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Synthesis of 5-Aminolevulinic Acid (ALA) and Its *t*-Butyl Ester for the Fluorescence Detection of Early Cancer

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5-Aminolevulinic acid and its derivatives, which are known to affect the early diagnosis and treatment of cancer, have been synthesized. Simple methods for the synthesis of 5-aminolevulinic acid (ALA), a precursor of porphyrins, have been developed in our laboratory for use in studies on the biosynthesis of porphyrins.

Key words: 5-Aminolevulinic acid, Fluorescence detection, ALA, PDT

INTRODUCTION

Cancer is a very common disease throughout the world, the incidence of which is still rising in most part of the world. Generally, only the early stages of cancer can be cured with a high probability (Cortese *et al.*, 1983). Therefore, tools for the screening and early diagnosis of cancer are necessary (Fontana *et al.*, 1991). Conventional white light endoscopy is typically insufficient for the early diagnosis of cancer. Therefore, 5-aminolevulinic acid (ALA) has been synthesized (Katsumi *et al.*, 2002; Andreas, 1984; Robert, 1949) for use in fluorescence diagnosis. As a precursor of heme synthesis 5-ALA is metabolized to protoporphyrin IX- a red fluorescent. Therefore, protoporphyrin IX accumulates in tumorous and premalignant tissue, and can be directly visualized by fluorescence bronchoscopy. 5-ALA is a naturally occurring substance, which is normally synthesized from succinyl CoA and glycine. This synthesis is negatively regulated by the presence of heme (Rudolf *et al.*, 1999) If 5-ALA is applied exogeneously, this negative feed-back mechanism is surpassed, with protoporphyrin IX is synthesized by heme biosynthesis. Fig. 1. shows a schematic demonstration of the principles of protoporphyrin IX accumulation within tumorous tissue.

Tumor-selectivity of a photosenstizer is related to distinctive features of tumor compared to normal tissue, such as increased low-density lipoprotein receptors,

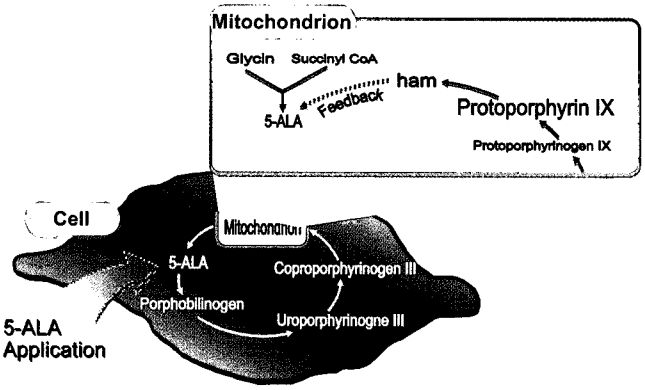
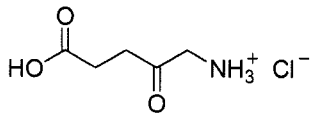
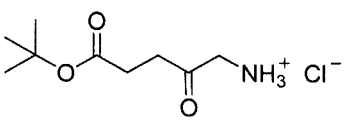


Fig. 1. Mechanism of 5-ALA induced protoporphyrin IX fluorescence

macrophages, an acidic environment and tumor stroma with a large interstitial space and leaky vasculature, increased lipids and newly-synthesized collagen. A photosensitizer bound with LDL enters into the cytoplasm *via* endocytosis, and is subsequently redistributed intra-



5-Aminolevulinic acid



5-Aminolevulinic acid *t*-butyl ester

Fig. 2. 5-Aminolevulinic acid and its derivative

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cellularly in the membrane domain, mitochondria, Golgi apparatus and lysosomes (Yoshihisa *et al.*, 2004).

Photodynamic diagnosis (PDD) utilizes the fluorescence detection of porphyrin derivatives induced in tumor tissue following the exogeneous injection of 5-ALA, which is very helpful in distinguishing tumor from surrounding normal tissue, which significantly contributes to subsequent clinical diagnosis and surgical section when visualizing the tumor margin.

MATERIALS AND METHODS

All reactions were carried out under an inert atmosphere (N_2) and at room temperature, unless other wise noted. Solvents and reagents were obtained commercially, and used without further purification. All reported yields are of the isolated products, and have not been optimized. Reactions were routinely monitored by thin-layer chromatography (TLC) on silica gel plates (pre-coated F_{254} Merck plates). Infrared spectra (IR) were measured on a Jasco FT-IR instrument. ^{1}H-NMR, ^{13}C-NMR were determined in $CDCl_3$ solution using a Varian Gemini 200 spectrometer. Peak positions are given in parts per million (δ) downfield from tetramethylsilane, used as the internal standard, with multiplicities reported in the usual manner and J values given in Hertz. Flash chromatography was performed using Merck 60-200 mesh silica gel. Mass spectrometry was performed at the KOREA Univ. Mass Spectroscopy center.

4-Methoxy-4-oxobutanoic acid (1)

Succinic anhydride (40.0 g) was added to a well stirred solution of methanol anhydride (25.0 mL) at 20~25°C. The reaction mixture was heated at 100~110°C for 3 h, cooled to room temperature and then concentrated at reduced pressure to give product **1** (50.2 g, 95%). The crude product was practically pure, and used for the next step without further purification. An analytical sample was obtained by recrystallization from EtOH/Hexane. ^{1}H-NMR (200 MHz, DMSO-d_6), δ (ppm): 12.15 (s, 1H), 3.64 (s, 3H), 2.54 (s, 4H); ^{13}C-NMR (50 MHz, DMSO-d_6) δ 177.3; 173.5; 52.0; 31.8; 28.9, MS (FAB); 133.05(M^++H^+).

Methyl 4-cyano-4-oxobutanoate (2)

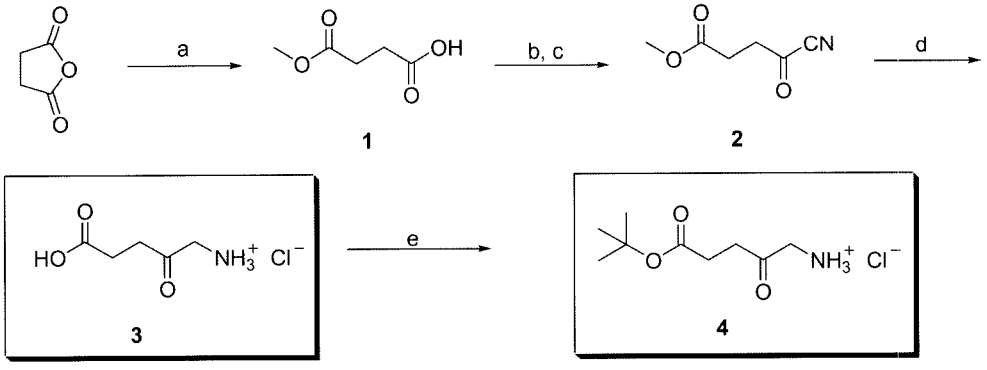
A stirred mixture of compound **1** (8.8 g) was chlorinated by the addition of thionyl chloride (10 mL) under N_2 gas at 35 °C for 5 h. The resulting reaction mixture was concentrated at reduced pressure, yielding the crude product, to which was then added a stirred solution of CuCN (5.5 g) in acetonitrile (20 mL). The reaction mixture was heated at 70°C for 8 h, cooled to room temperature and then filtered through celite, and the organic solution then concentrated at reduced pressure to yield the desired product **2** (6.4 g, 68%). ^{1}H-NMR (200 MHz, DMSO-d_6), δ (ppm): 3.67 (s, 3H), 2.79 (t, 2H), 2.58 (t, 2H), ^{13}C-NMR (50 MHz, DMSO-d_6) δ 175.3; 173.1; 113.2; 50.0; 42.9; 27.4, MS (FAB); 142.05 (M^++H^+).

5-Aminolevulinic acid hydrochloride (3)

A stirred mixture of compound **2** (5.4 g) was hydrogenated by the addition of Pd/C (0.5 g) in 6M HCl under a pressure of 125 psi at room temperature for 18 h. The resulting mixture was filtered through celite, and the organic solution concentrated at reduced pressure, yielding the crude product. The crude product was purified, by recrystallization with EtOH/2-propanol, to yield the pure product **3** as a solid (5.7 g, 89%). ^{1}H-NMR (200 MHz, D_2O), δ (ppm): 4.13 (d, 2H), 2.90 (t, 2H), 2.72 (t, 2H), ^{13}C-NMR (50 MHz, DMSO-d_6) δ 172.3; 48.2; 35.2; 31.0, ESI MS: m/z 168.04[M]$^+$.

t-Butyl 5-aminolevulinate (4)

Compound **3** (1.6 g) was dissolved in water at room temperature. To this solution was added 2M NaOH until a



Reagent: a) MeOH, reflux, 3 h b) $SOCl_2$, 35°C, 5 h c) CuCN, ACN, 70°C, 6 h
d) Pd/C, H_2, EtOH, rt, 18 h e) 2-methylpropene, H_2SO_4

Scheme 1. Syntheses of 5-aminolevulinic acid and its derivative

pH 7 was obtained, and the whole mixture was then extracted with Et_2O (3*10 mL), dried over $MgSO_4$, and concentrated at reduced pressure to yield the crude product. A stirred mixture of the crude product, 2-methylpropene and H_2SO_4 (0.1 mL) in Et_2O (10 mL). The reaction mixture was stirred at room temperature for 10 h, with its subsequent addition to 2M HCl (1.5 mL). After 2 h, the reaction mixture was concentrated at reduced pressure. The crude product was purified by recrystallization from EtOH to yield the pure product **4** as a solid (1.3 g, 58%). ^{1}H-NMR (200 MHz, D_2O), δ (ppm): 4.13 (d, 2H), 2.93 (t, 2H), 2.71 (t, 2H), 1.23 (s, 9H), ^{13}C-NMR (50 MHz, DMSO-d_6) δ 173.3; 82.2; 48.2; 32.0; 29.2; 28.0, ESI MS: m/z 225.08 $[M]^+$.

RESULTS AND DISCUSSION

Cancer is a very serious health problem, with early therapy offering the only chance of a cure. Apart from non-invasive screening methods there is also the need for better bronchoscopic techniques. 5-Aminolevulinic acid can be applied for the simple diagnosis of cancer. Therefore, a simple method for the synthesis of 5-ALA, using cheap starting materials and a very simple refining process, has been developed in our laboratory. The induced fluorescence of this compound is useful in the planning of photodynamic therapy and diagnosis.

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