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**Applicazioni di Radicali Solfonilici e
Isotiocianati Foto-Rilasciati**

**Applications of Photo-Released Sulfonyl
Radicals and Isothiocyanates**

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Abstract

La fotochimica si occupa dello studio delle reazioni chimiche, dei processi e dei meccanismi attivati dall'interazione tra luce e materia. Analizza l'effetto della luce sulle molecole, che può indurre sia la formazione di nuove strutture sia la loro frammentazione in componenti più semplici. Durante il mio tirocinio, ho avuto modo di approfondire entrambi questi aspetti, che saranno descritti nelle due sezioni principali di questa tesi.

La prima parte è dedicata alla spirociclizzazione indotta da luce visibile per la sintesi di azaspiro[4,5]trienoni, a partire da *N*-aril propiolammidi e arilazo solfoni. In questo approccio, la luce agisce sugli arilazo solfoni generando solfonil radicali, che sono coinvolti nella formazione di spiro composti con potenziale rilevanza biologica.

La seconda parte introduce il concetto di *photocaging* e dei cosiddetti gruppi fotoprotettivi, che verranno applicati alla sintesi di foto-pseudoGlucosinolati. Questi ultimi sono progettati per rilasciare isotiocianati in seguito all'irraggiamento con luce visibile.

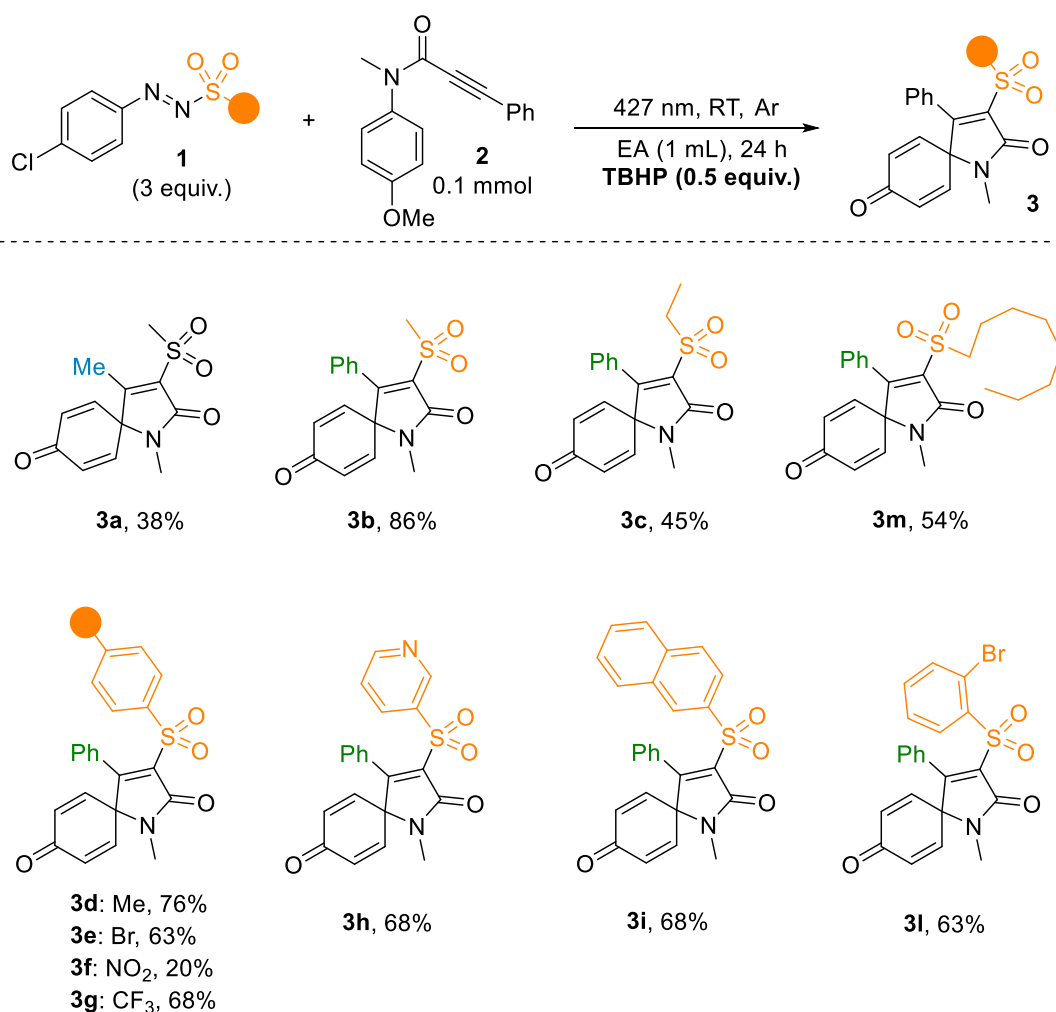
1. Spirociclizzazione Guidata da Luce Visible via Arilazo Solfoni

Le reazioni fotochimiche sono diventate uno strumento sempre più importante in chimica organica, poiché generalmente necessitano di condizioni blande grazie all'utilizzo della luce visibile, considerata come un reagente "pulito" in quanto non lascia tracce. Tuttavia, la sintesi fotochimica di molecole organiche risulta impegnativa per diversi motivi. Le molecole organiche tendono ad assorbire solo fotoni nella regione dell'ultravioletto (UV), ma questi non sono molto abbondanti nella radiazione solare che penetra l'atmosfera. Ciò ha per lungo tempo limitato lo sviluppo di processi fotochimici su larga scala, in particolare in ambito industriale. Inoltre, la maggior parte dei composti organici sono incolori, rendendo difficile il loro assorbimento nella regione del visibile. Ciononostante, l'utilizzo di luce visibile per promuovere passaggi chiave nella sintesi organica è cresciuto rapidamente negli ultimi anni.¹

La fotochimica è stata recentemente presa in considerazione per la sintesi di composti spirociclici, strutture ricorrenti in svariati prodotti naturali, farmaceutici e materiali funzionali.¹ In particolare, gli azaspiro[4,5]trienoni sono composti rilevanti, grazie alle loro notevoli applicazioni sintetiche e attività biologica.²⁻⁵ Nel corso degli anni, numerose strategie di spirociclizzazione sono state sviluppate. Generalmente, gli approcci tradizionali si focalizzano su reazioni di ciclizzazione intramolecolare.⁶ Tuttavia, molti di questi metodi soffrono di alcune limitazioni come reagenti poco disponibili, procedure di work-up monotone, condizioni di reazione estreme, e spesso richiedono l'utilizzo di catalizzatori metallici costosi.⁷ Pertanto, lo sviluppo di metodi efficaci per la reazione di spirociclizzazione è un obiettivo continuo in

chimica di sintesi, vista l'utilità di tali composti nella costruzione di *building blocks* ad alto valore sintetico. La fotochimica si è rivelata essere una via molto interessante per la sintesi di questi composti.

Nella prima parte di questa tesi verrà descritta l'applicazione degli arilazo solfoni per la sintesi di azaspiro[4,5]trienoni, tramite una strategia mediata da luce visibile e senza l'utilizzo di fotocatalizzatori. La parte iniziale del mio internato di tesi è consistita nell'ottimizzazione del protocollo sintetico, con l'obiettivo di determinare le condizioni di reazione più appropriate. Successivamente, sono stati sintetizzati e isolati alcuni azaspiro[4,5]trienoni, utilizzando diversi arilazo solfoni. Lo schema di reazione con le condizioni ottimizzate è riportato in Schema A1, insieme allo *scope* dei prodotti che sono stati isolati. Le condizioni ottimizzate sono le seguenti: acetato d'etile (EA) come solvente, atmosfera di Argon, agitazione a temperatura ambiente per 24 ore, irradiazione con una lampada da 427 nm e 0.5 equivalenti di *tert*-butil idroperossido (TBHP). Applicando questa procedura alla sintesi di azaspiro[4,5]trienoni, sono stati isolati dieci composti con rese fino all'86%.



Schema A1. Reazione nelle condizioni ottimizzate e *scope*

A causa del tempo limitato che ho avuto a disposizione per lo sviluppo del progetto, non ho potuto isolare una libreria di composti molto estesa. La mia attività si è focalizzata sullo *scope* degli arilazo solfoni, variando la parte solfonilica ($-\text{SO}_2\text{R}$), che ha portato alla sintesi dei composti **3b–3l**. La possibilità di utilizzare la luce solare per far avvenire questa reazione è stata confermata esponendo un vial di reazione alla luce del sole, da cui il prodotto **3b** è stato isolato con una buona resa.

Per ampliare lo *scope* delle molecole isolate, diversi reagenti devono ancora essere investigati, per determinare l'influenza dei reagenti sulla reattività e per introdurre nuovi sostituenti nella struttura finale. Per quanto riguarda gli arilazo solfoni, oltre alla parte solfonilica si possono cambiare i sostituenti sull'anello aromatico, introducendo gruppi elettron-attrattori o elettron-donatori. Inoltre, *N*-aril propiolammidi diversamente sostituite dovranno essere impiegate, modificando anche i sostituenti sull'anello aromatico legato all'atomo di azoto.

In conclusione, è stato sviluppato un metodo efficace per la sintesi di azaspiro[4,5]trienoni. Sebbene siano necessarie ulteriori analisi per consolidare i risultati ottenuti, i dati preliminari emersi si rivelano promettenti e delineano un approccio innovativo per la sintesi di composti spirociclici di potenziale interesse biologico.

2. Foto-pseudoGlucosinolati per il Rilascio Fotoindotto di Isotiocianati

I glucosinolati sono metaboliti secondari presenti nelle piante appartenenti alla famiglia delle *Cruciferae*.⁸ Quando i glucosinolati (GSL) vengono idrolizzati grazie all'azione di un enzima chiamato mirosinasi, generano una serie di composti bioattivi, tra cui gli isotiocianati (ITC). Gli ITC sono potenti elettrofili, e reagiscono prontamente con nucleofili all'azoto, allo zolfo e all'ossigeno. Metodi di estrazione dedicati permettono di isolare un numero limitato di glucosinolati dalle piante, ma in molti casi la sintesi organica rappresenta un aiuto cruciale per la produzione di queste molecole, in particolare di analoghi artificiali ai glucosinolati non presenti in natura.⁹

Gli isotiocianati presentano importante attività biologica, ma il loro potenziale come agenti terapeutici è limitato dalla loro inerente instabilità. Per affrontare queste limitazioni, la natura impiega i glucosinolati come profarmaci di ITC, ma è necessaria l'azione della mirosinasi, di conseguenza l'assenza di questo enzima negli animali ed esseri umani ne limita l'utilizzo in contesti terapeutici.^{9,10} Recentemente è stato sviluppato un approccio per la sintesi di pseudoglucosinolati, ovvero analoghi sintetici che non richiedono la presenza di mirosinasi per il processo di idrolisi, i quali rilasciano ITC ad opera di un enzima nitroreduktasi.¹⁰ Sebbene questo rappresenti un'interessante strategia, il rilascio di ITC rimane ancora strettamente legato

all'azione degli enzimi, e ciò può rappresentare un limite nel momento in cui questi non siano disponibili dove si vuole rilasciare l'isotiocianato.

L'attività che ho svolto in laboratorio si è concentrata sullo sviluppo di un nuovo metodo per il rilascio di ITC da analoghi dei glucosinolati senza l'azione di enzimi. In particolare, è stato studiato il rilascio di queste sostanze tramite un processo mediato dalla luce. In questo approccio, il *photocaging* appare come una strategia efficace.

La fotofarmacologia utilizza la luce esterna per attivare piccole molecole, in una specifica regione di interesse e in un momento preciso. Questo fenomeno può essere ottenuto in modo reversibile (*photoswitching*) o irreversibile (*photocaging*). Nel caso del *photocaging*, una molecola bioattiva viene legata covalentemente a un gruppo fotoprotettivo (PPG), che la rende temporaneamente inattiva. In seguito ad irraggiamento con una specifica lunghezza d'onda, che dipende dal PPG utilizzato, la molecola viene rilasciata nella sua forma attiva in modo irreversibile (processo detto *uncaging*) ed è libera di attuare la sua funzione biologica.¹¹

Le *photocages* sono molecole sensibili alla luce costituite da due componenti: un gruppo fotosensibile (gruppo fotoprotettivo, PPG) e una sostanza target specifica, che viene rilasciata in seguito ad irraggiamento. Nelle gabbie, i target sono protetti chimicamente dai PPG, e solo nel momento in cui vengono rilasciati le loro proprietà vengono ripristinate.¹² Per avere un'ampia visione su quali siano i migliori gruppi fotoprotettivi per l'attività studiata, sono stati scelti i quattro seguenti: dietilammino cumarina (DEAC), gruppo dimetossinitrobenzilico (DMNB), gruppo α -metil-nitropiperonilossimetilico (MeNPOM), e infine un boro-dipirrometene (BODIPY). Un problema che accomuna alcuni dei PPG più frequenti riguarda l'utilizzo di luce ad alta energia per la loro rimozione, che tipicamente richiede lunghezze d'onda inferiori a 400 nm, che possono danneggiare i tessuti biologici. Una soluzione diretta a questo problema, come lo spostamento batocromico della banda di assorbimento del PPG attraverso un'estensione del sistema π , ha come conseguenza lo svantaggio di rendere il PPG più grande e meno polare, diminuendone la solubilità in acqua.¹³

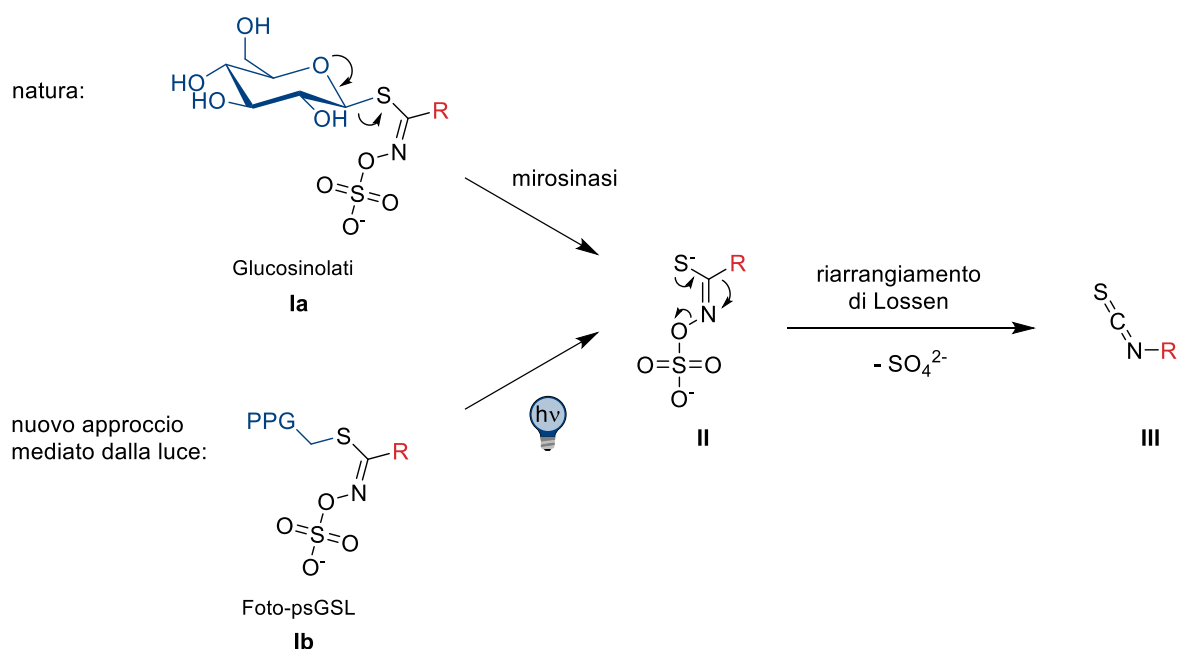
La strategia di *photocaging* è stata usata molto per l'attivazione delle biomolecole, rendendo in tal modo possibile la regolazione di processi biologici con alta precisione tramite controllo spazio-temporale. La luce offre infatti numerosi vantaggi rispetto ad altri tipi di stimoli, grazie al controllo di lunghezza d'onda, intensità e durata dell'irraggiamento, e ciò permette di rilasciare la molecola target con molta precisione.¹⁴

La marcatura delle biomolecole è un metodo comune utilizzato per scopi bioanalitici. Può avvenire con materiali radioattivi o composti che presentino assorbimento e/o fluorescenza

dalla regione dell'UV fino al vicino infrarosso dello spettro elettromagnetico. I fluorofori organici sono in grado di interagire con il composto d'interesse attraverso legami covalenti o non covalenti, generando addotti fluorescenti. La lunghezza d'onda dell'emissione può variare da valori relativamente brevi fino a regioni spettrali molto estese, in funzione della natura del marcatore impiegato.¹⁵ La marcatura attivata da luce visibile è una strategia che permette di avere un controllo molto preciso sull'identificazione dei residui nelle catene polipeptidiche. Sfruttando il concetto del *photocaging*, i marcatori biologici possono essere rilasciati in seguito ad irraggiamento, in tal modo permettendo la regolazione di processi biologici con un elevato controllo spazio-temporale.

La reattività degli ITC è stata usata per il sequenziamento e la marcatura delle proteine con pigmenti fluorescenti. Gli ITC sono elettrofili e la loro attività biologica è collegata alla loro reattività con i tioli per formare un addotto tiocarbamoilico instabile. Tuttavia, in ambiente basico reagiscono anche con residui amminici, formando un addotto ITC-ammina stabile.¹⁶

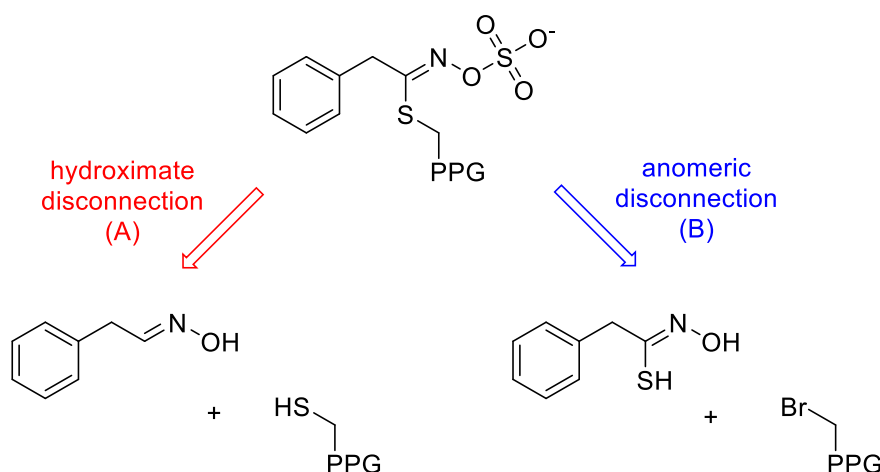
La seconda parte della tesi si propone di applicare il concetto di *photocaging* al fine di modulare in modo controllato l'attività degli pseudoglucosinolati. In particolare, questa applicazione ha come risultato la sintesi di foto-pseudoGlucosinolati (foto-psGSL), analoghi dei glucosinolati in grado di rilasciare ITC in seguito ad irraggiamento (Schema A2).



Schema A2. Meccanismo di rilascio dell'isotiocianato dai glucosinolati naturali e nuovo rilascio mediato dalla luce dai foto-pseudoGlucosinolati

I foto-psGSL possono essere funzionalizzati con un opportuno gruppo fluoroforo, in modo che l'ITC che viene rilasciato porti il frammento fluorescente. Un vantaggio chiave di questo approccio è che l'ITC fluorescente può essere rilasciato con un elevato controllo spazio-temporale irraggiando con un'appropriata lunghezza d'onda, per avviare il processo di *uncaging*. Tale proprietà lo rende facilmente rilevabile tramite spettroscopia di fluorescenza. Questa strategia può essere applicata, data la reattività degli ITC, per investigare la presenza di residui di cisteina o lisina all'interno delle catene polipeptidiche. Il foto-psGSL può essere indirizzato selettivamente verso la regione di interesse e, successivamente, attivato tramite irraggiamento alla lunghezza d'onda specifica, determinando il rilascio dell'ITC fluorescente. Tale rilascio consente una marcatura mirata, facilitando l'identificazione dei residui bersaglio.

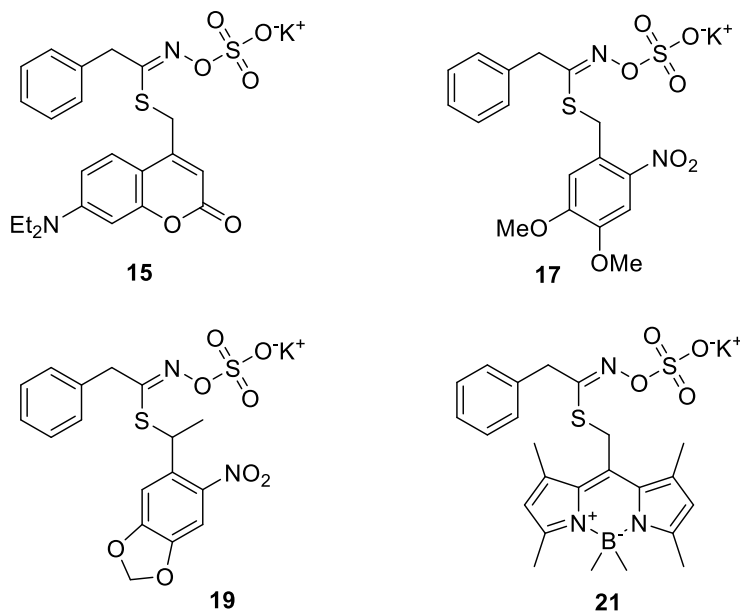
Due approcci sono stati sviluppati per la sintesi di pseudoglucosinolati, basati su due vie retrosintetiche (Schema A3). Due disconnessioni sono state considerate, una sul frammento idrossimoilico (A) e l'altra sul centro anomerico (B).⁹



Schema A3. Vie retrosintetiche per la sintesi di pseudo-glucosinolati

Per studiare il processo di *uncaging*, le molecole isolate (Schema A4) sono state irraggiate usando una specifica lunghezza d'onda dipendente dal PPG usato. Dopodiché, i campioni irraggiati sono stati analizzati con LC-MS. La rimozione del gruppo fotoprotettivo si è dimostrata efficace per i composti **15**, **17** e **19**, per i quali il rilascio di isotiocianato è stato successivamente analizzato. Il composto **21** invece non ha rilasciato ITC dopo irraggiamento, suggerendo che il processo di *uncaging* non è avvenuto. L'ITC rilasciato non è stato rilevato probabilmente a causa della sua instabilità, per cui i campioni sono stati trattati con una soluzione di ammoniaca e con una soluzione di glicina, nucleofili in grado di reagire con ITC

e determinarne la presenza. I prodotti della reazione tra ITC e ammoniaca o glicina sono stati rilevati successivamente, confermando il rilascio di ITC.



Schema A4. Foto-pseudoglucosinolati sintetizzati

Delle quattro molecole sintetizzate, solo tre hanno dimostrato di rilasciare efficacemente ITC. Il BODIPY foto-psGSL non ha in nessun caso rilasciato isotiocianato. Quest'ultimo risultato è sfavorevole e limitante, poiché tra i quattro PPG impiegati il BODIPY è quello che assorbe a lunghezze d'onda più elevate. Tale proprietà lo rende molto interessante in particolare per applicazioni biologiche, in quanto l'assorbimento di questo gruppo è vicino alla regione della finestra biologica, e di conseguenza meno dannoso per i sistemi biologici.¹⁵

A causa del tempo ridotto a disposizione, l'applicazione dei foto-psGSL per la marcatura non è stata testata nei sistemi biologici. I foto-psGSL possono essere funzionalizzati con un gruppo fluorescente appropriato, in modo che, in seguito ad irraggiamento, l'ITC che viene rilasciato mantenga il gruppo fluorescente diventando in questo modo facilmente rilevabile tramite spettroscopia di fluorescenza. Esperimenti futuri si focalizzeranno sulla sintesi di foto-psGSL che possano rilasciare ITC fluorescente.

In conclusione, quattro foto-psGSL sono stati progettati e sintetizzati. I composti isolati si sono dimostrati essere degli ottimi analoghi e un'alternativa sintetica ai glucosinolati naturali. La sintesi dei foto-psGSL fluorescenti non è stata portata a termine, tuttavia i risultati ottenuti pongono le basi per ulteriore ricerca in questo campo e per la futura applicazione di queste molecole in contesti biologici per la marcatura di peptidi e proteine.

Visible-Light-Driven Dearomative Spirocyclization via Arylazo Sulfones

1. Introduction

1.1 Photochemistry

Photochemical processes are induced by the interaction between light and matter. Thanks to photochemistry, some processes which would not happen thermally can indeed be carried out because of the modification of the behaviour of the molecules as a result of the absorption of light. Light can indeed be seen as a “reagent” which activates the substrate, without leaving any traces thus favouring the isolation of products. A photochemical reaction needs a light source, which can be in the ultraviolet or visible region. In the latter case, sunlight would be sufficient to promote the reaction.

The photochemical synthesis of complex organic molecules is challenging for different reasons. Organic molecules tend to absorb only photons in the ultraviolet (UV) region, which are not abundant in the solar radiation that penetrates the atmosphere. This has constrained the rate of development in large-scale industrial photochemical synthesis, because energy-efficient sources of UV light have only recently become available. Additionally, UV photons are quite high in energy, on the order of a C–C bond, and can cause considerable unproductive decomposition reactions to occur.¹⁷ Moreover, most organic compounds are colourless, making it hard for them to absorb in the visible region. Nonetheless, the use of solar and visible light to promote key photochemical steps in organic synthesis has rapidly grown in recent years. Photochemical reactions have become an important tool for organic chemists, as they generally require mild conditions and visible (solar) light can be conveniently adopted. However, there is a limit to these types of reactions: only when processes are catalysed by visible-light-absorbing photocatalysts, or when using coloured organic compounds, can photochemistry play an important role.¹

Photoredox catalysis has been successfully employed as a strategy to use visible light in organic synthesis. This approach consists in the use of a photocatalyst that absorbs radiation and promotes the activation of colourless compounds under redox conditions. However, the cost of the catalyst (often a transition metal-based complex) as well as the difficult work up procedure are a significant limitation. An alternative method consists in the functionalisation of one of the colourless reagents with groups capable to promote reactivity in the visible range, inducing

the formation of the desired reactive intermediate. These functional groups are defined as dyedauxiliary groups (DGs) since they are responsible for the colour and the photoreactivity of the molecule, promoting photochemical reactions.¹⁸

Spirocyclic compounds

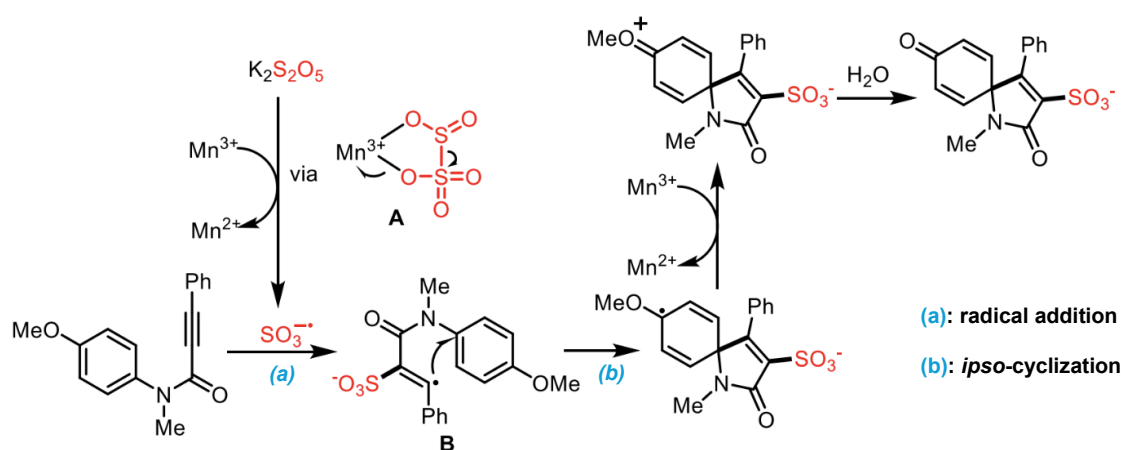
Spirocyclic compounds are recurrent molecular frameworks in various natural products, pharmaceuticals and functional materials.¹ They have recently received significant attention because of their unique structural properties, and biological relevance as building blocks for the synthesis of more complex structures.^{7,19} In particular, azaspiro[4,5]trienones are pivotal because of their remarkable biological activity, such as antibacterial, antitumoral, and antioxidant, and their diverse synthetic applications.^{2–5} Notably, this framework has also been recognized as a constrained tamoxifen mimic, displaying significant anticancer potential against human breast cancer cell lines MCF-7 and MDA-MB-231.²⁰

Sulfur-containing skeletons, in turn, are found in many important drugs and natural products. Among them, sulfones and their derivatives are useful organosulfur compounds, and have been widely applied in organic synthesis, agrochemicals, flame retardant materials and pharmaceuticals.²¹ Consequently, the synthesis of sulfonated spiro[4,5]trienone derivatives becomes an interesting topic. Much effort has been made to synthesize these compounds, however the selective preparation of spirocyclic sulfonyl compounds has proven to be a challenging task.¹⁹

1.2 Spirocyclization processes

The development of efficient spirocyclization methodologies has been a continuous objective in synthetic chemistry, given the utility of spirocyclic compounds in constructing valuable molecular building blocks.^{7,19} Over the years, numerous strategies have been devised to achieve this transformation. Generally, traditional approaches are mainly focused on intramolecular cyclization reactions such as electrophilic substitution on *N*-acyliminium or thionium ions, oxidative spiro-cyclization of phenol derivatives, radical *ipso*-carbocyclization, *ipso*-Friedel–Crafts/Michael addition cascade reactions, or transition-metal-catalysed intramolecular dearomatization strategies.^{6,22,23} Dearomatization reactions provide a powerful strategy for the synthesis of useful hydrocarbons and alicyclic frameworks from readily available aromatic compounds.²⁴ Recently, many efforts have been made towards the synthesis of functionalized azaspiro[4,5]trienones via cascade *ipso*-spirocyclization of *N*-arylpropiolamides, involving either electrophilic activation or radical addition (Scheme 1).

This strategy was efficiently exploited to introduce a variety of functional groups on the azaspiro[4,5]trienone framework. In contrast, only a handful of reports exist for the synthesis of azaspiro[4,5]decanes via cascade cyclization.²⁵ Nonetheless, most of these protocols depend on toxic metal reagents and strong oxidants, they suffer from limitations such as unready available starting materials, tedious workup procedures and harsh reaction conditions.⁷ This highlights the urgent need for synthetic methodologies that are straightforward, safe and environmentally sustainable. In this context, photocatalytic approaches driven by visible light have emerged as versatile and sustainable alternatives.⁶



Scheme 1. Example of radical addition and *ipso*-spirocyclization as reported from Zhang et al.¹⁹

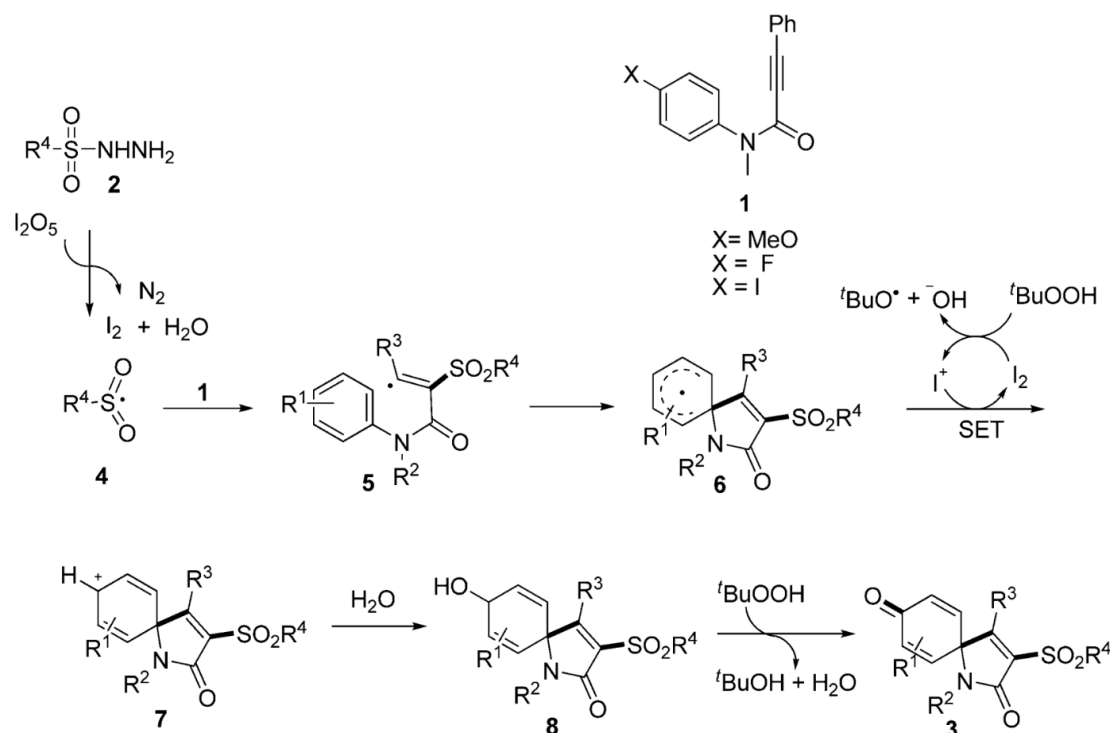
In the following paragraphs, I will shortly report some works that have recently been published for the synthesis of spirocyclic compounds, after which I will examine the visible-light-mediated approach that will be the centre of this thesis.

1.2.1 Thermal or metal catalysed methods

In 2014, Wang et al.²⁴ proposed a silver-catalysed difunctionalization of *N*-(*p*-methoxyaryl)propionamides coupled with dearomatization, which was applied to the preparation of a variety of phosphorylated azadecenones. The silver salt (AgNO_3) employed to catalyse the carbon phosphonation of arylalkyne alkynoate coupled with dearomatization was cheap and nontoxic, however the synthetic protocol still requires the use of a metal catalyst.

In 2015, Wen et al.⁷ proposed a direct oxidative spirocyclization of arylpropionamides with sulfonylhydrazides leading to 3-sulfonated azaspiro[4,5]trienones, under metal-free conditions using I_2O_5 as an oxidizing agent. Basing their work on the difunctionalization of alkynes as an efficient way of forming C–C and C–heteroatom bonds in a single operation, they were able to introduce useful organic frameworks into the spiro[4,5]trienone structure. A possible reaction pathway is described in Scheme 2. Initially, the single-electron oxidation of sulfonylhydrazide

2 mediated by I_2O_5 formed sulfonyl radical **4** and I_2 . Next, the selective addition of sulfonyl radical **4** to arylpropiolamide **1** gave the vinyl radical **5**. Furthermore, intramolecular spirocyclization of vinyl radical **5** with an aryl ring generated the radical intermediate **6**, which produced the corresponding cyclohexadienyl cation **7** via a single-electron-transfer process. Subsequently, the nucleophilic attack of H_2O to cation **7** afforded intermediate **8**. Finally, oxidation of intermediate **8** by *tert*-butyl hydroperoxide (TBHP) would lead to the formation of the desired sulfonated azaspiro[4,5]trienone **3**.



Scheme 2. Proposed reaction mechanism by Wen et al.⁷

Similarly, in 2020 Nair et al.²⁵ reported an efficient synthesis of azaspiro[4,5]decanes and azaspiro[4,5]trienones by the radical spirocyclization of *N*-benzylacrylamides or *N*-arylpropiolamides. These reactions proceed under metal-free conditions and involve *in situ* generation of aryl sulfonyl radicals from DABCO(SO₂)₂ and aryl diazonium salts.

In 2015, Yugandhar et al.²⁰ came up with a solution-phase parallel synthesis of azaspiro[4,5]trienones by performing tandem Ugi four-component condensation (U4CC), involving substituted *p*-anisidines, aldehydes, 3-alkyl/aryl-propionic acids, and isocyanides, and iodine-mediated *ipso*-iodocyclization in one-pot.

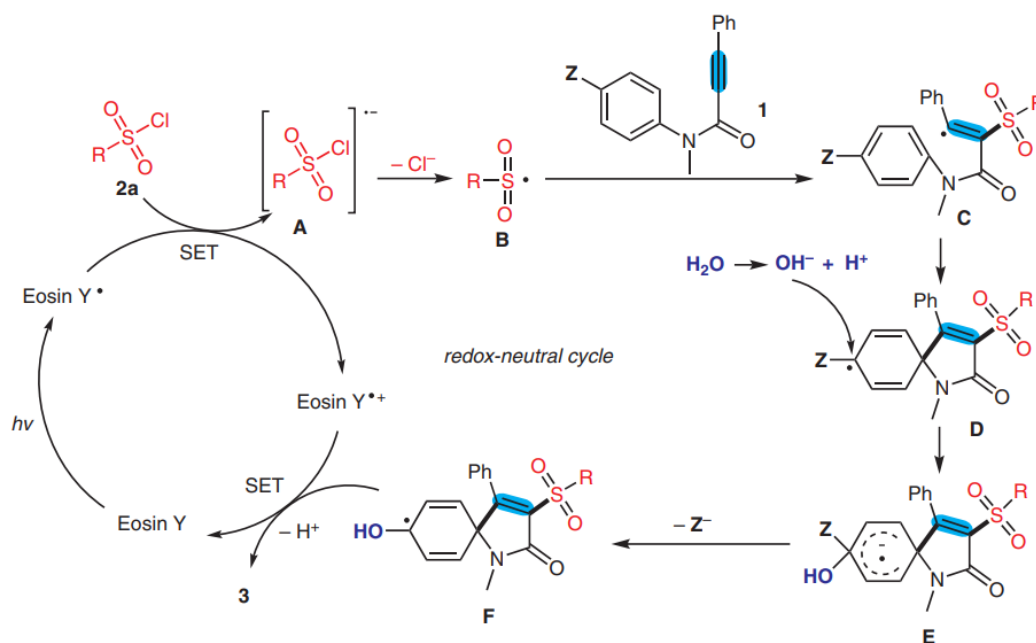
In 2023, Zhang et al.¹⁹ proposed a radical sulfonation–*ipso*-cyclization cascade promoted by $Mn(OAc)_3 \cdot 2H_2O$, using functionalized alkynes or alkenes and potassium metabisulfite for the generation of spirocyclic sulfonates. They also demonstrated a modular synthesis approach for

the preparation of various azaspiro[4,5]trienone-based sulfonamides and sulfonate esters. While this approach was particularly efficient, affording yields of up to 98%, it still requires the use of a manganese salt, which does not make it a sustainable process. The mechanism was already reported in Scheme 1.

1.2.2 Photochemical methods

In 2017, Wei et al.⁶ developed a mild and convenient visible-light-induced method for the construction of 3-sulfonyl and 3-sulfenyl azaspiro[4,5]trienones through metal-free difunctionalization of alkynes with sulfinic acids or thiols at room temperature, using inexpensive and non-toxic organic dyes (Eosin Y and Na₂-Eosin Y) as photocatalysts.

In 2018, Liu et al.²⁶ reported a strategy to synthesize diverse 3-sulfonylspiro[4,5]trienones using sulfonyl chlorides. This *ipso*-carbosulfonylation of alkynes proceeds by visible-light catalysis under transition metal-free conditions. The proposed reaction mechanism is reported in Scheme 3. Eosin Y* reduces sulfonyl chloride **2a** into radical anion **A** and Eosin Y^{•+} radical cation through single electron transfer (SET). Then, radical anion **A** forms a sulfonyl radical **B**, which reacts on the α position of the carbonyl group in propiolamide **1** affording the more stable intermediate **C**, along with formation of a new carbon–carbon bond. Next, the intermediate **D** is produced by *ipso*-cyclization and dearomatization of intermediate **C**. Attack of the hydroxyl ion, derived from H₂O, to intermediate **D** furnishes the intermediate **E**. Subsequently, the removal of the Z group from intermediate **E** produces the radical intermediate **F**, which finally undergoes SET and deprotonation to afford the target product **3**.

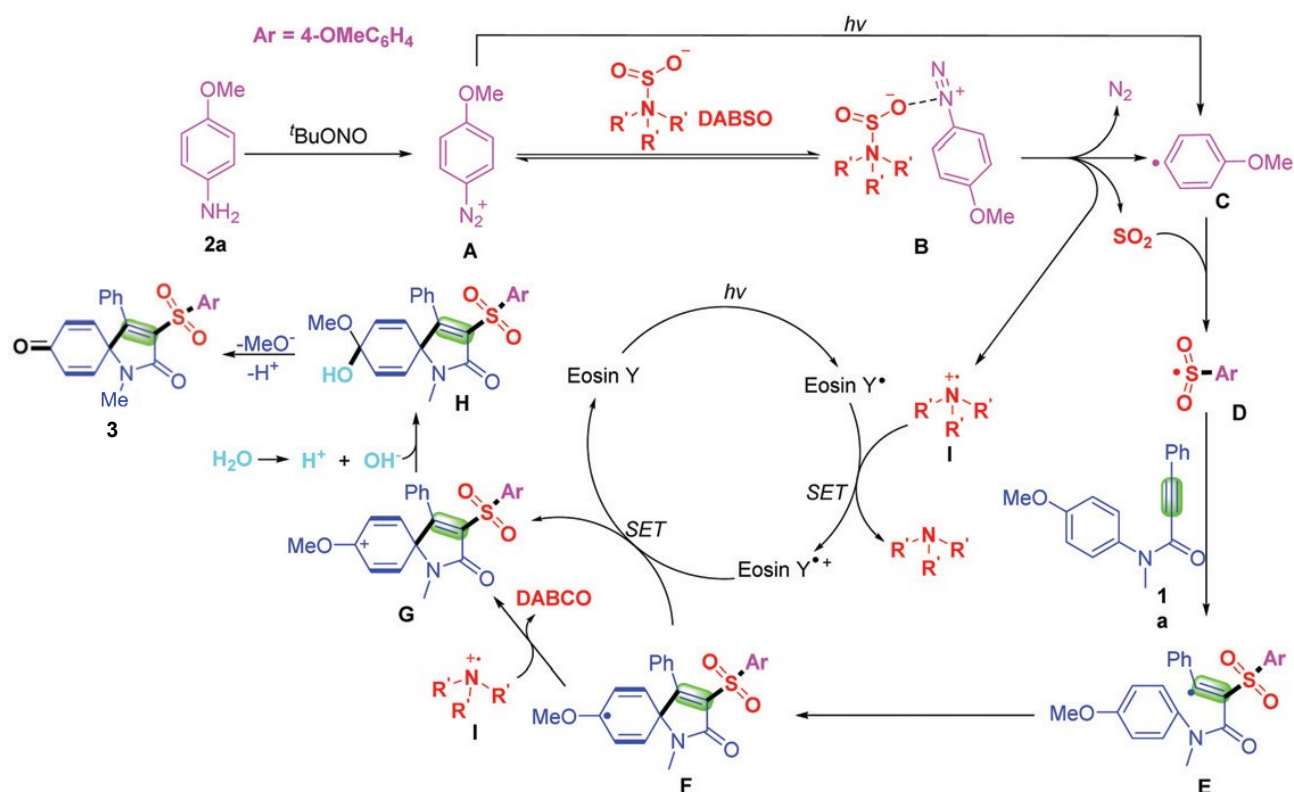


Scheme 3. Proposed reaction mechanism by Liu et al.²⁶

In 2018, Sahoo et al.²⁷ devised a visible light-induced (blue LED) radical cascade to effect selenylative spirocyclization of *N*-aryl alkynamides at room temperature under oxygen atmosphere and with no external photocatalyst, to obtain 3-selenospiro[4,5]trienones. This methodology was further extended for the synthesis of 3-iodospiro[4,5]trienones in the presence of readily available *N*-iodosuccinimide as a halogen source.

In 2019, Liu et al.²¹ reported a visible-light-induced sulfonylation/*ipso*-cyclization of *N*-arylpropiolamides with arylamines and DABCO(SO₂)₂, affording a variety of sulfonated spiro[4,5]trienones under transition-metal-free conditions. The conversion proceeds through a radical pathway involving arylsulfonyl radical generation, sulfonylation, and subsequent *ipso*-cyclization. Traditionally, sulfones have been synthesized via alkylation of sodium sulfinates or oxidation of thioethers; however, the oxidative routes are often incompatible with substrates bearing sensitive functional groups. To overcome these limitations, several methodologies have recently been developed employing alternative sulfonyl sources such as DABCO(SO₂)₂, thiourea dioxide, or sodium sulfites. These approaches, which are typically transition-metal-catalysed or radical-mediated, circumvent the direct handling of sulfur dioxide. Among them, DABCO(SO₂)₂ has emerged as one of the most convenient sulfonylating agents. The proposed reaction mechanism, based on radical inhibition experiments, is reported in Scheme 4. This sulfonylation/*ipso*-cyclization reaction proceeds via a radical process and undergoes arylsulfonyl radical formation, sulfonylation, and *ipso*-cyclization. Aromatic amines, acting as an aryl source, go through diazotization and decomposition to generate aryl radicals. The *in situ* formed sulfonyl radicals are trapped by *N*-aryl propiolamides to produce the target products.

Under no visible light conditions, aryldiazonium cation **A** combined with DABSO via electrostatic interaction to give complex **B**. Then, complex **B** went through homolytic cleavage of the N–S bond and a SET process to furnish the *para*-methoxyaryl radical **C**, sulfur dioxide and radical cation **I**. Subsequently, sulfur dioxide was trapped by the aryl radical **C** to furnish the sulfonyl radical **D**. The addition of radical **D** to the carbon–carbon triple bond in propiolamide **1a** afforded the vinyl radical intermediate **E**, which underwent *ipso*-carbocyclization to give intermediate **F**. Next, radical **F** was oxidized by the intermediate **I** via a SET process to generate the cation intermediate **G** and DABCO. Then, H₂O underwent hydrolysis with the action of DABCO to form a hydroxyl ion, which attacked the cation **G** to provide intermediate **H**. Finally, the release of the OMe group and then deprotonation led to the terminal product **3**.²¹



Scheme 4. Proposed reaction mechanism by Liu et al.²¹

In 2020, Liu et al.²⁸ proposed a novel and convenient photo-mediated halogenated spirocyclization of *N*-(*p*-methoxyaryl)propiolamides. The photolysis of phenyliodine bis(trifluoroacetate) ($\text{PhI}(\text{OCOCF}_3)_2$) as an iodination reagent led to iodinated *ipso*-cyclization under the irradiation of a xenon lamp, while brominated *ipso*-cyclization or chlorinated *ipso*-cyclization was achieved by irradiating a mixture of $\text{PhI}(\text{OCOCF}_3)_2$ and KBr/KCl under a blue LED. This protocol utilizes light as the safe and clean energy source and does not require any external photocatalyst.

In 2023, Manna et al.²⁹ proposed a visible-light mediated synthesis of 3-alkylated coumarins and unsaturated spirocyclic compounds through difunctionalization of activated alkynes with boronic acid. The reaction employs a hypervalent iodine reagent and ruthenium photocatalyst. The spirolactones thus obtained were subjected to another mode of visible-light-mediated radical addition cascade cyclization to access various fused spirocyclic compounds. Boronic acids were found to be an effective alkyl source as they generate alkyl radicals in the presence of a hypervalent iodine reagent and photocatalyst.

In 2024, Luo et al.³⁰ eliminated the need for photocatalysts and external additives, providing an operable and mild method for halogen radical-enabled dearomatization of *N*-

arylpropiolamides under oxygen atmosphere at room temperature. Among the various dearomatization strategies, halofunctionalization is one of the most studied reaction types, and on the other hand, the visible light induced synthesis strategy has emerged as a powerful platform for facilitating a variety of organic transformations under mild conditions due to excellent features such as safety and sustainability.

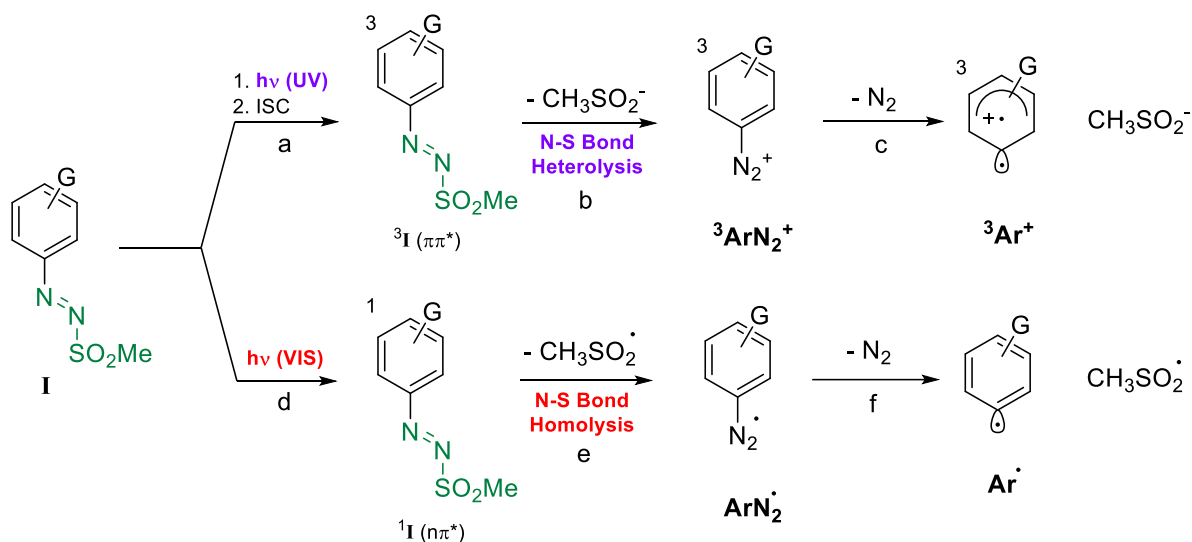
1.3 Visible-light-promoted spirocyclization using arylazo sulfones

Several strategies have been proposed for the synthesis of spirocyclic molecules, most of them related to the *ipso*-cyclization and subsequent dearomatization occurring on aromatic rings, under oxidative and transition metal-catalyzed conditions. Many have also investigated the visible light-mediated synthesis of spirocompounds, with or without the use of metals and photocatalysts. The use of light is an interesting alternative to traditional synthetic pathways, and with our approach, by taking advantage of arylazo sulfones' photochemistry, the spirocyclization can be carried out without a photocatalyst.

Arylazo sulfones are thermally stable-coloured compounds, but should be stored well away from light, because of their photoreactivity. They can be smoothly prepared from the corresponding anilines (via conversion to diazonium salts followed by coupling with sulfinate salts). The transformation proceeds from a colourless organic precursor (aniline) to a less stable diazonium salt, still colourless. This intermediate subsequently undergoes conversion into a sulfone derivative, which is both coloured and stable, and is characterized by the presence of a photolabile bond. The synthesis can also occur via oxidation of *N*-sulfonylaryl hydrazines, in turn generated from aryl hydrazine.^{1,18}

Regarding the photophysics of these compounds, arylazo sulfones exhibit two absorption maxima, located in the UV (300–360 nm, $\epsilon = 10000\text{--}20000\text{ M}^{-1}\text{cm}^{-1}$) and in the visible region (400–450 nm, $\epsilon = 100\text{--}200\text{ M}^{-1}\text{cm}^{-1}$), assigned to a $\pi\pi^*$ and a $n\pi^*$ transition, respectively.¹⁸ These substrates have been moderately described as suitable precursors of chemical intermediates, including aryl radicals and cations, under mild and (photo)catalyst-free conditions.¹ The observed photoreactivity of arylazo sulfones is in fact dependent on the populated excited state in a wavelength-dependent fashion. Therefore, upon UV irradiation, the generated $^1\pi\pi^*$ state undergoes intersystem crossing (ISC) to the corresponding triplet ($^3\pi\pi^*$, Scheme 5, path *a*), followed by heterolysis of the N–S bond releasing a diazonium salt ($^3\text{ArN}_2^+$, *b*). Upon dediazonation (*c*), the diazonium salt is converted in a triplet phenyl cation ($^3\text{Ar}^+$) with methanesulfinate anion as the counterion. On the other hand, visible-light exposition

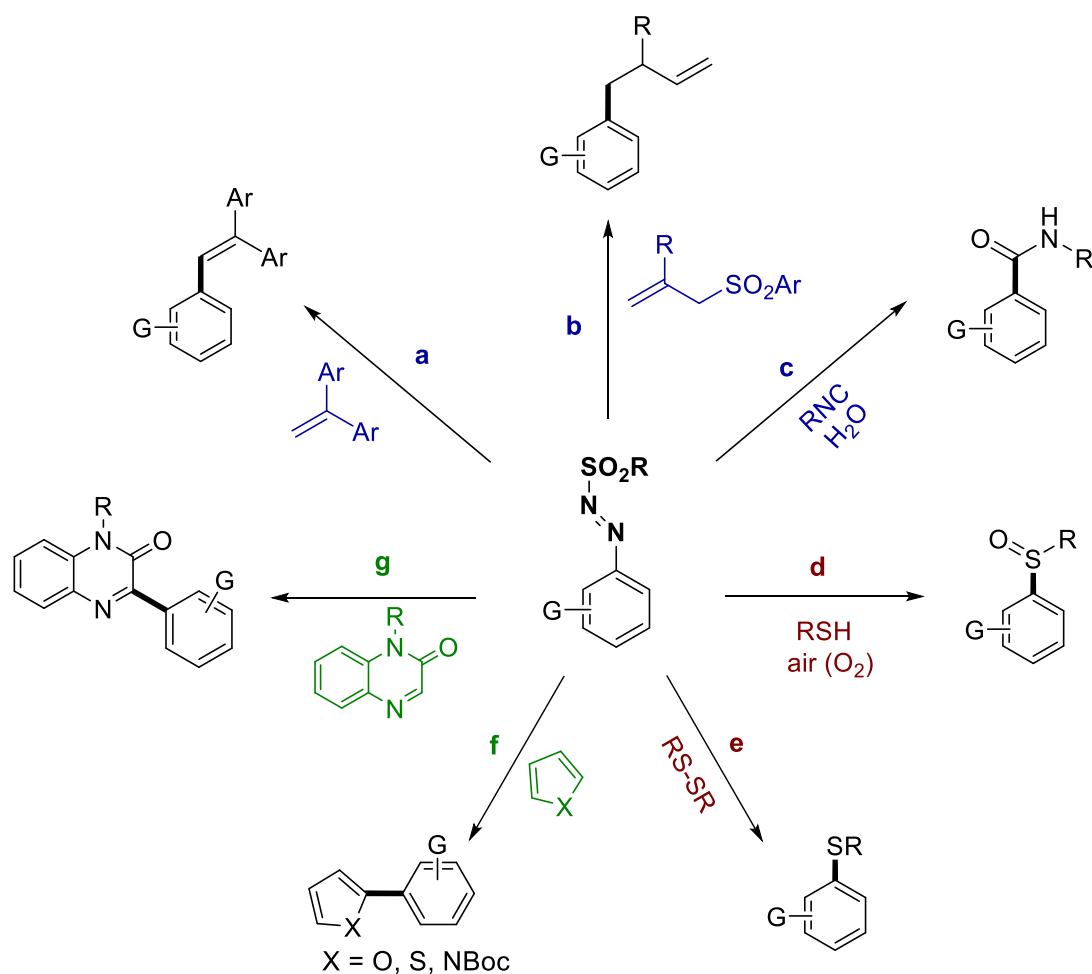
populates selectively the $^1\pi\pi^*$ state of **I** and homolysis of the N–S bond generates, after nitrogen loss from the diazenyl radical $\text{Ar-N}_2^\bullet$, an aryl ($\text{Ar}^\bullet$)/methanesulfonyl ($\text{CH}_3\text{SO}_2^\bullet$) radical pair (**e**, **f**). It is, however, believed that isomerization of the N=N bond from the *trans* to the (less stable) *cis* configuration plays a key role in the cleavage of the N–S bond. Both aryl cations and aryl radicals are accessible when a polychromatic light (e.g., sunlight) is used as the energy source.¹⁸



Scheme 5. Photochemistry of arylazo sulfones

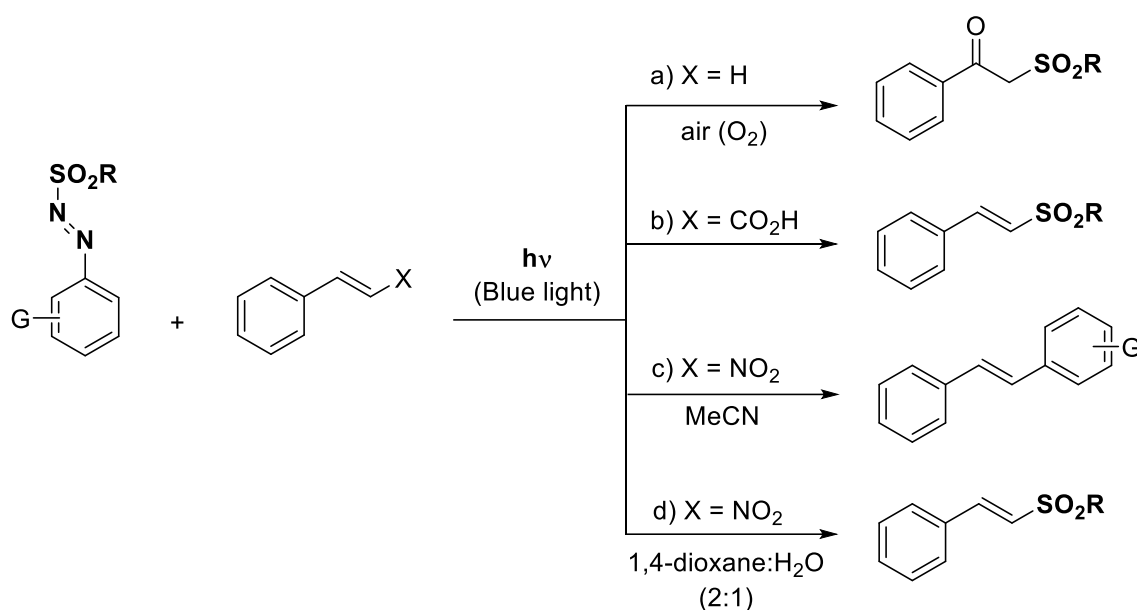
Arylazo sulfones have many diverse applications in synthesis, as they can be successfully used in C–C or C–heteroatom bond formation with various substrates. They have been employed in several light-activated reactions, and a few examples are reported in the following paragraphs. An interesting application of arylazo sulfones concerns the formation of $\text{Ar-C}(\text{sp}^2)$ bonds through the arylation of alkenes (Scheme 6, path *a*).³¹ Then, the allylation of arenes has always suffered the use of harsh conditions and the contamination of the final product by heavy metals. Therefore, the use of arylazo sulfones as starting materials results in a greener and milder approach to the synthesis of these structures (path *b*).^{32,33} A visible light-driven, metal-free synthetic way to aromatic amides was achieved via radical arylation of isonitriles, using arylazo sulfones as suitable precursors of aryl radicals (path *c*).³⁴

Notably, arylazo sulfones can be used for the synthesis of Ar–S bonds. Different approaches have been outlined, like the formation of unsymmetrical differently functionalised aryl sulfoxides from commercially available thiols and the synthesis of aryl sulfides from disulfides (paths *d*, *e*).³⁵ Arylazo sulfones have also been employed for the visible light-driven formation of new Ar–Ar bonds with different heteroaromatics (paths *f*, *g*), thus avoiding the use of metal catalysts.^{1,37}



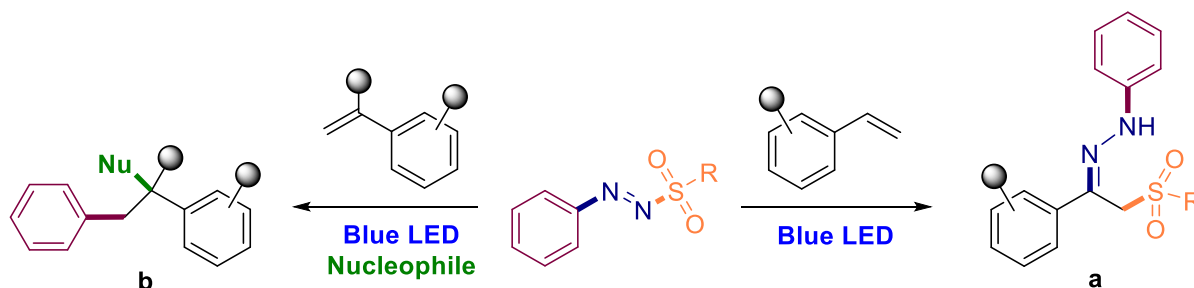
Scheme 6. Selected reactivity of arylazo sulfones, upon light irradiation with different sources

Besides the use of diazenyl or aryl radicals, the methanesulfonyl radical is of particular interest in chemical transformations, notably in sulfonylation reactions (Scheme 7). In 2019, Wei et al. reported the visible light-induced sulfonylation of styrenes with arylazo sulfones to achieve several β -oxo sulfones (path *a*).³⁸ In 2020, Yadav studied the irradiation of arylazo sulfones with cinnamic acid. The proposed protocol afforded (*E*)-vinyl sulfones via sulfonylation/decarboxylation (path *b*).³⁹ Finally, when arylazo sulfones are photolyzed, β -nitrostyrenes undergo a solvent selective arylation or sulfonylation. When the arylation is performed in acetonitrile it affords stylobenes in good to moderate yields (path *c*), while in a dioxane/water mixture the sulfonyl radical reacts with the nitroalkene forming (*E*)-vinylsulfones (path *d*).⁴⁰



Scheme 7. Reactivity of arylazo sulfones: sulfonylation reactions

Arylazo sulfones were also employed in the vicinal difunctionalization of styrenes (Scheme 8), by performing a visible-light-mediated regioselective synthesis of α -sulfonyl arylhydrazones **a** and 1,2-alkoxyarylated products **b** from readily available styrenes.⁴¹



Scheme 8. Double functionalization of sp^2 bonds with arylazo sulfones⁴¹

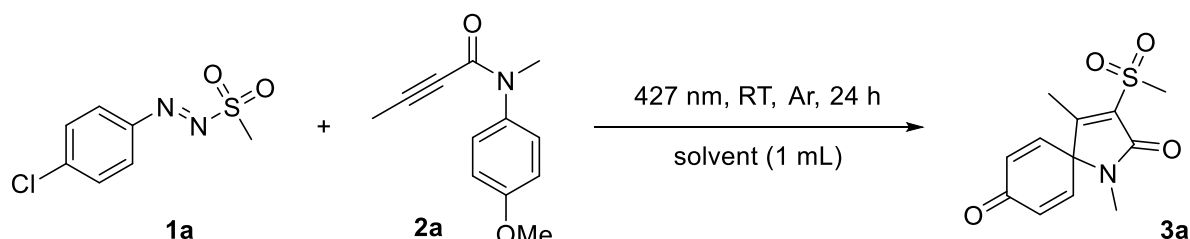
1.4 Aim of the Thesis

In this thesis, the application of arylazo sulfones as sulfonylating agents for the photochemical transformation of *N*-aryl propiolamides in 3-sulfonyl-azaspiro[4,5]trienones is described. The proposed strategy is a visible-light-mediated and photocatalyst-free synthetic pathway. Following an initial screening to determine the optimal reaction conditions, as detailed in Chapter 2, the optimised parameters will be applied to explore the reaction scope through the synthesis of a series of different molecules, in that way assessing the influence of different reagents on the performance of the reaction.

2. Results and Discussion

2.1 Optimization

My investigation started taking as reference the reaction between 4-chlorophenylazo sulfone **1a** and propiolamide **2a** at room temperature, irradiating with a 427 nm lamp for 24 hours. This process was performed under argon atmosphere, using deoxygenated ethyl acetate as solvent (Table 1, entry 1).



At first, different solvents were tested, with the results summarized in Table 1. After TLC analysis (eluent mixture: CyHex/EA 1:1), entries 2 and 3 were chosen to be purified by column chromatography, delivering a 25% and 27% yield of the desired product **3a**, respectively.

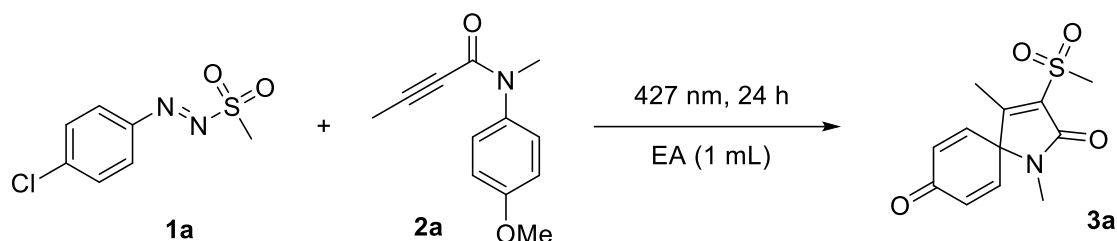
entry	1 (mmol)	2 (mmol)	solvent	3a (%)
1	0.3 (3 equiv.)	0.1 (1 equiv.)	EA	34
2	0.3	0.1	MeCN/H ₂ O 4:1	25
3	0.3	0.1	MeCN	27
4	0.3	0.1	DMC	14*
5	0.3	0.1	DCM	-
6	0.3	0.1	THF	-
7	0.3	0.1	EtOH	-

*NMR yield

Table 1. Optimization step 1: solvents

The product obtained from entry 4 was not isolated and the yield was calculated from the NMR spectrum by adding a reference compound to the crude reaction product. From the peaks of the reference compound and the peaks of the product in the NMR spectrum, the approximate NMR yield of the compound was calculated to be 14%. We observed the presence of residual starting material at the end of the reaction, indicating that it did not reach completion.

Keeping ethyl acetate as the solvent, different reaction conditions were investigated. Changes in temperature, irradiation intensity, varying the amount of amide were tested and the results are reported in Table 2.



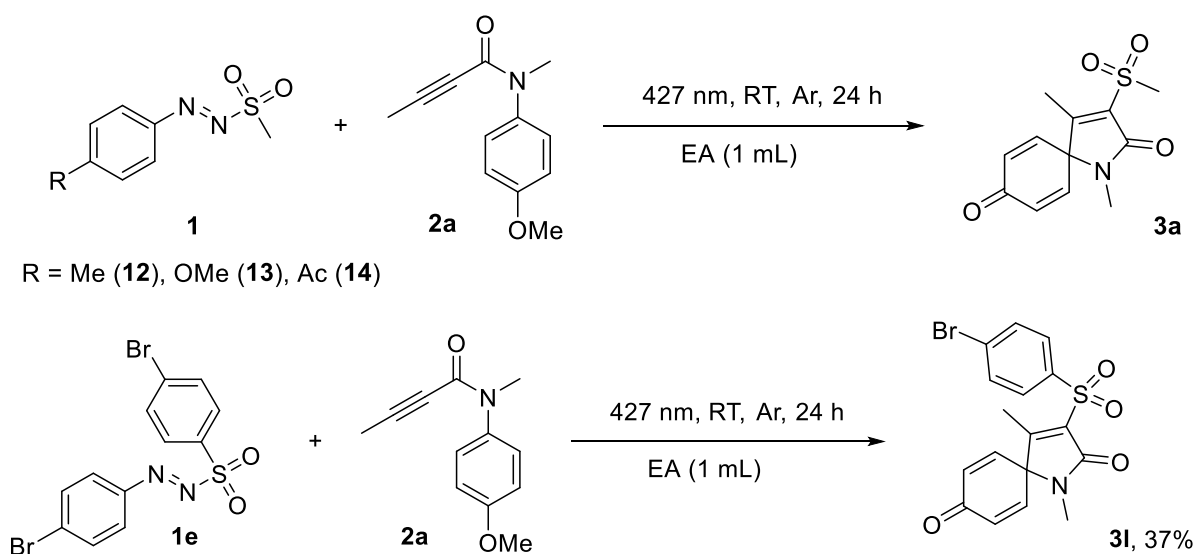
entry	1 (mmol)	2 (mmol)	conditions	3a (%)*
1	0.3 (3 equiv.)	0.05 (0.5 equiv.)	Ar	33
2	0.3	0.1 (1 equiv.)	40°C	8
3	0.3	0.1	427 nm 25%	7
4	0.3	0.1	stirring	9

*NMR yield

Table 2. Optimization step 2: different conditions

After 24 hours, by the TLC analysis it was observed that there was some unreacted amide leftover. Therefore, we wanted to see if all of the amide could be consumed by using half of the amount that had been used, while keeping constant the quantity of arylazo sulfone. Even though the yield is compatible with entry 1 (Table 1), the TLC still showed unreacted amide. A considerable difference can be seen for the last three entries where the yield is significantly lower, therefore heating and reduced intensity of irradiation are not conditions that we will keep in the future.

To this point, the conditions from entry 1 (Table 1) were kept, and for the next optimization step we tried using different arylazo sulfones, changing substituent in the *para* position to the sulfonyl group on the benzene ring. A sulfone bearing a novel sulfonyl moiety (**1e**) was also employed, resulting in the formation of the new product **3l**.



entry	1 (mmol)	2 (mmol)	R	solvent	3 (%)*
1	0.3 (3 equiv.)	0.1 (1 equiv.)	Me	EA	18
2	0.3	0.1	OMe	EA	8
3	0.3	0.1	Ac	EA	11

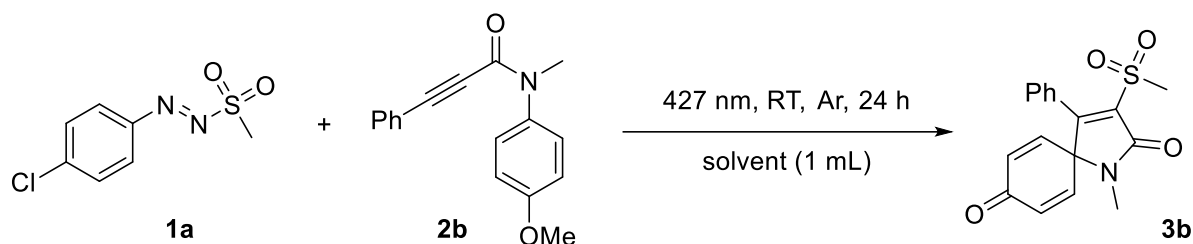
*NMR yield

Table 3. Optimization step 3: different R group in arylazo sulfone

The yields with the differently substituted arylazo sulfones were still low, while using arylazo sulfone **1e** resulted in a 37% NMR yield of **3l**. To this moment, the optimization didn't bring many improvements, and the yield never went over 40%, therefore we decided to change one of the two reagents.

Since *N*-(4-methoxyphenyl)-*N*-methylbut-2-ynamide **2a** revealed to be inefficient in all the previous entries, I decided to switch to *N*-(4-methoxyphenyl)-*N*-methyl-3-phenylpropiolamide **2b** as a new reagent, which ensured better reactivity (Table 4, entry 15). I so embarked in a new investigation of the parameters that can affect the reactivity, retracing a similar way used for **2a**. Firstly, different solvents were tested, then the conditions were modified to see how the yield would change.

Four of the solvents used in the initial phase of the optimization (Table 1) were kept. Acetonitrile is polar and aprotic, and it is fully miscible with water and most organic solvents. However, it is rather toxic and it is associated with environmental and safety concerns. Dichloromethane is a moderately polar halogenated solvent, its volatility and ability to dissolve most organic compounds may be seen as advantages, but it is toxic and potentially carcinogenic, while also posing some environmental concerns due to halogenation. Dimethyl carbonate is moderately polar, aprotic and it has a relatively low toxicity. It is considered as a "green" solvent, making it a great solution to green chemistry principles. Finally, ethyl acetate is a moderately polar and aprotic solvent, it is biodegradable and has a relatively lower toxicity.^{42,43} As a result, ethyl acetate and dimethyl carbonate are a better choice compared to more toxic acetonitrile and dichloromethane, as they represent interesting options when thinking about the sustainability aspect, especially when compared to halogenated solvents. Ethyl acetate was employed directly from the commercial bottle, which can be regarded as 'dry' ethyl acetate in the majority of entries (Table 4). For entry 5, however, an aqueous ethyl acetate (EA(aq)) system was prepared by adding equal amounts of water and ethyl acetate to a flask, shaking the mixture, and subsequently allowing the phases to separate. The upper organic layer (ethyl acetate) was collected and used as the solvent for the reaction.



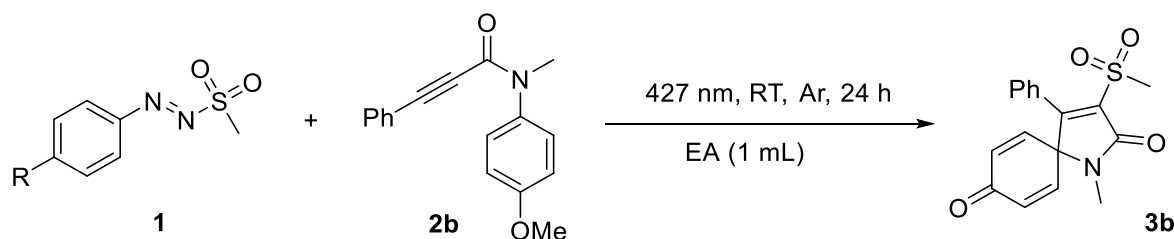
entry	1 (mmol)	2 (mmol)	solvent	conditions	3b (%) [*]
1	0.3 (3 equiv.)	0.1 (1 equiv.)	EA	-	62
2	0.3	0.1	MeCN	-	58
3	0.3	0.1	DCM	-	53
4	0.3	0.1	DMC	-	73
5	0.3	0.1	EA(aq)	-	72
6	0.3	0.1	EA	stirring	86 ^{**}
7	0.3	0.1	EA	40°C	66
8	0.3	0.1	EA	427 nm 25%	44
9	0.3	0.05 (0.5 equiv.)	EA	-	79

^{*}NMR yield ^{**}isolated yield

Table 4. Optimization step 4: new amide, different solvents and conditions

Normal EA was elected as the reaction medium, taking into consideration the comparable effect of DMC on the reaction and similar eco-friendly behaviors. Different conditions were applied for entries 6–9, like heating, stirring and decreased irradiation. Entry 6 with added stirring had a 65% NMR yield, from the TLC analysis the spot of the product was clean therefore it was chosen to be purified by column chromatography delivering **3b** with 86% yield.

Different arylazo sulfones were again tested, changing the substituent in the *para* position to the sulfonyl group on the benzene ring, with average NMR yields.



R = Me (**24**), OMe (**25**), Ac (**26**)

entry	1 (mmol)	2 (mmol)	R	solvent	3b (%) [*]
1	0.3 (3 equiv.)	0.1 (1 equiv.)	Me	EA	56
2	0.3	0.1	OMe	EA	59
3	0.3	0.1	Ac	EA	60

^{*}NMR yield

Table 5. Optimization step 5: different R group in arylazo sulfone

Finally, introducing *tert*-butyl hydroperoxide (TBHP) as additional oxidant brought significant improvements. In the beginning, 3 equivalents were used, then the amount was decreased to 2 equivalents and stirring was added (Table 6, entry 3). With the aim to use the smallest quantity of TBHP as possible, we tried using 0.5 equivalents (Table 6, entry 4) and to our delight the NMR yield in these conditions and the one with 2 equivalents of TBHP was the same, therefore 0.5 equivalents of TBHP were kept for the final optimized conditions.

entry	1 (mmol)	2 (mmol)	conditions	3b (%)*
1	0.3 (3 equiv.)	0.1 (1 equiv.)	TBHP (3 equiv.)	69
2	0.3	0.1	TBHP (2 equiv.)	81
3	0.3	0.1	TBHP (2 equiv.) + stirring	86
4	0.3	0.1	TBHP (0.5 equiv.) + stirring	86

*NMR yield

Table 6. Optimization step 6: addition of TBHP

The definitive reaction conditions were finally chosen to be the ones of entry 4: ethyl acetate for solvent, 24 hours reaction time, deoxygenation with argon and stirring at room temperature under irradiation with a 427 nm lamp, with 0.5 equivalents of TBHP.

With the optimized reaction conditions in hand, we explored the substrate scope of the reaction.

2.2 Reaction scope

Keeping the same amide **2b**, several arylazo sulfones with a different sulfonyl moiety were used for the synthesis of spirocycles. The yields refer to the isolated products. The used arylazo sulfones are reported in Figure 1.

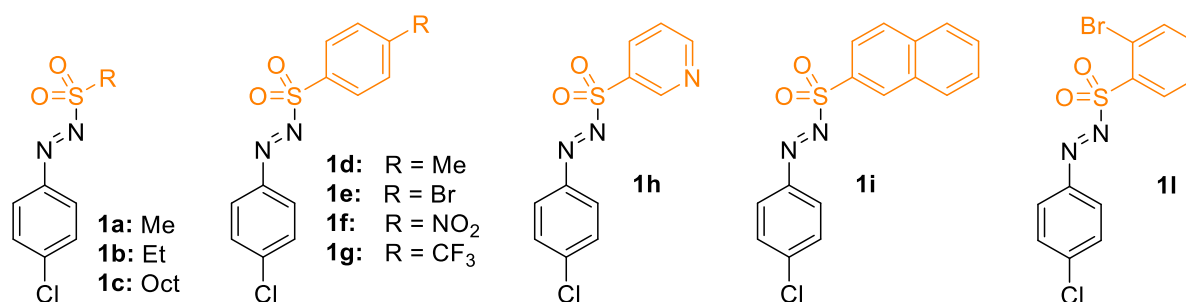
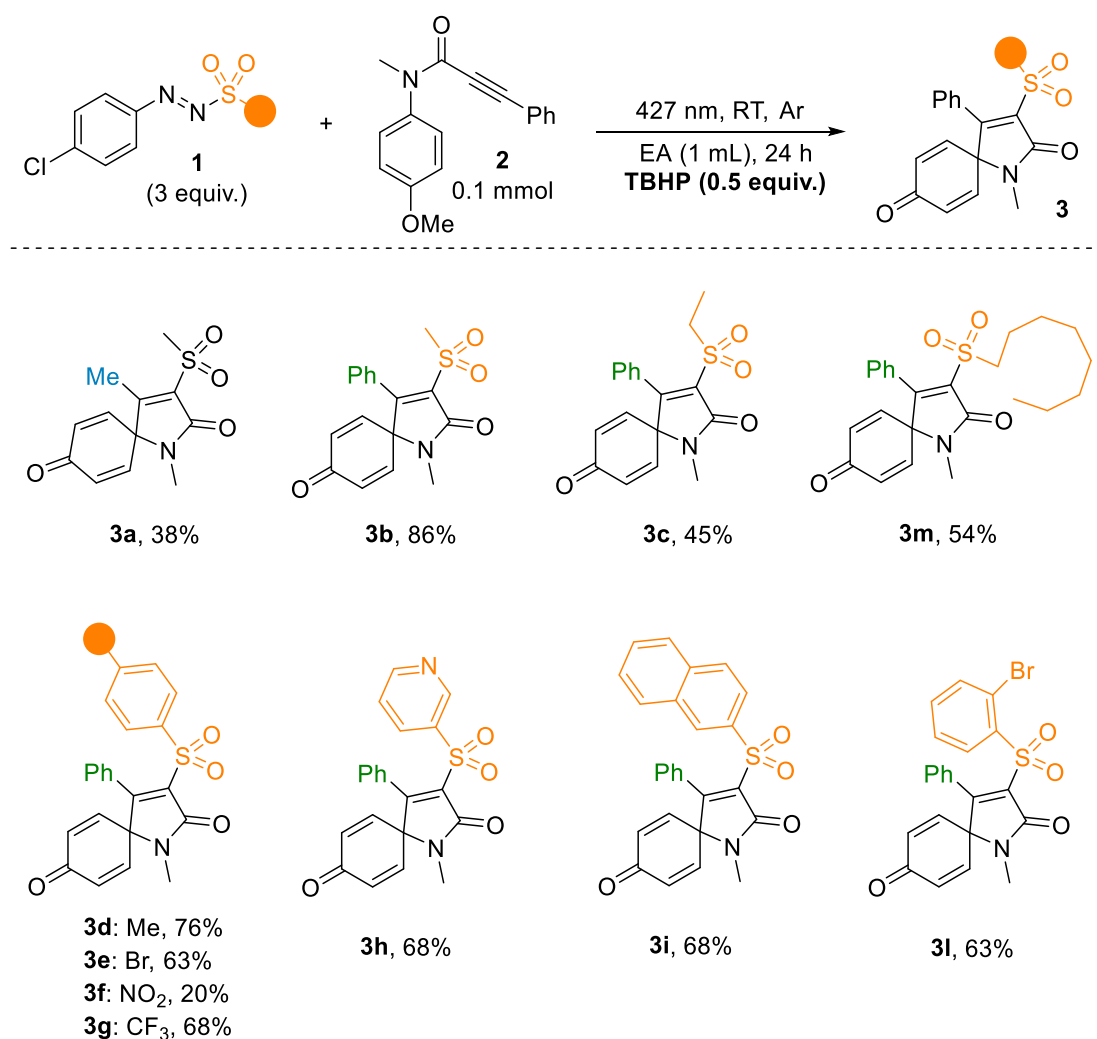


Figure 1. Arylazo sulfones used for the scope

The scope is reported in Scheme 9.



Scheme 9. Scope of arylazo sulfones

The variation of the R substituent on the -SO₂R moiety influenced the reaction outcome. Most compounds were isolated in yields ranging from 60 to 80% with alkyl-substituted sulfones giving slightly lower yields, particularly in the case of **3c**. On the other hand, aryl-substituted sulfones generally performed well. Compound **3f**, however, was isolated in only 20% yield and could not afford a good result, representing a limitation in the sulfone scope. Overall, these results are encouraging, as they show that the reaction proceeds efficiently across a range of sulfone substrates.

2.3 Solar reaction

The reaction was subsequently tested using natural sunlight as the illumination source. The reaction vial was prepared following the same procedure as for the experiments conducted in the photoreactor, with the exception that no magnetic stirring bar was included, as stirring was not possible outdoors. The reaction time under sunlight was observed to be longer than that

under artificial light from the lamp, which is attributable to differences in light intensity between the two sources. The yield was nevertheless quite good and comparable to the yields of the reactions performed in the photoreactor boxes.

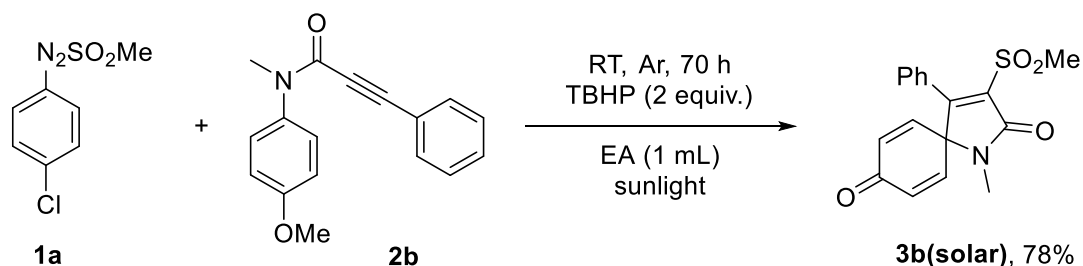
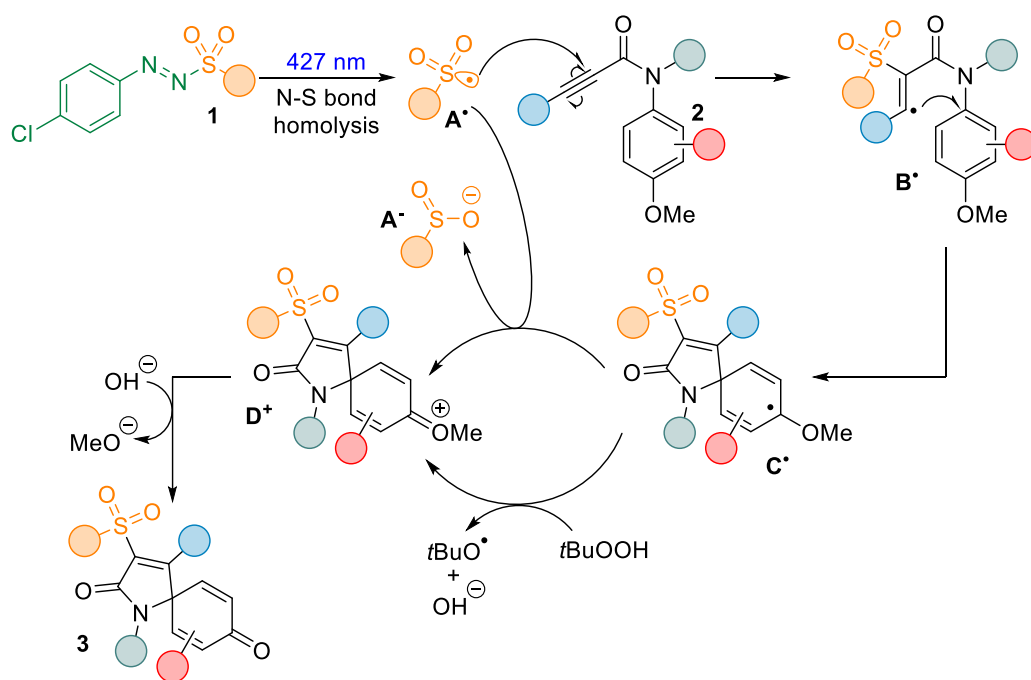


Figure 2. Set-up for solar reaction

2.4 Proposed mechanism

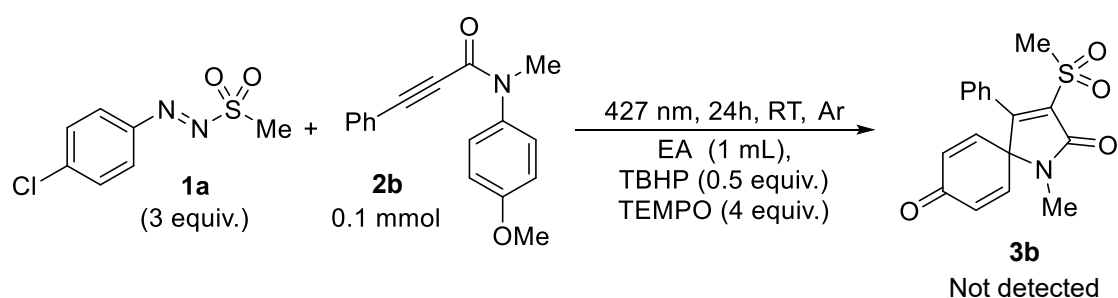
Based on previous works and observations,²⁶ the proposed reaction mechanism is reported in Scheme 10.

Upon irradiation of the arylazo sulfone **1**, the N–S bond goes through homolytic cleavage, forming a diazenyl radical and sulfonyl radical (**A•**). The sulfonyl radical then reacts with the α -position of the propiolamide **2**, forming a new C–S bond. The vinyl radical-adduct (**B•**) rapidly undergoes an *ipso*-spirocyclization with the *N*-(4-methoxy)phenyl moiety, resulting into the spiro-radical intermediate **C•**. Finally, **C•** is oxidized to the cation intermediate **D⁺**, a step which can be mediated by *tert*-butyl hydroperoxide (*t*BuOOH) or an additional **A•** delivering **A⁺**, and then converted to the final product **3**.



Scheme 10. Proposed mechanism for the preparation of 3-sulfonyl-azaspiro[4,5]trienones

The addition of TEMPO as a radical scavenger in the reaction between arylazo sulfone **1a** and propiolamide **2b** completely suppresses the formation of product **3b**, suggesting a radical reactivity nature of the process. To address the possibility of a radical chain propagation, the quantum yields for the formation of **3** (Φ) and the consumption of **2** (Φ_{-1}) were determined under reaction conditions. In both cases, values below 1 were obtained ($\Phi = 0.208$, $\Phi_{-1} = 0.192$), indicating that a chain propagation contribution to the mechanism may be safely excluded.



Scheme 11. Trapping experiment with TEMPO

3. Summary and Outlook

In recent years, the drive toward the development of eco-sustainable synthetic protocols has particularly highlighted photochemical reactions that employ visible light. Many strategies have been proposed for the synthesis of spirocyclic compounds, with an increasing number of photochemical methods that have been introduced.

The encouraging biological profile of spirocyclic compounds led us to identify an efficient method to synthesize a moderate number of derivatives. The aim of this thesis was to develop a visible-light-mediated strategy for the synthesis of 3-sulfonyl-azaspiro[4,5]trienones. The proposed method is an effective spirocyclization, simple and eco-friendly thanks to the use of light. Moreover, the mild conditions and the absence of a photocatalyst make this method particularly interesting. The arylazo sulfones and the *N*-aryl propiolamides are reagents of easy synthesis and purification, as they are both derived from non-expensive commercially available starting materials.

The initial part of the work consisted in a step of optimization to find the most appropriate reaction conditions. After switching from amide **2a** to amide **2b**, the yield increased significantly, therefore prompting us to choose **2b**. The optimization was carried out successfully to determine the best conditions, and subsequently a scope was performed to isolate a number of azaspiro[4,5]trienones. Due to the limited time that I have worked on this project, I was not able to isolate an extensive library of compounds. My activity focused on using different sulfonyl moieties on the arylazo sulfones (-SO₂R) to obtain compounds **3b–3l**. The possibility of using solar light was confirmed by exposing a vial to sunlight and checking the course of the reaction, which proved successful.

The hypothesized mechanism goes through a radical addition of the sulfonyl radical to the triple bond of the *N*-aryl propiolamide, after irradiation of the arylazo sulfone, and subsequent *ipso*-cyclization of the alkenyl radical to form the spirocyclic intermediate, which is oxidized by TBHP to the final molecule. To confirm the proposed mechanism, experiments in the presence of radical inhibitors still have to be conducted.

To broaden the scope of isolated molecules, different reagents have yet to be investigated, to introduce new substituents in the final products. Besides from the sulfonyl moiety of the arylazo sulfone, substituents on the aromatic ring with electron-donating and electron-withdrawing properties should be used. Several types of differently *N*-substituted propiolamides have to be employed, and different substituents on the *N*-aryl moiety also have to be examined to assess their influence on the reaction.

In conclusion, an efficient method for the synthesis of azaspiro[4,5]trienones was developed. While some more in-depth analysis has to be conducted, the current state of the results I achieved is promising and introduces a new method for the synthesis of biologically relevant spirocyclic molecules.

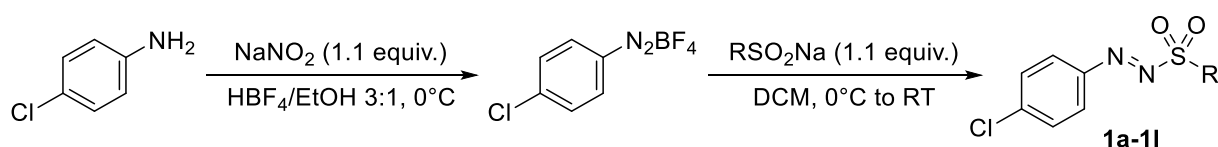
4. Experimental Section

4.1 General

Analytical thin layer chromatography (TLC) plates (silica gel 60Å F254) were visualized either with a UV lamp (254 nm), or by submersion in the chosen stain for TLC. The products were purified by flash column chromatography using an Isolera apparatus (Biotage) and a SiO₂ cartridge (eluant: cyclohexane/ethyl acetate mixture). Proton ¹H NMR spectra were recorded on a Bruker Avance NEO 400 MHz or Bruker Avance 300 MHz, and proton-decoupled carbon ¹³C{¹H} NMR spectra were recorded either at 101 MHz or 75 MHz. Proton-decoupled fluorine ¹⁹F{¹H} NMR spectra were recorded at 376 MHz. Chemical shifts (δ) are reported in parts per million (ppm) relative to residual solvent as the internal reference. The NMR yields were calculated using 1,3,5-trimethoxybenzene as an external reference compound. The crude reaction product was dried in vacuo to remove all the solvent, then 1,3,5-trimethoxybenzene (0.1 mmol, 16.8 mg) was added to the vial and finally the chosen deuterated solvent was added. The NMR spectrum was analysed and the comparison between the peaks of the reference compound and the peaks of the product afforded the approximate NMR yield of the compound. The photochemical experiments were carried out in an EvoluChemTM PhotoRedox monobox equipped with a 427 nm Kessil lamp as the light source and a fan system as the cooling system, and placed on a stirrer plate. The arylazo sulfones and the amide **2a** used for the photochemical reactions were available in the lab as pure compounds.

4.2 Synthesis of arylazo sulfones

Arylazo sulfones employed in this thesis have been prepared by following a two-step procedure already reported in literature (Scheme S1).³⁴ The list of arylazo sulfones employed in the present work is depicted in Figure S1. Full characterization of compounds **1a-1e** has been already reported in literature.^{44,45}



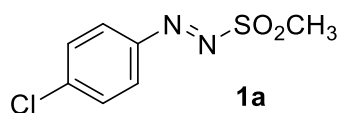
Scheme S1. Two-step synthesis of arylazo sulfones.

Synthesis of arenediazonium tetrafluoroborates (step a). A 100 mL round-bottom flask was charged with the chosen arylamine (10 mmol, 1.0 equiv.), EtOH (2 mL) and HBF₄ (48%aq, 6 mL). After stirring for 15 minutes, an aqueous solution of NaNO₂ (1.1 equiv., in minimum quantity of H₂O) was added dropwise at 0 °C. The mixture was stirred for additional 30 minutes at 0 °C, then 50 mL of diethyl ether were added to precipitate the arenediazonium tetrafluoroborate, which was filtered and washed with additional diethyl ether. The crude was employed in the next step without further purification.

Synthesis of arylazo sulfones (step b). In a 250 mL round-bottom flask, the previously prepared arenediazonium tetrafluoroborate (10 mmol, 1.0 equiv.) was suspended in DCM (100 mL) and stirred at 0 °C; then the chosen sulfinic acid (1.1 equiv.) was added to the reaction system in one portion. The suspension was allowed to warm up to room temperature and stirred overnight. The resulting mixture was filtered, and the solvent was removed under vacuum. The crude was revived with cold DCM, the product was precipitated by adding *n*-Hexane, and then filtered and dried in vacuo.

4.2.1 Arylazo sulfones characterizations and spectra

1-(4-chlorophenyl)-2-(methylsulfonyl)diazene (**1a**)⁴⁶

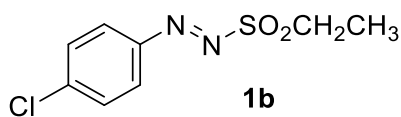


1a

¹H NMR (400 MHz, CDCl₃) δ 7.91 (d, *J* = 8.8 Hz, 2H), 7.57 (d, *J* = 8.8 Hz, 2H), 3.22 (s, 3H). ¹³C NMR (75 MHz, CDCl₃) 147.3, 141.8, 130.2, 125.8, 34.9. **m.p.** = 116–118 °C. Compound unstable

upon HRMS analysis

1-(4-chlorophenyl)-2-(ethylsulfonyl)diazene (**1b**)⁴⁴

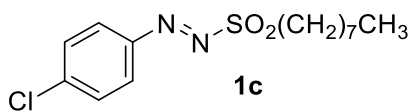


1b

¹H NMR (300 MHz, CDCl₃) δ 8.10–7.76 (m, 2H), 7.68–7.45 (m, 2H), 3.48 (q, *J* = 7.5 Hz, 2H), 1.50 (t, *J* = 7.5 Hz, 3H). ¹³C NMR (75 MHz, CDCl₃) δ 147.8, 141.9, 130.3, 125.8,

43.1, 7.6. **m.p.** = 76–78 °C (Hexane). Compound unstable upon HRMS analysis

1-(4-chlorophenyl)-2-(octylsulfonyl)diazene (**1c**)⁴⁴

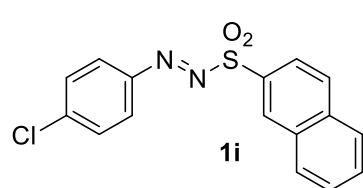


1c

¹H NMR (300 MHz, CDCl₃) δ 7.90 (d, *J* = 8.8 Hz, 2H), 7.56 (d, *J* = 8.7 Hz, 2H), 3.45–3.38 (m, 2H), 1.92 (tt, *J* = 7.9, 6.4 Hz, 2H), 1.54–1.42 (m, 2H), 1.34–1.23 (m, 8H), 0.86 (t, *J* =

6.1 Hz, 3H). ¹³C NMR (75 MHz, CDCl₃) δ 147.8, 141.8, 130.3, 125.8, 48.3, 31.8, 29.0, 28.7, 22.7, 14.2. **m.p.** = 62–64 °C (Hexane). Compound unstable upon HRMS analysis

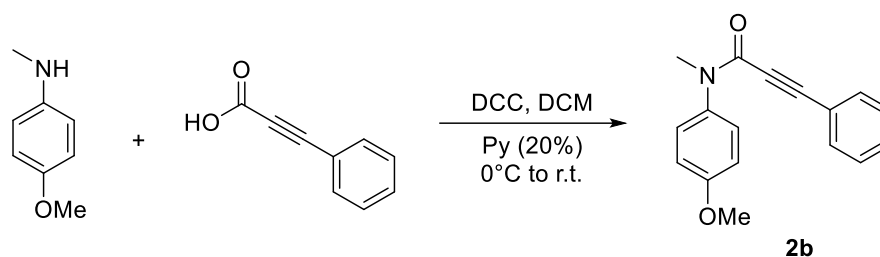
1-(4-chlorophenyl)-2-(naphthalen-2-ylsulfonyl)diazene (**1i**)⁴⁴



¹H NMR (300 MHz, CDCl₃) δ 8.61 (d, *J* = 1.8 Hz, 1H), 8.05 – 8.00 (m, 2H), 7.98 – 7.87 (m, 2H), 7.78 – 7.63 (m, 4H), 7.48 – 7.42 (m, 2H). ¹³C NMR (75 MHz, CDCl₃) δ 147.6, 141.5, 136.0, 133.0, 132.2, 130.0, 129.7, 129.5, 128.2, 128.0, 125.9,

124.4. **m.p.** = 117–119 °C (Hexane). Compound unstable upon HRMS analysis.

4.3 Synthesis of *N*-substituted-*N*-aryl propiolamides



In a 150 mL round-bottom flask, 4-methoxy-*N*-methylaniline (5.0 mmol, 1.0 equiv.) and phenylpropionic acid (5.0 mmol, 1.0 equiv.) were dissolved in DCM (30 mL) at 0°C, then dicyclohexylcarbodiimide (DCC) (7.5 mmol, 1.5 equiv.) was added dropwise, followed by pyridine (1 mmol, 0.2 equiv.). The resulting mixture was stirred at 0°C for 1 hour, then, removed from the ice bath, for 12 hours at room temperature. The mixture was filtered and washed with DCM (3 × 50 mL), the organic layers were collected and extracted with brine (2 × 50 mL). The organic layer was dried over Na₂SO₄, the solvent was removed in vacuo and the residue was purified by silica gel flash column chromatography (0-30% EA in CyHex). The purified compound **2b** was obtained as a white solid (0.985 g, 75%).

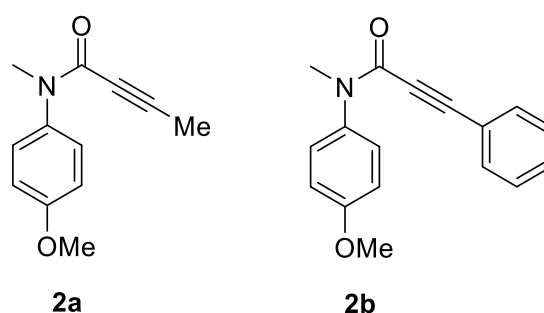
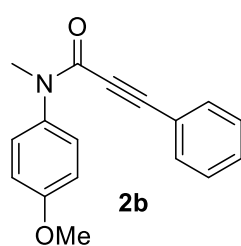


Figure S1. *N*-methyl-*N*-aryl propiolamides employed in the present thesis.

N-(4-methoxyphenyl)-*N*-methyl-3-phenylpropiolamide (**2b**)

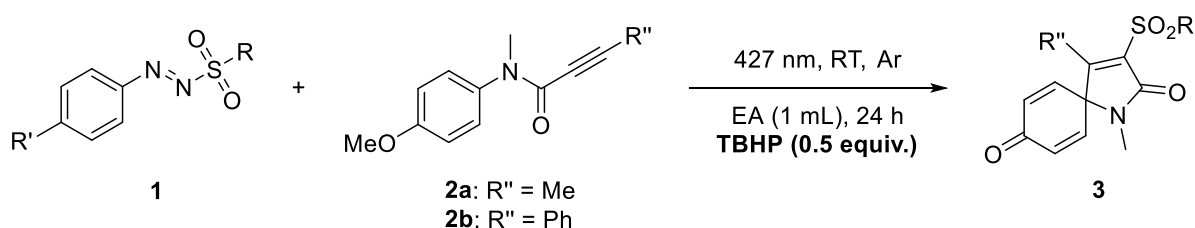
Starting from 686 mg (5.0 mmol, 1.0 equiv) of 4-methoxy-*N*-methylaniline and 733 mg of phenylpropionic acid (5.0 mmol, 1.0 equiv). Compound **2b** was obtained in 0.985 g as a white solid (yield 75%).



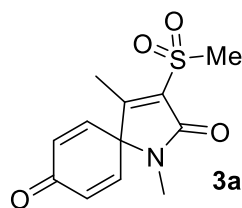
¹H NMR (200 MHz, CDCl₃) δ 7.29 (d, *J* = 6.5 Hz, 5H), 7.24 – 7.14 (m, 3H), 7.02 – 6.91 (m, 2H), 3.87 (s, 3H), 3.37 (s, 3H). **¹³C{¹H} NMR** (75 MHz, CDCl₃) δ 159.2, 154.7, 136.2, 132.5, 130.0, 128.7, 128.4, 120.6, 114.4, 91.0, 82.7, 55.7, 36.7. Spectroscopic data of **2b** are in accordance with the literature.⁴⁷

4.4 Photochemical procedure for the synthesis of azaspiro[4.5]trienones (3a–3l).

A 4 mL vial equipped with a magnetic stirrer was charged with the chosen arylazo sulfone **1** (0.3 mmol, 3 equiv) and *N*-(4-methoxyphenyl)-*N*-methyl propiolamide **2a** or **2b** (0.1 mmol, 1 equiv) which were mixed together in ethyl acetate (1 mL). The solution was deoxygenated by purging Argon for approximately 5 minutes, then 5.5 μL of *tert*-butyl hydroperoxide (TBHP 70% in H₂O) (0.05 mmol, 0.5 equiv.) were added, and the vial was capped. The solution was irradiated for 24 hours at room temperature by means of a 427 nm Kessil Lamp under continuous stirring. The photolyzed solution was checked by TLC (1:1 CyHex/EA) and the crude was purified by silica gel flash column chromatography (Hex/EA as eluent mixture) furnishing desired compound **3**.



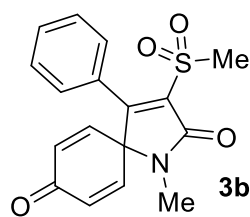
1,4-dimethyl-3-(methylsulfonyl)-1-azaspiro[4.5]deca-3,6,9-triene-2,8-dione (3a).



Starting from 65.6 mg of **1a** (0.3 mmol, 3 equiv.), 20.3 mg of **2a** (0.1 mmol, 1 equiv.). Purification via silica gel chromatography (Hex/EA 30:70) afforded **3a** in 38% yield (10.2 mg, pale yellow solid).

R_f = 0.1 (CyHex/EA 1:1). **¹H NMR** (300 MHz, CDCl₃) δ 6.65 (d, *J* = 10.2 Hz, 2H), 6.36 (d, *J* = 10.2 Hz, 2H), 3.33 (s, 3H), 2.88 (s, 3H), 2.23 (s, 3H). **¹³C NMR** (75 MHz, CDCl₃) δ 183.3, 164.5, 162.0, 143.0, 134.8, 134.7, 68.5, 42.6, 26.6, 12.0.

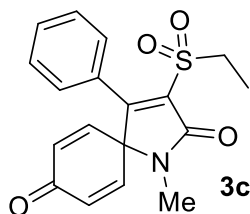
1-methyl-3-(methylsulfonyl)-4-phenyl-1-azaspiro[4.5]deca-3,6,9-triene-2,8-dione (**3b**).



Starting from 65.6 mg of **1a** (0.3 mmol, 3 equiv.), 26.5 mg of **2b** (0.1 mmol, 1 equiv.). Purification via silica gel chromatography (Hex/EA 30:70) afforded **3b** in 86% yield (28.5 mg, pale yellow solid).

$R_f = 0.1$ (CyHex/EA 1:1). $^1\text{H NMR}$ (300 MHz, CDCl_3) δ 7.46 – 7.31 (m, 4H), 7.24 – 7.21 (m, 1H), 6.51 (s, 4H), 3.32 (s, 3H), 2.92 (s, 3H). $^{13}\text{C NMR}$ (75 MHz, CDCl_3) δ 183.2, 164.2, 162.0, 142.2, 135.8, 134.6, 131.0, 128.3, 128.3, 128.2, 68.3, 42.6, 26.5. **HRMS** (ESI-TOF) m/z : calcd. for $\text{C}_{17}\text{H}_{16}\text{NO}_4\text{S}^+ [\text{M}+\text{H}]^+$: 330.0795, found: 330.0791.

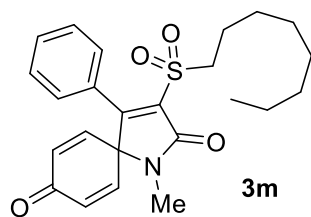
3-(ethylsulfonyl)-1-methyl-4-phenyl-1-azaspiro[4.5]deca-3,6,9-triene-2,8-dione (**3c**).



Starting from 69.8 mg of **1b** (0.3 mmol, 3 equiv.), 26.5 mg of **2a** (0.1 mmol, 1 equiv.). Purification via silica gel chromatography (Hex/EA 35:65) afforded **3c** in 45% yield (15.4 mg, pale yellow solid).

$R_f = 0.1$ (CyHex/EA 1:1). $^1\text{H NMR}$ (300 MHz, CDCl_3) δ 7.45 – 7.32 (m, 3H), 7.25 – 7.18 (m, 2H), 6.55 – 6.45 (m, 4H), 3.52 (q, $J = 7.4$ Hz, 2H), 2.92 (s, 3H), 1.33 (t, $J = 7.4$ Hz, 3H). $^{13}\text{C NMR}$ (75 MHz, CDCl_3) δ 183.1, 164.2, 163.6, 142.2, 142.2, 134.6, 130.9, 128.4, 128.2, 128.1, 68.6, 48.4, 26.5, 6.6. **HRMS** (ESI-TOF) m/z : calcd. for $\text{C}_{18}\text{H}_{18}\text{NO}_4\text{S}^+ [\text{M}+\text{H}]^+$: 344.0951, found: 344.0950.

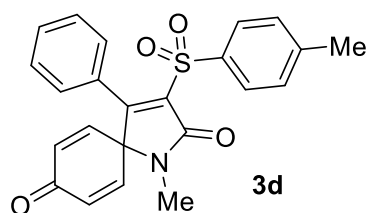
1-methyl-3-(octylsulfonyl)-4-phenyl-1-azaspiro[4.5]deca-3,6,9-triene-2,8-dione (**3m**).



Starting from 95.0 mg of **1c** (0.3 mmol, 3 equiv.), 26.5 mg of **2a** (0.1 mmol, 1 equiv.). Purification via silica gel chromatography (Hex/EA 1:1) afforded **3c** in 54% yield (23.1 mg, pale yellow solid).

$R_f = 0.2$ (CyHex/EA 1:1). $^1\text{H NMR}$ (300 MHz, CDCl_3) δ 7.4 – 7.3 (m, 3H), 7.2 – 7.2 (m, 2H), 6.5 – 6.5 (m, 4H), 3.5 – 3.4 (m, 2H), 2.9 (s, 3H), 1.8 – 1.7 (m, 2H), 1.4 – 1.2 (m, 10H), 0.9 – 0.8 (m, 3H). $^{13}\text{C NMR}$ (75 MHz, CDCl_3) δ 183.1, 164.3, 163.0, 142.3, 134.5, 134.5, 130.8, 128.4, 128.1, 128.1, 68.5, 53.9, 31.8, 29.1, 29.0, 28.4, 26.5, 22.7, 21.8, 14.2. **HRMS** (ESI-TOF) m/z : calcd. for $\text{C}_{24}\text{H}_{29}\text{NO}_4\text{S}^+ [\text{M}+\text{H}]^+$: 428.1890, found: 428.1876

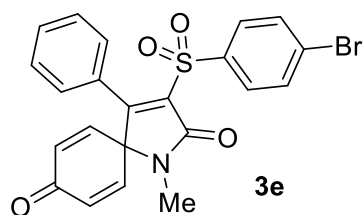
1-methyl-4-phenyl-3-tosyl-1-azaspiro[4.5]deca-3,6,9-triene-2,8-dione (3d).



Starting from 81.8 mg of **1d** (0.3 mmol, 3 equiv.), 26.5 mg of **2a** (0.1 mmol, 1 equiv.). Purification via silica gel chromatography (Hex/EA 60:40) afforded **3d** in 76% yield (30.4 mg, pale yellow solid).

R_f = 0.3 (CyHex/EA 1:1). $^1\text{H NMR}$ (300 MHz, CDCl_3) δ 7.96 – 7.87 (m, 2H), 7.44 – 7.31 (m, 5H), 7.18 – 7.10 (m, 2H), 6.48 – 6.39 (m, 4H), 2.82 (s, 3H), 2.44 (s, 3H). $^{13}\text{C NMR}$ (75 MHz, CDCl_3) δ 183.2, 163.7, 161.7, 145.7, 142.4, 137.0, 136.3, 134.4, 130.4, 129.8, 129.4, 128.8, 128.1, 128.0, 68.4, 26.5, 21.9. **HRMS** (ESI-TOF) m/z : calcd. for $\text{C}_{23}\text{H}_{20}\text{NO}_4\text{S}^+$ $[\text{M}+\text{H}]^+$: 406.1108, found: 406.1104.

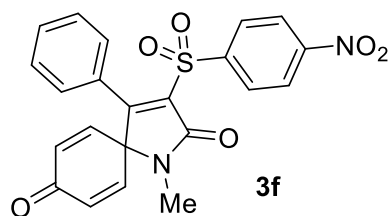
3-((4-bromophenyl)sulfonyl)-1-methyl-4-phenyl-1-azaspiro[4.5]deca-3,6,9-triene-2,8-dione (3e).



Starting from 121.2 mg of **1e** (0.3 mmol, 3 equiv.), 26.5 mg of **2b** (0.1 mmol, 1 equiv.). Purification via silica gel chromatography (Hex/EA 60:40) afforded **3e** in 63% yield (30.6 mg, pale yellow solid).

R_f = 0.3 (CyHex/EA 1:1). $^1\text{H NMR}$ (300 MHz, CDCl_3) δ 7.94 – 7.87 (m, 2H), 7.71 – 7.65 (m, 2H), 7.46 – 7.34 (m, 3H), 7.18 – 7.10 (m, 2H), 6.49 – 6.39 (m, 4H), 2.83 (s, 3H). $^{13}\text{C NMR}$ (75 MHz, CDCl_3) δ 183.1, 163.5, 162.6, 142.1, 138.2, 136.5, 134.6, 132.5, 131.0, 130.7, 130.2, 128.5, 128.2, 127.9, 68.5, 26.5.

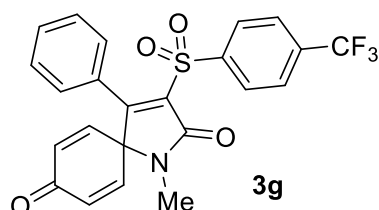
1-methyl-3-((4-nitrophenyl)sulfonyl)-4-phenyl-1-azaspiro[4.5]deca-3,6,9-triene-2,8-dione (3f).



Starting from 92.8 mg of **1f** (0.3 mmol, 3 equiv.), 26.5 mg of **2b** (0.1 mmol, 1 equiv.). Purification via silica gel chromatography (Hex/EA 60:40) afforded **3f** in 20% yield (8.5 mg, pale yellow solid).

R_f = 0.2 (CyHex/EA 1:1). $^1\text{H NMR}$ (400 MHz, Chloroform-d) δ 8.42 – 8.34 (m, 2H), 8.30 – 8.22 (m, 2H), 7.50 – 7.38 (m, 3H), 7.21 – 7.15 (m, 2H), 6.51 – 6.39 (m, 4H), 2.83 (s, 3H). $^{13}\text{C NMR}$ (101 MHz, CDCl_3) δ 182.9, 164.1, 163.3, 151.3, 144.7, 141.7, 135.8, 134.8, 131.1, 131.1, 128.4, 128.2, 127.9, 124.3, 68.7, 26.6. **HRMS** (ESI-TOF) m/z : calcd. for $\text{C}_{22}\text{H}_{17}\text{N}_2\text{O}_6\text{S}^+$ $[\text{M}+\text{H}]^+$: 437.0802, found: 437.0809.

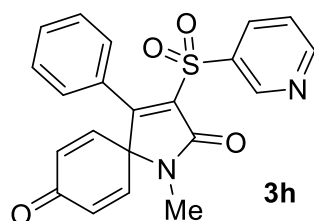
1-methyl-4-phenyl-3-((4-(trifluoromethyl)phenyl)sulfonyl)-1-azaspiro[4.5]deca-3,6,9-triene-2,8-dione (3g).



Starting from 98.6 mg of **1g** (0.3 mmol, 3 equiv.), 26.5 mg of **2b** (0.1 mmol, 1 equiv.). Purification via silica gel chromatography (Hex/EA 70:30) afforded **3g** in 68% yield (31.3 mg, pale yellow solid).

R_f = 0.3 (CyHex/EA 1:1). **¹H NMR** (300 MHz, CDCl₃) δ 8.18 (d, *J* = 8.2 Hz, 2H), 7.80 (d, *J* = 8.2 Hz, 2H), 7.48 – 7.35 (m, 3H), 7.18 – 7.12 (m, 2H), 6.45 (s, 4H), 2.83 (s, 3H). **¹³C NMR** (75 MHz, CDCl₃) δ 183.0, 163.4, 163.4, 142.7, 141.9, 136.2, 136.1, 135.7, 134.6, 130.0, 128.4, 128.2, 127.9, 126.3 (q, *J* = 3.7 Hz), 123.2 (q, *J* = 272.7 Hz), 68.6, 26.5. **¹⁹F NMR** (376 MHz, CDCl₃) δ -63.2. **HRMS** (ESI-TOF) *m/z*: calcd. for C₂₃H₁₇F₃NO₄S⁺ [*M*+*H*]⁺: 460.0825, found: 460.0816.

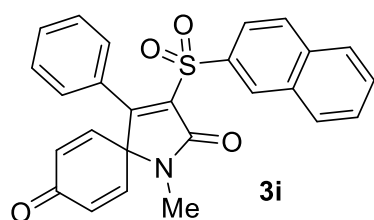
1-methyl-4-phenyl-3-(pyridin-3-ylsulfonyl)-1-azaspiro[4.5]deca-3,6,9-triene-2,8-dione (3h).



Starting from 84.5 mg of **1h** (0.3 mmol, 3 equiv.), 26.5 mg of **2b** (0.1 mmol, 1 equiv.). Purification via silica gel chromatography (Hex/EA 30:70) afforded **3h** in 68% yield (27.4 mg, pale yellow solid).

R_f = 0.1 (CyHex/EA 1:1). **¹H NMR** (300 MHz, CDCl₃) δ 9.13 – 9.07 (m, 1H), 8.88 – 8.82 (m, 1H), 8.49 – 8.41 (m, 1H), 7.52 – 7.36 (m, 4H), 7.21 – 7.13 (m, 2H), 6.49 – 6.39 (m, 4H), 2.82 (s, 3H). **¹³C NMR** (75 MHz, CDCl₃) δ 183.0, 163.4, 163.2, 154.7, 150.1, 141.9, 137.7, 136.1, 135.8, 134.6, 130.9, 128.3, 128.3, 127.9, 123.7, 68.5, 26.5. **HRMS** (ESI-TOF) *m/z*: calcd. for C₂₁H₁₇N₂O₄S⁺ [*M*+*H*]⁺: 393.0904, found: 393.0893.

1-methyl-3-(naphthalen-2-ylsulfonyl)-4-phenyl-1-azaspiro[4.5]deca-3,6,9-triene-2,8-dione (3i).



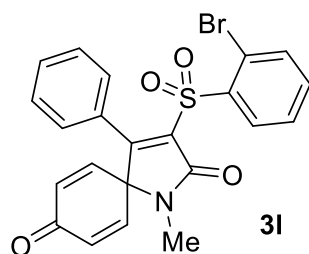
Starting from 99.2 mg of **1i** (0.3 mmol, 3 equiv.), 26.5 mg of **2b** (0.1 mmol, 1 equiv.). Purification via silica gel chromatography (Hex/EA 60:40) afforded **3i** in 68% yield (30.5 mg, pale yellow solid).

R_f = 0.1 (CyHex/EA 1:1). **¹H NMR** (300 MHz, CDCl₃) δ 8.68 – 8.57 (m, 1H), 8.04 – 7.90 (m, 4H), 7.68 – 7.59 (m, 2H), 7.45 – 7.35 (m, 3H), 7.21 – 7.15 (m, 2H), 6.47 – 6.39 (m, 4H), 2.81 (s, 3H). **¹³C NMR** (75 MHz, CDCl₃) δ 183.2, 163.7, 162.2, 142.4, 137.0, 136.2, 135.8, 134.5,

132.2, 131.8, 130.6, 130.0, 129.8, 129.4, 128.7, 128.1, 128.1, 128.0, 127.7, 123.6, 68.5, 26.5.

HRMS (ESI-TOF) m/z : calcd. for $C_{26}H_{19}NO_4S^+$ $[M+H]^+$: 442.1108, found: 442.1101.

3-((2-bromophenyl)sulfonyl)-1-methyl-4-phenyl-1-azaspiro[4.5]deca-3,6,9-triene-2,8-dione (3l).



Starting from 107.8 mg of **1l** (0.3 mmol, 3 equiv.), 26.5 mg of **2b** (0.1 mmol, 1 equiv.). Purification via silica gel chromatography (Hex/EA 60:40) afforded **3l** in 63% yield (29.6 mg, pale yellow solid).

1H NMR (300 MHz, DMSO) δ 8.23 (dd, $J = 7.6, 2.0$ Hz, 1H), 7.64 (dd, $J = 7.6, 1.7$ Hz, 1H), 7.52 – 7.28 (m, 7H), 6.55 – 6.46 (m, 4H), 2.81 (s, 3H). **^{13}C NMR** (75 MHz, $CDCl_3$) δ 183.3, 163.3, 163.1, 142.5, 138.4, 135.2, 135.0, 134.5, 134.4, 132.9, 131.0, 128.6, 128.3, 128.2, 128.0, 120.9, 68.2, 26.3.

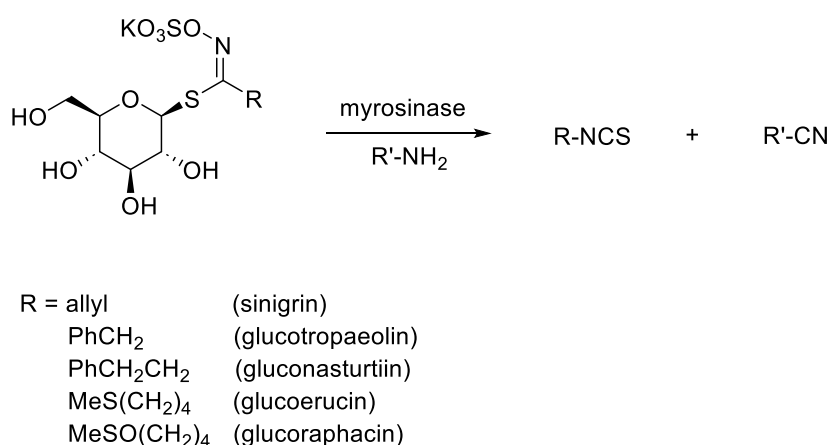
Photocaged Pseudo-Glucosinolates for the Light-Induced Release of Isothiocyanates

1. Introduction

1.1 Glucosinolates and Isothiocyanates

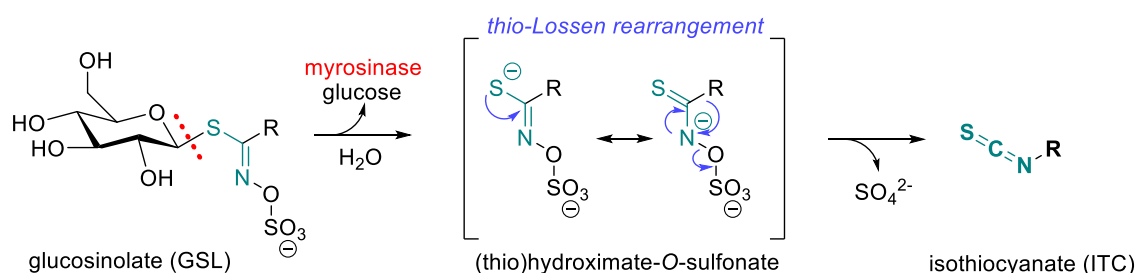
Isothiocyanates are hydrolysis products of glucosinolates (GSL), which are secondary plant metabolites found in high concentration in all plant families of the order *Brassicales* (also known as *Cruciferae*). These plants have been known to have a positive health impact ever since ancient time. Once glucosinolates are hydrolysed by the thioglucosidase enzyme called myrosinase, they generate a range of bioactive compounds. The breakdown products can include isothiocyanates and thiocyanates as well as nitriles and epithionitriles, depending on the reaction conditions. It is the isothiocyanates that are known to give both the pungent aromas and the beneficial bioactive effects.⁸

Chemically, GSLs are glycosidic thiohydroximate-*O*-sulfonates derived from amino acids: they are stored in the tissue of the plant that produces them, separated in different compartments from myrosinase. When the tissue of the plant gets damaged (for example because of cutting or chewing), the separating compartments are destroyed, thus the enzyme can cleave the thioglycosidic bond of the GSLs. Glucose and a thio-hydroximate-*O*-sulfonate aglycone are formed, and this latter intermediate undergoes a subsequent thio-Lossen rearrangement to form isothiocyanates (Scheme 1).¹⁰ The rearrangement reaction is displayed in Scheme 2.



Scheme 1. Glucosinolates as ITC and nitrile precursors⁴⁸

Isothiocyanates (ITCs) are powerful electrophiles. This property can be attributed to the central carbon atom of the $-N=C=S$ group, which reacts with nitrogen-, sulfur-, and oxygen-based nucleophiles. ITCs are known as active feeding deterrent agents, but they have raised interest in recent years due to their diverse biological activity, such as pollinator attraction, antimicrobial activities against bee-colony infecting fungi *Nosema ceranae*, enterohemorrhagic *Escherichia coli* (EHEC), methicillin-resistant *Staphylococcus aureus* (MRSA), *Pseudomonas aeruginosa*, and related biofilms. Moreover, multiple specific bioactivities in mammalian cells have been reported, which can be summarized as neuroprotective, cardioprotective, and chemoprotective.^{49–52} ITCs are an interesting class of anticarcinogenic phase II enzyme inducers. Evidence suggests that the major mechanisms of chemoprotection by isothiocyanates depend on the induction of phase II detoxification enzymes and the inhibition of phase I enzymes that are involved in the activation of certain carcinogens.⁵³



Scheme 2. Thio-Lossen rearrangement in glucosinolates⁹

Dedicated extractive methods enable the isolation of several GSLs from plant material, but in many cases, organic synthesis brings crucial help for the production of natural GSLs. At the same time, synthesis is the only way to elaborate a diversified range of *artificial* GSL analogues.⁹

While ITCs are compounds that display diverse bioactivity, including high cytotoxicity in certain concentrations, their potential as therapeutic agents is constrained by their inherent instability.^{9,10} To address these limitations, nature employs glucosinolates as prodrugs for ITCs, however, the absence of myrosinase in mammalian systems restricts the application of natural GSLs in therapeutic contexts.¹⁰

An approach was already undertaken in the synthesis of pseudoGlucosinolates (psGSLs), analogues which do not need the presence of myrosinase to trigger the release of isothiocyanate. It has recently been reported that the natural release mechanism of ITC from GSLs was successfully adapted to nitroreductase as the triggering enzyme. These

nitroreductase-responsive psGSLs were studied and showed potential for ITC-prodrug approaches, peptide labelling and for *in vivo* applications.¹⁰

The release of isothiocyanate from glucosinolates still strongly relies on the presence of enzymes: natural release of ITC is based on the presence of myrosinase, which only exists in plants, while the pseudoGlucosinolates described above depend on the activity of nitroreductases. This can be limiting when these enzymes are not available in the space and time they are needed to act in order to release ITC. Thus, our attention was drawn to developing a method which could release ITC from pseudoGlucosinolates without relying on enzymes, and the light-activated release of the target compounds caught our interest. A new and interesting approach, which has been the subject of this thesis, is to generate GSLs-like compounds that can release ITC upon light irradiation. In order to do this, photocaging appears as an efficient strategy to this approach.

1.2 Photopharmacology

The use of light to promote the controlled release of substances has advantageous features, including: light is a clean and inexpensive source of energy; light promoted reactions are more selective than thermally activated reactions; the controllability of light reactions is high so that the rate of target release can be controlled by simply varying the intensity and wavelength; light reactions have a wide range of applications as a consequence of the fact that they are most often temperature independent; long wavelength light is both less damaging to cells and more penetrable into cells, tissues and other organisms.¹²

Photopharmacology makes use of external light irradiation to activate small molecules in a specific region of interest at a chosen time and intensity. This can be achieved reversibly (photoswitching) or irreversibly (photocaging). In the photocaging strategy, a bioactive molecule is covalently attached to a photoprotecting group (PPG), which renders the target bioactive molecule inactive. Upon irradiation with a particular wavelength, the molecule is released in its active form in an irreversible manner and is free to exert its biological function locally.¹¹ PPGs provide spatial and temporal control over the release of various chemicals such as bioagents, acids, bases, ions, oxidants, insecticides, pheromones, fragrances, and many others.⁵⁴

There are some general requirements for designing an efficient PPG, which do not need to be fulfilled all at the same time. First, a PPG should have strong absorption at wavelengths well above 300 nm, preferably in the so called “phototherapeutic window” (650–900 nm), where irradiation is less likely to be absorbed by the biological entity and therefore shows better

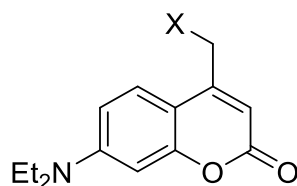
penetration through tissue.¹³ Moreover, the photoreaction should be clean and should occur with a high quantum yield, Φ_{rel} . The quantum yield Φ_{rel} is equal to the amount of released substrate divided by the number of photons, that were absorbed by the caged compound. Other important aspects of the PPG include high purity of the compound, low intrinsic activity and stability in the chosen media prior to and during photolysis.⁵⁴ The PPG should be soluble in the targeted media, but at the same time it may be required to pass through biological barriers such as cell membranes. Additionally, the PPG should show affinity to specific target components, such as binding sites on cancer cells or the active site of an enzyme. The photochemical byproducts released after irradiation should ideally be transparent at the irradiation wavelength, to avoid competitive absorption at the excitation wavelength, and they should be biocompatible and non-toxic.⁵⁴

A common issue with most widely used PPGs (*o*-nitrobenzene, coumarin and benzoin derivatives) is that their removal typically requires high-energy light (wavelengths lower than 400 nm), which can potentially lead to tissue damage. A direct solution to this problem, for example red-shifting the absorption band of the PPGs by extending the π -systems, has an inherent drawback of making the PPG molecules larger and less polar, hence decreasing their solubility in aqueous media.¹³

Photocages are photosensitive molecules that release target products upon light irradiation. These molecules consist of two components: a photosensitive group and a specific target substance that is released upon irradiation. In the “cages”, targets are chemically protected by the PPG, which undergoes photoreaction to release the target. Therefore, targets do not function until they are released at specific locations where their properties are restored.¹²

To have a broader view on which PPGs would perform better for the studied activity, we analysed a varied range of PPGs, among which the four following were chosen: diethylaminocoumarin (DEAC), dimethoxynitrobenzyl group (DMNB), α -methyl-nitropiperonyloxymethyl group (MeNPOM) and a BODIPY scaffold.

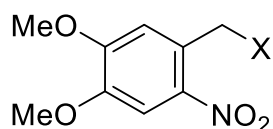
DEAC – 7-(diethylamino)-coumarin



Coumarins are very commonly used as PPGs. They were identified as secondary metabolites in a variety of plants in 1820. In 1984, it was discovered that phosphate is released from

(coumarin-4-yl)-methyl upon light irradiation,⁵⁵ and since that time, coumarin has been broadly used as a photoprotective moiety. Coumarin-based photocages have a wide variety of advantages, such as high molar absorption coefficients, high photolysis efficiencies, and rapid photolysis rates. In addition, the spectroscopic and photochemical properties of these substances can be tuned by changing substituents on the coumarin ring.¹² Clever modifications of the substituents at C6 and C7 in the coumarinyl group addressed low water solubility, and also helped to extend the absorption maximum of the group into the biologically benign range (≥ 350 nm).⁵⁴ As a result, coumarin and related derivatives have been increasingly used in the chemical and biomedical fields because of their high biocompatibility.

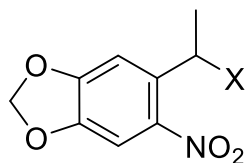
DMNB or NV – 4,5-dimethoxy-2-nitrobenzyl or 6-nitroveratryl



The non-fluorescent *o*-nitrobenzyl moiety is a common photoprotective group. Owing to their confirmed photolytic mechanism and excellent photolytic efficiency, increasing numbers of *o*-nitrobenzyl-based photocages have been prepared. Two methoxy groups were added to increase the absorbance at longer wavelengths ($\lambda > 350$ nm), leading to the 6-nitroveratryl (NV) moieties.¹²

o-Nitrobenzylic derivatives were proposed as general PPGs in 1970 and they are widely used groups to date, both in solution and in the solid phase. However, there were earlier reports on their “unfortunate” photochemistry, including the photoisomerization of *o*-nitro benzaldehyde into the corresponding nitrosobenzoic acid. Another more serious problem is the formation of nitrosoaldehyde that yields brown-coloured degradation products that absorb the incident light, thus creating an internal filter. Nevertheless, they remain one of the most frequently used PPGs at present.⁵⁴

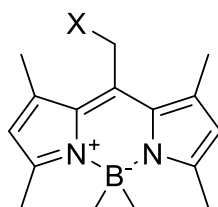
MeNPOM – α -methyl-(6-nitropiperonyloxymethyl)



Methylenedioxy derivatives are very close analogues to DMNB, but with slightly altered properties. This is probably due to a restriction in the freedom in aligning the nonbonding

orbitals of the oxygen with the aromatic π system.⁵⁴ Further addition of an α -methyl group leads to α -methyl-(6-nitropiperonyloxymethyl) (MeNPOM) groups. MeNPOM presents several advantages, such as increased photolytic efficiency upon irradiation at 365 nm, high stability of the caged molecules in aqueous media and release of acetophenone as a less toxic byproduct.⁵⁶

BODIPY – *meso*-methyl boron dipyrromethene



Owing to its narrow absorption band and good biocompatibility, the boron difluoride complex of a dipyrromethene, commonly called BODIPY, has become a promising candidate for visible light photocaging.¹³ One of the major appeals of repurposing BODIPY dyes as PPGs is the possibility to achieve red-shifted light absorption properties compared to most conventional PPGs, avoiding the requirement for photoexcitation with UV light. For other PPGs that natively absorb UV light, many efforts over decades have been undertaken to prepare derivatives with substituents that bathochromically shift the chromophore's absorbance. BODIPY PPGs are especially attractive for their ability to be activated by different wavelengths in the visible or by long-wavelength light in the biological window (650–900 nm).⁵⁷ In recent years, studies conducted on BODIPY-based photocages containing sulfonate modification, π -extension, and structural optimization have led to the development of many novel photocages having adjustable water solubilities, tuneable membrane permeabilities, and large quantum yields that have been utilized in a wider range of applications¹². The BODIPY core is very appealing because of its easy synthesis, near-ideal photochemical properties, a narrow absorption band and high ϵ values ($\sim 10^5 \text{ M}^{-1}\text{cm}^{-1}$). Moreover, by exchanging the conventional BF_2 unit of the BODIPY to a BMe_2 unit, an increase in the uncaging quantum yield was observed.¹³

1.3 Labelling of Biomolecules

Labelling is a common methodology used for bioanalytical purposes. It can be carried out with, for example, radioactive materials or compounds with absorption and/or fluorescence from the UV to the NIR region of the electromagnetic spectrum. In recent years, detection based on fluorescence techniques has received particular attention and remarkable progress has been made in this area.^{14–16,58} Fluorescence labelling can be extended over a wide range of

wavelengths using semiconductor nanocrystals, fluorescent proteins, or organic molecules, and we will focus on the latter.

Organic fluorophores can form covalent or non-covalent linkages with the sample to be analysed, producing the respective conjugates or complexes that can show fluorescence from short to very long wavelengths, depending on the marker used. Moreover, the development of fluorophores with absorption and emission at long wavelengths is of extreme importance for biological purposes, because longer wavelengths are not damaging to tissues and cells.¹⁵

The use of longer wavelengths is also required for multiplexing. Multiplexed bioassays are needed for simultaneous analysis of multiple targets. These assays can detect and record images from multiple chemicals, biomolecules, cells, and tissues. In comparison to labelling with a single biomarker, multiplexed detection of numerous targets improves diagnostic accuracy while saving time, sample volume, and cost. A key strategy in multiplexing involves optical encoding, which uses fluorescent signals to differentiate between targets. To make deep-tissue or *in vivo* analysis possible, longer wavelengths are preferred, especially in the near-infrared (NIR, 700–900 nm) and NIR-II (1000–1700 nm) regions. The advantage of using such wavelengths reduces light scattering and background autofluorescence compared to visible light. This makes it easier to visualize signals from within complex biological environments, improving the ability to study diverse chemical species in cells and tissues.⁵⁹

The covalent labelling of proteins is a widespread approach in medicinal chemistry and chemical biology. The formation of covalent bonds generally requires the presence of a nucleophilic amino acid residue in the protein and a small molecule equipped with an electrophilic centre. Usually cysteine and lysine are targeted, but in some cases tyrosine, threonine and serine might be modified, as well.⁶⁰ As previously stated, ITCs are electrophilic compounds, and their biological activity seems to be derived from their reaction with thiols to form an unstable thiocarbamoyl adduct. However, ITCs also react with amino moieties under alkaline pH forming a stable ITC-amine conjugate (namely thiourea derivatives). This reaction has been utilized for protein sequencing and protein labelling with a fluorescent dye.¹⁶

Fluorescein isothiocyanate (FITC, Figure 1) is a popular fluorescent labelling dye mainly used for preparing a variety of bioconjugates on lysine or cysteine residues. However, the low conjugation efficiency and the limited brightness of its conjugates are still limiting applications.⁶⁰ These issues can be attributed to the characteristics of the isothiocyanate group.⁶¹

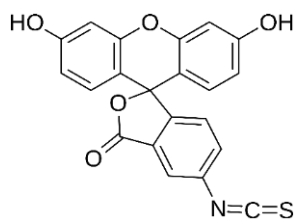


Figure 1. Fluorescein isothiocyanate (FITC)

ITCs have been demonstrated to covalently bind peptides via irreversible formation of thioureas upon reacting with lysine residues or via reversible formation of dithiocarbamates upon reacting with cysteine residues (Figure 2). The labelling selectivity between the targeted amino acids is mainly influenced by the pH of the surrounding media through the protonation state of the target amino acid sidechains. While the optimum pH for the thiourea formation has been shown to be between pH 9–11, dithiocarbamates form predominately at pH 6–8. Although dithiocarbamates might be the initially formed adducts, it is believed that they might function as temporary intermediate, reforming the ITC and finally leading to thiourea adducts as stable modifications under physiological conditions.¹⁶

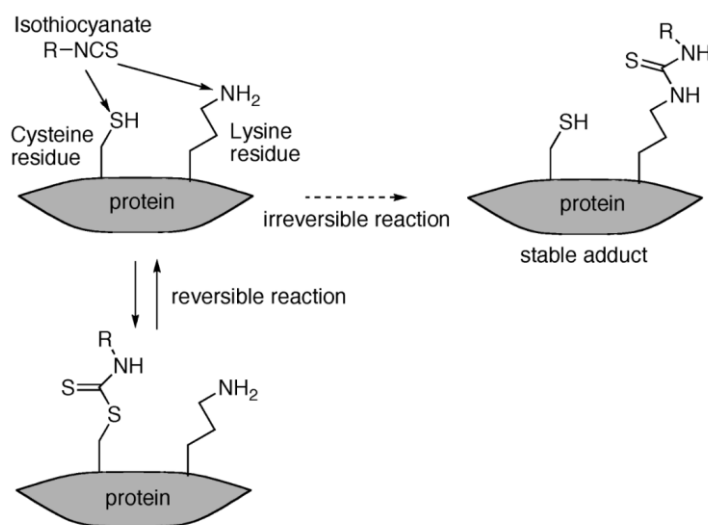


Figure 2. Reactivity of ITC with Cys or Lys in physiological conditions¹⁶

Several studies characterised the reactivity of a variety of isothiocyanates with small molecules, peptides and proteins, and showed that ITCs react up to one thousand times faster with thiol groups than with amino groups, rendering proteins with functional and structural cysteine residues particularly sensitive targets for modification. The reaction of isothiocyanates occurs predominantly with the deprotonated $R-S^-$ thiolate anion, and the deprotonated $R-NH_2$ or $R-NH$ of primary and secondary amines, respectively. As such, the reactivity of specific amines and thiols depends on their individual pK_a values and increases under basic conditions. Alkaline

buffers are required to optimise the reaction of isothiocyanates with amine groups. At physiological pH, neighbouring residues will have a major influence on the reactivity of specific thiols or amines. Positively charged histidine, lysine, and arginine lower the pK_a of thiol and amino groups thereby increasing the proportion of nucleophilic deprotonated forms.⁶² It has to be kept in mind that, whilst a wide variety of isothiocyanate-protein interactions can occur *in vitro*, it does not automatically follow that these occur in biological systems amongst a background of competing reactions.⁶²

Light-activated protein labelling allows precise control over when and where proteins are tagged, making this approach very useful for studying dynamic cellular processes. Proteins markers can be released through the photocaging strategy, which has been widely used for activating biomolecules, thereby enabling the regulation of biological processes with high spatial and temporal resolution. Light offers several advantages over other forms of stimuli, as it can be delivered with high precision through control of wavelength, intensity, and irradiation time.¹⁴

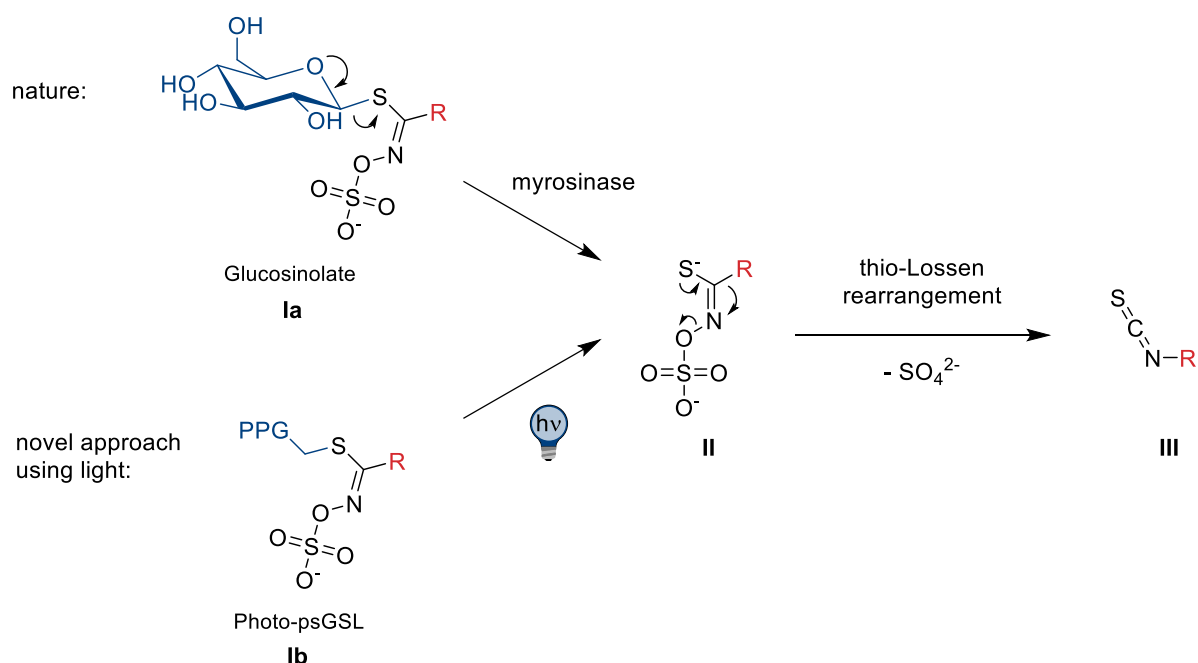
1.4 Aim of the Thesis

Within the scope of this thesis, we aim to capitalize on the concept of photocaging to gain control over the activity of glucosinolates. In particular, we propose combining glucosinolates and photocaging in the synthesis of artificial light-responsive analogues, referred to as photo-pseudoGlucosinolates (photo-psGSLs). The *photo*- indicates the photocage nature of the molecules, while the term *pseudo*- denotes their synthetic origin, to mimic the structure of natural glucosinolates found in plants.

Upon irradiation with a specific wavelength, which depends on the respective photoprotecting group used, the photo-psGSL **Ib** releases the key intermediate **II**, which subsequently undergoes a thio-Lossen rearrangement to generate isothiocyanate **III** (Scheme 3). The synthesized photo-pseudoGlucosinolates thus act as light-controllable precursors of isothiocyanate. Once the final molecules are synthesized, they will be subjected to irradiation studies to monitor the uncaging process and to study the release of isothiocyanate.

The ITC-releasing photo-pseudoGlucosinolates can eventually be functionalized with an appropriate fluorescent group, such that upon irradiation, the released ITC carries this fluorescent moiety and becomes readily detectable by standard fluorescence spectroscopy. A key advantage of this approach is that the fluorescent ITC can be released in a spatially and temporally controlled way by irradiation with the appropriate wavelength for the uncaging process, at the desired time. For instance, to probe the presence of cysteine or lysine residues

on polypeptide chains, the photo-psGSL molecule can be directed to the region of interest. Then, upon irradiation at the suitable wavelength, uncaging occurs, resulting in the release of the fluorescently tagged ITC, which enables selective labelling and facilitates the identification of the targeted residues.

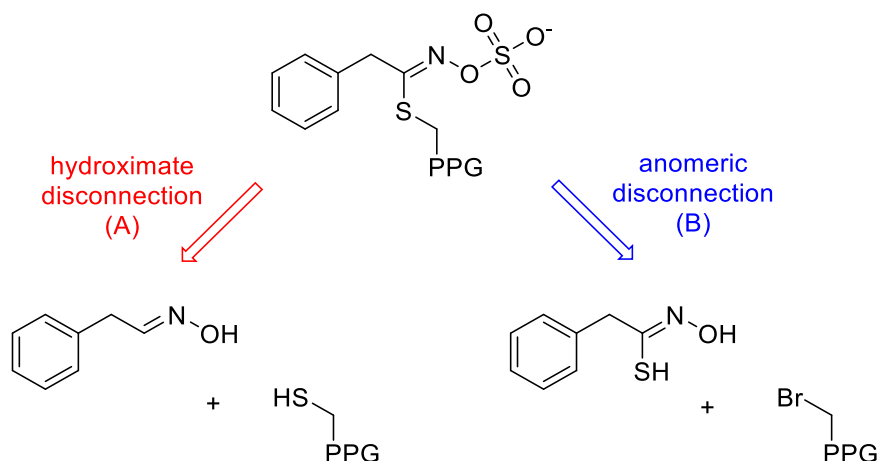


Scheme 3. Mechanism of release of isothiocyanate from natural glucosinolates and the novel light-mediated release from photo-psGSLs

2. Results and Discussion

Four photo-psGSL have been synthesized, with four different PPGs. For the design of pseudoGlucosinolates two major approaches have been developed (Scheme 4). They are based on a retrosynthetic scheme where the formation of a single specific bond affords the GSL skeleton: two types of disconnection have been considered, either on the hydroximoyl moiety (A) or onto the anomeric center (B).⁹

The anomeric disconnection has only been reported in one example in literature, with a rather low yield,⁶³ and it has not been further developed, while the hydroximate disconnection has proven to be more successful.⁹ The hydroximate disconnection has proven to be working well in three cases, but in one case (BODIPY) it did not afford the desired product therefore the anomeric disconnection pathway was used.



Scheme 4. Disconnection pathways for the synthesis of pseudo-glucosinolates

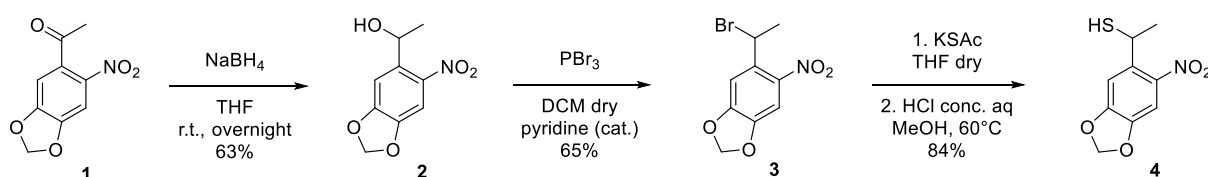
2.1 Synthetic considerations including the disconnection strategies from the introduction

First, the photoprotecting groups needed to be synthesized in order to make the final photo-psGSLs. The detailed experimental procedures are reported in the Experimental Section.

2.1.1 Synthesis of photoprotecting groups

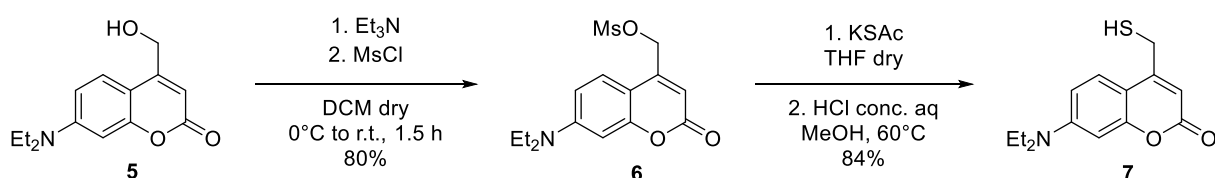
Synthesis of MeNPOM-SH (4)

According to the procedure developed by Smaga *et al.*,⁶⁴ ketone **1** was reduced using NaBH₄ to yield alcohol **2**. Next, bromination of alcohol **2** was carried out with PBr₃ to obtain the corresponding compound **3**.⁶⁵ Then, the two following steps of generation of thioester and following hydrolysis were carried out consecutively to obtain the final thiol **4** (84% yield).⁶⁶



Synthesis of DEAC-SH (7)

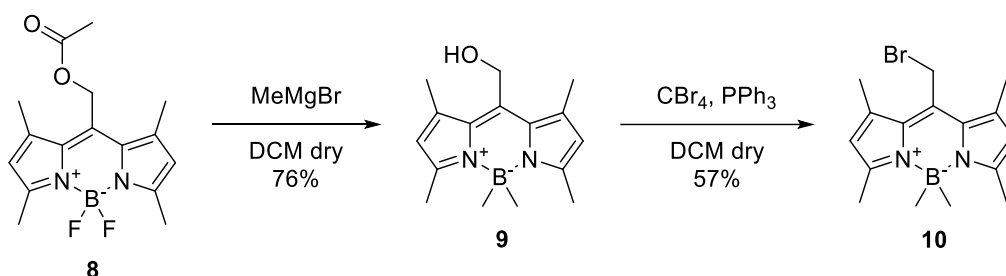
According to a literature procedure, coumarin alcohol **5** was converted into the mesylated compound **6**.⁶⁷ The thioester generation and subsequent hydrolysis were carried out following the same strategy used for compound **4**, which proved to work well, and thiol **7** was obtained in 84% yield.⁶⁶



Synthesis of Me-BODIPY-Br⁶⁸ (10)

Contrary to the previously established procedure, the synthesis of Me-BODIPY-SH could not be achieved successfully. Our initial approach involved adapting the strategy of generating the thioester intermediate followed by hydrolysis. While the thioester could be obtained without difficulty, the subsequent hydrolysis step consistently failed to yield the thiol. A variety of hydrolytic conditions were explored to overcome this issue. Acidic hydrolysis using HCl was found to be too harsh, resulting in cleavage of the boron atom. Consequently, milder conditions were investigated, including treatment with acetic acid as well as several basic conditions (NH₃, NaOH, KHCO₃, Na₂CO₃). All reactions were performed in a methanol–water mixture and heated at 60 °C overnight. The reaction mixtures were analysed by LC-MS, but the expected signal corresponding to Me-BODIPY-SH (*m/z* 286.1675) was not observed in any case. Realising that this synthetic route was unsuitable for the preparation of Me-BODIPY-SH, we instead employed Me-BODIPY-Br as the precursor for the generation of the BODIPY photo-psGSL. Therefore, the anomeric disconnection was used, which requires Me-BODIPY-Br and the thiohydroxamic acid.⁹

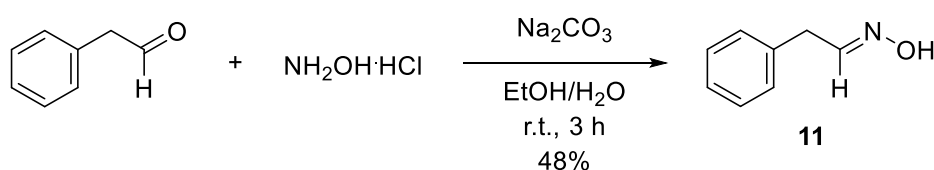
F-BODIPY-OAc **8**, previously synthesized by group members according to literature procedure,⁶⁹ was treated with a Grignard reagent to give Me-BODIPY-OH **9**. Then, the alcohol was treated with CBr₄ and PPh₃ to obtain the brominated compound **10** (57% yield).



2.1.2 Synthesis of hydroxylamines

Synthesis of phenyl acetaldoxime⁷⁰ (**11**)

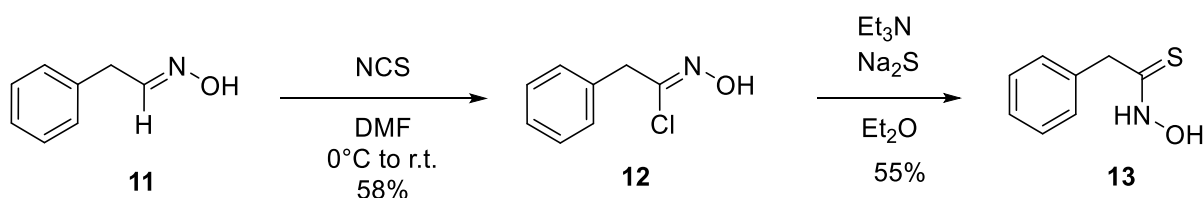
Phenylacetaldehyde was treated with hydroxylamine hydrochloride in basic environment in a 1:1 ethanol/water mixture. Through a simple condensation of a readily available aldehyde with hydroxylamine, oxime **11** was isolated, recrystallized and obtained in 48%.



Synthesis of *N*-hydroxy-2-phenylethanethioamide⁷¹ (**18**)

Thiohydroxamic acids are peculiar molecules with four different types of atoms next to one another (C, N, O, and S), which gives them an extensive reactivity and makes them very *N*-acidic.⁷¹ There are three main literature methods for the synthesis of thiohydroxamic acids. Thionation of hydroxamic acids with Lawesson's reagent or P₂S₅ and thioacylation of hydroxylamine derivatives are the most used procedures.^{72–74} On the contrary, the addition of sulfur nucleophiles to *N*-hydroxyimidoyl chlorides or nitrile oxides has received scarce attention, but proved to work successfully when used for the synthesis of compound **13**. This is also the chosen method to synthetically access thiohydroxamic acids from the glucosinolate family.^{9,75,76}

Phenylacetaldoxime **11** was chlorinated with NCS in DMF to provide *N*-hydroxyimidoyl chloride **12**, which was purified and obtained in 58% yield. Compound **12** was then treated with Et₃N and Na₂S to yield *N*-hydroxy-2-phenylethananimidothioic acid **13** (55%).

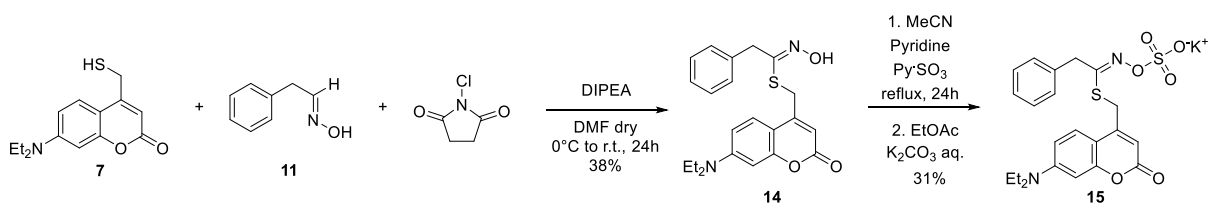


2.2 Synthesis of photo-psGLs

With the photoprotecting groups and hydroxylamines in hand, the following step involved the synthesis of photocaged pseudoGlucosinolates (photo-psGLs).

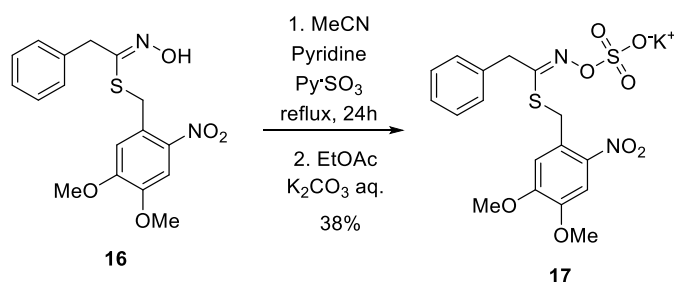
Synthesis of DEAC photo-psGSL (**15**)

According to the procedure reported in literature for the hydroximate disconnection,⁴⁸ coumarin thiol **7** and oxime **11** were converted into thiohydroximate **14** in the presence of NCS and DIPEA. Subsequently, compound **14** was sulfonated and treated with K₂CO₃ as a base to afford DEAC photo-pseudoGlucosinolate **15** in 31% yield. Compound **15** was obtained in a relatively low yield, and the sulfonation step required a reaction time of 24 hours. This could reflect the non-optimal conditions of the sulfonation step.



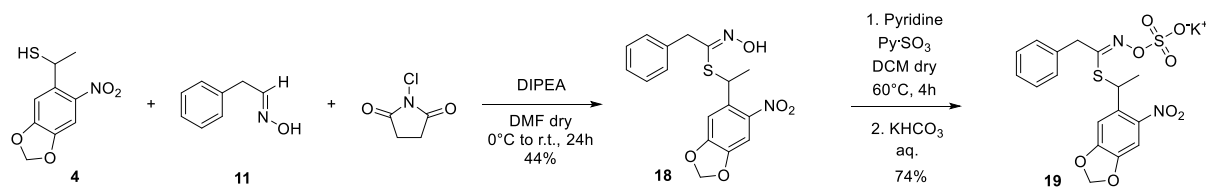
Synthesis of DMNB photo-psGSL (17)

DMNB thiohydroximate **16** had already been synthesized and isolated as a pure compound by group members, and therefore only needed to be sulfonated to generate the final photo-psGSL. Following the same literature procedure as for DEAC photo-psGSL,⁴⁸ thiohydroximate **16** was sulfonated and treated with K₂CO₃ to obtain the DMNB photo-pseudoGlucosinolate **17**. The relatively low yield of compound **17** (38%), together with the necessity of a very long reaction time for the sulfonation step to afford the desired product, clearly indicate that the conditions for the generation of the photo-psGSL are not optimal.



Synthesis of MeNPOM photo-psGSL (19)

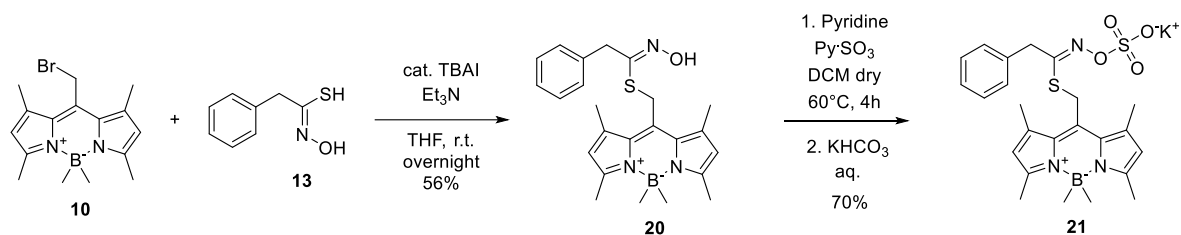
Next, MeNPOM photo-psGSL was synthesized. The first step followed the same procedure used for the two previous photo-psGSLs: α -methyl nitropiperonyl thiol **4** and oxime **11** reacted in the presence of NCS and DIPEA and thiohydroximate **18** was obtained in 44% yield. Then, considering the limited efficiency of the previously used sulfonation method, an alternative procedure found in literature was followed.¹⁰ Compound **18** was treated with pyridine and sulfur trioxide pyridine complex in DCM, then treated with KHCO₃ to afford MeNPOM photo-pseudoGlucosinolate **19**. This approach turned out to be significantly more effective, reducing the reaction time from 24 to 4 hours and increasing the yield to 74%.



Synthesis of Me-BODIPY photo-psGSL (21)

For the BODIPY photo-psGSL, the anomeric disconnection pathway was undertaken.⁹ According to the literature reported procedure for the disconnection, Me-BODIPY-Br **10** and *N*-hydroxy-2-phenylethanimidothioic acid **13** were converted into thiohydroximate **20** using Et₃N as a base and TBAI as a catalyst.⁷⁷ Even though this synthetic pathway was very scarcely

reported in literature, compound **20** was obtained with 56% yield. Then, compound **20** was sulfonated and treated with KHCO_3 to obtain the Me-BODIPY photo-pseudoGlucosinolate **21** in 70% yield.¹⁰



The synthesis of the photo-pseudoGlucosinolates afforded the desired target molecules. The first step of generation of the thiohydroximate was carried out in the same way for DEAC and MeNPOM photo-psGSLs, with the use of NCS and DIPEA, affording similar yields. For the BODIPY photo-psGSL, on the other hand, the anomeric disconnection pathway was undertaken. Initially, the conditions for the sulfonation step needed for the synthesis of the final compounds were not optimal, however a change in procedure could increase the yields and shorten the reaction time.

2.3 Spectroscopy: uncaging of the photo-psGSLs

To investigate the photochemical properties of the isolated photo-psGSLs, the molecules were irradiated with different wavelengths of light. The DMNB and MeNPOM groups have their absorption maxima around 360 nm, DEAC around 400 nm and Me-BODIPY around 500 nm. The stock solutions of photo-pseudoGlucosinolates were prepared with a concentration of 10 mM. Different conditions were tested, varying pH and irradiation time.

2.3.1 pH

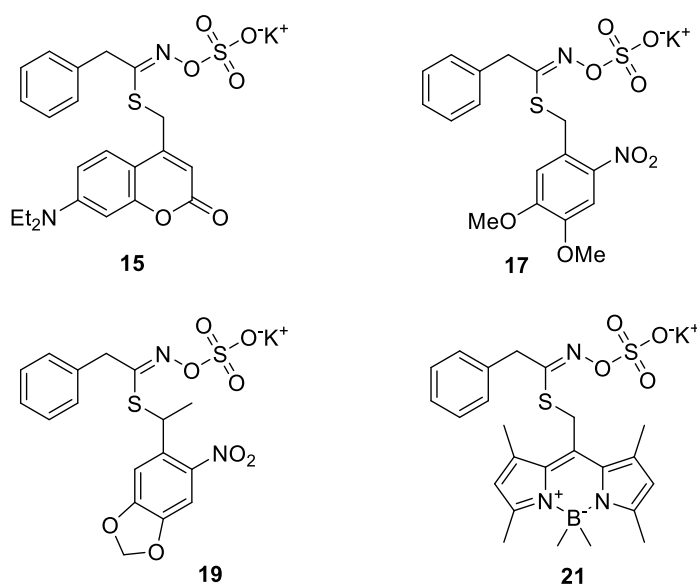
To analyse the influence of the pH on the uncaging process, the following buffer solutions were prepared: 10 mM MES buffer solution pH 5.5, 100 mM HEPES buffer solution pH 7, 100 mM Bicine buffer solution pH 8, 100 mM Bicine buffer solution pH 9.

The samples were prepared by adding different quantities of buffer solution and stock solution of photo-psGSL to a cuvette, with a final volume of approximately 2 mL. Then, they were analysed with a UV-Vis spectrometer. Some photo-psGSL had a low absorbance therefore the amount of stock solution added to the cuvette was increased, while the BODIPY derivative **21** had a very strong absorption therefore only 5 μL of stock solution were sufficient. Table 1 shows how the samples were prepared; this was done four times to cover the four pH values.

	stock sol. (μL)	buffer (mL)	final volume (mL)	photo-psGSL final conc. (μM)
15	20	1.99	2.01	100
17	40	1.99	2.03	197
19	40	1.99	2.03	197
21	5	1.995	2.00	25

Table 1. Preparation of samples at different pH values

Initially, measurements in the dark were carried out to obtain the absorption spectra of the different compounds. Then, after identifying the maximum absorption wavelength (λ_{max}) for the photo-psGSLs, the irradiation wavelengths were chosen (λ_{irr}), using the lamps available in the Specord UV-vis setup.⁷⁸ The data are reported in Table 2.



	λ_{max} (nm)	λ_{irr} (nm)
15	398	405
17	355	365
19	360	365
21	505	500

Table 2. Maximum absorption and irradiation wavelengths

Figure 3. Isolated photo-pseudoGlucosinolates

In the following pages the absorption spectra of the photo-psGSLs at different pH are reported, along with the trend of the absorbance over time during irradiation and the trend of absorbance at the λ_{max} over time.

Photo-psGSLs behaviour upon irradiation at pH 5.5

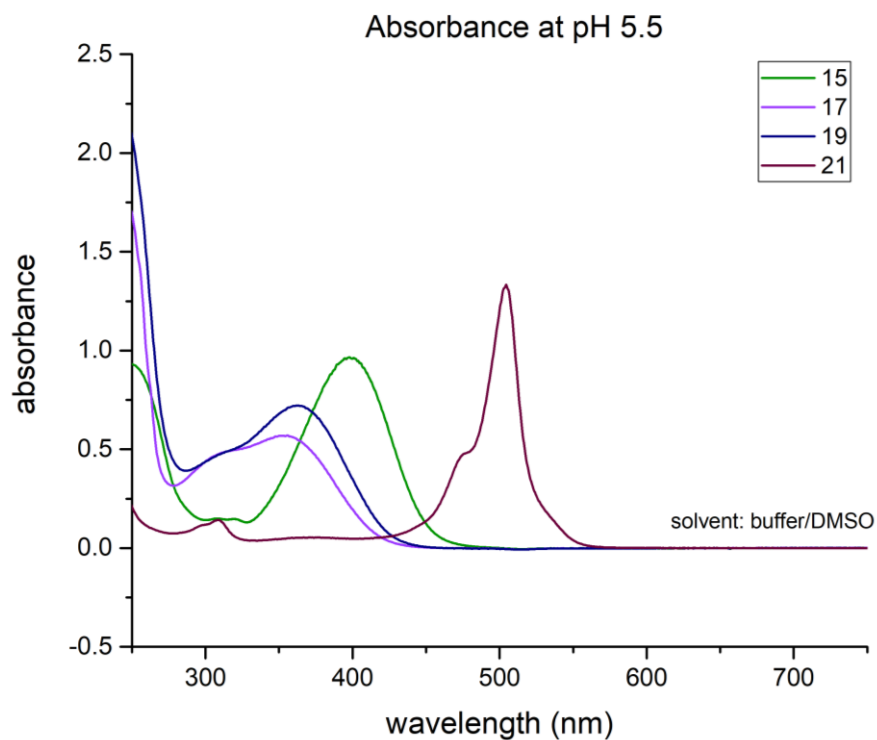


Figure 4a. Absorption spectra of photo-psGSLs at pH 5.5

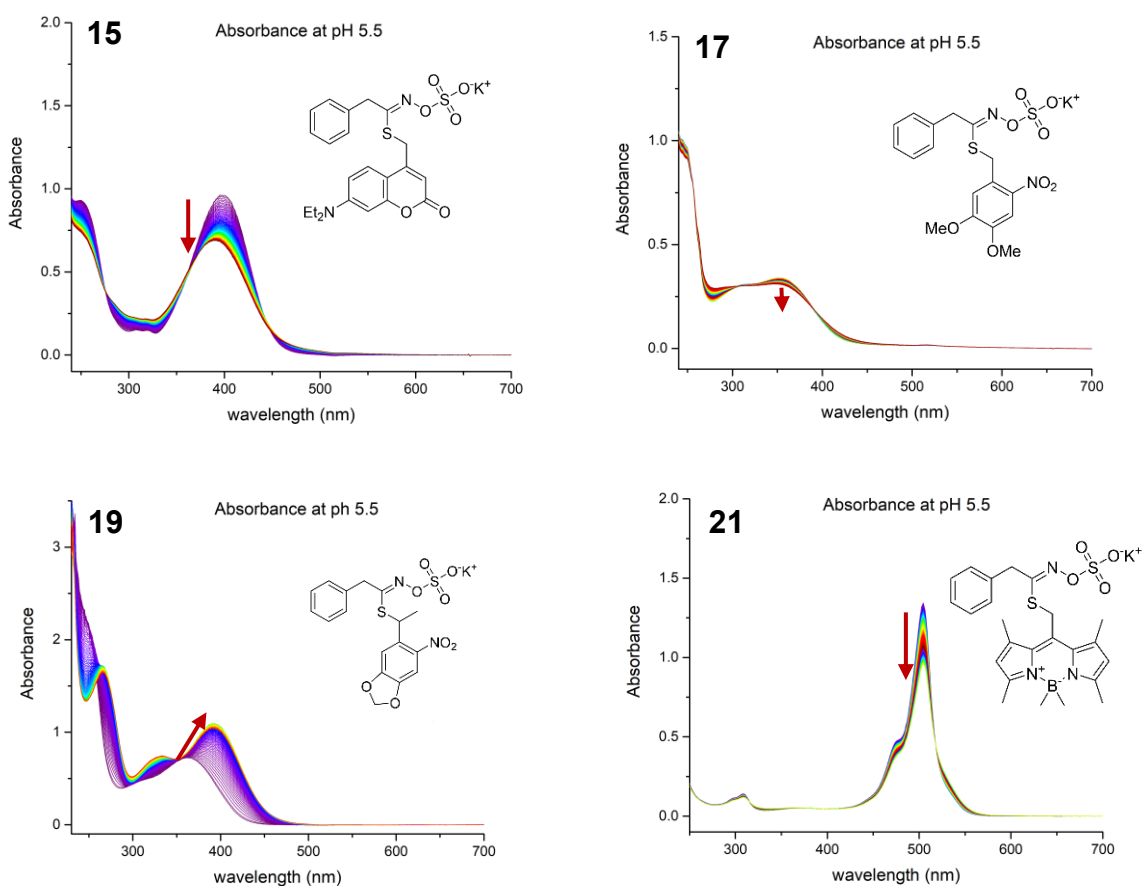


Figure 4b. Variation of absorption spectra of photo-psGSLs upon irradiation at pH 5.5

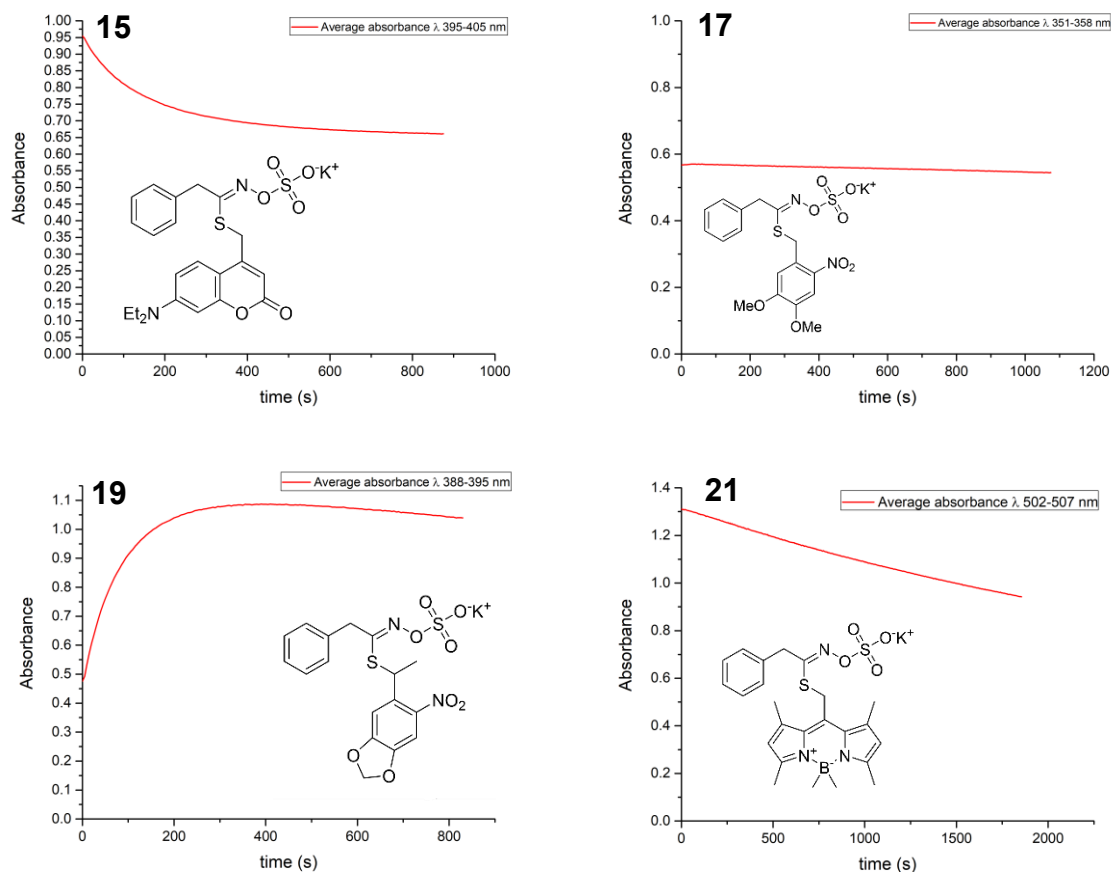


Figure 4c. Trend of absorption at average λ_{max} over time upon irradiation at pH 5.5

When analysing the measurements, a few aspects were striking: the DEAC derivative **15** and the MeNPOM derivative **19** show a visible variation in absorbance over time, even after being irradiated for a short period of time. It can be observed that **15** has a very slight blue shift of the λ_{max} while **19** is red-shifted to higher wavelengths. On the other hand, the DMNB derivative **17** and the BODIPY derivative **21** do not show the same behaviour: first of all, they need a longer irradiation time to show any changes in the spectra, and they are not shifted in any direction, the only thing that can be observed is a small decrease in absorbance.

Photo-psGSLs behaviour upon irradiation at pH 7

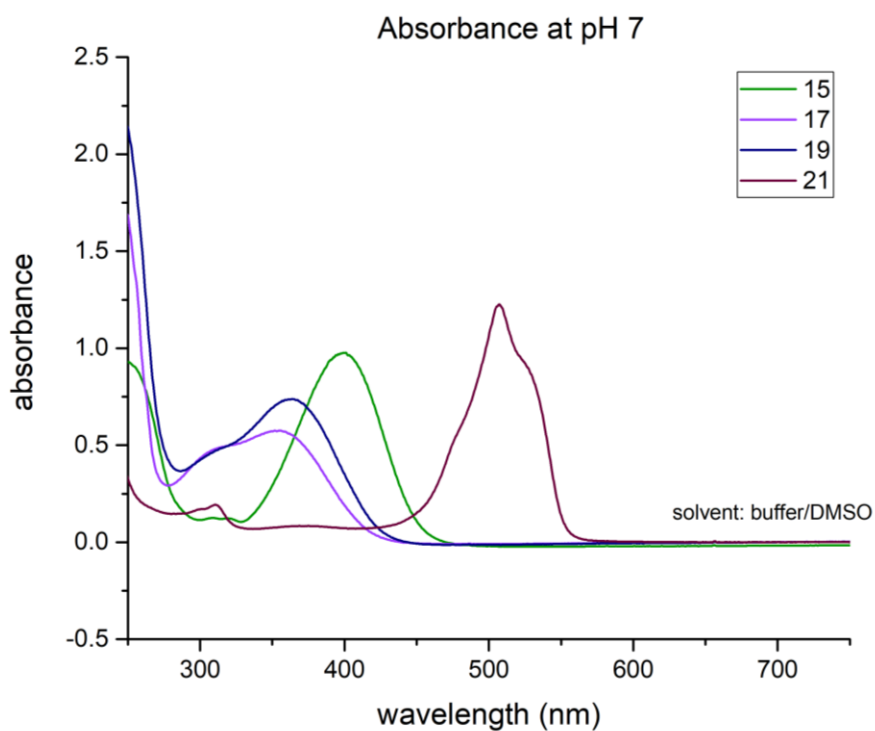


Figure 5a. Absorption spectra of photo-psGSLs at pH 7

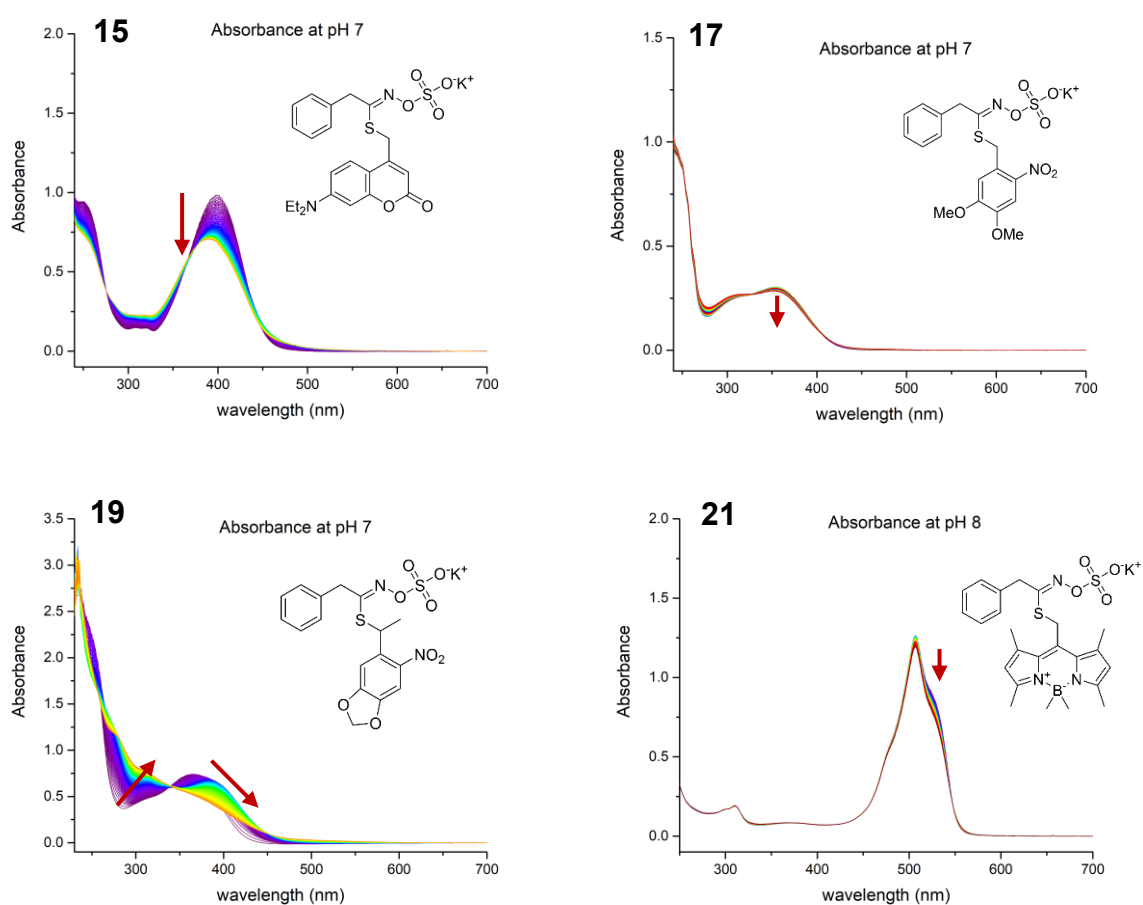


Figure 5b. Variation of absorption spectra of photo-psGSLs upon irradiation at pH 7

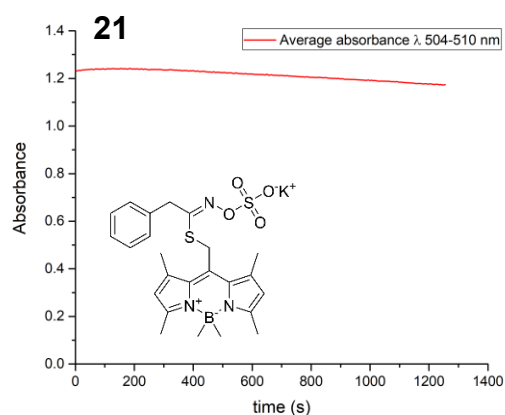
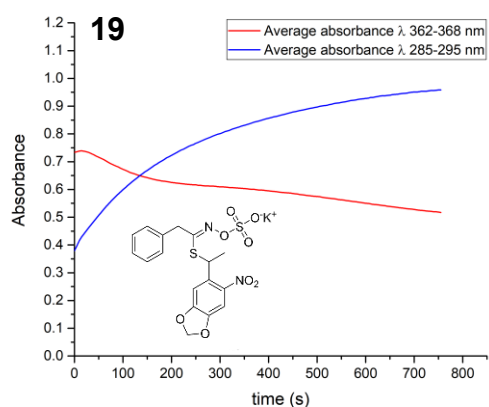
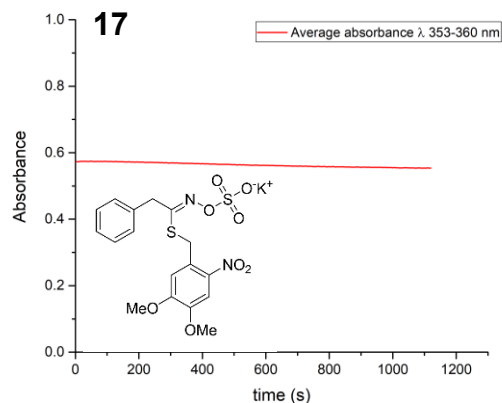
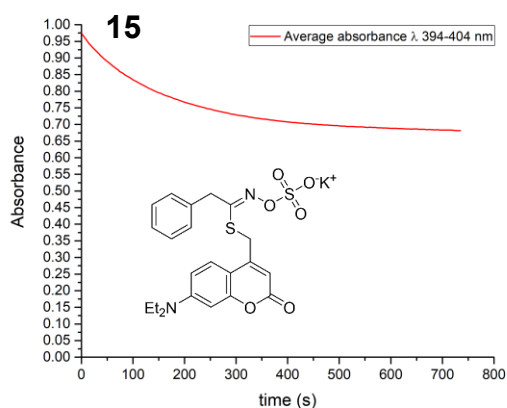


Figure 5c. Trend of absorption at average $\lambda_{\max}$ over time upon irradiation at pH 7

The data analysis highlighted that the trends are very similar to the ones under the previous conditions (pH 5), but the change in the spectrum of the MeNPOM derivative **19** is a bit different, the absorption maximum around 360 nm decreases while there is an increase in the 285–295 nm region.

Photo-psGSLs behaviour upon irradiation at pH 8

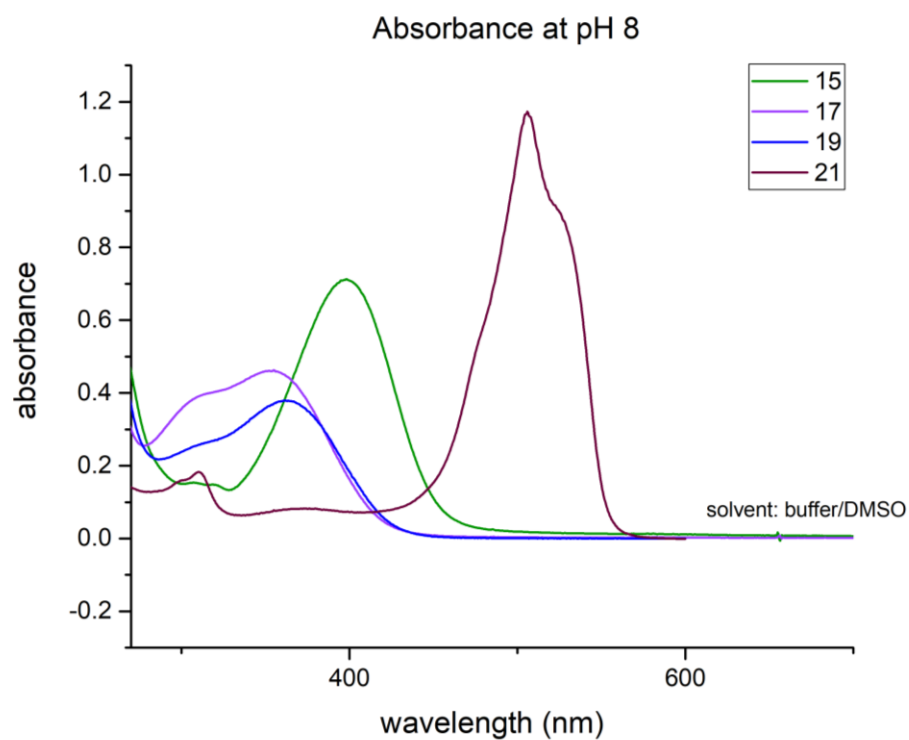


Figure 6a. Absorption spectra of photo-psGSLs at pH 8

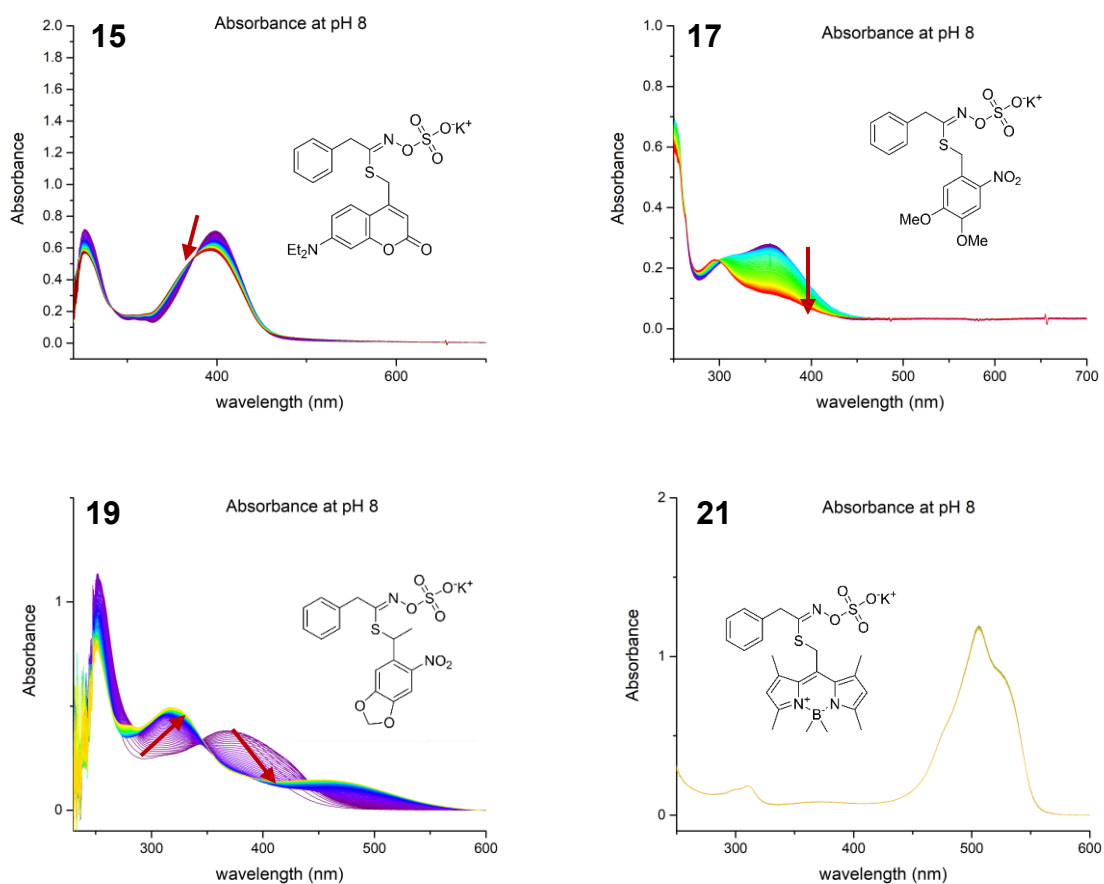


Figure 6b. Variation of absorption spectra of photo-psGSLs upon irradiation at pH 8

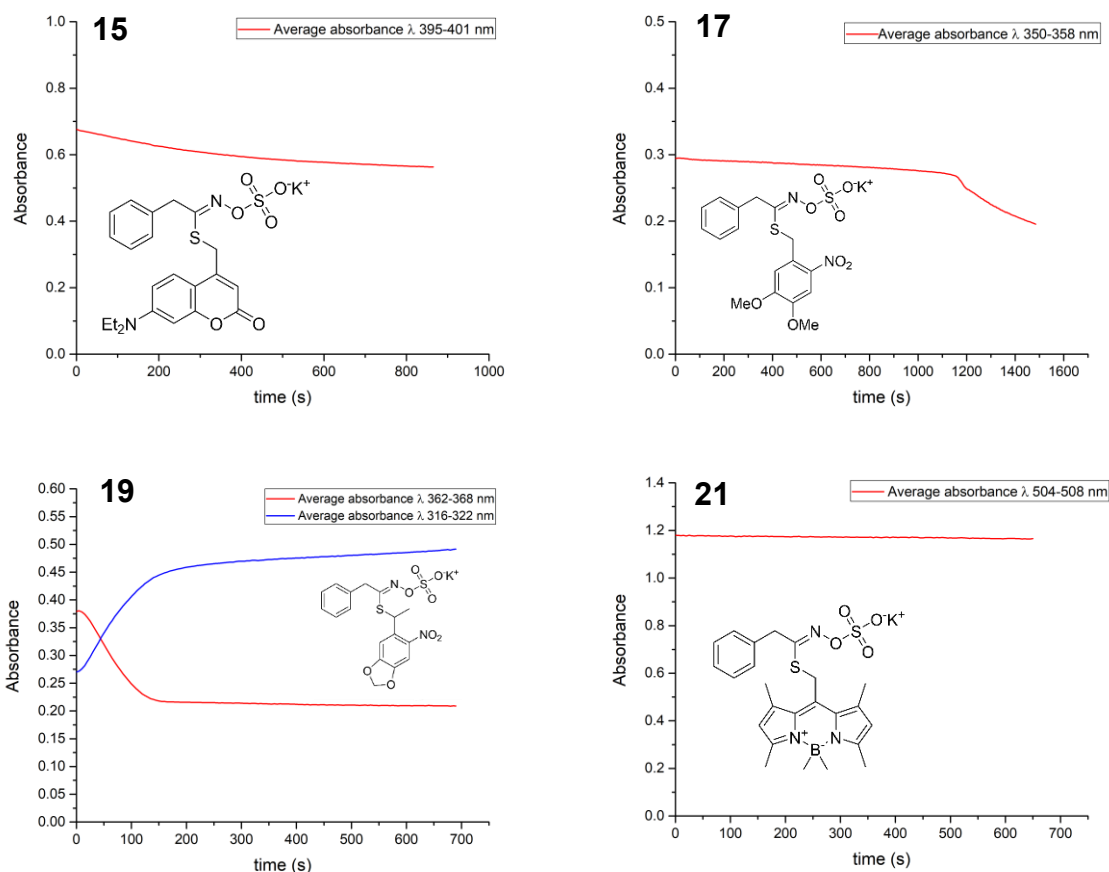


Figure 6c. Trend of absorption at average λ_{max} over time upon irradiation at pH 8

When analysing the measurements, a few aspects were noticed: under these conditions, the behaviour of DMNB derivative **17** differs from that observed at pH 5.5 and pH 7. In the latter cases, even after 20–25 minutes of irradiation, only a slight decrease in absorbance was recorded. In contrast, under the present conditions, the absorbance of **17** initially showed only a minor decrease, but after approximately 30 minutes of irradiation, a pronounced reduction in absorbance was observed, which continued steadily until the irradiation was terminated. In comparison, BODIPY photo-psGSL **21** exhibited no appreciable change throughout the measurement period.

Photo-psGSLs behaviour upon irradiation at pH 9

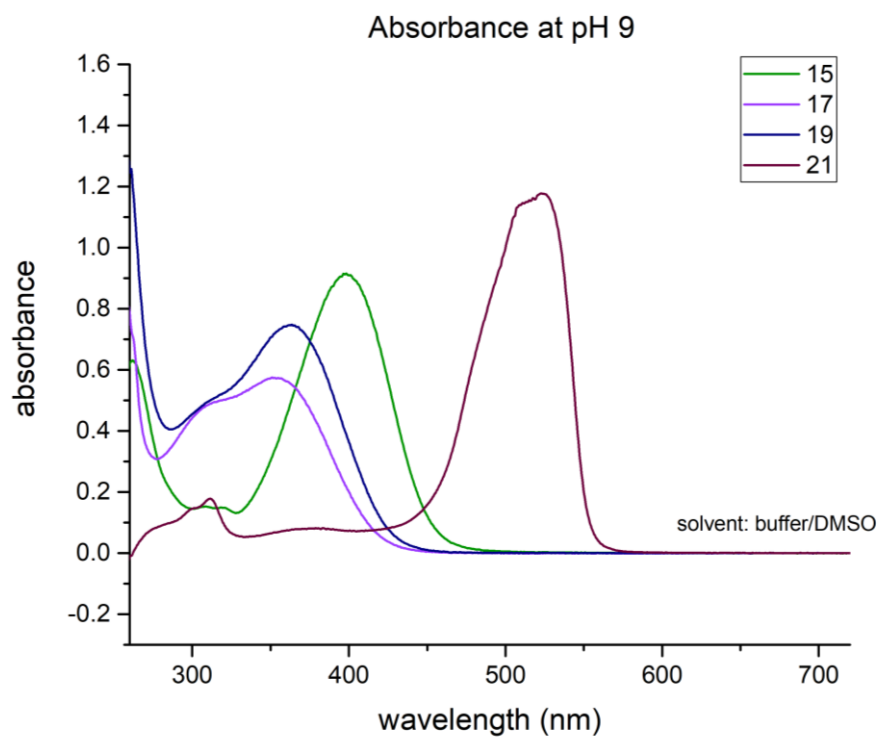


Figure 7a. Absorption spectra of photo-psGSLs at pH 9

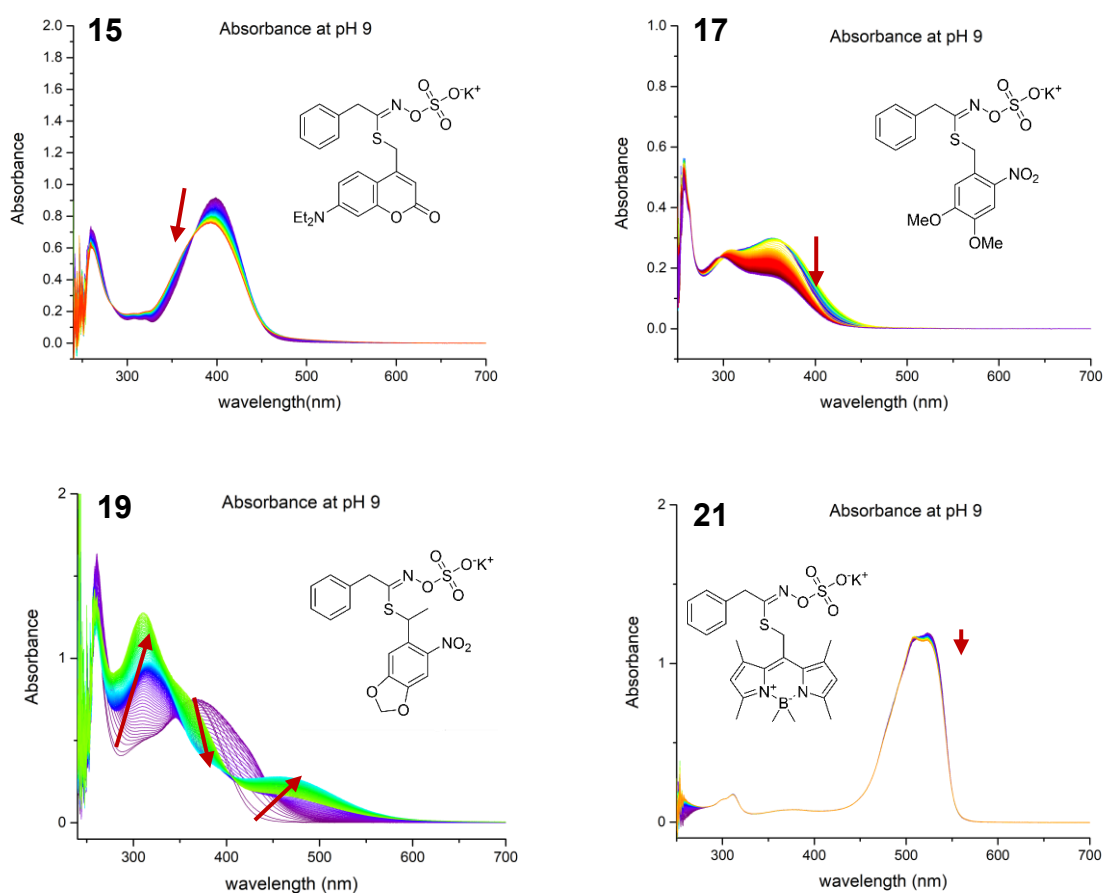


Figure 7b. Variation of absorption spectra of photo-psGSLs upon irradiation at pH 9

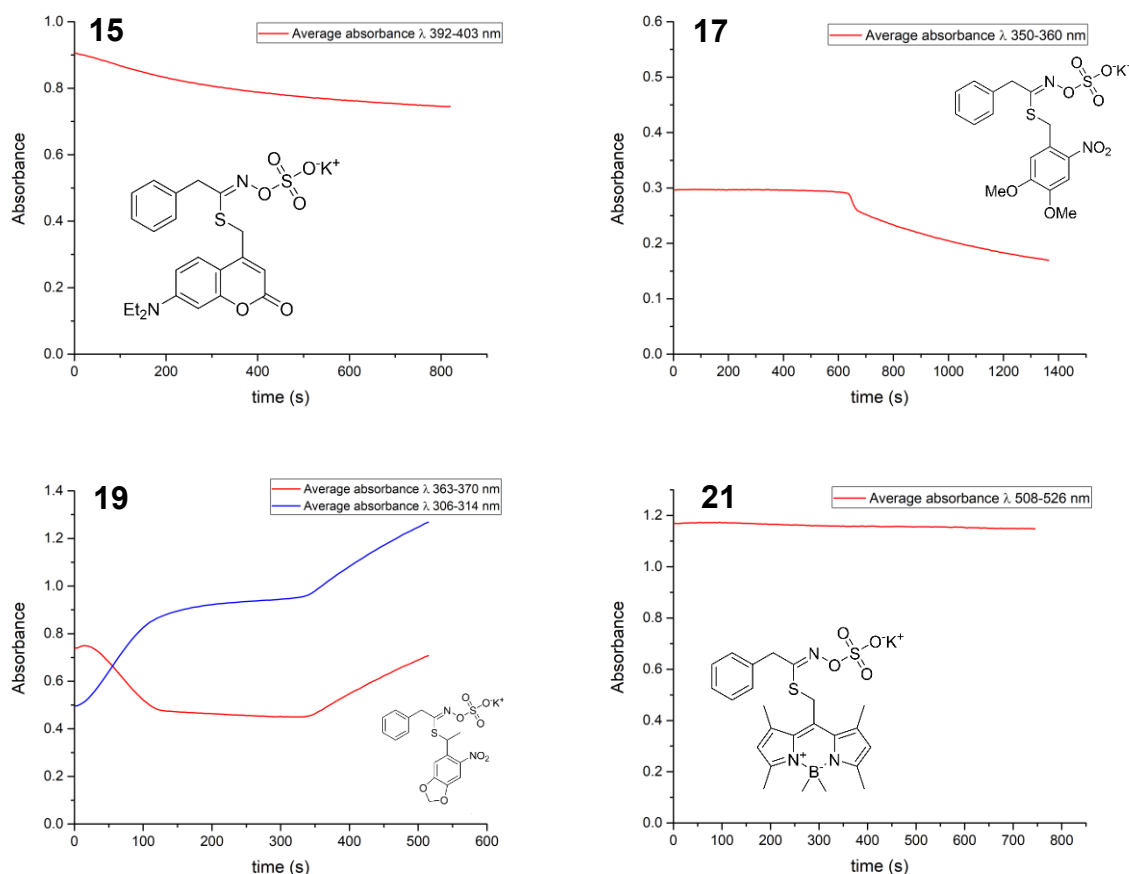


Figure 7c. Trend of absorption at average λ_{max} over time upon irradiation at pH 9

Upon analysing the measurements, the following features were found: at pH 9, photo-psGSLs **15** and **19** again displayed a similar trend, while **17** behaved consistently with what was observed at pH 8. Compound **21** showed no evidence of uncaging under these conditions. Notably, the average absorbance of **19** over time exhibited an unusual increase after approximately six minutes, rising sharply in an almost linear manner. To investigate this phenomenon, **19** was subjected to irradiation, and aliquots were collected at five-minute intervals over a total period of 25 minutes for analysis by LC–MS. However, these analyses did not provide further insight into the cause of the observed behaviour. The samples analyzed at both the initial (after 5 and 10 minutes) and final time intervals (after 20 and 25 minutes) displayed identical mass peaks, with no observable differences between them. The mass corresponding to the photoproduct (MeNPOM cation) was detected, but no additional species were observed.

2.3.2 Irradiation time

Photo-psGSLs **15** and **19** showed changes in spectra as soon as they were irradiated, but longer irradiation time was needed for **17** and **21**. DMNB derivative **17** did not show any significant

change in absorbance at pH 5.5 and 7 even if irradiated for longer (20–25 minutes), while at pH 8 and 9 following an initial period of stability there is a drop in absorbance. BODIPY derivative **21** was irradiated for 40 minutes and even though there was a slight decrease in absorbance no product of uncaging was observed. To support this, the following LC-MS trace of compound **21**, irradiated for 40 minutes at pH 7, is reported, which does not show anything other than the mass of the photo-psGSL (Figure 8).

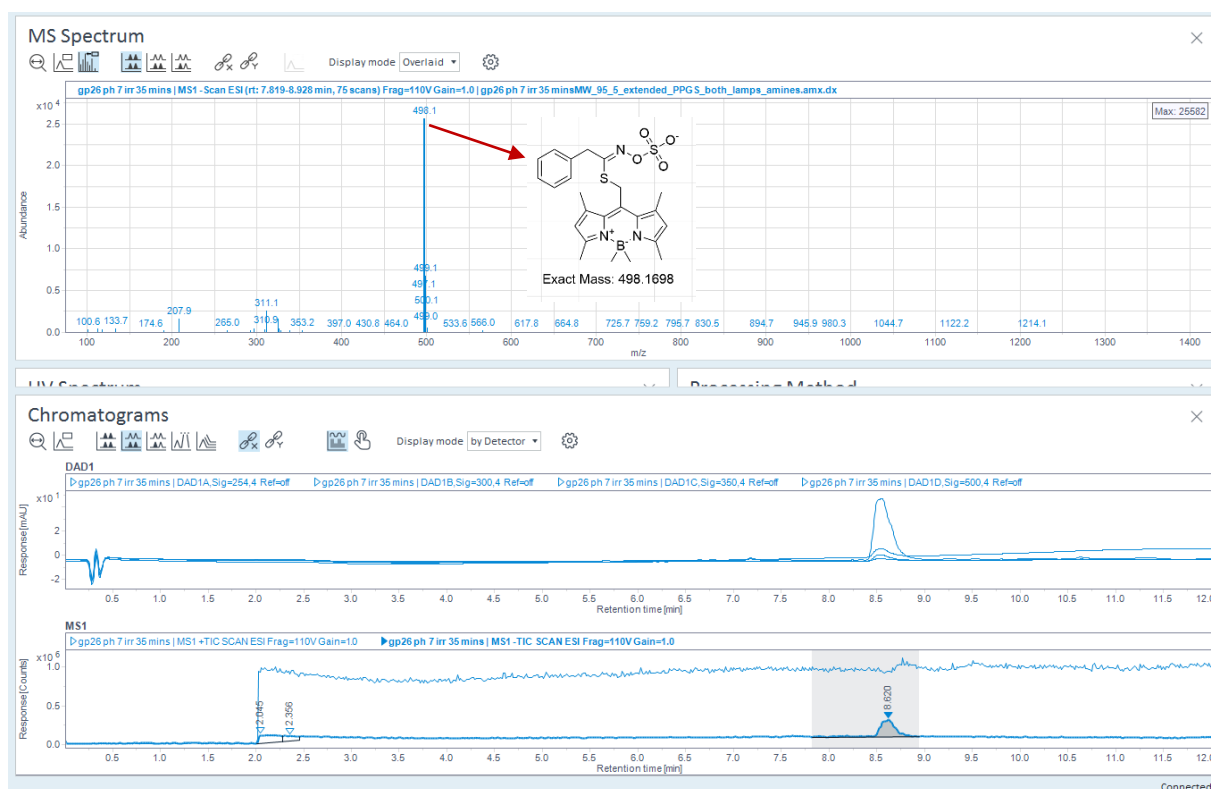


Figure 8. LC-MS trace of compound **21** after irradiating for 45 minutes

The removal of the PPG was successful for compounds **15**, **17** and **19**, therefore the molecules were tested for the release of isothiocyanate.

2.4 Addition of NH₃ and glycine to irradiated samples – trapping of ITC

After irradiation, the uncaging process is suspected to release a thiohydroximate intermediate which undergoes spontaneous thio-Lossen rearrangement, losing SO₄²⁻ and releasing isothiocyanate. All irradiated samples were analysed at the LC-MS and the mass of the released benzyl isothiocyanate could not be detected.

To exclude technical difficulties being the reason for the absence of the signal, we attempted to directly react the isothiocyanates with suitable nucleophiles. Specifically, the reaction of isothiocyanate and ammonia produces benzylthiourea, while with glycine the product is (benzylcarbamothioyl)glycine. To ascertain that the reactivity of isothiocyanate with ammonia

or glycine was a consequence of the irradiation of photo-psGSLs, these were treated with a solution of ammonia and a solution of glycine before irradiation. No benzylthiourea or (benzylcarbamothioyl)glycine were found, confirming that the ITC is not released before irradiation. Thus, the irradiated samples were treated with NH_3 (5 μL of 25% w/w conc. solution added to 1 mL of irradiated sample) and glycine (10 μL of 100 mM solution at pH 9 added to 1 mL of irradiated sample). After irradiation the solution in the cuvette was divided into two vials (1 mL each) and NH_3 was added to one, while Glycine was added to the other, then the samples were analysed at the LC-MS. The reaction with NH_3 proved to be quite fast and after just even less than one hour the mass of the benzylthiourea could be detected. On the other hand, the samples treated with glycine reacted more slowly and when analysed on the same day of irradiation they did not show any mass of the product, only by analysing the same samples the following day was the mass detected (Table 3). The pH value reported in the table is the initial value from the buffer solutions, however with the addition of ammonia the pH will likely be affected, but the change of pH was not measured.

A rough estimation of the final pH can be made by comparing the initial amount of buffer to the number of moles of ammonia or glycine added. The buffer volume was 1 mL: at pH 5.5, with a concentration of 10 mM, this corresponds to 0.01 mmol; the other buffer solutions (pH 7, 8, and 9) had a concentration of 100 mM, thus the quantity of buffer is 0.1 mmol. Upon addition of ammonia (5 μL of a 25% w/w solution, approximately 0.066 mmol), the buffer is neutralized, and the excess ammonia raises the pH. This suggests that the final pH will likely be basic enough to favour the formation of thiourea. In the case of glycine, however, the amount added (0.001 mmol) is insufficient to significantly alter the pH, especially at acidic conditions. As shown in Table 3, no adducts between the released isothiocyanate and glycine are observed at pH 5.5. Only at higher pH values (8 or 9), where the buffer itself maintains a basic environment, does the reaction between ITC and glycine proceed, confirming that a sufficiently basic pH is required for the formation of the adduct.

pH	+ NH_3 (same day analysis)				+ Glycine (24h analysis)			
	5.5	7	8	9	5.5	7	8	9
15	✓	✓	✓	✓	×	×	✓	✓
17	×	×	✓	✓	×	×	✓	✓
19	✓	✓	✓	✓	×	✓	✓	✓

Table 3. Presence or absence of ITC products after addition of NH_3 and glycine to irradiated samples of photo-psGSLs

The analysis was performed for all samples; however, for clarity, only two representative LC-MS traces are presented. Figure 9 corresponds to samples of photo-psGSL **19** with the addition of ammonia and glycine at initial pH 8, where the mass of the product of the reaction is highlighted.

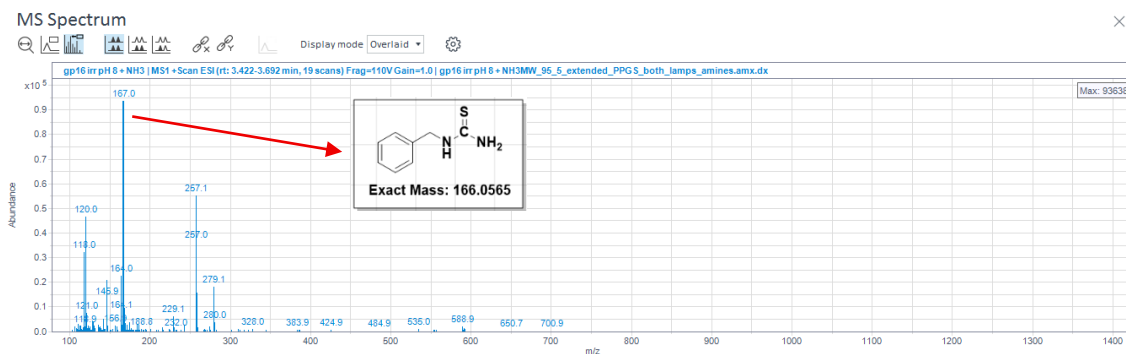


Figure 9a. LC-MS trace of irradiated compound **19** + NH₃

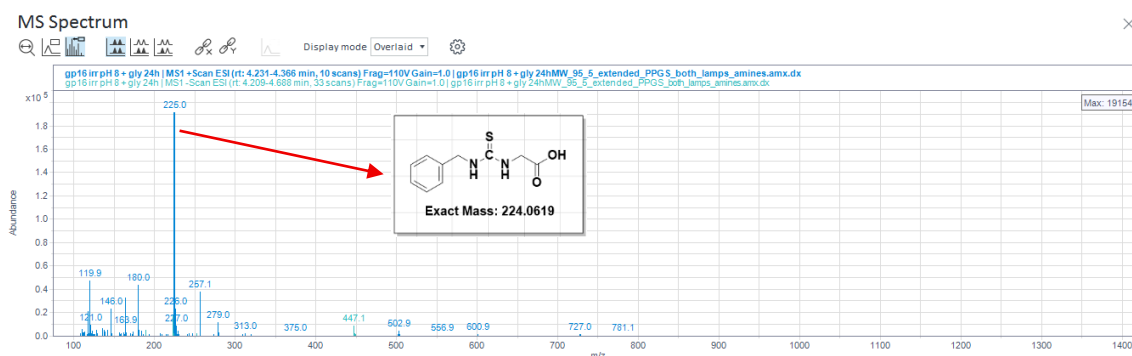


Figure 9b. LC-MS trace of irradiated compound **19** + glycine

2.5 Benzylthiourea calibration curve

Once it was established that the uncaging process released ITC, and that it reacted with ammonia to form benzylthiourea, an attempt was made to calculate the concentration of benzylthiourea formed from the irradiated photo-psGSLs by means of a calibration curve.

To make the calibration curve, a 200 μ M solution of benzylthiourea in 1:1 DMSO/water was prepared. The samples with lower concentration were prepared by diluting the 200 μ M solution to obtain the desired concentration. The samples were then analysed at the LC-MS using the same method that was used to analyse the irradiated photo-psGSL samples. The retention time of the peak of the benzylthiourea was from 3.45 to 3.90 minutes and the selected wavelength range to measure the integral was between 238 and 242 nm. The following results were obtained, and the corresponding calibration curve was plotted.

concentration (μM)	integral
200	14835
100	6597
50	3098
25	1675
12.5	890
6.25	458
3.125	250

Table 4. Concentrations and integrals of benzylthiourea solutions for calibration curve

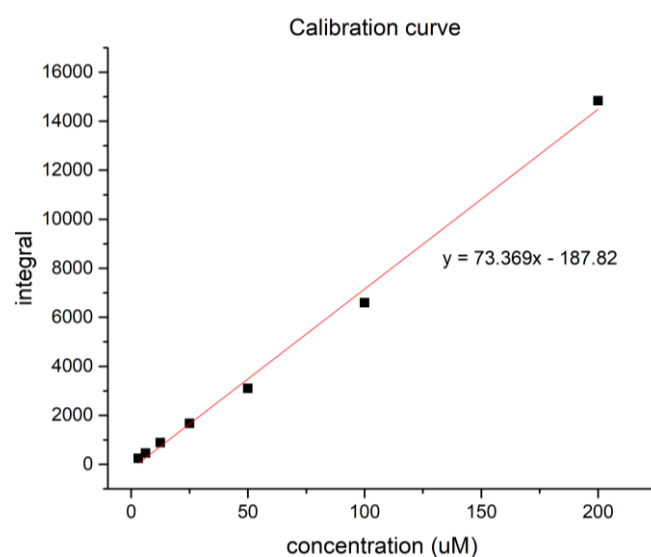


Figure 10. Calibration curve

With the calibration curve in hand, the concentration of the formed benzylthiourea was calculated from the samples of irradiated photo-pseudoGlucosinolates to which concentrated NH_3 was added, by using the linear equation of the calibration curve (Table 5).

To each irradiated sample 5 μL of concentrated NH_3 solution (25% w/w) were added and the samples were immediately analysed at the LC-MS. The integrals were measured in the same way, in the wavelength range between 238-242 nm. Photo-pseudoGlucosinolate **21** did not show any uncaging after irradiation therefore it was not included in Table 5.

The concentration of benzylthiourea formed from compound **19** at pH 5.5 was notably higher than that observed in the other analysed samples. Similarly, the concentration of benzylthiourea formed from compound **15** at pH 8 exceeded that recorded under other pH conditions. This result can be attributed to the fact that, during the initial measurement of **15**, no peak corresponding to benzylthiourea was detected; therefore, the analysis was repeated the following day, by which time a substantial amount of product had accumulated. Overall, this investigation served primarily as a qualitative assessment, providing only an approximate

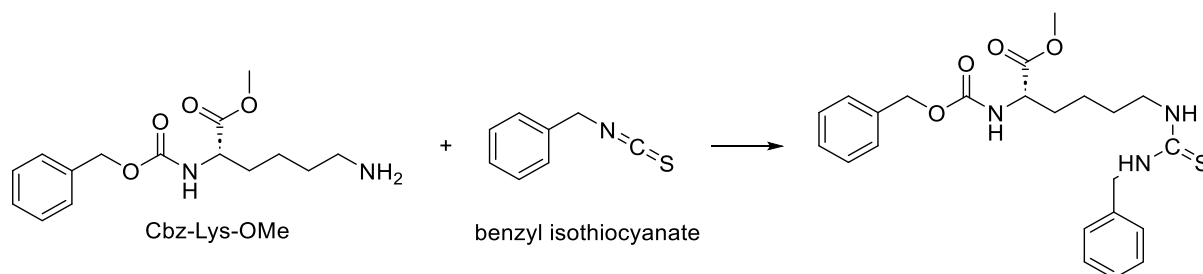
indication of the relative amounts of benzylthiourea formed from isothiocyanate under the various tested conditions.

	pH 5.5	pH 7	pH 8	pH 9
	integral			
15	993	630	1890	960
17	0	0	578	721
19	8604	2736	2038	2029
	benzylthiourea concentration (μM)			
15	16	11	28	16
17	0	0	10	12
19	120	40	30	30

Table 5. Calculated integrals and concentrations of benzylthiourea formed from the irradiated photo-psGSLs

2.6 Lysine labelling

Some preliminary studies were carried out to test the labelling of lysine by benzyl isothiocyanate, at different pH values and reaction conditions. This was done to study the standard reactivity of isothiocyanate with lysine, to have a reference reaction for the ITC release from psGSLs and its reactivity with lysine and proteins, which is the final aim of this project.



Scheme 5. Reactivity of Cbz-Lys-OMe with benzyl isothiocyanate

First, the buffers were prepared. The chosen pH values are 7, 8 and 9. The pH 7 buffer was prepared from a 100 mM solution of HEPES, regulating the pH with NaOH 1 M until the desired pH was reached. The buffer solutions at pH 8 and 9 were prepared from a 100 mM solution of Bicine. Then, the stock solutions of lysine (5 mM) and isothiocyanate (10 mM) were prepared.

With the buffer and the stock solutions in hand, the first samples were prepared to study the reactivity of the isothiocyanate with lysine.

Different parameters need to be taken into consideration. For example, the temperature at which to conduct the analysis, or the number of equivalents of benzyl isothiocyanate. The first experiments were carried out at room temperature and using 3 equivalents of benzyl isothiocyanate.

The samples were prepared in LC-MS vials as follows. The samples containing lysine were prepared by adding 20 μ L of Cbz-Lys-OMe, 30 μ L of isothiocyanate and 950 μ L of buffer to a vial. The samples without lysine were prepared by adding 20 μ L of DMSO, 30 μ L of isothiocyanate and 950 μ L of buffer to a vial.

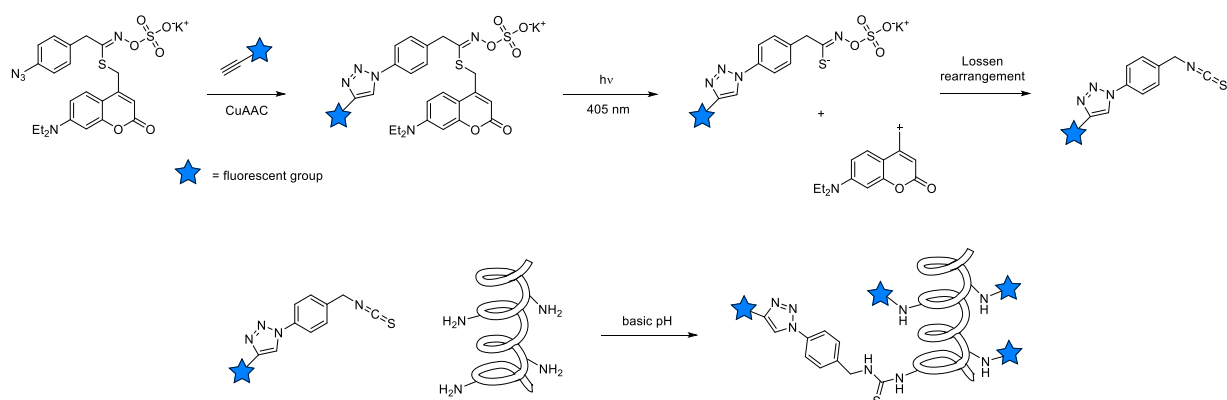
The samples were analysed by LC-MS at four time points, at intervals of 1.5 hours, followed by a final analysis on the following day (after overnight incubation). The preliminary results indicated that the most favourable conditions for labelling occurred at pH 9, whereas pH 7 and pH 8 exhibited minimal reactivity. At pH 9, benzyl isothiocyanate was observed to react with Cbz-Lys-OMe, however the amount of adduct formed remained low, and a substantial quantity of unreacted lysine was still present. The fact that the best results were obtained with pH 9 would explain why ammonia was a better trap than glycine in the experiments conducted in section 2.4, and is consistent with the fact that thioureas form prevalently at higher pH values. This experiment was intended as an initial screening performed while other reactions were ongoing. Further investigation of this approach has not yet been pursued, as subsequent experiments will utilise the final photocages as the isothiocyanate source for lysine labelling. Unfortunately, due to limited time, the labelling activity of the photo-psGSLs could not be investigated, but the results give hope for a possible future application of these compounds for the labelling of peptides and proteins.

2.7 Towards the synthesis of DEAC-N₃ photo-psGSL

Three out of the four isolated photo-psGSLs successfully released isothiocyanate upon light irradiation. The following step would be to render the photo-psGSLs fluorescent, by adding a chosen fluorescent group to the molecule, to employ these compounds for protein labelling. Therefore, the synthetic desired experiment displayed in Scheme 6 was designed.

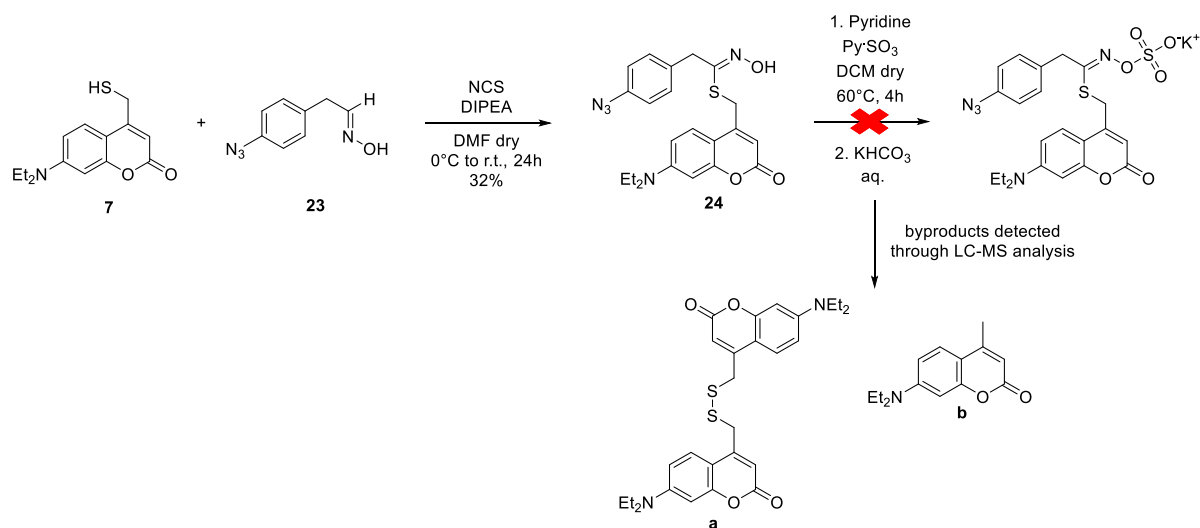
The azide group was chosen for its relevance in photoclick chemistry and its easy and quick reactivity with a triple bond. After isolating the photo-psGSL with the -N₃ group, this would react in a photoclick fashion with a commercially available fluorophore-bearing alkyne. The result is a photo-psGSL functionalized with a fluorescent moiety. After irradiation, the uncaging of the target isothiocyanate results in the release of a fluorescent ITC, which becomes readily detectable by standard fluorescence spectroscopy. Then, to probe the presence of

cysteine or lysine residues in the active site of an enzyme, the fluorescently tagged ITC will enable selective labelling and thus facilitate the identification of the targeted residues (Scheme 6).



Scheme 6. Design of light-activated labelling

To achieve this goal, I set out to synthesize DEAC-N₃ photo-psGSL (Scheme 7) from DEAC-SH and hydroxylamine **23**. The synthesis started out with 2-(4-azidophenyl)acetaldehyde **22** which was treated with hydroxylammonium chloride to obtain the corresponding oxime **23**. Next, DEAC-SH **7** and oxime **23** reacted in the presence of NCS and DIPEA for 24 hours to give thiohydroximate **24**. The sulfonation was attempted to obtain the final photo-psGSL but it did not afford the expected result. The reaction was analysed at the LC-MS, where two peaks corresponding to the mass of the coumarin dimer **a** and the coumarin photoproduct **b** were detected. The mass of the sulfonated product was not detected.



Scheme 7. Synthesis of DEAC-N₃ photo-psGSL

Unfortunately, it was not possible to further explore this pathway during the project. Future work will focus on optimizing the synthetic protocol and investigating the labelling activity, as

illustrated in Scheme 6. Notably, the release of isothiocyanate (ITC) from the previously synthesized photo-pseudoGlucosinolates (photo-psGSLs) appears promising for further experiments and potential applications of fluorescent labelling in biological systems.

3. Summary and Outlook

In this thesis, we aimed to synthesize a collection of photo-pseudoGlucosinolates as light-controllable precursors of isothiocyanate. Four photo-pseudoGlucosinolates were obtained, using the following photoprotecting groups: diethylaminocoumarin (DEAC), dimethoxynitrobenzyl group (DMNB), α -methyl-nitropiperonyloxymethyl (McNPOM) and a BODIPY scaffold. The synthesis occurred smoothly most of the time, though the synthesis of the BODIPY photo-psGSL required more time and attention.

To study the uncaging process, the molecules were irradiated using a specific wavelength based on the photoprotecting group, and they were subsequently analysed at the LC-MS. The released isothiocyanate could not be detected immediately at the LC-MS due to its instability, therefore the irradiated samples were treated separately with a glycine solution and an ammonia solution. The reaction between isothiocyanate and glycine or ammonia led to the formation of benzylthiourea and (benzylcarbamothioyl)glycine, respectively. These products were detected by LC-MS, thus confirming the successful release of ITC.

Different pH conditions were tested and they proved to affect the uncaging process of the compounds: in particular for the DMNB derivative **17**, which at lower pH (5.5 and 7) did not show any variation in the absorption spectrum, while pH 8 and 9 were effective in facilitating the uncaging process.

Another important factor in the uncaging of the photo-psGSL was the irradiation time: the compounds were initially irradiated for 10 minutes, but not all showed variations in the absorption spectra. Consequently, the irradiation time was increased for compounds **17** and BODIPY derivative **21** that did not uncage after only 10 minutes. Compound **17** needed more time to show a variation in absorbance, while compound **21** remained the same even after 40 minutes of irradiation, suggesting that it might simply not uncage in the conditions that were used.

Out of the four synthesized molecules, only three could efficiently release ITC. The BODIPY photo-psGSL did not in any circumstances show any release of isothiocyanate. This limitation is unfavourable, as among the four PPGs, BODIPYs are the ones that absorb at higher wavelengths (around 500 nm). This property makes them especially attractive for biological

applications, since absorption in this region of the visible spectrum is closer to the biological window and less harmful to biological systems.

Because of the limited time available, the application of the photo-pseudoGlucosinolates for labelling purposes could not yet be tested in biological systems. The ITC-releasing photo-psGSLs can be functionalized with an appropriate fluorescent group, such that upon irradiation, the released ITC carries this fluorescent moiety and becomes readily detectable by standard fluorescence spectroscopy. Future experiments will focus on the synthesis of photo-psGSLs able to release a fluorescent ITC upon irradiation. Moreover, for biological applications, water solubility of the compounds should be thoroughly studied.

In conclusion, several photo-pseudoGlucosinolates were designed and synthesized. Despite some initial drawbacks in the synthesis of BODIPY photo-psGSL, and later discovering it did not release ITC upon uncaging, significant progress has been made. The isolated compounds were found to be a very good synthetic alternative to natural glucosinolates, which are only found in plants. The synthesis of fluorescent photo-psGSLs could not be completed, however the activity I carried out in this part of the thesis lays the foundation for further research and future application of these molecules in biological environments. Overall, these findings will certainly contribute to the development of new photo-pseudoGlucosinolates, which will be used for labelling purposes in the future.

4. Experimental Section

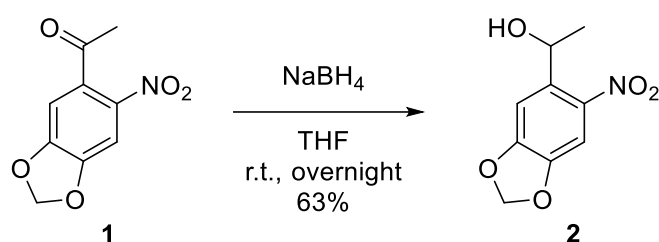
4.1 General

All reagents were bought from commercial suppliers and used without further purification. TLC was performed with the pre-coated Alugram Xtra SIL G/UV254 sheets and the spots were analysed with 254nm or higher wavelength light or with a KMnO₄ stain. Normal phase flash chromatography was performed either by hand or with a Biotage Isolera One flash chromatography instrument. Pre-packed Biotage Sfär normal phase columns or manually packed silica gel (Supelco, 60Å pore size, 40-60µm particle size) was used as a stationary phase. Fractions were analysed by TLC, LCMS, or NMR spectroscopy. UV/Vis spectra were recorded with an Analytik Jena Specord S600 spectrometer and samples were measured in 10x10mm quartz cuvettes. ¹H-NMR spectra were recorded at a frequency of 300 MHz or 400 MHz, and the ¹³C-NMR spectra at 75 MHz or 101 MHz. All ¹³C-NMR measurements were ¹H decoupled. The used solvents (CDCl₃, D₂O, DMSO-*d*₆, acetone-*d*₆) were all deuterated and used as reference peak in the analysed spectra. The chemical shift δ is shown in ppm, while the coupling constant J is shown in Hz. All spectra were analysed in the MestReNova program.

The obtained peaks were characterised as follows: singlet (s), doublet (d), doublet of doublets (dd), doublet of triplets (dt), triplet of doublets (td), quartet of doublets (qd) and multiplet (m). Analytics with LC-MS was performed with an Agilent 1260 Infinity II device, coupled with an InfinityLab LC/MSD iQ mass detector (ESI). The used solvents were MeCN and H₂O containing 0.1% formic acid, while the used column was an InfinityLab Poroshell 120 EC-C18 heated to 40°C with a flow rate of 0.5 mL per minute. Typically, an injection volume between 1 µL and 10 µL was chosen and a blank run was performed before each series of injections.

1.2 Synthesis of photoprotecting groups

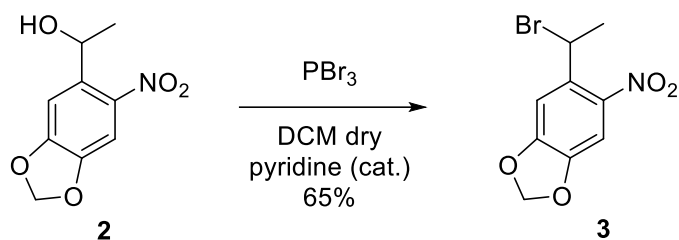
Synthesis of MeNPOM-OH⁶⁴ (2)



4',5'-Methylenedioxy-2'-nitroacetophenone **1** (3.20 g, 15.3 mmol, 1.0 equiv) was dissolved in THF (76 mL) and sodium borohydride (1.15 g, 30.7 mmol, 2.0 equiv) was added. The reaction mixture was stirred overnight at room temperature, the course of the reaction was monitored by TLC, NMR, and LC-MS. Since the analysis showed the presence of starting material, another 2.0 equivalents of sodium borohydride were added. When the reagent appeared to be completely consumed, the reaction was then quenched with 1 M HCl until the formation of gas had ceased. The solvent was partially removed *in vacuo* to reduce the amount of THF before pouring the mixture into brine, and extracting the organic components with DCM (3 × 20 mL). The combined organic layers were dried over sodium sulfate, filtered, and then concentrated under reduced pressure. The solid residue was purified via flash column chromatography (0-5% MeOH in DCM) to give 1-(6-nitrobenzo[d][1,3]dioxol-5-yl)ethan-1-ol as a yellow solid (2.03 g, 63%).

R_f = 0.3 (DCM). **¹H NMR** (300 MHz, CDCl₃) δ 7.46 (s, 1H), 7.27 (s, 1H), 6.11 (q, *J* = 1.1 Hz, 2H), 5.46 (dd, *J* = 6.2, 2.7 Hz, 1H), 2.25 (d, *J* = 2.8 Hz, 1H), 1.54 (d, *J* = 6.3 Hz, 3H). **¹³C NMR** (75 MHz, MeOD) δ 153.7, 148.2, 142.4, 141.1, 107.0, 105.5, 104.4, 66.2, 25.1. **IR** (cm⁻¹) 3392.2, 3339.1, 1616.1, 1511.9, 1499.4, 1480.1, 1320.0, 1251.6, 1028.8. **m.p.** 86°C.⁷⁹ The spectroscopic data matched the literature.⁷⁹

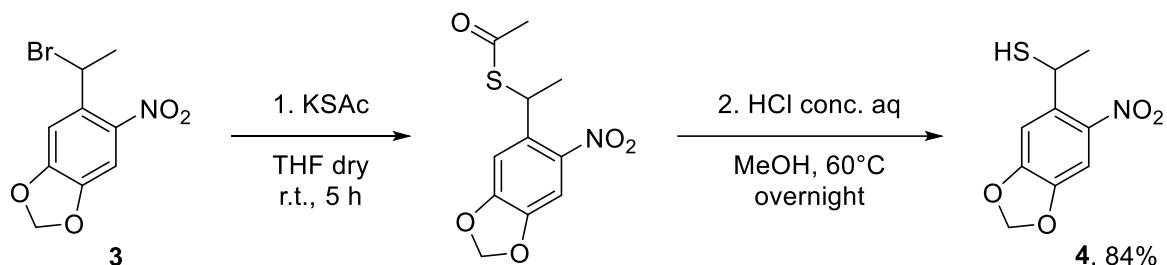
Synthesis of MeNPOM-Br⁸⁰ (3)



1-(6-Nitrobenzo[*d*][1,3]dioxol-5-yl)ethan-1-ol **2** (1.02 g, 4.85 mmol, 1 equiv) was added in a round bottom flask under inert atmosphere and dissolved in dry DCM (20 mL). Then, PBr_3 (0.175 mL, 1.85 mmol, 0.4 equiv) was added dropwise to the solution at 0°C, and finally a catalytic amount of pyridine (10 μL) was added. The reaction mixture was kept at 0 °C for 15 minutes, then the ice bath was removed and the stirring continued at room temperature for 1.5 hours. When the reagent was fully consumed the reaction mixture was quenched with a saturated solution of NaHCO_3 (20 mL). The aqueous and organic layers were separated, and the aqueous phase was washed twice with DCM (10 mL). The organic layers were collected, washed with brine (10 mL), and dried over sodium sulphate. The solvent was removed at the rotary evaporator and the crude product 5-(1-bromoethyl)-6-nitrobenzo[*d*][1,3]dioxole was used in the following step without further purification (0.87 g crude product, 65%).

R_f = 0.8 (7:3 pentane/EA). $^1\text{H NMR}$ (300 MHz, CDCl_3) δ 7.34 (s, 1H), 7.27 (s, 1H), 6.12 (s, 2H), 5.89 (q, J = 6.8 Hz, 1H), 2.03 (d, J = 6.8 Hz, 3H). $^{13}\text{C NMR}$ (75 MHz, MeOD) δ 153.3, 149.1, 142.9, 135.3, 109.0, 105.7, 104.9, 43.6, 27.3. IR (cm^{-1}) 2979.5, 2912.0, 1617.0, 1518.7, 1504.2, 1480.1, 1254.5, 1034.6. **m.p.** 76.1-77.8°C.⁶⁵ The spectroscopic data matched the literature.⁶⁵

Synthesis of MeNPOM-SH⁶⁶ (4)

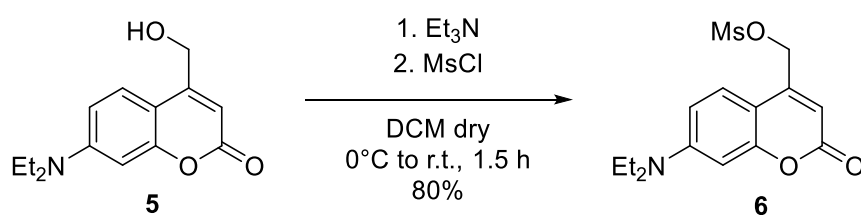


To a solution of 5-(1-bromoethyl)-6-nitrobenzo[*d*][1,3]dioxole **3** (0.72 g, 3.65 mmol, 1.0 equiv) in dry THF (25 mL) was added potassium thioacetate (0.37 g, 4.38 mmol) and the reaction mixture was stirred for 5 hours at room temperature. The solvent was removed *in vacuo* and

the residue was partitioned between EtOAc (25 mL) and H₂O (25 mL). The separated organic layer was washed with brine (20 mL), dried over Na₂SO₄, and concentrated *in vacuo*. The residue was dissolved in MeOH (50 mL), and 37% aqueous HCl (3.5 mL) was added. The reaction was kept stirring at 60 °C overnight, and monitored by TLC until completion, before the solvent was removed *in vacuo*. The product 1-(6-nitrobenzo[*d*][1,3]dioxol-5-yl)ethane-1-thiol (0.50 g) was used without further purification and obtained in 84% yield.

R_f = 0.84 (1:1 pentane/EtOAc). **¹H NMR** (300 MHz, CDCl₃) δ 7.33 (s, 1H), 7.17 (s, 1H), 6.10 (s, 2H), 4.90 (dd, *J* = 6.9, 5.5 Hz, 1H), 2.25 (d, *J* = 5.5 Hz, 1H), 1.65 (dd, *J* = 7.0, 0.7 Hz, 3H). **¹³C NMR** (75 MHz, CDCl₃) δ 152.0, 146.7, 141.8, 137.9, 107.1, 105.0, 103.2, 103.1, 33.0, 25.4. **HR-MS** (ESI⁺) *m/z*: calcd. for C₉H₉NO₄SN⁺ [M+Na]⁺: 250.0144, found: 250.0143. **IR** (cm⁻¹) 2968.9, 2927.4, 1617.0, 1498.4, 1485.9 1323.9, 1257.4, 1025.9. **m.p.** 66-68°C

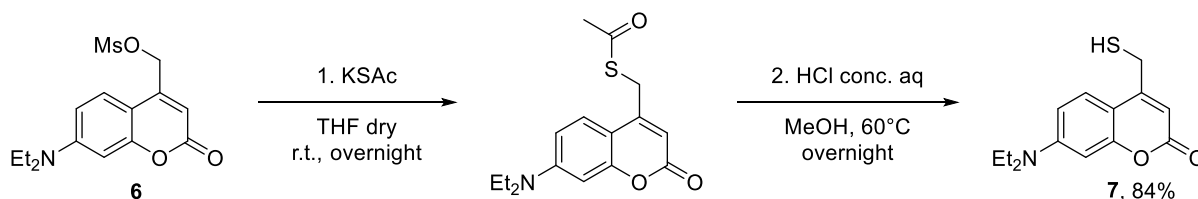
Synthesis of DEAC-OMs⁶⁷ (6)



7-(Diethylamino)-4-(hydroxymethyl)-2*H*-chromen-2-one **5** (1.01 g, 4.06 mmol, 1.0 equiv) was dissolved in dry DCM (20 mL) in a water-ice bath and Et₃N (0.85 mL) and MsCl (0.5 mL) were added sequentially. The mixture was stirred at 0°C for 5 minutes and at room temperature for 1.5 hours. When the reaction was monitored by TLC (1:1 EtOAc/pentane) and once completed, the mixture was washed with 1M H₃PO₄ (2 × 12 mL), saturated NaHCO₃ (12 mL), and water (2 × 12 mL). The aqueous phase was washed with DCM, then the combined organic phases were washed with brine and dried over MgSO₄. Evaporation of the organic phase afforded (7-(diethylamino)-2-oxo-2*H*-chromen-4-yl)methyl methanesulfonate as an orange solid (1.06 g, 80%), that was used in the next step without further purification.

R_f = 0.67 (1:1 pentane/EtOAc). **¹H NMR** (300 MHz, CDCl₃) δ 7.32 (d, *J* = 9.0 Hz, 1H), 6.60 (dd, *J* = 9.0, 2.6 Hz, 1H), 6.53 (d, *J* = 2.5 Hz, 1H), 6.17 (s, 1H), 5.30 (d, *J* = 1.1 Hz, 2H), 3.42 (q, *J* = 7.1 Hz, 4H), 3.10 (s, 2H), 1.22 (t, *J* = 7.1 Hz, 6H). **¹³C NMR** (75 MHz, CD₃CN) δ 161.8, 157.2, 149.1, 126.2, 67.8, 40.0, 38.1, 12.5, 2.2, 1.9, 1.6, 1.3, 1.0, 0.8, 0.5. **HR-MS** (ESI⁺) *m/z*: calcd. for C₁₅H₁₉NO₅SN⁺ [M+Na]⁺: 348.0876, found: 348.0875. **IR** (cm⁻¹) 1713.4, 1602.6, 1339.3, 1168.7, 1060.7, 1000.9, 815.7, 512.0, 501.4. **m.p.** 126-128°C

Synthesis of DEAC-SH⁶⁶ (7)

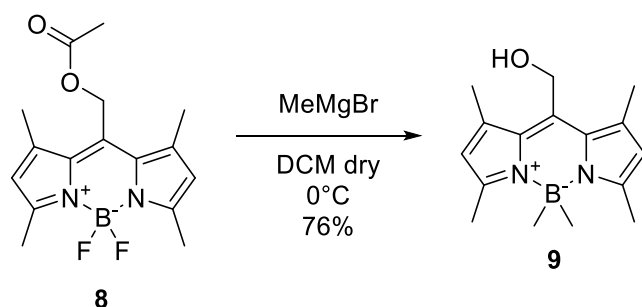


To a solution of DEAC-OMs **6** (0.91 g, 2.79 mmol, 1.0 equiv) in dry THF (50 mL) was added potassium thioacetate (394 mg, 3.35 mmol) and the reaction mixture was stirred overnight at room temperature. The solvent was removed *in vacuo* and the residue was partitioned between EtOAc (10 mL) and H₂O (10 mL). The separated organic layer was washed with brine (10 mL) and then dried with Na₂SO₄, and concentrated *in vacuo*.

The resulting residue was dissolved in MeOH (50 mL), and 35% aqueous HCl (3 mL) was added. After being stirred for 12 hours at 60 °C, the solvent was removed *in vacuo*. The crude product was recrystallized in a mixture of pentane/EtOAc (5 mL of EtOAc to dissolve the product then 20 mL of pentane to make it precipitate) and filtered on a Buchner funnel to obtain the pure product 7-(diethylamino)-4-(mercaptomethyl)-2*H*-chromen-2-one (0.62 g, 84%).

R_f = 0.74 (1:1 pentane/EtOAc). **¹H NMR** (300 MHz, CDCl₃) δ 7.69 (d, *J* = 8.8 Hz, 1H), 7.52 (d, *J* = 6.5 Hz, 1H), 7.18 (s, 1H), 6.42 (s, 1H), 3.81 (d, *J* = 8.1 Hz, 2H), 3.49 (q, *J* = 7.1 Hz, 4H), 1.91 (t, *J* = 8.0 Hz, 1H), 1.28 (t, *J* = 7.2 Hz, 6H). **¹³C NMR** (75 MHz, MeOD) δ 161.6, 156.1, 156.1, 128.8, 118.7, 115.7, 54.1, 25.1, 10.9. **HR-MS** (ESI⁺) *m/z*: calcd. for C₁₄H₁₇NO₂SN⁺ [M+Na]⁺: 286.0872, found: 286.0872. **IR** (cm⁻¹) 2975.6, 2536.9, 2463.6, 1717.3, 1616.1, 984.5. **m.p.** 169-172 °C

Synthesis of Me-BODIPY-OH⁶⁸ (9)

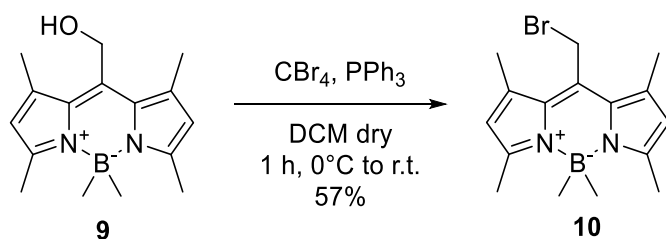


To a solution of F-BODIPY-OAc **8** (1.85 g, 5.76 mmol, 1.0 equiv) in anhydrous DCM (200 mL), 3 M methyl magnesium bromide (30 mL, 86.4 mmol, 15 equiv) was added under a N₂ atmosphere and the reaction was stirred until the starting material was consumed. Additions of the Grignard reagent are done portion-wise at 0 °C – this should help to obtain the product

without further purification. The reaction was then quenched with NH₄Cl (150 mL, aq. sat.), still at 0°C, until no more development of gas was observed. Ethyl acetate was added, and the organic layer was washed 3 times with ammonium chloride (100 mL), with brine (100 mL), and dried over Na₂SO₄. The solvent was removed in vacuo and the crude product (1,3,5,5,7,9-hexamethyl-5*H*-4λ⁴,5λ⁴-dipyrrolo[1,2-*c*:2',1'-*f*][1,3,2]diazaborinin-10-yl)methanol (1.18 g, 76%) was used for the next step without further purification.

R_f = 0.79 (7:3 pentane/EtOAc). **¹H NMR** (400 MHz, DMSO-*d*₆) δ 6.15 (s, 2H), 4.73 (s, 2H), 2.47 (s, 6H), 2.40 (s, 6H), 0.11 (s, 6H). **¹³C NMR** (101 MHz, DMSO) δ 151.4, 151.3, 140.5, 137.5, 137.4, 130.5, 122.0, 54.6, 40.2, 40.2, 39.9, 39.7, 39.5, 39.3, 39.1, 38.9, 16.2, 16.1, 15.5, 15.4. **HR-MS** (ESI⁺) *m/z*: calcd. for C₁₆H₂₃BN₂O⁺ [M]⁺: 270.1903, found: 270.2009. **IR** (cm⁻¹) 3220.5, 1562.1, 1516.7, 1298.8, 1145.5, 942.1, 796.5. **m.p.** 187-190°C⁸¹

Synthesis of Me-BODIPY-Br⁶⁸ (10)

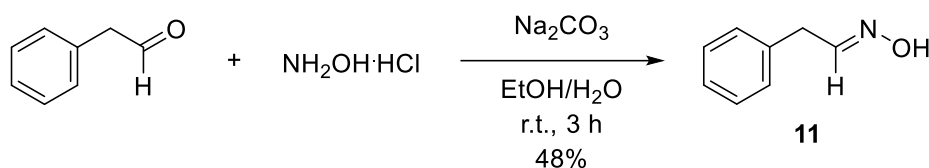


Me-BODIPY-OH **9** (0.50 g, 1.84 mmol, 1.0 equiv) was dissolved in dry DCM (60 mL) under inert atmosphere. The reaction mixture was cooled down to 0°C using an ice-water bath before adding sequentially CBr₄ (1.03 g, 3.08 mmol, 1.66 equiv) and PPh₃ (0.80 g, 3.08 mmol, 1.66 equiv). After adding all the reagents, the reaction mixture was removed from the ice bath and warmed to room temperature. Stirring was kept for about 1 hour and the course of the reaction was monitored by TLC (pentane). The solvent was evaporated and the product was dried at the vacuum line. The crude product was purified by flash column chromatography (0-35% DCM in pentane) and 10-(bromomethyl)-1,3,5,5,7,9-hexamethyl-5*H*-4λ⁴,5λ⁴-dipyrrolo[1,2-*c*:2',1'-*f*][1,3,2]diazaborinine was isolated as a dark purple shiny solid (0.35 g, 57%).

R_f = 0.5 (pentane). **¹H NMR** (300 MHz, CDCl₃) δ 6.09 (s, 2H), 4.78 (s, 2H), 2.58 (s, 6H), 2.46 (s, 6H), 1.54 (s, 2H), 0.18 (d, *J* = 16.5 Hz, 6H). **¹³C NMR** (75 MHz, CDCl₃) δ 153.2, 137.6, 136.6, 129.6, 122.9, 121.8, 26.0, 18.0, 16.7, 16.4. **IR** (cm⁻¹) 2909.1, 1560.1, 1513.9, 1300.8, 1151.3, 941.1. **m.p.** 177.5-179°C

1.3 Synthesis of hydroxilamines

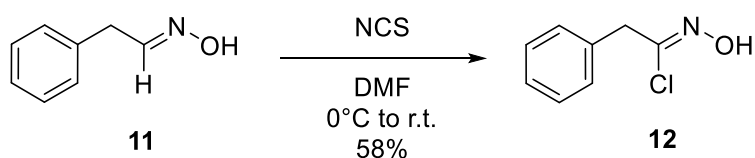
Synthesis of phenylacetaldoxime (**11**)⁸²



An aqueous solution of $\text{Na}_2\text{CO}_3\cdot 10\text{H}_2\text{O}$ (2.99 g) was dissolved in the minimum amount of water (*ca.* 15 mL) and was added dropwise to a suspension of phenylacetaldehyde (2.44 g, 20 mmol, 1 equiv) and hydroxylamine hydrochloride (1.54 g, 22 mmol, 1.1 equiv) in a 1:1 mixture of $\text{H}_2\text{O}/\text{EtOH}$ (60 mL total). After stirring at room temperature for 3 hours, the mixture was extracted twice with EtOAc (40 mL), the combined organic layers were washed with H_2O (30 mL) and brine (30 mL), dried over anhydrous Na_2SO_4 , and the solvent was evaporated *in vacuo* to get phenylacetaldehyde oxime (2.20 g crude product). The crude product was purified by recrystallization with a 1:1 mixture of EtOAc and heptane: the solid was completely dissolved under heating at 85°C , then the flask was transferred to an ice bath to precipitate the product, which was then filtered on a Buchner funnel. The final product was obtained as a white solid (1.05 g recrystallized product, 48% yield).

$R_f = 0.46$ (8:2 pentane/EtOAc). $^1\text{H NMR}$ (300 MHz, CDCl_3) δ 7.36 – 7.21 (m, 6H), 3.79 (d, $J = 5.5$ Hz, 1H), 3.56 (d, $J = 6.3$ Hz, 1H). $^{13}\text{C NMR}$ (75 MHz, CDCl_3) δ 151.0, 150.8, 136.7, 136.2, 129.0, 128.9, 128.9, 128.9, 127.0, 126.8, 36.0, 31.9. **HR-MS** (APCI⁺) m/z : calcd. for $\text{C}_8\text{H}_{10}\text{NO}^+$ [$\text{M}+\text{H}$]⁺: 136.0757, found: 136.0757. **IR** (cm^{-1}) 3176.2, 3084.6, 2852.2, 1660.4, 1600.6, 1054.9, 747.3, 686.5. **m.p.** $89.8\text{--}93.5^\circ\text{C}$ ⁸³

Synthesis of *N*-hydroxy-2-phenylacetimidoyl chloride⁷¹ (**12**)

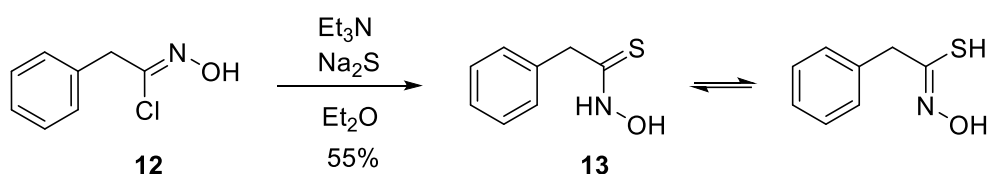


To hydroxylamine **11** (0.50 g, 3.73 mmol, 1.0 equiv) in DMF (4 mL) at 0°C was added NCS (0.53 g, 4.0 mmol, 1.1 equiv) in 5 portions over one hour. The flask was removed from the ice bath and the reaction mixture was stirred at room temperature for 2 hours, poured into iced water (15 mL), and extracted with EtOAc. The organic layer was separated, washed with water (20 mL), dried over MgSO_4 , and concentrated *in vacuo*. The residue was purified by flash

column chromatography (100% pentane to 80% pentane – 20% EtOAc) to obtain *N*-hydroxy-2-phenylacetimidoyl chloride **12** (0.365 g, 58%).

R_f (7:3 pentane/EtOAc) = 0.73. ¹H NMR (400 MHz, CDCl₃) δ 7.78 (d, *J* = 0.8 Hz, 1H), 7.41 – 7.22 (m, 5H), 3.81 (s, 2H). ¹³C NMR (101 MHz, CDCl₃) δ 141.1, 134.6, 129.2, 129.2, 129.1, 128.8, 128.8, 128.5, 127.8, 127.8, 127.6, 77.5, 77.2, 76.8, 43.0, 32.2, 28.5. **IR** (cm⁻¹) 3373.9, 3311.2, 3280.3, 3030.6, 2918.7, 1635.3, 1602.6, 1495.5, 1455.0, 1421.3, 1115.6, 987.3, 700.0. **m.p.** 84-85°C⁸⁴

Synthesis of *N*-hydroxy-2-phenylethanimidothioic acid⁷¹ (**13**)

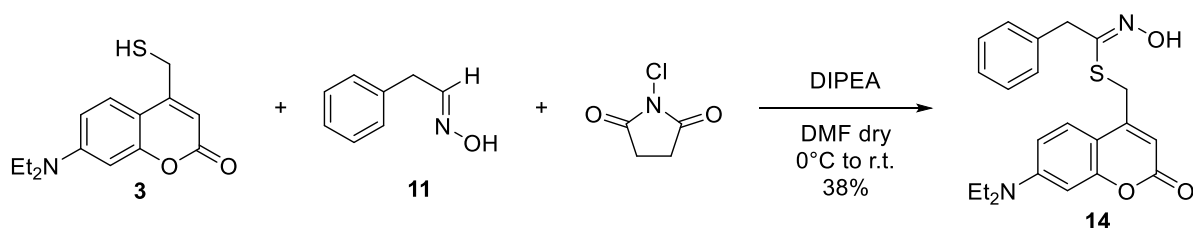


To a stirred solution of *N*-hydroxy-2-phenylacetimidoyl chloride **12** (0.35 g, 2.1 mmol, 1.0 equiv) in Et₂O (27 mL) was added an aqueous solution of Na₂S (0.50 g dissolved in 10 mL of water, 6.4 mmol) followed by Et₃N (0.3 mL, 2 mmol, 1 equiv). The reaction mixture was stirred for 2 hours and once completed poured into a separatory funnel. The Et₂O was separated from the aqueous, which was acidified to pH = 2 with 1 M HCl and extracted with dichloromethane (2 × 20 mL). The organic layers were dried over MgSO₄ and concentrated in vacuo to yield the product *N*-hydroxy-2-phenylethanimidothioic acid, which was used without further purification (190 mg crude, 55%).

R_f = 0.77 (1:1 EA/pentane). ¹H NMR (400 MHz, CDCl₃) δ 7.58 – 7.02 (m, 5H), 3.75 (s, 2H). ¹³C NMR (101 MHz, CDCl₃) δ 130.0, 129.2, 128.1, 128.0, 118.0, 77.5, 77.4, 77.2, 76.8, 45.3, 44.1, 43.4, 43.3, 40.1, 23.7. **IR** (cm⁻¹) 3031.6, 2250.5, 1496.5, 1455.0, 730.9, 694.3. **m.p.** 73-74.2°C⁸⁵

1.4 Synthesis of photo-pseudoGlucosinolates

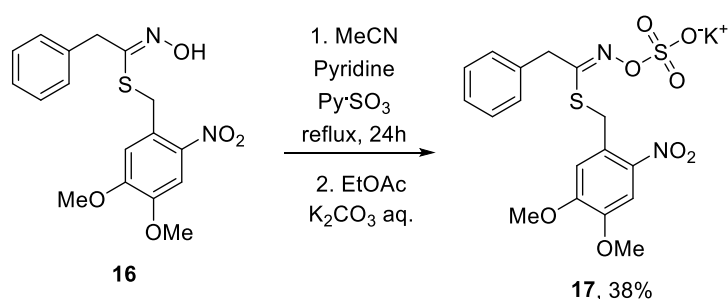
Synthesis of DEAC-thiohydroximate¹⁰ (**14**)



(diethylamino)-2-oxo-2*H*-chromen-4-yl)methyl)thio)-2-phenylethylidene)amino sulfate **15** (22 mg, 31%) as a dark yellow solid.

R_f = 0.15 (9:1 DCM/MeOH). **¹H NMR** (400 MHz, MeOD) δ 7.40 – 7.17 (m, 5H), 7.07 (d, *J* = 9.1 Hz, 1H), 6.58 (dd, *J* = 9.1, 2.6 Hz, 1H), 6.44 (d, *J* = 2.6 Hz, 1H), 5.89 (s, 1H), 4.13 – 4.05 (m, 2H), 3.97 (s, 2H), 3.45 (q, *J* = 7.1 Hz, 4H), 1.19 (t, *J* = 7.1 Hz, 6H). **¹³C NMR** (101 MHz, MeOD) δ 164.0, 160.1, 157.5, 152.9, 136.8, 132.4, 129.9, 129.9, 129.6, 128.4, 126.8, 69.1, 40.2, 39.9, 31.6, 31.0, 30.1, 24.9, 24.0, 14.4, 12.7, 11.4. **HR-MS** (ESI[−]) *m/z*: calcd. for C₂₂H₂₃N₂O₆S₂[−] [M][−]: 475.1007, found: 475.1003. **IR** (cm^{−1}) 3530.1, 3470.3, 3388.3, 2971.8, 2928.4, 1710.6, 1613.2, 1524.5, 1418.4, 1271.8, 1233.3. **m.p.** 85–86°C

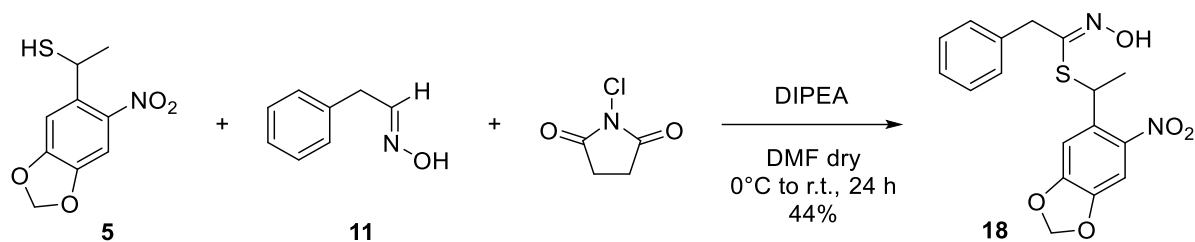
Synthesis of DMNB photo-pseudoGlucosinolate⁴⁸ (**17**)



Thiohydroximate **16** (48 mg, 0.132 mmol, 1.0 equiv) was treated successively with CH₃CN (0.8 mL), pyridine (10 μL, 0.12 mmol) and pyridine–sulfur trioxide complex (53 mg, 0.33 mmol, 2.5 equiv), and the mixture refluxed for 16 hours (81°C). The solution was cooled to ca. 60 °C, treated with EtOAc (0.5 mL), then a solution of K₂CO₃ (25 mg, 0.18 mmol) in H₂O (0.5 mL) was added with rapid stirring. Once effervescence had ceased (ca. 10 minutes), a thick precipitate was formed. The solvent was removed *in vacuo* and purified by flash column chromatography (DCM/MeOH, MeOH 2% → 20%) to give (1-((4,5-dimethoxy-2-nitrobenzyl)thio)-2-phenylethylidene)amino sulfate **17** (24 mg, 38%), as an orange solid.

R_f = 0.15 (9:1 DCM/MeOH). **¹H NMR** (400 MHz, CD₃CN) δ 7.55 (s, 1H), 7.31 – 7.17 (m, 5H), 6.81 (s, 1H), 4.34 (s, 2H), 3.84 (d, *J* = 6.8 Hz, 6H), 1.30 – 1.21 (m, 1H). **¹³C NMR** (101 MHz, CD₃CN) δ 157.9, 154.4, 149.3, 148.1, 142.6, 136.8, 129.7, 129.3, 128.5, 127.9, 127.3, 114.6, 109.6, 57.2, 56.9, 39.9, 33.0. **HR-MS** (ESI[−]) *m/z*: calcd. for C₁₇H₁₇N₂O₈S₂[−] [M][−]: 441.0430, found: 441.0432. **IR** (cm^{−1}) 3474.1, 3070.1, 2937.1, 1710.6, 1520.6, 1272.8, 1228.4, 1055.8, 794.5.

Synthesis of MeNPOM thiohydroximate¹⁰ (**18**)

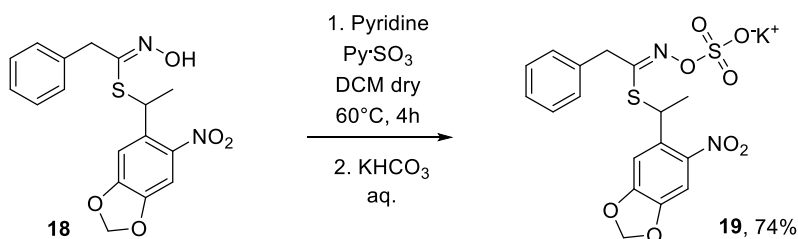


N-Chlorosuccinimide (123 mg, 0.92 mmol, 1.1 equiv) was added in 5 portions to a solution of oxime **11** (110 mg, 0.81 mmol, 1.0 equiv) in anhydrous DMF (0.6 mL) under argon atmosphere and at 0°C. The reaction mixture was warmed up to room temperature and stirred under light exclusion for 2 hours. A solution of thiol **5** (271 mg, 1.19 mmol, 1.5 equiv) and DIPEA (212 mg, 1.64 mmol, 2.0 equiv) in anhydrous DMF (2 mL) was added dropwise to the pre-cooled reaction mixture at 0°C and under argon atmosphere. The resulting solution was warmed to room temperature and stirring continued for 24 hours, before dilution with EtOAc (10 mL) and H₂O (10 mL).

The resulting mixture was then extracted with EtOAc (3 × 10 mL). The combined organic layers were washed with brine (3 × 10 mL), dried over anhydrous Na₂SO₄, filtered and the solvent was removed in vacuo. The resulting residue was purified by flash column chromatography (100 % DCM to 80% DCM – 20% MeOH) to afford 1-(6-nitrobenzo[*d*][1,3]dioxol-5-yl)ethyl-*N*-hydroxy-2-phenylethanimidothioate **15** (130 mg, 44%).

R_f = 0.27 (DCM). ¹H NMR (400 MHz, Acetone-*d*₆) δ 10.48 (s, 1H), 7.40 – 6.98 (m, 7H), 6.20 (p, *J* = 1.2 Hz, 2H), 5.28 (q, *J* = 7.0 Hz, 1H), 3.63 (d, *J* = 15.7 Hz, 1H), 3.47 (d, *J* = 15.7 Hz, 1H), 1.46 (d, *J* = 7.0 Hz, 3H). ¹³C NMR (101 MHz, Acetone-*d*₆) δ 152.8, 149.9, 147.9, 137.5, 136.5, 129.2, 129.1, 127.5, 108.7, 105.4, 104.4, 40.1, 38.9, 23.9. HR-MS (APCI⁺) *m/z*: calcd. for C₁₇H₁₇N₂O₅S⁺ [M+H]⁺: 361.0856, found: 361.0853. IR (cm⁻¹) 3261.0, 2913.0, 1617.0, 1519.6, 1483.0, 1333.5, 1257.4, 1034.6, 702.9. *m.p.* 154-157°C

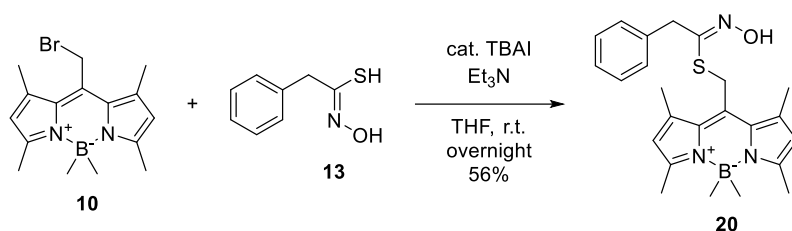
Synthesis of MeNPOM photo-pseudoGlucosinolate¹⁰ (**19**)



Sulfur trioxide pyridine complex (230 mg, 1.45 mmol, 5.3 equiv) and anhydrous pyridine (0.2 mL, 2.48 mmol) were added to a solution of thiohydroximate **18** (98 mg, 0.27 mmol, 1.0 equiv) in anhydrous DCM (3 mL) and the reaction mixture was stirred for 4 hours at 60°C. After cooling down the reaction mixture to 23°C, an aqueous solution of KHCO₃ (580 mg dissolved in 1 mL water, 21.5 equiv) was added and the mixture was stirred vigorously for an additional 30 minutes at 23°C. The solvents were removed under reduced pressure and the resulting residue was purified by flash column chromatography (100% DCM to 80% DCM – 20% MeOH) to afford potassium (1-((1-(6-nitrobenzo[d][1,3]dioxol-5-yl)ethyl)thio)-2-phenylethylidene)amino sulfate **19** (96 mg, 74%) as a dark yellow solid.

R_f = 0.24 (9:1 DCM/MeOH). **¹H NMR** (400 MHz, MeOD) δ 7.37 (s, 1H), 7.27 – 7.11 (m, 6H), 6.11 (s, 2H), 5.07 (q, J = 6.9 Hz, 1H), 3.83 (d, J = 16.1 Hz, 1H), 3.55 (d, J = 16.1 Hz, 1H), 1.43 (d, J = 7.0 Hz, 3H). **¹³C NMR** (101 MHz, MeOD) δ 159.9, 153.7, 148.7, 143.3, 136.5, 136.4, 129.7, 129.0, 128.0, 109.0, 106.1, 104.8, 40.1, 39.4, 24.0. **HR-MS** (ESI⁻) m/z : calcd. For C₁₇H₁₅N₂O₈S₂⁻ [M]⁻: 439.0273, found: 439.0275. **IR** (cm⁻¹) 3499.2, 3461.6, 2924.5, 1518.7, 1484.0, 1257.4, 1062.6, 1033.7, 810.0. **m.p.** 68-70°C

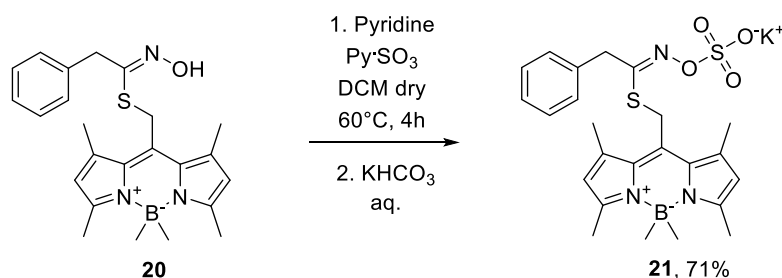
Synthesis of Me-BODIPY thiohydroximate^{77,86} (**20**)



Me-BODIPY-Br (163 mg, 0.49 mmol, 1.0 equiv), TBAI (20 mg, 0.05 mmol, 0.1 equiv), and Et₃N (70 mL, 0.5 mmol, 1.0 equiv) were added to a stirred solution of thiohydroximic acid **13** (82 mg, 0.5 mmol, 1.0 equiv) in anhydrous THF (5 mL). The reaction mixture was stirred overnight at room temperature. The reaction mixture was diluted with EtOAc (15 mL), washed with sat. aq. Na₂S₂O₃ (10 mL) and extracted with EtOAc (2 × 10 mL). The combined organic phase was washed with H₂O (15 mL), 1 M HCl (10 mL), and brine (15 mL), dried over Na₂SO₄, and concentrated under reduced pressure. The crude benzylated product was purified by silica gel column chromatography (100% pentane to 80% pentane – 20% EA) to afford (1,3,5,5,7,9-hexamethyl-5H-4λ⁴,5λ⁴-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-10-yl)methyl-N-hydroxy-2-phenylethanimidothioate **20** (116 mg, 56%).

R_f = 0.48 (DCM). **¹H NMR** (400 MHz, Acetone-*d*₆) δ 10.58 (s, 1H), 7.47 – 7.27 (m, 5H), 6.07 (d, *J* = 1.0 Hz, 2H), 4.27 (s, 2H), 3.99 (s, 2H), 2.40 (s, 6H), 2.20 (d, *J* = 0.9 Hz, 6H), 0.12 (d, *J* = 6.8 Hz, 6H). **¹³C NMR** (101 MHz, Acetone-*d*₆) δ 153.0, 151.8, 138.2, 137.5, 137.4, 130.9, 129.7, 129.6, 127.9, 123.2, 39.5, 28.1, 16.6, 15.7. **IR** (cm⁻¹) 3242.7, 2929.3, 1693.2, 1560.1, 1512.9, 1304.6, 1175.4, 1144.6. **m.p.** No melting, decomposition

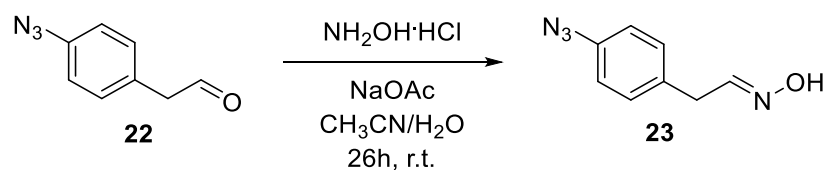
Synthesis of Me-BODIPY photo-pseudoGlucosinolate¹⁰ (21)



Sulfur trioxide pyridine complex (202 mg, 1.27 mmol, 6.3 equiv) and anhydrous pyridine (0.2 mL, 2.48 mmol) were added to a solution of thiohydroximate **20** (82 mg, 0.20 mmol, 1.0 equiv) in anhydrous DCM (3 mL) and the reaction mixture was stirred for 4 hours at 60°C. After cooling down the reaction mixture to 23°C, an aqueous solution of KHCO₃ (0.48 g dissolved in 1 mL water, 23.8 equiv) was added and the mixture was stirred vigorously for an additional 30 minutes at 23°C. The solvents were removed under reduced pressure and the resulting residue was purified by flash column chromatography (100% DCM to 70% DCM – 30% MeOH) to afford potassium (1-(((1,3,5,5,7,9-hexamethyl-5*H*-4λ⁴,5λ⁴-dipyrrolo[1,2-*c*:2',1'-f][1,3,2]diazaborinin-10-yl)methyl)thio)-2-phenylethylidene)amino sulfate **26** (74 mg, 70%) as a dark red solid.

R_f = 0.17 (9:1 DCM/MeOH). **¹H NMR** (400 MHz, MeOD) δ 7.49 – 7.27 (m, 5H), 6.01 (d, *J* = 1.0 Hz, 2H), 4.27 (s, 2H), 4.04 (s, 2H), 2.38 (s, 6H), 2.12 (d, *J* = 0.8 Hz, 6H), 0.10 (d, *J* = 11.8 Hz, 6H). **¹³C NMR** (101 MHz, MeOD) δ 161.1, 153.6, 138.5, 136.4, 136.3, 131.3, 130.1, 130.0, 128.6, 39.9, 29.2, 16.6, 15.8. **IR** (cm⁻¹) 3470.3, 2934.2, 1559.2, 1505.2, 1302.7, 1172.5, 1029.8. **m.p.** No melting, decomposition

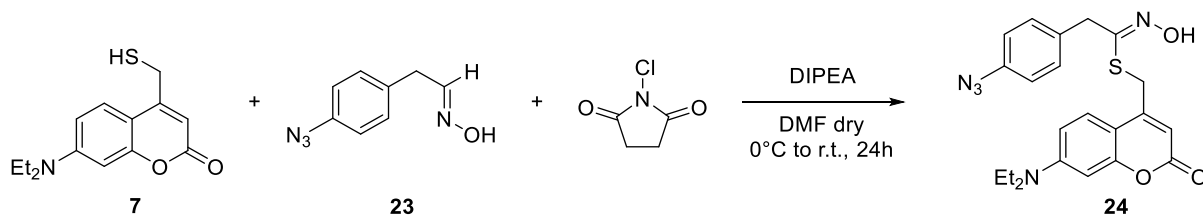
Synthesis of 2-(4-azidophenyl)-acetaldehyde oxime¹⁰ (23)



A solution of 2-(4-azidophenyl)acetaldehyde **22** (150 mg, 0.93 mmol, 1.0 equiv) in CH₃CN (2.3 mL) was added to hydroxylammonium chloride (78 mg, 1.12 mmol, 1.2 equiv) and NaOAc (76 mg, 0.93 mmol, 1.0 equiv) in water (1 mL) at room temperature and the mixture was stirred for 26 hours. Afterwards, the mixture was extracted with DCM (3 × 40 mL). The combined organic phases were dried over Na₂SO₄, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography (100% pentane to 65% pentane – 35% EtOAc) yielding 2-(4-azidophenyl)-acetaldehyde oxime **23** as an off-white crystalline solid (95 mg, 58%).

R_f = 0.32 (1:5 EtOAc /pentane). **¹H NMR** (400 MHz, CDCl₃) δ 8.66 (s, 1H), 7.25 – 7.18 (m, 2H), 7.03 – 6.94 (m, 2H), 6.87 (t, *J* = 5.4 Hz, 1H), 3.72 (d, *J* = 5.3 Hz, 2H). **¹³C NMR** (101 MHz, CDCl₃) δ 150.6, 150.5, 138.9, 138.7, 133.4, 132.9, 132.1, 131.0, 130.4, 130.3, 129.8, 119.5, 119.5, 119.2, 119.1, 77.5, 77.2, 76.8, 35.4, 31.1. **HR-MS** (ESI⁺) *m/z*: calcd. For C₈H₉N₄O⁺ [M+H]⁺: 177.0771, found: 177.0769. **IR** (cm⁻¹) 3204.2, 2862.8, 2416.4, 1505.2, 1421.3, 907.3, 823.5, 729.9. **m.p.** 90-92°C

Synthesis of DEAC-N₃ thiohydroximate¹⁰ (**24**)



N-Chlorosuccinimide (67 mg, 0.50 mmol, 1.1 equiv) was added in 5 portions to a solution of oxime **23** (68 mg, 0.81 mmol, 1.0 equiv) in anhydrous DMF (2 mL) under argon atmosphere and at 0°C. The reaction mixture was warmed up to room temperature and stirred under light exclusion for 2 hours. A solution of DEAC-SH **7** (169 mg, 0.64 mmol, 1.5 equiv) and DIPEA (115 mg, 0.89 mmol, 2.0 equiv) in anhydrous DMF (2 mL) was added dropwise to the pre-cooled reaction mixture at 0°C and under argon atmosphere. The resulting solution was warmed to room temperature and stirring continued for 24 hours, before dilution with EtOAc (10 mL) and H₂O (10 mL). The resulting mixture was then extracted with EtOAc (3 × 10 mL). The combined organic layers were washed with brine (3 × 10 mL), dried over anhydrous Na₂SO₄, filtered and the solvent was removed in vacuo. The resulting residue was purified by flash column chromatography (100 % pentane to 40% pentane – 60% EtOAc) to afford (7-(diethylamino)-2-oxo-2*H*-chromen-4-yl)methyl-2-(4-azidophenyl)-*N*-hydroxyethanimidothioate **24** (89 mg, 32%).

R_f = 0.32 (6:4 pentane/EtOAc). **HR-MS** (ESI⁺) m/z: calcd. for C₂₂H₂₃N₅O₃SNa⁺ [M+Na]⁺: 460.1413, found: 460.1414

The ¹H- and ¹³C- NMR spectra after purification were not satisfactory. IR spectroscopy as well as determination of melting point could not be performed, for the entire sample had been used in the sulfonation step. Given the time constraints, a mass spectrometric analysis was submitted, which confirmed the molecular mass of the expected product. Therefore, the subsequent sulfonation step was attempted, however it failed.

List of abbreviations

BODIPY	boron-dipyrromethene
DEAC	diethylaminocoumarin
DIPEA	<i>N,N</i> -diisopropylethylamine
DCC	<i>N,N'</i> -dicyclohexylcarbodiimide
DCM	dichloromethane
DMC	dimethylcarbonate
DMF	dimethylformamide
DMSO	dimethylsulfoxide
DMNB	dimethoxynitrobenzyl
EA/EtOAc	ethyl acetate
ESI	electron spray ionisation
GSL	glucosinolate
HEPES	2-[4-(2-hydroxyethyl)piperazin-1-yl]ethane-1-sulfonic acid
HRMS	high resolution mass spectrometry
ITC	isothiocyanate
LC-MS	liquid chromatography – mass spectrometry
LED	light emitting diode
MALDI-TOF	matrix assisted laser desorption/ionization – time of flight
MeNPOM	α -methyl-nitropiperonyloxymethyl
MeCN	acetonitrile
MES	2-(<i>N</i> -morpholino)ethanesulfonic acid
<i>m/z</i>	mass-to-charge ratio
NMR	nuclear magnetic resonance
PPG	photoremovable protecting group
ppm	parts per million
SET	single electron transfer
TBAI	tetra- <i>n</i> -butylammonium iodide
TBHP	<i>tert</i> -butyl hydroperoxide
TEMPO	(2,2,6,6-tetramethylpiperidin-1-yl)oxyl
THF	tetrahydrofuran
TLC	thin layer chromatography
UV/Vis	ultraviolet and visible light

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