UNIVERSITAT DE BARCELONA

Facultat de Farmàcia
i Ciències de l'Alimentació

FACULTAT DE FARMÀCIA I CIÈNCIES DE L'ALIMENTACIÓ

Degree's Final Project

Pharmacy Degree

SYNTHESIS OF RED-SHIFTED AZOBENZENE AMINO ACIDS FOR SOLID PHASE PEPTIDE SYNTHESIS

CÈSAR SÁNCHEZ AMORÓS

Main field: Organic Chemistry

Secondary fields: Pharmaceutical Chemistry, Physical Chemistry and Instrumental Techniques

Department of Pharmacology, Toxicology, and Therapeutic Chemistry

Faculty of Pharmacy and Food Science

University of Barcelona (UB)

Barcelona, June 2024

ABBREVIATIONS

^{1}H-NMR: Proton Nuclear Magnetic Resonance

^{13}C-NMR: Carbon-13 Nuclear Magnetic Resonance

ACN: Acetonitrile

Alloc: Allyloxycarbonyl

CCiTUB: Scientific and Technological Centers of the University of Barcelona

DAE: Diarylethene

DBDMH: 1,3-Dibromo-5,5-dimethylhydantoin

DCE: 1,2-Dicloroethane

DIPEA: *N,N*-Diisopropylethylamine

DMSO: Dimethylsulfoxide

Fmoc: 9-Fluorenylmethoxycarbonyl

Fmoc-OSu: *N*-(9-Fluorenylmethoxycarbonyloxy)succinimide

HTI: Hemithioindigo

***p*-TsOH**: *para*-Toluensulfonic Acid

RT: Room Temperature

SDG: Sustainable Development Goals

SPPS: Solid Phase Peptide Synthesis

***t*-Bu**: *tert*-Butyl

THF: Tetrahydrofuran

TLC: Thin Layer Chromatography

UV: Ultra Violet

ABSTRACT

One of the main global health concerns of the current era is the appearance and rise of antimicrobial resistance, aiming to potentially surpass other main causes of mortality such as cancer or traffic accidents in the years to come. Bacteria are gaining antimicrobial resistances primarily due to misuse and overuse of antibiotics, so the rush to develop new strategies to overcome resistance, like photopharmacology, is a major necessity.

Photopharmacology offers a promising approach by giving precise spatiotemporal control over the activity of molecules using light. Therefore, these molecules can be inactivated after exposure to light once released to the environment, reducing their impact and the chances of resistance emerging.

To accomplish it, photoswitchable groups are added to biologically active molecules. However, the isomerization of current photoswitches takes place in the UV range of the electromagnetic spectrum, creating safety concerns for *in vivo* applications. Thus, there's an urgent need for new photoswitches that respond to visible light.

A potential solution that holds promise involves using azobenzenes, photoswitchable molecules whose activity can be regulated with visible light. An azobenzene that by substituting its *ortho*-positions allows using visible light, could serve for solid phase peptide synthesis to assemble a peptide that could be used to fight back antimicrobial resistance.

Key concepts: *photopharmacology, azobenzene, solid phase peptide synthesis*

Síntesi d'aminoàcids azobenzens operats amb llum vermella per a síntesi de pèptids en fase sòlida

Una de les majors preocupacions globals de la salut actuals, és l'aparició i augment de resistències antimicrobianes, on podrien arribar a superar altres causes principals de mortalitat com el càncer o els accidents de trànsit en els pròxims anys. Les bactèries estan adquirint aquestes resistències principalment degut a l'ús inadequat i excessiu d'antibiòtics, per la qual cosa la urgència de desenvolupar noves estratègies per superar les resistències, com la fotofarmacologia, és una necessitat.

La fotofarmacologia ofereix un enfocament prometedor donant un control precís espaciotemporal sobre l'activitat de les molècules mitjançant la llum. Aquestes, poden ser inactivades amb l'exposició a la llum un cop alliberades al medi ambient, reduint el seu impacte i les possibilitats d'aparició de resistències.

Per aconseguir-ho, grups fotocommutables són afegits a les molècules biològicament actives. No obstant, la isomerització dels fotocommutables actuals té lloc en el rang UV de l'espectre electromagnètic, creant preocupacions de seguretat per a aplicacions *in vivo*. Per això, hi ha una necessitat urgent de nous fotocommutadors que responguin a llum visible.

Una possible solució implica l'ús d'azobenzens, molècules fotocommutables l'activitat de les quals es pot regular amb llum visible. Un azobenzè que substituint les seves posicions *orto* permeti l'ús de llum visible, podria servir per a la síntesi de pèptids en fase sòlida per assemblar un pèptid que pogués utilitzar-se per combatre la resistència antimicrobiana.

Conceptes clau: *fotofarmacologia, azobenzè, síntesi de pèptids en fase sòlida*

INTEGRATION OF FIELDS

The main field of work for this project is **Organic Chemistry**, since the main objective the synthesis, purification and characterization of red-shifted azobenzenes amino acids, which will be used for Solid Phase Peptide Synthesis.

Different instrumental techniques were used to purify and determine the identity of all compounds. These include Silica Flash Column Chromatography, Thin Layer Chromatography and NMR. Thus, one of the secondary fields of this work is **Physical Chemistry and Instrumental Techniques**.

The other secondary field of this project is **Pharmaceutical Chemistry**, as the target azobenzenes have potentially therapeutic use when coupling them with an antibiotic or any other bioactive compound to regulate their activity.

SUSTAINABLE DEVELOPMENT GOALS

The development of a photoswitchable molecule that can be used to fight the global concern of antimicrobial resistance is related to several **Sustainable Development Goals (SDG).**

SDG 3, Good Health and Well-Being, includes promoting research and development of treatments to address diseases. Therefore, by combating a major global threat such as antimicrobial resistance, we are providing new tools and strategies to avoid its alarming growth.

SDG 5 consists of promoting **Gender Equality**, which in this case also applies to the scientific field. Within the laboratory where the experiments were conducted, there is equitable treatment for both men and women, encouraging the participation of the latter, thus putting an end to gender discrimination and building a more equal society.

SDG 6 aims to ensure **Clean Water and Sanitation**. Since most antibiotics usually end up in the sewer system of densely populated areas, they start generating resistance in aquatic microorganisms and those in their hosts. The development of antibiotics that can deactivate after their use will largely diminish the accumulation of active antimicrobials in the environment.

INDEX

1. INTRODUCTION

1.1. Photopharmacology

Photopharmacology is, a field at the intersection of chemistry, biology, and photonics, aiming at controlling the activity of biologically active molecules using light. This approach offers precise spatiotemporal control over drug action, presenting a promising solution to many challenges in traditional pharmacology, including off-target effects, limited selectivity, and poor temporal control.[1]

There are several types of photopharmacological approaches, such as photocaging, photodynamic therapy and photoswitchable molecules. The latter, also known as photoswitches, undergo a reversible isomerization upon exposure to light of specific wavelengths. These molecules can isomerize between states in response to light, each with different properties, such as solubility, steric hindrance or dipole moment. By incorporating photoswitches into biologically active compounds, we can modulate their physicochemical properties therefore modifying the pharmacological effect.

One of the main advantages of photopharmacology consists in its ability to achieve spatiotemporal control over drug action and activity. By directing visible light to specific anatomical sites or cellular compartments, we can activate or deactivate drugs incorporating photoswitchable molecules. This spatial precision is valuable in complex biological systems, where traditional drugs may lack specificity and affect unintended targets producing adverse effects to the patient. By adjusting the duration and intensity of light exposure, we can regulate the duration of the activity of biologically active molecules, optimizing treatments and also minimizing the risk of adverse effects. The applications of photopharmacology span a broad range of fields such as neuroscience, oncology, and treating infectious diseases, our main focus for this work.[1-5]

All in all, photopharmacology is a field that represents a great change in drug discovery, offering control over drug action. With continued and new advancements in photoswitch design, light delivery technologies, and therapeutic applications, the field of photopharmacology has huge potential to revolutionize the way we treat diseases and improve patient outcomes.[3]

1.2. Photoswitches

Photoswitches or molecular photoswitches (PSs) are chromophores capable of isomerizing by illumination at a specific wavelength between two (or more) species with different absorption spectra at their photostationary states. In photopharmacology, these molecules are incorporated into biologically active compounds. When these are exposed to light, they transition between distinct states, potentially altering the activity of the attached drug. This allows researchers to gain control over drug action, giving spatial and temporal modulation of pharmacological effects.[5]

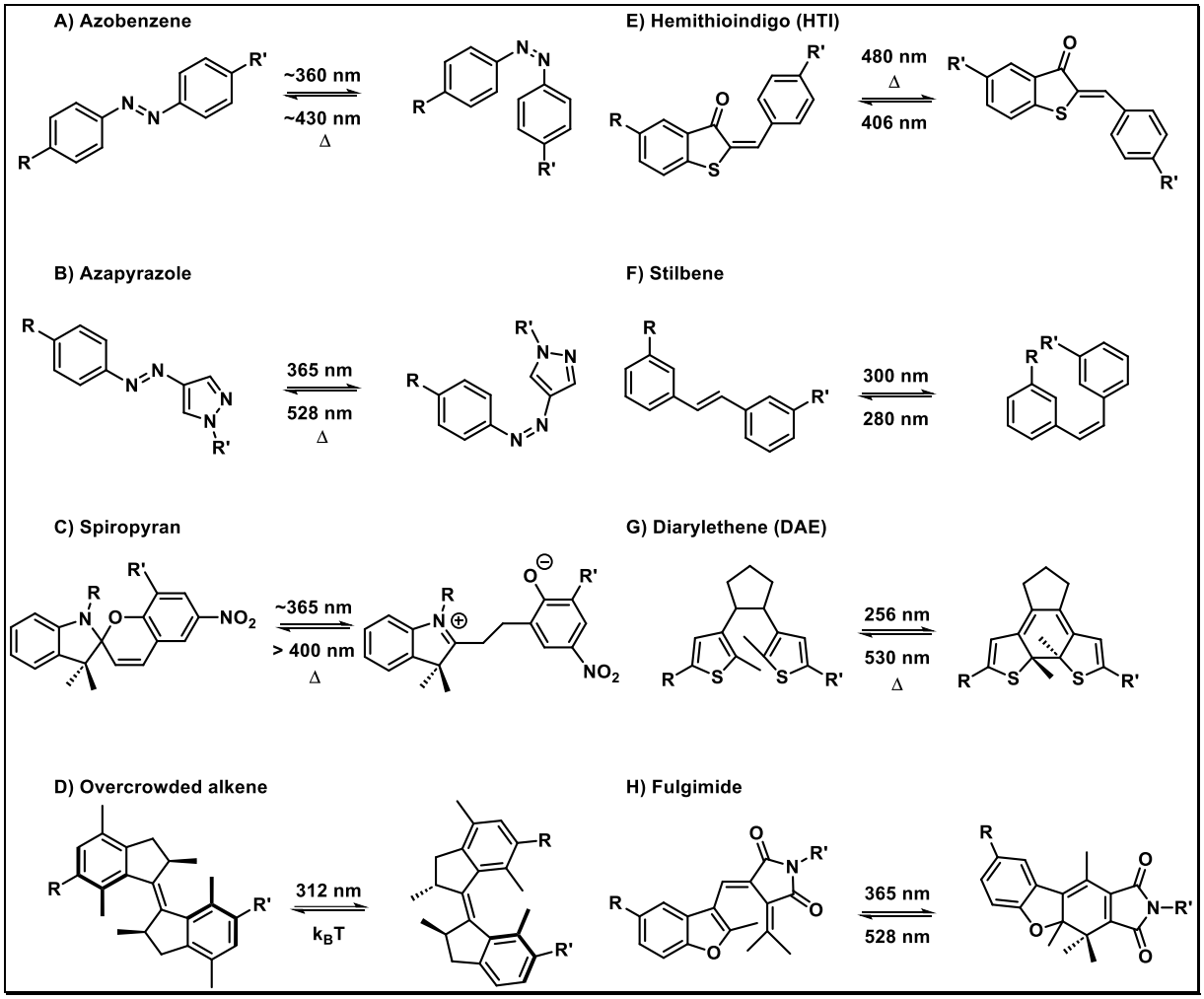


Figure 1. *Structure and isomerization of common photoswitches in photopharmacology.*

1.3. Azobenzenes

Azobenzenes represent a class of photoswitchable molecules widely used in photopharmacology for their ability to undergo reversible isomerization, where they transition between their *trans*- and *cis*- configuration upon exposure to light of specific wavelengths. This reversible process makes them valuable tools for controlling the activity of biologically active compounds with non-invasive high precision.

In their *trans* configuration, azobenzenes, which consists of two phenyl rings joined by a N=N bond scaffold, show a flat/planar structure with low steric hindrance. Upon absorption of light in the UV region of the electromagnetic spectrum, azobenzenes undergo a *trans*-to-*cis* isomerization. In their *cis* configuration, they display a higher dipolar moment, shorter distance between the two ends of the scaffold and both aromatic rings cannot be coplanar. This phenomenon allows us to control the shape and conformation of the azobenzene molecule and a drug bound to it, thereby modulating its interaction with surrounding biomolecules and influencing its biological activity.[6-12]

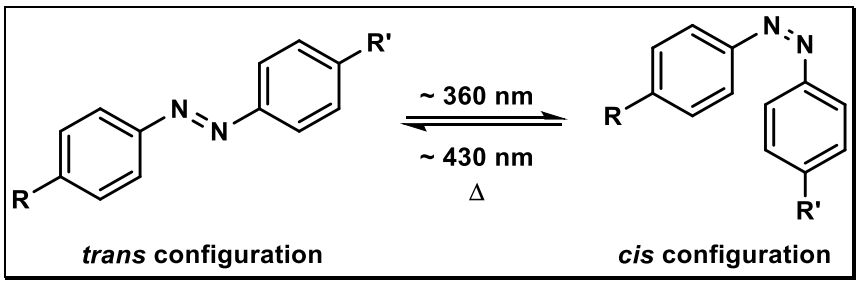


Figure 2. *Azobenzene isomerization.*

1.4. Red-shifted azobenzenes

Red-shifted azobenzenes refer to modified azobenzenes that exhibit isomerization under light of longer wavelengths, typically in the visible (400-650 nm) or even near-infrared regions of the spectrum (beyond 650 nm).[6,7]

This red-shifted absorption spectrum is achieved through structural modifications that alter the electronic properties of the azobenzene core, such as the introduction of electron-donating or electron-withdrawing substituents, or even both (push-pull systems). The design of azobenzene derivatives that undergo isomerization under red light has been recently achieved with tetra-*ortho*-chlorinated and tetra-*ortho*-methoxylated azobenzenes, which all respond to wavelengths of red light, whereas tetra-*ortho*-fluorinated azobenzenes, shift at the green region of the spectrum.[6,7,15,22]

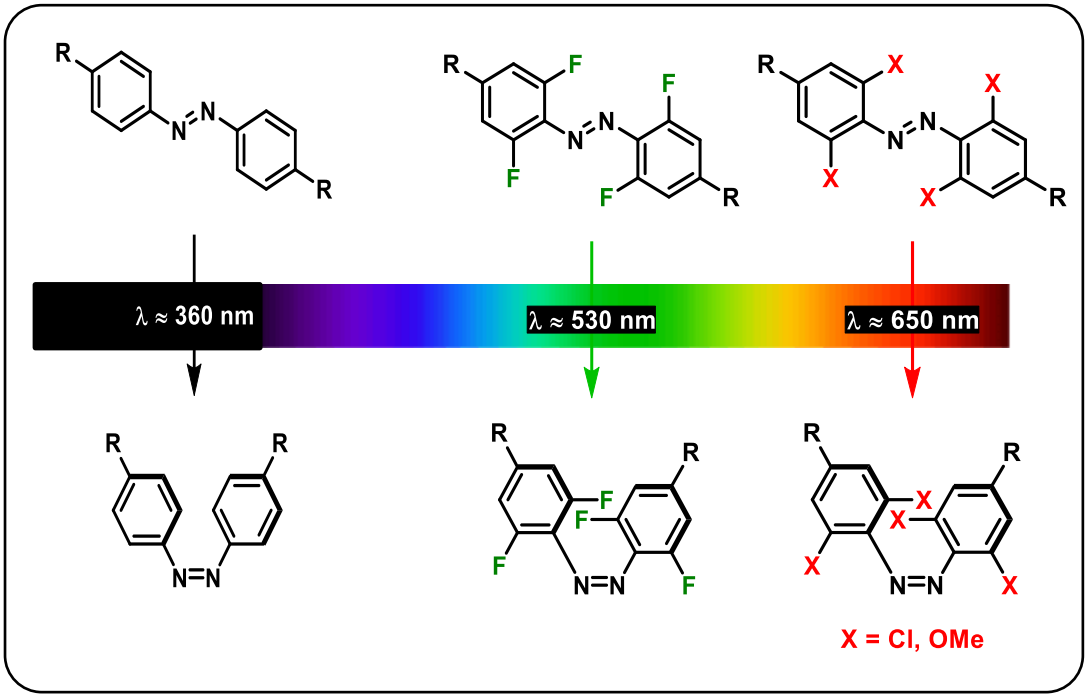


Figure 3. *Isomerization of different substituted azobenzenes in the UV spectrum.*

The recent discovery and application of red light-switchable azobenzene molecules are expected to initiate major breakthroughs in photopharmacology. It addresses several limitations associated with traditional UV-responsive azobenzenes, particularly in the context of *in vivo* applications. The advantages of using visible light over UV light for photoswitching include an increased tissue-penetration depth and reduced phototoxicity, minimizing photodamage to irradiated tissues. Therefore, the development of redshifted azobenzenes is key to obtaining highly precise control of biological systems with light.[6-14]

1.5. Photoswitchable antibiotics

We aim to create antibiotics with light-responsive properties to achieve precise control over their activity for combating antimicrobial resistance.[4,5,15] These antibiotics contain photoswitchable moieties, and as mentioned above, undergo reversible *cis-/trans*-isomerization upon exposure to light of specific wavelengths.[6-8,13] By switching between different isomeric states, photoswitchable antibiotics can be activated or deactivated in a controlled manner, allowing for spatiotemporal modulation of their antimicrobial effects. This allows to target and eradicate pathogenic bacteria while minimizing damage to endogenic microbiota or surrounding tissues, which can also contribute to decreasing environmental accumulation of the active compound, leading to a reduced appearance of resistance.[13,15]

Moreover, Feringa and collaborators successfully developed photoresponsive, antibacterial diaminopyrimidines bearing azobenzene photoswitches. For the first time, these compounds allowed for the full *in situ* photocontrol of antibacterial activity with green and violet light, making it possible to trigger both the activation as well as deactivation of the antibacterial agent in the presence of bacteria.[15]

1.6. Solid Phase Peptide Synthesis (SPPS)

Solid Phase Peptide Synthesis (SPPS) is a crucial method for the efficient assembly of peptides. SPPS involves the stepwise addition of amino acids onto a solid support, usually a resin, enabling the synthesis of peptides in a controlled manner and greatly improving efficiency when compared to synthesis in solution.[16]

The most common SPPS strategy (Fmoc/*t*-Bu) use Fmoc (9-fluorenylmethyloxycarbonyl) as the temporary N-terminus protecting group, safeguarding the amino group of the incoming amino acid and ensuring a selective coupling. This strategy provides greater flexibility and control during peptide assembly, minimizes side reactions and simplifies the purification processes.[16]

The synthesis begins with anchoring the first Fmoc-protected amino acid to the solid support. Subsequent amino acids are then added sequentially, each with their Fmoc protection group, after deprotection of the previously incorporated amino acid. Coupling reactions are facilitated by coupling reagents, and after each coupling step, the Fmoc group is removed under mildly basic conditions using piperidine (a nucleophilic amine), revealing the free amino group for the next coupling reaction. This cyclic process of coupling and deprotection is repeated until the desired peptide sequence is achieved.[16]

In conclusion, Solid Phase Peptide Synthesis, coupled with Fmoc protection strategy, provides a robust and efficient method for the assembly of long and complex peptidic structures.

1.7. Photoswitchable peptides

Currently in this era, misuse and overuse of antibiotics have turned bacterial resistance into one of the major concerns to global public health.[16] Therefore, antimicrobial peptides play a crucial role as a first line of defense against pathogenic bacteria.

Peptides have well-defined structures due to specific sequences of amino acids that fold into unique three-dimensional shapes. This folding is driven by interactions such as hydrogen bonds, hydrophobic interactions, Van der Waals forces and ionic bonds. These defined structures are crucial for their biological functions.

Introducing a photoswitch into peptide structures allows for precise control over their conformation and activity using light. There are two main strategies for incorporating photoswitches into peptides: 1) photoswitchable amino acids, which are the focus of this thesis as mentioned through points 1.2 to 1.6; and 2) photoswitchable staples, which involves attaching a photoswitchable molecule between two positions within a peptide, effectively “stapling” the peptide into a specific conformation. The latter method is particularly useful for stabilizing or inducing specific secondary structures, such as α-helices or beta-sheets.[18-20]

2. OBJECTIVES

The objective of this thesis is to achieve and optimize the synthesis of a photoswitchable azobenzene amino acid that can be operated using red light. Previous reports show that tetra-*ortho*-chlorinated azobenzenes are not compatible with piperidine, as it can perform a nucleophilic attack on the *ortho*-chlorinated positions of the azobenzene. Thus, the challenge is to find new compatible tetra-*ortho*-substituted red-shifted azobenzenes stable under Fmoc deprotection conditions using piperidine.[21,22] Therefore, the target compound is a tetra-*ortho*-methoxylated azobenzene **2** (Figure 4). Methoxide is a worse leaving group than chloride, making methoxylated azobenzenes more robust substrates towards nucleophilic aromatic substitution.

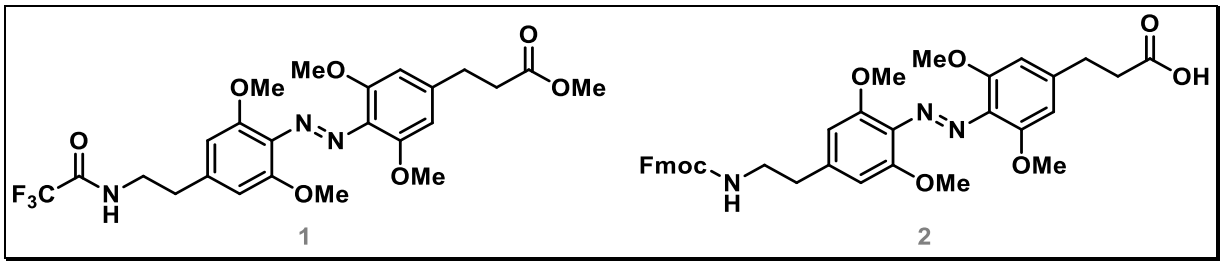


Figure 4. *Target trifluoroacetylated amino ester **1** and tetra-ortho-methoxylated azobenzene amino acid **2**.*

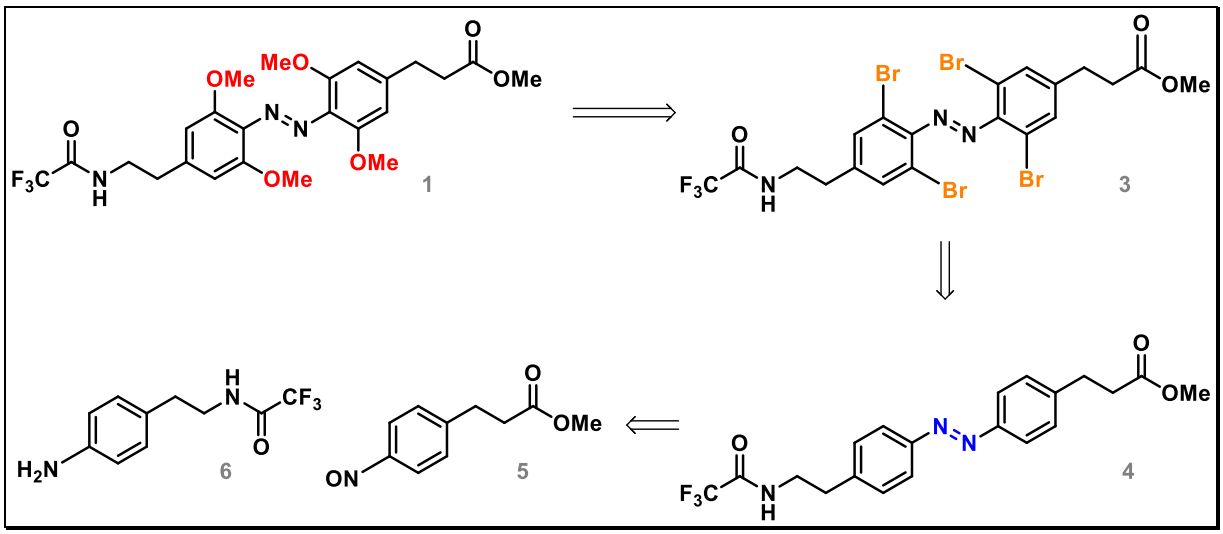
To achieve this feat, the following objectives have been defined:

- Optimize the synthesis of the trifluoroacetylated amino ester **1**.
- Screen conditions for the synthesis of the Fmoc-protected amino acid **2**.

3. RESULTS AND DISCUSSION

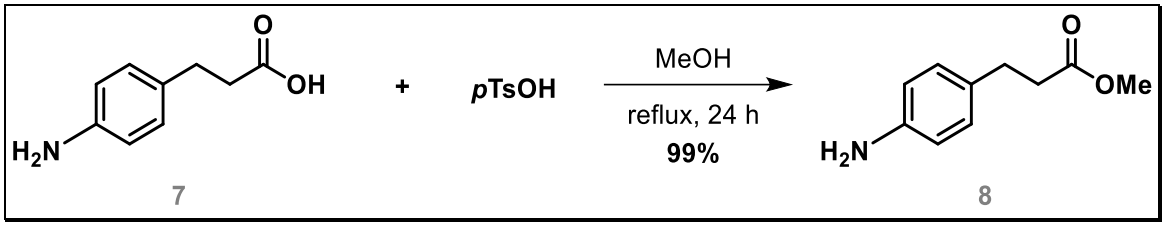
3.1. Synthesis of the tetra-*ortho*-methoxylated azobenzene

Starting from previous works of the group, a retrosynthetic plan was proposed to achieve the synthesis of the target compound **1** (Scheme 1). It is known from previous works from our group that the optimal obtention of the methoxylated azobenzene was via the corresponding azobenzene brominated in the all *ortho* positions.[22] The synthesis of the tetra-*ortho*-brominated compound was through a palladium-catalyzed C-H activation of the parent azobenzene.[21-23] The synthesis of the non-substituted azobenzene had been already optimized in previous works performing the Baeyer-Mills reaction, a condensation reaction between an aniline and an aryl nitroso compound.[21]



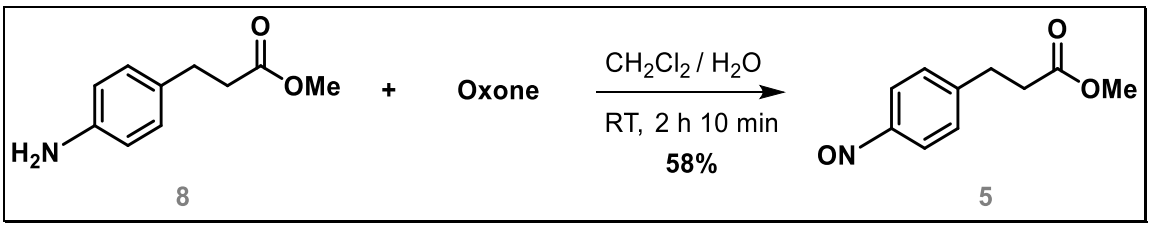
Scheme 1. *Retrosynthetic proposal to obtain the target tetra-ortho-methoxylated azobenzene **1**.*

First, to get the starting material compounds for the Bayer-Mills reaction, the corresponding synthesis of nitroso compound **5** and the trifluoroacetamide **6** are needed. In order to obtain compound **6**, we performed esterification of compound **7** in methanol under *p*TsOH catalysis to furnish **8** in excellent yield (Scheme 2).



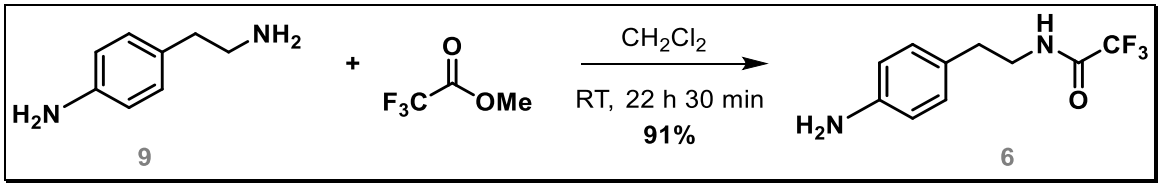
Scheme 2. *Esterification of carboxylic acid **7**.*

After the obtention of ester **8**, Oxone was used as an oxidizing agent in a biphasic CH_2Cl_2 / H_2O system to obtain nitroso compound **5** (Scheme 3). The reaction had to be checked by TLC every hour, to monitor consumption of the starting materials, as if left more time to react, it can over-oxidize the nitroso compound to a nitro function and the subsequent reaction would not be possible. After the reaction was over, compound **5** was isolated after extraction without any chromatographic step. A yield of 58% for the oxidation of aniline **8** was achieved.



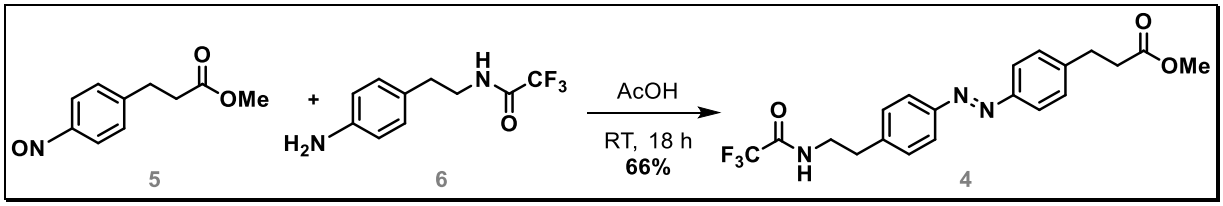
Scheme 3. *Oxidation of aniline **8**. Conversion to nitroso compound **5**.*

With compound **5** in hand, it was time to synthesize trifluoroacetamide **6**. Having aniline compound **9** as starting material in a CH_2Cl_2 solution, the reaction was set up with the conditions described in Scheme 4. It is worth mentioning that this reaction is highly selective, due to the aniline having more resonance and being less nucleophilic than the amine on compound **6**. A yield of 91% was achieved for the reaction.



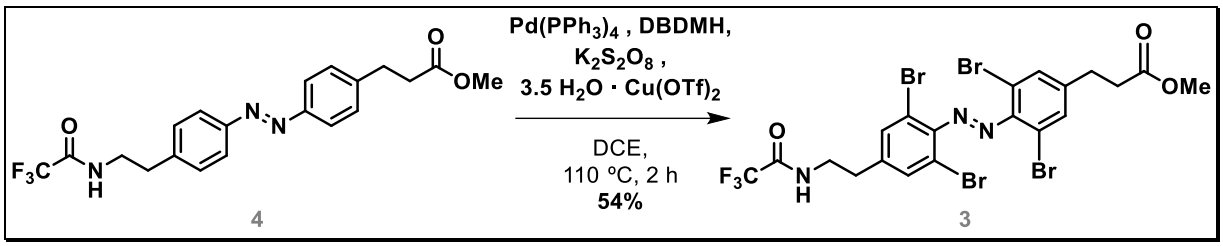
Scheme 4. *Synthesis of the trifluoroacetamide **6**.*

Now that the starting compounds to synthesize the non-substituted azobenzene were obtained, the next goal was obtaining compound **4** via the Baeyer-Mills reaction.[21,22] To carry out this reaction, nitroso compound **5** was dissolved in acetic acid under N_2 and aniline **6** was added slowly and left it stirring overnight (Scheme 5). A 66% yield was obtained for the reaction.



Scheme 5. *Synthesis of azobenzene **4**.*

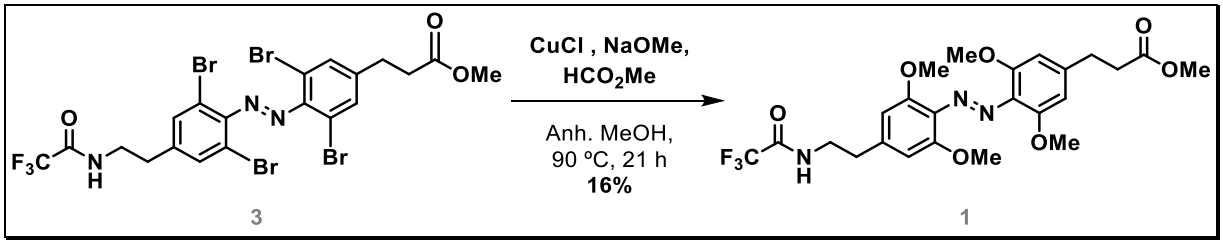
With the non-substituted azobenzene **4** in our hands, palladium-catalyzed C-H *ortho*-bromination of the azobenzene was used to functionalize all four *ortho* positions in compound **4**. Described conditions by our group that were already optimized previously were used to obtain compound **3** (Scheme 6). The use of $Cu(OTf)_2$ as a co-catalyst, prevents the undesired benzylic bromination of the substrate, and the addition of H_2O is key to achieve consistently good results.[22] A 66% yield was obtained for the reaction.



Scheme 6. *Synthesis of tetra-ortho-brominated azobenzene **3** via C-H activation.*

For the final step in the obtention of the tetra-*ortho*-methoxylated azobenzene **1**, there was a struggle to obtain a good yield (Scheme 7).[25] From previous works from our group, it is known that better yields can be obtained with the copper-catalyzed methoxylation of the

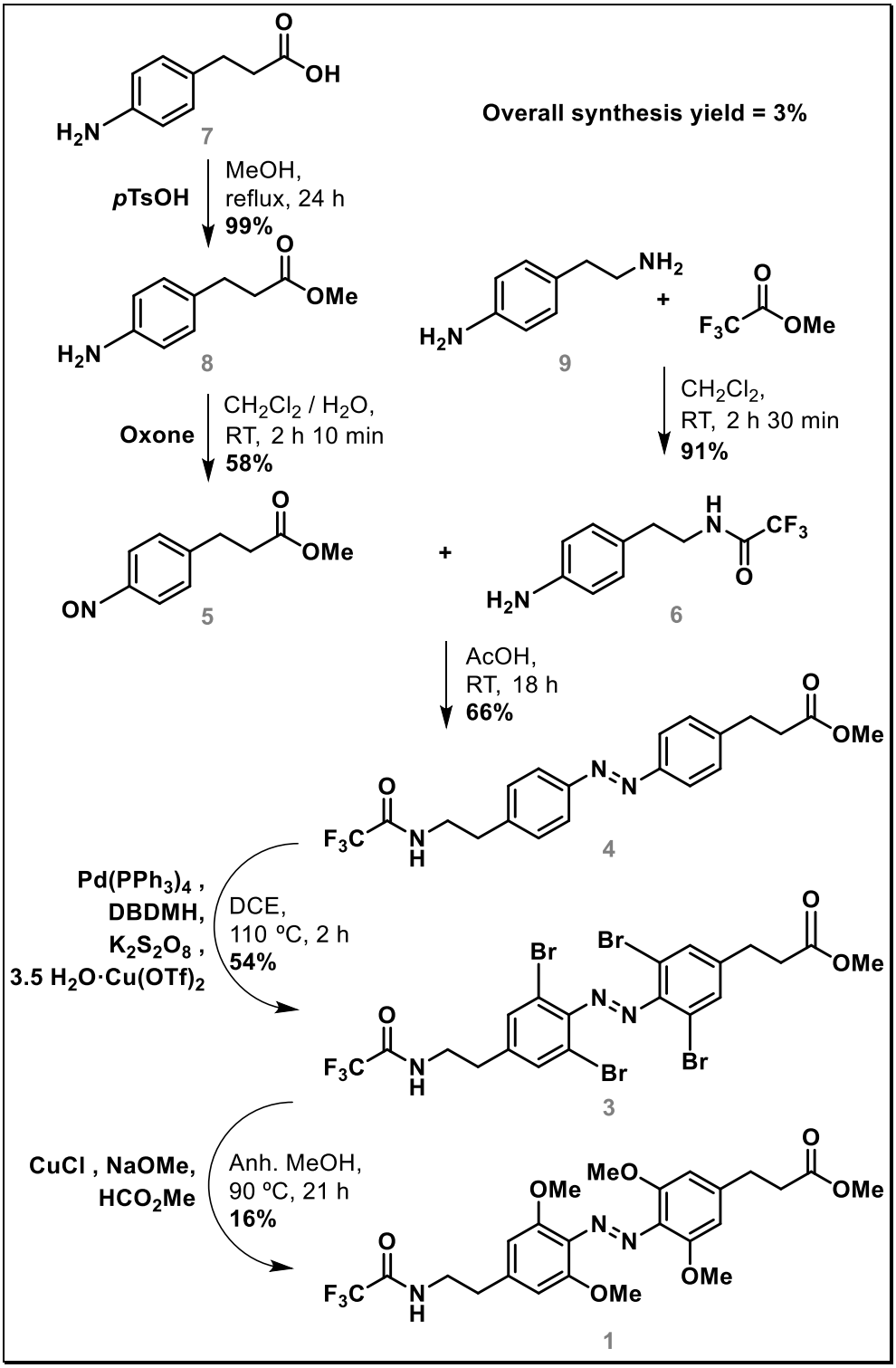
tetra-ortho-brominated azobenzene **3**,[22] but in my hands, only a 16% yield was obtained. Since 4 substitutions on *ortho*-positions must occur, intermediates that have not undergone all the substitutions are observed. Another issue may be due to the degradation by hydrolysis of the trifluoroacetamide and the ester. A solution to this problem would be to use the most anhydrous conditions possible, or once the reaction is done, directly introduce the protecting group for future SPPS to take advantage of this hydrolysis, which is the step that needs to be done prior to the protection of the amino acid group with Fmoc.



Scheme 7. *Copper-catalyzed methoxylation to obtain target tetra-ortho-methoxylated azobenzene **1**.*

To summarize the synthetic route performed, the first step was the esterification of aniline **7**, followed by the oxidation of the aniline **8**, to obtain nitroso compound **5**. On the other hand, aniline **6** was synthesized by monotrifluoroacetamidation of diamine **9**. The Baeyer-Mills reaction was performed with compounds **5** and **6** as starting materials for the formation of the azobenzene **4**. Then, Palladium-catalyzed C-H bromination was carried out prior to the subsequent methoxylation, finally furnishing the target tetra-*ortho*-methoxylated azobenzene **1**.

Overall, from the starting materials until the obtention of the target methoxylated azobenzene **1**, a synthesis with an overall yield of 3% was achieved (Scheme 8).



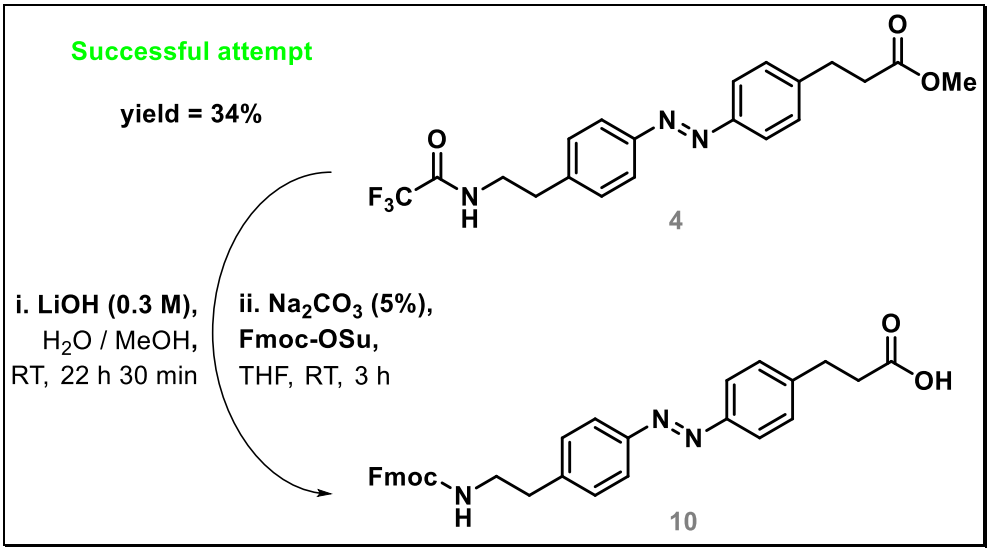
Scheme 8. *General synthetic pathway of the target methoxylated azobenzene* ***1*** *and its overall yield.*

3.2. Protecting group exchange for future SPPS applications

After the obtention of the target compound **1** (Scheme 8), the goal is to test and optimize the yields of the reprotection with Fmoc group for red-shifted azobenzenes. Non-substituted and tetra-*ortho*-methoxylated azobenzenes obtained on the synthesis of the work were used to test different conditions to optimize this protection strategy.

3.2.1. Protection with Fmoc of the non-substituted azobenzene

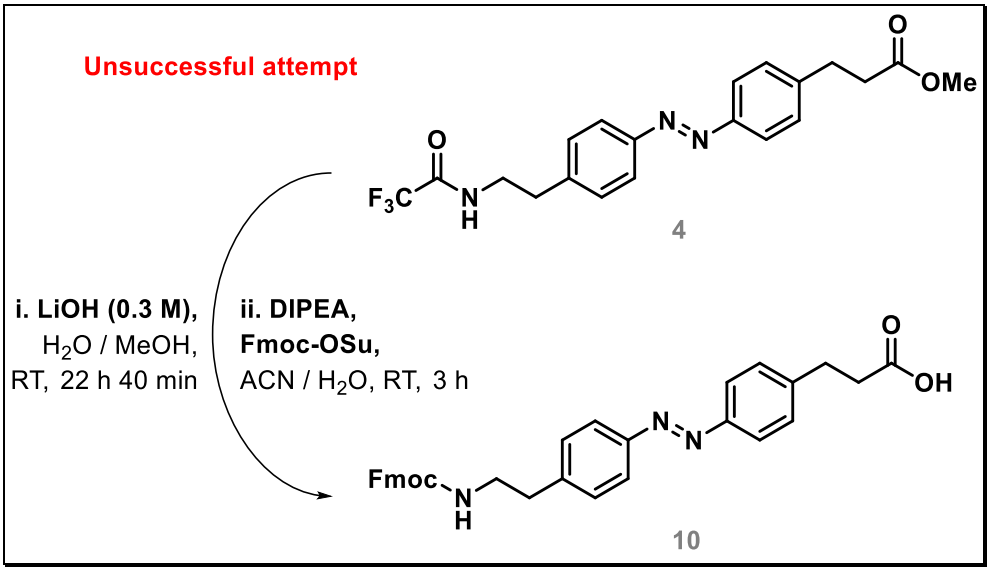
To start off, hydrolysis of the two functional groups in non-substituted azobenzene **4** was performed, for the subsequent protection with Fmoc under conditions described in Scheme 9.



Scheme 9. *Successful reprotection attempt with Fmoc for non-substituted compound **4**.*

Although the product was very insoluble and difficult to purify, the target compound **10** was obtained successfully. A 34% yield was obtained.

Considering the low yield of product **10** obtained during the one-pot hydrolysis and Fmoc protection of compound **4**, a decision was made to try alternative conditions for the second step of the sequence. For instance, DIPEA was used as a base instead, but in our hands this method failed to provide the desired product (Scheme 10).

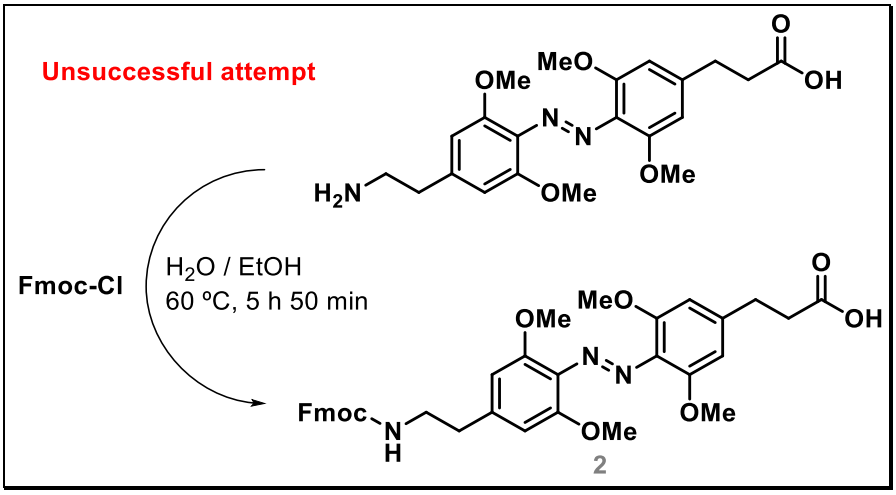


Scheme 10. *Unsuccessful reprotection attempt with Fmoc for non-substituted compound **4** under different conditions from attempt of Scheme 9.*

It is important to point out that due to Fmoc being a base-labile group and run under strong basic conditions, pH levels were checked while setting up the reaction to match the pH in reported precedents.

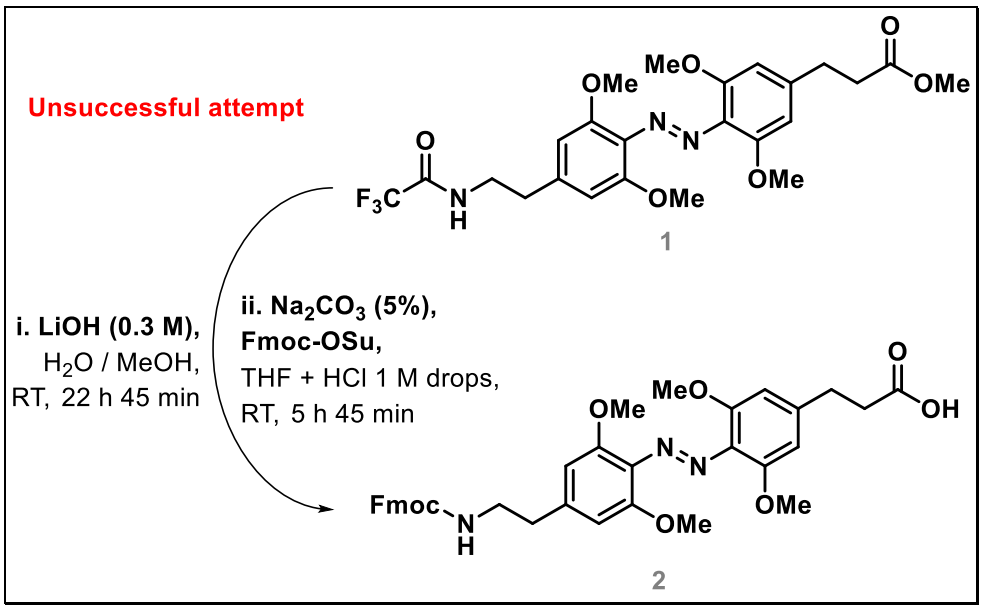
3.2.2. Protection with Fmoc of the tetra-*ortho*-methoxylated azobenzene

Since it was suspected that compound **8** was being hydrolyzed during its syntheses, it was decided to take advantage of the situation and directly attempt Fmoc protection of the crude under conditions described in Scheme 11. However, we could not identify the target product **2** by ^{1}H-NMR, so it was defined as an unsuccessful attempt.



Scheme 11. Unsuccessful reprotection attempt with Fmoc for the hydrolyzed methoxylated azobenzene.

Thus, the plan of using the conditions of the successful attempt of Scheme 9 on the tetra-ortho-methoxylated azobenzene **1** was tested, and alike the previous attempt, the obtention of the target compound **2** was not achieved (Scheme 12).

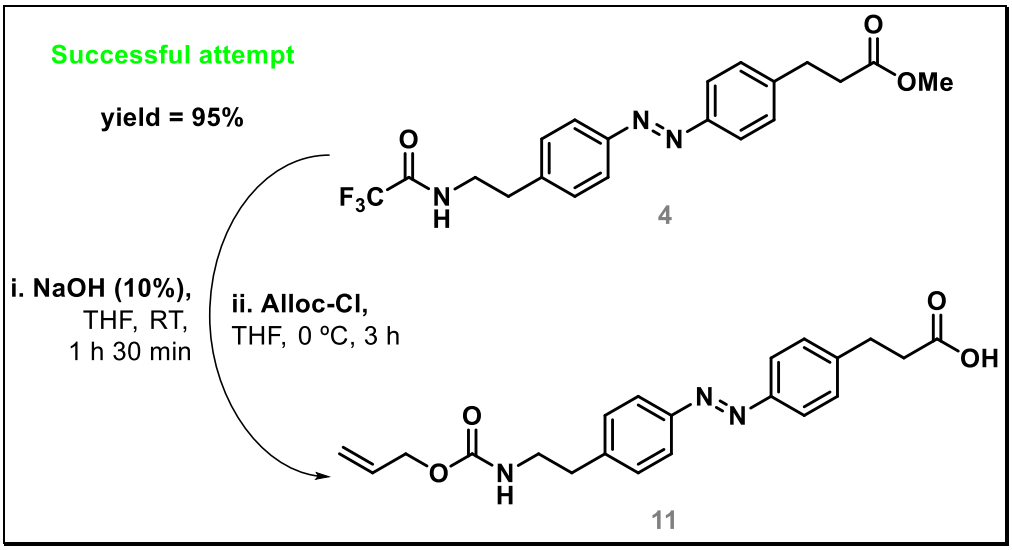


*Scheme 12. Unsuccessful reprotection attempt with Fmoc for methoxylated azobenzene **1** under different conditions from attempt of Scheme 11.*

3.2.3. Protection with Alloc of the non-substituted azobenzene

Due to the inability to obtain the desired result from the previous attempts with Fmoc protection of the tetra-*ortho*-methoxylated azobenzene, an alternative protecting group that could be interesting to try on the azobenzenes is Alloc, as it is completely compatible with Fmoc strategies. After the protection is done, catalytic amounts of $Pd(PPh_3)_4$ in the presence of phenylsilane ($PhSiH_3$) can be used in neutral conditions for the removal of the Alloc group,[21-23] instead of the use of piperidine for Fmoc. Moreover, once the photoswitch is introduced in the peptide and the Alloc group is removed, the product would be indistinguishable from the one that would have been obtained if the Fmoc-protecvted amino acid had been used.

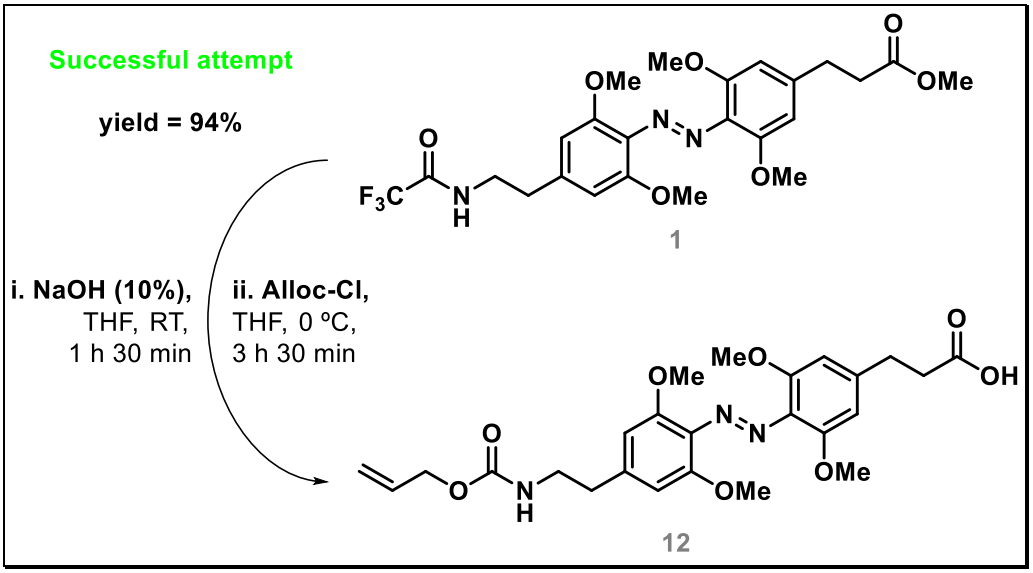
Our first attempt with Alloc protection of the non-substituted azobenzene **4** (Scheme 13) resulted on a high yield reaction (95%). These results made us decide to try these conditions[21] on the methoxylated azobenzene.



Scheme 13. *Successful reprotection attempt with Alloc for non-substituted compound* ***4****.*

3.2.4. Protection with Alloc of the tetra-*ortho*-methoxylated azobenzene

Therefore, similar conditions were tested for the Alloc protection of the tetra-*ortho*-methoxylated azobenzene **1** (Scheme 14). A successful attempt with Alloc instead of Fmoc group was achieved, reaching a yield of 94%.



Scheme 14. *Successful reprotection attempt with Alloc for methoxylated azobenzene **1**.*

4. CONCLUSIONS

The focus of this project was to achieve and synthesize red-shifted azobenzenes amino acids for Solid Phase Synthesis, and although photochemical characterization and UV Vis spectra could not be performed due to the lack of time of my thesis within the group, the obtention of the target tetra-*ortho*-methoxylated azobenzene **1** was achieved and protection with groups commonly used for SPPS ended with successful results.

The desired tetra-*ortho*-methoxylated azobenzene **1** was obtained despite the yield for the copper-catalyzed methoxylation step was lower than expected. Future efforts will require an optimization of the last stages of the synthesis to develop an efficient scale up of the process.

For the attempts of protection with Fmoc and Alloc of the non-substituted azobenzene **4** and methoxylated azobenzene **1**, protection with Alloc group holds more promise and resulted in much higher yields (Scheme 13 and 14) to obtain the protected azobenzene amino acid, that also works as a protecting group for SPPS. The removal of Alloc requires Pd,[26] therefore Alloc or other Pd-labile protecting groups should be avoided in the sequence during SPPS before the tetra-*ortho*-methoxylated azobenzene amino acid is introduced.

5. MATERIALS AND METHODS

5.1. General information

All solvents and reagents used were purchased from commercial suppliers and used without further purification.

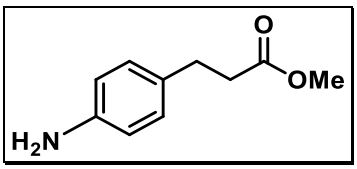
^{1}H-NMR spectra were obtained at room temperature on a Bruker 400 MHz spectrometer in the NMR Unit of CCiTUB (Scientific and Technological Centre of the University of Barcelona). ^{13}C-NMR spectra were obtained at 100 MHz. All NMR spectra were processed using MestReNova Lite NMR software. Chemical shifts are reported in parts per million (ppm) and coupling constants (*J*) are reported in Hz. Splitting patterns are reported as follows: singlet (s), doublet (d), triplet (t), quadruplet (q), quintuplet (quint), doublet of doublets (dd), doublet of doublets of doublets (ddd), multiplet (m), etc. NMR signals were assigned using the appropriate 2D NMR experiments, such as HSQC.

TLC analysis was carried out on aluminum sheets coated with silica gel and visualized using UV light.

ChemDraw software was used to draw and schematize compounds and reactions.

5.2. Synthesis and characterization

5.2.1. Methyl 3-(4-aminophenyl)propanoate (8)



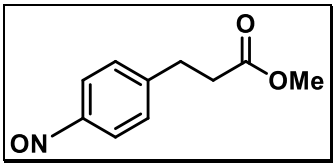
A solution of commercially available 4-(aminophenyl)propionic acid **7** (2.00 g, 12.1 mmol) and p-TsOH (2.50 g, 14.5 mmol) in MeOH (37 mL) was stirred at reflux overnight. After cooling to room temperature, the solvent was evaporated under vacuum. The crude product was partitioned between saturated aqueous $NaHCO_3$ (20 mL) and EtOAc. Layers were separated and the aqueous fraction extracted again with more EtOAc (2 × 20 mL). The final product was

obtained as a brown solid (2.15 g, 99 %). The product matched the one reported in the literature.[22]

^{1}H-NMR (400 MHz, $CDCl_3$): δ 6.98 (d, *J* = 8.4 Hz, 2H), 6.63 (d, *J* = 8.4 Hz, 2H), 3.66 (s, 3H), 2.84 (t, *J* = 8 Hz, 2H), 2.57 (t, *J* = 8 Hz, 2H) ppm.

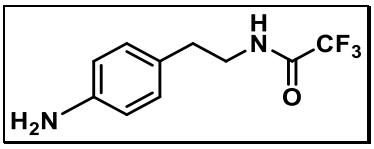
NH_2 protons were not observed.

5.2.2. Methyl 3-(4-nitrosophenyl)propanoate (5)



A mixture of aniline **8** (1.00 g, 5.58 mmol) in CH_2Cl_2 and Oxone (3.43 g, 11.2 mmol) in H_2O (30 mL) was stirred at room temperature. After 2 h, layers were separated and the aqueous fraction extracted again with more CH_2Cl_2 (3 × 20 mL). Organic layers were combined and washed with 1 M HCl, $NaHCO_3$ sat., H_2O and brine and dried (Na_2SO_4). The solvent was evaporated under vacuum and the final product was obtained as a brownish green solid (630 mg, 58 %). The product thus obtained was used without further purification.

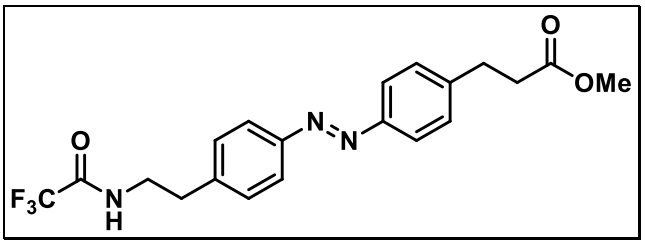
5.2.3. *N*-(4-Aminophenethyl)-2,2,2-trifluoroacetamide (6)



To a stirring solution of compound **9** (2.05 g, 15.0 mmol) in CH_2Cl_2 (75 mL), methyl trifluoroacetate (1.5 mL, 15.0 mmol) was added. After 23 h at room temperature, the solvent was removed under vacuum and the crude product was purified by silica flash column chromatography (CH_2Cl_2 / MeOH, 100:0 to 98:2), affording product the title compound as a yellow solid (3.56 g, 91 %). The product matched the one reported in the literature.[22]

^{1}H-NMR (400 MHz, $CDCl_3$): δ 6.97 (d, *J* = 8.4 Hz, 2H), 6.66 (d, *J* = 8.4 Hz, 2H), 6.30 (bs, 1H), 3.64 (s, 2H), 3.55 (m, 2H), 2.76 (t, *J* = 7.2 Hz, 2H) ppm.

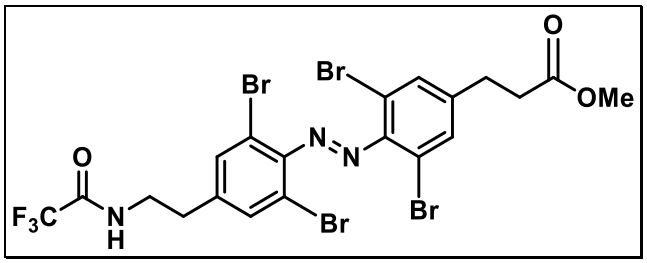
5.2.4. Methyl (*E*)-3-(4-((4-(2-(2,2,2trifluoroacetamido)ethyl)phenyl)diazenyl)phenyl) propanoate (4)



To a stirring solution of compound **5** (630 mg, 3.26 mmol) in AcOH (16 mL) under N_2 compound **6** (757 mg, 3.26 mmol) was added. After 18 h stirring at room temperature the solvent was removed under vacuum and the crude product was purified by silica flash column chromatography (hexane / EtOAc, 85:15 to 60:40), affording the title product as an orange solid (980 mg, 66 %). The product matched the one reported in the literature.[22]

^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.87 (m, 4H), 7.35 (m, 4H), 6.31 (s, 1H), 3.69 (s, 3H), 3.66 (m, 2H), 3.04 (t, *J* = 7.6 Hz, 2H), 2.98 (t, *J* = 7.6 Hz, 2H), 2.69 (t, *J* = 7.6 Hz, 2H) ppm.

5.2.5. Methyl (*E*)-3-(3,5-dibromo-4-((2,6-dibromo-4-(2-(2,2,2-trifluoroacetamido)ethyl)phenyl)diazenyl)phenyl)propanoate (3)

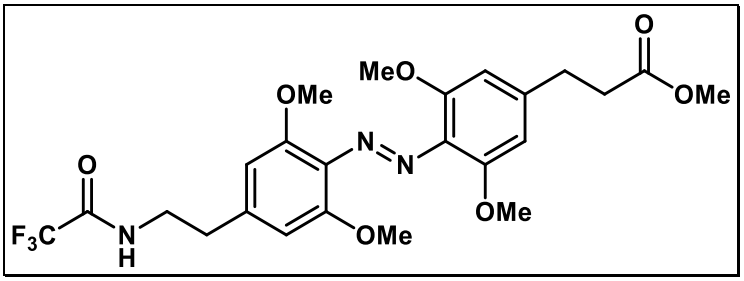


To a thick-wall glass reaction tube charged with compound **4** (240 mg, 0.59 mmol), $Pd(PPh_3)_4$ (68 mg, 0.059 mmol), DBDMH (506 mg, 1.77 mmol), $K_2S_2O_8$ (191 mg, 0.71 mmol) and $Cu(OTf)_2$ · 3.5 H_2O (107 mg, 0.30 mmol) was added DCE (7.5 mL) before the tube was sealed under air

and the mixture stirred at 110 °C. After 2 h, the mixture was allowed to cool to room temperature. The reaction mixture was filtered over Celite®, washed with EtOAc and the filtrate was concentrated under vacuum. The crude product was purified by silica gel column chromatography (hexane / EtOAc, 90:10) to obtain the title compound as a red solid (215 mg, 54 %). The product matched the one reported in the literature.[22]

^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.55 (s, 2H), 7.52 (s, 2H), 6.58 (bs, 1H), 3.70 (s, 3H), 3.63 (m, 2H), 2.96 (t, *J* = 7.6 Hz, 2H), 2.92 (t, *J* = 7.6 Hz, 2H), 2.67 (t, *J* = 7.6 Hz, 2H) ppm.

5.2.6. Methyl (*E*)-3-(4-((2,6-dimethoxy-4-(2-(2,2,2-trifluoroacetamido)ethyl)phenyl)diazenyl)-3,5-dimethoxyphenyl)propanoate (1)

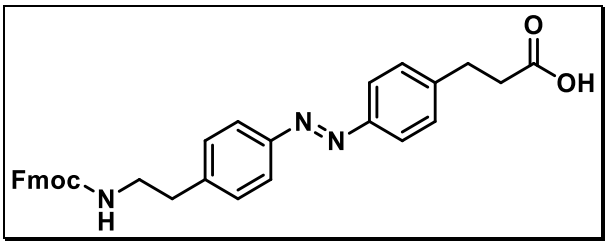


To a thick-wall glass reaction tube charged with compound **3** (215 mg, 0.297 mmol), CuCl (5.5 mg, 0.059 mmol) and NaOMe (80 mg, 1.49 mmol) and methyl formate (29 µL, 0.48 mmol) was added MeOH (7.4 mL) before the tube was sealed and the mixture stirred at 90 °C. After 21 h the mixture was allowed to cool to room temperature. The reaction mixture was filtered over Celite® with the aid of EtOAc, and the filtrate was concentrated under reduced pressure. The crude product was purified by silica gel column chromatography (hexane / EtOAc, 50:50 to 0:100) to obtain the title compound as a red solid (24 mg, 16 %). The product matched the one reported in the literature.[22]

^{1}H-NMR (400 MHz, $CDCl_3$): δ 6.70 (bs, 2H), 6.50 (s, 2H), 6.46 (s, 2H), 3.82 (s, 6H), 3.80 (s, 6H), 3.68 (s, 3H), 3.55 (m, 2H), 3.95 (t, *J* = 7.6 Hz, 2H), 2.88 (t, *J* = 7.6 Hz, 2H), 2.65 (t, *J* = 7.6 Hz, 2H) ppm.

cis isomer is also observed in the ^{1}H-NMR.

5.2.7. (*E*)-3-(4-((4-(2-((((9H-Fluoren-9-yl) methoxy)carbonyl)amino)ethyl)phenyl)diazenyl)phenyl)propanoic acid (10)

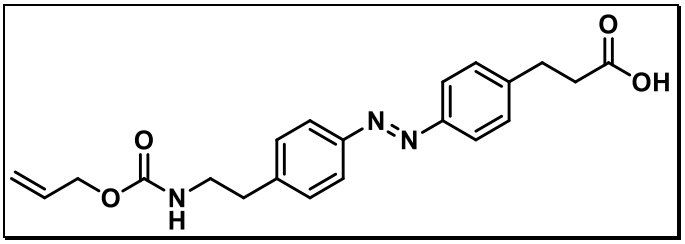


To a stirring solution of compound **4** (20 mg, 0.049 mmol) in a mixture of H_2O / MeOH (1:3, 3.3 mL), an aqueous 5% LiOH (0.82 mL, 0.245 mmol) was added. After 23 h at room temperature, the solvent was removed under vacuum. Then, to a stirring solution of aqueous 5% Na_2CO_3 solution (0.6 mL) charged with the crude amino acid of the first reaction, Fmoc-Osu (24.8 mg, 0.074 mmol) in THF (0.6 mL) was added. After 3 h at room temperature the solvent was removed under vacuum. The crude product was purified by silica gel column chromatography (hexane / EtOAc, 80:20 to 60:40 + AcOH) to obtain the title compound as a yellow solid (9 mg, 34 %).

^{1}H-NMR (400 MHz, DMSO-d_6): δ 7.89 (d, *J* = 7.6 Hz, 2H), 7.78 (d, *J* = 7.6 Hz, 4H), 7.66 (d, *J* = 7.6 Hz, 2H), 7.38 (m, 8H), 4.30 (d, *J* = 6.8 Hz, 2H), 4.20 (t, *J* = 6.8 Hz, 1H), 3.28 (q, *J* = 12.8, 6.8 Hz, 2H),2.91 (t, *J* = 7.2 Hz, 2H), 2.81 (t, *J* = 7.2 Hz, 2H) 2.55 (t, *J* = 7.2 Hz, 2H) ppm.

CO_2H nor NH is were observed by ^{1}H-NMR.

5.2.8. (*E*)-3-(4-((4-(2-(((Allyloxy)carbonyl)amino)ethyl)phenyl)diazenyl)phenyl)propanoic acid (11)



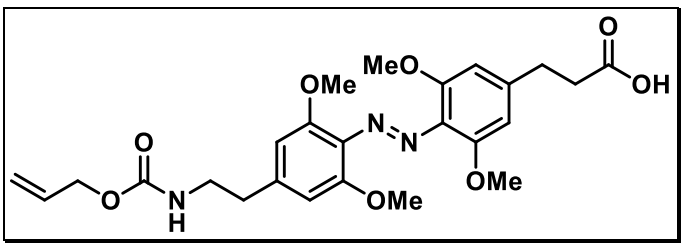
To a flask charged with a solution of compound **4** (100 mg, 0.245 mmol) in THF (0.13 mL) was added 10% aqueous NaOH (0.70 mL). The resulting mixture was stirred at room temperature under air. After 1.5 h the mixture was cooled to 0 °C before adding Alloc-Cl (31 µL, 0.294 mmol). The flask was sealed under air and the reaction mixture was stirred for 3 h at 0 °C. The reaction was quenched with 10% aqueous HCl (1 mL) and extracted with EtOAc (3 × 25 mL). The organic layer was washed with H_2O (3 × 20 mL), dried ($MgSO_4$) and concentrated under vacuum. Analysis of the crude material confirmed full conversion into the desired product and was used without further purification as a yellow solid (89 mg, 95 %).

^{1}H-NMR (400 MHz, DMSO-d_6): δ 12.13 (bs, 1H) 7.80 (m, 4H), 7.45 (d, *J* = 8.4 Hz, 2H), 7.41 (d, *J* = 8.4 Hz, 2H), 7.34 (t, *J* = 5.6 Hz, 1H), 5.88 (ddt, *J* = 12.0, 10.8, 5.2 Hz, 1H), 5.25 (dq, *J* = 17.2, 2.0 Hz, 1H), 5.15 (dq, *J* = 10.8, 1.6 Hz, 1H), 4.45 (dt, *J* = 5.2, 1.6 Hz, 2H), 3.28 (q, *J* = 6.4 Hz, 2H), 2.92 (t, *J* =7.6 Hz, 2H), 2.82 (t, *J* =7.6 Hz, 2H), 2.60 (t, *J* =7.6 Hz, 2H) ppm.

^{13}C-NMR (100 MHz, DMSO-d_6): δ 173.6 (CO_2H), 155.9 ($NHCO_2$), 150.5 (C_{Ar}), 150.4 (C_{Ar}), 144.8 (C_{Ar}), 143.2 (C_{Ar}), 133.8 ($CH_2CH=CH_2$), 129.7 ($C_{Ar}H$), 129.3 ($C_{Ar}H$), 122.5 ($C_{Ar}H$ ×2), 116.9 ($CH_2CH=CH_2$), 64.2 ($CH_2CH=CH_2$), 41.5 (CH_2CH_2NH), 35.3 ($CH_2CH_2CO_2H$), 34.9 (CH_2CH_2NH), 30.2 ($CH_2CH_2CO_2H$) ppm.

HRMS results are pending.

5.2.9. (*E*)-3-(4-((4-(2-(((Allyloxy)carbonyl)amino)ethyl)-2,6-dimethoxyphenyl)diazenyl)-3,5-dimethoxyphenyl)propanoic acid (12)



To a flask charged with a solution of compound **1** (80 mg, 0.151 mmol) in THF (0.06 mL) was added 10% aqueous NaOH (0.25 mL). The resulting mixture was stirred at room temperature under air. After 1.5 h the mixture was cooled to 0 °C before adding Alloc-Cl (16 µL, 0.152

mmol). The flask was sealed under air and the reaction mixture was stirred for 3.5 h at 0 °C. The reaction was quenched with 10% aqueous HCl (1 mL) and extracted with EtOAc (3 × 25 mL). The organic layer was washed with H_2O (3 × 20 mL), dried ($MgSO_4$) and concentrated under vacuum. The crude product was purified by silica gel column chromatography (hexane / EtOAc, 80:20 + AcOH) to obtain the title compound as a black product (72 mg, 94 %).

^{1}H-NMR (400 MHz, DMSO-d_6): δ 7.33 (t, *J* = 5.6 Hz, 1H), 6.67 (s, 2H), 6.61 (s, 2H), 5.90 (m, 1H), 5.26 (dq, *J* = 17.4, 2.0 Hz, 1H), 5.16 (dq, *J* = 10.4, 2.0 Hz, 1H), 4.47 (dt, *J* = 5.2, 1.6 Hz, 2H), 3.71 (s, 6H), 3.70 (s, 6H), 3.30 (m, 2H), 2.85 (t, *J* = 7.2 Hz, 2H), 2.75 (t, *J* = 7.2 Hz, 2H), 2.60 (t, *J* = 7.2 Hz, 2H) ppm.
CO_2H is not observed.

^{13}C-NMR (100 MHz, DMSO-d_6): δ 173.8 (*C*O_2H), 155.9 (NH*C*O_2), 151.6 (*C*$_{Ar}$O), 151.6 (*C*$_{Ar}$O), 143.0 (*C*$_{Ar}$C), 141.4 (*C*$_{Ar}$C), 133.9 (CH_2*C*H=CH_2), 131.9 (*C*$_{Ar}$N), 116.9 (CH_2CH=*C*H_2), 105.7 (*C*$_{Ar}$H), 105.3 (*C*$_{Ar}$H), 64.2 (*C*H_2CH=CH_2), 56.1 (O*C*H_3), 56.0, (O*C*H_3), 41.5 (CH_2*C*H_2NH), 35.9 (*C*H_2CH_2NH), 35.2 (*C*$H_2CH_2CO_2$H), 31.1 (CH_2*C*H_2CO_2H) ppm.

HRMS results are pending.

6. BIBLIOGRAPHY

1. Velema, W. A.; Szymanski, W.; Feringa, B. L. Photopharmacology: beyond proof of principle. *J. Am. Chem. Soc.* **2014**, *136*, 2178. https://doi.org/10.1021/ja413063e.

2. Babii, O.; Afonin, S.; Berditsch, M.; Reiβer, S.; Mykhailiuk, P. K.; Kubyshkin, V. S.; Steinbrecher, T.; Ulrich, A. S.; Komarov, I. V. Controlling Biological Activity with Light: Diarylethene-Containing Cyclic Peptidomimetics. *Angew. Chem. Int. Ed.* **2014**, *53,* 3392. https://doi.org/10.1002/anie.201310019.

3. Fuchter, M. J. On the Promise of Photopharmacology Using Photoswitches: A Medicinal Chemist's Perspective. *J. Med. Chem.* **2020**, *63*, 11436. https://doi.org/10.1021/acs.jmedchem.0c00629.

4. Lewis, K. New approaches to antimicrobial discovery. *Biochem. Pharm.* **2017**, *134*, 87. https://doi.org/10.1016/j.bcp.2016.11.002.

5. Albert, L.; Vázquez, O. Photoswitchable peptides for spatiotemporal control of biological functions. *Chem. Commun.* **2019**, *55*, 10192. https://doi.org/10.1039/c9cc03346g.

6. Konrad, D. B.; Frank, J. A.; Trauner, D. Synthesis of redshifted azobenzene photoswitches by Late-Stage functionalization. *Chem. Eur. J.* **2016**, *22*, 4364. https://doi.org/10.1002/chem.201505061.

7. Samanta, S.; Beharry, A. A.; Sadovski, O.; McCormick, T. M.; Babalhavaeji, A.; Tropepe, V.; Woolley, G. A. Photoswitching Azo Compounds in Vivo with Red Light. *J. Am. Chem. Soc.* **2013**, *135*, 9777. https://doi.org/10.1021/ja402220t.

8. Zhu, M.; Zhou, H. Azobenzene-based small molecular photoswitches for protein modulation. *Org. Biomol. Chem* **2018**, *16*, 8434. https://doi.org/10.1039/c8ob02157k.

9. Zhu, Y.; Li, P.; Liu, C.; Jia, M.; Luo, Y.; He, D.; Liao, C.; Zhang, S. Azobenzene quaternary ammonium salt for photo-controlled and reusable disinfection without drug resistance. *Chin. Chem. Lett.* **2023**, *34*, 107543. https://doi.org/10.1016/j.cclet.2022.05.057.

10. Merino, E. Synthesis of azobenzenes: the coloured pieces of molecular materials. *Chem. Soc. Rev.* **2011**, *40*, 3835. https://doi.org/10.1039/c0cs00183j.

11. Volarić, J.; Buter, J.; Schulte, A. M.; Van Den Berg, K.-O.; Santamaría-Aranda, E.; Szymanski, W.; Feringa, B. L. Design and Synthesis of Visible-Light-Responsive

Azobenzene building blocks for chemical biology. *J. Org. Chem.* **2022**, *87*, 14319. https://doi.org/10.1021/acs.joc.2c01777.

12. Cheng, H.; Zhang, S.; Qi, J.; Liang, X.; Yoon, J. Advances in application of Azobenzene as a trigger in biomedicine: molecular design and spontaneous assembly. *Adv. Mater.* **2021**, *33*, 2007290. https://doi.org/10.1002/adma.202007290.

13. Velema, W. A.; Van Der Berg, J. P.; Hansen, M. J.; Szymanski, W.; Driessen, A. J. M.; Feringa, B. L. Optical control of antibacterial activity. *Nat. Chem.* **2013**, *5*, 924. https://doi.org/10.1038/nchem.1750.

14. Hansen, M. J.; Lerch, M. M.; Szymanski, W.; Feringa, B. L. Direct and versatile synthesis of Red-Shifted azobenzenes. *Angew. Chem. Int. Ed.* **2016**, *55*, 13514. https://doi.org/10.1002/anie.201607529.

15. Wegener, M.; Hansen, M. J.; Driessen, A. J. M.; Szymanski, W.; Feringa, B. L. Photocontrol of Antibacterial Activity: Shifting from UV to Red Light Activation. *J. Am. Chem. Soc.* **2017**, *139*, 17979. https://doi.org/10.1021/jacs.7b09281.

16. Isidro-Llobet, A.; Álvarez, M.; Albericio, F. Amino Acid-Protecting Groups. *Chem. Rev.* **2009**, *109*, 2455. https://doi.org/10.1021/cr800323s.

17. World Health Organization: WHO. *Antimicrobial resistance*. https://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance.

18. Woolley, G. A. Photocontrolling Peptide α Helices. *Acc. Chem. Res.* **2005**, *38*, 486. https://doi.org/10.1021/ar040091v.

19. Hüll, K.; Morstein, J.; Trauner, D. *In vivo* photopharmacology. *Chem. Rev.* **2018**, *118*, 10710. https://doi.org/10.1021/acs.chemrev.8b00037.

20. Lāce, I.; Bazzi, S.; Uranga, J.; Schirmacher, A.; Diederichsen, U.; Mata, R. A.; Simeth, N. A. Modulating secondary structure motifs through Photo-Labile peptide staples. *ChemBioChem* **2023**, *24*. https://doi.org/10.1002/cbic.202300270.

21. Just-Baringo, X.; Yeste-Vázquez, A.; Moreno-Morales, J.; Ballesté-Delpierre, C.; Vila, J.; Giralt, E. Controlling Antibacterial Activity Exclusively with Visible Light: Introducing a Tetra-ortho-Chloro-Azobenzene Amino Acid. *Chem. Rev.* **2021**, *27*, 12987. https://doi.org/10.1002/chem.202102370.

22. Ruiz-Soriano A, Lamelza L, Pizzamiglio E, Just-Baringo X. Synthesis of Tetra-ortho-Methoxylated Azobenzene Photoswitches via Sequential Catalytic C−H Activation and Methoxylation. *ChemRxiv* **2023** https://doi.org/10.26434/chemrxiv-2023-dr111.

23. Liu, Q.; Luo, X.; Wei, S.; Wang, Y.; Zhu, J.; Liu, Y.; Quan, F.; Zhang, M.; Xia, C. Palladium-catalyzed direct ortho C X bond construction via C H activation of azobenzenes: Synthesis of (E)-1,2-b(2,6-dibromo(chloro)-phenyl)diazene. *Tetrahedron Lett.* **2019**, *60*, 1715. https://doi.org/10.1016/j.tetlet.2019.05.056.

24. Müller-Deku, A.; Thorn-Seshold, O. Exhaustive catalytic ortho-Alkoxylation of azobenzenes: flexible access to functionally diverse Yellow-Light-Responsive photoswitches. *J. Org. Chem.* **2022**, *87*, 16526. https://doi.org/10.1021/acs.joc.2c02214.

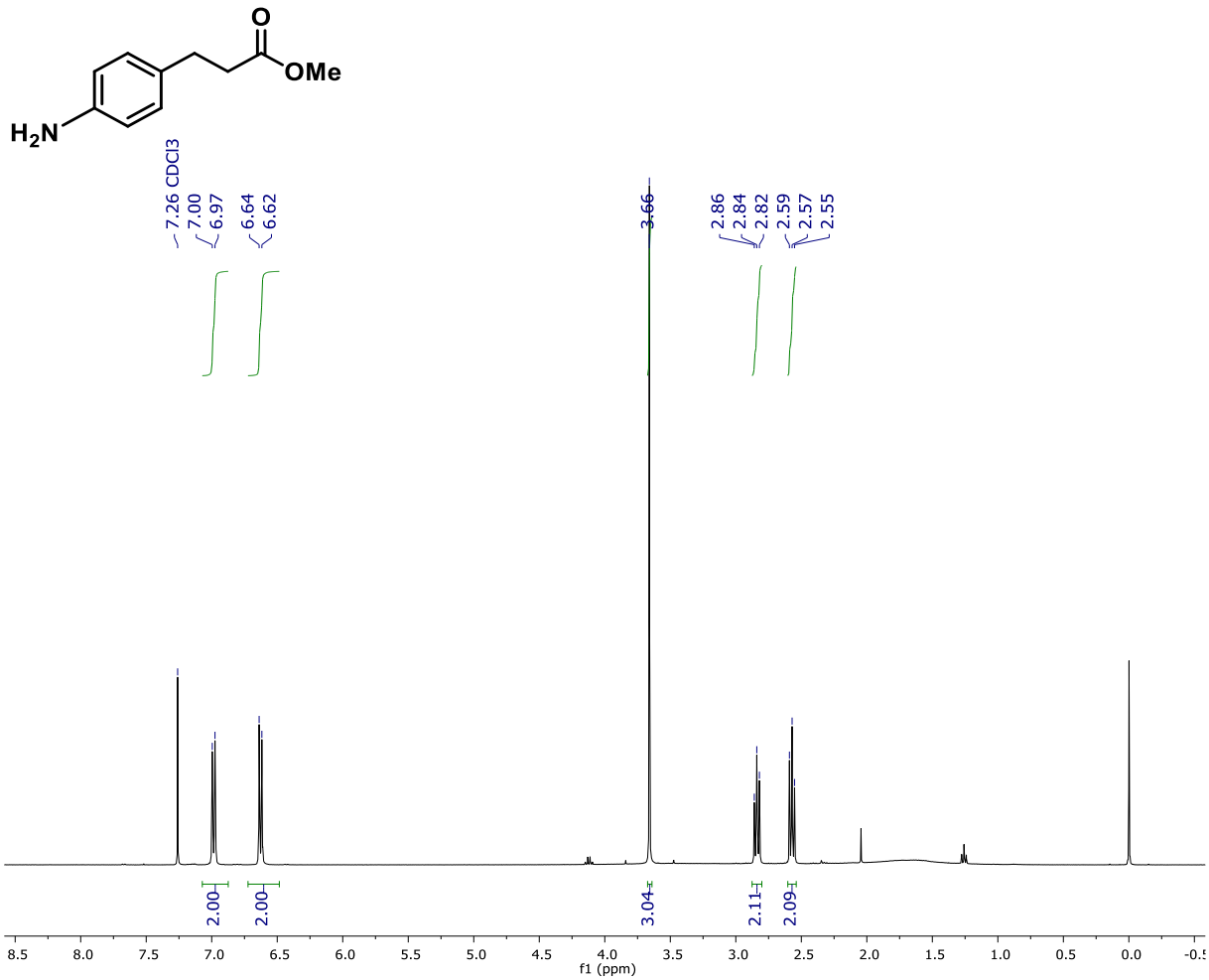
25. Ghorai, M. K.; Das, K.; Kumar, A. An efficient synthetic route to substituted tetrahydropyrimidines by $Cu(OTf)_2$-mediated nucleophilic ring-opening followed by the [4+2] cycloaddition of N-tosylazetidines with nitriles. *Tetrahedron Lett.* **2009**, *50*, 1105. https://doi.org/10.1016/j.tetlet.2008.12.035.

26. Thieriet, N.; Alsina, J.; Giralt, E.; Guibé, F.; Albericio, F. Use of Alloc-amino acids in solid-phase peptide synthesis. Tandem deprotection-coupling reactions using neutral conditions. *Tetrahedron Lett.* **1997**, *38*, 7275. https://doi.org/10.1016/s0040-4039(97)01690-0.

7. ANNEX NMR SPECTRA

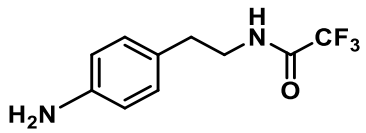
Methyl 3-(4-aminophenyl)propanoate (8)

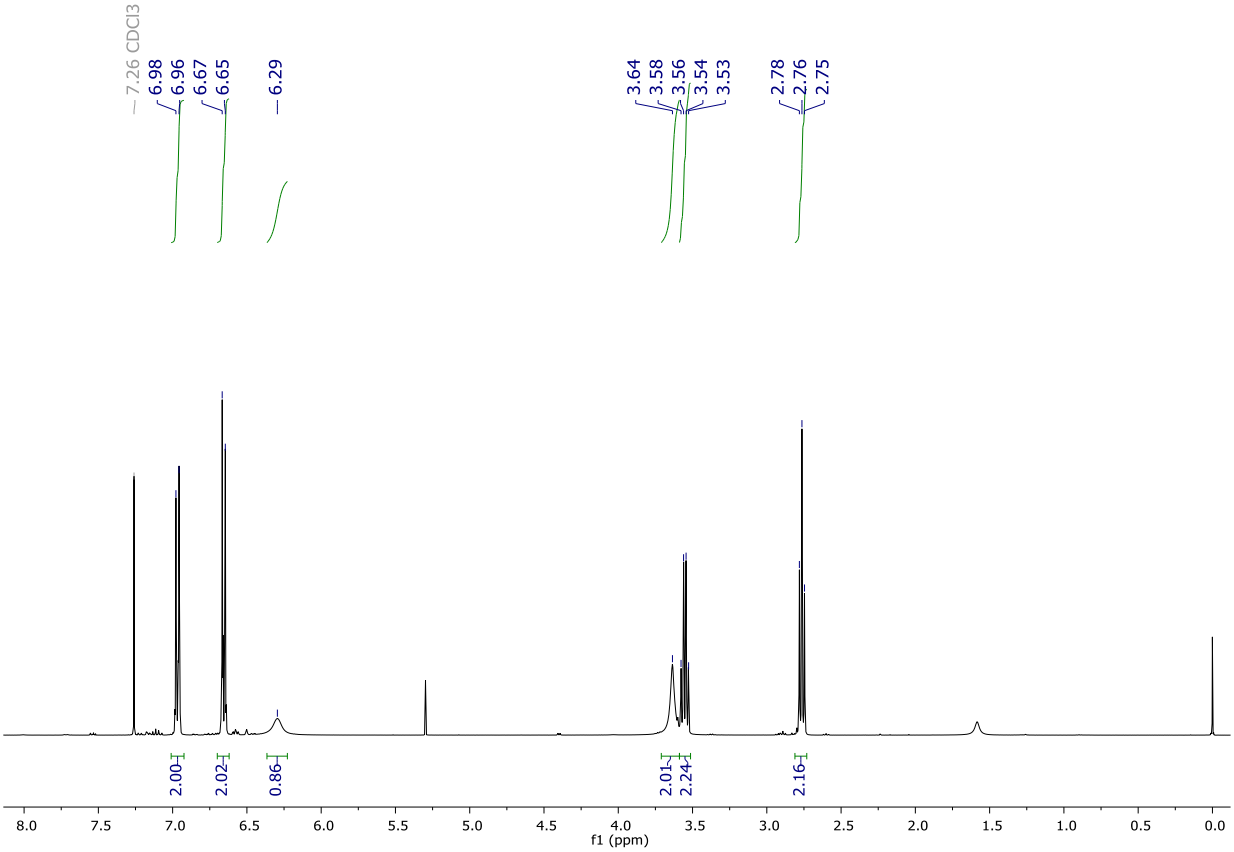
^{1}H-NMR (400 MHz, $CDCl_3$):



N-(4-aminophenethyl)-2,2,2-trifluoroacetamide (6)

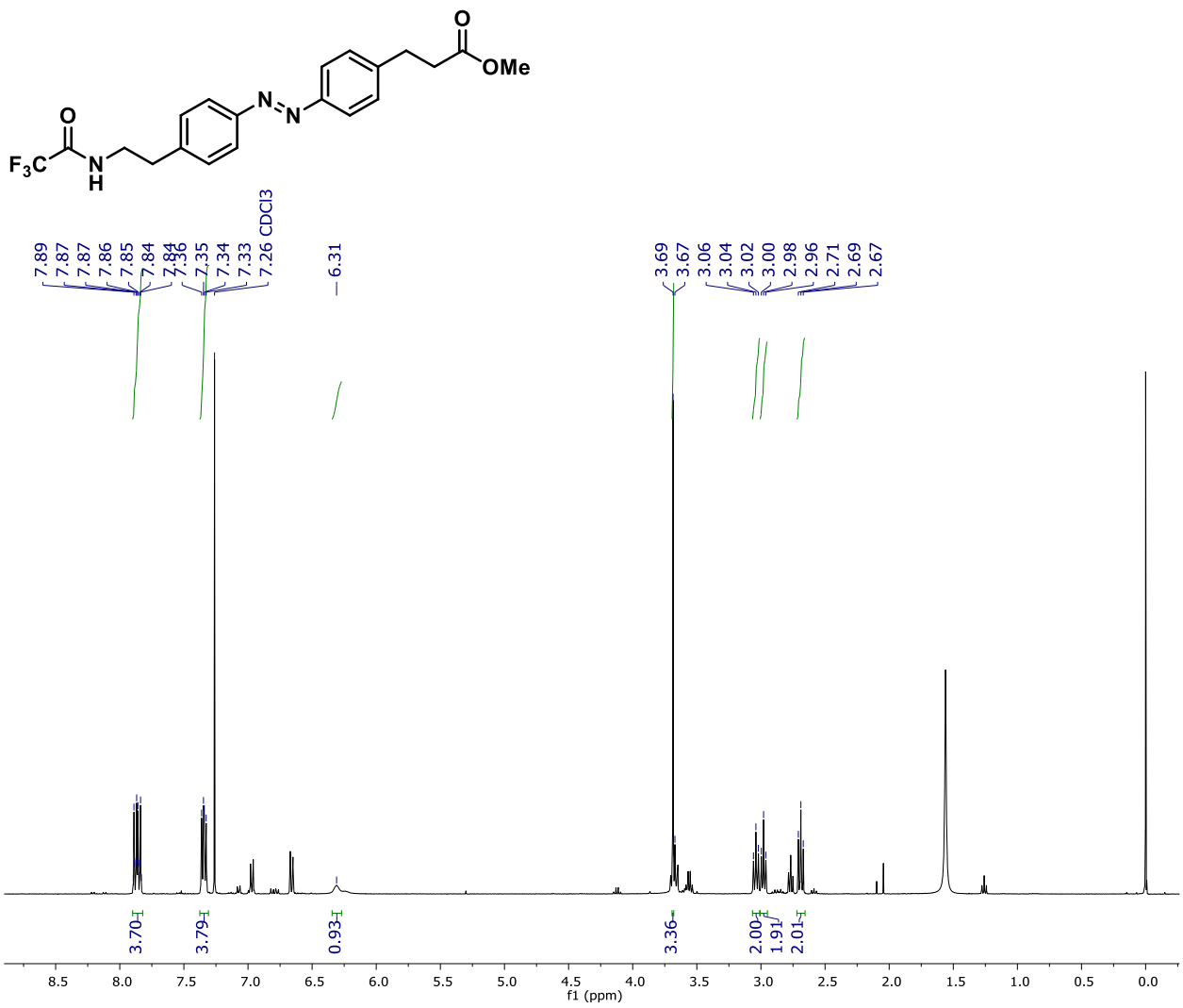
^{1}H-NMR (400 MHz, $CDCl_3$):





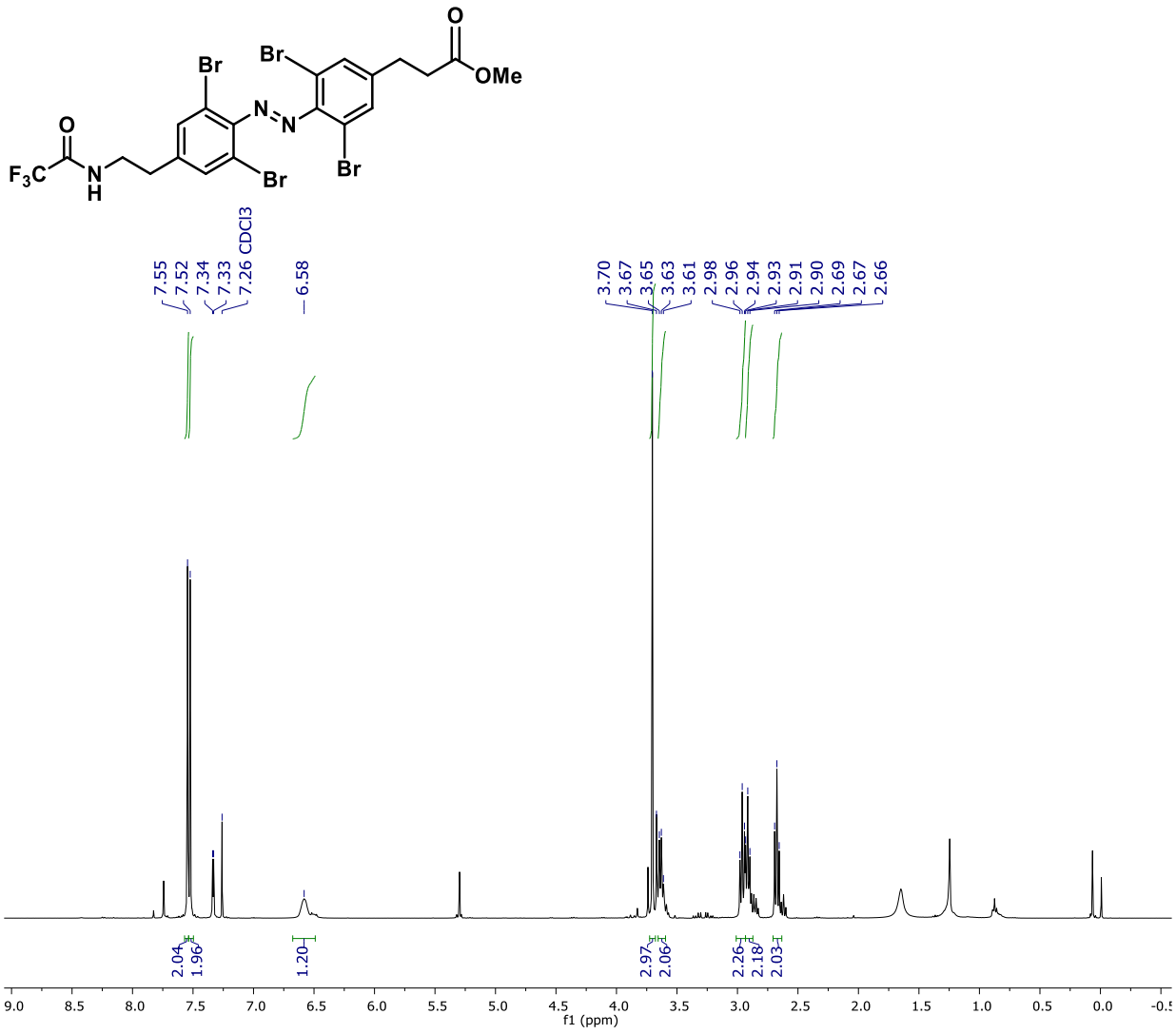
Methyl (*E*)-3-(4-((4-(2-(2,2,2trifluoroacetamido)ethyl)phenyl)diazenyl)phenyl)propanoate (4)

^{1}H-NMR (400 MHz, $CDCl_3$):



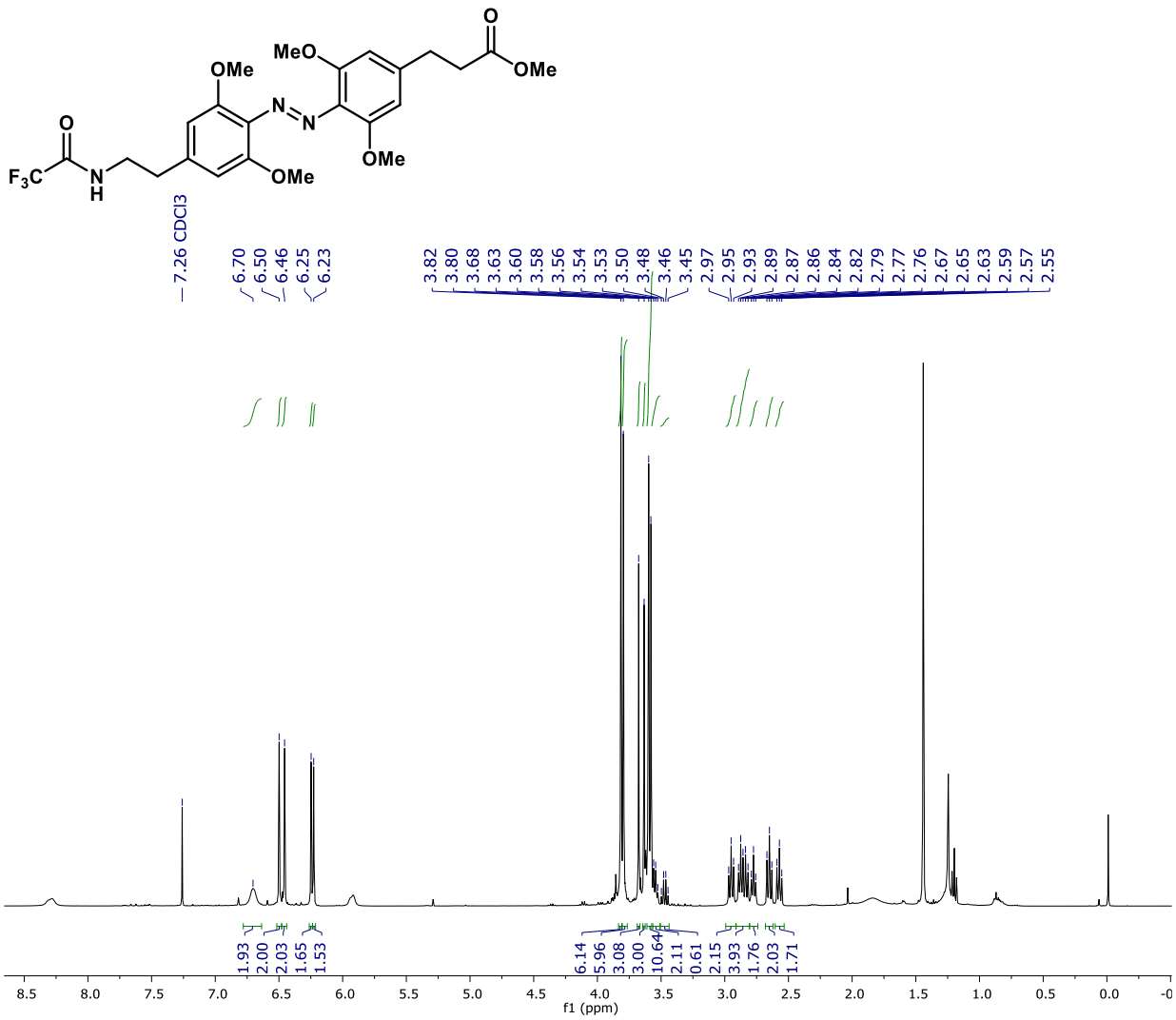
Methyl (*E*)-3-(3,5-dibromo-4-((2,6-dibromo-4-(2-(2,2,2-trifluoroacetamido)ethyl)phenyl)diazenyl)phenyl)propanoate (3)

^{1}H-NMR (400 MHz, $CDCl_3$):



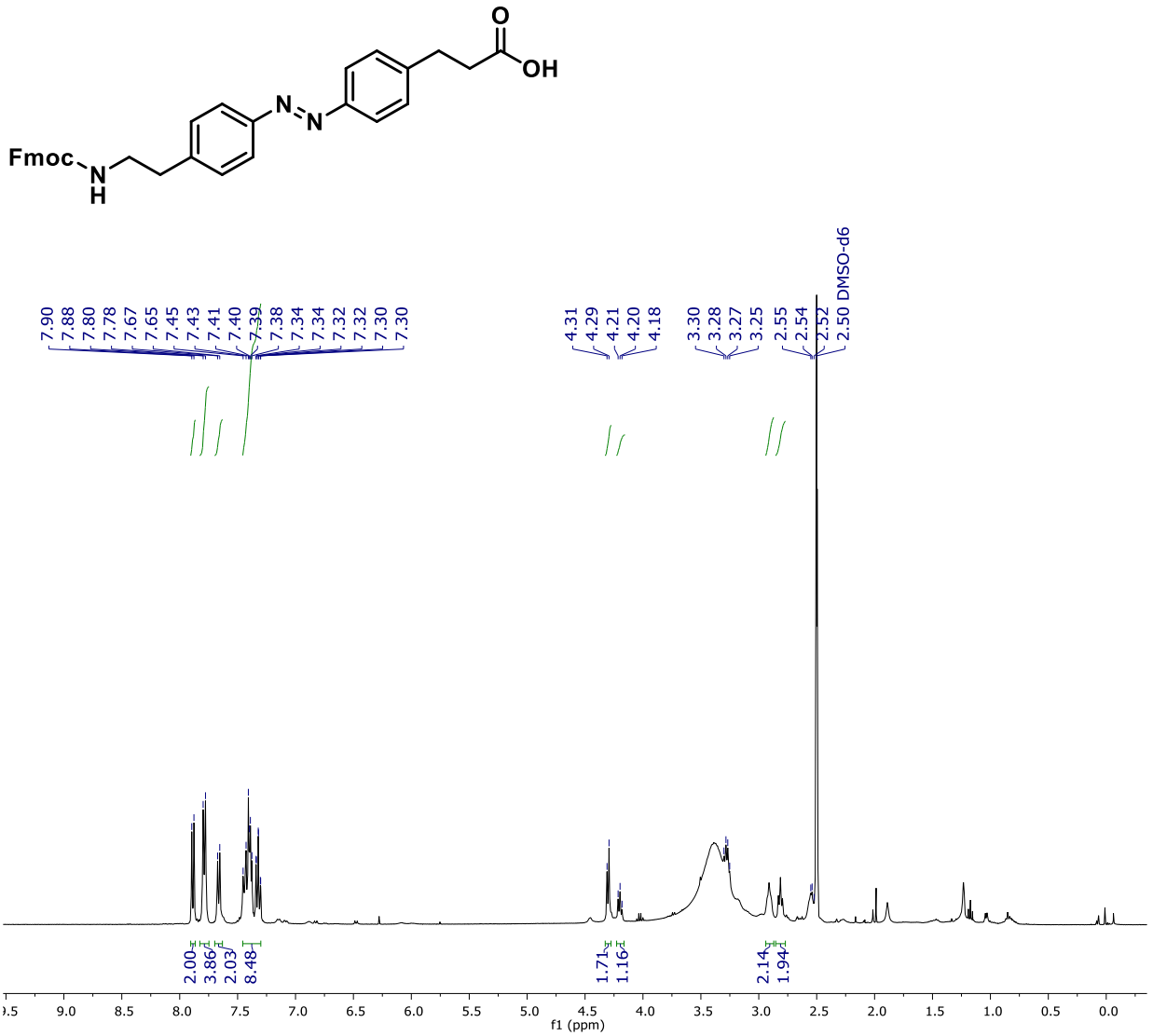
Methyl (*E*)-3-(4-((2,6-dimethoxy-4-(2-(2,2,2-trifluoroacetamido)ethyl)phenyl)diazenyl)-3,5-dimethoxyphenyl)propanoate (1)

^{1}H-NMR (400 MHz, $CDCl_3$):



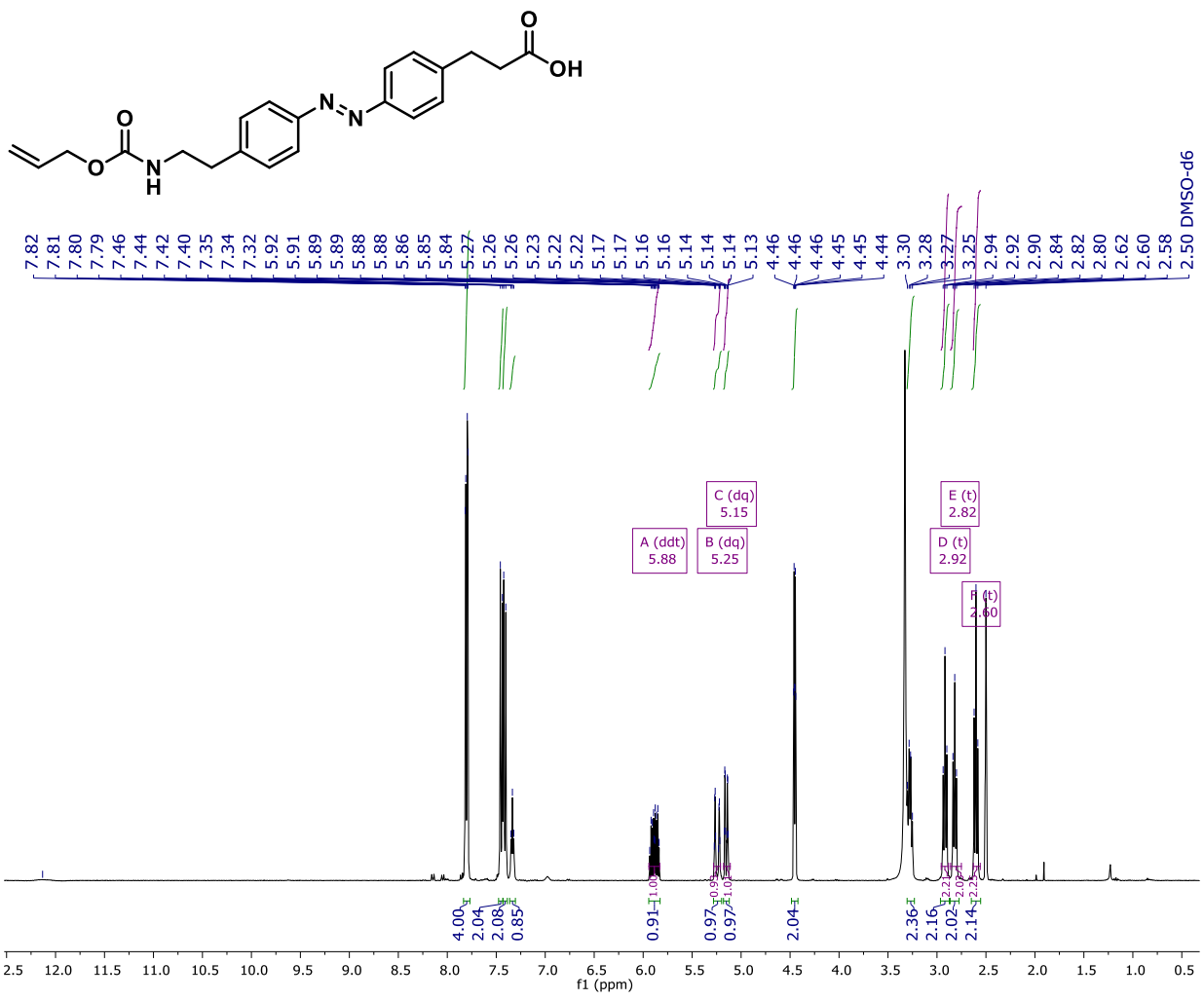
Methyl (*E*)-3-(4-((4-(2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)ethyl)-2,6-dimethoxyphenyl)diazenyl)-3,5-dimethoxyphenyl)propanoate (10)

^{1}H-NMR (400 MHz, DMSO-d_6):

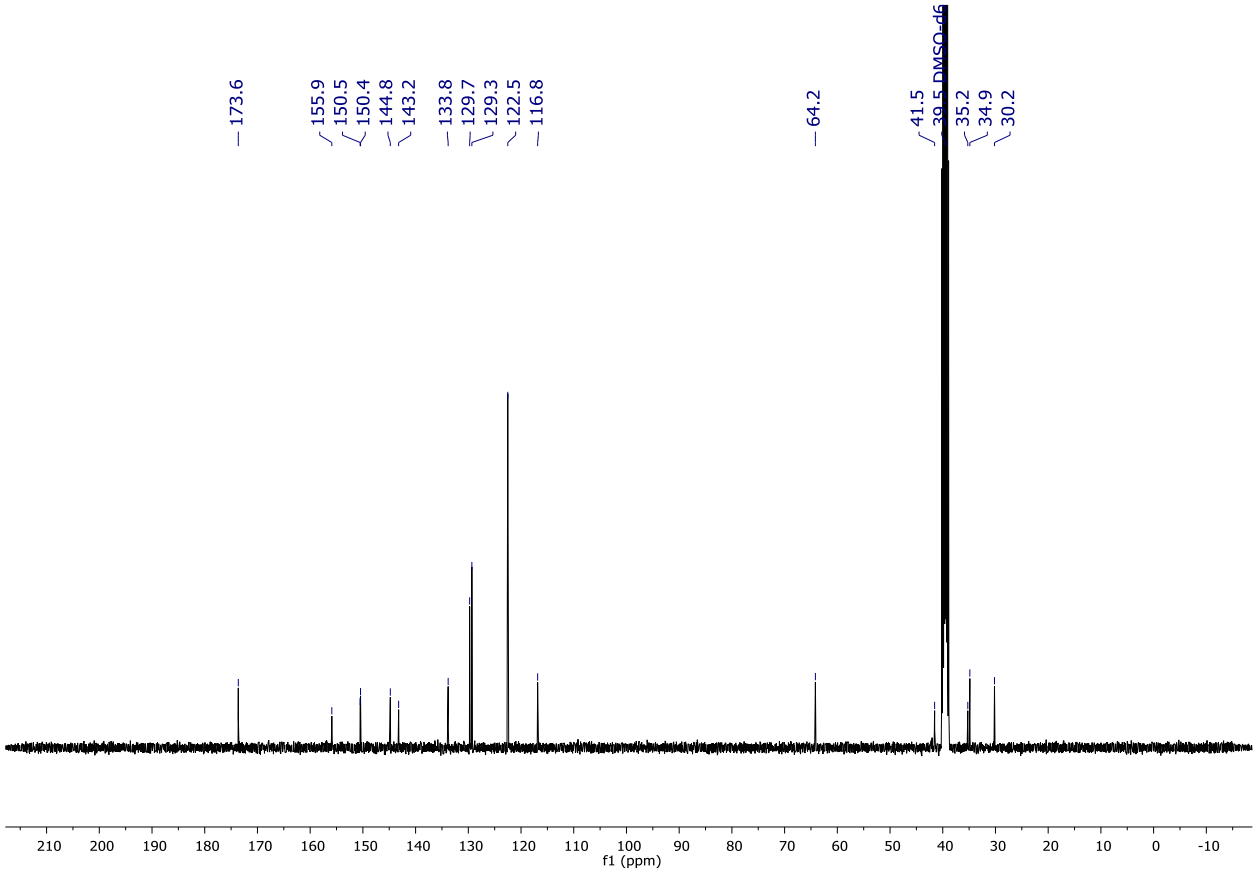


(*E*)-3-(4-((4-(2-(((Allyloxy)carbonyl)amino)ethyl)phenyl)diazenyl)phenyl)propanoic acid (11)

^{1}H-NMR (400 MHz, DMSO-d_6):

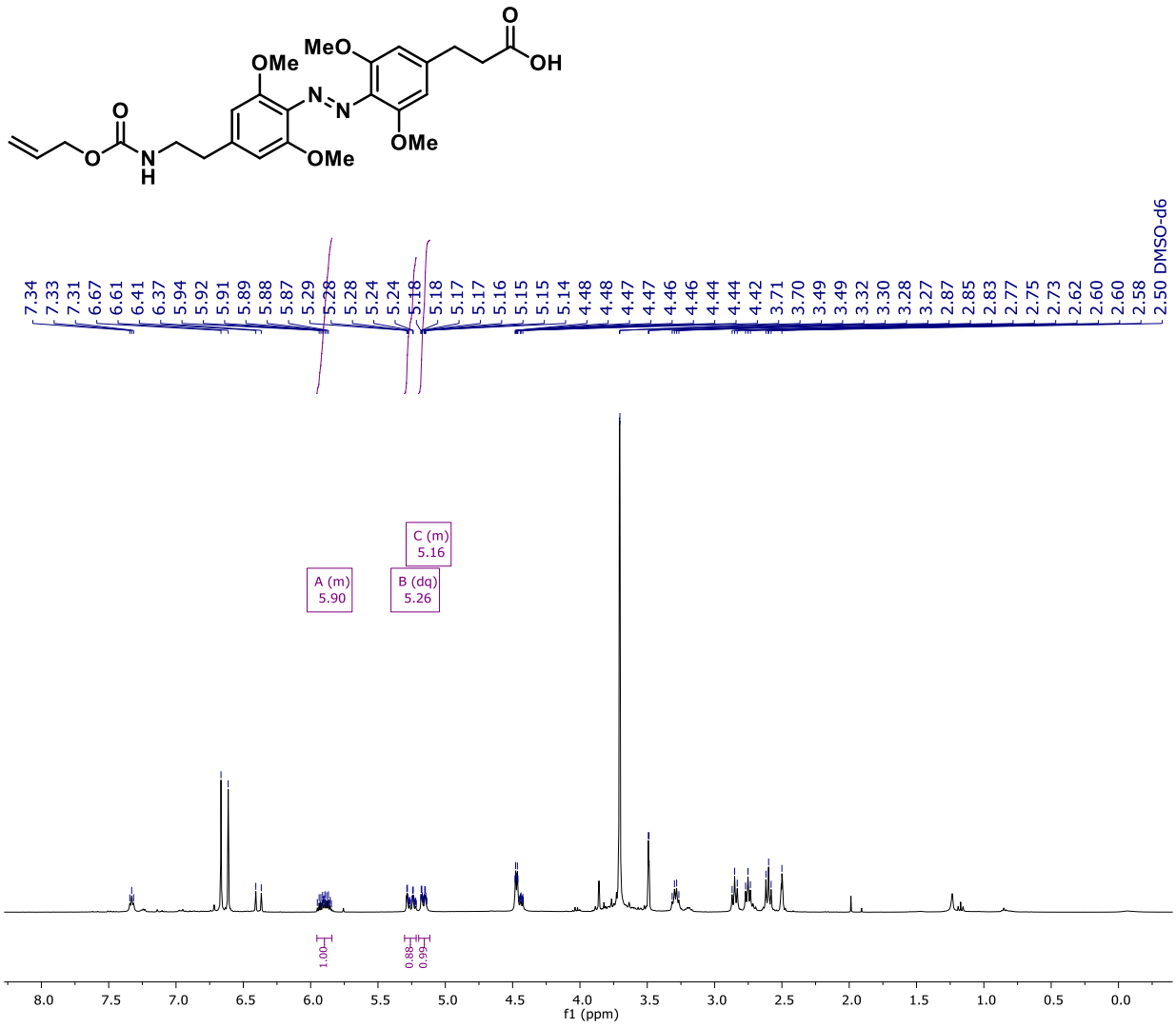


^{13}C-NMR (100 MHz, DMSO-d_6):



(*E*)-3-(4-((4-(2-(((Allyloxy)carbonyl)amino)ethyl)-2,6-dimethoxyphenyl)diazenyl)-3,5-dimethoxyphenyl)propanoic acid (12)

^{1}H-NMR (400 MHz, DMSO-d_6):



^{13}C-NMR (100 MHz, DMSO-d_6):