Synthesis of Quenched Fluorophores for Iminoboronate Chemistry

BSc Research Project

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Abstract

Designing synthetic routes to make fluorescent dyes for protein labeling to investigate biological systems has already come a long way. In this research project a lucifer dye with an anthranilhydrazide handle **4** was synthesized and its potential as a quenched dye in iminoboronate chemistry was investigated. Other hydrazide compounds were also tested for their fluorescence when introduced with boronic acids. Several control compounds were synthesized to investigate the alkylation synthesis step. The control compound with a propargyl functionality **1** and the anthranilic hydrazide **3** were able to be synthesized. The azide control compound **2** was not synthesized due difficulties with the alkylation step. The lucifer dye 1 was synthesized in very low yield. The product by itself was fluorescence, while the boron complex was less fluorescence, meaning it is not suitable as a quenched dye. The fluorescence of various hydrazide and boronic acid combinations was strongly influenced by the pH of the solutions, the buffer and oxidation by peroxynitrite.

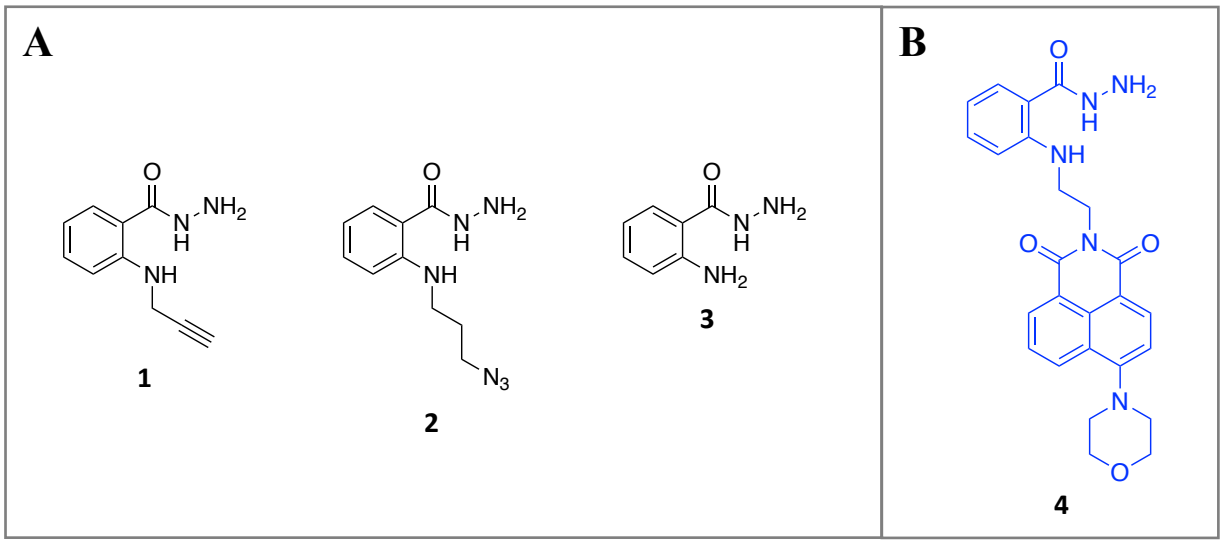


Figure 1: Target compounds; A) Control compounds; B) Main compound, lucifer dye

Introduction

Several methods exist to study biological systems. One of these methods is by using fluorescent proteins (FP). One famous example is the discovery of the green fluorescent protein (GFP), which was discovered and isolated from a jellyfish. Fluorescent proteins will emit visible lights under specific light wavelengths, which makes them easily detectable for fluorescence microscopy[1]. The downside is that these proteins are bulky and can influence the 3D-structure of the protein of interest (POI) when introduced as a tag[2].

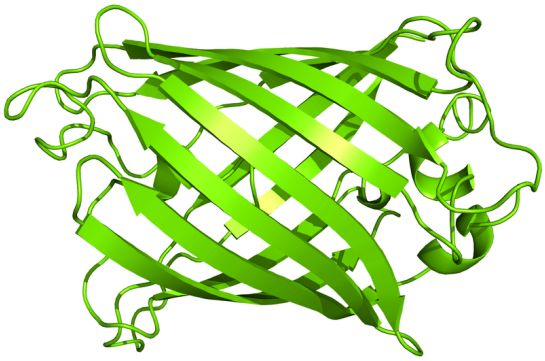


Figure 2: GFP structure[3]

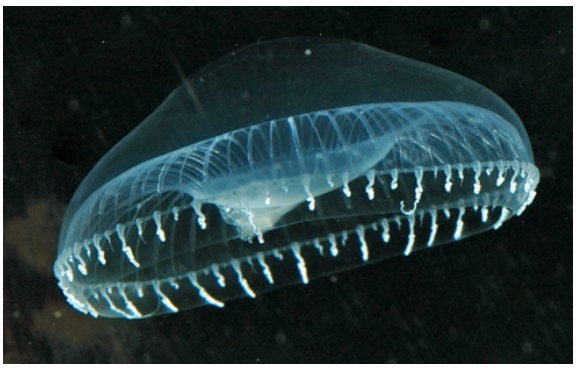


Figure 3: Jellyfish with GFP[1]

A smaller alternative is using synthetic dyes, which can be introduced to the POI via bio-orthogonal handles. A few options that are commonly used are: affinity-based protein labeling[4], adding unnatural amino acids[5] or modifying the N-terminus[6].
With affinity-based labeling two functional groups are presents. One acts as a tag, which is non-fluorescent. The other is a reactive group that covalently reacts with the POI and releases the ligand, making the tag fluorescent[7] (Figure 4A). Another way is to modify the POI by adding unnatural fluorescent amino acids (Figure 4B). These modifications could be performed in specialized *E. coli*[5]. The last example is modifying the N-terminus of a protein by adding a fluorescent tag to methionine[8] (Figure 4C).

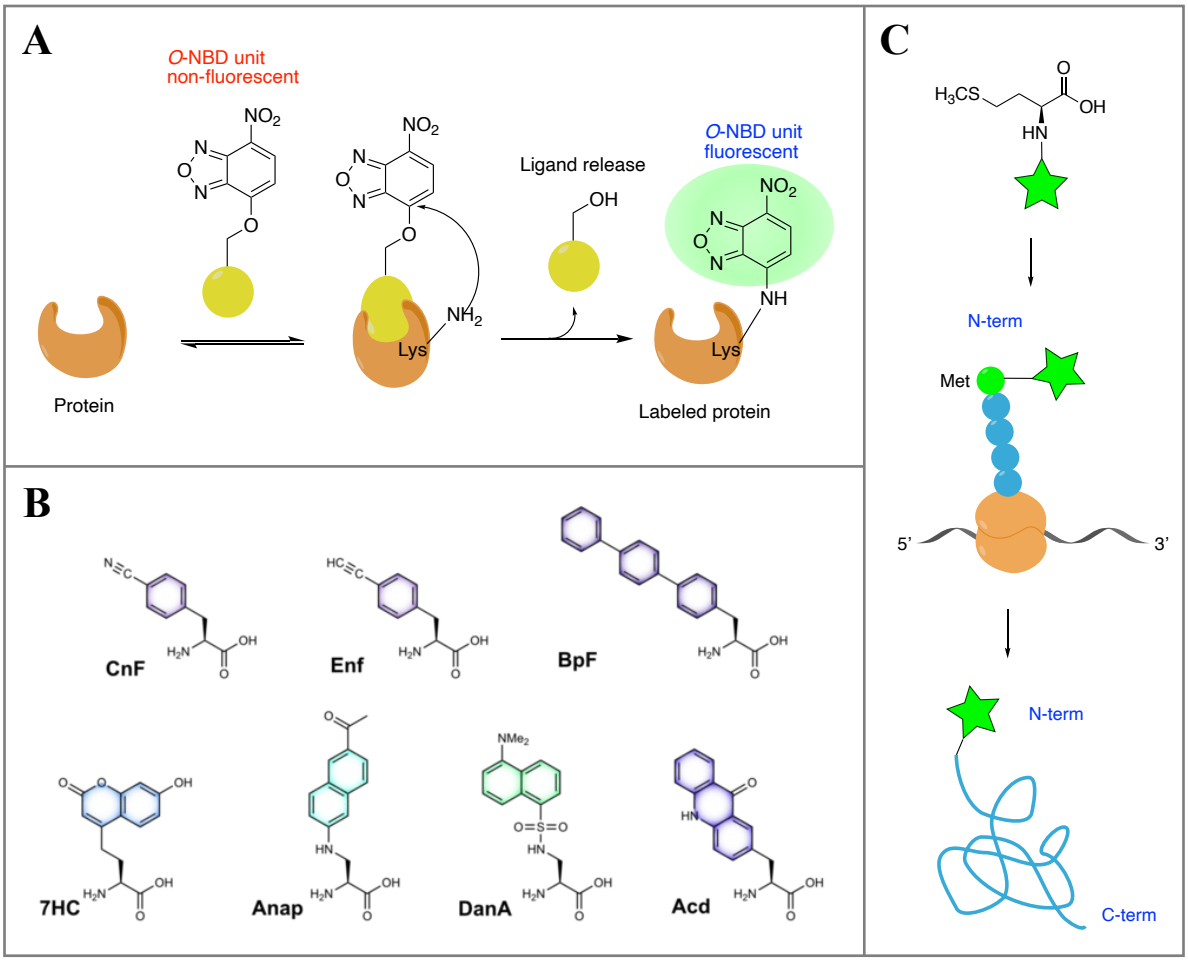


Figure 4: A) affinity-based labeling; B) unnatural fluorescent amino acids[9]; C) N-terminus modification

The problem with these methods is that the protein can only be visualized when the labeling reaction occurs. Not only that, but side reactions with other compounds present can also cause background labeling, making it very hard to read what is happening in a system.
The solution to this problem could be quenched dyes[10]. In this project the possibilities of a naphthalimide dye with an anthranilhydrazide handle will be researched. The general idea is building a ligand with a 2-fPBA moiety which selectively bind to the POI. The quenched lucifer dye will be used to selectively bind to the 2-fPBA handle which forms a boron complex[11] (Figure 5). Here the boron acts as a sensor and will block the quenching mechanism, making the complex fluorescent.

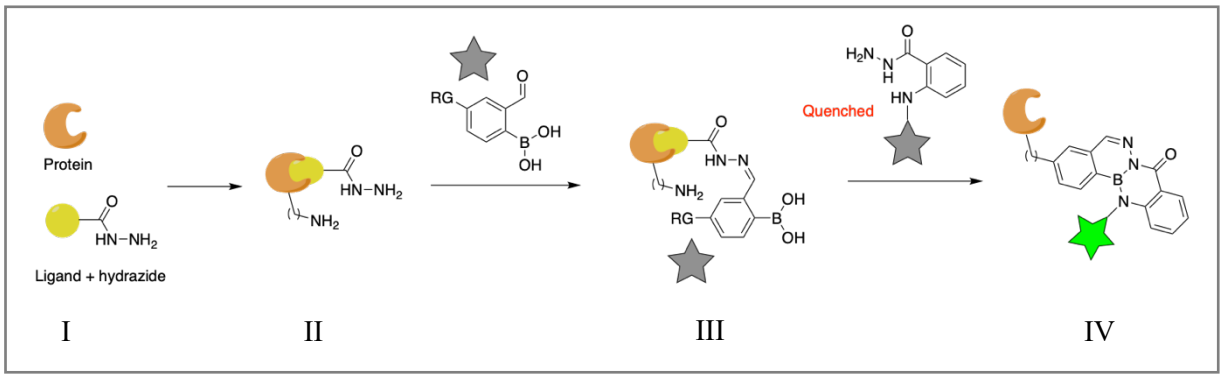
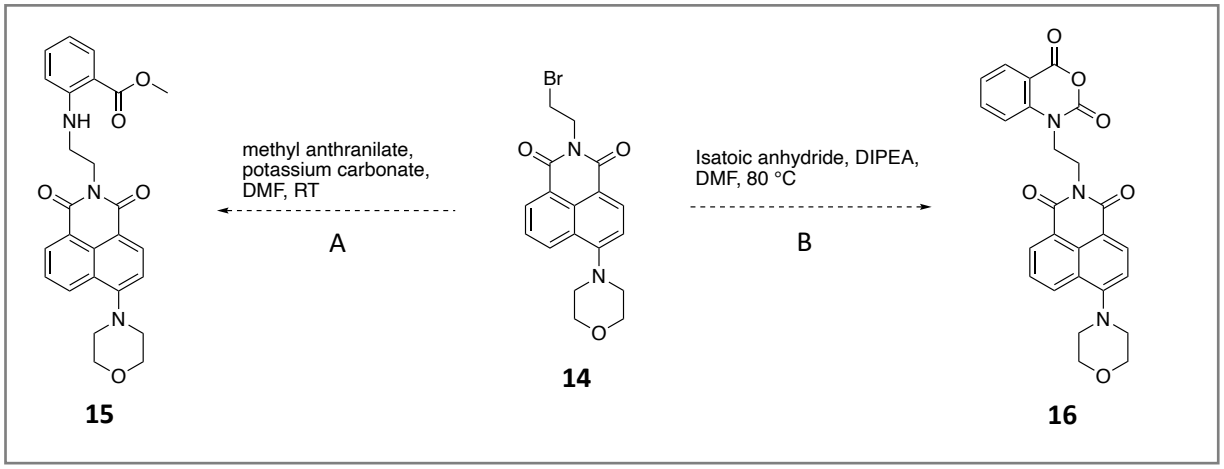


Figure 5: I) POI and ligand with a hydrazide; II) Ligand binds to POI; III) Boronic acids attaches, non-fluorescent; IV) Iminoboronate complex is formed, fluorescent

Hannes Hovorka has explored several synthesis routes to synthesize a quenched naphthalimide dye in his master research project. One of his target dyes was compound **4**[12] which might be accessible from key intermediates **15** and **16**. Attempts to synthesize **15** and **16** by alkylating were not successful, due incomplete conversions or not being able to isolate the product (Scheme 1).



Scheme 1: bromine alkylation; A) methyl anthranilate; B) isatoic anhydride

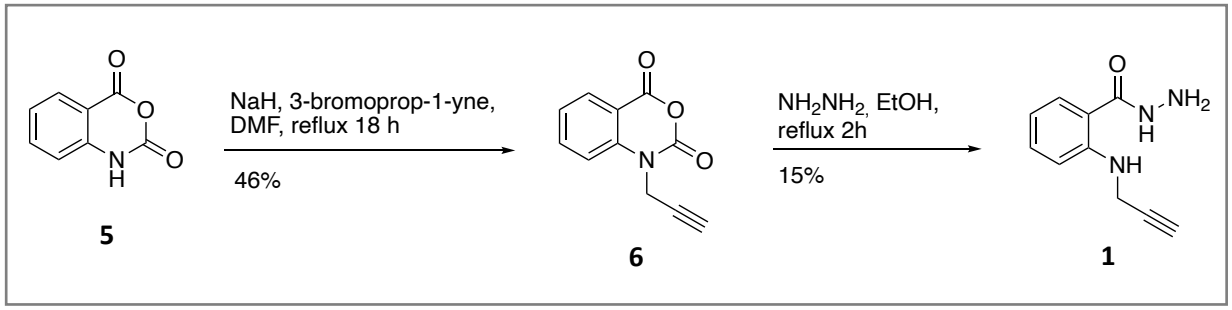
Aim of this project

This project is a continuation in an attempt synthesize several anthranilate derivatives (Figure 1). Three of these will be control compounds **1**, **2** and **3** and the main compound being a lucifer dye **4**. In the second part of this project the fluorescence of the synthesized compounds will be tested by forming iminoboronate complexes and adding peroxynitrite.

Results and discussion

Synthesis control compound (1)

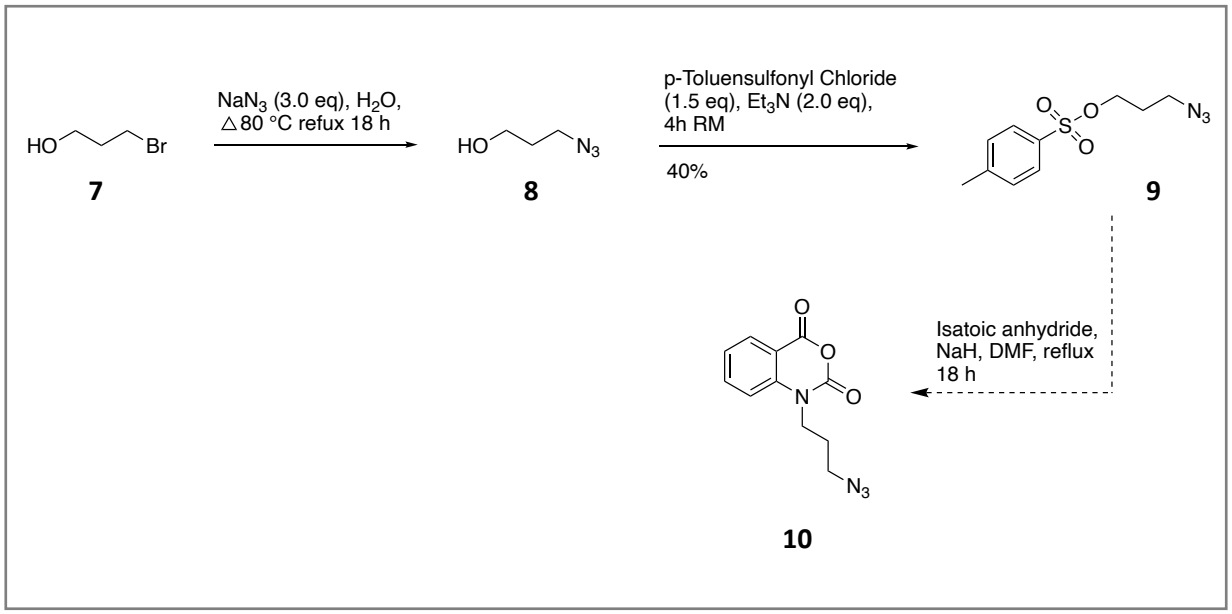
Control compound **1** was synthesized by first performing an alkylation. Sodium hydride was used as a base to deprotonate the isatoic anhydride **5** to make it a better nucleophile. A S_N2 reaction had resulted in the formation of **6** with a yield of 46%. Next, a hydrazide formation was performed. The final product **1** was purified via recrystallization and yielded 15%. To increase the yield, the purification step could be optimized.



Scheme 2: Synthesis of propargyl control group

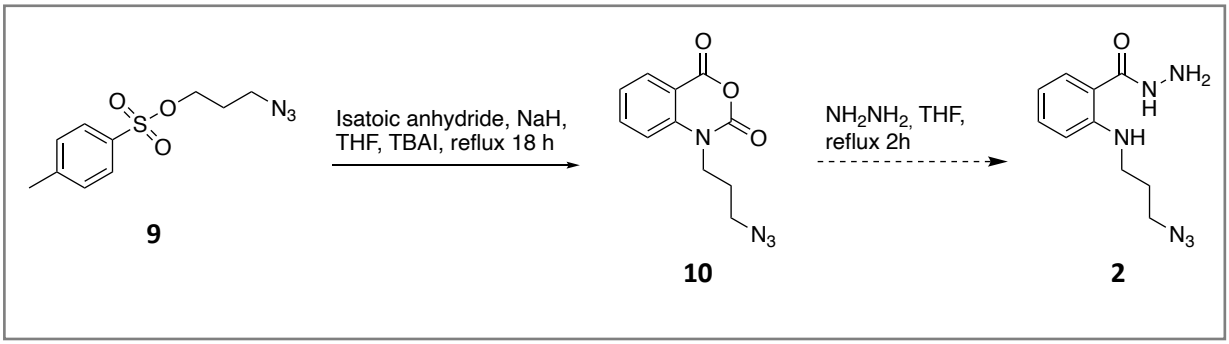
Synthesis control compound (2)

For this control compound a spacer had to be made. The spacer was then used to test the synthesis route of the alkylation step. In the first step a substitution reaction from a bromide **7** to an azide **8** was performed. Next an esterification was performed to transform the alcohol group to a p-toluenesulfonate functional group, which will act as a leaving group in the next step. Compound **9** was used as an electrophile for an alkylation with isatoic anhydride. For this step the same conditions were used as for control compound **1**. TLC showed a new compound had formed, but no full conversion was obtained. It was attempted to isolate the newly formed compound through column chromatography, but the alkylation product degraded. Another compound that was formed was a hydrolysis side product, where the anhydride had opened and formed an acid.



Scheme 3: Synthesis of azide control group

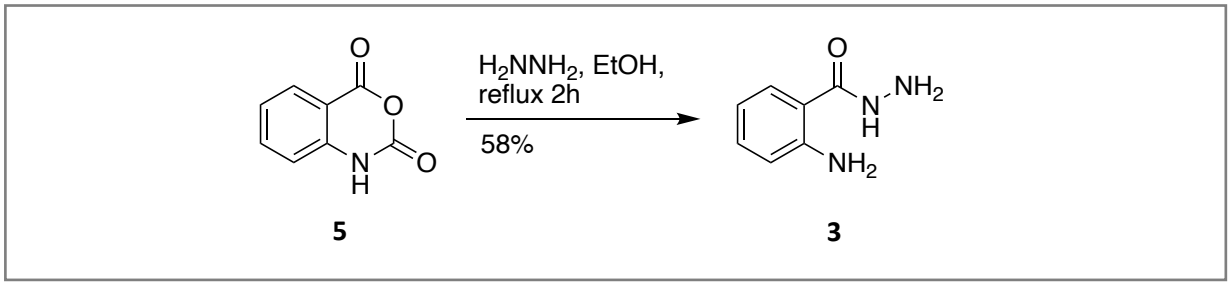
It was tried to optimize the synthesis route to **10** by changing the reaction conditions (Scheme 3). The solvent was changed from DMF to THF for a lower reflux temperature and TBAI was added as a catalyst. The p-toluenesulfonate will be substituted, forming an alkyl iodide, which is more reactive. Again, incomplete conversion was observed. Hydrazine was added to the reaction mixture to see if hydrazide formation would occur, but TLC showed no change. The alkylation might not have occurred due **9** not being a good electrophile. Too much steric hindrance lowers the S_N2 reaction and will favor the elimination side. Also, the leaving group of the propargyl control compound was next to a SP hybridized atom. This has less steric hindrance and because of overlapping orbitals it becomes more reactive[13]. With this control group, the leaving groups is next to an SP2 hybridized atom, giving more hindrance and no overlap in orbitals.



Scheme 4: Optimized synthesis route

Synthesis control compound (3)

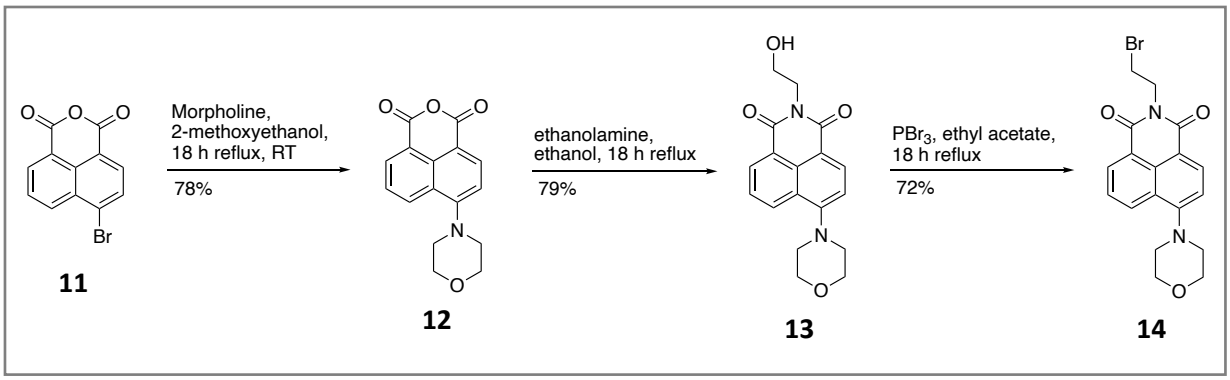
This reaction was performed to test the hydrazide formation with isatoic anhydride and as a control for the fluorescence studies. TLC showed full conversion was obtained and the product was purified via recrystallization.



Scheme 5: Synthesis anthranilic hydrazide control compound

Synthesis lucifer dye (4)

In the previous attempt to synthesis the naphthalimide dye had failed at the alkylation step. The first steps to create the spacer was copied from Hannes procedures[12]. Next a proposed mechanism and another synthesis route was explored for the alkylation. To synthesize **12** the synthesis started with an amination of **11** with morpholine. Next the anhydride was refluxed with ethanolamine in ethanol to yield **13**. The alcohol was then converted to a bromine **14** with phosphorus tribromide. No deviations from the copied procedure were observed.



Scheme 6: Synthesis of bromine lucifer dye (14)

From **14** two different alkylation routes have been theorized to make **15** and **16**. Isatoic anhydride and methyl anthranilate (MA) have been used for these attempts, but they were not successful due no full conversions or not being able to isolate the product. Hannes described in his 'future prospects' a different synthetic route to synthesize **4** by using NaH as a base instead of DIPEA with the alkylation step. The carbonyl **15** will not be further investigated due the lowered reactivity of the carbonyl to perform the hydrazide formation.

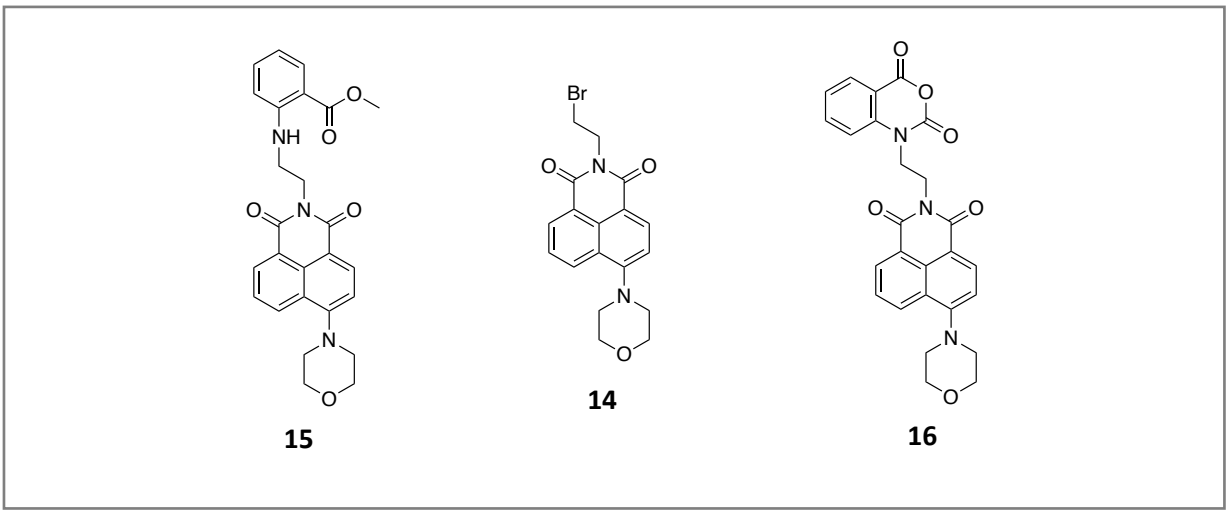


Figure 6: (from left to right) MA alkylation product, bromine, isatoic anhydride alkylation product

To synthesize **16** isatoic anhydride was dissolved in DMF and sodium hydride was added. After an hour of stirring **14** was added. After 18 hours of stirring, several spots were visible on the TLC (Figure 7). Spot *A* corresponds to bromide **14**, which indicated that no full conversion was obtained. Spot *B* and *D* could be formed due a hydrolyzation with water that was still present in the starting material **14**. This could be present by not properly drying the compound. After performing LCMS tests, spot *C* was identified as product **16** and isolated with a yield of 3%. Next a hydrazide formation was performed with hydrazine in ethanol. The product was characterized via LCMS. No further test could be performed due low amount of product.
Based on the literature, it was hypothesized that anhydride **16** should be more fluorescent than the aminobenzoic acid derivative **B** (Figure 7B), which resembles the hydrazide **4** product. Strikingly, the reverse is observed. The closed ring shows little to no fluorescence, while the opened ring shows some fluorescence. This could indicate that the desired product will not act as a quenched dye and therefore not work.

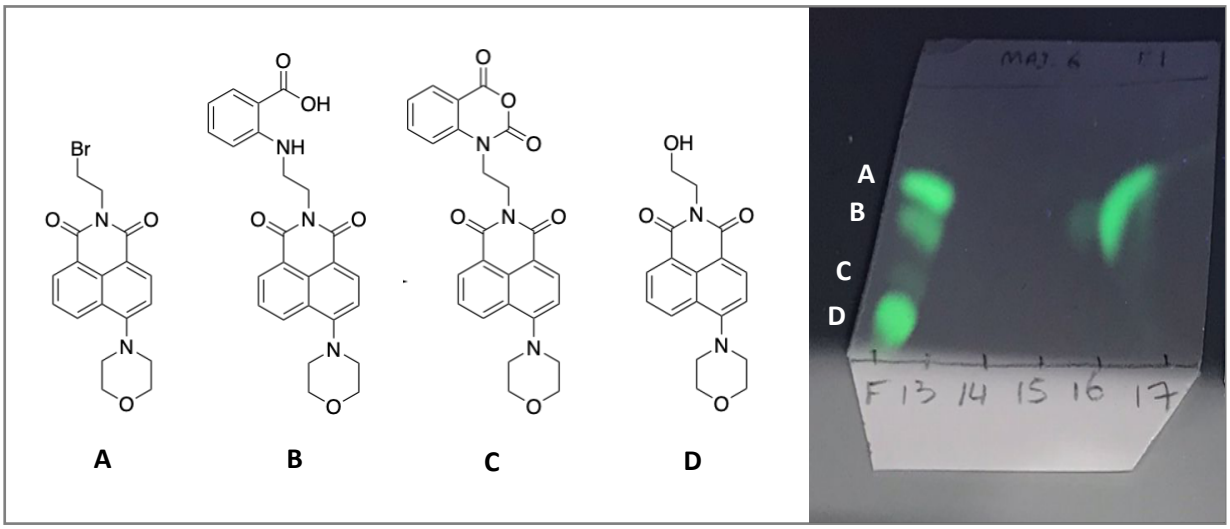
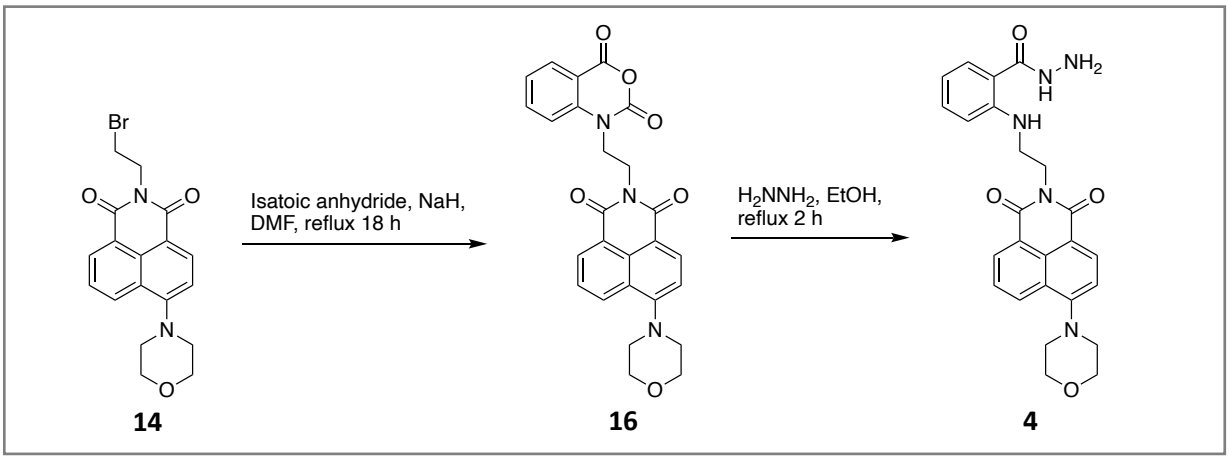
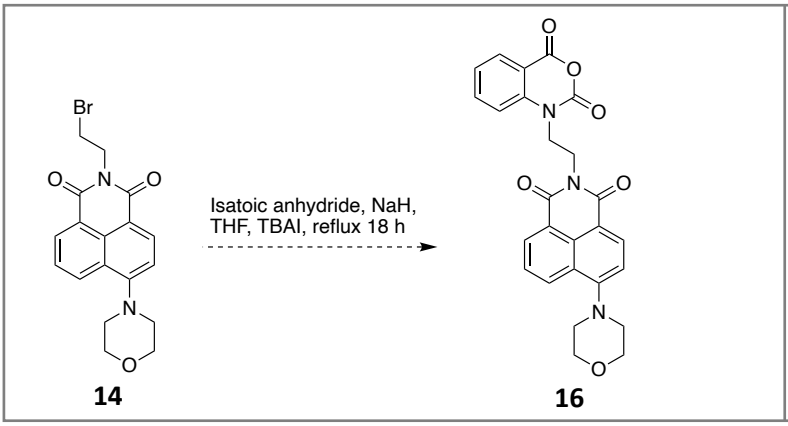


Figure 7: TLC after 18 hours reflux of alkylation step



Scheme 7: Hydrazide formation of lucifer dye

The same optimalization procedure as control compound **2** was also used for this alkylation step by changing the solvent and adding TBAI as a catalyst. After 18 hours of stirring, no conversion was observed on the LCMS. Some alkylation might have occurred, but it was immediately hydrolyzed, resulting in the aminobenzoic acid derivative side product. Almost no S_N2 reaction had occurred due probably same reasons as mentioned with control compound **2**. Based on the LCMS a significant amount of elimination side product was formed (Figure 8).



Scheme 8: Optimization attempt

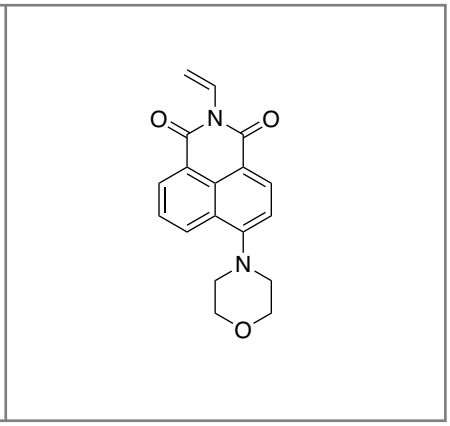


Figure 8: elimination side product

Fluorescence test

The lucifer dye **4** should act as a quenched dye, meaning by itself it should be non-fluorescent. By adding (2-formylphenyl)boronic acid, which by itself is also non-fluorescent, it will form a boron complex that is fluorescent in theory (Figure 9). However, compound **4** is by itself fluorescent and after forming the boron complex it lost some of its fluorescence. From these results, no other attempts were performed to optimize the synthesis route to **4**. And since there is not enough of compound **4**, no further tests were conducted.

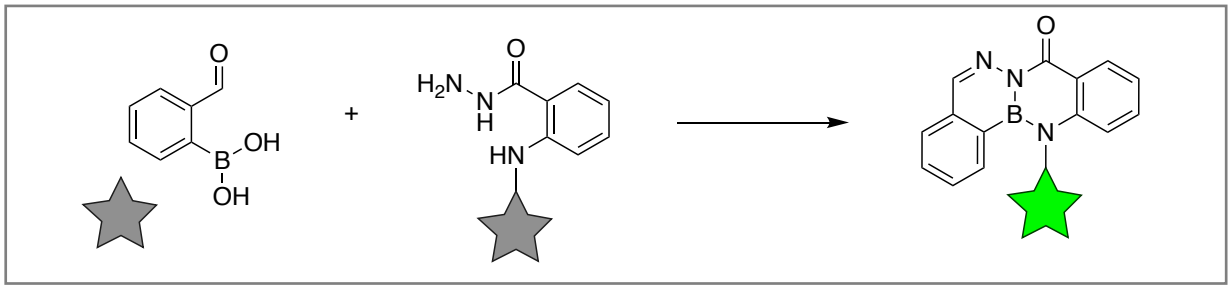


Figure 9: Iminoboronate formation of boronic acid with a hydrazide

For the control compounds **1** and **3** fluorescence test have been performed. By themselves they were fluorescent, so it would be interesting to know how they would behave when a complex was formed. To examine the effects of quenched dyes more, compounds that were synthesized by Hannes himself will also be tested for their fluorescence (Figure 10). Three different boronic acids (**17**, **18**, **19**) had been used to make the boron complexes.

Stock solutions of the hydrazides and boronic acids were made in acetonitrile. Of each, one to one was be added in various base solutions (lithium hydroxide, sodium hydroxide and sodium chloride) and HEPES buffer. The boronic acid used in this reaction acts as a Lewis acid. Water could coordinate to the boron which could result in bending of the molecule. The loss of the planar form could influence the fluorescence, so by adding a base this effect could be lessened.

To perform two tests the base solutions were made in 1:1 H_2O/DMSO to reproduce the conditions of Hannes experiment and in 100% H_2O.

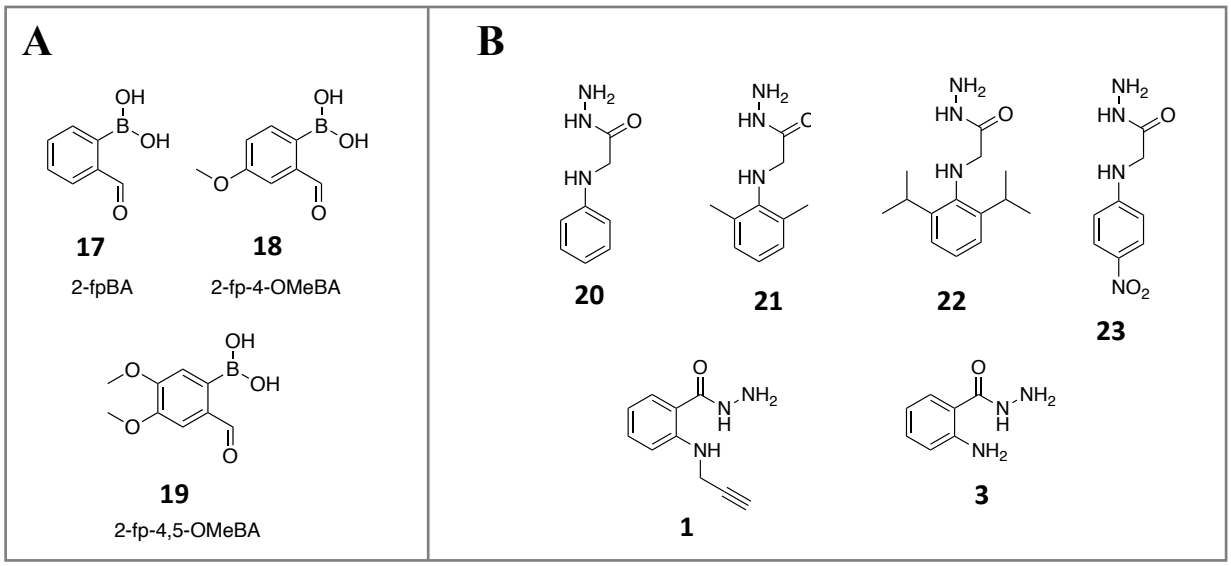


Figure 10: A) boronic acids; B) Hydrazides made by Hannes

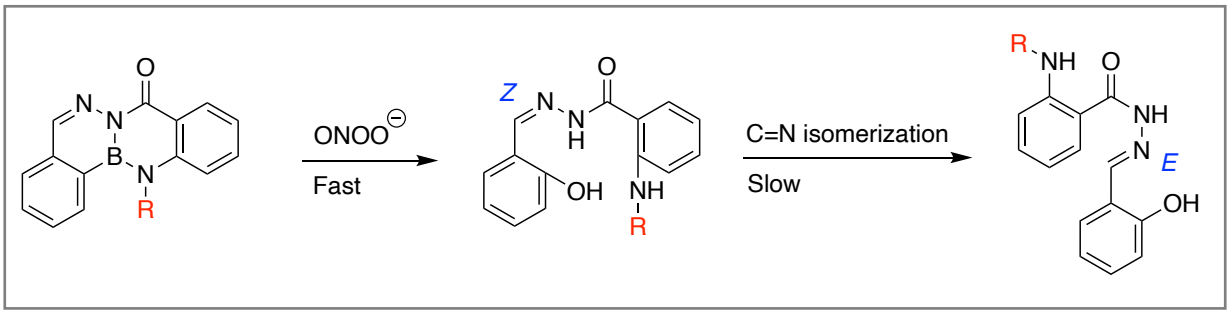
The wells of the plate had been filled as depicted on table 1. The bottom row of blanks only contains the hydrazide in the base solutions. The plates were incubated at 45 °C for one hour and then taken for measurements in the plate reader.

		2-fpBA (**17**)				2-fp-4-OMeBA (**18**)				2-fp-4,5-OMeBA (**19**)			
PLATE 1		1	2	3	4	5	6	7	8	9	10	11	12
20	A	LiOH	NaOH	NaCl	Buffer	LiOH	NaOH	NaCl	Buffer	LiOH	NaOH	NaCl	Buffer
21	B	LiOH	NaOH	NaCl	Buffer	LiOH	NaOH	NaCl	Buffer	LiOH	NaOH	NaCl	Buffer
22	C	LiOH	NaOH	NaCl	Buffer	LiOH	NaOH	NaCl	Buffer	LiOH	NaOH	NaCl	Buffer
23	D	LiOH	NaOH	NaCl	Buffer	LiOH	NaOH	NaCl	Buffer	LiOH	NaOH	NaCl	Buffer
1	E	LiOH	NaOH	NaCl	Buffer	LiOH	NaOH	NaCl	Buffer	LiOH	NaOH	NaCl	Buffer
3	F	LiOH	NaOH	NaCl	Buffer	LiOH	NaOH	NaCl	Buffer	LiOH	NaOH	NaCl	Buffer
4	G	LiOH	NaOH	NaCl	Buffer	LiOH	NaOH	NaCl	Buffer	LiOH	NaOH	NaCl	Buffer
Blanks	H	1	1	1	1	3	3	3	3	4	4	4	4

Table 1: Plate 1 well distribution

Plate 1 (1:1 H_2O/DMSO)

An absorbance spectrum was taken of the plate. The absorption at 325 nm gave strong fluorescence, so further measurements were taken with it. Next a fluorescence spectrum was taken of plate 1 (Table 1). After the measurements were ready, peroxynitrite was added in each well. The oxidation of the complexes is very fast and creates the *Z* isomer. The C=N isomerization to a form the *E* isomer is a slower process (Scheme 9).[14] The Z isomer is more fluorescent than the E isomer, so overtime the complexes will lose it fluorescence.[15] Again, fluorescence spectrum was taken and compared (Figure 11B).



Scheme 9: Iminoboronate complex reaction with peroxynitrite

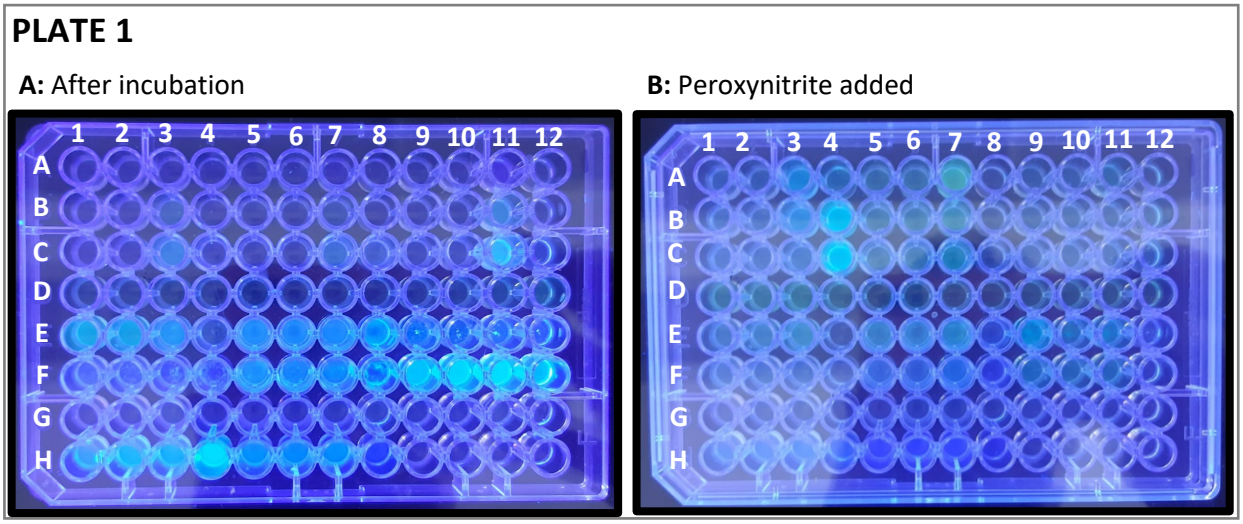


Figure 11: Plate 1, (1:1 H_2O/DMSO), UV 365 nm

There were a few interesting findings when the boron complexes had formed and when the peroxynitrite was added. Of plate 1A, well H4 contains a blank of **1** in buffer, which is fluorescent by itself. Well E4 contains a complex of **1** and **17** in buffer. When the complex was formed it lost its fluorescence (Figure 12). This is an example of an 'turn-off' dye. This is not a suitable dye to label a protein as it turn off when it is bound, making the protein not detectible.

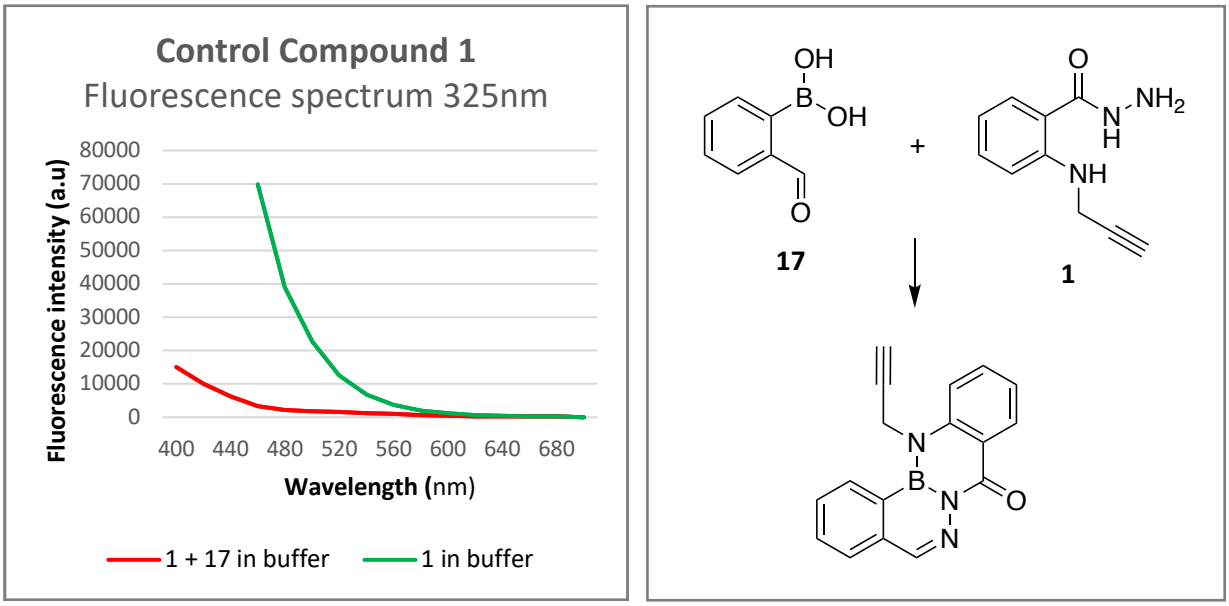


Figure 12: 1 and 17 formed complex fluorescence spectrum

The use of peroxynitrite also gave interesting results. In plate 1A, well B4 contains the complex formed form hydrazide **21** and 2-fPBA **17** and well C4 contains the complex formed from hydrazide 22 and 2-fPBA **17** complex. By themselves they were not fluorescent, but in plate 1B when peroxynitrite was added it started to become fluorescent (Figure 13).

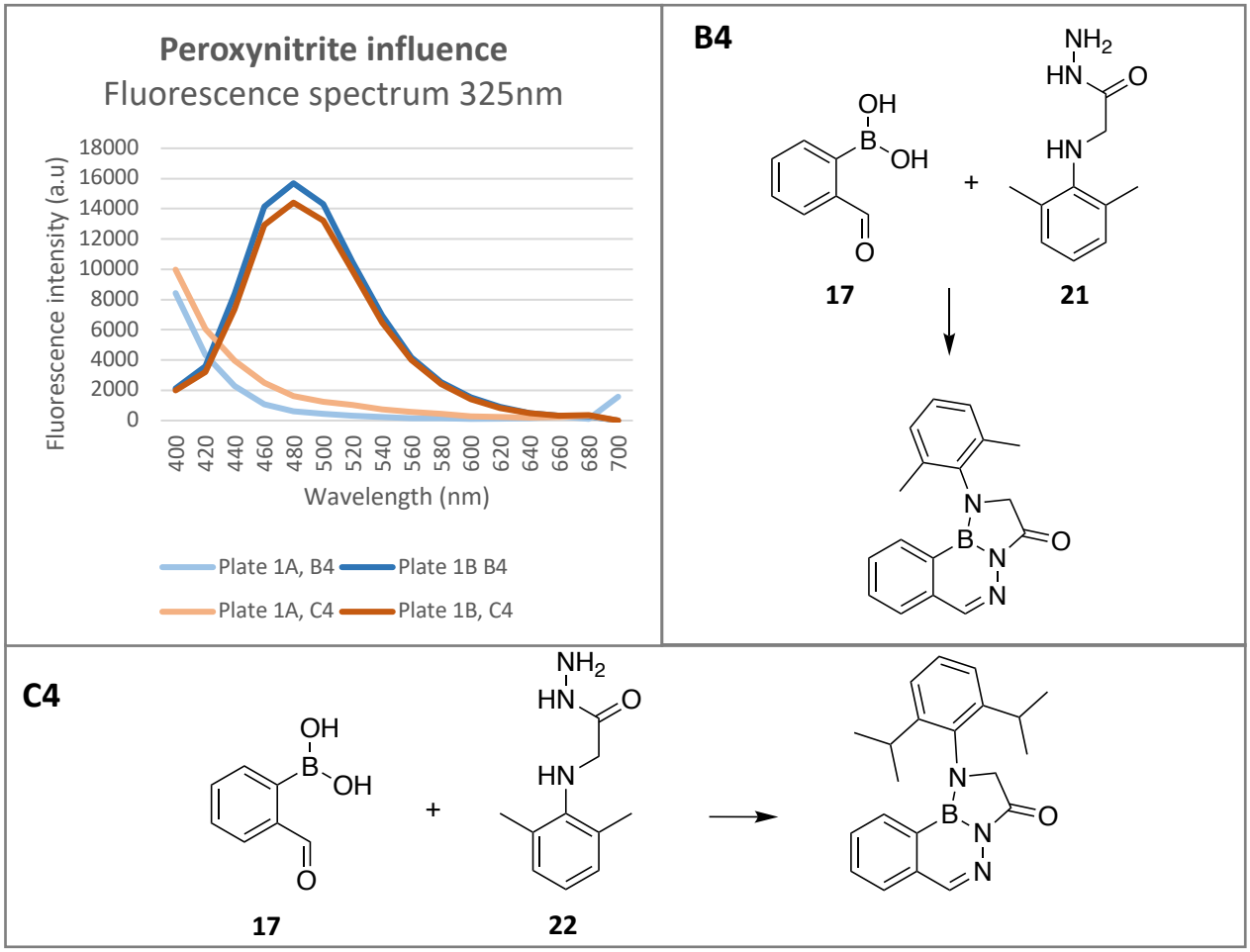


Figure 13: Influence of peroxynitrite on iminoboronate complexes

Plate 2 (100% H_2O)

The base solutions were made in 100% water, while the HEPES buffer was still the same. Aggregation could be induced by adding the reagents in a water solution. Also, most proteins in biological systems are present in mainly aqueous solutions. So, by using water, the system could be better mimicked. In this plate no blanks were present, so row H was empty. Here the same procedure was followed to measure the spectra.

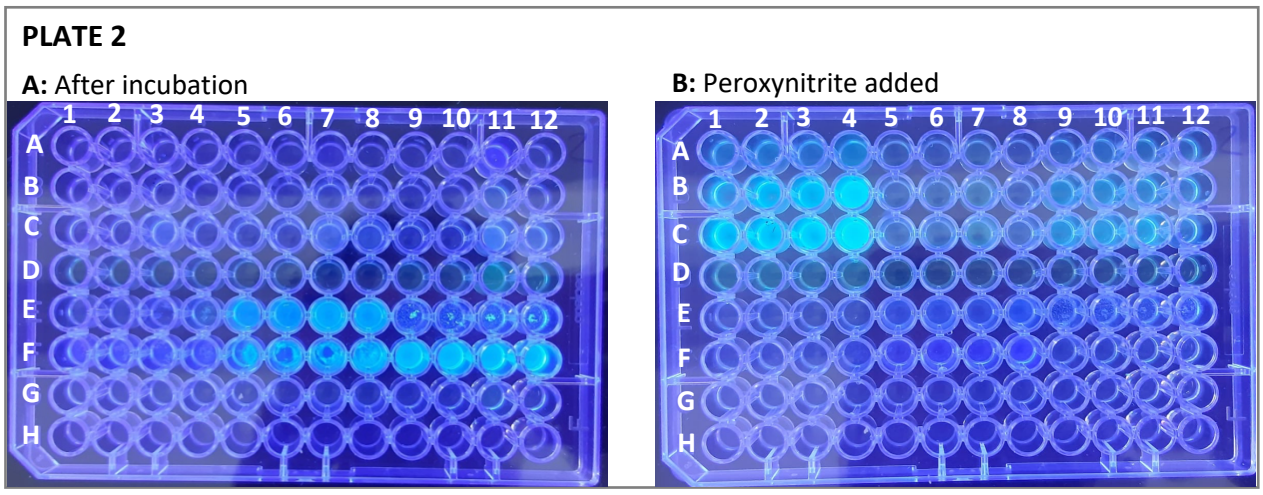


Figure 14: Plate 2, (100% H_2O), UV 365 nm

		2-fpBA (17)				2-fp-4-OMeBA (18)				2-fp-4,5-OMeBA (19)			
PLATE 2		1	2	3	4	5	6	7	8	9	10	11	12
20	A	LiOH	NaOH	NaCl	Buffer	LiOH	NaOH	NaCl	Buffer	LiOH	NaOH	NaCl	Buffer
21	B	LiOH	NaOH	NaCl	Buffer	LiOH	NaOH	NaCl	Buffer	LiOH	NaOH	NaCl	Buffer
22	C	LiOH	NaOH	NaCl	Buffer	LiOH	NaOH	NaCl	Buffer	LiOH	NaOH	NaCl	Buffer
23	D	LiOH	NaOH	NaCl	Buffer	LiOH	NaOH	NaCl	Buffer	LiOH	NaOH	NaCl	Buffer
1	E	LiOH	NaOH	NaCl	Buffer	LiOH	NaOH	NaCl	Buffer	LiOH	NaOH	NaCl	Buffer
3	F	LiOH	NaOH	NaCl	Buffer	LiOH	NaOH	NaCl	Buffer	LiOH	NaOH	NaCl	Buffer
4	G	LiOH	NaOH	NaCl	Buffer	LiOH	NaOH	NaCl	Buffer	LiOH	NaOH	NaCl	Buffer
	H												

Table 4: Plate 2 well distribution

With plate containing the water solutions the anthranilate derivatives **1** (row E) and **3** (row F) were fluorescent when the iminoboronate complexes were formed, but they lost some of their fluorescence when peroxynitrite was added (Figure 15).
The combination of hydrazides **21** and **22** with boronic acid **17** were significant more fluorescent when peroxynitrite was added, compared unhydrolyzed compound. The best results can be seen on at a wavelength of 365 nm in buffer (Figure 14). There was also visible change at the absorption wavelength of 325 nm (Figure 16).

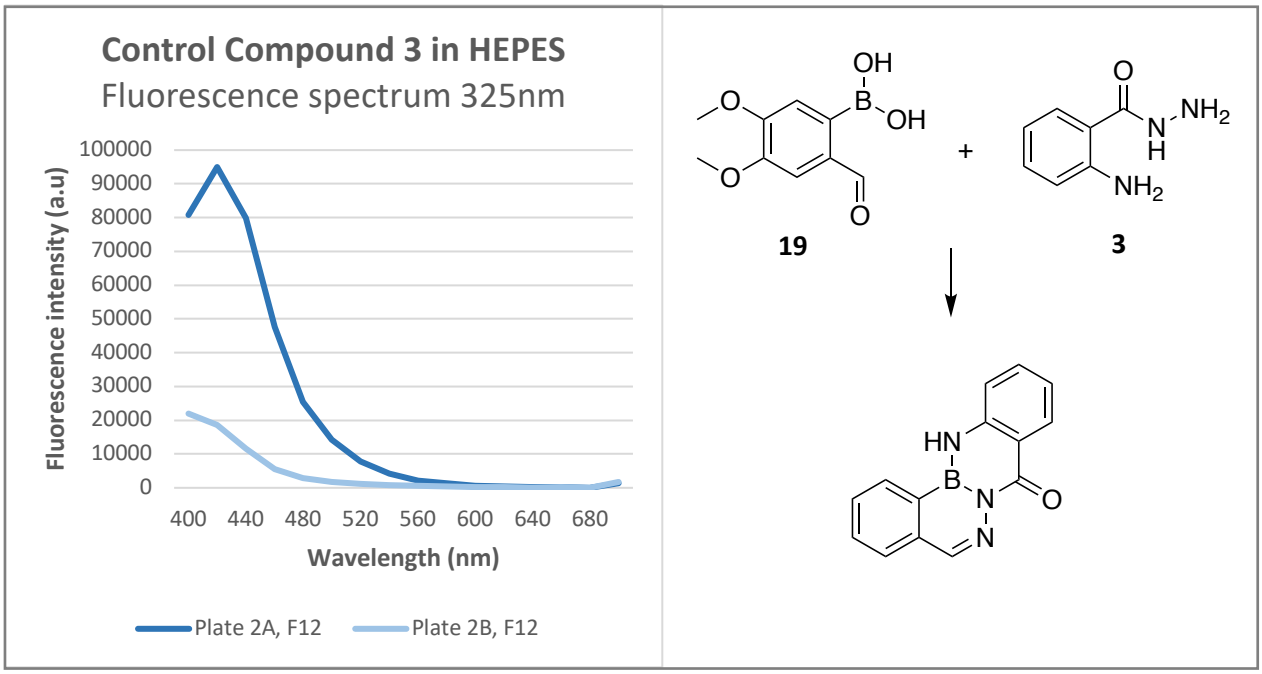


Figure 15: Influence of peroxynitrite on control group 3

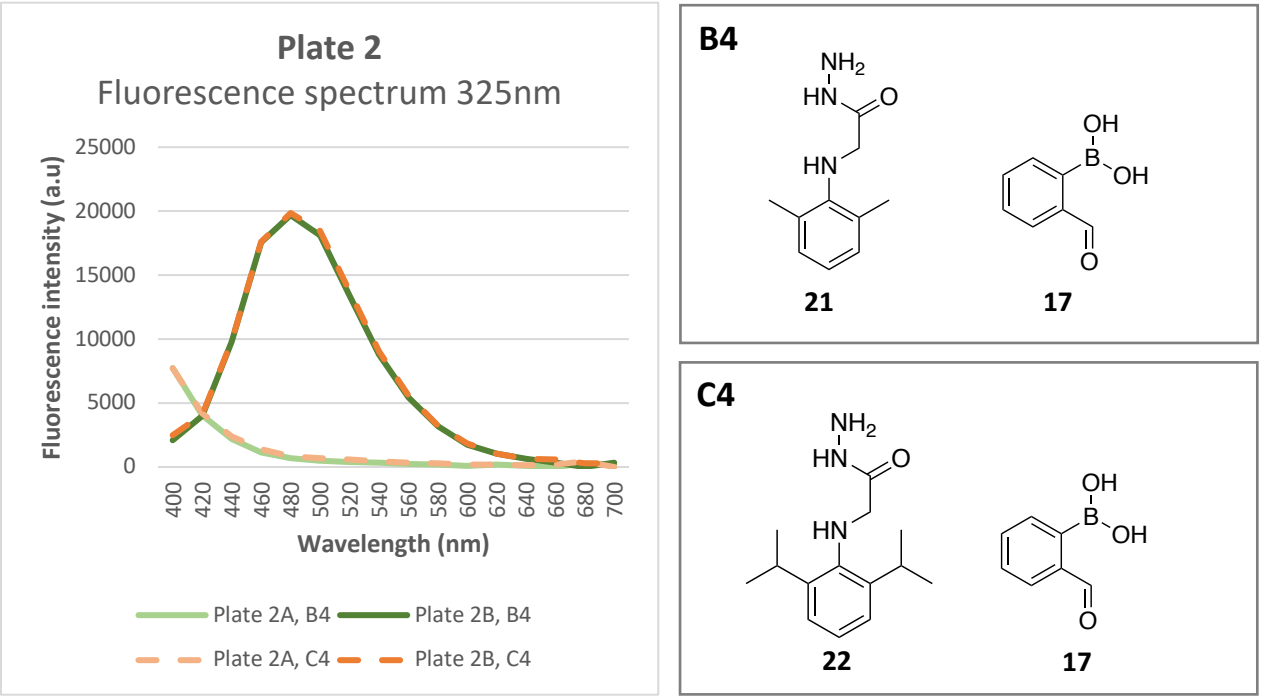


Figure 16: Influence of peroxynitrite on complex 21+17 and 22+17 in buffer, emission after excitation at 325 nm

Overall, the pictures of the plates at wavelength 365 nm gives different results as for the fluorescent spectra at 325 nm. The complexes give a variety of different results depending on de base solutions or buffer. Having de bases dissolved in water/DMSO of just water will influence the results as well.

Conclusion

The propargyl control group **1** and the anthranilic hydrazide control group **3** were successfully synthesized. The purification step should be optimized to gain a higher yield. The azide control compound **2** was not synthesized due having difficulties with the alkylation step. The compound by itself is also not stable, so isolating and storing would cause a problem.
The main target, lucifer dye **4**, was able to be synthesized, but in very small amounts, due also having difficulties with the alkylation step. The synthesis might be optimized, however the product does not act as a quenched dye. By itself it is fluorescent, while the desired product should not be. When a boronic acid is introduced to form an iminoboronate complex it is losing its fluorescence, which is the exact opposite of what is desired. Based on these results, compound 4 does not function as a quenched dye for protein labeling.
The results of the fluorescence test do not give one conclusive answer. Based on the boronic acids and hydrazides some combinations give strong fluorescence, while other combinations don't. It also depends in which base solution or buffer the complex is present. The complexes in H_2O/DMSO (1:1) solutions give different results than the 100% H_2O solutions. Adding peroxynitrite to the complexes could also influence the fluorescence in some cases.

Literature References

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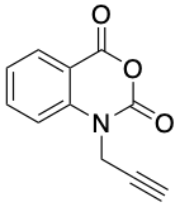
Supporting Information

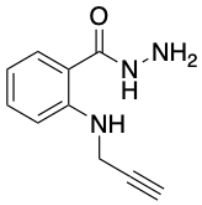
Experimentals

1-(prop-2-yn-1-yl)-2H-benzo[d][1,3]oxazine-2,4(1H)-dione (6)



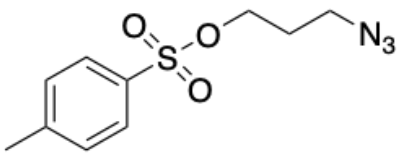
Sodium hydride (0.16 g, 6.7 mmol, 1.1 eq) was added to a solution of isatoic anhydride (1.00 g, 6.1 mmol, 1.0 eq) in anhydrous DMF (19 mL) and stirred for 1 h at room temperature. Propargyl Bromide (0.80 g, 6.7 mmol, 1.1 eq) was added to the reaction mixture and the mixture stirred overnight at room temperature. The reaction mixture was poured over ice water (100 mL) to precipitate the crude product which was filtered, washed with cold water, and dried under vacuum. The solids were purified by recrystallization with methanol and ethyl acetate, filtered, and dried under vacuum to yield (X) white crystals (0.56 g, 46%). 1H NMR (400 MHz, DMSO-d6) δ 8.02 (dd, J = 7.8, 1.6 Hz, 1H), 7.89 (ddd, J = 8.7, 7.3, 1.6 Hz, 1H), 7.49 (d, J = 8.5 Hz, 1H), 7.41 – 7.32 (m, 1H), 4.86 (d, J = 2.5 Hz, 2H), 3.40 (t, J = 2.5 Hz, 1H). 13C NMR (400 MHz, DMSO-d6) δ 158.90, 147.72, 140.91, 137.58, 130.06, 124.51, 115.44, 112.30, 78.02, 76.47, 34.79. HRMS-ESI+ (m/z): [M]+ calculated for $C_{11}H_7NO_3$, 201.04259; found, 202.04987.

2-(prop-2-yn-1-ylamino)benzohydrazide (1)



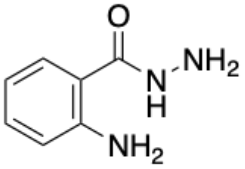
6 (0.43 g, 2.2 mmol, 1.0 eq) was dissolved in absolute ethanol (3 mL) and cooled to 0 °C with an ice bath. Hydrazine (69.1 mg, 2.2 mmol, 1.0 eq) was added and the reaction mixture was refluxed for four h. The mixture was then concentrated in vacuo and purified by recrystallization with methanol to yield X as a white powder (61.8 mg, 15%). 1H NMR (400 MHz, DMSO-d6) δ 9.58 (s, 1H), 7.70 (t, J = 5.9 Hz, 1H), 7.46 (dd, J = 7.8, 1.6 Hz, 1H), 7.27 (t, J = 8.3,1.6 Hz, 1H), 6.73 (d, J = 8.3 Hz, 1H), 6.59 (t, J = 8.3, 1H), 4.39 (s, 2H), 3.97 (dd, J = 5.9, 2.4 Hz, 2H), 3.09 (t, J = 2.4 Hz, 1H). 13C NMR (400 MHz, DMSO-d6) δ 168.80, 148.25, 132.40, 128.23, 115.73, 115.49, 112.08, 82.14, 73.74, 32.17. HRMS-ESI+ (m/z): [M]+ calculated for $C_{10}H_{11}N_3O$, 189.09021; found, 190.09749.

3-azidopropyl 4-methylbenzenesulfonate (9)



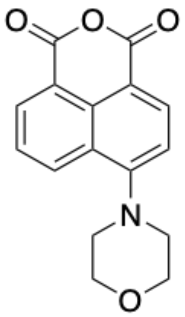
Sodium azide (2.0 g, 30 mmol, 3.0 eq) was dissolved in water (12.5 mL). 3-Bromo-1-propanol (1.4 g, 10 mmol, 1.0 eq) was added to the solution and refluxed at 80 °C overnight. The reaction mixture was washed with diethyl ether (4x) and the combined organic layers were dried over sodium sulfate, filtered, and concentrated in vacuo to obtain a clear oil. This was dissolved in DCM (11 mL) and cooled to 0 °C. Triethylamine (2.8 mL, 20 mmol, 2.0 eq) and p-toluenesulfonyl chloride (2.9 g, 15 mmol, 1.5 eq) were slowly added to the solution. After stirring for four h at room temperature, the mixture was quenched with water and diluted with ethyl acetate. The organic layer was washed with 2M HCl (2x), brine (2x), dried over sodium sulfate, filtered, and concentrated in vacuo to obtain a clear oil. The crude was purified with column chromatography (EtOAc/Pentane) to yield compound X a clear oil (1.0 g, 40%). 1H NMR (400 MHz, CDCl3) δ 7.78 (d, J = 8.1 Hz, 2H), 7.35 (d, J = 8.1 Hz, 2H), 4.09 (t, J = 6.2 Hz, 2H), 3.36 (t, J = 6.2 Hz, 2H), 2.44 (s, 3H), 1.87 (p, J = 6.2 Hz, 2H). 13C NMR (400 MHz, CDCl3) δ 145.04, 132.73, 129.93, 127.86, 67.02, 47.27, 28.41, 21.60.

2-aminobenzohydrazide (3)



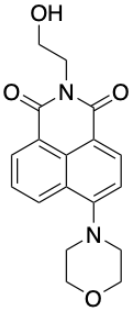
Isatoic anhydride (0.49 g, 3.0 mmol, 1.0 eq) was dissolved in absolute ethanol (6 mL) and cooled to 0 °C with an ice bath. Hydrazine (96.1 mg, 3.0 mmol, 1.0 eq) was added and the reaction mixture was stirred over the weekend at room temperature. The mixture was then concentrated in vacuo and purified by recrystallization with ethanol to yield X as a white powder (0.26 g, 58%). 1H NMR (400 MHz, DMSO-d6) δ 9.42 (s, 1H), 7.38 (dd, J = 7.9, 1.5 Hz, 1H), 7.09 (ddd, J = 8.4, 7.1, 1.5 Hz, 1H), 6.66 (dd, J = 8.4, 1.2 Hz, 1H), 6.45 (ddd, J = 8.0, 7.1, 1.2 Hz, 1H), 6.29 (s, 2H), 4.34 (s, 2H). 13C NMR (101 MHz, DMSO-d6) δ 168.96, 149.79, 131.96, 128.06, 116.63, 115.09, 114.13.

6-morpholino-1H,3H-benzo[de]isochromene-1,3-dione (12)



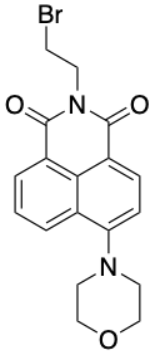
6-bromo-1H,3H-benzo[de]isochromene-1,3-dione (0.83 g, 3.0 mmol, 1.0 eq) and morpholine (0.52 g, 6.0 mmol, 2.0 eq) were dissolved in 2-methoxyethanol (13 mL) and refluxed overnight. The reaction mixture was cooled to room temperature, filtered, washed with cold water and dried under vacuum to yield orange crystals (0.67 g, 79%). 1H NMR (400 MHz, DMSO-d6) δ 8.54 (dd, J = 8.5, 1.2 Hz, 1H), 8.48 (dd, J = 8.5, 1.2 Hz, 1H), 8.41 (d, J = 8.2 Hz, 1H), 7.83 (t, J = 8.5, 7.3 Hz, 1H), 7.36 (d, J = 8.2 Hz, 1H), 3.89 (t, J = 4.6 Hz, 4H), 3.26 (t, J = 4.6 Hz, 4H). 13C NMR (400 MHz, DMSO-d6) δ 156.86, 134.62, 132.98, 132.35, 132.18, 126.82, 125.60, 119.87, 115.71, 112.14, 66.55, 53.32.

2-(2-hydroxyethyl)-6-morpholino-1H-benzo[de]isoquinoline-1,3(2H)-dione (13)



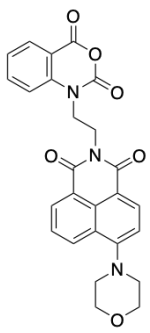
6-morpholino-1H,3H-benzo[de]isochromene-1,3-dione (0.65 g, 2.3 mmol, 1.0 eq) and ethanolamine (0.15 mL, 2.5 mmol, 1.1 eq) were dissolved in ethanol (12 mL) and refluxed overnight. The mixture was diluted with water, cooled on ice, filtered, washed with cold water and dried under vacuum to yield a yellow powder (0.59 g, 79%). 1H NMR (400 MHz, DMSO-d6) δ 8.50 - 8.43 (m, 2H), 8.38 (d, J = 8.1 Hz, 1H), 7.79 (t, J = 7.9 Hz, 1H), 7.34 (d, J = 8.1 Hz, 1H), 4.77 (t, J = 6.0 Hz, 1H), 4.11 (t, J = 6.5 Hz, 2H), 3.89 (t, J = 4.5 Hz, 4H), 3.58 (q, J = 6.5 Hz, 2H), 3.20 (t, J = 4.5 Hz, 4H). 13C NMR (400 MHz, DMSO-d6) δ 164.13, 163.62, 155.83, 132.56, 131.07, 130.90, 129.64, 126.55, 125.74, 123.21, 116.51, 115.52, 66.64, 58.29, 53.49, 42.10.

2-(2-bromoethyl)-6-morpholino-1H-benzo[de]isoquinoline-1,3(2H)-dione (14)



2-(2-hydroxyethyl)-6-morpholino-1H-benzo[de]isoquinoline-1,3(2H)-dion (0.59 g, 1.8 mmol, 1.0 eq) and Phosphorus tribromide (0.86 mL, 9.0 mmol, 5.0 eq) were dissolved in ethyl acetate (18 mL), put under nitrogen and refluxed overnight. The reaction mixture was quenched in a cold sodium bicarbonate solution, filtered, washed with cold water and dried under vacuum to yield a yellow powder (0.51 g, 72%). 1H NMR (400 MHz, CDCl3) δ 8.60 (dd, J = 7.3, 1.2 Hz, 1H), 8.54 (d, J = 8.1 Hz, 1H), 8.44 (dd, J = 8.5, 1.2 Hz, 1H), 7.72 (dd, J = 8.5, 7.3 Hz, 1H), 7.23 (d, J = 8.1 Hz, 1H), 4.59 (t, J = 7.2 Hz, 2H), 4.02 (t, J = 4.5 Hz, 4H), 3.66 (t, J = 7.2 Hz, 2H), 3.27 (t, J = 4.5 Hz, 4H). 13C NMR (400 MHz, CDCl3) δ 164.21, 163.69, 155.92, 132.88, 131.50, 130.42, 129.99, 126.16, 125.89, 122.96, 116.70, 115.01, 66.93, 53.43, 41.08, 27.91.

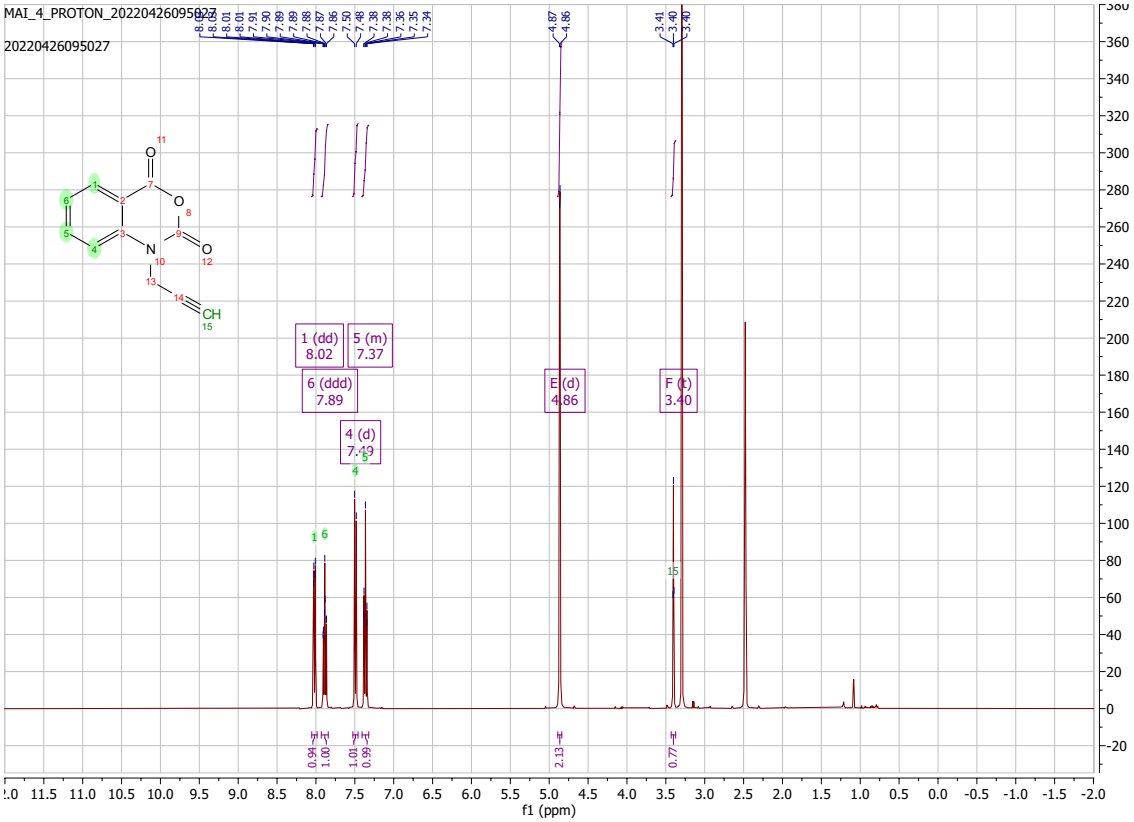
1-(2-(6-morpholino-1,3-dioxo-1H-benzo[de]isoquinolin-2(3H)-yl)ethyl)-2H-benzo[d][1,3]oxazine-2,4(1H)-dione (16)

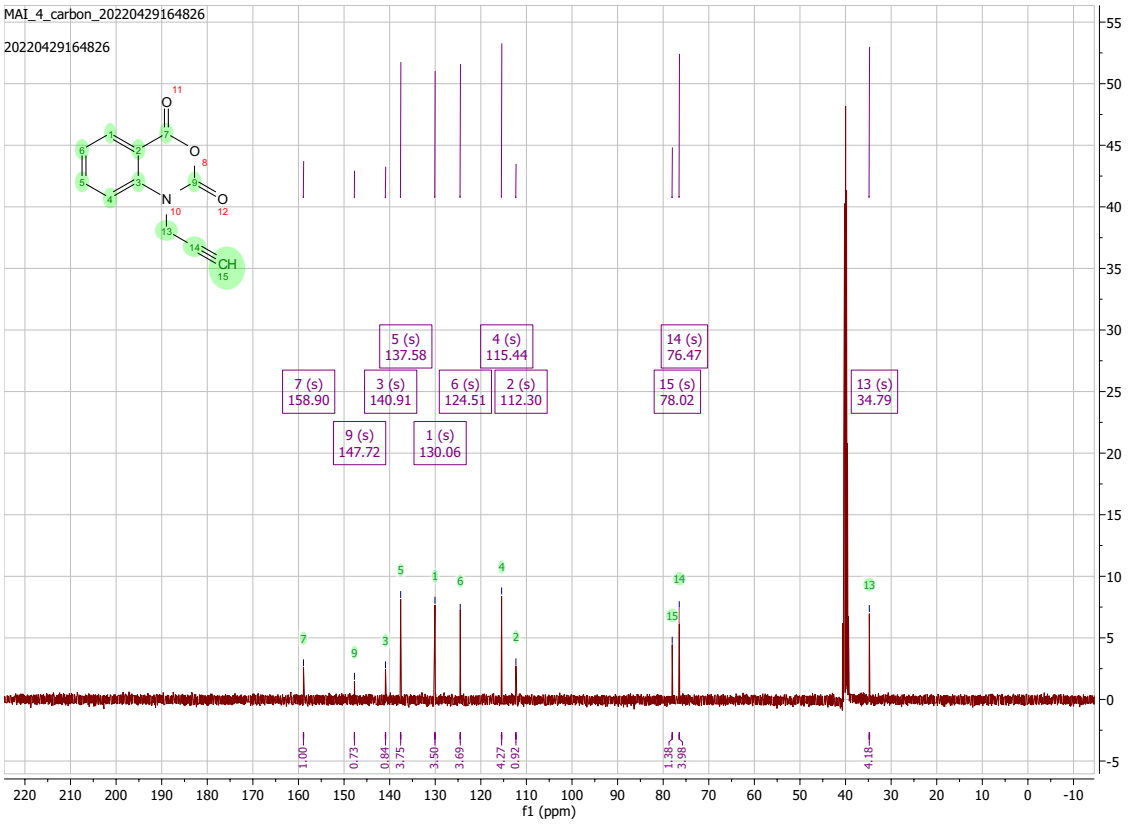


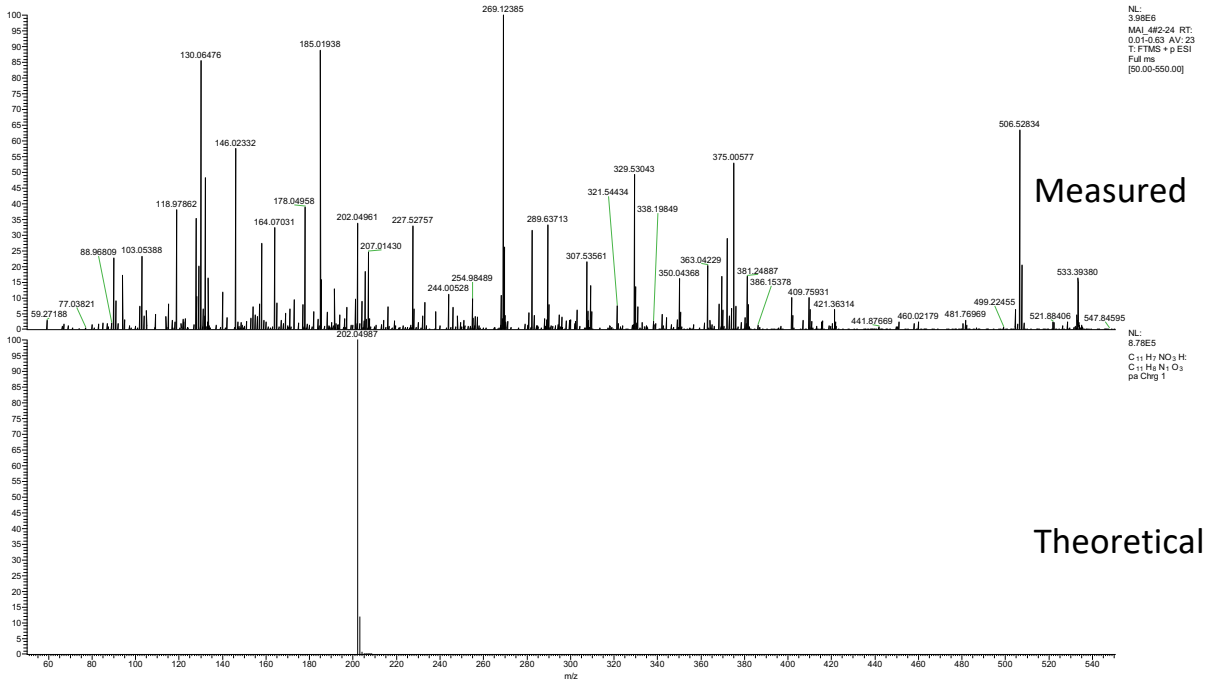
In a microwave vial, sodium hydride (24 mg, 0.9 mmol, 1.5 eq) was added to a solution of isatoic anhydride (98 mg, 0.6 mmol, 1.0 eq) in anhydrous DMF (4 mL) and stirred for 1 h at room temperature under nitrogen atmosphere. 2-(2-bromoethyl)-6-morpholino-1H-benzo[de]isoquinoline-1,3(2H)-dione (0.16 g, 0.4 mmol, 1.0 eq) was added to the reaction mixture and stirred overnight at room temperature. The reaction mixture was quenched in ice water (20 mL). The precipitate was filtered and washed with cold water. Ethyl acetate was added and new precipitate was formed. This was filtered, washed with cold pentane. The residue was purified via silica column chromatography (EtOAc/Pen) to yield a yellow powder (6.4 mg, 3%)

Data Obtained

1-(prop-2-yn-1-yl)-2H-benzo[d][1,3]oxazine-2,4(1H)-dione (6)



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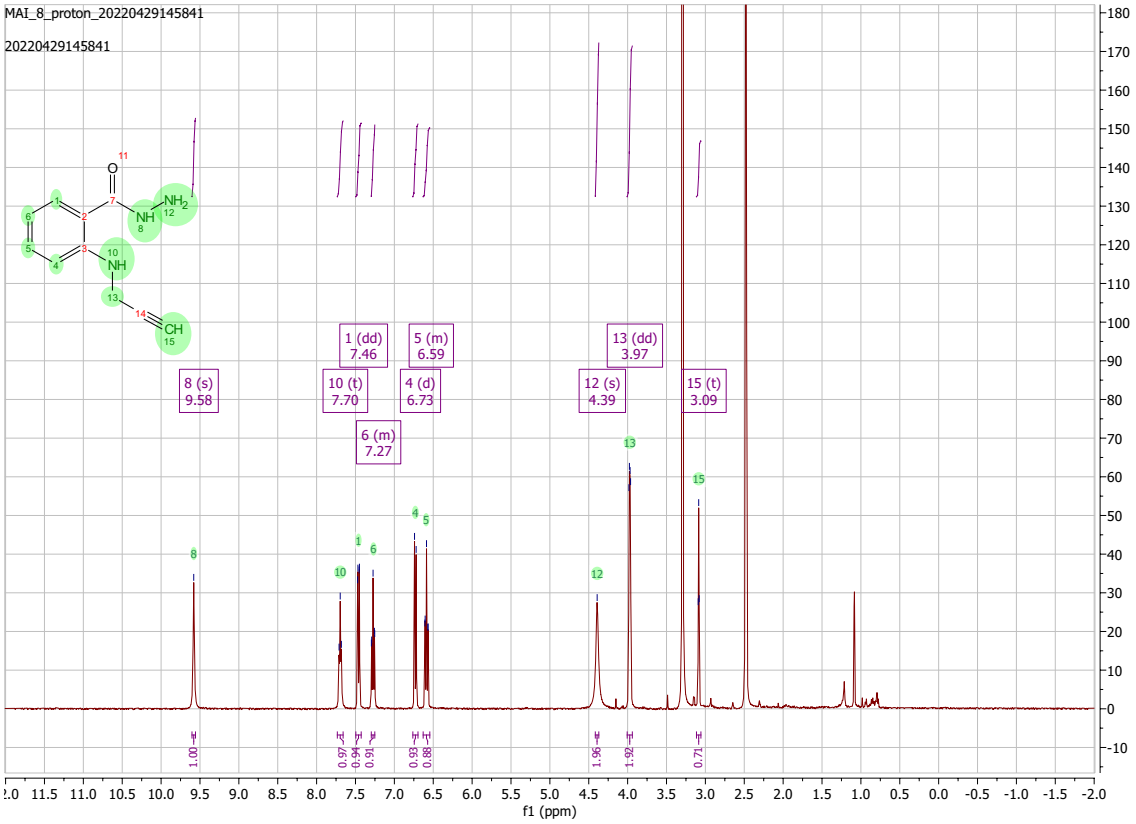
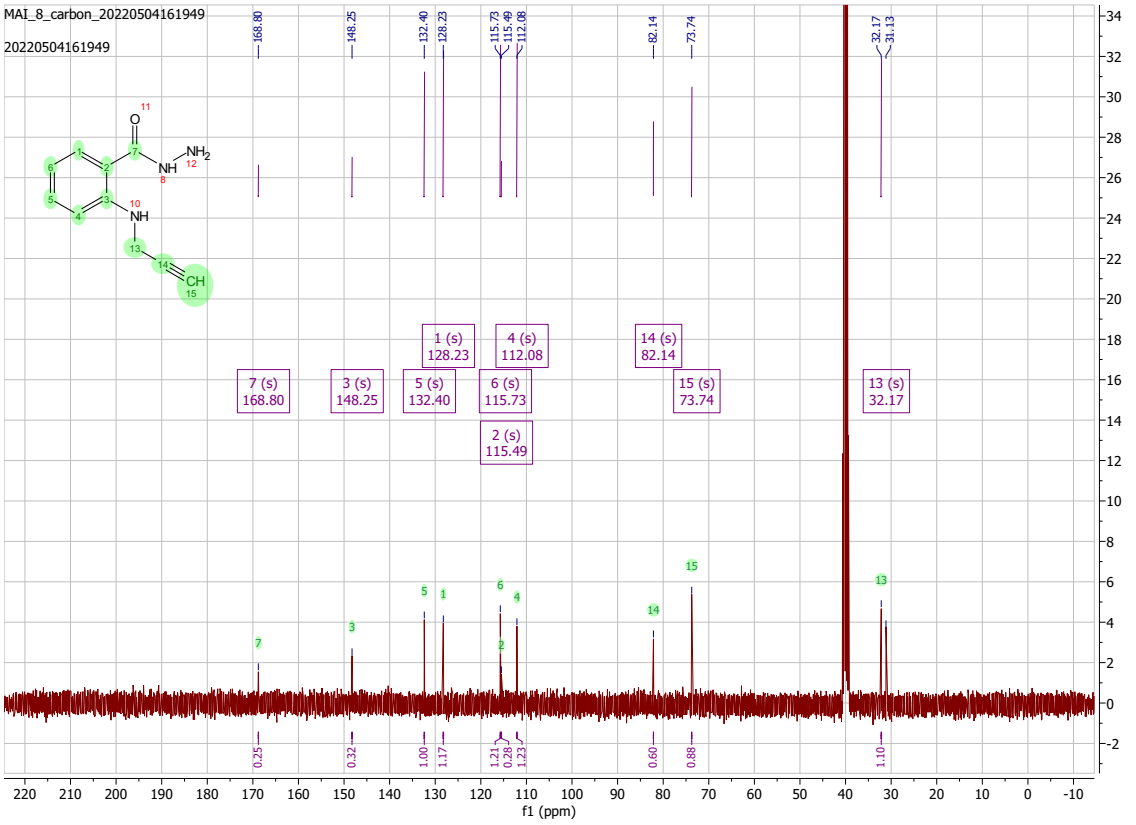
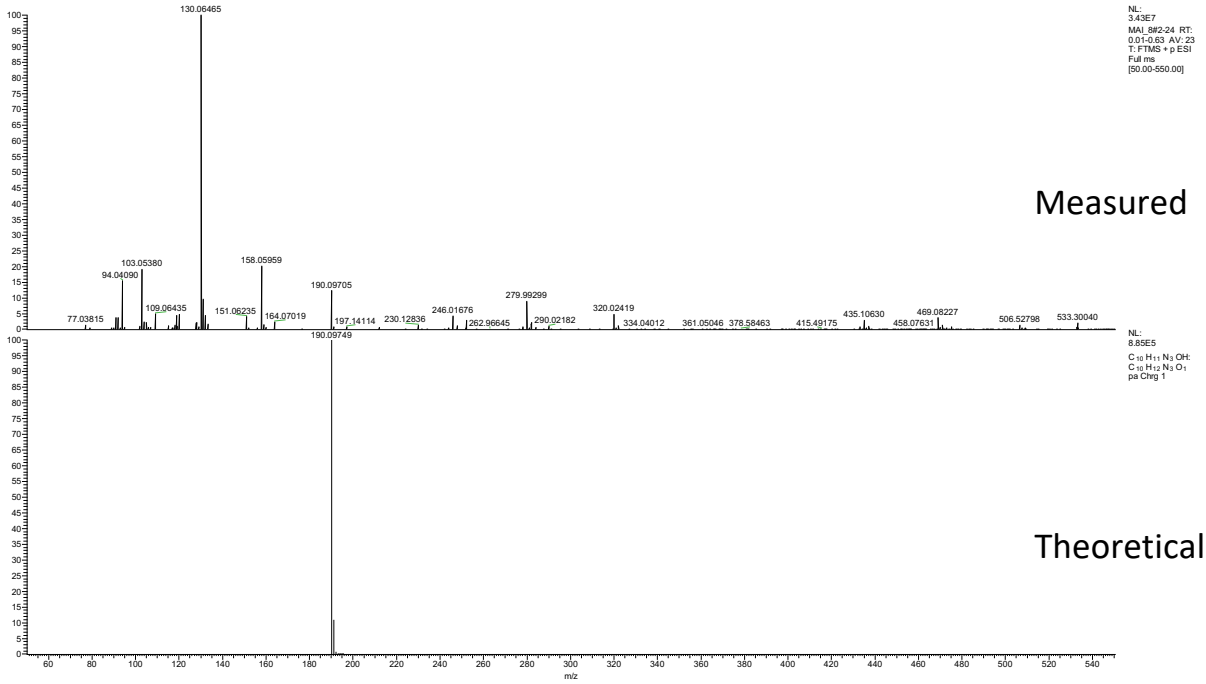
m/z

2-(prop-2-yn-1-ylamino)benzohydrazide (1)



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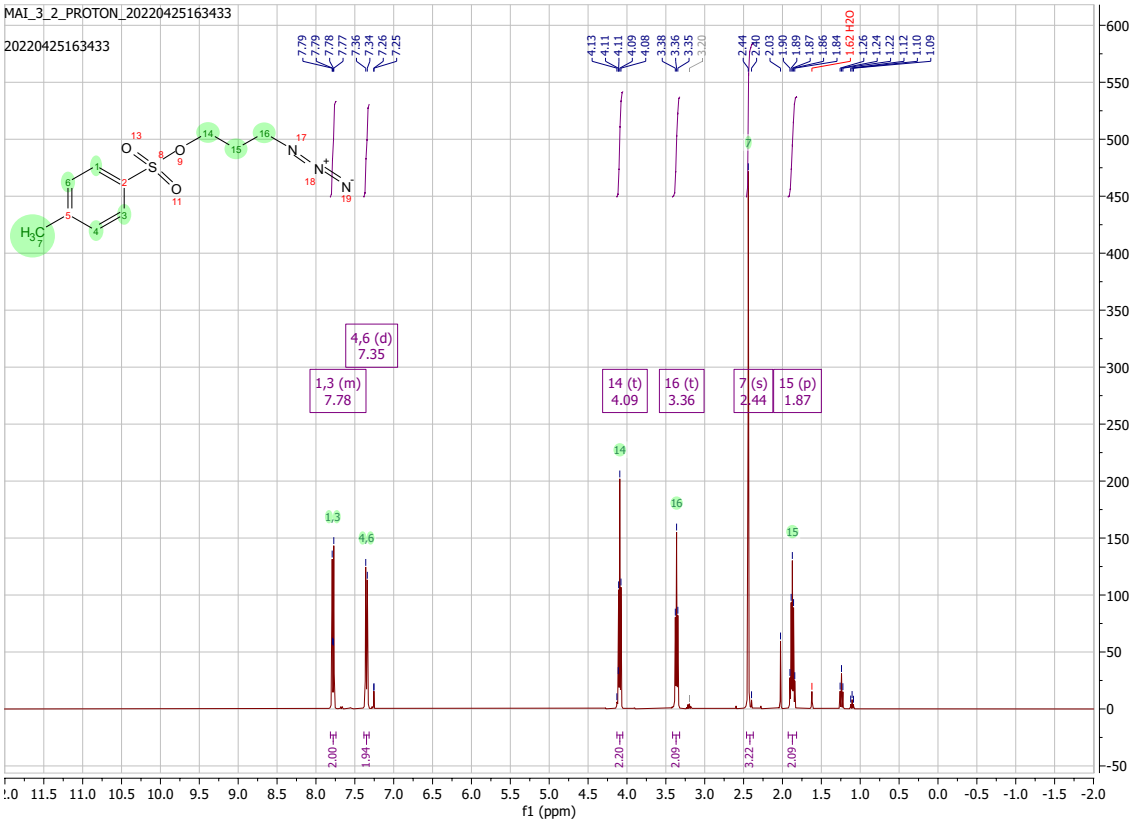
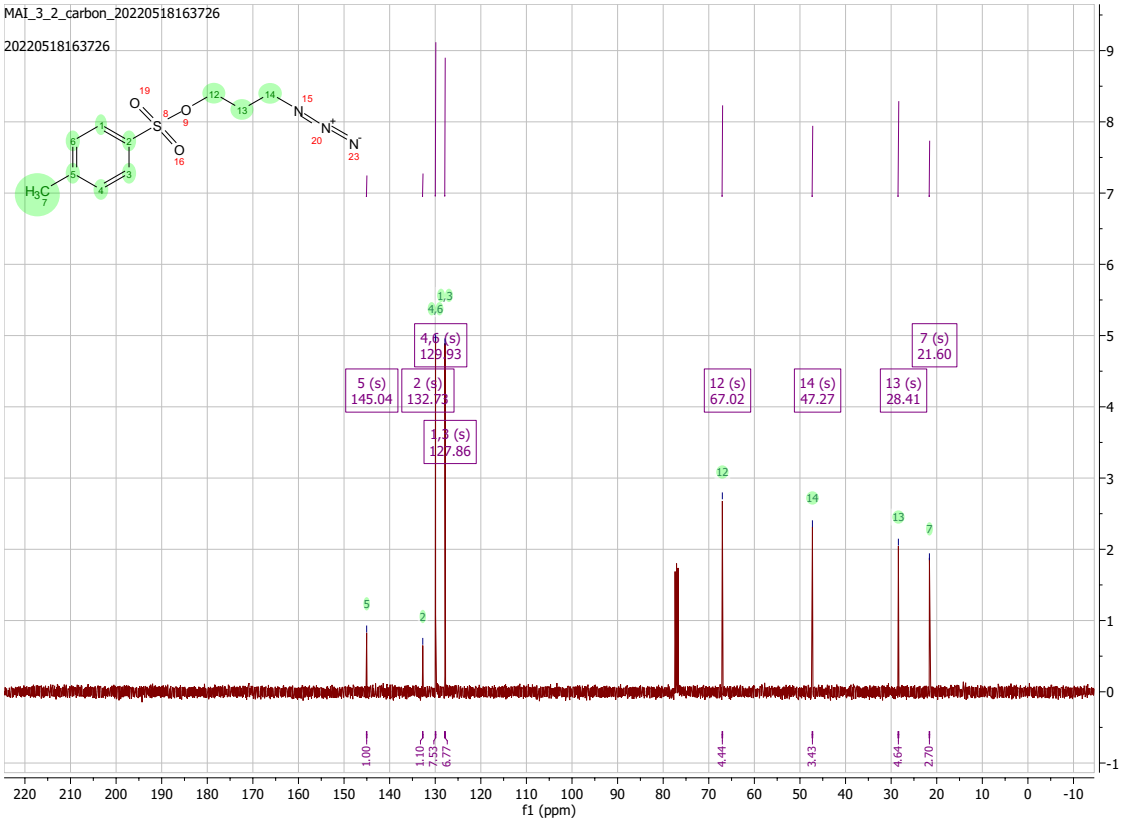
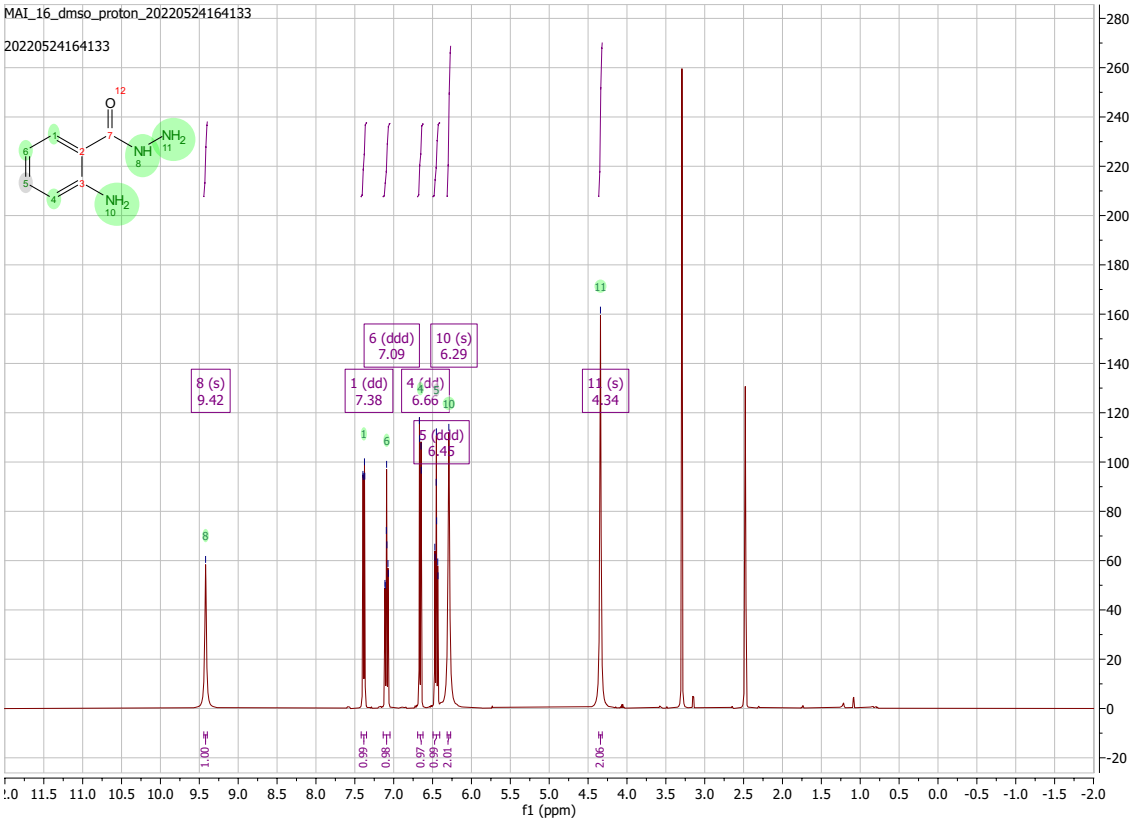
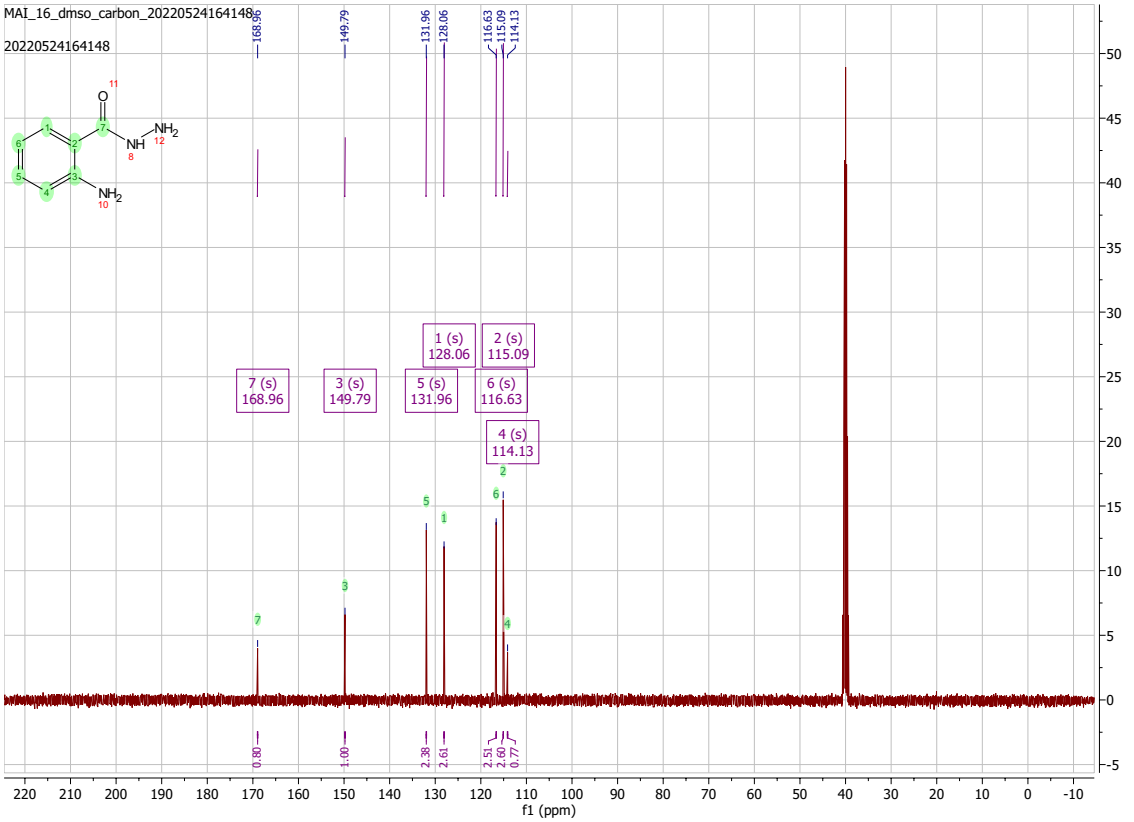
3-azidopropyl 4-methylbenzenesulfonate (9)



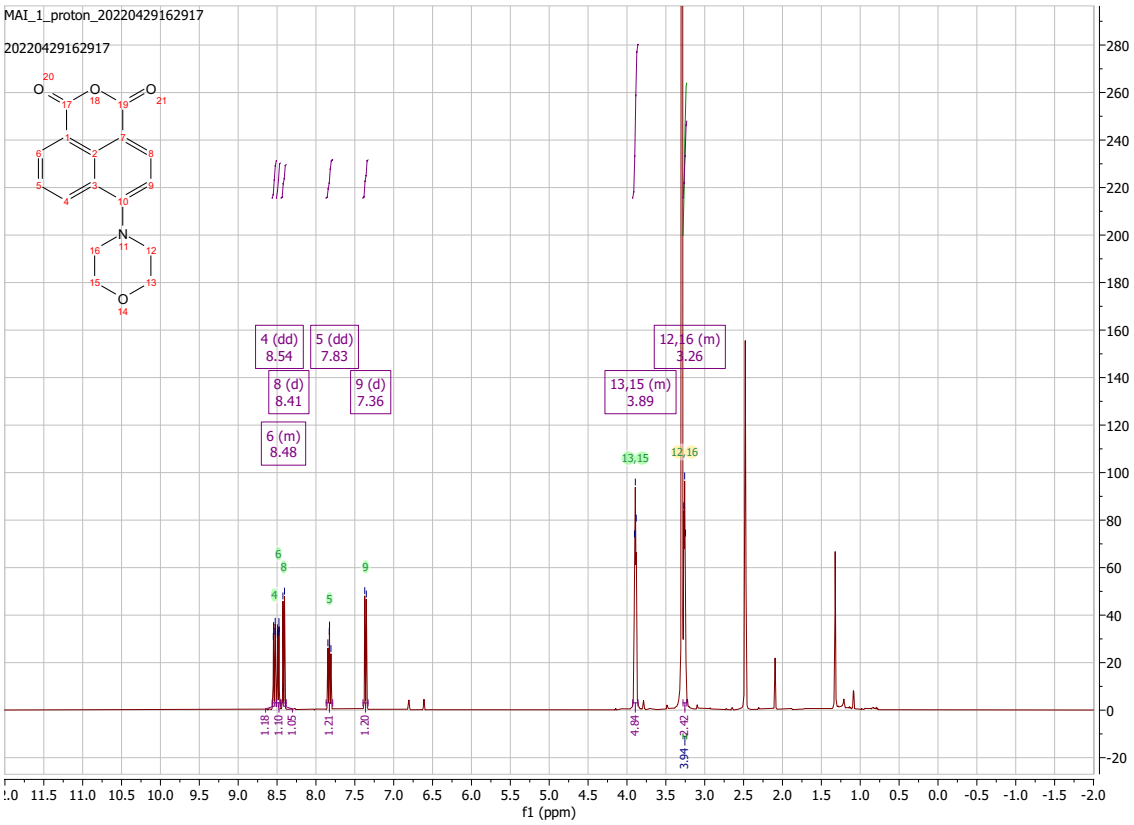


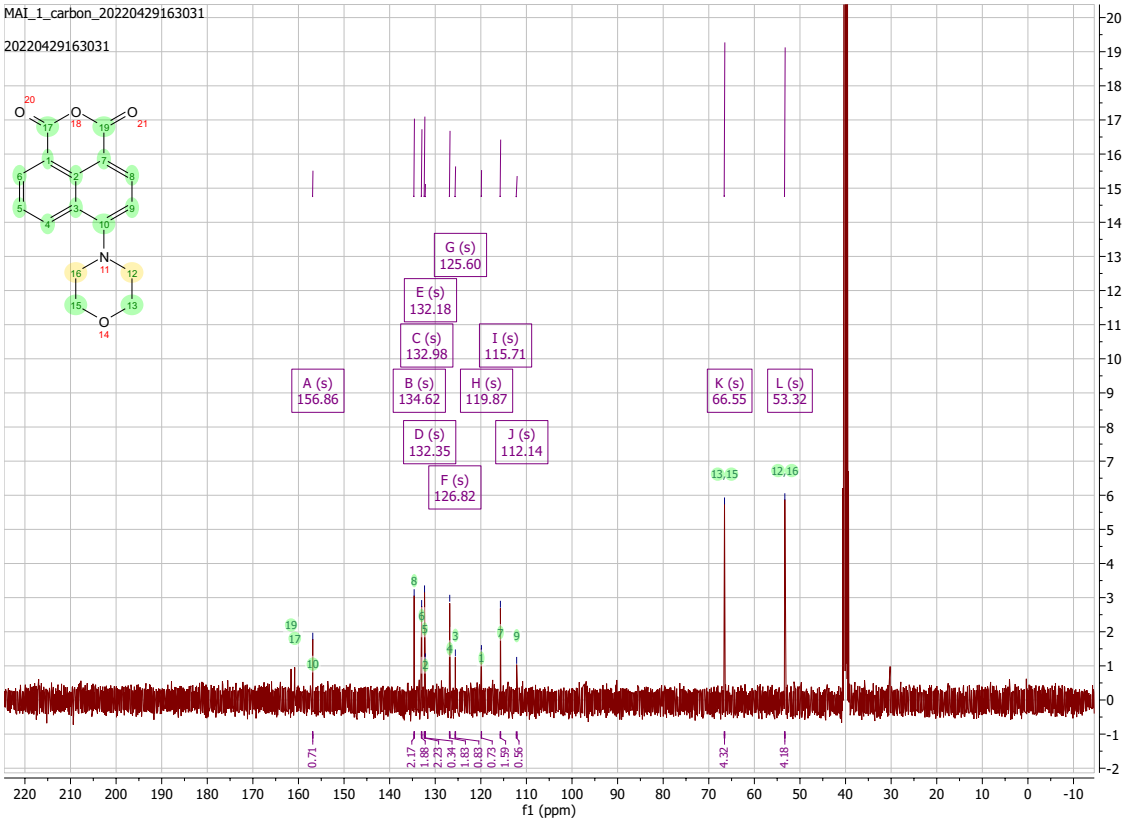
2-aminobenzohydrazide (3)



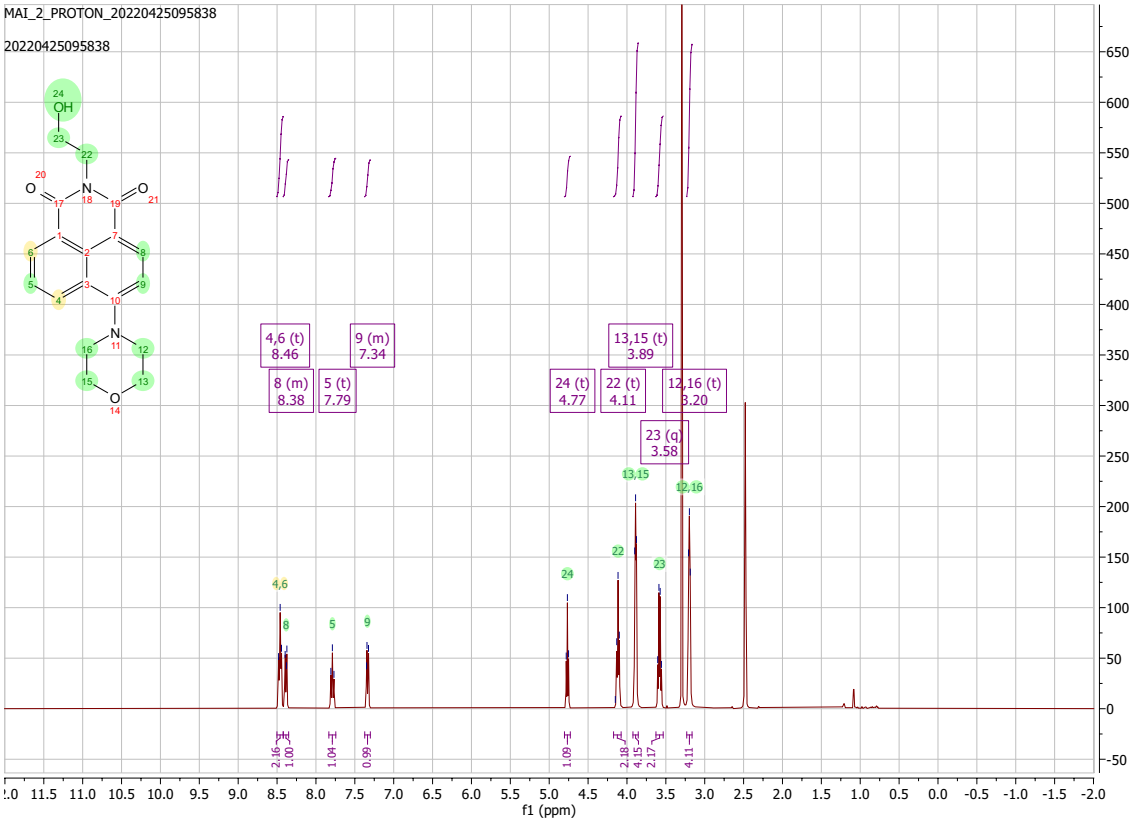


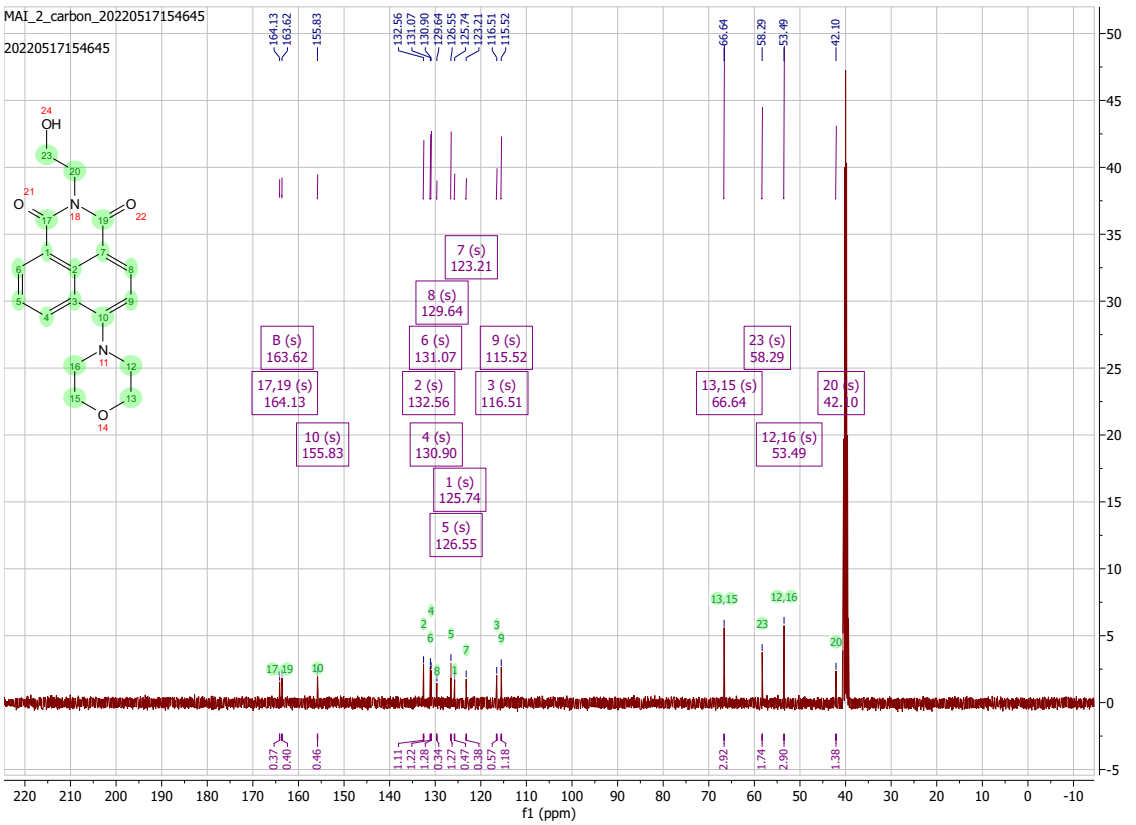
6-morpholino-1H,3H-benzo[de]isochromene-1,3-dione (12)





2-(2-hydroxyethyl)-6-morpholino-1H-benzo[de]isoquinoline-1,3(2H)-dione (13)





2-(2-bromoethyl)-6-morpholino-1H-benzo[de]isoquinoline-1,3(2H)-dione (14)