Synthesis of an organocatalysis activated fluorophore compound and the evaluation of its organocatalytic action.

BSc thesis

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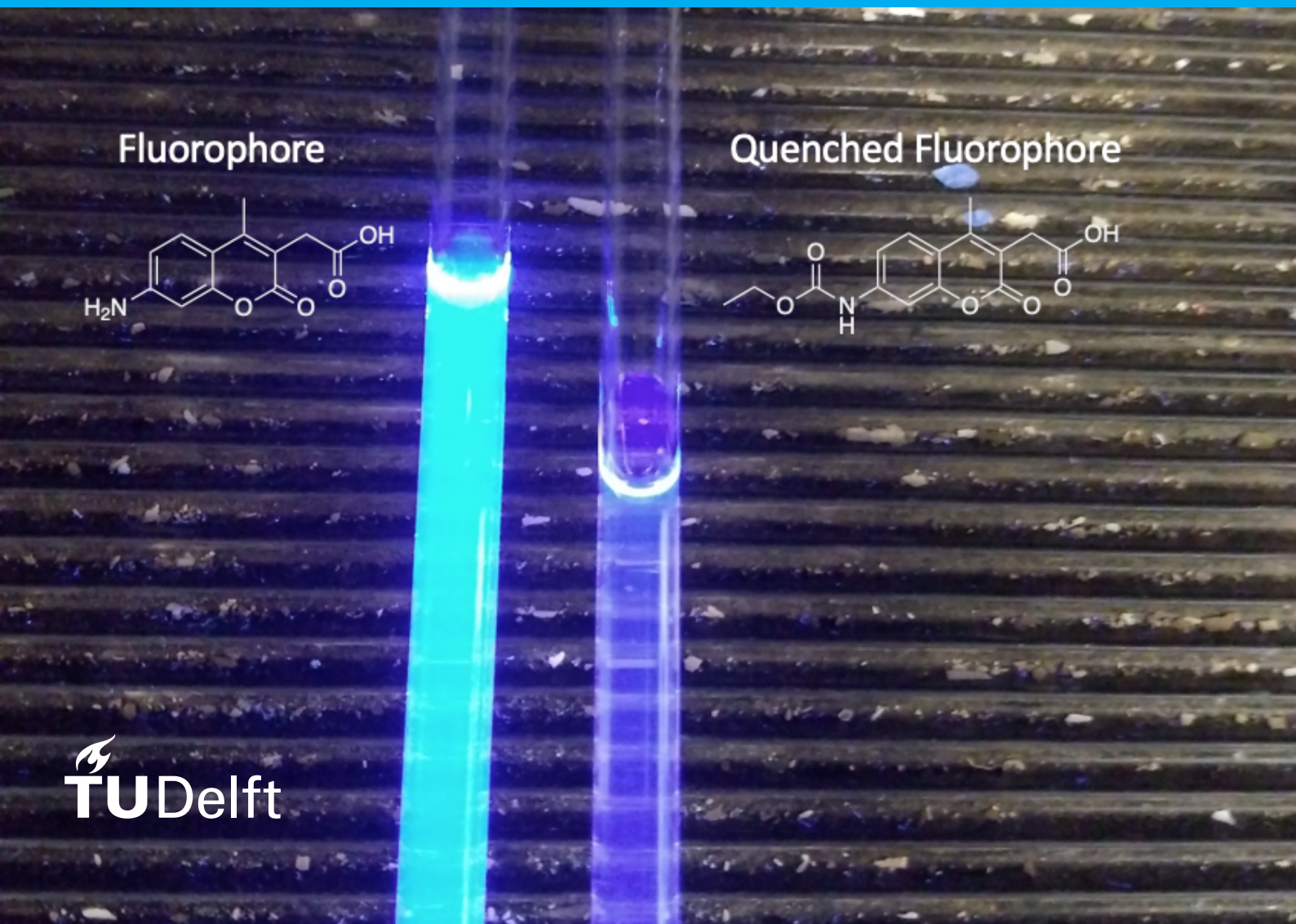
At the Advanced Soft Matter group



TU Delft

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by

Emma D. Hazekamp

to obtain the degree of Bachelor of Science
at the Delft University of Technology,
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Emma D. Hazekamp
Delft, June 2019

Abstract

Signal transduction is a rudimentary and specific way of communication in cells, in which a signal triggers a specific response. However, mimicking these processes in organic materials has been a major challenge. Although, some organocatalysts have been shown to be responsive to specific signals, only limited research has been done using organocatalysis in biological systems. A potentially bio-compatible organocatalytic reaction is the DABCO catalysed nucleophilic substitution reaction of a vinylphosphonate with N- and S- terminal nucleophiles. To evaluate the bio-compatibility of the catalytic reaction, this study focused on the preparation of a catalytic active profluorophore. Hereby, the profluorophore becomes active (fluorescent) upon catalysis in the presence of cells. To do so, 7-amino-4-methyl-3-coumarinylacetic acid and 7-amino-1-methylquinolin-1-ium will be synthetically quenched by acetyl amide formation and are thereafter linked to the catalytic active component (3-hydroxyprop-1-en-2-ylphosphonate). Signals, such as amides and thiols can be used to trigger the reaction and hence releasing the quenched fluorophore.

7-amino-4-methyl-3-coumarinylacetic acid was successfully quenched using ethyl chloroformate, however transesterification of the catalytic active compound could not be achieved at various conditions. 7-amino-1-methylquinolin-1-ium was synthesised according to literature and compared to a reference, confirming the compound. Many attempts have been undertaken to further modify the fluorophore with the catalytic compound. Unfortunately, non of the proposed synthetic pathways, such as quenching with ethyl chloroformate, resulted in the envisioned product. Based on the obtained results, it is recommended to explore other synthetic strategies such as the usage of disuccinimidyl carbonate to quench the fluorophores.

Contents

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Introduction

Signal transduction one of the primary processes (i.e. replication, growth and metabolism and protein synthesis) used by living cells, when responding to changes in their environment.[1–3] Change in pH, temperature and concentration are examples of external cues that could start a cascade of signals inside living cells.[1, 2] Figure 1.1 is a simplified illustration of a signal pathway in a cell, showing how a ligand (i.e. signal) forms a receptor-ligand complex. Where-after, a cascade of signals will trigger a specific response to the original signal (signal-transduction). For example apoptosis would be the cells response on finding defects in its DNA.[3] Additionally, another example is the storage of glucose as glucogen, which can be rapidly converted in glucose in times of famine.[3] Organic materials do not have such a rudimentary and specific way of communicating.[2] Though, some organocatalysts, which react to specific signals (e.g. light and chemical signals), have been designed to resemble signal transduction in cells.[4]

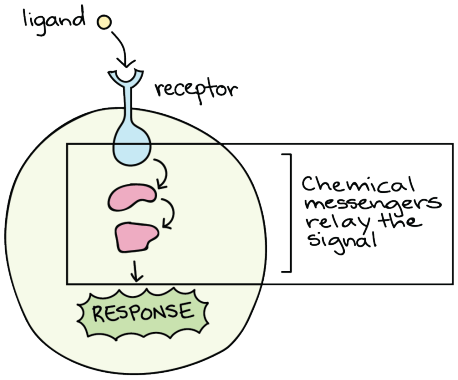
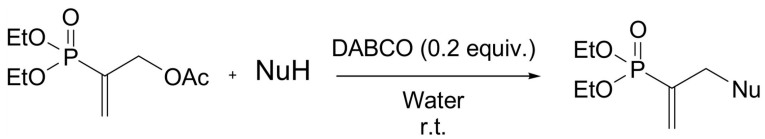


Figure 1.1: Schemetic of signal transduction in a cell, which illustrates how a external signal can trigger a response inside the cell.[3]

Even though organocatalysts use mild conditions and are less toxic than organometallic catalysts they can be harmful for cells.[5] The catalyst can interfere with the internal and tissue processes of living systems.[6] In addition to the toxicity of the catalysts, it is important that the catalysis proceeds in water under biological conditions (e.g. physiological pH, high salt content and the presence of amino acids) and that the products produced are non-toxic and do not interfere with the cell's processes.[6]

Scheme 1.1: DABCO catalysed nucleophilic substiution reaction of diethyl(α-acetoxymethyl) vinylphosphonate (DVP) with N- and S terminal nucleophiles.[7]



A potentially biocompatible organocatalytic reaction is the DABCO catalysed nucleophilic substiution re-

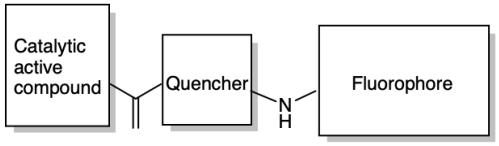
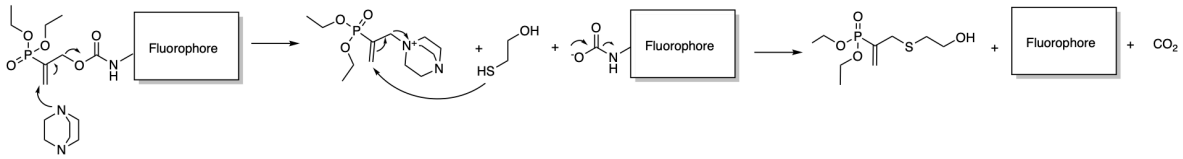


Figure 1.2: Schemetic of the profluorophore, the fluorophore is quenched and linked to a catalytic active compound which will release the parent fluorophore after addition of a catalyst/signal.

action of diethyl(α-acetoxymethyl) vinylphosphonate (DVP) with N- and S- terminal nucleophiles (scheme 1.1).[7] This reaction, first shown by Maffei *et al* [7], will be evaluated in an biological environment (in the presence of cells). To do so, an profluorophore will be developed by modification of another vinylphosphonate, 3-hydroxyprop-1-en-2-ylphosphonate, with an fluorophore. Figure 1.2 illustrates the envisioned profluorophore. The fluorophore is quenched and linked to the catalytic active compound. The catalytic active compound will release the fluorophore upon addition of the catalyst, which will attack the double bond of the catalytic active compound (scheme 1.2).

Scheme 1.2: Mechanism of the organocatalytic substitution reaction, which releases the fluorophore after the introduction of a catalysist (signal).



A specific drug delivery system based on enzymatic activity has been designed by Tanihara *et all.*[8] An antibacterial drug is released when the linker molecule is cleaved by the enzyme, thrombin.[8] As thrombin is only active in microbial-infected wounds, it functions as a very specific signal.[8] The antiseptic will only release in the area of the wound, making sure the drug is released at the right time and place.[8] Additionally, Versteegen *et all.* combined tumor-antibody's and a tumour treating drug with a linker molecule.[9] The linker can be cleaved by a probe (the signal), creating another specific way of delivering drugs.[9] This shows that signal responsive materials are very useful in the medical world and that more possible applications can be explored.

The quenching of a fluorophore can be done in two ways: static and dynamic quenching. The former forms a complex when the fluorophore is in the ground state. The latter is formed during the lifetime of the excited state of the fluorophore.[10, 11] A few examples of quenching a profluorophore are: C=N isomerisation, alkynation, addition of heavy atoms (e.g. Cl, Br and I) and acetyl amide formation.[12–15] Forming an acetyl amide complex with a coumarin will restrict the electron-rich and charge-transport properties on the coumarinic ring, resulting in reduction of the fluorescence of the coumarin.[16, 17] Both 7-amino-4-methyl-3-coumarinylacetic acid (AMCA) and 7-amino-1-methylquinolin-1-ium (AMQ), will function as parent fluorophores and will be quenched and connected to the catalytic active compound.

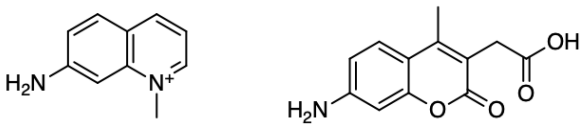
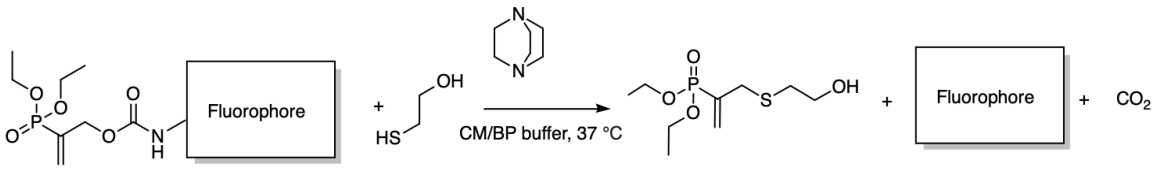


Figure 1.3: AMQ (left) and AMCA (right) will function as parent fluorophores and will be quenched and connected to the catalytic active compound.

In summary, the aim of the thesis is to synthesise and characterize a profluorophore that will release the fluorophore on catalyst-mediated nucleophillic substitution. In scheme 1.3 the mechanism of the catalytic fluorophore release can be seen. This reaction will be monitored in both the presence and absence of cells by observing the fluorescent development.

Scheme 1.3: Organocatalytic substitution reaction of profluorophore with SH- nucleophile and DABCO as catalyst/signal, leading to the release of fluorophore and carbondioxide



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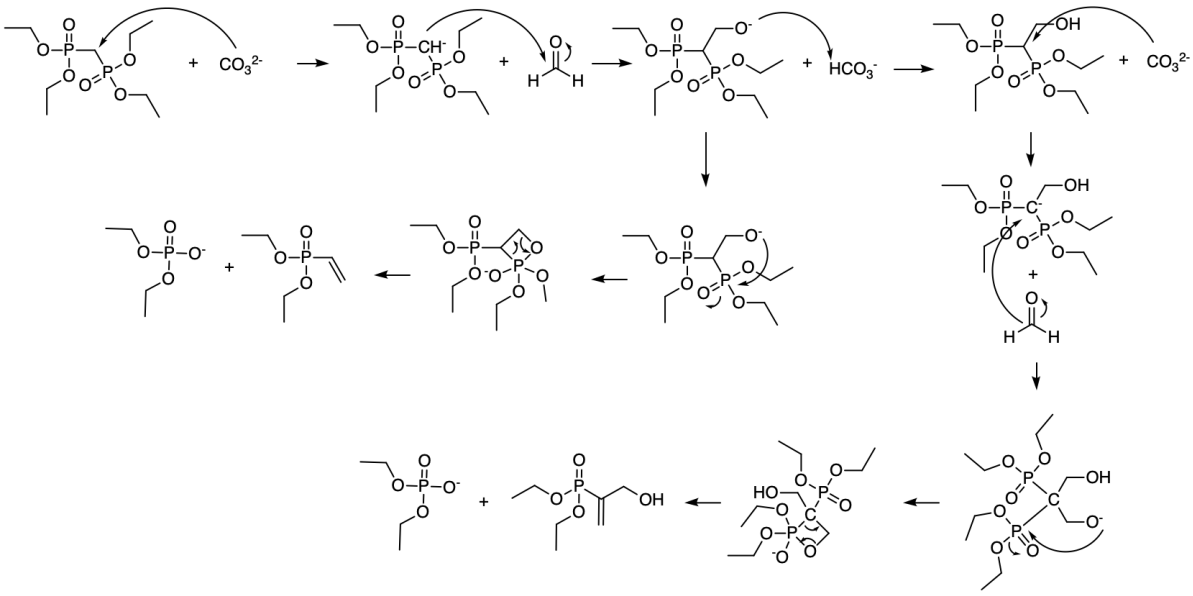
Materials and methods

2.1. Synthesizing 3-hydroxyprop-1-en-2-ylphosphonate (HYP).

The procedure followed to synthesize HYP was taken from the work of Rambaud *et all.*[18] Tetraethylmethyldienediphosphonate (30 mmol; 8.64 g) and thirty percent aquaeous formaldehyde (200 mmol) were added to a 50 ml roundbottom flask, in nitrogen atmosphere. After slow addition of potassium carbonate (60 mmol; 8,29 g) in 10 ml water, the reaction mixture was stirred under reflux at 100 °C for an hour. The mixture was extracted with chloroform, washed with brine and dried over magnesiumsulfate. After destillation 3-hydroxyprop-1-en-2-ylphosphonate was obtained. The mechanism of this reaction is shown in scheme 2.1.

Scheme 2.1: Reaction mechanism of HYP, catalysed by potassium carbonate.



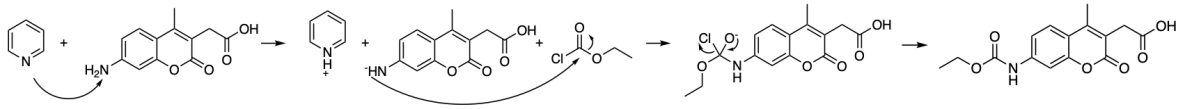
2.2. Synthesis of the AMCA linker molecule.

The fluorophore 7-amino-4-methyl-3-coumarinylacetic acid (AMCA) was quenched with both methyl- and ethyl chloroformate. The procedures followed were adapted from Zadlo *et all.*, Zeng *et all.* and Smith *et all.* [13, 19, 20]

AMCA (303.3 mg; 1.30 mmol), pyridine (123.4 mg; 1.56 mmol) and dimethylformamide (20 ml) were added to a 50 ml two-neck-roundbottomflask and cooled to 0 °C. Ethyl chloroformate (169.4 mg; 1.56 mmol) and dimethylformamide (5 ml) were slowly added and the mixture was stirred for 1.5 hours at room temperature. The product in the mixture was protonated by addition of acetic acid (pH 3) and subsequently extracted with dichloromethane and water. After evaporation the product was recrystallized in both methanol

and ethanol. The reaction was followed with thin layer chromatography (DCM:MEOH, 9:1) and after evaporation of the solvent an H-NMR of the crude product was analysed. In addition to ethyl chloroformate, dichlorometane and pyridine, this reaction has been executed with methyl chloroformate as reactant, dimethylformamide and dimethylsulfoxide as solvent and with triethylamine as catalyst. The mechanism of this reaction is shown in scheme 2.2.

Scheme 2.2: Reaction mechanism of the AMCA linker molecule, using pyridine as catalyst to quench AMCA with ethyl chloroformate.

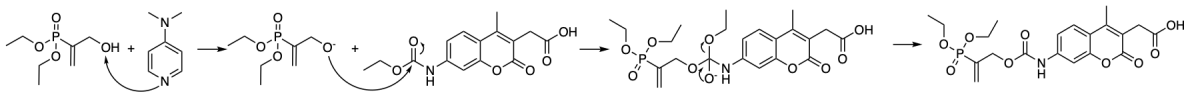


2.3. Transesterfication of the AMCA linker molecule with HYP.

Transesterfication of the linker molecule with HYP to synthesize the pro-fluorophore has been performed in two ways. Firstly, the transestrfication was catalyzed with 4-dimethylaminopyridine. Secondly, bromotrimethylsilane, was used to attach a better leaving group to the ester, which thereafter can be replaced by HYP.

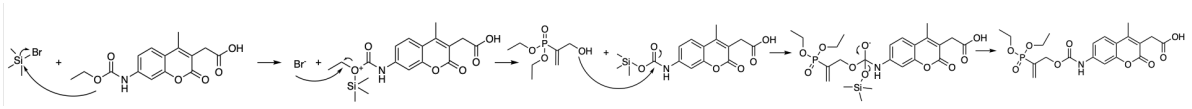
The procedure followed was adapted from the work of Schie *et all.*[21] Linker (50 mg; 0.173 mmol), 4-dimethylaminopyridine (7,2 mg; 0.059 mmol) and 5 ml dioxane were added to a 5 ml roundbottomflask. After addition of HYP (31.4 mg; 0.207 mmol) in 5 ml dioxane everything was stirred under reflux for two days at 120 °C and followed with thin layer chromatography (DCM:MeOH, 9:1). After evaporation of the solvent the crude product was analyzed with H-NMR. The mechanism of this reaction is shown in scheme 2.3.

Scheme 2.3: Reaction mechanism of the transesterfication of the AMCA linker molecule with HYP, using DMAP as catalyst.



The procedure followed was adapted from the work of Jung *et all.* and Thiem *et all.*[22, 23] Linker (50 mg; 0.173 mmol) and 5 ml of ethanol were added to a 25 ml roundbottomflask. After addition of bromotrimethylsilane (39.7 mg; 0.259 mmol) in 5 ml ethanol, the solution was stirred for 20 hours at 50 °C and followed by thin layer chromatography (PE:EtOAc, 5:1). The solution was quenched with sodium bicarbonate and the product was extracted with chloroform. After evaporation of the solvent the crude product was analyzed with H-NMR. The mechanism of this reaction is shown in scheme 2.4.

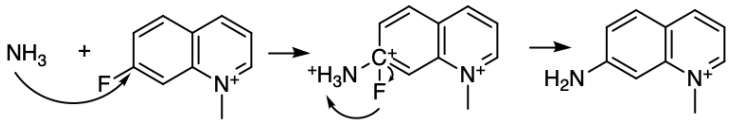
Scheme 2.4: Reaction mechanism of the esterfication of the AMCA linker molecule with HYP, using bromotrimethylsilane as a better leaving group.



2.4. Synthesis of 7-amino-1-methylquinolin-1-ium (AMQ).

The procedure followed was taken from Jager *et all.*[24] 7-fluoro-1-methylquinolinium iodide (6.92 mmol; 2 g) and 89 ml ethanol were added to a 250 ml roundbottomflask. After the addition of 78.8 ml of 30% ammonia solution (20 equivalents), made from 59.2 ml of 2.33 Molar ammonium solution and 19.6 ml of water, the solution was heated to 60 °C for 1 hour. After evaporation of the solvent the product was recrystallized multiple times in absolute ethanol and analysed by H-NMR. The mechanism of this reaction is shown in scheme 2.5.

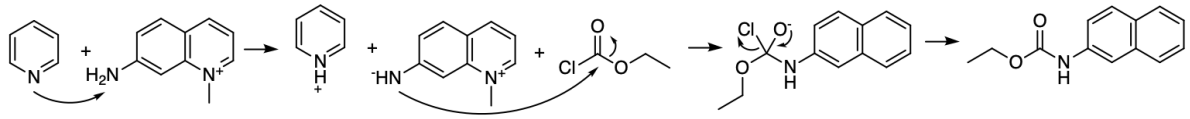
Scheme 2.5: Reaction meachnism of the fluorophore 7-amino-1-methylquinolin-1-ium (AMQ).



2.5. Synthesis of AMQ linker molecule.

The quenching of the fluorophore 7-amino-1-methylquinolin-1-ium (AMQ) with ethyl chloroformate was a similar procedure as the quencing of AMCA and was based on the work of Zadlo *et all.*, Zeng *et all.* and Smith *et all.* [13, 19, 20]. AMQ (50 mg; 0.314 mmol), pyridine (29.8 mg; 0.337 mmol) and acetonitril (5 ml) were added to a 50 ml roundbottomflask and cooled to 0 °C. Ethyl chloroformate (40.9 mg; 0.337 mmol) and acetonitril (5 ml) were slowly added and the mixture was stirred for 3.5 hours at room temperature and followed with thin layer chromatography (DCM:MEOH, 9:1). After evaporation of the solvent the crude product was analysed with H-NMR. In addition to pyridine and acetonitril, this reaction has been executed with dimethylformamide and tetrahydrofuran/water (1:1) as solvents and with triethylamine and potassium carbonate as catalyst. The mechanism of this reaction is shown in scheme 2.6.

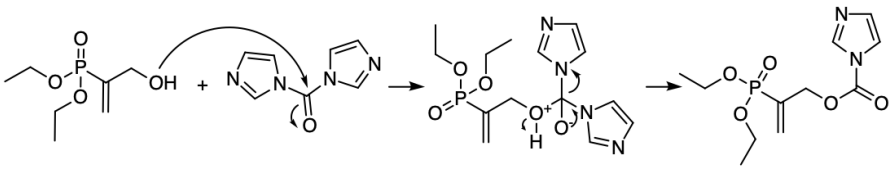
Scheme 2.6: Reaction mechanism of the AMQ linker molecule, using pyridine as catalyst.



2.6. One-pot synthesis of the AMQ profluorophore

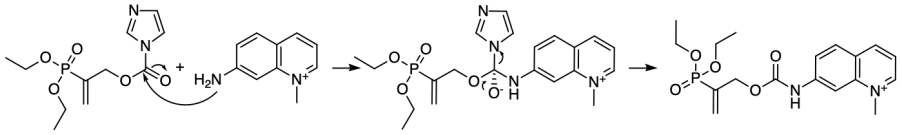
Another procedure, based on the work of Deng *et all.* and Matzen *et all.* [25, 26], to synthesise the profluorophore is a one-pot synthesis that consists of two steps. In the first step, 3-hydroxyprop-1-en-2-ylphosphonate (60.98 mg; 0.314 mmol) and acetonitril (2.5 ml) are added in a 50 ml roundbottomflask. Secondly, 1,1-carbonyldiimidazole (61.11 mg; 0.377 mmol) in acetonitril (2.5 ml) was added and the mixture was stirred for 2 hours, while being followed by TLC (DCM:MeOH, 9:1). The mechanism of this reaction is shown in scheme 2.7.

Scheme 2.7: The reaction mechanism of the first step in the one-pot synthesis of the AMQ profluorophore.



After 2 hours a mixture of triethylamine (31.75 mg; 0.314 mmol), AMQ (50 mg; 0.314 mmol) and acetonitril (5 ml) was added and stirred under room temperature for 18 hours. The crude product was extracted with chloroform and water and both layers were evaporated and their products checked with H-NMR. The mechanism of this reaction is shown in scheme 2.8.

Scheme 2.8: The reaction mechanism of the second step in the synthesis pathway of the AMQ profluorophore.



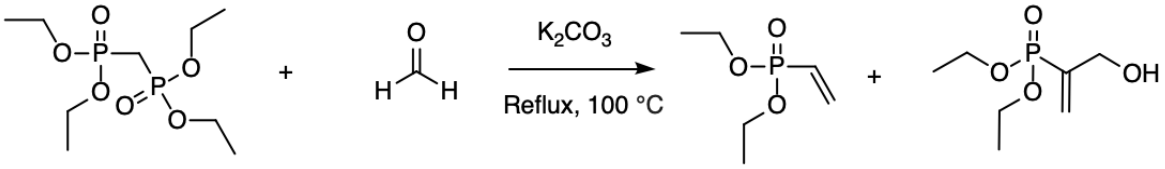
3

Results and discussion

3.1. Analysis of 3-hydroxyprop-1-en-2-ylphosphonate (HYP).

In order to develop the profluorophore, 3-hydroxyprop- 1-en-2-ylphosphonate (HYP) had to be synthesised, according to scheme 3.1.

Scheme 3.1: Synthesis pathway of HYP, resulting in two products (HYP on the right), which were separated with distillation.



After work-up, proton nuclear magnetic resonance (NMR) spectroscopy has been used to evaluate the reaction. The H-NMR spectrum has been appended in appendix A.1, and the assignment of the peaks have been classified in table 3.1. Comparing the peaks of the two protons attached to the double-bond (10) to the work of Rambaud *et all.*[18], from which the procedure was taken, it was concluded that the product was indeed 3-hydroxyprop-1-en-2-ylphosphonate. A yield of 20 % was obtained after distillation. The low yield may be due to the vacuum leak during the distillation, making it harder to separate HYP from the side product. In a second attempt of making HYP the pressure remained low (0.4 mmHg) and a yield of 40 % was obtained.

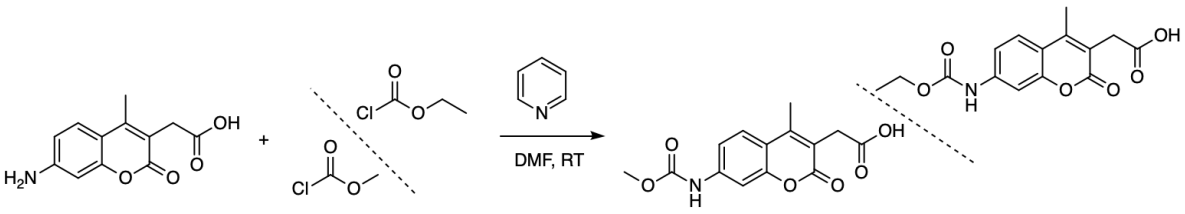
Table 3.1: H-NMR peaks of HYP assigned by multiplicity, relative intensity and attribution.

δ (ppm)	Multiplicity	Relative intensity	Attribution
1.33	triplet	6H	7+9
4.10	quintet	4H	6+8
4.30	quartet	2H	11
6.00	quartet	2H	10

3.2. Analysis of linker molecule.

The second step in the development of the profluorophore is the quenching of 7-amino-4-methyl-3-coumarinylacetic acid (AMCA) with methyl chloroformate (scheme 3.2). This reaction has been tried multiple times and the different reaction conditions have been tabulated in table 3.2. In the first synthesis the product was too impure to confirm whether the linker had been formed. The scale-up (2) had problems dissolving and resulted in no product, after 7 hours of stirring. As the solubility of AMCA in DCM was poor, both DMF and DMSO were used as solvent. Unfortunately, those two reactions showed no product after overnight stirring. It is likely that the hydroxilation on the newly attached linker occurred, resulting in an unstable molecule. The carbonyl group

Scheme 3.2: Synthesis pathway of the linker molecule by quenching AMCA with either methyl or ethyl chloroformate.



leaves and AMCA remains unchanged. In order to increase stability and thus avoiding hydroxilation, ethyl chloroformate was used to synthesise the linker.

Table 3.2: The different combinations of reactant, solvent and catalyst used for the synthesis of the linker molecule.

$n0$	Reactant	Solvent	Catalyst	(AMCA:catalyst:reactant)	Product
1	methyl	DCM	Et_3N	1:1.3:1.2	Minor
2	methyl	DCM	Et_3N	1:1.3:1.2	No
3	methyl	DMF	Pyridine	1:1.2:1.2	No
4	methyl	DMSO	Pyridine	1:1.2:1.2	No
5	ethyl	DMF	Pyridine	1:1.2:1.2	Yes

The crude product of the linker molecule has been analysed by H-NMR and compared with the H-NMR of AMCA. The peaks have been classified in table 3.3 and H-NMR spectra of both AMCA and the linker have been appended in Appendix A.2 and A.3. The appearance of the ethyl (20) and methyl (23) ester-group, the shift of the aromatics (1,6 and 3) and the proton on the amide validates the formation of the ethyl linker. Since, the appearance of the AMCA linker had been confirmed by H-NMR, the reaction mixture had been recrystallized to purify the linker. The crude product was first recrystallized in ethanol and the particles formed were filtered from the solution and analyzed by H-NMR. After evaporation of ethanol, the product was recrystallized in methanol and both the particles that formed after recrystallization and the solution have been analyzed in H-NMR. In appendix A.4 all three spectra can be compared. Both the recrystallized particles and particles after evaporation of the methanol solution, were pure enough to use for the next step. The two combined resulted in a yield of 41 %.

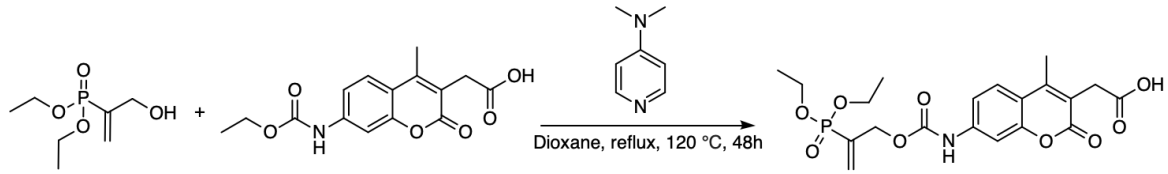
Table 3.3: H-NMR peaks of the linker molecule assigned by multiplicity, relative intensity and attribution.

δ (ppm)	Multiplicity	Relative intensity	Attribution
1.25	triplet	3H	22
2.33	singlet	3H	11
3.55	singlet	2H	13
4.16	triplet	2H	20
7.40	doublet	1H	3
7.54	singlet	1H	1
7.70	doublet	1H	6
10.10	singlet	1H	17
12.40	singlet	1H	15

3.3. Analysis of the transesterfication of the linker molecule with HYP.

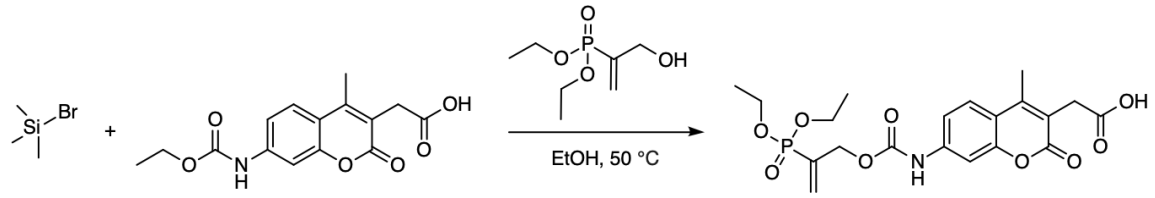
After the successful preparation and purification of the AMCA linker molecule in the previous step, the next step in the synthesis of profluorophore has been attempted. Hereby, transesterification was used to remove the ethyl group and replacing it with HYP. As, transesterification is slow, the reaction was left for 48 hours at reflux in the presence of DMAP (scheme 3.4).

Scheme 3.3: Synthesis pathway of the transesterfication of HYP and the linker molecule to synthesise the profluorophore.



After 48 hours the crude product was analysed with H-NMR (Appendix A.5), from which was concluded that both the starting compounds were still present and no reaction had occurred. Since, transesterification did not show promising results, another strategy had been envisioned. Subsequently, the linker was treated with bromotrimethylsilane (scheme 3.4), which acts as a better leaving group on the linker molecule.[27]

Scheme 3.4: Synthesis pathway of HYP and the linker molecule, using bromotrimethylsilane as a better leaving group



The analysis of the H-NMR spectrum of the crude product (Appendix A.6) is classified in table 3.4. Unfortunately, instead of reacting with the ester group of the linker molecule the bromotrimethylsilane reacted with the alcohol group on the other side of AMCA. Then, ethanol (the solvent) attacked the carbonyl, making bromotrimethylsilane leave and esterficating the alcohol group on AMCA (figure 3.1). In appendix A.7 the spectra of both the linker molecule and the crude product of the transesterfication are compared. The disappearance of the alcohol group (15) and the increased multiplicity of the ester peaks (20 and 22 t/m 24) confirms the esterfication of the alcohol group.

δ (ppm)	multiplicity	relative intensity	attribution
1.20	sextet	9H	22+24
2.33	singlet	3H	12
3.65	singlet	2H	13
4.11	octet	4H	20+23
7.23	sextet	1H	3
7.56	singlet	1H	1
7.77	doublet	1H	6
10.14	singlet	1H	17

Table 3.4: H-NMR peaks of the crude product, after addition of bromotrimethylsilane, assigned by multiplicity, relative intensity and attribution.

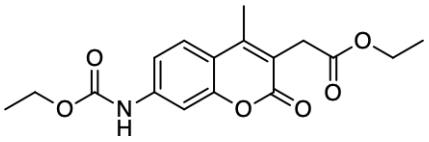


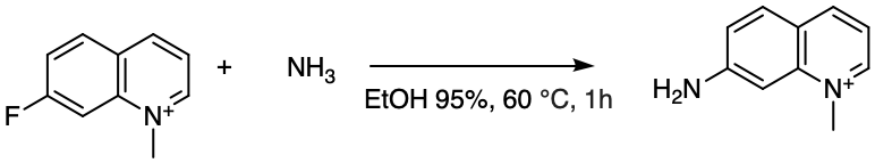
Figure 3.1: Structure of the molecule synthesized after addition of bromotrimethylsilane.

Since, bromotrimethylsilane attacks both carbonyl groups on AMCA, changing the solvent to for example DCM would not improve the reaction, as the bromotrimethylsilane could have reacted with either of the sides of AMCA. When HYP is added it could react with the bromotrimethylsilane on the alcohol side of AMCA, altering the fluorophore. Additionally, as AMCA was difficult to solubilize (only soluble in DMSO and DMF), it is likely that the profluorophore will not be soluble in water. To conclude, a different fluorophore with a better solubility and only one reactive group is needed.

3.4. Analysis of 7-amino-1-methylquinolin-1-ium (AMQ).

7-amino-1-methylquinolin-1-ium (AMQ) is a water soluble fluorophore with only one reactive group making this fluorophore a suitible replacement for AMCA. In order to be able to synthesise the AMQ linker, it was necessary to first synthesise AMQ from the precursor 7-fluoro-1-methylquinolinium iodide (scheme 3.5).

Scheme 3.5: Synthesis pathway of the fluorophore 7-amino-1-methylquinolin-1-ium (AMQ).



The crude product was recrystallised in absolute ethanol three times and a full characterisation has been done to analyse the product. In table 3.5 the H-NMR peaks (Appendix B.1) of the recrystallised product have been assigned. In Appendix B.2 the product was compared to 7-amino-1-methylquinolin-1-ium provided by W. Jager [24], which shows two similar spectra. Therefore, it was concluded that the product formed is indeed AMQ.

Table 3.5: H-NMR peaks of AMQ assigned by multiplicity, relative intensity and attribution.

δ (ppm)	Multiplicity	Relative intensity	Attribution
4.56	singlet	3H	11
7.68	triplet	2H	7+9
7.94	triplet	1H	1
8.40	doublet	1H	10
9.12	doublet	1H	2
9.32	doublet	1H	6

Additionally, to fully characterize the molecule C-NMR (Appendix B.3), which peaks are assigned in table 3.6, COSY (Appendix B.4), HSQC (Appendix B.5) and MS (appendix B.6) have been taken.

Table 3.6: C-NMR peaks of AMQ assigned by shift and attribution.

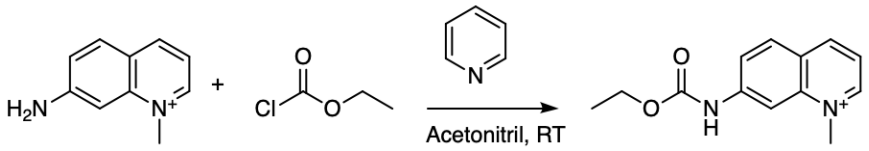
δ (ppm)	Attribution
45.15	j
98.80	c
119.18	g
122.19	a
124.86	e
132.10	k
164.03	d
149.12	i
164.72	f
198.56	b

There remains one noticeable peak that has not been assigned. This peak has a shift of 4.12 and is coupled to a carbon (shift of 56.94) in the HSQC (appendix B.5). The peak has a relative intensity of three protons and could be an extra methyl group. The H-NMR spectrum of the reference molecule showed the same unidentified peak and after taking a mass spectrum of the reference compound (appendix B.7) it showed that both the molecule and the reference compound shared the same impurity. To be sure that the impurity was not in one of the reactants, they were all checked with H-NMR and came out clean. Despite the impurity, the synthesised AMQ was used in order to proceed with the project.

3.5. Analysis of linker molecule

After the successful preparation of AMQ in the previous step, AMQ was quenched with ethyl chloroformate (scheme 3.6). The reaction has been tried multiple times and the different reaction conditions have been tabulated in table 3.7, using a stronger base with every synthesis. It was attempted to recrystallise the product, however the product either immediately dissolved (ethanol, methanol, isopropanol and chloroform) or does not dissolve completely after heating (ethyl acetate, acetone, dichloro-methane and dioxane). Unfortunately,

Scheme 3.6: Synthesis pathway of the AMQ linker molecule, which is quenched by ethyl chloroformate.



non of the attempts to synthesise the linker molecule were successful and the H-NMR of all the three attempts (Appendix B.8) showed that AMQ was still present (clearly visible in the aromatic region) and no product was formed. Consequently, another procedure to synthesise the AMQ profluorophore is needed and the results of this procedure are discussed in the next section.

Table 3.7: The different combinations of solvent and catalyst used for the synthesis of the AMQ linker molecule.

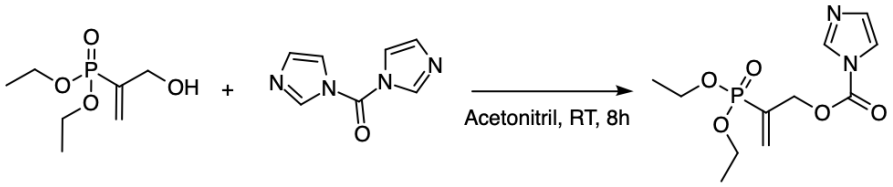
*n*0	Solvent	Catalyst	(AMQ:catalyst:reactant)	Product
1	acetonitril	pyridine	1:1.2:1.2	No
2	DMF	Et_3N	1:1.2:1.2	No
3	THF/H_2O (1:1)	K_2CO_3	1:1.2:1.2	No

3.6. Analysis of one pot synthesis

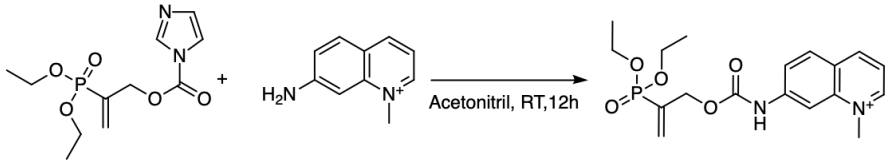
As all the linker attempts did not succeed another procedure to synthesise the AMQ profluorophore was envisioned. The procedure consists of two steps. Firstly, 1,1-carbonyldiimidazole (CDI) and HYP are mixed to synthesise a carbamate with an imidiazole as leaving group (scheme 3.7). Secondly, AMQ is introduced to the mixture and will react with the carbamate and make the imidiazole leave(scheme 3.8).

During the synthesis of the first step (scheme 3.7) in the one pot synthesis of the AMQ profluorophore, the TLC's made showed that HYP had reacted and that the next step (scheme 3.8) could be carried out. The second step reacted overnight and after the workup, both the organic and water layer were examined. The peaks of the crude product of the organic layer have been tabulated in table 3.8 and the H-NMR spectrum has been appended in appendix B.9.

Scheme 3.7: First step in the one-pot synthesis of the AMQ profluorophore.



Scheme 3.8: Step two in the synthesis pathway of the AMQ profluorophore.



In appendix B.10 the product has been compared to HYP and AMQ. The shift of protons on the double bond (10), the shift of the protons on the carbon before the hydroxyl group (11) and the disappearance of the proton on the hydroxyl group (12) confirms that the hydroxyl group on HYP has been replaced. When comparing AMQ to the product, it can be concluded that AMQ did not react with HYP and that the desired product was not synthesized. Unfortunately, the nucleophilic imidiazole (side product) attacked the double bond of

HYP making the newly attached carbamate leave and creating a HYP-imidiazole (figure 3.2). Conclusively, to make the profluorophore primary, secondary and tertiary amines need to be avoided.

δ (ppm)	Multiplicity	Relative intensity	Attribution
1.20	triplet	36H	7+9
3.94	quartet	4H	6+8
4.75	doublet	2H	11
5.71+6.04	doublets	2H	10
6.92	singlet	1H	14
7.12	singlet	1H	13
7.63	singlet	1H	16

Table 3.8: H-NMR peaks of the product of the one pot synthesis assigned by multiplicity, relative intensity and attribution

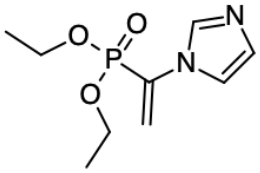


Figure 3.2: Structure of the HYP-imidiazole.

4

Conclusion

The quenching of AMCA has been arduous, as after multiple attempts of synthesising the linker molecule with methyl chloroformate it was concluded that it simply does not work. On the contrary, ethyl chlorformate does work, successfully quenched AMCA and could be used for the next step. Unfortunately, during the transesterfication of the AMCA linker molecule the bromotrimethylsilane attacked the alcohol group on the other side of the molecule. Therefore, it was concluded that another fluorophore (AMQ) needed to be used. Eliminating the alcohol group on the opposite side of the molecule, would enable transesterfication of HYP with the quenched fluorophore.

After synthesising 7-amino-1-methylquinolin-1-ium (AMQ), the same method as with the AMCA linker molecule was used to make the AMQ linker molecule. The identity of the impurity remains uncontested and may have played a role in not successfully synthesising the AMQ linker molecule.

The one pot synthesis of the AMQ profluorophore did not work out due to the strong nucleophilic character of the imidazole, which replaced the imidiazole carbamate. Thus to synthesise the profluorophore a less reactive phosgene derivative is needed.

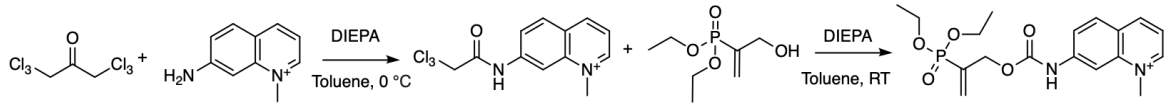
The aim of the project was to monitor the release of the fluorophore in both presence and absence of cells. As the synthesis of the profluorophore turned out to be very time consuming, a working profluorophore has yet to be made and the release of the fluorophore could not be monitored.

4.1. Recommendations

After synthesising the profluorophores it is important to evaluate their solubility in buffer solution with high glucose content, as cells live in such an environment.[6] As AMCA was not soluble in water, it remains to be seen if the profluorophore will be water soluble. However, in cell work many fluorophores are co-solubilized with small amounts of DMSO, which cells can withstand.[28, 29] AMQ was very soluble in water and will very likely be water soluble as profluorophore, as HYP is hydrophilic as well. However, as there was an impurity problem with AMQ, it needs to be further investigated before proceeding with the profluorophore synthesis.

After monitoring whether the catalytic release of the fluorophore in absence of cells is successful, the release can be monitored in the presence of cells. The reaction will have to deal with physiological pH (7.4), a high salt content and the presence of amino acids.[6, 30] Amino acids like lysine and cysteine have a nucleophilic N- and S- terminus and could react with HYP, making them unfit for the production of proteins. Another point to take into account is the generation of carbon dioxide during the release of fluorophore, as too much carbon dioxide might poison the cells.

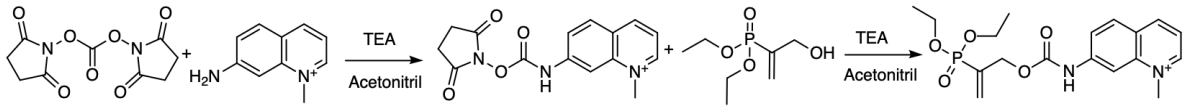
Scheme 4.1: Proposed synthesis pathway by using tri-phosgene instead of the phosgene derivative: ethyl chloroformate.



The carbamates in this study are synthesised using chloroformates (phosgene derivatives) and secondary amines, as this is reported as a simple and effective procedure.[31] Additionally, the phosgene derivatives

are a replacement for tri-phosgene which decomposes in the toxic phosgene when exposed to water.[32] As primary, secondary and tertiary amines need to be avoided in the further synthesis of the profluorophore there are two new options proposed for the synthesis of the profluorophore. Both tri-phosgene (scheme 4.1) and disuccinimidyl carbonate (DSC)(scheme 4.2) have leaving groups that will not attack the formed carbamate and are possible candidates for the synthesis of the profluorophore.

Scheme 4.2: Proposed synthesis pathway by using DSC instead of ethyl chloroformate.



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A

Appendix AMCA

A.1. H-NMR spectrum of 3-hydroxyprop-1-en-2-ylphosphonate (HYP).

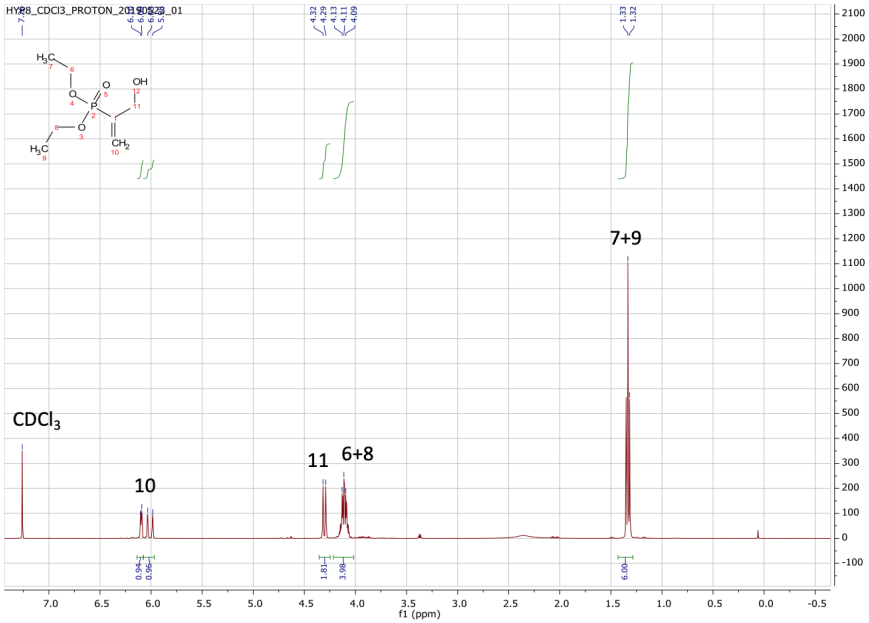

Figure A.1: H-NMR spectrum of HYP in chloroform, with peak assignment

A.2. H-NMR spectrum of the AMCA linker molecule.

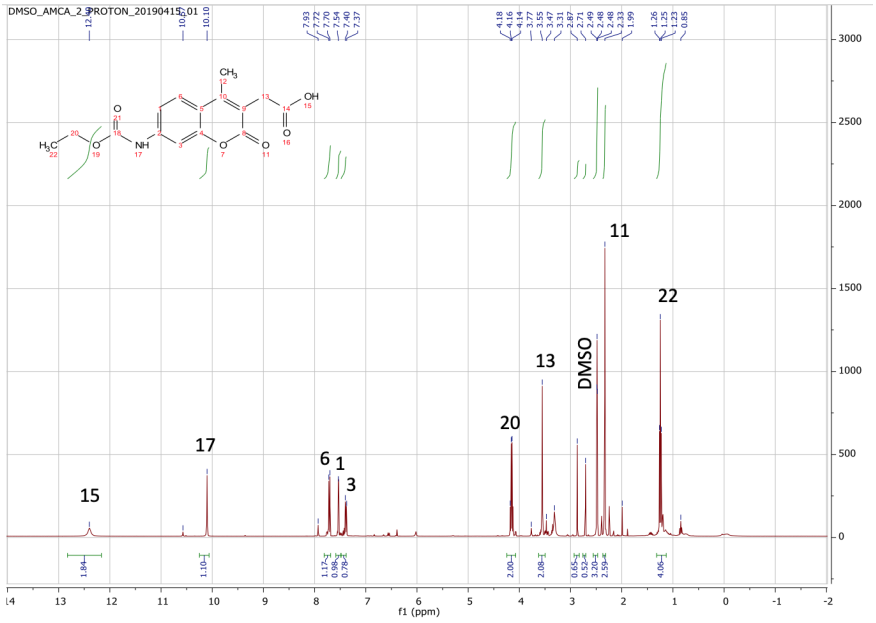

Figure A.2: H-NMR spectrum of the AMCA linker molecule in DMSO, with peak assignment.

A.3. H-NMR spectrum of AMCA and the AMCA linker molecule.

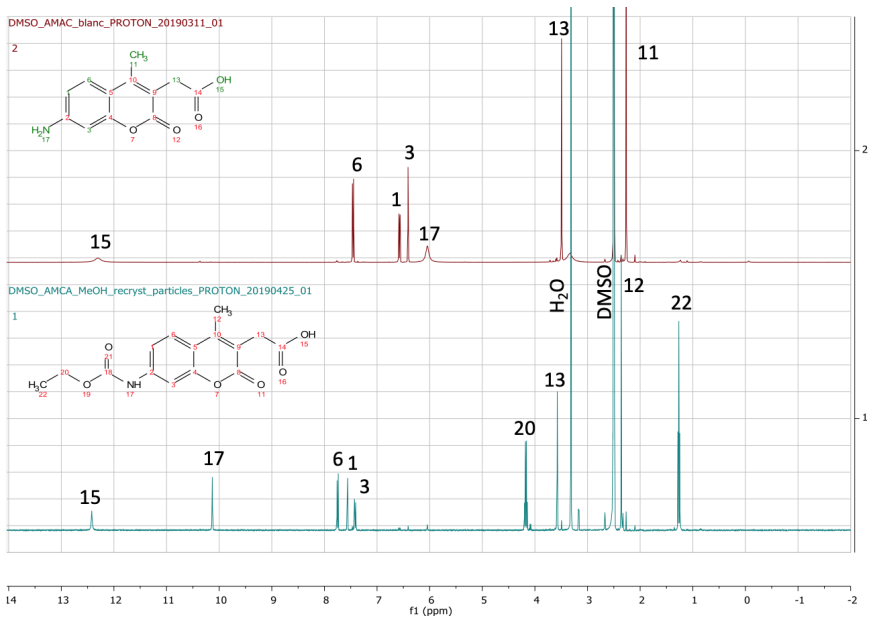

Figure A.3: Comparison on the H-NMR spectra of AMCA (1) and the ethyl linker (2) in DMSO, with peak assignment.

A.4. H-NMR spectrum of AMCA and the recrystallization of the AMCA linker molecule.

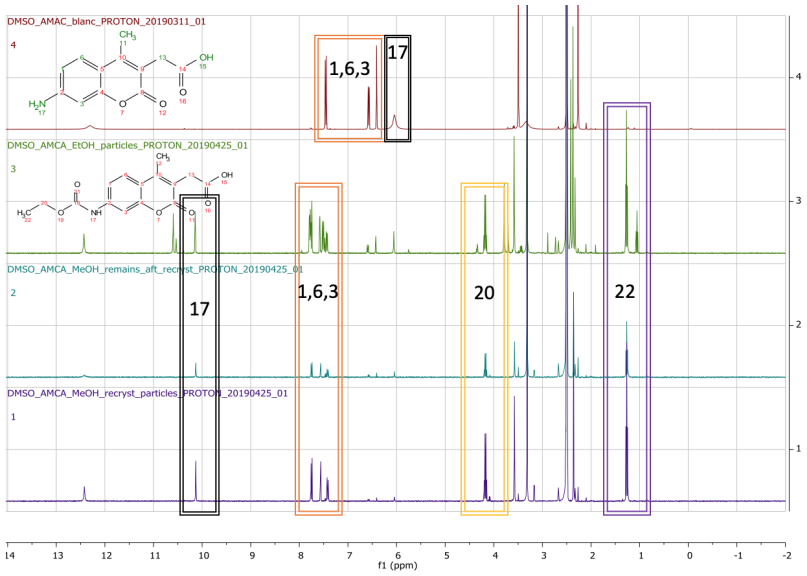

Figure A.4: H-NMR spectra of the purification steps of the ethyl linker in DMSO. From top to bottom: AMCA, ethanol recrystallization particles, methanol solution (after evaporation of the solvent and methanol recrystallization particles. The boxes show the shifts of the aromatics and amide and the appearance the attached methyl and ethyl.

A.5. H-NMR spectrum of the transesterfication of HYP and the AMCA linker molecule.

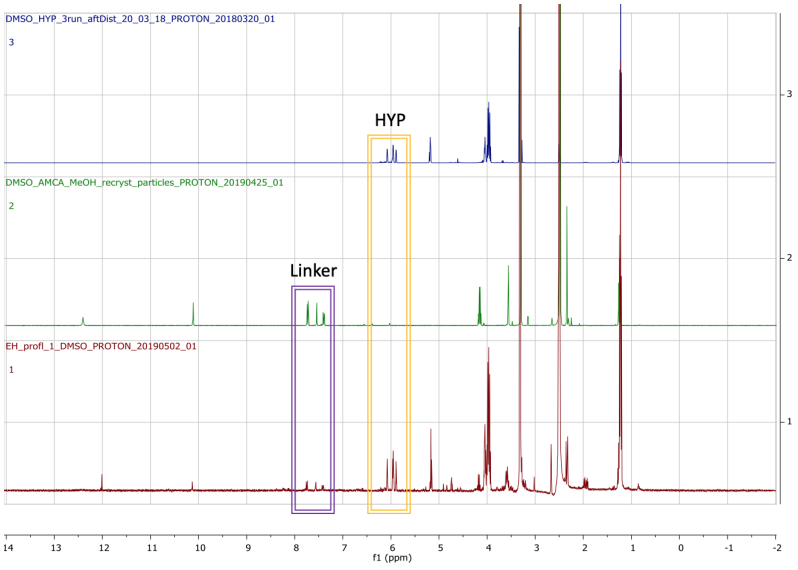

Figure A.5: H-NMR spectra of the transesterfication of the linker molecule and HYP with DMAP. From top to bottom: spectrum of HYP, spectrum of the linker molecule and the spectrum of the crude product after transesterfication, all in DMSO. The two boxes show that both HYP (yellow box) and the AMCA linker molecule (purple box) are still present.

A.6. H-NMR spectrum of transesterfication with TMSBr.

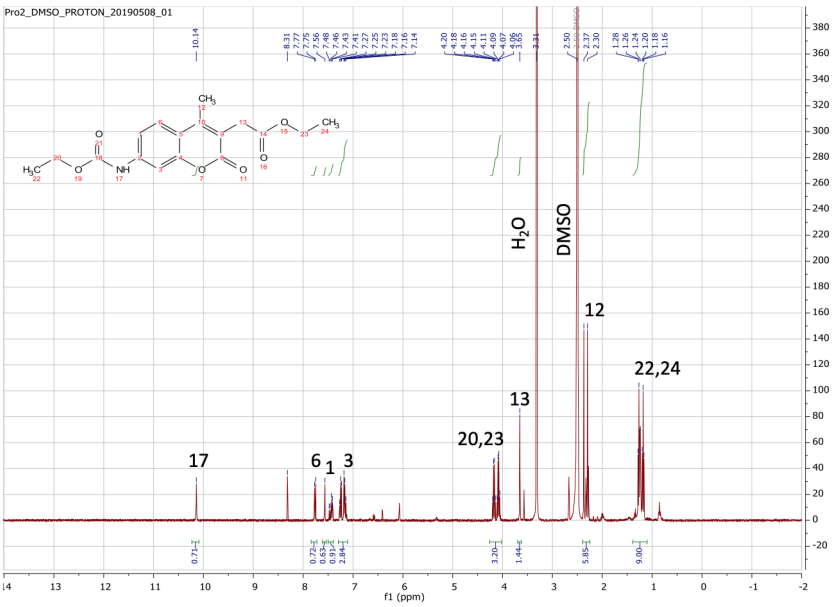

Figure A.6: H-NMR spectrum of the crude product after transesterfication with TMSBr in DMSO, with peak assignment.

A.7. H-NMR spectra of the AMCA linker molecule and the crude product.

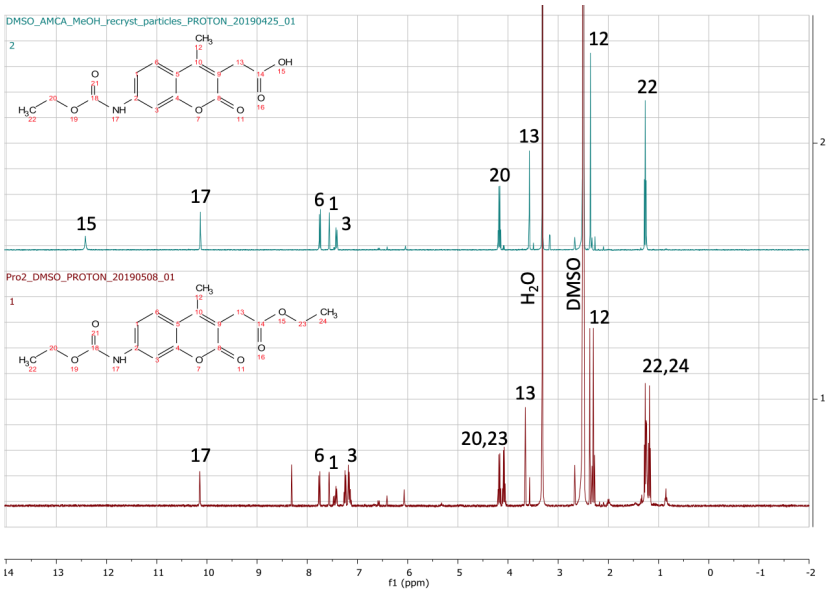

Figure A.7: Comparison of the H-NMR spectra of the AMCA linker molecule (2) and the attempted profluorophore (1), both in DMSO and with peak assignment.

B

Appendix AMQ

B.1. H-NMR spectrum of 7-amino-1-methylquinolin-1-ium (AMQ).

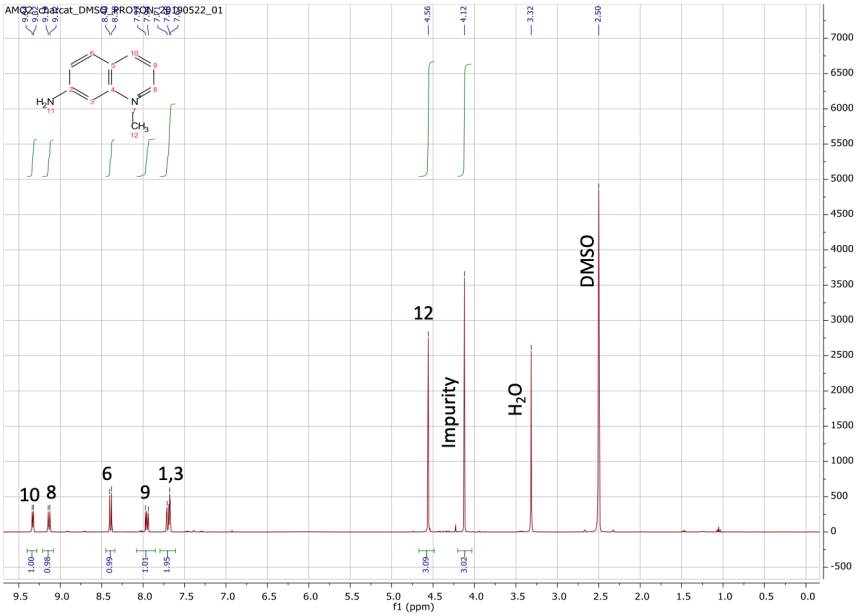

Figure B.1: H-NMR spectrum of the product in DMSO, with peak assignment.

B.2. H-NMR spectrum of the recrystallized product compared to a AMQ reference.

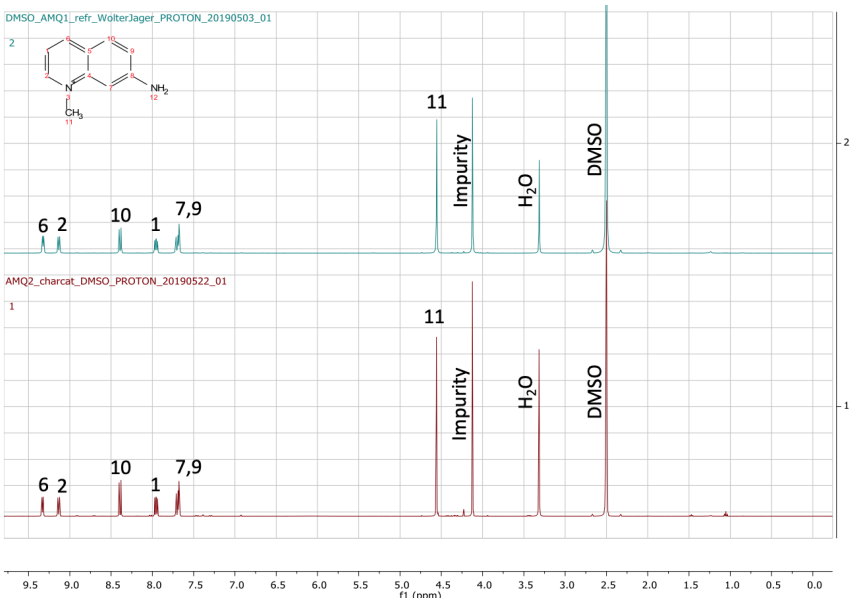

Figure B.2: Comparison of the H-NMR spectra of the AMQ reference (2) and the product (1), both in DMSO and with peak assignment.

B.3. C-NMR spectrum of 7-amino-1-methylquinolin-1-ium (AMQ).

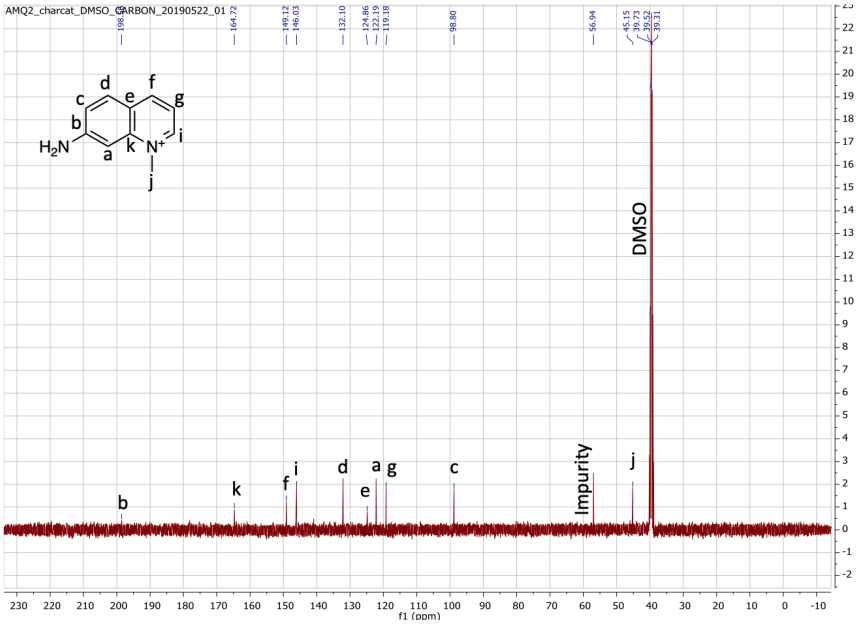

Figure B.3: C-NMR of the product in DMSO, with peak assignment.

B.4. COSY of 7-amino-1-methylquinolin-1-ium (AMQ).

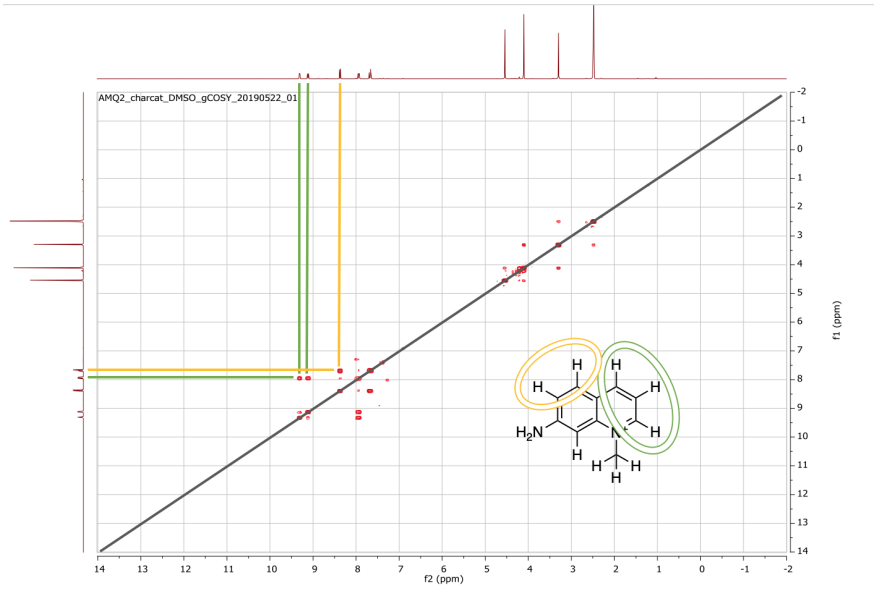

Figure B.4: COSY of the product in DMSO, the lines indicate the protons that can see their neighbours.

B.5. HSQC of 7-amino-1-methylquinolin-1-ium (AMQ).

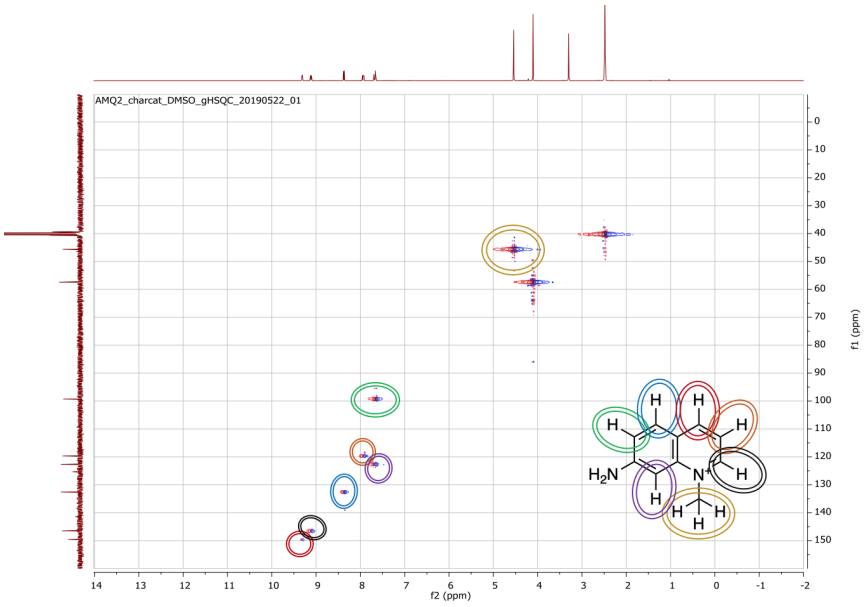

Figure B.5: HSCQ of the product in DMSO, the coloured rings couple the protons to their linked carbon atom.

B.6. Mass pectrum of AMQ

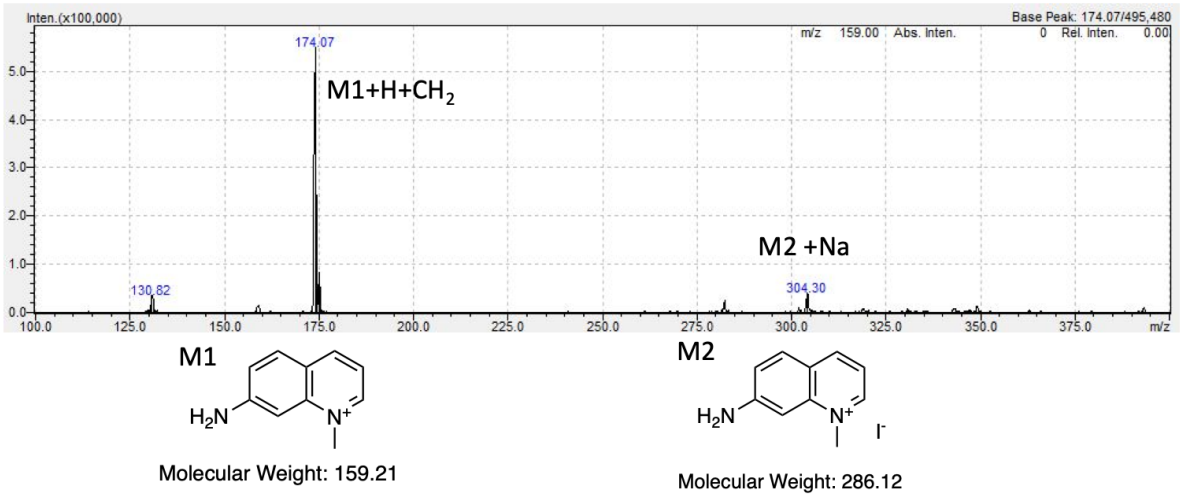

Figure B.6: Mass spectrum of 7-amino-1-methylquinolin-1-ium (AMQ).

B.7. MS

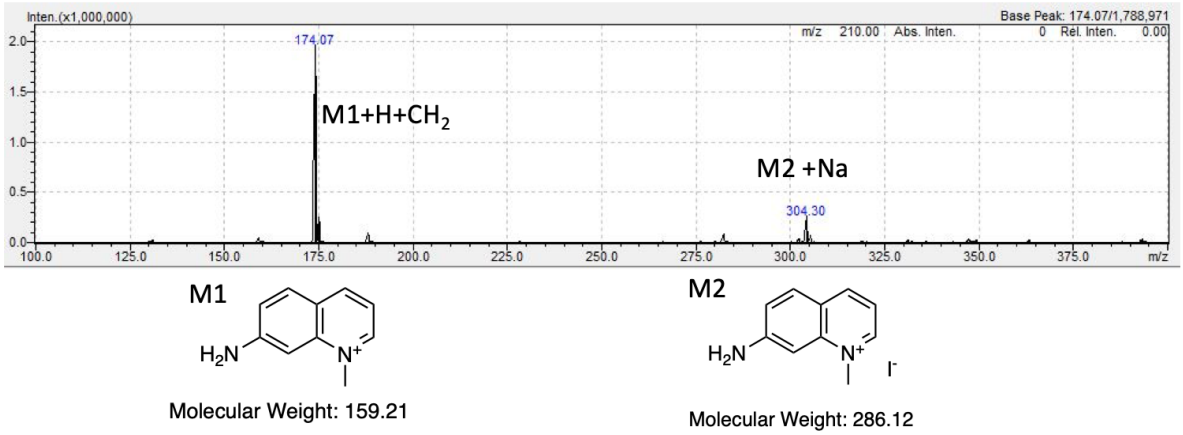

Figure B.7: Mass specturm of reference molecule

B.8. H-NMR spectra of all three AMQ linker molecule attempts.

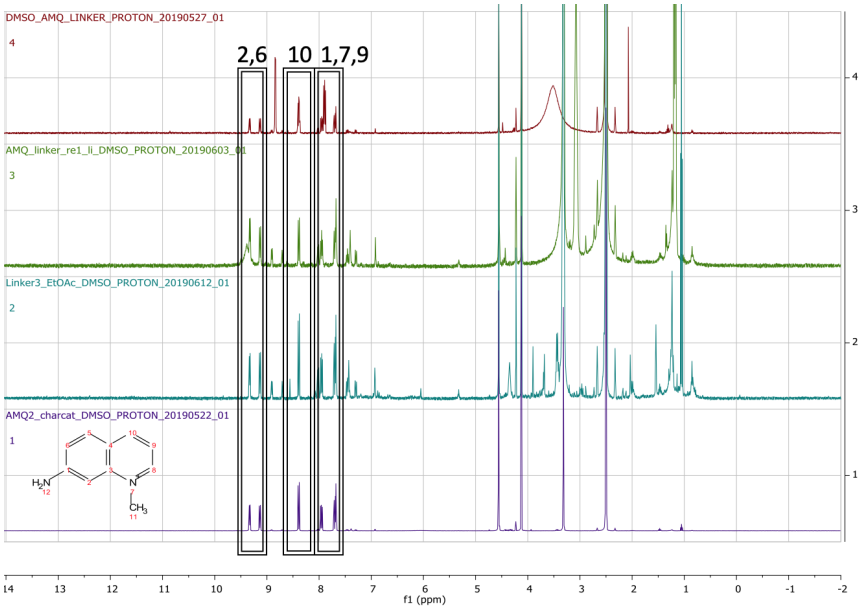

Figure B.8: Comparison of the three linker attempts to the starting compound AMQ. From top to bottom: first attempt, second attempt third attempt and starting material AMQ, all in DMSO.

B.9. H-NMR spectrum of the first step in the one-pot synthesis.

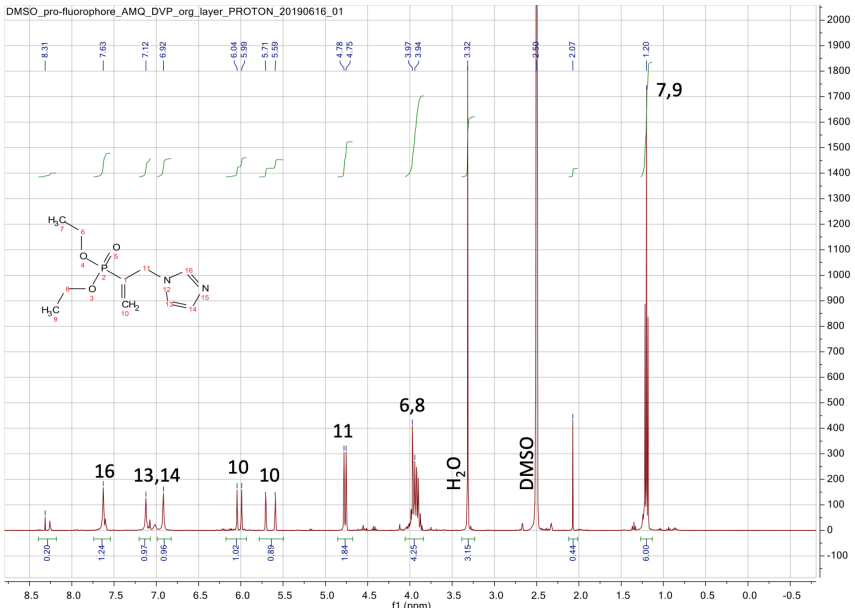

Figure B.9: H-NMR spectrum of the product in DMSO, with peak assignment.

B.10. H-NMR spectrum of the second step in the one-pot synthesis.

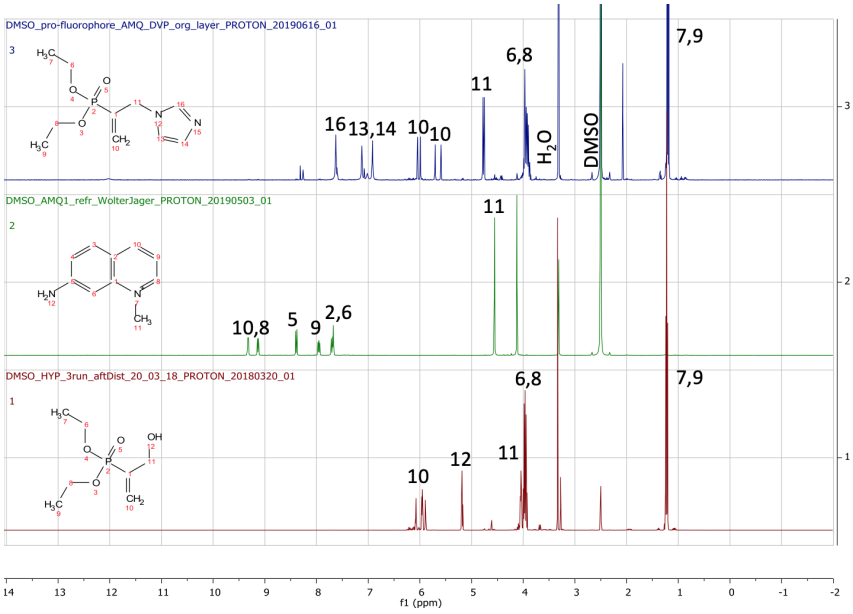

Figure B.10: Comparison of the product (3) to the starting materials AMQ (2) and HYP (1).