A modular approach to the synthesis of stretched luciferin analogues for use in near infrared optical imaging

A thesis by

Steven James Pacman

Submitted in partial fulfilment for the degree of Doctor of Philosophy

Department of Chemistry

University College London

I, Steven James Pacman confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Signed.....
Date.....

Abstract

The introduction to this thesis covers the original work by McElroy, which established the requirements for light emission from firefly luciferin, and then the eventual elucidation of it chemical structure and synthesis.

The mechanism of light emission is then discussed and a brief overview of some of the uses of bioluminescence in biology is discussed. The limitations of firefly luciferin are described along with some of the methods and attempts to address the limitations

The results and discussion section describes the various attempts and progress towards a new alkyne bridged luciferin analogue. The various attempts to produce a new synthetic route to infraluciferin are described along with the successful route. The synthesis of a new analogue using the new synthetic route to infraluciferin is then presented.

The future work and conclusions section give a summary of the work left that still warrants more attention and the successful results that were attained.

The experimental section provides procedures and data for all the relevant compounds from the research carried out.

Impact Statement

Bioluminescence imaging (BLI) is a powerful method to visualise molecular and cellular features, *in vitro* and *in vivo* noninvasively.

The enzyme luciferase produced by the firefly is able to emit light chemically from its small molecule substrate D-luciferin. Introduction of these two components into cells or whole animals produces light that can be captured by sensitive detectors. The simplicity of this imaging method has led to it becoming one of the most popular methods for *in vivo* monitoring of numerous diseases and cellular functions.

The technique has been held back because the light emitted by D-luciferin is at λ_{max} 557 nm, which is absorbed and scattered by tissue and haemoglobin, thus limiting its use in mammalian tissue.

Previous work within the research group has led to an analogue of D-luciferin called infraluciferin that emits light at λ_{max} 706 nm, which is able to penetrate tissue and haemoglobin. The original synthesis however was limited to small quantities of material, which prohibited large scale in vivo studies. The synthesis did not allow for other analogues to be produced from the same method.

A new synthetic route to infraluciferin was developed which can produce multi gram quantities, and enough material was produced to conduct a

large scale multiparametric in vivo mouse study. The new synthetic route was also used to produce a new infraluciferin analogue.

Acknowledgments

Firstly I would like to thank Prof. Jim Anderson for giving me the opportunity to work in his research group, and for the help and guidance throughout the course of the PhD. I would also like to thank Dr Helen Grounds for all her help and patience.

I would also like to thank all of the Anderson group in particular Dr Chris Rundell for wealth of chemical knowledge and amusing bluntness! Also Oli and James who were always there when a few drinks were called for.

I would like to thank all the staff of the CIB building at UCL, especially Kersti from mass spec, Abil from NMR and Tony from stores and all the guys in the teaching lab.

I would like to say thank you to Laurence Tisi and the EPSRC for the financial support, which made the project possible.

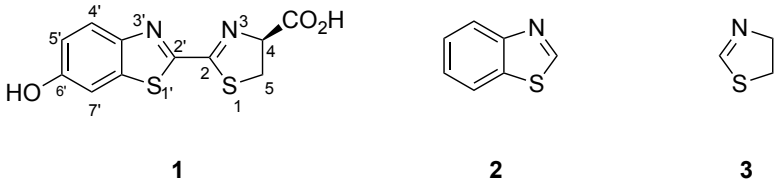
Finally I like to thank my friends and family, in particular my wife Bev for always sticking by me and keeping me going. My mum for always believing in me, and lastly my son Obi for always making me laugh.

Table of Contents

Introduction

1.0 The structure of luciferin

The structure of luciferin **1** can be described as a benzothiazole moiety **2** that is directly attached to a thiazoline moiety **3** (figure 1). Both structures are linked via the carbon at the C2 positions, with the benzothiazole having an alcohol at the C6' position and the thiazoline having a carboxylic acid at the C5 position

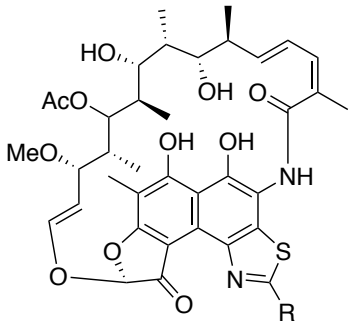


1 **2** **3**

Figure 1: Luciferin moieties

1.1 Benzothiazoles in nature

The benzothiazole moiety **2** is relatively rare in natural products, but has been observed ranging in complexity from benzothiazole itself (first isolated in 1967 from the volatiles of American cranberries *Vaccinium macrocarpon* Ait. *var*. Early Black), to more complex molecules such as the thiazo-rifamycins **4** (figure 2). [1]



4

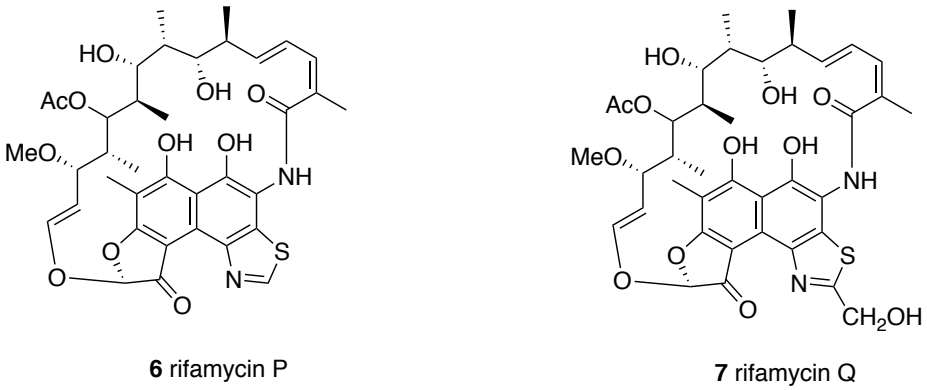
Figure 2: thiazo-rifamycin structure

Since the initial isolation, benzothiazole has been isolated from a number of other sources including: - the tail gland of the red deer *Cervus elaphus*, sulfur volatiles in wines, volatile fraction of French oak wood used in the aging of wine, and was also seen in the aroma fraction of tea leaves, as well as in the flavor compound produced by the fungi *Aspergillus clavatus*. [2–4]

1.2 Rifamycins

The rifamycins are a group of naturally occurring antibiotics that are synthesized by the bacterium *Nocardia mediterranei*. They are a subclass of a larger family known as the ansamycins. Rifamycins are particularly effective against mycobacteria, and are therefore used to treat diseases such as tuberculosis and leprosy. Rifamycins were first isolated in 1957 from a fermentation culture of the bacterium *Nocardia mediterranei* at the Lepetit laboratory in Milan,[5] seven rifamycins were discovered, including the parent molecule rifamycin S **5**.[6] Cricchio and coworkers reported the

isolation of two benzothiazole containing rifamycins from mutant strains of *Nocardia mediterranei*.[7] These rifamycins are known as thiazo-rifamycins, namely rifamycin P **6** and rifamycin Q **7** (figure 3).



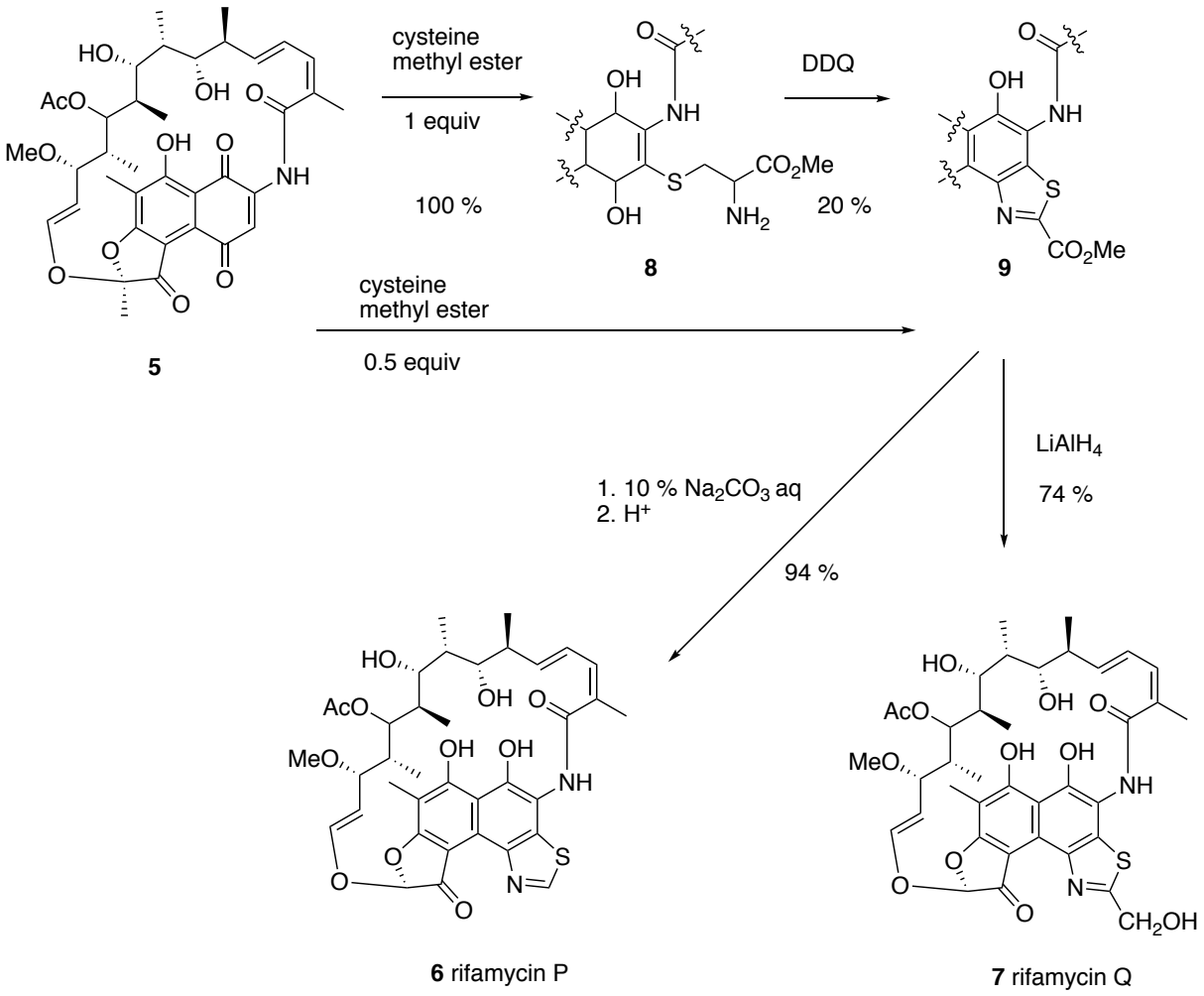
6 rifamycin P

7 rifamycin Q

Figure 3: rifamycin P and Q

As part of the same research Cricchio and coworkers described the synthesis of rifamycin P and rifamycin Q from rifamycin S (Scheme 1). By reacting rifamycin S with cysteine methyl ester hydrochloride, intermediate **8** was obtained quantitatively, and then oxidized using 2,3-dichloro-5,6-dicyano-1,4- benzoquinone (DDQ) to give the benzothiazole motif **9** in 20% yield. The same intermediate **9** could be obtained directly by reacting rifamycin S with only 0.5 equivalent of cysteine methyl ester hydrochloride, using the excess of the rifamycin S quinone as an oxidant. The common intermediate, methyl benzothiazole-2-carboxylate **9**, gave benzothiazole (rifamycin P) **6** by alkaline hydrolysis and decarboxylation,

or rifamycin Q **7** by reduction using lithium aluminium hydride.

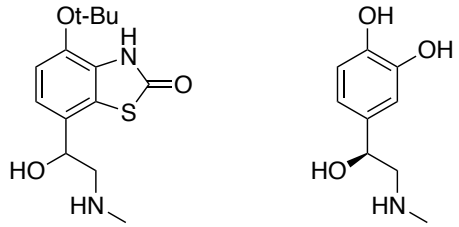


Scheme 1: synthesis of rifamycin P and Q

1.3 Benzothiazoles from Marine Sources

A novel β2- adrenoceptor-selective agonist was isolated in 1999 by a Japanese group from the marine sponge *Dysidea* sp., the benzothiazole S1319 **10** (figure 4).[8] β2-Adrenoceptor agonists are used as anti-asthmatic

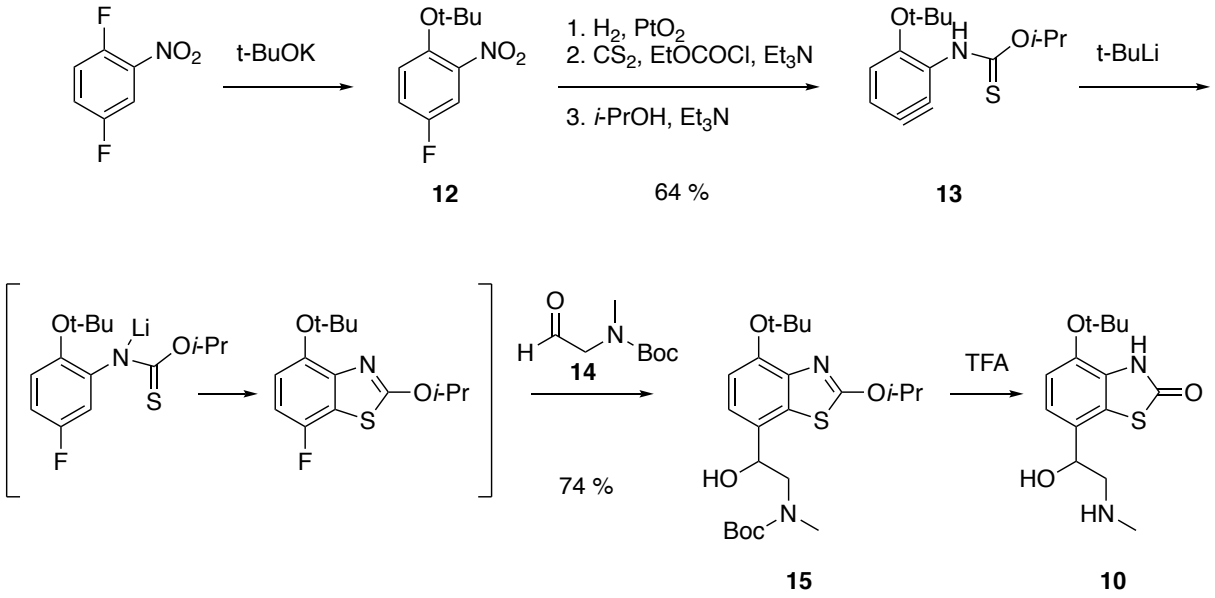
drugs, and S1319 **10** was the first example of a sponge-derived bronchodilator. Structurally, the molecule is closely related to the endogenous ligand adrenaline **11** (figure 4)**.**



10 **11**

Figure 4: S1319 and adrenaline

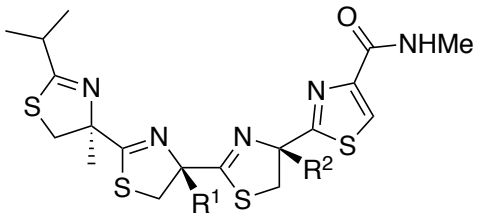
The syntheses of this naturally occurring bronchodilator has been reported,[9,10] that involved a directed lithiation/benzyne-mediated cyclization reaction (scheme 2). Aryl ether **12** was prepared efficiently from the commercially available 2,5- fluoronitrobenzene by displacement of the more activated fluoride via an S_NAr reaction with potassium *tert*-butoxide. Reduction of the nitro group to the aniline, followed by conversion to the isothiocyanate and addition of 2-propanol allowed the formation of thiocarbamate cyclization precursor **13** in 64% over three steps. Exposure of **13** to directed-lithiation/benzyne-mediated cyclization conditions, followed by quenching of the anion by addition of the readily prepared Boc-protected sarcosine-derived aldehyde **14** gave the expected benzylic alcohol **15**. The final step to produce S1319 **10** was achieved by global deprotection using trifluoroacetic acid.



Scheme 2: Synthesis of S1319

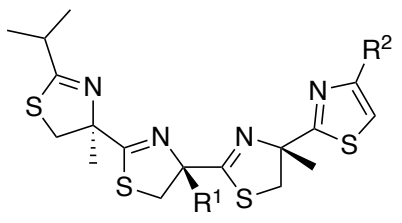
1.4 Thiazolines in nature

Biologically active Thiazonlines found in natural products are often multiple and directly linked, such as tantazoles **16**, mirabazoles **17**, and thiangazole **18** (figure 5).



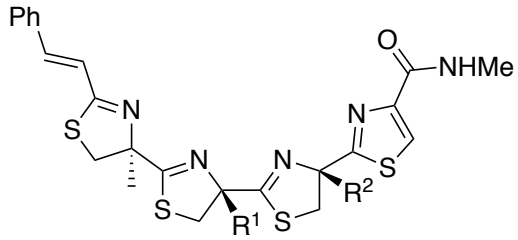
16

(-)-Tantazole A, R^1= H, R^2 = Me
(-)-Tantazole B, R^1= Me, R^2 = Me
(-)-Tantazole C, R^1= Me, R^2 = H



17

(-)-Mirabazole A, R^1= H, R^2 = Me
(-)-Mirabazole B, R^1= Me, R^2 = Me
(-)-Mirabazole C, R^1= Me, R^2 = H



18

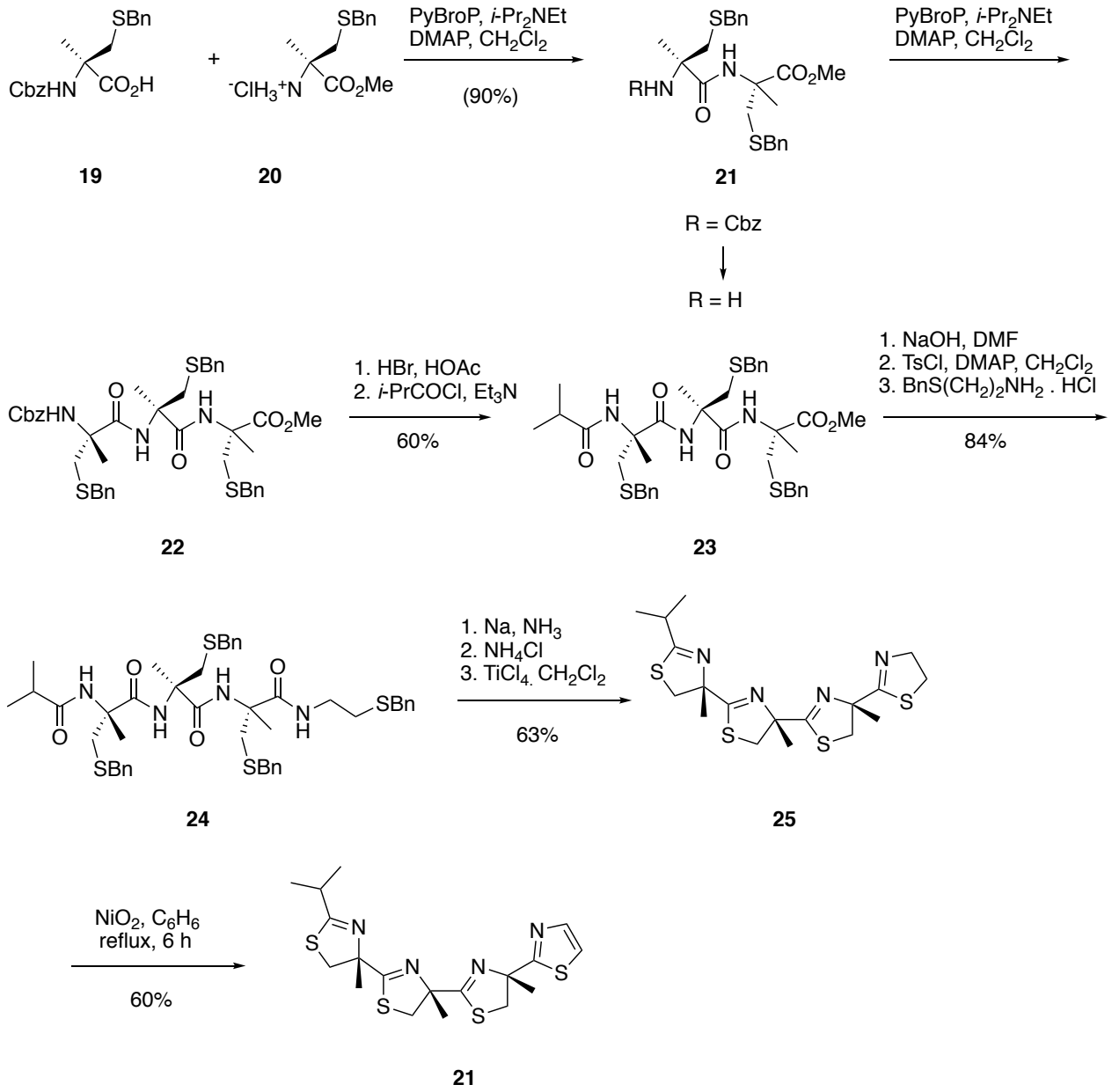
(-)-Thiangazole A,

Figure 5: tantazoles, mirabazoles, and thiangazole structures

Mirabazoles and tantazoles were isolated from blue- green algae *Scytonemea mirabile* and are studied for their cytotoxic and anticancer properties.[11] Thiangazole was isolated from a metabolyte of *Polyangium spec.* strain P13007 and studied for its anthelmintic and antiviral properties.[12,13] Due to their unusual structure and their biological properties, these compounds have aroused great interest for organic chemists, and many have been target for total synthesis. More than one thiazoline ring (2, 3, or 4) is contained in these compounds, and in most cases the starting sulfur-containing precursor used for thiazoline ring formation is the 2-methylcysteine **19** and **20**, which is available from the chiral pool as the

(*R*) or (*S*) enantiomer with a free or protected thiol function.

S-Benzyl-protected amino thiols were used by Heathcock for preparation of thiazolines in the total synthesis of (-)- Mirabazole C **21** (scheme 3) [14]



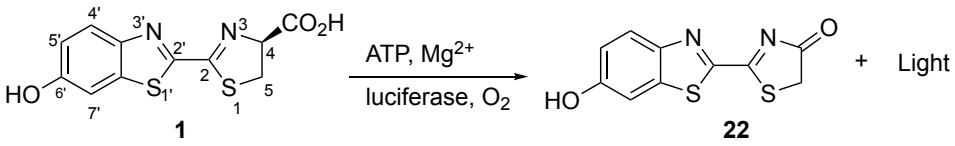
Scheme 3: Mirabazole C synthesis

(R)-N-(Carbobenzyloxy)-S-benzyl-2-methylcysteine **19** and the hydrochloride salt of the corresponding methyl ester **20** were coupled using bromotris(pyrroli- dino)phosphonium hexafluorophosphate (PyBroP)6 as the condensation reagent to obtain the dipeptide **21**. The carbobenzyloxy group was removed by treatment with HBr in acetic acid, and the resulting amine was coupled with **19** to afford tripeptide **22**. Once again the carbobenzyloxy group was removed and the resulting amine was acylated with isobutyryl chloride to obtain **23**. Saponification of the methyl ester gave the free acid, which was treated successively with p-toluenesulfonyl chloride and S-(benzylamino)ethanethiol to obtain **24**. The benzyl groups were removed by treatment owith sodium in ammonia and the resulting tetrathiol treated with titanium tetrachloride in DCM to obtain dihydromirabazole. The terminal thiazoline ring was oxidized by nickel peroxide 7 to (-)-mirabazole C **21**.

1.5 Discovery and light emission requirements

The compound that is responsible for the light emitted from the firefly of the Lampyridae family is known as D-luciferin **1.** Its bioluminescent properties (emitting light at λ_{max} 558 nm) have been widely used in small animal studies for the visualisation of various cellular functions.

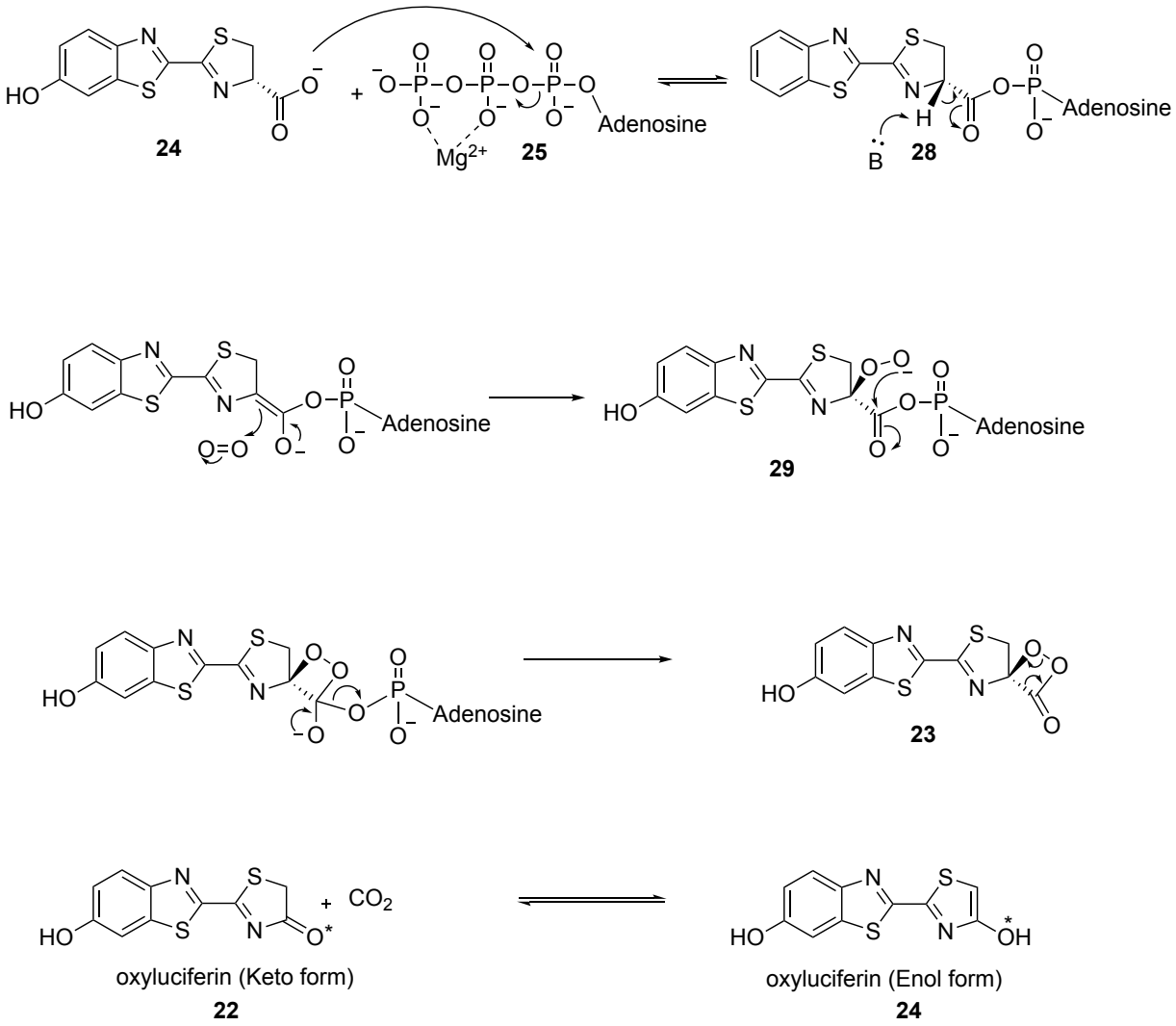
McElroy was the first to report that extracts from the firefly were capable of emitting light for short periods in the yellow/green spectrum, and to show that addition of adenosine triphosphate (ATP) could prolong the light emission. He hypothesised that the energy required for light emission came from the phosphate bond and building on these early studies he continued to identify the role of ATP, Mg^{2+}, O_2 and the enzyme luciferase as being essential for light emission in the firefly (Scheme 4). [15]



Scheme 4: McElroy's proposed light emission

1.6 Mechanism of bioluminescence from D-luciferin

A detailed mechanistic study of the process of light emission involving D-luciferin proposed that a dioxetanone intermediate **23** is a key step in the process (scheme 5).[16]



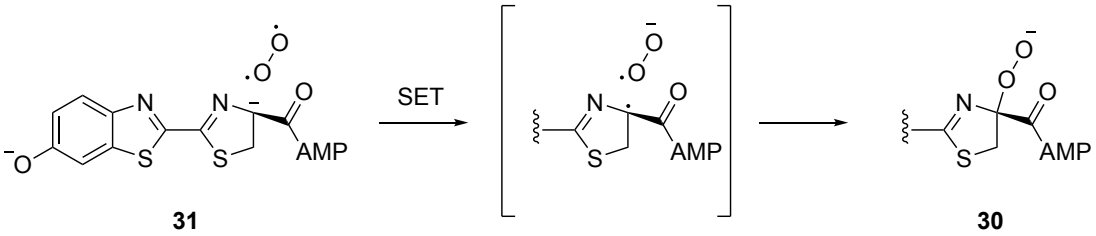
Scheme 5: Bioluminescent mechanism of D-luciferin [16]

Once bound in the luciferase active site the anion of luciferin 2**4** takes part in nucleophilic attack on the α-phosphoryl group of the carboxylate ATP-Mg^{2+} complex **25**, to form the adenylated luciferin **28**. Proton abstraction from the adenylated luciferin allows the molecule to interact with molecular oxygen to form the hydroperoxide anion **29**, which is able to cyclise and form dioxetanone **23**, as AMP is a good leaving group. The 4-

membered ring formed is unstable and the O-O bond is weak which results in the collapse of the ring with release of CO_2 and the formation of oxyluciferin **22** in an exited state. It is the relaxation of this molecule to the ground state which releases a photon of light. Whether the light is emitted from the keto **22** or enol form **24**, or whether each is responsible for different wavelengths are the subject of various studies, which are ongoing.

1.7 SET oxidation mechanism

Branchini *et al.* have proposed a single electron transfer mechanism, which they have supported with EPR experiments confirming the presence of the superoxide anion seen in **30 (**scheme 6**).**



31 **30**

Scheme 6: SET oxidation Mechanism

This mechanism which still leads to the dioxetanone **23** from **30** is more plausible given that molecular oxygen exists as triplet oxygen shown in **31**, and attack by a nucleophile (scheme 5) leads to a spin forbidden process.[17]

1.8 Bioluminescence in biology

The light emitted from various bioluminescent sources has been utilised to visualise a multitude of cellular functions, which would either not be possible or would be highly invasive in vivo. The luciferin luciferase system has for example been used in reporter gene assays to study G-protein coupled receptors. By placing a luciferase gene under the control of the desired promoter sequence; it is possible to monitor the gene expression controlled by that promoter by luciferase expression and subsequent light emission with D-luciferin.[18] Apoptosis and proteosome activity have been studied by generating a pro-luciferin analogue which emits lights that is dependent upon the enzymatic activity which occurs via the specific proteases under investigation.[19 20]

Fluorescence has been used as an optical reporter by genetically encoding florescent proteins in a similar fusion to that used for the luciferase enzyme. Modified florescent dyes can also be constructed in such a way that they are able to be cleaved by specific enzymes under investigation.[21] Bioluminescence however has some advantages over fluorescence; most importantly an external photon is required to excite the florescent molecule for light emission to occur as opposed to the biologically catalysed reaction for the luciferin/luciferase system. It is this external process that limits the

fluorescence reporter method, as haemoglobin can absorb visible light.[22] Another disadvantage is that as well exciting the fluorophore any other chromophores could be excited which results in high background signal to noise ratio. The external light source can also damage these molecules causing damage to cells or even cell death. Auto-luminescence and photo toxicity are not an issue with bioluminescence as well as having a high signal to noise ratio.

The most commonly used bioluminescent system is D-luciferin and beetle luciferase, but coelenterazine with renilla reniformis luciferases have been used in the past. Coelenterazine however has some disadvantages to D-luciferin; it is a much larger molecule and less water soluble. It is also more toxic than D-luciferin and this limits its use in vivo. Because coelenterazine does not require activation via adenylation there is a tendency for auto activation and therefore higher background luminescence.[23] For these reasons the D-luciferin with firefly luciferase is the most used system as a bioluminescent reporter in biological sciences.

1.9 Limitations of firefly luciferin

The Luciferase/D-luciferin system for imaging of biological events is not with out its limitations. Although relatively inexpensive and efficient it is only suitable for small animals as the natural firefly bioluminescence

system produces only yellow-green light (λ_{max} = 560 nm). Cellular components and other endogenous molecules such as hemoglobin, absorb visible light.[24] [22] Therefore, a large portion of the light emitted by luciferase is absorbed by the surrounding tissue and will not penetrate through the animal for detection.

The tissue attenuation of the light generated by the reaction of luciferin with luciferase can be overcome by a red and far-red emission that greatly improves the detection in small animal imaging.[10] Near- infrared (near-IR) light (650-900 nm) is less well absorbed by these endogenous molecules and is able to further penetrate through tissue. Modifying either the luciferase enzyme or the natural substrate D-luciferin can lead to a red-shifted emission.

Diverse factors can impact the wavelength of light emitted with D-luciferin. For example, the presence of divalent cations, such as Cd^{2+} and Zn^{2+}, can red-shift the peak wavelength of light emitted with firefly luciferase. Additionally, lowering the pH of the solution to ~6, or increasing the temperature of the solution from ambient to 37 °C also red shift the emission profile. Finally, the origin of the luciferase can impact the wavelength of light emitted. The railroad worm luciferase from the lateral lanterns of Pyrearinus termifiuminans catalyzes emission of green light (542 nm), while the luciferase from the head lanterns of results in red

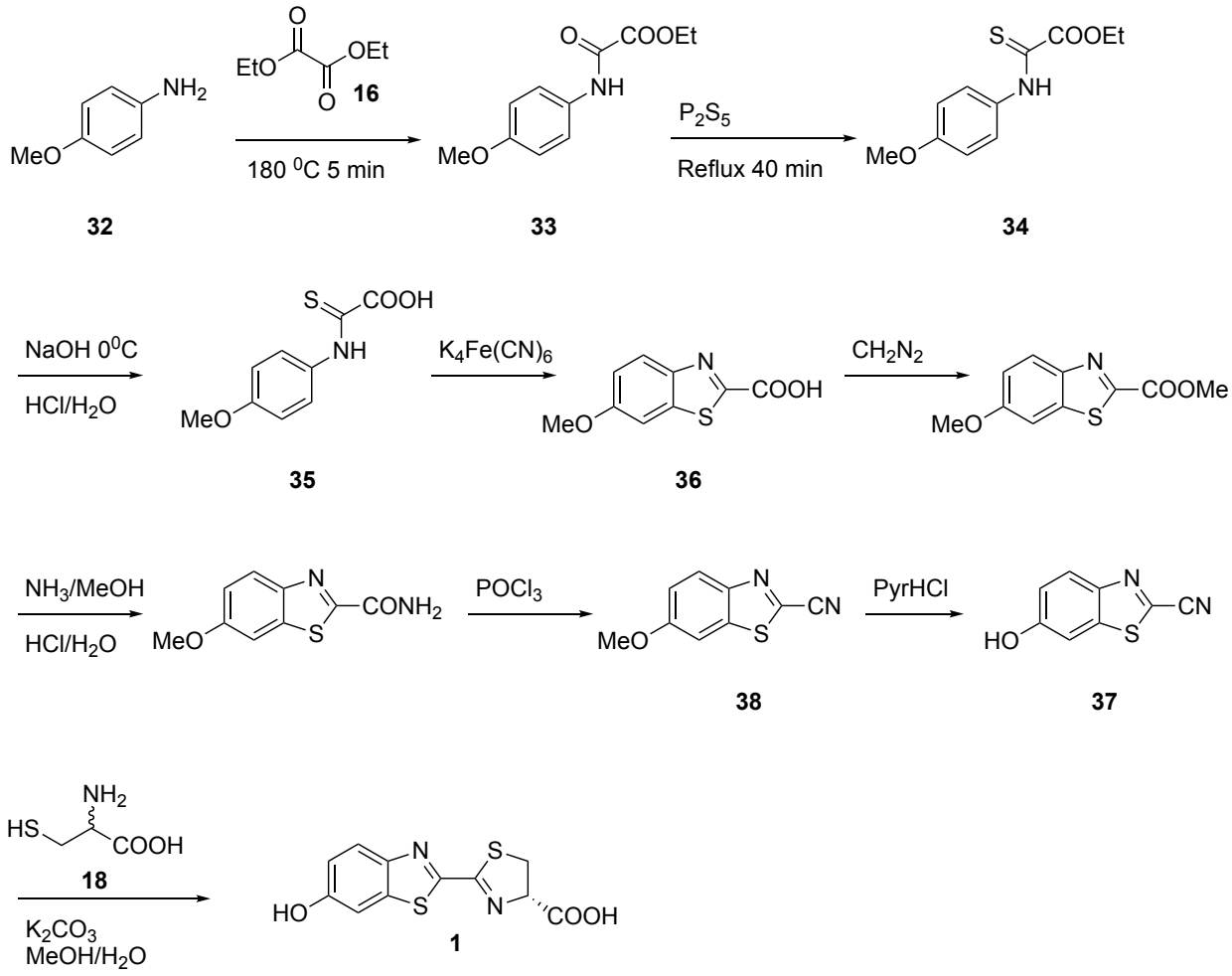
light emission (628 nm).[25] In a laboratory situation the environmental parameters can be readily manipulated. Living organisms however hold the concentration of divalent cations, pH, and temperature to their optimum levels. Therefore, the luciferase and luciferin are the preferred targets for altering the emission profile in vivo.

1.10 Synthesis of Luciferin

The empirical formula of luciferin was deduced in 1957 by elemental analysis that was conducted on luciferin isolated by Bitler and McElroy from 15,000 fireflies, which yielded 9 mg of crystalline D-luciferin.[26] The first synthesis was reported in 1961 by Seliger *et al* confirmed that the molecule contained both a benzothiazole and thiazoline ring. With the use of L or D-cysteine they produced both enantiomers and showed that the natural L isomer did not emit light when subjected to the protocols developed previously of adding luciferase, ATP, Mg^{2+} in the presence of oxygen to the substrate. In fact they found that L-luciferin inhibits the emission of light. The luciferase enzyme is unable to distinguish between the two enantiomers and adenylates both molecules equally but the L enantiomer is not oxidised and remains bound in the active site. [27] [28]

Luciferin (D/L-2-(6-hydroxy-2-benzothiazol-yl-)-Δ-thiazoline-4-carboxylic acid) was first synthesised by White et al. In this procedure, *p*-anisidine **32**

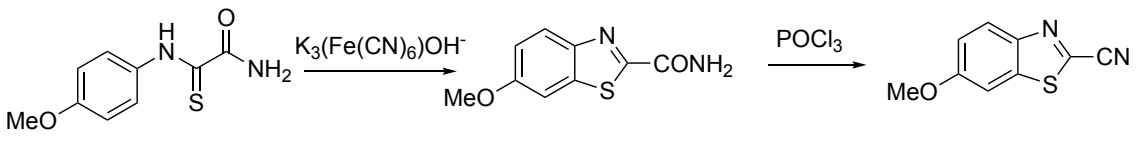
is the starting material that, through intermediates **33** and **34**, is transformed into the thioacid **35**, in turn cyclized to 6- methoxybenzothiazole-2-carboxylic acid **36.** From this benzothiazole derivative, 2-cyano-6-hydroxybenzothiazole **37** is prepared in four steps. Compound **37** is the key intermediate for the synthesis of **1** that can be obtained almost quantitatively by reaction with D-cysteine (Scheme X). [28,29]



Scheme 7: First synthesis of luciferin

The overall yield of D-luciferin **1** from *p*-anisidine **32** is 9% through nine steps. The synthetic approach described by White *et al*. is still more or less used for the preparation of **1** and related compounds. As previously stated, 2-cyano-6- hydroxybenzothiazole **37** is the key intermediate for the synthesis of **1** and the most reliable procedure to obtain this compound is the demethylation of 2-cyano-6-methoxy derivative **38.**

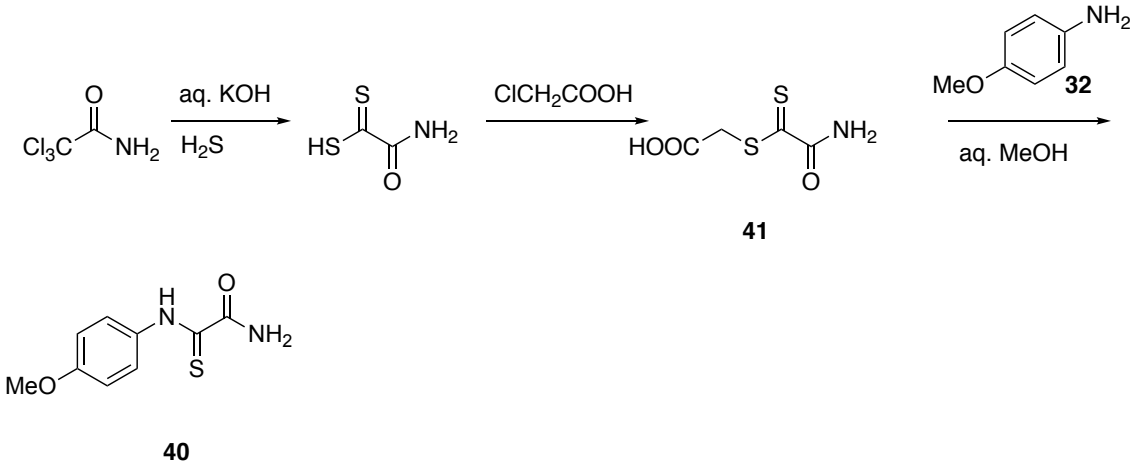
This reaction can be most efficiently accomplished by fusion at 220 °C with pyridinium hydrochloride (Py.HCl). In these conditions, the labile 2-nitrile moiety is kept intact and alternative procedures lead to hydrolysis of the cyano group. Other procedures are described for the synthesis of 2-cyano-6-methoxybenzotiazole **38**. According to Seto *et al.*[30] 6-methoxybenzothiazole-2-carboxyamide **39** is prepared from the 4-methoxythioxanilinamide **40** by oxidative cyclization with alkaline $K_3[Fe(CN)_6]$. The transformation of compound **10** into the nitrile **38** has been carried out essentially as reported by White *et al*.[31] (scheme 8).



40 **39** **38**

Scheme 8: Synthesis of nitrile 38

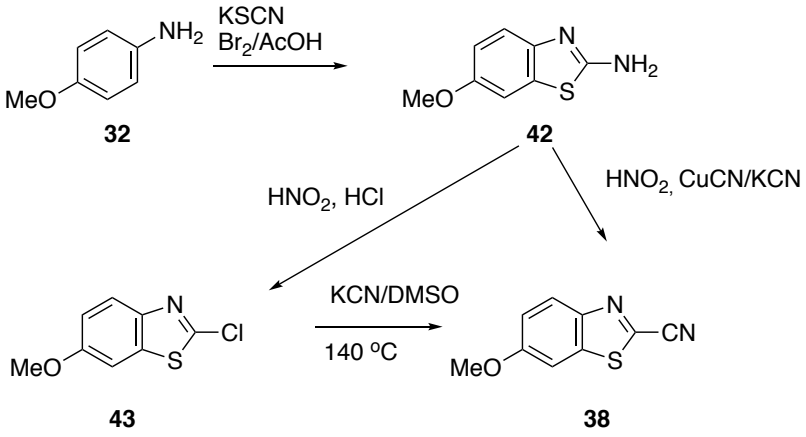
This experimental protocol has been applied to the preparation of 5-10 g of D-luciferin **1**.[32] The preparation of 4-methoxythioxanilinamide **40**, according to Seto *et al* [30] can be carried out in good yields from *p*-anisidine **32** and carbamoylthiocarbonylthioacetic acid **41**. However, this compound is unstable and has to be prepared *in situ*, as described in detail by Bowie.[32] The experimental procedure allows **40** to be prepared from *p*-anisidine 32 with an overall 39% yield (scheme 9)**.**



Scheme 9: Synthesis of 4-methoxythioxanilinamide 40

According to another synthetic approach, 2-amino-6-methoxybenzothiazole **42** can be prepared from *p*-anisidine **32**, [33] and different routes can lead to 2-cyano-6-methoxybenzotiazole **38** using a classical Sandmeyer reaction. In a first synthesis,[33,34] 2-chloro-6- methoxybenzothiazole **43** was prepared

by reaction of compound **42** with nitrous acid and HCl. Reaction of compound **43** with KCN in DMSO afforded the nitrile **38** (scheme 10).



Scheme 10: improved synthesis of 38

Conditions of formation of 2-chloro derivative **43** were improved using isoamyl nitrite and copper (II) chloride in polyethylene glycol 200 as solvent and yields were improved to 56%.[35] More recently, the Sandmeyer reaction was carried out by direct introduction of cyanide with CuCN/KCN and following this approach a 41% yield was obtained. [35]

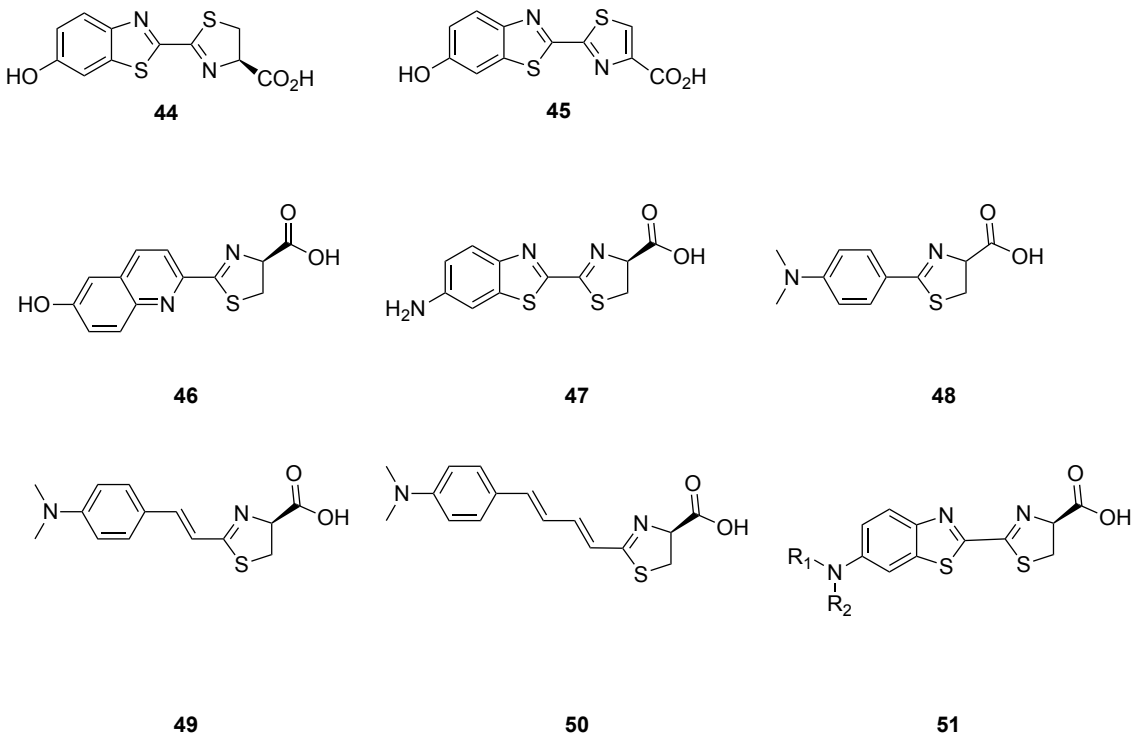
1.11 Red-shifting firefly bioluminescence via luciferin

The emission of longer wavelengths than D-luciferin can be achieved either through bioluminescence resonance energy transfer (BRET), which uses the energy of the excited state oxyluciferin to excite a second fluorophore that will then emit light at an even longer wavelength.[36] This technique is

often used to measure protein-protein interactions, since the donor luciferase and the acceptor fluorophore must be in close proximity. By either directly labeling firefly luciferase with a near-IR fluorophore,[37] or conjugating the luciferin to a near-IR dye,[38] BRET can be a source of near-IR light. The need to modify luciferase with a near-IR dye limits its use as a genetically encodable reporter. Attaching a near-IR dye to the luciferin will limit water solubility and cell permeability of the luciferin.[36] Reported analogues which include fluorophores to give a maximum emission > 600 nm up to 675 nm.[15] The donor luciferin-acceptor fluorophore configuration red shifts emission of the donor luciferin by intramolecular bioluminescence resonance energy transfer to the acceptor fluorophore. There is however a loss of signal intensity with BRET that may counter any benefit from the red shift in peak wavelength.

A more direct method to increase the wavelength of light emitted is by chemical modification of the luciferin substrate itself. The energy difference between the excited and ground states of the molecule determines the wavelength of light emitted.[39] A number of analogues of luciferin have been reported in the literature (scheme 11). Both L-luciferin **44** and dehydroluciferin **45** do not produce light with luciferase and actually competitively inhibit the production of light when D-luciferin is also present.[11]

Replacing the 6′-hydroxyl of D-luciferin with a more electron donating amino group red shifts the peak wavelength of light emitted.[40] The three hydroxyl positional isomers of luciferin, O-methylluciferin, 5,5'-dimethylluciferin and decarboxyluciferin were all inactive.[12] A number of analogues have been reported to display considerably red-shifted bioluminescence spectra these include D-quinolylluciferin (**46**, λ_{max} = 608 nm) [5] and 6-aminoluciferin (**47**, λ_{max} = 605 nm). Alkyl amino substituents are more strongly electron-donating than either the original hydroxyl or the amine, which red shifted the peak emission wavelength even more. Maki *et al.* have reported amino derivatives in which the benzothiazole unit has been replaced with a conjugated aromatic ring (**48**, **49**, **50**). [14] The bioluminescence spectra of the more conjugated compounds is reported to be red-shifted (**48**, λ_{max} = 445 nm; **49**, λ_{max} = 565 nm; **50**, λ_{max} = 688 nm) although the emission intensity is decreased 1000-10000 times compared to luciferin. Miller *et al.* report the synthesis of some amino derivatives of luciferin 1. [15] They found that the unsubstituted amino derivative **51** gave a maximum emission at λ_{max} = 593 nm compared to the 605 nm reported by White. By changing the substitution on the amino group the maximum emission could be red-shifted further (**51**, R^1= Et, ^{i}Pr or ^{n}Bu, R^2=H, λ_{max} = 607 nm; R^1, R^2= Me, λ_{max} = 607 nm).

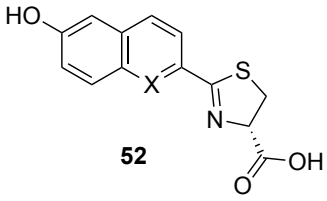


Scheme 11: Analogues of luciferin reported to red-shift light with luciferase.

Branchini et al. was one of the first to alter the core of D-luciferin. They showed that it was possible to exchange the benzothiazole of D-luciferin for either a naphthalene or quinolone **52**. [41] These luciferins emit light upon treatment with firefly luciferase at 524 nm and 608 nm, respectively, albeit at a lower intensity than D-luciferin. Subsequently, others have developed electronically modified luciferins by substituting single atoms in either the benzothiazole or thiazoline rings of D-luciferin (scheme 12).[42–44]All remain substrates for luciferase and display altered emission profiles, but none improve on the bioluminescence obtained with D-luciferin.

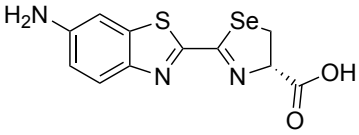
Iwano et al. completely removed the benzothiazole in favor of a simpler

aromatic system with extended π-conjugation to the thiazoline ring **53**. [45] This strategy produced the first example of a peak emission wavelength in the near-IR, at 675 nm. However, as with all the other synthetic substrates, shifting the peak wavelength is accompanied by a loss of signal intensity. While all of these new substrates demonstrate how promiscuous luciferase is, no example of a synthetic luciferin has shown improvement over WT luciferase and D-luciferin in terms of quantum yield under saturating luciferin and ATP conditions.

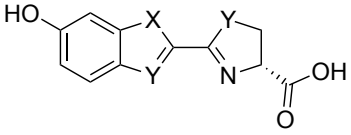


52

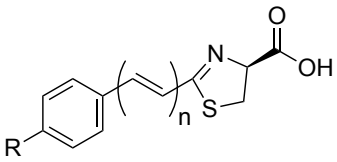
D-Naphthylluciferin X = CH (524 nm)
D-Quinolylluciferin X = N (608 nm)



$BiLH_2$ X = NH Y = N (577 nm)
$BoLH_2$ X = O Y = N (561 nm)
$BtLH_2$ X = S Y = CH (523 nm)
$BfLH_2$ X = O Y = CH (519 nm)



X = NH Y = S (578 nm)
X = NH Y =NH (460 nm)



53

R = OH n = 1 (530 nm)
R = OH n = 2 (640 nm)
R = NMe_2 n = 1 (560 nm)
R = NMe_2 n = 2 (675 nm)

Scheme 12: Analogues of luciferin 2.

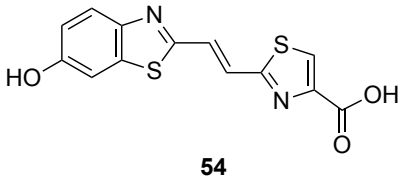
1.12 Properties of D-luciferin and its analogues

An added benefit of many synthetic luciferins is increased cell permeability and/or higher affinity for luciferase. When used in live cells and organisms, firefly luciferase will be retained inside the cell. Therefore, a luciferin must first cross the membrane in order to emit light. D-luciferin is small and relatively polar, therefore it is capable of moderate diffusion across cell membranes. However, D-luciferin only has a modest affinity for luciferase (Km = ~7 μM) and is thus unlikely to reach saturating conditions when used in live cells and organisms. Many of the synthetic luciferins developed thus far show-increased hydrophobicity relative to D-luciferin, which should increase cell permeability. Cyclic alkylamino luciferins also have increased affinity for luciferase (Km < 0.1 μM), allowing sufficient substrate to enter the cells to saturate the enzyme. [44]

1.13 Scope of research

The aim of this project is to synthesise analogues of the bioluminescent molecule luciferin, which is found in the firefly. Previous synthetic successes in the research group have produced an analogue of luciferin known as infraluciferin **54** (figure 6) that emits light at a wavelength that is able to penetrate body tissues (at 706 nm it is the furthest red shifted

analogue to date), and therefore has the potential to be used for deep tissue in vivo imaging. [46]



54

Figure 6 infraluciferin

Our first aim was to improve on the original synthetic route (which is detailed in chapter 3) to obtain larger quantities of material for further biological assays, and also produce a route that would allow for other analogues to be more easily accessed from the same methodology. One the reasons for the development of new analogues is that infraluciferin, as well as all the other analogues produce by various research groups which red shifted the wavelength of light emitted, have suffered from reduced quantum yields.

There has been extensive work carried out on the effect that the luciferase enzyme plays in colour tuning of luciferin. It would seem that the degree of deprotonation of the phenolic group at C-6 can affect the keto binding interaction at the other end of the molecule, by charge being delocalised through the extended π-system. We believe that the increased conjugation

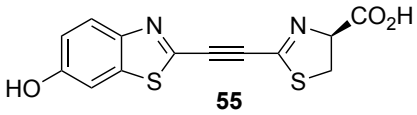
of infra-luciferin compared to D-luciferin is responsible for the shift in light emission towards the red spectrum.[47]

The shape of the luciferin molecule is of interest, as it will affect the fit of the substrate in the active site. Continuing on from the previously synthesized infraluciferin **54** we would like to investigate the luciferin analogue with an alkyne bridge connecting the benzothiazole and thiazoline rings. This would have an extended π-system retaining the conjugation through the molecule (which is vital for light emission) but a subtly different shape being linear instead of bent, and could lead to different binding interactions within the active site.[46]

Results and Discussion

2 Alkyne bridged luciferin analogue

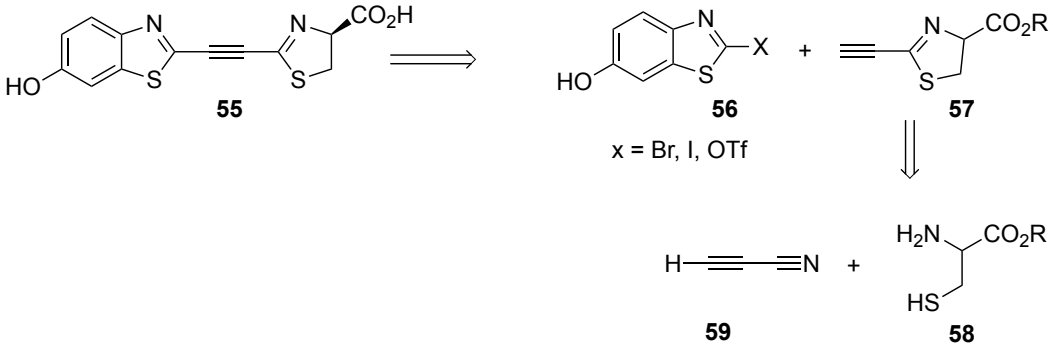
2.1 Sonogashira route to alkyne bridged luciferin



55

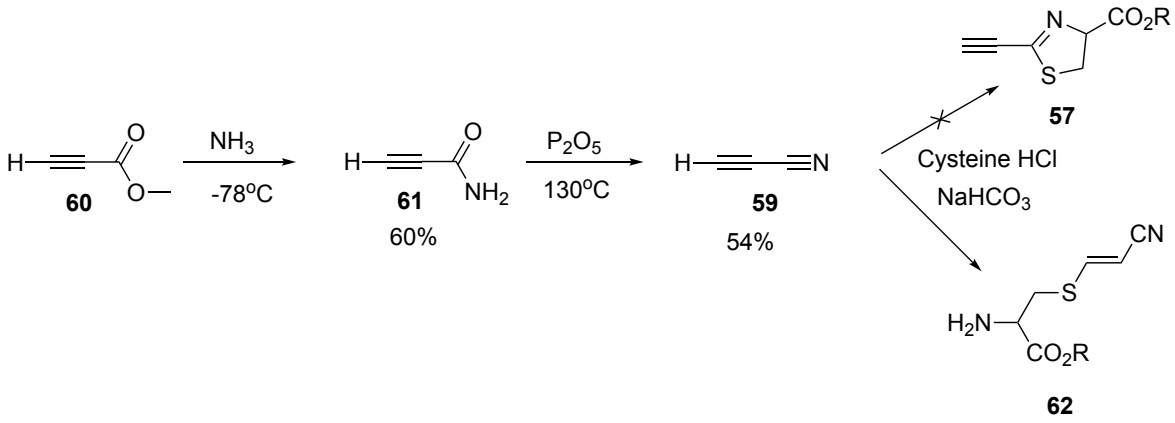
Figure 7: Alkyne bridged luciferin analogue

To synthesize the luciferin analogue **55** we proposed a Sonogashira disconnection to yield **56** and **57**. The thiazoline with a terminal alkyne **57** could be produced from the condensation reaction of cysteine **58** with propiolonitrile **59** (scheme 13). The Sonogashira reaction, which generally utilizes palladium and copper to catalyze the reaction between terminal alkynes and aryl halides, would be used to couple **56** and **57**.



Scheme 13: Proposed Sonogashira route to 55

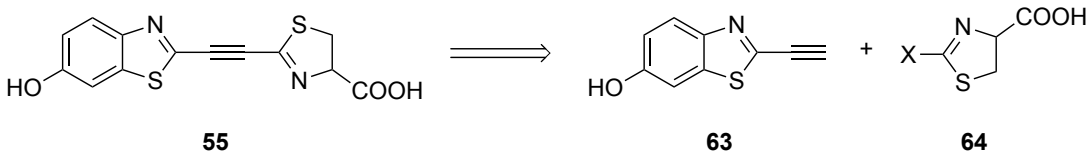
For this route the focus was first on the synthesis of the thiazoline **57**, and for this we needed to produce propiolonitrile **59.** Following the method used by Halter *et al* for the synthesis of this nitrile, the methyl ester propiolate **60** was converted to the amide using liquid ammonia to give the propiolamide **61**, this was then treated with phosphorus pentoxide to yield propiolonitrile **59**.[48] It was hoped that **59** could be reacted with cysteine to form the thiazoline with a terminal alkyne **57**, this was unsuccessful and the result of repeated attempts showed a double bond in the ^{1}H NMR [δ 7.51 (d, *J* = 10.5 Hz, 1H), 5.60 (d, *J* = 10.5 Hz, 1H] that suggested conjugate addition to **59** possibly by the sulfur of cysteine to give **62** (scheme 14).



Scheme 14: Attempted synthesis of thiazoline 57

2.2 Alternative Sonogashira route to alkyne bridged luciferin

An alternative disconnection places the terminal acetylene on the benzothiazole **63**. This terminal acetylene could then be coupled to a preformed thiazoline with a good leaving group **64** *via* the Sonogashira reaction (scheme 15).

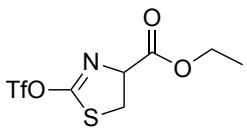


55 **63** **64**

Scheme 15: Proposed second route to 55

Although there is no literature precedent for the Sonogashira reactions with thiazolines it would be an ideal reaction, and there is vast literature precedence for coupling terminal alkynes to various substrates bearing either a halogen or triflate as a leaving group, to form a carbon-carbon bond.

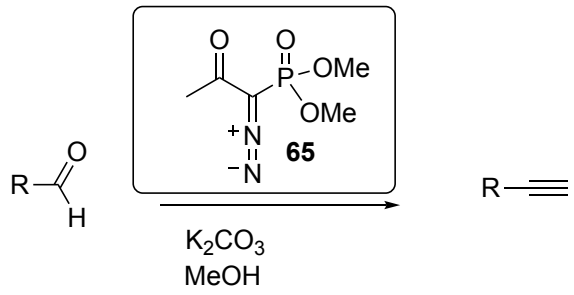
A possible thiazoline had been produced by Schmitz and Romo which had ethyl ester attached as well as a triflate **64** (figure 8). [49]



64

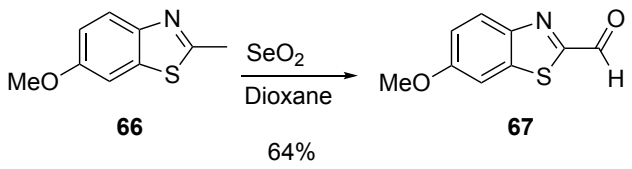
Figure 8: Thiazoline with leaving group

There are several reagents, which are capable of converting aldehydes in to our desired alkyne, including the Corey-Fuchs and the Seyfeth-Gilbert reagents. However the Ohira-Bestmann reagent **65** is able to produce the functional group inter conversion under mild conditions (scheme 16). [50] [51, 52]



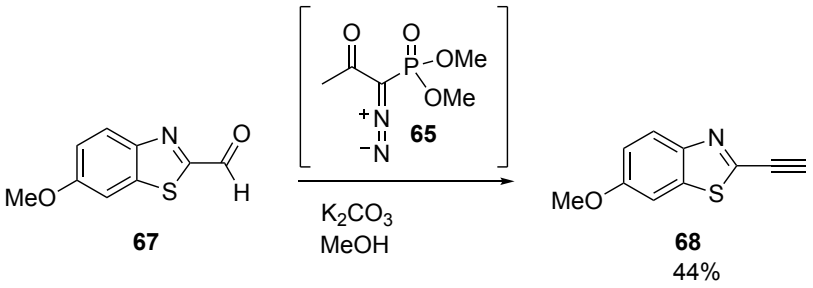
Scheme 16: Conversion of aldehyde to terminal alkyne using the Ohira-Bestmann reagent

As we have previously produced suitable benzothiazoles with attached aldehydes within the research group it was decided to attempt this route. The commercially available 6-methoxy-2- methylbenzothiazole **66** was treated with selenium dioxide to give the aldehyde **67** (64% yield, Lit. yield[53] 50 %) (scheme 17). [53]



Scheme 17: Synthesis of benzothiazole bearing terminal alkyne

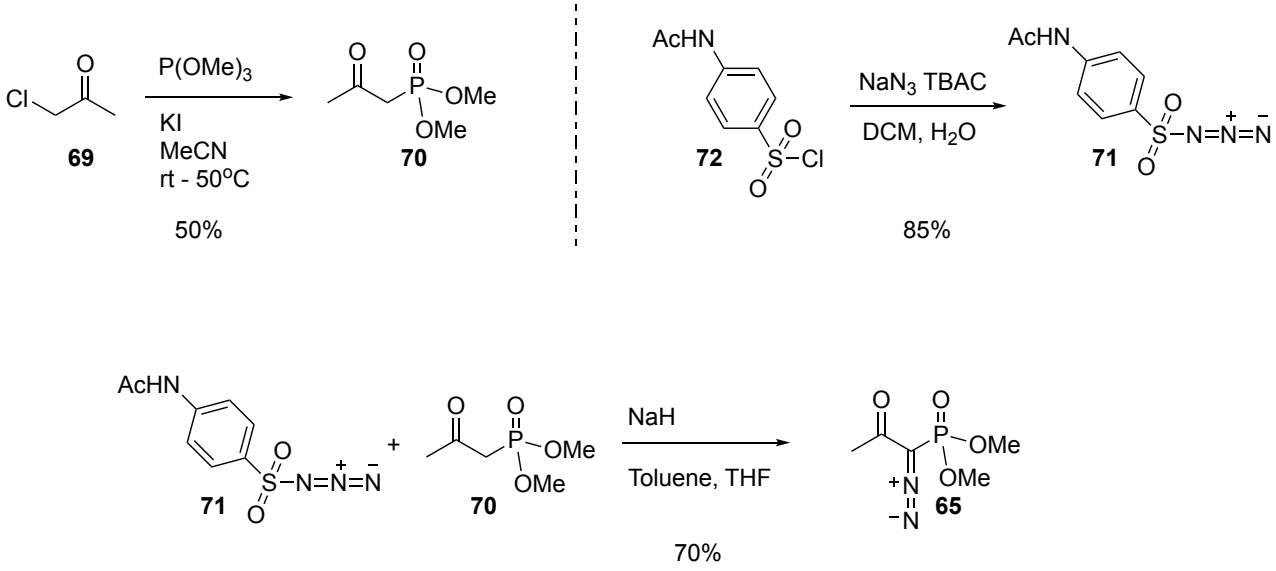
The aldehyde **67** was converted to the alkyne using conditions previously utilised by Muller to give the novel alkyne **68** in 44 % yield after purification by column chromatography (scheme 18).[51] Alkyne formation is clearly seen in ^{1}H NMR by loss of the aldehyde peak at δ 10.10 and the appearance of the terminal alkyne peak at δ 3.56.



Scheme 18: aldehyde to alkyne formation

The Ohira-Bestmann reagent **65** was chosen, as the conditions employing potassium carbonate as a milder base were more suitable for use with the benzothiazole structure. The synthesis of the Ohira-Bestmann reagent was completed in three steps according to the procedure by Pietruszka and Witt.[52] Starting with chloroacetone **69** and trimethyl phosphite to yield the Horner–Wadsworth–Emmons type product dimethyl 2-oxopropylphosphonate **70**. The azide **71** was formed from

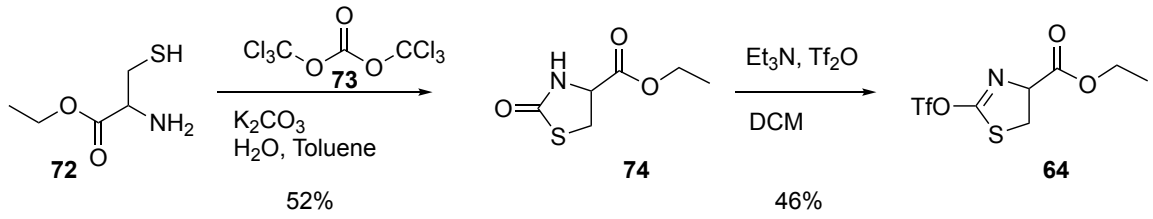
acetamidobenzenesulfonyl chloride **72** under phase transfer conditions using tertiarybutylammonium chloride (TBAC). Azide **71** was then utilised as a diazo transfer reagent for the previous oxophosphonate product **70**, following deprotonation with sodium hydride to give the Ohira-Bestmann reagent **65** (scheme 19). Yields for all three steps were within 5-10% of that stated in the literature. The final product **65** was judged to be >90% by ^{1}H NMR and as noted in the literature was used as isolated in further reactions.



Scheme 19: Synthesis of the Ohira-Bestmann reagent [52]

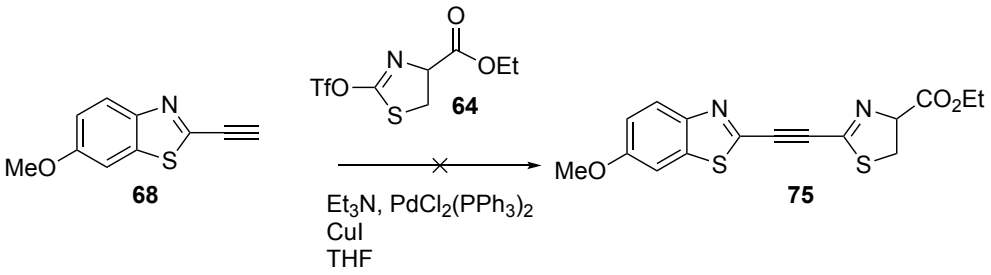
With the alkyne in hand our attention was turned to the synthesis of the thiazoline to be coupled with the alkyne. Following the procedure by Schmitz and Romo treatment of cysteine ethyl ester **72** with triphosgene **73** gave the thiazolidinone **74**. The desired thiazoline **64** with a triflate leaving

group was formed by treatment of **74** with triethylamine and triflic anhydride (49 % yield, Lit. yield [49] 56 % over 2 steps) (scheme 20).[49]



Scheme 20: Synthesis of thiazoline with triflate leaving group

With both halves of the luciferin analogue in hand a Sonogashira coupling was attempted under standard literature conditions (scheme 21).[54] However, these initial reactions were unsuccessful, yielding only decomposed starting material.



Scheme 21: Sonogashira coupling reaction[54]

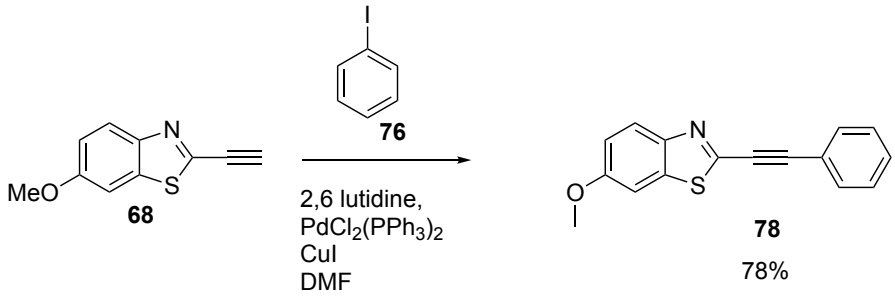
A survey of Sonogashira coupling reactions showed that subtle changes to the base, solvent and temperature could have drastic effects on the reaction, either increasing yields or obtaining a reaction product where previously none was observed. A series of Sonogashira reactions were conducted in

an attempt to find conditions that would afford the desired compound **75** (Table 1).[55] No product was observed under any of the conditions screened, giving either un-reacted starting material or decomposition.

Table 1: Conditions for Sonogashira coupling reaction

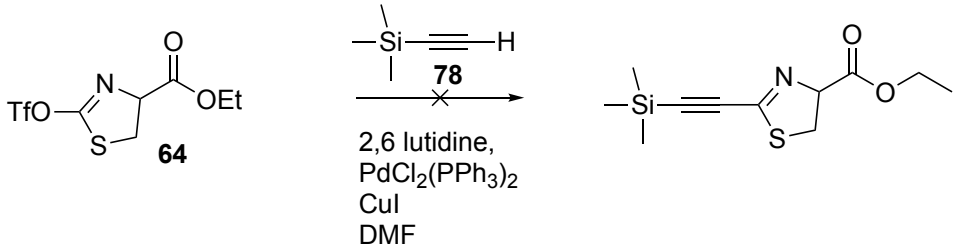
Catalyst	Equivalents	Base	Solvent	Temp °C
$PdCl_2(PPh_3)_2$/CuI	5-10 % / 10 %	Et_3N	THF	Rt-120
$PdCl_2(PPh_3)_2$/CuI	5-10 % / 10 %	Et_3N	THF	rt - 120
$Pd(PPh_3)_4$/CuI[54]	5-10 % / 10 %	2,6 lutidine	DMF	rt- 120
$Pd(PPh_3)_4$/CuI[54]	5-10 % / 10 %	2,6 lutidine	Dioxane	rt- 65
$(PPh_3)_2Pd(OAc)_2$	5-10 % / 10 %	NaOAc	DMF	40-60

With no encouraging results from these reactions, and the quantity of alkyne **68** depleted it was decided that it would be best to test the reactivity of the alkyne **68** and the thiazoline **64** separately. Under standard Sonogashira conditions (Scheme 22) the alkyne **68** was reacted with iodobenzene **76** which gave **77** in good yields.



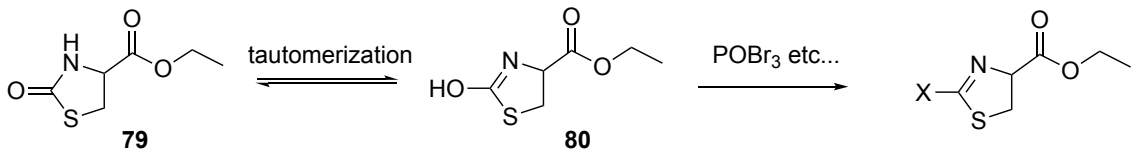
Scheme 22: Sonogashira test reaction with 68

Reaction of thiazoline **64** and terminal acetylene **78** under standard Sonogashira conditions was unsuccessful (scheme 23).



Scheme 23: Sonogashira test reaction with thiazoline 64

Further examination of the literature found few examples of triflates as leaving groups in Sonogashira reactions. It was decided to attempt to synthesise the desired thiazoline with a halide as the leaving group. There are numerous examples of halogenations of alcohols in the literature, which would be possible from **79** as the structure can potentially tautomerize to **80** (scheme 24). [56] [57]



Scheme 24: Halogenation of 79/80

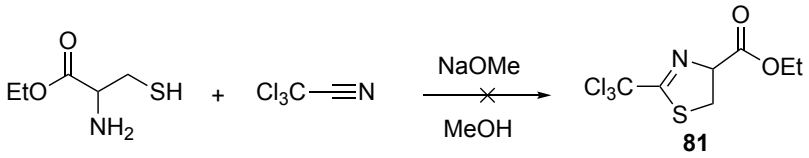
Various attempts at halogenation were unsuccessful (Table 2).[57 58 56 59 60]

In all the attempts no product was able to be isolated, with either starting material unreacted, formation of the thiazole, degradation or a complex mixture of all three

Table 2: Conditions for halogenations of 79/80

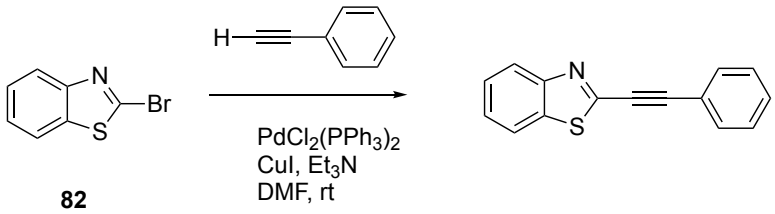
Reagent	Solvent	Temp °C	Result
$POBr_3$[56]	None	0-130	SM-azole/degraded
CCl_4/PPh_3	Acetonitrile	0-65	SM-azole
Bu_4NBr/P_2O_5	Toluene	0-100	SM-azole/degraded
Oxalyl Chloride/DMF	DCM	Rt-45	SM-complex mixture
PPh_3/CBr_4[61]	Toluene	0-125	SM-azole/degraded
$POBr_3$[57]	Toluene	0-135	SM-azole/degraded
$PPh_3/BrCCl_3$	DCM	rt	azole
$PPh_3/Br_3CCOCBr_3$	DCM	rt	azole
PPh3/Br3CCO$_2$Et	DCM	rt	azole

There is literature precedence for a CCl_3 group acting as a leaving group and we proposed that thiazoline **81** could be synthesized as shown in scheme 25.[62] Attempts to synthesize this compound did not produce the product, giving only unreacted starting materials and degraded products.



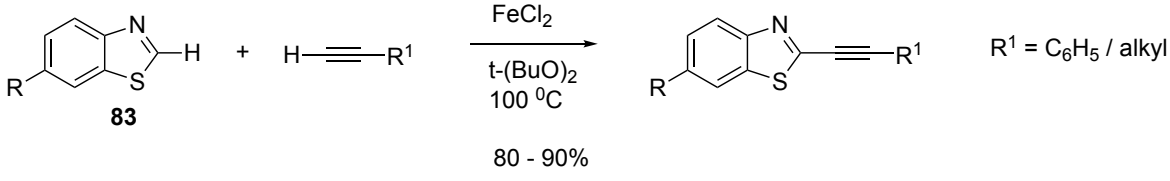
Scheme 25: Attempted thiazoline formation

There are numerous examples in the literature which show a benzothiazole with a halide **82** taking part in a Sonogashira coupling reaction (scheme 26).[63]



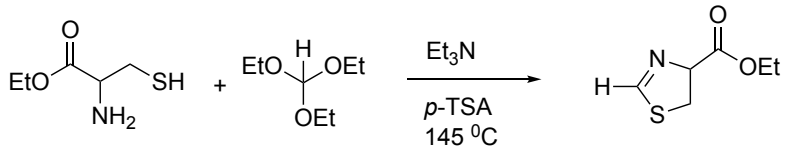
Scheme 26: Sonogashira coupling with benzothiazole halide

There is also a literature example of C-H activation with a benzothiazole bearing a proton **83** being directly coupled to an alkyne (scheme 27). [64]



Scheme 27: C-H activation coupling of benzothiazole and alkyne

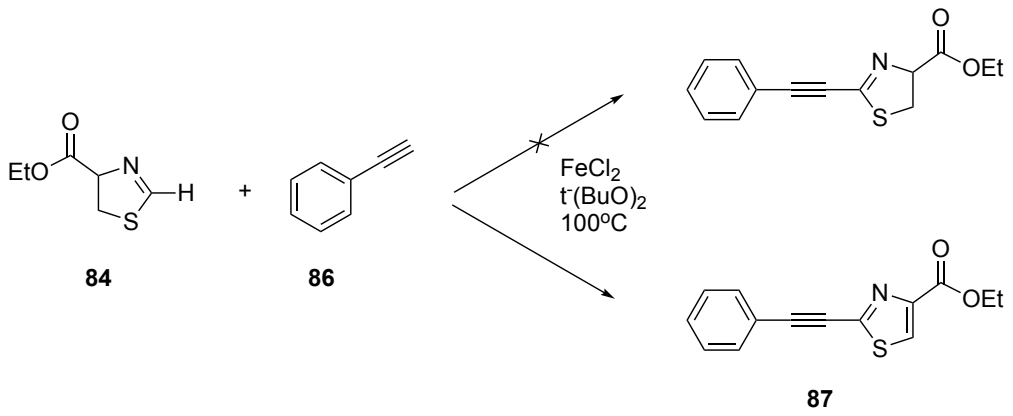
With these studies in mind we hypothesized that the thiazoline **84** might show the same reactivity as benzothiazole **83** to give a thiazoline with the alkyne attached, and then undergo a Sonogashira coupling with a benzothiazole/halide such as **82**. The precursor thiazoline was synthesized from cysteine ethyl ester **72** and triethylformate **85**, in quantitative yields using the method by Emtenäs (Scheme 28). [65]



72 **85** 100% **84**

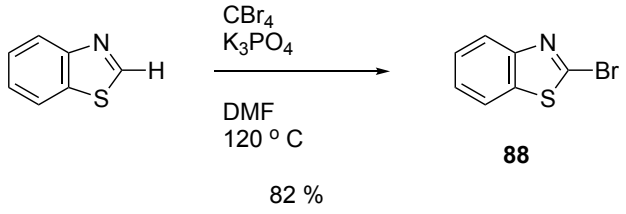
Scheme 28: Synthesis of thiazoline 84 [65]

The C-H activation reaction was attempted with **84** and phenyl acetylene **86** as a test reagent but as feared the facile thiazoline was oxidized to the thiazole **87** (Scheme 29) as seen by ^{1}H NMR with the peak at around δ 8.2



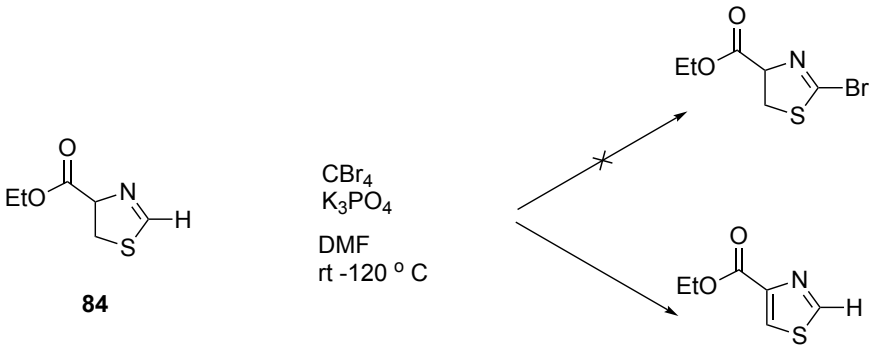
Scheme 29: Coupling of 84 via C-H activation[64]

With the alkyne attachment to **84** unsuccessful and a supply of this material still available a literature search found that work by Popov *et al* had halogenated a benzothiazole **88** (scheme 30) [61]



Scheme 30: Halogenation of benzothiazole

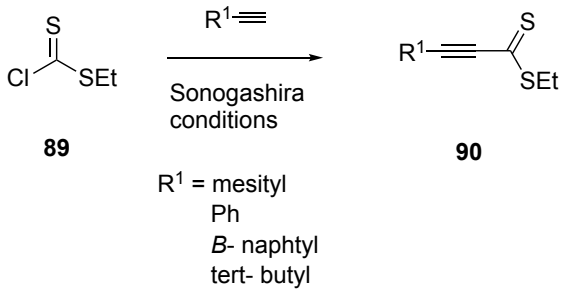
This method was trailed with thiazoline **84** but again thiazole formation was observed within a highly complex mixture, and none of the desired product was able to be isolated (scheme 31).



Scheme 31: Halogenation of thiazoline 84

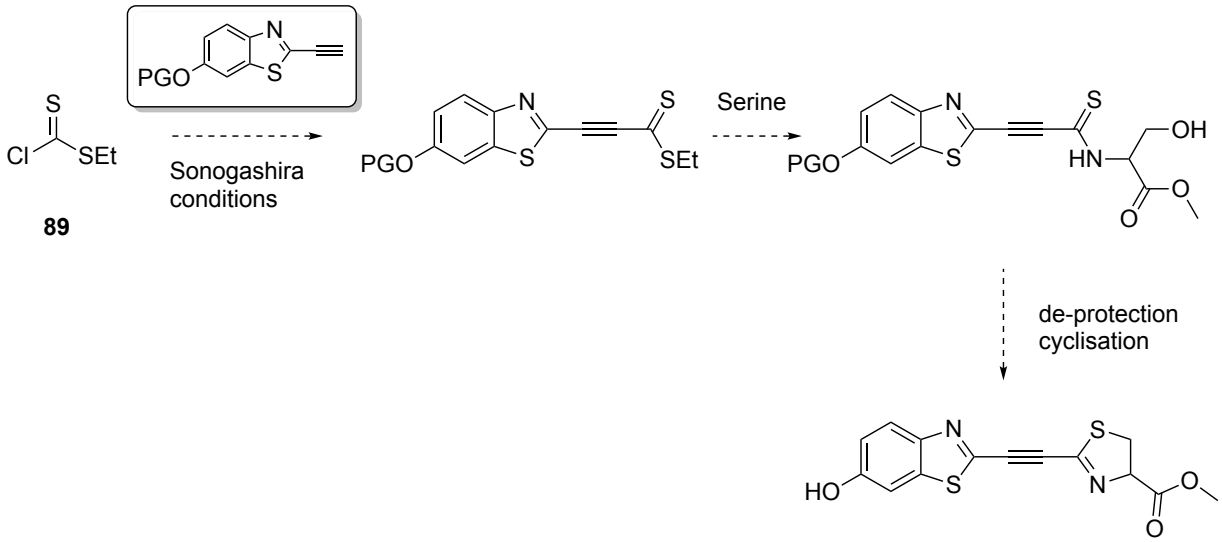
2.3 Dithioester Sonogashira route to alkyne bridged luciferin

With it clear that any manipulation involving a complete thiazoline will result in formation of a thiazole, it was decided that a route was needed where thiazoline formation was left until the last step. At this point in the research a new synthetic route to infraluciferin was complete which involved a dithioester, and importantly the cyclisation to form the thiazoline at the end of the synthesis. Under this premise a further literature search was conducted, and work by Thiono et al showed that **89** can undergo coupling reactions with alkynes via a Sonogashira reaction to give alkynes **90** attached to a dithioester (scheme 32). [66]



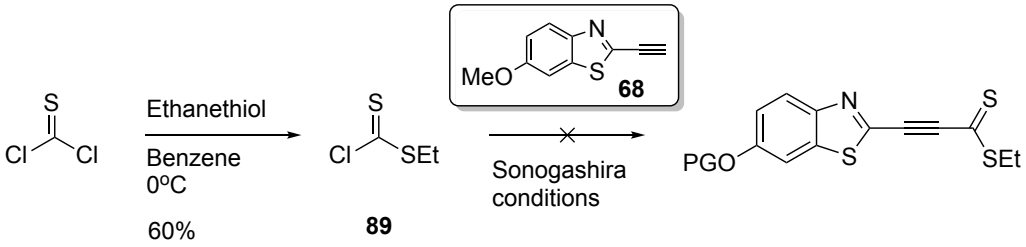
Scheme 32: Thiono et al Sonogashira reaction

We had by this point demonstrated that dithioesters can undergo substitution reactions with serine to give a precursor to a thiazoline moiety.[67] From **89** a similar procedure to that used for the new route to infraluciferin (section 3) could be followed e.g. adding in serine then cyclizing with DAST etc. (scheme 33).



Scheme 33. Proposed new route to 55

Following the protocols by Thiono et al the dithioester **89** was produced and then the coupling of this compound to our previously produced benzothiazole **68** bering an alkyne was attempted (scheme 34).



Scheme 34: Sonogashira coupling of 89 and 68

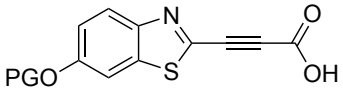
All attempts to add in our alkyne failed to give any product, only SM and some degradation was observed (table 3).

Table 3: Sonogashira coupling reaction conditions

Catalyst	Eq	Base	Solvent	Temp °C	Result
$Pd(PPh_3)_4$/CuI	5-10 % / 10%	2,6 lutidine	DMF	rt	SM/Complex mixture
$PdCl_2(PPh_3)_2$/CuI/PPh_3	5-10 % / 10%	Et_3N	DMF	rt	SM/Complex mixture
Direct addition		n-BuLi	THF	-78 – 0 °C	Degradation/complex mixture

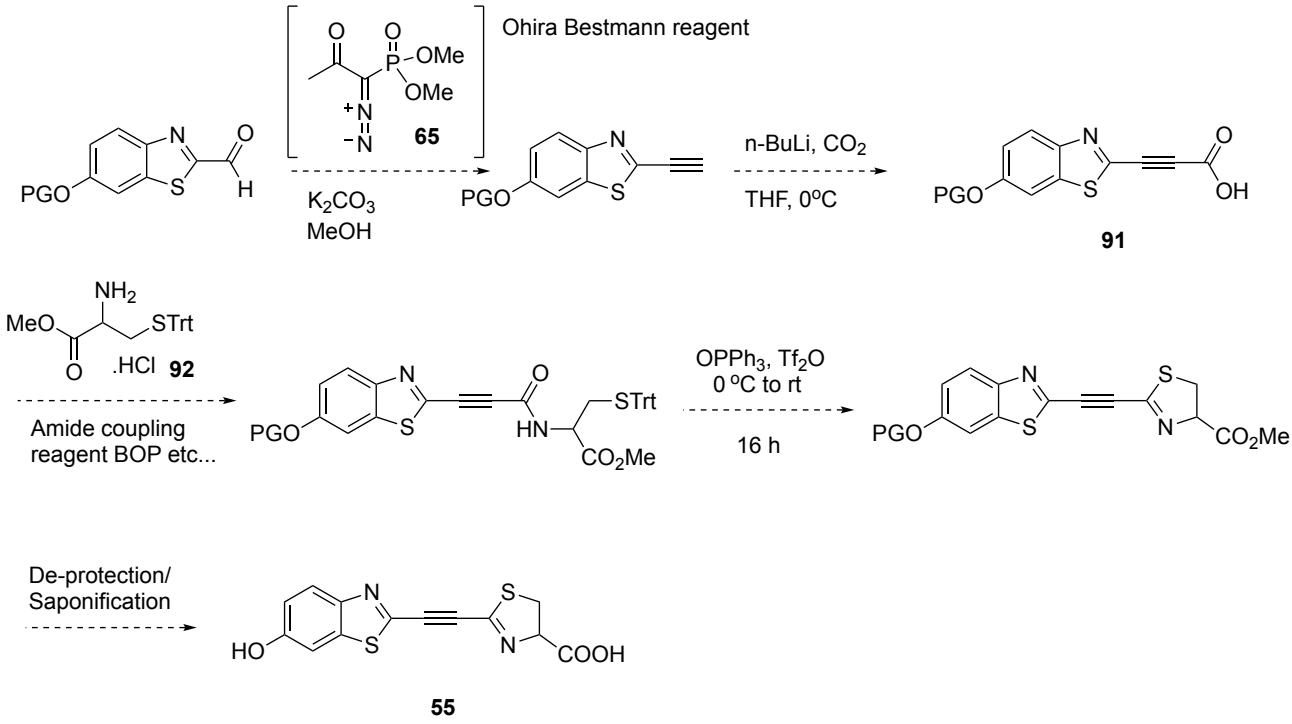
2.4 Linear synthesis to alkyne bridged luciferin

Having tried several convergent type routes to produce the alkyne bridged luciferin it was decided to attempt the synthesis of the analogue with a route similar to the original synthesis of infraluciferin. This would mean producing the intermediate **91** (figure 8) which would then be coupled with the cysteine derivative **92** and cyclised using the Hendrickson reagent (scheme 35) as per the procedure detailed in chapter **3** (scheme 42). [46]



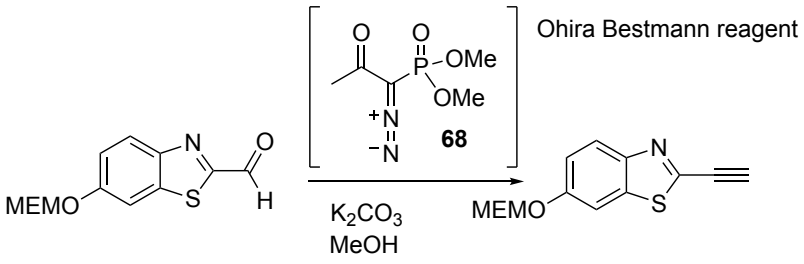
91

Figure 8: alkyne bridged luciferin intermediate



Scheme 35: Proposed linear route to alkyne bridged luciferin

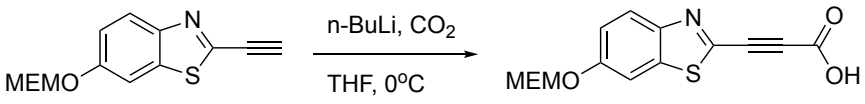
By this stage in the research the new synthetic route to infraluciferin was complete, and had shown that the MEM protecting group could be removed at the end of the synthesis without affecting the final molecule (scheme 70). This successful synthesis would guide us to use this protecting group for other routes, and thus we was able to use **93** as our starting point for this analogue. By treating **93** with our Ohira Bestmann reagent **68** we produced the alkyne **94** (scheme 36).



93 60% **94**

Scheme 36: synthesis of intermediate 94

Following standard conditions for the formation of a carboxylic acid using n-BuLi as a base and CO_2 gave the desired intermediate **95** in a 55 % yield (scheme 37). [68]

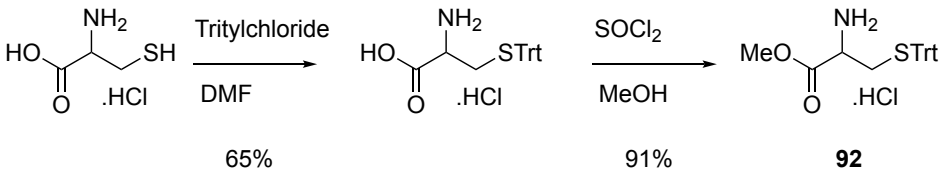


55%

94 **95**

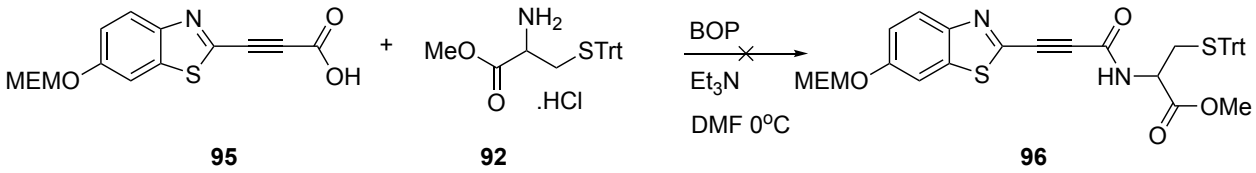
Scheme 37: Carboxylic acid formation

The necessary trityl and methyl ester protected amino acid cysteine **92** was produced in two steps (scheme 38).[69]



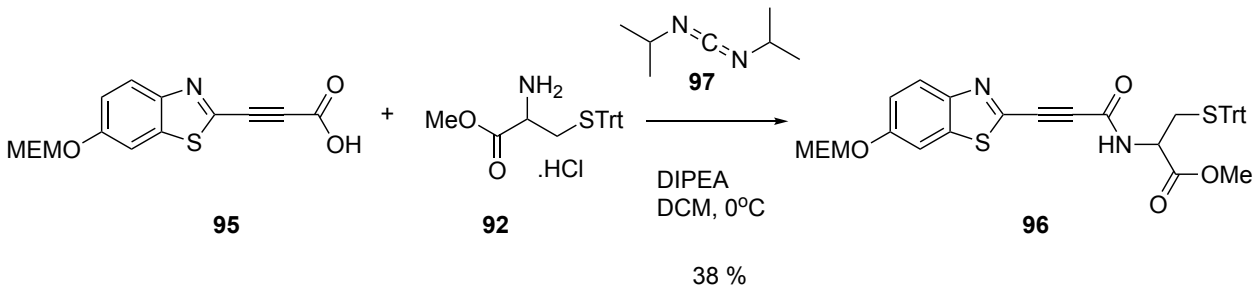
Scheme 38: synthesis of protected cysteine methyl ester 92

Using the protocols from the original synthesis of infraluciferin to couple **95 w**ith **92** using BOP (scheme 42) failed to give any product **96** (scheme 39).[46]



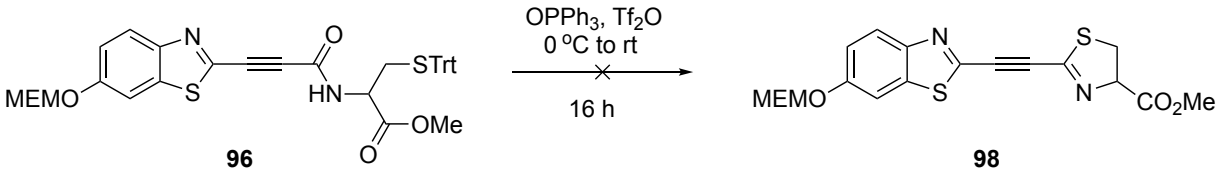
Scheme 39: Peptide coupling via Bop

Another amide coupling reagent (diisopropylcarbodiimide) **97** was used which did give the desired product **96** which is purified by column chromatography in EtOAc/Hex 1:1 to a sweet smelling waxy yellow solid (scheme 40)



Scheme 40: amide coupling of 95 and 92

Unfortunately time constraints only allowed for a few attempts at the cyclisation using the Hendrickson reagent, and these failed to give the desired compound **98** (scheme 41)



Scheme 41: Cyclisation to thiazoline using the Hendrickson reagent

3 New synthetic route to infra-luciferin

3.1 Original Synthesis

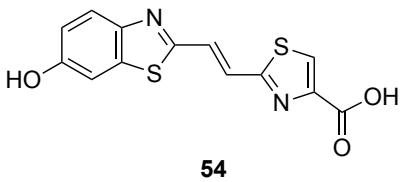
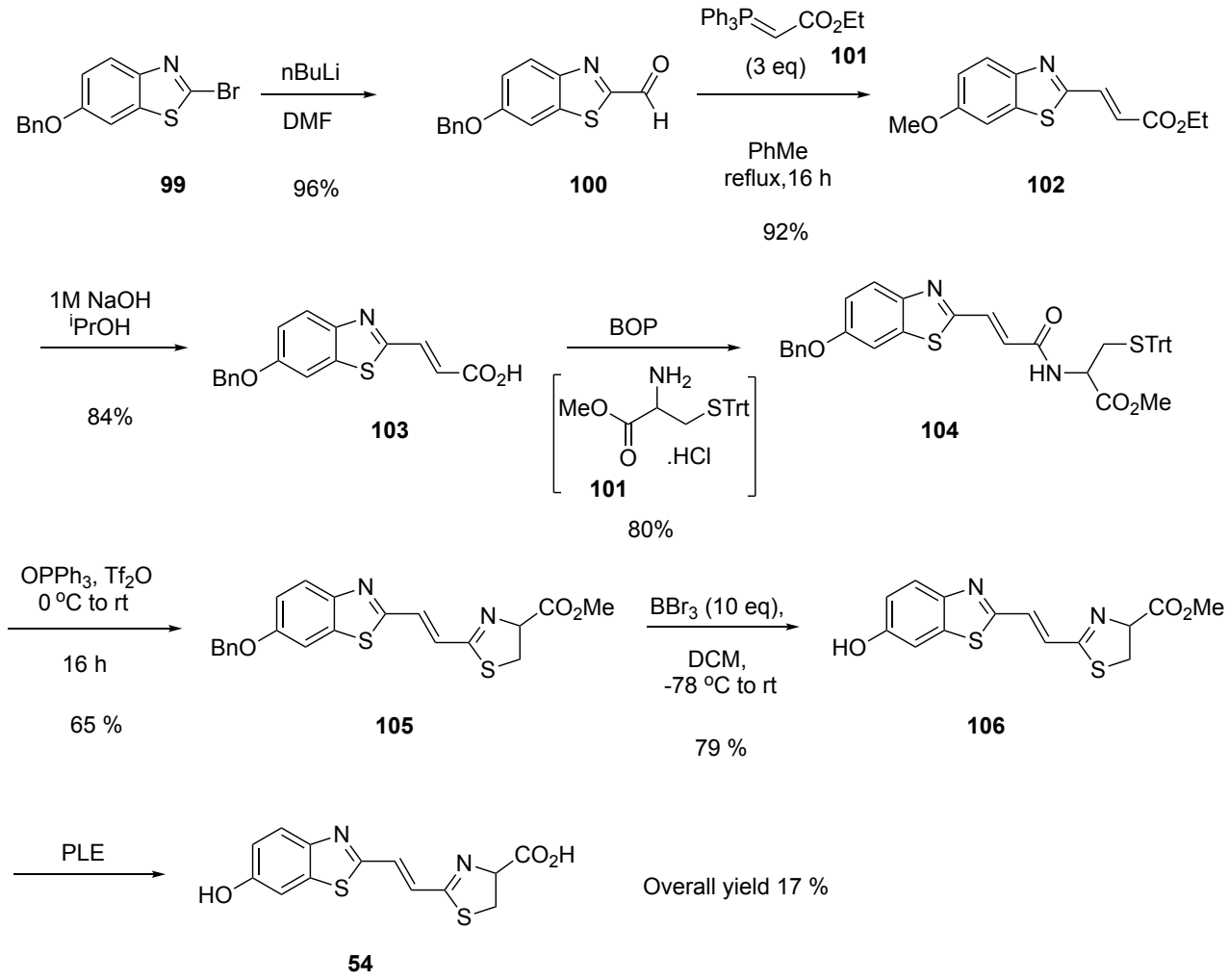


Figure 9: Infra-luciferin

Following the successful synthesis of the trans-alkene infraluciferin **54**, and confirmation that the compound is the furthest red shifted analogue to date, we attempted to improve the synthetic route.[46] The published synthetic route to infraluciferin (scheme 42) began with lithium halogen exchange of benzyl protected benzothiazole **99** and subsequent addition of DMF to form the aldehyde **100**. A Wittig reaction between the aldehyde **100** and the reagent **101** gave the ester **102** containing the desired double bond. The ester was converted to the free acid **103** via saponification with NaOH. With the double bond in place, the free carboxylic acid underwent an amide coupling with trityl protected cysteine methyl ester, using BOP as the coupling reagent to give **104,** a precursor to the desired thiazoline moiety. Cyclisation with $OPPh_3/Tf_2O$ (Hendrickson reagent) gave the thiazoline **105** in a reasonable 65% yield. However the reaction was extremely capricious, often failing to give any usable amount of product and would not scale up beyond a few tens of milligrams. The penultimate step of the synthesis was deprotection using BBr_3 to give **106**. This is a harsh reagent and on scale up past 10 – 20 mg the thiazoline can undergo major degradation resulting in a drastic loss of material, which at a late stage in the synthesis was extremely costly. The final step is saponification of the ester to the free acid, with pig liver esterase to preserve any enantomeric excess [46]



Scheme 42: Original route to infraluciferin 54

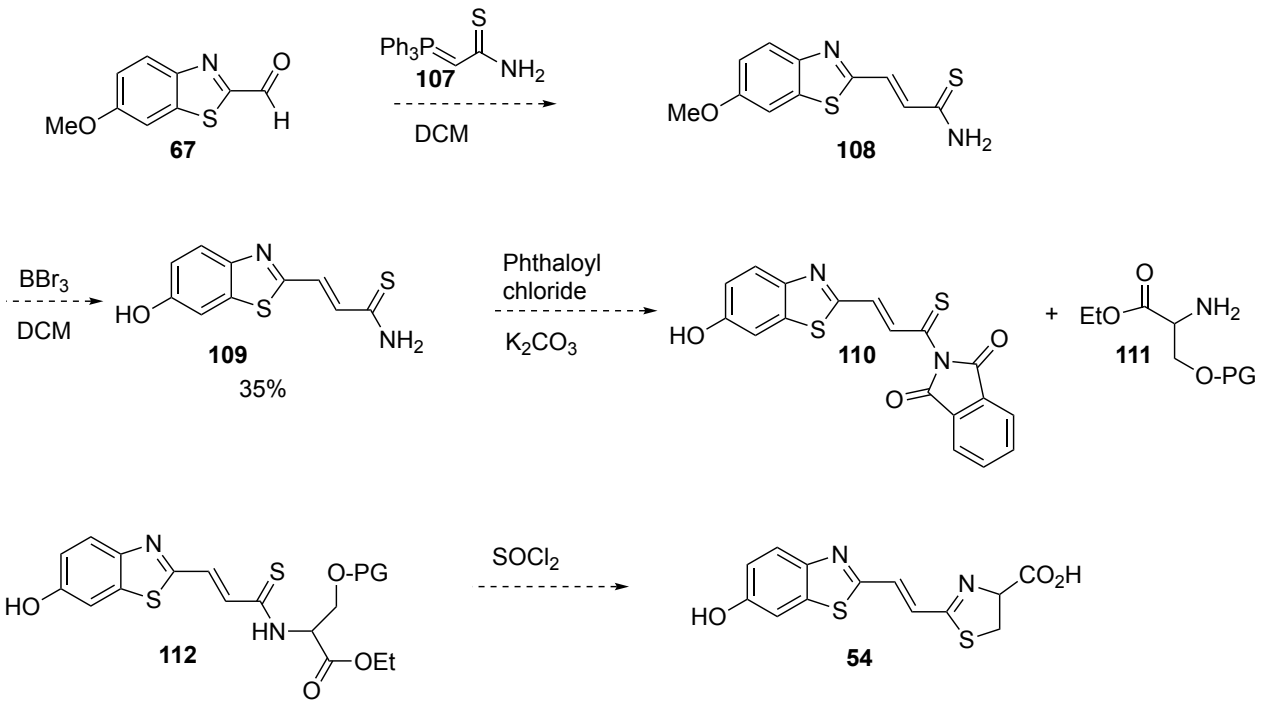
The original synthetic route gave an overall yield of around 17 %, however because of the problems encountered this meant that at most only 10 –20 mg were able to be produced from any one batch, thus limiting the material available for biological testing.

It was also hoped that the single enantiomer (D) could be obtained from this route by starting with D-cysteine, as this is the natural form of luciferin found in the firefly and has been the subject of debate regarding the effect

of the L enantiomer in terms of light emission/inhibition and conversion.[28,70,71] Unfortunately racemization occurred at the cyclisation step with $OPPh_3/Tf_2O$ (Hendrickson reagent), and therefore if another synthetic route could be found then the single enantiomer may also be obtained.

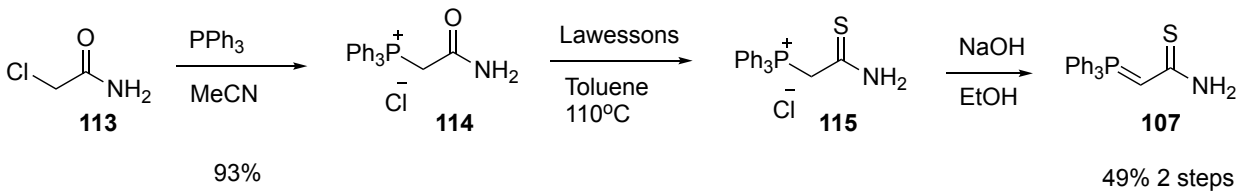
3.2 First attempt at Alternative route to infralucifern

To circumvent the unreliable cyclisation using the Hendrickson reagent and breakdown of the thiazoline ring by BBr_3 from the original synthesis, we proposed a new route (Scheme 43). This new route would utilize the previously synthesised aldehyde **67** in a Wittig reaction with **107** to form the thioamide **108**. Deprotection of the methoxy group prior to the formation of the thiazoline ring to give **109** would prevent thiazoline degradation, which occurs upon late stage removal of the methyl group from the benzothiazole. Conversion of the primary thioamide to a better leaving group such as phthaloyl chloride in **110** would allow for a peptide coupling with serine as either the ethyl or methyl ester **111** (with the alcohol protected if required) to give the penultimate **112**. Deprotection of the hydroxyl group if required, followed by conversion to a chloride by standard thionyl chloride procedure would allow cyclisation forming the thiazoline ring, and simultaneous hydrolysis of the ester to give **54** without the use of the Hendrickson reagent (scheme 43).



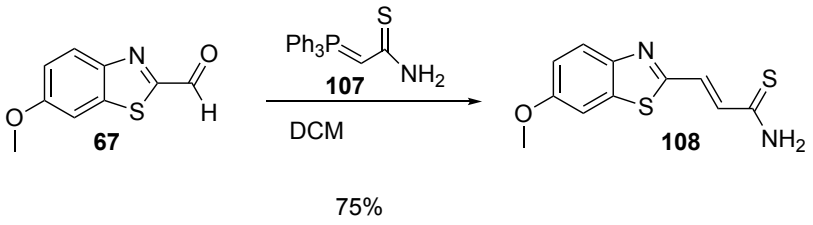
Scheme 43: Proposed new route to infraluciferin 54

The known Witting reagent **107** was synthesized in three steps from 2-chloroacetamide **113** and triphenylphosphine. The phosphonium salt **114** produced from this reaction was subjected to Lawesson's reagent to induce thiation of the oxygen to give the thioamide **115**. Finally sodium hydroxide was used to convert the crude salt to the Wittig reagent **107** (49% yield, Lit. yield[72] 49% mp 180-185°C, lit mp 182-185°C) (scheme 44).



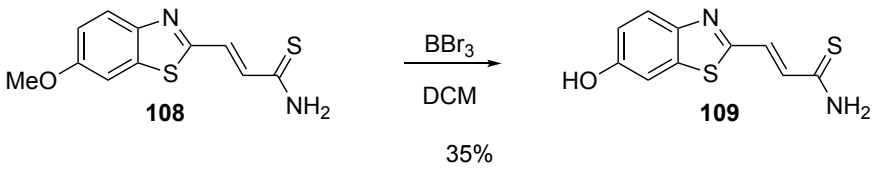
Scheme 44: Synthesis of the Wittig reagent 107 [72]

Treatment of the aldehyde **67** with the Wittig reagent **107** gave the α,β-unsaturated thioamide **108** in 75 % yield (Scheme 45). Interestingly the ratio of alkenes obtained was 2:1 *trans* to *cis*, but on leaving the mixture as a solid sitting in the presence of sun light the ratio was altered to more than 90% of the trans isomer. This was determined by analysis of the alkene protons by ^{1}H NMR; *Trans* 7.76 (d, *J* = 15.4 Hz, 1H), δ 7.26 (d, *J* = 15.4 Hz, 1H). *Cis* δ 6.68 (d, *J* = 12.7 Hz, 1H), δ 6.61 (d, *J* = 12.7 Hz, 1H)



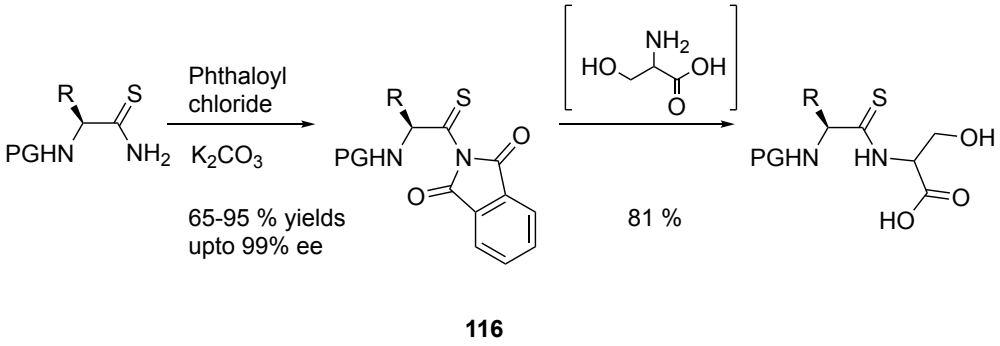
Scheme 45: Wittig reaction between 67 and 107

The free hydroxyl group on the benzothiazole is required in the final compound and previous attempts to de-protect after the formation of the thiazoline ring formation have resulted in degradation. To avoid degradation of the thiazoline ring it was decided therefore to proceed with unveiling the hydroxyl once alkene formation was complete. Demethylation of **108** using boron tribromide gave **109** in 35 % yields with approx 30 – 40 % recovered starting material (scheme 46). [31]



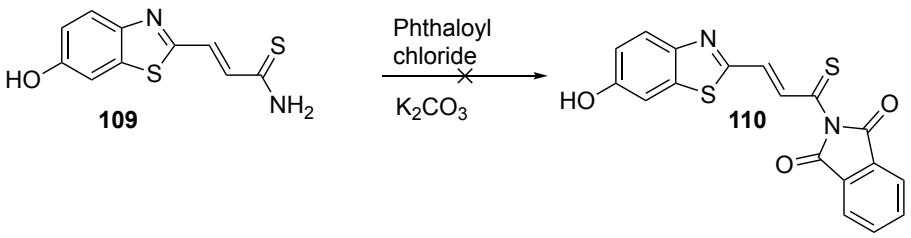
Scheme 46: Demethylation of 108

Brain *et al* have shown that primary thioamides can be converted in to suitable leaving groups for displacement by an incoming amine to give the desired thioamide by using phthaloyl chloride **116** (scheme 47) [73]



Scheme 47: Previous example of thioamide by amine displacement

Phenol **109** was treated with phthaloyl chloride **116** in an attempt to form **110** (scheme 48), to provide a good leaving group for peptide coupling with serine. However this product was not seen from proton NMR and mass spec showed no product with a molecular weight near the 366.41 of the desired product, only degraded fragments.



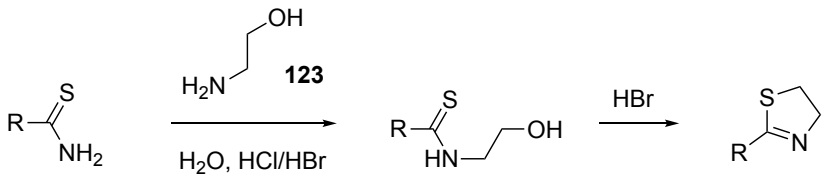
Scheme 48: leaving group synthesis

There is also literature president for this reaction where pyridine is used in place of potassium carbonate. This however was also unsuccessful, only showing fragmented products by mass spec (table 4).

Table 4: Leaving group attachment

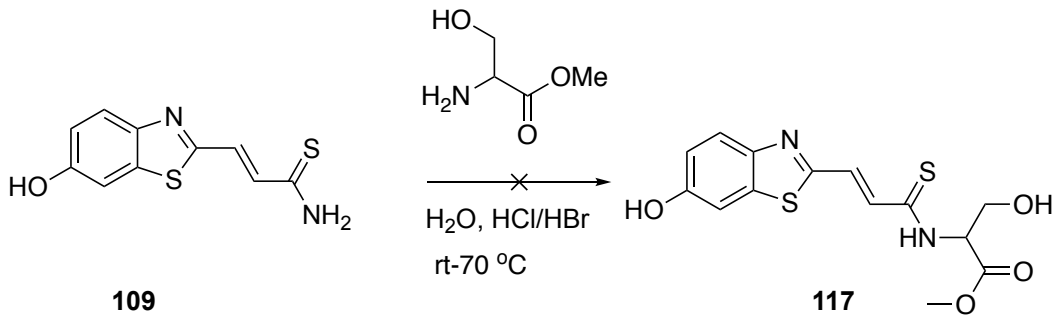
Base	Solvent	Time hrs	Temp °C	Result
K_2CO_3	THF	2-3	0-rt	Degraded SM and recovered reagent
Pyridine	THF	2-3	0-rt	Degraded SM and recovered reagent

Following the unsuccessful attempts to convert the thioamide **109** to a good leaving group, a further literature search revealed direct addition protocols for amino-alcohols to thioamides. In fact under acidic conditions formation of the thiazoline group occurred in situ. (Scheme 49)[74]



Scheme 49: Addition of aminoalcohol to thioamide and thiazoline formation

Treatment of **109** with serine methyl ester under the same acidic conditions was attempted in order to give the uncyclised precursor **117** (scheme 50).

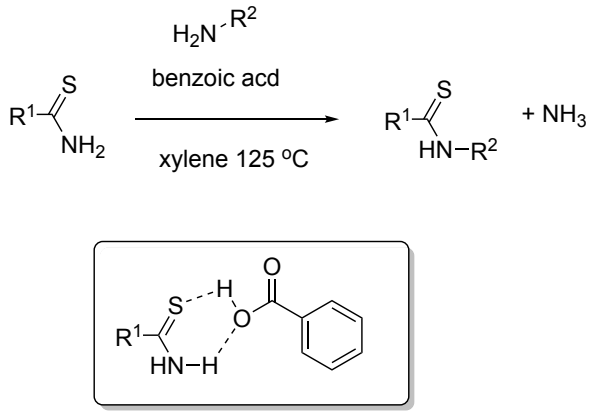


Scheme 50: Direct addition of serine to 109

All attempts were unsuccessful only benzothiazole peaks being discernable from the complex NMR spectra. The alkene and the thioamide portions of the starting material were degraded and separation of any compounds from the crude mixture was not possible

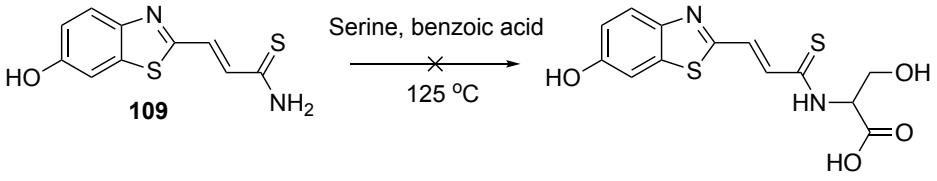
Benzoic acid has been shown by J. Wu et al to enable transamidation between thioamides and several amines. It has been proposed that benzoic acid can act a catalyst forming intermolecular hydrogen bonds with the

thioamide, allowing the amine to attack the thioamide to give the product and ammonia (Scheme 51) [75]



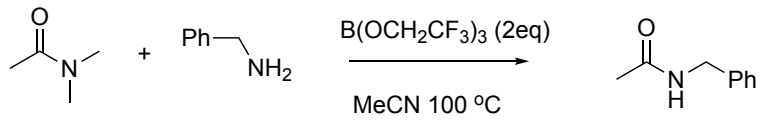
Scheme 51: Transamidation using benzoic acid and proposed intermediate

Trials with this route proved unsuccessful with only stating materials recovered. (Scheme 52)



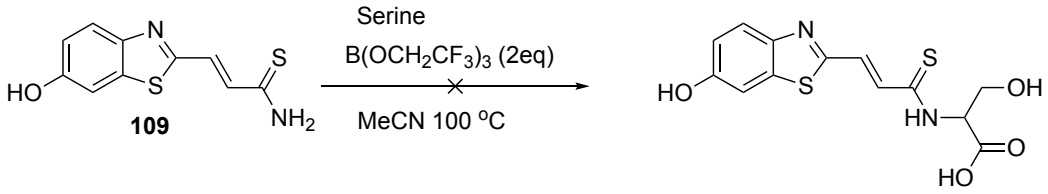
Scheme 52: Transamidation of 109 using benzoic acid

One final attempt was made at transamidation using the boron reagent $B(OCH_2CF_3)_3$ which has been shown by the Sheppard group at UCL to be effective for the transamidation of DMF (scheme 53). [76]



Scheme 53: Transamidation of DMF using $B(OCH_2CF_3)_3$

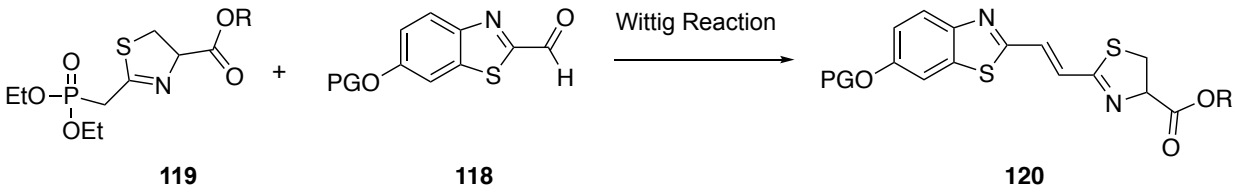
Attempts at transamidation using $B(OCH_2CF_3)_3$ reagent with **109** were unsuccessful showing only degraded material (scheme 54).



Scheme 54: Transamidation of 109 using $B(OCH_2CF_3)_3$

3.3 Convergent Dithioester route to infra-luciferin

With attempts at producing infraluciferin **54** from **109** unsuccessful an alternative route to **54** was sought. As the compound **118** is a complete half of luciferin and has the aldehyde functional group, a more convergent route where by a complete thiazoline moiety with a phosphonate attached **119** would enable a simple Wittig reaction to produce infraluciferin needing only deprotection instantly **120** (scheme 55).

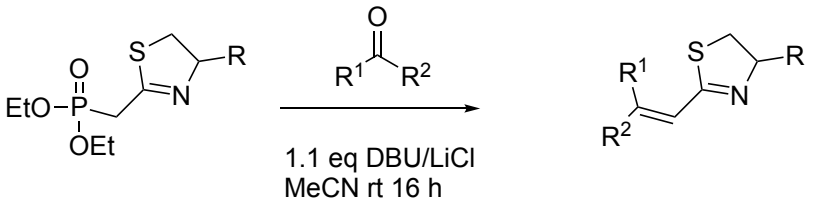


119 **118** **120**

Scheme 55: Proposed convergent synthesis via Wittig reaction

A literature search reveled that work done by Masson *et al* and a few others had indeed made use of **119** albeit with their R group being alkyl, benzyl and not acids or esters. [77 78 66]

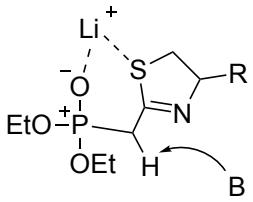
A particular example by Masson was the coupling of **121** to simple aldehydes where R = H, Et, Ph, Bn, R^1 = H and R^2 = Me, Ph, *i*-Pr (scheme 56)



121

Scheme 56: Wittig reaction of 121 with simple aldehydes

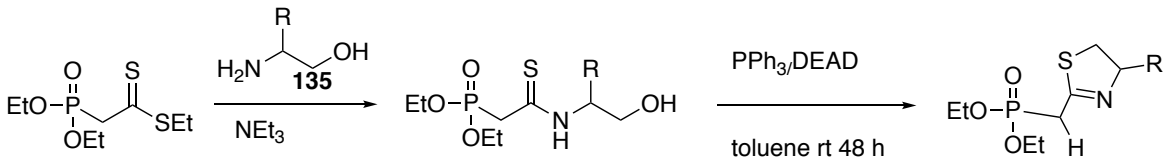
The use of DBU as a mild base and LiCl as a catalyst is ideal for use with our often-facile synthetic intermediate molecules. The lithium ion coordinates to either the nitrogen or sulfur of the thiazoline and the oxygen of the phosphonate, making the protons of the CH_2 more acidic and easily removed via DBU (scheme 57). [77]



121

Scheme 57: Proposed activation using LiCl

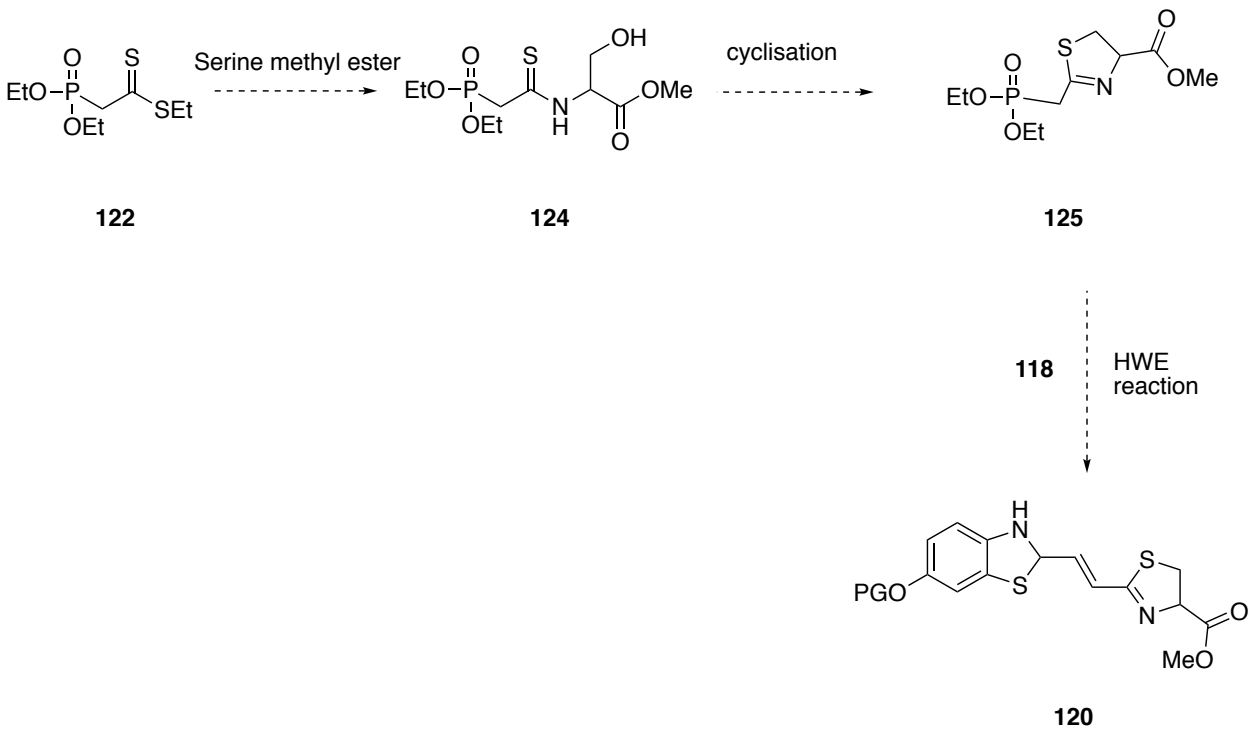
A further literature found that various amino alcohols had been reacted with phosphonodithioacetate **122** and then cyclised via an intramolecular Mitsunobu reaction to give the thiazoline Wadsworth Horner Emmons reagent **123** R = H, Et, Ph, Bn (scheme 58) [79]



122 **123**

Scheme 58: Synthesis of thiazoline phosphonate

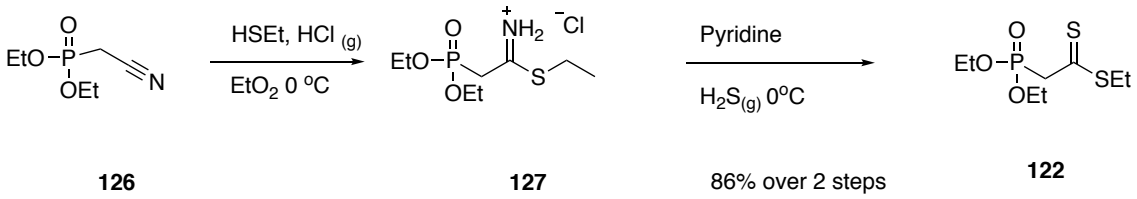
All that is required for a similar route to infraluciferin is to replace the amino alcohol with serine methyl or ethyl ester to give **124**, cyclise to yield the desired thiazoline **125** and perform the Wittig coupling with **118** (scheme 59).



Scheme 59: Proposed route to infra-luciferin *via* phosphonate intermediate

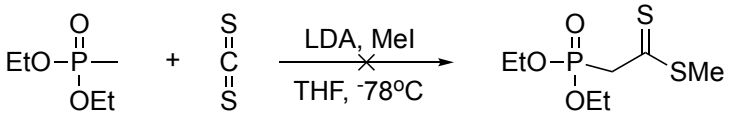
The phosphonodithioacetate **122** was synthesised in two steps without the need for any purification by a modified procedure of that carried out by Marvel *et al* (scheme 60).[80] Our synthesis began with diethylcyanomethyl phosphonate **126,** which was reacted with ethanethiol in diethyl ether whilst passing through a stream of HCl gas to give the salt of imine **127**. The intermediate imine **127** was then dissolved in pyridine and a stream of

hydrogen sulfide gas was passed through the solution and after an aq. work up the final product **122** was obtained. Both reactions were successfully carried out on large (5-10g) scale, and without the need for any purification (>95 % by H^1 NMR) and the whole process could be achieved in two days.



Scheme 60: Synthesis of phosphonodithioacetate 122

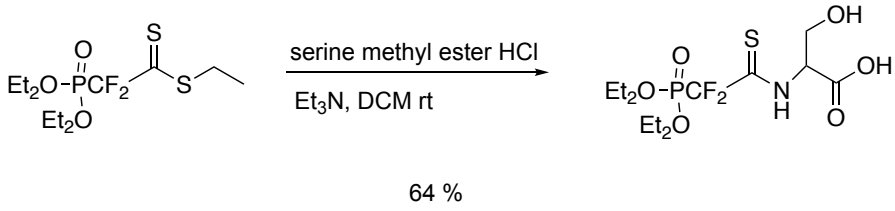
Due to the hazards associated with using these gases, and their corrosive nature and foul stench, an alternative route was attempted to produce **122** that avoided using HCl and H_2S gas cylinders and the foul stench of ethanethiol. This reaction produced the methyl ester instead of the ethyl ester but the product required purification by column chromatography and was found to be unstable on silica resulting in degradation, and no pure product was isolated (scheme 61) [81]



Scheme 61: Alternate synthesis of a phosphonodithioacetate 134

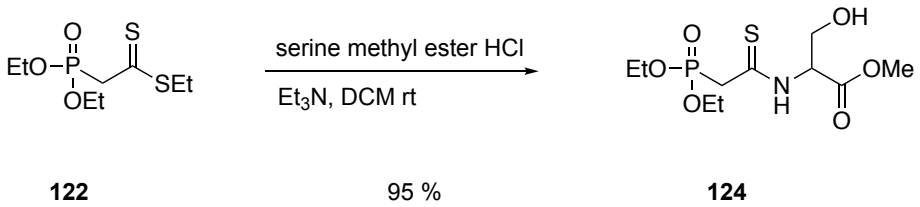
It was decided that because of the scalability and purity of the product produced **122** (scheme 60) this route was the most suitable.

With multiple grams of pure **122** in hand the next stage was for the substitution by serine methyl ester Vazeux *et al.* had previously described the substitution of serine methyl ester on a similar dithioester (scheme 62) [81]



Scheme 62: Established substitution conditions of serine to dithioester

The reaction with our substrate took 48 h to go to completion as monitored by ^{31}P NMR **122** δ 17.14 to give the product **124** δ 21.41 in yields of 95 % or greater (scheme 63). It is worth noting that **124** is extremely reactive and has to be kept below 0 °C and used within 48 h or noticeable amounts of degradation are observed.

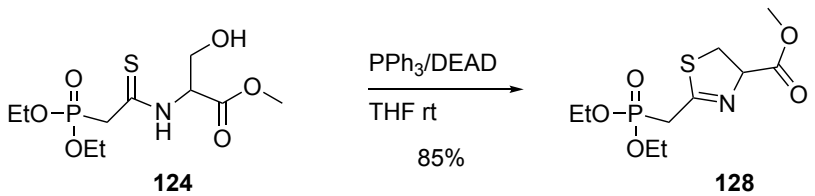


122 95 % **124**

Scheme 63: Addition of serine methyl ester

Analysis by ^{1}H NMR showed δ 5.2 (CH from serine) and δ 5.49 (CH_2 from phosphonate), mass spec gave m/z 314 consistent with the formation of the product, which was purified by column chromatography. This reaction was very scalable and the entire amount of product from a batch of >10 g of **122** could be used whilst maintaining high yields.

The first option from this point was to cyclise **124** to give the HWE reagent with the thiazoline preformed. Masson *et al*. had previously attached an amino alcohol to **122** and then performed a Mitsunobu type reaction to form a thiazoline, this method was attempted and gave **128** in excellent yields (Scheme 64). ^{1}H NMR showed a clear shift from δ 4.0 to δ 3.2 for the CH_2 which originated from serine and the formed the thiazoline ring, a mass spec of m/z 296 was consistent with the formation of the thiazoline**.** [77]

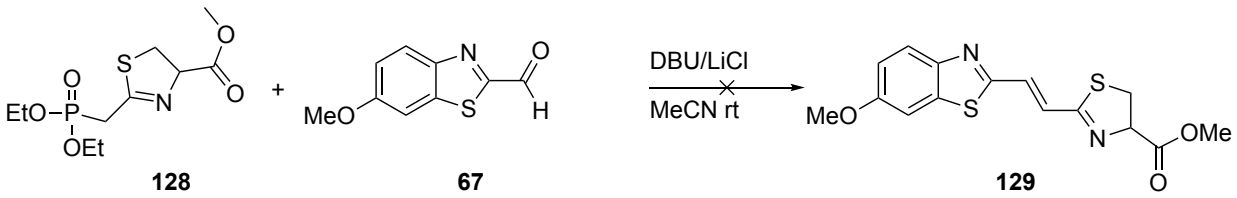


124 **128**

Scheme 64: Formation of thiazoline 128

This reaction also works on lager scales (>1g) and gave **128** in excellent yields (~85 %) after purification by column chromatography.

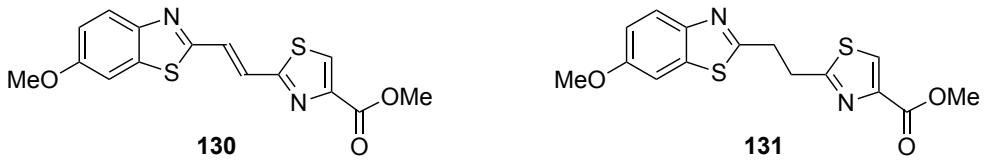
With the thiazoline half of infraluciferin attached to a phosphonate the next step was to investigate the HWE reaction with the benzothiazole portion of luciferin. The same conditions were used from the Masson paper, which employed DBU and lithium chloride as mild reagents to form the double bond whilst not affecting the thiazoline ring (scheme 65)**.** [77]



128 **67** **129**

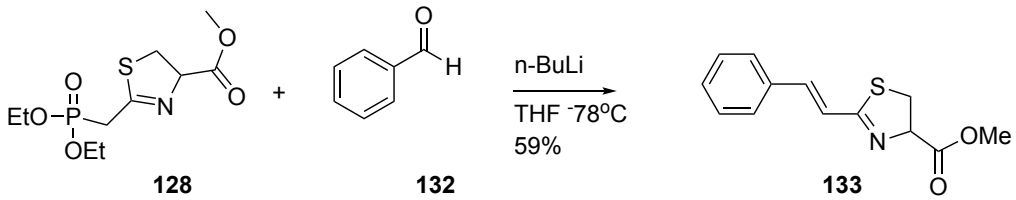
Scheme 65: HWE using mild conditions

This reaction failed to give **129** and instead the thiazole **130** was isolated from the reaction, the thiazole clearly identified by ^{1}H NMR with the loss of the signal at CH_2 δ 3.2 and formation of a new signal for the CH of the azole at δ 8.21. The reduced alkene **131** was also formed as a trace product < 5 %, ^{1}H NMR showing the two CH_2 protons at δ 3.63 and the thiazole CH at δ 8.06 (scheme 66).



Scheme 66: Unwanted products from DBU/LiCl HWE reaction

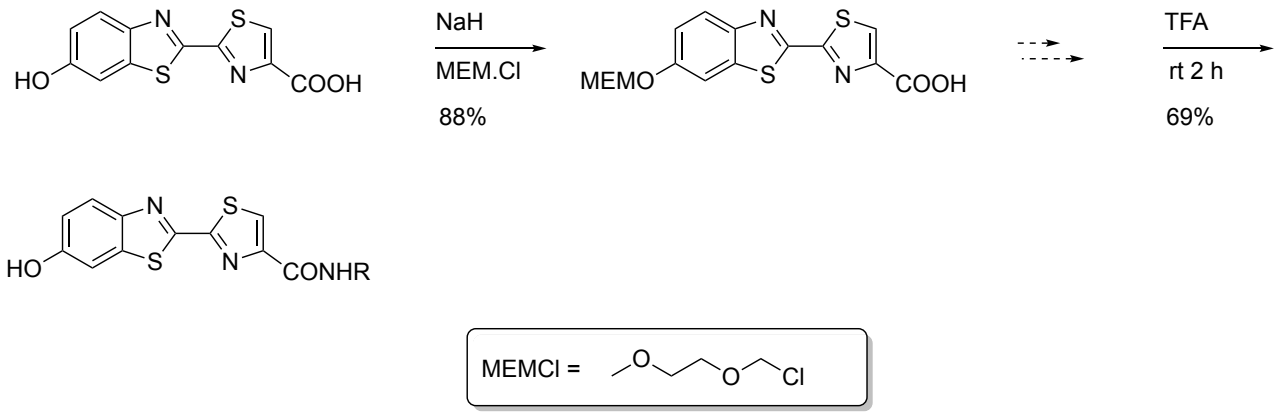
As the use of the reversible base DBU proved to be problematic resulting in azole formation with our substrate, it was decided that the use of a non-reversible base such n-BuLi might be more suitable to form the ylide for the HWE reaction. The supply of aldehyde **67** was now depleted so benzaldehyde **132** was used as a test the aldehyde for the reaction which gave the desired alkene in **133** 59 % yield (scheme 67)**.**



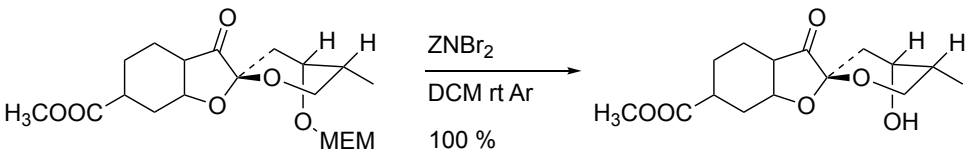
Scheme 67: HWE using n-BuLi

With this reaction being successful it was decided to try these conditions with **128** and a benzothiazole bearing an aldehyde. At this stage it was also decided to investigate the use of a different protecting group instead of methoxy group, as we knew unmasking the methyl ether to give the free alcohol using BBr_3 had been unreliable from previous syntheses. The protecting group 2 –methoxyethoxymethyl (MEM) protecting group had

been previously used by Santaniello et al and also Branchini *et al* for the synthesis of luciferin analogues, albeit with the azole instead of the thiazoline (scheme 68). [82] Deprotection of the MEM group had been induced using neat TFA, and we believed the thiazoline to be stable to acid this would be a viable protecting group. Better still the removal of MEM group had been shown by Williams et al to proceed in quantitative yields with $ZnBr_2$ (scheme 69).[83] [84]

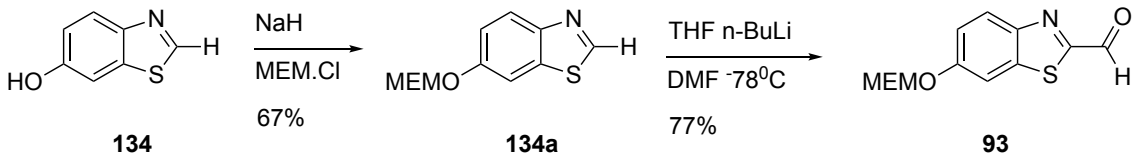


Scheme 68: Santaniello et al MEM protection/deprotection



Scheme 69: Williams et al MEM deprotection via $ZnBr_2$

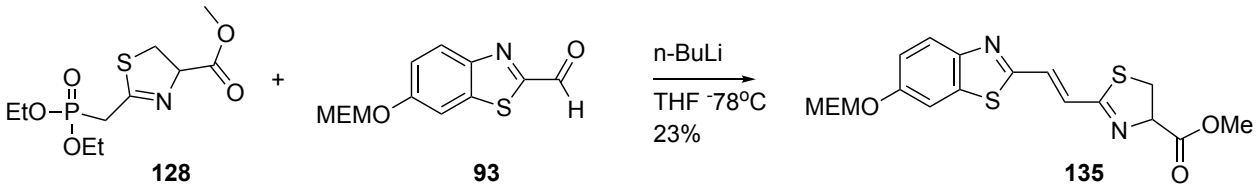
The required benzothiazole aldehyde **93** was prepared in 2 steps starting from **134** MEM protection was followed by deprotonation of the most acidic proton of the benzothiazole and quenching with DMF. Aldehyde **93** was isolated in 77 % yields after column chromatography (scheme 70).



134 **134a** **93**

Scheme 70: MEM protection and aldehyde formation

With both halves of the molecule complete the HWE reaction utilizing n-BuLi was attempted (scheme 71).



128 **93** **135**

Scheme 71: HWE using n-BuLi and MEM protected aldehyde

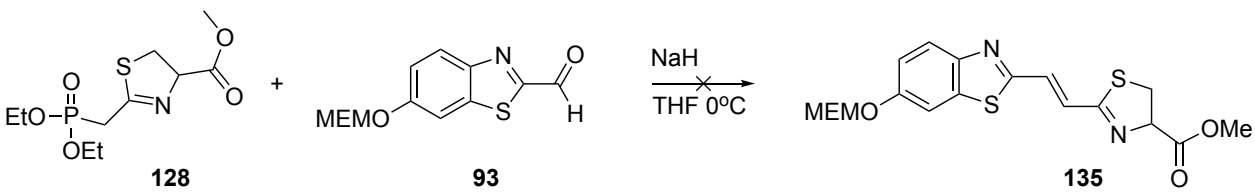
Interestingly the first attempts at this reaction which were quench with saturated $NaHCO_3$ whilst at -78 °C failed to give any product, but once the reaction was allowed to stir at rt for 2 hrs before quenching the product **135** was formed, albeit it 23 % yield.

Given the low yields of the reaction some optimization was carried out by altering conditions, but no significant results were observed (table 5).

Table 5: ylide formation using *n*-BuLi

Temp °C for addition of base	Time at rt before quench	Result
-78 - -50	0	No product complex mixture
-78 - -50	20 min	Degraded SM and trace product
-78 - -50	45 min	Degraded SM and trace product
-78 - -50	1.5 h	Degraded SM and 10-15 % product
-78 - -50	2 h	Degraded SM and 23 % product
-78 - -50	2.5 h	Degraded SM and trace product

The use of another nonreversible base was then considered and sodium hydride was trialed in this reaction (scheme 72).

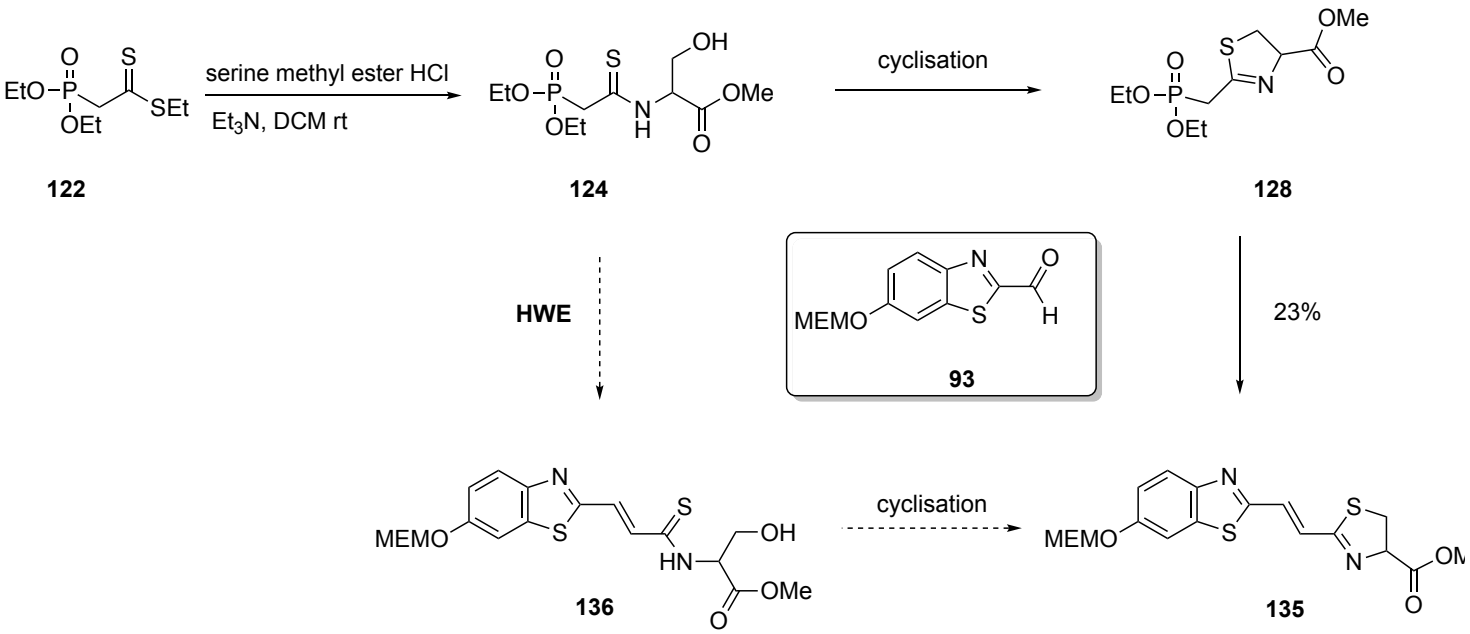


128 93 135

Scheme 72: HWE using NaH and MEM protected aldehyde

The use of sodium hydride gave little or no product within a highly complex mixture of UV active compounds.

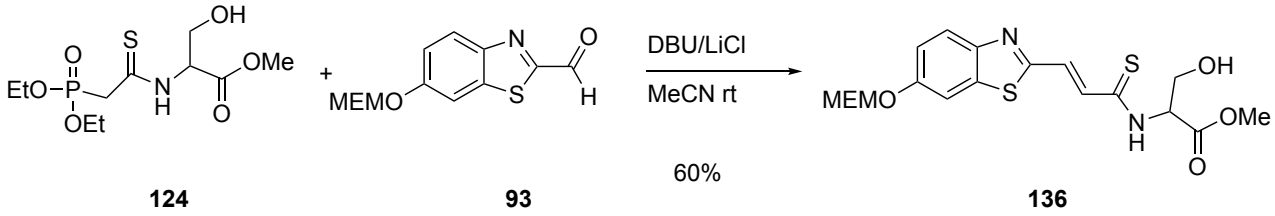
The literature conditions we were utilizing for the HWE reaction had all used thiazolines with either alkyl groups or an alcohol attached, our molecule had an ester in this position and this was possibly making the thiazoline susceptible to dehydrogenation to give a thiazole. For this reason and with the supply of phosphonate **128** consumed, it was decided to perform the HWE reaction with the un-cyclised HWE reagent **124** and the aldehyde **93** (Scheme 73).



122 124 128 93 136 135

Scheme 73: Proposed alternate HWE using un-cyclised phosphonate 124

For this reaction the original conditions from Masson's work using DBU/LiCl were used (scheme 74).[77]

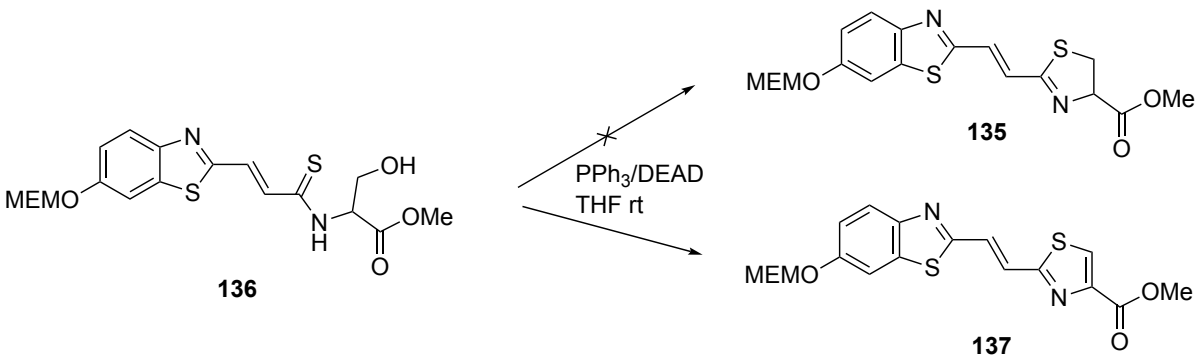


124 **93** **136**

Scheme 74: HWE with un-cyclised phosphonate 1124 using mild DBU/LiCl

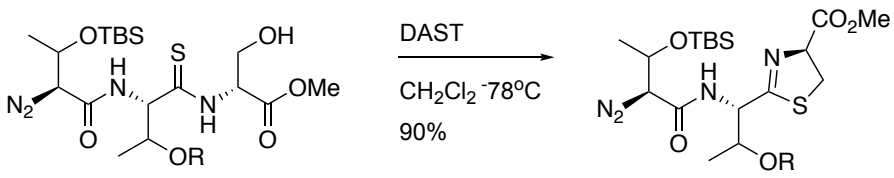
Pleasingly this reaction proceeded to give the product **136** in 60% yield with around 20 % recovered starting material plus some base line material, all of which were easily separated by column chromatography. However **136** was found to unstable even at 0 °C and degraded to a complex mixture of UV active compounds, therefore **136** was purified immediately and used in the next step within 24 hrs.

For the formation of the thiazoline from **135** the same protocol was followed as that for conversion of **124** to **128** (scheme 64).[77] Unfortunately after several attempts this reaction failed to give **135** and only ever resulted in producing the thiazole **137** (scheme 75).



Scheme 75: Cyclisation to thiazoline using DEAD

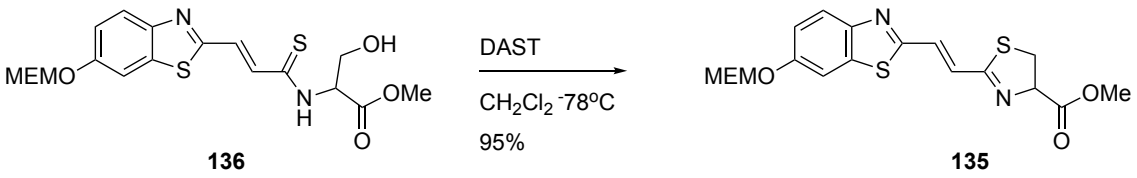
A literature search for the formation of thiazolines yielded a procedure using the reagent DAST. This method was specifically used by Nicolaou to form a thiazoline with an ester attached without the formation of a thiazole (scheme 76).[85]



Scheme 76: Nicolaou thiazoline formation

As HF is a by-product of the reaction and could potentially form the azole, the reaction was quenched at -78 °C with ammonium chloride to form ammonium fluoride and HCl, which effectively prevents azole formation. Ammonium fluoride is water soluble and is removed with all by products by aqueous extraction.

These conditions were used with our intermediate **136** (scheme 77) and gave excellent results, eventually being conducted on a 500 mg scale to give 95 % yields of **135**.

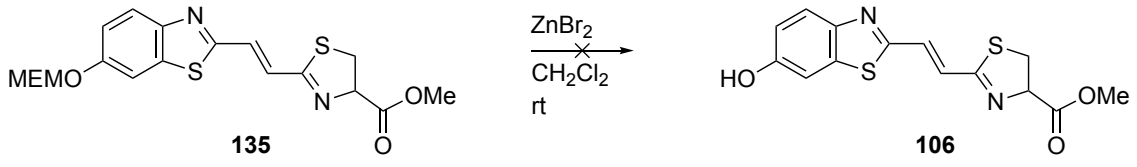


136 **135**

Scheme 77: Cyclisation to thiazoline using DAST

Not only was this reaction very scalable with consistently high yields but the crude product after extracting the aqueous phase with EtOAc was pure enough by ^{1}H NMR (>95 % and no thiazole observed by ^{1}H NMR) to need no further purification for use in the next step.

An attempt was made to remove the MEM protecting group, using $ZnBr_2$ that Williams (scheme 69)[83] had used to give quantitative yields (scheme 78).

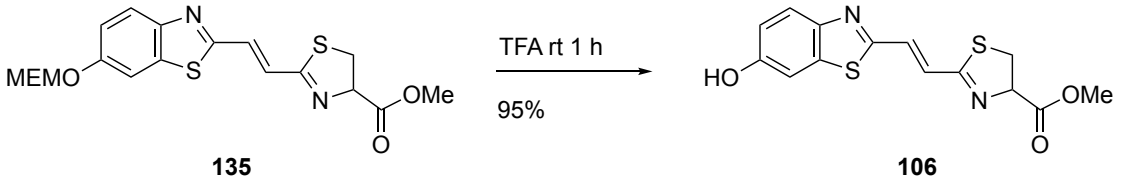


135 **106**

Scheme 78: $ZnBr_2$ MEM deprotection

This reaction failed and gave back only SM and some degraded material; presumably the nitrogen or sulfur atoms coordinate to the zinc and inhibit the deprotection of the MEM group. MEM deprotection occurs via coordination of zinc to 2 of the oxygen atoms in the MEM chain.

Another method was used for the deprotection of the MEM group as used by Branchini and Santaniello, which was to stir in neat TFA (scheme 79).[84]

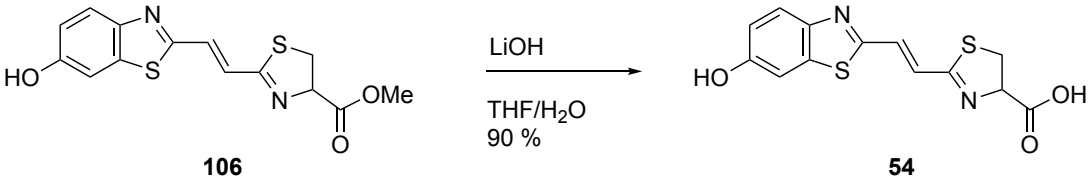


135 **106**

Scheme 79: TFA deprotection of MEM

Initially the exact protocols of Branchini and Santaniello (stirring the substrate in neat TFA at rt for 3 – 4 h, then add H_2O and stirred for 15 min and extracted with EtOAc). This resulted in a 33% yield of material isolated, where there was possibly degradation of our benzothiazole, also TFA was still present as observed via ^{19}F NMR. The reaction was optimised by stirring in neat TFA at rt for 1 hr then quenched with $NaHCO_3$ at 0 °C for 15 min extracted with EtOAc to give 95% of pure **106** with no trace of TFA or thiazole. This reaction was reproducible and could be scaled to give >350 mg of pure product **106** in consistent yields (~ 95 %)

For the final conversion of ester **112** to the free acid to give infraluciferin **54** saponification with LiOH was used (scheme 80).



Scheme 80: Saponification with LiOH

However on closer examination of the final product there was around 1 – 3 % contamination with the thiazoleazole **138** observed by ^{1}H NMR signal at δ 8.21.

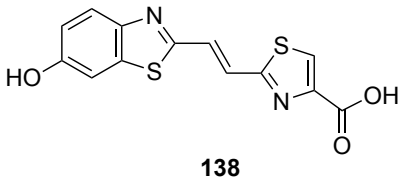


Figure 10: infraluciferin Azole contaminant

Unfortunately even 1 % of this compound drastically reduces light emission from the luciferase enzyme, as this molecule is believed to inhibit the active site and emits no light itself. The initial reaction called for 2 equivalents of LiOH and had a reaction time of 30 minutes. All solvents were flushed with argon as we were aware that the oxygen can lead to dehydrogenation with infraluciferin to give the thiazole. This however did

not prevent the formation of the thiazole. Re-crystallization from MeOH was used to obtain pure infraluciferin, but only around 20 % of the material was returned. Optimization of the LiOH equivalents was carried out to obtain pure infra luciferin from the initial saponification by LiOH (table 6)

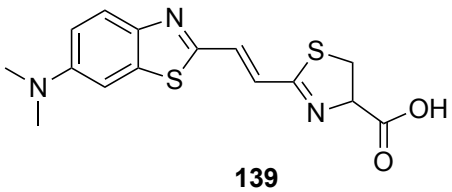
Table 6: Saponification reaction conditions

Eq of LiOH	Reaction Time min	Result
2	30	90 % infra 1 -3% azole
1.5	30	55 % infra 30 % ester 1 -3% azole
2	20	50 % infra 30 % ester 1-2 % azole
2	10	50 % infra 30 % ester 1 % azole
2	5	50 % infra 30 % ester
2.5	5	62 % infra 30 % ester
3	5	77 % infra 20 % ester
3.5	5	85 % infra 10 % ester
4.3	5	90 % infra

This reaction was scaled up to > 150 mg with consistent yields and without formation the thiazole. Over 600 mg of pure infraluciferin was produced using these protocols.

4 Convergent Dithioester route to dimethyl-infra-luciferin

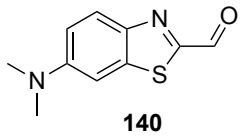
With a successful route to infraluciferin complete it was decided to test the new route to synthesise further analogues. It has been shown that by replacing the hydroxyl group on the benzothiazole portion of the molecule the wave length of light emitted can be altered, in particular work carried out by Miller *et al* showed that having NH_2 on luciferin red-shifted light emission by 36 nm, MeNH 52 nm and Me_2N 66 nm.[86] As the dimethylamine appears to red-shift the furthest and having two methyl groups avoids the need for protecting groups this analogue was chosen as the first analogue to attempt **139**.[86]



139

Figure 11: Dimethylamino infraluciferin

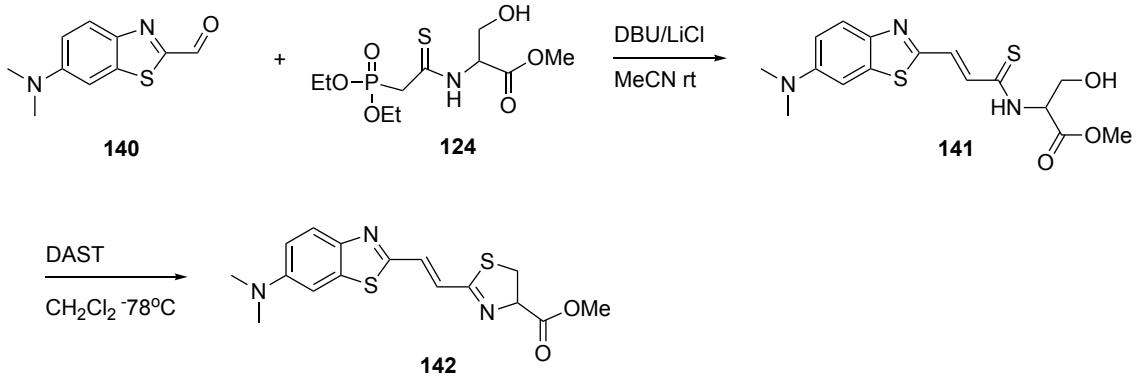
To utilise the synthesis previously developed for infraluciferin the aldehyde **140** first needed to be synthesized.



140

Figure 12: Dimethylamino benzothiazole aldehyde

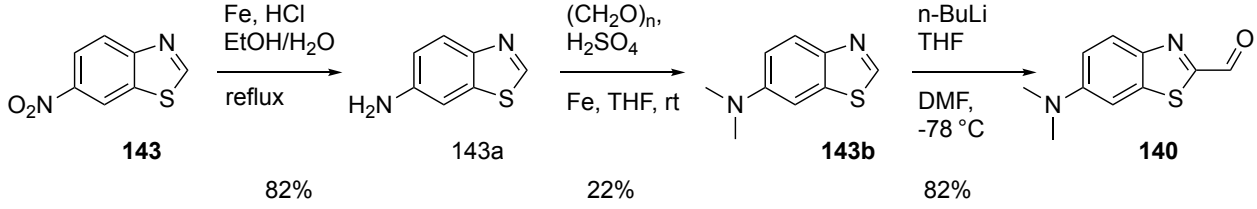
This aldehyde would then be reacted with phosphonate **124** for a HWE reaction under the same conditions previously used, followed by cyclisation using DAST to give **142** (scheme 81).



140 **124** **141** **142**

Scheme 81 Proposed route to 142

Fortunately the desired aldehyde had been previously produced in three steps by Ono *et al* (scheme 82). [87]



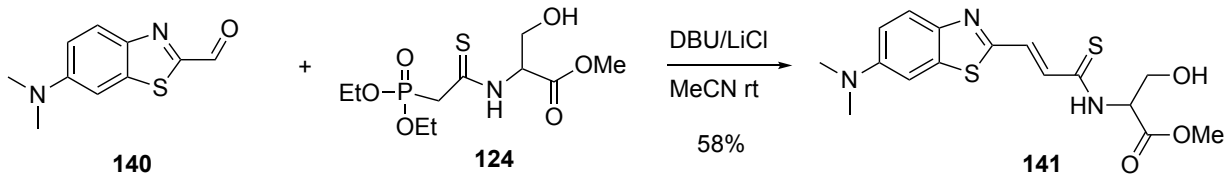
143 143a **143b** **140**

82% 22% 82%

Scheme 82: Three step synthesis of aldehyde 140

The first step known as the Bechamp reduction was achieved in an 82% yield (lit. 91%). The second methylation step was low yielding 22% (lit. 26%) but could be scaled up to multi gram quantities (and the starting materials are much cheaper than that of infraluciferin) giving white crystals after column chromatography. The formylation of **143** proceeds with an 82 % yield after crystallizing from EtOAc (lit. 97%), the literature procedure did not purify the material, it was used as the crude product obtained.

With the desired aldehyde **140** in hand the HWE reaction with **124** was attempted (scheme 83).

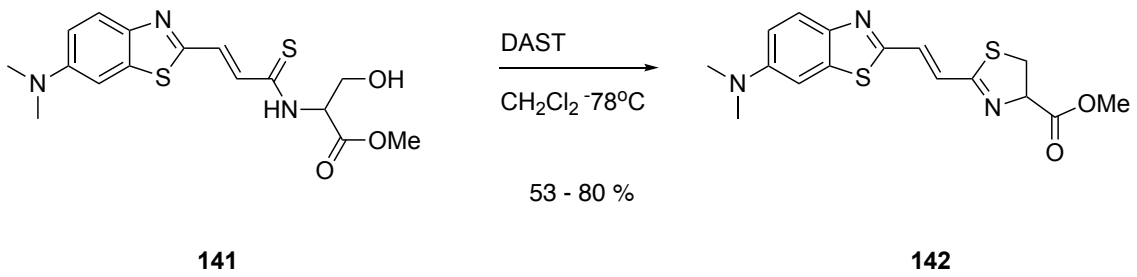


Scheme 83: HWE coupling reaction of 140 and 124

Pleasingly this reaction worked and in yields equal to that as the synthesis of infraluciferin, 58 % after column chromatography. This reaction was scaled up to > 200 mg of **140** without effecting yields.

The cyclisation of infraluciferin utilized DAST as the reagent to cyclize **136** to give **135 (**scheme 77**),** this method was again used for the

conversion of **141** to **142** (scheme 84). The first attempts gave low yields 12 % as opposed to 83 % for infraluciferin (scheme 77).



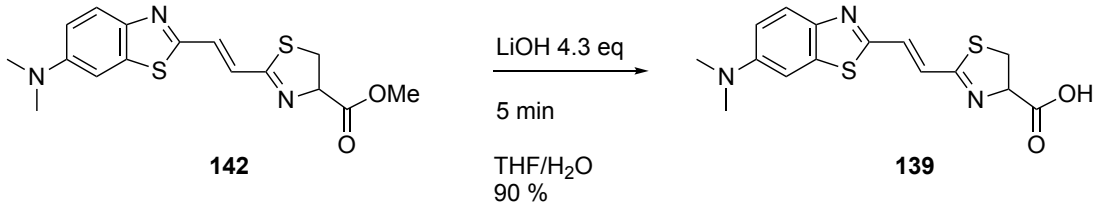
Scheme 84: DAST cyclisation

The original protocols for infraluciferin used 1.2 eq of DAST stirred at -78 °C for 30 min then worked up immediately. As this method showed incomplete conversion by TLC for the dimethylamino substrate **141**, optimization was conducted (table 7).

Table 7: DAST cyclisation of 141

Eq of DAST	Reaction Time at – 78 °C	Reaction Time at rt	Result
1.2	30 min		12 % product + SM
1.2	30 min	15 min	~20 % product + SM
2	30 min	15 min	~30 % product + SM
2	1 h	15 min	~40 % product + SM
2	2.5 h	15 min	~40 % product + SM
2	2.5 h	O/N	~40 % product + SM
3	2.5 h	15 min	~50 % product + SM
4	2.5 h	15 min	>80 % product

With the ester of our dimethyl amino infraluciferin complete the final saponification to the free acid using the protocols developed previously were used (scheme 85).



Scheme 85: Saponification with LiOH

Future Work and conclusions

5.1 Future Work

As is the case with any research project of this nature some aspects require more time invested. It was with this project that time constraints meant that two main areas needed further investigation. Firstly the synthesis of the alkyne bridged luciferin **32** that was an on going project through out the course of the three years, only became as close to completion as it did in the final few weeks of the time that I had available. Therefore I feel that with the final linear synthetic route outlined in Scheme **30** warrants further investigation, and with a few minor synthetic alteration the analogue could be produced.

Secondly one of the goals was to produce the single enantiomer of infraluciferin **104**, some brief work was carried out which utilised the successful new route outlined in chapter 3. However no meaningful data was able to be collected with the time and materials available, however with an efficient route to racemic infraluciferin now complete this would be an ideal starting point with which to attempt to isolate the single enantiomer.

5.2 Conclusions

Focusing on the successes form the time spent on this doctoral research, the new efficient convergent route to the furthest red shifted luciferin analogue infraluciferin has proved to be worthwhile by itself. With over 600 mg produced this has allowed for a large scale parametric in vivo study to be under way which would not have been possible via the original route. This has also resulted in two publications [88,89] and a third pending. Not has this route opened up the use of infraluciferin to more research but has already allowed for the new analogue dimethylinfraluciferin. Hopefully the new route will be utilised to produce even more analogues.

During the course of the research several attempts were made to produce the alkyne-bridged luciferin **32**, finally producing a route with real promise, and at the least has eliminated the other more conventional approaches.

Experimental

6.1 General Experimental

Unless otherwise stated, all reactions were carried out under an atmosphere of nitrogen. All glassware was flame dried under a stream of nitrogen before use. Cooling to 0 °C was effected using an ice-water bath. Reactions were monitored by thin layer chromatography (TLC) using Polygram Sil G/UV254 0.25 mm silica gel precoated plastic plates with fluorescent indicator. Sheets were visualised using ultraviolet light (254 nm), ninhydrin or KMnO4, as appropriate. Flash chromatography was performed using Fluorochem silica

gel 60, 35-70 μ. The liquid phase was analytical grade 40-60 petroleum ether (pet. Ether) and ethyl acetate (EtOAc) unless otherwise stated. Removal of solvents (in vacuo) was achieved using a Vacuubrand diaphragm pump or house vacuum and Büchi rotary evaporators. All NMR data was collected using a Bruker AMX 300 MHz, Bruker AVANCE III 400 MHz, Bruker AVANCE 500 MHz or Bruker AVANCE III 600 MHz. Data was manipulated directly using Bruker XwinNMR (version 2.6) Mnova (version 9.1.0). Reference values for residual solvents were taken as δ = 7.27 (CDCl3) and 2.51 ppm (DMSO-d6) for 1H NMR; δ = 77.16 ppm (CDCl3) for 13C NMR. Multiplicities for coupled signals were denoted as: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br. = broad,

apt. = apparent and dd = double doublet etc. Coupling constants (J) are given in Hz and are uncorrected. Where appropriate, COSY, DEPT, 2HMBC, HMQC and NOE experiments were carried out to aid assignment.

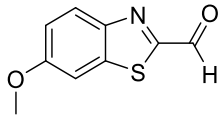
Mass spectroscopy data was collected on a Thermo Finnigan Mat900xp (EI/CI) VG-70se (FAB) and Waters LCT Premier XE (ES) instruments. Infrared data was collected using a Perkin-Elmer 1600 FTIR machine as a thin film unless otherwise stated. Elemental analysis was performed on an Exeter Analytical Inc. EA440 horizontal load analyser. Melting points are uncorrected and were recorded on a Stuart Scientific SMP3 system.

6.2 Purification of Solvents and Reagents

Commercial solvents and reagents were used as supplied or purified in accordance with standard procedures, as described below. THF, Et2O and Toluene were obtained from solvent towers, where the degassed solvent was passed through a 7 micron filter under 4 bar pressure.

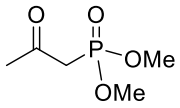
6.3 Experimental Procedures

6-methoxybenzo[d]thiazole-2-carbaldehyde 67 [50]



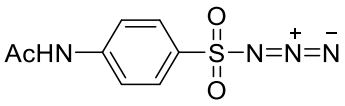
To a solution of 6-methoxy-2-methylbenzothiazole **66** (1.00 g, 5.58 mmol) in dioxane (30 mL) was added selenium dioxide (0.620 g, 5.58 mmol) and the reaction mixture was brought to reflux (105-110 °C). The progress of the reaction was monitored by TLC and after 4 h at reflux the reaction was allowed to cool to room temperature, and left to stir over night. The mixture was filtered through celite and washed with ethyl acetate, then concentrated *in vacuo* to give 1.17 g of orange solid. Purification by column chromatography (100% $CHCl_3$) gave 6-methoxybenzo[d]thiazole-2-carbaldehyde **67** as yellow crystals mp 120 °C (0.690 g, 64%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.10 (s, 1H,CO*H*), 8.11 (d, *J* = 9.1 Hz, 1H, Ar*H*), 7.39 (d, *J* = 2.5 Hz, 1H, Ar*H*), 7.21 (dd, *J* = 9.1, 2.5 Hz, 1H, Ar*H*), 3.93 (s, 3H, OCH_3). NMR data was in agreement with that reported in the literature.[50]

Dimethyl (2-oxopropyl)phosphonate 70 [52]



To a solution of potassium iodide (3.00 g, 18.1 mmol) in MeCN (4.5 mL) and acetone (3.5 mL) to which chloroacetone **69** (1.73 g, 18.1 mmol) was added and stirred at rt for 1 hr. Trimethylphosphite (2.24 g, 18.1 mmol) was added dropwise and stirred at rt 16 hrs. The mixture was then heated at 50 ^{0}C for 30 min to complete conversion and filtered through celite *in vacuo* to give 3.65 g brownish oil. Purification by column chromatography (100% EtOAc) gave dimethyl (2-oxopropyl) phosphonate **70** as a clear oil (1.50 g, 50%, Lit yield 62-70%) ^{1}H NMR (600 MHz, $CDCl_3$) δ 3.74 (d, J = 11.3 Hz, 6H, POCH_3), 3.06 (d, J = 22.8 Hz, 2H, POCH_2), 2.28 (s, 3H, COCH_3). ^{1}H NMR data was in agreement with that reported in the literature.[52]

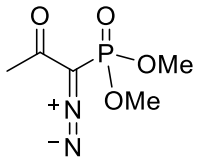
4-acetamidobenzenesulfonyl azide 71 [52]



To a solution of acetamidobenzenesulfonyl chloride **72**(4.00 g, 17.1 mmol) in DCM (32 mL), tetrabutylammonium chloride (TBAC) (14 mg, 0.04mL) was added. Sodium azide (NaN_3) (1.60 g, 25.7 mmol) was dissolved in H_2O (8 mL) and added to the reaction flask, the mixture was stirred overnight at rt. The DCM was separated from the aqueous layer and

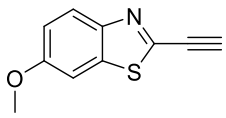
washed with H_2O (3 x 10mL), dried ($MgSO_4$) then concentrated *in vacuo* to give 4-acetamidobenzenesulfonyl azide **71** as a white solid (3.00 g, 72 %, lit yield 91%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.91 (d, *J* = 8.8 Hz, 2H, Ar*H*), 7.78 (d, *J* = 8.8 Hz, 2H, Ar*H*), 7.57 (s, 1H, N*H*), 2.26 (s, 1H). ^{1}H NMR data was in agreement with that reported in the literature.[52]

Dimethyl (1-diazo-2-oxopropyl)phosphonate (Ohira Bestmann reagent) 65 [52]



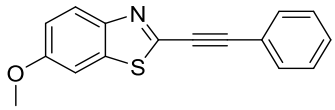
To a solution of dimethyl (2-oxopropyl) phosphonate **70** (0.557 g, 3.35 mmol) in toluene (3 mL) and cooled to 0 °C, was added sodium hydride (NaH) (8 mg, 3.10 mmol) and 4-acetamidobenzenesulfonyl azide **71** (0.74 g, 3.10 mmol) dissolved in THF (1 mL), the reaction mixture was stirred at rt overnight. The mixture was diluted with hexane (5 mL) and filtered through celite, washing with Et_2O (10 mL), then concentrated *in vacuo* to give dimethyl (1-diazo-2-oxopropyl)phosphonate **65** as a slightly yellow oil (0.668 g, 70 %, lit yield 77%) which could be used without further purification. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.82 (d, *J* = 11.9 Hz, 6H, POCH_3), 2.25 (s, 3H, COCH_3). ^{1}H NMR data was in agreement with that reported in the literature.[52]

2-ethynyl-6-methoxybenzo[d]thiazole 68 [51]



To a solution of 6-methoxybenzo[d]thiazole-2-carbaldehyde **67** (0.193 g, 1.00 mmol) in methanol (10 mL) with potassium carbonate (0.276 g, 2.00 mmol) and stirred for 10 minutes. Dimethyl (1-diazo-2-oxopropyl) phosphonate (Ohira Bestmann reagent) (0.230 g, 1.20 mmol) was dissolved in methanol (5 mL) and added to the reaction flask, and the reaction stirred for 4 hrs. Ethyl acetate (10 mL) was used to diluted the reaction mixture and 5% sodium bicarbonate/H_2O (20 mL) added and extracted with EtOAc (3 x 20 mL), the EtOAc fractions were combined and washed with brine (30 mL) and dried with $MgSO_4$ then concentrated *in vacuo* to give 0.200 g of brown oil. Purification by column chromatography (1:1 $CHCl_3$/EtOAc) gave 2-ethynyl-6-methoxybenzo[d]thiazole **68** as a tan powder (0.189 g, 44%); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.95 (d, *J* = 9.0 Hz, 1H, Ar*H*), 7.29 (d, *J* = 2.5 Hz, 1H, Ar*H*), 7.13 (dd, *J* = 9.0, 2.5 Hz, 1H, Ar*H*), 3.89 (s, 3H, COCH_3), 3.56 (s, 1H, C*H*). ^{1}H NMR data was in agreement with that reported in the literature.

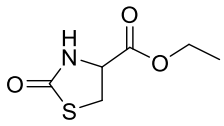
6-methoxy-2-(phenylethynyl)benzo[d]thiazole 78



To a solution of t etrakis (triphenylphosphine) palladium ($Pd(PPh_3)_4$) (14 mg, 0.012 mmol) in DMF (1 mL) (which was degassed by bubbling nitrogen through the solvent for 10 mins) was added iodobenzene **76** (48 mg, 0.24 mmol). To this reaction mixture was added **68** (50 mg, 0.26 mmol) dissolved in DMF (1mL), followed by copper (I) iodide (5 mg, 0.024 mmol) and lutidine (129 mg, 1.2 mmol). The reaction mixture was stirred at rt 16 hrs and the reaction quenched with H_2O (10 mL) and extracted with EtOAc (10 mL x3), the combined organic layers were washed with brine and dried with $MgSO_4$ then concentrated *in vacuo* to give 0.200 g of brown solid, Purification by column chromatography (100% $CHCl_3$) gave 6-methoxy-2-(phenylethynyl)benzo[d]thiazole **78** as a yellow powder (0.054 g, 78%); ^{1}H NMR (600 MHz, Chloroform-*d*) δ 7.96 (d, *J* = 8.9 Hz, 1H, Ar*H*), 7.65 – 7.62 (m, 2H, Ar*H*), 7.43 – 7.38 (m, 3H, Ar*H*), 7.32 (d, *J* = 2.3 Hz, 1H,Ar*H*), 7.13 (dd, *J* = 9.1, 2.5 Hz, 1H, Ar*H*), 3.91 (s, 3H, OC*H*$_3$). ^{13}C NMR (151 MHz, $CDCl_3$) δ 158.8 (s), 147.6 (s), 146.1 (s), 137.1 (s), 132.2 (s), 129.84 (s), 128.7 (s), 124.27 (s), 121.4 (s),

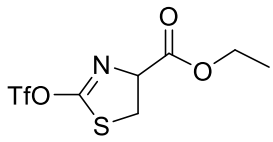
116.6 (s), 103.5 (s), 95.3 (s), 82.91 (s), 55.9 (s). m/z (EI) 220 M+H HRMS $C_{16}H_{11}NOS$ calcd. 266.0640, found 266.0645

Ethyl 2-oxothiazolidine-4-carboxylate 74 [90]



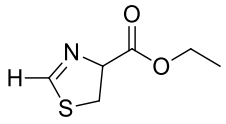
To a solution of cysteine ethyl ester HCl **72** (6.00 g, 32.3 mmol) in H_2O (40 mL) and cooled to 0 $^{\circ}$C, $NaHCO_3$ (2.72 g, 32.3) was added in portions over 30 mins, then K_2CO_3 was added at a rate that kept the reaction mixture below 5 ^{0}C. Triphosgene **73** (4.78 g, 16.14 mmol) was dissolved in THF (12 mL) and added via a pressure equalized dropping funnel over 2 hrs, then stirred overnight. THF was removed *in vacuo* and the aqueous solution was extracted with DCM (3x 30 mL) and the combined organic fractions washed once with brine, then dried with Na_2SO_4 and concentrated *in vacuo* to give ethyl 2-oxothiazolidine-4-carboxylate **74** as a yellowish oil (3.16 g, 55%) which can be used without further purification ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.26 (s, br, 1H, N*H*), 4.43 (t, 1H, COC*H*NH), 4.27 (q, 2H, CH_3CH_2O), 3.70 (dd, *J* = 11.3, 8.3 Hz, 1H, $CHCH_2S$), 3.63 (dd, *J* = 11.3, 5.1 Hz, 1H, $CHCH_2S$), 1.32 (s, 3H, CH_3). ^{1}H NMR data was in agreement with that reported in the literature.

Ethyl 2-(((trifluoromethyl)sulfonyl)oxy)-4,5-dihydrothiazole-4-carboxylate 64 [91]



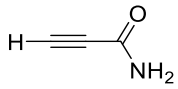
Neat triflic anhydride (1.82 g, 6.46 mmol) was slowly added o a solution of ethyl 2-oxothiazolidine-4-carboxylate **74** (0.942 g, 5.38 mmol) and Et_3N (1.50 mL, 10.8 mmol) in DCM (20 mL) at -78 ^{0}C and stirred for 1 hr. The reaction was quenched with saturated $NaHCO_3$ (20 mL) the organic layer separated and washed with brine (10 mL), then dried (Na_2SO_4) and concentrated *in vacuo* to give 1.27 g of yellowish oil. Purification by column chromatography (3:1 Hexane/EtOAc) gave ethyl 2-(((trifluoromethyl)sulfonyl)oxy)-4,5-dihydrothiazole-4-carboxylate **64** as a slightly yellow oil (0.746 g, 46 %) ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.05 (d, *J* = 7.5 Hz, 1H, COC*H*NH), 4.39 – 4.32 (m, 2H, COCH_2CH$_3$), 3.89 (dd, *J* = 11.8, 7.9 Hz, 1H, CHCH_2S), 3.57 (dd, *J* = 11.9, 1.0 Hz, 1H, CHCH_2S), 1.34 (t, *J* = 7.2 Hz, 3H, CH_3). ^{1}H NMR data was in agreement with that reported in the literature.

Ethyl 4, 5-dihydrothiazole-4-carboxylate 84 [65]



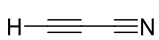
Neat Et_3N (1.25 mL, 9 mmol) was added drop wise to a solution of cysteine ethyl ester HCl **72** (2.01 g, 10.00 mmol) in 1,2 dichloroethane (DCE) (15 mL) at 0 °C, and stirred for 10 mins. To the reaction flask equipped with a soxhlet extractor filled with 4 A molecular sieves was added triethylorthoformate (2.22 g, 15 mmol) and DCE (15 mL) and the mixture refluxed at 145°C for 2 hrs. A catalytic amount of *p*-toluenesulfonic acid was then added reflux continued for 18 hrs. The mixture was then cooled to rt, toluene (5 mL) added and the mixture cooled to -15°C. The precipitate was then filtered and solvent concentrated *in vacuo* to give ethyl 4, 5-dihydrothiazole-4-carboxylate **84** (1.59 g, 100%) as a brown oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.03 (s, 1H), 5.08 (t, *J* = 7.5 Hz, 1H, COC*H*NH), 4.26 (q, *J* = 1.2 Hz, 2H, $COCH_2CH_3$), 3.50 (m, 2H, $CHCH_2S$), 1.33 (t, 3H, CH_3). ^{1}H NMR data was in agreement with that reported in the literature.

Propiolamide 61 [48]



Liquid ammonia (approx. 14 mL, 0.59 mmol) was added to a reaction flask which was cool to -78 °C and fitted with a dry ice condenser. Methylpropiolate **60** (4.13 mL, 50mmol) was added drop wise and the reaction mixture and allowed to stir at -78°C for 2 hrs. The dry ice condenser was removed and the reaction flask was placed in a water bath and allowed to warm to rt whilst the excess NH_3 evaporated. Et_2O (30 mL) was added then the solution filtered and concentrated *in vacuo.* Recrystallization from DCM (50 mL) gave propiolamide **61** as white crystals (2.06 g, 60 %). ^{1}H NMR (500 MHz, CD_3CN) δ 6.71 (s, 1H, N*H*$_2$), 6.26 (s, 1H, N*H*$_2$), 3.26 – 3.19 (m, 1H, C*H*). ^{1}H NMR data was in agreement with that reported in the literature.

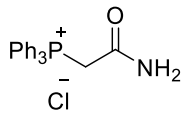
Propiolonitrile 59 [92]



Into a pestle and mortar was ground propiolamide **61** (1.00 g, 14.50 mmol) with sand (approx. 1.00g) and phosphorous pentoxide (3.00 g, 21.75 mmol) until a thick paste was formed. This mixture was transferred to a reaction

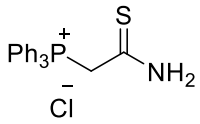
flask fitted with vacuum distillation apparatus fitted and the flask heated to 130 ^{0}C under vacuum (approx. 30-40 mbar) and the distillate allowed to collect in a receiving flask cooled to -78 $^{\circ}$C over 2 hrs to give propiolonitrile **59** as a clear oil (0.400 g, 54%). No further purification was needed. ^{1}H NMR (500 MHz, D_2O) δ 4.70 (s, 1H). ^{1}H NMR data was in agreement with that reported in the literature.

(2-amino-2-oxoethyl) triphenylphosphonium chloride 114 [72]



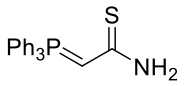
A solution of 2-chloroacetamide **113** (2.00g, 21.40 mmol) and triphenylphosphine in acetonitrile MeCN (10 mL) was heated to 80 $^{\circ}$C overnight. The reaction mixture was cooled to rt and the solids broken up, TBME (10 mL) added and the slurry was filtered and was with TBME(10 mL) to obtain (2-amino-2-oxoethyl) triphenylphosphonium chloride **114** as a white solid mp 180-185 °C (Lit mp 182-185 °C) which was dried *in vacuo* (7.1 g, 93 %). ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.83 (s, 1H, NH_2), 7.78 (m, 15H, Ar*H*), 5.57 (s, 1H, NH_2), 5.09 (d, J = 14.3 Hz, 2H, PCH_2CO). All data was in agreement with the literature. [72]

(2-amino-2-thioxoethyl) triphenylphosphonium chloride 115 [72]



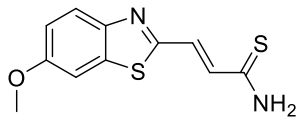
To a solution of (2-amino-2-oxoethyl) triphenylphosphonium chloride **114** (7.14 g, 20 mmol) in toluene (75 mL) was added Lawessons reagent (8.10 g, 20 mmol). The reaction mixture was heated to 175 °C for 4 hrs and the left to stir at rt overnight. The toluene was decanted off and the residue recrystallized from acetone twice, heating to 45 ^{0}C each time, and then recrystallized from EtOH (45 mL) at 45 ^{0}C to yield (2-amino-2-thioxoethyl) triphenylphosphonium chloride **115** as a white solid mp 270-275 °C (Lit mp 273-275 °C) (4.70 g, 64%). ^{1}H NMR (500 MHz, DMSO) δ 7.72 – 7.45 (m, 15H, Ar*H*), 3.81 (d, *J* = 28.2 Hz, 2H, PCH_2CO). All data was in agreement with the literature. [72]

2-(triphenyl-λ^5-phosphanylidene)ethanethioamide 107 [72]



To a solution of (2-amino-2-thioxoethyl) triphenylphosphonium chloride **115** (4.562 g, 12.27 mmol) in EtOH (40 mL), NaOH (0.58 g, 14.72 mmol) in H_2O (25 mL) was added and stirred for 15 min then the reaction flask was place in a freezer for 3hrs, the mixture was filtered and washed with cold EtOH to yield 2-(triphenyl-λ^5-phosphanylidene)ethanethioamide **114** as an off white solid mp 230-235 °C (Lit mp 232-235 °C) (3.27 g, 80%). ^{1}H NMR (300 MHz, DMSO) δ 7.75 – 7.44 (m, 15H, Ar*H*), 6.60 (s, 2H, N*H*$_2$), 3.82 (d, *J* = 28.2 Hz, 1H, PPh$_3$C*H*CS). All data was in agreement with the literature. [72]

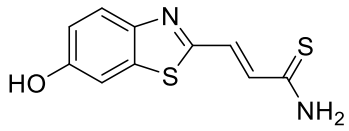
(E)-3-(6-methoxybenzo[d]thiazol-2-yl)prop-2-enethioamide 108



A solution of 6-methoxybenzo[d]thiazole-2-carbaldehyde **67** (0.149 g, 0.77 mmol) and 2-(triphenyl-λ^5-phosphanylidene)ethanethioamide **107** (0.258 g, 0.77 mmol) in DCM (10 mL) were stirred at rt overnight. The reaction mixture were concentrated *in vacuo* to give 0.370 g of dark red solid which shows approx. a 50/50 ratio of E:Z isomers from the crude ^{1}H NMR, if this

crude solid is left to sand in the presence of sunlight the ratio appears to covert to >95% of the desired E isomer. Purification by column chromatography (2:1 hexane/EtOAc) gave (E)-3-(6-methoxybenzo[d]thiazol-2-yl)prop-2-enethioamide **108** as a red solid (0.146 g, 75 %) mp 170-175 °C. ^{1}H NMR (500 MHz, DMSO) δ 9.68 (d, *J* = 16.39 Hz, 2H, N*H*$_2$), 7.93 (t, *J* = 10.2 Hz, 1H, Ar*H*), 7.76 (d, *J* = 15.4 Hz, 1H, CHC*HNCS*), 7.71 (d, *J* = 2.5 Hz, 1H, Ar*H*), 7.26 (d, *J* = 15.4 Hz, 1H, NCSC*HC*H), 7.14 (m, 1H. Ar*H*), 3.84 (s, 3H, OC*H*$_3$). ^{13}C NMR (151 MHz, $CDCl_3$) δ 160.1 (s, S*C*NH$_2$), 157.9 (s, N*C*S), 147.9 (Ar*C*OCH$_3$), 146.4 (s, Ar*C*N), 137.1 (s, Ar*C*S), 133.4 (s, *C*H), 133.3 (s, CH), 124.0 (Ar*C*), 116.7 (Ar*C*), 104.7 (Ar*C*), 55.8 (s, O*C*H$_3$). m/z (EI) 220 M+H HRMS $C_{11}H_{12}N_2OS_2$ calcd. 251.0313, found 251.0310

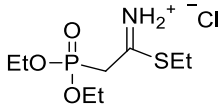
(E)-3-(6-hydroxybenzo[d]thiazol-2-yl) prop-2-enethioamide 109



To a solution of (*E*)-3-(6-methoxybenzo[d]thiazol-2-yl)prop-2-enethioamide **108** (0.100 g, 0.400 mmol) in DCM (5 mL) and cooled to -78 °C was added boron tribromide (BBr_3) as a 1 M solution in DCM (1.2 mL, 1.2 mmol) and the reaction allowed to warm to rt overnight. The reaction

was quenched with 5% $NaHCO_3$ and then extracted with EtOAc x 3 (10 mL), the combined organic fractions were washed with brine and dried with $MgSO_4$, then concentrated *in vacuo* to give 0.94 g of brown solid. Purification by column chromatography (1:1 hexane/EtOAc) gave (E)-3-(6-hydroxybenzo[d]thiazol-2-yl)prop-2-enethioamide **109** (0.030 g, 32 %) as a tan solid. ^{1}H NMR (600 MHz, DMSO) δ 9.69 (d, *J* = 189.3 Hz, 2H, N*H*$_2$), 7.85 (d, *J* = 8.8 Hz, 1H, Ar*H*), 7.75 (d, *J* = 15.4 Hz, 1H, CHC*HNCS*), 7.41 (d, *J* = 2.4 Hz, 1H, Ar*H*), 7.21 (d, *J* = 15.4 Hz, 1H, NCSC*HC*H), 7.01 (dd, *J* = 8.8, 2.4 Hz, 1H, Ar*H*).

Diethylphosphono thioimido acetate hydrochloride 127 [80]

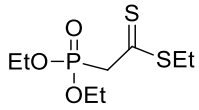


To a solution of diethylcyanomethyl phosphonate **126** (5.00 g, 28.23 mmol) in Et_2O (25 mL) cooled to 0°C was added ethanethiol (5.26 g, 84.68 mmol). HCl gas was passed through the reaction mixture for approx. 3.5 hrs keeping the reaction flask at 0°C. The reaction was monitored by ^{31}P NMR showing a peak at δ 15.64 for the product. Once conversion was complete as seen by ^{31}P NMR the contents were concentrated *in vacuo* to give **127**

6.20 g of yellow viscous oil, this product cannot be purified and is used in the next reaction as is.

^{1}H NMR (300 MHz, $CDCl_3$) δ 4.32 – 4.18 (m, 4H, OCH_2CH_3), 3.70 (d, J = 22.5 Hz, 2H, PCH_2), 3.57 – 3.46 (m, 1H, CH_2CH_3), 1.45 (t, J = 7.4 Hz, 3H, CH_2CH_3), 1.37 (t, J = 7.1 Hz, 6H, OCH_2CH_3). All data was in agreement with the literature. ^{1}H NMR all data was in agreement with the literature.

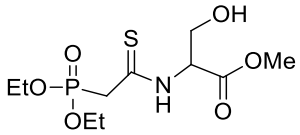
ethyl 2-(diethoxyphosphoryl)ethanedithioate 122 [80]



To a reaction flask containing **127** (6.20 g, 22.5 mmol) and cooled to 0°C was added pyridine (15 mL). Whilst keeping the reaction flask at 0°C hydrogen sulfide gas was passed through the solution, there was observed an immediate colour change to vivid orange and the stream of gas was continued for approx. 2 hours. Completion of the reaction was confirmed by ^{31}P NMR showing a peak at δ 17.94 for the product. Ice cold H_2O (5 mL) was added and the mixture stirred for 5 minutes. HCl/H_2O 3:1 (20 mL) was added and the aqueous phase was extracted with Et_2O (3 x 20 mL) and the organic phase washed once with HCl/H_2O 3:1 (20 mL) dried with $MgSO_4$ and concentrated *in vacuo* to give **122** 4.3 g of orange oil

^{1}H NMR (300 MHz, $CDCl_3$) δ 4.16 (dq, *J* = 14.2, 7.1 Hz, 4H), 3.80 (d, *J* = 22.7 Hz, 2H), 3.23 (q, *J* = 7.4 Hz, 2H), 1.34 (m, 9H). ^{1}H NMR data was in agreement with the literature.

Methyl 2-(2-(diethoxyphosphoryl)ethanethioamido)-3-hydroxypropanoate 124

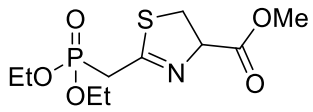


To a solution serine methyl ester in DCM (50 mL) neat Et_3N (0.61 mL 4.41 mmol) was added with stirring until completely dissolved**.** A solution of Ethyl 2-(diethoxyphosphoryl)ethanedithioate **122** (1.03 g, 4.01 mmol) in DCM (10 mL) was added drop wise to the reaction flask. The reaction was stirred at rt for 2 days until ^{31}P NMR showed complete conversion to the product. The reaction mixture was concentrated *in vacuo* to give yellowish oil, which when dissolved in EtOAc precipitated excess serine methyl ester formed as a white solid that was filtered off and the mother liquor purified by column chromatography (100 % EtOAc - 5% MeOH – 10% MeOH) to give methyl 2-(2-(diethoxyphosphoryl)ethanethioamido)-3-hydroxypropanoate **124 (**0.70 g, 56%) as a yellow oil.

^{1}H NMR (300 MHz, $CDCl_3$) δ 4.17 (m, 5H, OCH_2 + CH_2OH), 4.00 (dd, *J* = 11.8, 2.8 Hz, 1H, CH_2OH), 3.82 (s, 1H, OCH_3), 3.49 (m, 2H, PCH_2), 1.35

(m, 6H, CH_2CH_3). ^{13}C NMR (151 MHz, $CDCl_3$) δ 63.25 (d, *J* = 140 Hz, OCH_2CH_3), 61.39 (s, CH_2OH), 60.81 (s, NHCH), 52.94 (s, OCH_3), 45.10 (d, *J* = 127.1 Hz, PCH_2), 16.47 (s, CH_2CH_3). IR ν_{max} (thin film) 3310 – 3243, 2982, 1741, 1512, 1225, 1010. m/z (EI) HRMS $C_{14}H_{21}NOPS_2$ calcd.314.0802, found 314.0803

methyl 2-((diethoxyphosphoryl)methyl)-4,5-dihydrothiazole-4-carboxylate 128

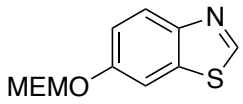


A solution of methyl 2-(2-(diethoxyphosphoryl)ethanethioamido)-3-hydroxypropanoate **124** (1.46 g, 4.66 mmol) in THF (100 mL) was treat with triphenylphosphine (2.20 g, 8.40 mmol). After stirring a rt for 5 min neat DEAD (1.32 mL, 8.40 mmol) was added dropwise. After stirring at rt for 4 hrs the solution was concentrated in *vacuo.* Purification was archived by flash chromatography (5% MeOH/EtOAc) to give **128** (1.38 g, 85 %) as a yellowish oil

1H NMR (300 MHz, CDCl3) δ 5.08 (dd, J = 15.1, 9.4 Hz, ^1H, $CHCH_2$), 4.25 – 4.03 (m, 4H, CH_3CH_2O), 3.80 (s, 3H, CH_3O), 3.71 – 3.50 (m, 2H,

SC*H*$_2$CH), 3.31 – 3.10 (m, 2H, C*H*$_2$P), 1.33 (t, J = 7.1 Hz, 6H, CH_3CH_2O). ^{13}C NMR (151 MHz, CDCl3) δ 171.10 (s, *C*O), 165.67 (s, S*C*N), 77.75 (s, *C*HCH$_2$), 62.90 (t, J = 6.0 Hz, PO*C*H$_2$CH$_3$), 52.93 (s, *C*H$_3$O), 36.54 (s, *C*H$_2$CH), 33.46 (d, J = 137.7 Hz, *C*H$_2$P), 16.49 (d, J = 4.4 Hz, *C*H$_3$CH$_2$). IR νmax (thin film) 2980, 1742, 1255, 1023. m/z (EI) 220 M+ H HRMS ($C_{10}H_{18}NO_5PS$+H) calcd. 296.0721. found 296.0719

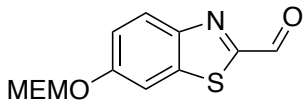
6-((2-methoxyethoxy)methoxy)benzo[*d*]thiazole 134a [84]



A solution of **134** (1 g, 6.60 mmol) in THF (80 mL) was treated cooled to 0 °C and NaH (316 mg, 13.20 mmol) added in portions. The reaction mixture was stirred at rt for 1 h then cooled to 0 °C and 2-methoxyethoxymethyl.Cl added drop wise and stirred at rt over night. The reaction mixture was quenched with 5% KCO_3 in H_2O (160 mL) and extracted with EtOAc (80 mL x3) and the organic fractions were combined and washed with brine and dried with $MgSO_4$ filtered and concentrated. Purification was achieved by flash chromatography (1:1 EtOAc/Hex) to give **134a** (833 mg, 53 %) as orange oil

1H NMR (300 MHz, CDCl3) δ 8.86 (s, 1H, C*H*NCS), 8.02 (d, J = 8.9 Hz 1H, CHC*H*CO), 7.65 (s, 1H, C*H*CO), 7.22 (dd, J = 8.9 Hz, 2.4Hz 1H, C*H*Ar), 5.34 (s, 2H, OC*H*O), 3.86 (dd, J = 5.6 Hz, 3.7 Hz 2H, 2H, CH_2CH_2O), 3.57 (dd, J = 5.5 Hz, 3.7 Hz 2H, OCH_2CH_2), 3.37 (s, 3H, CH_3O). ^{1}H NMR data was in agreement with the literature

6-((2-methoxyethoxy)methoxy)benzo[*d*]thiazole-2-carbaldehyde 93 [84]

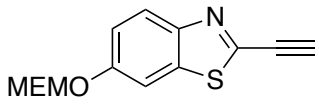


A solution of *n*-BuLi (3.83 mmol) in THF (20 mL) was cooled to -78 °C and a solution of **134a** (833 mg, 3.48 mmol) in THF (5 mL) was added drop wise and the mixture warmed to -50 °C for 1 h. The reaction mixture was cooled to -78 °C and DMF (1.08 mL, 13.92 mmol) was added drop wise and the reaction was stirred at -78 °C for 2 h. The reaction was quenched saturated $NaHCO_3$ (50 mL) and extracted with EtOAc (50 mL x 3). The organic phase dried with $MgSO_4$ and concentrated *in vacuo*. Purification was achieved by flash chromatography (2:1-1:1 EtOAc/Hex) to give **93** (672 mg, 72 %) as an orange oil

1H NMR (300 MHz, CDCl3) δ 10.11 (s, 1H, C*H*CO), 8.12 (d, J = 9 Hz 1H, CHC*H*CO), 7.67 (s, 1H, C*H*CO), 7.31 (dd, J = 9 Hz, 2.4Hz 1H, C*H*Ar),

5.38 (s, 2H, OC*H*O), 3.87 (m, 2H, 2H, $CH_2C\mathit{H}_2O$), 3.58 (m, 2H, $OC\mathit{H}_2CH_2$), 3.38 (s, 3H, $C\mathit{H}_3O$). ^{1}H NMR data was in agreement with the literature

2-ethynyl-6-((2-methoxyethoxy)methoxy)benzo[*d*]thiazole 94

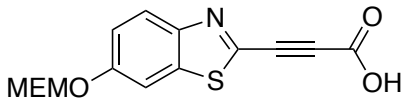


To a solution of **93** (742 mg, 2.78 mmol) in methanol (25 mL) with potassium carbonate (768 mg, 5.56 mmol) and stirred for 10 minutes. Dimethyl (1-diazo-2-oxopropyl) phosphonate **68** (Ohira Bestmann reagent) (640 mg, 3.33 mmol) was dissolved in methanol (5 mL) and added to the reaction flask, and the reaction stirred for 4 hrs. Ethyl acetate (20 mL) was used to diluted the reaction mixture and 5% sodium bicarbonate/H_2O (40 mL) added and extracted with EtOAc (3 x 40 mL), the EtOAc fractions were combined washed with brine and dried with $MgSO_4$ then concentrated *in vacuo*. Purification by column chromatography (1.5:1 Hex/EtOAc) gave **94** as a orange waxy solid (387 mg, 60%)

1H NMR (300 MHz, $CDCl_3$) δ 7.96 (d, J = 8.8 Hz 1H, CHC*H*CO), 7.56 (s, 1H, C*H*CO), 7.23 (dd, J = 9 Hz, 2.4Hz 1H, C*H*Ar), 5.35 (s, 2H, OC*H*O), 3.86 (m, 2H, 2H, $CH_2C\mathit{H}_2O$), 3.58 (m, 2H, $OC\mathit{H}_2CH_2$), 3.57 (s, 1H, C*H*C),

3.38 (s, 3H, C*H*$_3$O). ^{13}C NMR (151 MHz, $CDCl_3$) δ 156.57 (1C), 148.00 (1C), 145.60 (1C), 136.69 (1C), 124.54 (1C, CHAr), 118.01 (1C, CHAr) 106.91 (1C, CHAr), 94.00 (1C, CH_2), 83.59 (1C), 71.66 (1C, CH_2), 68.019 (1C, CH_2), 59.20 m/z (EI) 220 M+ HRMS $C_{13}H_{13}NO_3S_s$ calcd. 264.0694, found 264.0702

3-(6-((2-methoxyethoxy)methoxy)benzo[*d*]thiazol-2-yl)propiolic acid 95

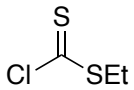


A solution of **94** (369 mg, 1.58 mmol) in THF (6 mL) was cooled to -78 °C and *n*-BuLi (1.74 mmol) was added drop wise then left to stir for 30 min. A stream of CO_2 was passed through CaCl and bubbled through the reaction mixture for 30 min. The reaction was warmed to rt and then made acidic (~pH 3) with 2M HCl then extracted with Et_2O (10 mL x 3). The combined organic fractions were made basic with 5% Na_2CO_3/H_2O solution and extracted with H_2O (10 mL x 3). The combined aqueous fractions were again acidified and extracted with Et_2O (10 mL x 3) washed with brine and dried with $NaSO_4$ filtered and concentrated in *vacuo* to give **95** (169 mg, 39 %) as an off white solid mp 71-76 °C

1H NMR (300 MHz, CDCl3) δ 8.07 (d, J = 9 Hz 1H, CHC*H*CO), 7.56 (s, 1H, C*H*CO), 7.28 (m, 1H, C*H*Ar), 5.36 (s, 2H, OC*H*O), 3.88 (m, 2H, 2H,

CH_2CH_2O), 3.62 (m, 2H, OCH_2CH_2), 3.40 (s, 3H, CH_3O). ^{13}C NMR (151 MHz, $CDCl_3$) δ 157.34 (1C), 153.87 (1C), 147.74 (1C), 143.50 (1C), 137.46 (1C), 125.00 (1C CHAr), 118.98 (1C CHAr), 106.71 (1C CHAr), 93.99 (1C CH_2), 84.86 (1C), 78.50 (1C), 71.71 (1C CH_2), 68.00 (1C CH_2), 59.09 (1C CH_3) m/z (EI) 220 HRMS $C_{14}H_{13}NO_5S_s$ calcd. 308.0593, found 308.0595

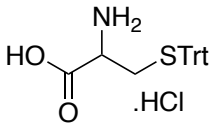
ethyl carbonochloridodithioate 89



A solution of thiophosgene (6.02g, 52.4 mmol) in benzene (10 mL) was cooled to 0 °C and ethanethiol (3.88 mL, 52.4 mmol) in benzene (7 mL) was added drop wise. The reaction mixture was stirred at 0 °C over night and then concentrated in *vacuo*. . Purification was achieved by fractional distillation to give **89** (485 mg, 60 %) as red oil.

1H NMR (300 MHz, CDCl3) δ 3.20 (q, J = 7.1 Hz 1H, CH_2), 1.37 (t, 7.4, CH_3) ^1H NMR data was in agreement with the literature

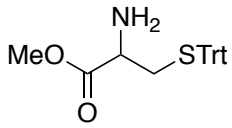
S-tritylcysteine 92 [46]



A solution of DL-cysteine. HCl (600 mg, 3.8 mmol) in DMF (3 mL) was treated with tritylmethyl chloride (1.62g, 5.81 mmol) and stirred at rt for 2 days. To the reaction flask was added 10 % NaOAc/H_2O (25 mL) which produced a white precipitate that was filtered off and washed with H_2O. The material obtained was dissolved in acetone and heated to 50 °C for 1 h then cooled to rt, filtered and rinsed with acetone then Et_2O and dried in *vacuo* to give **92** (870 mg, 65 % lit 89%) as a white solid

1H NMR (300 MHz, DMSO) δ 7.22 (m, 15H Ar), 2.91 (m, 1H C*H*), 2.58 (dd, J= 4.4 Hz, 12 Hz, 1H C*H*2) 2.49(dd 9 Hz, 12Hz, 1H CH_2) mp 190-195 °C (lit 195°C) ^{1}H NMR data was in agreement with the literature

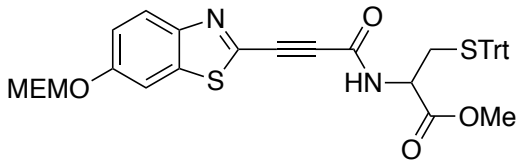
methyl S-tritylcysteinate 92a [46]



A solution of **92** (870 mg, 2.47 mmol) in MeOH (18 mL) was cooled to 0 °C and thionylchloride (1.35 mL, 18.57 mmol) was added drop wise. The reaction mixture was warmed to rt then concentrated in *vacuo* to give **90a** (910 mg, 89 % lit 97%) as a white solid

1H NMR (300 MHz, DMSO) δ 7.27-7.39 (m, 15 H Ar), 3.85 (dd, J= 6 Hz, 6 Hz 1H C*H*), 3.70 (s, 3H, CO_2CH_3) 2.58 (m, 2H CH_2) mp 73-76 °C (lit 78°C) ^{1}H NMR data was in agreement with the literature

methyl *N*-(3-(6-((2-methoxyethoxy)methoxy)benzo[*d*]thiazol-2-yl)propioloyl)-*S*-tritylcysteinate 96

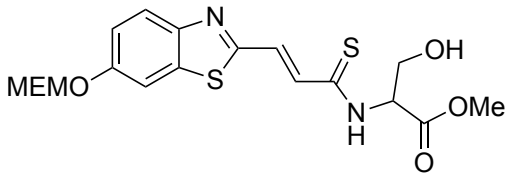


A solution of **95** (176 mg, 0.429 mmol) in DCM (8 mL) was treated with DIPEA (75 μL, 0.429 mmol). To the reaction mixture was added **92a** and the solution cooled to 0 °C, then diisopropylcarbonimide (64 μL, 0.409 mmol) was added and left at rt over night. The reaction was quenched with saturated NH_4Cl (4 mL) and extracted with DCM dried with $NaSO_4$ filtered and concentrated in *vacuo.* Purification was achieved by flash chromatography (1:1 EtOAc/Hex) to give **96** (54 mg, 27 %) as a yellow waxy solid

^{1}H NMR (600 MHz, $CDCl_3$) δ 8.07 (d, J = 9 Hz 1H, CHC*H*CO), 7.56 (s, 1H, C*H*CO), 7.28 (m, 1H, C*H*Ar), 5.36 (s, 2H, OC*H*O), 3.88 (m, 2H, 2H, CH_2CH_2O), 3.62 (m, 2H, OCH_2CH_2), 3.40 (s, 3H, CH_3O). ^{13}C NMR (151 MHz, $CDCl_3$) δ 168.89 (1C), 157.06 (1C), 151.31 (1C), 148.25 (1C),

144.23 (1C), 143.23 (1C), 137.44 (1C), 129.59 (6 C Trt), 128.26 (6 C Trt), 127.18 (3 C Trt), 125.04 (1 C Ar), 118.92 (1 C Ar), 93.96 (1 C CH_2), 71.65 (1 C CH_2), 68.08 (1 C CH_2), 60.64 (1 C), 59.21 (1C CH_3), 53.08 (1C CH_3), 51.65 (1C CH), 33.50 (1C CH_2), 29.83 (1C), 21.21 (1C), 20.30 (1C), 19.86 (1C), 14.33 (1C) m/z (EI) 220 M+ H HRMS $C_{37}H_{34}N_2O_6S_2$ calcd. 677.1937, found 677.1873

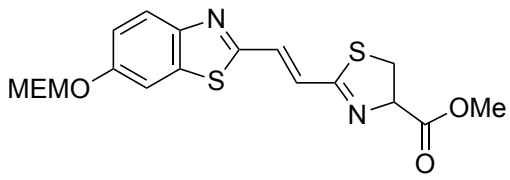
methyl-(*E*)-(3-(6-((2-methoxyethoxy)methoxy)benzo[*d*]thiazol-2-yl)prop-2-enethioyl)serinate 136



A solution **93** methyl 2-(2-(diethoxyphosphoryl)ethanethioamido)-3-hydroxypropanoate (274 mg, 0.874 mmol) in MeCN (4mL) was treated with DBU (146 mg, 0.961 mmol) and LiCl (41 mg, 0.961 mmol). After stirring at rt for 5 min a solution of **124** 6-(*β*-Methoxyethoxymethyl ether)-2-formylbenzothiazole in MeCN (4 mL) was added dropwise and stirred as rt for 3.5 hrs. The solution was filtered and concentrated in *vacuo.* Purification was achieved by flash chromatography (75 % EtOAc/Hex) to give **136** (188 mg, 50 %) as an orange oil

^{1}H NMR (600 MHz, $CDCl_3$) δ 8.95 (br. s, 1H, CH_2OH) 8.02 (d, *J* = 9.0 Hz, 1H, C*H* Ar), 7.95 (d, *J* = 15.1 Hz, 1H, CHC*H*CS), 7.60 (d, *J* = 7.25 Hz, 1H, C*H* Ar), 7.45 (d, *J* = 15.1 Hz, 1H, SNCC*HCH*), 7.25 (d, *J* = 2.5 Hz, 1H, C*H* Ar), 5.39 (m, 1H, CH_2CHNH), 5.36 (s, 2H,OCH_2O), 4.2 (dd, *J* = 11.7, 3.3 Hz, 2H, CH_2OH), 3.86 (m, 2H, CH_3OCH_2), 3.85 (s, 3H, $COOCH_3$), 3.58 (m, 2H, CH_2CH_2O), 3.38 (s, 3H, CH_3O). ^{13}C NMR (151 MHz, $CDCl_3$) δ 192.83 (1C), 170.01 (1C), 163.02 (1C), 156.84 (1C), 135.56 (1C), 134.99(1C, CH), 131.04 (1C, CH), 123.26 (1C, CHAr), 119.13 (1C, CHAr), 107.44 (1C), 94.00 (1C), 77.3 (1C CH), 71.63(1C, CH_2), 68.15(1C, CH_2), 62.07(1C, CH_2), 60.49(1C, CH), 59.21 (1C, CH_3), 53.17 (1C, CH_3), 14.32(1C). IR ν_{max} (thin film) 3100-3500, 2925, 1740, 1597, 1194, 1077, 991, 822, 732. m/z (EI) 220 M+ H HRMS $C_{36}H_{234}N_2O_6S_2$ calcd. 427.0998, found 427.0999

methyl (*E*)-2-(2-(6-((2-methoxyethoxy)methoxy)benzo[*d*]thiazol-2-yl)vinyl)-4,5-dihydrothiazole-4-carboxylate 135

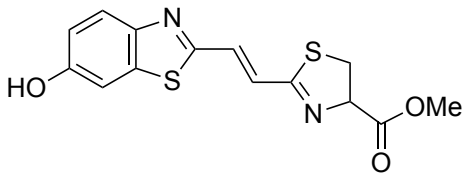


A solution of **136** (230 mg, 0.54 mmol) in DCM (3 mL) cooled to -78 °C was treated with DAST (85 μL 0.64 mmol). After stirring at -78 °C for 30 min the reaction was quenched with saturated aqueous NH_4Cl solution (3.5 mL). The layers were separated and the aqueous layer was re-extracted with DCM (2 × 10 mL). The combined organic layers were washed with brine (30 mL), dried Na_2SO_4, and concentrated. Purification was achieved by flash chromatography (75 % EtOAc/Hex) to give **135** (185 mg, 83 %) as an orange waxy solid.

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.93 (d, *J* = 9.0 Hz, 1H, C*H* Ar), 7.57 (d, *J* = 2.4 Hz, 1H, C*H* Ar), 7.41 (d, *J* = 16.2 Hz, 1H, NSCC*H*CH), 7.33 (d, *J* = 16.2 Hz, 1H, CHC*H*CNS), 7.20 (dd, *J* = 9.0, 2.46 Hz, 1H, C*H* Ar), 5.34 (s, 2H, OCH_2O), 5.25 (t, *J* = 9.3 Hz, 1H, NC*H*CH_2), 3.88 – 3.82 (m, 5H, COOCH_3, CH_3OCH_2), 3.73 – 3.60 (m, 2H, SCH_2CH), 3.57 (m, 2H, CH_2CH_2O), 3.38 (s, 1H, CH_3O).

^{13}C NMR (151 MHz, $CDCl_3$) δ 170.95(1C), 169.33(1C), 162.61(1C), 156.37(1C), 149.24(1C), 136.49(1C), 135.04(1*C*,CH), 129.27(1C, *C*H), 124.51(1C. CHAr), 117.77(1*C,* CHAr), 107.44(1*C, CH*Ar), 93.99 (1C, CH_2), 78.14 (1C, *C*H), 71.66 (1C, *C*H_2), 68.01 (1C, *C*H_2), 59.20 (1C, *C*H_3), 53.13(1C, *C*H_3), 35.07(1C, *C*H_2). IR ν_{max} (thin film) 2900, 1737, 1597, 1452, 1199, 1102, 991 m/z (EI) 220 M+ H HRMS $C_{18}H_{20}N_2O_5S_2$ calcd. 409.0892, found 409.0894

methyl-(*E*)-2-(2-(6-hydroxybenzo[*d*]thiazol-2-yl)vinyl)-4,5-dihydrothiazole-4-carboxylate 106 [46]

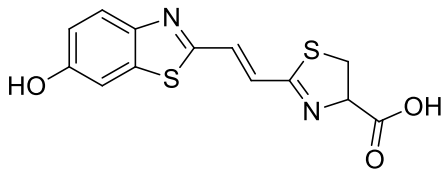


A solution of **135** (440 mg, 0.198 mmol) in neat TFA (14 mL) was stirred at rt for 1 h. The reaction mixture was cooled to 0 °C and saturated $NaHCO_3$ (200 mL) was added until a pH of 7-8 was attained. Extracted with EtOAc (3 x 50 mL) and the combined organic fractions were washed with brine, filtered and concentrated to give **106** as a yellowish solid (298 mg, 95 %).

^{1}H NMR (600 MHz, MeOD) δ 7.82 (d, J = 8.9 Hz, 1H, CHAr), 7.40 (d, J = 16.1 Hz, 1H, CHCH), 7.29 (d, J = 2.5 Hz, 1H, CHAr), 7.29 (d, J = 16.1 Hz,

1H,CHCH), 7.03 (d, J = 8.9Hz, 1H, CHAr), 5.33 (t, J = 9.0 Hz, 1H), 3.76 – 3.66 (m, 2H, SCH_2).) ^{1}H NMR data was in agreement with the literature

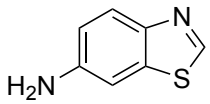
(*E*)-2-(2-(6-hydroxybenzo[*d*]thiazol-2-yl)vinyl)-4,5-dihydrothiazole-4-carboxylic acid 54 [46]



A solution of infraluciferin methyl ester **106** (50 mg, 0.156 mmol) in THF/H_2O (2.4/1.2 mL) was treated with LiOH (8 mg, 0.372 mmol). After stirring at rt for 5 min H_2O (20 mL) was added and the aqueous solution was washed with EtOAc (20 mL). The aqueous phase was then made basic (pH 3) with 2M HCl, and then extract with EtOAc (20 mL x 3). The organic extractions were combined and dried with $MgSO_4$ filtered and concentrated to give infraluciferin **544** as a free flowing orange powder (44 mg, 92 %).

^{1}H NMR (600 MHz, MeOD) δ 7.82 (d, J = 8.9 Hz, 1H), 7.40 (d, J = 16.1 Hz, 1H), 7.31 (d, J = 2.5 Hz, 1H), 7.30 (d, J = 16.1 Hz, 1H), 7.03 (dd, J = 8.9, 2.4 Hz, 1H), 5.30 (t, J = 9.0 Hz, 1H), 3.76 – 3.66 (m, 2H). ^{1}H NMR data was in agreement with the literature.

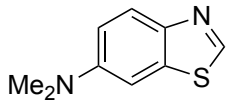
benzo[*d*]thiazol-6-amine 143a [87]



A solution of HCl (3 mL, 34 mmol) in EtOH/H_2O (80/20 mL) was added 6-nitrobenzothiazole **143** (3.90 g, 21.65 mmol) and iron powder (5.6 g, 101 mmol). After stirring at 114 °C for 2 the solution was filtered and rinsed with EtOH. A solution of 10 % $NaCO_3$/H_2O was added and then extracted with EtOAc. The organic extractions were combined and dried with $MgSO_4$ filtered and concentrated to give **143a** as abrown solid (3.25 g, 82 % lit 92%).

1HNMR (400 MHz, CDCl3) d 8.70 (s, 1H), 7.89 (d, J = 8.8 Hz, 1H), 7.17 (d, J = 2.4 Hz, 1H), 6.87 (dd, J = 8.8, 2.4 Hz, 1H), 3.85 (br s, 2H). ^{1}H NMR data was in agreement with the literature.

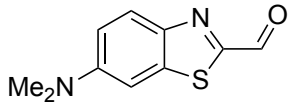
***N*,*N*-dimethylbenzo[*d*]thiazol-6-amine 143b** [87]



To a solution of 6-aminobenzothiazole **143a** (2.68 g, 17.84 mmol) in THF (70 mL) was added formaldehyde/H_2SO_4 (13/14 mL) iron powder (7.97 g, 143 mmol) was then added and the mixture was stirred at rt for 3.5 h. NaOH/H_2O (5 g / 90 mL) was added to the reaction mixture until basic. The aqueous phase was extracted with EtOAc and the organic phase dried with $MgSO_4$ and concentrated *in vacuo* to give 4.25 g of orange/brown waxy solid. Purification was achieved by flash chromatography (1:1 EtOAc/Hex) to give **143b** (680 mg, 22 %) as white crystals.

^{1}H NMR (400 MHz, CDCl3) d 8.67 (s, 1H), 7.95 (d, J = 8.8 Hz, 1H), 7.15 (d, J = 2.4 Hz, 1H), 7.00 (dd, J = 8.8, 2.4 Hz, 1H), 3.04 (s, 6H). ^{1}H NMR data was in agreement with the literature.

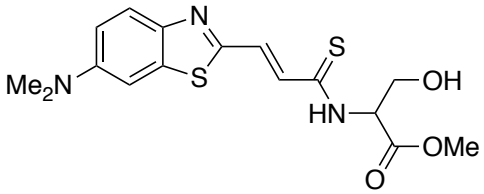
6-(dimethylamino)benzo[*d*]thiazole-2-carbaldehyde 140 [87]



A solution of *n*-BuLi (3.98 mmol) in THF (12 mL) was cooled to -78 °C and a solution of **143b** (676 mg, 3.79 mmol) in THF (6 mL) was added drop wise and the mixture warmed to -50 °C for 1 h. The reaction mixture was cooled to -78 °C and DMF (1.17 mL, 15 mmol) was added dropwise and the reaction was stirred at -78 °C for 2 h. The reaction was quenched with H_2O (35 mL) and extracted with DCM (50 mL x 3). The organic phase dried with $NaSO_4$ and concentrated *in vacuo* to give **140** (760 mg, 82 %) of orange crystals which were >95 % pure by ^{1}H NMR

^{1}H NMR (400 MHz, $CDCl_3$) d 10.06 (s, 1H), 8.03 (d, J = 10.0 Hz, 1H), 7.07– 7.04 (m, 2H), 3.12 (s, 6H) ^{1}H NMR data was in agreement with the literature.

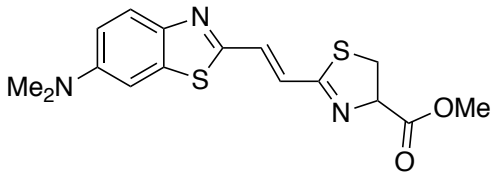
Methyl-(*E*)-(3-(6-(dimethylamino)benzo[*d*]thiazol-2-yl)prop-2enethioyl)serinate 141



A solution **124** methyl 2-(2-(diethoxyphosphoryl)ethanethioamido)-3-hydroxypropanoate (68 mg, 0.22 mmol) in MeCN (1 mL) was treated with DBU (36 mg, 0.24 mmol) and LiCl 10 mg, 0.24 mmol). After stirring at rt for 5 min a solution of **140** in MeCN (1 mL) was added drop wise and stirred as rt for 3.5 hrs. The solution was filtered and concentrated in *vacuo.* Purification was achieved by flash chromatography (1:1 EtOAc/Hex) to give **141** (27 mg, 34 %) as an orange oil

^{1}H NMR (600 MHz, $CDCl_3$) δ 8.13 (s, 1H, CHAr), 9.95 (d, J = 15.6 Hz, 1H, CHCH), 7.86 (d, J = 9.4 Hz, 1H, CHAr), 7.16 (d, J = 15.6 Hz, 1H, CHCH), 6.97 (d, J = 11.2, 1H, CHAr), 5.46 (s, 1H, CHNH), 4.16 (m, 2H, $CHCH_2OH$), 3.86 (s, 3H, OCH_3), 3.06 (s, 6H, CH_3NCH_3. m/z (EI) 220 M+ HRMS $C_{16}H_{19}N_3O_3S_2$ calcd. 366.0946, found 366.0950

methyl (*E*)-2-(2-(6-(dimethylamino)benzo[*d*]thiazol-2-yl)vinyl)-4,5-dihydrothiazole-4-carboxylate 142

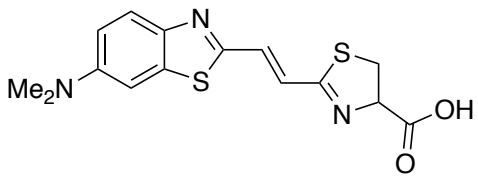


A solution of **141** (55 mg, 0.150 mmol) in DCM (15 mL) cooled to 0 °C was treated with DAST (80 µL 0.602 mmol). After stirring at 0 °C for 1 h

40 min the reaction was warmed at rt for 15 min and quenched with saturated aqueous NH4Cl solution (3.5 mL). The layers were separated and the aqueous layer was re-extracted with DCM (2 × 10 mL). The combined organic layers were washed with brine (30 mL), dried Na_2SO_4, and concentrated. Purification was achieved by flash chromatography (1:1 EtOAc/Hex) to give **142** (43 mg, 83 %) as an orange waxy solid.

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.85 (d, J = 9.1 Hz 1H, CHAr), 7.41 (d, J = 16.1 Hz, 1H, CHCH), 7.24 (d, J = 16.1 Hz, 1H, CHCH), 7.03 (d, J = 2.5 Hz, 1H, CHAr), 6.95 (dd, J = 9.1 Hz, 2.5 Hz, 1H, CHAr), 5.24 (t, 9.3 Hz 1H, CHN), 3.86 (s, 3H, OCH_3), 3.66 (m, 2H, $CHCH_2OH$), 3.06 (s, 6H, CH_3NCH_3. ^{13}C NMR (151 MHz, $CDCl_3$) δ 171.10 (1C), 169.54 (1C), 159.25 (1C), 149.67 (1C), 145.76 (1C), 137.76 (1C), 135.64 (1*C*,CHCH), 127.70(1C, *C*HCH), 124.08(1C, CHAr), 113.73(1*C,* CHAr), 102.28(1*C, CH*Ar), 78.24 (1C, CH), 53.09 (1C, *C*H_3), 40.95 (6C, N*C*H_3), 35.01 (1C, *C*H_2). m/z (EI) 220 M+ HRMS $C_{16}H_{17}N_3O_2S_2$ calcd. 348.0840, found 348.0842

(*E*)-2-(2-(6-(dimethylamino)benzo[*d*]thiazol-2-yl)vinyl)-4,5-dihydrothiazole-4-carboxylic acid 139



A solution of **142** (41 mg, 0.118 mmol) in THF/H_2O (2.4/1.2 mL) was treated with LiOH (12 mg, 0.507 mmol). After stirring at rt for 5 min H_2O (20 mL) was added and the aqueous solution was washed with EtOAc (20 mL). The aqueous phase was then made basic (pH 3) with 2M HCl, and then extract with EtOAc (20 mL x 3). The organic extractions were combined and dried with $MgSO_4$ filtered and concentrated to give **139** as a free black solid (35 mg, 90 %).

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.87 (d, J = 8.5 Hz 1H, CHAr), 7.46 (d, J = 15.8 Hz, 1H, CHCH), 7.25 (d, J = 15.8 Hz, 1H, CHCH), 7.03 (s, 1H, CHAr), 6.96 (dd, J = 8.1 Hz, 2.5 Hz, 1H, CHAr), 5.32 (t, 9.1 Hz1H, CHNH), 3.71 (m, 2H, $CHCH_2OH$), 3.06 (s, 6H, CH_3NCH_3. ^{13}C NMR (151 MHz, $CDCl_3$) δ 170.02 (1C), 158.81 (1C), 149.25 (1C), 145.74 (1C), 141.46 (1C), 138.01 (1C), 136.03 (1*C*,CHCH), 124.19(1C, *C*HCH), 119.37(1C, CHAr), 113.65(1*C,* CHAr), 102.29(1*C, CH*Ar), 78.83 (1C, CH), 40.095 (6C, N*C*H_3), 29.83 (1C, *C*H_2). IR ν_{max} (thin film) 2922, 1719, 1593, 1351, 1200, 906, 729 m/z (EI) 220 HRMS $C_{15}H_{15}N_3O_2S_2$ calcd. 334.0684, found 334.0689

Appendices

7.1 Abbreviations

aq.	aqueous
DCM	dichloromethane
DMSO	dimethylsulfoxide
g	gram
h	hour
Hz	hertz
IR	infrared spectroscopy
M	mole per litre
Mg	milligram
MHz	megahertz
mL	millilitre
mmol	millimole
mp	melting point
MS	mass spectrometry
NMR	nuclear magnetic resonance
rt	room temperature

7.2 References

(1) K. Anjou, E. V. S. Acta Chem. Scand. 1967, 21, 2076. doi:10.3891/ACTA.CHEM.SCAND.21-2076. 1967, p 2076.

(2) Edrizzi, B. R. F.; Agno, F. R. M.; Adocco, D. E. B.; Icolini, G. I. N.; Ersini, G. I. V. Aging Effects and Grape Variety Dependence on the Content of Sulfur Volatiles in Wine. **2007**, 10880–10887.

(3) Farmacologiche, R.; Negri, M.; Mario, C.; Sud, N.; Paone, G. Analysis of Benzothiazole in Italian Wines Using Headspace Solid-Phase Microextraction and Gas Chromatography - Mass Spectrometry. **2000**, 1239–1242.

(4) Littleton, B. C.; Sinsheimer, J. E. New Volatile Constituents of Black Tea Aroma. **1975**, *23* (5), 999–1003.

(5) P.Sensi, P.Margalith, M.T.Timbal, Farmaco1959, 14, 146. P.

(6) P. Margalith, P. Sensi P. Margalith, P. Sensi, G. P. D.-A. 1961. P.

(7) R. Cricchio, P. Antonini, G. C. Lancini, G. Tamborini, R. J. White, E. Martinelli, Tetrahedron 1980, 36, 1415. R.

(8) H. Suzuki, K. Shindo, A. Ueno, T. Miura, M. Takei, M. S.; H. Fukamachi, J. Tanaka, T. Higa, Bioorg. Med. Chem. Lett. 1999, 9; 1361. H.

(9) H. Suzuki, K. Shindo, A. Ueno, M. Takei, H. Fukamachi, T. H.; 1999., W. P. 9909018-A. H.

(10) T. Okita, N. Otsuka, J. P. 2005187357-A. 2005. T.

(11) Carmeli, S.; Moore, R. E.; Patterson, G. M. L.; Corbett, T. H.; Valeriote, F. A. J. Am. Chem. Soc. 1990, 112, 8195.

(12) Jurkiewicz, E.; Jansen, R.; Kunze, B.; Trowitzsch-Kienast, W. AntiViral Chem. Chemother. 1992, 3, 189.

(13) Jansen, R.; Kunze, B.; Reichenbach, H.; Jurkiewicz, E.; Husmann, G.; Ho¨fle, G. Liebigs Ann. Chem. 1992, 357.

(14) Walker, M. A.; Heathcock, C. H. J. Org. Chem. 1992, 57, 5566.

(15) McElroy, W. D. The Energy Source for Bioluminescence in an Iisolated System. *Proc. Natl. Acad. Sci. U. S. A.* **1947**, No. 17, 342–345.

(16) Marques, S. M.; Esteves Da Silva, J. C. G. Firefly Bioluminescence: A Mechanistic Approach of Luciferase Catalyzed Reactions. *IUBMB Life* **2009**, *61* (January), 6–17.

(17) Branchini, B. R.; Behney, C. E.; Southworth, T. L.; Fontaine, D. M.; Gulick, A. M.; Vinyard, D. J.; Brudvig, G. W. Experimental Support for a Single Electron-Transfer Oxidation Mechanism in Firefly Bioluminescence. *J. Am. Chem. Soc.* **2015**, *137* (24), 7592–7595.

(18) Hill, S. J.; Baker, J. G.; Rees, S. Reporter-Gene Systems for the Study of G-Protein-Coupled Receptors. *Curr. Opin. Pharmacol.* **2001**, *1* (5), 526–532.

(19) Close, D. M.; Xu, T.; Sayler, G. S.; Ripp, S. In Vivo Bioluminescent Imaging (BLI): Noninvasive Visualization and Interrogation of Biological Processes in Living Animals. *Sensors* **2011**, *11* (1), 180–206.

(20) Moravec, R. A.; O'Brien, M. A.; Daily, W. J.; Scurria, M. A.; Bernad, L.; Riss, T. L. Cell-Based Bioluminescent Assays for All Three Proteasome Activities in a Homogeneous Format. *Anal. Biochem.* **2009**, *387* (2), 294–302.

(21) Fernández-Suárez, M.; Ting, A. Y. Fluorescent Probes for Super-Resolution Imaging in Living Cells. *Nat. Rev. Mol. Cell Biol.* **2008**, *9* (12), 929–943.

(22) Zhao, H.; Li, C. C.; Pardo, J.; Chu, P. C.; Liao, C. X.; Huang, J.; Dong, J. G.; Zhou, X.; Huang, Q.; Huang, B.; et al. A Novel E3 Ubiquitin Ligase TRAC-1 Positively Regulates T Cell Activation. *J. Immunol.* **2005**, *174* (9), 5288–5297.

(23) Welsh, D. K.; Noguchi, T. Cellular Bioluminescence Imaging. *Cold Spring Harb. Protoc.* **2012**, *7* (8), 852–866.

(24) Weissleder, R.; Ntziachristos, V. Shedding Light onto Live Molecular Targets. *Nat. Med.* **2003**, *9* (1), 123–128.

(25) Viviani, V. R.; Silva, A. C. R.; Perez, G. L.; Santelli, R. V.; Bechara, E. J. H.; Reinach, F. C. Cloning and Molecular Characterization of the cDNA for the Brazilian Larval CIick Beetle Pyrearinus Termifiuminans Luciferase. *Photochem. Photobiol.* **1999**, *70* (2), 254–260.

(26) Bitler, B.; Mcelroy, W. D. The Preparation and Properties of Crystalline Firefly Lucif Erinl Preparation of Crude Luciferin. *Arch. Biochem. Biophys.* **1957**, *72*, 358–368.

(27) McElroy, W. D.; Hastings, J. W.; Coulombre, J.; Sonnenfeld, V. The Mechanism of Action of Pyrophosphate in Firefly Luminescence. *Arch. Biochem. Biophys.* **1953**, *46* (2), 399–416.

(28) Seliger, H. H.; McElroy, W. D.; White, E. H.; Field, G. F. Stereo-Specificity and Firefly Bioluminescence, a Comparison of Natural and Synthetic Luciferins. *Proc. Natl. Acad. Sci. U. S. A.* **1961**, *47* (1), 1129–1134.

(29) White, E. H.; Rapaport, E.; Hopkins, T. a; Seliger, H. H. Chemi- and Bioluminescence of Firefly Luciferin. *J. Am. Chem. Soc.* **1969**, *91* (1), 2178–2180.

(30) Seto, S.; Ogura, K.; Nishiyama, Y. A Convenient Synthetic Method of 2-Carbamoyl-6-Methoxybenzothiazole , One of Intermediates for the Synthesis of FireflyLuciferin. *Bull. Chem. Soc. Jpn.* **1963**, *981* (1924), 331–333.

(31) White, E. H.; McCapra, F.; Field, G. F. The Structure and Synthesis of Firefly Luciferin. *J. Am. Chem. Soc.* **1963**, *85* (3), 337–343.

(32) Bowie, L. J. Synthesis of Firefly Luciferin and Structural Analogs. *Arch. Biochem. Biophys.* **1970**, *141* (668).

(33) Stuckwisch, B. C. G. Derivatives of Z-Amino-6-Methoxybenzothiazole. *J. Am. Chem. Soc* **1949**, *71* (10), 3417.

(34) White, E. H.; Woelther, H.; Field, G. F.; McElroy, W. D. Analogs of Firefly Luciferin. *J.Org.Chem* **1965**, *30*, 2344–2348.

(35) Nomoto, T.; Toya, Y.; Kanamori, N.; Yoda, B. Synthetic Reactions in PEG: PEG-Assisted Synthesis of 2-Cyano-6-Methoxy-Benzothiazole, A Key Intermediate For The Synthesis of Firefly Luciferin. *Biosci. Biotech. Biochem.* **1993**, *57* (9), 1561–1562.

(36) Pfleger, K. D. G.; Dromey, J. R.; Dalrymple, M. B.; Lim, E. M. L.; Thomas, W. G.; Eidne, K. A. Extended Bioluminescence Resonance Energy Transfer (eBRET) for Monitoring Prolonged Protein-Protein Interactions in Live Cells. *Cell. Signal.* **2006**, *18* (10), 1664–1670.

(37) Arranz, A.; Ripoll, J. Advances in Optical Imaging for Pharmacological Studies. *Front. Pharmacol.* **2015**, *6*.

(38) Yoshida, H.; Kojima, T.; Kurokouchi, K.; Takahashi, S.; Hanamura, H.; Kojima, M.; Poole, A. R.; Ishiguro, N. Relationship between Pre-Radiographic Cartilage Damage Following Anterior Cruciate Ligament Injury and Biomarkers of Cartilage Turnover in Clinical Practice: A Cross-Sectional Observational Study. *Osteoarthr. Cartil.* **2013**, *21* (6), 831–838.

(39) Naumov, P.; Ozawa, Y.; Ohkubo, K.; Fukuzumi, S. Structure and Spectroscopy of Oxyluciferin, the Light Emitter of the Firefly Bioluminescence. *J. Am. Chem. Soc.* **2009**, *131* (32), 11590–11605.

(40) White, E. H.; Wörther, H.; Seliger, H. H.; McElroy, W. D. Amino Analogs of Firefly Luciferin and Biological Activity Thereof 1. *J. Am. Chem. Soc.* **1966**, *88* (9), 2015–2019.

(41) Branching, B. R.; Hayward, M. M.; Bamford, S.; M. Brennan, P.; Lajiness, E. J. Naphthyl- and Quinolylluciferin: Green and Red Light Emitting Firefly Luciferin Analogues. *Photochem. Photobiol.* **1989**, *49* (5), 689–695.

(42) Conley, N. R.; Dragulescu-Andrasi, A.; Rao, J.; Moerner, W. E. A Selenium Analogue of Firefly D-Luciferin with Red-Shifted Bioluminescence Emission. *Angew. Chemie - Int. Ed.* **2012**, *51* (14), 3350–3353.

(43) Mccutcheon, D. C.; Paley, M. A.; Steinhardt, R. C.; Prescher, J. A. Expedient Synthesis of Electronically Modi Fi Ed Luciferins for Bioluminescence Imaging. *J. Am. Chem. Soc.* **2012**, *134*, 7604–7607.

(44) Harwood, K. R.; Mofford, D. M.; Reddy, G. R.; Miller, S. C. Identification of Mutant Firefly Luciferases That Efficiently Utilize Aminoluciferins. *Chem. Biol.* **2011**, *18* (12), 1649–1657.

(45) Iwano, S.; Obata, R.; Miura, C.; Kiyama, M.; Hama, K.; Nakamura, M.; Amano, Y.; Kojima, S.; Hirano, T.; Maki, S.; et al. Development of Simple Firefly Luciferin Analogs Emitting Blue, Green, Red, and near-Infrared Biological Window Light. *Tetrahedron* **2013**, *69* (19), 3847–3856.

(46) Jathoul, A. P.; Grounds, H.; Anderson, J. C.; Pule, M. a. A Dual-Color Far-Red to Near-Infrared Firefly Luciferin Analogue Designed for Multiparametric Bioluminescence Imaging. *Angew. Chemie Int. Ed.* **2014**, *53* (48), 13059–13063.

(47) Orlova, G.; Goddard, J. D.; Brovko, L. Y. Theoretical Study of the Amazing Firefly Bioluminescence: The Formation and Structures of the Light Emitters. *J. Am. Chem. Soc.* **2003**, *125* (7), 6962–6971.

(48) Halter, R. J.; Fimmen, R. L.; Mcmahon, R. J.; Peebles, S. A.; Kuczkowski, R. L.; Stanton, J. F.; May, R. V. Microwave Spectra and Molecular Structures of (Z) -Pent-2-En-4-Ynenitrile and Maleonitrile. *J. Am. Chem. Soc* **2001**, No. 11, 12353–12363.

(49) Schmitz, W. D.; Romo, D. A New Route to 2-Substituted A2-Thiazolines: Stille Cross-Couplings of 2-Bromo-A2-Thiazolines. *Tetrahedron Lett.* **1996**, *37* (28), 4857–4860.

(50) Chen, Y. L. 6-(Substituted)methylene-Penicillanic and 6-(Substituted)hydroxymethylpenicillanic Acids and Derivatives Thereof. EP0150984A2, 1989.

(51) Müller, S.; Liepold, B.; Roth, G.; Bestmann, H. An Improved One-Pot Procedure for the Synthesis of Alkynes from Aldehydes. *Synlett* **1996**, 521–522.

(52) Pietruszka, J.; Witt, A. Synthesis of the Bestmann-Ohira Reagent. *Synthesis (Stuttg)*. **2006** (24), 4266–4268.

(53) Newsome, J. J.; Colucci, M. A.; Hassani, M.; Beall, D.; Moody, C. J. Benzimidazole- and Benzothiazole-Quinones : Excellent Substrates for NAD(P)H : Quinone Oxidoreductase 1. *Org. Biomol. Chem.* **2007**.

(54) Langille, N. F.; Dakin, L. a.; Panek, J. S. Sonogashira Coupling of Functionalized Trifloyl Oxazoles and Thiazoles with Terminal Alkynes: Synthesis of Disubstituted Heterocycles. *Org. Lett.* **2002**, *4* (15), 2485–2488.

(55) Chinchilla, R.; Nájera, C. The Sonogashira Reaction: A Booming Methodology in Synthetic Organic Chemistry. *Chem. Rev.* **2007**, *107*, 874–922.

(56) Jacobi, P. a.; Liu, H. Iterative Synthesis of Semicorrins, Tripyrrolines, and Higher Analogues. *J. Am. Chem. Soc.* **1999**, *121* (9), 1958–1959.

(57) Melzer, B.; Plodek, A.; Bracher, F. Total Synthesis of the Marine Pyridoacridine Alkaloid Demethyldeoxyamphimedine. *J. Org. Chem.* **2014**, *79* (15), 7239–7242.

(58) Kijrungphaiboon, W.; Chantarasriwong, O.; Chavasiri, W. Two Efficient Reagent Systems for the Preparation of N-Heteroaromatic Halides. *Tetrahedron Lett.* **2012**, *53*, 674–677.

(59) Kato, Y.; Okada, S.; Tomimoto, K.; Mase, T. A Facile Bromination of Hydroxyheteroarenes. *Tetrahedron Lett.* **2001**, *42*, 4849–4851.

(60) Tongkate, P.; Pluempanupat, W.; Chavasiri, W. Hexabromoacetone and Ethyl Tribromoacetate: A Highly Efficient Reagent for Bromination of Alcohol. *Tetrahedron Lett.* **2008**, *49* (7), 1146–1148.

(61) Popov, I.; Do, H.; Daugulis, O. In Situ Generation and Trapping of Aryllithium and Arylpotassium Species by Halogen , Sulfur , and Carbon Electrophiles. *J. Org . Chem.* **2009**, No. 6, 8309–8313.

(62) Aditya, A.; Kodadek, T. Incorporation of Heterocycles into the Backbone of Peptoids to Generate Diverse Peptoid-Inspired One Bead One Compound Libraries. *ACS Comb. Sci.* **2012**, *14*, 164–169.

(63) Lu, L.; Yan, H.; Sun, P.; Zhu, Y.; Yang, H.; Liu, D.; Rong, G.; Mao, J. Synthesis of Internal Alkynes through the Pd-Catalyzed Coupling of Heteroaryl Halides with Terminal Alkynes. *European J. Org. Chem.* **2013**, *2013* (9), 1644–1648.

(64) Patil, S. S.; Jadhav, R. P.; Patil, S. V.; Bobade, V. D. Ligand and Solvent-Free Iron Catalyzed Oxidative Alkynylation of Azoles with Terminal Alkynes. *Tetrahedron Lett.* **2011**, *52* (43), 5617–5619.

(65) Emtenäs, H.; Carlsson, M.; Pinkner, J. S.; Hultgren, S. J.; Almqvist, F. Stereoselective Synthesis of Optically Active Bicyclic β-Lactam

Carboxylic Acids That Target Pilus Biogenesis in Pathogenic Bacteria. *Org. Biomol. Chem.* **2003**, *1*, 1308–1314.

(66) Hartke, K.; Kunze, O.; Hoederath, W. A Simple Route to 2-Alkenethioic O -Esters and 2-Alkenedithioic Esters [Thiono- and Dithioesters. *Synthesis.* **1985**, No. 10, 960–961.

(67) Anderson, J. C.; Grounds, H.; Jathoul, A. P.; Murray, J. A. H.; Pacman, S. J.; Tisi, L. *RSC Adv.* **2017**, *7*, 3975–3982.

(68) Chalifoux, W. A.; Mcdonald, R.; Ferguson, M. J.; Tykwinski, R. R. Supporting Information for " T -Butyl Endcapped Polyynes : Evidence of Reduced Bond Length Alternation ." *Angew. Chemie - Int. Ed.* **2009**, *48* (42), 7915–7919.

(69) Rudolph, J.; Theis, H.; Hanke, R.; Endermann, R.; Johannsen, L. Seco -Cyclothialidines : New Concise Synthesis , Inhibitory Activity toward Bacterial and Human DNA Topoisomerases , and Antibacterial Properties. *J. Med. Chem.* **2001**, 619–626.

(70) Niwa, K.; Nakamura, M.; Ohmiya, Y. Stereoisomeric Bio-Inversion Key to Biosynthesis of Firefly D-Luciferin. *FEBS Lett.* **2006**, *580* (22), 5283–5287.

(71) Lembert, N. Firefly Luciferase Can Use L-Luciferin to Produce Light. *Biochem. J.* **1996**, *317* (1), 273–277.

(72) Smolii, O. B.; Bezverhnii, V. D.; Drach, B. S. Triphenyl(thiocarbamoylmethylene)phosphorane, a Promising Reagent for Heterocyclizations. *Russ. J. Gen. Chem.* **2001**, *71* (6), 981–982.

(73) Brain, C. T.; Hallett, A.; Ko, S. Y. Thioamide Synthesis: Thioacyl-N-Phthalimides as Thioacylating Agents. *J. Org. Chem.* **1997**, *62* (12), 3808–3809.

(74) Pathak, U.; Bhattacharyya, S.; Mathur, S. RSC Advances Selective Thioacylation of Amines in Water : A Convenient Preparation of Secondary Thioamides and Thiazolines. *RSC Adv.* **2014**, *5*, 4484–4488.

(75) Wu, J. W.; Wu, Y. D.; Dai, J. J.; Xu, H. J. Benzoic Acid-Catalyzed Transamidation Reactions of Carboxamides, Phthalimide, Ureas and Thioamide with Amines. *Adv. Synth. Catal.* **2014**, *356* (11–12), 2429–2436.

(76) Lanigan, R. M.; Starkov, P.; Sheppard, T. D. Direct Synthesis of Amides from Carboxylic Acids and Amines Using B(OCH 2 CF 3) 3. *J. Org. Chem.* **2013**, *78* (9), 4512–4523.

(77) Leflemme, N.; Marchand, P.; Gulea, M.; Masson, S. Synthesis of New Asymmetric Phosphonylated Thiazolines and Their Use in Olefination Reactions. *Synthesis (Stuttg).* **2000**, No. 8, 1143–1147.

(78) Collignon, È.; Maddaluno, J.; Masson, S.; Al-badri, H. Synthesis of New Phosphono-Substituted Dihydrothiopyrans via Hetero Diels ± Alder Reaction , under Thermal and High Pressure Conditions. *Tetrahedron* **2000**, *56*.

(79) Le Roy-Gourvennec, S.; Masson, S. Functionalised A,β-Unsaturated Thioamides via Horner-Emmons Reactions. *Synthesis (Stuttg).* **1995**, *1995* (11), 1393–1396.

(80) Marvel, C. S.; de Radzitzky, P.; Brader, J. J. An Improved Preparation of Dithioesters and Some Reactions and Spectral Properties of These Compounds. *J. Am. Chem. Soc.* **1955**, *77* (22), 5997–5999.

(81) Pfund, E.; Lequeux, T.; Masson, S.; Vazeux, M. Synthesis of Thiazolines Linked to a Difluoromethylphosphonate Diester via Dithioester Chemistry. *Org. Lett.* **2002**, *4* (5), 843–846.

(82) Meroni, G.; Rajabi, M.; Santaniello, E. Luciferase-Catalyzed Bioluminescent Activity. **2009**, *2009* (i), 265–288.

(83) Williams, D. R.; Sit, S. Y. Total Synthesis of (+)-Phyllanthocin. *J. Am. Chem. Soc.* **1984**, *106* (10), 2949–2954.

(84) Branchini, B. R.; Murtiashaw, M. H.; Carmody, J. N.; Mygatt, E. E.; Southworth, T. L. Synthesis of an N-Acyl Sulfamate Analog of

Luciferyl-AMP: A Stable and Potent Inhibitor of Firefly Luciferase. *Bioorganic Med. Chem. Lett.* **2005**, *15* (17), 3860–3864.

(85) Nicolaou, K. C.; Nevalainen, M.; Zak, M.; Bulat, S.; Bella, M.; Safina, B. S. Synthetic Studies on Thiostrepton: Construction of Thiostrepton Analogues with the Thiazoline-Containing Macrocycle. *Angew. Chem. Int. Ed. Engl.* **2003**, *42* (29), 3418–3424.

(86) Reddy, G. R.; Thompson, W. C.; Miller, S. C. Robust Light Emission from Cyclic Alkylaminoluciferin Substrates for Firefly Luciferase. *J. Am. Chem. Soc.* **2010**, *132* (39), 13586–13587.

(87) Ono, M.; Hayashi, S.; Kimura, H.; Kawashima, H.; Nakayama, M.; Saji, H. Push-Pull Benzothiazole Derivatives as Probes for Detecting Amyloid Plaques in Alzheimer's Brains. *Bioorganic Med. Chem.* **2009**, *17* (19), 7002–7007.

(88) Anderson, J. C.; Grounds, H.; Jathoul, A. P.; Murray, J. A. H.; Pacman, S. J.; Tisi, L. Convergent Synthesis and Optical Properties of near-Infrared Emitting Bioluminescent Infra-Luciferins. *RSC Adv.* **2017**, *7* (7), 3975–3982.

(89) Woodhouse, J. L.; Assmann, M.; Parkes, M. A.; Grounds, H.; Pacman, S. J.; Anderson, J. C.; Worth, G. A.; Fielding, H. H. Photoelectron Spectroscopy of Isolated Luciferin and Infraluciferin Anions in Vacuo: Competing Photodetachment, Photofragmentation

and Internal Conversion. *Phys. Chem. Chem. Phys.* **2017**, *19* (34), 22711–22720.

(90) Shaffer, C.; Fan, R.; Lewis, M.; Kowalczyk, J. (R)-[(2-Oxo-4-Thiazolidinyl)methyl]triphenylphosphonium Iodide: A Wittig Reagent for the Synthesis of Cysteine-Derived Alkenes. *Synthesis (Stuttg).* **2011**, *2011* (23), 3859–3865.

(91) Kelley, C.; Zhang, Y.; Parhi, A.; Kaul, M.; Pilch, D. S.; LaVoie, E. J. 3-Phenyl Substituted 6,7-Dimethoxyisoquinoline Derivatives as FtsZ-Targeting Antibacterial Agents. *Bioorg. Med. Chem.* **2012**, *20* (24), 7012–7029.

(92) Halter, R. J.; Fimmen, R. L.; McMahon, R. J.; Peebles, S. A.; Kuczkowski, R. L.; Stanton, J. F. Microwave Spectra and Molecular Structures of (Z)-Pent-2-En-4-Ynenitrile and Maleonitrile. *J. Am. Chem. Soc* **2001**, *123* (49), 12353–12363.