A Convenient Synthetic Method of 2-Cyano-6-methoxybenzothiazole, —A Key Intermediate for the Synthesis of Firefly Luciferin

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(Received October 14, 1991)

The title compound was prepared in one step from commercially available 2-amino-6-methoxybenzothiazole by using the Sandmeyer cyanation reaction. The result enabled us to synthesize firefly luciferin effectively from this amino compound through three steps in 36% overall yield.

Firefly luciferin (**1**) has been attracting much interest in view of its utility for the ultratrace bioluminescent analysis of many biologically important materials[1] as well as its necessity for mechanistic studies of firefly bioluminescence.[1] Recently, a series of luciferin derivatives modified at the 6-position have synthesized from luciferin (**1**).[8] These new luciferins are useful as substrates for the bioluminescent assay of hydrolytic enzymes and are effectively applied to bioluminescent immunoassay.[8]

By now, three major synthetic routes have been reported for luciferin (**1**):[2-5] 8 steps from *p*-anisidine (**2**) (first synthesis by White et al.,[2] in 1963, overall yield, 5.8%); 5 steps from **2** (Seto et al.,[3] in 1963, 6.5%); 4 steps from 2-amino-6-methoxybenzothiazole (**3**) (improved synthesis by White et al.,[4] in 1965, 21%) (see Fig. 1). The key intermediate in all these synthetic schemes is 2-cyano-6-methoxybenzothiazole (**4**), which is condensed with D-cysteine after removal of the methyl group at position 6 to give **1** in 2 steps. A main drawback in all of these syntheses was that the preparation of **4** was in poor total yields from each starting material (9.9%, 6 steps from **2**;[2] 21%, 3 steps from **2**;[3] 33%, 2 steps from **3**[4]). Although the improved method by White et al. was the shortest approach of highest yield utilizing commercially available **3**, the method by Seto et al. proved to be a very convenient and useful route for large-scale preparations.[4,5] In this method, however, extra 2 steps of reactions were necessary to prepare the reagent; (carbamoylthiocarbonylthio)acetic acid (**5**), and therefore, the sequence practically involved 5 steps in total to afford **4**.

In the course of synthetic studies to obtain a large amounts of the firefly luciferin derivatives, we required several tens of grams of **4** as starting material and commenced to improve the synthetic method of **4**.

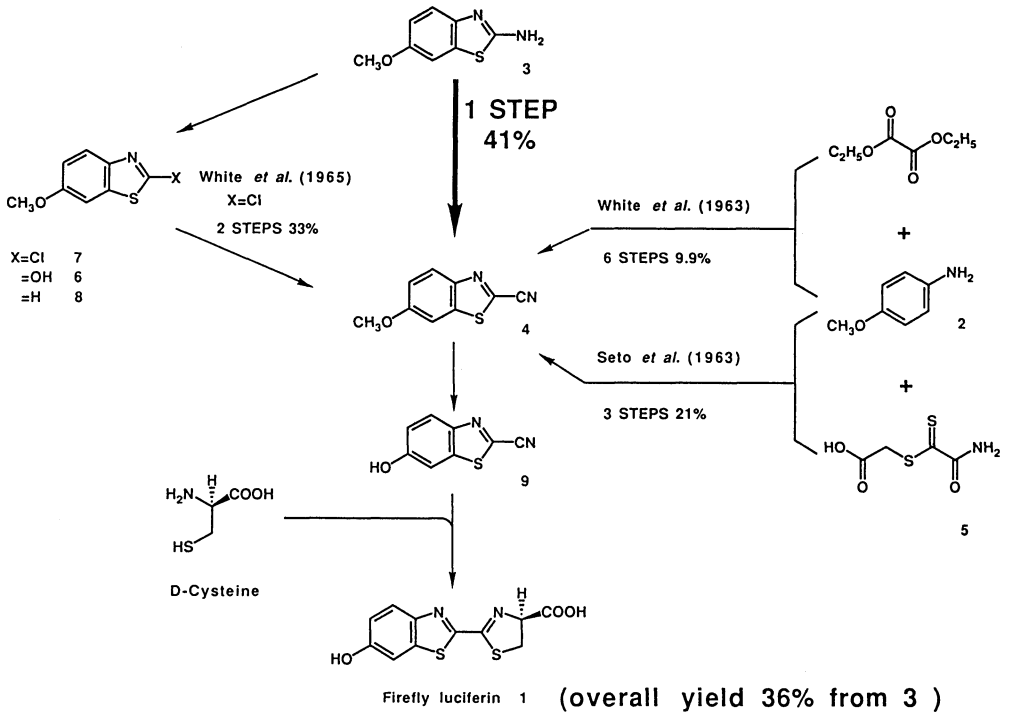

Fig. 1. Synthetic pathways of firefly luciferin (**1**).

The commercially available amino compound **3** appeared to be one of the most promising candidate for a starting compound, but no effort has been reported on direct conversion of **3** to **4** by Sandmeyer cyanation reaction. We now wish to present convenient reaction conditions, which gave **4** by one step from **3** in fairly good yield, and was applicable to large-scale (up to ca. 10 g of **3**) synthesis.

We first applied the cyanation conditions that converted *o*-toluidine into *o*-tolunitrile[6] and found that **3** was recovered unchanged. As the amine **3** showed poor solubility in dilute hydrochloric acid, the diazotizing conditions seemed to be crucial. We employed the mixed solvent (acetic acid–formic acid), which was used as solvent for the Sandmeyer chlorination of **3**,[4,11] and diazotized the amine **3** by adding hydrochloric acid and $NaNO_2$ to the solution at 0 °C. When the diazotized solution was kept at room temp, 2-hydroxy-6-methoxybenzothiazole (**6**) (main product), 2-chloro-6-methoxybenzothiazole (**7**) (by-product), and 6-methoxybenzothiazole (**8**) (trace) were detected on silica-gel TLC (solvent: 5% MeOH–CH_2Cl_2). No starting material **3** was detected on TLC, which suggested that the diazotization reaction proceeded sufficiently. To suppress the formation of by-products of chlorinated compound **7** and reduced compound **8**, sulfuric acid–acetic acid system was used for the diazotization instead of hydrochloric acid–acetic acid–formic acid system, and satisfactory results were obtained.

The solution of the diazonium salt was added to a mixture of the cyano complex, which was prepared by dissolving CuCN in aqueous KCN (3 equivalents of CuCN), and base, which was excess to the acids in the diazonium solution.[7] These cyanation conditions of the diazonium salt were examined in detail. The results are shown in Table 1.

It was reported that the pH of the solution during the Sandmeyer reaction was of importance for the yield of the nitrile, and the yield considerably increased by buffering the reaction mixture with $NaHCO_3$ or acetate buffer.[7,10] Indeed almost no product **4** was obtained without adding base for buffering. Addition of mild bases led to tolerable yields (Run 8, 13), whereas addition of NaOH gave **4** in poor yield (Run 1).

Although CuCN used in the Sandmeyer cyanation was usually freshly prepared[6,10] or specially prepared and stored according to the method of Barber et al.,[9,10] we obtained **4** in comparable yield by using commercially available CuCN, and therefore we employed it in all reactions. Large excess (at least 3 equivalents) of CuCN to the arylamine **3** was necessary to increase the yields (Run 2—4, 6—9), whereas the reaction temp (Run 4 & 5, 8 & 10, 9 & 12) and the concentration of the cyano complex solution (Run 10 & 11) had only little effect.

The maximum yield (41%) from 0.5 mmol of **3** (90 mg) was accomplished by using 12 equivalents of CuCN to **3** and $NaHCO_3$ for buffering (Run 9). Large-scale synthesis of **4** from 50 mmol of **3** (9 g) was successful and we were able to prepare about 3 g of **4** one step in a few days, though the yield slightly decreased (32%, Run 14).

Finally, conversion of the amine **4** into luciferin (**1**) according to the literature[3] was examined. 2-Cyano-6-hydroxybenzothiazole (**9**) and luciferin (**1**) were obtained in 96% and 93% yields respectively. Consequently, firefly luciferin (**1**) was effectively synthesized from commercially available 2-amino-6-methoxybenzothiazole (**3**) through three steps in 36% overall yield.

Synthetic studies from **4** of the firefly luciferin derivatives, which are useful for the bioluminescent assay of carboxylic esterases, phosphatases and glucosidases, are

Table 1. Preparation of the Cyano Compound **4**

Run[a]	Base for neutralization		$CuCN–H_2O$[b]	$KCN–H_2O$[b]	Temp	Isolated yield of **4**
	mmol		mmol ml	mmol ml	°C	%
1	NaOH,	38[c]	3—0.5	9—0.5	0	12
2	Na_2CO_3,	28	0.75—2	2.25—1	0	29
3	Na_2CO_3,	28	1.5—2	4.5—1	0	33
4	Na_2CO_3,	28	6—2	18—1	0	33
5	Na_2CO_3,	28	6—2	18—1	rt	35
6	$NaHCO_3$,	36	0.75—2	2.25—1	0	33
7	$NaHCO_3$,	36	1.5—2	4.5—1	0	36
8	$NaHCO_3$,	36	3—2	9—1	0	39
9	$NaHCO_3$,	36	6—2	18—1	0	41
10	$NaHCO_3$,	36	3—2	9—1	rt	37
11	$NaHCO_3$,	36	3—1	9—0.5	rt	38
12	$NaHCO_3$,	36	6—2	18—1	rt	35
13	AcONa,	43	3—2	9—1	0	29
14	$NaHCO_3$,	1800	302—100	905—100	0	32

a) In Run 1—13, diazotization were carried out from **3** (0.5 mmol), by adding AcOH (12 mmol), 6 mol dm^{-3} H_2SO_4 (3 mmol), and 2 mol dm^{-3} $NaNO_2$ (1 mmol). In Run 14, the reaction was carried out in 100 fold scale of Run 1—13. b) The cyano complex solution was prepared by adding aqueous KCN to CuCN suspension in H_2O. The base (solid) was then added to this solution. c) Dissolved in 2 ml of H_2O.

now in progress.

Experimental

All melting points were measured on a Mitamura Riken mp apparatus and uncorrected. ^{1}H NMR spectra were recorded on a JEOL GSX-270 and an FX-200 spectrometer. Chemical shifts (δ) are given in ppm from internal TMS and coupling constants (J) in Hz. IR spectra were taken on a JASCO IR-700, an IR-810, and an FT/IR-8300 infrared spectrometer. UV spectra were obtained on a JASCO UVIDEC-660 and a Hitachi 228 spectrometer. Mass spectra were measured on a JEOL JMS DX-705L, a DX-300, and a D100 instrument. The specific rotation of firefly luciferin (**1**) was measured on a JASCO DIP-181 digital polarimeter. 2-Amino-6-methoxybenzothiazole (**3**) was obtained from Tokyo Kasei Co. Copper(I) cyanide was CP grade from Nacalai Tesque Inc. Pyridinium chloride was first grade from Wako Chemical Co. D-Cysteine hydrochloride monohydrate was purchased from Sigma Chemical Co. The other chemicals were of reagent grade.

2-Cyano-6-methoxybenzothiazole (4): Commercial CuCN (0.54 g) was suspended in water (2.0 ml) with stirring and the mixture was cooled in an ice bath. To the resulting suspension, a solution of KCN (1.18 g) in water (1.0 ml) was added dropwise to keep the temperature of the reaction below room temp and the mixture stirred, while the CuCN entered into colorless solution. The solution was held at about 0 °C before use. On the other hand, 2-amino-6-methoxybenzothiazole (**3**) (90.1 mg) was mixed with AcOH (0.7 ml) and 6 mol dm^{-3} H_2SO_4 (0.5 ml), and the resulting colorless solution was cooled in an ice-salt bath. To the solution was added 2 mol dm^{-3} aqueous $NaNO_2$ (0.5 ml) dropwise with stirring. The addition required about 15 min and the resulting brown-yellow diazonium solution was then kept stirring and cooling for 1 h. In the cyano complex solution prepared previously was suspended $NaHCO_3$ (3.0 g) with vigorous stirring and the mixture was cooled in an ice bath. To the resulting suspension was slowly added the cold diazonium solution in about 15 min, and then the mixture was kept stirring with cooling for 30 min. The mixture was filtered through a glass filter with the aid of Celite and the precipitate was extracted thoroughly with ether (about 150 ml). The organic layer was separated and the aqueous layer was extracted three times with ether (10 ml each). The combined extracts were dried over anhydrous $MgSO_4$ and evaporated under vacuum. The residual red-brown solid (82.7 mg) was purified by silica-gel TLC with 10% hexane–CH_2Cl_2 to give the nitrile **4** (39.3 mg, 41%) as pale yellow needles which was used for the next step without further purification. An analytical sample was obtained by recrystallizing the product from CH_2Cl_2–hexane; mp 129 °C (lit, 129—130 °C[3]); IR (KBr) 2220, 1590, 1470, 1430, 1260, 1220, 810 cm^{-1}; MS m/z 190 (M^+); UV (MeOH) λ_{max} (ε) 320 (15400), 262 (6600) nm; (MeOH–HCl) 320 (15200), 262 (6600) nm; (MeOH–NaOH) 315 (14000), 262 (6100) nm; ^{1}H NMR ($CDCl_3$) δ=3.93 (3H, s), 7.24 (1H, dd, J=9.0, 2.4 Hz), 7.36 (1H, d, J=2.4 Hz), 8.08 (1H, d, J=9.0 Hz). Calcd for $C_9H_6ON_2S$: C, 56.82; H, 3.18; N, 14.73%. Found: C, 56.81; H, 2.94; N, 14.58%.

The large-scale synthesis was carried out as follows. A cyano complex solution was prepared from CuCN suspension (27.00 g, suspended in 100 ml of H_2O) and aqueous KCN (59.00 g, dissolved in 100 ml of H_2O) in a similar manner as described above. A diazonium solution was also prepared from **3** (9.00 g), AcOH (70.0 ml), 6 mol dm^{-3} H_2SO_4 (50.0 ml), and 2 mol dm^{-3} $NaNO_2$ (50.0 ml). In the cyano complex solution was suspended $NaHCO_3$ (150.0 g) with vigorous stirring, and to the resulting suspension the cold diazonium solution was added slowly in about 65 min with cooling in an ice bath. The mixture was kept stirring with cooling for 30 min, and then filtered through a glass filter with the aid of Celite. The precipitate was extracted thoroughly with ether (about 1000 ml), and the ether extract was dried over anhydrous $MgSO_4$ and evaporated under vacuum. The residual red-brown solid (11.85 g) was purified by silica-gel column chromatography with 50% hexane–CH_2Cl_2 to give the nitrile **4** (3.08 g, 32%) as pale yellow small needles. The above aqueous filtrate was extracted three times with ether (100 ml each) and the combined extracts were dried and evaporated under vacuum. The residual red-brown oil (0.14 g) was purified by silica-gel TLC with CH_2Cl_2 to give the nitrile **4** (19.6 mg, 0.21%) and 6-methoxybenzothiazole (**8**) (78.1 mg, 0.95%) as pale red plates.

2-Chloro-6-methoxybenzothiazole (7): MS m/z 201 & 199 (M^+); ^{1}H NMR ($CDCl_3$) δ=3.87 (3H, s), 7.07 (1H, dd, J=9.3, 2.4 Hz), 7.23 (1H, d, J=2.3 Hz), 7.82 (1H, d, J=9.3 Hz).

2-Hydroxy-6-methoxybenzothiazole (6): MS m/z 181 (M^+); ^{1}H NMR ($CDCl_3$) δ=3.81 (3H, s), 6.84 (1H, d, J=2.4 Hz), 7.06 (1H, d, J=8.8 Hz), 7.12 (1H, dd, J=8.8, 2.4 Hz), 9.69 (1H, bs).

6-Methoxybenzothiazole (8): MS m/z 165 (M^+); ^{1}H NMR ($CDCl_3$) δ=3.88 (3H, s), 7.12 (1H, dd, J=9.3, 2.5 Hz), 7.39 (1H, d, J=2.5 Hz), 8.01 (1H, d, J=9.3 Hz), 8.82 (1H, s).

2-Cyano-6-hydroxybenzothiazole (9): After a mixture of the methoxy compound **4** (0.98 g) and pyridinium chloride (2.00 g) was heated with stirring in a sealed test tube under nitrogen atmosphere at 200 °C for 45 min, saturated aqueous $NaHCO_3$ (about 5 ml) was added to the reaction mixture. The pH of the resulting suspension was adjusted nearly 7 by additional aqueous $NaHCO_3$, then the pale yellow precipitate was collected, washed with water and dried under vacuum. The aqueous filtrate was extracted three times with ether (15 ml each) and the organic layer was dried over anhydrous $MgSO_4$ and evaporated under vacuum. The residual brown powder and the precipitate previously obtained were combined and chromatographed on a silica-gel column with CH_2Cl_2 to give **9** (0.87 g, 96%) as pale yellow small needles and unchanged starting material **4** (0.02 g, 2% of recovery) as colorless needles. The product was used for the next step without further purification. Fractional recrystallization from MeOH afforded pure **9** as pale yellow needles (81% from the chromatographically separated **9**); mp 183—184 °C (sealed tube) (lit, 205—207 °C decomp[3]); IR (KBr) 3170, 2230, 1605, 1465, 1435, 1280, 1250, 1165, 1145, 835 cm^{-1}; MS m/z 176 (M^+); UV (MeOH) λ_{max} (ε) 322 (14900), 263 (7500) nm; (MeOH–HCl) 322 (15400), 262 (7700) nm; (MeOH–NaOH) 377 (16500), 282 (6400) nm; ^{1}H NMR ($CDCl_3$) δ=5.72 (1H, bs), 7.17 (1H, dd, J=9.0, 2.4 Hz), 7.36 (1H, d, J=2.4 Hz), 8.08 (1H, d, J=9.0 Hz). Calcd for $C_8H_4ON_2S$: C, 54.54; H, 2.29; N, 15.90%. Found: C, 54.69; H, 2.12; N, 15.75%.

Firefly Luciferin [D-(−)-Luciferin, (*S*)-Luciferin] (1): The nitrile **9** (176.0 mg) and D-cysteine hydrochloride monohydrate (177.0 mg) was dissolved in a mixed solvent of MeOH (4.0 ml) and water (2.0 ml) under a nitrogen atmosphere. To the resulting solution was added K_2CO_3 (140.0 mg) and the mixture was stirred under a nitrogen atmosphere. After 5 min,

the reaction was complete. The pH of the reaction mixture was adjusted to about 3 with 1 mol dm^{-3} HCl, then a few ml of MeOH was removed from the reaction mixture under vacuum. The precipitates formed were collected and washed with water to give **1** as pale yellow crystalline powder (245.0 mg, 88%), which was pure enough for elemental analysis. Evaporation of the combined filtrate yielded the second precipitates of **1** (13.0 mg, 5%), which showed a single spot on silica-gel TLC (AcOEt : MeOH : H_2O=5 : 1.1 : 1 as developing solvent); mp 185.5—186 °C (decomp) (lit, 200—202 °C[3]); IR (KBr) 3370, 1705, 1615, 1575, 1560, 1505, 1435, 1280, 1220, 1205, 890 cm^{-1}; MS (FAB) *m/z* 281 (MH^+), 235; UV (MeOH) λ_{max} (ε) 328 (17900), 269 (7400) nm; (MeOH–HCl) 331 (17000), 269 (7400) nm; (MeOH–NaOH) 385 (18000), 283 (6400) nm; ^{1}H NMR (d_6-DMSO) δ=3.61—3.82 (2H, m), 5.41 (1H, dd, *J*=9.3, 8.3 Hz), 7.07 (1H, dd, *J*=8.8, 2.2 Hz), 7.45 (1H, d, *J*=2.2 Hz), 7.96 (1H, d, *J*=8.8 Hz), 10.24 (1H, s), 13.23 (1H, bs). Calcd for $C_{11}H_8O_3N_2S_2$: C, 47.13; H, 2.88; N, 9.99%. Found: C, 47.12; H, 2.81; N, 9.84%. $[\alpha]_D^{27}$ −32.5° (*c* 1.20, *N,N*-dimethylformamide) (lit, $[\alpha]_D^{22}$ −36° (*c* 1.2, *N,N*-dimethylformamide)).[2]

Financial support from the Science and Technology Agency is gratefully acknowledged.

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