Organic LETTERS

Letter

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The First One-Pot Synthesis of L-7-Iodotryptophan from 7-Iodoindole and Serine, and an Improved Synthesis of Other L-7-Halotryptophans

Duncan R. M. Smith,[†,⊥] Tom Willemse,[†,‡,§,⊥] Danai S. Gkotsi,[†] Wim Schepens,[∥] Bert U. W. Maes,[§] Steven Ballet,[‡] and Rebecca J. M. Goss*[,†]

[†]School of Chemistry and BSRC, University of St. Andrews, St. Andrews, KY16 9ST, U.K.

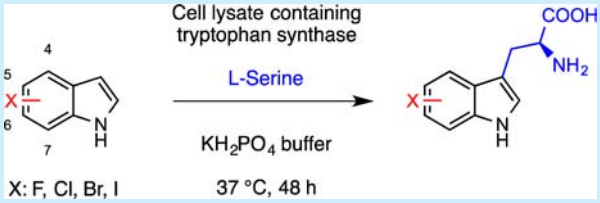
[‡]Departments of Chemistry and Bio-engineering Sciences, Vrije Universiteit Brussel, Pleinlaan 2, B-1050 Brussels, Belgium

[§]Organic Synthesis, University of Antwerp, Groenenborgerlaan 171, B-2020 Antwerp, Belgium

[∥]Janssen Pharmaceutica, CREATe, Turnhoutseweg 30, B-2340 Beerse, Belgium

Supporting Information

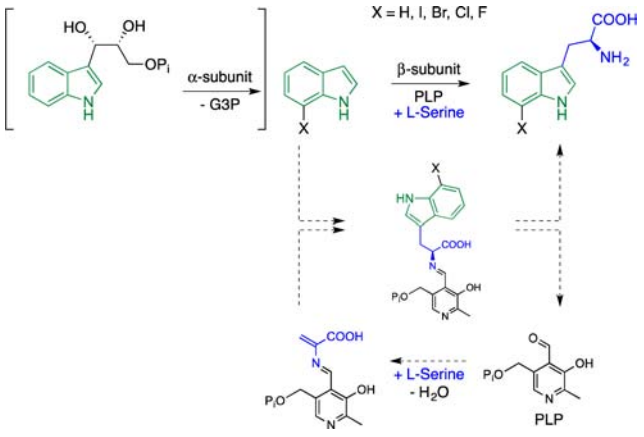
ABSTRACT: A simple and scalable one-pot biotransformation enables direct access to L-halotryptophans, including L-7-iodotryptophan, from the corresponding haloindoles. The biotransformation utilizes an easy to prepare bacterial cell lysate that may be stored as the lyophilizate for several months and utilized as a catalyst as and when required.



Tryptophan is a key residue in numerous important natural products including daptomycin,[1] the calcium dependent antibiotic,[2a,b] conotoxin,[3] and the uridyl peptide antibiotics.[4] Tryptophan is also a biosynthetic precursor in notable bioactive compounds such as the anticancer agent staurosporine,[5] the ergot alkaloids,[6] indole alkaloids such as vindoline[7] and vinblastine,[8] and epipolythiodiketopiperazine alkaloids such as (+)-gliocladins[9] and (+)-bionectins.[10] The 7-halotryptophan motif is found in a number of biologically active natural products, such as rebeccamycin[11a] and pyrrolnitrin.[11b] Furthermore, 7-halotryptophans are a tool used in combinatorial biosynthesis or chemogenetic approaches to natural product analogue generation, such as in recent work from the Goss group on pacidamycin analogues[11c,d] and the O'Connor group on plant-derived indole alkaloid natural products.[11e,f] Ready access to halogenated analogues is important in both the total synthesis and directed biosynthesis of such compounds, enabling the study of structure−activity relationships.[11c,e,12a−c] Tryptophan also has a central role in metabolism, protein structure, and signaling, and analogues are frequently used to probe enzyme function or alter enzyme properties. The low natural abundance of tryptophan within proteins (approximately 1%) and its essential role in protein structure and function make it ideally suited for this role. By replacing tryptophan in proteins with halotryptophans, it has been possible to probe π−cation interactions[13] and study folding pathways.[14,15] The introduction of a halogen provides the ability to tune the properties of tryptophan, altering fluorescence properties and lipophilicity while providing a chemically functionalizable, selectable handle, which can be modified even under mild aqueous conditions.[16]

The tryptophan synthase α-subunit converts indole-3-glycerol-phosphate to indole, with the loss of glyceraldehyde-3-phosphate (G3P). The β-subunit converts indole and L-serine to L-tryptophan, using pyridoxal phosphate (PLP) as a cofactor.[20a,b] When haloindoles are exogenously provided, the α-subunit mechanism (shown in brackets in Scheme 1) is bypassed; however, the β-subunit is not functional in the absence of the α-subunit.[20b,c]

Scheme 1. Mechanism of Tryptophan Synthase



For the first time, we report here a one-pot synthesis of L-7-iodotryptophan. The incorporation of a carbon−iodine bond is particularly attractive due to its high reactivity. The introduction of iodophenylalanine into proteins has been used as an important chemical biology tool to enable post-translational synthetic modification,[17] and ready access to synthetically useful quantities of halotryptophans (in particular

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Table 1. Analysis of Tryptophan Synthase (TS) Lysates

lysate	*E. coli* host	lysis buffer	total protein (mg/mL)[a]	TS activity (U/mL)[b]	relative TS amount[c]	L-7-iodotryptophan yield
1	CB149	A	4	350	0.21	n/a
2	BL21	A	4	550	0.46	51%
3	BL21	B	9	750	1.00	35%

[a]Determined by Bradford assay, reported to nearest mg/mL. [b]Determined by DMAB assay, reported to nearest 50 U/mL. [c]Determined by SDS-PAGE visualization, normalized to the largest amount and reported to 2dp. See SI for details of lysis buffers and protein analysis techniques.

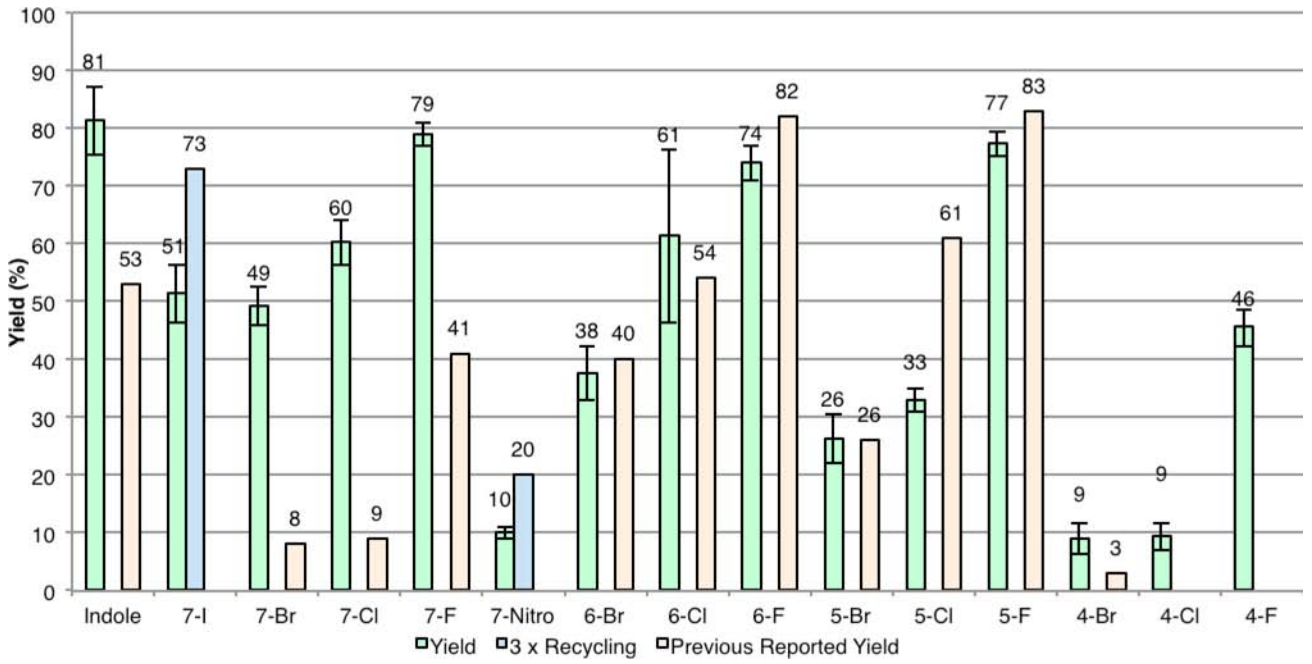

Figure 1. Yields of L-halotryptophans, synthesized from the corresponding haloindole at 2 mmol scale using the procedure detailed in this letter, excluding 7-iodoindole which was performed at a 1 mmol scale. Yields are based on the amount of indole used, and biotransformations were repeated in at least triplicate (excluding 3× recycling). Standard deviations are indicated by error bars.

iodotryptophans) is desirable. The need for halotryptophans has stimulated many synthetic studies. However, despite advances since the first synthesis of 7-chlorotryptophan reported by Rydon and Tweddle,[18] the best synthetic methods currently available are multistep, lack generality, or require specialized procedures. Typically these multistep syntheses generate racemic halotryptophans, and enantiopure halotryptophans are accessed through enzymatic deracemization, resulting in a maximum 50% yield of the desired product in the final step.[19a−d] Conversely, biotransformation using tryptophan synthase generates enantiopure L-halotryptophans in a single step, and we have demonstrated that close to quantitative conversions may be achieved by carrying out multiple passes with the enzyme.[20b]

The enzyme, tryptophan synthase, can be utilized to access a range of tryptophan derivatives mediating the β-replacement reaction of serine with a variety of indole derivitives.[20a−c] Tryptophan synthase consists of two subunits, α and β, and uses PLP as the cofactor. The α-subunit provides the indole from indole 3-glycerolphosphate and channels it through a tunnel to the β-subunit where the condensation between indole and L-serine occurs.[21a,b] The reaction of the α-subunit may be bypassed by the provision of indole or an indole analogue as the substrate (Scheme 1).

To develop a procedure that would be attractive to and readily applicable by synthetic organic chemists we wished to avoid the need for involved protein purification. We previously reported a straightforward process that utilizes a readily prepared and stored bacterial cell lysate[20b] and demonstrated that the lysate may be lyophilized and stored at 5 °C for several months without significant loss of activity.[20c] The lysate may be utilized as a catalyst as and when required. The lysate is contained in dialysis tubing during the reaction and so can be easily removed before workup.

Our previously reported procedure enabled ready access, in good yields, to a wide variety of halotryptophans. However, yields for the 7-halotryptophans were moderate to low and it had not been possible to use this method to access even trace quantities of L-7-iodotryptophan, even through our series of previous attempts to rationally reengineer the tryptophan synthase. Here we report a new procedure, utilizing a lysate prepared from a different *Escherichia coli* host strain. This procedure enables, for the first time, access to good quantities of L-7-iodotryptophan, as well as dramatically improved yields for the L-7-halotryptophans and good yields for other 4-, 5-, and 6-halotryptophans.

Tryptophan synthase from *Salmonella enterica* is commercially available encoded on plasmid pSTB7 in *E. coli* CB149 (ATCC 37845).[22a−c] Through exploring various *E. coli* hosts, we determined that not only were good levels of production and activity of the required enzyme achievable in *E. coli* BL21(DE3) (Table 1) but also surprisingly the resultant lysate gave considerably better yields for 7-halotryptophans and for the first time enabled us to access iodotryptophan (Figure 1).

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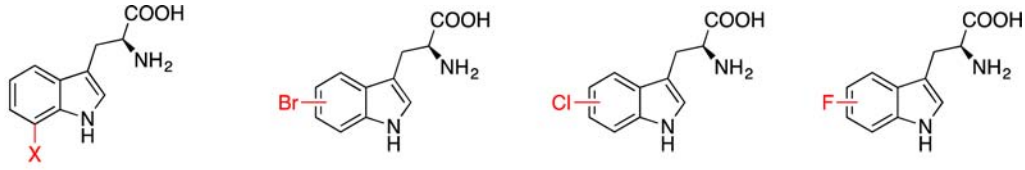

1 X = I: 51 ± 5%
5 X = NO_2: 10 ± 2%
15 X = H: 81 ± 6%

2 7-Br: 49 ± 3%
6 6-Br: 28 ± 5%
9 5-Br: 26 ± 4%
12 4-Br: 9 ± 3%

3 7-Cl: 60 ± 4%
7 6-Cl: 61 ± 15%
10 5-Cl: 33 ± 2%
13 4-Cl: 9 ± 2%

4 7-F: 77 ± 2%
8 6-F: 74 ± 3%
11 5-F: 77 ± 2%
14 4-F: 46 ± 3%

Figure 2. Structures and corresponding yields of L-halotryptophans.

We set out to compare the catalytic ability of our new and readily prepared *E. coli* BL21(DE3)/pSTB7 lysate with *E. coli* CB149/pSTB7 lysate, carrying out all biotransformations in triplicate. Reactions were simply set up by adding serine and indole or an indole analogue to a buffered aqueous solution containing PLP and the crude lysate. Following 48 h of reaction, any unreacted indole was extracted into ethyl acetate and the aqueous phase containing the tryptophan analogue was separated from unreacted serine using a small plug of reversed phase silica (for full details, see Supporting Information (SI)). In both systems the yields were generally inversely proportional to the size of the halogen, and as previously observed[20c] the new lysate could be stored for several months following preparation and utilized as a catalyst at will, without appreciable loss of activity. Yields for the 7-halotryptophans were significantly elevated using the BL21(DE3) lysate, and production of the other halotryptophans was comparable to previously reported yields.

While the two different lysates gave very similar yields for the 5- and 6-substituted tryptophans, the yields for the 7-halotryptophans were significantly elevated using the new lysate, making this simple preparation a very attractive method for accessing multigram quantities of these compounds (Figure 2).

We sought to investigate reasons behind the altered substrate specificity and the cause of the enhanced yields, but neither total protein production levels nor tryptophan synthase activity measurements could be correlated with the improved production of 7-halotryptophans. Sequencing at both the genetic and protein level demonstrated that no advantageous mutation had occurred. There is a clear qualitative difference between the lysates (Table 1). While intriguing, further investigations into subtle changes in protein folding, likely to be causing the altered substrate specificity, are nontrivial and are beyond the scope of this study, which aims to present a useful preparative procedure.

This striking result means that it is now possible to access L-7-iodotryptophan in up to 58% yield in just one step and to obtain a 73% conversion following three recycles in which the unreacted iodoindole was extracted and reincubated twice more with fresh lysate. It was also possible to access 7-nitrotryptophan in 11% yield (showing a modest improvement to 20% by recycling). Reactions were typically carried out using 200−500 mg of indole (2 mmol), but could be readily scaled up without need for a fermenter to enable conversions of around 2.5 g of haloindole (see SI).

In addition to allowing the facile synthesis of enantiopure L-iodotryptophan, the modified procedure for the preparation of cell lysate containing tryptophan synthase reported in this letter also improved upon the previously reported yields of several other halotryptophans (Figure 1). The crude protein extract is readily prepared, and it has previously been reported that this can be lyophilized for ease of storage.[20c]

The modified procedure for the preparation of the cell lysate containing tryptophan synthase has improved yields of L-halotryptophans and allowed access to L-7-halotryptophans in yields of 50% or more.

L-7-Iodotryptophan has become accessible using this simple, scalable, one-pot biotransformation for the first time. This method provides a convenient means of accessing an expanded range of enantiopure L-halotryptophans from commercially available starting materials.

■ ASSOCIATED CONTENT

Supporting Information

Full experimental procedures including the preparation of the lysates, characterization of compounds, and ^{1}H and ^{13}C NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: rjmg@st-andrews.ac.uk.

Author Contributions

⊥These authors contributed equally.

Notes

The authors declare no competing financial interest.

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