

## 2-Cyano-6-methoxybenzothiazole, a Key Intermediate for the Synthesis of Firefly Luciferin via PEG-Mediated Sandmeyer Reaction\*<sup>1</sup>

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As an application of PEG-mediated Sandmeyer synthetic reaction developed in our laboratory, the title compound, that is a key intermediate of an important, bioluminescent analytical reagent, firefly luciferin, was prepared from commercially available 2-amino-6-methoxybenzothiazole. Using the compound firefly luciferin can be synthesized effectively from the amino compound in 47% overall yield.

### 1 Introduction

Firefly luciferin (FL), a bioluminescent substrate of firefly emission, has a versatile utility for bioluminescent ultrasensitive analyses of many biological trace components and for investigating mechanisms of bioluminescent phenomena. There are four main approaching routes to FL, all of which pass through a key intermediate, 2-cyano-6-methoxybenzothiazole (CMB); White et al.<sup>1)</sup> reported 6-step synthesis of FL from *p*-anisidine (AN) (total yield, 5.8%), Seto et al.,<sup>2)</sup> 5-steps from AN (6.5%), White et al.,<sup>3)</sup> 4-steps from commercially available 2-amino-6-methoxybenzothiazole (AMB) (13.9%), and Toya et al.,<sup>4)</sup> 3-steps from AMB (36%).

However, their overall yields of CMB from the starting materials were very low. Highest yield was obtained by Toya et al. in 44% from AMB.<sup>4)</sup> Now we describe a novel, effective synthesis of the cyano compound CMB from AMB as an application of the polyethylene glycol (PEG)-mediated Sandmeyer reactions developed in our laboratory.<sup>5,6)</sup>

### 2 Materials and Methods

#### 2.1 Chemicals

2-Amino-6-methoxybenzothiazole (AMB) and 2-aminobenzothiazole (ABT) were purchased from Tokyo Chem. Co. PEG #200

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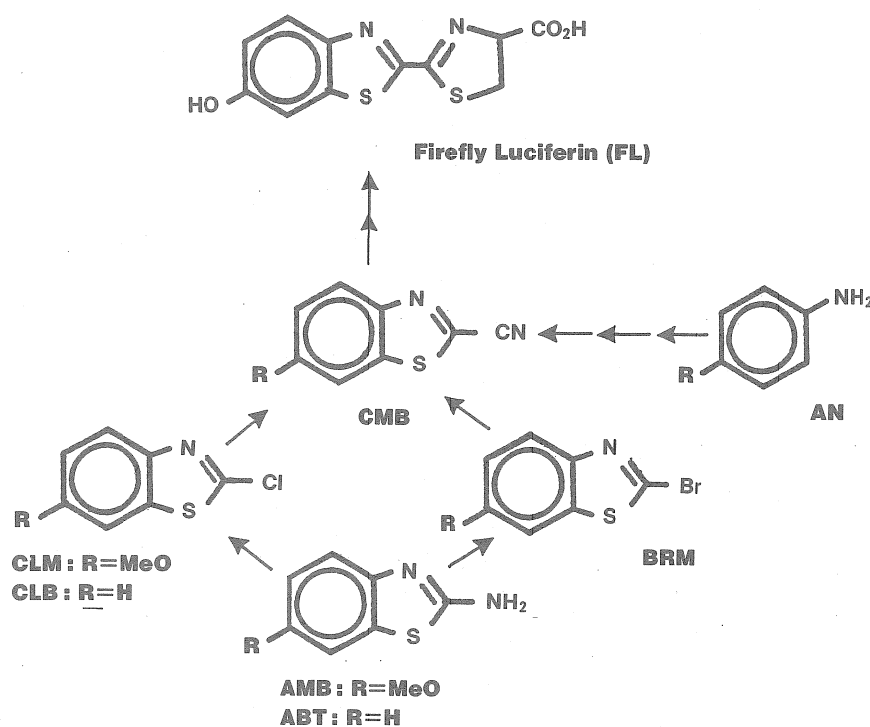


Fig. 1. Synthetic Route to Firefly Luciferin.

(mean mw, 190-200) was purchased from Nacalai Tesque (chem. pure grade) and dehydrated by azeotropic distillation with benzene before use.  $\text{CH}_3\text{CN}$  (Nacalai Tesque) was distilled from  $\text{CaH}_2$ . Dimethyl sulfoxide (DMSO), tetraglyme,  $\text{CH}_3\text{COOH}$ ,  $\text{HCOOH}$ ,  $\text{CuCl}$ ,  $\text{CuCl}_2$ ,  $\text{CuBr}_2$ ,  $(\text{CH}_3)_2\text{CHCH}_2\text{CH}_2\text{ONO}$ ,  $\text{NaNO}_2$ ,  $\text{KCN}$ , and  $\text{NaCN}$  were purchased from Nacalai Tesque. Silica gel (Kieselgel 60 PF<sub>254</sub>) was purchased from Merck.  $\text{HCl}$  was purchased from Wako Chem. Co.

## 2.2 Instrumentation

IR spectra were measured by JASCO A-100; NMR, by JEOL JNM-MH-100 (100 MHz); MS, by Shimadzu GCMS-QP1000 (Silicon OV-17 in glass column 1.0 m, column temp: 100-

240°C, inj. temp. 240°C, He 40 ml/s, ionization 70 eV). Mps. were uncorrected.

## 2.3 Synthetic Reactions

*2-Chloro-6-methoxybenzothiazole (CLM).* Copper (II) chloride (1.6 g; 12 mmol) was dried by heating at 110°C with a teflon stirrer bar in a recovery flask for 1 h. PEG (5 g) and  $\text{CH}_3\text{CN}$  (150 ml) were added to dissolve the chloride. Under argon stream isoAmONO (2 ml; 15 mmol) was added and the resulted mixture was stirred at room temperature. 2-Amino-6-methoxybenzothiazole (AMB) (1.8 g; 10 mmol) was dissolved in  $\text{CH}_3\text{CN}$  (50 ml) and PEG (5 g) under sonication at room temperature. Into the diazotized solution the solution of AMB was added dropwisely under argon streams during

more than 10 min. Then the resulted solution was stirred at 65°C for 3 h.

After cooling down to room temperature, the resulted mixture was poured into 20% HCl (1 l) and the product was extracted by chloroform. The residue was purified by sublimation under vacuum (40°C). Colorless needles (CLM), mp 52.0-53.0°C, yield 83.5%. Lit.<sup>3)</sup> mp 52.5-53.0°C, yield 30%. IR and NMR spectra were identical to those of the authentic sample.<sup>3)</sup> MS, *m/z* 201. Beilstein test, positive.

Maximal conditions were searched as shown in Table 1.

2-Chlorobenzothiazole (CLB) was prepared from 2-aminobenzothiazole (ABT) by the similar procedure in the synthesis of the chloro compound (CLM) by using CuCl<sub>2</sub>. Pale yellow liq., yield 94.8%. IR and NMR spectra were identical to those of the authentic sample.<sup>7,8)</sup>

2-Bromo-6-methoxybenzothiazole (BRM) was prepared from AMB, by the similar procedure in the synthesis of the chloro compound (CLM) by using CuBr<sub>2</sub> instead of CuCl<sub>2</sub>. Colorless fine needles, BRM, mp 59.0-

60.5°C, yield 79.3%. Lit.<sup>3)</sup> mp 52.5-53.0°C, yield 33%. Elemental anal. for C<sub>8</sub>H<sub>6</sub>BrNOS requires C, 39.36, H, 2.47, and N, 5.74%. Found, C, 39.10, H, 2.44, and N, 5.71%. IR and NMR spectra were identical to those of the authentic sample synthesized after White.<sup>3)</sup> MS, *m/z* 245. Beilstein test, positive.

Maximal conditions were searched as shown in Table 2.

2-Cyano-6-methoxybenzothiazole (CMB). NaCN (30.0 mg; 0.55 mmol) was dissolved in DMSO (5 ml) under argon streams at 100°C during 15 min and the resulted solution was cooled to 50°C. Solution of BRM (122 mg; 0.5 mmol) in DMSO (5 ml) was added dropwisely under argon gas. Then the resulted mixture was stirred at 110°C for 5 h.

After cooling down the mixture was diluted by ether (15 ml) and extracted by ether (40 ml) and water (30 ml). The residual solid was purified by silica gel TLC (ether—petr. ether, 1:2). Colorless needles (CMB), 67.0 mg (70.4%), mp 128.0-131.0°C. Lit.<sup>3)</sup> 129-131°C. IR and NMR spectra were identical to those of

Table 1. Synthesis of 2-Chloro-6-methoxybenzothiazole (CLM)

Substrate AMB/mg (/mmol)	CuCl <sub>2</sub> /mg (/mmol)	isoAmONO/ $\mu$ l (/mmol)	Solvents CH <sub>3</sub> CN/ml-PEG/g	Temp./°C	Yield <sup>a</sup> /mg (/%)
180 (1.0)	161 (1.2)	200 (1.5)	15	3.0	r. t. 63.3 (31.7)
180 (1.0)	161 (1.2)	200 (1.5)	15	3.0 <sup>b</sup>	r. t. — <sup>c</sup> (29.0)
180 (1.0)	161 (1.2)	200 (1.5)	20	1.0	r. t. 106.8 (53.5)
180 (1.0)	161 (1.2)	400 (3.0)	20	1.0	r. t. 100.3 (50.3)
180 (1.0)	322 (2.4)	200 (1.5)	20	1.0	r. t. 129.0 (64.6)
180 (1.0)	161 (1.2)	200 (1.5)	20	1.0	65 157.3 (78.8)
1800 (10.0)	1600 (12.0)	2000 (15.0)	200	10.0	65 1670.0 (83.5)

a) Isolated yields.

b) Tetraglyme was used instead of PEG.

c) Not isolated yield; GLC yield.

the authentic sample synthesized after White.<sup>3)</sup>

Maximal conditions were searched as shown in Table 3.

### 3 Results and Discussion

#### 3.1 Sandmeyer halogenation

Nitrosyl complex of anhydrous  $\text{CuX}_2$  is supposed to halogenate aromatic amines effectively through diazonium dihalocuprates salts as follows,<sup>9)</sup>

**Table 2.** Synthesis of 2-Bromo-6-methoxybenzothiazole (BRM)

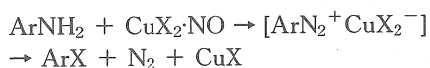
Substrate AMB/mg (/mmol)	CuBr <sub>2</sub> /mg (/mmol)	isoAmONO/ $\mu\text{l}$ (/mmol)	Solvents CH <sub>3</sub> CN/ml-PEG/g	Temp./ $^{\circ}\text{C}$	Time /h	HX	Yield <sup>a</sup> /mg (/%)
180 (1.0)	268 (1.2)	200 (1.5)	20 1.0	65	3.0	20% HCl	—
180 (1.0)	268 (1.2)	200 (1.5)	20 1.0	65	1.0	—	—
180 (1.0)	268 (1.2)	200 (1.5)	20 1.0	65	1.0	10% HBr	146.5 (60.0)
180 (1.0)	268 (1.2)	200 (1.5)	20 1.0	65	1.0	5N H <sub>2</sub> SO <sub>4</sub>	111.0 (45.5)
180 (1.0)	268 (1.2)	200 (1.5)	20 1.0	65	0.5	10% HBr	101.9 (41.7)
180 (1.0)	268 (1.2)	200 (1.5)	20 1.0	65	0.25	10% HBr	95.9 (39.3)
180 (1.0)	268 (1.2)	200 (1.5)	20 1.0	55	1.0	10% HBr	166.0 (43.2)
180 (1.0)	268 (1.2)	200 (1.5)	20 1.0	40	5.0	10% HBr	166.0 (68.0)
180 (1.0)	534 (2.4)	200 (1.5)	20 1.0	40	5.0	10% HBr	183.6 (75.2)
180 (1.0)	534 (2.4)	200 (1.5)	20 1.0	40	9.0	10% HBr	156.5 (64.1)
180 (1.0)	534 (2.4)	200 (1.5)	20 1.0	50	3.0	10% HBr	193.5 (79.3)

a) Isolated yield.

**Table 3.** Synthesis of 2-Cyano-6-methoxybenzothiazole (CMB)

Substrate BRM/mg (/mmol)	Reagent/mg (/mmol; /eq.)	Solvents DMSO/ml	Temp./ $^{\circ}\text{C}$	Time /h	Yield <sup>a</sup> /mg (/%)
122 (0.5)	KCN 35.8 (0.55; 1.1)	10.0	140	1.0	43.4 (45.6)
122 (0.5)	KCN 35.8 (0.55; 1.1)	10.0	110	10.0	60.5 (63.6)
122 (0.5)	NaCN 30.0 (0.55; 1.1)	10.0	110	5.0	67.0 (70.4)
122 (0.5)	NaCN 60.0 (1.1; 2.2)	10.0	110	5.0	44.7 (47.0)

a) Isolated yield.



In the present work, anhydrous  $\text{CuCl}_2$  and isoAmONO were used to produce the nitrosyl complex, which chlorinate 2-aminobenzothiazoles in  $\text{CH}_3\text{CN}$ -PEG # 200 mixture. The amine (1 mmol) and  $\text{CuCl}_2$  dissolve in  $\text{CH}_3\text{CN}$  (5 mL) about halfly, but it would be unpractical to use too much solvent.

Addition of PEG to this mixture could dissolve them completely.  $\text{CH}_3\text{CN}$ -PEG (20:1) gave best results. This reaction conditions yielded 78.8% of CLM from 1 mmol of substrate AMB and 83.5% from 10 mmol of AMB and yielded 94.8% of CLB from 1 mmol of ABT. This reaction conditions also yielded 79.3% of bromination product BRM from 1 mmol of AMB when  $\text{CuBr}_2$  was used instead of  $\text{CuCl}_2$ .

### 3. 2 Cyanation of the halogeno compounds

White et al.<sup>3)</sup> reported synthesis of 2-cyano-6-methoxybenzothiazole (CMB) from the reaction of CLM and KCN in DMSO at 140°C in 49% yield. In the present works, we used PEG instead of DMSO for the cyanation of the chloro compound, however, yields could not be improved. Hence, we tried another cyanation route via the bromo compound BRM.

In general, cyanation of bromide is afforded at lower temperature than that of chloride,<sup>10)</sup> as observed in this work. NaCN worked better than KCN did. Under these conditions the cyano compound CMB was obtained in 70.4% yield from BRM.

### 3. 3 Synthesis of firefly luciferin (FL)

As shown in our previous report,<sup>4)</sup> firefly luciferin (FL) can be obtained in 84.5% yield

from CMB. Hence, present method can give a synthetic route of the luciferin in 47.3% overall yield from the commercial amino compound AMB.

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PEG 介助の Sandmeyer 反応によるホタルルシフェリン重要合成中間体,  
2-シアノ-6-メトキシベンゾチアゾールの合成

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PEG を用いる合成反応の一環として開発したアリールアミンの効率的な Sandmeyer 反応を2-アミノ-6-ベンゾチアゾールに適用して、高収率でホタルルシフェリン合成の重要中間体、2-シアノ-6-メトキシベンゾチアゾールを2段階、総収率56%で合成することに成功した。このことは、ホタルルシフェリンをアミノ化合物から47%の総収率で合成できることを意味する。