A convenient enzymatic synthesis of L-halotryptophans†

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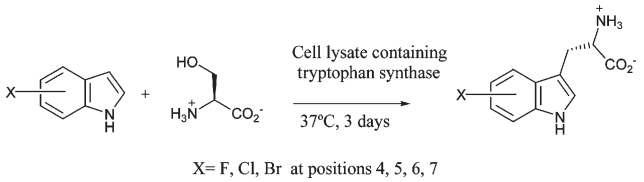
A scalable and general biotransformation for the generation of a series of L-halotryptophans using the lysate of a commercially available microorganism containing tryptophan synthase.

The essential amino acid tryptophan is a biosynthetic and synthetic precursor for many naturally occurring nonribosomal peptides and alkaloids.[1] Several medicinally important compounds including the anticancer agents Rebeccamycin and Diazonamide A incorporate chlorinated tryptophans,[2,3] and the biosynthesis of the antifungal, pyrrolnitrin, proceeds *via* the cleavage of 7-chlorotryptophan.[4] The seemingly trivial incorporation of a halogen into a natural product to make new analogues can often result in dramatic changes in the compounds bioactivity and bioavailability.[5] A general, rapid and convenient synthesis of halotryptophans is important to provide starting material for synthetic and biosynthetic incorporation into such natural products. Furthermore, the generation of such halogenated analogues could enable their selective functionalisation through such reactions as the Suzuki and the Sonogashira coupling. Additionally, fluorinated amino acids are a valuable labeling tool in the structural elucidation of proteins.[6]

The requirement for halotryptophans has stimulated many synthetic studies but despite many advances since the first synthesis of 7-chlorotryptophan reported by Rydon and Tweddle,[7] the best methods currently available are multi-step, lack generality or require specialised procedures. Syntheses of enantiopure halotryptophans, reported to date, generally involve de-racemisation in the final step using either an *N*-acylase[8] or through the formation and separation of diastereoisomers.[9] Two notable exceptions are the elegant, but by no means atom efficient, palladium-mediated heteroannulation of a Schöllkopf chiral auxiliary,[10] and the technically specialised electrochemical oxidation of proline to 5-hydroxyproline followed by Fischer indole synthesis.[11] In contrast the direct enzymatic alkylation of indole with serine constitutes a direct and potentially efficient approach and has been demonstrated to be useful in the preparation of a number of tryptophan analogues.[12] This approach has not been widely adopted because of the need to purify tryptophan synthase.

We report a general, one-step synthesis of fluoro, chloro, bromo and methyltryptophans using a readily prepared bacterial cell lysate (Scheme 1). *Escherichia coli* pretransformed with pSTB7, a high copy number plasmid expressing tryptophan synthase from *Salmonella enterica*, is commercially available (ATCC 37845).

In the first step pyridoxal phosphate (PLP) **1** forms a Shiff base with L-serine **2** followed by enzyme mediated dehydration to yield intermediate **3** (Scheme 2). Intermediate **5** results from the

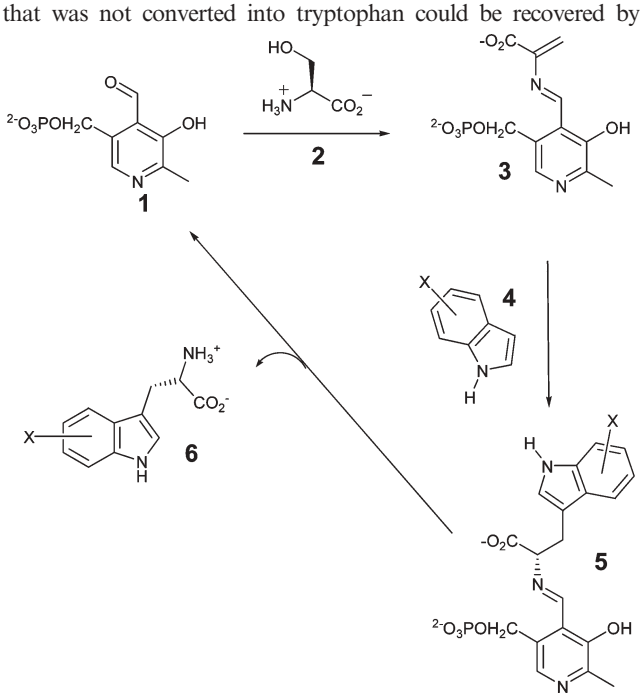


Scheme 1 Synthesis of substituted tryptophans.

nucleophilic attack of indole **4** on **3**. **5** Undergoes subsequent hydrolysis to release PLP **1** and tryptophan **6**.

The cell lysate may be made by sonicating a pellet of *E. coli* pSTB7 suspended in buffer. Once obtained, the lysate may be stored at 5 °C for periods of up to two weeks and treated as a synthetic reagent. The biotransformation is readily achieved by addition of an aliquot of cell lysate to a suspension of haloindole and serine in buffer agitated for 3 d at 37 °C.[13] After the reaction, aggregated proteins remaining in the mixture may be removed by filtration. Any remaining indole may be extracted into diethyl ether prior to the aqueous phase being concentrated and the tryptophan purified by reverse phase silica chromatography. This simple one-step procedure furnished yields of up to 63%, see Table 1. Enantiomeric purity was confirmed using a binol derived chiral shift reagent for amino acids.[14]

Furthermore, it was determined that almost all of the indole that was not converted into tryptophan could be recovered by



Scheme 2 The tryptophan synthase mediated conversion of serine and haloindole (where X = F, Cl, Br) to tryptophan with PLP.

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Table 1 Yields for the transformation of indole analogue and serine to tryptophan

Haloindole	Yield after one cycle with enzyme (%)
7-Fluoro	41
6-Fluoro	62, 82[a]
5-Fluoro	63[b]
7-Chloro	9
6-Chloro	54
5-Chloro	50, 61[a]
7-Bromo	8
6-Bromo	40
5-Bromo	16, 26[a]
4-Bromo	3
7-Methyl	50
6-Methyl	38
5-Methyl	42
4-Methyl	12
2-Methyl	41
Indole	53

[a] Yields reported for cell-free lysate contained within dialysis tubing. [b] Improved to 100% yield after extracting unreacted indole and exposing it to two further cycles of reaction.[c] All products were fully characterised, see electronic supplementary information, ESI.

extraction into diethyl ether. By reintroducing this material to the lysate and serine mixture we were able to obtain almost quantitative yields over the course of three cycles. The synthesis of tryptophans by cell lysate contained within dialysis tubing, tied to form a bag, was also investigated. This method had the effect of concentrating the enzyme and we observed a further improvement in yield, attributed to improved stabilisation of the enzyme. The contained cell lysate could be removed from the reaction, by removal of the dialysis bag. The contained enzyme proved still to be active and could be reused as catalyst in further reactions. Using dialysis tubing, the reactions were scaled up to enable the generation of gram quantities of tryptophans in one pot.

This one-step enzymatic synthesis compares very favourably with chemical syntheses reported in the literature. Syntheses of racemic 5-fluoro and 6-fluoro tryptophan starting in three steps from the corresponding indoles have overall yields of 79 and 61% respectively.[15,16] A two step synthesis 6-bromo D-tryptophan involving an acylase to effect de-racemisation is reported in overall 36% yield.[8]

A variety of factors contribute to the trend in yields. In the series of halotryptophans the major contribution to the efficiency of the reaction is from steric factors. The shorter the carbon–halogen bond the greater the ease of conversion. For example 5-fluoro, 5-chloro and 5-bromo indole are converted to tryptophan in yields of 63, 54 and 16% respectively. In order to reach the active site of the β subunit of tryptophan synthase, indole must first enter the α subunit then pass through a 25 Å long tunnel. A turn in this tunnel has been noted to prevent the passage of Nile red, a molecule 12 × 6 × 3.4 Å in dimension (Fig. 1).[17]

Indoles substituted at the 7 and 4 positions will have the greatest widths (6.0 Å for Br, 5.8 Å for methyl, 5.8 Å for Cl, 5.3 Å for F) compared to the width of indole unsubstituted in the 4 or 7 position (5.0 Å). Comparatively lower yields in each series of halotryptophans for the 7 and 4 analogues could be postulated to be due to steric difficulties with passage of these molecules through the enzyme's tunnel to the active site.

To summarise, the general and scalable synthesis of a range of tryptophan analogues, using a readily prepared cell-free lysate, is reported. The efficiency of the reaction is predominantly related to steric factors with the 5 and 6-substituted indoles bearing smaller substituents resulting in excellent yield of the analogue. Our procedure requires no purification of the enzyme and makes the reaction readily accessible to synthetic chemists. The generality of the transformation enables the rapid preparation of synthetically useful quantities of halotryptophans under environmentally friendly conditions. By recycling the unconsumed indole and reintroducing this to fresh enzyme almost quantitative yields of several of the halotryptophans may be obtained.

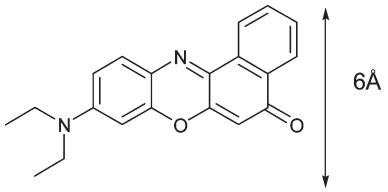

Fig. 1 Nile red.

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