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A convenient one-step synthesis of L-aminotryptophans and improved synthesis of 5-fluorotryptophan

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ABSTRACT

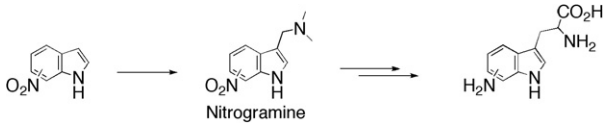
A one-pot biotransformation for the generation of a series of L-aminotryptophans using a readily prepared protein extract containing tryptophan synthase is reported. The extract exhibits remarkable stability upon freeze-drying, and may be stored and used for long periods after its preparation without significant loss of activity.

Tryptophan is an essential amino acid that plays a crucial role in the structure and function of proteins. Due to its low natural abundance of approximately 1%, it is ideally suited for replacement with analogues to probe function or alter enzyme properties. For example, incorporation of fluoro- and amino-substituted tryptophans into proteins has been used to probe π-cation interactions[1] or to study folding pathways.[2] The incorporation of an electron-donating group such as an amine into an indole ring system has a significant effect upon its electronic and physical properties, which, in turn, influence the absorption and emission characteristics of tryptophan.[3,4] This has been exploited in the modification of green-fluorescent protein to generate a variant with a significant red-shifted emission.[5]

In addition to its proteinogenic role, tryptophan is also the precursor for numerous metabolites in eukaryotes such as plant alkaloids and the neurotransmitter serotonin.[6] Of particular interest are microbial tryptophan-containing natural products that exhibit a range of biological activities.[7] In the non-ribosomal peptide gramicidin, fluorotryptophan derivatives have been used to study the orientation of the antibiotic in the membrane using ^{19}F NMR and to investigate the effects on ion channel function.[8] Interestingly, no natural products containing an aminotryptophan appear to have been isolated to date. However, precursor-directed biosynthesis and mutasynthesis provide convenient methods for accessing novel natural products derivatives.[9] The opportunity of making designer aminotryptophan-containing natural products arises. For these reasons, a convenient preparation of amino and fluorotryptophans is required.

Whilst a wealth of syntheses of halotryptophans are reported in the literature, there are very few reports of the generation of aminotryptophans. Typically, aminotryptophans are prepared from their corresponding nitroindoles via nitrogramine (Scheme 1). Reduction of the nitro group is performed either before or after ester hydrolysis. Racemic mixtures of 4-, 5-, 6- and 7-aminotryptophans have been prepared in this manner.[10] Using enantiomerically pure starting material, Moriya et al. were able to obtain 6-amino-D-tryptophan from D-tryptophan via nitration followed by reduction.[11] A procedure using tryptophanase has also been described for the synthesis of 5-aminotryptophan.[12] The synthesis of L-amino-tryptophans using tryptophan synthase has been alluded to; however, no details have been published.[2,3]

The enzyme tryptophan synthase can be utilised to access a range of tryptophan derivatives using serine and indole derivatives (Scheme 2).[13,14] Tryptophan synthase consists of two subunits, α and β, and uses pyridoxal phosphate (PLP) as co-factor. The α sub-



Scheme 1. Chemical synthesis of racemic aminotryptophans.

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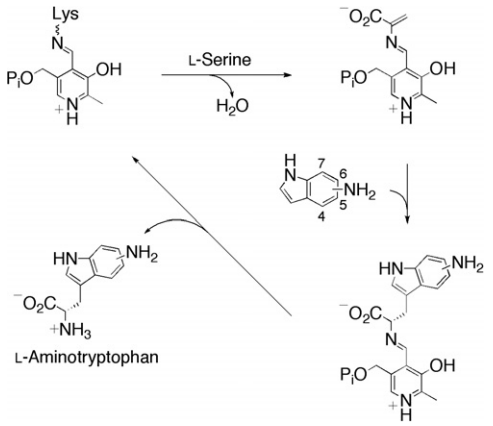

Scheme 2. Tryptophan synthase-mediated conversion of serine and aminoindole to give aminotryptophan. The reaction cycle starts with enzyme-bound PLP. Serine replaces the active site lysine and undergoes dehydration to give the aminoacrylate intermediate. The ensuing β-addition by aminoindole yields co-factor bound aminotryptophan that is liberated by transamination with the active site lysine.

unit provides the indole from indole 3-glycerolphosphate and channels it through a tunnel to the β subunit where the condensation between indole and serine occurs.[15] The reaction cycle of the β subunit is shown in Scheme 2. We recently reported a convenient one-step synthesis of a series of methyl- and halotryptophans utilising a readily prepared bacterial protein extract, rather than the purified enzyme improving the ease of utility of this reaction by organic chemists.[13] We also demonstrated that yields were significantly higher when the extract was contained within a dialysis bag.

Escherichia coli pre-transformed with pSTB7, a high copy number plasmid expressing tryptophan synthase from *Salmonella enterica*, is commercially available (ATCC 37845).[16] The cell lysate was prepared as previously described.[13] Typically, 80 ml protein extract was obtained from 2 L of a *E. coli*/pSTB7 overnight culture.[17] Aliquots of the extract were either stored at −80 °C or lyophilised. Freeze-dried material was stored at 4 °C and reconstituted, to its original volume, with water before use. For the biotransformation, the crude extract containing tryptophan synthase was sealed into a dialysis bag and introduced into buffered aqueous solution of serine, the corresponding indole, and the co-factor PLP.[18] After three days of incubation at 37 °C with gentle shaking, indoles were removed by extraction with ethylacetate or ether. The aqueous phase was concentrated in vacuo and loaded onto C_{18} reverse-phase silica gel. Serine and PLP were removed by washing with water, and the tryptophan derivative was then eluted in methanol.[19]

Both 4- and 7-aminotryptophans were obtained in excellent yield (Table 1, entries **1** and **4**). Indoles with the amino substituent in the 5- or 6-position were converted less efficiently (Table 1, entries **2** and **3**). However, if large quantities are required, the extracted indole derivative can be reintroduced into a fresh biotransformation mixture. By recycling the indole two times, we were able to obtain very good overall yields even for poorly converting substrates. Interestingly, the yields for 4- and 7-substituted methyl or halotryptophans were significantly lower than those for the corresponding 5- and 6-substituted tryptophans.[13] This is the opposite of our observation for the amino derivatives. At present, it is unclear whether the observed substituent effects are reflective of changes in reactivity of the indole or if they are the result of interactions with the enzyme.

Table 1
Yields of tryptophan derivatives obtained through biotransformation

Entry	Tryptophan	Conditions	Yield[a] (%)
1	7-Amino	Crude extract	65
2	6-Amino	Crude extract	35
3	5-Amino	Crude extract	37
4	4-Amino	Crude extract	70
5	5-Fluoro	Crude extract	83
6	5-Fluoro	Freeze-dried, 1 day storage	77 ± 12[b]
7	5-Fluoro	Freeze-dried, 2 months storage	72 ± 19[b]

[a] Yields are relative to theoretical maximum based on the amount of indole used for the biotransformation.
[b] Yields from three independent experiments with standard deviation.

We also wanted to investigate how freeze-drying would affect the activity of the protein extract. For this purpose, the crude extract was prepared as before but PMSF and β-mercaptoethanol were omitted from the lysis buffer.[17] Aliquots were lyophilised immediately after cell lysis and removal of the cell debris. The freeze-dried material was redissolved in the original volume of water and then used as described above. For up to 2 months of storage at 4 °C, the crude extract retained all of its activity relative to non-lyophilised extract stored at −80 °C (Table 1, entries **5**–**7**). Notably, the yields for two out of three independent experiments were above 80%.

This method provides a convenient and scalable means of accessing L-aminotryptophans from commercially available starting materials. The crude protein extract containing tryptophan synthase is readily prepared and may be freeze-dried for storage. It is then utilised as a reagent by simply resuspending it in water.

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Supplementary data

^{1}H NMR and ^{13}C NMR spectra for aminotryptophans. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.07.053.

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17. Pelleted cells were washed with saturated NaCl, then resuspended in lysis buffer for cell lysis by sonication. Cell debris was removed by centrifugation. Lysis buffer: Tris–HCl (0.5 M), β-mercaptoethanol (10 mM), EDTA (5 mM), PMSF (1 mM), pH 7.8.
18. Per 100 ml potassium phosphate buffer (0.1 M, pH 7.8): serine (0.127 g, 1.21 mmol), aminoindole (0.160 g, 1eq), PLP (0.8 mg), 2 ml protein extract in dialysis tubing. Ref. 13 gives a detailed procedure.
19. Analytical data: *5-Fluoro-L-tryptophan hydrochloride:* ^{1}H NMR (D_2O, 400 MHz) δ 3.10–3.15 (dd, 1H, J = 7.2, 15.6 Hz), 3.16–3.22 (dd, 1H, J = 5.6, 15.6 Hz), 4.08 (t, 1H, J = 6.2 Hz), 6.76–6.81 (dt, 1H, J = 2.4, 9.2 Hz), 7.07 (d, 1H, J = 2.4 Hz), 7.10 (s, 1H), 7.18–7.22 (dd, 1H, J = 4.6, 9.0 Hz); MS (EI, *+ve*) m/z 222.95 (M^+). *4-Amino-L-tryptophan:* ^{1}H NMR (D_2O, 400 MHz) δ 3.25 (dd, 1H, J = 4.8, 16.4 Hz), 3.37 (dd, 1H, J = 5.6, 16.0 Hz), 4.03 (t, 1H, J = 6.2 Hz), 7.03–7.06 (m, 2H), 7.22 (s, 1H), 7.37 (dd, 1H, J = 0.40, 8.0 Hz); ^{13}C NMR (D_2O, 75 MHz) δ 25.2, 53.6, 104.0, 112.8, 114.3, 118.9, 121.1, 121.5, 126.8, 137.5, 172.5; HRMS (ESI, *+ve*) m/z 220.1084 $[M+H]^+$, Calcd for $C_{11}H_{14}N_3O_2$ m/z 220.1081. *5-Amino-L-tryptophan:* ^{1}H NMR (D_2O, 400 MHz) δ 3.18–3.35 (m, 2H), 4.05 (t, 1H, J = 6.4 Hz), 7.00 (d, 1H, J = 8.4 Hz), 7.25 (s, 1H), 7.40 (d, 1H, J = 8.8 Hz), 7.50 (s, 1H); ^{13}C NMR (D_2O, 75 MHz) δ 24.9, 52.9, 106.5, 112.0, 112.7, 115.9, 121.3, 126.3, 127.0, 135.3, 171.5; HRMS (ESI, *+ve*) m/z 220.1079 $[M+H]^+$, Calcd for $C_{11}H_{14}N_3O_2$ m/z 220.1081. *6-Amino-L-tryptophan:* ^{1}H NMR (D_2O, 400 MHz) δ 3.21 (dd, 1H, 7.6, 15.6 Hz), 3.27 (dd, 1H, J = 5.6, 15.6 Hz), 3.99 (t, 1H, J = 6.0 Hz), 6.91 (d, 1H, J = 8.0 Hz), 7.20 (s, 1H), 7.32 (s, 1H), 7.58 (d, 1H, J = 8.4 Hz); ^{13}C NMR (D_2O, 75 MHz) δ 25.0, 53.0, 106.0, 106.5, 113.5, 119.1, 123.7, 126.3, 126.7, 135; HRMS (ESI, *+ve*) m/z 220.1079 $[M+H]^+$, Calcd for $C_{11}H_{14}N_3O_2$ m/z 220.1081. *7-Amino-L-tryptophan:* ^{1}H NMR (D_2O, 400 MHz) δ 3.22–3.34 (m, 2 H), 4.10 (t, 1H, J = 5.6 Hz), 7.01 (s, 1H), 7.07 (t, 1H, J = 7.6 Hz), 7.23 (s, 1H), 7.58 (d, 1H, J = 8.0 Hz); ^{13}C NMR (D_2O, 75 MHz) δ 24.9, 52.8, 100.1, 107.3, 113.8, 116.1, 118.9, 119.2, 126.2, 128.8, 171.7; HRMS (ESI, *+ve*) m/z 220.1078 $[M+H]^+$, Calcd for $C_{11}H_{14}N_3O_2m/z$ 220.1081.