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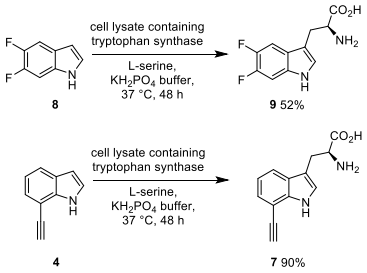
Michael J. Corr, Duncan R. M. Smith and Rebecca J. M. Goss*



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One-Pot Access to L-5,6-Dihalotryptophans and L-Alknyltryptophans Using Tryptophan Synthase

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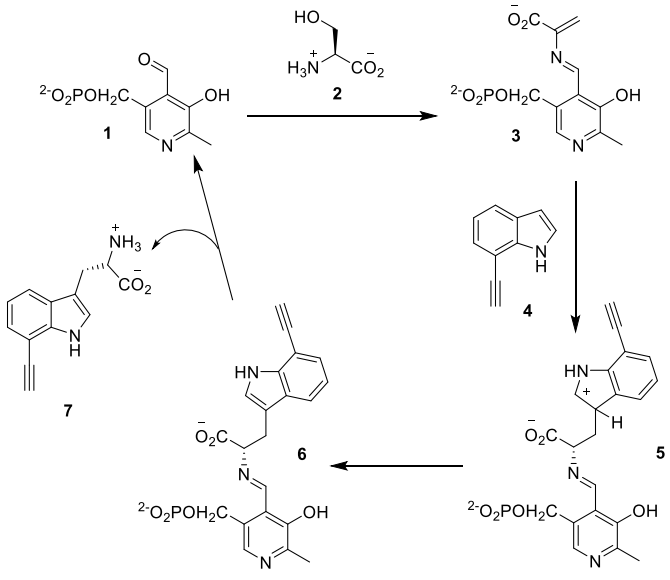
ABSTRACT

We report, for the first time, the use of tryptophan synthase in the generation of L-dihalotryptophans and L-alkynyltryptophans. These previously unpublished compounds will be useful tools in the generation of probes for chemical biology, in biosynthetic diversification and as convenient building blocks for synthesis.

1. Introduction

Tryptophan is an essential amino acid and a synthetic and biosynthetic precursor for many natural products of alkaloid and non-ribosomal peptide origin. These include the anticancer agent staurosporine[1] and diazonamide A,[2] indole alkaloids such as vindoline[3] and vinblastine,[3] and antibiotics such as daptamycin[4] and the calcium-dependent antibiotic (CDA).[5] Furthermore, tryptophans are essential to the structure and function of proteins and, though present in proteins at only 1.2% frequency, tryptophan is largely attributed to conferring UV absorbance and fluorescence in these biomolecules.[6] The ability to functionalise tryptophan is attractive as it provides a handle for selectively tuning the properties of the free amino acid or the amino acid as a component of a natural product or a peptide. We have previously demonstrated a new paradigm in natural product analogue generation, Genochemetics,[7] in which gene installation enables the biosynthetic introduction of a chemically orthogonal handle into a natural product, enabling selective synthetic diversification. Specifically, we demonstrated that we could engineer the production of the antibiotic pacidamycin bearing a 7-chlorotryptophan, the halogen handle enabled synthetic diversification through Suzuki-Miyaura cross coupling chemistry to produce a new array of natural product analogues.[7]

The requirement for halotryptophans has stimulated many synthetic studies. Since the first chemical synthesis of 7-chlorotryptophan reported by Rydon and Tweddle,[8] despite many advances, the best methods currently available are multi-step, lack generality or require specialised procedures. The use of the enzyme tryptophan synthase represents a convenient alternative, enabling the biotransformation of serine **2** and a substituted indole to give the corresponding substituted tryptophan (Scheme 1). We have previously shown the utility of tryptophan synthase to convert haloindoles and nitroindoles to the corresponding tryptophans,[9-11] and have even used this approach to access sterically demanding 7-iodotryptophan.[11] Here, we report for the first time, the generation of 5,6-dihalotryptophans and



Scheme 1: Mechanism of tryptophan synthase showing how it may be harnessed to enable access to analogues such as L-7-alkynyltryptophan **7**

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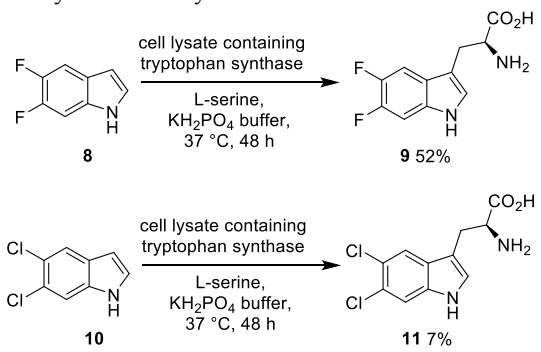
alkynyltryptophans using tryptophan synthase.

2. Tryptophan Synthase

Naturally, the α-subunit of tryptophan synthase converts indole-3-glycerolphosphate to indole, with the loss of glyeraldehyde-3-phosphate, the β-subunit then utilizes this indole along with L-serine **2** and converts it to L-tryptophan, *via* the replacement reaction outlined in scheme 1, using pyridoxal phosphate (PLP) as a co-factor. When substituted indoles are provided exogenously, the α-subunit is bypassed and the β-subunit can be used to convert the indoles to the corresponding substituted-tryptophans.

To further explore extension of substrate scope, we turned our attention to di-halotryptophans (Scheme 2). We subjected commercially-available 5,6-difluoroindole **8** to the tryptophan synthase reaction and were pleased to see the reaction proceed to give the corresponding 5,6-difluoro-L-tryptophan **9** in 52% yield (an average of 3 isolated yields) on a 2 mmol scale. The unreacted starting indole from the reaction could be recovered and subjected to additional rounds of reaction with tryptophan synthase. After 3 rounds of biotransformation with tryptophan synthase, 5,6-difluoro-L-tryptophan **9** could be isolated in a synthetically useful 71% yield on a 2 mmol scale.

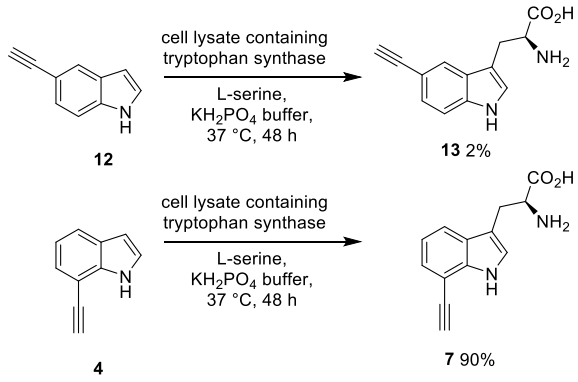


Scheme 2: Synthesis of 5,6-dihalotryptophans

With commercially-available and sterically more demanding 5,6-dichloroindole **10**, the reaction proved more difficult than for the difluoro-derivative. 5,6-Dichloro-L-tryptophan **11** was isolated in 7% yield (average of 4 isolated yields) after 2 mmol scale reaction with tryptophan synthase. Again, recycling of the unreacted starting material for 3 rounds with tryptophan synthase gave 25% isolated yield of **11**. With the dihalotryptophans in hand, we decided to explore the possibility of using this reaction to process an alkynyl indole (Scheme 3). Incorporation of an alkyne "tag" into a small molecule, protein or natural product can enable selective diversification and tagging,[12] through an alkyne-azide cycloaddition reaction, commonly known as "click chemistry".[13]

We wanted to see if alkyne-substituted indoles were amenable to the tryptophan synthase reaction. 5-Ethynylindole **12** was synthesised from 5-iodoindole following literature procedure.[14] When substrate **12** was subjected to the tryptophan synthase reaction, alkyne-tryptophan **13** was isolated in low 2% yield (average of 3 isolated yields) on a 2 mmol scale.

In an analogous manner to **12**, 7-ethynylindole **4** was synthesized from 7-iodoindole. When subjected to the tryptophan synthase reaction on a small scale (0.9 mmol), the reaction worked with a much greater efficiency than for the 5-substituted indole and expected product **7** was isolated in 90% yield. We were unable to carry out the biotransformations on a larger scale and in triplicate due to limited availability of the starting 7-iodoindole. To our knowledge, the synthesis of alkynyl tryptophans **7** and **13** have not previously been reported.



Scheme 3: Synthesis of ethynyltryptophans.

3. Conclusions

We have expanded the scope of the tryptophan synthase reaction previously reported[9-11] to include the synthesis of di-halotryptophans and shown the first examples of the enzyme carrying out the biotransformation of alkyne-containing indoles. Although the conversion of 5-ethynylindole **13** was poor, 7-ethynylindole **7** underwent surprisingly high conversion to the corresponding tryptophan. Perhaps reengineering of the enzyme might enable useful one step access to other terminal alkyne-containing tryptophans. These new compounds show that tryptophan synthase can be used for the production of synthetically useful building blocks for subsequent chemical diversification or as useful platforms for precursor directed biosynthesis.

4. Experimental

4.1. General

All reagents were purchased from commercial suppliers and were used without further purification unless otherwise stated.

Proton NMR (^{1}H), fluorine NMR (^{19}F{^{1}H}) and carbon NMR (^{13}C) were recorded on a Bruker Advance 500 (500 MHz) or a Bruker Avance II (400 MHz). Using a deptq sequence or an HSQC experiment with multiplicity editing, the ^{13}C NMR signals were assigned to CH_3, CH_2, CH and C. The NMR experiments were carried out in deuterochloroform ($CDCl_3$), deuterated methanol (CD_3OD), deuterated water (D_2O) or deuterated DMSO (d_6-DMSO). The chemical shifts (δ) are quoted in parts per million (ppm). Multiplicities are abbreviated as s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; b, broad for the ^{1}H NMR, ^{19}F{^{1}H} NMR and ^{13}C NMR spectra. Coupling constants are reported in Hertz (Hz).

High and low resolution mass spectra were recorded at the EPSRC National Mass Spectrometry Service, Swansea or at the University of St Andrews on a Waters Micromass LCT time of flight mass spectrometer coupled to a Waters 2975 HPLC system or on an Orbitrap ELOS pro.

Flash chromatography was performed using Davisil silica gel LC60A (40-63 micron). Thin layer chromatography (TLC) was performed using aluminium sheets of silica gel 60 F254 and was visualised under a Mineralight model UVGL-58 lamp (254 nm). The plates were developed with acidic methanolic vanillin solutions, ethanolic phosphomolybdic acid solutions or basic potassium permanganate solutions.

Purification of the ethynyltryptophans was carried out on a Biotage Isolera Four using reverse-phase SNAP C18 12 g column cartridges. The purification was carried out using water/methanol on the following gradient: 12 mL/min elution, 2% methanol/water for 1.15 min, 2→15% methanol/water for 1.15 min, 15% methanol/water for 5.15 min, 15→95% methanol/water for 15 min, 95% methanol/water for 3.45 min. The collection wavelength was set a 254 nm. Collection of the peaks occurring from approx. 4.3 min to 12.0 min gave the resulting ethynyltryptophans.

Freeze drying was carried out on a Scanvac CoolSafe™ freeze dryer.

Dialysis tubing cellulose membrane (average flat width 33 mm) was purchased from Sigma Aldrich. An approximately 20 cm length of tubing was immersed in water prior to use. One end was tied in a knot. The thawed cell lysate was inserted into the tied tubing using a pipette. Excess air was removed from inside the tubing and the other end was tied to give a cylinder of membrane sealed at both ends. The off-cuts of the knots were removed to give a tube length of approximately 5 cm for use in the reaction.

4.1. L-5,6-Difluorotryptophan 9

L-Serine **2** (0.262 g, 2.5 mmol, 1.25 eq) was dissolved in KH_2PO_4 reaction buffer (0.1 M, pH 7.8, 95 mL). A solution of 5,6-difluoro-1*H*-indole **8** (0.306 g, 2.0 mmol, 1.0 eq) in methanol (5 mL) was added to the flask containing the reaction buffer with vigorous stirring. Tryptophan synthase cell lysate was thawed in a 37 °C water bath, then stored at 0 °C. The cell lysate (3 mL) was stored in a cellulose dialysis bag and added to the reaction flask. The biotransformation was carried out at 37 °C, shaking at 180 rpm for 2 days. The aqueous layer was collected and washed with ethyl acetate (2 x 50 mL). The aqueous layer was concentrated to half its volume, then purified on C18 silica. The aqueous layer was loaded onto 30 g C18 silica, which was then washed with 200 mL water before elution of the product in methanol. The methanolic fractions were collected and the solvent was removed *in vacuo*. The residue was dissolved in water (50 mL) and lyophilised to give L-5,6-difluorotryptophan **8** (245 mg, 51%) as a white solid; $[\alpha]_D$ -12.4° (c 0.51, MeOH); ^{1}H NMR (500 MHz, D_2O) δ = 3.23 (1H, dd, *J*(H,H)= 15.5, 7.1 Hz, CH_AH$_B$), 3.28 (1H, dd, *J*(H,H)= 15.5, 5.4 Hz, CH$_A$$H_B$), 4.18 (1H, dd, *J*(H,H)= 6.9, 5.5 Hz, CH), 7.19 (2H, overlapping m, ArH), 7.28 (1H, dd, *J*(H,F)= 11.2, 7.7 Hz, ArH); ^{13}C NMR (125 MHz, D_2O) δ = 25.6 (CH_2), 53.3 (CH), 99.5 (d, *J*(C,F)= 21.7 Hz, CH), 104.4 (d, *J*(C,F)= 19.5 Hz, CH), 106.6 (d, *J*(C,F)= 5.6 Hz, C), 121.7 (d, *J*(C,F)= 8.1 Hz, C) 126.4 (d, *J*(C,F)= 3.5 Hz, CH), 131.2 (d, *J*(C,F)= 10.9 Hz, C), 145.8, (dd, *J*(C,F)= 198.6, 15.0 Hz, CF), 147.6 (dd, *J*(C,F)= 200.0, 15.3 Hz, C), 172.0 (CO); ^{19}F{^{1}H} NMR (470 MHz, D_2O) δ = -148.4 (d, *J*(F,F) = 21.1 Hz), -144.9 (d, *J*(F,F) - 21.1 Hz); HRMS (ESI): *m/z* calcd for $C_{11}H_{11}F_2N_2O_2^+$ $[M+H]^+$: 241.0783; found 241.0782.

The reaction was repeated two more times to give L-5,6-difluorotryptophan **9** (307 mg, 55%) and (233 mg, 49%). Over three runs, this gave an average yield of 52%.

4.2. L-5,6-Dichlorotryptophan 11

Using the biotransformation procedure reported above for **9**, 5,6-dichloro-1*H*-indole **10** (0.372 g, 2.0 mmol, 1.0 eq) gave 5,6-dichlorotryptophan **11** (33 mg, 6%) as a white solid; $[\alpha]_D$ -14.7° (c 0.53, MeOH); ^{1}H NMR (500 MHz, D_2O) δ = 3.21 (1H, dd, *J*(H,H)= 15.5, 7.1 Hz, CH_AH$_B$), 3.28 (1H, dd, *J*(H,H)= 15.3, 5.0 Hz, CH$_A$$H_B$), 4.13 (1H, dd, *J*(H,H)= 7.2, 5.3 Hz, CH), 7.18 (1H, s, ArH), 7.44 (1H, s, ArH), 7.57 (1H, s, ArH); ^{13}C NMR (125 MHz, D_2O) δ = 25.7 (CH_2), 53.5 (CH), 106.3 (C), 113.0 (CH), 118.9 (CH), 122.4 (C), 124.7 (C), 126.3 (C), 127.1 (CH), 135.0 (C), 172.3 (CO); HRMS (ESI): *m/z* calcd for $C_{11}H_{11}Cl_2N_2O_2^+$ $[M+H]^+$: 273.0192; found: 273.0190.

The reaction was repeated three more times to give L-5,6-dichlorotryptophan **11** (68 mg, 12%), (30 mg, 5%) and (26 mg, 5%). Over four runs, this gave an average yield of 7%.

4.3. *tert*-Butyl 5-iodo-1*H*-indole-1-carboxylate

Di-*tert*-butyl dicarbonate (3.4 mL, 14.8 mmol, 1.2 eq) was added to a solution of 5-iodoindole (3 g, 12.3 mmol, 1.0 eq) and 4-DMAP (0.3 g, 2.5 mmol, 0.2 eq) in DCM (100 mL) at r.t. under nitrogen and stirred for 2.5 h. The solvent was removed *in vacuo*. Purification by column chromatography using silica gel (1:19 ethyl acetate:hexane) gave *tert*-butyl 5-iodo-1*H*-indole-1-carboxylate (4.2 g, 100%) as a white solid; ^{1}H NMR (500 MHz, $CDCl_3$) δ = 1.68 (9H, s, CH_3), 6.50 (1H, dd, *J*(H,H)= 3.7, 0.6 Hz, ArH), 7.56 (1H, d, *J*(H,H)= 3.7Hz, ArH), 7.58 (1H, dd, *J*(H,H)= 8.7, 1.8 Hz, ArH), 7.90 (1H, d, *J*(H,H)= 1.8 Hz, ArH), 7.93 (1H, bd, *J*(H,H)= 8.7 Hz, ArH); ^{13}C NMR (125 MHz, $CDCl_3$) δ = 28.3 (CH_3), 84.3 (C), 86.8 (C), 106.4 (CH), 117.2 (CH), 126.8 (CH), 129.9 (CH), 132.8 (CH), 133.0 (C), 134.7 (C), 149.6 (CO).

4.4. 5-Ethynyl-1*H*-indole 12

Ethynyltrimethylsilane (2.6 mL, 18.5 mmol, 1.5 eq) was added to a solution of *bis*(triphenylphosphine)palladium (II) dichloride (216 mg, 0.3 mmol, 2.5 mol%), copper (I) iodide (117 mg, 0.6 mmol, 5 mol%) and *tert*-butyl 5-iodo-1*H*-indole-1-carboxylate (4.22 g, 12.3 mmol, 1.0 eq) in triethylamine (100 mL) at r.t under nitrogen and stirred for 18 h. The reaction mixture was filtered through celite and the solvent was removed *in vacuo*. The residue was dissolved in methanol (100 mL) and potassium carbonate (3.4 g, 24.6 mmol, 2.0 eq) was added. The reaction was stirred at r.t. for 4 h. The reaction mixture was diluted with water (200 mL) and extracted with diethyl ether (2 x 200 mL). The combined organic layers were dried over anhydrous sodium sulfate and the solvent removed *in vacuo*. Purification by column chromatography using silica gel (1:19 ethyl acetate:hexane) gave 5-ethynyl-1*H*-indole **12** (1.04 g, 60% over 2 steps) as a pale brown oil; ^{1}H NMR (500 MHz, $CDCl_3$) δ = 3.01 (1H, s, CH), 6.56 (1H, dd, *J*(H,H)= 3.2, 2.0 Hz, ArH), 7.25 (1H, dd, *J*(H,H)= 3.2, 2.4 Hz, ArH), 7.34-7.35 (2H, m, ArH), 7.84-7.85 (1H, m, ArH), 8.24 (1H, bs, NH); ^{13}C NMR (125 MHz, $CDCl_3$) δ = 74.8 (CH), 85.5 (C), 103.1 (CH), 111.3 (CH), 113.4 (C), 125.4 (CH), 125.5 (CH), 126.2 (CH), 127.8 (C), 135.8 (C); HRMS (EI): *m/z* calcd for $C_{10}H_7N_1^{+\bullet}$ $[M]^{+\bullet}$: 141.0578; found: 141.0583.

4.5. L-5-Ethynyltryptophan 13

L-Serine **2** (0.262 g, 2.5 mmol, 1.25 eq) was dissolved in KH_2PO_4 reaction buffer (0.1 M, pH 7.8, 95 mL). A solution of 5-ethynyl-1*H*-indole **12** (0.282 g, 2.0 mmol, 1.0 eq) in acetonitrile (5 mL) was added to the flask containing the reaction buffer with vigorous stirring. Tryptophan synthase cell lysate was thawed in a 37 °C water bath, then stored at 0 °C. The cell lysate (3 mL) was stored in a cellulose dialysis bag and added to the reaction flask. The biotransformation was carried out at 37 °C, shaking at 180 rpm for 2 days. The aqueous layer was collected and washed with ethyl acetate (2 x 50 mL). The aqueous layer was

concentrated to half its volume, then purified using a Biotage column. The aqueous layer was loaded onto a SNAP C18 12 g cartridge, then 200 mL water was eluted from the cartridge before loading onto the Biotage Isolera 4 for the purification gradient. The fractions were collected and the solvent was removed *in vacuo*. The residue was dissolved in water (50 mL) and lyophilised to give L-5-ethynyltryptophan **13** (8.3 mg, 1.8%) as a white solid; $[\alpha]_D$ +64.7° (c 0.37, MeOH); ^{1}H NMR (500 MHz, CD_3OD) δ = 3.13 (1H, dd, *J*(H,H)= 15.1, 9.0 Hz, CH_AH_B), 3.26 (1H, s, CH), 3.44-3.48 (1H, m, CH_AH_B), 3.81 (1H, dd, *J*(H,H)= 9.0, 4.2 Hz, CH), 7.22 (1H, dd, *J*(H,H)= 8.5, 1.5 Hz, ArH), 7.24 (1H, s, ArH), 7.32 (1H, dd, *J*(H,H)= 8.4, 0.5 Hz, ArH), 7.91 (1H, bs, ArH); ^{13}C NMR (125 MHz, CD_3OD) δ = 28.6 (CH_2), 56.8 (CH), 75.6 (CH), 86.4 (C), 110.2 (C), 112.5 (CH), 114.1 (C), 124.0 (CH), 126.4 (CH), 126.5 (CH), 128.4 (C), 138.2 (C), 151.0 (CO); MS (ESI) 229 (100) $[M+H]^+$, 212 (90); HRMS: *m/z* calcd for $C_{13}H_{13}N_2O_2$ $[M+H]^+$: 229.0972; found: 229.0970.

The reaction was repeated two more times to give L-5-ethynyltryptophan **13** (5.9 mg, 1.3%) and (7.2 mg, 1.6%). Over three runs, this gave an average yield of 1.6%.

4.6. *tert*-Butyl 7-iodo-1*H*-indole-1-carboxylate

Following the procedure reported above, 7-iodoindole was converted to *tert*-butyl 7-iodo-1*H*-indole-1-carboxylate; ^{1}H NMR (500 MHz, $CDCl_3$) δ = 1.68 (9H, s, CH_3), 6.54 (1H, d, *J*(H,H)= 3.7 Hz, ArH), 6.95 (1H, dd, *J*(H,H)= 7.7, 7.7 Hz, ArH), 7.51 (1H, d, *J*(H,H)= 3.7 Hz, ArH), 7.56 (1H, dd, *J*(H,H)= 7.7, 0.9 Hz, ArH), 7.85 (1H, dd, *J*(H,H)= 7.7, 0.8 Hz, ArH); ^{13}C NMR (125 MHz, $CDCl_3$) δ = 28.3 (CH_3), 78.6 (C), 84.4 (C), 107.1 (CH), 121.1 (CH), 124.6 (CH), 129.3 (CH), 133.8 (C), 137.2 (CH), 137.7 (C), 148.3 (CO); HRMS (ESI): *m/z* calcd for $C_{13}H_{18}I_1N_2O_2^+$ $[M+NH_4]^+$: 361.0407; found: 361.0408.

4.7. 7-Ethynyl-1*H*-indole 4

Following the procedure reported above, *tert*-butyl 7-iodo-1*H*-indole-1-carboxylate was converted to 7-ethynyl-1*H*-indole **4** (0.15 g, 70%) as a brown oil; ^{1}H NMR (500 MHz, $CDCl_3$) δ = 3.42 (1H, s, CH), 6.62 (1H, dd, *J*(H,H)= 3.1, 2.2 Hz, ArH), 7.12 (1H, dd, *J*(H,H)= 7.9, 7.3 Hz, ArH), 7.27-7.28 (1H, m, ArH), 7.41 (1H, d, *J*(H,H)= 7.3 Hz, ArH), 7.70 (1H, d, *J*(H,H)= 7.9 Hz, ArH), 8.51 (1H, bs, NH); ^{13}C NMR (125 MHz, $CDCl_3$) δ = 80.5 (C), 81.4 (CH), 103.5 (CH), 105.0 (C), 119.9 (CH), 122.3 (CH), 124.7 (CH), 126.1 (CH), 127.6 (C), 137.1 (C); HRMS (EI): *m/z* calcd for $C_{10}H_7N_1^{+\bullet}$ $[M]^{+\bullet}$: 141.0578; found: 141.0582.

4.8. L-7-Ethynyltryptophan 7

Using the procedure reported above for **13**, 7-ethynyl-1*H*-indole **4** (0.128 g, 0.9 mmol, 1.0 eq) gave L-7-ethynyltryptophan **7** (0.19 g, 90%) as a white solid; $[\alpha]_D$ +64.2° (c 0.55, MeOH); ^{1}H NMR (500 MHz, d_6-DMSO) δ = 2.83 (1H, bs, CH_AH_B), 3.20 (1H, bd, *J*(H,H)= 13.2 Hz, CH_AH_B), 3.32 (1H, bs, CH), 4.41 (1H, s, CH), 6.96 (1H, dd, *J*(H,H)= 7.6, 7.5 Hz, ArH), 7.19-7.20 (2H, m, ArH), 7.61 (1H, bd, *J*(H,H)= 7.8 Hz, ArH), 11.03 (1H, bs, NH); ^{1}H NMR (500 MHz, CD_3OD) δ = 3.05-3.10 (1H, m, CH_AH_B), 3.39-3.43 (1H, m, CH_AH_B), 3.72-3.74 (1H, m, CH), 3.74 (1H, s, CH), 7.01 (1H, dd, *J*(H,H)= 7.8, 7.5 Hz, ArH), 7.24 (1H, s, ArH), 7.25 (1H, d, J(H,H)= 7.3 Hz, ArH), 7.75 (1H, dd, *J*(H,H)= 8.0, 0.8 Hz, ArH); ^{13}C NMR (125 MHz, CD_3OD) δ = 30.0 (CH_2), 57.1 (CH), 80.8 (C), 81.2 (C), 82.2 (CH), 106.6 (C), 111.6 (C), 119.8 (CH), 120.9 (CH), 125.7 (CH), 126.6 (CH), 128.7 (C), 138.9 (C), 177.5 (CO); MS (ESI) 229 (100) $[M+H]^+$; HRMS: *m/z* calcd for $C_{13}H_{13}N_2O_2$ $[M+H]^+$: 229.0972; found: 229.0969.

L-7-Ethynyltryptophan **7** exhibited some unusual NMR behavior; When the ^{1}H NMR spectrum was recorded in CD_3OD, the signal for the alkyne proton at δ_H 3.74 ppm gave a decreased integral to what was expected (0.2 rather than 1.0). Increasing the relaxation time of the NMR spectrum (from 1 s to 4 s) did not increase the intensity of the signal as may have been expected, but removed the signal for the alkyne proton entirely. When the ^{13}C NMR was recorded, the alkyne signals were visible at δ_C 81.21 and 82.23 ppm, along with a new, broader peak at δ_C 80.76 ppm. When the spectrum was recorded in d_6-DMSO, the alkyne proton signal was apparent at δ_H 4.41 ppm and had no decrease in the NMR integration, relative to the aromatic protons. However, when the ^{13}C NMR was recorded in d_6-DMSO, two alkyne signals were observed (at δ_C 80.82 and 84.09 ppm), but this time, the carbon signals for the tryptophan side chain has disappeared. We are unsure what the cause of this unusual NMR behaviour. L-5-ethynyltryptophan **13** exhibited no such behaviour in the NMR spectra.

Enantiopurity analysis

The enantiopurity of the generated tryptophan derivatives was analysed using Marfey's Reagent (1-fluoro-2,4-dinitrophenyl-5-L-alanine amide, FDAA).

The tryptophan was dissolved in HCl solution (1M, 10 mg/mL). A vial containing the tryptophan (50 μL), FDAA (1% *w/v* in actone, 100 μL) and $NaHCO_3$ solution (1M, 70 μL) was mixed thoroughly and incubated at 37 °C for 1 h. The reaction mixture was diluted (10 μL in 490 μL water) and centrifuged (13,000 rpm, 5 min) before UPLC analysis.

The UPLC analysis was carried out in acetonitrile/0.1% TFA in water at a gradient of 20% acetonitrile to 90% acetonitrile over 4.7 minutes. A 3:1 mixture of L-tryptophan to D-tryptophan was used as a standard. Two blank runs using acetone were conducted between each tryptophan sample. In all of the tryptophans analysed (with the exception of L-5-ethynyltryptophan **13**), only a single peak for the L-enantiomer was observed (see supporting information). In the case of L-5-ethynyltryptophan **13**, decomposition appeared to occur when subjected to the analysis conditions. Given the similar optical rotations between **7** and **13**, we are confident that no racemization of the stereocentre had occurred during the biotransformation reaction.

Use of DL-serine

The biotransformation was carried out on indole using either L-serine (2.5 eq) or DL-serine (2.5 eq). The number of equivalents of serine was doubled from a normal biotransformation to ensure that enough L-serine was present for the reaction to go to full conversion (for the case of DL-serine). In both reactions, comparable quantities of L-tryptophan were isolated; L-tryptophan (0.120 g, 29%) was isolated from the reaction with L-serine, L-tryptophan (0.153 g, 37%) was isolated from the reaction with DL-serine. Analysis of the enantiopurity of the tryptophans using Marfey's reagent showed that in both cases, only L-typtophan was produced. The use of DL-serine is possible in the reaction and shows no detriment to yield or enantiopurity.

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Supplementary Material

Supplementary data (^{1}H, $^{19}F\{^{1}H\}$ and ^{13}C NMR, as well as Marfey's reagent analysis) associated with this article can be found, in the online version, at xxxx.

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