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

A thesis by
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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..........................................................................
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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 its 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, \textit{in vitro} and \textit{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 \textit{in vivo} monitoring of numerous diseases and cellular functions.

The technique has been held back because the light emitted by D-luciferin is at $\lambda_{\text{max}}$ 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 $\lambda_{\text{max}}$ 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 \textit{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.

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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.

![Figure 1: Luciferin moieties](image)

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 thiao-rifamycins 4 (figure 2). ¹
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*. These rifamycins are known as thiazo-rifamycins, namely rifamycin P 6 and rifamycin Q 7 (figure 3).

![Figure 3: rifamycin P and Q](image)

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). 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).

![Figure 4: S1319 and adrenaline](image)

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\(_{N}\)Ar 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.
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).
Figure 5: tantazoles, mirabazoles, and thiangazole structures

Mirabazoles and tantazoles were isolated from blue-green algae *Scytonema 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-methylecysteine 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 with 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 $\lambda_{\text{max}}$ 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).\textsuperscript{15}

![Scheme 4: McElroy’s proposed light emission](image)

**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).\textsuperscript{16}
Scheme 5: Bioluminescent mechanism of d-luciferin\textsuperscript{16}

Once bound in the luciferase active site the anion of luciferin 24 takes part in nucleophilic attack on the $\alpha$-phosphoryl group of the carboxylate ATP-Mg\textsuperscript{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).

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. 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.

Fluorescence has been used as an optical reporter by genetically encoding fluorescent proteins in a similar fusion to that used for the luciferase enzyme. Modified fluorescent dyes can also be constructed in such a way that they are able to be cleaved by specific enzymes under investigation. Bioluminescence however has some advantages over fluorescence; most importantly an external photon is required to excite the fluorescent 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.\textsuperscript{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 phototoxicity 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.\textsuperscript{23} For these reasons the D-luciferin with firefly luciferase is the most used system as a bioluminescent reporter in biological sciences.

\textbf{1.9 Limitations of firefly luciferin}

The Luciferase/D-luciferin system for imaging of biological events is not without 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 ($\lambda_{\text{max}} = 560$ nm). Cellular components and other endogenous molecules such as hemoglobin, absorb visible light. 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. 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 termifiumans catalyzes emission of green light (542 nm), while the luciferase from the head lanterns of results in red
light emission (628 nm). 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. 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. 

Luciferin (D/L-2-(6-hydroxy-2-benzothiazol-yl-)-\(\Delta\)-thiazoline-4-carboxylic acid) was first synthesised by White et al. In this procedure, \(p\)-anisidine
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-methoxysthioxanilinamide 40 by oxidative cyclization with alkaline K₃[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).
This experimental protocol has been applied to the preparation of 5-10 g of D-luciferin 1.\textsuperscript{32} The preparation of 4-methoxythioxanilinamide 40, according to Seto et al\textsuperscript{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 \textit{in situ}, as described in detail by Bowie.\textsuperscript{32} The experimental procedure allows 40 to be prepared from p-anisidine 32 with an overall 39% yield (scheme 9).

According to another synthetic approach, 2-amino-6-methoxybenzothiazole 42 can be prepared from p-anisidine 32,\textsuperscript{33} and different routes can lead to 2-cyano-6-methoxybenzotiazole 38 using a classical Sandmeyer reaction. In a first synthesis,\textsuperscript{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,\textsuperscript{37} or conjugating the luciferin to a near-IR dye,\textsuperscript{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.\textsuperscript{36} Reported analogues which include fluorophores to give a maximum emission > 600 nm up to 675 nm.\textsuperscript{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.\textsuperscript{39} A number of analogues of luciferin have been reported in the literature (scheme 11). Both L-luciferin\textsuperscript{44} and dehydroluciferin\textsuperscript{45} do not produce light with luciferase and actually competitively inhibit the production of light when D-luciferin is also present.\textsuperscript{11}
Replacing the 6'-hydroxyl of D-luciferin with a more electron donating amino group red shifts the peak wavelength of light emitted. The three hydroxyl positional isomers of luciferin, O-methyllyluciferin, 5,5'-dimethyllyluciferin and decarboxyluciferin were all inactive. A number of analogues have been reported to display considerably red-shifted bioluminescence spectra these include D-quinolyllyluciferin (46, $\lambda_{\text{max}} = 608$ nm) and 6-aminoluciferin (47, $\lambda_{\text{max}} = 605$ 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). The bioluminescence spectra of the more conjugated compounds is reported to be red-shifted (48, $\lambda_{\text{max}} = 445$ nm; 49, $\lambda_{\text{max}} = 565$ nm; 50, $\lambda_{\text{max}} = 688$ 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. They found that the unsubstituted amino derivative 51 gave a maximum emission at $\lambda_{\text{max}} = 593$ 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, $R^1 = \text{Et, } \text{Pr or } \text{nBu}$, $R^2 = \text{H}$, $\lambda_{\text{max}} = 607$ nm; $R^1$, $R^2 = \text{Me}$, $\lambda_{\text{max}} = 607$ 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. 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). 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 $\pi$-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.

![Scheme 12: Analogues of luciferin 2.](image-url)
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.\(^\text{46}\)

![Figure 6 infraluciferin](image)

**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 \(\pi\)-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.\textsuperscript{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 infra-luciferin \textbf{54} we would like to investigate the luciferin analogue with an alkyne bridge connecting the benzothiazole and thiazoline rings. This would have an extended $\pi$-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.\textsuperscript{46}
Results and Discussion

2 Alkyne bridged luciferin analogue

2.1 Sonogashira route to alkyne bridged luciferin

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 propiononitrile 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 propiolamid 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 \([\delta 7.51 (d, J = 10.5 \text{ Hz}, 1\text{H}), 5.60 (d, J = 10.5 \text{ Hz}, 1\text{H})]\) 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).

![Scheme 15: Proposed second route to 55](image)

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). 

![Figure 8: Thiazoline with leaving group](image)
There are several reagents, which are capable of converting aldehydes into 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).  

![Scheme 16: Conversion of aldehyde to terminal alkyne using the Ohira-Bestmann reagent](image)

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. yield53 50 %) (scheme 17).  

![Scheme 17: Synthesis of benzothiazole bearing terminal alkyne](image)
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).\textsuperscript{51} Alkyne formation is clearly seen in $^1$H NMR by loss of the aldehyde peak at $\delta$ 10.10 and the appearance of the terminal alkyne peak at $\delta$ 3.56.

\begin{center}
\textbf{Scheme 18: aldehyde to alkyne formation}
\end{center}

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.\textsuperscript{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

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).  

\[
\begin{align*}
\text{Scheme 20: Synthesis of thiazoline with triflate leaving group}
\end{align*}
\]

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

\[
\begin{align*}
\text{Scheme 21: Sonogashira coupling reaction}
\end{align*}
\]

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).\textsuperscript{55} No product was observed under any of the conditions screened, giving either un-reacted starting material or decomposition.

<table>
<thead>
<tr>
<th>Catalyst</th>
<th>Equivalents</th>
<th>Base</th>
<th>Solvent</th>
<th>Temp °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \text{PdCl}_2(\text{PPh}_3)_2/\text{CuI} )</td>
<td>5-10 % / 10 %</td>
<td>( \text{Et}_3\text{N} )</td>
<td>THF</td>
<td>RT-120</td>
</tr>
<tr>
<td>( \text{PdCl}_2(\text{PPh}_3)_2/\text{CuI} )</td>
<td>5-10 % / 10 %</td>
<td>( \text{Et}_3\text{N} )</td>
<td>THF</td>
<td>rt - 120</td>
</tr>
<tr>
<td>( \text{Pd}(\text{PPh}_3)_4/\text{CuI}^{54} )</td>
<td>5-10 % / 10 %</td>
<td>2,6 lutidine</td>
<td>DMF</td>
<td>rt- 120</td>
</tr>
<tr>
<td>( \text{Pd}(\text{PPh}_3)_4/\text{CuI}^{54} )</td>
<td>5-10 % / 10 %</td>
<td>2,6 lutidine</td>
<td>Dioxane</td>
<td>rt- 65</td>
</tr>
<tr>
<td>( \text{(PPh}_3)_2\text{Pd(OAc)}_2 )</td>
<td>5-10 % / 10 %</td>
<td>NaOAc</td>
<td>DMF</td>
<td>40-60</td>
</tr>
</tbody>
</table>

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
Various attempts at halogenation were unsuccessful (Table 2). 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.

**Scheme 24: Halogenation of 79/80**

Table 2: Conditions for halogenations of 79/80

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Solvent</th>
<th>Temp °C</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>POBr$_3$</td>
<td>None</td>
<td>0-130</td>
<td>SM-azole/degraded</td>
</tr>
<tr>
<td>CCl$_4$/PPh$_3$</td>
<td>Acetonitrile</td>
<td>0-65</td>
<td>SM-azole</td>
</tr>
<tr>
<td>Bu$_4$NBr/P$_2$O$_5$</td>
<td>Toluene</td>
<td>0-100</td>
<td>SM-azole/degraded</td>
</tr>
<tr>
<td>Oxalyl Chloride/DMF</td>
<td>DCM</td>
<td>Rt-45</td>
<td>SM-complex mixture</td>
</tr>
<tr>
<td>PPh$_3$/CB$_4$</td>
<td>Toluene</td>
<td>0-125</td>
<td>SM-azole/degraded</td>
</tr>
<tr>
<td>POBr$_3$</td>
<td>Toluene</td>
<td>0-135</td>
<td>SM-azole/degraded</td>
</tr>
<tr>
<td>PPh$_3$/BrCCl$_3$</td>
<td>DCM</td>
<td>rt</td>
<td>azole</td>
</tr>
<tr>
<td>PPh$_3$/Br$_3$CCOCBr$_3$</td>
<td>DCM</td>
<td>rt</td>
<td>azole</td>
</tr>
<tr>
<td>PPh$_3$/Br$_3$CCO$_2$Et</td>
<td>DCM</td>
<td>rt</td>
<td>azole</td>
</tr>
</tbody>
</table>

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. Attempts to synthesize this compound did not produce the product, giving only unreacted starting materials and degraded products.
There are numerous examples in the literature which show a benzothiazole with a halide 82 taking part in a Sonogashira coupling reaction (scheme 26).

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).
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

![Scheme 28: Synthesis of thiazoline 84](image)

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).
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
We had by this point demonstrated that dithioesters can undergo substitution reactions with serine to give a precursor to a thiazoline moiety. 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 32: Thiono et al Sonogashira reaction**

**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>
<thead>
<tr>
<th>Catalyst</th>
<th>Eq</th>
<th>Base</th>
<th>Solvent</th>
<th>Temp °C</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pd(PPh₃)₄/CuI</td>
<td>5-10 % / 10%</td>
<td>2,6 lutidine</td>
<td>DMF</td>
<td>rt</td>
<td>SM/Complex mixture</td>
</tr>
<tr>
<td>PdCl₂(PPh₃)₂/CuI/PPh₃</td>
<td>5-10 % / 10%</td>
<td>Et₃N</td>
<td>DMF</td>
<td>rt</td>
<td>SM/Complex mixture</td>
</tr>
<tr>
<td>Direct addition</td>
<td></td>
<td>n-BuLi</td>
<td>THF</td>
<td>-78 – 0 °C</td>
<td>Degradation/complex mixture</td>
</tr>
</tbody>
</table>

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

![Figure 8: alkyne bridged luciferin intermediate](image)

![Scheme 35: Proposed linear route to alkyne bridged luciferin](image)
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).

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

Scheme 37: Carboxylic acid formation
The necessary trityl and methyl ester protected amino acid cysteine 92 was produced in two steps (scheme 38).\textsuperscript{69}

![Scheme 38: synthesis of protected cysteine methyl ester 92]

Using the protocols from the original synthesis of infraluciferin to couple 95 with 92 using BOP (scheme 42) failed to give any product 96 (scheme 39).\textsuperscript{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.\textsuperscript{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\textsubscript{3}/Tf\textsubscript{2}O (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\textsubscript{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 \textsuperscript{46}
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.\textsuperscript{28,70,71} Unfortunately racemization occurred at the cyclisation step with OPPh\textsubscript{3}/Tf\textsubscript{2}O (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 infraluciferin

To circumvent the unreliable cyclisation using the Hendrickson reagent and breakdown of the thiazoline ring by BBr\textsubscript{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 infrafaluciferin 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 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 1H 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).
Brain et al have shown that primary thioamides can be converted into suitable leaving groups for displacement by an incoming amine to give the desired thioamide by using phthaloyl chloride 116 (scheme 47)\(^{73}\).

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.
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

<table>
<thead>
<tr>
<th>Base</th>
<th>Solvent</th>
<th>Time hrs</th>
<th>Temp °C</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\text{K}_2\text{CO}_3)</td>
<td>THF</td>
<td>2-3</td>
<td>0-rt</td>
<td>Degraded SM and recovered reagent</td>
</tr>
<tr>
<td>Pyridine</td>
<td>THF</td>
<td>2-3</td>
<td>0-rt</td>
<td>Degraded SM and recovered reagent</td>
</tr>
</tbody>
</table>

Following the unsuccessful attempts to convert the thioamide \(\textbf{109}\) 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) \(^{75}\)

\[
\begin{array}{c}
\text{R}^1\text{S} \quad \text{NH}_2 \\
\text{H}_2\text{N}^+ \text{R}^2 \\
\text{benzoic acid} \\
\text{xylene 125 °C} \\
\text{R}^1\text{S} \quad \text{HN}^+ \text{R}^2 \\
+ \text{NH}_3
\end{array}
\]

Scheme 51: Transamidation using benzoic acid and proposed intermediate

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

One final attempt was made at transamidation using the boron reagent \(\text{B(OCH}_2\text{CF}_3)\)) which has been shown by the Sheppard group at UCL to be effective for the transamidation of DMF (scheme 53). \(^{76}\)
Attempts at transamidation using $\text{B(OCH}_2\text{CF}_3)_3$ reagent with 109 were unsuccessful showing only degraded material (scheme 54).

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).
A literature search revealed 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 \(R = H, \text{Et}, \text{Ph}, \text{Bn}, R^1 = H\) and \(R^2 = \text{Me}, \text{Ph}, i-\text{Pr}\) (scheme 56)

\[
\begin{align*}
\text{EtO} & \quad \text{O} \\
\quad & \quad \text{OEt} \\
\quad & \quad \text{N} \\
119 \\
\end{align*}
\]

\[
\begin{align*}
\text{EtO} & \quad \text{O} \\
\quad & \quad \text{OEt} \\
\quad & \quad \text{N} \\
\text{PGO} & \quad \text{H} \\
\text{PGO} & \quad \text{O} \\
\quad & \quad \text{S} \\
118 \\
\end{align*}
\]

\[
\begin{align*}
\text{Wittig Reaction} \\
\text{PO} \\
\quad & \quad \text{EtO} \\
\quad & \quad \text{OEt} \\
\quad & \quad \text{N} \\
\text{PGO} & \quad \text{H} \\
\text{PGO} & \quad \text{O} \\
\quad & \quad \text{S} \\
120 \\
\end{align*}
\]

Scheme 55: Proposed convergent synthesis via Wittig reaction

\[
\begin{align*}
\text{O} \\
\quad & \quad \text{EtO} \\
\quad & \quad \text{OEt} \\
\quad & \quad \text{N} \\
\text{R} \\
\text{119} \\
\end{align*}
\]

\[
\begin{align*}
\text{O} \\
\quad & \quad \text{EtO} \\
\quad & \quad \text{OEt} \\
\quad & \quad \text{N} \\
\text{R} \\
\text{118} \\
\end{align*}
\]

\[
\begin{align*}
\text{O} \\
\quad & \quad \text{EtO} \\
\quad & \quad \text{OEt} \\
\quad & \quad \text{N} \\
\text{R} \\
\text{120} \\
\end{align*}
\]

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 CH₂ more acidic and easily removed via DBU (scheme 57). ⁷⁷

![Scheme 57: Proposed activation using LiCl](image)

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

![Scheme 58: Synthesis of thiazoline phosphonate](image)
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). 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\textsuperscript{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\textsubscript{2}S 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).\textsuperscript{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)

![Scheme 62: Established substitution conditions of serine to dithioester](image)

The reaction with our substrate took 48 h to go to completion as monitored by $^{31}$P NMR 122 $\delta$ 17.14 to give the product 124 $\delta$ 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.
Scheme 63: Addition of serine methyl ester

Analysis by $^1$H NMR showed $\delta$ 5.2 (CH from serine) and $\delta$ 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 $\delta$ 4.0 to $\delta$ 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.

Scheme 64: Formation of thiazoline 128
This reaction also works on larger 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).  

![Scheme 65: HWE using mild conditions](image)

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$_2$ $\delta$ 3.2 and formation of a new signal for the CH of the azole at $\delta$ 8.21. The reduced alkene 131 was also formed as a trace product < 5 %, $^1$H NMR showing the two CH$_2$ protons at $\delta$ 3.63 and the thiazole CH at $\delta$ 8.06 (scheme 66).
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).

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 BBr₃ 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). 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).

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).

![Scheme 70: MEM protection and aldehyde formation](image)

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

![Scheme 71: HWE using n-BuLi and MEM protected aldehyde](image)

Interestingly the first attempts at this reaction which were quench with saturated NaHCO₃ 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

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

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

![Scheme 72: HWE using NaH and MEM protected aldehyde](image_url)
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).

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). 

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). Unfortunately after several attempts this reaction failed to give 135 and only ever resulted in producing the thiazole 137 (scheme 75).
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).\(^{85}\)

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.

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).

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

\[\text{Scheme 79: TFA deprotection of MEM}\]

Initially the exact protocols of Branchini and Santaniello (stirring the substrate in neat TFA at rt for 3 – 4 h, then add H\textsubscript{2}O 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 \textsuperscript{19}F NMR. The reaction was optimised by stirring in neat TFA at rt for 1 hr then quenched with NaHCO\textsubscript{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 \textbf{112} to the free acid to give infraluciferin \textbf{54} saponification with LiOH was used (scheme 80).

![Scheme 80: Saponification with LiOH](image)

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

![Figure 10: infraluciferin Azole contaminant](image)

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

<table>
<thead>
<tr>
<th>Eq of LiOH</th>
<th>Reaction Time min</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>30</td>
<td>90 % infra 1 -3% azole</td>
</tr>
<tr>
<td>1.5</td>
<td>30</td>
<td>55 % infra 30 % ester 1 -3% azole</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>50 % infra 30 % ester 1-2 % azole</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>50 % infra 30 % ester 1 % azole</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>50 % infra 30 % ester</td>
</tr>
<tr>
<td>2.5</td>
<td>5</td>
<td>62 % infra 30 % ester</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>77 % infra 20 % ester</td>
</tr>
<tr>
<td>3.5</td>
<td>5</td>
<td>85 % infra 10 % ester</td>
</tr>
<tr>
<td>4.3</td>
<td>5</td>
<td>90 % infra</td>
</tr>
</tbody>
</table>

Table 6: Saponification reaction conditions
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$_2$N 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 $^{139,86}$

![Figure 11: Dimethylamino infraluciferin](image)

To utilise the synthesis previously developed for infraluciferin the aldehyde $^{140}$ first needed to be synthesized.
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).

Scheme 81 Proposed route to 142

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

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](image)

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

<table>
<thead>
<tr>
<th>Eq of DAST</th>
<th>Reaction Time at –78 °C</th>
<th>Reaction Time at rt</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.2</td>
<td>30 min</td>
<td></td>
<td>12 % product + SM</td>
</tr>
<tr>
<td>1.2</td>
<td>30 min</td>
<td>15 min</td>
<td>~20 % product + SM</td>
</tr>
<tr>
<td>2</td>
<td>30 min</td>
<td>15 min</td>
<td>~30 % product + SM</td>
</tr>
<tr>
<td>2</td>
<td>1 h</td>
<td>15 min</td>
<td>~40 % product + SM</td>
</tr>
<tr>
<td>2</td>
<td>2.5 h</td>
<td>15 min</td>
<td>~40 % product + SM</td>
</tr>
<tr>
<td>2</td>
<td>2.5 h</td>
<td>O/N</td>
<td>~40 % product + SM</td>
</tr>
<tr>
<td>3</td>
<td>2.5 h</td>
<td>15 min</td>
<td>~50 % product + SM</td>
</tr>
<tr>
<td>4</td>
<td>2.5 h</td>
<td>15 min</td>
<td>&gt;80 % product</td>
</tr>
</tbody>
</table>
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/Cl) 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

\[
\begin{array}{c}
\text{O} \\
\text{S} \\
\text{N} \\
\text{H} \\
\text{O}
\end{array}
\]

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 \textit{in vacuo} to give 1.17 g of orange solid. Purification by column chromatography (100% CHCl\textsubscript{3}) gave 6-methoxybenzo[d]thiazole-2-carbaldehyde 67 as yellow crystals mp 120 °C (0.690 g, 64%); \textsuperscript{1}H NMR (500 MHz, CDCl\textsubscript{3}) \(\delta\) 10.10 (s, 1H, COH), 8.11 (d, J = 9.1 Hz, 1H, ArH), 7.39 (d, J = 2.5 Hz, 1H, ArH), 7.21 (dd, J = 9.1, 2.5 Hz, 1H, ArH), 3.93 (s, 3H, OCH\textsubscript{3}). NMR data was in agreement with that reported in the literature.\textsuperscript{50}

Dimethyl (2-oxopropyl)phosphonate 70

\[
\begin{array}{c}
\text{O} \\
\text{P} \\
\text{OMe}
\end{array}
\]

University College London 91
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 °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, POC$_3$H$_3$), 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 $^{52}$

![4-acetamidobenzenesulfonyl azide](image)

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$_2$O (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$_2$O (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, ArH), 7.78 (d, $J = 8.8$ Hz, 2H, ArH), 7.57 (s, 1H, NH), 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$^{52}$**

![Image of the compound](image)

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$_2$O (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

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$_2$O (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 \textit{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$_3$) $\delta$ 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, CH$H$). $^1$H NMR data was in agreement with that reported in the literature.
6-methoxy-2-(phenylethynyl)benzo[d]thiazole 78

To a solution of tetrakis (triphenylphosphine) palladium (Pd(PPh₃)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 H₂O (10 mL) and extracted with EtOAc (10 mL x3), the combined organic layers were washed with brine and dried with MgSO₄ then concentrated *in vacuo* to give 0.200 g of brown solid, Purification by column chromatography (100% CHCl₃) gave 6-methoxy-2-(phenylethynyl)benzo[d]thiazole 78 as a yellow powder (0.054 g, 78%); ¹H NMR (600 MHz, Chloroform-δ) δ 7.96 (d, J = 8.9 Hz, 1H, ArH), 7.65 – 7.62 (m, 2H, ArH), 7.43 – 7.38 (m, 3H, ArH), 7.32 (d, J = 2.3 Hz, 1H,ArH), 7.13 (dd, J = 9.1, 2.5 Hz, 1H, ArH), 3.91 (s, 3H, OCH₃). ¹³C NMR (151 MHz, CDCl₃) 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**

![Ethyl 2-oxothiazolidine-4-carboxylate](image)

To a solution of cysteine ethyl ester HCl 72 (6.00 g, 32.3 mmol) in H$_2$O (40 mL) and cooled to 0 °C, NaHCO$_3$ (2.72 g, 32.3) was added in portions over 30 mins, then K$_2$CO$_3$ was added at a rate that kept the reaction mixture below 5 °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$_2$SO$_4$ 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, NH), 4.43 (t, 1H, COCHNH), 4.27 (q, 2H, CH$_3$CH$_2$O), 3.70 (dd, $J = 11.3, 8.3$ Hz, 1H, CHCH$_2$S), 3.63 (dd, $J = 11.3, 5.1$ Hz, 1H, CHCH$_2$S), 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

Neat triflic anhydride (1.82 g, 6.46 mmol) was slowly added to a solution of ethyl 2-oxothiazolidine-4-carboxylate 74 (0.942 g, 5.38 mmol) and Et₃N (1.50 mL, 10.8 mmol) in DCM (20 mL) at -78 °C and stirred for 1 hr. The reaction was quenched with saturated NaHCO₃ (20 mL) the organic layer separated and washed with brine (10 mL), then dried (Na₂SO₄) 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 %) ¹H NMR (600 MHz, CDCl₃) δ 5.05 (d, J = 7.5 Hz, 1H, COCHNH), 4.39 – 4.32 (m, 2H, COCH₂CH₃), 3.89 (dd, J = 11.8, 7.9 Hz, 1H, CHCH₂S), 3.57 (dd, J = 11.9, 1.0 Hz, 1H, CHCH₂S), 1.34 (t, J = 7.2 Hz, 3H, CH₃). ¹H NMR data was in agreement with that reported in the literature.
Ethyl 4, 5-dihydrothiazole-4-carboxylate 84

Neat Et₃N (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 Å 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. ¹H NMR (300 MHz, CDCl₃) δ 8.03 (s, 1H), 5.08 (t, J = 7.5 Hz, 1H, COCHNH), 4.26 (q, J = 1.2 Hz, 2H, COCH₂CH₃), 3.50 (m, 2H, CHCH₂S), 1.33 (t, 3H, CH₃). ¹H NMR data was in agreement with that reported in the literature.

Propiolamide 61
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, 50 mmol) 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 NH₃ evaporated. Et₂O (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 %). ¹H NMR (500 MHz, CD₃CN) δ 6.71 (s, 1H, NH₂), 6.26 (s, 1H, NH₂), 3.26 – 3.19 (m, 1H, CH). ¹H NMR data was in agreement with that reported in the literature.

**Propionitrile 59**

Into a pestle and mortar was ground propiolamide 61 (1.00 g, 14.50 mmol) with sand (approx. 1.00 g) 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 °C under vacuum (approx. 30-40 mbar) and the distillate allowed to collect in a receiving flask cooled to -78 °C over 2 hrs to give propionlonitrile 59 as a clear oil (0.400 g, 54%). No further purification was needed. 1H NMR (500 MHz, D2O) δ 4.70 (s, 1H). 1H NMR data was in agreement with that reported in the literature.

(2-amino-2-oxoethyl) triphenylphosphonium chloride 114

\[
\begin{align*}
\text{Ph}_3\text{P}^+ - &\text{O} \\
&\text{NH}_2 \\
&\text{Cl}
\end{align*}
\]

A solution of 2-chloroacetamide 113 (2.00g, 21.40 mmol) and triphenylphosphate in acetonitrile MeCN (10 mL) was heated to 80 °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 %). 1H NMR (300 MHz, CDCl₃) δ 9.83 (s, 1H, NH₂), 7.78 (m, 15H, ArH), 5.57 (s, 1H, NH₂), 5.09 (d, J = 14.3 Hz, 2H, PCH₂CO). All data was in agreement with the literature.
(2-amino-2-thioxoethyl) triphenylphosphonium chloride 115

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 °C each time, and then recrystallized from EtOH (45 mL) at 45 °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%). ¹H NMR (500 MHz, DMSO) δ 7.72 – 7.45 (m, 15H, ArH), 3.81 (d, J = 28.2 Hz, 2H, PCH₂CO). All data was in agreement with the literature.
2-(triphenyl-\(\lambda^5\)-phosphanylidene)ethanethioamide 107

\[
\begin{array}{c}
\text{Ph}_3\text{P} \equiv \text{S} \\
\downarrow \\
\text{NH}_2
\end{array}
\]

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\(\text{2}O\) (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-\(\lambda^5\)-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) \(\delta\) 7.75 – 7.44 (m, 15H, Ar\(\text{H}\)), 6.60 (s, 2H, \(\text{NH}_2\)), 3.82 (d, \(J = 28.2\) Hz, 1H, P\(\text{Ph}_3\)CHCS). All data was in agreement with the literature. \(^7\)2

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

\[
\begin{array}{c}
\text{O} \\
\text{N} \\
\downarrow \\
\text{S} \\
\downarrow \\
\text{NH}_2
\end{array}
\]

A solution of 6-methoxybenzo[d]thiazole-2-carbaldehyde 67 (0.149 g, 0.77 mmol) and 2-(triphenyl-\(\lambda^5\)-phosphanylidene)ethanethioamide 107 (0.258 g, 0.77 mmol) in DCM (10 mL) were stirred at rt overnight. The reaction mixture were concentrated \textit{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) $\delta$ 9.68 (d, $J$ = 16.39 Hz, 2H, NH$_2$), 7.93 (t, $J$ = 10.2 Hz, 1H, ArH), 7.76 (d, $J$ = 15.4 Hz, 1H, CHCHNCS), 7.71 (d, $J$ = 2.5 Hz, 1H, ArH), 7.26 (d, $J$ = 15.4 Hz, 1H, NCSCCHCH), 7.14 (m, 1H. ArH), 3.84 (s, 3H, OCH$_3$). $^{13}$C NMR (151 MHz, CDCl$_3$) $\delta$ 160.1 (s, SCNH$_2$), 157.9 (s, NCS), 147.9 (ArCOCH$_3$), 146.4 (s, ArCN), 137.1 (s, ArCS), 133.4 (s, CH), 133.3 (s, CH), 124.0 (ArC), 116.7 (ArC), 104.7 (ArC), 55.8 (s, OCH$_3$). m/z (EI) 220 M+H HRMS C$_{11}$H$_{12}$N$_2$OS$_2$ 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) $\delta$ 9.69 (d, $J = 189.3$ Hz, 2H, NH$_2$), 7.85 (d, $J = 8.8$ Hz, 1H, ArH), 7.75 (d, $J = 15.4$ Hz, 1H, CHC(HNCS)), 7.41 (d, $J = 2.4$ Hz, 1H, ArH), 7.21 (d, $J = 15.4$ Hz, 1H, NC(SCH)H), 7.01 (dd, $J = 8.8$, 2.4 Hz, 1H, ArH).

**Diethylphosphono thioimido acetate hydrochloride 127**

To a solution of diethylcyanomethyl phosphonate 126 (5.00 g, 28.23 mmol) in Et$_2$O (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 $\delta$ 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.

\[ ^1H \text{ NMR (300 MHz, CDCl}_3 \] \( \delta \) 4.32 – 4.18 (m, 4H, OCH\(_2\)CH\(_3\)), 3.70 (d, \( J = 22.5 \) Hz, 2H, PCH\(_2\)), 3.57 – 3.46 (m, 1H, CH\(_2\)CH\(_3\)), 1.45 (t, \( J = 7.4 \) Hz, 3H, CH\(_2\)CH\(_3\)), 1.37 (t, \( J = 7.1 \) Hz, 6H, OCH\(_2\)CH\(_3\)). All data was in agreement with the literature. \(^1H\) 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 \( \delta 17.94 \) for the product. Ice cold H\(_2\)O (5 mL) was added and the mixture stirred for 5 minutes. HCl/H\(_2\)O 3:1 (20 mL) was added and the aqueous phase was extracted with Et\(_2\)O (3 x 20 mL) and the organic phase washed once with HCl/H\(_2\)O 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\(_3\)) \(\delta\) 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\(_3\)N (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\(_3\)) \(\delta\) 4.17 (m, 5H, OC\(_2\)H\(_2\)+C\(_2\)H\(_2\)OH), 4.00 (dd, \(J = 11.8, 2.8\) Hz, 1H, C\(_2\)H\(_2\)OH), 3.82 (s, 1H, OCH\(_3\)), 3.49 (m, 2H, PCH\(_2\)), 1.35
(m, 6H, CH₂CH₃). ¹³C NMR (151 MHz, CDCl₃) δ 63.25 (d, J = 140 Hz, OCH₂CH₃), 61.39 (s, CH₂OH), 60.81 (s, NHCH), 52.94 (s, OCH₃), 45.10 (d, J = 127.1 Hz, PCH₂), 16.47 (s, CH₂CH₃). IR νmax (thin film) 3310 – 3243, 2982, 1741, 1512, 1225, 1010. m/z (EI) HRMS C₁₄H₂₁NOPS₂ 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, CHCH₂), 4.25 – 4.03 (m, 4H, CH₃CH₂O), 3.80 (s, 3H, CH₂O), 3.71 – 3.50 (m, 2H,
SCH$_2$CH), 3.31 – 3.10 (m, 2H, CH$_2$P), 1.33 (t, J = 7.1 Hz, 6H, CH$_3$CH$_2$O).

$^{13}$C NMR (151 MHz, CDCl3) $\delta$ 171.10 (s, CO), 165.67 (s, SCN), 77.75 (s, CHCH$_2$), 62.90 (t, J = 6.0 Hz, POCH$_2$CH$_3$), 52.93 (s, CH$_3$O), 36.54 (s, CH$_2$CH), 33.46 (d, J = 137.7 Hz, CH$_2$P), 16.49 (d, J = 4.4 Hz, CH$_3$CH$_2$). IR $\nu_{\text{max}}$ (thin film) 2980, 1742, 1255, 1023. m/z (EI) 220 M+ H HRMS (C$_{10}$H$_{18}$NO$_5$PS+H) calcd. 296.0721. found 296.0719

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

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$_2$O (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
$^1$H NMR (300 MHz, CDCl3) δ 8.86 (s, 1H, CHNCS), 8.02 (d, J = 8.9 Hz 1H, CHCHCO), 7.65 (s, 1H, CHCO), 7.22 (dd, J = 8.9 Hz, 2.4 Hz 1H, CHar), 5.34 (s, 2H, OCHO), 3.86 (dd, J = 5.6 Hz, 3.7 Hz 2H, 2H, CH₂CH₂O), 3.57 (dd, J = 5.5 Hz, 3.7 Hz 2H, OCH₂CH₂), 3.37 (s, 3H, CH₃O). $^1$H NMR data was in agreement with the literature

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

\[\text{MEMO} \quad \text{O}\]

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 NaHCO₃ (50 mL) and extracted with EtOAc (50 mL x 3). The organic phase dried with MgSO₄ 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, CHCO), 8.12 (d, J = 9 Hz 1H, CHCHCO), 7.67 (s, 1H, CHCO), 7.31 (dd, J = 9 Hz, 2.4 Hz 1H, CHar),
5.38 (s, 2H, OCHO), 3.87 (m, 2H, 2H, CH₂CH₂O), 3.58 (m, 2H, OCH₂CH₂), 3.38 (s, 3H, CH₃O). ¹H NMR data was in agreement with the literature

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

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

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/H₂O (40 mL) added and extracted with EtOAc (3 x 40 mL), the EtOAc fractions were combined washed with brine and dried with MgSO₄ 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, CDCl₃) δ 7.96 (d, J = 8.8 Hz 1H, CHCHCO), 7.56 (s, 1H, CHCO), 7.23 (dd, J = 9 Hz, 2.4Hz 1H, CHAr), 5.35 (s, 2H, OCHO), 3.86 (m, 2H, 2H, CH₂CH₂O), 3.58 (m, 2H, OCH₂CH₂), 3.57 (s, 1H, CHC),
3.38 (s, 3H, CH$_3$O). $^{13}$C NMR (151 MHz, CDCl$_3$) $\delta$ 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$_3$S$_5$ calcd. 264.0694, found 264.0702

3-(6-((2-methoxyethoxy)methoxy)benzo[d]thiazol-2-yl)propionic 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$_2$O (10 mL x 3). The combined organic fractions were made basic with 5% Na$_2$CO$_3$/H$_2$O solution and extracted with H$_2$O (10 mL x 3). The combined aqueous fractions were again acidified and extracted with Et$_2$O (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) $\delta$ 8.07 (d, J = 9 Hz 1H, CHCHCO), 7.56 (s, 1H, CHCO), 7.28 (m, 1H, CHAr), 5.36 (s, 2H, OCHO), 3.88 (m, 2H, 2H,
CH$_2$CH$_2$O), 3.62 (m, 2H, OCH$_2$CH$_2$), 3.40 (s, 3H, CH$_3$O). $^{13}$C NMR (151 MHz, CDCl$_3$) $\delta$ 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$_5$S$_5$ calcd. 308.0593, found 308.0595

**ethyl carbonochloridodithioate 89**

![ethyl carbonochloridodithioate 89](image)

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) $\delta$ 3.20 (q, $J = 7.1$ Hz 1H, CH$_2$), 1.37 (t, 7.4, CH$_3$). $^1$H NMR data was in agreement with the literature

**S-tritylcysteine 92**

University College London
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\textsubscript{2}O (25 mL) which produced a white precipitate that was filtered off and washed with H\textsubscript{2}O. 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\textsubscript{2}O 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 CH), 2.58 (dd, J= 4.4 Hz, 12 Hz, 1H CH\textsubscript{2}) 2.49(dd 9 Hz, 12Hz, 1H CH\textsubscript{2}) mp 190-195 °C (lit 195°C) 1H NMR data was in agreement with the literature

methyl S-tritylcysteinate 92a\textsuperscript{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 CH), 3.70 (s, 3H, CO₂CH₃) 2.58 (m, 2H CH₂) mp 73-76 °C (lit 78°C) ¹H NMR data was in agreement with the literature

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

![Chemical Structure](image)

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 diisopropylcarbonimid (64 μL, 0.409 mmol) was added and left at rt over night. The reaction was quenched with saturated NH₄Cl (4 mL) and extracted with DCM dried with Na₂SO₄ 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

¹H NMR (600 MHz, CDCl₃) δ 8.07 (d, J = 9 Hz 1H, CHCHCO), 7.56 (s, 1H, CHCO), 7.28 (m, 1H, CHAr), 5.36 (s, 2H, OCHO), 3.88 (m, 2H, 2H, CH₂CH₂O), 3.62 (m, 2H, OCH₂CH₂), 3.40 (s, 3H, CH₃O). ¹³C NMR (151 MHz, CDCl₃) 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 CH₂), 71.65
(1 C CH₂), 68.08 (1 C CH₂), 60.64 (1 C), 59.21 (1C CH₃), 53.08 (1C CH₃),
51.65 (1C CH), 33.50 (1C CH₂), 29.83 (1C), 21.21 (1C), 20.30 (1C), 19.86
(1C), 14.33 (1C) m/z (EI) 220 M+ H HRMS C₃₇H₃₄N₂O₆S₂ calcd. 677.1937,
found 677.1873

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

![Chemical Structure]

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$_3$) $\delta$ 8.95 (br. s, 1H, CH$_2$O) 8.02 (d, $J = 9.0$ Hz, 1H, CH Ar), 7.95 ( d, $J = 15.1$ Hz, 1H, CHCHCS ), 7.60 ( d, $J = 7.25$ Hz, 1H, CH Ar), 7.45 ( d, $J = 15.1$ Hz, 1H, SNCCCHCH), 7.25 ( d, $J = 2.5$ Hz, 1H, CH Ar ), 5.39 (m, 1H, CH$_2$CHNH), 5.36 (s, 2H, OCH$_2$O), 4.2 (dd, $J = 11.7, 3.3$ Hz, 2H, CH$_2$OH), 3.86 (m, 2H, CH$_3$OCH$_2$), 3.85 (s, 3H, COOCH$_3$), 3.58 (m, 2H, CH$_2$CH$_2$O), 3.38 (s, 3H, CH$_3$O). $^{13}$C NMR (151 MHz, CDCl$_3$) $\delta$ 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 $\nu_{\text{max}}$ (thin film) 3100-3500, 2925, 1740, 1597, 1194, 1077, 991, 822, 732. m/z (El) 220 M+ H HRMS C$_{36}$H$_{234}$N$_2$O$_6$S$_2$ 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 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 Na₂SO₄, and concentrated. Purification was achieved by flash chromatography (75 % EtOAc/Hex) to give 135 (185 mg, 83 %) as an orange waxy solid.

¹H NMR (600 MHz, CDCl₃) δ 7.93 (d, J = 9.0 Hz, 1H, CH Ar), 7.57 (d, J = 2.4 Hz, 1H, CH Ar), 7.41 (d, J = 16.2 Hz, 1H, NSCCHCH), 7.33 (d, J = 16.2 Hz, 1H, CHCHCNS), 7.20 (dd, J = 9.0, 2.46 Hz, 1H, CH Ar), 5.34 (s, 2H, OCH₂O), 5.25 (t, J = 9.3 Hz, 1H, NCHCH₂), 3.88 – 3.82 (m, 5H, COOCH₃, CH₃OCH₂), 3.73 – 3.60 (m, 2H, SCH₂CH), 3.57 (m, 2H, CH₂CH₂O), 3.38 (s, 1H, CH₃O).

¹³C NMR (151 MHz, CDCl₃) δ 170.95(1C), 169.33(1C), 162.61(1C), 156.37(1C), 149.24(1C), 136.49(1C), 135.04(1C,CH), 129.27(1C, CH), 124.51(1C, CHAr), 117.77(1C, CHAr), 107.44(1C, CHAr), 93.99 (1C, CH₂), 78.14 (1C, CH), 71.66 (1C, CH₂), 68.01 (1C, CH₂), 59.20 (1C, CH₃), 53.13(1C, CH₃), 35.07(1C, CH₂). IR νmax (thin film) 2900, 1737, 1597, 1452, 1199, 1102, 991 m/z (El) 220 M+ H HRMS C₁₈H₂₀N₂O₅S₂ calcd. 409.0892, found 409.0894
methyl-(E)-2-(2-(6-hydroxybenzo[d]thiazol-2-yl)vinyl)-4,5-dihydrothiazole-4-carboxylate 106

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 NaHCO₃ (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%).

¹H 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 = 8.9 Hz, 1H, CHAr), 5.33 (t, J = 9.0 Hz, 1H), 3.76 – 3.66 (m, 2H, SCH₂). ¹H NMR data was in agreement with the literature on

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

A solution of infraluciferin methyl ester 106 (50 mg, 0.156 mmol) in THF/H₂O (2.4/1.2 mL) was treated with LiOH (8 mg, 0.372 mmol). After stirring at rt for 5 min H₂O (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 MgSO₄ filtered and concentrated to give infraluciferin 544 as a free flowing orange powder (44 mg, 92%).
$^1$H NMR (600 MHz, MeOD) $\delta$ 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[\textit{d}]thiazol-6-amine 143a** $^{87}$

![benzo[d]thiazol-6-amine 143a](image)

A solution of HCl (3 mL, 34 mmol) in EtOH/H$_2$O (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$_2$O was added and then extracted with EtOAc. The organic extractions were combined and dried with MgSO$_4$ filtered and concentrated to give 143a as brown solid (3.25 g, 82 % lit 92%)

$^1$HNMR (400 MHz, CDCl$_3$) $\delta$ 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.
To a solution of 6-aminobenzothiazole 143a (2.68 g, 17.84 mmol) in THF (70 mL) was added formaldehyde/H$_2$SO$_4$ (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$_2$O (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, CDCl$_3$) 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 \textit{n}-BuLi (3.98 mmol) in THF (12 mL) was cooled to -78 °C and a solution of \textbf{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$_2$O (35 mL) and extracted with DCM (50 mL x 3). The organic phase dried with NaSO$_4$ and concentrated \textit{in vacuo} to give \textbf{140} (760 mg, 82 %) of orange crystals which were >95 % pure by \textsuperscript{1}H NMR

\textsuperscript{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) \textsuperscript{1}H NMR data was in agreement with the literature.

\textbf{Methyl-\textit{(E)-}(3-(6-(dimethylamino)benzo[\textit{d}]thiazol-2-yl)prop-2enethioyl)serinate 141}
A solution of 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$_3$) $\delta$ 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$_2$OH), 3.86 (s, 3H, OCH$_3$), 3.06 (s, 6H, CH$_3$NCH$_3$. m/z (EI) 220 M$^+$. HRMS C$_{16}$H$_{19}$N$_3$O$_3$S$_2$ 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 Na2SO4, and concentrated. Purification was achieved by flash chromatography (1:1 EtOAc/Hex) to give 142 (43 mg, 83 %) as an orange waxy solid.

1H NMR (600 MHz, CDCl3) 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, OCH3), 3.66 (m, 2H, CHCH2OH), 3.06 (s, 6H, CH3NCH3. 13C NMR (151 MHz, CDCl3) δ 171.10 (1C), 169.54 (1C), 159.25 (1C), 149.67 (1C), 145.76 (1C), 137.76 (1C), 135.64 (1C,CHCH), 127.70(1C, CHCH), 124.08(1C, CHAr), 113.73(1C, CHAr), 102.28(1C, CHAr), 78.24 (1C, CH), 53.09 (1C, CH3), 40.95 (6C, NCH3), 35.01 (1C, CH2). m/z (El) 220 M+ HRMS C16H17N3O2S2 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$_2$O (2.4/1.2 mL) was treated with LiOH (12 mg, 0.507 mmol). After stirring at rt for 5 min H$_2$O (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$_3$) $\delta$ 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 Hz 1H, CHNH), 3.71 (m, 2H, CHCH$_2$OH), 3.06 (s, 6H, CH$_3$NCH$_3$). $^{13}$C NMR (151 MHz, CDCl$_3$) $\delta$ 170.02 (1C), 158.81 (1C), 149.25 (1C), 145.74 (1C), 141.46 (1C), 138.01 (1C), 136.03 (1C, CHCH), 124.19(1C, CHCH), 119.37(1C, CHAr), 113.65(1C, CHAr), 102.29(1C, CHAr), 78.83 (1C, CH), 40.095 (6C, NCH$_3$), 29.83 (1C, CH$_2$). IR $\nu_{\text{max}}$ (thin film) 2922, 1719, 1593, 1351, 1200, 906, 729 m/z (EI) 220 HRMS C$_{15}$H$_{15}$N$_3$O$_2$S$_2$ calcd. 334.0684, found 334.0689
7.1 Abbreviations

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<td>aq.</td>
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<tr>
<td>DCM</td>
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