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CHEMICAL CONFIRMATION OF THE STRUCTURE OF A MUTAGENIC AMINOPHENYLNORHARMAN, 9-(4'-AMINOPHENYL)-9*H*-PYRIDO[3,4-*b*]INDOLE : AN AUTHENTIC SYNTHESIS OF 9-(4'-NITROPHENYL)-9*H*- PYRIDO[3,4-*b*]INDOLE AS ITS RELAY COMPOUND

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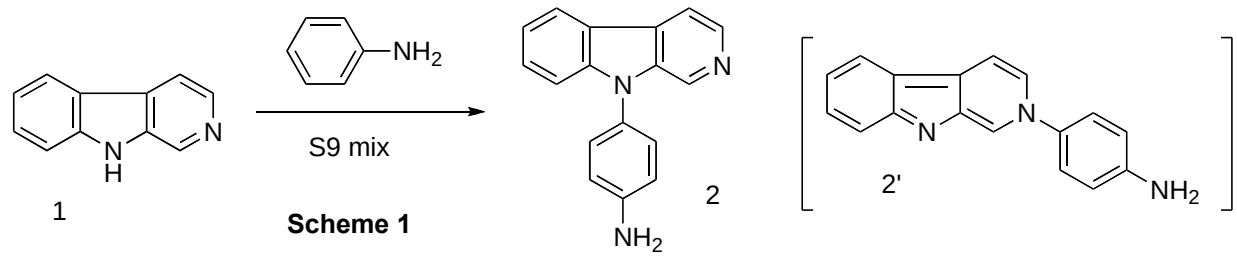
Abstract – 9-(4'-Aminophenyl)-9*H*-pyrido[3,4-*b*]indole **2** is a mutagenic compound produced by non-mutagenic norharman **1** and aniline in the presence of S9 mix. 9-(4'-Nitrophenyl)-9*H*-pyrido[3,4-*b*]indole **4**, the relay compound for synthesis of **2**, was synthesized starting from ethyl indole-2-aldehyde **12** via initial *N*-(4-nitro)phenylation of the indole nucleus, elongation of the 2-aldehyde substituent, and then construction of the pyridine nucleus in order to ensure the nitrogen substitution in **2**.

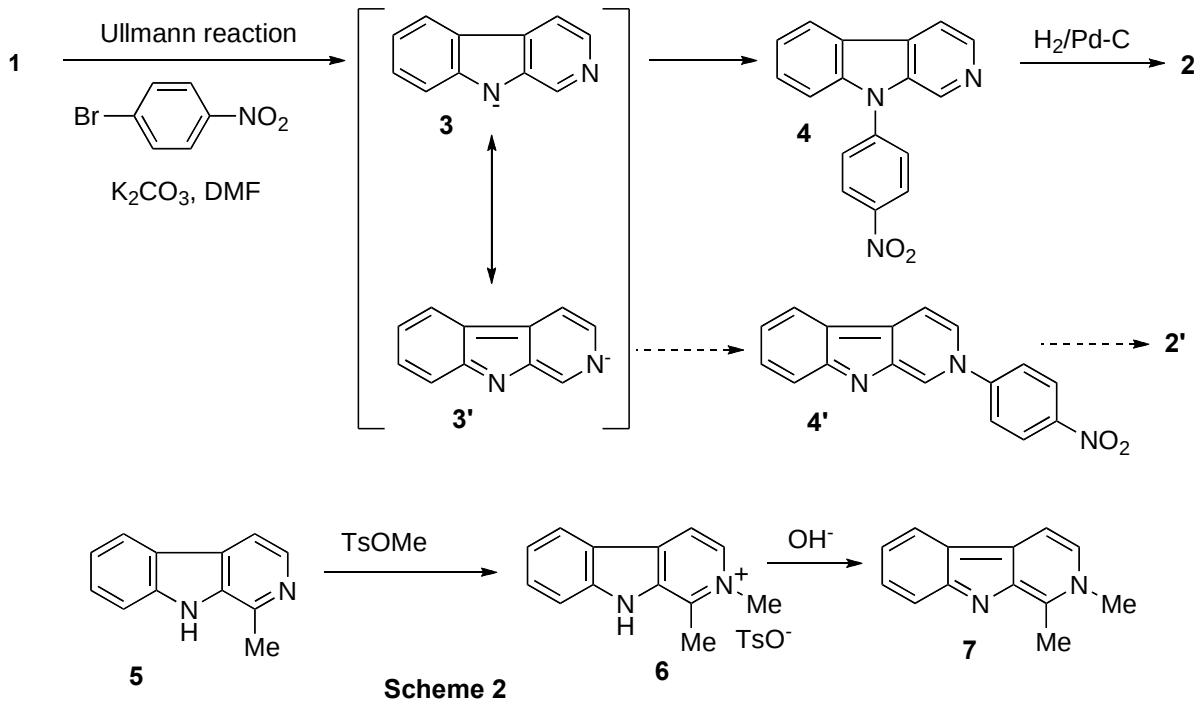
INTRODUCTION

Sugimura et al.[1] reported that norharman **1** (9*H*-pyrido[3,4-*b*]indole, β-carboline) itself is not mutagenic to *Salmonella* strains, but becomes mutagenic to *S. typhimurium* TA98 and YG1024 with S9 mix in the presence of non-mutagenic aromatic amines such as aniline and *o*-toluidine. In a



Scheme 1

subsequent report[2] they isolated mutagenic compound **2** produced by the reaction between norharman and aniline with S9 mix (Scheme 1). In order to elucidate the structure, one of the potential structures, 9-(4'-aminophenyl)-9*H*-pyrido[3,4-b]indole **2**, was synthesized[2] via Ullmann reaction of norharman **1** with 4-bromonitrobenzene, followed by catalytic hydrogenation. The synthetic sample was identical to the natural one and the spectral data of the product supported the structure of **2** but not **2'**. The synthetic strategy was based on the fact that Ullmann reaction of indoles with aryl halides proceeded at its NH position.[3] However, if the reaction occurs on the pyridine nitrogen of **1** via its basicity or another resonance structure **3'**, the product should be compound **4'** (Scheme 1 and 2), whose structure would be much more unstable than the structure **4**, as it has neither benzene, indole, nor pyridine aromaticity any longer. Thus, such a compound is thought to be difficult to produce. On the other hand, it was recently reported[4] that N_A-methylammonium harman **6** derived from harman **5** was basified to yield the compound **7**, whose skeleton is the same as those of **2'** and **4'** (Scheme 2). In this paper we report the authentic synthesis of the relay compound **4** in order to ensure the nitrogen substitution of the substituted phenyl group in **2**.

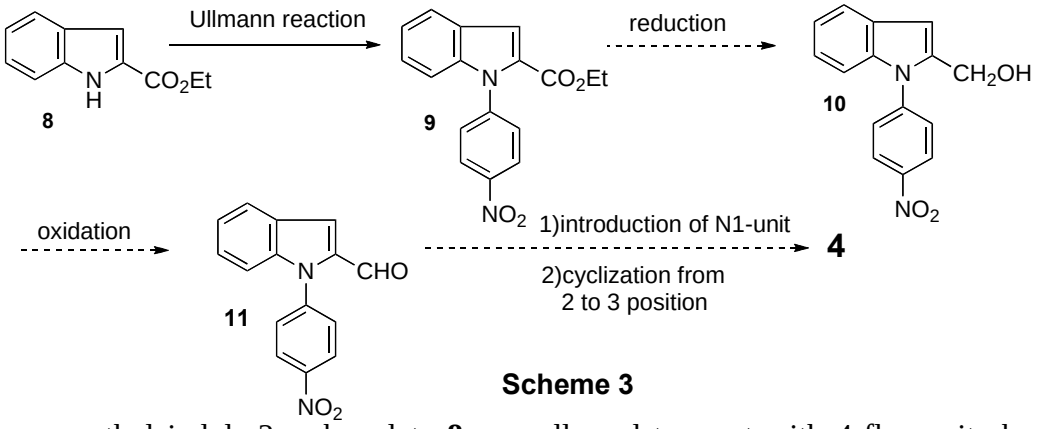


Scheme 2

RESULTS AND DISCUSSION

The synthetic strategy for the synthesis of the relay compound **4** was designed as shown in Scheme 3. The key point is the initial (4-nitro)phenylation at the 1-nitrogen position in the indole nucleus, followed by elongation of the 2-substituent and cyclization to form the pyridine nucleus.

The usual construction of the pyridine ring in the indole nucleus for synthesis of the 9*H*-pyrido[3,4-*b*]indole nucleus is cyclization of the 3-substituent of the tryptamine derivative to the 2-position of the indole nucleus as seen in the Bischler-Napieralski reaction, Pictet-Spengler reaction and so on. On the other hand, there are few methods for cyclization of the 2-substituent to the 3-position of the indole nucleus. Several years ago we developed a method for 9*H*-pyrido[3,4-*b*]indole synthesis of the latter type in the course of the synthetic study of 4-oxo-β-carboline.[5] We applied this method in the present strategy.

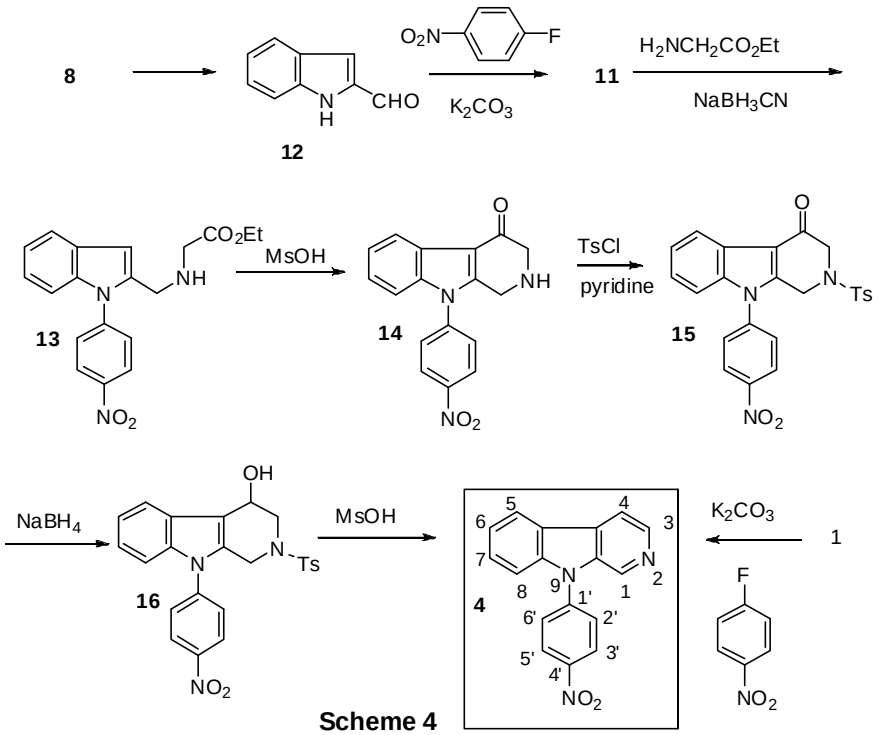


Scheme 3

For this purpose, ethyl indole-2-carboxylate **8** was allowed to react with 4-fluoronitrobenzene to give ethyl *N*-(4'-nitrophenyl)indole-2-carboxylate **9**. However, the reduction of ester carbonyl of **9** with $LiAlH_4$ was not successful (Scheme 3). Thus, the reaction scheme to prepare the aldehyde **11** had to be changed. The synthetic route was changed as in Scheme 4.

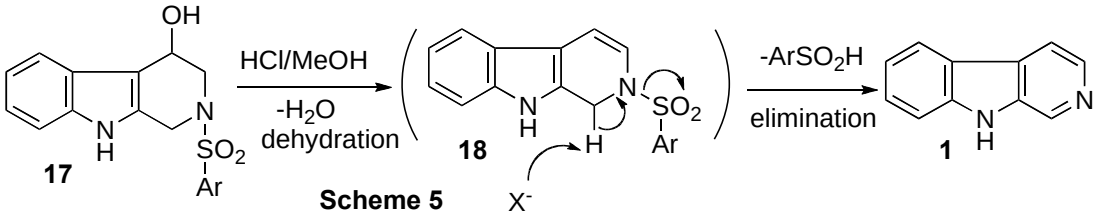
The first *N*-(4-nitro)phenylation of indole-2-carboxaldehyde[5] **12** prepared from **8**, which we feared to proceed with difficulty due to the sensitive reactivity of aldehyde functionality, went much better than expected (51% yield). The *N*-(4'-nitrophenyl)indole-2-carboxaldehyde **11** thus obtained was allowed to react with ethyl aminoacetate and then sodium cyanoborohydride to give the *N*-indolic aminoacetate **13**. The cyclization of **13** with methanesulfonic acid gave the cyclized amino ketone **14**. The aminoketone **14** was treated with tosyl chloride in the presence of pyridine to give the corresponding tosylamide **15** in good yield. The subsequent process of cyclic amino ketone resembling **14** to the target 9*H*-pyrido[3,4-*b*]indole nucleus has already been developed.[6]

The reduction of the ketone of **15** to the hydroxyl group with a large excess amount of sodium borohydride proceeded to give the alcohol **16** in good yield. The last and important dehydration and aromatization processes (two successive β-eliminations) were examined for the present reaction.



Scheme 4

The reaction[6] involved dehydration of the alcohol and β-elimination process around the sulfonyl group with HCl in MeOH, as shown in Scheme 5. In the present case the reaction did not proceed well with HCl in MeOH in several trials, probably due to its insolubility.



Scheme 5

After several acidic conditions were tried, the alcohol **16** was allowed to react with methanesulfonic acid. The target compound **4** was finally obtained from the basic layer in this reaction (20% yield). The product was identified with the already[2] and freshly synthesized sample directly via the Ullmann reaction from **1** and 4-bromo-(or 4-fluoro)nitrobenzene in the presence of K_2CO_3. It was proved that the Ullmann reaction of **1** proceeded at the indolic NH position even on the 9*H*-pyrido[3,4-*b*]indole nucleus. It is worth noting that the Ullmann reaction of 1 with 4-fluoronitrobenzene without K_2CO_3 did not proceed at all. This means that the formation of nitrogen anion is necessary for Ullmann reaction of indole and pyridine nitrogen cannot take Ullmann reaction directly. Thus, the structure **2** was chemically determined. Using this scheme, it may be possible to develop a new strategy for 9*H*-pyrido[3,4-*b*]indole synthesis that involves cyclication of the 2-substituent toward the 3-position of the indole skeleton.

EXPERIMENTAL

All melting points were measured on a hot stage micro-melting points apparatus (Yanagimoto) and are uncorrected. Elemental analyses were conducted with a Yanaco CHN CORDER MT-6. The ^{1}H-NMR spectra were measured with a Bruker Ultrashield^{TM} 400 Plus (400MHz) spectrometer. Deuteriochloroform was used as the solvent with tetramethylsilane as an internal reference. MS spectra were measured on JEOL JMS-GC-mate II and JEOL JMS-600H spectrometers. IR spectra were recorded on a Shimadzu FTIR-8400S spectrometer. For column chromatography, Silica gel 60 (70-230 mesh ASTM; Merck) was used.

1-(4'-Nitrophenyl)indole-2-carboxaldehyde 11

A solution of indole-2-carboxaldehyde[5] **12** (1.80 g, 12.4 mmol), 4-fluoronitrobenzene (5.19 g, 36.8 mmol), and powdered anhydrous K_2CO_3 (5.14 g, 37.2 mmol) in anhydrous DMF (27 mL) was heated with stirring at 100 ºC for 1.5 h. The reaction mixture was poured onto water (150 mL), and extracted with AcOEt. The organic layer was washed with water, dried over $MgSO_4$, and evaporated *in vacuo* to dryness. The residue (6.38 g) was chromatographed over SiO_2 with toluene as eluent to give the target compound **11** (1.70 g, 51%). Recrystallization of a part of the compound from a mixture of AcOEt and hexane gave pale yellow columns, mp 170-172 ºC. *Anal*. Calcd for $C_{15}H_{10}N_2O_3$: C, 67.67; H, 3.79; N, 10.52. Found: C, 67.98; H, 3.95; N, 10.43. MS ($C_{15}H_{10}N_2O_3$): *m/z*266 (M^+). IR ν max(KBr)cm^{-1}: 1683 (sh), 1672 (CO). ^{1}H-NMR ($CDCl_3$) δ : 7.23-7.58 (6H, m, $C_{3,5,6,7,2',6'}$-H), 7.83 (2H, m, C_4-H), 8.41 (2H, d, *J*=8.0 Hz, $C_{3'}$,$C_{5'}$-H), 9.89 (1H, s, CHO).

Ethyl [1-(4'-Nitrophenyl)indole-2-ylmethyl]aminoacetate 13

To a muddy solution of 1-(4'-nitrophenyl)indole-2-carboxaldehyde **11** (724 mg, 2.77 mmol) and ethyl aminoacetate hydrochloride (1.12 g, 8.16 mmol) in ethanol (30 mL) was added triethylamine (1.17 mL, 8.16 mmol) and $NaBH_3CN$ (685 mg, 10.9 mmol) successively with stirring under ice-cooling. The reaction mixture (muddy state) was stirred under ice-cooling for 15 min and then at rt for an additional 3 h. Then, the reaction mixture was poured onto water and extracted with AcOEt. The organic layer was washed with brine, dried over $MgSO_4$ and evaporated *in vacuo* to dryness to give a pale yellow residue. The crude products were chromatographed over SiO_2. Elution with toluene, followed by toluene-AcOEt (10:1), gave the target compound **13** (713 mg, 74%) as a pale yellow oil. MS ($C_{19}H_{19}N_3O_4$): *m/z* 353 (M^+). HRMS: Calcd for $C_{19}H_{19}N_3O_4$, 353.1376; Found, 353.1378. IR ν max($CHCl_3$)cm^{-1}: 3684, 3620 (NH), 1734 (C=O). ^{1}H-NMR δ :1.23 (3H, t, *J*=8.0 Hz, -$CH_2C\underline{H}_3$), 3.41 (2H, s, -CH_2NH-), 3.94 (2H, s, -NCH_2CO-), 4.12 (2H, *J*=8.0 Hz, -O$\underline{CH}_2CH_3$), 6.75 (1H, s, C_3-H), 7.18-7.26 (3H, m, $C_{5,6,7}$-H), 7.63 (1H, m, C_4-H), 7.73 (2H, *J*=8.0 Hz, $C_{2',6'}$-H), 8.41 (2H, $C_{3',5'}$-H).

9-(4'-Nitrophenyl)-1,2,3,9-tetrahydro-9*H*-pyrido[3,4-*b*]indole-4-one 14

A mixture of ethyl [1-(4'-nitrophenyl)indole-2-ylmethyl]aminoacetate **13** (513 mg, 1.45 mmol) and methanesulfonic acid (7 mL) was stirred at 45 ºC for 45 min, and then 70 ºC for 1 h. The reaction mixture was poured onto water (50 mL), basified with K_2CO_3, and extracted with AcOEt. The organic layer was washed with water and dried over $MgSO_4$. Evaporation of the solvent *in vacuo* to dryness gave a solid (425 mg). This solid was chromatographed over SiO_2 (12 g). Elution with $CHCl_3$, followed by AcOEt, gave a small amount of the starting material and unknown compounds. Further elution with a mixture of AcOEt and EtOH (10:1) gave the target compound **14** (298 mg, 67%). A part of the sample was recrystallized from acetone to give pale yellow fine needles, mp 215-217 ºC (decomp). MS ($C_{17}H_{13}N_3O_3$): *m/z*307 (M^+, 25% of base peak), 252(base peak). HRMS: Calcd for $C_{17}H_{13}N_3O_3$, 307.0957; Found,307.0964. IR ν max(KBr)cm^{-1}:3326(NH), 1649(CO). ^{1}H-NMR δ : 2.23 (1H, br.s, NH), 3.67 (2H, s, C_3-H), 4.13 (2H, s, C_1-H), 7.21-7.45 (3H, m, $C_{6,7,8}$-H), 7.62 (2H, d, *J*=8.0 Hz, $C_{2',6'}$-H), 8.30 (1H, m, C_5-H), 8.50 (2H, d, *J*=8.0 Hz, $C_{3',5'}$-H).

9-(4'-Nitrophenyl)-2-tosyl-1,2,3,9-tetrahydro-9*H*-pyrido[3,4-*b*]indole-4-one 15

To a suspension of 9-(4'-nitrophenyl)-1,2,3,9-tetrahydro-9*H*-pyrido[3,4-*b*]indole **14** (265 mg, 0.862 mmol) in pyridine (7 mL) was added TsCl (493 mg, 2.59 mmol) under ice-cooling. The mixture was stirred under ice-cooling for 15 min and at rt for an additional 1 h. The reaction mixture was poured onto water, extracted with $CHCl_3$, washed with dil. HCl aq. and water, and dried over $MgSO_4$. Evaporation of the solvent *in vacuo* to dryness gave the target compound **15** (349 mg, 88%). A part of the compound was recrystallized from a mixture of DMF and EtOH to give almost colorless very fine needles, mp 253-258 ºC (decomp). *Anal*. Calcd for $C_{24}H_{19}N_3O_5S$: C, 62.46; H, 4.15; N, 9.11: Found: C; 62.42, H; 4.18, N; 8.58. MS: *m/z* 461 (M^+,15% of base peak), 306 (base peak). HRMS:Calcd for $C_{24}H_{19}N_3O_5S$, 461.1045; Found,461.1043. IR ν max(KBr)cm^{-1}:1664(CO). ^{1}H-NMR δ : 2.50 (3H, s, arom-CH_3), 4.11 (2H, s, C_3-H), 4.76 (2H, s, C_1-H), 7.20-7.45 (7H, m, arom-H), 7.87 (1H, dd, *J*=8.0 and 2.0 Hz, C_5-H), 7.92 (2H, d, *J*=8.0 Hz, Ts-ortho-H), 8.56 (2H, d, *J*=8.0 Hz, $C_{3',5'}$-H).

9-(4'-Nitrophenyl)-2-tosyl-2,3,4,9-tetrahydro-9*H*-pyrido[3,4-*b*]indole-4-ol 16

The tosyl ketone **15** (33 mg, 0.0715 mmol) was added to a mixture of $CHCl_3$ (1.5 mL) and MeOH (4 mL). To the resulting suspension was added $NaBH_4$ (270 mg, 7.15 mmol) under ice-cooling to prevent generation of heat at the beginning and then the whole was stirred for 4.5 h at rt. The reaction mixture was poured onto water and extracted with $CHCl_3$. The organic layer was washed with brine and dried over $MgSO_4$. Evaporation of the solvent *in vacuo* to dryness gave the target alcohol **16** as yellowish powder (31 mg, 94%). This sample showed one spot on TLC (SiO_2, toluene-AcOEt = 2:1), and was

used for the next reaction. A part of the powder was recrystallized from acetone-MeOH to give pale yellow powder, mp 175-177 ºC (decomp). *Anal*. Calcd for $C_{24}H_{21}N_3O_5S$: C, 62.19; H、4.57; N, 9.07. Found: C, 61.83; H, 4.68; N, 8.85. MS: 463 (M^+, 5.9% of the base peak), 252 (base peak). HRMS; Calcd, 463.1202; Found, 463.1208. IR ν max(KBr)cm^{-1}: 3482(OH). ^{1}H-NMR δ : 2.44 (3H, s, arom-CH_3), 3.16,3.96,4.55 (4H, aliph-H), 5.12 (1H, br. d, *J*=12.0 Hz, C_4-H), 7.24-7.78 (10H, m, arom-H), 8.46 (2H, d, *J*=8.0 Hz, $C_{3'}$, $C_{5'}$-H).

9-(4'-Nitrophenyl)-9*H*-pyrido[3,4-*b*]indole 4 from 9-(4'-nitrophenyl)-2-(toluene-4''-sulfonyl)-2,3,4,9-tetrahydro-9*H*-pyrido[3,4-*b*]indole-4-ol 16

The above-mentioned alcohol **16** (40 mg, 0.086 mmol) was dissolved in methanesulfonic acid (3 mL) and stirred for 4 h at rt. The reaction mixture was poured onto water and extracted out with AcOEt. The aqueous layer was basified with K_2CO_3 and extracted with AcOEt. The organic layer was washed with water and dried over $MgSO_4$. Evaporation of the solvent *in vacuo* to dryness gave the crude product. The crude product was purified with column-chromatography [SiO_2 (8 g), $CHCl_3$] to give yellow powder (5 mg, 20%), mp 188-190 ºC. This sample was identified with the relay compound[2] derived from Ullmann reaction of norharman **1** and 4-bromonitrobenzene (or 4-fluoronitrobenzene) as described below, based on their NMR spectra and TLC behavior. ^{1}H-NMR δ : 7.44 (1H, m, C_6-H), 7.56-7.62 (2H, m, $C_{7,8}$-H), 7.85 (2H, d, *J*=8 Hz, $C_{2',6'}$-H), 8.05 (1H, d, *J*=4.0 Hz, C_4-H), 8.23 (1H,d, *J*=8.0 Hz, C_5-H), 8.53 (2H, d, *J*=8.0 Hz, $C_{3',5'}$-H), 8.59 (1H, br. d, *J*=4.0 Hz, C_3=H), 8.95(1H, s, C_1-H).

9-(4'-Nitrophenyl)-9*H*-pyrido[3,4-*b*]indole 4 *via* Ullmann reaction[2] from norharman 1

A mixture of norharman **1** (40 mg, 0.238 mmol), 4-fluoronitrobenzene (66 mg, 0.476 mmol) and powdered anhydrous K_2CO_3 (99 mg, 714 mmol) was added to DMF (3 mL) and the whole was heated at 100 ºC with stirring for 3 h. The reaction mixture was poured onto water (60 mL) and extracted with AcOEt. The organic layer was washed with water, dried over $MgSO_4$, and evaporated *in vacu*o to dryness. The resulting mass was purified over column chromatography (SiO_2, $CHCl_3$) to give the target compound (79 mg, quantitative). This sample was recrystallized from $CHCl_3$-MeOH and then treated with $CHCl_3$ to give pale yellow needles, mp 192-192.5 ºC. The sample obtained from recrystallization from $CHCl_3$-MeOH contained MeOH in its crystals. The crystals were dried at 100 ºC *in vacuo* over night for elemental analysis. *Anal*. Calcd for $C_{17}H_{11}N_3O_2$: C; 70.58, H; 3.83, N; 14.53. Found: C; 70.60, H; 3.97, N; 14.53.

Ethyl 1-(4'-nitrophenyl)indole-2-carboxylate 9

In anhydrous DMF (3 mL) was added ethyl indole-2-carboxylate (299 mg, 1.58 mmol), 4-

fluoronitrobenzene (417 mg, 3 mmol), and powdered anhydrous K_2CO_3 (304 mg, 2.2 mmol). The whole was heated at 100 ºC under stirring for 14.5 h. The reaction mixture was poured onto water, and extracted with AcOEt. The organic layer was washed with water, dried over $MgSO_4$, and evaporated to dryness *in vacuo*. The residue (702 mg) was chromatographed over SiO_2 and eluted with toluene to give the target compound **9** (266 mg, 54%). A part of this compound was recrystallized from AcOEt-hexane to give pale yellow plates, mp 133-135 ºC. *Anal*. Calcd for $C_{17}H_{14}N_2O_4$: C, 65.80; H, 4.55; N, 9.03. Found: C, 65.99; H, 4.64; N, 8.76. MS ($C_{17}H_{14}N_2O_4$): 310 (M^+). IR ν max(KBr)cm^{-1}: no NH, 1704 (CO). ^{1}H-NMR δ : 1.29 (3H, t, *J*=8.0 Hz, -$CH_2C\underline{H}_3$), 4.25 (2H, t, *J*=8.0 Hz, -$OC\underline{H}_2CH_3$), 7.13 (1H, d, *J*=1.5 Hz, C_3-H), 7.23-7.36 (3H, m, indolic Hs), 7.52-7.56 (3H, m, $C_{2'}$,$C_{6'}$,an indolic H), 7.76 (1H, d, *J*=9.0 Hz, C_4-H), 8.40 (2H, d, *J*=9.0 Hz, $C_{3'}$,$C_{5'}$-H).

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