FUNCTIONALIZATION OF BODIPY-DYE SCAFFOLD USING CLICK-CHEMISTRY REACTIONS

by

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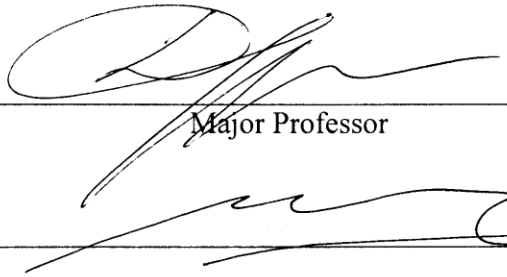

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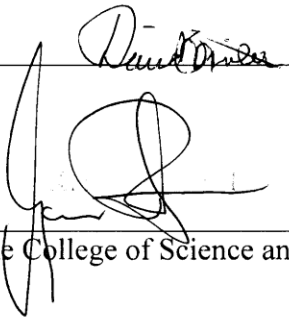

For The College of Science and Engineering

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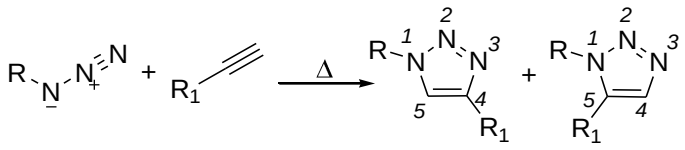
CHAPTER 1. 'Click Chemistry' and BODIPY Dyes

Heteroatom-containing moieties are among the most prevalent structural motifs found in many molecules of natural and synthetic origin. Therefore, the ability to synthesize such moieties with high selectivity and efficiency constitutes an important area of modern synthetic chemistry. Recently, a set of reactions has been identified that features readily available starting materials, easily attainable, non-demanding reaction conditions as well as facile, non-chromatographic isolation and purification of the products. This set has been coined as 'click chemistry' reactions by K. Barry Sharpless in 2001.[1] In recent years, the original meaning of the 'click' component appeared to be drastically diminished, as the efficiency of the reaction as well as the ease of isolation and purification of the products have not been given priority. It is rather the formation of particular moieties, such as triazoles and isoxazoles, for example, that is often referred to as 'click chemistry'.

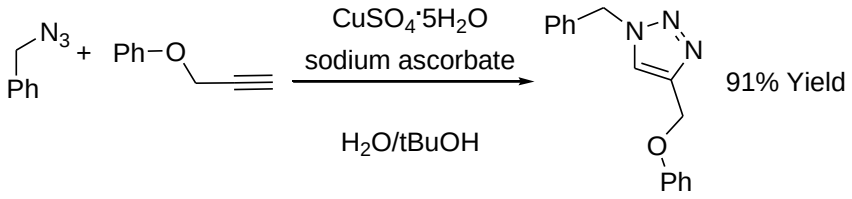
1.1. Alkyne – azide cycloadditions: triazole formation

One of the most popular click-type reaction features a formation of a triazole-group via a [3+2] cycloaddition between an alkyne and an azide (Scheme 1.1).



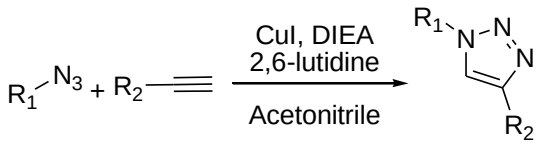
Scheme 1.1 Huisgen cycloaddition

The reaction was discovered by Huisgen and co-workers in 1967 and is now known as the Huisgen cycloaddition.[2] The triazole group is known for its thermal and chemical stability and hence it is of interest for a variety applications. The lack of wide synthetic utility of this reaction after its disclosure in the late 60s could be primarily attributed to the lack of selectivity in the formation of the 1,4- and 1,5-regiosiomeric product mixture. It has been recently discovered that the use of copper salts in the reaction not only decreases the reaction time and diminishes the need for heat, but also allows for a complete control of the regiochemistry, as it produces 1,4-disubstituted triazoles upon reaction of azides with terminal alkynes (Scheme 1.2).[3]



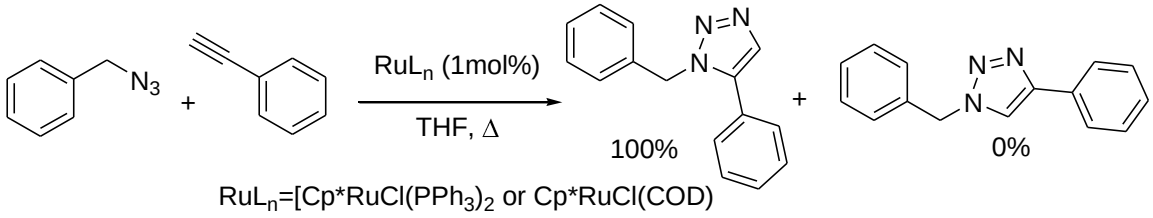
Scheme 1.2 Copper catalyzed cycloaddition

Since the first report by Sharpless' group, a number of conditions have been reported for the triazole formation. Specifically, Ghadiri's group developed non-aqueous conditions (Scheme 1.3).[4] Application of nitrogenous bases, which could also serve as ligands for copper species, significantly increased the number of possibilities for the reaction conditions as well as the substrate scope of this cycloaddition.



Scheme 1.3 Triazole formation under non-aqueous conditions

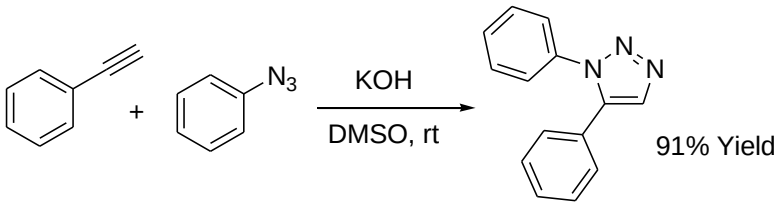
Fokin and coworkers have recently demonstrated that the application of ruthenium-based catalysts, instead of copper ones, allows one to obtain 1,5-disubstituted triazoles as sole cycloaddition products (Scheme 1.4).[5]



Scheme 1.4. Ruthenium-catalyzed 1,5-disubstituted triazole formation

This ruthenium-based triazole formation also allowed the use of internal alkynes (only external alkynes have been shown to undergo copper-catalyzed cycloadditions with azides).

Furthermore, the formation of 1,5-disubstituted triazoles was recently shown under basic conditions in the absence of a metal catalyst (Scheme 1.5).[6] The scope of this reaction appeared to be limited to aryl azides and aryl acetylenes, as the alkyl acetylenes failed to react most likely due to their lower acidity.



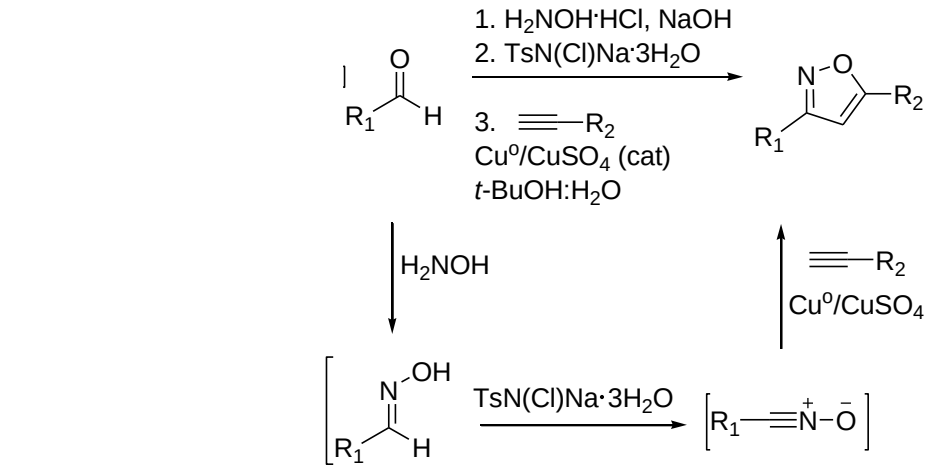
Scheme 1.5 Base-mediated, metal-free triazole formation

Collectively, metal-catalyzed alkyne – azide cycloaddition is a unique and versatile reaction in which the regiochemistry and reactivity can be easily controlled by a proper choice of the catalyst.

1.2. Alkyne – nitrile oxide cycloaddition: isoxazole formation

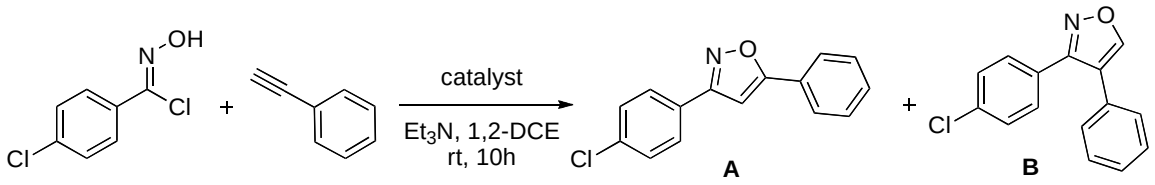
The use of aldoximes to create isoxazoles is another important tool that can be used to introduce both nitrogen and oxygen into a system. The synthesis of the isoxazole group has been hindered in the past due to instability of the nitrile oxide, side reactions, and low yields. Recent advances have helped bring this potentially useful reaction to the forefront of synthetic chemistry.

One approach (Scheme 1.6) is the *in situ* generation of a nitrile oxide *via* a reation of an aldehyde with hydroxylamine, followed by the treatment with chloramine-T trihydrate.[7] The subsequent addition of an alkyne in the presence of a copper catalyst, the isoxazole formation proceeds in acceptable yield.



Scheme 1.6 Isoxazole synthesis from aldehydes

Similar to the triazole formation, the isoxazole formation is not limited to just copper catalysts. Fokin's group demonstrated that ruthenium-based catalysts are capable of favoring the formation of 1,5-disubstituted isoxazoles.[8] Instead of generating a nitrile oxide from the aldehyde in one-pot, the authors showed that the hydroxybenzimidoyl chloride served as a suitable, easy to handle precursor for the nitrile oxide.



Catalyst	Conversion, %[a]	
	A	B
none	72	0
5 mol% [Cp*RuCl(cod)]	3	95
10 mol% [Cp*RuCl(cod)]	3	97

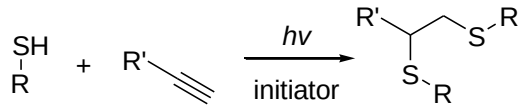
a – determined by GC

Scheme 1.7 Ruthenium catalyzed isoxazole formation

It is of interest to point out that in the absence of the catalyst, the 3,5-disubstituted isoxazole (Scheme 1.6 **A**) was formed as the only cycloaddition product. However, in the presence of the ruthenium catalyst, *i.e.*, [Cp*RuCl(cod)], the 3,4-regioisomer **B** was obtained exclusively. A number of other ruthenium catalysts were screened, but all were less efficient, as the mixtures of **A** and **B** were obtained at lower conversions. This ruthenium catalyzed process was also shown to work for internal alkynes, thus resulting in the formation 3,4,5-trisubstituted isoxazoles. Mild reaction conditions and simple filtration as a work-up step could be noted as the most attractive features of the this procedure.

1.3. Thiol-yne 'click chemistry'

A third example of click-type of reactions that builds the heteroatom-carbon bond library is thiol-yne.[9] This reaction has been given relatively little attention, but should provide a viable complement to other click-type reactions. Currently, the photochemical thiol-yne reaction is the most widely used type (Scheme 1.8).[10]

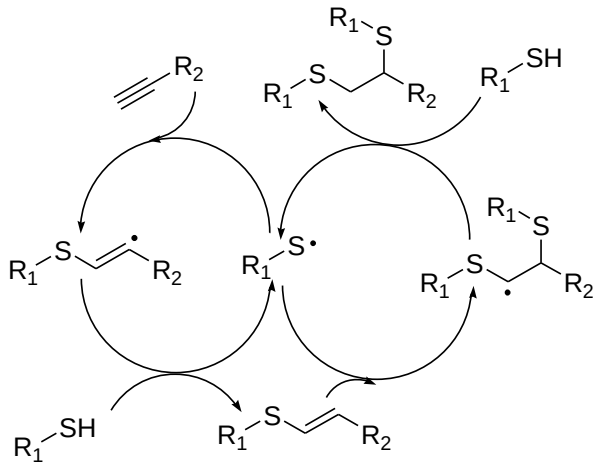


Scheme 1.8. A representative example of thiol-yne reaction

The thiol-yne reaction features many attributes of a true click-chemistry paradigm: high yields, high reaction rates, and large scope of reactants. In addition, these

reactions can be performed at ambient conditions and usually occur with no side products, which facilitates the product isolation and purification.

Several initiators have been shown to be suitable for this transformation, and 2,2-dimethoxy-2-phenylacetophenone (DMPA) is one of the most commonly used initiators. From the mechanistic point of view (Scheme 1.9), the reaction is believed to proceed *via* the RS• radical, and it also features alkene intermediates *en route* to the fully saturated product.[10]



Scheme 1.9 Proposed mechanism for the thiol-yne click reaction

Current applications of the thiol-ene/yne chemistry primarily focus on hydrogel formations,[10] polymer functionalization,[11] and synthesis of macrocycles.[12]

1.4. Modifications and Applications of BODIPY Dyes

The design, synthesis and uses of small fluorescent molecules are among the most active areas of modern research. Easily accessible fluorescent molecules should

significantly aid in the development of numerous applications related to various fields of sciences, engineering and medicine. Therefore, facile functionalization of a basic dye might create an array of versatile fluorescent probes with a wide range of applications. Among many fluorescent dyes, 4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene (BODIPY) dyes (Figure 1.1) have been attracting a lot of attention.

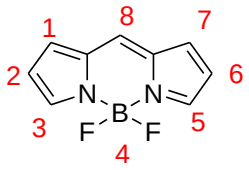


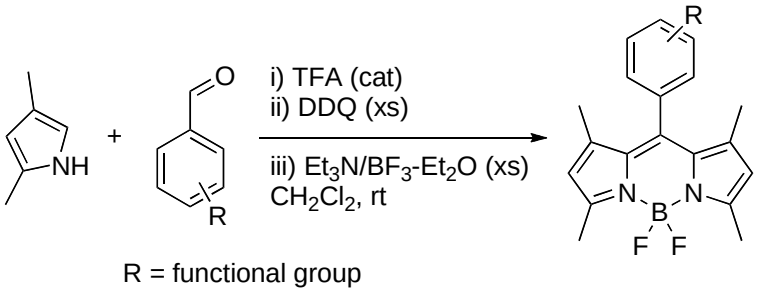
Figure 1.1 Structure of the BODIPY core

These dyes are stable under physiological conditions, and they are fairly insensitive to variations in pH and polarity of the media.[13] In addition, sharp excitation and emission bands, high quantum yields, and high photostability contribute to the increasing popularity of BODIPY dyes for numerous biological applications as fluorescent labels. Importantly, the spectroscopic properties of BODIPY dyes can be altered by structural modification of the dye.[13,14] Thus, a great deal of studies focuses on developing synthetic routes to introduce substitutents on the BODIPY core at virtually every position, *i.e.*, 1 – 8, including modifications at the boron atom (Figure 1.1).

In general, structural and functional diversity of BODIPY dyes can be achieved *via* several routes: (a) synthesis of the dyes using distinctly modified starting materials, and (b) introducing modifications onto the existing BODIPY dye.

One of the main methods to prepare BODIPY dyes is based on the condensation of various aldehydes with pyrroles under acidic conditions, followed by oxidation with

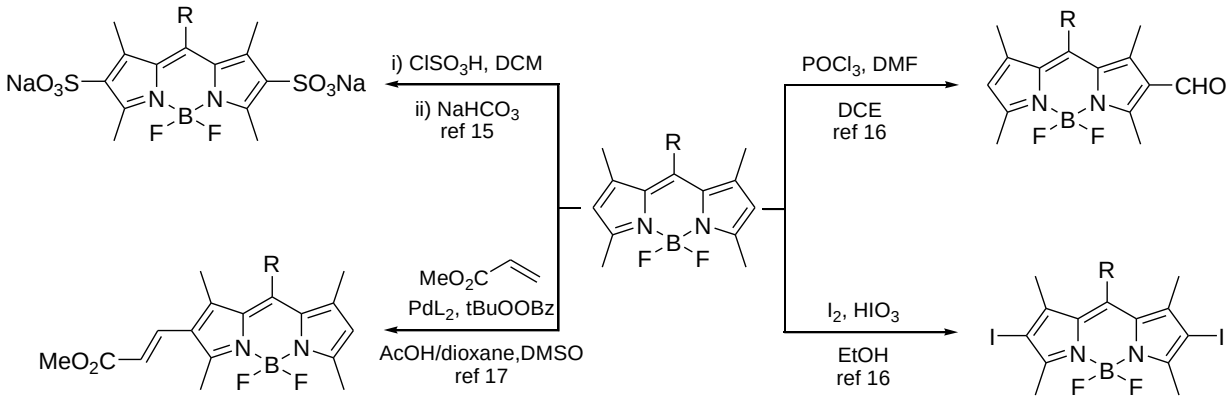
DDQ, and finally installation of the BF_2-moiety using BF_3-etherate and triethylamine as a base (Scheme 1.10).[13]



Scheme 1.10 A typical synthesis of a BODIPY dye.[13]

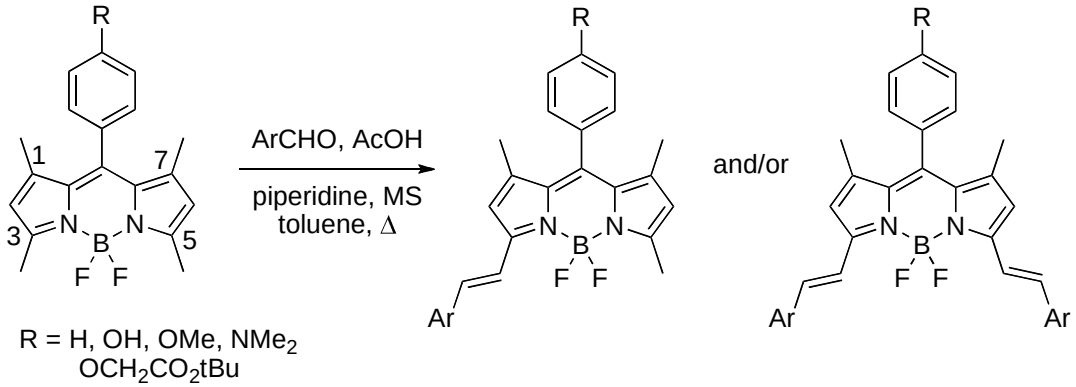
In general, this BODIPY synthesis can be carried in one pot or each intermediate can be isolated, purified and subjected to the next step without significant impact on the overall yield. The use of substituents, usually methyl groups, in the 2 position as well as both positions 2 and 4, on the pyrrole prevents the undesired polymerization reaction. The yields of the final BODIPY dye depend on the nature of the substitutent on the benzaldehyde, and usually range between 20 and 70 %.

Functionalization of the 2 and 6 positions on the BODIPY core can be achieved in a variety of ways, and several representative approaches are shown in Scheme 1.11. These functionalized BODIPY dyes can be transformed into more structurally elaborate dyes.



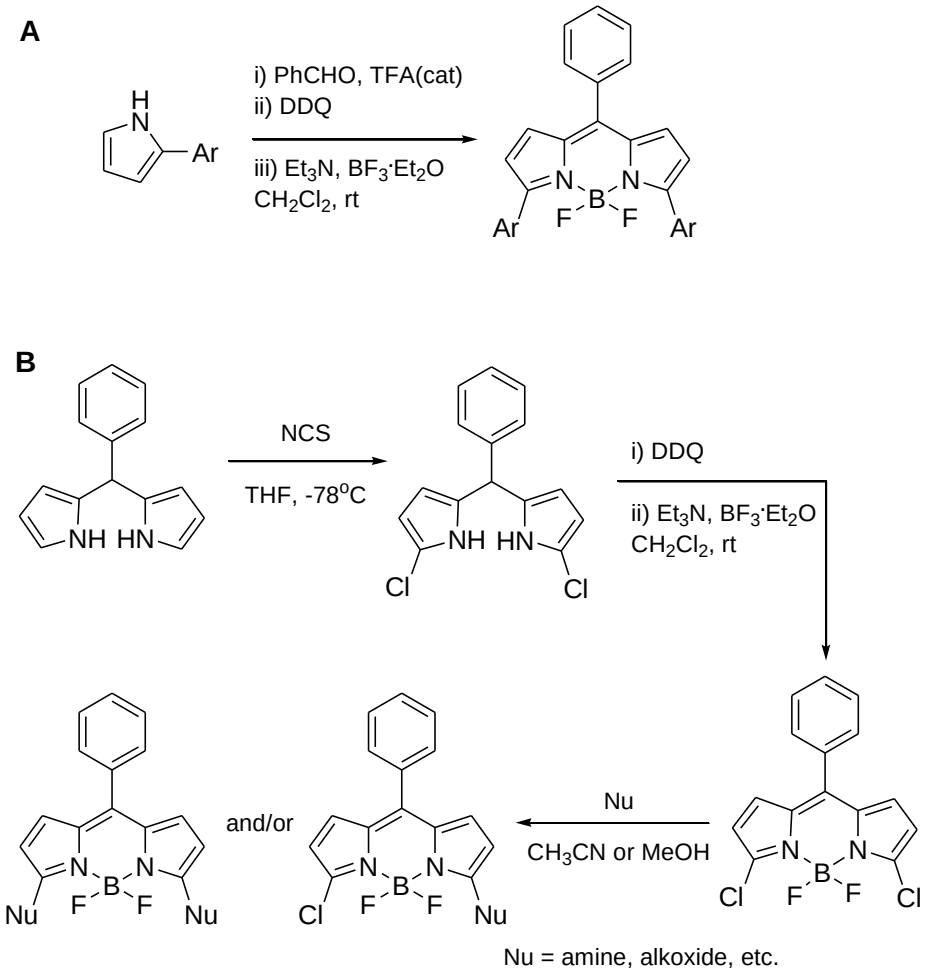
Scheme 1.11 BODIPY modification at the 2 and 6 positions

In view of the commercial availability of 2,4-dimethylpyrrole and 2,4-dimethyl-3-ethylpyrrole, the corresponding 3,5-dimethyl-containing BODIPY scaffolds are very prevalent in literature. These methyl-containing BODIPY dyes can be further functionalized using Knoevenagel condensation (Scheme 1.12).[18] Although the methyl groups at 3 and 5-positions are the most reactive (*i.e.*, most acidic, and easily deprotonated under the given reaction conditions), some recent accounts demonstrated that under some conditions, the methyl substitutents at 1- and 7-positions could be functionalized as well.[19]



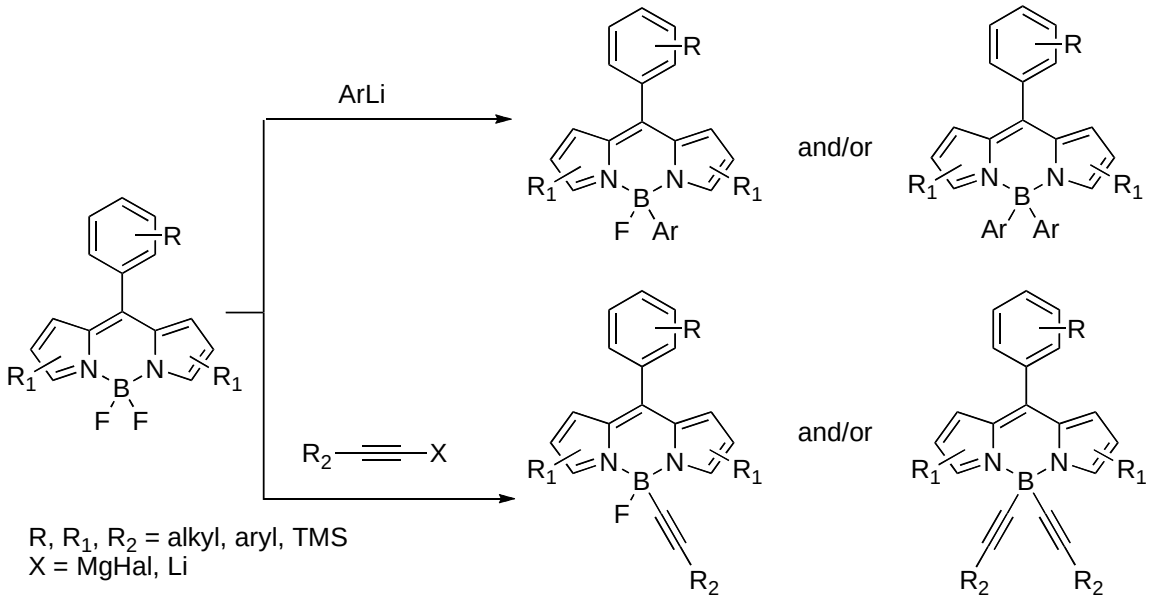
Scheme 1.12 BODIPY modification at the 3 and 5 methyl groups *via* Knoevenagel Condensation

In addition, the incorporation of substitutents in positions 3 and 5 can be accomplished by the use of 2-substituted pyrroles,[20] which are subsequently converted into the BODIPY dyes (Scheme 1.13A). Furthermore, 3,5-disubstituted BODIPY dyes could be prepared from the 3,5-dihalo-containing BODIPY dyes *via* a nucleophilic aromatic substitution (Scheme 1.13B).



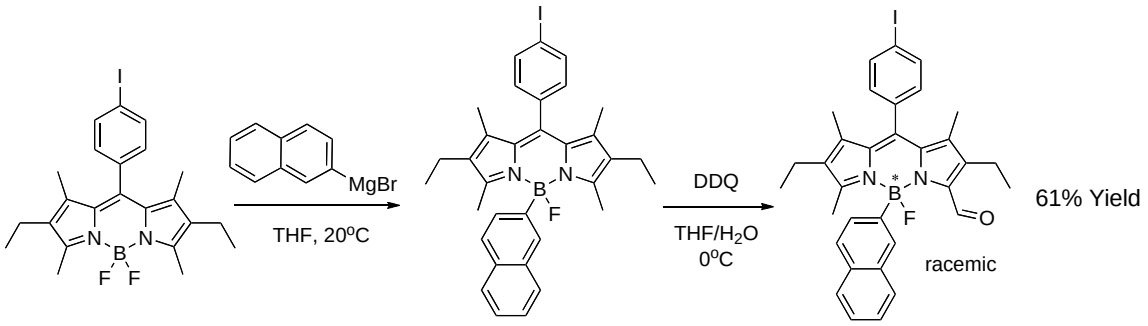
Scheme 1.13 BODIPY modification at the 3,5 positions

Several approaches were developed for the modification of the boron center. Using Grignard reagents or organolithium reagents the fluorine atoms can be replaced with ethynyl or aryl subunits (Scheme 1.14).[21]



Scheme 1.14 Dye substitutions on boron

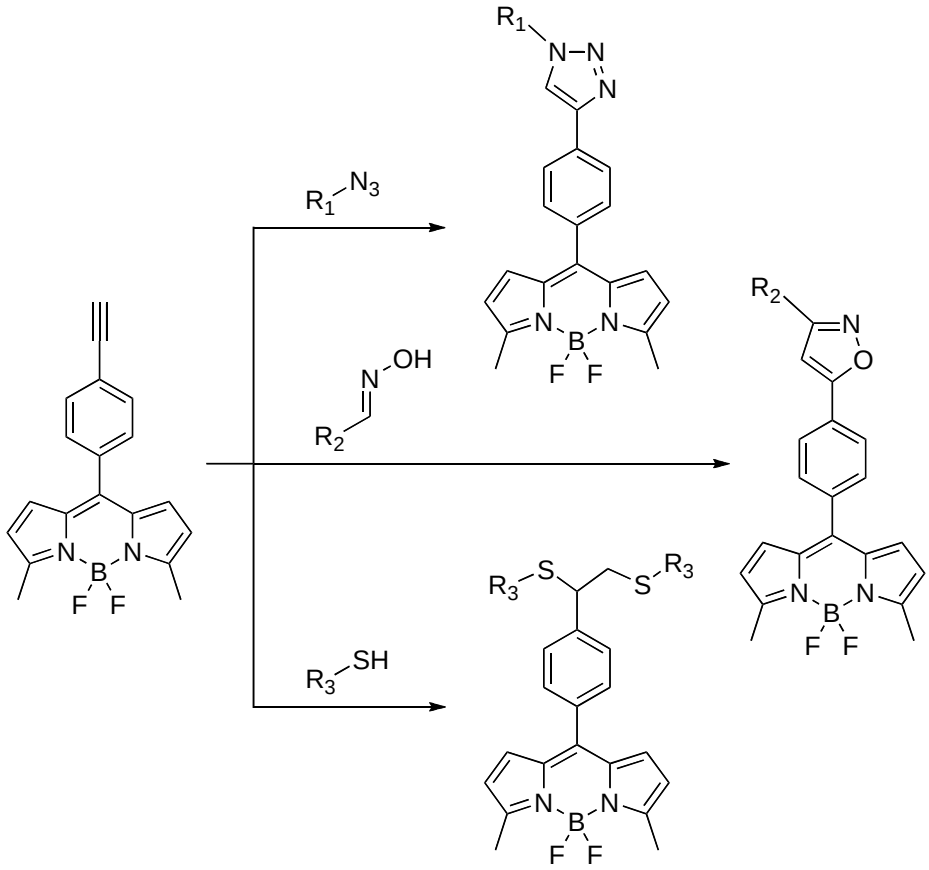
It is of interest to note that chiral (at B-center) BODIPY dyes were recently prepared *via* a monosubstitution at the boron using a Grignard reagent, followed by oxidation of the 3-methyl substituent with DDQ (Scheme 1.15).[21] The racemate was resolved by chiral HPLC (using a Chiralcel-OD column), and the circular dichroism spectra confirmed the presence of two enantiomers (the absolute stereochemistry was not determined).



Scheme 1.15 Synthesis of BODIPY dye with a stereocenter at boron

It is important to note that although the nature of the substituents and their position on the BODIPY skeleton are known to be major factors in controlling fluorescent properties of the BODIPY dyes, the synthetic efficiency of the reactions reduces drastically as the structural complexity increases.

The brief survey of literature on the modification of BODIPY dyes indicated to us that the availability of a single alkyne-containing BODIPY scaffold might be a sufficiently, interesting and useful tool to create several structurally and functionally diverse fluorescent dyes using ‘click chemistry’ reactions (Scheme 1.16).



Scheme 1.16 Proposed modifications of BODIPY scaffold using 'click chemistry' reactions

Although, some triazole-containing BODIPY dyes have been reported in the literature[22], the application of click-methodology for modification of the BODIPY dyes remains a fairly unexplored area of research. Our efforts in this area are presented in the subsequent chapters.

CHAPTER 2. Synthesis of 5-Iodo-1,4-Disubstituted Triazoles

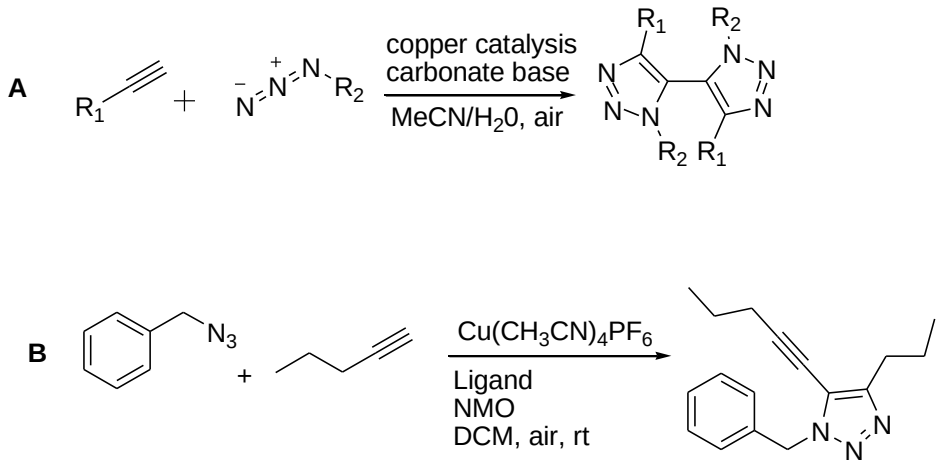
2.1. Base and concentration effects on the formation of 5-iodo triazoles

One of the leading synthetic tools with applications that go beyond those of organic chemistry is the copper-promoted/catalyzed alkyne-azide cycloaddition, more commonly known as 'click-chemistry'.[23] From the synthetic point, these 'click' cycloadditions are distinguished by mild conditions, high tolerance to many functional groups, and high yields.[23] In the case of CuI-promoted alkyne-azide cycloadditions, the presence of a base is needed to facilitate the formation of copper acetylides, which are believed to be the active species for the reaction with the azide. It was also suggested that the base could prevent acetylide aggregation, which helps the reaction proceed forward.[24]

Earlier studies in the group demonstrated that when the reaction of an alkyne-BODIPY with an azide was done under aqueous conditions, 5-H BODIPY was the only cycloaddition product. However, under non-aqueous conditions, a small amount of a triazole-BODIPY that contained an iodo-group at the position 5 of the triazole-ring was also isolated.

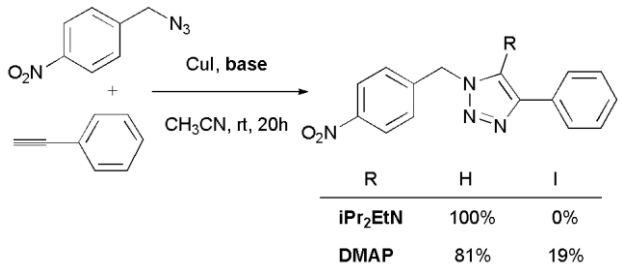
In general, it has been demonstrated that the nature of the base as well as additives might have a dramatic impact on the cycloaddition product distribution. For example, in the presence of inorganic bases, such as potassium carbonate (Scheme 2.1 A), in addition to 1,4-disubstituted triazole, the triazole dimer product was produced.[25] Also, addition of Cu ligands, such as *N,N,N'*-trimethylethylenediamine, allowed for the formation of 1,4,5-trisubstituted-1,2,3-triazoles (Scheme 2.1 B).[26] The two most used

bases for reactions done under non-aqueous condition are either Et_3N or i-Pr_2EtN (Hünig's base); providing 1,4-disubstituted 1,2,3-triazoles as the major or only cycloaddition products.[23]



Scheme 2.1. Effect of inorganic base (**A**) and copper ligands (**B**) on the product distribution in alkyne-azide cycloaddition.

During our model studies on copper-promoted alkyne-azide reaction, we observed that when 4-dimethylaminopyridine (DMAP) was used instead of Hünig's base, a formation of *ca*. 20% of 5-iodo-1,4-triazole was noted (Scheme 2.2).



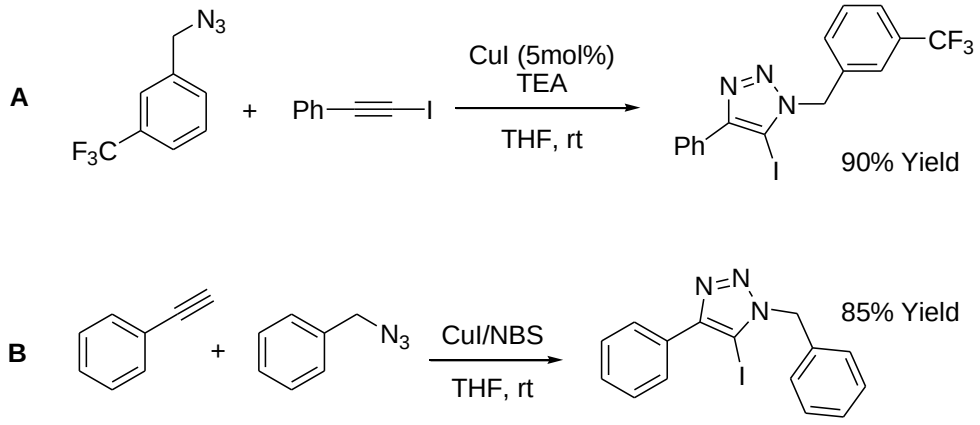
R	H	I
iPr$_2$EtN	100%	0%
DMAP	81%	19%

Scheme 2.2. Base-specific distribution of 5-R-triazoles.

The ratio between the 5-H and 5-I products was determined from ^{1}H NMR of the crude mixture. Specifically the CH_2-protons of 5-I-triazole appear as a singlet (5.8 ppm) downfield relative to the CH_2-protons of 5-H-triazole (5.7 ppm) and therefore, an unambiguous evaluation of the composition could be achieved.

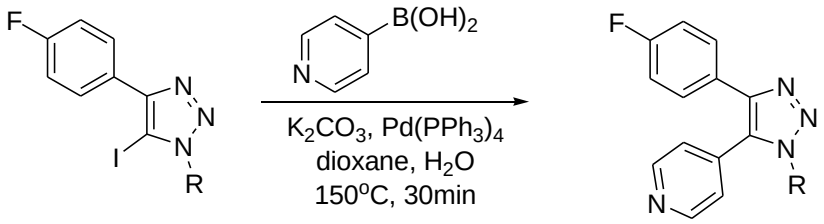
While iodo-containing triazoles are occasionally reported as minor products in different copper-catalyzed and copper-promoted alkyne-azide cycloadditions, there are no reports however on the ability of organic bases, such as DMAP, to control the product distributions in the alkyne-azide cycloadditions.[27] It is important to note though that DMAP was recently shown to accelerate 1,4-triazole formation catalyzed by copper-carbene ligands, without inducing the formation of any other cycloaddition products.[28]

It is worth pointing out that iodo-triazole can be prepared directly *via* an *in situ* formation of iodo-acetylenes (Scheme 2.3A).[29] This procedure showed a lot of potential due to its convenience and the fact that it only yielded the 5-iodo-triazole product. It was proposed that the integrity of the C-I bond was retained through out the reaction. In addition, *in situ* generation of Cu-triazoles in the presence of electrophilic iodine was also shown to introduce iodine into the 5-position of the triazole ring (Scheme 2.3B).[30]



Scheme 2.3. *In Situ* formation of 5-iodo-triazoles

The 5-iodo-triazoles can be used in a number of different cross-coupling reactions. In particular, in the case of Suzuki coupling, various heteroaromatics were prepared using 5-iodo-triazoles (Scheme 2.4).[31] These coupled products were found to be potent kinase inhibitors and they have the potential to combat diseases and inflammation.[32] Carbanucleoside analogues were prepared using halogen containing triazoles,[33] and showed promise in treating an influenza virus and the hepatitis virus.

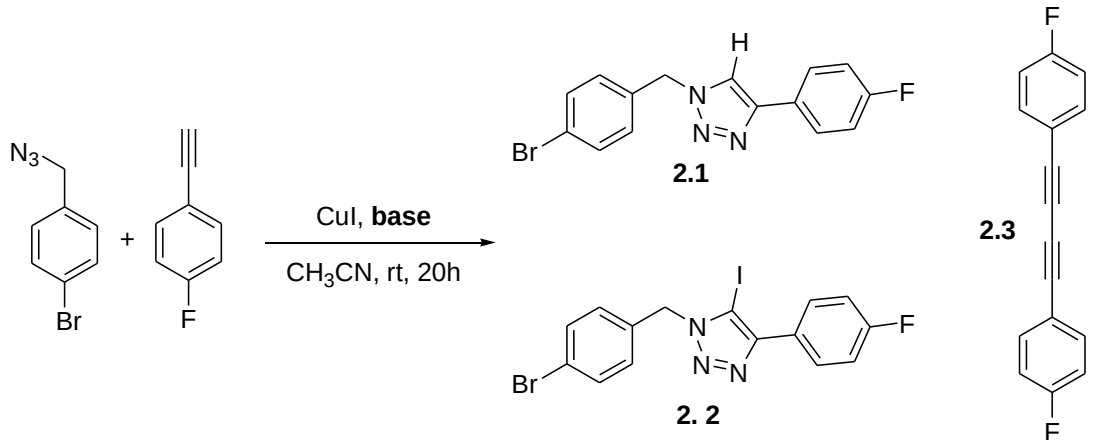


Scheme 2.4. Cycloaddition-Suzuki relay *via* the formation of 5-iodo-triazole.[31]

Additional uses of the 5-iodo-triazole moiety stem from the interactions featuring C-Hal compounds, which are significant for modulating various molecular, biomolecular, and supramolecular recognition processes.[34] Preliminary results show that halogen bonds built into protein kinase inhibitors play an important part in inhibiting interactions with protein kinase. Arguably, using a strong halogen bond's selectivity for the carbonyl oxygen on polypeptide backbones, inhibitors could be constructed that are less inclined to drug resistance.

In view of the aforementioned reports, it was of interest to elaborate on our initial findings (Scheme 2.2) as an interesting possibility to synthesize iodo-triazoles as the major cycloaddition product by simply switching the base, *i.e.*, from i-Pr_2EtN to DMAP. As a model reaction, the flouro-containing alkyne was chosen for the convenience of

Table 2.1 Base/concentration effects on the 5-iodo-triazole formation.



Entry	[alkyne], mM	Base	Product distribution, %[b]		
			2.1	**2.2**	**2.3**
1	1000	DMAP	88	12	-
2	1000	Pyridine	84	16	-
3	1000	DMA	100	-	-
4	1000	iPr_2EtN	100	-	-
5	35	DMAP	64	30	6
6	3.5	DMAP	21	58	21
7	3.5	DMAP[c]	-	23	77
8	3.5	DMAP[d]	-	44	56
9	3.5	DMAP[e]	-	96	4
10	35	DMA	100	-	-
11	3.5	DMA	36	64	-
12	3.5	Pyridine	76	24	-
13	35	iPr_2EtN	100	-	-
14	3.5	iPr_2EtN	100	-	-

[a]Ratios: alkyne/azide/base/CuI – 1.0/1.0/1.0/1.0; [b]Determined by ^{19}F NMR on a crude reaction mixture; [c]Ratios: alkyne/azide/base/CuI – 1.0/1.5/1.0/1.0; [d]Ratios: alkyne/azide/base/CuI – 1.0/3.0/1.0/1.0; [e]Ratios: alkyne/azide/base/CuI – 1.0/3.0/0.3/1.0.

identifying the product distribution using ^{19}F NMR due to non-overlapping resonances of compounds **2.1** (-112.7 ppm), **2.2** (-111.9 ppm) and **2.3** (-107.9 ppm) (Table 2.1).

In view of the base-dependant nature of this cycloaddition, common aromatic bases, such as pyridine and N,N-dimethylaniline (DMA) that are structurally related to DMAP were examined. After screening several reaction conditions (Table 2.1), it was discovered that three products were obtained: 5-H-triazole **2.1**, 5-iodo-triazole **2.2**, and the diyne **2.3**. The product distribution appeared to be controlled by the identity of the base and the alkyne concentration.

Among various bases screened (Table 2.1, entries 1-4), it was found that at high reaction concentrations, only DMAP and pyridine promoted the formation of the iodo-containing triazole **2.1** (entries 1 and 2). The formation of the iodo-triazole correlated with the alkyne concentration under DMAP-promoted reactions. By lowering the alkyne concentration from 1.0 M to 3.5 mM (entries 1, 5, and 7), the 5-I-triazole was obtained as the major or only cycloaddition product (at 3.5mM), while **2.1** was not observed.

With respect to other bases used, it appeared that only in the cases of pyridine and DMA, the formation of the 5-iodo product **2.2** was observed, while the product distribution was concentration independent when the Hunig's base was used (Table 2.1, entries 4, 13, 14). Notably, both DMA and pyridine produced appreciable amounts of 5-H-triazole **2.1**. However, DMA turned out to be superior to pyridine, as at 3.5 mM, DMA afforded 5-I-triazole as the major cycloaddition product (entry 11), whereas pyridine still favored 5-H product **2.1** (entry 12). In addition, the conversions with DMA and pyridine were inferior to those of DMAP. After considering all of these results, DMAP was chosen as the most efficient base in promoting the formation of 5-I-triazoles.

We also investigated the distribution of 5-*I*-triazole and 5-*H*-triazole as a function of time. The ratio of the two products was found to be time independent at both concentrations of 1.0 M and 3.5 mM of the alkyne, while using equimolar amounts of azide, DMAP, and CuI. No 5-*H*-triazole was ever detected at 3.5 mM. Also, when **2.1** was reacted with an equimolar mixture of CuI and DMAP for 20 h, **2.1** was recovered unchanged, and 5-I-triazole **2.2** was not detected. Hence, it is plausible that the formation of 5-I-triazole takes place *via* a distinct Cu-intermediate, which is stabilized by DMAP.

Next, the effect of CuI concentration was investigated. Increasing the amount of CuI to 2.0 eq. had no apparent impact on the rate of formation of the iodo-triazole **2.1**. But as was projected, decreasing CuI content led to lower conversions. Also, the use of CuCl proved to be inefficient in promoting the cycloaddition.

Furthermore, the cycloaddition product distribution was found to be fairly insensitive to the nature of the solvent. Among the solvents screened (CH_3CN, THF, DMSO), CH_3CN seemed the most efficient in terms of product conversion and distribution.

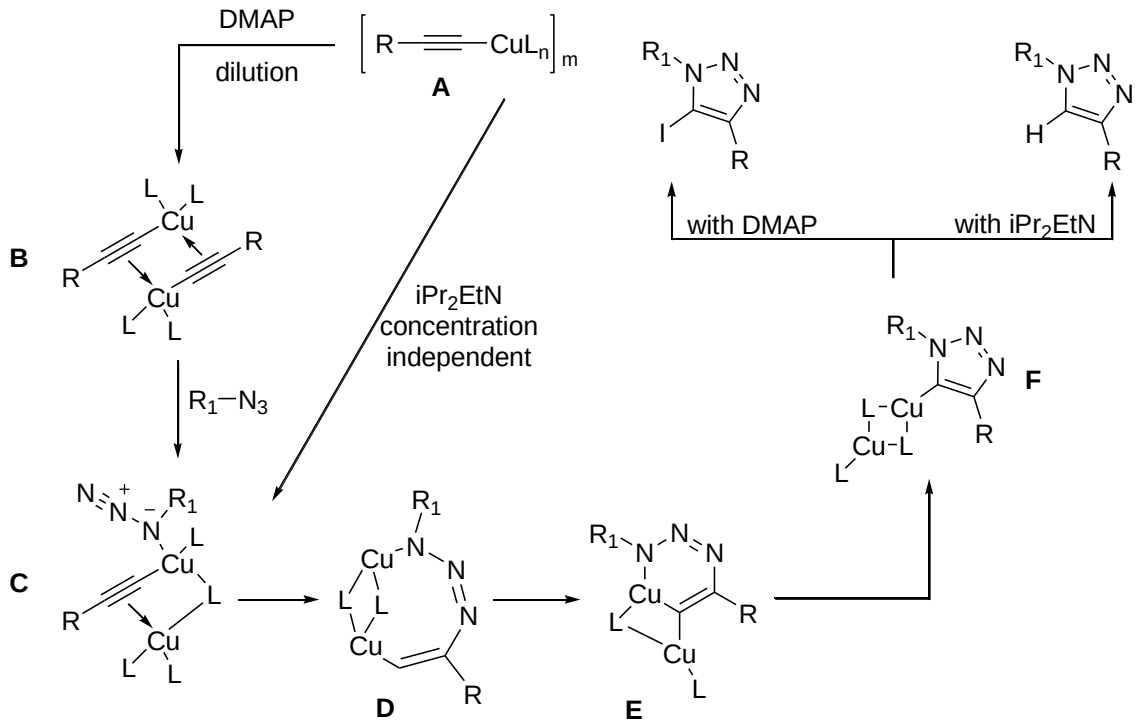
Even though the 5-iodo-triazole **2.2** was obtained as the main cycloaddition product, the major product of the reaction was the diyne **2.3** (Table 2.1, entry 7). This side reaction for some Cu-catalyzed and Cu-promoted alkyne-azide cycloadditions was previously reported,[24] however the effect of neither the base nor concentration had been examined. In an attempt to suppress the formation of **2.3**, the reactions were conducted using degassed CH_3CN and/or under inert atmosphere (N_2), albeit with no success, as the amount of the diyne product was largely unaffected. After some experimentation, the concentration of the azide was found to be one of the complementary factors in

controlling the formation of 5-iodo-triazole. Increasing concentration of the azide to 3.0 eq. (Table 2.1, entry 8) increased the amount of **2.2**, while reducing the diyne **2.3** formation. It is interesting that in the absence of the azide, DMAP promoted a clean formation of the diyne **2.3**.

To further suppress the formation of **2.3**, the concentration of DMAP was varied. It appeared that increased amounts of **2.3** were correlated with increasing the concentrations of DMAP, which could be expected due to an enhanced rate of deprotonation of 4-fluorophenylacetylene. Alternatively, the formation of **2.3** was significantly suppressed at 0.3 eq. of DMAP (Table 2.1, entry 9). However, 5-I-triazole **2.2** was obtained as the major product and the only cycloaddition product, though the rate of the reaction was inhibited. The formation of the diyne **2.3** was not promoted by either DMA nor by pyridine, which is consistent with both of these bases being weaker than DMAP.

We have also screened the effect of bases and concentrations on the CuI-promoted cycloaddition between phenylacetylene and benzyl azide, *i.e.*, electronically unbiased substrates, and the results appeared in correlation with the trends observed in Table 2.1.

Although the mechanistic details of a typical CuI-catalyzed/promoted alkyne-azide cycloaddition has been reported in literature,[35] the exact mechanism still needs to be clarified. By taking together our optimization studies (Table 2.1) and literature accounts, some mechanistic insight about the formation of 5-I-triazoles under our conditions can be proposed (Scheme 2.5).



Scheme 2.5. A tentative mechanism for the base-controlled 5-iodo-triazole formation

It is largely accepted that copper-complexes could form various aggregates,[24] and therefore at high concentrations it is likely that these aggregates, **A**, might be responsible for promoting the alkyne-azide cycloaddition leading to the formation of 5-H triazoles (Scheme 2.5). Upon dilution it is possible, the Cu-acetylides **A** disaggregate to form a bis-copper acetylide complex **B** (Scheme 2.5). This complex **B** might then be responsible for the formation of the diyne **2.3** (Table 2.1), which becomes the major product in dilute media. This observation is supported by the mechanistic rationale for the terminal alkyne homocoupling (known as the Glaser alkyne homocoupling),[36] which is proposed to involve bis-copper acetylides. Additionally, DMAP, a strongly coordinating, sterically unbiased ligand (in comparison with Hünig's base) for Cu-complexes should assist in stabilization of **B**. This proposal is in accord with our observation that decreasing the

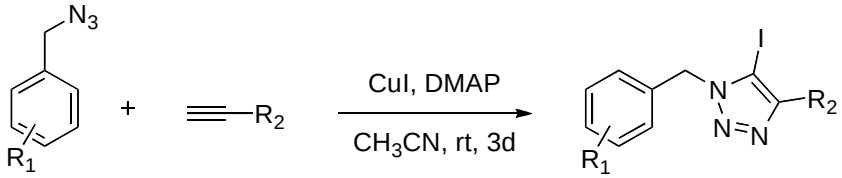
amount of DMAP decreased the alkyne homocoupling. Apparently, compared to DMAP, other bases are inferior in helping to stabilize **B**, and therefore, the formation of the diyne **3** was not observed.

Further, azides could act as ligands, which would help in the stabilization of the subsequent copper complexes. It could then be expected that the increase in the amount of azide would disrupt complex **B** and promote a formation of complex **C**. The relative inability of pyridine and DMA to stabilize **B**, compared to DMAP, allows the azide to compete for the coordination site and, arguably, lead to **C**. This should explain the formation of **2.2** and the lack of **2.3** (Table 2.1, entries 11 and 12).

The subsequent cascade probably proceeds from **D** to **E** to **F**, all of which are reminiscent to those suggested for the formation of the 5-H triazoles.[37, 23] Finally, DMAP, pyridine and DMA, might provide extra stabilization of one or more of the **D**, **E** and **F** complexes, (which was suggested for pyridine[37]) and allow for an efficient intramolecular delivery of iodine to furnish 5-I-triazole, whereas the bulkier ligand iPr_2EtN does not,[37] thus leading to 5-H-triazole.

Having the optimized conditions, we examined the scope of the DMAP-promoted 5-iodo-triazoles (Table 2.2).

Table 2.2. Synthesis of 5-iodo-triazoles.[a]



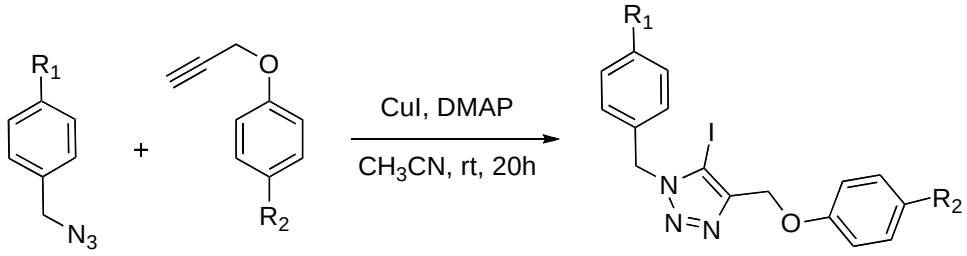
Entry	5-I-triazole	R_1	R_2	Isolated Yield, %
1[b]	**2.4**	H	C_6H_5	77
2	**2.5**	Br	4-F-C_6H_4	24
3	**2.6**	4-MeO	C_6H_5	37
4	**2.7**	4-NO_2	C_6H_5	65
5[c]	**2.7**	4-NO_2	C_6H_5	78

[a]Conditions: [alkyne] = 3.5 mM, ratios: alkyne/azide/DMAP/CuI – 1.0/2.0/0.3/1.0; [b]Reaction time 48 h; [c]3.0 eq of azide.

The slow reaction rates were due to low concentrations that were required to achieve the exclusive formation of 5-I-triazole. Thus, in order to achieve appreciable conversions and since product distribution was time independent, we increased the reaction times to 72 h. Chromatography-free removal of the reagents and unreacted starting materials afforded iodo-triazoles in low to moderate yields (entries 1-4). Increasing the amount of the azide from 2.0 eq. to 3.0 eq. had a marginal impact on the yield (entries 4 versus 5).

In addition, we examined the formation of 5-iodo-1,4-disubstituted triazoles using propargyl-containing substrates under the established set of reaction conditions (Table 2.3).

Table 2.3. Syntheses of 5-iodo-triazole from propargyl-containing substrates

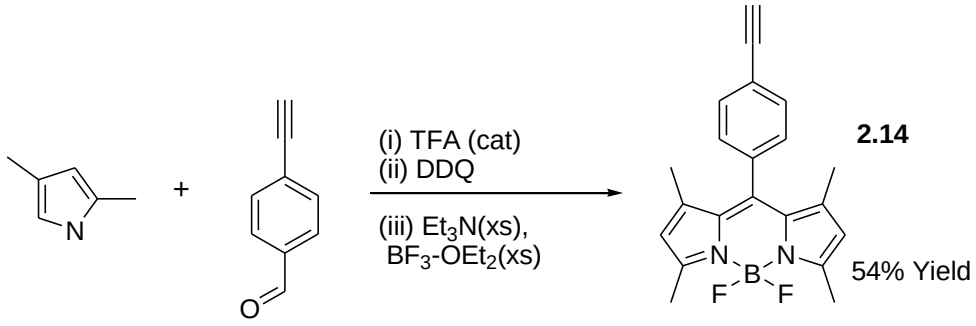


Entry	5-I-triazole	R_1	R_2	Isolated Yield, %
1	**2.8**	NO_2	H	22
2	**2.9**	tBu	MeO	34
3	**2.10**	tBu	NO_2	34
4	**2.11**	H	MeO	39
5	**2.12**	NO_2	MeO	23
6	**2.13**	MeO	MeO	33

The unreacted starting materials were easily recovered, using flash column chromatography. Overall, these reactions appeared to be less efficient than the arylacetylene series (Table 2.2). Importantly, DMAP and low alkyne concentration were still the determining factors in facilitating the formation of the iodo-triazoles. No alkyne homocoupling products were observed. The effect of varying other conditions, *i.e.*, concentrations of DMAP and CuI as well as the solvent, was virtually identical to the results obtained with 4-fluoro-phenylacetylene.

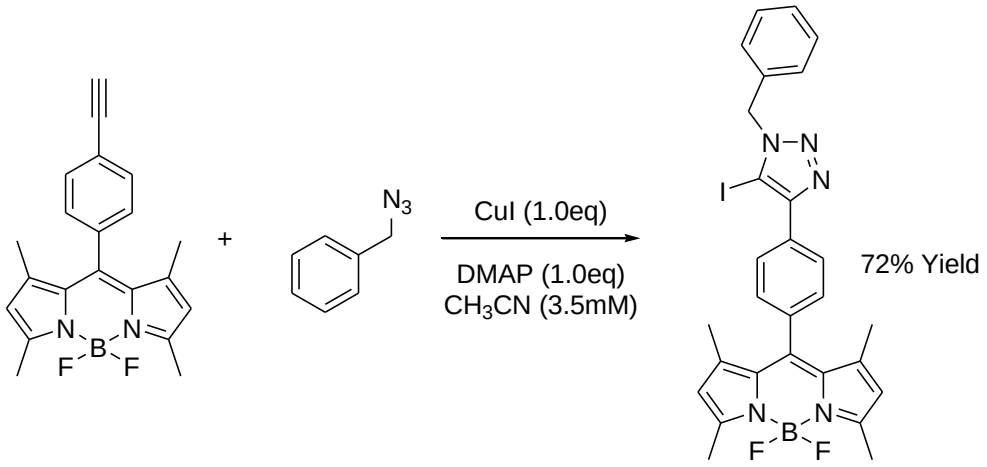
2.2. Synthesis of 5-iodo-triazole-containing BODIPY dyes

With the optimized conditions for the 5-iodo-triazole, attention was turned to the possibility of creating the 5-I triazole with a BODIPY dye as the main product.[14] Based on the proposed modular approach for the facile functionalization of the BODIPY scaffold (Scheme 1.11), we prepared the alkyne-containing BODIPY dye **2.14** using 4-ethynylbenzaldehyde and 2,4-dimethyl pyrrole (Scheme 2.6).



Scheme 2.6 Synthesis of alkyne-BODIPY **2.14**.

Using benzyl azide, copper iodide, and DMAP the formation of the 5-I triazole was attempted (Scheme 2.7). Under the optimized conditions established during the model study (Table 2.1), no cycloaddition product was formed, but instead only the di-alkyne BODIPY dimer formed. After some optimization of the reaction conditions, *i.e.*, changing the concentration to 35 mM from 3.5 mM, the reaction afforded the desired 5-I-containing dye in 72% yield as the only product.



Scheme 2.7 5-iodo-triazole formation with BODIPY dye

2.3. Conclusions

In summary, we found that the identity of the organic base as well as the concentration of the alkyne, played the major roles in determining the nature of the products in the CuI-promoted alkyne-azide cycloaddition. Low concentrations of the alkyne and the use of DMAP led to the formation of 5-I-triazoles as the only cycloaddition products. This methodology was applied for the synthesis of the 5-iodo-triazole-containing BODIPY dyes.

2.4. Experimental section

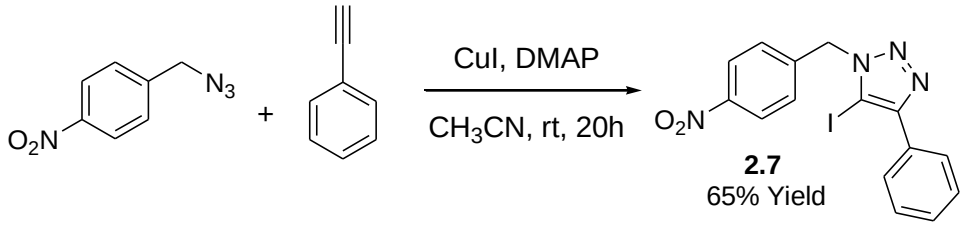
2.4.1. Materials and Methods

All reagents and solvents were from commercial sources (Sigma-Aldrich, Acros, Matrix Scientific, or Alfa Aesar). They were of the highest grade possible and were used as received. Azides were prepared from the corresponding bromides upon reaction with

NaN_3 in DMSO. Reactions were monitored by TLC (silica gel 60 F254), and the spots were visualized by UV or I_2. Column chromatography was performed using silica gel (230-400 mesh). NMR spectra were recorded on a Varian Mercury-300, and chemical shifts are reported in parts per million relative to internal standard tetramethylsilane (δ = 0.00 ppm) for ^{1}H NMR and ^{13}C NMR spectra taken in $CDCl_3$, and relative to DMSO (δ = 39.52 ppm) for ^{1}H NMR and ^{13}C NMR spectra taken in DMSO, and chemical shifts for ^{19}F NMR were externally referenced to the fluorine peak of 10-[*4-Aminophenyl*]-2,8-*diethyl*-5,5-*difluoro*-1,3,7,9-*tetramethyldipyrrolo*[1,2-c:2,1-f][1,3,2]*diazaborinin*-4-*ium*-*5-uide* ^{19}F-NMR ($CDCl_3$, 282.5 MHz): -145.29 (q, *J* = 35Hz).[38] The structure of the 5-iodo-triazole is determined by not observing the 5-H proton which appears at 7.62 ppm.

2.4.2. Synthesis of 5-iodo-containing triazoles

General procedure A



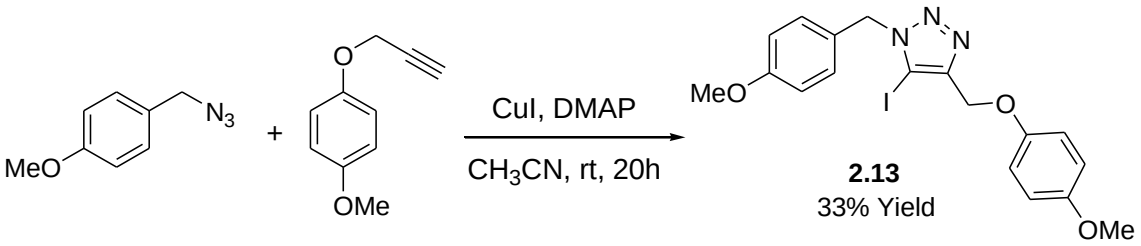
2.7
65% Yield

A one neck round bottom flask was charged with a stirring bar, CH_3CN (45 ml), 4-nitrobenzyl azide (56.0 mg, 0.318mmol), phenylacetylene (17.6 ml, 0.159 mmol), and CuI (30.3 mg, 0.159 mmol) and stirring initiated. DMAP (5.8 mg, 0.0477 mmol) was added as a solid in one portion, the flask was capped, and stirred at room temperature for 20 h in the dark. Subsequently, the reaction mixture was diluted with CH_2Cl_2 (50 ml) and extracted with 1M HCl (2 x 10 ml) and water (10 ml). Organic layer was dried over

$MgSO_4$ and volatiles removed in vacuo. The residue was redissolved in CH_2Cl_2 (2 ml) and hexane (25 ml) was gradually added. The cloudy solution was placed on ice, and product **2.7** was isolated as a light yellow solid upon filtration (42 mg, 65 %).

^{1}H NMR (300 MHz, DMSO-d_6): δ = 8.25 (2H, d, *J* = 8.5 Hz), 7.90 (2H, d, *J* = 8.5 Hz), 7.47 (5H, m), 5.91 (2H, s), 5.52 (2H, s), 5.05 (2H, s), 3.78 (3H, s), 3.76 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 149.86, 147.86, 143.59, 131.04, 129.40, 129.20, 127.61, 124.71, 83.02, 53.49; HRMS (EI): $[M]^+$ *m/z* calcd for $C_{15}H_{11}IN_4O_2$ 405.9927, found 405.9937.

General procedure B

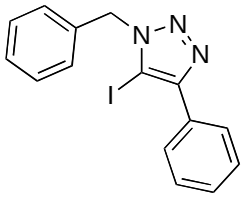


2.13
33% Yield

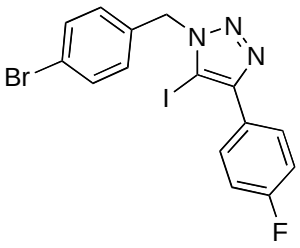
A one neck round bottom flask was charged with a stirring bar, CH_3CN (88 ml), 4-methoxybenzyl azide (50 mg, 0.308 mmol), the alkyne (50 mg, 0.308 mmol) and CuI (59 mg, 0.308 mmol) and stirring initiated. DMAP (38 mg, 0.308 mmol) was added as a solid in one portion, the flask was capped, and stirred at room temperature for 72 h in the dark. Subsequently, the reaction mixture was diluted with CH_2Cl_2 (100 ml) and extracted with 1M HCl (2 x 50 ml), brine (2 x 50 ml) and water (50 ml). Organic layer was dried over $MgSO_4$, and volatiles removed in vacuo. The residue was subjected to column chromatography (EtOAc / hexanes - 30 / 70) to give product **2.13** as a light yellow solid (46 mg, 33 %).

^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.25 (2H, d, *J* = 8.8 Hz, overlap with residual $CHCl_3$), 6.95 (2H, d, *J* = 9.2 Hz), 6.86 (2H, d, *J* = 8.8 Hz), 6.82 (2H, d, *J* = 9.2 Hz), 5.52 (2H, s), 5.05 (2H, s), 3.78 (3H, s), 3.76 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 159.97, 154.53, 152.62, 148.13, 129.73, 126.37, 116.57, 114.83, 114.47, 80.55, 62.83, 55.93, 55.54, 54.11; HRMS (EI): $[M]^+$ *m/z* calcd for $C_{18}H_{18}IN_3O_3$ 451.0393, found 451.0392.

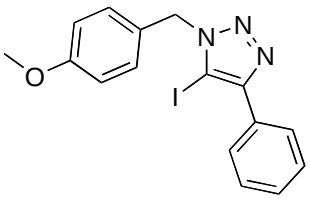
2.4 was prepared according to general procedure A as a yellow solid in 77 % yield; ^{1}H NMR (300 MHz, $CDCl_3$): d = 7.94 (2H, d, *J* = 8.2 Hz), 7.44 (8H, m), 5.69 (2H, s).[21a]



2.5 was prepared according to general procedure A as a yellow solid in 24 % yield; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.90 (2H, m), 7.50 (2H, d, *J* = 8.3), 7.16 (4H, m), 5.62 (2H, s); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 163.20 (d, J_{C-F} = 248.8 Hz), 149.89, 133.40, 132.38, 129.78, 129.687 (d, J_{C-F} = 8.3 Hz), 126.45 (d, J_{C-F} = 4.2Hz), 122.99, 115.87 (d, J_{C-F} = 21.8 Hz), 76.37, 54.05; ^{19}F NMR (282 MHz, $CDCl_3$): δ = -111.9 (m); HRMS (EI): $[M]^+$ *m/z* calcd for $C_{15}H_{10}BrFIN_3$ 456.9087, found 456.9089.

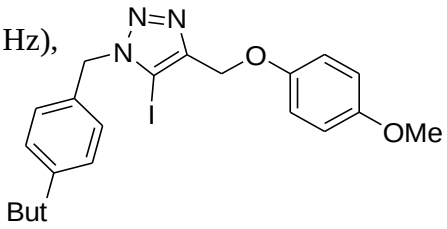


2.6 was prepared according to general procedure A as a yellow solid in 37 % yield; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.92 (2H, d, *J* = 6.9 Hz), 7.46 (3H, m), 7.29 (2H, d, *J* = 8.7 Hz), 6.88 (2H, d, *J* = 8.7 Hz), 5.61 (2H, s), 3.79 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$): δ =



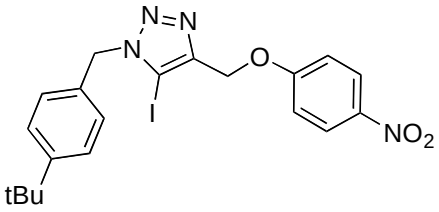
159.93, 150.40, 130.44, 129.68, 128.82, 128.77, 127.69, 126.58, 114.46, 76.45, 55.55, 54.23; HRMS (EI): $[M]^+$ *m/z* calcd for $C_{16}H_{14}IN_3O$ 391.0182, found 391.0176.

2.9 was prepared according to general procedure B as a white solid in 34 % yield;



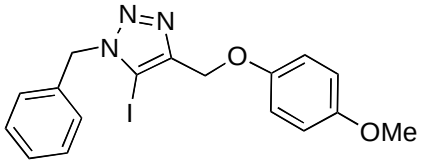
^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.36 (2H, d, *J* = 8.5 Hz), 7.23 (2H, d, *J* = 8.5 Hz), 6.96 (2H, d, *J* = 9.2 Hz), 6.83 (2H, d, *J* = 9.2 Hz), 5.55 (2H, s), 5.06 (2H, s), 3.76 (3H, s), 1.30 (9H, s); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 154.54, 152.64, 151.89, 148.06, 131.31, 127.98, 126.07, 116.59, 114.84, 80.87, 62.87, 55.93, 54.21, 31.50; HRMS (EI): $[M]^+$ *m/z* calcd for $C_{21}H_{24}IN_3O_2$ 477.0913, found 477.0909.

2.10 was prepared according to general procedure B as a yellow solid in 34 % yield;



^{1}H NMR (300 MHz, DMSO-d_6): d = 8.27 (2H, d, *J* = 9.2 Hz), 7.43 (2H, d, *J* = 8.1 Hz), 7.32 (2H, d, *J* = 9.2 Hz), 7.21 (2H, d, *J* = 8.1 Hz), 5.66 (2H, s), 5.29 (2H, s), 1.29 (9H, s); ^{13}C NMR (75 MHz, DMSO-d_6): δ = 163.97, 151.34, 147.02, 141.87, 132.88, 128.10, 126.56, 126.26, 116.10, 87.12, 63.07, 31.71; HRMS (EI): $[M]^+$ *m/z* calcd for $C_{20}H_{21}IN_4O_3$ 492.0658, found 492.0648.

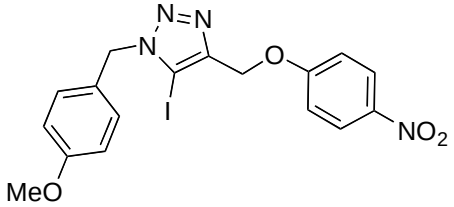
2.11 was prepared according to general procedure B as a pale yellow solid in 39 % yield;



^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.35 (3H, m), 7.23 (2H, m), 6.97 (2H, d, *J* = 9.5 Hz), 6.82 (2H, d, *J* = 9.5 Hz), 5.59 (2H, s), 5.07 (2H, s), 3.76 (3H, s); ^{13}C NMR

(75 MHz, $CDCl_3$): d = 154.55, 152.59, 148.21, 134.30, 129.16, 128.81, 128.10, 116.61, 114.84, 80.84, 62.86, 55.93, 54.51; HRMS (EI): $[M]^+$ *m/z* calcd for $C_{17}H_{16}IN_3O_2$ 421.0287, found 421.0291.

2.12 was prepared according to general procedure B as a yellow solid in 23 % yield;



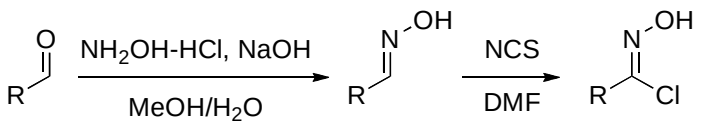
^{1}H NMR (300 MHz, DMSO-d_6): 8.21 (2H, d, *J* = 9.1 Hz), 7.25 (2H, d, *J* = 9.1 Hz), 7.19 (2H, d, *J* = 8.5 Hz), 6.92 (2H, d, *J* = 8.5 Hz), 5.56 (s, 2H), 5.22 (s, 2H), 3.71 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6): 163.98, 159.77, 147.04, 141.86, 129.91, 127.70, 126.57, 116.07, 114.83, 86.79, 63.06, 57.25, 55.82; HRMS (EI): $[M]^+$ *m/z* calcd for $C_{17}H_{15}IN_4O_4$ 466.0138, found 466.0124.

CHAPTER 3. Synthesis of Isoxazole-Containing BODIPY Dyes

3.1. Synthetic studies on isoxazole formation using BODIPY scaffold

As a complement to the triazole formation, we decided to explore the modification/functionalization of BODIPY dyes by incorporating an isoxazole moiety onto an alkyne-BODIPY scaffold. The cycloaddition between acetylenes and nitrile oxides was discovered several decades ago,[39] but gained little interest due to poor yields and side reactions. Recent improvements allowing for the regioselective formation of products and good yields promoted an interest in isoxazole synthesis.[40]

Nitrile oxides can be synthesized by an oxidation of the corresponding aldoximes with N-chloramine T or *via* a reaction of imidoyl chlorides with a base. Adopting the literature procedure for the isoxazole formation from the corresponding aldoximes and alkyne proved to be ineffective.[7] Hence, it was decided to carry on the isoxazole synthesis *via* the imidoyl chloride route. The preparation of the chlorides was accomplished by a two-step procedure starting with commercially available aldehydes (Scheme 3.1) according to the reported protocol.[40] The aromatic imidoyl chlorides were stored over time with no signs of decomposition, while the aliphatic analogues decomposed rapidly. In view of the exploratory nature of this research it was decided to explore the isoxazole synthesis using aromatic congeners.



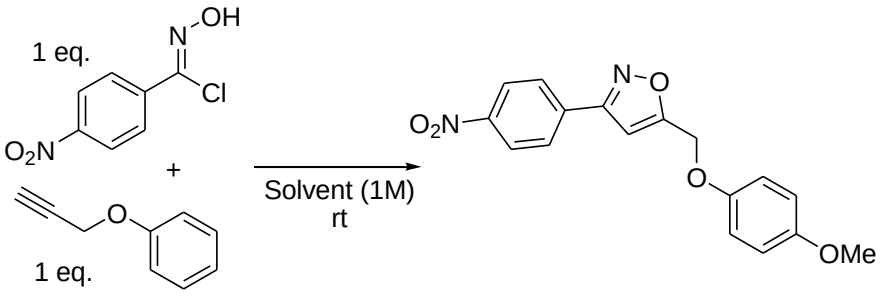
Scheme 3.1. Formation of imidoyl chlorides (R = alkyl, aryl).

Readily available starting materials, with non-overlapping ^{1}H resonances in the aromatic region, were chosen for the initial model study (Table 3.1). Due to a relatively high reactivity of imidoyl chlorides, the reaction was performed without any metal source and without the base (Table 3.1, entries 1-4). The choice for the specific solvents was governed by the solubility of the imidoyl chloride. About 20% of the isoxazole, as judged by crude ^{1}H NMR, was obtained in both DMSO and DMF, whereas less than 10% conversion was obtained in CH_3CN, and in CH_2Cl_2 virtually no isoxazole formation was noted.

In the presence of equimolar amounts of both CuI and DMAP, a more efficient production of the isoxazole was observed. After some experimentation (Table 3.1, entries 5-6), it was determined that an excess of the imidoyl chloride is necessary to produce the isoxazole.

Unlike triazole formation (Chapter 2), the synthesis of isoxazoles turned out to be largely insensitive to the nature of the base, as well as the concentration of the reaction mixture. In view of this, we decided to examine the possibility of forming the isoxazole moiety as a way of functionalizing BODIPY dyes, using the alkyne-containing BODIPY dye as the starting material.

Table 3.1. Model study on the isoxazole formation

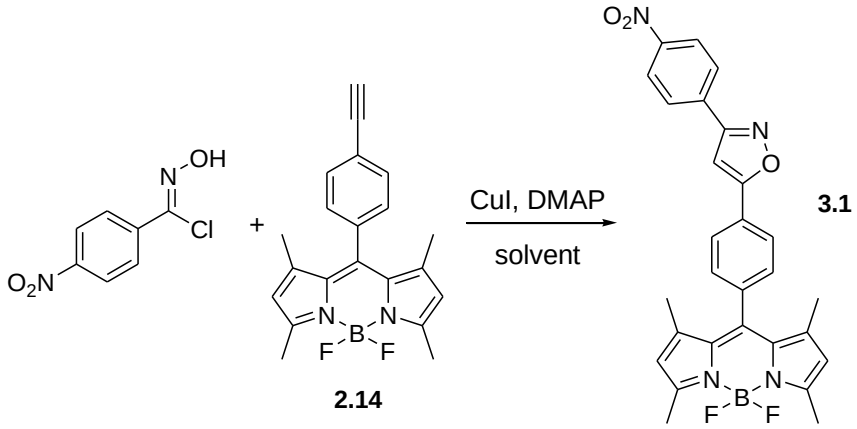


Entry	CuI, eq	Base, eq	Solvent	Isoxazole, %[a]
1	-	-	DMF	23
2	-	-	DMSO	20
3	-	-	CH_3CN	7
4	-	-	CH_2Cl_2	<2
5	1.0	DMAP, 1.0	DMF	58
6[b]	1.0	DMAP, 1.0	DMF	70

a - determined from ^{1}H NMR of the crude reaction mixture; b - 3 eq. of imidoyl chloride was used.

Since 4-nitro-phenyl imidoyl chloride (Table 3.1) was available, the initial screening of the reaction conditions was done using this substrate (Table 3.2). Performing the reaction in either CH_2Cl_2 or DMF, and using Et_3N as the base in both cases, revealed that the desired isoxazole-containing dye could be obtained with appreciable conversions. However, the pure product could not be isolated even after several chromatography columns and/or preparative TLCs. The effect of DMAP in various solvents was examined (Table 3.2).

Table 3.2. Initial condition screening for an isoxazole-containing BODIPY dye



entry	solvent[a]	3.1, %[b]
1	CH_2Cl_2	-[c]
2	DMF	-[c]
3	CH_3CN[d]	-[c]
4	CH_3CN[e]	44

a – concentration of the alkyne is 1 M; b – determined from ^{1}H NMR of the crude reaction mixture; c – complex mixture of products; d – concentration of the alkyne is 0.5 M; e – concentration of the alkyne is 35 mM.

The 1,4-stereochemistry of the product was confirmed by literature reports that describe the proton on the isoxazole for the 1,4-regioisomer appears around 6.5 ppm[7] in ^{1}H NMR whereas the proton on the 1,5-regioisomer appears around 8.5 ppm[8].

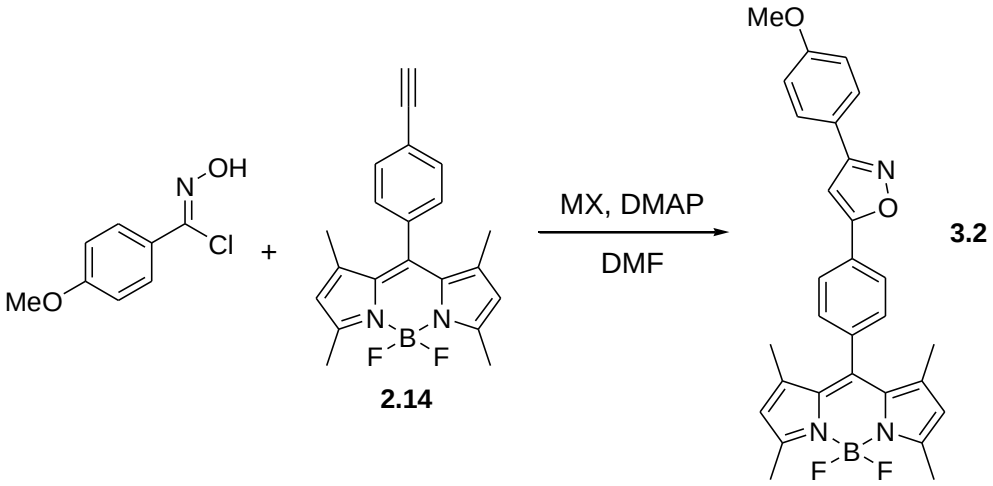
Due to low solubility of BODIPY alkyne, the maximum concentration in CH_3CN was 0.5 M, whereas in both DMF and CH_2Cl_2 the maximum concentration tested was 1 M. In CH_2Cl_2, although the product was detected, the reaction proved to be ineffective because an inseparable mixture of the products was formed, irrespective of the concentration of imidoyl chloride, and presence/absence of CuI (Table 3.2, entry 1). In

DMF, the reaction was equally inefficient (Table 3.2, entry 2). Although isoxazole formation in CH_3CN at 0.5 M produced a complex mixture (Table 3.2, entry 3), whereas dilution of the reaction to 35 mM produced the desired dye (Table 3.2, entries 3 and 4), without formation of unidentified side products. Even though the isoxazole-click BODIPY was obtained, the diyne-BODIPY dimer was observed as the major product.

Despite not very favorable outcomes, it was decided to probe the scope of this reaction, using alkyne-BODIPY dye **2.14** as a scaffold. In order to contrast the electron-withdrawing nature of the nitro group of **3.1**, the isoxazole formation using the methoxy-containing imidoyl chloride was tested next (Table 3.3). Surprisingly, no product was obtained using conditions established for the NO_2-containing analogue.

After screening various parameters, *i.e.*, solvent, concentration of DMAP and alkyne, the nature of the metal salt was identified as the major factor in controlling the outcome of the reaction. Specifically, the utilization of $InCl_3$, $Sc(OTf)_3$, and $ZnBr_2$ in DMF, led to a gradual increase of the conversion from 30 to 64% (Table 3.3, entries 2-4). Upon further experimentation, it was established that increasing the imidoyl chloride concentration resulted in a fairly clean conversion to the isoxazole-containing dye **3.2**. (Table 3.3, entry 5). It appeared that $ZnBr_2$ was also able to catalyze the reaction of **3.1** with **2.14** (Scheme of Table 3.2), albeit at a significantly lower efficiency. While the mechanism of the reaction is not known, $ZnBr_2$ could be considered a novel catalyst for the isoxazole formation on BODIPY scaffold, which is fairly insensitive to the electronic demands of the imidoyl chlorides.

Table 3.3. Effect of the catalyst on the formation of isoxazole-containing BODIPY dye

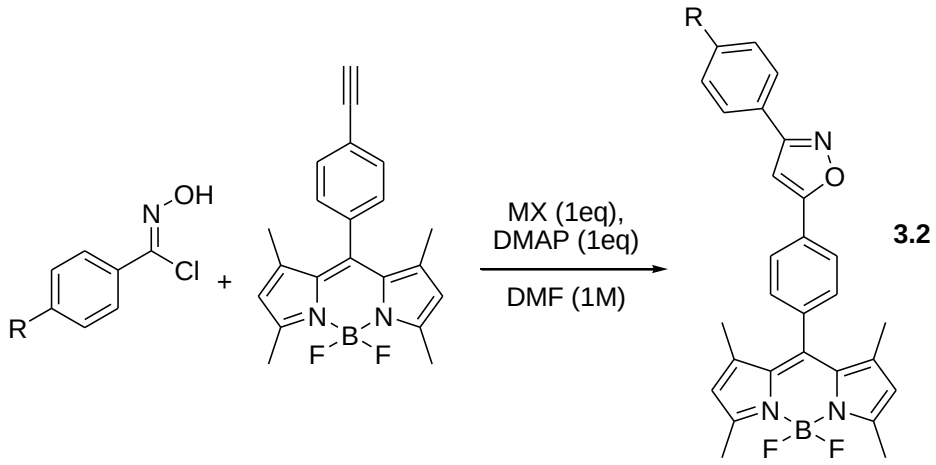


Entry	MX, 1 eq.	Yield of 3.2[b], %
1	CuI	0
2	$InCl_3$	30
3	$Sc(OTf)_3$	40
4	$ZnBr_2$	64
5[c]	$ZnBr_2$	95

a – concentration of the alkyne is 1 M; b – determined from ^{1}H NMR of the crude reaction mixture; c– 1.5 eq of imidoyl chloride.

In view of the above results, the effect of CuI *versus* $ZnBr_2$ on the synthesis of several isoxazole-containing BODIPY was investigated since copper is the most widely used metal catalyst according to literature reports for the isoxazole formation. Although there appeared to be no strict correlation between the nature of the substituent and the efficiency of the reaction as a function of the catalyst, $ZnBr_2$ could be considered as a better general catalyst than CuI (Table 3.4).

Table 3.4. Substituent effect in the synthesis of isoxazole-containing BODIPY dyes



entry	R (dye)	MX	Isolated yield, %
1	H (**3.5**)	$ZnBr_2$	16
2	H (**3.5**)	CuI	-
3	Br (**3.3**)	$ZnBr_2$	52
4	Br (**3.3**)	CuI	-
5	OMe (**3.2**)	$ZnBr_2$	56
6	OMe (**3.2**)	CuI	-
7	CF_3 (**3.4**)	$ZnBr_2$	22
8	CF_3 (**3.4**)	CuI	27
9	CN (**3.6**)	$ZnBr_2$	28
10	CN (**3.6**)	CuI	-
11	NO_2 (**3.1**)	$ZnBr_2$	24
12	NO_2 (**3.1**)	CuI	60
13	C_6F_5 (**3.7**)	$ZnBr_2$	77
14	C_6F_5 (**3.7**)	CuI	21

Moderate conversions were obtained using $ZnBr_2$ with phenyl-containing or electron-donating group containing imidoyl chlorides (Table 3.4, entries 1, 5), whereas virtually no formation of the isoxazole BODIPY was obtained with CuI used as the

catalyst. In the case of the C_6F_5-containing imidoyl chloride, the reaction was quite efficient with $ZnBr_2$, while a complex mixture, that nonetheless contained a substantial amount of product, was found when CuI was used (Table 3.4, entries 13 and 14).

With electron-withdrawing groups, such as CF_3 and CN, distinct results were noted. Specifically, with CF_3-containing imidoyl chloride, the isoxazole formation was slightly more facile in the presence of CuI (60% conversion) than in the presence of $ZnBr_2$ (42 % conversion) (Table 3.4, entries 7 and 8). However, in the case of CN-containing substrate, CuI proved completely inefficient (Table 3.4, entry 10), whereas in the presence of $ZnBr_2$, a moderate conversion was noted (Table 3.4, entry 9). Obviously, an expanded substrate set would be required to draw any firm conclusions.

In an attempt to increase structural diversity of isoxazole-containing BODIPY dyes, the synthesis of several imidoyl chlorides was attempted based on literature procedures,[40] and according to Scheme 3.1, from the commercially available aldehydes (Figure 3.1). It turned out that only pyrene-, biphenyl-, and thiophene-containing imidoyl chlorides (Figure 3.1, top row) could be prepared, and the conversion of other aldehydes into the corresponding imidoyl chlorides failed (Figure 3.1, bottom row).

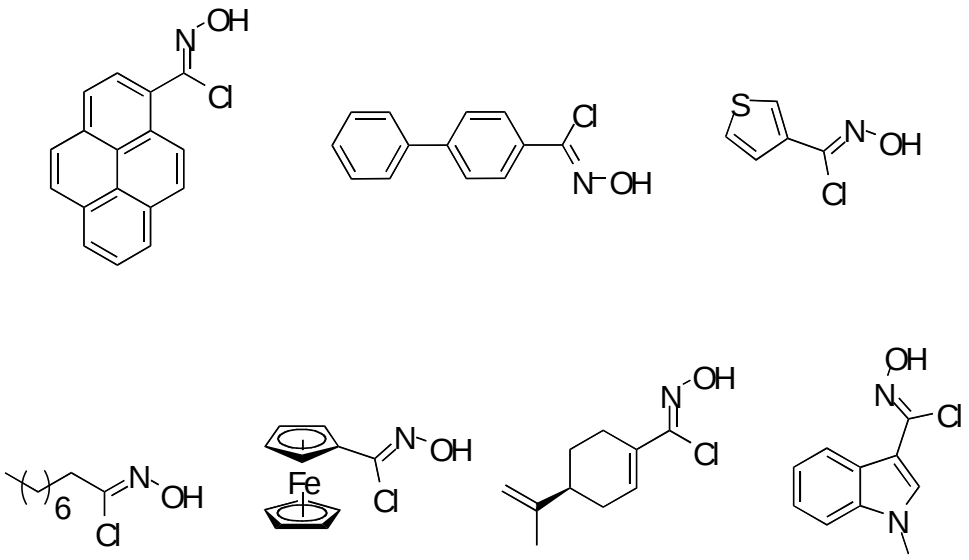
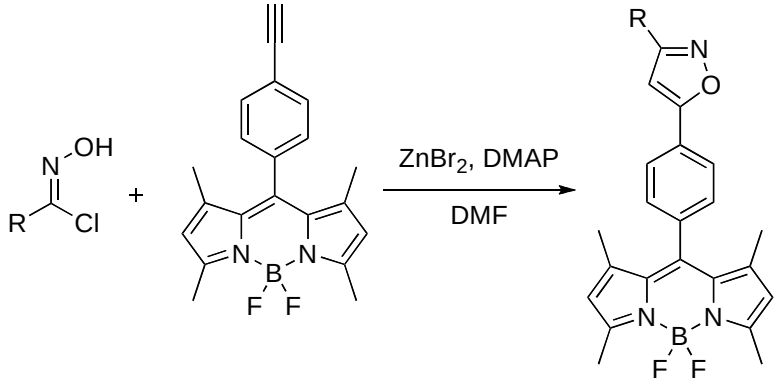
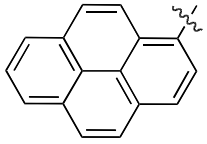
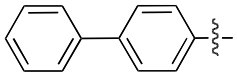
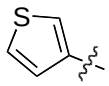


Figure 3.1. Structures of imidoyl chlorides

Subsequently, the synthesis of pyrene-, biphenyl and thiophene-containing isoxazoles using $ZnBr_2$/DMAP combination was performed (Table 3.5). Although all reactions provided the desired isoxazole-BODIPY dyes in moderate yields, only the thiophene-containing BODIPY **3.10** could be obtained in a pure form. In the other two cases, *i.e.*, **3.8** and **3.9** small amounts of unidentified impurities, *ca*. 10%, could not be removed even upon sequential chromatography columns.

Table 3.5. Synthesis of aryl-containing isoxazole-click-BODIPY dyes



dye	R	Isolated yield, %
3.8		19[a]
3.9		18[a]
3.10		38

a – ~85-90% purity

The identity of all pure isoxazole-containing dyes was confirmed by ^{1}H NMR and high resolution MS. Although the chemical shifts in the ^{13}C NMR of all dyes were largely unaffected by the nature of the substituent, the behavior of the methyl-groups at the 4- and 12-positions on the BODIPY core was unpredictable (Figure 3.2).

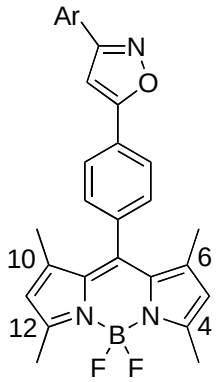


Figure 3.2. Numbering of the methyl groups on an isoxazole-containing BODIPY dye

The resonance for the methyl-groups in the positions 6 and 10 were observed at 14.9 ppm, and it was insensitive to the identity of the dye. The other two methyl groups at the 4 and 12 positions exhibited resonance at 29.9 ppm. However, this resonance was only observed for dyes **3.3**, **3.5**, **3.6** and **3.8**. The relative intensity of the 29.9-peak was similar to that of the 14.9-peak for **3.3** and **3.8** dyes, whereas for the **3.5** and **3.6** dyes, it was drastically smaller. The 29.9 ppm peak was absent in the ^{13}C NMR of all other dyes. This might be attributed to low d1 relaxation time (1 sec) during the acquisition of the ^{13}C NMR spectra and might be observed if the relaxation time was increased to 15 sec.

3.2. Interactions of isoxazole-BODIPY dyes with soluble amyloid oligomers

Soluble oligomers of amyloid peptides, specifically Aβ1-42, are suggested as one of the main neurotoxic species responsible for impairment of neuronal function.[41] Recognitions of various conformations of these peptides, *i.e.*, random coil, α-helix and β-sheet, using small fluorescent molecular probes is an underdeveloped challenging area of modern research, however, *in vitro* studies on small molecule – Aβ1-42 interactions

might prove useful in understanding structural changes that might be responsible for the neurotoxicity of these oligomers. It was recently demonstrated that triazole-containing BODIPY dyes could detect and differentiate between distinct conformations of soluble oligomers of Aβ1-42.[42]

In this light, we examined whether triazole to isoxazole substitution would result in dyes that would be able to recognize soluble Aβ1-42 oligomers. Several of the prepared isoxazole-containing dyes were tested as a proof of principle experiments. Specifically, in aqueous buffer, **3.10** dye (Figure 3.3) exhibited similar spectroscopic behavior to typical triazole-containing BODIPY dyes, *i.e*., the aggregates were confirmed by the presence of multiple peaks in the emission spectra.

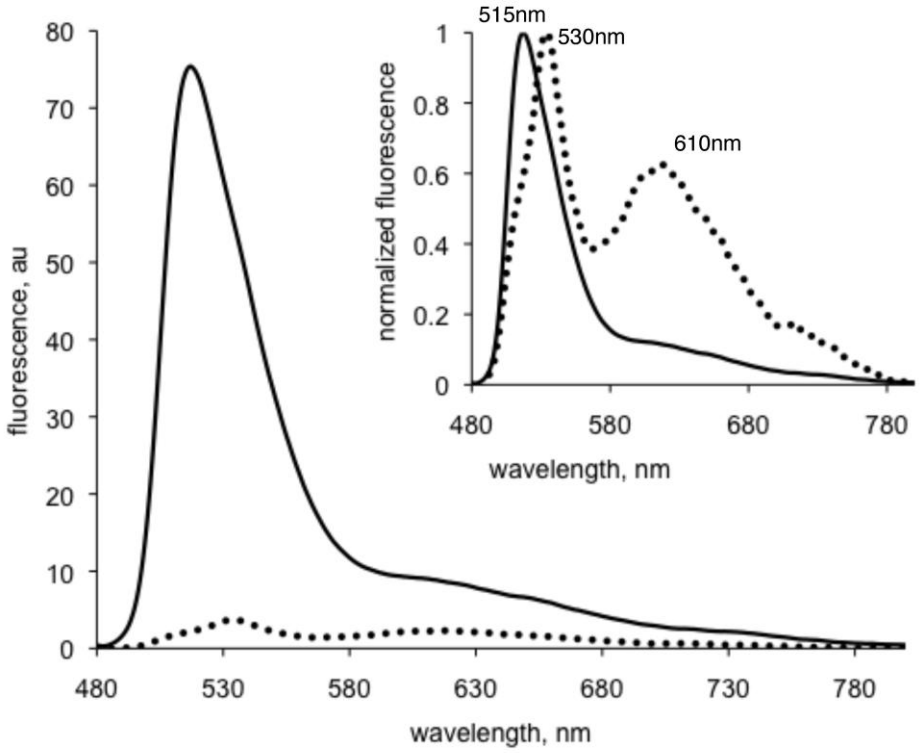


Figure 3.3. Fluorescence spectra of dye **3.10** (8μM) in the presence (solid line) and in the absence (dotted line) of 25μM of β-sheet rich soluble Aβ1-42 oligomers. Inset: normalized spectra. Buffer: 10mM TRIS/NH_4Cl, pH 8.6, DMSO 0.8 % (v/v).

Although the isoxazole-containing dyes appeared to be more soluble in aqueous buffer than the triazole-containing BODIPY dyes, the emission intensity of the dye was quite low. However, in the presence of the aggregated, β-sheet-rich Aβ1-42 oligomers, the fluorescence intensity drastically enhanced (Figure 3.3). Upon interaction, the dye's aggregates seemed to disaggregate, as the 610 nm peak disappeared, and the 530 nm shifted to 515 nm (Figure 3.3, inset). This phenomenon is similar to the triazole congeners.

Next, the interaction of **3.10** and **3.5** dyes with soluble Aβ 1-42 oligomers, which were prepared according to the procedure developed in our laboratory, was assessed by measuring the fluorescence of the dye in the presence of unordered or ordered Aβ 1-42 species, *i.e.*, F, and compared to the fluorescence of the dye in the absence of the Aβ 1-42, *i.e.*, F_o. The data were displayed as F/F_o. Specifically, titration of the isoxazole-click-BODIPY dyes, **3.10** and **3.5**, into solutions that contained unordered and ordered Aβ1-42 soluble oligomers, revealed that substituent at the position 3 of the isoxazole ring plays a significant role in the recognition process (Figure 3.4).

Thiophene-containing dye, **3.10**, exhibited high levels of sensitivity (F/F_o) in recognizing largely unordered conformation of the Aβ oligomers as well as the ordered one. For example, *ca*. 10-fold increase in the fluorescence intensity in the presence of the unordered conformation and *ca*. 40-fold increase in the presence of β-sheet-rich Aβ species (Figure 3.4A) are in the ranges of the fluorescence enhancements provided by some of the triazole-click-BODIPY. However, **3.5** BODIPY dye exhibited a marginal differentiation between the unordered and ordered conformations of Aβ1-42 oligomers (Figure 3.4B).

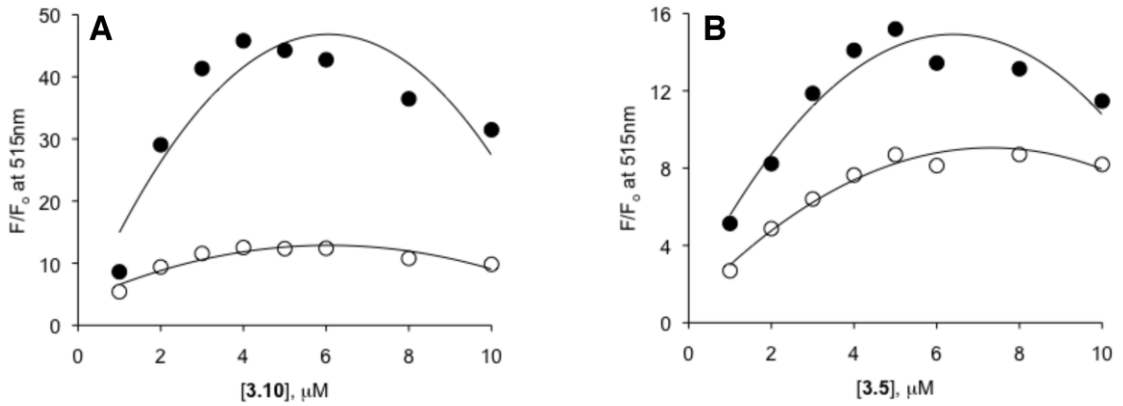


Figure 3.4. Interaction of **3.10** (**A**) and **3.5** (**B**) dyes with unordered (open symbols) and ordered (solid symbols) soluble Aβ1-42 oligomers (25μM). Buffer: 10mM TRIS/NH_4Cl, pH 8.6, DMSO ≤ 1 % (v/v).

Although preliminary and limited in scope, the aforementioned results indicate that isoxazole-click-BODIPY dyes could be viable fluorescence probes for detecting soluble amyloid oligomers, and superior probes could be obtained *via* a modification of the substituents on the isoxazole-ring.

3.3. Conclusions

Modification of a BODIPY scaffold *via* isoxazole tether had been demonstrated for the first time. The reaction conditions exhibited high substrate specificity. It was discovered that $ZnBr_2$ is a viable general catalyst for the formation of the isoxazole moiety. Some of the isoxazole-click BODIPY dyes were tested for their ability to recognize distinct conformations of the soluble amyloid oligomers.

3.4. Experimental Section

3.4.1. Materials and methods

All reagents and solvents were from commercial sources (Sigma-Aldrich, Acros or Matrix Scientific), they were of highest grade possible, and were used as received. Absorbance and fluorescence measurements were performed on Agilent 8453 UV–vis instrument and Shimadzu RF-5301PC, respectively, using 1 cm quartz cells with a resolution of 1 nm. Fluorescence measurements were carried out as follows: excitation and emission slit widths were 3 mm and 3 mm. Samples were excited at the absorption maxima. Spectra were smoothed using manufacture provided software.

NMR spectra were recorded on a Varian Mercury-300 and chemical shifts are reported in part per million: for ^{1}H NMR and ^{13}C NMR spectra are reported relative to internal tetramethylsilane (δ = 0.00 ppm), chemical shifts for ^{19}F NMR were externally referenced to the fluorine peak of 10-[*4-Aminophenyl*]-2,8-*diethyl*-5,5-*difluoro*-1,3,7,9-*tetramethyldipyrrolo*[1,2-c:2,1-f][1,3,2]*diazaborinin*-4-*ium*-*5*-*uide* ^{19}F-NMR ($CDCl_3$, 282.5 MHz): -145.29 (q, *J* = 35Hz).[38]

3.4.2. Synthesis of isoxazole-containing BODIPY dyes

General procedure A: synthesis of isoxazole-containing BODIPY dyes using $ZnBr_2$.

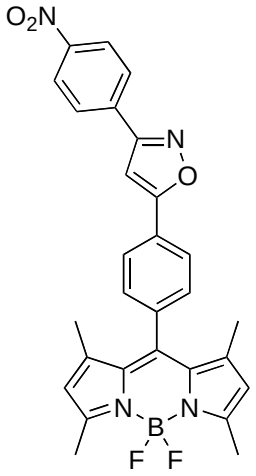
A vial was charged with a stirring bar, $ZnBr_2$ (13 mg, 0.0568 mmol), DMF (57 μL), alkyne (20 mg, 0.0568 mmol), imidoyl chloride (17 mg, 0.0852 mmol), DMAP (7 mg, 0.0568 mmol), the vial was capped and stirred at room temperature for 18 hours in

the dark. Subsequently, the reaction mixture was diluted with CH_2Cl_2 (2 mL) and extracted with 1M HCl (2 x 2 mL). The organic layer was dried over $MgSO_4$ and the solvent was removed under vacuo. The residue was subjected to column chromatography (EtOAc/Hexanes – 30/70) to give the product as a powder.

General procedure B: synthesis of isoxazole-containing BODIPY dyes using CuI.

A vial was charged with a stirring bar, CuI (11 mg, 0.0568 mmol), DMF (57 μL), alkyne (20 mg, 0.0568 mmol), imidoyl chloride (17 mg, 0.0852 mmol), DMAP (7 mg, 0.0568 mmol), the vial was capped and stirred at room temperature for 18 hours in the dark. Subsequently, the reaction mixture was diluted with CH_2Cl_2 (2 mL) and extracted with 1M HCl (2 x 2 mL). The organic layer was dried over $MgSO_4$ and the solvent was removed under vacuo. The residue was subjected to column chromatography (EtOAc/Hexanes – 30/70) to give the product as a powder.

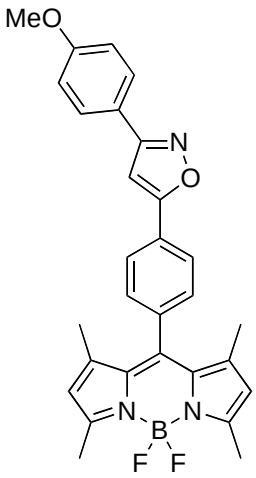
*Synthesis of **3.1***. The compound was synthesized according to general procedure A and B in 24% and 60% yield respectively as a dark red solid.



^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.38 (d, *J* = 8.7 Hz, 2H), 8.08 (d, *J* = 8.7 Hz, 2H), 8.02 (d, *J* = 8.0 Hz, 2H), 7.47 (d, *J* = 8.0 Hz, 2H), 7.01 (s, 1H), 6.01 (s, 2H), 2.57 (s, 6H), 1.44 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 170.7, 161.6, 156.3, 151.8, 149.0, 143.0, 140.2, 137.8, 135.2, 131.3, 129.4, 127.8, 126.8, 124.6, 121.8, 98.5,

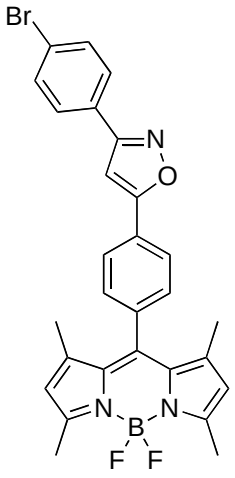
29.9, 14.9; ^{19}F (282 MHz, $CDCl_3$): δ = -145.64 (q, *J* = 33.7 Hz); HRMS calcd for $C_{28}H_{23}BF_2N_4O_3$ 512.1831, found 512.1833.

Synthesis of ***3.2***. The compound was synthesized according to general procedure A in 56% yield as a dark red solid.



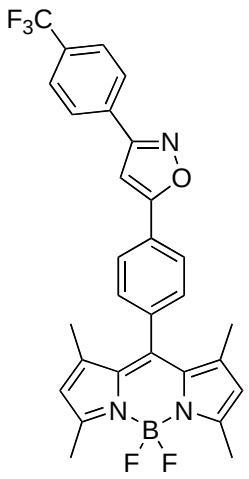
^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.99 (d, *J* = 8.0Hz, 2H), 7.82 (d, *J* = 8.7Hz, 2H), 7.43 (d, *J* = 8.0 Hz, 2H), 7.02 (d, *J* = 8.7 Hz, 2H), 6.89 (s, 1H), 6.00 (s, 2H), 3.88 (s, 3H), 2.57 (s, 6H), 1.44 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 169.4, 163.0, 161.4, 156.2, 143.2, 140.6, 137.2, 131.4, 129.2, 129.1, 128.5, 128.4, 126.7, 121.7, 121.6, 114.6, 98.3, 55.6, 14.9; ^{19}F (282 MHz, $CDCl_3$): δ = -145.67 (q, *J* = 33.8 Hz); HRMS calcd for $C_{29}H_{26}BF_2N_3O_2$ 497.2086, found 497.2089.

Synthesis of **3.3**. The compound was synthesized according to general procedure A/B in 52% yield as a dark red solid.



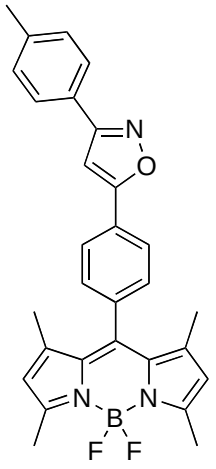
^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.98 (d, *J* = 8.3 Hz, 2H), 7.75 (d, *J* = 8.5 Hz, 2H), 7.63 (d, *J* = 8.0 Hz, 2H), 7.43 (d, *J* = 8.5 Hz, 2H), 6.92 (s, 1H), 5.99 (s, 1H), 2.56 (s, 6H), 1.42 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 169.97, 162.5, 156.2, 143.1, 137.4, 133.1, 132.5, 131.7, 131.4, 129.2, 128.5, 128.04, 126.7, 124.8, 121.7, 98.3, 29.9, 14.9; ^{19}F (282 MHz, $CDCl_3$): δ = -145.63 (q, *J* = 33.7 Hz); HRMS calcd for $C_{28}H_{23}BBrF_2N_3O$ 545.1086, found 545.1083.

*Synthesis of **3.4***. The compound was synthesized according to general procedure A and B in 22% and 27% yield respectively as a dark red solid.



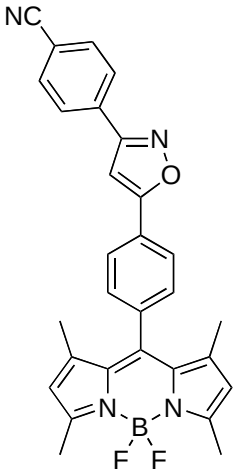
^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.01 (m, 4H), 7.77 (d, *J* = 8.2 Hz, 2H), 7.47 (d, *J* = 8.2 Hz, 2H), 6.98 (s, 1H), 6.01 (s, 2H), 2.57 (s, 6H), 1.43 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 170.3, 162.2, 156.3, 143.1, 140.3, 137.6, 132.6, 132.1, 131.3, 129.3, 128.8, 128.0, 127.4, 126.8, 126.3, 121.7, 98.4, 14.9; ^{19}F NMR (282 MHz, $CDCl_3$): δ = -145.66 (q, *J* = 33.7 Hz, 2F), -63.26 (s, 3F); HRMS calcd for $C_{29}H_{23}BF_5N_3O$ 535.1854, found 535.1867.

*Synthesis of **3.5***. The compound was synthesized according to general procedure A in 36% yield as a dark red solid.



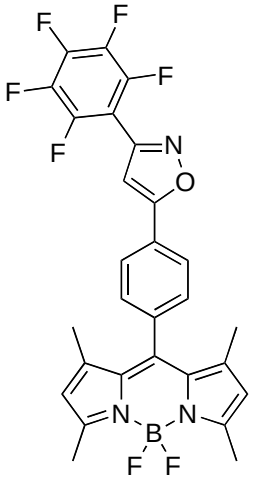
^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.99 (d, *J* = 8.0 Hz, 2H), 7.77 (d, *J* = 8.0 Hz, 2H), 7.44 (d, *J* = 8.0 Hz, 2H), 7.31 (d, *J* = 8.0 Hz, 2H), 6.92 (s, 1H) 6.00 (s, 2H), 2.57 (s, 6H), 2.43 (s, 3H), 1.44 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 169.5, 163.3, 156.2, 143.2, 140.6, 140.5, 137.2, 131.4, 129.9, 129.2, 128.4, 126.9, 126.7, 126.3, 121.7, 98.4, 29.9, 21.7, 14.9; ^{19}F (282 MHz, $CDCl_3$): δ = -145.65 (q, *J* = 33.7 Hz); HRMS calcd for $C_{29}H_{26}BF_2N_3O$ 481.2137, found 481.2135.

*Synthesis of **3.6***. The compound was synthesized according to general procedure A in 28% yield as a dark red solid.



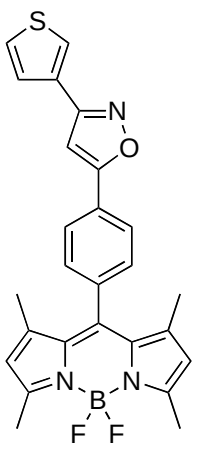
^{1}H NMR (300 MHz, $CDCl_3$): δ= 8.07 (m, 4H) 7.81 (d, *J* = 8.5 Hz, 2H), 7.47 (d, *J* = 8.5 Hz, 2H), 6.97 (s, 1H), 6.01 (s, 2H), 2.57 (s, 6H), 1.43 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): δ= 170.6, 161.9, 156.3, 143.0, 140.3, 137.7, 133.4, 133.1, 131.3, 129.4, 127.8, 127.6, 126.8, 121.8, 118.5, 114.1, 98.3, 29.9, 14.9; ^{19}F (282 MHz, $CDCl_3$): δ = -145.66 (q, *J* = 33.7 Hz); HRMS calcd for $C_{29}H_{23}BF_2N_4O$ 492.1933, found 492.1947

*Synthesis of **3.7***. The compound was synthesized according to general procedure A and B in 77% and 21% yield respectively as a dark red solid.



^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.01 (d, *J* = 8.1 Hz, 2H), 7.48 (d, *J* = 8.1 Hz, 2H), 6.94 (s, 1H), 6.01 (s, 2H), 2.57 (s, 6H), 1.43 (s, 6H); ^{13}C NMR (300 MHz, $CDCl_3$): δ = 170.1, 156.3, 152.5, 146.9, 143.5, 143.1, 140.2, 137.9, 135.5, 131.3, 129.4, 127.5, 126.9, 121.8, 101.4 (overlap), 14.9; ^{19}F (282 MHz, $CDCl_3$): δ = -137.48 (d, *J* = 15.9 Hz, 2F), -145.64 (q, *J* = 31.7 Hz, 2F), -150.06 (t, *J* = 21.8 Hz, 1F), -159.94 (m, 2F); HRMS calcd for $C_{28}H_{19}BF_7N_3O$ 557.1509, found 557.1524.

*Synthesis of **3.10***. The compound was synthesized according to general procedure A in 38% yield as a dark red solid.

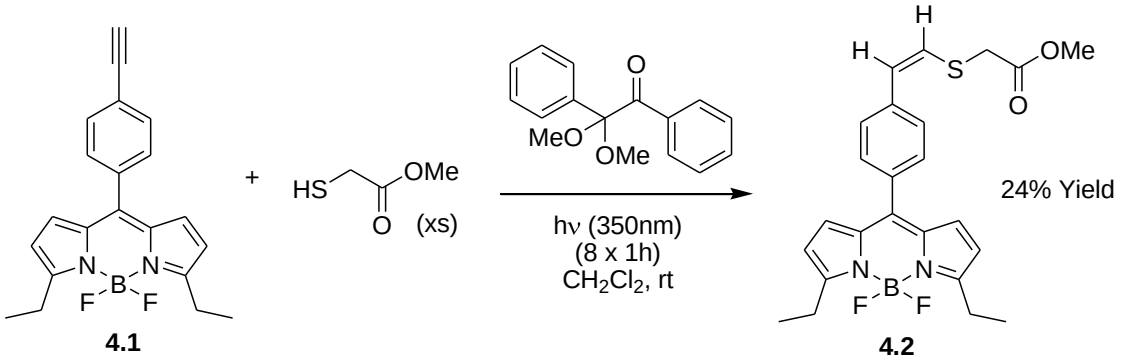


^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.98 (d, *J* = 8.0 Hz, 2H), 7.80 (m, 1 H), 7.59 (m, 1H), 7.45 (m, 3H), 6.85 (s, 1H), 6.00 (s, 2H), 2.57 (s, 6H), 1.43 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 169.4, 159.2, 156.2, 143.2, 140.5, 137.3, 131.4, 130.4, 129.2, 128.2, 127.2, 126.7, 126.2, 125.1, 121.7, 98.8, 29.9, 14.9; ^{19}F (282 MHz, $CDCl_3$): δ = -145.67 (q, *J* = 31.7 Hz); HRMS calcd for $C_{26}H_{22}BF_2N_3OS$ 473.1545, found 473.1550.

Chapter 4. Application of Thiol-yne Reaction for BODIPY Functionalization

4.1. Preliminary evaluation of thiol-yne click reaction as a tool for modifying BODIPY dyes

In order to expand the repertoire of click reactions as a way of increasing structural and functional diversity of BODIPY dyes, we have investigated thiol-yne reaction as a way of modifying a BODIPY scaffold. The initial experiment, performed in collaboration with Ms Laramie Jameson (Scheme 4.1), revealed that the alkyne BODIPY dye **4.1** could be converted into the corresponding ester **4.2**, using methyl thioglycolate as the "thiol" component.



Scheme 4.1. Reaction of alkyne-BODIPY with a thiol

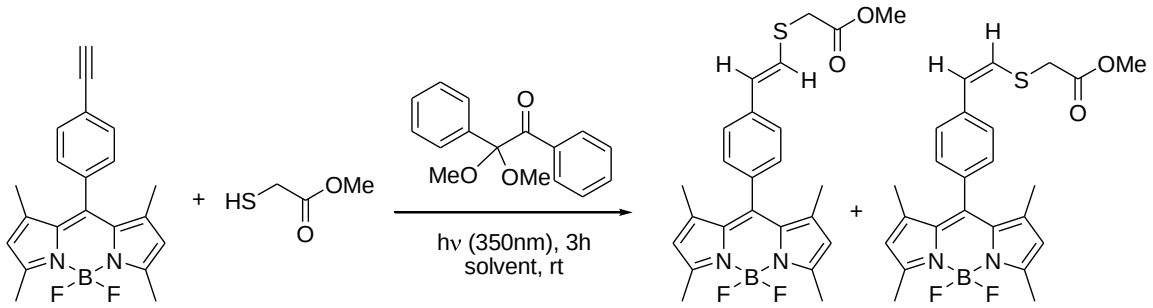
Unlike reported literature accounts[43], the above reaction appeared to be sluggish, and appreciable conversion was obtained only after 8 hour of irradiation. The progress of the reaction was monitored by TLC, and after 6 hours of irradiation, there appeared to be no appreciable change in the relative intensity of **4.1** as compared to the intensity of other

spots. The reaction mixture was subjected to preparative TLC to give **4.2**. It is of interest to note that although an excess of thiol (*ca*. 30 eq. relative to **4.1**) was used, the alkene was the only product isolated. Some experimentation indicated that the reaction was insensitive to the amount of the thiol used, as **4.2** was the only product formed. In addition, only the *cis*-alkene, a thermodynamically less stable product, was obtained. Also, a small amount of the BF_2-free alkyne-BODIPY was isolated: apparently, the BF_2-moeity might have been cleaved under irradiation. Although not very efficient, this trial reaction demonstrated that thio-yne-based chemistry could be made applicable to BODIPY dyes.

In view of somewhat promising results on the application of thiol-yne reaction towards functionalization of BODIPY scaffold (Scheme 4.1), we decided to explore this reaction further. Our initial screening of the reaction conditions started with the solvent and indicated that solvent had a significant impact on the stereochemistry of the reaction (Table 4.1). It should be pointed out that the reaction was fairly inefficient, as ca. 10-15 % conversion were noted after 3 hours of irradiation. In view of small scale (about 5 mg based on the alkyne BODIPY), and low conversions of the reaction, no isolation of the products was attempted.

The reaction performed in CH_2Cl_2 (Table 4.1, entry 1) immediately highlighted the difference between **4.1** and **2.14**: whereas an exclusive formation of *cis*-product was observed in the case of **4.1**, an equal mixture of *cis*/*trans* products was obtained in the case of **2.14**. The identical result was obtained in benzene (entry 2). However, when

Table 4.1. Solvent effect on the thiol-yne reaction[a]



2.14 **4.3** **4.4**

Entry	Solvent[b]	4.3 / 4.4 ratio[c]
1	CH_2Cl_2	1 / 1
2	C_6H_6	1 / 1
3	CH_3CN[d]	1 / 5
4	EtOH[d]	1 / 5
5	THF	2 / 1
6	DMSO	2 / 1
7	EtOAc	5 / 1

a – conversion of <15% were obtained in all cases; b - concentration of the reaction mixture was 0.7M; c – determined by integration of the alkene protons in crude ^{1}H NMR, trans: δ = 6.98 (d, *J* = 16.0 Hz, 1H); 6.61 (d, *J* = 16.0 Hz, 1H) cis: δ = 6.54 (d, *J* = 12.0 Hz, 1H); 6.42 (d, *J* = 12.0 Hz, 1H) c - concentration of the reaction mixture was 0.35M, the reaction was performed under stirring.

CH_3CN and EtOH were used as solvents (entries 3 and 4, respectively), the cis-dye **4.4** was obtained as the major product. It is not clear at the moment whether the change of the solvent polarity could cause the shift in selectivity. Especially, since due to poor solubility of the **2.14** dye in these solvents, the concentration was reduced 2-fold, as compared to CH_2Cl_2 and benzene (entries 1 and 2, respectively). Even upon increasing the amount of solvent, the reactions in CH_3CN and EtOH were heterogeneous, and they

were stirred for the duration of the irradiation (unlike the reactions in the other solvents). To our surprise, when the reaction was performed in THF (entry 5), the *trans*-dye **4.3** was obtained as the major isomer. Similar level of selectivity was noted in DMSO (entry 6). Importantly, in EtOAc (entry 7), a significantly higher selectivity in the formation of **4.3** was noted.

4.2. Conclusions

The above results highlight an interesting phenomenon on previously unknown/not reported solvent-controlled *cis/trans* selectivity in thiol-yne reactions. It appeared that by simply switching from EtOH to EtOAc, for example, it was possible to completely switch between *cis* and *trans* isomers of the BODIPY dyes. However, further work will need to be carried out to confirm the solvent effect as well as to improve on the overall efficiency of the reaction.

4.3. Experimental Section

4.3.1. Materials and Methods

All reagents and solvents were from commercial sources (Sigma-Aldrich, Acros or Matrix Scientific), they were of highest grade possible, and were used as received. TLC monitoring and separation was performed on glass plates (TLG-R10011-323 SilicaPlates) from Silicycle, Inc. Photochemical experiments were performed using a 100W long

wavelength UV lamp from UVP, Inc. NMR spectra were recorded on a Varian Mercury-300 and chemical shifts are reported in part per million: for ^{1}H NMR spectra are reported relative to internal tetramethylsilane (δ = 0.00 ppm), chemical shifts for ^{19}F NMR were externally referenced to the fluorine peak of 10-[*4-Aminophenyl*]-2,8-*diethyl*-5,5-*difluoro*-1,3,7,9-*tetramethyldipyrrolo*[1,2-c:2,1-f][1,3,2]*diazaborinin*-4-*ium*-*5*-*uide* ^{19}F-NMR ($CDCl_3$, 282.5 MHz): -145.29 (q, *J* = 35Hz).[38]

4.3.2. Synthesis of Sulfur-containing Dyes

*Synthesis of **4.2***:

A 1 ml screw cap vial was charged with **4.1** (6.0 mg, 17.2 μmol), methyl thioglycolate (50 μl, 0.56 mmol), 2,2-dimethoxy-2-phenylacetophenone (1.0 mg, 3.9 μmol), followed by 0.2 ml of CH_2Cl_2. The vial was exposed to 350 nm wavelength radiation for 8 hours in 1-hour intervals; after each 1-hour time period, the radiation source was removed, and the progress of the reaction was checked by TLC (hexane/EtOAc – 7/3). After 8 hours, the reaction mixture was directly transferred onto a TLC plate (hexane/EtOAc – 7/3), and the product was isolated by scraping the corresponding band on the TLC plate, followed by extraction with CH_2Cl_2/MeOH (9/1 v/v) mixture. Subsequent filtration through cotton and removal of volatiles in vacuo gave 1.9 mg (24 % yield) of **4.2** as a red solid.

^{1}H NMR (300MHz, $CDCl_3$): δ = 7.56 (d, *J* = 7.9 Hz, 2 H), 7.50 (d, *J* = 7.9 Hz, 2H), 6.78 (d, *J* = 4.2 Hz, 2H), 6.58 (d, *J* = 11.0 Hz, 1H), 6.47 (d, *J* = 11.0 Hz, 1H), 6.36 (d, *J* =

4.2Hz, 2H), 3.78 (s, 3H), 3.54 (s, 2H), 3.08 (q, *J* = 7.5Hz, 4H), 1.35 (t, *J* = 7.5Hz, 6H); ^{19}F NMR (282MHz, $CDCl_3$): δ = - 144.69 (q, *J* = 33.8Hz).

Effect of Solvent on the cis/trans-Dye Formation: A Typical Procedure

A 1 ml screw cap vial was charged with **2.14** (5.0 mg, 14.2 μmol), methyl thioglycolate (1.3 μl, 14.2 μmol), 2,2-dimethoxy-2-phenylacetophenone (4.0 mg, 14.2 μmol), followed by 20 μl of solvent. In case of EtOH and CH_3CN, the vial was also charged with a stirring bar, and the reaction mixture was rigorously stirred for the duration of irradiation. After 3 hours, the reaction vial was washed with CH_2Cl_2 (2 ml), and the volatiles were removed in vacuo. The residue was analyzed by ^{1}H NMR. Specifically, ^{1}H resonances of olefinic protons were examined: trans: δ = 6.98 (d, *J* = 16.0 Hz, 1H); 6.61 (d, *J* = 16.0 Hz, 1H) cis: δ = 6.54 (d, *J* = 12.0 Hz, 1H); 6.42 (d, *J* = 12.0 Hz, 1H)

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Presentations

Nicholas W. Smith; Polenz, Bradley P.; Deal, Eric; Boyd, Connor; Dzyuba, Sergei V. "Click-BODIPY dyes as fluorescent probes for amyloidogenic biomolecules." 240th ACS National Meeting. Boston, MA, United States. August 22-26, 2010.

ABSTRACT

FUNCTIONALIZATION OF BODIPY-DYE SCAFFOLD USING CLICK-CHEMISTRY REACTIONS

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The design, synthesis and uses of small fluorescent molecules are among most active areas of modern research especially with 4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene (BODIPY) dyes. The spectroscopic properties of the BODIPY dyes can be altered by structural modification of the dye. One of the leading synthetic tools with many numerous applications is the copper-promoted alkyne-azide cycloaddition, commonly known as 'click-chemistry'. Knowing the modification of BODIPY dyes, the use of a single alkyne-containing BODIPY scaffold might be an interesting tool to create several structurally and functionally-diverse fluorescent dyes using 'click chemistry'-type of reactions (Chapter 1).

Earlier cycloaddition studies in the group found a small amount of 5-I-triazole-BODIPY as a side product. It was found that low concentrations of the alkyne and the use of DMAP led to the formation of 5-I-triazoles as the only cycloaddition products. This

methodology was applied for the synthesis of the 5-iodo-triazole-containing BODIPY dyes (Chapter 2).

Modification of a BODIPY scaffold *via* isoxazole tether was demonstrated for the first time. It was discovered that $ZnBr_2$ is a viable general catalyst for the formation of the isoxazole moiety. Some of the isoxazole-click BODIPY dyes were tested for their ability to recognize distinct conformations of the soluble amyloid oligomers (Chapter 3).

Using photochemistry to facilitate the tio-yne reaction it appeared that by simply switching solvents, it was possible to completely switch between *cis* and *trans* isomers of the BODIPY dyes (Chapter 4).