New Syntheses of Racemic Tryptophans

Urs Hengartner,* Andrew D. Batcho, John F. Blount, Willy Leimgruber, Mary Ellen Larscheid, and John W. Scott

Chemical Research Department, Hoffmann-La Roche Inc., Nutley, New Jersey 07110

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o-Nitrotoluenes are readily converted into the acrolein derivatives **4** and **12**, which are useful intermediates for the preparation of tryptophans. Thus, Michael addition of diethyl formamidomalonate to **4** gives the pyrrolidines **5**, while condensation of **12** with methyl nitroacetate affords the dinitro esters **13**. Both **5** and **13** are reduced catalytically to afford, upon hydrolysis, racemic tryptophans in good to excellent yield.

A continuing interest in tryptophans has led over the years to a variety of syntheses.[1] These are either approaches in which the alanyl side chain is attached to a preformed indole or routes in which the indole nucleus is formed late in the synthetic scheme. The latter methods usually involve cyclization into an aromatic ring (typically a Fischer indole cyclization), leading, in the case of 4- and 6-substituted tryptophans, to mixtures of isomers. In this paper, we describe two new and efficient syntheses of racemic tryptophans with indole formation at the penultimate step to give tryptophans with an unambigous substitution pattern. The compounds of particular interest to us were 6-substituted tryptophans, the D enantiomers of which have potential value as nonnutritive sweeteners.[2]

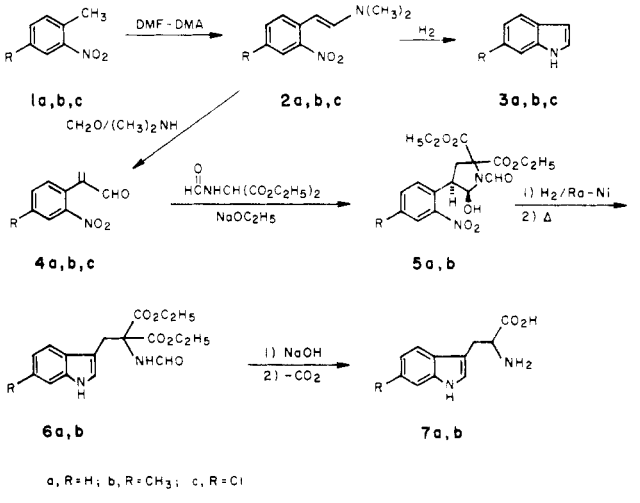
Results and Discussion

In 1971 Batcho and Leimgruber[3] reported a new indole synthesis (Scheme I). *o*-Nitrotoluenes **1** on heating with *N*,*N*-dimethylformamide dimethyl acetal (DMF-DMA) were readily converted to the enamines **2**. Reduction of the nitro group was accompanied by in situ ring closure to give good to excellent yields of a variety of indoles **3**.

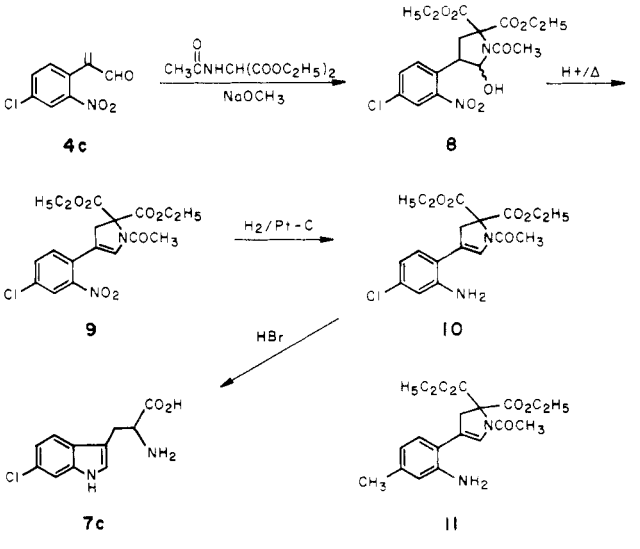
It has now been found that the enamines **2** cleanly undergo Mannich reaction with formaldehyde and dimethylamine in acetic acid/methanol to give the crystalline atropaldehydes **4a–c** in good yields. These compounds proved to be excellent substrates for Michael reaction with acylamidomalonates.[4] Sodium ethoxide catalyzed addition of diethyl formamidomalonate to **4a** proceeded smoothly in ethanol at 5 °C to give the adduct **5a**, which crystallized from the reaction mixture as a single diastereoisomer in 90% yield. The cis configuration of the phenyl and hydroxyl groups was established by an X-ray crystallographic structure determination of **5a**. Catalytic hydrogenation of the nitro group of **5a** with Raney nickel in methanol followed by heating of the crude product in toluene to complete the indole ring closure gave, in 97% yield, the tryptophan precursor **6a** identical with material prepared from indole as described by Hellmann.[5] Basic hydrolysis and decarboxylation[5] gave racemic tryptophan (**7a**) in 64% overall yield from *o*-nitrotoluene (**1a**). Similarly, the commercially available nitroxylene **1b** was converted to 6-methyltryptophan (**7b**)[6,7] in 55% yield.

A variation of this approach was used to convert the atropaldehyde **4c** to racemic 6-chlorotryptophan (**7c**) (Scheme II). Sodium methoxide catalyzed reaction of **4c** with diethyl acetamidomalonate in benzene gave the

Scheme I



Scheme II



(1) For reviews of synthetic routes to tryptophan, see (a) J. P. Greenstein and M. Winitz, "Chemistry of the Amino Acids", Vol. 3, Wiley, New York, 1961, pp 2316–47; (b) T. Kaneko, Y. Izumi, I. Chibata, and T. Itoh, *Synth. Prod. Util. Amino Acids*, 206–19 (1974).

(2) (a) E. C. Kornfeld, J. M. Sheneman, and T. Suarez, German Offen. 1917844 (1969); *Chem. Abstr.*, **72**, 30438 (1970); (b) E. C. Kornfeld, T. Suarez, R. Edie, D. R. Brannon, D. Fukuda, J. Sheneman, G. C. Todd, and M. Secondino, Abstracts, 167th National Meeting of the American Chemical Society, Los Angeles, CA, Apr 1974, Med. Chem. 41.

(3) (a) A. D. Batcho and W. Leimgruber, German Offen. 2057840 (1971); *Chem. Abstr.*, **75**, 63605 (1971); (b) W. Leimgruber and A. D. Batcho, Abstracts of Papers, 3rd International Congress of Heterocyclic Chemistry, Tohoku University, Sendai, Japan, Aug 1971, p 462.

(4) Michael addition of diethyl acylamidomalonate to α,β-unsaturated carbonyl compounds has been reported by, *inter alia*, (a) D. A. Cox, A. W. Johnson, and A. B. Mauger, *J. Chem. Soc.*, 5024 (1964); (b) B. J. Magerlein, *J. Med. Chem.*, **15**, 1255 (1972).

(5) H. Hellmann, *Z. Physiol. Chem.*, **284**, 163 (1949).

(6) H. R. Snyder and F. J. Pilgrim, *J. Am. Chem. Soc.*, **70**, 3787 (1948).

(7) H. N. Rydon, *J. Chem. Soc.*, 705 (1948).

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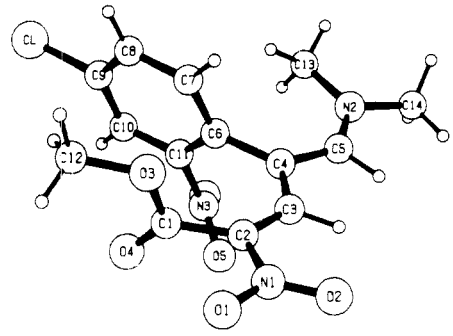
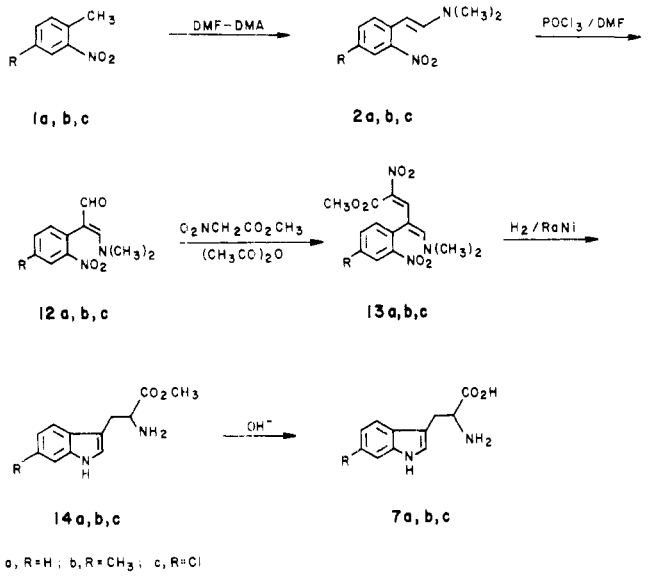


Figure 1. Stereoscopic drawing of **13c**.

Scheme III



hydroxypyrrolidine **8** which dehydrated readily in the presence of *p*-toluenesulfonic acid to give the nitropyrroline **9** in 95% yield. Hydrogenation of **9** over sulfided platinum on carbon in methanol/tetrahydrofuran proceeded without loss of chlorine and afforded the aminopyrroline **10** in 80% yield. Hydrolysis, decarboxylation and rearrangement of **10** were effected in one pot with 2 N hydrobromic acid at 95 °C to give 6-chlorotryptophan (**7c**)[8] in 60% overall yield from **1c**. It should be noted, however, that the last transformation (**10** → **7c**) is not generally applicable; when we attempted to prepare 6-methyltryptophan (**7b**) by this modified route, we found that acid hydrolysis of **11** led to partial destruction of the molecule. Although we were finally able to convert **11** to **7b** by a stepwise process (50% yield) of saponification of the ester groups, decarboxylation, and basic amide hydrolysis, we consider the route outlined in Scheme I to be superior.

Our second approach to use the enamines **2a–c** as tryptophan precursors is shown in Scheme III. Vilsmeier formylation of the enamines **2a–c** with phosphorus oxychloride and dimethylformamide gave, after basic workup, the 3-(dimethylamino)acroleins **12a–c** in good yield. Although these compounds are homogeneous, the configuration of the double bond is not established. Reaction of **12a–c** with methyl nitroacetate in acetic anhydride at 80–95 °C gave the crystalline dienes **13a–c**. TLC and NMR analysis indicated that only one of the four possible double bond isomers was formed. The configuration shown was established by an X-ray crystallographic analysis of **13c**. A stereoscopic drawing of compound **13c** is given in Figure 1. A planar conjugated system exists from the nitro to the dimethylamino group; presumably for steric reasons, the carbomethoxy and aromatic groups are out of the plane and thus out of conjugation. In addition, the aromatic nitro group is twisted out of the plane of the phenyl ring.

The conversion of the dienes **13a–c** to the tryptophan methyl esters **14a–c** required reduction of the two nitro groups and one of the double bonds with concomitant formation of the indole nucleus. This was accomplished by high-pressure hydrogenation with Raney nickel in methanol/tetrahydrofuran.

The crude esters **14a–c** were saponified to afford racemic tryptophans **7a–c** in 40–43% yield from the nitrotoluenes **1a–c**. Although the overall yields are lower for this route than for the other synthetic sequence, it proved to be very convenient for the rapid preparation of a variety of racemic tryptophans.

Experimental Section

General Methods. Melting points were taken on a Thomas-Hoover capillary melting point apparatus and are not corrected. Spectral measurements were taken by members of the Physical Chemistry Department of Hoffmann-La Roche using the following instruments: ^{1}H NMR spectra were recorded on Varian A-60 and HA-100 instruments and are reported in parts per million downfield from internal tetramethylsilane. Infrared spectra were obtained on a Beckman IR-9 or Digilab FTS-14 spectrometer. Ultraviolet spectra were recorded on a Cary Model 14 spectrophotometer. Mass spectra were obtained on Jeolco OISG or CEC 21-110 instruments. Elemental analyses were carried out under the supervision of Dr. F. Scheidl of our Microanalytical Laboratory.

2-(2-Nitrophenyl)acrolein (4a). A stirred mixture of 50 g (0.365 mol) of 2-nitrotoluene (**1a**), 56.0 g (0.47 mol) of *N,N*-dimethylformamide dimethyl acetal, and 200 mL of *N,N*-dimethylformamide was heated under nitrogen for 32 h, the pot temperature being raised gradually from 130 to 150 °C while the methanol formed during the reaction was removed by continuous distillation through a Vigreux column. The NMR spectrum of a sample verified the complete conversion of the starting 2-nitrotoluene. The dark red solution of the enamine **2a**[3] was added at 4 °C over 1 h to a mixture of 58 g (0.52 mol) of 40% aqueous dimethylamine, 120 g (1.48 mol) of 37% aqueous formaldehyde, 90 g of acetic acid, and 300 mL of methanol.

The mixture was stirred at 4 °C for 30 min, and then the methanol was removed in vacuo on a rotary evaporator. A 1.5-L sample of water was added at 10 °C over 1 h and the suspension stirred at 4 °C for 2 h. The crystalline precipitate was collected, washed with water, and dried to afford 55.8 g (86%) of atropaldehyde **4a** as brown crystals, mp 52.5–54 °C. The analytical sample was prepared by recrystallization from ethanol, followed by evaporative distillation at 120 °C (0.1 mm): white crystals, mp 54.5–56 °C; IR ($CHCl_3$) 1708, 1533, 1354 cm^{-1}; NMR ($CDCl_3$) δ 6.36 (s, 1), 6.53 (s, 1), 7.2–8.1 (m, 4), 9.64 (s, 1); mass spectrum, *m/e* 177 (M^+).

Anal. Calcd for $C_9H_7NO_3$: C, 61.02; H, 3.98; N, 7.91. Found: C, 61.24; H, 4.06; N, 8.15.

2-(4-Methyl-2-nitrophenyl)acrolein (4b). 2-Nitro-1,4-dimethylbenzene (**1b**; 45.4 g, 0.30 mol) was processed in the manner

(8) H. N. Rydon and J. C. Tweedle, *J. Chem. Soc.*, 3499 (1955).

described above to give, after recrystallization from ethanol, 46.0 g (80%) of **4b**, mp 58.5–60 °C. A sample was recrystallized from ethanol and evaporatively distilled at 105 °C (0.05 mm): white crystals, mp 59–61 °C; IR ($CHCl_3$) 1705, 1540, 1353 cm^{-1}; NMR ($CDCl_3$) δ 2.43 (s, 3), 6.29 (s, 1), 6.48 (s, 1), 7.16 (d, 1, J = 8 Hz), 7.45 (d, 1, J = 8 Hz), 7.88 (s, 1), 9.63 (s, 1); mass spectrum, m/e 191 (M^+).

Anal. Calcd for $C_{10}H_9NO_3$: C, 62.83; H, 4.75; N, 7.33. Found: C, 62.91; H, 4.63; N, 7.35.

2-(4-Chloro-2-nitrophenyl)acrolein (4c). 4-Chloro-2-nitrotoluene (**1c**; 126 g, 0.734 mol) was processed in the manner described above to give 144.3 g (93%) of **4c**, mp 87–88 °C. A sample was recrystallized from chloroform/hexane and evaporatively distilled at 100 °C (0.05 mm): white crystals, mp 88.5–90 °C; IR ($CHCl_3$) 1705, 1535, 1350 cm^{-1}; NMR ($CDCl_3$) δ 6.42 (s, 1), 6.58 (s, 1), 7.26 (d, 1, J = 8 Hz), 7.68 (dd, 1, J = 8 and 2 Hz), 8.12 (d, 1, J = 2 Hz), 9.71 (s, 1); mass spectrum, m/e 211 (M^+).

Anal. Calcd for $C_9H_6ClNO_3$: C, 51.08; H, 2.86; N, 6.62; Cl, 16.75. Found: C, 51.29; H, 3.02; N, 6.63; Cl, 16.83.

Diethyl *cis*-1-Formyl-5-hydroxy-4-(2-nitrophenyl)-pyrrolidine-2,2-dicarboxylate (5a). To a stirred, ice bath cooled mixture of 42.5 g (0.24 mol) of atropaldehyde **4a** and 50.4 g (0.25 mol) of diethyl formamidomalonate in 200 mL of dry ethanol was added over 15 min 4 mL of a 1 M solution of sodium ethoxide in ethanol. The mixture was stirred at 5 °C for 1 h as the Michael adduct **5a** crystallized. After the suspension had been stored for 4 h at −20 °C, the product was collected by filtration, washed with ethanol (−60 °C), and dried to yield 82.4 g (90%) of off-white crystals, mp 109–111 °C. The analytical sample was prepared by recrystallization from ethanol: white crystals, mp 113–115 °C; IR ($CHCl_3$) 3580, 1747, 1675, 1530, 1354 cm^{-1}; NMR ($CDCl_3$) δ 1.32 (t, 6, J = 7 Hz), 2.8–3.3 (m, 2), 3.7–4.6 (m, 6), 5.81 (t, 0.15, J = 4 Hz) and 5.99 (t, 0.85, J = 4 Hz) (CHOH, 5:1 mixture of rotamers; 2 doublets, J = 4 Hz, after addition of D_2O), 7.3–7.9 (m, 4 H), 8.46 (s, 1 H).

Anal. Calcd for $C_{17}H_{20}N_2O_8$: C, 53.68; H, 5.30; N, 7.36. Found: C, 53.62; H, 5.38; N, 7.36.

The same reaction carried out in benzene with sodium ethoxide gave a mixture of epimers (NMR: singlets at δ 8.46 and 8.53, CHO).

Diethyl *cis*-1-Formyl-5-hydroxy-4-(4-methyl-2-nitrophenyl)pyrrolidine-2,2-dicarboxylate(5b). This compound was prepared by the above described procedure for **5a**. Base-catalyzed addition of 48.9 g (0.24 mol) of diethyl formamidomalonate to 46.0 g (0.24 mol) of atropaldehyde **4b** afforded 88.0 g (93%) of **5b**, mp 113–114 °C. The analytical sample was prepared by recrystallization from ethanol: white crystals, mp 120–122 °C; IR ($CHCl_3$) 3580, 1746, 1673, 1534, 1355 cm^{-1}; NMR ($CDCl_3$) δ 1.29 (t, 6, J = 7 Hz), 2.37 (s, 3), 2.7–3.2 (m, 2), 3.6–3.9 (m, 1), 4.06 (d, 1, J = 4 Hz, OH), 4.24 and 4.28 (2 q, 4, J = 7 Hz), 5.76 (t, 0.15, J = 4 Hz) and 5.96 (t, 0.85, J = 4 Hz) (5:1 mixture of rotamers), 7.32 (d, 1, J = 8 Hz), 7.48 (d, 1, J = 8 Hz), 7.64 (s, 1), 8.42 (s, 1).

Anal. Calcd for $C_{18}H_{22}N_2O_8$: C, 54.82; H, 5.62; N, 7.10. Found: C, 54.98; H, 5.56; N, 7.10.

Ethyl 2-Formamido-3-(3-indolyl)-2-carbethoxypropionate (6a). Compound **5a** (50.0 g, 0.13 mol) in 500 mL of methanol and 1 mL of acetic acid was hydrogenated in a rocking autoclave at room temperature and 200 psi in the presence of 6 g of Raney nickel. After the hydrogen uptake (3 equiv) was complete, the filtered reaction mixture was concentrated in vacuo to a volume of 140 mL. Toluene (600 mL) was added, and the remaining methanol was removed by azeotropic distillation. The mixture was heated at reflux for an additional 30 min and then allowed to cool to room temperature. The precipitate was collected, washed with toluene, and dried to yield 42.3 g (97%) of white crystals, mp 179–180 °C (lit.[5] mp 179 °C). The analytical sample was prepared by recrystallization from ethanol: mp 180–181 °C; IR (KBr) 3370, 3318, 1745 cm^{-1}; NMR (Me_2SO-d_6) δ 1.13 (t, 6, J = 7 Hz), 3.60 (s, 2), 4.05 (q, 4, J = 7 Hz), 6.8–7.5 (m, 5), 7.87 (s, 1), 8.33 (br s, 1), 10.69 (br s, 1); UV max (2-PrOH) 221 nm (ϵ 36 700), 273 (5700), 281 (6270), 289 (5580).

Anal. Calcd for $C_{17}H_{20}N_2O_5$: C, 61.44; H, 6.07; N, 8.43. Found: C, 61.51; H, 6.10; N, 8.39.

Ethyl 2-Formamido-3-(6-methyl-3-indolyl)-2-carbethoxypropionate (6b). Compound **5b** (88.0 g, 0.22 mol) in 800 mL of methanol and 1 mL of acetic acid was hydrogenated in the presence of 8 g of Raney nickel. The mixture was concentrated in vacuo, and the solid residue was taken up in benzene (460 mL) and heated at reflux for 2 h as the water formed was removed with a Dean–Stark apparatus. Hexane (390 mL) was added, and the mixture was allowed to stand at room temperature overnight. The precipitate was collected, washed with benzene/hexane (1:2) and dried to give 69.5 g (90%) of white crystals, mp 133–134.5 °C (sinters at 113 °C). The analytical sample was prepared by recrystallization from benzene/hexane: mp 114–116 °C (recrystallization from ethanol/water gave crystals with mp 135–136 °C); IR ($CHCl_3$) 3480, 3405, 1740, 1690 cm^{-1}; NMR ($CDCl_3$) δ 1.20 (t, 6, J = 7 Hz), 2.38 (s, 3), 3.82 (s, 2), 4.20 (q, 4, J = 7 Hz), 6.8–7.5 (m, 5), 8.11 (s, 1), 8.33 (br s, 1); UV max (2-PrOH) 223 nm (ϵ 39 300), 276 (5700), 282 (5980), 293 (5220).

Anal. Calcd for $C_{18}H_{22}N_2O_5$: C, 62.42; H, 6.40; N, 8.09. Found: C, 62.51; H, 6.34; N, 8.08.

Tryptophan (7a). A mixture of 40 g (0.12 mol) of compound **6a** and 24.0 g (0.60 mol) of sodium hydroxide in 240 mL of water was heated at reflux for 18 h. A 48-mL sample of glacial acetic acid was added to the solution, and heating was continued for an additional 8 h. The suspension was allowed to stand at 0 °C overnight, and the white precipitate was collected, washed with ice-cold water, and dried to yield 24.7 g (100%) of crude **7a**. Recrystallization from 80% aqueous acetic acid afforded, after drying at 100 °C (0.05 mm), 21.2 g (86%) of white crystals, mp 292 °C dec (lit.[5] mp 293 °C), which was identical (UV, IR, NMR, TLC) with an authentic sample (Eastman).

Anal. Calcd for $C_{11}H_{12}N_2O_2$: C, 64.69; H, 5.92; N, 13.72. Found: C, 64.86; H, 6.06; N, 13.86.

6-Methyltryptophan (7b). Compound **6b** (68.5 g, 0.20 mol) was converted in the same manner to give 35.8 g (83%) of recrystallized **7b** as white crystals, mp 298 °C dec (lit.[6] mp 298–300 °C), which was identical (UV, IR, NMR, TLC) with an authentic sample (Fluka).

Anal. Calcd for $C_{12}H_{14}N_2O_2$: C, 66.04; H, 6.47; N, 12.84. Found: C, 66.22; H, 6.52; N, 12.95.

Diethyl 1-Acetyl-4-(4-chloro-2-nitrophenyl)-2,3-dihydro-1*H*-pyrrole-2,2-dicarboxylate (9). A solution of 143.0 g (0.676 mol) of 2-(4-chloro-2-nitrophenyl)acrolein (**4c**) in 600 mL of benzene was added over 90 min to a stirred suspension of 148.5 g (0.684 mol) of diethyl acetamidomalonate and 0.80 g of sodium methoxide in 600 mL of benzene. After the mixture was stirred for an additional 30 min, 3.8 g of *p*-toluenesulfonic acid monohydrate was added, and the mixture was heated at reflux for 40 min, using a Dean–Stark apparatus to remove the water. The orange benzene solution was washed with saturated $NaHCO_3$ and brine, dried with anhydrous Na_2SO_4, and concentrated in vacuo to yield 278.5 g of **9**, mp 90–95 °C. Recrystallization from ethanol (500 mL) gave 264.6 g (95%) of deep yellow crystals, mp 96–98 °C. A sample was recrystallized again from ethanol: mp 96.5–98 °C; IR ($CHCl_3$) 1743, 1727, 1670, 1528 cm^{-1}; NMR ($CDCl_3$) δ 1.36 (t, 6, J = 7 Hz), 2.24 (s, 3), 3.43 (d, 2, J = 1.5 Hz), 4.32 (q, 4, J = 7 Hz), 6.74 (br s, 1), 7.36 (d, 1, J = 8 Hz), 7.57 (dd, 1, J = 8 and 2 Hz), 7.83 (d, 1, J = 2 Hz); UV max (2-PrOH) 215 nm (ϵ 17 600), 268 (13 080), 285 infl (12 200), 349 (2850); mass spectrum, m/e 410 (M^+).

Anal. Calcd for $C_{18}H_{19}N_2O_7Cl$: C, 52.63; H, 4.66; N, 6.82; Cl, 8.63. Found: C, 52.82; H, 4.50; N, 6.94; Cl, 8.49.

Diethyl 1-Acetyl-4-(2-amino-4-chlorophenyl)-2,3-dihydro-1*H*-pyrrole-2,2-dicarboxyate (10). A solution of 82.2 g (0.20 mol) of compound **9** in 300 mL of tetrahydrofuran and 700 mL of methanol was hydrogenated in the presence of 4 g of 5% sulfided platinum on carbon (Engelhardt) at room temperature and 1500 psi for 22 h. The reaction mixture was concentrated, and the solid residue (76 g) was recrystallized from ethanol (320 mL) to afford 61.4 g (81%) of light orange crystals, mp 151.5–153 °C. The analytical sample was prepared by a second recrystallization from ethanol: white crystals, mp 152–153 °C; IR ($CHCl_3$) 1750, 1735, 1675, 1625 cm^{-1}; NMR ($CDCl_3$) δ 1.30 (t, 6, J = 7 Hz), 2.23 (s, 3), 3.51 (d, 2, J = 2 Hz), 4.00 (br s, 2), 4.28 (q, 4, J = 7 Hz), 6.6–7.1 (m, 4); UV max (2-PrOH) 211 nm (ϵ 18 990), 244 (22 700), 282 (14 600), 293 (13 120), 325 (11 220); mass spectrum, m/e 380 (M^+).

Anal. Calcd for $C_{18}H_{21}N_2O_5Cl$: C, 56.77; H, 5.56; N, 7.36; Cl, 9.31. Found: C, 57.06; H, 5.61; N, 7.40; Cl, 9.43.

6-Chlorotryptophan (7c). A mixture of 60 g (0.16 mol) of compound **10** and 450 mL of 2 N aqueous HBr was heated under nitrogen at 95 °C for 30 h. The solution was concentrated in vacuo and the residual salt was taken up in 300 mL of water. The solution was brought to pH 5.5 with 4 N NaOH, and the mixture was chilled in an ice bath for 1 h. The precipitate was collected, washed with cold water, and dried to give 34.8 g of **7c**. Recrystallization from glacial acetic acid (360 mL) afforded, after drying at 100 °C (0.2 mm), 30.8 g (82%) of white crystals, mp 280 °C dec (lit.[8] mp 285–286 °C), which was identical (UV, IR, NMR, TLC) with an authentic sample.[8]

Anal. Calcd for $C_{11}H_{11}N_2O_2Cl$: C, 55.36; H, 4.65; N, 11.74; Cl, 14.85. Found: C, 55.62; H, 4.66; N, 11.91; Cl, 14.78.

3-(Dimethylamino)-2-(4-chloro-2-nitrophenyl)acrolein (12c). A stirred mixture of 53.8 g (0.31 mol) of 4-chloro-2-nitrotoluene (**1c**), 50.0 g (0.42 mol) of *N,N*-dimethylformamide dimethyl acetal, and 175 mL of *N,N*-dimethylformamide was heated under nitrogen for 22 h at 135 °C, while the methanol formed was removed by continuous distillation through a Vigreux column. At the end of the reaction period the temperature was raised to 155 °C. The dark red solution of the enamine **2c**[3] was then added at 10–15 °C over 30 min to a mixture of 62 g (0.40 mol) of phosphorus oxychloride and 145 mL of *N,N*-dimethylformamide (prepared by adding the acid chloride slowly at 15 °C to the dimethylformamide). The orange solution was stirred at room temperature for 1 h. A 1-kg sample of ice was then added, followed by a solution of 80 g (2.0 mol) of sodium hydroxide in 300 mL of water. The mixture was heated at 60 °C for 20 min and allowed to stand at room temperature overnight. The precipitate was collected, washed with water, and dried to afford 73.1 g (92%) of **12c** as orange crystals, mp 145–147 °C. The analytical sample was prepared by recrystallization from benzene: light orange crystals, mp 146–147.5 °C; IR ($CHCl_3$) 1610, 1595 cm^{-1}; NMR ($CDCl_3$) δ 2.87 (br s, 6), 6.98 (s, 1), 7.16 (d, 1, J = 8 Hz), 7.51 (dd, 1, J = 8 and 2 Hz), 7.87 (d, 1, J = 2 Hz), 8.90 (s, 1); mass spectrum, m/e 254 (M^+); UV max (2-PrOH) 287 nm (ϵ 27 620).

Anal. Calcd for $C_{11}H_{11}N_2O_3Cl$: C, 51.88; H, 4.35; N, 11.00; Cl, 13.92. Found: C, 51.86; H, 4.43; N, 10.87; Cl, 13.84.

3-(Dimethylamino)-2-(2-nitrophenyl)acrolein (12a). A mixture of 50.0 g (0.36 mol) of 2-nitrotoluene (**1a**), 56.0 g (0.47 mol) of *N,N*-dimethylformamide dimethyl acetal, and 190 mL of *N,N*-dimethylformamide was heated under nitrogen for 22 h at 140–145 °C, while the methanol formed was continuously removed by distillation. The resulting solution of **2a** was added at 10–15 °C over 30 min to a mixture of 71.3 g (0.46 mol) of phosphorus oxychloride and 165 mL of *N,N*-dimethylformamide. After the mixture had been stirred for 1 h at room temperature, 1.1 kg of ice was added, followed by a solution of 92 g (2.3 mol) of sodium hydroxide in 345 mL of water. The mixture was heated at 60 °C for 2 h and then refrigerated overnight. The precipitate was collected, washed with water, and dried to yield 65.0 g (81%) of **12a** as orange crystals, mp 116–118.5 °C (lit.[9] mp 116–119 °C). The analytical sample was prepared by recrystallization from benzene: mp 116.5–118.5 °C; IR ($CHCl_3$) 1610, 1590 cm^{-1}; NMR ($CDCl_3$) δ 2.80 (br s, 6), 6.93 (s, 1), 7.2–7.9 (m, 4), 8.90 (s, 1); UV max (EtOH) 289 nm (ϵ 28 600).

Anal. Calcd for $C_{11}H_{12}N_2O_3$: C, 60.00; H, 5.49; N, 12.72. Found: C, 59.78; H, 5.67; N, 12.56.

3-(Dimethylamino)-2-(4-methyl-2-nitrophenyl)acrolein (12b). 2-Nitro-1,4-dimethylbenzene (**1b**; 50.0 g, 0.33 mol) was processed in a similar manner, affording 67.9 g (88%) of **12b** as yellow-orange crystals, mp 117–119 °C. The analytical sample was prepared by recrystallization from ethanol: mp 119–121 °C; IR ($CHCl_3$) 1605 cm^{-1}; NMR ($CDCl_3$) δ 2.38 (s, 3), 2.83 (br s, 6), 6.94 (s, 1), 7.08 (d, 1, J = 8 Hz), 7.34 (d, 1, J = 8 Hz), 7.68 (s, 1), 8.90 (s, 1); mass spectrum, m/e 234 (M^+); UV max (EtOH) 288 nm (ϵ 28 200).

Anal. Calcd for $C_{12}H_{14}N_2O_3$: C, 61.53; H, 6.02; N, 11.96. Found: C, 61.68; H, 5.83; N, 12.02.

Methyl (2*E*,4*Z*)-5-(Dimethylamino)-2-nitro-4-(4-chloro-2-nitrophenyl)-2,4-pentadienoate (13c). A mixture of 73.1 g (0.29 mol) of **12c**, 34.0 g (0.29 mol) of methyl nitroacetate[10] and 600 mL of acetic anhydride was stirred and heated at 90–95 °C for 45 min. The reaction mixture was allowed to cool to room temperature, and the crystalline product was collected by filtration. The filtrate was concentrated in vacuo, and the residue was slurried with ether (200 mL) and filtered. The combined materials were washed with ether and dried to give 91.0 g (89%) of red-orange crystals, mp 193–197 °C. Recrystallization from benzene/*N,N*-dimethylformamide (6:1; 1725 mL) afforded 78.8 g (77%) of **13c**, mp 198–200 °C; IR (KBr) 1725, 1625 cm^{-1}; NMR (Me_2SO-d_6) δ 2.88 (br s, 6), 3.15 (s, 3), 7.33 (d, 1, J = 8 Hz), 7.75 (dd, 1, J = 8 and 2 Hz), 7.98 (s, 2), 8.10 (d, 1, J = 2 Hz); mass spectrum, m/e 355 (M^+); UV max (EtOH) 219 nm (ϵ 18 400), 250 infl (11 400), 436 (39 400).

Table I. Crystal Data

	5a	13c
formula	$C_{17}H_{20}N_2O_8$	$C_{14}H_{14}ClN_3O_6$
fw	380.35	355.73
space group	$P\bar{1}$	$P\bar{1}$
a, Å	9.078 (2)	11.895 (6)
b, Å	10.465 (1)	12.213 (12)
c, Å	10.714 (1)	12.350 (6)
α, deg	98.34 (1)	109.46 (6)
β, deg	98.96 (1)	107.02 (3)
γ, deg	108.94 (1)	93.41 (8)
Z	2	4
d_{calcd}, g cm^{-3}	1.358	1.483
μ(Cu Kα), cm^{-1}	9.4	24.8

Anal. Calcd for $C_{14}H_{14}N_3O_6Cl$: C, 47.27; H, 3.97; N, 11.81; Cl, 9.97. Found: C, 47.34; H, 4.09; N, 11.81; Cl, 9.96.

Methyl (2*E*,4*Z*)-5-(Dimethylamino)-2-nitro-4-(2-nitrophenyl)-2,4-pentadienoate (13a). A mixture of 63.0 g (0.29 mol) of **12a** and 35.7 g (0.30 mol) of methyl nitroacetate in 600 mL of acetic anhydride was heated at 85 °C for 2 h. Workup as described above and recrystallization from benzene/*N,N*-dimethylformamide (2:1) afforded 74.9 g (81%) of **13a** as red-orange crystals, mp 211–214 °C; IR (KBr) 1710, 1625 cm^{-1}; NMR (Me_2SO-d_6) δ 2.84 (br s, 6), 3.08 (s, 3), 7.3–8.2 (m, 6); mass spectrum, m/e 321 (M^+); UV max (EtOH) 250 nm (ϵ 12 000), 438 (43 600).

Anal. Calcd for $C_{14}H_{15}N_3O_6$: C, 52.33; H, 4.71; N, 13.08. Found: C, 52.50; H, 4.79; N, 13.07.

Methyl (2*E*,4*Z*)-5-(Dimethylamino)-2-nitro-4-(4-methyl-2-nitrophenyl)-2,4-pentadienoate (13b). A mixture of 67.5 g (0.29 mol) of **12b** and 34.3 g (0.29 mol) of methyl nitroacetate in 540 mL of acetic anhydride was heated at 60 °C for 2 h. Workup as described above and recrystallization from benzene/*N,N*-dimethylformamide (3:1) afforded 75.1 g (78%) of **13b** as red-orange crystals, mp 213–215 °C; IR (KBr) 1735, 1725, 1630 cm^{-1}; NMR (Me_2SO-d_6) δ 2.41 (s, 3), 2.8 (br s, 6), 3.03 (s, 3), 7.16 (d, 1, J = 8 Hz), 7.48 (d, 1, J = 8 Hz), 7.85 (s, 1), 7.95 (s, 1), 7.99 (s, 1); mass spectrum, m/e 335 (M^+); UV max (EtOH) 252 nm (ϵ 11 700), 439 (43 000).

Anal. Calcd for $C_{15}H_{17}N_3O_6$: C, 53.73; H, 5.11; N, 12.53. Found: C, 53.93; H, 4.99; N, 12.65.

6-Chlorotryptophan (7c). A mixture of 20.0 g (0.056 mol) of compound **13c** and 8 g of Raney nickel in 250 mL of methanol and 250 mL of tetrahydrofuran was hydrogenated in a rocking autoclave at 1500 psi for 22 h. The filtered solution was concentrated in vacuo and the crude 6-chlorotryptophan methyl ester **14c** (14.4 g, ca. 70% pure) was dissolved in 75 mL of methanol. Sodium hydroxide (4.5 g) was added, and the mixture was stirred at 50 °C for 3 h. The brown solution was again concentrated, the solid residue was taken up in 50 mL of water, 125 mL of ethyl acetate was added, and the two-phase system was stirred while the aqueous layer was adjusted to pH 5.5 with acetic acid. The mixture was allowed to stand at 0 °C overnight. The precipitate was collected, washed with ethyl acetate (100 mL), ether (50 mL), and ice-cold water (50 mL), and dried to give 8.8 g of crude **7c**. GC analysis of its tris(trimethylsilyl) derivative confirmed the absence of tryptophan. Recrystallization from glacial acetic acid yielded, after drying at 120 °C (0.1 mm), 7.80 g (58%) of off-white crystals, mp 278 °C dec (lit.[8] mp 285–286 °C), which was identical

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(10) S. Sifniades, U.S. Patent 3 761 510 (1973); *Chem. Abstr.*, **80**, 14 551 (1974); S. Sifniades, *J. Org. Chem.*, **40**, 3562 (1975).

Table II. Experimental Details

	5a	13c
cryst size, mm	0.05 × 0.20 × 0.5	0.12 × 0.20 × 0.30
max θ, deg	57	57
no. of rflctns	2506	4316
no. of obsd rflctns	1641	3199
abs cor	none	none
least squares	full matrix	block diagonal (two blocks)
refinement	anisotropic	anisotropic
heavier atoms	isotropic (fixed)	isotropic (fixed)
hydrogen atom	0.081	0.048
final R	0.085	0.047
final R_w	0.3	0.2
final difference ma--largest peak, e Å^{-3}		

(UV, IR, NMR, TLC) with an authentic sample.[8]

Anal. Calcd for $C_{11}H_{11}N_2O_2Cl$: C, 55.36; H, 4.65; N, 11.74; Cl, 14.85. Found: C, 55.45; H, 4.60; N, 11.64; Cl, 14.87.

Tryptophan (7a). Compound **13a** (20 g, 0.062 mol) was hydrogenated and worked up as described above to yield 7.98 g (63%) of recrystallized **7a**, mp 291 °C dec (lit.[5] mp 293 °C), which was identical (UV, IR, NMR, TLC) with an authentic sample (Eastman).

Anal. Calcd for $C_{11}H_{12}N_2O_2$: C, 64.69; H, 5.92; N, 13.72. Found: C, 64.64; H, 5.86; N, 13.67.

6-Methyltryptophan (7b). Compound **13b** (70 g, 0.21 mol) was hydrogenated and worked up as described above to yield 27.6 g (61%) of recrystallized **7b**, mp 297 °C dec (lit.[6] mp 298–300 °C), which was identical (UV, IR, NMR, TLC) with an authentic sample (Fluka).

Anal. Calcd for $C_{12}H_{14}N_2O_2$: C, 66.04; H, 6.47; N, 12.84. Found: C, 66.08; H, 6.62; N, 12.88.

Crystallography. Crystals of **5a** were prepared from ethanol, and the crystals of **13c** were obtained from *N,N*-dimethylformamide/benzene. Crystal data for **5a** and **13c** are listed in Table I. The intensity data were measured on a Hilger-Watts four-circle diffractometer (Ni filtered Cu Kα radiation, θ–2θ scans, pulse-height discrimination). Both structures were solved by a multiple-solution procedure.[11] Details of the analyses are summarized in Table II.

Registry No. **1a**, 88-72-2; **1b**, 89-58-7; **1c**, 89-59-8; **2a**, 32991-03-0; **2c**, 32989-56-3; **4a**, 71463-16-6; **4b**, 71463-17-7; **4c**, 71463-18-8; **5a**, 71463-19-9; **5b**, 71463-20-2; **6a**, 64258-95-3; **6b**, 71463-21-3; **7a**, 54-12-6; **7b**, 2280-85-5; **7c**, 17808-21-8; **9**, 71463-22-4; **10**, 71463-23-5; **12a**, 53868-36-3; **12b**, 70082-60-9; **12c**, 71463-24-6; **13a**, 71463-25-7; **13b**, 71463-26-8; **13c**, 71463-27-9; **14c**, 71463-28-0; *N,N*-dimethylformamide dimethyl acetal, 4637-24-5; diethyl formamidomalonate, 6326-44-9; methyl nitroacetate, 2483-57-0.

Supplementary Material Available: Tables of the final atomic parameters, bond lengths, and bond angles for compounds **5a** and **13c** (8 tables, 9 pages). Ordering information is given on any current masthead page.

(11) G. Germain, P. Main, and M. M. Woolfson, *Acta Crystallogr., Sect. A*, **27**, 368 (1971).

An Improved Synthesis of Agaritine[1]

Lawrence Wallcave,* Donald L. Nagel,* Chitta R. Raha, Hwan-Soo Jae, Susan Bronczyk, Robert Kupper, and Bela Toth

Eppley Institute, University of Nebraska Medical Center, Omaha, Nebraska, 68105

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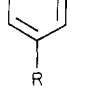
L-Glutamic acid 5-[2-[4-(hydroxymethyl)phenyl]hydrazide] (agaritine, **4a**), a compound present in *Agaricus bisporus*, the commercial edible mushroom, was synthesized for the bioassay of its possible tumorigenic properties. The mixed anhydride derived from 1-benzyl *N*-(benzyloxycarbonyl)-L-glutamate and ethyl chloroformate reacted with 4-carboxyphenylhydrazine (**1a**) to form the benzyl ester of *N*-(benzyloxycarbonyl)-L-glutamic acid 5-[2-(4-carboxyphenyl)hydrazide] (**3**). Reduction of **3** with BH_3/THF gave the corresponding 4-(hydroxymethyl)phenyl derivative (**5a**) which on hydrogenolysis in THF over Pd/C gave **4a**. The overall yield from **1a** was 25%, some 25-fold higher than previously obtained.

The amino acid L-glutamic acid 5-[2-[4-(hydroxymethyl)phenyl]hydrazide] (**4a**), called agaritine by its discoverer,[2] is a constituent of edible mushrooms classified as *Agaricus bisporus*. These are the ordinary mushrooms of commerce in the Western hemisphere. Because hydrazine and many of its derivatives have marked physiological activity, including the ability to induce cancers in laboratory animals,[3] a bioassay of agaritine for potential tumorigenic activity is presently underway in this Institute. More than 1 kg of agaritine may be required during the course of the bioassay and it seemed desirable to obtain these quantities by synthesis rather than by isolation from mushrooms. An earlier synthesis[4] was on a scale sufficient to provide proof of structure, but the yield of product based on 4-carboxyphenylhydrazine (**1a**) was only 1%.



1a, R = HOOC-
b, R = $HOCH_2$-

Furthermore, ion-exchange column chromatography was used in the last step and required 5 L of packing to purify 1 g of crude agaritine. Scale-up of this synthesis to the

(1) This work was done under contract (N01 CP33278) with the Public Health Service (NIH-NCI).
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(3) B. Toth, *Cancer Res.*, **35**, 3693 (1975).
(4) R. B. Kelly, E. G. Daniels, and J. W. Hinman, *J. Org. Chem.*, **27**, 3229 (1962).

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