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Synthesis of 2-D-*L*-Tryptophan by Sequential Ir-Catalyzed Reactions

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Abstract

Herein, we report a practical synthesis of 2-D-*L*-tryptophan *via* sequential Ir-catalyzed C–H borylation, and Ir-catalyzed C-2-deborylative deuteration steps. In this synthetic sequence, deprotection of the Boc and methyl ester groups proved challenging, due to replacement of deuterium with hydrogen. However, mild deprotection conditions were developed to avoid this D/H scrambling. Further, 2-D-*L*-Tryptophan is stable in many buffers used for biological studies.

Graphical Abstract

Keywords

L-Tryptophan; C–H Borylation; Deborylative deuteration; Metal-Catalysis; Deuterium labeling

1. Introduction:

Deuterated compounds possess many uses in synthetic organic, biological and medicinal chemistries. In synthetic chemistry, deuterated compounds are used (i) to study reaction mechanisms through experimentally observed kinetic isotopic effects,¹ and by deuterium labeling experiments,² (ii) as analytical standards in quantitative analysis of C-deuterated drugs,³ (iii) to alter chemoselectivity and regioselectivity in the synthesis of natural products,⁴ and (iv) to aid in the structural elucidation of peptides.⁵ In biological systems, deuterium can enhance absorption, distribution, metabolism and excretion properties (ADME) of bioactive molecules,^{6,7} which can lower the doses and subsequent toxicity of drugs.⁸ In fact, a deuterium-containing drug, deutetetrabenazine, was approved recently to

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5. Supplementary data:

¹H NMR, ¹³C NMR and ²H NMR for new compounds **12** and **5** and can be found at <https://doi.org/10.1016/j.tet.2019.02.054>.

treat chorea associated with Huntington's disease.⁹ Deudextromethorphan (AVP-786), another deuterium-containing drug candidate, is currently in phase II and III clinical trials for the treatment of residual schizophrenia, intermittent explosive disorder, neurobehavioral disinhibition in patients with traumatic brain injury, and agitation in patients with Alzheimer's dementia.^{10,11}

Various deuterated tryptophan (Trp) analogs have been used to study: (i) protein structure by neutron scattering,¹² (ii) amino acid metabolism,¹³ and (iii) enzymatic mechanisms.¹⁴ Though these studies have been predominantly conducted with perdeuterated⁵ probes containing or deuterium at C4 and C5 positions,¹⁴ C-2-deuterated L-Trp might provide more insights into the biosynthesis of Trp-derived natural products, particularly in steps that involve indole C2–C3 bond cleavage.¹⁵ However, no strategy exists to selectively generate 2-D-Trp. Traditional methods to incorporate deuterium on aromatic and heteroaromatic compounds, such as hydrogen-deuterium exchange and metal-halogen exchange followed by deuteration, are not suitable for selective C-2 deuteration of Trp, as those methods suffer from poor selectivity, harsh reaction conditions, and poor functional group tolerance.¹⁶ A minor amount of deuteration at C-2 (7%) was observed of Trp in an attempt to regioselectively deuterate the C-4 position using photochemical conditions; though optimization of this side reaction was not pursued.¹⁷ A possible synthesis of Trp-derivatives with 2-D substitution on the indole ring might involve electrophilic substitution at the C-3 carbon of C-2-deuterated indole with *L*-Serine followed by enzymatic resolution.¹⁸ However, this strategy suffers complete erosion of enantioselectivity in the first step and requires prior deuteration of the indole.¹⁹ In contrast, we envisioned that a more practical approach might involve late-stage deuteration of a C-2-metallated Trp derivative.²⁰ Herein, we describe the selective synthesis of 2-D-*L*-Trp starting from *L*-Trp, using sequential Ir-catalyzed reactions and SiO₂-assisted global deprotection.

2. Results and Discussion:

We envisioned that protected Trp (**1**) could convert to the C2-deuterated analog via sequences involving either (i) bromination and subsequent Pd-catalyzed debromodeuteration,²¹ or (ii) C2-selective Ir-catalyzed C–H borylation, then Ir-catalyzed deborylative deuteration. Though both 2-Br-Trp and 2-Bpin-Trp are known, the subsequent C–D bond forming reactions have not been tested on Trp derivatives. Finally, acid-mediated global deprotection would furnish the target compound (Scheme 1), though acid-catalyzed D/H scrambling of the C-2 position would ultimately complicate this reaction.

Synthetic work commenced by esterification of *L*-Trp (**6**)²² followed by N-acetylation.²³ Initial attempts to brominate *N*-acetyl-*L*-tryptophan methyl ester at 2-position provided low yield of desired product. In contrast, use of a trifluoroacetyl protecting group enabled bromination in modest yield.²⁴ However, Pd-catalyzed debromodeuteration did not provide deuterated tryptophan (**9**) using previously reported conditions (Scheme 2).^{21,25}

After exploring the Pd-catalyzed route, we focused on a second strategy involving sequential Ir-catalyzed borylation of arenes^{26–29} and deborylative deuteration.^{28b} *N*-Boc-*L*-Trp methyl ester (**10**) was subjected to Ir-catalyzed microwave-assisted borylation conditions with

B₂Pin₂ to provide 2-Bpin-Trp derivative (**11**) as the major product, along with a small quantity of the 2,7-diborylated product, which was separated by column chromatography. The 2-Bpin-Trp derivative (**11**) was then treated with [Ir(OMe)(cod)]₂ in THF/D₂O (6:1) to provide 2-D-*L*-Trp methyl ester (**12**, Scheme 3) in 90% purity, with nondeuterated N-Boc-Trp-OMe (**10**) remaining as an inseparable impurity.

Subsequent deprotection proved challenging due to an unexpected C–D to C–H scrambling event under acidic conditions. Specifically, removal of the Boc group with aqueous HCl facilitated D replacement with H to produce *L*-Trp methyl ester (**13**).²⁷ Similar D/H scrambling occurred, using TFA in dichloromethane in the presence of tertiary butyl cation scavengers (dimethyl sulfide and 1,2-ethane dithiol), though these conditions did not remove the Boc group.²⁸ However, treatment of compound **12** with SiO₂ in D₂O²⁹ at 140 °C for 10 h not only suppressed D/H scrambling, but also both removed the Boc group and hydrolyzed the methyl ester to provide the target compound 2-D-*L*-Trp (**5**) in excellent yield (Scheme 4).

Despite the instability towards strong acids, compound **5** is stable to many buffers relevant for biological studies. To establish the stability of the compound 2-D-*L*-Trp (**5**) in acidic, basic and buffer solutions, we treated the 90% pure compound with 1.0 N HCl, 1.0 N NaOH and with various buffers that are generally used in biological studies. By ¹H NMR of the crude reaction mixtures, scrambling occurred in 1.0 N HCl. However, no D/H scrambling occurred in 0.1 N Na₂HPO₄, 1.0 N NaOH, TBS, PBS, HEPES buffers (Table 1). As such, 2-D-*L*-Trp (**5**) should serve use in biological studies.

3. Conclusion:

In conclusion, we prepared 2-D-*L*-Trp (**5**) using a selective C–H activation and functionalization strategy *via* Ir-catalyzed borylation and selective deborylative deuteration. To overcome D scrambling during acidic deprotection and work up, we identified neutral conditions for deprotection that retained D at the C2 position. This deuterated probe is stable in several media used for biological studies and should serve many uses in chemical biology.

4. Experimental Section:

Section A: General Considerations

Unless otherwise noted, all reactions were performed using oven-dried glassware under an atmosphere of dry N₂. Ir-catalyzed deborylative deuteration reaction was performed in a resealable 15 mL screw-top vial sealed with a PTFE septum. All other reactions were performed in round-bottom flasks sealed with rubber septa. Stainless steel syringes were used to transfer air- or moisture-sensitive liquid reagents. Reactions were monitored by thin-layer chromatography (TLC) on UNIPLATE™ Silica Gel HLF 250-micron glass plates precoated with 230–400 mesh silica impregnated with a fluorescent indicator (250 nm) and visualized by UV irradiation (254 nm). A CombiFlash® RF-4× purification system was used for chromatographic purifications. Silica gel was purchased from Sorbent Technologies (cat. #30930M-25, 60 Å, 40–63 μm). Unless otherwise noted, reagents were purchased from commercial sources and used as received. Anhydrous acetonitrile (CH₃CN), methanol

(MeOH), dichloromethane (DCM), tetrahydrofuran (THF), toluene, and triethylamine (NEt₃) were dispensed from a solvent purification system, in which the solvent was dried by passage through two columns of activated alumina under argon.

Melting points were measured using a Thomas-Hoover melting point apparatus. Proton nuclear magnetic resonance (¹H NMR) spectra, and carbon nuclear magnetic resonance (¹³C NMR) spectra were recorded on a Bruker 500 AVANCE spectrometer (500 and 126 MHz, respectively) or a Bruker 400 AVANCE spectrometer (400 and 101 MHz, respectively). Chemical shifts (δ) for protons are reported in parts per million (ppm) and are respectively referenced to the proton resonance of residual MeOH or H₂O in the NMR solvent (δ = 3.31 ppm, and 4.87) and for D₂O (δ = 4.79 ppm). Chemical shifts (δ) for carbon are reported in ppm and are referenced to the carbon resonances of the CDCl₃ solvent peak (δ = 77.16 ppm). NMR data are represented as follows: chemical shift (ppm), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, sept = septet, m = multiplet), coupling constant in Hertz (Hz), and integration. Exact mass determination was obtained by the following method: electron impact ionization (EI) on a ZG analytical ZAB mass spectrometer or electrospray ionization (ESI) on a SI-3 Waters LCT Premier™ mass spectrometer.

Section B: Experimental Procedures

Compounds **7**, **8**,^{22,24} **10**, and **11**²⁹ were prepared based on the literature procedure.

Methyl (tert-butoxycarbonyl)-L-tryptophanate-2-D (12)—To a 20 mL microwave vial were added 2-BPin-WBoc-*N*-tryptophan methyl ester (**11**) (0.60 g, 1.35 mmol, 1 equiv) and [Ir(cod)(OMe)]₂ (18.0 mg, 0.027 mmol, 2 mol%). The vial was sealed, evacuated and backfilled with N₂ three times. Dry THF (6.0 mL) was added, followed by D₂O (1.0 mL). The reaction mixture was stirred at 80 °C for 2.5 h. Before unsealing the vial, the pressure was released by slowly inserting an open needle. Water (10.0 mL) was added to the reaction mixture and the aqueous layer was extracted with EtOAc (3 × 20.0 mL). The combined organic layer was then washed with brine (20.0 mL), dried over MgSO₄, filtered, and adsorbed onto 2.0 g silica gel. Purification by silica gel chromatography (EtOAc/hexanes: 0% to 40%) yielded a tan solid (352.0 mg, 82% yield with 90% compound **12** and 10% nondeuterated *N*-Boc-Trp-OMe (**10**) impurity). m.p.: 145–146 °C. ¹H NMR (400 MHz, MeOD) δ 7.53 (d, *J* = 8.0 Hz, 1 H), 7.34 (d, *J* = 8.0 Hz, 1H), 7.11 (t, *J* = 8.0 Hz, 1 H), 7.03 (t, *J* = 8.0 Hz, 1 H), 4.44 (t, *J* = 8.0 Hz, 1H), 3.65 (s, 3H), 3.27 (dd, *J* = 8.0 Hz, 4.0 Hz, 1H), 3.14 (dd, *J* = 8.0 Hz, 8.0 Hz, 1H), 1.39 (s, 9H). ¹³C NMR (126 MHz, CDCl₃) δ 172.8, 155.2, 136.0, 127.6, 122.2, 119.6, 118.7, 111.2, 110.0, 79.9, 54.1, 52.3, 28.3, 27.9. ²H NMR (126 MHz, MeOH) δ 7.06. HRMS (ESI) calculated for C₁₇H₂₁DN₂O₄K [M+K]⁺ m/z 358.1279, found m/z 358.1295, 4.5 ppm.

(S)-2-Ammonio-3-(1H-indol-3-yl-2-d)propanoate (5)—To a solution of Methyl (tert-butoxycarbonyl)-*L*-tryptophanate-2-D (**12**) (10.0 mg, 0.031 mmol, 1.0 equiv) in DCM (1.0 mL) was added silica gel (66.0 mg, 60 Å). The solvent was removed under reduced pressure to adsorb compound onto silica gel. In a 5 mL round bottom flask equipped with a water condenser, the adsorbed material was suspended in 1.0 mL D₂O and heated to 140 °C for 10 h. The reaction mixture was cooled to room temperature, then filtered by Buckner funnel

under vacuum and washed with H₂O (1.0 mL) and DCM (3 × 2.0 mL) to remove the silica gel. The filtrate was diluted with H₂O (3.0 mL) to separate layers. The aqueous layer was further extracted with DCM (2 × 3.0 mL) to remove any organic impurities present in the solution. The aqueous layer was then dried under vacuum to provide the desired compound **5** as a white solid (5.7 mg, 90%). m.p.: 245–246 °C. ¹H NMR (400 MHz, D₂O) δ 7.71 (d, *J* = 8.0 Hz, 1 H), 7.51 (d, *J* = 8.0 Hz, 1 H), 7.27 (t, *J* = 8.0 Hz, 1 H), 7.19 (t, *J* = 8.0 Hz, 1H), 4.02 (dd, *J* = 8.0, 4.0 Hz, 1H), 3.47 (dd, *J* = 16.0, 8.0 Hz, 1H), 3.23 (dd, *J* = 16.0, 8.0 Hz, 1H). ¹³C NMR (126 MHz, D₂O) δ 174.7, 136.25, 126.6, 124.9, 122.0, 119.4, 118.4, 111.9, 107.3, 55.0, 26.4, 7.1. ²H NMR (126 MHz, MeOH) δ 7.18. HRMS (ESI) mass calculated for C₁₁H₁₁DN₂O₂ [M-H]⁺ *m/z* 204.0883, found *m/z* 204.0891, 3.9 ppm.

Procedure for 2-D-*L*-Trp (5**) Stability Test**—In a 1-dram vial, 2-D-*L*-Trp (**5**, 0.05 M) was added to the reagent (1.0 N HCl, 0.1 M Na₂HPO₄, 1.0 N NaOH, TBS buffer, PBS buffer, HEPES buffer) and stirred for 24 h at room temperature. The solvent was removed *in vacuo* to obtain crude material as solid. The material was re-dissolved into D₂O to obtain a ¹H NMR spectrum.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Scheme 1:
Synthetic Plan to Synthesize 2-D-L-Trp (5)

Conditions:

- 10 mol% $\text{Pd}(\text{dba})_2$, SPhos, MeOD
 K_2CO_3 , MeCN, $100\text{ }^\circ\text{C}$, MW, 2.5 h
- 0.5 mol% $[\text{Pd}(\text{allyl})(\text{Cl}_2)]_2$, IMes·HCl
 MeOD, Cs_2CO_3 , PhMe, $40\text{ }^\circ\text{C}$, 16 h
- 1.0 mol% $\text{Pd}(\text{OAc})_2$, Ad_2PBn , MeOD
 K_3PO_4 , $80\text{ }^\circ\text{C}$, 16 h

Scheme 2:

Attempted Preparation of Protected 2-D-*L*-Tnp Analog by Pd-Catalyzed
 Debromodeuteration

Scheme 3:

Preparation of 2-D-*L*-Trp Methyl Ester (12) Using Ir-Catalyzed C–H Borylation and Deuteration Reactions

^a by ¹HNMR of the crude reaction mixture

Scheme 4:
Deprotection of *N*-Boc-2-D-*L*-Trp Methyl Ester (12)

Table 1:Stability Data for 2-D-*L*-Trp (5)

Entry#	Reagent	%D ^a
1	1.0 N HCl	72%
2	0.1 M Na ₂ HPO ₄ (pH 7.4)	89%
3	1.0 N NaOH	90%
4	^b TBS Buffer	90%
5	^c PBS Buffer	90%
6	^d HEPES Buffer	90%
7	^e Tris Buffer	90%

^aby ¹HNMR of the crude reaction mixture.^b50 mM Tris, 150 mM NaCl^c8 mM Na₂HPO₄, 2 mM KH₂PO₄, 2.7 mM KCl, 13.7 mM NaCl^d50 mM HEPES, 150 mM NaCl^e50 mM Tris-HCl, 200 mM NaCl, 5% Glycerol