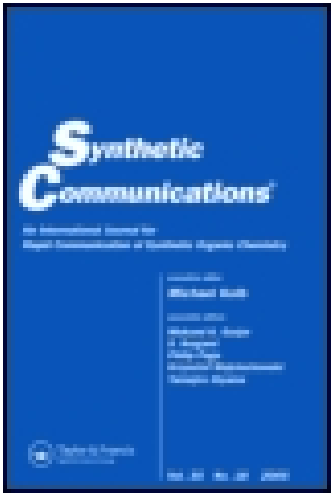

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Synthesis of Dye-Labeled Lysine Derivatives

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SYNTHESIS OF DYE-LABELED LYSINE DERIVATIVES

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Abstract: The N^{ε}-dabcyl-N^{α}-(9-fluorenylmethoxy)-carbonyllysine was prepared by reaction of lysine-Cu^{2+} complex with the *N*-hydroxysuccinimide (HOSu) activated ester of [4-(4′-dimethylamino)phenylazo]benzoic acid (dabcyl acid) followed by treatment with EDTA and acylation with Fmoc-OSu, and the N^{α}-*tert*-butyloxycarbonyl-N^{ε}-dabcyllysine was prepared by reaction of N^{α}-*tert*-butyloxycarbonyllysine with dabcyl-OSu.

An increased awareness of the importance of biochemical medicines in people's health has led to an interest in labeled amino acids[1], which can be directly attached to many sorts of biochemical medicines (peptides, proteins, substrates of enzymes and antagonists, etc) and applied to detecting and analyzing the medicine binding with the acceptor and its subsequent metabolism. It is to be hopped these labeled materials will play an important role in studying the pharmacology, synthesis and screening of new biomedical medicines.

In this communication, we report the synthesis of two dabcyl-labeled lysine derivatives, Fmoc-Lys(Dabcyl)-OH **1** and Boc-Lys(Dabcyl)-OH **2**.

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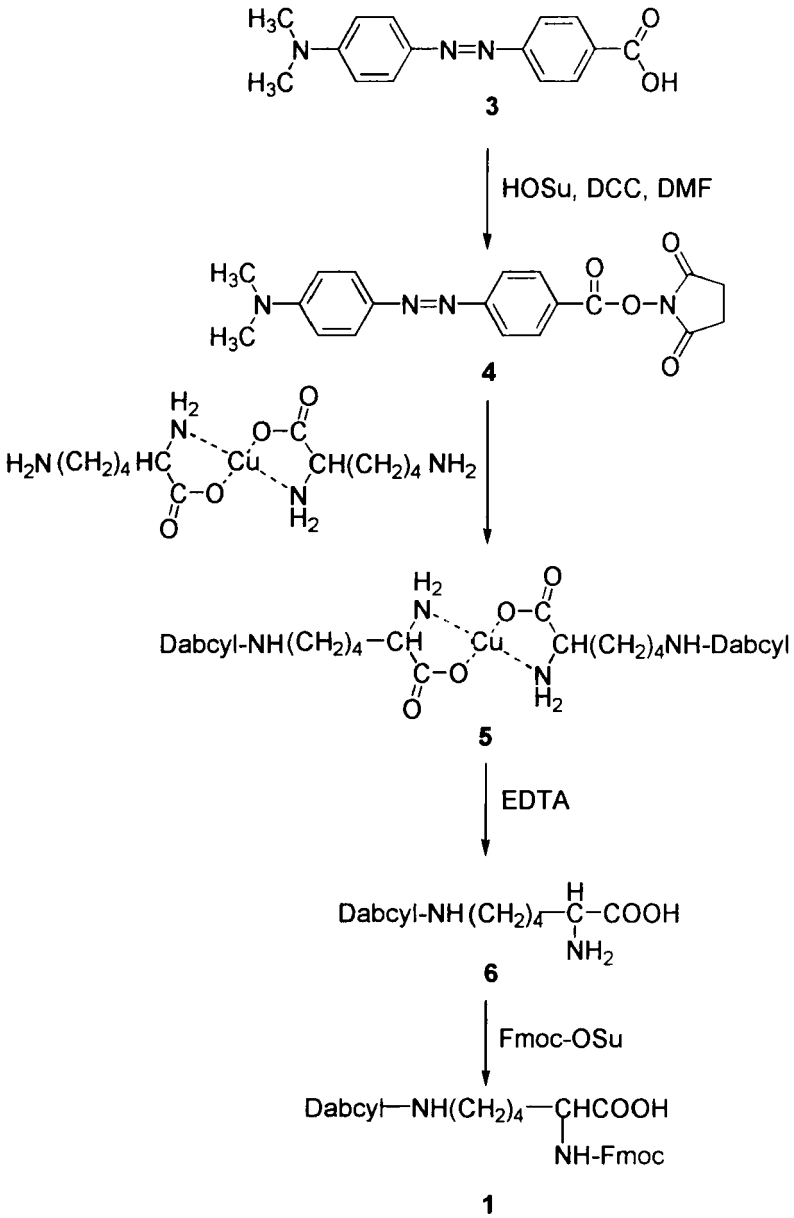
The preparation of **1** is shown in scheme 1. The dabcyl acid **3** was reacted with *N*-hydroxysuccinimide (HOSu) and 1,3-dicyclohexylcarbodiimide (DCC) in DMF to give the activated ester **4**, which was then reacted with lysine-Cu^{2+} complex prepared by refluxing an aqueous solution of lysine hydrochloride with cupric carbonate, to form N^{ε}-dabcyllysine-Cu^{2+} complex **5**. Treating this complex with a saturated solution of EDTA as its disodium salt afforded N^{ε}-dabcyllysine **6**, which was then acylated with Fmoc-OSu to give **1**. Scheme 2 illustrates the preparation of Boc-Lys(Dabcyl)-OH **2.** Reaction of the activated ester **4** with Boc-Lys-OH **10**, obtained from lysine by N^{ε}-acylation of the Cu^{2+} complex with Z-Cl followed by N^{α}-acylation with Boc-N_3 and catalytic hydrogenolysis, gave the target compound **2**.

Experimental

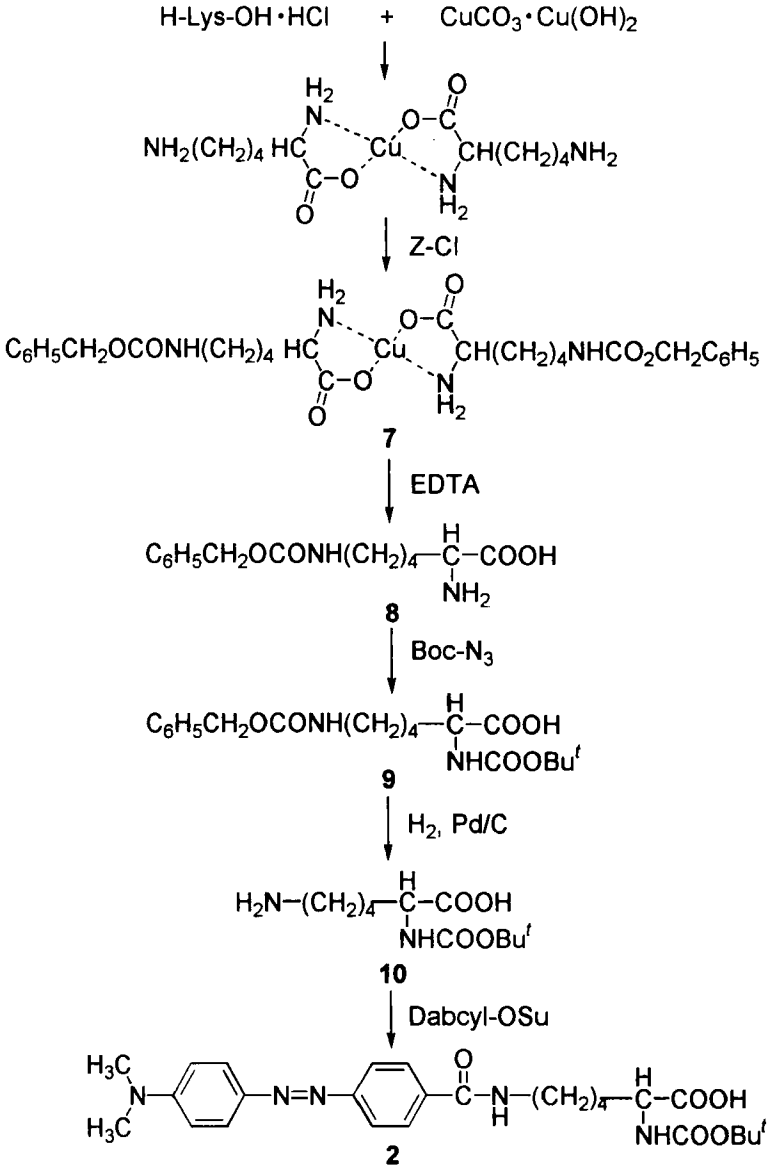
Melting points were measured in capillary tubes and are uncorrected. IR spectra were recorded on a Nicolet Magna FT 550 spectrometer. ^{1}H NMR spectra were obtained on a Varian Inova 400 MHz Spectrometer. Elemental analyses were performed using a Carlo-Erba Model 1110 instrument.

1. Preparation of N^{ε}-dabcyl-N^{α}-(9-fluorenylmethoxy)-carbonyllysine 1

1.1 Dabcyl-*N*-hydroxysuccinimide ester (Dabcyl-OSu) 4 2.2 g (8.2 mmol) of dabcyl acid (prepared following the procedure described by Zhmurova[2]) was dissolved in hot DMF (50 mL) and the resultant solution allowed to cool to room temperature. To this solution was added *N*-hydroxysuccinimide (HOSu) (1.2 g, 10 mmol), then DCC (2.0 g, 10 mmol) in DMF (6 mL), with stirring. The suspension was stirred overnight at room temperature, and the dicyclohexylurea removed by filtration. The filtrate was evaporated under reduced pressure, and the residue was precipitated with ether.



Scheme 1. The synthesis of Fmoc-Lys(Dabcyl)-OH **1**



Scheme 2. The synthesis of Boc-Lys(Dabcyl)-OH **2**

The product was washed further with 2-propanol and dried to give red solid **4** (2.2 g, 74%), m.p. 200-1°C. IR, ν_{max} (cm^{-1}): 1737, 1767 (CO), 1603 (-N=N-). ^1H NMR($CDCl_3$): δ2.92-2.98(bs, 4H, CH_2CH_2), 3.14(s, 6H, $(CH_3)_2N$-), 6.79-8.22(m, 8H, Ar-H).

1.2 N^{ε}-Dabcyllysine 6 To a solution of L-lysine hydrochloride (1.08 g, 5.88 mmol) in water (7 mL), was added $CuCO_3 \cdot Cu(OH)_2 \cdot H_2O$ (0.86 g, 3.60 mmol). The stirred mixture was heated to reflux. After two hours of boiling, the undissolved cupric carbonate was removed from the hot mixture by filtration and was washed with hot water (5 mL). The combined filtrate and washings were cooled to r.t. and $NaHCO_3$ (0.41 g, 4.93 mmol) was added. To the cold mixture at 0-5°C was added dropwise Dabcyl-OSu activated ester **4** (1.80 g, 4.91 mmol) dissolved in DMF (50 mL) under vigorous stirring over a period of about 2h. The contents then stirred overnight at room temperature. The precipitate was collected by filtration and washed successively with water, acetone and ether, before being dried in air to give the cupric complex **5**. The finely powdered complex was suspended in excess of saturated EDTA solution and stirred overnight at r.t. The precipitate was collected by filtration and again stired with EDTA, filtered, washed with water, and dried to give 1.5 g of **6** (77.0%).

1.3 N^{ε}-dabcyl-N^{α}-(9-fluorenylmethoxy)-carbonyllysine 1 N^{ε}-dabcyl-lysine **6** (1.15 g, 2.89 mmol) was suspended in CH_3CN / DMF / H_2O (6:2:1) and stirred at 0°C. The pH of the solution was adjusted to 8~8.5 by the addition of solid sodium bicarbonate (0.25 g, 3.00 mmol), then Fmoc-OSu (1.17 g, 3.47 mmol) was added in portions over a period of about 1h. After stirring overnight at room temperature, the reaction mixture was extracted with ether (20mL × 2). The aqueous layer was acidified to pH3 by the addition of 6M hydrochloric acid. The precipitate that formed was collected by filtration and washed successively with brine and water. The crude product was chromatographed (chloroform / methanol = 50 : 1) to give 0.7 g of pure **1** (yield 40%), TLC analysis showed a

single sport, Rf = 0.30 (silica gel plate, chloroform / methanol = 5:1), m.p. 132-134°C. Elemental anal. calcd. for $C_{36}H_{37}N_5O_5$: C, 69.77; H, 6.02; N, 11.30. Found: C, 69.93; H, 6.05; N, 11.19. IR, $\nu_{max}(cm^{-1})$: 3417(NH); 1688, 1632 (CO). ^1H NMR(acetone-d_6): δ 1.28(bs, 3H, NH, NH, OH), 1.52-1.62(m, 2H, γ-CH_2), 1.66-1.75(m, 2H, δ-CH2), 1.79-1.89(m, 1H, β-CH), 1.90-2.00(m, 1H, β-CH), 3.14(s, 6H, $N(CH_3)_2$), 4.22-4.27(m, 2H, α-CH and C9-H of the fluorene), 4.32-4.35(m, 2H, OCH_2), 4.37(t, 2H, ε-CH_2), 6.85-8.08(m, 16H, Ar-H).

2. Preparation of N^{α}-*tert*-Butyloxycarbonyl-N^{ε}-dabcyllysine 2

2.1 N^{ε}-Benzyloxycarbonyllysine 8 This was prepared by stirring the N^{α}-*tert*-Butyloxycarbonyl-N^{ε}-dabcyllysine-Cu^{2+} complex **7**, which was obtained by the acylation of the Cu^{2+}-lysine complex with benzyl chloroformate according to the procedure described by Schlögl[3], with a saturated solution of EDTA as its disodium salt, yield 74%, m.p. 226-229°C.

2.2 N^{ε}-Benzyloxycarbonyl-N^{α}-*tert*-Butyloxycarbonyllysine 9 The N^{α}-protection of **8** was accomplished using *tert*-butyl azidoformate (prepared according to the literature description[4]) following a standard procedure[5] gave **9** in 96% yield.

2.3 N^{α}-*tert*-Butyloxycarbonyllysine 10 To a solution of **9** (11.7 g, 0.031 mol) in methanol (50 mL) containing 1g of Pd-C catalyst, hydrogen gas was bubbled in at 50-55°C with stirring, until the starting material disappeared (as evidenced by TLC). The catalyst was removed by filtration and the filtrate was evaporated to dryness. The residue was dried in vacuum over P_2O_5 to give **10** 5.7 g (75%), m.p. 190-194°C.

2.4 N^{α}-*tert*-Butyloxycarbonyl-N^{ε}-dabcyllysine 2 To a vigorously stirred solution of **10** (2.4 g, 9.74 mmol in 30 mL water) was added a solution of **4** (3.0 g, 8.19 mmol) in DMF (80 mL) dropwise at 0-5°C. After stirring at room temperature overnight, the reaction mixture was acidified cautiously to pH3 with

2M hydrochloric acid whilst cooling with an ice-bath. The orange precipitate that formed was collected by filtration, washed several times with water, dried in air, and chromatographed (chloroform / methanol = 50 : 1) to give 2.4 g of **2** (59%). TLC, Rf = 0.14 (silica gel plate, chloroform/methanol = 5 : 1). Elemental anal calcd for $C_{26}H_{35}N_5O_5$: C, 62.76; H, 7.09; N, 14.07; Found: C, 62.76; H, 7.16; N, 14.07. IR, ν_{max} (cm^{-1}): 3286 (NH), 1745, 1686, 1606 (CO). ^1H NMR (DMSO-d_6) δ 1.37 (s, 9H, $(CH_3)_3C$), 1.42-1.78 (m, 6H, $(CH_2CH_2CH_2)$), 3.08 (s, 6H, $N(CH_3)_2$), 3.25-3.29 (m, 2H, ε-CH_2), 3.82-3.88 (m, 1H, α-CH), 6.85 (d, 2H, Ar-H), 7.05-7.07 (d, 1H, α-NH), 7.80-7.83 (m, 4H, Ar-H), 7.98 (d, 2H, Ar-H), 8.55-8.57 (t, 1H, ε-NH), 12.39 (s, 1H, COOH).

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