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A history of the syntheses of D-luciferin, D-aminoluciferin and D-thioluciferin

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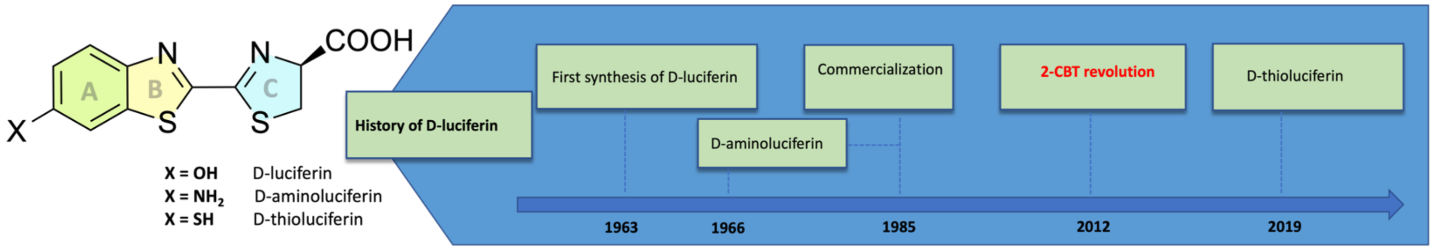
Abstract

D-Luciferin is the cornerstone of modern bioluminescence technologies, with applications spanning molecular biology, chemical biology, medical imaging, and diagnostics. Since its structural elucidation and first total synthesis in the early 1960s, sustained synthetic innovation has transformed D-luciferin from a rare natural product into a widely accessible and broadly used chemical tool. This review presents a comprehensive and chronological account of the chemical syntheses of D-luciferin, with particular emphasis on how successive synthetic strategies developed over time. The evolution of benzothiazole construction, and the now canonical 1,2-aminothiol click reaction with D-cysteine is traced in detail. We show that despite the many different syntheses reported, they all employ 2-cyanobenzothiazoles (2-CBTs) as common intermediates, to achieve the synthesis of the various luciferins. We further demonstrate that the progress made in accessing D-luciferin, stemmed largely from effective synthetic efforts to forging 2-CBTs. The emergence of C6-modified luciferins is also highlighted, notably D-aminoluciferin and D-thioluciferin, and how improved synthetic access to these 2-CBT analogues enabled new bioluminescence technologies.



Keywords: D-luciferin, bioluminescence, D-aminoluciferin, D-thioluciferin, 2-cyanobenzothiazoles

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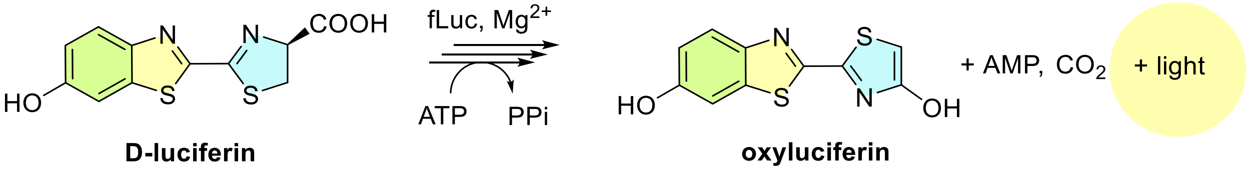
1. Introduction

One of the most well-known examples of bioluminescence is that of the North American firefly (*Photinus pyralis*).[1] Among the various bioluminescent systems, the firefly luciferin–luciferase reaction is the most extensively studied, with research spanning over 50 years.[2-4] In this system, two essential components are involved: D-luciferin, the light-emitting molecule, and firefly luciferase (fLuc), the enzyme that catalyses the oxidation of luciferin. This oxidation results in the formation of an excited-state molecule called oxyluciferin, which releases light as it returns to its ground state. It requires ATP, Mg^{2+}, and oxygen to function, emitting light at approximately 560 nm (Scheme 1). This particular bioluminescent reaction has a relatively high quantum yield (~40%) and the small molecule substrate, D-luciferin, has good water solubility and low toxicity, making it ideal for biological applications.[5-9]



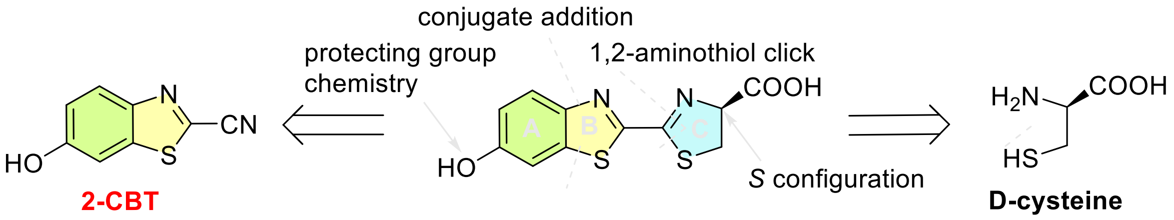
Scheme 1. Condensed mechanism of the D-luciferin bioluminescence reaction.

The structural elucidation of D-luciferin in the early 1960s represented a landmark achievement in natural products chemistry. White, McCapra, Field and co-workers established that the naturally occurring substrate was a chiral benzothiazole C2-linked thiazoline carboxylic acid. White *et al.* immediately undertook to confirm the structure by synthesising D-luciferin a few years later.[10-12] In recent times, rapid synthetic access to D-luciferin remains essential for the sustained development of bioluminescent assays and imaging technologies. As a result, the synthesis of D-luciferin evolved toward more robust, scalable and shorter sequences. Additionally, several non-natural D-luciferin analogues have also been prepared, the most notable of which are the C6 analogues, amino- and thioluciferin.

In the chemical synthesis of D-luciferin and its analogues in general, there are three important considerations; i) The basicity/nucleophilicity of the C6-hydroxyl/thiol/amine, ii) the C2 bridge between the benzothiazole (B-ring) and thiazole carboxylic acid (C-ring), and iii) the configuration of the stereocenter of the thiazoline carboxylic acid bearing carbon.

2. Syntheses of D-Luciferin

A unifying feature of D-luciferin syntheses is the use of a 2-cyanobenzothiazole (2-CBT) intermediate. This motif also enables a highly efficient 1,2-aminothiol click reaction between the 2-CBT nitrile functionality with D-cysteine, forming the thiazoline ring under mild, aqueous conditions. The near-quantitative yield of this reaction has shifted synthetic attention upstream, instead, placing emphasis on efficient, high-yielding routes to 2-CBTs (Scheme 2).

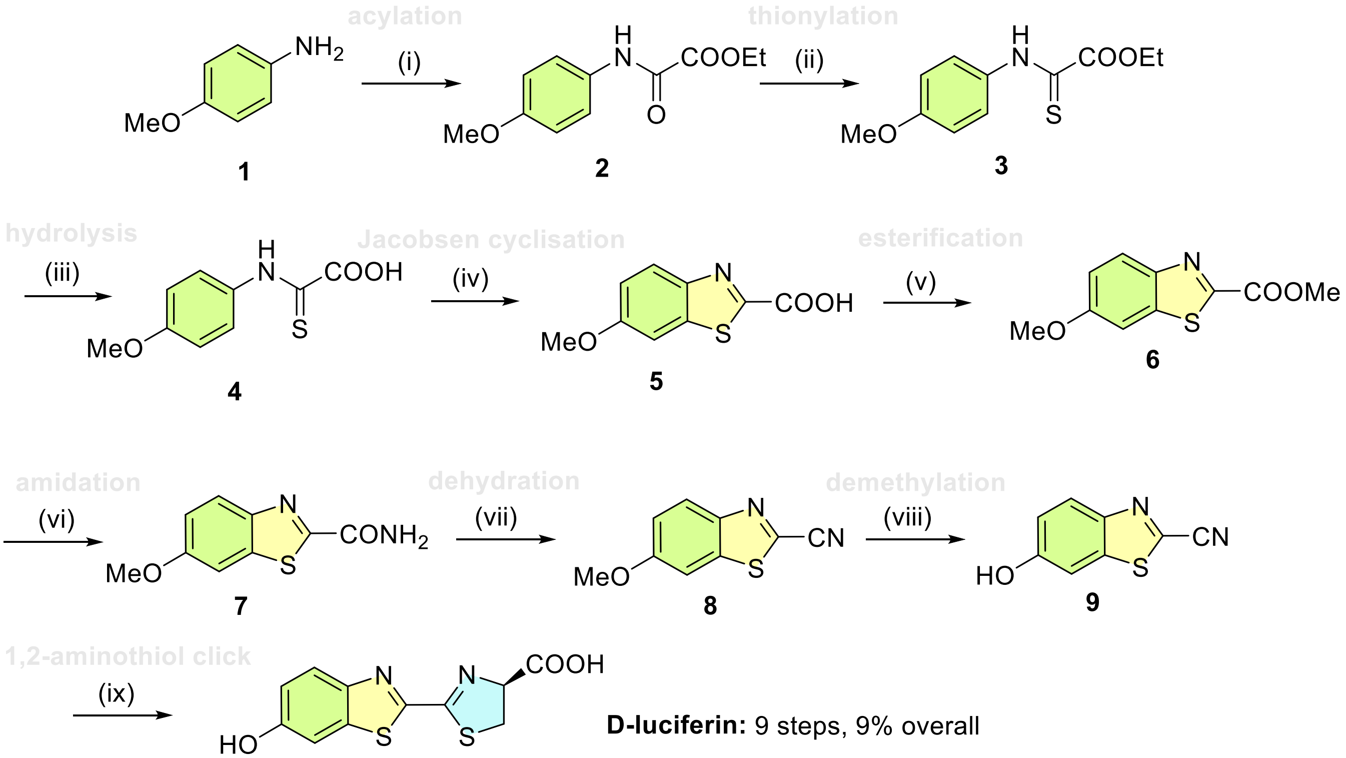


Scheme 2. Synthetic strategies toward D-luciferin.

Syntheses typically involve the formation of the 2-CBT core from either an aniline or quinone, all while maintaining a protected phenolic hydroxyl which, subsequent to 2-CBT formation, is unmasked using standard protecting group (PG) chemistry (Scheme 2).[13]

2.1. White et al. synthesis of D-luciferin (1961)

The first total synthesis described by White *et al.* in 1961 starts from *p*-anisidine **1** (Scheme 3).[10] Several functional interconversions are carried out to prepare the anilide **2** and then thioamide **3**. In this strategy, the thioamide is a key intermediate in the synthesis and provides the scaffolding for the 2-CBT B-ring via a Jacobsen cyclisation of compound **4** to benzothiazole **5**.[14] The benzothiazole carboxylic acid **5** is then converted to the methyl ester **6**, which readily undergoes nucleophilic acyl substitution to afford thioamide **7**. dehydration of the C2-amide of **7** then produces 2-CBT **8** with the required nitrile functionality. A condensation reaction between D-cysteine and 2-CBT **9** forms the thiazoline-thiazole system through a 1,2-aminothiol click reaction[15] to produce the C-ring and D-luciferin, in an overall yield of 9%.

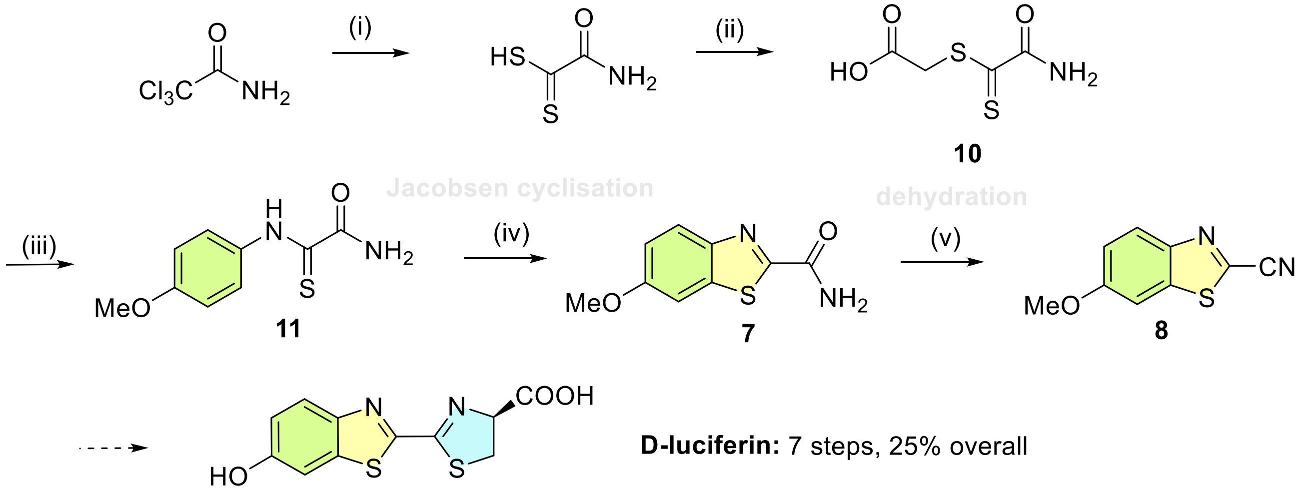


Scheme 3. Synthesis of D-luciferin from *p*-anisidine **1**.[10, 13] Reagents and conditions: (i) ethyl oxalate, 180 °C, 5 min (58%), (ii) P_2S_5, reflux, 40 min, (iii) NaOH, 0 °C then HCl, (iv) $K_3Fe(CN)_6$/ OH^-, <10 °C, 15 min (76% crude, no isolation in steps ii – iv), (v) CH_2N_2, 0 °C, 15 min (40%), (vi) anhydrous NH_3/MeOH, heat, 30 min (100%), (vii) $POCl_3$, reflux, 15 min (56%), (viii) Py·HCl, 200 °C, 1 h (62%), (ix) D-cysteine (in situ from D-cystine/liquid NH_3/Na, rt, 10 min) and **9** in H_2O/MeOH, rt, 30 min (94%).

In White’s synthesis of D-luciferin, the final step involving the D-cysteine addition proved to be highly efficient with near quantitative yield. The poor overall yield for the sequence was instead due to the lower efficiency in the 2-CBT preparation. Unsurprisingly, most of the subsequent syntheses of luciferin differ only in their construction of the 2-CBT core.

2.2. Seto et al. synthesis of D-luciferin (1963)

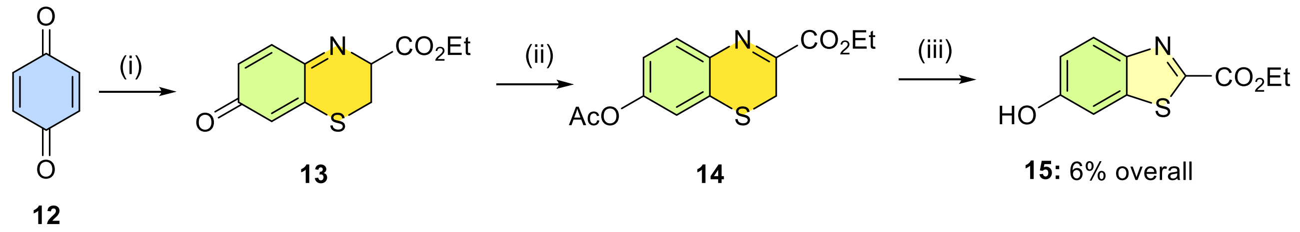
Seto *et al.* developed a shorter, seven-step alternate synthetic route (Scheme 4), which forms the thioamide **11** directly.[16] By using carbamoylthiocarbonylthioacetic acid **10**, the thioamide **11** could be prepared without passing through the anilide which would require further thionylation. As with the White synthesis, dehydration of the C2-amide of **7**, afforded 2-CBT **8**, with the chemistry post 2-CBT formation remaining unchanged from the seminal White synthesis.[17] The Seto synthesis of D-luciferin had a higher overall yield but shared many elements with the White synthesis, notably, the Jacobsen cyclisation was an integral part of the sequence.



Scheme 4. Synthesis of D-luciferin by Seto and coworkers. Reagents and conditions: (i) KOH (aq), H_2S, (ii) $ClCH_2CO_2H$, (iii) *p*-anisidine, EtOH (aq), rt, 24 h (29%), (iv) $K_3[Fe(CN)_6]$, NaOH, rt, 1 h (65%), (v) $POCl_3$, Δ, 1.5 h (63%).

2.3. McCapra et al. formal synthesis of D-luciferin (1975)

Following the White and Seto syntheses, which both involved the Jacobsen cyclisation as a key step, significant insights into the biosynthetic pathway of D-luciferin was reported by Frank McCapra, who had initially worked together with Emil White on the original synthesis. McCapra *et al.* highlighted that the benzothiazole, which at the time was considered an unusual ring system among natural products, could be accessed from *p*-benzoquinone (*p*-BQ) **12**.[17] McCapra and coworkers went on to synthesize the carbamoyl benzothiazole (Scheme 5) by reacting *p*-BQ with cysteine, followed by oxidation and cyclisation of the quinone to the corresponding quinonoid **13**. Acetylation of the quinonoid then generated the carbamoyl benzothiazole **15** via rearrangement of **14**. Although the conversion of the acetylated quinonoid **14** to benzothiazole **15** is described by McCapra as a rearrangement reaction, a methylene carbon is clearly lost, and so it might be considered a type of ring contraction as well. McCapra and others have suggested that the process involves an initial decarboxylation followed by oxidation and rearrangement.

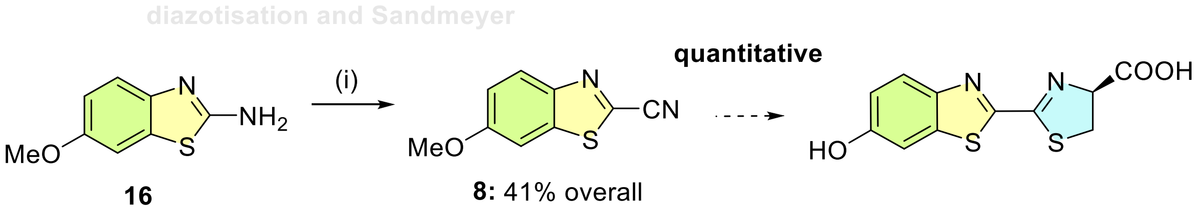


Scheme 5. Biomimetic synthesis of benzothiazole **15** by McCapra and coworkers.[18] Reagents and conditions: (i) Cysteine ethyl ester, H_2O, rt, 8 h (30%), (ii) Ac_2O, pyridine (70%), (iii) O_2, NaOEt, EtOH (30%).

It is, however, unclear whether the carbon atoms at the C2 position in the resulting benzothiazole is derived from the carbon atom at the C2 position of cysteine. McCapra *et al.* published several articles around this time further confirming the benzoquinone and cysteine-based biosynthesis of D-luciferin. Having proved that the benzothiazole core could be formed using the chemistry shown in scheme 5, Frank McCapra paved the way for the development of subsequent biomimetic synthesis, although these biomimetic sequences would only emerge years later.

2.4. Toya et al. synthesis of D-luciferin (1992)

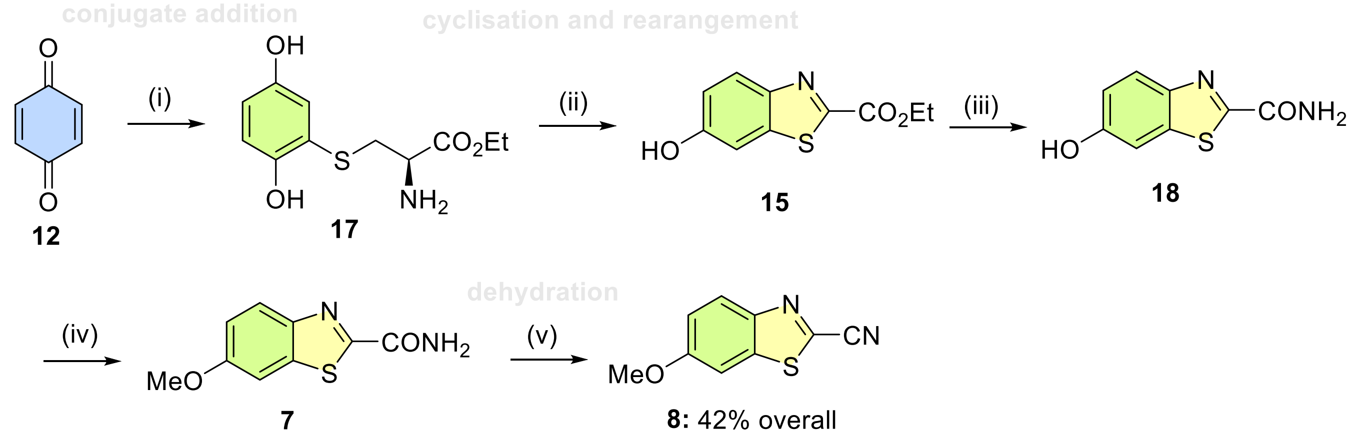
Toya *et al.* made use of commercially available 2-amino-6-methoxybenzothiazole **16** and, via activation through Sandmeyer conditions, directly substituted the amine for the essential cyanide group using CuCN and KCN (Scheme 6). This approach reportedly improved on the overall yield, when compared to other routes at the time.[13, 18] Access to the C2 nitrile through substitution presented an interesting alternative to the dehydration of carbamides. The benzothiazole core however, was still accessed using the same seminal chemistry, i.e. Jacobsen-type cyclisations, that is, until the 2-CBT revolution of the early 2000s.



Scheme 6. Synthesis of 2-cyano-6-methoxybenzothiazole by Toya and coworkers. Reagents and conditions: (i) HNO_2, CuCN/KCN, 0 °C, 1 h (41%).

2.5. Santaniello et al. synthesis of D-luciferin (2010)

Santaniello and co-workers expanded on the biomimetic synthesis developed by Frank McCapra. Their oxidative cyclisation approach delivered 2-cyano-6-methoxybenzothiazole **8** in substantially improved overall yield, which is comparable to the diazotization strategy reported by Toya *et al*. Despite the similar overall yields, a notable improvement of this sequence is the avoidance of toxic cyanides (Scheme 7).



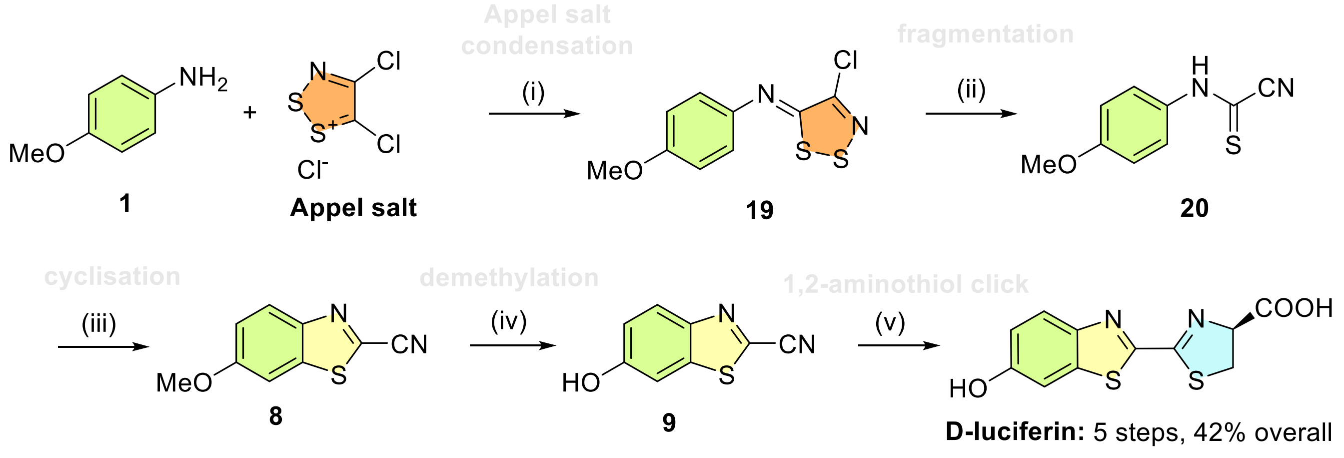
Scheme 7. Synthesis of benzothiazole by Santaniello and coworkers. Reagents and conditions: (i) L-Cysteine ethyl ester hydrochloride, MeOH:H_2O (1:1), rt, 1 h, (ii) $K_3(FeCN)_6$, NaOH, EtOH, rt, 1 h (68%), (iii) NH_4OH, EtOH, reflux, 5 h (98%), (iv) MeI, K_2CO_3, DMF, reflux, 1 h (92%), (v) $POCl_3$, pyridine, DCM, rt, 12 h (72%).

In this sequence, *p*-BQ **12** was reacted with L-cysteine ethyl ester hydrochloride to produce (*R*)-2-amino-3-(2,5-dihydroxyphenylsulfanyl) propanoate **17**. Treatment of **17** with $K_3(FeCN)_6$ produced the cyclized benzothiazole **15**, which was further transformed to produce the 2-cyano-6-methoxybenzothiazole **8**. Interestingly, the methylation of the phenolic hydroxyl could be achieved in the presence of the carboxyamide functionality, although the authors also report the direct methylation of the C6-hydroxyl of **15** as well. It was presumably necessary to methylate the hydroxyl due to the potential chlorine substitution under these conditions.

2.6. McCutcheon et al. synthesis of D-luciferin (2012, 2015): The 2-CBT revolution

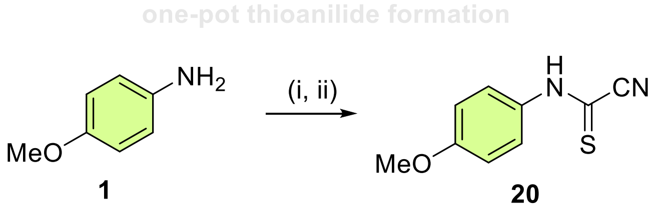
The synthetic breakthrough that revolutionised luciferin chemistry in the twenty-first century cannot be understood without acknowledging the foundational contributions of Charles W. Rees and Appel salt chemistry.[19-21] Rees' systematic exploration of 4,5-dichloro-1,2,3-dithiazolium chloride, more commonly known as Appel salt, throughout the 1980s and 1990s established this reagent as a uniquely versatile synthon for heterocycle construction, particularly benzothiazoles.[21] Rees and coworkers demonstrated that anilines could be efficiently converted into *N*-aryliminodithiazoles, which undergo controlled sulfur extrusion to afford thioformamides which, in turn, are key precursors for subsequent cyclisation reactions. These studies laid the mechanistic and practical groundwork that later enabled rapid access to 2-CBTs.

In the context of luciferin synthesis, Rees' chemistry provided a fundamentally new solution to the long-standing problem of inefficient B-ring construction. Appel salt allowed direct installation of both sulfur and nitrile functionality in a concise, modular fashion. Although Rees himself did not apply this chemistry to luciferins, his work effectively supplied the missing synthetic logic required to modernize the synthesis of D-luciferin. This latent potential was realized in 2012 by McCutcheon and Prescher *et al.*, who adapted Appel salt condensation chemistry directly to luciferin synthesis (Scheme 8).



Scheme 8. McCutcheon *et al.* synthesis of D-luciferin from *p*-anisidine.[22] Reagents and conditions: (i) DCM, pyridine (2 eq), rt, 3 h (99%), (ii) DBU (3 eq), DCM, 0° C – rt, 30 min (97%), (iii) $PdCl_2$ (10 mol %), CuI (50 mol %), TBAB (2 eq), DMF:DMSO (1:1), 120 °C, 2 h (87%), (iv) Pyr·HCl (10 eq), neat, 190 °C, 1 h (61%), (v) D-cysteine, K_2CO_3, H_2O:MeOH, rt, 20 min (86%).

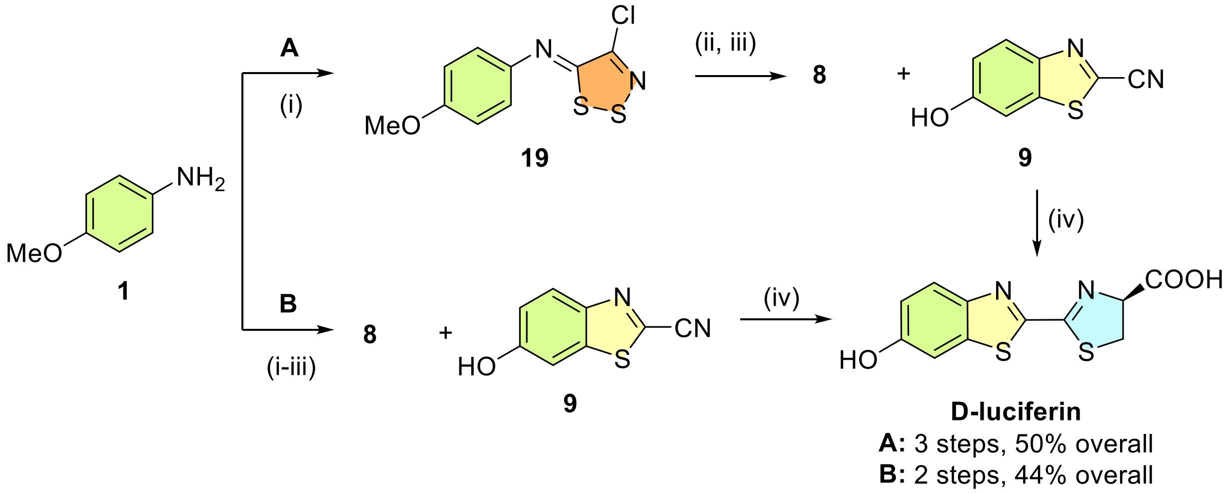
Condensation of *p*-anisidine **1** with Appel salt generated the corresponding dithiazole intermediate **19**, which could be cleanly fragmented using DBU to afford cyanoformanilide **20**. Interestingly, Nyoni *et al.* had demonstrated several years prior that DBU could be utilized as a thiophilic base to extrude sulfur.[23] The mechanism involves nucleophilic attack by the DBU amidine nitrogen at the dithiazole S2 ring sulfur, resulting in the dithiazole ring opening to afford an acyclic disulfide. A second equivalent of DBU cleaves the disulfide bond to give the cyanoformanilide **20** and a neutral DBU-sulfane as a byproduct. Palladium- and copper-mediated intramolecular C–S coupling then delivered the key intermediate 6-methoxy-2-cyanobenzothiazole **8** in a markedly improved yield relative to all prior approaches. Demethylation furnished 6-hydroxy-2-cyanobenzothiazole **9**, which underwent near-quantitative condensation with D-cysteine to afford D-luciferin. This route reduced the overall step count by four relative to the nine steps of the White synthesis and increased the overall yield to approximately 42%.[10] Building on this success, McCutcheon and co-workers further refined the sequence by eliminating chromatographic separations and replacing DBU with sodium thiosulfate as a milder, more scalable sulfur-extrusion reagent (Scheme 9).[24] This one-pot protocol condensed imine formation and dithiazole fragmentation into a single operation, raising the overall yield of D-luciferin to approximately 60% while substantially improving operational simplicity.



Scheme 9. One pot condensation synthesis of D-luciferin employed by McCutcheon *et al*.[24] Reagents and conditions: (i) Appel salt, DCM, pyridine (2 eq), rt, 3 h, (ii) $Na_2S_2O_3$ (3 eq), H_2O, rt, 3 h (86%).

The culmination of these synthetic improvements was the recognition that the poorly scalable metal-catalyzed cyclisation step itself could be bypassed. Drawing inspiration from earlier thermolysis studies by Rakitin and Rees[25] on related dithiazole systems, McCutcheon demonstrated that direct thermal extrusion of sulfur from the Appel salt-derived intermediate could generate 2-cyano-6-methoxybenzothiazole **8** or 2-cyano-6-hydroxybenzothiazole **9**, without transition-metal catalysis (Scheme 10A). When combined with in situ imine formation, this strategy delivered the shortest synthesis of D-luciferin from simple anilines reported to date (Scheme 10B). In route A, thermolysis is promoted by high reaction temperatures and subsequent treatment with Pyr·HCl and, as a result, benzothiazoles **8** (20%) as well as **9** (61%) were isolated (Scheme 10A).

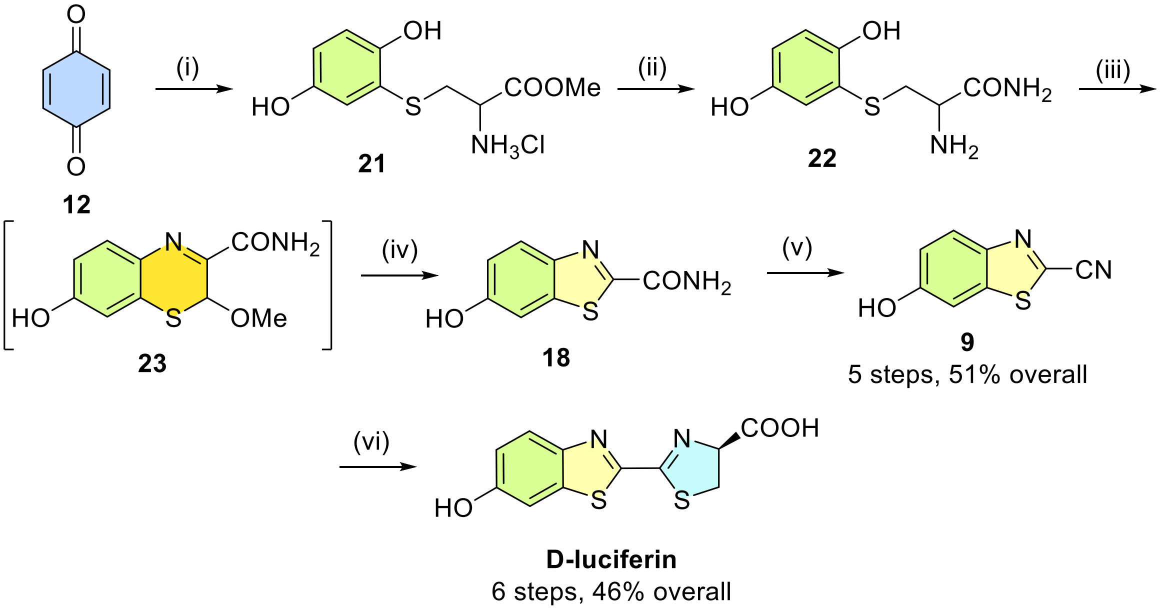
The broader impact of this Appel salt-based paradigm extends well beyond D-luciferin itself. By enabling rapid, high-yielding access to diversely substituted 2-CBTs, this chemistry underpins the synthesis of many modern luciferin analogues, including C6-modified derivatives and conjugatable probes. In this sense, Rees' fundamental heterocyclic chemistry and the later translational work of McCutcheon and Prescher collectively transformed luciferin synthesis from a target-specific endeavor into a general, more broadly applicable technology.



Scheme 10. Condensed synthesis of D-luciferin via Appel salt condensation and thermolysis.[24] Reagents and conditions **(A):** (i) Appel salt, DCM, pyridine (2 eq), rt, 3 h, (96%), (ii) Sulfolane, 180 °C, 20 min, (iii) Pyr·HCl (10 eq), 180 °C, 1 h, **8** (20%) and **9** (61%). **(B):** (i) Appel salt, sulfolane, 40 °C, 3 h, (ii) 180 °C, 20 min, (iii) Pyr·HCl (10 eq), 180 °C, 1 h, **8** (21%) and **9** (51%), (iv) D-cysteine, K_2CO_3, H_2O:MeOH, rt, 20 min (86%).

2.7. Nishikawa et al. biomimetic synthesis of D-luciferin (2024)

Recently, Nishikawa and coworkers returned to the biomimetic approach and reported a one-pot, six-step synthesis of starting from *p*-BQ (**12**) and L-cysteine methyl ester (Scheme 11).[26, 27]



Scheme 11. One-pot synthesis of D-luciferin.[27] Reagents and conditions**:** (i) L-Cysteine methyl ester hydrochloride, MeOH, rt, 20 min, (ii) 28% NH_3, H_2O, rt, 30 min, (iii) *p*-BQ, MeOH, rt, (iv) *p*-PQ, aq. HCl, rt, 3.5 h, (v) TFAA, pyridine, rt, 1 h, (vi) D-cysteine hydrochloride, K_2CO_3, MeOH:H_2O, rt, 20 min (46% overall).

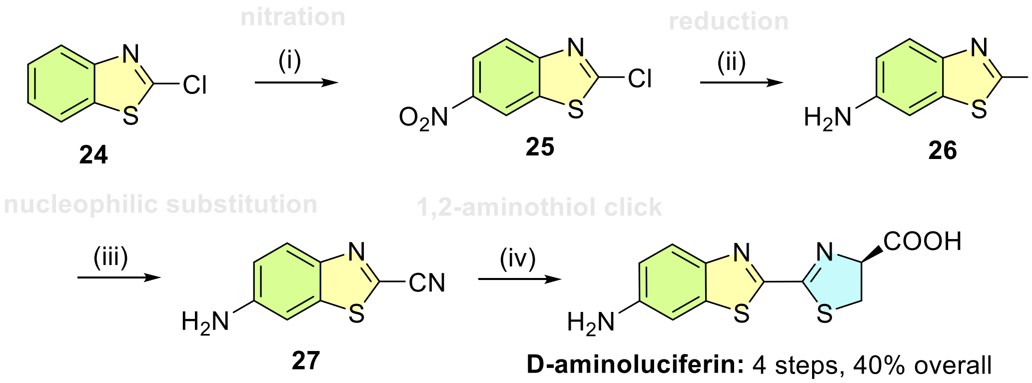
Their reported one-pot synthesis produces D-luciferin in an overall yield of 46% while making use of inexpensive starting materials (Scheme 11). Additionally, a single extractive work-up is only needed at the end of the final step and the sequence completely avoids the use of metallic reagents. The synthesis by Nishikawa *et al.* is the only one-pot synthesis and the only synthesis not to require metallic reagents or forcing conditions.

3. Syntheses of D-Aminoluciferin

D-Aminoluciferin was the first reported example of a bioluminescent active, non-naturally occurring D-luciferin analogue.[9] It was shown to react with firefly luciferase in a decarboxylative bioluminescent reaction. Unlike the characteristic yellow-green light produced by D-luciferin, the C6-amino analogue emits red light, independent of the pH of the reaction media. D-Aminoluciferin is the foundation for many bioluminescent probes and bioluminescent technologies.[6, 28-30]

3.1. White et al. synthesis of D-aminoluciferin (1966)

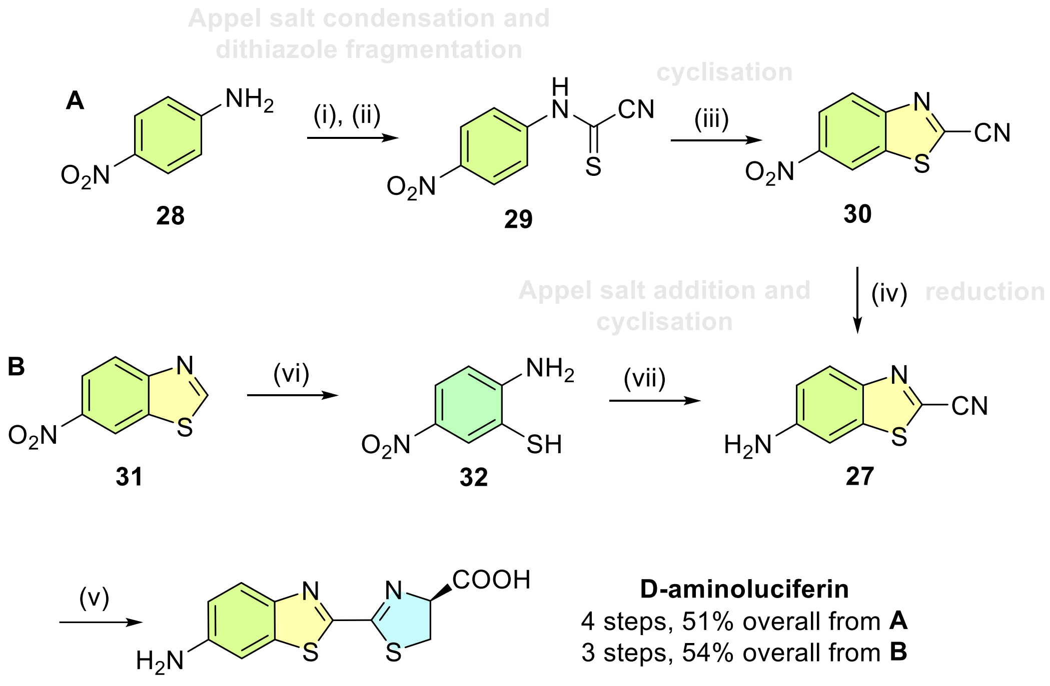
The synthesis of D-aminoluciferin follows a similar pathway to that of D-luciferin but incorporates an amino functional group instead of the hydroxyl at the 6-position of the benzothiazole ring. The first reported synthesis of this bioluminescent non-naturally occurring analogue was completed shortly after the first total synthesis of D-luciferin. The amino C6-amino analogue was prepared from 2-chlorobenzothiazole **24** which was further nitrated to afford 2-chloro-6-nitrobenzothiazole **25**. Interestingly, in this sequence, the nitro was first reduced before nitrile substitution at C2. The overall yield was 40% starting from 2-chlorobenzothiazole (**24**, Scheme 12).



Scheme 12. White synthesis of D-aminoluciferin.[9] Reagents and conditions**:** (i) H_2SO_4, KNO_3, rt, 1 h (95%), (ii) EtOH, CH_3COOH, H_2O, Fe, 80 °C, 2 h (75%), (iii) KCN, DMSO, 120 °C, 1 h (60%), (iv) D-cysteine, NH_3, Na, MeOH, rt, 2 h, (93%).

3.2. McCutcheon et al. synthesis of D-aminoluciferin (2015)

The innovative use of Appel salt in the preparation of 2-CBT, initially introduced by Prescher, McCutcheon and coworkers,[24] was also applied to D-aminoluciferin. In this sequence *p*-nitroaniline **28** was condensed with Appel salt followed by treatment with sodium thiosulfate to produce the (4-nitrophenyl)carbamothioyl cyanide **29**. Palladium and copper mediated cyclisation produced 2-cyano-6-nitrobenzothiazole **30**, followed by reduction of the nitro group and subsequent D-cysteine condensation to produce the desired D-aminoluciferin with an overall yield of 51% (Scheme 13A). The authors were able to further improve their synthetic route by installing a thiol group ortho to the nitro group in the starting material *p*-nitroaniline, thus enabling facile access to 2-CBT **27** and D-aminoluciferin in three steps with an overall yield of 54% (Scheme 13B). Notably, this is the shortest and highest yielding synthesis of D-aminoluciferin to date.



Scheme 13. McCutcheon *et al.* synthesis of D-aminoluciferin from **(A)** *p*-nitroaniline and **(B)** hydrazine.[24] Reagents and conditions: (i) Appel salt, MeCN:THF (2:1), rt, 1 h, (ii) $Na_2S_2O_3$ (3 eq), H_2O, rt, 2 h (80%), (iii) $PdCl_2$ (10 mol %), CuI (50 mol %), TBAB (2 eq), DMF:DMSO (1:1), 130 °C, 3 h (74%), (iv) Zn, NH_4Cl, MeOH, rt, 30 min (95%), (v) D-cysteine, K_2CO_3, MeCN:H_2O (4:1), rt, 20 min (91%), (vi) $NH_2NH_2.H_2O$, EtOH, rt, 12 h, (vii) Appel salt, DCM, reflux, 12 h (62%).

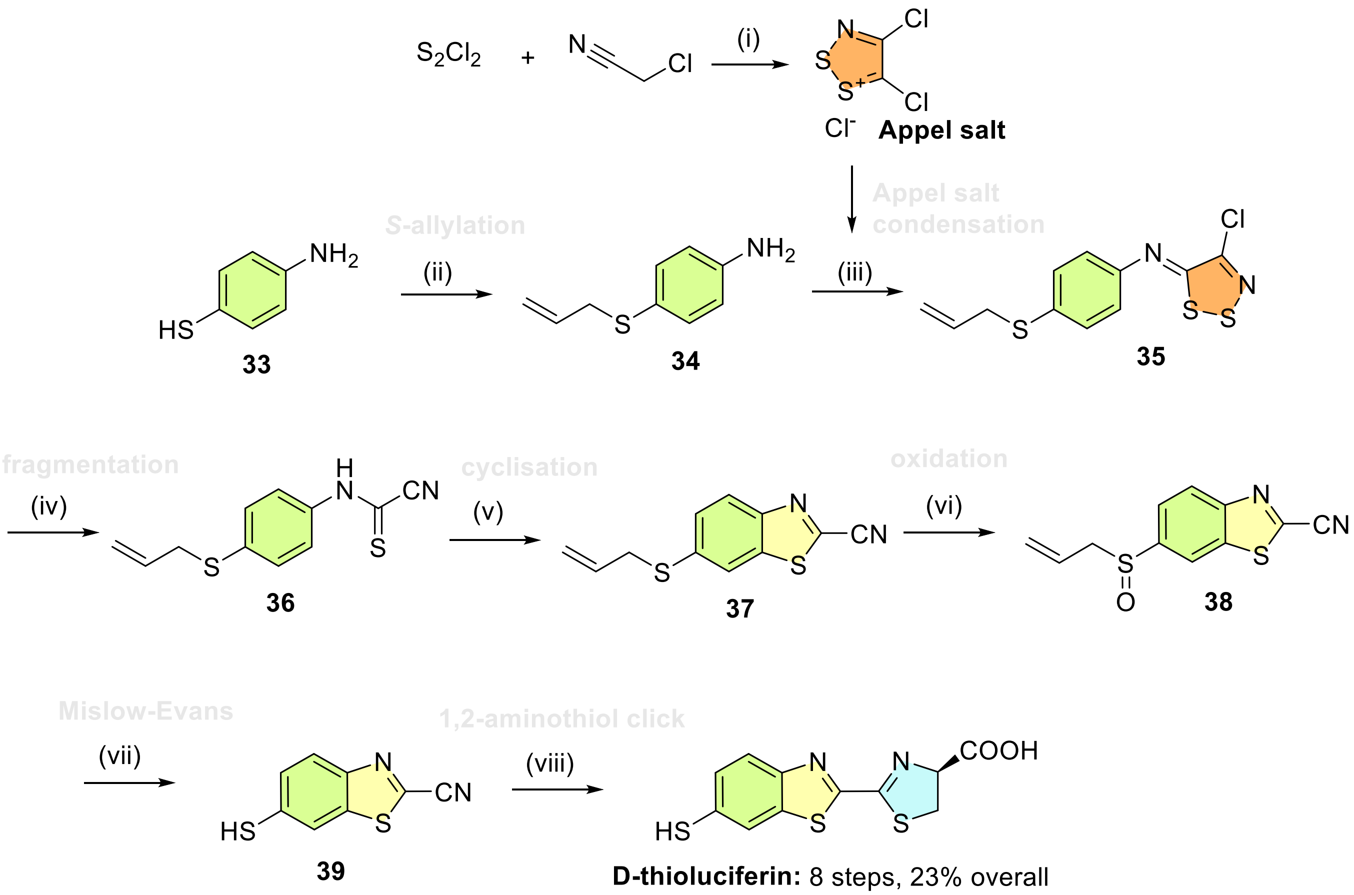
4. Syntheses of D-Thioluciferin

Much like D-aminoluciferin, which enabled the growth and development of amino- and amide-based bioluminescent probes, the C-6 thio analogue was also intended to expand D-luciferin bioluminescence to biological sulfur chemistry. To date, there are only two reports on the synthesis of D-thioluciferin. In both accounts, D-thioluciferin is reported to have a much lower emission when compared to D-luciferin, with the thiol functionality being easily oxidised to the homodisulfide form.[31-33] Together with the lower emission, D-thioluciferin was found to bind tightly to luciferase making it a useful addition to the D-luciferin toolkit.

4.1. Pirrung et al. synthesis of D-thioluciferin (2019)

Pirrung *et al.* published their synthesis of D-thioluciferin starting from commercially available 4-thioaniline. This synthetic route comprises of a sequence of steps incorporating the general luciferin synthesis developed by Prescher[22, 24] as well as a Mislow-Evans[34] type rearrangement mediated by triphenylphosphite.

Pirrung and co-workers employed an *S*-allyl protecting group in their synthetic strategy. The sequence begins with 4-thioaniline **33**, which is *S*-allylated to form **34** which is further reacted with Appel salt to generate the dithiazolimine **35**. This intermediate then undergoes DBU-mediated dithiazole fragmentation to yield thioformamide **36**. As in the original work by McCutcheon *et al*, a copper(I) iodide/palladium(II) chloridecatalysed C–S cross-coupling of thioformamide **36** produces the *S*-allylated 2-CBT **37**. Subsequent non-asymmetric oxidation of **37** using sodium periodate affords sulfoxide **38**, which undergoes a Mislow–Evans-type *[2,3]*-sigmatropic rearrangement. This is followed by triphenylphosphite [$P(OPh)_3$]-mediated cleavage of the sulfenate intermediate to give thiobenzothiazole **39**. The final step involves a standard luciferin condensation with D-cysteine to afford the desired product, D-thioluciferin (Scheme 14). Overall, this route produces D-thioluciferin in an overall yield of 23%, over eight steps.

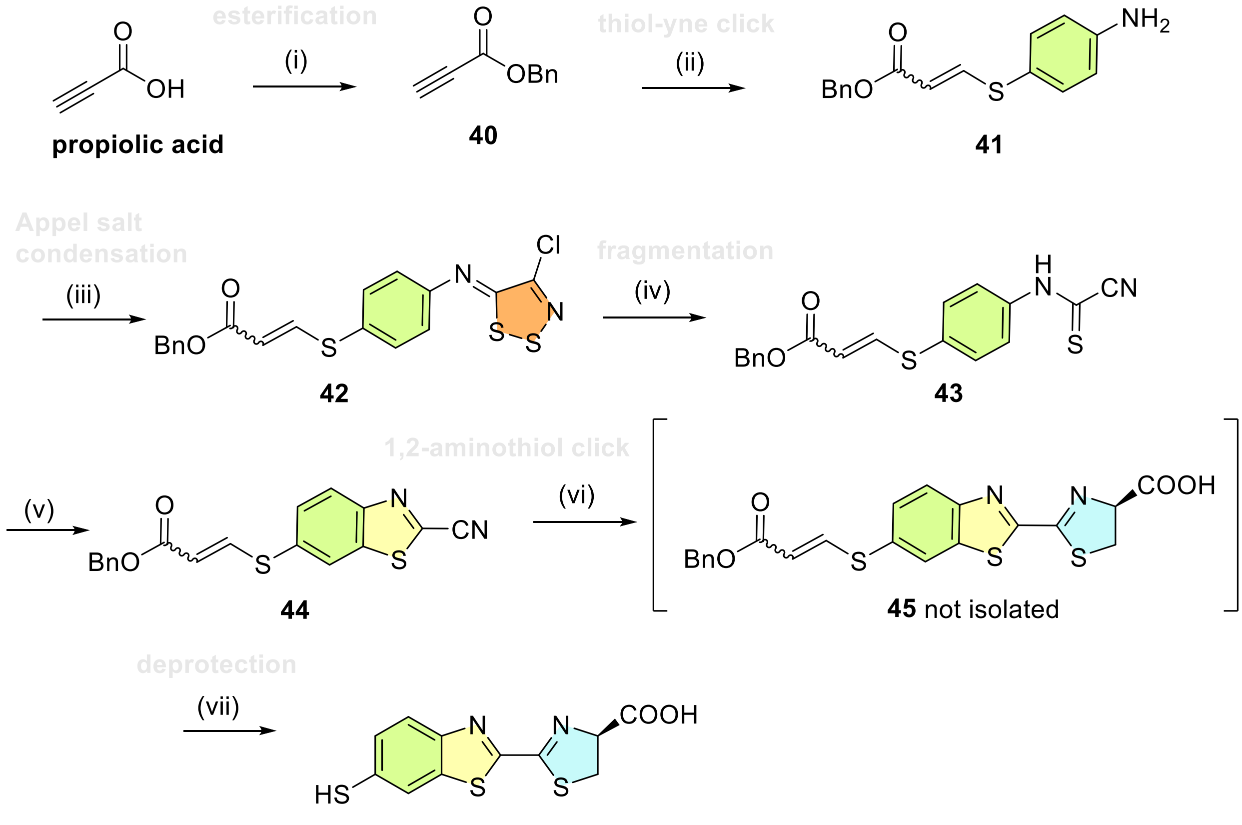


Scheme 14. Linear synthetic route for the synthesis of D-thioluciferin. Reagents and conditions: (i) DCM, rt, 24 h, (ii) Allyl bromide, K_2CO_3, acetone, rt, 18 h (90%), (iii) Appel salt, DCM, anhydrous pyridine, rt, 3 h (77%), (iv) DCM, DBU, -5 °C, 1 h (83%), (v) $PdCl_2$ (10 mol %), CuI (50 mol %), TBAB (2 eq), DMF:DMSO (1:1), 120 °C, 1 h (65%), (vi) DCM:MeOH (1:5), $NaIO_4$, 0 °C, 12 h (90%), (vii) $P(OPh)_3$, THF, H_2O, 72 °C, 18 h (79%), (viii) D-Cysteine, K_2CO_3, MeOH:H_2O, rt, 30 min (87%).

4.2. Rylands et al. synthesis of D-thioluciferin (2020)

In 2020 Marwaan Rylands and Anwar Jardine reported a similar synthesis of D-thioluciferin, using vinyl sulfides as protecting groups, instead of allyl sulfides as reported by Pirrung and coworkers.[35] The Rylands *et al.* synthesis relies on a benzyl thioacrylate as a sulfur protecting group. As such, using thiol-yne click chemistry, thioacrylate **41** was obtained via a chemoselective reaction between benzyl propiolate **40** (prepared from propiolic acid) and *p*-aminothiophenol. This resulted in a *E/Z* thioacrylate mixture of **41**. The cis isomer was isolated and subsequently reacted with Appel salt to yield *N*-aryliminodithiazole **42**. Subsequent nucleophilic attack of thiophilic DBU afforded cyanoformanilide **43**. Thereafter, intramolecular C-S coupling using palladium chloride formed the cyclised 2-CBT **44**. Following the addition of 2 mol equivalents of D-cysteine, a one pot D-cysteine condensation as well as thioacrylate deprotection of **45** released D-thioluciferin in an overall yield of 14% (Scheme 15).[35]

A notable limitation of this sequence when compared to the *S*-allyl strategy of Pirrung and coworkers, relates to the configurational instability of the thioacrylate **41**. It was found that in the presence of organic bases, such as DBU, the pure isolated cis isomer undergoes conversion to a mixture of *E/Z* isomers, following a well reported Michael addition-elimination mechanism.[36] The uncaging of the D-thioluciferin is presumably isomer dependant.



D-thioluciferin: 7 steps, 14% overall

Scheme 15. Synthetic route toward D-thioluciferin. Reagents and conditions: (i) Cs_2CO_3, BnBr, DMF, 0 °C – rt, 30 min (99%), (ii) *p*-aminothiophenol, DMF, 24 h, 0 °C, 24 h (39%), (iii) Appel salt, pyridine, DMF, rt, 3 h (99%), (iv) DBU, DMSO, rt, 1.1 h (61%), (v) $PdCl_2$, CuI, TBAB, DMSO, 120 °C, 4 h (62%), (vi) D-cysteine, K_2CO_3, DMSO, 0 °C – rt, 15 min (98%).

5. Conclusions

Over six decades, the synthesis of D-luciferin has evolved from an impressive multistep endeavor, into a streamlined process, focusing on scalability and avoiding toxic reagents. Central to this evolution has been the identification and exploitation of the 2-CBT intermediate and the extraordinary efficiency of the 1,2-aminothiol click reaction. All the reported syntheses of D-luciferin, D-aminoluciferin and D-thioluciferin incorporate these two unifying features. Initially, advances in the synthesis of D-luciferin focused on improving access to 2-CBTs from anilides and thioanilides. As the biosynthesis of D-luciferin started unravelling, new biomimetic approaches to 2-CBT and D-luciferin were reported. D-luciferin synthesis, which started as a nine-step synthesis with a 9% overall yield, can now be produced in a one-pot reaction with an overall yield of 46%. Extensions of the chemistry developed to access D-luciferin have also enabled access to C6 analogues such as D-aminoluciferin and D-thioluciferin, each with unique bioluminescent properties and applications. These analogues underscore how synthetic ingenuity directly shapes technological capability. The history reviewed here demonstrates that advances in small-molecule synthesis remain foundational to the continued growth of the science of bioluminescence.

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