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Synthesis of deuterium labelled diclofenac with improved isotopic enrichment

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A five-step synthesis of deuterium-labelled diclofenac starting from 2-phenyl[$^2H_5$] acetic acid is described. The synthesis prevents deuterium from scrambling during the reaction. It offers the labelled compound with over 99% isotopic enrichment. It also provides a possible alternative route for the synthesis of deuterium-labelled 4′-hydroxydiclofenac, which is the principal human metabolite of diclofenac.

Keywords: deuterium label; diclofenac; improved isotopic enrichment

Introduction

The synthesis of deuterium-labelled diclofenac[1] starting from [$^2H_5$]-bromobenzene was described in the previous publications.[2–5] ^{1}H-NMR analysis revealed that during the cyclization reaction there was some scrambling of deuterium into the other aryl ring[2,3] (average of about one deuterium per molecule). Mass spectrometry indicated a complicated distribution of mass ions. It is desirable to synthesize an internal standard with improved isotopic enrichment (preferably as a single isotopomer).

Results and discussion

Although Friedel–Crafts reaction under mild condition will not result in significant loss or scrambling of deuterium label, the unusual high temperature (150–155°C) cyclization in the previous synthesis of deuterated diclofenac[2] and 4′-hydroxy diclofenc[3] was apparently the main factor that was responsible for the scrambling of deuterium.

The new synthesis of 2-(2-(2,6-dichlorophenylamino)phenyl [$^2H_4$])acetic acid (**6**, deuterium labeled diclofenac) is shown in Scheme 1. In our synthesis, we chose a new route to avoid the Friedel–Crafts reaction used in the previous paper.[2,3] The experiments were conducted similarly to the procedure described for synthesis of 4′-hydroxy diclofenac.[3] The key of the synthesis is a two-step iodination of phenyl acetic acid-d5 to form isotopically pure 2-(2-Iodophenyl[$^2H_4$])acetic acid (**3**), avoiding the high temperature Friedel–Crafts reaction used in previous synthesis. Small amounts of isomers were formed and separated by chromatography after formation of amide. To further prevent the loss of deuterium, trifluoroacetic acid-d was used as a solvent, providing a deuterium environment for the reaction. Compound (**3**) was obtained with isotopic enrichment of 99.4%. Standard conversion of 2-iodophenylacetic acid-d4 (**3**) to *N*,*N*-dimethylamide (**4**)[6] via the acid chloride was followed by Ullmann coupling of (**4**) and 2,6-dichloroaniline using activated copper powder with no deterioration of isotopic purity. The final hydrolysis step required very carefully controlled conditions to avoid cyclization of the product and this was achieved by heating an ethanol solution of (**5**) with 1.0 M NaOH under gentle reflux under argon. The overall yield for the new synthesis was a satisfactory 19%.

^{1}H-NMR analysis of compound (**6**) revealed that the compound has over 99% deuterium enrichment. Mass spectrometry indicated the following distribution of mass ions: 298 (M+4), 97.6%; 297 (M+3), 2.4%; 296 (M+2), 295 (M+1) and 294 (M+0), 0%. The compound provided an excellent internal standard in LC-MS-MS studies.

Experimental

All reagents were obtained from Sigma-Aldrich and CDN Isotope. Mass spectra were recorded using a Quattro micro API mass spectrometer. ^{1}H-NMR spectra were recorded on a Bruker 300 MHz instrument. Chemical purities were determined by an Agilent 1200 HPLC with a XDB-C18 column, 5 μm, 4.6 × 150 mm.

2-(2-Iodophenyl[$^2H_4$])acetic acid (3)

To a solution of phenylacetic acid-d5 (5.0 g, 35 mmol) in trifluoroacetic acid-d (60 mL) in an aluminum wrapped flask, was added $Tl(OCOCF_3)_3$ (38.5 g, 71 mmol). A yellow solution was formed instantaneously. The resulting reaction mixture was stirred at RT overnight and then treated with a solution of KI (13.5 g, 81 mmol) in water (100 mL). A dark blue-purple solution formed. After stirring for 15 min, $Na_2S_2O_5$ was added until the solution was mustard yellow (about 8.4 g of $Na_2S_2O_5$ was used). The reaction mixture was then cooled in an ice bath and basified

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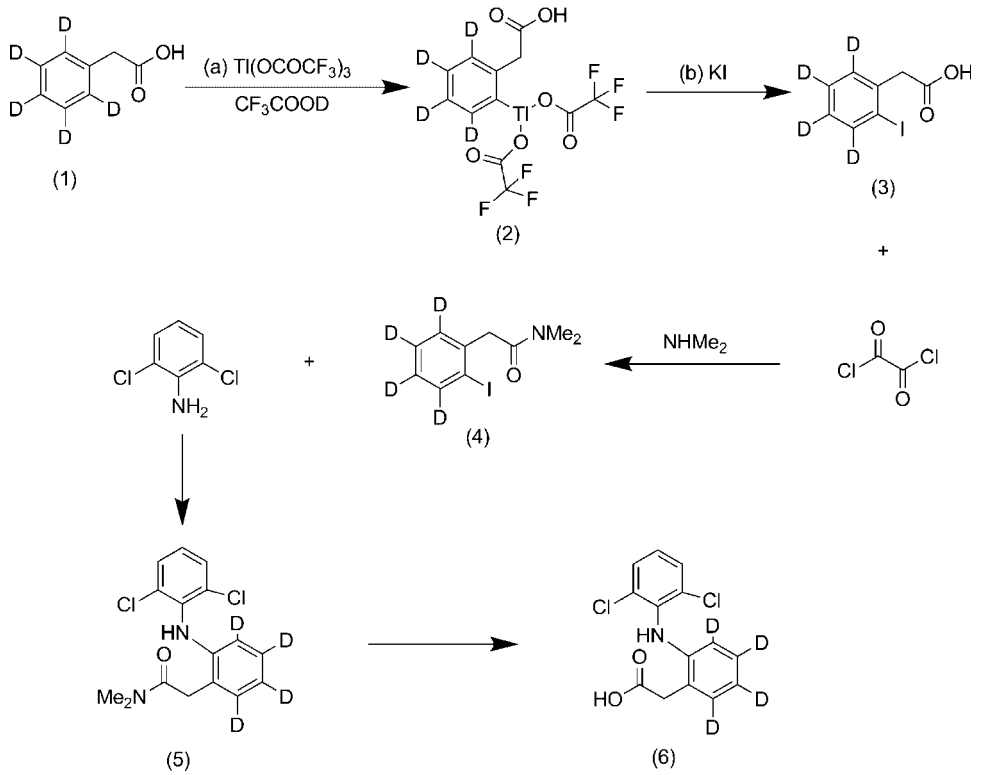

Scheme 1.

with 25% NaOH until pH 9–10, some precipitate started to form and was removed by filtering through a thin layer of Celite. The filtercake was washed with water (3 × 65 mL), the filtrate was cooled in an ice bath and treated slowly with 25% HCl untill pH 4–5. Product started to precipitate and was extracted with diethylether (6 × 400 mL). The combined ether extracts were dried over Na_2SO_4 and concentrated under reduced pressure to afford 7.2 g (76.4%) of (**3**) as an off-white solid.

^{1}H-NMR ($CDCl_3$) ppm:δ3.85 (s, 2H, Ar-C$\underline{H}_2$).

2-(2-Iodophenyl[$^2H_4$])-*N*,*N*-dimethylacetamide (4)

After drying by Azeotropic distillation with toluene, Compound (**3**) (6.3 g, 24 mmol) was dissolved in freshly distilled dichloromethane (141 mL) and was treated with oxalyl chloride (6.0 g, 47 mmol) dropwise under N_2 with stirring at RT. Two drops of DMF were added and carbon dioxide started to evolve immediately. The reaction mixture was stirred at RT for 1 h. TLC analysis showed that the reaction was completed. The reaction mixture was evaporated to dryness under nitrogen, redissolved in dry dichloromethane (30 mL) and evaporated again to remove residual oxalyl chloride. The yellow residue was dissolved in dry dichloromethane (45 mL) and added dropwise to an ice cooled two-phase mixture of dichloromethane (581 mL), water (409 mL), and dimethylamine (141 mL, 40% w/w in water). The mixture was further stirred for 1 h, maintaining the internal temperature of 0–2°C. The reaction mixture was slowly warmed up to room temperature overnight. Layers were separated and the aqueous layer was extracted with dichloromethane (2 × 100 mL). The combined dichloromethane layers were washed with 1 M HCl (2 × 50 mL), saturated Na_2CO_3 solution (2 × 150 mL), water (2 × 150 mL), dried over Na_2SO_4, and concentrated under vacuum to give 10.6 g of yellow oil. The crude product was purified by chromatography on silica gel (300 g) column, eluted with EtOAc/hexanes (400 mL, 1:4; 3.5 L, 3:7), to afford (**4**) as yellow oil (6.0 g, 87%).

^{1}H-NMR ($CDCl_3$) ppm:δ3.80 (s, 2H, Ar-C$\underline{H}_2$), 3.03 (d, J = 9.0 Hz, 6H, –CON(C$\underline{H}_3$)$_2$).

2-(2-(2,6-Dichlorophenylamino)phenyl[$^2H_4$])-*N*,*N*-dimethylacetamide (5)

To a mixture of (**4**) (1.0 g, 3 mmol) and 2,6-dichloroaniline (0.8 g, 5 mmol) was added 20 mL of dry toluene. The mixture was evaporated to dryness. This was repeated one more time. To the residue was added anhydrous K_2CO_3 (0.4 g, 3 mmol), CuI (45.0 mg, 0.2 mmol), activated Cu[7] (147.0 mg, 2 mmol), and dry toluene (21 mL). The reaction mixture was refluxed with a Dean–Stark trap filled with dry molecular sieves (4 Å) for 19 h. The reaction mixture was filtered and the filtercake was washed with EtOAc (4 × 25 mL). The pale yellow filtrate was washed with saturated Na_2CO_3 (25 mL) and water (2 × 20 mL). The combined water washings were back extracted with EtOAc (45 mL). The combined organic layers were dried over Na_2SO_4. Concentration under reduced pressure afforded an off-white solid (1.8 g), which was then purified by chromatography on silica gel (50 g) column, eluted with EtOAc/hexanes (1:4) to afford (**5**) as yellow oil (487.1 mg, 43.6%).

^{1}H-NMR ($CDCl_3$) ppm:δ7.80 (br, s, 1H, Ar-N$\underline{H}$-Ar), 7.34 (d, J = 9.0 Hz, 2H, Ar-$\underline{H}$), 6.97 (t, J = 9.0 Hz, 3H, Ar-$\underline{H}$), 3.86

(s, 2H, Ar(d4)-C$\underline{H}_2$), 3.22 (s, 3H, –CON(C$\underline{H}_3$)$_2$), 3.00 (s, 3H, –CON(C$\underline{H}_3$)$_2$).

2-(2-(2,6-Dichlorophenylamino)phenyl[$^2H_4$])acetic acid (6)

To a suspension of (**5**) (0.4 g, 1 mmol) in ethanol (14 mL) was added a solution of NaOH (0.4 g) in water (11 mL). The reaction mixture was purged with argon for 20 min, stirred under argon and under reflux for 12 h, and then cooled to RT. The pH of the pale-pink solution was first adjusted to 8 with 3 M HCl, and then to 6 with saturated NH_4Cl. The reaction mixture was extracted with EtOAc (3×25 mL). The combined EtOAc extracts were washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure to afford a pink solid (0.47 g), which was purified by chromatography on silica gel (30 g) column, eluted with CH_2Cl_2/MeOH (97:3). The desired product (**6**) was obtained as a pale pink solid (261.2 mg, 65.6%).

^{1}H-NMR ($CDCl_3$) ppm:δ7.31 (d, $J = 9.0$ Hz, 2H, Ar-$\underline{H}$), 6.98 (t, $J = 9.0$ Hz, 1H, Ar-$\underline{H}$), 6.80 (br, s, 1H, Ar-N$\underline{H}$-Ar), 3.85 (s, 2H, Ar-C$\underline{H}_2$). MS (EI, 70ev) *m/z* (%) 254(30), 298(100), 621(15).

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