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Synthesis, characterization and computational studies of 2-cyano-6-methoxybenzothiazole as a firefly-luciferin precursor

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Abstract: A novel approach to the synthesis of 2-cyano-6-methoxybenzothiazole via the Cu-catalyzed cyanation of 2-iodo-6-methoxybenzothiazole was developed. $K_4[Fe(CN)_6]$ was used as a source of cyanide, and a Cu/ N,N,N',N' -tetramethylethylenediamine (TMEDA) system was utilized as a catalyst. This approach is scalable and can be practiced with operational benign. The most stable conformation of 2-cyano-6-methoxybenzothiazole was delineated using the density functional theory (DFT)/B3LYP method with 6-311++G(d, p) basis set.

Keywords: 2-cyano-6-methoxybenzothiazole; 2-iodo-6-methoxybenzothiazole; Cu catalyst; cyanation; DFT; firefly luciferin; $K_4[Fe(CN)_6]$.

Introduction

Firefly luciferin is a common substrate in biological imaging [1] that originates from some insects [2]. It is widely applied in life sciences [3–5]. The chemical preparation of firefly luciferin has been described previously [6–9]. In particular, 2-cyano-6-methoxybenzothiazole has been converted to 2-cyano-6-hydroxybenzothiazole followed by the reaction with cysteine [6] (Scheme 1). Typical routes to 2-cyano-6-methoxybenzothiazole include the classical Rosenmund-von Braun [10] and Sandmeyer [11] reactions. These methods proceed with low atom economy and require toxic reagents such as KCN [12], NaCN [13], $Zn(CN)_2$ or TMSCN [14] that are also difficult to handle in a large-scale synthesis [15, 16].

The Cu-catalyzed cyanation of aryl halides to benzonitrile derivatives has been reported [13]. Various copper catalyst systems with bidentate ligands [17–23] have been

developed. In 2004, Beller and co-workers introduced $K_4[Fe(CN)_6]$ as a low-cost and eco-friendly source of cyanide [19]. Due to its strong CN bond, the catalyst deactivation was prevented through a slow release of cyanide ion [24]. Herein, a Cu-catalyzed cyanation of 2-iodo-6-methoxybenzothiazole for the synthesis of 2-cyano-6-methoxybenzothiazole was introduced. $K_4[Fe(CN)_6]$ was applied as a source of cyanide, and CuI in the presence of N,N,N',N' -tetramethylethylenediamine (TMEDA) [25] was used as part of the catalyst system. Density functional theory (DFT) calculations were made with the structural parameters calculated using the B3LYP/6-311++G(d, p) method [26–29].

Results and discussion

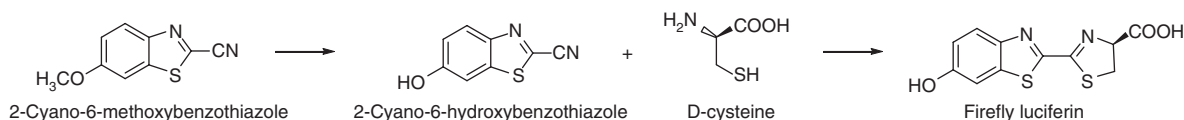
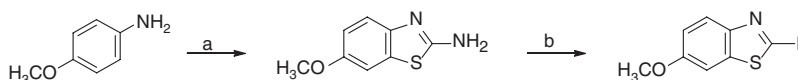
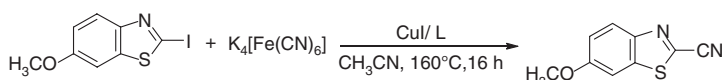
2-Amino-6-methoxybenzothiazole as a starting material was synthesized from *p*-anisidine as shown in Scheme 2 [30] and subsequently converted into 2-iodo-6-methoxybenzothiazole using a simple and efficient one-pot sequential diazotization-iodination method.

Reagents and conditions: (a) $-CH_3COOH$, Br_2 , KSCN, 20 h, temperature $<35^\circ C$, yield 85%; (b) $-H_2SO_4$, $NaNO_2$, KI, $0^\circ C$, 30 min, yield 80%.

The one-pot cyanation of 2-iodo-6-methoxybenzothiazole to 2-cyano-6-methoxybenzothiazole was achieved using 0.25 mmol of $K_4[Fe(CN)_6]$, 0.25 mmol of CuI and 3 mmol of TMEDA in acetonitrile at $160^\circ C$ (Scheme 3). In addition, 1 mmol of mystril trimethyl bromide (MTMAB) was used as a phase transfer agent. The presence of a phase-transfer catalyst is essential for a successful cyanation reaction. Under these conditions, 2-cyano-6-methoxybenzothiazole was produced in a 90% yield. In the presence of 0.18 mmol of $K_4[Fe(CN)_6]$, the cyanation still proceeded in a 90% yield, indicating that all six cyanide ions in the molecule are utilized in the reaction. On the other hand, increasing the amount of $K_4[Fe(CN)_6]$ to more than 0.25 mmol resulted in a moderate yield of 60%, presumably due to the catalyst deactivation. In addition, a decrease in the CuI loading to 0.1 mmol from 0.25 mmol, resulted in a poor yield of 40%, even after an increase of the reaction time to 40 h.

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**Scheme 1** Synthesis of firefly luciferin.**Scheme 2** Synthesis of 2-iodo-6-methoxybenzothiazole from *p*-anisidine.**Scheme 3** Cyanation reaction of 2-iodo-6-methoxybenzothiazole.

The optimized geometric structures of 2-cyano-6-methoxybenzothiazole were calculated using the B3LYP 6-311++G(d, p) method. This molecule has C1 full point group and 51 fundamental modes of vibrations. It exists in two stable conformers, I and II. Conformer III is the transition state structure (Figure 1). Conformer I with the dihedral angle (C7-C6-O15-C16) of 1.4° is 0.17 kcal/mol more stable than conformer II and 2.02 kcal/mol more stable than conformer III. The dihedral angle (C7-C6-O15-C16) of the second stable structure is 179.98° .

Conclusions

2-Cyano-6-methoxybenzothiazole, a synthetic intermediate of firefly luciferin, was prepared by a high-yield Cu-catalyzed cyanation of 2-iodo-6-methoxybenzothiazole. $\text{K}_4[\text{Fe}(\text{CN})_6]$ was applied as an eco-friendly cyanide source. The cyanation reaction was performed efficiently by applying the catalytic system of CuI/TMEDA that is non-toxic and environmentally friendly. The structure of 2-cyano-6-methoxybenzothiazole was characterized using computational studies.

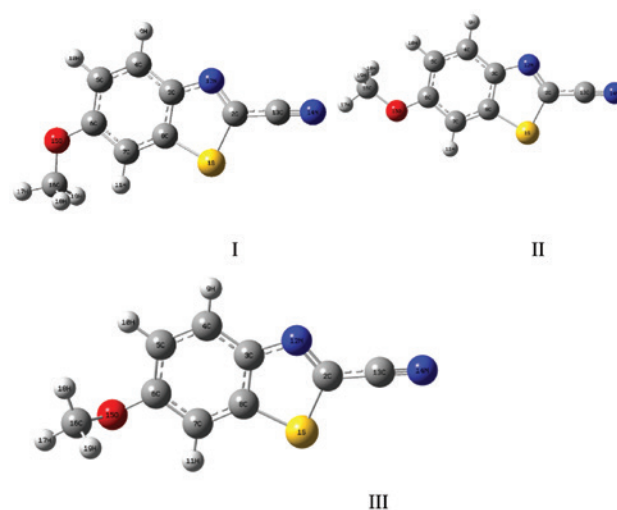
Experimental

All chemicals and solvents were supplied by Merck (Germany) and used without purification. The Fourier transform infrared (FT-IR) spectra were recorded on an Alpha Centauri FT-IR (Bruker, Germany) spectrophotometer using KBr pellets. The ^1H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectra were determined on a Mercury-300 MHz (Bruker, Germany) instrument. The mass spectra were obtained on

an Agilent 575 mass spectrometer equipped with a quadrupole analyzer. 2-Amino-6-methoxybenzothiazole was synthesized according to the method described by Stuckwisch [30].

Synthesis of 2-iodo-6-methoxybenzothiazole

A solution of 2-amino-6-methoxybenzothiazole (1.81 g, 0.01 mol) in water (3 mL) was stirred at 0°C and treated with acetic acid (3 mL) and sulfuric acid (6 M, 4.5 mL). The mixture was stirred for 1 h. The resultant clear solution was treated dropwise for 15 min at 0°C with an aqueous solution (3 mL) of NaNO_2 (0.70 g, 0.01 mol). After stirring for 1 h, an aqueous solution (3 mL) of KI (1.65 mg, 0.01 mol) was added slowly for 30 min, until the evolution of nitrogen gas ceased. The product was extracted with EtOAc (3×12 mL), and the combined organic layers were washed with a 10% aqueous solution of Na_2SO_4 , dried and concentrated. The residue of 2-iodo-6-methoxybenzothiazole was

**Figure 1** Conformers of 2-cyano-6-methoxybenzothiazole.

crystallized from ethanol; ^1H NMR (300 MHz, $\text{DMSO}-d_6$): δ 3.67 (s, 3H), 6.87 (m, 1H), 7.41 (m, 1H), 7.71 (m, 1H); IR: 2932, 1651, 1239, 1208 (C-N), 1053, 875 cm^{-1} ; MS: m/z 291.0 (M^+). Anal. Calcd for $\text{C}_8\text{H}_6\text{INOS}$: C, 33.01; H, 2.15; N, 4.87. Found: C, 32.27; H, 2.08; N, 4.81.

Synthesis of 2-cyano-6-methoxybenzothiazole from 2-iodo-6-methoxybenzothiazole

A solution of CuI (47.6 mg, 0.25 mmol), $\text{K}_4[\text{Fe}(\text{CN})_6]$ (90 mg, 0.18 mmol) and TMEDA (348 mg, 3 mmol) in dry acetonitrile (5 mL) was stirred for 30 min and then treated with MTMAB (34 mg, 1 mmol) and 2-iodo-6-methoxybenzothiazole (290 mg, 1 mmol). The mixture was stirred under argon atmosphere at room temperature for 30 min and then under reflux at 160°C for 16 h. After cooling to room temperature, the mixture was extracted with diethyl ether (3×5 mL). The extract was dried with anhydrous MgSO_4 , filtered and concentrated. The red-brown solid residue was purified by column chromatography on silica gel eluting with hexane/ CH_2Cl_2 , 1:10, to give 2-cyano-6-methoxybenzothiazole in the form of pale yellow needles; yield 90%; mp $128\text{--}130^\circ\text{C}$; ^1H NMR (300 MHz, CDCl_3): δ 3.87 (s, 3H), 7.11 (m, 1H), 7.23 (m, 1H), 7.52 (m, 1H); ^{13}C NMR (75 MHz, $\text{DMSO}-d_6$): δ 160.6, 147.3, 141.3, 135.6, 125.6, 118.6, 113.1, 103.5, 55.6; IR: 3030, 2932, 2240, 1651, 1280, 1207, 1034, 872 cm^{-1} ; MS: m/z 190.1 (M^+). Anal. Calcd for $\text{C}_9\text{H}_6\text{N}_2\text{OS}$: C, 56.83; H, 3.18; N, 14.73. Found: C, 56.68; H, 3.01; N, 14.42.

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