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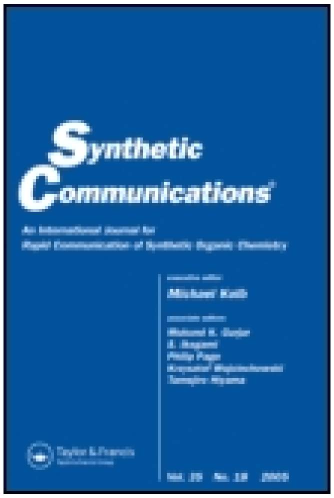

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SYNTHESIS OF L-INDOSPICINE

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SYNTHESIS OF L-INDOSPICINE

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ABSTRACT

Preparation of L-indospicine in eight steps by a reaction sequence starting with L-lysine.

Key Words: L-indospicine; Non-protein amino acid; Arginine analog

L-Indospicine [2(S)-2,7-diamino-7-iminohepatanoic acid] **(1)** is the amidino analog of L-arginine **(2)**, and is found in the seed and leaf of several tropical legumes in the genus *Indigofera*, including the creeping indigo plant (*Indigofera spicta* Forsk), and *Indigofera linnaei*[1] (Figure 1). Several studies have investigated the biological effects of L-indospicine. Exposure to L-indospicine resulted in the inhibition of protein synthesis and subsequent inhibition of DNA synthesis in several different cell systems.[2–5] L-Indospicine also inhibited the in vitro activity of rat liver arginase, and may be an inhibitor of both the constitutive form of nitric oxide synthase (NOS) in vascular endothelium, and the inducible form of NOS in macrophages.[6] Due to the structural similarity between L-arginine and L-indospicine, L-indospicine appears to have the capacity to be charged to arginyl-tRNA and to be incorporated into nascent protein chains in place of L-arginine.[7] L-Canavanine,

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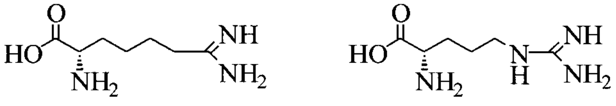


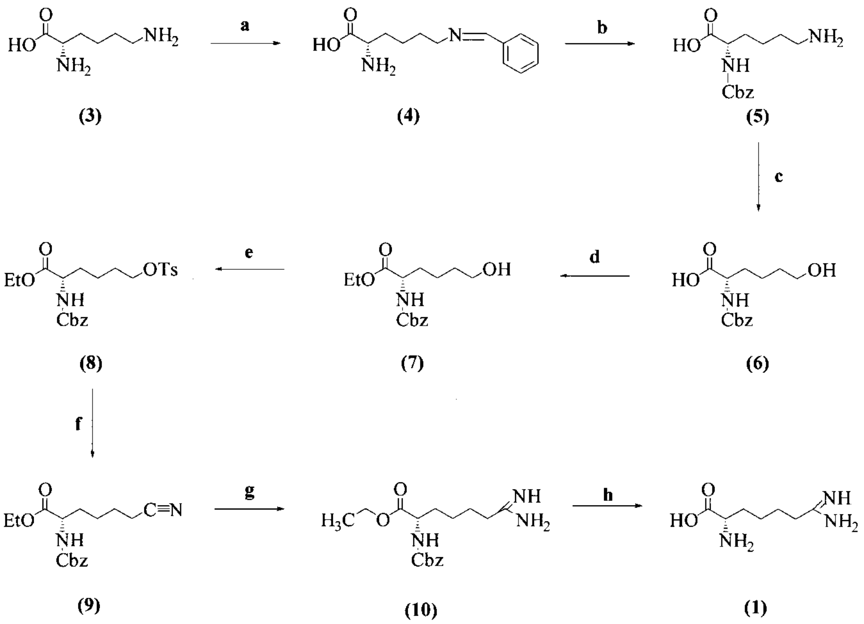
(1) **(2)**

Figure 1.

another L-arginine analog, is currently in development as an anticancer agent;[8] it is postulated that the antineoplastic properties of L-canavanine result from its capacity to be charged to arginyl-tRNA and incorporated into protein in place of L-arginine. Studies indicate that L-indospicine may be charged to arginyl-tRNA and incorporated into protein in place of L-arginine more efficiently than L-canavanine.[9] Consequently, the anticancer properties of L-indospicine are currently under active investigation.[10]

Despite the interesting and promising biological activity of L-indospicine, research on the compound is limited because of the difficulty in obtaining sufficient quantities of the pure L-enantiomer by synthetic methods. Currently, the only detailed literature reports on the total synthesis of indospicine are for the synthesis of DL-indospicine;[11,12] L-indospicine can be subsequently separated through acylation and enzymatic resolution.[11] The overall yield of DL-indospicine obtained by this method was only 2%. Although the yields of the acylation and enzymatic resolution steps were not reported, it is likely that the overall yield of the reaction was less than 1%.[11] In 1996, a short note describing a chirally pure synthesis of L-indospicine and the biological effects of L-indospicine on NOS was published by Feldman and Chi.[13] Unfortunately, this synthesis cannot be readily repeated, since the authors do not provide full experimental details for the synthesis, or report the spectral data for structure confirmation.

To our knowledge, this is the first report to fully describe a total synthesis of L-indospicine. This synthesis, drawing on the work of Culvenor and coworkers[12] and Baldwin and colleagues,[14] was developed to facilitate the characterization of the biological effects of this interesting L-arginine analog. The synthesis of L-indospicine was achieved from readily available L-lysine in eight steps via L-aminoadipic acid (Scheme 1). It was necessary to protect only the α-amino moiety of L-lysine. Initially, the ε-amino was masked by formation of the benzylidene imine **(4)**, and the α-amino group was protected with the benzyloxycarbonyl group. The α-imine was then selectively hydrolyzed to yield **(5)**. In a key transformation, the ε-amino group of **(5)** was converted to a hydroxyl group to form Cbz-protected L-α-aminoadipic acid **(6)**. The carboxylic acid moiety was then protected as an ethyl ester **(7)**, and the ε-hydroxyl function was tosylated **(8)**. After displacement of the



Scheme 1. a) 2M LiOH, 4°C, benzaldehyde; b) (i) EtOH/1M NaOH, benzyl chloroformate, −20°C; (ii) HCl, 50°C; c) 4M NaOH, 60°C, pH 9.5, sodium nitroprusside; d) EtOH, H_2SO_4, reflux; e) (i) *p*-toluenesulfonyl chloride/pyridine, 4°C, (ii) HCl; f) EtOH/KCN/H_2O, 90°C; g) (i) CH_2Cl_2, EtOH/HCl (g), (ii) MeOH, NH_3 (g), 0°C; h) 6M HCl, rt.

tosyl group with cyanide ion to afford **(9)**, the Pinner synthesis was utilized to form the amidino analog **(10)**. Finally, **10** was deprotected by hydrolysis in aqueous hydrochloric acid to yield L-indospicine **(1)** in 6% overall yield.

EXPERIMENTAL

^{1}H and ^{13}C NMR spectra were determined using a Varian 300 MHz NMR. All spectra were referenced, and chemical shifts determined using tetramethylsilane (TMS) as the internal standard. Melting points were determined on a Fisher–Johns visual melting point apparatus, and are uncorrected. Infrared spectra (IR) were obtained using a Bio-Rad Excalibur infrared spectrophotometer. MALDI (matrix-assisted laser desorption ionization) experiments were carried out on a Kratos KOMPACT SEQ. The mass spectroscopy (MS) experiments were performed at the University of Kentucky Mass Spectroscopy Laboratory, Lexington, KY. Amino acid analysis was done on a Hewlet-Packard 1100 amino acid analyzer fitted with a Pickering PCX 5200 column. Elemental analyses were conducted by Atlantic Microlabs (Norcross, GA) using automated combustion tube analysis. Optical rotation measurements were determined on a Perkin Elmer 241 polarimeter.

2(S)-2-Amino-6-(benzylideneamino)hexanoic Acid (4)

L-Lysine monohydrochloride **(3)** (0.3 mol) was dissolved in 2 M LiOH (0.3 mol) and cooled to 4°C. Benzaldehyde (0.31 mol) was slowly added, and the cooled solution was carefully shaken. The reaction mix was allowed to stir for two hours at 4°C. After the addition of 250 mL of cold ethanol, the solution was filtered. The resulting white precipitate was suspended in 60 mL of cold ethanol and filtered again. The product was dried in vacuo to yield 0.19 mol (63.3%) of **(4)**; m.p. 187–89°C, lit. (14). Found: 185–186°; ^{1}H NMR (300 MHz, D_2O) δ 3–1.5m (2H, γ-CH_2), 1.6–1.8 m (4H, β, δ-CH_2), 3.0t (2H, ε-CH_2), 3.5m (1H, α-CH), 3.6m (1H, CH–Ar), 7.6–7.8m (5H, Ar) ppm. ^{13}C NMR (D_2O) δ 20.7 (γ-CH_2) 25.8 (δ-CH_2), 31.0 (β-CH_2), 38.2 (ε-CH_2), 54.1 (α-CH), 74.2 (CH–Ar), 127.2 (Ar), 127.9 (Ar), 130.3 (Ar), 133.9 (Ar), 179 (CO) ppm.

2(S)-2-(Benzyloxycarbonylamino)-6-aminohexanoic Acid (5)

N-ε-Benzylidene L-lysine **(4)** (0.316 mol) was dissolved in 600 mL of an ethanol–1 M NaOH solution (1 : 1). The solution was cooled to −20°C. Over the period of one hour, precooled benzyl chloroformate (0.316 mol) was added alternatively in portions with 1 M NaOH in ethanol (2.5 : 1). The temperature was allowed to rise to −5°C, and 50 mL of precooled HCl was added. After stirring for five minutes, the reaction mixture was warmed to 50°C for five minutes and then concentrated under vacuum. The pH of the solution was adjusted to 6.2 and the solution was allowed to stand for 14 h at 4°C. The brown precipitate was separated, and the remaining solution evaporated. The resulting white precipitate was recrystallized from water to yield 0.275 mol (86.7%) of the product; TLC (silica) $R_f = 0.15$ (95% ethanol); ^{1}H NMR (300 MHz, D_2O) δ 1.4–1.5m (2H, γ-CH_2), 1.6–1.9m (4H, β, δ-CH_2), 3.0t (2H, ε-CH_2), 4.0m (1H, α-CH), 5.1s (2H, CH_2–Ar), 7.5m (5H, Ar) ppm. ^{13}C NMR (D_2O) δ 21.8 (γ-CH_2), 26.0 (δ-CH_2), 30.9 (β-CH_2), 39.0 (ε-CH_2), 54.1 (α-CH), 66.6 (CH_2–Ar), 127.4 (Ar), 128.0 (Ar), 128.5 (Ar), 136.3 (Ar), 157.6 (NCO), 179.0 (CO) ppm.

2(S)-2-(Benzyloxycarbonylamino)-6-hydroxyhexanoic Acid (6)

Compound **(5)** (25 mmol) was dissolved in 50 mL of water, the solution warmed to 60°C, and 4 M NaOH was added to adjust the pH to 9.5. Sodium nitroprusside dihydrate (31 mmol) was slowly added with stirring. The reaction was stirred at 60°C for 6.5 h with periodic additions of NaOH to maintain the pH at 9.5. The mixture was filtered through celite, and the filtrate acidified to pH 1 and extracted with ethyl acetate (3 × 100 mL). The combined organic extracts were washed with brine, dried over anhydrous $MgSO_4$, and evaporated, to yield 13 mmol of the crude oil (52.2%). MS: $(m/z) = 304$ $(M + K^+)$; $(m/z) = 320$ $(M + Na^+)$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.4–1.9m (6H, γ,β,δ-CH_2), 3.6t (2H, ε-CH_2), 4.4m (1H, α-CH), 5.1s (2H, CH_2–Ar), 5.6d (1H, NH), 6.2–6.5s (1H, OH), 7.4m (5H, Ar) ppm. ^{13}C NMR ($CDCl_3$) δ 22.1 (γ-CH_2), 32 (β-CH_2), 32.5 (δ-CH_2), 53.8 (α-CH), 61.5 (ε-CH_2), 67.0 (CH_2–Ar), 128.1 (Ar), 128.2 (Ar), 128.5 (Ar), 136.3 (Ar), 155.9 (NCO), 179.5 (CO) ppm.

Ethyl 2(S)-2-(Benzyloxycarbonylamino)-6-hydroxyhexanoate (7)

Crude **(6)** (11.3 mmol) was dissolved in 70 mL of absolute ethanol. Sulfuric acid (1.4 mL) was added and the solution heated under reflux for 5 h. The mixture was neutralized with 2 M NaOH and the ethanol evaporated on a rotary evaporator. The resulting oil was taken up into water and extracted with ethyl acetate (3 × 50 mL). After silica gel column chromatography (100 : 1, chloroform : methanol), 7.9 mmol of the product was obtained (73%). TLC (silica): $R_f = 0.21$ (80 : 1, chloroform : methanol); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.2t (3H, CH_3), 1.35–1.45m (2H, γ-CH_2), 1.5–1.9m (4H, β, δ-CH_2), 2.1s (1H, OH), 3.6t (2H, ε-CH_2), 4.2q (2H, CH_2–O), 4.35m (1H, α-CH), 5.1s (2H, CH_2–Ar), 5.5d (1H, NH), 7.3m (5H, Ar) ppm. ^{13}C NMR ($CDCl_3$) δ 14.2 (CH_3), 21.5 (γ-CH_2), 32 (β-CH_2), 32.4 (δ-CH_2), 53.9 (α-CH), 61.4 (CH_2–O), 62.3 (ε-CH_2), 67.0 (CH_2–Ar), 128.1 (Ar), 128.2 (Ar), 128.5 (Ar), 136.3 (Ar), 156 (NCO), 172.6 (CO) ppm.

Ethyl 2(S)-2-(Benzyloxycarbonylamino)-6-tosylhexanoate (8)

A solution of **(7)** (34.3 mmol) in 25 mL of dry pyridine was cooled to −25°C. The temperature was kept below 0°C and *p*-toluenesulfonyl chloride (69 mmol) was slowly added. The mixture was stirred for six hours at 4°C and then acidified to pH 1 with aqueous HCl. The mixture was extracted with chloroform, dried over anhydrous $MgSO_4$, and purified by silica gel column chromatography (80 : 1 chloroform : methanol). 26.4 mmol (77% yield) of the product was obtained; MS: $(m/z) = 502$ $(M + K^+)$; $(m/z) = 486$ $(M + Na^+)$; TLC (silica) $R_f = 0.18$ (200 : 1, chloroform : methanol); IR ($CHCl_3$): 3431, 3020, 1718, 1509 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.2t (3H, CH_3), 1.3 (2H, γ-CH_2), 1.5–1.8m (4H, β, δ-CH_2), 2.4s (3H, CH_3–Ar), 3.9t (2H, ε-CH_2), 4.1q (2H, CH_2–O), 4.2m (1H, α-CH), 5.1s (2H, CH_2–Ar), 5.2d (1H, NH), 7.3m (5H, Ar), 7.7m (5H, Ar) ppm. ^{13}C NMR ($CDCl_3$) δ 14.1 (CH_3), 21.1 (γ-CH_2), 21.6 (Ar–CH_3), 28.3 (δ-CH_2), 32.0 (β-CH_2), 53.5 (α-CH), 61.5 (CH_2–O), 67.0 (CH_2–Ar), 69.9 (ε-CH_2), 128.7 (Ar), 128.2 (Ar), 129.8 (Ar), 133 (Ar), 136.2 (Ar), 144.7 (Ar), 155.8 (NCO), 172.1 (CO) ppm.

Ethyl 2(S)-2-(Benzyloxycarbonylamino)-6-cyanohexanoate (9)

26.5 mmol of **(8)** was dissolved in 10 mL of ethanol. A solution of KCN (78.5 mmol) in 10 mL of water was slowly added. The mixture was kept at 90°C for two hours, the ethanol was evaporated, and the resulting solution was acidified with aqueous HCl. The mixture was extracted with diethyl ether (3 × 20 mL) and the combined organic extracts were washed with water, brine, and dried over anhydrous sodium sulfate. After evaporation and purification by silica gel chromatography (chloroform : methanol gradient 100 : 1 to 1 : 1), 7.4 mmol (28%) of the ester product **(9)** was isolated along with 16 mmol (60%) of the free acid, which could be subsequently reesterified with ethanol to afford a second crop of **(9)** (total yield: 88%); MS: $(m/z) = 318$ $(M + K^+)$; $(m/z) = 357$ $(M + Na^+)$; TLC (silica) $R_f = 0.21$ (100 : 1, chloroform : methanol); ^1H NMR (300 MHz, $CDCl_3$) δ 1.3t (3H, CH_3), 1.4–2.0m (6H, γ, β, δ-CH_2), 2.3t (2H, ε-CH_2), 4.2q (2H, CH_2–O), 4.3m (1H, α-CH), 5.1s (2H, CH_2–Ar), 5.35d (1H, NH), 7.3m (5H, Ar) ppm. ^{13}C NMR ($CDCl_3$) δ 14.1 (CH_3), 17.0 (ε-CH_2), 24.2 (γ-CH_2), 24.8 (β-CH_2), 32 (δ-CH_2), 53.4 (α-CH), 61.7 (CH_2–O), 67.0 (CH_2–Ar), 119.3 (CN), 128.1 (Ar), 128.2 (Ar), 128.5 (Ar), 136.1 (Ar), 155.8 (NCO), 172.6 (CO) ppm.

Ethyl 2(S)-2-(Benzyloxycarbonylamino)-7-diamino-7-iminoheptanoate (10)

Compound **(9)** (1.4 mmol) was dissolved in 10 mL dry methylene chloride under nitrogen. Anhydrous ethanol (1.5 mL) was added and dry HCl gas was bubbled through the solution until it was saturated. The reaction was stirred at room temperature overnight. The following morning, more dry HCl gas was bubbled through the reaction mixture and the solution was allowed to stir at room temperature for an additional five hours. At this time, the solvent was evaporated and anhydrous methanol was added and evaporated. This procedure was repeated an additional two times. The resulting residue was dissolved in anhydrous methanol under nitrogen and the solution cooled to 0°C. Ammonia gas was bubbled through the solution until it was saturated. The reaction was stirred at 0°C for six hours and allowed to stand at 4°C overnight. TLC (silica) analysis (25 : 1, chloroform : methanol) indicated the reaction had gone to completion. The reaction mixture was evaporated and the residue dissolved in hot distilled water, decolorized with charcoal, filtered and evaporated. The resulting product was purified via column chromatography (7 : 1, chloroform : methanol +1% acetic acid) to afford 0.67 mmol (48%) of **(10)**. A small, but significant amount of methyl 2(S)-2,7-diamino-7-iminoheptanoate (the transesterification product) was recovered during the column chromatographic separation, which was subsequently hydrolyzed to L-indospicine **(1)**. MS: $(m/z) = 336$ $(M+1)$; TLC (silica) $R_f = 0.15$ (7 : 1, chloroform : methanol +1% glacial acetic acid; ^{1}H NMR (300 MHz, CD_3OD) δ 1.3t (3H, CH_3), 1.4–1.8m (6H, γ,β,δ-CH_2), 2.4t (2H, ε-CH_2), 4.2q (2H, CH_2–O), 4.2m (1H, α-CH), 5.1s (2H, CH_2–Ar), 7.3m (5H, Ar) ppm. ^{13}C NMR (CD_3OD) δ 14.7 (CH_3), 26.3 (γ-CH_2), 27.7 (δ-CH_2), 32.2 (β-CH_2), 33.4 (ε-CH_2), 55.3 (α-CH), 62.5 (CH_2–O), 67.8 (CH_2–Ar), 128.8 (Ar), 129.0 (Ar), 129.5 (Ar), 138.1 (Ar), 158.6 (NCO), 172.6 (C=N), 174.3 ppm.

2(S)-2,7-Diamino-7-iminoheptanoic Acid Dihydrochloride (L-Indospicine) (1)

Compound **(10)** (0.2 mmol) was dissolved in 5 mL of 6M HCl and allowed to stir at room temperature for 72 h. The solvent was evaporated, the residue was taken up in methanol, and the methanol evaporated. This procedure was repeated two more times. Aqueous methanol was used to crystallize 0.146 mmol (73%) of the extremely hygroscopic product. MS (m/z): 174.09 $(M+1)$; optical rotation ($[\alpha]_D^{22}$) lit. $+18 \pm 1$ (c. 1 in 5N HCl) [172]; Found: $+18.5 \pm 0.6°$ (c. 0.25 in 5N HCl); amino acid analysis $R_f = 60.6$ min (Pickering PCX 5200 amino acid analysis column, $T = 40° \pm 20°$, flow rate = 0.30 mL/min, with gradient pH elution); Anal. Calc. for $C_7H_{17}Cl_2N_3O_2 \cdot (3/4)H_2O \cdot (1/4)\ CH_3OH$: C, 33.48; H, 7.51; N, 15.28. Found: C, 33.41; H, 7.06; N, 14.90 (Note: Natural L-Indospicine hydrochloride has been shown to crystallize with 0.75 molecule of water per L-indospicine molecule[15]). ^{1}H NMR (300 MHz, CD_3OD) δ 1.8m (2H, γ-CH_2), 1.9m (2H, δ-CH_2), 2.1m (β-CH_2), 2.6m (2H, ε-CH_2), 4.1m (1H, α-CH), 8.5s and 9.0s ($CNH_2(NH)$) ppm. ^{13}C NMR (CD_3OD) δ 25.5

(γ-CH_2), 27.8 (β-CH_2), 31.1 (δ-CH_2), 33.3 (ε-CH_2), 53.8 (α-CH), 171.6 (C=O), 172.8 (C=N) ppm.

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