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ENZYMATIC SYNTHESIS OF CHLORO-L-TRYPTOPHANS

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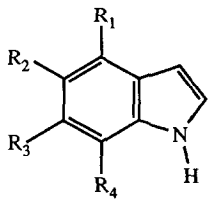
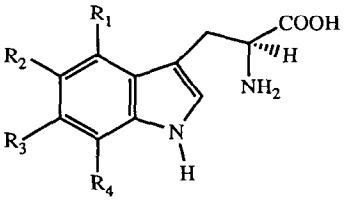
Abstract: 4-Chloro-L-tryptophan (**1a**), 5-chloro-L-tryptophan (**1b**), 6-chloro-L-tryptophan (**1c**) and 7-chloro-L-tryptophan (**1d**) were prepared from 4-, 5-, 6- and 7-chloroindoles (**2a-d**) by reaction with L-serine using tryptophan synthase. The chlorotryptophans were optically pure (>99% e.e).

After 6-chloro-D-tryptophan was identified as a nonnutritive sweetener,[1] there has been an increasing interest in chemical and biological properties of chlorotryptophans. 4-Chloro-L-tryptophan was found in the protein of pea seeds,[2] and 5-chloro-D-tryptophan was isolated from the hydrolyzate of the antibiotic longicatenamycin.[3] 7-Chlorotryptophan was found to be a substrate of L-tryptophan-2,3-dioxygenase from *Pseudomonas aureofaciens*.[4] 4-Chlorotryptophan has been synthesized from 2-chloro-6-nitrotoluene using the Batcho-Leimgruber indole synthesis, as well as from 4-chloroindole *via* gramine.[2,5] Fischer indole sythesis was used for the preparation of 5- and 7-chlorotryptophan.[2-6] 6-Chloro-D-tryptophan was prepared from D-tryptophan *via* 6-nitro-D-tryptophan.[7] Even though a number of L-tryptophan derivatives have been prepared using either microorganism or isolated enzymes,[8] the enzymatic preparation of chloro-L-tryptophans has not been reported. We report here the convenient one-pot synthesis of chloro-L-tryptophans using *Salmoonella typhimurium* tryptophan synthase.



2a-d



1a-d

1a, **2a**: R_1=Cl, R_2=R_3=R_4=H

1b, **2b**: R_2=Cl, R_1=R_3=R_4=H

1c, **2c**: R_3=Cl, R_1=R_2=R_4=H

1d, **2d**: R_4=Cl, R_1=R_2=R_3=H

Because of the low solubility of chloroindoles[9] in the buffer solution, **2a-d** were dissolved in toluene (100mg/10ml) and added to the buffer containing the enzyme and L-serine.[10] The bi-phasic solution was shaken for a week at 37°, and briefly heated (80°) to stop the reaction. After extraction with ether to recover unreacted chloroindoles, and filtering, concentration of the aqueous layer precipitated **1a-d** with 35-60% yields (80-90% based on the recovered chloroindoles) at 4°, due to their low solubility in water.[11] The optical purity of **1a-d** was measured by HPLC using a chiral

column, and was greater than 99% e.e.[12]

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9. 7-Chloroindole has been synthesized from *o*-chloroaniline accoring to Rydon and Tweddle.[5] The other chloroindoles are commercially available.
10. A typical reaction mixture consisted of chloroindole (100 mg), L-serine (15 mM), PLP (30 mM), potassium phosphate buffer (100 mM, pH 8), NaCl (16.2 mM), tryptophan synthase (20-40 international units), and NaN_3 (2 mM).
11. Each compound was characterized by ^{1}H NMR **1a**: mp 252-254°C; ^{1}H NMR (Methanol-D_4, 300Mhz, ppm) 7.28(dd, 1H, J=7.6, 1.4Hz, H-7), 7.20(s, 1H, H-2), 7.00(t, 1H, J=7.6Hz, H-6), 6.97 (dd, 1H, J=7.6, 1.4Hz, H-5), 3.66 (dd, 1H, J=5.1, 8.3Hz, α-H), 3.58(dd, 1H, J=5.1, 13.1Hz, β-H), 3.08(dd, 1H, J=8.2, 14.0Hz, β-H') **1b**: mp 246-248°C; ^{1}H NMR (Methanol-D_4, 300Mhz, ppm) 7.73(d, 1H, J=2.0Hz, H-4), 7.31(d, 1H, J=8.6Hz, H-7), 7.20(s, 1H, H-2), 7.05(dd, 1H, J=2.0, 8.6Hz, H-6), 3.54(dd, 1H, J=4.4, 8.4Hz, α-H), 3.26(dd, 1H, J=4.4, 13.9Hz, β-H), 2.89(dd, 1H, J=8.3, 14.3Hz, β-H') **1c**: mp 240-241°C; ^{1}H NMR (Methanol-D_4, 300Mhz, ppm) 7.67(d, 1H, J=8.3Hz, H-4), 7.34(d, 1H, J=1.6Hz, H-7), 7.18(s, 1H, H-2), 6.99(dd, 1H, J=1.9, 8.5Hz, H-5), 3.54(dd, 1H, J=4.5, 8.3Hz, α-H), 3.27(dd, 1H, J=4.5, 14.3Hz, β-H), 2.93(dd, 1H, J=8.2, 14.3Hz, β-H') **1d**: mp 230-232°C; ^{1}H NMR (Methanol-D_4, 300Mhz, ppm) 7.67(dd, 1H, J=0.8, 7.8Hz, H-4), 7.24(s, 1H, H-2), 7.12(dd, 1H, J=0.8, 7.8Hz, H-6), 7.01(t, 1H, J=7.7Hz, H-5), 3.56(dd, 1H, J=4.5, 8.2Hz, α-H), 3.29(dd, 1H, J=4.5, 14.4Hz, β-H), 2.95(dd, 1H, J=8.2, 14.4Hz, β-H')
12. Column: Cu-proline (Serva). Eluent: 10mM $CuSO_4$ at 1mL/minute Detector: 270nm. **1a**; $[\alpha]^{25}_D$= -21.8 (c=0.5, 1N HCl) **1b**; $[\alpha]^{25}_D$= -44.1(c=0.5, 1N HCl) **1c**; $[\alpha]^{25}_D$= -27.5 (c=0.5, 1N HCl) **1d**; $[\alpha]^{25}_D$= +5.1(c=0.5, 1N HCl)