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A FACILE SYNTHESIS OF RACEMIC N^{α}-FMOC-2-PHENYLTRYPTOPHAN

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ABSTRACT: A multigram synthesis of racemic N^{α}-Fmoc-2-phenyltryptophan (**6**) from 3-diethylaminomethyl-2-phenylindole (**2**) and ethyl nitroacetate is described.

2-Substituted tryptophans represent a very important class of unnatural amino acids, and have been used in the syntheses of a variety of functionally and structurally modified isopeptides.[1] They have also been prepared to study their serotoninergic activities,[2] or incorporated as structural fragments of polycyclic indoles.[3] 2-Bromo-derivatives of this system are found in numerous sea organisms and obtained synthetically by electrophilic bromination of appropriately protected tryptophans.[4, 5]

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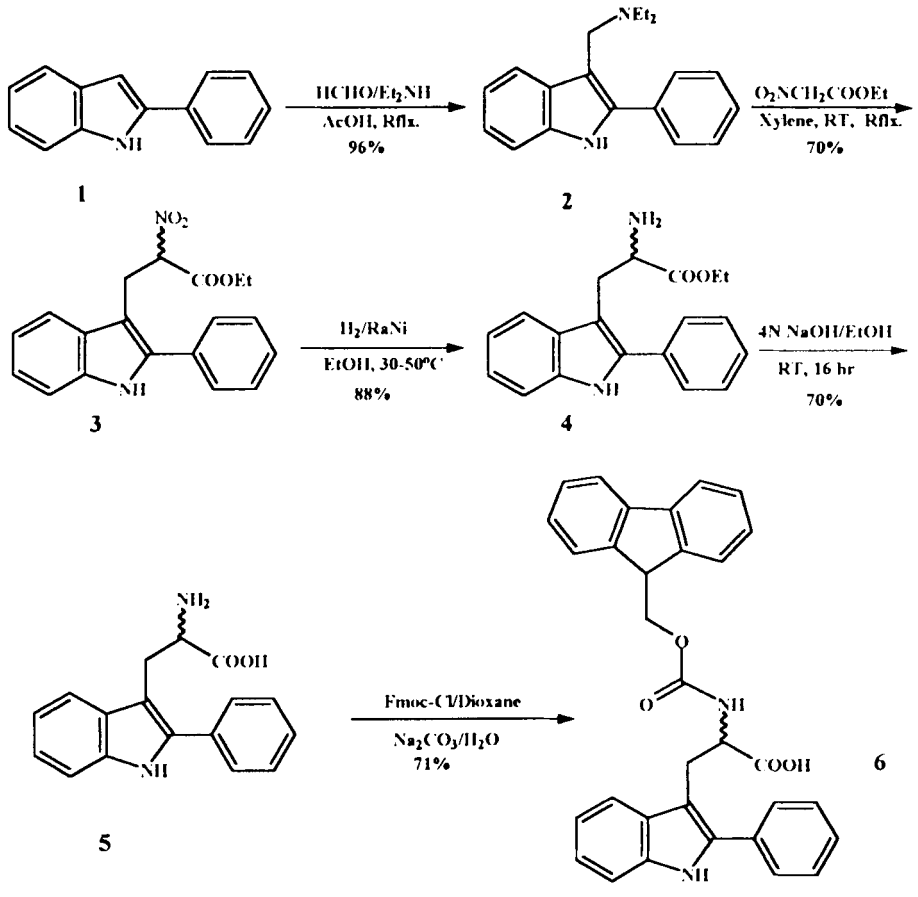
In one of our projects we needed large quantities of N^{α}-Fmoc-2-phenyltryptophan (**6**). For practical purposes we had to develop a synthetic method which would meet the key industrial requirements such as good scaleability and reproducibility. Ideally the material should be produced in a short number of steps, and the substrates should be inexpensive and readily available. Conceptually, the N-unprotected (D,L)-2-phenyltryptophan (**5**) could be synthesized either by, a) transition metal catalyzed cross coupling of an appropriately protected tryptophan with an appropriately substituted benzene, e.g. Suzuki[6] or Stille[7] reactions, or b) introduction of the amino acid functionality onto 2-phenylindole. The second option seemed to meet our requirements more closely since it did not require elaborate protection/deprotection, activation of the 2-position in tryptophan, or both.[1, 3-5] Among those methods,[8-14] nucleophilic substitution of the dialkylamine of 2-phenyl-3-dialkylaminomethylindole[15] (2-phenylgramine) represented the most straightforward approach towards the synthesis of this compound. Variations upon the synthesis of racemic 2-phenyltryptophan (**5**) were described by Kissman and Witkop[16] using the "gramine" method. However, we and others[14] were not able to obtain satisfactory results using any of their routes.

We wish to report a facile, reproducible, and scaleable method for the preparation of 2-phenyltryptophan (**5**) and its N^{α}-Fmoc derivative (**6**). We have found in this laboratory that the condensation of 2-phenylethylgramine (**2**) with ethyl nitroacetate furnishes an intermediate **3** which could be easily and

reproducibly transformed into the title compound (**6**), as shown in the scheme below.

Thus, reaction of ethyl nitroacetate with 3-diethylaminomethyl-2-phenylindole (**2**) in boiling xylene or toluene in the absence of any basic catalyst

SCHEME



gives in a good yield ethyl 2-nitro-3-(2-phenylindol-3-yl)propionate (**3**). In order to prevent the formation of the bis-adduct,[17] the reagents, prior to reflux, were stirred for one hour to complete the formation of the gramine salt of ethyl nitroacetate,[18] and then during reflux the liberated diethylamine was continuously removed by passing a relatively fast stream of argon through the reaction mixture. The reaction was easy to follow by TLC, and usually was complete after refluxing for two hours. In contrast to Kissman's and Witkop's report,[16] catalytic hydrogenation using Raney Nickel as catalyst, but not Pd/C, afforded the amino ester (**5**). The reaction was almost quantitative and went to completion within a short period of time (approximately 3 hr). This intermediate was of sufficient purity to be used in the next step. Stirring of the crude ester **4** in 4N NaOH/EtOH at reflux temperature overnight assured complete hydrolysis. The 2-phenyltryptophan (**5**) was obtained as a white crystalline material after acidification with glacial acetic acid to pH 4.8-5.3. Again, this step rendered highly pure material which could be used in the next step without any further purification. The conversion of **5** to its N^{α}-Fmoc derivative (**6**) was carried out as reported by Carpino and Han.[19] The yields and purity were typically high and the final product was additionally purified by recrystallization from toluene.

In summary, we have developed an efficient, reproducible and easily scaleable synthesis of 2-phenyltryptophan (**5**) and its N^{α}-Fmoc derivative (**6**) consisting of a condensation of 2-phenylethylgramine (**2**) with ethyl nitroacetate

followed by a Raney Nickel catalyzed hydrogenation of purified **3**. The overall yield was approximately 40%. The yield was usually higher (45+%) when the intermediates were used without purification.

EXPERIMENTAL SECTION:

Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. IR spectra were obtained using a Mattson FT IR spectrometer. ^{1}H NMR spectra were recorded on a Brucker 250 MHz spectrometer using TMS as an internal standard. HPLC analyses were performed on a Shimadzu chromatograph, model SPD-10A, equipped with a Beckman Ultrasphere-XL column (C8, 3μm, 4.6 x 70 mm, mobile phase AcCN/H_2O/AcOH-1000:990:10, flow rate 1 ml/min.), and UV detector set at 254 nm. TLC analyses were performed on precoated HPTLC-HLF UV 254 glass plates purchased from Analtech using the indicated solvent systems. Components were visualized under UV light. Microanalyses were performed by the Galbraith Laboratories, Knoxville, TN 37950, USA. All reagents were purchased from Aldrich Chemical Company, Inc. and were used without further purification.

3-Diethylaminomethyl-2-phenylindole (2).

This compound was obtained from 2-phenylindole (**1**) in 96% yield as a thick oil *via* the procedure of Kissman and Witkop.[16]

Ethyl 2-Nitro-3-(2-phenylindol-3-yl)propionate (3).

2 (54.5g, 0.20 mol) and ethyl nitroacetate (26.6g, 0.20 mol) in xylene or toluene (200 mL) under an argon atmosphere were stirred at room temperature. Formation of a white precipitate was observed after approximately 10 minutes. Stirring at this temperature was continued for one hour. Then the argon inlet tube was lowered below the surface level of the solution, the gas flow was increased to a steady stream while heating to reflux. The white solid dissolved and evolution of diethylamine commenced (as monitored with wet pH paper). After approximately two hours at reflux the evolution of diethylamine ceased and the solution became completely clear. TLC (SiO_2, CH_2Cl_2) indicated complete disappearance of the starting material (a characteristically wide fluorescent spot of R_f - 0.20), and a new spot corresponding to the product (R_f - 0.87), appeared. The heating was turned off and a white precipate formed after stirring overnight at room temperature. The solids were filtered off and washed with hexane (2 x 50 mL), to give **3**, 31.2g. The filtrate was concentrated *in vacuo* on a Büchi rotary evaporator and the oily residue was purified by column chroamtography on silica gel using CH_2Cl_2/Hexane (8:2), to give the second crop of **3**, 15.0g. Finally the product was recrystallized from toluene/hexane (8:2), and dried at RT/0.2 mm Hg overnight.

46.2g (68.6%); mp 100 -102°C. Lit.[16] mp 111-112°C.

^{1}H NMR (DMSO-d_6) δ 11.38 (s, 1, NH), 7.70-7.30 (m, 7, Ar), 7.14 and 7.07 (t, J=7.5 Hz, 2, Ar), 5.82 (t, J=7.5 Hz, 1, CH), 3.84 and 3.73 (dd, J=7.5 and 15.0 Hz, 2, CH_2O), 3.95 (m, 2, CH_2).

IR (KBr): 3420, 3030, 2920, 1750 and 1580 cm^{-1}.

The product could also be isolated after removal of solvents *in vacuo* either by a) bringing to crystallization by agitating with spatula of the oily residue in hexane at low temperature, or b) column chroatography SiO_2, *vide supra.*

Ethyl (D,L)-2-Amino-3-(2-phenylindol-3-yl)propionate (4)

To a solution of **3** (32g, 0.095 mol) in ethanol (1.0 L) in a 2 L Parr Shaker bottle was added a 50% aqueous slurry of Raney Nickel (5 scoops, approximately 20g). The bottle was connected to the shaker. The system was purged with argon and hydrogen (3 x to 30 psi), respectively. Finally the system was charged with hydrogen to 50 psi, the shaker was turned on, and the flask was warmed with a heat gun to 50°C. Shaking was continued for one hour then reheated to 50° and shaking was continued until the theoretical amount of hydrogen was absorbed (a pressure drop to 29 psi, approximately 3 hrs). The system was purged with argon and the bottle disconnected. The catalyst was then filtered off ***(Caution the spent catalyst is highly pyrophoric)*** and the filtrate concentrated *in vacuo* on a Büchi. TLC (SiO_2, CH_2Cl_2) indicated complete disappearance of the spot at R_f - 0.87 (starting material) and the appearance of a characteristic new spot of R_f - 0.18. The product has R_f - 0.71 in CH_2Cl_2/MeOH/TEA (8:2:0.15) solvent system. 25.7g (88.1%); viscous oil.

The product was of sufficient purity to be used directly in the next step. It would solidify, however, after standing for a prolonged period of time (approximatelly two weeks), or during agitation. Recrystallization from hexane/ether (9:1) renders material of mp 106-108°C. Lit.[16], mp 110-111°C.

^{1}H NMR (DMSO-d_6) δ 11.19 (s, 1, NH), 7.70 (d, J=7.5 Hz, 2, Ar), 7.60-7.25 (m, 5, Ar), 7.09 and 6.99 (t, J=7.5 Hz, 2, Ar), 3.89-3.70 (m, 2, CH_2O), 3.64 (t, J=7.5 Hz, 1, CH), 3.18 and 3.07 (dd, J=7.5 and 15.0 Hz, 2, CH_2), 1.77 (s, 2, NH_2), 0.94 (t, J=7.5 Hz, Me).

IR (KBr): 3450, 3030, 2940 and 1790 cm^{-1}.

(D,L)-2-Phenyltryptophan (5).

To **4** (24.2g, 0.078 mol) in ethanol (50 mL), sodium hydroxide (16g, 0.4 mol) in water (100 mL) was added. The clear solution was stirred at reflux overnight. TLC (SiO_2, in CH_2Cl_2/MeOH/TEA - 8:2:0.15) indicated complete consumption of the starting material and formation of a new spot at the origin. Water (500 mL), the reaction mixture was extracted with ethyl ether (2 x 50 mL). The aqueous layer was treated with charcoal. Traces of diethyl ether were removed by evaporation *in vacuo* on a Büchi rotoevaporator, (15 minutes at 40°C). The charcoal was removed by filtration through a glass fiber filter. The filtrate was cooled approximately to 5°C and acidified with glacial acetic acid to pH 5.2. A white precipitate formed instantly. The slurry was cooled for two hours in a freezer. The precipitate was filtered off, washed with cold water (3 x 50 mL) and dried *in vacuo* at 40°C.

20.3g (92.3%); grayish -white crystalline powder.

Sample recrystallized from MeOH/toluene (8:2) had a mp 210-215°C (sintering at 185°). Lit.[16] mp 219-222°C (sintering above 195°C).

This material was used in the next step without further recrystallization.

^{1}H NMR (DMSO-d_6) δ 11.24 (s, 1, NH), 7.80-6.80 (m, 9, Ar), 3.60 (m, 2, CH, CH_2), 3.02 (dd, J=8.5 and 13.7 Hz, 1, C$\underline{H_2}$).

IR (KBr): 3620, 3430, 3200-2000, 1620 cm^{-1}.

(D,L)-N^{α}-Fmoc-2-phenyltryptophan (6).

5 (25g, 0.089 mol) and sodium carbonate (23.5g, 0.22 mol) in water (250 mL) were gently heated until a clear solution resulted. The clear solution was coled on ice bath and the fluorenylmethyl chloroformate (Fmoc-Cl), (23g, 0.089 mol) in dioxane (150 mL) was added dropwise over one hour. The cooling bath was removed and the reaction mixture was stirred for three hours. Water (3.0 L) was added, the inslubile material was removed by filtration. The solution was acidified to pH 3 with conc. HCl. The white precipitate that formed was filtered off, washed with cold water (2 x 50 mL), dried *in vacuo* and recrystallized from toluene.

32g (71.5%); mp is not well defined.

^{1}H NMR (DMSO-d_6) δ 11.17 (s, 1, NH), 7.90-6.90 (m, 18, Ar), 4.2 (pq, 1, CH), 4.19 (brs, m, 3, NH, CH), 3.40-3.15 (m, CH_2), (superimosed on the residual water signal).

IR (KBr): 3420, 3040, 2980, 1710 cm^{-1}.

HPLC (peak area) 98.9% purity, retention time 7.5 min.

Elemental Analysis for $C_{32}H_{26}N_2O_4 \cdot ½H_2O$

	Calculated	Found
	%C 75.13	75.55
	%H 5.31	5.43
	%N 5.47	5.44
Karl-Fischer Water	1.47%	
TLC	R_f - 0.9 (SiO_2, CH_2Cl_2/H_2O/TEA - 8:2:0.15).	

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