)_2Cl_2$ (63 mg, 2 mol%) and *n*-butylamine (4 mL). The solution was frozen followed by addition of CuI (17 mg, 2 mol%). The mixture was evacuated and purged with argon. The degassing procedure was repeated two more times and the mixture and then stirred at 45°C for 45 min in an argon atmosphere. The products were evaporated under reduced pressure and the residue was purified by column chromatography on silica gel. The appropriate fractions, which were eluted with hexanes were combined and evaporated under reduced pressure to give homo coupling dimer **163h** as a viscous colorless oil (200 mg, 40%).

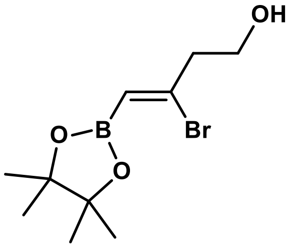
*R*f: 0.59 (System A).

δ_H ($CDCl_3$): 0.90 (6 H, t, *J* = 6.8), 1.27–1.44 (12 H, m), 1.48–1.58 (4 H, m), 2.26 (4 H, t, *J* = 7.0).

δ_C ($CDCl_3$): 14.0, 19.2, 22.5, 28.3, 28.5, 31.1, 65.2.

HRMS (EI) found 218.20336, $C_{16}H_{26}$ requires 218.20345.

4.2.8 (*Z*)-3-Bromo-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-3-buten-1-ol



165a

To a cooled (ice–water bath) solution of boron tribromide (8.00 g, 31.9 mmol) in dry dichloromethane (20 mL) was added drop-wise a solution of 3-butyn-1-ol (1.50 g, 21.4 mmol) in dry dichloromethane (20 mL). After the addition was completed the solution was brought to room temperature and stirred for 3 h. Upon cooling (ice–water bath), a solution of pinacol (3.85 g, 32.6 mmol) and dry triethylamine (5.97 mL, 42.8 mmol) in dry dichloromethane (20 mL) was added drop-wise. After the reaction mixture was stirred for 12 h at room temperature, the products were extracted with brine solutions

(3×20 mL). The organic layer was dried over anhydrous $MgSO_4$ and evaporated under reduced pressure to give the crude product, which was purified by column chromatography on silica gel (eluted with 0–60% ethyl acetate in hexanes) to give the *title compound* **165a** as a colorless oil (2.17 g, 37%). The product contains *ca*. 14% inseparable *E*-isomer as indicated by NMR spectra.

R_f (System D): 0.44

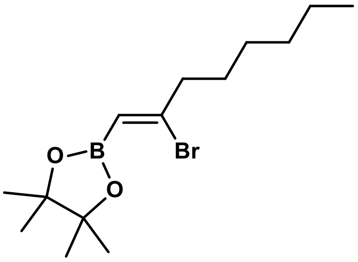
δ_H ($CDCl_3$): 1.306 and 1.308 (together 12 H), 2.77 (2.3 H, including signals from the *E*-isomer, t, *J* = 5.8), 3.84 (2 H, dt, *J* = 1.0 and 5.9), 6.01 (1 H, s).

δ_C ($CDCl_3$): 24.8, 48.3, 60.2, 83.8, 140.4.

δ_B ($CDCl_3$): 29.2 (*Z*-isomer), 22.5 (*E*-isomer).

Mass (ESI positive) found 299.1, $C_{10}H_{18}BBrNaO_3^+$ requires 299.04.

4.2.9 (*Z*)-2-Bromo-1-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1-octene



165b

To a cooled (ice–water bath) solution of boron tribromide (6.82 g, 27.2 mmol) in dry dichloromethane (30 mL) was added drop-wise a solution of 1-octyne (3.0 g, 27.2 mmol) in dry dichloromethane (30 mL). After the addition was completed the solution was brought to room temperature and stirred for 3 h. Upon cooling (ice–water bath), a solution of pinacol (6.42 g, 54.3 mmol) and dry triethylamine (3.21 mL, 23.0 mmol) in dry dichloromethane (20 mL) was added drop-wise. After the reaction mixture was stirred for 12 h at room temperature, the products were extracted with brine solutions

(3×20 mL). The organic layer was dried over anhydrous $MgSO_4$ and evaporated under reduced pressure to give the crude product, which was purified by column chromatography on silica gel (eluted with 0–30% of ethyl acetate in hexanes) to give the *title compound* as a colorless oil (2.00 g, 23%). The product contains *ca*. 8% inseparable *E*-isomer as indicated by the ^{1}H NMR spectrum.

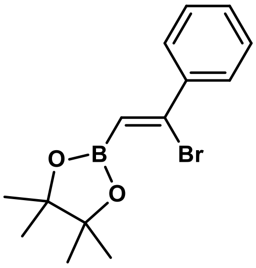
R_f (System D): 0.71.

δ_H ($CDCl_3$): 0.89 (3 H, t, *J* = 6.9), 1.31 (18 H, m), 1.58 (2 H, m), 2.52 (2 H, t, *J* = 7.5), 5.88 (1 H, s).

δ_C ($CDCl_3$): 14.0, 22.5, 24.8, 28.0, 28.2, 31.5, 45.3, 83.6, 145.3.

δ_B ($CDCl_3$): 29.1.

4.2.10 (*Z*)-*α*-Bromo-*β*-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-styrene

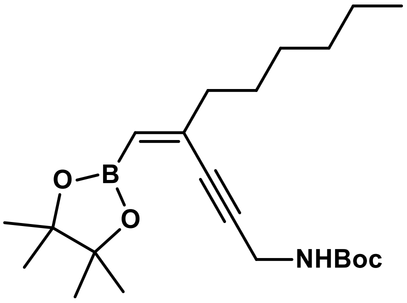


165c

To a cooled (ice–water bath) solution of boron tribromide (14.7 g, 58.7 mmol) in dry dichloromethane (60 mL) was added drop-wise a solution of phenylacetylene (6.0 g, 58.7 mmol) in dry dichloromethane (60 mL). After the addition was completed the solution was brought to room temperature and stirred for 3 h. Upon cooling (ice–water bath), a solution of pinacol (6.94 g, 58.7 mmol) and triethylamine (18 mL, 0.13 mol) in dry dichloromethane (60 mL) was added drop-wise. After the reaction mixture was stirred for 12 h at room temperature, the products were extracted with brine solutions (3×50 mL). The organic layer was dried over anhydrous $MgSO_4$ and evaporated under reduced pressure to give the crude product (12.0 g, 66%). The compound tends to decompose upon purification by column chromatography on silica gel, and thus was used without further purification.

*R*f (System D): 0.77.

4.2.11 (*Z*)-2-(*N*-Boc-amino-2-propyne)-1-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1-octene



170

To a solution of (*Z*)-2-bromo-1-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1-octene **165b** (200 mg, 0.631 mmol) and *N*-Boc-propargylamine (156 mg, 1.00 mmol) in dry DMF (3.0 mL) was added diisopropylethylamine (0.60 mL, 3.44 mmol). The solution was frozen in liquid nitrogen followed by addition of $Pd(PPh_3)_4$ (91 mg, 0.0788 mmol) and CuI (60 mg, 0.316 mmol). After evacuation while the mixture was frozen, the reaction mixture was purged with nitrogen. This degassing procedure was repeated two more times, and the mixture was then stirred at 45°C for 3 h. The products were evaporated under reduced pressure and the residue was purified by column chromatography on silica gel (0–40% EtOAc in hexane) to give the mono alkyne coupled product **170** as a light yellow oil (46 mg, 0.117 mmol, 18%).

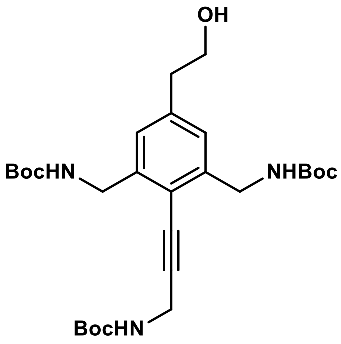
*R*f (system D): 0.72.

δ_H (DMSO-d$_6$): 0.85 (3 H, t, *J* = 6.8), 1.20 (12 H, s), 1.24 (6 H, m), 1.39 (9 H, s), 1.45 (2 H, m), 2.13 (2 H, t, *J* = 7.3), 3.88 (2 H, d, *J* = 5.6), 5.49 (1 H, s), 7.12 (1 H, t, *J* = 5.5).

δ_C (DMSO-d$_6$): 14.4, 22.5, 25.0, 27.9, 28.5, 28.6, 31.5, 78.6, 82.5, 83.2, 92.1, 124.7, 142.4, 155.6.

Mass (ESI positive) found 414.4, $C_{22}H_{38}BNNaO_4^+$ requires 414.28.

4.2.12 3-[2,6-Bis(*N*-*tert*-butyloxyaminomethyl)-4-(2-hydroxyethyl)]phenyl-2-propyn-*N*-*tert*-butyloxyamine



168b

To a solution of (*Z*)-3-bromo-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-3-buten-1-ol **165a** (400 mg, 1.44 mmol) and *N*-Boc-propargylamine (786 mg, 5.06 mmol) in dry DMF (7.0 mL) was added diisopropylethylamine (1.2 mL, 6.89 mmol). The solution was frozen in liquid nitrogen followed by addition of Pd(PPh$_3$)$_4$ (376 mg, 0.326 mmol, 6 mol% with respect to *N*-Boc-propargylamine) and CuI (247 mg, 1.30 mmol, 25 mol% with respect to *N*-Boc-propargylamine). After evacuation while the mixture was frozen, the reaction mixture was purged with nitrogen. This degassing procedure was repeated two more times, and the mixture was stirred at 45°C overnight in a nitrogen atmosphere. The products were then evaporated under reduced pressure and the residue was purified by column chromatography on silica gel (20–60% EtOAc in hexane) to give the aromatized product **168b** as a light yellow solid (120 mg, 16%).

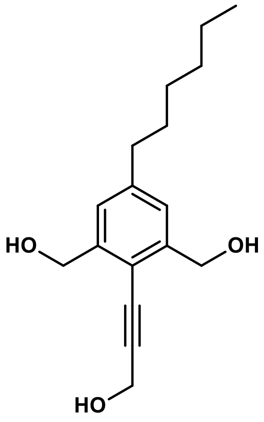
*R*f: 0.66 (system G).

δ_H (DMSO-d$_6$): 1.41 (s, 27 H), 2.69 (2 H, d, *J* = 6.9), 3.56 (2 H, d, *J* = 6.9), 4.02 (2 H, d, *J* = 5.5), 4.21 (4 H, 5.6), 6.97 (2 H, s), 7.30 (2 H, t, *J* = 5.9, ex), 7.40 (1 H, t, *J* = 5.5, ex).

δ_C (DMSO-d$_6$): 28.6, 28.7, 31.1, 39.8, 42.6, 62.4, 77.3, 78.3, 78.7, 97.8, 116.9, 125.3, 139.6, 141.9, 155.9, 156.2.

MS (ESI$^+$) found 556.2, $C_{28}H_{43}N_3NaO_7^+$ requires 556.3.

4.2.13 3-(2,6-Dihydroxymethyl-4-hexyl)prop-2-yn-1-ol



168e

To a solution of (*Z*)-2-bromo-1-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1-octene **165b** (150 mg, 0.475 mmol) and propargyl alcohol (93 mg, 1.66 mmol) in dry DMF (3.0 mL) was added diisopropylethylamine (0.20 mL, 1.15 mmol). The solution was frozen in liquid nitrogen followed by addition of Pd(PPh$_3$)$_4$ (100 mg, 0.0865 mmol, 5 mol% with respect to propargyl alcohol) and CuI (60 mg, 20 mol%). After evacuation while the mixture was frozen, the reaction mixture was purged with nitrogen. This degassing procedure was repeated two more times, and the mixture was then stirred at

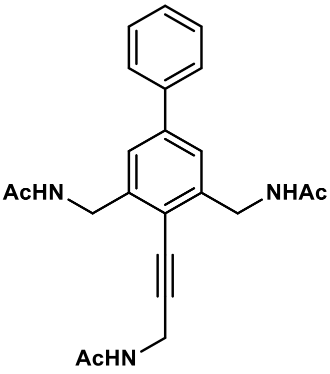
45°C overnight in a nitrogen atmosphere. The products were evaporated under reduced pressure and the residue was purified by column chromatography on silica gel (0–6% MeOH in dichloromethane) to give the aromatized product **168e** as a light yellow solid (57 mg, 43%).

R$_f$: 0.57 (system I).

HR-MS (MALDI-TOF positive) found 299.1604, $C_{17}H_{24}NaO_3^+$ requires 299.16231. δ_H (DMSO-d$_6$): 0.86 (3 H, t, *J* = 6.4), 1.29 (6 H, m, br), 1.56 (2 H, m), 2.60 (2 H, t, *J* = 7.6), 4.34 (2 H, d, *J* = 5.9), 4.60 (4 H, d, *J* = 5.4), 5.19 (2 H, t, *J* = 5.5, OH), 5.32 (1 H, t, *J* = 5.9, OH), 7.21 (2 H, s).

δ_C (DMSO-d$_6$): 14.4, 22.5, 28.8, 31.3, 31.6, 36.0, 50.1, 61.7, 79.0, 99.1, 114.6, 124.5, 142.7, 144.2.

4.2.14 3-[2,6-Bis(*N*-acetamidomethyl)-4-phenyl]phenyl-2-propyn-*N*-acetylamine



168k

To a solution of crude (*Z*)-*α*-bromo-*β*-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-styrene **165c** (400 mg, 1.29 mmol) and *N*-acetylpropargylamine (445 mg, 4.59 mmol) in dry DMF (6.0 mL) was added diisopropylethylamine (0.75 mL, 4.31 mmol). The solution was frozen followed by addition of Pd(PPh$_3$)$_4$ (224 mg, 0.194 mmol, 4 mol% with respect to *N*-acetylproporgylamine) and CuI (147 mg, 0.772 mmol, 17 mol% with respect to *N*-acetylproporgylamine). After evacuation while frozen, the reaction mixture was purged with nitrogen. This degassing procedure was repeated two more times, and the mixture was then stirred at 45°C overnight in a nitrogen atmosphere. The products were evaporated under reduced pressure and the residue was purified by column chromatography on silica gel (0–12% MeOH in dichloromethane) to give the aromatized product **168k** as a pale yellow solid (70 mg, 14% based on crude alkene). M.p. 169°C (decomposed) (dichloromethane–hexane).

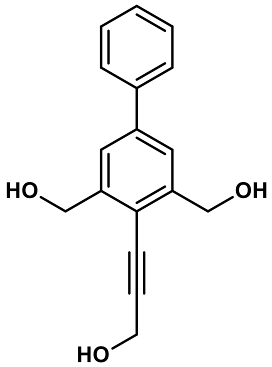
*R*f: 0.61 (system I).

δ_H (DMSO-d$_6$): 1.89 (3 H, s), 1.92 (6 H, s), 4.19 (2 H, d, *J* = 5.13), 4.43 (4 H, d, *J* = 5.5), 7.38 – 7.52 (5 H, m), 7.61 (2 H, d, *J* = 7.6), 8.35 (2 H, t, *J* = 5.4, NH), 5.43 (1 H, t, *J* = 5.0, NH).

δ_C (DMSO-d$_6$): 22.9, 23.0, 29.7, 41.6, 77.3, 98.4, 119.3, 124.2, 127.1, 128.3, 129.6, 139.97, 140.00, 142.3, 169.8, 169.9.

HR-MS (MALDI-TOF positive) found 414.1741; $C_{23}H_{25}N_3NaO_3^+$ requires 414.17881.

4.2.15 3-(2,6-Dihydroxymethyl-4-phenyl)-2-propyn-1-ol



168i

To a solution of crude (*Z*)-*α*-bromo-*β*-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-styrene **165c** (2.00 g, 6.47 mmol) and propargyl alcohol (2.00 g, 33.7 mmol) in dry DMF (30.0 mL) was added diisopropylethylamine (11.3 mL, 64.9 mmol). The solution was frozen followed by addition of Pd(PPh$_3$)$_4$ (400 mg, 0.346 mmol, 1 mol% with respect to propargyl alcohol) and CuI (258 mg, 1.36 mmol, 4 mol% with respect to propargyl alcohol). After evacuation while frozen, the reaction mixture was purged with nitrogen. This degassing procedure was repeated two more times, and the mixture was then stirred at 45°C overnight in a nitrogen atmosphere. The products were evaporated under reduced pressure and the residue was purified by column chromatography on silica gel (0–10% MeOH in dichloromethane) to give the aromatized product **168i** as a light yellow solid (350 mg, 20% based on crude alkene).

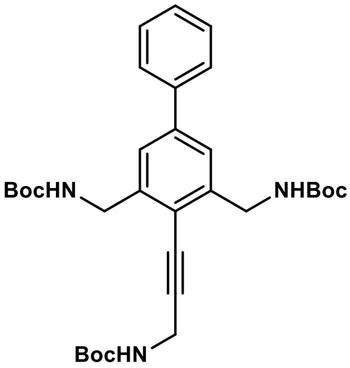
*R*f: 0.57 (system I).

M.p. 181–184°C (ethanol). Found in material recrystallized from ethanol: C, 76.34%; H, 5.80%. $C_{17}H_{16}O_3$ requires C, 76.10%; H, 6.01%.

δ_H (DMSO-d$_6$): 4.39 (2 H, d, *J* = 5.6), 4.69 (4 H, d, *J* = 5.6), 5.34 (2 H, t, *J* = 5.6, ex), 5.39 (1 H, t, *J* = 5.6, ex), 7.38 (1 H, t, *J* = 7.3), 7.50 (2 H, t, *J* = 7.5), 7.66 and 7.68 (together 4 H).

δ_C (DMSO-d$_6$): 50.1, 61.7, 78.7, 100.7, 116.4, 122.8, 127.0, 128.2, 129.5, 139.9, 140.5, 145.0.

4.2.16 3-[2,6-Bis(*N*-*tert*-butyloxyaminomethyl)-4-phenyl]phenyl-2-propyn-*N*-*tert*-butyloxyamine



168j

To a solution of crude (*Z*)-*α*-bromo-*β*-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)styrene **165c** (400 mg, 1.29 mmol) and *N*-Boc-propargylamine (607 mg, 3.91 mmol) in dry DMF (6.0 mL) was added diisopropylethylamine (1.3 mL, 7.46 mmol). The solution was frozen followed by addition of $Pd(PPh_3)_4$ (224 mg, 0.194 mmol, 5 mol% with respect to *N*-Boc-propargylamine) and CuI (147 mg, 0.774 mmol, 20 mol%). After evacuation while frozen, the reaction mixture was purged with nitrogen. This degassing procedure was repeated two more times, and the mixture was then stirred at 45°C overnight in a nitrogen atmosphere. The products were evaporated under reduced pressure and the residue was purified by column chromatography on silica gel (0–60% EtOAc in hexane) to give the aromatized product **168j** as a colorless solid (198 mg, 27% based on crude alkene).

R_f: 0.57 (system D).

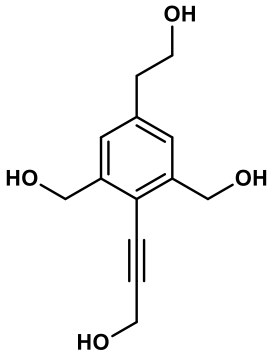
M.p. 114–116°C (dichloromethane–hexane). Found in material recrystallized from dichloromethane–hexane: C, 67.77%; H, 7.66%; N, 7.26%. $C_{32}H_{43}O_6$ requires C, 67.94%; H, 7.66%; N, 7.43%.

δ_H (DMSO-d$_6$): 1.42 and 1.43 (together 27 H), 4.07 (2 H, d, *J* = 5.3), 4.30 (4 H, d, *J* = 5.6), 7.41–7.58 (10 H, m).

δ_C (DMSO-d$_6$): 28.64, 28.69, 42.7, 77.1, 78.4, 78.8, 99.2, 118.5, 123.3, 126.9, 128.3, 129.6, 139.8, 140.4, 142.7, 155.9, 156.3.

HR-MS (MALDI-TOF positive) found 604.2734; $C_{32}H_{43}KN_3O_6^+$ requires 604.27834.

4.2.17 3-(2,6-Dihydroxymethyl-4-(2-hydroxyethyl))prop-2-yn-1-ol



168a

To a solution of (*Z*)-3-bromo-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-3-buten-1-ol **165a** (400 mg, 1.44 mmol) and propargyl alcohol (391 mg, 6.97 mmol) in dry DMF (7.0 mL) was added diisopropylethylamine (2.0 mL, 11.5 mmol). The solution was frozen followed by addition of $Pd(PPh_3)_4$ (376 mg, 0.326 mmol, 5 mol% with respect to propargyl alcohol) and CuI (247 mg, 1.30 mmol, 19 mol% with respect to propargyl alcohol). After evacuation while frozen, the reaction mixture was purged with nitrogen. This degassing procedure was repeated two more times, and the mixture was then stirred at 45°C overnight in a nitrogen atmosphere. The products were evaporated under reduced pressure and the residue was purified by column chromatography on silica gel (0–10% MeOH in dichloromethane) to give the aromatized product **168a** as a light yellow solid (126 mg, 37%).

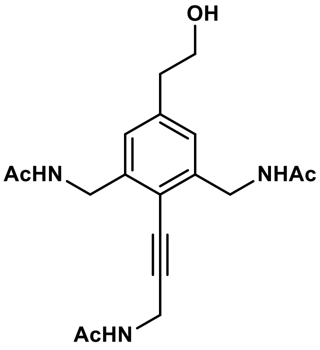
R_f: 0.33 (system J).

δ_H (DMSO-d$_6$): 2.74 (2 H, t, *J* = 7.0), 3.61 (2 H, dt, *J* = 5.2 and 7.0, ex), 4.34 (2 H, d, *J* = 5.8), 4.59 (4 H, d, *J* = 5.3), 4.66 (1 H, t, *J* = 5.2, ex), 5.20 (2 H, t, *J* = 5.4, ex), 5.32 (1 H, t, *J* = 5.4, ex), 7.24 (2 H, s).

δ_C (DMSO-d$_6$): 49.5, 50.1, 61.6, 62.5, 79.0, 99.2, 114.8, 125.2, 139.9, 144.1.

HR-MS (MALDI-TOF positive) found 259.0914; $C_{13}H_{16}NaO_4^+$ requires 259.09408.

4.2.18 3-[2,6-Bis(*N*-acetamidomethyl)-4-phenyl]phenyl-2-propyn-*N*-acetylamine



168c

To a solution of (*Z*)-3-bromo-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-3-buten-1-ol **165a** (100 mg, 0.36 mmol) and *N*-acetylpropargylamine (141 mg, 1.45 mmol) in dry DMF (3.0 mL) was added diisopropylethylamine (0.2 mL). The solution was frozen followed by addition of $Pd(PPh_3)_4$ (83 mg, 0.0719 mmol, 5 mol% with respect to *N*-acetylpropargylamine) and CuI (55 mg, 0.289 mmol, 20 mol% with respect to *N*-acetylproparagylamine). After evacuation under vacuum, the reaction mixture was purged with nitrogen. This degassing procedure was repeated two more times, and the mixture was then stirred at 45°C overnight in a nitrogen atmosphere. The products were evaporated under reduced pressure and the residue was purified by column chromatography on silica gel (0–12% MeOH in dichloromethane) to give the aromatized product **168c** as a light yellow solid (50 mg, 39%).

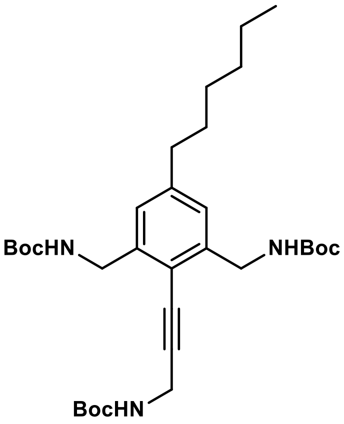
R_f: 0.25 (system J).

HR-MS (MALDI-TOF positive) found 382.1736; $C_{19}H_{25}N_3NaO_4^+$ requires 382.17373.

δ_H (DMSO-d_6): 1.87 (3 H, s), 1.90 (6 H, s), 2.70 (2 H, t, *J* = 6.9), 3.57 (2 H, t, *J* = 6.9), 4.14 (2 H, d, *J* = 5.2), 4.33 (4 H, d, *J* = 5.6), 7.01 (2 H, s), 8.26 (2 H, t, *J* = 5.8), 8.39 (1 H, t, *J* = 5.1).

δ_C (DMSO-d_6): 22.82, 22.87, 22.99, 29.6, 39.6, 41.4, 62.4, 77.5, 97.0, 117.7, 126.4, 140.0, 141.5, 169.69, 169.73.

4.2.19 3-[2,6-Bis(*N*-*tert*-butyloxyaminomethyl)-4-hexyl]phenyl-2-propyn-*N*-*tert*-butyloxyamine



168f

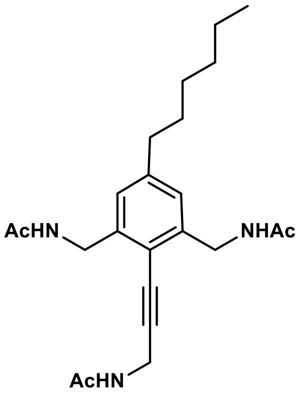
To a solution of (*Z*)-2-bromo-1-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1-octene **165b** (0.500 g, 1.58 mmol) and *N*-Boc-propargylamine (0.860 g, 5.54 mmol) in dry DMF (6.0 mL) was added diisopropylethylamine (0.96 mL, 5.51 mol). The solution was frozen followed by addition of $Pd(PPh_3)_4$ (320 mg, 0.277 mmol, 5 mol% with respect to *N*-Boc-propargylamine) and CuI (210 mg, 1.10 mmol, 20 mol% with respect to *N*-Boc-propargylamine). After evacuation while frozen, the reaction mixture was purged with nitrogen. This degassing procedure was repeated two more times, and the mixture was then stirred at 45°C overnight in a nitrogen atmosphere. The products were evaporated under reduced pressure and the residue was purified by column chromatography on silica gel (10–60%, EtOAc–hexane) to give the aromatized product **168f** as a light yellow wax (142 mg, 16%).

R_f: 0.66 (system D). The product contains a contaminant that is inseparable by column chromatography as indicated by NMR spectra.

δ_H (DMSO-d_6) contains the following signals: 0.85 (3 H, t, *J* = 6.3), 4.01 (2 H, d, *J* = 5.4), 4.20 (4 H, d, *J* = 5.4), 6.94 (2 H, s), 7.29 (2 H, t, *J* = 6.3), 7.39 (1 H, br).

HR-MS (MALDI-TOF positive) found 596.3688; $C_{32}H_{51}N_3NaO_6^+$ requires 596.36756.

4.2.20 3-[2,6-Bis(*N*-acetamidomethyl)-4-hexyl]phenyl-2-propyn-*N*-acetylamine



168g

To a solution of (*Z*)-2-bromo-1-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1-octene **165b** (130 mg, 0.410 mmol) and *N*-acetylpropargylamine (140 mg, 1.44 mmol) in dry DMF (3.0 mL) was added diisopropylethylamine (0.2 mL, 1.15 mmol). The solution was frozen followed by addition of $Pd(PPh_3)_4$ (83 mg, 0.072 mmol, 5 mol% with respect to *N*-acetylpropargylamine) and CuI (54 mg, 0.28 mmol, 20 mol% with respect to *N*-acetylpropargylamine). After evacuation while frozen, the reaction mixture was purged with nitrogen. This degassing procedure was repeated two more times, and the mixture was then stirred at 45°C overnight in a nitrogen atmosphere. The products were evaporated under reduced pressure and the residue was purified by column chromatography on silica gel (0–8% MeOH in dichloromethane) to give the aromatized product **168g** as a light yellow solid (40 mg, 24%).

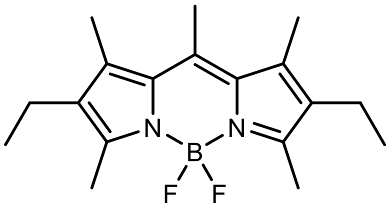
R_f: 0.56 (system I).

δ_H (DMSO-d$_6$): 0.86 (3 H, t, *J* = 6.5), 1.28 (6 H, m), 1.52 (2 H, m), 1.87 (3 H, s), 1.90 (6 H, s), 2.54 (2 H, t, *J* = 6.9), 4.14 (2 H, d, *J* = 5.1), 4.33 (4 H, d, *J* = 5.6), 6.98 (2 H, s), 8.25 (2 H, t, *J* = 5.5), 8.38 (1 H, t, *J* = 5.1).

δ_C (DMSO-d_6): 14.4, 22.5, 22.9, 23.0, 28.8, 29.6, 31.2, 31.6, 35.7, 41.5, 77.6, 96.8, 117.5, 125.8, 141.5, 142.8, 169.7.

HR-MS (MALDI-TOF positive) found 422.2424; $C_{23}H_{33}N_3NaO_3^+$ requires 422.24196.

4.2.21 4,4-Difluoro-1,3,5,7,8-pentamethyl-2,6-diethyl-4-bora-3a,4a-diaza-*s*-indacene



173

A solution of distilled acetyl chloride (0.405 mL, 5.68 mmol) in dry dichloromethane (20 mL) was added drop-wise to a stirred degassed solution of 3-ethyl-2,4-dimethylpyrrole (2.20 mL, 16.23 mmol) in dry dichloromethane (10 mL). After the mixture was heated under reflux for 3 h, the solvents were evaporated under reduced pressure. The residue was co-evaporated with dry toluene (3×20 mL) and then re-dissolved in dry dichloromethane (30.0 mL), followed by addition of dry triethylamine (2.26 mL, 16.23 mmol). After 15 min boron trifluoride diethyl etherate (2.03 mL, 16.23 mmol) was added and the mixture was stirred under reflux for 3 h. Upon cooling to room temperature, the products were extracted successively with water (30 mL) and brine (3×30 mL). The organic layer was separated, dried ($MgSO_4$) and evaporated under reduced pressure. The residue was purified by column chromatography on silica gel. The appropriate fractions, which were eluted with dichloromethane–hexane (50:50 v/v), were combined and evaporated under reduced pressure to give the *title compound* as an orange solid (0.78 g, 30%).

R_f (System F): 0.90

δ_H ($CDCl_3$): 1.06 (6 H, t, J = 7.5), 2.34 (6 H, s), 2.42 (4 H, q, J = 7.5), 2.52 (6 H, s), 2.61 (3 H, s).

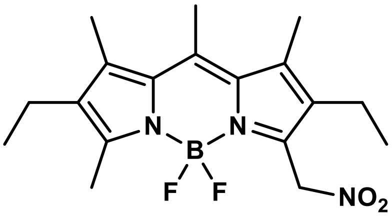
δ_C ($CDCl_3$): 12.4, 14.4, 14.9, 16.9, 17.1, 131.6, 132.3, 136.3, 139.7, 151.8

δ_B ($CDCl_3$): 0.66 (t, *J* = 33)

δ_F ($CDCl_3$): -146.0 (q, *J* = 33)

HR-FAB found $[M+H]^+$ =318.19648, $C_{18}H_{25}BF_2N_2$ requires 318.20788

4.2.22 4,4-Difluoro-3-nitromethyl-1,5,7,8-tetramethyl-2,6-diethyl-4-bora-3a,4a-diaza-*s*-indacene



174a

To a solution of 4,4-difluoro-1,3,5,7,8-pentamethyl-2,6-diethyl-4-bora-3a,4a-diaza-*s*-indacene **173** (100 mg, 0.31 mmol) in dichloromethane (20 mL, dichloromethane was first dried by heating in the presence of phosphorus pentoxide and distilled, and then 0.004 % water (v/v) was added), a solution of cupric nitrate trihydrate in acetonitrile (0.41 *M*, 0.38 mL, 0.5 mol. equiv., acetonitrile was first dried by heating in the presence of calcium hydride and distilled, and then 0.004 % water (v/v) was added) was added. After the mixture was stirred for 3 h, the products were evaporated under reduced pressure. The residue was re-dissolved in dichloromethane (20 mL) and extracted with water (3×15 mL). The organic layer was separated, dried ($MgSO_4$) and evaporated under reduced pressure. The residue was purified by column chromatography on silica gel. The appropriate fractions, which were eluted with dichloromethane–hexane (50:50 v/v), were combined and evaporated under reduced pressure to give the *title compound* as a red solid (61 mg, 54%).

R_f: 0.87 (system F).

M.p. 196–200°C (dichloromethane–hexanes).

δ_H ($CDCl_3$): 1.07 (3 H, t, *J* = 7.6), 1.10 (3 H, t, *J* = 7.6), 2.39 (3 H, s), 2.40 (3 H, s), 2.43 (2 H, q, *J* = 7.6), 2.48 (2 H, q, *J* = 7.6), 2.55 (3 H, s), 2.70 (3 H, s), 5.72 (2 H, s).

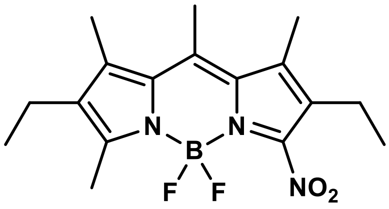
δ_C ($CDCl_3$): 13.0, 14.1, 14.5,14.7, 14.9, 17.1, 17.2, 17.3, 69.4, 131.8, 133.6, 133.9, 134.6, 135.6, 140.9, 142.2, 159.9.

δ_B ($CDCl_3$): 0.47 (t, *J* = 33.3).

δ_F ($CDCl_3$): -143.6 (q, *J* = 33.3).

HRMS (EI) found 363.19288, $C_{18}H_{24}BF_2N_3O_2$ requires 363.19296.

4.2.23 4,4-Difluoro-3-nitro-1,5,7,8-tetramethyl-2,6-diethyl-4-bora-3a,4a-diaza-*s*-indacene



174b

To a solution of 4,4-methyl-1,3,5,7,8-pentamethyl-2,6-diethyl-4-bora-3a,4a-diaza-*s*-indacene **173** (100 mg, 0.31 mmol) in dry dichloromethane (20 mL), a solution of cupric nitrate trihydrate (380 mg, 5 mol. equiv.) in dry acetonitrile (10 mL) was added. After the mixture was stirred for 5 min, the products were evaporated under reduced pressure. The residue was re-dissolved in dichloromethane (30 mL) and extracted with water (3×20 mL). The organic layer was separated, dried ($MgSO_4$) and evaporated under reduced pressure. The residue was purified by column chromatography on silica gel. The appropriate fractions, which were eluted with dichloromethane–hexane (70:30 v/v), were combined and evaporated under reduced pressure to give the *title compound* as a red solid (42 mg, 38%).

*R*f: 0.42 (System F).

M.p. 212–216°C (dichloromethane–hexanes).

δ_H ($CDCl_3$): 1.09 (3 H, t, *J* = 7.5), 1.16 (3 H, t, *J* = 7.5), 2.37 (3 H, s), 2.41 (3 H, s), 2.46 (2 H, q, *J* = 7.5), 2.64 (3 H, s), 2.72 (2 H, q, *J* = 7.5), 2.73 (3 H, s).

δ_C ($CDCl_3$): 13.4, 13.7, 14.1, 14.2, 14.9, 17.1, 17.9, 18.3, 130.0, 130.2, 130.4, 137.6, 138.8, 141.5, 142.7, 144.3, 166.8.

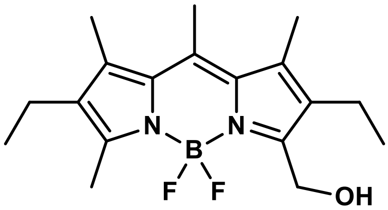
δ_B ($CDCl_3$): 0.30 (t, *J* = 28.2).

δ_F ($CDCl_3$): -144.6 (q, *J* = 29.2).

HRMS (EI) found 349.17713, $C_{17}H_{22}BF_2N_3O_2$ requires 349.17731.

It is noted that this reaction gave a mixture of 4,4-difluoro-3-nitro-1,5,7,8-tetramethyl-2,6-diethyl-4-bora-3a,4a-diaza-*s*-indacene **174b** and 4,4-difluoro-1,5,7,8-tetramethyl-2,6-diethyl-4-bora-3a,4a-diaza-*s*-indacene-3-carboxyaldehyde **174d** (with a total mass of 78 mg) in a ratio of 6.3:1 as determined by ^{1}H NMR. Therefore, the overall yield of the *title compound* is 62%. In addition to the pure 4,4-difluoro-3-nitro-1,5,7,8-tetramethyl-2,6-diethyl-4-bora-3a,4a-diaza-*s*-indacene **174b** (42 mg), separation of the rest of product **174b** from contaminating aldehyde **174d** was rather difficult.

4.2.24 4,4-Difluoro-3-hydroxymethyl-1,5,7,8-tetramethyl-2,6-diethyl-4-bora-3a,4a-diaza-*s*-indacene



174c

To a solution of 4,4-difluoro-1,3,5,7,8-pentamethyl-2,6-diethyl-4-bora-3a,4a-diaza-*s*-indacene **173** (50 mg, 0.16 mmol) in dichloromethane (8 mL, dichloromethane was first dried by heating in the presence of phosphorus pentoxide and distilled, and then 0.02 % water (v/v) was added), a solution of cupric nitrate trihydrate (4 mg, 0.1 mol. equiv.)

and calcium nitrate tetrahydrate (371 mg, 10 mol. equiv.) in acetonitrile (17 mL, acetonitrile was first dried by heating in the presence of calcium hydride and distilled, and then 0.02 % water (v/v) was added) was added. After the mixture was stirred for 24 h, the products were concentrated under reduced pressure and the residue was re-dissolved in dichloromethane (15 mL) and washed with water (3×10 mL). The organic layer was separated, dried ($MgSO_4$) and evaporated under reduced pressure. The residue was purified by column chromatography on silica gel. The appropriate fractions, which were eluted with dichloromethane, were combined and evaporated under reduced pressure to give the *title compound* as a red solid (26 mg, 50%).

R_f: 0.32 (System F).

M.p. 177–179°C (dichloromethane– hexanes).

δ_H ($CDCl_3$): 1.08 (3 H, t, *J* = 7.5), 1.12 (3 H, t, *J* = 7.4), 2.38 (6 H, s), 2.44 (2 H, q, *J* = 7.5), 2.52 (2 H, q, *J* = 7.4), 2.54 (3 H, s), 2.67 (3 H, s), 4.76 (2 H, s).

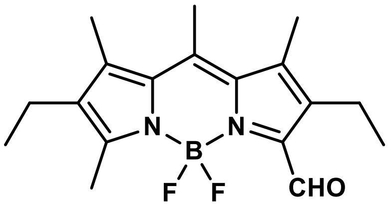
δ_C ($CDCl_3$):12.7, 14.2, 14.6, 14.7, 15.8, 17.0, 17.1, 17.2, 55.06, 131.6, 132.1, 133.1, 134.0, 135.5, 138.9, 141.7, 149.2, 155.8.

δ_B ($CDCl_3$): 0.70 (t, *J* = 34.5).

δ_F ($CDCl_3$): -142.7 (q, *J* = 34.2).

HRMS (EI) found 334.20272, $C_{18}H_{25}BF_2N_2O$ requires 334.20280.

4.2.25 4,4-Difluoro-1,5,7,8-tetramethyl-2,6-diethyl-4-bora-3a,4a-diaza-*s*-indacene-3-carboxyaldehyde



174d

To a solution of 4,4-difluoro-3-nitromethyl-1,5,7,8-tetramethyl-2,6-diethyl-4-bora-

3a,4a-diaza-*s*-indacene **173** (50 mg, 0.14 mmol) in dichloromethane (10 mL, dichloromethane was first dried by heating in the presence of phosphorus pentoxide and distilled, and then 0.004 % water (v/v) was added), a solution of cupric nitrate trihydrate (172 mg, 5 mol. equiv.) in acetonitrile (5 mL, acetonitrile was first dried by heating in the presence of calcium hydride and distilled, and then 0.004 % water (v/v) was added) was added. After the mixture was stirred for 3 h, the products were evaporated under reduced pressure. The residue was re-dissolved in dichloromethane (15 mL) and extracted with water (3×10 mL). The organic layer was separated, dried ($MgSO_4$) and evaporated under reduced pressure. The residue was purified by column chromatography on silica gel. The appropriate fractions, which were eluted with dichloromethane–hexane (70:30 v/v), were combined and evaporated under reduced pressure to give the *title compound* as a red solid (19 mg, 42%).

R_f: 0.45 (System F).

M.p. 215–219°C (dichloromethane–hexanes).

δ_H ($CDCl_3$): 1.09 (3 H, t, *J* = 7.6), 1.12 (3 H, t, *J* = 7.5), 2.36 (3 H, s), 2.42 (3 H, s), 2.47 (2 H, q, *J* = 7.6), 2.63 (3 H, s), 2.73 (3 H, s), 2.84 (2 H, q, *J* = 7.5), 10.37 (1 H, s).

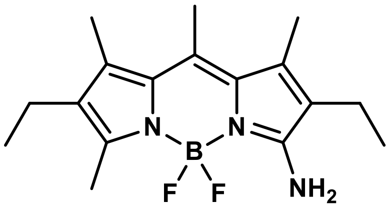
δ_C ($CDCl_3$): 13.1, 13.5, 14.3, 14.6, 14.9, 17.2, 17.6, 17.8, 132.0, 133.3, 136.4, 136.9, 137.4, 138.9, 141.9, 142.0, 163.9, 186.0.

δ_B ($CDCl_3$): 0.62 (t, *J* = 33.1).

δ_F ($CDCl_3$): -138.1 (q, *J* = δ33.0).

HRMS (EI) found 332.18780, $C_{18}H_{23}BF_2N_2O$ requires 332.187150.

4.2.26 4,4-Dimethyl-3-amino-1,5,7,8-tetramethyl-2,6-diethyl-4-bora-3a,4a-diaza-*s*-indacene



175

To a solution of 4,4-difluoro-3-nitro-1,5,7,8-tetramethyl-2,6-diethyl-4-bora-3a,4a-diaza-*s*-indacene **174b** (57 mg, 0.17mmol) in MeOH-EtOAc (10 mL, 9:1 v/v), palladium on activated charcoal (10% loading, 6 mg) was added and the mixture was stirred in a hydrogen atmosphere (1 atm) for 2 h at room temperature. The resulting mixture was filtered through a bed of celite and the filtrate was concentrated under reduced pressure. The crude residue was purified by flash chromatography on silica gel. The appropriate fractions, which were eluted with dichloromethane, were combined and evaporated under reduced pressure to afford the *title compound* (43 mg, 87%) as an orange solid.

M.p. 239-243°C (dichloromethane–hexanes).

δ_H (DMSO-d$_6$): 0.97 and 0.98 (6 H, two triplets, *J* = 7.5), 2.23 (3 H, s), 2.26 (3 H, s), 2.27 (3 H, s), 2.33 (2 H, q, *J* = 7.5), 2.41 (2 H, q, *J* = 7.5), 2.45 (3 H, s), 7.32 (2 H, s, br).

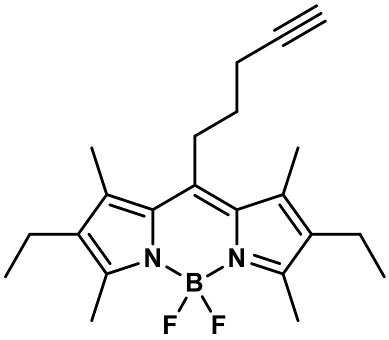
δ_C (DMSO-d$_6$): 11.7, 13.4, 14.5, 14.7, 15.6, 15.9, 16.1, 17.1, 125.3, 126.1, 126.9, 127.0, 128.1, 130.1, 136.0, 140.9, 158.6.

δ_B (DMSO-d$_6$): 0.78 (br).

δ_F (DMSO-d$_6$): -144.9 (br).

HRMS (EI) found 319.20310, $C_{17}H_{24}BF_2N_3$ requires 319.20313.

4.2.27 4,4-Difluoro-4-bora-1,3,5,7-tetramethyl-2,6-diethyl-8-(hex-5-ynyl)-3a,4a-diaza-*s*-indacene



181

A solution of hex-5-ynoyl chloride **180** (84 μL, 0.68 mmol) in dry dichloromethane (3.0 mL) was added drop-wise to a stirred solution of 3-ethyl-2-4-dimethyl pyrrole (0.22 mL, 1.63 mmol) in dry dichloromethane (6 mL). After the mixture was heated under reflux for 4 h, the reaction mixture was cooled to room temperature followed by addition of dry triethylamine (1.8 mL, 12.9 mmol). After the mixture was stirred for 30 min under reflux, boron trifluoride diethyl etherate (1.6 mL, 13.0 mmol) was added and the reaction mixture was stirred for 4 h under reflux. Upon cooling to room temperature, the reaction mixture was poured into saturated aqueous sodium hydrogen carbonate solution (20 mL) and extracted with dichloromethane (3×30 mL). The combined organic layers were dried ($MgSO_4$) and evaporated under reduced pressure. The residue was purified by column chromatography on silica gel. The appropriate fractions, which were eluted by ethyl acetate–hexane (20:80, v/v), were combined and evaporated under reduced pressure to give the *title compound* as a red solid (83 mg, 33%).

R_f: 0.45 (System C).

δ_H ($CDCl_3$): 1.07 (6 H, t, J = 7.5), 1.83–1.91 (2 H, m), 2.07 (1 H, t, J = 2.7), 2.38 (6 H, s), 2.39–2.45 (6 H, m), 2.52 (6 H, s), 3.15 (2 H, ddd, J = 4.7, 4.7, and 8.7).

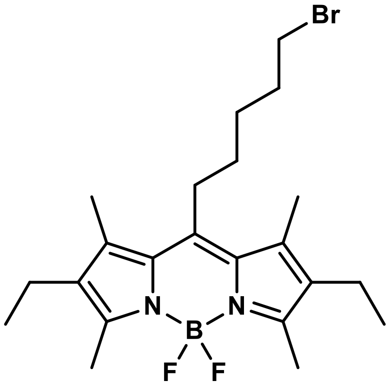
δ_C ($CDCl_3$): 12.4, 13.5, 14.8, 17.2, 18.9, 27.4, 29.8, 69.8, 82.9, 131.0, 132.7, 135.7, 143.6, 152.4.

δ_B ($CDCl_3$): 0.61 (t, J = 33.4).

δ_F ($CDCl_3$): −145.9 (q, *J* = 33.4), −145.8 (q, *J* = 33.4).

$C_{22}H_{30}BF_2N_2^+$ requires 371.24701, ESI [M + H]+ found 371.2477.

4.2.28 4,4-Difluoro-4-bora-1,3,5,7-tetramethyl-2,6-diethyl-8-(pent-5-bromide) 3a,4a-diaza-*s*-indacene



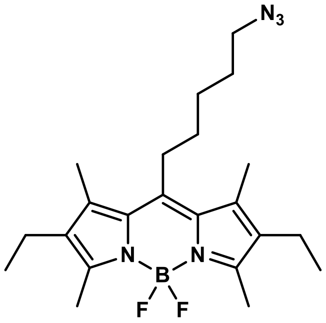
183

A solution of bromo-hexanoyl chloride **182** (1.24 mL, 8.10 mmol) in dry dichloromethane (60 ml) was added drop-wise to a stirred solution of 3-ethyl-2-4-dimethyl pyrrole (2.1 mL, 16.23 mmol) in dry dichloromethane (20 mL). After the mixture was heated under reflux for 4 h, the reaction mixture was cooled to room temperature followed by addition of dry trimethylamine (2.34 mL, 16.77 mmol). After the mixture was stirred for 30 min under reflux, boron trifluoride diethyl etherate (2.0 mL, 16.20 mmol) was added and the reaction mixture was stirred for 4 h under reflux. Upon cooling to room temperature, reaction mixture was diluted with saturated $NaHCO_3$ solution (20 mL) and products were extracted with dichloromethane (3×50 mL) and the combined organic layer was washed with saturated $NaHCO_3$ solution (2×20 mL). The organic layers were dried ($MgSO_4$) and evaporated under reduced pressure. The residue was purified by column chromatography on silica gel. The appropriate fractions, which were eluted by ethyl acetate–hexane (20:80, v/v), were combined and evaporated under reduced pressure to give the *title compound* a red solid (1.2 g, 2.84 mmol, 35%)

$\delta_H(CDCl_3)$: 1.07 (6 H, t, *J* = 7.6), 1.66–1.70 (4 H, m), 1.94–1.96 (2 H, m), 2.35 (6 H, s), 2.42 (4 H, q, *J* = 7.2), 2.51 (6 H, s), 3.02 (2 H, m), 3.46 (2 H, t, *J* = 6.4).

$\delta_C(CDCl_3)$: 12.4, 13.4, 14.8, 17.2, 28.3, 28.5, 30.8, 32.2, 33.4, 130.8, 132.6, 135.5, 144.2, 152.2.

4.2.29 4,4-Difluoro-4-bora-1,3,5,7-tetramethyl-2,6-diethyl-8-(5-azidopentyl)-3a,4a-diaza-*s*-indacene



184

To a solution of 4,4-difluoro-4-bora-1,3,5,7-tetramethyl-2,6-diethyl-8-(5-bromopentyl)-3a,4a-diaza-*s*-indacene **183** (30 mg, 0.0663 mmol) in water (1.0 mL)–DMF (100 μL) was added NaN_3 (6.46 mg, 0.0994 mmol) and the reaction mixture was heated in a CEM microwave reactor at 100°C for 10 min under stirring. Upon cooling, the products were diluted with water (2 mL) and extracted with ethyl acetate (2×6 mL). The organic layers were dried ($MgSO_4$) and evaporated under reduced pressure. The crude products were purified by column chromatography on silica gel. The appropriate fractions, which were eluted with ethyl acetate–hexanes (20:80, v/v), were combined and evaporated under reduced pressure to give the *title compound* as a red solid (22 mg, 80%).

R_f: 0.38 (System C):

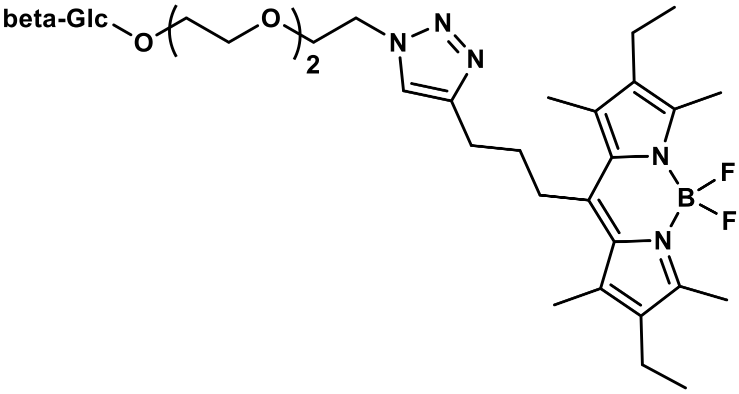
δ_H(CDCl$_3$): 1.07 (6 H, t, *J* = 7.5), 1.56–1.73 (6 H, m), 2.35 (6 H, s), 2.42 (4 H, q, *J* = 7.5), 2.52 (6 H, s), 3.02 (2 H, m), 3.34 (2 H, t, *J* = 6.6).

δ_C(CDCl$_3$): 12.4, 13.4, 14.8, 17.2, 27.3, 28.3, 28.7, 31.3, 51.3, 130.9, 132.7, 135.3, 144.2, 152.2.

δ_B(CDCl$_3$): 0.61 (t, *J* = 33.5).

δ_F(CDCl$_3$): -146.0 (q, *J* = 33.5), -145.9 (q, *J* = 33.5).

4.2.30 Conjugation between *β*-Glc-PEG$_2$-N$_3$ (176) and 4,4-difluoro-4-bora-1,3,5,7-tetramethyl-2,6-diethyl-8-(hex-5-ynyl)-3a,4a-diaza-*s*-indacene (181)



185

To a solution of *β*-Glc-PEG$_2$-N$_3$ **176** (5.0 mg, 0.015 mmol) in water (100 μL) was added a solution of 4,4-difluoro-4-bora-1,3,5,7-tetramethyl-2,6-diethyl-8-(hex-5-ynyl)-3a,4a-diaza-*s*-indacene **181** (6.0 mg, 0.016 mmol) in DMF (200 μL), a solution of cupric sulfate (50 μL, 13 mol%, stock solution prepared by dissolving 200 mg cupric sulfate pentahydrate in 20 mL of water), and a solution of sodium L-ascorbate (75 μL, 25 mol%, stock prepared by dissolving 200 mg of sodium L-ascorbate in 20 mL of water). The reaction mixture was then heated in a CEM microwave at 80°C for 3 min and was diluted with water (2 mL) upon cooling. The products were extracted with diethyl ether (2×6 mL) and the aqueous solution was purified by FPLC column chromatography on C18 reverse phase silica gel (1×30 cm). The column was eluted with a linear gradient of mixture of acetonitrile and water (40 to 80% acetonitrile over 100 min). The appropriate fractions were combined and lyophilized to give the *title compound* as a red sponge solid (7.5 mg, 71%).

R_f: 0.60 (System K).

δ_H(CD_3OD): 1.07 (6 H, t, J = 7.6), 1.96–2.02 (2 H, m), 2.26 (6 H, s), 2.45 (6 H, s), 2.45 (4 H, q, J = 7.6), 2.95 (2 H, t, J = 7.1), 3.08–3.12 (2 H, m), 3.18 (1 H, dd, J = 7.6 and 8.6, H-2), 3.25–3.39 (3 H, m, overlap with CD_3OD solvent residue), 3.60–3.68 (10 H, including CH_2, a dd with J = 5.7 and 12 for H-6a, and one of the two protons in the PEG-CH2 attached to the glycsidic linkage), 3.78 – 3.97 (4 H, including a dd with J = 2.1 and 12 for H-6b, and the other proton in the PEG-CH_2 attached to the glycsidic linkage), 3.90 (2 H, t, J = 5.0, CH_2), 4.25 (1 H, d, J = 7.8, H-1), 4.59 (2 H, t, J = 5.0, CH_2), 7.92 (1 H, s, CH triazole).

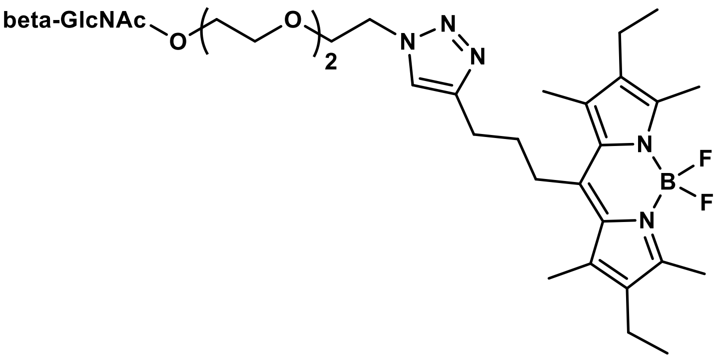
δ_C(CD_3OD): 11.1, 12.1, 13.8, 16.5, 25.0, 27.3, 31.5, 49.9, 61.3 (C6), 68.2, 69.0, 69.9, 70.0, 70.2 (C5), 73.7 (C2), 76.53 and 76.56 (C3 and C4), 103.0 (C1), 123.4, 130.7, 132.5, 136.0, 144.5, 146.3, 151.8.

δ_B (CD_3OD): 0.54 (t, J = 33.3).

δ_F (CD_3OD): -146.5 to -146.2 (m)

$C_{34}H_{53}BF_2N_5O_8^+$ requires 708.39553, ESI [M+H]+ found 708.3932.

4.2.31 Conjugation between *β*-GlcNAc-PEG$_2$-N$_3$ (177) and 4,4-difluoro-4-bora-1,3,5,7-tetramethyl-2,6-diethyl-8-(hex-5-ynyl)-3a,4a-diaza-*s*-indacene (181)



186

To a solution of *β*-GlcNAc-PEG$_2$-N$_3$ **177** (5.0 mg, 0.013 mmol) in water (100 μL) was added a solution of 4,4-difluoro-4-bora-1,3,5,7-tetramethyl-2,6-diethyl-8-(hex-5-ynyl)-3a,4a-diaza-*s*-indacene **181** (5.8 mg, 0.016 mmol) in DMF (200 μL), a solution of cupric sulfate (50 μL, 15 mol%, stock prepared by dissolving 200 mg cupric sulfate pentahydrate in 20 mL of water), and a solution of sodium L-ascorbate (75 μL, 29 mol%, stock prepared by dissolving 200 mg of sodium L-ascorbate in 20 mL of water). The reaction mixture was then heated in a CEM microwave at 80°C for 3 min and was diluted with water (2 mL) upon cooling. The products were extracted with diethyl ether (2×6 mL) and the aqueous solution was purified by FPLC column chromatography on C18 reverse phase silica gel (1×30 cm). The column was eluted with a linear gradient of mixture of acetonitrile and water (40 to 80% acetonitrile over 100 min). The appropriate fractions were combined and lyophilized to give the *title compound* as a red sponge solid (7.0 mg, 72%).

R_f: 0.53 (System K).

δ_H(CD_3OD): 1.06 (6 H, t, *J* = 7.6), 1.95 (3 H, s), 1.96 (2 H, m), 2.25 (6 H, s), 2.45 (6 H, s), 2.45 (4 H, q, *J* = 7.6), 2.94 (2 H, t, *J* = 7.1), 3.06–3.10 (2 H, m), 3.23 (1 H, ddd, *J* = 2.2, 5.7 and 9.5, H5), H4 overlaps with deuterated methanol solvent residue, 3.43 (1 H, dd, *J* = 8.6 and 10, H3), 3.54–3.70 (9 H, m, 3×CH_2, H2, H6a, and one of the protons of the PEG-CH_2 attached to the gycosidic linkage), 3.85–3.91 (4 H, including a dd, *J* = 2.2 and 10.3 for H6b, the other proton of the PEG-CH_2 attached to gycosidic linkage, and CH_2), 4.38 (1 H, d, *J* = 8.2, H1), 4.58 (2 H, t, *J* = 5.0), 7.91 (1 H, s).

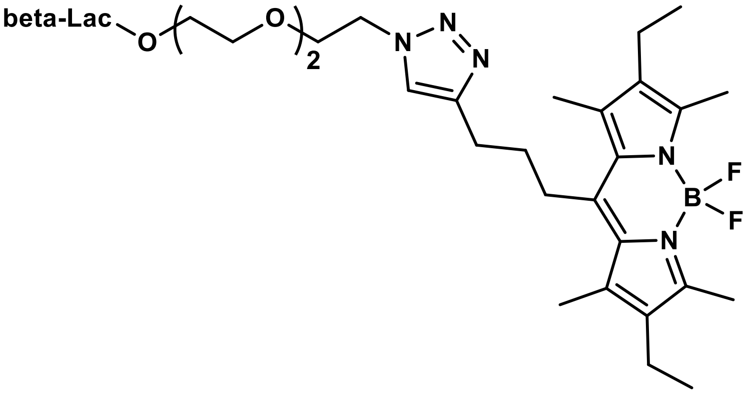
δ_C(CD_3OD): 11.1, 12.1, 13.8, 16.5, 21.6, 25.0, 27.3, 31.5, 49.9, 55.9 (C2), 61.4 (C6), 68.6, 69.0, 70.0, 70.1, 70.2, 70.7 (C4), 74.8 (C3), 76.6 (C5), 101.3 (C1), 123.3, 130.7, 132.6, 136.0, 144.5, 146.3, 151.8, 172.2.

$\delta_B(CD_3OD)$: 0.53 (t, *J* = 33.3).

$\delta_F(CD_3OD)$: -146.5 to -146.2 (m).

$C_{36}H_{56}BF_2N_6O_8^+$ requires 749.42207, ESI $[M+H]^+$ found 749.4232.

4.2.32 Conjugation between *β*-Lac-PEG$_2$-N$_3$ (178) and 4,4-difluoro-4-bora-1,3,5,7 tetramethyl-2,6-diethyl-8-(hex-5-ynyl)-3a,4a-diaza-*s*-indacene (181)



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To a solution of *β*-Lac-PEG$_2$-N$_3$ **178** (5.0 mg, 0.010 mmol) in water (100 μL) was added a solution of 4,4-difluoro-4-bora-1,3,5,7-tetramethyl-2,6-diethyl-8-(hex-5-ynyl)-3a,4a-diaza-*s*-indacene **181** (4.5 mg, 0.011 mmol) in DMF (200 μL), a solution of cupric sulfate (50 μL, 20 mol%, stock prepared by dissolving 200 mg cupric sulfate pentahydrate in 20 mL of water), and a solution of sodium L-ascorbate (75 μL, 37 mol%, stock prepared by dissolving 200 mg of sodium L-ascorbate in 20 mL of water). The reaction mixture was then heated in a CEM microwave at 80°C for 3 min and was diluted with water (2 mL) upon cooling. The products were extracted with diethyl ether (2×6 mL) and the aqueous solution was purified by FPLC column chromatography on C18 reverse phase silica gel (1×30 cm). The column was eluted with a linear gradient of mixture of acetonitrile and water (40 to 80% acetonitrile over 100 min). The appropriate fractions were combined and lyophilized to give the *title compound* as a red sponge solid (7.4 mg, 85%).

R_f: 0.36 (System K).

δ_H (CD_3OD): 1.07 (6 H, t, J = 7.4), 1.99 (2 H, ddd, J = 7.2, 11.5, and 12.0), 2.26 (6 H, s), 2.45 (6 H, s), 2.46 (4 H, q, J = 7.5), 2.96 (2 H, t, J = 7.0), 3.08–3.13 (2 H, m, CH_2), 3.24 (1 H, t, J = 8.5, H2Glc), 3.32–3.63 (multiple unresolved signals, including deuterated methanol solvent residue signals), 3.69 – 3.85 (4 H, m, H6Glc and H6Gal), 3.88 – 3.95 (m, including 1×CH_2 and one of the two protons of the PEG-CH_2 attached to the glycosidic linkage), 4.26 (1 H, d, J = 7.8, H1Glc), 4.35 (1 H, d, J = 7.5 H1Gal), 4.59 (2 H, t, J = 4.9, CH_2), 7.93 (1 H, s, triazole).

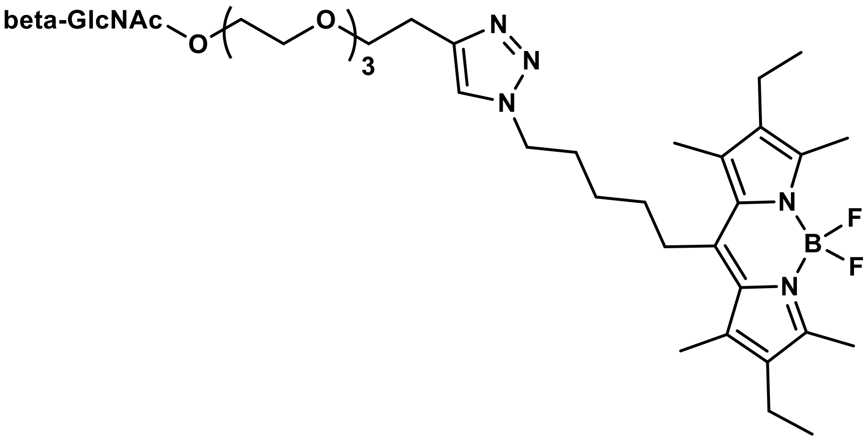
δ_C (CD_3OD): 11.1, 12.1, 13.8, 16.5, 25.0, 27.3, 31.5, 49.9, 60.5, 61.6, 68.3, 68.9, 69.0, 70.0, 70.1, 71.1, 73.3, 73.4, 74.9, 75.0, 75.7, 79.2, 123.4, 130.7, 132.6, 136.0, 144.5, 146.2, 151.8.

δ_B (CD_3OD): 0.53 (t, J = 33.2).

δ_F (CD_3OD): -146.5 to -146.2 (m).

$C_{40}H_{63}BF_2N_5O_{13}^+$ requires 870.44835, ESI [M+H]+ found 870.4497.

4.2.33 Conjugation between *β*-GlcNAc-PEG$_3$-propyne (179) and 4,4-difluoro-4-bora-1,3,5,7-tetramethyl-2,6-diethyl-8-(5-azidopentyl)-3a,4a-diaza-*s*-indacene (184)



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To a solution of *β*-GlcNAc-PEG$_3$-propyne **179** (5.0 mg, 0.013 mmol) in water (100 μL) was added a solution of 4,4-difluoro-4-bora-1,3,5,7-tetramethyl-2,6-diethyl-8-(hex-5-ynyl)-3a,4a-diaza-*s*-indacene **184** (6.4 mg, 0.015 mmol) in DMF (200 μL), a solution of cupric sulfate (50 μL, 15 mol%, stock prepared by dissolving 200 mg cupric sulfate pentahydrate in 20 mL of water), and a solution of sodium L-ascorbate (75 μL, 30 mol%, stock prepared by dissolving 200 mg of sodium L-ascorbate in 20 mL of water). The reaction mixture was then heated in a CEM microwave at 80°C for 3 min and was diluted with water (2 mL) upon cooling. The products were extracted with diethyl ether (2×6 mL) and the aqueous solution was purified by FPLC column chromatography on C18 reverse phase silica gel (1×30 cm). The column was eluted with a linear gradient of mixture of acetonitrile and water (40 to 80% acetonitrile over 100 min). The appropriate fractions were combined and lyophilized to give the *title compound* as a red sponge solid (7.7 mg, 73%).

R_f: 0.56 (System K).

δ_H (CD_3OD): 1.07 (6 H, t, *J* = 7.6), 1.49–1.57 (2 H, m, CH_2), 1.63–1.71 (2 H, m, CH_2), 1.96 (3 H, s), 1.98–2.07 (2 H, m, CH_2), 2.35 (6 H, s), 2.45 (6 H, s), 2.46 (4 H, q, *J* = 7.6), 2.99–3.03 (2 H, m, CH_2), 3.24–3.35 (2 H, m, H4 and H5, overlap with deuterated methanol solvent residue), 3.45 (1 H, dd, *J* = 8.3 and 10.0, H3), 3.60 – 3.74 (13 H, m, H2, H6a, one of the protons of the PEG-CH_2 attached to glycosidic linkage, and five other CH_2 of PEG), 3.88 (1 H, dd, *J* = 2.1 and 11.7, H6b), 3.92 (1 H, dt, *J* = 4.3 and 7.7, the other proton of the PEG-CH_2 attached to glycosidic linkage), 4.45 (2 H, t, *J* = 7.0, CH_2), 4.49 (1 H, d, *J* = 8.4, H1), 4.66 (2 H, s, CH_2), 8.01 (1 H, s, triazole proton).

δ_C (CD_3OD): 11.1, 12.2, 13.8, 16.5, 21.6, 26.4, 27.8, 29.5, 30.8, 49.7, 55.9 (C1), 61.4 (C6), 63.5, 68.4, 69.3, 70.1, 70.2, 70.3, 70.7 (C4), 74.9 (C3), 76.6 (C5), 101.5 (C1), 123.7, 130.7, 132.5, 136.0, 144.6, 144.8, 151.7, 172.4.

δ_B (CD_3OD): 0.54 (t, *J* = 33.0).

δ_F (CD_3OD): -146.6 to -146.3 (m).

$C_{39}H_{62}BF_2N_6O_9^+$ requires 807.46394, ESI $[M+H]^+$ found 807.4652.

4.2.34 Liposome preparation

Conjugate **185** (2.0 mg, 2.8 μmol) was dissolved in chloroform (1 mL) and evaporated under reduced pressure. The residue was dried further *in vacuo* for 1 h. To the dried material, HPLC grade water (3 mL) pre-heated at 60°C, followed by 10 glass beads (2 mm diameter), were added, and the mixture was gently swirled for 2 min. After incubation at 70°C for 1 h, the mixture was slowly cooled down to room temperature over 70 min to give liposomes that were analyzed by bright field and fluorescent confocal microscopy.

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