A New Synthesis of 2-Cyano-6-hydroxybenzothiazole, the Key Intermediate of D-Luciferin, Starting from 1,4-Benzoquinone

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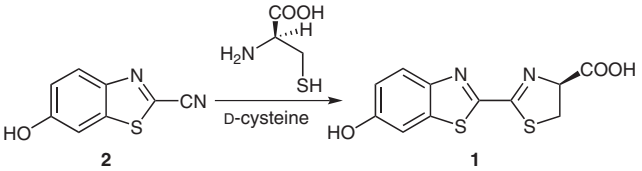
Abstract: 2-Cyano-6-hydroxybenzothiazole is the key intermediate for the synthesis of D-luciferin, the natural substrate of firefly luciferases. A new synthesis of 2-cyano-6-hydroxybenzothiazole has been realized starting from the reaction of 1,4-benzoquinone with L-cysteine ethyl ester, followed by oxidation–cyclization of the intermediate ethyl (*R*)-2-amino-3-(2,5-dihydroxyphenylsulfanyl)propanoate hydrochloride to 2-carbethoxy-6-hydroxybenzothiazole. A suitable protection of this intermediate and a conversion to the corresponding nitrile gave, after deprotection, 2-cyano-6-hydroxybenzothiazole (32% yield from 1,4-benzoquinone). This nitrile reacts with D-cysteine to afford D-luciferin at room temperature in nearly quantitative yield (90–95%).

Key words: D-luciferin, 1,4-benzoquinone, firefly luciferase, 2-cyano-6-hydroxybenzothiazole

The luciferase from the North American firefly *Photinus pyralis* (PpyLuc) catalyzes the conversion of D-luciferin [(*S*)-2-(6′-hydroxy-2′-benzothiazolyl)-Δ^2-thiazoline-4-carboxylic acid (**1**)] to oxyluciferin in the presence of ATP, Mg^{2+}, and oxygen with production of a yellow-green light characterized by a broad emission spectrum and a peak at 560 nm (Scheme 1).[1]

Fireflies are only a part of the big family of bioluminescent insects that can emit light with wavelengths going from yellow-green to red, but all luciferases use the same substrate, namely D-luciferin (**1**).[2] The chemical structure of D-luciferin (**1**), isolated from firefly tails, was proposed in 1961[3] and later confirmed by synthesis.[4]

This synthetic approach (Scheme 2) remains yet the only and unsurpassable method for the preparation of D-luciferin (**1**), that is obtained in a nearly quantitative yield (90–95%) by reaction at room temperature of 2-cyano-6-hydroxybenzothiazole (**2**) with an aqueous solution of D-cysteine.

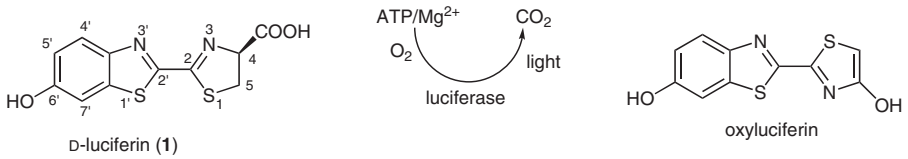


Scheme 2 Synthesis of D-luciferin (**1**) from 2-cyano-6-hydroxy benzothiazole (**2**)

Most of the synthetic procedures currently available for the preparation of the key intermediate 2-cyano-6-hydroxybenzothiazole (**2**) start from *p*-anisidine (**3**)[4–10] (Scheme 3).[11] All these routes[12] allow the preparation of the intermediate 2-cyano-6-methoxybenzothiazole (**7**), that can be demethylated with pyridinium hydrochloride (200 °C, 62%) to the nitrile **2**.[4]

We describe here a new synthesis of 2-cyano-6-hydroxybenzothiazole (**2**), the immediate precursor for the synthesis of D-luciferin (**1**), starting from 1,4-benzoquinone (**8**).[13] Our synthetic approach was planned to be more versatile than the previously existing ones. In fact, starting from 1,4-benzoquinones containing different groups, the preparation of variously substituted analogues of the nitrile **2** would become feasible. By this approach, luciferin-like compounds, potentially endowed with new bioluminescent properties in the luciferase-catalyzed light emission,[14] would be available.

The reaction of 1,4-benzoquinone (**8**) with L-cysteine ethyl ester affords different products, depending on the experimental conditions.[15–17] However, ethyl (*R*)-2-amino-3-(2,5-dihydroxyphenylsulfanyl)propanoate hydrochloride (**9**) has been isolated and characterized.[18] Under controlled conditions, the oxidation of the ester **9** can lead to the benzothiazolyl ester **10**.[19] We have followed essential-



Scheme 1 Luciferase-catalyzed production of bioluminescence

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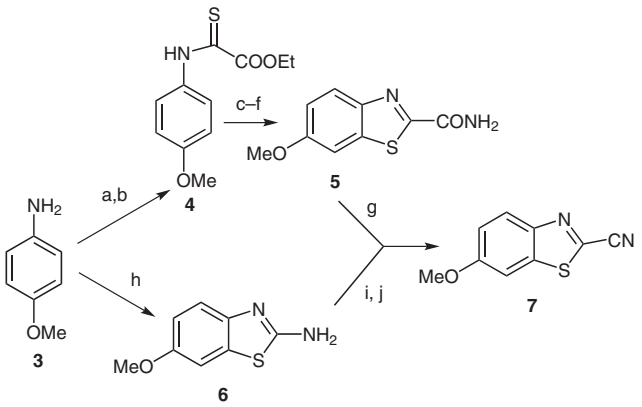
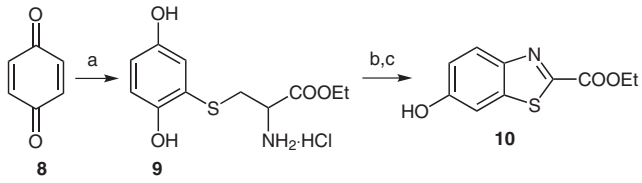


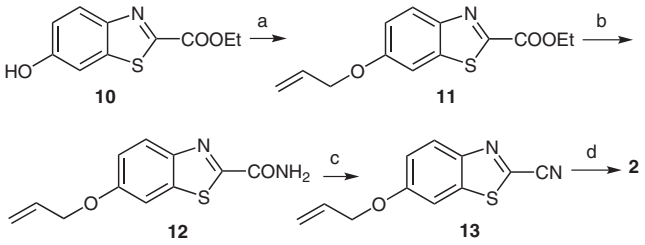
Scheme 3 Synthetic approaches to the preparation of 2-cyano-6-methoxybenzothiazole (**7**) from *p*-anisidine (**3**). *Reagents and conditions*: (a) ethyl oxalate, 180 °C, 5 min, 58%; (b) P_2S_5, xylene, reflux; (c) NaOH, 0 °C; then HCl; (d) $K_3Fe(CN)_6$, NaOH, <10 °C, 15 min (76% crude, no isolation in steps b–d); (e) CH_2N_2, 0 °C, 15 min, 40%; (f) anhyd NH_3–MeOH, heat, 30 min, 100%; (g) $POCl_3$, reflux, 15 min, 56%; (h) KSCN, Br_2, AcOH, 35 °C, 10 h, 87%; (i) $CuBr_2$ in PEG 200, MeCN, isoamyl nitrite, 65 °C, 3 h, 79.3%; (j) NaCN, DMSO, 110 °C, 5 h, 70.4%.

ly both procedures[18,19] with minor modifications of the published protocols (Scheme 4). Briefly, we have prepared the ester **9** in methanol–water, and the residue from workup[20] was directly oxidized with $K_3Fe(CN)_6$ in an aqueous ethanol solution of NaOH.[21] The residue obtained after workup (90% yield) was dissolved in ethanol and treated with 1 N HCl. The required ester **10** was obtained in 60% yields after column chromatography.[22]



Scheme 4 Synthesis of ester **10** from 1,4-benzoquinone (**8**). *Reagents and conditions*: (a) L-cysteine ethyl ester hydrochloride, MeOH–H_2O, r.t., 1 h, 96% (crude); (b) $K_3Fe(CN)_6$, NaOH, aq EtOH, r.t., 1 h, 90% (crude); (c) 1 N HCl, EtOH, r.t., 24 h, 60% (after column chromatography).

We then studied different protections of the ester **10** bearing in mind that these groups should be resistant to the conditions required for the transformation of an amide into a nitrile. Also the final deprotection of the intermediate protected nitrile was considered with attention, due to sensitivity of the nitrile group to hydrolytic and reducing conditions.[23] It turned out that the 6-*O*-allyl ether was the most suitable protecting group, meeting all previous requirements.[24] The synthetic approach is described in Scheme 5.[25] Accordingly, the 6-*O*-allyl ester **11** was prepared from the ester **10** and converted to the amide **12** smoothly and almost quantitatively using concentrated ammonia in ethanol.[26,27] Then, the amide **12** was transformed into the nitrile **13** using phosphorus oxychloride as dehydrating agent under controlled conditions.[28,29]



Scheme 5 Synthesis of the nitrile **2** from the ester **10**. *Reagents and conditions*: (a) allyl bromide, DMF, K_2CO_3, reflux, 1 h, 96%; (b) conc NH_4OH–EtOH, reflux, 5 h, 95%; (c) $POCl_3$, pyridine, imidazole, CH_2Cl_2, from –10 °C to r.t., 12 h, 81%; (d) $Pd(PPh_3)_4$, $NaBH_4$ in THF, r.t., 24 h, 83%.

By this procedure, the allyl protecting group remained essentially intact and the nitrile **13** was obtained in 81% yield. The final deprotection was successfully achieved following a described procedure[30] (83% yields).[31,32]

In conclusion, we have prepared 2-cyano-6-hydroxybenzothiazole (**2**), the pivotal intermediate for the preparation of D-luciferin (**1**), by a more versatile approach with respect to the classical ones that start from *p*-anisidine (**3**).[4–10] In our approach, starting from 1,4-benzoquinone (**8**), the 6-*O*-allyl ester **11** has been prepared and this intermediate transformed into the nitrile **2** (32% overall yield). These yields can favorably be compared with previous procedures (6–29%), considering also that a few of these protocols involve the use of poisonous cyanide.[8–10]

Acknowledgment

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(11) The nitrile **7** has also been prepared from the corresponding *N*-aryliminodithiazole under microwave irradiation; see:

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(20) According to the original procedure, extraction with EtOAc removed most of the impurities, and evaporation of the solvents afforded the hygroscopic ester **9**. In our hands, samples of different preparations showed variable purity for the final product that contained also variable amount of H_2O. Therefore, the recovery (96%) refers to the weight of the final product.
(21) In the case of 4-hydroxybenzothiazole derivatives similar to compound **9**, the oxidation proceeds efficiently with $K_3Fe(CN)_6$, in the presence of $ZnSO_4$; see: Greco, G.; Panzella, L.; Napolitano, A.; D'Ischia, M. *Tetrahedron Lett.* **2009**, *50*, 3095.
(22) The one-pot procedure for the preparation of the benzothiazolyl ester **10** starting from 1,4-benzoquinone **8** proceeds less satisfactorily (35–45%).
(23) Among protecting groups, we have tested *tert*-butyldimethylsilyl, tetrahydropyranyl, and 2-methoxy ethoxymethyl (MEM) ethers that did not survive or were partially hydrolyzed in the conditions required for the conversion of the 2-carboxyamide into the 2-nitrile function. Benzyl was an effective protecting group in the transfor-mation of amide **12** into nitrile **13**, but removal of the protecting group by hydrogenolysis of 2-cyano-6-*O*-benzyl-oxy benzothiazole caused the reduction of the nitrile moiety.
(24) For an application of allyl ethers as protecting groups and to the methods available for their removal, see: Hu, Y.-J.; Dominique, R.; Das, S. K.; Roy, R. *Can. J. Chem.* **2000**, *78*, 838; and references cited therein.
(25) The progress of all reactions and column chromatography were monitored by TLC (PE–EtOAc, 8:2) using silica gel 60 F_{254} precoated plates with a fluorescent indicator (Merck). Purification of products by chromatography was performed using silica gel 60 (230–400 mesh, Merck). All compounds gave satisfactory elemental analysis.
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(27) **Ester 11**
To a solution of the ester **10** (1.20 g, 5.38 mmol) in DMF (15 mL), K_2CO_3 was added (1.11 g, 8.07 mmol) and the mixture stirred at r.t. (30 min). After addition of allyl bromide (0.746 mL, 8.07 mmol) the reaction was refluxed for 1 h. After H_2O addition, extraction with EtOAc, washing of the organic solution with aq NaCl, drying, and evaporation of solvent, the product is purified by chromatography (PE–EtOAc, 9:1). The 6-*O*-allyl ester **11** is obtained as a yellow solid (1.353 g, 96% yield); mp 97–99 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.48 (t, 3 H, *J* = 8.7 Hz), 4.53 (q, 2 H, *J* = 8.7 Hz), 4.63 (d, 2 H, *J* = 5.2 Hz), 5.35 (dd, 1 H, *J* = 1.5, 10.5 Hz), 5.45 (dd, 1 H, *J* = 1.8, 17.5 Hz), 6.05 (m, 1 H), 7.18 (dd, 1 H, *J* = 2.4, 9.1 Hz), 7.36 (d, 1 H, *J* = 2.4 Hz), 8.10 (dd, 1 H, *J* = 2.4, 9.1 Hz). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 14.90, 63.00, 69.50, 104.50, 118.05, 118.50, 126.30, 132.90, 139.00, 148.00, 156.00, 158.90, 161.00.
Amide 12
Compound **11** (1.10 g, 4.198 mmol) was added to a solution containing concentrated aq NH_3 (20 mL) in EtOH (50 mL) and the solution refluxed (5 h). After evaporation at reduced pressure, EtOAc was added and the solution passed through a column of Florisil (20 g). Evaporation of the solvent left the pure amide **12** as a yellow solid (0.929 g, 95% yield); mp 173–175 °C.
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(29) **2-Cyano-6-*O*-allyloxybenzothiazole (13)**
A solution containing compound **12** (0.950 g, 4.077 mmol) and imidazole (0.277 g, 4.077 mmol) in anhyd pyridine (25 mL) was cooled to –10 °C under nitrogen. Through a syringe, a solution of phosphorous oxychloride (0.746 mL, 8.154 mmol) in CH_2Cl_2 (5 mL) was slowly added, and the resulting solution was kept in ice under stirring (1 h). The temperature was slowly raised to ambient and stirred for additional 12 h, then CH_2Cl_2 and H_2O were sequentially added. The organic phase was separated, washed with H_2O, dried over Na_2SO_4 and evaporated. The crude was purified by chromatography (PE–EtOAc, 9:1), obtaining compound **13** (0.713 g, 81%) as a yellow solid; mp 74–75 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 4.62 (d, 2 H, *J* = 5.2 Hz), 5.35 (dd, 1 H, *J* = 1.5, 10.5 Hz), 5.45 (dd, 1 H, *J* = 1.8, 17.5 Hz), 6.07 (m, 1 H), 7.21 (dd, 1 H, *J* = 2.4 Hz), 7.36 (d, 1 H, *J* = 2.4, 9.1 Hz), 8.05 (dd, 1 H, *J* = 2.4, 9.1 Hz). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 69.50, 104.10, 113.70, 118.20, 118.90, 126.20, 132.80, 133.50, 137.80, 147.00, 159.70.
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(32) **2-Cyano-6-hydroxybenzothiazole (2)**
To a solution of compound **13** (0.600 g, 2.777 mmol) in anhyd THF under N_2, $Pd(PPh_3)_4$ (0.02 mmol) and $NaBH_4$ (0.422 g, 11.1 mmol) were added sequentially. After 24 h at r.t., the reaction was filtered and the solvent evaporated to leave a residue that was purified by column chromatography (PE–EtOAc, 8:2). The nitrile **2** was obtained as a pale yellow solid (0.406 g, 83%). Chemico-physical data were in agreement with those reported previously.[9,10]