Deuteration of Indole Compounds: Synthesis of Deuterated Auxins, Indole-3-acetic Acid-d5 and Indole-3-butyric Acid-d5

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ABSTRACT: In this study, we describe a practical and facile synthesis of deuterium-labeled indoles via acid-catalyzed hydrogen−deuterium exchange. 3-Substituted indoles were efficiently deuterated through treatment with 20 wt % D2SO4 in CD3OD at 60−90 °C. A deuterium incorporation reaction of 3-unsubstituted indoles was accomplished through treatment with CD3CO2Da t 150 °C. The in situ preparation of a 20 wt % D2SO4/CH3OD/D2O solution enabled a large-scale and low-cost synthesis of auxins, indole-3-acetic acid-d5 and indole-3-butyric acid-d5.

INTRODUCTION

Deuterium compounds are widely used as an efficient tool to understand the biosynthesis of natural products, reaction mechanism, pharmacokinetics, and metabolism of bioactive compounds, by taking advantage of their features in NMR and mass spectroscopies. Besides their use in chemical kinetics, deuterated drugs have recently attracted much attention because of the deuterium pharmacokinetic isotope effect, which lowers the rate of metabolism and the distribution of metabolites. Indeed, deutetrabenazine has recently been approved by the United States Food and Drug Administration, and several deuterated compounds are currently being tested in clinical trials. An indole skeleton is one of the most common structures observed in biologically active molecules. Thus, deuterated indole compounds are in demand not only by organic chemists but also by bioorganic chemists. Particularly, polydeuterium-labeled indoles are useful for tracing the biotransformation of bioactive indoles. To access a deuterium-labeled indole, a hydrogen−deuterium (H−D) exchange reaction is considered more effective than the multistep synthesis from originally deuterium-labeled small synthons. However, 3-unsubstituted indoles cannot be exposed to acidic conditions because of their high reactivity at the 3-position. The polydeuteration of indoles, including 3-unsubstituted indoles, has been achieved using a transition metal catalyst. Although such catalytic conditions are effective for small-scale preparations, the required pyrophoric and flammable reagents and/or high-pressure conditions might not be suitable for large-scale synthesis.

In the course of our study on the biological role and biosynthesis of auxins such as indole acetic acid (IAA, 1) and indole butyric acid (IBA, 2) in plants, we required deuterium-labeled IAA (3) and IBA (4). Thus, we attempted to prepare a labeled compound via Fischer indole synthesis as a key step from commercially available nitrobenzene-d5 (Scheme 1).
Although we successfully prepared the desired deuterium-labeled IBA (4), the deuteration degree was dramatically reduced under the Fischer indole synthesis condition, 1% H$_2$SO$_4$ in the CH$_3$OH solution.\textsuperscript{11} To our surprise, all deuteriums attached to the indole at C4–C7 positions were partially replaced by protons. The H–D replacement at the C2 position, usually reactive under acidic conditions, also occurred and demonstrated a slight D incorporation (C2:13%, C4:95%, C5:49%, C6:91%, and C7:76% D incorporation). These results indicate that the deuteriums of IBA methyl ester 5 and CH$_3$OH proton were exchanged under acidic conditions. This CD$_3$OD promoted the incorporation of deuteriums at the C2 position, usually reactive under acidic conditions, and demonstrated a slight D incorporation (C2:13%, C4:95%, C5:49%, C6:91%, and C7:76% D incorporation). These results indicate that the deuteriums of IBA methyl ester 5 and CH$_3$OH proton were exchanged under acidic conditions. This inspired a new idea for preparing deuterium-labeled indoles: indole compounds would incorporate deuterium from CD$_3$OD in the presence of an appropriate acid catalyst. This method could be applicable to biologically important indole compounds, such as IAA (1), tryptophan (6), and other substituted indoles. In this manuscript, we describe a practical and facile method to prepare deuterium-labeled indoles via acid-catalyzed H–D exchange reactions.

### RESULTS AND DISCUSSION

On the basis of the aforementioned idea, we first attempted the deuteration of IBA (2). Thus, 2 was dissolved in a variety of H$_2$SO$_4$/CD$_3$OD solutions, and the reaction was monitored by $^1$H NMR spectra. The treatment of 2 with 2.4 wt % H$_2$SO$_4$ in CD$_3$OD promoted the incorporation of deuteriums at the C2 and C4–C7 positions, even at room temperature, albeit with low D content. Upon continuous stirring at 60 °C for 65 h, IBA CD$_3$ ester 7 with good D incorporation (77% average D incorporation at C2 and C4–C7) was obtained (Table 1, entry 1).

| entry | H$_2$SO$_4$ (%) | time (h) | D content (%)$^a$ | yield (%)$^b$ |
|-------|----------------|----------|-------------------|--------------|
| 1     | 2.4            | 65       | 77$^c$            | n.d.$^d$     |
| 2     | 9.7            | 110      | 91$^c$            | n.d.$^d$     |
| 3     | 20             | 45       | 82$^c$            | 98           |
| 4$^f$ | 20             | 45       | 81$^c$            | n.d.$^d$     |
| 5     | 20$^g$         | 18       | 97$^c$            | 99           |
| 6$^g$ | 20$^g$         | 18       | 93$^c$            | 93           |

$^a$Indicated as the average D incorporation at C2 and C4–C7.
$^b$Isolated yield. D content was calculated based on the $^1$H NMR spectra of the reaction mixture. $^c$Not determined. D content was calculated based on the $^1$H NMR spectra of the isolated product.
$^d$With light shielding.
$^e$CD$_3$OD was used instead of H$_2$SO$_4$.
$^f$The reaction was performed in a 0.3 M solution.

The use of 9.7 wt % H$_2$SO$_4$ accelerated D incorporation. After reaction at 60 °C for 110 h, the average D incorporation reached a high of 91% (entry 2). Using 20 wt % D$_2$SO$_4$ instead of H$_2$SO$_4$ as the acid catalyst accelerated the reaction rate and improved D incorporation (97% average D incorporation, entry 5). Increasing the IBA concentration from 0.1 to 0.3 M slightly decreased the yield and D incorporation (93% average D incorporation, entry 6).

Once we achieved optimized reaction conditions, the substrate scope in the H–D exchange reaction was investigated, and the results are summarized in Table 2. 3-Substituted indole compounds, such as IAA (1), 1-tryptophan (6), N$_2$O-dimethyl-IBA (12), indomethacin (14), and yohimbine hydrochloride (16), were effectively deuterated in good yields with high D incorporation (>91% yield, >95% average D incorporation at C2, C4–7, entries 1–3 and 5–7). The H–D exchange reaction of 1-tryptophan (6, >99% ee) was relatively slower than those of other indoles, presumably due to the presence of the amino group (entry 2). The optical purity was determined by chiral high-performance liquid chromatography (HPLC) analysis and was almost maintained (98% ee). When 6 was treated at 90 °C under the same acidic conditions, the reaction time was shortened to 20 h, and the corresponding deuterated tryptophan CD$_3$ ester (9) was obtained in 99% yield without reducing the optical purity (>99% ee, entry 3). As predicted, introduction of an electron-withdrawing group reduced the reactivity; however, the reaction proceeded well and provided deuterated indole 11 with good D incorporation except for the C7 position (entry 4). 3-Substituted N-methylindole (12) was smoothly deuterated with high D incorporation (entry 5). An acyl group on indole nitrogen was removed under this reaction condition and then the H–D exchange reaction proceeded smoothly because of increasing the electron density (entry 6). In this reaction, C2 methyl protons were replaced with deuterium.\textsuperscript{13} As a result, it was found that various 3-substituted indoles can be effectively deuterated with a high degree of deuterium. Meanwhile, the H–D exchange reaction of carbazole (18) was remarkably slow and required more than 120 h at 90 °C to complete the reaction. Repeating the H–D exchange reaction resulted in increased D incorporation, but the yield decreased (entries 8, 9). Treatment of harmine hydrochloride (20), a naturally occurring β-carboline alkaloid, under this H–D exchange reaction provided deuterated harmine 21 in good yield (entry 10). However, only C6 and C8 protons were selectively exchanged to deuterium due to low electron density at other positions. Contrary to the results of 3-substituted indoles, C3-unsubstituted indoles, such as 2-methylindole (22) and 5-methylindole (23), resulted in complex mixtures under these conditions (entries 10 and 11). High nucleophilicity at the C3 position could cause side reactions.

Therefore, for the H–D exchange reaction of 3-unsubstituted indoles, we considered using weaker Bronsted acid, such as acetic acid and substituted acetic acid, which could act as both an acid catalyst and a source of deuterium (Table 3). After screening several reaction conditions, we found that treating 3-unsubstituted indoles with CD$_3$CO$_2$D at 150 °C effectively promotes the incorporation of deuteriums at C2–C7 positions. Using more-acidic α-halogenated acetic acids, such as CH$_2$CO$_2$D, CCl$_3$CO$_2$D, and CF$_3$CO$_2$D, resulted in the production of indole dimers and oligomers.\textsuperscript{17} The H–D exchange reactions of 3-unsubstituted indoles with CD$_3$CO$_2$D
Table 2. H–D Exchange Reaction of Indoles and Carbazole

| entry | substrate | temp. (°C) | time | D content (%)a | yield (%)b |
|-------|-----------|------------|------|----------------|------------|
| 1     | ![Image](image1) | 60         | 40 h | ![Image](image2) | 99°        |
| 2     | ![Image](image3) | 60         | 130 h| ![Image](image4) | 99°        |
| 3     | ![Image](image5) | 90         | 20 h | ![Image](image6) | 99°        |
| 4     | ![Image](image7) | 90         | 20 h | ![Image](image8) | 92°        |
| 5     | ![Image](image9) | 60         | 27 h | ![Image](image10) | 99°        |
| 6     | ![Image](image11) | rt         | 12 h | ![Image](image12) | 91°        |
| 7     | ![Image](image13) | 90         | 20 h | ![Image](image14) | 91         |
| 8     | ![Image](image15) | 90         | 120 h| ![Image](image16) | 90         |
| 9     | ![Image](image17) | 90         | 120 h + 48 h | ![Image](image18) | 61         |
| 10    | ![Image](image19) | 90         | 24 h | ![Image](image20) | 94         |
| 11    | ![Image](image21) | rt         | 5 min| complex mixture | complex mixture |
| 12    | ![Image](image22) | rt         | 5 min| complex mixture | complex mixture |

aD content was calculated based on the 1H NMR spectra of the isolated product. bIsolated yield. cIsolated yield of CD3 ester. d98% ee. eThe reaction was conducted in a sealed tube. f>99% ee. gReactions were performed in a 0.05 M solution. hD content was calculated based on 1H NMR spectroscopy after N-methylation. iAfter isolating deuterated carbazole (70% average D incorporation), the H–D exchange reaction of this product was performed again.
Table 3. H–D Exchange Reaction of 3-Unsubstituted Indoles

![Diagram of indole structure with H–D exchange reaction]

| entry | D content (%) | yield (%) |
|-------|---------------|-----------|
| 1     | 77            |           |
| 2     | 81            |           |
| 3     | 92            |           |
| 4     | 96            |           |
| 5     | 57            |           |
| 6     | 78            |           |

Reactions were performed in CD3CO2D (0.1 M) in a sealed tube at 150 °C for 110 h. D content was calculated based on the 1H NMR spectra of the isolated product. Isolated yield.

are summarized in Table 3. The condition was found to be applicable to the indole itself and the substituted indoles bearing an alkyl group at each position. After reacting for 110 h, the corresponding deuterated indoles were obtained with moderate to good D incorporation and yield. In all cases, the C3 position of the indole was highly reactive, and the H–D exchange occurred even when dissolved in CD3CO2D and CDCl3 and also during purification by silica gel column chromatography. Therefore, the C3 deuterium was partially protonated during the isolation process. Introducing an alkyl group at the C2 and C3 positions suppressed that side reaction due to their steric hindrance. In the reaction of 2-methylindole, methyl protons were replaced with deuterium (entry 3), whereas the alkyls on the benzene ring were intact (entries 4–6).

Table 4. Synthesis of Polydeuterated IAA and IBA in an Economical Way

| entry | substrate | solvent | time (h) | D content (%) | yield (%) |
|-------|-----------|---------|----------|---------------|-----------|
| 1     | CH(OCH3)3 | CH(OCH3)3/D2O | 5        | 97            | 99        |
| 2     | CH(OCH3)3 | CH(OCH3)3/D2O | 5        | 97            | 95        |
| 3     | CH(OCH3)3 | CH(OCH3)3/D2O | 14       | 97            | 94        |

Reactions were performed on a 1 gram scale of the substrate. 

In summary, we developed a practical and facile protocol for preparing polydeuterium-labeled indoles through the H–D exchange reaction. These procedures are operationally and economically feasible and can be performed not only by organic chemists but also by researchers in other fields. We believe that the results are valuable to encourage researchers to further investigate chemical biology and drug development. In our laboratories, we are currently investigating the metabolism of indole plant hormones using deuterium-labeled 3-substituted indoles in quantities greater than 1 g.

**CONCLUSIONS**

In summary, we developed a practical and facile protocol for preparing polydeuterium-labeled indoles through the H–D exchange reaction. These procedures are operationally and economically feasible and can be performed not only by organic chemists but also by researchers in other fields. We believe that the results are valuable to encourage researchers to further investigate chemical biology and drug development. In our laboratories, we are currently investigating the metabolism of indole plant hormones using deuterium-labeled indoles.

**EXPERIMENTAL SECTION**

**General Information.** All reagents and solvents were purchased from Tokyo Chemical Industry Co., Ltd.; FUJIFILM Wako Pure Chemical Corporation; Kanto Chemical Co., Ltd.; Sigma-Aldrich Co., LLC; and ISOTEC and used without further purification. Unless otherwise noted, all reactions were conducted without any inert gas. Chromatography was carried out with Wakogel C-200 silica gel (FUJIFILM Wako Pure Chemical Corporation, granule, 0.075–0.150 mm). NMR spectra were recorded at 600, 500, and 400 MHz for 1H and 150, 125, and 100 MHz for 13C on JEOL JNM-ECA600, -ECA500, and -ECZ400R spectrometers.

(7/3, 0.2 M) at 95 °C for 5 h followed by saponification with aqueous LiOH effectively provided deuterated IBA (4, 97% average D incorporation at C2, C4–C7) in 99% yield (Table 4, entry 1). Next, we turned our attention to the use of HC(OCH3)3 as a source of CH3OD and the in situ preparation of this solvent system (entry 2). Indeed, a D2SO4/CH3OD/D2O mixture was prepared by treating HC(OCH3)3/D2O with D2O in the presence of D2SO4, followed by removing HCO2CH3 via common distillation in an Ar atmosphere. In this media, the reaction of 2 proceeded as smoothly as under original conditions and successfully provided deuterated IBA (4) in a high yield with a high D content (95% yield, 97% average D incorporation). The effective deuteration of IAA (1) with this procedure was also attained (entry 3). The reaction conditions provide economical ways to access deuterium-labeled 3-substituted indoles in quantities greater than 1 g.
respectively. Chemical shifts were reported in parts per million (ppm, δ) relative to the residual solvent peaks of CD3OD (3.31 ppm for 1H NMR), 49.0 ppm for 13C NMR) and (CD3)2CO (2.05 ppm for 1H NMR, 206.26 ppm for 13C NMR), and coupling constants (J values) were given in Hertz. High-performance liquid chromatography was carried out with a PU-2089 plus HPLC pump (JASCO), an LC–NetII/ADC (JASCO), and a UV–2075 plus UV/vis detector (JASCO). High-resolution mass spectra (HRMS) were measured on a JEOL Accu TOF T-100 equipped with an electrospray ionization (ESI) unit.

Preparation of Deuterated 3-Substituted Indole CD3 Ester (Tables 1 and 2). A solution of 3-substituted indoles with 20 wt % D2SO4 in CD3OD (0.1 M) was heated at 60 °C in an NMR tube or 90 °C in a sealed tube. The reaction was monitored by 1H NMR spectroscopy. After the reaction was completed, the reaction mixture was slowly poured into a saturated aqueous NaHCO3 solution (10 mL). The resulting organic extracts were washed with brine, dried over anhydrous Na2SO4, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel with hexane/AcOEt (3:7, UV 282 nm, diethylamine) = 3:7, UV 282 nm, 1.0 mL/min, 25 °C, L-isomer retention time 1.0 h, D isomer retention time 0.5 h.

Deuterated IBA CD3 Ester (7). Treatment of IBA (2) (10.7 mg, 0.053 mmol) with 20 wt % D2SO4 in CD3OD at 60 °C afforded deuterated IBA CD3 ester 7 (11.8 mg, 99% yield, 97% average D incorporation at C2 and C4).

Deuterated N-methyl-IBA CD3 Ester (8). Treatment of IIA (1) (8.8 mg, 0.050 mmol) with 20 wt % D2SO4 in CD3OD at 60 °C afforded deuterated IIA CD3 ester (9.9 mg, 99% yield).

Deuterated t-Trp CD3 Ester (9). Treatment of t-triptophan (6) (11.2 mg, 0.055 mmol) with 20 wt % D2SO4 in CD3OD at 90 °C in a sealed tube afforded deuterated t-Trp CD3 ester (9) (12.5 mg, 99% yield, >99% ee).

Deuterated 2-(5-Methoxy-2-methylindole-3-acetic Acid) CD3 Ester (15). Treatment of indomethacin (14) (17.9 mg, 0.05 mmol) with 20 wt % D2SO4 in CD3OD at room temperature afforded deuterated 2-(5-methoxy-2-methylindole-3-acetic acid) CD3 ester (15) (10.8 mg, 91% yield).

Deuterated Yohimbine (17). Treatment of yohimbine hydrochloride (16) (39.1 mg, 0.1 mmol) with 20 wt % D2SO4 in CD3OD at room temperature afforded deuterated yohimbine (17) (32.8 mg, 91% yield).
N-Methylation of Deuterated Indole. To a solution of deuterated indole (24) (15.1 mg, 0.13 mmol) in THF (1.3 mL) was added NaH (60% oil dispersion, 8 mg, 0.20 mmol) at 0 °C under an Ar atmosphere. After being stirred at 0 °C for 20 min, methyl iodide (17 μL, 0.27 mmol) was added and stirred at rt for 1 h. The reaction was quenched with a saturated aqueous NH₄Cl solution (5 mL) and extracted with AcOEt (5 mL × 3). The combined organic extracts were washed with brine, dried over anhydrous Na₂SO₄ and concentrated under reduced pressure to give crude N-methylindole (31). Deuterium content was calculated based on the ¹H NMR spectra of this crude mixture. ¹H NMR (600 MHz, acetone-d₆) δ 7.57−7.53 (0.86H), 7.39−7.35 (0.68H), 7.21−7.19 (0.02H), 7.18−7.12 (0.58H), 7.06−7.00 (0.72H), 6.41 (s, 0.57H), 3.82 (s, 3H).

Deuterated 1-Ethylindole (25). A solution of 1-ethylindole (20.5 mg, 0.14 mmol) in CD₃CO-D₂ (1.4 mL, 0.1 mL) was heated at 150 °C to afford deuterated 1-ethylindole (25) (16.6 mg, 81% yield). ¹H NMR (400 MHz, acetone-d₆) δ 7.56−7.53 (0.74H), 7.44−7.40 (0.53H), 7.28 (d, J = 3.2 Hz, 0.12H), 7.16−7.11 (0.49H), 7.03−6.98 (0.26 H), 6.43−6.41 (0.81 H), 4.24 (q, J = 7.4 Hz, 2H), 1.41 (t, J = 7.4 Hz, 3H). ¹C NMR (100 MHz, CDCl₃) δ 135.6, 128.5, 126.7 (t, J = 27.8 Hz, 121.2 (t, J = 11.1 Hz), 120.9, 119.1, 109.1, 100.8, 40.9, 15.4. HRMS (ESI-TOF) m/z [M + H]⁺ calcd for [C₁₉H₁₆D₅N⁺] 148.1066, found 148.1069.

Deuterated 2-Methylindole (26). A solution of 2-methylindole (22) (14.7 mg, 0.11 mmol) in CD₃CO-D₂ (1.1 mL, 0.1 mL) was heated at 150 °C to afford deuterated 2-methylindole (26) (13.8 mg, 92% yield). ¹H NMR (500 MHz, CD₂Cl₂) δ 7.39−7.35 (0.34H), 7.23 (s, 0.30H), 6.99−6.96 (0.04H), 6.93−6.89 (0.10H), 6.09 (s, 1H), 2.38−2.35 (m, 0.14H). ¹C NMR (125 MHz, CD₂Cl₂) δ 137.9, 136.4, 130.5, 121.1−118.8 (3C), 110.9 (t, J = 24.4 Hz), 100.1 (m, 12.7, 15.4. HRMS (ESI-TOF) m/z [M + H]⁺ calcd for [C₁₉H₁₄D₄N⁺] 137.1096, found 137.1094.

Deuterated 4-Methylindole (27). A solution of 4-methylindole (12.4 mg, 0.10 mmol) in CD₃CO-D₂ (1.0 mL, 0.1 mL) was heated at 150 °C to afford deuterated 4-methylindole (27) (13.0 mg, 96% yield). ¹H NMR (500 MHz, CD₂Cl₂) δ 7.21−7.16 (0.11H), 6.97 (s, 0.67H), 6.80−6.76 (0.04H), 6.45 (s, 0.53H), 2.50 (s, 3H). ¹C NMR (125 MHz, CD₂Cl₂) δ 137.2, 130.3, 129.1, 124.8−124.1, 122.4−121.5, 119.7 (t, J = 20.3 Hz), 109.5 (t, J = 19.2 Hz), 106.0−100.1, 18.9. HRMS (ESI-TOF) m/z [M + H]⁺ calcd for [C₁₉H₁₄D₅N⁺] 135.0971, found 135.0970.

Deuterated 5-Methylindole (28). A solution of 5-methylindole (23) (13.1 mg, 0.10 mmol) in CD₃CO-D₂ (1.0 mL, 0.1 mL) was heated at 150 °C to afford deuterated 5-methylindole (28) (7.7 mg, 57% yield). ¹H NMR (600 MHz, CD₂Cl₂) δ 7.31 (s, 0.07H), 7.24 (s, 0.33H), 7.16−7.14 (0.02H), 6.93−6.89 (0.03H), 6.32 (s, 0.28H), 2.39 (m, 3H). ¹C NMR (125
Exchange Reaction of 3-Substituted Indole (Table 4).

To a mixture of HC(OCH3)3 (51 mL, 0.47 mol) and D2O at rt for 20 min, generated HCO2CH3 (28.6 mL, 0.47 mol) was removed by general distillation under an Ar atmosphere to prepare 20 wt % D2SO4 in CH3OD/D2O (7/3) solution. A solution of IAA (1) or IBA (2) (1.0 g) in 20 wt % D2SO4 in a CH3OD/D2O (7/3) solution (0.2 M) was heated at 95 °C for 18 h. The mixture was cool to ambient temperature and poured into a 6 wt % LiOH aqueous solution (100 mL). After being stirred at rt for 1 h, 6 N HCl was added to the mixture to be an acidic solution (pH = 2). The resulting mixture was extracted with Et2O (100 mL × 3). The combined organic extracts were washed with brine (100 mL), dried over anhydrous Na2SO4, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (hexane/AcOEt = 1/1) to provide deuterated IAA (3) or IBA (4) (97% average D incorporation at C2 and C4–C7).

Deuterated IBA (4). A solution of IBA (2) (1.0 g, 4.92 mmol) in 20 wt % D2SO4 in a CH3OD/D2O (7/3) solution (24.6 mL, 0.2 M) was heated at 95 °C for 5 h to provide deuterated IBA (4) (967 mg, 95% yield, 97% average D incorporation at C2 and C4–C7) as colorless crystals. 1H NMR (600 MHz, CD3OD) δ 7.55 (s, 0.05H), 7.32 (s, 0.03H), 7.07 (s, 0.03H), 7.01 (s, 0.03H), 6.98 (s, 0.03H), 2.79 (t, J = 7.8 Hz, 2H), 2.34 (t, J = 7.5 Hz, 2H), 1.99 (t, J = 7.8, 7.5 Hz, 2H). 13C NMR (150 MHz, CD3OD) δ 177.8, 138.1, 128.7, 122.7 (t, J = 27.3 Hz), 121.7 (t, J = 23.0 Hz), 119.0 (t, J = 23.0 Hz), 118.9 (t, J = 23.0 Hz), 115.4, 111.8 (t, J = 24.5 Hz), 34.5, 26.9, 25.5. HRMS (ESI-TOF) m/z [M + H]+ calcd for [C10H13D3NO3]^+ 209.1338, found 209.1332.

Deuterated IAA (3). A solution of IAA (1) (1.0 g, 5.77 mmol) in 20 wt % D2SO4 in a CH3OD/D2O (7/3) solution (28.6 mL, 0.2 M) was heated at 95 °C for 14 h to provide deuterated IAA (3) (959 mg, 94% yield, 95% average D incorporation at C2 and C4–C7) as brown solid. 1H NMR (600 MHz, CD3OD) δ 7.54 (s, 0.03H), 7.33 (s, 0.03H), 7.17 (s, 0.03H), 7.09 (s, 0.02H), 7.01 (0.03H), 3.73 (s, 2H). 13C NMR (150 MHz, CD3OD) δ 176.5, 137.9, 128.6, 124.4 (t, J = 27.3 Hz), 121.9 (t, J = 23.0 Hz), 119.3 (t, J = 24.5 Hz), 119.0 (t, J = 24.5 Hz), 111.8 (t, J = 23.0 Hz), 108.7, 31.9. HRMS (ESI-TOF) m/z [M + H]+ calcd for [C10H13D3NO3]^+ 209.1338, found 209.1332.

ASSOCIATED CONTENT

† Supporting Information
The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.1c02940.

Details of the results and 1H and 13C NMR spectra and MS spectra for all deuterated compounds (PDF)

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Notes
The authors declare no competing financial interest.

ACKNOWLEDGMENTS
This work was supported by a Kanagawa University Grant for Joint Research. The authors thank Haruka Yamamoto and Misaki Shimada for their contributions at the early stage of this study.

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